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Supplemental information

KRAS^{G12C}-independent feedback activation of wild-type RAS constrains KRAS^{G12C} inhibitor efficacy

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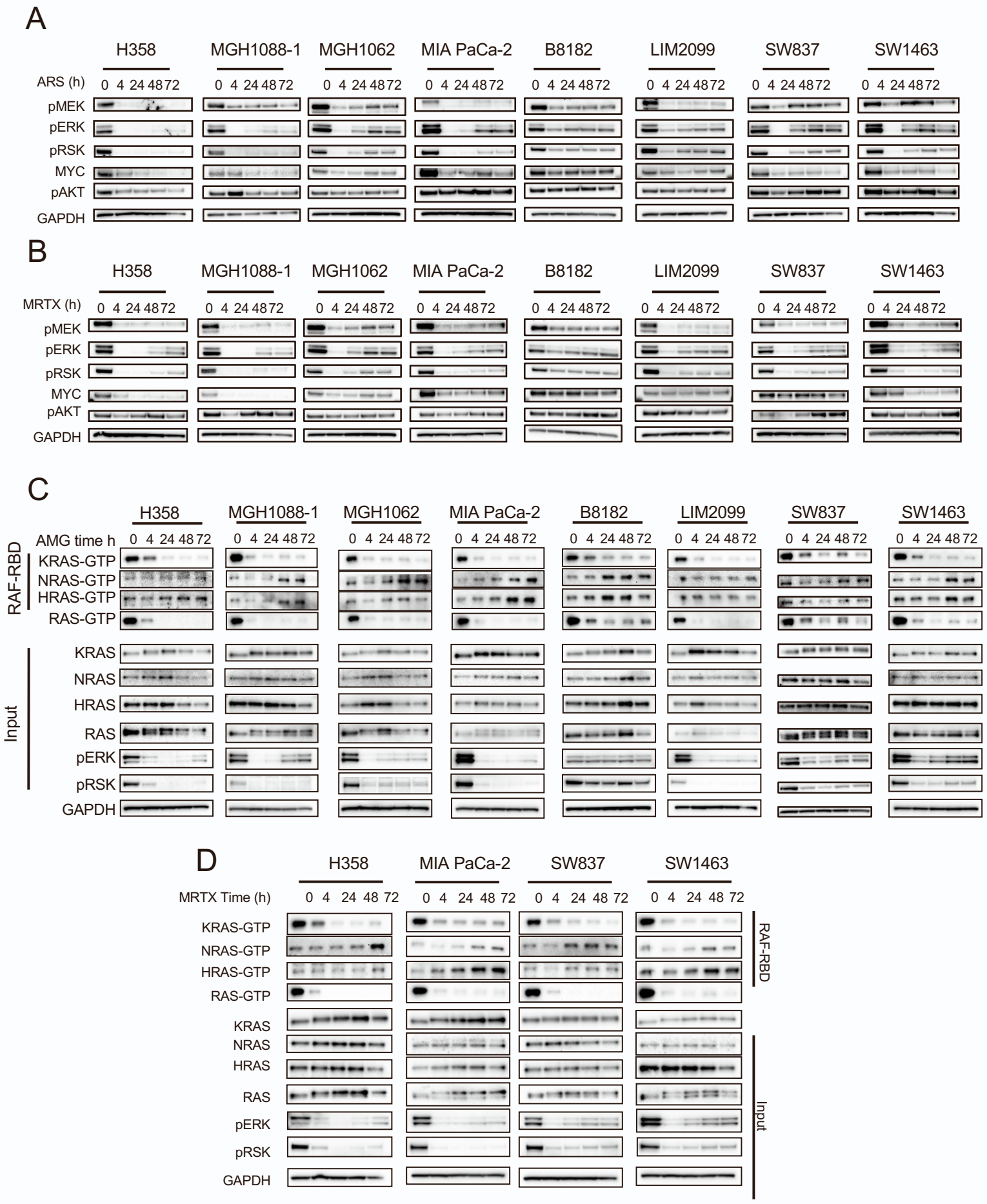
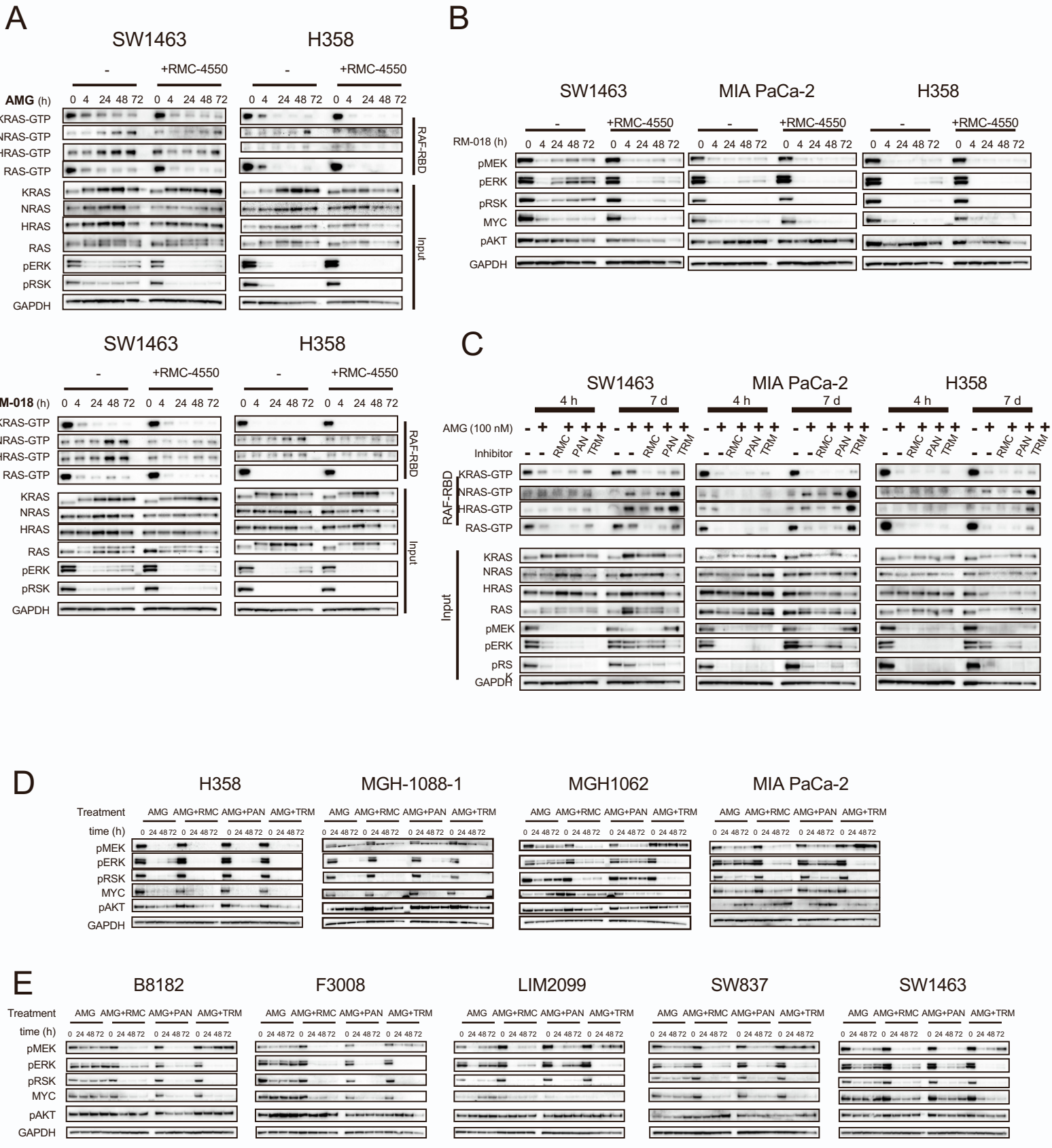
Supplemental Figure 1 (Related to Fig 1 and 2)



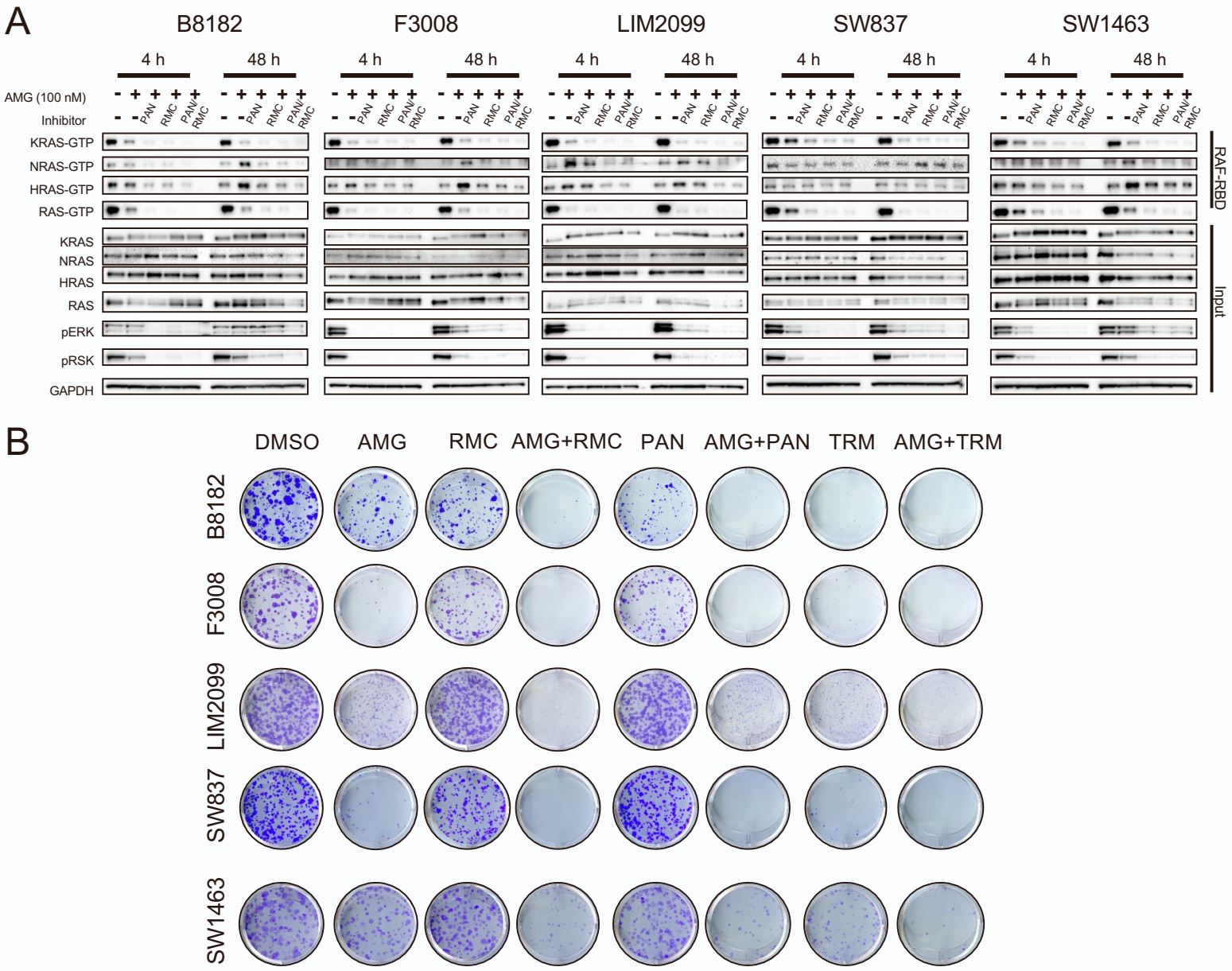
Supplemental Figure 1 KRAS^{G12C} inactive GDP state inhibitors are prone to adaptive feedback reactivation of the MAPK pathway, related to Figures 1 and 2 (A, B) KRAS-^{G12C} mutant cell lines were treated with (A) ARS-1620(10 μM) or (B) MRTX849 (100 nM) for 0, 4, 24, 48, and 72 h. Blot analysis was performed for phospho- (p)MEK, pERK, pRSK, pAKT, and total MYC with GAPDH as a loading control C) Cell lines were treated with AMG 510 (100 nM) for 0, 4, 24, 48, and 72 h and lysates were subject to a RAF-RBD pulldown and blot analysis of KRAS, NRAS, HRAS and total RAS as well as pERK, pRSK and GAPDH for input samples D) Cell lines were treated with MRTX849 (100 nM) for 0, 4, 24, 48, and 72 h and lysates were subject to a RAF-RBD pulldown and blot analysis of KRAS, NRAS, HRAS and total RAS as well as pERK, pRSK and GAPDH for input samples

Supplemental Figure 2 (related to figure 3)



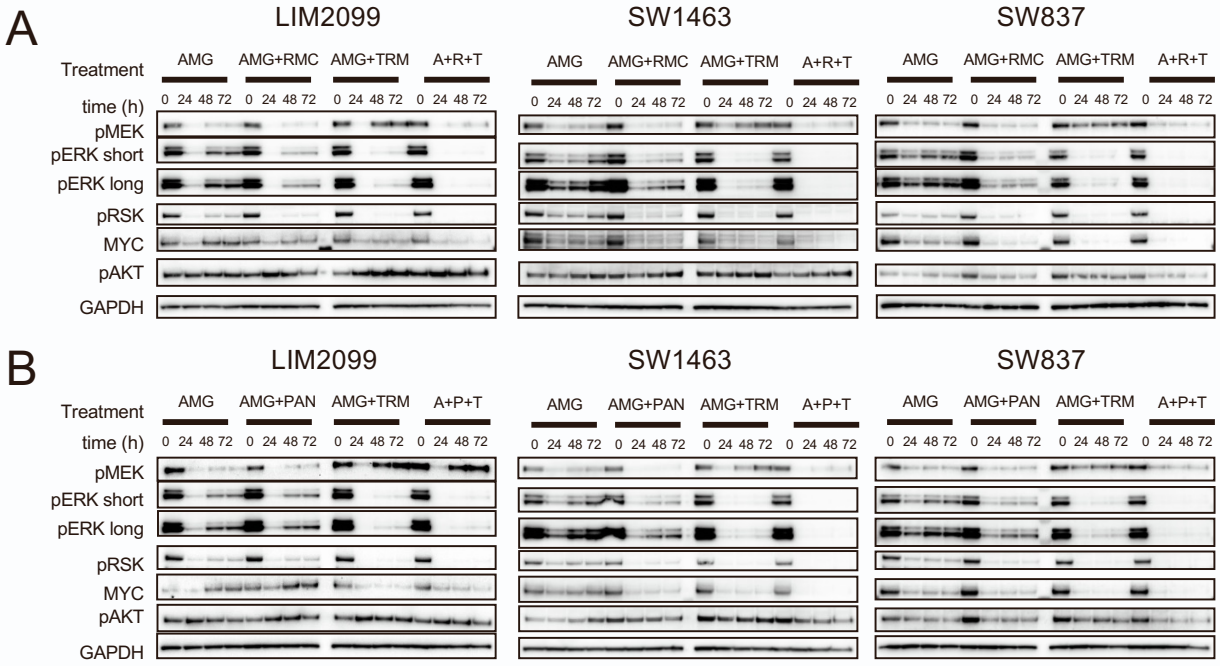
Supplemental Figure 2 Vertical combination strategies abrogate adaptive response to KRAS^{G12C} inhibitors in NSCLC and CRC, Related to Figure 3 (A) SW1463 or H358 cells were treated with AMG 510 (100 nM) or RM-018 (100 nM) alone or in combination with the SHP2 inhibitor RMC-4550 for 4, 24, 48, or 72 h and lysates were subject to a RAF-RBD pulldown and blot analysis of KRAS, NRAS, HRAS and total RAS as well as pERK, pRSK and GAPDH for input samples (B) SW1463, MIA PaCa-2 and H358 cell lines were treated with RM-018 (100 nM) alone or in combination with the SHP2 inhibitor RMC-4550 (1 μM) for 0, 4, 24, 48, and 72 h. Blot analysis was performed for phospho- (p)MEK, pERK, pRSK, pAKT, and total MYC with GAPDH as a loading control (C) SW1463, MIA PaCa-2 and H358 cell lines were treated with AMG 510 (100 nM) alone or in combination with the EGFR mAb panitumumab (30ug/mL), RMC-4550 (1 uM), or the MEK inhibitor trametinib (10 nM) for 4 h or 7 d and lysates were subject to a RAF-RBD pulldown and blot analysis of KRAS, NRAS, HRAS and total RAS as well as pERK, pRSK and GAPDH for input samples. (D, E) Indicated cell lines were treated with AMG 510 (100 nM) alone or in combination with the panitumumab (30ug/mL), RMC-4550 (1 uM), or trametinib (10 nM) for 0, 24, 48, and 72 h. Blot analysis was performed for phospho- (p)MEK, pERK, pRSK, pAKT, and total MYC with GAPDH as a loading control

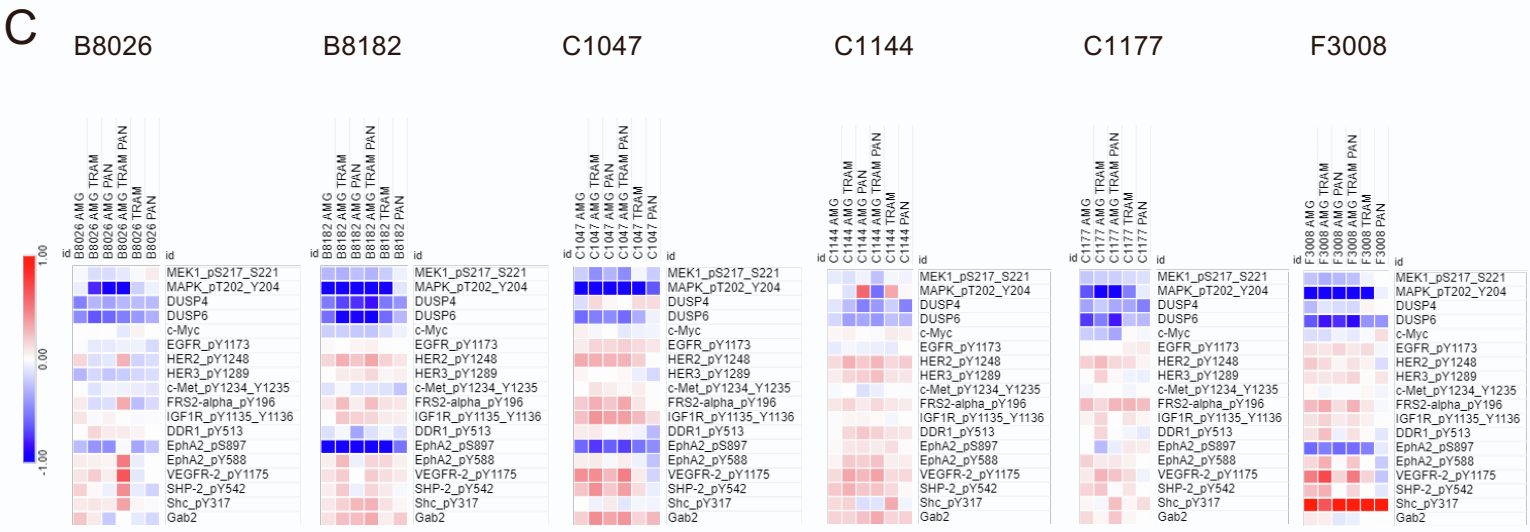
Supplemental Figure 3 (Related to Figure 3)



Supplemental Figure 3, Vertical combination strategies overcome adaptive feedback reactivation of RAS MAPK signaling in CRC, Related to Figure 3 (A) B8182, F3008, LIM2099, SW837, and SW1463 cell lines were treated with AMG 510 (100 nM) alone or in combination with the EGFR mAb panitumumab (30ug/mL), the SHP2 inhibitor RMC-4550 (1 uM), or the MEK inhibitor trametinib (10 nM) for 4 or 48 h and lysates were subject to a RAF-RBD pulldown and blot analysis of KRAS, NRAS, HRAS and total RAS as well as pERK, pRSK and GAPDH for input samples. (B) B8182, F3008, LIM2099, SW837, and SW1463 cell lines were treated with AMG 510 (100 nM) alone or in combination with the panitumumab (30ug/mL), RMC-4550 (1 uM), trametinib (10 nM) for 14 days and then stained with crystal violet.

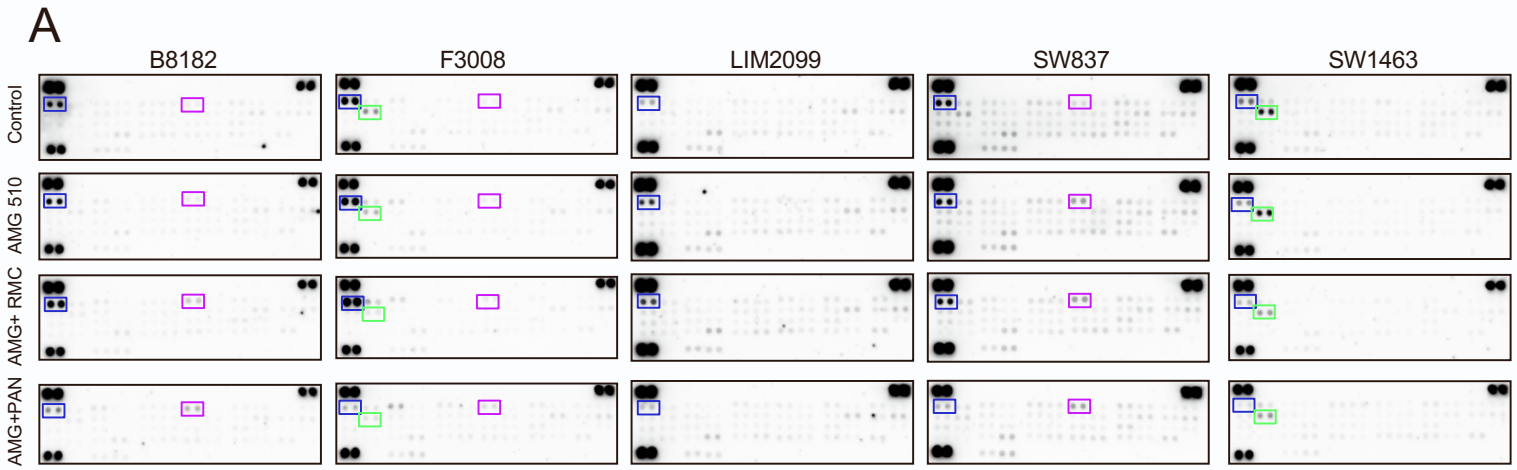
Supplemental Figure 4 (Related to figure 4 and 5)

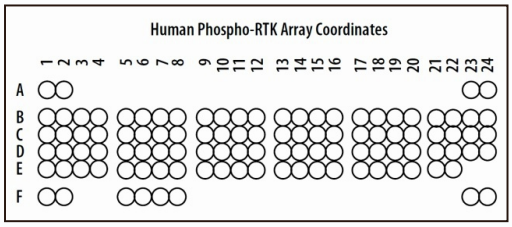




Supplemental Figure 4 EGFR and MEK Doublet and Triplet combination strategies enhance the efficacy of KRAS^{G12C} inhibition in CRC *in vitro* and *in vivo*, related to Figures 4 and 5 (A,B) LIM2099, SW837, and SW1463 cell lines were treated with AMG 510 alone or in combination with panitumumab or RMC-4550, or trametinib for 24, 48, and 72 h. Blot analysis was performed for phospho- (p)MEK, pERK, pRSK, pAKT, and total MYC with GAPDH as a loading control (C) RPPA analysis of KRAS^{G12C} CRC PDX tumors treated with AMG 510 (100 mg/kg), panitumumab (0.5 mg) or trametinib (1 mg/kg) alone or in combination for 3 d

Supplemental Figure 5 (Related to figure 5)

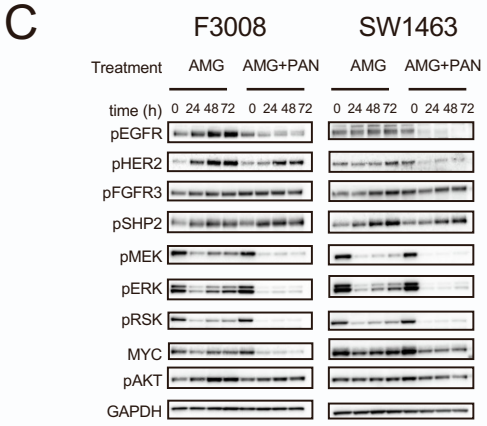


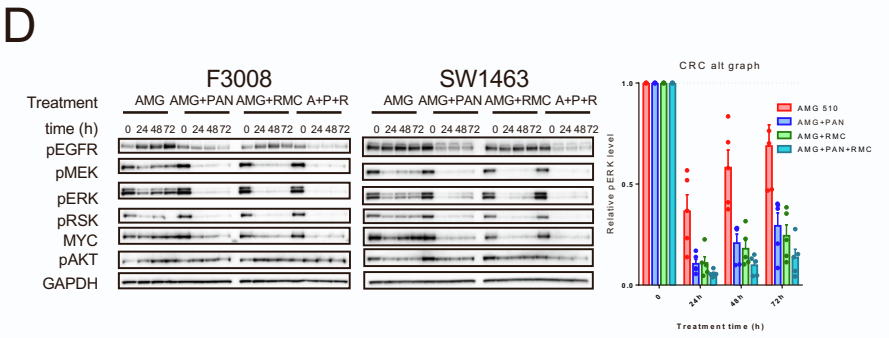


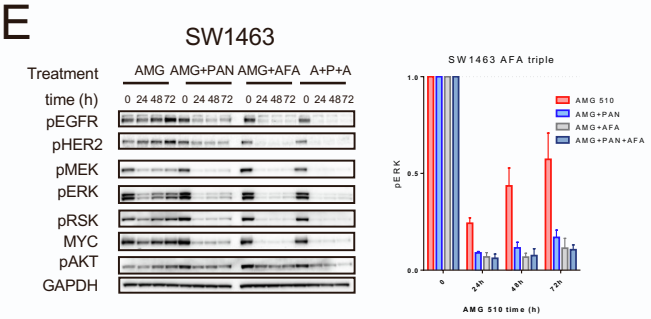
EGFR C-MET FGFR3

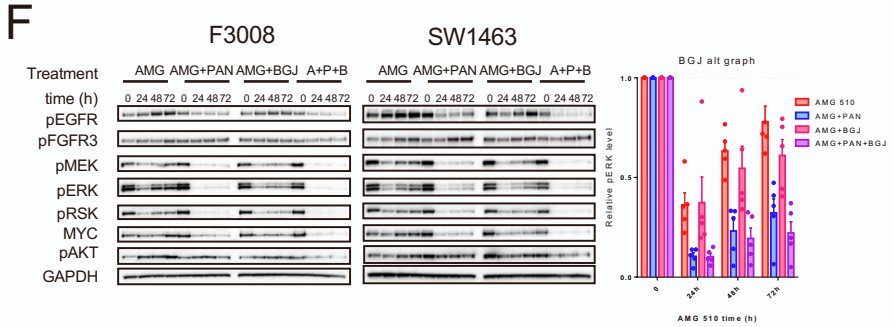
Coordinate	Receptor Family	RTK/Control	Coodinate	Receptor Family	RTK/Control
A1, A2	Reference Spots	——	D1, D2	Tie	Tie-2
A23, A24	Reference Spots	——	D3, D4	NGF R	TrkA
B1, B2	EGF R	EGF R	D5, D6	NGF R	TrkB
B3, B4	EGF R	ErbB2	D7, D8	NGF R	TrkC
B5, B6	EGF R	ErbB3	D9, D10	VEGF R	VEGF R1
B7, B8	EGF R	ErbB4	D11, D12	VEGF R	VEGF R2
B9, B10	FGF R	FGF R1	D13, D14	VEGF R	VEGF R3
B11, B12	FGF R	FGF R2α	D15, D16	MuSK	MuSK
B13, B14	FGF R	FGF R3	D17, D18	Eph R	EphA1
B15, B16	FGF R	FGF R4	D19, D20	Eph R	EphA2
B17, B18	Insulin R	Insulin R	D21, D22	Eph R	EphA3
B19, B20	Insulin R	IGF-I R	D23, D24	Eph R	EphA4
B21, B22	Axl	Axl	E1, E2	Eph R	EphA6
B23, B24	Axl	Dtk	E3, E4	Eph R	EphA7
C1, C2	Axl	Mer	E5, E6	Eph R	EphB1
C3, C4	HGF R	HGF R	E7, E8	Eph R	EphB2
C5, C6	HGF R	MSP R	E9, E10	Eph R	EphB4
C7, C8	PDGF R	PDGF Rα	E11, E12	Eph R	EphB6
C9, C10	PDGF R	PDGF Rβ	E13, E14	Insulin R	ALK
C11, C12	PDGF R	SCF R	E15, E16	——	DDR1
C13, C14	PDGF R	Flt-3	E17, E18	——	DDR2
C15, C16	PDGF R	M-CSF R	E19, E20	Eph R	EphA5
C17, C18	RET	c-Ret	E21, E22	Eph R	EphA10
C19, C20	ROR	ROR1	F1, F2	Reference Spots	——
C21, C22	ROR	ROR2	F5, F6	Eph R	EphB3
C23, C24	Tie	Tie-1	F7, F8	——	RYK
			F23, F24	Control (-)	PBS

RTK	Occurences	Present In
EGFR	5	B8182, F3008, LIM2099, SW837, SW1463
EphB2	5	B8182, F3008, LIM2099, SW837, SW1463
HER2	4	F3008, LIM2099, SW837, SW1463
HER3	4	B8182, F3008, SW837, SW1463
RYK	4	F3008, LIM2099, SW837, SW1463
FGFR3	3	B8182, F3008, SW837
c-MET	3	B8182, F3008, SW1463
EphB3	2	LIM2099, SW837
FGFR1	2	F3008, LIM2099
Insulin-R	2	LIM2099, SW837
ROR1	2	F3008, LIM2099
Tie-1	2	F3008, LIM2099
EphA10	1	LIM2099









Supplemental Figure 5 $KRAS^{G12C}$-mutant CRC models demonstrate RTK heterogeneity that limits the efficacy of $KRAS^{G12C}$ inhibition, related to Figure 5 (A, B) RTK array expression analysis of CRC cell lines treated with AMG 510 alone or in combination with panitumumab or RMC-4550 for 72 h (C) F3008 and SW1463 cell lines were treated with AMG 510 alone or in combination with panitumumab for 24, 48, or 72, Blot analysis was performed for phospho- (p)EGFR, HER2, FGFR3, SHP2, MEK, pERK, pRSK, pAKT, and total MYC with GAPDH as a loading control (D, F) F3008 and SW1463 cell lines were treated with AMG 510 alone or in combination with panitumumab or RMC-4550 and combined densitometry analysis was performed for pERK normalized to GAPDH for all 5 CRC cell lines in Fig 5 and Fig S5 (E) SW1463 cell lines were treated with AMG 510 alone or in combination with panitumumab or the pan-HER inhibitor afatinib and densitometry analysis was performed for pERK normalized to GAPDH