Supporting Information

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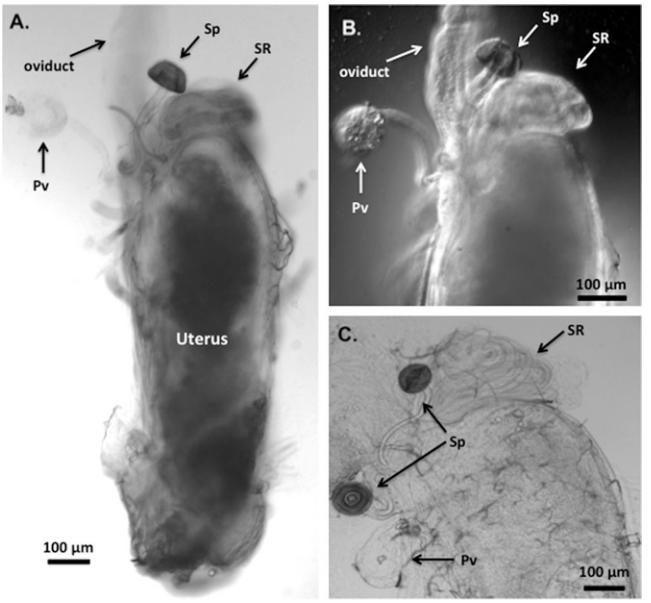

Fig. S1. The *D. melanogaster* lower female RT (SSOs and uterus). (*A*) Nomarski image at low magnification of a mated female RT. (*B*) Magnified Nomarski image showing the anterior portion of the lower female RT. (*C*) Magnified bright-field image of the anterior lower RT. Images were taken in the presence (*C*) and absence (*A* and *B*) of a coverslip. Pv, parovaria; Sp, spermathecae; SR, seminal receptacle. The ovaries are located anterior to the uterus. One (of two) parovaria is shown in *A*–*C*, and one (of two) spermatheca is shown in *A* and *B*.

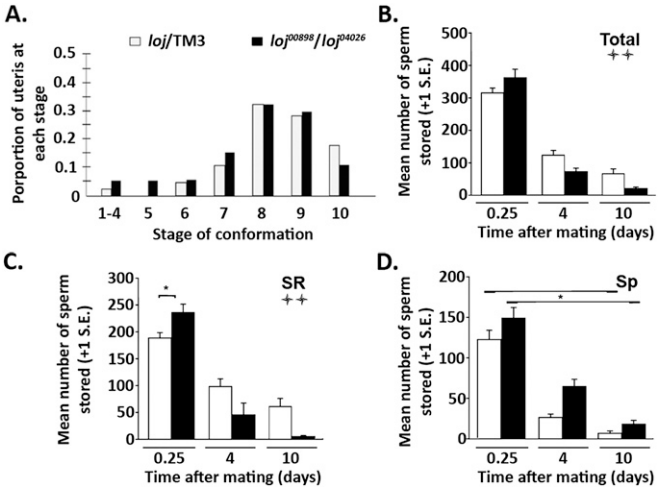

Fig. S2. Sterile, egg-retention mutants do not excessively retain stored sperm. (*A*) Distribution of the uterine conformational stages at 35 min after the start of mating in loj^{04026}/loj^{00898} transheterozygous mutant females compared with their sibling controls [Wilcoxon test (Rank sums), $P = 0.38$; $N_{loj} = 37$, $N_{TM3} = 46$]. Total sperm stored in the SSOs (*B*), in the seminal receptacle (*C*), and in the spermathecae (*D*) in *loj* mutant females compared with their sibling controls. The *loj* mutant and control females stored similar numbers of total and spermathecal sperm at 6 h postmating, although significantly more sperm are stored in the *loj* mutants' SRs at 6 h postmating (significant difference indicated by *; *B*: $t = -1.69$, df = 26, $P = 0.103$; *C*: $t = -2.73$, df = 32, $P = 0.01$; *D*: $t = -1.47$, df = 27, $P = 0.16$). Examination of sperm depletion from storage in *loj* mutant females showed that the total number of stored sperm (*B*: $F_{1,58} = 52.53$, $P < 0.0005$), as well as sperm stored in the seminal receptacle (*C*: $F_{1,62} = 34.83$, $P < 0.0005$), had been depleted at significantly greater rates than in control females. Sperm depletion from storage in the spermathecae did not differ between *loj* mutant and control females at 4 d after mating but was greater in *loj* mutant females by 10 d after mating (*D*: genotype × time $F_{1,68} = 5.33$, $P = 0.024$; *t* test, significant difference indicated by *, 4 d: $t = 1.12$, df = 25.5, $P = 0.28$; **t* test, 10 d: $t = -2.93$, df = 34, $P = 0.006$). Significance of the genotype factor, indicating differences in sperm depletion between *loj* mutant and control females, is reported in the figure as follows: * = $0.005 < P < 0.05$ (an additional explanation of the statistical analysis is provided in *Materials and Methods*). Sp, spermathecae; SR, seminal receptacle. Sample sizes for sperm counts range from $n = 9$–20 (Table S1).

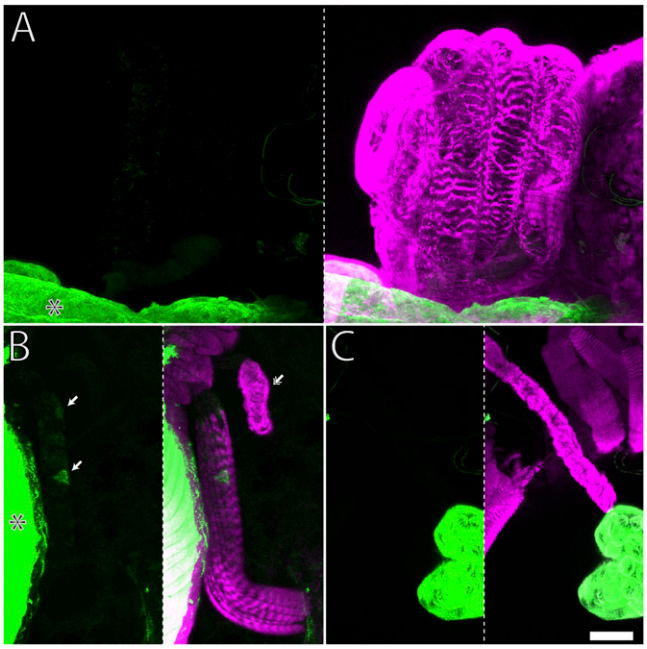

Fig. S3. Expression of OAMB in the seminal receptacle (*A*), spermathecae (*B*), and parovaria (*C*) in *oamb*-GAL4/UAS-mCD8-GFP females. (*Left*) *oamb*-driven GFP (green) expression alone. (*Right*) GFP overlayed with phalloidin-stained muscle (magenta). Oviduct epithelium, indicated by an asterisk (*), expresses GFP highly, but the seminal receptacle and spermathecae do not show repeatable high levels of expression. Occasionally, spermathecal epithelial cells express low levels of GFP (arrows). The parovaria show high levels of GFP expression. The parovaria stalk in *B* is labeled (double arrow). (Scale bar: 25 μm.)

Table S1. Sample sizes of sperm counts by genotype and time point

Fig.	Sperm storage	Time AM, d	Female genotype	Sample size
1*B*	Total	0.25	$tβh^{M18}$/FM7	13
1*B*	Total	0.25	$tβh^{M18}/tβh^{M18}$	15
1*B*	Total	4	$tβh^{M18}$/FM7	15
1*B*	Total	4	$tβh^{M18}/tβh^{M18}$	14
1*B*	Total	10	$tβh^{M18}$/FM7	11
1*B*	Total	10	$tβh^{M18}/tβh^{M18}$	11
1*C*	SR	0.25	$tβh^{M18}$/FM7	13
1*C*	SR	0.25	$tβh^{M18}/tβh^{M18}$	15
1*C*	SR	4	$tβ^{M18}$/FM7	15
1*C*	SR	4	$tβh^{M18}/tβh^{M18}$	14
1*C*	SR	10	$tβh^{M18}$/FM7	11
1*C*	SR	10	$tβh^{M18}/tβh^{M18}$	11
1*D*	SP	0.25	$tβh^{M18}$/FM7	13
1*D*	SP	0.25	$tβh^{M18}/tβh^{M18}$	15
1*D*	SP	4	$tβh^{M18}$/FM7	15
1*D*	SP	4	$tβh^{M18}/tβh^{M18}$	14
1*D*	SP	10	$tβh^{M18}$/FM7	11
1*D*	SP	10	$tβh^{M18}/tβh^{M18}$	11
1*F*	Total	0.25	Df(2R)42/Gla	8
1*F*	Total	0.25	tdc^{RO54}/SM5	10
1*F*	Total	0.25	tdc^{RO54}/Df(2R)42	7
1*F*	Total	4	Df(2R)42/Gla	10
1*F*	Total	4	tdc^{RO54}/SM5	11
1*F*	Total	4	tdc^{RO54}/Df(2R)42	9
1*F*	Total	10	Df(2R)42/Gla	13
1*F*	Total	10	tdc^{RO54}/SM5	14
1*F*	Total	10	tdc^{RO54}/Df(2R)42	15
1*G*	SR	0.25	Df(2R)42/Gla	8
1*G*	SR	0.25	tdc^{RO54}/SM5	10
1*G*	SR	0.25	tdc^{RO54}/Df(2R)42	7
1*G*	SR	4	Df(2R)42/Gla	10

Table S1. Cont.

Fig.	Sperm storage	Time AM, d	Female genotype	Sample size
1*G*	SR	4	tdc^{RO54}/SM5	11
1*G*	SR	4	tdc^{RO54}/Df(2R)42	9
1*G*	SR	10	Df(2R)42/Gla	14
1*G*	SR	10	tdc^{RO54}/SM5	15
1*G*	SR	10	tdc^{RO54}/Df(2R)42	15
1*H*	SP	0.25	Df(2R)42/Gla	8
1*H*	SP	0.25	tdc^{RO54}/SM5	10
1*H*	SP	0.25	tdc^{RO54}/Df(2R)42	7
1*H*	SP	4	Df(2R)42/Gla	10
1*H*	SP	4	tdc^{RO54}/SM5	11
1*H*	SP	4	tdc^{RO54}/Df(2R)42	9
1*H*	SP	10	Df(2R)42/Gla	13
1*H*	SP	10	tdc^{RO54}/SM5	14
1*H*	SP	10	tdc^{RO54}/Df(2R)42	15
2*B*	Total	0.25	$OAMB^{286}$/TM6	13
2*B*	Total	0.25	$OAMB^{286}$/$OAMB^{286}$	11
2*B*	Total	4	$OAMB^{286}$/TM6	12
2*B*	Total	4	$OAMB^{286}$/$OAMB^{286}$	12
2*B*	Total	10	$OAMB^{286}$/TM6	11
2*B*	Total	10	$OAMB^{286}$/$OAMB^{286}$	12
2*C*	SR	0.25	$OAMB^{286}$/TM6	15
2*C*	SR	0.25	$OAMB^{286}$/$OAMB^{286}$	15
2*C*	SR	4	$OAMB^{286}$/TM6	15
2*C*	SR	4	$OAMB^{286}$/$OAMB^{286}$	12
2*C*	SR	10	$OAMB^{286}$/TM6	15
2*C*	SR	10	$OAMB^{286}$/$OAMB^{286}$	13
2*D*	SP	0.25	$OAMB^{286}$/TM6	13
2*D*	SP	0.25	$OAMB^{286}$/$OAMB^{286}$	12
2*D*	SP	4	$OAMB^{286}$/TM6	12
2*D*	SP	4	$OAMB^{286}$/$OAMB^{286}$	12
2*D*	SP	10	$OAMB^{286}$/TM6	11
2*D*	SP	10	$OAMB^{286}$/$OAMB^{286}$	12
3*B*	Total	0.25	loj^{00898}/TM3	19
3*B*	Total	0.25	loj^{00898}/loj^{04026}	9
3*B*	Total	4	loj^{00898}/TM3	17
3*B*	Total	4	loj^{00898}/loj^{04026}	16
3*B*	Total	10	loj^{00898}/TM3	17
3*B*	Total	10	loj^{00898}/loj^{04026}	12
3*C*	SR	0.25	loj^{00898}/TM3	20
3*C*	SR	0.25	loj^{00898}/loj^{04026}	14
3*C*	SR	4	loj^{00898}/TM3	18
3*C*	SR	4	loj^{00898}/loj^{04026}	19
3*C*	SR	10	loj^{00898}/TM3	17
3*C*	SR	10	loj^{00898}/loj^{04026}	12
3*D*	SP	0.25	loj^{00898}/TM3	19
3*D*	SP	0.25	loj^{0898}/loj^{0898}	10
3*D*	SP	4	loj^{00898}/TM3	17
3*D*	SP	4	loj^{00898}/loj^{04026}	19
3*D*	SP	10	loj^{00898}/TM3	19
3*D*	SP	10	loj^{00898}/loj^{04026}	17

SP, spermathecae; SR, seminal receptacle.