Supporting Information

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SI Text

RNA Isolation and RT-PCR Protocol. Total RNA was extracted using TRIzol according to the manufacturer's instructions (Invitrogen) from various mouse tissues from adult mice. First strand cDNA synthesis was performed with 2.5 μg total RNA, random hexamers, and M-MLV reverse transcriptase (Promega). A similar amount (2.5 μg) of total RNA was subjected to the above mentioned conditions in the absence of M-MLV reverse transcriptase. For PCR assays, 2 μL reaction mixture (from a total of 40 μL) obtained from the first strand cDNA synthesis reaction were used. The following primer pairs were used for the RT-PCR analyses: WT Bub1: Bub-1 (Forward): 5′-AATGCTCTGTCAGCTCATCTGTGG-3′ Bub-1 (Reverse): 5′-AGAAGCAGGAAGGTCCTTGTGTGA-3′ Bub1-β-geo: Bub-1 (Forward): 5′-AATGCTCTGTCAGCTCATCTGTGG-3′ 1665r (Reverse): 5′-TTTCCCAGTCACGACGTTGT-3′ Ribosomal protein L38: RPL38 (forward): 5′-TTCGGTTCTCATCGCTGTGAGTGT-3′ RPL38 (reverse): 5′-TCTTGACAGACTTGGCATCCTTCC-3′.

Reversal of Gene Trap. The presence of a floxed splice acceptor (SA with lox-P sites on either side) allowed us to generate mice that were lacking the SA sequence (indicated as SA−) within the gene trap. To generate SA negative (SA−) mice, we crossed the Bub1 mutants to transgenic mice that expressed Cre-recombinase under the control of mouse protamine 1 promoter for Cre-mediated excision of the SA sequences and reexpression of Bub1 in spermatocytes (1). Female mice lacking the SA sequence were generated from germ line compound mutants using this strategy. As shown in Fig. S1 (last 3 lanes), loss of the SA results in a smaller PCR product (that was further confirmed by sequencing) with primers that are specific for the gene trap.

β-Galactosidase Staining of Embryos. WT and Bub1$^{+/m}$ embryos were fixed with 1% paraformaldehyde and stained in a solution containing X-gal (2 mM $MgCl_2$, 0.01% sodium deoxycholate, 0.02% Nonidet P-40, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 0.1 M phosphate buffer, pH 7.3). Genotypes of embryos were determined from genomic DNA isolated from yolk sacs.

1. O'Gorman S, Dagenais NA, Qian M, Marchuk Y (1997) Protamine-Cre recombinase transgenes efficiently recombine target sequences in the male germ line of mice, but not in embryonic stem cells. *Proc Natl Acad Sci USA* 94:14602–14607.

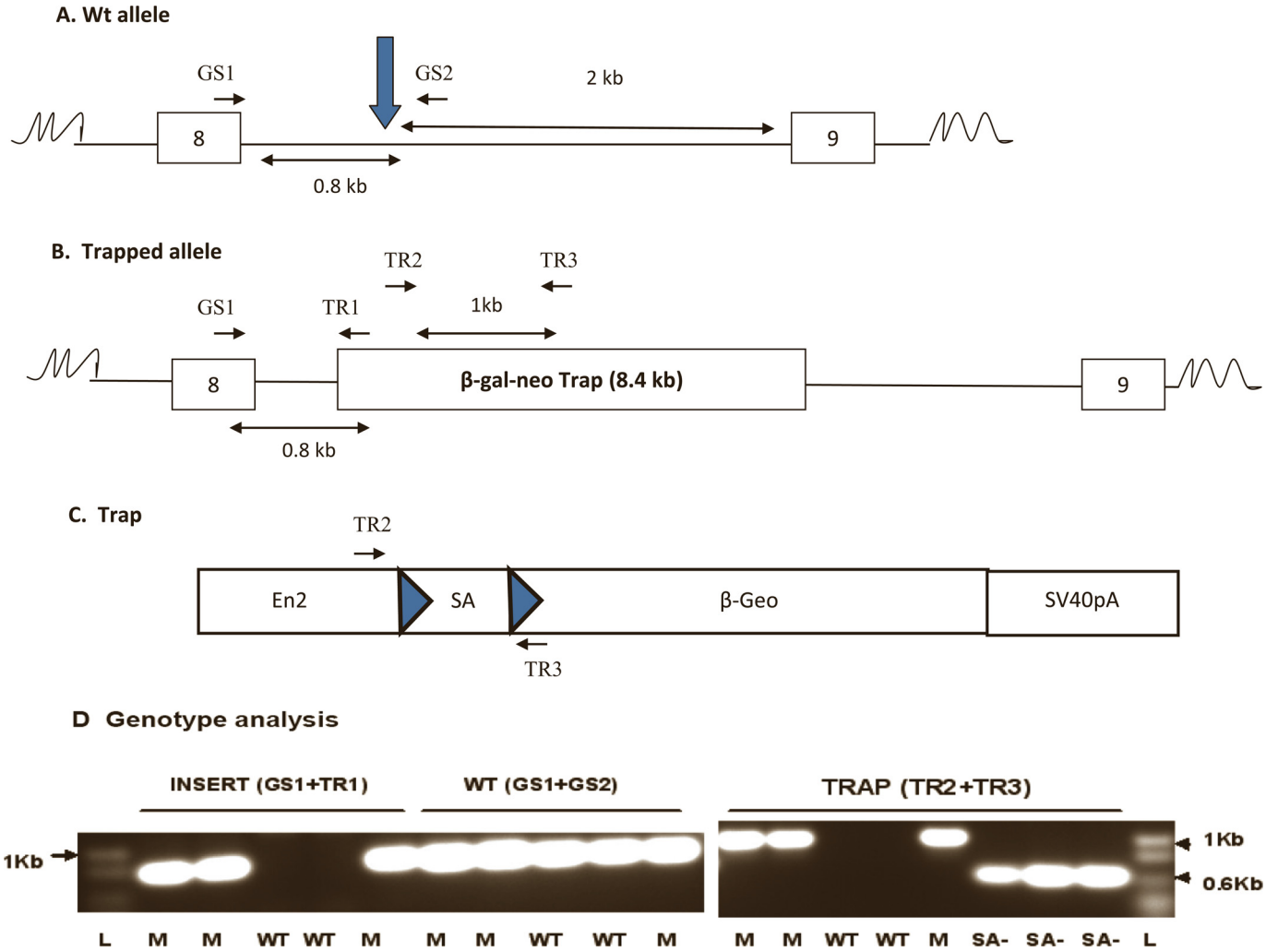

Fig. S1. Schematic representation of the Bub1 gene disruption and genotype analysis of mutant offspring. (*A* and *B*) Structure of the WT and mutant alleles at the gene-trap insertion site. (*C*) Organization of the gene trap containing a portion of *engrailed 2* intron (En2) and its floxed (triangles) SA, the β-galactosidase-neomycin fusion gene, and a SV40 polyadenylation signal. The location of the various primers used for the genotype analysis are represented by arrows (GS1, GS2, Bub1-specific primers; TR1-TR3, gene-trap-specific primers). The approximate length of the insertion site within the intron and PCR products are indicated by lines with arrow heads. (*D*) Genotype analysis of Bub1 mutant mice. Representative results of the PCR-based genotype analysis are shown for the indicated primer pair shown within brackets. The last 3 lanes refer to the SA− offspring obtained from intercrosses between Cre-transgenic-Bub1 mutants. The loss of SA leads to a smaller PCR product in the SA− offspring obtained from Cre-recombinase-positive heterozygotes (*D*, last 3 lanes).

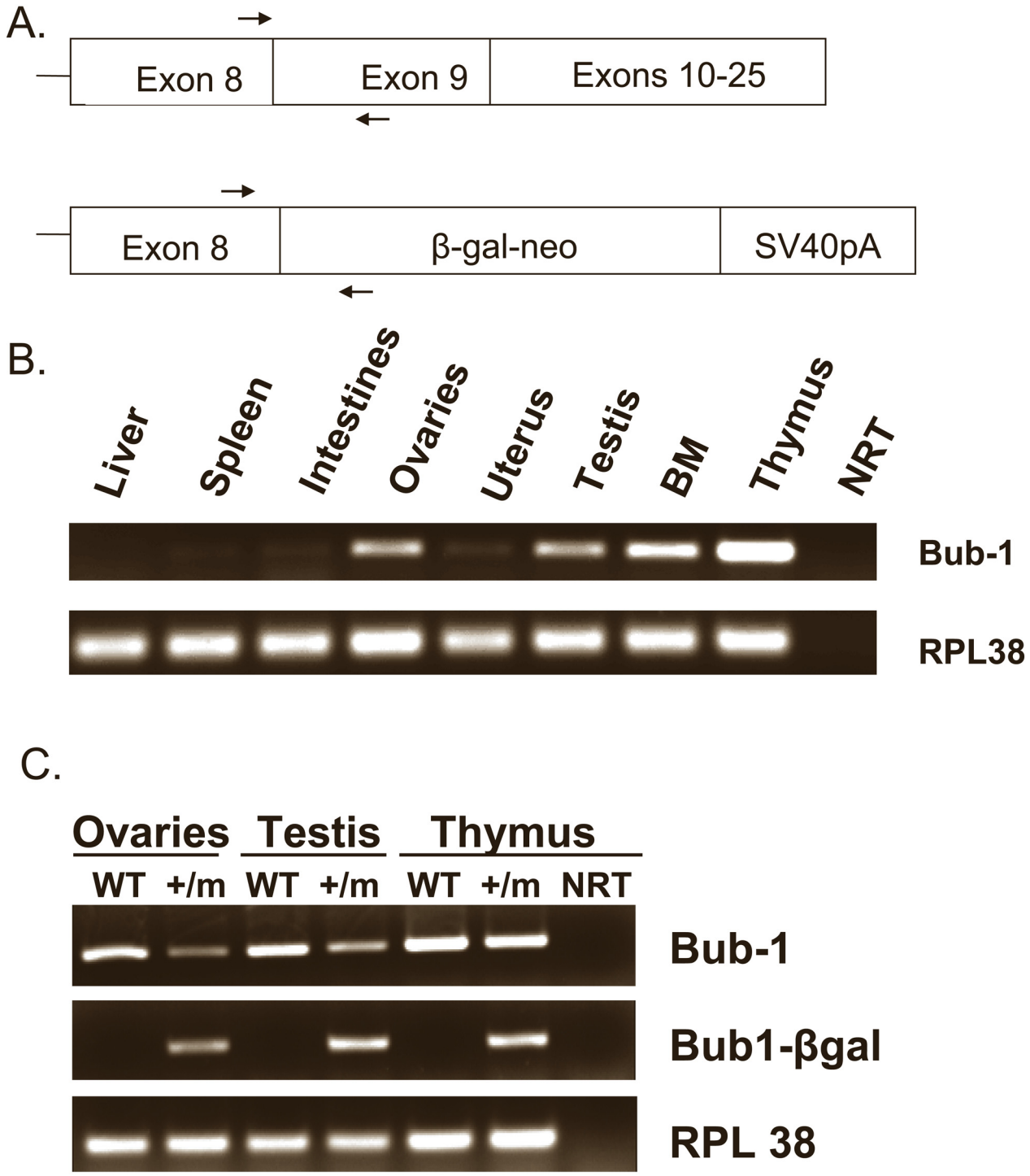

Fig. S2. Expression analysis of Bub1 and Bub1-βgal fusion mRNA in WT and mutant mouse tissues. (*A*) Structure of the WT (*Upper*) and mutant mRNA (*Lower*) species are shown. The relative positions of primers used for RT-PCR analysis are represented by arrows. (*B*) Expression of Bub1 mRNA in various mouse tissues. Rpl38, ribosomal protein 38. (*C*) Relative expression of WT and mutant Bub1 mRNAs in Bub1$^{+/m}$ mice in specific tissues. The primers used for the RT-PCR analyses are indicated in *A*.

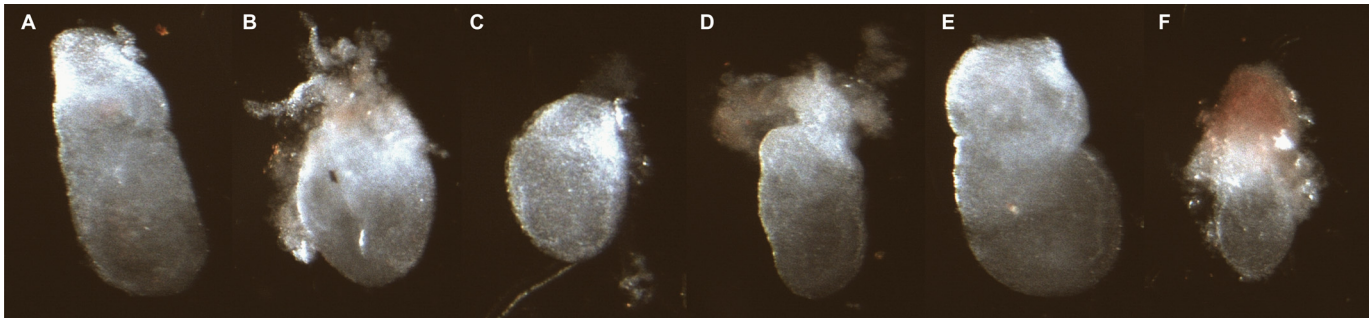

Fig. S3. Bub1 mutation leads to embryonic arrest. A representative example of embryonic arrest phenotypes obtained from a cross between a mutant female and a WT male at 7.5 dpc is shown above. A total of 8 implantation sites were noted, of which 2 were empty indicating loss of embryos after implantation. In the above example, embryos in *A* and *E* were scored as normal with developmental features appropriate for E7.5, and embryos in *B*–*D* and *F* were scored as arrested.

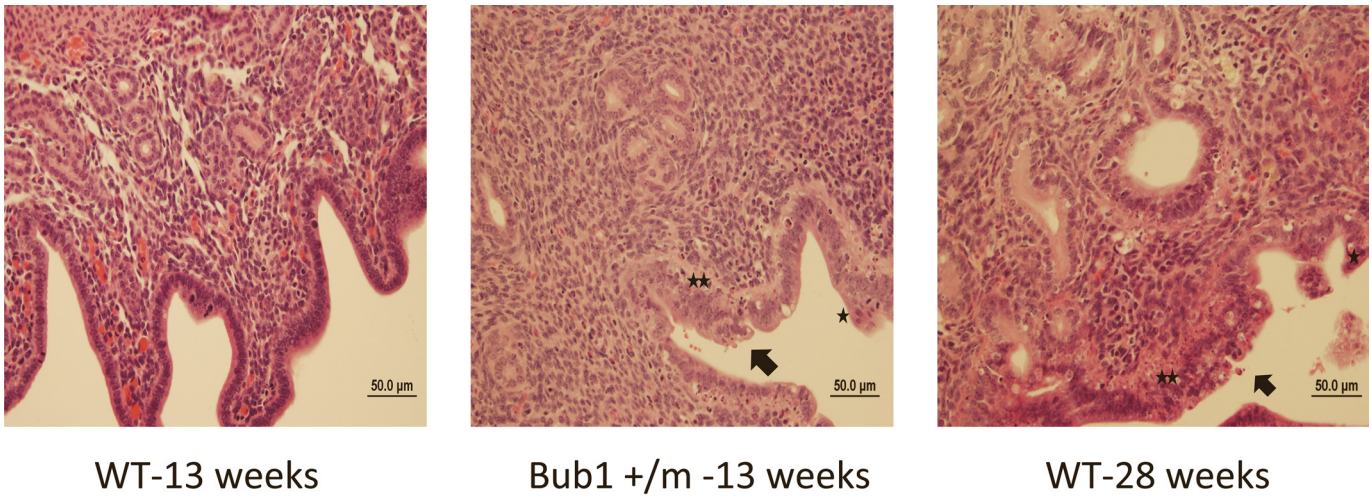

Fig. S4. Comparison of uteri from diestral Bub1 mutant and WT females. H&E stained sections of uteri from age matched mutant and WT females are shown above. Note the similarities between the younger mutants and older WT female that show a mild disarray of luminal epithelial cells (block arrow), rare luminal epithelial proliferation, multifocal luminal epithelial apoptosis(*), multifocal superficial stromal necrosis (**), mild neutrophilic endometrial stromal infiltrates, moderate endometrial stromal cellular density, mild deep glandular dilation, and fluid retention. In the WT section the epithelial apoptosis, multifocal superficial stromal necrosis and neutrophilic endometrial infiltrates are not as prominent.

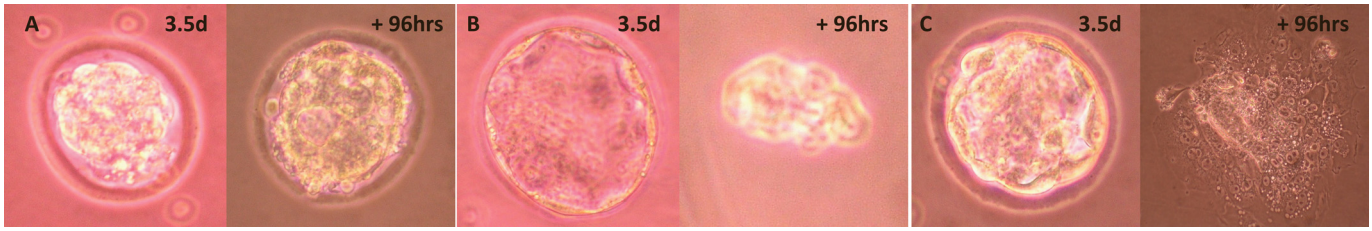

Fig. S5. Bub1 mutation leads to embryonic arrest in preimplantation embryos. A representative example of embryonic arrest phenotypes of blastocysts obtained from Bub1 mutant females crossed to WT males is shown above. A total of 6 blastocysts were cultured in ES cell medium, and only one of the blastocysts (shown in *C*) grew in culture whereas others (*A* and *B*) were arrested.

Table S1. Chromosome number distribution in oocytes and zygotes from Bub1 WT and Bub1$^{+/m}$ heterozygous females

Total number of chromosomes*	Chromosome karyotype†	Bub1 WT oocytes	Bub1$^{+/m}$ oocytes	Bub1 WT zygotes	Bub1$^{+/m}$ zygotes
14	12c + 4m		1		
14.5					
15					
15.5	15c + 1m		1		
16	16c				1
16.5					
17	17c		1	2	1
17.5	17c + 1m		3		
18	18c		6		7
18.5	18c + 1m		3		
19	18c + 2m		1		
	19c	2	6	1	6
19.5	19c + 1m		3		
20	18c + 4m		1		
	19c + 2m	4	1		
	20c	68	17	60	9
20.5	19c + 3m		2		
	20c + 1m	1	7		
21	18c + 6m		1		
	20c +2m		1		
	21c		6		13
21.5	19c + 5m		1		
	20c + 3m		2		
	21c + 1m		3		
22	21c + 2m		2		
	22c		2	4	5
22.5	22c + 1m		2		
23	23c				2
23.5	23c + 1m		1		
24	23c + 2m		1		
	24c				2
24.5	20c + 9m		1		
	22c + 5m		1		
25	24c + 2m		1		
	25c				1
40				3	
Total		75	78	70	47

*For the maternal complement.

†Whole chromosomes or dyads are indicated by ″c.″ Single chromatids or half chromosomes (or monads) are indicated by ″m″.

Table S2. Aneuploidy frequencies in germ cells of Bub1 heterozygous male mice

Female strain	Male strain	Total cells	Paternal chromosome number (%)			
			<20	20	21–25	40
Metaphase II spermatocytes						
	Bub1 WT	127	7 (5.5)	120 (94.5)	0 (0.0)	0 (0.0)
	$Bub1^{+/m}$	109	4 (3.7)	105 (96.3)	0 (0.0)	0 (0.0)
Zygotes						
B6C3F1	Bub1 WT	157	8 (5.1)	148 (93.0)	1 (0.6)	0 (0.0)
B6C3F1	$Bub1^{+/m}$	109	2 (1.8)	106 (96.3)	1 (0.9)	0 (0.0)

$Bub1^{+/-}$ males were mated with B6C3F1 females and zygotes were collected ≈30 hr after the induction of superovulation. Bub1 WT males from the same litters of the heterozygotes males were used as controls. Metaphase II spermatocytes were collected from testis from 4 of the same males that had been used for the mating experiments