Supplemental Information

SI-Table 1: Wild-type sperm storage in the DSR. Wild-type levels of sperm storage were determined by counting the sperm inside the DSR at 4 hr or 20 hr after mating of virgin females (y^1w^1) with males of genotype of *Protamine-GFP/CyO; CG34110*^{MB00722}*/TM3* (*CyO* and *TM3* are balancer chromosomes that show wild-type activity with respect to sperm storage). In our experiments, similar numbers of sperm were found at the 4 and 20-hr time points (average 330 and 329, respectively). An explanation for this is that at the 20-hr time point, sperm loss from the DSR is balanced out by the extra 0.5 - 1 hr available for the sperm to enter the DSR, as compared to the 4-hr time point. A recent report showed that 84% of the females have ejected the inseminated sperm within 5 hr of mating (Manier *at al.*, Science 328, 354-357, 2010). For these reasons, quantification of sperm storage is routinely done at 4 hr after mating (data in Fig. 6).

	Wild-type sperm numbers in the DSR at 4 hr after mating	**Wild-type sperm numbers in the DSR at 20 hr after mating**
1	275	365
2	301	377
3	404	316
4	344	374
5	351	376
6	331	205
7	281	319
8	392	302
9	267	
10	353	
	Average: 330; ST DEV: 48	Average: 329; ST DEV: 59

Supplemental Table 2:
Distribution of peptides originating from FAP50 and inner arm dyneins in fractions of the flagellar proteome[‡]

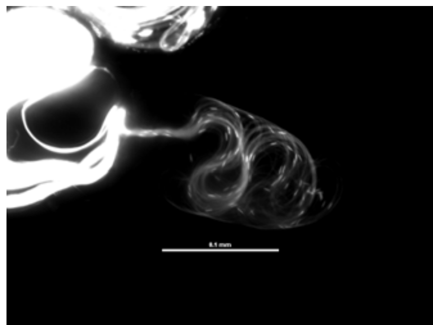
		M+M	ExAx	KCl Ex
Protein				
FAP50		**0**	**31**	**1**
Inner arm I1/f subunits	1α HC	0	6	103
	1β HC	0	4	64
	IC140	0	1	26
	IC138	0	0	22
	IC97 (FAP94)	0	2	13
	Tctex2b	3	0	4
*"Monomeric" inner arm dynein heavy chains**	DHC2	6	35	34
	DHC3	0	1	1
	DHC4	0	11	0
	DHC5	1	10	26
	DHC6	5	5	22
	DHC7[§]	1	32	33
	DHC8	1	2	23
	DHC9	3	25	6
	DHC11	0	6	3
*Other "monomeric" inner arm dynein subunits**	actin	9	13	18
	p28	8	13	16
MBO2		0	25	0

[‡]This table shows, for the listed proteins, the number of peptides identified in each of three flagellar fractions analyzed in the *C. reinhardtii* flagellar proteome study of Pazour et al. (2005): "M+M," detergent-soluble membrane-plus-matrix fraction; "ExAx," proteins remaining in the axoneme after extraction with 0.6 M KCl; "KCl Ex," proteins extracted from the axoneme by 0.6 M KCl. Peptides for MBO2 are also included. Data are from http://labs.umassmed.edu/chlamyfp/index.php. Most inner arm dynein subunits are substantially solubilized by 0.6 M KCl, whereas FAP50 and MBO2 remain tightly bound to the axoneme.

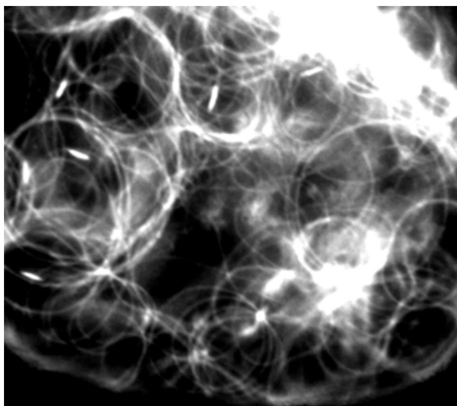
*Inner arm dyneins are subdivided into I1/f and "monomeric" dyneins (King and Kamiya, 2009, Axonemal dyneins: assembly, structure and force generation. *In* The *Chlamydomonas* Source Book, 2nd Edition. Volume 3: Cell Motility and Behavior. G.B. Witman, editor. Elsevier, San Diego. pp.131-208).

§Combines data for Joint Genome Institute *C. reinhardtii* genome version 2 gene models C_2600003 and C_2600004.

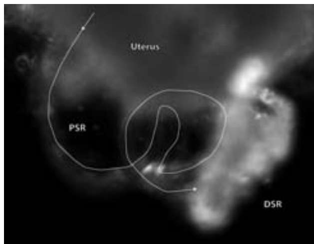
SI-Movie 1: Sperm exit movement from the SV into the ED. The wild-type sperm carried both the head and tail GFP markers. The movie was obtained by imaging the ED at 2 - 4 min after the initiation of copulation. The movie recorded events trigged by mating, from the opening of the SV to sperm movement into the ED. Passing sperm are readily visible in the narrow connecting tubule that connects the two bilateral SVs to the ED. The sperm heads appear as specks which are at the moving front. Nikon image counting software counted ~200 sperm heads passing through the CT to enter the ED while the sperm moving front advanced up to the anterior ~1/8 ED length as shown in Fig. 4B; the tail ends of these sperm have not entered the ED at this time based on the length measurements of the flagellar portions that were inside the ED. This was recorded at 5 frames per second (fps). The scale bar is 0.1 mm.


Movie1

SI-Movie 2: Circular foci of sperm movement in the uterus. The wild-type sperm carried both the head and tail GFP markers. This video was obtained at 15 - 30 min after mating. The sperm population in the uterus is moving with circular-shaped flagellar bends. The heads are generally dragged behind by their flagella. Images like this are difficult to obtain because the sperm mass is frequently too numerous to provide a clear image, even after repetitive mating of the same male with multiple females. This uterus happened to have a low level of sperm. The video was recorded at 8.95 fps.


Movie2

SI-Movie 3: Wild-type sperm passing through the entire PSR without making turns. The movie was taken at 45 min after mating completion. The attached still image of SI-Movie 3 (below) is used to show the convoluted PSR tubule that the sperm go through to enter the folded DSR on the right, which already contained a lot of moving sperm (bright fluorescence). There are a few immotile sperm in the PSR lumen, possibly caused by damage incurred during tissue dissection. Three sperm heads can be seen moving steadily through the entire PSR tubule without making any turns. The distances between these passing sperm heads were measured and found to be frequently shorter than the full-length sperm. The sperm in the video carried only the head GFP marker because flagellum-associated GFP was found to obscure the visibility of the head due to multiple sperm going through the PSR at the same time. After SI-Movie 3 was recorded, additional sperm (~10) went through the PSR without making any turns, and the distances between adjacent heads in this group of entering sperm were also shorter than the full-length sperm. This provides evidence that sperm move through the PSR as overlapping parallel arrays. It is worth noting that the circular PSR loop is usually positioned to have spatial overlap with the folded DSR on the right. This spatial overlap could give an illusion of some sperm moving out of the DSR and then backing into the DSR. The video was recorded at 2.44 fps.

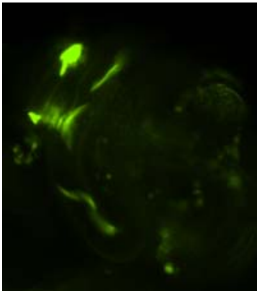


Movie3

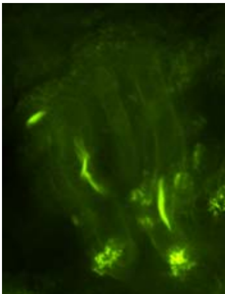
SI-Movie 4 and SI-Movie 5: Comparison of movement of wild-type sperm (Movie 4) with *Pkd2 lobo* double mutant sperm (Movie 5) inside the DSR. The sperm were marked with head GFP only (for a better comparison with the double mutant sperm, which could only be marked with head GFP due to the lack of GFP markers on the X chromosome). To reduce the number of wild-type sperm in the DSR, the same male was mated repeatedly with 6 virgin females before the last mated female was dissected and used for imaging. Movie 4 and Movie 5 were taken immediately after dissection at the same frame speed (5 fps) and the head speed was determined by Nikon image tracking software from different samples and three different video frames of the same sample. The head speeds for wild-type (*Protamine-GFP/CyO; CG34110*^{MB00722}*/TM3*) and double mutant sperm (genotype, *Protamine-GFP Pkd2*^{KO67}*/+ Pkd2*^{MB06703}*; CG34110*^{MB00722}*/CG34110*^{lobo}) are similar with no significant differences in the t-test (p-value=0.32; average head speeds, 93 μm/s and 84 μm/s, respectively). The movement observed here in the absence of the flagellum GFP marker is inadequate for revealing the actual mode of sperm movement. The actual sperm movement can be seen in SI-Movie 6, although the speed can not be compared due to different image acquisition conditions. UV exposure during imaging was found to reduce moving speeds within 4

min of initial exposure. Thus, unless the images were taken under identical conditions, the speeds could not be compared directly.

	Speeds of head movement (μm/s) wild-type sperm		Speeds of head movement (μm/s) *Pkd2 lobo* double mutant
1-1	92.6	4-1	92.4
1-2	92.2	4-2	98.3
1-3	70.2	4-3	105.1
1-4	125.7	4-4	97.7
2-1	53.0	5-1	73.4
2-2	64.7	5-2	90.0
2-3	94.9	5-3	107.1
2-4	79.0	5-4	57.9
3-1	92.8	6-1	73.5
3-2	96.0	6-2	63
3-3	137.4	6-3	66.6
3-4	119.4		
	Average = 93 μm/s; n=12 St dev 25		Average = 84 μm/s; n=11 St dev 18



Movie 4 (wild-type sperm in DSR)



Movie 5 (The *Pkd2 lobo* double mutant sperm in DSR)

SI-Movie 6: **Wild-type sperm movement in the DSR.** The wild-type sperm carried both the head and tail GFP markers. Consecutive mating of one male with multiple females was carried out to reduce the number of wild-type sperm inside the DSR. Sperm movement was captured on the same or the next day. Inside the DSR, sperm move in folded form (also see Fig. 5H, I) by propagating hairpin-shaped flagellar bends. The head is mostly being dragged backwards by the flagellum. Occasionally, the sperm pauses and switches to head-leading movement. Thus, this video shows the bi-

directional movement of the *Drosophila* sperm inside the DSR. The video was recorded at 14.29 fps.