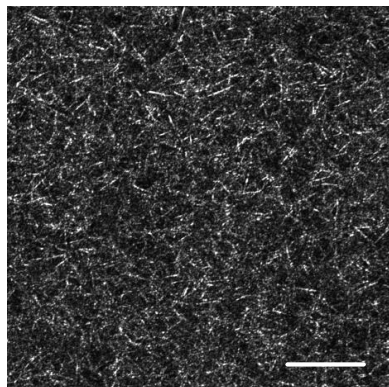


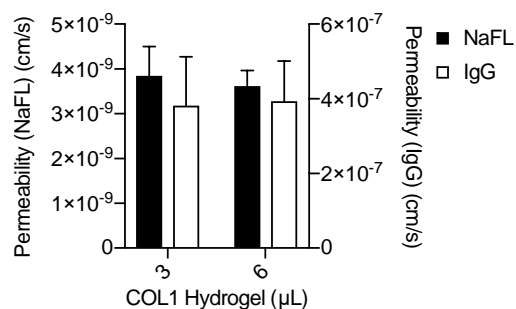
# Efflux Pump Substrates Shuttled to Cytosolic or Vesicular Compartments Exhibit Different Permeability in a Quantitative Human Blood–Brain Barrier Model

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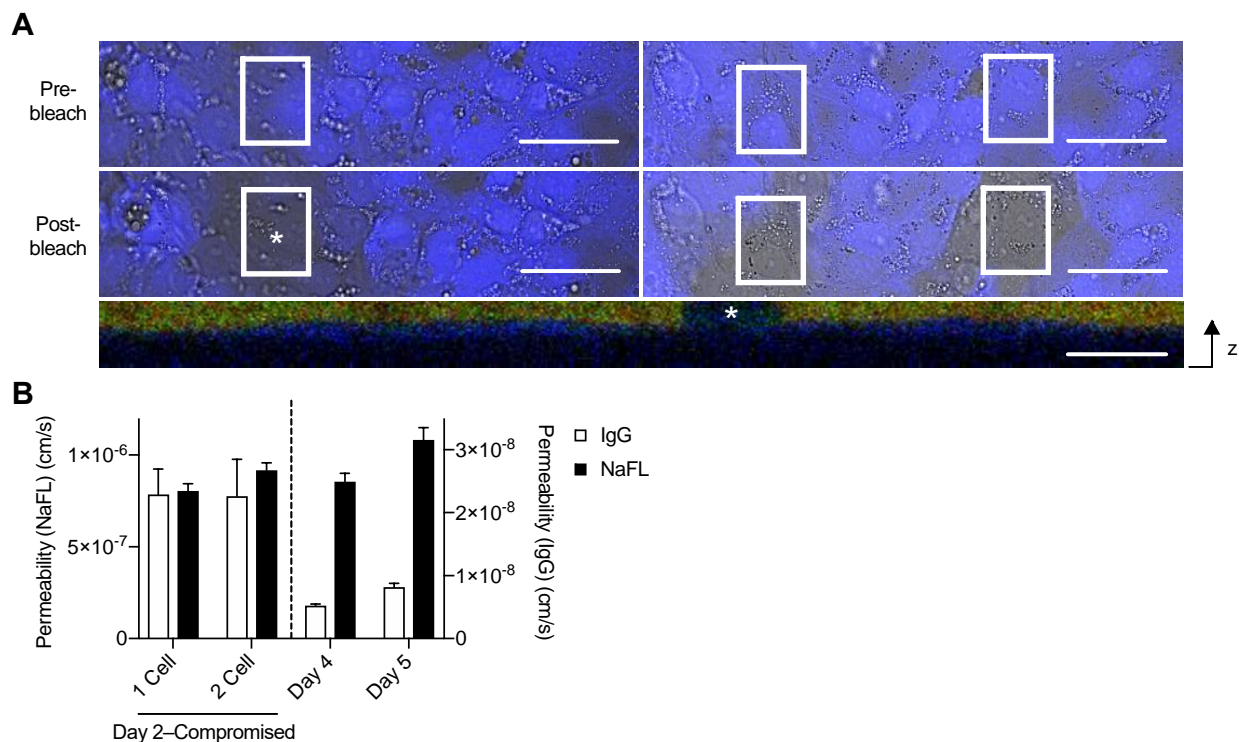
## SUPPORTING INFORMATION



**SI Figure 1.** Representative confocal reflection microscopy image of a 5 mg/mL COL1 hydrogel formed at 37 °C. Scale bar is 20  $\mu\text{m}$ .



**SI Figure 2.** NaFL and IgG permeability of hiPSC-BMECs grown on COL1 hydrogels of 3 or 6  $\mu\text{L}$ . Statistical significance was determined using a Student's t test. Values are mean  $\pm$  SEM of four individual hydrogels from two independent differentiations.



**SI Figure 3.** Analysis of NaFL and IgG permeability following photobleaching of hiPSC-BMEC monolayers using confocal microscopy. (A) A comparison of hiPSC-BMEC monolayers, positive for live-cell CB blue staining (blue), before (Pre-bleach) and after (Post-bleach) the photobleaching of one (left) or two (right) regions (white boxes). Figures are composite fluorescent and brightfield (greyscale) images. Scale bar is 40  $\mu\text{m}$ . A vertical profile of Post-bleach cells with one photobleached region is shown in the bottom panel, where NaFL (green) and IgG (red) help identify the rounded, CB-negative compromised cell (indicated with a \*). Scale bar is 20  $\mu\text{m}$ . (B) Permeability values of compromised monolayers and Day 4 and Day 5 uncompromised monolayers. Statistical significance was determined using a Student's t test. Values are mean  $\pm$  SEM of four individual hydrogels from two independent differentiations.