Loss of *DDRGK1* Causes Shohat Type SEMD by increasing SOX9 Ubiquitination

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SUPPLEMENTARY INFORMATION

Tables S1 and S2

Figures S1-S3

Table S1. *Ddrgk1*$^{-/-}$ mice are embryonic lethal between E11.5 and E12.5.

Time	*Ddrgk1*$^{+/+}$	*Ddrgk1*$^{+/-}$	*Ddrgk1*$^{-/-}$	Total
3 weeks	17 (34%)	33 (66%)	0	50
E14.5	6 (42.9%)	8 (57.1%)	0 (0%)	14
E13.5	3 (50%)	3 (50%)	0 (0%)	6
E12.5	21 (32.3%)	37 (56.9%)	7 (10.8%)	65
E11.5	11 (25%)	20 (45.5%)	13 (29.5%)	44

Table S2. Primer list.

Primer	Sequence
sox9a_pCS2 F	TCTTTTTGCAGGATCCCGTCCATCTACGGTGTTCG
sox9a_pCS2 R	TCACTATAGTTCTAGATCAGTGCACATTCAGACGT
ddrgk1_pCS2 F	TCTTTTTGCAGGATCCCCAAACTGTACCTGCAGCAGG
ddrgk1_pCS2 R	TCACTATAGTTCTAGATGTAGGTCATGCGCTGCT
Ddrgk1 sgRNA F	CACCGCCAGCGCGCCTGCGGG ATC
Ddrgk1 sgRNA_R	AAACGATCCCGCAGGCGCGCTGGC
T7_Ddrgk1 sgRNA F	TTAATACGACTCACTATAGGGCCAGCGCGCCTGCGGG
T7_Ddrgk1 sgRNA_R	AAAAGCACCGACTCGGTGCC

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Shohat type SEMD patient LCLs express a mixture of two aberrant *DDRGK1* RNA species that both result in a premature stop codon. (A, B) Chromatograms of *DDRGK1* cDNA and schematic of wildtype and mutant RNA species from (A) control and (B) patient LCLs. Patient LCLs show aberrant splicing at intron 3 and a mixture of two mutant RNA species because of the c.408+1G>A mutation. The first mutant RNA species found in patient LCLs is a readthrough of intron 3, while the second mutant RNA species is the result of activation and use of a cryptic splice donor site. Both RNA species have a premature stop codon.

Figure S2. Generation of *Ddrgk1*$^{-/-}$ mice by CRISPR/Cas9 gene editing. (A) Schematic of the *Ddrgk1*^{2BL} allele showing the 310 bp c.-249_61del mutation deleting a portion of the 5'UTR and the first exon of the *Ddrgk1* gene. (B) Chromatogram of an E11.5 *Ddrgk1*$^{2BL/2BL}$ (Ddrgk1$^{-/-}$) mouse showing the deleted region. (C) RT-PCR of total RNA from E11.5 WT (n = 3) and *Ddrgk1*$^{-/-}$ (n = 3) limb buds. Values represented as means ± S.E.M (**P < 0.01; two-tailed t-test). (D) Immunoblots of total cell lysates from E11.5 WT (n = 3) and *Ddrgk1*$^{-/-}$ (n = 3) limb buds. The immunoblot probing for α-tubulin is also shown in figure 4E. (E) Whole mount preparations of E11.5 WT and *Ddrgk1*$^{-/-}$ embryos.

Figure S3. DDRGK1 does not affect the translation of SOX9. Even when translation is inhibited, overexpression of *Ddrgk1* increases levels of SOX9. 293T cells were transiently transfected with plasmids expressing His-tagged *Ub*, FLAG-tagged *Sox9* and Myc-tagged *Ddrgk1* and 24 hr later the cells were treated with 10 µg/mL of the protein synthesis inhibitor cycloheximide for 6 hr. The immunoblot is representative of two independent experiments with technical replicates.

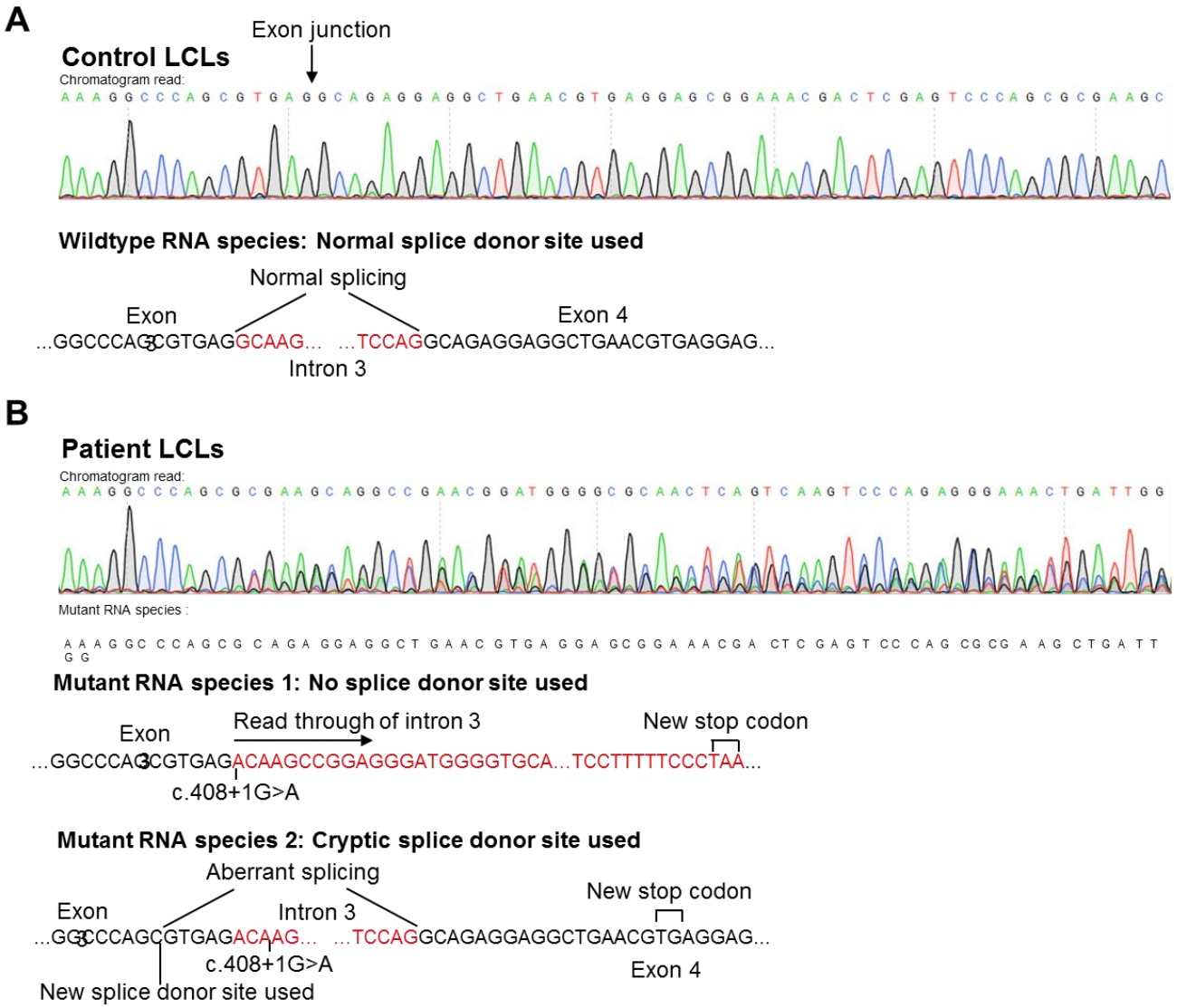
A
Exon junction
Control LCLs
Chromatogram read:
A A A G G C C C A G C G T G A G G C A G A G G A G G C T G A A C G T G A G G A G C G G A A A C G A C T C G A G T C C C A G C G C G A A G C
Wildtype RNA species: Normal splice donor site used
Normal splicing
Exon
Exon 4
...GGCCCAGCGTGAGGCAAG... ...TCCAGGCAGAGGAGGCTGAACGTGAGGAG...
Intron 3
B
Patient LCLs
Chromatogram read:
A A A G G C C C A G C G C G A A G C A G G C C G A A C G G A T G G G G C G C A A C T C A G T C A A G T C C C A G A G G G A A A C T G A T T G G
Mutant RNA species :
A A A G G C C C A G C G C A G A G G A G G C T G A A C G T G A G G A G C G G A A A C G A C T C G A G T C C C A G C G C G A A G C T G A T T G G
Mutant RNA species 1: No splice donor site used
Exon
Read through of intron 3
New stop codon
...GGCCCAGCGTGAGACAAGCCGGAGGGATGGGGTGCA...TCCTTTTTCCCTAA...
c.408+1G>A
Mutant RNA species 2: Cryptic splice donor site used
Aberrant splicing
New stop codon
Exon
Intron 3
...GGCCCAGCGTGAGACAAG... ...TCCAGGCAGAGGAGGCTGAACGTGAGGAG...
c.408+1G>A
Exon 4
New splice donor site used

Figure S1

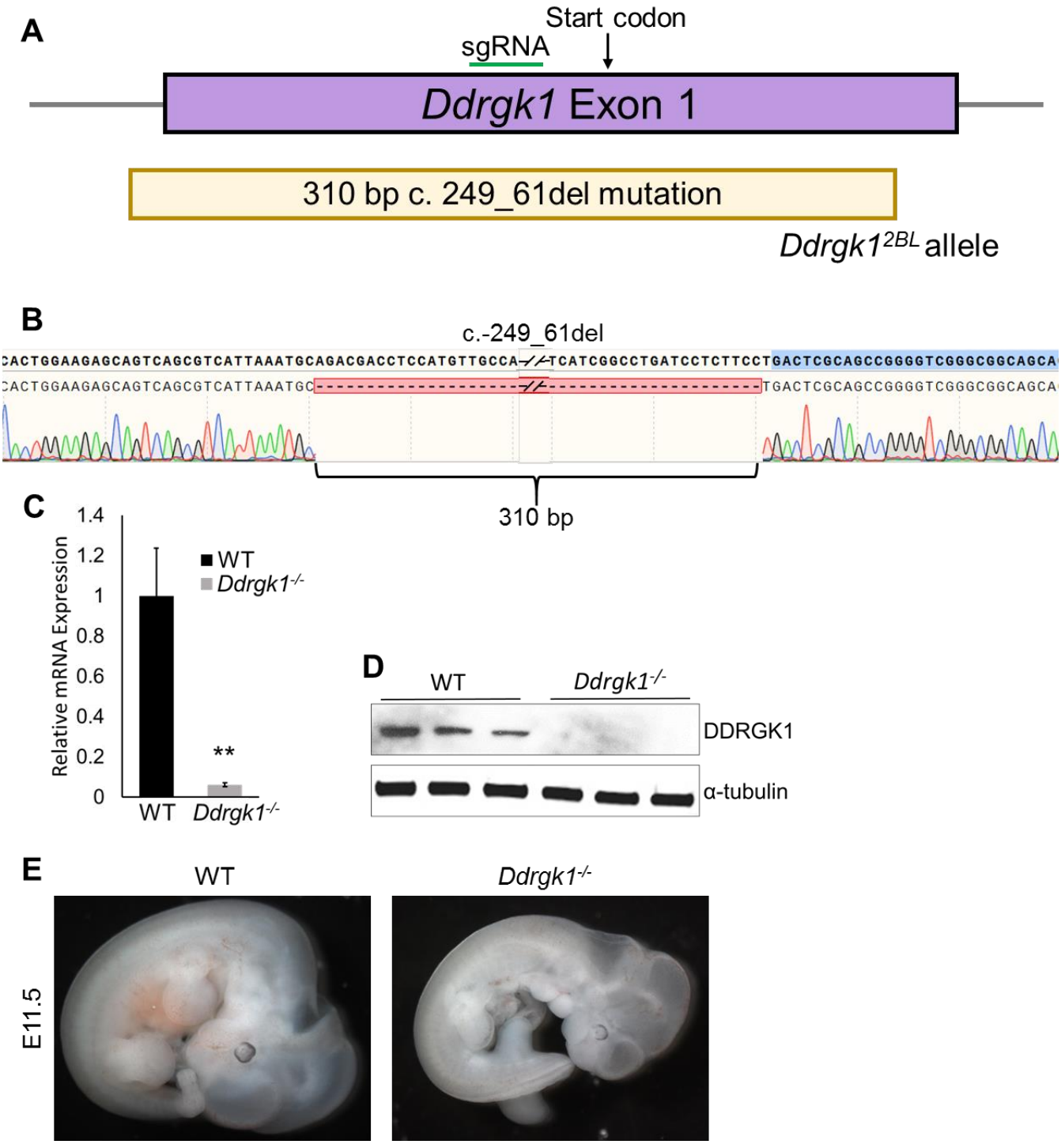
A
Start codon
sgRNA
Ddrgk1 Exon 1
310 bp c. 249_61del mutation
Ddrgk1 2BL allele
B
c.-249_61del
CACTGGAAGAGCAGTCAGCGTCATTAAATGCAGACGACCTCCATGTTGCCA--TCATCGGCCTGATCCTCTTCCTGACTCGCAGCCGGGGTCGGGCGGCAGCA
CACTGGAAGAGCAGTCAGCGTCATTAAATGC----------TGACTCGCAGCCGGGGTCGGGCGGCAGCA
310 bp
C
Relative mRNA Expression
1.4
1.2
1
0.8
0.6
0.4
0.2
0
WT
Ddrgk1-/-
**
WT
Ddrgk1-/-
D
WT
Ddrgk1-/-
DDRGK1
α-tubulin
E
WT
Ddrgk1-/-
E11.5

Figure S2

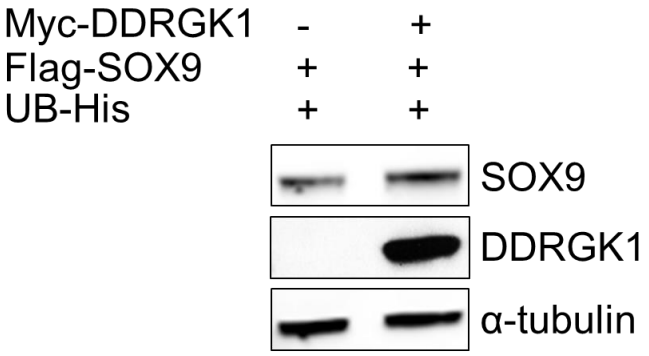

Figure S3