Supplementary information:

Figure S1: Cumulative distribution of the number of reads per sgRNA in a single A375 experiment. The dotted line indicates that less than 7.5% of the sgRNAs are covered by less than 100 reads**.**

Figure S2: Comparison of hits from CRISPR-based screens for vemurafenib resistance. Venn diagram showing the overlap between our list of 33 hits with the top 100 hits from previous CRISPRko screens performed with GeCKOv1 [1], [2]. GeCKOv2 [3] and Avana [3] sgRNA libraries.

Figure S3: Proposed mechanisms for vemurafenib resistance caused by deletion of Mitogen-activated protein kinase (MAPK) signalling pathway related hits in the screen. Both TGF-β (non-Smad pathway) and receptor tyrosine kinase (RTK) activate MAPK through the formation of Grb2/SOS/Ras complex. Loss of specified genes (red boxes) contributes towards the constitutive activation of the MAPK pathway and lead to excessive cell proliferation. Details are indicated in the text.

Table S1: Sequences of primers used for amplifying sgRNA Brunello library and for NGS. NGS-Lib-Fwd primers contain 1-10bp staggered nucleotides and the NGS-Lib-Rev primers provide unique barcodes. Nucleotides indicated in bold are the unique barcodes of the reverse primers.

Table S2: Read count file of the 76,441 sgRNA in the Brunello library targeting 19,114 genes.

Table S3: MAGeCK Enriched genes rank after 14 days of vemurafenib treatment. This excel file contains two tabs, where the first tab contains all the MAGeCK Enriched genes rank of 19,114 genes and the second tab only shows the MAGeCK Enriched genes rank with the p-value less than 0.05 and the read count of the all 4 sgRNAs for each gene are shown.

Table S4: Comparison of the top 33 hits with top 100 enriched genes obtained in screens with Gecko v1 [1], [2], Gecko v2 [3] and Avana [3] sgRNA libraries.

Table S5: STRING analysis of top 33 genes that regulate vemurafenib resistance.

Table S6: Gene Ontology (GO) analysis of the top 33 genes that regulate vemurafenib resistance using the online tools DAVID and REVIGO.

Supplemental references

[1]   O. Shalem *et al.*, "Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells," 2014.

[2]   W. Li *et al.*, "MAGeCK enables robust identification of essential genes from genome-scale CRISPR/Cas9 knockout screens," *Genome Biol.*, vol. 15, no. 12, pp. 1–12, 2014.

[3]   J. G. Doench *et al.*, "Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9," *Nat. Biotechnol.*, vol. 34, no. 2, pp. 184–191, 2016.