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Supplementary Material

corresponding to:

3`UTR Stop Cassette Knock into the *Wnt4* locus increases mRNA expression and Leads to Ovarian Cyst Formation

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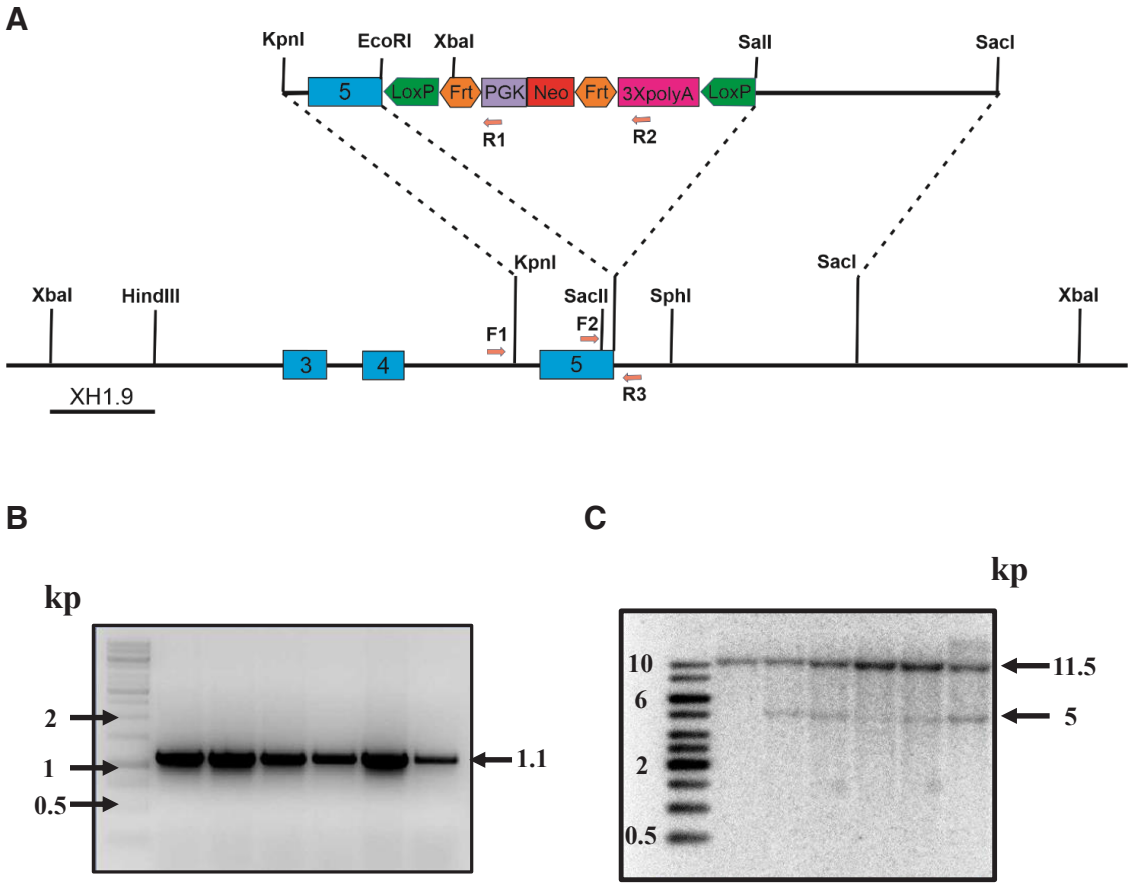
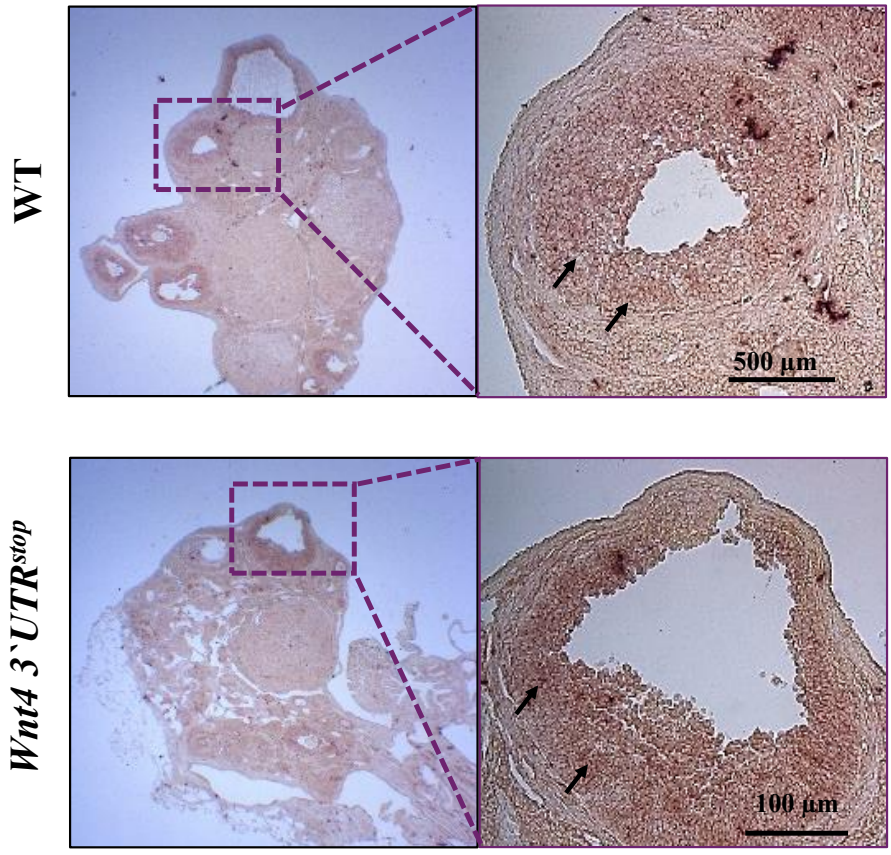
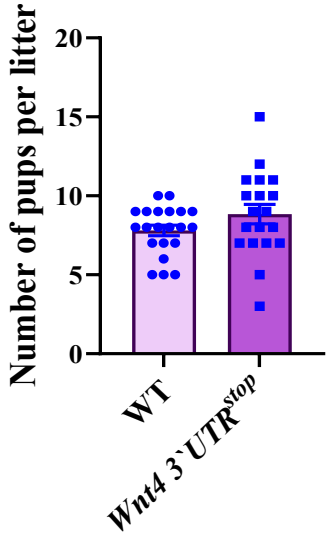


Suppl. Fig. 1. Schematic representation of the *Wnt4 3`UTR*^{stop} targeting. (A) The arms showing homology to the endogenous *Wnt4*, and the exchange cassette that was inserted after stop codon of the *Wnt4* exon5 are indicated. **(B)**. Six putative ones out of 300 ES clones were screened by PCR using primers F1 and R1, while F1 is located upstream of the 5`-homologue arm, and R1 is located at PGK promoter region. The size of amplicon is about 1.1kb. Genotyping primers for wild type allele F2 and R2 as well as for targeted allele F2 and R3 are also indicated. **(C)** Southern blot analysis confirmed six correct targeted ES clones. Genomic DNA samples were digested with XbaI. A genomic fragment XbaI-HindIII corresponding to nucleotides 38289-40137 was used as probe. Gene targeting results in the insertion of an ectopic XbaI site at the Frt site and leads to the generation of a gene-targeted fragment of around 5 kb, while the size of the wild-type allele is around 11.5 kb in Southern blotting.



**Suppl. Fig. 2. Wnt4 3`UTR$^{stop}$ modified allele results on activation of *Wnt4* expression in ovary of two-three months old mice.** *Wnt4* mRNA *in situ* hybridization (brown color, black arrows) of three month-old WT and *Wnt4 3`UTR^{stop};Wnt4 3`UTR^{stop}* ovaries. Scale bars = 500 and 100 µm.



**Suppl. Fig. 3. Deregulated *Wnt4 3`UTR* does not affect male fertility.** Number of pups per litter. Data are presented as means ± SEM, n=12 (WT) and n=19 (*Wnt4 3`UTR^{stop};Wnt4 3`UTR^{stop}*).

SUPPL. TABLE 1

PRIMERS SEQUENCES USED FOR RT-QPCR

Gene	*Primer sequence*
Wnt4	*F: 5'-CTGGAGAAGTGTGGCTGTGA-3'*
	R: 5'-GGACGTCCACAAAGGACTGT-3'
Hsd17b1	*F: 5'-TTGTTTGGGCCGCTAGAAG-3'*
	R: 5'-CACCCACAGCGTTCAATTCA-3'
Hsd3b1	*F: 5'-CAGGAGCAGGAGGGTTTGTG-3'*
	R: 5'-GTGGCCATTCAGGACGAT-3'
Cyp19	*F: 5'-GCAATCCTGAAGGAGATCCA-3'*
	R: 5'-CCGTCAATTACGTCATCCT-3'
Amh	*F: 5'-CGCTATTTGGTGCTAACCGTG-3'*
	R: 5'-CTTGCAGCTGATCGATGCTCA-3'
Amhr	*F: 5'-CCCAACATCCCATCCACTT-3'*
	R: 5'-GCTGAAAGGCAGTTCTCTGG-3'
Lhcgr	*F: 5'-GCCCTGAGCCCTGCGACTGC-3'*
	R: 5'-AAAGCGTTCCCTGGTATGGTGGTT-3'
Fshr	*F: 5'-TGTCATTGCTCTAACAGGGTCT-3'*
	R: 5'-TGGTGAGCACAAATCTCAGTTC-3'
GAPDH	*F: 5'-AGAACATCATCCCTGCATCC-3'*
	R: 5'-CAGTGAGCTTCCCGTTCAG-3'