

SUPPLEMENTARY INFORMATION**Pregnenolone sulphate-independent inhibition of TRPM3 channels by progesterone**

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Supplementary figure legends

Supplementary Figure I. PregS-evoked calcium signals in HEK 293 cells depend on exogenous TRPM3 expression. Mean data from intracellular Ca²⁺ measurement experiments comparing responses evoked by 1 μM PregS in cells over expressing TRPM3 (Tet+), control non-induced (Tet-) cells, or wild-type (WT) HEK 293 cells (N/n = 24-36/3). For example traces, see Supplementary Fig. IV.

Supplementary Figure II. Effects of additional progesterone analogues on TRPM3-mediated Ca²⁺-influx. Data were generated by intracellular Ca²⁺ measurement. Mean normalised data are shown for the effects of 25 μM each of pregnanolone (5β) or allopregnanolone (5α) on Ca²⁺ responses to 10 μM PregS (N/n = 9/3 for each).

Supplementary Figure III. Additional 2-dimensional chemical structures. Chemical structures of 17-hydroxy progesterone, 21-hydroxy progesterone, pregnanolone, allopregnanolone, 17β-oestradiol, aldosterone, cortisol, and nifedipine.

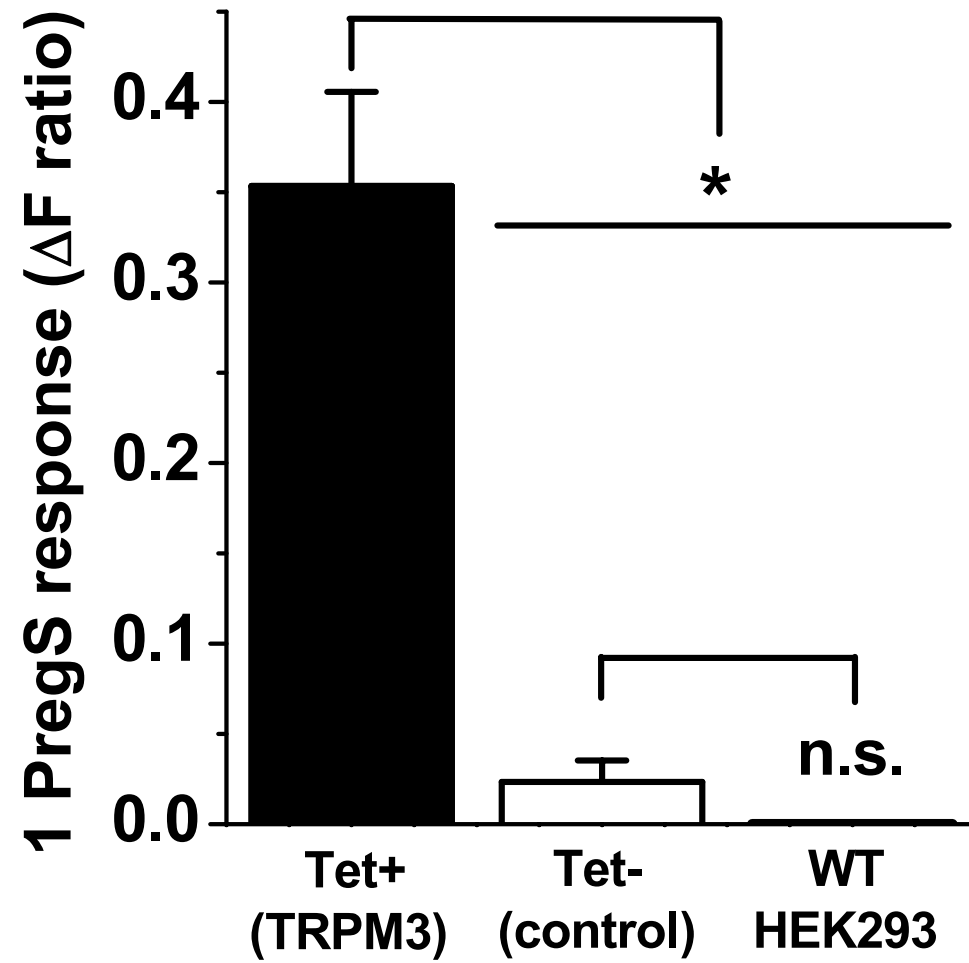
Supplementary Figure IV. TRPM3 activity in the absence of an exogenous agonist. a, b. Data were generated by intracellular Ca²⁺ measurement. **a.** Example traces showing the effect of 1 μM PregS in cells over-expressing TRPM3 (Tet+), control (Tet-) cells, or wild-type (WT) HEK 293 cells (N = 8-12). **b.** Mean data comparing basal Ca²⁺ levels in the indicated groups (N/n = 24-36/3). **c, d.** Whole-cell voltage-clamp was performed in cells over-expressing TRPM3 (Tet+) or control (Tet-) cells. Statistical comparisons were made between data from these two types of cell. **c.** Mean data for basal currents recorded in cells over-expressing TRPM3 (Tet+) or control (Tet-) cells. **d.** Mean data for the currents stimulated by 100 μM PregS in Tet+ and Tet- cells in the recordings shown in **c** (n = 3).

Supplementary Figure V. Detection of agonist-independent TRPM3 activity. Data were generated by intracellular Ca²⁺ measurement. **a, b.** Cells were incubated in Ca²⁺-free SBS for 30

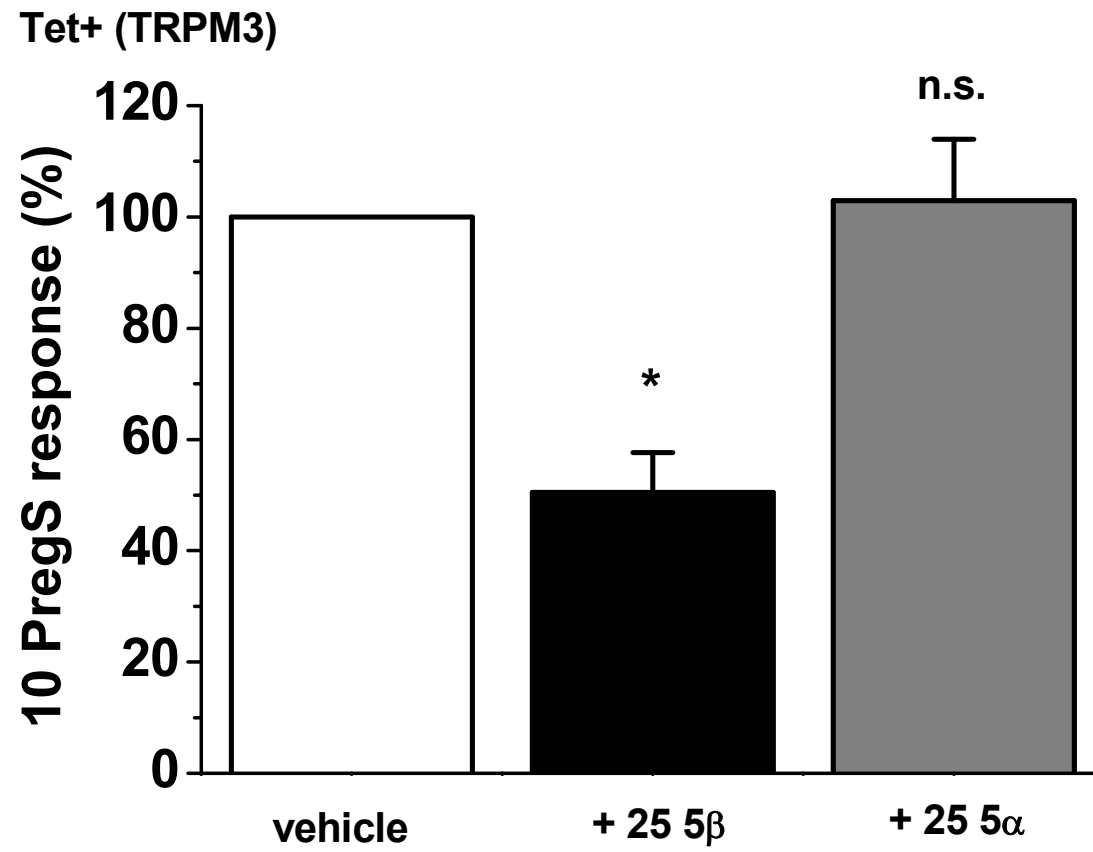
min and then Ca^{2+} was returned (“add-back”) at 1, 3 or 5 mM in Tet+ (TRPM3) (**a**) or Tet- (control) (**b**) cells (N=4 for each condition).

Supplementary Figure VI. A-type potassium current in granulosa cells. Example whole-cell patch-clamp recording of an endogenous potassium current in freshly isolated bovine granulosa cells. The voltage paradigm is depicted in the inset.

Supplementary Figure VII. PregS potency at room temperature and 37 °C. Data are from intracellular Ca^{2+} measurements in cells over-expressing TRPM3 (Tet+). Concentration-response curves for PregS at room temperature (Room temp., white circles) or at 37 °C (black circles). The fitted Hill equations had EC_{50} and slope values of: 1.21 μM and 1.34 (Room temp.), and 1.83 μM and 1.19 (37 °C) (N/n = 9/3 for each).

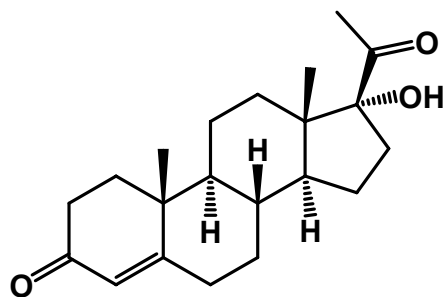


Supplementary figure I

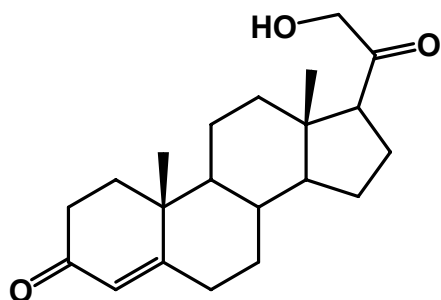


Supplementary figure II

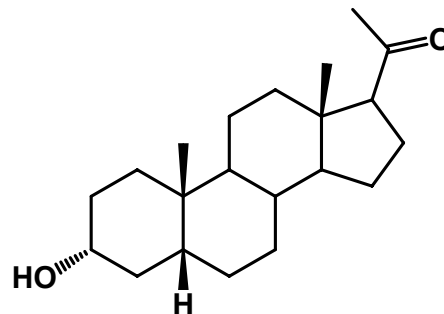
17-OH progesterone



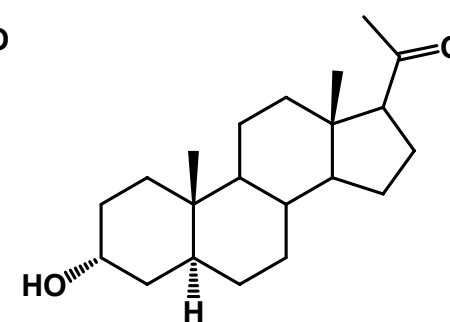
21-OH progesterone



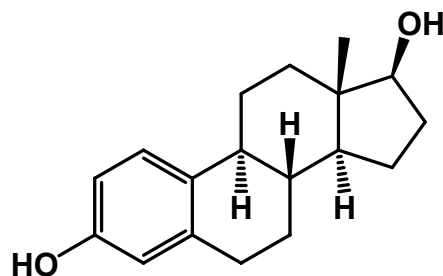
pregnanolone (5 β)



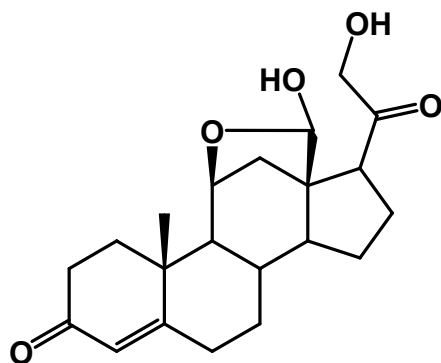
allopregnanolone (5 α)



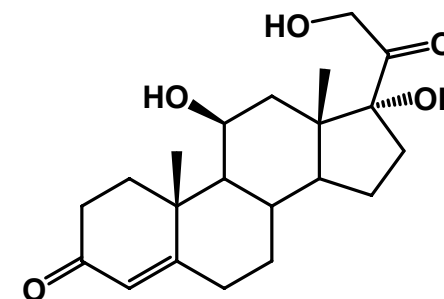
17 β -oestradiol



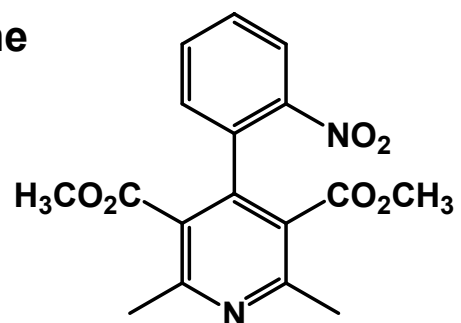
aldosterone

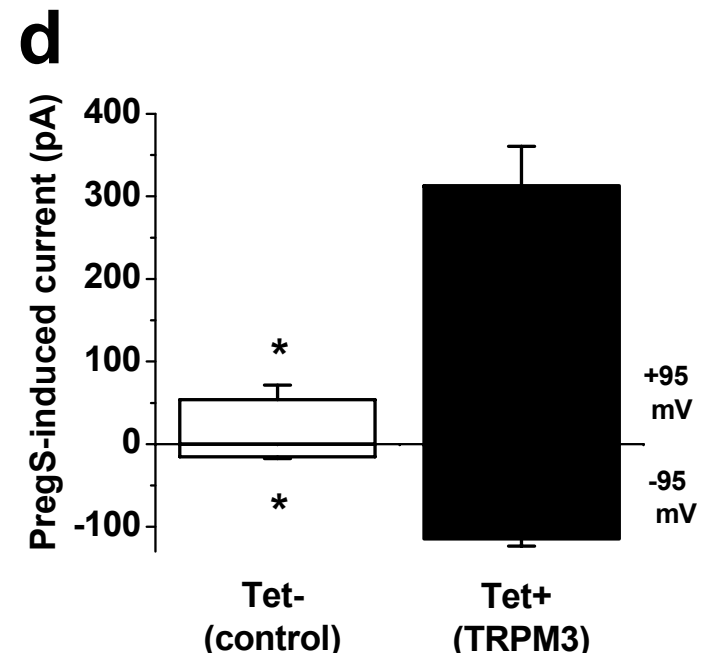
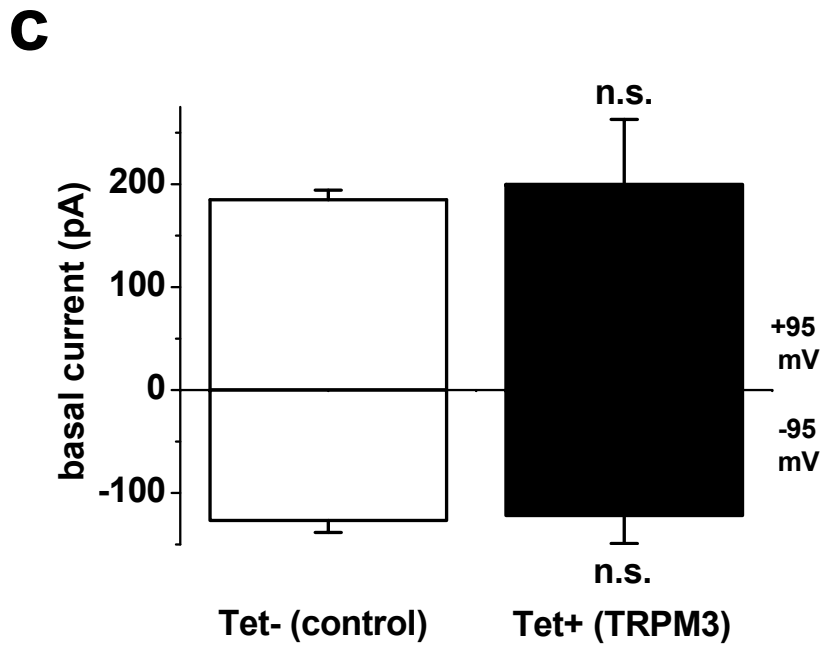
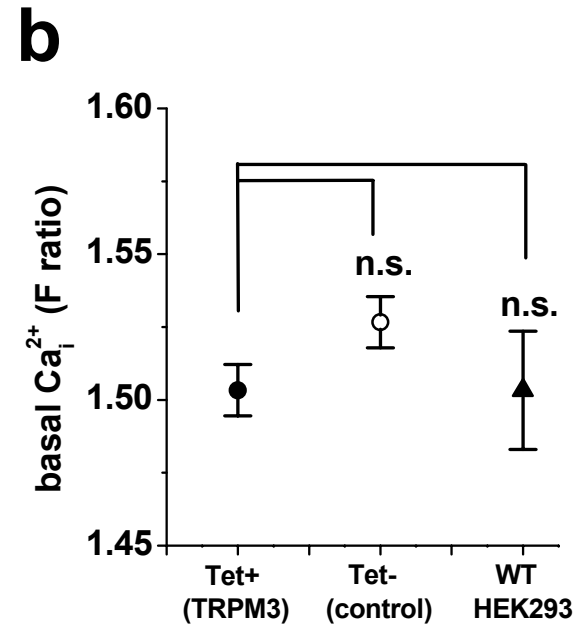
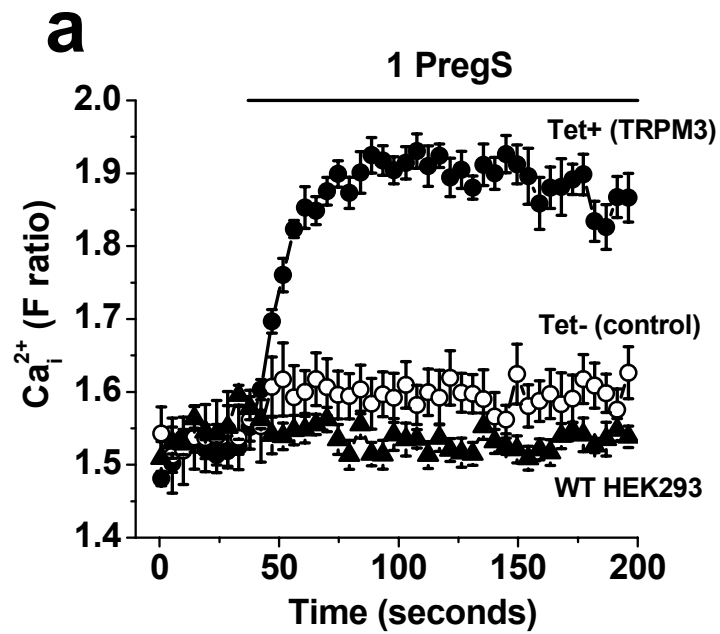


cortisol

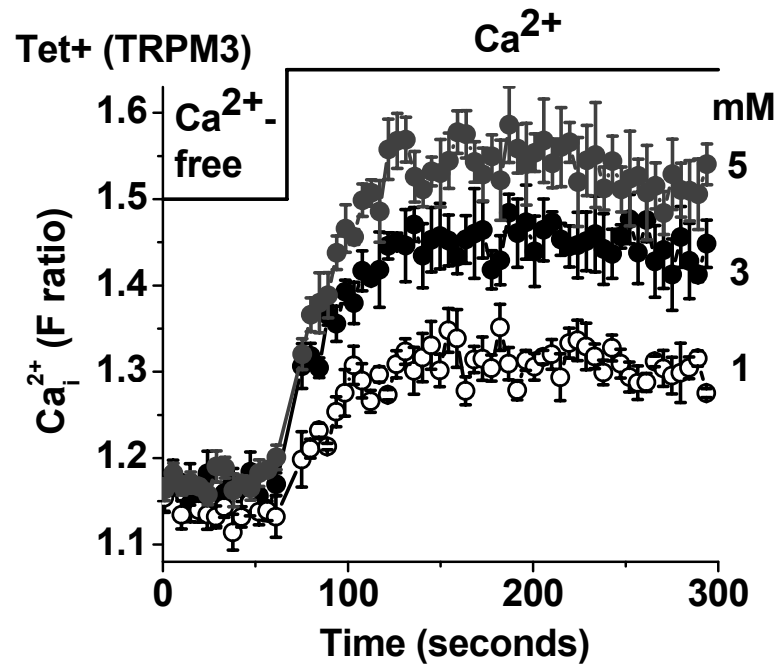
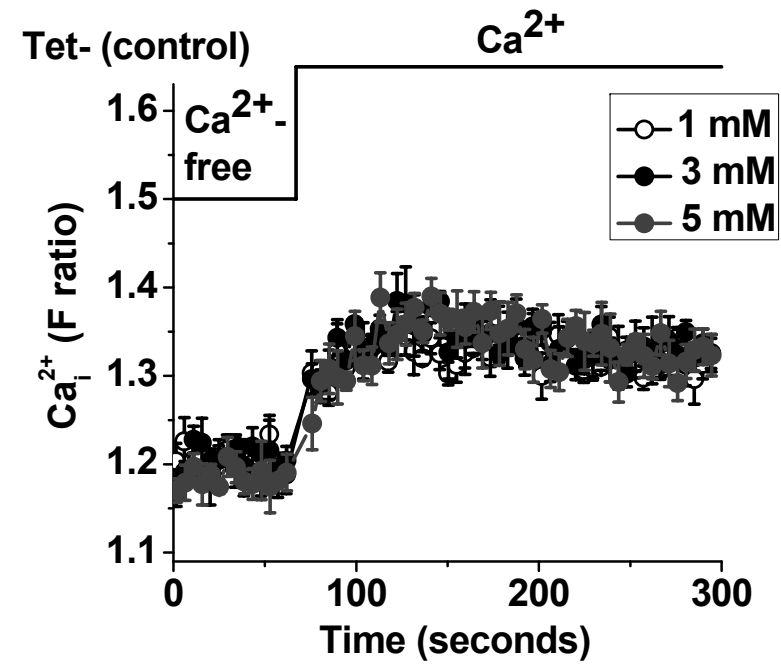


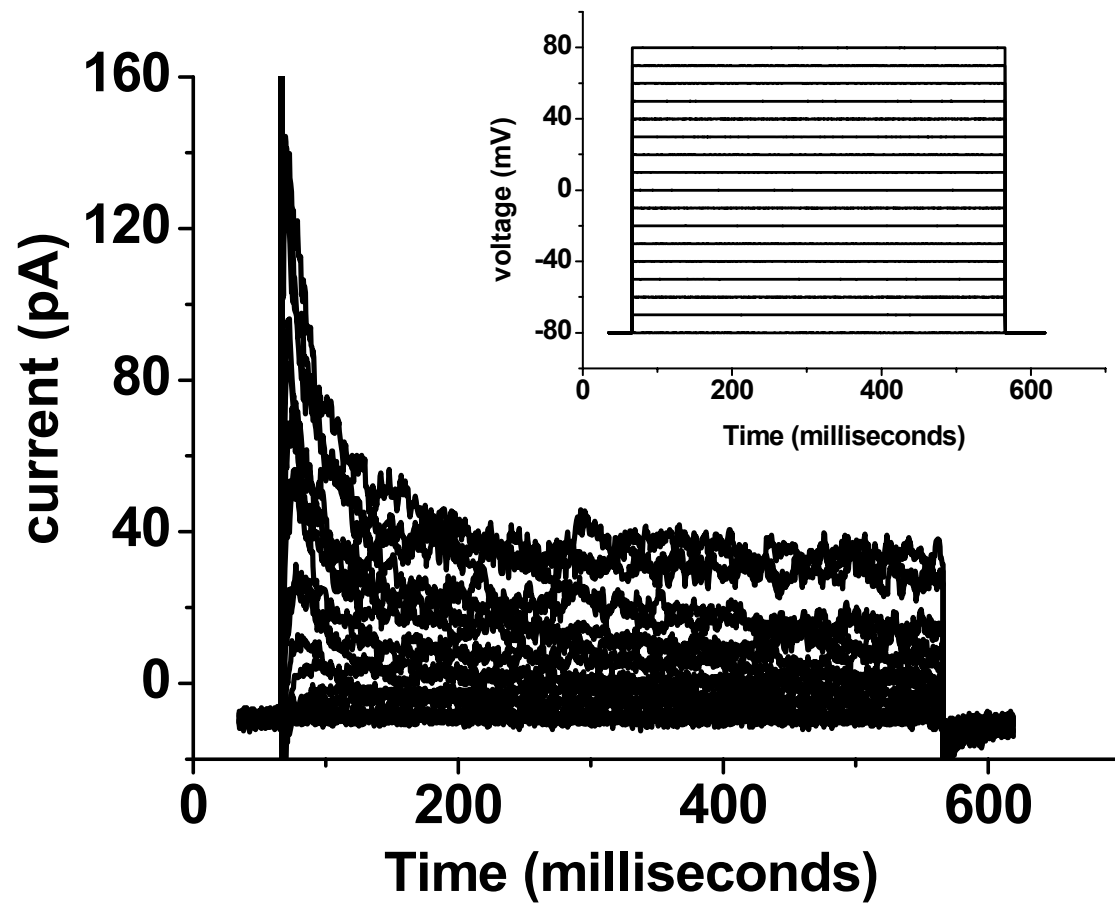
nifedipine



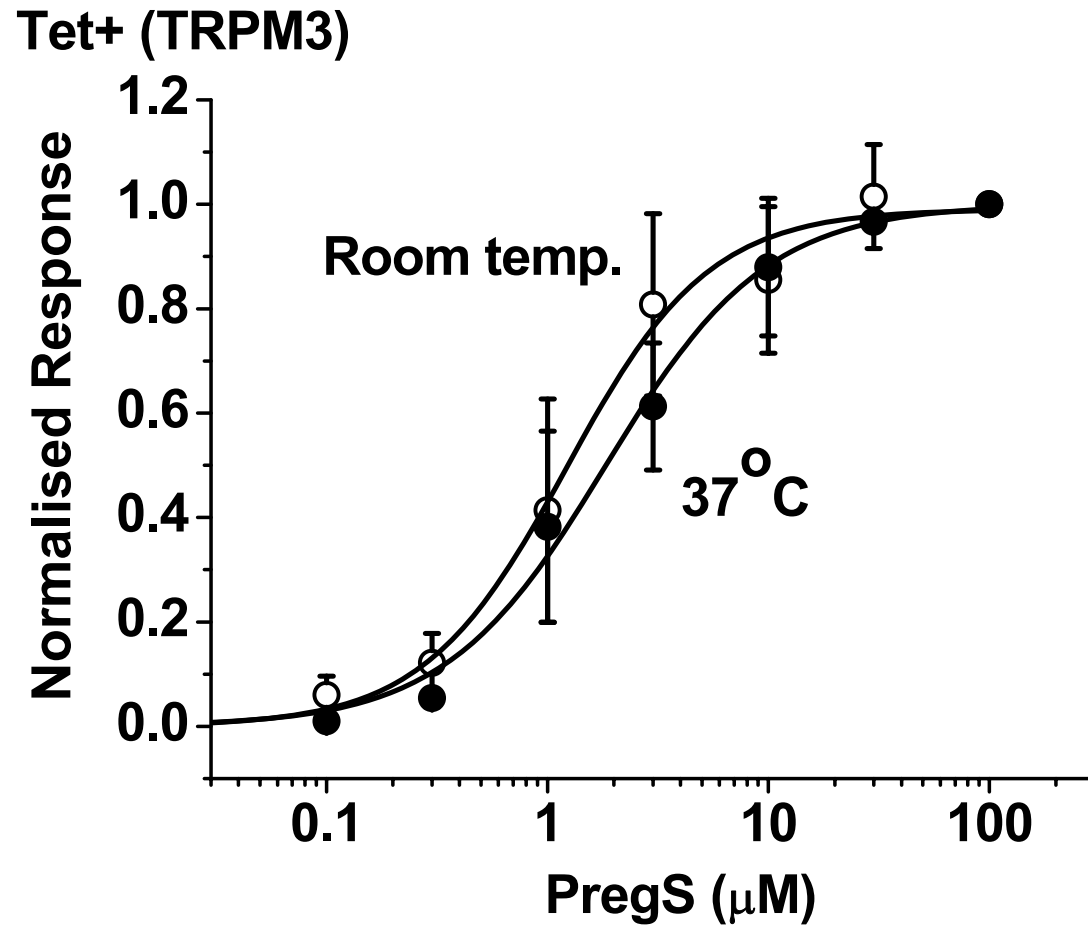


Supplementary figure IV

a**b**



Supplementary figure VI



Supplementary figure VII