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Supplemental Information

Overcoming Resistance to Dual Innate Immune and MEK Inhibition Downstream of *KRAS*

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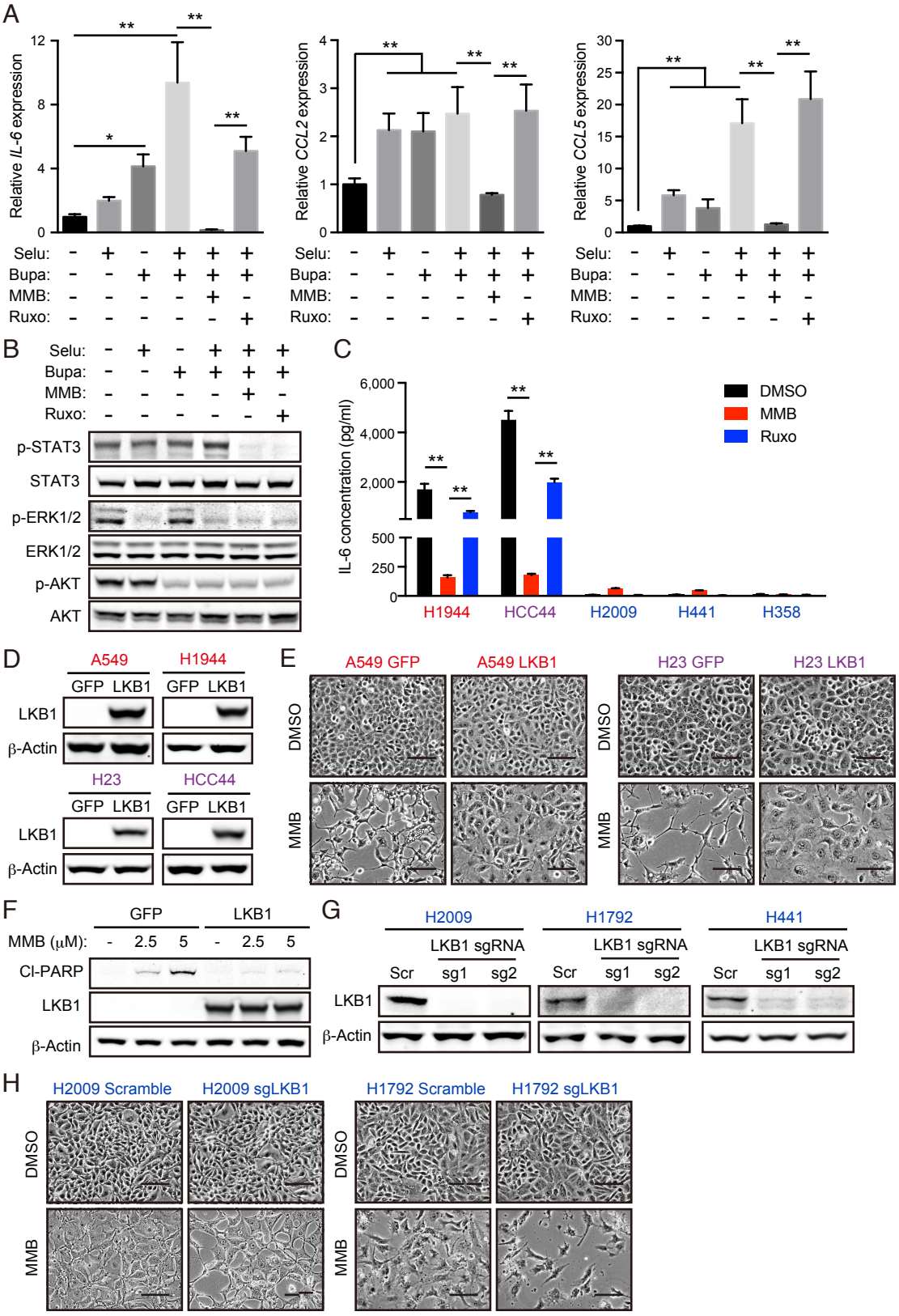

Figure S1. Feedback cytokine induction and MMB sensitivity of KL cells (Related to Figure 1)

(A) qRT-PCR of *IL-6*, *CCL2* and *CCL5* in A549 cells treated with 1 μM selumetinib (Selu), 1 μM buparlisib (Bupa), 2.5 μM momelotinib (MMB) and/or 1 μM ruxolitinib (Ruxo) for 48 hr (n = 4).

(B) Immunoblot (IB) of the indicated proteins in A549 cells treated with 1 μM Selu, 1 μM Bupa, 2.5 μM MMB and/or 1 μM Ruxo for 48 hr.

(C) ELISA of human IL-6 levels in conditioned medium derived from H1944, HCC44, H2009, H441 and H358 cells treated with 2.5 μM MMB or 1 μM Ruxo for 24 hr (n = 2). Red cell lines: KL. Purple cell lines: KLP. Blue cell lines: KP.

(D) IB of the indicated proteins in A549, H1944, H23 and HCC44 cells transduced with the indicated vector. Cells were cultured in serum-free and glucose-free medium for 1 h. Red cell lines: KL. Purple cell lines: KLP.

(E) Phase-contrast images of A549 and H23 cells transduced with the indicated vector and treated with 2.5 μM MMB. Scale bars: 100 μm. Red cell lines: KL. Purple cell lines: KLP.

(F) IB of the indicated proteins in A549 cells transduced with the indicated vector and treated with indicated concentration of MMB for 24 hr.

(G) IB of the indicated proteins in H2009, H1792 and H441 cells transduced with the indicated sgRNA. Blue cell lines: KP.

(H) Phase-contrast images of H2009 and H1792 cells transduced with the indicated vector and treated with 2.5 μM MMB. Scale bars: 100 μm. Blue cell lines: KP.

All quantitative data are represented as mean ± S.D; *$p < 0.05$, **$p < 0.01$.

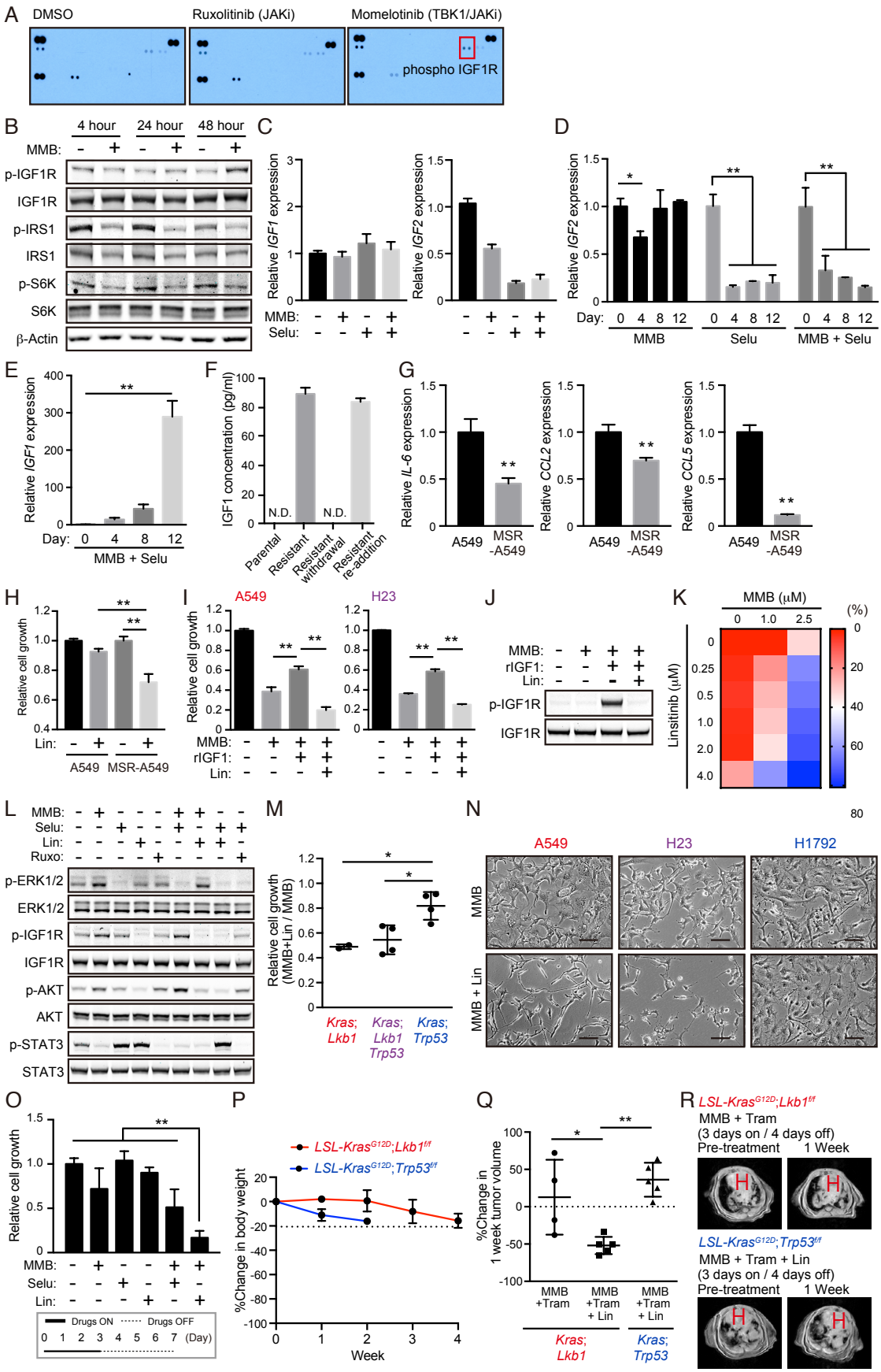
A
DMSO
Ruxolitinib (JAKi)
Momelotinib (TBK1/JAKi)
phospho IGF1R
B
4 hour
24 hour
48 hour
MMB:
p-IGF1R
IGF1R
p-IRS1
IRS1
p-S6K
S6K
β-Actin
C
Relative IGF1 expression
Relative IGF2 expression
MMB:
Selu:
D
Relative IGF2 expression
Day: 0 4 8 12
MMB
Selu
MMB + Selu
E
Relative IGF1 expression
Day: 0 4 8 12
MMB + Selu
F
IGF1 concentration (pg/ml)
N.D.
N.D.
Parental
Resistant
Resistant withdrawal
Resistant re-addition
G
Relative IL-6 expression
Relative CCL2 expression
Relative CCL5 expression
A549
MSR -A549
H
Relative cell growth
Lin:
A549
MSR-A549
I
A549
H23
Relative cell growth
MMB:
rIGF1:
Lin:
J
MMB:
rIGF1:
Lin:
p-IGF1R
IGF1R
K
MMB (μM)
0 1.0 2.5
(%)
Linsitinib (μM)
0
0.25
0.5
1.0
2.0
4.0
0
20
40
60
80
L
MMB:
Selu:
Lin:
Ruxo:
p-ERK1/2
ERK1/2
p-IGF1R
IGF1R
p-AKT
AKT
p-STAT3
STAT3
M
Relative cell growth (MMB+Lin / MMB)
Kras; Lkb1
Kras; Lkb1 Trp53
Kras; Trp53
N
A549
H23
H1792
MMB
MMB + Lin
O
Relative cell growth
MMB:
Selu:
Lin:
Drugs ON
Drugs OFF
0 1 2 3 4 5 6 7 (Day)
P
LSL-KrasG12D;Lkb1f/f
LSL-KrasG12D;Trp53f/f
%Change in body weight
Week
Q
%Change in 1 week tumor volume
MMB +Tram
MMB +Tram + Lin
MMB +Tram + Lin
Kras; Lkb1
Kras; Trp53
R
LSL-KrasG12D;Lkb1f/f
MMB + Tram
(3 days on / 4 days off)
Pre-treatment
1 Week
H
LSL-KrasG12D;Trp53f/f
MMB + Tram + Lin
(3 days on / 4 days off)
Pre-treatment
1 Week
H

Figure S2. IGF1R pathway activation in response to MMB and MEK inhibition (Related to Figure 2)

(A) Receptor tyrosine kinase arrays of A549 cells treated with 5 μM ruxolitinib (Ruxo) or 5 μM MMB for 24 hr. Red box indicates phospho-IGF1R signals.

(B) IB of the indicated proteins in A549 cells treated with 5 μM MMB for the indicated time.

(C) qRT-PCR of *IGF1* and *IGF2* in A549 cells treated with 5 μM MMB and/or 1 μM Selu for 48 hr (n = 4).

(D) qRT-PCR of *IGF2* in A549 treated with 5 μM MMB and/or 1 μM Selu for the indicated time (n = 2).

(E) qRT-PCR of *IGF1* in A549 treated with 5 μM MMB and 1 μM Selu for the indicated time (n = 2).

(F) ELISA of human IGF1 levels in conditioned medium derived from A549, MSR-A549, MSR-A549 withdrawal, and MSR-A549 re-addition cells. N.D.: Not detected (n = 2).

(G) qRT-PCR of *IL-6*, *CCL2* and *CCL5* in A549 and MSR-A549 cells (n = 4).

(H) Relative cell growth of A549 and MSR-A549 cells in the presence of 1 μM linsitinib (Lin) for 72 hr (n = 3).

(I) Relative cell growth of A549 and H23 cells in the presence of 2.5 μM MMB with or without 1 μM Lin and/or 100 ng/ml recombinant IGF1 for 72 hr (n = 3).

(J) IB of the indicated proteins in A549 treated with 2.5 μM MMB with or without 1 μM Lin and/or 100 ng/ml recombinant IGF1 for 24 hr.

(K) Percent inhibition in A549 cells at each concentration of MMB and Lin for 96 hr.

(L) IB of the indicated proteins in A549 cells treated with 2.5 μM MMB, 1 μM Selu, 1 μM Lin, and/or 1 μM Ruxo for 24 hr.

(M) Relative cell growth of KL (Red: A549 and H1944 cells), KLP (Purple: HCC44, H23, H2122, and H1355 cells), and KP (Blue: H2009, H1792, H441, and H358 cells) NSCLC lines treated with 2.5 μM MMB and 1 μM Lin for 72 hr normalized to 2.5 μM MMB treated cells.

(N) Phase-contrast images of A549, H23, and H1792 cells treated with 2.5 μM MMB with or without 1 μM Lin for 96 hr. Scale bars: 100 μm. Red cell lines: KL. Purple cell lines: KLP. Blue cell lines: KP.

(O) Relative cell growth of A549 treated with 2.5 μM MMB, 1 μM Selu and/or 1 μM Lin in accordance to the indicated schedule (lower) for 1 week (n = 9).
(P) Percent change in mouse body weight of *LSL-Kras*^{G12D};*Lkb1*$^{f/f}$ and *LSL-Kras*^{G12D};*Trp53*$^{f/f}$ mice during MMB + Tram + Lin combination therapy (3 days on and 4 days off) (each group; n = 5).
(Q) Percent change in MRI tumor volume of *LSL-Kras*^{G12D};*Lkb1*$^{f/f}$ or *LSL-Kras*^{G12D};*Trp53*$^{f/f}$-induced lung cancer 1 week following MMB + Tram or MMB + Tram + Lin therapy (treatment schedule 3 days on and 4 days off). MMB 10 mg/kg, Tram 2 mg/kg, Lin 7.5 mg/kg.
(R) Representative MRI images from *LSL-Kras*^{G12D};*Lkb1*$^{f/f}$ mice treated with MMB + Tram therapy (upper) and *LSL-Kras*^{G12D};*Trp53*$^{f/f}$ mice treated with MMB + Tram + Lin therapy (lower) for the indicated time. H indicates heart.
All quantitative data are represented as mean ± S.D; *$p < 0.05$, **$p < 0.01$.

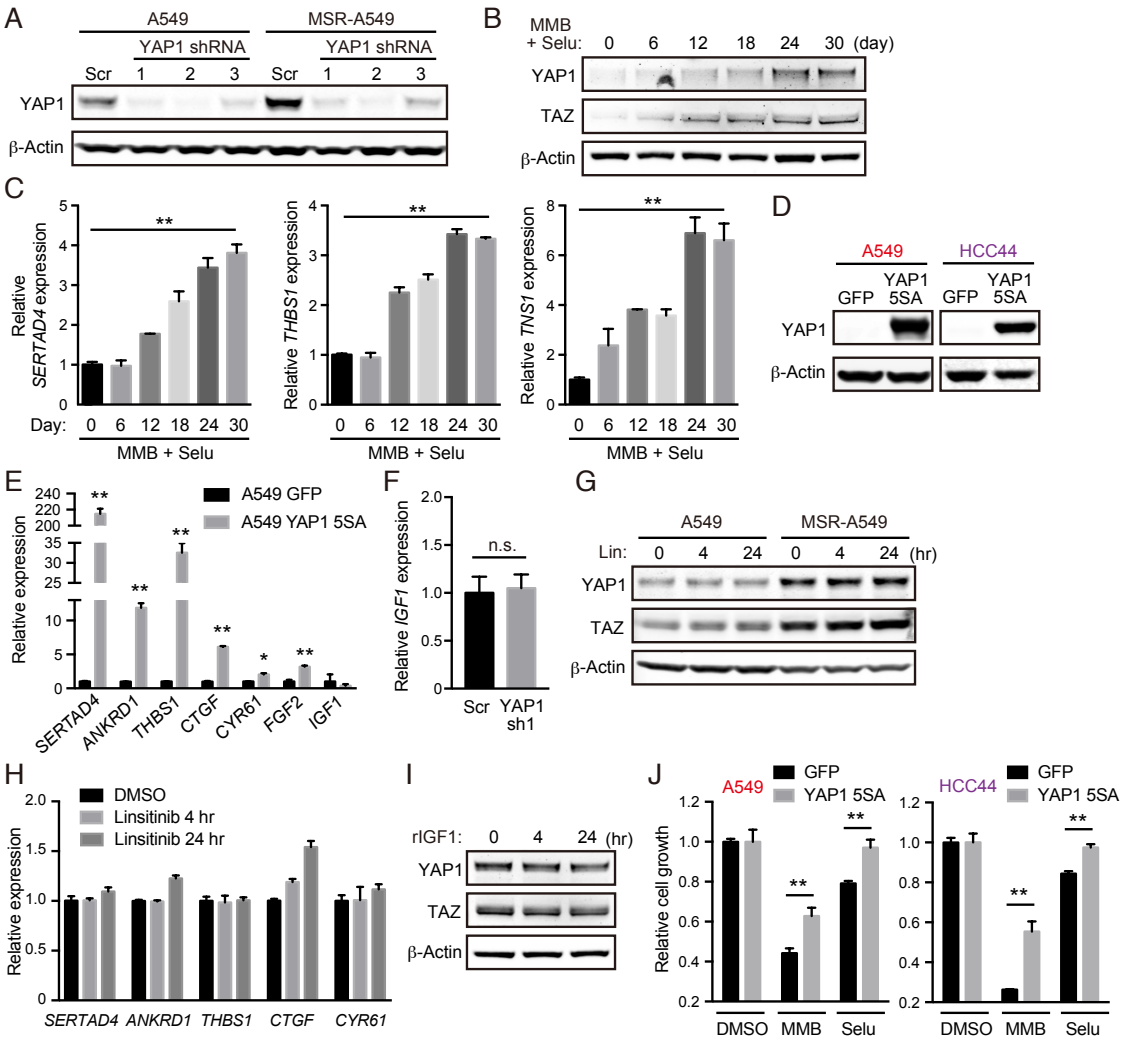

Figure S3. YAP1 pathway activation in response to MMB and MEK inhibition (Related to Figure 3)

(A) IB of the indicated proteins in A549 and MSR-A549 cells transduced with the indicated shRNA.

(B) IB of the indicated proteins in A549 treated with 5 μM MMB and 1 μM Selu for the indicated time.

(C) qRT-PCR of *SETAD4, THBS1,* and *TNS1* in A549 treated with 5 μM MMB and 1 μM Selu for the indicated time (n = 2).

(D) IB of the indicated proteins in A549 and HCC44 cells transduced with the indicated vector.

(E) qRT-PCR of *SERTAD4*, *ANKRD1*, *THBS1*, *CTGF*, *CYR61*, *FGF2,* and *IGF1* in A549 cells transduced with the indicated vector (n = 2).

(F) qRT-PCR of *IGF1* in MSR-A549 cells transduced with the indicated shRNA

(n = 4). n.s.: Not significant.

(G) IB of the indicated proteins in A549 and MSR-A549 cells treated with 1 μM Lin for the indicated time.

(H) qRT-PCR of *SERTAD4*, *ANKRD1*, *THBS1*, *CTGF* and *CYR61* in MSR-A549 cells transduced with 1 μM Lin for the indicated time (n = 2).

(I) IB of the indicated proteins in A549 treated with 100 ng/ml recombinant IGF1 for the indicated time.

(J) Relative cell growth of A549 and HCC44 transduced with the indicated vector in the presence of 2.5 μM MMB or 1 μM Selu for 72 hr.

All quantitative data are represented as mean ± S.D; *p < 0.05, **p < 0.01.

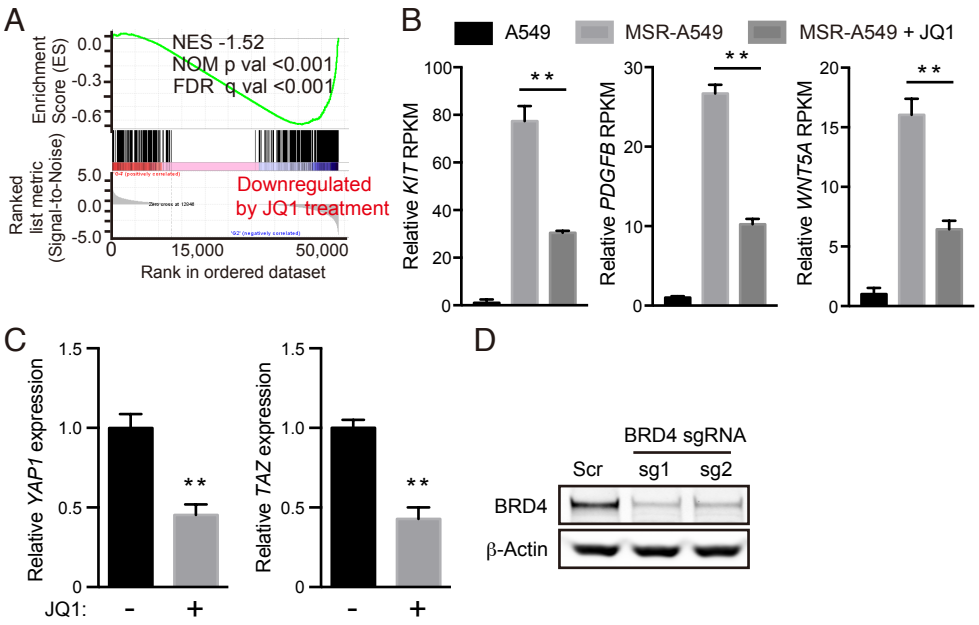

Figure S4. BET inhibition suppresses transcriptional adaptation to MMB and MEK inhibition (Related to Figure 4)

(A) GSEA analyses of MSR-signature (overlap of genes upregulated by RNA-seq and enriched by histone H3K27 acetylation ChIP-seq in MSR-A549 cells compared with A549 cells) in DMSO vs. JQ1 treated A549 (See STAR methods). NES: normalized enrichment score. NOM: nominal. FDR: false discovery rate.

(B) Relative RPKM (Reads per kilobase million) values of *KIT*, *PDGFB* and *WNT5A* in A549 and MSR-A549 cells treated with or without 200 nM JQ1 for 24 hr (n = 3).

(C) qRT-PCR of *YAP1* and *TAZ* in MSR-A549 cells treated with or without 500 nM JQ1 for 24 hr (n = 4).

(D) IB of the indicated proteins in A549 transduced with the indicated sgRNA.

All quantitative data are represented as mean ± S.D; **p < 0.01.

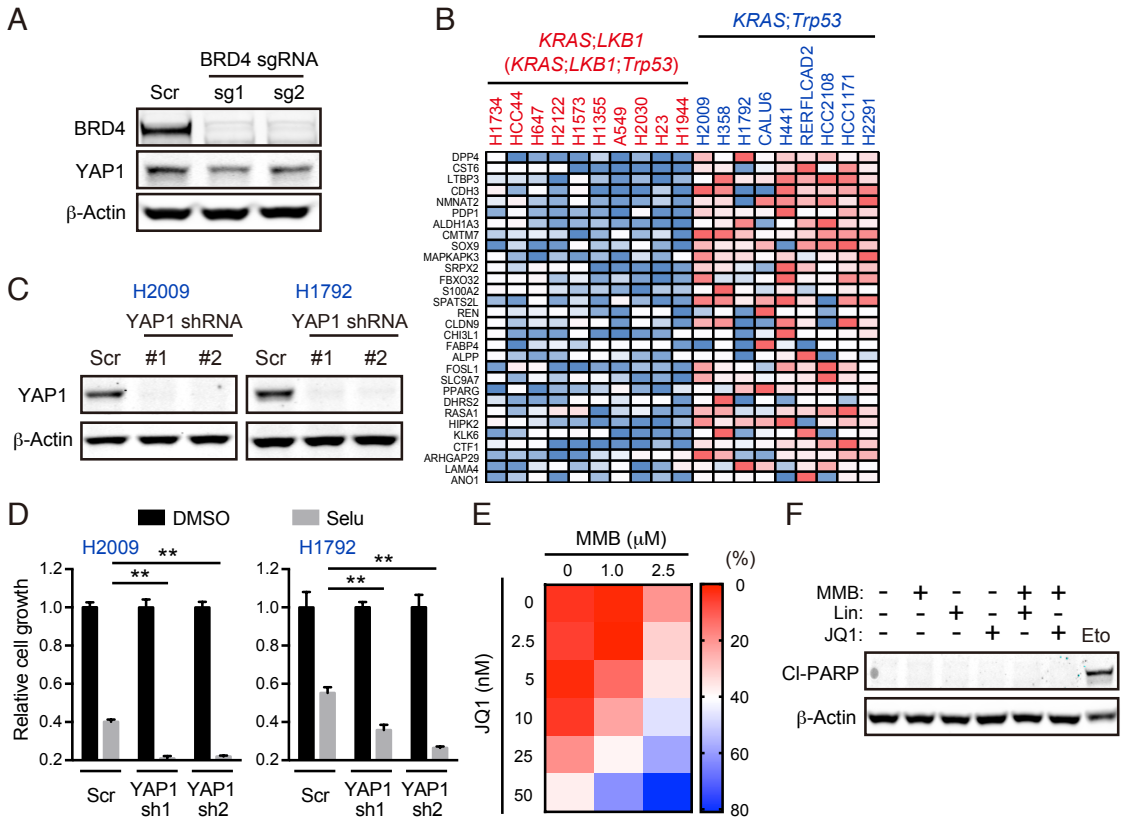

Figure S5. YAP1 activation in KP cells contributes to baseline resistance (Related to Figure 5)

(A) IB of the indicated proteins in H2009 transduced with the indicated sgRNA.

(B) Heat map of RPKM values of highly expressed genes in KP lung adenocarcinoma cell lines (blue) compared with in KL and KLP lung adenocarcinoma cell lines (red) provided by Cancer Cell Line Encyclopedia (CCLE) (See STAR methods).

(C) IB of the indicated proteins in H2009 and H1792 cells transduced with the indicated shRNA.

(D) Relative cell growth of H2009 and H1792 transduced with the indicated shRNA in the presence of 1 μM Selu for 96 hr (n = 3).

(E) Percentage inhibition in H2009 cells at each concentration of MMB and JQ1 for 96 hr.

(F) IB of the indicated proteins in mouse embryonic fibroblast (MEF) treated with 2.5 μM MMB, 1 μM Lin and/or 500 nM JQ1 for 24 hr, and treated with 10 μM etoposide (Eto) for 48 hr as a positive control for apoptosis induction.

All quantitative data are represented as mean ± S.D; **p < 0.01

Table S3, related to Figure 5
KRAS, *LKB1*, and *TP53* mutational status of TCGA samples studied.

TCGA sample ID	*KRAS*	*STK11/LKB1*	*TP53*
TCGA-55-7726	Mut		Mut
TCGA-95-7567	Mut		Mut
TCGA-55-7576	Mut		Mut
TCGA-50-5941	Mut		Mut
TCGA-44-6777	Mut		Mut
TCGA-99-7458	Mut		Mut
TCGA-97-7554	Mut		Mut
TCGA-55-7911	Mut		Mut
TCGA-78-7145	Mut		Mut
TCGA-05-4405	Mut		Mut
TCGA-55-7907	Mut		Mut
TCGA-05-4430	Mut		Mut
TCGA-95-7039	Mut		Mut
TCGA-50-5933	Mut		Mut
TCGA-69-7980	Mut		Mut
TCGA-49-4505	Mut		Mut
TCGA-64-1677	Mut		Mut
TCGA-64-5775	Mut		Mut
TCGA-97-7938	Mut		Mut
TCGA-55-7283	Mut		Mut
TCGA-64-5778	Mut		Mut
TCGA-78-7148	Mut	Mut	
TCGA-73-7498	Mut	Mut	
TCGA-44-6776	Mut	Mut	
TCGA-78-7161	Mut	Mut	
TCGA-05-4417	Mut	Mut	
TCGA-53-7813	Mut	Mut	
TCGA-91-6849	Mut	Mut	
TCGA-86-7713	Mut	Mut	
TCGA-80-5608	Mut	Mut	
TCGA-50-5932	Mut	Mut	
TCGA-35-3615	Mut	Mut	
TCGA-64-5774	Mut	Mut	
TCGA-78-7167	Mut	Mut	
TCGA-50-7109	Mut	Mut	
TCGA-44-7671	Mut	Mut	
TCGA-55-6983	Mut	Mut	
TCGA-55-6970	Mut	Mut	

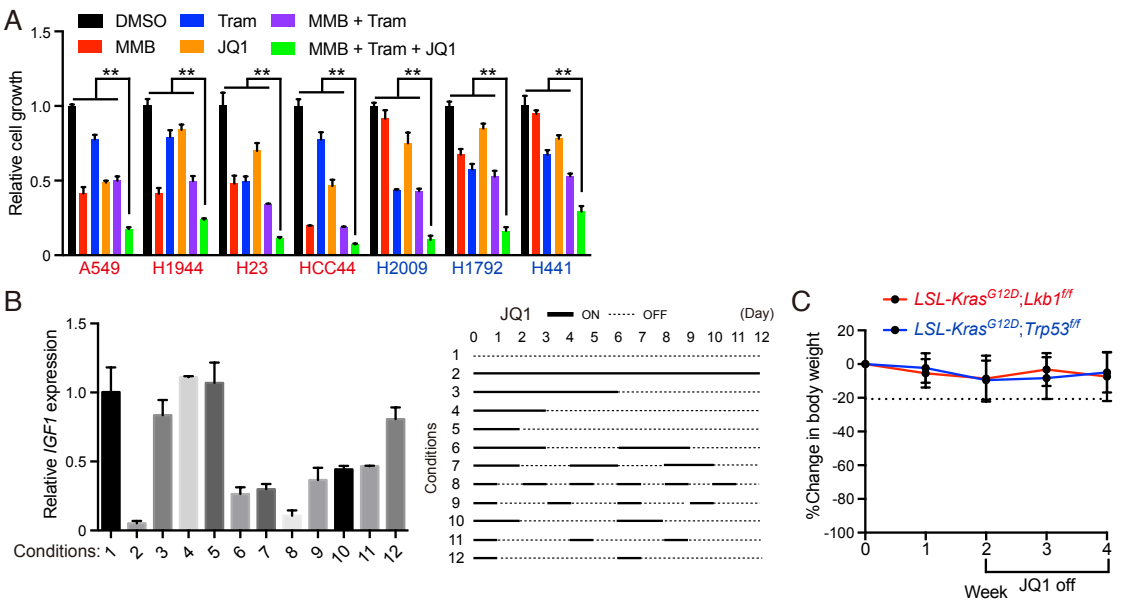

Figure S6. Development of triple combination MMB, Tram, and JQ1 therapy schedule (Related to Figure 6)

(A) Relative cell growth of A549, H1944, HCC44, H23, H2009, H1792, and H441 cells in the presence of 2.5 μM MMB, 10 nM Tram and/or 200 nM JQ1 for 72 hr (n = 3). Red cell lines: KL and KLP. Blue cell lines: KP.

(B) qRT-PCR of *IGF1* in A549 continuously treated with 5 μM MMB and 1 μM Selu and intermittently treated with 200 nM JQ1 in accordance to the indicated schedule (right) for 12 days (n = 2).

(C) Percent change in mouse body weight of *LSL-Kras*^{G12D};*Lkb1*$^{f/f}$ and *LSL-Kras*^{G12D};*Trp53*$^{f/f}$ mice during MMB + Tram + JQ1 combination therapy (each group; n = 7). All quantitative data are represented as mean ± S.D; **p < 0.01.

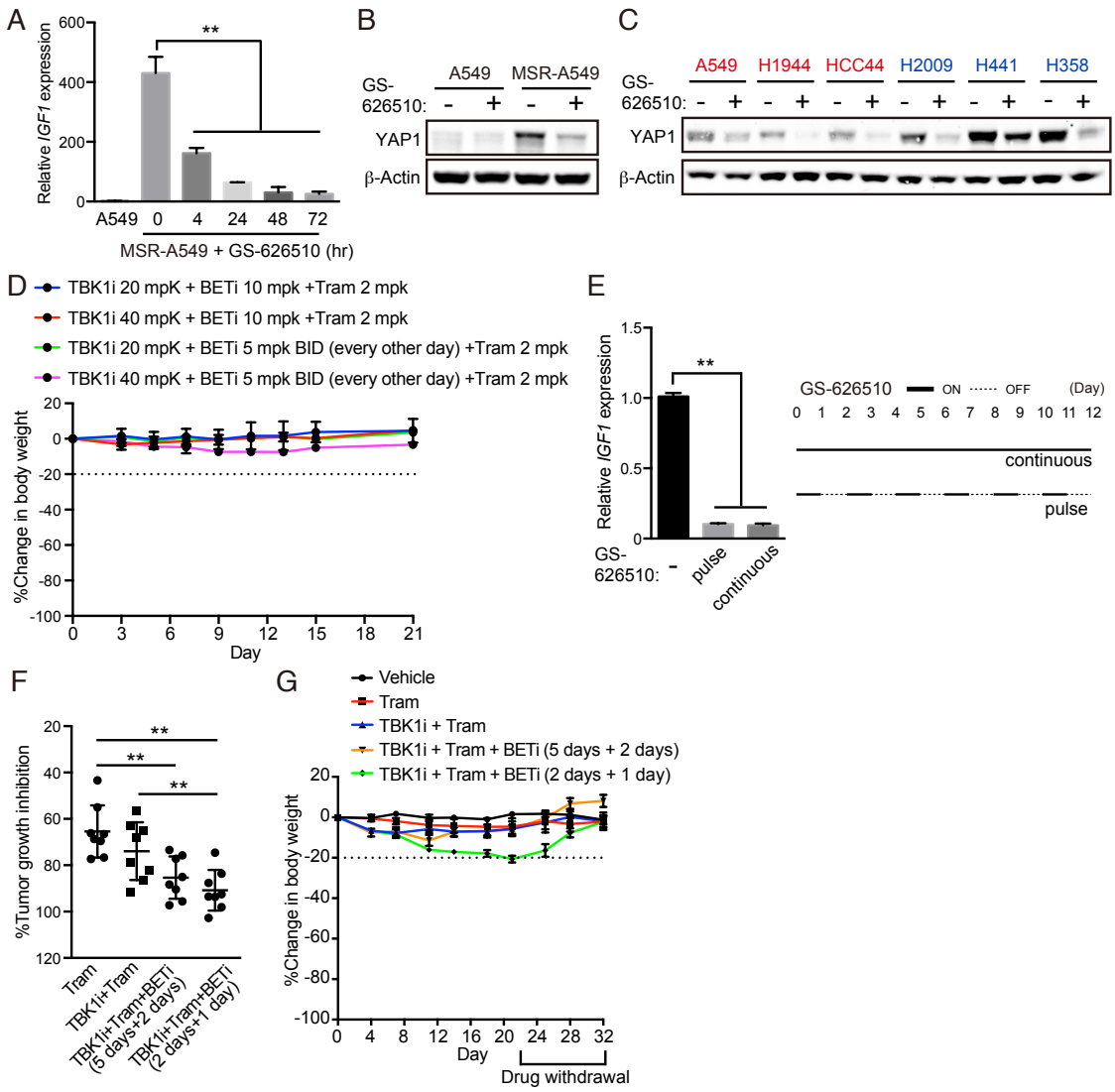

Figure S7. Development of triple combination TBK1i, Tram, and BETi therapy schedule (Related to Figure 7)

(A) qRT-PCR of *IGF1* in A549 and MSR-A549 cells treated with 200 nM GS-626510 for the indicated times (n = 2).

(B) IB of the indicated proteins in A549 and MSR-A549 cells treated with or without 200 nM GS-626510 (BETi) for 24 hr.

(C) IB of the indicated proteins in A549, H1944, HCC44, H2009, H441, and H358 cells treated with or without 500 nM GS-626510 for 24 hr. Red cell lines: KL. Blue cell lines: KP.

(D) Percent change in mouse body weight of mice during combination therapy in accordance to the indicated schedule (each group; n = 10). TBK1i = Compound

1. mpk: mg per kg.

(E) qRT-PCR of *IGF1* in MSR-A549 continuously treated with 200 nM GS-626510 or intermittently treated with 200 nM GS-626510 in accordance to the indicated schedule (right) for 12 days (n = 2).

(F) Percent tumor growth inhibition of DFCI366 PDX following pulse treatment of 40 mg/kg Compound 1, 2 mg/kg Tram, and/or 40 mg/kg GS-626510 in accordance to the indicated schedule (See figure 7F) for 22 days.

(G) Percent change in mouse body weight of mice during pulse treatment in accordance to the indicated schedule (See figure 7F) for 22 days, and 10 days after drug withdrawal (each group; n = 8).

All quantitative data are represented as mean ± S.D; **p < 0.01.

Table S4, related to STAR Methods
Primers for qRT-PCR, ChIP-qPCR, construction of sh/sgRNA-expressing vectors and mycoplasma monitoring.

For qRT-PCR

	Forward (5'-3')	Reverse (5'-3')
human IL6	ATTCAATGAGGAGACTTGCCTGGT	ACTCATCTGCACAGCTCTGGCTTG
human CCL2	CCCCAGTCACCTGCTGTTAT	TGGAATCCTGAACCCACTTC
human CCL5	CCAGCAGTCGTCTTTGTCAC	CTCTGGGTTGGCACACACTT
human SERTAD4	TGGCAGGATCACATTACAGG	TAAAGGATGTGGGCTCGTTC
human THBS1	TTGTCTTTGGAACCACACCA	CTGGACAGCTCATCACAGGA
human TNS1	TGAGATACCCCTGAGGAACG	CACAGTCGGGAGAGGAGAAG
human GLIS2	AACGCCAGGTACAAGATGCT	GCTTAAAGCGGTCACTGGAG
human CDKN2C	TGCACAAAATGGATTTGGAA	GGGCAGGTTCCCTTCATTAT
human SGK1	AGGGCAGTTTTGGAAAGGTT	CAGGAAAGGGTGCTTCACAT
human MYO1C	ATCCCATTATGAGCCAGTGC	CATCATTGGGTTTGATGCAG
human FGF2	GGGGGTGGAGATGTAGAAGA	GGTTCACGGATGGGTGTCT
human PHGDH	GTCATCAACGCAGCTGAGAA	AGGCACATGATCATTCCACA
human CTGF	AGGAGTGGGTGTGTGACGA	CCAGGCAGTTGGCTCTAATC
human ANKRD1	AGTAGAGGAACTGGTCACTGG	TGGGCTAGAAGTGTCTTCAGAT
human CYR61	CCTCGGCTGGTCAAAGTTAC	GAGGCTCCATTCCAAAAACA
human TAZ	CATCTCAACCCCATCATCCT	GAGCTGCTCTGCCTGAGTCT
human 36B4	CAGATTGGCTACCCAACTGTT	GGAAGGTGTAATCCGTCTCCA

For sh/sgRNA vector

	Target sequence (5'-3')
LKB1 sgRNA1	GTACTCCATCACCATATACG
LKB1 sgRNA2	CTTCAAGGTGGACATCTGGT
BRD4 sgRNA1	GAGCAGGTATTGCAGTTGGT
BRD4 sgRNA2	AGTCGAACTGTCACTGTCCG
pLKO.1 YAP1 shRNA1	CAGGTGATACTATCAACCAAA
pLKO.1 YAP1 shRNA2	CGACCAATAGCTCAGATCCTT

For ChIP-qPCR

	Forward (5'-3')	Reverse (5'-3')
human IGF1 promoter	CCTCATCGCAGGAGAAAAAG	CCCTGAAGTGACTGGGGTAA

For Mycoplasma detection

	Forward (5'-3')	Reverse (5'-3')
	ACACCATGGGAGCTGGTAAT	CTTCWATCGACTTYCAGACCCAAGGC