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Tyrosine-protein kinase Yes controls endothelial junctional plasticity and barrier integrity by regulating VE-cadherin phosphorylation and endocytosis

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Supplementary Information

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Supplementary table

Supplementary table 1. Analysis of *Yes1*, *Src* and *Cdh5* expression levels from single cell RNA sequencing data

Comparison of expression levels of *Yes1*, *Src*, and *Cdh5* in arterial, venous, capillary ECs, and tip cells present at P6 and P10 in retinal vessels analyzed by single cell RNA-sequencing. Differential gene expression was analyzed by comparing each individual EC subtype to all other ECs present at the same age. PCT 1 and PCT 2 denote the percentage of ECs in the compared groups where the gene of interest is detected (PCT 1, EC subtype; PCT2, all other age-matched ECs). Log2 FC denoted the average log2 fold change of the comparisons, positive values indicate that the gene of interest is more highly expressed in the EC subtype compared to all other age-matched ECs. Unadjusted p-values and p-values adjusted by Bonferroni correction using all genes present in the dataset are shown in the last two columns. Corresponding graphs of the gene expression are shown in Extended Data Fig. 1h-j.

Subtype	Age	Gene	PCT 1	PCT 2	Log2 FC	P-value	Adjusted p-value
Arterial ECs	**P6**	***Yes1***	58%	75%	-0.19	0.05	1.00
	P10	***Yes1***	57%	73%	-0.07	0.06	1.00
Capillaries	**P6**	***Yes1***	67%	77%	0.24	7.65×10^{-8}	2.47×10^{-3}
	P10	***Yes1***	73%	71%	0.16	0.20	1.00
Tip Cells	**P6**	***Yes1***	80%	73%	0.51	0.03	1.00
	P10	***Yes1***	76%	71%	0.32	5.84×10^{-3}	1.00
Proliferative ECs	**P6**	***Yes1***	82%	69%	-0.29	1.54×10^{-11}	4.98×10^{-7}
	P10	***Yes1***	71%	72%	-0.44	1.49×10^{-4}	1.00
Venous ECs	**P6**	***Yes1***	69%	75%	-0.25	0.25	1.00
	P10	***Yes1***	71%	72%	-0.35	0.13	1.00
Arterial ECs	**P6**	***Src***	13%	39%	-0.40	2.39×10^{-4}	1.00
	P10	***Src***	12%	44%	-0.87	1.44×10^{-5}	0.46
Capillaries	**P6**	***Src***	41%	37%	0.30	5.78×10^{-6}	0.19
	P10	***Src***	45%	39%	0.29	0.02	1.00
Tip Cells	**P6**	***Src***	27%	40%	-0.15	0.03	1.00
	P10	***Src***	42%	42%	0.12	0.65	1.00
Proliferative ECs	**P6**	***Src***	41%	36%	-0.28	8.93×10^{-12}	2.88×10^{-7}
	P10	***Src***	44%	42%	-0.29	0.02	1.00
Venous ECs	**P6**	***Src***	42%	37%	0.21	0.10	1.00
	P10	***Src***	43%	42%	-0.09	0.58	1.00
Arterial ECs	**P6**	***Cdh5***	98%	99%	0.60	1.23×10^{-5}	0.40
	P10	***Cdh5***	98%	99%	0.59	1.84×10^{-5}	0.59
Capillaries	**P6**	***Cdh5***	99%	99%	0.25	4.99×10^{-4}	1.00
	P10	***Cdh5***	99%	99%	0.21	0.02	1.00
Tip Cells	**P6**	***Cdh5***	99%	99%	0.34	1.01×10^{-5}	0.33
	P10	***Cdh5***	99%	99%	-0.28	0.01	1.00
Proliferative ECs	**P6**	***Cdh5***	99%	99%	-0.51	1.85×10^{-16}	5.98×10^{-12}
	P10	***Cdh5***	98%	99%	-0.38	9.33×10^{-4}	1.00
Venous ECs	**P6**	***Cdh5***	100%	99%	-0.06	0.33	1.00
	P10	***Cdh5***	100%	99%	-0.07	0.48	1.00

Supplementary videos

Supplementary video 1. Live cell imaging of HUVECs transfected with control or *YES1* siRNA in scratch wound healing assay. Images were taken every 15 min for 10 h. Migration of individual cells was tracked in ImageJ and their migratory routes are shown in color coded tracks (red, 1st row; yellow, 2nd row, green, 3rd row.

Supplementary video 2. VE-cadherin dynamics shown by live imaging of HUVECs expressing GFP-tagged VE-cadherin and transfected with control siRNA. Images were taken every 52 sec with confocal microscope. Examples of endocytosis of VE-cadherin vesicles are highlighted in boxed areas. Timestamps are min:sec. Scale bar, 10 µm.

Supplementary video 3. VE-cadherin dynamics shown by live imaging of HUVECs expressing GFP-tagged VE-cadherin and transfected with *YES1* siRNA. Images were taken every 52 sec with confocal microscope. Examples of the dynamics of VE-cadherin clusters are highlighted in boxed areas. Timestamps are min:sec. Scale bar, 10 µm.

Supplementary video 4. VE-cadherin dynamics shown by live imaging of HUVECs expressing GFP-tagged VE-cadherin and transfected with *SRC* siRNA. Images were taken every 15 sec with confocal microscope. Examples of endocytosis of VE-cadherin vesicles are highlighted in boxed areas. Timestamps are min:sec. Scale bar, 10 µm.

Supplementary video 5. Live cell imaging of control and *YES1* silenced HUVECs stained with SiR-Actin dye. Images were taken every 1 min for 3 h. Scale bar, 50 µm.

Supplementary video 6. Live cell imaging of migrating control HUVECs. Actin stress fibers visualized by SiR-Actin staining. Timestamps are min:sec. Scale bar, 10 µm.

Supplementary video 7. Live cell imaging of migrating *YES1* silenced HUVECs. Actin stress fibers visualized by SiR-Actin staining. Arrows indicate the break of stress fibers. Timestamps are min:sec. Scale bar, 10 µm.

Supplementary video 8. Live cell imaging of *SRC* silenced HUVECs during migration. Actin stress fibers visualized by SiR-Actin staining. Time stamps are min:sec. Scale bar, 10 µm.

Supplementary video 9. Intra-vital imaging of VEGFA induced leakage in the dermal vessels in control mice. Images were taken every 2 sec with confocal microscope and motion-corrected in ImageJ. Scale bar, 50 µm.

Supplementary video 10. Intra-vital imaging of VEGFA induced leakage in the dermal vessels in *Yes1* iECKO mice. Images were taken every 2 sec and motion corrected in ImageJ. Scale bar, 50 µm.

Supplementary video 11. Video showing the appearance of individual leakage points in dermal vessels in control mice.

Supplementary video 12. Video showing the appearance of individual leakage points in dermal vessels in *Yes1* iECKO mice.