

## Expanded View Figures

**Figure EV1. Characterization of pre- and post-FFX (FOLFIRINOX) models.**

- A Schematic illustration of the biopsy strategy and downstream applications.
- B GLUT1 IHC of embedded and sectioned organoids (p30 in both PDO lines). Scale bar = 50  $\mu$ m.
- C Growth curves of ID188 (p27–29) and ID211 (p26–28) in 3D culture measured using CellTiter Glo assay for five consecutive days. Shown is the mean  $\pm$  SD of three independent replicates. Unpaired t-test \*\*\*\* $P < 0.0001$ .
- D Western blots of E-cadherin, GATA6, and KRT81 in ID188 (p5+33) and ID211 (p9+15) 2D lines. Actin = loading control.  $n = 2$ .
- E *Vimentin* and *SNAI2* mRNA expression retrieved from RNA-seq experiments in ID188 and ID211 organoids. Shown is the mean  $\pm$  SD of two technical replicates.

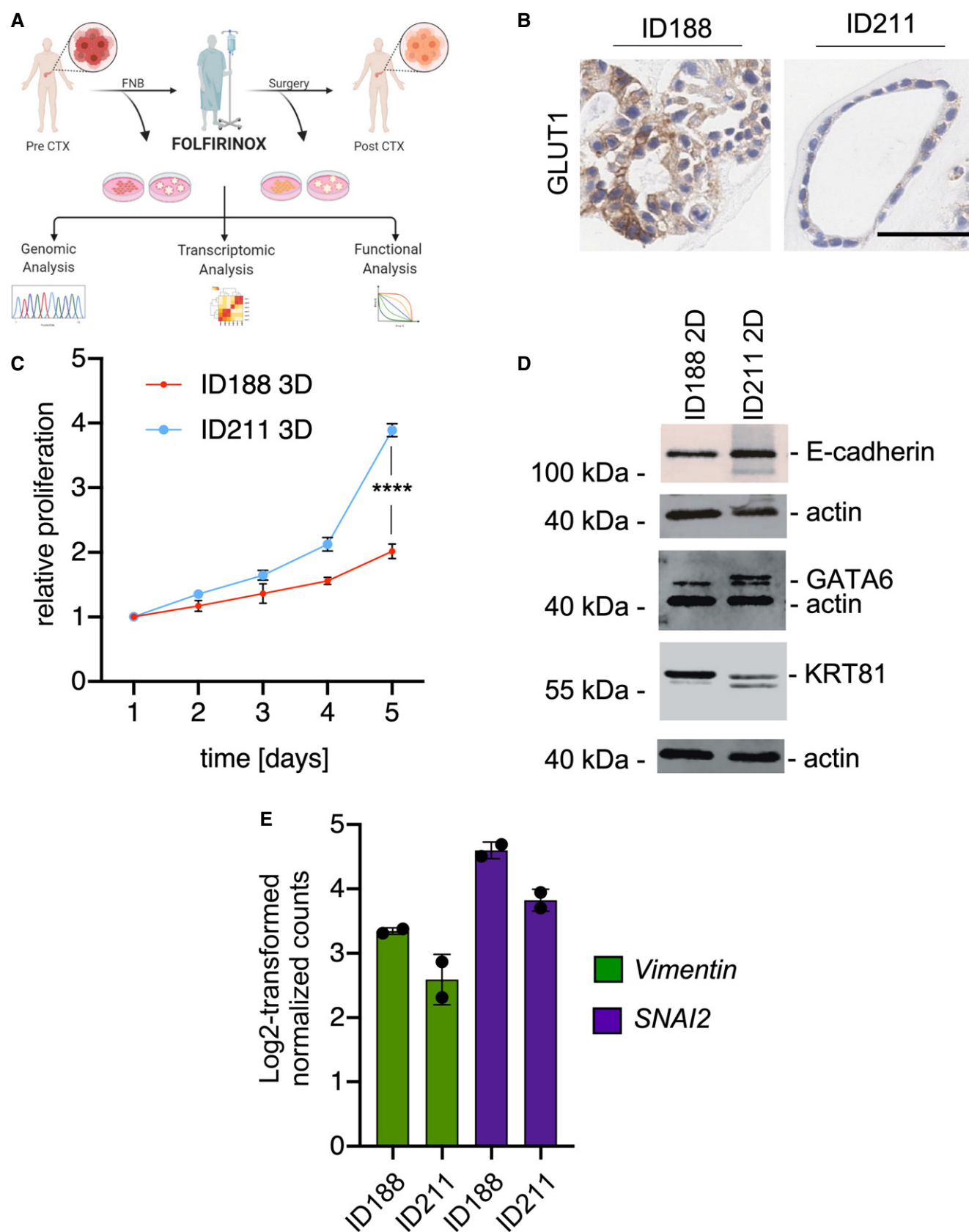
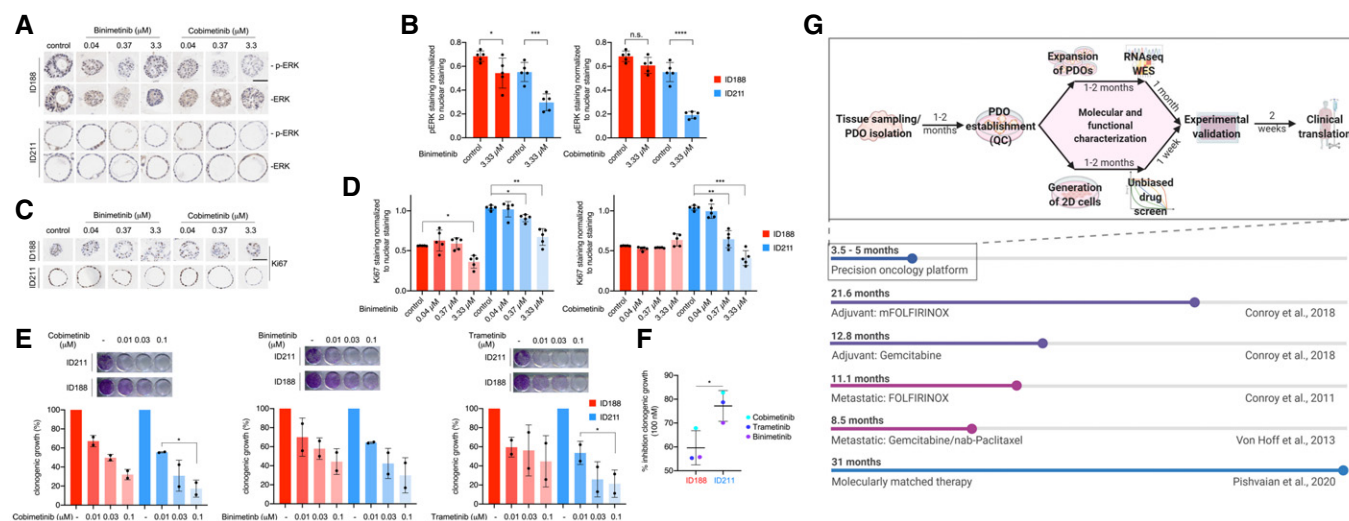


Figure EV1.



**Figure EV2. MEKi response.**

- A pERK IHC staining of embedded and sectioned organoids of ID188 (p30) and ID211 (p30) upon 24 h of binimetinib and cobimetinib treatment. Scale bar = 50 μm.
- B Quantification of the pERK staining normalized to nuclear staining. Shown is the mean ± SD of  $n = 5$  organoid images. Paired  $t$ -test: n.s. = not significant, \* $P < 0.05$ , \*\*\* $P < 0.001$ , and \*\*\*\* $P < 0.0001$ .
- C Ki67 IHC staining of embedded and sectioned organoids of ID188 (p30) and ID211 (p30) upon 24 h of binimetinib and cobimetinib treatment. Scale bar = 50 μm.
- D Quantification of the Ki67 staining normalized to nuclear staining. Shown is the mean ± SD of  $n = 5$  organoid images. ANOVA test: \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ .
- E Clonogenic assay of the indicated 2D lines (ID188 p5+29–36, ID211 p9+13–16). Upper panel: illustration of one out of two clonogenic assays. Lower panel: quantification; Shown is the mean ± SD of  $n = 2$  independent experiments. ANOVA test: \* $P < 0.05$ .
- F Percent inhibition of clonogenic growth at 100 nM trametinib, binimetinib, or cobimetinib was calculated. Unpaired  $t$ -test: \* $P < 0.05$ . Shown is the mean ± SD of  $n = 3$  biological replicates.
- G Timeline from tissue sampling to clinical translation of our longitudinal pipeline in context of the median survival observed with standard of care therapies.