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Supplemental Information

SUMO-Specific Protease 2 Is Essential for Suppression of Polycomb Group Protein-Mediated Gene Silencing during Embryonic Development

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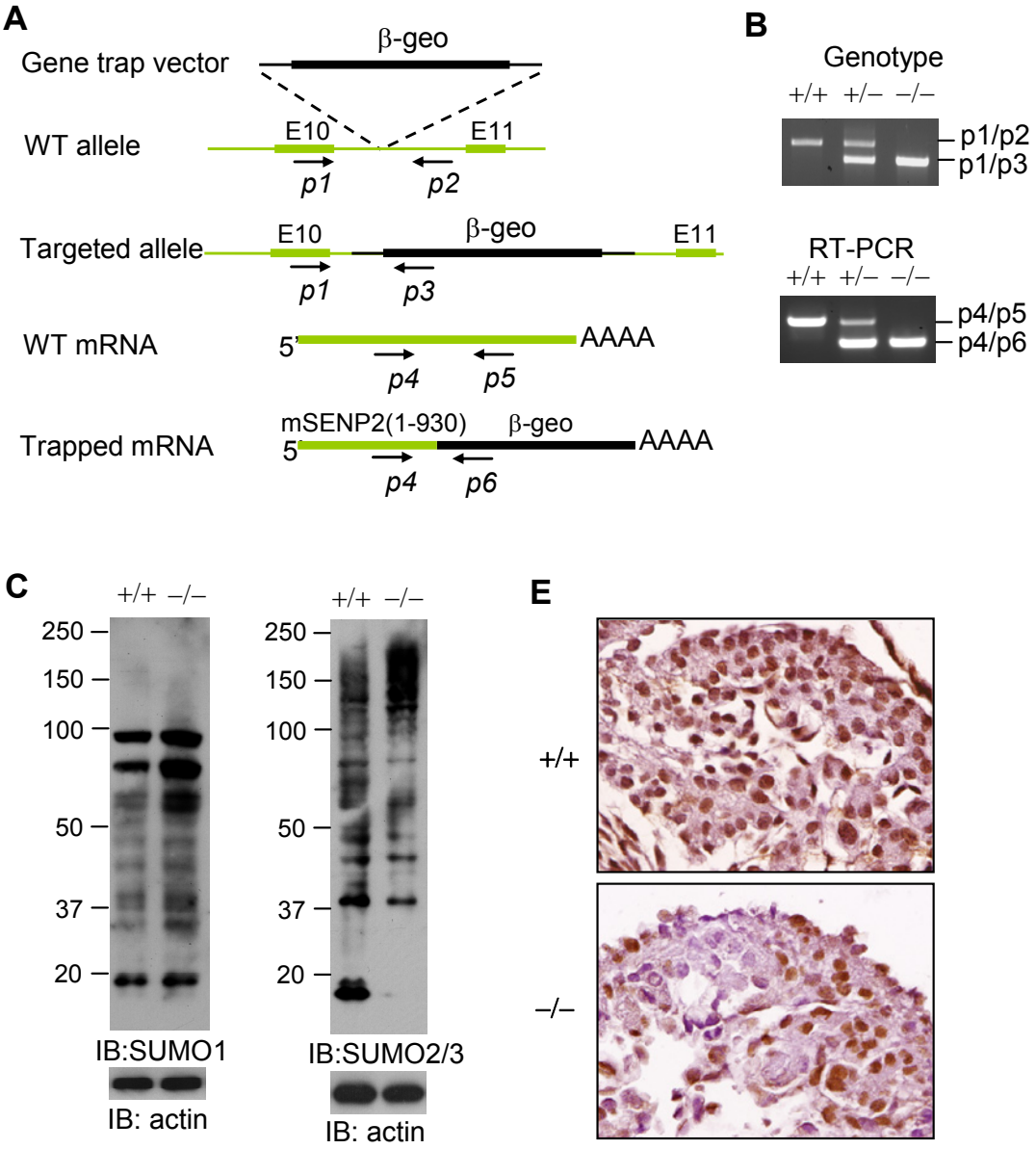

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Stage	Total (litters)	+/+	+/-	-/- (Dead)
E9.5	36 (4)	8	19	9(1)
E10.5	162(19)	42	93	27(16)
E11.5	35 (5)	10	21	4(2)
E13.5	42 (7)	13	29	0

Figure S1. Generation and analysis of mice with targeted mutation of *SENP2* gene. **A,** Structures of *SENP2* genomic DNA, the gene trap vector, the targeted allele, SENP2 WT mRNA, and trapped SENP2 mRNA. Locations of the PCR primers used for genotyping and RT-PCR are indicated. **B,** PCR analysis of genomic DNA obtained from

live progeny of *SENP2*+/- intercrosses and RT-PCR of total RNA from embryos. **C,** Western blot analysis of embryo extracts with anti-SUMO-1or anti-SUMO-2/3 antibodies. **D**. Numbers of the observed live and dead (in parentheses) *SENP2-/-* embryos to the total number of embryos analyzed at different stages of development. **E**. Immunostaining with anti-PCNA in heart sections from *SENP2*+/+ and *SENP2-/-* embryos at E10.5 revealed reduced proliferation in the *SENP2-/-* embryos.

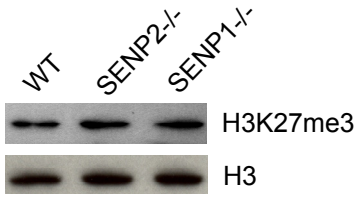

Figure S2. Mutation of SENP2 does not alter H3K27 methylation pattern. The lysates from SENP2+/+ (WT), SENP2-/-, and SENP1-/- MEF cells were analyzed by immunoblotting with anti-H3K27me3 or H3 antibodies.

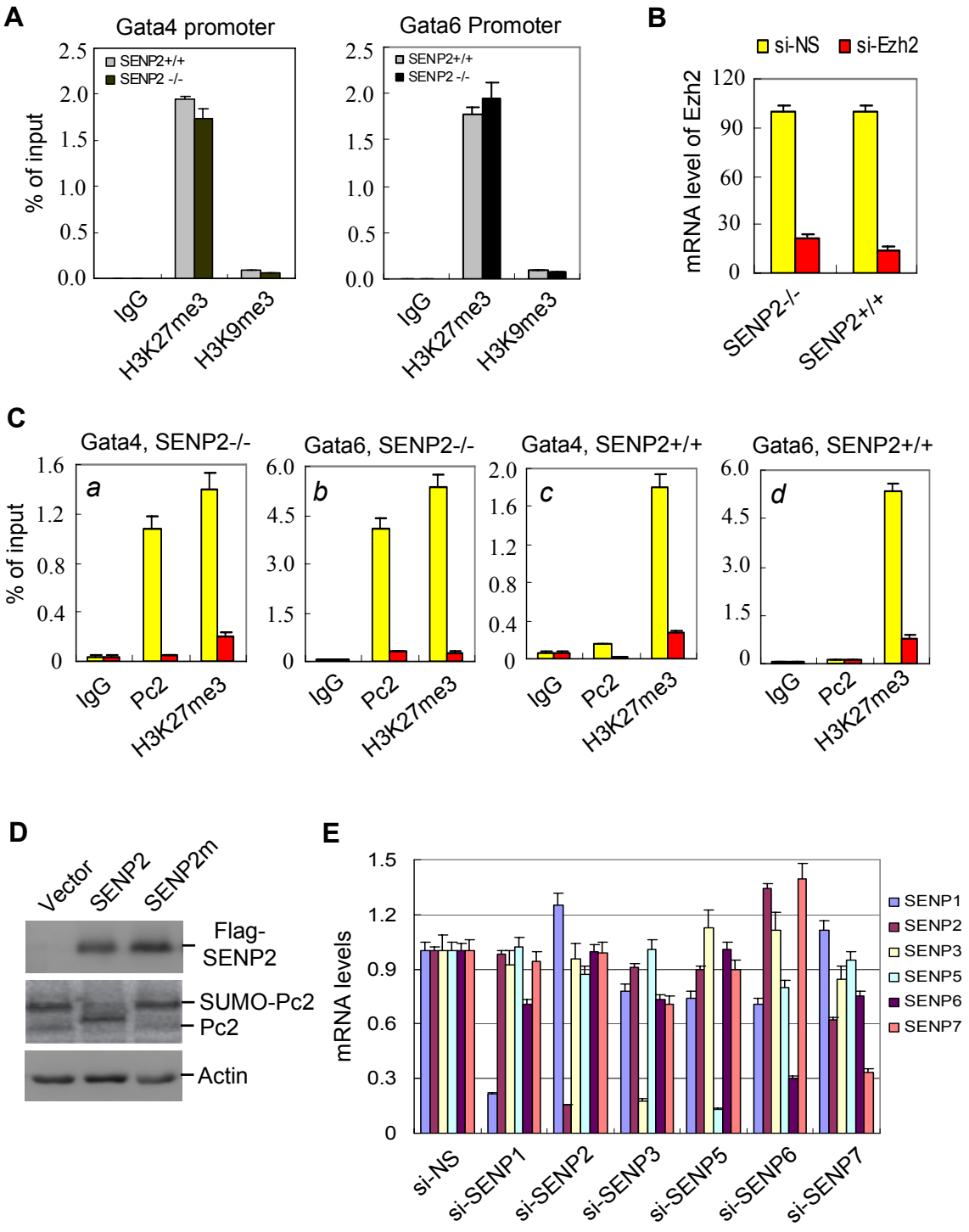

Figure S3. SENP2 regulates Pc2 occupancy via H3K27me3. A. H3K27me3 and H3K9me3 enrichment at the locus of *Gata4* and *Gata6* promoter were analyzed by a qChIP assay in SENP2+/+ and -/- MEF cells. Data are shown in means±s.d. of three independent transfection experiments. **B.** Ezh2 mRNA was measured by real-time PCR in the SENP2-/- and SENP2+/+ MEF cells transfected with siRNA against Ezh2 (si-Ezh2: shRNA sequence AGCTCAAGAGGTTCAGAAG) or non-specific control (si-NS). **C.** Pc2 and H3K27me3 occupancy at the *Gata4* and *Gata6* promoter in SENP2-/- MEF cells

(*a* and *b*) and SENP2+/+ MEF cells (*c* and *d*) were analyzed by a qChIP assay as indicated. Pc2 binding to the promoters of Gata4 and Gata6 was much higher in si-NS SENP2-/- MEF cells (*a* and *b*) than that in si-NS SENP2+/+ MEF cells (*c* and *d*) presumably due to enhanced SUMOylation of Pc2 in SENP2-/- MEF cells (see Figure 5B). "Yellow" indicates si-NS and "red" indicates si-Ezh2 cells. Data are shown in means±s.d. of three independent transfection experiments. **D.** The expression level of SENP2, Pc2, and SUMO-Pc2 in SENP2-/- MEF cells transfected with pBabe-SENP2 or pBabe-SENP2 mutant. **E**. SENP transcripts were measured by using real-time PCR in 293 cells transfected with siRNA as indicated. The mRNA level is shown in means±s.d. of three independent transfection experiments.

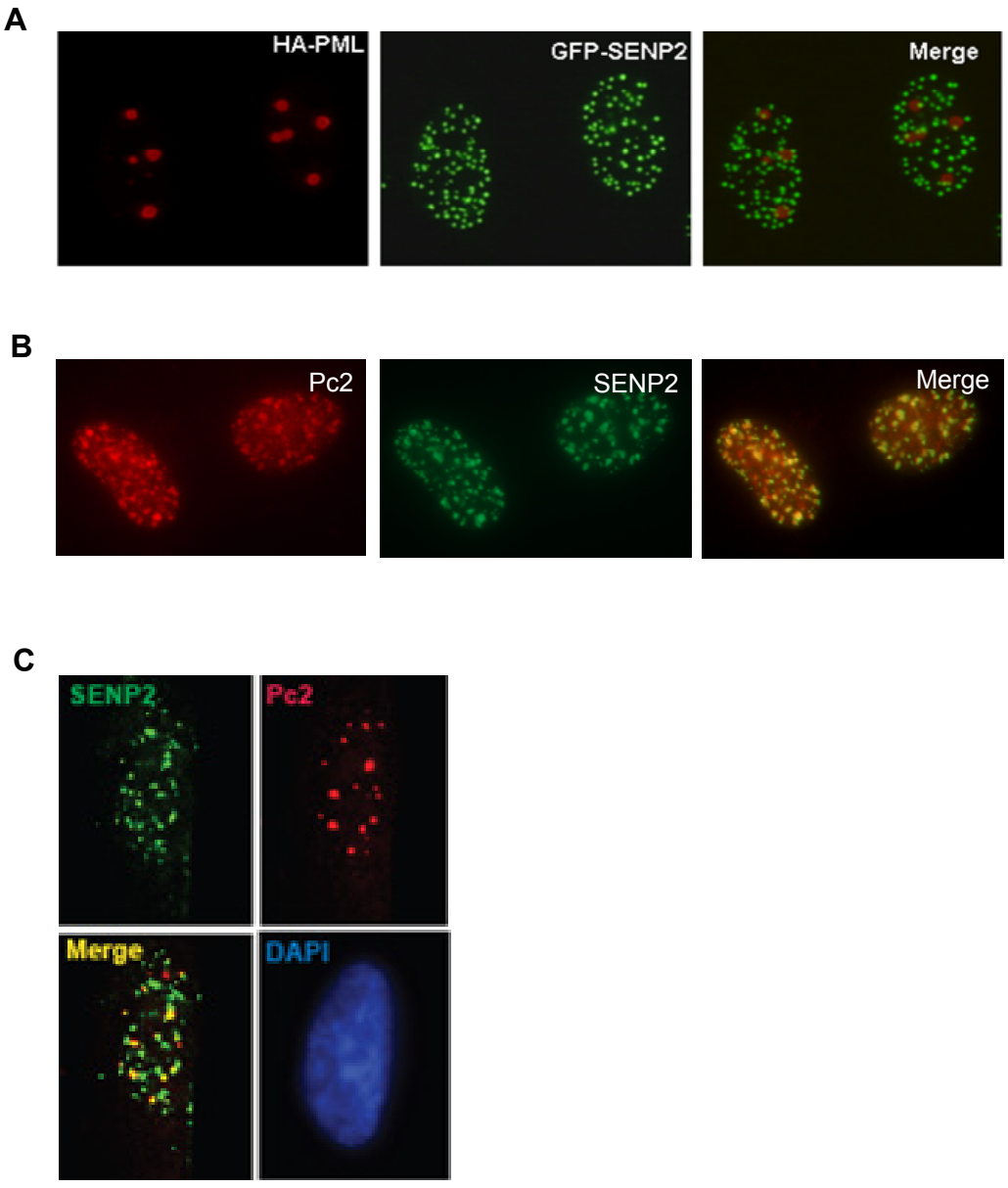

Figure S4. Co-localization of SENP2 with Pc2 in the nucleus. A. SENP2 and PML do not co-localize in the nucleus of COS-1 cells transfected with GFP-SENP2 and HA-PML. **B.** Co-localization of SENP2 with Pc2 in HeLa cells transfected with GFP-SENP2 and

Flag-Pc2. **C**. Co-localization of endogenous SENP2 with Pc2 in HUVEC cells.

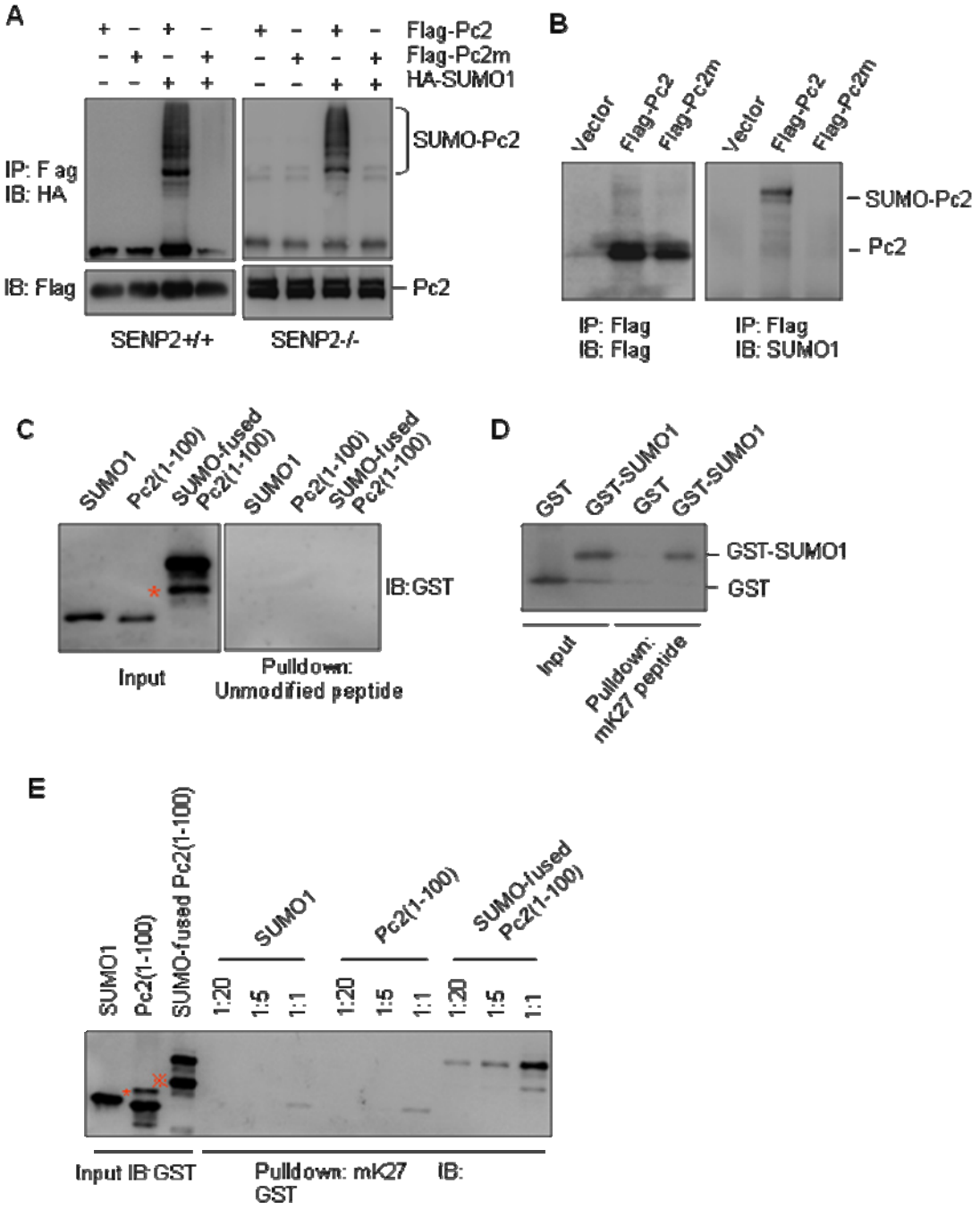

Figure S5. SUMOylation specifically enhances binding of Pc2 to H3K27me3.
A. The immunoprecipitates with anti-Flag (IP) from transfected SENP2+/+ or -/- (control for **Figure 6A**) MEF cells as indicated were detected by immunoblotting with anti-HA and anti-Flag (IB). **B.** The immunoprecipitates with anti-Flag (IP) from transfected SENP2 -/- MEF cells with Flag-Pc2 or Flag-Pc2 mutant were detected by immunoblotting with anti-Flag or anti-SUMO1 (IB). **C.** Biotinylated histone H3 unmodified peptides were incubated with GST-SUMO1, GST-Pc2(1-100), or GST-SUMO1-fused Pc2(1-100) recombinant proteins in the presence of streptavidin-conjugated sepharose beads. The inputs and precipitates were detected by immunoblotting (IB) with anti-GST. "*" indicates

a degraded band. **D.** Biotinylated histone H3 peptides that were tri-methylated on K27 (mK27), were incubated with GST and GST-SUMO1 recombinant proteins in the presence of streptavidin-conjugated sepharose beads. Inputs and precipitates by mK27 peptides were detected with anti-GST. **E.** *In vitro* titration binding assay shows that SUMOylation facilitates binding of Pc2 chromodomain to tri-methylated H3K27. Biotinylated histone H3 peptides that were tri-methylated on K27 (mK27), were incubated with titrated amount of GST-SUMO1, GST-Pc2(1-100), or GST-SUMO1-fused Pc2(1-100) recombinant proteins in the presence of streptavidin-conjugated sepharose beads. The precipitates were detected by immunoblotting (IB) with anti-GST. "*" indicates a non-specific band. "□" indicates a degraded band.

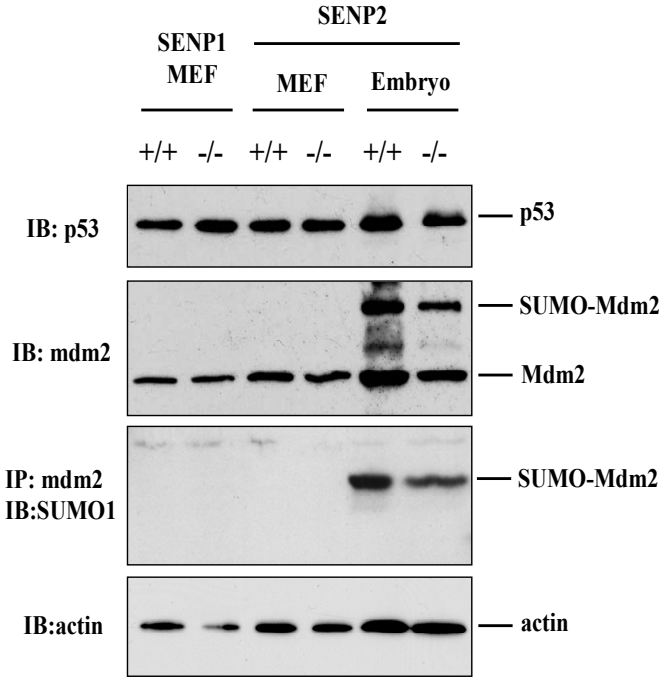

Figure S6. The p53 protein level and Mdm2 SUMOylation state were analyzed in SENP1 and SENP2 MEF cells (+/+ and -/-), and SENP2 embryos (+/+ and -/-).

Table S1. Primers for real time PCR

Gene	Forward primer	Reverse primer
mSENP2	AAGAAGTGTCTGCCCGACTT	TGGCCCAGGGCATTACTGATTT
mBmp6	AGCAGAGTCGCAACCGGTC	GGGTGCAATGATCCAGTCCT
mGAPDH	TTCACCACCATGGAGAAGGC	CCCTTTTGGCTCCACCCT
mGata4	GTCGTAATGCCGAGGGTGA	TCCTTCCGCATTGCAAGAG
mGata6	GACGGCACCGGTCATTACC	ACAGTTGGCACAGGACAGTCC
mHoxa1	ATTCCCACTCGAGTTGTGGTCCAA	AGGTTTCCAGAGTAAACAGCGGGA
mHoxa4	TATACCCGGCAGCAAGTCTTGGAA	GATCGCATCTTGGTGTTGGGAAGT
mHoxa7	AGACGCTGGAACTGGAGAAGGAAT	TCTTCCACTTCATGCGCCGATTCT
mHoxa11	AGCGAGAGTTCTTCTTCAGCGTCT	TTTGACTTGACGGTCGGTGAGGTT
mHoxb2	GCTGGAGAAGGAGTTCCACTTCAA	GCTTGTGTTTCATGCGTCGGTTCT
mHoxb4	CCTGGATGCGCAAAGTTCA	CGCGTCAGGTAGCGATTGTA
mHoxb7	CCCGAACAAACTTCTTGCGCCTTT	CAGTGCATGTTGAAGGAACTCGGT
mHoxb13	CATTCTGGAAAGCAGCGTTTG	TGTTGGCTGCATACTCCCG
mHoxc6	TAACCCTTCCTTATCCTGCCACCT	TGCTCCATAGGTCGAGAAATGCCT
mHoxc10	TGACAGCAAAGAGCGGAAGGAAGA	AATGGTCTTGCTAATCTCCAGGCG
mHoxc12	TCTGGTTTCGCCGTTGAAC	AGATTCAAGCGGTCCGAGAGT
mHoxd13	AAAGGGTGCCTTACACCAAACTGC	ACAGTGTCTTTGGCTTGGAGACGA
hSENP2	AGCCTGGTGGTGATTGACCTAAGA	AGCTGTTGAGGAATCTCGTGTGGT
hHoxa2	TGCAGCATCTGAATTACTAAAAACA	CCA AATAAAAGAAGGCAAAACC
hHoxa4	CCACAAACTGCCCAACACCAAGAT	GTGTGGGCTCTGAGTTTGTGCTTT
hHoxa7	AGAAGGAGTTCCACTTCAACCGCT	AGATCTTAATCTGGCGCTCGGTGA
hHoxa11	AACGGGAGTTCTTCTTCAGCGTCT	ACTTGACGATCAGTGAGGTTGAGC
hHoxb4	AGGTCTTGGAGCTGGAGAAGGAAT	GGTGTTGGGCAACTTGTGGTCTTT
hHoxb7	AGACCCTGGAGCTGGAGAAAGAAT	ATGCGCCGGTTCTGAAACCAAATC
hHoxb13	TACGCTGATGCCTGCTGTCAACTA	AGTACCCGCCTCCAAAGTAACCAT
hHoxc6	AGGACCAGAAAGCCAGTATCCAGA	ATTCCTTCTCCAGTTCCAGGGTCT
hHoxc10	TGAAATCAAGACGGAGCAGAGCCT	TTGCTGTCAGCCAATTTCCTGTGG
hHoxc12	AGGGAACTCTCAGACCGCTTGAAT	AGAGCTTGCTCCCTCAACAGAAGT
hHoxd13	ATGTGGCTCTAAATCAGCCGGACA	AGATAGGTTCGTAGCAGCCGAGAT
hGata4	TCTCAGAAGGCAGAGAGTGTGTCA	GGTTGATGCCGTTCATCTTGTGGT
hGAPDH	CATGTTCGTCATGGGTGTGAACCA	AGTGATGGCATGGACTGTGGTCAT