Severe Preeclampsia is Associated with Alterations in Cytotrophoblasts of the Smooth Chorion

T. Garrido-Gomez\textsuperscript{1,2,3,4}, K. Ona\textsuperscript{1,2}, M. Kapidzic\textsuperscript{1,2}, M. Gormley\textsuperscript{1,2}, C. Simón\textsuperscript{3,4,5}, O Genbacev\textsuperscript{1,2,§}, S.J. Fisher\textsuperscript{1,2,6,7,*}

\textsuperscript{1}Center for Reproductive Sciences, \textsuperscript{2}Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California San Francisco, San Francisco, CA, 94143, \textsuperscript{3}Fundación Instituto Valenciano de Infertilidad (FIVI), Instituto Universitario IVI, INCLIVA, Valencia University, \textsuperscript{4}Igenomix, Parc Científic Universitat de València, Valencia, Spain, \textsuperscript{5}Department of Obstetrics and Gynecology, School of Medicine, Stanford University, \textsuperscript{6}The Eli & Edythe Broad Center for Regeneration Medicine and Stem Cell Research, \textsuperscript{7}Department of Anatomy, University of California San Francisco, San Francisco, CA 94143

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\textbf{*Corresponding author:} S.J. Fisher, University of California, San Francisco, Box 0665 San Francisco, CA 94143

Tel. 415-476-5297, email, sfisher@cgl.ucsf.edu

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**Summary Statement**

Here we show that cytotrophoblasts of the smooth chorion undergo a significant expansion in preeclampsia, which we theorize may be an attempt to compensate for a loss of placental function.

**Abstract**

Preeclampsia (PE), which affects ~8% of first pregnancies, is associated with faulty placentation. Extravillous cytotrophoblasts (CTBs) fail to differentiate properly, contributing to shallow uterine invasion and deficient spiral artery remodeling. We studied the effects of severe PE (sPE) on the smooth chorion portion of the fetal membranes. The results showed a significant expansion of the CTB layer. The cells displayed enhanced expression of stage-specific antigens that extravillous CTBs normally up regulate as they exit the placenta. Transcriptomics revealed the dysregulated expression of many genes (e.g., placental proteins, markers of oxidative stress). We confirmed a sPE-related increase in production of PAPPA1, which releases IGF-1 from its binding protein. IGF1 enhanced proliferation of smooth chorion CTBs, a possible explanation for expansion of this layer, which may partially compensate for the placental deficits.
Introduction

Human placentation involves a remarkable series of interactions between embryonic/fetal trophoblasts and maternal cells (Burton and Jauniaux, 2015; Maltepe and Fisher, 2015). In most of the villous placenta (chorion frondosum), cytotrophoblasts (CTBs) fuse to form a multinucleated epithelium that is perfused with maternal blood. Thus, they are ideally positioned to transport growth-promoting substances to the embryo/fetus, which they exchange for spent material. However, near the uterine wall, CTBs adopt a different fate. They leave the villi, forming columns of mononuclear cells, which anchor the placenta to the uterus, which they subsequently invade. The intricacies of CTB interactions with maternal cells that reside within the uterine wall involve many layers of cell-cell interactions. Within the interstitial compartment, they mingle with decidual and immune cells, eventually penetrating as far as the inner third of the myometrium. Invasive CTBs also remodel the uterine vasculature. They open up the spiral arteries, enabling placental perfusion, which substantially increases as they line and enlarge these vessels. They also breach uterine veins, which establishes venous return.

Defects in placentation are mechanistically related to several of the most clinically significant pregnancy complications. The CTB differentiation pathway that leads to uterine invasion appears to be particularly vulnerable, perhaps due to the unusual cell-cell interactions that occur, its explosive nature and the exceptional plasticity of the cells. Defects in this CTB subpopulation are associated with several pregnancy complications, but most consistently, preeclampsia (PE). In this syndrome, CTB invasion of the interstitial compartment is frequently shallow (Brosens et al., 1970). Similar patterns are observed during placentation in nonhuman primates, including the baboon and macaque (Enders et al., 2001), suggesting that this may not be a major determinant. Instead, failed vascular invasion is thought to be the critical defect, a theory that is bolstered by the fact that restricting blood flow to the uterus and placenta creates some of the clinical signs in animal models (Abitbol et al., 1977; Granger et al., 2006). As compared to normal pregnancy, many fewer spiral arteries show evidence of CTB remodeling, and the process is often less robust in those that do. Accordingly, they retain fundamental aspects of pre-pregnancy anatomy, which precludes carrying the amount of blood that the placenta needs to develop and function properly. Currently, development of PE is thought to involve a two-stage process in which abnormal placentation, the instigator, leads to a maternal inflammatory response (Redman and Sargent, 2005).
In comparison to the trophoblast components of the chorion frondosum, the CTBs that reside in the chorion laeve or smooth chorion (sch) have received little attention. schCTBs comprise the outer surface of the fetal membranes, external to the amnion and its stroma, which is shared with the smooth chorion. Here, this subpopulation of CTBs forms a second interface with the decidua, which they appear to invade, albeit more superficially than CTBs that emigrate from the chorion frondosum, which are found throughout the decidua basalis and inner third of the muscular portion of the uterine wall. Additionally, schCTBs do not invade uterine blood vessels. Thus, vascular remodeling is confined to the decidua basalis.

Very little is known about the functions of these cells. A recent review suggested that they might play an active role, via their invasive activity, in fusion of the fetal membranes with the parietal decidua (Genbacev et al., 2015). This CTB subpopulation could also be involved in rupture of the fetal membranes at birth. In this regard, inducible nitric oxide synthase and cyclooxygenase-2 contribute to the induction of apoptosis in these cells (Yuan et al., 2006; Yuan et al., 2009), as does an imbalance in the production and elimination of reactive oxygen species (Yuan et al., 2008). Here we tested the hypothesis that the defects in the chorion frondosum that are associated with PE extend to the smooth chorion. Surprisingly, the results showed changes consistent with the novel concept that, in this syndrome, the later population of CTBs expands, which could compensate for anatomical and functional deficits in the placenta.

Results

The CTB layer of the smooth chorion expands in sPE. In cases where the fetal membranes were intact, H&E staining revealed a single layer of amniotic epithelial (AMNION EP) cells that was connected to the smooth chorion via a shared stromal compartment. The outer CTB portion was in direct contact with the decidua parietalis. During early second trimester of normal pregnancy, the smooth chorion contained numerous villous remnants, termed ghost villi (GV) (Fig. 1A). The CTB layer (schCTB) was ill defined and in some areas the fetal cells intermingled with the decidua. As gestation proceeded, the number of GV declined precipitously (Fig. 1B). By the end of the second trimester, few-to-none of these structures were visible. The schCTB layer had a more condensed appearance, and there was a defined border between the fetal cells and the decidua (Fig. 1C). It is likely that the observed changes are secondary to several factors. One is the loss of a direct blood supply. Another is establishment of the utero-placental circulation at the end of the first trimester of pregnancy, which results in a significant rise in oxygen tension. Placental perfusion is most robust at the center, creating oxidative stress at the margins (Burton et al., 2010).
We also analyzed tissue sections of the intact fetal membranes from 5 sPE and 5 preterm labor cases with no signs of infection (nPTB) (25-34 wks). The maternal and neonatal characteristics are summarized in Table S1. Higher power magnifications of the photomicrographs revealed greater detail (Fig. 1) and lower power magnifications enabled assessment of nearly the entire membrane (Fig. S1). There were no notable differences between the appearances of the amniotic epithelium in the two pregnancy complications. In nPTB, some areas of the stroma appeared to be more loosely organized than in the equivalent layer from sPE cases (compare Fig. 1D, E). The major finding was the morphological resemblance of the smooth chorion from sPE cases to early and mid-second trimester samples (compare Fig. 1A, B, D). In addition to an expansion of the schCTB population, the layer was less organized and numerous GV were evident, which appeared with less frequency in samples from nPTB cases that had morphological features of late second trimester samples (compare Fig. 1C, E). In some nPTB samples, the adjacent decidual layer was also somewhat disorganized. Thus, in sPE, the schCTB layer of the fetal membranes retained morphological features of the earlier gestation samples.

To confirm and extend these findings we double immunostained fetal membrane samples with anti-cytokeratin (CK), which labeled CTBs and amniocytes, and anti-vimentin, which reacted with cells of the stromal layer of the membranes and the decidua (Fig. 2). As previously reported, amniocytes in some samples also gave a vimentin signal (Behzad et al., 1995). The results confirmed the intermixing of the schCTB layer and decidua in early second trimester samples and the reduction in GV as pregnancy neared the third trimester (Fig. 2A). CK immunolocalization confirmed the morphological differences between the sPE and nPTB samples (Fig. 2B) that were observed in the H&E stained specimens (Fig. 1D, E). Next, we quantified two major features of the schCTB layer during the second trimester of normal pregnancy and in sPE vs. nPTB. On average there was an approximate 5x decrease in the width of this layer during the second trimester (Fig. 2C). Measuring this parameter in sPE samples showed that the width of the schCTB layer was comparable to that of early second trimester specimens. In this regard, nPTB samples were similar to the mid and late second trimester specimens. As to GV, there was a dramatic reduction during the second trimester of normal pregnancy (Fig. 2D). In sPE, the schCTB layer contained ~2x the number of GV as compared to the nPTB samples, and the frequency was higher than in the mid and late second trimester specimens. Together these results suggested that the schCTB layer of the fetal membranes in sPE retained the morphological features of the early second trimester samples. In nPTB, this layer had the morphology of late second trimester samples from normal pregnancy.
sPE is associated with increased schCTBs expression of a combination of villous and extravillous CTB stage-specific antigens. Next we examined the expression of antigens that are misregulated in the basal plate region in pregnancies that are complicated by sPE. Specifically, invasive CTBs down regulate the expression of HLA-G (Lim et al., 1997), misexpress several integrins (Zhou et al., 1993), and up regulate E-cadherin (Zhou et al., 1997). These changes are associated with a failure of CTBs within the uterine wall to express vascular-type antigens (Zhou et al., 1997).

As to the smooth chorion in the second trimester of normal pregnancy, a subset of CTBs toward the decidual interface reacted with anti-HLA-G, -integrin α4 and E-cadherin (Fig. 3A, B, C). In general, the number of cells that immunostained was reduced (HLA-G and integrin α4) or nearly absent (E-cadherin) by late 2nd trimester. At this stage, immunopositive cells were most often found at the decidual boundary. We failed to detect expression of vascular-type molecules that vCTBs express within the uterine wall and blood vessels—PECAM, VCAM, VE-cadherin and VEGF-A by schCTBs (data not shown). Thus, this subpopulation of CTBs has similarities and differences from invasive extravillous cells.

In comparison to the second trimester of normal pregnancy, the proportion of cells that reacted with an antibody that specifically recognized HLA-G was higher in sPE (Fig. 3D). The fraction of cells that expressed this antigen in nPTB was similar to that observed in the late second trimester samples. With regard to the integrin α4 and E-cadherin expression patterns in the pregnancy complications that we studied, the major finding was up regulated expression in sPE as compared to nPTB (Fig. 3E, F). For the three antigens whose expression we studied, the immunopositive cells were oriented toward the decidua rather than the amnion. As in the second trimester samples from normal pregnancies, no expression of the vascular-type antigens we assayed was detected in the pregnancy complication groups (data not shown). Together, these results suggested that schCTBs in sPE vs. nPTB have more wide-spread expression of the stage-specific antigens that the extravillous subpopulation normally up regulate in columns and the superficial decidua.

During the second trimester of normal pregnancy isolated schCTBs are more invasive than villous CTBs. In these experiments, we quantified invasion by using a Matrigel penetration assay in which CTBs are plated on matrix-coated filters and the number of cells that reach the underside are counted. First, we compared the behavior of schCTBs and their villous (v) counterparts from the same donors. During the second trimester of normal pregnancy, CTBs from the smooth chorion were more invasive than vCTBs at all the gestational ages that
were examined (Fig. 4A, B). The invasiveness of CTBs isolated from both compartments decreased during the second trimester.

**sPE vs. nPTB is associated with an increase schCTB invasiveness.** Next we asked if the morphological and antigenic alterations that were observed in sPE were accompanied by functional changes. The maternal and infant characteristics of the samples that went into this analysis are included in Table S1. In sPE, schCTBs were once again more invasive than vCTBs and as compared to schCTBs and vCTBs in nPTB (Fig. 4A, B). Although variability impacted the statistical significance, CTBs from the sPE smooth chorion tended to mimic the invasion levels of the second trimester samples. In contrast, comparable levels of invasion were observed in the later second trimester, sPE and nPTB vCTB samples. Thus, these data suggested either an autocrine or a paracrine braking mechanism that restrains schCTB invasion *in vivo* and that sPE is associated with increased invasiveness of these cells, perhaps overcoming this barrier. We hypothesize that the sPE-related changes in the schCTBs subpopulation may be, at least in part, a compensatory mechanism for functional deficits in vCTBs, particularly with regard to differentiation along the extravillous pathway.

**sPE impacts schCTB gene expression.** To gain a better understanding of the molecular mechanisms behind the observed schCTB alterations in sPE, we used a laser capture microdissection approach to isolate this population of cells from the fetal membranes of sPE and nPTB pregnancies (n=4/group). The maternal and neonatal associated with the samples used for microarray analysis are summarized in, Table S2. There were no differences in maternal characteristics other than elevated blood pressure and proteinuria in the sPE group. Neonatal weights were also lower in this group.

Microarray analyses enabled a global comparison of the cells’ gene expression patterns in the two conditions. In sPE schCTBs, 116 genes were up-regulated (2-fold and higher) and 133 genes were down-regulated (2-fold and higher) as compared to nPTB (Fig. 5, 50 most highly differentially expressed; Fig. S2, complete list). The most highly up regulated genes were CSH1 (HPL, 26-fold), glutathione S-transferase alpha 3 (GSTA3; 13-fold) and PAPPA1 (9-fold). Two related molecules, PAPPA antisense RNA 1 and PAPPA2, were also up regulated (7-fold and 4-fold, respectively). Another placental lactogen, choricran somatomammotropin hormone 2, was up regulated 7-fold. Thus, several placenta-specific proteins were modulated in sPE as was GSTA3, which functions in hormone production and detoxification. Upregulation of TGFBR3L (6-fold), which binds TGFβ family members via its heparan sulfate chains, suggests the possibility of enhanced growth factors signaling in sPE.
Conversely, we found up regulation, in nPTB, of immune molecules, guanylate binding proteins 5 and 4 (GBPs; 20-fold and 9-fold, respectively), CXCL 10 (9-fold), ICAM 1 (6-fold), CD3D (6-fold), CCL5 (6-fold), CCL2 (5-fold), CD24 (5-fold) and CCL13 (4-fold). As a group they have many interesting immune-related functions. GBP4 dampens interferon-induced viral responses (Hu et al., 2011) and GBP5 is an interferon-inducible inhibitor of viral infectivity including HIV (Krapp et al., 2016). These chemokine-related and adhesion molecules promote chemotaxis of monocytes/macrophages, T cells, NK cells and dendritic cells; T cell adhesion to endothelial cells; and angiogenesis. ICAM strengthens intercellular adhesion, and CD3D is a component of the T-cell receptor. These data suggested that inflammation and immune processes were activated in nPTB even when there were no clinical signs of overt infection. Other genes that were down regulated in schCTBs from sPE as compared to nPTB (e.g., BMP2, 7-fold; FGFR2, 5-fold) may be related to aberrations in growth factor signaling.

We also compared gene expression of schCTBs in sPE and nPTB to the equivalent population of cells that were laser captured from fetal membranes, which were obtained during the second trimester of normal pregnancy (Fig. S3A). In general, the gene expression patterns of the nPTB samples were more similar to the second trimester schCTBs than those of the equivalent sPE population. Hierarchical clustering failed to entirely separate the second trimester and nPTB samples. In contrast, the equivalent cells from sPE cases clustered together (Fig. S3B). Thus, although the schCTB layer of the smooth chorion had morphological features of early second trimester samples, global transcriptional profiling showed major differences at a molecular level.

In comparison to normal second trimester schCTBs, the mostly highly up regulated genes in sPE samples (Fig. S4) were MT1H (23-fold) and MT1G (13-fold), evidence of oxidative stress, which was consistent with the vascular constriction that was observed in the adjacent decidual compartment (data not shown). Corneodesmosin, which is related to cornification of epithelial layers, was also highly up regulated (10-fold) in sPE. Whether or not this is functionally related to the expansion of the schCTB layer in these cases remains to be determined. PAPPA1 and GSTA3 expression was also higher than in normal second trimester samples (8-fold and 4-fold, respectively). With regard to genes whose expression was down regulated in sPE vs. second trimester samples, several chemokine family members were in this category, suggesting that their upregulation in nPTB may not be related to this condition, e.g., CD24 (10-fold), CXCL 10 (6-fold) and CCL2 (6-fold). Interestingly, expression of NPPB (11-fold), which acts to decrease blood pressure, was also reduced in sPE. Thus, immunolocalization of stage-specific antigens, invasion assays and gene expression data pointed to schCTB dysregulation in sPE.
We also compared gene expression of schCTBs in nPTB vs. the equivalent population of cells that were laser captured from fetal membranes that were obtained during the second trimester of normal pregnancy (Fig. S5). The most highly up regulated genes included a serine peptidase inhibitor (SPINK1; 12-fold), HLA-DR (12-fold) and BMP-2 (7-fold). The most highly down regulated genes included histone H2AM (11-fold) and CGA (8-fold). Overall, small nuclear RNAs were highly differentially expressed in this dataset.

**Validation of the differentially expressed genes at the protein level.** Next, we asked whether the highly up regulated mRNAs were accompanied by an equally dramatic increase in expression at the protein level. Accordingly, we immunolocalized CSH1 (HPL) in tissue sections of the fetal membranes from sPE and nPTB cases (n=4/group; Table S1). Little immunoreactivity was detected in the CTB layer of the smooth chorion from pregnancies that were complicated by nPTB. In contrast, sPE was associated with strong anti-CSH1 immunoreactivity in a subset of CTBs that tended to be located near the decidual junction (Fig. 6A). Essentially the same result was obtained for GSTA3. However, a greater proportion of the CTB population was immunopositive (Fig. 6B).

**PAPPA1 stimulated proliferation of schCTBs.** Finally, we were interested in determining if any of the up regulated molecules could be functionally related to the observed sPE-associated increase in the number of CTBs that resided within the smooth chorion. Given its ability to cleave IGFBP4 and-5, releasing the bound IGF-1 (Gaidamauskas et al., 2013), we were interested in the expression and function of PAPPA1. Immunolocalization of this antigen in the fetal membranes showed high CTB-associated immunoreactivity in sPE as compared to largely background staining in nPTB (Fig. 6C). Additionally, we assayed CTB secretion of PAPPA1 into the conditioned medium. In sPE, villous and schCTB release of PAPPA1 significantly increased as compared to the same populations in nPTB (Fig. 6D). Thus, this pregnancy complication is associated with highly up regulated production and secretion of PAPPA1 by CTBs of the placenta and smooth chorion.

To determine the consequences, we isolated schCTBs and vCTBs (n=3 second trimester samples/group) and cultured them in the presence of exogenous IGF-1. As compared to base-line (no addition), IGF-1 (2 and 10 ng) increased BrDU incorporation by schCTB, but not vCTBs (Figure 6E). Together these data suggested that sPE-associated increases in PAPPA1 could be at least partially responsible for the observed expansion of the schCTB layer in this pregnancy complication. Alternatively, enhanced expression of PAPPA1 and its effects on CTB proliferation could be attributable to a delay or halting of differentiation such
that, in sPE, the later gestation smooth chorion has features that are more typical of second trimester samples.

Discussion

It has been nearly 50 years since PE was linked to malformations of the maternal-fetal interface, in particular, deficient CTB remodeling of the uterine vasculature (Brosens et al., 1970). In normal pregnancy and in PE, very little is known about the other region where CTBs interface with the decidua—the smooth chorion. We studied this portion of the fetal membranes, during the second trimester of normal pregnancy, in relationship to sPE and nPTB. At a morphological level, two major features of the smooth chorion changed in the interval between early to late second trimester. The width of the CTB layer dramatically decreased along with the number of ghost villi. In sPE, the smooth chorion retained characteristics of the early gestation normal samples—a wide CTB layer with numerous ghost villi that were absent in the equivalent region of the nPTB samples, which resembled the morphology of the smooth chorion in the late second trimester of normal pregnancy.

At a molecular level, schCTBs were a distinct CTB subpopulation. Initially, we examined the cells’ expression of stage-specific antigens that villous CTBs modulate as they exit the chorion frondosum and invade the decidua. In line with the fact that there was no morphological evidence of uterine blood vessel invasion, the cells did not express the vascular-type cell adhesion molecules that they normally up regulate within the uterine wall, including PECAM, VCAM, VE-cadherin and VEGF-A (Zhou et al., 1997). They also did not express integrin $\alpha_1$, which plays an important role in invasion (Damsky et al., 1994). During the second trimester of normal pregnancy, they immunostained for a combination of antigens that are expressed by villous and invasive/extravillous CTBs: E-cadherin, integrin $\alpha_4$ and HLA-G. In general, expression of these molecules in normal pregnancy tended to be more widespread throughout the CTB layer in early second trimester samples than in later gestation when fewer cells were antibody-reactive. In this regard, HLA-G and integrin $\alpha_4$ expression were confined to a subset of CTBs that were in direct contact with the decidua. Thus, CTBs of the smooth chorion had an antigenic profile that resembled cells in proximal regions of columns that have initiated differentiation along the invasive pathway.

Previous studies reported similarities (e.g., EGF receptor expression (Bulmer et al., 1989)) and differences (e.g., lectin staining (Lalani et al., 1987)) between schCTBs and vCTBs. Additionally, production of renin (Poisner et al., 1981; Symonds et al., 1968) and its
substrate, angiotensinogen (Lenz et al., 1993), is concentrated in schCTBs. Yeh et al. (Yeh et al., 1989) described two mononuclear CTB populations in the chorion laeve. The first is vacuolated cells, with a clear cytoplasm, that is rich in pinocytotic vesicles and lipid droplets. They react with antibodies that recognize human placental lactogen and placental alkaline phosphatase. The role of the latter molecule in absorption led the authors to suggest that these cells might play a role in this process. The second population of cells has an eosinophilic cytoplasm, which lacked vacuoles, and they express neither antigen. Both schCTB subtypes failed to express other molecules that are characteristic of vCTBs, including prolactin, pregnancy specific beta 1 glycoprotein and hCG beta. The antigen positive and negative cells that we describe, which did not clearly correspond to the either of these populations, may be a mixture of the two.

The significant morphological changes that were associated with the CTB layer of the smooth chorion in sPE were accompanied by alterations in the expression of E-cadherin, integrin α4 and HLA-G. Specifically a large proportion of the CTBs expressed these antigens as compared to the same subpopulation in either the late second trimester of normal pregnancy or in nPTB. Thus, sPE is associated with morphological and molecular alterations in the CTB layer of the smooth chorion that were reminiscent of the villous immaturity involving the chorion frondosum that was noted many years ago (Hustin et al., 1984).

The gestation-related and sPE-associated differences in the CTB subpopulation of the smooth chorion suggested the possibility of functional alterations. Accordingly, we investigated this possibility by assaying their invasive ability. With regard to normal pregnancy, second trimester schCTBs were more invasive than vCTBs isolated from the same placenta. Additionally, we observed a gestational-age related decline in invasiveness. We speculate that this surprising difference may be indicative of an active role for these cells in attaching the fetal membranes to the decidua parietalis. Whether the decidua capsularis is lost in the process or fuses with rest of the decidua during this process is uncertain (Benirschke et al., 2012). The mechanisms that restrain schCTB invasion in vivo are unknown, but could include enhanced expression of PAI-1 by decidual cells in this region (Floridon et al., 2000).

Using the same experimental design, we investigated the impact of sPE on the invasiveness of schCTBs vs. vCTBs. Bolstering our finding that CTBs from the chorion are more invasive that their villous counterparts, we observed the same phenomenon in sPE and nPTB samples. Furthermore, schCTBs from the placentas of sPE patients were significantly more invasive than those from the placentas of women who experienced preterm birth. We
speculate that this may reflect an attempt to form more extensive interactions with maternal
cells perhaps for absorptive purposes. The enhanced invasiveness of schCTBS in sPE
together with the expansion of this layer described above may be in response to the
pathological alterations of the chorion frondosum and invasive/extravillous CTBs that are
associated with this pregnancy complication.

Global transcriptional profiling revealed the gene expression patterns of schCTBs in sPE as
compared to nPTB (and the second trimester of normal pregnancy). Previously, we used this
approach to compare vCTB gene expression in the same pregnancy complications
immediately after the cells were isolated and as they differentiated along the invasive
pathway over 48 h in culture (Zhou et al., 2013). A comparison of the two datasets yielded
new insights. First, the number and magnitude of changes in gene expression that were
evident in the schCTB dataset were far greater than those observed for vCTBs. This finding
suggested that sPE has very significant effects on the chorion laeve as well as the chorion
frondosum. Second, there was no overlap in the schCTB and vCTB genes that were
dysregulated in sPE and the same was true for nPTB. This result suggested that these are
two very different CTB subpopulations in normal pregnancy and in their transcriptional
responses to sPE and nPTB. For example, many of the PSGs were up regulated in vCTBs
isolated from the placentas of sPE cases (Zhou et al., 2013). In contrast, only PSG11 was
modulated (4-fold increase) in the equivalent schCTB dataset. Together these finding
suggested that schCTBs are active participants rather than passive bystanders to the
placenta’s role in pregnancy. In this regard it is interesting to note the geometry of the
placenta and the very large surface area formed by the smooth chorion. Our results
suggested the importance of considering this “second front” where fetal and maternal cells
directly interact, which, in addition to the chorion frondosum, could play an important role in
governing pregnancy outcome.

As to the dysregulated expression of specific genes, CSH1 (HPL) was the most highly up
regulated schCTB transcript in sPE. In vCTBs, another transcript from this locus, GH2, was
the most highly expressed gene (Zhou et al., 2013). In contrast to both findings, analyses of
placentas from sPE cases showed that transcription from the entire locus (GH2, CSH1,
CHS2 and CSHL1) was down regulated (Mannik et al., 2012). HPL, which is secreted into
maternal blood, regulates maternal metabolic adaptation to pregnancy. Consistent with its
role in promoting intrauterine growth, it is also found in the fetal circulation (Handwerger and
Freemark, 2000). Our data suggested that schCTBs may be a particularly important source
of the latter fraction. The GSTA3 transcript was also highly up regulated. This enzyme, a
member of the glutathione S transferase family, plays an important role in the generation of
intermediate metabolites in the biosynthesis of progesterone and testosterone (Johansson and Mannervik, 2001). Its enhanced expression in sPE suggested that schCTBs up regulate steroid production. In a rat model of this pregnancy complication, treatment with 17-hydroxyprogesterone improved uterine perfusion (Amaral et al., 2015). Whether our observation is related in terms of increased GSTA3 expression as being an attempt to up regulate blood flow to the placenta remains to be determined. Finally, schCTBs highly expressed PAPPA1 (and PAPPA2) in sPE vs. nPTB. This result was in accord with previous reports of elevated levels of this proteinase in maternal serum and/or placentas from PE cases (Kramer et al., 2016). Our investigation of increased PAPPA1 production suggested an autocrine role in expansion of the schCTB layer in sPE. Also, these data might be indicative of the smooth chorion retaining features of a more immature state. Finally, this analysis highlighted genes that were up regulated in nPTB. They included GBP4 and -5, which play important roles in inflammasome assembly and function (Shenoy et al., 2012; Tyrkalska et al., 2016), possible signals of the beginning of an inflammatory response despite the absence of clinical indicators.

We also compared gene expression of schCTBs in sPE and nPTB to schCTBs in the second trimester of normal pregnancy. In sPE, the highly up regulated genes included MT1H and MT1G (Fig. S4), metal-binding proteins that protect cells from oxidative stress (You et al., 2002). Thus, our results suggested that the smooth chorion is also affected by reduced uterine perfusion. We were surprised to find a strong upregulation of corneodesmosin in sPE. This molecule is the major component of desmosomes in the stratum corneum of the skin (Ishida-Yamamoto and Igawa, 2015). This raised the possibility that components of a desquamation-like process might be involved in separation of the smooth chorion from the uterus. In the skin, SPINK family members, serine proteinase inhibitors, prevent degradation of corneodesmosomes by inhibiting the actions of kallikreins. Interestingly SPINK1 was the most highly up regulated gene in schCTBs from nPTB vs. the second trimester of normal pregnancy (Fig. S5). In the second trimester comparison, natriuretic peptide B was the most highly down regulated gene in sPE. Deletion in mice results in impaired cardiac remodeling (Tamura et al., 2000), raising the possibility that this peptide could be involved in aspects of maternal vascular responses to pregnancy that are impaired in sPE.

In summary, we studied the impact of sPE on the CTB layer of the smooth chorion. In comparison to the equivalent region in nPTB, we found a large number of differences at morphological and functional levels. We also found that sPE had very different effects on the chorion frondosum and the chorion laeve, possible evidence that the CTBs in the two areas are different subpopulations of cells. The results also raised the possibility of crosstalk
between the two extraembryonic regions that enabled the smooth chorion to compensate for deficits in the villous placenta. In this new light, we proposed that CTBs that reside in the fetal membranes play a larger role in governing pregnancy outcome than was previously appreciated.

Materials and Methods

Human Tissue Collection. The UCSF Institutional Review Board approved this study. Informed consent was obtained from all donors. Samples were collected within 1 h of the procedure, washed in phosphate-buffered saline (PBS) and kept in cytowash medium (DME/H-21 Medium, 1% Glutamine Plus, 1% Penicillin/Streptomycin, 0.1% Gentamycin) supplemented with 2.5% FBS, placed on ice prior to processing. The second trimester samples were classified as early (15.5 to 17.6wks); mid (18 to 22.3wks) or late (22.4 to 24 wks). The clinical characteristics of the sPE and nPTB pregnancies are summarized in Table S1 and S2.

Immunolocalization. We published the methods we used (Genbacev et al., 2016). The antibodies we employed are listed in Table S3 along with the working concentrations.

Morphological evaluations of the smooth chorion. All biopsies (~10 x 20-40 mm) were obtained either 5-10 cm (second trimester) or 10-15 cm (term) from the chorionic plate, from random areas of the smooth chorion (≥ 3/case). They were washed in PBS, fixed with 4% paraformaldehyde for 30 min and infiltrated with 5-15% sucrose. The samples were rolled end-to-end, embedded in OCT, and frozen in liquid nitrogen. Tissue sections from second trimester, sPE and nPTB samples (n=5/each group) were stained with Hematoxylin and Eosin.

Immunolocalization of cytokeratin and vimentin was performed as described (Genbacev et al., 2016). Tilescan images of the entire tissue section were generated using a Leica DMI6000 B fluorescence microscope (Leica Microsystems) and LAS software. This method enabled evaluation of large area of the tissue (~10-30 x 1 mm wide). All measurements were done in Image J (version 1.45s). Three sections from two areas of early second trimester (n=3), mid second trimester (n=4), late second trimester (n=3), sPE (n=5) or nPTB (n=5) specimens were examined. The width of the CTB layer was measured from the inner edge of CTBs adjacent to the stroma to the outer edge that was adjacent to the decidua. Ten
random measurements were made over the entire length of the samples. In parallel, ghost villi in the same images were counted by using Image J.

**Isolation of villous cytotrophoblasts.** vCTBs were isolated from second and third trimester human placentas by published methods (Fisher et al., 1989; Kliman and Feinberg, 1990). Primary CTBs were from floating (second trimester and term) and anchoring villi (second trimester), which were dissected from the placentas. The isolated cytotrophoblasts were ≥90% pure as shown by staining for cytokeratin 7.

**Isolation of schCTBs.** After extensive washing with 1 X PBS (Ca++Mg++ Free) supplemented with 1% penicillin-streptomycin 100X, 0.3% fungizone 250 ug/ml and 1% gentamicin 50mg/ml, the amnion and smooth chorion were manually separated along their shared stromal plane. Next, the decidua parietalis was removed and discarded. The chorionic CTB layer was minced into small pieces (3-4mm) and subjected to a series of enzyme digestion steps. The first incubation (15-30 min) was in PBS (10 ml/gram of tissue) containing 3.5 mg collagenase, 1.2 mg DNase, 6.9mg hyaluronidase and 10 mg BSA. The supernatant was discarded. Then, the tissue was incubated for 20-40 min in PBS containing Trypsin 6.9 mg trypsin, 20 mg EDTA, 12 mg DNase per 100 ml (tissue wt: dissociation buffer volumen = 1:8). Enzyme activity was stopped by adding an equal volume of cytowash containing 10% FBS. The cell suspension was filtered through a 70 μm sterile strainer and centrifuged at 1200 xg for 7 min. A second collagenase digestion was performed by adding a 7X volume of the collagenase digestion buffer (see above), calculated on the basis of the weight of the cell pellet, followed by incubation for 15-30 min. The cell suspension was collected a second time by centrifugation. The cell pellets from the trypsin and second collagenase digestions were combined and purified over a Percoll gradient as described above for vCTBs.

**Invasion assays.** CTB invasion was quantified by using methods that we published (Genbacev et al., 2016). The entire experiment was repeated 3-4 times per group. Two inserts were evaluated for each sample type.

**Laser Microdissection (LMD).** We used laser capture microdissection to isolate schCTBs from normal second trimester, sPE or nPTB smooth chorion samples (n=4/each group). Portions of the smooth chorion, separated from the amnion as described above, were washed repeatedly in cold PBS to remove blood. Unfixed specimens were rolled end-to-end, placed in cryomolds containing OCT, frozen over a
dry ice/ethanol slurry, and stored at -80°C. The blocks were sectioned at 20 μm by using a Leica CM3050 cryostat, mounted on UV treated PEN-membrane slides (ThermoFisher Scientific), and stored under ice prior to laser capture microdissection later that day. Immediately prior to the procedure, sections were immersed in cold PBS until the OCT dissolved (~1 min), dipped in 0.1% toluidine blue for 30 s, washed in cold PBS, dehydrated (30 s/treatment) in a graded ethanol series (75%, 95%, 100%), then rapidly dried with compressed nitrogen. All solutions were made with nuclease-free water. schCTBs were laser dissected (Leica LMD 7000) and collected directly into RLT Plus Buffer (Qiagen RNeasy Plus Micro kit).

Total RNA was isolated according to the manufacturer’s protocol and concentrations were measured photometrically (NanoDrop 2000c). RNA integrity was determined via microfluidic phoresis (Agilent Bioanalyzer 2100). The samples were stored at -80°C.

**Microarray analyses.** Global gene profiling was accomplished by using the GeneChip HuGene 2.0 ST array (Affymetrix). Sample processing and hybridization were done according to protocols that were devised by the UCSF Gladstone (NHLBI) Genomics Core Facility. Gene level expression data quality was confirmed, normalized (RMA) and summarized GEO Accession no. GSE91189 (Affymetrix Expression Console Software). Significant differential expression was determined by statistical analysis of false discovery rate (FDR < 0.05) and absolute linear fold change >2 (Transcriptome Analysis Console Software).

**PAPPA1 ELISA.** Sch and vCTB conditioned medium was isolated from sPE (n=3) and control nPTB (n=3) samples. The PAPPA1 concentrations of technical replicated were assayed by using a commercial ELISA kit (LifeSpan BioSciences) according to the manufacture’s instructions. Sample values (pg/ml) were extrapolated from the standard curve.

**IGF-1-induced CTB proliferation.** Freshly isolated second trimester vCTBs and schCTBs were plated at a density of 8,400 cells per well of a 96-well plate coated with Matrigel diluted 1:2 in medium containing various concentrations of human recombinant IGF-1 (cat. no. 291-G1-200, R&D Systems, USA): 0, 2, 10, 50 and 75 ng/mL. BrDU (60 μM) was added simultaneously. After 16 h, BrDU incorporation was assessed according to the manufacturer’s directions (ab126556, Abcam, UK). The entire experiment was repeated three times with duplicate or triplicate technical replicates.
Statistical analysis. Data were shown as the mean ± SEM. Students’ onetailed t-distribution was used to compare the mean values among groups. Statistical significance was defined as a p value of < 0.05.
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Competing interests

Authors declare no conflict of interest.

Author contributions

T.G.G., K.O. and M.K. performed the experiments. M.G. prepared the samples for global transcriptional profiling and analyzed the data. C.S., O.G. and S.F. designed the experiments. O.G. and S.F. guided their execution. All authors contributed to the interpretation of the data. T.G.G., K.O. and M.G. prepared the figures and tables. T.G.G. and S.F. wrote the manuscript, which the other authors helped edit.

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Fig. 1. The cytotrophoblast (CTB) layer of the smooth chorion expands in sPE. Tissue sections from samples of this region, during the second trimester of normal pregnancy,
and specimens from non-infected preterm birth (nPTB) and severe preeclampsia (sPE) were stained with hematoxylin and eosin (H&E). (A) During early second trimester, the smooth chorion contained numerous ghost villi (GV). The CTB layer (schCTB) was disorganized, intermingling with the decidua. (B) As gestation proceeded, fewer GV were observed. (C) By the end of the second trimester, few (to no) GV were visible. The schCTBs became a defined layer adjacent to the decidua. (D) In sPE, the morphology of the smooth chorion resembled that of samples from the first half of the second trimester (compare Figure 1A, B and D). (E) In contrast, the morphology of this region in nPTB was comparable to that of late second trimester specimens (compare C and E). In some cases, the stroma was more loosely organized than in the equivalent layer from sPE cases (compare D and E). n = 5/group. EP, epithelium. Scale bar = 500 μm.
Fig. 2. Immunolocalization of cytokeratin (CK) confirmed expansion of the schCTB layer and retention of ghost villi in sPE. The samples were double immunostained. Anti-CK labeled CTBs and amniotic epithelial cells. Anti-vimentin (Vim) reacted with the...
decidua, the stromal layer of the fetal membranes and, in some samples, the amniotic epithelium. (A) The results confirmed intermixing of the schCTB layer and decidua in early second trimester samples, the reduction in GV as pregnancy advanced, and the reorganization into a defined layer by late second trimester. (B) CK immunolocalization also confirmed the morphological differences between the sPE and nPTB samples that were observed in the H&E stained specimens (see Fig.1D, E). (C) Quantification revealed an approximate 5-fold decrease in the width of the schCTB layer during the second trimester interval examined. The width of the schCTB layer in sPE was comparable to that of early second trimester specimens and nPTB samples were similar to the late second trimester samples. (D) Quantification revealed a dramatic reduction of GV during the second trimester of normal pregnancy. In sPE, the schCTB layer contained fewer GV than the early gestation samples, but more than in nPTB. n = 5/group. (C and D) Two areas of the same sample, denoted by individual colors, were analyzed. *p < 0.05; **p < 0.01; ***p < 0.001; n.s., not significant. a, amnion. Scale bars = 100 μm.
Fig. 3. In sPE, a subpopulation of schCTBs showed strong staining for stage-specific antigens, which invasive/extravillous CTBs up regulate as they emigrate from the placenta. (A) In early second trimester of normal pregnancy, a subset of CTBs throughout the smooth chorion, reacted with anti-HLA-G. By late second trimester, fewer cells immunostained. (B) The same general pattern was observed for anti-integrin α4 immunoreactivity. (C) Numerous schCTBs reacted with anti-E-cadherin in early second trimester. This subpopulation was largely absent in samples from the late second trimester of normal pregnancy. (D) As compared to second trimester and nPTB samples, the proportion of cells that reacted with anti-HLA-G was higher in sPE. (E) Expression of integrin α4 and (F) E-cadherin was up regulated in sPE as compared to nPTB. The images are representative of the analysis of a minimum of 3 sections from different samples (n = 5). a, amnion; Dec, decidua. Scale bars = 100 μm.
Fig. 4. Quantification of cytotrophoblast invasion: smooth chorion vs. chorionic villi and sPE vs. nPTB. Invasion was assayed by using a Matrigel penetration assay in which cells are plated on Matrigel-coated filters and the number of cells that reached the filter underside were counted. The overall approach was to compare the behavior of cytotrophoblasts isolated from the two compartments. (A, raw data; B, quantification)

With the exception of nPTB samples, cytotrophoblasts from the smooth chorion (schCTB) were more invasive than their villous counterparts (vCTBs). During the second trimester of normal pregnancy, the invasiveness of CTBs isolated from both compartments decreased with advancing gestational age. sPE was associated with increased invasiveness of schCTBs as compared to the comparable population of cells isolated from the placentas of nPTB cases such that the levels were similar to second trimester cells. n = 3-4 CTB preparations isolated from different placentas per experimental group (denoted by colors). Samples were assayed in duplicate. Bars = mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.
| schCTBs Symbol | sPE | nPTB | Title | Δ |
|----------------|-----|------|-------|---|
| CSH1           |     |      | chorionic somatomammotropin hormone 1 | 26 |
| GSTA3          |     |      | glutathione S-transferase alpha 3  | 13 |
| T1560          |     |      | T1560 protein                          | 12 |
| PAPPRA         |     |      | pregnancy-associated plasma protein A, pappalysin 1 | 9 |
| C2158C6.3      |     |      | putative novel transcript             | 8 |
| HTRA4          |     |      | HtrA serine peptidase 4              | 8 |
| PDIA3P1        |     |      | protein disulfide isomerase family A, member 3 pseudogene 1 | 8 |
| PAPPA-AS1      |     |      | PAPPA antisense RNA 1                | 7 |
| CHS2           |     |      | chorionic somatomammotropin hormone 2 | 7 |
| RP11-78C3.1    |     |      | novel transcript                      | 6 |
| RN12-63P       |     |      | RNA, U2 small nuclear RNA 63, pseudogene | 6 |
| TGFBR3L        |     |      | transforming growth factor, beta receptor III-like | 6 |
| RP11-327117.2  |     |      | novel transcript                      | 5 |
| PAPPA2         |     |      | pappalysin 2                          | 4 |
| AC004878.2     |     |      | novel transcript                      | 4 |
| LOC389765      |     |      | kinesin family member 27 pseudogene  | 4 |
| LINC01028      |     |      | long intergenic non-coding RNA 1028  | 4 |
| SCARN9L        |     |      | small Cajal body-specific RNA 9-like  | 4 |
| FARP3          |     |      | fatty acyl-CoA reductase 2 pseudogene | 4 |
| SNORD114-28    |     |      | small nucleolar RNA, C/D box 114-28  | 4 |
| GDPD1          |     |      | glycerolphosphate dehydrogenase domain containing 1 | 4 |
| CHRM5          |     |      | cholinergic receptor, muscarinic 5    | 4 |
| KCNJ16         |     |      | potassium inwardly-rectifying channel, subfamily J, member 16 | 4 |
| RP11-246A10.1  |     |      | novel transcript                      | 4 |
| RCEC           |     |      | reversion-inducing cysteine-rich protein with kazal motifs | 4 |
| CCL13          |     |      | chemokine (C-C motif) ligand 13       | 4 |
| KLHL2-IT1      |     |      | KLHL2 intronic transcript 1 (non-protein coding) | 5 |
| CD24           |     |      | CD24 molecule                         | 5 |
| BCL2A1         |     |      | BCL2-related protein A1               | 5 |
| FGFR2          |     |      | fibroblast growth factor receptor 2   | 5 |
| HRASLS2        |     |      | HRAS-like suppressor 2                | 5 |
| ITGA2          |     |      | integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor) | 5 |
| CCL2           |     |      | chemokine (C-C motif) ligand 2        | 5 |
| CCL5           |     |      | chemokine (C-C motif) ligand 5        | 6 |
| CD3D           |     |      | CD3d molecule, delta (CD3-TCR complex) | 6 |
| LINC0278       |     |      | long intergenic non-coding RNA 278    | 6 |
| SNORD75        |     |      | small nucleolar RNA, C/D box 75       | 6 |
| MR548K         |     |      | microRNA 548k                        | 6 |
| ICAM1          |     |      | intercellular adhesion molecule 1     | 6 |
| RN66-767P      |     |      | RNA, U6 small nuclear 767, pseudogene | 6 |
| ORL1           |     |      | oxidized low density lipoprotein (lectin-like) receptor 1 | 7 |
| BMP2           |     |      | bone morphogenetic protein 2          | 7 |
| RP11-67K17.4   |     |      | putative novel transcript             | 7 |
| RN66-874P      |     |      | RNA, U6 small nuclear 874, pseudogene | 7 |
| PMAIP1         |     |      | phosphol-12-myrst delaying-acid-induced protein 1 | 8 |
| CXCL310        |     |      | chemokine (C-X-C motif) ligand 10     | 9 |
| GBP4           |     |      | guanylate binding protein 4            | 9 |
| SNORD59A       |     |      | small nucleolar RNA, C/D box 59A      | 12 |
| RN66-1154P     |     |      | RNA, U6 small nuclear 1154, pseudogene | 14 |
| GBP5           |     |      | guanylate binding protein 5            | 20 |
Fig. 5. Global transcriptional profiling of cytotrophoblasts from the smooth chorion revealed sPE-associated aberrations in gene expression. The cells were isolated by laser capture microdissection. Then RNA was prepared and gene expression analyzed by using an Affimatrix Microarray platform. The relative expression levels of the 50 most highly differentially expressed genes (severe preeclampsia, sPE) vs. non infected preterm birth (nPTB) are shown as a heat map (red, up regulated; blue, down regulated). The fold changes are shown on the right ($\Delta$). $n = 4$ sPE samples and 4 nPTB samples.
Fig. 6. Confirmation of the microarray results: protein level and functional data. CSH1 (HPL), GSTA3 and PAPPA1 immunoreactivity corroborated differential expression at the RNA level. The identity of cytotrophoblasts in the smooth chorion (schCTBs) was confirmed by cytokeratin (CK) expression. (A) Little to no signal for CSH1 (HPL) was detected in cases of non-infected preterm birth (nPTB). In contrast, a subset of schCTBs interspersed among the immunonegative cells stained strongly with an antibody that recognized this molecule. (B) In sPE, the same pattern of differential expression was observed for GSTA3 except that immunoreactivity was more widespread among the
schCTBs and the signal was associated with cells that were adjacent to the decidua (dec) parietalis. (C) Immunolocalization of PAPP in the fetal membranes showed high CTB associated immunoreactivity in sPE as compared to largely background staining in nPTB. The images are representative of the analysis of a minimum of 3 sections from different areas of smooth chorion biopsies for each case (n = 4/group). Cytokeratin (CK7) expression confirmed trophoblast identity and DAPI staining enabled visualization of nuclei. a, amnion; dec, decidua. Scale bar: 100 μm. (D) ELISA quantification of CTB PAPPA1 secretion into the culture medium. In sPE, villous (v)CTB and schCTB release of PAPPA1 significantly increased as compared to the same subpopulations of cells in nPTB. n= 3/group. (E) Isolated schCTBs and vCTBs (n=3 second trimester samples/group) were cultured in the presence of exogenous IGF-1. As compared to baseline (no addition), IGF-1 (2 and 10 ng) increased BrDU incorporation by schCTB, but not vCTBs. *p < 0.05; **p < 0.01.
Supplemental Figure 1. Low power micrograph of H&E stained fetal membranes showed that severe preeclampsia (sPE) was associated with expansion of the CTB layer of the smooth chorion (schCTB), a phenomenon which was not observed in equivalent samples from cases of non-infected preterm birth. n=5/group; scale bar: 500 μm.
| Symbol       | Title                                                                 | Δ  |
|--------------|-----------------------------------------------------------------------|----|
| CSH1         | chorionic somatomammotropin hormone 1                                 | 26 |
| GSTA3        | glutathione S-transferase alpha 3                                     | 13 |
| T1560        | T1560 protein                                                         | 12 |
| PAPPA        | pregnancy-associated plasma protein A, pappalysin 1                   | 9  |
| XX-C2158C6.3 | putative novel transcript                                             | 8  |
| HTRA4        | HtrA serine peptidase 4                                              | 8  |
| PDIA3P1      | protein disulfide isomerase family A, member 3 pseudogene 1           | 8  |
| PAPPA-AS1    | PAPPA antisense RNA 1                                                | 7  |
| CSH2         | chorionic somatomammotropin hormone 2                                 | 7  |
| RP11-78C3.1  | novel transcript                                                      | 6  |
| RNU2-63P     | RNA, U2 small nuclear 63, pseudogene                                 | 6  |
| TGFBR3L      | transforming growth factor, beta receptor III-like                    | 6  |
| RP11-327J17.2| novel transcript                                                      | 5  |
| PAPPA2       | pappalysin 2                                                          | 4  |
| AC004878.2   | novel transcript                                                      | 4  |
| LOC389765    | kinesin family member 27 pseudogene                                  | 4  |
| LINC01028    | long intergenic non-protein coding RNA 1028                          | 4  |
| SCARNA9L     | small Cajal body-specific RNA 9-like                                  | 4  |
| FAR2P3       | fatty acyl CoA reductase 2 pseudogene                                | 4  |
| SNORD114-28  | small nucleolar RNA, C/D box 114-28                                  | 4  |
| GDPD1        | glycerophosphodiester phosphodiesterase domain containing 1          | 4  |
| CHRM5        | cholinergic receptor, muscarinic 5                                   | 4  |
| KCNJ16       | potassium inwardly-rectifying channel, subfamily J, member 10        | 4  |
| RP11-246A10.1| novel transcript                                                      | 4  |
| RECK         | reversion-inducing-cysteine-rich protein with kazal motifs           | 4  |
| PDIA5        | protein disulfide isomerase family A, member 5                        | 4  |
| PSG11        | pregnancy specific beta-1-glycoprotein 11                            | 4  |
| RAB9BP1      | RAB9B, member RAS oncogene family pseudogene 1                       | 4  |
| LINC01338    | long intergenic non-protein coding RNA 1338                          | 4  |
| ADAM12       | ADAM metallopeptidase domain 12                                      | 4  |
| FBXO9        | F-box protein 9                                                       | 4  |
| DGCR11       | DiGeorge syndrome critical region gene 11 (non-protein coding)       | 3  |
| SNORD8       | small nucleolar RNA, C/D box 8                                       | 3  |
| MAP7D3       | MAP7 domain containing 3                                             | 3  |
| SLC23A2      | solute carrier family 23 (ascorbic acid transporter), member 2       | 3  |
| LIFR         | leukemia inhibitory factor receptor alpha                             | 3  |
| HYAL4        | hyaluronoglucoaminidase 4                                            | 3  |
| AC004878.8   | novel transcript                                                      | 3  |
| GPR126       | G protein-coupled receptor 126                                       | 3  |
| FGG          | fibrinogen gamma chain                                               | 3  |
| HEBX         | hexosaminidase B (beta polypeptide)                                  | 3  |
| RNU6-989P    | RNA, U6 small nuclear 989, pseudogene                               | 3  |
| POTEE        | POTE ankyrin domain family, member E                                 | 3  |
| POTEF        | POTE ankyrin domain family, member F                                 | 3  |
| MIR924       | microRNA 924                                                         | 3  |
| RP4-693M11.3 | novel transcript, antisense to DNAL1                                 | 3  |
| NAIP         | NLR family, apoptosis inhibitory protein                             | 3  |
| DDX43        | DEAD (Asp-Glu-Ala-Asp) box polypeptide 43                            | 3  |
| SNORA6       | small nucleolar RNA, H/ACA box 6                                     | 3  |
| MEP1A        | meprin A, alpha (PABA peptide hydrolase)                             | 3  |
| sPE | Symbol       | Title                                                                 | Δ |
|-----|-------------|----------------------------------------------------------------------|---|
|     | PDPR        | pyruvate dehydrogenase phosphatase regulatory subunit                 | 3 |
|     | BGN         | biglycan                                                              | 3 |
|     | LOC440173   | uncharacterized LOC440173                                            | 3 |
|     | LOC101926943| uncharacterized LOC101926943                                         | 3 |
|     | RNU12       | RNA, U12 small nuclear                                                | 3 |
|     | RNA5SP435   | RNA, 5S ribosomal pseudogene 435                                     | 3 |
|     | SH3BP5-AS1  | SH3BP5 antisense RNA 1                                                | 3 |
|     | CTD-2363C16.1 | novel transcript                                                       | 3 |
|     | C8orf48     | chromosome 8 open reading frame 48                                   | 3 |
|     | LRRC17      | leucine rich repeat containing 17                                     | 3 |
|     | RNU7-123P   | RNA, U7 small nuclear 123 pseudogene                                  | 3 |
|     | ZNF283      | zinc finger protein 283                                               | 3 |
|     | KCNJ2       | potassium inwardly-rectifying channel, subfamily J, member 2          | 3 |
|     | ITM2C       | integral membrane protein 2C                                          | 3 |
|     | SUN3        | Sad1 and UNC84 domain containing                                     | 3 |
|     | LOC220729   | succinate dehydrogenase complex, subunit A, flavoprotein (Fp) pseudogene | 2 |
|     | KRT223P     | keratin 223 pseudogene                                               | 2 |
|     | CXorf58     | chromosome X open reading frame 58                                    | 2 |
|     | LOC100506922| basic proline-rich-like                                              | 2 |
|     | EXO5        | exonuclease 5                                                         | 2 |
|     | SMAD4       | SMAD family member 4                                                 | 2 |
|     | ENG         | endoglin                                                              | 2 |
|     | RP11-288L9.1 | putative novel transcript                                             | 2 |
|     | TSPAN10     | tetraspanin 10                                                       | 2 |
|     | LOC100506746| uncharacterized LOC100506746                                         | 2 |
|     | MIR3942     | microRNA 3942                                                        | 2 |
|     | MT1F        | metallocionine 1F                                                     | 2 |
|     | MKLN1-AS    | MKLN1 antisense RNA                                                  | 2 |
|     | MAGEH1      | melanoma antigen family H, 1                                         | 2 |
|     | LOC101928062| uncharacterized LOC101928062                                         | 2 |
|     | PCBD2       | pterin-4 alpha-carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1) 2 | 2 |
|     | TDRD6       | tudor domain containing 6                                            | 2 |
|     | ZNF594      | zinc finger protein 594                                               | 2 |
|     | ICA1L       | islet cell autoantigen 1,69kDa-like                                   | 2 |
|     | SPATA17     | spermatogenesis associated 17                                         | 2 |
|     | NBPF1       | neuroblastoma breakpoint family, member 1                            | 2 |
|     | PTPRQ       | protein tyrosine phosphatase, receptor type, Q                       | 2 |
|     | RNU6-806P   | RNA, U6 small nuclear 806, pseudogene                                | 2 |
|     | RPS6KA6     | ribosomal protein S6 kinase, 90kDa, polypeptide 6                     | 2 |
|     | RP11-317G6.1 | novel transcript, antisense to HERC1                                 | 2 |
|     | SLC29A3     | solute carrier family 29 (equilibrative nucleoside transporter), member 3 | 2 |
|     | CABLES1     | Cdk5 and Abl enzyme substrate 1                                       | 2 |
|     | ATP8A2      | ATPase, aminophospholipid transporter, class I, type 8A, member 2     | 2 |
|     | MIR4759     | microRNA 4759                                                        | 2 |
|     | HDAC5       | histone deacetylase 5                                                | 2 |
|     | TGFIF2LY    | TGFβ-induced factor homeobox 2-like, Y-linked                         | 2 |
|     | EOGT        | EGF domain-specific O-linked N-acetylglucosamine (GlcNAc) transferase | 2 |
|     | SCG2        | secretogranin II                                                     | 2 |
|     | ERVV-2      | endogenous retrovirus group V, member 2                               | 2 |
|     | TCEA3       | transcription elongation factor A (SII), 3                            | 2 |
| sPE | nPTB | Symbol | Title | Δ |
|-----|------|--------|-------|---|
|     |      | TNKS2  | tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase 2 | 2 |
|     |      | RNU6-858P | RNA, U6 small nuclear 858, pseudogene | 2 |
|     |      | RP11-545P7.4 | novel transcript, antisense to C12orf52 | 2 |
|     |      | LINC01003 | long intergenic non-protein coding RNA 1003 | 2 |
|     |      | CROCCP3 | ciliary rootlet coiled-coil, rootletin pseudogene 3 | 2 |
|     |      | ZFYVE9  | zinc finger, FYVE domain containing 9 | 2 |
|     |      | RNU6-77P  | RNA, U6 small nuclear 77, pseudogene | 2 |
|     |      | HABP4  | hyaluronan binding protein 4 | 2 |
|     |      | OR10A3 | olfactory receptor, family 10, subfamily A, member 3 | 2 |
|     |      | RP1-50J22.4 | putative novel transcript | 2 |
|     |      | SH3BP5  | SH3-domain binding protein 5 (BTK-associated) | 2 |
|     |      | SPATA17-AS1 | SPATA17 antisense RNA 1 | 2 |
|     |      | USP50  | ubiquitin specific peptidase 50 | 2 |
|     |      | DCLRE1C  | DNA cross-link repair 1C | 2 |
|     |      | PROSC  | proline synthetase co-transcribed homolog (bacterial) | 2 |
|     |      | RP11-585P4.5 | novel transcript, antisense to GLIPR1 | 2 |
|     |      | CTD-2501M5.1 | novel transcript | 2 |
|     |      | KIAA1958 | KIAA1958 | 2 |
|     | 0    | NNMT  | nicotinamide N-methyltransferase | 2 |
|     | 0    | RNA5SP356 | RNA, 5S ribosomal pseudogene 356 | 2 |
|     | 0    | RNU6-557P | RNA, U6 small nuclear 557, pseudogene | 2 |
|     | 0    | LOC100129617 | uncharacterized LOC100129617 | 2 |
|     | 0    | P2RY6  | pyrimidinergic receptor P2Y, G-protein coupled, 6 | 2 |
|     | 0    | RNU6-1157P | RNA, U6 small nuclear 1157, pseudogene | 2 |
|     | 0    | TRIM22  | tripartite motif containing 22 | 2 |
|     | 0    | MAST4  | microtubule associated serine/threonine kinase family member 4 | 2 |
|     | 0    | HLA-B  | major histocompatibility complex, class I, B | 2 |
|     | 0    | CD80  | CD80 molecule | 2 |
|     | 0    | HLA-A  | major histocompatibility complex, class I, A | 2 |
|     | 0    | IL10RA  | interleukin 10 receptor, alpha | 2 |
|     | 0    | EPHA5-AS1 | EPHA5 antisense RNA 1 | 2 |
|     | 0    | RP11-217B7.2 | novel transcript | 2 |
|     | 0    | NPPB  | natriuretic peptide B | 2 |
|     | 0    | HMMR  | hyaluronan-mediated motility receptor (RHAMM) | 2 |
|     | 0    | LOC102723672 | uncharacterized LOC102723672 | 2 |
|     | 0    | PSTPIP2  | proline-serine-threonine phosphatase interacting protein 2 | 2 |
|     | 0    | ITK  | IL2-inducible T-cell kinase | 2 |
|     | 0    | RNA5SP234 | RNA, 5S ribosomal pseudogene 234 | 2 |
|     | 0    | CASP4  | caspase 4, apoptosis-related cysteine peptidase | 2 |
|     | 0    | MIR1204  | microRNA 1204 | 2 |
|     | 0    | LINC01057  | long intergenic non-protein coding RNA 1057 | 2 |
|     | 0    | CNN3  | calponin 3, acidic | 2 |
|     | 0    | SNAR-C2  | small ILF3/NF90-associated RNA C2 | 2 |
|     | 0    | TMEM52B  | transmembrane protein 52B | 2 |
|     | 0    | RNA5SP88  | RNA, 5S ribosomal pseudogene 88 | 2 |
|     | 0    | RASA3  | RAS p21 protein activator 3 | 2 |
|     | 0    | KRT17P1  | keratin 17 pseudogene 1 | 2 |
|     | 0    | NUAK2  | NUAK family, SNF1-like kinase, 2 | 2 |
|     | 0    | RNA5-8SP3 | RNA, 5.8S ribosomal pseudogene 3 | 2 |
|     | 0    | SNORA84  | small nucleolar RNA, H/ACA box 84 | 2 |
| sPE | Symbol   | Title                                                                 | Δ  |
|-----|----------|----------------------------------------------------------------------|----|
|     | CLDN6    | claudin 6                                                             | -2 |
|     | RNA5SP22 | RNA, 5S ribosomal pseudogene 22                                       | -2 |
|     | EPCAM    | epithelial cell adhesion molecule                                      | -2 |
|     | IFITM1   | interferon induced transmembrane protein 1                            | -2 |
|     | RN6U-707P| RNA, U6 small nuclear 707, pseudogene                                 | -2 |
|     | CD47     | CD47 molecule                                                          | -2 |
|     | LOC391003| PRAME family member-like                                               | -2 |
|     | TAP1     | transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)            | -2 |
|     | SLC44A3  | solute carrier family 44, member 3                                    | -2 |
|     | YBX3P1   | Y box binding protein 3 pseudogene                                    | -2 |
|     | HIST1H2AE| histone cluster 1, H2ae                                               | -2 |
|     | RN6U-74P | RNA, U6 small nuclear 74, pseudogene                                  | -2 |
|     | IL4R     | interleukin 4 receptor                                                 | -2 |
|     | RP11-290L1.3| novel transcript antisense to PHLDA1                                  | -2 |
|     | SLAMF8   | SLAM family member 8                                                   | -2 |
|     | IL2RG    | interleukin 2 receptor, gamma                                          | -3 |
|     | PLAUR    | plasminogen activator, urokinase receptor                              | -3 |
|     | RP11-10J5.1| putative novel transcript                                             | -3 |
|     | VTRNA1-3 | vault RNA 1-3                                                          | -3 |
|     | RP11-212I21.2| novel transcript                                                      | -3 |
|     | SNORD50B | small nucleolar RNA, C/D box 50B                                       | -3 |
|     | IRF1     | interferon regulatory factor 1                                         | -3 |
|     | CD96     | CD96 molecule                                                          | -3 |
|     | MIR3529  | microRNA 3529                                                         | -3 |
|     | C6orf48  | chromosome 6 open reading frame 48                                    | -3 |
|     | DRAM1    | DNA-damage regulated autophagy modulator 1                             | -3 |
|     | HIST1H3F | histone cluster 1, H3f                                                 | -3 |
|     | PLAU     | plasminogen activator, urokinase                                      | -3 |
|     | NEAT1    | nuclear paraspeckle assembly transcript 1 (non-protein coding) (microRNA 612) | -3 |
|     | RP4-694A7.2| putative novel transcript                                             | -3 |
|     | SNORD52  | small nucleolar RNA, C/D box 52                                       | -3 |
|     | CCL4     | chemokine (C-C motif) ligand 4                                         | -3 |
|     | CD48     | CD48 molecule                                                          | -3 |
|     | XXbac-BPG252P9| novel transcript antisense to IER3                                    | -3 |
|     | RNY4P20  | RNA, Ro-associated Y4 pseudogene 20                                   | -3 |
|     | RN6U-1316P| RNA, U6 small nuclear 1316, pseudogene                                | -3 |
|     | ANKR5D4  | ankyrin repeat domain 54                                               | -3 |
|     | SKA3     | spindle and kinetochore associated complex subunit 3                   | -3 |
|     | GZMB     | granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1) | -3 |
|     | RN4U-62P | RNA, U4 small nuclear 62, pseudogene                                  | -3 |
|     | RN6U-1187P| RNA, U6 small nuclear 1187, pseudogene                                | -3 |
|     | RP1-239B22.5| antisense to KCNJ11 and overlapping to a novel gene                  | -3 |
|     | GBP1P1   | guanylate binding protein 1, interferon-inducible pseudogene 1        | -3 |
|     | SQDRL    | sulfide quinone reductase-like (yeast)                                 | -3 |
|     | RN6U-226P| RNA, U6 small nuclear 226, pseudogene                                 | -3 |
|     | CXCL2    | chemokine (C-X-C motif) ligand 2                                       | -3 |
|     | HIST1H2AK| histone cluster 1, H2ak                                                | -3 |
|     | MIR28    | microRNA 28                                                            | -3 |
|     | MIR3671  | microRNA 3671                                                          | -3 |
|     | RP11-102L12.2| novel transcript antisense to RASGRP1                                | -3 |
| Symbol   | Title                                                                 | Δ  |
|----------|----------------------------------------------------------------------|----|
| RNU6-1330P | RNA, U6 small nuclear 1330, pseudogene                             | -3 |
| AREG     | amphiregulin                                                        | -3 |
| HIST1H4L | histone cluster 1, H4I                                             | -3 |
| GBP2     | guanylate binding protein 2, interferon-inducible                   | -3 |
| C10orf55 | chromosome 10 open reading frame 55                                | -3 |
| SOCS3    | suppressor of cytokine signaling 3                                 | -3 |
| HIST1H3I | histone cluster 1, H3i                                             | -3 |
| MIR1323  | microRNA 1323                                                       | -3 |
| LOC541472 | uncharacterized LOC541472                                          | -4 |
| SNORD16  | small nucleolar RNA, C/D box 16                                     | -4 |
| SOCS1    | suppressor of cytokine signaling 1                                 | -4 |
| SIPA1L1  | signal-induced proliferation-associated 1 like 1                   | -4 |
| RP11-92K15.1 | novel transcript                                                       | -4 |
| FOSL1    | FOS-like antigen 1                                                  | -4 |
| MIR302A  | microRNA 302a                                                       | -4 |
| RNU6-890P | RNA, U6 small nuclear 890, pseudogene                              | -4 |
| GBP1     | guanylate binding protein 1, interferon-inducible                   | -4 |
| SNORA29  | small nucleolar RNA, H/ACA box 29                                   | -4 |
| SNORD63  | small nucleolar RNA, C/D box 63                                     | -4 |
| JUNB     | jun B proto-oncogene                                                | -4 |
| CCL4L2   | chemokine (C-C motif) ligand 4-like 2                               | -4 |
| SLAMF7   | SLAM family member 7                                                | -4 |
| KRT17    | keratin 17                                                          | -4 |
| RNU6-638P | RNA, U6 small nuclear 638, pseudogene                              | -4 |
| CCL13    | chemokine (C-C motif) ligand 13                                     | -4 |
| KLHL22-IT1 | KLHL22 intronic transcript 1 (non-protein coding)                  | -5 |
| CD24     | CD24 molecule                                                       | -5 |
| BCL2A1   | BCL2-related protein A1                                              | -5 |
| FGFR2    | fibroblast growth factor receptor 2                                 | -5 |
| HRASLS2  | HRAS-like suppressor 2                                              | -5 |
| ITGA2    | integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)        | -5 |
| CCL2     | chemokine (C-C motif) ligand 2                                      | -5 |
| CCL5     | chemokine (C-C motif) ligand 5                                      | -6 |
| CD3D     | CD3d molecule, delta (CD3-TCR complex)                              | -6 |
| LINC00278 | long intergenic non-protein coding RNA 278                         | -6 |
| SNORD75  | small nucleolar RNA, C/D box 75                                     | -6 |
| MIR548K  | microRNA 548k                                                       | -6 |
| ICAM1    | intercellular adhesion molecule 1                                   | -6 |
| RNU6-767P | RNA, U6 small nuclear 767, pseudogene                              | -6 |
| OLR1     | oxidized low density lipoprotein (lectin-like) receptor 1           | -7 |
| BMP2     | bone morphogenetic protein 2                                        | -7 |
| RP1-67K17.4 | putative novel transcript                                      | -7 |
| RNU6-874P | RNA, U6 small nuclear 874, pseudogene                              | -7 |
| PMAIP1   | phorbol-12-myristate-13-acetate-induced protein 1                   | -8 |
| CXCL10   | chemokine (C-X-C motif) ligand 10                                   | -9 |
| GBP4     | guanylate binding protein 4                                         | -9 |
| SNORD59A | small nucleolar RNA, C/D box 59A                                    | -12 |
| RNU6-1154P | RNA, U6 small nuclear 1154, pseudogene                            | -14 |
| GBP5     | guanylate binding protein 5                                         | -20 |
Supplemental Figure 2. Global transcriptional profiling of cytotrophoblasts from the smooth chorion revealed sPE-associated aberrations in gene expression as compared to the equivalent subpopulation of cells from cases of non-infected preterm birth. The heatmap lists genes that were differentially expressed by 2-fold or greater. The fold changes are shown on the right (△).
Supplemental Figure 3. Overview of the global transcriptional profiling data for cytotrophoblasts of the smooth chorion in severe preeclampsia (sPE), non-infected preterm birth (nPTB) and the late second trimester of normal pregnancy (2nd Late). (A) Overlap of the differentially expressed genes in the three sample groups. Circle sizes are proportional to the number of differentially expressed genes. (B) Hierarchical clustering failed to completely separate the nPTB and Late 2nd trimester samples. In contrast, the sPE data clustered together.
| Symbol  | Title                                                                 | 2nd | sPE |
|---------|------------------------------------------------------------------------|-----|-----|
| MT1H    | metallothionein 1H                                                     |     | 23  |
| MT1G    | metallothionein 1G                                                     |     | 13  |
| KRT24   | keratin 24                                                             |     | 11  |
| CDSN    | corneodesmosin                                                        |     | 10  |
| PAPPA   | pregnancy-associated plasma protein A, pappalyasin 1                   |     | 8   |
| OR2T5   | olfactory receptor, family 2, subfamily T, member 5                   |     | 6   |
| RP11-78C3.1 | novel transcript                                             |     | 5   |
| PAPPA-AS1 | PAPPA antisense RNA 1                                         |     | 5   |
| KRT223P | keratin 223 pseudogene                                                |     | 5   |
| TRAJ59  | T cell receptor alpha joining 59 (non-functional)                    |     | 4   |
| ATP13A4-AS1 | ATP13A4 antisense RNA 1                                    |     | 4   |
| NRCAM   | neuronal cell adhesion molecule                                        |     | 4   |
| MTHFD1  | formyltetrahydrofolate synthetase                                     |     | 4   |
| SCEL    | sciellin                                                               |     | 4   |
| PTPRQ   | protein tyrosine phosphatase, receptor type, Q                        |     | 4   |
| RP11-407H12.8 | novel transcript                                       |     | 4   |
| GSTA3   | glutathione S-transferase alpha 3                                     |     | 4   |
| RNY4P23 | RNA, Ro-associated Y4 pseudogene 23                                   |     | 4   |
| T1560   | T1560 protein                                                         |     | 4   |
| LOC103352541 | uncharacterized LOC103352541                                      |     | 4   |
| LOC100130476 | uncharacterized LOC100130476                                       |     | 4   |
| PRKXP1  | protein kinase, X-linked, pseudogene 1                                |     | 4   |
| RNU12   | RNA, U12 small nuclear                                                |     | 3   |
| SNORD48 | small nucleolar RNA, C/D box 48                                       |     | 3   |
| RNU6-1065P | RNA, U6 small nuclear 1065, pseudogene                              |     | 3   |
| MIR3908 | microRNA 3908                                                         |     | 3   |
| LOC255187 | uncharacterized LOC255187                                           |     | 3   |
| TTC21B-AS1 | TTC21B antisense RNA 1                                               |     | 3   |
| MIR630  | microRNA 630                                                          |     | 3   |
| PL2A4A  | phospholipase A2, group IVA (cytosolic, calcium-dependent)            |     | 3   |
| LOC101928461 | uncharacterized LOC101928461                                       |     | 3   |
| RNU5F-4P | RNA, U5F small nuclear 4, pseudogene                                  |     | 3   |
| RP11-65J21.1 | novel transcript                                      |     | 3   |
| LPAL2   | lipoprotein, Lp(a)-like 2, pseudogene                                 |     | 3   |
| SNORA6  | small nucleolar RNA, H/ACA box 6                                      |     | 3   |
| DNAH11  | dynein, axonemal, heavy chain 11                                      |     | 3   |
| AQP9    | aquaporin 9                                                           |     | 3   |
| IL13RA2 | interleukin 13 receptor, alpha 2                                      |     | 3   |
| TGFB3L  | transforming growth factor, beta receptor III-like                    |     | 3   |
| LINC01338 | long intergenic non-protein coding RNA 1338                        |     | 3   |
| LINC00330 | long intergenic non-protein coding RNA 330                          |     | 3   |
| RNU6-732P | RNA, U6 small nuclear 732, pseudogene                                |     | 3   |
| FCGR1C  | Fc fragment of IgG, high affinity Ic, receptor (CD64), pseudogene    |     | 3   |
| SERPINA3 | serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3 |     | 3   |
| KDR     | kinase insert domain receptor (a type III receptor tyrosine kinase)  |     | 3   |
| IGHD2-8 | immunoglobulin heavy diversity 2-8                                     |     | 3   |
| ICA1L   | islet cell autoantigen 1,69kDa-like                                   |     | 3   |
| RNA5SP444 | RNA, 5S ribosomal pseudogene 444                                     |     | 3   |
| RP11-458D21.1 | novel transcript                                       |     | 3   |
| SMAD4   | SMAD family member 4                                                  |     | 3   |
| Symbol     | Title                                                                 | Δ  |
|------------|----------------------------------------------------------------------|----|
| HAS2       | hyaluronan synthase 2                                                | 3  |
| RN7SKP260  | RNA, 7SK small nuclear pseudogene 260                                | 3  |
| RNU4-76P   | RNA, U4 small nuclear 76, pseudogene                                  | 3  |
| RP11-246K15.1 | novel transcript           | 3  |
| SORBS1     | sorbin and SH3 domain containing 1                                   | 3  |
| ARG1       | arginase 1                                                            | 3  |
| FBXO9      | F-box protein 9                                                       | 3  |
| RNU6-540P  | RNA, U6 small nuclear 540, pseudogene                                | 3  |
| SCARNA17   | small Cajal body-specific RNA 17                                      | 3  |
| LOC101927483 | uncharacterized LOC101927483                                    | 3  |
| CSH1       | chorionic somatomammetropin hormone 1                                 | 3  |
| POTEF      | POTE ankyrin domain family, member F                                  | 3  |
| MUM1L1     | melanoma associated antigen (mutated) 1-like 1                        | 2  |
| RNF185-AS1 | RNF185 antisense RNA 1                                                | 2  |
| RNU5B-4P   | RNA, USB small nuclear 4, pseudogene                                  | 2  |
| LL0XNC01-237 | putative novel transcript                | 2  |
| RNU6-453P  | RNA, U6 small nuclear 453, pseudogene                                | 2  |
| RNU6-65P   | RNA, U6 small nuclear 65, pseudogene                                 | 2  |
| RP11-347J14.8 | putative novel transcript                | 2  |
| AC008440.10 | putative novel transcript                | 2  |
| OR5M1      | olfactory receptor, family 5, subfamily M, member 1                   | 2  |
| RNU6-1043P | RNA, U6 small nuclear 1043, pseudogene                               | 2  |
| RP11-459O1.2 | putative novel transcript                | 2  |
| RNU7-80P   | RNA, U7 small nuclear 80 pseudogene                                   | 2  |
| IPW        | imprinted in Prader-Willi syndrome (non-protein coding)              | 2  |
| MIR4540    | microRNA 4540                                                        | 2  |
| RNU2-11P   | RNA, U2 small nuclear 11, pseudogene                                 | 2  |
| RNU7-51P   | RNA, U7 small nuclear 51 pseudogene                                   | 2  |
| RP11-875H7.1 | novel transcript                  | 2  |
| PPP1R3C    | protein phosphatase 1, regulatory subunit 3C                         | 2  |
| ANKFN1     | ankyrin-repeat and fibronectin type III domain containing 1          | 2  |
| PROSP      | protein S pseudogene (beta)                                          | 2  |
| SCEL-AS1   | SCEL antisense RNA 1                                                 | 2  |
| AC004878.2 | novel transcript                                                      | 2  |
| RNU12-2P   | RNA, U12 small nuclear 2, pseudogene                                 | 2  |
| LOC102724050 | uncharacterized LOC102724050                                      | 2  |
| MIR4307    | microRNA 4307                                                        | 2  |
| RNU6-919P  | RNA, U6 small nuclear 919, pseudogene                                | 2  |
| MKLN1-AS   | MKLN1 antisense RNA                                                   | 2  |
| LOC101926943 | uncharacterized LOC101926943                                    | 2  |
| ATP13A4    | ATPase type 13A4                                                      | 2  |
| LOC102724077 | uncharacterized LOC102724077                                   | 2  |
| OR1S1      | olfactory receptor, family 1, subfamily S, member 1                  | 2  |
| KRTAP6-2   | keratin associated protein 6-2                                       | 2  |
| LOC730081  | uncharacterized LOC730081                                            | 2  |
| FOXJ3      | forkhead box J3                                                      | 2  |
| CSH2       | chorionic somatomammetropin hormone 2                                 | 2  |
| RP11-661A12.4 | 7SK RNA                     | 2  |
| OR2T34     | olfactory receptor, family 2, subfamily T, member 34                 | 2  |
| VPS37D     | vacuolar protein sorting 37 homolog D (S. cerevisiae)                 | 2  |
| Symbol | Title | Δ |
|--------|-------|---|
| OSER1-AS1 | OSER1 antisense RNA 1 (head to head) | 2 |
| C7orf69 | chromosome 7 open reading frame 69 | 2 |
| LRRC17 | leucine rich repeat containing 17 | 2 |
| RNA5SP255 | RNA, 5S ribosomal pseudogene 255 | 2 |
| RP11-111F5.3 | putative novel transcript | 2 |
| MIR3152 | microRNA 3152 | 2 |
| RNU4ATAC5P | RNA, U4atac small nuclear 5, pseudogene | 2 |
| MIR548B | microRNA 548b | 2 |
| RNA5SP222 | RNA, 5S ribosomal pseudogene 222 | 2 |
| LOC101927468 | uncharacterized LOC101927468 | 2 |
| RNU6-1286P | RNA, U6 small nuclear 1286, pseudogene | 2 |
| LINC01003 | long intergenic non-protein coding RNA 1003 | 2 |
| SNORD32A | small nucleolar RNA, C/D box 32A | 2 |
| RNU6-206P | RNA, U6 small nuclear 206, pseudogene | 2 |
| IGIP | IgA-inducing protein | 2 |
| MAMDC2 | MAM domain containing 2 | 2 |
| RIMBP3B | RIMS binding protein 3B | 2 |
| RNA5SP347 | RNA, 5S ribosomal pseudogene 347 | 2 |
| UGCCG | UDP-glucose ceramide glucosyltransferase | 2 |
| ERICH2 | glutamate-rich 2 | 2 |
| MXD1 | MAX dimerization protein 1 | 2 |
| TCERG1L-AS1 | TCERG1L antisense RNA 1 | 2 |
| AFF1 | AF4/FMR2 family, member 1 | 2 |
| KIAA1377 | KIAA1377 | 2 |
| PTGER3 | prostaglandin E receptor 3 (subtype EP3) | 2 |
| LOC102724763 | uncharacterized LOC102724763 | 2 |
| RNU6-478P | RNA, U6 small nuclear 478, pseudogene | 2 |
| WFIKKN1 | WAP, follistatin/kazal, immunoglobulin, kunitz and netrin domain containing 1 | 2 |
| TGF2LY | TGFbeta-induced factor homeobox 2-like, Y-linked | 2 |
| C12orf55 | chromosome 12 open reading frame 55 | 2 |
| FIIRE | fire intergenic repeating RNA element | 2 |
| LINC00202-2 | long intergenic non-protein coding RNA 202-2 | 2 |
| PPM1N | protein phosphatase, Mg2+/Mn2+ dependent, 1N (putative) | 2 |
| RP11-408J6.1 | novel transcript, intronic to SEMA6D | 2 |
| NRG4 | neuregulin 4 | 2 |
| RNA5SP90 | RNA, 5S ribosomal pseudogene 90 | 2 |
| RNU4-80P | RNA, U4 small nuclear 80, pseudogene | 2 |
| LOC100506746 | uncharacterized LOC100506746 | 2 |
| SNORD37 | small nucleolar RNA, C/D box 37 | 2 |
| CCDC18 | coiled-coil domain containing 18 | 2 |
| ESCO2 | establishment of sister chromatid cohesion N-acetyltransferase 2 | 2 |
| HIST1H4I | histone cluster 1, H4i | 2 |
| KRT17P2 | keratin 17 pseudogene | 2 |
| MBOAT2 | membrane bound O-acyltransferase domain containing 2 | 2 |
| PTER | phosphotriesterase related | 2 |
| RP11-681L8.1 | novel transcript, sense overlapping to STPG2 | 2 |
| TSPAN15 | tetraspanin 15 | 2 |
| RP11-48B3.3 | novel transcript | 2 |
| AC097721.2 | novel transcript | 2 |
| ANXA8 | annexin A8 | 2 |
| Symbol    | Title                                                                 | \( \Delta \) |
|-----------|----------------------------------------------------------------------|-------------|
| ISYNA1    | inositol-3-phosphate synthase 1                                      | -2          |
| MFSD12    | major facilitator superfamily domain containing 12                  | -2          |
| NEDD4L    | neural precursor cell expressed, developmentally down-regulated 4-like, E3 ubiquitin protein ligase | -2          |
| SEPT6     | septin 6                                                             | -2          |
| SPNS2     | spinster homolog 2 (Drosophila)                                      | -2          |
| CHEK1     | checkpoint kinase 1                                                  | -2          |
| RAB11FIP1 | RAB11 family interacting protein 1 (class I)                        | -2          |
| SIX4      | SIX homeobox 4                                                       | -2          |
| SPINT1    | serine peptidase inhibitor, Kunitz type 1                           | -2          |
| TIMM50    | translocase of inner mitochondrial membrane 50 homolog (S. cerevisiae) | -2          |
| SLC38A9   | solute carrier family 38, member 9                                  | -2          |
| ATP6VOA4  | ATPase, H+ transporting, lysosomal V0 subunit a4                     | -2          |
| TMEM52B   | transmembrane protein 52B                                           | -2          |
| COL4A5    | collagen, type IV, alpha 5                                          | -2          |
| ERVH48-1  | endogenous retrovirus group 48, member 1                             | -2          |
| MIR301A   | microRNA 301a                                                       | -2          |
| MT-TT     | mitochondrially encoded tRNA threonine                               | -2          |
| GREB1L    | growth regulation by estrogen in breast cancer-like                 | -2          |
| BLNK      | B-cell linker                                                       | -2          |
| HCP5      | HLA complex P5 (non-protein coding)                                  | -2          |
| LOC100288637 | OTU deubiquitinase 7A pseudogene                                    | -2          |
| TGFb1     | transforming growth factor, beta 1                                  | -2          |
| TPTE2     | transmembrane phosphoinositide 3-phosphatase and tensin homolog 2   | -2          |
| TTC7B     | tetratricopeptide repeat domain 7B                                  | -2          |
| INTS6     | integrator complex subunit 6                                        | -2          |
| MIR1-2    | microRNA 1-2                                                        | -2          |
| RP2       | retinitis pigmentosa 2 (X-linked recessive)                         | -2          |
| SNORD10   | small nucleolar RNA, C/D box 10                                      | -2          |
| WNT6      | wingless-type MMTV integration site family, member 6                 | -2          |
| RNU6-208P | RNA, U6 small nuclear 208, pseudogene                               | -2          |
| RNU6-879P | RNA, U6 small nuclear 879, pseudogene                               | -2          |
| AC093375.1| novel transcript                                                     | -2          |
| ITGB6     | integrin, beta 6                                                    | -2          |
| LOC102725166 | uncharacterized LOC102725166                                         | -2          |
| MYBL2     | v-myb avian myeloblastosis viral oncogene homolog-like 2             | -2          |
| RNU6-1340P| RNA, U6 small nuclear 1340, pseudogene                              | -2          |
| RP11-577N17.5 | putative novel transcript                                           | -2          |
| SH2D4A    | SH2 domain containing 4A                                            | -2          |
| SNORD50A  | small nucleolar RNA, C/D box 50A                                     | -2          |
| ARL15     | ADP-ribosylation factor-like 1                                       | -2          |
| PARP8     | poly (ADP-ribose) polymerase family, member 8                       | -2          |
| PLK1      | polo-like kinase 1                                                  | -2          |
| SLC15A2   | solute carrier family 15 (oligopeptide transporter), member 2        | -2          |
| SLC22A11  | solute carrier family 22 (organic anion/urate transporter), member 11 | -2          |
| DNMT1     | DNA (cytosine-5-)-methyltransferase 1                                | -2          |
| C5orf30   | chromosome 5 open reading frame 30                                  | -2          |
| HAND1     | heart and neural crest derivatives expressed 1                       | -2          |
| LAMC2     | laminin, gamma 2                                                    | -2          |
| SIRPB1    | signal-regulatory protein beta 2                                     | -2          |
| METTL7A   | methyltransferase like 7A                                            | -2          |
| sPE | Symbol | Title | Δ |
|-----|--------|-------|---|
| 2nd | MIR619 | microRNA 619 | -2 |
| 2nd | SLC6A4 | solute carrier family 6 (neurotransmitter transporter), member 4 | -2 |
| 2nd | AC011558.5 | novel transcript, antisense to CNN2 | -2 |
| 2nd | AIM2 | absent in melanoma 2 | -2 |
| 2nd | CHI3L1 | chitinase 3-like 1 (cartilage glycoprotein-39) | -2 |
| 2nd | HCST | hematopoietic cell signal transducer | -2 |
| 2nd | KIAA1217 | KIAA1217 | -2 |
| 2nd | LOC101928076 | uncharacterized LOC101928076 | -2 |
| 2nd | MAN1A1 | mannosidase, alpha, class 1A, member 1 | -2 |
| 2nd | MIR3916 | microRNA 3916 | -2 |
| 2nd | GINS1 | GINS complex subunit 1 (Psf1 homolog) | -2 |
| 2nd | C6orf226 | chromosome 6 open reading frame 226 | -2 |
| 2nd | HJURP | Holliday junction recognition protein | -2 |
| 2nd | KIF4A | kinesin family member 4A | -2 |
| 2nd | LYPD3 | LY6/PLAUR domain containing 3 | -2 |
| 2nd | SIGLEC14 | sialic acid binding Ig-like lectin 14 | -2 |
| 2nd | FERMT1 | fermitin family member 1 | -2 |
| 2nd | ANKRD10-IT1 | ANKRD10 intronic transcript 1 (non-protein coding) | -2 |
| 2nd | ITM2A | integral membrane protein 2A | -2 |
| 2nd | KIF13B | kinesin family member 13B | -2 |
| 2nd | RASGRP3 | RAS guanyl releasing protein 3 (calcium and DAG-regulated) | -2 |
| 2nd | SMS | spermine synthase | -2 |
| 2nd | GBP1P1 | guanylate binding protein 1, interferon-inducible pseudogene 1 | -2 |
| 2nd | FFAR3 | free fatty acid receptor 3 | -2 |
| 2nd | GAS2L3 | growth arrest-specific 2 like 3 | -2 |
| 2nd | SORT1 | sortilin 1 | -2 |
| 2nd | ZNF66 | zinc finger protein 66 | -2 |
| 2nd | IRF1 | interferon regulatory factor 1 | -2 |
| 2nd | GEN1 | GEN1 Holliday junction 5 flap endonuclease | -2 |
| 2nd | SPRY2 | sprouty homolog 2 (Drosophila) | -2 |
| 2nd | FEZ1 | fasciulation and elongation protein zeta 1 (zygin I) | -2 |
| 2nd | MND1 | meiotic nuclear divisions 1 homolog (S. cerevisiae) | -2 |
| 2nd | ENAH | enabled homolog (Drosophila) | -2 |
| 2nd | RNU6-572P | RNA, U6 small nuclear 572, pseudogene | -2 |
| 2nd | TMEM194B | transmembrane protein 194B | -2 |
| 2nd | C5orf34 | chromosome 5 open reading frame 34 | -2 |
| 2nd | RP11-64D22.5 | novel transcript | -2 |
| 2nd | CDH2 | cadherin 2, type 1, N-cadherin (neuronal) | -2 |
| 2nd | GUCY1A3 | guanylate cyclase 1, soluble, alpha 3 | -2 |
| 2nd | INTS4L1 | integrator complex subunit 4-like 1 | -2 |
| 2nd | CDC48 | cell division cycle associated 8 | -2 |
| 2nd | DUSP10 | dual specificity phosphatase 10 | -2 |
| 2nd | RP5-1087E8.3 | putative novel transcript | -2 |
| 2nd | TPM4 | tropomyosin 4 | -2 |
| 2nd | GNG5P2 | guanine nucleotide binding protein (G protein), gamma 5 pseudogene 2 | -2 |
| 2nd | GBP3 | guanylate binding protein 3 | -2 |
| 2nd | PCDH10 | protocadherin 10 | -2 |
| 2nd | GPR115 | G protein-coupled receptor 115 | -2 |
| 2nd | BCAT1 | branched chain amino-acid transaminase 1, cytosolic | -2 |
| 2nd | ZNF431 | zinc finger protein 431 | -2 |
| Symbol       | Title                                                                 | Δ  |
|-------------|----------------------------------------------------------------------|----|
| DTWD2       | DTW domain containing 2                                             | -2 |
| CENPU       | centromere protein U                                                 | -2 |
| ANXA3       | annexin A3                                                          | -2 |
| SPC25       | SPC25, NDC80 kinetochore complex component                          | -2 |
| GGH         | gamma-glutamyl hydrolase (conjugase, polyglycaminogluamyl hydrolase) | -2 |
| ITGA6       | integrin, alpha 6                                                   | -2 |
| RP11-184E9.1| novel transcript                                                    | -2 |
| CAPN6       | calpain 6                                                           | -2 |
| KPNA2       | karyopherin alpha 2 (RAG cohort 1, importin alpha 1)                | -2 |
| RFC3        | replication factor C (activator 1) 3, 38kDa                         | -2 |
| SH3RF2      | SH3 domain containing ring finger 2                                 | -2 |
| RMI2        | RecQ mediated genome instability 2                                  | -2 |
| IL32        | interleukin 32                                                      | -2 |
| RNU6-583P   | RNA, U6 small nuclear 583, pseudogene                              | -2 |
| RP11-108P20.4| novel transcript                                                | -2 |
| NUAK2       | NUAK family, SNF1-like kinase, 2                                    | -2 |
| RNU6-1316P  | RNA, U6 small nuclear 1316, pseudogene                             | -2 |
| RNA5SP82    | RNA, 5S ribosomal pseudogene 82                                     | -2 |
| TNF         | tumor necrosis factor                                               | -2 |
| LOC101929579| uncharacterized LOC101929579                                        | -2 |
| ASF1B       | anti-silencing function 1B histone chaperone                        | -2 |
| CNN3        | calpain 3, acidic                                                   | -2 |
| CYP2D6      | cytochrome P450, family 2, subfamily D, polypeptide 6               | -2 |
| SPOPL       | speckle-type POZ protein-like                                       | -2 |
| POT1-AS1    | POT1 antisense RNA 1                                                | -2 |
| GEMIN2      | gem (nuclear organelle) associated protein 2                        | -2 |
| REEP4       | receptor accessory protein 4                                        | -2 |
| TRANK1      | tetracliopeptide repeat and ankyrin repeat containing 1            | -2 |
| ICAM1       | intercellular adhesion molecule 1                                   | -2 |
| LINC01036   | long intergenic non-protein coding RNA 1036                         | -2 |
| LOC101927841| uncharacterized LOC101927841                                       | -2 |
| ARHGAP11B   | Rho GTPase activating protein 11B                                    | -2 |
| HCAR3       | hydroxycarboxylic acid receptor 3                                   | -2 |
| SNAR-D      | small ILF3/NF90-associated RNA D                                    | -2 |
| ECT2        | epithelial cell transforming 2                                      | -2 |
| SPATA18     | spermatogenesis associated 18                                       | -2 |
| ZNF554      | zinc finger protein 554                                             | -2 |
| GLDC        | glycine dehydrogenase (decarboxylating)                             | -2 |
| SCCPDH      | saccharopine dehydrogenase (putative)                               | -2 |
| RNF128      | ring finger protein 128, E3 ubiquitin protein ligase                 | -2 |
| SLC4A4      | solute carrier family 44, member 3                                  | -2 |
| DTL         | denticule E3 ubiquitin protein ligase homolog (Drosophila)           | -2 |
| RNU6-801P   | RNA, U6 small nuclear 801, pseudogene                              | -2 |
| CXCL2       | chemokine (C-X-C motif) ligand 2                                    | -2 |
| ARHGAP23P1  | Rho GTPase activating protein 23 pseudogene 1                       | -2 |
| RP11-203H2.2| novel transcript                                                    | -2 |
| MIR521-1    | microRNA 521-1                                                      | -2 |
| MIR3671     | microRNA 3671                                                       | -2 |
| MKI67       | marker of proliferation Ki-6                                        | -2 |
| RPS3A       | ribosomal protein S3A                                               | -2 |
| Symbol | Title | Δ |
|-------|-------|---|
| TK1   | thymidine kinase 1, soluble | -2 |
| KRT16P4 | keratin 16 pseudogene 4 | -2 |
| LOC101927260 | uncharacterized LOC101927260 | -2 |
| RP11-1166P10. | Rho GTPase activating protein 23 (ARHGAP23) pseudogene | -2 |
| KIF15 | kinesin family member 15 | -2 |
| KIF23 | kinesin family member 23 | -2 |
| STIL | SCL/TAL1 interrupting locus | -2 |
| TTK | TTK protein kinase | -2 |
| COBL | cordon-bleu WH2 repeat protein | -3 |
| HIST2H2BA | histone cluster 2, H2ba (pseudogene) | -3 |
| HMGA1 | high mobility group AT-hook 1 | -3 |
| YBX3P1 | Y box binding protein 3 pseudogene 1 | -3 |
| FANCD2 | Fanconi anemia, complementation group D2 | -3 |
| TLR2 | toll-like receptor 2 | -3 |
| CDH5 | cadherin 5, type 2 (vascular endothelium) | -3 |
| GATSL2 | GATS protein-like 2 | -3 |
| FCGBP | Fc fragment of IgG binding protein | -3 |
| RN6-580P | RNA, U6 small nuclear 580, pseudogene | -3 |
| TMEM63A | transmembrane protein 63A | -3 |
| MIR622 | microRNA 622 | -3 |
| TRD3 | T cell receptor delta joining 3 | -3 |
| KIF20B | kinesin family member 20B | -3 |
| FAM169A | family with sequence similarity 169, member A | -3 |
| BTG2 | BTG family, member 2 | -3 |
| TPT | transmembrane phosphatase with tensin homology | -3 |
| GBP1 | guanylate binding protein 1, interferon-inducible | -3 |
| CLSPN | clasin | -3 |
| MIR103B2 | microRNA 103b-2 | -3 |
| RNU6-971P | RNA, U6 small nuclear 971, pseudogene | -3 |
| RNU6-151P | RNA, U6 small nuclear 151, pseudogene | -3 |
| ITGB4 | integrin, beta 4 | -3 |
| KRT17P1 | keratin 17 pseudogene 1 | -3 |
| CDCP1 | CUB domain containing protein 1 | -3 |
| PBK | PDZ binding kinase | -3 |
| KIAA0101 | KIAA0101 | -3 |
| TAGLN | transgelin | -3 |
| FOXO4 | forkhead box O4 | -3 |
| RNU6-850P | RNA, U6 small nuclear 850, pseudogene | -3 |
| RAD51 | RAD51 recombinase | -3 |
| CDKN3 | cyclin-dependent kinase inhibitor 3 | -3 |
| RP11-517O13.1 | novel transcript, antisense to TIMM9 | -3 |
| ATP6V1C2 | ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C2 | -3 |
| CEP55 | centrosomal protein 55kDa | -3 |
| PRR11 | proline rich 11 | -3 |
| TP63 | tumor protein p63 | -3 |
| CD47 | CD47 molecule | -3 |
| EFNB2 | ephrin-B2 | -3 |
| ARHGAP18 | Rho GTPase activating protein 18 | -3 |
| PRC1 | protein regulator of cytokinesis 1 | -3 |
| P2RY6 | pyrimidinergic receptor P2Y, G-protein coupled, 6 | -3 |
| Symbol       | Title                                           | Δ   |
|--------------|-------------------------------------------------|-----|
| CKAP2        | cytoskeleton associated protein 2               | -3  |
| CKS2         | CDC28 protein kinase regulatory subunit 2       | -3  |
| KRAS         | Kirsten rat sarcoma viral oncogene homolog      | -3  |
| RNA5SP282    | RNA, 5S ribosomal pseudogene 282                | -3  |
| CCDC81       | coiled-coil domain containing 81                | -3  |
| BLM          | Bloom syndrome, RecQ helicase-like              | -3  |
| CKAP2L       | cytoskeleton associated protein 2-like          | -3  |
| HSD17B1      | hydroxysteroid (17-beta) dehydrogenase 1        | -3  |
| ANKRD36BP2   | ankyrin repeat domain 36B pseudogene 2          | -3  |
| KRT18P15     | keratin 18 pseudogene 15                        | -3  |
| SGOL1        | shugoshin-like 1 (S. pombe)                     | -3  |
| SNAR-C2      | small ILF3/NF90-associated RNA C2               | -3  |
| RNU6-226P    | RNA, U6 small nuclear 226, pseudogene          | -3  |
| MIR15B       | microRNA 15b                                    | -3  |
| LOC102724842 | uncharacterized LOC102724842                   | -3  |
| AC078883.4   | novel transcript                                | -3  |
| RNU6-531P    | RNA, U6 small nuclear 531, pseudogene          | -3  |
| RP11-49I4.3  | novel transcript                                | -3  |
| EXO1         | exonuclease 1                                   | -3  |
| MIR520E      | microRNA 520e                                   | -3  |
| KRT23        | keratin 23 (histone deacetylase inducible)      | -3  |
| KDM4B        | lysine (K)-specific demethylase 4B              | -3  |
| COL15A1      | collagen, type XV, alpha 1                      | -3  |
| RNA5SP330    | RNA, 5S ribosomal pseudogene 330               | -3  |
| RP11-524H19.2| putative novel transcript                       | -3  |
| LOC101927700 | uncharacterized LOC101927700                   | -3  |
| AGL          | amylo-alpha-1, 6-glucosidase, 4-alpha-glucanotransferase | -3  |
| CCL4         | chemokine (C-C motif) ligand 4                  | -3  |
| RNU6-83P     | RNA, U6 small nuclear 83, pseudogene           | -3  |
| CENPF        | centromere protein F, 350/400kDa               | -3  |
| SLC7A6       | solute carrier family 7 (amino acid transporter light chain, α+L system), member 6 | -3  |
| MIR498       | microRNA 498                                    | -3  |
| KRT18P49     | keratin 18 pseudogene 49                        | -3  |
| SNORA53      | small nucleolar RNA, H/ACA box 53              | -3  |
| CDCA2        | cell division cycle associated 2               | -3  |
| GPR137B      | G protein-coupled receptor 137B                | -3  |
| LOC101927202 | uncharacterized LOC101927202                   | -3  |
| MYH10        | myosin, heavy chain 10, non-muscle             | -3  |
| SMPDL3A      | sphingomyelin phosphodiesterase, acid-like 3A  | -3  |
| SFN          | stratfin                                        | -3  |
| NUF2         | NUF2, NDC80 kinetochore complex component       | -3  |
| NRN1L        | neuritin 1-like                                 | -3  |
| LAMB4        | laminin, beta 4                                 | -3  |
| OCLN         | occludin                                        | -3  |
| PLAU         | plasminogen activator, urokinase               | -3  |
| CACNG4       | calcium channel, voltage-dependent, gamma subunit 4 | -3  |
| MCM6         | minichromosome maintenance complex component 6 | -3  |
| RNU7-43P     | RNA, U7 small nuclear 43 pseudogene            | -3  |
| PGAP1        | post-GPI attachment to proteins 1              | -3  |
| AGR2         | anterior gradient 2                             | -3  |

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| sPE  | 2nd | Symbol            | Title                                           | Δ  |
|------|-----|-------------------|-------------------------------------------------|----|
| LBR  | -3  | lamin B receptor  |                                                 |    |
| KRT18P54 | -3 | keratin 18 pseudogene 54 |                          |    |
| CSF3R | -3  | colony stimulating factor 3 receptor (granulocyte) |                     |    |
| KIAA1524 | -3 | KIAA1524          |                                                 |    |
| RRM2  | -3  | ribonucleotide reductase M2 |                          |    |
| HNRNPA1P33 | -3 | heterogeneous nuclear ribonucleoprotein A1 pseudogene 33 | |    |
| RBMS3 | -3  | RNA binding motif, single stranded interacting protein 3 |                 |    |
| PLK4  | -3  | polo-like kinase 4 |                                               |    |
| MIR548AL | -3  | microRNA 548al  |                                                 |    |
| ORC6  | -3  | origin RNA, subunit 6 |                                |    |
| RNU6-780P | -3 | RNA, U6 small nuclear 780, pseudogene |               |    |
| CASC5 | -3  | cancer susceptibility candidate 5 |                          |    |
| BIRC5 | -3  | baculoviral IAP repeat containing 5 |                        |    |
| LMNB1 | -3  | lamin B1         |                                                 |    |
| RNU6-803P | -3 | RNA, U6 small nuclear 803, pseudogene |               |    |
| RN7SKP42 | -3 | RNA, 7SK small nuclear pseudogene 42 |              |    |
| FGFR2 | -3  | fibroblast growth factor receptor 2 |                       |    |
| ZDHHC2 | -3  | zinc finger, DHHC-type containing 2 |                      |    |
| KRT18P10 | -3  | keratin 18 pseudogene 10 |                     |    |
| RNU6-560P | -3 | RNA, U6 small nuclear 560, pseudogene |               |    |
| CENPK | -3  | centromere protein K |                                   |    |
| AFAP1L2 | -3 | actin filament associated protein 1-like 2 |                 |    |
| SNORD35A | -3 | small nucleolar RNA, C/D box 35A |                      |    |
| NLRP2 | -3  | NLR family, pyrin domain containing 2 |                      |    |
| SLC43A2 | -3 | solute carrier family 43 (amino acid system L transporter), member 2 |     |    |
| NLRP7 | -3  | NLR family, pyrin domain containing 7 |                       |    |
| RNASSP474 | -3 | RNA, 5S ribosomal pseudogene 474 |                 |    |
| TMC7  | -3  | transmembrane channel-like 7 |                                |    |
| TPX2  | -3  | TPX2, microtubule-associated |                                  |    |
| AREG  | -3  | amphiregulin |                                             |    |
| CCNA2 | -3  | cyclin A2 |                                          |    |
| CCNB2 | -3  | cyclin B2 |                                         |    |
| EPCAM | -3  | epithelial cell adhesion molecule |                        |    |
| CENPH | -3  | centromere protein H |                                   |    |
| CLU   | -3  | clusterin |                                      |    |
| DRAM1 | -3  | DNA-damage regulated autophagy modulator 1 |                  |    |
| GPR82 | -3  | G protein-coupled receptor 82 |                      |    |
| SLC7A5 | -3  | solute carrier family 7 (amino acid transporter light chain, L system), member 5 | |    |
| HELLS | -3  | helicase, lymphoid-specific |                          |    |
| CST6  | -3  | cystatin E/M |                                      |    |
| RP11-1M18.1 | -3 | novel transcript |                               |    |
| KIF11 | -3  | kinesin family member 11 |                             |    |
| HAPLN1 | -3 | hyaluronan and proteoglycan link protein 1 |                 |    |
| MEST  | -3  | mesoderm specific transcript |                     |    |
| RNU6-308P | -3 | RNA, U6 small nuclear 308, pseudogene |               |    |
| PLS1  | -3  | plastin 1 |                                      |    |
| CCNB1 | -4  | cyclin B1 |                                       |    |
| PVRL2 | -4  | poliovirus receptor-related 2 (herpesvirus entry mediator B) |  |    |
| GPR37 | -4  | G protein-coupled receptor 37 (endothelin receptor type B-like) | |    |
| SNORD16 | -4 | small nucleolar RNA, C/D box 16 |                       |    |
| Symbol | Title | Δ |
|--------|-------|---|
| XAGE3  | X antigen family, member 3 | -4 |
| TOP2A  | topoisomerase (DNA) II alpha 170kDa | -4 |
| CD274  | CD274 molecule | -4 |
| RNU6-79P | RNA, U6 small nuclear 79, pseudogene | -4 |
| FAR2P2 | fatty acyl CoA reductase 2 pseudogene 2 | -4 |
| RAD51AP1 | RAD51 associated protein 1 | -4 |
| TINAGL1 | tubulointerstitial nephritis antigen-like 1 | -4 |
| COL1A2 | collagen, type I, alpha 2 | -4 |
| DIAPH3 | diaphanous-related formin 3 | -4 |
| OLR1   | oxidized low density lipoprotein (lectin-like) receptor 1 | -4 |
| DDAH1  | dimethylarginine dimethylaminohydrolase 1 | -4 |
| HSD17B2 | hydroxysteroid (17-beta) dehydrogenase 2 | -4 |
| MIR7641-2 | microRNA 7641-2 | -4 |
| DSCC1  | DNA replication and sister chromatid cohesion 1 | -4 |
| COL3A1 | collagen, type III, alpha 1 | -4 |
| RNU6-223P | RNA, U6 small nuclear 223, pseudogene | -4 |
| TP53INP1 | tumor protein p53 inducible nuclear protein 1 | -4 |
| AC106053.1 | novel transcript | -4 |
| SERPINF1 | serpin peptidase inhibitor, clade F1 | -4 |
| RNU6-623P | RNA, U6 small nuclear 623, pseudogene | -4 |
| BUB1   | BUB1 mitotic checkpoint serine/threonine kinase | -4 |
| KIF14  | kinesin family member 14 | -4 |
| PDIA6  | protein disulfide isomerase family A, member 6 | -4 |
| RNU6-387P | RNA, U6 small nuclear 387, pseudogene | -4 |
| TYMS   | thymidylate synthetase | -4 |
| RNU6-1195P | RNA, U6 small nuclear 1195, pseudogene | -4 |
| ARHGAP11A | Rho GTPase activating protein 11A | -4 |
| DLGAP5 | discs, large (Drosophila) homolog-associated protein 5 | -4 |
| CTD-2306M5.1 | novel transcript | -4 |
| MIR218-1 | microRNA 218-1 | -4 |
| SHCBP1 | SHC SH2-domain binding protein 1 | -4 |
| CENPE  | centromere protein E, 312kDa | -4 |
| HIST1H3E | histone cluster 1, H3e | -4 |
| C10orf55 | chromosome 10 open reading frame 55 | -4 |
| MELK   | maternal embryonic leucine zipper kinase | -4 |
| SNORD75 | small nucleolar RNA, C/D box 75 | -4 |
| FMOD   | fibromodulin | -4 |
| RNU6-736P | RNA, U6 small nuclear 736, pseudogene | -4 |
| PABPC4L | poly(A) binding protein, cytoplasmic 4-like | -4 |
| LINCO1237 | long intergenic non-protein coding RNA 1237 | -4 |
| RNU6-606P | RNA, U6 small nuclear 606, pseudogene | -4 |
| GBP2   | guanylate binding protein 2, interferon-inducible | -4 |
| AP001615.9 | putative novel transcript | -4 |
| CDC6   | cell division cycle 6 | -4 |
| SKA3   | spindle and kinetochore associated complex subunit 3 | -4 |
| NCAPG  | non-SMC condensin I complex, subunit G | -4 |
| EPPK1  | epiplakin 1 | -4 |
| TSPAN2 | tetraspanin 2 | -4 |
| RNU6-375P | RNA, U6 small nuclear 375, pseudogene | -4 |
| SNORD2 | small nucleolar RNA, C/D box 2 | -4 |
| Symbol      | Title                                                                 | Δ  |
|-------------|------------------------------------------------------------------------|----|
| RN6U-1318P  | RNA, U6 small nuclear 1318, pseudogene                                | -5 |
| ASPM        | asp (abnormal spindle) homolog, microcephaly associated (Drosophila)  | -5 |
| CDK1        | cyclin-dependent kinase 1                                              | -5 |
| SIGLEC6     | sialic acid binding Ig-like lectin 6                                  | -5 |
| VIT         | vitrin                                                                | -5 |
| RN6U-1170P  | RNA, U6 small nuclear 1170, pseudogene                                | -5 |
| AIM1        | absent in melanoma 1                                                  | -5 |
| NUSAP1      | nucleolar and spindle associated protein 1                            | -5 |
| RN6U-1188P  | RNA, U6 small nuclear 1188, pseudogene                                | -5 |
| RNA5SP88    | RNA, 5S ribosomal pseudogene 88                                       | -5 |
| PMAIP1      | phorbol-12-myristate-13-acetate-induced protein 1                      | -5 |
| NRK         | Nik related kinase                                                    | -5 |
| BRIP1       | BRCA1 interacting protein C-terminal helicase 1                        | -5 |
| PAGE4       | P antigen family, member 4 (prostate associated)                      | -5 |
| RP4-694A7.2 | putative novel transcript                                             | -5 |
| HMMR        | hyaluronan-mediated motility receptor (RHAMM)                         | -5 |
| KIF18A      | kinesin family member 18A                                             | -5 |
| NDC80       | NDC80 kinetochore complex component                                    | -5 |
| DEPDC1      | DEP domain containing 1                                               | -5 |
| RN6U-54P    | RNA, U4 small nuclear 54, pseudogene                                  | -5 |
| KRT17       | keratin 17                                                            | -5 |
| HIST1H3B    | histone cluster 1, H3b                                                | -5 |
| HGF         | hepatocyte growth factor (hepapoietin A; scatter factor)               | -6 |
| SUCNR1      | succinate receptor 1                                                  | -6 |
| SNORD111    | small nucleolar RNA, C/D box 111                                      | -6 |
| HIST1H3F    | histone cluster 1, H3f                                                | -6 |
| HIST1H3I    | histone cluster 1, H3i                                                | -6 |
| CLDN6       | claudin 6                                                             | -6 |
| LRP2        | low density lipoprotein receptor-related protein 2                    | -6 |
| MIR205HG    | MIR205 host gene (non-protein coding)                                  | -6 |
| CCL2        | chemokine (C-C motif) ligand 2                                        | -6 |
| CXCL10      | chemokine (C-X-C motif) ligand 10                                     | -6 |
| MIR1323     | microRNA 1323                                                         | -6 |
| PRSS12      | protease, serine, 12 (neurotyspin, motopsin)                          | -6 |
| LPHN3       | latrophilin 3                                                         | -6 |
| F5          | coagulation factor V (proaccelerin, labile factor)                     | -7 |
| EGFL6       | EGF-like-domain, multiple 6                                            | -7 |
| CDO1        | cysteine dioxygenase type 1                                           | -7 |
| ITGA2       | integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)          | -8 |
| RN6U-1330P  | RNA, U6 small nuclear 1330, pseudogene                                | -8 |
| CD24        | CD24 molecule                                                         | -10|
| HIST1H2AM   | histone cluster 1, H2am                                              | -10|
| NPPB        | natriuretic peptide B                                                | -11|
| MIR515-1    | microRNA 515-1                                                       | -26|
Supplemental Figure 4. Global gene expression profiles of schCTBs isolated by laser capture microdissection from the fetal membranes: severe preeclampsia (sPE) as compared to the late second trimester of normal pregnancy (2nd) (n=4/group). The heatmap lists genes that were differentially expressed by 2-fold or greater.
| nPTB | Symbol | Title | 2^{nd} | Δ |
|------|--------|-------|--------|---|
| 12SPINK1 | serine peptidase inhibitor, Kazal type 1 | | | 12 |
| HLA-DRB1 | major histocompatibility complex, class II, DR beta 1 | | | 12 |
| RNU6-1154P | RNA, U6 small nuclear 1154, pseudogene | | | 10 |
| RNU6-638P | RNA, U6 small nuclear 638, pseudogene | | | 7 |
| BMP2 | bone morphogenetic protein 2 | | | 7 |
| KLHL22-IT1 | KLHL22 intronic transcript 1 (non-protein coding) | | | 6 |
| HK2 | hexokinase 2 | | | 5 |
| LRRC15 | leucine rich repeat containing 15 | | | 5 |
| RNU1-124P | RNA, U1 small nuclear 124, pseudogene | | | 5 |
| PPP1R3C | protein phosphatase 1, regulatory subunit 3C | | | 5 |
| MIR302A | microRNA 302a | | | 5 |
| RP11-407H12.8 | novel transcript | | | 4 |
| SNORA29 | small nucleolar RNA, H/ACA box 29 | | | 4 |
| RNU6-540P | RNA, U6 small nuclear 540, pseudogene | | | 4 |
| RNU6-1014P | RNA, U6 small nuclear 1014, pseudogene | | | 4 |
| RNU5F-4P | RNA, U5F small nuclear 4, pseudogene | | | 4 |
| SNORD59A | small nucleolar RNA, C/D box 59A | | | 4 |
| SCEL | sciellin | | | 4 |
| ATP13A4 | ATPase type 13A4 | | | 3 |
| AC008269.2 | putative novel transcript | | | 3 |
| CES1 | carboxylesterase 1 | | | 3 |
| GJB3 | gap junction protein, beta 3, 31kDa | | | 3 |
| LOC101928461 | uncharacterized LOC101928461 | | | 3 |
| DDIT4 | DNA-damage-inducible transcript 4 | | | 3 |
| RP11-48B3.5 | novel transcript, antisense to ZBTB10 | | | 3 |
| RP11-138H8.2 | novel transcript | | | 3 |
| HRASLS2 | HRAS-like suppressor 2 | | | 3 |
| SNORD48 | small nucleolar RNA, C/D box 48 | | | 3 |
| FCGR1C | Fc fragment of IgG, high affinity Ic, receptor (CD64), pseudogene | | | 3 |
| SNORD68 | small nucleolar RNA, C/D box 68 | | | 3 |
| MIR1204 | microRNA 1204 | | | 3 |
| SERPINA3 | serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3 | | | 3 |
| RNU7-9P | RNA, U7 small nuclear 9 pseudogene | | | 3 |
| RNU4-21P | RNA, U4 small nuclear 21, pseudogene | | | 3 |
| RNU6-249P | RNA, U6 small nuclear 249, pseudogene | | | 3 |
| FAM86EP | family with sequence similarity 86, member A pseudogene | | | 3 |
| LINC00152 | long intergenic non-protein coding RNA 152 (LOC101930489) | | | 3 |
| SNORD33 | small nucleolar RNA, C/D box 33 | | | 3 |
| RNASSP155 | RNA, 5S ribosomal pseudogene 155 | | | 3 |
| KLF9 | Kruppel-like factor 9 | | | 3 |
| RNU7-62P | RNA, U7 small nuclear 62 pseudogene | | | 3 |
| RNU4-76P | RNA, U4 small nuclear 76, pseudogene | | | 3 |
| LOC101930489 | uncharacterized LOC101930489 | | | 3 |
| ADM | adrenomedullin | | | 3 |
| GFPT2 | glutamine-fructose-6-phosphate transaminase 2 | | | 3 |
| RP11-458D21.1 | novel transcript | | | 3 |
| MT1M | metallothionein 1M | | | 3 |
| SNORD61 | small nucleolar RNA, C/D box 61 | | | 3 |
| GK-AS1 | GK antisense RNA 1 | | | 3 |
| C15orf48 | chromosome 15 open reading frame 48 | | | 3 |
| nPTB 2<sup>nd</sup> | Symbol | Title |
|-------------------|--------|-------|
| RNU7-80P | RNA, U7 small nuclear 80 pseudogene | 3 |
| IRS2 | insulin receptor substrate 2 | 3 |
| RNA5SP468 | RNA, 5S ribosomal pseudogene 468 | 3 |
| ZNF812 | zinc finger protein 812 | 2 |
| OR10G7 | olfactory receptor, family 10, subfamily G, member 7 | 2 |
| SV2B | synaptic vesicle glycoprotein 2B | 2 |
| SRGAP2 | SLIT-ROBO Rho GTPase activating protein 2 | 2 |
| PER1 | period circadian clock 1 | 2 |
| RNU7-26P | RNA, U7 small nuclear 26 pseudogene | 2 |
| RNU6-1230P | RNA, U6 small nuclear 1230, pseudogene | 2 |
| KRTAP6-2 | keratin associated protein 6-2 | 2 |
| RNU7-51P | RNA, U7 small nuclear 51 pseudogene | 2 |
| RNU7-136P | RNA, U7 small nuclear 136 pseudogene | 2 |
| RSF1-IT1 | RSF1 intronic transcript 1 (non-protein coding) | 2 |
| DUSP5 | dual specificity phosphatase 5 | 2 |
| RP11-33I11.2 | putative novel transcript | 2 |
| MMP10 | matrix metallopeptidase 10 (stromelysin 2) | 2 |
| RP11-10J5.1 | putative novel transcript | 2 |
| RP11-812E19.9 | novel gene similar to an immunoglobulin heavy variable 3/OR16 gene | 2 |
| RNA5SP287 | RNA, 5S ribosomal pseudogene 287 | 2 |
| RP11-38L15.2 | novel transcript | 2 |
| RNA5SP255 | RNA, 5S ribosomal pseudogene 255 | 2 |
| IL23A | interleukin 23, alpha subunit p19 | 2 |
| SNORA15 | small nucleolar RNA, H/ACA box 15 | 2 |
| RP11-549L6.3 | putative novel transcript | 2 |
| CELSR3-AS1 | CELSR3 antisense RNA 1 (head to head) | 2 |
| RNY3P8 | RNA, Ro-associated Y3 pseudogene 8 | 2 |
| AC068491.2 | putative novel transcript | 2 |
| TRAJ14 | T cell receptor alpha joining 14 | 2 |
| APOOP5 | apolipoprotein O pseudogene 5 | 2 |
| IRF2BP2 | interferon regulatory factor 2 binding protein 2 | 2 |
| RN7SKP140 | RNA, 7SK small nuclear pseudogene 140 | 2 |
| CTD-2501M5.1 | novel transcript | 2 |
| RP11-212I21.2 | novel transcript | 2 |
| RNA5SP489 | RNA, 5S ribosomal pseudogene 489 | 2 |
| MIR378F | microRNA 378f | 2 |
| RNA5SP185 | RNA, 5S ribosomal pseudogene 185 | 2 |
| DLEU2L | deleted in lymphocytic leukemia 2-like | 2 |
| INTS4 | integrator complex subunit 4 | 2 |
| LOC101927468 | uncharacterized LOC101927468 | 2 |
| B3GALTL | beta 1,3-galactosyltransferase-like | 2 |
| MIR1537 | microRNA 1537 | 2 |
| CTB-4E7.1 | novel transcript | 2 |
| GEM | GTP binding protein overexpressed in skeletal muscle | 2 |
| MIR551A | microRNA 551a | 2 |
| RNU2-71P | RNA, U2 small nuclear 71, pseudogene | 2 |
| RN7SKP142 | RNA, 7SK small nuclear pseudogene 142 | 2 |
| RNA5SP460 | RNA, 5S ribosomal pseudogene 460 | 2 |
| RN4U4-41P | RNA, U4 small nuclear 41, pseudogene | 2 |
| RNU4ATAC10P | RNA, U4atac small nuclear 10, pseudogene | 2 |
| nPTB | 2nd | Symbol    | Title                                                                 | Δ   |
|------|-----|-----------|----------------------------------------------------------------------|-----|
|      |     | VTRNA1-1  | vault RNA 1-1                                                        | 2   |
|      |     | MIR365A   | microRNA 365a                                                        | 2   |
|      |     | MIR4463   | microRNA 4463                                                        | 2   |
|      |     | RN7U-59P  | RNA, U7 small nuclear 59 pseudogene                                  | 2   |
|      |     | RP11-400L8.2 | novel transcript                           | 2   |
|      |     | KIF15     | kinesin family member 15                                              | -2  |
|      |     | HIST1H2BE | histone cluster 1, H2be                                              | -2  |
|      |     | LOC102723627 | uncharacterized LOC102723627                              | -2  |
|      |     | SLC40A1   | solute carrier family 40 (iron-regulated transporter), member 1    | -2  |
|      |     | TIA1      | TIA1 cytotoxic granule-associated RNA binding protein               | -2  |
|      |     | CPNE2     | copine II                                                            | -2  |
|      |     | WDHD1     | WD repeat and HMG-box DNA binding protein 1                         | -2  |
|      |     | FERMT1    | fermitin family member 1                                             | -2  |
|      |     | SPC25     | SPC25, NDC80 kinetochore complex component                           | -2  |
|      |     | PLK4      | polo-like kinase 4                                                   | -2  |
|      |     | KIAA1549  | KIAA1549                                                             | -2  |
|      |     | LRRC28    | leucine rich repeat containing 28                                   | -2  |
|      |     | AP000704.5 | putative novel transcript                                            | -2  |
|      |     | COLEC12   | collectin sub-family member 12                                       | -2  |
|      |     | DNA2      | DNA replication helicase/nuclease 2                                  | -2  |
|      |     | IGSF10    | immunoglobulin superfamily, member 10                               | -2  |
|      |     | LGSN      | lensin, lens protein with glutamine synthetase domain               | -2  |
|      |     | LOC101928054 | uncharacterized LOC101928054                           | -2  |
|      |     | ZNF43     | zinc finger protein 43                                               | -2  |
|      |     | CD274     | CD274 molecule                                                       | -2  |
|      |     | CDK1      | cyclin-dependent kinase 1                                            | -2  |
|      |     | ACPP      | acid phosphatase, prostate                                           | -2  |
|      |     | CTA-398F10.2 | novel transcript                           | -2  |
|      |     | FOCAD     | focadhesin                                                           | -2  |
|      |     | MTRN2L6   | MT-RNR2-like 6                                                       | -2  |
|      |     | GREB1L    | growth regulation by estrogen in breast cancer-like                  | -2  |
|      |     | CDH2      | cadherin 2, type 1, N-cadherin (neuronal)                            | -2  |
|      |     | LOC101927700 | uncharacterized LOC101927700                                   | -2  |
|      |     | CS        | citrate synthase                                                     | -2  |
|      |     | LOC100287834 | uncharacterized LOC100287834                                 | -2  |
|      |     | ZNF229    | zinc finger protein 229                                              | -2  |
|      |     | GEN1      | GEN1 Holliday junction 5 flap endonuclease                           | -2  |
|      |     | TGFB3     | transforming growth factor, beta 3                                   | -2  |
|      |     | LINC01355 | long intergenic non-protein coding RNA 1355                        | -2  |
|      |     | RN7SKP163 | RNA, 7SK small nuclear pseudogene 163                               | -2  |
|      |     | RP3-368A4.5 | novel transcript, sense intronic FTX                               | -2  |
|      |     | SLC44A2   | solute carrier family 44 (choline transporter), member 2            | -2  |
|      |     | TMEM117   | transmembrane protein 117                                            | -2  |
|      |     | CD24      | CD24 molecule                                                        | -2  |
|      |     | AC016644.1 | novel transcript                                                     | -2  |
|      |     | RP11-710F7.2 | novel transcript                           | -2  |
|      |     | SYDE2     | synapse defective 1, Rho GTPase, homolog 2 (C. elegans)              | -2  |
|      |     | LOXL1     | lysyl oxidase-like 1                                                | -2  |
|      |     | TNFRSF21  | tumor necrosis factor receptor superfamily, member 21               | -2  |
|      |     | TYMS      | thymidylate synthetase                                              | -2  |
| nPTB | 2nd | Symbol     | Title                                                                 | Δ  |
|------|-----|------------|----------------------------------------------------------------------|----|
| BST1 |     | bone marrow stromal cell antigen 1                              | -2 |
| SMG8 |     | SMG8 nonsense mediated mRNA decay factor                        | -2 |
| RNU6-858P | RNA, U6 small nuclear 858, pseudogene                        | -2 |
| GINS2 |     | GINS complex subunit 2 (Psf2 homolog)                           | -2 |
| LOC400541 | uncharacterized LOC400541                                  | -2 |
| SLC39A11 | solute carrier family 39, member 11                           | -2 |
| DNMT1 |     | DNA (cytosine-5-)-methyltransferase 1                           | -2 |
| AP001615.9 | putative novel transcript                               | -2 |
| AC092597.3 | novel transcript                                           | -2 |
| ZNF554 |     | zinc finger protein 554                                         | -2 |
| ATP6V1C2 | ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C2      | -2 |
| SESN3 |     | sestrin 3                                                        | -2 |
| DTWD2 |     | DTW domain containing 2                                         | -2 |
| RRM2 |     | ribonucleotide reductase M2                                      | -2 |
| KGFLP2 | keratinocyte growth factor-like protein 2                    | -2 |
| PPP1R9A | protein phosphatase 1, regulatory subunit 9A                 | -2 |
| DGCR11 | DiGeorge syndrome critical region gene 11 (non-protein coding) | -2 |
| EXO1 |     | exonuclease 1                                                     | -2 |
| AGAP6 |     | ArfGAP with GTPase domain, ankyrin repeat and PH domain 6       | -2 |
| DTL |     | denticleless E3 ubiquitin protein ligase homolog (Drosophila)   | -2 |
| EFN2 |     | ephrin-B2                                                        | -2 |
| CASP6 |     | caspase 6, apoptosis-related cysteine peptidase                  | -2 |
| COL14A1 | collagen, type XIV, alpha 1                                  | -2 |
| LOC283299 | uncharacterized LOC283299                                   | -2 |
| LOC101929579 | uncharacterized LOC101929579                               | -2 |
| COL1A1 |     | collagen, type I, alpha 1                                        | -2 |
| ZNF329 |     | zinc finger protein 329                                          | -2 |
| NUF2 |     | NUF2, NDC80 kinetochore complex component                        | -2 |
| PHC1 |     | polyhomeotic homolog 1 (Drosophila)                             | -2 |
| GNG5P2 | guanine nucleotide binding protein (G protein), gamma 5 pseudogene 2 | -2 |
| TPX2 |     | TPX2, microtubule-associated                                    | -2 |
| ASH1L-IT1 | ASH1L intronic transcript 1 (non-protein coding)            | -2 |
| ARHGAP11B | Rho GTPase activating protein 11B                           | -2 |
| KDM4B |     | lysine (K)-specific demethylase 4B                               | -2 |
| TUSC3 |     | tumor suppressor candidate 3                                    | -2 |
| RAB11FIP4 | RAB11 family interacting protein 4 (class II)                | -2 |
| KIF18A |     | kinesin family member 18A                                       | -2 |
| XRCC2 |     | X-ray repair complementing defective repair in Chinese hamster cells 2 | -2 |
| RAB9BP1 | RAB9B, member RAS oncogene family pseudogene 1              | -2 |
| GPR126 |     | G protein-coupled receptor 126                                   | -2 |
| SH3RF2 |     | SH3 domain containing ring finger 2                              | -2 |
| DIAPH3 |     | diaphanous-related formin 3                                     | -2 |
| FZD3 |     | frizzled class receptor 3                                        | -2 |
| PPP2R3A | protein phosphatase 2, regulatory subunit B, alpha            | -2 |
| FANCD2 |     | Fanconi anemia, complementation group D2                         | -2 |
| CCNB1 |     | cyclin B1                                                       | -2 |
| DDAH1 |     | dimethylarginine dimethylaminohydrolase 1                        | -2 |
| RNU6-208P | RNA, U6 small nuclear 208, pseudogene                       | -2 |
| ATP10D |     | ATPase, class V, type 10D                                        | -2 |
| RNU2-58P | RNA, U2 small nuclear 58, pseudogene                        | -2 |
| Symbol  | Title                                                                 | \(\Delta\) |
|---------|----------------------------------------------------------------------|----------|
| ORC6    | origin recognition complex, subunit 6                               | -2       |
| SOX4    | SRY (sex determining region Y)-box 4                                | -2       |
| SLC38A9 | solute carrier family 38, member 9                                   | -2       |
| ANKR10-IT1 | ANKR10 intronic transcript 1 (non-protein coding)                 | -2       |
| GPR82   | G protein-coupled receptor 82                                        | -2       |
| BUB1    | BUB1 mitotic checkpoint serine/threonine kinase                     | -2       |
| PGAP1   | post-GPI attachment to proteins                                     | -2       |
| AFAP1L2 | actin filament associated protein 1-like 2                           | -2       |
| MYO6    | myosin VI                                                            | -2       |
| SUSD1   | sushi domain containing 1                                            | -2       |
| CYP2D6  | cytochrome P450, family 2, subfamily D, polypeptide 6                | -2       |
| LMNB1   | lamin B1                                                             | -2       |
| ZNF99   | zinc finger protein 99                                               | -2       |
| SPOPL   | speckle-type POZ protein-like                                        | -2       |
| RP11-48B3.3 | novel transcript                                             | -2       |
| TMEM254-AS1 | TMEM254 antisense RNA 1                                            | -2       |
| SLC23A2 | solute carrier family 23 (ascorbic acid transporter), member 2      | -2       |
| COL15A1 | collagen, type XV, alpha 1                                          | -2       |
| NLRP7   | NLR family, pyrin domain containing 7                                | -2       |
| CD200R1 | CD200 receptor 1                                                     | -2       |
| RP11-574K11.5 | putative novel transcript                                          | -2       |
| TTC7B   | tetra/tri-cysteine repeat domain 7B                                 | -2       |
| PBX1    | pre-B-cell leukemia homeobox 1                                       | -2       |
| CD36    | CD36 molecule (thrombospondin receptor)                             | -2       |
| CEP55   | centrosomal protein 55kDa                                            | -2       |
| BRC2    | breast cancer 2, early onset                                         | -2       |
| LOC441601 | septin 7 pseudogene                                        | -2       |
| MIR15B  | microRNA 15b                                                        | -2       |
| PCDH10  | protocadherin 10                                                     | -2       |
| RNU6-1330P | RNA, U6 small nuclear 1330, pseudogene                              | -3       |
| RNU6-879P | RNA, U6 small nuclear 879, pseudogene                              | -3       |
| RNU6-850P | RNA, U6 small nuclear 850, pseudogene                              | -3       |
| RP4-778K6.3 | putative novel transcript                                      | -3       |
| AC004160.4 | putative novel transcript                                       | -3       |
| ZNF594  | zinc finger protein 594                                             | -3       |
| NBPF1   | neuroblastoma breakpoint family, member 1                           | -3       |
| RNU6-801P | RNA, U6 small nuclear 801, pseudogene                              | -3       |
| F2RL1   | coagulation factor II (thrombin) receptor-like 1                   | -3       |
| LOC643072 | uncharacterized LOC643072                                       | -3       |
| HYAL4   | hyaluronoglucosaminidase 4                                          | -3       |
| ARHGAP23P1 | Rho GTPase activating protein 23 pseudogene 1                   | -3       |
| C15orf41 | chromosome 15 open reading frame 41                                | -3       |
| AC097721.2 | novel transcript                                               | -3       |
| CABLES1 | Cdk5 and Abl enzyme substrate 1                                     | -3       |
| RNASSP282 | RNA, 5S ribosomal pseudogene 28                                    | -3       |
| HSD17B2 | hydroxysteroid (17-beta) dehydrogenase 2                           | -3       |
| CCNB2   | cyclin B2                                                           | -3       |
| PDI6    | protein disulfide isomerase family A                                | -3       |
| RN7SKP116 | RNA, 7SK small nuclear pseudogene 116                           | -3       |
| CDH5    | cadherin 5, type 2 (vascular endothelium)                           | -3       |
| nPTB | Symbol | Title | Δ |
|------|--------|-------|---|
| TFPI2 | tissue factor pathway inhibitor 2 | -3 |
| LILRA6 | leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 6 | -3 |
| ZNF681 | zinc finger protein 681 | -3 |
| RNU4-16P | RNA, U4 small nuclear 16, pseudogene | -3 |
| CENPU | centromere protein U | -3 |
| GSG2 | germ cell associated 2 (haspin) | -3 |
| LINC00308 | long intergenic non-protein coding RNA 308 | -3 |
| RNU6-83P | RNA, U6 small nuclear 83, pseudogene | -3 |
| DPP4 | dipeptidyl-peptidase 4 | -3 |
| SNORD113-1 | small nucleolar RNA, C/D box 113-1 | -3 |
| XAGE3 | X antigen family, member 3 | -3 |
| RNU6-1188P | RNA, U6 small nuclear 1188, pseudogene | -3 |
| RP11-49H1.3 | novel transcript | -3 |
| MXRA5 | matrix-remodelling associated 5 | -3 |
| GUCY1A3 | guanylate cyclase 1, soluble, alpha 3 | -3 |
| PSG9 | pregnancy specific beta-1-glycoprotein 9 | -3 |
| RNASSP424 | RNA, 5S ribosomal pseudogene 424 | -3 |
| CTD-2562J17.7 | novel transcript | -3 |
| CCDC81 | coiled-coil domain containing 81 | -3 |
| KIAA0101 | KIAA0101 | -3 |
| ASPN | asporin | -3 |
| LOC101929607 | uncharacterized LOC101929607 | -3 |
| LOC644919 | uncharacterized LOC644919 | -3 |
| RP1-69D17.3 | putative novel transcript | -3 |
| NDC80 | NDC80 kinetochore complex component | -3 |
| DSSC1 | DNA replication and sister chromatid cohesion 1 | -3 |
| RP11-337C18.8 | novel transcript | -3 |
| ARHGAP11A | Rho GTPase activating protein 11A | -3 |
| SHCBP1 | SHC SH2-domain binding protein 1 | -3 |
| LPHN3 | latrophilin 3 | -3 |
| GPR115 | G protein-coupled receptor 115 | -3 |
| NUSAP1 | nucleolar and spindle associated protein 1 | -3 |
| RNU6-512P | RNA, U6 small nuclear 512, pseudogene | -3 |
| GATSL2 | GATS protein-like 2 | -3 |
| SNORD114-28 | small nucleolar RNA, C/D box 114-28 | -3 |
| LOC102724842 | uncharacterized LOC102724842 | -3 |
| RNU6-960P | RNA, U6 small nuclear 960, pseudogene | -3 |
| SNORD114-26 | small nucleolar RNA, C/D box 114-26 | -3 |
| KIF14 | kinesin family member 14 | -3 |
| CLSPN | claspin | -3 |
| NAIP | NLR family, apoptosis inhibitory protein | -3 |
| FAR2P1 | fatty acyl CoA reductase 2 pseudogene 1 | -3 |
| CTD-2306M5.1 | novel transcript | -3 |
| ASPM | asp (abnormal spindle) homolog, microcephaly associated (Drosophila) | -3 |
| ELOVL2 | ELOVL fatty acid elongase 2 | -3 |
| HENLS | helicase, lymphoid-specific | -3 |
| HAPLN1 | hyaluronan and proteoglycan link protein 1 | -3 |
| RNU6-971P | RNA, U6 small nuclear 971, pseudogene | -3 |
| MELK | maternal embryonic leucine zipper kinase | -3 |
| LOC101929381 | uncharacterized LOC101929381 | -3 |
| nPTB 2nd | Symbol       | Title                                                                 | Δ   |
|---------|--------------|------------------------------------------------------------------------|-----|
| RN7SKP42 | DNA, 7SK small nuclear pseudogene 42                               | -3  |
| PABPC4L  | poly(A) binding protein, cytoplasmic 4-like                        | -3  |
| RNU6-623P | RNA, U6 small nuclear 623, pseudogene                              | -3  |
| POT1-AS1 | POT1 antisense RNA 1                                               | -3  |
| CDYL2    | chromodomain protein, Y-like 2                                     | -3  |
| RNU6-780P | RNA, U6 small nuclear 780, pseudogene                              | -3  |
| KAL1     | Kallmann syndrome 1 sequence                                        | -3  |
| ERBB3    | v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 3     | -4  |
| ZNF283   | zinc finger protein 283                                            | -4  |
| FMO5     | fibromodulin                                                       | -4  |
| LAMA2    | laminin, alpha 2                                                   | -4  |
| PDIA5    | protein disulfide isomerase family A, member 5                      | -4  |
| METTL7A  | methyltransferase like 7A                                          | -4  |
| CENPE    | centromere protein E, 312kDa                                       | -4  |
| F5       | coagulation factor V (proaccelerin, labile factor)                  | -4  |
| ANKRD36BP2 | ankyrin repeat domain 36B pseudogene 2                          | -4  |
| HNRNPA1P33 | heterogeneous nuclear ribonucleoprotein A1 pseudogene 33            | -4  |
| MEST     | mesoderm specific transcript                                       | -4  |
| CKAP2    | cytoskeleton associated protein 2                                   | -4  |
| KIF11    | kinesin family member 11                                           | -4  |
| DPT      | dermatopontin                                                      | -4  |
| CASC5    | cancer susceptibility candidate 5                                   | -4  |
| MIR580   | microRNA 580                                                       | -4  |
| VIT      | vitrin                                                              | -4  |
| CENPH    | centromere protein H                                                | -4  |
| RNU6-1170P | RNA, U6 small nuclear 1170, pseudogene                            | -4  |
| SCARNA9L | small Cajal body-specific RNA 9-like                                | -4  |
| RNU6-77P | RNA, U6 small nuclear 77, pseudogene                               | -4  |
| RAD51    | RAD51 recombinase                                                   | -4  |
| RNU6-375P | RNA, U6 small nuclear 375, pseudogene                             | -4  |
| MIR205HG | MIR205 host gene (non-protein coding)                               | -4  |
| RNU6-595P | RNA, U6 small nuclear 595, pseudogene                             | -4  |
| AC009262.2 | putative novel transcript                                           | -5  |
| RNU6-308P | RNA, U6 small nuclear 308, pseudogene                             | -5  |
| FGF7     | fibroblast growth factor 7                                          | -5  |
| BRIP1    | BRCA1 interacting protein C-terminal helicase 1                    | -5  |
| RNU6-151P | RNA, U6 small nuclear 151, pseudogene                            | -5  |
| LRP2     | low density lipoprotein receptor-related protein 2                 | -5  |
| TSPAN2   | tetraspanin 2                                                       | -5  |
| NPPB     | natriuretic peptide B                                              | -5  |
| HIST1H3B | histone cluster 1, H3b                                              | -5  |
| RNU2-63P | RNA, U2 small nuclear 63, pseudogene                               | -5  |
| RNU6-560P | RNA, U6 small nuclear 560, pseudogene                            | -6  |
| COL1A2   | collagen, type I, alpha 2                                           | -6  |
| MIR515-1 | microRNA 515-1                                                     | -6  |
| COL3A1   | collagen, type III, alpha 1                                         | -6  |
| PRSS12   | protease, serine, 12 (neurotrypsin, motopsin)                       | -6  |
| HGF      | hepatocyte growth factor (hepapoietin A; scatter factor)            | -7  |
| RNU6-606P | RNA, U6 small nuclear 606, pseudogene                            | -7  |
| RP11-308B16.2 | putative novel transcript                                     | -7  |
| Symbol   | Title                                                                 | \( \Delta \) |
|----------|----------------------------------------------------------------------|---------------|
| RNU2-29P | RNA, U2 small nuclear 29, pseudogene                                  | -7            |
| RNU6-989P| RNA, U6 small nuclear 989, pseudogene                                | -7            |
| LOC389765| kinesin family member 27 pseudogene                                  | -8            |
| CGA      | glycoprotein hormones, alpha polypeptide                              | -8            |
| PDIA3P1  | protein disulfide isomerase family A, member 3 pseudogene 1           | -8            |
| RNU6-79P | RNA, U6 small nuclear 79, pseudogene                                 | -10           |
| HIST1H2AM| histone cluster 1, H2am                                              | -11           |
| RP11-318G21.4 | putative novel transcript | -17          |

**Supplemental Figure 5.** Global gene expression profiles of schCTBs isolated by laser capture microdissection from the fetal membranes: non infected preterm birth (nPTB) as compared to the late second trimester of normal pregnancy (2\(^{nd}\)) \( (n=4/group) \). The heatmap lists genes that were differentially expressed by 2-fold or greater.
Table S1. Maternal and neonatal characteristics (morphology, immunolocalization, invasion and proliferation experiments).

|                              | sPE (n=7)* | nPTB (n=5)* | P value** |
|------------------------------|------------|-------------|-----------|
| Maternal age (years)         | 27.86 (2.94) | 30.20 (2.22) | > 0.05    |
| BMI, Kg/m²                   | 29.90 (2.12) | 24.06 (1.19) | > 0.05    |
| Systolic blood pressure, mmHg| 150.5 (6.58) | 116.5 (4.34) | < 0.01    |
| Diastolic blood pressure, mmHg| 88 (3.56)   | 68.2 (3.86)  | < 0.01    |
| Proteinuria                  | +1 to +3    | 0 or NA     | < 0.05    |
| Gestational age at delivery (week) | 30.93 (1.42) | 32.87 (0.66) | > 0.05    |
| Birth weight, g              | 1264.29 (307.02) | 1957.6 (154.19) | < 0.05    |

* mean ± SD  
** 2-tailed Student’s t-test  
NA: Not available
Table S2. Maternal and neonatal characteristics (transcriptional profiling).

|                          | sPE (n=4)* | nPTB (n=4)* | P value** |
|--------------------------|------------|-------------|-----------|
| Maternal age (years)     | 29.0 (3.34)| 31.7 (2.3)  | > 0.05    |
| BMI, Kg/m²               | 29.95 (3.59)| 25.0 (1.89)| > 0.05    |
| Systolic blood pressure, mmHg | 152.0 (6.58)| 117.8 (7.82)| < 0.02    |
| Diastolic blood pressure, mmHg | 91.62 (3.25)| 72.7 (4.97)| < 0.02    |
| Proteinuria              | +1 to +3   | 0 or NA     | < 0.05    |
| Gestational age at delivery (weeks) | 26.0 (3.76)| 32.4 (0.82)| > 0.05    |
| Birth weight (grams)     | 908.75 (177.82)| 2083.3 (207.87)| < 0.01    |

* mean ± SD
** 2-tailed Student’s t-test
NA: Not available
Table S3. Antibodies

| Antibody       | Catalog Number/ Clone | Source                        | Concentration (µg/ml) |
|----------------|------------------------|-------------------------------|----------------------|
| Vimentin       | V4630                  | Sigma Aldrich                 | 72.6                 |
| CK7            | 7D3                    | Damsky et al., 1992*          | 26                   |
| HLA-G          | 4H84                   | McMaster et al., 1998**       | 20                   |
| ITGA4          | NBP1-77333             | Novus Biologicals             | 10                   |
| E-cadherin     | 36/E-cadherin          | BD Biosciences                | 2.5                  |
| hPL            | MCA331                 | Serotec                       | 50                   |
| GSTA3          | sc-100547              | Santa Cruz Biotechnology      | 1                    |
| PAPP-A         | ab203683               | Abcam                         | 10                   |
| anti-rabbit secondary Ab | A21206                    | Life Technologies             | 2                    |
| anti-goat secondary Ab  | A11055                   | Life Technologies             | 2                    |
| anti-mouse secondary Ab | A21907                   | Life Technologies             | 2                    |
| anti-rat secondary Ab  | 712-025-153            | Jackson ImmunoResearch        | 15                   |

* Damsky C, et al 1992. Distribution patterns of extracellular matrix components and adhesion receptors are intricately modulated during first trimester cytotrophoblast differentiation along the invasive pathway, in vivo. J Clin Invest. 89: 210-22

** McMaster M, et al 1998. HLA-G isoforms produced by placental cytotrophoblasts and found in amniotic fluid are due to unusual glycosylation. J Immunol. 160(12) 5922-8.