Supplementary Figure 1: Electron microscopy of MBP-Gli DDM-solubilized in the absence of asolectine. White arrows indicate dissociated proteins, and the black arrow indicates proteins associated in rosette. The inset shows typical rosettes at higher magnification.
SUPPLEMENTARY METHODS

HEK cell expression and sedimentation analysis

Cells were transfected using calcium phosphate, and immunolabeled (anti-HA antibody Sigma-Aldrich H6908, secondary antibody coupled to FITC Vector FI-1000). Sucrose gradients (11 ml, 37 000g for 26 hours, Beckman SW 41 rotor) of the Glvi protein were run in 2 mM DDM (n-dodecyl-β-D-maltoside, Sigma), 50 mM sodium acetate, pH 4, after 3 hours metabolic labeling and solubilization in 40 mM DDM buffer. Fractions were immunoprecipitated with anti-HA antibody Sigma-Aldrich H9658, analyzed by 4-12% SDS-PAGE (apparent molecular weight of 32 kDa). Gradients of AChBP and 40 mM DDM-solubilized AChBP (ring)-5HT3 chimera were run respectively in PBS and PBS containing 2 mM DDM.

Electrophysiology

For ionic exchange experiments, current-voltage relationships were measured by applying inverted voltage ramps (from +50 to -100 mV in 200 ms) at the current plateau. Liquid junction potentials were corrected. For cell-attached patches, pipette was filled with NaCl solution buffered with MES. Single channel events were detected by the half amplitude threshold criterion using the program TAC (Bruxton Corporation) at a final bandwidth at 1 kHz. Expression and two-electrode voltage-clamp in Xenopus oocytes were performed with external solutions (in mM): 100 NaCl, 2.5 KCl, 2 CaCl2, 1 MgCl2, 10 MES, pH brought to 9.0 with NaOH and then adjusted to final values with HCl.

E. coli expression experiments

Negative staining electron microscopy: DDM-solubilized MBP-Glvi samples were supplemented with 10 µg/ml bacitracin (Sigma). Ten ml drops were then deposited onto freshly carbon-coated EM grids. Negative staining was achieved by washing the grids with few drops of 1% (wt/vol) uranyl acetate in water and dried with filter paper. Grids were observed in a Philips EM12 electron microscope (FEI Company, Eindhoven, the Netherlands) fitted with a LaB6 filament and operating at 80 keV. Micrographs were taken at x 28,000 magnification on Kodak electron microscope films. Films were digitalized at high resolution (1200 dpi) and prepared for publication using Adobe Photoshop.

Cross-linking experiments: chemical cross-linking was performed for 10 min at 37°C in PBS containing (in mM) 50 iodoacetamide and 2 DDM.

Homology Modeling

Sequence alignment of the α7nAChR and Glvi-pLGIC was generated using clustal X after removal of the 138 cytoplasmic α7 residues. M1-M4 were inferred from Torpedo nAChR model9. The Glvi-pLGIC homology model was built with Modeller6v2 using the “model” routine, and the alignment from Fig 1a.

Supplementary Figure 2: a) Erev values as a function of external Na concentration measured after progressive substitution of NaCl with mannitol, on HEK cells expressing the Glvi protein (Mean of 3 experiments). The curve corresponds to the Goldmann-Hodgkin-Katz equation. b) Typical I-V curves recorded at different pHs, on a single HEK cell expressing the Glvi protein.