

Expanded View Figures

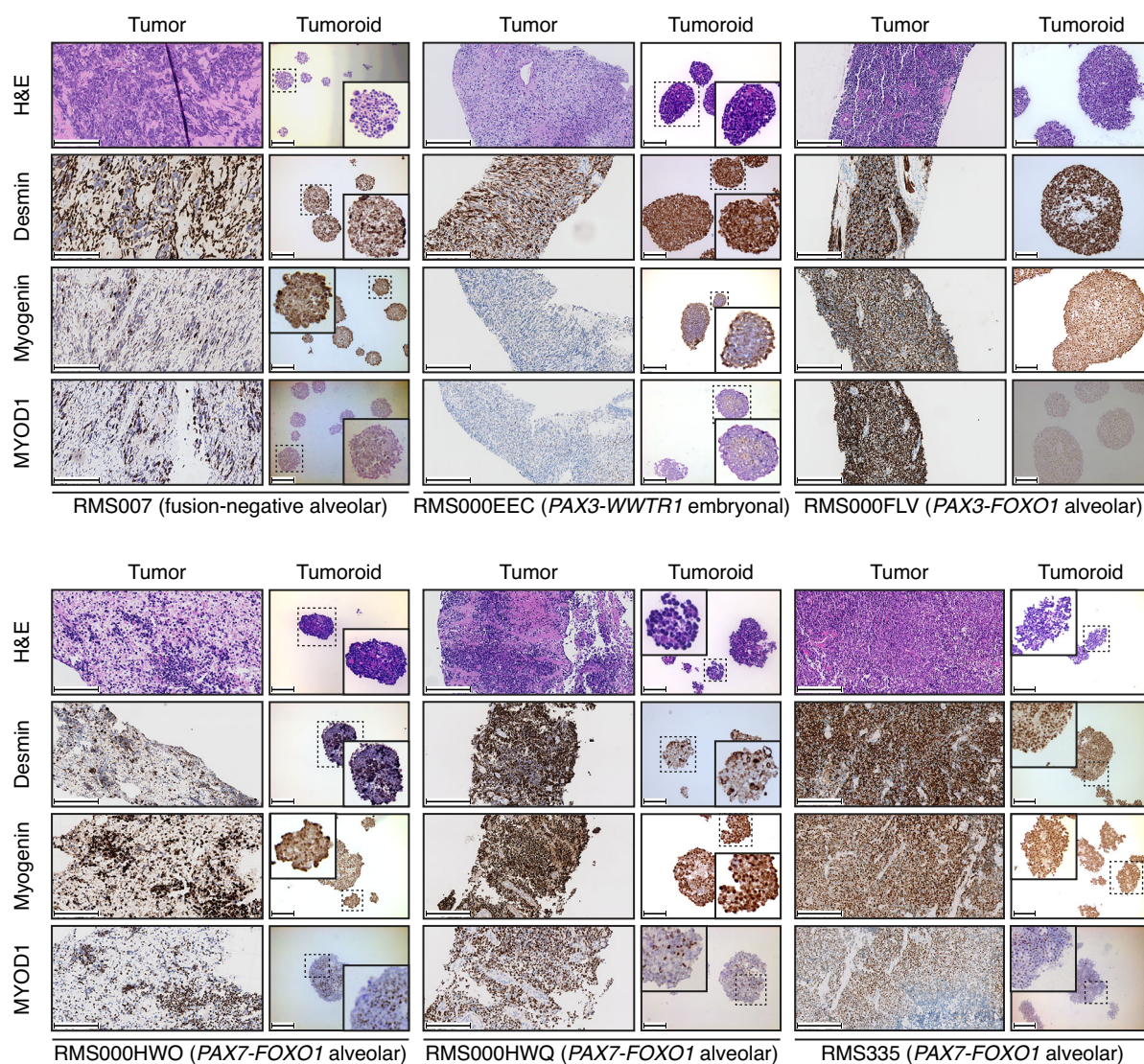


Figure EV1. Retained marker protein expression (additional figures).

Morphological (via H&E) and immunohistochemical (IHC) comparison of RMS tumors and derived RMS tumoroid models shows retained marker protein (Desmin, Myogenin, and MYOD1) expression and cellular morphology. Scale bars equal 200 μ m (RMS007, RMS000HWO, RMS335) or 100 μ m (RMS000EEC, RMS000FLV, RMS000HWQ). For RMS tumoroid models that form small spheres a zoom-in image of a sphere was inserted.

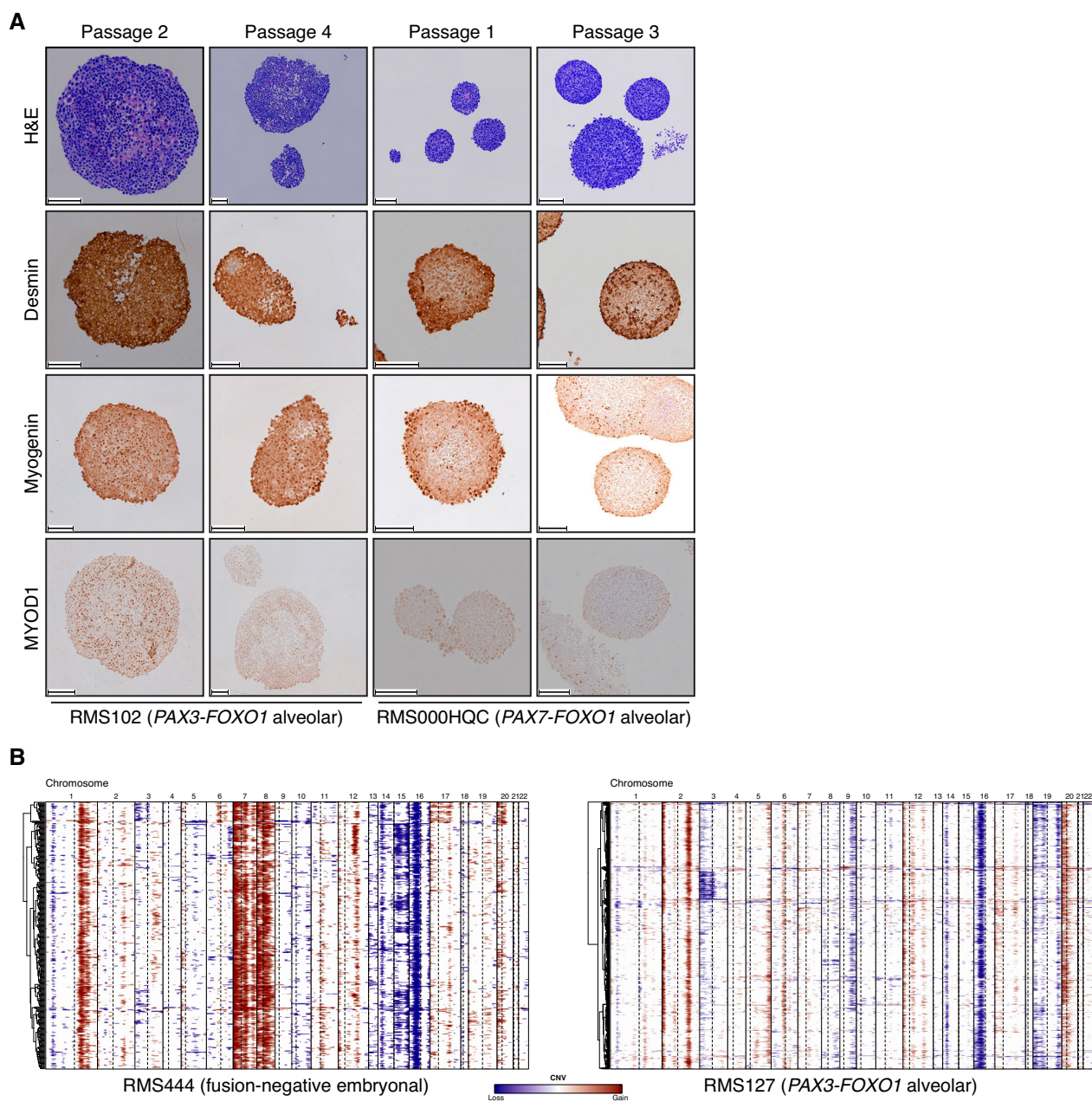


Figure EV2. Marker protein expression during early passages of culturing and single-cell RNA-seq inferred per cell CNV profiles.

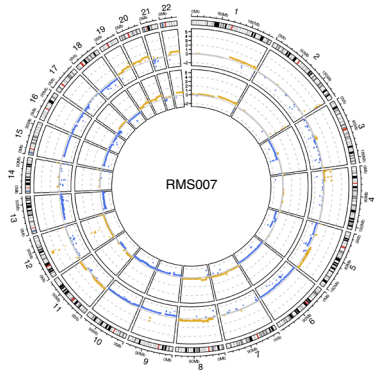
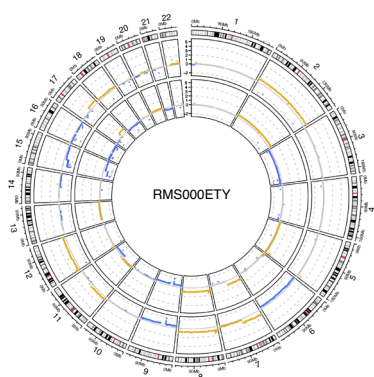
A H&E and immunohistochemistry (IHC) stainings of early passage RMS102 (passage 2 and passage 4 – passage 6 used for drug screening) and RMS000HQC (passage 1 and passage 3 – passage 5 used for drug screening). Scale bars equal 100 μ m.

B Heatmaps showing the inferred CNV profiles of single cells (y-axis) from the RMS127 and RMS444 tumoroid models, respectively. Chromosome arms are delineated by dotted lines where applicable.

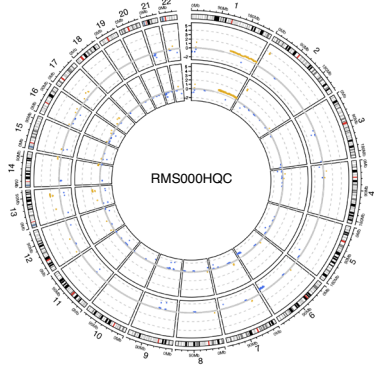
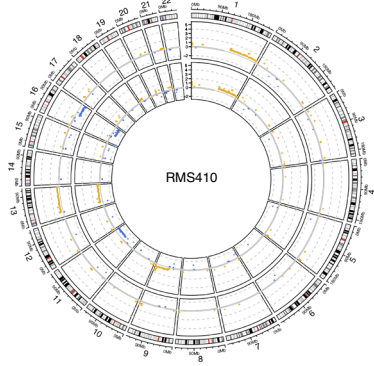
Figure EV3. RMS tumoroid models molecularly resemble the tumor they are derived from (additional figures).

- A Examples of circular copy number plots of fusion-negative RMS with embryonal (RMS000ETY) or alveolar (RMS007) histology. Outer circle depicts the tumoroid model, inner circle depicts the tumor.
- B Examples of circular copy number plots of fusion-positive RMS with alveolar histology and *PAX3-FOXO1* fusion (RMS410) or *PAX7-FOXO1* fusion (RMS000HQC). Outer circle depicts the tumoroid model, inner circle depicts the tumor.
- C Contribution of averaged single base substitution (SBS) profiles for RMS tumors (upper row) and tumoroid models (lower row).
- D Clustered correlogram of SBS profiles detected in RMS tumors (T) and tumoroid models (O).
- E Comparison of total number of mutations (synonymous and nonsynonymous) in RMS tumors and tumoroid models per fusion-type (fusion-negative $n = 4$, fusion-positive = 15, Wilcoxon test, $P = 0.15$ for tumors, $P = 0.02$ for tumoroid models). Data are shown as boxplots: The central line in the box represents the median; the upper and lower limits of the box represent the third and first quartile, respectively; the whiskers represent the minimum (bottom) and maximum (top) values excluding outliers, which are plotted as individual points.

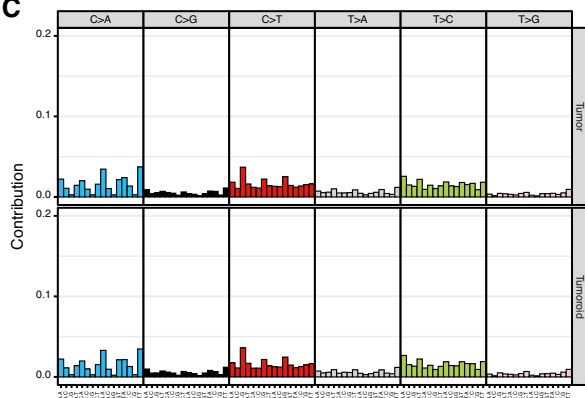
A



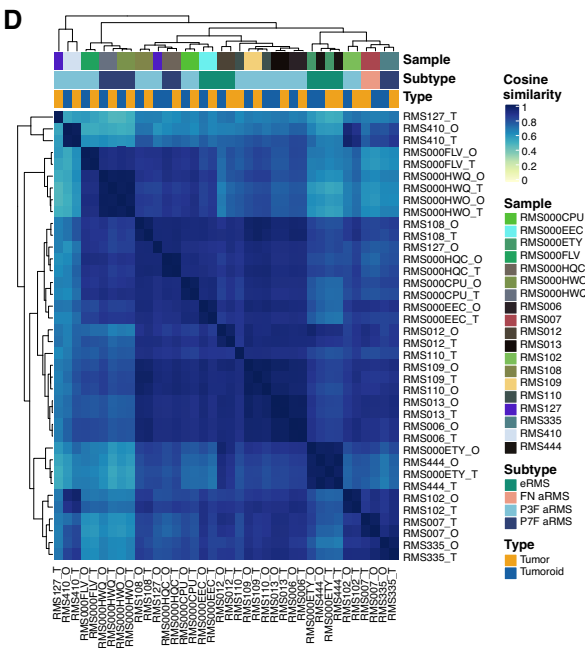
B



C



D



E

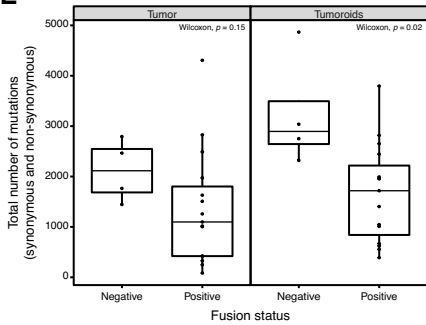


Figure EV3.

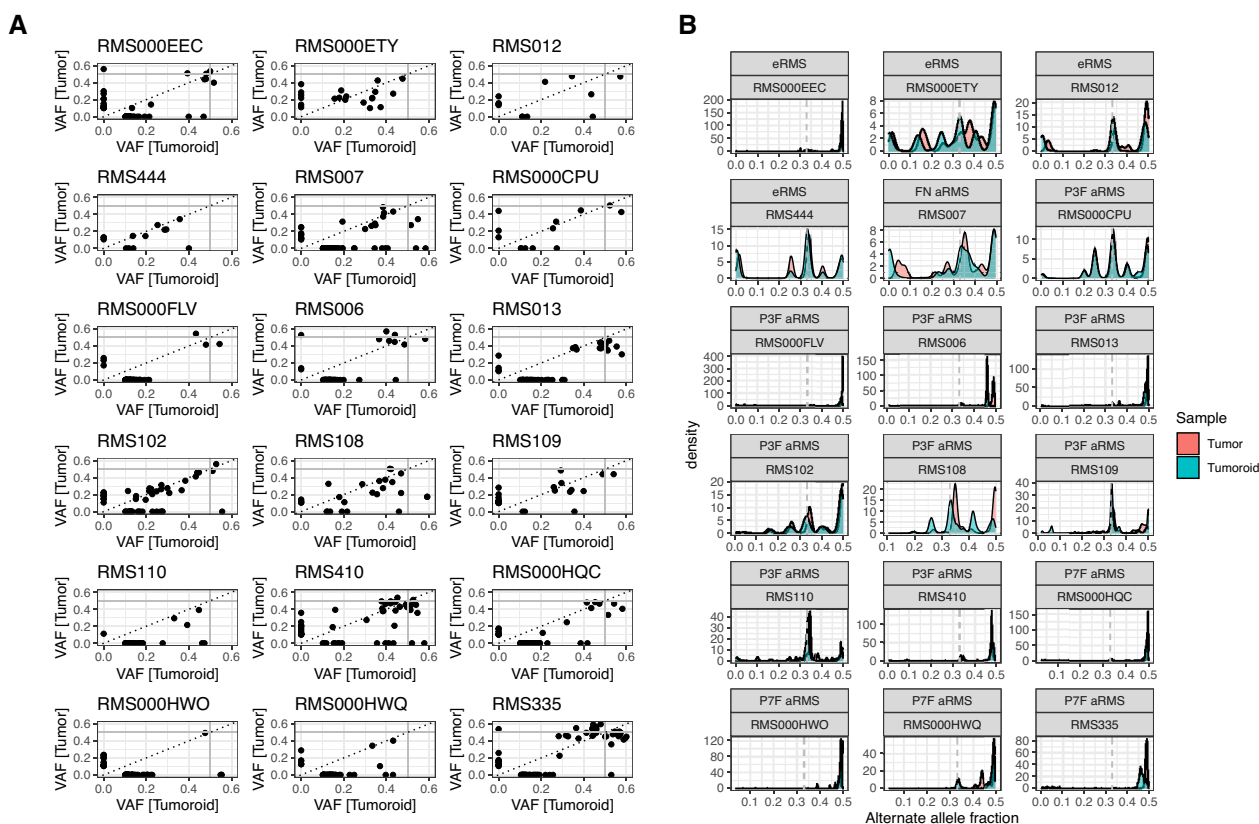
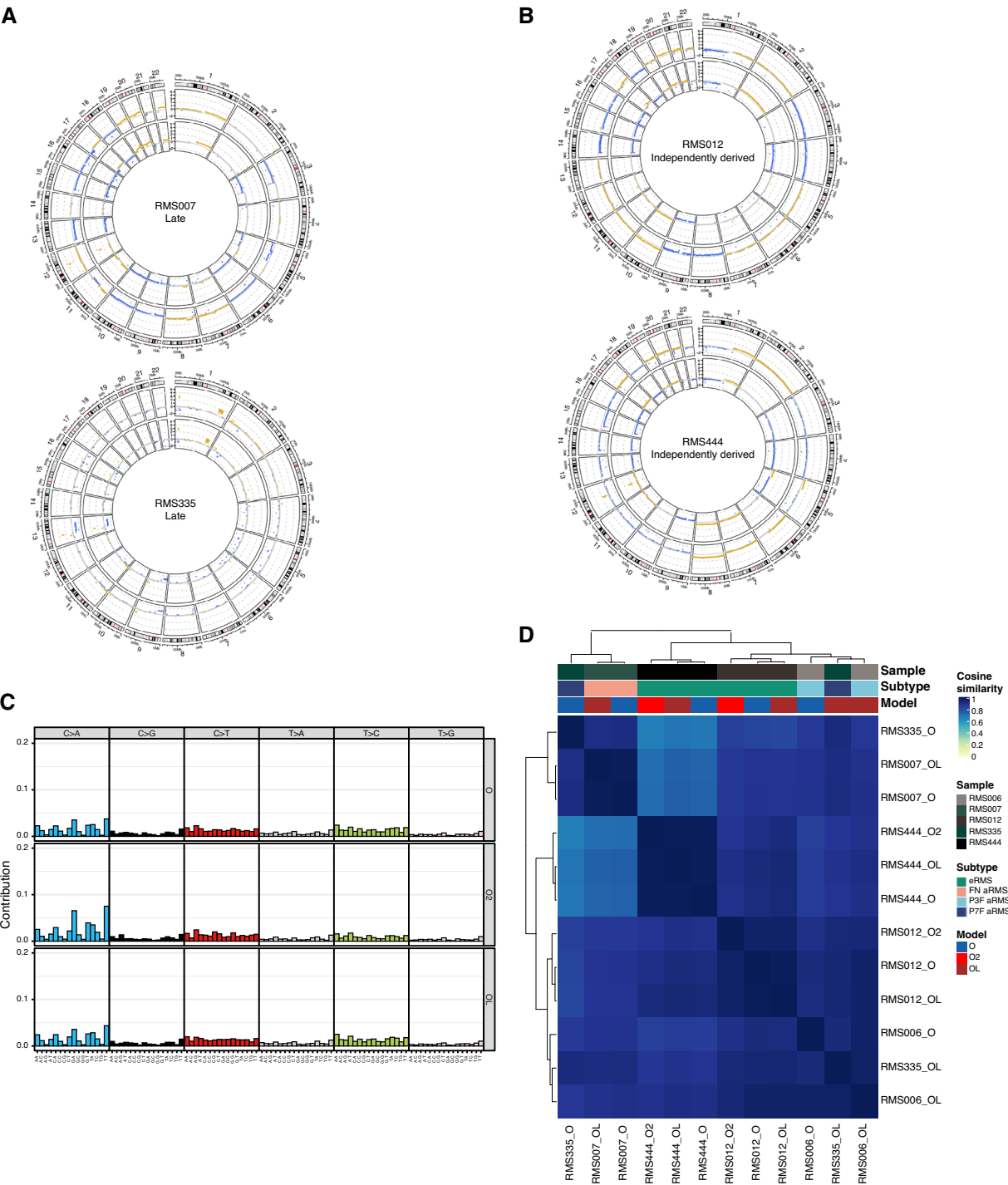


Figure EV4. Established RMS tumoroid models maintain the clonal composition of the RMS tumor they were derived from to a large extent.

A Correlation plot of variant allele fraction (VAF) of single-nucleotide variants (SNVs) detected in RMS tumors and derived tumoroid models per pair.
B Density plots of alternate allele (B-allele) fraction over the entire genome per RMS tumor (red) and derived RMS tumoroid model (blue) per pair.



A

Indel Spectrum

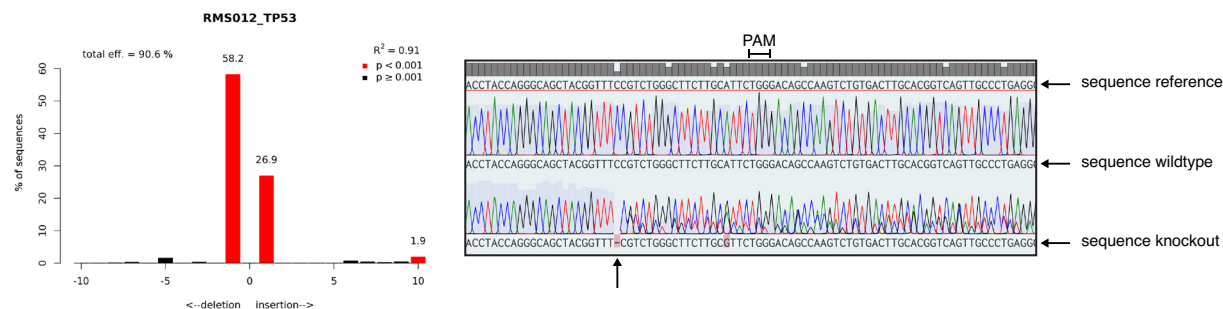
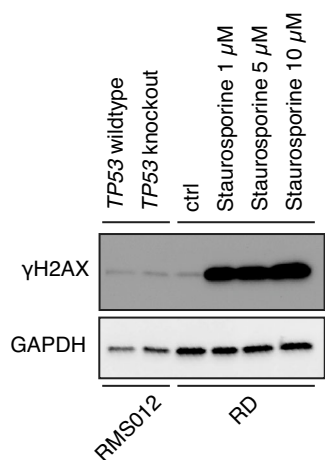
**B**

Figure EV6. RMS tumoroid models can be molecularly edited using CRISPR/Cas9 with P53-deficient eRMS being more sensitive to the checkpoint kinase inhibitor prexasertib (additional figures).

A Left panel: Inferred Indel Spectrum from Sanger sequencing using the TIDE tool (<https://tide.nki.nl>). Right panel: Sanger sequencing around Cas9 cut site (arrow at the bottom) in *TP53* gene. Protospacer Adjacent Motif (PAM) sequence indicated.

B Western Blotting analysis of γ H2AX (Ser-139) as proxy for DNA double-strand breaks in untreated RMS012 tumoroid cells (*TP53* wildtype and *TP53* knockout). RMS cell line RD treated with staurosporine served as positive control while GAPDH served as loading control.

Source data are available online for this figure.