

Chaperone-mediated autophagy sustains hematopoietic stem cell function

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Supplementary Discussion

Discussion

Other components of the proteostasis network have been reported to contribute to HSC homeostasis and function^{1,2}. Metabolic deregulation with massive accumulation of lipid droplets and increased glycolysis occurs upon macroautophagy blockage in neutrophil precursors³. This metabolic phenotype is in clear contrast with the reduced glycolysis observed in CMA-deficient HSC. In further support that both autophagic pathways are non-redundant, loss of CMA in HSC cannot be compensated for by macroautophagy in these cells. Different from other cell types (i.e. fibroblasts, hepatocytes) that respond to CMA blockage with an upregulation of macroautophagy activity to preserve cellular proteostasis^{4,5}, HSC fall in the group of cells (also including retinal cells or activated T cells^{6,7}) in which macroautophagy activity remains unaltered upon CMA blockage. Differences in timing, signaling pathways and substrate recognition may be responsible for the inability of these proteolytic systems to compensate for each other in HSC.

As expected for a proteolytic system, the consequences of CMA failure in HSC may go beyond the changes in lipid metabolism reported in this work. Our comparative proteomic analysis demonstrate that CMA-deficient cells are unable to carry out the overall proteome remodeling required during the process of activation. Failure to timely modulate levels of proteins involved in processes such as cell cycle, cytoskeletal organization, or in the regulation of other proteostasis components, are likely to contribute to the functional impairment of HSC. The selectivity and timing of CMA degradation is likely behind the cell type-specific functions described for this type of autophagy. For example, CMA facilitates T cell activation by directly degrading proteins that actively repress T cell activation⁶, whereas here we uncovered that CMA partakes in regulation of the metabolic state of HSC upon activation. Interestingly, the mechanisms and metabolic pathways regulated by CMA appear also to be cell type and context specific as, for example, CMA participates in maintenance of pluripotency of embryonic stem cells also through metabolic

changes but in that case by limiting α -ketoglutarate production, which ultimately affects activity of DNA demethylases involved in pluripotency⁸.

Supplementary References

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Fig. 3h

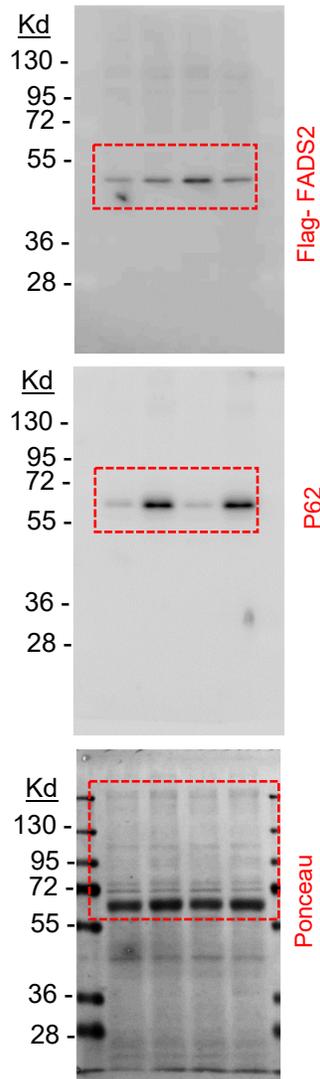
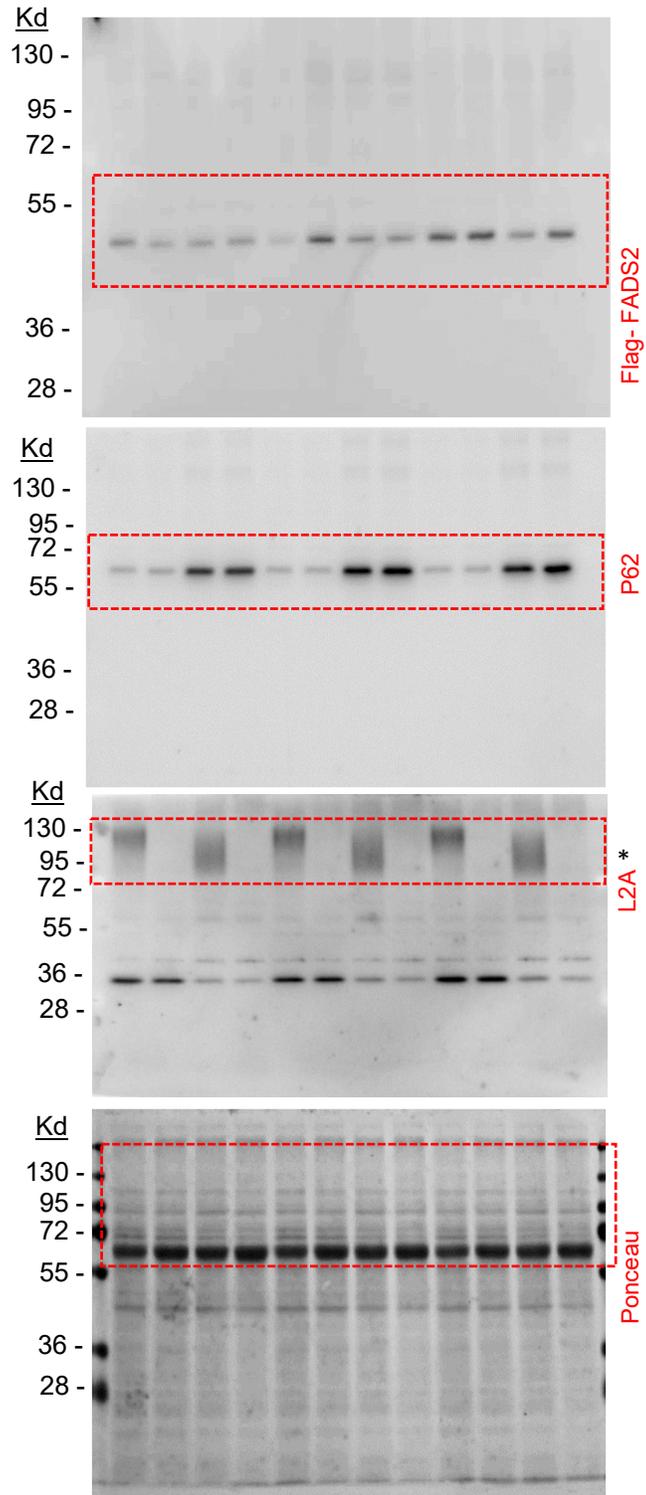
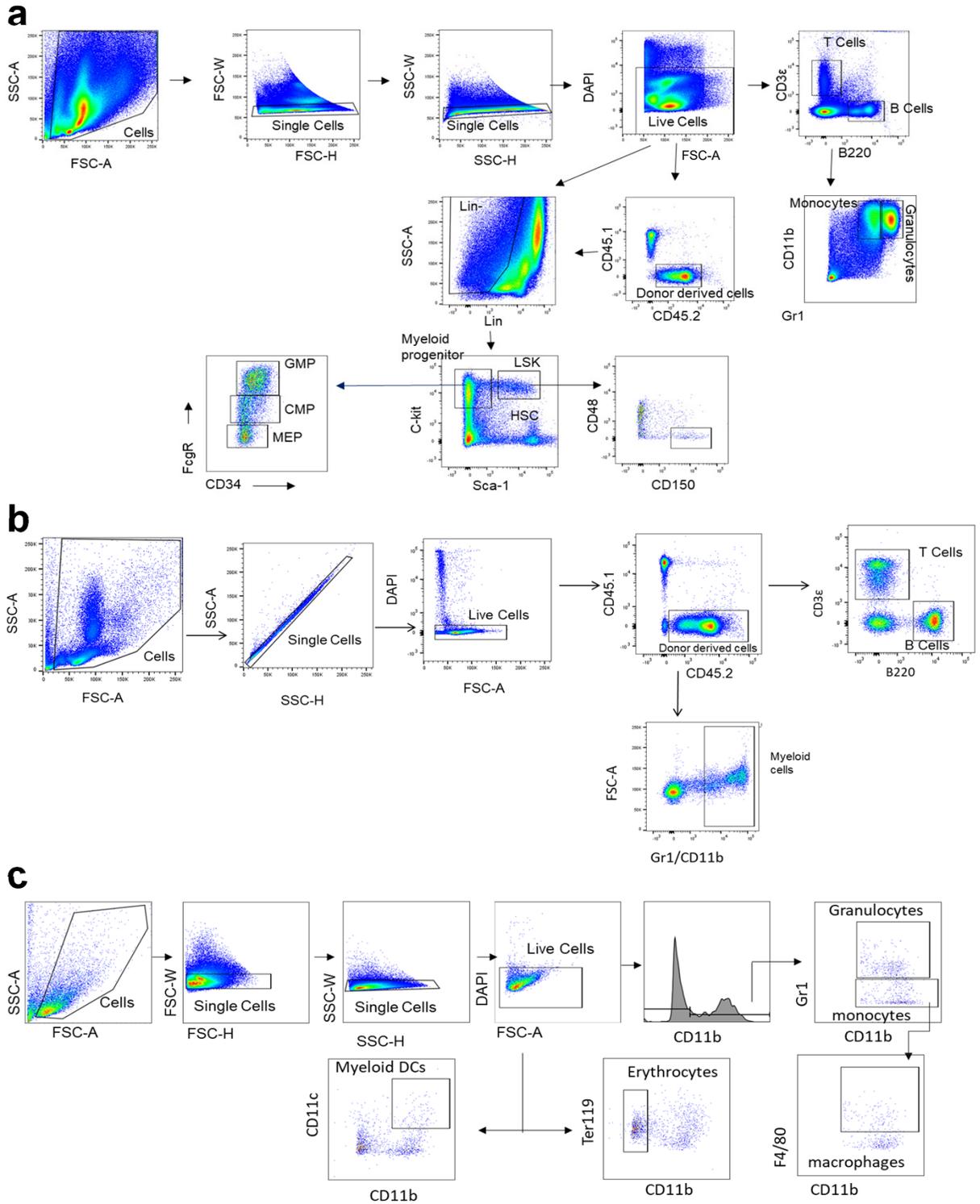


Fig. 3i



Supplementary Fig. 1. Uncropped gels from Main Fig. 3. Molecular weight markers are indicated on the side. Ponceau staining of the transferred membrane was used as control for even protein loading per lane. Dotted red boxes mark cropped areas shown in the main figure.



Supplementary Fig. 2. Gating strategies used in main and Extended Data figures. a, Gating strategy in bone marrow. LSK gating were used in Fig. 2g-r, Fig. 3a-d,g,j,k,n-o, Fig. 4d-g,j-m, Fig. S3e,l, Fig. S6b-d,i-l, Fig. S7a-l, Fig. S9j. Lin- gating were used in Fig. S3d. Myeloid progenitor gating were used in Fig. 1c, Fig. S2f-l, 4b-h, Fig. S6f. GMP gating were used in Fig. S1c. T cells, B cells, monocytes and granulocytes in bone marrow gating were used in Fig. S3c. HSC and Lin- population in transplanted recipient mice bone marrow gating were used in Fig. 1h, 2d, Fig. S3h. HSC in mice bone marrow gating were used in all the other panels. **b**, Gating strategy for donor derived total cells, T cells, B cells and Myeloid cells from peripheral blood of the transplanted recipient mice were used in Fig. 1i, 4h, S3g, S3i-k, S8b-4c **c**, Gating strategy for cells recovered from colonies at the end of LTC-IC: Granulocytes, monocytes, macrophages, erythrocytes and myeloid Dendritic cells (DCs) in Fig. S9o.