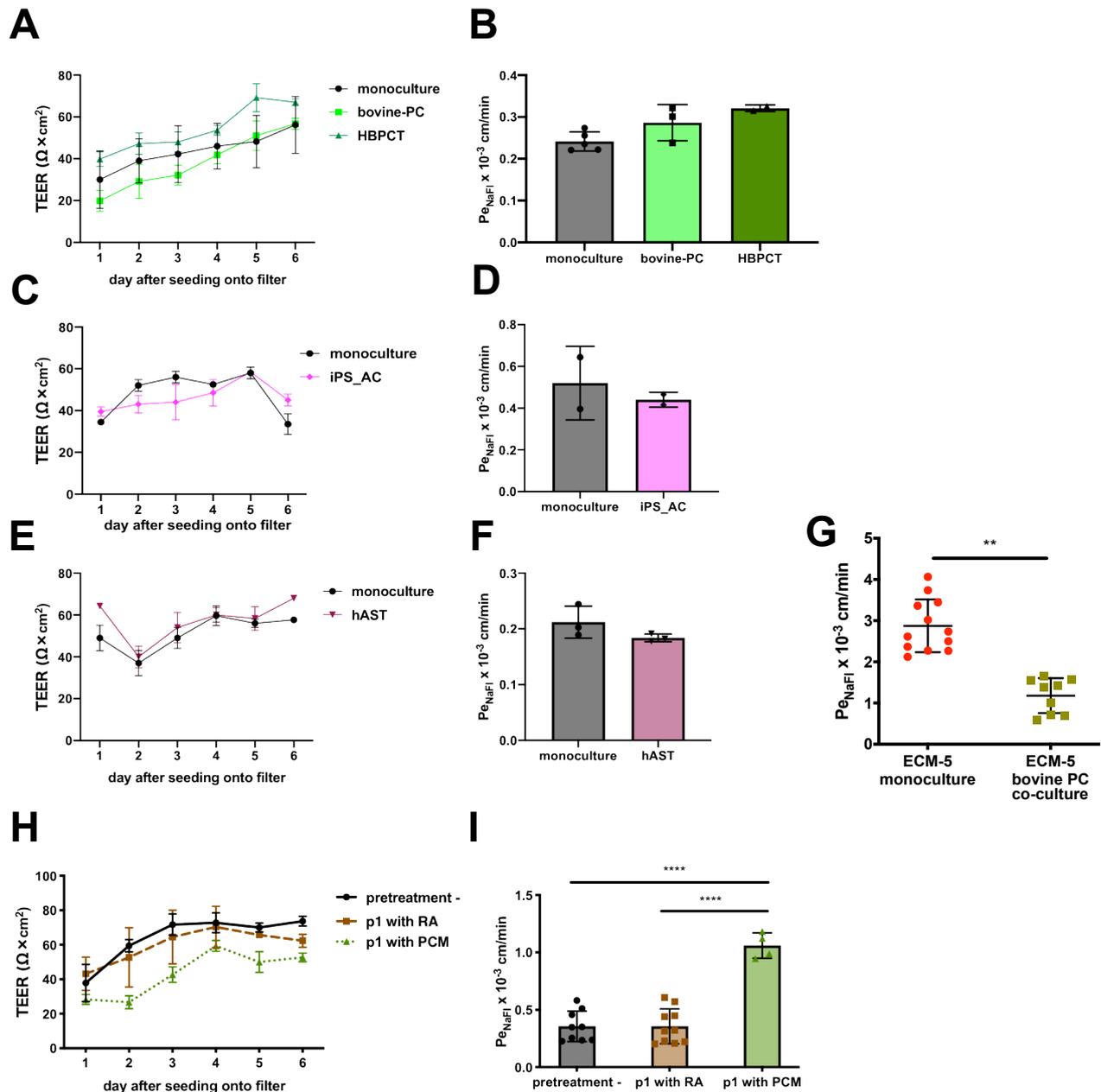# Supplementary Figure 4

Supplementary Figure 4. The effect of astrocyte or pericyte co-culture and ECM-5 medium on barrier characteristics of EECM-BMEC-like cell monolayers from additional hiPSC clones and the effect of RA pretreatment or pericyte conditioned medium on EPCs.

TEER (A, C, E) and permeability to sodium fluorescein (B, D, F, G) of EECM-BMEC-like cell monolayers is shown. EECM-BMEC-like cells were seeded onto 0.4 µm pore size Transwell filters and co-cultured for 6 days with bovine pericytes (bovine PC; A and B, light green), a human brain pericyte cell line (HBPCT) (A and B, dark green), a human astrocyte cell line (hAST) (C and D, dark red), hiPSC-derived astrocytes (iPS_AC) (E and F, pink). Monoculture condition was used as a control (black). (A, C, E) Plotted data are mean TEER values ± SD. (B, D, F) Bars show the mean permeability coefficients (Pe) ± SD. (A-E) Representative data from donor 1 are shown. At least 2 independent differentiations performed on 2 distinct filters were used in this study. (G) Permeability of sodium fluorescein of EECM-BMEC-like cell monolayers cultivated in ECM-5 medium in the presence and absence of bovine pericytes. EECM-BMEC-like cells from donor 2 (passage 3-5) were seeded onto 0.4 µm pore size Transwell filters and co-cultured for 6 days with bovine pericytes. Monoculture condition using ECM-5 medium was used as a control (black). Bars show the mean permeability coefficients (Pe) ± SD. Data are overlaid from at least 3 independent differentiations each performed in triplicate per conditions. Statistical analysis: unpaired t-test ($P < 0.01$ = **) (H) TEER and (I) permeability of sodium fluorescein of EECM-BMEC-like cells from donor 2 at passage 2 are shown. EPCs (after EPC purification by MACS) were cultured in the presence or absence of RA for 3 days (brown) or bovine pericyte conditioned medium (PCM) for 6 days (green) and then EECM-BMEC-like cells at passage 2 were cultured on 0.4 µm pore size Transwell filters over 6 days. Data are from 3 independent differentiations for RA treatment each performed in at least triplicate, and from 2 independent differentiations for PCM condition each performed in duplicate. Statistical analysis: one-way ANOVA followed by Tukey's multiple comparison test. ($p < 0.0001$ = ****).