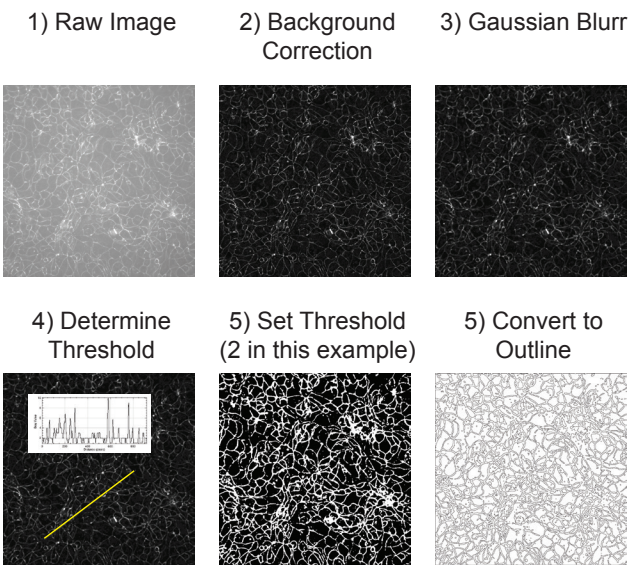

Figure S1: Image pipeline for tight junction quantification

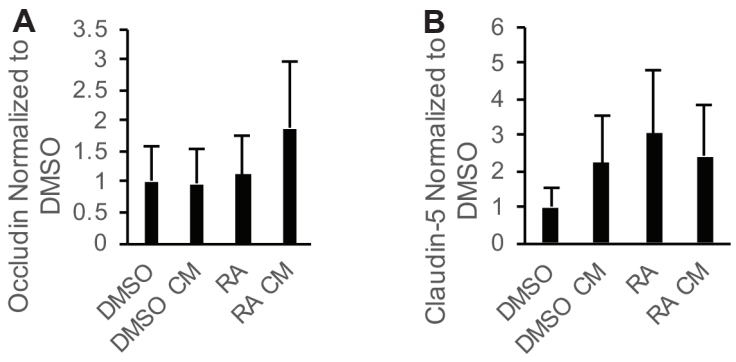

Figure S2: BMEC progenitor purification supplementary information. A) Densitometry quantification of occludin band intensities from three independent differentiations from Western blots of D8 BMEC progenitors exposed to the indicated treatments from D6-D8. ANOVA test failed. B) Densitometry quantification of Claudin-5 band intensities from three independent differentiations from Western blots of D8 BMEC progenitors exposed to the indicated treatments from D6-D8. ANOVA test failed.

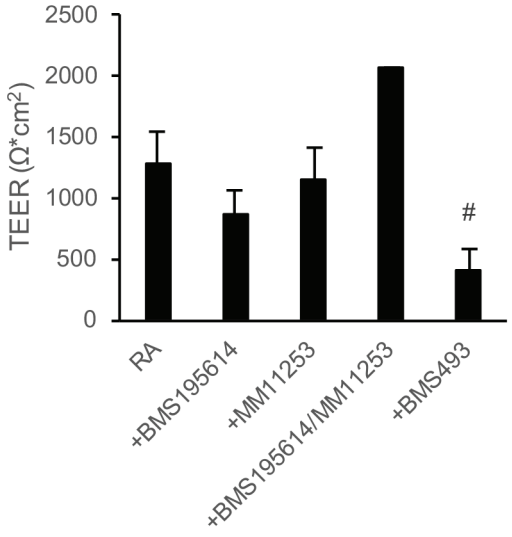

Figure S3: D10 TEER following small molecule antagonism of RARα/γ receptors from D6-D8 of the differentiation. iPSC-derived BMECs were treated at D6 for one hour with EC medium + 5 μM BMS195614 (RARα antagonist; n = 3), 5 μM MM11253 (RARγ antagonist; n = 3), combined 5 μM BMS195614/5 μM MM11253 (n = 2), or 5 μM BMS493 (pan-RAR antagonist; n=3). BMECs were subsequently treated with 10 μM RA +/- the indicated small molecule antagonists from D6-D8 of the differentiation. BMECs were subcultured onto Collagen/Fibronectin-coated filters in the absence of RA. Error bars represent standard error of the mean. ANOVA followed by Dunnett's test; # p = 0.057 vs. RA.

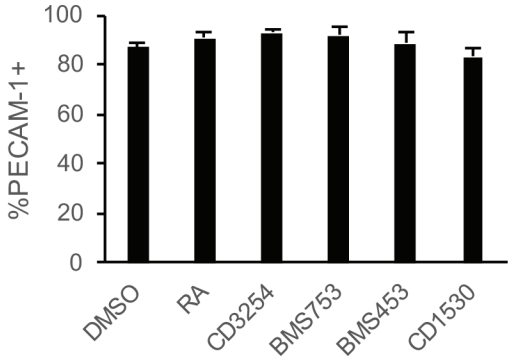

Figure S4: RA-associated nuclear hormone receptor agonists affect on PECAM-1+ cell distribution. Cells were treated D6-D8 with DMSO, 10 μM RA, 10 μM CD3254, 10 μM BMS753, 10 μM BMS453, or 10 μM CD1530 and PECAM-1+ distribution assayed at D8 by flow cytometry. Values represent averages of three technical replicates (N = 3) in single differentiation. Error bars represent standard deviation. ANOVA test failed.

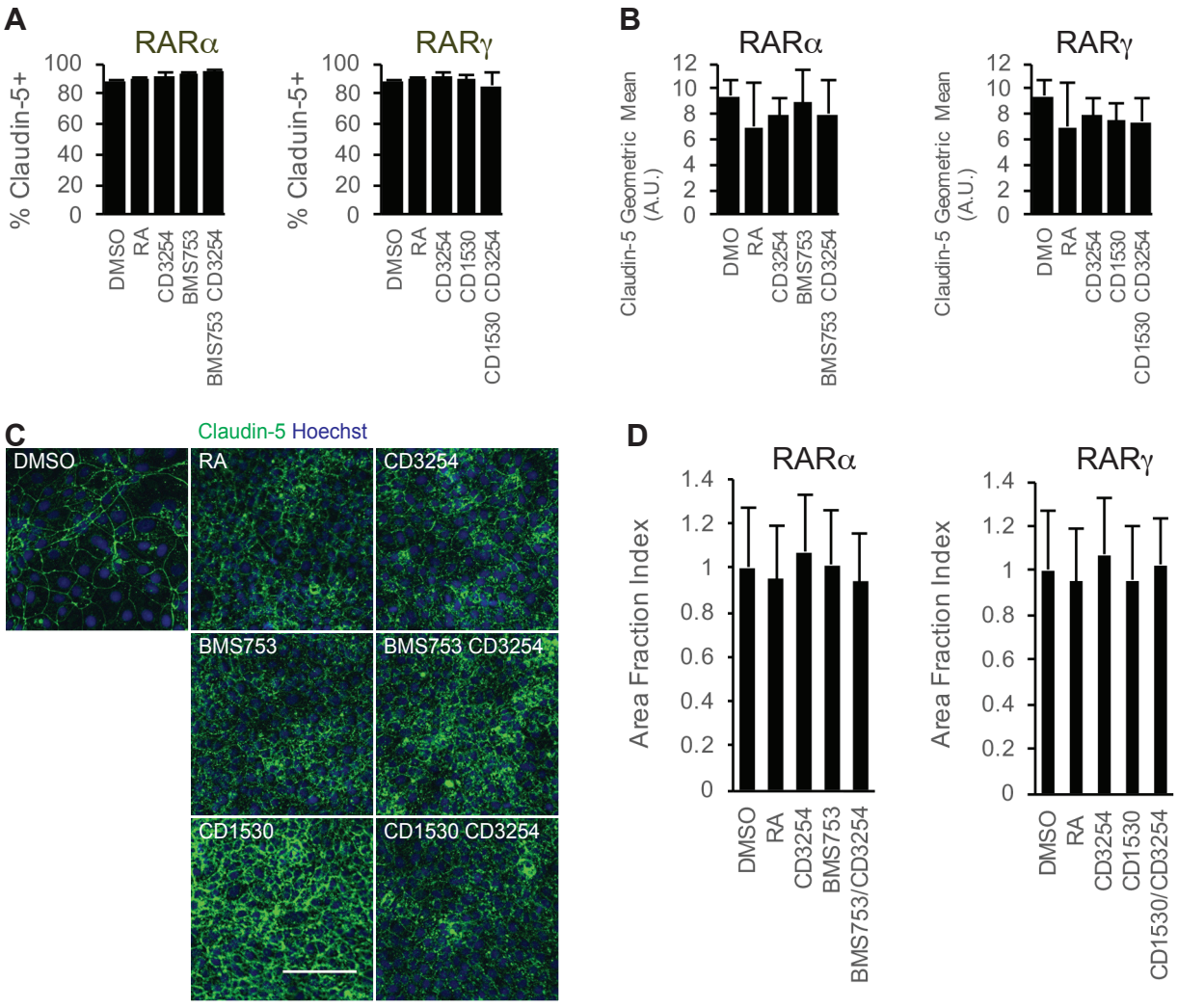

Figure S5: Changes in tight junction proteins following RAR/RXRα coactivation. A) Percentage of Claudin-5+ cells as assessed by flow cytometry in D10 iPSC-derived BMECs following D6-D9 treatment with the indicated RA-signaling associated nuclear hormone receptor agonists; n = 3. Error bars represent standard error mean. ANOVA failed to identify statistically significant differences between the groups. Left: RARα agonist combinations. Right: RARγ agonist combinations. B) Geometric mean of Claudin-5 fluorescence levels in Claudin-5+ cells as measured by flow cytometry in D10 iPSC-derived BMECs following D6-D9 treatment with the indicated RA-signaling associated nuclear hormone receptor agonists; n = 3. Error bars represent standard error of the mean. ANOVA test failed. Left: RARα agonist combinations. Right: RARγ agonist combinations. C) Representative Claudin-5 immunocytochemistry images for iPSC-derived BMECs at D10 following D6-D9 treatment with the indicated RA-signaling associated nuclear hormone receptor agonists; n = 3. Claudin-5 is indicated in green and Hoechst counterstain in blue. Scale bar represents 100 μm. D) Area fraction index quantification of Claudin-5 immunofluorescent images in panel C. Error bars represent standard deviation. N = 11 technical replicates averaged over three independent

differentiations. ANOVA test failed. Left: RARα agonist combinations. Right: RARγ agonist combinations.

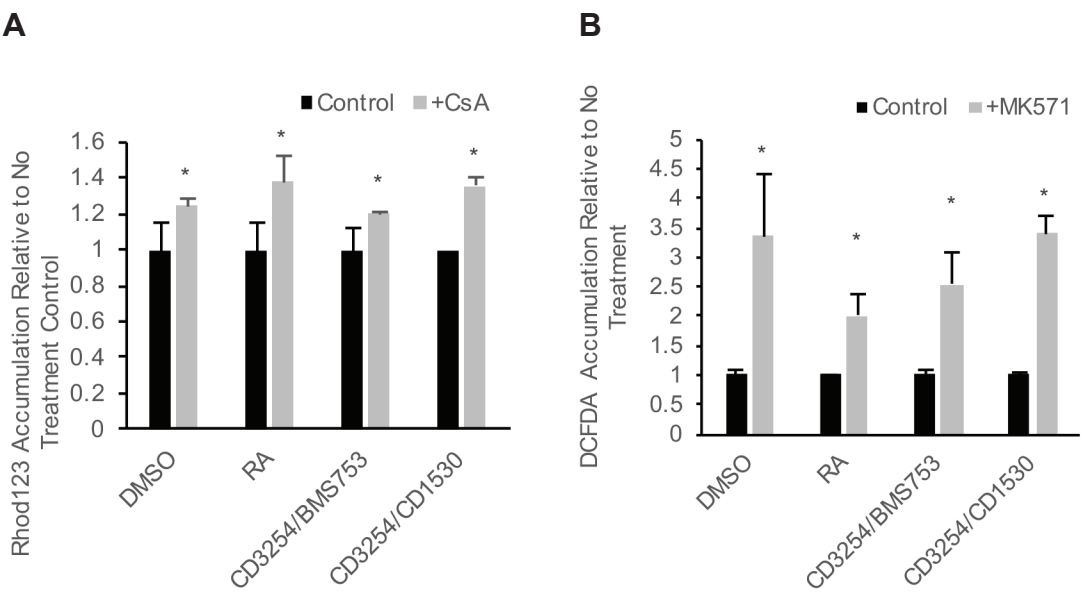

Figure S6: Effect of RAR/RXRα coactivation on P-glycoprotein and MRPefflux transporter activity. A) Representative results for accumulation of the P-glycoprotein substrate Rhodamine 123 in iPSC-derived BMECs in the presence and absence of the P-glycoprotein inhibitor CsA over three independent differentiations following D6-D9 treatment with RA, CD3254/BMS753, or CD3254/CD1530. Error bars represent standard deviation of three technical replicates (N = 3) from one representative differentiation. ANOVA comparison of the percentage increase in Rhodamine 123 accumulation following CsA inhibition between control and RA associated small molecules failed. B) Representative results for accumulation of the MRP substrate DCFDA in iPSC-derived BMECs in the presence and absence of the MRP inhibitor MK571 over three independent differentiations following D6-D9 treatment with RA, CD3254/BMS753, or CD3254/CD1530. Error bars represent standard deviation of three technical replicates (N = 3) from one representative differentiation. ANOVA comparison of percentage increase in DCFDA accumulation following MK571 inhibition between control and RA associated small molecules failed. * $p < 0.05$ vs. no inhibitor treatment, Student t-test.