Supplemental Methods

Antibodies and Cytokines

Rabbit anti-phospho-AKT (S473)(D9E), anti-AKT, anti-phospho-SHP2 (Y580), anti-phospho-KIT (Y719), and anti-Gab2 were purchased from Cell Signaling Technologies (Danvers, MA). Rabbit anti-SHP2 (C-18) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse anti-p85α (AB6) was purchased from Millipore (Billerica, MA). Mouse anti-β-actin (AC-74) was purchased from Sigma (St. Louis, MO). PE-conjugated anti-annexin V antibody and 7-amino actinomycin D (7-AAD) were purchased from BD Pharmingen (San Diego, CA). Murine and human recombinant interleukin-3 (IL-3), interleukin-6 (IL-6), stem cell factor (SCF), thrombopoietin (TPO), FMS-like tyrosine kinase 3 ligand (FLT3L), macrophage colony stimulating factor (M-CSF) were purchased from Peprotech (Rocky Hill, NJ). Retronectin was purchased from Takara (Madison, WI).

Expression of WT and mutant KIT receptors in 32D cells and primary HSC/Ps

32D cells and primary LDMNC were transduced with WT or chimeric KIT receptors as described previously [1,2]. Briefly, retroviral supernatants for transduction of 32D cells and primary LDMNC were generated using Phoenix ecotropic packaging cell line transfected with retroviral vector plasmids using a calcium phosphate transfection kit (Invitrogen, Carlsbad, CA). Supernatants were collected 48 hours after transfection and filtered through 0.45-μm membranes. 32D cells were infected twice at 24 hour intervals with 2 mL high-titer virus supernatant in the presence of 10 ng/mL IL-3 and 8 μg/mL polybrene. LDMNCs were incubated in prestimulation medium (IMDM plus 20% FBS and 2% Penicillin-Streptomycin) supplemented with 100 ng/mL SCF, 100 ng/mL TPO, 50 ng/mL FLT3L, and 4 ng/mL recombinant IL-6 for two days. After 48 hours,

prestimulated LDMNCs were infected with the viral supernatants in the presence of same cytokine cocktails and 20% FBS on fibronectin fragments in 6-well non-tissue culture plate. Forty-eight hours after infection, either 32D cells or primary LDMNCs expressing vector, WT KIT, KITD814V or chimeric KIT receptors were sorted to homogeneity based on EGFP expression and utilized to perform all the experiments

Analysis of apoptosis and cell cycle

Serum and growth factor starved cells (2 X10^5/well) were incubated in 24 well-plate in 0.5 mL complete medium in the presence or absence of growth factors and inhibitors at indicated concentrations for 48 hours at 37ºC. Cells were harvested and washed with PBS containing 0.2% BSA. Cells were subjected to apoptosis assay with Annexin V: PE Apoptosis Detection Kit I (BD Biosciences Pharmingen, San Diego, CA) according to the manufacturer's protocol. For cell cycle assay, starved and incubated cells were stained with propidium iodide (PI) for 10 min at room temperature. Stained cells were analyzed on a FACS flow cytometer (BD Biosciences, San Jose, CA) and data was analyzed using the CellQuest software or ModFit LT for Mac (Verity Software House, Topsham, ME).

Legends for Supplemental Figures

Supplemental Figure 1. Pharmacokinetic analysis of mice treated with a SHP2 inhibitor II-B08. Mice received 100 mg/kg II-B08 intraperitoneally each day X 7 days followed by collection of blood at 30 min, 1 hour, 4 hours, and 24 hours after the first injection. A serum sample was also collected 24 hours after the final (7^{th}) injection (168 hours after the first injection). A Cmax of 155 µM +/- 24.7 was determined. Twenty-four hours after 1^{st} dose, a trough concentration of 0.14 µM +/- 0.002 was found, which was about 100-fold lower than effective concentrations of II-B08 *in vitro* (~10 µM). However, after a cumulative dose of 700 mg/kg (7 doses of 100 mg/kg over 7 days), we found a steady state concentration of 6.4 µM +/- 6.3.

Supplemental Figure 2. (A) 6-8 weeks old *SHP2fl/fl/Cre*- (referred as WT) and *SHP2fl/fl/Cre*+ (referred as *SHP2-/-*) mice were treated with 3 doses of poly I:C (300 µg) on alternative days. After 2 weeks of final injection, DNA was isolated from peripheral blood and analyzed by PCR. *Cre*-mediated deletion of SHP2 was detected as a 400-bp fragment and that of WT SHP2 was observed as a 1.1-kb fragment. **(B) & (C)** Primary BM derived cells from WT and *SHP2-/-* mice were transduced with bicistronic retrovirus expressing WT KIT or KITD814V and EGFP. Transduced cells were sorted to homogeneity based on EGFP expression. SHP2 deletion was analyzed by western blotting with anti-SHP2 antibody (B). Representative flow micrograph indicates similar expression of WT KIT or KITD814V in WT or *SHP2-/-* mice (C). **(D)** Primary BM derived cells expressing WT KIT or KITD814V from WT or *SHP2-/-* mice were starved and subjected to proliferation assay in the presence or absence of SCF (50 ng/mL) by thymidine incorporation. Bars denote the mean thymidine incorporation (Mean ± SD) from one of three independent experiments performed in triplicate. *$p < 0.01$, WT-WT KIT vs. *SHP2-/-*-WT KIT or

WT-KITD814V vs *SHP2-/-*-KITD814V. **(E)** Primary BM derived cells from WT (SHP2+/+) or SHP2fl/fl mice bearing tamoxifen Cre were transduced with retrovirus encoding WT KIT or KITD814V and cells were sorted to homogeneity based on EGFP expression. Deletion of SHP2 in cells cultured in the presence of tamoxifen was confirmed by western blot analysis using an anti-SHP2 antibody (Left panel). Transduced cells were starved and subjected to proliferation assay in the absence of growth factors by thymidine incorporation (Right panel). Bars denote the mean thymidine incorporation (Mean ± SD) from one experiment performed in quadruplicate. *p < 0.01, WT-KITD814V vs. *SHP2-/-*-KITD814V.

Supplemental Figure 3. SHP2 inhibitor II-B08 treatment show significantly reduced growth of cells bearing KITD814V. (A) 32D cells expressing WT KIT or KITD814V were starved and subjected to proliferation assay in the presence of IL-3 (10 ng/mL) and indicated amounts of II-B08 by thymidine incorporation. Bars denote the mean thymidine incorporation (Mean ± SD) performed in triplicate. *p <0.05. **(B)** 6-8 week old *SHP2fl/fl/Cre-* (referred as WT) and *SHP2fl/fl/Cre+* (referred as *SHP2-/-*) mice were treated with 3 doses of poly I:C (300 µg) on alternative days. After 2 weeks, BM derived cells from WT and *SHP2-/-* mice were transduced with bicistronic retrovirus expressing WT KIT and EGFP, and transduced cells were sorted to homogeneity based on EGFP expression. WT or *SHP2-/-* cells expressing WT KIT were starved and subjected to proliferation assay in the presence of SCF (50 ng/mL) and indicated amounts of II-B08 by thymidine incorporation. Bars denote the mean thymidine incorporation (Mean ± SD) performed in triplicate. No significant difference in the growth of WT or *SHP2-/-* cells bearing WT KIT was observed in the presence of II-B08.

Supplemental Figure 4. SHP2 inhibitor II-B08 treatment has no effect on the hematologic profile in treated mice. Mice received 100 mg/kg II-B08 intraperitoneally each day X 7 days. Mice were harvested after 24 hours of final injection and peripheral blood, bone marrow, spleen and thymus were analyzed. No significant difference in peripheral blood cell counts **(A)**, spleen and liver weights **(B)**, and cellularity of bone marrow, spleen and thymus **(C)** were observed in mice treated with II-B08 compared to DMSO. n=4.

Supplemental Figure 5. SHP2 inhibitor II-B08 treated mice do not show drug related tissue or cellular toxicity. (A) Mice received 100 mg/kg II-B08 intraperitoneally each day X 7 days. After 24 hours of final injection, bone marrow, spleen and thymus were harvested. Flow cytometric analysis of cells from bone marrow, spleen and thymus derived from mice treated with DMSO or II-B08. No significant difference in various blood lineages was observed in mice treated with II-B08 compared to DMSO. **(B)** Histopathologic analysis of mice treated with II-B08 compared to DMSO. Bone marrow, liver, kidney and heart from the mice treated with DMSO or II-B08 were harvested, fixed in 10% buffered formalin, sectioned, and stained with hematoxylin and eosin. Shown are representative tissue sections from mice treated with DMSO or II-B08. No compound related changes were observed in these mice as determined by a board certified pathologist, Dr. Sandusky. n=4.

Supplemental Figure 6. SHP2 inhibitor II-B08 treatment has no further effect on the hematologic profile in SHP2 deficient mice. WT or *SHP2-/-* mice received 100 mg/kg II-B08 intraperitoneally each day X 7 days. After 24 hours of final injection, peripheral blood, bone marrow, spleen and thymus were harvested and analyzed. No significant difference in WBC

counts, bone marrow and spleen cellularity was observed in mice treated with II-B08 compared to DMSO.

Supplemental Figure 7. SHP2 inhibitor II-B08 treatment has no effect on cycling of cells bearing WT KIT or KITD814V. 32D cells bearing WT KIT or KITD814V were starved and treated with indicated concentrations of II-B08 for 48 hours. Assays were performed in the presence of IL-3 (10 ng/mL) for cells bearing WT KIT and in the absence of growth factors for cells bearing KITD814V. Cells were stained with propidium iodide followed by flow cytometric analysis. Percentage of cells in S-phase is indicated. No significant difference in the cycling of cells bearing WT KIT or KITD814V was observed upon II-B08 treatment (n=2).

Supplemental Figure 8. p85α and SHP2 form a constitutive protein complex with oncogenic KIT receptor in cell lines derived from human mastocytosis patients. (A) HMC 1.2 cells bearing activating human KIT mutation KITD816V were starved for 8 hours in serum- and growth factor-free medium followed by incubation with or without II-B08 (50 μM) for 1 hour. After incubation, equal amount of protein lysates were subjected to immunoprecipitation with an anti-SHP2 antibody followed by western blot analysis using an anti-p85α, anti-KIT or anti-SHP2 antibodies as indicated. Similar results were observed in two independent experiments.

Supplemental Figure 9. Schematic of mutant KITD814V receptors. (A) Wild-type (WT) and oncogenic chimeric KIT receptors were constructed by replacing the extracellular ligand binding domain of murine WT KIT or KITD814V with the ligand binding domain of human M-CSF receptor. A naked chimeric receptor (KITD814V-F7) was constructed in which seven critical tyrosines (Y) at indicated positions were changed to phenylalanine (F) using KITD814V as a

template. In the single tyrosine add-back mutant KITD814V-Y719 receptor, phenylalanine at tyrosine 719 in the KITD814V-F7 receptor was restored to tyrosine.

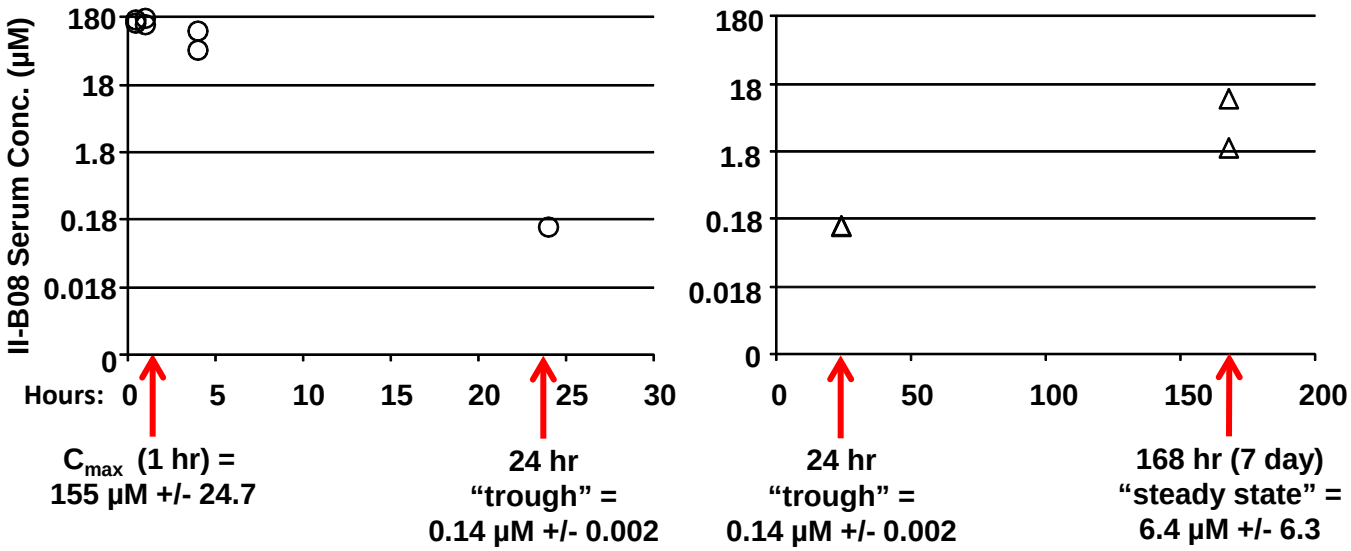
Supplemental Figure 10. SHP2 inhibitor II-B08 treatment suppress the activation of downstream targets in cell line derived human mastocytosis patient. (A) HMC 1.2 cells bearing activating human KIT mutation KITD816V were starved for 8 hours in serum- and growth factor-free medium followed by incubation with or without II-B08 (25 μM and 50 μM) for 1 hour. After incubation, equal amount of protein lysates were subjected to western blotting with anti-phospho-SHP2 (Y580), anti-phospho-AKT, anti-phospho-ERK, anti-ERK, and anti-β-actin antibodies as indicated. Similar results were observed in two independent experiments. **(B)** 32D cells bearing oncogenic KITD814V were injected into syngenic C3H/HeJ mice through tail vein. After 48 hours, mice were treated with either vehicle DMSO (n=2) or II-B08 (50 mg/kg body weight, n=2) at 24 hour intervals for 14 days. After 24 hours of final treatment, splenocytes were harvested from mice, lysed in lysis buffer, and equal amount of protein lysates were subjected to western blot analysis using anti-phospho-ERK or anti-ERK antibodies as indicated.

Supplemental References

1. Munugalavadla V, Sims EC, Borneo J, Chan RJ, Kapur R. Genetic and pharmacologic evidence implicating the p85 alpha, but not p85 beta, regulatory subunit of PI3K and Rac2 GTPase in regulating oncogenic KIT-induced transformation in acute myeloid leukemia and systemic mastocytosis. Blood. 2007;110:1612-1620.

2. Mali RS, Ramdas B, Ma P, et al. Rho kinase regulates the survival and transformation of cells bearing oncogenic forms of KIT, FLT3, and BCR-ABL. Cancer Cell;20:357-369.

Supplemental Figure 1



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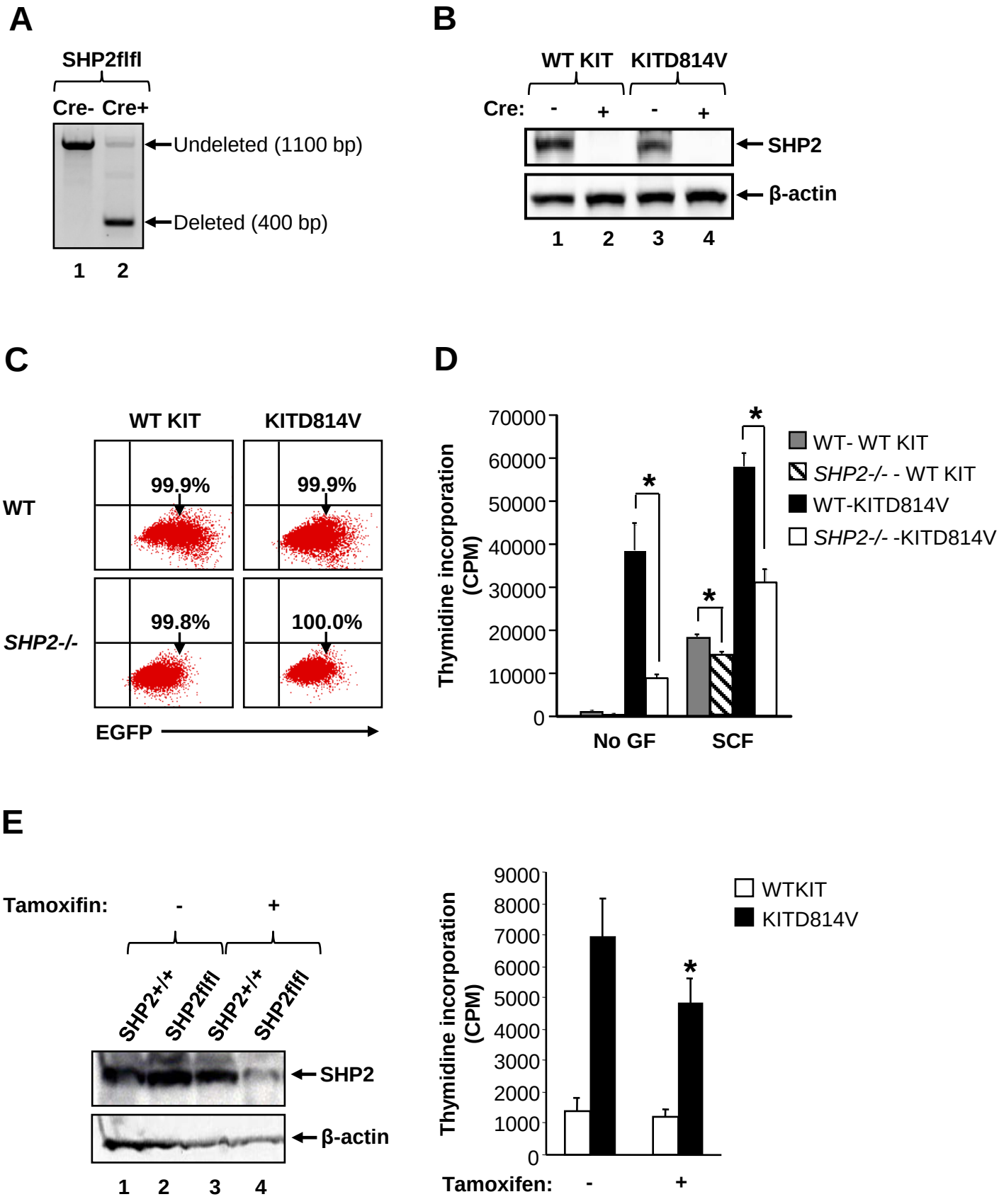

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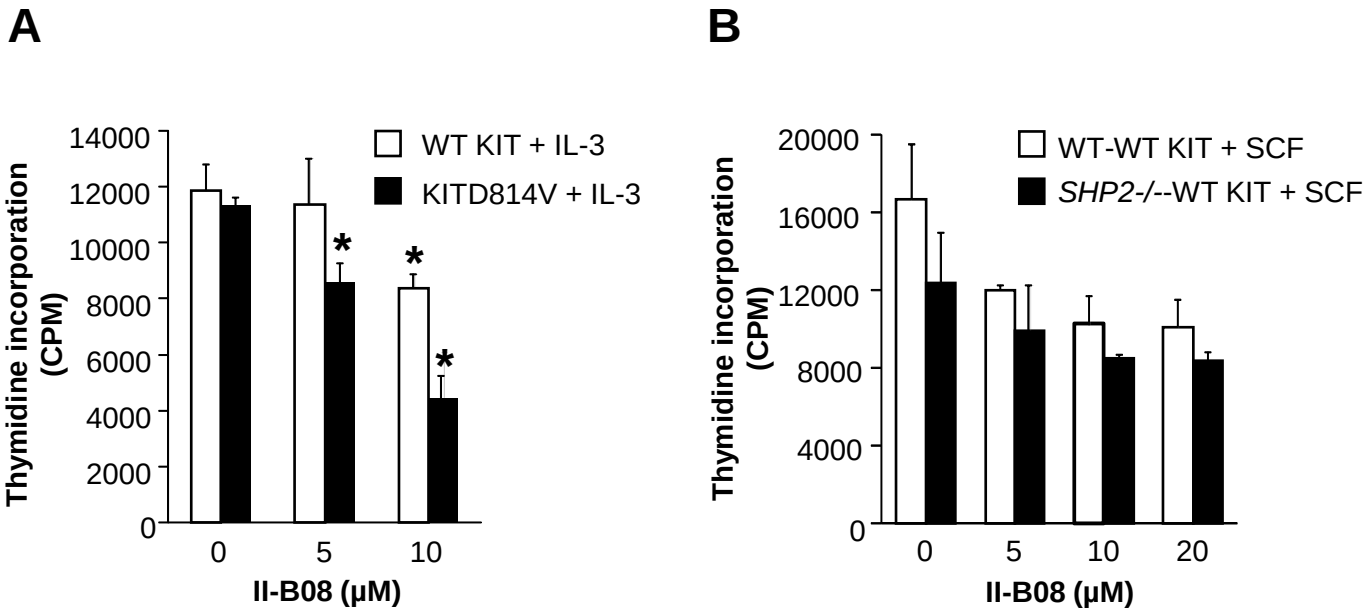

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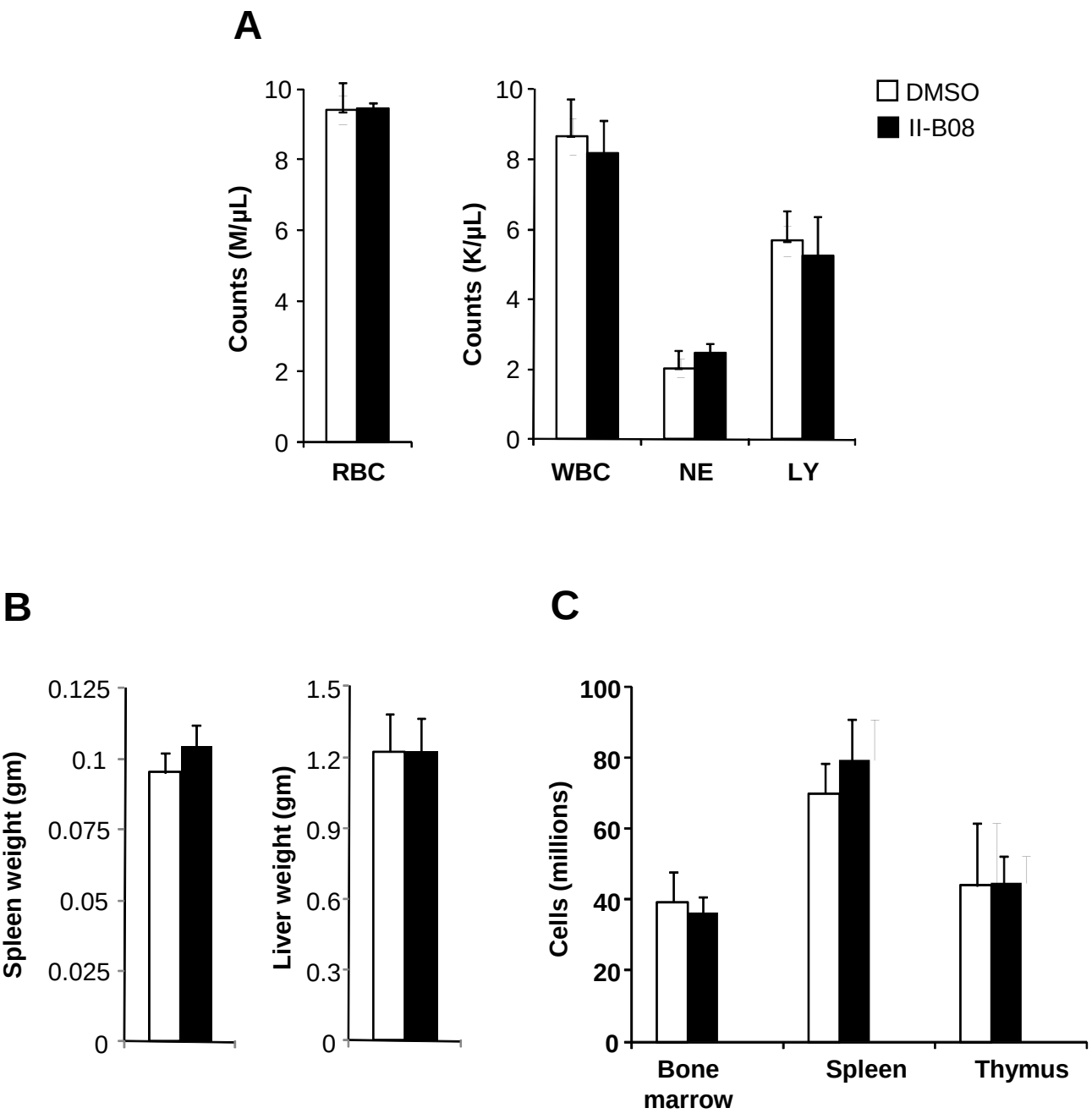

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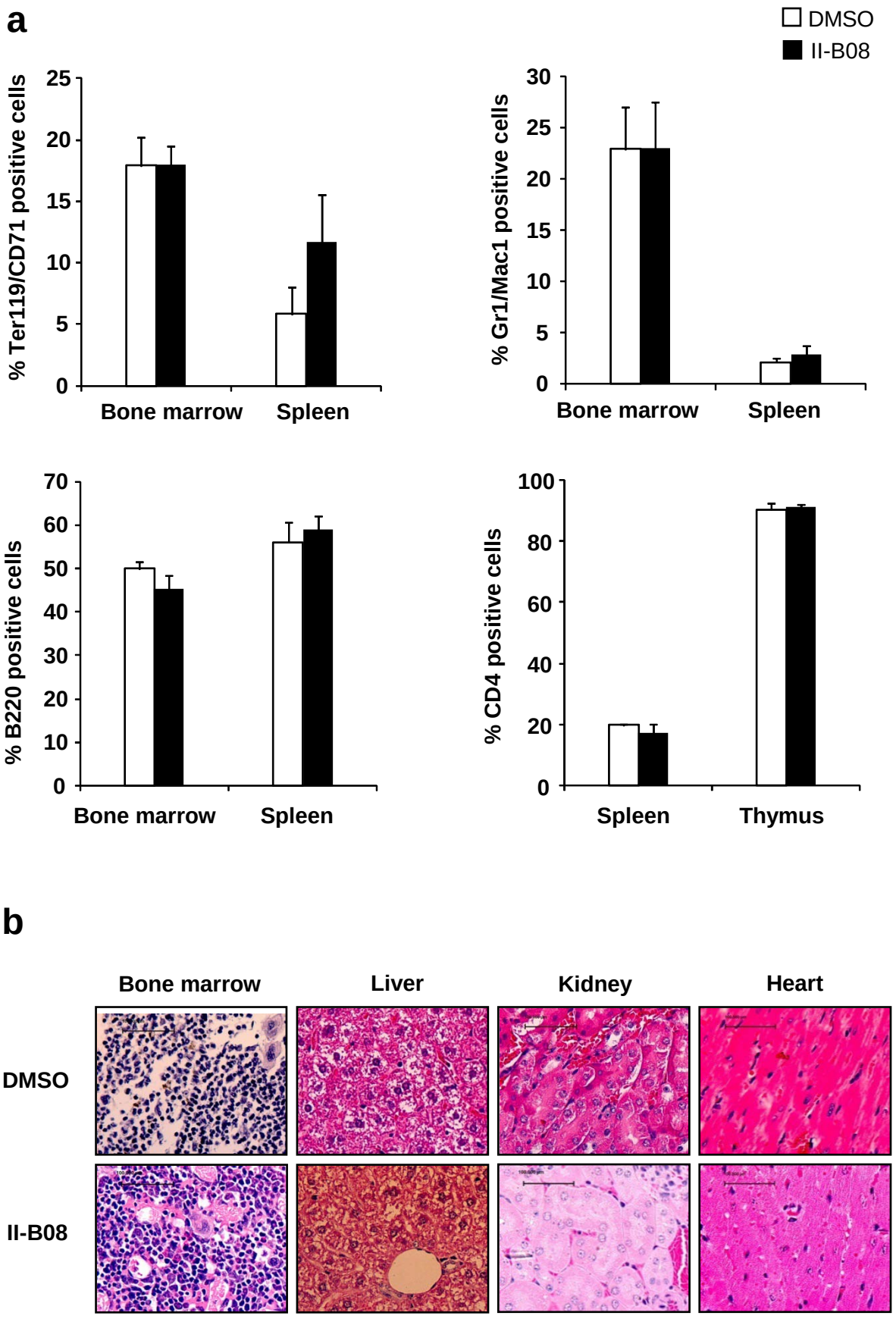

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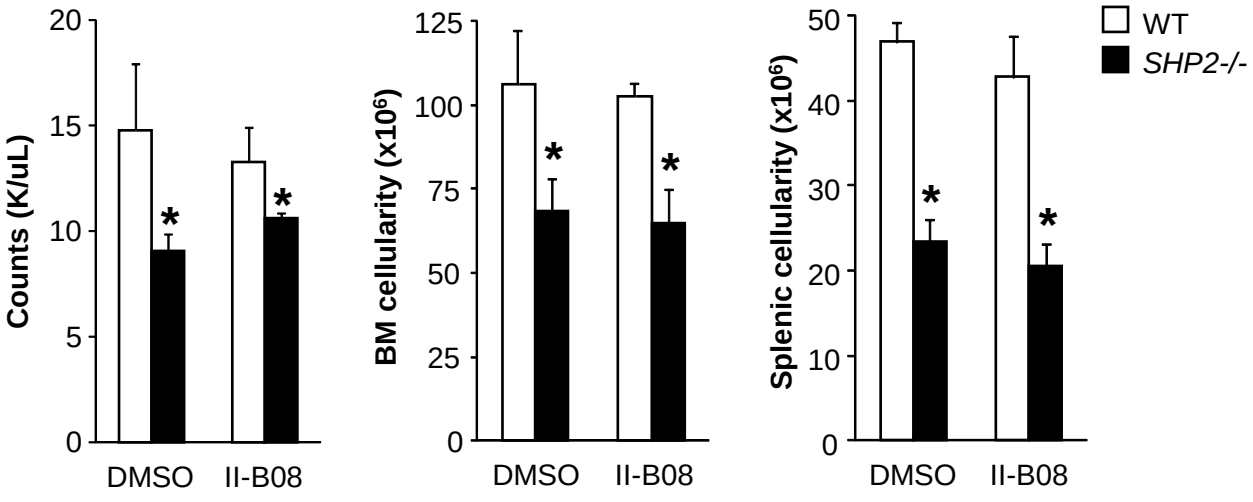

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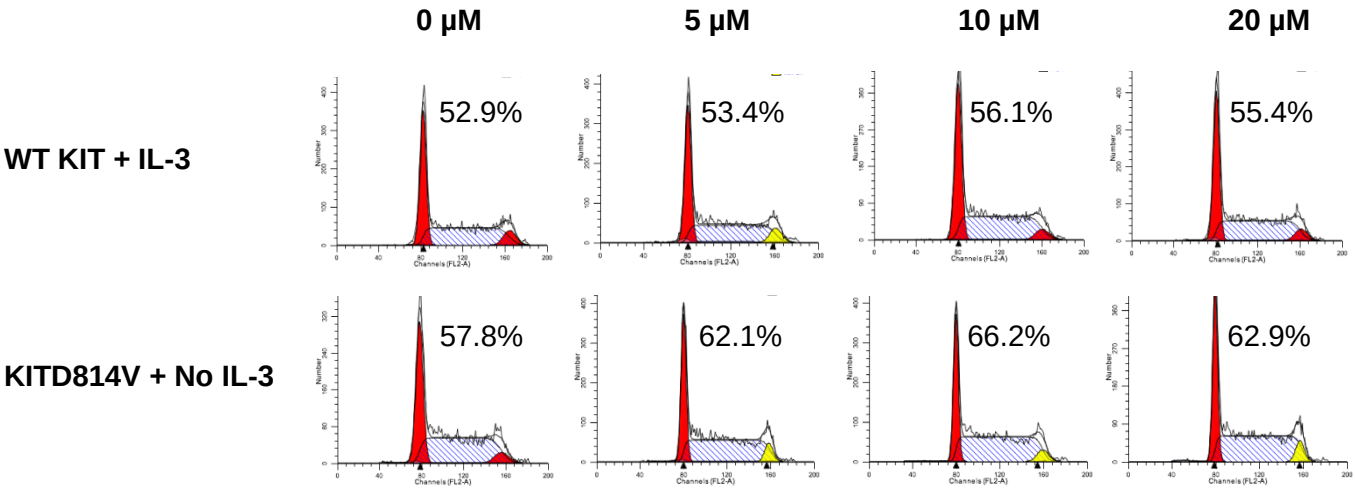

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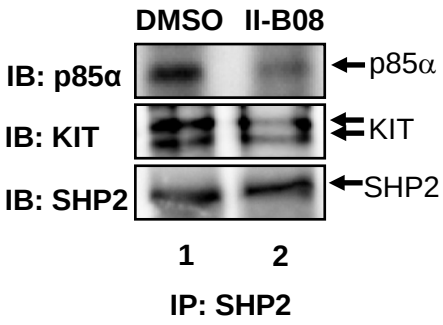
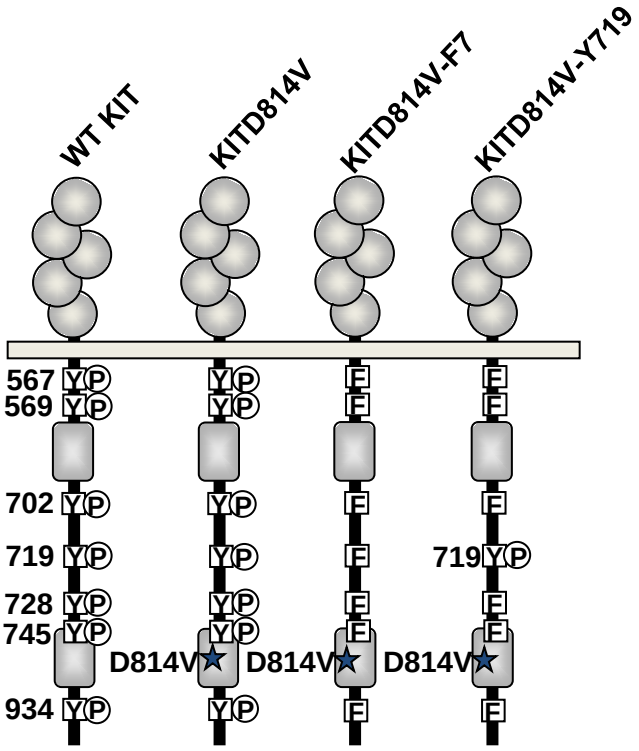


Supplemental Figure 9



Supplemental Figure 10