Supplementary information

Mouse adult hematopoietic stem cells actively synthesize ribosomal RNA

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Running title: Ribosome biogenesis in HSCs

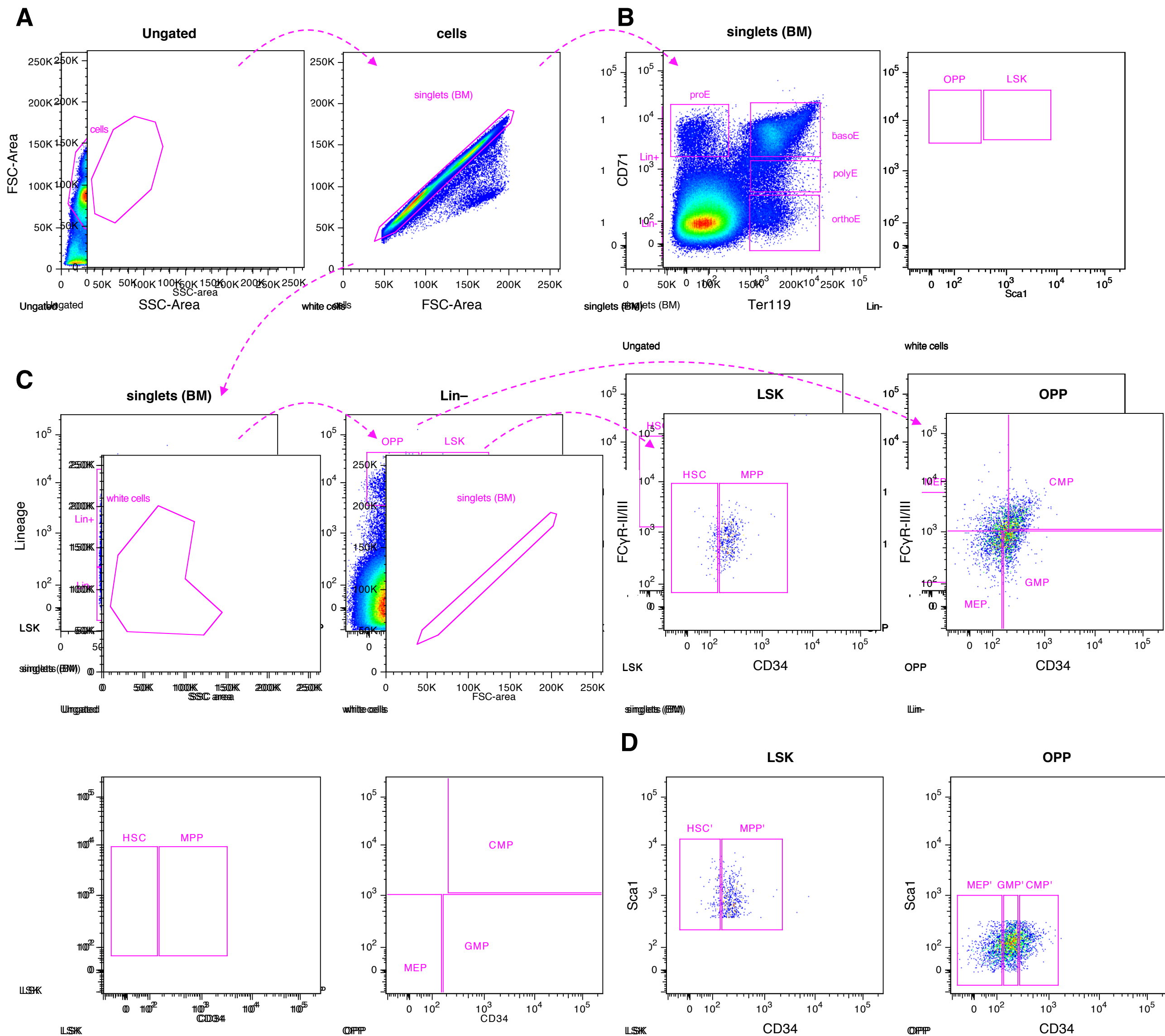

Figure S1: Gating strategies for flow cytometry analyses

A. For all flow cytometry analyses, cell debris and doublets were eliminated using forward and side scatter plots as indicated. **B.** For analysis of the erythroid lineage, singlets (BM) were plotted using CD71 and Ter119 and the indicated gate were drawn to separate pro-erythroblasts (proE;), CD71^{+} Ter119^{low}), basophilic erythroblasts (basoE; CD71^{+} Ter119^{+}), late basophilic and polychromatophilic erythroblasts (polyE; CD71^{low} Ter119^{+}) and orthochromatophilic erythroblasts (orthoE; CD71^{-} Ter119^{+}). **C.** For analyses on the immature hematopoietic populations, singlets (BM) were plotted using Lineage markers and forward-scatter to separate Lin^{+} and Lin^{-} cells. Lin- cells were then plotted using Sca1 and cKit to separate Lin^{-} Sca1^{-} cKit^{+} oligopotent progenitors (OPP) and Lin^{-} Sca1^{+} cKit^{+} (LSK) population enriched for stem cells and multipotent progenitors. HSC and MPP were further isolated from the LSK population based on CD34 expression while CMP, GMP and MEP were isolated from the OPP population using FCγR-II/III and CD34 expression. **D.** For Flow-FISH experiments, FCγR-II/III couldn't be used and CMP, GMP and MEP populations were defined based on CD34 expression as indicated.

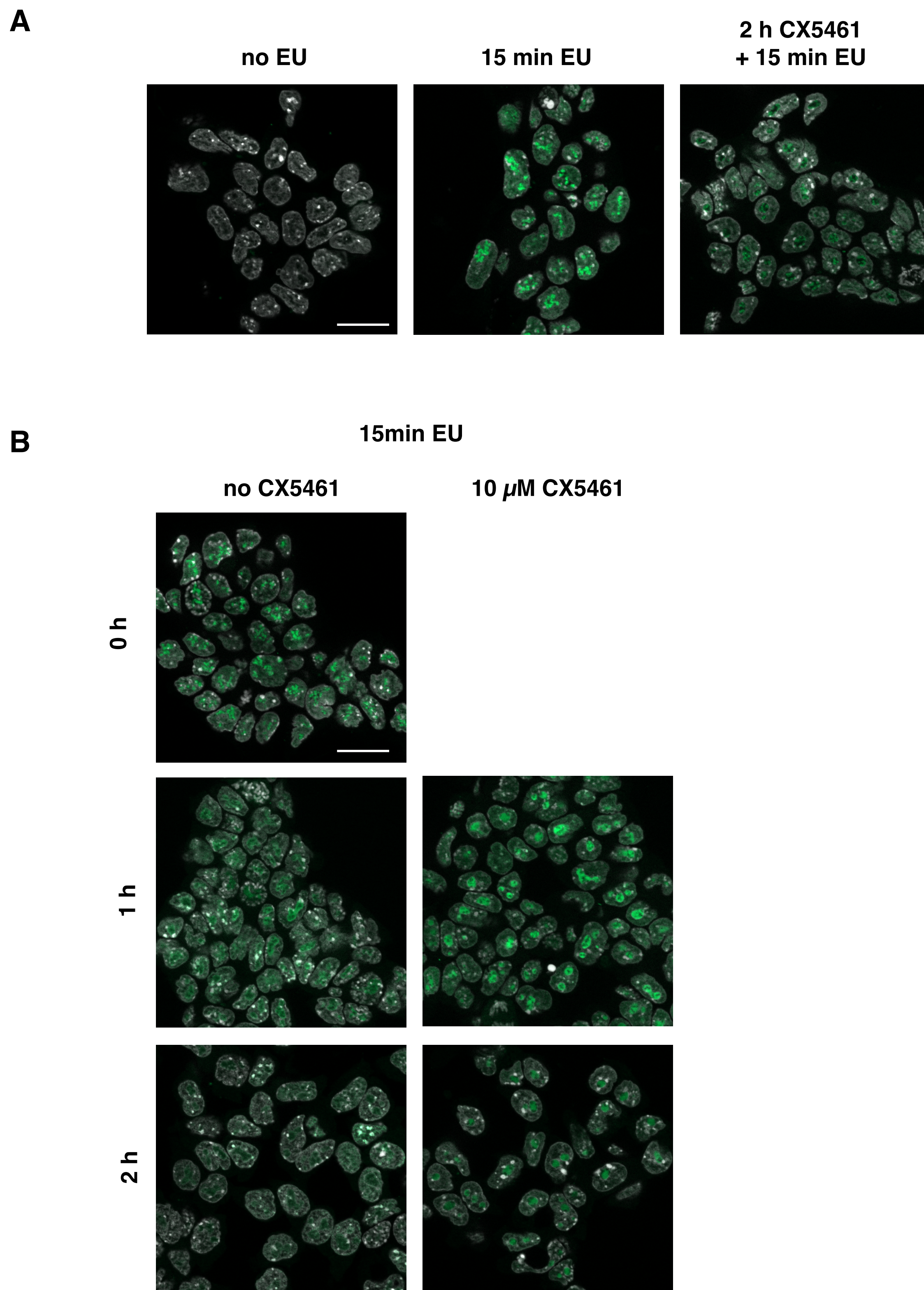

Figure S2: CX-5461 treatment blocks processing of previously synthesized rRNA
A. Embryonic stem cells were incubated in the presence or absence of 10 μM CX-5461 for 2h, and then incubated for 15 min in the presence or absence of 1 mM EU. Cells incubated with neither compound were used as controls. **B.** Embryonic stem cells were incubated in the presence of 1 mM EU for 15 min, and were then incubated in the presence or absence of 10 μM CX-5461 up to 2h. Cells were analyzed at the indicated time-points after EU incubation. EU staining (green) was revealed using Click-iT chemistry, and nuclei were stained with Hoechst (white). Bar: 10 μm.

Antibody information			
Antigen	**Fluorochrome**	**Clone**	**Supplier**
CD71	Biotin	C2	BD Biosciences
B220	Biotin	RA3-6B2	BioLegend
Nk1.1	Biotin	PK136	BD Biosciences
Gr-1	Biotin	RB6-8C5	BD Biosciences
Ter119	Biotin	TER-119	BD Biosciences
CD3ε	Biotin	145-2C11	BD Biosciences
CD11c	Biotin	N418	BioLegend
Mac1	Biotin	M1/70	BioLegend
Sca-1	PeCy7	D7	BD Biosciences
Sca-1	BV510	D7	DB
cKit	APC	2B8	BioLegend
cKit	PeCy7	2B8	BioLegend
CD34	FITC	RAM34	eBioscience
CD34	eFluor660	RAM34	eBioscience
FCγR-II/III	PE	2.4G2	BD Biosciences
Streptavidin	Pacific Blue	–	Invitrogen
Puromycin	Alexa647	2A4	Seedhom et al., 2016

Table S1: Antibodies
List of antibodies used for surface marker staining and intra-cellular puromycin staining.

	Volume per well	Comments
Solution A		
Formamide	5µL	
2X SSC	2,5µL	
tRNA (10mg/mL)	2,5µL	SIGMA R1753
H2O (*QS* 21,25µL)	8,75µL	
FISH probe (50ng/µL)	2,5µL	
	final vol. 21,25µL	
Solution B		
4X SSC, 20% dextran sulfate	25µL	
BSA 10mg/mL	1,25µL	
VRC 200mM	2,5µL	SIGMA R3380
	final vol. 28,75µL	
Solution (A+B)	**final vol. 50µL**	heat solution A 5min at 95°C before mixing

Table S2: FISH staining buffer preparation
For preparation of FISH staining buffer, solutions A and B were prepared separately as indicated. Solution A was heated for 5min at 95°C, and mixed with solution B for stainings.

Supplementary References

Seedhom, M. O., Hickman, H. D., Wei, J., David, A. and Yewdell, J. W. (2016). Protein Translation Activity: A New Measure of Host Immune Cell Activation. The Journal of Immunology 197, 1498–1506.