Retrovirus
293-T17 cells (kindly provided by Dr. Rick Van Etten, Tufts-New England Medical Center, Boston, MA) were grown in DMEM (Gibco, Carlsbad, CA) containing 10% FBS. Cells were transfected with MIG-EPOR or MIG-JAK2V617F using Fugene 6 Transfection Reagent (Roche, Indianapolis, IN). To estimate viral titers NIH-3T3 cells (ATCC, Manassas, VA) were incubated for 48 hours with graded amounts of viral supernatant with 8 μg/ml polybrene (Hexadimethrine Bromide, Sigma-Aldrich, St. Louis, MO). Cells were analyzed for GFP expression by flow cytometry (BD FACS Aria™, San Diego, CA). Titers were estimated by plotting the proportion of GFP-positive cells versus retroviral supernatant.

Cell culture
Ba/F3 and 32D cells (ATCC) were grown in RPMI-1640 (Gibco) supplemented with 10% FBS (HyClone, Logan, UT), L-glutamine, penicillin/streptomycin, and 10% WEHI-3B (ATCC) conditioned media (WEHI-CM). Ba/F3 or 32D-EPOR cells were created with viral supernatant containing MIG-EPOR in the presence of polybrene. Cells were selected in medium containing 3 U/ml of human erythropoietin (EPO) (Procrit, Amgen Inc, Thousand Oaks, CA) instead of WEHI-CM as the source of growth factor for stable EPOR expression. For generating stable JAK2V617F/Ba/F3–EPOR or JAK2V617F/32D-EPOR, cells were infected with viral supernatant containing MIG-JAK2V617F. For stable JAK2V617F, cells were selected in medium containing no growth factors. To assess CYT387 sensitivity, Ba/F3-EPOR and Ba/F3-EPOR-JAK2V617F cells were cultured for 3 days with 3 U/ml EPO (for Ba/F3-EPOR) or without EPO (for Ba/F3-EPOR-JAK2V617F) in the presence of a CYT387 gradient, at which point the cells were subjected to an MTS assay (Cell Titer 96® Solution; Promega, Madison, WI). For trypan blue exclusion, Ba/F3-EPOR-JAK2V617F cells were cultured in the presence of graded concentrations of CYT387 for 48 hours, stained with trypan blue, and counted manually on a hemacytometer.

Immunoblotting
For immunoblot analysis, cells were cultured in RPMI-1640 containing 0.5% bovine serum albumin (BSA) (Sigma) with CYT387 for 12 hours (24 hours for cleaved caspase 3 blot). Following incubation, cells were lysed in cell lysis buffer (Cell Signaling Technologies, Danvers, MA) supplemented with protease inhibitor cocktail (Roche), phosphatase inhibitor cocktail 2, Aprotinin, Na3VO4, Phenyl-methane-sulfonyl-fluoride, and Amino-ethyl-benzene-sulfonyl-fluoride (Sigma). Equal amounts of whole cell extracts were subjected to SDS-PAGE and transferred to a polyvinylidene-fluoride membrane (PVDF) (Millipore, Billerica, MA). Membranes were probed with antibodies specific for phospho-JAK2 (Tyr1007/1008), phospho-STAT5, STAT5, phospho-ERK1/2 (197G2), ERK1/2 (Cell Signaling Technologies), JAK2 (HR-758) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), cleaved caspase 3 (Cell Signaling Technologies), tubulin (Santa Cruz Biotechnology, Inc.), or β-actin (JLA20) (Calbiochem, La Jolla, CA).

Bone marrow transplantation
Standard techniques were used. Balb/c donor mice (6 to 9 weeks old) (Charles River Laboratories, Wilmington, MA) were retro-orbitally injected with 5-Fluorouracil (100 mg/kg) (Fluorouracil injection, GeneraMedix Inc., Liberty Corner, NJ). Five days later, bone marrow was harvested from the femur and tibia. Cells were plated at 5 x 105 cells/ml in DMEM

supplemented with penicillin/streptomycin, L-glutamine (Gibco), 15% FBS, 15% WEHI-CM, 7 ng/ml IL-3, 12 ng/ml IL-6, 56 ng/ml stem cell factor (SCF) (Peprotech, Rocky Hill, NJ) and 3 µg/ml polybrene and cultured for 24 hours. Subsequently, cells were infected twice over a 48 hour period with medium containing viral supernatant (MIG-JAK2V617F). Twenty-four hours after the second round of infection, 1 x 106 viable cells were injected retro-orbitally into lethally irradiated (2 x 450 cGy) syngeneic recipients (n=42). Blood counts were determined weekly starting at day 15 using a Vet abc™ animal blood counter (HESKA®, Fort Collins, CO). On day 32 after bone marrow transplantation (start of CYT387 administration) 6 mice were euthanized for analysis.

Flow cytometry

White cells from bone marrow and spleen were stained with conjugated monoclonal antibodies as described[6]. PE-Cy7 labeled antibodies for staining of lineage positive cells were CD3e (145-2011), CD11b (M1/70), CD45R (RA3-6B2), Ly-6G (RB6-8C5), CD4 (RM4-5), CD8 (53-6.7) (BD Pharmingen™),Ter-119 (TER-119) and CD-90.1 (H1551) (eBioscience, San Diego, CA). Antibodies for progenitor cell staining were APC-Cy7-conjugated CD117 (2B8), Pe-Cy5-conjugated CD34 (RAM34) (eBioscience) and PE-conjugated CD-71 (C2) (BD Pharmingen™). Antibodies for lineage positive cells were APC-Cy7-conjugated CD19 (ID3), PerCp-Cy5.5-conjugated B220 (RA3-6B2), PE-Cy7-conjugated CD3 (145-2C11) and APC-conjugated Gr1 (RB6-8C5) and PE-conjugated CD-41 (MWReg30) (BD Pharmingen™). Cells were analyzed by multicolor flow-cytometry.

Mutagenesis screen

The selection for CYT387 resistant clones was performed as described[13]. Briefly, Ba/F3-EPOR-JAK2V617F cells were treated overnight with N-ethyl-N-nitrosourea (ENU; 50 µg/mL) (Sigma), pelleted, resuspended, and distributed into 96-well plates (2x105 cells/well) in media containing CYT387. Wells were visually inspected for cell growth every 5 days throughout the 38-day experiment. The contents of wells exhibiting outgrowth were expanded in the presence of CYT387, protein was extracted as above and DNA was extracted (Qiagen, Valencia, CA). JAK2 cDNA was amplified using M13-tagged PCR primers: forward 5′-gtaaaacgacggccagt-3′, reverse 5′-caggaaacagctatgacc-3′; forward1 5′-cccttgaacctcctcgttcgacc-3′, reverse1 5′-gctgaatgaatctgcgaaatc-3′; forward2 5′- tcacattttaacccggaagc-3′, reverse2 5′-tccttagggctgcatcgtag-3′; forward3 5′- gcagccctaaggacttcaac-3′, reverse3 5′-tttgatgaaaggtgggttcc-3′; forward4 5′-aagacaggagaacggggaac-3′, reverse4 5′-tgtcatgctgcaaggatttc-3′; forward5 5′-ctgaagagcacctccgagac-3′, reverse5 5′-ctgctggtctctgagtgaagg-3′. PCR products were bi-directionally sequenced (Agencourt Bioscience Corporation, Beverley, MA) and chromatograms were analyzed using Mutation Surveyor software (SoftGenetics, State College, PA).

Percentage Inhibition	Kinases
Less than 50% at 1μM CYT387 **(IC50>1μM)**	71 kinases ADRBK1 (GRK2), ADRBK2 (GRK3), ALK, BLK, CAMK2A (CaMKII alpha), CAMK4 (CaMKIV), CDC42 BPA (MRCKA), CHEK1 (CHK1), CLK3, CSK, CSNK1G1 (CK1 gamma 1), CSNK1G2 (CK1 gamma 2), CSNK2A1 (CK2 alpha 1), CSNK2A1 (CK2 alpha 1), DYRK3, EPHA2, EPHA3, EPHB3, ERBB2 (HER2), FGFR1, FRK (PTK5), GRK7, GSK3A (GSK3 alpha), GSK3B (GSK3 beta), IGF1R, INSR, IRAK4, ITK, JAK3, LCK, LTK (TYK1), LYN A, MAP2K1 (MEK1), MAP4K4 (HGK), MAPK11 (p38 beta), MAPK12 (p38 gamma), MAPK14 (p38 alpha), MAPK3 (ERK1), MAPKAPK2, MAPKAPK5 (PRAK), MET (cMet), NEK2, NTRK1 (TRKA), PAK2 (PAK65), PAK6, PASK, PDGFRB (PDGFR beta), PDK1, PHKG2, PIM1, PLK1, PRKACA (PKA), PRKCA (PKC alpha), PRKG1, PTK6 (Brk), RET, ROCK1, RPS6KA3 (RSK2), RPS6KA5 (MSK1), SRC, SRMS (Srm), SRPK1, SRPK2, STK22B (TSSK2), STK23 (MSSK1), STK25 (YSK1), STK3 (MST2), STK6 (Aurora A), TAOK2 (TAO1), YES1, ZAP70.
More than 50% at 1μM CYT387and Less than 50% at 0.1μM **(0.1<IC50<1μM)**	51 kinases ABL1, AMPK A1/B1/G1, AURKB (Aurora B), AURKB (Aurora B), AURKC (Aurora C), BRAF, BRSK1 (SAD1), CDK1/cyclin B, CDK1/cyclin B, CDK5/p35, CLK1, CLK2, CSF1R (FMS), DAPK3 (ZIPK), EPHB1, FES (FPS), FGFR3, FGR, FLT1 (VEGFR1), FLT1 (VEGFR1), FLT3, HIPK4, IKBKB (IKK beta), KDR (VEGFR2), MAP3K9 (MLK1), MAP4K2 (GCK), MAPK1 (ERK2), MAPK10 (JNK3), MAPK9 (JNK2), MARK1 (MARK), MINK1, MST1R (RON), MST4, MYLK2 (skMLCK), NEK9, NTRK1 (TRKA), NTRK2 (TRKB), PAK4, PDGFRA (PDGFR alpha), PDGFRA (PDGFR alpha), PLK3, PRKCG (PKC gamma), PRKD2 (PKD2), PRKX, RAF1 (cRAF) Y340D Y341D, ROS1, RPS6KB1 (p70S6K), SGK (SGK1), SGKL (SGK3), STK22D (TSSK1), SYK,
Less than 50% at 0.1μM CYT387 **(IC50<0.1μM)**	6 kinases CDK2/cyclin A, MAPK8 (JNK1), PRKCN (PKD3), PRKD1 (PKCμ), ROCK2, TBK1

Table S1. CYT387 was tested at two concentrations (0.1 and 1μM) using the SelectScreen™ Profiling Service (Invitrogen)

	average (STDV) (range)	**average** (STDV) (range)
Hours	**25mg/Kg**	**50mg/Kg**
1	**7.1** (2.9) (4.5-12.2)	**32.1** (3.5) (28.5-36.8)
3	**4.4** (2.1) (1.1-6.3)	**9.3** (1.7) (7.3-11.9)
6	**0.5** (0.6) (0.2-1.6)	**1.5** (1.1) (0.1-2.9)
9	**0.01** (0.01) (0-0.02)	**0.4** (0.4) (0.05-1.1)
12	**0.01** (0.02) (0-0.04)	**0.9** (0.9) (0.06-2.3)

Table S2. Plasma CYT387 concentration (μM) at indicated time points after single oral dose of 25 mg/Kg or 50 mg/Kg in mice
STDV (standard deviation).

	mouse	Reticulin Fibrosis Grading (0-3)	Average	Std. Error	t-test vs. 0 mg/kg
0 mg/kg	401	1.0	1.5	0.2	
	403	2.0			
	412	1.0			
	413	2.0			
	414	1.0			
	437	2.0			
25 mg/kg	408	1.0	1.0	0.3	0.30
	409	1.0			
	433	2.0			
	436	0.0			
	439	1.0			
	441	1.0			
50 mg/kg	420	1.0	0.5	0.2	0.04
	421	0.0			
	425	0.0			
	429	0.0			
	448	1.0			
	450	1.0			

Table S3. Humerus sections from six mice in each treatment group were stained for reticulin fibers and scored in a blinded fashion on a 0 to 3 scale with 0 indicating normal tissue and 3 indicating extreme fibrosis

The mean and standard error of the six values from each group were calculated and a paired t-test was performed between 25 mg/kg vs. 0 mg/kg and 50 mg/kg vs. 0 mg/kg. the p-value from each t-test is reported.

		average (range) (STDV)	average (range) (STDV)	average (range) (STDV)	double sided T-test	double sided T-test
Tissue	**Cell Type**	**0 mg/Kg group (n=6)**	**25 mg/Kg group (n=6)**	**50 mg/Kg group (n=6)**	**50 mg/Kg versus 0 mg/Kg**	**25 mg/Kg versus 0 mg/Kg**
Bone marrow	CD19+/B220+ cells	4% (1.4% - 9.2%) (2.9%)	4.7% (1.2% - 9.1%) (2.9%)	7.5% (2.2% - 11.2%) (3.6%)	0.09	0.7
Bone marrow	CD3+ cells	0.61% (0.5% - 0.8%) (0.14%)	0.68% (0.4% - 1%) (0.23%)	0.95% (0.6% - 1.3%) (0.24%)	**0.02**	0.6
Bone marrow	Gr1 cells	79.7% (73% - 83%) (4%)	73.9% (64% - 80%) (5.8%)	71.6% (65% - 79%) (5.5%)	**0.01**	0.1
Bone marrow	CD41+ cells	0.6% (0.4% - 1.3%) (0.3%)	0.8% (0.5% - 1.2%) (0.2%)	1% (0.6% - 1.5%) (0.3%)	**0.05**	0.4
Bone marrow	CD71+ cells	0.3% (0.2% - 0.4%) (0.07%)	0.3% (0.1% - 0.6%) (0.1%)	1.6% (0.6% - 1.8%) (0.6%)	**0.01**	0.8
Bone marrow	Ter119+ cells	1.6% (1% - 2.5%) (0.5%)	1.9% (0.6% - 3.6%) (1%)	5.6% (3.9% - 7.6%) (1.3%)	**0.003**	0.6
Bone marrow	cKit+ CD34-	0.2% (0.1% - 0.2%) (0.04%)	0.3% (0.2% - 0.4%) (0.07%)	0.4% (0.3% - 0.5%) (0.08%)	**0.003**	**0.05**
Bone marrow	cKit+ CD34+	0.17% (0.09% - 0.2%) (0.05%)	0.18% (0.08% - 0.2% (0.06%)	0.2% (0.2% - 0.3%) (0.07%)	**0.006**	0.6
Spleen	CD19+/B220+ cells	15.6% (5.5% - 26.5%) (7.7%)	14.8% (9.4% - 21.7%) (5.6%)	19.5% (2.1% - 33.1%) (12%)	0.5	0.8
Spleen	CD3+ cells	12.4% (7% - 20.4%) (6.7%)	14.8% (5% - 48%) (17.1%)	22.1% (2% - 41%) (14.8%)	0.1	0.7
Spleen	Gr1 cells	59% (45% - 74%) (10%)	61% (16% - 78%) (24%)	37% (13% - 77%) (27%)	0.1	0.8
Spleen	CD41+ cells	1.2% (0.9% - 1.8%) (0.4%)	2.1% (1.2% - 2.8%) (0.5%)	5% (2.1% - 9%) (2.7%)	**0.01**	**0.009**
Spleen	CD71+ cells	1.2% (0.6% - 2.9%) (0.8%)	0.3% (0.2% - 0.5%) (0.1%)	0.2% (0.07% - 0.39%) (0.1%)	**0.02**	**0.04**
Spleen	Ter119+ cells	7.3% (3.3% - 21.3%) (6.9%)	4.3% (2.1% - 7.2%) (2.3%)	5% (2% - 10%) (3.2)	0.4	0.3
Spleen	cKit+ CD34-	0.5% (0.4% - 0.6%) (0.06%)	0.5% (0.4% - 0.6%) (0.06%)	0.1% (0% - 0.58%) (0.2%)	**0.008**	0.3
Spleen	cKit+ CD34+	0.15% (0.04% - 0.21%) (0.06%)	0.09% (0.06% - 0.16%) (0.04%)	0.04% (0% - 0.15%) (0.06%)	**0.01**	0.1

Table S4. Overview of Flow Cytometry data of mice

Statistically significant p values ($p < 0.05$) are bold. ND (not done). Percentage of individual cell populations are percent of total spleen or bone marrow cells.

	average in pg/ml (STDV) (range)				double sided T-test		
Cytokine	normal mice	0 mg/KG	25 mg/Kg	50 mg/Kg	p (0 mg/Kg vs. normals)	p (50mg/Kg vs. 0mg/Kg)	p (50 mg/Kg vs. normals)
G-CSF	271 (72) (137-393)	220 (226) (12-728)	322 (247) (66-690)	404 (147) (170-749)	0.6	0.05	0.06
GM-CSF	11 (4.5) (9-20)	5 (4) (3-12)	5 (3) (3-12)	17 (13) (3-49)	0.07	0.003	0.2
IFN-γ	2.9 (0.6) (1-3)	21 (29) (3-96)	3 (0.1) (3-4)	3 (0.7) (3-5)	0.1	0.05	0.2
IL-10	10 (4) (3-19)	14 (17) (3-52)	5 (4) (3-15)	4 (5) (3-20)	0.6	0.1	0.08
Il-12 p40	74 (52) (3-150)	22 (40) (3-118)	23 (22) (3-68)	58 (80) (6-300)	0.1	0.1	0.6
IL-12 p70	18 (13) (3-44)	24 (27) (3-79)	24 (21) (3-71)	5 (6) (3-23)	0.3	0.09	0.3
IL-13	674 (279) (200-1124)	515 (507) (27-1475)	582 (507) (35-1290)	795 (725) (27-2760)	0.6	0.2	0.6
IL-15	40 (60) (7-197)	330 (586) (12-1124)	61 (40) (17-121)	63 (103) (7-311)	0.2	0.2	0.6
IL-17	3 (0.1) (3-4)	6 (2) (3-11)	3 (1.6 (3-7)	3 (0.1) (3-4)	0.02	0.001	0.6
IL-1α	619 (263) (360-993)	216 (184) (47-570)	388 (242) (110-691)	634 (331) (37-1242)	0.04	0.001	0.6
IL-1β	11 (5) (6-19)	24 (9) (14-45)	36 (29) (6-83)	9 (8) (3-28)	0.02	0.1	0.5
IL-2	6 (6) (3-21)	13 (25) (3-75)	3 (1) (3-6)	5 (3) (3-11)	0.5	0.3	0.5
IL-3	3 (0.1) (3-4)	7 (5) (3-20)	3 (1) (3-7)	3 (0.1) (3-4)	0.09	0.03	0.5
IL-4	3 (0.1) (3-4)	14 (15) (3-51)	5 (2) (3-10)	6 (4) (3-15)	0.1	0.1	0.1
IL-5	10 (2) (7-12)	17 (27) (3-90)	4 (2) (3-7)	8 (2) (4-15)	0.5	0.3	0.5
IL-6	6 (2) (3-8)	101 (110) (16-369)	45 (35) (12-105)	54 (42) (7-125)	0.08	0.3	0.01
IL-7	7 (8) (3-28)	114 (246) (3-749)	33 (22) (3-58)	16 (43) (3-167)	0.3	0.2	0.5
IL-9	24 (13) (7-43)	74 (56) (9-149)	27 (10) (7-48)	37 (25) (3-106)	0.08	0.1	0.4
IP-10	117 (19) (93-151)	438 (294) (203-1007)	196 (31) (143-233)	212 (101) (129-549)	0.03	0.04	0.04
KC	9 (7) (3-21)	95 (180) (5-567)	19 (20) (3-59)	58 (28) (3-107)	0.1	0.5	0.01
LIF	(3 (0.9) (3-5)	38 (43) (3-106)	5 (6) (3-23)	4 (2) (3-8)	0.1	0.04	0.5
M-CSF	11 (6) (5-24)	49 (87) (3-131)	11 (4) (7-14)	10 (6) (3-26)	0.3	0.2	0.6
MCP-1	20 (9) (10-37)	70 (44) (3-125)	33 (13) (20-61)	38 (6) (26-49)	0.03	0.06	0.03
MIG	285 (134) (170-553)	1602 (988) (875-1967)	845 (343) (433-1303)	1268 (1889) (313-7032)	0.01	0.5	0.1
MIP-1α	41 (12) (21-64)	187 (271) (21-870)	46 (29) (25-100)	42 (25) (13-112)	0.2	0.1	0.5
MIP-1β	83 (19) (48-112)	259 (306) (70-982)	151 (101) (31-333)	105 (77) (15-305)	0.2	0.1	0.4
Rantes	15 (8) (3-26)	46 (44) (8-131)	36 (25) (9-80)	30 (31) (6-124)	0.1	0.3	0.2
TNF-α	5 (1) (3-7)	38 (36) (12-105)	12 (6) (7-26)	9 (5) (5-28)	0.08	0.05	0.09
VEGF	5 (2) (3-10)	22 (22) (3-64)	5 (4) (3-12)	5 (4) (3-16)	0.1	0.05	0.5

Table S5. Cytokine and chemokine concentrations in the plasma of mice treated vehicle, 25 mg/kg, or 50 mg/kg CYT387 as well as normal controls

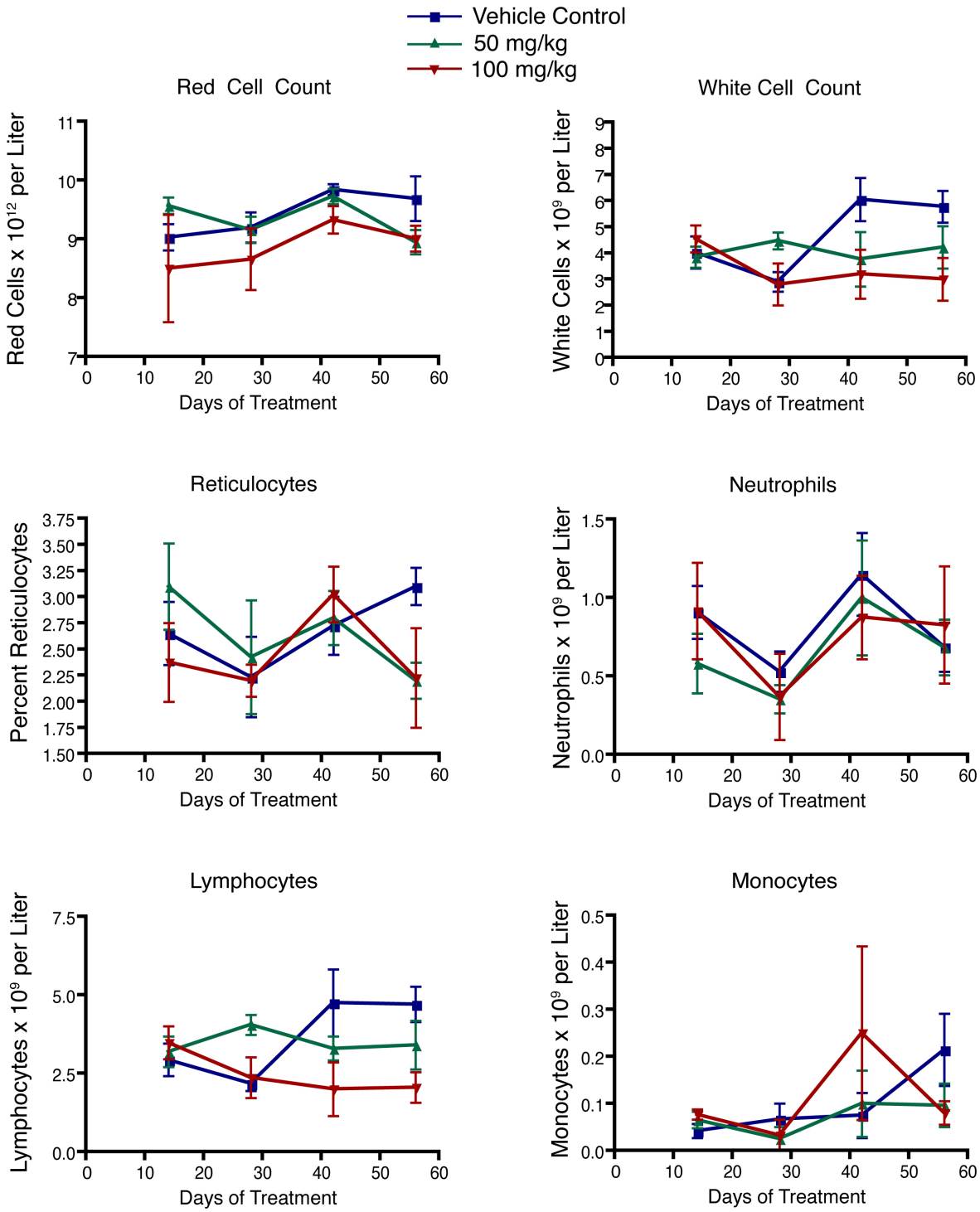

Figure S1. Balb/c mice were treated with vehicle control, 50 mg/kg, or 100 mg/kg CYT387 twice daily by oral gavage for 56 days

At days 14, 28, 42, and 56, peripheral blood was drawn and assessed for levels of red cells, white cells, reticulocytes, neutrophils, lymphocytes, and monocytes. Values represent mean ± s.e.m. n = 4 mice per group.

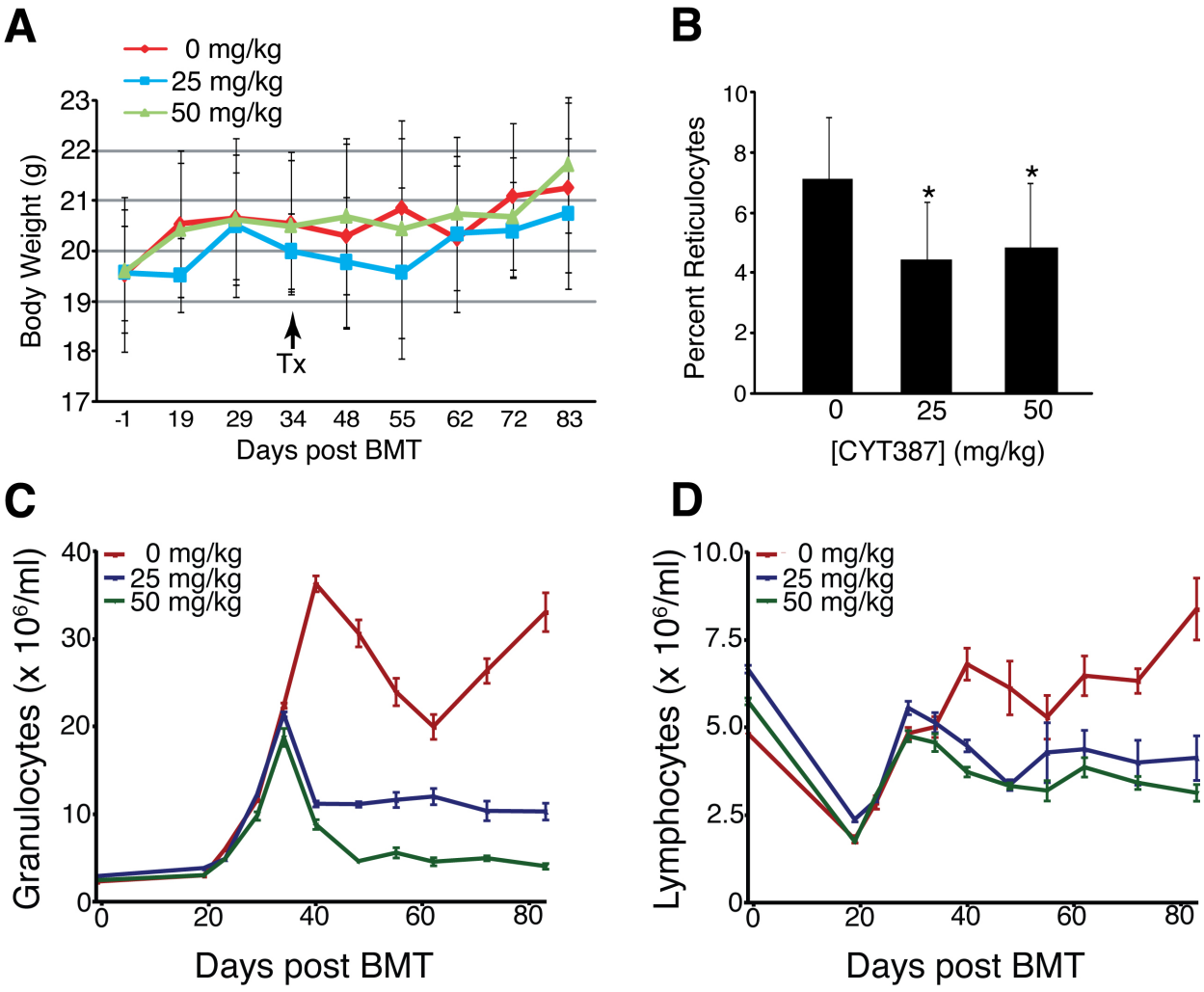

Figure S2. Balb/c mice were subjected to bone marrow transplant with bone marrow donor cells retrovirally-transduced to express JAK2^{V617F}

Thirty-four days after transplant, mice exhibited symptoms of myeloproliferative neoplasm as measured by elevated white blood cell counts and hematocrit. Mice were divided into three groups and initiated on twice daily oral gavage administration of vehicle control, 25 mg/kg CYT387, or 50 mg/kg CYT387 (n = 12 per group). (A) Body weight of mice was measured each week over the 83 day course of the experiment. (B) At day 83 post bone marrow transplant, all mice were sacrificed and reticulocyte counts were measured. (C) Peripheral blood was analyzed each week over the 83 day course of the experiment and concentrations of granulocytes for each treatment group are reported. (D) Peripheral blood was analyzed each week over the 83 day course of the experiment and concentrations of lymphocytes for each treatment group are reported. Values represent mean ± s.e.m. * indicates $p < 0.05$.

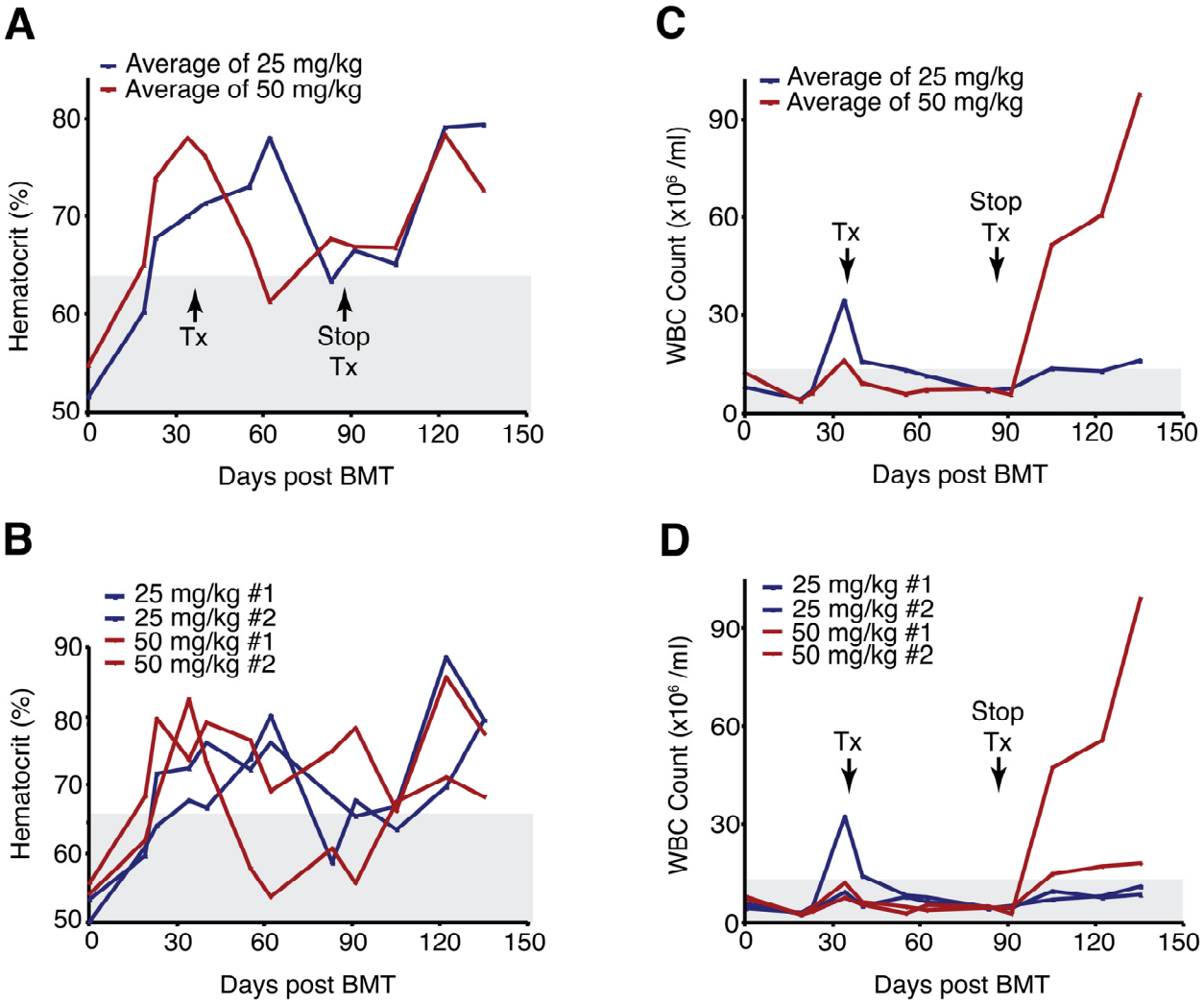

Figure S3. Balb/c mice were subjected to bone marrow transplant with bone marrow donor cells retrovirally-transduced to express JAK2^{V617F}

Thirty-four days after transplant, mice exhibited symptoms of myeloproliferative neoplasm as measured by elevated white blood cell counts and hematocrit. Mice were divided into three groups and initiated on twice daily oral gavage administration of vehicle control, 25 mg/kg CYT387, or 50 mg/kg CYT387 (n = 12 per group). At day 83 post-bone marrow transplant, CYT387 therapy was discontinued in two mice from the 25 mg/kg and two mice from the 50 mg/kg treatment groups. These mice were monitored for an additional 52 days. (A) Average hematocrit levels in 25 mg/kg and 50 mg/kg groups. (B) Hematocrit levels in each mouse in the 25 mg/kg and 50 mg/kg groups. (C) Average white blood cell levels in 25 mg/kg and 50 mg/kg groups. (D) White blood cell levels in each mouse in the 25 mg/kg and 50 mg/kg groups.

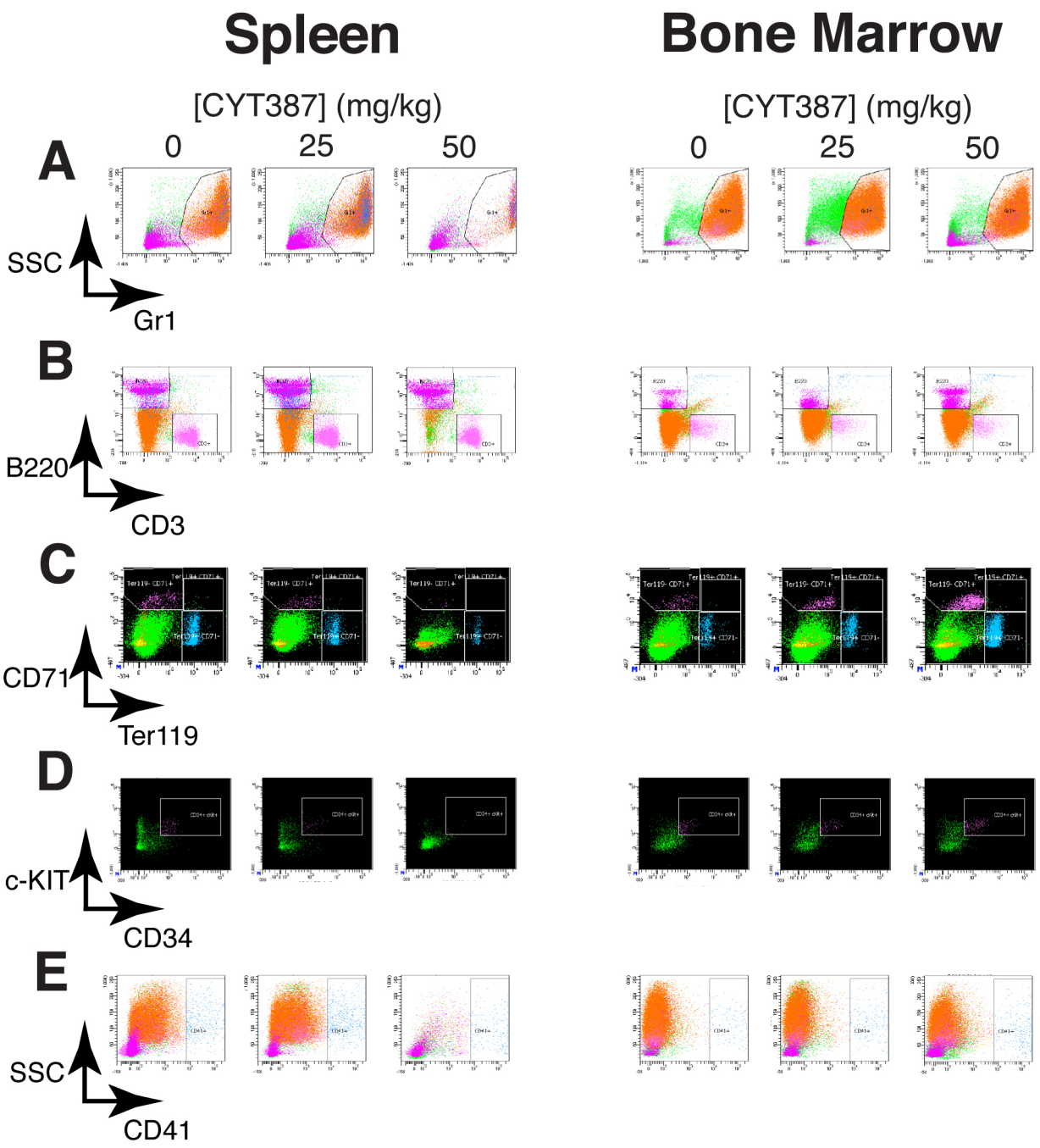

Figure S4. Effect of CYT387 on hematopoietic cell lineages in myeloproliferative neoplasm *in vivo*

Representative dot plots of FACS analysis presented in Fig. 4 for (A) SSC/Gr1 (Granulocytes), (B) B220 (B-cells)/CD3 cells (T-cells), (C) CD71 (early erythroid progenitors)/Ter119 (late erythroid progenitors), (D) cKit+CD34- (stem cells/ early progenitors)/cKit+CD34+ (late progenitors), (E) SSC/CD41 (megakaryocytes).