

Supplemental Data Part 1

Construction of the *Fam83h^{Tr}* Mouse Allele and First Molar Analyses.

Supplemental File 1:

Figure S1. Generating and Genotyping the *Fam83h^{Tr/Tr}* Mouse.

Figure S2. Wild-Type Mouse *Fam83h* Gene Sequence and Structure.

Figure S3. Knockin intermediate following removal of selection genes by Flp-FRT recombination.

Figure S4. Final *Fam83h^{TR}* Knockin Sequence.

Figure S5. bSEMs of mandibular molars at 7-weeks.

Figure S6. Histology of *Fam83h^{+/+}* (WT), *Fam83h^{Tr/+}*, and *Fam83h^{Tr/Tr}* Molars at Days 5, 11, and 14.

Figure S7. SDS-PAGE of enamel proteins at D5, D11, and D14.

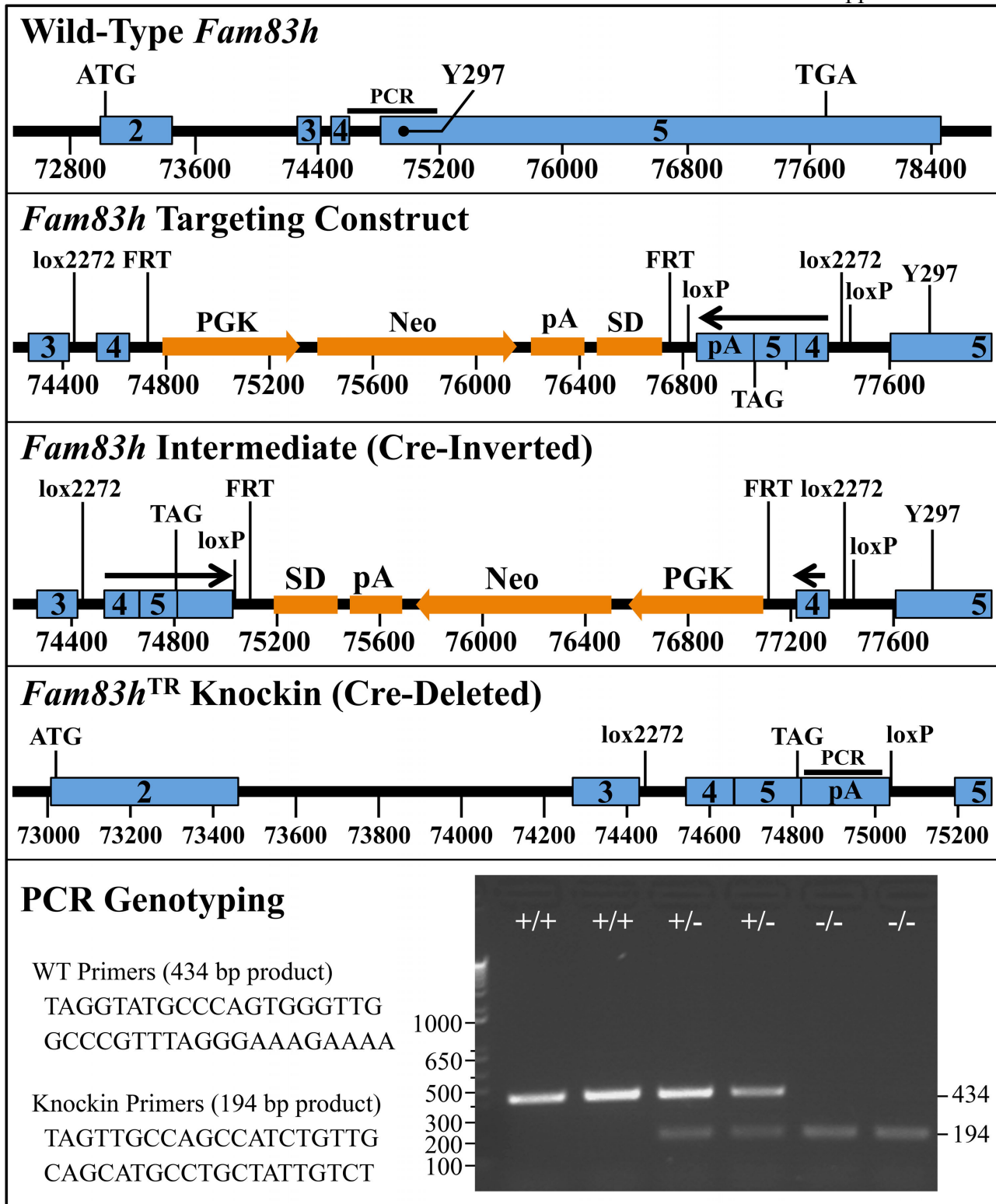


Fig. S1. Generating and Genotyping the *Fam83h*^{Tr/Tr} Mouse. Wild-Type *Fam83h* has 4 coding exons (2 through 5). The goal was to replace the codon for Y297 (TAT) with a translation termination codon (TAG). The *Fam83h* Targeting Construct contained a large insert in Intron 4 that included selection genes (PGK, Phosphoglycerate kinase promoter; Neo, Neomycin) and an inverted segment of the *Fam83h* cDNA from the beginning of Exon 4 to a TAG codon that replaced TAT codon for Tyr²⁹⁷, which was followed by a novel sequence for transcription termination (pA). The PGK-neo cassette was flanked by FRT sites so that it could be deleted using FLPe recombinase (Flp-FRT recombination). Exon 4 and the inverted mini cDNA were flanked by pairs of loxP and lox2272 sites in a head-to-head orientation. Cre mediated recombination between the lox2272 sites inverted DNA sequences between them and produced a loxP or lox2272 pair in a head-to-tail orientation (*Fam83h* Intermediate). Subsequent Cre-mediated recombination between the head-to-tail pair deleted the Exon 4 sequence and the selection cassette, generating the *Fam83h*^{TR} knockin mouse.

Fig. S2. Wild-Type Mouse *Fam83h* Gene Sequence and Structure.

Exon 1a: 93 nucleotides (NT), all are non-coding. Gene: 1-93. Exon 1a is not found on the mouse *Fam83h* cDNA ref seq [NM_001168253.1; *Mus musculus* family with sequence similarity 83, member H (*Fam83h*), transcript variant 1, mRNA], but is found on EST BY722193.1. Transcript variant 1 represents the longer transcript of 2 *Fam83h* variants. Both variants 1 and 2 encode the same protein. Here we start the numbering of nucleotides in the *Fam83h* gene at the first nucleotide of Exon 1a.

ACCTGGAGCGACTCCTCGAAACAGGAGGCCTGGGAAGGAAGCCTCAG
ATGAGAGTTGGTCTTGGACCCTCAGCTCCACCCCTGGGACTAACAG

Intron 1a: 4745 NT (actually longer as it would include exon 1b and intron 1b); Gene: 94-4838.

GTGGGCCACGTGAGGGTCCCTTAGCCCTTGCTTTAATTTAAAGGGGGTCTGGACTCCCAATCATGGCAGTAGTCAGGTCCCACACCCCATCCAGGAGCCAAAGCTAGACTC
TTCTTTATCTAGACTTTTGAAGGCTCAGTGTCTTCACCAGCCCACGGTGTGGCCCAAGTGCTTCATGTGGGCTAGGGTGGGCCCTTCATCTCTGGCAAACCTCTGTCTGGC
AGGGTTCGTCCGGGTAGGCTGGGGCTCCAGTGGTCACTGGGTGGGATGACCTATGGCCAGTGAGGGTCTGGGCAGTTAGGGCTGGCTGGTGCATTGGGATGCAGCTCT
TGGCTCTGTGGCCAGTTTGAAGTTCCCGCGACCAGAGACTGAGGGTGGGGTAAAGGGGGGGGCTTGGTGACACCCAGGCAGGACCAGGCAAGTCTCAAGCAGCAGTTTAG
CTACTGAGAGCCGCCCCAGTTTCTCAGTCTCCCTCCAGTCTGGCCAGTGAAGTCTGTGGCTGTAAAGTCTCGCACCCCTGTGTGAGCGTGTAGTCAAGCCTCTCGGCTA
GGGTGAGCCACTGTGCCGCCAAGTCTCTATAAACTCTCTGGTGTGGCCAGTGCACAGAGTGGGACCTGTGGGGCTATAGGCAGAGCCAGGGCCCTGGGCTGTCTACC
GGGGTGGCGAGATCAGCCAAGGGGTAGCACAGCCACTTGGGACAGCGGGGAGCAGAGGGCTGAACCTGGCTGAGAGCAAGCAACCTCTGTGGCCCTGGGATGTCTCTGA
GCTCCCTAACAGGCCAGTTGATGAGCCAGGCCCTGGGGAGCAGACATCTGGAGAAAAGGCACTGGATTGAGGGCCATGTCTGGGAAACGAGGGCAGCTCGAAAGCCCC
GAGGCAAGAGGGTGGGCAGGGCCAGCGGGCAGCTGGGCAGCAGGGCCCTGAGGCTGAGCCCCGCCCCCAACACAGGGGGCCCTCAGGACCAGTGGGGTGGCCCCGAC
AGAGCAGTGAACAGCACTCAGCCGGCTCCTCAGGACACTCCAGGGCCACCAGGCCAGAGCAGCTACAGGCAGCCTGGAGGCCAGTTCAGGACATTGCTTTGGGATCCGAG
CTGGTGTGGCCCTGAGCACATGGAGGGCTGTGGAGCTGGGGCCCCATGCCAGCCCTGGGCCCTGAATTGCTGAGACTCCACGAAGTCCAGCTGTGCCTAGCCAGGAGC
AGCTGCTGCTGGAGGATCGGAGGCGGAGGTCAGCTGCAGATGCAGCTGTGGCAGGAGGAGCAGCTGTGGCTGCAGCAGCTGCAGGAGGAGCAGCTGTGGCTGCAGCAGCT
GCAGGAGGAGCAGGCCTGGGTGCACATGGAGGGGCTGCAGCTGGCTGTGGCCCTGGAGCAGCTCCGGAGTGGAGGGCTCGAGGCAGTCAAACTCAAGGCCAGGTAAGGCCT
AGAGGGGTATGGGGCAGGGGCTCGGGCAGTGCAGTACAGTGGACACTCAAGCATCTTATAGTGTGTGAAGGATGGGAAGCCAGGTTGGCTGTAGAACGCTGTGGGGCTG
TCCACCAAGAGGGGCTAGTGTCTACTACATAGGATGTTGCCTGGGTGAAGGACAGCAAGCCTGCCAGACCACCAATGCTTGGTAGGCAAGTTCATTGAGGAGGGAACTT
GCTTGGGGATGGAGTGTGAGGGGACCAAGTAAAGTACAGTACAGGATGTGTCAGGGCGGTAGGGTGTGTGTGGGGGGAGGGCCGGGTGCCACAGCTGATAGACAACACTCA
GAACCAAGAAACAGTCCCTACCCCAAGCATCTTGACAACCTTCTCGTCTGGTCCACCCCGCTGCCCGCTGCTTACTGCAGCTTGGTGGCCAGGCTGAGATCACAGAGG
CAGAACGCAATGTGGTACCCATTTATCTCATTCCAGGAGGAAGACTGGCCTCAGCCAGCAGCTGGGGCTATTTTGGGCAACTAGGATTCACCTCAGGCTAGTGTGGAGTCA
CCTACTGCTGTACAGCCAGGGAATCTAAGATGTGGGCTGCTGTGGGTCTGGGGTGGTCCCTGACAGCGGGTACAGACTCCAGGCAAGTTCGGTGAAGAGACAGTCT
GGGAAGATGTTACAGCTGTGGCAGAATGAATGGGACTCAGAGCAGAGGGGCGATAAAGGCTGCAGGAGGGTAGGCACAGGGGCGAGGGAGACCTTTTTCTGATGCTCTGCA
GGAGCCAGCCAGGCTGGCTGTAAACCTTATCCTTCTCGAGGCTGTACAGACAACAGCTGAAGGGGAAGGCAGGACTGTGGGATGGTGAATAGACAACCCTTAGCA
TGAAGATTCCCAACTCAGAATCAAGGCTGCTTTTTCTCAATATTGCTTTGTTGCTGTGTTGTCGTTCCAGACAGGGTTTCTGTGTAACCCCTGGCTGTCTGGAATC
ACTCTGTAGACCAGGCTGGCCTCAGACTCAGAGATCCGCTGCCTCTGCCTCTGAGTGTGTTGGCAGTGTGCGTCCACACGACCTGCTTCGATATTTTTTTAAGCTAT
CTGCCCTCCAACTCCTCCTTACCCTAACACTGTACCCTCCTGAGCACTGTACCCACACCCCTTCTCCAGCTCTAGAAGTTCGGGGTGCAGTGGACATCTGAGCTTTGAGT
CACAACTATCATTCCACGCCCACTGAAAGATCTCTTGAAACCACTTGGAGAGAAAGGGTTTCTGTGTGCTAGTGGTCTCGTGGTCAAGTGTGGGGTAGTGCCTGGA
GGTTCAGAGGGGCACTCACCAGCAGTAGCTCGAAGCTTGGACCAGGGAGGAGCGCCTGCCCTTCCCTGATGCAGCTTTGGGCCCCCTTCTTAGTGTCTATTCTCCTGG
GGTGGGAAGGAAAAGTCCAGGGATGTTACATTCAGGAGGGCAGCGAACAAGCAGTGGGAATACTGCCCGGCCCTGGATGAACATGCAGCAGGAGCATAGACCTCCAGCA
TGTCACCCAGCCAGCTTCTTGGTCCACCTGCCCGCCCTGGTGGTCTAAGGATGTGCCAGTAGCTCGAGGAAGGATACCTAGCAAGAGGATGTGTCCCTTTATGCTGGTAC
CCCCGCTCTTACAATTTCTGGATCTGCCTGTCTTCTGTGTTCTAACACCATCCTCTAAATCGCTGGGCTAGAAGCTCAACAGCCTGAGCTCTGCGGACTTGCTTTCAACTA
CTGCCAACGTGGGTCTGTGAACACAGCCCTCCCCACCCGCAAACTCTTCTTCTTGGCCCTGGGAATCTCTGTGTCTGGTCTTGGAAAGCAATGCCCTTCTCCCTAGT
ATACAAGGCAGGCCCCCATTCACTCCTTGGAAAGAACTTCCGCTCCTGTTTTAGTAGGGTCTCCAGTGCCTAGAACCCTCACAGGGCCAATGAATAGGAAAGCGGG
TAGTGCAATCGCTGACTCCATACACTTATGTACCCACTAGGCTGGGGCCGAGGGCTCCAGAGTGCATTTGGGGATGGAGTTAATGAAATGTTATCTTAAAGTAGGACCAG
AAAGTGAAGTGAAGTAAAGTGTCTTACGGACAATGTTTCAGGCAGGTGGCAGACTGAGAATCTAGGACAGGAAAGTGGTAGGTAGGAAAGCTGGCATGTGACTGA
GTTTCAGGGCACGCGATGCGTAGAGGAGTGGGTTGGAGGGAGGCTGGGTAGATTAGGGCTTAGGAGGAACCTAAGTCTTAGTGTACGGGTGATGTGTTGGGTCGATACA
ACCATAGGTTCCCTGTCTGTAGAGGATCCAGTACTTGCAGCATCTTGGGTGCAGCCCTTTTTAATGTGCATGTATTAAGACGGGACCTTTGTGTCTCAGTTGGTTTGCAC
AGGAAAGGATCAGTGGGTGCCAGGACGACTGTTGGAGCAGTTTAGAGAGTATCTCATGTGAGATGGGACTTGGGGGTACGATTTGGTTTGGGGATCTGTGTGGC
AGAGGGGCTTCTGCTGGCGGATCACTCCAGATTCCCTGTGCCTGAGGGTCCATGGGTACGGGTGAGTGCAGGAAACCTTTATGGTTGGGCAATGAGTGGGAGGTTCC
GTGGAGTTCCTATGTGTGCGTCTCATGCGCGAAGATGAGCCTGCGGGGAGTGGACTGGGAAACTAGAGGTGTGTCTGCAACGGTAGGGCGGTCACTTGTGAGATGGG
TACCAAAACACCCCCCGCCCCCGCTCTGAGACCTCACTGCCTCACTGCCTCTCGCAACACAGAGCCAGAAGCTCCGCCCTCGCGTCGAGCTGGCCAGTTTCTCT
AGGCACTAAGCCGAACTCCGAGTTACCCCAACTCCGGGGCAGGGTCCAGGGTCCGAGTAGGGCTTTGAGCGATCGGTTTTCAATAGGTTCAAGGGGCTCAGTGT
CCGGAAGACAGGCTTAGAAAATCCGAGCGGGCTGGCCAGGGTCCGAGCGCAGCTCTGGGGCTCCACCAGAGCCTCAGGGTCCGGGTGGGGTGTGGGCTGGGGC
GCCAGTGAAGCCACTGGAAACCCGGGATCGCGGGCGGGTCCGGGGCGGAGGGCGCAGGAATGGGGCGCTGGGGTGGGGCCGCCCGCCAGGTTGCTGCCGGAC
AGCAGGAGGCAGAGCGCGGGCGGGGCGGGGCGGGCCGT

Exon 1b: 125 NT. All are non-coding; Gene: 4839-4963.

GAATCAGGCCGAACGGGCGGGCGGGCCGCGGGGGAGTGGCTGTCCGACGGACGGGACCGCG
AGGCCGCTGGGCGGCGGTGGGCTCCTGCTGCCCTGTGCCGAAACCCGCTCACCTGGCCAG

Intron 1b: 2811 NT; Gene: 4964-7774.

GTAGGGCTCCCGCCCCGAGCGGGCCGGGCACAGCACGGGCGGGGAGGCACACGGGACGGGAGGCTCGGGCCTCCGCGCGGTGCCACCTGGGTCTGGTCTCTGGACCTGGG
 CCGGGCGGTGCAGTGAGCGCGTCTTTGGGACTGGGTGGGAGACAGGAGACTGCGCTCCGGGGCAGTGGGTGAGGGATTTTCCAGGGTCACGCTGGCAAGTAGGAGACTGA
 ACAGTGCAGATTTCGCTCTGGCGGCTAGGTATTGTGACGACATGACCTGTACCTTTCCCGGAGCCTCTCCCGAGCCGATGAACCGGGGTGTCTAGGGCTGGGTCTTTGTG
 TGGAAGGCAGGAAAACTGGACTCTCTAACTCCTGATCTCATATCCCTGGACTGGATCGGCAGATAAGATACCTGGCCTGGGTAGCCGCTAACTTCTGGGGTACAGTAAC
 ACCTTTGGGCCTTGCAGTGCCTAACCTGAAAGCTGTATAATTCATCGACTTCCGGTCTTCTAGGTTCTTCTGTCCCCCACAACCCCAATCTTCTGTATCACAAATCATCC
 TGTATCTCTTTGTGTAGTAGCCTAAGGTCTCAACCATGGATCCCCCTCCAGACCCAGGAACACTACTACCTCTGCCAGACAGGAGAGGAAAGGGTCATTTTCTTGCC
 CTACTTGTGGTGAAGGGCTGGGGCTAGCTTCCAGGTAGGAGGTGAGGAACAAAAATCTCTGTGAGTCACTGGTTTGGGTGGGATAGGCTCTCTTAAAGCAGGGCCCCA
 CTTGGATAGGCAGATGTCAGTGAGCAGGCTGTTGGATTTCATCGCTGATAGGAGTGGTACCAGCCCCGCTATCGTCAAGGGGGGATGTGTACCAGTGAATGATGTAAAGGC
 ATTTAAGGTGCTGTGTGACTTCTGTGAGGTGAGTACCACAGTGGCCGCTCAGGACTAAGGCCACTTCCCAACCCATGTGTCTCTGAGGGTCTCACCAGTGGCTAGACAGT
 ATCAGTTCTCAGTCTTATACAAGGAAGTGTCCCTCAGCGTGAGCATGCTAGGATCCCCAGGTAGTGTGGGAATGCTGCCTAGGAGAGCTGGCTCCAGTCTGAGACCTGTCC
 AAGCAGACAGGCCCGCATCAGACCCAGGTGCTCTGCTTCCATCTCCCGAGAAGTGTGCCACGCCCTCACATTCAGACAGGAAGGAAAAAGCAACAGCCTTAGGTACAGGGC
 AATGCCCCAGAGGGAAGTCGGAGCACTTCTTAGGGCCAGTAGACATAGGAGTGTCCCCACCTCCAATCTCTGTACACTTGGACTTGTCTAATGTGTACGCCATGCCCCCT
 AGGGGTGGCAGACACTGTAGCTCCCTGGGTATACACAGCAGAGTCCCGCTGGACCCCAACGTCATTGAACAAGTATCCCCATAATACAGAGCTGCCATCTGTGTCAGCATC
 GCTGGCCCACTCTCAAGGCCTCTCTCTGGGCAGAAATACTTCCCTAGTTTGGACTATCATCCAGGAACCTGTGTGTAGGCAGGGCAAGAGTCCCAAGCCATTTCAA
 ACCTGTGAGCAGTGGGAATGGTATGAGCCTGCCCTAAGGCAACTTCCAGGCCAGAGGCCACCAGGTTTGCAGGCGTTTGGTGTGACTTCTAATGCCTTCTCAACCACA
 AGCTTCTGCTACTCGCACTCATGGCCAGGACCTCTGATGTGACATGAACGGTTTCTCGGGCACATACTGCCAGTGAACCTCAGACAGGCTGGACCTGGGCTAGCCCG
 TGACATCTGGCTTCCAAGGCAGGAGTCACTCTCATGAGCGCACATGCAGCAGTGTGTGTCACACACACACACACCAGCCACTAGAGAACAGTGAATGCTGATCCTGAG
 GCACCTGTGATGGGGACGCTGACAGAACTTGGGACGAGGCTTGTAGCAAAGTCTTCCCTTATTCTAGTGTATTAGGGAGAGGGTGGAGATCCGGGGCCTGGGTATTG
 GCAATGGCTCTGTGCCATCAGGATGGGTGAGTGTGAGTCTGGGAGGTTATAAGAAGAGACCCTACAGGGAAGTGTGAGTTCCTCAAGGAAGTGTGGCCAGGGAGGTTTAGC
 TGAGGTGAGACGGAAGCAAGGGTGAAGATTTTTCAGAGAGGTGCAAGGTTGAGGAGTACAGGACAGGAGATCAGTGAAGGGACTGTGGGCAGATGCAGGTGTGGACTGAGGAA
 AGAGGTGTGGGTGGGGCTTAAGGCCATCATCCCTGGATGAGTCAATCTGCCTCCACTGTGCTCACCTTGGGCCAGGTATGATGTCTGCCACCCCTCCAGCTCTGTGCAG
 TGAAGGACACAGGCTAGAGCAAAAGGGGGGGGGGGAAGGAATAATCTTTAACTGGCCTGCATTTGTATCTTAACCAAACTTATCTTTACAGGAAAAGGGAACCACAG
 AGTAAATGGCCGTGAATGTATTGACTCTGAAGTAAGGGAGGTGGGACCTTACCTGTATTGGGGTGGGACAAACATTGGCAGTCACTGAGACTGATAGAAACAAGGTG
 TCAGCCCAGGAAGGGGATTAAGGGCTGAGTACCAGTGGCCATGTCTTACCAGCTAGGCACATGCTACGGCCTGTGGTGTGCTTCCAGAGTCTGTCTCACTGGGGCTCAG
 AAAAATCCCGGCATAAGCTAAACCAAGATCCAGCAGCCACATCAGCCGTGTGTGCCACTTGTGGCATGAGGTAAGTGGGTCTACTGCCCTAGTTAATGCATTTTCC
 TGCCCTCCAG

Exon 2: First Coding Exon: Gene: 7775-8236; cDNA: 126-587; Protein: 1-149.

GCCCCTGGCCCCAACATGGCCCCGTCGCTCCAGAGCAGCTCGCAGGGGGACAACCCACTGGCACCT

M A R R S Q S S S Q G D N P L A P

GGGTACCTGCCACCTCACTACAAAGAATATTACCGCCTAGCGGTGGATGCATTGACTGAGGGTGGG

G Y L P P H Y K E Y Y R L A V D A L T E G G

CCAGAAGCCTACAACCGCTTCTTGGCATCTGAGGGGGCACCTGACTTCCTGTGCCCTGAGGAACTG

P E A Y N R F L A S E G A P D F L C P E E L

GAACACGTGAGCCGCCACCTGCAGCCCCACAGTATGTGGCCCGGGAACCCCTGAAGGCACCCCA

E H V S R H L Q P P Q Y V A R E P P E G T P

TCTGATGTGGACATGGACGGGTCCTCAGGCACCTACTGGCCAGTGAACCTCAGACCAGGCTGTGCCT

S D V D M D G S S G T Y W P V N S D Q A V P

GAGCTGGACCTGGGCTGGCCCCCTCACATTTGGCTTCCAAGGCACAGAGGTCACCACACTGGTACAG

E L D L G W P L T F G F Q G T E V T T L V Q

CCGCCGCCACCTGACAGCCCCAGCATCAAAGATGAAGCTCGGAGGATGATCCGCTCTGCCAGCAG

P P P P D S P S I K D E A R R M I R S A Q Q

Intron 2: 801 NT. Gene: 8237-9037.

GTGCATCTCACGGCTTGAAGGATGGTGAAGGCAGCCCAAGCCGGTTTTGAAGCATGAATAGGAGGGTGTGGGAGATATAAAAGCATTCCTGGTGCCT
 AGGATGCCAGGGCTGGCCAGCCAGACCTCGGCAGTAGGGTTGCCCTGTAGAGTCTGCTCATTATCACCTGGGTCTTGGGTAGCGGGTCTGTAGACATTC
 AGTATCTAGGATCATGGCTGTACCCAGAACTCAGGGTCTTCCAGCTGAGAGCACCCACCCAGGCAGTGGTAAACTGAAAAACCAAGTGTTTAAG
 CCAGGAAGACTGGCTCATGCCTGTAATCCTAGCGCCAGGGAGGTGGAGGCCAGCCTGGGCTACACGGTAACAGACCAGTCCCTAATAAGAAACAGGAACA
 CTCTACAAAACCCAGGAATATTAAGAGAGGTAGCAAGGCCAGATGGTAAAGGCCTAGCTTCTGCCCTGTCCAGGAAGTTGACAAAACCGCACCTTGAC
 CACTTGGGGTCTGAACACACGGAGCTGCAGATAATCCCCCTCTGAATGAAGCACCCATAAAAGTATAGCCCCTGCTTCTGCTTCAAGCTCCCTCAAGGGCTA
 CCAAGTGTCAATTTGTGTGTGTGTGAGACCTTCTGCTGGGTCCCTTCTTCCCCCACCTCCCAATTCATGACCAGTCTCTCTGGGGCAGGCTAATGG
 TCATTTTTATCGTCTTGTGGGTGAAAAATGTGGCCAGAGATGGGCAGGATGACCAGGCCCTTCCCTCCCGCTGACCTGATACCCACCTCCAG

Exon 3: 165 NT; Gene: 9038-9202; cDNA: 588-752; Protein 150-204.

GTGGTAGCTGTGGTGGATGGACATGTTTACCGACGTGGATCTGCTCAGTGAGGTGCTA
 V V A V V M D M F T D V D L L S E V L
 GAGGCCGCTGCAAGGCGAGTCCCGGTCTACATTCTGCTGGATGAAATGAACGCCAG
 E A A A R R V P V Y I L L D E M N A Q
 CACTTCCTAGACATGGCCGACAAGTGTGCGTCAACCTGCATCATGTGGAC
 H F L D M A D K C R V N L H H V D

Intron 3: 69 NT. Gene: 9203-9271.

GTGAGTGACCAAGCCAGGGGAGTGGAGGAAAATATCTTTGGTGAAGACCTGACTCCCTCTCTCCAG

Exon 4: NT; Gene: 9272-9396; cDNA: 588-752; Protein 205-246.

TTCCTGCGTGTGCGCACAGTGGCAGGTCCTACTTACTACTGCCGCACTGGGAAGTCTTTCAAG
 F L R V R T V A G P T Y Y C R T G K S F K
 GGCCATCTAAAGGAGAAGTTCTTGCTTGTGGACTGTGCCGTAGTGATGAGCGGCAGTTA TAG
 G H L K E K F L L V D C A V V M S G S Y S

Intron 4: 191 NT. Gene: 9397-9587. (Green highlight = PCR genotyping primers) This first pair is specific for the wild-type allele and generates a 434 bp amplification product.

STATGCCAGTGGGTTGCCCTTAGCCCTGGCCCTTCCCACCTAGTCCCTCCGTGGTCCCACTTCCCTTCCATAAAGCTGTCTTGGCCAGCCACC
 AGCCCAACCCCTTTCAGATCCCTGATTATCCACCTAGTGCCTTTAGCCTCTGTGGCCCTATTTCCCTCCCTGACTCCTGCCATCCTGTCCACAG

Exon 5: 3658 NT; Gene: 9588-13245; cDNA: 753-4535; Protein 247-1209.

The bold **T** marks the site of translation termination in *Fam83h*^{TR}.

CTTCATGTGGTCCCTTCGAGAAAATCCACCGCAGCCTGGCTCATGTGTTCCAGGGAGAGTGTGGTCTCCAGC
 F M W S F E K I H R S L A H V F Q G E L V S S
 TTCGACGAAGAGTTCGCGATCCTCTTCGCACAGTCCGAGCCACTGGTGCCCTCAGCCGGGGCGCTAGCC
 F D E E F R I L F A Q S E P L V P S A G A L A
 CGCATGGATGCCTAT**T**GCGCTAGCTCCATACTCTGGGGCTGGGCCCCTGGTGGGCGTCCCCGGGGTTGGA
 R M D A Y A L A P Y S G A G P L V G V P G V G
 GCACCAACACCTTT**TTTCTTCCCTAAACGGGC**GCACCTCCTATTTCCACCGCCAGGGAAGAAGGCCTG
 A P T P F S F P K R A H L L F P P P R E E G L
 GGCTTCCCCTCTTTCCTAGACCCTGACCGCCACTTCTGTGGCTTTCCGCCGAGAGGAGCTGCAGAGA
 G F P S F L D P D R H F L S A F R R E E L Q R
 ATGCCTGGGGGTGCTTTGGAGCCTCACACAGGGCTCCGGCCACTGGCGCGCCCAACTGAGGCTGGGCCG
 M P G G A L E P H T G L R P L A R P T E A G P
 TTCGGAGAGCTCGCGGGCCCCCGGGCTTCTTCCAGTCAAGGCACCTGGAAATGGATGCCTTCAAGCGG
 F G E L A G P R G F F Q S R H L E M D A F K R
 CATAGCTACGCAACACCCGATGGAGCTGGAGCAGTGGAGAACTTTGCAGCGGCACGGCAGGTGTCACGA
 H S Y A T P D G A G A V E N F A A A R Q V S R
 CAAACATTCCTCAGTCACGGTGGATGACTTCCGTTTCCAGACCAGCCACTTCCAACGGGACCAGCTCTAT
 Q T F L S H G D D F R F Q T S H F Q R D Q L Y

CAGCAGCATTACCAGTGGGACCCACAGTTTGTCTCTGCGCGCCACAGGGCCTCTTCGAGAAGCTTCGT
Q Q H Y Q W D P Q F A P A R P Q G L F E K L R
GCAGGCCGACCTGGCTTTGCGGACCCTGATGACTTTGCCTTAGGTGCTGGTCACCGCTTCCCAGAACTC
A G R P G F A D P D D F A L G A G H R F P E L
GGTGCTGATGTGCACCAACGGCTGGAATACGTGCCATCCAGCGCATCTCGGGAGGTACGCCACGGCTCG
G A D V H Q R L E Y V P S S A S R E V R H G S
GATCCGGCCTTTGGACCCAGCCCCGTGGTCTAGAGCCCAGTGGAGCCTCGCGTCCCAATCTGGGCCAG
D P A F G P S P R G L E P S G A S R P N L G Q
CGTTTTCCATGCCAAGCAACCTTGAGACAAGGCCTGGACACCGCTTCGGAGGCAGAACCTGAGCGCAGG
R F P C Q A T L R Q G L D T A S E A E P E R R
GGCGGACCCGAGGGCCGGGCGGGCTGCGTCACTGGCGCCTTGCCTCCTACCTGAGCGGCTGCCACGGT
G G P E G R A G L R H W R L A S Y L S G C H G
GACGGTGGGGAGGAGGGTCTACCAATGGAGGCTGAGGCTTGTGAAGACGAGGTGCTGGCTCCCGGAGGC
D G G E E G L P M E A E A C E D E V L A P G G
CGGGACCTGCTCCCCTCCGCCTTCCGCACTCCTGCAGCCTTCCCAGCTAAGGGACCAAAGCCGGGCTCA
R D L L P S A F R T P A A F P A K G P K P G S
GGAAGCGGTGGTGGTGACAGCTCCGAGCGAGAGGGCCAGAAGAGACAAGCCTGGCTAAGCAGGACTCC
G S G G G D S S E R E G P E E T S L A K Q D S
TTCCGCTCTCGCTTGAACCCGCTCATCCAGCGCAGCTCCAGGTTGCGCTCATCACTCATCTTTGCGTCC
F R S R L N P L I Q R S S R L R S S L I F A S
CAGGCTGAGGGTGTGTTGGGACCGCAGCAGCCACCCTGAAAAAGTACAGCTGATGCACAAAGAACAA
Q A E G A V G T A A A T T E K V Q L M H K E Q
ACAGTCAGTGAAACTCTGGGTCCCAGCGGAGAGGCTGTTTCGTTCCAGCGCCTCGGCCAAAGTGGCGGAG
T V S E T L G P S G E A V R S S A S A K V A E
CTCCTGGAGAAATACAAGGGCCCTGCCCGGACCCCTGGCGGTGCAGGAGGTGCCGTCACCTCCTCCAGC
L L E K Y K G P A R D P G G A G G A V T S S S
CACAGCAAGGCTGTAGTGTCCCAGGCCTGGCGGGAGGAGGTGGTAGCACCAGGAGGAGCGGGAAGTAA
H S K A V V S Q A W R E E V V A P G G A G T E
CGCCGCAGCCTTGAGAGTTGCTTGCTTGACCTGCGCGATTCTTTGCCAGCAGTTGCACCAGGAGGCA
R R S L E S C L L D L R D S F A Q Q L H Q E A
GAGCGACACCCAGGAGCCGCTTCGCTCACTGCTGCGCAACTGCTCGACACCCTGGGCGGCACTGACCGC
E R H P G A A S L T A A Q L L D T L G G T D R
CTGCCATCACGCTTCTCCTCCTCCGCCAGGGCCGCTCCTTGTCTCCACAAGGTCGAGATAGCCCTCCGCCA
L P S R F L S A Q G R S L S P Q G R D S P P P
GAAGGGCTTGGGACACACCAGCTGCCTTATTCTGAGCCAAAGGGAAACCCACCCAGCTTACCCTGAG
E G L G T H Q L P Y S E P K G N P T P A Y P E

CGCAAGGGGAGCCCTACCCCAGCTTACCCTGAGCGCAAGGGGAGCCCTACCCCAGCTTACCCTGAGCGC
 R K G S P T P A Y P E R K G S P T P A Y P E R
 AAGGGGAGCCCTACCCCAGCTTACCCTGAGCGCAAGGGGAGTCCTACCCAAGCCTACCCTGAGCGCAAG
 K G S P T P A Y P E R K G S P T Q A Y P E R K
 GGGAGCCCCACGTCTGGATTTCCCAATCGGAGGGGCAGCCCAACCACAGGATTGATGGAGCAGAAGGGA
 G S P T S G F P N R R G S P T T G L M E Q K G
 AGTCCCACTTCAACCTACCCAGACCGCAGGGGCAGTCCGGTGCCCCAGTGCCTGAGCGCAGGGGTAGT
 S P T S T Y P D R R G S P V P P V P E R R G S
 CCAGTACCCCCTGTGCCCGAGCGCAGAGGCAGTCTCACTTTCGCTGGGGAGTCTTCGAAGACTGGGCCT
 P V P P V P E R R G S L T F A G E S S K T G P
 ACAGAGGAGGTGTCTAGTGGCCCCATGGAAGTCCTGCGAAAGGGTTCTCTCCGCCTCAGGCAGCTGCTG
 T E E V S S G P M E V L R K G S L R L R Q L L
 AGCCCCAAGAATGAGAGGCGTGGGGAGGATGAGGGCAGCTTCCCAACTCCGCAGGAAAATGGGCAGCCC
 S P K N E R R G E D E G S F P T P Q E N G Q P
 GAGAGCCCCCGGCGGCCCTCGCTGAGTCGGGGTGACAGCACAGAGGCTGCTGCAGAGGAGAGAGGCTCG
 E S P R R P S L S R G D S T E A A A E E R G S
 AGGGTCCGCCTAGCTTCAGCTACAGCCAATGCTCTGTACAGCAGCAATCTGCGAGATGACACTAAGGCC
 R V R L A S A T A N A L Y S S N L R D D T K A
 ATTCTGGAGCAAATTAGTGCCCACGGCCAGAAGCACCGCGGGTCCCTGCTCCAGGTCCAGCCCACAGC
 I L E Q I S A H G Q K H R G V P A P G P A H S
 AGTCCTGACGTAGGTGTCCTCAACAACCTGCAGGAGACTTGGCCCCAGACATGTCCGACAAGGACAAATGT
 S P D V G R P T T A G D L A P D M S D K D K C
 TCAGCTATCTTCCGCTCAGACAGCCTAGGGACACAAGGCCGGCTCAGCCGCACCCTGCCTGGCAGTGCA
 S A I F R S D S L G T Q G R L S R T L P G S A
 GAGGAGCGAGACCGGCTCCTTCGCCGCATGGAGAGCATGCGCAAAGAGAAGCGTGTCTACAGTCGCTTC
 E E R D R L L R R M E S M R K E K R V Y S R F
 GAAGTCTTCTGCAAAAAGGACGAAGCTGGCAGTAGTGGGGCAGGAGACAACCTTGGCAGATGAGGACACC
 E V F C K K D E A G S S G A G D N L A D E D T
 AGGGACAGTAAAATGGGCAAATTTGTCCCCAAGATCCTGGGCACATTCAAAGCAAAAAATGA
 R D S K M G K F V P K I L G T F K S K K *

TCTCCTGGTCTCGGAGGCCAGGACTCTGCATCACTGCCATACTGAACCTACAATACCCATCTGGAGTGGTGGCTGTCAGGTTAGTGTCAAAGCAGTTTGGGACC
 TAGCTGACAACCACCTGAACTGAGCTCCACTTGAATTCGCCTCGCAGCCGCACTGCCTCTCACTCTTGGCTTTTCCATGAGGGGCTTAGCCCCCTCCACCTGGTG
 CCTTTCCTCATACCTACCCCCACCCAGCCTCCACCATCTCCTGGGCTCAGGTCTACTTTGGGTCAATATTTACCTCAGGGATCCATCTCTCTGCCTTATGC
 TCCAGTTTTTCAAGAACTCTATCACTTTGTGCCTCAGTTCCTCTTAAGGCCCTTTATCTCAGGGCTTTTCTCAGGGTTTCCATCTCATGGCTCCTGGTCCCTC
 TCCCTCTGGTGACAGCCTCCTCCCATCTCTCATCTCTATCTCTCGGGAACACACCTTGCCTGCTCCCACTCTGACTCCTCACTGCCTCATAGCACTTTCTT
 GGCATCTGCCCTTACTGTGTGGATGGCAGAGTTGGCCTCGGGCAGTTTAAGGGAAGAGAGGGATGCTGGCTAGGACAGACTGTTGAAAGTAATTGCTAAAC
 ACCTAGGGCTCCCCACCTCCCCGCTCAGGCCATGCCTGCCTCTCCAGCAAGTTTCTGGGTAGCACTTGAAGAGGACCAAGATTGGGAGCACTGGGCTTT
 GCAGTACAATAAAGGAATCGGGACAGACGTTCTGTGA

* * *

Fig. S3. Knockin intermediate following removal of selection genes by Flp-FRT recombination. Nothing was modified upstream of intron 3.

Exon 3: 165 NT; Gene: 9038-9202; cDNA: 588-752; Protein 150-204.

GTGGTAGCTGTGGTGATGGACATGTTTACCGACGTGGATCTGCTCAGTGAGGTGCTA
 V V A V V M D M F T D V D L L S E V L
 GAGGCCGCTGCAAGGCGAGTCCCGGTCTACATTCTGCTGGATGAAATGAACGCCAG
 E A A A R R V P V Y I L L D E M N A Q
 CACTTCCTAGACATGGCCGACAAGTGTGCGGTCAACCTGCATCATGTGGAC
 H F L D M A D K C R V N L H H V D

Intron 3: Formerly 69 NT, now 137 NT after insertion of 68 NT (gray highlight) containing a lox2272 site (underlined).

GTGAGTGACCAAGCCAGGGGAGTGGAGGGAAAATATCTTTTAAAGGCGCGCCATAACTTCGTATAAAGT
ATCCTATACGAAGTTATGGAGTGGAGGGAAAATATCTTTGGTGAAGACCTGACTCCCCTCTCTTCCAG

Exon 4: NT; Gene: 9272-9396 cDNA: 588-752 Protein 205-246.

TTCCTGCGTGTGCGCACAGTGGCAGGTCCTACTTACTACTGCCGCACTGGGAAGTCTTTCAAG
 F L R V R T V A G P T Y Y C R T G K S F K
 GGCCATCTAAAGGAGAAGTTCTTGTGTTGTGGACTGTGCCGTAGTGATGAGCGGCAGTTA **TAG**
 G H L K E K F L L V D C A V V M S G S Y S

Intron 4: 980 NT comprised of 191 bp of wild-type sequence plus 789 bp of insert (gray highlight). Green highlight is WT 5' genotyping primer annealing site. Magenta highlight is the *Fam83h*^{TR} genotyping primer annealing sites, which generate a 194 bp amplification product. The reverse complement of the Exon 4 coding sequence is in ***bold italic*** (font 12) and the reverse complement of coding sequence from Exon 5 is in ***bold italic*** (font 10).

GTATGCCAGTGGGTTGCCCTTAGCCCTGGCCCTTCCCACCTAGTCCTCCGTGGTCCCCTTCCCTTCCATAAAGCTGTCCTTA
 ATTTAAATTCGCCAGAAGTTCTTATACTTACTAGAGAATAGGAACTTCGGAATAGGAACTTCTTTAATTAAGGCGCGCCATCGA
 TATAACTTCGTATAATGTATGCTATACGAAGTTATCCATAGAGCCCACCGCATCCC**CAGCATGCCTGCTATTGTCTT**CCCAATC
 CTCCCCCTTGCTGTCTGCCCCACCCACCCCCAGAATAGAATGACACCTACTCAGACAATGCGATGCAATTTCTCATTTTA
 TTAGGAAAGGACAGTGGGAGTGGCACCTTCCAGGGTCAAGGAAGGCACGGGGGAGGGGCAAA**CAACAGATGGCTGGCAACTAGA**
 AGGCACAC**TAGGCATCCATGCGGGCTAGCGCCCCGGCTGAGGGCACCAGTGGCTCCGACTGTGCGAAGAGGATGCGGAACTCTT**
CGTCGAAGCTGGAGACCAACTCTCCCTGGAACACATGAGCCAGGCTGCGGTGGATTTTCTCGAAGGACCACATGAAGCTATAA
CTGCCGCTCATCACTACGGCACAGTCCACAAGCAAGAATTCTCCTTTAGATGGCCCTTGAAAGACTTCC
CAGTGCGGCAGTAGTAAGTAGGACCTGCCACTGTGCGCACACGCAGGAACTGGAAGAGAGGGGAGTCAGGTCTT
 CACCAAAGATATTTCCCTCCACTCCCCTGGATAACTTCGTATAGGATACTTTATACGAAGTTATAGTACTGCGGCCGATAAC
 TTCGTATAGCATAACATTATACGAAGTTATAGTACTTCTTCCATAAAGCTGTCTTGGCCCCAGCCCACCAGCCCAACCCCTTT
 CAGATCCCTGATTATCCACCTAGTGCCTTAGCCTCTGTGGCCCTATTTCTCTCCCTGACTCCTGCCATCCTGTCCACAG

Exon 5: Same as wild-type sequence (not shown) and contains a reverse primer for the WT genotyping amplification.

* * *

Fig. S4: Final *Fam83h*^{TR} Knockin Sequence.

Exon 3: 165 NT; same as wild-type *Fam83h*. There are no modifications upstream of intron 3.

GTGGTAGCTGTGGTGATGGACATGTTTACCGACGTGGATCTGCTCAGTGAGGTGCTA
 V V A V V M D M F T D V D L L S E V L
 GAGGCCGCTGCAAGGCGAGTCCCGGTCTACATTCTGCTGGATGAAATGAACGCCAG
 E A A A R R V P V Y I L L D E M N A Q
 CACTTCCTAGACATGGCCGACAAGTGTGCGCTCAACCTGCATCATGTGGAC
 H F L D M A D K C R V N L H H V D

New Intron 3: Contains the 5' 40 NT from wild-type intron 3 followed by 46 bp insert (gray highlight) containing *Lox2272* site (underlined), then 3' 89 NT from Intron 3 (italicized sequences are duplicates).

GTGAGTGACCAAGCCAGGGGAGTGGAGGGAAAATATCTTTTAAAGGCGCGCCATAACTTCGTATAAAAGTAT
CCTATACGAAGTTATCCAGGGGAGTGGAGGGAAAATATCTTTGGTGAAGACCTGACTCCCCTCTCTCCAG

Exon 4/5^{TR}: Exon 4 and the beginning of exon 5 are fused (gray highlight). Translation termination occurs at the modified codon: g.9741T>G; c.906T>G; p.Tyr297*. After the translation stop codon is an inserted sequence containing a polyadenylation signal (underlined), and an inserted loxP site, and is followed by the 3' 86 nucleotides of Intron 4.

TTCTTGCCTGTGCGCACAGTGGCAGGTCCTACTTACTACTGCCGACTGGGAAGTCTTTCAAGGGCCATCTA
 F L R V R T V A G P T Y Y C R T G K S F K G H L
 AAGGAGAAGTTCTTGCTTGTGGACTGTGCCGTAGTGATGAGCGGCAGTTATAGCTTTCATGTGGTCCTTCGAG
 K E K F L L V D C A V V M S G S Y S F M W S F E
 AAAATCCACCGCAGCCTGGCTCATGTGTTCCAGGGAGAGTTGGTCTCCAGCTTCGACGAAGAGTTCCGCATC
 K I H R S L A H V F Q G E L V S S F D E E F R I
 CTCTTCGCACAGTCGGAGCCACTGGTGCCCTCAGCCGGGGCGCTAGCCCGCATGGATGCCTAG
 L F A Q S E P L V P S A G A L A R M D A *

Unique downstream untranslated sequence (394 bp) with polyadenylation signal (underlined) prior to old Exon 5. Magenta highlight is the *Fam83h*^{TR} genotyping primer annealing sites, which generate a 194 bp amplification product. The segment between Exon 3 and old Exon 5 was confirmed by DNA sequencing.

TGTGCCTTCTAGTGGCAGCCATCTGTTGTTTGCCCTCCCGTGCCTTCTTGACCTGGAAGGTGCCACTCCACTGTCTTTCTTAATAAAATGA
 GGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGCAAGACAATAGCAGGCA
TGCTGGGATGCGGTGGGCTCTATGGATAACTTCGTATAGCATACATTATACGAAGTTATAGTACTTCTCCATAAAGCTGCCTTGCCCCAGCCCA
 CCAGCCCAACCCCTTTGAGATCCCTGATTATCCACCTAGTGCCTTTAGCCTCTGTGGCCCTATTTCTCTCCCTGACTCCTGCATCTGTCCACAG

Wild-Type Exon 5 is still intact, but is now downstream of the in-frame translation termination codon and polyadenylation signal from the flipped 5' end of Exon 5 with the premature termination codon. The 3' PCR genotyping primer anneals (green highlight) to *Fam83h*^{TR}, but the 5' primer does not.

CTTCATGTGGTCCTTCGAGAAAATCCACCGCAGCCTGGCTCATGTGTTCCAGGGAGAGTTGGTCTCCAGC
 F M W S F E K I H R S L A H V F Q G E L V S S
 TTCGACGAAGAGTTCCGCATCCTCTTCGCACAGTCGGAGCCACTGGTGCCCTCAGCCGGGGCGCTAGCC
 F D E E F R I L F A Q S E P L V P S A G A L A
 CGCATGGATGCCTATGCGCTAGCTCCATACTCTGGGGCTGGGCCCCTGGTGGGCGTCCCCGGGGTTGGA
 R M D A Y A L A P Y S G A G P L V G V P G V G
 GCACCAACACCTTTTCTTTCCCTAAACGGGGCACCCTCCTATTCCCACCGCCCAGGGAAGAAGGCCTG
 A P T P F S F P K R A H L L F P P P R E E G L

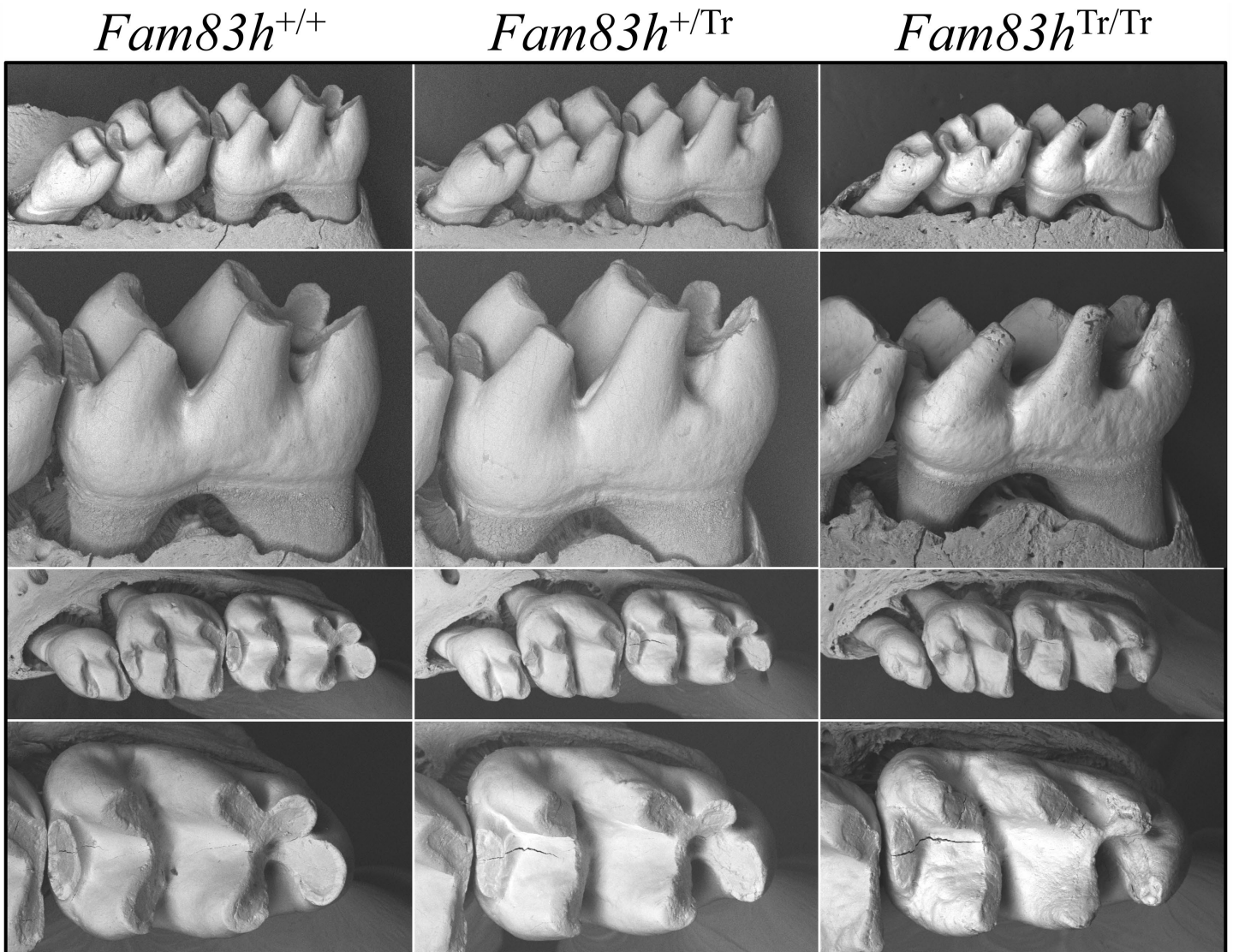


Fig. S5. bSEMs of mandibular molars at 7-weeks. The *Fam83h*^{*Tr/Tr*} molars appeared to be somewhat smaller, had a mildly rougher surface, but did not appear to undergo the rapid attrition characteristic of the human ADHCAI phenotype. (2WT; 6Het; 8Null)

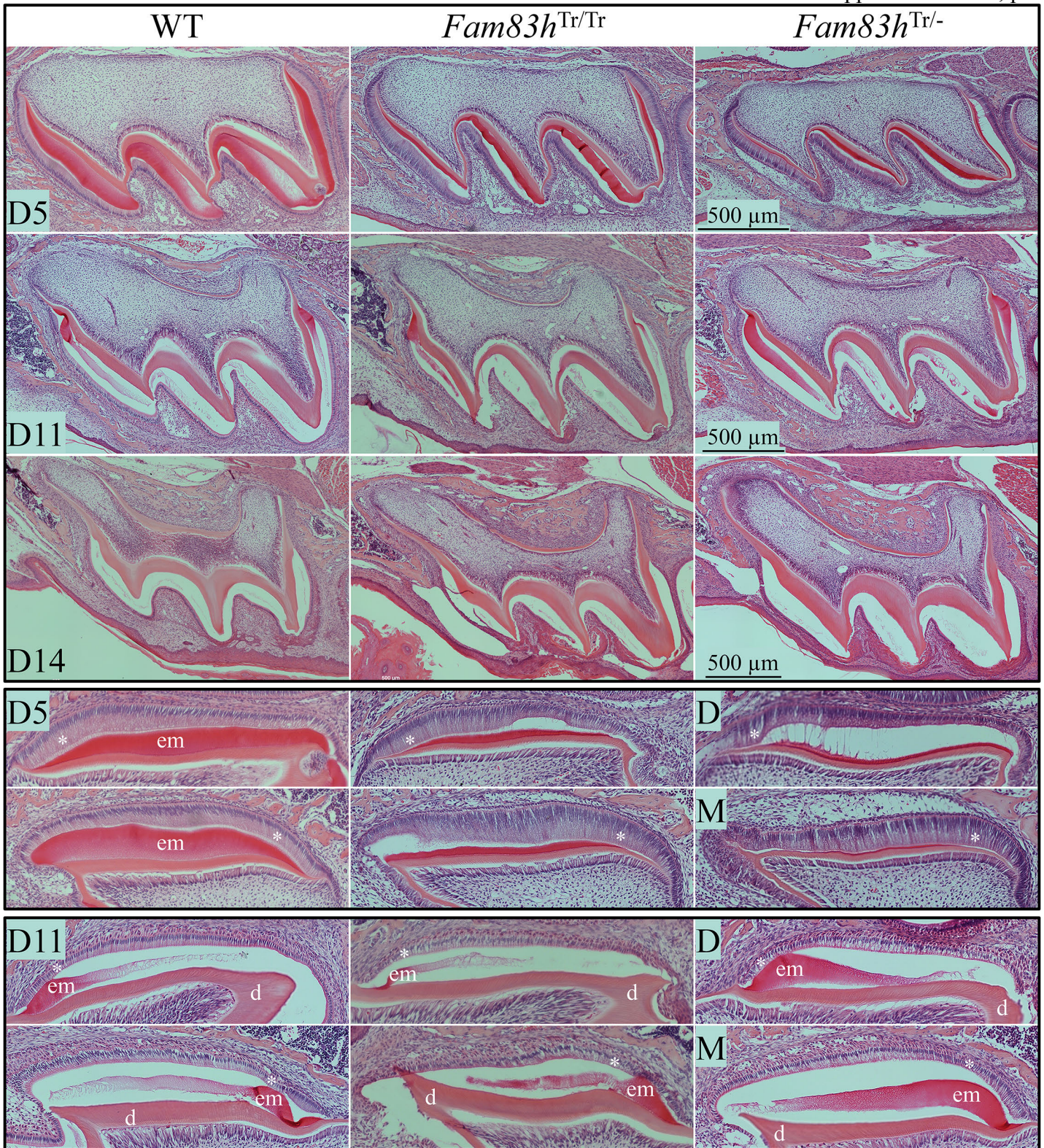


Fig. S6. Histology of *Fam83h^{+/+}* (WT), *Fam83h^{Tr/Tr}*, and *Fam83h^{Tr/-}* Molars at Days 5, 11, and 14. **Key:** d, dentin; em, enamel matrix; *, ameloblasts. The *Fam83h^{Tr/-}* genotype refers to a mouse that contains one *Fam83h* allele that expressed the truncated protein and one *Fam83h* null allele. This mouse expresses half the amount of truncated FAM83H protein as the *Fam83h^{Tr/Tr}* mouse.

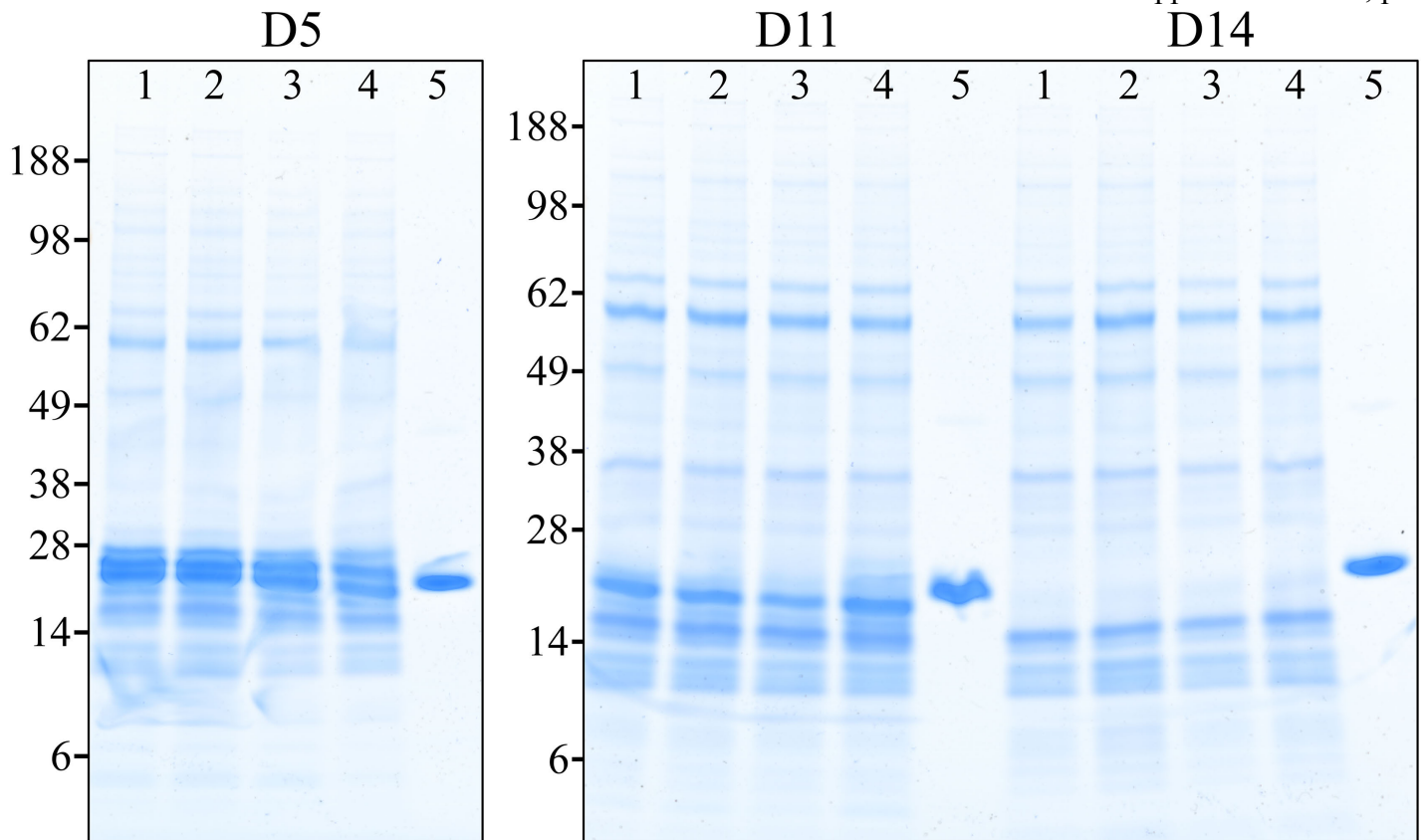


Fig. S7. SDS-PAGE of enamel proteins at D5, D11, and D14. **Lane 1:** *Fam83h^{+/+}*; **Lane 2:** *Fam83h^{+/Tr}*; **Lane 3:** *Fam83h^{Tr/Tr}*; **Lane 4:** *Fam83h^{-/Tr}*; **Lane 5:** rM179. About 15% of the extracted proteins from a single molar were run down each lane and visualized by Coomassie Brilliant Blue staining. D5 assays secretory stage enamel; D11 assays maturation stage enamel; and D14 is immediately prior to eruption.