Supplementary Figures (1-11)

Therapy-induced hypoxia contributes to AML drug-resistance through BMX Kinase upregulation

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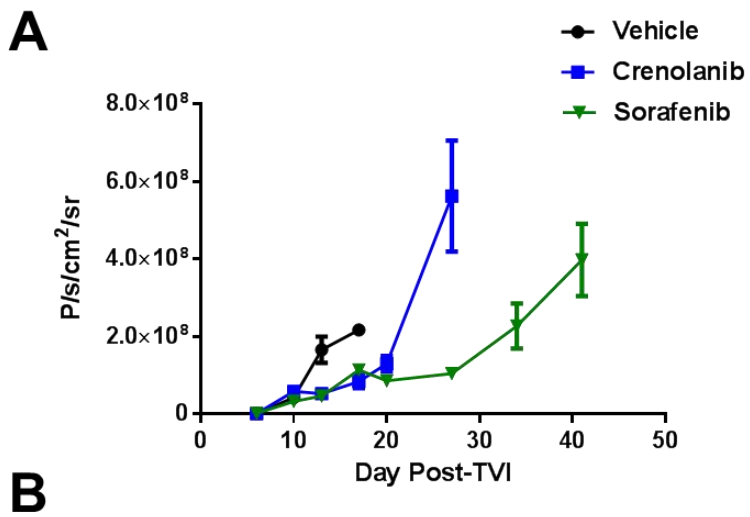
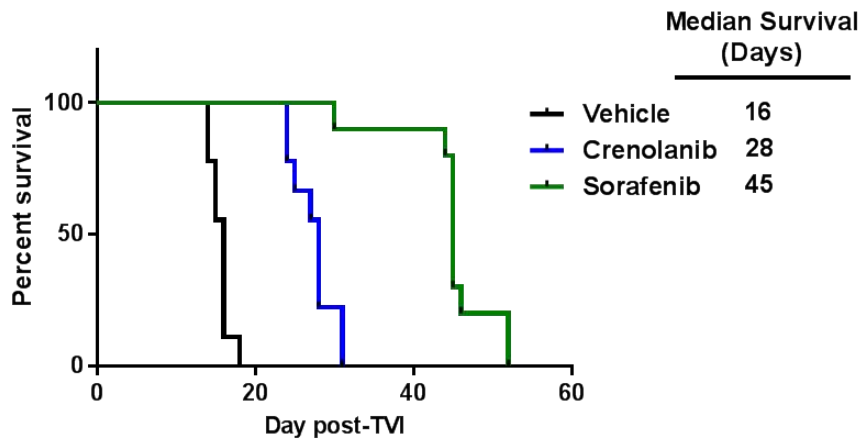
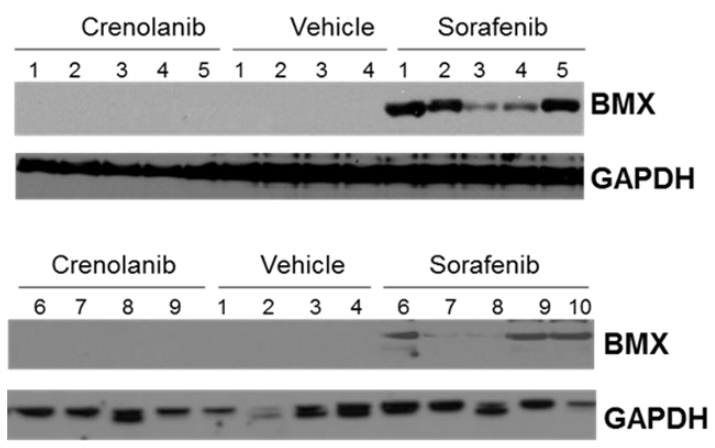
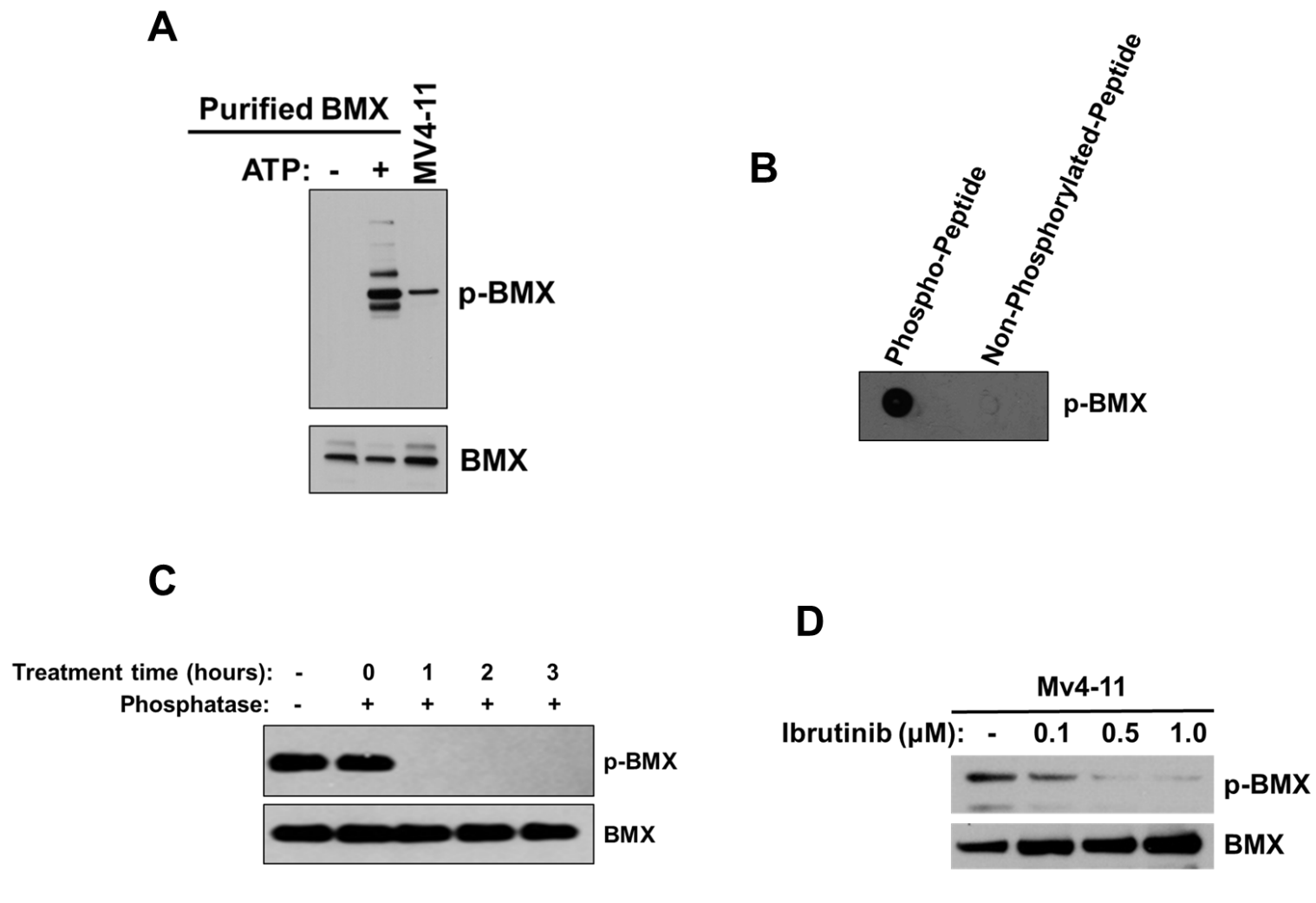




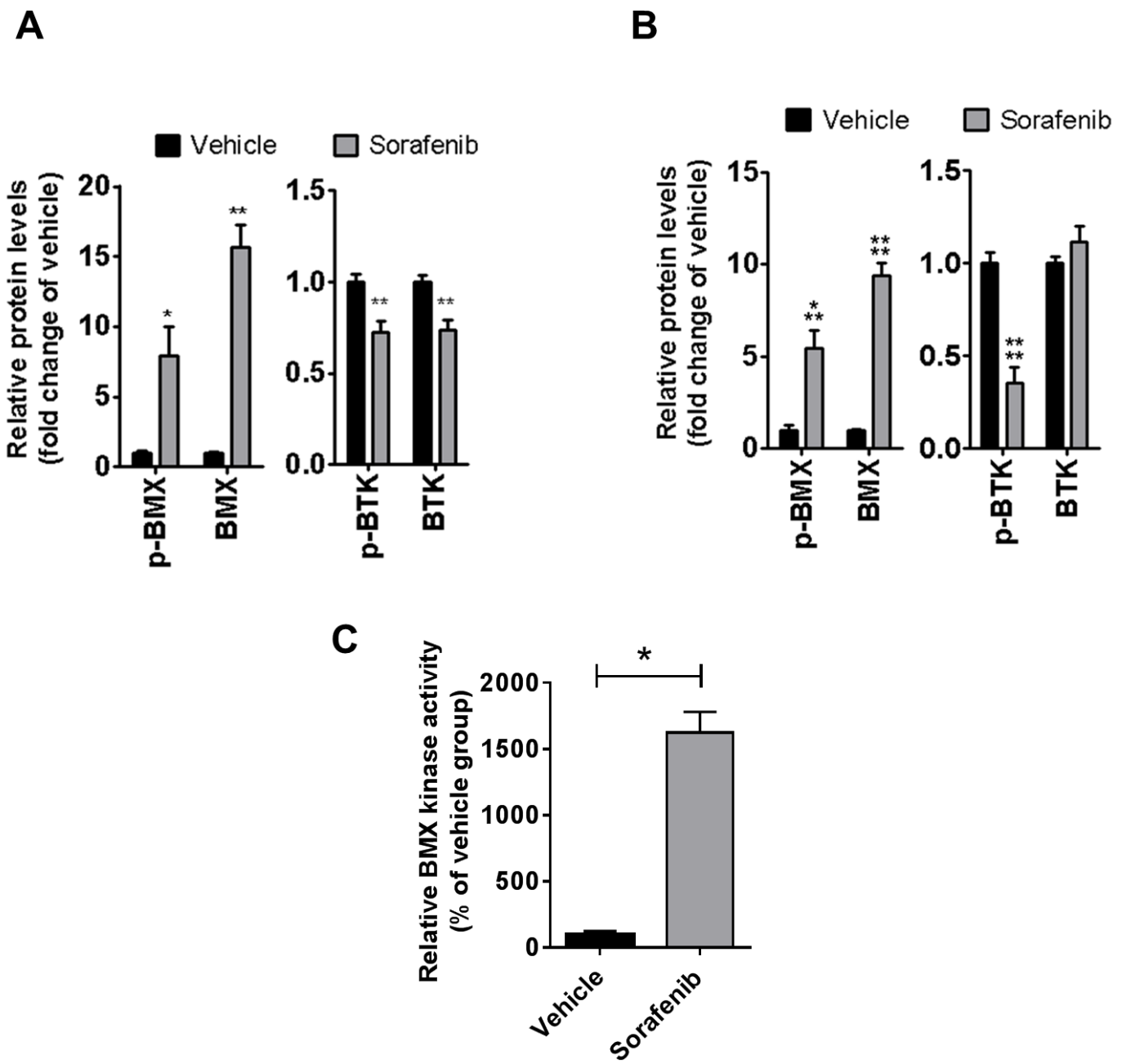


	Animal ID (as shown in Supplementary Table 3)		
Lane #	Crenolinib	Vehicle	sorafenib
1	4378	4372	4374
2	4355	4366	4356
3	4370	4353	4376
4	4359	4344	4349
5	4346		4371
6	4351		4368
7	4347		4363
8	4358		4365
9	4354		4352
10			4377

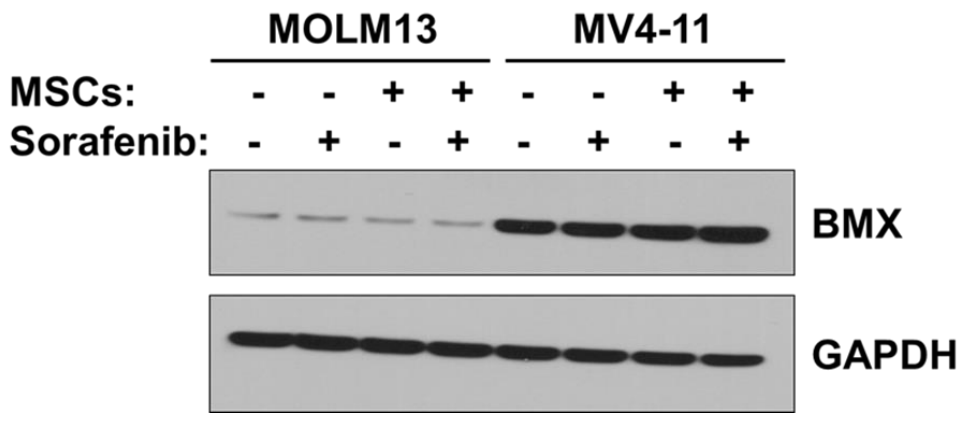
Supplementary Figure 1: BMX upregulation during sorafenib resistance *in vivo.* (A) Pilot survival study of crenolanib and sorafenib treatment in a MOLM13 cell xenograft in NSG mice. Treatment started 10 days post tail vein injection (TVI) of MOLM13 cells with vehicle, crenolanib (15 mg/kg twice daily), or sorafenib (60 mg/kg once daily). Mice were treated until signs of leukemic progression (N=9 per treatment group). (Left panel) Mean (± SD) whole body bioluminescence signal over the duration of treatment is shown for each treatment group. (Right panel) Kaplan-meier analysis of animal survival in the different treatment groups. (**B**) In a repeat treatment study, MOLM13 leukemic cells were obtained from the bone marrow of mice treated with vehicle (N=4; day 17 post-TVI), crenolanib (N=9, day 24 post-TVI) or sorafenib (N=10, day 40 post TVI) and western blot analysis of indicated proteins was performed.



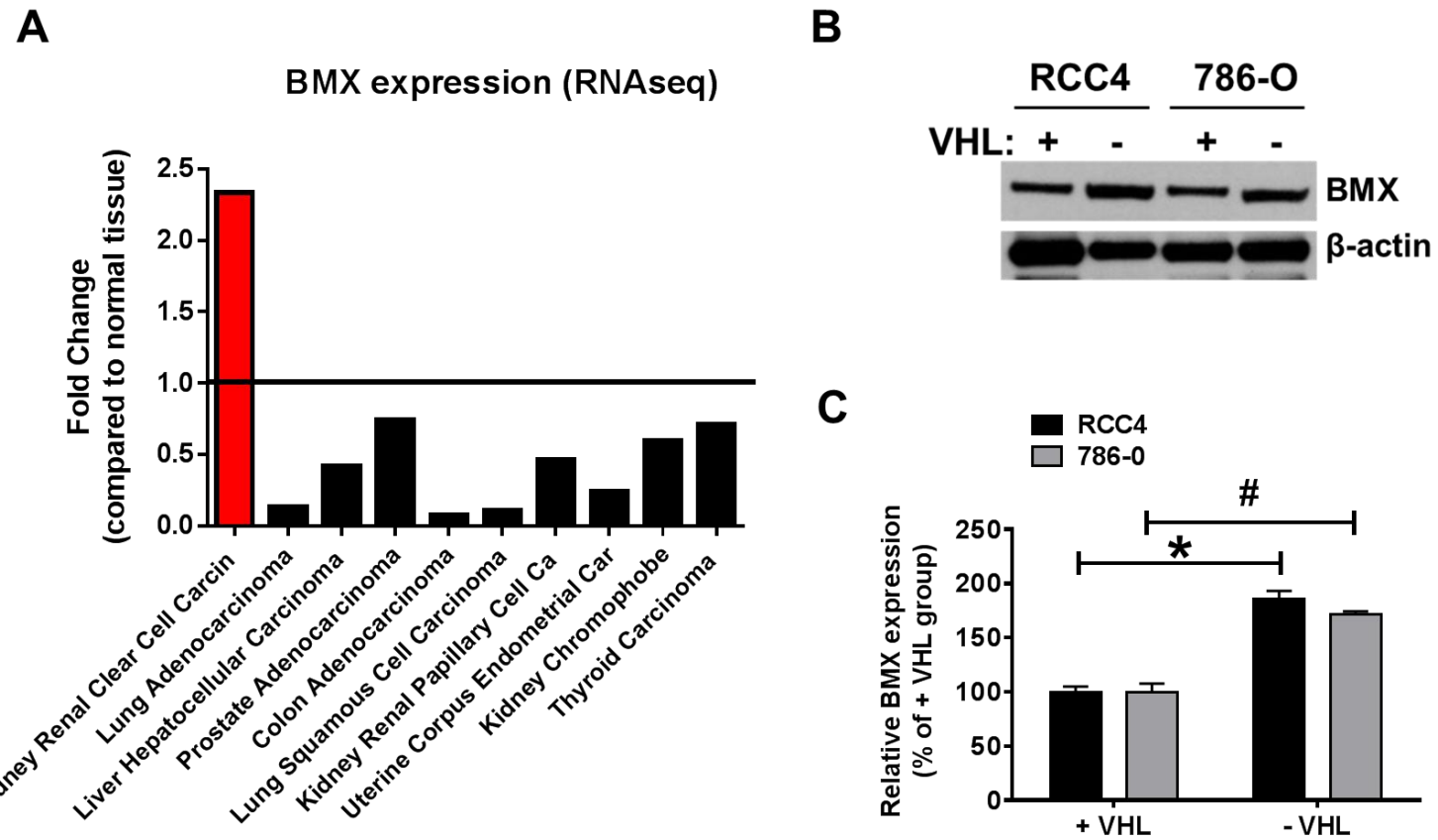
Supplementary Figure 2. Characterization of phospho-BMX (tyr-194) antibody. (**A**) Purified recombinant human BMX protein was incubated in a kinase buffer in the presence or absence of ATP followed by western blot analysis (lane 1 and 2). The Phospho-tyr-194 antibody specifically detected active BMX (+ATP). This antibody also detected active BMX in whole cell lysates from MV4-11 cells (lane 3). (**B**) Representative dot blots showing the specificity of the phospho-BMX antibody. (**C**) Whole cell lysates from MV4-11 cells were treated with lambda Protein Phosphatase, followed by western blot analysis of total and phospho-BMX. (**D**) MV4-11 cells were treated with Ibrutinib for 1 hour followed by whole cell lysate collection and western blot analysis. Ibrutinib treatment reduced the levels of p-BMX in Mv4-11 cells. Data from two independent experiments.



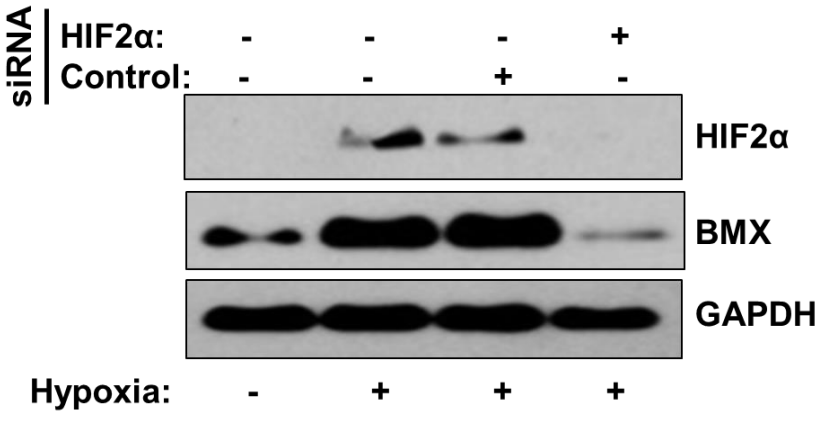
Supplementary Figure 3. Sorafenib induces BMX upregulation and activation in a MOLM13 FLT3-ITD+ mouse model of sorafenib resistance. MOLM13 cells were administered to NSG mice by tail vein injection (TVI) and treatment was started on day 10 post-TVI. After 10 days (**A**) or 30 days (**B**) of sorafenib treatment, BMX is activated in bone marrow leukemic cells, whereas p-BTK is downregulated. Quantitation of protein expression shown in Figure 2C and 2D. **p<0.05, ***p<0.005, ****p<0.0005 by Welch's t-test. (**C**) BMX was immunoprecipitated from cells derived from vehicle or sorafenib treated mice (N=5 per treatment group) followed by in vitro kinase assay, which showed that BMX kinase activity is elevated in the AML cells derived from sorafenib treated mice as compared to the vehicle treated group (*P = 0.0005, Welch's t-test).



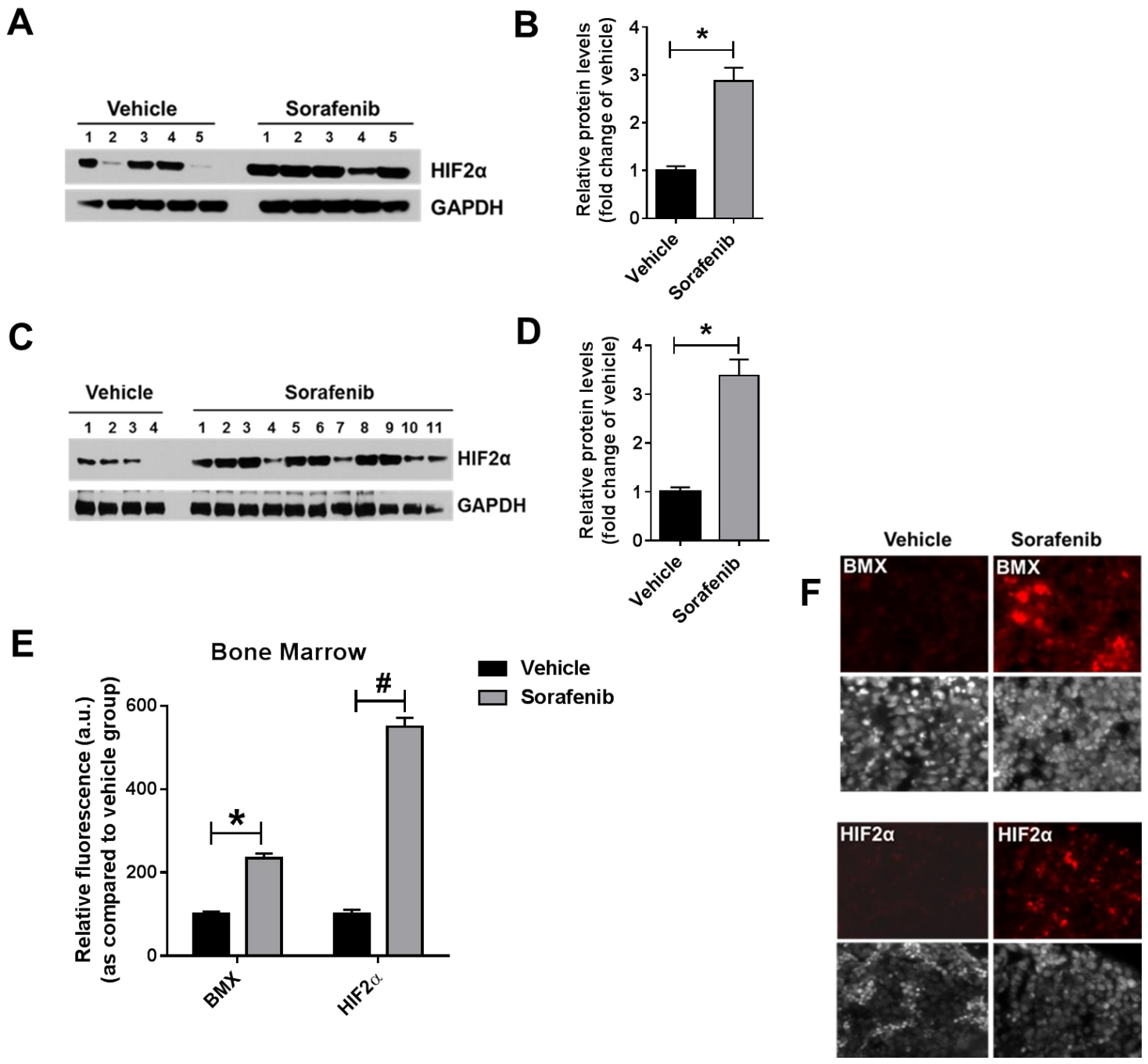
Supplementary Figure 4. BMX expression during sorafenib treatment *in vitro*. Indicated AML cell lines were grown on MSCs or alone, followed by treatment with sorafenib for 24 hours. Whole cell lysates were used for western blot analysis of indicated proteins, which showed no significant increase in BMX expression under these conditions. Representative blot of 2 independent experiments.



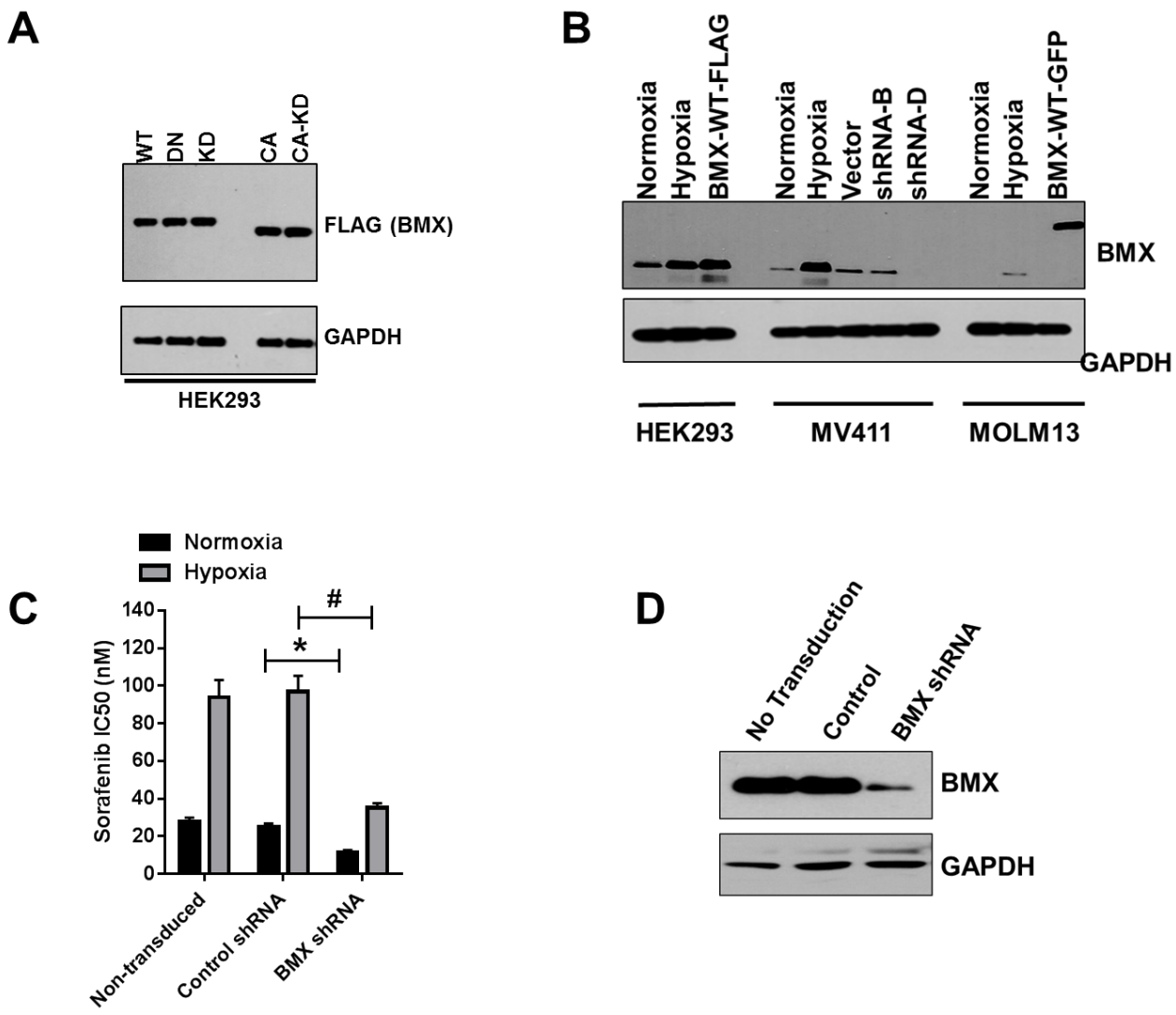
Supplementary Figure 5. BMX expression is elevated in renal cell carcinoma. (**A**) BMX expression in indicated patient cancer samples derived from The Cancer Genome Atlas (TCGA). (**B**) BMX protein expression in indicated renal cell carcinoma cell lines (VHL: Von Hippel-Lindau). Representative blot from 2 independent experiments (**C**) Densitometric analysis of data in panel B, showing increased expression of BMX in RCC cells lines (N=3) (*P = 0.0008 and $^{\#}$P **=** 0.0057, Welch's t-test).



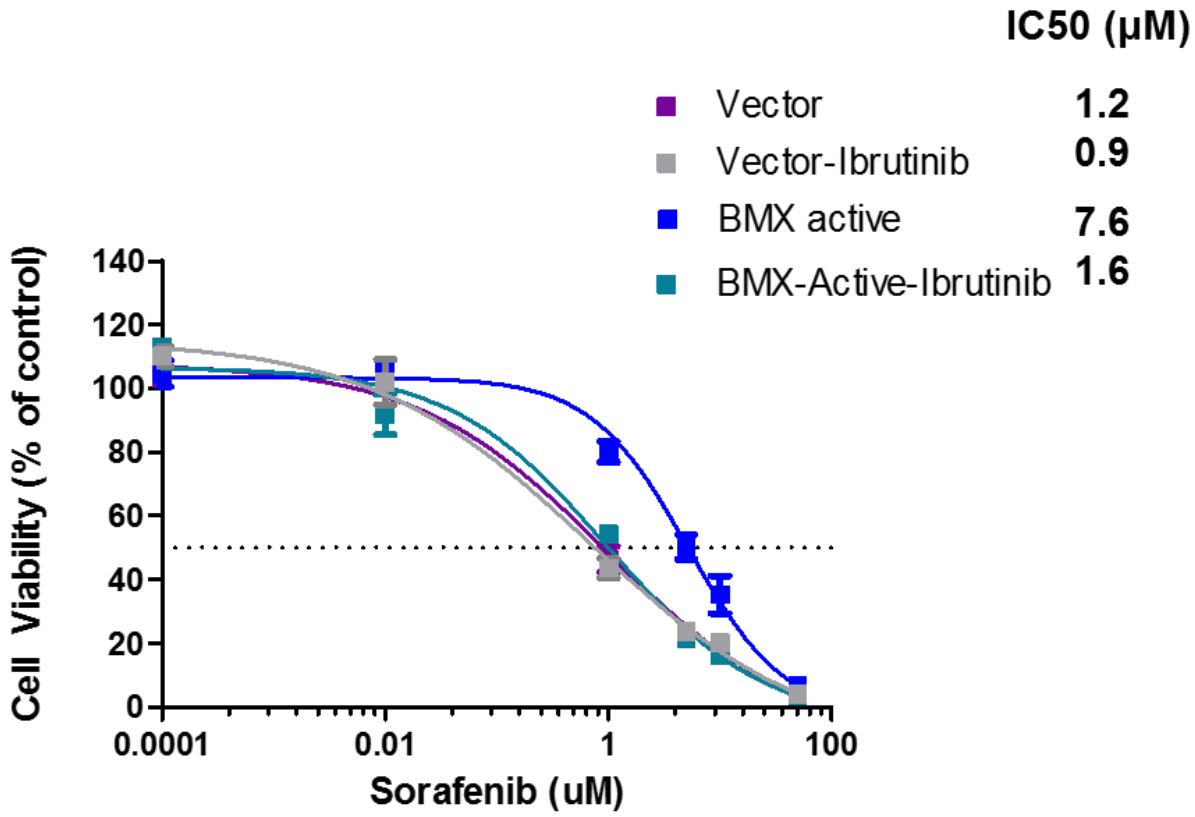
Supplementary Figure 6. BMX upregulation is HIF2α dependent. HEK293 cells were transfected with control or HIF2α siRNA, followed by hypoxia treatment and western blot analysis. HIF2α knockdown abrogated hypoxia mediated BMX upregulation. Representative blots from at least two independent experiments.



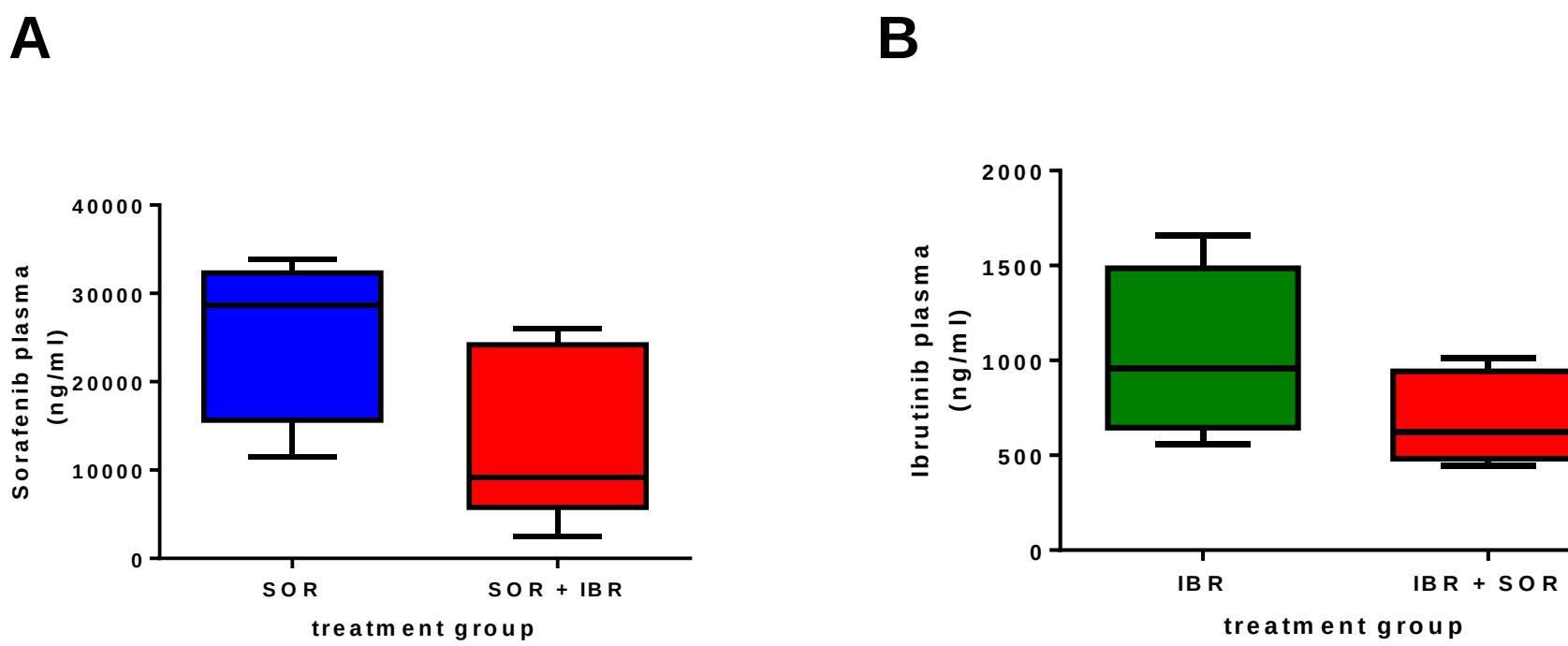
Supplementary Figure 7. Sorafenib induces HIF2α pregulation in a MOLM13 FLT3-ITD+ xenograft model of sorafenib resistance. (A) MOLM13 cells were administered to NSG mice by tail vein injection (TVI), and 10 days post-TVI mice were treated with vehicle or sorafenib (60 mg/kg once daily for 5 days per week). After 10 days (**A-B**) or 30 days (**C**-**D**) of sorafenib treatment, MOLM13 cells were isolated from bone marrow, followed by western blot analysis of indicated proteins. (B) Densitometric analysis of data in panel A (*P = 0.0014, Welch's t-test). (D) Densitometric analysis of data in panel C (*P < 0.0001, Welch's t-test). (**E-F**) Bone marrow from vehicle or sorafenib treated mice were used for immunohistochemical analysis of BMX and HIF2α protein levels in the leukemic cells. (E) Image J software was used to quantify the relative expression of indicated proteins as compared to the vehicle group (set as 100%); N = 4 per treatment group (*P = 0.0003 and #P < 0.0001, Welch's t-test). (F) Representative images (lower panels show nuclei staining) at 40X magnification. More than 3 fields were analyzed per sample per mice. These results indicate that BMX and HIF2α expression is induced in MOLM13 cells in the bone marrow during sorafenib treatment.



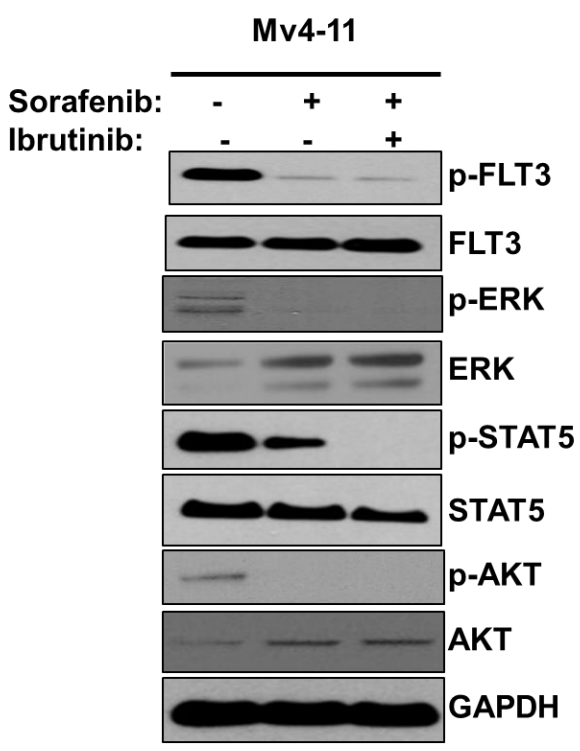
Supplementary Figure 8. BMX knockdown abrogates hypoxia-induced sorafenib resistance in MV4-11 cells. (**A**) Western blot showing the expression of Flag-tagged BMX in HEK293 cells (**B**) Western blot showing the expression of endogenous BMX under normoxic and hypoxic conditions as well as the knockdown and overexpression of BMX in indicated cell lines. (**C,D**) MV4-11 cells were transduced with either a control or BMX shRNA lentiviral particles followed by treatment with sorafenib under normoxic or hypoxic conditions. (C) After 72 hours treatment with sorafenib, MTT assays were performed followed by measurement of IC50 values for sorafenib. The graph shows the cumulative IC50 values from 3 independent experiments (*P = 0.0075 and $^{\#}$P = 0.013. Welch's t-test). (D) Representative western blot of 3 independent experiments showing BMX knockdown as compared to control group.



Supplementary Figure 9. Ibrutinib treatment reverses sorafenib resitance mediated by active BMX. HEK293 cells were transiently transfected with either empty vector or constitutively active BMX plasmids. Post-transfection, cells were treated with increasing concentration of sorafenib or 100 nM Ibrutinib for 1 hour followed by sorafenib + ibrutinib for 48 hours. In a MTT cell viability assay, sorafenib resistance (BMX active group) is reversed by ibrutinib treatment (BMX-active-ibrutinib group). Representative of 2 independent experiments.



Supplementary Figure 10. Plasma concentrations in mice treated with sorafenib (SOR) alone, ibrutinib (IBR) alone, or the combination. Plasma concentrations of (**A**) sorafenib (P = 0.104, Welch's t-test) or (**B**) Ibrutinib (P = 0.172, Welch's t-test) are not significantly altered during combination treatment *in vivo*. N = 5 per treatment group.



Supplementary Figure 11. Ibrutinib treatment reduces STAT5 activation during sorafenib treatment in MV411 cells. MV411 cells were treated with the indicated kinase inhibitors for 1 hour followed by whole cell lysate collection and western blot analysis. Representative blot of 3 independent experiment.

Supplementary Tables (1-5)

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