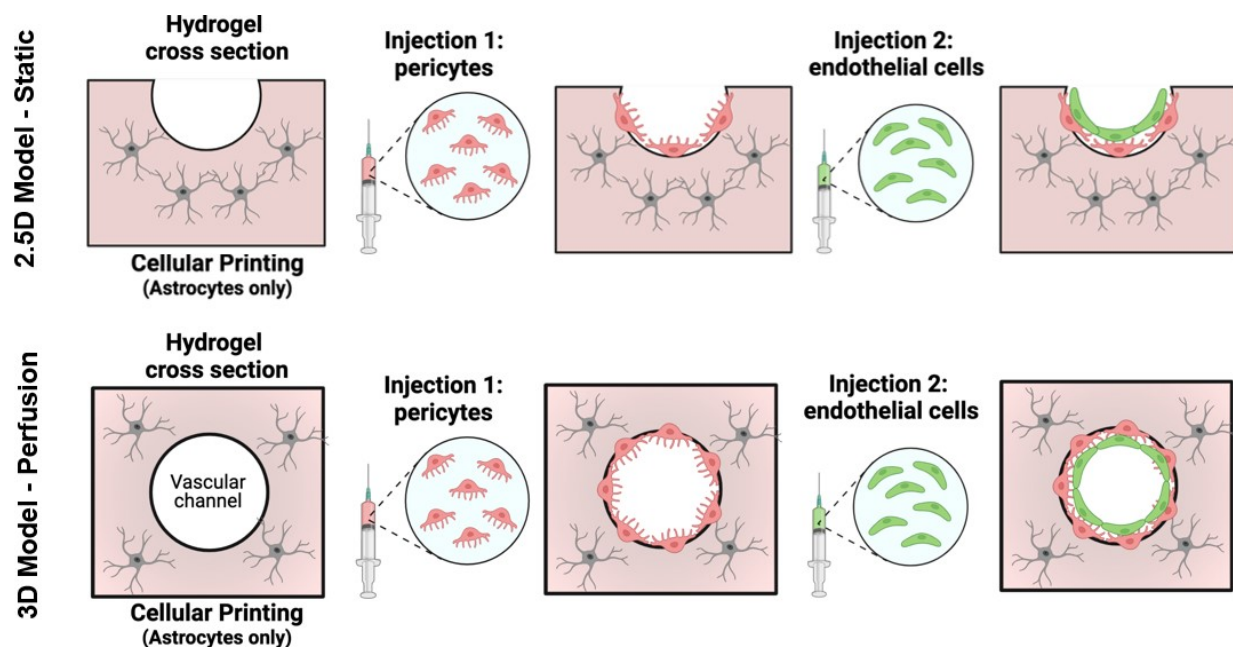


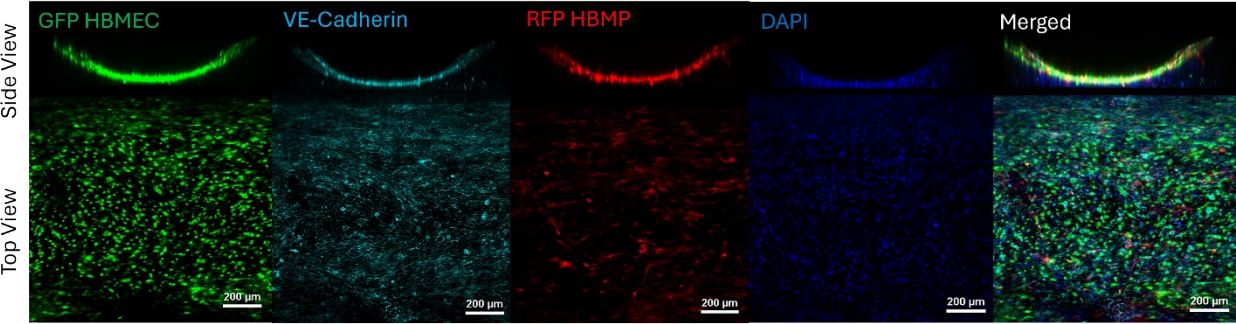
## Supplementary Figures



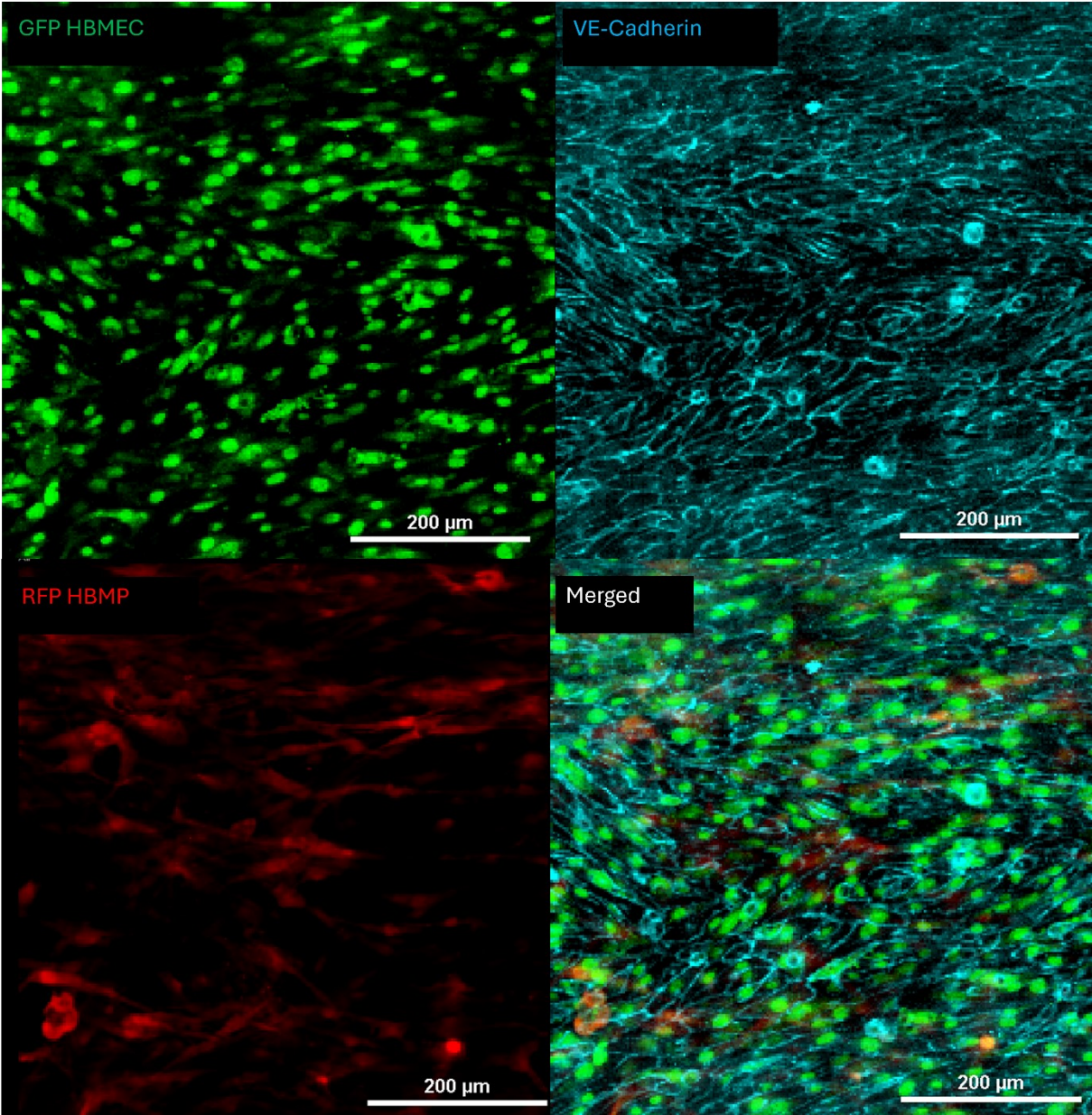
**Supplementary Figure 1. Experimental overview of 2.5D (static) and 3D (perfusion) models.**

Astrocytes (HBAs) are printed in the bulk of 2.5D and 3D hydrogels, followed by sequential seeding of pericytes (HBMPs) and endothelial cells (HBMECs).

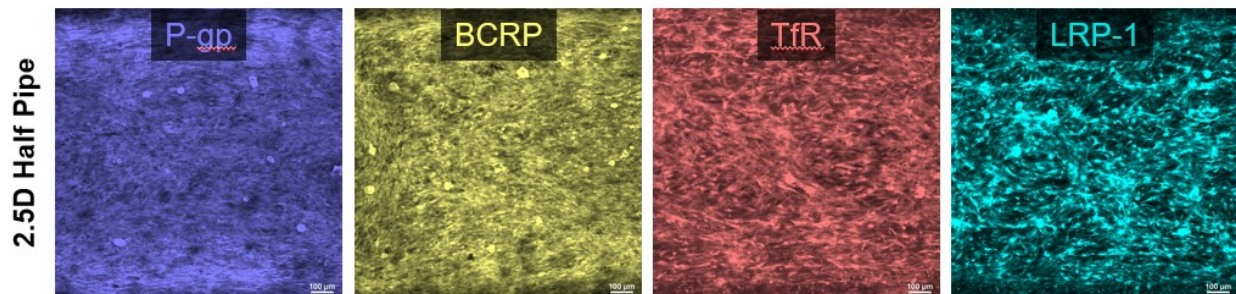
a. Visualization of tri-culture sequential seeding of RFP HBMP and GFP HBMEC with endothelial barrier formation



b. Magnified tri-culture 2.5D model "top view"



**Supplemental Figure 2. Sequential seeding of HBMP followed by seeding of HBMECs for tri-culture 2.5D half-pipe model.** RFP HBMPs were first seeded into open channels of 3D serpentine hydrogels for three days. GFP HBMECs are seeded after three days up to day 6, scale bar = 200  $\mu\text{m}$ . (a) The side view (top) and top view (bottom) of the 2.5D half-pipe model demonstrates consistent layers of GFP HBMEC, VE-Cadherin (cell-cell endothelial junctions), RFP HBMP, and DAPI (nuclei) throughout half-pipe model. (b) Magnified image shows cell-cell tight endothelial cell junctions (blue) positioned above the HBMP (red) layer.



**Figure 3. Presence of BBB transport proteins in 2.5D static culture model.** Transporters such as P-gp, BCRP, TfR, and LRP-1 can be found in tri-culture conditions within a 2.5D static culture model, scale bar = 100  $\mu\text{m}$ .