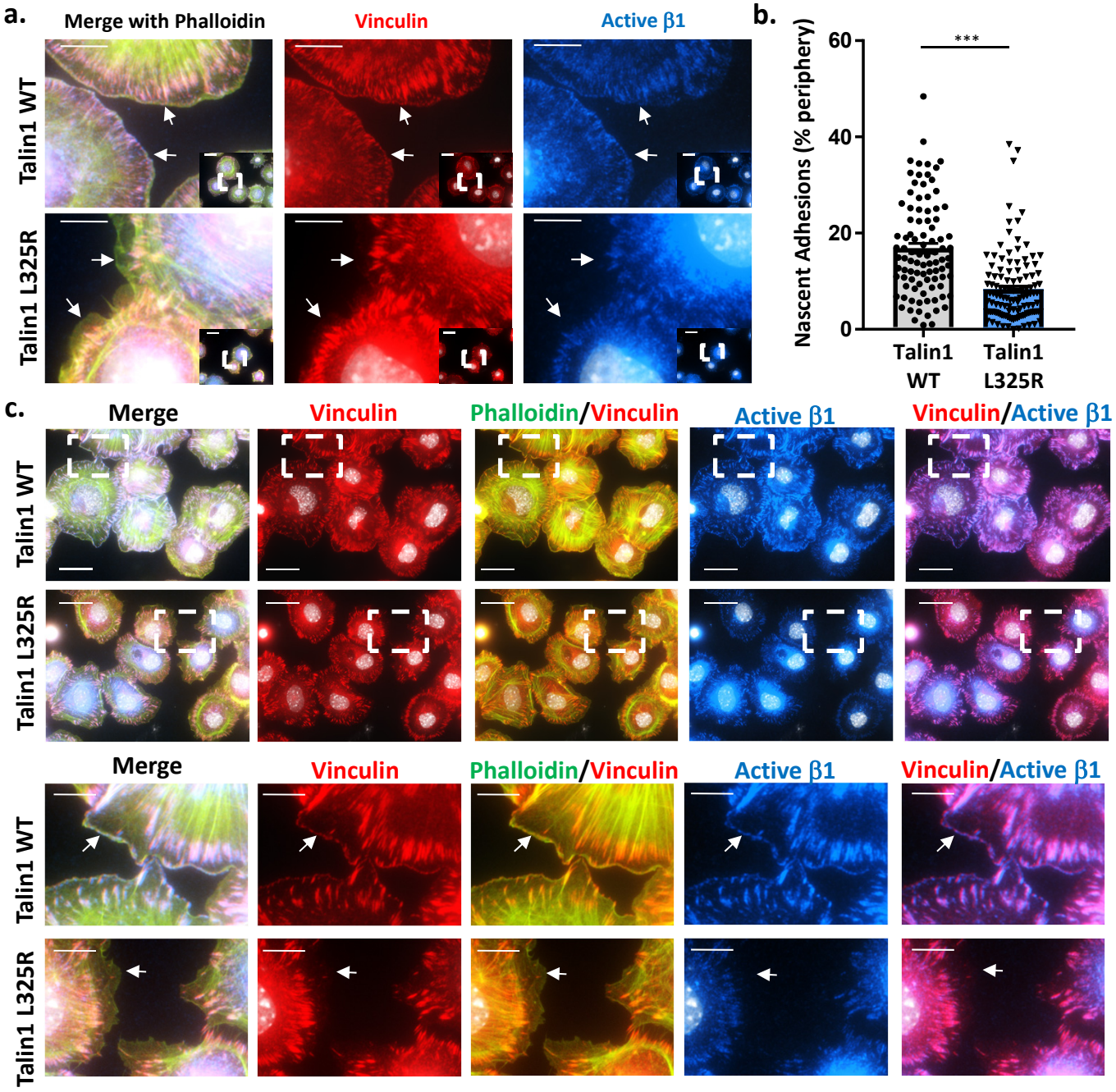
Supplemental Table I

Antibodies

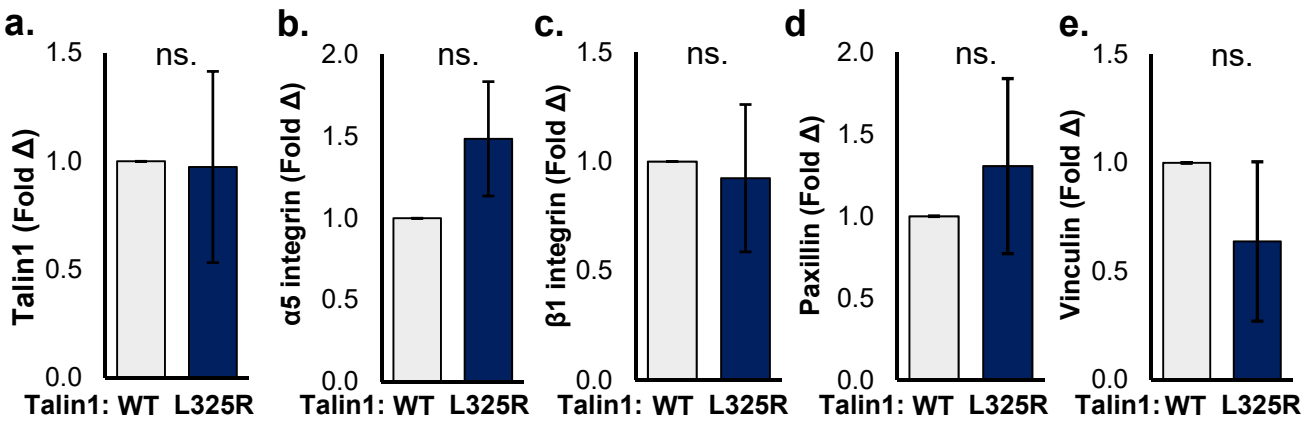
Target antigen	Vendor or Source	Catalog #	Working concentration
α5 integrin	Abcam	ab150361	1:1000(WB) 1:250 (ICC)
β1 integrin	Santa Cruz	sc-374429	1:1000 (WB)
Active β1 integrin (9EG7)	BD Biosciences	#553715	1:250 (ICC)
β-tubulin	Cell Signaling Technology	#2128	1:000 (WB)
AKT 1/2	Santa Cruz	sc-1619	1:500 (WB)
P-AKT (Ser473)	Cell Signaling Technology	#4060	1:1000 (WB)
CD31	Santa Cruz	Sc-1506	4 μg/mL (IHC)
CD68	Cell Signaling Technology	#76437	1:400 (IHC)
P-eNOS (Ser1177)	Cell Signaling Technology	#9571	1:500 (WB)
ERK1/ERK2	Santa Cruz	sc-94	1:5000 (WB)
P-p44/42 MAPK (ERK1/2) (Y204)	Cell Signaling Technology	#4370	1:1000 (WB)
Fibronectin	Sigma Aldrich	F3648	1:5000(WB)
GAPDH	Cell Signaling Technology	#2118	1:5000 (WB)
GST	Santa Cruz	sc-1511	1:5000 (WB)
ICAM-1	Santa Cruz	sc-138	1:200 (WB)
ILK	Cell Signaling Technology	3856	1:000 (WB)
Mac2	Accurate Chemical	CL8942AP	0.1 μg/mL (IHC)
NF-κB (p65 subunit)	Cell Signaling Technology	#4764	1:1000 (WB) 1:200 (ICC) 1:200 (IHC)
Phospho-NF-κB (Ser536, p65 subunit)	Cell Signaling Technology	#3033	1:1000 (WB)
Smooth Muscle Actin	Sigma Aldrich	C6198	2.5 μg/ml (IHC)
Paxillin	Cell Signaling Technology	#2542	1:1000 (WB)
Paxillin	Abcam	Ab32115	1:100 (ICC)
Talin1	Cell Signal Technology	#4021	1:1000 (WB) 1:500 (ICC)
Tensin 1	Sigma Aldrich	SAB4200283	1:1000 (WB) 1:500 (ICC)
VCAM-1	Abcam	Ab134047	1:1000 (WB) 1:100 (IHC)
Vinculin	Sigma Aldrich	V4139	1:400 (ICC)

qRT-PCR Primers

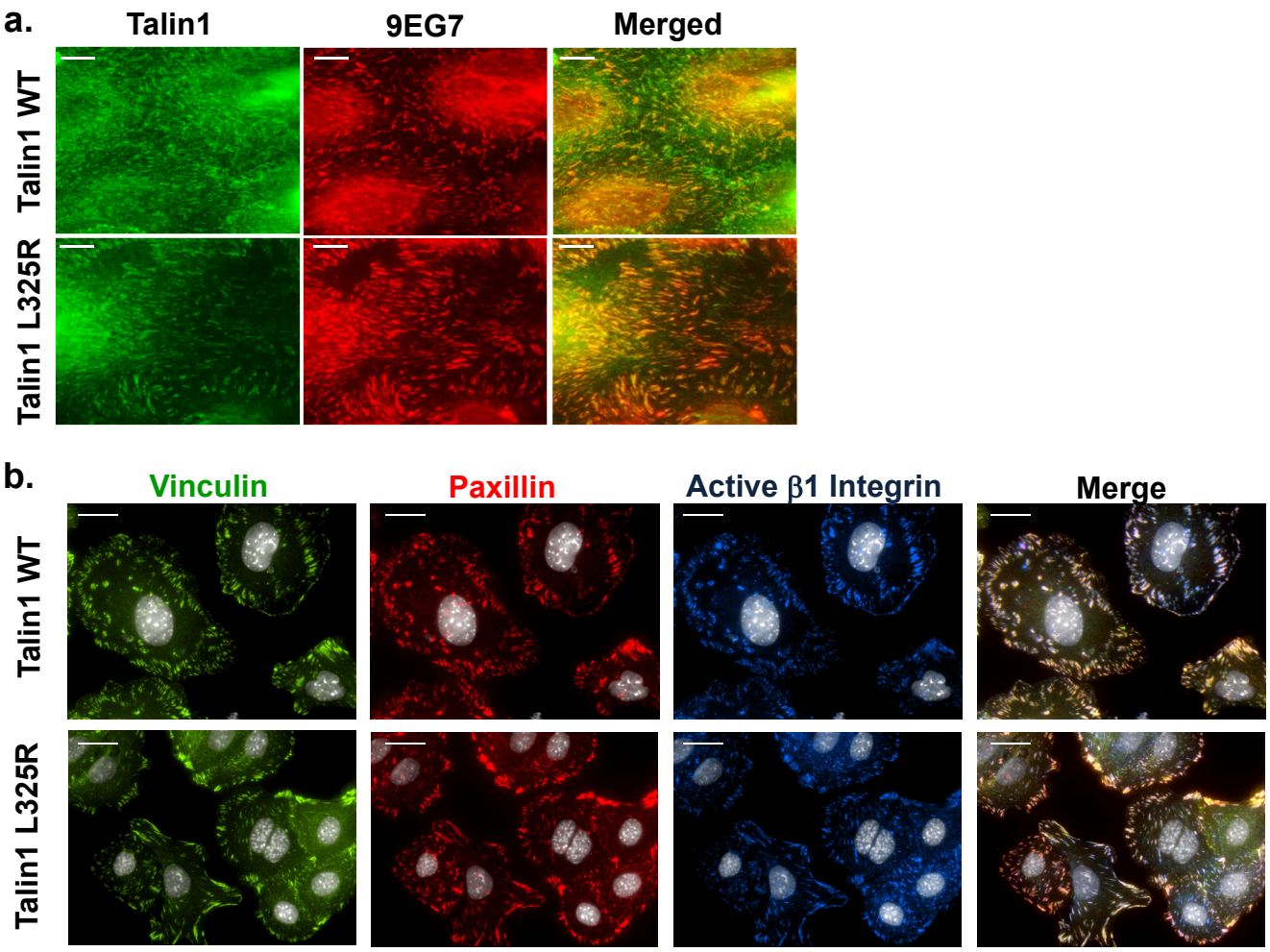
Gene	Species	Forward	Reverse
β2-microglobulin	Mouse	TTCTGGTGCTTGTCTCACTGA	CAGTATGTTCGGCTTCCCATTC
FN1	Mouse	ATGTGGACCCCTCCTGATAGT	GCCCAGTGATTTCAGCAAAGG
ICAM-1	Mouse	CTGGCTGTCACAGAACAGGA	AAAGTAGGTGGGGAGGTGCT
KLF-2	Mouse	AGAATGCACCTGAGCCTGCTAG	AATTTCCCCGAAAGCCTGC
Rpl13a	Mouse	GGGCAGGTTCTGGTATTGGAT	GGCTCGGAAATGGTAGGGG
VCAM-1	Mouse	TCAAAGAAAGGGAGACTG	GCTGGAGAACTTCATTATC



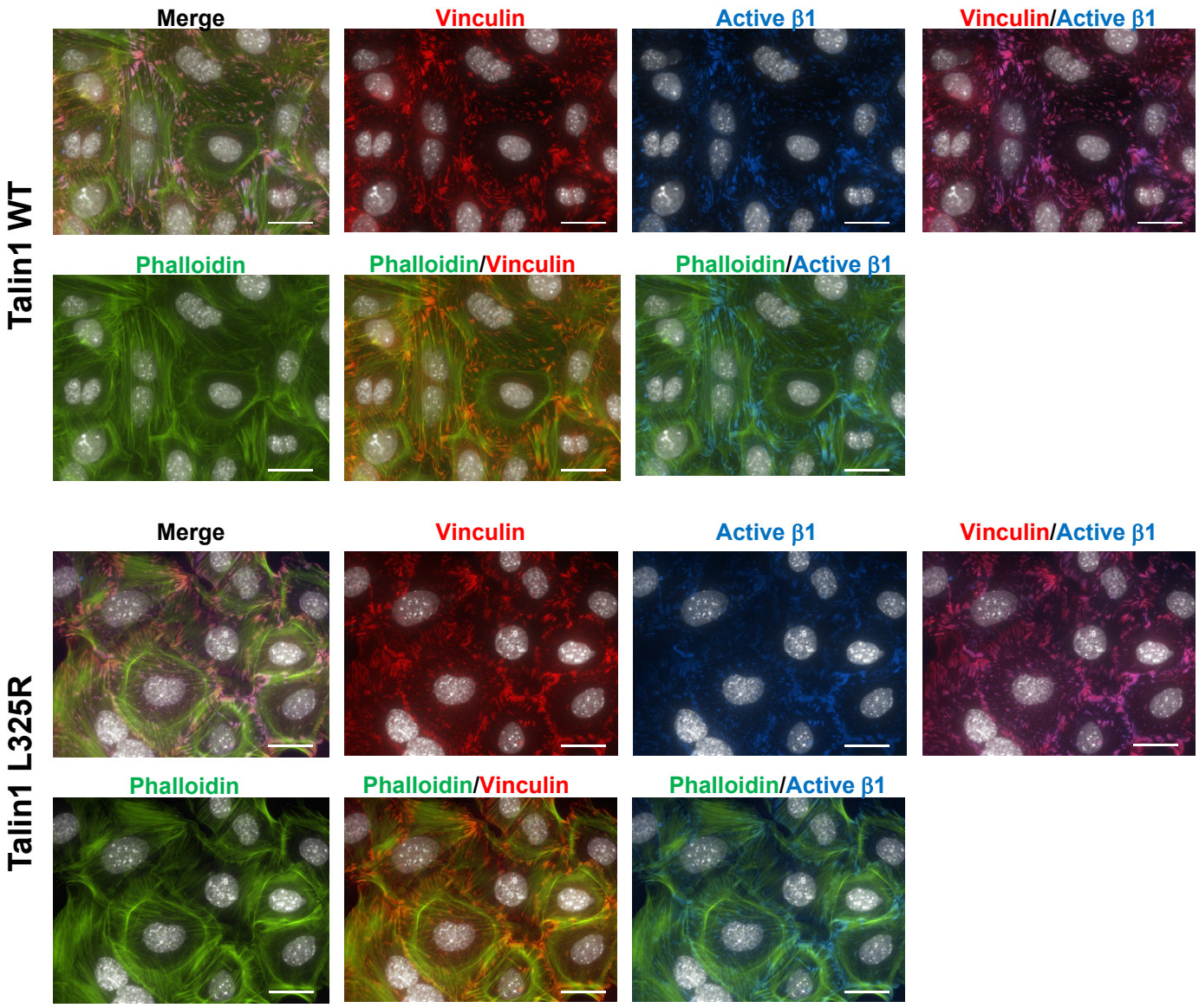
Supplemental Figure I. Talin1 L325R Mutation Limits Nascent Adhesion Formation. Talin1 WT and Talin1 L325R cells were plated on fibronectin for 30 minutes and stained for actin (phalloidin, green), vinculin (red), and active (high affinity) β1 (blue). a) Representative micrographs are shown with 60x inset. Scale bars = 10 μm and 20 μm in inset. b) Nascent adhesions were identified as vinculin and β1 integrin positive within 1 μm of the cell edge. Percent of the cell periphery positive for nascent adhesions (active β1) were quantified for individual cells from each of three independent experiments. c) Additional images of altered nascent adhesion formation between Talin1 WT and Talin1 L325R endothelial cells. Representative micrographs are shown with 60x inset. Scale bars = 10 μm and 20 μm in inset. Representative micrographs from one of three independent experiments are shown. ***$p<0.001$. Statistical analysis was performed using Student's t-test.



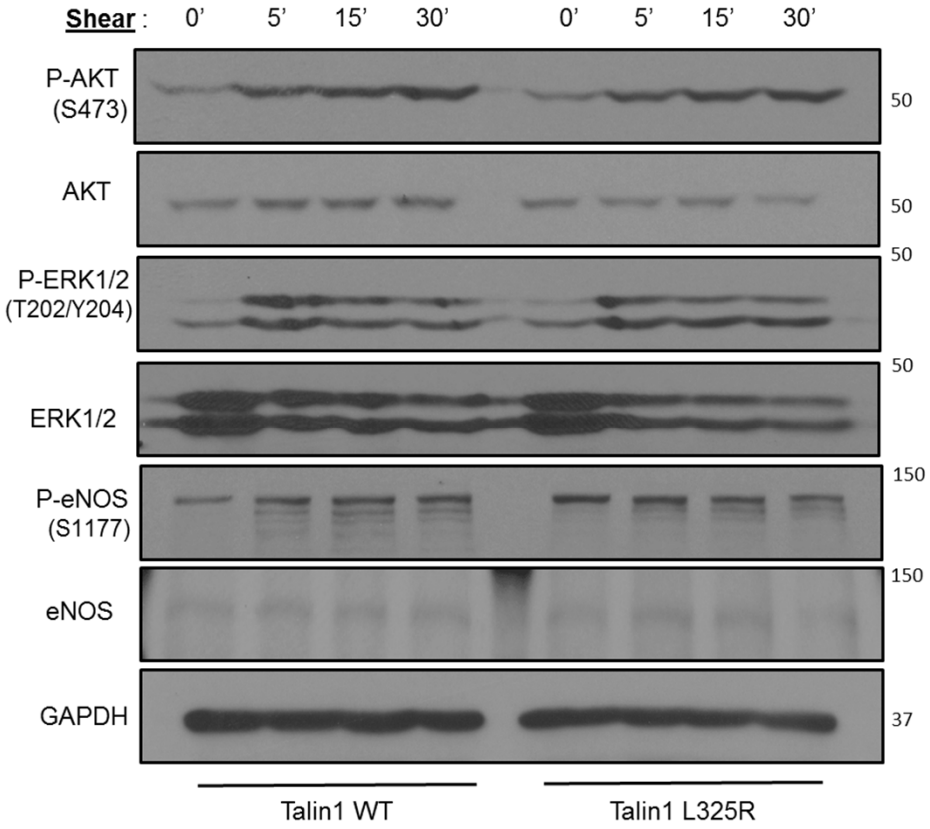
Supplemental Figure II. Talin1 L325R Mutation Does Not Alter Focal Adhesion Structure. Talin1 WT and Talin1 L325R endothelial cells were plated on fibronectin, and focal adhesions were extracted. Quantification of the Western blots for focal adhesion proteins shown in Figure 1F are provided, including a) talin1, b) α5, c) β1, d) paxillin, and e) vinculin. n=4. n.s. indicates not significant. Statistical analysis was performed using Student's t-test.



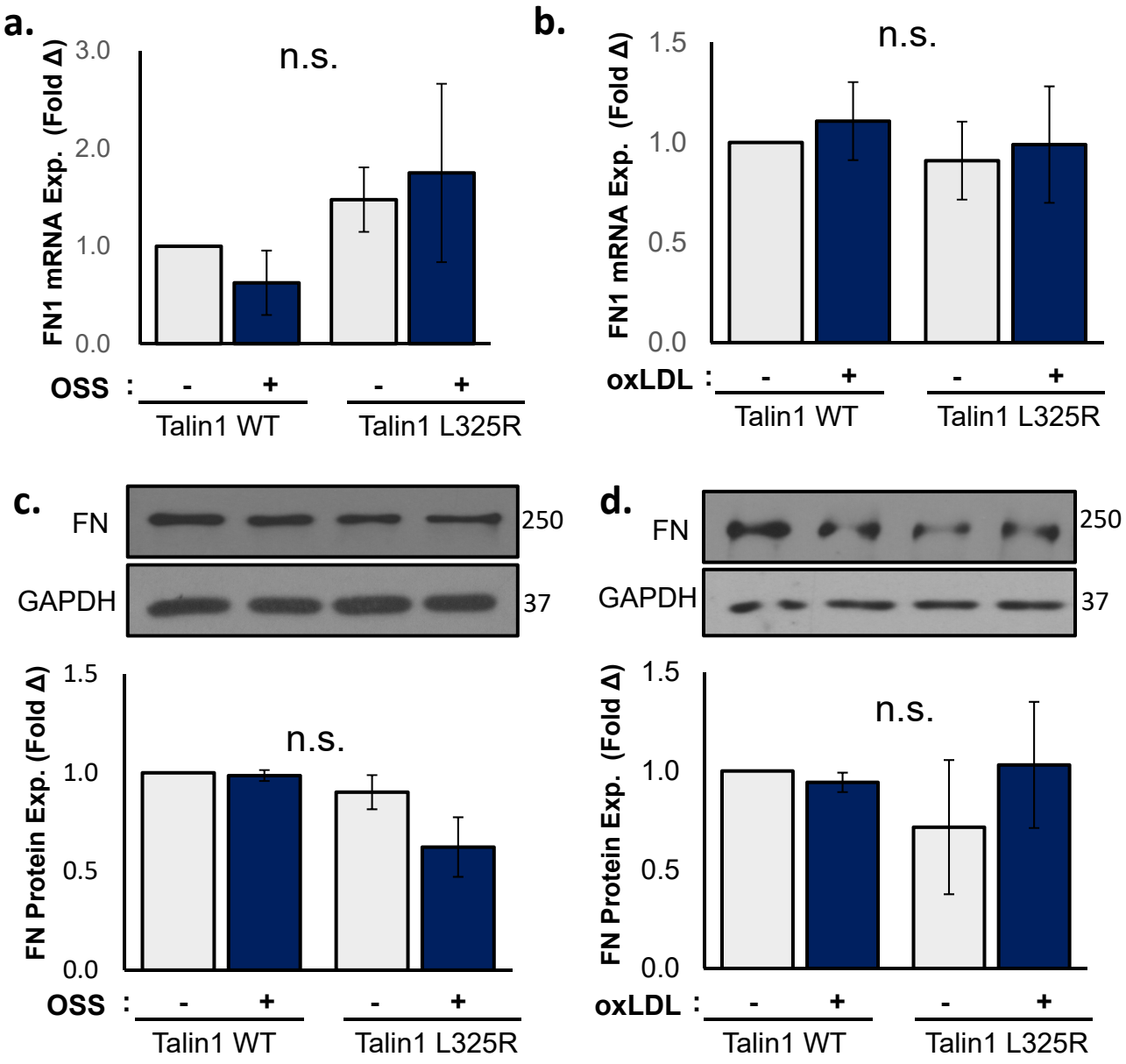
Supplemental Figure III. Talin1 L325R Mutation Does Not Alter Focal Adhesion Structure. a) Talin1 WT and Talin1 L325R MLECs were plated on fibronectin for 120 min, and talin1 (green) recruitment into focal adhesions (9EG7 (active β1 integrins), red) was assessed by immunocytochemistry. Representative images are shown. n=3. b) Talin1 WT and Talin1 L325R MLECs were plated as in (a), and the recruitment of the focal adhesion proteins vinculin (green), paxillin (red), and active β1 integrin (blue) was assessed by immunocytochemistry. Scale bars = 20 μm. Representative images are shown. n=3.



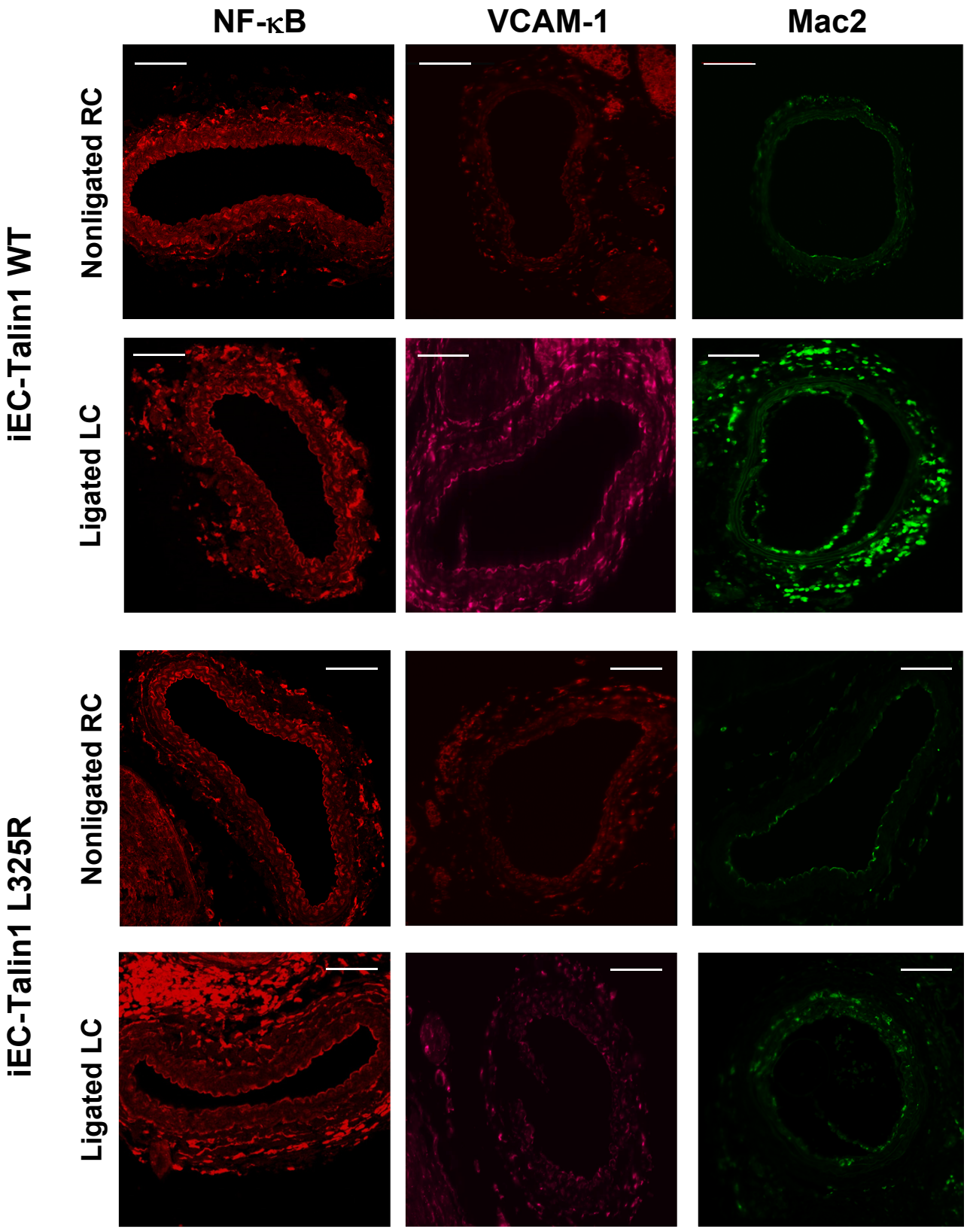
Supplemental Figure IV. Talin1 WT and Talin1 L325R Endothelial Cells Show Similar Focal Adhesion Formation. Talin1 WT and Talin1 L325R cells were plated on fibronectin for 120 minutes and stained for actin (phalloidin, green), vinculin (red), and active (high affinity) β1 (blue). Representative 60X micrographs are shown. Scale bars = 20 μm. Representative micrographs from one of three independent experiments are shown.



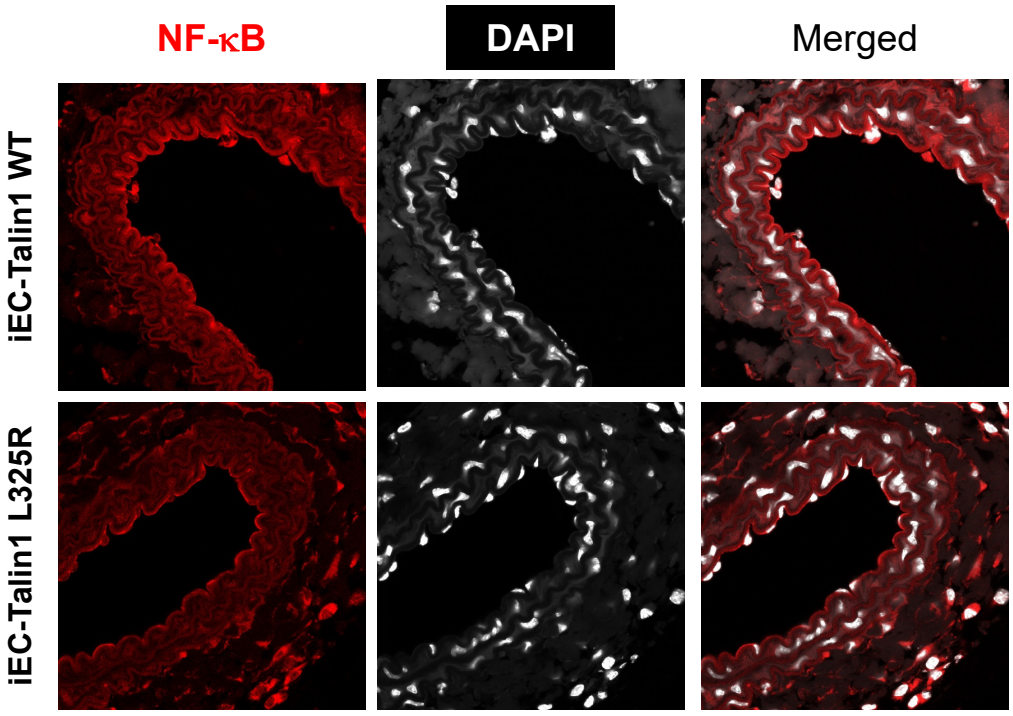
Supplemental Figure V. Talin1-dependent Integrin Activation isn't required for Shear Stress-Induced AKT/ERK/eNOS signaling. Talin1 WT and Talin1 L325R cells were exposed to acute onset of laminar shear stress for the indicated time points. Activation of known shear stress-sensitive pathways, such as extracellular signal-regulated kinase 1/2 (ERK1/2), Akt, and endothelial nitric oxide synthase (eNOS), was assessed by Western blotting using phospho-specific antibodies. Representative images are shown. n=3.



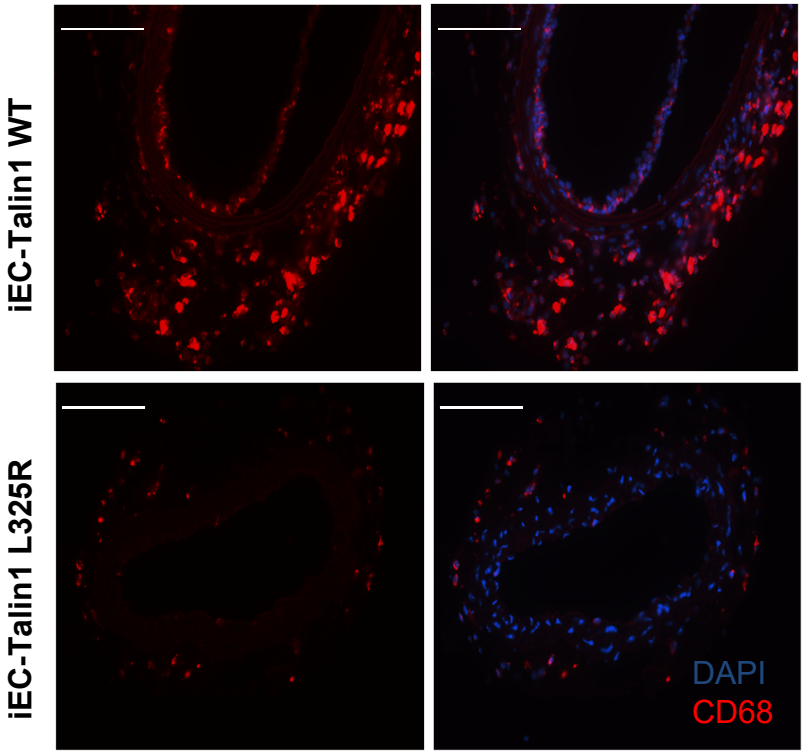
Supplemental Figure VI. Intact Fibronectin Expression in Talin1 L325R Cells . Talin1 WT and Talin1 L325R MLECs were plated on basement membrane proteins (diluted matrigel) and either (a,c) exposed to OSS (18 hrs) or (b,d) treated with oxLDL (100 μg/ml, 24 hrs). Fibronectin (FN) expression was determined by (a/b) qRT-PCR normalized to Rpl13a and β2-macroglobulin or by (c/d) Western blot normalized to GAPDH. n=3. Statistical analysis utilized Two-Way ANOVA with Bonferroni posttest. n.s. indicates not significant.

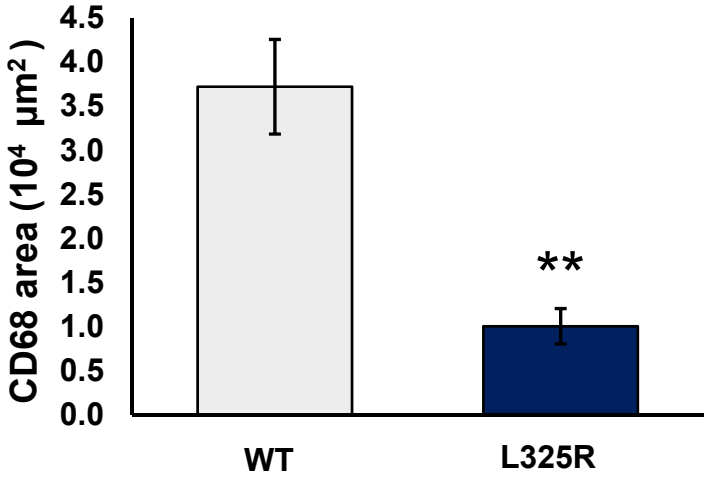


Supplemental Figure VII. Lack of inflammation in the unligated right carotids. Seven days after partial carotid ligation of the left carotid, inflammation in the carotid arteries was assessed by immunocytochemistry for NF-κB (red), VCAM-1 (red), and Mac2 (green). Right carotid controls show very limited inflammatory markers in this model. Scale bars = 100 μm. Representative images are shown. n=5-6.

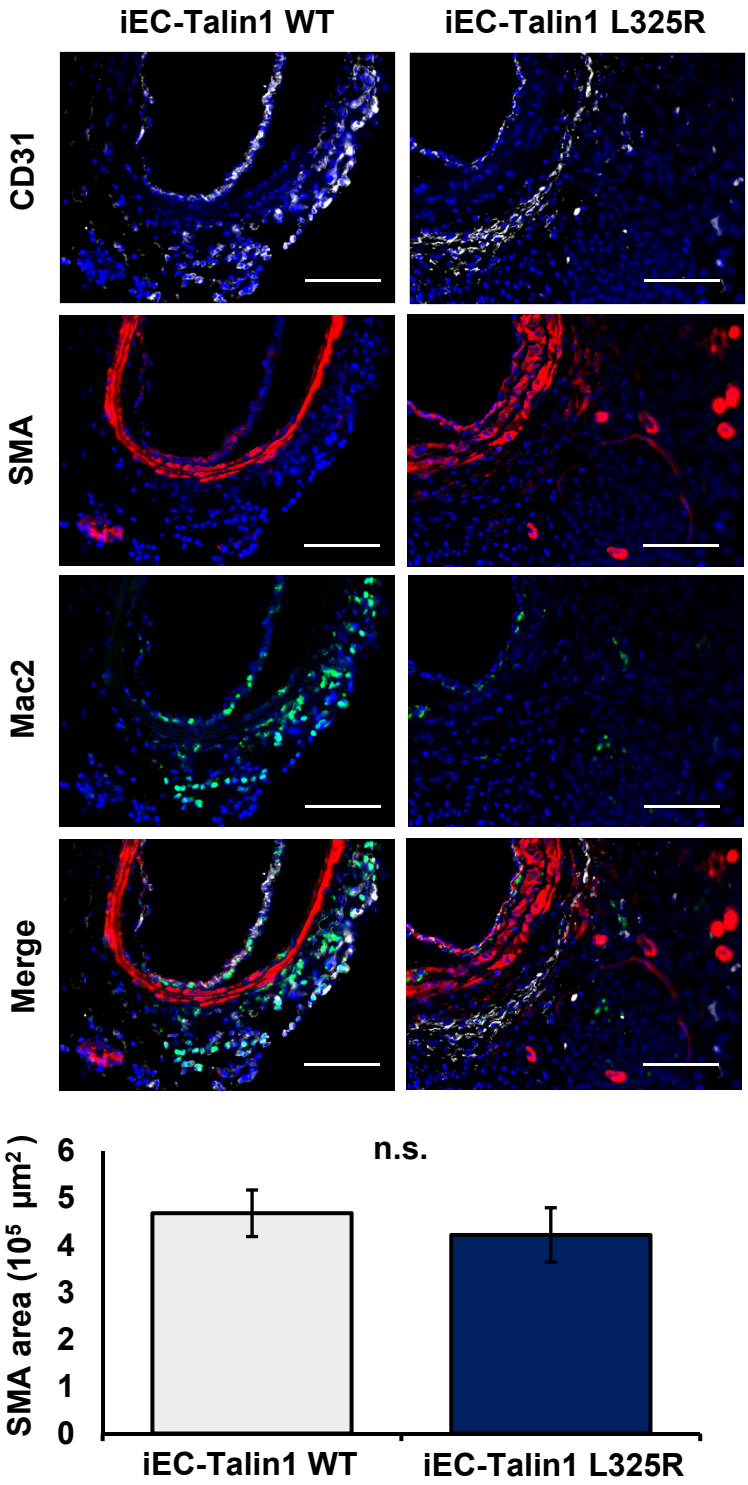


Supplemental Figure VIII. Individual channels for NF-κB localization. Seven days after partial carotid ligation in the left carotid, NF-κB (red) activation was assessed by nuclear localization, visualized by colocalization with the nuclear stain DAPI (pseudocolored white). Representative images are shown. n=5-6.





Supplemental Figure IX. Reduced Macrophage Accumulation in Mice Harboring Talin1 L325R in Endothelium. Seven days after partial carotid ligation in the left carotid, immunohistochemistry was performed for CD68 (red) to visualize myeloid cells in this tissue. Representative images are shown. n=5-6. Values are means ±SE. **p<0.01 compared with iEC-Talin1 WT. Scale bars = 100 μm. Student's t-test was used for statistical analysis.



Supplemental Figure X. Impaired Talin1-Dependent Integrin Activation Does Not Affect Endothelial Coverage or Smooth Muscle Cell Content. Seven days after partial carotid ligation, the left carotid was examined by immunohistochemistry for endothelial cells (CD31, white), smooth muscle cells (SMA, red), macrophages (Mac2, green), and counterstained with DAPI (blue) to show nuclei. Representative images are shown. n=5-6. Values are means ±SE. n.s. indicates not significant. Scale bars = 100 μm. Student t test was used for statistical analysis.