

. . . **TCTGAGACTCTTCTTGGACCGAGACCTCATGACACTTCTTATTTCAGAA****GT**GAGATAT  
 ATATAAACACCTCAATTATAACCTTGGCACTACATTGACATGTGTCTCTCAATTCTGTAG  
 TTTCAAACCAGTAAATAGTTTTAATGCGTATCTGGTAATGGTTAAACAGCAGCATTGTTT  
 TCTGTTGGATATCAGAAGGATATGATATCAGAAAATTGCAGGGGAGAGAGCAAATAAGT  
 TAGTGGGGATTCTCCTTGAGCCCTTTGCTCCCCAGAGCCCTGGAAATTGCAGTTGTCTTG  
 ACATAGCCTA**GGTACC**TTTAAAGATTTTTAAAGATATATTTGTACTTGTCACTTAACGGC  
 TGATTAACACTCAGGGAAGCAATGATTATTATTCATTTGTACTATAAATATGGATTGTTG  
 CTGCCCTTCTTTTCTTTTCATCCTTCTTTCCCTTCTTCCCTTCTCCTTTTTTCTTTCAA  
 ATAGATTTCTATTATGAAAAATTAGTACATGCATGGGATAAAAACTACAAAATAGTATAAA  
 AGTAATTACAGTAAAAGGCAAGTGCCCTTTCACCTTGCACTCCAGTTATCCACCTGGA  
 GGAAAGCGCTGTTACATCCAGAAAATAGTCCGTGCATATCCAAGCATAACACATACATAC  
 ACACACACGCGCACATACATACATACACATGCACA**CCCCCCCCCCCC**ACTTCGTTAC  
**ACAAAATGTGAAGGTA**CTTATGTACTATATTTGAACTTTCC**TTTTTTTTTTTTTTTTTTTT**  
**TTTT**ACATACGTATACATGTGCCATGTTGGTGTGCTGCACCCATTAACTCGTCATTTACA  
 TTAGGTATATCTCCTAATGCTATCCCTCCCCTCTCCCCCACTCCACGATAGGCCCTGGT  
 GTGTGATGTTCCCCTTCCCTGTGTCCAAGTGTCTCATTGTTCAATTCCCACCTATGAGTG  
 AGAACATGCGGTGTTGGTTTTTTGTCTTGTGATAGTTTGTGAGAAATGGTGGTTTCCA  
 GCTTCATCCATGTCCCTACAAAGGACATGAACTCATCCCTTTTTATGGCTGCATTAGTAT  
 TCCACATTTTCTTAATCCAGTCTATCATTGATGGACATTTGGGTGGTTCCAAGTCTTTG  
 CTGTTGTGAATGTGTAACCTAACACTGTATCTTGTTCATAACGTTATATCGATATATA  
 GGTCTGGCTCATTTTTTTGATTACTATTTAGAACTGCATCATATGGCTGTAACCTTAATTTG  
 TTTAATCCATGCTTCATGGTGGACAATTAGTTTGTTTACAGTGTTCAGTGAACATTCTT  
 GTGCATACATCTTTGCTCCTATATGCAAGTGTATCTATAAGATTAATAGACTTTATTTTT  
 TAAATTAAGAGAAATGTCTACTTCATTTGTCTTATATCCTAGTTCTCGTTAGGTGTGGGA  
 TCAAAAATGTGTTACGCTAGTA**GCTAGC**GCACATCCAGGTTTCTCTGCATGGGTGTTGTGT  
 GTCCGAATCTTCAGGTGTGCTGCTGGAACAGCTTGTGGAGCACAGCTGGGTATTCCCTCA  
 GAGAGGTATAGCTTTAGCTCCTTATTCTACAACAAGAGAGCTGGCTATTGTCAATGACC  
 TCAAGCTTCTGTTTTAAAGGCTTAATCTTGTTAGGCTCTCTAAAATTTTGCTTTTTTTCT  
 TTTTTATTGAGT**AG**AAAACATTTCTCAGG**C**TGCTAAGCTGGAATAAAATCCACTTACCTG  
**TATGTTAAATGATTTAG**TAAGCTGGCACCATTTCATCGCCAAACGCCGAACCCCAAGATT  
**CAAGAGCAGTACCGCAGGATTGGAGCGGATCC**CCCATCAAGATATGGACTTCCAAGCAG  
**GGAGAGGGCATGGTGAAGCTGCTGGATGAATTGTCCCCAACACAGGT**ATGGTGTTCCTT  
 CATTAAACATGTAACCTAGATTAGTTCTTTTTCGAAGTAGTTATGAAATTCAAAAGAGTAGTAG  
 ATGCTAAATGCTTCATATGTACTGGCAACTCAATTTGTGGTGTTCCTACATAATACATGG  
 TCACTTAATTTAGGTCTCCAGGCGTTATCCCTTGACCTTTAGCCTTTGCTATGACTTCTT  
 ACATTACAAGTAAAAAAAAGCTAAGGATTTGGGACATG . . .

**Figure S1. Sequence of clone C22 insert.** Primers used for PCR amplification introduced restriction sites for XbaI and SalI immediately up- and downstream of the shown sequence, respectively. The part of exon 3 and the entire exon 4 (light blue highlighting) are present. The extension of exon 4 caused by aberrant splicing is highlighted in light green. The c.315-48C polymorphism is highlighted in red and underlined. Restriction sites for BspH1, KpnI, NheI and BamHI are shown in magenta. The GT and AG dinucleotides at the 5' and 3' splice sites are shown in bold, underlined letters. Note that the oligo-C and -T stretches (yellow highlighting) are 13 and 23 nucleotides in length compared to 11 and 24, respectively, in the reference sequence (NT\_025028).

**GGCGCGCC** GAGCAATTGAGGTCCGTTGATGCCAACATAGGGCTGGCCTAGAGACCAGAGT  
 CAGTGAAGAAGTGGAGGCCCTCTGTGCCTGAGGAGACAGCCCTGTGAGGGCAGCATCGGGC  
 CTTCCGGTTCTTATCAGCTGAAGTAATTTCTGCCCGGAGCAAGTGAAAGTGTGGCAGTGA  
 ACTGTTTTATCTTGTGTAG**GAAGCCAAAAACGGGCATATTGATGTTAAACATGGGAGGCC**  
**CGAAACCCTTGGAGAAGTTCAAGACTTTCTTCAGAGGCTCTTCCTGGACCGAGAC****TCATG**  
**ACACTTCCTATT****CAGAA****GT**GAGATATATATAAACACCTCGATTATAACCTTGGCACTACAT  
 TGACATGTGTCTCTCAATTCTGTAGTTTCAAACCAGTAAATAGTTTTAATGCGTATCTGGT  
 AATGGTTAAACAGCAGCATTGTTTTCTGTTGGATATCAGAAGGATATGATATCAGAAAATT  
 GCAGGGGGAGAGAGCAAATAAGTTAGTGGGGATTCTCCTTGAGCCCTTTGCTCCCCAGAGC  
 CCTGGAAATTGCAGTTGTCTTGACATAGCCTA**GGTACC**TTTAAAGATTTTTAAAGATATAT  
 TTGTACTTGTCACTTAACGGCTGATTAACACTCAGGGAAAGCAATGATTATTATTCATTTGT  
 ACTATAAATATGGATTGTTGCTGCCCTTCTTTTCCTTTTCATCCTTCTTTCCCTTCTTCCTT  
 CTCTCCTTTTTCTTTCAAATAGATTTCTATTATGAAAAATTAGTACATGCATGGGATAAA  
 AACTACAAATAGTATAAAAAGTAATTACAGTAAAAGGCAAGTGTCCCTTTCACCTTGCACCTC  
 CCAGTTATCCACCTGGAGGAAAGCGCTGTTACATCCAGAAATAGTCCGTGCATATCCAAGC  
 ATACACATACATACACACACACACGCGCACATACATACATACACACATGCACA**CCCCCCCC**  
**CCCCC**ACTTCGTTACACAAATGTGAAGTACTTATGTACTATATTTGAAACTTTCCTTTTTT  
**TTTTTTTTTTTTTTTTTTTT**ACATACGTATACATGTGCCATGTTGGTGTGCTGCACCCATTAA  
 CTCGTCATTTACATTAGGTATATCTCCTAATGCTATCCCTCCCTCTCCCCCACTCCACG  
 ATAGGCCCTGGTGTGTGATGTTCCCTTCTGTGTCCAAGTGTCTCATTGTTCAATTCCC  
 ACCTATGAGTGAGAACATGCGGTGTTGGTTTTTGTCTTGTGATAGTTTGTCTGAGAATG  
 GTGGTTCCAGCTTCATCCATGTCCCTACAAAGGACATGAACTCATCCCTTTTTATGGCTG  
 CATTAGTATTCCACATTTTCTTAATCCAGTCTATCATTGATGGACATTTGGGTTGGTTCCA  
 AGTCTTTGCTGTTGTGAATGTGTAACCTTAACACTGTATCTTGT'TTCATAACGTTATATCG  
 ATATATAGGTCTGGCTCATTTTTTGATTACTATTTAGAACTGCATCATATGGCTGTAACCTT  
 AATTTGTTAATCCATGCTTCATGGTGGACAATTAGTTTGT'TTACAGTGTGTCAGTGAACA  
 TTCTTGTGCATACATCTTTGCTCCTATATGCAAGTGTATCTATAAGATTAATAGACTTTAT  
 TTTTTAAATTAAGAGAAATGTCTACTTCATTTGTCTTATATCCTAGTTCTCGTTAGGTGTG  
 GGATCAAATGTGTTACGCTAGTA**GCTAGC**GCACATCCAGGTTTCTCTGCATGGGTGTTGT  
 GTGTCTGAATCTTCAGGTGTGCTGCTGGAACAGCTTGTGGAGCACAGCTGGGTATTCTC  
 AGAGAGGGTATAGCTTTAGCTCCTTATTCTACAACAAGAGAGCTGGCTATTGTCAATGACC  
 TCAAGCTTCTGTTTTAAAGGCTTAATCTTGTTAGGCTCTCTAAAATTTTTGCTTTTTTTCTT  
 TTTTATTGAGT**AG**AAAACATTTCTCAGG**C**TGCTAAGCTGGAATAAAATCCACTTACCTGTA  
**TGTTAAATGATTT****AG****TAAGCTGGCACCATT****CATCGCCAAACGCCGAACCCCCAAGATTCAA**  
**GAGCAGTACCGCAGGATTGGAGGCGGAT****CC**CCATCAAGATGTGGACTTCCAAGCAAGGAG  
**AAGGCATGGTGAAGCTGCTGGATGAGTTATCCCCTGCCACAG****GT**GTGCTCTTCTTCTTAGT  
 GCTGGGCTAGGCTCGTTACAGTAGGACTCAGGGTCAGGGGTGGACTGGGGTGGGCTGGGGG  
 CACCCCTGTAATCCAGCACTCGGGGTGTGGGGGGCAAAGGCAAGAAGATTC**TTAATTAA**

F3

F2

F1

**Figure S2. Sequence of chimeric exon3-4 fragment used for the targeting construct.** Fragments F1 and F3 were generated by DNA synthesis which introduced restriction sites for *Ascl* at the beginning of F3 and for *Pacl* at the end of F1 (Italic letters, underlined). Fragment F2 was generated by PCR from the human genomic clone C22, The three fragments were joined via restriction sites for *KpnI* and *NheI* (underlined). Exon parts from mouse are distinguished from human parts by a darker blue highlighting. The extension of exon 4 caused by aberrant splicing is shaded in light green. The c.315-48C polymorphism is highlighted in red and underlined.

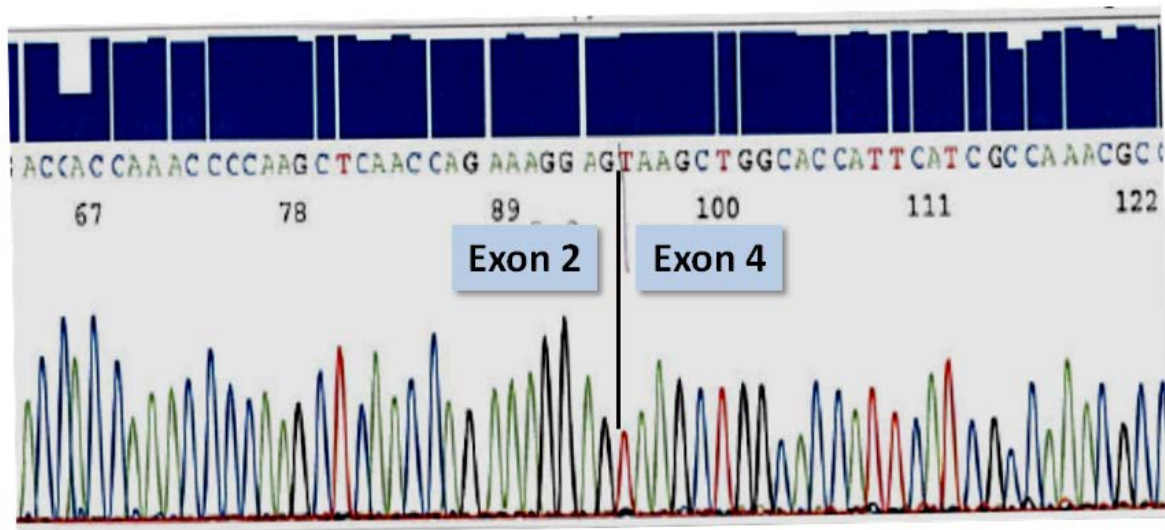


Figure S3. Sequencing of cDNA from clone II-1C1 showing skipping of exon 3 on the humanized FECH allele. Only the relevant exon-exon junction is shown.

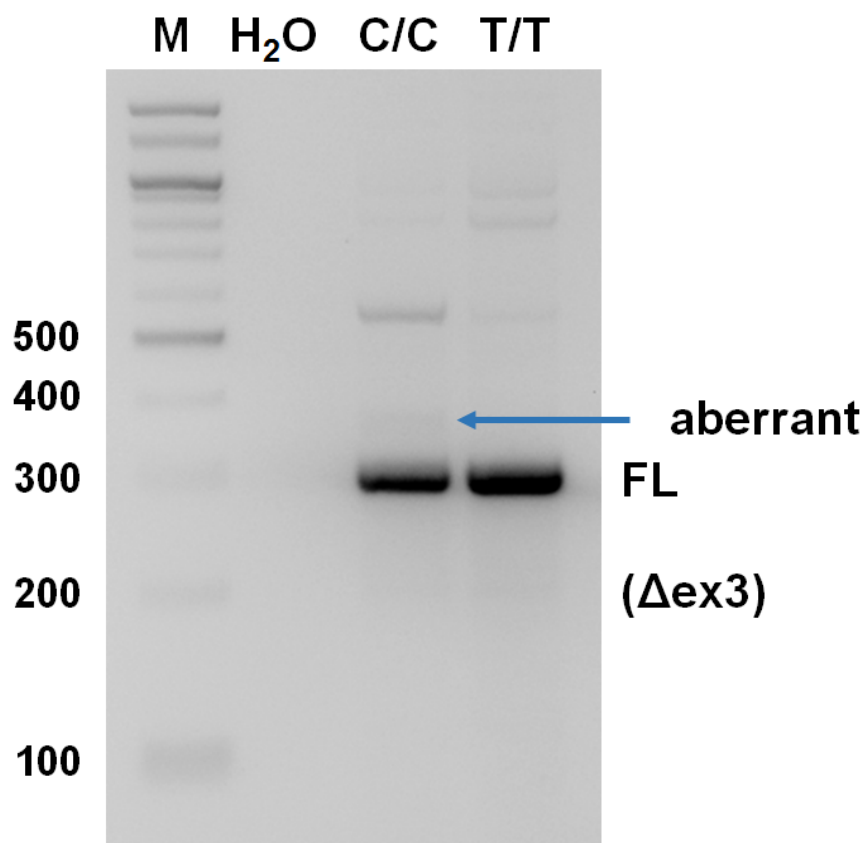


Figure S4. Use of the aberrant 3' splice site and lack of exon 3 skipping in immortalized human lymphoblast cells homozygous for the c.315-48C and c.315-48T FECH alleles. Total RNA from the lymphoblast cells (Barman-Aksözen et al., 2013) was subjected to RT-PCR with primers binding in exons 2 and 4, and the products were analyzed on a 1.5% agarose/TBE gel. The aberrant splicing product is faintly visible in C/C cells, but its quantity is reduced by nonsense-mediated mRNA decay.

```

Emi exon 3      TTCAAGAAAATGTCTGACTGACGTGGCCACAGACCGTTGTGAGGGAGGCGTTTTTCCTCT
Emi ex3 fw     -----CT
Emi ex3 re     -----

Emi exon 3      TGGTGTGCTTCTTTCCAAATGACTCTGGTTGTTTTTCATGTTGTCAAAAATTAACCAA
Emi ex3 fw     TGGTGTGCTTCTTTCCA-ATGACTCTGGTTGTTTTTCATGTTGTCAAAAATTAACCAA
Emi ex3 re     -----TCAAAAATTAACCAA
                    *****

Emi exon 3      ACAGGTTAGCACGTAAGTGTACTCTTCCATGGTATGTTATATTGCCAGCCAGACTGTGGA
Emi ex3 fw     ACAGGTTAGCACGTAAGTGTACTCTTCCATGGTATGTTATATTGCCAGCCAGACTGTGGA
Emi ex3 re     ACAGGTTAGCACGTAAGKKTACTNNTCCATGGTATGTTAWWTTGCCAGCCAGACTGKGG
                    *****

Emi exon 3      GGCTAGGGATACTACTAAACACCCACAGGAGAGTCCTCTACAGTGAAGGGAAGGCAAACA
Emi ex3 fw     GGCTAGGGATACTACTAAACACCCACAGGAGAGTCCTCTACAGTGAAGGGAAGGCAAACA
Emi ex3 re     GGCTAGGGATACTACTAAACMCCCMCAGGAGAGTCCTYTACAGTGAAGGGAAGGCAAACA
                    *****

Emi exon 3      GAACCTATTTAGCTTAGGAATAATTTCCATGTGACCAAAGAGACGGGAACAGATGAAAG
Emi ex3 fw     GAACCTATTTAGCTTAGGAATAATTTCCATGTGACCAAAGAGACGGGAACAGATGAAAG
Emi ex3 re     GAACCTATTTAGCTTAGGAATAATTTCCATGTGACCAAAGAGACGGGAACAGATGAAAG
                    *****

Emi exon 3      AGCAAGCGATAGACTCGTCGAGGTGTTCAAATAAAAGGGGAACACTTCTCCGTGGCACAG
Emi ex3 fw     AGCAAGCGATAGACTCGTCGAGGTGTTCAAATAAAAGGGGAACACTTCTCCGTGGCACAG
Emi ex3 re     AGCAAGCGATAGACTCGTCGAGGTGTTCAAATAAAAGGGGAACACTTCTCCGTGGCACAG
                    *****

Emi exon 3      AGCTCACTCTGTGGTAGACTGTGCCGAGTATGTGAAGAATACAGGAAGTTCACCAAATAA
Emi ex3 fw     AGCTCACTCTGTGGTAGACTGTGCCGAGTATGTGAAGAATACAGGAAGTTCACCAAATAA
Emi ex3 re     AGCTCACTCTGTGGTAGACTGTGCCGAGTATGTGAAGAATACAGGAAGTTCACCAAATAA
                    *****

Emi exon 3      TGTTGAAGGTAAGATGTTAGGCTTTAATTTTAGCATAACGAAGCAACCTTAACCTCTGGAA
Emi ex3 fw     TGTTGAAGGTAAGATGTTAGGCTTTAATTTTAGCATAACGAAGCAACCTTAACCTCTGGAA
Emi ex3 re     TGTTGAAGGTAAGATGTTAGGCTTTAATTTTAGCATAACGAAGCAACCTTAACCTCTGGAA
                    *****

Emi exon 3      AGCCAGACCTGCGTACTTAAACGAAGCACTAGCACCTACAGGTCTTTTGGCCACTGAACA
Emi ex3 fw     AGCCAGACCTGCGTACTTAAACGAAGCACTAGYACCTACAGGTCTTTTGGCCACTGAACA
Emi ex3 re     AGCCAGACCTGCGTACTTAAACGAAGCACTAGCACCTACAGGTCTTTTGGCCACTGAACA
                    *****

Emi exon 3      ATTTTTGATTTCTAAAACCTTCTGTCTCTAAAGAATTGGATCTTTGTTCAAGATTGACAG
Emi ex3 fw     ATTTTTGATTTCTAAAACCTTCTGTCTCTAAAGAATTGGATCTTTGTTCAAGATTGACAG
Emi ex3 re     ATTTTTGATTTCTAAAACCTTCTGTCTCTAAAGAATTGGATCTTTGTTCAAGATTGACAG
                    *****

Emi exon 3      AAAAAAATAGATATCAGTACTGGCGCGCCGAGCAATTGAGGTCCGTTGATGCCAACATAG
Emi ex3 fw     AAAAAAATAGATATCAGTACTGGCGCGCCGAGCAATTGAGGTCCGTTGATGCCAACATAG
Emi ex3 re     AAAAAAATAGATATCAGTACTGGCGCGCCGAGCAATTGAGGTCCGTTGATGCCAACATAG
                    *****

Emi exon 3      GGCTGGCCTAGAGACCAGAGTCAGTGAAGAAGTGGAGGCCCTCTGTGCCTGAGGAGACAG
Emi ex3 fw     GGCTGGCCTAGAGACCAGAGTCAGTGAAGAAGTGGAGGCCCTCTGTGCCTGAGGAGACAG
Emi ex3 re     GGCTGGCCTAGAGACCAGAGTCAGTGAAGAAGTGGAGGCCCTCTGTGCCTGAGGAGACAG
                    *****

Emi exon 3      CCCTGTGAGGGCAGCATCGGGCCTTCGGGTTCTTATCAGCTGAAGTAATTTCTGCCCGG
Emi ex3 fw     CCCTGTGAGGGCAGCATN-GNCCTTCGGGTTCTTATCAGCTGAAGTAATTTCTGCCMCGG
Emi ex3 re     CCCTGTGAGGGCAGCATCGGGCCTTCGGGTTCTTATCAGCTGAAGTAATTTCTGCCCGG
                    *****

Emi exon 3      AGCAAGTAAAAGTGTGGCAGTGAAGTGTGTTTATCTTGTGTAGGAAGCCAAAAACGGGCAT
Emi ex3 fw     AGCAAGTAAAAGTGTGGCAGTGAAGTGTGTTTATCTTGTGTAGGAAGCCAAAAACGGGCAT
Emi ex3 re     AGCAAGTAAAAGTGTGGCAGTGAAGTGTGTTTATCTTGTGTAGGAAGCCAAAAACGGGCAT
                    *****
    
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Emi exon 3      ATTGATGTTAAACATGGGAGGCCCGAACCCCTTGGAGAAGTTCAAGACTTCTTCAGAG
Emi ex3 fw     ATTGATGTTAAACATGGSAGGCCCGAACCCCTTGGAGAAGTTCAAGACTTCTTCAGAG
Emi ex3 re     ATTGATGTTAAACATGGGAGGCCCGAACCCCTTGGAGAAGTTCAAGACTTCTTCAGAG
*****
Emi exon 3      GCTCTTCCTGGACCGAGACCTCATGACACTTCCTATTCAGAAGTGAGATATATAAACA
Emi ex3 fw     GCTCTTCCTGGACCGAGACCTCATGACACTTCCTATTCAGAAGTGRGATTTWTTAAACM
Emi ex3 re     GCTCTTCCTGGACCGAGACCTCATGACACTTCCTATTCAGAAGTGAGATATATAAACA
*****
Emi exon 3      CCTCGATTATAACCTTGGCACTACATTGACATGTGTCTCTCAATTCTGTAGTTTCAAACC
Emi ex3 fw     CCTCGATTATAACCTTGGCACTACATTGA-----
Emi ex3 re     CCTCGATTATAACCTTGGCACTACATTGACATGTGTCTCTCAATTCTGTAGTTTCAAACC
*****
Emi exon 3      AGTAAATAGTTTTAATGCGTATCTGGTAATGGTTAAACAGCAGCATTGTTTTCTGTTGGA
Emi ex3 fw     -----
Emi ex3 re     AGTAAATAGTTTTAATGCGTATCTGGTAATGGTTAAACAGCAGCATTGTTTTCTGTTGGA

Emi exon 3      TATCAGAAGGATATGATATCAGAAAATTGCAGGGGGAGAGAGCAAATAAGTTAGTGGGGA
Emi ex3 fw     -----
Emi ex3 re     TATCAGAAGGATATGATATCAGAAAATTGCAGGGGGAGAGAGCAAATAAGTTAGTGGGGA

Emi exon 3      TTCTCCTTGAGCCCTTTGCTCCCCAGAGCCCTGGAAATTGCAGTTGTCTTGACATAGCCT
Emi ex3 fw     -----
Emi ex3 re     TTCTCCTTGAGCCCTTTGCTCCCCAGAGCCCTGGAAATTGCAGTTGTCTTGACATAGCCT

Emi exon 3      AGGTACCTTTAAAGATTTTTAAAGATATATTTGTACTTGTCACTTAACGGCTGATTAACA
Emi ex3 fw     -----
Emi ex3 re     AGGTACCNNTAAN-----

Emi exon 3      CTCAGGGAAGCAATGA
Emi ex3 fw     -----
Emi ex3 re     -----

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**Figure S5. Sequencing of a 1276 bp PCR fragment encompassing exon 3 of the humanized FECH allele.** Exon 3 with surrounding intronic sequences was amplified from genomic DNA of an Emi/wt (C57BL/6J) mouse. The theoretical sequence is shown on top of this CLUSTAL W alignment. Primer sequences are highlighted with a gray background. The sequences obtained from the purified PCR product by using the same forward and reverse primers are shown below. Exon 3 is indicated in blue with the BspH1 site in magenta.

```

Emi exon 4          AAGTGTGGGATCAAAATGTGTTACGCTAGTAGCTAGCGCACATCCAGGTTTCTCTGCATG
Emi pre ex4 fw    -----GGTTTCTCTGCATG
nir-mfech-int4-2  -----
                                *****

Emi exon 4          GGTGTTGTGTGTCCTGAATCTTCAGGTGTGCTGCTGGAACAGCTTGTGGAGCACAGCTGG
Emi pre ex4 fw    GGTGTTGTGTGTCCTGAATCTTCAGGTGTGCTGCTGGAACAGCTTGTGGAGCACAGCTGG
nir-mfech-int4-2  -----
                                *****

Emi exon 4          GTATTCCTCAGAGAGGGTATAGCTTTAGCTCCTTATTCTACAACAAGAGAGCTGGCTATT
Emi pre ex4 fw    GTATTCCTCAGAGAGGGTATAGCTTTAGCTCCTTATTCTACAACAAGAGAGCTGGCTATT
nir-mfech-int4-2  -----
                                *****

Emi exon 4          GTCAATGACCTCAAGCTTCTGTTTTAAAGGCTTAATCTTGTTAGGCTCTCTAAAATTTTG
Emi pre ex4 fw    GTCAATGACCTCAAGCTTCTGTTTTAAAGGCTTAATCTTGTTAGGCTCTCTAAAATTTTG
nir-mfech-int4-2  -----
                                *****

Emi exon 4          CTTTTTTCTTTTTTATTGAGTAGAAAAACATTTCTCAGGCTGCTAAGCTGGAATAAAATC
Emi pre ex4 fw    CTTTTTTCTTTTTTATTGAGTAGAAAAACATTTCTCAGGCTGCTAAGCTGGAATAAAATC
nir-mfech-int4-2  -----
                                *****
                                *****

Emi exon 4          CACTTACCTGTATGTAAATGATTTAGTAAGCTGGCACCATTATCGCCAAACGCCGAAC
Emi pre ex4 fw    CACTTACCTGTATGTAAATGATTTAGTAAGCTGGCACCATTATCGCCAAACGCCGAAC
                                *****
                                *****

Emi exon 4          CCCCAGATTCAAGAGCAGTACCGCAGGATTGGAGGCGGATCCCCATCAAGATGTGGAC
Emi pre ex4 fw    CCCCAGATTCAAGAGCAGTACCGCAGGATTGGAGGCGGATCCCCATCAAGATGTGGAC
nir-mfech-int4-2  -----
                                *****

Emi exon 4          TTCCAAGCAAGGAGAAGGCATGGTGAAGCTGCTGGATGAGTTATCCCCTGCCACAGGTGT
Emi pre ex4 fw    TTCCAAGCAAGGAGAAGGCATGGTGAAGCTGCTGGATGAGTTATCCCCTGCCACAGGTGT
nir-mfech-int4-2  -----
                                *****

Emi exon 4          GCTCTTCTTCTTAGTGCTGGGCTAGGCTCGTTACAGTAGGACTCAGGGTCAGGGGTGGAC
Emi pre ex4 fw    GCTCTTCTTCTTAGTGCTGGGCTAGGCTCGTTACAGTAGGACTCAGGGTCAGGGGTGGAC
nir-mfech-int4-2  -----
                                *****

Emi exon 4          TGGGGTGGGCTGGGGGCACCCCTGTAATCCCAGCACTCGGGGTGTGGGGGGCAAAGGCA
Emi pre ex4 fw    TGGGGTGGGCTGGGGGCACCCCTGTAATCCCAGCACTCGGGGTGTGGGGGGCAAAGGCA
nir-mfech-int4-2  -----
                                *****

Emi exon 4          AGAAGATTCTTAATTAATAACTTCGTATAATGTATGCTATACGAAGTTAT-----
Emi pre ex4 fw    AGAAGATTCTTAATTAATAACTTCGTATAATGTATGCTATACGAAGTTATTTTAATTA
nir-mfech-int4-2  -----
                                *****

Emi exon 4          -----GTGTGTGTGTGTGTGTGTGTGTGT
Emi pre ex4 fw    GGCGCGCCGCGCCCTAGGCTGCACTAGCAGAGTGTGTGTGTGTGTGTGTGTGTGT
nir-mfech-int4-2  -----CAGAGNGTGTGTGTGTGTGTGTGTGT
                                *****

Emi exon 4          GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT
Emi pre ex4 fw    GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT
nir-mfech-int4-2  -----GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT
                                *****

Emi exon 4          GAGTTCACAGGCACTATCCTCTGACTGTGAACTTCAAAAATGACTTCCAGTGCTACA
Emi pre ex4 fw    GAGTTCACAGGCACTATCCTCTGACTGTGAACTTCAAAAATGACTTCCAGTGCTACA
nir-mfech-int4-2  -----
                                *****

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```

Emi exon 4          AGTAAACAAACTGAAATGCCAGAAAGCGTTTCAAAGGAAGTTTAAATTAAGTTGAGCT
Emi pre ex4 fw     -----
nir-mfech-int4-2   AGTAAACAAACTGAAATGCCAGAAAGCGTTTCAAAGGAAGTTTAAATTAAGTTGAGCT
                    *****

Emi exon 4          AGTTTATAAAGAAGAGGCAATATTCCGTGCACAGAGGGTAAGACTTGCTGTGGCCTTTTC
Emi pre ex4 fw     -----
nir-mfech-int4-2   AGTTTATAAAGAAGAGGCAATATTCCGTGCACAGAGGGTAAGACTTGCTGTGGCCTTTTC
                    *****

Emi exon 4          TTCTAGTTGGATGGTAAATAGCTGGGCATTTAGAGAAGTTGTTGGCTGGTGTCACTGGGC
Emi pre ex4 fw     -----
nir-mfech-int4-2   TTCTAGTTGGATGGTAAATAGCTGGGCATTTAGAGAAGTTGT-GGC-----
                    *****

Emi exon 4          AGCCTTCAGTCACACTTTCAGCCAACAGCACC
Emi pre ex4 fw     -----
nir-mfech-int4-2   -----
    
```

**Figure S6. Sequencing of a 1071 bp PCR fragment encompassing exon 4 of the humanized FECH allele.** Exon 4 with surrounding intronic sequences was amplified from genomic DNA of an Emi/wt (C57BL/6J) mouse. The theoretical sequence (Emi exon 4) does not match the sequences obtained with the two primers in a highly repetitive region downstream of exon 4, but the two sequences obtained from the PCR product show a good overlap. Primer sequences are highlighted with a gray background. Exon 4 is indicated in blue with the BamH1 site in magenta. The extra 63 bp between the aberrant and correct 3' splice sites is highlighted in light green, and the c.315-48C nucleotide is shown in red and underlined.

**Table S1. Oligonucleotides used in this paper**

Name	Sequence (5' to 3')	Amplicon(s)
<b>Genotyping murine/humanized <i>Fech</i> gene:</b> <sup>1</sup>		
FECH mm Fw	GAGGAGACAGCCCTGTGAGGG	313 bp (mouse) 271 bp (humanized allele)
FECH mm Re	CAGGTGCTGCCGTGACCACGG	
FECH hs re	GTGCCAAGGTTATAATTGAGG	
<b>Genotyping (sex):</b>		
Sry 5'	AGCTCTTACACTTTAAGTTTTGACTTC	600 bp (male)
Sry 3'	GCAGCTCTACTCCAGTCTTGCC	no band (female)
<b>amplification/cloning of C22:</b>		
FECH XbaI Fw	GTACATCTAGATCTGAGACTCTTCTTGACCG	2157 bp
FECH SalI Re	GTACAGTCGACCATGTCCCAAATCCTTAGCTT	
<b>RT-PCR of correctly &amp; aberrantly spliced <i>Emi</i> mRNA:</b> <sup>2</sup>		
Emi spec fw-3	GAGACCTCATGACACTTCCT	169 bp (aberrant)
Emi spec rv-2	GGGGATCCGCCTCCAATC	106 bp (correct) (exons 3-4)
<b>RT-PCR of total mouse <i>Fech</i> transcripts:</b>		
Emi ex2 fw	GGAGAAGGTACATCATGCCAAGAC	386 bp (aberrant)
Mm-FECH-ex4-Re	CTGTGGCAGGGGATAACTC	323 bp (correct) 203 bp (ex 3 skipped) (exons 2-4)
<b>RT-PCR of human <i>FECH</i> transcripts:</b>		
Fech ex2 Hs Fw	CACAGAAACAGCCCAGCATG	378 bp (aberrant)
FECH ex4 rev	GACAATTCATCCAGCAGCTTC	315 bp (correct) 195 bp (ex 3 skipped) (exons 2-4)

<sup>1</sup> The wild-type mouse and humanized alleles, but not the Pas allele, are cleavable by Bsp HI.

<sup>2</sup> Nucleotides shown in red are human-specific.

## References

Barman-Aksözen, J., Béguin, C., Dogar, A. M., Schneider-Yin, X. and Minder, E. I. (2013). Iron availability modulates aberrant splicing of ferrochelatase through the iron- and 2-oxoglutarate dependent dioxygenase *Jmjd6* and *U2AF65*. *Blood Cells, Molecules, and Diseases* **51**, 151-161.