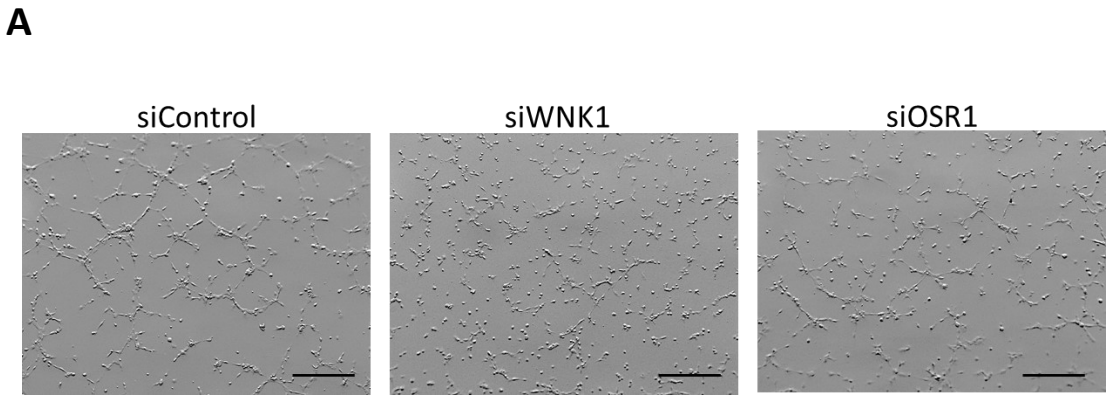
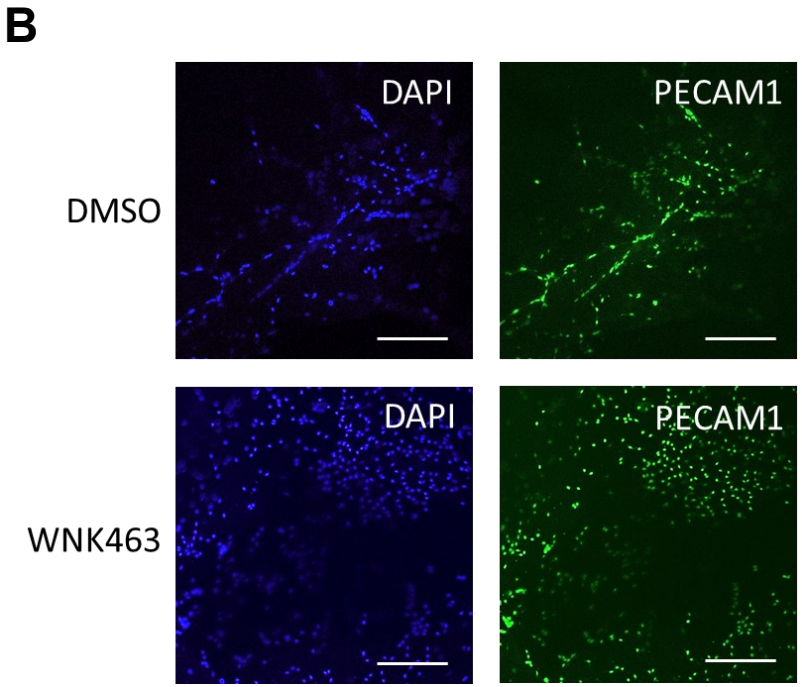
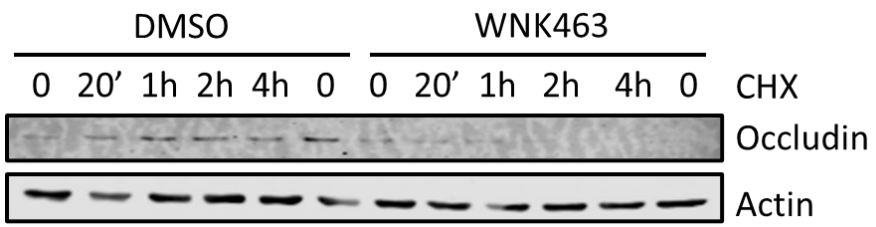
Supplementary Figure 1

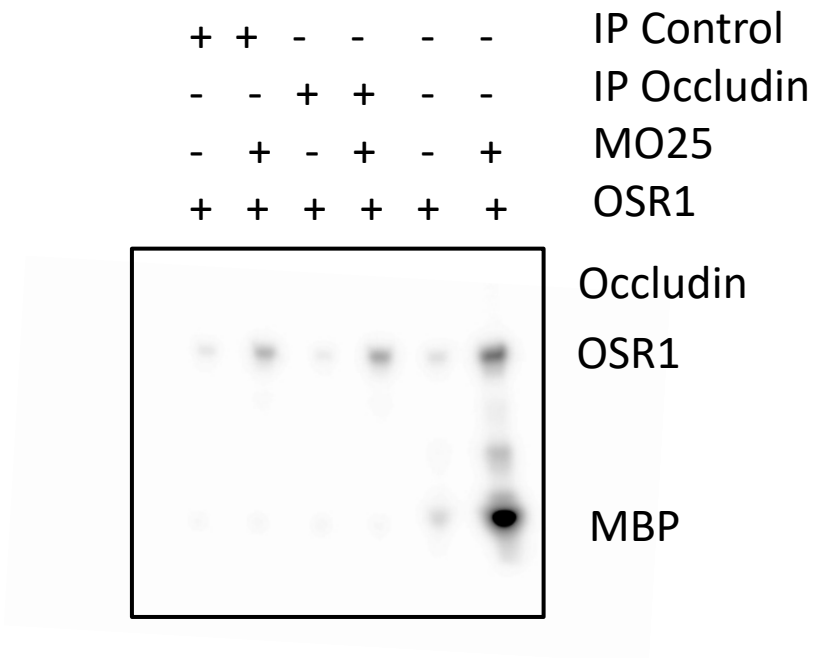




Supplementary Figure 2

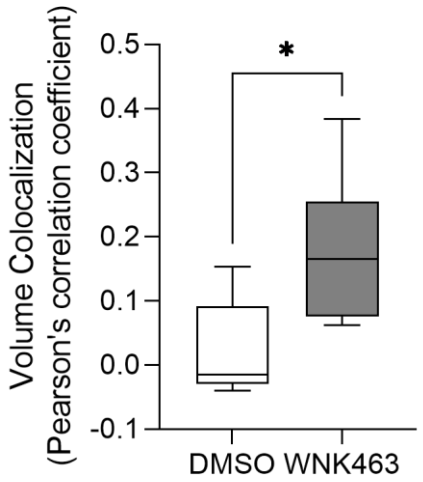


Supplementary Figure 3



Supplementary Figure 4

A



Supplementary Figure 5

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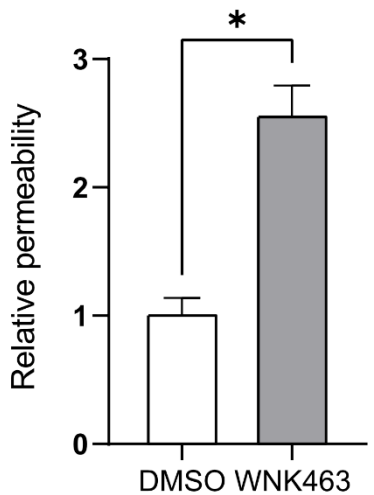
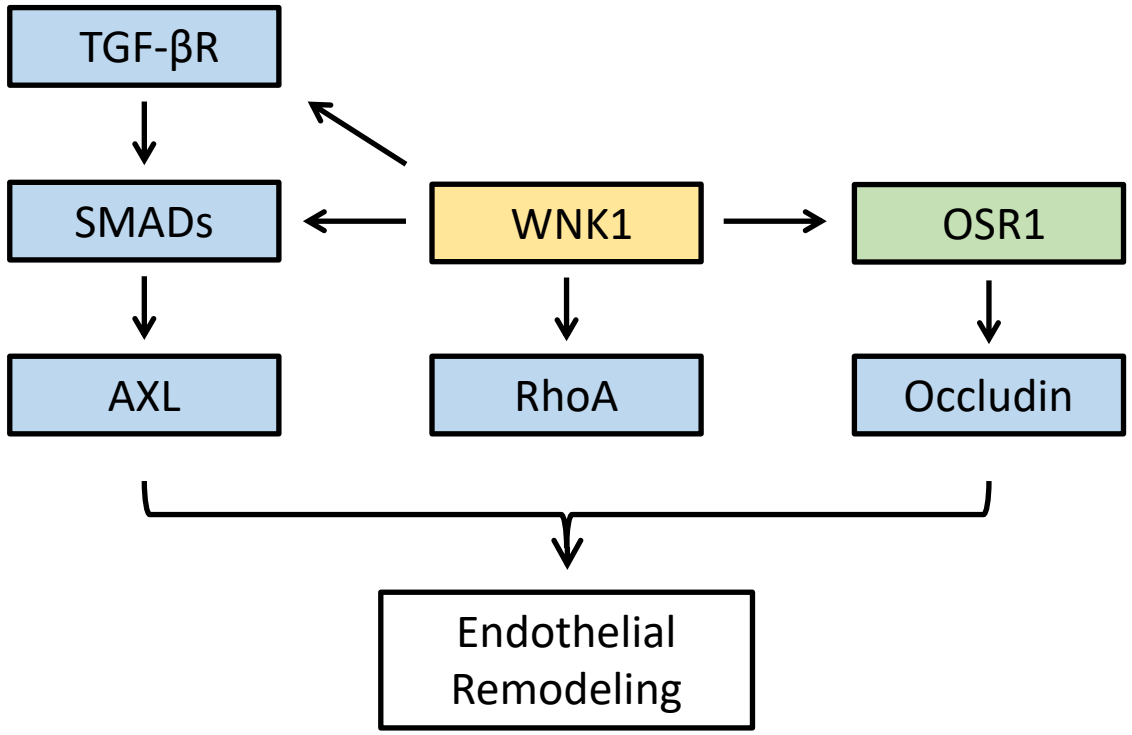


Supplementary Figure 6

A



Legends for Movies S1 and S2

Supplemental Movie S1 and S2. Real-time imaging of cord formation in primary HUVECs. Real-time imaging of cord formation showed that primary HUVEC cells treated with WNK463 (Movie S2) compared to DMSO (Movie S1) exhibited defects in cell migration; n=3

Legends for Datasets S1 to S6

Figure S1. Sprouting angiogenesis is decreased with WNK463 treatment. **A**) Representative bright-field images of cord formation in HUVECs treated with siWNK1 or siOSR1 show reduced cord formation compared to siControl; Scale=1 mm. **B**) The endothelial marker PECAM staining of ex-vivo mouse aortic ring slice culture after 6 days of WNK463 treatment show decreased sprouting angiogenesis and cord formation compared to DMSO control; Scale = 1 mm, n=3.

Figure S2. Regulation of tight junction occludin by WNK1/OSR1 Western blot show occludin protein expression upon 4 hour-time course treatment of cycloheximide (CHX: 100 µg/ml) in HUVECs pre-treated with DMSO control or WNK463 (1µM) for 24 hours.

Figure S3. OSR1 does not phosphorylate occludin *in vitro*. Autoradiograph show *in vitro* kinase assay performed with flag-occludin immunoprecipitated from HEK cells, 9 µg GST-OSR1 and 0.83 mg/ml MBP as substrate in the presence or absence of 4µM GST-MO25 (master regulator of OSR1).

Figure S4. Co-localization of OSR1 and occludin increases upon WNK1 inhibition. A) Imunofluorescence staining of OSR1 and occludin in HUVECs treated with DMSO control or WNK463 (1 µM) and pre-treated with 0.5M NaCl for 5 min show colocalization between OSR1 and occludin measured as Pearson's correlation coefficient; n=6.

Figure S5: **Inhibition of WNK1 decreases junctional maturity. A**) Relative endothelial permeability measured by Transwell permeability assay in HUVECs treated with DMSO or WNK463 (1 µM); n=4. * $p<0.05$ by Student's 2-tailed *t*- test.

Figure S6: **A**) Schematic representation of cooperation between WNK1 and TGF-β signaling components