Ernesto et al., http://www.jcb.org/cgi/content/full/jcb.201412041/DC1

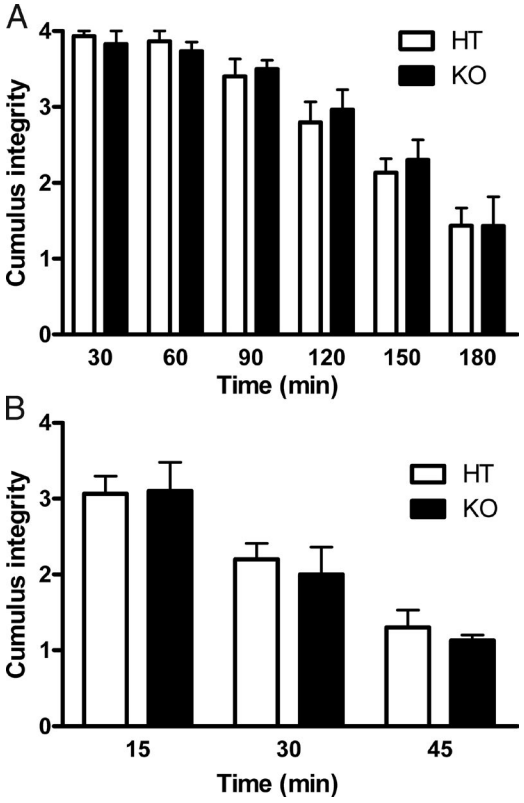

Figure S1. **Effect of CRISP1 on cumulus integrity.** (A) COC from *Crisp1*$^{+/-}$ (HT) or *Crisp1*$^{-/-}$ (KO) animals were incubated up to 180 min and their integrity was evaluated at different intervals. Cumulus integrity was classified as 4 when COC were intact, as 0 when eggs were completely denuded of cumulus cells, and as 1, 2, or 3 for intermediate stages. (B) COC from *Crisp1*$^{+/-}$ and *Crisp1*$^{-/-}$ animals were incubated in the presence of hyaluronidase during 15, 30, or 45 min and cumulus integrity was analyzed as described in A. Results represent the mean ± SEM of three independent experiments.

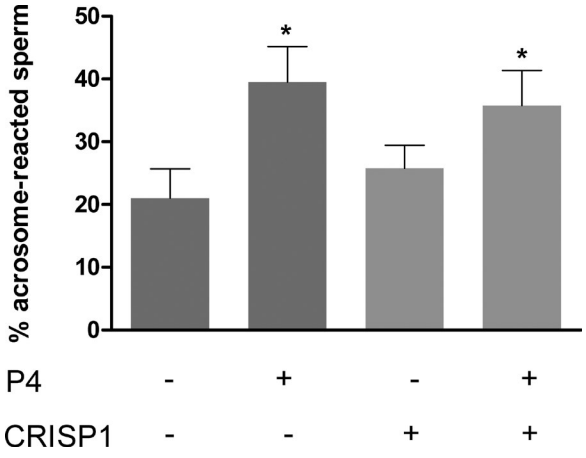

Figure S2. **Effect of CRISP1 on the occurrence of spontaneous or progesterone-induced AR.** Capacitated sperm were exposed to 10 µM CRISP1 and/or 15 µM progesterone and their acrosomal status was analyzed by staining the cells with Coomassie brilliant blue. Results represent the mean ± SEM of four independent experiments in which at least 350 sperm per experiment were analyzed. *, $P < 0.05$ vs. control without progesterone.

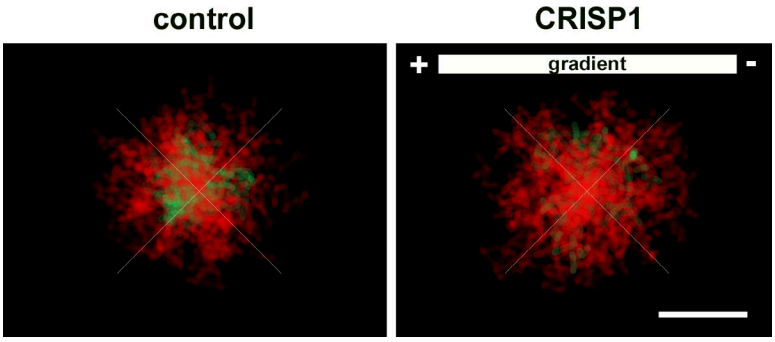

Figure S3. **Trajectories observed for mouse sperm in the modified Zigmond chamber.** All tracked sperm trajectories from a representative experiment were plotted positioning the first point of each trajectory in the origin. Traces were analyzed using the Processing 2 software. Hyperactivated trajectories are represented in green whereas both linear and transitional trajectories are represented in red. (left) Sperm trajectories in medium. (right) Sperm trajectories in a CRISP1 gradient along the x (horizontal) axis. Bar, 100 µm.

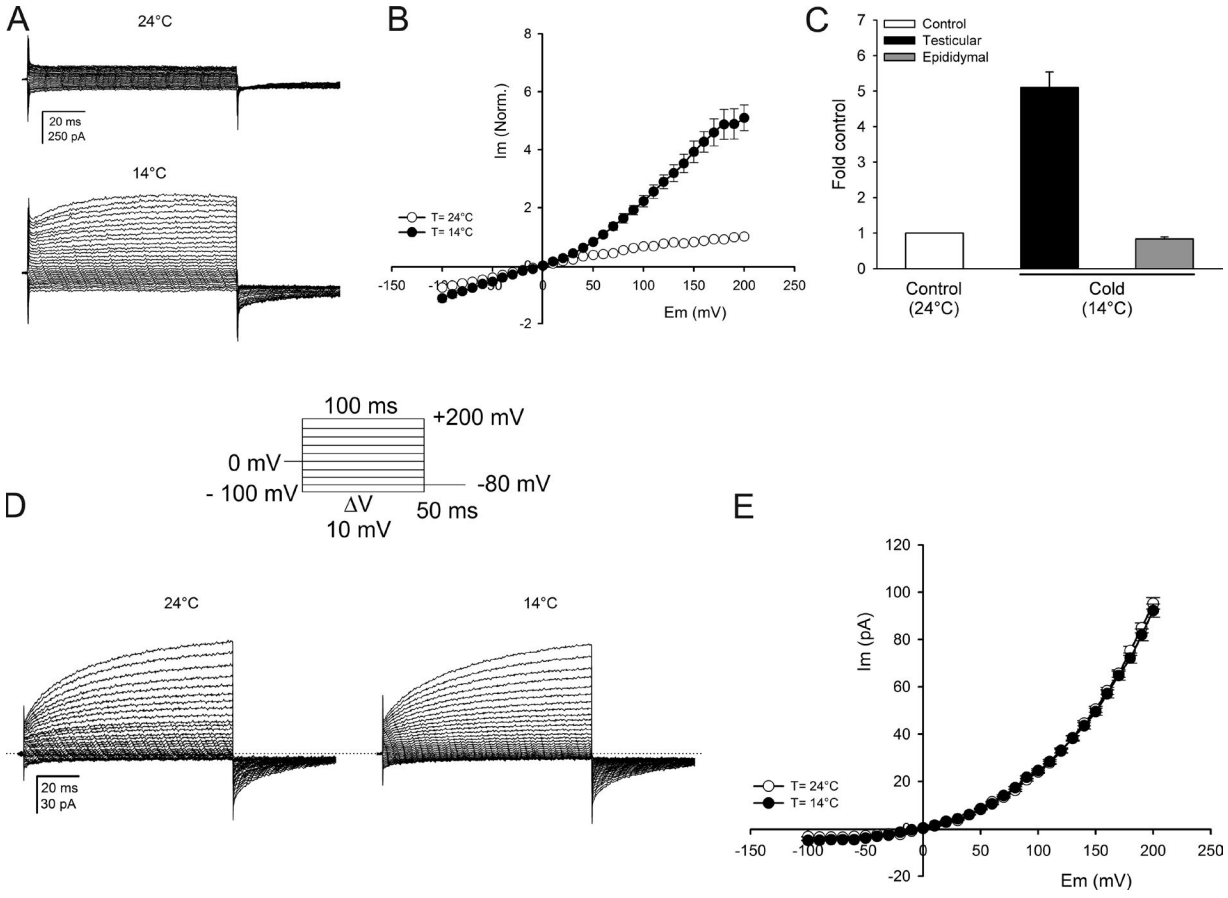

Figure S4. **Cold-activated TRPM8 currents in testicular sperm.** (A) Representative whole-cell currents were measured using TRPM8-recording conditions and the voltage protocol shown in D at the indicated temperatures. The current obtained was responsive to cold temperature and voltage when recorded from testicular sperm. (B) The I-V relationship shows the cold temperature activated effect on the TRPM8 currents. (C) A temperature change from 24°C to 14°C resulted in a fivefold current activation (Q_{10} = 5). Data represent the mean ± SEM of four different sperm. (D and E) When epididymal sperm under the same experimental conditions as in A were used, we failed to record cold-activated currents such as those reported previously (Gibbs et al., 2011; Martínez-López et al., 2011).

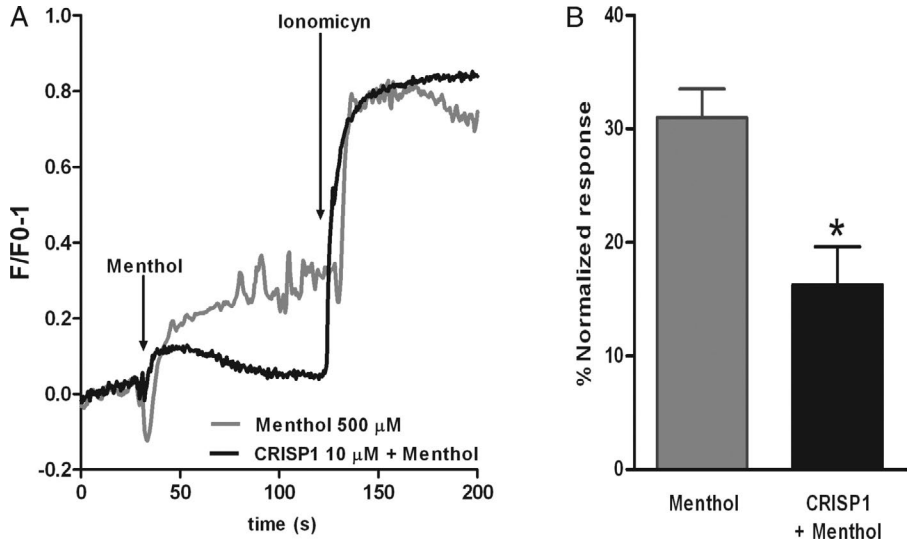

Figure S5. **Effect of CRISP1 on menthol-induced increase in intracellular Ca^{2+}.** Motile noncapacitated sperm were loaded with the fluorescent Ca^{2+} indicator Fluo-3 AM, and fluorescence intensity was measured before and after addition of menthol (500 μM). (A) Corresponding representative traces showing the menthol-induced fluorescence changes in the absence or presence of 10 μM CRISP1. Menthol $[Ca^{2+}]i$ responses were observed in 55 ± 7% of control sperm (n = 3 independent experiments and 127 cells analyzed). This response was inhibited (53.0 ± 4.3%; n = 3) by 10 μM CRISP1 (58 ± 9% cells responded; n = 3 independent experiments; 103 cells analyzed). (B) Summary of experiments as in A. Intracellular Ca^{2+} increases induced by menthol ± CRISP1 were normalized with respect to those induced by ionomycin (100%). Data represent the mean ± SEM of three independent experiments. *, P < 0.05 vs. menthol.

Table S1. **Effect of CRISP1 on sperm motility**

Treatment	VCL	ALH	LIN	VSL	STR	VAP	HA
	μm/s	*μm/s*	*%*	*μm/s*	*%*	*μm/s*	*%*
Medium	226.9 ±11.2	4.8 ± 0.3	27.5 ± 0.5	59.4 ± 2.8	50.4 ± 1.0	117.5 ± 3.7	24.3 ± 3.3
CRISP1	198.3 ± 9.8[a]	4.1 ± 0.2[b]	27.2 ± 0.6	50.8 ± 3.1[b]	47.2 ± 2.1	109.5 ± 4.1[c]	17.2 ± 3.3[a]

CASA analysis was performed in sperm incubated during the last 15 min of capacitation either in the absence or presence of 10 μM CRISP1. VCL, curvilinear velocity; ALH, amplitude of lateral head displacement; LIN, linearity; VSL, straight line velocity; STR, straightness; VAP, mean path velocity; HA, hyperactivated sperm. n = 7.
[a]P < 0.001 vs. medium.
[b]P < 0.005.
[c]P < 0.05.

References

Gibbs, G.M., G. Orta, T. Reddy, A.J. Koppers, P. Martínez-López, J.L. de la Vega-Beltràn, J.C. Lo, N. Veldhuis, D. Jamsai, P. McIntyre, et al. 2011. Cysteine-rich secretory protein 4 is an inhibitor of transient receptor potential M8 with a role in establishing sperm function. *Proc. Natl. Acad. Sci. USA.* 108:7034–7039. http://dx.doi.org/10.1073/pnas.1015935108

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