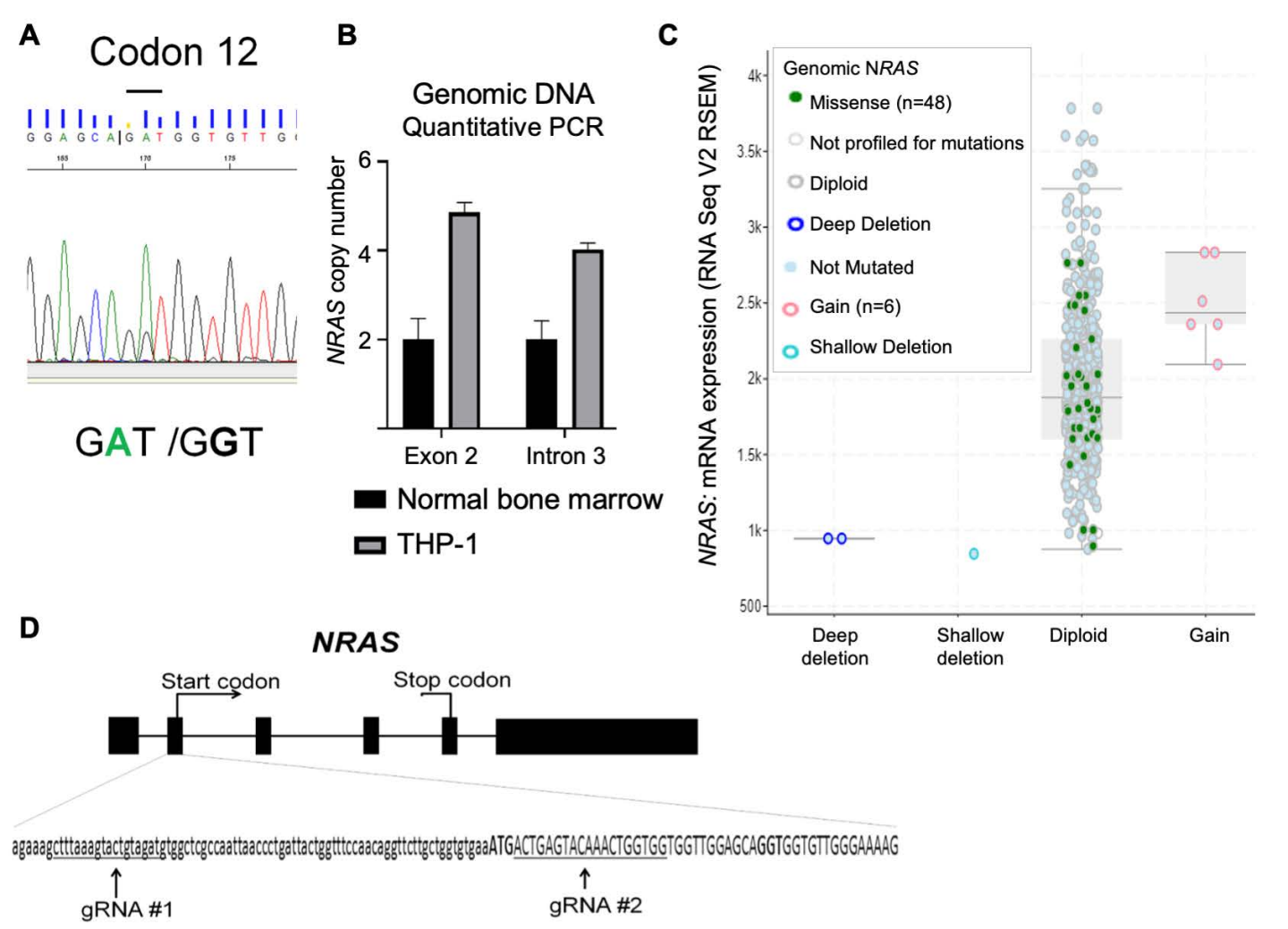
Supplemental Materials

List of Supplementary Tables



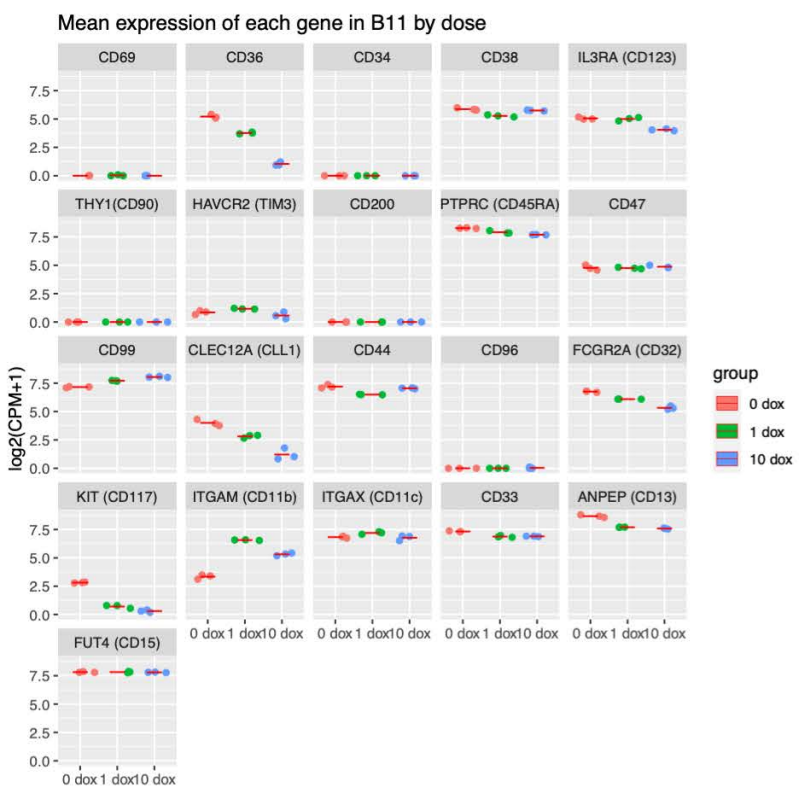
Supplemental Figure S1

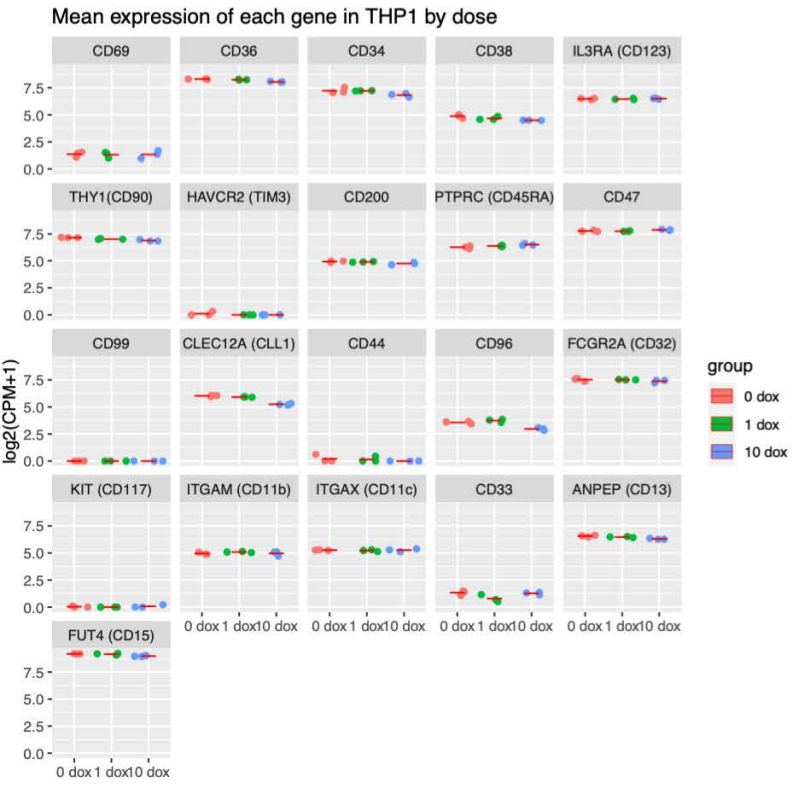
Supplemental Figure Legends

Supplemental Figure S1.

G12D mutation in THP-1 cells. **A,** Direct sequencing of the *NRAS* locus in THP-1 cells. Both the G12D mutation (GAT) and wild-type (GGT) alleles were detected. The *NRAS* locus was amplified by PCR and sequenced with the following primers: Forward: 5'-ggccgatattaatccggtgt-3' and Reverse: 5'-cactgggcctcacctctatg-3'. **B,** *NRAS* copy number in THP1 cells was determined by TaqMan PCR of genomic DNA. Copy Number Assays (Hs05807163_cn, Hs00006651_cn, Thermo Fisher Scientific, Waltham, MA). TaqMan™ Copy Number Reference Assay, human, *TERT* (Thermo Fisher Scientific, Waltham, MA) was used for normalization. Control cells were de-identified peripheral blood mononuclear cells obtained from G-CSF mobilized peripheral blood (MPB) donors from the University of Minnesota Leukemia Tissue Bank according to protocols approved by the University of Minnesota Institutional Review Board. **C,** *NRAS* transcript levels were compared in the cBioPortal dataset of 600 AML cases. The genomic status of *NRAS* alleles is indicated. Among the 600 cases, 54 have activating alterations in *NRAS*: 48 missense mutations and 6 amplifications. **D,** Targeted sequence of the *NRAS* locus for guide RNAs; gRNA1 was used to make a large deletion, which included the start codon. **E,** Derivative cell lines were submitted for Sanger sequencing. The nucleotide sequence and mutation status of the $NRAS^{G12D}$ and $NRAS^{WT}$ loci in the three derivative cell lines are shown for E5, B11, and G6. Lower case letters indicate 5' UTR; upper care letters indicate coding region; underlined letters indicate gRNA targeted site; gray letters indicate deletions. Bold "GGT" indicates wild-type allele. Bold, red "GAT" indicates mutation that leads to the *G12D* variant. E5: five *NRAS* alleles were detected: one endogenous $NRAS^{WT}$ allele and four endogenous *NRAS* variants. The $NRAS^{WT}$ allele had an insertion and deletion (in/del) in the 5'UTR but retained functional coding sequence. Another *NRAS* allele had a large deletion that spanned from the 5' UTR into much of the coding region. Three additional

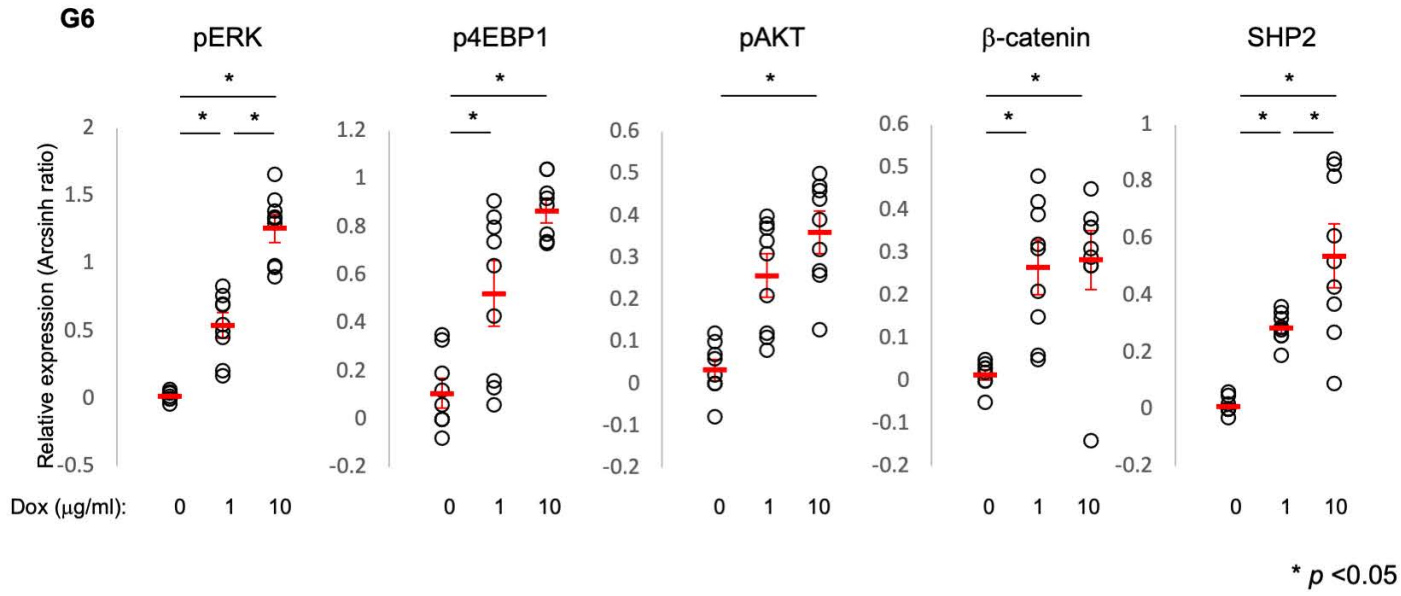
endogenous *NRAS*^{G12D} alleles had genetic modifications at the 5'-UTR (the gRNA1 targeted site). However, of these three altered *NRAS*^{G12D} alleles, the deletion only impacted the coding region in one of them. B11: three *NRAS*^{WT} and two *NRAS*^{G12D} alleles were detected. Four of these alleles had deletion mutations in the coding sequences that spanned 2-6 base pairs (bp). G6: two *NRAS*^{WT} and three *NRAS*^{G12D} alleles were detected. Each of these five alleles had deletion mutations in the coding sequence.

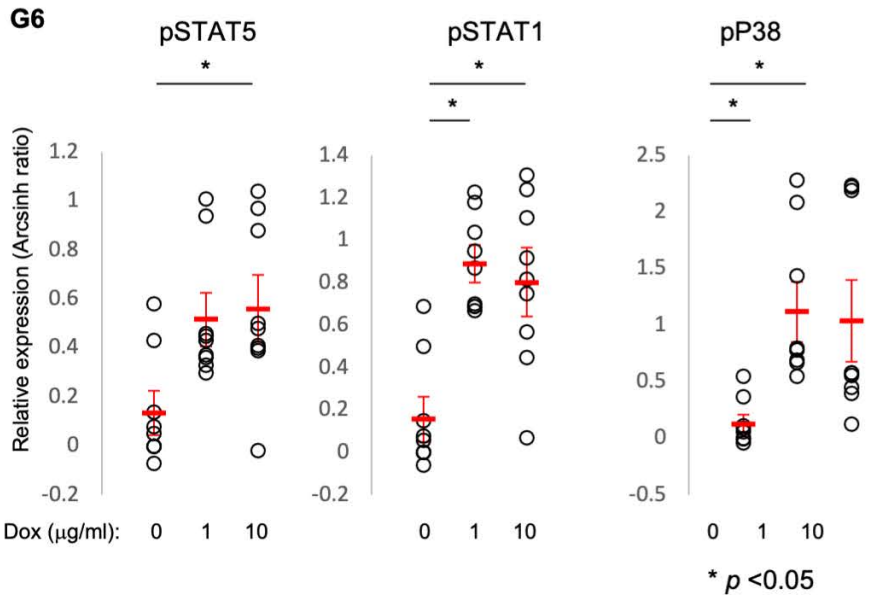




Supplemental Figure S2.

The expression of genes encoding LSC-associated cell surface markers. B11 and THP-1 parental cells were plated in liquid culture with 0, 1, and 10 µg/ml Dox for 96 hours and submitted for RNA sequencing.





Supplemental Figure S3.

Levels of canonical RAS effectors and mediator of self-renewal correlate with NRAS^{G12V} levels. G6 cells were plated in liquid culture with 0, 1, and 10 μg/ml of Dox. After 96 hours in culture, cells were labeled with antibodies to signaling intermediates and analyzed by CyTOF. The arcsinh ratio of expression (relative to the 0 μg/ml Dox control) are displayed. The arcsinh ratio is the standard metric for comparison of CyTOF data.[50,51] Each sample was plated in 6 replicates. See Supplementary Table S9 for full list of *p* values.