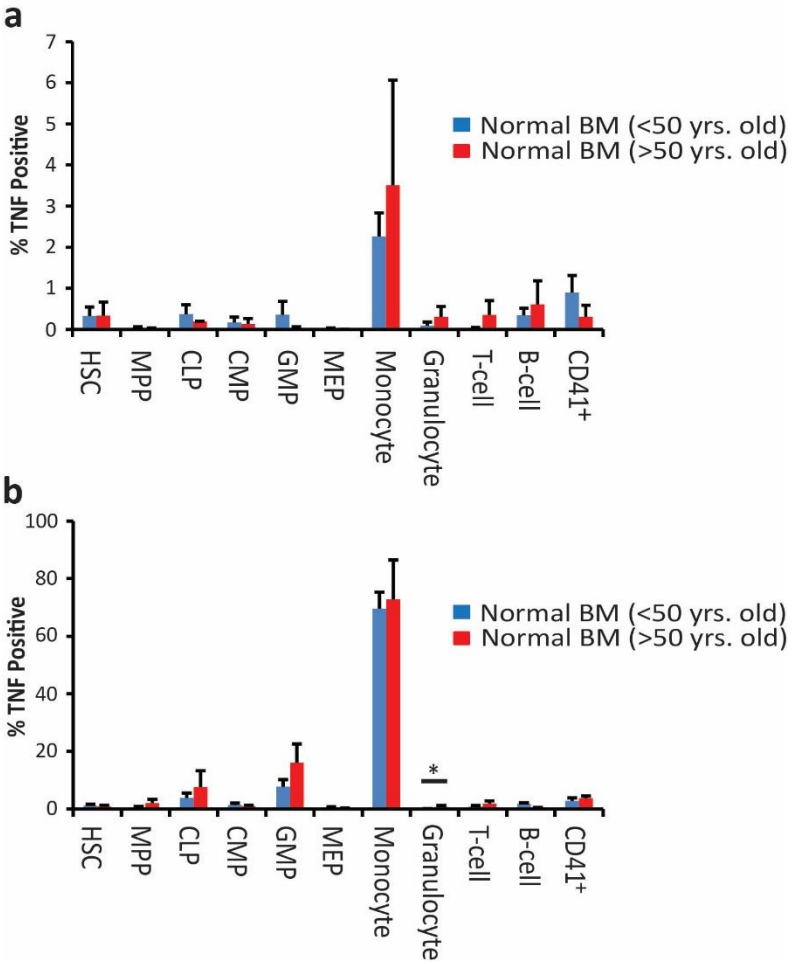
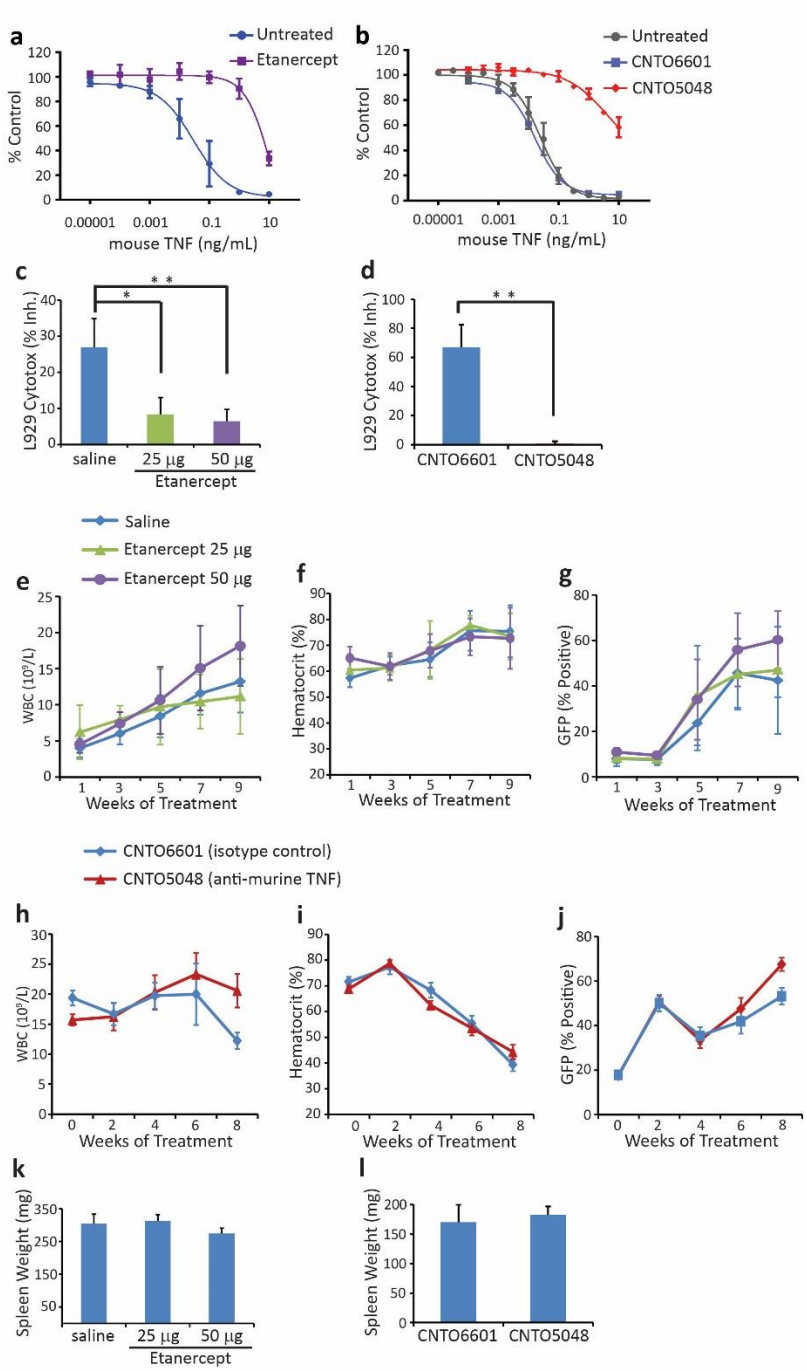
**Supplemental Figures**

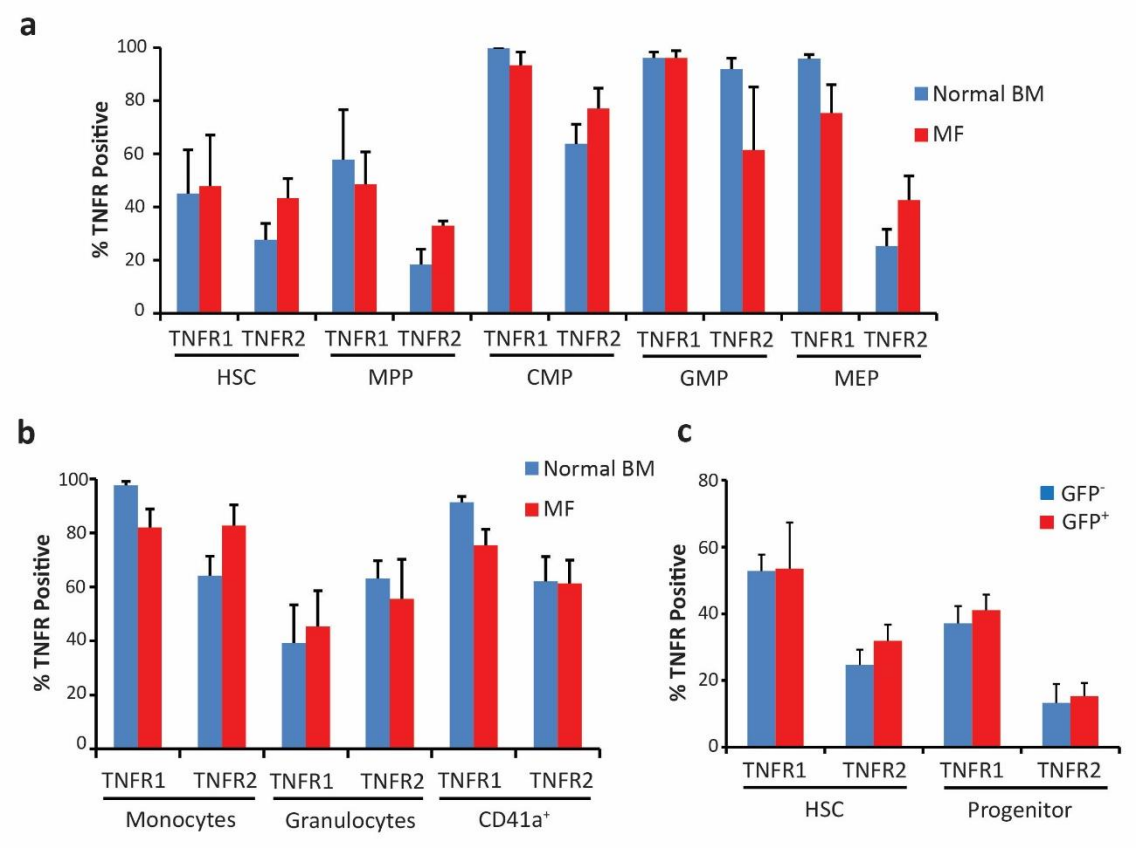



**Supplemental Figure 1. TNF expression is equivalent in normal control groups stratified by age.** (a,b) Data from normal BM controls (Fig. 1b,c) were divided into 2 groups based on the age of the donor (<50 or >50 years old). (a) There was no difference in TNF expression between the age groups for cells treated with brefeldin A only. (B) There was no difference in any of the groups treated with brefeldin A and LPS, except for the granulocyte population, although the absolute magnitude of TNF expressing cells in this group was extremely low (<50 = 0.1% vs. >50 = 0.7% $TNF^{+}$).*$p<0.05$

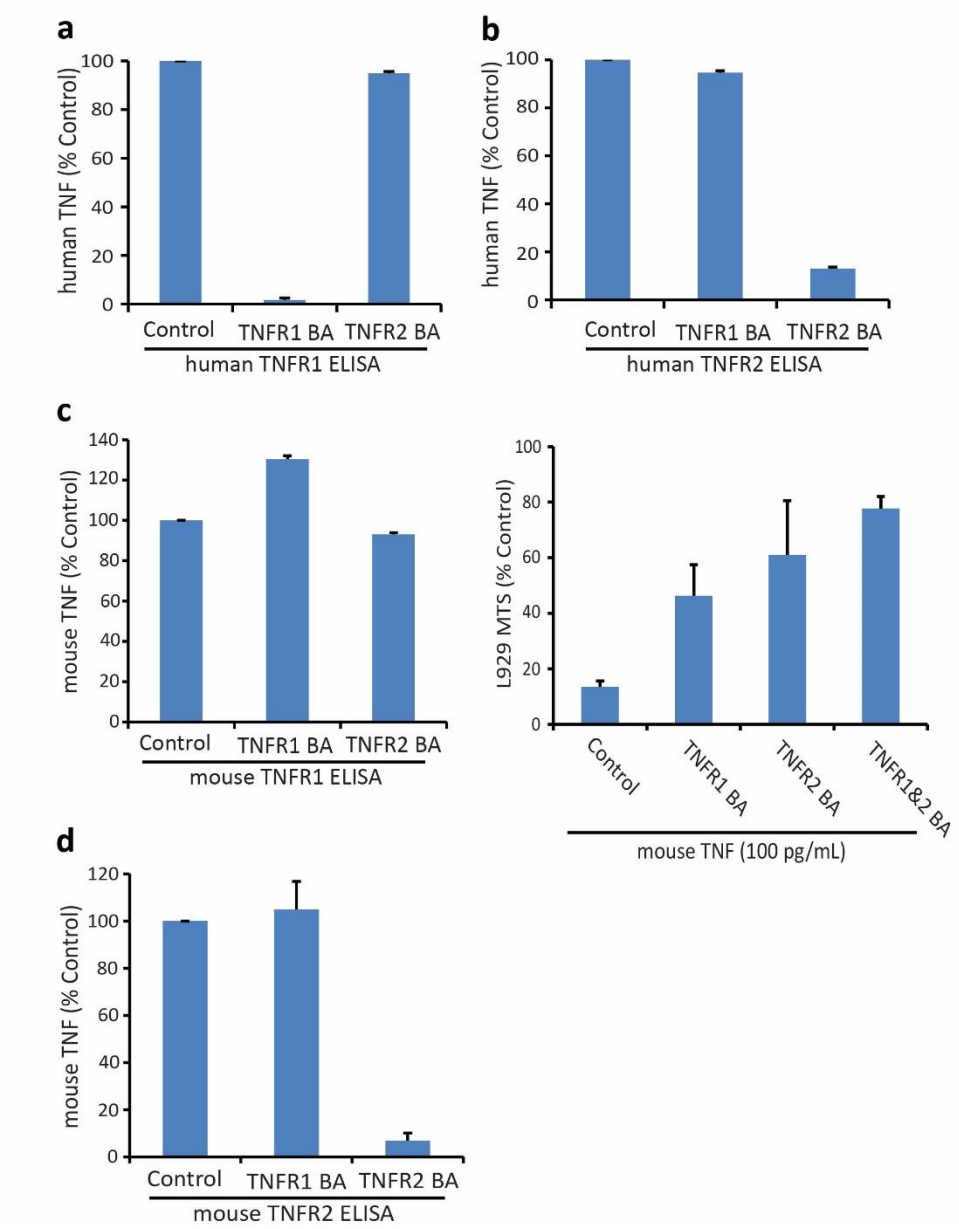



**Supplemental Figure 2. Non-selective anti-TNF therapy does not reduce disease burden in MPN mice.** (a) Etanercept (1 µg/ml) protected cells from the cytotoxicity of mouse TNF in L929 cells. (b) Mouse TNF cytotoxicity was inhibited by the murine version of infliximab (CNTO5048, 1 µg/mL) but not by the isotype control (CNTO6601, 1 µg/mL). (c,d) At the end of the study neutralization of TNF in plasma of mice treated with (c) etanercept or (d) CNTO5048 was confirmed by testing sterilized plasma samples from mice in the L929 cell TNF cytotoxicity assay. (e-g) Mice were treated every other day with saline (n=9), 25 µg etanercept (n=5) or 50 µg etanercept (n=12) by subcutaneous injection. Blood counts were analyzed every other week following sub-mandibular collection and the proportion of $GFP^+$ cells was

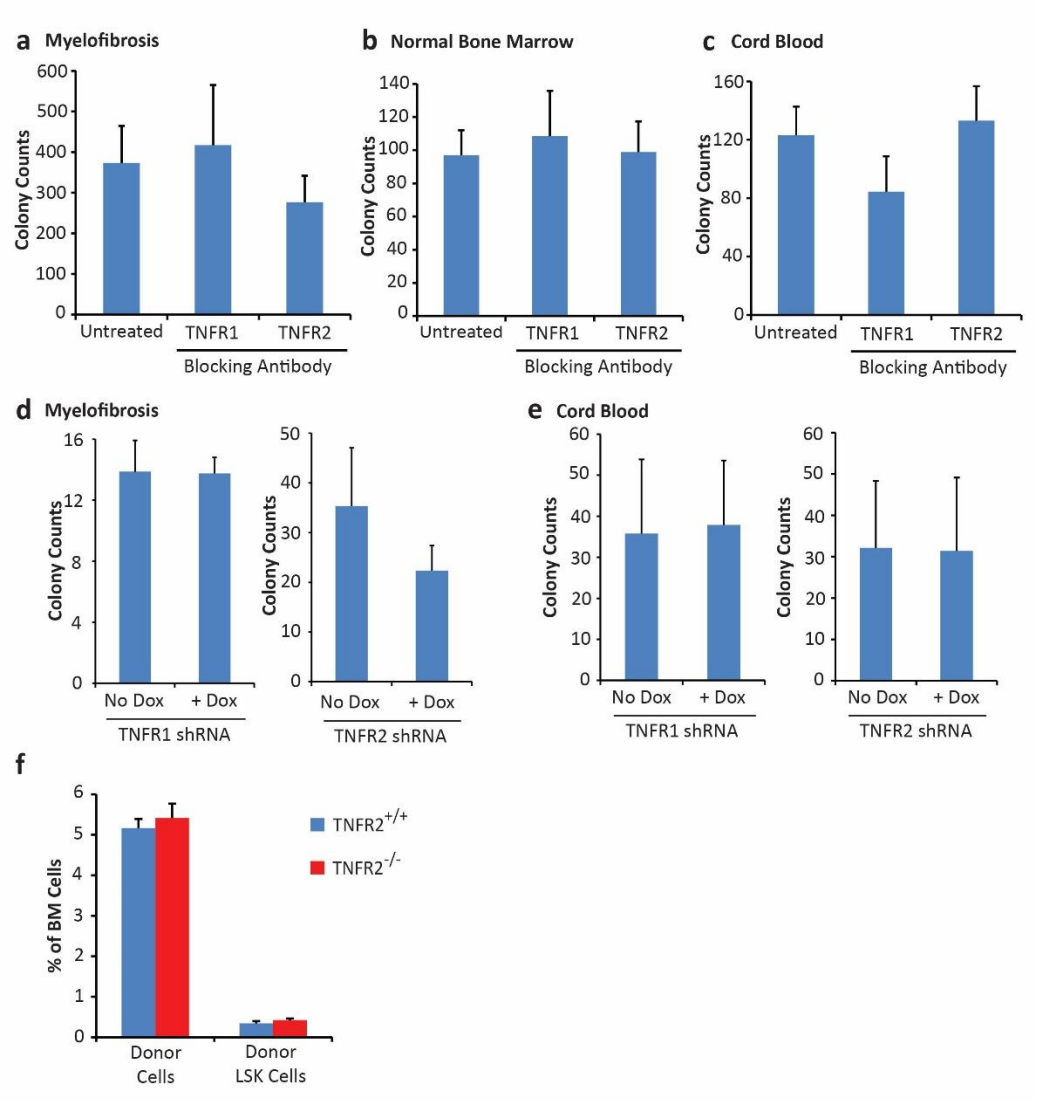
quantified as a percentage of total white blood cells (WBCs). There was no significant difference between the treatment groups with respect to (e) WBC counts, (f) hematocrit or (g) JAK2$^{V617F}$ allele burden as measured by GFP expression. (h-j) Mice were treated with a monoclonal antibody selective for mouse TNF (CNTO5048; n=11) or isotype control (CNTO6601; n=11) at 8 mg/kg with intraperitoneal dosing twice a week. There was no significant difference between the treatment groups with respect to (h) WBC counts, (i) hematocrit or (j) JAK2$^{V617F}$ allele burden. There was no significant difference in spleen weight between the treatment groups in the (k) etanercept study or (l) CNTO5048 study. *p<0.05, **p<0.005



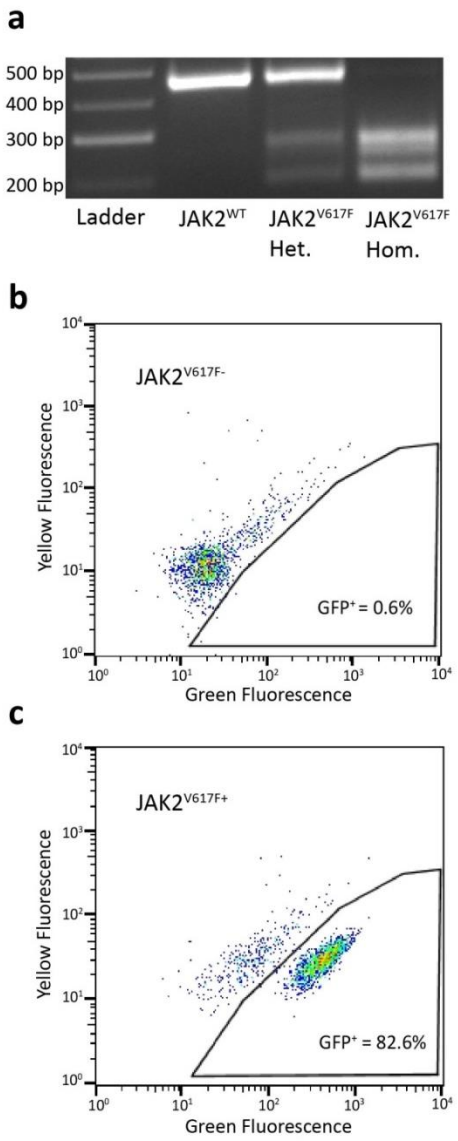

**Supplemental Figure 3. TNFR1 and TNFR2 expression is comparable between MF cells and BM controls and in mouse JAK2$^{V617F+}$ compared to JAK2$^{V617F-}$ progenitor cells.** (a,b) TNFR1 and TNFR2 receptor expression was measured in (a) primitive and (b) mature hematopoietic cell subsets from MF patient samples (n=3 for primitive and n=5 for mature populations) and normal BM (n=5). There were no significant differences in receptor expression. (c) Assessment of TNFR1 and TNFR2 expression in BM HSCs ($Lin^-Kit^+CD48^-CD150^+$) and bulk progenitor cells ($Lin^-Kit^+CD48^-CD150^-$) from MPN mice also showed no difference between JAK2$^{V617F+}$ and JAK2$^{V617F-}$ cells.



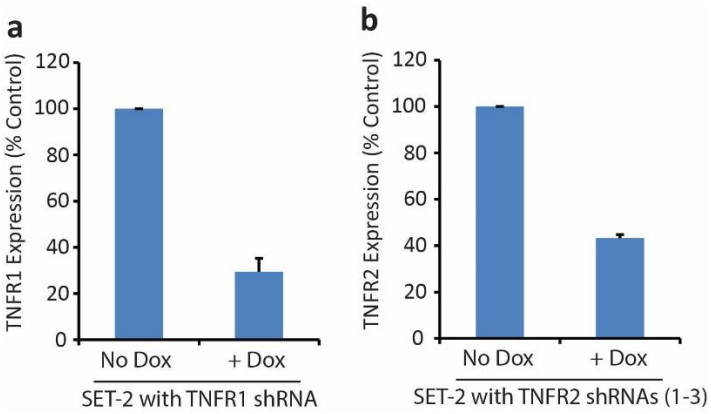

**Supplemental Figure 4. TNFR1 and TNFR2 BAs selectively block TNFR1 or TNFR2 respectively.** In ELISA assays with human TNFR1 or TNFR2, (A) the TNFR1 BA (10 µg/mL) inhibits TNF (10 ng/mL) binding to plates coated with TNFR1 protein (25ng) but not TNFR2 protein (25ng) and (B) the TNFR2 BA (10 µg/mL) inhibits TNF binding to plates coated with TNFR2 protein (25 ng) but not TNFR1 protein (25 ng). (C) The mouse TNFR1 BA (10 µg/mL) did not inhibit binding of mouse TNF (10 ng/mL) in the ELISA assay but reduced mouse TNF cytotoxicity in the L929 cell assay. (D) The mouse TNFR2 BA (10 µg/mL) inhibited binding of mouse TNF (10 ng/mL) to plates coated with TNFR2 protein (25 ng) but not TNFR1 protein (25 ng).




**Supplemental Figure 5. Colony numbers from human samples and BM homing of murine donor cells.** (a-c) Colony counts from $CD34^+$ cells from (a) myelofibrosis, (b) normal BM or (c) cord blood samples treated with or without TNF receptor blocking antibodies. (d,e) Colony counts from $CD34^+$ cells infected with TNF receptor shRNAs from (d) myelofibrosis samples or (e) human cord blood. Additional details provided in the legend for Figure 2. (f) BM cells from 5-FU-treated $TNFR2^{+/+}$ or $TNFR2^{-/-}$ mice were stained with the lipophilic dye DiO and transplanted into irradiated recipients. After 48 hours, BM cells were harvested from recipient mice and the percent of DiO positive (donor cells) and LSK cells was determined by flow cytometry.




**Supplemental Figure 6. Genotyping JAK2 in hematopoietic colonies.** (a) In DNA isolated from human colonies, digestion of a JAK2 PCR product with BsaXI identifies JAK2$^{WT}$ colonies with a single band at 460 bp, JAK2$^{V617F}$ heterozygous colonies with 3 bands at 460, 241 and 189 bp, and JAK2$^{V617F}$ homozygous colonies with 2 bands at 241 and 189 bp. (b,c) Mouse colonies are analyzed by flow cytometry where cells from (b) JAK2$^{V617F-}$ colonies lack GFP expression and cells from (c) JAK2$^{V617F+}$ colonies are predominantly GFP$^{+}$.




**Supplemental Figure 7. TNFR shRNAs reduce mRNA expression of TNFRs in the JAK2$^{V617F+}$ SET-2 cell line.** (a) SET-2 cells infected with a doxycycline inducible TNFR1 shRNA, show reduced expression of TNFR1 mRNA by qPCR analysis in the presence of doxycycline (200 ng/mL). (b) TNFR2 mRNA expression was inhibited by a mixture of 3 different doxycycline inducible shRNAs in SET-2 cells when treated with doxycycline (200 ng/mL).

**Supplemental Tables**

**Supplemental Table 1.** MF samples

| **TNF expression** | | | |
|---|---|---|---|
| **Mutation** | **Current Treatment** | **Age** | **Sex** |
| CALR | fedratinib | 68 | F |
| JAK2$^{V617F}$ | ruxolitinib | 67 | F |
| JAK2$^{V617F}$ | no current therapy | 78 | F |
| JAK2$^{V617F}$ | ruxolitinib | 73 | M |
| JAK2$^{V617F}$ | ruxolitinib | 63 | M |
| **TNF receptor expression** | | | |
| **Mutation** | **Current Treatment** | **Age** | **Sex** |
| JAK2$^{V617F}$ | busulfan | 80 | F |
| triple negative | recently stopped pacritinib and darbapoietin | 79 | F |
| MPL | pegylated interferon and aranesp | 78 | M |
| JAK2$^{V617F}$ | pegylated Interferon | 50 | M |
| JAK2$^{V617F}$ | ruxolitinib and 5-azacitidine | 77 | M |
| **Clonogenic assay with TNF receptor blocking antibodies** | | | |
| **Mutation** | **Current Treatment** | **Age** | **Sex** |
| JAK2$^{V617F}$ | ruxolitinib | 63 | M |
| JAK2$^{V617F}$ | baracitinib | 77 | F |
| JAK2$^{V617F}$ | hydroxyurea | 62 | F |
| JAK2$^{V617F}$ | ruxolitinib | 60 | M |

| **Clonogenic assay with TNF receptor shRNAs** | | | |
|---|---|---|---|
| **Mutation** | **Current Treatment** | **Age** | **Sex** |
| $JAK2^{V617F}$ | anegralide | 61 | M |
| $JAK2^{V617F}$ | ruxolitinib | 68 | F |
| $JAK2^{V617F}$ | ruxolitinib | 76 | M |
| $JAK2^{V617F}$ | ruxolitinib | 68 | F |
| **XIAP and MAPK8 RNA expression** | | | |
| **Mutation** | **Current Treatment** | **Age** | **Sex** |
| $JAK2^{V617F}$ | none | 67 | F |
| triple negative | none | 80 | F |
| $JAK2^{V617F}$ | none | 60 | M |
| CALR | ruxolitinib | 47 | F |
| JAK2 and MPL negative | recently stopped ruxolitinib | 67 | M |
| **cIAP immunofluorescence** | | | |
| **Mutation** | **Current Treatment** | **Age** | **Sex** |
| $JAK2^{V617F}$ | none | 79 | M |
| $JAK2^{V617F}$ | none | 70 | F |
| $JAK2^{V617F}$ | none | 77 | F |
| $JAK2^{V617F}$ | none | 57 | M |
| **cIAP histology** | | | |
| **Mutation** | **Current Treatment** | **Age** | **Sex** |
| $JAK2^{V617F}$ | hydroxyurea | 69 | M |
| $JAK2^{V617F}$ | pegylated interferon | 70 | F |
| $JAK2^{V617F}$ | unknown | 69 | F |
| $JAK2^{V617F}$ | unknown | 60 | F |
| CALR | none | 68 | M |
| CALR | hydroxyurea and anagrelide | 67 | M |
| CALR | hydroxyurea | 86 | M |
| CALR | unknown | 82 | F |
| **Clonogenic assay with birinapant** | | | |
| **Mutation** | **Current Treatment** | **Age** | **Sex** |
| $JAK2^{V617F}$ | none | 55 | F |
| $JAK2^{V617F}$ | none | 68 | M |
| $JAK2^{V617F}$ | momelotinib | 85 | M |
| **NF-kB reporter assay** | | | |
| **Mutation** | **Current Treatment** | **Age** | **Sex** |
| $JAK2^{V617F}$ | ruxolitinib | 69 | M |
| $JAK2^{V617F}$ | thalidimide and hydroxyurea | 82 | F |
| $JAK2^{V617F}$ | ruxolitinib and 5-azacitidine | 77 | M |
| MPL | ruxolitinib | 77 | M |
| **XIAP and MAPK8 ectopic expression** | | | |

| Mutation | Current Treatment | Age | Sex |
|---|---|---|---|
| $JAK2^{V617F}$ | ruxolitinib | 46 | M |
| $JAK2^{V617F}$ | ruxolitinib and hydroxyurea | 82 | F |
| $JAK2^{V617F}$ | ruxolitinib and lenalidomide | 76 | M |
| **cIAP immunofluorescence after XIAP ectopic expression** | | | |
| **Mutation** | **Current Treatment** | **Age** | **Sex** |
| $JAK2^{V617F}$ | ruxolitinib and hydroxyurea | 82 | F |
| $JAK2^{V617F}$ | ruxolitinib and lenalidomide | 76 | M |
| MPL | ruxolitinib | 77 | M |

**Supplemental Table 2.** Normal bone marrow samples

| **TNF expression** | | |
|---|---|---|
| **Patient information / sample type** | **Age** | **Sex** |
| Referred for neutrophilia, BM biopsy was normal | 45 | F |
| Seen for leukocytosis, BM biopsy was normal | 47 | F |
| Burkitt's lymphoma, no BM involvement | 18 | M |
| Idiopathic thrombocytopenic purpura, no BM involvement | 27 | F |
| Referred for potential PV, BM biopsy was normal | 53 | M |
| Referred for leukocytosis, BM biopsy was normal | 60 | F |
| Referred for mastocytosis, BM biopsy was normal | 24 | F |
| Diffuse large B-cell lymphoma, no BM involvement | 39 | M |
| Diffuse large B-cell lymphoma, no BM involvement | 64 | F |
| Hodgkin's lymphoma, no BM involvement | 23 | M |
| **TNF receptor expression** | | |
| **Patient information / sample type** | **Age** | **Sex** |
| Diffuse large B-cell lymphoma, no BM involvement | 26 | M |
| Diffuse large B-cell lymphoma, no BM involvement | 31 | F |
| Diffuse large B-cell lymphoma, no BM involvement | 63 | M |
| Diffuse large B-cell lymphoma, no BM involvement | 55 | M |
| Follicular lymphoma, no BM involvement | 67 | F |
| Diffuse large B-cell lymphoma, no BM involvement | 58 | F |
| **Clonogenic assay with TNF receptor blocking antibodies** | | |
| **Patient information / sample type** | **Age** | **Sex** |
| Femoral head sample | 42 | M |
| Femoral head sample | 74 | M |
| Femoral head sample | 62 | M |
| **XIAP and MAPK8 RNA expression** | | |
| **Patient information / sample type** | **Age** | **Sex** |
| Macrocytosis, BM biopsy was normal | 55 | F |
| Referred for leukocytosis, BM biopsy was normal | 22 | F |
| Diffuse large B-cell lymphoma, no BM involvement | 82 | M |

| **cIAP immunofluorescence** | | |
|---|---|---|
| **Patient information / sample type** | **Age** | **Sex** |
| Referred for leukocytosis, BM biopsy was normal | 54 | M |
| Diffuse large B-cell lymphoma, no BM involvement | 82 | M |
| Diffuse large B-cell lymphoma, no BM involvement | 77 | M |
| **Clonogenic assay with birinipant** | | |
| **Patient information / sample type** | **Age** | **Sex** |
| Femoral head sample | 55 | M |
| Femoral head sample | 68 | M |
| Femoral head sample | 85 | F |
| **NF-kB reporter assay** | | |
| **Patient information / sample type** | **Age** | **Sex** |
| Femoral head sample | 47 | M |
| Femoral head sample | 88 | F |
| Femoral head sample | 68 | M |

**Supplemental Table 3.** Human Lineage Markers

| Compartment | Markers |
|---|---|
| HSC | CD34+ CD38- CD90+ CD45RA- |
| CMP | CD34+ CD38+ CD123+ CD45RA- |
| GMP | CD34+ CD38+ CD123+ CD45RA+ |
| MEP | CD34+ CD38+ CD123- CD45RA- |
| CLP | CD34+ CD38+ CD7+ or CD10+ |
| Monocyte | CD45+CD14+CD16- |
| Granulocyte | CD45+CD14-CD16+ |
| T lymphocyte | CD45+CD3+ |
| B lymphocyte | CD45+CD19+ |

**Supplemental Table 4.** Antibodies for Lineage Discrimination in Human Samples

| Name | Vendor | Catalog # |
|---|---|---|
| CD45RA FITC | BD Biosciences | 347723 |
| CD34 PE-Cy7 | BD Biosciences | 348791 |
| CD38 APC | BD Biosciences | 340439 |
| CD45 APC-H7 | BD Biosciences | 641399 |
| CD7 FITC | BD Biosciences | 340737 |
| CD10 PE | BD Biosciences | 340921 |
| CD123 PE | BD Biosciences | 340545 |
| CD90 PerCP-Cy 5.5 | BD Biosciences | 561557 |
| CD14 PE | BD Biosciences | 562691 |
| CD16 PE-Cy7 | BD Biosciences | 557744 |
| CD3 FITC | BD Biosciences | 555332 |
| CD19 PerCP-Cy5.5 | BD Biosciences | 561295 |
| CD41a APC | BD Biosciences | 559777 |
| TNF-α V450 | BD Biosciences | 561311 |

| | | |
|---|---|---|
| TNFR1 AF700 | Novus Biologicals | NBP2-37724AF700 |
| TNFR2 PE | Invitrogen | TNFR7504 |

**Supplemental Table 5.** Mouse Lineage Markers

| Compartment | Markers |
|---|---|
| HSC-enriched | Lin- c-kit+ CD48- CD150+ |
| CMP | Lin- c-kit+ CD34+ FcγRII/RIII$^{Lo}$ |
| GMP | Lin- c-kit+ CD34+ FcγRII/RIII$^{Hi}$ |
| MEP | Lin- c-kit+ CD34- FcγRII/RIII$^{Lo}$ |

**Supplemental Table 6.** Antibodies for Lineage Discrimination in Murine Samples

| Name | Vendor | Catalog # |
|---|---|---|
| c-kit (CD117) APC-eFluor780 | eBioscience | 41-1171-82 |
| CD48 PerCP-eFluor710 | eBioscience | 46-0481-82 |
| CD150 APC | eBioscience | 17-1501-81 |
| FcγRII/RIII (CD16/32) PE-Cy7 | eBioscience | 25-0161-82 |
| CD34 PE | BD Biosciences | 551387 |
| TNFα eFluor450 | eBioscience | 48-7321-82 |
| Lineage antibody cocktail | BD Biosciences | 51-9006957 |
| Lineage Cell Depletion Kit | Miltenyi Biotec | 130-090-858 |
| B220 PE-Cy7 | eBioscience | 25-0452-81 |
| GR1 APC | eBioscience | 17-5931-82 |
| CD4 APC-eFluor780 | eBioscience | 47-0042-82 |
| CD11b PE | eBioscience | 12-0112-82 |
| TNFR1 APC | Novus Biologicals | NB110-85469APC |
| TNFR2 PE | BD Biosciences | 550086 |

**Supplemental Table 7.** TNF Receptor Blocking Antibodies

| Species | Target | Vendor | Catalog # |
|---|---|---|---|
| Human | TNFR1 | R&D Systems | MAB225 |
| Human | TNFR2 | R&D Systems | MAB726 |
| Mouse | TNFR1 | R&D Systems | MAB430 |
| Mouse | TNFR2 | R&D Systems | MAB426 |

**Supplemental Table 8.** TNF Receptor shRNA Details

| Target | Sequence |
|---|---|
| TNFR1 | CCACAGAGCCTAGACACTGAT |
| TNFR2 | CCTGGGAATGCAAGCATGGAT |
| TNFR2 | CCCTTCTCCAAGGAGGAATGT |
| TNFR2 | CACCCTGGAATCAAGATGTCA |
| | |
| Hairpin Insert | ACCGGCCACAGAGCTTAGACATTGATGTTAATATTCATAGCATCAGTGTCTAGGCTCTGTGGTTTT |
| | |
| Vector | pRSIT12-U6Tet-sh-CMV-TetRep-2A-TagRFP-2A-Puro |

**Supplemental Table 9.** CD45 Discrimination Antibodies

| Name | Vendor | Catalog # |
|---|---|---|
| CD45.1-APC, clone A20 | eBioscience | 17-0453-81 |
| CD45.2-PE, clone 104 | eBioscience | 12-0454-82 |

**Supplemental Table 10.** qPCR Primers

| Species | Target | Forward Primer | Reverse Primer |
|---|---|---|---|
| human | XIAP | GGGGTTCAGTTTCAAGGACA | CGCCTTAGCTGCTCTTCAGT |
| human | MAPK8 | TTGGAACACCATGTCCTGAA | ATGTACGGGTGTTGGAGAGC |
| human | GUS | GAAAATATGTGGTTGGAGAGCTCATT | CCGAGTGAAGATCCCCTTTTTA |
| mouse | Xiap | TTGGAACATGGACATCCTCA | TGCCCCTTCTCATCCAATAG |
| mouse | Mapk8 | AGAAACTGTTCCCCGATGTG | TGATGTATGGGTGCTGGAGA |
| mouse | Gapdh | GGCATTGCTCTCAATGACAA | TGTGAGGGAGATGCTCAGTG |

**Supplemental Methods**

**JAK2$^{V617F}$ murine MPN**

Murine MPN was induced by retroviral infection of BM cells isolated from 5-fluorouacil (5-FU; APP Pharmaceuticals, Inc., Shaumburg, IL) treated Balb/c (The Jackson Laboratory, Bar Harbor, ME) donor mice with MSCV-IRES-JAK2$^{V617F}$-GFP as described.[1] Balb/c female recipient mice (6-8 weeks old) were subject to a split dose 900 Gy whole-body irradiation with an RS 2000 X-ray irradiator (Rad Source, Suwanee, GA) and injected via tail vein with 3 x $10^5$ transduced cells/mouse BM cells. Sample sizes were chosen based on previous experiments characterizing the disease model.[1] All animal studies were performed under an approved protocol from the University of Utah Institutional Animal Care and Use Committee.

**Treatment of mice with MPN with anti-TNF therapeutics**

MPN was induced in Balb/c mice as described in the "JAK2$^{V617F}$ murine MPN" methods. In the etanercept (Enbrel®; Amgen, Thousand Oaks, CA) study, mice were treated by subcutaneous injection with normal saline (n=9, control), 25 μg etanercept (n=5) or 50 μg etanercept (n=12) every other day. In a separate experiment we used CNTO5048[2], which is a monoclonal IgG2 antibody specific for mouse TNF, and CNTO6601, the isotype-matched IgG2 negative control (both kindly provided by Janssen Research & Development, Radnor, PA). CNTO5048 and CNTO6601 were both administered at 8 mg/kg by intraperitoneal injection twice a week, with 11 mice in each dose group. Mice were randomized based on initial GFP measurements to ensure that the mean starting values were equal between groups. In both studies, blood counts were analyzed every other week with a HemaTrue® veterinary blood analyzer (Heska, Loveland, CO) following sub-mandibular collection and the proportion of GFP$^+$ was quantified as a percentage of total WBCs with a Guava easyCyte flow cytometer (Millipore, Billerica, MA). The investigators were not blinded to the dose group. Following completion of the study mice were euthanized by exsanguination and subjected to autopsy. Plasma was isolated and used to determine the pharmacodynamic activity of the anti-TNF agents by measuring TNF cytotoxicity in L929 cells.[3] L929 cells were treated with sterilized plasma samples or recombinant mouse TNF (R&D Systems) in the presence of 2 μg/mL actinomycin D (Sigma-Aldrich). Toxicity was determined after 20 hours by addition of an MTS reagent (CellTiter 96®, Promega, Madison, WI) and absorbance at 490 nm was read with an Epoch plate

reader (BioTek, Winooski, Vermont). The L929 cells were confirmed to be mycoplasma free, but had not been authenticated.

**Competitive repopulation assay**

We used B6.SJL-Ptprca Pepcb/BoyJ mice expressing Ptprc$^{a}$ (CD45.1)[4], as TNFR2$^{+/+}$ mice and the TNFR2 deficient strain B6.129S2-Tnfrsf1btm1Mwm/J (TNFR2$^{-/-}$)[5] that express the standard Ptprc$^{b}$ (CD45.2). BM cells were harvested from equal numbers of 5-FU treated CD45.1 and TNFR2$^{-/-}$ mice aged 6-8 weeks. Lineage depletion was performed using a Direct Lineage Cell Depletion Kit, (Miltenyi Biotec) with separation on an autoMACS Pro Separator (Miltenyi Biotec), prior to infection with the MSCV-IRES-JAK2$^{V617F}$-GFP retrovirus. The percentage of GFP$^{+}$ cells from each mouse strain was measured by FACS and equal numbers of Lin$^{-}$GFP$^{+}$ cells from each strain were combined. A total of 5,000 Lin$^{-}$ (~10% GFP$^{+}$) cells, from each strain, were transplanted along with 200,000 CD45.1 whole BM cells, by tail vein injection, into 8 CD45.1 female recipient mice (6-8 weeks old) which had been subjected to 11.5 Gy whole-body irradiation. To monitor disease, flow cytometry of peripheral blood cells following sub-mandibular collection was performed and the proportion of GFP$^{+}$ cells in CD45.1$^{+}$ and CD45.2$^{+}$ fractions was determined.

**Bone marrow homing**

TNFR2$^{+/+}$ or TNFR2$^{-/-}$ (see description above) donor mice were treated with 5-FU 5 days prior to harvest. BM cells from donor mice were stained with the carbocyanine dye DiO (5 µM; Molecular Probes, Eugene OR) for 20 minutes at 37 °C. TNFR2$^{+/+}$ recipient mice were subject to a split dose, 11.5 Gy whole-body irradiation. Donor cells (500,000 per mouse) were transplanted by tail vein injection into 3 recipient mice for each donor cell type. After 48 hours, BM cells were isolated from recipient mice and analyzed for DiO expression by flow cytometry. In addition, markers for Sca-1, c-Kit and mature lineages were included for the identification of Lin$^{-}$, Sca-1$^{+}$, c-Kit$^{+}$ (LSK) cells.

**JAK2$^{V617F}$ genotyping**

Colonies from human CD34$^{+}$ cells were genotyped by isolating DNA from individual colonies, amplifying JAK2 by nested PCR and digesting with BsaXI as described.[6] DNA was isolated with an Allprep DNA/RNA Microkit (Qiagen, Hilden, Germany). The first PCR step was performed with forward primer gtcaagcctgtttgactggcattattc and reverse primer caatgttatgttgaactgccataatc. The second PCR step was performed with 1.5 µL of DNA template from step 1 with forward primer gggtttcctcagaacgttga and reverse primer tcattgctttccttttttcacaa, which amplified a 460-bp reaction product. The product from step 2 was digested with 0.75 U of BsaXI (New England Biolabs, Ipswich, MA) overnight at 37 °C and the product was run on a 2% agarose gel. Since the JAK2$^{V617F}$ mutation removes the BsaXI restriction site, the homozygous JAK2$^{V617F}$ mutants were identified by a single 460-bp band, JAK2$^{WT}$ colonies displayed a 241-bp and a 189-bp band, while the JAK2$^{V617F}$ heterozygous mutants displayed all 3 bands (Supplemental Figure 3A). Genotyping for JAK2$^{V617F}$ in mouse BM derived colonies was performed by plucking single colonies, washing with PBS to remove methylcellulose and analyzing GFP expression on a Guava easyCyte flow cytometer (EMD Millipore, Darmstadt, Germany). Colonies were scored as JAK2$^{V617F+}$ when >50% of the cells from a colony were positive for GFP expression, although the majority were <1% or >80% GFP$^{+}$ (Supplemental Figure 3B, C).

**Immunofluorescence for cIAP**

Cells were washed in PBS and spun onto positively charged glass slides (VWR, Radnor, PA). Cells were fixed on the slide by incubation with 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA) for 20 minutes at room temperature. Cells were then blocked and permeabilized by addition of PBS with 5% bovine serum albumin fraction V (BSA, Thermo Fisher Scientific) and 0.1% Triton X-100 (Sigma-Aldrich) for 15 minutes at room temperature. The cIAP1/2 rabbit IgG polyclonal antibody (Thermo Fisher Scientific; Catalog # PA5-20066) was diluted in PBS/5%BSA/0.1% Triton X-100 to a final concentration of 10 μg/mL and incubated with cells at 4°C for 16 hours. Slides were washed 5 times with PBS and then Alexa Fluor 488 or 594 goat anti-rabbit secondary antibody was added at 4 μg/mL and incubated for 90 minutes at room temperature. Slides were washed 5 times with PBS and then 4', 6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich) was added to the slides at 2 μg/mL in PBS and incubated for 5 minutes at room temperature. Slides were washed 3 times with PBS and then a No. 1.5 cover slip was mounted to each slide with a drop of ProLong Gold antifade reagent (Thermo Fisher Scientific). Slides were imaged with an Axioskop 2 (Carl Zeiss Microscopy, Jena, Germany) with a 40X objective or a Leica TCS SP8 X (Leica Microsystems, Wetzlar, Germeny) with a 63X objective. Quantification was performed by manually delineating cytoplasmic regions and measuring the pixel intensity with Adobe Photoshop (Adobe Systems Inc., San Jose, CA) or with Imaris image analysis software (Bitplane, Zurich, Switzerland).

**Histology of human bone marrow biopsies**

Bone marrow specimens were fixed overnight with AZF (Acetic Zinc Formalin) and decalcified for 6 hours with Gooding and Stewart's decalcification fluid. Four-μm sections were cut with a Leica rotary microtome and air dried on plus glass slides at room temperature. Slides were melted in the oven at 60 °C for 30 minutes to melt the paraffin. Staining procedures were performed with a Ventana BenchMark ULTRA (Roche, Basel, Switzerland) automated staining system. Slides were de-paraffinized with EZ Prep solution (Roche) and pretreated with CC1 cell conditioning solution (Roche) for 36 minutes for antigen retrieval. The cIAP1/2 polyclonal antibody (Catalog # PA5-20066, ThermoFisher Scientific) was applied at 1.7 μg/mL for 32 minutes at 37 °C. Detection was performed with the ultraView Universal DAB Detection Kit (Roche). Hematoxylin counterstain was then applied for 8 minutes. Slides were removed from the autostainer and placed in a $dH_2O$/DAWN mixture (1 mL of DAWN, 500 mL of $H_2O$) to remove residual oil from the autostainer. Slides were dehydrated in graded alcohols (70%, 95% and 100%) and dipped 10 times each in 4 changes of xylene and cover slipped.

**qPCR expression analysis**

RNA was isolated from $CD34^+$ cell pellets ($5 \times 10^4 - 2 \times 10^5$ cells/pellet) with an RNeasy Micro Kit (Qiagen) including an on-column DNA digestion step according to the manufactures instructions. For each sample cDNA conversion was performed with iScript Reverse Transcription Supermix (Bio-Rad, Hercules, CA). Expression data was generated by performing qPCR with target specific primers (Supplemental Table of qPCR primers) and SsoFast EvaGreen Supermix (Bio-Rad) on a C1000 Thermal Cycler (Bio-Rad). Expression was normalized by identifying the Ct value for each condition and normalizing the result of test genes with the housekeeping gene GUS by calculating the ΔCt (GUS Ct – test gene Ct). Normalized expression is then calculated as $2^{\Delta Ct}$.

**Microarray data analysis**

CEL files from the output of Affymetrix GeneChip Command Console were used as input for determining differential gene expression among the various treatments. One of the samples (Untreated-$GFP^+$

replicate #3) was excluded because it failed QC parameters according to the Bioconductor affyQCReport package.[7] The data were normalized using the GCRMA[8] linear models and empirical Bayesian methods for assessing differential expression in microarray experiments and fold-change in expression was assessed relative to the Untreated-GFP$^-$ group.

**NF-κB Reporter Assay**

MF CD34$^+$ cells were infected with lentiviral particles containing the Cignal Lenti NF-κB Reporter (Qiagen), that has tandem repeats of an NF-κB transcriptional response element, or a negative control reporter. Cells were incubated with virus for 16 hours and then resuspended in fresh medium. At 72 hours post infection, cells were transferred to 96-well white plates (ThermoFisher Scientific). Cells were treated with TNFR blocking antibodies at 10 μg/mL for 30 minutes prior to stimulation with TNF (1 ng/mL). Addition of ONE-Glo luciferase substrate (50 μL, Promega) was performed at the indicated timepoints after stimulation and luminescence was read on a GloMax-Multi Detection System (Promega).

**References (Supplemental Methods)**


1. Bumm TG, Elsea C, Corbin AS, Loriaux M, Sherbenou D, Wood L*, et al.* Characterization of murine JAK2V617F-positive myeloproliferative disease. *Cancer research* 2006 Dec 1; **66**(23)**:** 11156-11165.

2. Lei B, Dawson HN, Roulhac-Wilson B, Wang H, Laskowitz DT, James ML. Tumor necrosis factor alpha antagonism improves neurological recovery in murine intracerebral hemorrhage. *Journal of Neuroinflammation* 2013; **10**(1)**:** 103.

3. Shiau MY, Chiou HL, Lee YL, Kuo TM, Chang YH. Establishment of a consistent L929 bioassay system for TNF-alpha quantitation to evaluate the effect of lipopolysaccharide, phytomitogens and cytodifferentiation agents on cytotoxicity of TNF-alpha secreted by adherent human mononuclear cells. *Mediators of Inflammation* 2001; **10**(4)**:** 199-208.

4. Janowska-Wieczorek A, Majka M, Kijowski J, Baj-Krzyworzeka M, Reca R, Turner AR*, et al.* Platelet-derived microparticles bind to hematopoietic stem/progenitor cells and enhance their engraftment. *Blood* 2001; **98**(10)**:** 3143-3149.

5. Erickson SL, de Sauvage FJ, Kikly K, Carver-Moore K, Pitts-Meek S, Gillett N*, et al.* Decreased sensitivity to tumour-necrosis factor but normal T-cell development in TNF receptor-2-deficient mice. *Nature* 1994 Dec 08; **372**(6506)**:** 560-563.

6. Steensma DP. JAK2 V617F in myeloid disorders: molecular diagnostic techniques and their clinical utility: a paper from the 2005 William Beaumont Hospital Symposium on Molecular Pathology. *The Journal of molecular diagnostics : JMD* 2006 Sep; **8**(4)**:** 397-411; quiz 526.

7. Huber W, Carey VJ, Gentleman R, Anders S, Carlson M, Carvalho BS*, et al.* Orchestrating high-throughput genomic analysis with Bioconductor. *Nature methods* 2015 Feb; **12**(2)**:** 115-121.

8. Wu Z, Irizarry RA, Gentleman R, Martinez-Murillo F, Spencer F. A Model-Based Background Adjustment for Oligonucleotide Expression Arrays. *Journal of the American Statistical Association* 2004; **99**(468)**:** 909-917.