Supplemental Materials

Molecular Biology of the Cell

Wiedmann et al.

Supplementary Materials for

N-glycosylation-dependent regulation of $hK_{2P}17.1$ currents

Felix Wiedmann[1,2,3], Daniel Schlund[1], Niels Voigt[4,5], Antonius Ratte[1,2,3], Manuel Kraft[1,2,3], Hugo A. Katus[1,2,3], Constanze Schmidt[1,2,3]

[1]Department of Cardiology, University of Heidelberg, Heidelberg, Germany; [2]DZHK (German Center for Cardiovascular Research), partner site Heidelberg /Mannheim, University of Heidelberg, Germany; [3]HCR, Heidelberg Center for Heart Rhythm Disorders, University of Heidelberg, Heidelberg, Germany; [4]Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Georg-August University Göttingen, Germany; [5]DZHK (German Center for Cardiovascular Research), partner site Göttingen, University Göttingen, Germany.

Corresponding author:

PD Dr. med. Constanze Schmidt, FESC

Department of Cardiology

Medical University Hospital Heidelberg

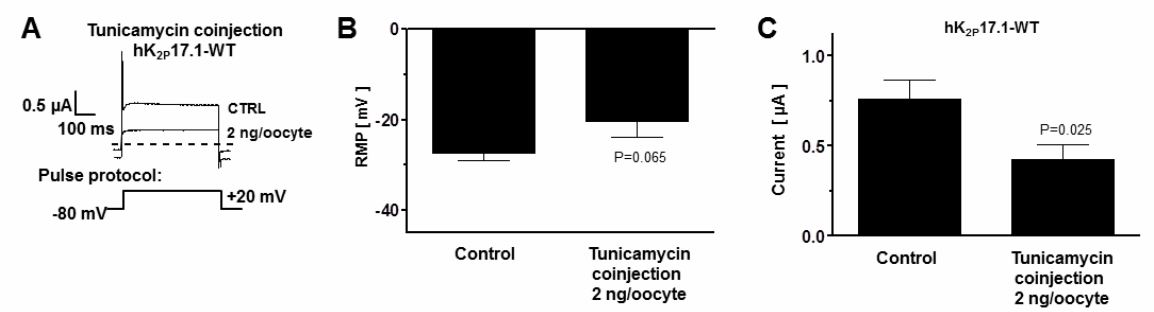
Im Neuenheimer Feld 410

D-69120 Heidelberg, Germany

Tel.: ++49 6221 568187

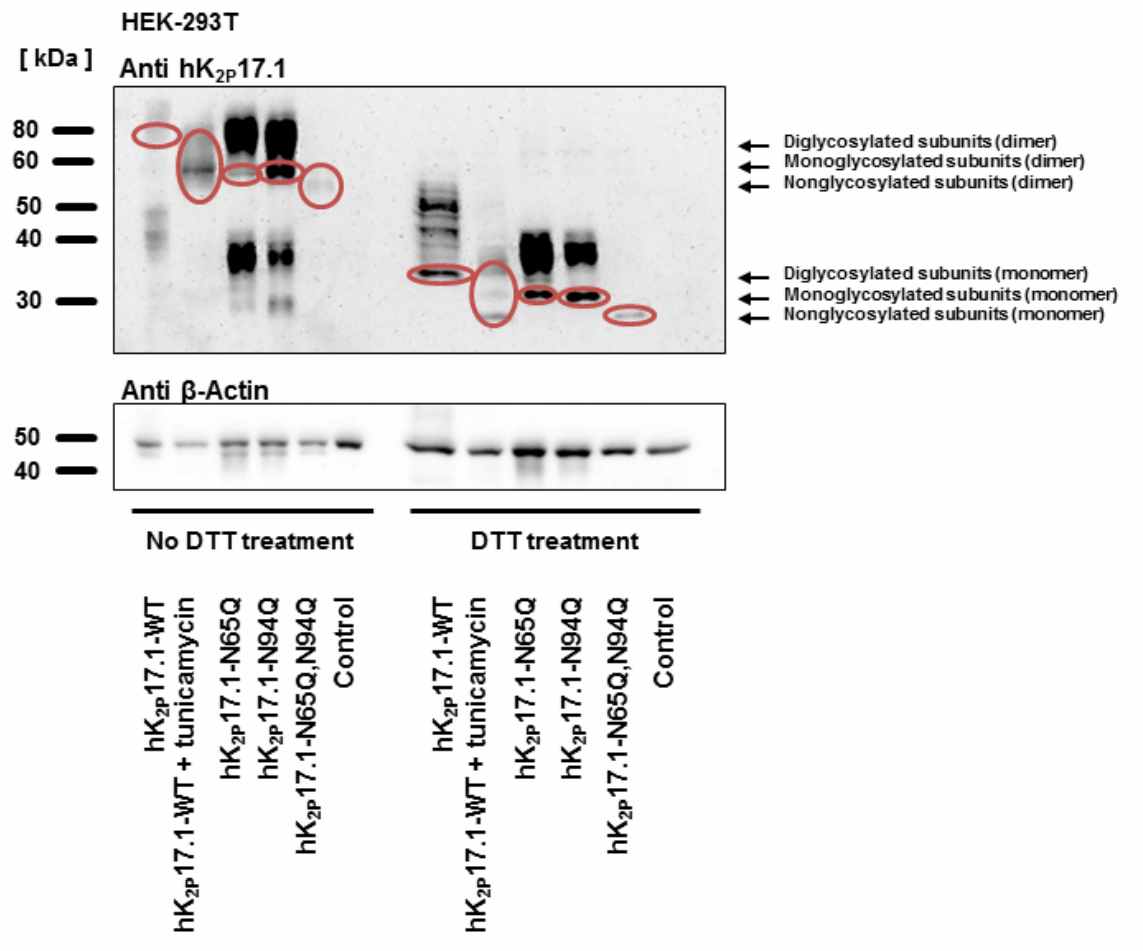
Fax: ++49 6221 56 5724

E-Mail: Constanze.Schmidt@med.uni-heidelberg.de



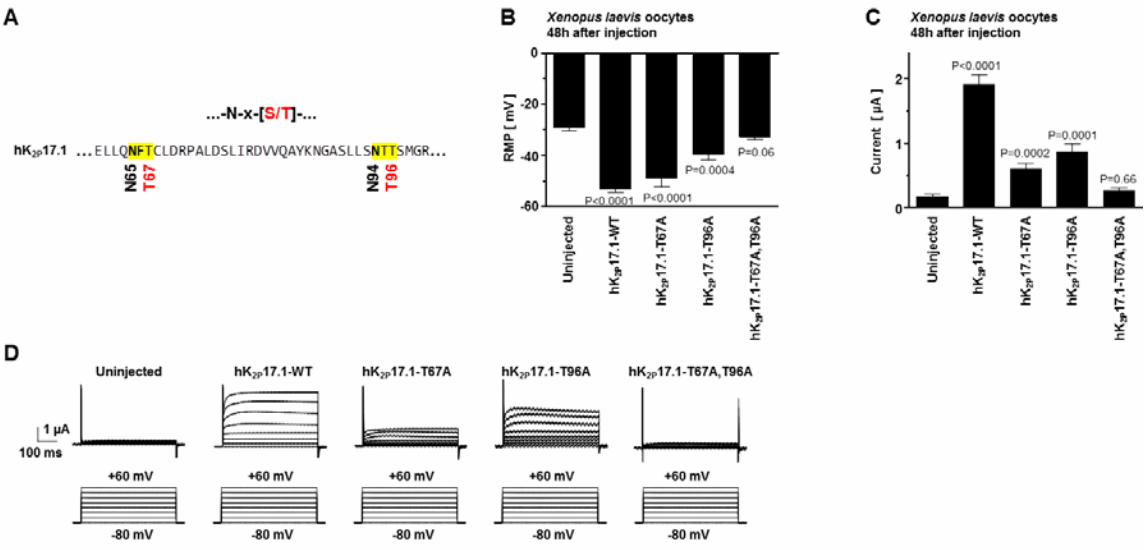
Supplemental figure 1: Intracytoplasmic injection of tunicamycin reduces currents of $hK_{2P}17.1$ channels, heterologously expressed in *Xenopus* laevis oocytes

To probe whether the route of tunicamycin administration influences its effect on $hK_{2P}17.1$ channels, tunicamycin was coinjected together with the cRNA and measurements were performed 48h after injection. (A) Representative current traces of *Xenopus* oocytes injected with $hK_{2P}17.1$-WT cRNA and 2ng tunicamycin are compared to control cells (CTRL) injected with $hK_{2P}17.1$-WT cRNA plus the vehicle DMSO. (B) Resting membrane potentials (RMP) of *Xenopus* oocytes after coinjection with tunicymycin showed a trend towards depolarization, compared to control cell. (C) After coninjection with tunicamycin $hK_{2P}17.1$ currents were significantly diminished. Measurements were performed using the pulse protocol provided. Data are presented as mean ± SEM. P-values of two-tailed students t-tests are given as inserts. Dashed lines indicate 0 mV.



Supplemental figure 2: Disruption of hK$_{2P}$17.1 *N*-glycosylation by asparagine to glutamine mutagenesis does not prevent channel dimerization

hK$_{2P}$17.1-WT, hK$_{2P}$17.1-N65Q, hK$_{2P}$17.1-N94Q or hK$_{2P}$17.1-N65Q,N94Q channel subunits were heterologously expressed in HEK-239T cells and subjected to SDS-PAGE either without (left) or with (right) DTT treatment. For the purpose of clear presentation specific bands are marked in red. Note that hK$_{2P}$17.1-N65Q, hK$_{2P}$17.1-N94Q and hK$_{2P}$17.1-N65Q,N94Q mutant constructs can be detected as dimers, too. Non-transfected negative control experiments and samples of cells grown in the presence of the antibiotic *N*-glycosylation inhibitor tunicamycin (1 µg/ml) have been included as denoted.



Supplement figure 3: Disruption of $hK_{2P}17.1$ *N*-glycosylation by alanine-mutagenesis of the *N+2* amino acid of the *N*-glycosylation consensus motive

(A) Amino acid (AA) sequence of $hK_{2P}17.1$, threonine residues 67 and 96 as essential parts of the N-glycosylation consensus motives (N-x-[S/T], where x can be any AA except proline) around asparagine 65 and 94 are highlighted. (B) *Xenopus* oocytes expressing $hK_{2P}17.1$-WT, $hK_{2P}17.1$-T67A or $hK_{2P}17.1$-T96A display resting membrane potential (RMP)hyperpolarization while expression of $hK_{2P}17.1$-T67A,T96A double mutant constructs does not result in statistically significant changes of the RMP.(C) Single mutant channel subunits $hK_{2P}17.1$-T67A and $hK_{2P}17.1$-T96A give rise to outward potassium currents, when expressed in *Xenopus* oocytes. After injection of $hK_{2P}17.1$-T67A,T96A cRNA potassium currents did not differ from uninjected oocytes. (D)Representative families of current traces, elicited by the pulse protocols depicted. All measurements were performed 48h after cRNA injection using the pulse protocol provided. Data are presented as mean ± SEM. P-values of two-tailed students t-tests vs. uninjected cells are given as inserts. Dashed lines indicate 0 mV.