

Fig 2-S2

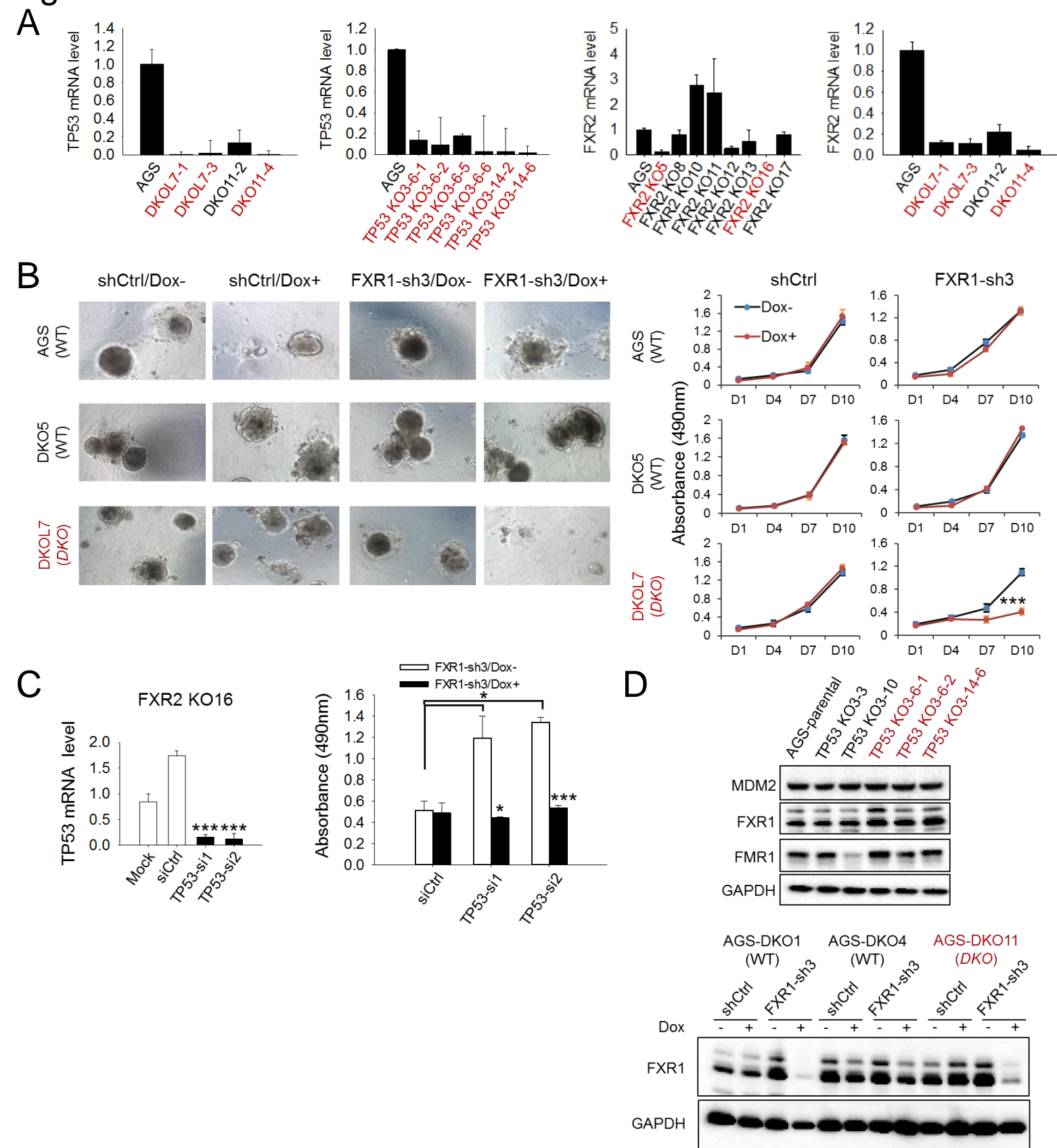


Figure 2—Figure Supplement 2. Effect of FXR1 knockdown on cell proliferation in CRISPR-Cas9-engineered clones.

(A) *TP53* and/or *FXR2* expression in *TP53/FXR2* double knockout (DKO) clones (left), *TP53* single knockout (KO) clones (middle), and *FXR2* single KO clones (right) by q-RT-PCR. The mRNA level in AGS parental cells is set as 1. The wild type clones are in black, and the KO clones are in red.

(B) AGS parental cell, clone DKO5 (copy number normal, WT), or double knockout clone DKOL7 (*TP53/FXR2* double knockout) stably expressing control shRNA (shCtrl) or FXR1-sh3 are cultured on Matrigel for one day followed by Dox treatment for up to 10 days. Left, organoids are imaged at the last time point. Right, cell growth in organoids is measured by MTS assay at indicated time points. Data represent the mean \pm s.d. of three independent experiments. The cell proliferation rate in MTS was determined by measuring absorbance at 490nm (Y axis).

(C) p53 deficiency is required for FXR1's function in proliferation regulation. Left, *TP53* siRNAs-mediated silence of expression. siCtrl, control non-coding siRNA. Right, effect of *TP53* siRNA and the combination with FXR1 knockdown on cell proliferation in *FXR2* knockout clone KO16. Data represent the mean \pm s.d. of three independent experiments. The cell proliferation rate in MTS was determined by measuring absorbance at 490nm (Y axis).

The mRNA level shown in q-RT-PCR is fold change normalized to GAPDH mRNA level, data represent mean \pm s.d. of three independent experiments.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

(D) Protein level of FXR1, FMR1 and MDM2 in AGS normal (WT) and *TP53* knockout (KO) or *TP53/FXR2* double KO (DKO) cells by immunoblotting. The knockout clones are marked in red. GAPDH is used as an internal control.