Supporting information

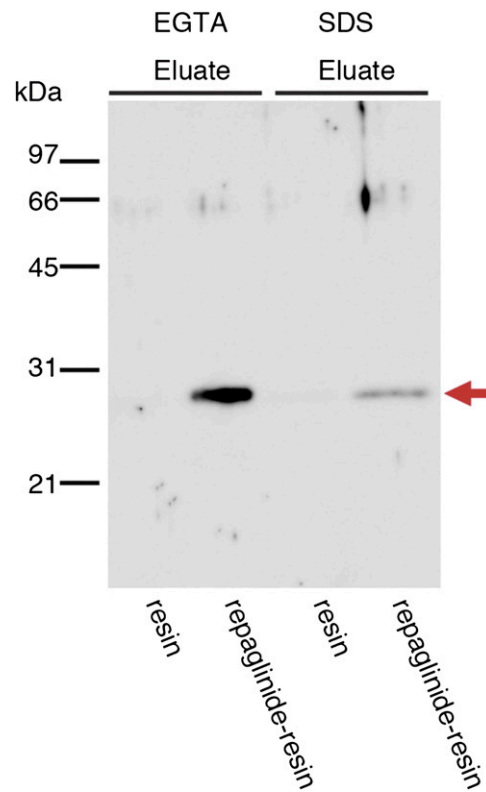## Mizuno et al. 10.1073/pnas.1217018109

Fig. S1. Calaxin specifically binds to repaglinide. Sperm axonemal proteins were extracted and mixed with EAH Sepharose beads conjugated with or without repaglinide in the presence of Ca^{2+}. The two left lanes show an initial elution with buffer containing EGTA; the two right lanes show a second elution from resins using a solution containing SDS after an initial elution. Presence of calaxin was probed by anti-calaxin antibody. Calaxin was specifically bound to re-paglinide-Sepharose and eluted with EGTA. The red arrow shows the position of calaxin.
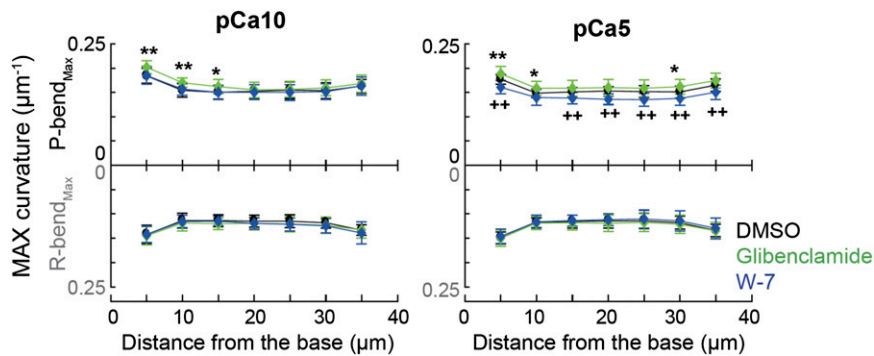
Fig. S2. Curvature along the length of the flagellum in the demembranated sperm model. Reactivation was carried out in the presence of DMSO, 200 μM glibenclamide, or 100 μM W-7. Maximum flagellar curvature was plotted for P-bends or R-bends against the distance from the base of flagellum. $n = 30$ from triplicate experiments. $*P < 0.01$, $**P < 0.001$ (DMSO vs. glibenclamide), $^{++}P < 0.001$ (DMSO vs. W-7).

Maximum curvature of P-bend ($P\text{-bend}_{Max}$) = C_{max}

Maximum curvature of R-bend ($R\text{-bend}_{Max}$) = $|C_{min}|$

Asymmetric index = $P\text{-bend}_{Max}$ / $R\text{-bend}_{Max}$
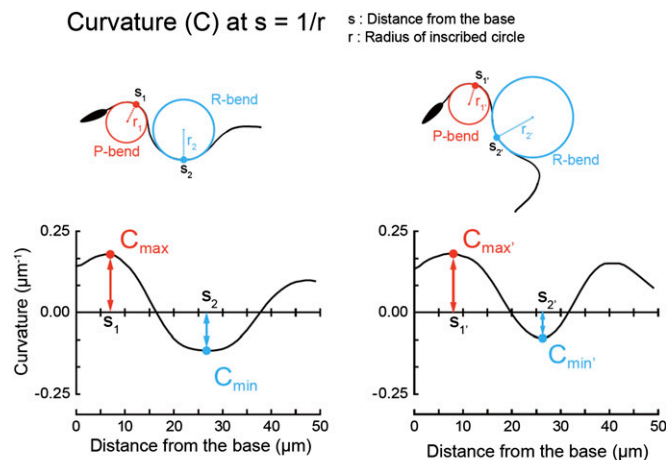
Fig. S3. Definition of parameters for flagellar bending and asymmetry of intact sperm. Flagellar curvature (C) was defined as the reciprocal of radius of inscribed circle. Because the direction of R-bend is reverse to R-bend, flagellar curvature of R-bend shows negative value. Maximum flagellar curvature for P-bend or R-bend ($P\text{-bend}_{Max}$ or $R\text{-bend}_{Max}$) was defined as C_{max} or absolute value of C_{min}, respectively. Asymmetric index was calculated as the ratio of maximal curvatures of both bends ($P\text{-bend}_{Max}/R\text{-bend}_{Max}$).

$$\text{Asymmetric index} = \text{P-bend}_{Max} / \text{R-bend}_{Max} \text{ at } 15 \ \mu\text{m}$$

Fig. S4. Definition of parameters for flagellar bending and asymmetry of demembranated sperm model. Curvature and maximum flagellar curvature were defined in the same way as Fig. S3. Curvatures of 20 frames of flagellar waveforms were plotted against the distance from the base of a flagellum. Asymmetric index was calculated as the ratio of maximal curvatures of both bends (P-bend$_{Max}$/R-bend$_{Max}$) at 15 μm.

Table S1. Composition of the reactivation solutions for demembranated sperm

Solution	Total CaCl$_2$ concentration, M	Calculated pCa*
pCa10	3.90×10^{-5}	10.04
pCa9	3.32×10^{-4}	9.04
pCa8	1.44×10^{-3}	8.03
pCa7	2.31×10^{-3}	7.02
pCa6	2.48×10^{-3}	6.03
pCa5	2.51×10^{-3}	4.99
pCa4	2.61×10^{-3}	4.02

Components other than CaCl$_2$ include 2.5 mM EGTA, 50 mM Tris, 2 mM MgSO$_4$, 0.15 M K-acetate, 1 mM DTT, 1 mM ATP, and 20 μM cAMP. The pH of all reactivation solutions was adjusted to pH 8.0.
*Calculated by CALCON.

Table S2. Composition of the ATP solutions for in vitro microtubule sliding

| Solution	Total CaCl$_2$ concentration, M	Calculated pCa*
pCa10	2.00×10^{-5}	10.04
pCa5	2.00×10^{-3}	5.32

Components other than CaCl$_2$ include 2 mM EGTA, 10 mM Hepes, 4 mM MgSO$_4$, 50 mM K-acetate, 1 mM DTT, 0.05% methylcellulose, 1 mM ATP, and 1 μM Taxol. The pH of all solutions was adjusted to pH 8.0.
*Calculated by CALCON.
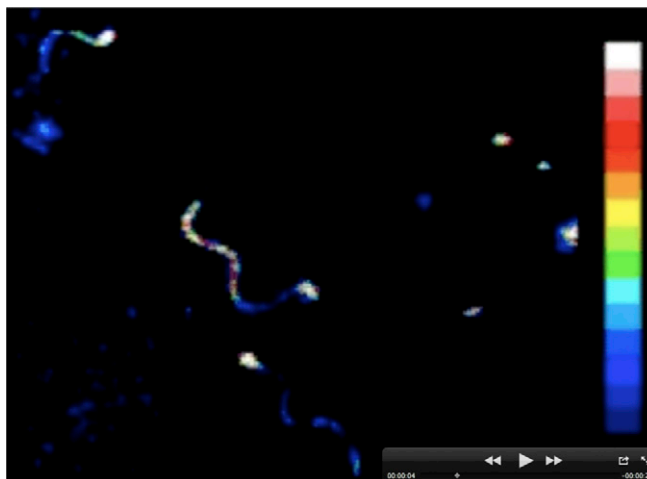
Movie S1. Ca^{2+} imaging of control sperm during chemotactic movements. Fifty frames were recorded per second. The movie plays at 0.6× speed. (Color bar scale, 6,500–16,000 a.u.) (QuickTime, Apple Inc.: ~2.1 MB).

Movie S1
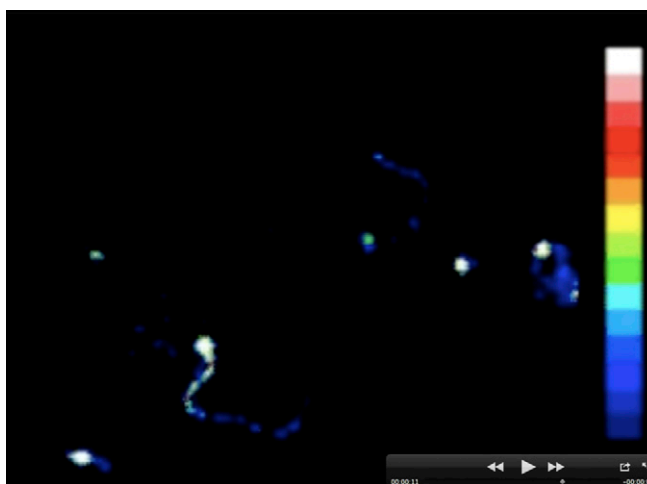
Movie S2. Ca^{2+} imaging of sperm treated with 150 μM repaglinide during chemotactic movements. Repaglinide-treated sperm showed normal increases and decreases in intracellular Ca^{2+} concentration despite the fact that they could not execute clear turn movements. Fifty frames were recorded per second. The movie plays at 0.6× speed. (Color bar scale, 6,500–16,000 a.u.) (QuickTime: ~1.2 MB).
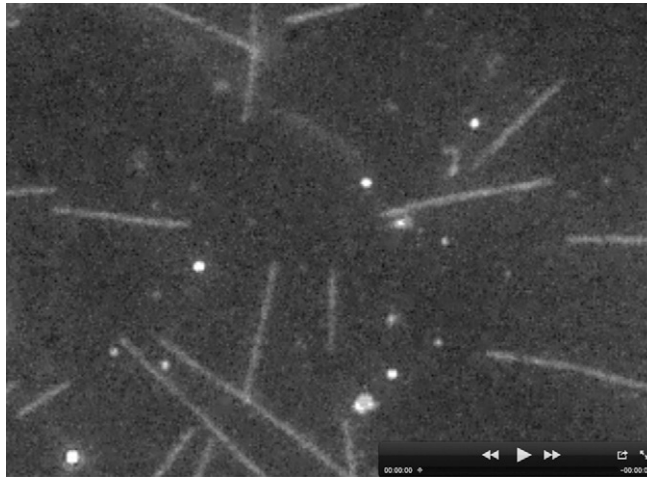
Movie S2

Movie S3. Chemotactic turn movement of control sperm. Magenta circle indicates chemoattractant position. Two hundred frames were recorded per second. The movie plays at 0.15× speed. (QuickTime: ~0.45 MB).
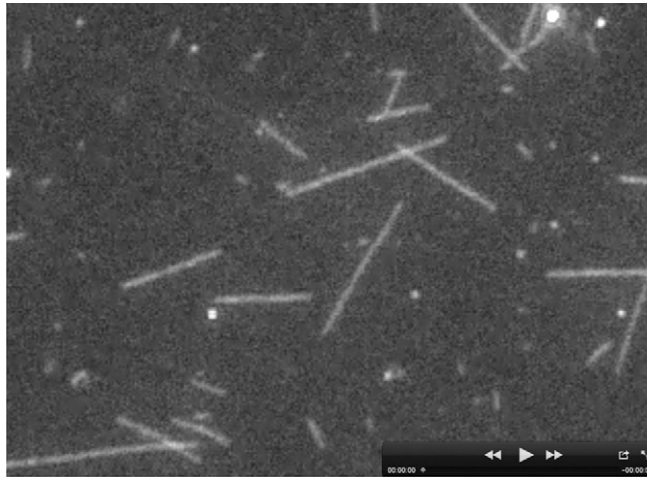
Movie S3

Movie S4. Chemotactic turn movement of sperm treated with 150 μM repaglinide. Repaglinide-treated sperm make no clear turn movement due to unstable flagellar asymmetry. Magenta circle indicates chemoattractant position. Two hundred frames were recorded per second. The movie plays at 0.15× speed. (QuickTime: ~2.0 MB).

Movie S4

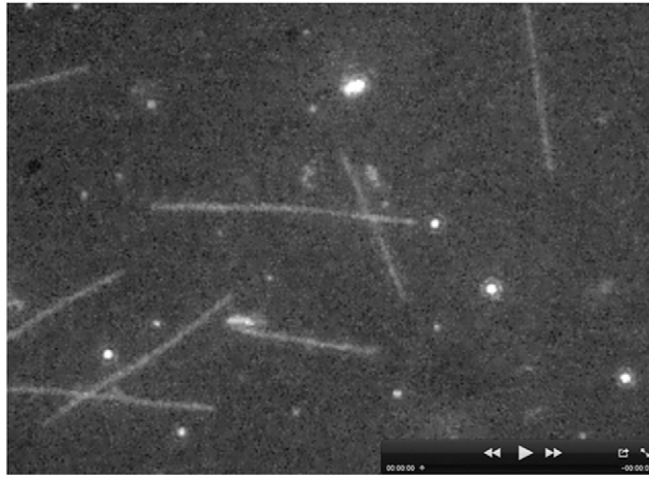**Movie S5.** Translocation of microtubules by outer-arm dyneins at a Ca^{2+} concentration of 10^{-10} M (pCa10) in the absence of calaxin. The movie plays at real speed. (QuickTime: ~1.2 MB).
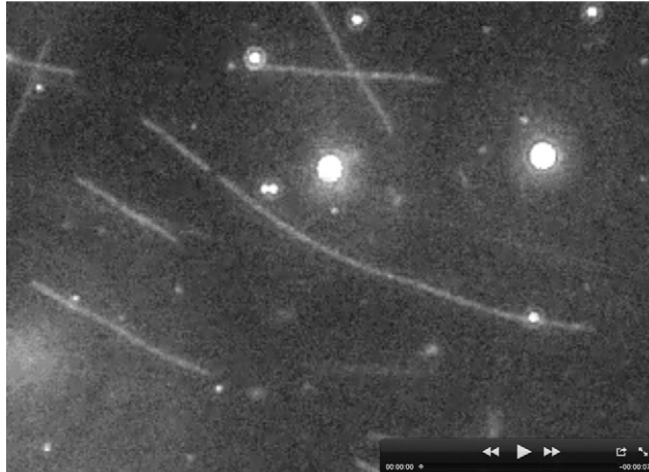
Movie S5

Movie S6. Translocation of microtubules by outer-arm dyneins at pCa10 in the presence of calaxin. The movie plays at real speed. (QuickTime: ~1.2 MB).

Movie S6

Movie S7. Translocation of microtubules by outer-arm dyneins at a Ca^{2+} concentration of 10^{-5} M (pCa5) in the absence of calaxin. The movie plays at real speed. (QuickTime: ~1.2 MB).

Movie S7

Movie S8. Translocation of microtubules by outer-arm dyneins at pCa5 in the presence of calaxin. The movie plays at real speed. (QuickTime: ~1.2 MB). Translocation is significantly suppressed by calaxin at pCa5.

Movie S8