STOML3 assembles into ring-like membrane structures that organize mechanosensitive ion channels

Angela Tzu-Lun Huang[1], Clément Verkest[4], Stefan G Lechner[4], Gary R. Lewin[1,2,3*]

1 Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Germany ; 2 German Center for Mental Health (DZPG); 3 Institute for Chemistry and Biochemistry, Freie Universität Berlin, Germany ; 4 Department of Anesthesiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

STOML3 is a membrane protein for mechanosensitivity

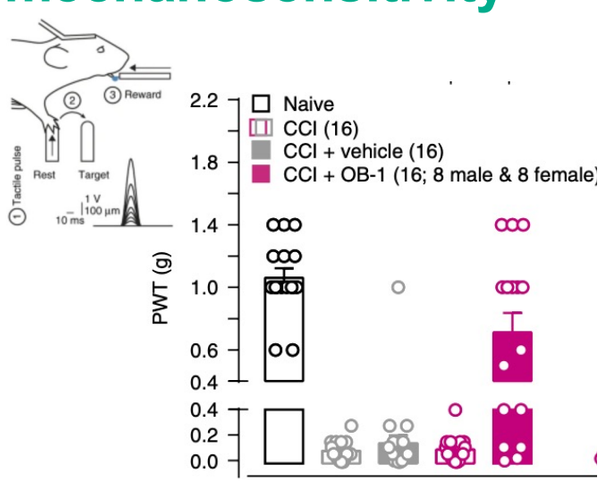
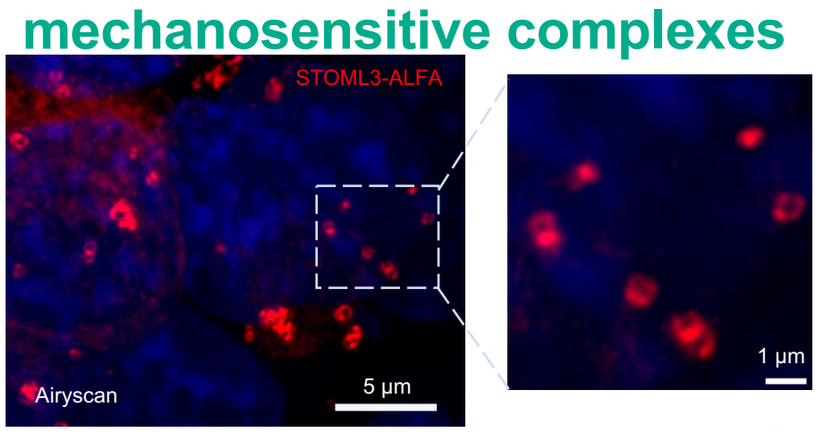


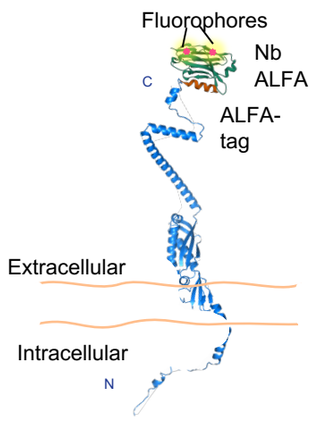
Fig. 1 Small-molecule inhibition of STOML3 reverses pathological mechanical hypersensitivity. Wetzel et al. *Nature Neuronscience* 2017.

STOML3 is involved in sensing mechanical force on the skin.

Stomatin-like protein-3 (STOML3) is a peripheral membrane protein that was discovered by screening for mammalian orthologs of the first mechanosensitive complex proteins that were identified in *C. elegans*[1,2]. It has been suggested to alter membrane stiffness and associates with mechaonsensitive ion channels and modulates them[3]. Here, we further examined how STOML3 associates with mechanosensitive ion channels using cell biology, structural and biochemical studies.

STOML3 oligomerizes into higher order mechanosensitive complexes





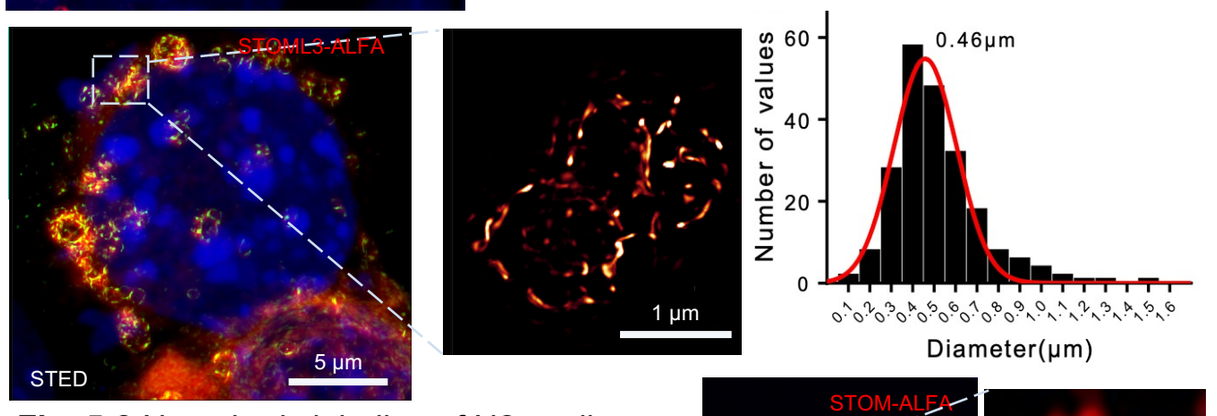


Fig. 5-8 Nano body labeling of N2a cell membrane over-expressing STOML3-ALFA coupled with super-resolution microscopies show ring formation with different diameters. STOM-ALFA exhibits puncta like structure on the membrane. DAPI staining in blue.

MINFLUX single molecule super-resolution study of STOML3

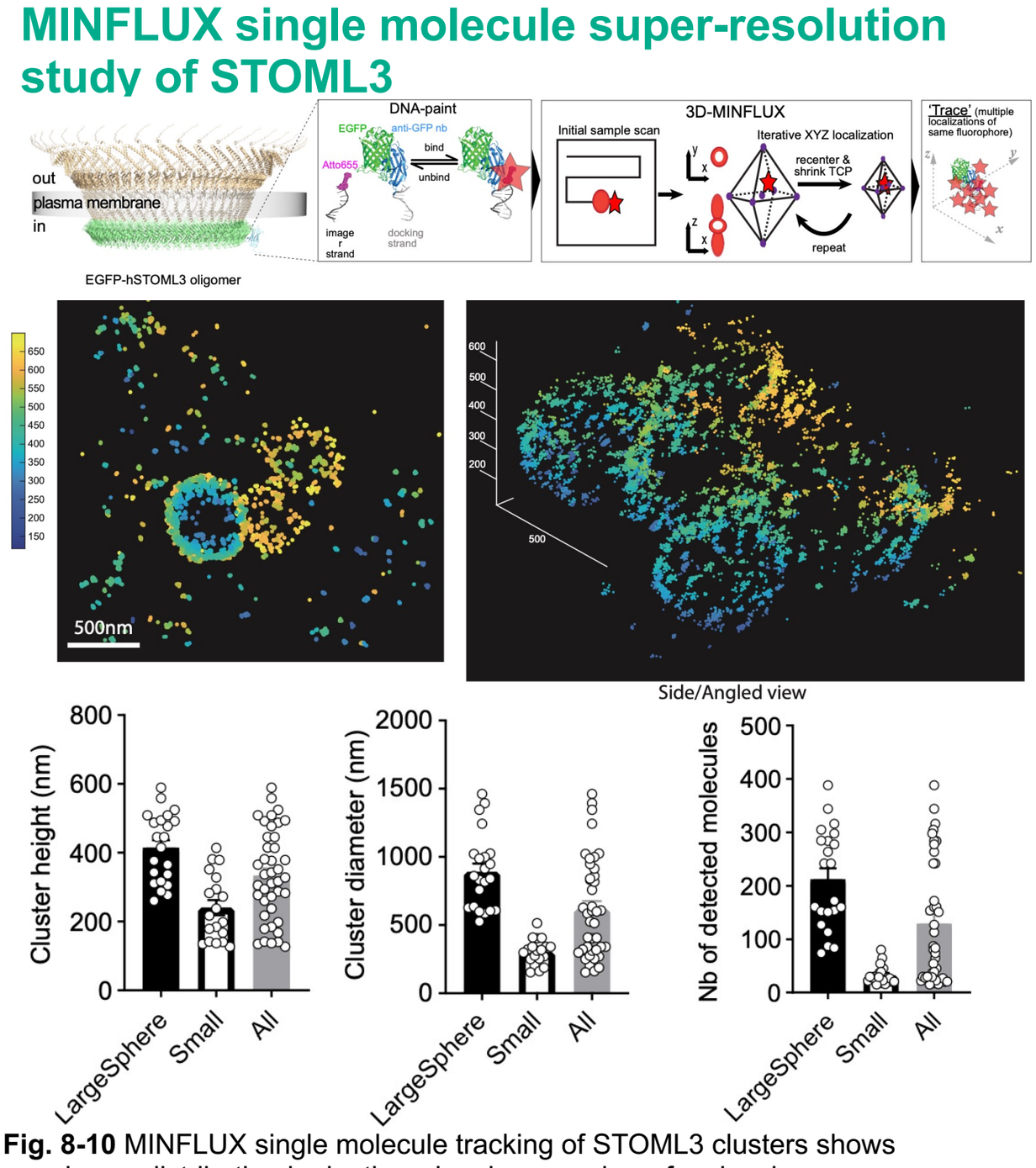
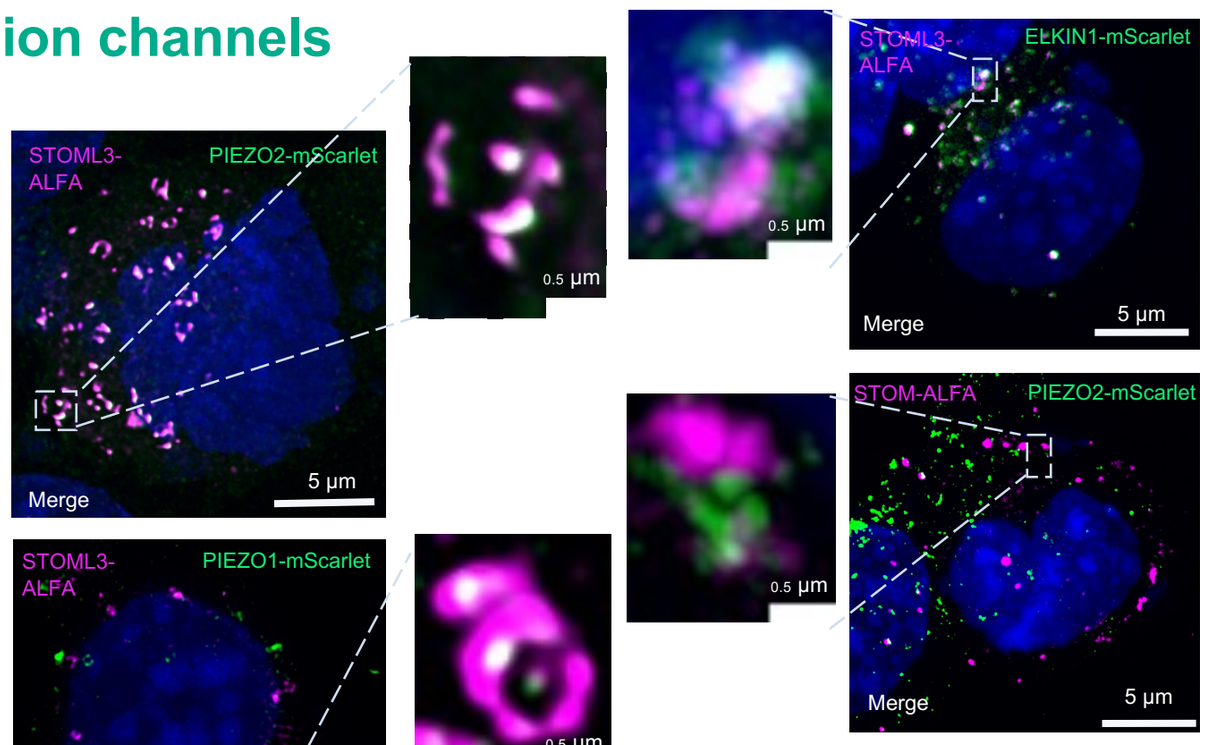


Fig. 8-10 MINFLUX single molecule tracking of STOML3 clusters shows membrane distribution in depth and various number of molecules.

STOML3 colocalizes with mechanosensitive ion channels



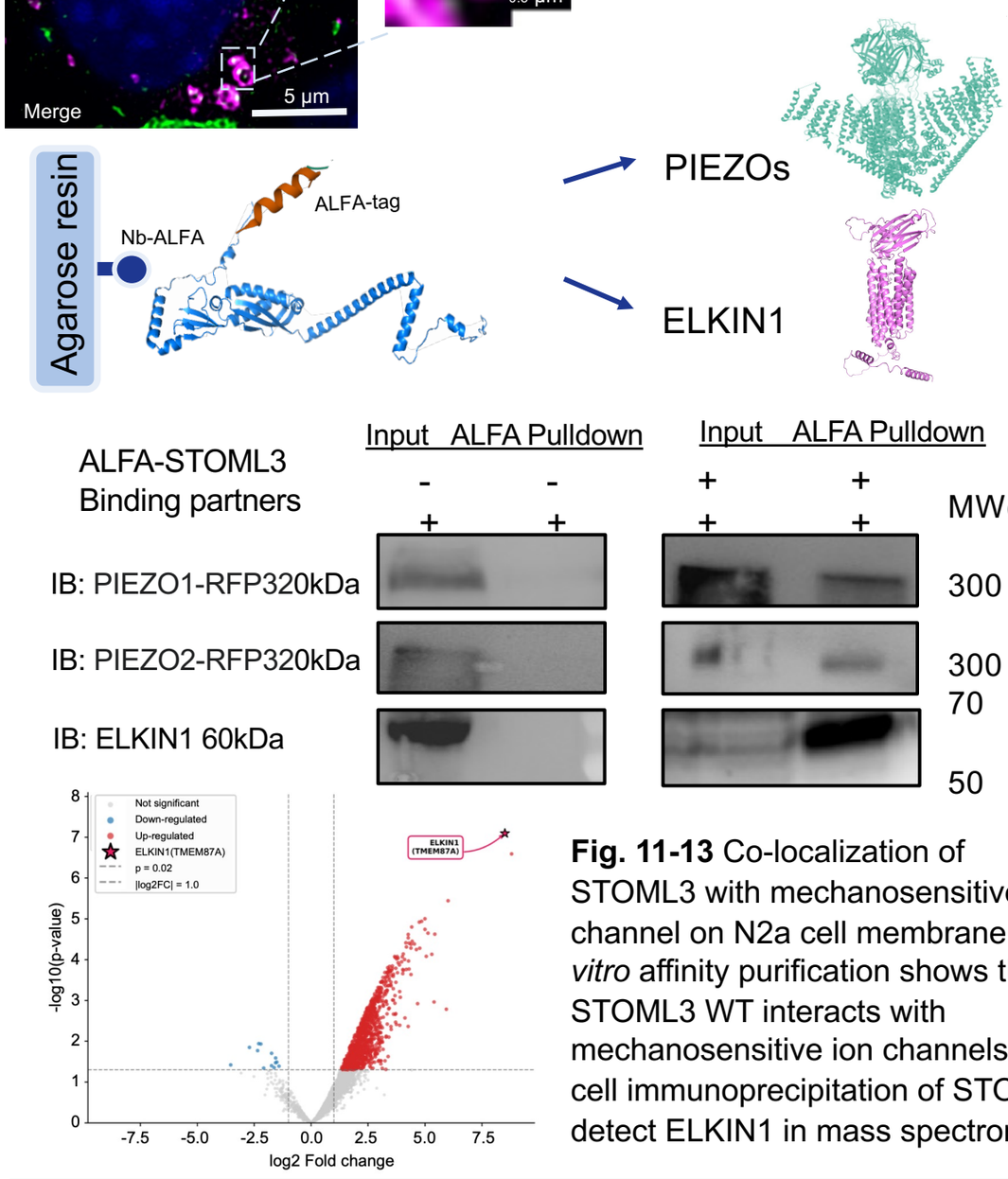


Fig. 11-13 Co-localization of STOML3 with mechanosensitive ion channel on N2a cell membrane. *In vitro* affinity purification shows that STOML3 WT interacts with mechanosensitive ion channels. HEK cell immunoprecipitation of STOML3 detect ELKIN1 in mass spectrometry.

Inhibiting STOML3 ring formation reduces ion channel interactions

Fig. 14-16 STOML3 mechanotransduction mutation abolishes the ring formation on N2a cell membrane. *In vitro* affinity purification shows that STOML3 mutation or small-molecule inhibition disrupt interacts with mechanosensitive ion channels.

References

1. Wetzel, C., Hu, J., Riethmacher, D. *et al.* A stomatin-domain protein essential for touch sensation in the mouse. *Nature* 445, 206–209 (2007).
2. Chalfie M, Sulston J. Developmental genetics of the mechanosensory neurons of *Caenorhabditis elegans*. Dev. Biol. 1981;82:358–370.
3. Qi, Y., Andolfi, L., Frattini, F. *et al.* Membrane stiffening by STOML3 facilitates mechanosensation in sensory neurons. *Nat Commun* **6**, 8512 (2015).