EXTENDED DATA

Extended Data Figure Legends

Extended Data Figure 1: Isolation of haematopoietic progenitor cell populations by flow cytometry and histograms showing protein synthesis in vivo relative to HSCs from the same mice. a-e, One hour after OP-Puro administration to mice we observed no effect on bone marrow cellularity (1 femur and 1 tibia; a; n=7 PBS treated and n=9 OP-Puro mice) or the frequencies of $CD150^{+}CD48^{-}$LSK HSCs (b; n=4 PBS treated, n=6 OP-Puro treated), Annexin V^{+} bone marrow cells (c; n=4 PBS treated, n=6 OP-Puro treated), Annexin V^{+} HSCs (d; n=4 PBS treated, n=6 OP-Puro treated), or HSCs in $S/G_2/M$ phase of the cell cycle (e; n=3/treatment; a-e each reflect 2 or 3 independent experiments). f-i, Representative flow cytometry plots showing the markers and gating strategies used to isolate CMPs[42], GMPs[42], and MEPs[42] (f), pro-B[43], pre-B[43], and IgM^{+} B cells (g), Gr-1^{+} myeloid cells (h), $CD3^{+}$ T cells (h), and $CD71^{+}Ter119^{+}$ erythroid progenitors (i). Each panel also shows OP-Puro incorporation histograms for each cell population relative to HSCs after 1 hour of OP-Puro incorporation in vivo. The level of background fluorescence from PBS treated controls is overlaid in black. j, Data from Figure 1h showing protein synthesis in various haematopoietic cell populations relative to unfractionated bone marrow cells on a log2 scale (n=15 mice from 9 independent experiments). All data represent mean±s.d. Two-tailed Student's t-tests were used to assess statistical significance in a-e. The statistical significance of differences relative to HSCs in j was assessed using a

repeated measures one way ANOVA followed by Dunnett's test for multiple comparisons. # indicates statistical comparison to HSCs (#, $p<0.05$; ##, $p<0.01$; ###, $p<0.001$).

Extended Data Figure 2: OP-Puro containing polypeptides are not degraded within 30 minutes, the degradation that occurs over 24 hours is blocked by bortezomib, and OP-Puro administration does not induce cell death. a, OP-Puro fluorescence in haematopoietic cells after one hour of OP-Puro administration in vivo followed by a 30 minute ex vivo incubation on ice or at 37°C (n=11 mice from 4 independent experiments). b, OP-Puro fluorescence in haematopoietic cells 24 hours after OP-Puro administration in vivo. Treatment with bortezomib one hour before OP-Puro administration increased OP-Puro fluorescence in every cell population 24 hours later (n=3 independent experiments; total number of mice/treatment are shown in the panel). c, Frequency of Annexin V^+ cells in each cell population one hour after OP-Puro administration in vivo relative to the same cells from untreated mice (n=7 mice/treatment from 2 independent experiments). All data represent mean±s.d. To assess the statistical significance of treatment effects within the same cell population we performed two-tailed Student's t-tests (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$). To assess the statistical significance of differences between HSCs and other cell populations in panel a, we performed a repeated measures one way ANOVA followed by Dunnett's test for multiple comparisons (#, $p<0.05$; ##, $p<0.01$; ###, $p<0.001$).

Extended Data Figure 3: Cyclophosphamide and G-CSF treatment drives certain cells into cycle and increases protein synthesis. a, Frequency of dividing cells in $S/G_2/M$ phases of the cell cycle (>2N DNA content; n=5 mice from 3 independent experiments). b, Total protein isolated from 50,000 unfractionated bone marrow cells or Gr-1^+ cells in G_0/G_1 or $S/G_2/M$ measured by BCA assay (n=3). c, Total RNA content in 15,000 cells from each stem/progenitor cell population (n=3 mice). d, The frequency of cycling (KI-67^+) HSCs increased dramatically

after cyclophosphamide/G-CSF treatment (n=5 untreated mice and n=6 Cy/G-CSF treated mice from 2 independent experiments, p<0.001). e, Frequency of KI-67$^+$ cells in haematopoietic cell populations before and after cyclophosphamide/G-CSF treatment (n=5 untreated and n=6 Cy/G-CSF treated mice for BM, HSC and MPP, n=3 mice/treatment for other cell populations). f,g Protein synthesis in G_0/G_1 and $S/G_2/M$ cells from untreated mice or mice treated with cyclophosphamide followed by two days of G-CSF (n=10 mice/treatment from 6 independent experiments). These data are the same as shown in Figures 3b, 3d, and 3e, shown together in this panel for comparison purposes. The data are plotted on a linear scale in (f) and on a log2 scale in (g). All data represent mean±s.d. To assess the statistical significance of treatment effects within the same cell population (panels a,b, e-g) we performed two-tailed Student's t-tests (*, p<0.05; **, p<0.01; ***, p<0.001). To assess the statistical significance of differences between HSCs and each other cell population (panels a,c,f,g) we performed a repeated measures one way ANOVA followed by Dunnett's test for multiple comparisons (#, p<0.05; ##, p<0.01; ###, p<0.001).

Extended Data Figure 4: Differences in protein synthesis among haematopoietic stem/progenitor cells are not fully explained by differences in cell division, cell diameter, pS6 levels, rRNA or total RNA content. a-f Scatter plots show the relative rates of protein synthesis (per hour) in each cell population (from Fig. 1h) plotted against the frequency of dividing cells (a; from Extended Data Fig. 3a), cell diameter (b; from Fig. 3f), 18S rRNA content (c; from Fig. 3g), 28S rRNA content (d; from Fig. 3g), total RNA content (e; from Extended Data Fig. 3c) and pS6 levels (f; from Fig. 5a normalized to β–Actin). For each parameter, regressions were performed using all populations excluding HSCs and 95% confidence intervals were determined. R^2 values are shown in each plot. Rates of protein synthesis are plotted on a linear scale (left panels) and on a log2 scale (right panels). Note that HSCs were outliers with respect to each regression. CD150$^+$CD48$^-$LSK cells were used to determine HSC rates of protein

synthesis, cell diameter, and % $S/G_2/M$ while $CD48^-LSK$ cells (HSCs/MPPs) were used to determine 18S, 28S, total RNA, and pS6 levels (as these measurements required more cells).

Extended Data Figure 5: *Rpl24*$^{Bst/+}$ mice have normal frequencies of lymphoid and myeloid lineage progenitors and do not express increased p53 or p21^{cip1} in adult haematopoietic cells. a, Bone marrow (2 femurs and 2 tibias; n=5 wild-type and n=6 *Rpl24*$^{Bst/+}$ mice from 4 experiments), spleen (n=3 wild-type mice and n=4 *Rpl24*$^{Bst/+}$ mice from 2 experiments), and thymus cellularity (n=3 wild-type and n=4 *Rpl24*$^{Bst/+}$ mice from 2 experiments). b, White blood cell, red blood cell, and platelet counts (n=5 wild-type and n=6 *Rpl24*$^{Bst/+}$ mice from 4 experiments). c-f, The frequencies of B (c), myeloid (d), and T (e) lineage cells in the bone marrow and spleen (f) of *Rpl24*$^{Bst/+}$ and control mice (n=3 wild-type and n=4 *Rpl24*$^{Bst/+}$ mice from 2 experiments). g-h, The frequencies of T lineage progenitors in the thymus of *Rpl24*$^{Bst/+}$ and control mice (n=3 wild-type and n=4 *Rpl24*$^{Bst/+}$ mice from 2 experiments). DN1/ETP cells were $CD4^-CD8^-CD44^+CD25^-$. DN2 cells were $CD4^-CD8^-CD44^+CD25^+$. DN3 cells were $CD4^-CD8^-CD44^-CD25^+$. DN4 cells were $CD4^-CD8^-CD44^-CD25^-$. i, The frequencies of Annexin V^+ HSCs and MPPs in *Rpl24*$^{Bst/+}$ versus littermate control mice (n=3 wild-type and n=4 *Rpl24*$^{Bst/+}$ mice from 3 experiments). j, Western blot for Rpl24 and β-Actin using 30,000 cells from each haematopoietic cell population. Differences in β-Actin between lanes represent differences in β-Actin content per cell (1 representative blot from two independent experiments). k, Donor B cell, T cell and myeloid cell engraftment when $5x10^5$ donor bone marrow cells were transplanted along with $5x10^5$ recipient bone marrow cells into irradiated recipient mice (n=4 independent experiments with a total of 17 recipients for wild-type cells and 20 for *Rpl24*$^{Bst/+}$ cells). These transplant recipients are the same as those shown in Fig. 4e. l, The frequency of donor cells in the bone marrow 20 hours after transplanting $1x10^5$ donor LSK cells from *Rpl24*$^{Bst/+}$ or wild-type control mice into irradiated recipient mice (n=3 recipients/donor). The horizontal line represents the level of background detected in an untransplanted control. m,

Western blot for p53 using $5x10^5$ Lineage$^-$ bone marrow cells from wild-type or *Rpl24*$^{Bst/+}$ mice, or $5x10^5$ bone marrow cells from a *p53*$^{-/-}$ mouse (1 representative blot from two experiments). n, Western blot for p21^{cip1} using 285,000 LSK cells from the bone marrow of adult wild-type or *Rpl24*$^{Bst/+}$ mice, or 142,500 LSK cells from a wild-type mouse that received 540 rad of total body irradiation 3-4 hours before being euthanized (1 representative blot from three independent experiments). All data represent mean±s.d. Two-tailed Student's t-tests were used to assess statistical significance (*p<0.05, **p<0.01, ***p<0.001).

Extended Data Figure 6: *Rpl24*$^{Bst/+}$ and *Pten*-deficient progenitors form colonies with normal cellularity but *Rpl24*$^{Bst/+}$ impairs the development of haematopoietic neoplasms after *Pten* deletion. a,b, The percentage of HSCs (a) or bone marrow cells (b) that formed colonies in methylcellulose within 14 days of culture (n=3 mice/genotype in 3 independent experiments with 16 HSCs or 3200 bone marrow cells tested/mouse/experiment). c,d, The average number of cells per GM or GEMM colony derived from single HSCs (c) or bone marrow cells plated at clonal density (d) (n=4 independent experiments). e, The average number of cells per GM or GEMM colony derived from individual HSCs of the indicated genotypes 15 days after plating (n=2 independent experiments). f, Frequency of Annexin V$^+$ CD4$^+$CD8$^+$ thymocytes (n=5 independent experiments). g, Representative histograms of OP-Puro fluorescence in HSCs of the indicated genotypes. h, Mass of spleens and thymuses 2 weeks after pIpC administration (n=7 independent experiments). i, Representative photographs of thymuses 2 weeks after pIpC administration to wild-type, *Mx1-Cre; Pten*$^{fl/fl}$, *Rpl24*$^{Bst/+}$, and *Mx1-Cre; Pten*$^{fl/fl}$; *Rpl24*$^{Bst/+}$ mice. j, HSCs in the spleen 2 weeks after pIpC administration (n=7 independent experiments). k, Haematoxylin and eosin stained spleen and thymus sections from mice 2 weeks after pIpC administration or when they were sacrificed due to illness. All data represent mean±s.d. In panels a-d and f, two-tailed Student's t-tests were used to assess statistical significance relative to wild-type, *p<0.05, **p<0.01. To assess statistical significance in panel e we performed a one

way ANOVA followed by Tukey's t-tests for multiple comparisons (relative to wild-type *, p<0.05; ***, p<0.001 and relative to *p53*$^{+/-}$ #, p<0.05; ##, p<0.01). To compare the statistical significance of differences among genotypes in panel h and j we performed a one way ANOVA followed by Dunnett's test for multiple comparisons relative to *Pten*-deficient (**, p<0.01; ***, p<0.001).

Extended Data Figure 7: *Rpl24*$^{Bst/+}$ and *Pten*-deficient MPPs have relatively normal reconstituting activity. a,b, 100 donor CD150^{-}CD48^{-}LSK MPPs from *Mx1-Cre; Pten*$^{fl/fl}$ versus control mice (a; n=3 independent experiments with a total of 13 recipients/genotype) or *Rpl24*$^{Bst/+}$ versus control mice (b; n=3 independent experiments with a total of 14 recipients of wild-type cells and 13 recipients of *Rpl24*$^{Bst/+}$ cells) were transplanted along with 3x10^{5} recipient-type bone marrow cells into irradiated recipient mice. Donor cell engraftment levels in the peripheral blood were assessed at 3, 5, and 7 weeks after transplantation. All data represent mean±s.d. Two-tailed Student's t-tests were used to assess statistical significance relative to wild-type, *p<0.05.

EXTENDED DATA REFERENCES

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