Supplemental Figure Legends

Supplemental Fig 1A. Wild type follistatin allele, targeting construct and modified allele

Wild type follistatin allele (top), targeting construct with replacement of intron sequence with Neo cassette (middle) and modified allele after homologous recombination are shown.

Supplemental Fig 1B. Sequencing of resulting mRNA

Once larger mRNA species were detected (see Fig 1 northern), the larger mRNA species were reverse transcribed and cloned for sequencing. We found that a cryptic splice acceptor site within the neo cassette was used to create the longer sequence but a stop codon was still created at the end of exon 5 so that only the FST288 protein isoform could be translated.

Supplemental Fig 2. The number of secondary and antral atretic follicles at 42 days, 98 days and 250 days.

Atretic follicles were identified using criteria reported by *Devine et al.(Biol of Reprod, 63:1245-1252, 2000)* with modifications, including: more than five pyknotic, darkly stained and shrunken granulosa cells/follicle, alteration in the oocyte shape from its normal round appearance, and lack of an intact layer of granulosa cells surrounding and in contact with the surface of the oocyte, all of which must be in the same section. The follicle was only counted if it contained an oocyte with its nucleus to prevent from double

counting. Atretic follicles in which the oocyte nucleus was not readily apparent were not counted.

Fig 2A, 2C and 2E show the numbers of atretic secondary (left bars) or antral (right bars) follicles at 42 days, 98 days and 250 days, respectively. Fig 2B, 2D and 2F show the atretic per follicle number as a percentage of total follicles counted for the same specimens. White bars show WT and black bars show FST288-only.

As expected from the reduced numbers of healthy follicles in FST2888-only ovaries shown in manuscript Fig 5B, the number of secondary atretic follicles was greater in FST288-only mice, but the number of antral atretic follicles was not different. When plotted as percent of total, neither secondary or antral atretic follicle number was not different. However, compared to other ages, more follicles are atretic at 42 days for both genotypes. These data suggest that increased atresia may be one mechanism whereby the excess secondary follicles in FST288-only ovaries are lost after puberty as shown in manuscript Fig 5. In addition, this increased atresia seems to extend to older females since the number of atretic secondary follicles was significantly greater in 250 day old FST288-only females.

For antral follicles the situation is less clear. At 98 and 250 days, non-atretic antral follicles are fewer in FST288 only females (manuscript Fig.5A). Although there is a tendency for more atretic antral follicles in FST288-only females, these differences are

not significant. Thus, most of the atresia resulting from increased number of follicles in FST288-only ovaries appears to be confined to the pre-antral follicle stage.

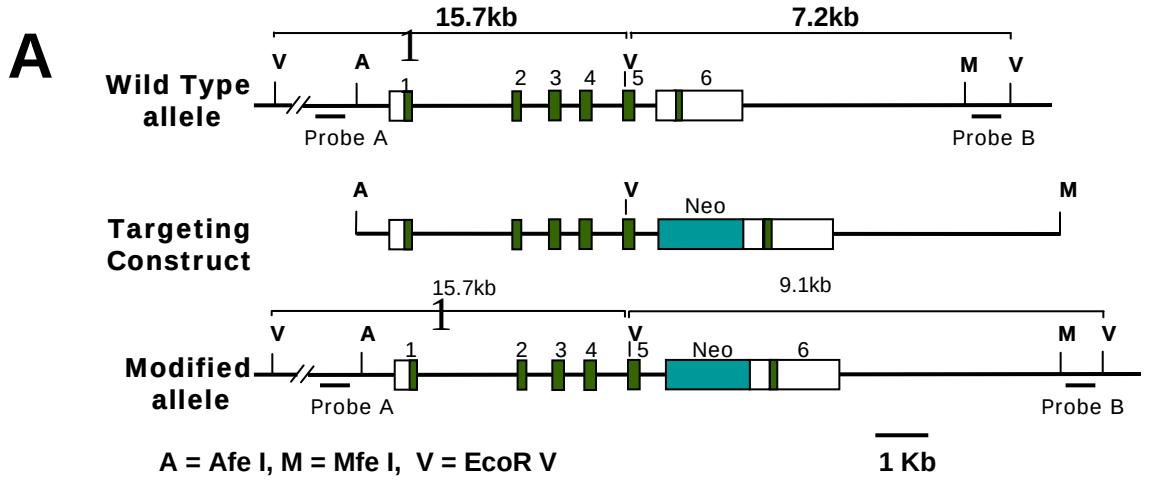
Supplemental Table 1. Sequences of primers to genotype FST288-only mice are shown. The PCRs for WT and FST288 alleles are run separately using the program: 95C for 5min, 35 cycles of 95C for 30 sec, 57 C for 1 min and 72 C for1 min, followed by 72 C for 3min.

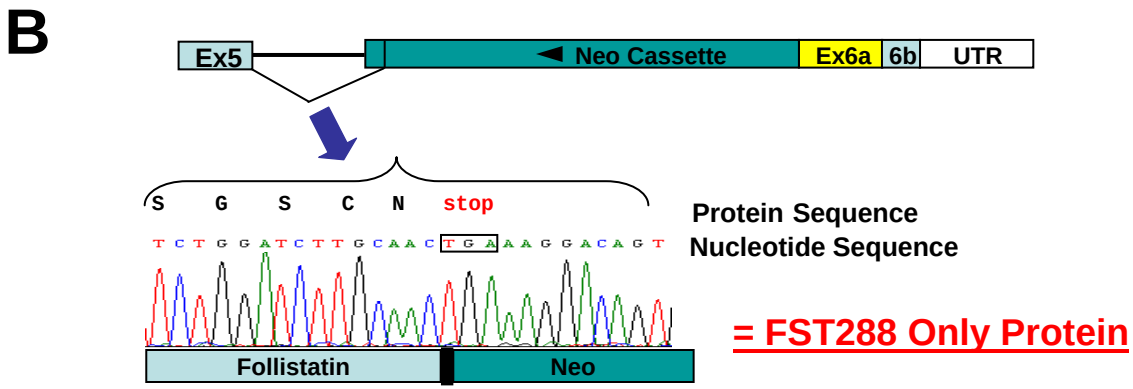
Allele Detected	Forward primer	Reverse primer
Wild Type *Fst*	CTGTGCCAGTGACAATGCCAC	CAATGGCTCAGGTTTTACAGGCAGAT
FST288-only	GGAGTAGAAGGTGGCGCGAAGG	GGTTTGGGCCTCTGCAGTTACG

Supplemental Table 2. Sequences of primers used for SYBR qPCR. The PCR program was: 95 C for 10 min, 40 cycles of 95C for 30 sec., 54 C for 50 sec, 72 C for 60 sec, followed by melt curve.

Gene Amplified	Forward Primer	Reverse Primer
Follistatin	TGGCCATGATCTTGCTGT	CCTTGACTTCTAAAAAGGGATTCA
*Inhβ*A (Activin A subunit)	ATCATCACCTTTGCCGAGTC	ACAGGTCACTGCCTTCCTTG
*Inhβ*B (Activin B subunit)	CAGTCTGCCACCCACACA	CCATTTGTCACCGCATCC
SMAD2	GCCCCAACTGTAACCAGAGA	GCCAGAAGAGCAGCAAATTC
SMAD3	TCAAGAAGACGGGGCAGTT	AGCCGACCATCCAGTGAC
RPL19	CCTGAAGGTCAAAGGGAATGTGTT	GCTTTCGTGCTTCCTTGGTCTTA

Supplemental Fig 1





Supplemental Fig 2