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Supplemental Information

Overcoming Adaptive Resistance to KRAS and MEK Inhibitors by Co-targeting mTORC1/2 Complexes in Pancreatic Cancer

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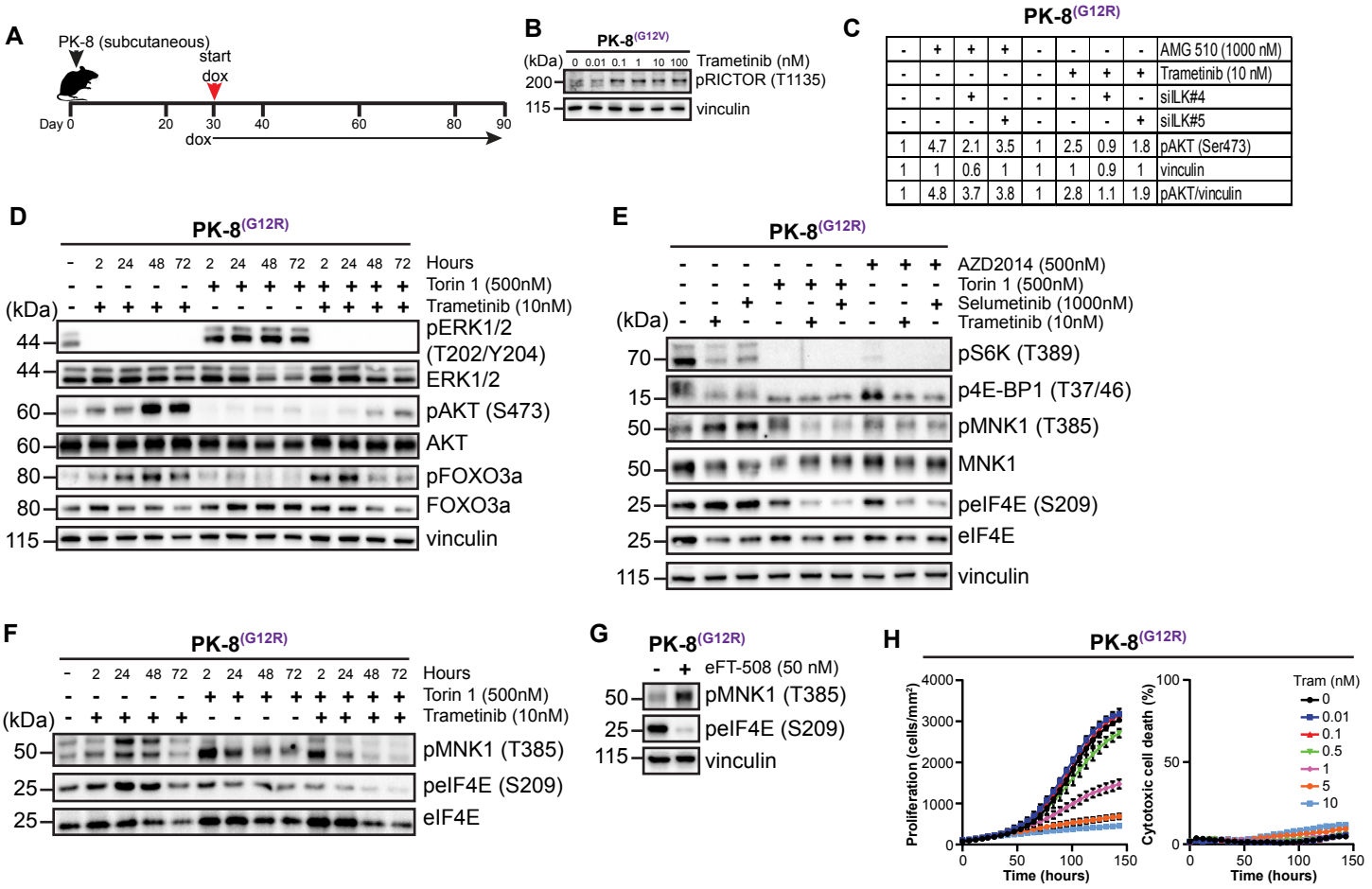

Figure S1. Combination of KRAS or MEK inhbitors with mTORC1/2 inhibitors prevents activation of compensatory pathways in PDAC cells and enhances toxicity, Related to Figures 1 to 5. (A) Schematic showing the experimental design and timeline of administration of doxycycline for the inducible shRNA-mediated knockdown of KRAS in the PK-8 xenografts described in Fig. 1A. **(B)** Immunoblotting of Rictor phosphorylation in PK-8 cells cultured with escalating concentrations of trametinib for 72 hours. **(C)** Table showing densitometry values levels for levels of expression of pAKT in MIA PaCa-2 cells transfected with 2 independent siRNAs targeting ILK described in Fig. 2D. **(D)** Immunoblotting of ERK, AKT and FOXO3a phosphorylation in PK-8 cells incubated with 10 nM trametinib and 500 nM torin-1, alone and in combination, for the indicated times. **(E)** Immunoblotting of S6K, 4E-BP1, Mnk and eIF4E phosphorylation in PK-8 cells incubated with MEK inhibitors (Trametinib, 10 nM; Selumetinib, 1000 nM) and TORC1/2 inhibitors (Torin-1, 500 nM, AZD2014, 500 nM), alone and in combination for 72 hours. **(F)** Immunoblotting of Mnk and eIF4E phosphorylation in PK-8 cells incubated with 10 nM trametinib and 500 nM torin-1, alone and in combination, for the indicated times. **(G)** Immunoblotting of Mnk and eIF4E phosphorylation in PK-8 cells incubated with 50 nM of the MNK inhibitor, eFT-508, for 72 hours. **(H)** Kinetic evaluation of cell proliferation and cytotoxic cell death of PK-8 cells cultured with escalating concentrations of trametinib.

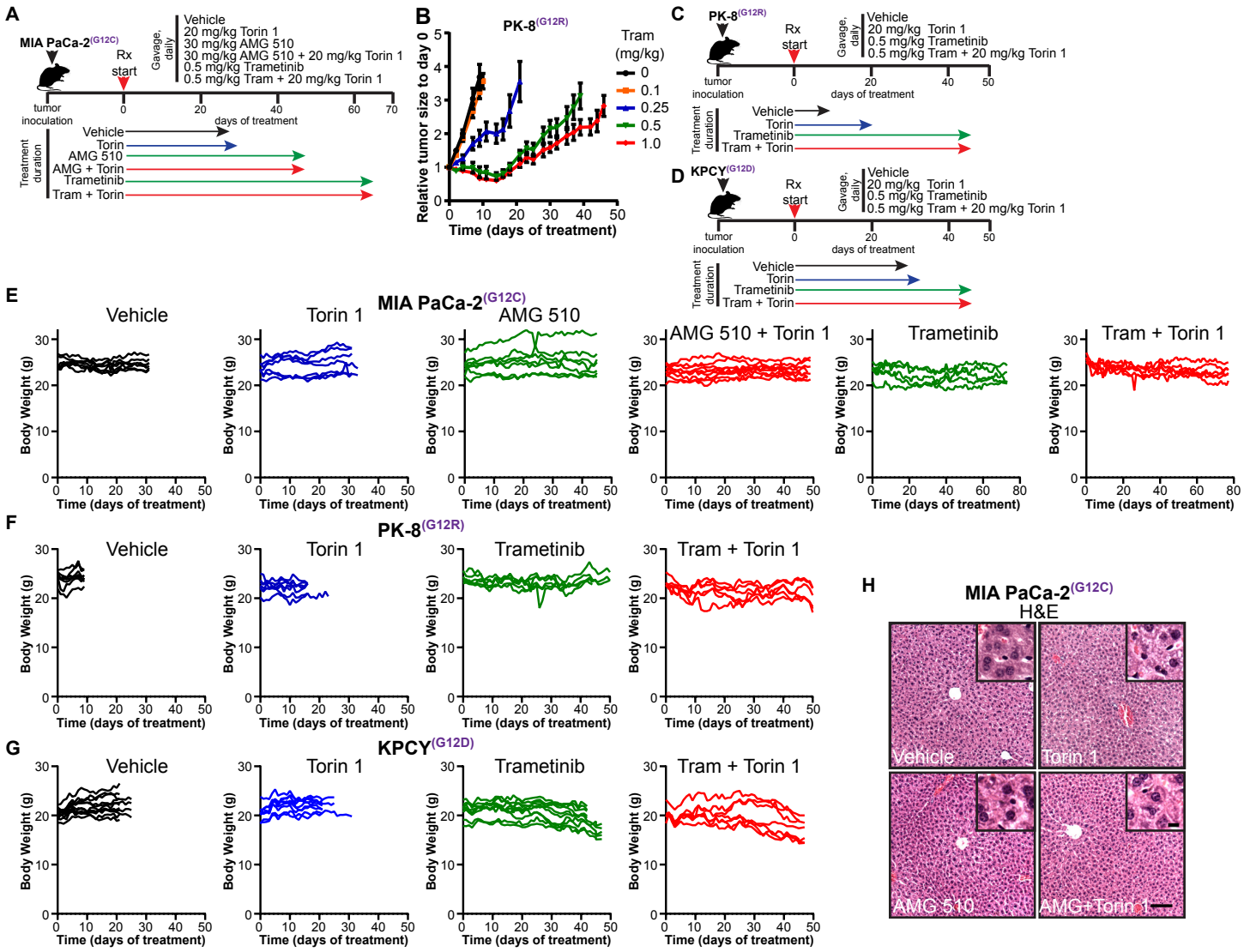

Figure S2. Combined inhibition of KRAS or MEK and mTORC1/2 in vivo in PDAC tumor models is well-tolerated, Related to Figure 6. (A) Schematic showing the experimental design, drug administration timelines and doses used for the MIA PaCa-2 tumor model presented in Figure 6. **(B)** Tumor growth curve of PK-8 xenografts (6 to 8 mice per group, mean ± SEM) administered trametinib at the indicated doses. Statistical analysis by two-way ANOVA. **(C-D)** Schematic showing the experimental design, drug administration timelines and doses used for the **(C)** PK-8 and **(D)** KPCY PDAC tumor models presented in Figure 6. **(E-G)** Graphs showing the individual daily body weight measurements for mice with **(E)** MIA PaCa-2, **(F)** PK-8 and **(G)** KPCY PDAC tumors and treated as indicated. **(H)** Representative images of liver tissue sections from mice with MIA PaCa-2 xenografts administered AMG 510 and torin, alone an in combination, and stained for H&E. Scale bar = 100 μm; Inset, 10 μm.

A **Presence of metastases in animals with PK-8$^{(G12R)}$ xenografts administered increasing doses of trametinib.**

Presence of Metastases (n = 8/group)		
Treatment Arm	**Lung**	**Lymph Node**
Vehicle	0	0
0.1 mg/kg Tram	0	0
0.25 mg/kg Tram	4	0
0.5 mg/kg Tram	5	4
1.0 mg/kg Tram	3	2

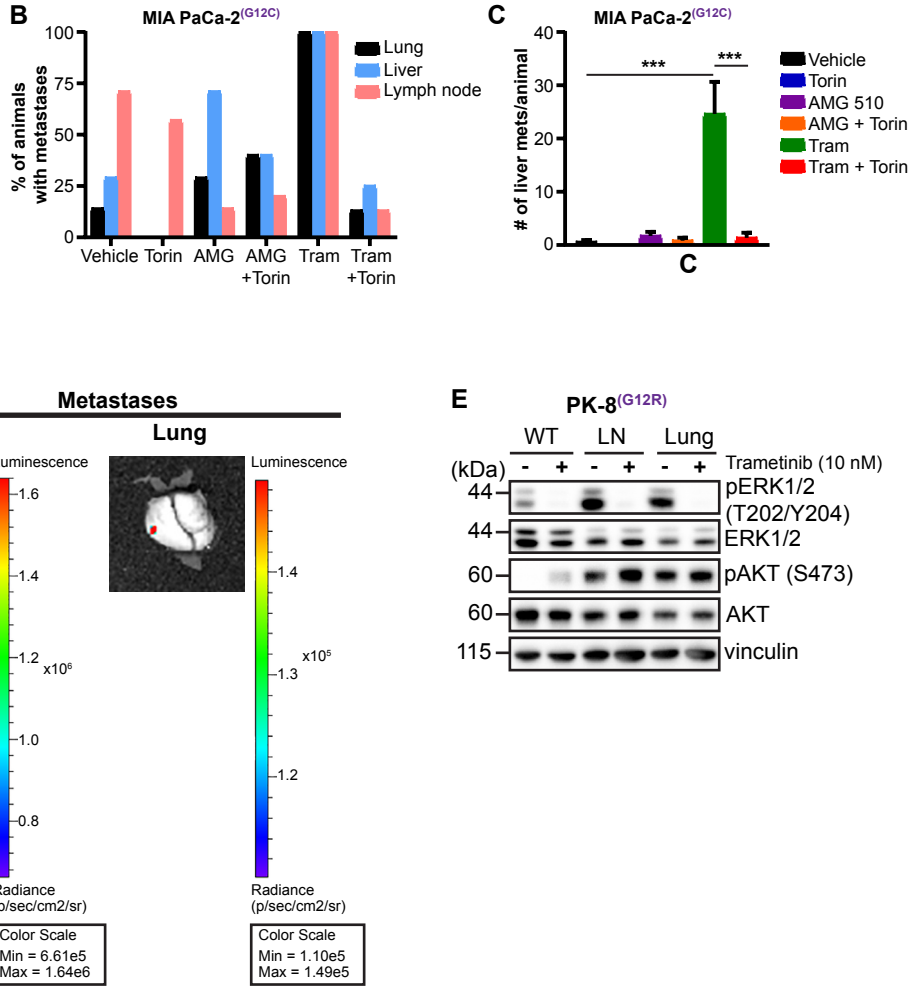

Figure S3. Co-targeting KRAS-MEK and mTORC1/2 effectively prevents the formation of metastases by PDAC tumors <u>in vivo</u>, Related to Figure 6. (A) Table showing the presence of lung and lymph node metastases in mice with PK-8 xenografts treated with increasing doses of trametinib. **(B)** Quantification of the percentage of animals with MIA PaCa-2 tumors treated as described in Figure 6A that showed grossly visible metastases to the lung, liver and lymph nodes. n = 6 to 9 mice per group. **(C)** Quantification of the number of grossly visible liver metastases observed in tumors from animals treated as described in panel B. Statistical analysis by 1-way ANOVA. ***$P < 0.001$. **(D)** Bioluminescence images of a mouse with a PK-8 xenograft treated with 0.5 mg/kg trametinib at endpoint, showing the primary tumor in the live animal and metastases to the lung and lymph node immediately after euthanasia. **(E)** Immunoblotting of ERK and AKT phosphorylation in wild type (WT) PK-8 cells and PK-8 cell lines derived from metastases to the lymph node (LN) and lung (Lung) of PK-8 xenografts treated with trametinib. Cells were cultured with (+) or without (-) trametinib (10 nM) for 72 hours.

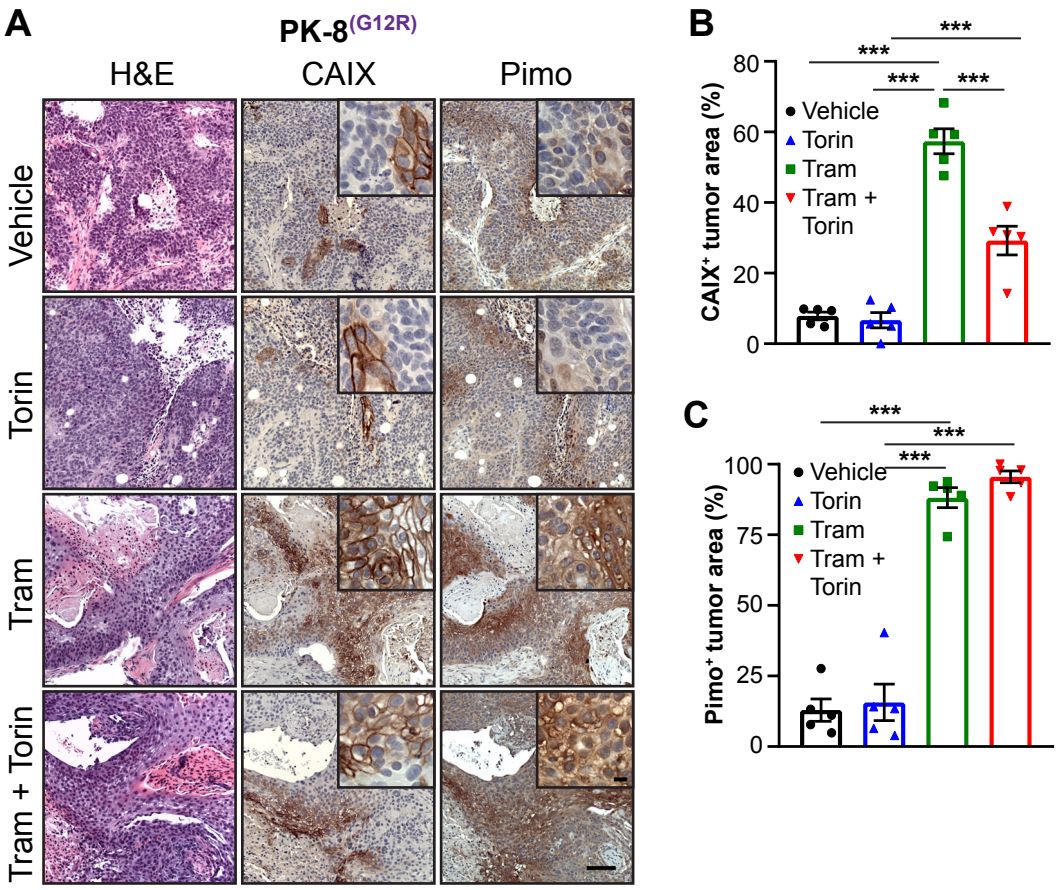

Figure S4. PDAC tumors from animals treated with trametinib, alone and in combination with torin, exhibit extensive hypoxia, Related to Figure 7. (A) Representative images of tumor tissue sections from PK-8 xenografts administered trametinib and torin, alone an in combination, and stained for H&E, CAIX, and pimonidazole. Scale bar = 100 µm; Inset, 10 µm. **(B-C)** Quantification of CAIX (**B**, n = 5, each 5 fields) and pimonidazole (**C**, n = 5, each 5 fields). Statistical analysis by 2-way ANOVA. ***$P < 0.001$.

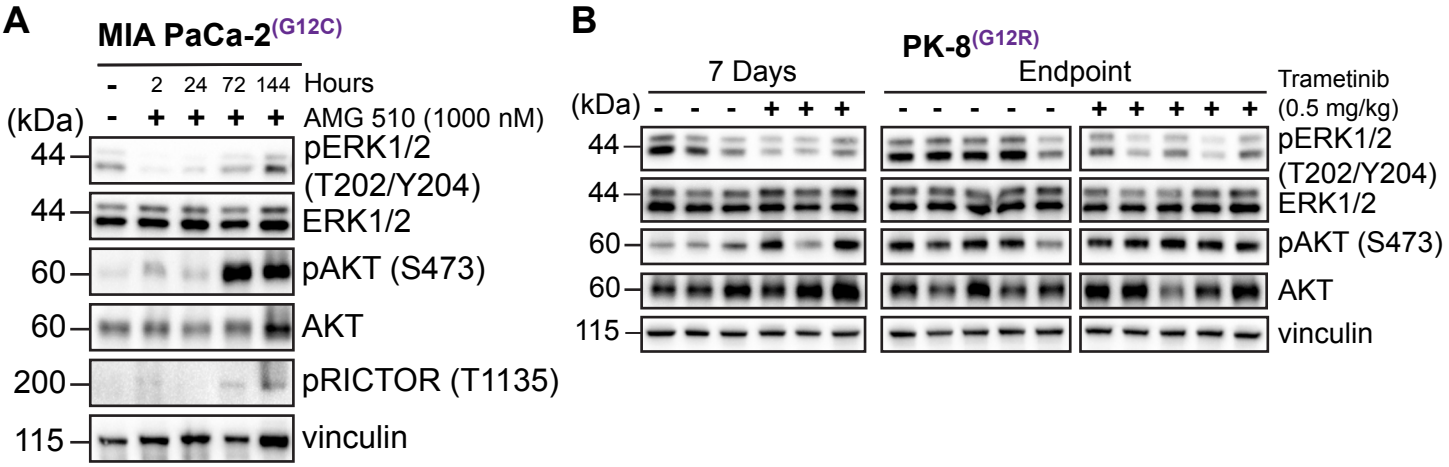

Figure S5. Long-term treatment with trametinib in vitro and in vivo results in sustained phosphorylation and activation of AKT, and suppression of pERK in PDAC cells, Related to Figure 7. (A) Immunoblotting of ERK, AKT and Rictor phosphorylation in MIA PaCa-2 cells cultured with escalating concentrations of the $KRAS^{G12C}$ inhibitor AMG510 for 7 days. **(B)** Immunoblotting of resected PK-8 tumors (n = 3-5 mice per group) for the indicated proteins. Lysates are from tumors harvested after 7 days of treatment and at the tumor volume endpoint for each group. While samples were loaded across multiple gels due to the large number of individual samples to be assessed, Western blots for all samples were imaged simultaneously using identical exposure times to allow for comparison of samples across membranes.