

1 **Additional file 4**

2 **Supplementary Methods**

3 **Endothelial barrier function analysis**

4 Endothelial barrier function in response to a specific stimulus can be measured in a fully
5 standardized manner by continuously recording changes in trans-endothelial electric
6 resistance using Electric Cell-substrate Impedance Sensing (ECIS) [1, 2]. The barrier
7 function of HCAEC was recorded in real-time using the ECIS Z Theta system (Applied
8 Biophysics) and the associated software v.1.2.126 PC, as described [3]. HCAEC seeded
9 (80,000- 90,000 cells/well) into rat tail collagen type I coated and stabilized 8W10E+
10 electrode chamber arrays were grown up to tight monolayer stage. Treatments were then
11 carried out by replacing the medium with fresh medium without or with stimuli (Wnt5A,
12 Wnt5A+sFRP1, Wnt5A+WIF1). The barrier function measurements were conducted in Ohms
13 every 5 min at multiple frequencies ranging from 62.6 Hz to 64 kHz, normalized to its value
14 at time zero, and plotted with respect to time. 40 electrodes present in each of the eight wells
15 of 8W10E+ array traced the cells at 40 different locations in each well and the measurements
16 were averaged.

17 **References**

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