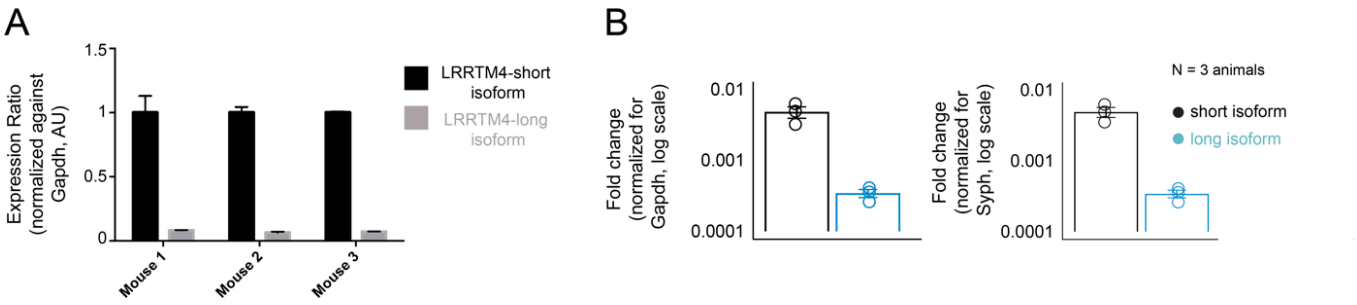
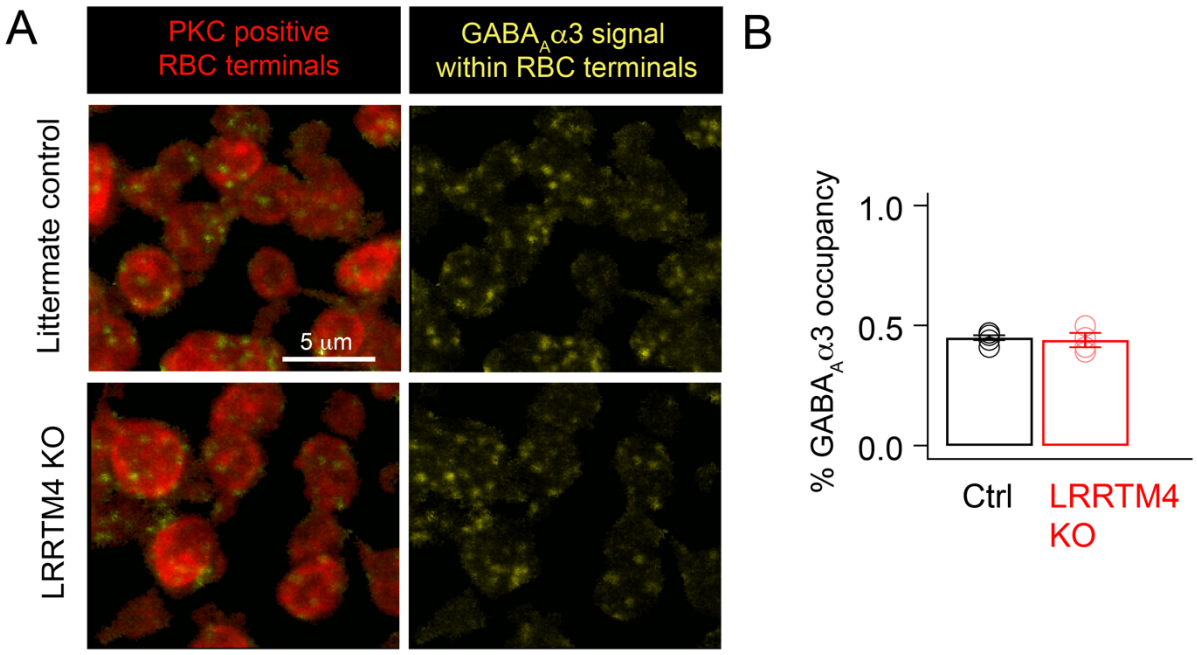
Supplementary Figure 1 (Related to Figure 1)



Supplementary Figure 1 (related to Figure 1): Short isoform of LRRTM4 is enriched in the mouse retina.

(**A**) Expression ratio of the short (black) and long (grey) isoforms of LRRTM4 from retinas of three different wildtype animals by real-time quantitative PCR. LRRTM4 expression was normalized against Gapdh (Glyceraldehyde-3-Phosphate Dehydrogenase) expression. (**B**) Bar plots depicting the expression levels of LRRTM4 isoforms in mouse retina (averaged across 3 animals) by real-time quantitative-PCR. The levels are normalized for Gapdh (Glyceraldehyde-3-Phosphate Dehydrogenase; *Left* graph) or Synaptophysin (Syph; *Right* graph) and represented in log-scale. Similar to the rest of the brain, the short isoform of LRRTM4 is relatively more abundant in the retina. N=3 mice, each with three technical replicates (*Left* graph: short vs long isoform expression p= 0.0074; *Right* graph: short vs long isoform expression p=0.0045, two-tailed unpaired T-test).

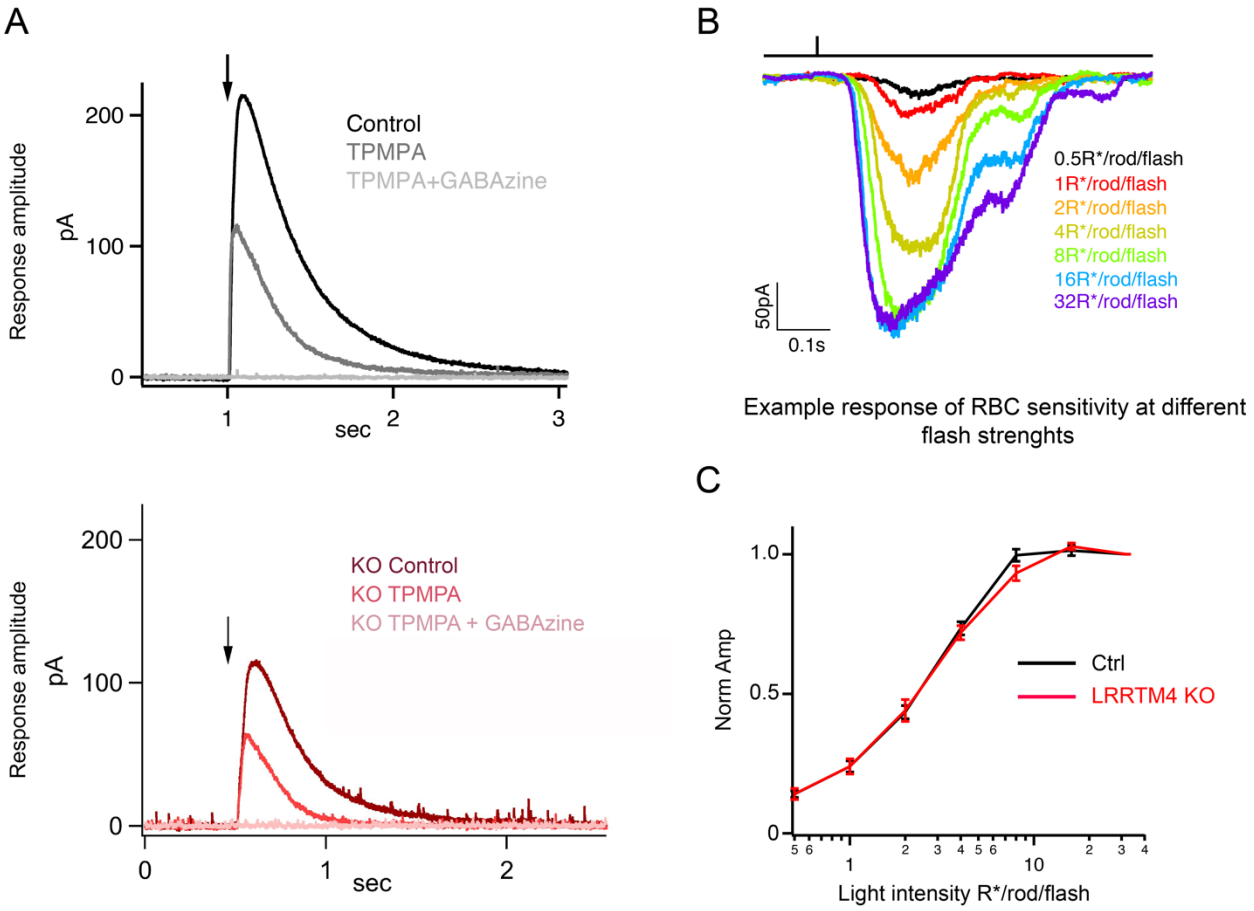
Supplementary Figure 2 (Related to Figure 2)



Supplementary Figure 2 (related to Figure 2): Rod bipolar cell GABA receptor expression in LRRTM4 deficient retina.

(**A**) Labeling of α3-subunit containing GABA receptors ($GABA_A\alpha3$; yellow) within protein kinase C (PKC, red) labeled rod bipolar cell (RBC) terminals in littermate control (Ctrl) and LRRTM4 knockout (KO) retinas. Labeling across genotypes is comparable. (**B**) The percent (%) receptor occupancy of $GABA_A\alpha3$ within PKC positive RBC terminals is equivalent across LRRTM4 KO (red) and Ctrl (black) RBC boutons. N=2 pairs of littermate control and KO animals.

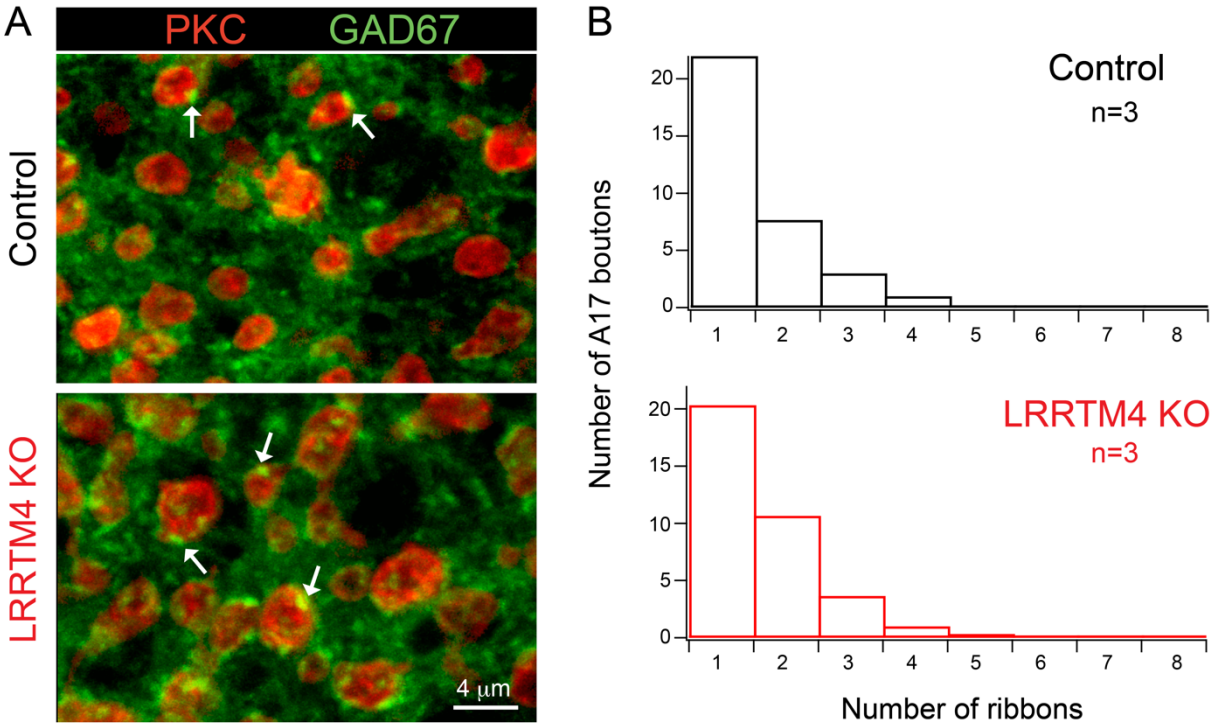
Supplementary Figure 3 (Related to Figure 2)



Supplementary Figure 3 (related to Figure 2): Functional responses of Rod bipolar cells in LRRTM4 deficient retina.

(**A**) Example of the evoked response of a rod bipolar cell (RBC) after GABA puff application at its axon terminal. Control: Top panel (black trace); LRRTM4 knockout: Bottom panel (red trace). Arrows mark time of puff application. $GABA_A$ and $GABA_C$ receptor mediated components of the evoked response were pharmacologically isolated by application of TPMPA ($GABA_C$ receptor antagonist) and GABAzine ($GABA_A$ receptor antagonist). After application of TPMPA the $GABA_A$ component is revealed (TPMPA) which is eliminated upon addition of GABAzine (TPMPA+GABAzine). (**B**) Whole cell voltage-clamp recordings of RBCs held at -60mV (chloride reversal potential) to measure light-evoked excitatory currents, indicative of photoreceptor input onto RBC dendrites. Family of response curves demonstrating the sensitivity of a RBC to increasing flash strengths at scotopic light levels that stimulate rod photoreceptors. (**C**) Graphs plotting the normalized response amplitude (Norm Amp) of RBCs to increasing flash strengths in control (Ctrl) and LRRTM4 KO retinas. Response profiles of littermate control (black trace) and LRRTM4 knockout (KO; red trace) were comparable. Number of recorded cells: 11 for Ctrl and 9 for KO; N>3 pairs of littermate control and KO animals.

Supplementary Figure 4 (Related to Figure 3)

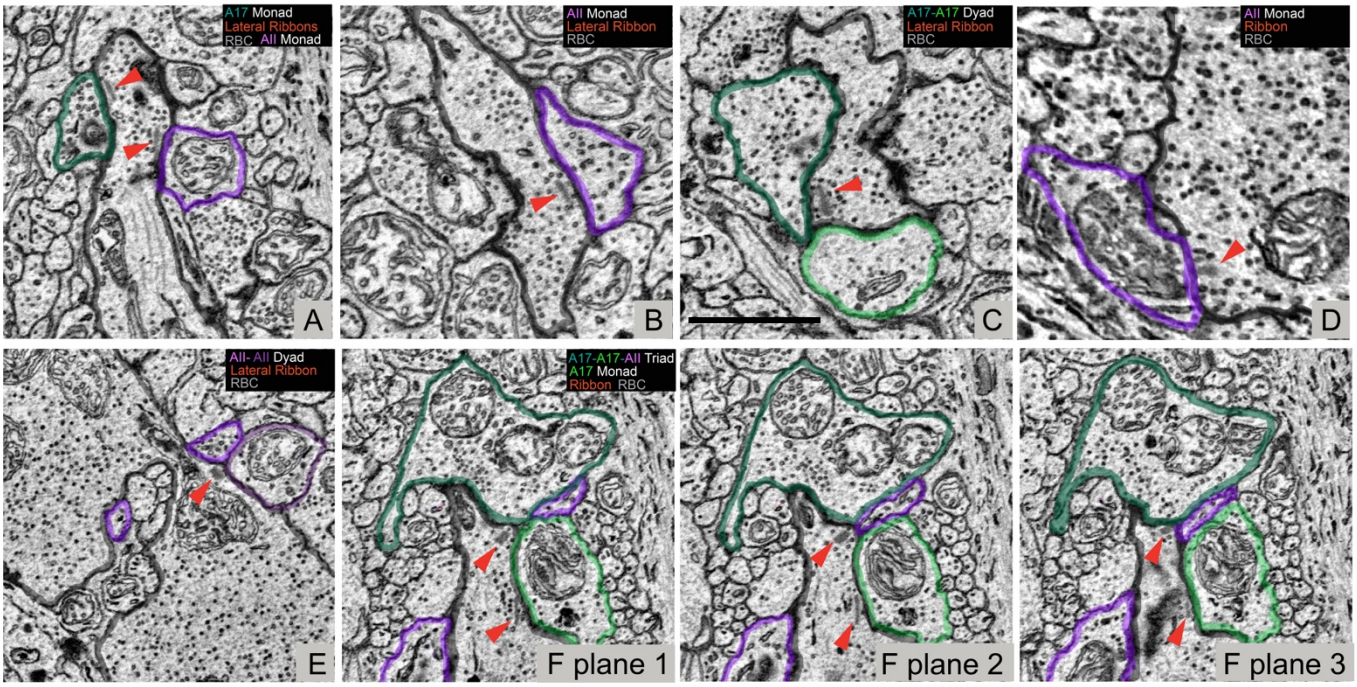


Supplementary Figure 4 (related to Figure 3): Profiles and synaptic input of A17 boutons in LRRTM4 deficient retina.

(**A**) Profiles of GAD67 positive (green) A17 varicosities contacting PKC positive (red) rod bipolar boutons in adult LRRTM4 KO and littermate control retinas. Example A17 varicosities contacting rod bipolar boutons marked with arrows.

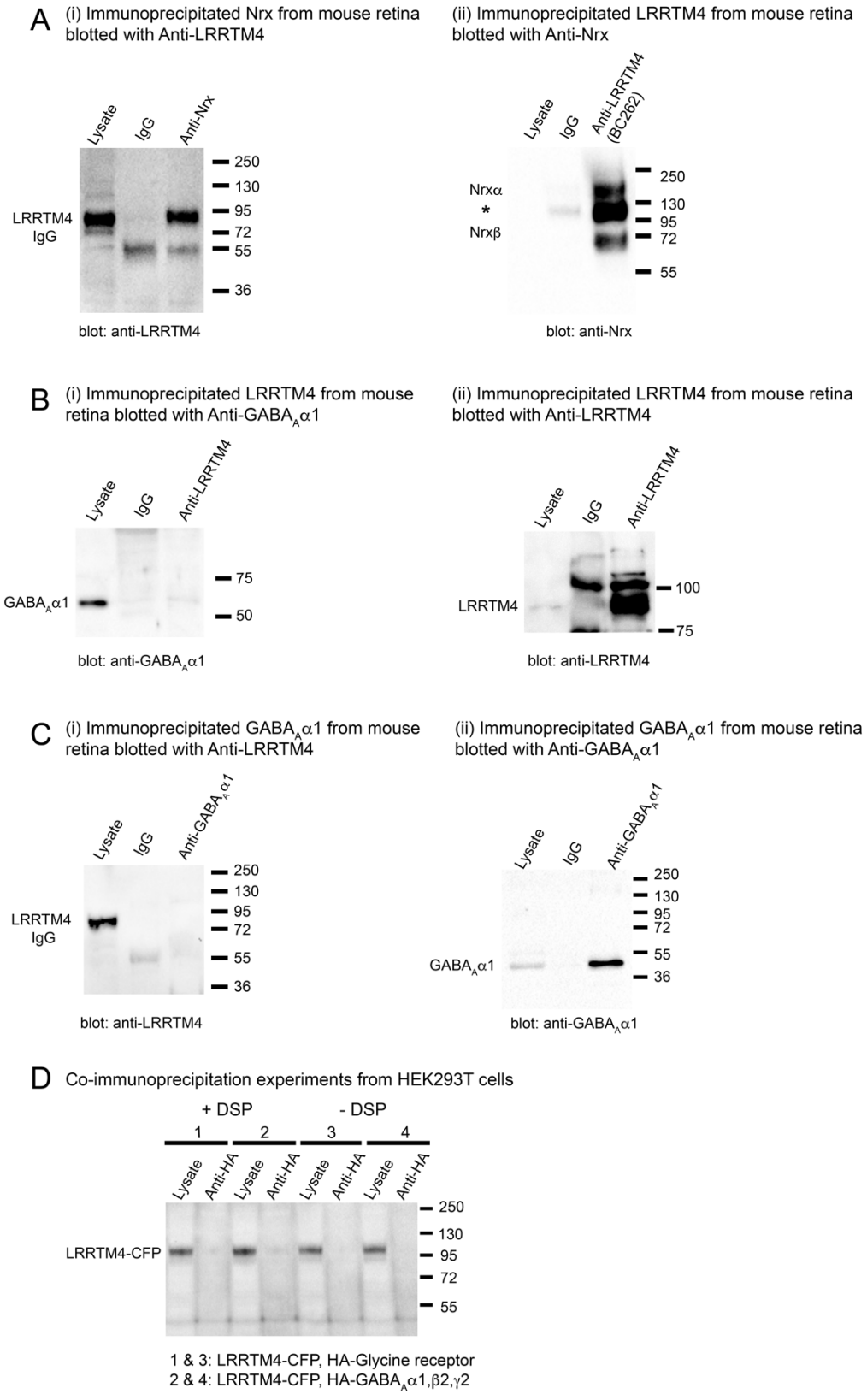
(**B**) Histograms of the average number of ribbons apposed to A17 boutons contacting rod bipolar cells in littermate control (black) and LRRTM4 knockout (KO, red) retinas. The distributions are comparable across genotypes. Three rod bipolar cells of littermate control and of LRRTM4 KO retinas (n=3 each) were reconstructed fully by serial block face scanning electron microscopy. Number of rod bipolar cell ribbons apposed to A17 boutons is not affected by loss of LRRTM4.

Supplementary Figure 5 (Related to Figure 3)



Supplementary Figure 5 (related to Figure 3): EM profiles of mis-organized dyads in adult LRRTM4 KO retina.
(A) Single plane scanning electron microscopy (EM) image of an adult LRRTM4 KO RBC terminal (outlined in grey) with two ribbons (red arrowheads) apposed to a single A17 (outlined in green) and a single AII (outlined in magenta) process.
(B) Single plane EM profile of another AII monad synapse within an adult LRRTM4 KO RBC terminal.
(C) Single plane EM image of an A17-A17 dyad within an adult LRRTM4 KO RBC terminal.
(D) Single plane EM profile of an AII monad synapse within an adult LRRTM4 KO RBC terminal.
(E) Single plane EM image of an AII-AII dyad synapse within an adult LRRTM4 KO RBC terminal.
(F) Three consecutive planes (plane 1-3) showing a three-partner "triad" ribbon synapse within an adult LRRTM4 KO RBC terminal. One of the A17 partners of this triad is also apposed to a lateral ribbon site (A17 monad).
Scale bar = 1.5 μm.

Supplementary Figure 6 (Related to Figures 2-4)



Supplementary Figure 6 (related to Figures 2-4): Biochemical interactions of retinal LRRTM4.

(A) (i) Immunoprecipitation from mouse retinal lysate by anti-pan Neurexin (Nrx) antibody but not control IgG coimmunoprecipitated LRRTM4 (n= at least 3 independent experiments/animals). (ii) Immunoprecipitation from mouse retinal lysate by anti-LRRTM4 (BC262) antibody but not control IgG coimmunoprecipitated Nrxs (n= at least 3 independent experiments/animals. *Note: The band at 130K.Da is a non-specific band that is recognized by the anti-pan Nrx antibody used for blotting).

(B) (i) An anti-LRRTM4 antibody did not coimmunoprecipitate $GABA_A\alpha1$ from mouse retinal lysate. (ii) LRRTM4 was immunoprecipitated efficiently by the antibody used for immunoprecipitation (n= at least 3 independent experiments/animals).

(C) (i) An anti-$GABA_A\alpha1$ antibody did not coimmunoprecipitate LRRTM4 from mouse retinal lysate. (ii) $GABA_A\alpha1$ was immunoprecipitated efficiently by the antibody used for immunoprecipitation (n= at least 3 independent experiments/animals).

(D) Coimmunoprecipitation from transfected HEK 293T cells. HEK 293T cells were transfected with the indicated plasmids. An anti-HA antibody did not coimmunoprecipitate LRRTM4-CFP. The experiments were done with/without pretreatment of the cells with a cross-linker DSP (2mM) (n=2 independent experiments).

Note: Each experiment was repeated with complexiolyte-48 (3X), DDM (2X), TritonX-100 (1X). For $GABA_A\alpha1$ interaction with LRRTM4, crosslinker (BS3) was also tried at least two times.