Supporting Information

Tateno et al. 10.1073/pnas.1317113110

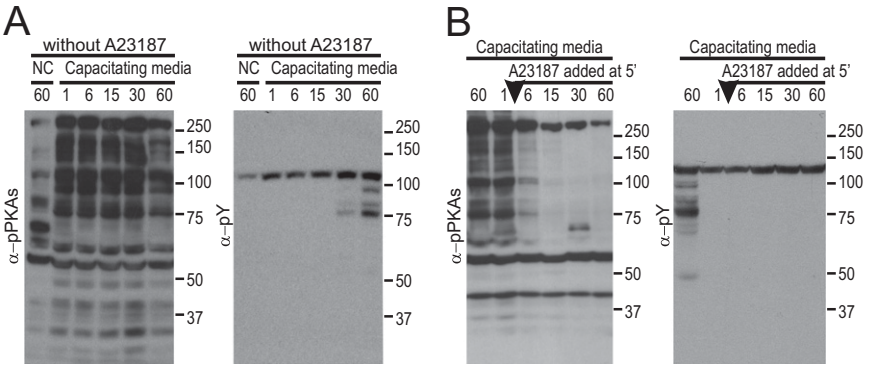


Fig. S1. Comparison of PKA activity and protein phosphorylation in spermatozoa with or without ionophore treatment. (*A*) In control spermatozoa in capacitating medium (no ionophore treatment), PKA was activated quickly and remained active for 60 min; protein phosphorylation began in 30 min after start of incubation. In HCO_3-free, noncapacitating medium (NC), neither PKA activation nor protein phosphorylation occurred. (*B*) When ionophore was added to HCO_3^--containing (capacitating) medium 1 min after the start of sperm incubation and spermatozoa were incubated continuously in the presence of ionophore, both PKA activity and protein phosphorylation were blocked. Western blot analyses were performed using antiphospho PKA substrates (α-pPKAs) or antiphosphotyrosine (α-pY) antibodies.

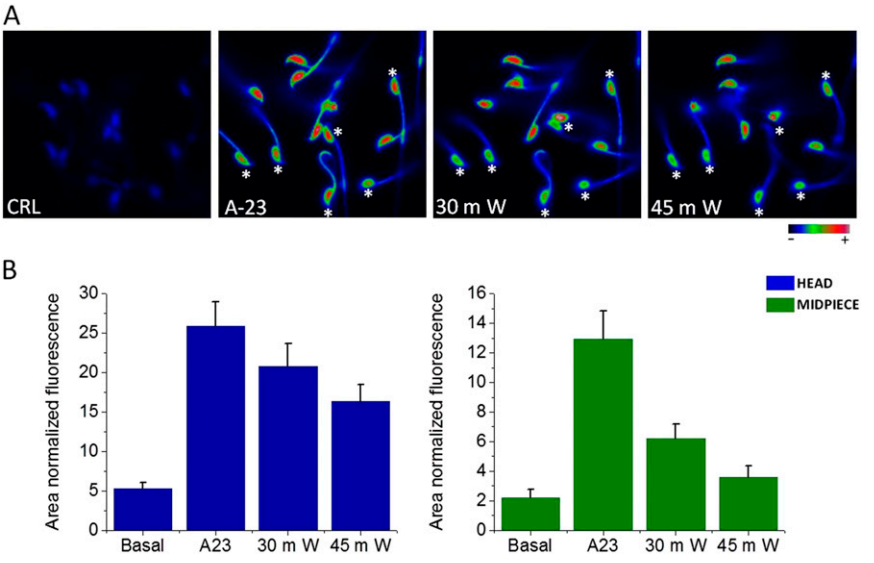


Fig. S2. Intracellular Ca^{2+} concentration decreases after removing ionophore. (*A*) Frames captured from movies showing that intracellular Ca^{2+} concentration increases by ionophore treatment and decreases after washing. CRL, before A23187 treatment; A-23, the end of 10 min ionophore treatment; 30 mW and 45 mW, 30 and 45 min after washing to remove ionophore, respectively. The color scale from red to blue is shown, where red represents the higher and blue the lower intracellular Ca^{2+} concentrations. (*B*) Quantification of fluorescence intensity in the head and midpiece regions. The total fluorescence was normalized by the area of the head and midpiece, respectively.

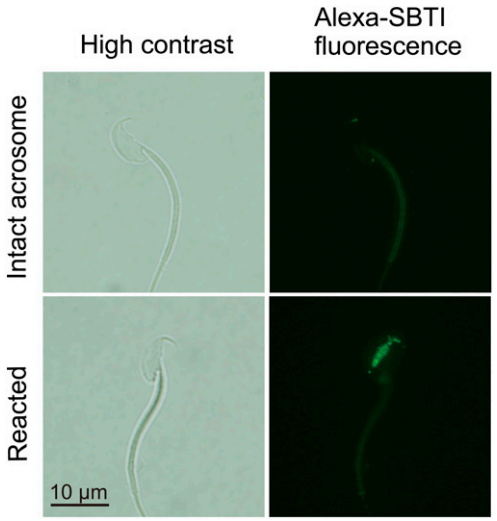
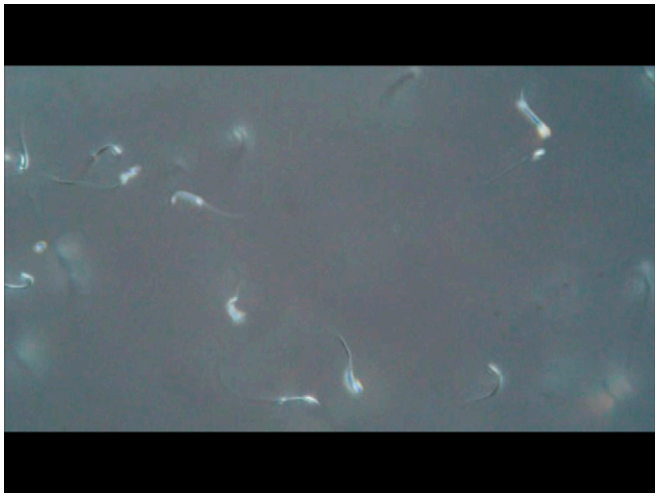


Fig. S3. Dual sperm staining for flow cytometry. Representative images of spermatozoa stained with both Alexa-soybean trypsin inhibitor and propidium iodide (PI) for acrosomal status assessment by flow cytometry. Nuclei of live spermatozoa remained dark for the PI channel. Live acrosome-reacted (*Lower Right*) spermatozoa had green fluorescence in their acrosomal cap region. Intact live sperm remained unstained for both dyes (*Upper Right*). The *Upper Left* and *Lower Left* panels are the respective phase contrast images.



Movie S1. Control sperm. Control spermatozoa in capacitating TYH medium at 5 min of incubation.

Movie S1

Movie S2. Control sperm. Control spermatozoa in capacitating TYH medium at 30 min of incubation.

Movie S2

Movie S3. Control sperm. Control spermatozoa in capacitating TYH medium at 60 min of incubation.

Movie S3

Movie S4. Control sperm. Control spermatozoa in capacitating TYH medium at 120 min of incubation.

Movie S4

Movie S5. Ionophore treatment. Spermatozoa recorded immediately before addition of ionophore.

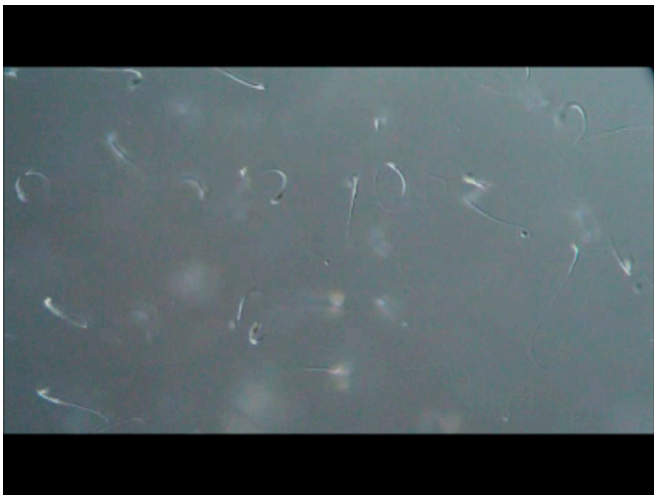
Movie S5

Movie S6. Ionophore treatment. Spermatozoa recorded at 10 min of incubation with 20 μM A23187.

Movie S6

Movie S7. Ionophore treatment. Spermatozoa recorded at 5 min after washing.

Movie S7



Movie S8. Ionophore treatment. Spermatozoa recorded at 30 min after washing.

Movie S8

Movie S9. Ionophore treatment. Spermatozoa recorded at 60 min after washing.

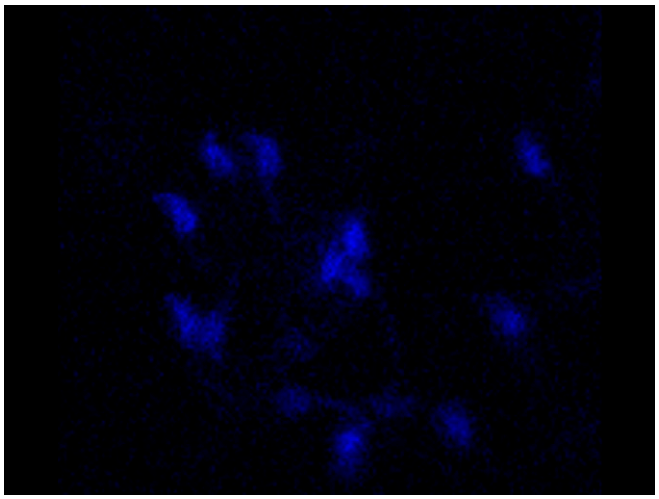
Movie S9

Movie S10. Calcium-free media. Spermatozoa incubated in TYH in the absence of calcium immediately before addition of ionophore.

Movie S10

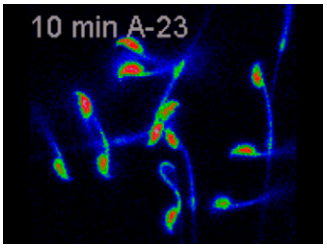
Movie S11. Calcium-free media. Spermatozoa incubated in TYH in the absence of calcium at 10 min of incubation with 20 μM A23187.

Movie S11



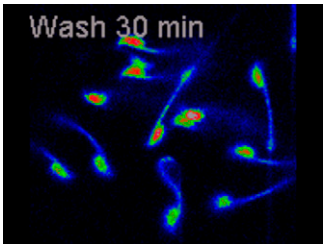
Movie S12. Calcium imaging. Film showing Fluo-4 fluorescence recordings of spermatozoa at the moment of A23187 addition. Notice that the tail started to move in those spermatozoa in which $[Ca^{2+}]_i$ decreased.

Movie S12



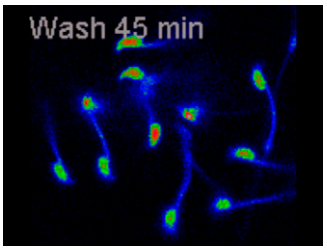
Movie S13. Calcium imaging. Film showing Fluo-4 fluorescence recordings of spermatozoa at 10 min after addition of A23187. Notice that the tail started to move in those spermatozoa in which $[Ca^{2+}]_i$ decreased.

Movie S13



Movie S14. Calcium imaging. Film showing Fluo-4 fluorescence recordings of spermatozoa at 30 min after washing of A23187. Notice that the tail started to move in those spermatozoa in which $[Ca^{2+}]_i$ decreased.

Movie S14



Movie S15. Calcium imaging. Film showing Fluo-4 fluorescence recordings of spermatozoa at 45 min after washing of A23187. Notice that the tail started to move in those spermatozoa in which $[Ca^{2+}]_i$ decreased.

Movie S15