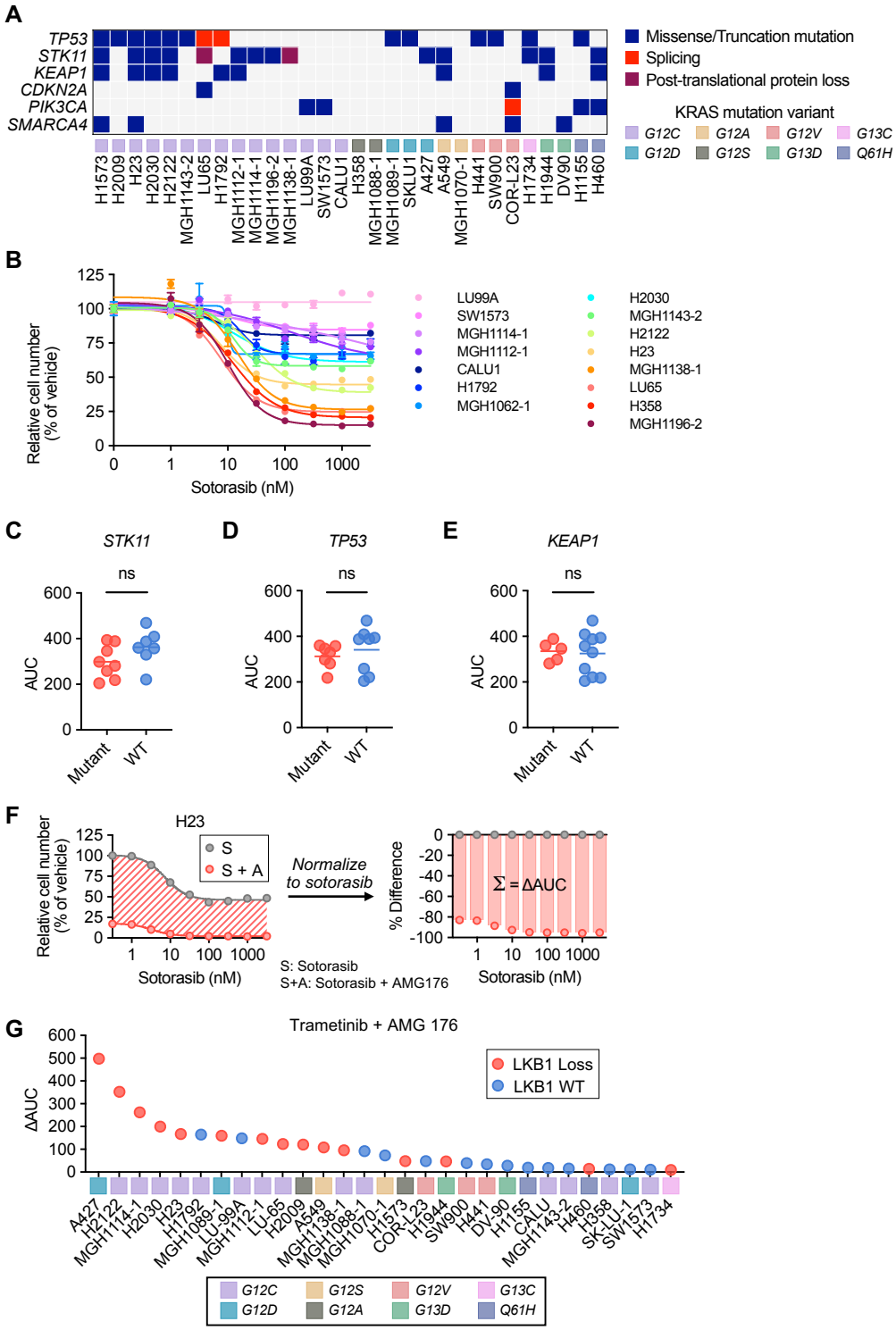
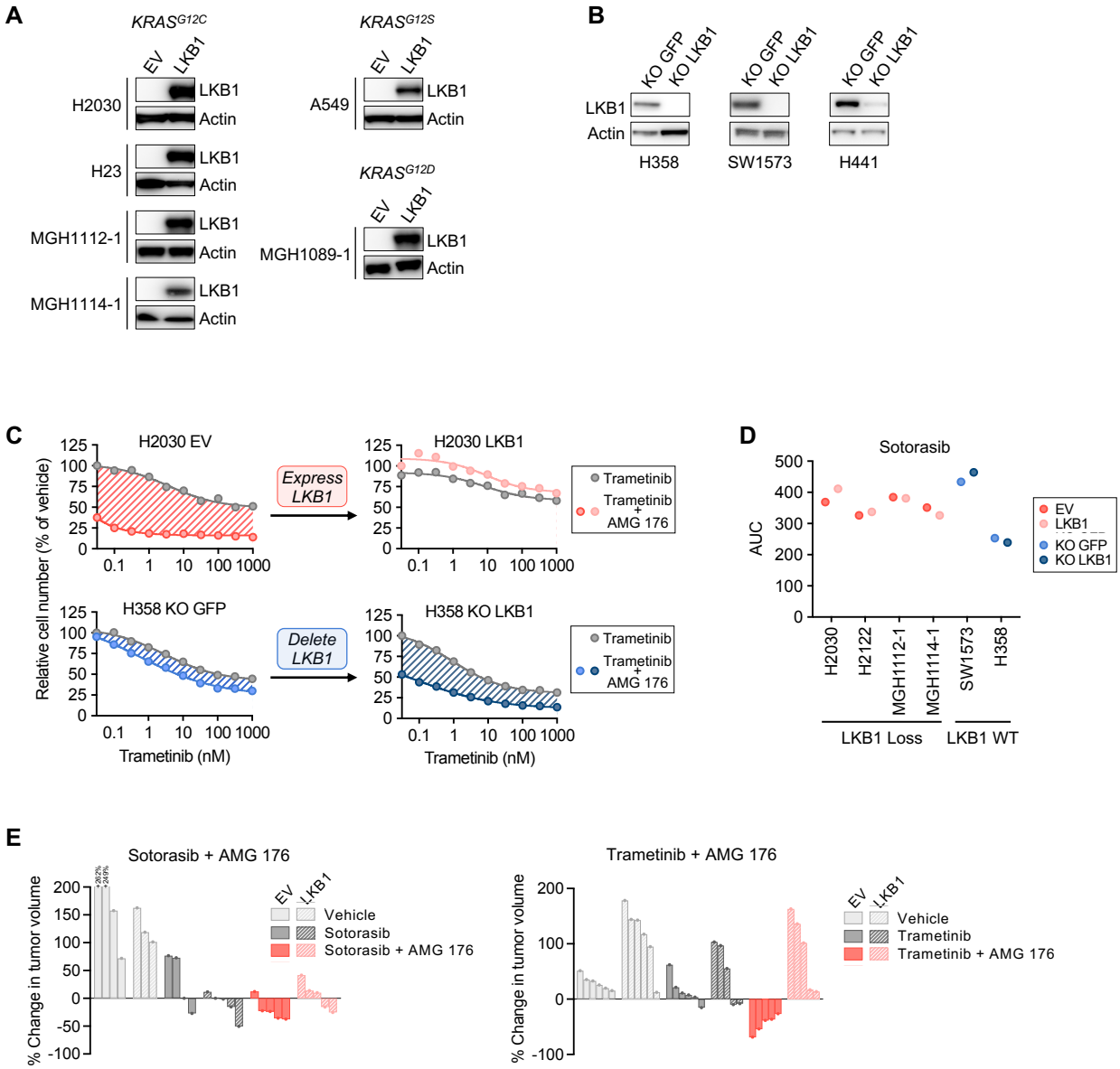


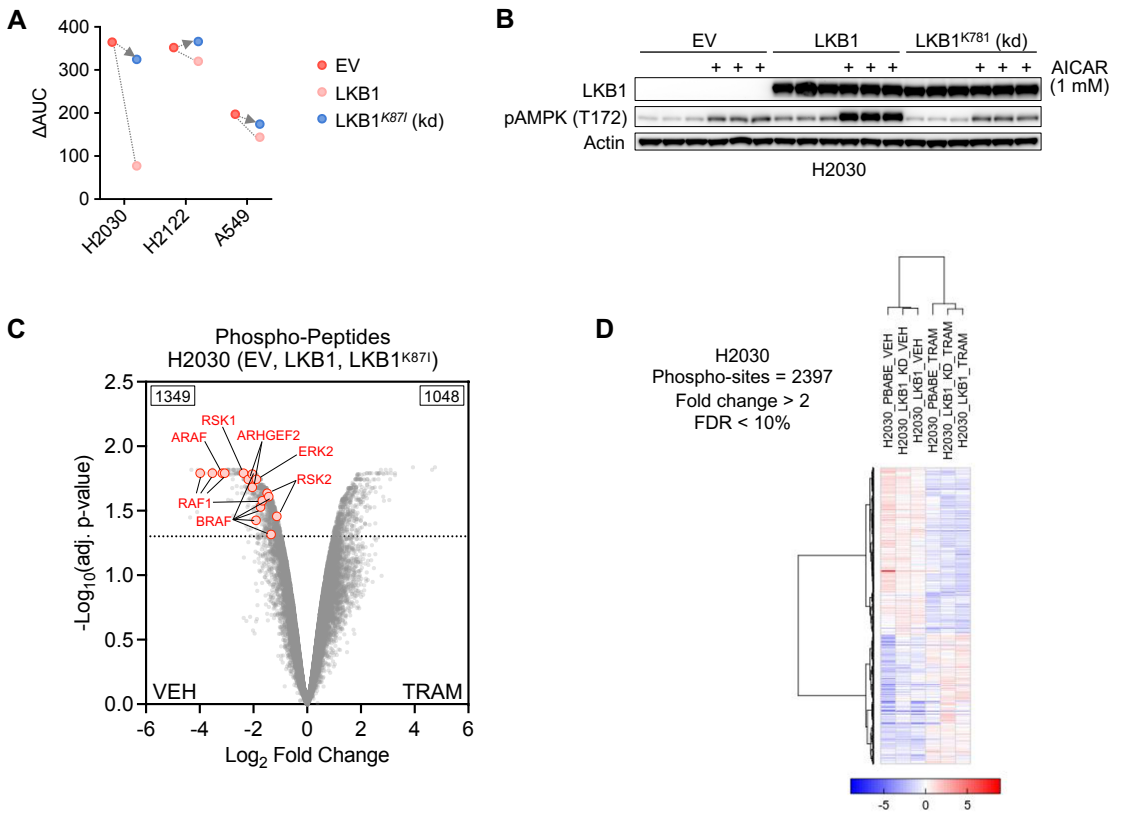
Supplemental Figure 1. *In vitro* sensitivity of *KRAS*^{G12C}-mutant NSCLC cell lines to sotorasib and trametinib. A. *KRAS* variant and co-occurring mutations of cell lines used in this study. **B.** Sotorasib sensitivity of *KRAS*^{G12C}-mutant NSCLC cell lines. Cells were treated with sotorasib for 3 days and viability was determined by CellTiter-Glo (CTG). **C-E.** Comparison of sotorasib sensitivity (quantified by AUC of sotorasib dose response curve) of *KRAS*^{G12C}-mutant cell lines grouped by common recurrent co-occurring mutations. **F.** Method for calculating ΔAUC, a metric quantifying the additional effect on cell viability upon adding AMG 176 to sotorasib or trametininb. S = sotorasib, A = AMG176. **G.** Relative increase in efficacy of trametinib + AMG 176 compared to trametinib alone.

Supplemental Figure 1



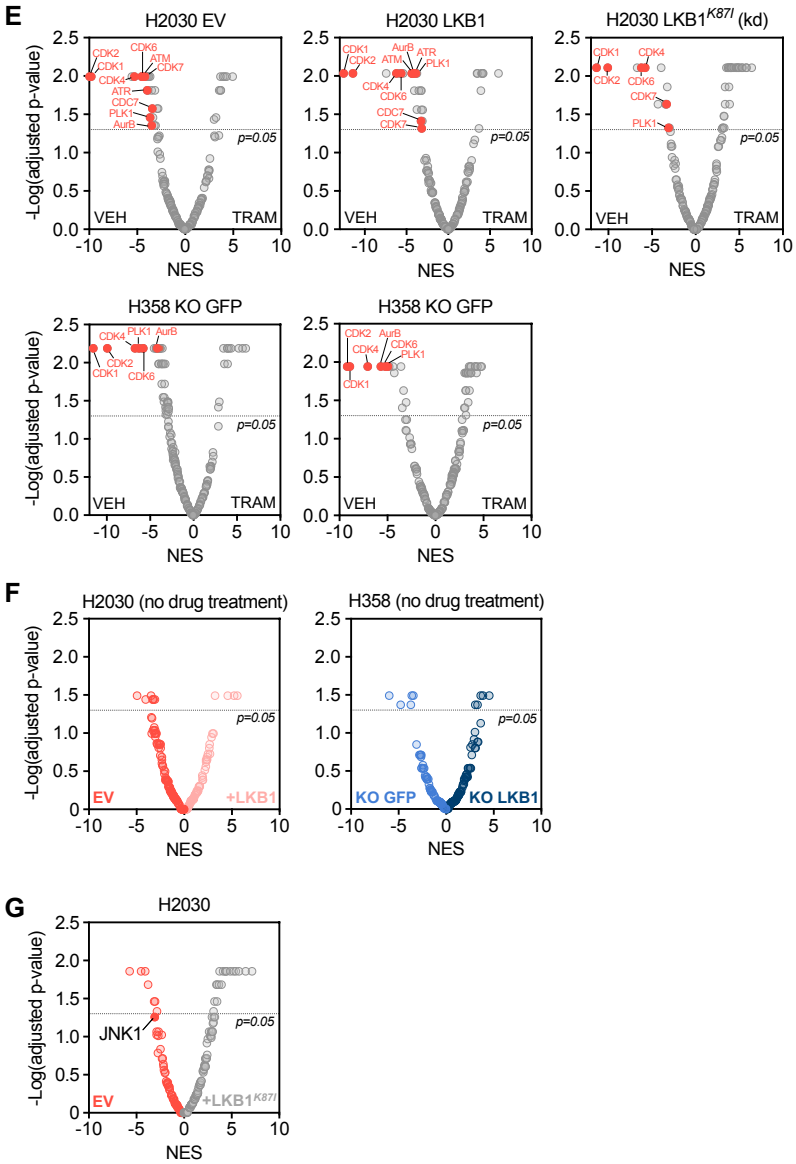
Supplemental Figure 2. LKB1 loss sensitizes to co-inhibition of KRAS/MEK and MCL-1 signaling. A. Expression of LKB1 in LKB1-deficient *KRAS*-mutant NSCLC cell lines used in this study. (EV = empty pBABE vector control) **B.** CRISPR deletion of LKB1 (KO LKB1) in LKB1 wild-type *KRAS*-mutant NSCLC cell lines used in this study. (KO GFP = CRISPR guide targeting GFP as negative control) **C.** Dose response curves (CTG) for H2030 EV, H2030 LKB1, H358 KO GFP, and H358 KO LKB1 cell lines after treatment with increasing doses of trametinib with or without a fixed dose of 1 μM of AMG 176. **D.** Re-expression of LKB1 in LKB1-deficient cells, or LKB1 deletion in LKB1 wild-type cells does not change sensitivity to sotorasib alone (expressed as AUC of sotorasib dose response curve). **E.** Waterfall plots showing tumor response at 3.5 weeks of H2030 *KRAS*^{G12C}-mutant NSCLC xenograft tumors treated with sotorasib (30 mg/kg), trametinib (3 mg/kg), or in combination with AMG 176 (50 mg/kg).

Supplemental Figure 2

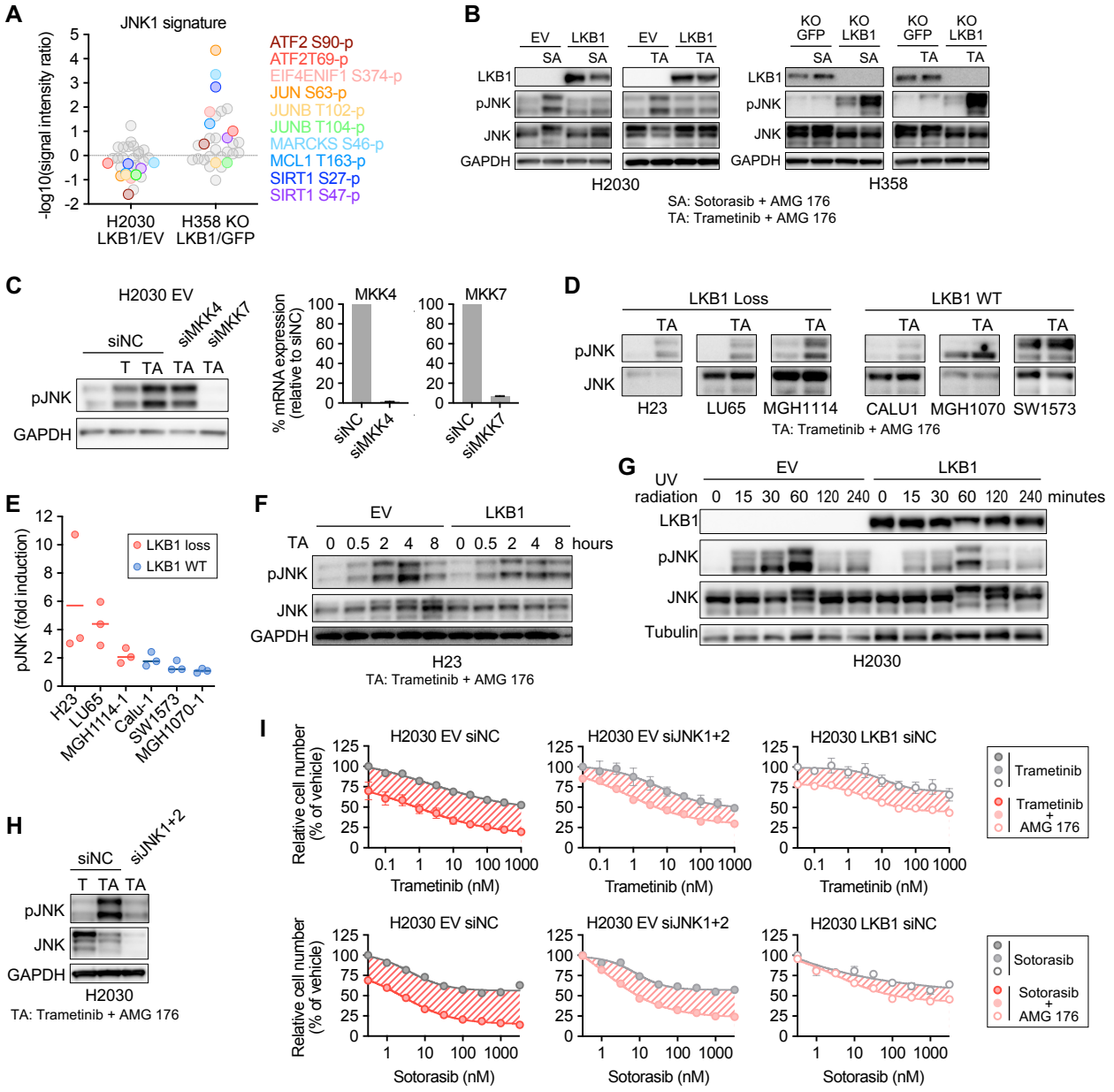


Supplemental Figure 3. Phospho-proteomic analysis reveals JNK activation in LKB1-deficient *KRAS*-mutant cells after trametinib treatment. A. Quantification of ΔAUC from CTG assays of *KRAS*-mutant NSCLC cell lines with LKB1 loss (EV), LKB1 restoration (LKB1), or restoration of a kinase dead version of LKB1 (LKB1^{K87I} kd) after treatment with trametinib alone or trametinib + 1 μM AMG 176. **B.** Western blot assessment of LKB1 and pAMPK (T172) in H2030 EV, H2030 LKB1, and H2030 kinase-dead LKB1 after treatment with 1 mM AICAR for 8 hours. **C-D.** Differential levels of phospho-peptides between trametinib and vehicle treatment in H2030 EV, H2030 LKB1, and H2030 LKB1 kd cell lines (grouped). Number of differentially quantified phospho-peptide and proteins with fold-change > 2-fold and adjusted p value < 0.05 are indicated.

Supplemental Figure 3

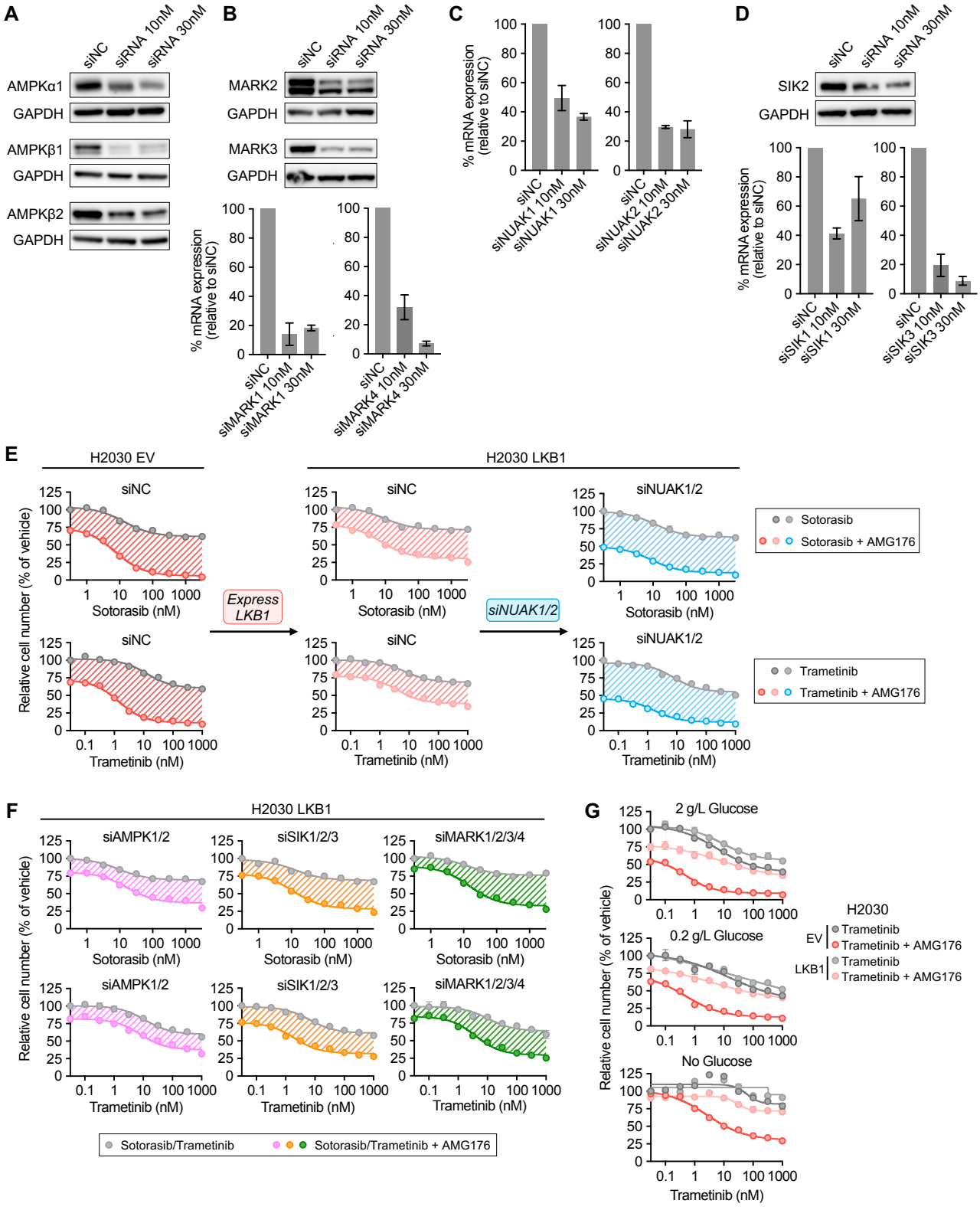


Supplemental Figure 3 (continued). E. Volcano plots showing differentially enriched signatures between trametinib and vehicle treatment. Phospho-peptide signatures were calculated using ssGSEA2.0/PTM-SEA. **F.** Differentially enriched phospho-peptide signatures in paired isogenic cell lines in the absence of drug treatment. **G.** Differentially enriched signatures between H2030 LKB1^{K87I} kinase-dead and H2030 EV control cells after trametinib treatment.



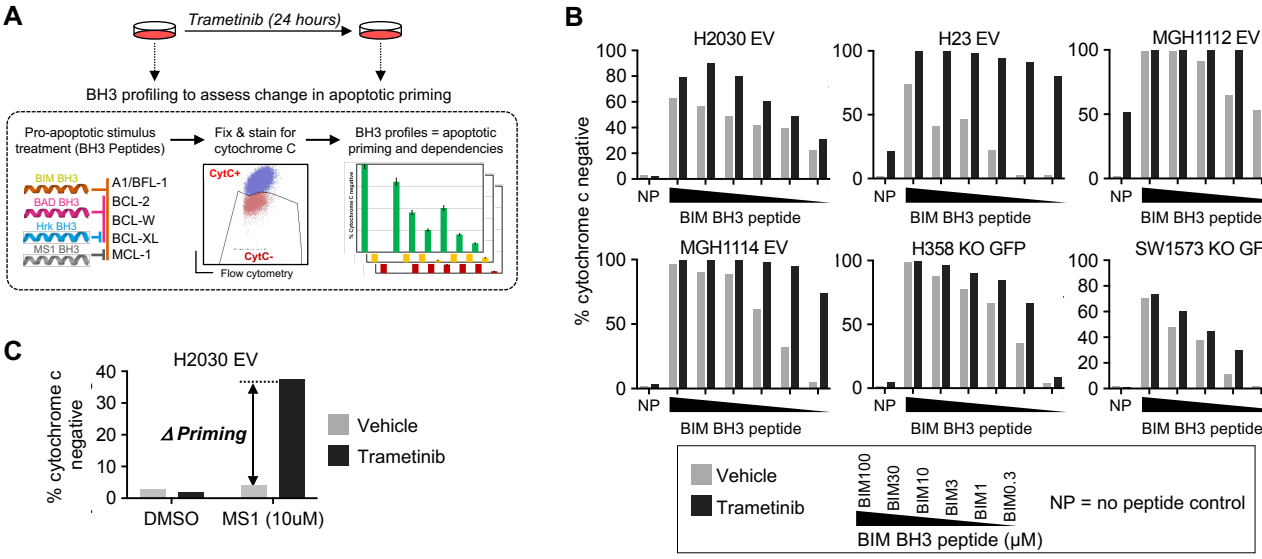
Supplemental Figure 4. JNK activation in LKB1-deficient cells underlies MCL-1 dependence. A. Ratio of individual phospho-peptide sites that make up JNK1 signature between isogenic paired cell lines after trametinib treatment. **B.** Western blot analysis of isogenic KRAS-mutant NSCLC cell lines after treatment with 1 μM sotorasib + 1 μM AMG 176 (SA) or 0.1 μM trametinib + 1 μM AMG 176 (TA) for 8 hours. SA: sotorasib + AMG 176. **C.** Left: Effect of siRNA knock-down of MKK4 and MKK7 on pJNK in H2030 EV cells treated with 0.1 μM trametinib (T) for 24 h or 0.1 μM of trametinib for 24 h followed by 1 μM AMG 176 (TA) for 4 hours. Right: mRNA expression of MKK4 and MKK7 after siRNA knockdown was determined by RT-qPCR. **D.** Phosphorylation of JNK in KRAS mutant NSCLC cell lines treated with 0.1 μM of trametinib + 1 μM of AMG 176 (TA) for 8 hours. **E.** Fold induction of phospho-JNK in LKB1-deficient or wild-type KRAS-mutant NSCLC cell lines after treatment with 0.1 μM trametinib + 1 μM AMG 176 for 8 hours. Data shown are quantification of densitometry levels from western blots of phospho-JNK normalized to total JNK, in drug-treated compared to vehicle cells. **F.** Time-course of JNK phosphorylation in H23 EV and LKB1 cells treated with 0.1 μM trametinib + 1 μM AMG 176. **G.** JNK phosphorylation in H2030 EV and LKB1 cells after UV irradiation for 0-4 hours. **H.** JNK phosphorylation in H2030 EV cells with siRNA knockdown of JNK1+2 after treatment with 0.1 μM of trametinib for 24 hours (T) or 0.1 μM of trametinib for 24h + AMG 176 for 4 hours (TA). **I.** H2030 EV cells with siRNA knockdown of JNK1+2 (or siNC negative control) or H2030 LKB1 cells were treated with trametinib or sotorasib alone or in combination with a fixed dose of 1 μM of AMG 176. Cell viability was determined by CTG after 3 days.

Supplemental Figure 4



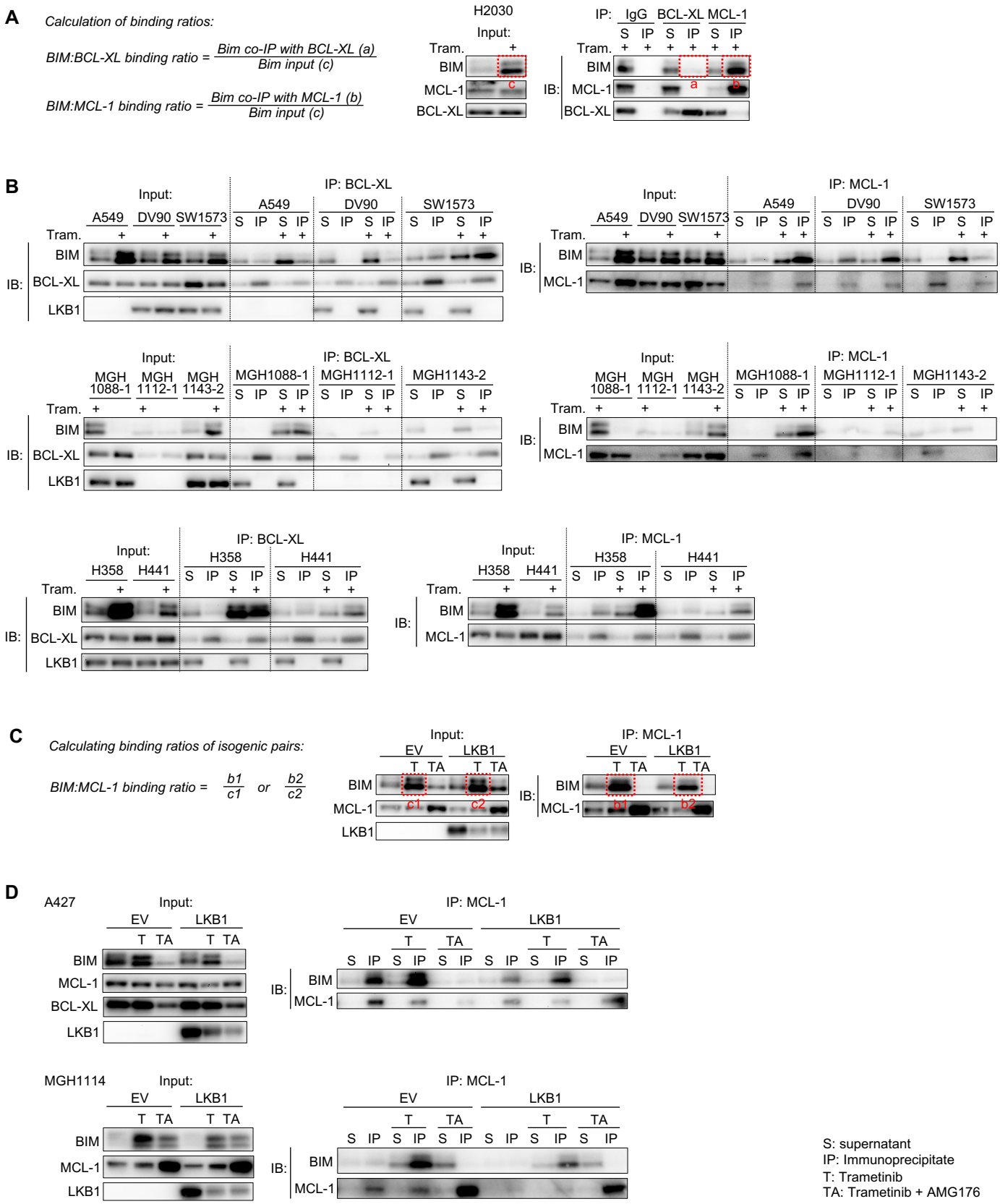
Supplemental Figure 5. Suppression of JNK activation by LKB1 is mediated by NUAK kinases. A-D. siRNA knockdown of AMPK-family assessed by western blot or RT-qPCR. **E-F.** Viability assessment H2030 LKB1 cells with corresponding siRNA knockdown after treatment with sotorasib/trametinib alone or in combination with 1 μM AMG 176. **G.** Viability assessment of H2030 EV or LKB1 cells in culture media containing no glucose, 0.2 g/L glucose, or 2 g/L glucose.

Supplemental Figure 5



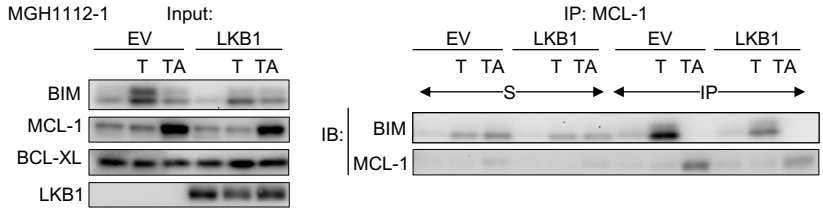
Supplemental Figure 6. LKB1 deficiency increases BIM:MCL-1 interaction and creates an MCL-1 dependent state. A. Schematic of BH3 profiling experimental setup. The change in priming (ΔPriming) is measured before and after treatment with trametinib (0.1 μM) as depicted in Fig. S6C. **B.** Overall priming of *KRAS*-mutant NSCLC cells treated with vehicle or 0.1 μM of trametinib for 16 hours, as determined by BH3 profiling with titration of BIM BH3 peptide. **C.** Illustration of method for calculating ΔPriming (increase or decrease in priming between vehicle and drug treated cells) from BH3 profiling results. **D.** Change in BCL-XL dependence (HRK BH3 peptide at 100μM dose) upon treatment of isogenic cell lines with trametinib. **E.** Expression of MCL-1 and BCL-XL in *KRAS*-mutant NSCLC cell lines grouped according to sensitivity to trametinib/sotorasib + MCL-1 inhibitor. **F.** Relative protein expression level of MCL-1 and BCL-XL in *KRAS* NSCLC cell lines grouped by LKB1 status from CCLE proteomic database.

Supplemental Figure 6

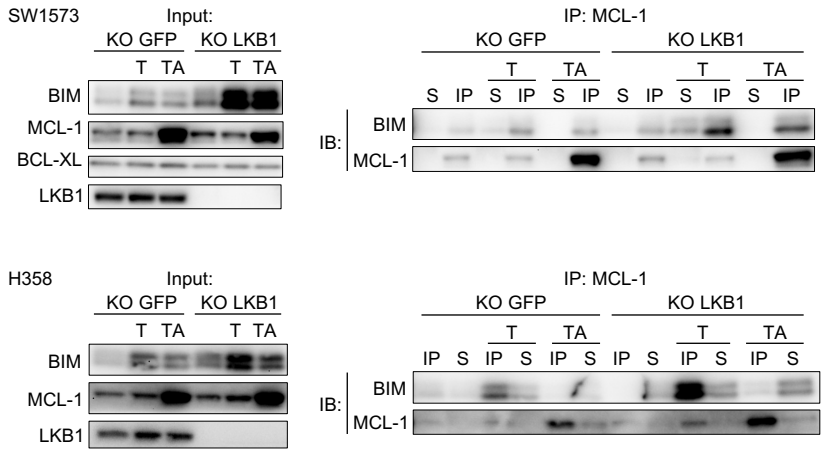


Supplemental Figure 7

(Panel D continued)

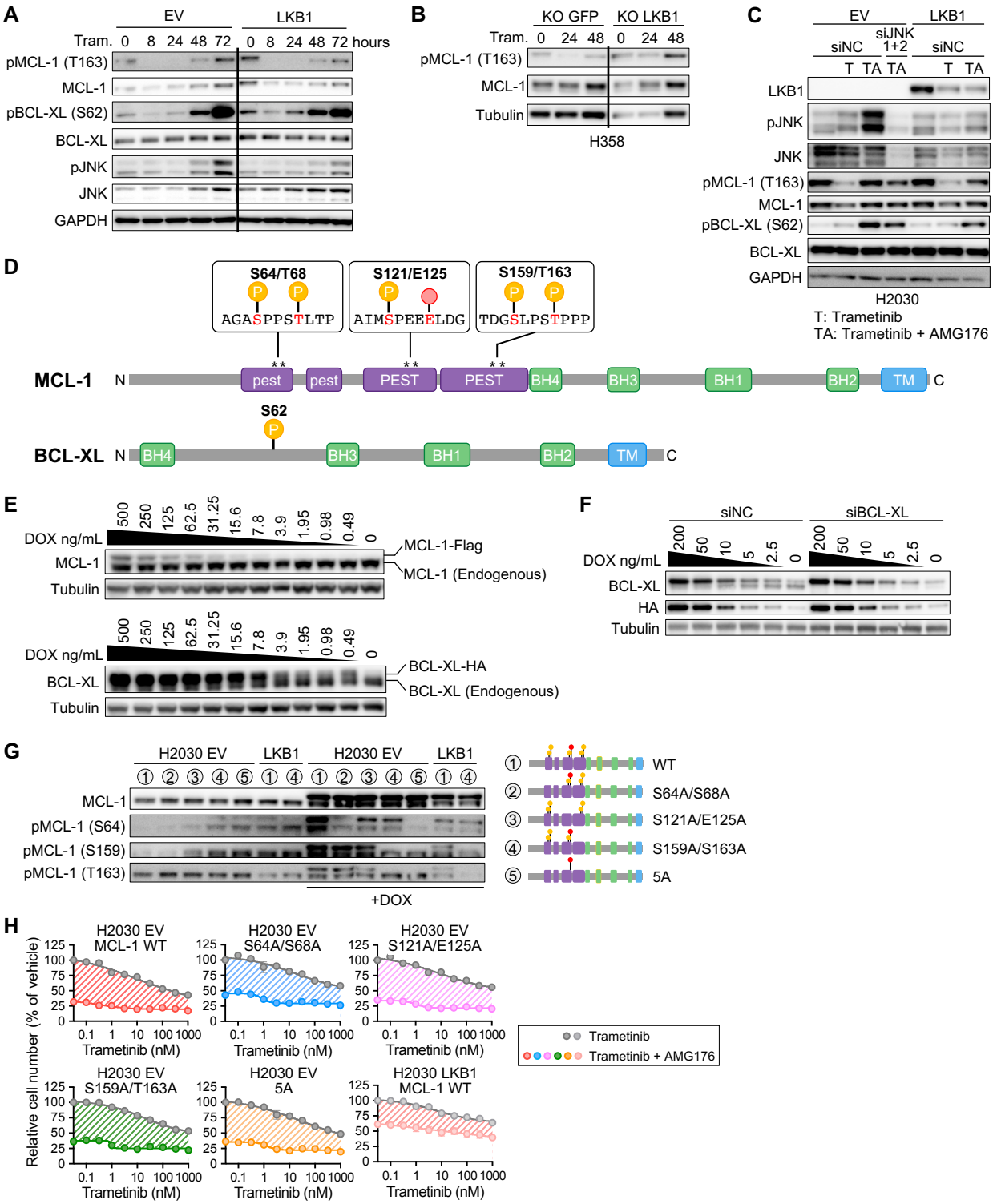


E



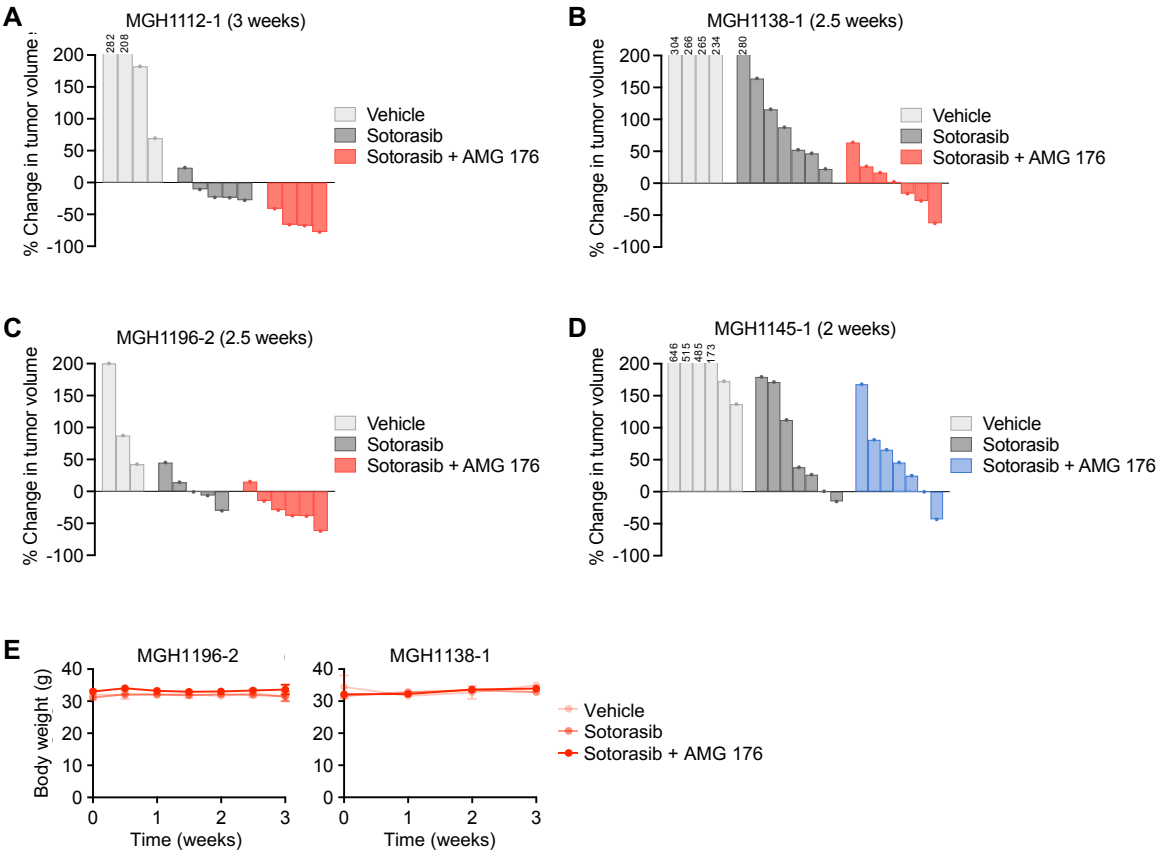
Supplemental Figure 7. LKB1 deficiency correlates with BIM:MCL-1 protein binding. A. Co-IP assessment of BIM bound to MCL-1 and BCL-XL binding in H2030 cells after treatment with 0.1 μM of trametinib for 24 hours. Method for calculating BIM:MCL-1 and BIM:BCL-XL binding ratios is shown. Input and IP protein bands were quantified from the same blot membrane. **B.** Co-IP assessment of BIM bound to MCL-1 and BCL-XL binding in *KRAS*-mutant NSCLC cell lines after treatment with 0.1 μM of trametinib for 24 hours. **C.** Method for calculating BIM: MCL-1 and BIM-BCL-XL binding ratios in isogenic cell lines. Input and IP protein bands were quantified from the same blot membrane. Blot is reprinted from Fig. 3C for illustration purposes. **D-E.** Co-IP assessment of BIM bound to MCL-1 in isogenic cell lines after treatment with 0.1 μM of trametinib for 24 hours (T), or 0.1 μM of trametinib for 24 hours + 1 μM of AMG 176 for 4 hours (TA).

Supplemental Figure 7 (continued)



Supplemental Figure 8. JNK phosphorylates BCL-XL S62, altering BIM:BCL-XL binding and driving an MCL-1 dependent state. **A-B.** Time-course of phosphorylation of MCL-1 and BCL-XL in H2030 EV/LKB1 and H358 KO GFP/LKB1 cells treated with 0.1 μM of trametinib. Blots shown in each panel are from a single membrane with non-relevant intervening lanes removed (indicated by solid vertical line). **C.** JNK1/2 knockdown in H2030 EV cells decreases drug-induced BCL-XL phosphorylation to a similar level as in H2030 LKB1 cells. After siRNA transfection, cells were treated with 0.1 μM trametinib for 48h or trametinib for 48h followed by 1 μM AMG 176 for 4 hours. **D.** Schematic of phospho-sites in MCL-1 and BCL-XL phosphorylated by JNK. MCL-1 E125 is a phospho-mimetic site, indicated by red. **E.** Western blot of H2030 EV cells with inducible WT MCL-1-Flag and BCL-XL-HA cultured in media containing various concentrations of Doxycycline (DOX). **F.** Western blot of H2030 EV cells with inducible BCL-XL WT and siRNA knockdown of BCL-XL (or negative control) cultured in media containing various concentrations DOX. **G.** MCL-1 phospho-mutants lack corresponding phosphorylation bands. **H.** Viability of H2030 EV cell lines expressing inducible wild-type MCL-1 or phospho-site mutants (with concurrent knockdown of endogenous MCL-1) after treatment with trametinib or trametinib + 1 μM AMG 176 for three days.

Supplemental Figure 8



Supplemental Figure 9. In vivo response of *KRAS*-mutant NSCLC PDX models to sotorasib +AMG 176. A-D. Waterfall plots showing tumor response H2030 *KRAS*^{G12C}-mutant NSCLC PDX tumors treated with sotorasib (30 mg/kg) alone or in combination with AMG 176 (50 mg/kg) at indicated time points. **E.** Animal body weights of MGH11196-2 and MGH1138-1 models treated with sotorasib or sotorasib + AMG 176.

Supplemental Figure 9

Cell line	IC50/nM	EMAX
MGH1143-2	13	43
MGH1196-2	11	91
MGH1138-1	15	83
MGH1114-1	N.A.	879
MGH1112-1	150	46
MGH1062-1	11	31
H2122	34	61
H1792	17	34
SW1573	13	17
LU99A	N.A.	N.A.
CALU1	7	19
H358	12	82
H23	8	57
H2030	12	45
LU65	8	78
H2030	12	45
LU65	8	78

Supplemental Table 1

Gene symbol	Protein	Forward 5'->3'	Reverse 5'->3'
map2k4	MKK4	TGCAGGGTAAACGCAAAGCA	CTCCTGTAGGATTGGGATTCAGA
map2k7	MKK7	CCACGTCATTGCCGTTAAGC	GCACGATGTAGGGGCAGTC
nuak1	NUAK1	AAGGCACCTACGGCAAAGTC	GTCTGATGTGAACCATGTCTTGT
nuak2	NUAK2	CGCCCAAGCCCCTAATGAAG	TCCCTCCGTATGTGCATCAGA
sik1	SIK1	CTCCGGGTGGGTTTTTACGAC	CTGCGTTTTGGTGACTCGATG
sik3	SIK3	CGTATCGGCTACTACGAGATCG	GGGGTGGCAAAGCATCTTC

Supplemental Table 2