Figure S1



Supplementary Figure S1. Expression of *Htr1b* in osteoblasts is necessary for leukemia progression. Related to Figure 1. (A) Representative images of in vivo leukemia progression in MLL/AF9-dsRed injected wild-type (WT) mice at the indicated days. Histograms: ds-Red emitted fluorescence (n=5-14 mice/day) and mRNA levels in bone (femur, n=5 mice/day); one-way ANOVA. (B) In vivo leukemia progression (fluorescence counts) in WT MLL/AF9 mice treated with vehicle (n=4) or PTH (n=7). (C) mRNA cyclins (Cyc) and collagen 1 (Collal) levels in bone (femur) of mice in (B). (D) mRNA relative levels of the 14-known serotonin receptors in primary mouse osteoblasts (n=12 femur samples). (E) Representative images of Htr1b^{+/-} vs Htr1b^{+/-} littermates injected with MLL/AF9 dsRed cells; nuclei stained with 4,6-diamidino-2-phenylindole staining (DAPI). Scale 100µm. (F-I): Left panels: In vivo quantification of leukemia progression (dsRed fluorescence); right panels: complete blood counts (CBC) at harvest in: (F) Htr1b^{-/-} (n=29 flux; n=8 CBC) vs. Htr1b^{+/+} (n=13 flux; n=5 CBC) mice; (G) mice lacking Htr1b expression in mesenchymal/stromal progenitor cells ($Htr1b_{Lep-R}^{-/-}$, n=8 vs. $Htr1b_{Lep-R}^{+/+}$, n=4); (H) in committed osteoblast progenitors (Htr1bc-osb-/-, n=7 vs. Htr1bc-osb+/+, n=11 -the 4 Htr1bc-osb-/- mice dead are independently depicted with red squares and the only $Htr1b_{c-osb}^{+/+}$ alive is shown with a white circle-; or in (I) differentiated osteoblasts (Htr1bd-osb^{-/-}, n=5 vs. Htr1bd-osb^{+/+}, n=7 flux and n=3 CBCs). (J) Recombination efficiency percentage in CollaCre (n=2) versus OCN Cre (n=3) lines for the Htr1b fl/fl locus; unpaired t-test. (K) In vivo quantification of leukemia progression in mice kept on doxycycline (DOX) diet (*Htr1b* _{Osx}^{+/+}; n=6) or with DOX- removed 24h after MLL/AF9 injection (no-DOX; *Htr1b* _{Osx}-/-; n=9). (L) *In vivo* quantification of leukemia progression on the indicated days for the two survivor mice (#1 and #2) in the no-DOX group. (M) Radioligand competitive-binding assay on *Htr1b*-overexpressing-HEK293T membranes with [³H]-5-HT (25nM, 41.3 Ci/mmol) competed out with SB9 at the indicated concentrations, logEC₅₀: 1µM (see Table 1). (N) mRNA cyclins (Cyc) and collagen 1 (Collal) levels in bone (femur) of WT mice injected with MLL/AF9-dsRed cells and treated with either vehicle (n=8) or SB9 (n=8). Osb, osteoblast; NE, neutrophils; LY, lymphocytes; MO, monocytes; EO, eosinophils and BA, basophils. All data are represented as mean \pm SEM. Statistical analysis done with two-way ANOVA unless otherwise stated.