Suppl. Fig. 1. Introduction of affinity epitopes into the TM3-4 loop of hsGlyRα1. *A.* Schematic representation of human GlyRα1 with affinity epitopes (GlyRα1-HA-T7). The TM3-4 loop is shown as amino acid sequence with conserved motifs (C1-3) and variable regions (V1, 2). Small, non-charged tags (HA, T7) were introduced into variable regions to retain structural and functional integrity of the receptor. *B.* Confocal immunofluorescence of HEK293 cells, transfected with human GlyRα1 or GlyRα1-HA-T7, using an anti-HA-antibody (anti-HA) or mAb4a (anti-GlyRα). Representative cells are shown. *C.* Western blot with mAb4a after fractionation of HEK293 cells transfected with hsGlyRα1-HA-T7 or hsGlyRα1 and of untransfected HEK293 cells (GlyR variants: black arrow). Membrane (M), nuclear (N) and cytoplasmic (C) compartments are provided. The subunits are similarly distributed. *D.* Whole-cell current recordings of hsGlyRα1-HA-T7 transfected HEK293 cells in voltage clamp (-60 mV) with application of saturating glycine concentration (2 mM). Five cells were measured. Taken together, the introduction of small epitope tags into the TM3-4 loop did not significantly affect distribution or function of the receptor.

Supplementary figure 1