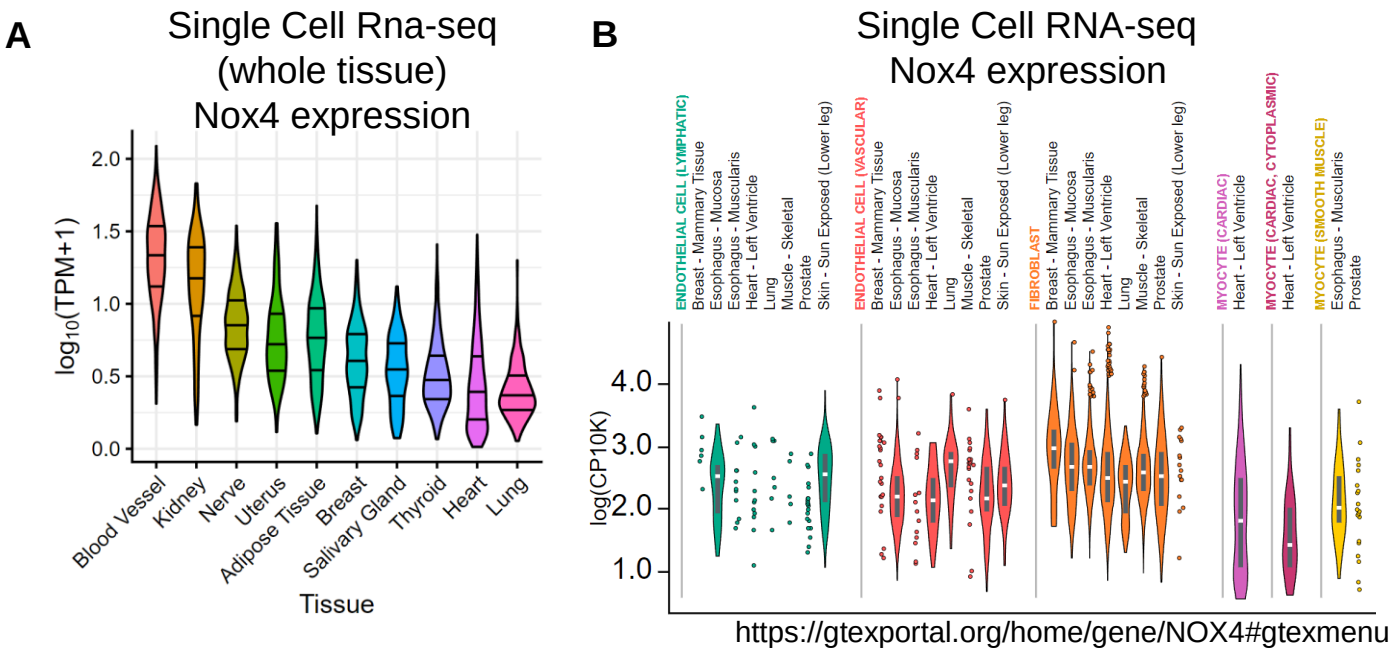
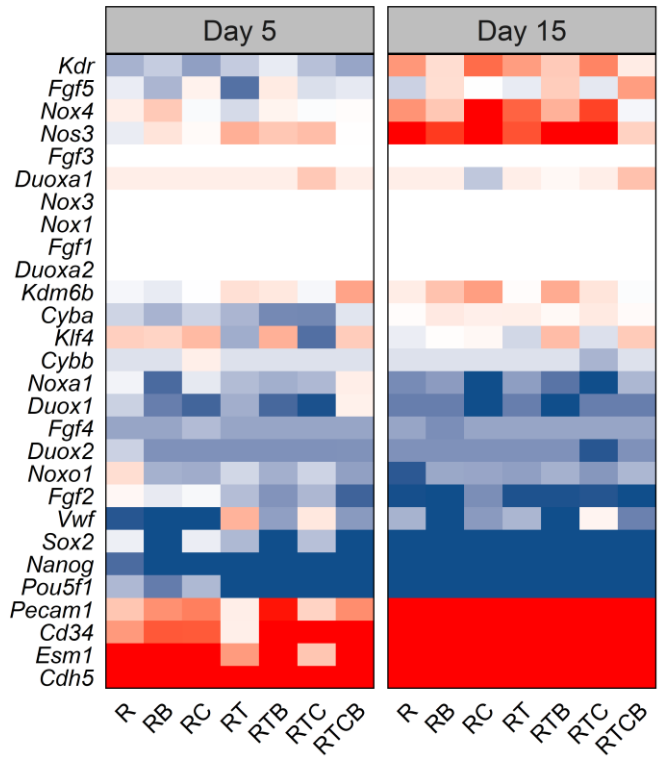
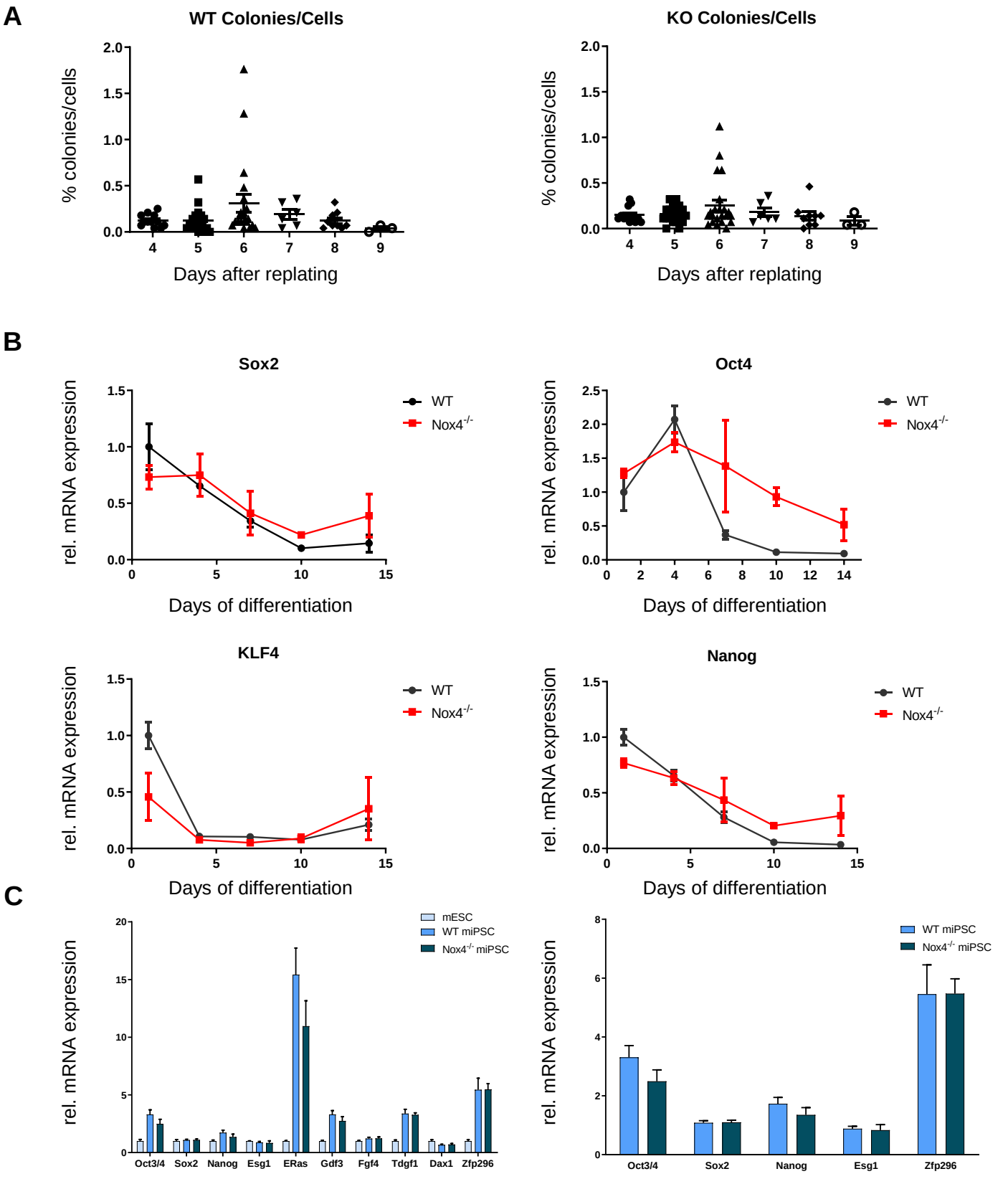


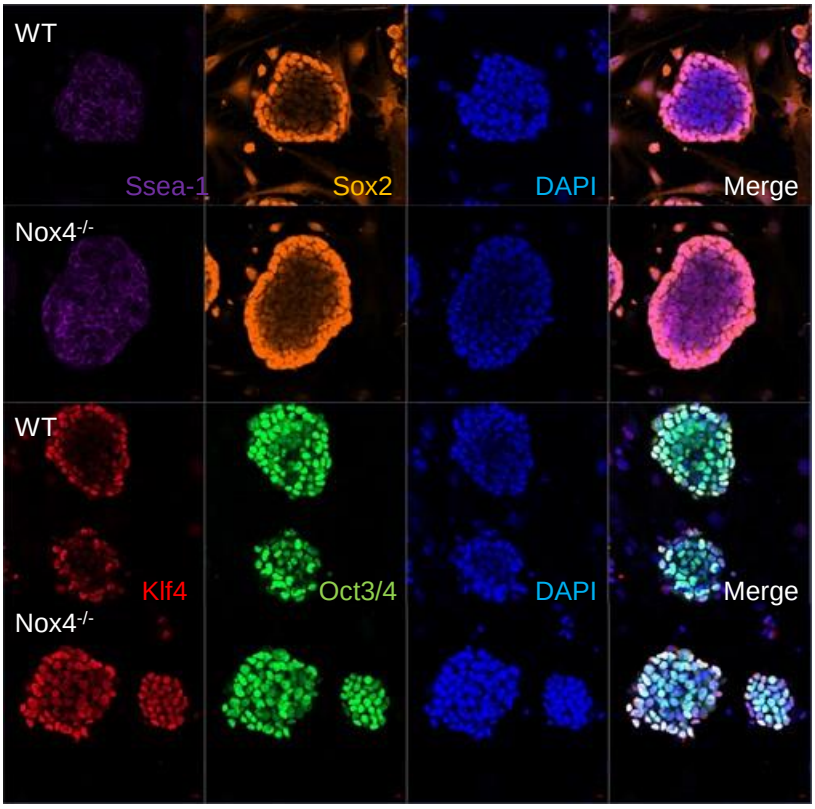


Supplement Figure 1. Nox4 is highly expressed in vascular tissue, endothelial cells & expression increases in endothelial differentiation (A) Bulk RNA sequencing data from GTEx database indicating Nox4 is highly expressed in blood vessels. (B) GTEx Single Cell RNA sequencing indicating Nox4 highest expression in endothelial cells (C) RNA-Sequencing data adapted and re-analysed from Belt et al. A Heatmap of differentially expressed NADPH oxidase isoforms, endothelial markers & stemness markers in different differentiation conditions on Day 5 & 15, displayed as log2fc normalised on iPSC control. Supplements used by Belt et al.: R=Rock inhibitor, RB=Rock inhibitor/BMP4, RC=Rock inhibitor/cyclic-amp, RT=Rock inhibitor/ TGF inhibitor, RTB=Rock inhibitor/TGF inhibitor/BMP4, RTC=Rock inhibitor/TGF inhibitor/cyclic-amp, RTCB=Rock inhibitor/TGF inhibitor/cyclic-amp/BMP4.

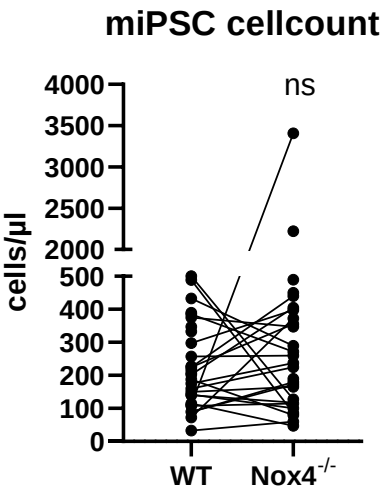


Supplement Figure 2. Comparison of mESC vs. miPSC. (A) Reprogramming efficiency shows no difference between WT & Nox4-/-. (B) Stem cell genes decrease expression in the course of differentiation (n=3). (C) qPCR indicating no difference in stem cell marker expression between mESC & miPSC (n=2-5) (left panel) and no difference between WT & Nox4-/- (n=4) (right panel).

A

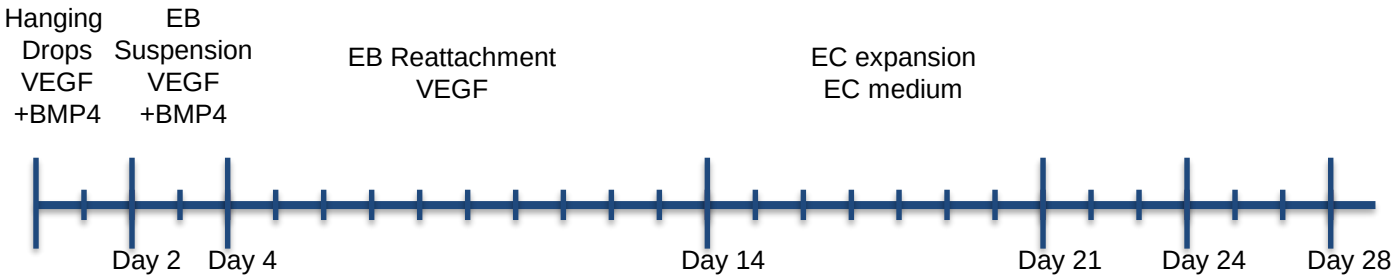


B

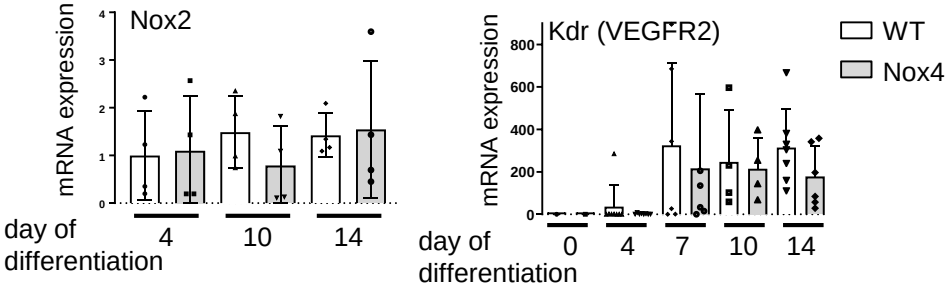


Supplement Figure 3. miPSC WT vs Nox4-/- comparison (A) Representative immunofluoresence staining of stem cell markers in WT & KO miPSC. (B) No Proliferation differences between WT & Nox4 knockout (comparing 33 passages).

A **time scale of forced in vitro endothelial differentiation**



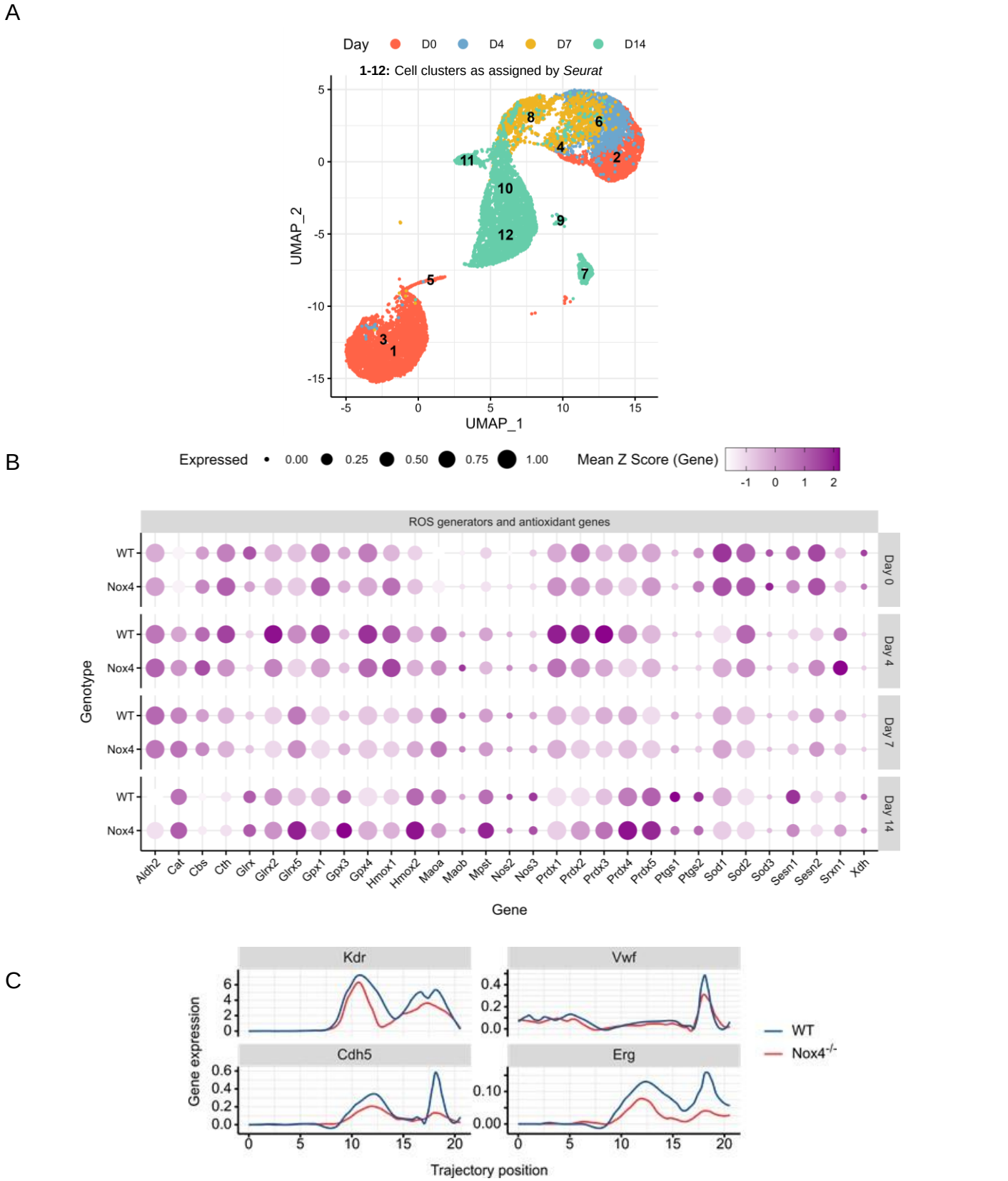
A **mRNA expression of Nox2 and Kdr (VEGFR2)**



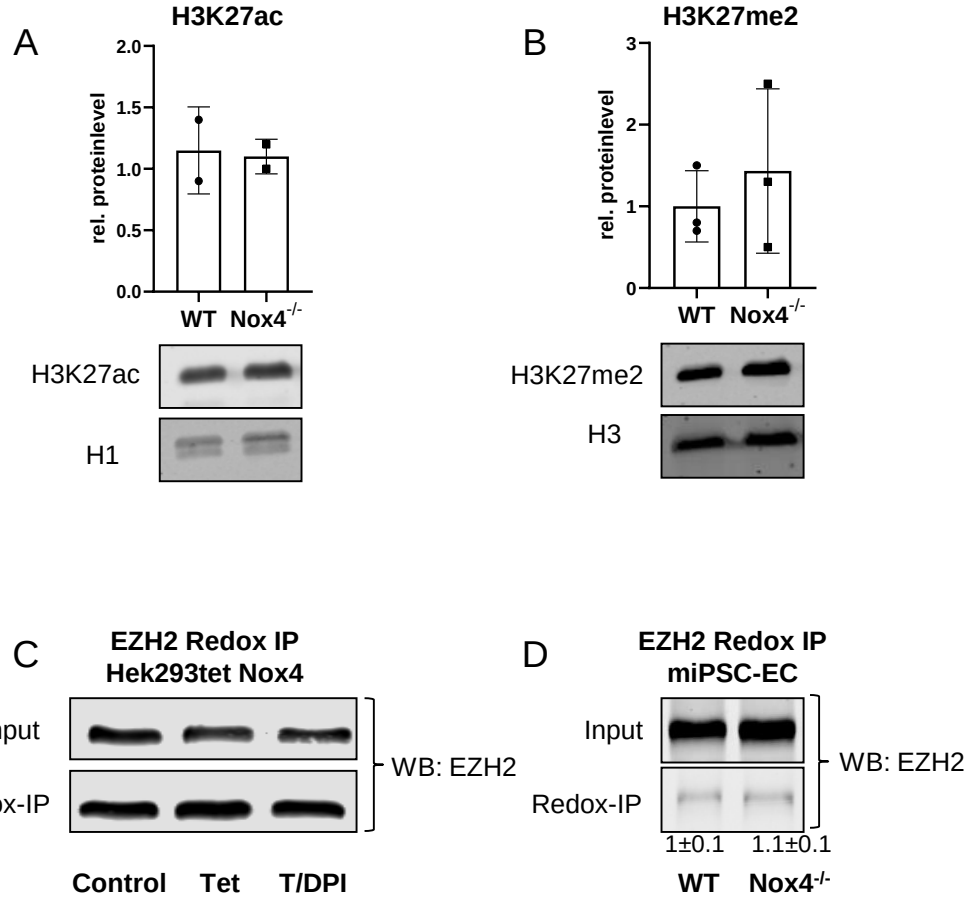
No ct found for:

Nox1
Nox3
Duox1
Duox2

Supplement Figure 4: (A) time scale of forced in vitro endothelial differentiation; (B) Nox2 and Kdr mRNA expression in the course of differentiation. Real-time qPCR for Nox2 and Kdr. . (n=5-7)



Supplement Figure 5. UMAP plot with excluded Feeder clusters & ROS related genes in course of differentiation. (A) Uniform manifold approximation and projection (UMAP) plots of cells at days 0, 4, 7 and 14 of differentiation from induced pluripotent stem cells to endothelial cells, indcluding Feeder (fibroblasts) in Cluster 1,3,5. (B) Percentages of wild-type and Nox4-knockout cells from each day of differentiation expressing subsets of marker genes for ROS generation and antioxidants, in combination with Z-score normalised expression of each gene across all cells. (C) Normalised expression of *Kdr*, *Vwf*, *Cdh5* and *Erg* along the trajectory of endothelial differentiation in Nox4-knockout and wild-type cells.



Supplement figure 6. Abundance of chromatin marks and oxidation state of methyltransferase. (A) Western Blot for H3K27ac & me2 in WT vs. Nox4$^{-/-}$ (n=2-3). (B) BIAM switch redox assay of EZH2 in Nox4 overexpressing human embryonic kidney cell, Tetracyclin 1µg/ml, 24h, DPI 3 µM, 3h. (C) BIAM switch redox assay of EZH2 indicates no difference in oxidation state in WT miPSC-ECs vs Nox4$^{-/-}$.