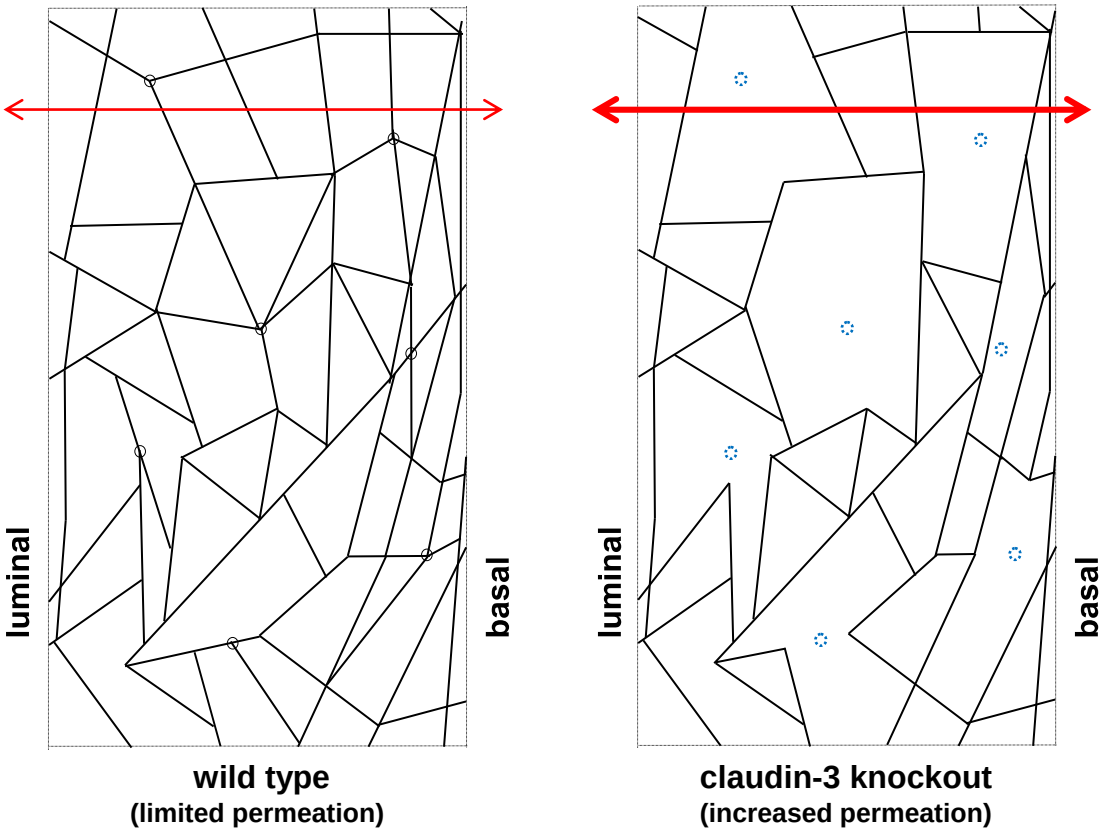

Supplementary Figure 3
Scheme of a mesh network of intercellular strands (cf. Fig. 2c) composed of tight junction particles mainly formed by claudin oligomers in cell-cell contacts (top view). Lines, TJ strands; double arrows, paracellular diffusion plane; circles, Cldn3-dependent branching points; blue dotted circles indicate missing branch points due to Cldn3 deficiency, resulting in larger meshes (polygons encompassing blue dotted circles) as seen in Fig. 2 of the manuscript

The number of branching points in freeze-fracture electron microscopy is drastically reduced in Cldn3 deficiency resulting in enhanced mesh areas in the network of TJ strands formed by Cldn oligomers. This suggests a function of Cldn3 in the branching of TJ strands. A reduced number of intercellular strands, which represent the paracellular diffusion barrier[1], causes enlarged meshes in the network. Cldn5, the dominating claudin at the BBB, generates the majority of strands, whereas Cldn3 may have a more subtle function. Both claudins oligomerize homophilically and heterophilically[2]. Heterophilic binding is sterically different due to sequence differences in the extracellular loops of these two claudins[3] and may cause the branching. Consequently, homophilic Cldn5 association is occasionally interrupted by heterophilic association with Cldn3 in branching points.

[1]Krause G, Winkler L, Mueller SL, et al. Structure and function of claudins. *Biochim Biophys Acta Biomembr* 2008; 1778: 631-645
[2]Piontek J, Fritzsche S, Cording J, et al. Elucidating the principles of the molecular organization of heteropolymeric tight junction strands. Cell Mol Life Sci 2011; 68: 3903-3918
[3]Gehne N, Lamik A, Lehmann M, et al. Cross-over endocytosis of claudins is mediated by interactions via their extracellular loops. PLoS One 2017; 12: 21