

A*Tgm3^{Btlr}*

Exon 3

AAAGCCAACAGTGGCAATAATCTGGCCATTGCTATTGCCAGTCCTGTCAGTGCTCCCATCGGATTGTACACACTG 130024502
 -K--A--N--S--G--N--N--L--A--I--A--I--A--S--P--V--S--A--P--I--G--L--Y--T--L- 113

AGTGTTGAGATCTCCCTCCAGGGGCCAGGCGCTCCCTCTGAAACTTGGCACGTTTATAATGCTCTTCAACCCGTGG 130024577
 -S--V--E--I--S--S--R--G--R--A--S--S--L--K--L--G--T--F--I--M--L--F--N--P--W- 138

Intron 3-4

a

TTGCAAGgtaggtctttaagcacggcattccccacacatccctgatagaacaatgagtgtgaaaaagagagatg 130024652
 -L--Q-- 140

[gctaacctgtgccccgtatgggcttagatctgggtgg](#) 130024689

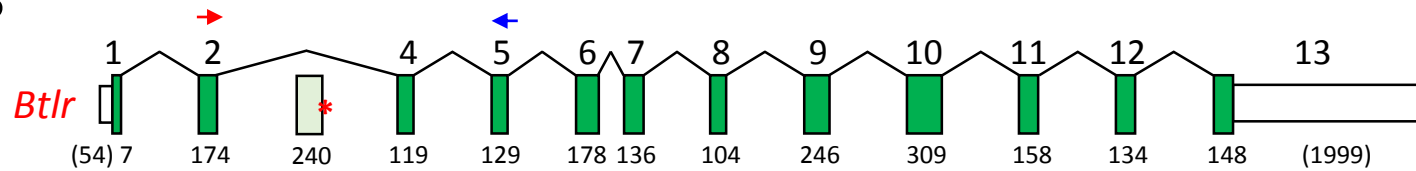
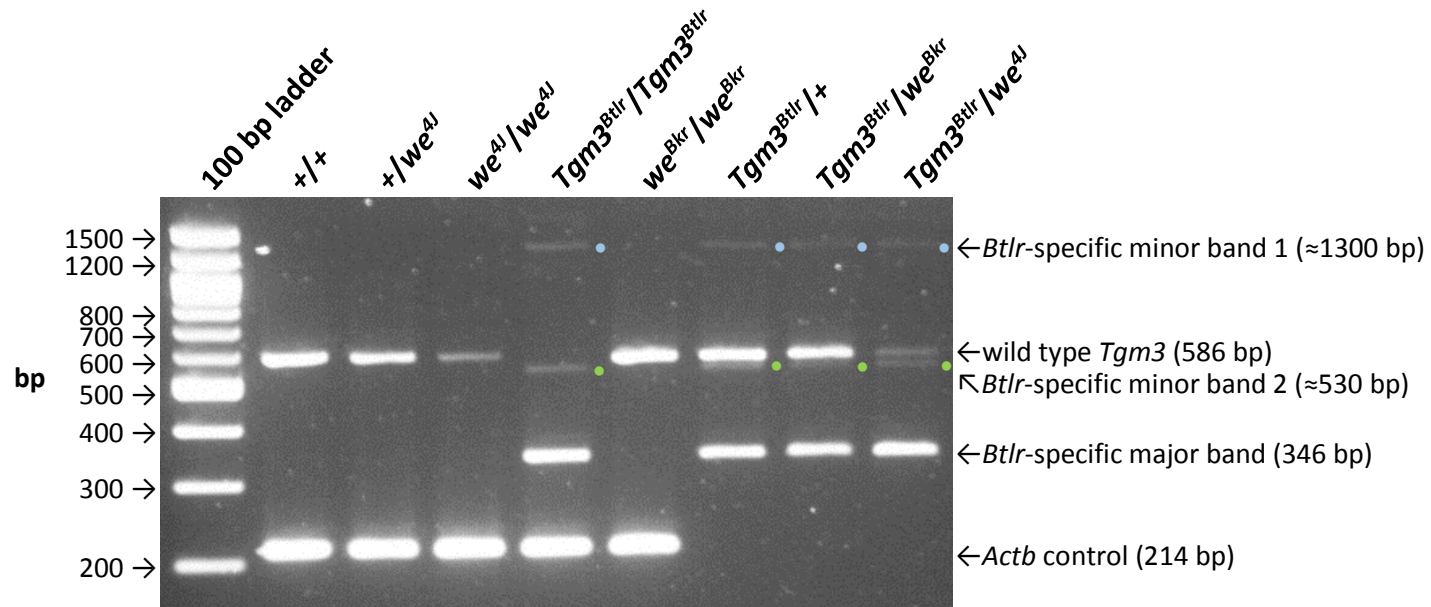
B**C**

Figure S5. Analysis of *Tgm3* mRNA from mutant skin shows that the *Tgm3^{Btlr}* transcript skips Exon 3, and that the *we^{4J}* transcript is unstable. **(A)** The *Tgm3^{Btlr}* mutation, an EtNU-induced G to A transition made by Beutler and colleagues (Won, Moresco & Beutler 2013, ref. 7) at position 2:130024585, is shown in red. Sequences in Exon 3 are shown in black, upper-case letters; sequences from Intron 3-4 are in blue, lower-case letters. Alternate codons are highlighted in yellow, and single-letter amino acid abbreviations are shown below each codon. The primer sequences used for sequence verification of the *Tgm3^{Btlr}* defect in our lab are underlined. **(B)** The *Tgm3^{Btlr}* mutation destroys the splice donor at the 3' end of Exon 3 (indicated by a red asterisk), and is predicted by Won, Moresco & Beutler to cause the skipping of Exon 3 in the processed transcript, as diagrammed here. Poly-A⁺ RNA from skin was copied into cDNA and amplified using forward and reverse primers that anneal with Exon 2 (5' GGGCGCATCACACAGACAAGTTCTCCAG 3', depicted by the red arrow) and Exon 5 (5' GGGTCATTTCTGCGAGCCACATCAGTCA 3', depicted by the blue arrow) of *Tgm3*, respectively. **(C)** While cDNA templates from wild type, *we^{4J}*, and *we^{Bkr}* homozygotes yielded a 586 bp amplicon as expected for the normal splicing of Exons 2, 3, 4 and 5; templates from *Tgm3^{Btlr}* homozygotes instead yielded a prominent amplicon 240 bp shorter, consistent with the skipping of Exon 3. The identity of these 586 and 346 bp amplicons was confirmed by sequence analysis. Two low-intensity bands were also amplified from *Tgm3^{Btlr}* homozygotes or heterozygotes, highlighted here with blue dots (for "minor band 1") or green dots (for "minor band 2"). We have not determined the DNA sequence of these two minor bands, but it may be notable that band 1's length is consistent with alternative splicing of Exons 2-5 such that Intron 3-4 (673 bp) is retained. The lower intensity of the 586 bp product in *we^{4J}* homozygotes and *we^{4J}/+* heterozygotes suggests that the *we^{4J}* transcript is unstable compared with wild type, *we^{Bkr}* and *Tgm3^{Btlr}* transcripts. [Primers that anneal within Exon 4 of the mouse β actin (*Actb*) gene (5' CCCAGCCATGTACGTAGCCATCCA 3' and 5' GAAGCTGTAGCCACGCTCGGTTCAG 3') were used together with *Tgm3* primers in the first five samples to provide an internal loading control.] This observation is supported by comparison of band intensities in *Tgm3^{Btlr}/+*, *Tgm3^{Btlr}/we^{Bkr}*, and *Tgm3^{Btlr}/we^{4J}* heterozygotes, where the *Tgm3^{Btlr}*-specific major band serves as an internal control for stable *Tgm3* transcript levels. These data suggest that the *we^{4J}* transcript is likely the target of nonsense-mediated decay (Chang, Imam & Wilkinson 2007, ref. 15).