Supplementary Figure and Supplementary Dataset legends:

Identification of a combination of transcription factors that synergistically increases endothelial cell barrier resistance

Filip Roudnicky[1], Bo Kyoung Kim[2], Yanjun Lan[1], Roland Schmucki[3], Verena Küppers[2], Klaus Christensen[1], Martin Graf[1], Christoph Patsch[1], Mark Burcin[1], Claas Aiko Meyer[1#], Peter D. Westenskow[2*#], Chad A Cowan[4,5,6*#].

*Corresponding authors: ccowan@bidmc.harvard.edu, peter.westenskow@roche.com

Supplementary Material and methods

Analysis of published gene expression datasets. Raw Affymetrix expression files were downloaded from GEO (GSE35802 [1], GSE48209 [2], GSE56777 [3], GSE47067 [4, 5]) and analyzed using the Partek Genomic Suite. Probeset data were normalized by making pre-background adjustments for GC correction and probe sequence bias, and then correcting for RMA background and carrying out quantile normalization. Normalized probe set data were $\log_2$ transformed and summarized using the median polish method. Differential expression was determined using an ANOVA model. Data from a study by Nolan *et al.* [4], where brain ECs were compared to several other tissues, were analyzed using RankProdIt [6], a rank based meta-analysis tool that we used to generate a consensus brain EC gene signature. Additionally, a literature search was conducted to find evidence of TFs that are associated with EC barrier resistance formation [7-15] or induction of molecules involved in barrier formation (e.g. tight junctions [7-9, 12, 16-27]).

Supplementary Figure and Supplementary Dataset legends:

Supplementary Figure 1. Expression of transcription factors in freshly isolated endothelial cells from different organs with upregulation in brain endothelial cells. TF expression levels were compared between brain ECs and ECs of other vascular beds using data from three different studies (GSE47067 [4], GSE48209 [2], GSE35802 [1], GSE56777 [3, 5]). Data from a study by Nolan *et al*. (GSE47067 [4]), where brain ECs were compared to several other tissues, was analyzed using RankProdIt [6], a rank based meta-analysis tool, to generate a consensus brain EC fold-change in expression. The heatmap shows genes with the greatest fold difference. Genes were sorted based on RankProdIt fold-change (FC>1.5) in GSE47067 [4].

Supplementary Figure 2. Relative mRNA expression of EC transporter genes as compared to empty vector adenovirus control. Columns represent mean ± SD. *=*P* or FDR<0.05, **= *P* or FDR<0.01, ***= *P* or FDR<0.001. All experiments were performed in triplicates.

Supplementary Dataset 1. Fold-change in expression of genes in freshly isolated ECs from the BBB versus ECs from different organs as calculated using RankProdIt analysis. Raw microarray expression data from GSE47067 [4] of ECs from different vascular beds was analyzed using the Partek Genomics suite to calculate the fold-change in gene expression between ECs from brain versus other ECs of other vascular beds. RankProdIt values were calculated as a consensus summary fold-change.

Supplementary Dataset 2. List of human and mouse transcription factors as identified by the FANTOM consortium [28].

Supplementary Dataset 3. Fold-change expression of transcription factors that had a FC>1.5 in GSE47067 from studies with freshly isolated ECs from the BBB versus ECs from different organs. GSE47067 [4], GSE48209 [2], GSE35802 [1], GSE56777 [3, 5].

Supplementary Dataset 4. Evidence from literature or expression that transcription factors are relevant to induction of high-barrier resistance of ECs. The list denotes gene symbols, expression evidence from previous studies among 67 TFs that were identified from: GSE47067 [4] or literature evidence.

Supplementary Dataset 5. RNA-seq differential expression analysis at 48 h after transduction of hPSC-ECs with adenovirus overexpressing transcription factors at 80 MOI. FPKM (Fragments per Kilobase of transcript per Million mapped reads) values are listed for each gene and replicate.

Supplementary Dataset 6. RNA-seq differential expression analysis at 48 h after transduction of hPSC-ECs with adenovirus overexpressing transcription factor combinations at 20 MOI. RPKM (Reads per Kilobase of transcript per Million mapped reads) values are listed for each gene and replicate.

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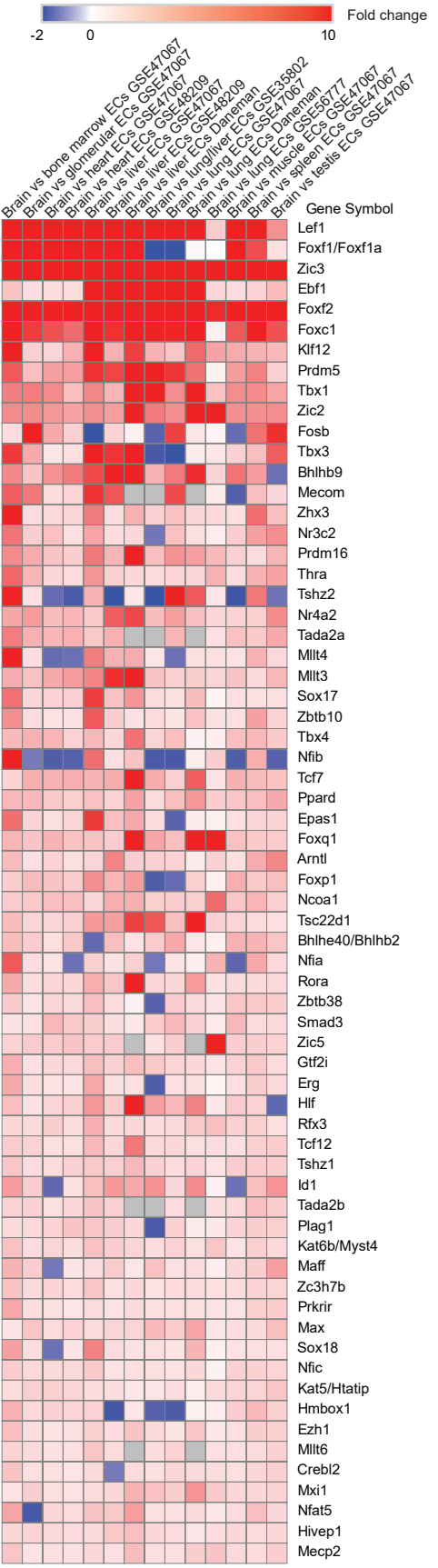
Supplementary Figure S1.



Supplementary Figure S2.