WT Sidt1 ssDNA mice 1-32	GATGACCTGGATGTGGTCCGGAGAGACCAGATCCCTGTCTTCTGAGCACCCAGCATCACAGGGGGAAGAC GATGAttTGGAcGTcGTtaGacGcGAtCAGATCCCTGTCTTCTGAGCACCCAGCATCACAGGGGCAAGAC  96 nt Myc(3X) GATGACCTGGATGTGGTCCGGAGAGACCAGATCCCTGTCTTCTGAGCACCCAGCATCACAGGGGTAAGAC	
	96 nt Myc(3X)	
WT Sidt2 ssDNA	CCTCCACCCACCCTTAGGTTTTGCTGACGTTGGATGACGACTTGGACACAGTACAGCGGGACAAGATCTATGTC CCTCCAgCCACCCTTAGGTTTTGCTGACGTTGGATGACGAtcTcGAtACcGTtCAGCGCGACAAGATCTATGTC	
mice 1,2,4, 10,13,17,22, 24,25,29,31	CCTCCAgCCACCCTTAGGTTTTGCTGACGTTGGATGACGAtcTcGAtaCcGTtCAGCGcGACAAGATCTATGTC	nt HA(3X) TTCTAGCA nt HA(3X)
mouse 26	CCTCCACCCACCCTTAGGTTTTGCTGACGTTGGATGACGATCTCGATACTGTTCAGCGCGCGACAAGATCTATGTC	TTCTAGCA

Figure S4. Sequence analysis of recovered Sidt1 alleles.

A) SidT1 sequence of mouse om11 (Figure S3) produced with the commercial oligo shows that the sgRNA1 associated PAM mutation introduced by the ssDNA oligo was not incorporated into the knock-in locus, while the sgRNA2 associated PAM mutation was incorporated. B) SidT1 sequence analysis of truncation allele from outcrossed  $F_2$  progeny (T142, T165, and T166) derived from  $F_0$  mouse 2 (Figure 3) shows that the truncation is associated with an 8-10bp inverted insertion (yellow highlighted region) and a 39 bp deletion. The expected location of the single-strand nicks is shown (V).