Occludin phosphorylation and ubiquitination regulate tight junction trafficking and vascular endothelial growth factor (VEGF)-induced permeability

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. S490AOcc mutant inhibits TJ trafficking.
Immunocytochemistry demonstrated that VEGF disrupted continuous TJ staining in WtOcc transfected cells, whereas transfection of the S490AOcc mutant prevented the VEGF induced TJ disruption. These changes were quantified and presented in supplemental figure S3. EV; empty vector, Wt; WtOcc, S490A; S490AOcc. Scale bar = 10 μm.

Figure S2. Occludin-ubiquitin chimera induces TJ trafficking.
BRECs were transfected with these chimeric mutants for immunocytochemistry. Both Occ-Ub and S490A-Ub presented intracellular puncta, and cell border staining of TJ proteins was fragmented in Occ-Ub or S490A-Ub transfected cells. EV; empty vector, Wt; WtOcc. Scale bar = 10 μm.

Figure S3. S490A inhibits VEGF-induced TJ disruption, and Occ-Ub or S490A-Ub increases it.
(A-C) Immunostaining of BRECs transfected with each occludin mutant were performed after VEGF treatment (50 ng/ml, 15min). Cell border staining of occludin (A), claudin-5 (B) and ZO-1 (C) was quantified. Wt; WtOcc, S490A; S490AOcc.

Figure S1

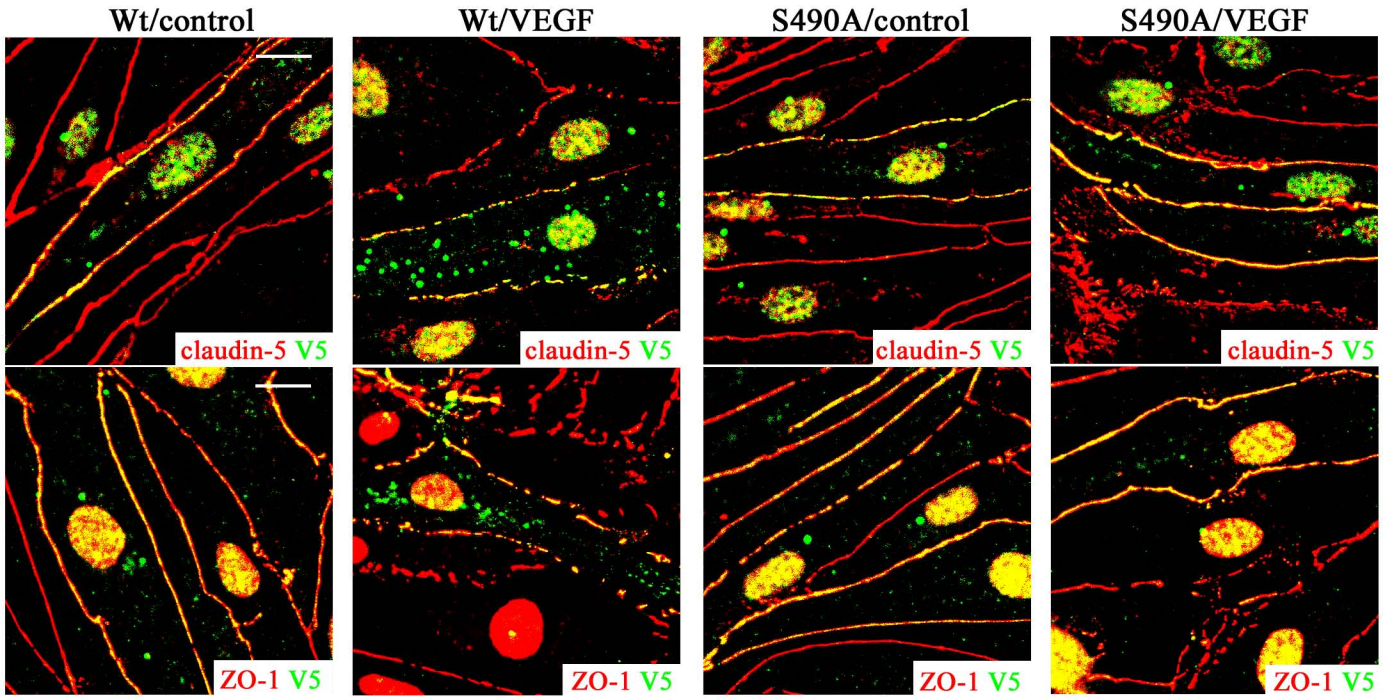

Wt/control
Wt/VEGF
S490A/control
S490A/VEGF
claudin-5 V5
claudin-5 V5
claudin-5 V5
claudin-5 V5
ZO-1 V5
ZO-1 V5
ZO-1 V5
ZO-1 V5

Figure S2

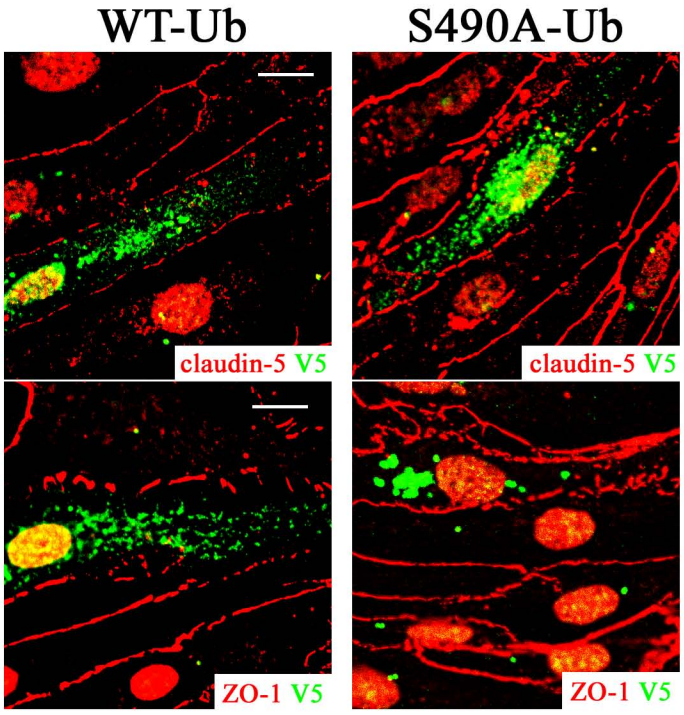
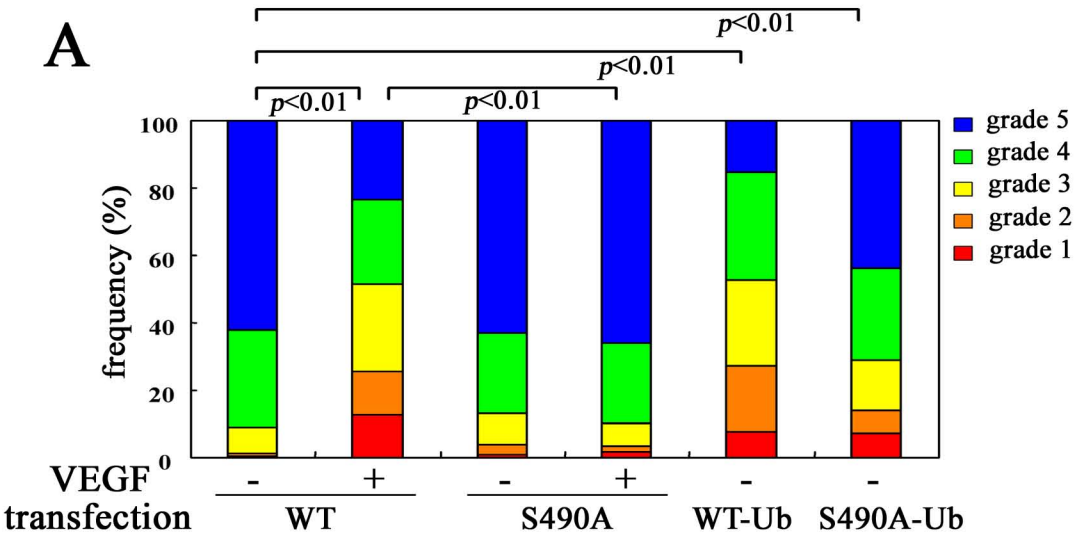


Figure S3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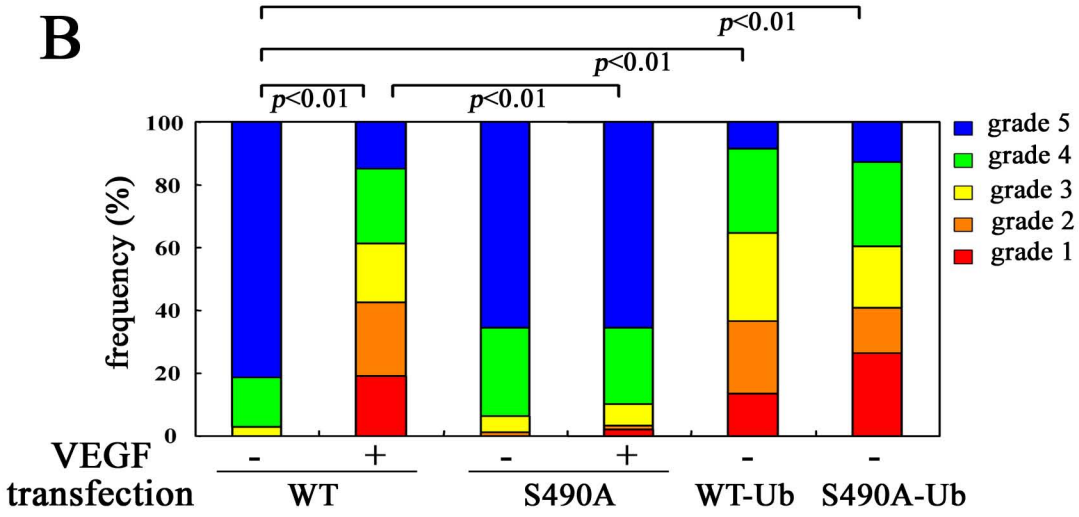
p<0.01
p<0.01
p<0.01
frequency (%)
100
80
60
40
20
0
grade 5
grade 4
grade 3
grade 2
grade 1
VEGF transfection
-
+
-
+
-
-
WT
S490A
WT-Ub
S490A-Ub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p<0.01
p<0.01
p<0.01
frequency (%)
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80
60
40
20
0
grade 5
grade 4
grade 3
grade 2
grade 1
VEGF transfection
-
+
-
+
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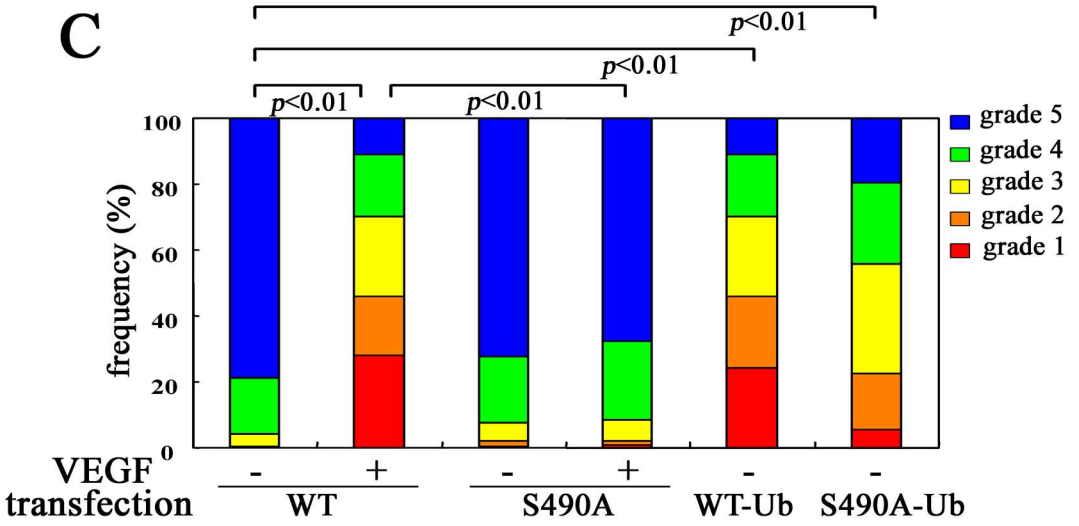
-
WT
S490A
WT-Ub
S490A-Ub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p<0.01
p<0.01
frequency (%)
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80
60
40
20
0
grade 5
grade 4
grade 3
grade 2
grade 1
VEGF transfection
-
+
-
+
-
-
WT
S490A
WT-Ub
S490A-Ub