Supplemental Materials

Molecular Biology of the Cell

Funaki et al.

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The Arf GAP SMAP2 Is Necessary for the Organized Vesicles Budding from the Trans-*Golgi*-network and Subsequent Acrosome Formation in Spermiogenesis

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Supplemental Table S1. Sperm motility

SMAP2 genotype	(-/-)	(+/-)	*p* value
Number of mice examined	3	4	-
Number of sperm examined per mouse	237	226	-
Moving sperm (%)	17±2	49±14	0.02
Moving forward sperm (%)	9.0±1.2	31±8.1	0.01
VAP (μm/sec)	145±7	188±24	0.03
VSL (μm/sec)	76±3.5	108±10	0.004
VCL (μm/sec)	299±21	392±40	0.02
ALH (μm)	19±2.3	24±0.7	0.01
BCF(Hz)	40±1.7	35±0.9	0.007
LIN (%)	30±1.5	29±0.9	-
STR (%)	58±2.1	59±2.5	-
Elongation (%)	56±2.1	53±4.8	-

Sperm samples were loaded onto a microslide, and the parameters of sperm motility were measured using an IVOS TOX automated system (Hamilton Thorne, Beverly, MA).
VAP, average path velocity; VSL, straight-line velocity; VCL, curvilinear velocity; ALH, amplitude of lateral head; BCF, beet cross frequency, LIN, linearity; STR, straightness.

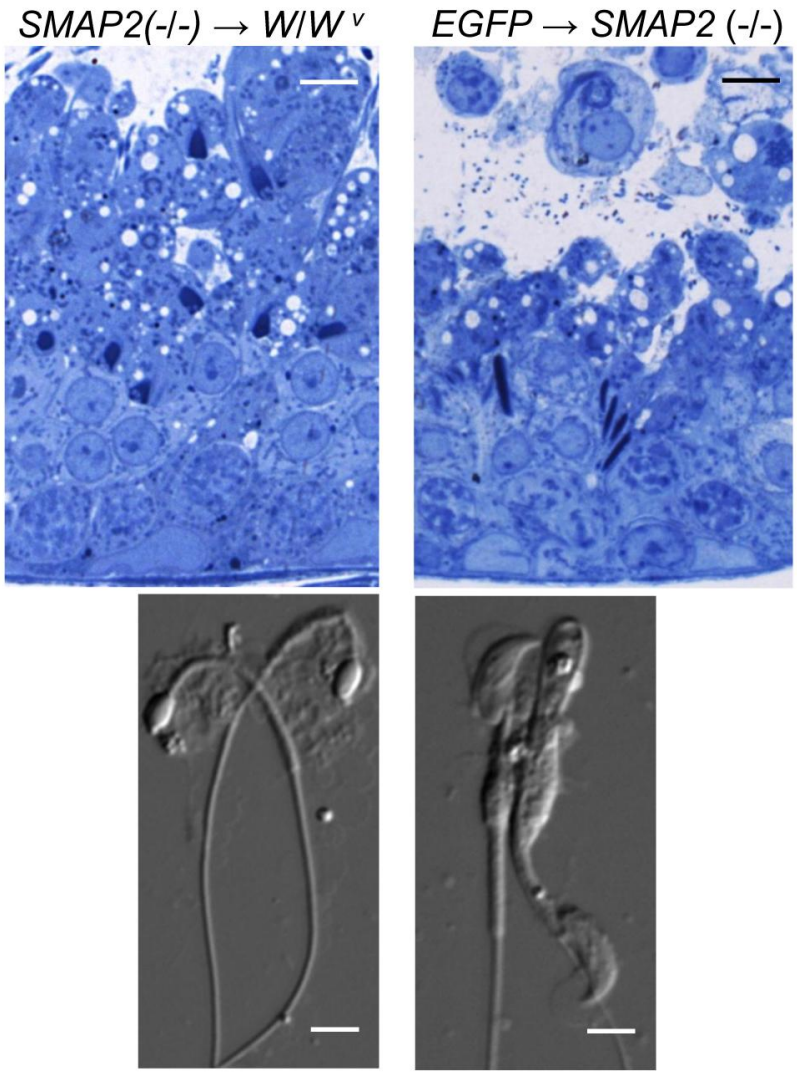
Supplemental Table S2. *In vitro* fertilization

Experiment no.	ID of male mice used	*SMAP2* genotype of sperm [1)]	Number of oocytes used [2)]	Number of 2-cell embryos (%) [3)]
1	#4	(-/-)	59	1 (1.7%)
2	#21	(-/-)	62	6 (9.7%)
3	#33	(-/-)	63	0
4	#37	(-/-)	67	5 (7.5%)
5	#38	(-/-)	55	0
6	#24	(+/-)	55	16 (29%)
7	#25	(+/-)	73	32 (44%)

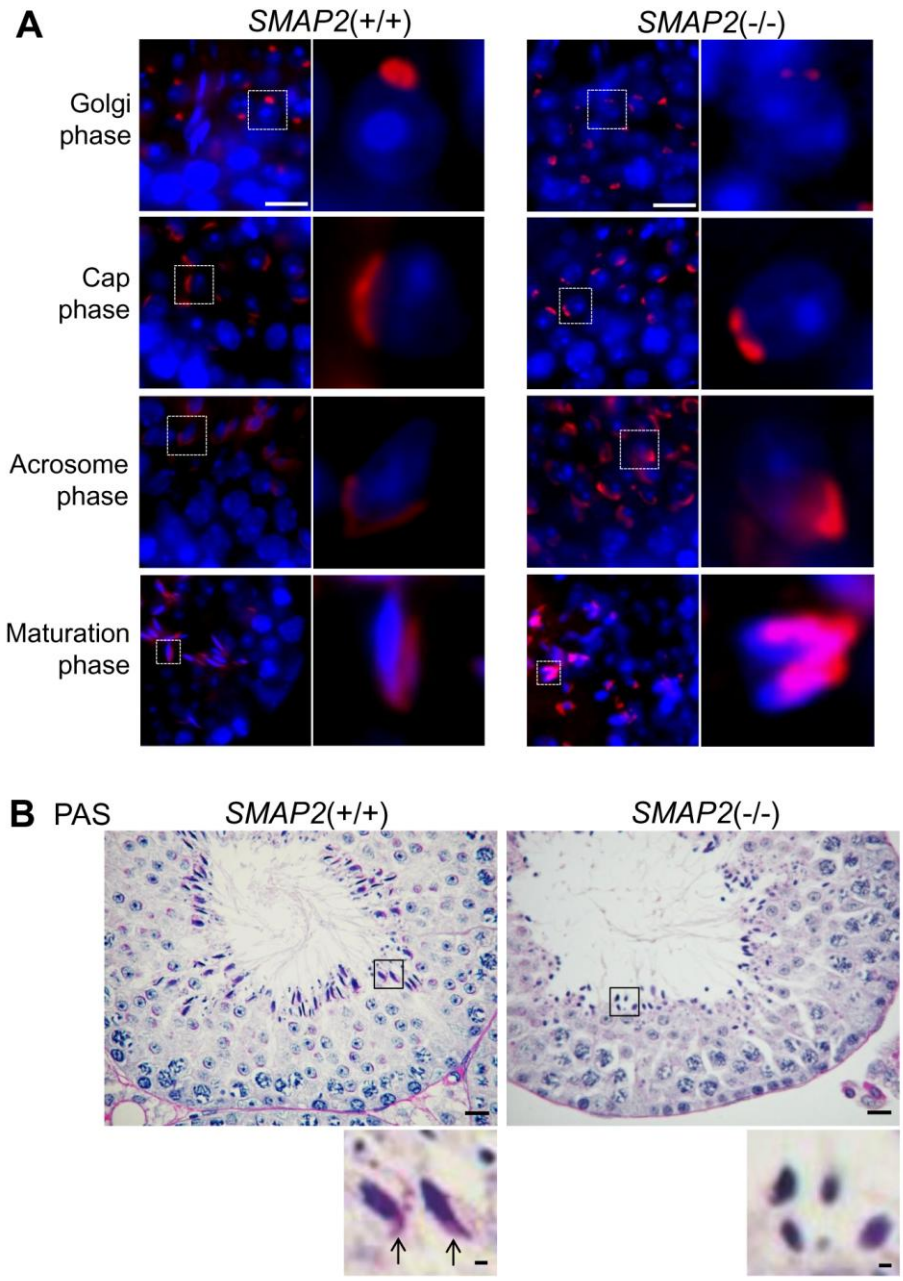
[1)] In *in vitro* fertilization, the final concentration of sperm was 200 cells/μl.
[2)] For one experiment, normal appearing oocytes were collected from two female BDF1 mice.
[3)] Number of 2-cell embryos was counted at 24hr after fertilization.

Supplemental Table S3. Intracytoplasmic sperm injection

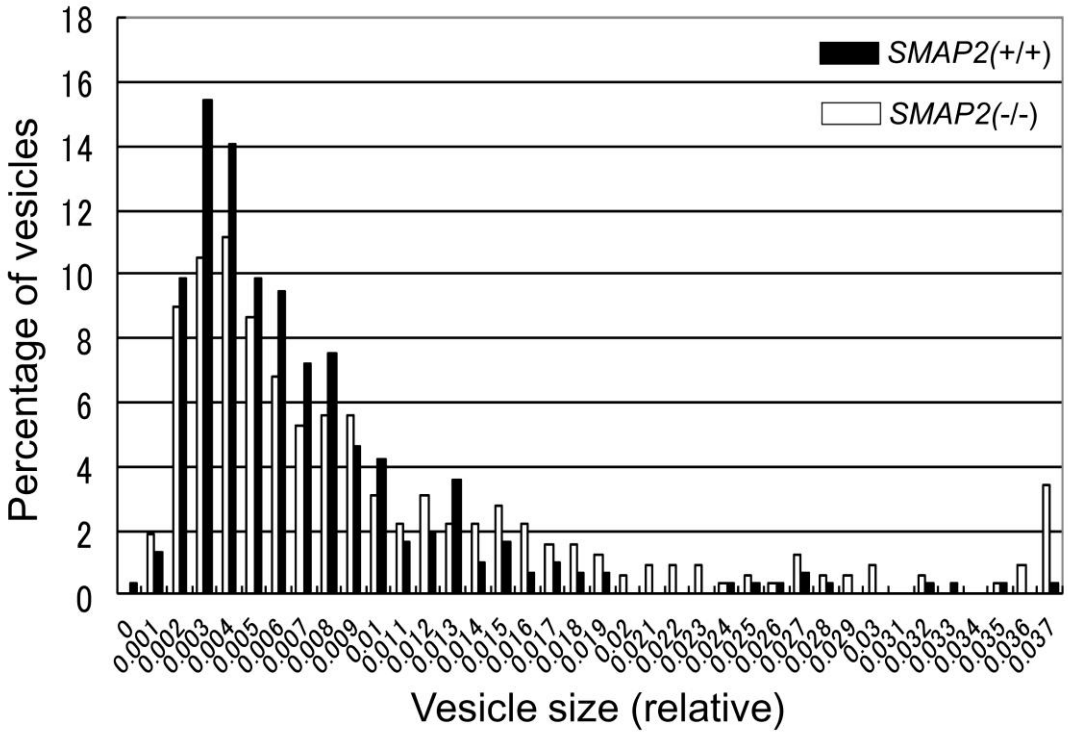
Experiment no.	ID of male mice used	*SMAP2* genotype of sperm	Number of oocytes used	Number of 2-cell embryos (%)	Number of embryos transferred (%)	Number of embryos implanted (%)	Number of offsprings (%)
1	KI	(-/-)	70	62 (89%)	62	56 (90%)	38 (61%)
2	HM	(-/-)	61	39 (64%)	39	21 (54%)	22 (56%)
3	AO	(+/-)	35	25 (71%)	25	16 (64%)	10 (40%)
4	NO	(+/-)	33	25 (76%)	25	22 (88%)	19 (76%)



Supplemental Figure S1. Spermatogonial stem cell transplantation experiments. In the left panels, spermatogonia were prepared from *SMAP2*(-/-) testes and injected into the seminiferous tubules of testes of W/W^{v} mice. In the right panels, spermatogonia were prepared from testes of pCXN-EGFP transgenic mice, and implanted into the seminiferous tubules of busulfan-treated *SMAP2*(-/-) testes. Three months later, testes were removed and examined. The upper panels are toluidine blue-stained sections (bars, 10 μm); the lower panels are Nomarsky images of isolated sperm (bars, 5 μm).



Supplemental Figure S2. PNA- and PAS-staining of testis sections. (A) Testes from wild-type and *SMAP2*(-/-) mice were cryo-sectioned and processed for PNA (red) and DAPI (blue) staining. Seminiferous tubules that contained cells corresponding to Golgi, cap, acrosome and maturation phases are shown. Bars: 10 μm. (B) PAS staining of seminiferous tubules. Normally shaped acrosomes were detected by PAS staining in the elongated spermatids of wild-type but not *SMAP2*(-/-) testes. Bars: 10 μm, (upper), 1 μm (lower).



Supplemental Figure S3. Size distribution of proacrosomal vesicles that were observed in *SMAP2*(+/+) (closed bars) and (-/-) (open bars) germ cells at the Golgi phase. Using the photos as shown in Figure 5A, diameters were measured for each proacrosomal vesicle. Percentages of vesicle numbers are plotted for each vesicle diameter.