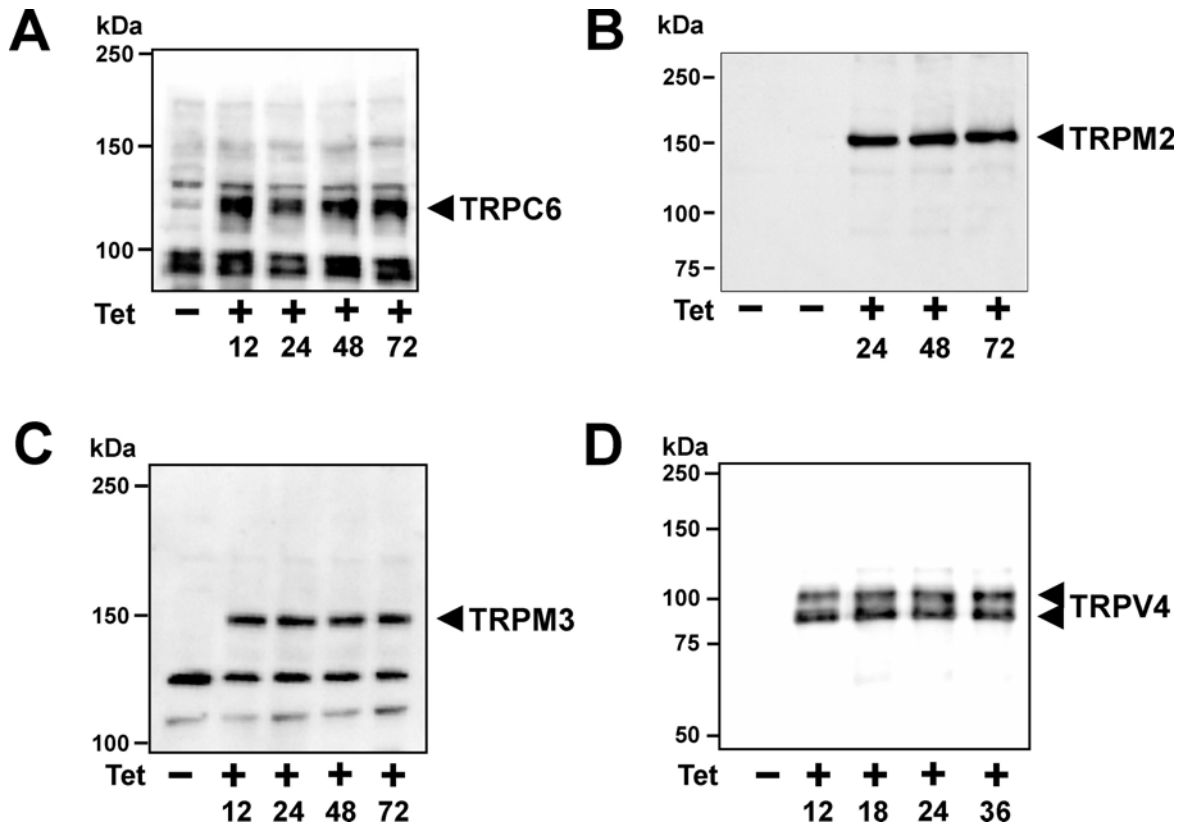


**Figure S1: Biochemical characterisation of the stably TRP channel-expressing cell lines.**

For the Western blot analysis of the tetracycline-inducible expression of TRP channels, membrane proteins extracted from cell line stably expressing of TRPC6 (A), TRPM2 (B), TRPM3 (C) and TRPV4 (D) grown in the absence or presence of tetracycline (2.5 mg/ml) were separated by SDS-PAGE electrophoresis and transferred onto nitrocellulose membranes as previously described Kraft et al. (2006). Immobilized proteins were visualized by using specific antibodies. The anti-TRPC6 antibody was from Millipore (Schwabach, Germany). The generation and characterization of the antibodies directed against TRPM2, TRPM3 and TRPV4 have been previously described (Grimm *et al.*, 2003; Kraft *et al.*, 2006; Reiter *et al.*, 2006). The anti-TRPV4 antibody visualized two bands, the glycosylated and unglycosylated TRPV4 protein, described by Xu et al (Xu *et al.*, 2006).

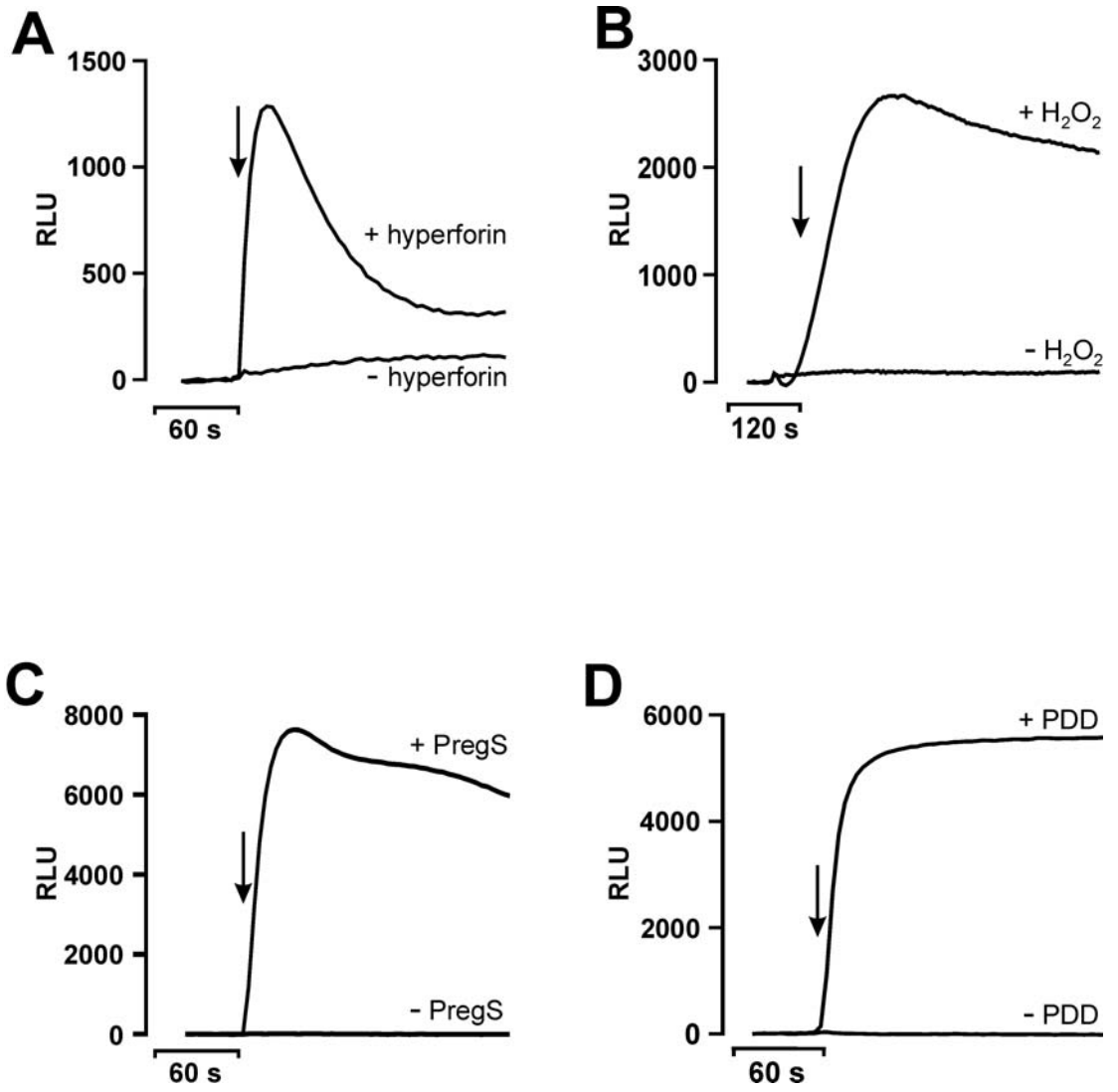


**Figure S2: Functional characterisation of the stable TRP channel-expressing cell lines.**

Cells stably expressing the TRP channel in a tetracycline-dependent manner were seeded and expression was induced by the addition of tetracycline (2.5 mg/ml). For calcium imaging, cells were loaded with Fluo-4. Fluo-4-dependent fluorescence was recorded in the presence or absence of the specific stimulus (arrow). A) TRPC6-expressing cells were stimulated with hyperforin (10 µM). B) TRPM2-expressing cells were stimulated with hydrogen peroxide ($H_2O_2$; 5 mM). C) TRPM3-expressing cells were stimulated with pregnenolone sulphate (PregS; 35 µM). D) TRPV4-expressing cells were stimulated with 4α-phorbol-didecanoate (PDD; 5 µM). Shown are representative traces recorded from 10,000 cells. Subsequently to

the initial validation of functional expression, optimal time periods in the presence of tetracycline were determined. The optimal expression levels of TRPC6, TRPM2, TRPM3 and TRPV4 were achieved 72, 24, 72 and 20 h after expression induction by tetracycline, respectively (data not shown). The incubation time have been determined experimentally and represent a compromise between optimal signal resulting from expression level and signal-to-noise ratio determined by the loss of cells during loading and washing procedures due to increased intracellular calcium concentration in TRP channel expressing cells leading to rounding and displacing of the cells.

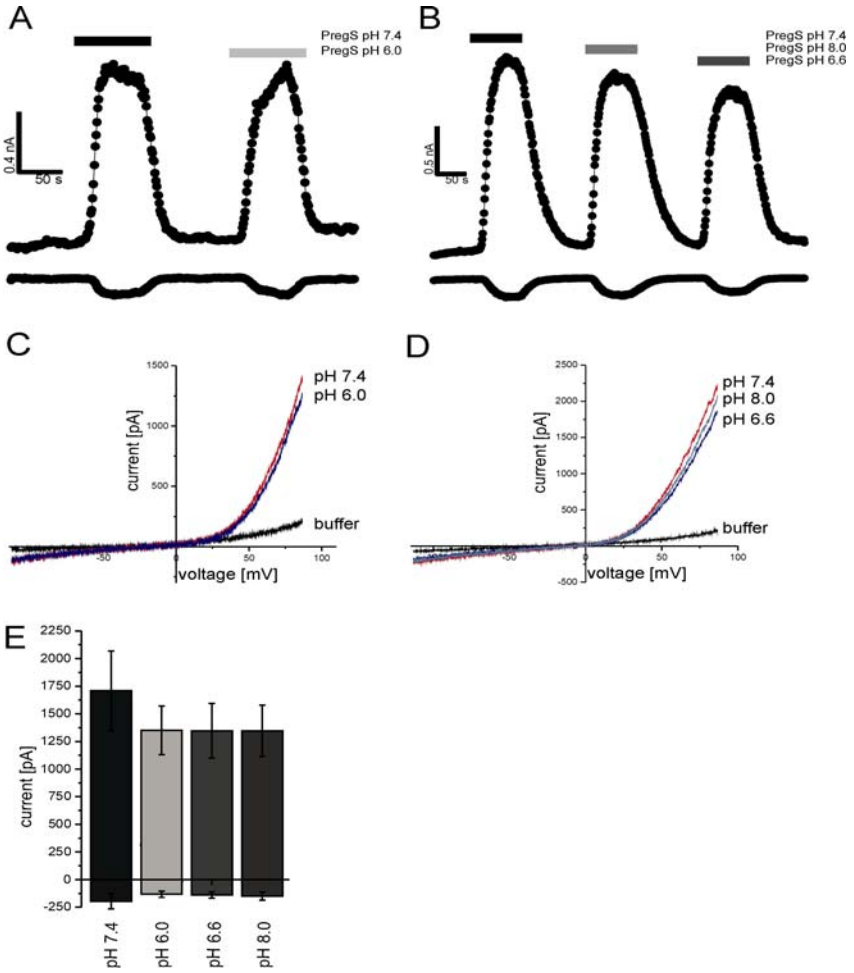


**Figure S3: Activation of TRPM3 by pregnenolone sulphate is independent of pH**. **A** Currents of TRPM3 at membrane potentials of -80 (upper trace) and +80 mV (lower trace) recorded during extracellular application of the TRPM3 activator pregnenolone sulphate (35 µM) diluted in extracellular solutions with pH adjusted to 7.4 or 6.0 as indicated. **B** Currents obtained under comparable experimental conditions as used in (A), however the pH of the extracellular solutions was adjusted to pH 7.4, 8.0 or 6.6 as indicated. **C/D** Current-voltage relationship from experiments shown in **A/B,** respectively, show that the pregnenolone sulphate-dependent stimulation of TRPM3 is independent of extracellular pH. **E** Statistical analysis of experiments performed at pH 7.4 (n = 5), pH 6.0 (n = 7), pH 6.6 (n = 6) and pH 8.0 (n = 6).

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