ETV2 functions as a pioneer factor and regulates the endothelial lineage

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**Abstract**

**Background:** Genetic mutations perturb the multipotent progenitors, which results in congenital cardiovascular disease. Therefore, it is essential to decipher the pioneer factors and the regulatory pathways that govern the specification and differentiation of mesodermal progenitors and use this information to develop targeted therapies to promote cardiovascular regeneration. Etv2 as an essential transcription factor for the development of cardiac, endothelial and hematopoietic lineages. In the present study, we used ES/EB differentiation and MEF reprogramming systems, to define Etv2 as a novel pioneer factor that relaxes the closed chromatin and drives endothelial development.

**Results:** Using the *iHA-Etv2* ES cell line, we engineered a mouse that inducibly overexpresses ETV2. The bulk RNA-seq, single cell RNA-seq data and ATAC-seq experiments showed that inducing Etv2 in MEFs and ES/EBs activated the downstream endothelial marker genes and promoted the development of endothelial lineages, supporting the notion that Etv2 functioned as a master regulator to drive the endothelial lineage development in different cellular contexts. We found that similar to other known pioneer factors, Etv2 was intrinsically able to target and bind the nucleosomes, and this capability appeared to be independent of the cellular context. To further define the mechanism, we performed Etv2, Brg1 and H3K27ac ChIP-seq analyses during MEF reprogramming and ES/EB differentiation. We found that Brg1 maintains and stabilizes the binding of Etv2 on the nucleosome, and Etv2 requires Brg1 to activate downstream genes during reprograming.

**Conclusion:** In these studies, we defined Etv2 as a novel pioneer factor that relaxed the closed chromatin and promoted the endothelial lineage in both ES/EB differentiation and MEF reprogramming. The definition of these mechanisms will enhance our understanding of cardiovascular development and regeneration and serve as a platform for therapeutic applications for patients with congenital or aging related cardiovascular diseases.

**Introduction**

Ischemic heart and vascular diseases are common and deadly and result in considerable morbidity and mortality1. Progression of these diseases results in myocardial infarction requiring vascular bypass grafting or limb amputation and these therapeutic interventions have significant limitations and morbidity. Therefore, new therapies are warranted. One strategy is to enhance or facilitate vascular development and requires the definition of the earliest mechanisms that govern endothelial and vascular fate determination.

During development, pioneer transcription factors have the essential and unique role of opening new regulatory chromatin landscapes on genomic DNA2. Compared with the non-pioneer transcription factors, the pioneer factors can recognize their target DNA sequences in closed heterochromatin and trigger the remodeling of the adjacent chromatin landscape to provide accessibility to non-pioneer transcription factors3. Through the relaxation of the chromatin state, these pioneer factors enable other transcription factors to sequentially access their binding motifs through histone modifications and collectively activate gene expression resulting in reprogramming the cell fate4. A limited number of pioneer factors have been defined and all of these factors have been shown to have important roles in various biological processes. Examples of pioneer factors include pluripotent factors OSK (Oct4, Sox2, and Klf4) for iPSC reprogramming, FoxA for hepatocyte reprogramming5, GATA3 for mesenchymal to epithelial transition (MET)6, PU.1 and C/EBPa for macrophage differentiation7, Pax7 for pituitary development8, Ebf1 for B cell lineage specification9 and Ascl1 for neurogenesis10.

Studies from our laboratory and others have identified ETV2 as an essential transcription factor for the development of cardiac, endothelial and hematopoietic lineages11–20. Our laboratory has previously shown, using the 3.9kb *Etv2-Cre* and *Rosa-EYFP* reporter alleles, that *Etv2*-EYFP progenitors daughter endocardial/endothelial and hematopoietic lineages in the WT background20. Furthermore, when crossed into the *Etv2* mutant background, the *Etv2-*EYFP progenitor cells daughtered cardiomyocytes in the absence of *Etv2*. *Etv2* has been shown to be responsive to BMP, WNT and NOTCH signaling pathways and synergizes with FOXC2 to regulate the endothelial program by directly targeting *Lmo2, Cdh5*, *Tie2* and *Flk1*13,15,21*.* Importantly, the ability of ETV2 to reprogram fibroblasts to endothelial cells points to its master regulatory role and supports the notion that ETV2 functions as a pioneer factor in endothelial development22.

The mammalian SWI/SNF-related chromatin-remodeling complex consists of one of two different ATPases (brahma or BRM vs. brahma related gene-1 or Brg1) along with ten or more Brg1 associated factor subunits (BAFs)23. Using gene disruption strategies, BRM has been shown not to be essential for hematoendothelial development24,25. In contrast, the global knockout of Brg1 results in early lethality prior to embryo implantation25. In addition, the conditional knockout of Brg1 using the *Tie2-Cre* transgenic mouse line results in midgestational lethality with perturbed hematoendothelial development. Previous studies have also established a mechanism whereby pioneer factors function in the recruitment of Brg1, which facilitates the opening of chromatin to promote transcription factor binding and potentiates transcriptional activation of gene expression26. In this study, we found that Brg1 maintains and stabilizes the binding of Etv2 on the chromatin, and Etv2 requires Brg1 to activate downstream genes during reprogramming and ES/EB differentiation. These studies provide an important platform for the design of new therapeutic strategies to promote vasculogenesis for the treatment of cardiovascular diseases.

**Results**

**Etv2 reprograms fibroblasts into an endothelial cell population**

Using the *iHA-Etv2* ES cell line, we engineered a mouse that inducibly overexpresses ETV2. We isolated embryonic fibroblasts from this mouse line and demonstrated that these cell populations uniformly expressed fibroblast markers (Thy1.2, CD44 and CD29) and lacked hematoendothelial (HE) expression by FACS (in the absence of Dox) (Supplementary Figure 1a). Using western blot analysis, we further demonstrated that ETV2 was robustly expressed within 3 hrs post-Dox treatment (Supplementary Figure 1b). ETV2 overexpression resulted in more than a 50-fold increase in cells expressing FLK1/TIE2 by FACS (Supplementary Figure 1c - 1f). At day 7 post-Etv2 induction, we found Ac-LDL uptake, positive sprouting assays and an induction of ETV2 downstream endothelial target expression (Supplementary Figure 1g - 1m).

To further investigate the molecular dynamics of Etv2 inducible reprogramming, we captured and sequenced 3,539, 2,936 and 7,202 high quality single cells from 24 hours, 48 hours and 7 days post-induction of Etv2 in mouse embryonic fibroblasts (MEFs). We also performed single cell RNA-seq of 948 undifferentiated MEF cells as well as 827 sorted Flk1+ cells from day 7 reprogrammed cells. The dimension reduction analysis by scVI27, followed by uniform manifold approximation and projection (UMAP) and *k*-means clustering identified seven distinct cell clusters (Figure 1a, and Figure 1c-1d). We noted that during the first 48 hours of reprogramming, even though Etv2 was significantly up-regulated as early as 24 hours, endothelial markers such as Kdr, Lmo2, Emcn, Cdh5 and Sox18 were significantly activated only in a subpopulation of cells (cluster 1 for MEFs in day 1 cells and cluster 2 for day 2 cells) (Figure 1d, Supplementary Figure 2a and 2b). The pathway analysis suggested that the cell cycle process and chromatin organization related genes were significantly up-regulated in cluster 1 MEF cells, and may be required for the initiation of Etv2 induced reprogramming process (Supplementary Figure 2c). At day 7 of reprogramming, the FACS analysis showed that 17% of the cells were Flk1+ cells (Supplementary Figure 1c and 1d). The scRNA-seq showed that the Flk1+ cells at day 7 formed a unique cell population (cluster 7). The endothelial marker genes such as Lmo2 and Emcn were robustly expressed in the cluster 7 cells, while the fibroblast markers such as Cd44 and Fosl1 were down-regulated (Figure 1f). The pathway analysis confirmed that transcripts with functions related to vasculature development and blood vessel development were more abundantly expressed in cluster 7 compared with the remaining clusters (Figure 1g). In summary, the scRNA-seq analysis supported the hypothesis that Etv2 overexpression in MEFs activated the downstream endothelial marker genes and promoted the development of endothelial lineages.

The embryoid body (EB) formation from ES cells has been broadly used for studying the role of Etv2 in endothelial development28. To investigate the molecular programs that are commonly or differentially responsive to the Etv2 induction in EBs and MEFs, we induced Etv2 at day 2.5 developing EBs (prior to the onset of endogenous Etv2 expression) and performed bulk RNA-seq of Flk1+ cells at 3 hours post-induction (Figure 1b and Supplementary Figure 3). Compared with the gene expression profiles in D2.5 EBs without induction, we identified 2,324 and 2,328 genes that were up- and down-regulated, respectively. Among them, 554 and 1,507 genes were up- and down-regulated in both EBs and MEFs (Supplementary Figure 4a and 4b). As expected, the commonly up-regulated genes were closely related to vasculature development, including endothelial markers Myct1 and Sox18, while the commonly down-regulated genes were significantly associated with translation and RNA processing (Supplementary Figure 4c - 4f).

To examine the chromatin accessibility changes in Etv2 induced cell differentiation, we performed ATAC-seq (Assays for Transposase-Accessible Chromatin with high-throughput sequencing) at 24 hours, 48 hours and 7 days post induction in MEFs and 3 hours post-induction in EB (Figure 1a and 1b). The analysis of transcription factors (TF) associated chromatin accessibility analysis suggested that although there existed significant batch effects between MEFs and EBs (PC1 in Figure 1h), the Flk1+ cells from day 7 MEFs and day 2.5 EBs post-Etv2 induction share a common global chromatin accessibility pattern (PC2 in Figure 1h), including 113 and 246 transcription factors whose chromatin accessibility that were commonly up-regulated and down-regulated in MEFs and EBs, respectively (Supplementary Figure 5a - 5d). By integrating the RNA-seq and ATAC-seq datasets from MEFs and EBs, we identified 13 TFs whose expression levels and TF associated accessibility were consistently increased in Flk1+ cells in both MEFs and EBs, and 18 TFs, which were decreased in Flk1+ cells (Figure 1i and Supplementary 5e and 5f). It is interesting that mesodermal factors such as Msx2, Eomes and Foxk2 were among the genes that were consistently down-regulated in both MEFs and EBs, suggesting the critical role of Etv2 as a suppressor of non-endothelial lineage development.

In the *in vivo* reprogramming study, Etv2 expression level was significantly increased within 2 days following LAD coronary artery ligation induced injury and markedly increased following the delivery of Dox inducible retroviral overexpression of ETV2 seven days following delivery and LAD coronary artery ligation induced injury, while no Etv2 was detectable in the unperturbed heart (left ventricle) (Supplementary Figure 5a and 5b). *iEtv2* overexpression (using a retroviral construct that we generated) resulted in increased endomucin labeled vasculature (EdU positive) compared to controls seven days following delivery and injury (Supplementary Figure 5c and 5d).

Collectively, these results demonstrated that induction of Etv2 was able to promote the specification and cell differentiation toward the endothelial lineage and the suppression of non-endothelial lineages, in two very distinct cellular environments: MEFs and EBs, supporting the notion that Etv2 functioned as a master regulator to drive the endothelial lineage development.

**Etv2 targets nucleosomes during reprogramming**

To investigate how Etv2 binding reshapes the genomic accessibility landscape and drives the endothelial lineage differentiation in different cellular environments, we performed Etv2 ChIP-seq at 24 hours, 48 hours and 7 days post-Etv2 induction in reprogrammed MEFs, and Etv2 ChIP-seq at 3 hours and 12 hours post-Etv2 induction in developing EBs at day 2.5. The initial Etv2 binding events were captured using Etv2 ChIP-seq at 24 hours in MEFs and 3 hours in EBs, resulting in 18,024 and 131,001 peaks, respectively (Figure 2a). The 11,751 common Etv2 peaks overlapped between MEFs and EBs and represented the majority (65.2%) of the ETV2 peaks in EBs, and were located predominately in the promoter region. In contrast, the EB and MEF specific Etv2 peaks were more likely to be distributed at the distal intergenic regions (Figure 2b). One of the key features of pioneer factors is their capability of targeting the nucleosomes. To test whether Etv2 was able to target the nucleosome, we first examined the nucleosome profiles of the 24 hours Etv2 ChIP-seq peaks in undifferentiated MEFs using published histone H3 ChIP-seq and MNase-seq29. We divided the Etv2 ChIP-seq peaks into four quartiles based on the mean MNase-seq signals of the central 200-bp region, and we used the first (lowest mean signal) and the fourth quartile (highest mean signal) to represent the nucleosome free region (NFR) and the nucleosome (Figure 2c and Supplementary Figure 7a). Thus, the nucleosome quartile represented the Etv2 peaks with their summits located at the nucleosome centers, suggesting that Etv2, like other reported pioneer factors, was also able to target the nucleosome during MEF reprogramming. Similarly, we divided the Etv2 ChIP-seq peaks at 3h post-induction in day 2.5 EB into NFR (5,291 peaks) and nucleosome (8,843 peaks) groups according to the local V-plot and fragment size profiles of ATAC-seq day 2.5 EB without Etv2 induction (Supplementary Figure 7b-7d). We found that similar to the MEF reprogramming, Etv2 also targeted the nucleosome centers at day 2.5 EB differentiation (Figure 2d). Our results in MEFs and EBs suggested that Etv2 was intrinsically able to target and bind the nucleosomes, and this capability appeared to be independent of cellular context. Sequence motif analysis identified a common GGAAAT motif that was significantly more enriched in NFR regions compared with the nucleosomes in both MEFs and EBs (Fisher's adjusted p-value=6.0E-05 and 6.8E-5) (Figure 2e and Supplementary Figure 8a). This motif has additional terminal "AT" nucleotides compared with the canonical Etv2. These findings suggested that Etv2, similar to other pioneer factors, such as Oct4, Sox2 and Klf4, were able to target nucleosome-enriched sites using partial or degenerate motifs, and targeted their full canonical motif in nucleosome-depleted sites3.

Moreover, we found that the Etv2 targeted nucleosome regions were characterized by low Brg1 ChIP-seq signals at 24 hours post induction in MEFs and 3 hours post-induction in EBs, suggesting that the initial recognition of the nucleosome may not require Brg1 (Figure 2c and 2d). Interestingly, the Etv2 targeted nucleosomes had a distinct H3K27ac surrounding pattern in MEFs and EBs: Etv2 targeted H3K27ac depleted nucleosomes in MEFs and H3K27ac enriched ones in EBs, suggested that Etv2 recognized the nucleosomes regardless of H3K27ac enrichment.

**Etv2 physically binds to the nucleosome**

To determine if Etv2 directly binds to nucleosomes as a pioneer factor, we identified a nucleosome-enriched site in the genome of both mESCs and MEFs that is efficiently targeted by Etv2, focusing on the Lmo2 locus that is an important downstream target of Etv2, relevant to HE lineage development (Figure 2f). Our bioinformatics analysis showed that Lmo2 is not expressed in both day 2 EB and MEFs before Etv2 induction, suggesting that Etv2 binding precedes Lmo2 activation. Additionally, we selected a region upstream of Lmo2 that is highly enriched for nucleosomes in both cell types, as measured by MNase-seq and ATAC-seq (Figure 2f). We generated a 159-bp PCR product containing Etv2 binding motifs (Cy5-labeledLmo2-DNA), which was assembled into nucleosomes (Figure 2g) by salt gradient dilution with purified recombinant mouse histones. As expected, recombinant ETV2 protein bound to Cy5-labeledLmo2-DNA probes without any histones (Figure 2h). Furthermore, when we tested the ability of Etv2 to bind to in vitro assembled Lmo2 nucleosomes, we observed a shift in the EMSA (Figure 2i). Altogether, these data suggest that Etv2 physically binds nucleosomes *in vitro*, further supporting our hypothesis that it functions as a pioneer factor.

**Brg1 maintains and stabilizes the binding of Etv2**

To further elaborate how Etv2 induction drives the endothelial lineage development during the reprogramming of MEFs and ES/EB differentiation, we performed Etv2 ChIP-seq at 48 hours and 7 days post-Etv2 induction in reprogramming MEFs and Etv2 ChIP-seq at 12 hours post-induction in EBs. In total, we identified 154,468 and 19,651 non-overlapping Etv2 peaks in MEFs and EBs, respectively. Similar to the OSK-binding sites during the reprogramming toward iPS29, the majority (more than 80%) of the unique Etv2 peaks were only present at early stages (day 1 for MEFs and 3 hours for EBs) but absent during the latter stages (day 7 for MEFs and 12 hours for EBs), suggesting that most of the initial Etv2 binding events were short-lived and were not sustained during differentiation (Supplementary Figure 9a and 9b). We divided the Etv2 peaks into "early", "late" and "sustained" groups according to whether Etv2 peaks were present in the early stage, the late stage or both stages (Figure 3a and 3b). Although we previously found that the majority of initial Etv2 binding events were independent of Brg1, the "late" Etv2 peaks were associated with increasing levels of Brg1 in the late stage of EBs and MEFs, while the "sustained" Etv2 peaks were associated with the highest levels of Brg1 in the early stage. Note that the establishment of "late" Etv2 peaks were also coupled with increasing H3K27ac enrichment, in contrast to the "sustained" Etv2 peaks that were initially located at a high H3K27ac environment. These results suggested a significant role for Brg1 on the maintenance and stabilization of the binding of Etv2 during the reprogramming period and facilitation of Etv2 activation of downstream endothelial programs. Indeed, we found there were significant overlap between the "late" Etv2 peaks, as well as the nearby genes (Figure 3c and 3d). Moreover, the "late" Etv2 peaks were located near a higher proportion of endothelial genes, in contrast to the "early" Etv2 peaks (Supplementary Figure 8d). Similar to Ascl1 induced neural reprogramming30, we also found the significant phasing events around the Etv2 binding motifs in the Flk1+ cells in both MEFs and EBs (Supplementary Figure 10)30. In summary, these results suggested that Brg1 maintained and stabilized Etv2 binding, promoted the local H3K27ac enrichment, and activated the downstream endothelial genes.

**Etv2 requires Brg1 to activate downstream genes during reprogramming**

Brg1 is required for ATP-dependent pioneer factors such as GATAs to open the chromatin6. To examine whether Brg1 is also required by Etv2 to perform its pioneer function, we first showed that Etv2 physically bind to Brg1 by GST pulldown experiments (Supplementary Figure 11). Next, we knocked down Brg1 in iHA-Etv2 MEFs using shRNAs 48 hours prior to the doxycycline induction of Etv2, and continued inducing Etv2 at 48 hours, 4 days and 6 days post induction (Supplementary Figure 12a). We found that the Brg1 RNA levels and the Flk1+ cell populations were significantly reduced at 24 hours, 48 hours and 7 days post induction, compared with control Etv2 induced MEF reprogramming (Supplementary Figure 12b and 12c). Moreover, we found that the expression levels of several downstream endothelial genes were also significantly reduced at 7 days post induction when Brg1 was knocked down, suggesting that Brg1 is required for Etv2 to function as a pioneer factor by opening the chromatin and activating the downstream endothelial target genes (Supplementary Figure 12e-12n).

To further examine global impact of Brg1 knockdown the pioneer function of Etv2, we performed the ATAC-seq and scRNA-seq before Etv2 induction (D0), 1 day and 7 days post induction of Etv2 in Brg1 KD MEFs. We found that 81.3% of sustained Etv2 ChIP-seq peaks that are present at both day 1 and day 7 post induction of Etv2 in control MEFs, have significantly reduced chromatin accessibility (as shown by the ATAC-seq reads intensity) at day 7 in Brg1 KD MEFs (Figure 4a). Meanwhile, the chromVAR analysis also showed that after inducing the Etv2, the Brg1 KD MEFs at day 7 have significantly less Etv2 motif containing ATAC-seq reads, compared with control MEFs at day 7, and Flk1+ cell population from control MEFs at day 7 (Figure 4b). Moreover, the Etv2 binding sites affected by the Brg1 KD were more likely located at the distal intergenic regions (Figure 4c). These results supported our previous observation that Brg1 is critical for maintaining the chromatin accessibility of sustained Etv2 binding sites during the Etv2 induced reprogramming.

To further examine how Brg1 KD affect the chromatin accessibility surrounding the Etv2 binding sites at the first 24 hours of post Etv2 induction, we identified 1,204 Etv2 binding sites that were closed in control MEFs and became open at day 1 post Etv2 induction in control MEFs. The aggregated V-plot of showed that these Etv2 binding sites were still closed and occupied by nucleosomes at day 1 post Etv2 induction in Brg1 KD MEFs. These findings supports the notation that Etv2 may also requires Brg1 to open the closed chromatin region and fulfil the pioneer function.

The scRNA-seq data showed that knocking down Brg1 in MEFs significantly promote the cell proliferation31. After correcting for the cell cycle effects, the Brg1 KD MEFs and control MEFs showed overlapped cell population and knocking down Brg1 did not perturbed the expression of Etv2 downstream genes (Supplementary Figure 14). The scRNA-seq at day 7 post Etv2 induction in Brg1 KD MEFs showed reduced expression levels of key mesodermal genes such as Kdr, though Etv2 expression levels were unchanged, and the Flk+ cells in Brg1 KD MEFs and control MEFs are separate populations (Figure 4e-4h). These results suggested that Brg1 is required for Etv2 to properly activate the downstream genes during the reprogramming.

Overall, these results suggested that Etv2 requires Brg1 to maintain and stabilize the binding events, which is critical for Etv2 to activate the downstream endothelial genes and fulfill its pioneer function (Figure 4i).

**Discussion**

In the present study, we used two distinct biological systems: ES/EB differentiation and MEF reprogramming, to define the role for Etv2 as a pioneer factor that drives endothelial development. To our knowledge, no other reprogramming study has used these powerful systems together to define pioneer factors and reprogramming capabilities. Even though these two model systems have very different global expression, chromatin accessibility and epigenetic profiles, we surprisingly found similar molecular programs and downstream genes that were regulated by Etv2 induction. We showed that Etv2 cooperates with Brg1, an ATPase of the SWI/SNF chromatin remodeling enzyme, and together they function to relax the closed chromatin and convert the nucleosome-enriched to the nucleosome-depleted region during the endothelial lineage development in both MEFs and EBs. The definition of these mechanisms will enhance our understanding of cardiovascular development and regeneration and serve as a platform for therapeutic strategies for patients with congenital or aging related cardiovascular diseases.

**Figure 1. Etv2 promotes the endothelial program in both MEFs and EBs. (a)** Schematic of the reprogramming strategy in iHA-Etv2 MEFs by overexpression of Etv2 with doxycycline (Dox). **(b)** Schematic of the differentiation of embryoid body (EB) and induction of Etv2 at day 2.5 of differentiation. **(c-d)** The UMAP plot shows the scRNA-seq of 948 undifferentiated MEFs, 3,539 reprogrammed cells at 24 hours, 2,936 cells at 48 hours and 7,202 cells at 7 days and 827 Flk1+ cells at 7 days post-induction of Etv2 in MEFs. The dimension reduction analysis by scVI, followed by uniform manifold approximation and projection (UMAP) and k-means clustering identified seven distinct cell clusters. The color represents **(c)** the cell sources and **(d)** cell clusters. **(e)** The expression profiles of Etv2 and Kdr (Flk1). **(f)** The volcano plot of genes differentially expressed between cluster 1 and cluster 7. The *p*-values were determined by Wilcoxon ran sum test of the normalized read counts. **(g)** The biological process that are significantly associated with the up-regulated in genes in cluster 7 (Flk1+ cells at day 7 of reprogramming) compared with cluster 1 (undifferentiated MEFs). **(h)** The PCA of the TF deviations of the ATAC-seq of MEF reprogramming (MEF, 24 hours, 48 hours and 7 days post induction) and EB differentiation (2.5 day and 3 hours post induction). The TF deviations were inferred by chromVAR. **(i)** The 31 transcription factors which expression levels and motif associated chromatin accessibility consistently showed directional change in both EB and MEF (13 up-regulated TFs and 18 down-regulated TFs).

**Figure 2. Etv2 targets nucleosomes during reprogramming. (a)** The Venn diagram shows 131,001 and 18,024 Etv2 ChIP-seq peaks at 24 hours post-induction in MEF reprogramming and 3 hours post induction in day 2.5 EB, respectively. There are 11,751 common Etv2 peaks overlapped between MEFs and EBs. **(b)** The genomic distribution of EB specific, MEF specific and common Etv2 peaks. The EB and MEF specific Etv2 peaks were more likely distributed at the distal intergenic regions. **(c)** The heatmap shows the read density of MNase-seq, Brg1 ChIP-seq and H3K27ac ChIP-seq in MEFs, surrounding 131,001 Etv2 ChIP-seq peaks at 24 hours post-induction during MEF reprogramming. The Etv2 peaks were divided into four quartiles based on the mean MNase-seq signals of the central 200-bp region. The first (lowest mean signal) and the fourth quartile (highest mean signal) were used to represent the nucleosome free region (NFR) and nucleosome. **(d)** The heatmap shows the ratio of NFR / nucleosome read density, read density of Brg1 ChIP-seq and H3K27ac ChIP-seq at EB day 2.5, surrounding 18,024 Etv2 ChIP-seq at 3 hours post-induction. The Etv2 peaks were divided into NFR (5,291 peaks) and nucleosome (8,843 peaks) groups according to the local V-plot and fragment size profiles of ATAC-seq day 2.5 EB without Etv2 induction. **(e)** Sequence motif analysis by DREME and CentriMo identified a common GGAAAT motif that are significantly more enriched in NFR regions compared with the nucleosomes in both MEF and EB (Fisher's adjusted p-value=6.0E-05 and 6.8E-5).

**Figure 3.** **Brg1 maintains and stabilize the binding of Etv2. (a)** The heatmap shows the fold enrichment of Etv2 ChIP-seq, Brg1 ChIP-seq and H3K27ac ChIP-seq, at 24 hours, 48 hours and 7 days post-induction of Etv2. We also included the Brg1 and H3K27ac ChIP-seq analyses of undifferentiated MEFs. Each site is centered at the 154,468 non-overlapping Etv2 ChIP-seq summits during MEF reprogramming. The Etv2 peaks into "early", "late" and "sustained" groups according to whether Etv2 peaks were present in 24 hours post-induction (early stage), 48 hours post-induction (late stage) or both stages. **(b)** The heatmap shows the fold enrichment of Etv2 ChIP-seq, Brg1 ChIP-seq and H3K27ac ChIP-seq, at 3 hours and 12 hours post-induction of Etv2 in day 2.5 EB. We also included the Brg1 and H3K27ac ChIP-seq analyses at day 2.5 EB without induction. Each site is centered at the 19,651 non-overlapping Etv2 ChIP-seq summits during EB differentiation. The Etv2 peaks classified into "early", "late" and "sustained" groups according to whether Etv2 peaks were present in 3 hours post-induction (early stage), 12 hours post-induction (late stage) or both stages. **(c-d)** The Venn diagram shows the overlap between late Etv2 peaks in MEFs and EBs, as well as the overlap between the genes near the late Etv2 peaks.

**Figure 4.** **Etv2 requires Brg1 to activate downstream genes during reprogramming. (a)** The heatmap shows the piled up ATAC-seq signal surrounding the summit of 12,170 sustained Etv2 ChIP-seq peaks that are present at day 1 and day post induction of Etv2 in control MEFs. The ATAC-seq data include control MEF (D0), day 7 post induction, and Flk1+ cells from day 7 post induction in control MEFs. We also include the ATAC-seq data from D0 (before induction) and day 7 post induction of Brg1 KD (knockdown) MEFs. The sustained Etv2 peaks were divided into two groups: open(red) or closed (black) at day 7 post induction in Brg1 KD MEFs. **(b)** The heatmap shows the transcription factors which motif associated chromatin accessibility are significantly changed at day 7 post Etv2 induction in control MEFs (whole MEFs or Flk1+ cells), or the Brg1 KD MEFs (chromVAR adjusted *p*-value<1e-200). The color red and blue indicate the enrichment and the deficient of the transcription factor associated ATAC-seq reads in associated conditions, respectively. **(c)** The genomic distribution of open Etv2 peaks and closed Etv2 peaks at 7 days post induction in Brg1 KD MEFs. These Etv2 peaks are present in both day 1 and day 7 post induction in control MEFs. **(d)** The aggregated V-plot of 1,204 Etv2 binding sites that were closed in control MEFs and became open at day 1 post Etv2 induction in control MEFs. Top left: control MEF; top right: Brg1 KD MEF; bottom left:24 hours post induction in control MEF; bottom right: 24 hours post induction in Brg1 KD MEFs. **(e-h)** The UMAP plot shows the scRNA-seq of 8,838 cells from day 7 post induction in control MEFs, 1,502 Flk1+ cells from day 7 post induction in control MEFs, 8,248 cells from day 7 post induction in Brg1 KD MEFs, and 8,034 cells at day 7 in Brg1 KD MEFs (without induction). The dimension reduction analysis by scVI, followed by uniform manifold approximation and projection (UMAP). The color represents **(e)** the cell sources and **(f-h)** the expression levels. **(i)** The model of Etv2 induced reprogramming. Etv2 targets the nucleosome during the early stage of reprogramming. Brg1 helps to maintain and stabilize the Etv2 binding near the endothelial genes, coupled with the increase of local H3K27ac levels.

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