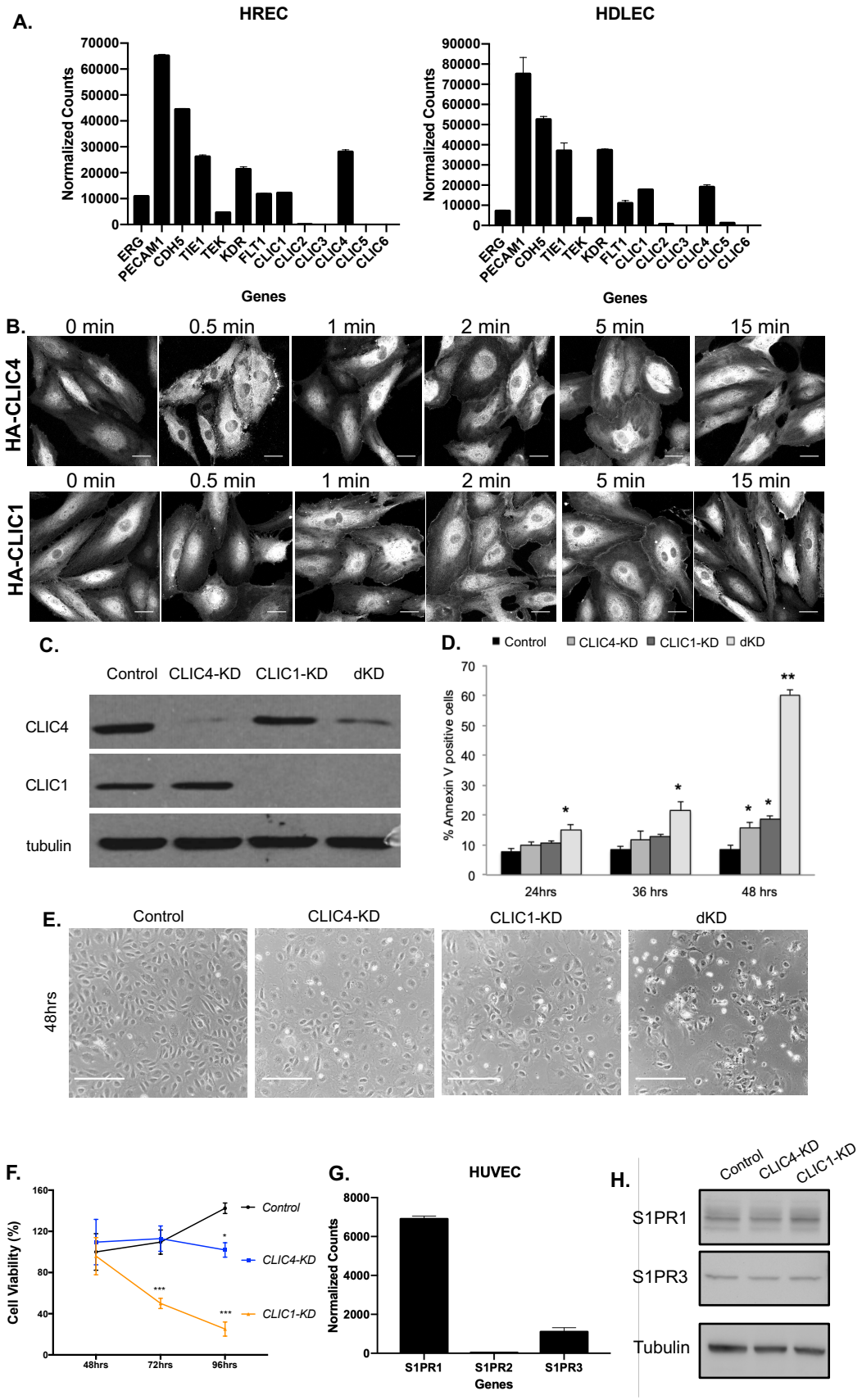

Figure S1. CLIC1 and CLIC4 promote endothelial cell survival and localize to the plasma membrane in response to S1P. (A) RNA sequencing for *CLIC1* and *CLIC4* expression in human retinal endothelial cells (HRECs) and human dermal lymphatic endothelial cells (HDLECs). **(B)** HUVECs were infected with HA-CLIC4 or HA-CLIC1, and immunofluorescent staining for the HA tag was performed at the indicated timepoints after addition of 1 μM S1P. Confocal microscopy images are representative of three independent experiments. Scale

bar, 30 μm**. (C to F)** HUVECs were lentivirally infected with CLIC1 shRNA or CLIC4 shRNA, confirmed 48 hours later by Western blot (C). In (D), cells were assessed for apoptosis by annexin V staining. Results are presented as percent positive at 24, 36 and 48 hours after infection. Data are from n=3 experiments. **P*<0.05 and ***P*<0.01 by student's t-test. Representative light microscopy images of the cell lines (representative of n=5) are shown at 48 hours after infection (E). Scale bar, 250μm. In (F), cell viability was assessed by MTT assay at 48, 72, and 96 hours after infection. Results are presented as percentage of absorbance (570nm) normalized to that in control cells at 48 hours. Data are from n=3 experiments. **P*<0.05, ***P*<0.01, and ****P*<0.001 by ANOVA, compared to controls at the corresponding time point. **(G and H)** Expression of S1PRs in HUVECs at the mRNA level by RNA sequencing (G) and after loss of CLIC1 or CLIC4 at the protein level by Western blotting (H). Data are mean ± SEM from (G) or representative of (H) n=3 experiments.

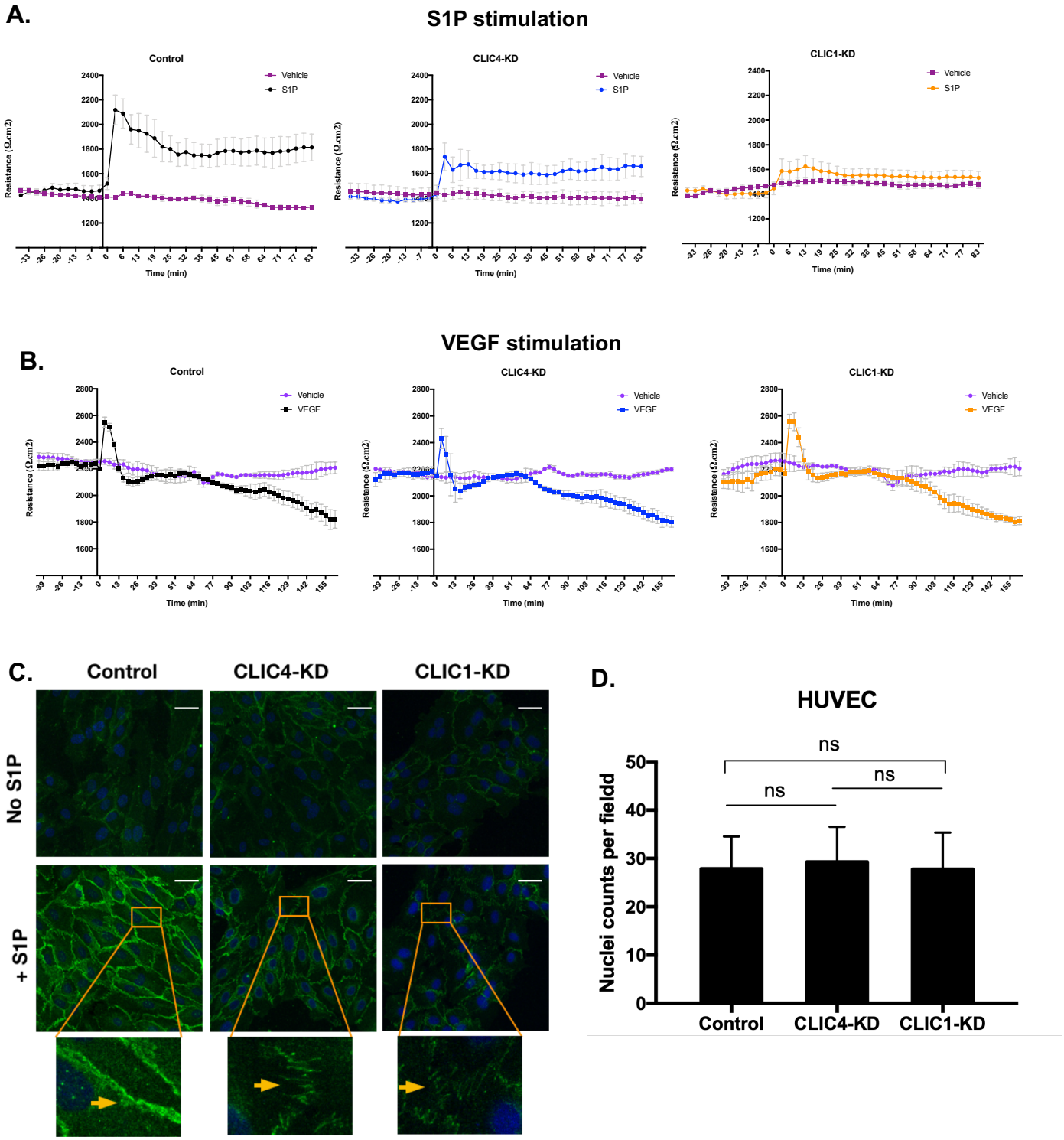

Figure S2. CLICs regulate S1P-driven barrier integrity and VE-cadherin junction formation. (A and B) HUVECs – control or knocked down for CLIC1 or CLIC4 – were serum starved for 2 hours then stimulated with vehicle control (BSA), 1 μM S1P (A), or 100 ng/mL VEGF (B) at time 0. Trans-endothelial electrical resistance (TEER) was monitored for 2 hours after treatment. Resistance is the basal resistance reading, and graphs are representative of four independent experiments. **(C and D)** HUVECs described in (A) were serum starved for 3 hours, then stimulated with 1 μM S1P for 30 min, then fixed and stained for VE-cadherin and DAPI. Arrows in the representative confocal microscopy images (C) mark the abnormal endothelial junctions in CLIC-KD cells compared to control cells. DAPI-positive nuclei counts were quantified from n=3 experiments (D). $^{ns}P>0.05$ (not significant) by ANOVA with Bonferroni correction for multiple comparisons test.

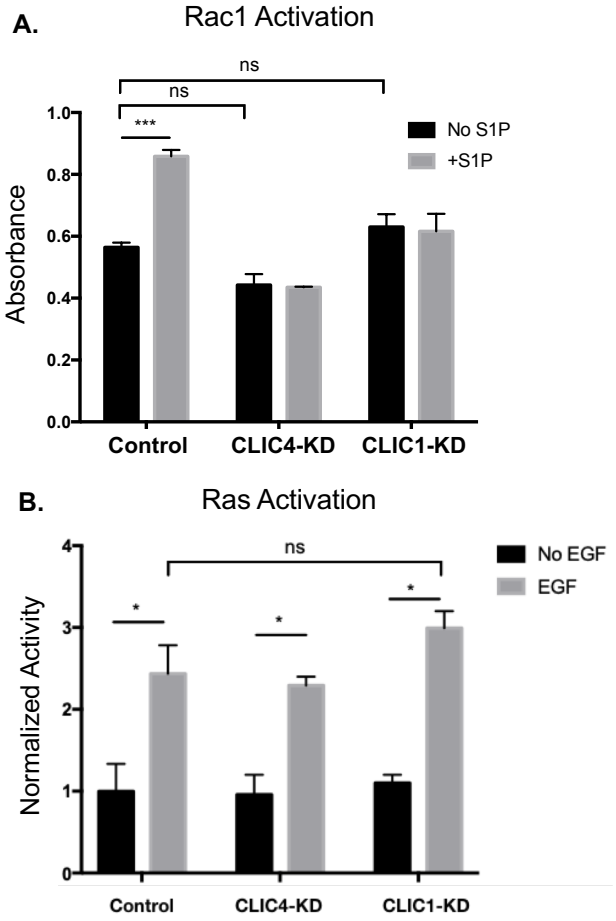

Figure S3. Loss of CLICs did not alter basal Rac1 activity or EGF-induced Ras activation. (A) HUVECs – control or knocked down for CLIC1 or CLIC4 – were serum starved for 3 hours then stimulated with 1 μM S1P for 5 min. Rac1 activity was then measured in lysates by G-LISA assay with absorbance at 490nm. **(B)** HUVECs described in (A) were serum starved for 3 hours then treated with 100 ng/mL EGF for 10 min. Ras activity was measured in lysates by G-LISA assay. Results were normalized to those in unstimulated cells. In both panels, data are means ± SEM from n=3 experiments; **P*<0.05 and ****P*<0.001 by ANOVA analysis with Bonferroni correction for multiple comparisons test.

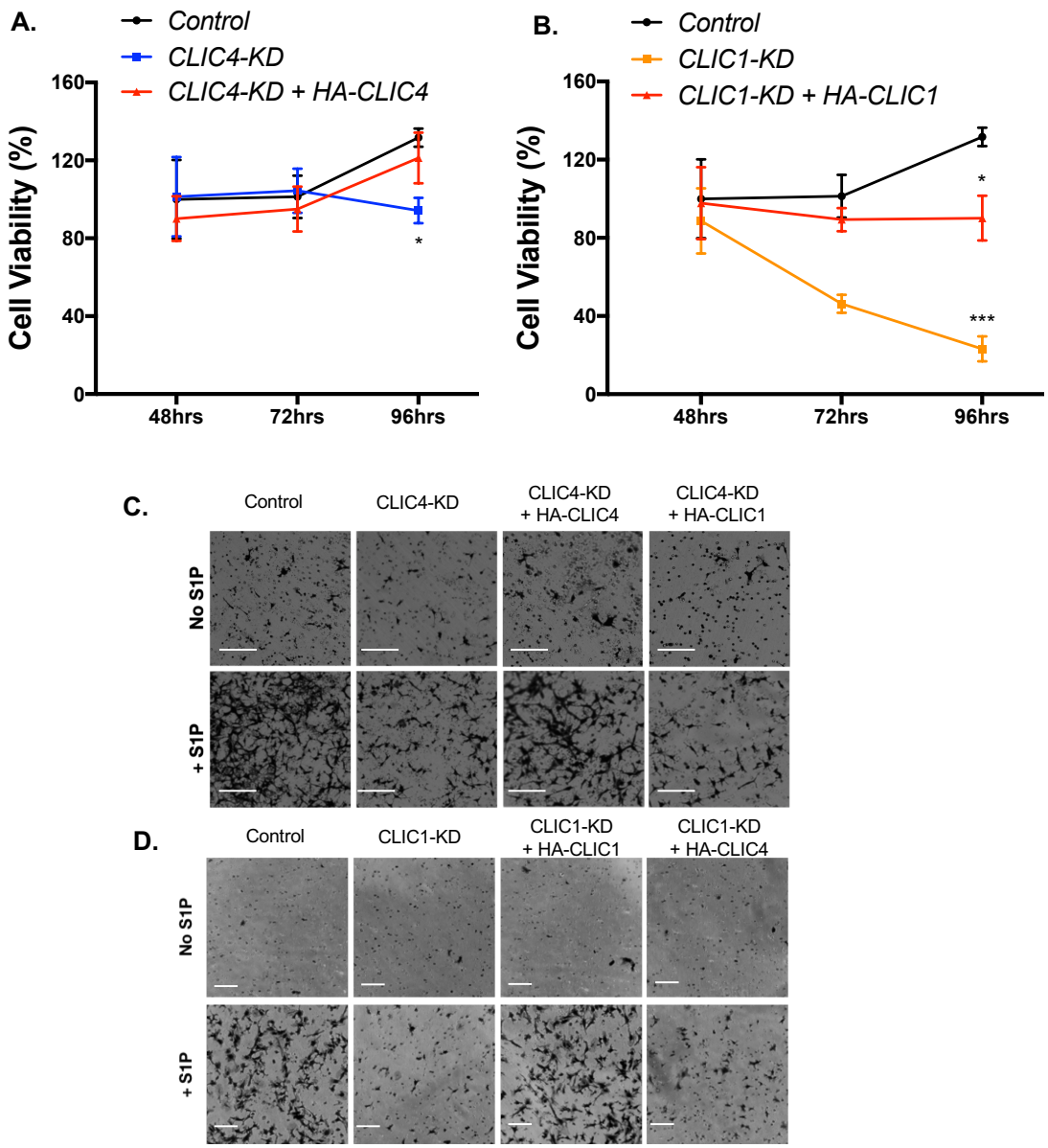

Figure S4. Ectopic expression of CLIC1 and CLIC4 rescues endothelial cell viability and S1P-mediated migration. (A and B) Viability of HUVECs – control or knocked down and rescued for CLIC1 or CLIC4 as indicated – assessed by MTT assay at 48, 72, and 96 hours after shRNA/vector infection. Data are means ± SEM from n=3 experiments. **P*<0.05 and ****P*<0.001 by ANOVA with Bonferroni correction for multiple comparisons test, compared to control at each time point. **(C and D)** Boyden chamber assay assessing the migration of HUVECs – control or knocked down and rescued for CLIC1 or CLIC4 as indicated – towards 1 μM S1P over 6 hours after prior serum starvation for 3 hours. Images of migrated, crystal violet-stained cells are representative of three independent experiments. Scale bars, 250μm.