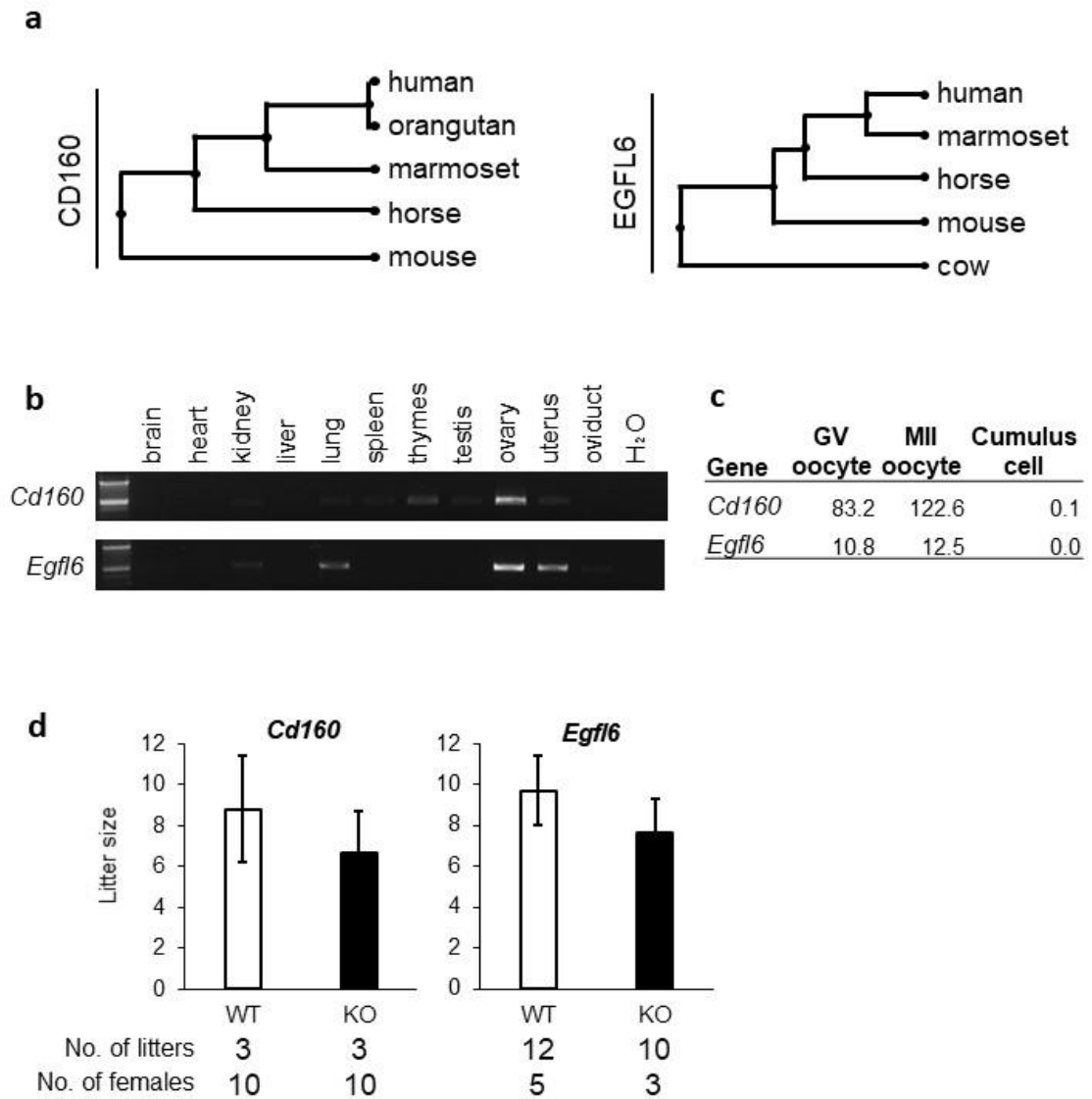**Figure S1.** Conservation of *Cd160* and *Egfl6* between species, tissue expression analysis, and KO mice fertility. (a) Phylogenetic trees. (b) RT-PCR analysis using multiple tissues. (c) Expression levels in germinal vesicle (GV) oocytes, metaphase II (MII) oocytes and cumulus cells. (d) Average litter size of control and KO females.

Table S1. Primers used for RT-PCR analysis.

Gene	Upstream	Downstream	Band size (bp)
Oosp1	GTCTGCAATGTGCCGACCAC	CTCAGACCTGCTCGTTTCAG	464
Oosp2	AGTCTGCCGGATGGGGGTCT	TCCGAGAGGCAGAACACTGCAA	374
Oosp3	ATGAAGGCCTTCGTTGCTTC	AGTACCAGGAACCAGCATTG	470
Cd160	GCTGGCAATTGTGAACTTCC	AACTGAGAGTGCCGTTGATA	428
Egfl6	GGTGCAAGTTCGGTGAGTGT	CGGGTATTGAGGCAATTGGC	517
Actb	CATCCGTAAAGACCTCTATGCCAAC	ATGGAGCCACCGATCCACA	171

Table S2. sgRNAs used for generating knockout mice. sgRNAs used for generating *Oosp* family knockout mice targeted the gene's upstream (U) and downstream (D) regions. EP: electroporation

Gene	sgRNA (5'→3')		PAM	Mutation
Oosp1	–		–	COMP tm1b
Oosp2	GTGCGTGTATTTGAGTTACA		CGG	-11/-11
Oosp3	GCTGCCCAGTAACTTCCATA		AGG	-26/-26
Oosp family	U	TATGCCACTATCTACTGAGG	AGG	-64159/ -64159
	D	ATGCTGCTGTGGCAAGTCAA	CGG	
Cd160	AGGGCACAGCAGCTTTGGCC		AGG	-20/-20
Egfl6	CAGCCTGGGGTCTGTCAGTA		TGG	+1/+1

Table S3. Efficiency of generating knockout mice. EP: electroporation.

Gene	method	No. of transferred egg	GMO / pups genotyped
Oosp1	COMP tm1b	*ES	*ES 24/28
Oosp2	Plasmid injection	87	2/19 (11%)
Oosp3	Plasmid injection	102	3/26 (12%)
Oosp family	EP	93	7/33 (21%)
Cd160	Plasmid injection	72	1/3 (33%)
Egfl6	Plasmid injection	75	1/6 (17%)

Table S4. Primers used for genotyping analysis and conditions of PCR.

Gene	Allele	Forward primer	Reverse primer	Annealing	Elongation	Band Size (bp)
Oosp1	WT	GTGCCGACCACTGGTTC CATC	GCACTGTTACAGCACAG CCTCTC	65℃,30s	72℃,30s	532
	KO	CACACCTCCCCCTGAAC CTGAAAC	GCACTGTTACAGCACAG CCTCTC	65℃,30s	72℃,30s	331
Oosp2	WT	GTCCATCCTTTCGTCAC AGCTCC	CATGCATATGGATCAGT TAAAGGACC	60℃,30s	72℃,30s	406
	KO	GTCCATCCTTTCGTCAC AGCTCC	CATGCATATGGATCAGT TAAAGGACC	60℃,30s	72℃,30s	395
Oosp3	WT	AACTTCTGAGTGTTTTG	ATTATCCTATACATGGC AAG	60℃,30s	72℃,30s	577
	KO	AACTTCTGAGTGTTTTG	ATTATCCTATACATGGC AAG	60℃,30s	72℃,30s	551
Oosp family	WT	TGCCTACCTCCTGTCCT AACAACCTG	CTTGCAGTGATTCTGTT GTCTCTG	60℃,30s	72℃,30s	746
	KO	TGCCTACCTCCTGTCCT AACAACCTG	CACTGATAGTTATTTTC ATAGGTATTACAAAAGG	60℃,30s	72℃,30s	366
Cd160	WT	AACACGGGCTATTCACC AAAGGAGC	GACTTTGTGAAATGCCT TGGCTC	60℃,30s	72℃,30s	653
	KO	AACACGGGCTATTCACC AAAGGAGC	GACTTTGTGAAATGCCT TGGCTC	60℃,30s	72℃,30s	633
Egfl6	WT	CTAACTCAGAATTCT TTATCCACAGTCCAT	GTGAAGTGTCAACCACT CTGGACACTGCAC	60℃,30s	72℃,30s	304
	KO	CTAACTCAGAATTCT TTATCCACAGTCCAT	GTGAAGTGTCAACCACT CTGGACACTGCAC	60℃,30s	72℃,30s	305