Description of Additional Supplementary Files

File: Supplementary Data 1

Description: Differentially expressed genes (DEGs) in TP53-WT and TP53-KO organoids under nutlin treatment. The analysis was conducted to assess changes in gene expression upon induction of TP53-KO and subsequent nutlin treatment. DEGs were identified by comparing the gene expression profiles between TP53-WT and TP53-KO organoids in the presence of nutlin and between control and nutlin treatment in TP53-WT organoids. Total annotated genes were 21,451. There were 3,974 significantly different expressed genes (ANOVA, unadjusted *P* value <0.05) between TP53-WT and TP53-KO in the presence of nutlin and significantly different expressed genes between control and nutlin treatment in TP53-WT organoids. The fold changes in gene expression were log2-transformed.

File: Supplementary Data 2

Description: Quantitative analysis of gRNA depletion in CRISPR-Cas9 library screen. Guide RNA (gRNA) abundance was assessed by counting unique gRNA sequences and normalizing the counts against the average counts from a control set of 1,000 non-targeting gRNAs. Log fold changes (LFC) for each gRNA were calculated separately for TP53-WT and TP53-KO conditions, by taking the log base2 of the ratio of normalized gRNA frequencies at Day21 or Day36 to Day7 and Day0. The average LFC of gRNA frequencies at late time points versus early time points was assessed for the Tumor-dependent plot. *P* value were then calculated for all guides. Those with *P* value smaller than 0.05 are shown as significant in both. The average gRNA frequencies at late time points

between TP53-WT and TP53-KO background were assessed for *TP53*-dependent plot. Guides with *P* value smaller than 0.05 are shown.

File: Supplementary Data 3

Description: gRNA sequences used for validation and primary data for figure 4 Genetic Validation of the Brunello CRISPR Knockout Screen

File: Supplementary Data 4

Description: Quantitative analysis for pharmacological validations of the Brunello CRISPR knockout screen.

File: Supplementary Data 5

Description: Somatic mutations in driver genes in F147T and F130T organoids. Patient-matched normal organoids were used as control.