

## SUPPLEMENTAL FIGURE LEGENDS

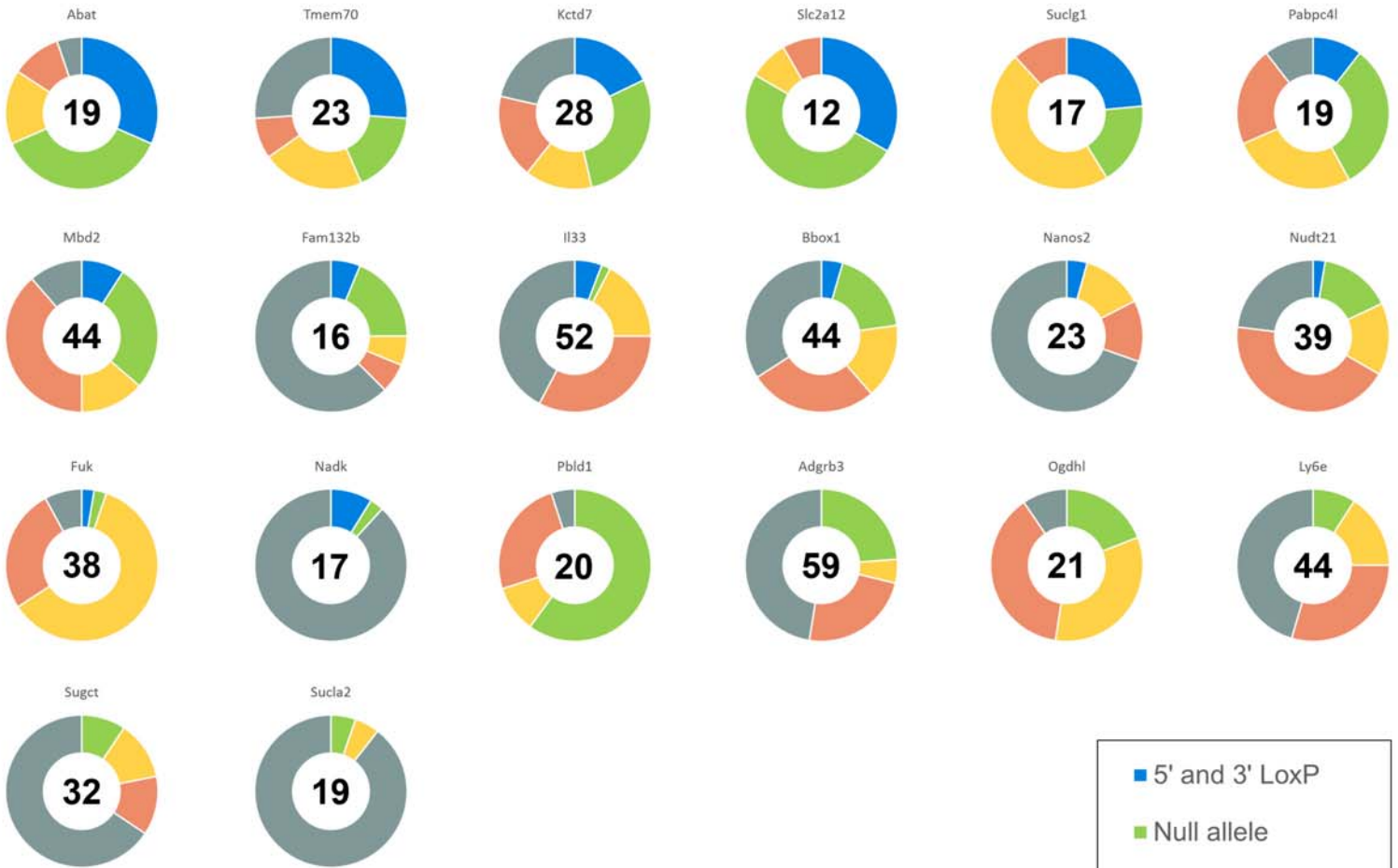
**Supplemental Figure 1 A-D.** Individual conditional KO symmetric and asymmetric ssODN and lssODN targeting attempts. (A) Symmetric homology arm design attempts for the 20 genes attempted. (B) Paired comparison of the symmetric and asymmetric homology arm design attempts for *Il1r1* and *Eif2s2*. A red circle circumscribing the total number of mice genotyped indicates an asymmetric design attempt. (C) Asymmetric homology arm design attempts for an additional 8 genes. (D) lssODN-mediated attempts for 4 genes, which included *Eif2s2*.

**Supplemental Figure 2 A-B.** Examples of mutagenized LoxP sites identified upon TA cloning PCR products from LoxP PCR reactions and Sanger sequencing resulting clones from founder mice. (A) Example of truncated LoxP site, and missing sgRNA target sequence in HDR founder from *Mbd2* 5' LoxP site targeting. (B) Examples of base changes and deletions in three different founders from *Il1r1* 5' LoxP site targeting. Endogenous sequence in blue, sgRNA target site (split) in black underline, *Bam*HI sequence in gold, LoxP sequence in green, PAM site in red underline.

**Supplemental Figure 3 A-B.** Designs for creating and genotyping conditional alleles through CRISPR-mediated targeting with lssODN donors. (A) Schematic for illustrating lssODN conditional targeting designs. Cas9 (gray) complexed with sgRNA (dark blue) binds to complementary DNA (blue) on the target strand after recognition of the PAM site (red). A double-stranded DNA template is purchased with 100-200 bp homology arms. The 5' homology arm begins with a GGG and ends with the 5' sgRNA cut site. The 3' homology arm terminates at an appropriate cDNA primer. (B) Genotyping schemes for detecting floxed and null alleles. Orange triangles represent LoxP sites, with representative homology sequence color coded on blue DNA strand. Genotyping for a null allele uses primers P1 and P3 from each LoxP genotyping reaction, which can also detect a full-length wild-type product (not shown), due to the smaller distance between LoxP sites than typical designs from ssODN targeting attempts.

Figure S1

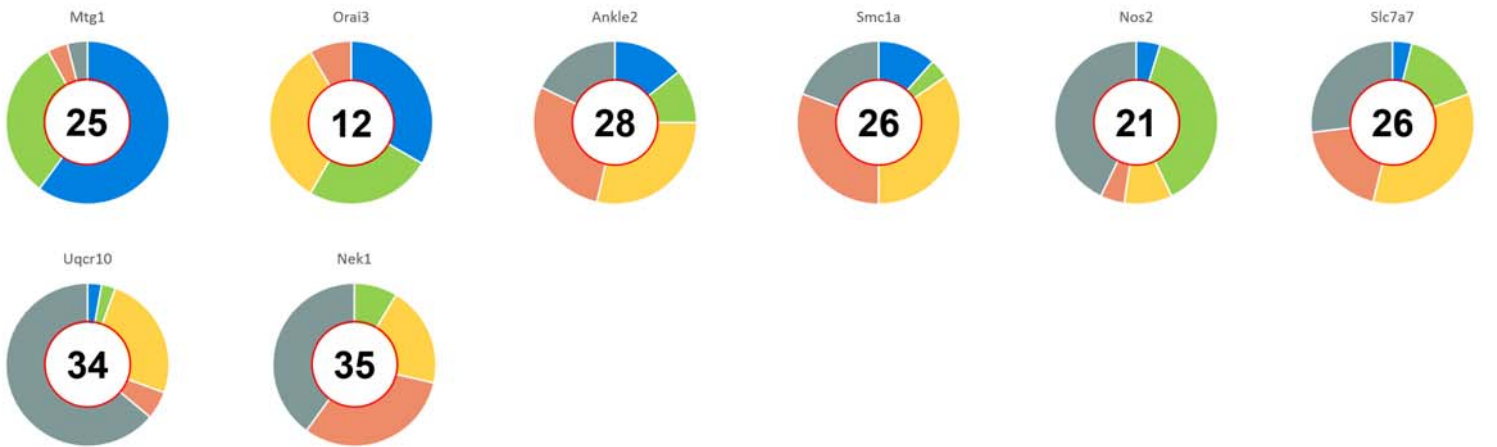
**A.**



**B.**



**C.**



**D.**

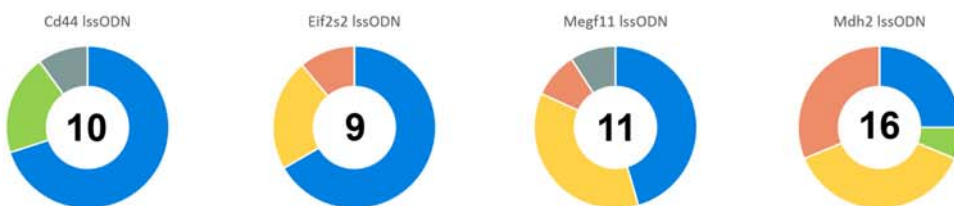


Figure S2

**A. 5' loxP Site in *Mbd2* putative founder:**

ssODN donor sequence

sgRNA

BamHI

loxP

sgRNA

AGTTCTCAGCAGTGAGCTGTGTGTGCAGTAGCAGCATGCGCAGCACGGATCCATAACTTCGTATAGCATAACATTATACGAAGTTATTTTCGGTGTTAAG  
AGTTCTCAGCAGTGAGCTGTGTGTGCAGTA-----GCATACATTATACGAAGTTATTTTCGGTGTTAAG

Genomic loxP site sequence

**B. 5' loxP Site in *Il1r1* putative founders:**

ssODN donor sequence

sgRNA

BamHI

loxP

sgRNA

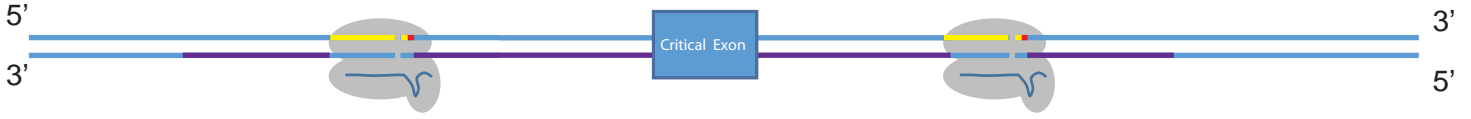
CATTAGAACAATGAGTAATTGCTCGATGCAGTAATTGCTCGATGCGGATCCATAACTTCGTATAGCATAACATTATACGAAGTTATTCCTCTGGGATTAAG  
CATTAGAACAATGAGTAATTGCTCGATGCAGTAATTGCTCGATGCGGATCCATAACTTCATATAGCATAACATTATACGAAGTTATTCCTCTGGGATTAAG  
CATTAGAACAATGAGTAATTGCTCGATGCAGTAATTGCTCGATGCGGATCCATAACTTCGTATA-C-TACATTATACGAAGTTATTCCTCTGGGATTAAG  
CATTAGAACAATGAGTAATTGCTCGATGCAGTAATTGCTCGATGCGGA-CCATAACTTCGTATAGCATAACATTATACGAAGTTATTCCTCTGGGATTAAG

Genomic loxP site sequences: 3 different founders

Figure S3

**A.**

Genomic locus



dsDNA template

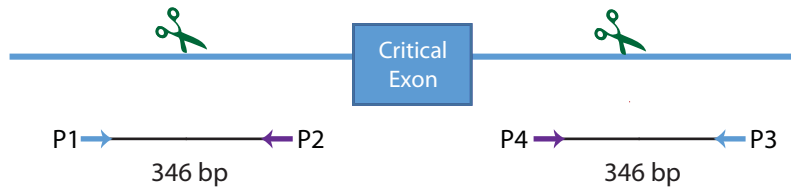


**B.**

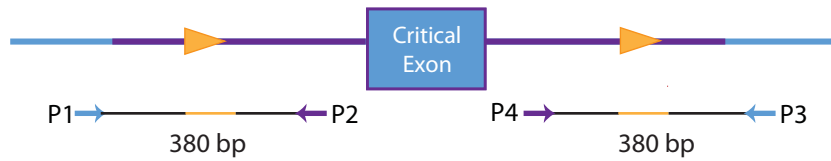
5' LoxP

3' LoxP

WT Allele



LoxP Allele



Null Allele

