Supplemental Materials

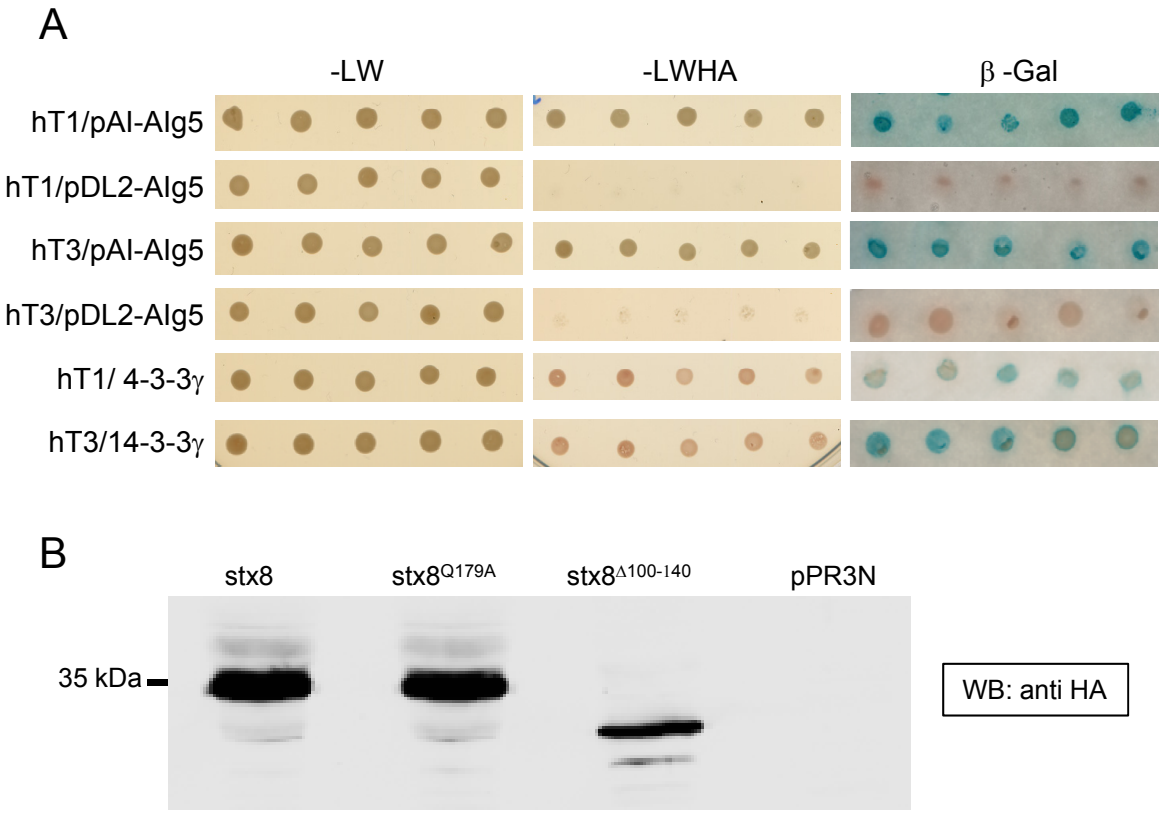
Molecular Biology of the Cell

Renigunta et al.

Supplementary Table 1

Constructs used in our study

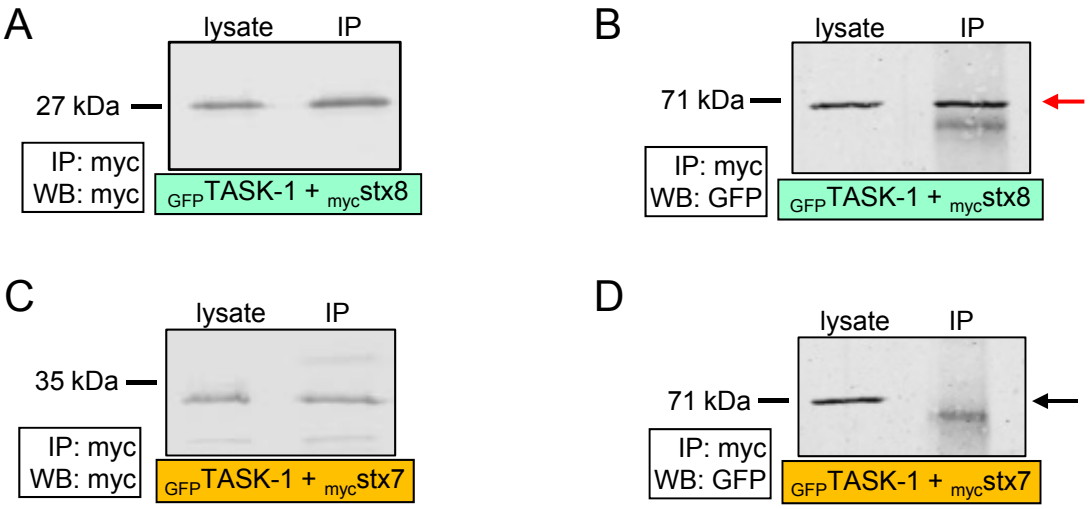
Gene	**Xenopus Oocytes**	**Patch-clamp in CHO cells**	**Imaging in HeLa and CHO cells**	**Yeast-two-hybrid analysis**	**Protein Biochemistry in HeLa cells**	**Antibody uptake assay in COS cells**
TASK-1	^{NQ}hTASK-1 -pSGEM	^{NQ}hTASK-1 -pCDNA3.1 $_{myc}$rTASK-1 -pCDNA3.1	^{NQ}hTASK-1 -pEGFP-C1 ^{NQ}hTASK-1 -pmCherry-C1	TASK-1-pBT3N	^{NQ} hTASK-1 -pEGFP-C1	rTASK-1^{HA} -pCDNA3.1
TASK-3	hTASK-3 -pSGEM			TASK-3-pBT3N		
Syntaxin-8	stx8 -pSGEM	$_{myc}$Stx8-pCDNA3.1	Stx8-pmCherry-C1	Stx8-pPR3N	$_{myc}$Stx8 -pCDNA3.1	Stx8 -pCDNA3.1
Syntaxin-7	stx7 -pSGEM	$_{myc}$Stx7-pCDNA3.1			$_{myc}$Stx7 -pCDNA3.1	Stx7 -pCDNA3.1
Vti1b	vti1b-pSGEM					
Vamp8	vamp8-pSGEM					
AP180C	AP180C-pSGEM					
2xFYVE			2xFYVE-pEGFP			
Rab5			Rab5 -pDsRedMonomer			
Clathrin (LC)			Clathrin-pEGFP			
ALG5				pAI-Alg5 pDL2-Alg5		
14-3-3γ				14-3-3γ-pPR3N		



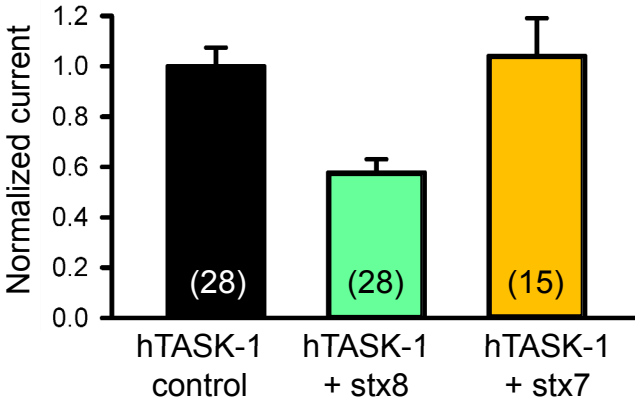
Supplementary Figure 1. Tests for the expression of bait and prey proteins in the NMY51yeast strain.

(**A**) Membrane yeast-two-hybrid direct interaction experiments using TASK-1 or TASK-3 Cub fusion proteins as bait, and 14-3-3γ or Alg5 NubG-HA fusion proteins as prey. The control vectors pAI-AIg5 and pDL2-AIg5 were used as positive and negative controls, respectively (upper rows). The growth of yeast in the LWHA drop-out plates and the blue coloring on the β–Gal assay clearly indicate that bait proteins are expressed at the membrane. An additional control was provided by 14-3-3γ (lower rows). 14-3-3γ has been shown previously to interact with TASK-1 and TASK-3 (Rajan *et al.* J. Physiol. 2002;545:13-26). We have now also detected 14-3-3γ in our recent membrane yeast-two-hybrid screen of a human brain library (together with stx8). This finding further supports the correct expression of the bait proteins TASK-1 and TASK-3 in yeast.

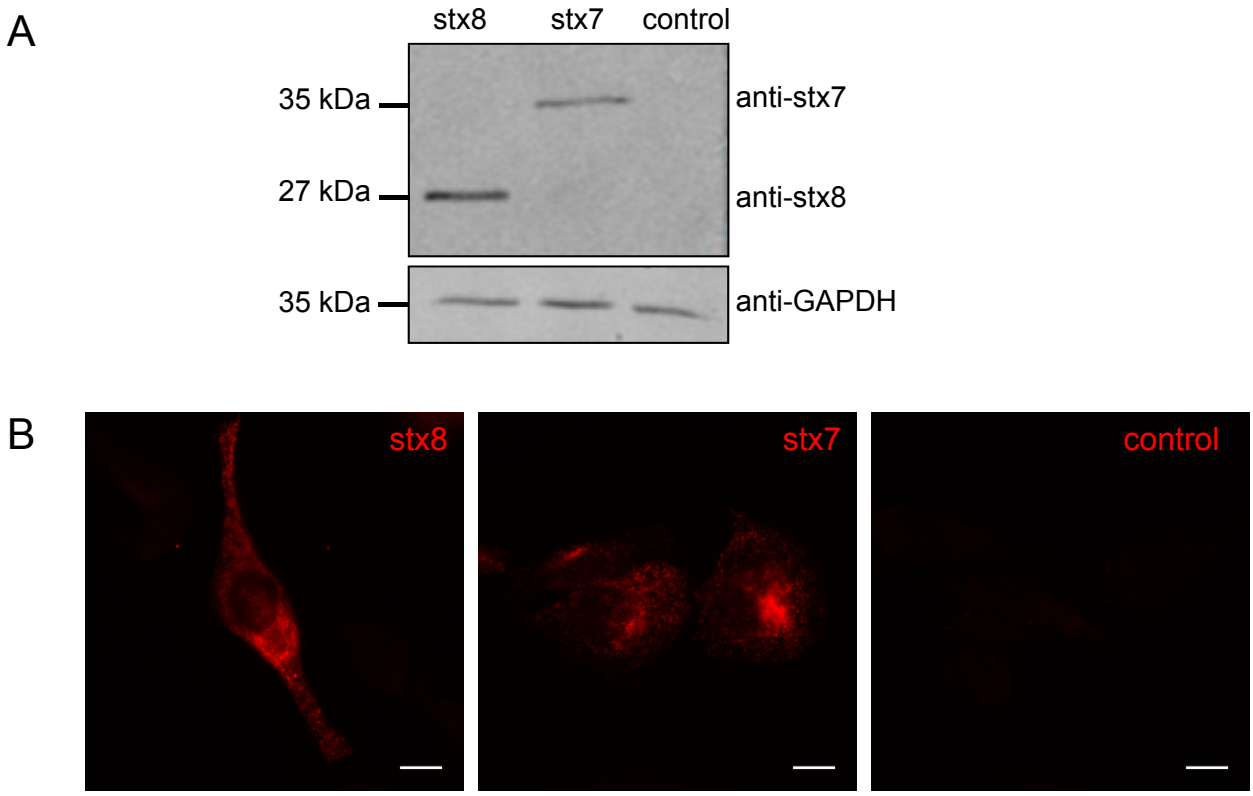
(**B**) Western blot of stx8 and stx8 mutants used as prey proteins. NMY51 yeast expressing NubG-HA fusion proteins were lysed according to the manufacturer's instructions and the lysates were analyzed on SDS polyacrylamide gels. The separated proteins were blotted, probed with anti-HA antibodies (Sigma; 1:1000) and visualized with Alexa Fluor 800 secondary antibodies (LI-COR Biosciences; 1: 5000). Immunoreactivity was detected using an infrared fluorescence imaging system (Odyssey, LI-COR Biosciences). Thus, the results shown here confirm the correct expression of the bait and prey proteins in yeast.



Supplementary Figure 2. Co-immunoprecipitation of stx8 and TASK-1 expressed in HeLa cells. (**A** and **B**) Co-IP of GFP-tagged human TASK-1 and myc-tagged and stx8. (**C** and **D**) Co-IP of GFP-tagged human TASK-1 and myc-tagged and stx7. The cell lysate was precipitated with anti-myc antibodies and the Western blot of the precipitate was probed with anti-myc (**A** and **C**) or with anti-GFP antibodies (**B** and **D**). $_{GFP}$TASK-1 was co-immunoprecipiated with $_{myc}$stx8 (**B**, red arrow) but not with $_{myc}$stx7 (**D**, black arrow). The origin of the (unspecific) lower band seen in **C** and **D** is unknown.



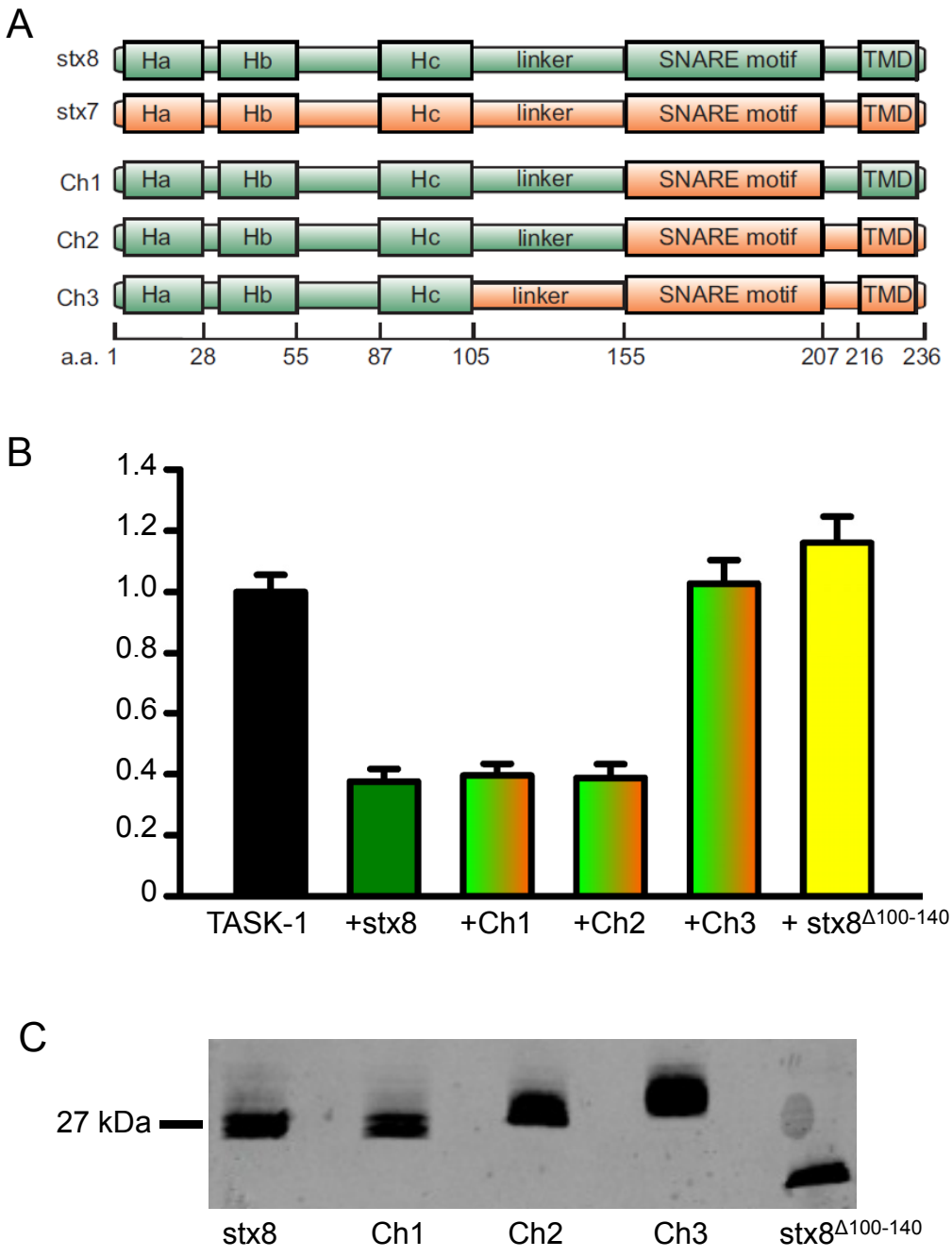
Supplementary Figure 3. Effects of stx8 on human TASK-1 currents expressed in CHO cells. For experiments with human TASK-1 (hTASK-1) we used the ^{NQ}TASK-1 mutant (Zuzarte *et al.*, J.Physiol. 2009;587,929-952), in which the first three amino acids at the N-terminus, MKR, were replaced by MNQ. This mutant displays a higher current amplitude due to deletion of a retention signal (KR). Co-transfection of stx8 in CHO cells had the same effect on human ^{NQ}TASK-1 channels as on wild-type rat TASK-1 channels.



Supplementary Figure 4. Tests for equal expression of stx8 and stx7. CHO cells cultured in 10 cm plates were transfected with rTASK1, pEGFP-C1 and myc-tagged stx8 or stx7. 24 h later the cells were dissociated with trypsin/EDTA (Life technologies) and the samples were subdivided for use in either protein biochemistry (**A**) or immunohistochemistry experiments (**B**).

(**A**) Western Blotting. CHO cells were lysed with Triton X-100 and lysates were analyzed on SDS polyacrylamide gels. The separated proteins were probed with anti-myc (1:1000, Santa Cruz) antibodies and visualized with Alexa Fluor 800 secondary antibodies (1: 5000, LI-COR Biosciences) . Immunoreactivity was detected using an infrared fluorescence imaging system (Odyssey, LI-COR Biosciences). Lysates obtained from mock-transfected CHO cells were used as negative control. GAPDH served as a loading control (lower row).

(**B**) Immunohistochemistry. CHO cells were seeded onto glass cover slips (Menzel GmbH, Saarbrücken, Germany) and fixed 24 h later with 4% PFA in PBS for 15 min at room temperature, washed three times in PBS and blocked with staining buffer (10% (v/v) goat serum (PAA), 2% (w/v) BSA (Carl Roth GmbH, Karlsruhe, Germany) and 0.01% Triton X-100 in PBS) for 1 h at RT. Subsequently, the cells were stained with mouse anti-myc antibody (Sigma Aldrich, St. Louis, US) 1:1000 in staining buffer for 60 min at room temperature, washed three times in PBS with 0.01% Triton X-100 and stained with goat anti-mouse Alexa Fluor 594 secondary antibody (Life technologies) 1:500 in staining buffer for 60 min at room temperature. After washing the cells three times in PBS with 0.01% Triton X-100 the cover slips were mounted with Mowiol 4-88 (Carl Roth GmbH) on microscope slides (Menzel GmbH). Cells were finally visualized with a Nikon Eclipse Ti microscope equipped with a 60x objective (Plan Apo VC 60x Oil DIC N2, Nikon); images were processed with NIS Elements AR 4 software (Nikon). The scale bar is 10 µm. Panels (**A**) and (**B**) show that the expression level of stx8 and stx7 in CHO cells was similar.

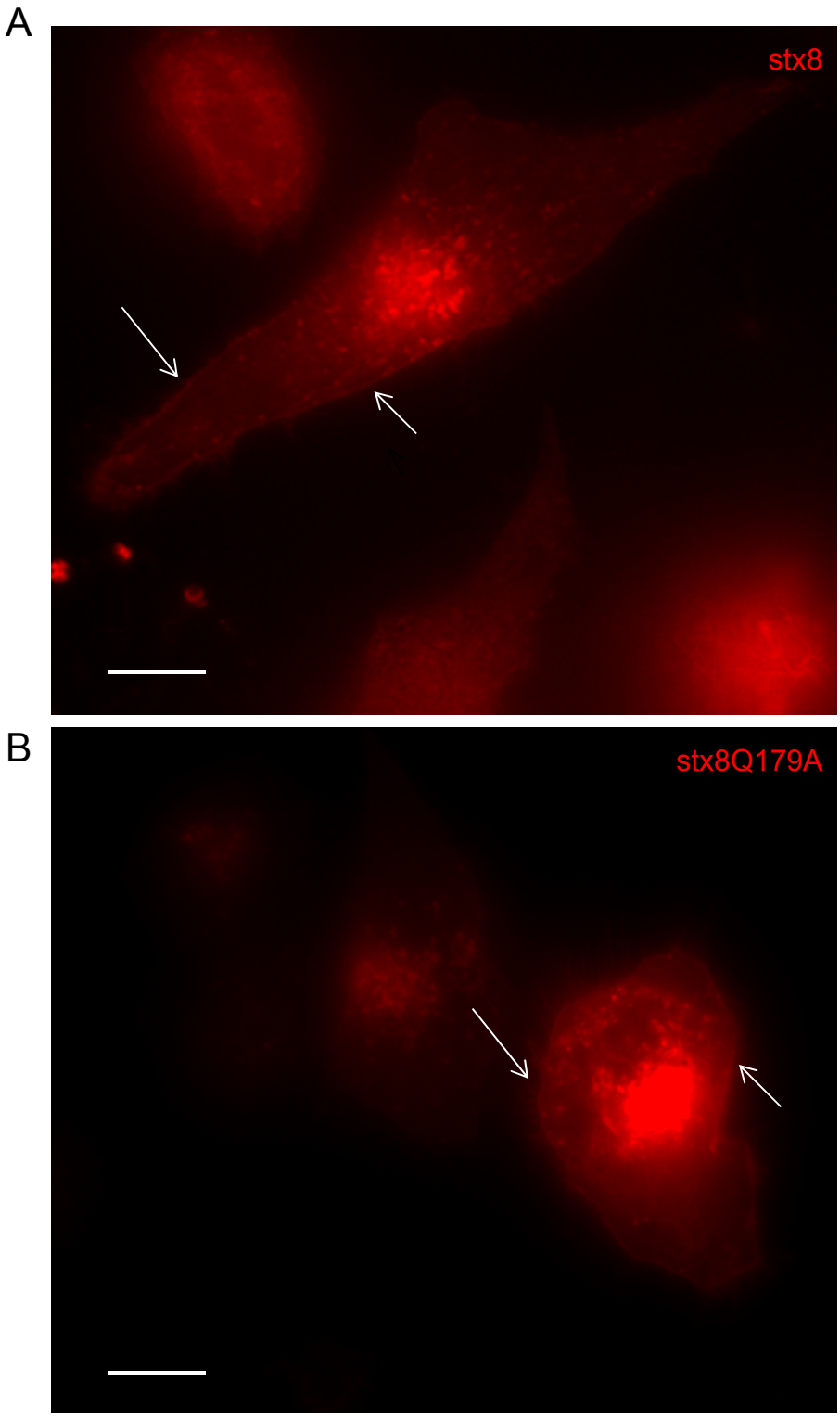


Supplementary Figure 5. Myc-tagged stx8 and stx8/stx7 chimeras had the same effect on TASK-1 current amplitude as un-tagged constructs.

(**A**) Topology of the stx8/stx7 chimeras (identical to Figure 3A).

(**B**) Co-expression of myc-tagged TASK-1 and myc-tagged stx8-derived constructs in *Xenopus* oocytes. The effect of co-expression of myc-stx8, myc-stx8/stx7 chimeras and the myc-stx8$^{\Delta 100\text{-}140}$ construct on TASK-1 current. Since the current amplitude varied between different batches of oocytes the currents measured after co-expression of the stx8-based constructs were normalized to the TASK-1 currents measured in the same batches of oocytes.

(**C**) Western blot of *Xenopus* oocyte lysates using an anti-myc antibody; the lysates were from the same batches of oocytes with which the current measurements were performed.



Supplementary Figure 6. Live-cell images of mCherry-tagged stx8 (A) and stx8Q179A (B) expressed in HeLa cells. The images were taken 48 h after transfection. The expression of the endosomal SNARE proteins in the plasma membrane can be clearly seen (arrows). Scale bars, 10 μm.