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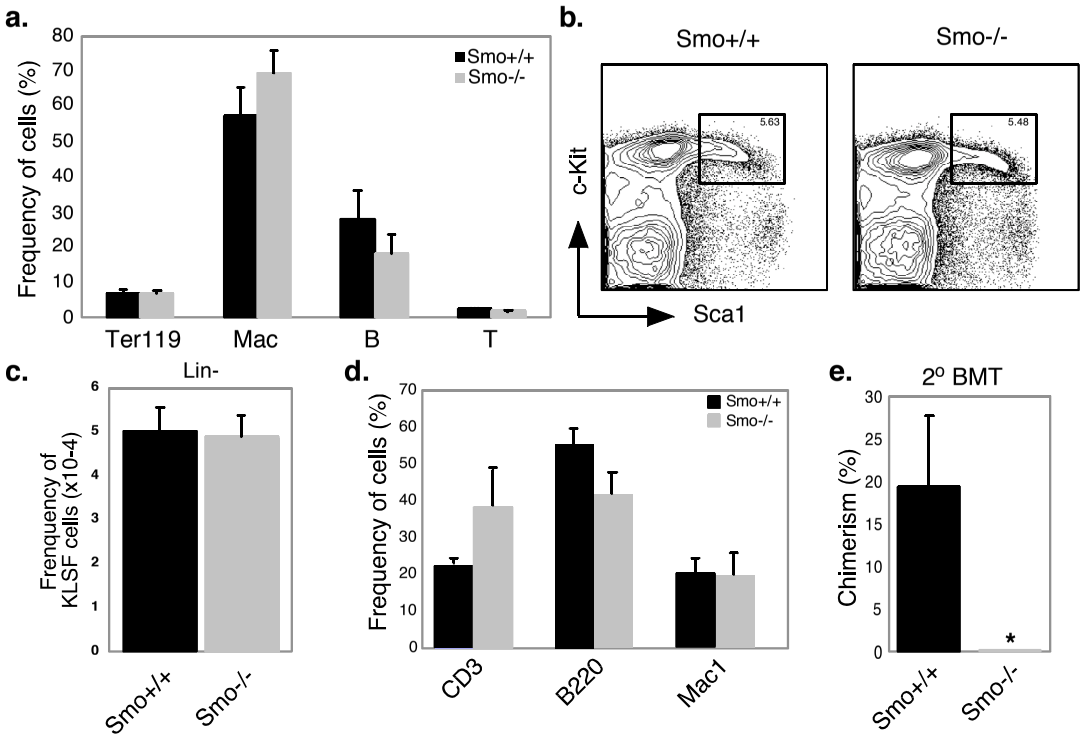
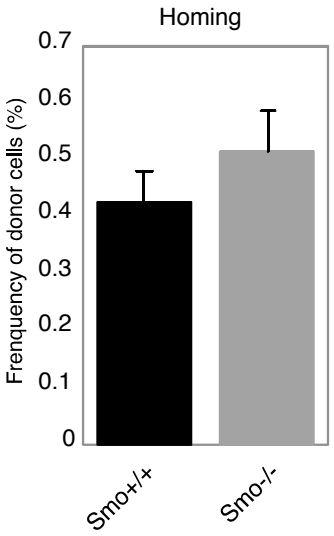
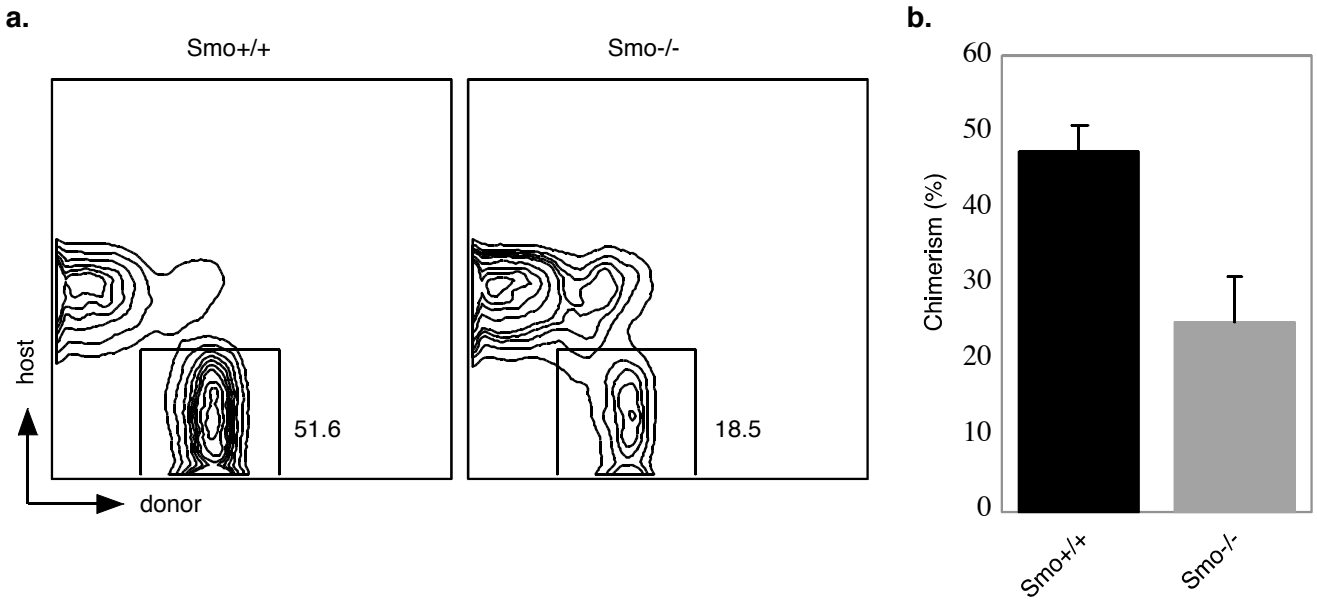


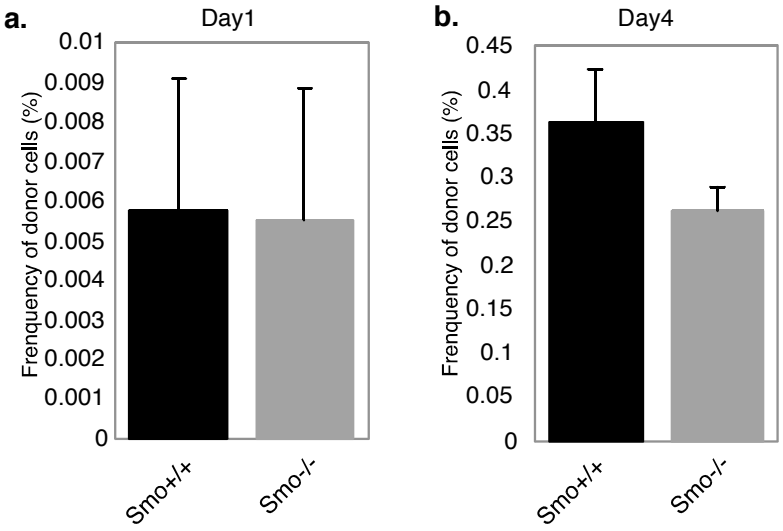
Supplementary Figure 1: Deletion of Smoothened does not alter the frequency of hematopoietic lineages
(a) Bone marrow cells from control or Smo -/- mice were analyzed for frequency of cells of distinct hematopoietic lineages. Whole bone marrow cells were stained with erythroid (Ter119), myeloid (Mac1), B (B220) or T(CD4 and/or CD8) markers and analyzed by FACS. Data shown is an average of results from five mice. (b) Bone marrow cells from control or Smo-/- mice were analyzed for frequency of KLSF cells. Dot plots are shown for one representative control Smo+/+ (left) and Smo -/-(right). (c) Average frequency of KLSF cells in control (n=10) and Smo -/- mice (n=10). Error bars show s.e.m. (d) Contribution to differentiated lineages from control Smo+/+ and Smo-/- cells following long-term bone marrow transplantation in peripheral blood. Results are representative of two independent experiments, with 4-6 mice per cohort per experiment. (e) Secondary competitive repopulation analysis of control (Smo+/+) and Smoothened knockout (Smo-/-) mice was carried out using $1X10^6$ bone marrow cells from primary recipients isolated at 24 weeks and analyzed for donor chimerism in secondary recipients at 24 weeks. Graph of average donor derived chimerism after long-term reconstitution (6 mice in each cohort p=0.04).



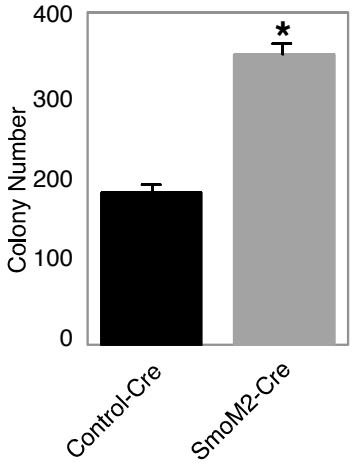
Supplementary Figure 2: Homing of hematopoeitic cells is not altered in absence of Smo
2×10^6 whole bone marrow cells from Smo+/+ or Smo-/- mice were transplanted into lethally irradiated recipients. The graph shows average of donor chimerism after 6 hours (n=4 for control, n=5 for Smo-/- mice, p=0.4).



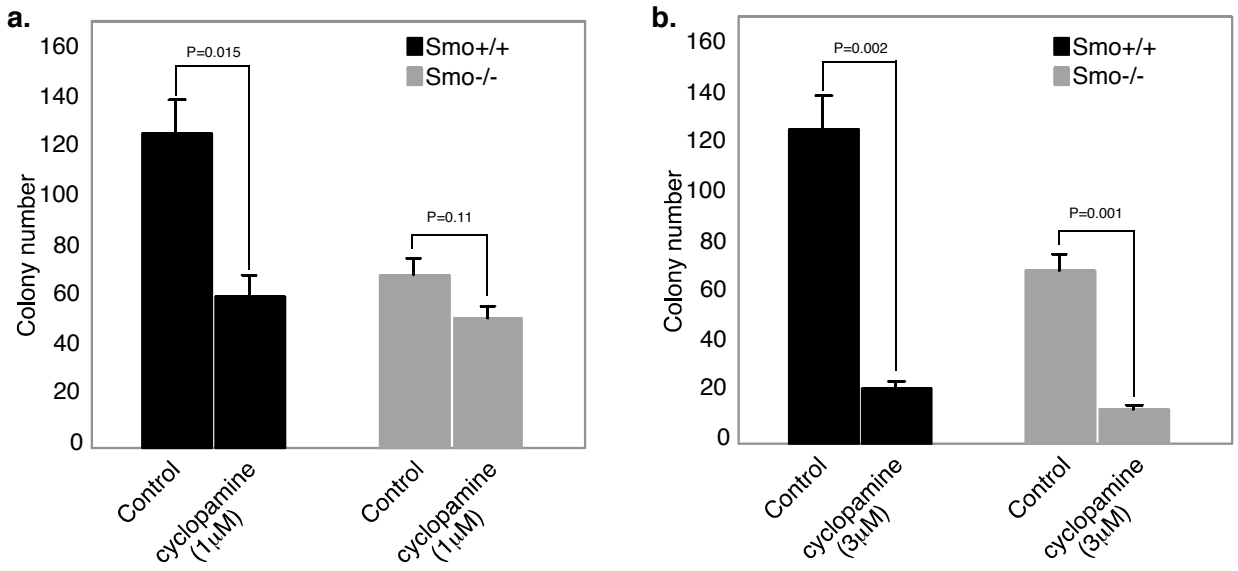
Supplementary Figure 3: Comparative reconstitution of whole bone marrow from Smo+/+ and Smo-/- mice (a) 200,000 bone marrow cells from Smo+/+ or Smo-/- mice were transplanted together with 200,000 competing bone marrow cells into lethally irradiated recipients. Plots show representative donor derived chimerism at 7 weeks. (b) Graph of average donor chimerism at 7 weeks (data is an average of 7 mice in each cohort, p=0.008).



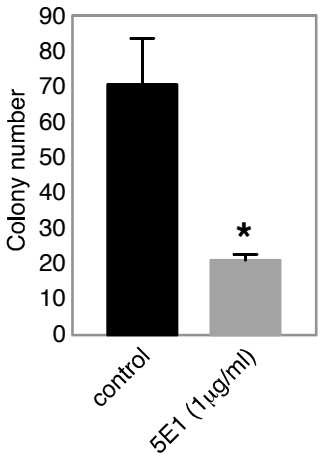
Supplementary Figure 4: Homing of BCR-ABL transduced Smo+/+ and Smo-/- cells are equivalent (a-b) 30,000 KLSF cells from Smo+/+ or Smo-/- mice infected with BCR-ABL were transplanted together with 200,000 competing bone marrow cells into lethally irradiated recipients. Recipient mice were sacrificed (a) 1 day or (b) 4 days after transplantation and analyzed for donor chimerism. Graphs show the average percentage of donor derived cells after 1 day (4 mice in each cohort for a total of 8 mice, p=0.96) and 4 days (mice in each cohort for a total of 8 mice, p=0.2).



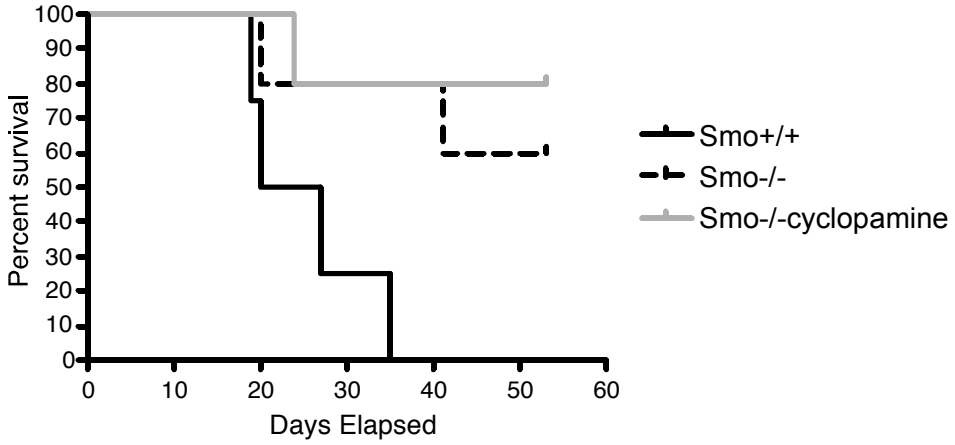
Supplementary Figure 5: Post-embryonic activation of SmoM2 enhances BCR-ABL induced growth. Bone marrow KLSF cells were harvested from wild type and SmoM2 mice, infected with MSCV-Cre (GFP) and MSCV-BCR-ABL (YFP) viruses. Sorted $YFP^{+}GFP^{+}$ cells were plated (600 cells/well) in methylcellulose media (M3434), and colonies counted after 7-10 days (p=0.004). This indicates that the effect of Smo on CML growth is not limited to activation due to embryonic development.



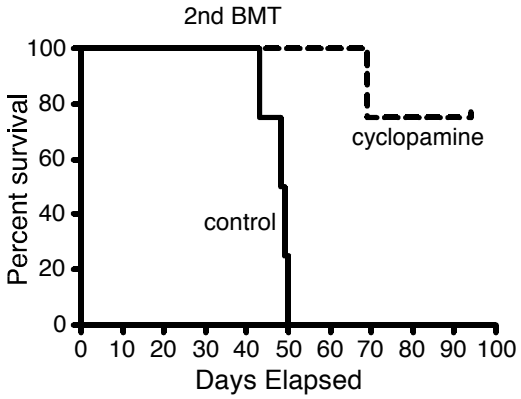
Supplementary Figure 6: Specificity of cyclopamine dose *in vitro*.
(a-b) Bone marrow KLSF cells from Smo+/+ and Smo-/- mice were infected with BCR-ABL and plated (350 cells/well) in methylcellulose media (M3434) in the presence of (a) control and cyclopamine (1μM) or (b) control and cyclopamine (3μM). Colonies were counted after 7-10 days. 1μM cyclopamine (concentration used in experiments) did not add any further effect to the loss of Smoothened, suggesting minimal off target effects. However, 3μM cyclopamine impaired the growth of Smo-/- cells possibly indicating off target effects.



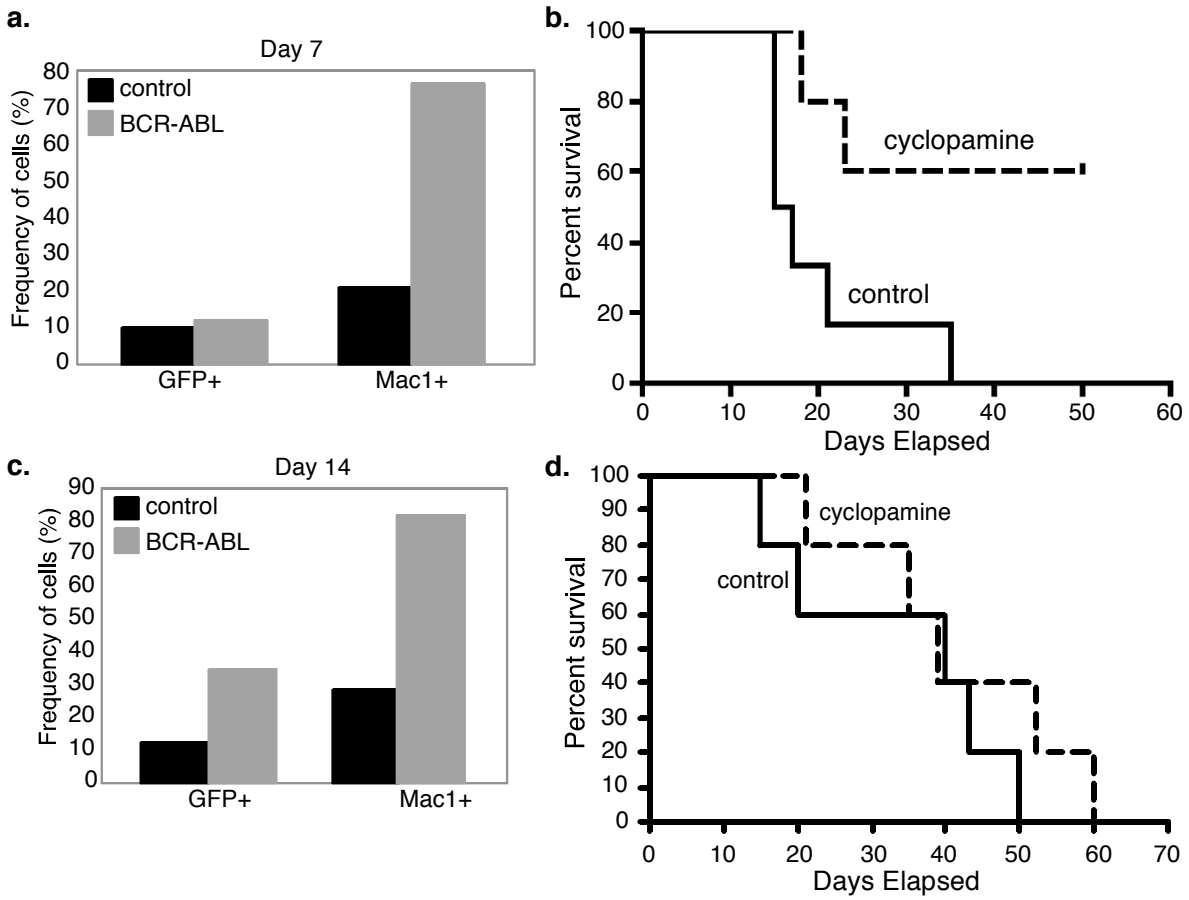
Supplementary Figure 7: Inhibition of Hh ligand impairs CML growth. KLS GFP+ cells from CML mice were sorted and plated (500cells/well) in methylcellulose media (M3434) in the presence of control or 5E1 antibody (1μg/ml). Colonies were counted after 7-10 days. Data shown is representative of 2 independent experiments (p=0.01).



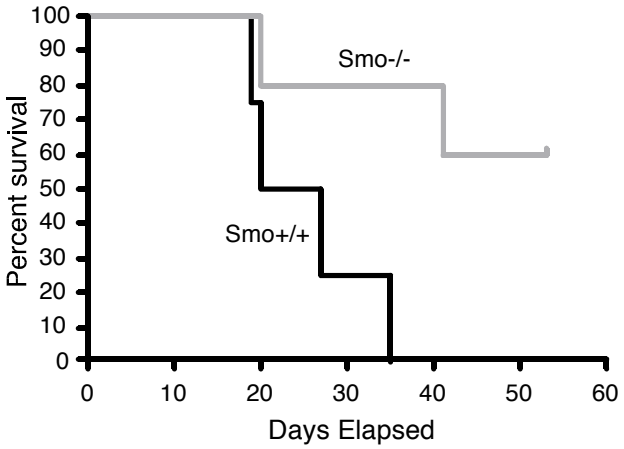
Supplementary Figure 8: Specificity of cyclopamine dose *in vivo*. 15,000 BCR-ABL infected Smo+/+ or Smo-/- KLSF cells were transplanted into lethally irradiated recipients along with 200,000 competing bone marrow cells. Subsequently, mice were treated with vehicle or cyclopamine as indicated and survival monitored. Smo+/+ vehicle data is same as in Supplementary Figure 11 (4 mice in each group for a total of 12 mice). Dose of cyclopamine used did not display additional impact beyond Smo loss, suggesting minimal off target effects.



Supplementary Figure 9: Cyclopamine treated CML display reduced self renewal and propagation in secondary hosts. BCR-ABL infected cells were transplanted into recipient mice and treated with vehicle or cyclopamine. Following CML formation, 2.5×10^6 CML cells from either pooled vehicle treated mice (5 CML samples) or cyclopamine treated mice (3 CML samples) were transplanted into secondary recipients and survival monitored ($p=0.007$). Analysis is shown of 4 recipients per group for a total of 8 mice.



Supplementary Figure 10: Efficacy of Hh blockade at different time points after CML development. Bone marrow KLSF cells were infected with either control vector or BCR-ABL and transplanted into recipient mice. (a,c) Peripheral blood cells from the recipient mice were analyzed at (a) 7 days and (c) 14 days for BCR-ABL-GFP$^+$ total cell chimerism and GFP$^+$myeloid (Mac1+) cell chimerism. (b,d) Survival curves of control or cyclopamine treated BCR-ABL mice after initiation of cyclopamine delivery at (b) 7 days (same as Figure 5, p value=.02) or (d) 14 days after transplant. Data shows analysis of 5 mice in each group for a total of 10 mice for d 14, and 30 mice for d7 over different experiments.



Supplementary Figure 11: Impaired growth of Imatinib resistant CML in Smo-/- cells. 15,000 BCR-ABL T3151 infected Smo+/+ or Smo-/- KLSF cells were transplanted into lethally irradiated recipients along with 200,000 competing bone marrow cells, and survival monitored (Data shows analysis of 4 mice in each group for a total of 8 mice p=0.025).

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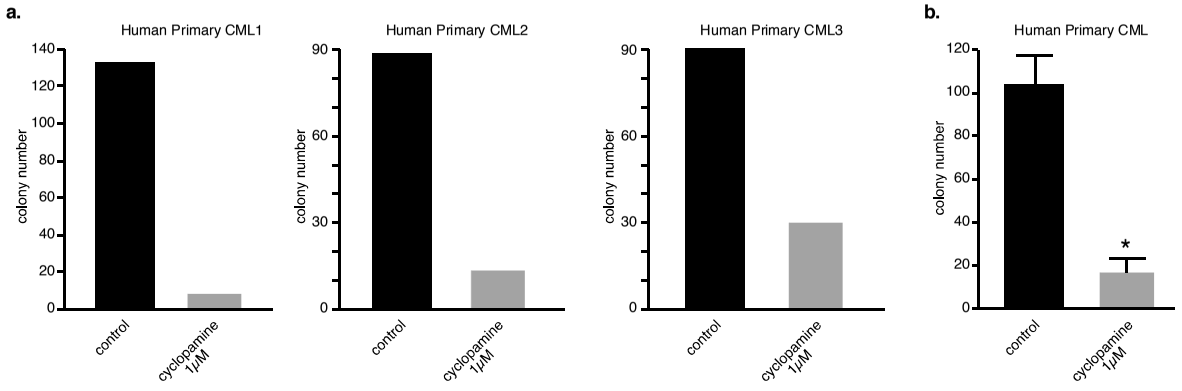
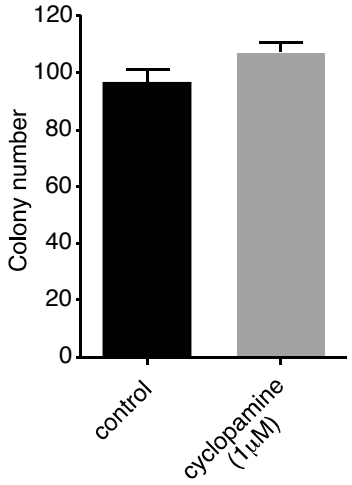


Supplementary Figure 12: Pharmacologic inhibition of Smoothened impairs growth of human CML cells.
(a) CD34+ cells were sorted from primary uncultured human blast crisis CML samples and plated in methylcellulose in the presence of tomatidine (1mM) or cyclopamine (1mM). Colonies were counted after 12-14 days. Data shown is for three independent bcCML patient samples. (b) Graph of the average number of colonies for the three individual patient samples (also shown in figure 4i).



Supplementary Figure 13: Impact of cyclopamine on human cord blood cells.
hCD34+ cells were sorted from primary human cord blood, plated ($1x10^5$/well) in methylcellulose in the presence of either tomatidine 1mM (control) or cyclopamine 1mM. Colonies were counted after 12-14 days. The graph shows results from 2 independent cord blood samples, each plated in triplicate ($p<0.05$)