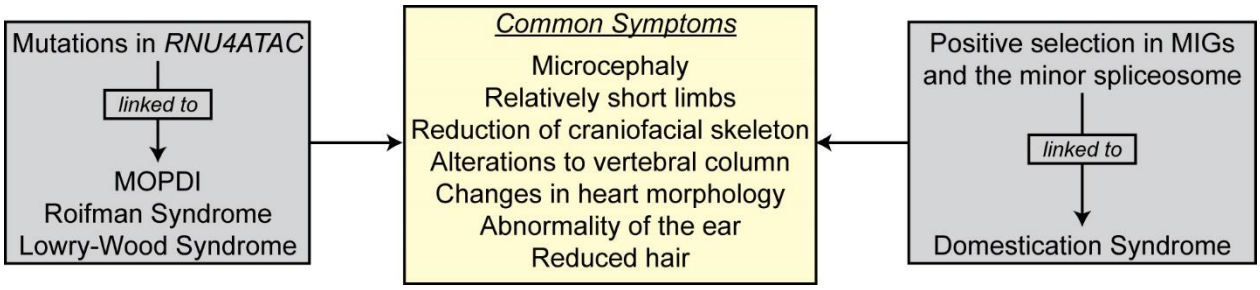

Figure S1 | Minor spliceosome-related diseases and domestication syndrome share overlapping symptoms. Schematic depicting common symptoms observed in MOPD1, Roifman syndrome, Lowry-Wood syndrome, and domestication syndrome.

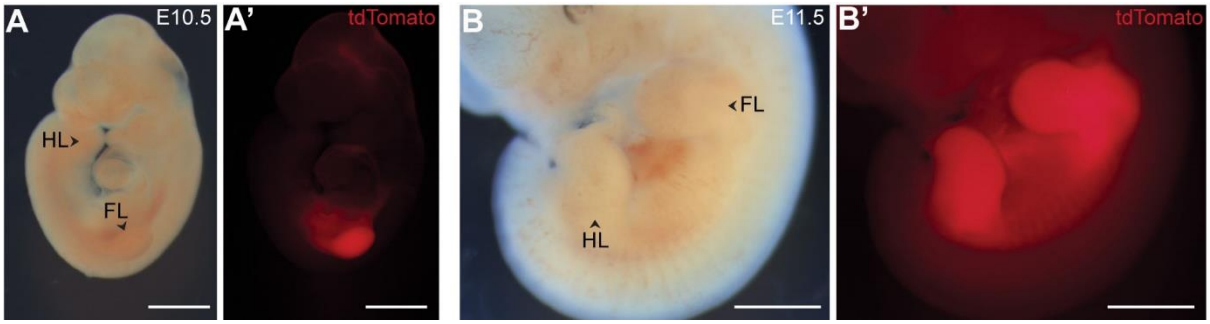

Figure S2 | *Prrx1*-Cre has delayed expression in the hindlimb relative to the forelimb. (A-B') Light image of mutant embryos **(A, B)** with red fluorescence imaging of tdTomato reporter **(A', B')** for Cre activity observed only in mutant forelimb (FL) at E10.5, FL and hindlimb (HL) at E11.5. Scale bars show 100 um.

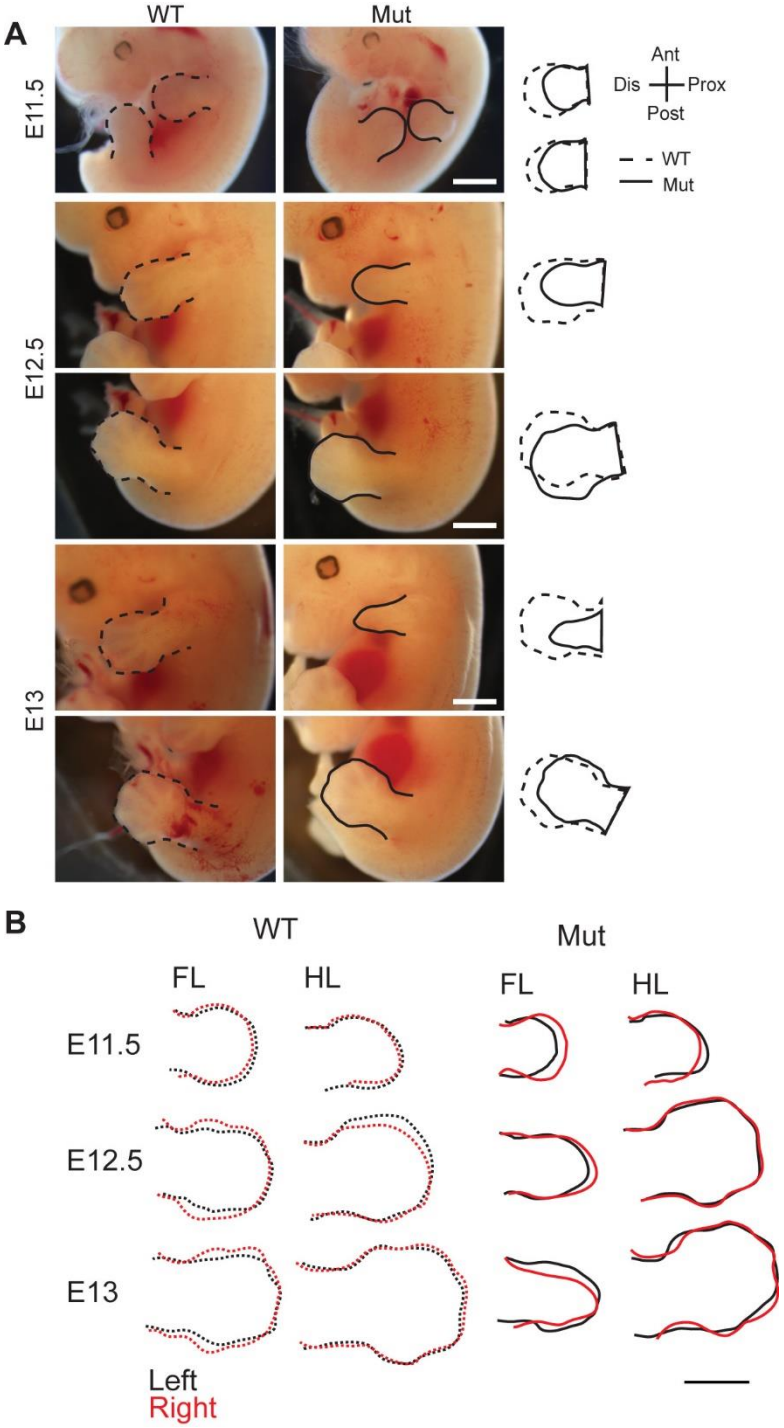

Figure S3 | U11 loss causes developmental defects to precipitate earlier in the forelimb versus hindlimb. (A) Light imaged embryos at E11.5, E12.5, and E13 with traces for WT (dashed) and mutant (mut; solid) forelimb (FL) and hindlimb (HL). **(B)** Overlay of representative traces from left (black) and right (red) FL and HL buds for WT and mutant at E11.5, E12.5, and E13. Scale bars show 100 um.

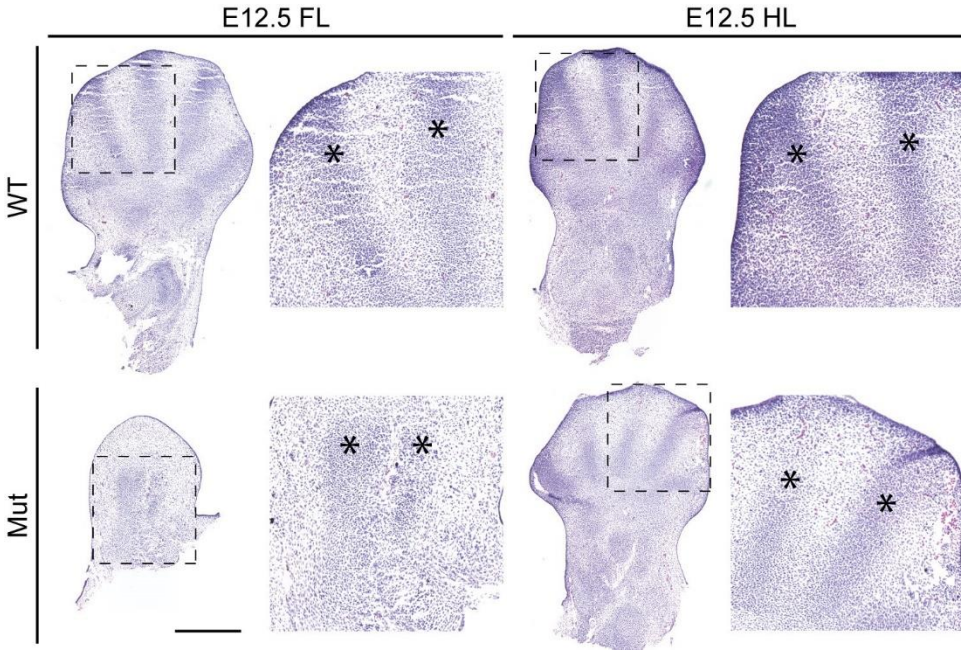

Figure S4 | Chondrogenesis is not delayed in E12.5 U11-null limbs. H&E stain of E12.5 WT (top) and mutant (Mut; bottom) forelimb (FL; left) and hindlimb (HL; right) with digital zoom for boxed regions. Asterisks represent regions of chondrogenesis. Scale bar shows 500 um.

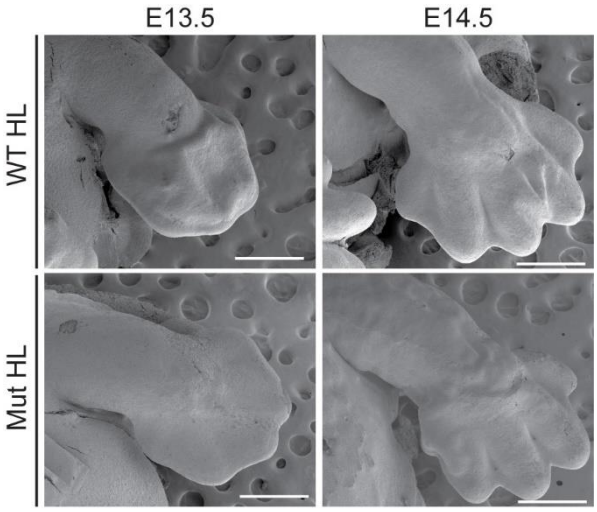

Figure S5 | The U11-null hindlimb does not show obvious morphological defects by E14.5. SEM imaging of E13.5 (left) and E14.5 (right) WT (top) and mutant (Mut; bottom) hindlimb (HL). Scale bars show 500 um.

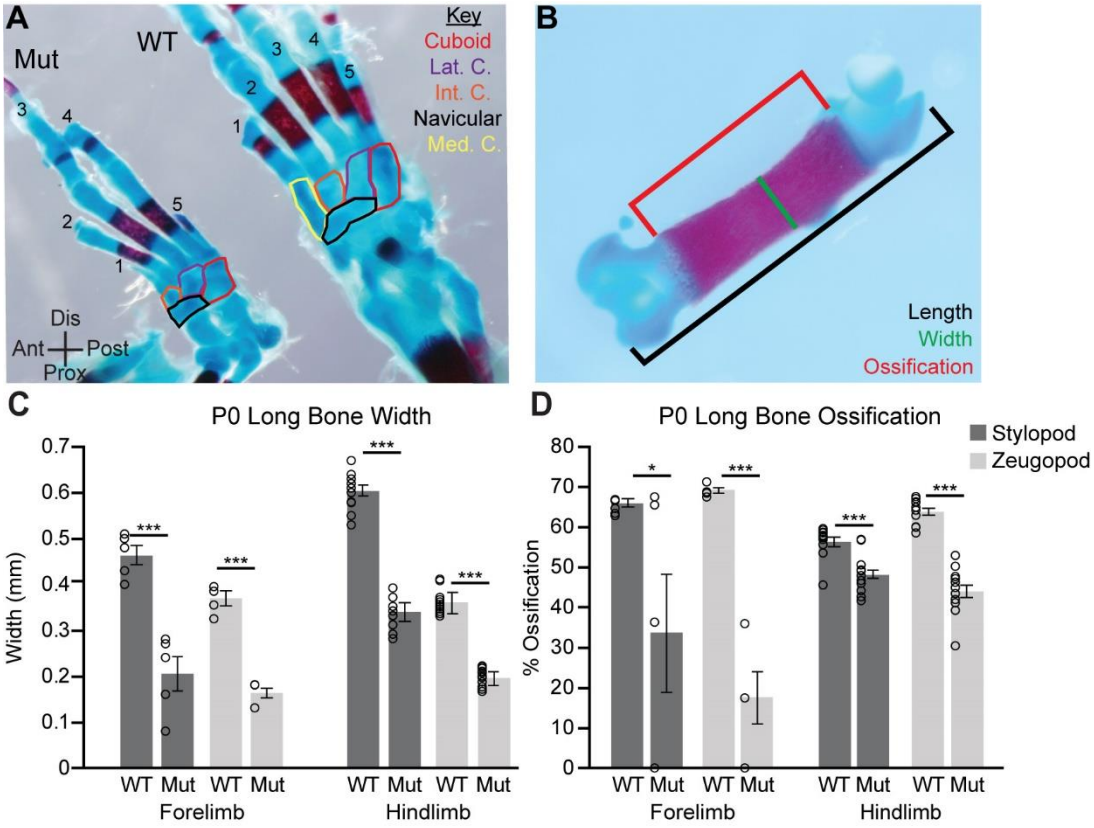

Figure S6 | U11 loss leads to compromised skeletal development at P0. (A) Skeletal preparation of mutant (mut; left) and WT (right) foot at P0 with colored traces to show metatarsals and numbers as digit labels. **(B)** Quantification schematic for long bone length, width, and ossification. **(C-D)** Bar chart showing quantification of long bone width **(C)** and ossification **(D)** for P0 WT and mutant FL and HL. Lat=lateral, Int=intermediate, Med=medial, C=cuneiform, Ant=anterior, Post=posterior, Prox=proximal, Dis=distal. Bar charts represent mean and error bars show standard error of mean. For details of statistical methods, see Table S6. *=p<0.05, **=p<0.01, ***=p<0.001.

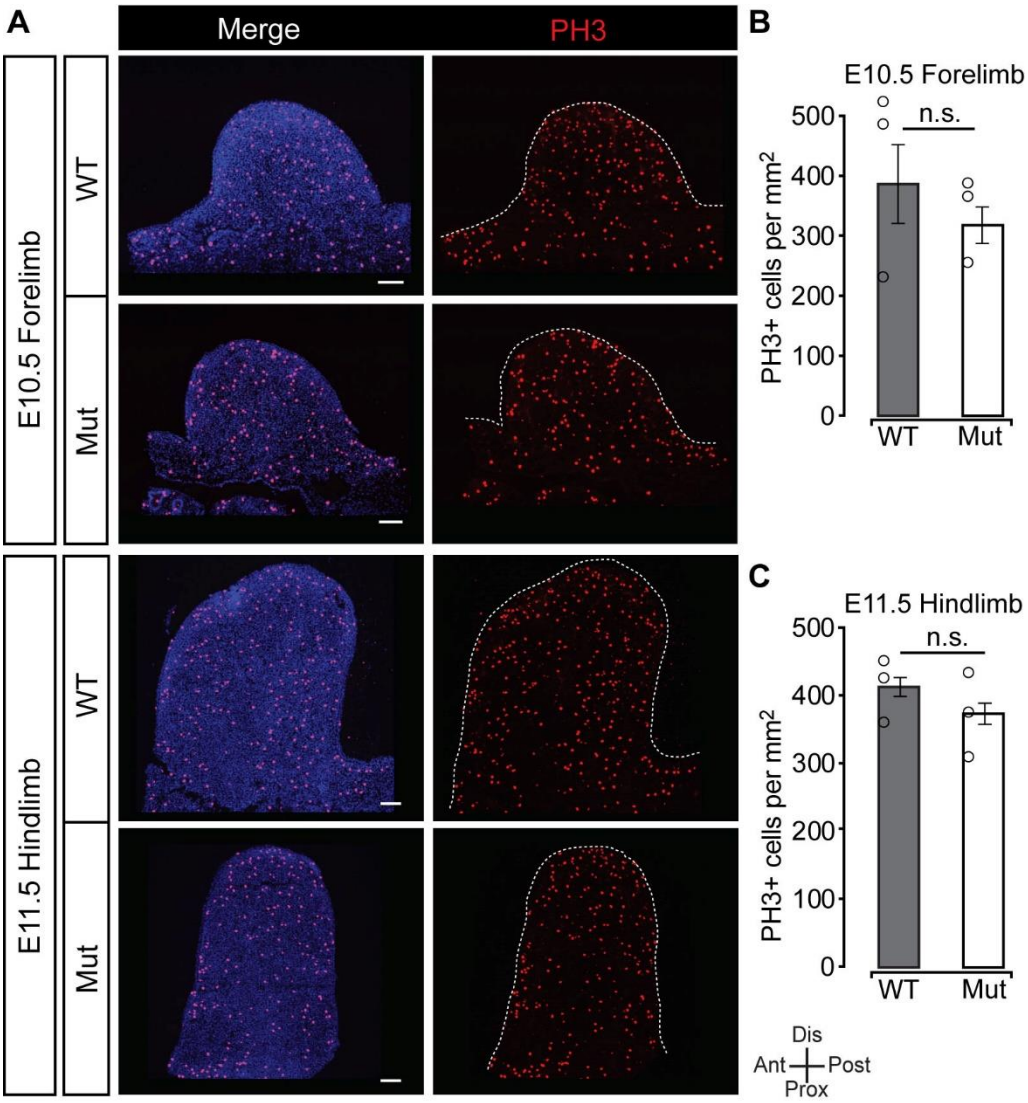

Figure S7 | Loss of U11 does not alter mitotic cells in the E10.5 forelimb or E11.5 hindlimb. (A-C) IF for PH3 counterstained with DAPI in the E10.5 forelimb (FL) and E11.5 hindlimb (HL) for WT and mutant (mut) **(A)** with quantification **(B-C)** normalized to limb bud area. Bar charts represent mean and error bars represent standard error of the mean. Scale bars represent 100 um. For details of statistical methods, see Table S6. n.s.=not significant.

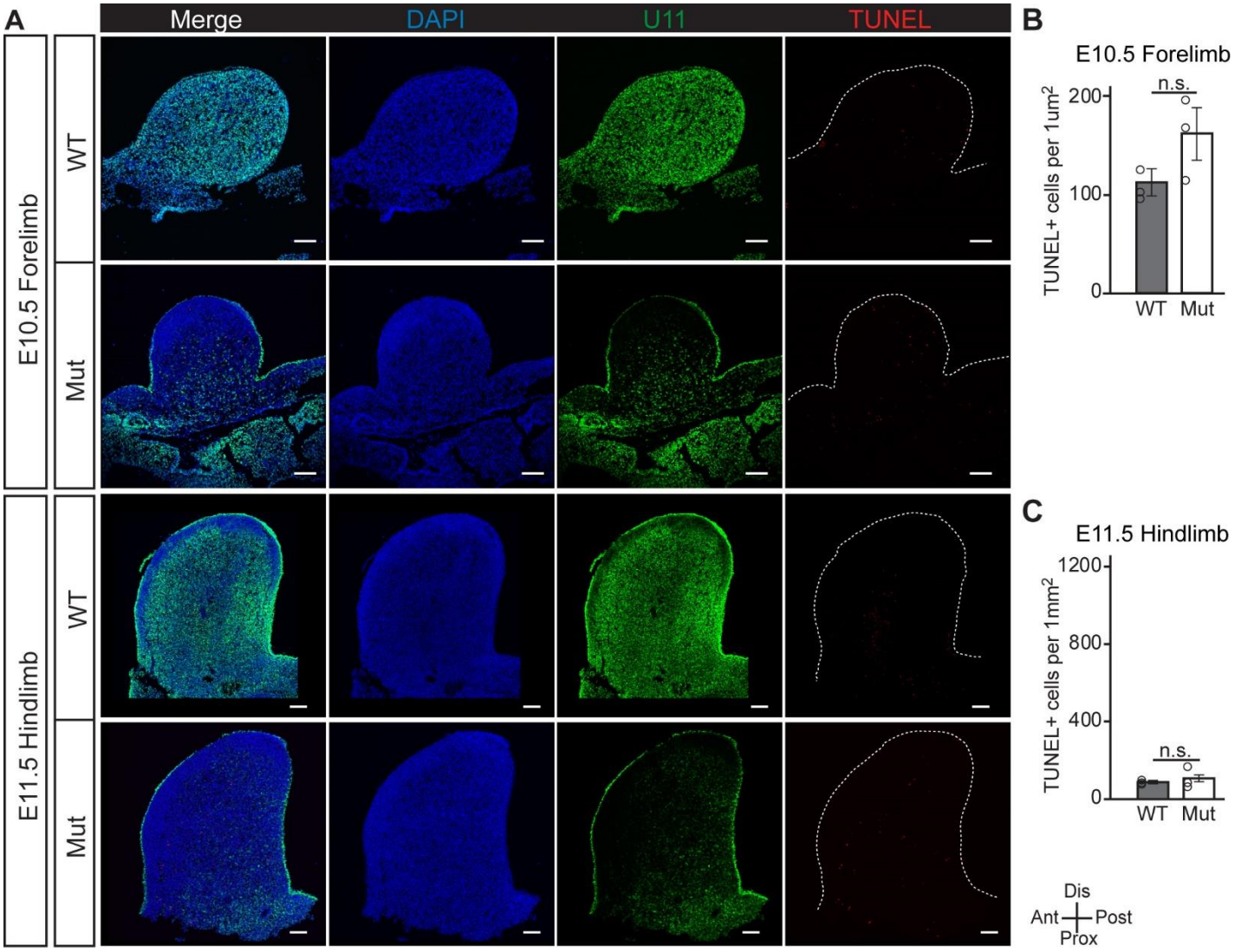

Figure S8 | U11 loss does not cause an increase in cell death in the E10.5 forelimb or E11.5 hindlimb. **(A-C)** FISH for U11 with TUNEL and counterstain for DAPI in E10.5 forelimb (FL) and E11.5 hindlimb (HL) for WT and mutant (mut) **(A)** with quantification **(B-C)** normalized to limb bud area. Bar charts represent mean and error bars represent standard error of the mean. Scale bars represent 100 um. For details of statistical methods, see Table S6. n.s.=not significant.

Table S1. Minor intron retention values

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Table S2. ORF Analysis

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Table S3. Effect of minor intron retention on open reading frame (ORF)

Sample	# MIGs w/ incr. minor intron retention	# ORF: Extension	# ORF: Premature Stop/Elongation	# ORF: Premature Stop/ Truncation	Of Premature Stop: # NMD	Of Premature Stop: # Novel Protein
E10.5 FL	21	1	0	20	20	0
E10.5 HL	2	0	1	1	1	1
E11.5 FL	134	1	2	131	118	15
E11.5 HL	15	0	0	15	14	1
Total (unique) #	152	2	3	147	134	16
Total (unique) %	100%	1.3%	2.0%	96.7%	88.1%	10.5%

Incr=increased, FL=forelimb, HL=hindlimb, NMD=nonsense-mediated decay

Table S4. DAVID Enrichment

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Table S5. Primer sequences used for qRT-PCR and WISH probes

Primer	Sequence (5' to 3')	Purpose
Rnu11 Forward	AAAGGGCTTCTGTCGTGAGTGGC	qRT-PCR
Rnu11 Reverse	CCGGGACCAACGATCACCAG	qRT-PCR
Rn7sk Forward	CTCCAAACAAGCTCTCAAGGTCCA	qRT-PCR
Rn7sk Reverse	ATGCAGCGCCTCATTTGGATGTGT	qRT-PCR
Shh Forward	ATGCTGCTGCTGCTGGCCAGATGT	WISH
Shh Reverse	GGGCCCCGAGTCGTTGTGCGGCGC	WISH
Sall1 Forward	CTCAGCTGTCAGAGCGCCTTGAAAATG	WISH
Sall1 Reverse	CAGGCTATCTTGGGAAGCGTCCGC	WISH
Hoxa11 Forward	GGTTCAGATCTCCGTGGTTAAG	WISH
Hoxa11 Reverse	GGCTCTTAGAAGATTGCCAGAA	WISH
Hoxa13 Forward	ATCCTTCAGACGCCAGCTCCTATA	WISH
Hoxa13 Reverse	TGGCTGATATCCTCCTCCGTTTGT	WISH
Fgf8 Forward	CAGGTCCTGGCCAACAAG	WISH
Fgf8 Reverse	AGCTCCCGCTGGATTCCT	WISH
Cyp26b1 Forward	TCTGCCCCTTTGCTCTTGGAAGAG	WISH
Cyp26b1 Reverse	CAGGGATCCCCTTCAGCTTTTCCT	WISH

Table S6. Summary of all statistical tests performed

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