

Expanded View Figures

Figure EV1. Integration and normalization of viability phenotypes in different screens.

- A Schematic workflow of the normalization process.
- B Schematic illustration of the gene-level batch adjustment to correct for differences across libraries. Each box in the plot corresponds to the phenotype observed in screens using the same sgRNA library when targeting a specific gene of interest. In case the phenotypes of one library differ significantly ($FDR < 5\%$; Benjamini–Hochberg) from the expected (median of all phenotypes for the same gene), the library is adjusted by subtracting the estimated difference between the mean of the library and the expected phenotype from each data point corresponding to that library.
- C Examples of two genes where batch effects are observed showing the effects of the normalization.
- D Example where an sgRNA library associated batch effect can, if not adjusted, lead to a false-positive interaction between PSMA1 and RNF43. Each dot represents one screening experiment. The RNF43 mutated group is comprised of 14 data points, the RNF43 wt group consists of 71 data points.
- E Four examples of genetic interactions reported previously where phenotypes are conserved through the normalization process. Each dot represents one screening experiment. The number of data points corresponding to each group are 7 (FZD5 knockout, RNF43 mut.), 12 (FZD5 knockout, RNF43 wt), 9 (all RAS wt groups) and 5 (all RAS mut. groups). Horizontal red lines indicate the group means.
- F Clustering of normalized CRISPR scores of all experiments used in the analysis based on genes shared across libraries. The heat map is similar to the one shown in Fig 1A with all columns labeled.

Data information: (B–D) Red dotted lines indicate the median CRISPR score across all libraries. Horizontal lines inside each box indicate the median. The upper and lower boundaries of each box represent the 75th and 25th percentile, respectively. Error bars indicate the 10th and 90th percentiles and outliers are depicted as circles.



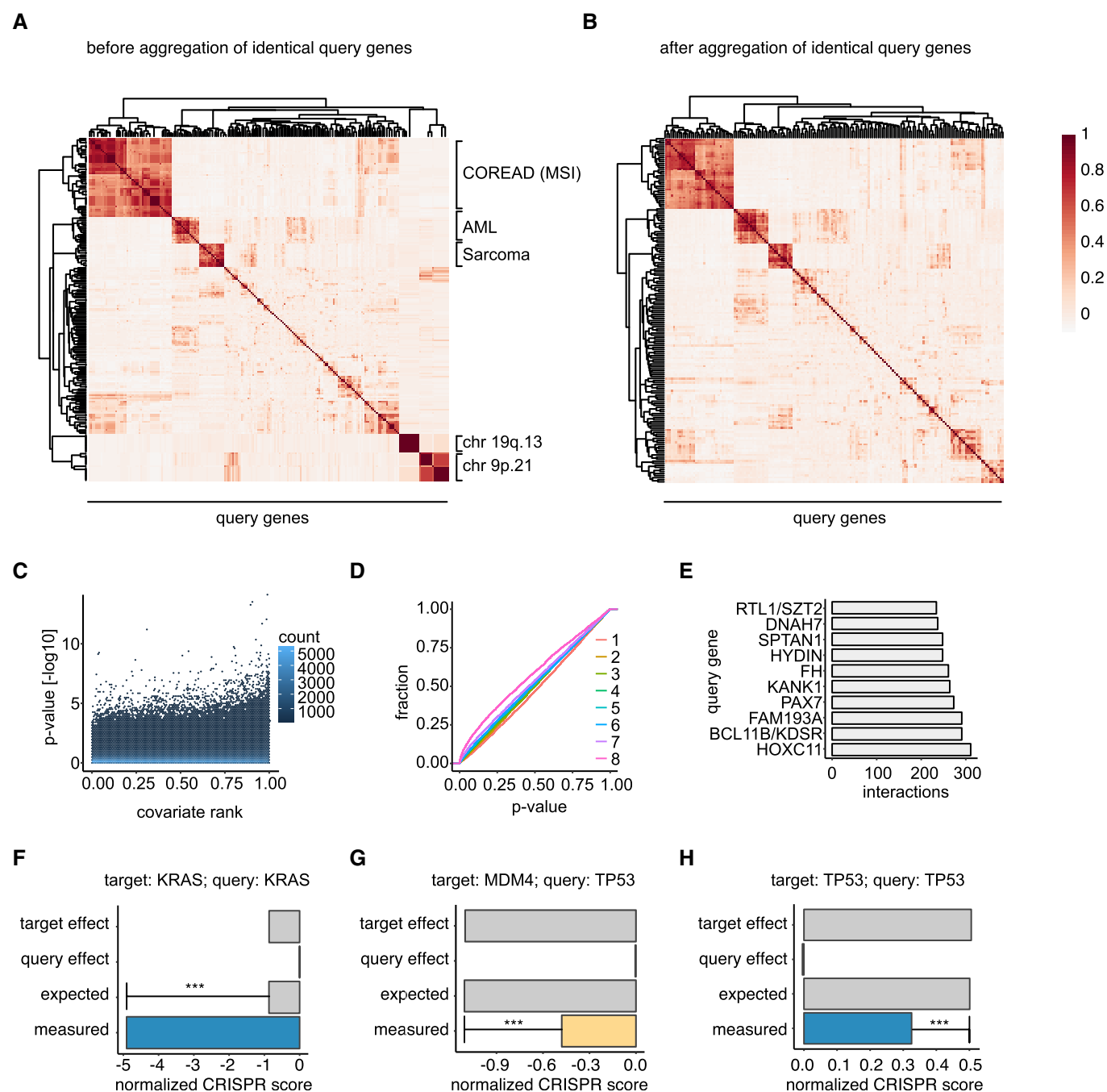


Figure EV2. Prediction of genetic interactions between gene pairs.

- A** Pairwise correlations between query genes. Clusters of highly correlating query genes are labeled indicating the source of the correlation.
- B** Query correlations after aggregation of fully (Pearson's correlation coefficient = 1) correlated query genes.
- C** Scatter plot of log₁₀-scaled *P*-values against the covariate rank of each gene pair as used for multiple testing correction using IHW. The covariate is the variance of CRISPR scores of the group of mutated cell lines.
- D** Empirical cumulative distribution function plot of *P*-values of genetic interactions based on the covariate.
- E** Ten query genes with the highest predicted interaction count. Multiple gene symbols separated by forward slashes indicate query genes that were aggregated due to high correlation.
- F–H** Epistasis plots for three examples of known genetic interactions. Blue color indicates a negative interaction, and yellow indicates a positive interaction. To determine a genetic interaction, the difference between the measured and the expected combined phenotype is quantified. Statistical significance was determined using a linear mixed effects model with the lmerTest method. ***, FDR < 0.0001.

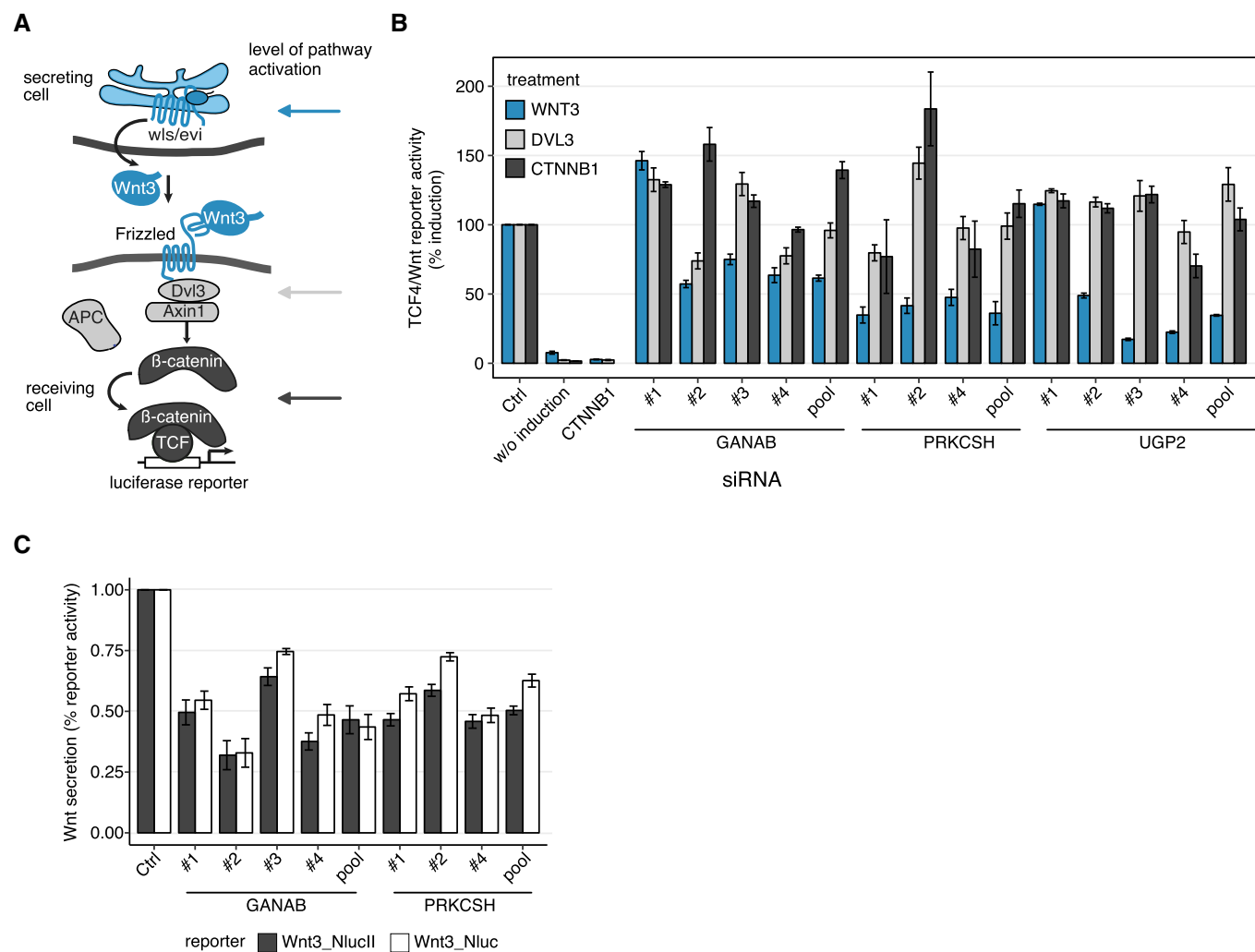


Figure EV3. Analysis of candidate regulators of Wnt/β-catenin signaling.

- A Schematic explaining the Wnt/TCF4 reporter assay used to determine the influence of candidate genes on Wnt/β-catenin signaling. Arrows indicate levels of pathway activation by overexpression of WNT3 (blue), DVL3 (gray), or CTNNB1/β-catenin (black).
- B Complete data for the Wnt activity assay presented in Fig 4B. Groups of bars indicate the effects of individual siRNAs targeting the candidate genes. Results are shown as averages of 3–4 independent experiments $\pm$ s.e.m.
- C Complete data for Wnt secretion assay presented in Fig 4C. Groups of bars illustrate the effect of individual siRNA perturbations. Results are shown as averages of three independent experiments $\pm$ s.e.m.

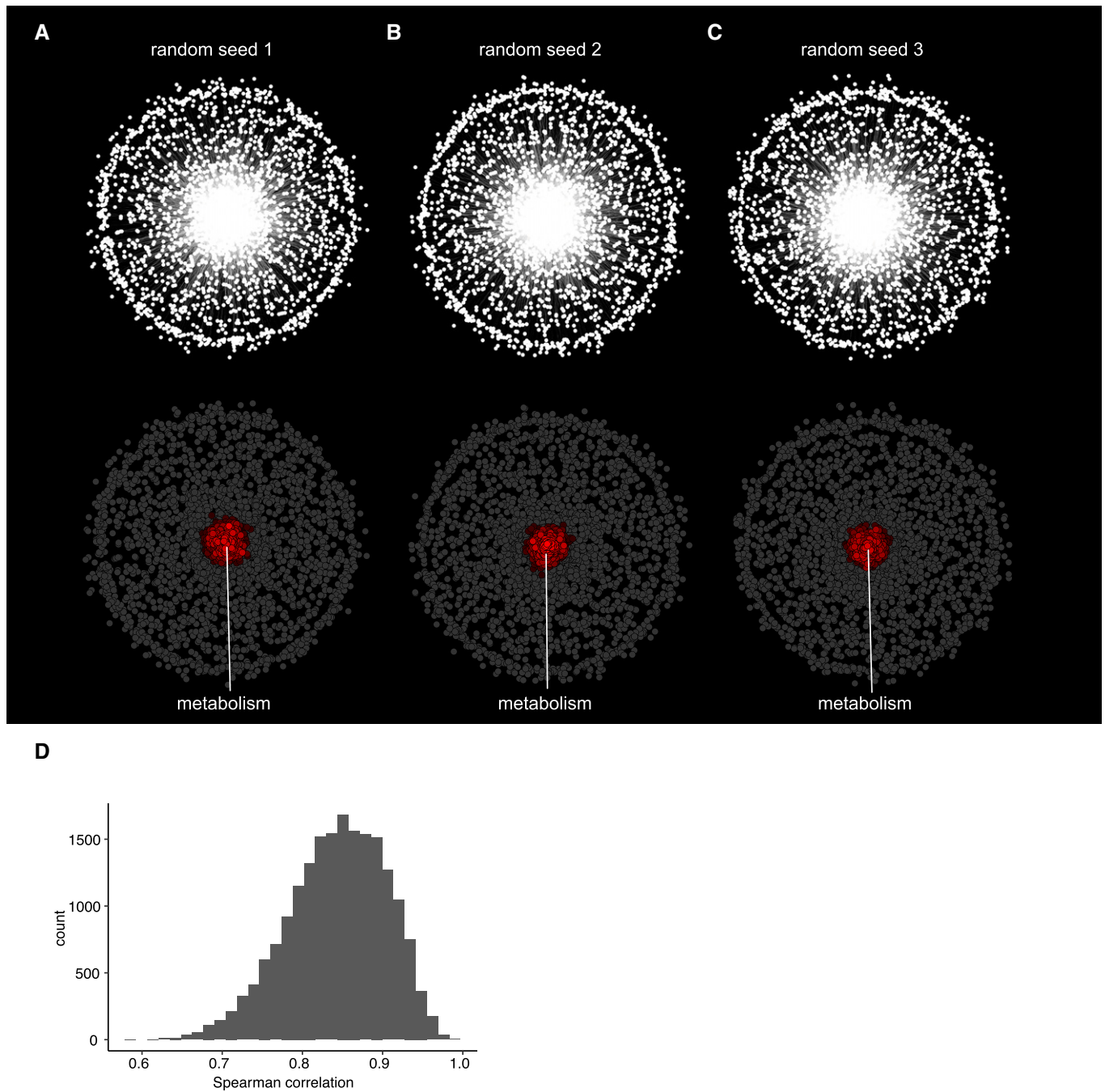


Figure EV4. Computational validation of the predicted genetic network.

A–C Genetic network after random permutation of edges using three different random seeds. A force-directed spring-embedded layout was applied to position nodes similar to the network shown in Fig 5. Each dot corresponds to a gene. Genes in the network as well as the number of edges were maintained.
 D Histogram of Spearman's correlation coefficients representing the edges of the genetic network shown in Fig 5D.