

<i>AMELX</i>	<i>ENAM</i>	<i>AMBN</i>	<i>MMP20</i>	<i>FAM83H</i>	<i>KLK4</i>	<i>SLC24A4</i>
<i>WDR72</i>	<i>ITGB6</i>	<i>ODAPH</i>	<i>COL17A1</i>	<i>LAMB3</i>	<i>GALNS</i>	<i>GPR68</i>
<i>ACPT</i>	<i>LAMA3</i>	<i>ODAM</i>	<i>AMTN</i>	<i>LAMC2</i>	<i>ITGB4</i>	<i>CNNM4</i>
<i>FAM20A</i>	<i>PEX1</i>	<i>PEX2</i>	<i>PORCN</i>	<i>LTBP3</i>	<i>DLX3</i>	<i>ORAI</i>
<i>STIM1</i>	<i>GJA1</i>	<i>ROGDI</i>	<i>AIRE</i>	<i>NHS</i>	<i>SLC4A4</i>	<i>SLC13A5</i>
<i>DSPP</i>	<i>CLDN1</i>	<i>CLDN16</i>	<i>ALPL</i>	<i>GPR98</i>	<i>RAI1</i>	<i>VDR</i>

**Table S1.** List of AI Candidate Genes. While the entire exome was analyzed, AI candidate genes were also specifically examined manually. No potential AI-causing mutations in the proband were observed in these AI candidate genes except *AMBN*.

	Forward Primer	Reverse Primer	Amplicon size
Exons 1-4	5'-TACAGAGAGAGCCCCAGGAA-3'	5'-TGAGAAAGTGCGTTGAGTCC-3'	292 bp
Exons 1-11	5'-TACAGAGAGAGCCCCAGGAA-3'	5'-TCCCTTGGGTGAATTCTGAT-3'	WT: 932/887 bp; null: 749/704 bp.
NLS-lacZ	5'-TACAGAGAGAGCCCCAGGAA-3'	5'-GAGGGGACGACGACAGTATC-3'	403 bp
<i>Gapdh</i>	5'-AGGCCGGTGCTGAGTATGTC-3'	5'-TGCCTGCTTCACCACCTTCT-3'	530 bp.

**Table S2.** Oligonucleotide primers used to check for *Ambn* expression in *Ambn<sup>lacZ</sup>* mice by RT-PCR. DNA sequencing confirmed the exact sizes of the amplicons (see Fig. 2).



**Figure S1.** Clinical Photos of Unaffected Family Members. The proband's mother (II:2) (age 38) and half-sister (III:2) (age 18) were heterozygous for the *AMBN* NG\_042078.1:g.19190T>C; NM\_016519.5:c.1061T>C; p.(Leu354Pro) mutation and exhibited very minor enamel pitting. The proband's brother in law (III:3) (age 20) had no *AMBN* mutation.

**Figure S2.** NCBI Reference Sequence NG\_042078.1 for Homo sapiens *AMBN* on chromosome 4 showing its intron-exon structure and AI-associated mutations. The exons are highlighted in yellow, with the corresponding amino acid sequences underneath. AI-causing mutations are highlighted in red. The AI causing *AMBN* mutations include:

- 1) *AMBN* 2347 bp deletion causing AI (c.294+139\_531+478del; p.Tyr99\_Glu177del) (Poulter et al., 2014).
- 2) *AMBN* splice junction mutation at the end of Intron 6 (c.532-1G>C; p.Leu178\_Ser190del) (Prasad et al., 2016).
- 3-4) *AMBN* compound heterozygous missense mutations causing AI in this report: NM\_016519.5:c.1061T>C p.(Leu354Pro); NM\_016519.5:c.1340C>T p.(Pro447Leu).
- 5) The *AMBN* single allele mutation NM\_016519.5:c.1069C>T; p.(Pro357Ser) was reported to cause a dominant condition with enamel and dentin defects (Lu et al., 2018), but the clinical phenotype exactly matched that of dentinogenesis imperfecta, a dominant condition caused by mutations in *DSPP*, a gene linked to *AMBN* on chromosome 4 that contains a large repetitive region that cannot be characterized by whole exome sequence analyses (Yang et al., 2016) (not caused by *AMBN* mutation).

**Exon 1:** 116 bp (Gene NT 5001 to 5116; cDNA NT 1 to 116; cDNA coding NT 1-15); 5 amino acids (Met<sup>1</sup> to Lys<sup>5</sup>)

GAAGTACAGAGCAAGTCCCACGCACAGTCCTGAAAAAATTTAATCTTCTTTTCTTAGAACTATCTTGGTTG  
GCATCATCAGGCCCTGAGAGCACAGTGC**ATG**TCAGCATCTAAG  
M S A S K

**Intron 1:** 953 bp (NT 5117 to 6069)

GTAAATGGGATTTTATGATTTCCATGTGTTTCCTGTAAATTCCTTTTTTCTATCTCCTGACAGCTTTTATAAAGAGGATTTATCTT  
CATTGTGACACCAGTAGCTGAATTATTGCTTGTGTGAATCCATTAGCATGTCCCAGAGATGACATGCCTAACACCCATTCAACTAAGAA  
AAAAAAAAGACTTTTTATTTATAGATTGTGTTTTTTTTAATGTTTTTGATTATAGTGCCTAATTCTTTTTAATATTAGAAGATGAGTA  
AATTTCTAGGCTATTCAGATCTCTATTTCATGTAAGAATAAAGAGCTTTTTGAATTTATAGAATAAATTTTAAAAGATAATTCATTTTA  
GGAAGAGGAAAAGCTAAATTTGAGACATATTTTCCACTAACATCAAAGAAAATTAAGATAGCCAATTTACTCTCATTGAAAAGAAAA  
TTTTGTCATGGTTACGTAGATTACCTAAACATGCAATGATGTACCCTCTATTCTGCAAATAAAGGTTTGTATAAAATGACTTAGTT  
GCATTACATTTACATGCATTTCAAATGTGTCCCAAAGGGTAAGCAAAGTATAAACTTCTGTGAATAAATACCTTATAGACCAATATT  
TTACTTAGTGGGAAGGATTTTTAAACTCATAATTCCTTTATGAAATGAAATATATCACAAAGAATATCGATGGAGGTTGTTCTCTACA  
TACTTATTTAGGCTGTAGATCCATTTAAAACGTGCTTAGATATTTTTAATTGCTTGTAAATTTATTTTCAGAAACTACTACATACAAA  
TCGGTTGTTTAGTAACATTTAGTTTTTGTGTTTTGTTCTTCTAACCTTTATCCCGGTGGTTTTTGTAAAGAGCAGAGACTCAGGCTCATT  
TTCAAAAATTGCTTTTTAACCATTCTGAATAAAAATTCATCTGATTTAATTCTCCAACCTTAATTATGTTTTAG

**Exon 2:** 69 bp (Gene: 6070 to 6138; cDNA 117 to 185; coding 16-84); 23aa (Ile<sup>6</sup> to Pro<sup>28</sup>) Signal Peptide cleavage is after Ala<sup>26</sup>.

ATTCCACTTTTCAA**ATGA**AGGACCTGATACTGATCCTATGCCTCCTGGAAATGAGTTTTGCAGTGCCG  
I P L F K **M** K D L I L I L C L L E M S F A V P

**Intron 2:** 3603 bp (NT 6139 to 9741)

GTAAGTCAGTCTTTAGAGTGTGTCCCAGAAAAGGTTATTGATTGTCGAACTCCAACAACACTGAAGGGATATGGACTGAGAGTCCAC  
GCATAGACTCACTTGAATTAATCCAGTGGTTTAGTCCTATTGAGTCAAAGCCATAGTTGCTCAGATGTCAGTCTTGAATCCAAAAG  
CGGTAAGTGATAGCCGGGTGCGGTGGCTCACGCCTGTAATCCAGCACTTTGGGAGGTTGAGGCAGGAGGATGACCTGAGGTGAGGAG  
CTTGAGACCAGCCTAGCCAACATGGCTAAACCCTGTCTCTACTAAAAATACAAAATTAGCCAGGCGTGGTGGTGGGCACCTGTAATC  
CCAGCTACTTGGGAGGCTGAGGCAGGAGAATTGCTTGAACCTGGGAGGCGGAGGTTGCAGTAAGCCAAGATTGTGCCACTACACTCCA  
GCCTGGGTGACGGAGTGAGACTCCATTTAAAAAAGCAGTAAGTGAAGTATGATATTTCTACTAAAAAGTTATTTAAA  
ATAGTCCCAAGACAATTTCTAGATCTTATTCCACTCATTCAAAGTTAAAAGCTTAGATAGTCTCCACAATGCATGGCGAAATTTTATT  
ATCAACTTATTGCTATCATTATAAAGTTGAAATGGAAAGAGGAAATTTCTTTGAAAAAATACATTTTCTTCATTTCAAAGAAGACTA  
TCTCTAAATTTCTATGGTTACAAAACCTCAGCTAGATAATTCATTTAAAGCTAGTAATTAAGTAACTAGCAAATTTACCTTGTAACATGAA  
ATTGTGTGACTGAGAGCTGAAGTTTCATAAATCAAACATATCATAGTGTGGGGCTAATTGTGTATTACAGTCCCACACCTGATCCA  
TTCTAGTATATATTGAAATGATCTCATACAAACACAATCTTTTGAACACTGAACTACTCCAGCCAACAGCATGCAATTTCTGTTATCAC  
AAAACTTTATTTGGCTGCTATATGCATTTAGCAAATATGTTGAGCAGCTGCCATGCATCCAGTAAATAAGACATGGAAAAATGAAAG  
TAAATAAGACATGATCTCTTGTCTCCAGTAGCTCACAGTTGGCCTCAAATGAACTTTAGAGTATTTAATTCCTTGAAGAAAAGGTT  
AGCTACTTGGTCTTCTTAAAGTGTGTTTAGAGAAAAGAGAATGGAAATGGAGAAAAGAGGTTGAAAAGCTGATTATAATGATGAAAACCTTA  
ATTCCCAAATAAAAATAGTCTCTGTTCCATCACGCAAGATGAATTTTAAATGAAAGAAAATTTGAGGGTGCCACCCTAGCTTACCTT  
TATATGATACTGACGTTGGGATGAGCCTCAGTCATTCATCAGTCTAACACATTGGCATTCTTTGAACCTATAATGATCCACACCT  
GAATCAATTGGGTCCACTCTGAGGAACAGAACAATTGCTTATTATTGCCAACCTATTGCCTGTCAAATCAGATGTTCTTCTCCTATTA  
ATTCTGCATGGGAAATGTATCCCCTATCTGTATACATTCGTGTAAATATTCTTTAAGTCATGACTTAGAGCAGATGGAAAGCAAAGTA

CACTTTGTTCTGTGGCTCAATCCAGGATGTGTTATTTATGCACTGGCAAATAGTGGGGAAGAAGTCTCTGCTCTCACGATTCCCCCAA  
ATTCTCAACAAGCTAACCACCACATACACACATACATGCACAATCACTCTTCTGTCTACACAGCTAACCTCTTTTCTACCCAGAGTA  
TTTGCTACACATTTGTAATGCCTTTCCCTCTATTCTTTTTTTTTTTTTTTTTTTTTTTTACAGTCTGGCTCTGTTGCCCGGGCTGGAGTGCA  
ATGCTGAGATCTCAGCTCACTGCCACCTCCTCCTTCTGGGTTCAAGCGATTCTCCTGCCTCAGCCTCCCAAGTAGCTGGGATTACAGG  
CACGTGTCACCACGCCAGCTAATTTTTGTATTTTTGTAGAGACGCGGTTTTCGCCATGTTGGCCAGGCTGATCTCAAATTCCTGACCT  
CATGATTTGCCACCTCAGCCTCCCAAAGTTCGGGATTAGTCTTGAGCCACCATGCCTGGCCCTTTCCCTCTATTCTTGTCCAAAAC  
TGAATTATTTAAAGAAATTCTCACTATGACCCAGAAGGTGGGATTTTACTTACATGAAAAAGGTGGTGGGTGGAAAAAGTAGGGGGTG  
TAATTCTTTAACTTCTTACTCATAAAGGAAAAATAATACTGATATATTTGGTAAACATTCCCTAAATCCTTGTGGTATAACTGAAGGA  
AAGACGGCATTACAACCCCTTGGTCTTAGAAAAGTGAGAATATCCAAAGAACCAGAGACTCTCAAATCCAATTTACTCTAATTCCTAC  
TCTTCCATTCGCCATGTTTTCTACCGGTATTTGTGTAAGTAATAAGGATTGGTTTTCAAAGAAAAATATATTTAACAAAAAACAAA  
AAACAAAAGGAAAAGGCTAGGCATGGTGGCTCATGCCTATCATCTGGCAGCTTTGGGAGGCCAAGGCAGGAGGATTACTTGAGCTCAG  
GAGTTTAAAGACAAGCCTGGGCAACATAGTGAGACCCCATCTCTATTTTTTAAAAAAGGAAGCCAGATGCAGTGGCTCACATCTGTAA  
TCCCAGCACTTTGGGAGGCTAAGGTGGGCGGATCACTTGAGCTCAGGAGTTCAAGACCAGCCTGGCCAACATGGTGAAACTCTGTCTC  
TACCAAAAATACAAAATTAGCCGGGCTTGGTGGTGCACACCTGTAATCCCAGCTACTTGGGAGGAGAATCACTTGAACCCAGCAGGC  
AGAGGTTGCAGTGAGCCGAGATCACGGTACTGCACTCCAGCCTGGGTGACACAGTGAAACTTAGTCTCAAAAAAAAAAAAAAAAAAGGAA  
AATATCTTACATTTTAAAAAGCTAATTGTATAGATAGGTATATTTTTGAACTTTTAAAGTGAACACATTAAGTATAATAGTATTTT  
GTGCTAGAATGCTTTTTTATTTAAGGAAAAAACCTTTGATATGATCCCAAAGTATAGTAGGTTTACACATAAAAATAATCTTTACTA  
AAAATGATAGGGAATTTGCTGAAGCCCTTTGAATGGGAATCCAGTTAGATTTTTTCATATGATTTTTCTGAATGTCATGATCCCATGCC  
ATGCTTTGTAACCAAAATCATATAGATTTGTCGAAAGCACATGAAAATTCAGCTTGGGTTTGTCCCTTTACTTTTTTCTGTGGTAA  
TTTTTAAAGAAAATAAAGTACTAGTAGTTTTCTATTTTTGTTGCTTTATTCTATTTCTCCTTGTGTGTAACTTAAATCATATTTGCT  
TTTTAAAGCAATGAAGCATTAAAGATGCAGACAAAGGATAGATGATGCAGGTGGGAGCAAATATCAAATACTATGTTGATTCTCCCAA  
ATAACTTATGCTCTTCTTCACTTCTGCCCAAGCACTCAAGTCATTTGCATAATAAACCAAAATGGTGGCCTCTCAGGTAGCCCGTATGT  
ACTGGAGCAATATCCTACGCCCAATGGGCATTGAAGGAAGTTTTGTACCAATAAGACTCAAATTTATTTCTTATTTTTCTTCAATTCAG

**Exon 3:** 51 bp (Gene 9742 to 9792; cDNA 186 to 236; coding 85-135); 17 aa (Phe<sup>29</sup> to Glu<sup>45</sup>).

**TTCTTTTCCTCAGCAATCTGGAACACCGGGTATGGCTAGTTTGAGCCTTGAG**

F F P Q Q S G T P G M A S L S L E

**Intron 3:** 1306 bp (NT 9793 to 11098)

GTATGTATTGTCAGAATTTTTAACATATTAACCTTTACATTGGTATATTTGTGGTACAGGAGAAATATAATCGTGTGCTTCTCAGTTTT  
CCTAGCCAGAAGTTTGTGGCTGTTATCCTGAGGAGTGCTAGAAAAGTTATGACATTTACTAGAGCTTTGTCTTATAATTCTACTGCAG  
TTTTAACTCTCAGGTGTTTTAAGTTTTAGAACATTGTTTCTATGCAAACCAAAGCAATATTAGTATTTAATGCCTTATATATAATAT  
GCACATTAATAAAATTACTACCTTAATTAATAATACATTGCTCCATACTCACAATAGTTTTATTTTTAATCCTGCTCCTTATACTATCT  
TACATGATAAAGTTCTCATGAAAAATTTGTACCTGTACAAAATTTAATTTTTTATAAGTCTGGTAAATAACATTAACATTGTAAT  
GATACTTCCCTGCATAAGTTGTACAGTGTATTTGTCTTATTGCAGTTCTCATGTAATATATATTCTGTTTAGAAAACCTCCAGCAAAACA  
GAAATTTTGGTCATGTCAATGAAAAATATTGACAGTTATATATAGAAAGATATTTATAAGATTATAAATTTTTTACAAGGTCTTGTGA  
AATTTCCATGTGTCATGGTAAGCTGGGGGAGTCAATACCTATTTGAGCTAGACGCTTATTTTACATGGAAGCTAATTTTCGCTGAGGG  
TTTTTTTTCTCACTTTTTAGCTTAACTCAATCCAAAGCATTCTAAATCTCTTCCCGTGAAAAAGTTAAATTTGGCATTCTGTTGAAAT  
GGTAGCATTCTCGATAATCTATCCAGATACTTAACATTTCTTTCAATAGTTTTTATTTTTAATATTTTGAAGATTTATTTTTATGGGA  
AAGAGGTAGAAGTTTTATTTGTTTGTCTTGTGTTGTTTCAAATTCCTCTTCTGGCTGAACTTGTAAGGGCAAAGTAAATTAAT  
GGGGAGAAAGAGTCAATGAAGCAAAGTCCTCACAATTACACTTATATTTCAATGGAATGAGCTATTTTCCCATGTTTCAATGTAACCT  
CCACTTAAATAATACTTTTTCTCCTTTAATTAATGATAAAAGCAACTGCATTATTTAATAGCAATAATAAACAATGGCAGAACCA  
AATTTAATATTTCCACCTTTTCAGTGATGATTTGTGTCTACTTTTCAATTTTATTAATTTGATTACACCAAACCTTTGTGTTGATAATGTC  
AAATATCATGAGAAATCAGTTGTGTCAAACACAGCATATGCGATAAACAGTAACCCACTTTTTTTTTCTTGATAG

**Exon 4:** 48 bp (Gene NT 11099 to 11146; cDNA 237 to 284; coding 136-183); 16 aa (Thr<sup>46</sup> to Gln<sup>61</sup>)

**ACAATGAGACAGTTGGGAAGTCTGCAGAGATTAAACACACTTTTCTCAG**

T M R Q L G S L Q R L N T L S Q

**Intron 4:** 1132 bp (NT 11147 to 12278)

GTAATCATATTTCTTATTGCAAGTATTCATGGTGGTGGTAGTGTAAATATTAGCAGCAGCATTATAATTCATCAAATTTATTTATGAA  
TAGACAATATACTAAAGATTGGAAGAACCAAGAAGTAAATGAGAGCCTAAAACAAAATAAGATACAGTGACAGGGAAAAACAAAAACA  
ATATTGAATTTTATTGAGTTAAGAAAAGAATCTTTTCCCTGCATTATTAATTTTATTATGTGGCACAAAACACAAGTGCTTAAAAAGCT  
AAATCTCAAACAATTTATGCAAATATTGCAGACTAGCTCAAACGAAATTTAAGTTCATTGAAAGGAAGAGTTCACCTACCCATTTGA  
TTTACTTATTTTATTTTATTTTATTTTATTTTATTTTATTTAGACAGAGTGTGCTCTGTTGCCAGGCTGAAGTGAATGGCATGATCTC  
GGCTCACTGAACCTCCATCTCCTGGGTTCAAGCAATTTCTCTGCTCAGCCTCCCAAGTAGCTGGGATTACAGGCATCTGCCACCAC  
GCCAGCTAAATTTTGTATTTTGTAGTAGACGGGTTTTGCCATGTTGCTAGGCTGATCTCGAATGCCTGACCTCAGGTGATCCAC  
CCACCTCGGCTTCCCAAAGTGTGGATTAGAGGCGTGAGCCACCAGTCCAGCCATACCTATCCATTTTAAATTAACCAACATCACT  
TTTTGCTTTTTGCTATATTTATGCTATGTGTTGATTTTTTAAAGAAATGAAAACAGGCCAGGAGCAGTGGCTCACGCCTGTAATTTCAAC  
ATTTTGGGAGGCCAAGGTGGGCGGATCATGAGGTCAAGAGATCAAGACCATCCTGGCCAACACGGTGAAACCCCGTCTCTACTAAAAA  
TACAGAAATTAGCTGGGCATGGTGGTGCCTGTAGACCCAGCTACTCAGGAGGCTGAGGCATGAGAATCGCTTGAACCCGGGAGG

TGGAGGATGCAGTGAGCTGAGATTGCGCCACTGCACTCCAGCCTGGCGACAGAGCAAGATTCCATCTCAAAAAAAAAAAGAAAAGGA  
AAAGGAAAACCAATATAACCAATGTTATATTTAACATTTAAATATAAGCATGTCTTTTTTTATCCATGTCTTTAG

**Exon 5:** 111 bp (Gene 12279 to 12389; cDNA 285 to 395; coding 184-294); 37 aa (Tyr<sup>62</sup> to Gln<sup>98</sup>)

TATTCTAGATACGGCTTTGGAAAATCATTTAATTCTTTGTGGATGCACGGTCTCCTC

Y S R Y G F G K S F N S L W M H G L L

CCACCACATTCTCTCTTCCATGGATGAGGCCAAGAGAACATGAAACTCAACAG

P P H S S L P W M R P R E H E T Q Q

**Intron 5:** 1771 bp (Gene NT 12390 to 14160) Red highlight indicates *AMBN* 2347 bp deletion causing AI (c.294+139\_531+478del; p.Tyr<sup>99</sup>\_Glu<sup>177</sup>del) (Poulter et al., 2014).

GTGAGTGAATAGCATCAATATGTTTGAACCTCAGGCTTTAAACCTCTTCTTACTTGCCTTCTTTAAGTTATGATATATTAGAAAACA  
GCCAAGTCCCTAGCATTAACAAAAAATTATTCTCGTGCCAAATAAATCGAAG CCTATAACTGCTTTTAGCGCTTAAGAAATATTTTAA  
ATTTAAACTATTTGCATATTTATTTAAATAGGAAGATACAGCCTCCAAAATATGAGACCTATATTAAAGATATCAGTAAACAATCC  
TCGAAAATCAAAGCAGGCTACATCCTGGACAATTTTCATTTGAATCAAGATTTCTTAAATAATTCCTCAAAGGCTGATTTCAAATGAT  
GACAATAATCCTGGTTAAATACCATAGCCAGGTGCGATGGCTGACGCCTGTAATCCCAGCATTTTGGGAGGCCAAGGTGGGTGGATC  
ACTTGAGGTGAGGATTCAGACCAGCCTGGCCAACATGGTGAACCCCGTCTCTACTAAAAATACAAAATTAGCCAGGCATGGTGG  
CAGGCACCTGTAATCTCAGCTACTCTCAGGAGGCTGAGGCAGGACAATAGCTTGAACCTGGGAGGCAGAGGCTGCAGTGAGCCGAGAT  
TGTGCCACTGCACTCCAGCCTGGGCAATAGAGTGAGACTCTGTCTCAAAAAAATTAAGAAATTTAAAAAAAAAAAAACATCATGAACC  
CTACAGAAGTCAATAAATATATCAATCTGTTAACCATTATTGATTAGGTGCCTAATAAATACATAAACCAAAATTGTGGTTTGGTAGTA  
AAAGCGAAGACATTTAAGAATGGCTCCAAGGATTGTGAAATTTTATCTTATCTGATTAGATAGGACAAGACAACATATTTCAAAGTG  
CTGAATTGTGACATGTGAGTAATGAATACTGCGTGAGCTCAGAGAGGGGTGATGGCTGGAGAGGCCAAGCAGAGCCCCAAATTGTTGC  
GAGACTTCCTTGCTGGTCTGAGGAGTTTACAATCAATCTACAAACAATAGGGAGCCATTCACCAAAAGTAATTTCTACAAGAAGTAAC  
AAAACATGGTGATTTTAACGGGGAAATTTTTTCGTTTAATATATGGACTCAGAACCAAGATTTAACAGGGAAATTTAGTCCAGGCGGT  
CTTCCACAGCAGGAGCATTACCACGAGGTACATTCAGTAGAACAATAAATTATGTTAATGGTGACTGTTTCTTAACTTAATTGTTTC  
CAAAGTGGACTCCATGATGCAAGAATTTGATTGGCATTTTAATTAATTACCCTTTATCCTTTCATTAACTCGAAAAATATATTAGGCA  
CCCACCATGGGCGAGGCATTTGTTAGGAGCTATGTTATGTACACGGGCTATAAAGAAAAACAAAACATATCATTCTCTCCGTTGAC  
TGGGGACAAAAGATTTATAACAAATAATATCATCCAGTGAGAAAGTGGTAAAAATAAGATACAGATACATACAGACATGAGGAA  
CTTAGAGAAGGACTGACTCATCTACCAGGAGGTTTCATGTAGTTCACCTTTACGCAATGGTGGTCCCAAAGGAGGTAGGGGAGG  
CACACACAAGACCTCCCCAAACAACATTAACACTAACTGGTGCTTGCTATGTAAACTCAACTTCAAGACAAGTCTCCTAGCCTCCCTT  
CCAGATAGAAAGCGCCCCAAGCCCCTTTGTTTAGAAATTTAGGCACCGTTGTTAATGAGCCATCCCTTCCTAACACTCTTTTCAA  
TTTCTCTGCAG

**Exon 6:** 237 bp (Gene 14161 to 14397; cDNA 396 to 632; coding 295-531); 79aa (Tyr<sup>99</sup> to Gln<sup>177</sup>)

TATGAATATTTCTTTGCCTGTGCATCCCCACCTCTCCCATCACAGCCATCCTTGAAGCCT

Y E Y S L P V H P P P L P S Q P S L K P

CAACAGCCAGGACTGAAACCTTTTCTCCAGTCTGCTGCTGCAACCACCAACCAGGCCACA

Q Q P G L K P F L Q S A A A T T N Q A T

GCCTGAAAGAAGCACTTCAAGCCTCCAATTCACCTGGGACATCTGCCCTTGCAGGAAGGA

A L K E A L Q P P I H L G H L P L Q E G

GAACTGCCTCTGGTTTCAGCAGCAGGTGGCACCATCAGATAAGCCACCAAAGCCTGAG

E L P L V Q Q Q V A P S D K P P K P E

**Intron 6:** 969 bp (Gene NT 14398 to 15366) The single nucleotide highlighted in red at the end of intron 6 was reported as a homozygous splice junction mutation causing AI: c.532-1G>C (p.Leu178\_Ser190del) (Prasad et al., 2016)

GTAATTCCTTTCTTGAAGTCAAGTCAAGATCAGCAATCACCAGCTAACCTAATAGAAGAAAACGAATTTGCAGGGCCTTTTAAATAATCGTA  
GCCTTCAGGCATAATACGTGATATAAATATATTCTTAATCAGTCTCCACATCTGAGATTTATTTTTTGGTAAAACGTAATATCCA  
TTCTATCTTTGGCCATCAGAGCAGGGCATGGATTCTAAAAGATACTGTGCCTATAAAAACCTGGGTTTCTTAGTGGCGAAAAGATGAA  
ACTCCCATAGGAAATAGAAATAAAACCCAGAGAGTCCAGGTTTCTTACTGTCTGCAAAAGAGGTTGACCCTATGCAAAAAGTATCTTGTAATAA  
ATTGATCCCTCAGCATAACAAGTAGGATTTTGCATTATTATCTCTCTGCAAAAGAGGTTGACCCTATGCAAAAAGTATCTTGTAATAA  
TGTAAGAAAAGTGTAAATGGATTAAAAAATAATGAAGGTACCAAGTAACCAGTATGTTGTGGGCATTTATCATTCCCCCATATCACC  
CTGCCTACAATCCTATAAAAAATATATATTATCAGCCTTTTTTAAAGAAAAGGAAGACAGATTTTTGAGAAGTTACAAAATTTGCCAGGAG  
TACACAAAATTAAGTGGCAGAGCCAGGGTTCAAACCAACCAGGTATGTTCCAGTTCACAAAATTCATTACTTTCTAATCTGCAAGAGA  
TAGCAAAGCCATGATAGATGTGCCTCATCTCCCTGCAGGTACATTTGAGTAATGTTAGTAAAAGACCATTGCAAACTCTTGGGTGATA  
CCTCCCCAAACACTAAAAGAAAAGTAAAATAATGAAACTCATTTTAATTGTTTTATTGTAATTGTAACAAGATAAGGATGTGATCAGT  
TCACTTTGTCTATTTTTTTATTTTTTAATGTCACCTTTGTCTATTTTTGTTTATTTTTTGTACTGATAATTTAATATTTATCTGTGATATA  
G

**Exon 7:** 39 bp (Gene 15367 to 15405; cDNA 633 to 671; coding 532-570); 13aa (Leu<sup>178</sup> to Ser<sup>190</sup>)

CTCCCAGGAGTAGATTTTGCTGATCCACAAGGTCCATCA

L P G V D F A D P Q G P S

**Intron 7:** 135 bp (Gene 15406 to 15540)

GTAAGTACAGATCTCAATGAGACACTTTCTGTATTTTATTTTTTAATTTTTTATTTGTTCACTTTTTTATTTTTATTTGTATTTAACTA  
CTTTGTTCATTTTTTACTGATAATTTTAATATTTATCTACAATATAG

**Exon 8:** 39 bp (Gene 15541 to 15579; cDNA 672 to 710; coding 571-609); 13 aa (Leu<sup>191</sup> to Ser<sup>203</sup>)

CTCCCAGGAATGGATTTTCCTGATCCACAAGGTCCATCA

L P G M D F P D P Q G P S

**Intron 8:** 135 bp (Gene 15580 to 15714)

GTAAGTACAGATCTCAGTGAGACACTTCTATTTTTTATTTTTATTTTTATTTGTTCACTTTGTCTATCTTTTATTTGCATTTATCTAT  
TTTGTTCTTTTTTACTGATAATTTTAATATTTATCTGTAATATAG

**Exon 9:** 39 bp (Gene 15715 to 15753; cDNA 711 to 749; coding 610-648); 13 aa (Leu<sup>204</sup> to Ser<sup>216</sup>)

CTCCCAGGATTGGATTTTGCTGATCCACAAGGTTCAACA

L P G L D F A D P Q G S T

**Intron 9:** 249 bp (Gene 15754 to 16002)

GTAAGTACAGATCTCAATGAGACACTGTCTTGCAAAAACACTGTCTCTATATTTTCAAAAAATAAGAGTGAATTTTTTTCATTGTCCCATT  
ATCCATGAATATGAAGTATAATGGGTTCCCTTTGTTCTCTTAAATATTAATACTTATTTTTCTATACTAATCCATCTGAAAATGTGTTT  
CATATACCTCAAAAATTACTGTTATGGGGATGTGCCTGTGAGAATTACTTATTCTGTTTTTCTACCATTTAAAG

**Exon 10:** 60 bp (Gene 16003 to 16062; cDNA 750 to 809; coding 649-708); 20 aa (Ile<sup>217</sup> to Pro<sup>236</sup>)

ATTTTCCAAATAGCCCGTTTGATTTCTCACGGACCAATGCCACAAAATAACAATCTCCA

I F Q I A R L I S H G P M P Q N K Q S P

**Intron 10:** 96 bp (Gene 16063 to 16158)

GTAAGTTTTTTTTAATACCACATCTCTGTTTGCAATTTACTTAAAAGTTTTTCCATTGGAAAATGCATTTTGTGATAATGATTGTATT  
TTATTTAG

**Exon 11:** 45 bp (Gene 16159 to 16203; cDNA 810 to 854; coding 709-753); 15 aa (Leu<sup>237</sup> to Leu<sup>251</sup>)

CTTTATCCAGGAATGTTGTACGTGCCTTTTGGAGCAAATCAATTG

L Y P G M L Y V P F G A N Q L

**Intron 11:** 416 bp (Gene 16204 to 16619)

GTAAGTCCATATTCTATAAAAAGTATTGTTTTTAATTAATTTTTATCTTAAAGAGCTAAAATTCAAAATCTAGGTCTCACACAAGAGA  
TTCCAAATAAACATTCTCTGAACACCTACATTAGTACTGAAATATTAAGGAGGCCAACTTCCATTTGGATAATCTTGGCCAGACACA  
CACACACACACACGCACACGCACACACACGCACACAAGGGTATTAAGAAAAAACAATTTTTAGATCTGAATTATCCGAGTTGACTAC  
ATTTATTCCATAATGACTCACCTCTCCACTTTGCCTCTGTACACAGCATTAAATATTAAGGTTAAGTGAAAAATAAATAACTTTTAAAC  
TTTGTCTTGAGTAGATAAGAAGGTAGCTGGTTCGTAATTTGACGCAATATTTCTTTTTGAACAG

**Exon 12:** 45 bp (Gene 16620 to 16664; cDNA 855 to 899; coding 754-798); 15 aa (Asn<sup>252</sup> to Ala<sup>266</sup>)

AATGCCCCCTGCCAGACTTGGCATCATGAGTTCAGAAGAAGTGGCA

N A P A R L G I M S S E E V A

**Intron 12:** 2263 bp (Gene 16665 to 18927)

GTGAGTAATGTCTTCTAACTCTTCTTAAAATAGTGGCCAGGGAAGAACAGTCACTTATGACTGGCTGCAAGAGACGTCTGCCATGAAT  
GTAGCCAAATGAGCATGGGACTCAGATTTTTCCACTCCAAAACACTAAGCTTAATTTCTGAAGCAATATCCAAAGATTTTCATCATGTTTC  
CTATGTAATCTTTTCATTTCTGGGTGATGACTATTCTTGAGCATAGAGTTTTGCCCCATTGCTTGTAGATGAACAGAGGAGAAAACCTCC  
CCTTGAAAGGCCGTCAATTGAACTATAAACACTTCAAACCAAAGGATACACGTGGTTCATACAGAGTTCTTGTCTTTTCTGGGACCAT  
TTAACCCCTCAAAACATCAAATTCACAGCATTACAAGTAATTCAGATCTCAAATTTGAAAGATCAAAAAGCAAAAAGAAAACCAAGTT  
TAAAATGTGATTATTATAAATGTCAATGTGCATTGTCAATGAGCATATACTCTTATTATGTTATCATTATCCATGCAAGTTTAAAGATT  
TGAGATCAATAAAGTCATACAACCTTGACTTTTTCCCTAAGAAAATTTTTGCCTGTTACATCTGTTAGAGCACAAAGGCAAGACAAAACA  
AAAGCTATATTGAATTAACAAATCACTGTCAACTACAGATCCTGTCAAGTTAACAAACACACACACATATTATCTGTGAAAGAAAAAT

TACATCTCAGTATTTTTGTGTGCCTTCCATGTGCCACACACATAGGAAAATACTGTAAACAAGACAAGGTAAGATTTTCGTTTTTGGC  
TGGGCGCAGTGGCTCATGCCTATAATCCCAGCACTTTGGGAGGCTGAAGCGGATGGATCACCCGAGATCAGGAGTTTGAGACCAGCCT  
GGCCAACATGGTAAAACCCCGTCTCCACTAAAAATACAAAATCAGCCAGGTTTAGTGGCGCGAGCCTGTAGTCGCAGCTACTCAGGA  
GGCTGAGGCAGGAGAATCACTCGAAACTGGGAGGCAGAGGTTTCAACGAGCTGAGATCACGCCACTGCACTCCAGCCTGGGCAACAGA  
GCAAGACCCTGCCTTAAAAAAAAAAAAAAAAAGTCTTGTTCAGGAACCTTACAGTCTAATGAATGGAAAAGATAGGTACAAGGGCAG  
ATACCTAATGTCAAATCATGGGCTAAAAGTAATAACAATTTTATTAGAGAGAAAGAGTCAGAGAGGAAGGGCAGTTAGCCCAGTCCA  
GGGATTACAGTATCATCCCTGGAGGAGATGATGTGAAAGCTGAGAGAGAATGAAGAGTCAGGAGAAAGGAGAAGGAAAGAGTATTGT  
ATCTATATAGCACCAATACAAGTGGCTGGAAGTTCAAAATTCAGTAAGTGTAAATGCCATCACATAGTATTAATTTCTATTCT  
GCCTCATTCCCAAAGATTTTGTAGAGCTTATGAAAAACACACAATAAATAAACAATAAATGAAAGTAGGATTTAAAATGAGTCAT  
AATTTCAAGAAAATCAGAATACAGATATGCAAGTCACACAGGCCCTGCTCAATTGCTACAAACAGCCTAAGATTTTGGACCCCATATTT  
CTGGCAGCAAAGGTAGAAAAAGAAACATGGTCAGTGTCTGTTATTCTATTTGTCCACAAGAAGAAAATTTTAGTTACTCCAGGGAAG  
TCACATTTTTTTTTCTTAAACTTTGTGCTTTAAAAAATTAAGTCTTAGCTGGGTGCAGTGTCTCACCCCTGTGATCCCAGCTACTAG  
GGAGGCTGAGGCAAGAAGATCACTTGAGCCTAGGAGTTCTAGACCAGCAGCCTGGGCCGTATAATGAGACCCTAAAAGAAAAAAAA  
GTACATGGAACCTTCAATTTGGGGGACACTGAAAAATGCCATTGACAAGATTCTCGCTCAACAAATGCAGTTATGAATTTTCAGTTCTTAT  
TTGTTTTGTGATGGTAGTACTCATTTCAACTGCTTGTTCCTTAAAATTAATTCAAACTCACCTTTGCCTCAAGTCTTGCTTCTGCC  
CTGTTATCTCCCTTTTACGGAAATCATGCCGAGATCCACCCAGATAAGTGCAGAATCCTCTTCATTATGTTTCAAAGCCAGTTTAGTG  
AGAAATGAGCTTACACAAAGCTATCAATCAGCCAACCTCCTATTCTCCACCAGTTTTTTAGAGATCCTTGGTGAATGTGACCAGGTAT  
AGCTGCATGGTATAGTTAATAGCATGTGATGATGGCATCTTTGACGAATGTTTTTTTTTCCAG

**Exon 13:** 1104 bp (Gene 18928 to 20031; cDNA 900 to 2003; coding 799-1344); 181 aa (Gly<sup>267</sup> to Pro<sup>447</sup>)  
Red highlight indicates *AMBN* compound heterozygous missense mutations causing AI in this report: NM\_016519.5:c.1061T>C  
p.(Leu354Pro); NM\_016519.5:c.1340C>T p.(Pro447Leu).

GGCGGGAGAGAAGACCCAATGGCCTATGGAGCCATGTTTCCAGGATTTGGAGGCATGAGGCCCGGCTTT  
G G R E D P M A Y G A M F P G F G G M R P G F  
GAGGGAATGCCCCACAACCAGCTATGGGCGGTGACTTCACTCTGGAATTTGACTCCCCAGTGGCTGCC  
E G M P H N P A M G G D F T L E F D S P V A A  
ACCAAAGGCCCTGAGAACGAAGAAGGAGGTGCACAAGGCTCCCCTATGCCGGAGGCCAACCAGACAAT  
T K G P E N E E G G A Q G S P M P E A N P D N  
CTAGAAAACCCAGCTTTCCTTACAGAGCTAGAACCTGCTCCCCACGCAGGGCTCCTTTGCTCTCCCTAAG  
L E N P A F L T E L E P A P H A G L L A L P K  
GATGACATTCCCGGCCTGCCAAGGAGCCCTTCCAGGGAAGATGAAGGGACTCCCCAGCGTCACCCAGCA  
D D I P G L P R S P S G K M K G L P S V T P A  
GCTGCTGACCCACTGATGACCCCTGAATTAGCTGATGTTTATAGGACCTACGATGCTGACATGACCACA  
A A D P L M T P E L A D V Y R T Y D A D M T T  
TCGCTGGATTTCCAGGAAGAAGCAACCATGGATACCACGATGGCCCCAAACTCTCTGCAAACATCCATG  
S V D F Q E E A T M D T T M A P N S L Q T S M  
CCAGGAAACAAAGCCCAGGAGCCCGAGATGATGCATGACGCATGGCATTTCOAAGAGCCCTGACAGCTC  
P G N K A Q E P E M M H D A W H F Q E P \*

TAAGATATTAGCTACTTTCTGTATGCACAAGCTTCCCAGCTTTGTCCCCACAGTGTACCTTTTTGCTAAAACA  
CTTATTACCCTTCTGCAGCAAAGGCATTAAGAGCGCTAAGCATATATTAATAAATGCAAGTGGCTAGAAATAG  
TGTAGGTCCCCTTCTTGCTTTCAATATCTTGTGAAATAAATGTGTCAATTGTCTCTGTGATTTAGAAACAC  
TATTAATAACATCAGAGCAAGGTTCTAAGGGTCTCAGCATTGATCATCACTTTTTCTTAGCTGTCTTAAGCA  
TTATAGAATTTCTCTTACCAGCATGACACTATTATATTCAGGAAACATGGCACTGCTTTTTTCTCTAAGCAA  
GGCAAATATCCTCATAATTCTAAGCTAATTCATTTAACTTTATTAATGGGGATTGGTGGAAAACCTCCTGAC  
TGGTATTACTGGGTTTGGTATAATTTAATTTAAAATTTCTCATTATAGAGTATTTTATTTAATCTAGTATAAA  
AAATCTAGCCTATTTTAAATAAAGAATTTTTTCTCACTGAAA

**Figure S3.** NCBI Reference Sequence NC\_000071.6 for *Mus musculus Ambn* on chromosome 5 in strain C57BL/6J. Important structural and functional features are noted. The *Ambn* region deleted in the *Ambn*<sup>lacZ</sup> knock-in is highlighted in yellow.

**Exon 1 (116 bp)** Gray highlight shows forward Exon 1 primer site for RT-PCR analyses. **ATG** is a weak, in-frame translation initiation site with a pyrimidine in the -3 position that appears to initiate translation by leaky scanning (Hinnebusch, Ivanov, & Sonenberg, 2016). Translation initiation at the leaky **ATG** near the end of Exon 1 adds 10 amino acids to the signal peptide, which are displayed in lower case.

...a purine at the -3 position from the AUG both is conserved and functionally predominates over other positions in all organisms. When an upstream AUG (uAUG) is in-frame with a downstream AUG without an intervening stop codon, leaky scanning may occur at some frequency to allow production of two protein isomers differing only by an N-terminal extension, with the longer form often targeted to a particular cellular compartment.

GAGAGATACAGAGAGAGCCCCAGGAAACAGTCCAGAAAAAATTAATCTTCTTTTCTTAGAACTGTTTTGATTGGCATCATCA  
GGCCTGGGAGCACAGTGA**ATG**TCAGCATCTAAG  
                                  m s a s k

**Intron 1 (778 bp)**

GTAAAATGTGATTTTATCATTCCATGTTGTTGCTATAAATTCCTTTTCTCCAGATAACCTTTACAAAAAGATTTATCTTCATTTATCACAAGATGCTGAATA  
ACAGCTTATTTCAACCATTAGAAATATTTCCAGAAAATATATTACCATTAACTAAGAAAATAAACTTATTTGTATGACTTTTTAAAACATGTTTGTGATTATAGTGATA  
GTGCTAATTTATAAAAGTAAAGAGGATAAGCACACCTGCTGATCAGATCTGCTTCCATTCGAGAGTGAACGACAAAATGCATCTCAAGATTATTCATTTTAGGAAGGA  
AAATCACTATCCAAGCAGATAAATATACTCAACTTTAGCTTTGAAGGCTGAGTTTTGCATGGTCTGCATTATGACCCAATCATAGGTTGATTTCAAATTCGAGTTA  
AACAAAGAATACAATTCGTAATGGTACTGAAGGATGCATTTTATGAAGCAAAGCATAGTGTTCAGGAATATTCCTGGAGGTTGTCTTAAACATTCATTCAAATG  
CAGCTTCATAAAAATAAGTACTCAGATCCATAGTTGGCTGCTCGTCAATTCCTGATAGCCACTGGTTTAGCAGCTTTTTGCTTTTGCATTTTAGCAGCTTTGCAAT  
TTTGAGTTTTTAACTGATTGTCTTTGGAAGAACAGCCACACGCTCTTAGAAATATACTTTGACCACTATGAATGCAATTTTGCCTGAGGTGATTTTACAACCCAAGT  
TGTTTTAG

**Exon 2 (69 bp)** encodes 18 amino acids (16 are from signal peptide) starting at the strong translation initiation codon (**ATG**). Use of the weak translation initiation codon in Exon 1 adds 10 amino acids to the n-region of the signal peptide, with the h-region and c-region being added after the strong initiator codon. Both translation initiation codons generate signal peptides that are cleaved at the red highlighted box and secrete identical proteins. Please see Figure 7 from (Hu et al., 1997)

ATTCCACTTTTTCAA**ATGA**AGGGCCTGATCCTGTTCCCTGTCCCTAGTGAAAATGAGCCTCGCCGTGCCG  
i p l f k M K G L I L F L S L V K M S L A V P

**Intron 2: (2405 bp)**

GTAAGTCCATCCCTTACCCGGGATCTCGGGCTCCTGCATCTGACTACAGCAAACCCAGGGACACTCTGTCTGACACTTCAGGCATGCACACTCACTCAG  
TTTGTAGCTCCAGTGAGTCAATGGTCAAGTGGCCACATGTCAGCCTTATCATCGAAAGGGGATAAGTAAATATAATTTTCTACTCTAAACAACAA  
TTTTGGAATATTTCAAAGGCAAATTTATGTCTTATGACACTCTTTAACAGGTAGAAGACTACAGCATCCTAGTTTTCATCATGTTTATTATCAAGT  
CCTTGCTAGCAGTATGAAATCGAAATGAAAAGGAAAAATATCTCTTGGGCAGGGGAGTATATGTCTCTTTATATTAGCAACATTATCAGATGGCTAT  
AGACTGTAGTTCAGTTAAAGCTAGTATTTAGCTGGCACATTTAGCTGTCTACTACCCAACAAGAAATTTTAAATGAAAGAAAAATGGAGGCTGGCT  
CTCTAACTTGCCCTTTGGATGATAACTGAGACTGGGTCCGCTTCAGCCATCAATCATCAATCTAAAACATTGGCATTTCTCTGAATATACAGTGATCCA  
CACATTAGTAATTTAGAGTACACTGAAACAATGGGAAATCACGTGTTTCTAAGTGAAGTGCCTGTCAGATCAGGTGTTTCTGCTTTAATTTCCAT  
AGGGAATGTATCCTCTGTCTATTCACATCCATGTAAAAAATGTTCCCTCTTAACTGGTGAAGTGGAGCAGATAGAAATCAGACTACCTTGCCCTGTAG  
TTAGAGTACTCCAAAATGTGGAAGTGGGCACTTACCAACAAAGTCAAGGAGGCTGTGCTCTATAATACCCCGAACATCTGAACATCTCCTCTGTTA  
CACACACTGTCTATGCACACATGCACATAGGCACACACAATCAGTCCCCTGTCTACACAACACTCATCTTTTTCTCTACCAGAAGTTTTACTGCACATT  
TTTAGTGCATTTCTTTAACACATACCCACAATGAATGTTTAGAAAATTTGCACTGTAATTAAGAAAATGGAGTACTTGCATAAAAAAATTAAGGG  
TTAAAAAAGTACAGGGTGTAAATACCTTAGCCTTACTCCTGAAGGATAATGGCCCCACTGTTCCATACTTGAGGGATGGATGGAGAGGCAGACA  
GACAGTACTTTTCAATATAAATGAAAGAAAAATTACAATACTAACACAACAAAAATGAGGATATCCATGAAATCAGAGAATTTCTCAAATCCAATTTT  
CTAGTAGGTAGAGTAACTTATAATGACCCAGCACATTGGAAGCTGGAAATATTTGTGAATTTAAGAAATTTAAATTTAATTTCTCTAATTTCTATTTCCAGGCTCCA  
GCCTCAGCTAATACCAAACCTTATTTAAAAATAATGAGTAATTTTGTGTAATTTAAATTTAATTTCTCTAATTTCTATTTCTCCAGGCTCCA  
CTCAGCAACACACTGGTATCTGTGCTTATTAACATAGAAATTTAAGATGTTTGAAGAGAAAGTATTTTATAATTTTGAAGATATTTGTAAA  
TATGTTTATGCTTCAACAGCATCCTACTAAGTACAAGAAATCTTTTCATTTAAAGAAAGATCCCCACTATCTGATCTGAACAAATTTAAATATATAA  
TTTTATTTATTTTATTTATGACATGTGTGTGTACATATGGGTATGTGCACATAAGTGTAGGTCCCCAAAGGTGTCAGAGGCATCAAATCCCCCTGAGA  
GCTGTCATTACAGATGGTTATGAGCCACTGACATGGATACTAGGAACCAACCCAGGACCTCTGGAAGAGCAACAGGGGCTGTTAATGTACCATAAG  
TCCAGCTCTGATCTAAAAAATTTAAACAGAGATCCACAAGCTTAACTTAAATAATCATTAATAATAGCACATAAGACTTGTTAAACCTTCTGAA  
TGGGAAGCAAGATAGGTTCTGTATATGATTTTCTCAATGAATAACCTCATACCATATCCCATACCAAAAGCACACCATTATAAAAAGCACAAAG





**Long Exon 6.** This 45 bp/15 amino acid segment distinguishes *Ambn* isoform 1 (NM\_001303431.1), which includes it during RNA splicing from *Ambn* isoform 2 (NM\_009664.2), which deletes this segment through use of an alternative 3' acceptor during RNA splicing. (Magenta is AMBN 89 Ab target sequence).

TATGAATATTCTTTGCCTGTGCATCCCCACCTCTCCCATCACAG  
Y E Y S L P V H P P P L P S Q

**Short Exon 6 (195 bp)** encodes 65 amino acids.

CCATCCTTGCAGCCTCACCCAGCCAGGACTGAAACCCTTCCCTCCAGCCCACTGCTGCAACCCGGTGTC  
P S L Q P H Q P G L K P F L Q P T A A T G V  
CAGGTCACACCCAGAAAGCCAGGGCCTCAGCCTCCAATGCACCCTGGACAGCTGCCCTTGCAGGAA  
Q V T P Q K P G P Q P P M H P G Q L P L Q E  
GGAGAGCTGATAGCACCCAGATGAGCCGCAGGTGGCACCATCCGAAAACCCACCAACACCTGAG  
G E L I A P D E P Q V A P S E N P P T P E

**Intron 6 (1004 bp)**

GTAATCTTTGGAGCCACCTAAGTTACTTCTTTAAAAATCAGATCAGGATCACCCACTAACACAATGAAACAAAAACCAACTGGTAGGGCATACTTAA  
ATAATCACATCTCTCAGACCTAACATGCTAAAAAATGAACACTTTATTAATATTTCTTTCATGAGACATAAATATCTATTTCAATCTTTTGCCATCAG  
AGATGTCAGGTTGCAAAAAGATCTGCATCTCTAACCTGGCTTAATGAGACCATTCTTAGGAATAGGACACCTCAGAGAAGTCCAGGCTCATCAGCTG  
GGGGTTTTAGTGTGGAAAAAGTATAGTACAGTGATTGTGTCTCTGCAGATAAAAATACAGCTATGCAAAATTACCTTGTAAATATGTAAGGAAGAG  
AACAAATGGACAGAAAAGGGAAGGTGCTATGTAACCTCAATACTATGTAGCATGCATTTAATATTTCTTTCATATCATCCTGACTACACCCTATAAAGA  
AAACATTAGGAACCTTTTAAGCCCAAGAAGGTGCCAGGCTTTGGACACACCTTTAATCCCAGGACTTGATCACAGGCAAAGAAAAGAGGATCTTTGA  
GGTCAAAGTGGACCTGATCCACAGATCGAATTCAGGATATCCAGAACTACACAGAGAAAACCTGACTTAAAAAAAATCAATAAATAAATAAACCTA  
AGCTTTGAGAGGCTACAAAGTTTTTCCAAGGATTACACTAGTGGCCGAGTAAAAATTGAAACCACTCAAGTATGTGCCAATTCCAACCTCATTACCAT  
TCCCTCTATAATGTAGAACAACCCAAAGATAGACATCTCATCTCCCTGTCATTCACTGGAGCCATGTCAATATAAGACAACCTTTAAAAGAATAGGTT  
ATCCCAAGACTTAGAGAATAAGACAGAGAGACATCTTAAACTGCTCTATTATAATCACAACCTAGAGGTGTACTCAGTTTGTCTGTTTTGAGTGACAG  
TTTTAATATTTCCCTCATGACACAG

**Exon 7 (39 bp)** encodes 13 amino acids.

GTACCAATAATGGATTTTGCTGATCCACAATTTCCAACC  
V P I M D F A D P Q F P T

**Intron 7 (621 bp)**

GTAAGTACAGATTTCAATAAGGTTCTATCCTGGAGAAAATATATATATGTGTGTGCTATATTTCAAAGATTTTGAATGAATTTTCTCTTTTCTTAT  
TTACCTATAGGTATAGAGTATTTCTGAGTTTCTTTTCTAAATATTAAGTGCTAAGTTTTCACACTAATCAATCAAAAAAATGTGTTCTTGGAACCTCT  
TAAAAGTCCCTCAGTATGTGCATATCATGTGTGTTCTTTTGTGATTGGGTTACGGAAGTCCACATTCTGTCAAAAGCATCAGGATGATATCCTCCAGA  
TGCATCTATTTGCCAAGAATTTCAATGAAATTCATGTTTTTAATTTGCCAGAGCTGTGTCTCTAGTTGCATATGTAGCAGAGGATGGCCTGTCAGCCAT  
CAATGGGAGGAGAGGCCCTTGGTATTGCAAGATCATATGCCCCAGTACAGGGGAATACTAGGGCCAGGAAGCGGGAGTGGGGGGTTGGGGAGCAGGG  
TGGGGGAGGGTATAGGGGGCTTTGGGGATAGCATTTGAAATGTAATGAAGAAAGTATTTAATAATAAAACAGTCTGTCAAGTGGAGGTTTGCCTGTT  
TTGACTGCCTTTTCTGCTTTCCACCTCTTAAAG

**Exon 8 (60 bp)** encodes 20 amino acids.

GTGTTCCAGATCGCCCGTTCAATATCTCGGGGACCAATGGCACACAACAAAGCATCCGCT  
V F Q I A R S I S R G P M A H N K A S A

**Intron 8 (99 bp)**

GTAAGTGTCTGCTTTCTACTTCCACATCTGTGTTTGAACCTACTTAAAAGGTTGATCTTGGGGAGATTCACTTTTTTATAACTATTTT  
GTTCTGCTCAG

**Exon 9 (45 bp)** encodes 15 amino acids.

TTTTACCCAGGAATGTTTTACATGTCTTATGGAGCAAACCAATTG  
F Y P G M F Y M S Y G A N Q L

**Intron 9 (371 bp)**

GTAAGTCCACATTTCTGTCAAAAAGCAGCAGTTAAAAATCAATAGCATCTTGAGGGCTAAAAACTGTAGAATCCATACCTAACACAAGAT  
ATTCCAAATATATAATCCACAAATACCTACAGTCATGATTTACATAGTAAAGTGGCACACTTTTCTATTTGCATAATTCCAAACAGAC  
ACAAACACAATCACATTAAGAAAAGGTTAATTTTTAAATCTGTTAAATTTGGCTCTATTTACTCAATAATAGTCAACTCTGTACTCTGC  
TCTGGACACAGAGCTATTGAGTCTAAGAGGATACTGAGTGGCTTTTGATATTGTCTTGGAGTAGATGGCTTGAGTTCATACAGTGACG  
CACTGTTTTCTTTTGAACAG

**Exon 10 (45 bp) encodes 15 amino acids.**

AATGCTCCTGCCAGAATTGGCTTCATGAGTTCAGAAGAAATGCCT  
N A P A R I G F M **S S E E M P**

**Intron 10 (1701 bp)**

GTGAGTACCACCTTCTAACACTTCTTAAAACAATGGCCAGGGAGAACAGCTGCTGTCTACCGGAGGCAATGACTGTAAAAGAACCCTAAAGGATTATTA  
GACTTCAATTCCCTCTATTCTAAAACCTGACAGCTCAATTCTGAAGCAGTATCAAAAAGATTTAAAACACTTCAGAGCTGAGAATGGTGGTGATGCACAGA  
ATCGCAGCCCTCAGGATGTGGAGGCAAGAAGATCAAGAAGAGTTTAAAGCCAGCCTTGACTTCATAAGACCCTGACTCAAAAATAATTGCTTTTTTCAT  
CACATTTTCTAATCATTTGTTTCCTGAGTGGTGGCTATTTTTGAGCCCAGGATTTTTGTTCCACTCCTCGTATATGAACAGAAGAGTCACCCTTCCAAG  
AGGAGTCAGTAGAGTGAACCACAAATCCTTCAAAGTGAAAAGATGTGTGGTTCATACAGATATCTTGCCTTTTAGGACTATTTAGCTCCCCCCCC  
CCCCGCCCTCATGTACCTATAGGATTTAGAACCTTAATGTATTTCTTTTGCTGCCATGACCATGGGAGGCATAAATAGGACTATTTATACTTGAGC  
AAAGTGTGTGATTTTGTCAATTGTTTGAAGTATTTTATTTTTTTTTTTGGGGGGGGGAAAAGGTTTTATTTTGTATTATGCTTCCAGATCACTGTTTATC  
AATGAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGG  
AAAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGG  
GAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGG  
ATATTTGAGGGATACTGAAAACATGTCCTCCAGACATTCCTCCTCAACCAAGGCATAACGAAGCTTGTGTTTTGTTTCATGATGATAGAGCCCTTTTCA  
ACTGCTTACTGTTTTCTTTTTTTTTTTAATTTTTTATTAGGTATTTTCTCATTACATTTCCAATGCTATCCCAAAAGTCCCCATACCCACCCT  
CCCACCCCTTACCCACCCTCCCTTTTTGGCCCTGGCGTTCCCTGTACTGGGCATATAAAAGTTTGAAGTCCAATGGGCCTCTCTTTCCAGTG  
ATGGCCGACTAGGCCATCTTTTGATACATATGCAGCTAGAGTCAAGAGCTCCGGGTACTGGTTAGTTCATAATGTTGTTCCACCTATCAGGTTACAG  
ATCCCTTTAGCTCCTTGGGTAATTTCTCTAGCTCCTCCATTGGGGCCCTGTGATCCATCCAATAGCTGACTGTGAACATCCACTTCTGTGGCAGAGT  
AGTAGTAGAGACCTGCCTTTCTGAAAGAGGCTTTCTCCTGCCAGATAAGTGGAGAATCCTCTCCCTATACTTCAAAGCCAGTTCTATAAGAAGTG  
TGTTTTCGACAAAGCAGTCAGCCAACCTCGCTGTTGTGTTAGTATTTATAGCTTCTCAATGAATATAATCATGTACAATTTGATGGAATGAATAACACAC  
AGTAATTTGTGTCACCTGATGGACGTTTTGTTTTCCAG

**Exon 11 (1074 bp) Gray highlighting shows the reverse Exon 11 primer annealing site for RT-PCR analyses.**

**TGA** is the translation termination codon. **AATAAA** sequences are transcription termination signals.

GGAGAAAGAGGAAGTCCCATGGCCTATGGAACCTCTGTTCCCAAGATTTGGAGGCTTCAGGCCAAACCCTTAGGAGACTG  
G E R G S P M A Y G T L F P R F G G F R Q T L R R L  
AATCAGAATTCACCCAAGGGAGGAGACTTTACTGTGGAAGTAGATTTCCCAAGTATCTGTTACCAAGGCCCTGAAAAA  
N Q N S P K G G D F T V E V D S P V S V T K G P E K  
GGAGAAGGTCCAGAAGGCTCTCCACTGCAAGAGGCCAACCCAGGCCAAACGGGAAAACCCCGCTCTCCTTTTACAAATG  
G E G P E G S P L Q E A N P G K R E N P A L L S Q M  
GCACCTGGGGCCCATGCAGGACTTCTTGCTTTCCCAATGACCACATCCCAGTATGGCAAGGGTTCCTGCAGGGCAA  
A P G A H A G L L A F P N D H I P S M A R G P A G Q  
AGACTCCTTGAGTACCCCTGCAGCTGCAGACCCACTGATCACCCCTGAATTAGCAGAAGTTTTATGAAACCTATGGT  
R L L G V T P A A A D P L I T P E L A E V Y E T Y G  
GCTGATGTTACCACACCCTGGGTGATGGAGAAGCAACCATGGATATCACCATGTCCCCAGACACTCAGCAGCCACTG  
A D V T T P L G D G E A T M D I T M S P D T Q Q P L  
CTACCTGGAAACAAAGTGCACCAGCCCCAGGTGCACAACGCATGGCGTTTTCCAAGAGCCCT**TGA**TAACCTTGACATAGC  
L P G N K V H Q P Q V H N A W R F Q E P \*

AGCTACTTTGTGTATGCACAAGCTTTCCAGCTTTGTCCTCATAGTACCTTGTGTCTAAAACACTTACTATCCTTCCGAGTGAAGGTGTTAAGAGCAC  
TAAGCATATATTAATAAATAACAAGTGGCTAGAAATAGTGTAGGTCCTTCTTGCTTCCATTCTTATCAA**AATAAA**ATGATCAACTGTCTCCGTGACT  
TAGAAATACTATCAATGATATCAGAGCAAGTCTAAGTGTGACACTTGGTGGTATAGCATGTAGCTGTCTTAGGCATATAAAATTTCTTACTACA  
TGACATTATTATGCCAGGAAATGTGACACTGCTTCTTCTACACAAAAGCACCTAGTTTTCAGAATTCCAAGTATTTCAATTTAAACCTTATTTAA  
TGGTATTGGTGGAAAACCCTGACTGCTATTACTGTTTCATATATGGATTTAAAATTTATTTATAGAATATTTATTTAATCTAGGAAAAGAAA  
GGCAATTGGCCTGTTTTA**AATAAA**GAATTTTTCTCAC

**Figure S4.** Sequence of the *Ambn*<sup>lacZ</sup> allele following deletion of the PGK driven neo selection cassette by FLP-FRT recombination

**Exon 1 (116 bp)** underlined ATG is a weak, in frame translation initiation site. Gray highlighting shows forward Exon 1 primer for RT-PCR analyses.

GAGAGATACAGAGAGAGCCCCAGGAAACAGTCCAGAAAAAATTAATCTTCTTTTCTTA  
 GAACTGTTTTGATTGGCATCATCAGGCCTGGGAGCACAGTGAATGTCAGCATCTAAG  
 X m s a s k

**Intron 1 (778 bp)**

GTAAATGTGATTTTATCATTTCATGTGTTTGTCTATAAATTCTCTTTTCTGTCTCCAGATAACCTTTACAAAAAGATTTAT  
 CTTTATTATCACAAGATGCTGAATAACAGCTTATTTCAACCATTAGAATATTCAGAAAAATATATTACCATTAACTAAGAAA  
 ATAACTTATTTGTATGACTTTTTAAACTATGTTTGTGATTATTAGTGATAGTGCTAATTTATAAAAGTAAAGAGGATAAGCA  
 CACCTGCTGATCAGATCTGCTTCCATTTCGAGAGTGAAGTGAACAAAATGCATCTCAAGATTATTCATTTTTAGGAAGGAAAATCA  
 CTATCCAAGCAGATAAATACTCAACTTTAGCTTGAAGGCTGAGTTTTGACATGGTTCTGCATTATGACCCAATCATAGGTTG  
 TATTTCAAATTTCTGAGTTTTAAACAAAGAATACAATTCTGTAAATGGTGACTGAAGGATGCATTTTTATGAAGCAAAGCATAGTGT  
 TCAAGGAATATTCCTGGAGGTTGTTCTTAAACATTCATTCAAATTCGAGCTTCATAAAAAATAAGTACTCAGATCCATAGTTTGG  
 CTGCTCGTCATTCCATTCTGGTACCCTAGTGGCGCCGCGATAGCCACTGGTTTTAGCAGCTTTTTGCTTTTGCATTTTTAGCACT  
 TTTGCATTTTTGAGTTTTTTAACTGATTGTCTTTGGAAGAACAGCCACACGCTCTTAGAAATATACTTTGACCACTATGAATG  
 CAATTTTGCTGAGGTGATTTTACAACCAACTGTGTTTTAG

**Exon 2.** The 3419 bp knocked in sequence is highlighted in cyan and begins at the strong *Ambn* translation initiation site (ATG). The annealing sites for the *Ambn-LacZR* (double underlined) RT-PCR primer is bold. The translation termination codon (TAA) and the two polyadenylation signals (**AATAAA**) in the 3' untranslated region are shown in bold.

ATTCACCTTTTCAAAAATGGCTCCCAAGAAGAAGAGGAAGGTGATGGAAGATCCCGTCGTTTTACAACGTCGTGACTGGGAA  
 i p l f k M A P K K K R K V M E D P V V L Q R R D W E  
 AACCTGGCGTTACCCAACCTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACC  
 N P G V T Q L N R L A A H P P F A S W R N S E E A R T  
 GATCGCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTCGCTGGTTTCCGGCACCAGAAGCGGTGCCGGAA  
 D R P S Q Q L R S L N G E W R F A W F P A P E A V P E  
 AGCTGGCTGGAGTGCATCTTCCCTGAGGCCGATACTGTCGTCGTCCCCTCAAACCTGGCAGATGCACGGTTACGATGCGCC  
 S W L E C D L P E A D T V V V P S N W Q M H G Y D A P  
 ATCTACACCAACGTAACCTATCCCATTACGGTCAATCCGCCGTTTGTTCACGGAGAATCCGACGGGTTGTTACTCGCTC  
 I Y T N V T Y P I T V N P P F V P T E N P T G C Y S L  
 ACATTTAATGTTGATGAAAGCTGGCTACAGGAAGCCAGACGCGAATTATTTTTGATGGCGTTAACTCGGCGTTTCATCTG  
 T F N V D E S W L Q E G Q T R I I F D G V N S A F H L  
 TGGTGCAACGGGCGCTGGGTTCGGTTACGGCCAGGACAGTCGTTTGCCTCTGAATTTGACCTGAGCGCATTTTTACGCGCC  
 W C N G R W V G Y G Q D S R L P S E F D L S A F L R A  
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 R V T L R L N V E N P K L W S A E I P N L Y R A V V E  
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 L H T A D G T L I E A E A C D V G F R E V R I E N G L  
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 L L L N G K P L L I R G V N R H E H H P L H G Q V M D

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M N R L T D D P R W L P A M S E R V T R M V Q R D R N

CACCCGAGTGTGATCATCTGGTCGCTGGGGAATGAATCAGGCCACGGCGCTAATCACGACGCGCTGTATCGCTGGATCAAA  
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S V D P S R P V Q Y E G G G A D T T A T D I I C P M Y

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A R V D E D Q P F P A V P K W S I K K W L S L P G E T

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R P L I L C E Y A H A M G N S L G G F A K Y W Q A F R

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G E V P L D V A P Q G K Q L I E L P E L P Q P E S A G

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F C I E L G N K R W Q F N R Q S G F L S Q M W I G D K

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K Q L L T P L R D Q F T R A P L D N D I G V S E A T R

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D T L A D A V L I T T A H A W Q H Q G K T L F I S R K

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T Y R I D G S G Q M A I T V D V E V A S D T P H P A R

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S Q Q Q L M E T S H R H L L H A E E G T W L N I D G F

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H M G I G G D D S W S P S V S A E F Q L S A G R Y H Y

CAGTTGGTCTGGTGTCAAAAA**TAA**TAATAAGCTAGCTGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTT  
Q L V W C Q K \*

TAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATA  
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TGCGGCCGCGGCGCGCCGGATCC

3' 529 bp from intron 4 (originally 882 bp) follows the lacZ knockin gene.

AGCTCCACTCCAATAACCCCAATCTTTGGGAGACTGAGACAGGAAGAATGGTTTGGAGTTGCGAGTCAGCCTGGGCTACACAGTTAATATCAAAGACA  
CATTTTAGTAATATTAATATGTGCTGGCAAATGAAAGTAGAAATAATATATCCAGTACTCGGGAGCTGATGCACAGAGAATGCAATTCAGGCCAAGC  
TGGGAACATGCTGAAGTATAAGCCTTCCTCAGAACACTAACAAAATAAGCCATCAAATATATACATTGTTACTTTAAGTAAATAAATTACTTATT  
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AATCTGAAATGTATGTATGCTTTCTTTGTCTCTGTCAG

### Exon 5 (111 bp; encodes 37 amino acids)

TATTCTAGACTTGGCTTTGGAAAAGCACTTAATAGTTTATGGTTGCACGGACTTCTC

Y S R L G F G K A L N S L W L H G L L

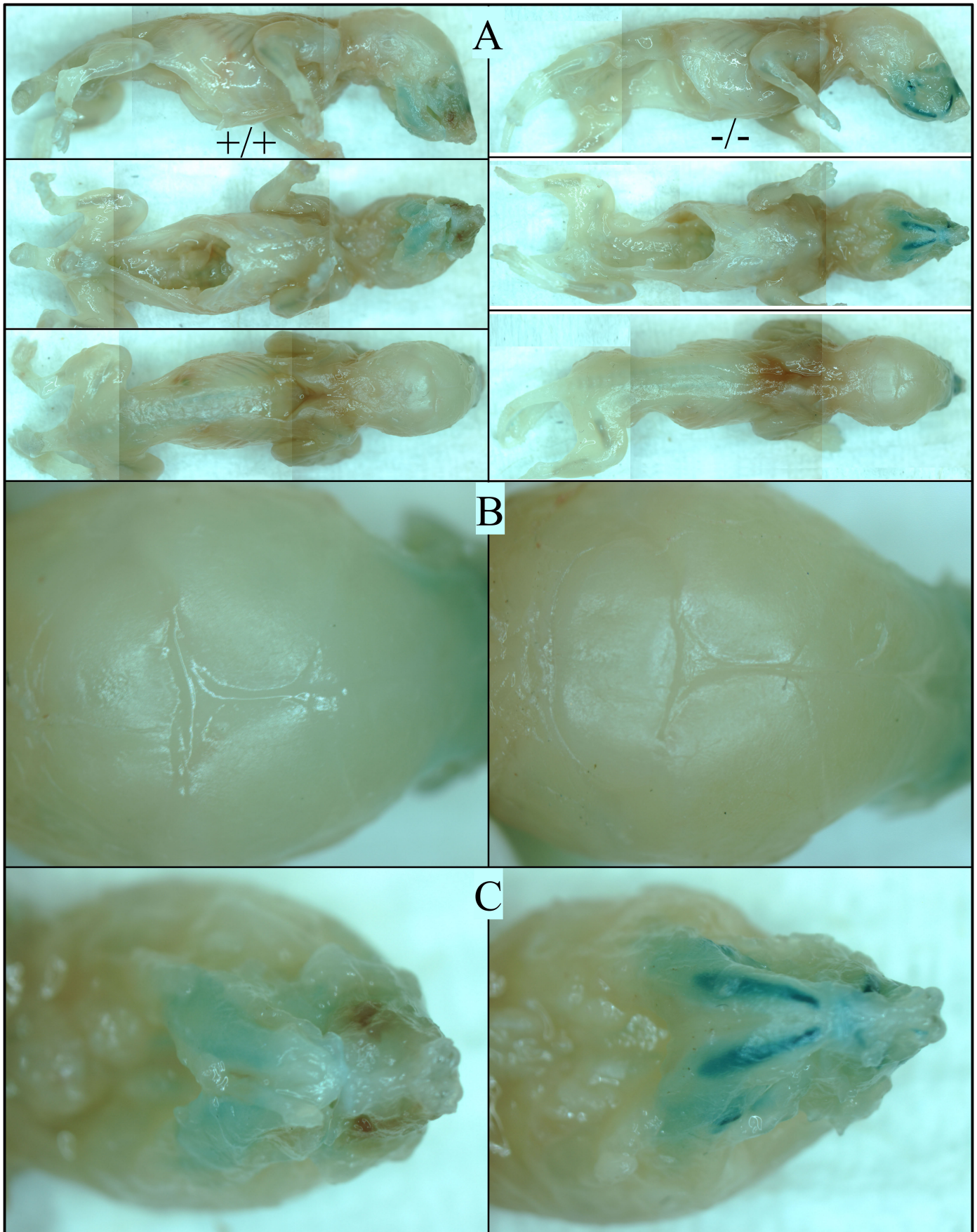
CCACCGCATAACTCTTTCCCATGGATAGGACCAAGGGAACATGAAACCCAGCAG

P P H N S F P W I G P R E H E T Q Q

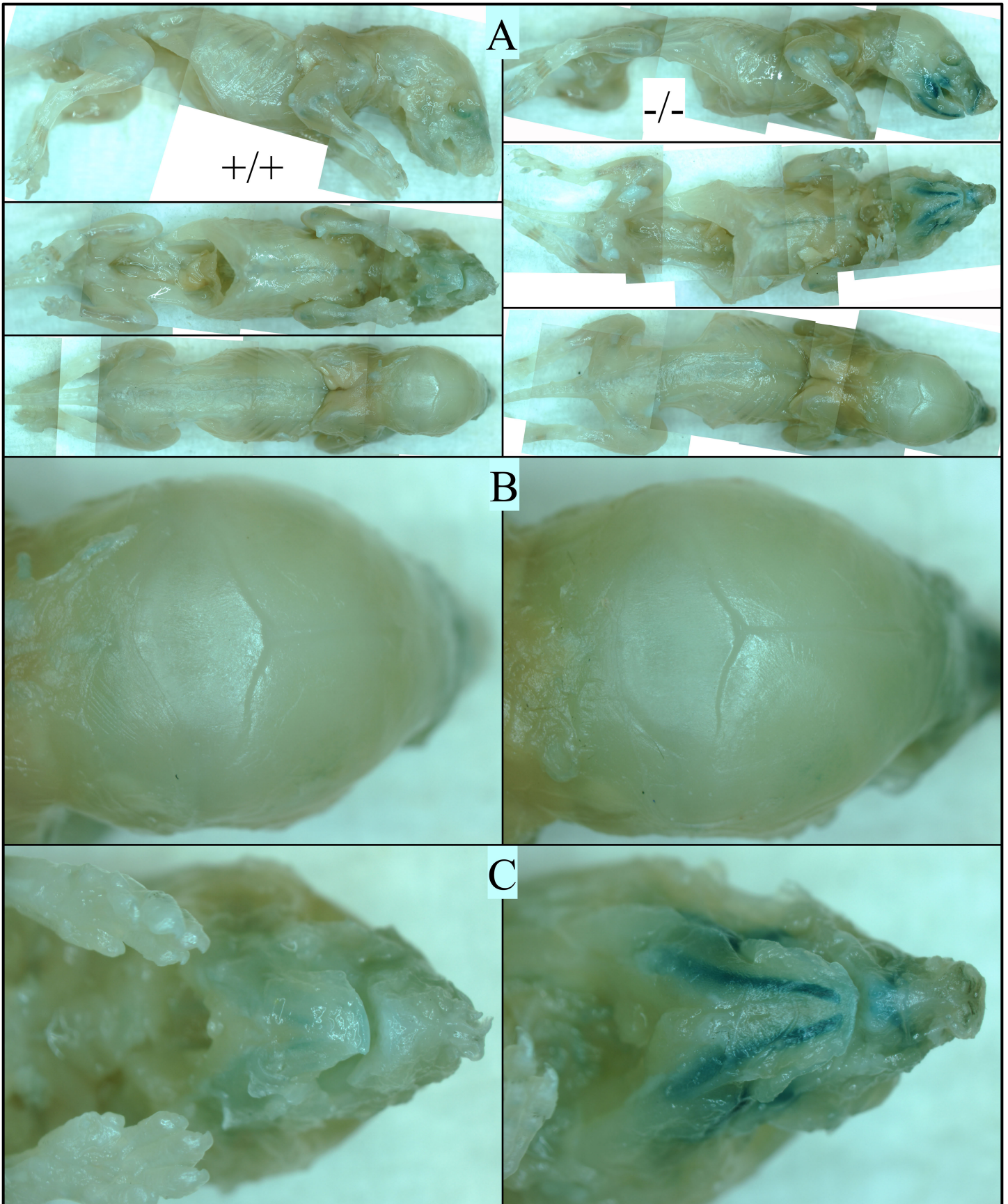
### Intron 5 (1528 bp)

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ACTTCACTTGATTGCTTCCAAAATAGATTTATGATGAAACATATAAGTATTTTAACTATCCTTTTAAATAATCACAAAATATTGTAAGTACCCTTCAT  
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CAATGGTCCCAACAAAGGTGGAGAGGTAAGTATGTTAAAACCTTCTCAAATACTTCTCTGAAGTGTACCTCTCCTGTGTAACCTCTGCTTTA  
AGACTACTCCCTTCCAAAACAGCAAGCCTCCAAGTACTTTGCTGTAGAAATCTAGGCACTGTGGTTCAATGAACCATCTCTTTCTGACATTCTTTTCA  
AATTTCTCTACAG

This is followed by the rest of the *Ambn* gene (Exon 6 to Exon 11), as detailed in the *Ambn* reference sequence: NC\_000071.6 Reference GRM38.p4 C57BL/6J.

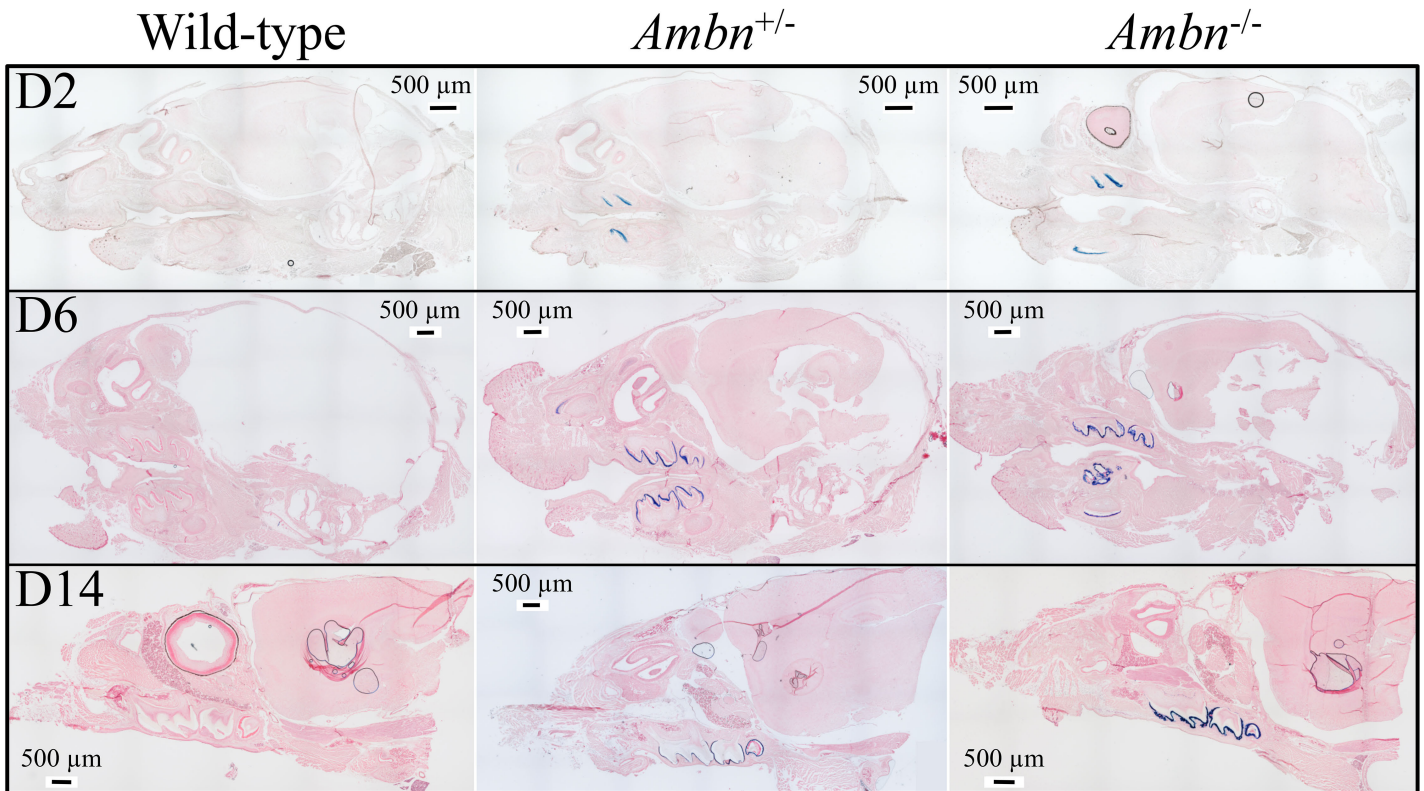


**Figure S5.** Day 2 whole mount X-gal staining. Skin and abdominal organs were removed. Staining was for 5 h. Wild-type mice (+/+) showed mild endogenous staining in vertebrate, the ends of arm bones, in the mandible, eye and nasal regions. The *Ambn<sup>lacZ/lacZ</sup>* knockin (-/-) mouse showed very strong staining in the incisor and molar regions. **A:** whole body; **B:** calvaria; **C:** Mandible.

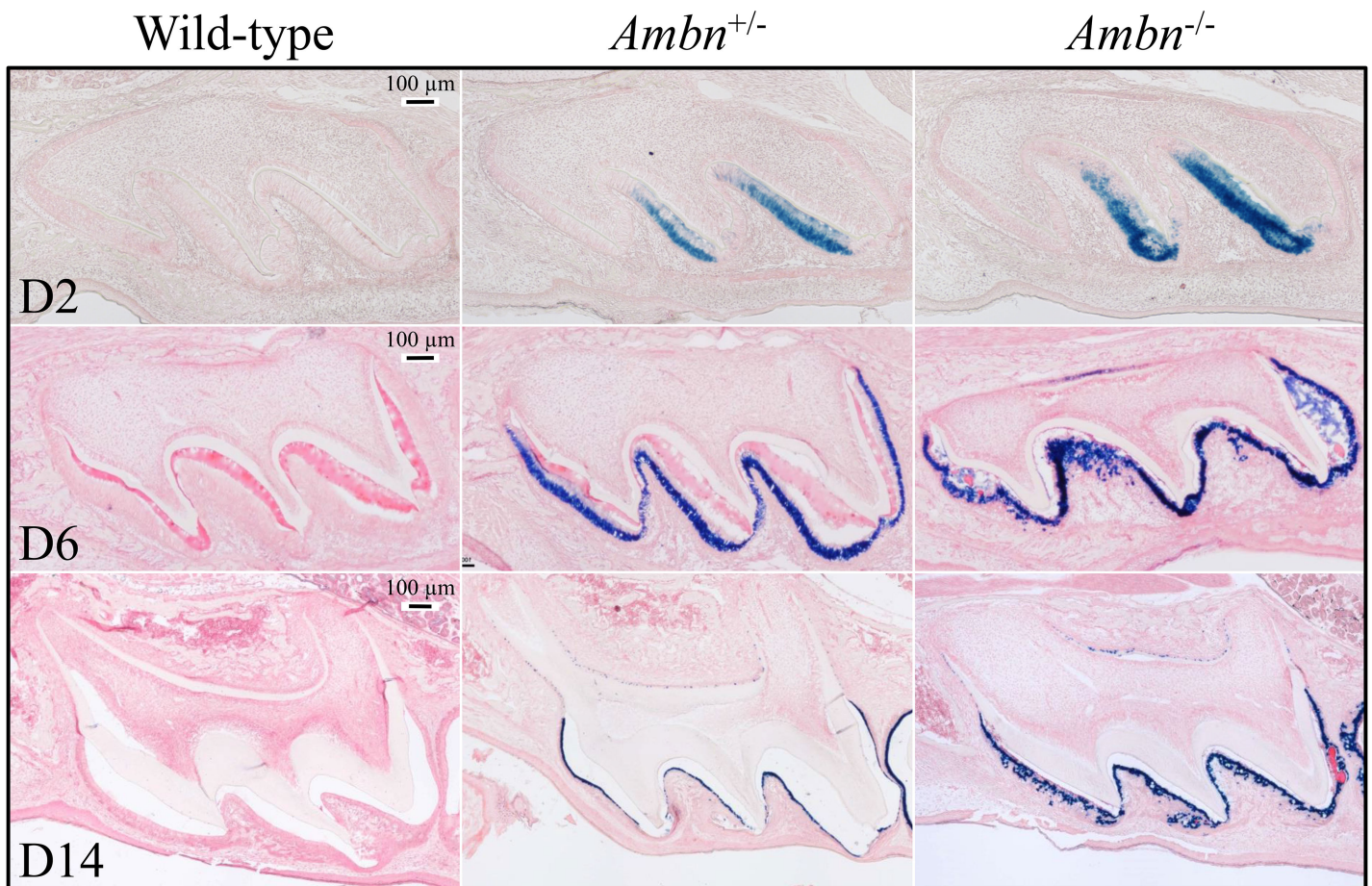


**Figure S6.** Day 6 whole mount X-gal staining. Skin and abdominal organs were removed. Staining was for 5 h. Wild-type mice (+/+) showed mild endogenous staining in vertebrate, the ends of arm bones, in the mandible, eye and nasal regions. The *Ambn*<sup>lacZ/lacZ</sup> knockin (-/-) mouse showed very strong staining in the incisor and molar regions. **A:** whole body; **B:** calvaria; **C:** Mandible.

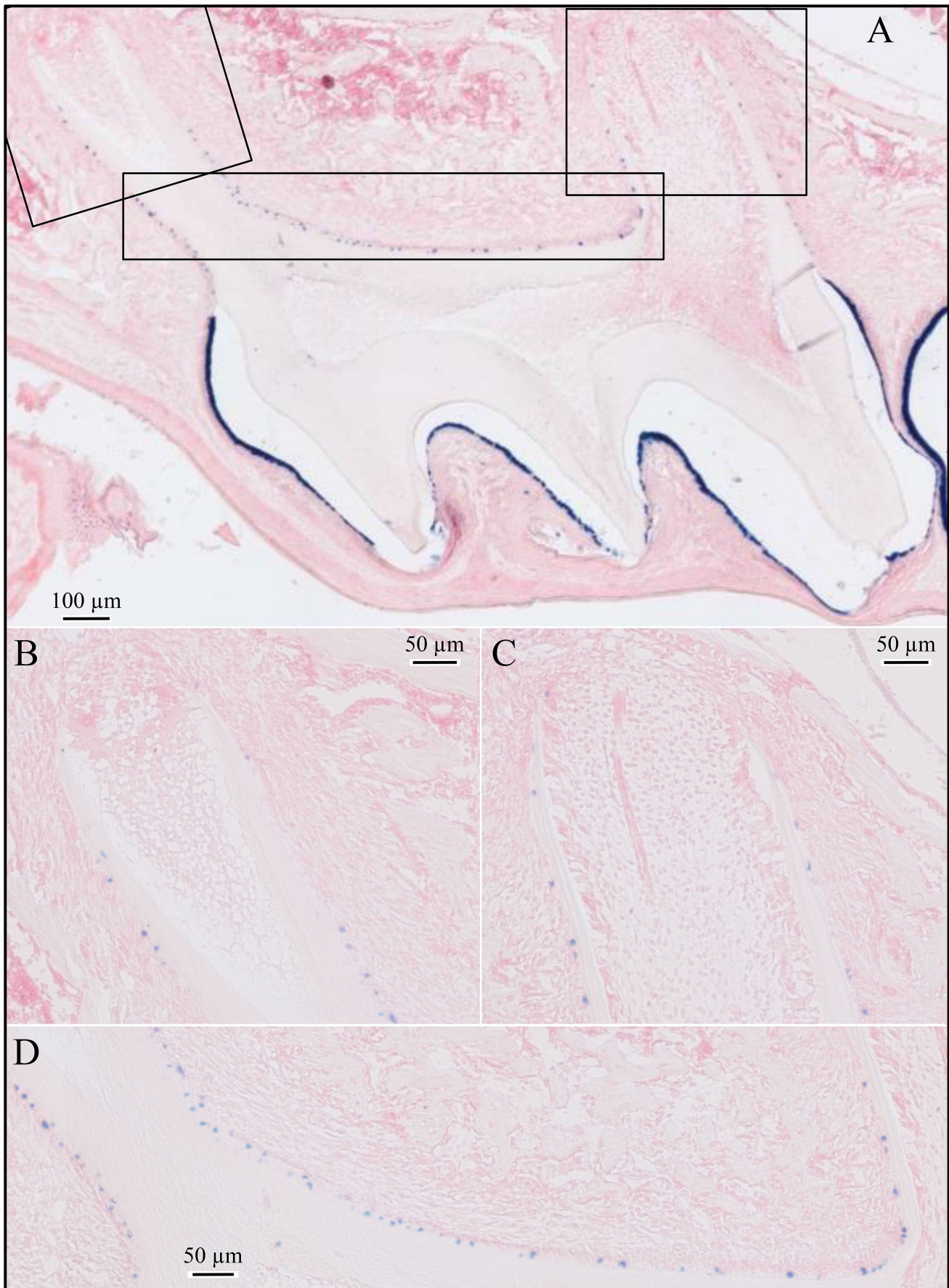




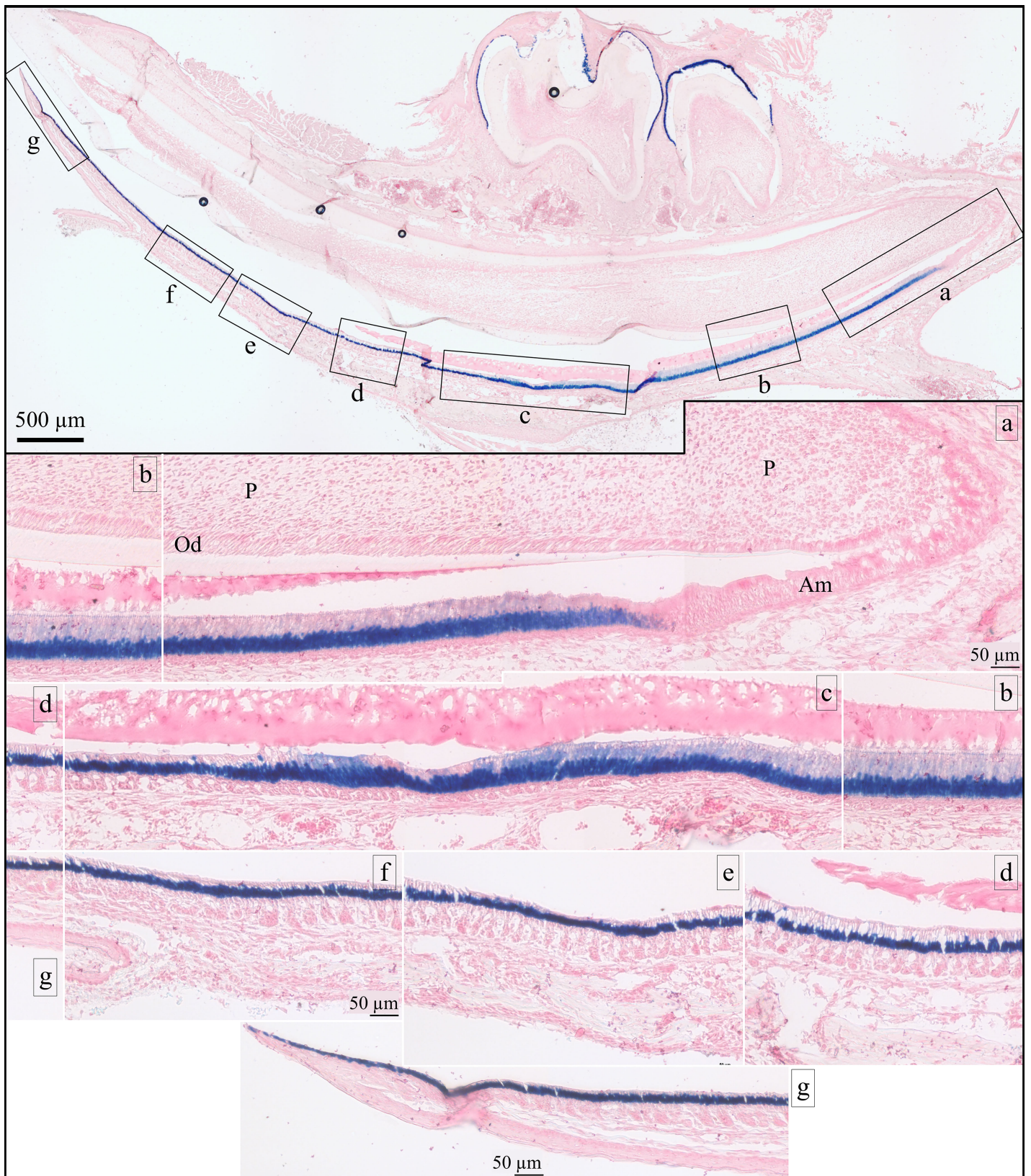
**Figure S7.** Mouse head sagittal section X-gal staining. Slides were stained in X-gal solution for 1 h at 45 °C and then counterstained with Nuclear Fast Red. Wild-type mice (+/+) showed no staining. The *Ambn* heterozygous (+/-) and *Ambn*<sup>lacZ/lacZ</sup> (-/-) mice showed strong staining in the ameloblast layer in the incisor and molar regions.



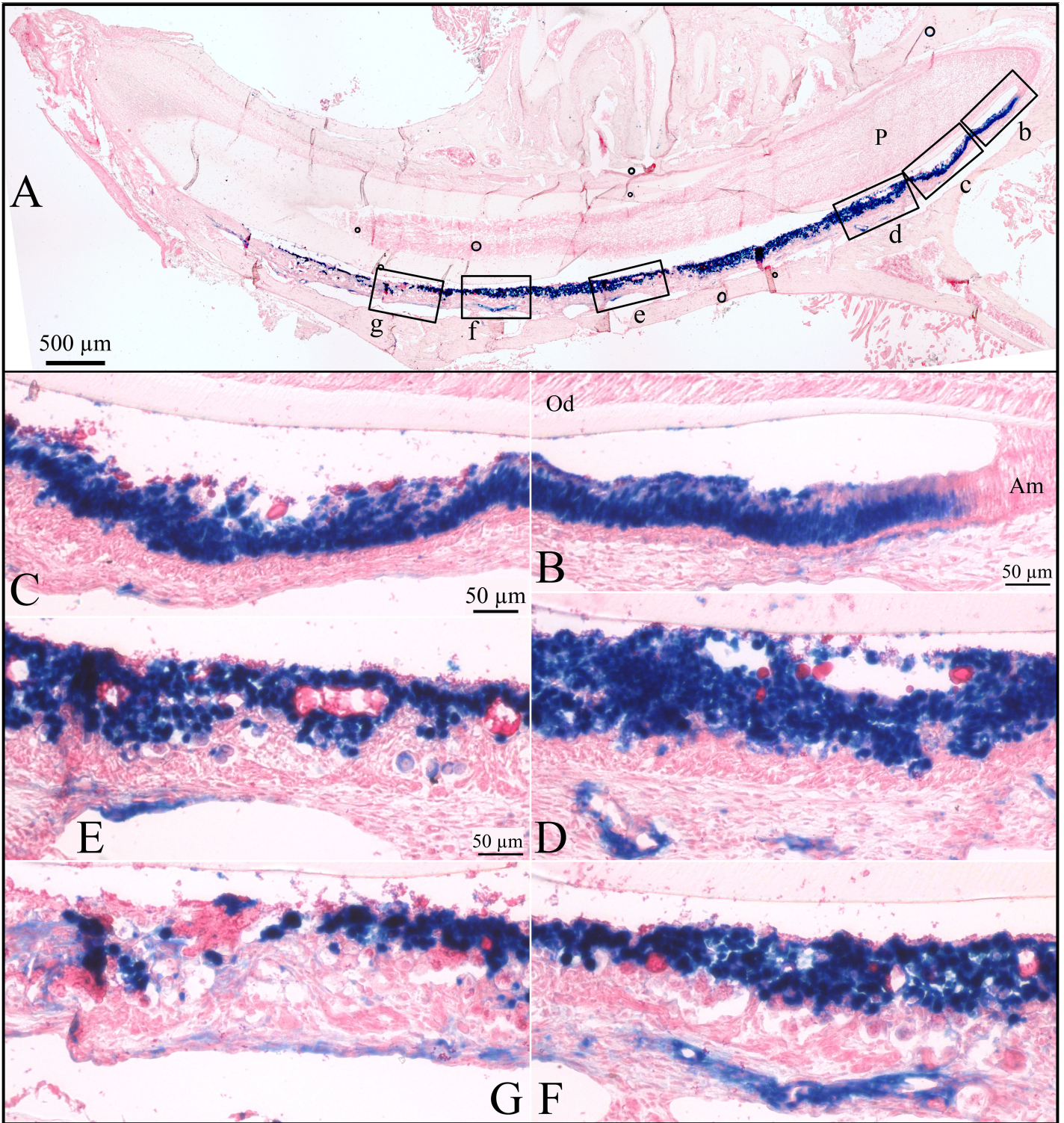
**Figure S8.** Maxillary first molar X-gal staining. Slides were stained in X-gal solution for 1 h at 45 °C and then counterstained with Nuclear Fast Red. Wild-type mice showed no staining. The *Ambn*<sup>+/*lacZ*</sup> heterozygous (+/-) and *Ambn*<sup>*lacZ*/*lacZ*</sup> homozygous knockin mice showed strong staining in the ameloblasts, and spotty staining along the outer root surface at postnatal day 14.



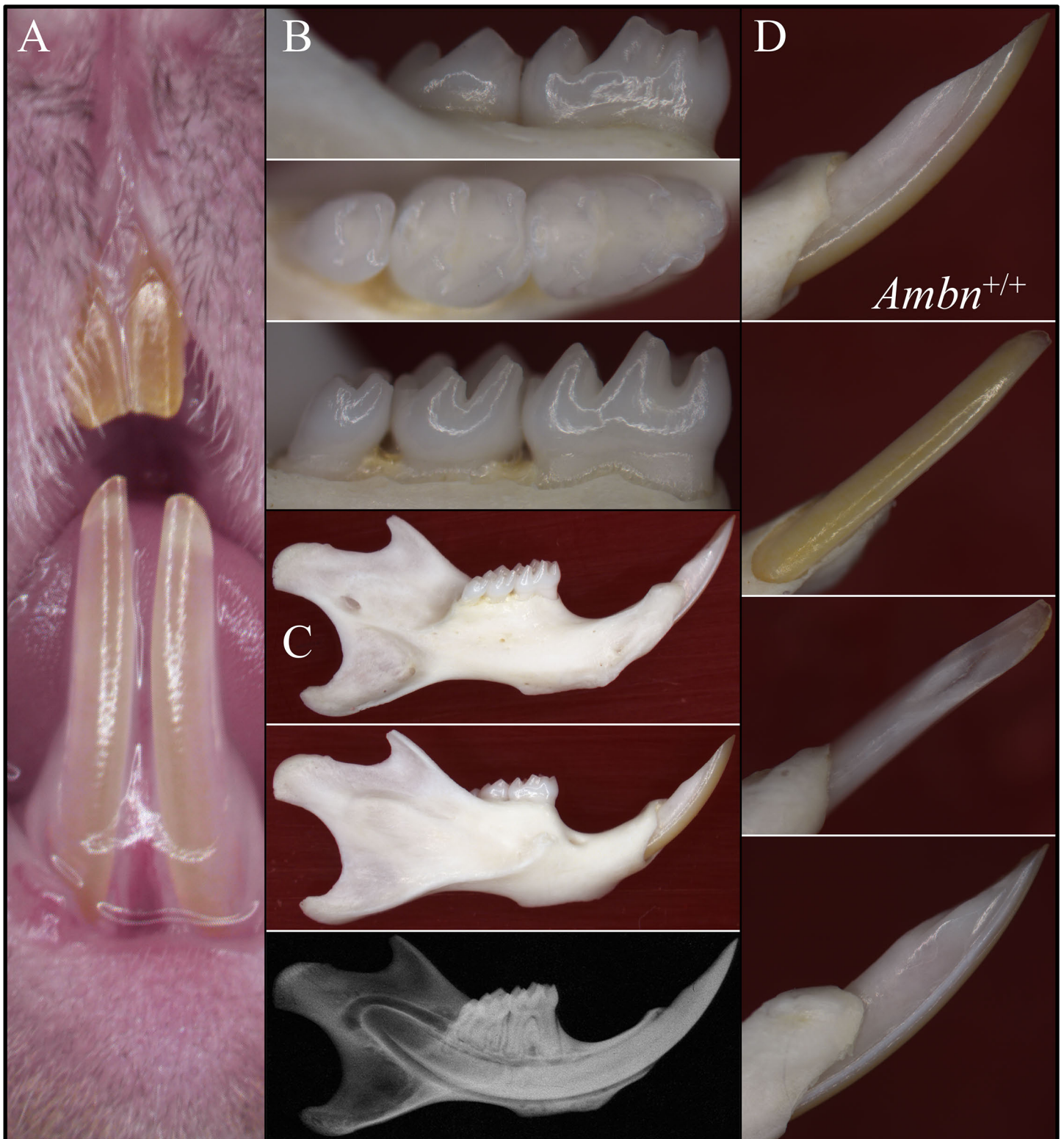
**Figure. S9.** Day 14 maxillary first molar X-gal staining in *Ambn*<sup>+/*lacZ*</sup> heterozygous mice. **A:** Maturation stage ameloblasts are strongly positive, odontoblasts are negative. **B-D:** Higher magnification of the boxed areas in A show relatively weak, spotty *Ambn* signal along the roots.



**Figure S10.** Day 14 mandibular incisor X-gal staining in *Ambn*<sup>+/*lacZ*</sup> (heterozygous) mouse. **Top:** Low magnification image of D14 *Ambn*<sup>+/*lacZ*</sup> mouse mandibular incisor. Boxes indicate positions of high magnification images shown below.



**Figure S11.** Mandibular incisor X-gal staining in *Ambn*<sup>lacZ/lacZ</sup> mouse. **A:** Low magnification image of 7-week *Ambn*<sup>-/-</sup> mouse mandibular incisor. Boxes indicate positions of high magnification images shown below. Note the strong staining in ameloblasts and the obvious ameloblast pathology.

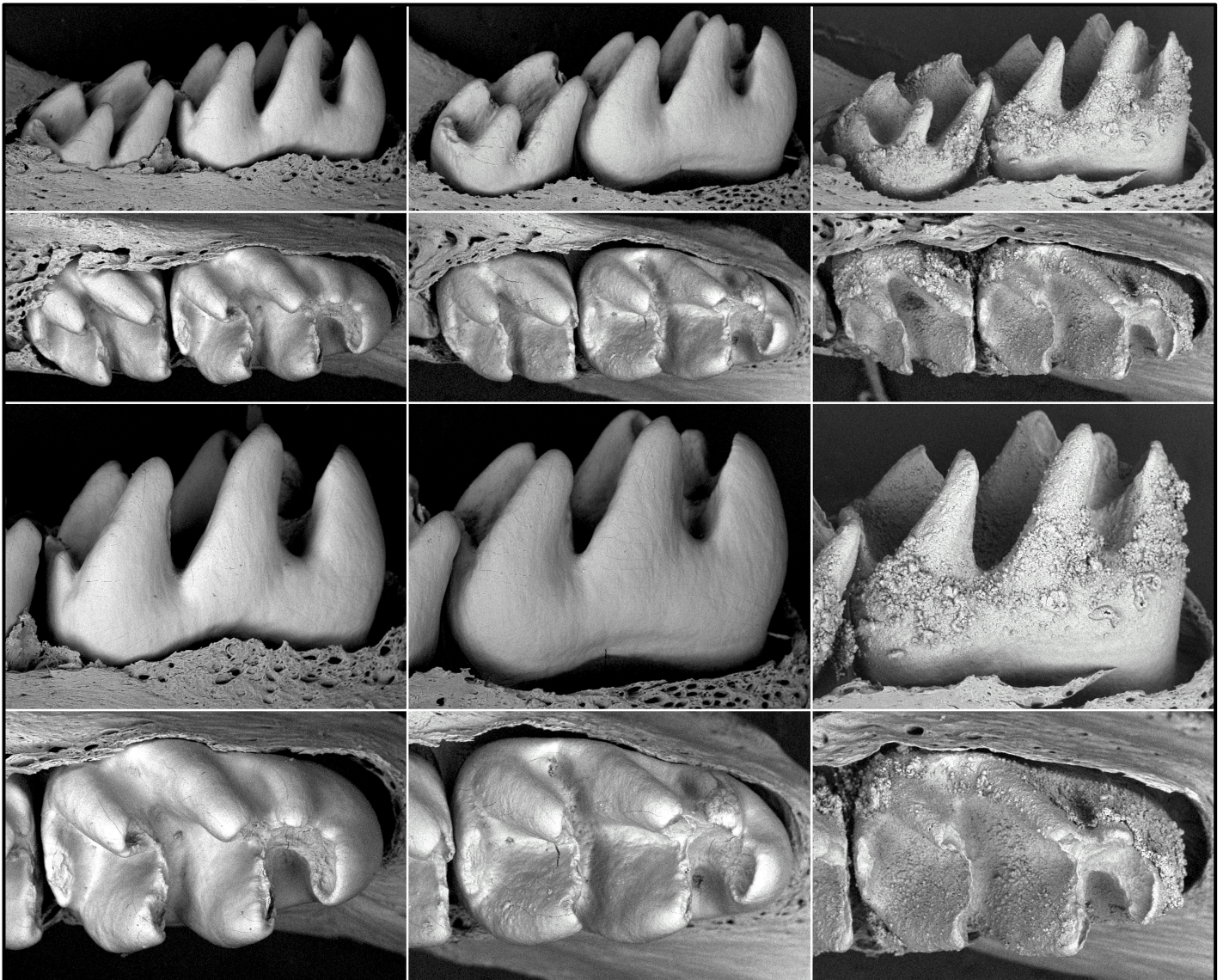


**Figure S12.** Dissecting microscope photographs of 7-week-old *Ambn*<sup>+/+</sup> mice. These images are for comparison to those of *Ambn*<sup>+/*lacZ*</sup> and *Ambn*<sup>*lacZ*/*lacZ*</sup> mice shown in Fig. 5. **A:** Frontal view of maxillary and mandibular incisors. **B:** Buccal (upper), occlusal (middle), and lingual (lower) views of mandibular molars. **C:** Lateral (top), medial (middle) views, and radiograph (bottom) of hemimandibles. **D:** Lingual (top), labial, lingual, and buccal (bottom) views of mandibular incisors.

Wild-type

*Ambn*<sup>+/-</sup>

*Ambn*<sup>-/-</sup>



**Figure S13.** Backscattered SEMs of Day 14 mandibular molars of *Ambn*<sup>+/+</sup> (Wild-type), *Ambn*<sup>+/*lacZ*</sup> (*Ambn*<sup>+/-</sup>) heterozygous, and *Ambn*<sup>*lacZ*/*lacZ*</sup> (*Ambn*<sup>-/-</sup>) null mice following soft tissue removal. These images show the molars immediately prior to their eruption into occlusion. The top and third rows are lingual views; the second and bottom rows are occlusal views. The null molars show thin cusps and surface accumulation of an irregular crusty material. Mild surface roughness of the heterozygous molar relative to the wild-type is most evident from the occlusal views.

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