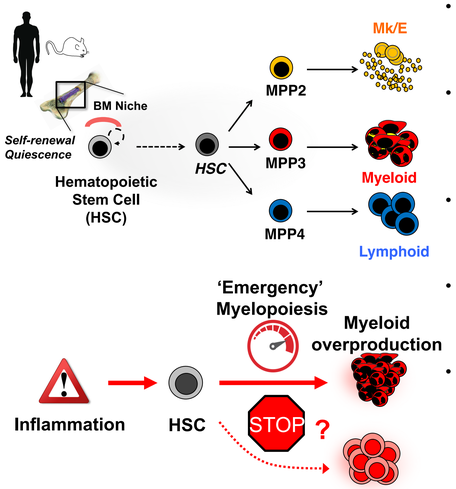
PU.1 enforces hematopoietic stem cell quiescence during chronic inflammation

James S. Chavez[1], Jennifer L. Rabe[1], Kelly C. Higa[2], Dirk Loeffler[3], Nouraiz Ahmed[3], Taylor S. Mills[1], Rachel L. Gessner[1], Zhonghe Ke[1], Beau M. Idler[1], Brett M. Stevens[1], Courtney Fleenor[4], Hideaki Nakajima[5], James Hagman[4], Hyunmin Kim[6], Timm Schroeder[3], Craig T. Jordan[1], James DeGregori[2], Eric M. Pietras[1]

[1]Division of Hematology; [2]Department of Biochemistry and Molecular Genetics; [6]Division of Medical Oncology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA; [3]Cell Systems Dynamics, ETH Zurich, Basel, Switzerland; [4]Department of Biomedical Research, National Jewish Health, Denver, CO, USA; 5Department of Immune and Stem Cell Regulation, Yokohama City University, Yokohama, Japan.

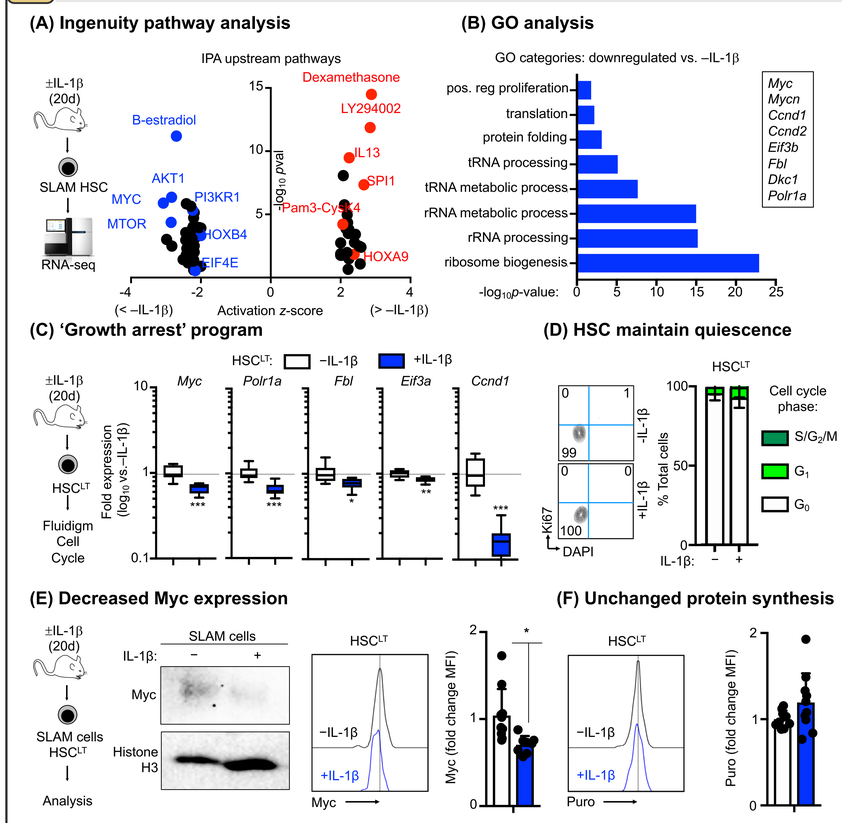
Introduction



- **Hematopoietic stem cell (HSC) quiescence promotes life-long blood output.**
- **HSC directly sense and respond to pro-inflammatory cytokines such as IL-1 by increasing myeloid output.**
- **However, HSC maintain quiescence during chronic inflammation.**
- **Aging and chronic inflammation are associated with leukemogenesis.**
- ***We hypothesize IL-1 triggers a 'braking' mechanism in HSC, thereby limiting their aberrant proliferation and expansion during chronic inflammation.***
- ***We propose HSC unable to engage this mechanism will outcompete 'less fit' HSC, ultimately promoting leukemogenesis.***

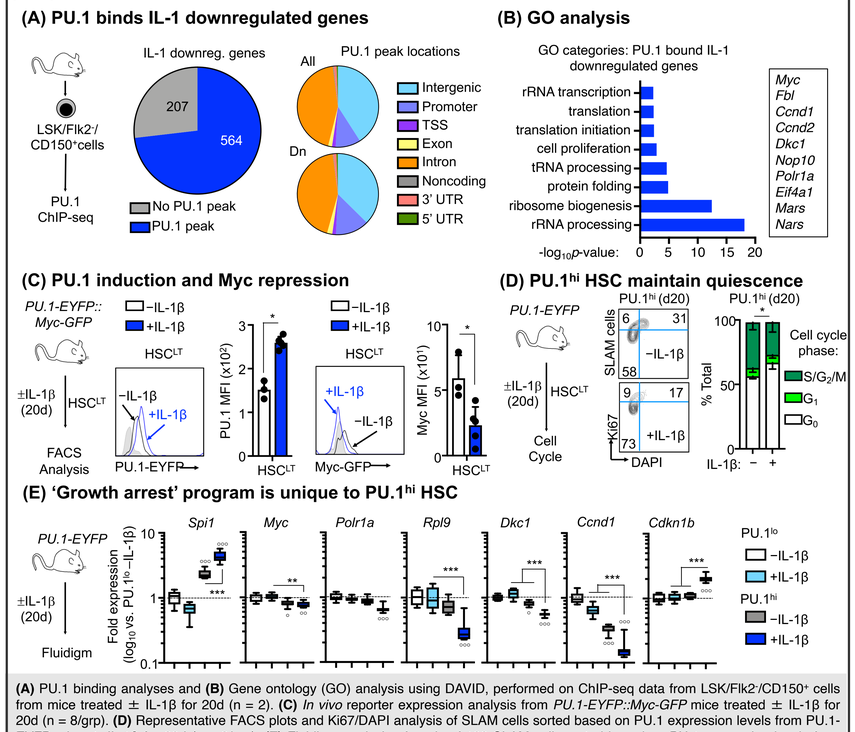
Results

1 IL-1 activates 'growth arrest' program in HSC



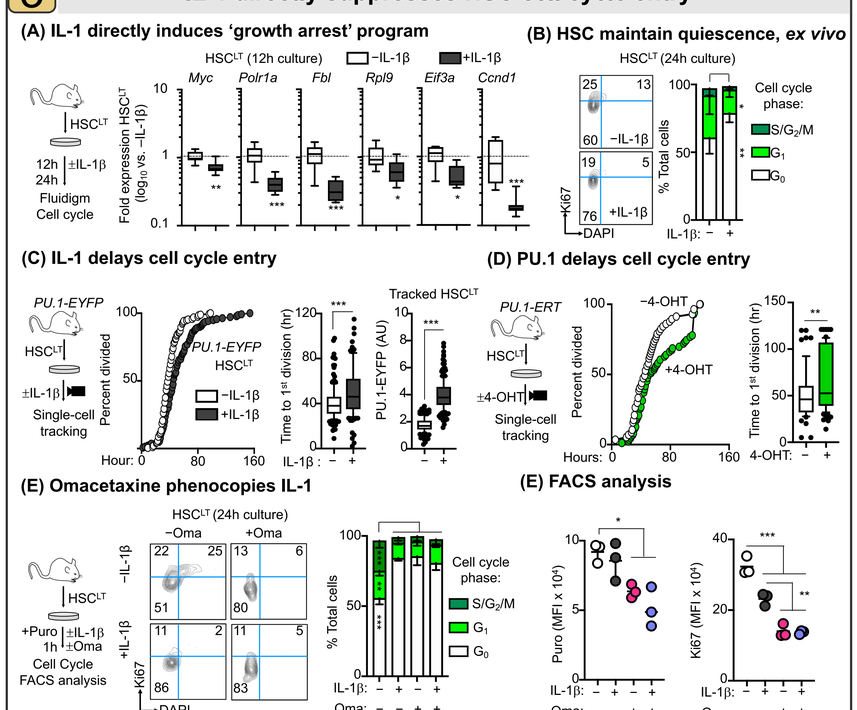
(A) Ingenuity pathway analysis (IPA) upstream regulators analysis and **(B)** Gene ontology (GO) analysis using DAVID, performed on RNA-seq data from SLAM HSC from mice treated ± IL-1β for 20d (n = 3-7/grp). **(C)** Fluidigm analysis of pools of 100 HSC^{LT} from mice treated ± IL-1β for 20d (n = 8/grp). **(D)** Representative FACS plots and Ki67/DAPI analysis of HSC^{LT} from mice treated ± IL-1β for 20d (n = 5/grp). **(E)** Myc protein expression in SLAM cells quantified by western blot (n = 5/grp) and in HSC^{LT} quantified by intracellular flow from mice treated 20d ± IL-1β (n = 9-10/grp). *p<0.05, **p<0.01, ***p<0.001 by Mann-Whitney u-test or ANOVA.

2 PU.1 induction is associated with 'growth arrest' program



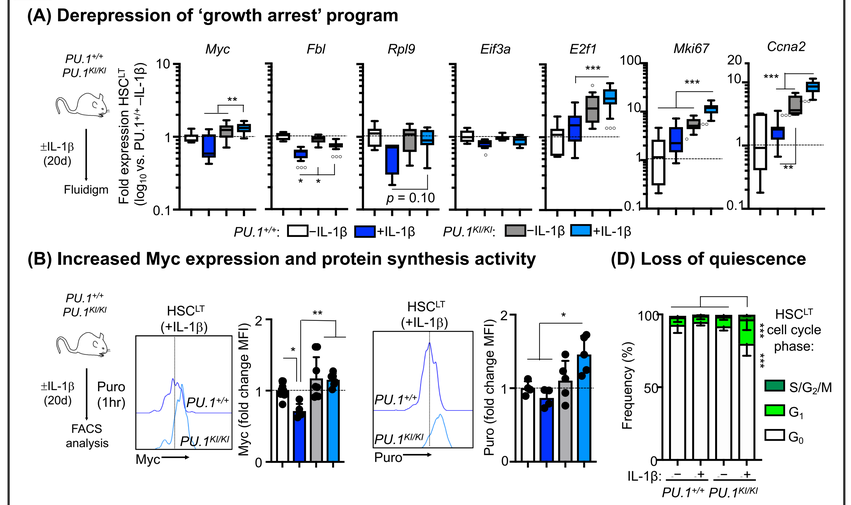
(A) PU.1 binding analyses and **(B)** Gene ontology (GO) analysis using DAVID, performed on ChIP-seq data from LSK/Flk2-/CD150+ cells from mice treated ± IL-1β for 20d (n = 2). **(C)** *In vivo* reporter expression analysis from *PU.1-EYFP::Myc-GFP* mice treated ± IL-1β for 20d (n = 8/grp). **(D)** Representative FACS plots and Ki67/DAPI analysis of SLAM cells sorted based on PU.1 expression levels from PU.1-EYFP mice ± IL-1β for 20d (n = 3/grp). **(E)** Fluidigm analysis of pools of 100 SLAM cells sorted based on PU.1 expression levels from *PU.1-EYFP* mice treated ± IL-1β for 20d (n = 8/grp). *p<0.05, **p<0.01, ***p<0.001 by Mann-Whitney u-test or ANOVA.

3 IL-1 directly suppresses HSC cell cycle entry



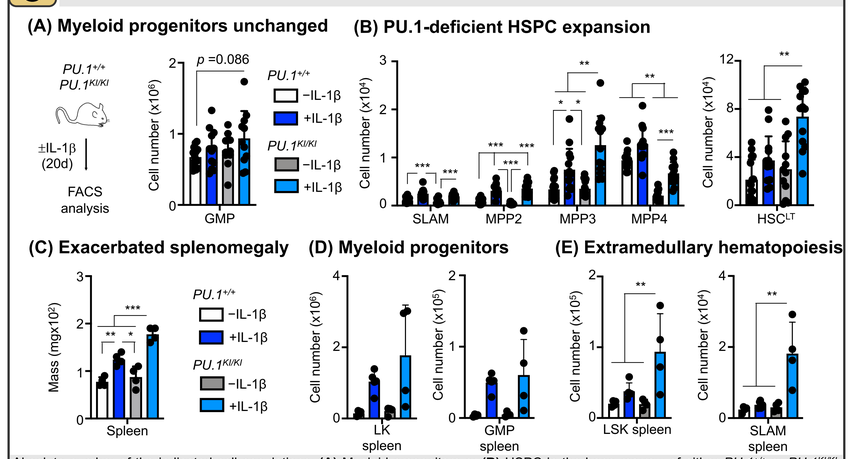
(A Fluidigm analysis of pools of purified 100 HSC^{LT} cultured ± IL-1β for 12h (n = 8/grp). **(B)** FACS plots and Ki67/DAPI analysis of purified HSC^{LT} cultured in ± IL-1β for 24h (n = 6/grp). **(C)** Division kinetics and PU.1 expression of HSC^{LT} cultured in ± IL-1β (n = 194-139/grp). **(D)** Division kinetics of HSC^{LT} cultured ± 4-OHT (n = 57-83/grp). **(E)** Ki67/DAPI analysis or **(F)** Ki67 and puromycin MFI of HSC^{LT} cultured in ± IL-1β and ± Omacetaxine (100nM) for 24h (n = 3/grp) *p<0.05, **p<0.01, ***p<0.001 by Mann-Whitney u-test or ANOVA.

4 Derepression of 'growth arrest' program in PU.1-deficient HSC



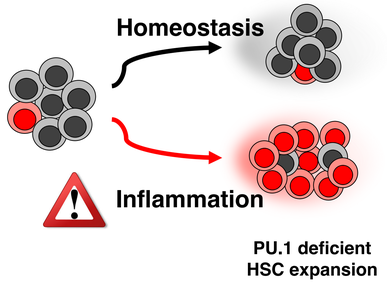
(A) Fluidigm analysis of pools of 100 HSC^{LT} from either *PU.1*$^{+/+}$ or *PU.1*$^{KI/KI}$ mice treated ± IL-1β for 20d (n = 8/grp). Representative FACS plots of HSC^{LT} and either **(B)** intracellular Myc (left) and protein synthesis rates (right) from *PU.1 +/+* or *PU.1 KI/KI* mice treated ± IL-1β for 20d (n = 5-7/grp). **(D)** Ki67/DAPI analysis of HSC^{LT} from either *PU.1*$^{+/+}$ or *PU.1*$^{KI/KI}$ mice treated 20d ± IL-1β (n = 5/grp). *p<0.05, **p<0.01, ***p<0.001 by Mann-Whitney u-test or ANOVA.

5 IL-1 induces expansion of PU.1-deficient HSPC



Absolute number of the indicated cell populations: **(A)** Myeloid progenitors or **(B)** HSPC in the bone marrow of either *PU.1*$^{+/+}$ or *PU.1*$^{KI/KI}$ mice treated ± IL-1β for 20d (n = 13-18/grp). **(C)** Weight of spleens from *PU.1*$^{+/+}$ or *PU.1*$^{KI/KI}$ mice treated ± IL-1β for 20d (n = 4-5/grp). Absolute number of the indicated cell populations: **(D)** myeloid progenitors and **(E)** HSPC in the spleen of mice from (C). *p<0.05, **p<0.01, ***p<0.001 by Mann-Whitney u-test or ANOVA.

Conclusions



- ***HSC engage a 'growth arrest' program during chronic inflammation.***
- ***The 'growth arrest' program is associated with induction of the myeloid transcription factor PU.1.***
- ***IL-1 directly activates the 'growth arrest' program in HSC cultured ex vivo.***
- ***IL-1 triggers derepression of 'growth arrest' program in PU.1 deficient HSC leading to their expansion.***
- ***Our data suggest a mechanism where PU.1 guards HSC from aberrant expansion during chronic inflammation and HSC unable to engage 'braking' mechanism gain a fitness advantage.***

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