

A compendium of synthetic lethal gene pairs defined by extensive combinatorial pan-cancer CRISPR screening

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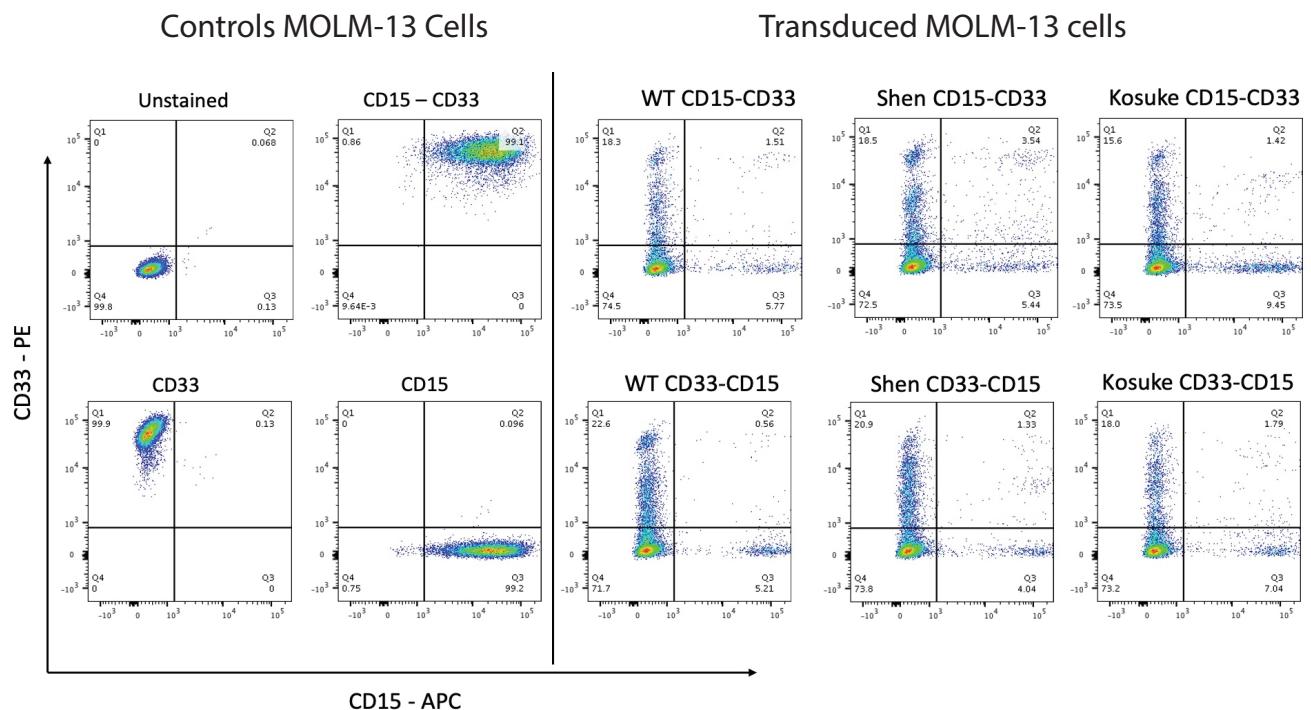
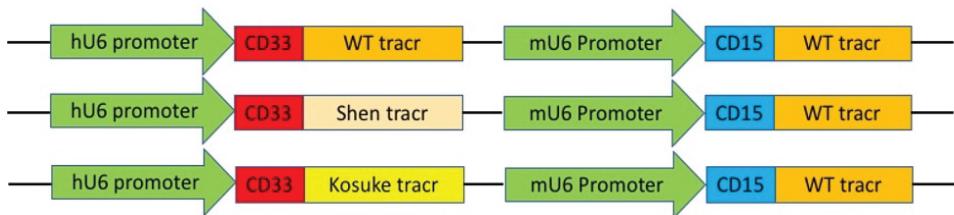
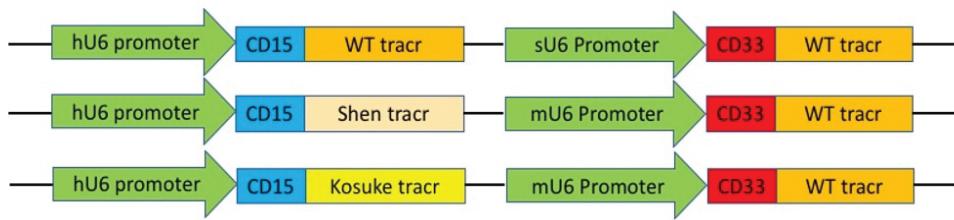
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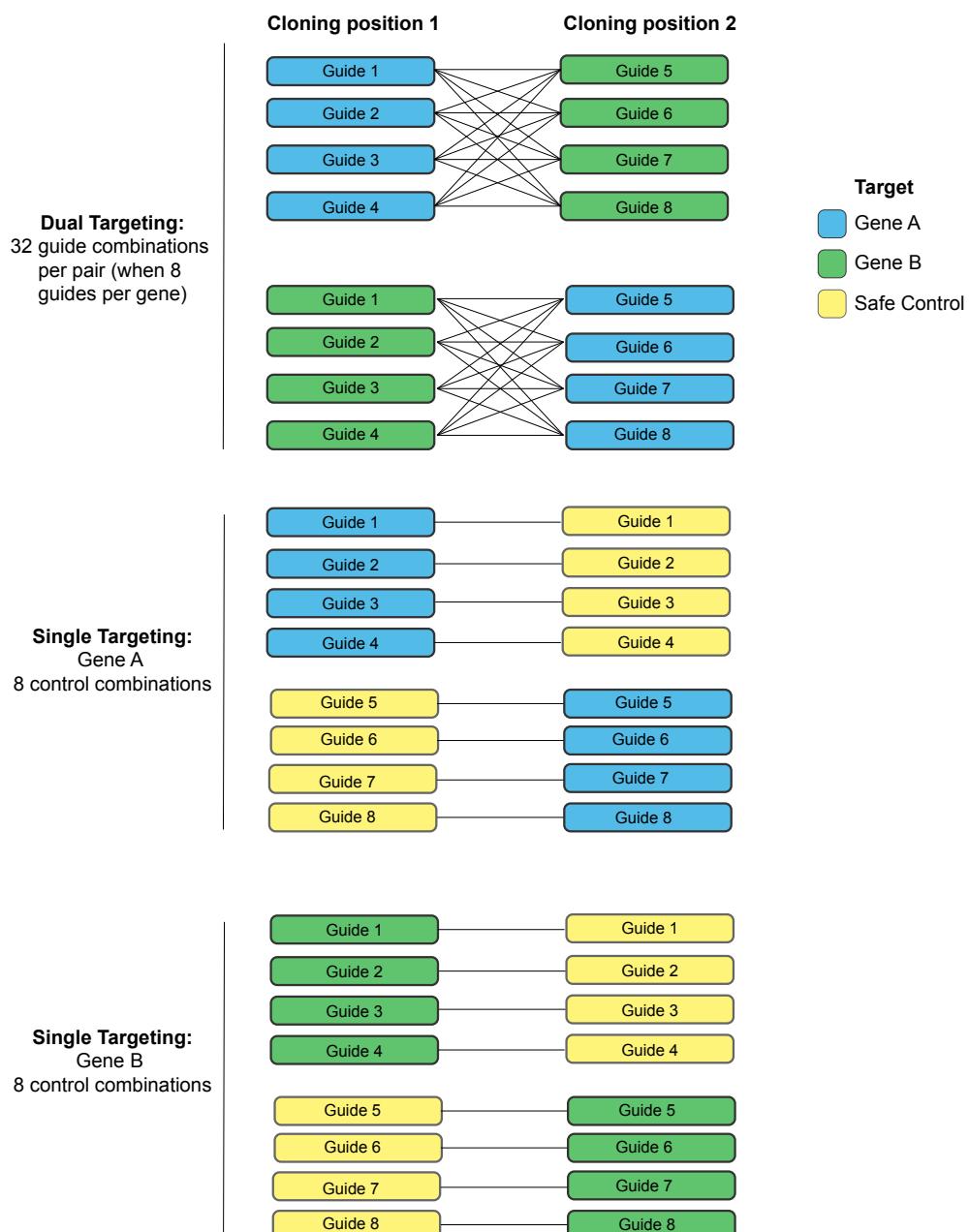
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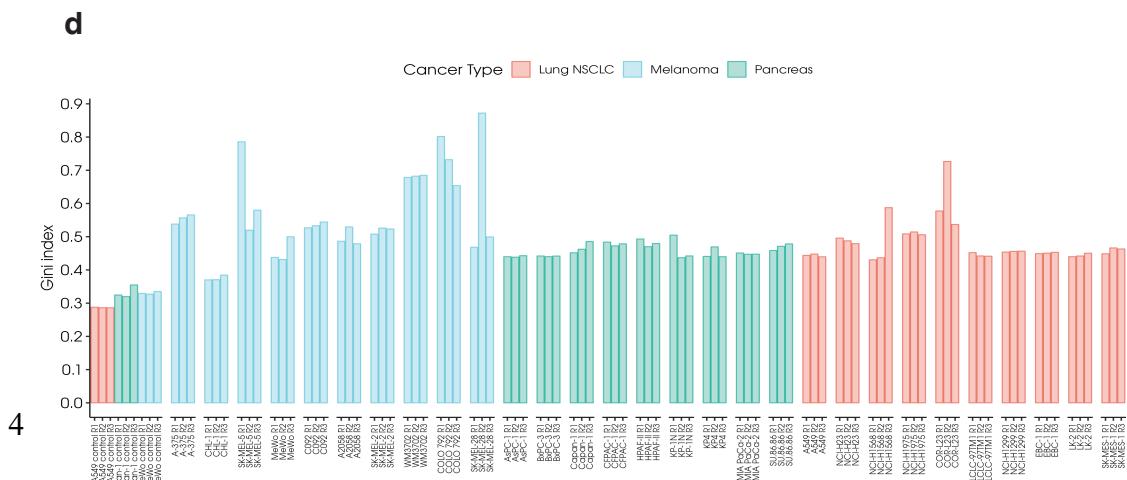
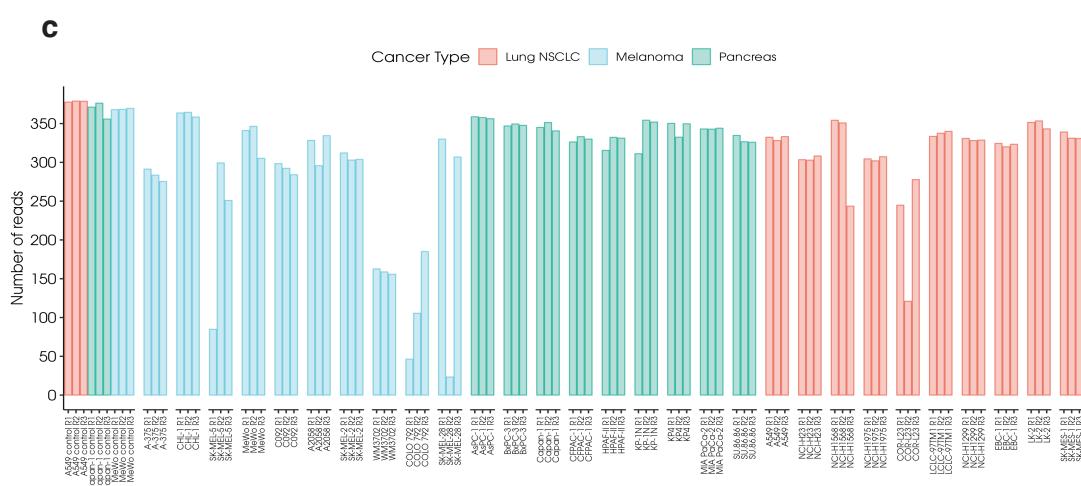
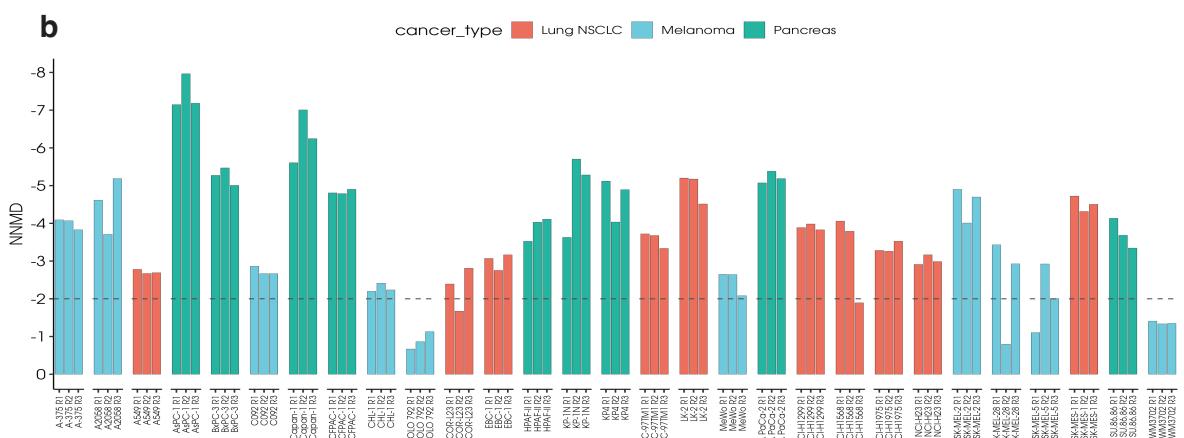
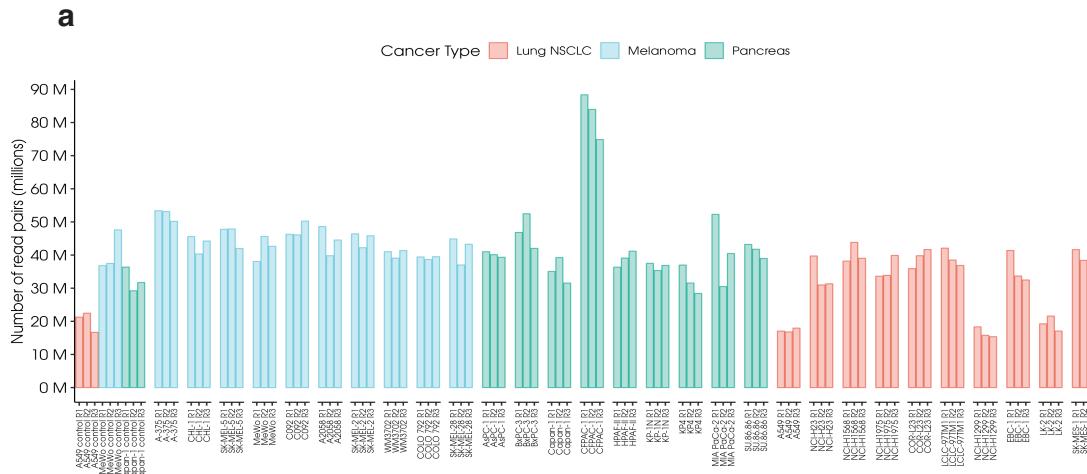


Additional File 2: Fig S1: Validation of the dual gRNA CRISPR system. Top: Vector designs showing the human (hU6) and mouse (mU6) U6 promoters and tracr sequences (Yusa and Shen)[1,2] arranged with the aim of reducing lentiviral recombination. Bottom: MOLM-13 cells express CD15 and CD33 on their cell surface. These cells were transduced with one of the vectors shown and analysed by flow cytometry. Controls and gating are shown on the left. There are some differences between the vectors used here and the one used for the final library (Additional File 3) but these experiments illustrate that the elements of the system work as expected.

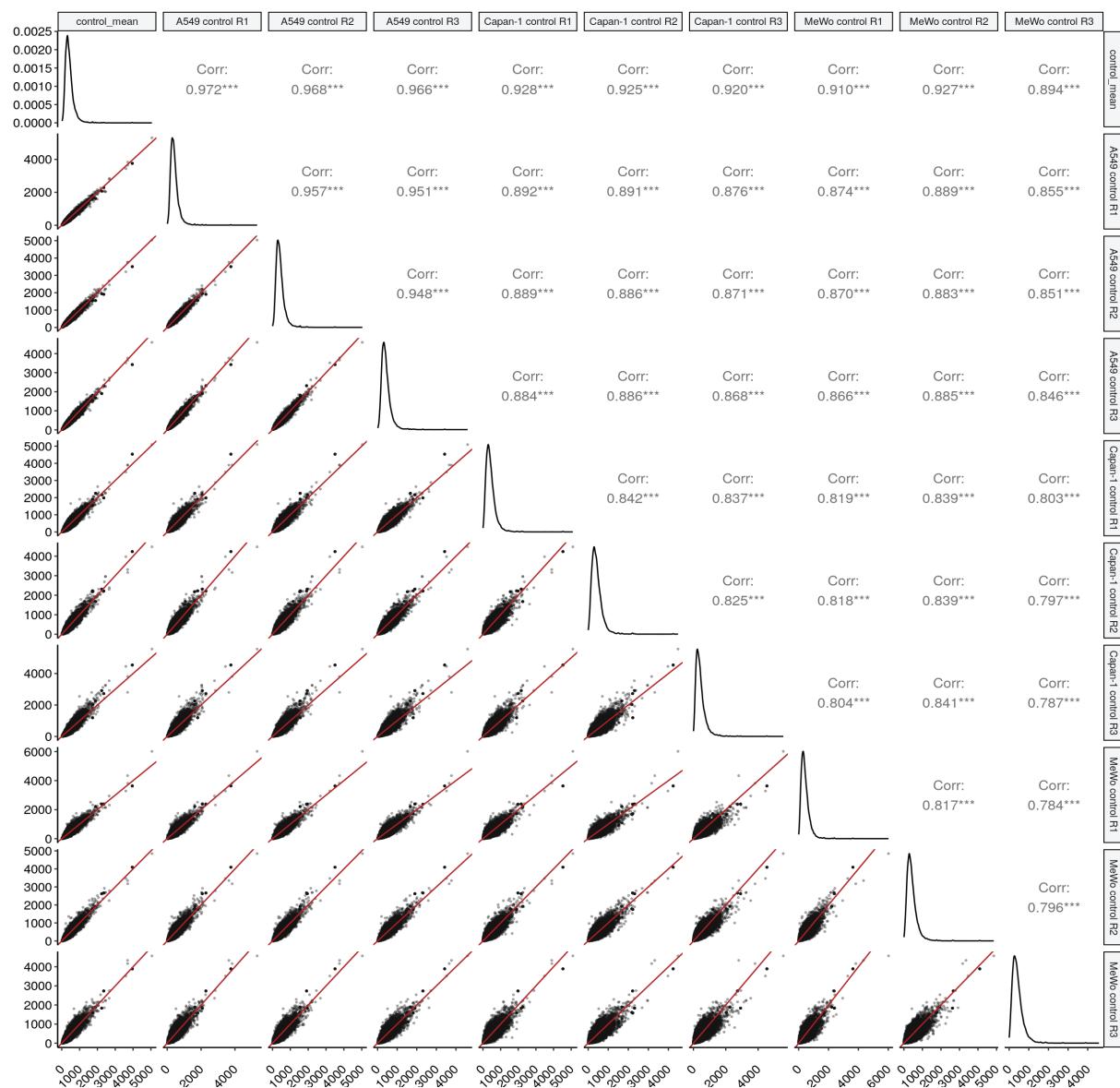
Schematic of guide layout for cloning



Additional File 2: Fig S2: Schematic of the guide layout/organisation in the library. The safe-targeting controls were collected from Morgens et al.,[3]. The guides used and their origin/source are provided in Additional File 1: Table S1.

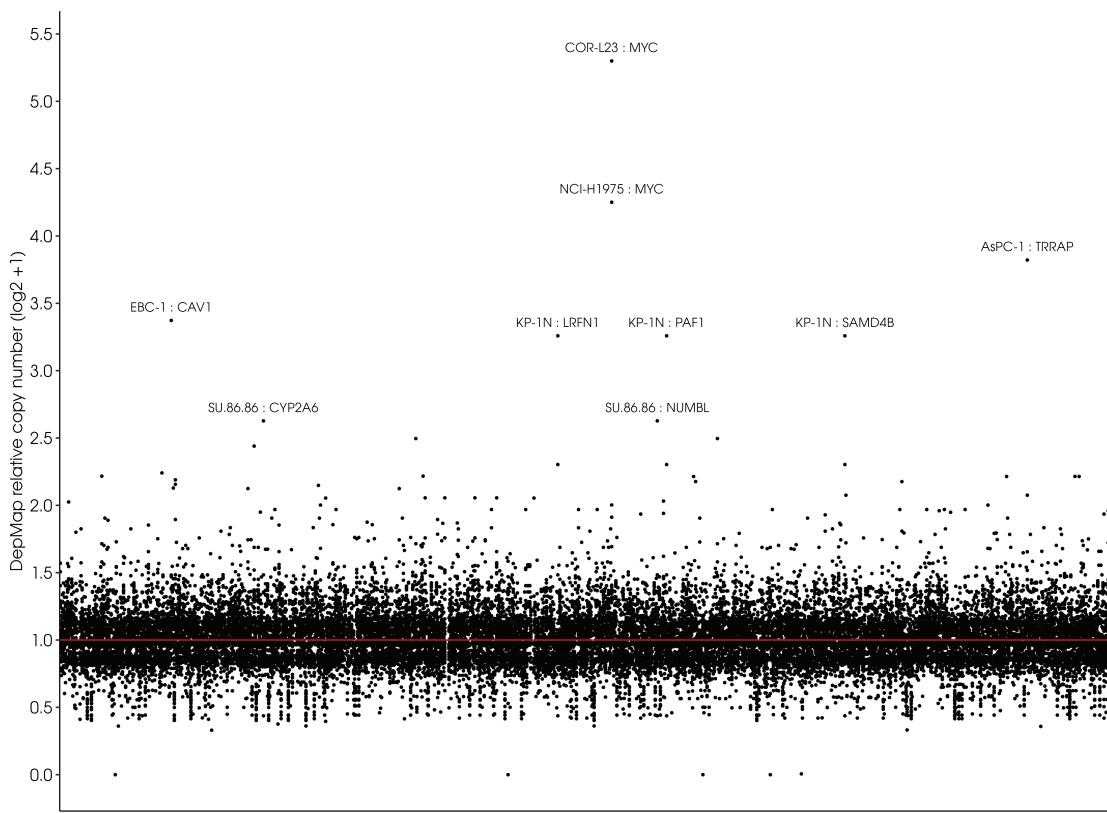


Additional File 2: Fig S3: Screen sequencing metrics. A. This graph shows each replicate for each cell line and the number of read pairs generated. B. Null-normalised mean difference (NNMD) of the normalised fold changes with a cutoff of -2 coloured by cancer type. C. Median read count (read pairs) per gRNA pair (calculated from normalised counts). D. This graph shows the Gini index for each replicate. The colour indicates the origin of the cell line from lung (red), melanoma (blue) and pancreas (green).

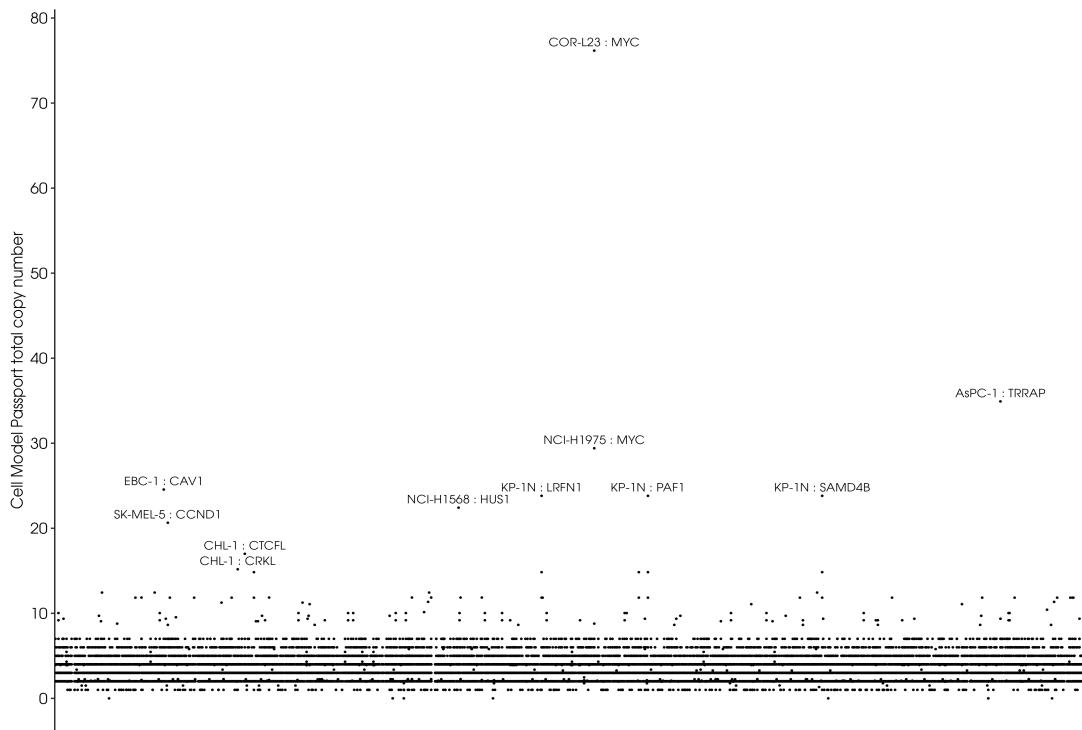


Additional file 2: Fig S4: Control Replicate Analysis. Spearman's correlation of the normalised counts between replicates from the three Cas9 wildtype lines (Capan1, A-549 and MeWo) and the averaged control (control_mean).

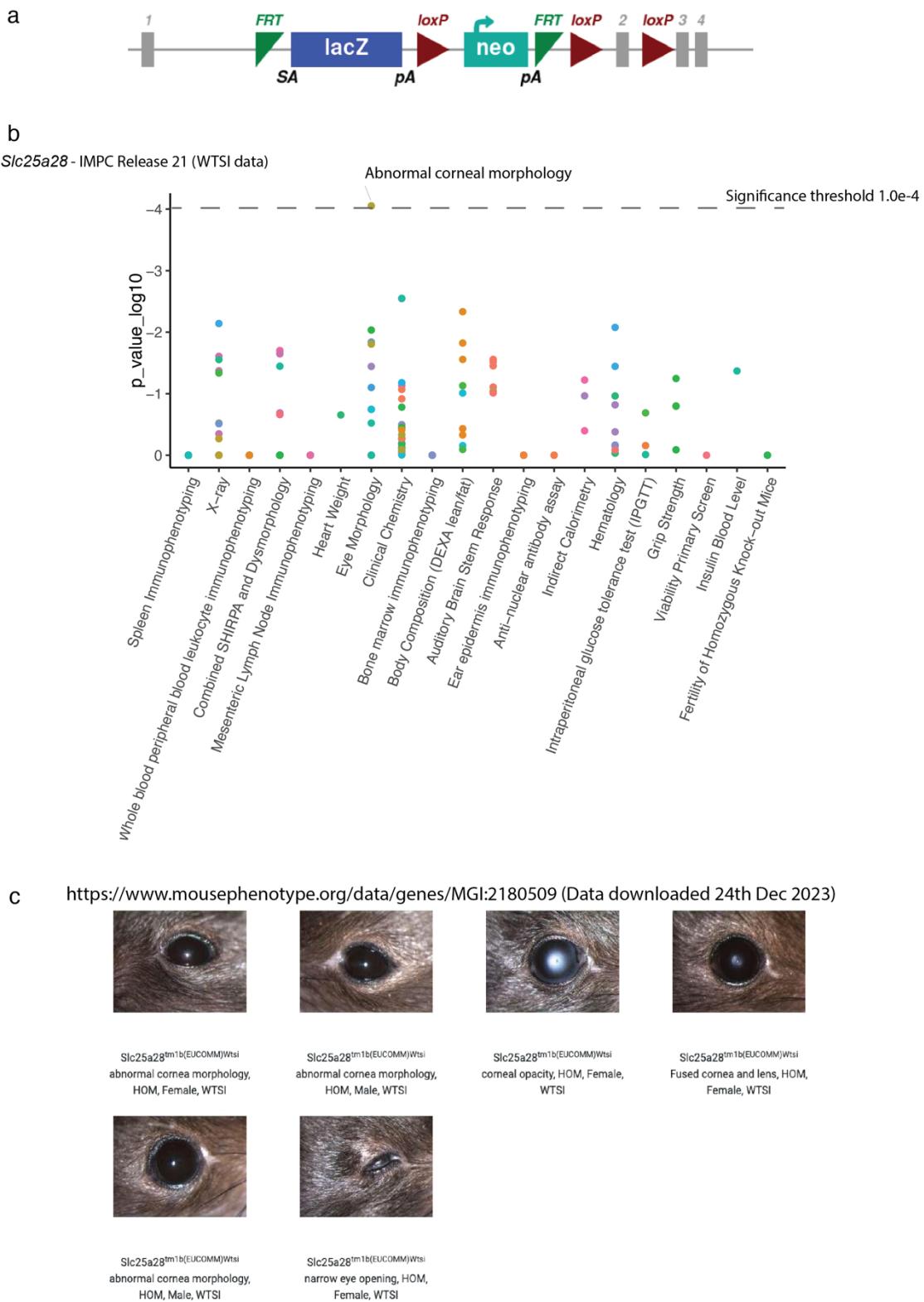
Broad DepMap



Cell Models Passport



Additional File 2: Fig S5: Genes in our combinatorial CRISPR library are generally not amplified in the cell lines we screened. Shown is the pooled copy number for the cell lines screened with genes targeted in the library and the cell lines in which they show a copy number alteration shown. The data comes from DepMap and the Cell Models Passport as indicated.



Additional file 2: Fig S6: Phenotyping of the *S/c25a28* knockout line. A. The design of the *S/c25a28* allele. Phenotyping was performed on the *tm1b* allele after Cre-mediated excision of the critical exon flanked by LoxP sites (exon 2)[4]. B. *S/c25a28* knockout animals were extensively phenotyped at the Wellcome Sanger Institute[5].

Shown are phenotypes tested and their P values. The data is from release 21 of the International Mouse Phenotyping Consortium (IMPC)[6] with P values calculated using Phenstat (mixed modelling)[7] or a Chi-squared test for categorical data. Note: the only significant phenotype is an eye defect, as shown. Each of the dots represents a different phenotyping test performed in the phenotypic area shown on the X-axis. C. Slit lamp analysis of *Slc25a28* knockout mouse mutants showing a range of presentations including fused cornea, narrowed eye and corneal opacity. Eye phenotyping was performed as described previously[5].

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