SUPPLEMENTARY APPENDIX

A second-generation 15-PGDH inhibitor promotes bone marrow transplant recovery independently of age, transplant dose and granulocyte colony-stimulating factor support

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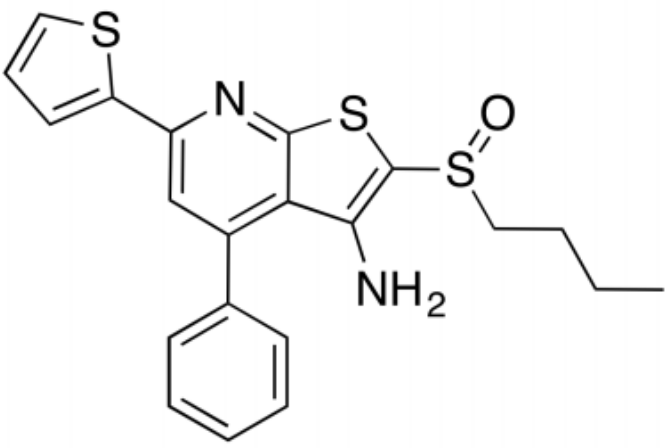
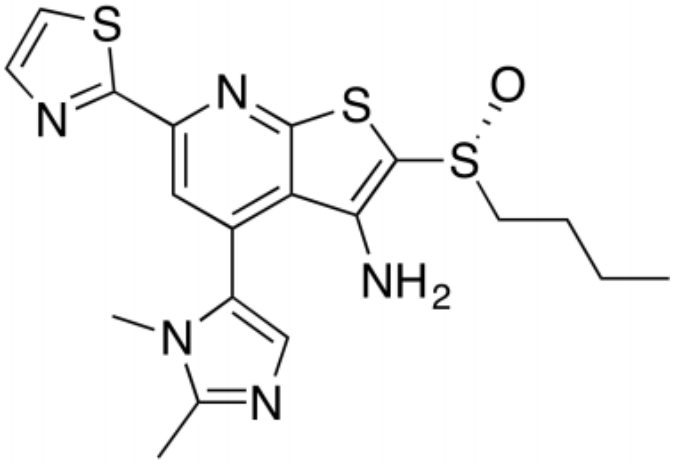
Figure S1



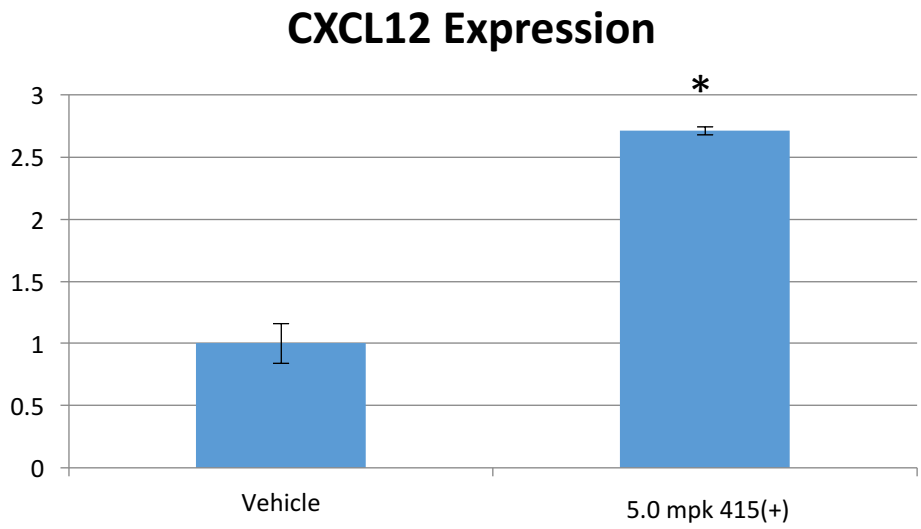
SW033291

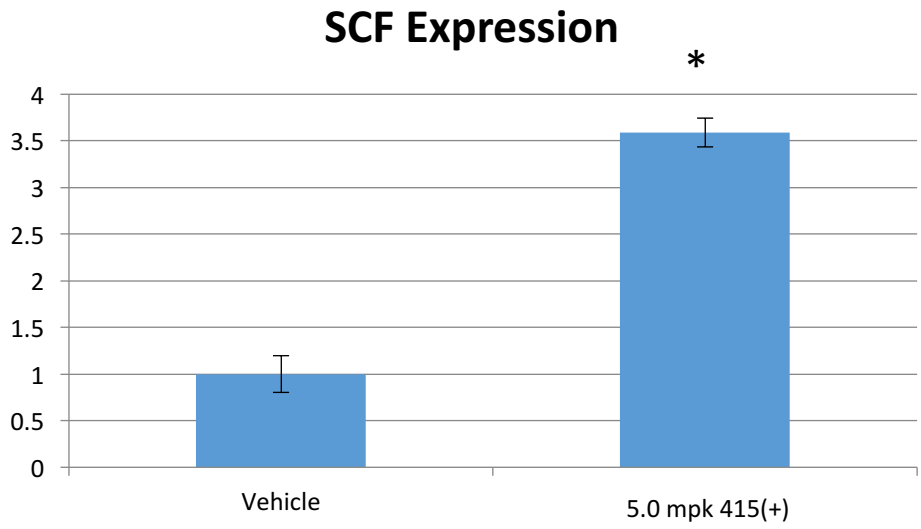


(+)-SW209415

Supp. 1: Structures of SW033291 and (+)-SW209415. SW209415 modifies SW033291 by substituting a dimethyl-imidazole in place of a previous phenyl group and substituting a thiazole ring in place of a prior thiophene.

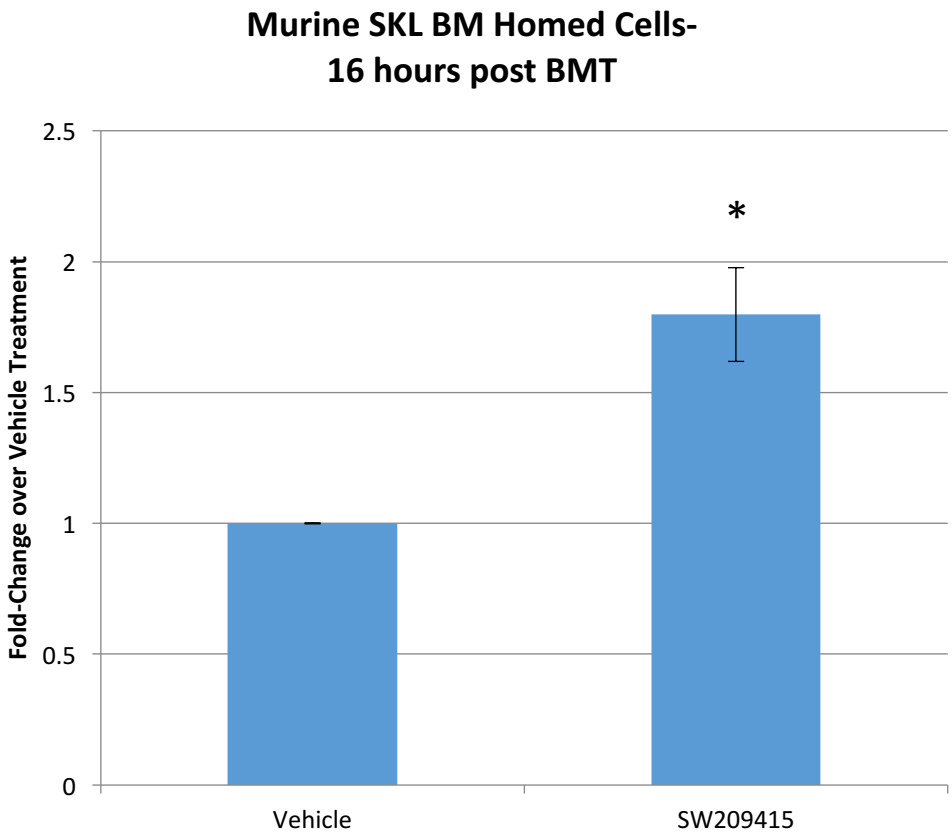
Figure S2





Supp. 2: Gene expression of SCF and CXCL12 in CD45(-) cells following five doses of (+)-SW209415. 8 week female C57/BL6J mice were treated I.P. twice daily for 5 doses with 5.0 mpk (+)-SW209415. Bone marrow was harvested 2 hours following the final dose. CD45(-) fraction was collected via magnetic separation (Miltenyi) and gene expression measured using Applied Biosystems TaqMan® primers and probe sets to mouse CXCL12 (Mm00445553_m1), SCF (Mm00442972_m1) and an endogenous control of GAPDH (Mm03302249_g1). N=3 mice/arm. Graphs show mean and SEM determinations.

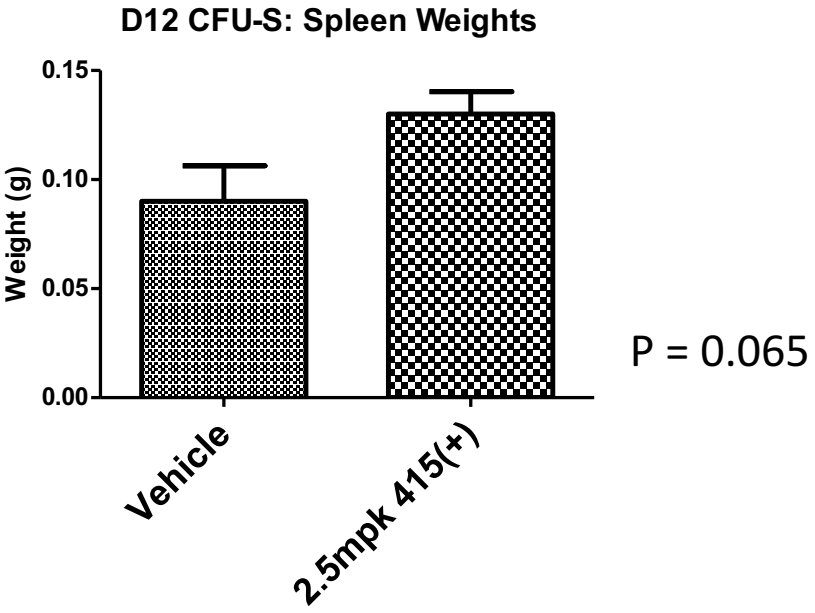
Figure S3



Supp. 3: (+)-SW209415 promotes homing of murine SKL cells

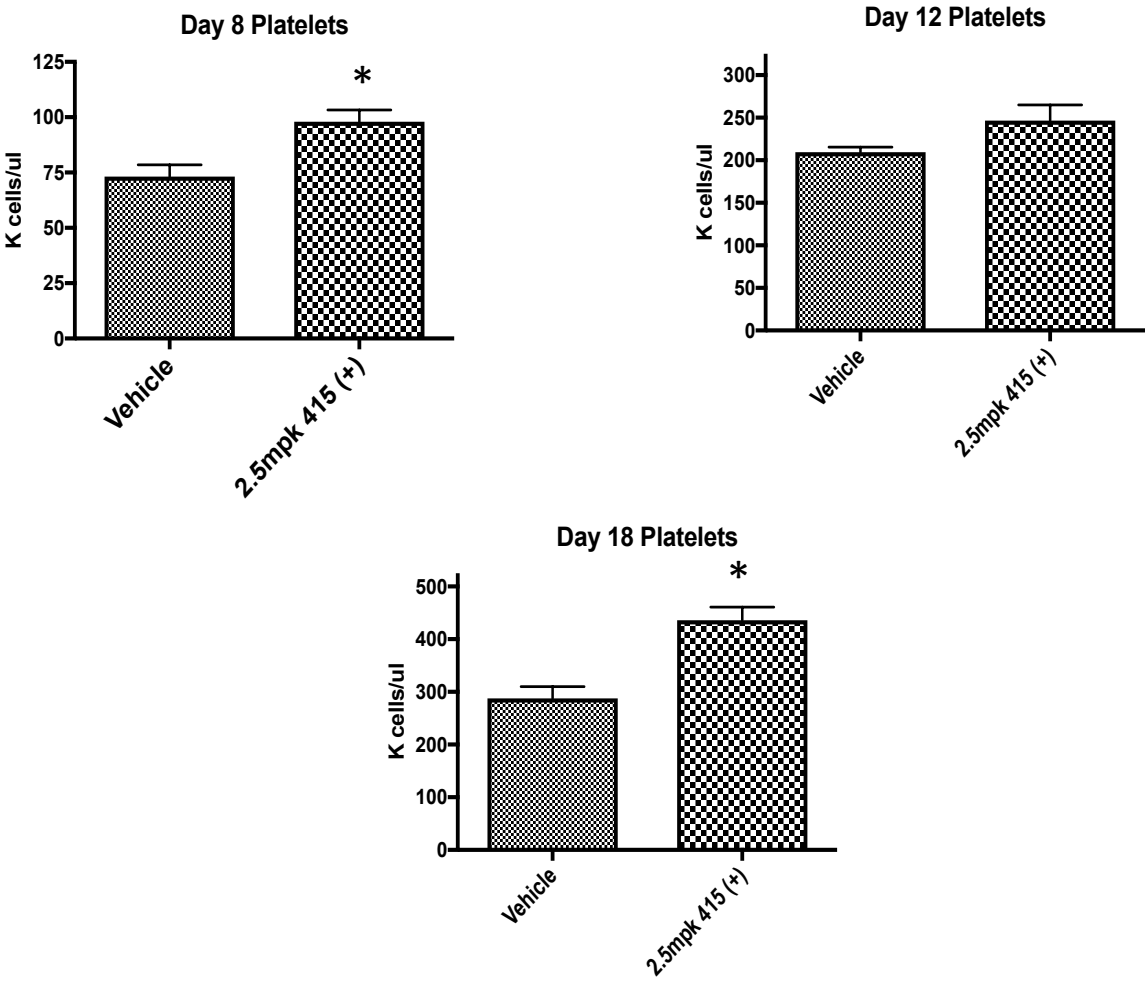
Lethally irradiated (11 Gy) 8 week old female C57/BL6J mice were transplanted with 10^7 CFSE labeled total bone marrow cells and treated with either Vehicle or 2.5 mpk (+)-SW209415 IP immediately post IR, post BMT, and 8 hours post BMT. Mice were harvested at 16 hours post BMT and CFSE+ cells were gated to measure lineage(-)/Sca1(+)/c-Kit(+) cells. Graphs show mean values and SEM. N=8 mice/arm. * Indicates student's t-test with p-value <0.05.

Figure S4



Supp. 4: Spleen weights of Vehicle and (+)-SW209415 treated spleens D12 post BMT for CFU-S Assay. Spleens from animals harvested in Figure 2B were weighed upon removal. Values were tabulated graphically with error bars corresponding to standard error of the means and compared using 2-tailed t-tests. N=6 mice/arm.

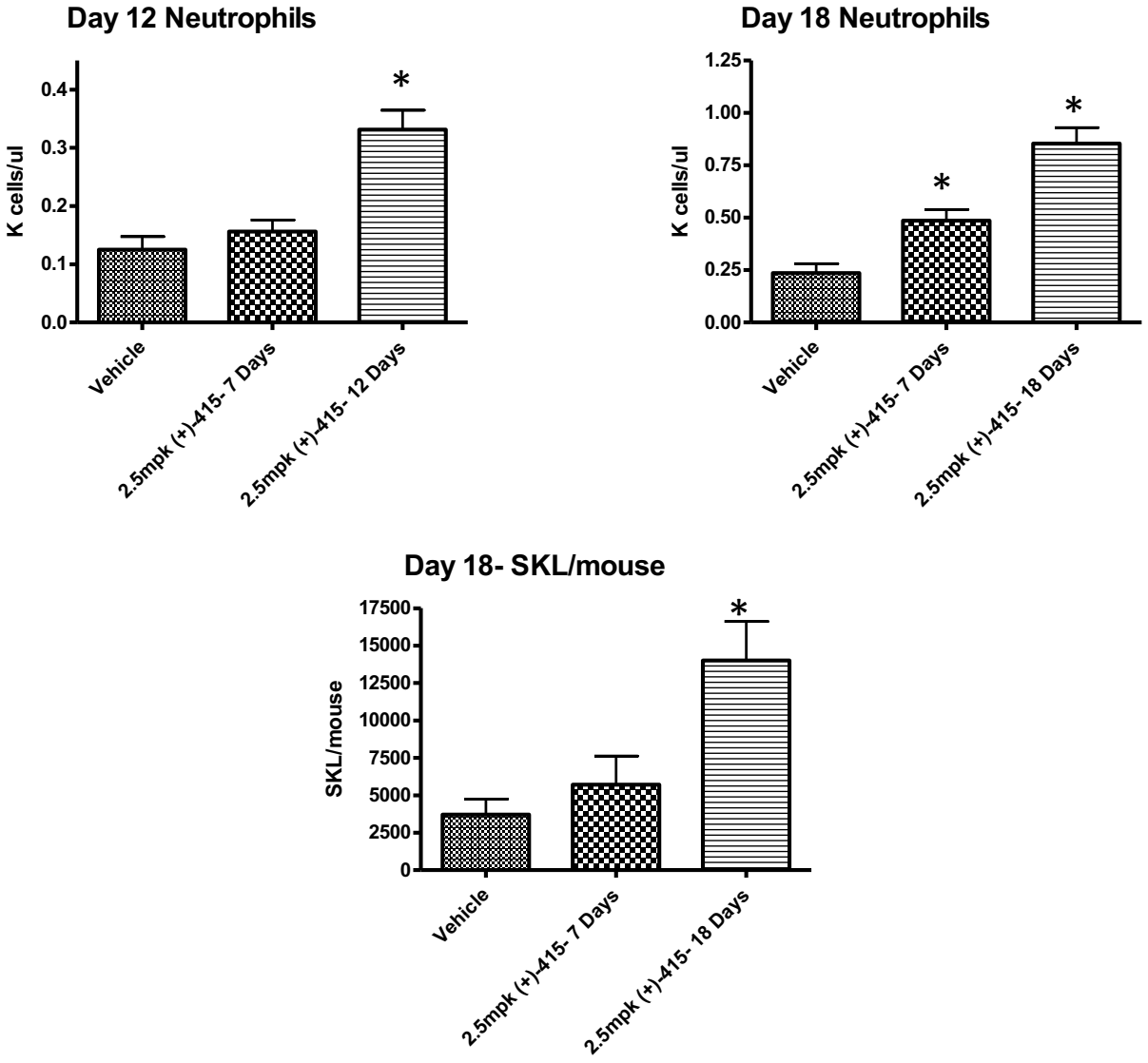
Figure S5



Supp. 5 (+)-SW209415 accelerates post-BMT platelet recovery

Lethally irradiated (11 Gy) 8 week old female C57/BL6J mice were transplanted with 500k total bone marrow cells and treated with either Vehicle, or 2.5 mpk (+)-SW209415 (IP, 2x daily) Graphs display means and SEM of peripheral blood platelet counts as measured on D8, D12, and D18 post BMT. N=13 mice/arm. * Indicates student's t-test with p-value <0.05.

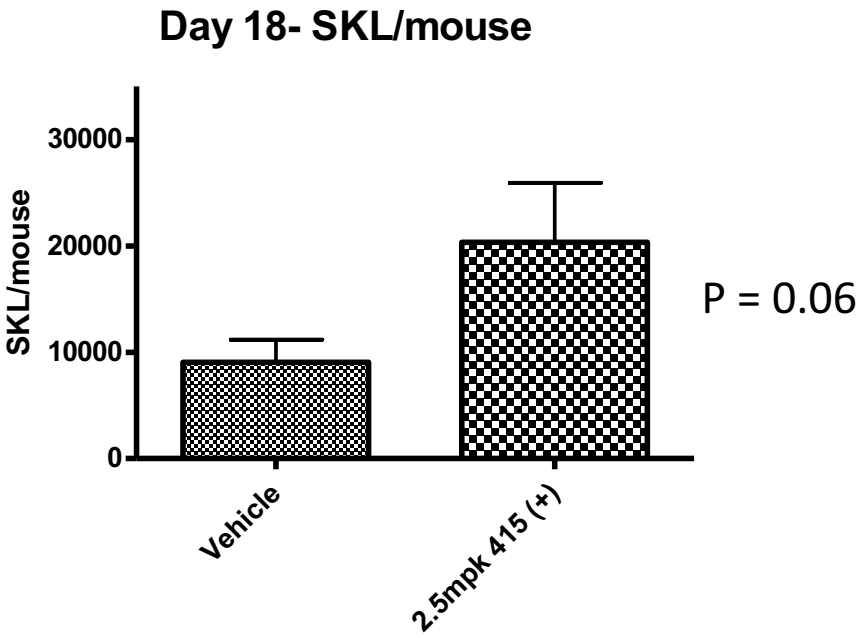
Figure S6



Supp. 6: Continuous 18-Day Dosing of (+)SW209415 is Optimal for Improving Hematopoietic Recovery Following BMT.

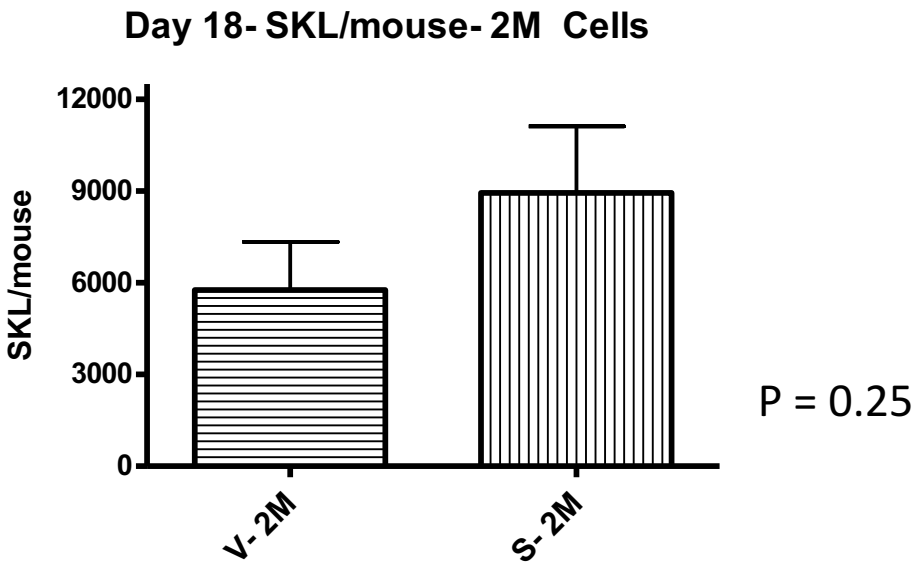
Lethally irradiated (11 Gy) 8 week old female C57/BL6J mice were transplanted with 500k total bone marrow cells and treated with twice daily Vehicle or 2.5 mpk (+)-SW209415 IP for either 7, 12 or 18 days. Graphs display means and SEM of peripheral blood neutrophil counts as measured on D8, D12, and D18 post BMT and bone marrow SKL cell numbers as determined in mice sacrificed on D18. N=8 mice/arm. * Indicates student's t-test with p-value <0.05.

Figure S7



Supp. 7: SKL/mouse on day 18 post BMT performed using aged donor and recipient mice.
Mice from Figure 4 were harvested at Day 18 and bone marrow SKL frequency measured via flow cytometry. Mean values are displayed graphically, with error bars corresponding to standard error of the means, and compared using 2-tailed t-tests. N=6 mice/arm.

Figure S8

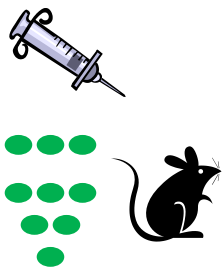


Supp. 8: SKL/mouse at 18 days post BMT performed with 2M cell transplant inoculum.
Mice from Figure 5 were harvested at Day 18 and bone marrow SKL frequency measured via flow cytometry. Values were tabulated graphically with error bars corresponding to standard error of the means and compared using 2-tailed t-tests. N=12 mice/arm.

Figure S9

Isolate CD34+ cells from human BM aspirate using Miltenyi magnetic separation kit

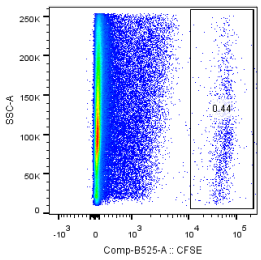
Label cells with 5 uM CFSE for 30 minutes at 37°



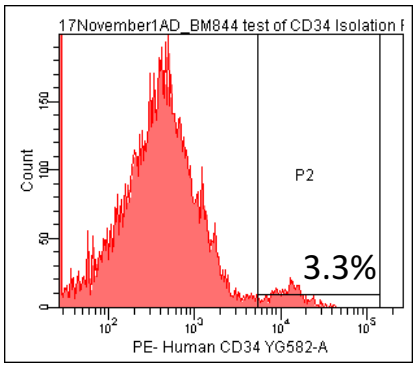
Transplant $5*10^4$ CD34(+) cells into 2.5 Gy NSG recipients

2.5mpk (+415) post IR, post BMT, 8 hours post BMT

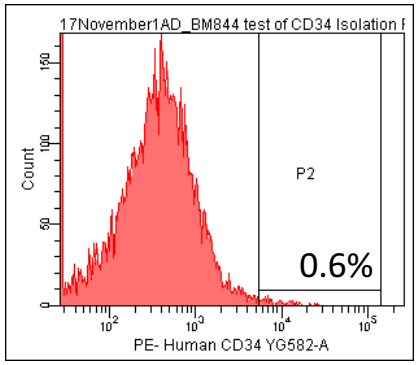
Sacrifice mice 16 hrs post transplant and measure CFSE- cells in whole marrow



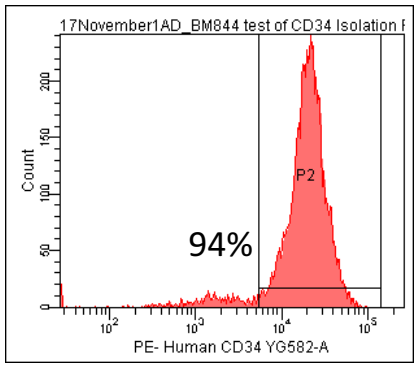
Total BM

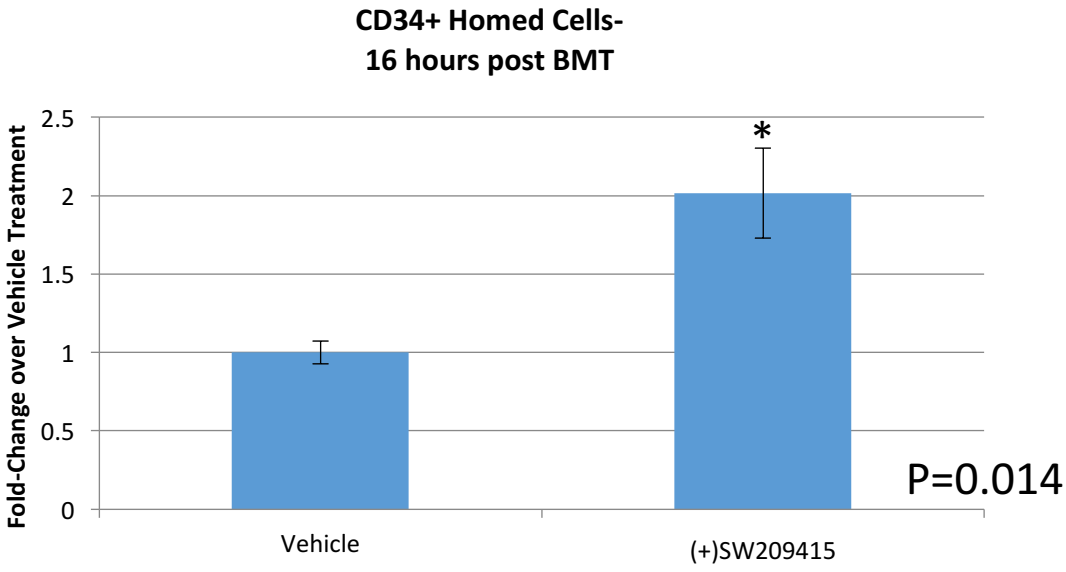


CD34(-)



CD34(+)

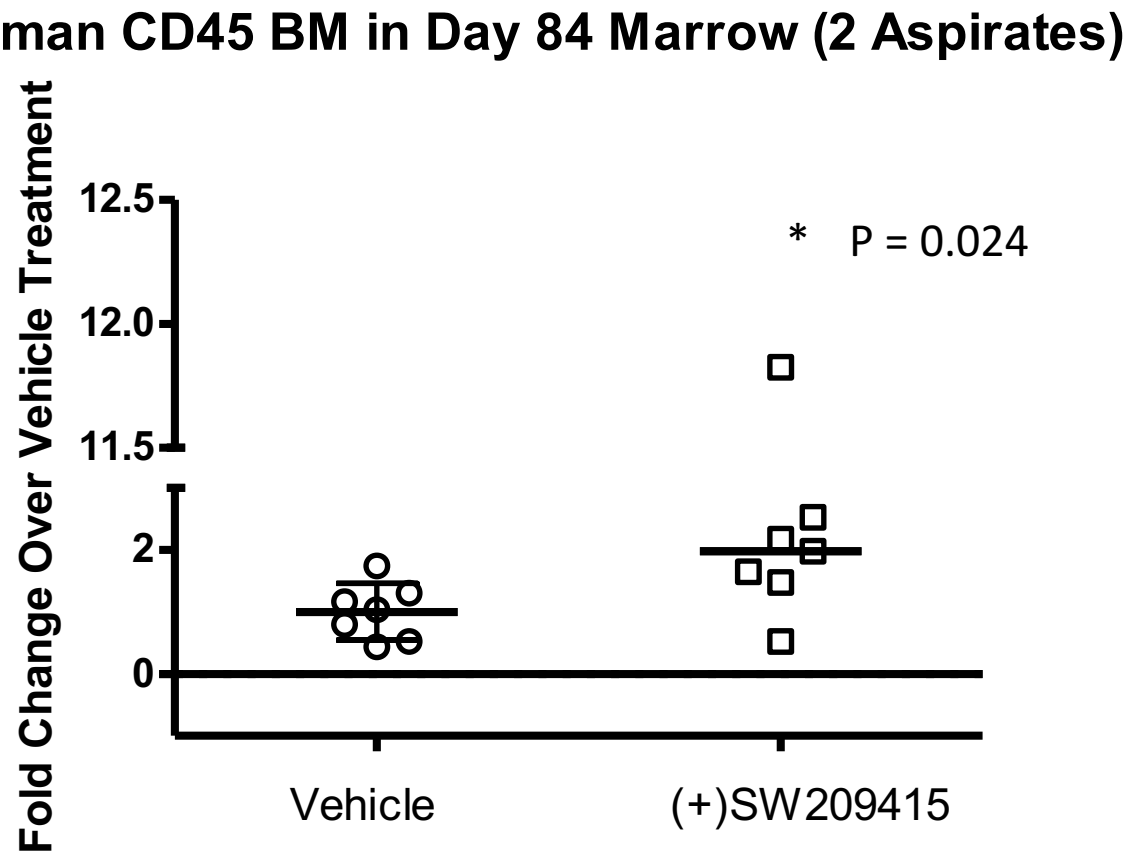




Supp. 9: (+)-SW209415 promotes homing of human CD34+ cells into NSG mice

Human CD34+ cells from two unique bone marrow aspirates (44/F and 45/F) were isolated using the Miltenyi CD34 separation kit. Cells were labeled with CFSE and 50k cells were transplanted into irradiated (2.5Gy) NSG mice and treated as described in Figure 2. Mice were harvested at 16 hours post BMT and CFSE+ measured. Graphs show mean values and SEM. N=9 mice/arm. * Indicates student's t-test with p-value <0.05.

Figure S10

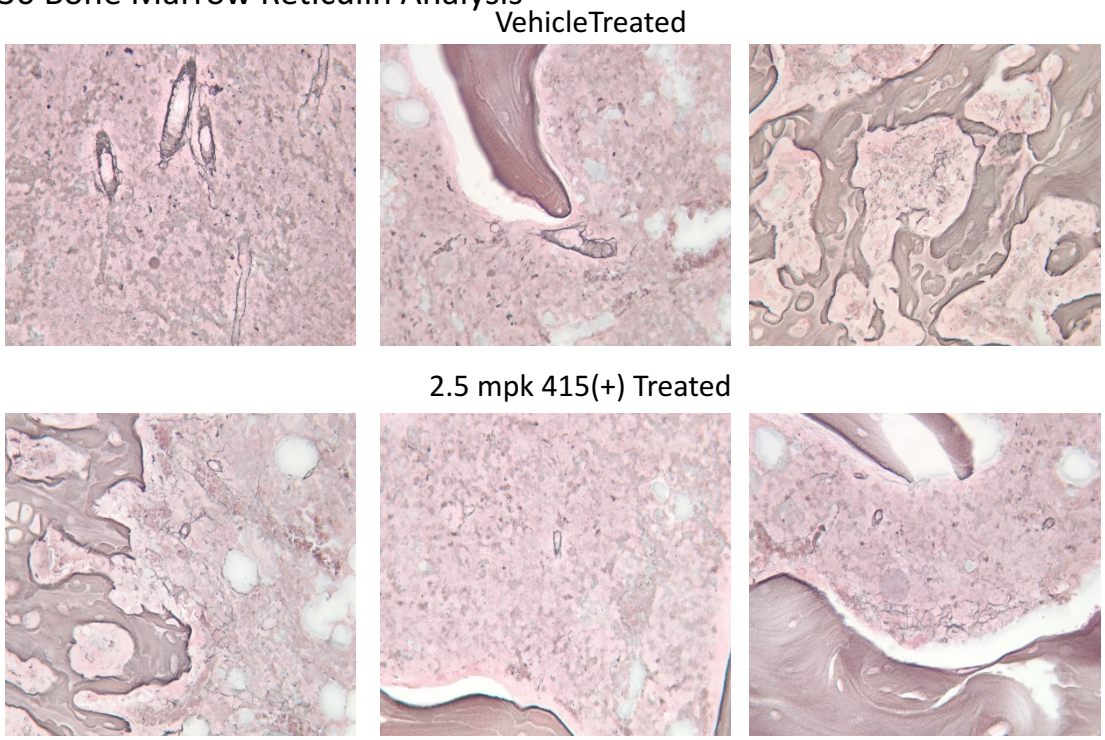


Supp. 10: (+)-SW209415 enhances long term human marrow engraftment in NSG mice.
Irradiated (2.5Gy) 8 week old male NSG mice were transplanted with $1*10^6$ white blood cells from two unique bone marrow aspirate donors (37/M and 41/M) and treated with either vehicle or 2.5mpk (+)-SW209415 2x daily for 21 days. Mice were sacrificed at Day 84 and the frequency of human CD45+ cells measured in total bone marrow. N=7 mice/arm. * indicates Mann-Whitney test with 2-tailed significance. Horizontal line = median values in each arm.

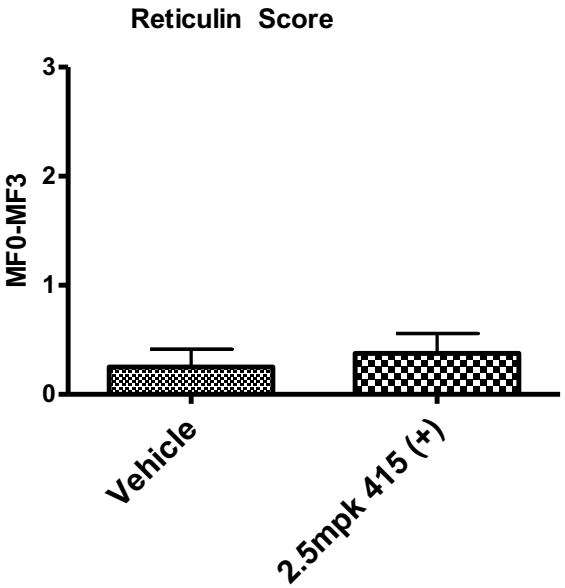
Figure S11

A.

Day 30 Bone Marrow Reticulin Analysis



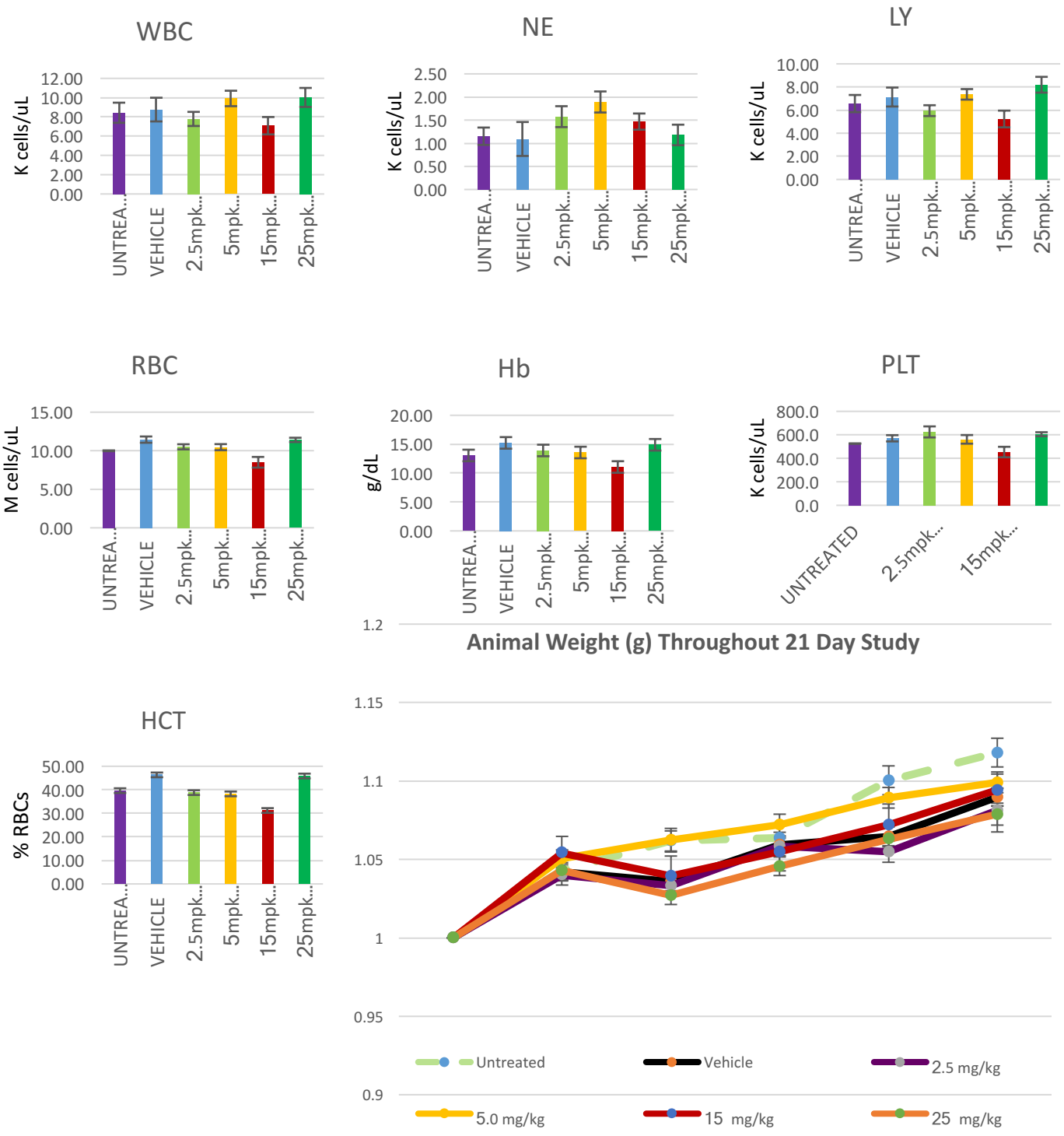
B.



Supp. 11: (+)-SW209415 has no effect on marrow fibrosis

8 week old female C57/BL6J mice were treated 2x daily with either Vehicle or 2.5 mpk (+)-SW209415 for 30 days. At day 30, reticulin staining (AML labs) was performed on femurs and tibias of animals and scored MF0-MF3 by a board certified pathologist blinded as to treatment group. Representative photomicrographs are shown in panel A with tabulated scores displayed as mean values with SEM in panel B. N=8 mice/arm.

Figure S12



Supp 12: (+)-SW209415 does not show off-target toxicity

8 week old male and female C57/BL6J mice were treated with increasing doses of (+)-SW209415 2x daily for 21 days. Animal weights were recorded 2x weekly. At D21 CBC counts were determined. Graphs show means and SEMs for weights and peripheral blood counts. Figure Legend- Plots of weights indicates dose of (+)-SW209415 aministered. Abbreviations on plots of blood counts are: WBC, white blood cells; NE, neutrophils; LY, lymphocytes; RBC, red blood cells; Hb, hemoglobin; PLT, platelets; HCT, hematocrit. N=8 mice/arm.

Table S1

	UNTREATED	VEHICLE	2.5mpk	5.0mpk	15mpk	25mpk
Glucose (mg/dL)	180.3	145.2	162.0	171.4	164.5	163.5
AST (GOT) (U/L)	217.7	305.0	251.0	244.2	275.2	296.8
ALT (GPT) (U/L)	133.7	232.7	178.0	183.7	200.3	178.0
Alkaline Phosphatase (U)	129.9	123.3	115.0	119.7	109.0	115.3
Total Bilirubin (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.3
Cholesterol (mg/dL)	90.4	101.5	102.7	97.0	102.0	91.3
Total Protein (g/dL)	5.35	5.3	5.4	5.3	5.4	5.3
Albumin 2.9 (g/dL)	3.06	3.1	3.1	3.0	3.1	3.0
Globulin (g/dL)	2.29	2.2	2.3	2.3	2.4	2.2
Urea Nitrogen (mg/dL)	28.1	28.2	27.8	27.6	27.8	26.2
Creatinine (mg/dL)	0.25	0.3	0.3	0.2	0.2	0.2
Phosphorous (mg/dL)	7.42	7.5	7.2	6.9	8.3	8.1
Calcium (mg/dL)	9.52	9.4	9.6	9.6	9.6	9.5
Sodium (mmol/L)	149.5	149.0	149.7	149.2	149.8	148.3
Potassium (mmol/L)	7.67	7.1	7.0	7.1	7.0	7.7
Chloride (mmol/L)	110.2	110.0	109.3	110.0	109.7	110.0
Bicarbonate (mmol/L)	27.5	22.7	27.0	26.0	27.0	31.3
Anion Gap (mmol/L)	19.4	23.3	20.2	20.2	20.2	14.7
Gamma-GT (U/L)	0.0	0.0	0.0	0.0	0.0	0.0
Triglycerides (mg/dL)	106.7	110.0	138.8	126.0	115.2	116.3
T4-VET (ug/dL)	6.81	8.2	17.3	6.3	6.1	6.0
A/G Ratio	1.35	1.4	1.4	1.3	1.3	1.4
Urea/Creat Ratio	116.6	110.2	109.2	118.0	132.0	122.2
Sodium/Potassium Ratio	19.5	21.3	21.3	21.0	21.5	19.3

Supplement Table 1: (+)-SW209415 does not show any off-target toxicity
8 week old male and female C57/BL6J mice were treated with increasing doses of (+)-SW209415 2x daily for 21 days. At D21 serum was sent for complete blood chemistry analysis. N=6 mice/arm.