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multimedia online data

SUPPLEMENTARY ONLINE DATA

2-APB-potentiated channels amplify CatSper-induced Ca^{2+} signals in human sperm

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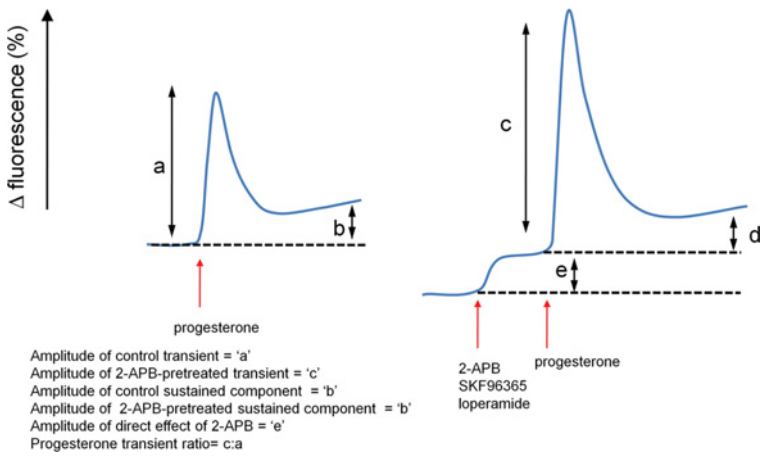

Figure S1 Diagrammatic illustration showing quantified components of $[Ca^{2+}]_i$ traces in control experiments (left) and after pre-treatment with 2-APB (right)

'a' and 'b' show transient and sustained response amplitudes under control conditions. 'c' and 'd' show transient and sustained response amplitudes in 2-APB and loperamide experiments. 'e' shows the amplitude of response to 2-APB or loperamide.

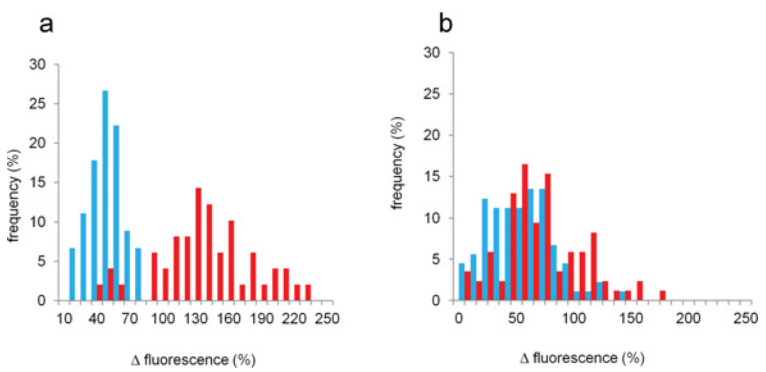

Figure S2 Amplitude distribution for single-cell progesterone transients recorded at the PHN from two pairs of experiments

In each graph, blue bars show the amplitude distribution for the control experiment and red bars show the distribution for a parallel experiment where cells were pre-treated with 5 μM 2-APB. In experiment (**a**), most cells show a large shift to the right after 2-APB pre-treatment but approximately 10 % are clustered at amplitudes similar to the control mean. In experiment (**b**), 2-APB increases the transient amplitude in only a subset of cells, the distribution peak remaining at a ΔF of ~70 %.

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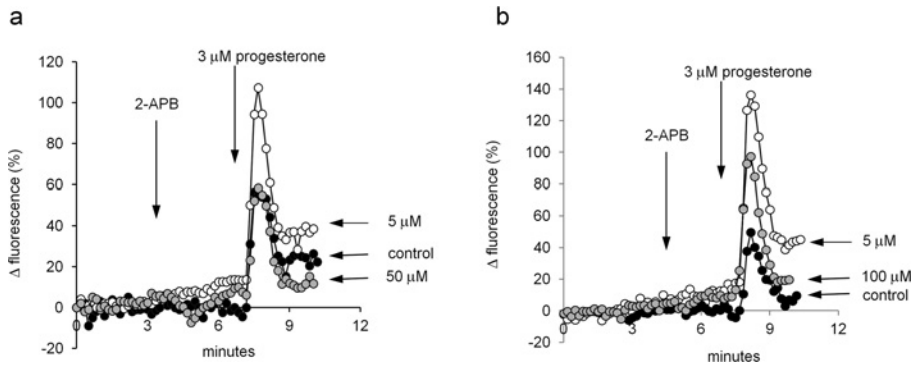

Figure S3 Dose-dependence of effect of pre-treatment with 2-APB on the $[Ca^{2+}]_i$ transient induced by 3 μM progesterone

2-APB (except controls) was added at the first arrow, progesterone (3 μM) was added at the second arrow. (**a**) Black circles, vehicle control; white circles, 5 μM 2-APB; grey circles, 50 μM 2-APB. (**b**) Black circles, vehicle control; white circles, 5 μM 2-APB; grey circles, 100 μM 2-APB.

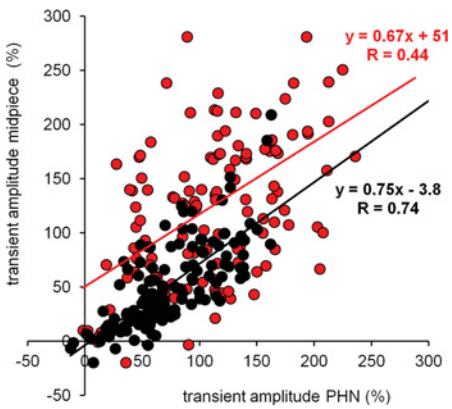

Figure S4 Relationship between the amplitude of the progesterone-induced $[Ca^{2+}]_i$ transient recorded at the PHN (*x* axis) and midpiece (*y* axis)

Under control conditions (black circles), midpiece amplitude is typically $\sim$75 % of amplitude at PHN ($y = 0.75\times - 3.8$). In 5 μM 2-APB-pretreated cells (red circles), both PHN and midpiece transients are larger but also the line of best fit is 'shifted' upward due to recruitment of an extra midpiece component in a sub-population of cells ($y = 0.67\times 51$). The results are from five pairs of experiments; each data set shows $>$130 cells.

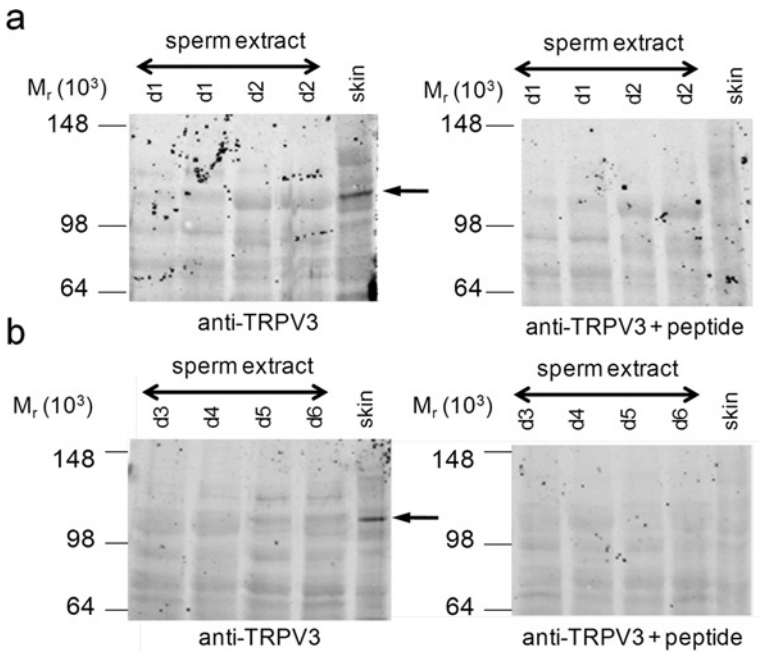

Figure S5 TRPV3 is not detectable in human sperm

(**a**) The left-hand panel shows the immunoblot for TRPV3 with sperm preparations from two different donors (d1 and d2). The final lane shows the positive control (human keratinocyte proteins; skin). The arrow on lane 5 shows the strong band corresponding to the predicted mass for TRPV3. The right-hand panel shows an identical blot, carried out in parallel, after pre-adsorbing the TRPV3 antibody with the antigenic peptide. The band detected in the positive control has been lost. (**b**) As for (**a**), but using four further donors (d3–d6).

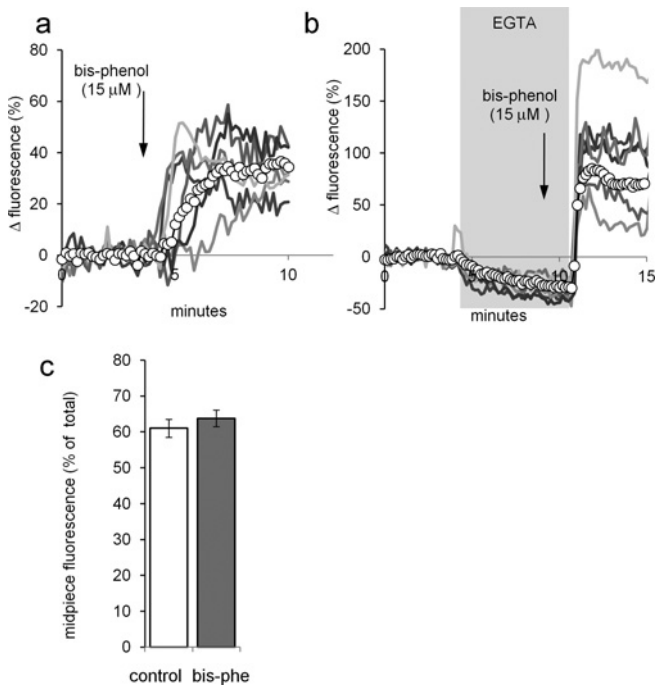

Figure S6 Activation of SOCs does not cause redistribution of STIM1

(**a**) Bis-phenol (15 μM), an inhibitor of Ca^{2+}-store ATPases, causes a sustained increase in $[Ca^{2+}]_i$. Six representative single-cell responses and the means for all cells in the experiment (circles) are shown. (**b**) In EGTA-buffered saline, bis-phenol fails to significantly increase $[Ca^{2+}]_i$, but upon reintroduction of 1.8 mM Ca^{2+}, a large prolonged $[Ca^{2+}]_i$ elevation was seen, indicating the activation of SOCs by store depletion. (**c**) Intensity of immunofluorescent staining of STIM1 in the sperm midpiece (as a proportion of total fluorescence of the sperm) under control conditions (white bar) and after incubation for 12 min in the presence of 15 μM bis-phenol (grey bar). Each bar shows the mean $\pm$ S.E.M. of fluorescence in 170 cells from three experiments.

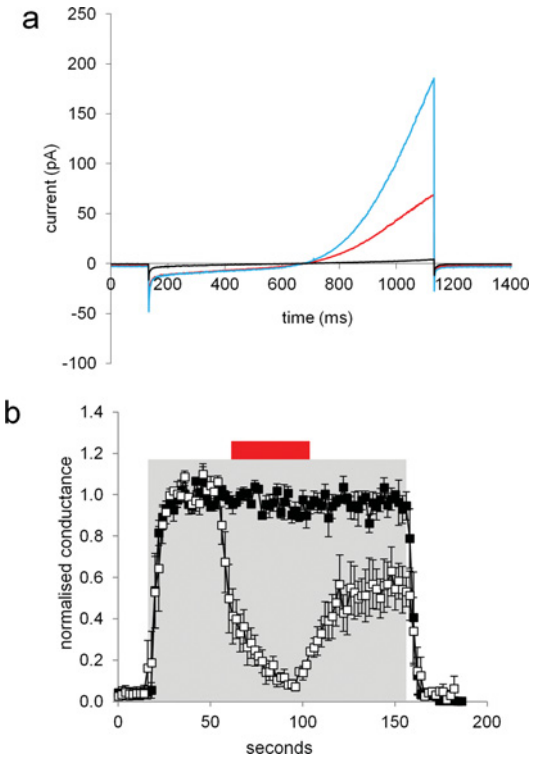

Figure S7 Loperamide does not enhance currents through CatSper channels

(**a**) Currents in a whole-cell clamped sperm induced by a 1 s voltage ramp from -80 mV to $+80$ mV. Black trace shows current in HS medium with 2 mM Ca^{2+} [1]. Blue trace shows current in DVF (divalent cation-free) medium. Red trace shows current in the presence of 10 μM loperamide. (**b**) Time-course of inhibition by 10 μM loperamide of outward conductance (normalized to maximum). Filled squares show control experiments where conductance is reversibly enhanced in DVF medium (shading). Open squares show the effect of 10 μM loperamide (red bar). Each line shows the mean of four experiments $\pm$ S.E.M. Conductance was calculated using $\delta I/\delta V$ at 70–80 mV.

REFERENCE

1 Lishko, P. V., Botchkina, I. L. and Kirichok, Y. (2011) Progesterone activates the principal Ca^{2+} channel of human sperm. Nature **471**, 387–391

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