Supplementary Figure 1, Generation and Functional Validation of a Conditional Mutant Nras$^{flox}$ Allele.

(A) Left panel. Schematic diagram of the targeting construct, WT Nras locus, and targeted allele. Right panel. Western blot analysis of N-Ras levels on colon tissue in Nras$^{2lox}$ mice with and without Fabpl-Cre expression.

(B) Efficient recombination of the Nras$^{flox}$ allele is visible in blood cells three weeks after pIpC injection. DNA fragments amplified from WT and recombined Nras$^{G12D/+}$ alleles run at 1.1 kb, while fragments amplified from the recombined Nras$^{flox}$ allele run at 356 bp.

(C) Sequencing of cDNA generated from bone marrow of Nras$^{G12D/+}$, Nras$^{G12D/-}$, and Nras$^{G12D/G12D}$ mice demonstrates biallelic expression in heterozygous mutant mice (left), but absence of the WT Nras expression in hemizygous (center) and homozygous (right) mutant mice.