

Supporting Information

Saito et al. 10.1073/pnas.1115086109

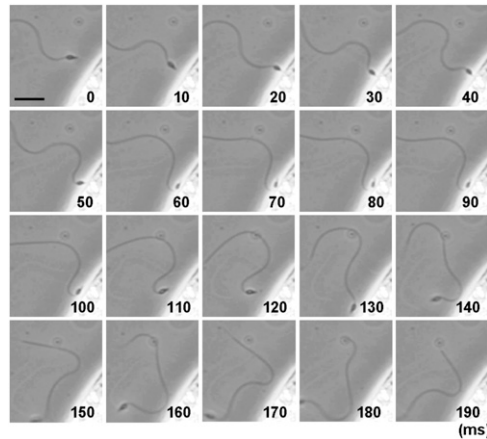


Fig. S1. Changes in flagellar waveform and in sperm swimming direction in self-insemination. When sperm bind to the self-VC, the sperm quit flagellar beating for a few seconds and become largely bended in flagella. Eventually, the sperm leave the VC of self-eggs. The interval between images is 10 ms (Scale bar, 10 μ m.)

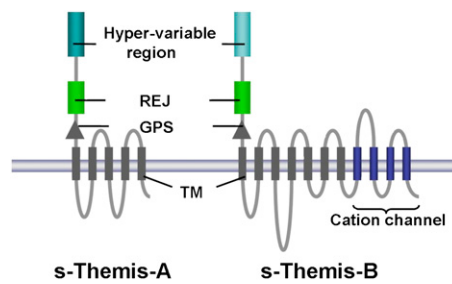


Fig. S2. Schematic diagram of the structures of s-Themis-A and s-Themis-B. s-Themis-A and s-Themis-B are 5-pass and 11-pass transmembrane proteins, respectively. REJ, receptor for egg jelly module; GPS, G protein-coupled receptor proteolytic site.

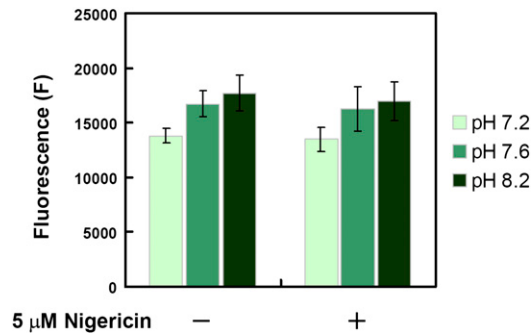
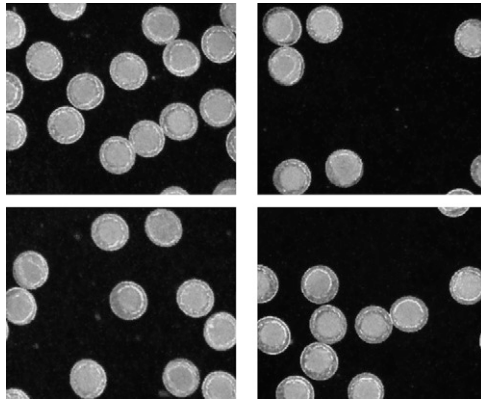


Fig. S3. Effect of pH_i on fluorescence of Ca^{2+} -bound Fluo-8H-AM within the sperm head region. Sperm, which had been preloaded with Fluo-8H-AM, were incubated in the presence or absence of 5 μ M nigericin, an H^+/K^+ ionophore, in ASW and subjected to measurement of the relative fluorescence as in Fig. 3. Note that the fluorescent change in sperm after the binding of sperm to the self-VC is three- to sixfold higher than the basal fluorescence level, which is considerably different from the fluorescent change affected by pH_i (less than 25%). These results indicate that the increase of fluorescence in the SI response (Fig. 3) is attributable to the increase of $[Ca^{2+}]_i$, but not to the difference in pH_i . The bars represent means \pm SEM ($n = 30$).



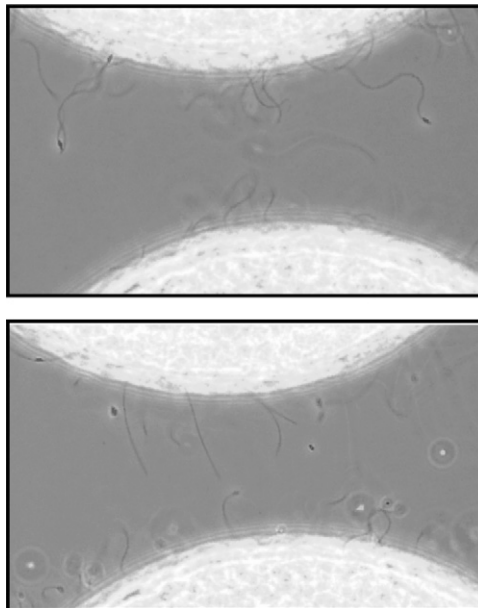
Movie S1. Movement of glycerinated eggs. The glycerinated eggs were rolling and moving at 1 min after the addition of nonself-sperm (A, *Upper Left*) or self-sperm (B, *Upper Right*). After 5 min, nonself-sperm-inseminated eggs continued their rotation and movement (C, *Lower Left*), but self-sperm-inseminated eggs showed little or no movement (D, *Lower Right*).

[Movie S1A](#)

[Movie S1B](#)

[Movie S1C](#)

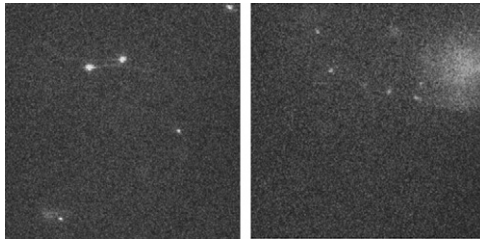
[Movie S1D](#)



Movie S2. Sperm behavior and motility during the binding to self- and nonself-eggs. Sperm were added to the egg suspension of self-egg (*Upper*) and nonself-egg (*Lower*). Sperm behaviors and motilities at 1 min (A, *Upper*) and 5 min (B, *Lower*) after insemination are shown. Note that the number of sperm bound to self-egg VC was decreased by the detachment of sperm from the VC and also that some attached sperm became quiescent. These movies are playing 0.1 times faster than normal.

[Movie S2A](#)

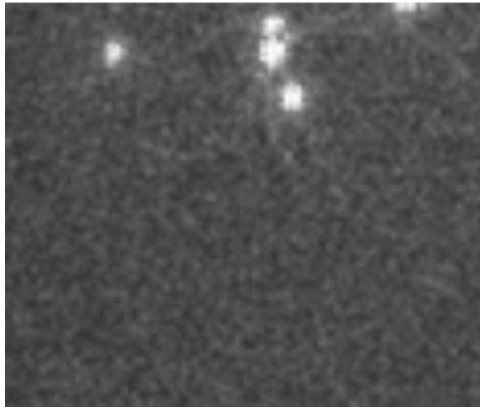
[Movie S2B](#)



Movie S3. $[Ca^{2+}]_i$ imaging of self-sperm before and after binding to the isolated VC. Sperm, which had been loaded with the Ca^{2+} indicator Fluo-8H-AM, swam toward the self-VC (A, *Left*) and nonself-VC (B, *Right*) and then attached to the VC. These movies are playing 0.8 times faster than normal. Note that $[Ca^{2+}]_i$ dramatically increased immediately after the binding of sperm to the self-VC but not after the binding of sperm to the nonself-VC.

[Movie S3A](#)

[Movie S3B](#)



Movie S4. $[Ca^{2+}]_i$ imaging of sperm detached from the self-VC. Sperm sometimes detached from the self-VC after binding. This movie is playing 0.6 times faster than normal.

[Movie S4](#)