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Supplemental Information

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Supporting Material

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Ulrike Breitinger,[1] Noha S. Farag,[2] Nourhan K. M. Ali,[1] and Hans-Georg A. Breitinger[1,*]

[1]Department of Biochemistry and [2]Department of Microbiology and Biotechnology, German University in Cairo, New Cairo, Egypt

*Correspondence: hans.breitinger@guc.edu.eg

Supplemental Material

Figure S1

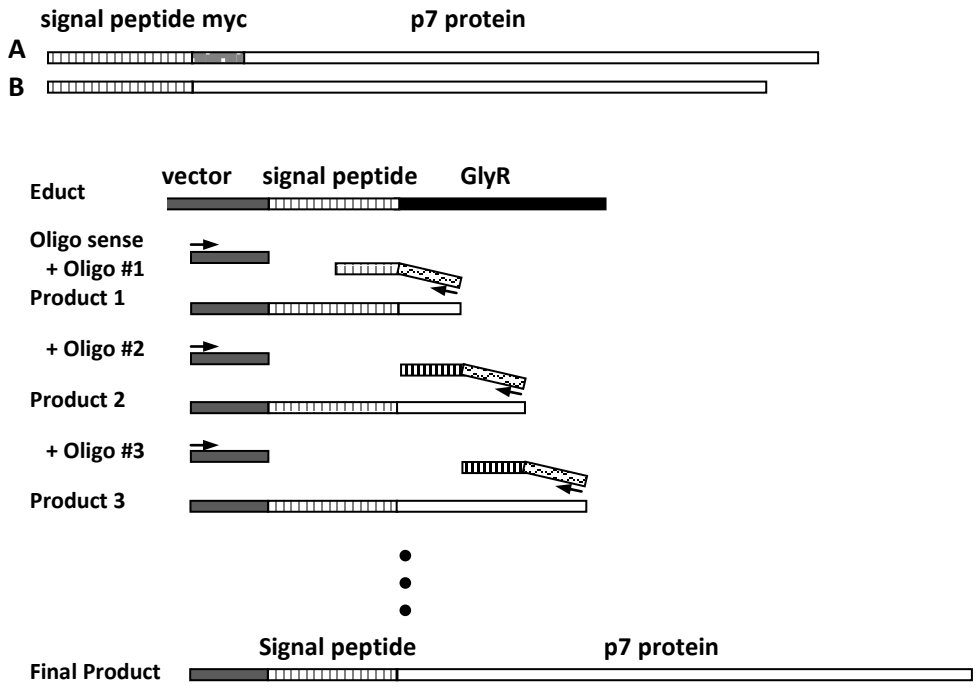

Figure 1 Generation of p7 protein constructs.

(A) p7 constructs including a myc tag (EQKLISEEDL) for Western Blot detection. (B) p7 products, lacking the myc tag, used in electrophysiological measurements. All constructs included a membrane-directing signal peptide. Dark gray – vector; light gray – signal peptide; black – glycine receptor; white – p7; dashes – ~20 base pairs non overlapping new sequence; arrows – sense and anti sense primers.

Materials and Methods - supplemental information

Generation of genotype-specific p7 cDNA contructs - PCR conditions

Standard PCR reactions were set up as follows: 0.5 ng of template DNA; 50 µM of each dATP, dCTP, dGTP, and dTTP; 0.25 µM of each primer, and 0.5 unit of high-fidelity *Taq* polymerase were completed to the end volume with supplied buffer (Roche Molecular Biochemicals, Mannheim, Germany). PCR conditions were 2 min at 95 °C for denaturation, followed by 26 cycles of 1 min at 95 °C, 0.5 min at 55 °C, and 1.0 min at 72 °C. The last cycle ended with a 2 min 72 °C amplification.

Membrane Preparation and Western Blot Analysis

HEK293 cells were harvested 3 days after transfection, taken up in PBS (1.5 mM KH_2PO_4; 6.5 mM Na_2HPO_4; 3.0 mM KCl; 137 mM NaCl), centrifuged (2000 x g, 10 min), and the pellet resuspended in 2 ml Buffer H (20 mM K-phosphate pH 7.4; 5 mM EDTA; 5 mM EGTA; protease inhibitors (c@mplete, Roche, Mannheim, Germany)). Cells were homogenized and centrifuged for 20 min at 17000 rpm twice and the final cell pellet (membrane fraction) taken up in 0.5 ml of Buffer B (Buffer H + 200 mM KCl). Protein concentration was determined by Bradford assay. For Western blot analysis, 15 µg of membrane fraction protein, or cell lysate were run on a 15 % SDS polyacrylamide gel (45 min, 120 V). Proteins were transferred onto nitrocellulose membrane using a semi-dry blotter (Bio-Rad, Munich, Germany). Blot membranes were blocked using bovine serum albumine. An alkaline phosphatase – conjugated anti c-myc antibody (SantaCruz, Heidelberg, Germany) was used to label myc-p7 protein and the blot visualized using 0.03 % nitro blue tetrazolium and 0.02 % 5-bromo-4-chloro-3-indolyl-phosphate in substrate buffer (100 mM Tris-HCl, pH 9.5; 100 mM NaCl; 5 mM $MgCl_2$).