Human Piezo1 membrane localization and gating kinetics are modulated by cholesterol levels

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The human mechanosensitive ion channel Piezo1 gates in response to membrane tension and regulates essential biological processes such as vascular development and erythrocyte volume homeostasis. Currently little is known regarding the plasma membrane localization and organization of Piezo1, but previous work suggests that membrane cholesterol content is a key determinant of Piezo1 function. Using a previously characterized Piezo1-GFP fusion protein (hP1-1591-GFP, Cox *et al.*, 2016), we investigated the effect of the cholesterol depleting agent methyl-β-Cyclodextrin (mβCD) on the membrane organization and the response of Piezo1 to mechanical force in HEK-293 cells. STORM super-resolution imaging revealed at the nanoscale that Piezo channels associate in the membrane as clusters, as previously inferred by electrophysiological data (Bae *et al.*, 2013). Both cluster size and diffusion rates, as determined using TIRF microscopy, were modulated by treatment with mβCD (5 mM). In addition, electrophysiological recordings in the cell-attached configuration revealed that mβCD caused a right-ward shift in the Piezo1 pressure-response curve and a delay in the initial response (*i.e.* increased latency). We suggest that cholesterol rich micro-domains host Piezo1 clusters and that this nanoscale membrane organization is essential for efficient Piezo1-mediated mechanotransduction. This is consistent with cholesterol rich domains acting as “membrane force foci”.

Bae C, Gnanasambandam R, Nicolai C, Sachs F, Gottlieb PA. (2013) *Proc Natl Acad Sci USA* **110**, E1162–E1168.

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