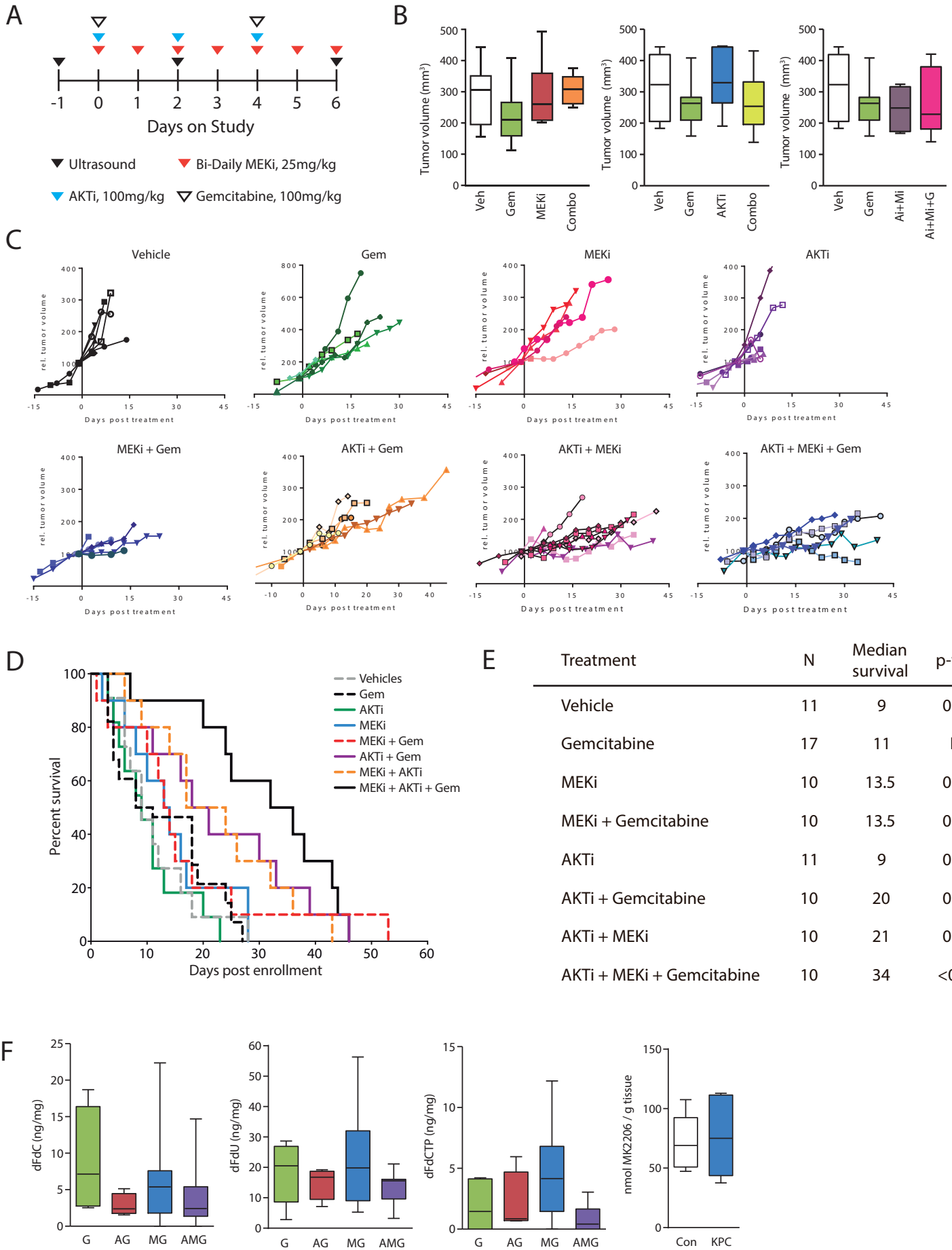
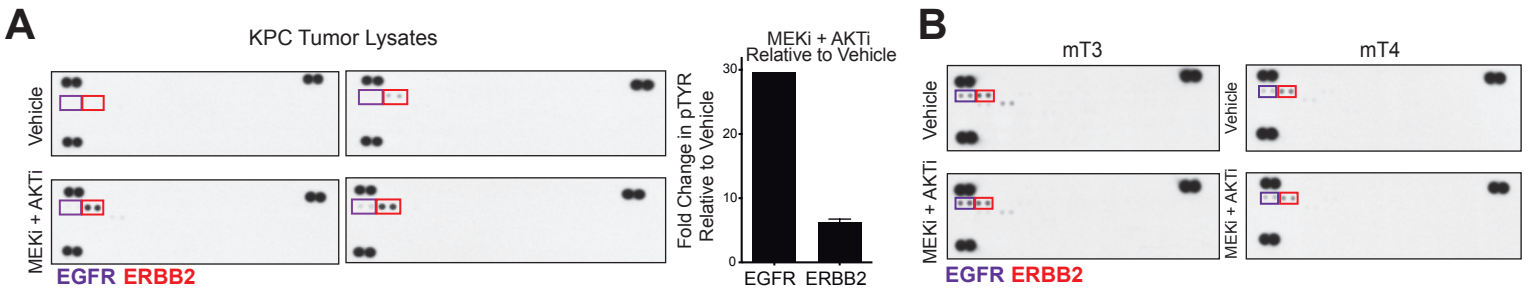


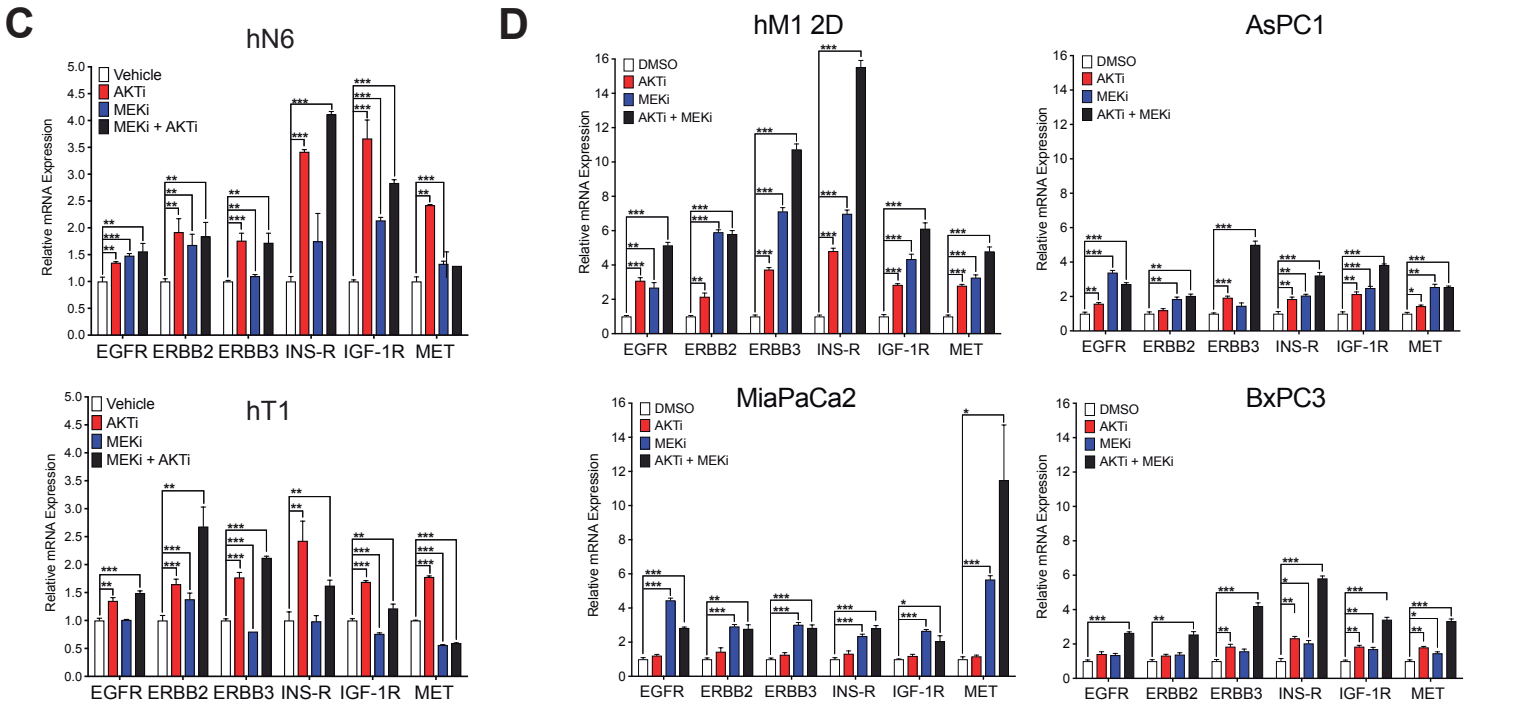
E

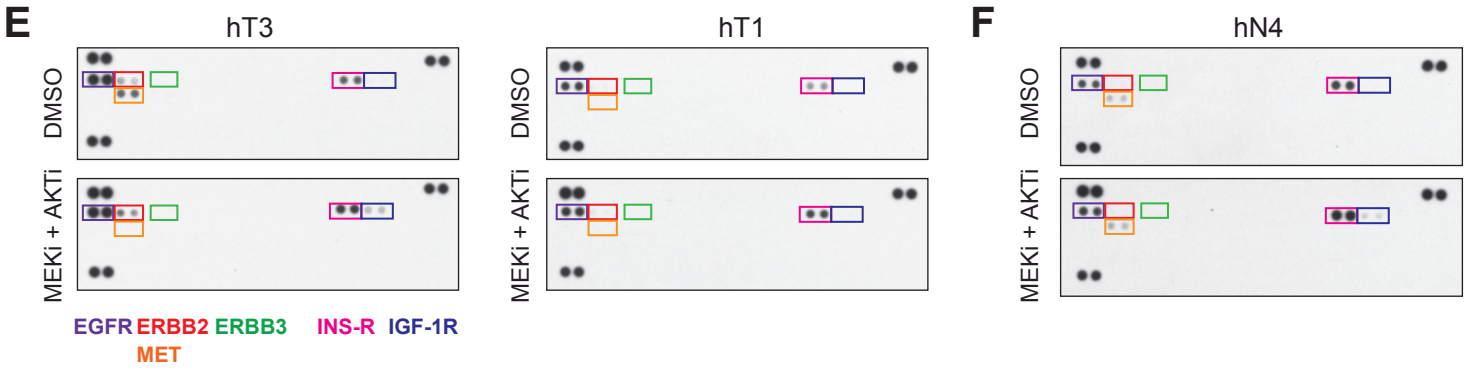
Treatment	N	Median survival	p-value
Vehicle	11	9	0.885
Gemcitabine	17	11	N/A
MEKi	10	13.5	0.563
MEKi + Gemcitabine	10	13.5	0.727
AKTi	11	9	0.333
AKTi + Gemcitabine	10	20	0.035
AKTi + MEKi	10	21	0.037
AKTi + MEKi + Gemcitabine	10	34	<0.001

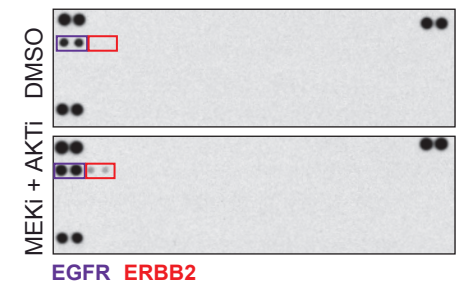
SUPPLEMENTARY FIGURE 1

Supplementary Figure S1.
A) Treatment schedule for *in vivo* studies with single agent, dual, and triple combinations using MEKi, AKTi, and Gemcitabine (Gem). MEKi and AKTi were given by oral gavage and Gem was administered intraperitoneally. Related to **figure 1B – G. B)** Pre-enrollment tumor volumes as measured by ultrasound indicating no significant differences between initial tumor volumes in the different cohorts. Related to **figure 1 B – G. C)** Representative individual relative tumor volumes for mice in each survival cohort are plotted as a function of the initial tumor volume upon treatment over time. Related to **figure 1 B – G. D)** Survival curves of KPC mice treated with vehicle, Gemcitabine (Gem), AKTi, MEKi, MEKi + Gem, AKTi + Gem, MEKi + AKTi, or with MEKi + AKTi + Gem. Related to **figure 1F**. **E)** Median survival of each cohort of KPC mice. p-values are measured relative to Gemcitabine monotherapy using log rank t tests. Related to **figure 1F**. **F)** Therapeutics do not alter drug pharmacokinetics. Intratumor levels of native gemcitabine dFdC, its inactivated metabolite dFdU, or its activated metabolite dFdCTP are not significantly different between survival cohorts ($n \geq 7$). MK2206 levels are not significantly different in KPC tumors (n=5) compared to pancreata from control PC animals (n=4). Related to **figure 1F.**









Fold induction after treatment

phospho-RTK	hM1 2D
EGFR	2.3
ERBB2	6.8

H

EGFR				
DRUG	DMSO	AKTi	MEKi	AKTi+MEKi
TOTAL	1.00	0.96	0.90	0.86
PHOSPHO	1.00	0.99	1.04	0.96

ERBB2				
DRUG	DMSO	AKTi	MEKi	AKTi+MEKi
TOTAL	1.00	1.20	1.08	1.19
PHOSPHO	1.00	1.47	0.98	1.22

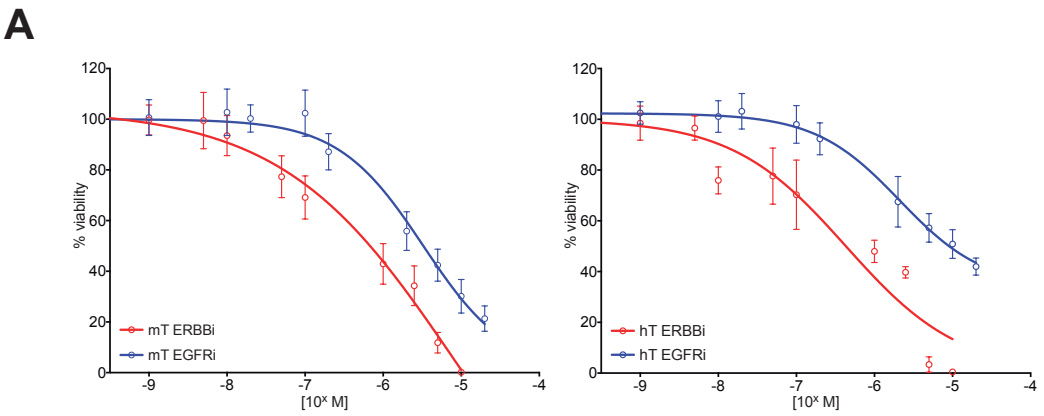
ERBB3				
DRUG	DMSO	AKTi	MEKi	AKTi+MEKi
TOTAL	1.00	1.29	1.07	1.26
PHOSPHO	1.00	1.39	1.04	1.65

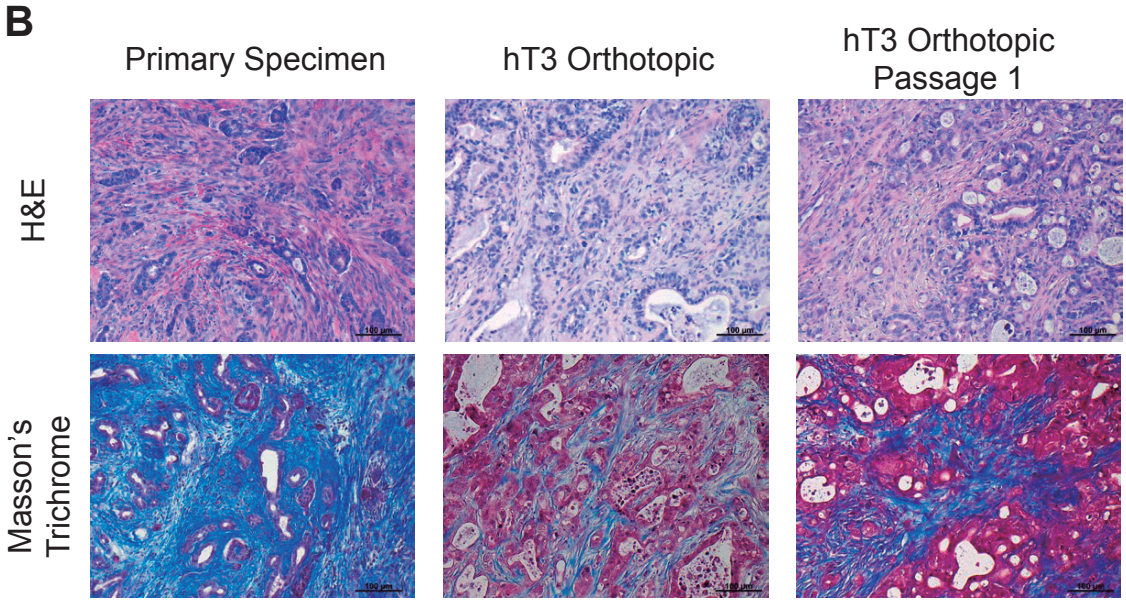
SUPPLEMENTARY FIGURE 2

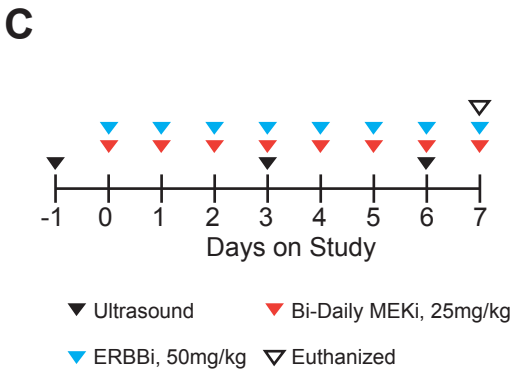
Supplementary Figure S2.

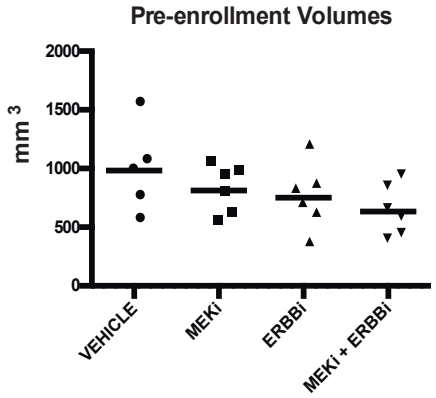
A) Lysates from KPC tumors treated for 7 days with Vehicle or with MEKi + AKTi (n = 2 mice per group) were applied to phospho-RTK arrays and for each phospho-protein, fold induction relative to experimental controls was determined. Quantification of changes in phosphorylation of Egfr and Erbb2 detected by phospho-RTK array is displayed on the right, with error bars indicating standard deviation of the means. Related **to figure 1G**.

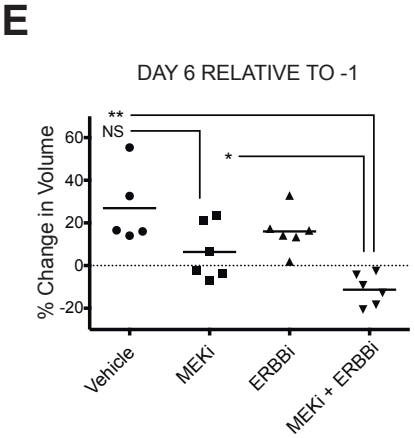
B) Phospho-RTK array on mouse tumor organoid lysates. Related to **figure 2H**. **C)** Changes in mRNA levels of ERBB family members and other RTKs in hN6 and hT1 organoids following 72 hours of single agent or combination treatment with MEK and AKT inhibitors. n = 3 per group (* $p<0.05$, ** $p<0.01$, and *** $p<0.001$). Related to **figure 3E, 3F**. **D)** Changes in mRNA levels of ERBB family members and other RTKs in human PDA monolayer cell lines following 48 hours of single agent or combination treatment with MEK and AKT inhibitors. Normalized, quantified, and plotted as in **B**. Related to **figure 3E**. **E)** Phospho-RTK arrays for the other hT organoids used (hT1 and hT3). Lysates (200 µg) obtained from an hT organoid culture treated with vehicle (DMSO) or MEKi + AKTi (1 µM each) for 48 hours were applied to Phospho-RTK arrays. Related to **figure 3G, 3H. F)** Phospho-RTK arrays for the other hN organoid used (hN4) as described in **D**. Related to **figure 3G, 3H**. **G)** Phospho-RTK arrays for hM1-2D cells treated with vehicle (DMSO) or MEKi + AKTi. Related to **figure 3G, 3H**. **H)** Quantification of the change following immunoblot of total and phosphorylated protein levels of EGFR, ERBB2, and ERBB3 in hM1 organoids after treatment with single and dual agent MEKi and AKTi relative to the vehicle control. Values were first normalized to the loading control HSP90. Related to **figure 4I**.

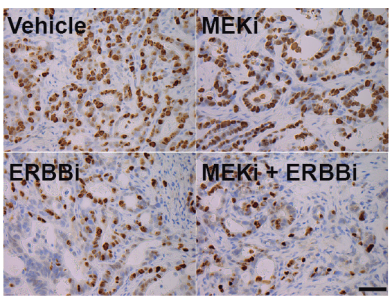


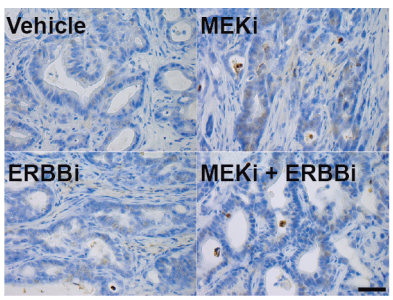












SUPPLEMENTARY FIGURE 3

Supplementary Figure S3

A) EGFRi and ERBBi dose response curves with their 95% confidence interval for hT and mT organoids (n = 3, each). For combination treatments, the EC50 for the first drug is listed. Related to figure 4. **B)** Hematoxylin and Eosin (H&E) and Masson's Trichrome staining of tumors from the primary patient specimen, the corresponding orthotopic graft of the human tumor organoid line hT3 and the subsequent passage of the orthotopic graft. Scale bars = 100 μM, **C)** Treatment schedule for *in vivo* studies with vehicle, MEKi, ERBBi, or MEKi + ERBBi. All drugs were given by oral gavage. **D)** Pre-enrollment volumes of the passage 1 hT3 orthotopic xenografts as quantified by ultrasound. **E)** Percentage of tumor growth shown as the total change between the pre-enrollment scan (Day -1) and the last ultrasound on Day 6 for the different treatment groups. **F)** Immunohistochemistry of Ki67 in the tumor sections. Related to lower left panel in **figure 5G.** Scale bars = 50 μM. **G)** Immunohistochemistry of cleaved caspase (CC3). Scale bars = 50 μM. Related to lower right panel in **figure 5G.**