Supplemental Figure 1

a

CASC15, Putative copy-number alterations from GISTIC

CASC15 mRNA Expression z-scores (RNA Seq V2 RSEM)

Hetloss  Diploid  Gain  Amp

**

**

C

LOC100132354, Putative copy-number alterations from GISTIC

LOC100132354 mRNA Expression z-scores (RNA Seq V2 RSEM)

Hetloss  Diploid  Gain  Amp
Supplemental Figure 5

(a) Graph showing relative CASC14 levels (LOG$_2$ n+1) for Melanocytes (n=2) and Melanoma cell lines (n=30).

(b) Box plot comparing relative CASC14 levels (LOG$_2$ n+1) for BRAF-wt (9) and BRAF-mt (20).
Supplemental Figure 6

(a) Diagram showing correlation between Exons 10-11 and Exon 8, with a correlation coefficient of $r=0.61^{**}$.

(b) Diagram showing correlation between Exons 11-12 and Exon 8, with a correlation coefficient of $r=0.70^{**}$.

(c) Diagram showing correlation between Exons 11-12 and Exons 10-11, with a correlation coefficient of $r=0.94^{**}$.
Nevus/Normal skin (n=15)
Stage I (10)
Stage II (7)
Stage III (8)
Other organ mets (11)
Brain/Lung mets (19)

Exons 10-11 vs. Exon 8

Relative levels (LOG\(_2\) n+1)

Stage IV

Supplemental Figure 7

a

b

r=0.83**

Log Rank: 1.08
p=0.3

High, n=38
Low, n=103

Overall survival

Years after LNM surgery

Log Rank: 1.08
p=0.3
Supplemental Figure Legends

Figure S1. The CASC15 locus is expressed in melanoma tumors. TCGA RNA sequencing (RNASeq Version 2, z-score +/- 1.5) and gene copy number (GISTIC2) data for the CASC15 (a) and LOC100132354 (b) lincRNA loci. Results show a positive correlation between CASC15 expression and CASC15 copy number status of 278 melanoma specimens. (**P<0.01, Mann-Whitney test).

Figure S2. Copy number alterations identified by SNP 6.0 of chromosome 6p in 78 metastatic melanoma specimens. The upper plot represents the CN value for 21,302 regions located on the short arm of the chromosome 6. The middle panel represents the CN value for 2,457 regions located between the region sub-band 3 and sub-band 2 of the region 2, band 2 of the short arm of the chromosome 6 (6p22.3-6p22.2). The lower panel represents the CN value for 250 regions located in the CASC15 locus.

Figure S3. Normal and pan-cancer CASC15 expression. (a) Illumina Body Map data RNA-seq data (EMBL-EBI Expression Atlas) from 16 normal human tissues was interrogated for CASC15, HOTAIR, and MALAT1 expression levels. Absolute CASC15 levels (FPKM values) were extremely low in normal tissues, and similar to the well-characterized HOTAIR lincRNA. The levels of the ubiquitous MALAT1 lincRNA are shown as a comparison. (b) TCGA RNAseq Version 2 data (RSEM values) from 23 different tumor types were downloaded from the cBioPortal (cbioportal.org). Median CASC15 levels are shown. Bars represent the range of values (Min to Max). AC: Adrenocortical Carcinoma (n=79), AML: Adult Myeloid Leukemia (n=173), BC: Bladder Urothelial Carcinoma (n=129), BrC: Breast Carcinoma (n=1098), CA: Colorectal Adenocarcinoma (n=365), CM: Cutaneous Melanoma (n=470), CSSC: Cervical Squamous Cell Carcinoma (n=253), DG: Diffuse Glioma (n=527), DLBCL: Diffuse Large B-Cell

1 Broad Institute TCGA Genome Data Analysis Center (2014): Analysis Overview for Skin Cutaneous Melanoma (Metastatic cohort) - 15 July 2014. Broad Institute of MIT and Harvard. doi:10.7908/C18P5Z9Z
Lymphoma (n=28), EC: Endometrial Carcinoma (n=170), GM: Glioblastoma Multiforme (n=166), HNSSC: Head and Neck Squamous Cell Carcinoma (n=498), KRCCC: Kidney Renal Clear Cell Carcinoma (n=532), KRPCC: Kidney Renal Papillary Cell Carcinoma (n=290), LHC: Liver Hepatocellular Carcinoma (n=269), LSCC: Lung Squamous Cell Carcinoma (n=501), LuA: Lung Adenocarcinoma (n=490), OSC: Ovarian Serous Cystadenocarcinoma (n=179), PaA: Pancreatic Adenocarcinoma (n=179), PrA: Prostatic Adenocarcinoma (n=487), Src: Sarcoma (n=261), TC: Thyroid Carcinoma (n=509), UC: Uterine Carcinosarcoma (n=57).

**Figure S4.** Multiple isoforms are transcribed from the CASC15 locus. UCSC genome browser representation of the CASC15 locus on chromosome 6p22.3 (GRCh37/hg19 assembly), along with RefSeq (release 58) and GENCODE v17 annotated genes, Human spliced ESTs, and CaptureSeq data of a foot fibroblast sample (Mercer, T.R., et al, Nature Biotechnology, 2011). The CR739395 EST and CaptureSeq transcripts assemblies aligning with CASC15 exon 1b are highlighted in red. Several ESTs and CaptureSeq isoforms also fully or partially align with splice variants identified in 3'-RACE experiments.

**Figure S5.** Expression of CASC14 in melanoma cell lines. (a) RT-qPCR detection of CASC14 (exon 3) levels in melanocytes (grey bars, n=2; ψ: pMEL-NRAS; π: primary melanocytes) and melanoma cell lines (black bars, n=30). (LOG2 (n+1) of 2^{(ddCq)} relative values) (b) CASC14 expression (RT-qPCR; exon 3) in BRAF BRAFwt (N=9) and BRAFmt (N=20) melanoma cell lines. BRAFmt includes V600E (N=16), V600K (N=3), and V600R (N=1). (LOG2 (n+1) of 2^{(ddCq)} relative values; Whiskers Min to Max; T-test, P>0.05).

**Figure S6.** Expression of CASC15 isoforms in melanoma cell lines. Dot blots depicting the correlation between the expression levels of CASC15 (a) exon 8- and 10-11-containing isoforms, (b) exon 8- and 11-12-containing isoforms, and (c) exon 10-11 and 11-12-containing isoforms. (RT-qPCR, N=30; LOG2 (n+1) of 2-(ddCq) relative values; Pearson r, **P<0.01).
**Figure S7.** Expression of CASC15 in melanoma specimens. (a) Dot blot depicting the correlation between CASC15 exon 8- and 10-11 isoforms in normal, nevus, and melanoma tumor specimens (RT-qPCR, LOG2 (n+1) of 2−(ddCq) relative values; Pearson r, **P<0.01, N=70.** (b) RT-qPCR detection of CASC15 expression levels (exon 10-11-containing isoforms) as a function of tumor stage. See **Table S1** for details. (LOG2 (n+1) of 2−(ddCq) relative values; T-test; **, P<0.01; Mean +/- SEM). (c) Kaplan-Meier curves representing the relationship between CASC15 expression in stage III melanoma LN mets and 10-year OS.

**Figure S8.** Efficiency of CASC15 knockdown is melanoma cell lines. Efficiency of CASC15 siRNA transfection in (a) WP, (b) M16, and (c) RKTJ-CB1 cells. (RT-qPCR, 2−(ddCq) value of siPool1 or siPool2 relative to siCTRL condition, T-test, **, P<0.01; Mean +/- SEM, N=4).

**Figure S9.** Effect of CASC15 knockdown on cell proliferation and baseline cell survival. (a) BrdU cell proliferation assays in WP, M16, and RKTJ-BI3 cells. Bars represent the average fold difference in the rate of DNA synthesis in siPool conditions relative to siCTRL (T-test, **, P<0.01; *, P<0.05; Mean +/- SEM, N=4). (b) Annexin V-PI assays in WP, M16, and RKTJ-BI3 cells. Bars represent the average fold difference in the percent number of cell death in siPool conditions relative to siCTRL (T-test, **, P<0.01; *, P<0.05; Mean +/- SEM, N=4 (N=3 for WP)).

**Figure S10.** Assessment of CASC15 expression in melanoma cells using the RNAscope Fluorescent Assay. Photographs are representative of CASC15 RNA-ISH staining of WP (a-b) and M16 (c-d) melanoma cells transfected with control siRNA (a, c) or CASC15 siRNA (b, d). Two fields per condition are presented. Zoom in of 40X photographs. Scale bars = 10µm. CASC15 cytoplasmic and nuclear expression was visualized using a FITC-labeled probe (green dots). Cell nuclei were counterstained with DAPI (blue). We observed robust CASC15 knockdown in both cytoplasmic and nuclear compartments upon siRNA treatment (b1-b2, d1-d2).
Supplemental Materials and Methods

Melanoma specimens and cell lines

Previously described YDFR.C, YDFR.SB3, and RKTJ-CB1 cell lines were derived from a mouse xenograft model of human melanoma brain metastasis (Izraely et al., 2012). The three cell lines used for siRNA knockdown studies (RKTJ-CB1, WP, and M16) were selected due to their high CASC15 expression levels as well as efficient CASC15 silencing upon siRNA treatment (Figure S8). RKTJ-CB1 brain metastasis cells are derived from a first round of intracardiac injection of parental RKTJ.C. cells (Izraely et al., 2012). WP (WP-0614-ME) cells are derived from a melanoma stage IV left frontal parietal brain lesion (Marzese et al., 2014). M16 (M16-ME) cells originate from a melanoma stage IV right anterior parietal brain lesion (Marzese et al., 2014). WP and M16 cells were also used for genome-wide exon array gene expression profiling (Figure 5d, Table S1). A list of specimens and cell lines used in this study is provided in Table S1.

DNA copy number profiling

The Contrast Quality Control algorithm was used for array quality control, with a minimal call rate >95%. The HapMap reference (release 28) was used as the reference model for CNA analyses of the 2367 intergenic domains (chromosome X domains excluded; Tables S2-S3) assessed in this study (Khalil et al., 2009). RefSeq (release 59) and GENCODE v17 databases were used to identify 6p-annotated lincRNAs.

Reverse transcription and quantitative real-time PCR (RT-qPCR)

For both cell line and tissue samples, the amount of cDNA was normalized to the SDHA housekeeping gene expression levels and analyzed with the delta-delta Cq (ddCq). For absolute RNA quantification, a 7-log serial dilution (10^1 to 10^7 copies) of a pME18SFL3 plasmid
containing the full-length CASC15 cDNA clone (Clone ID HRC13155, NBRC, Japan) was used to estimate RNA copy number per 15ng of input RNA. Determination of nuclear to cytoplasmic enrichment was performed using the following calculation: 

\[ \text{ddCq (Cytoplasm-Nucleus)} = \text{dCq (target gene-SDHA)}_{\text{cytoplasm}} - \text{dCq (target gene-SDHA)}_{\text{nucleus}} \]

A list of all qPCR primers used in this study is provided in Table S4.

**Rapid amplification of cDNA ends (RACE)**

Following the final round of amplification with inner primers, PCR products were separated by gel electrophoresis and extracted using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). Amplicons were then cloned into a pCR-2.1 vector (TOPO TA Cloning Kit, Life Technologies, Carlsbad, CA) and submitted to Sanger sequencing (Eurofins, Huntsville, AL) using M13 primers provided with the cloning kit.

**Melanoma tissue microarray (TMA)**

The previously described AJCC stage III melanoma TMA (Nguyen et al., 2011) includes 160 lymph node metastasis specimens (160 patients, duplicate cores) with well-annotated clinical follow-up. The TMA also contains tumor negative lymph nodes (negative control) and melanoma cell lines (positive control). Detached/damaged cores, cores with no evidence of tumor tissue, and cores with strong melanin expression were excluded from the analysis, leaving specimens from 141 patients.

**RNA in situ hybridization (RNA-ISH)**

For normal skin, CASC15 expression was assessed in the basal epidermal layer. A scoring scale assessing the percentage of tumor cells with 0, 1-3, 4-10, and >10 dots/cell was used to quantify CASC15 expression levels. For tissue imaging, sections were scanned using the Leica SCN400 scanner (Leica Biosystems, Buffalo Grove) and analyzed using the Aperio
ImageScope software (Leica Biosystems). For cellular immunoflorescence imaging, the Nuance Fx system (Perfin Elmer, Foster City, CA) was used for image acquisition and the Aperio ImageScope software (Leica Biosystems) for analysis.

**RNA interference**

Custom Mission siRNA pools (siPool1 and siPool2) and the Mission Universal Negative Control #1 (siCTRL) were obtained from Sigma-Aldrich, St.Louis, MO. CASC15-targeting sequences are provided in Table S4. WP, M16, and RKTJ-CB1 metastatic melanoma cell lines were seeded 24h before transfection at a density of 2-2.5 x 10^5 cells per 60mm dish, and subsequently transfected (24h) with jetPRIME reagent (VWR) at a final siRNA concentration of 30nM.

**Invasion assay**

WP, M16, and RKTJ-CB1 cells were transfected with siRNAs for 48h and then seeded into the upper chambers in duplicate at 2.5 x 10^4 cells per well. Cells were allowed to settle for 2h in complete medium (RPMI 1640 with 10% FBS) before the addition of serum-free RPMI 1640 in the upper chambers and complete medium in the lower chambers. After 24h, cells were fixed and stained in 2% crystal violet and 20% methanol in PBS. Photographs were taken under a Nikon Eclipse Ti microscope (Nikon) at 10X, with four random fields taken per chamber, for a total of eight fields per condition. Cells were counted by two independent investigators (L.L. and M.L.).

**Cell proliferation and apoptosis assays**

Analysis of DNA synthesis was done using the FITC BrdU Flow kit (BD Biosciences, San Diego, CA) following the manufacturer's instructions, with a 1h BrdU incorporation pulse. Quantification of apoptosis was performed using the FITC Annexin V Apoptosis Detection Kit
(BD Biosciences). A BD FACSVerse flow cytometer (BD Biosciences) was used for both assays that were performed 48h following siRNA transfection.

**mRNA sequencing and expression analysis**

The mRNA seQuant-iT RiboGreen RNA Assay (Life Technologies) was used to quantitate 1.0 µg of high quality total RNA (RIN > 8.0) extracted using the Ambion Paris Kit (Life Technologies). Libraries enriching for mRNA from the total RNA were prepared using the TruSeq Stranded mRNA Library Prep Kit (Illumina Inc., San Diego, CA) according to the manufacturer’s recommended protocol. The final mRNA libraries were quantitated using the Agilent High Sensitivity D1000 TapeStation system (Agilent Technologies, Santa Clara, CA) and Qubit dsDNA HS Assay kit (Life Technologies). The pooled libraries were sequenced on the Illumina HiSeq 2500 in rapid mode with 100 bp paired-end reads. An average sequencing depth of 75 million pass-filter reads was achieved per sample. The resulting bcl files for each condition were demultiplexed and converted to fastq files using the CASAVA v.1.8.2 software (Illumina Inc.). Sequencing quality was assessed using FastQC[^2] for each sample. Adapter trimming was performed using cutadapt[^3] with the adapter sequence provided by Illumina (AGATCGGAAGAGC). All samples were aligned to the hg19 reference genome downloaded from Illumina iGenome[^4] with bowtie2 (default parameters; (Langmead and Salzberg, 2012)). Raw counts were generated using the subread package’s featureCounts tool (Liao et al., 2013). The extracted feature counts were then normalized using DEseq2 package (Love et al., 2014).

**Statistics**

The Mann-Whitney or the Kruskal-Wallis tests were used to compare the non-parametric variables between two or more groups, respectively. The Student’s T-test was used to compare

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[^2]: [http://www.bioinformatics.babraham.ac.uk/projects/fastqc/](http://www.bioinformatics.babraham.ac.uk/projects/fastqc/)
[^3]: [https://github.com/marcelm/cutadapt](https://github.com/marcelm/cutadapt)
[^4]: [http://support.illumina.com/sequencing/sequencing_software/igenome.html](http://support.illumina.com/sequencing/sequencing_software/igenome.html)
parametric distributions between two groups. The Spearman’s rho correlation test was used to measure the correlations between CASC15 isoform expression levels (RT-qPCR), as well as the correlations between CASC15 expression and proliferative/invasive signature genes (HuEx). Kaplan-Meier and Log-Rank analyses were conducted to compare time of disease-free survival (DFS) and overall survival (OS) between high CASC15-expressing (>75th percentile) and low CASC15-expressing (≤75th percentile) lymph node metastasis specimens. Univariate and multivariate Cox proportional hazard models were used to assess survival and estimate hazard ratios (HR) adjusted for age, gender, primary tumor thickness, primary tumor ulceration, and number of positive lymph nodes (Table S6). The percentage of CASC15 positive cells per specimen was coded as a continuous variable. The Akaike information criterion (AIC) was used to estimate the predictive strength of multivariate models.

Supplemental References

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Nguyen T, Kuo C, Nicholl MB, *et al.* (2011) Downregulation of microRNA-29c is associated with hypermethylation of tumor-related genes and disease outcome in cutaneous melanoma. *Epigenetics: official journal of the DNA Methylation Society* 6:388-94.
Table S8. RNA sequencing analysis of proliferative and invasive gene signatures upon CASC15 knockdown

| Motif | Genes | M16 siPool2 | M16 siCTRL | RKTJ-BI3 siPool2 | RKTJ-BI3 siCTRL | WP siPool2 |
|-------|-------|-------------|------------|------------------|-----------------|------------|
| Motif 1 | PLP1  | 12624.24    | 16740.34   | 14710.28         | 19214.8         | 12587.77   |
| Motif 1 | EDNRB | 7489.09     | 13041.58   | 3252.63          | 5704.37         | 4009.9     |
| Motif 1 | LZTS1 | 7977.23     | 11771.63   | 3584.9           | 5442.67         | 3868.34    |
| Motif 1 | PHACTR1 | 100.58 | 241.32     | 93.8             | 273.23          | 232.3      |
| Motif 1 | GPR56 | 8581.64     | 10390.56   | 10109.36         | 16053.64        | 3856.55    |
| Motif 1 | AP1S2 | 9783.07     | 14374.91   | 3146.39          | 5936.1          | 3205.93    |
| Motif 1 | ERBB3 | 2993.42     | 4537.3     | 3926.21          | 5805.83         | 10478.92   |
| Motif 1 | INPP4B | 698.53  | 11771.63   | 3584.9           | 5442.67         | 3868.34    |
| Motif 1 | S100A1 | 100.58  | 241.32     | 93.8             | 273.23          | 232.3      |
| Motif 1 | GPRC5B | 1312.16    | 2119.78    | 1078.18          | 2208.89         | 765.87     |
| Motif 1 | ST3GAL6 | 3207.5    | 2811.61    | 1026.19          | 901.54          | 395.64     |
| Motif 1 | PLA1A | 498.29      | 457.46     | 27.12            | 19.6            | 45.37      |
| Motif 1 | CAPN3 | 2205.39     | 3536.44    | 1702.03          | 4020.04         | 441.92     |
| Motif 1 | PIR   | 1335.23     | 1067.7     | 1121.13          | 1172.46         | 326.67     |
| Motif 1 | SOX10 | 8588.1      | 13288.11   | 9378.14          | 15606.33        | 9993.45    |
| Motif 1 | IRF4  | 1551.15     | 1979.15    | 209.08           | 788.56          | 3112.46    |
| Motif 1 | GK    | 278.67      | 469.62     | 179.7            | 398.89          | 183.3      |
| Motif 1 | TFAP2A | 2683.38  | 3239.56    | 1491.82          | 1371.91         | 2721.36    |
| Motif 1 | GPRC5B | 1312.16    | 2119.78    | 1078.18          | 2208.89         | 765.87     |
| Motif 1 | ST3GAL6 | 3207.5    | 2811.61    | 1026.19          | 901.54          | 395.64     |
| Motif 1 | PLA1A | 498.29      | 457.46     | 27.12            | 19.6            | 45.37      |
| Motif 1 | CAPN3 | 2205.39     | 3536.44    | 1702.03          | 4020.04         | 441.92     |
| Motif 1 | PIR   | 1335.23     | 1067.7     | 1121.13          | 1172.46         | 326.67     |
| Motif 1 | SOX10 | 8588.1      | 13288.11   | 9378.14          | 15606.33        | 9993.45    |
| Motif 1 | Gene | Motif 1  | Motif 1  | Motif 1  | Motif 1  | Motif 1  |
|--------|------|----------|----------|----------|----------|----------|
| Motif 1 | TRAF4 | 1049.17  | 1117.18  | 1531.38  | 2787.63  | 1265.86  |
| Motif 1 | APOC1 | 14.76    | 7.81     | 3.39     | 1.15     | 0        |
| Motif 1 | CLCN7 | 3309.93  | 3099.81  | 1905.46  | 1780.02  | 1958.22  |
| Motif 1 | CDK5R1| 635.78   | 664.93   | 441.9    | 544.15   | 1088     |
| Motif 1 | ITPKB | 1039.95  | 1669.26  | 567.34   | 91.08    | 321.23   |
| Motif 1 | RAB38 | 2906.68  | 3066.82  | 528.92   | 1112.51  | 2833.88  |
| Motif 1 | PMEL  | 21810.28 | 26663.02 | 492.75   | 517.64   | 3349.3   |
| Motif 1 | TRPM1 | 490.91   | 748.26   | 0        | 0        | 2.72     |
| Motif 1 | APOE  | 310.05   | 315.1    | 176.31   | 306.66   | 968.22   |
| Motif 2 | CXCL8 | 796.34   | 466.14   | 142.4    | 28.82    | 213.24   |
| Motif 2 | PTX3  | 36.91    | 34.72    | 5.65     | 5.76     | 315.78   |
| Motif 2 | CEMIP | 1791.99  | 1143.22  | 617.07   | 2673.49  | 152.45   |
| Motif 2 | CXCL2 | 12       | 6.08     | 0        | 0        | 0.91     |
| Motif 2 | IL6   | 34.14    | 43.4     | 3.39     | 4.61     | 1.81     |
| Motif 2 | CDH2  | 726.21   | 1001.73  | 1184.42  | 1175.92  | 1039     |
| Motif 2 | CTGF  | 1303.86  | 1458.32  | 9147.59  | 3274.14  | 4017.16  |
| Motif 2 | TPM1  | 2916.83  | 3686.61  | 2331.54  | 2015.21  | 2487.25  |
| Motif 2 | CTGF  | 1303.86  | 1458.32  | 9147.59  | 3274.14  | 4017.16  |
| Motif 2 | TPM1  | 2916.83  | 3686.61  | 2331.54  | 2015.21  | 2487.25  |
| Motif 2 | FGF2  | 501.06   | 493.05   | 315.32   | 327.41   | 622.49   |
| Motif 2 | DKK1  | 2995.27  | 2045.99  | 82.5     | 55.34    | 857.51   |
| Motif 2 | PLAGL1| 0        | 0        | 3.39     | 4.61     | 8.17     |
| Motif 2 | CYR61 | 859.09   | 953.12   | 1062.36  | 338.94   | 1336.63  |
| Motif 2 | THBS1 | 764.97   | 1134.54  | 21423.48 | 8178.42  | 9503.44  |
| Motif 2 | ELL2  | 513.98   | 357.64   | 620.46   | 246.71   | 578.03   |
| Motif 2 | ADAM12| 176.25   | 294.27   | 573      | 863.5    | 74.41    |
| Motif 2 | PDGFC | 207.62   | 98.09    | 274.63   | 112.98   | 223.23   |
| Motif 2 | NTM   | 194.7    | 347.22   | 331.14   | 455.38   | 30.85    |
| Motif 2 | INHBA | 42.45    | 28.65    | 96.06    | 229.42   | 17.24    |
| Motif 2 | LOXL2 | 1188.51  | 889.75   | 2185.75  | 845.05   | 6744.88  |
| Motif 2 | DSE   | 110.73   | 96.35    | 103.98   | 100.3    | 21.78    |
| Motif 2 | NRP1  | 863.7    | 822.04   | 875.88   | 591.42   | 235.02   |
| Motif 2 | CDH13 | 4.61     | 6.94     | 1.13     | 0        | 39.93    |
| Motif 2 | DKK3  | 2057.75  | 1718.74  | 2.26     | 2.31     | 2627.9   |
| Motif 2 | TPM2  | 2175.86  | 1499.12  | 31.64    | 40.35    | 109.8    |
| Motif 2 | TAGLN | 41.52    | 32.12    | 20.34    | 28.82    | 34.48    |
| Motif 2 | AXL   | 104.27   | 190.97   | 136.75   | 168.32   | 1126.11  |
| Motif 2 | THY1  | 1.85     | 1.74     | 0        | 0        | 33.57    |
| Motif 2 | PODXL | 33.22    | 24.31    | 1792.45  | 570.67   | 1542.62  |
| Motif 2 | FBN1  | 190.09   | 130.21   | 120.93   | 58.8     | 177.85   |
| Motif 2 | AMIGO2| 0.92     | 0.87     | 29.38    | 16.14    | 20.87    |
| Motif 2 | EHD2  | 584.11   | 232.64   | 1950.67  | 516.48   | 931.02   |
| Motif 2 | FOXD1 | 155.02   | 144.1    | 396.69   | 463.45   | 384.75   |
| Motif 2 | NUAK1 | 134.72   | 149.3    | 387.65   | 267.46   | 303.99   |
| Motif 2 | TGFBI | 1745.86  | 1477.42  | 705.23   | 85.31    | 3440.95  |
| Motif 2 | Gene        | Motif 2 | Motif 2 | Motif 2 | Motif 2 | Motif 2 |
|--------|-------------|---------|---------|---------|---------|---------|
|        | TNFRSF11B   | 17.53   | 11.28   | 6.78    | 6.92    | 6.35    |
|        | WNT5B       | 44.29   | 45.14   | 22.6    | 5.76    | 360.25  |
|        | NID2        | 4.61    | 5.21    | 4.52    | 8.07    | 8.17    |
|        | SERPINE1    | 14.76   | 6.94    | 53.12   | 17.29   | 45.37   |
|        | LOX         | 585.95  | 405.38  | 93.8    | 65.71   | 606.16  |
|        | NID2        | 4.61    | 5.21    | 4.52    | 8.07    | 8.17    |
|        | SERPINE1    | 14.76   | 6.94    | 53.12   | 17.29   | 45.37   |
|        | WNT5B       | 44.29   | 45.14   | 22.6    | 5.76    | 360.25  |
|        | NID2        | 4.61    | 5.21    | 4.52    | 8.07    | 8.17    |
|        | SERPINE1    | 14.76   | 6.94    | 53.12   | 17.29   | 45.37   |
|        | LOX         | 585.95  | 405.38  | 93.8    | 65.71   | 606.16  |
|        | NID2        | 4.61    | 5.21    | 4.52    | 8.07    | 8.17    |
|        | SERPINE1    | 14.76   | 6.94    | 53.12   | 17.29   | 45.37   |
|        | WNT5B       | 44.29   | 45.14   | 22.6    | 5.76    | 360.25  |
|        | NID2        | 4.61    | 5.21    | 4.52    | 8.07    | 8.17    |
|        | SERPINE1    | 14.76   | 6.94    | 53.12   | 17.29   | 45.37   |
|        | LOX         | 585.95  | 405.38  | 93.8    | 65.71   | 606.16  |
|        | NID2        | 4.61    | 5.21    | 4.52    | 8.07    | 8.17    |
|        | SERPINE1    | 14.76   | 6.94    | 53.12   | 17.29   | 45.37   |
|        | LOX         | 585.95  | 405.38  | 93.8    | 65.71   | 606.16  |
|        | NID2        | 4.61    | 5.21    | 4.52    | 8.07    | 8.17    |
|        | SERPINE1    | 14.76   | 6.94    | 53.12   | 17.29   | 45.37   |
|        | LOX         | 585.95  | 405.38  | 93.8    | 65.71   | 606.16  |
|        | NID2        | 4.61    | 5.21    | 4.52    | 8.07    | 8.17    |
|        | SERPINE1    | 14.76   | 6.94    | 53.12   | 17.29   | 45.37   |
|        | LOX         | 585.95  | 405.38  | 93.8    | 65.71   | 606.16  |
|        | NID2        | 4.61    | 5.21    | 4.52    | 8.07    | 8.17    |
|        | SERPINE1    | 14.76   | 6.94    | 53.12   | 17.29   | 45.37   |
|        | LOX         | 585.95  | 405.38  | 93.8    | 65.71   | 606.16  |
|        | NID2        | 4.61    | 5.21    | 4.52    | 8.07    | 8.17    |
|        | SERPINE1    | 14.76   | 6.94    | 53.12   | 17.29   | 45.37   |
|        | LOX         | 585.95  | 405.38  | 93.8    | 65.71   | 606.16  |
| WP siCTRL | Genes*         | siPool2/siCTRL ratio |
|-----------|----------------|----------------------|
|           |                | M16                  | RKTJ-B13 | WP        |
| 25287.28  | PLP1           | 0.75412088           | 0.76557029 | 0.49779059 |
| 11249.96  | EDNRB          | 0.57424714           | 0.57019969 | 0.35643682 |
| 6020.87   | LZTS1          | 0.67766571           | 0.65866569 | 0.64248854 |
| 665.5     | PHACTR1        | 0.41679098           | 0.34330052 | 0.34906086 |
| 5992.29   | GPR56          | 0.82590736           | 0.62972385 | 0.64358534 |
| 5064.09   | AP1S2          | 0.68056565           | 0.53004329 | 0.63307129 |
| 13451.09  | ERBB3          | 0.65973597           | 0.67625301 | 0.77903872 |
| 1034.2    | INPP4B         | 0.86157433           | 1.08920881 | 0.71772384 |
| 762.29    | S100A1         | 1.16200165           | 1.17715772 | 0.8142308 |
| 1918.16   | DCT            | 0.60041546           | 0.46070619 | 0.69162635 |
| 1350.36   | CAPN3          | 0.62361867           | 0.42338633 | 0.32726088 |
| 500.51    | PIR            | 1.25056664           | 0.95622025 | 0.65267427 |
| 12967.18  | SOX10          | 0.64629959           | 0.60091899 | 0.77067257 |
| 4884.35   | IRF4           | 0.78374555           | 0.26514152 | 0.63723116 |
| 320.77    | GK             | 0.59339466           | 0.45050014 | 0.57143748 |
| 6027.32   | TFAP2A         | 0.82831619           | 1.08740369 | 0.45150415 |
| 1790.04   | GPRC5B         | 0.61900763           | 0.48810941 | 0.42785077 |
| 592.69    | ST3GAL6        | 1.14080545           | 1.13826342 | 0.66753277 |
| 64.52     | PLA1A          | 1.08925371           | 1.38367347 | 0.70319281 |
| 513.41    | SNCA           | 1.22552774           | 1.38740732 | 0.98622933 |
| 119.83    | CITED1         | 0.82149037           | 0.58983125 | 0.96169574 |
| 12427.95  | TYR            | 0.66892663           | 0.43434469 | 0.38807285 |
| 364.09    | RENBP          | 0.69464151           | 0.46409867 | 0.41121701 |
| 2665.7    | NSG1           | 0.71526926           | 0.6289702  | 0.4095097 |
| 453.5     | BCL2A1         | 1.24697921           | 0.77121004 | 0.59228225 |
| 1671.13   | GREB1          | 1.12946094           | 0.90543815 | 0.74988182 |
| 430.46    | GMPR           | 1.83750247           | 1.3535729  | 0.90224411 |
| 3128.42   | GPM6B          | 0.80123564           | 0.33942962 | 0.44320775 |
| 848.93    | RRAKD          | 1.21929462           | 0.96951873 | 1.10845417 |
| 7099.32   | MITF           | 0.71976031           | 0.55963097 | 0.50475398 |
| 878.43    | SLC45A2        | 0.59449754           | 0.6125768  | 0.52786221 |
| 114.3     | GALNT3         | 0.62171201           | 0.34288774 | 0.46045494 |
| 460.87    | GYG2           | 1.20808416           | #DIV/0!    | 1.31327706 |
| 1132.83   | MLANA          | 0.80388821           | 0.55238882 | 0.43335717 |
| 13440.03  | TYRP1          | 0.63734899           | 0.81671003 | 0.67921575 |
| 2246.3    | MREG           | 1.36604772           | 0.60995155 | 0.49646975 |
| 1094.12   | TNFRSF14       | 0.64701627           | 0.49817845 | 0.51503491 |
| 543.83    | GPR143         | 1.02073733           | 1.96521739 | 0.82593825 |
| 525.4     | PLXNC1         | 0.5375439            | 0.72696262 | 0.33679102 |
| 4801.39   | MBP            | 0.55098718           | 0.17504249 | 0.36891192 |
| Gene  | Log2FC | Log10FC1 | Log10FC2 | Log10FC3 |
|-------|--------|----------|----------|----------|
| TRAF4 | 0.939 | 0.549348 | 0.625659 | 0.625659 |
| APOC1 | 1.899 | 2.947826 | 0.625659 | 0.625659 |
| CLCN7 | 1.067 | 1.070471 | 1.153971 | 1.153971 |
| CDK5R1| 0.956 | 0.812092 | 0.907976 | 0.907976 |
| ITPKB | 0.623 | 0.651499 | 0.657482 | 0.657482 |
| RAB38 | 0.947 | 0.475429 | 0.583056 | 0.583056 |
| RGS20 | 1.042 | 1.079491 | 0.902839 | 0.902839 |
| PMEL  | 0.818 | 0.951916 | 1.048971 | 1.048971 |
| TRPM1 | 0.656 | 0.184407 | #DIV/0! | #DIV/0! |
| APOE  | 0.984 | 0.574936 | 0.9985 | 0.9985 |
| CXCL8 | 1.708 | 4.941013 | 1.31443 | 1.31443 |
| PTX3  | 0.894 | 2.793891 | 2.41051 | 2.41051 |
| CEMIP | 1.567 | 0.230811 | 0.298004 | 0.298004 |
| CXCL2 | 1.974 | #DIV/0! | #DIV/0! | #DIV/0! |
| IL6   | 0.787 | 0.735357 | 1.967391 | 1.967391 |
| CDH2  | 0.725 | 1.007228 | 0.590468 | 0.590468 |
| CTGF  | 0.894 | 2.793891 | 2.41051 | 2.41051 |
| TPM1  | 0.791 | 1.156971 | 1.20357 | 1.20357 |
| FGF2  | 1.016 | 0.963074 | 1.424756 | 1.424756 |
| DKK1  | 1.464 | 1.490784 | 2.704363 | 2.704363 |
| PLAG1 | #DIV/0! | 0.735357 | 1.266667 | 1.266667 |
| CYR61 | 0.901 | 3.13436 | 2.762088 | 2.762088 |
| THBS1 | 0.674 | 2.619513 | 2.036786 | 2.036786 |
| ELL2  | 1.437 | 2.514936 | 2.056095 | 2.056095 |
| ADAM12| 0.599 | 0.663578 | 0.183703 | 0.183703 |
| PDGFC | 2.116 | 2.430784 | 1.021881 | 1.021881 |
| NTM   | 0.560 | 0.727173 | 0.656243 | 0.656243 |
| INHBA | 1.481 | 0.418708 | 1.439065 | 1.439065 |
| LOXL2 | 1.336 | 2.586533 | 2.123468 | 2.123468 |
| DSE   | 1.149 | 1.036689 | 0.421929 | 0.421929 |
| NRP1  | 1.051 | 1.480978 | 1.15899 | 1.15899 |
| CDH13 | 0.664 | #DIV/0! | 2.548181 | 2.548181 |
| DKK3  | 1.197 | 0.978354 | 0.938133 | 0.938133 |
| TPM2  | 1.451 | 0.784139 | 1.134531 | 1.134531 |
| TAGLN | 1.292 | 0.705759 | 1.700197 | 1.700197 |
| AXL   | 0.546 | 0.812441 | 1.517648 | 1.517648 |
| THY1  | 1.063 | #DIV/0! | 2.602325 | 2.602325 |
| PODXL | 1.366 | 3.140597 | 1.435316 | 1.435316 |
| FBN1  | 1.459 | 2.056632 | 1.692519 | 1.692519 |
| AMIGO2| 1.057 | 1.820322 | 2.514458 | 2.514458 |
| EHD2  | 2.510 | 3.776854 | 2.767269 | 2.767269 |
| FOXD1 | 1.076 | 0.855949 | 1.53458 | 1.53458 |
| NUAK1 | 0.902 | 1.449376 | 1.421510 | 1.421510 |
| TGFBI | 1.182 | 8.266674 | 1.838949 | 1.838949 |
| Gene   | Ratio 1 | Ratio 2 | Ratio 3 |
|--------|---------|---------|---------|
| TNFRSF11B | 1.55407801 | 0.97976879 | 1.1482821 |
| WNT5B | 0.98116969 | 3.92361111 | 3.15179353 |
| NID2 | 0.88483685 | 0.56009913 | 0.98433735 |
| SERPINE1 | 2.12680115 | 3.07229612 | 3.78714524 |
| LOX | 1.44543391 | 1.4274844 | 1.30739367 |
| NNMT | 1.88637904 | 5.39334779 | 5.69767442 |
| PDGFRB | 1.86167147 | 2.09423052 | 2.80629409 |
| F3 | 1.11757755 | 2.52332196 | 1.24024236 |
| WNT5A | 0.9019026 | 0.55192958 | 0.51668771 |
| BGN | 1.03942652 | 0 | 0.8188178 |
| HS3ST3A1 | 1.46253602 | 1.39042308 | 4.55725191 |
| TCF4 | 0.85632 | 1.06037444 | 0.79014989 |
| ABCC3 | 1.70046083 | 0 | 2.73477999 |
| EGFR | #DIV/0! | 1.47071584 | 1.96918403 |
| COL5A1 | 0 | 3.08054523 | 2.04422638 |
| VEGFC | 1.7718894 | 2.10037175 | 0.98443396 |
| CRISPLD2 | 1.06340058 | 0.94879687 | 0.85754288 |
| PTGFR | 3.18809981 | #DIV/0! | 0.49051491 |
| STC2 | 1.16401536 | #DIV/0! | 1.63670852 |
| MICAL2 | 0.81826241 | 7.86086957 | 3.66863905 |

*Highlighted genes (selected for Figure 5D) follow the same trend in a
|        | LOG2 values |        |        |        |
|--------|-------------|--------|--------|--------|
|        | M16         | RKTJ-BI3 | WP     |        |
|        | -0.40713229 | -0.38539325 | -1.00638914 |        |
|        | -0.80025633 | -0.81046084 | -1.48828171 |        |
|        | -0.56135432 | -0.60238169 | -0.63825736 |        |
|        | -1.26260403 | -1.54245607 | -1.51844951 |        |
|        | -0.27594812 | -0.66720878 | -0.63579663 |        |
|        | -0.55519375 | -0.91581789 | -0.6596012  |        |
|        | -0.60003934 | -0.56436499 | -0.36023305 |        |
|        | -0.59855419 | -1.11808113 | -0.53193526 |        |
|        | -0.68126398 | -1.23995339 | -1.61148694 |        |
|        | -0.62972503 | -0.73475759 | -0.37581004 |        |
|        | -0.35154275 | -1.91516547 | -0.65011129 |        |
|        | -0.75293615 | -1.15040055 | -0.80733243 |        |
|        | -0.6919709  | -1.03472352 | -1.22482039 |        |
|        | -0.28368444 | -0.76162584 | -0.05634757 |        |
|        | -0.58008012 | -1.20308769 | -1.36560058 |        |
|        | -0.52565946 | -1.10749652 | -1.28202816 |        |
|        | -0.48344166 | -0.66893644 | -1.28803048 |        |
|        | -0.31970151 | -1.55881562 | -1.17394497 |        |
|        | -0.47441154 | -0.83745229 | -0.98634772 |        |
|        | -0.75025727 | -0.70703737 | -0.92176671 |        |
|        | -0.68568164 | -1.54419178 | -1.1188681  |        |
|        | -0.31493321 | -0.85624397 | -1.20637153 |        |
|        | -0.64984453 | -0.29210415 | -0.55805819 |        |
|        | -0.62812611 | -1.00526548 | -0.95725786 |        |
|        | -0.8955455  | -0.4600469  | -1.57007444 |        |
|        | -0.85990934 | -2.51422294 | -1.43865169 |        |
|       |       |       |       |
|-------|-------|-------|-------|
| -0.09061319 | -0.86420677 | -0.67654961 |
| 0.09462092   | 0.09824587   | 0.2066073   |
| -0.06467484  | -0.30028447  | -0.13927446 |
| -0.68269452  | -0.60790268  | -0.6049768  |
| -0.07737124  | -1.07269687  | -0.77829341 |
| -0.02330886  | -0.79852569  | -0.00217383 |
| 0.77262121   | 2.30480691   | 0.39443746  |
| 0.54988696   | 0.57607148   | 1.43530661  |
| 0.52320412   | 1.33052201   | 1.03990696  |
| -0.73951721  | -0.59166104  | -2.41655143 |
| 1.08176745   | 1.28142183   | 0.03122782  |
| -0.83459717  | -0.45962964  | -0.60769719 |
| 0.41768211   | 1.37101979   | 1.08642289  |
| 0.07132169   | 0.5665502    | 0.21286817  |
| 0.45050218   | 1.65120425   | 0.52136819  |
| 0.54584239   | 1.04028413   | 0.75917287  |
| 0.08061846   | 0.86419382   | 1.33024736  |
| 1.32814091   | 1.91718535   | 1.46846292  |
| 0.24085786   | 3.04730708   | 0.87888171  |
Highlighted genes (selected for Figure 5D) follow the same trend in all 3 cell lines (up- or down-regulated; ratio > or < 1)

|                  |                  |                  |
|------------------|------------------|------------------|
| -0.17651662     | -0.8362459       | -0.02277526      |
| 1.08868515      | 1.61931728       | 1.92111075       |
| 0.53150265      | 0.51347498       | 0.38669361       |
| 0.91561959      | 2.43118107       | 2.51037318       |
| 0.8965985       | 1.06642025       | 1.4886662        |
| 0.16037494      | 1.3353243        | 0.31062207       |
| -0.14895645     | -0.8574439       | -0.95263553      |
| 0.54847216      | 0.47552394       | 2.18816412       |

II 3 cell lines (up- or down-regulated; ratio > or < 1)