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SUPPLEMENTARY INFORMATION

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Cell-type-specific quantification of protein synthesis in vivo

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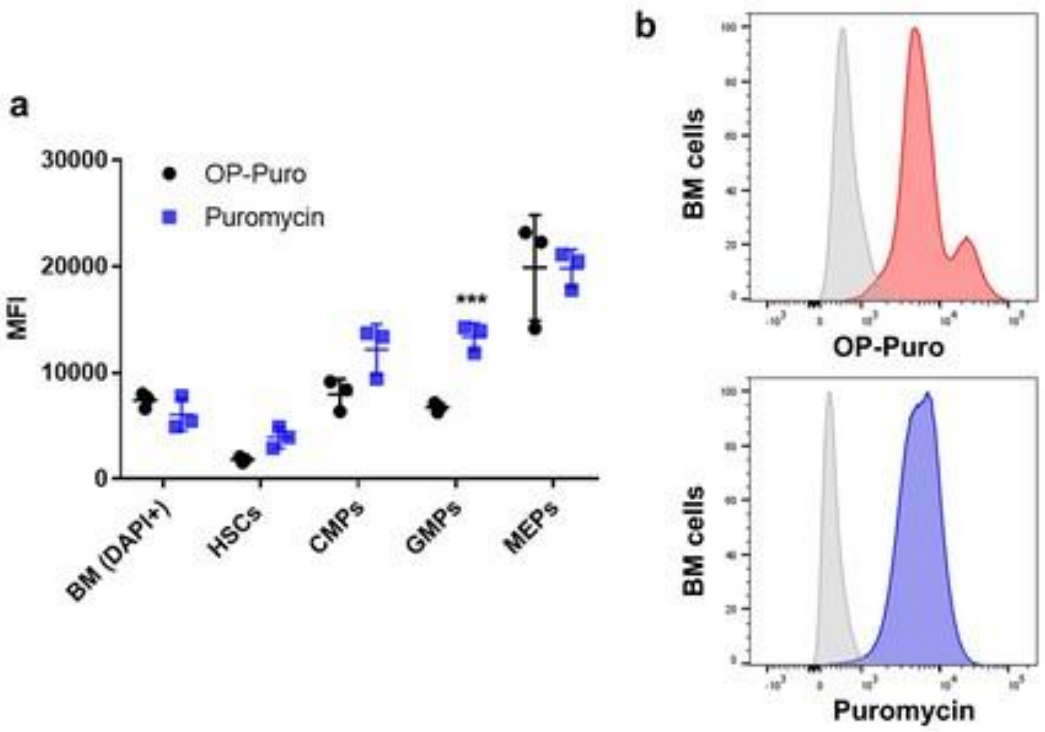
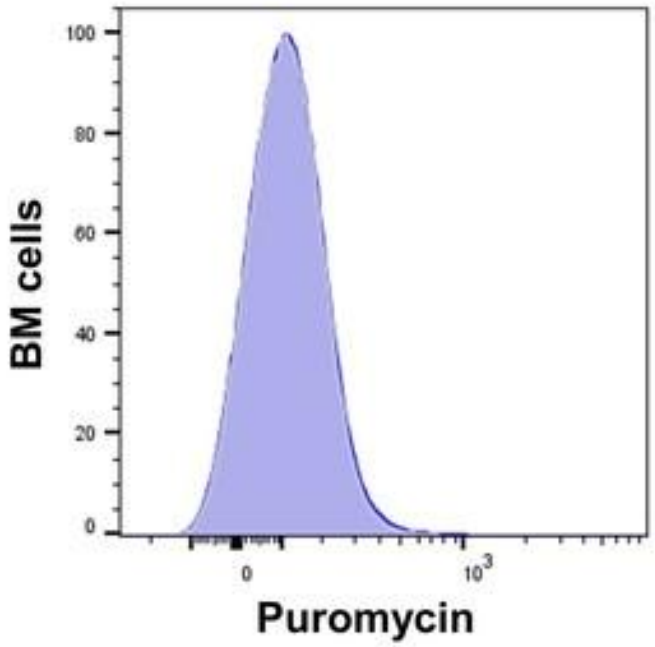


Supplementary Figure 1

Comparison between OP-Puro and anti-puromycin antibody mediated detection of protein synthesis within mouse bone marrow cells in vivo.

a, MFI of OP-Puro (black) and Puromycin (blue) in unfractionated bone marrow cells, HSCs, CMPs, GMPs, and MEPs (n = 3 for each method). Background fluorescence has been subtracted from each population. Data represent mean ± standard deviation. Statistical significance between methods within each cell type was assessed by Student's t-test.; ***P<0.001. b, Representative histograms show OP-Puro incorporation (red) and puromycin incorporation (blue) in unfractionated bone marrow cells compared to unfractionated bone marrow cells from a PBS injected control mouse (gray). All procedures in this protocol involving mice were approved by the UC San Diego Institutional Animal Care and Use Committee.



Supplementary Figure 2

The SUnSET method cannot be applied to measure protein synthesis *in vivo*.

A representative histogram shows the inability to detect puromycin on the cell surface of unfractionated bone marrow cells (blue). Unfractionated bone marrow cells from a PBS injected control mouse are shown in gray. All procedures in this protocol involving mice were approved by the UC San Diego Institutional Animal Care and Use Committee.

Supplementary Table 1. Absolute numbers of each population analyzed in the HSC samples

Sample	Events	Cells (DAPI$^+$)	CD150$^+$CD48$^-$Lin$^-$	Sca-1$^+$ckit$^+$ (HSCs)
1	1010438	1002022	793	72
2	1006636	997654	297	102
3	1177230	997191	120	90
4	1001144	995282	105	77
5	1009741	987164	113	77

Supplementary Table 2. Absolute numbers of each population analyzed in the CMP/GMP/MEP samples

Sample	Events	Cells (DAPI+)	ckit$^+$Lin$^-$ CD127$^-$	ckit$^+$Sca-1$^-$	CD34$^+$CD16/32$^{-/low}$ (CMPs)	CD34$^+$CD16/32^{high} (GMPs)	CD34$^-$CD16/32$^{-/low}$ (MEPs)
1	395431	391728	2320	1620	562	799	156
2	393526	378851	2453	1795	633	762	249
3	393846	388581	2766	2004	503	1118	234
4	425000	337415	1832	1346	481	562	199
5	398234	361318	1996	982	262	428	209

Supplementary Table 3. Frequencies related to parent (%) of each population analyzed in the HSC samples.

Sample	Events	Cells (DAPI$^+$)	CD150$^+$CD48$^-$Lin$^-$	Sca-1$^+$ckit$^+$ (HSCs)
1	83.6	99.2	0.079	9.08
2	80.6	99.1	0.030	34.3
3	85.3	99.3	0.012	75.0
4	83.5	99.4	0.011	73.3
5	83.2	97.8	0.011	68.1

Supplementary Table 4. Frequencies related to parent (%) of each population analyzed in the CMP/GMP/MEP samples.

Sample	Events	Cells (DAPI+)	ckit$^+$Lin$^-$ CD127$^-$	ckit$^+$Sca-1$^-$	CD34$^+$CD16/32$^{-/low}$ (CMPs)	CD34$^+$CD16/32^{high} (GMPs)	CD34$^-$CD16/32$^{-/low}$ (MEPs)
1	85.0	99.1	0.59	69.8	34.7	49.3	9.63
2	83.2	96.3	0.65	73.2	35.3	42.5	13.9
3	85.5	98.7	0.71	72.5	25.1	55.8	11.7
4	82.2	79.5	0.54	73.5	35.7	41.8	14.8
5	82.6	90.7	0.55	49.2	26.7	43.6	21.3