A role for BRG1 in the regulation of genes required for development of the lymphatic system

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ABSTRACT

Lymphatic vasculature is an important part of the cardiovascular system with multiple functions, including regulation of the return of interstitial fluid (lymph) to the bloodstream, immune responses, and fat absorption. Consequently, lymphatic vasculature defects are involved in many pathological processes, including tumor metastasis and lymphedema. BRG1 is an important player in the developmental window when the lymphatic system is initiated. In the current study, we used tamoxifen inducible Rosa26CreERT2-BRG1 floxed/floxed mice that allowed temporal analysis of the impact of BRG1 inactivation in the embryo. The BRG1 floxed/floxed/Cre-TM embryos exhibited edema and hemorrhage at embryonic day-13 and began to die. BRG1 deficient embryos had abnormal lymphatic sac linings with fewer LYVE1 positive lymphatic endothelial cells. Indeed, loss of BRG1 attenuated expression of a subset of lymphatic genes in-vivo. Furthermore, BRG1 binds at the promoters of COUP-TFII and LYVE1, suggesting that BRG1 modulates expression of these genes in the developing embryos. Conversely, re-expression of BRG1 in cells lacking endogenous BRG1 resulted in induction of lymphatic gene expression in-vitro, suggesting that BRG1 was both required and sufficient for lymphatic gene expression. These studies provide important insights into intrinsic regulation of BRG1-mediated lymphatic-gene expression, and further an understanding of lymphatic gene dysregulation in lymphedema and other disease conditions.

INTRODUCTION

The lymphatic system plays key role in development and in regulating tissue fluid, thereby maintaining tissue homeostasis [1, 2]. Its dysfunction results in life threatening diseases such as lymphedema, lymphangiectasia, lymphangioma, and lymphatic dysplasia [3]. Furthermore, tumor lymphangiogenesis has been implicated in cancer metastasis [4]. Blood vessels originate during embryonic development from mesodermally derived endothelial cell precursors. Endothelial sprouting and splitting contributes to the formation of mature network of blood vessels. Blood and lymphatic vessels develop from the same cell lineage but they perform very distinct and essential circulatory functions [1]. Subsequent to blood vessel formation, the lymphatic vasculature appears, perhaps indicating that lymphatics might have a blood vasculature origin. However, the molecular factor(s) that regulate this initial stage of lymphatic competence and establishment of the
lymphatic system, remain elusive. A clear nexus of signal transduction pathways, such as the VEGF-C/VEGFR-3 system and transcription factors including, PROX-1, SOX18 and COUP-TFII were shown to be critical for this process [1, 5].

BRG1 is the central catalytic subunit of the SWI/SNF chromatin-remodeling complex [6–9]. Numerous studies have placed SWI/SNF complex and its subunits at the nexus of transcriptional networks critical for many biological and disease processes [10–13]. BRG1 is required for peri-implantation development and heart formation [14–19]. More recently we established that BRG1 is highly and ubiquitously detectable in peri-gastrulation stage embryos. Global inactivation of BRG1 during peri-gastrulation causes growth retardation and ultimately embryonic mortality [20].

To explore the distinctive functional role(s) of BRG1, we inactivated BRG1 in the whole embryo during mid-to-late gestation stages of development. Embryos lacking BRG1 function(s) show a range of aberrant phenotypes that are typically associated with lymphatic defects and diminished expression of a sub-set of lymphatic genes, suggesting a novel and an essential role for BRG1 in the lymphatic system during development.

RESULTS AND DISCUSSION

BRG1 is detected across all stages of development

Previous reports established that BRG1 is essential for early embryogenesis and heart formation [16, 18, 21, 22]. Unlike transcription factors, which can display differential spatial-temporal expression patterns and thereby provide cellular specificity, BRG1 is ubiquitously expressed in peri-gastrulation stage embryos and acts as a global regulator of transcription [20]. To explore the role of BRG1 in later stages of development, we began by examining the spatiotemporal localization of BRG1 protein in mid-to-late gestation stage embryos. Immunohistochemistry analysis showed positive BRG1 staining localized to the cell nucleus of all organs examined including brain, heart, liver, kidney, placenta and lymphatic vessels (lymphatic endothelial cells) (Figure 1A–1F). The distribution of BRG1 staining was homogenous in the nuclei across a range of cell types in brain, heart, liver, kidney, placenta, and vasculature endothelial cells. This pattern was repeated over time across different developmental stages (E11.5 to E18.5) (Supplementary Figure 1), suggesting that BRG1 is expressed throughout development. Ubiquitous and abundant expression of BRG1 raised the possibility that BRG1 function(s) in a wide spectrum of developmental processes for embryonic growth and maintenance of tissue homeostasis beyond the early development stages when embryonic cells undergo rapid proliferation and differentiation.

BRG1 is essential during the mid-to-late stages of embryonic development

To study the implications of the loss of BRG1 function, we used a tamoxifen inducible Cre-lox system to identify roles of BRG1 in mid-to-late gestation stages of fetal development. The BRG1fl/fl/d/d dams were time mated with BRG1fl/fl/d/d/R26CreER-TM sires, pregnant dams were intraperitoneally injected with tamoxifen at E10.5 days post coitum (dpc.), and embryos were collected at E13.5 dpc. Alternatively, time mated pregnant mice received two consecutive doses at E12.5 and 13.5, and embryos were collected at E16.5dpc for morphological analysis. The BRG1fl/fl/d/d/R26CreER-TM (hereafter referred as BRG1fl/fl; embryos deficient for BRG1) embryos exhibited multiple morphologically distinct phenotypes when compared with BRG1fl/fl/d/d (hereafter referred as BRG1fl/fl; wild-type) embryos. (Supplementary Figure 2)

In embryonic day (E) 13.5 BRG1fl/fl embryos, the appearance of the conceptus was within normal limits for all litters collected (Figure 2). Normal patterning of the vitelline vessels (arrow) was observed on the exterior surface of the yolk sac. All vessels were filled with red blood cells. Removal of the extra embryonic membranes showed no abnormalities with the umbilical vessels (wide arrow). The placenta (asterisk) was normal in shape, color, and size. The developing embryo possessed features of gestation day 13.5 as characterized by clear indentation of the anterior footplate, while the posterior footplate was still webbed and absent of indentations. In contrast, conditional inactivation of BRG1 on E10.5 resulted in many irregularities on E13.5 (Figure 2). The vitelline vessels were present, but it was difficult to grossly distinguish if the vessel remodeling was affected (i.e. angiogenesis of the vitelline artery and veins). The vessels were small without signs of homogenous filling with red blood cells (RBCs, arrow). In addition, RBCs were not always visible in the umbilical vessels (double asterisk). The placenta was small, pale and the fetal surface was irregular and mottled. Embryos collected at this time point were smaller and paler than the WT and had blood filled spaces (arrow) on the surface of the embryo. Typically, these blood-filled spaces were localized to the lateral aspects of the embryo. The majority of the embryos were edematous with the degree ranging from mild to severe.

At E16.5 BRG1fl/fl, the appearance of the conceptus was within normal limits for all litters collected (Figure 2). Normal patterning of the vitelline vessels (arrow) was observed on the exterior surface of the yolk sac (arrow). RBCs filled the vitelline vessels. Removal of the extra embryonic membranes showed no abnormalities with the umbilical vessels. The placenta (asterisk) was normal in shape and size. The labyrinth (lab) and junctional zone (jz) were morphologically normal (*, asterisk). Features of gestation day 16.5 embryos were characterized by the end phalanges of forelimb and hind limb. The forelimb
Figure 1: BRG1 is ubiquitously expressed in the developing embryo. Immunohistochemistry analysis demonstrates high abundance nuclear staining of BRG1 (brown stain) in brain (A), heart (B) liver (C) kidney (D) placenta (E) and in endothelial cells of the lymphatic vessel (F) of developing wild-type embryos.
phalanges were nearly parallel and joined by skin proximally, but not near the distal tips. The hind limb phalanges were more divergent. In contrast, \(BRG1^{d/d}\) conceptus at E16.5 revealed many obvious abnormalities (Figure 2; →, arrows). The vitelline vessels were present, but it was difficult to grossly distinguish if vitelline vessel remodeling was affected. The vessels were thin without homogenous filling with RBCs (arrow). In addition, RBCs were not always visible in the umbilical vessels (arrow). The placenta was small, pale and the fetal surface was irregular and mottled (asterisk). Embryos were pale and exhibited blood filled spaces (wide arrow) on the surface of the embryo. In the E16.5 embryos the blood-filled spaces appeared to be more extensive than in the E13.5. The majority of the embryos were edematous with the degree ranging from moderate to severe.

![Image](image_url)

**Figure 2: BRG1 is necessary during mid-to-late stage embryogenesis.** Temporal deletion of \(BRG1^{fl/fl}\) alleles results multiple defects and led to embryonic mortality. The \(BRG1^{fl/fl}\) pregnant female mice time mated with male \(BRG1^{fl/fl}/R26CreER-TM\) mice were dosed with tamoxifen (100 mg/kg body weights) either at E10.5 (single dose) or at E12.5 and 13.5 (two consecutive doses). Embryo phenotype was then evaluated at E13.5 and 16.5 respectively. In E13.5 \(BRG1^{fl/fl}\) embryos, arrows indicate normal blood vessels. In the E13.5 \(BRG1^{d/d}\) embryos, arrows indicate defective blood vessels and hemorrhages with the asterisk indicating anemic placenta. In E16.5 \(BRG1^{d/d}\) embryos, thin arrow indicates edema and thick arrow indicates hemorrhages.
BRG1-deficient embryos showed abnormalities associated with lymphatic dysfunction

As development proceeds the lymphatic and blood vasculatures spread out in the whole body, and remain separated from each other. The adapter protein SLP76 and the tyrosine kinase SYK are mainly expressed by hematopoietic cells and have been shown to play a role in separating of blood and lymphatic vasculatures [23]. Indeed, mice lacking either SLP76 or SYK exhibit abnormal blood-lymphatic connections; embryonic hemorrhage and arteriovenous shunting [23].

Inactivation of BRG1 during mid-to-late stage embryonic development resulted in a range of abnormal phenotypes associated with defective lymphatic and blood vasculatures [24–27]. Most BRG1<sup>fl/fl</sup> embryos showed gross subcutaneous edema and swelling at E13.5 and 16.5 (Figure 3A, Supplementary Figure 2), and subsequently fetal mortality in all BRG1<sup>fl/fl</sup> embryos, as described previously by other researchers in different mouse models [24, 28, 29]. An array of abnormal phenotypes such as formation of subcutaneous edema, cerebral hemorrhage near the cardinal vein, and hemorrhaging throughout the body (Figure 3A, Supplementary Figure 2) were mostly seen in BRG1 mutant embryos. Blood vessels and the lymph sac were formed in BRG1<sup>fl/fl</sup> embryos; but smaller size of blood vessels and abnormally patterned lymphatic sacs were observed in BRG1<sup>d/d</sup> embryos (Figure 3B). ENDOMUCIN, also known as endothelial SIALOMUCIN, which interferes with the assembly of focal adhesion complexes and inhibits interaction between cells and the extracellular matrix, staining was comparable in the left internal jugular vein of both BRG1<sup>fl/fl</sup> and BRG1<sup>d/d</sup> embryos (Figure 3B, right panel upper and lower). Strikingly, staining for LYVE1, a cell surface protein expressed specifically by lymphatic endothelial cells [30, 31], co-localized with the lining of the lymphatic endothelial cells in lymphatic sac of the BRG1<sup>fl/fl</sup> embryos (Figure 3B; upper left panel). In contrast, diminished LYVE1 staining or loss of lymphatic endothelial cells were observed in the lymphatic sac of BRG1<sup>d/d</sup> embryos (Figure 3B; lower left panel). This finding suggests a role for BRG1 in developmental of lymphatic system.

Earlier studies have established the homeobox transcription factor PROX1 as a critical regulator of a number of lymphatic genes, including cell fate specification and maintenance of the LYVE1 positive lymphatic endothelial cells (LEC), and development of the murine lymphatic vasculature [32, 33]. To ascertain if BRG1 is necessary for PROX1 expression in lymphatic precursors in-vivo, we examined the localization pattern of PROX1 in BRG1<sup>fl/fl</sup> embryos, in comparison to BRG1<sup>fl/fl</sup> embryos. By E13.5, linear groups of PROX1-positive cells were clearly visible? visible lining the primary lymph sac in BRG1<sup>fl/fl</sup> embryos (Supplementary Figure 3, arrows). In contrast, lymphatic endothelial cells were mostly absent in BRG1<sup>d/d</sup> embryos (Supplementary Figure 3), although the staining intensity for PROX1 was comparable in the remaining cells to that seen in the lymphatic sac of the BRG1<sup>fl/fl</sup> embryo (Supplementary Figure 3, arrows). BRG1<sup>fl/fl</sup> embryos contained a round, spherical jugular vein completely filled with red blood cells. However, an abnormal structure lacking red blood cells was present in BRG1<sup>d/d</sup> embryos. These observations establish that the lack of BRG1 function results in a dysregulated lymphatic system from the pool of vascular endothelial cell precursors during mouse embryogenesis, confirming the pivotal role of BRG1 in the development of this system.

Specific sets of lymphatic genes were repressed in BRG1<sup>d/d</sup> embryos

Endothelial cells acquire LEC-specific gene signature during early development, leading to the subsequent assembly of the lymphatic vascular network. Thus, expression of LEC related genes are critical for both lymphatic network formation and function during mid-to-late stage of development, following their role in LEC fate specification [3]. To gain molecular insights into BRG1 dependent LEC related gene expression in BRG1<sup>d/d</sup> embryos, we searched for genes related to the lymphatic system in our genome wide RNA sequencing and microarrays analyses data sets of E7.5 and E8.5 BRG1<sup>fl/fl</sup> vs. BRG1<sup>fl/fl</sup> embryos [20]. The LEC related genes were differentially expressed in E7.5 and E8.5 BRG1<sup>d/d</sup> embryos prior to the appearance of the abnormal lymphatic phenotype and this could explain the defective defective function of lymphatic system during mid-to-late stages of BRG1<sup>d/d</sup> embryos. We found several lymphatic and blood endothelial-specific genes that were differentially expressed in BRG1<sup>d/d</sup> embryos compared to BRG1<sup>fl/fl</sup> embryos. Of particular interest, we found that LYVE1 and COUP-TFII (NR2F2), which are known lymphatic endothelial-specific marker genes and initially expressed during lymphatic endothelial cell specification in developing embryo [8, 34], were significantly down regulated (LYVE1 ~ 9.7 fold, COUP-TFII ~ 2.6 fold) in BRG1<sup>d/d</sup> embryos (Table 1; Figure 4A; upper panel, shows gene track of RNA sequencing signals). Additionally, we found several other genes associated with lymphatic system and immune responses were differentially expressed in microarray analysis of E8.5 BRG1<sup>d/d</sup> embryos (Table 1). As expected, PROX1, a transcription factor, that is involved in cell fate determination and function as a regulatory protein in the lymphatic system, was not detected in E7.5 and E8.5 global gene expression analyses because it is known not to be expressed at these early stages [35]. Using quantitative RT-PCR, we validated changes in expression of lymphatic genes by evaluating the mRNA level at E8.5 in BRG1<sup>d/d</sup> vs. BRG1<sup>fl/fl</sup> embryos. QPCR analysis shows attenuated expression level of selected lymphatic endothelial-related genes including LYVE1 and COUP-TFII in BRG1<sup>d/d</sup> embryos vs. BRG1<sup>fl/fl</sup> embryos.
Figure 3: BRG1-deficient embryos show hemorrhages. (A) Panels show gross morphology of the BRG1<sup>fl/fl</sup> and BRG1<sup>d/d</sup> embryos at the indicated developmental stages. White arrows – edema; yellow arrows – hemorrhage, lower panel of table shows number of BRG1<sup>d/d</sup> and BRG1<sup>fl/fl</sup> embryos that were analyzed for gross phenotype (B) Immunohistochemistry staining for ENDOMUCIN and LYVE1 identifies the jugular vein (jv) and the lymph sacs (ls) specifically in E13.5 BRG1<sup>fl/fl</sup> and BRG1<sup>d/d</sup> embryos. CS; carotid sinus, JLS; jugular lymphatic sac, LIJV; left internal jugular vein.
embryos (Figure 4A; lower panel), consistent with the genome wide transcriptome analyses. In addition, we also observed that immune response genes such as SWI/SNF2-related HILLS (SMARCA6), LY6E, and LY6I were also altered in the BRG1<sup>d/d</sup> embryos (Table 1, Supplementary Figure 4). These immune responsive genes were previously reported to regulate the expansion or survival of lymphoid cells [36]. Surprisingly, deletion of BRG1 did not change expression of other known lymphatic genes including SOX18, PODOPLANIN and EPHRIN (Figure 4A; lower panel) and general vascular endothelial cell differentiation markers such as VEGFR3, CD34 and CD44 (Figure 4B). This finding suggests that BRG1 expression is crucial for LEC maintenance, patterning and function of lymphatic vessels in mid-to-late embryos. However, vascular endothelial related genes such as TIE2 were significantly decreased in E8.5 BRG1<sup>d/d</sup> embryos accompanied by a lessor reduction of TIE1 (Figure 4B). VCAM expression was also significantly decreased in BRG1<sup>d/d</sup> vs. BRG1<sup>fl/fl</sup> embryos (Supplementary Figure 4, lower panel). Therefore, BRG1 stimulates expression of a subset of known lymphatic marker genes during the differentiation of cells along a vascular endothelial-specific pathway during mouse development.

**Key lymphatic genes LYVE1 and COUP-TFII are direct targets of BRG1**

To confirm the regulatory function of BRG1 on modulating expression of LYVE1 and COUP-TFII, chromatin immunoprecipitation experiments were performed using chromatin from E8.5 wild type mouse embryos, a developmental stage when endogenous expression of LYVE1 and COUP-TFII is detectable [34]. DNA fragments
were immunoprecipitated using anti-BRG1 antibody and control IgG, purified and tested for the presence of the BRG1 binding on the \textit{LYVE1} and \textit{COUP-TFII} promoters relative to the coding region. A clear enrichment of BRG1 over IgG was detected by QPCR on the tested loci of \textit{LYVE1} and \textit{COUP-TFII} promoter, confirming that BRG1 protein binds on the \textit{LYVE1} and \textit{COUP-TFII} promoters (Figure 5A–5B, left graphs). However, BRG1 binding was substantially lower at the coding region of the \textit{LYVE1} and \textit{COUP-TFII} genes (Figure 5A–5B, right panels). Taken together, these observations are consistent with the notion that BRG1 mediated transcriptional regulation during development is required for lymphatic system.

**BRG1 modulates expression of subset of lymphatic genes in cancer cells**

To investigate further the mechanism(s) of BRG1-dependent expression of lymphatic marker genes, we used cultured non-lymphatic adrenal gland/cortex derived SW13 cell line, which lack endogenous expression of BRG1 and the BRG1 dependent genes such as \textit{CD44} [37]. SW13 cells were transiently transfected for 24hrs with a BRG1 expression plasmid, and BRG1 protein and lymphatic marker mRNA expression analyzed by western blot and quantitative RT-PCR respectively (Figure 6A–6C). Transient expression of BRG1 in SW13 cells resulted in substantial up regulation of the lymphatic genes such as \textit{LYVE1}, \textit{COUP-TFII}, \textit{PROX1}, \textit{EPHB2}, \textit{PDPN} and \textit{VEGFR3} (Figure 6B, 6C). Surprisingly, \textit{SOX18} expression was repressed in SW13 cells expressing BRG1 (Figure 6B), while the expression level of the blood vascular endothelial genes \textit{TIE2}, \textit{CDH5} and canonical BRG1 target gene \textit{CD44} were increased (Figure 6C). In contrast, the expression levels of \textit{VEGFR2} and \textit{CD34} were repressed by BRG1 overexpression (Figure 6C). Thus, BRG1 specifically enhances a subset of lymphatic endothelial genes, which further supports its potential role in promoting the lymphatic endothelial differentiation program in development and in lymphangiogenesis within tumors [38, 39].

To extend these studies we used an \textit{in vitro} tube/vessel formation assay with a human umbilical vein endothelial cell (HUVEC) culture system to evaluate a functional role for BRG1. We observed that BRG1 silencing disrupted
the ability of HUVECs to form tube/vessel structures. HUVECs transfected with siRNA targeting BRG1 showed disorganized tube formation and less tubes per seeded cell population compared to cells transfected with scrambled control siRNA (Supplementary Figure 5).

In summary, we report that BRG1 function is required for the differentiation of lymphatic endothelial progenitor cells from blood vascular precursors in the developing embryo. BRG1 is essential for growth and maintenance of LECs in developing embryo and its deficiency contributes to a wide array of lymphatic defects and mortality. BRG1 overexpression in non-lymphatic SW13 cells resulted in the induction of a spectrum of lymphatic marker genes, a result that further supports the hypothesis that BRG1 activates expression of lymphatic genes in development. Mechanistically, BRG1 both directly and indirectly regulates genes that are critical for lymphatic system in the developing embryo [10, 11, 40]. Specifically, BRG1 directly binds on the promoters of key lymphatic genes such as the transcription factor Coup-TFII and target gene such as LYVE1.

Many critical developmental events during embryogenesis and subsequent organogenesis employ combinations of transcription factors, signal transduction

Figure 5: BRG1 is bound at the promoters of key lymphatic genes: Quantitative PCR was performed with primers designed from promoter and coding regions of the target genes (A) LYVE1 and (B) Coup-TFII after chromatin immunoprecipitation (ChIP) with BRG1 antibody and IgG using E8.5 embryos. The schematics above the QPCR data show ChIP primer locations upstream of transcription start site (TSS) and within an exon of the LYVE1 and Coup-TFII genes. The experiment was performed with at least three biological replicates P-value < 0.05.
Whole Cell Extract_40 ug of protein of each treatment loaded

1. Non transfected SW13 cells plus lipofectamine reagent
2. SW13 cells transfected with 1 ug of pcDNA3 empty vector
3. SW13 cells transfected with 1 ug of BRG1 plasmid
4. HeLa extract (positive control for BRG1 endogenous expression)
pathways, and chromatin remodeling proteins [1, 19, 41]. The role of BRG1 in promoting lymphatic endothelial cell specification is consistent with SWI/SNF components acting as developmental switches/fine tuners. Moreover, BRG1 is clearly determinative in a number of developmental processes—early embryogenesis, cardiovascular system, and neurogenesis—and is likely to depend on context-specific partner proteins and/or cofactors [14, 16, 19, 42]. Results of this study provide novel insights regarding development of the lymphatic system and the relatively unexplored aspects of chromatin/epigenetic regulation in this system. Further studies will need to focus on defining the cellular and molecular milieu in which BRG1 operates to activate lymphatic transcription, and may reveal new strategies for therapeutic stimulation or suppression of lymphangiogenesis [11].

**MATERIALS AND METHODS**

Materials and Methods are available in the online-only Data Supplement.

**Abbreviations**

BRG1: Brahma-related gene 1; COUP-TFII: Chicken ovalbumin upstream promoter transcription factor 2; DEG: Differentially expressed genes; LECs: Lymphatic endothelial cells; LYVE1: Lymphatic vessel endothelial hyaluronan receptor 1; PROX1: Prospero Homeobox 1; SMARCA4: SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily A, Member 4; SOX18: Sex Determining Region Y-Box 18; SWI/SNF: SWItch/Sucrose Non-Fermentable; VEGF: Vascular endothelial growth factor; VEGFR: Vascular Endothelial Growth Factor Receptor.
Table 1: Lymphatic endothelial related genes differentially expressed in Brg1d/d vs. Brg1fl/fl embryos

| Accession # | Primary Sequence Name | 7.5_Brg1 cKO/7.5_Control Fold Change | 7.5_Brg1 cKO/7.5_Control ANOVA p-value |
|-------------|-----------------------|--------------------------------------|---------------------------------------|
| NM_053247   | Lyve1                 | -9.74723123                          | 5.97E-09                              |
| NM_009868   | Cdh5                  | -2.982166891                          | 3.71E-07                              |

| Accession # | Primary Sequence Name | 8.5_Brg1 cKO/8.5_Control Fold Change | 8.5_Brg1 cKO/8.5_Control ANOVA p-value |
|-------------|-----------------------|--------------------------------------|---------------------------------------|
| NM_019391   | Lsp1                  | -2.9313                              | 0.0487                                |
| NM_009697   | Nr2f2                 | -2.5809                              | 0.0487                                |
| Z11886      | Notch1                | -2.5614                              | 0.0476                                |
| NM_007937   | Epha5                 | -2.4067                              | 0.0155                                |
| NM_008511   | Lmp                   | -2.3861                              | 0.033                                 |
| NM_008529   | Ly6e                  | -2.3109                              | 0.0079                                |
| AK021284    | Mll5                  | -2.0003                              | 0.0329                                |
| NM_008234   | Hells                 | -1.6854                              | 0.0289                                |
| NM_133743   | Lypd3                 | 3.9961                               | 0.0049                                |
| NM_033601   | Bcl3                  | 3.0254                               | 0.0398                                |
| NM_020498   | Ly6i                  | 2.8415                               | 0.0286                                |
| ENSMUST00000029673 | Efna3 | 2.6045  | 0.0274  |
| AK029978    | Prox2                 | 2.5298                               | 0.0362                                |
| NM_001033281 | Prdm6     | 1.8703                               | 0.0216                                |
| NM_007548   | Prdm1                 | 1.6187                               | 0.0249                                |

Green: Repressed genes; Red: Induced genes.

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CONFLICTS OF INTEREST

None.

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Footnotes

The online-only Data Supplement is available with this article at Oncotarget.

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