SUPPLEMENTARY TABLES

Supplementary Table 1. Rounds of selection used to design Avana and Asiago library.

Supplementary Table 2. sgRNAs in the six subpools of Avana library. Sequences annotated as "NO_CURRENT" are control sgRNAs.

Supplementary Table 3. sgRNAs in the six subpools of Asiago library. Sequences annotated as "NO_CURRENT" are control sgRNAs.

Supplementary Table 4. Screening data for vemurafenib in A375 cells for all biological replicates screened with Avana libraries (divided by subpools) as well as GeCKOv1 and GeCKOv2 libraries. The values provided are the $\log_2$-normalized counts per million.

Supplementary Table 5. RIGER analysis of vemurafenib screens using weighted-sum option.

Supplementary Table 6. STARS analysis of vemurafenib screens.

Supplementary Table 7. List of PanCancer genes.

Supplementary Table 8. Screening data for selumetinib in A375 cells for all biological replicates screened with Avana library. The values provided are the $\log_2$-normalized counts per million. Two replicates with subpool 5 failed during processing and were excluded from further analysis.

Supplementary Table 9. STARS analysis of selumetinib screens.

Supplementary Table 10. Negative selection screening data in HT29 and A375 cells with GeCKO libraries.

Supplementary Table 11. Negative selection screening data in HT29 and A375 cells with GeCKO libraries and the set of 291 core essential genes annotated by Hart and colleagues.

Supplementary Table 12. STARS analysis of the negative selection screening data for GeCKO and Avana libraries individually.

Supplementary Table 13. STARS analysis of the negative selection screening data for GeCKO and Avana libraries merged.

Supplementary Table 13. Screening data for 6-thioguanine screen in 293T, A375 and HT29 cells.

Supplementary Table 15. Screening data for interferon-gamma treatment of BV2 cells and output of STARS analysis.

Supplementary Table 16. Screening data for the tiling of resistance genes. sgRNAs that were present at low abundance in the early time point (ETP) were flagged and excluded from further analysis. Drug treatment abbreviations: AZD = selumetinib; PLX = vemurafenib; 6TG = 6-thioguanine.

Supplementary Table 17. Gini importance of individual features in the gradient-boosted regression tress model, Rule Set 2.

Supplementary Table 18. Screening data for off-target analysis of CD33 in MOLM13 cells.

Supplementary Table 19. Percent-active, delta-log-fold-change, and one-sided Welch's t-test p-value calculations for the CD33 off-target dataset that is used to calculate the CFD score. Note that in the calculation of the CFD score, a value of 1.0 is used for the NGG PAM, not the empirically-determined value of 0.91 that appears in Figure 5A.

Supplementary Table 20. Activity of sgRNAs designed against H2-D1 that have up to 6 mismatches to H2-K. CFD, CCTop and Hsu-Zhang scores for off-target sites are calculated and the measured activity against H2-K is provided as $\log_2$-fold-change (H2-K LFC).

Supplementary Table 21. sgRNAs in the Brunello library. A total of 19,114 genes are targeted with 4 sgRNAs per gene, for a total of 76,441 unique sequences (a very small number of genes with highly-homologous copies have fewer than 4 sgRNAs per gene). The library also contains 1,000 non-targeting sgRNAs.

Supplementary Table 22. sgRNAs in the Brie library. A total of 19,674 genes are targeted with 4 sgRNAs per gene, for a total of 78,633 unique sequences (a very small number of genes with highly-homologous copies have fewer than 4 sgRNAs per gene). The library also contains 1,000 non-targeting sgRNAs.

Supplementary Table 23. sgRNA sequences and primers used for individual follow-up experiments.