

SUPPLEMENTAL FIGURE LEGENDS

Fig. S1. Amino acid sequence of mouse 1810019J16Rik (Kdf1). The wild-type isoform is 397 amino acids long. *shd* mutant isoform 1 is the result of utilizing the new splice acceptor site, causing a longer open reading frame, coding for 445 amino acids. The frameshifted sequence is shown in red. *shd* mutant isoform 2 is the result of the splice machinery skipping exon 3, resulting in an in-frame deletion and a shorter protein (373 amino acids). The deleted amino acids are depicted by a red underline.

Fig. S2. 1810019J16Rik (Kdf1) protein sequence is well conserved amongst mammals. Black shading indicates strong conservation of specific residues across lineages, gray shading marks less conservation, no shading indicates no conservation. Sequence alignment was performed using Clustal Omega (Sievers et al., 2011). Shading was added using BoxShade. (http://www.ch.embnet.org/software/BOX_form.html). M_musculus = mouse; H_sapiens = human; R_norvegicus = rat; C_familiaris = dog; E_caballus = horse; B_taurus = cow.

Fig. S3. 1810019J16Rik (Kdf1) protein sequence is found across vertebrates, but the level of conservation falls off outside of mammals. Black shading indicates strong conservation of specific residues across lineages, gray shading marks less conservation, no shading indicates no conservation. Sequence alignment was performed using Clustal Omega (Sievers et al., 2011). Shading was added using BoxShade. (http://www.ch.embnet.org/software/BOX_form.html). M_musculus = mouse; H_sapiens = human; T_guttata = zebra finch; G_gallus = chicken; X_tropicalis = frog; T_nigroviridis = pufferfish; D_rerio = zebrafish.

Fig. S4. The Kdf1 protein is mislocalized in *shd* and gene trap alleles of Kdf1. The commercial antibody recognizes amino acid residues N-terminal to the mutation found in *shd*

mutants. Thus, the remaining staining in *shd* mutants may mark the mutant protein isoforms. Gene trap alleles are often hypomorphs, and the remaining staining in the gene trap homozygote may represent low levels of wild-type protein.

Fig. S5. An example of how animals were genotyped for *shd* and *p63* mutations. A,

Genotyping for the *shd* allele was carried out using the following primers on genomic DNA.

shd forward: 5' GCTGCACACCCAGTCTTAAA 3'

shd reverse: 5' GACTGTCAGCTCTAGGGACGAAGT 3'

This generate an uncut product of 147 bp. If the *shd* mutation is present, it will be cut by MlyI digest, removing ~20 bases. The small fragment runs off the gel.

B, Genotyping for *p63* was carried out as per JAX recommendations.

http://jaxmice.jax.org/protocolsdb/f?p=116:2:2081737814758151::NO:2:P2_MASTER_PROTOCOL_ID,P2_JRS_CODE:5248,003568

OL_ID,P2_JRS_CODE:5248,003568