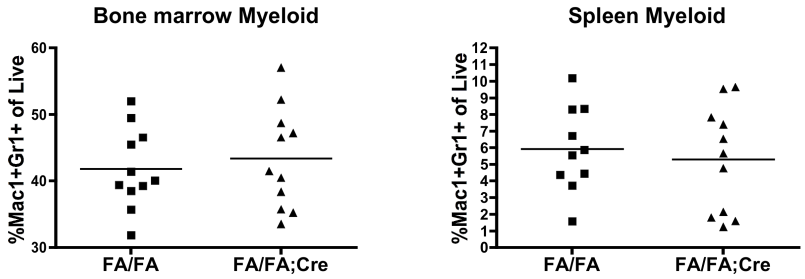
Supplementary Data

A.



B.

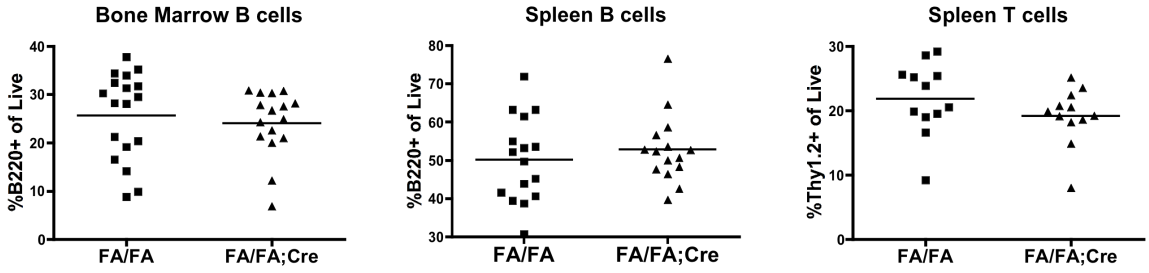


Figure S1: Mature myeloid and lymphoid populations in the bone marrow and spleen are unchanged in FA/FA;Cre mice. (A) Quantitation of myeloid flow cytometry shown in figure 1D (B) Quantitation of lymphoid flow cytometry shown in figure 1E. The Student's t-test was used to compare the two groups for each cell population.

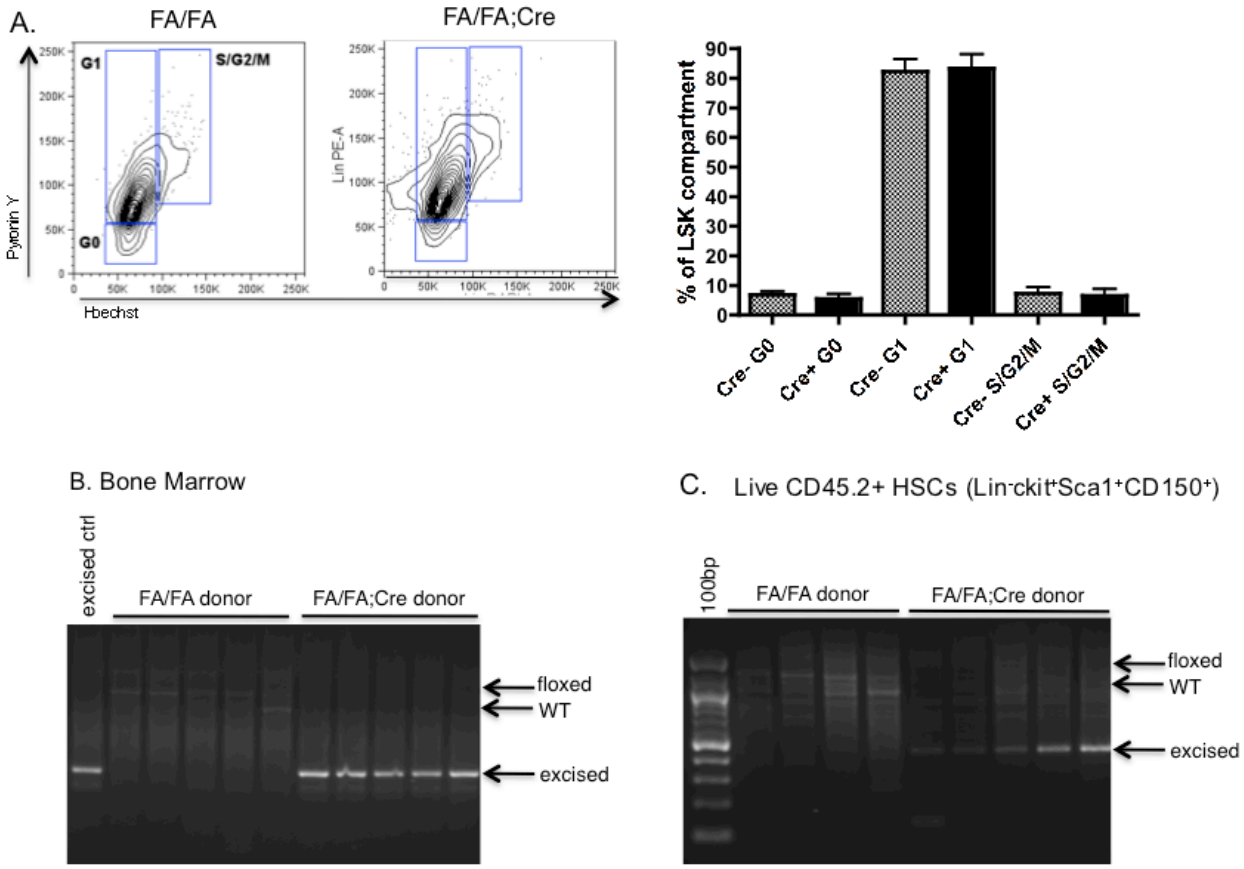
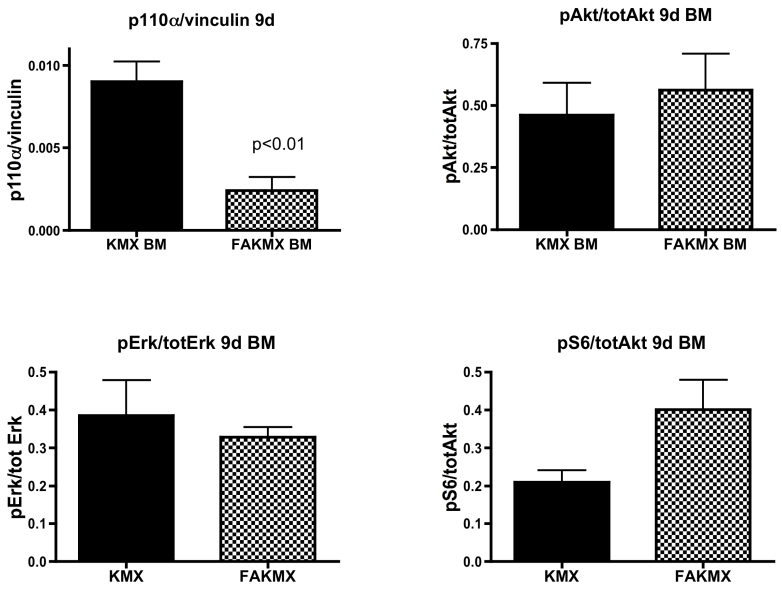


Figure S2: A. The cell cycle profile is unchanged in LSK and progenitor cells in FA/FA;Cre BM. Whole BM was harvested from 8-week old FA/FA;Cre mice and FA/FA littermate controls 4 weeks after pIpC treatment, stained with lineage antibodies, Hoechst, anti-Sca1 and anti-ckit antibodies, fixed overnight, stained with pyronin Y, and analyzed on the FACS Canto II instrument. The experiment was performed three times. The student's t-test was used to compare the two groups.

B-C. p110α is excised in recipient mice 16 weeks post-transplant. PCR was performed as previously described(1) on DNA extracted from whole BM (B) or sorted CD45.2+ LT-HSCs (C) from frozen BM samples from recipient mice sacrificed 16 weeks after transplantation in the competitive repopulation experiment shown in Figure 5B. The sizes of floxed, WT, and excised bands are indicated by arrows.

A.



B.

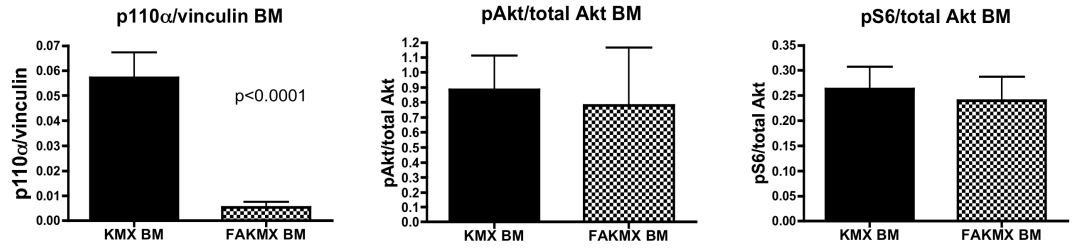


Figure S3: Quantification of Western blots in Figure 6D (A) and 6E (B): Signal intensities were normalized to the indicated loading controls. The student's t-test was used to compare the groups.

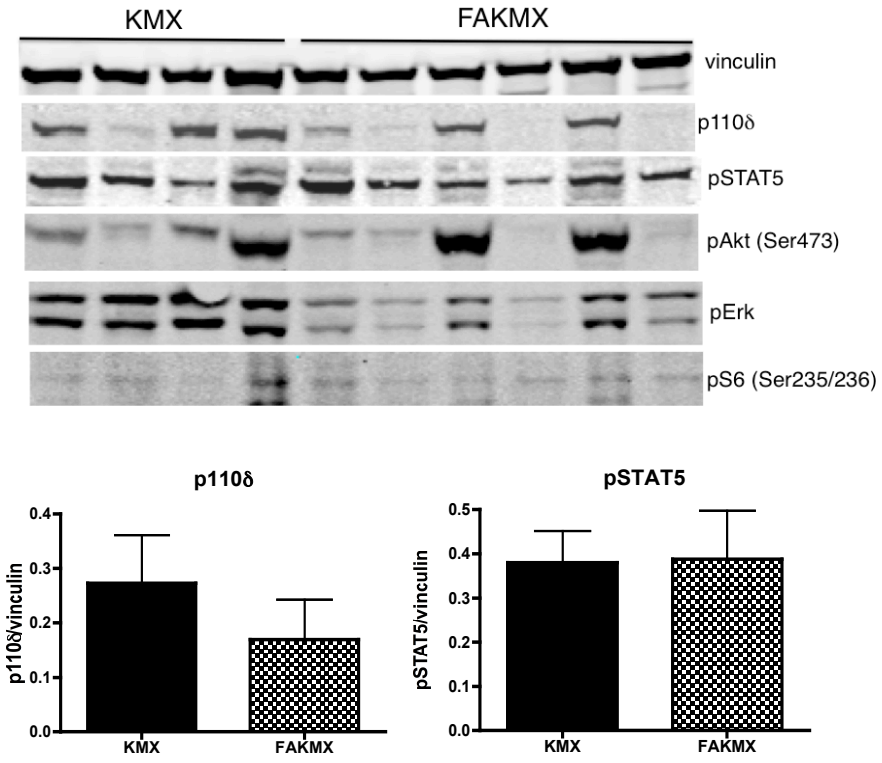
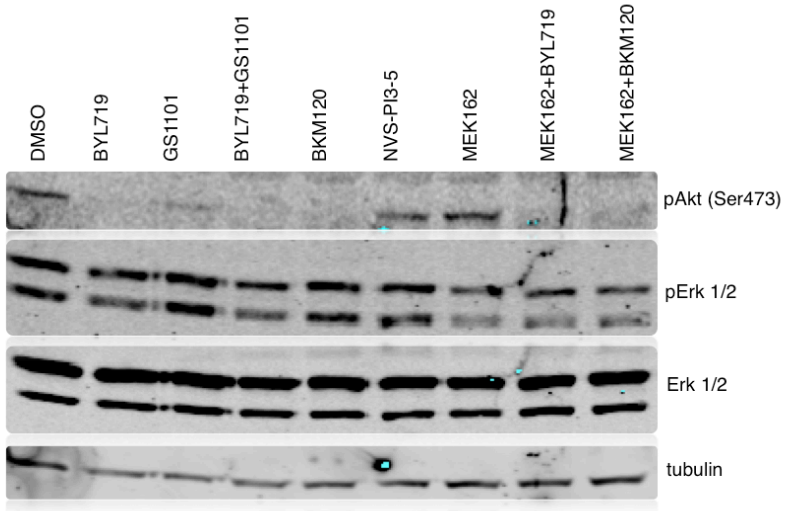


Figure S4: Western analysis of KMX and FAKMX BM lysates from moribund mice. The lanes were run on the same gel, but were non-contiguous. Quantification is shown below. Signal intensities normalized to the indicated loading controls.

A.



B.

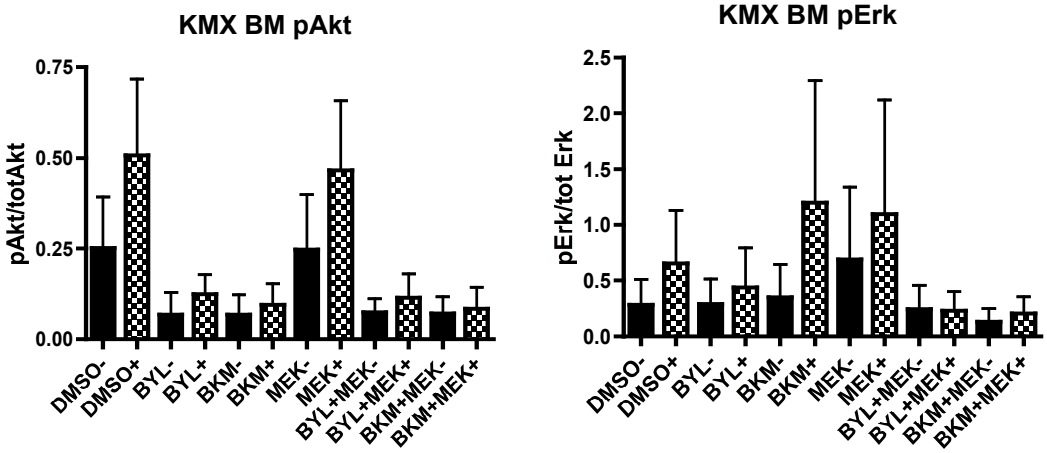
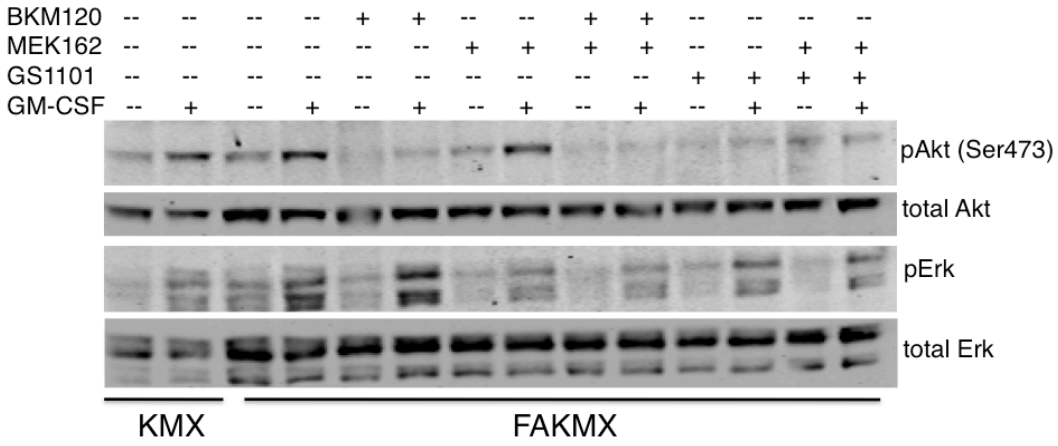


Figure S5: Drug treatment of BM from pIpC-treated Kras^{G12D};Mx1-Cre mice. BM was harvested from Kras^{G12D};Mx1-Cre (KMX) mice injected with pIpC at 4 weeks of age, 9 days following pIpC treatment. BM was incubated for one hour with the following pharmacologic inhibitors (dose in parentheses): BYL719 (1μM), GS1101 (1μM), BKM120 (1μM), NVS-PI35 (100nM), and MEK162 (100nM). The lanes were run on the same gel, but were non-contiguous. A. Representative Western blot of BM lysates after drug treatment in the absence of growth factors. The lanes were run on the same gel, but were non-contiguous. B. Quantification for the Western blot in Figure 7D: drug treatment with (+) and without (-) GM-CSF, 1ng/ml. Signal intensities were normalized to the indicated loading controls.

A.



B.

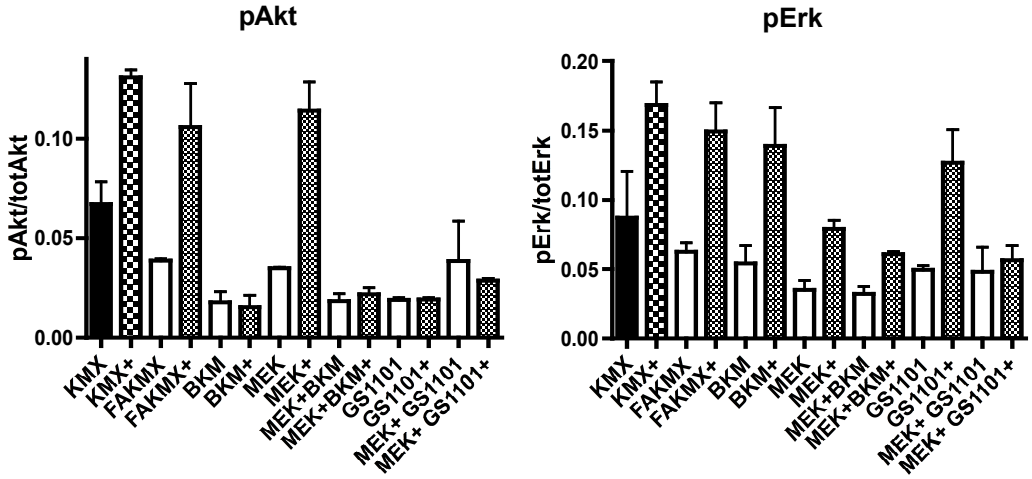
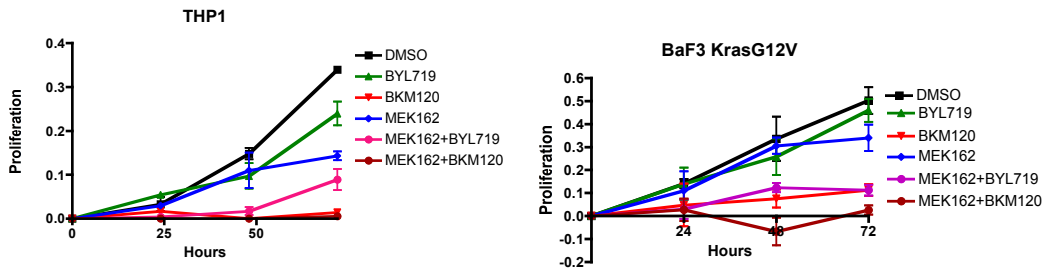


Figure S6: Drug treatment of BM from pIpC-treated p110$\alpha^{F/F}$;Kras^{G12D};Mx1-Cre (FAKMX) mice. BM was harvested from FAKMX mice injected with pIpC at 4 weeks of age, 9 days following pIpC treatment. BM was incubated for one hour with the following pharmacologic inhibitors (dose in parentheses): GS1101 (1μM), BKM120 (1μM), and/or MEK162 (100nM), and then stimulated with GM-CSF (1ng/ml) for 5 minutes. The lanes were run on the same gel, but were non-contiguous. A representative Western blot of drug treatment with (+) and without (-) GM-CSF is shown. Lysates from KMX BM treated with DMSO without or with GM-CSF (2 left lanes labeled "KMX" and "KMX+", respectively) were loaded on the same gel for comparison. Lanes labeled "FAKMX" and "FAKMX+" represent FAKMX BM treated with DMSO, without or with GM-CSF, respectively. The experiment was performed twice. Quantitation of signal intensities is shown below. Signal intensities were normalized to the indicated loading controls.

A.

B.



C.

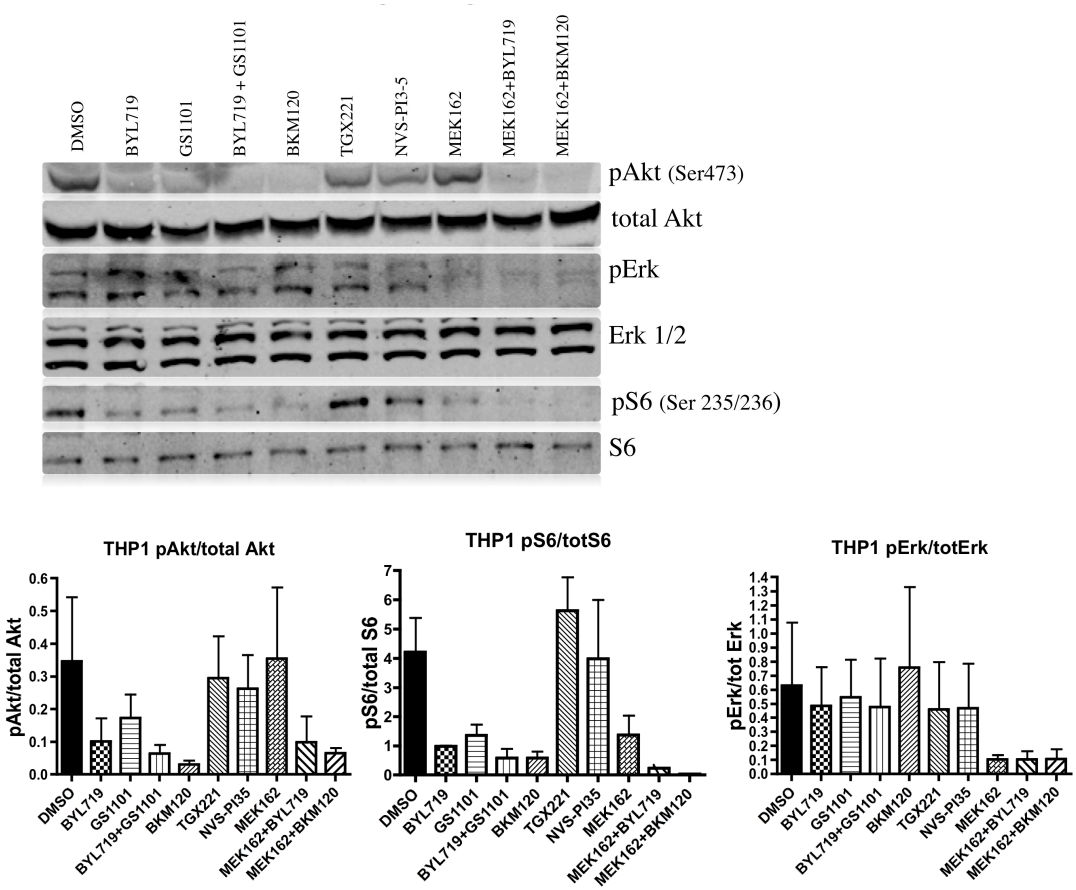


Figure S7: Treatment of cell lines with PI3K and MEK inhibitors individually and in combination. BaF3Kras G12V cells (A) or THP1 cells (B) were treated for 72 hours in RPMI 10%FCS without growth factors with the following pharmacologic inhibitors: BYL719 (1μM), BKM120 (1μM), and MEK162 (25nM). Proliferation was determined using the Aqueous One MTS proliferation assay. (C) Western blot on THP1 cells treated with PI3K isoform-selective inhibitors and combination. THP1 cells were cultured in the following inhibitors for one hour: BYL719 (1μM), GS1101 (1μM), TGX221 (1μM) BKM120 (1μM), NVS-PI35 (100nM), and MEK162 (25nM). The lanes were run on

the same gel, but were non-contiguous. The experiment was performed 3 times. Quantification is shown below. Signal intensities were normalized to the indicated loading controls. The one-way ANOVA test was used to compare the groups.

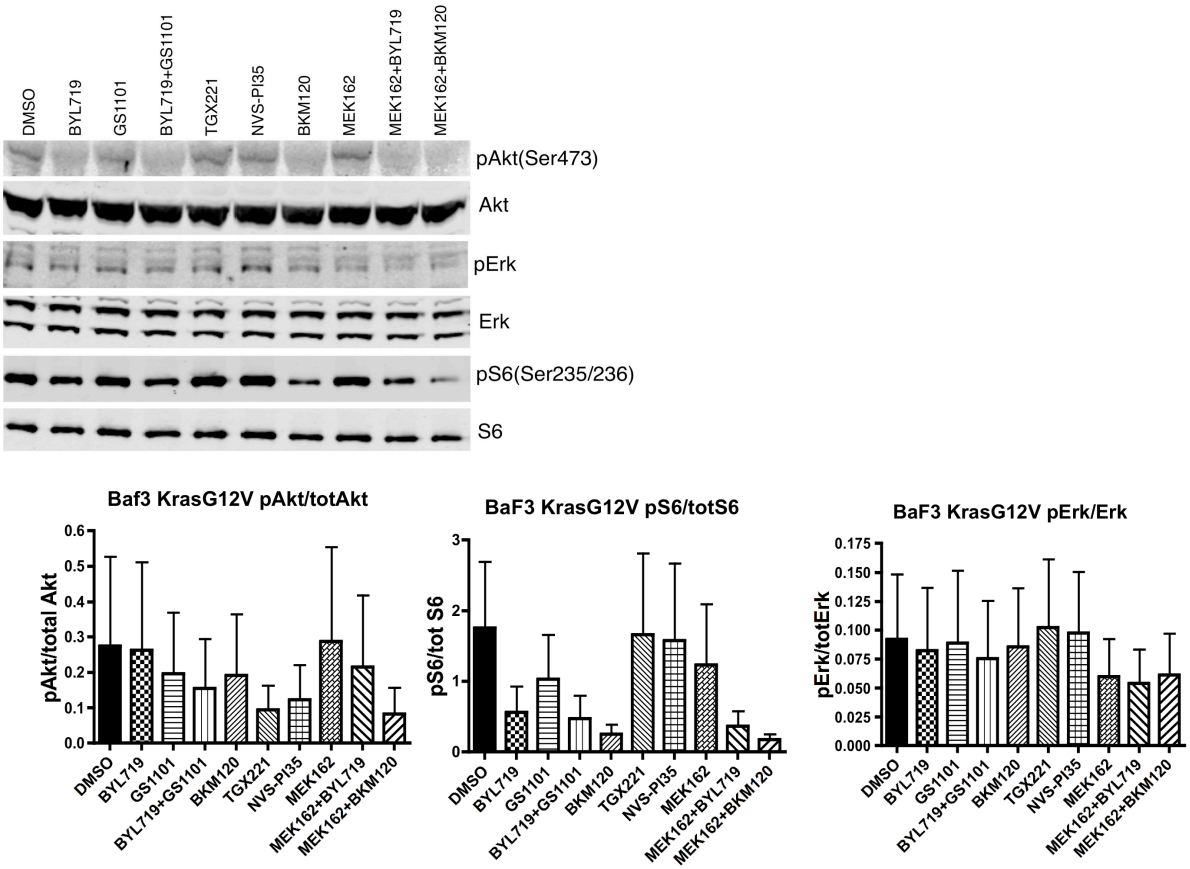


Figure S8: Western analysis of Akt and MAPK signaling in BaF3KrasG12V cells after drug treatment. BaF3KrasG12V cells were cultured in the following inhibitors for one hour: BYL719 (1μM), GS1101 (1μM), TGX221 (1μM) BKM120 (1μM), NVS-PI35 (100nM), and MEK162 (25nM). The lanes were run on the same gel, but were non-contiguous. The experiment was performed 3 times. Quantification of signal intensities is shown below, normalized to the indicated loading controls. The one-way ANOVA test was used to compare the groups.

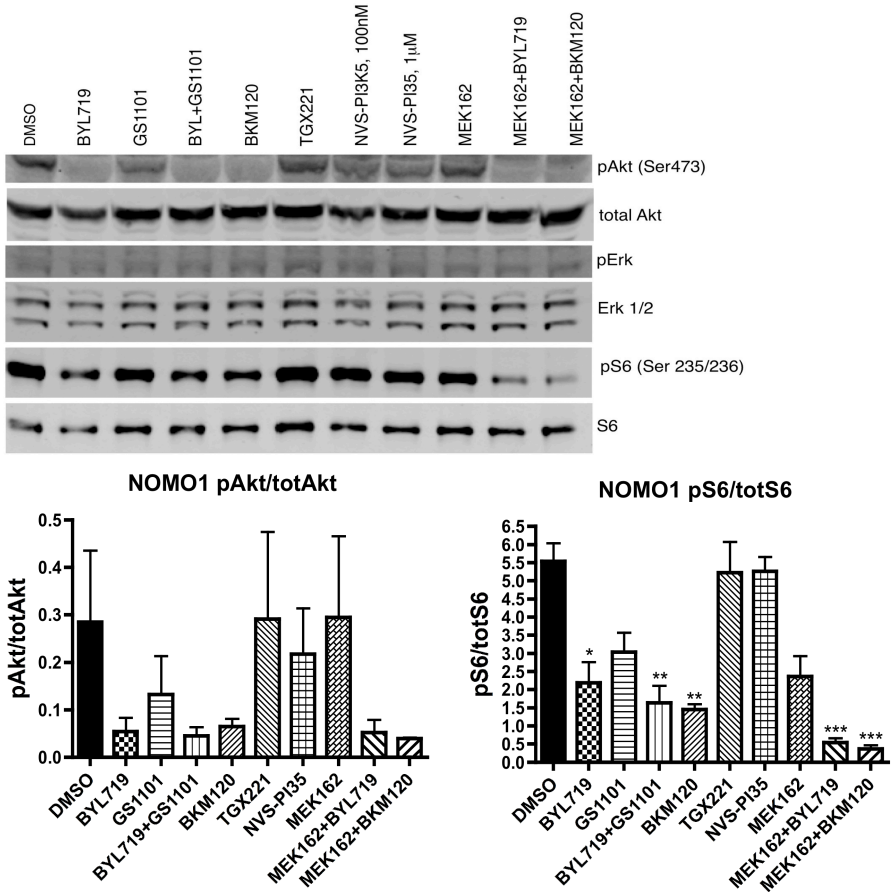


Figure S9: Western analysis of Akt and MAPK signaling in NOMO1 cells after drug treatment. Inhibitor treatment and Western analysis was performed as described for Figure S8. The lanes were run on the same gel, but were non-contiguous. The experiment was performed 3 times. Quantification of signal intensities is shown below, normalized to the indicated loading controls. The one-way ANOVA test was used to compare the groups, with the Bonferroni's post-test. *p<0.05, **p<0.01, ***p<0.001

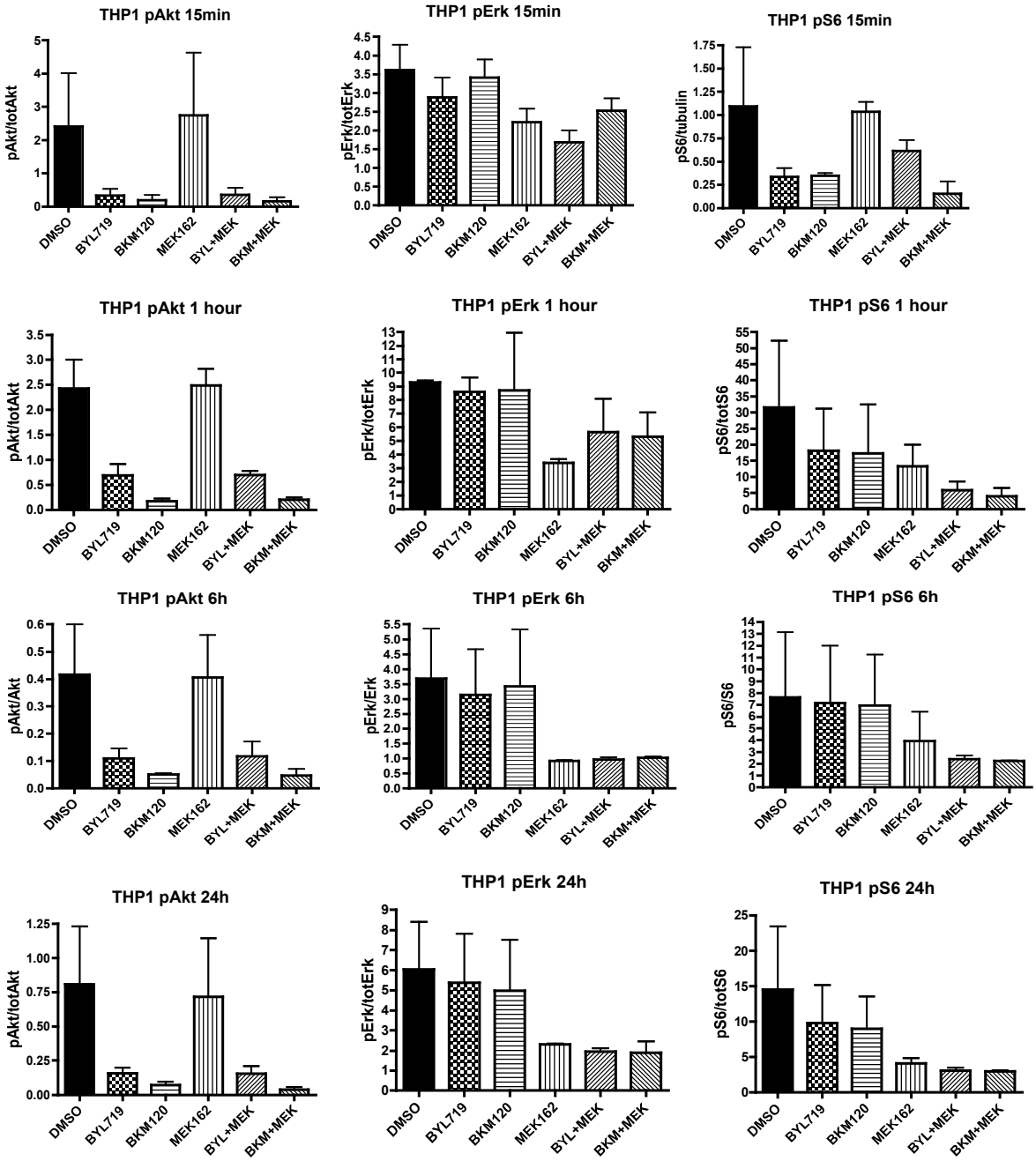
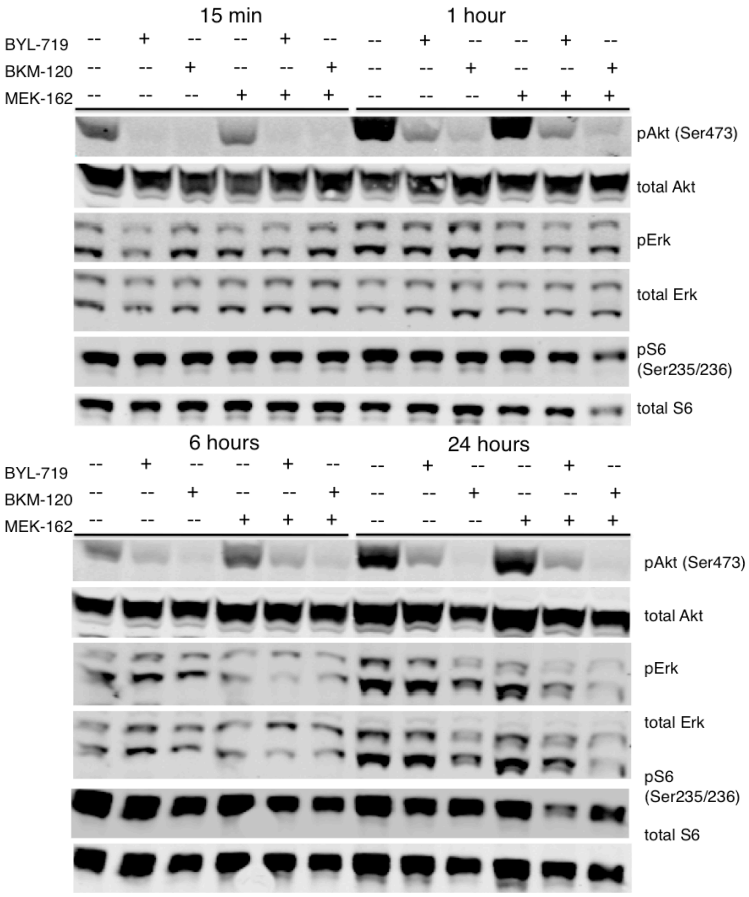
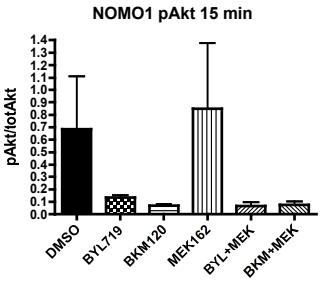
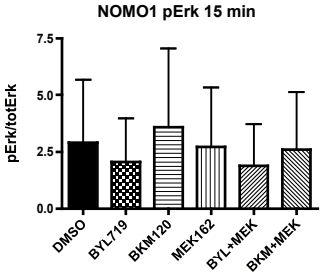
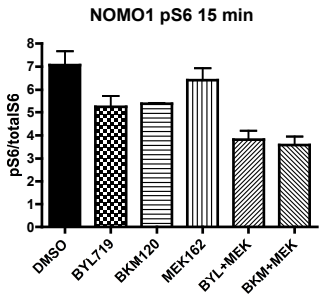


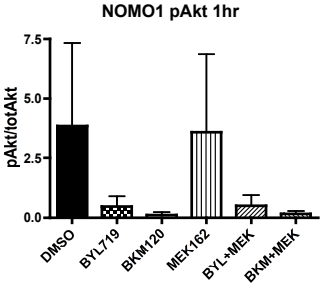
Figure S10: Quantification of THP1 time course western blot in Figure 8E. Signal intensities were normalized to the indicated loading controls.

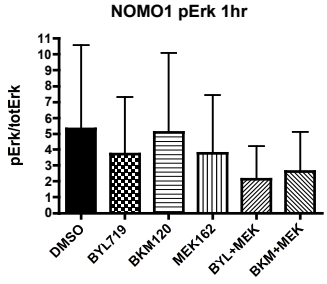

15 min
1 hour
BYL-719
BKM-120
MEK-162
pAkt (Ser473)
total Akt
pErk
total Erk
pS6 (Ser235/236)
total S6
6 hours
24 hours
BYL-719
BKM-120
MEK-162
pAkt (Ser473)
total Akt
pErk
total Erk
pS6 (Ser235/236)
total S6

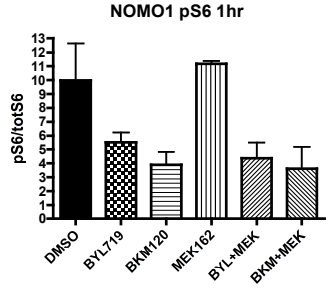

NOMO1 pAkt 15 min
pAkt/totAkt
DMSO
BYL719
BKM120
MEK162
BYL+MEK
BKM+MEK


NOMO1 pErk 15 min
pErk/totErk
DMSO
BYL719
BKM120
MEK162
BYL+MEK
BKM+MEK


NOMO1 pS6 15 min
pS6/totalS6
DMSO
BYL719
BKM120
MEK162
BYL+MEK
BKM+MEK


NOMO1 pAkt 1hr
pAkt/totAkt
DMSO
BYL719
BKM120
MEK162
BYL+MEK
BKM+MEK


NOMO1 pErk 1hr
pErk/totErk
DMSO
BYL719
BKM120
MEK162
BYL+MEK
BKM+MEK


NOMO1 pS6 1hr
pS6/totS6
DMSO
BYL719
BKM120
MEK162
BYL+MEK
BKM+MEK

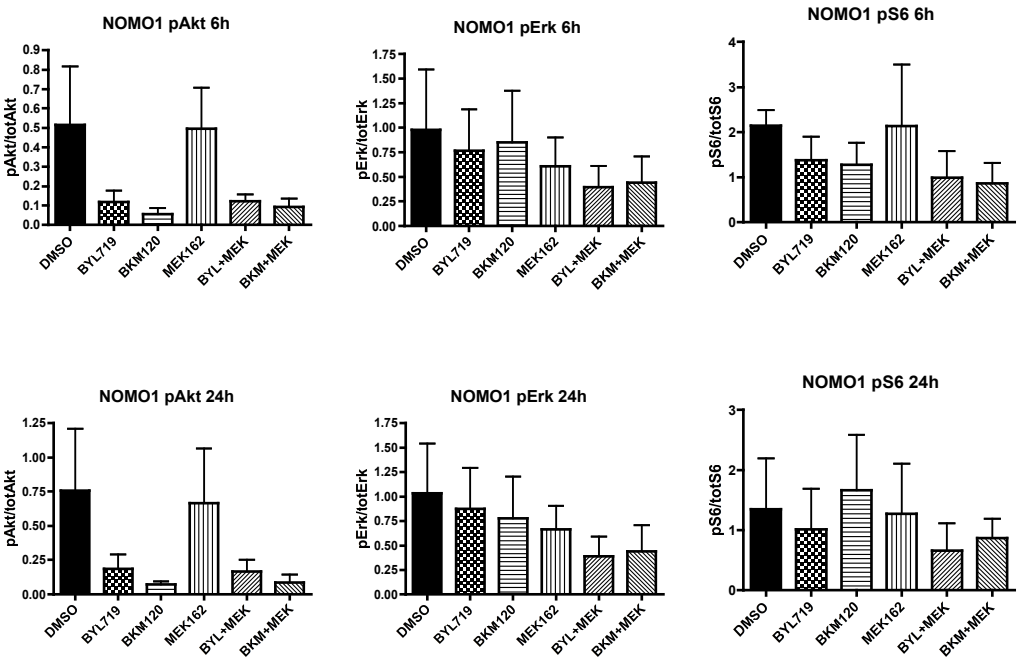
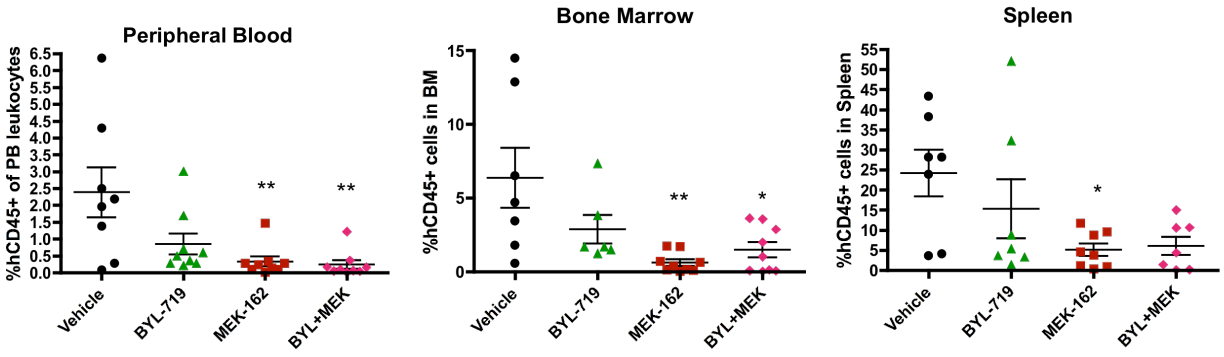


Figure S11: Time Course of inhibitor treatment of NOMO1 cells

NOMO1 cells were treated with the following inhibitors for the indicated time periods: BYL719 (1μM), BKM120 (1μM), and MEK162 (25nM). A representative Western blot is shown. The lanes were run on the same gel, but were non-contiguous. The experiment was performed 3 times. Quantitation is shown below. Signal intensities were normalized to the indicated loading controls.

A.



B.

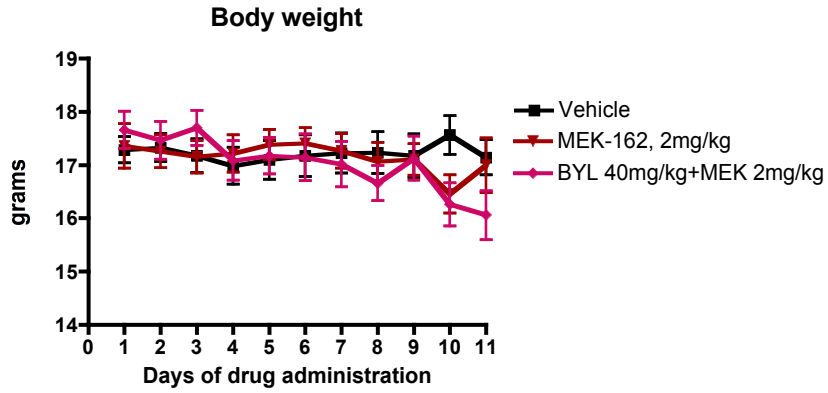


Figure S12: THP1 Xenograft Trial with BYL-719 and MEK-162 (A) Flow cytometry for human CD45+ cells in the peripheral blood, bone marrow, and spleen of NOG recipient mice confirms decreased disease burden in mice xenografted with THP1 cells after treatment with BYL-719 (40mg/kg/d), MEK-162 (10mg/kg/d), or the combination. The one-way ANOVA test with the Bonferroni multiple comparison post-test was used to compare the groups. *p<0.05, **p<001 (B) Body weights of mice treated with Vehicle, MEK-162 (2mg/kg/d), or the combination of BYL-719 (40mg/kg/d) and MEK-162 (2mg/kg/d). The differences were not significant at any of the time points by the one-way ANOVA test.