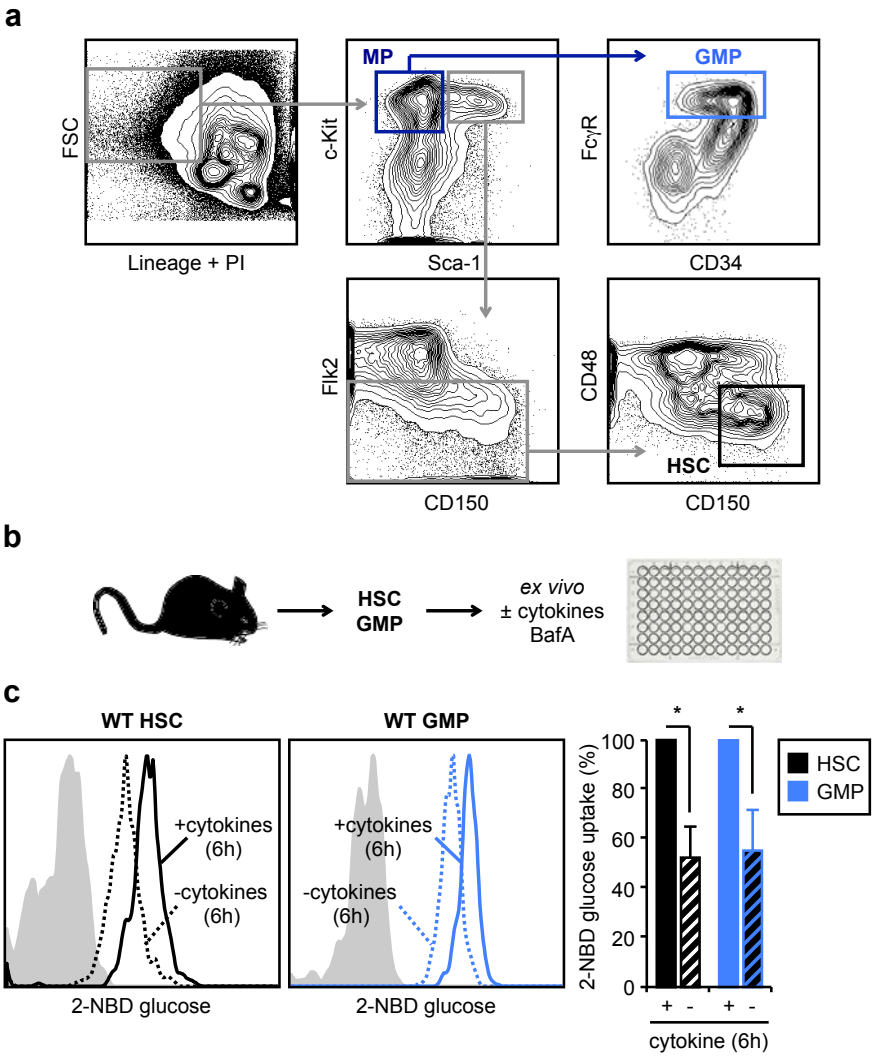
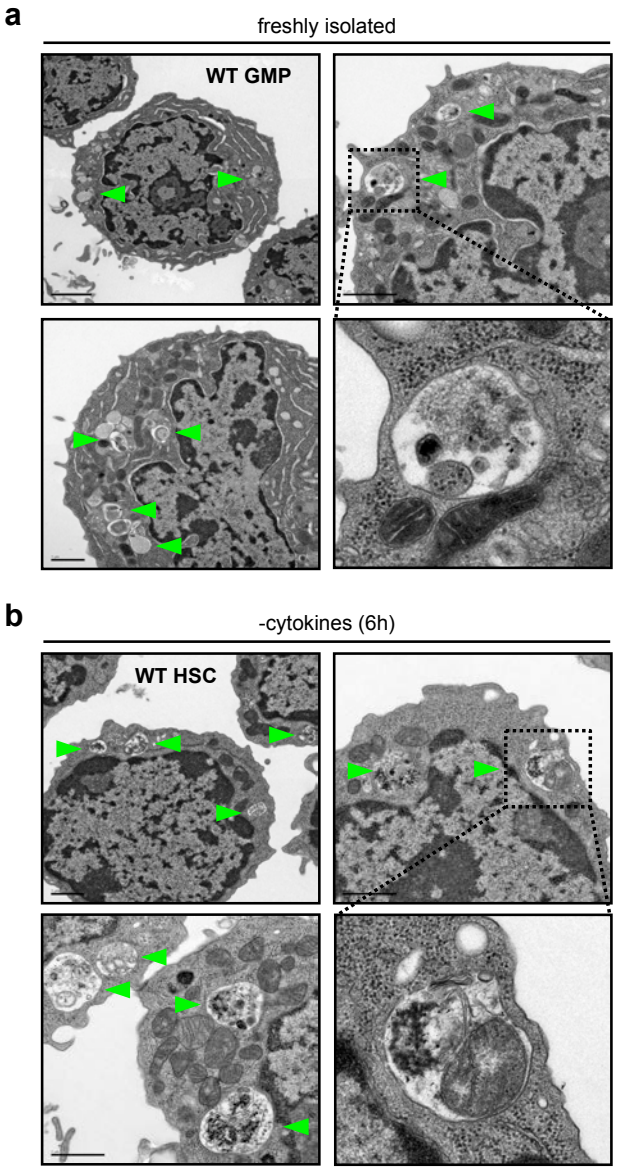
Supplementary Information

FoxO3a Directs a Protective Autophagy Program in Hematopoietic Stem Cells

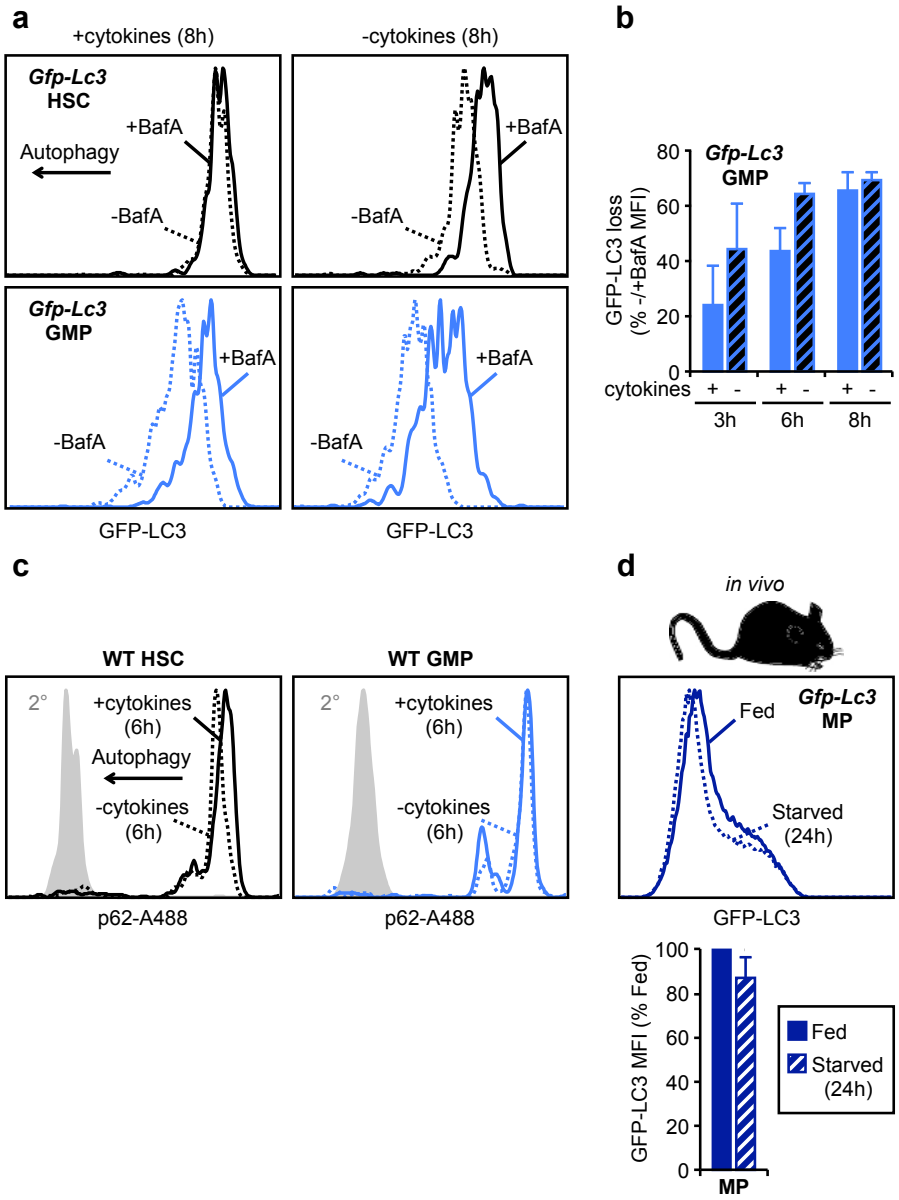
Warr, M.R., Binnewies, M., Flach, J., Garg, T., Malholtra, R., Debnath, J. & Passegué, E.



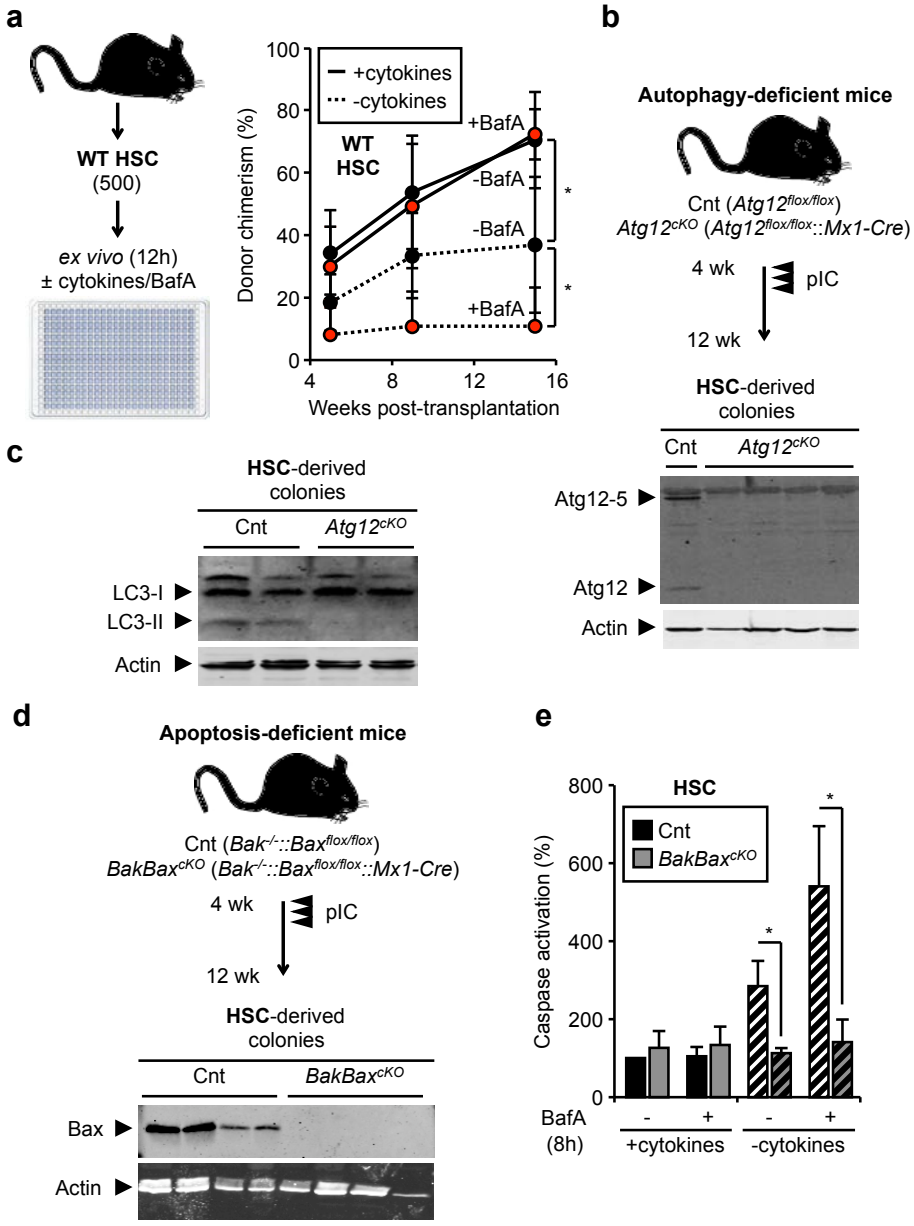
Supplementary Fig. 1 | Experimental strategy and evidence of metabolic stress following cytokines withdrawal in HSCs and GMPs. a, Gating strategy used to isolate HSCs ($Lin^-/Sca\text{-}1^+/c\text{-}Kit^+/Flk2^-/CD150^+/CD48^-$, black) and GMPs ($Lin^-/Sca\text{-}1^-/c\text{-}Kit^+/Fc\gamma R^+/CD34^+$, light blue). The bulk myeloid progenitor (MP) gate ($Lin^-/Sca\text{-}1^-/c\text{-}Kit^+$, dark blue) is also indicated. The example shows staining of unfractionated young adult C57Bl/6 wild type (WT) bone marrow cells. **b**, Experimental strategy to assess autophagy levels in purified HSCs and GMPs cultured *ex vivo* with or without (±) cytokines and BafA. **c**, Representative histograms and quantification of 2-NBD glucose uptake in HSCs and GMPs cultured for 6h ± cytokines (n = 3). Results are expressed as percentage of 2-NBD glucose uptake in -cytokines compared to +cytokines (set to 100%) conditions. Grey shades indicate the fluorescence level of cells cultured for 6h -2-NBD glucose, and hatching -cytokines conditions. All data represent mean ± s.d. $*P \leq 0.05$.



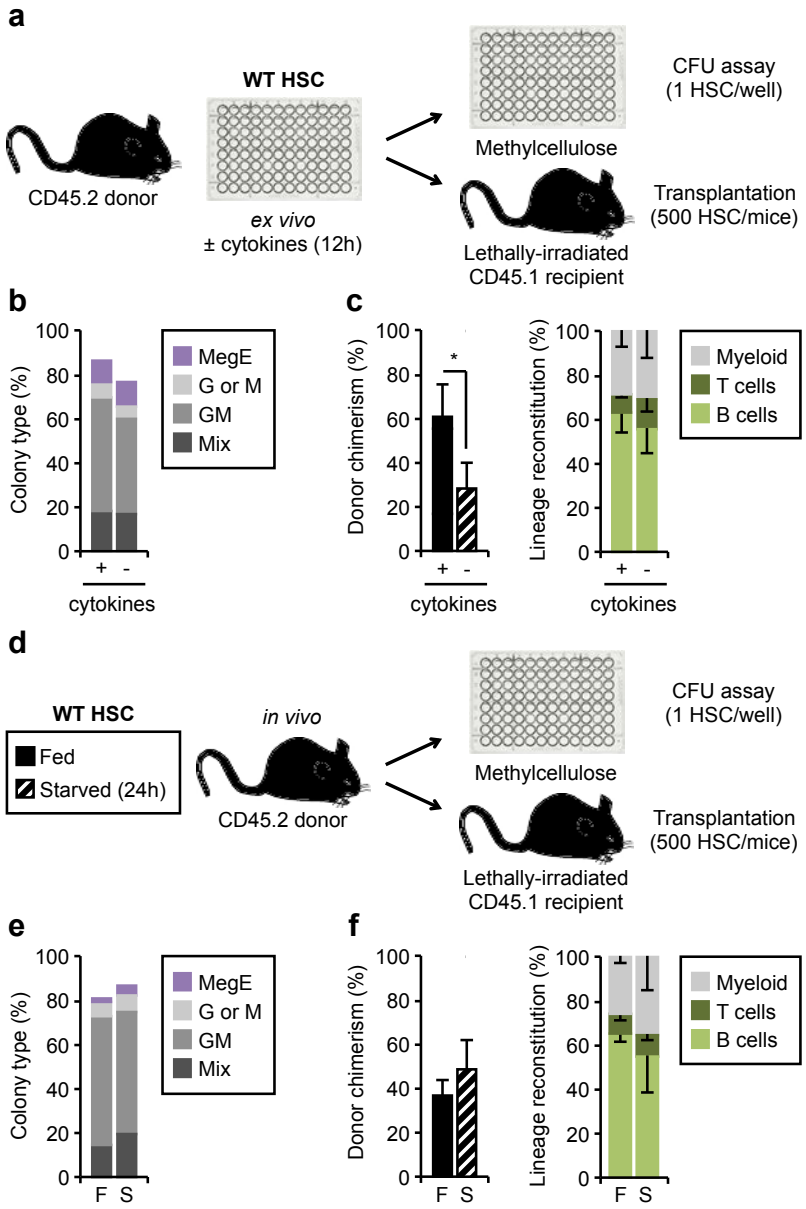
Supplementary Fig. 2 | Autophagy in GMPs and starved HSCs. a, **b**, Additional electron microscopy images showing autophagic vesicles (arrowheads) in freshly isolated WT GMPs (**a**) and HSCs cultured for 6h without (-) cytokines (**b**).



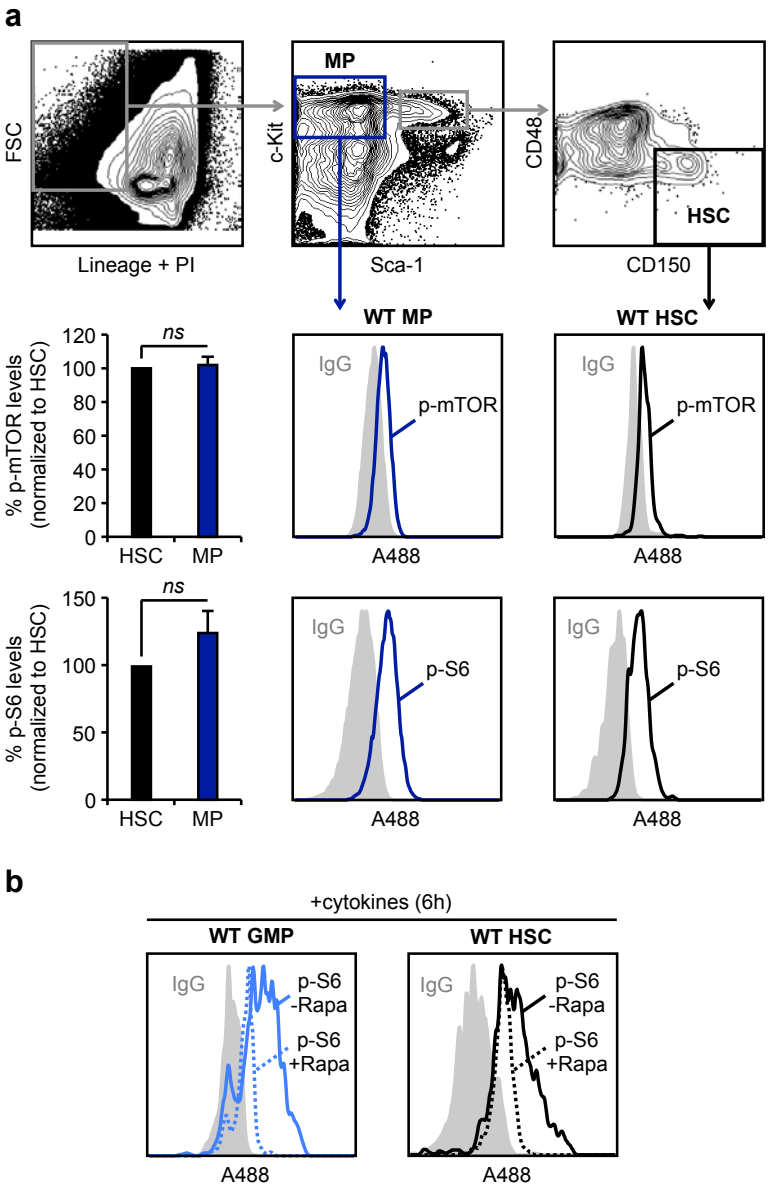
Supplementary Fig. 3 | Autophagy flux in HSCs and GMPs. a, Flow cytometry analyses of *Gfp-Lc3* HSCs (black) and GMPs (light blue) cultured for 8h with or without (±) cytokines and BafA. **b**, Quantification of GFP-LC3 loss in *Gfp-Lc3* GMPs cultured for 3h, 6h or 8h ± cytokines and BafA (n = 3). Results are expressed as percentage of GFP-LC3 mean intensity fluorescence (MFI) in -BafA compared to +BafA conditions. Hatching indicates -cytokines conditions. **c**, Intracellular flow cytometry analysis of p62 protein levels in WT HSCs and GMPs cultured for 6h ± cytokines. Cells stained with p62 specific antibodies followed by detection with an A488-conjugated secondary antibody, or with secondary alone (grey shade). **c**, Representative histograms and quantification of GFP-LC3 levels in the myeloid progenitor (MP, dark blue) compartment of *Gfp-Lc3* mice that have been fed or starved from food for 24h (n = 3). Results are expressed as percent of GFP-LC3 MFI in MPs of fed mice (set to 100%). All data represent mean ± s.d.



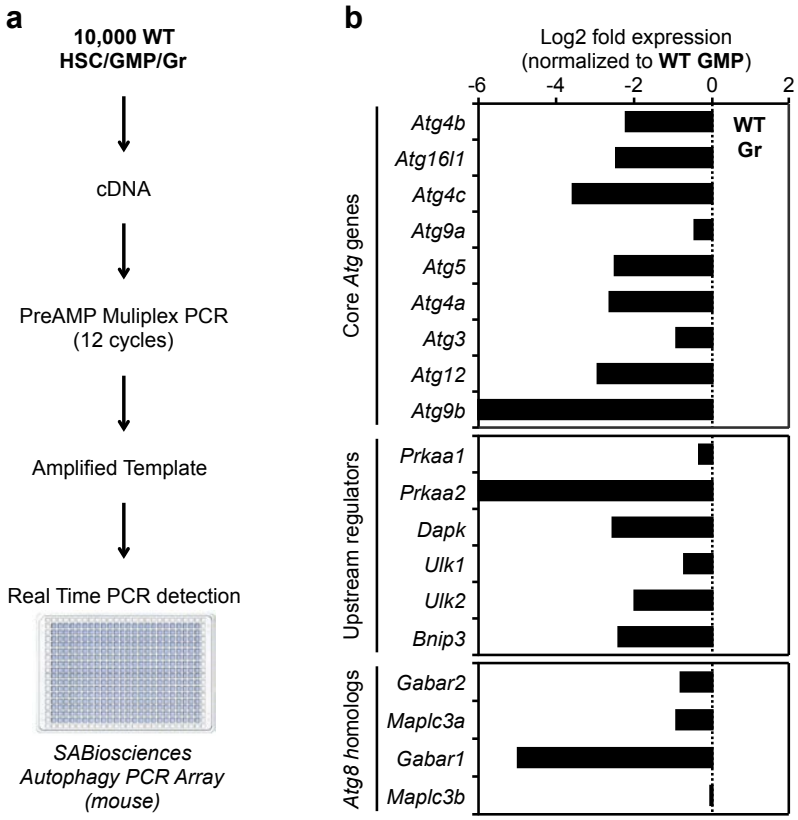
Supplementary Fig. 4 | *In vivo* validation and generation of autophagy- and apoptosis-deficient mice. a, Experimental strategy and donor-derived chimerism in the peripheral blood of recipient mice (CD45.1) transplanted with 500 HSCs (CD45.2) cultured for 12h with or without (±) cytokines and BafA (n = 5 mice per group). Red indicates +BafA conditions. **b**, **d**, Strategy to conditionally delete *Atg12* (**b**) and *Bak*/*Bax* (**d**) in adult HSCs. Floxed mice were crossed with *Mx1-Cre* mice to obtain hematopoietic-specific deletion following polyI/C (pIC) injections starting at 4 week of age. Mice were sacrificed for experiments on average 2 months post-pIC injection. **b**, **c**, **d**, Western blots performed on single HSC-derived colonies of the indicated genotypes grown for 7 days in methylcellulose. Immunoblots were probed with antibodies for murine Atg12 (**b**), LC3 (**c**) or Bax (**d**). Actin was used as a loading control. **e**, Apoptosis levels in control (Cnt) and *BakBax*^{cKO} HSCs cultured for 8h ± cytokines and BafA (n = 3). Results are expressed as percentage of caspase activation in Cnt HSCs cultured +cytokines (set to 100%).



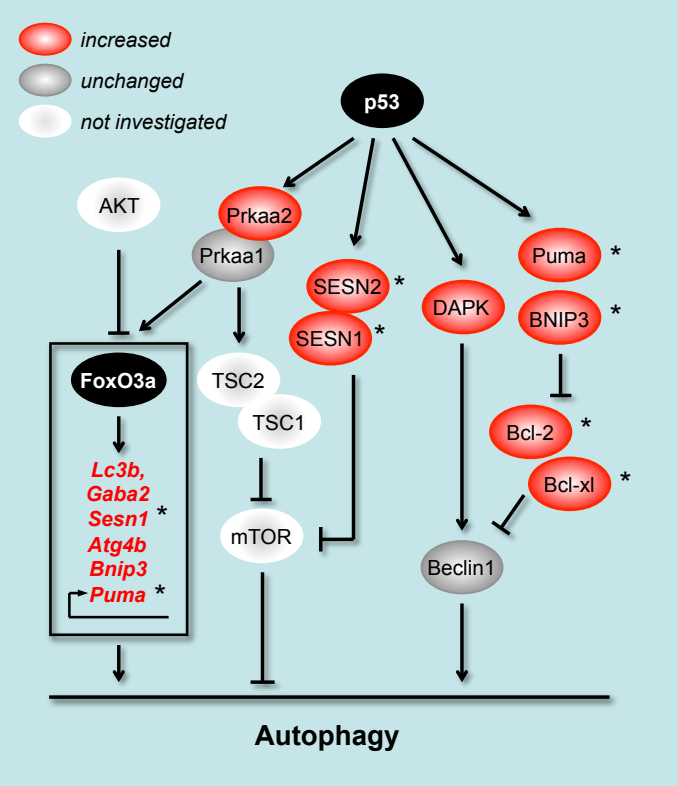
Supplementary Fig. 5 | Autophagy induction does not alter HSC functional properties. a, **d**, Strategies used to interrogate the functionality of WT HSCs that underwent autophagy induction either *ex vivo* (**a**), upon isolation and culture for 12h with or without (±) cytokines, or *in vivo* (**d**), upon isolation from mice that have been fed (F) or starved (S) from food for 24h. **b**, **e**, Colony forming unit (CFU) assays in methylcellulose. Single HSCs were either re-sorted after the 12h culture (**a**) or directly deposited (**d**) into 96-well plates and colony types were scored after 10 days of culture (n = 72 individual HSC per group). G: granulocyte; M: macrophage; GM: granulocyte/macrophage; MegE: megakaryocyte/erythrocyte; Mix: GMMegE. **c**, **f**, Transplantation into lethally irradiated congenic recipients. Pools of 500 HSCs were either individually cultured and transplanted (**a**) or directly transplanted (**d**) per mouse (n = 5 per group). The percentages of CD45.2$^+$ chimerism and donor-derived cells reconstituting the myeloid (Mac1$^+$), B (B220$^+$) or T (CD3$^+$) lineages are provided at 2 months post-transplantation. Hatching indicates -cytokines or starved conditions. All data represent mean ± s.d.



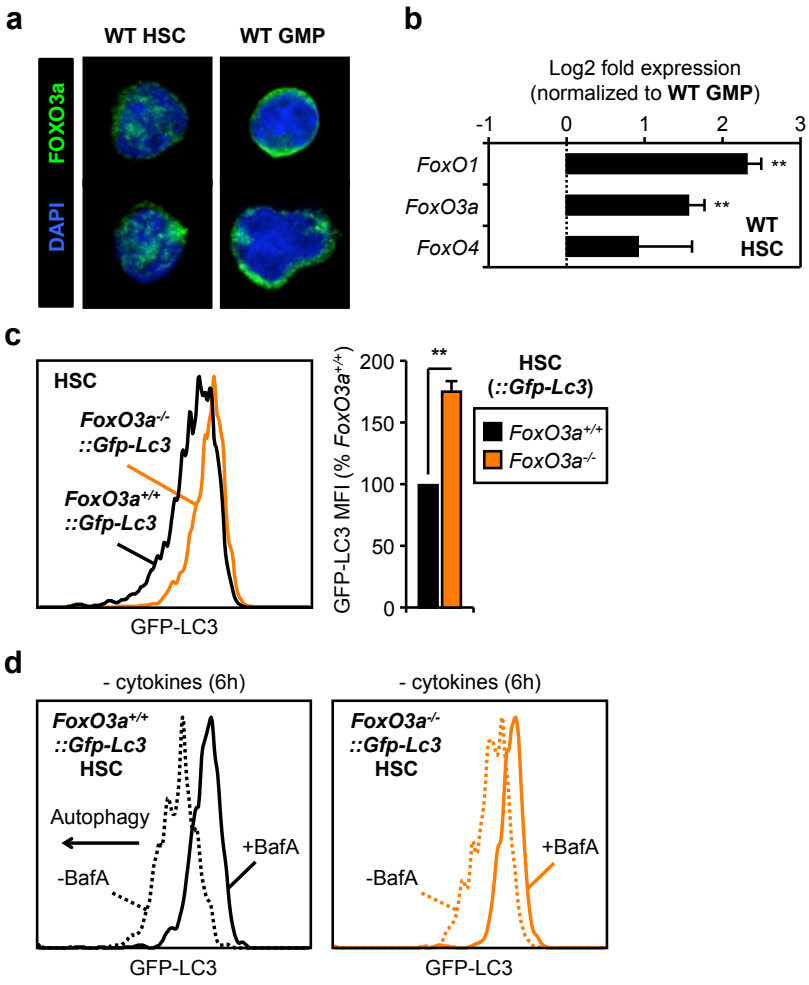
Supplementary Fig. 6 | Similar levels of mTORC1 activity in HSCs and myeloid progenitors. a, Gating strategy used to analyze phospho-mTOR (p-mTOR) and phospho-S6 (p-S6) levels by flow cytometry in HSCs ($Lin^-/Sca\text{-}1^+/c\text{-}Kit^+/CD150^+/CD48^-$, black) and bulk myeloid progenitors (MP: $Lin^-/Sca\text{-}1^-/c\text{-}Kit^+$, dark blue) in young adult C57Bl/6 WT mice. Representative plots of BM cells co-stained with control IgG (grey shade) or phospho-specific antibodies. Results are expressed as p-mTOR or p-S6 mean intensity fluorescence (MFI) divided by IgG MFI, and are normalized to HSCs (set to 100%) (n = 3). **b**, Representative histograms of p-S6 levels in HSCs and GMPs cultured for 6h with (+) cytokines and with or without (±) 20 µM rapamycin (Rapa). All data represent mean ± s.d.; *ns*: not significant.



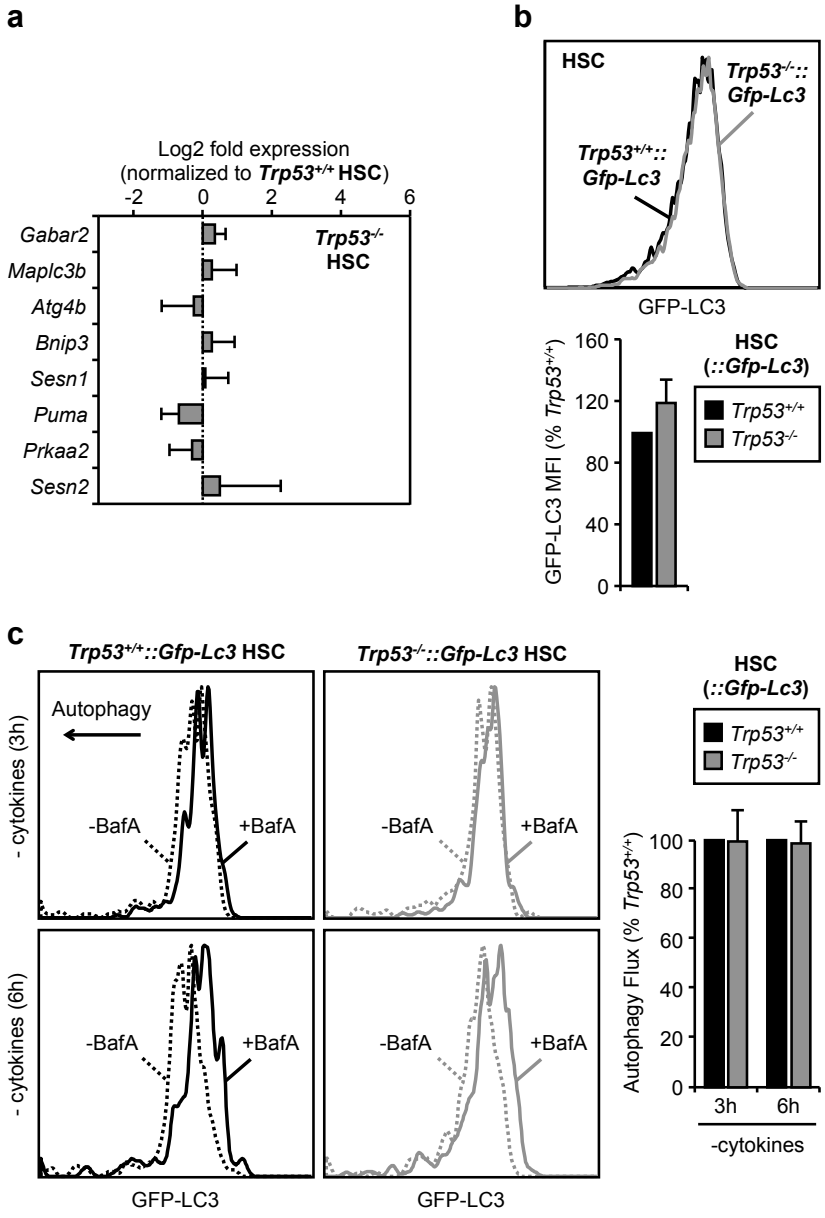
Supplementary Fig. 7 | Analysis of autophagy-related gene expression using SABiosciences autophagy PCR arrays. a, Experimental flow. **b**, Status of the autophagy machinery in WT granulocytes (Gr: $Gr1^+/Mac1^+$) (n = 2). Results are expressed as log2 fold expression compared to levels measured in WT GMPs (set to 0). Only selected genes are shown. All data represent means.



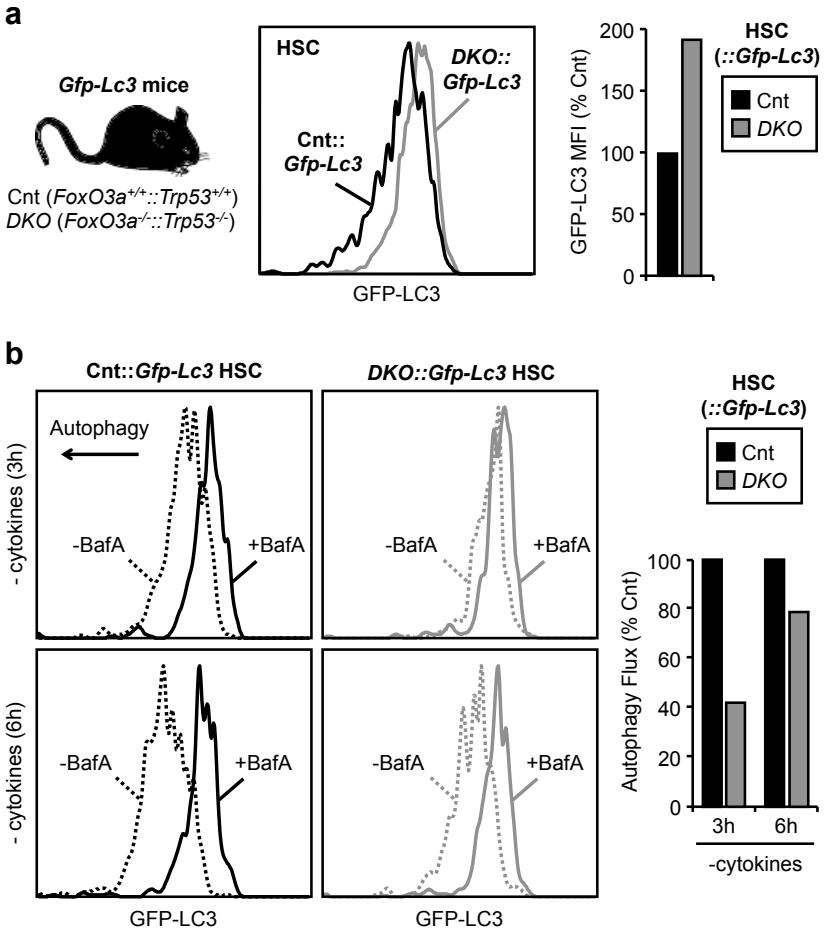
Supplementary Fig. 8 | Putative role for FoxO3a and p53 in driving a pro-autophagy gene expression program in HSCs. Summary of the changes in expression levels of autophagy-related genes (upstream regulator and *Atg8* homologs categories) observed in HSCs when compared to GMPs using both SABiosciences autophagy PCR arrays and complementary analyses on Affymetrix Gene ST 1.0 exon arrays (*unpublished*). The majority of the significantly upregulated genes are targets of p53 and the FoxO3a transcription factor. *denote genes identified by microarray analysis.



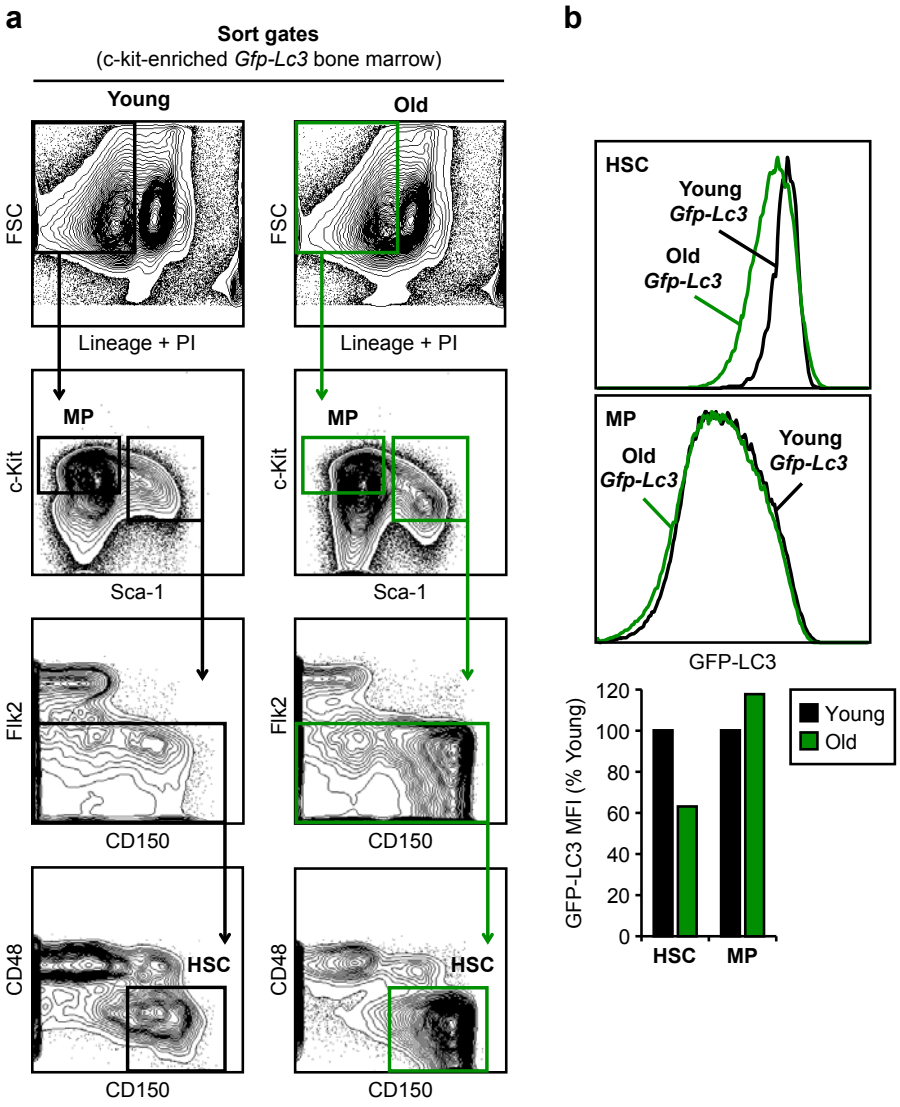
Supplementary Fig. 9 | FoxO3a is critical for rapid induction of autophagy in HSCs. a, Immunofluorescence detection of FoxO3a in WT HSCs and GMPs. **b**, qRT-PCR analysis of the expression level of FoxO family members in WT HSCs (n = 3). Results are expressed as log2 fold expression compared to levels measured in WT GMPs (set to 0). **c**, Steady state GFP-LC3 levels in the HSC compartment of *FoxO3a*$^{-/-}$*::Gfp-Lc3* (orange) and *FoxO3a*$^{+/+}$*::Gfp-Lc3* (black) mice (n = 3). Results are expressed as percent of GFP-LC3 mean intensity fluorescence (MFI) in control *FoxO3a*$^{+/+}$*::Gfp-Lc3* HSCs (set to 100%). **d**, Representative histograms of flow cytometry analyses of *FoxO3a*$^{+/+}$*::Gfp-Lc3* and *FoxO3a*$^{-/-}$*::Gfp-Lc3* HSCs cultured for 6h with or without (±) cytokines and BafA. All data represent mean ± s.d. $^{**}P \leq 0.01$.



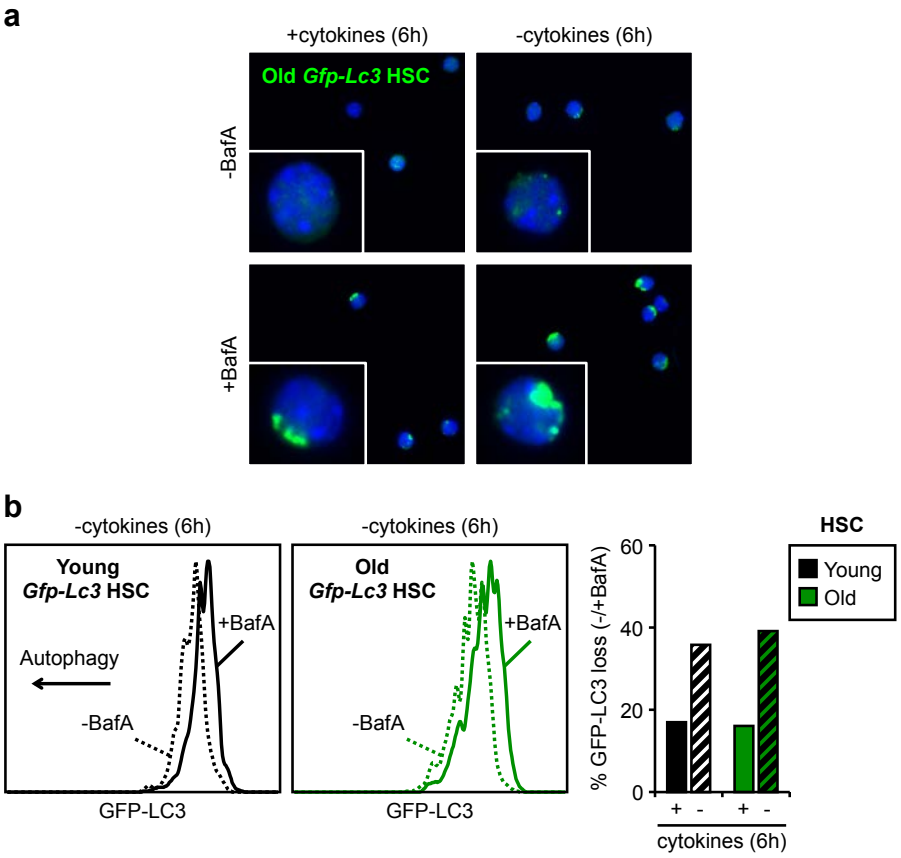
Supplementary Fig. 10 | p53 is dispensable for autophagy induction in wild type HSCs. a, qRT-PCR analyses of pro-autophagic genes in *Trp53*$^{-/-}$ HSCs (n = 3). Results are expressed as log2 fold expression compared to levels measured in *Trp53*$^{+/+}$ HSCs (set to 0). **b**, Steady state GFP-LC3 levels in the HSC compartment of *Trp53*$^{-/-}$*::Gfp-Lc3* (grey) and *Trp53*$^{+/+}$::*Gfp-Lc3* (black) mice (n = 3). Results are expressed as percent of GFP-LC3 mean intensity fluorescence (MFI) in control *Trp53*$^{+/+}$::*Gfp-Lc3* HSCs (set to 100%). **c**, Representative histograms and quantification of autophagy flux in *Trp53*$^{+/+}$::*Gfp-Lc3* and *Trp53*$^{-/-}$*::Gfp-Lc3* HSCs cultured for 3h or 6h without (-) cytokines and with or without (±) BafA (n = 3). Results are expressed as percentage of GFP-LC3 mean intensity fluorescence (MFI) in -BafA compared to +BafA conditions for each genotype, and are normalized to control *Trp53*$^{+/+}$::*Gfp-Lc3* HSCs (set to 100%) for each time point. All data represent mean ± s.d.



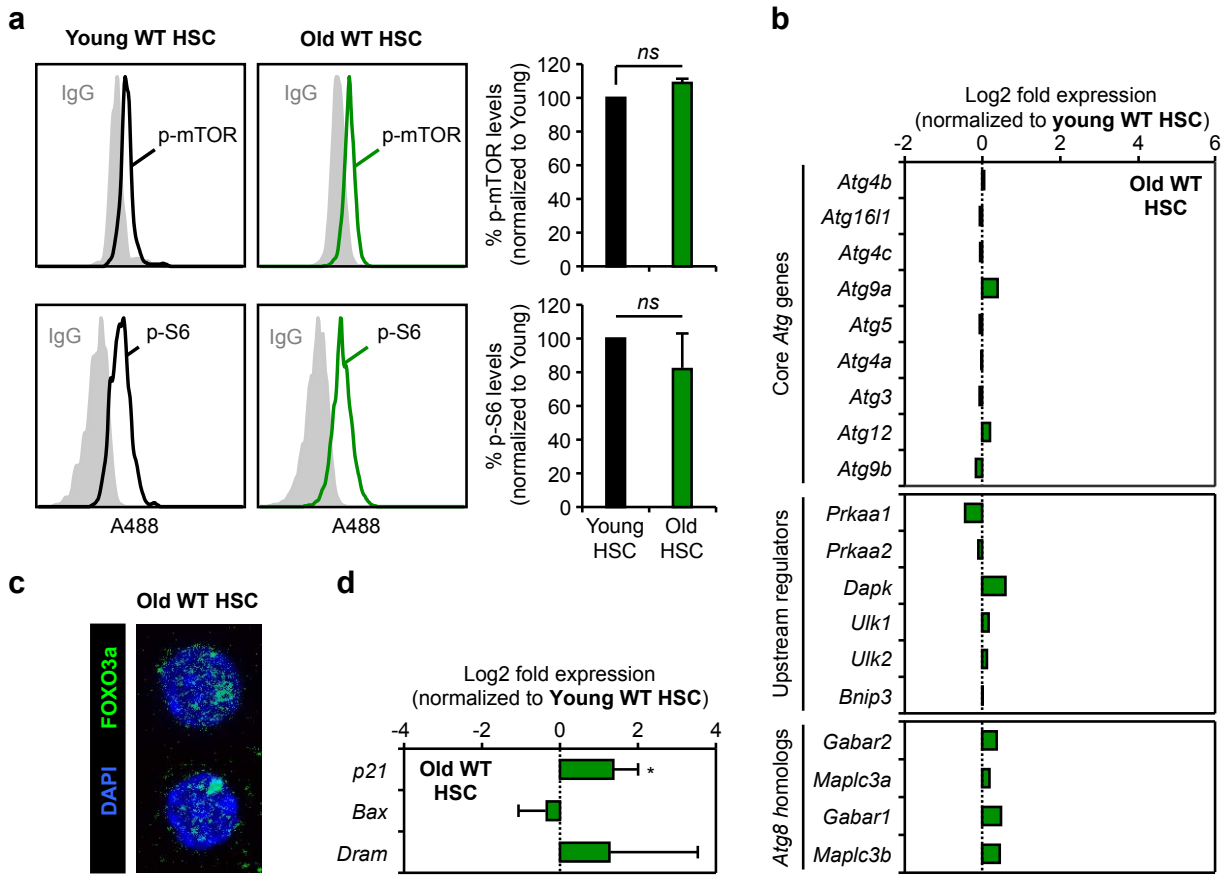
Supplementary Fig. 11 | p53 is dispensable for delayed autophagy induction in *FoxO3*$^{-/-}$ HSCs. a, Histograms and quantification of steady state GFP-LC3 levels in the HSC compartment of double knock out (DKO) *FoxO3*$^{-/-}$*::Trp53*$^{-/-}$*::Gfp-Lc3* (grey) and control (Cnt) *FoxO3*$^{+/+}$*::Trp53*$^{+/+}$*::Gfp-Lc3* (black) mice. Results are expressed as percent of GFP-LC3 mean intensity fluorescence (MFI) in Cnt::*Gfp-Lc3* HSCs (set to 100%). **b**, Histograms and quantification of autophagy flux in Cnt::*Gfp-Lc3* and *DKO*::*Gfp-Lc3* HSCs cultured for 3h or 6h without (-) cytokines and with or without (±) BafA. Results are expressed as percentage of GFP-LC3 mean intensity fluorescence (MFI) in -BafA compared to +BafA conditions for each genotype, and are normalized to Cnt::*Gfp-Lc3* HSCs (set to 100%) for each time point.



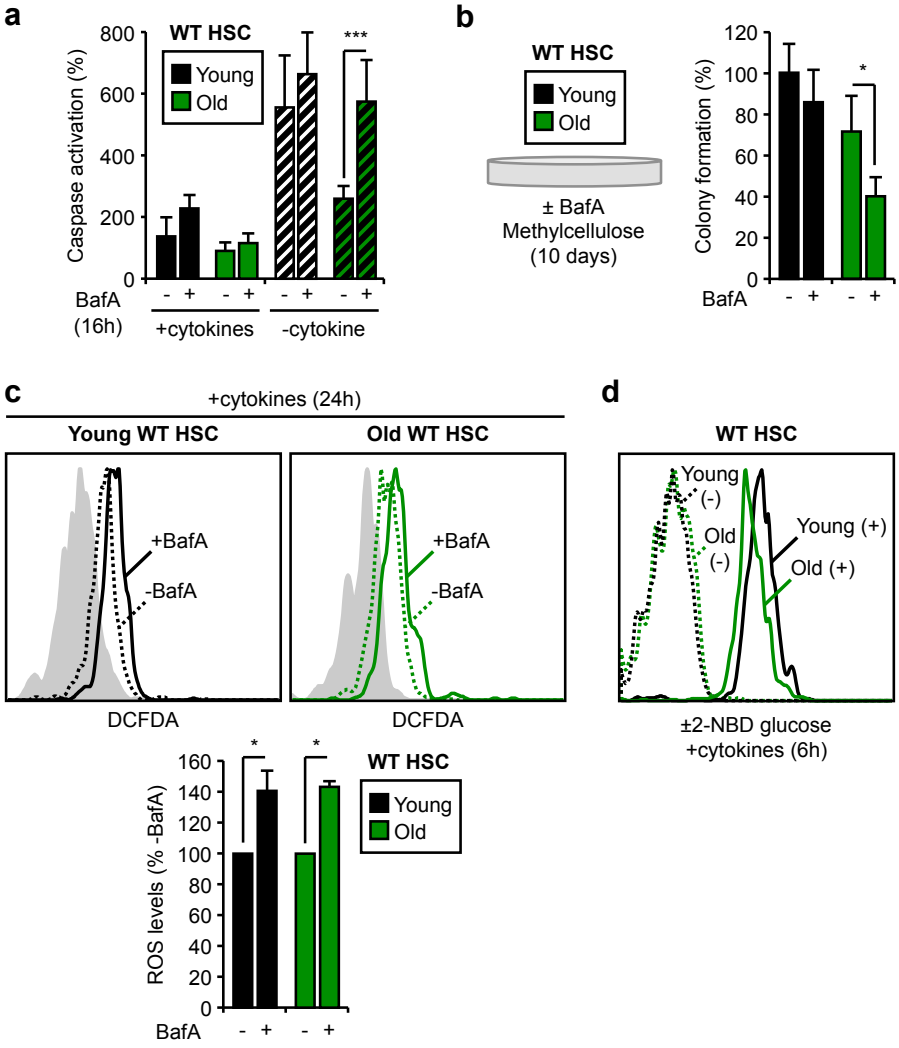
Supplementary Fig. 12 | High basal level of autophagy in old HSCs. a, Gating strategy used to isolate HSCs and investigate the myeloid progenitor (MP) compartment in the BM of young (6-12 weeks) and old (≥ 24 months) C57Bl/6 *Gfp-Lc3* mice. The example show staining of c-Kit enriched BM cells. **b**, Representative histograms and quantification of steady state GFP-LC3 levels in the HSC and MP compartments of young and old *Gfp-Lc3* mice. Results are expressed as percent of GFP-LC3 mean intensity fluorescence (MFI) in young populations (set to 100%).



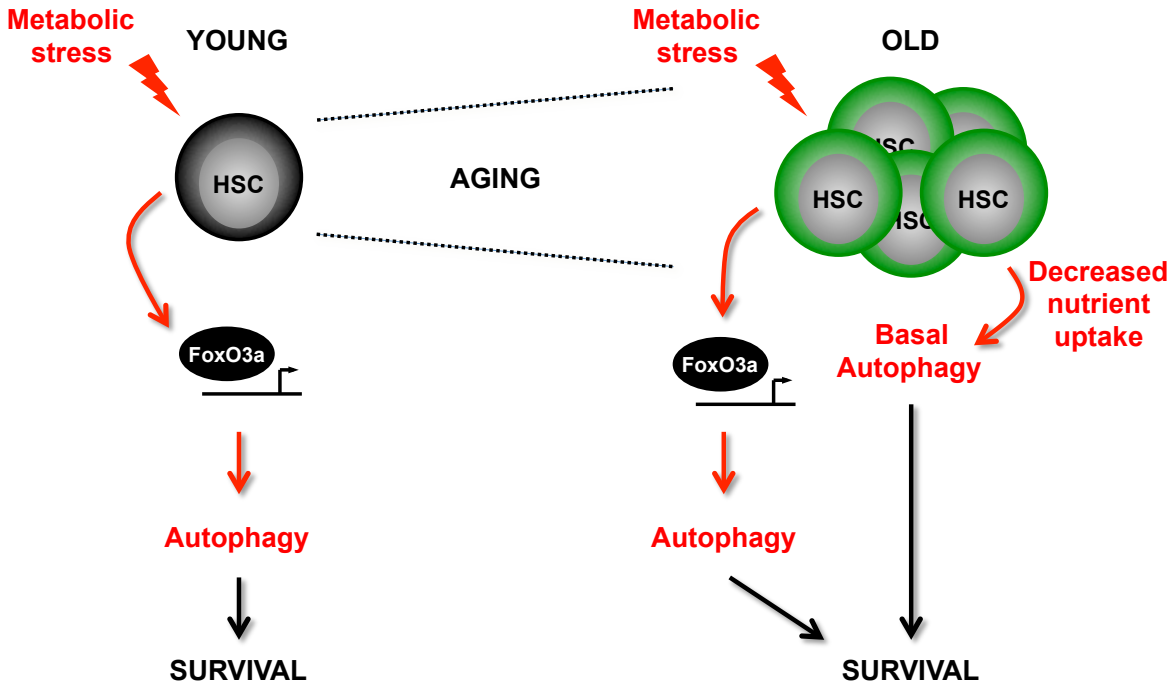
Supplementary Fig. 13 | Normal autophagy induction upon cytokines withdrawal in old HSCs. a, Fluorescent microscopy of old *Gfp-Lc3* HSCs cultured for 6h with or without (±) cytokines and BafA. **b**, Flow cytometry analyses of young (black) and old (green) *Gfp-Lc3* HSCs cultured for 6h ± cytokines and BafA. Results are expressed as percentage of GFP-LC3 mean intensity fluorescence (MFI) in -BafA compared to +BafA conditions. Hatching indicates -cytokine conditions.



Supplementary Fig. 14 | Similar levels of mTORC1, autophagy machinery, nuclear FoxO3a and p53 activity in young and old HSCs. a, Representative plots of phospho-mTOR (p-mTOR) and phospho-S6 (p-S6) levels in the HSC compartment of young (black) and old (green) C57Bl/6 WT mice (n = 3). BM cells were co-stained with control IgG (grey shade) or phospho-specific antibodies. Results are expressed as p-mTOR or p-S6 mean intensity fluorescence (MFI) divided by IgG MFI, and are normalized to young HSCs (set to 100%). **b**, Status of the autophagy machinery as measured by PCR arrays in old HSCs (n = 3). Results are expressed as log2 fold expression compared to levels measured in young HSCs (set to 0). **c**, Immunofluorescence detection of FoxO3a in old HSCs. **d**, qRT-PCR analysis of additional p53 direct targets in young and old HSCs (n = 3). Results are expressed as log2 fold expression compared to levels measured in young HSCs (set to 0). All data represent mean ± s.d. *ns*: not significant; $*P \leq 0.05$.



Supplementary Fig. 15 | Autophagy protects old HSCs. a, Apoptosis levels in young (black) and old (green) HSCs cultured for 8h (Fig. 5c) or 16h with or without (±) cytokines and BafA (n = 3). Results are expressed as percent caspase activation compared to young HSCs cultured for 8h +cytokines (set to 100%). Hatching indicates -cytokines conditions. **b**, Percent colony formation from young and old HSCs plated in methylcellulose ± BafA (n = 3). Colonies were counted after 10 days of culture and normalized to -BafA young HSCs (set to 100%). **c**, DCFDA-based detection of reactive oxygen species (ROS) levels in young and old HSCs cultured for 24h with (+) cytokines and with or without (±) BafA (n = 3). Grey shades indicate the fluorescence level of cells cultured for 24h and unstained with DCFDA. Results are expressed as percent of -BafA conditions (set to 100%). **d**, Representative plots of young and old HSCs cultured for 6h with (+) cytokines and with or without (±) 2-NBD glucose. All data represent mean ± s.d. $*P \leq 0.05$, $***P \leq 0.001$.



Supplementary Fig. 16 | Uncovered protective function of autophagy in young and old HSCs. FoxO3a directs a pro-autophagy gene expression program that allows HSCs to survive metabolic stress within the BM microenvironment. This autophagy program is conserved during physiological aging, and old HSCs actually require basal levels of autophagy to offset reduced nutrient uptake and maintain energy levels.

Gene	Fold Change	Gene	Fold Change	Gene	Fold Change
Akt1	0.2271	*Dapk1*	0.1695	*Rab24*	0.2931
Ambra1	0.1641	*1200002N14Rik*	0.3013	*Rb1*	0.3421
App	0.3875	*Eif2ak3*	0.3309	*Rgs19*	0.4154
Arsa	0.1114	*Eif4g1*	0.03	*Rps6kb1*	0.1222
Atg10	0.0337	*Esr1*	0.0138	*Snca*	1235.0301
Atg12	0.1286	*Fadd*	0.0072	*Sqstm1*	0.4194
Atg16l1	0.1782	*Fas*	75.2534	*Tgfb1*	0.0708
Atg16l2	0.5362	*Gaa*	0.0186	*Tgm2*	11.6362
Atg3	0.5271	*Gabarap*	0.8244	*Fam176a*	0.2952
Atg4a	0.1586	*Gabarapl1*	0.0313	*Tmem74*	0.0031
Atg4b	0.2134	*Gabarapl2*	0.5718	*Tmem77*	0.5837
Atg4c	0.0818	*Hdac1*	0.1503	*Tnf*	1.1023
Atg4d	0.4111	*Hgs*	0.2493	*Tnfsf10*	0.0201
Atg5	0.1753	*Hsp90aa1*	0.0151	*Trp53*	0.0188
Atg7	1.228	*Hspa8*	0.0437	*Trp73*	0.1228
Atg9a	0.7298	*Htt*	0.0005	*Ulk1*	0.6092
Atg9b	0.0026	*Ifna2*	0.2774	*Ulk2*	0.2509
Bad	0.1294	*Ifna4*	0.6233	*Uvrag*	0.1459
Bak1	0.0972	*Ifng*	65.5591		
Bax	0.0633	*Igf1*	2204.1115		
Bcl2	0.0209	*Ins2*	0.0642		
Bcl2l1	0.638	*Irgm1*	0.1919		
Becn1	0.2303	*Map1lc3a*	0.5296		
Bid	0.1355	*Map1lc3b*	0.9738		
Bnip3	0.1885	*Mapk14*	0.2232		
Casp3	0.2766	*Mapk8*	0.0313		
Casp8	0.0444	*Nfkb1*	0.1597		
Cdkn1b	1.0181	*Pik3c3*	0.0828		
Cdkn2a	106.1043	*Pik3cg*	0.5029		
Cln3	0.7817	*Pik3r4*	0.146		
Ctsb	0.5072	*Prkaa1*	0.7968		
Ctss	1.1358	*Prkaa2*	0.0003		
Cxcr4	0.5086	*Pten*	0.7859		

Supplementary Table 1 | Autophagy-related gene expression profile in granulocytes. Full data set obtained from the SABiosciences autophagy PCR arrays (n = 2). Results are expressed as log2 fold expression compared to levels measured in WT GMPs (set to 0).

Gene	Fold Change	*P* value	Gene	Fold Change	*P* value
Akt1	0.9714	0.8415	*Gabarapl1*	3.4655	0.0061*
Ambra1	1.2039	0.4361	*Gabarapl2*	1.9784	0.0015*
App	0.3213	0.0268*	*Hdac1*	1.1882	0.6393
Arsa	1.2441	0.3671	*Hgs*	1.3835	0.1786
Atg10	1.5336	0.0212*	*Hsp90aa1*	0.5856	0.0568
Atg12	0.6050	0.4731	*Hspa8*	0.6821	0.3697
Atg16l1	1.3670	0.4184	*Htt*	0.9866	0.9734
Atg16l2	1.6219	0.1942	*Ifna2*	3.0126	0.3444
Atg3	0.6062	0.0231*	*Ifna4*	4.0512	0.2510
Atg4a	0.7020	0.1721	*Ifng*	0.8635	0.6668
Atg4b	1.7904	0.0335*	*Igf1*	3258.4015	0.0576
Atg4c	1.2716	0.1795	*Ins2*	0.8135	0.8234
Atg4d	1.9424	0.1118	*Irgm1*	2.2118	0.0239*
Atg5	0.7883	0.4277	*Map1lc3a*	3.4933	0.0054*
Atg7	1.2083	0.5106	*Map1lc3b*	3.1939	0.0138*
Atg9a	1.1778	0.3920	*Mapk14*	1.0922	0.7580
Atg9b	0.4286	0.0341*	*Mapk8*	1.3565	0.2754
Bad	0.7165	0.0023*	*Nfkb1*	1.0337	0.7288
Bak1	0.3647	0.0027*	*Pik3c3*	0.8446	0.6713
Bax	0.5562	0.0800	*Pik3cg*	1.3304	0.0502
Bcl2	0.9697	0.9545	*Pik3r4*	1.9270	0.3089
Bcl2l1	4.1161	0.0834	*Prkaa1*	1.7612	0.2059
Becn1	0.7842	0.0629	*Prkaa2*	33.5246	0.0070*
Bid	0.2648	0.0037*	*Pten*	1.2835	0.2779
Bnip3	1.6521	0.1232	*Rab24*	1.0084	0.9488
Casp3	1.0880	0.6368	*Rb1*	0.8215	0.0895
Casp8	1.5745	0.2202	*Rgs19*	0.8656	0.4620
Cdkn1b	1.8649	0.0024*	*Rps6kb1*	1.3589	0.3000
Cdkn2a	3.1432	0.2914	*Snca*	1840.9019	0.0040*
Cln3	1.8882	0.0178*	*Sqstm1*	1.7913	0.0267*
Ctsb	1.2571	0.0319*	*Tgfb1*	0.5257	0.0002*
Ctss	0.4277	0.0900	*Tgm2*	663.8114	0.0032*
Cxcr4	0.3144	0.0832	*Fam176a*	5.8740	0.2998
Dapk1	6.8513	0.1902	*Tmem74*	0.9992	0.8929
1200002N14Rik	0.0652	0.0038*	*Tmem77*	1.5529	0.0018*
Eif2ak3	0.7720	0.7446	*Tnf*	0.4461	0.1002
Eif4g1	0.7870	0.0324*	*Tnfsf10*	5.7098	0.0098*
Esr1	1.5644	0.2571	*Trp53*	0.9211	0.9265
Fadd	4.8904	0.4586	*Trp73*	1.8099	0.4985
Fas	16.0487	0.1635	*Ulk1*	3.2135	0.0003*
Gaa	1.1506	0.3230	*Ulk2*	1.7011	0.1951
Gabarap	0.7998	0.1640	*Uvrag*	0.9855	0.9185

Supplementary Table 2 | Autophagy-related gene expression profile in HSCs. Full data set obtained from the SABiosciences autophagy PCR arrays (n = 3). Results are expressed as log2 fold expression compared to levels measured in GMPs (set to 0). * denote statistically significant *P* values.

Gene	Fold Change	*P* value	Gene	Fold Change	*P* value
Akt1	1.1830	0.2649	*Gabarapl1*	1.3240	0.2391
Ambra1	1.0171	0.8381	*Gabarapl2*	1.2417	0.3551
App	2.2452	0.0245*	*Hdac1*	0.7859	0.2338
Arsa	1.2377	0.1820	*Hgs*	1.1130	0.4873
Atg10	0.9933	0.9304	*Hsp90aa1*	0.9047	0.4715
Atg12	1.1549	0.6560	*Hspa8*	1.0069	0.8407
Atg16l1	0.9593	0.5776	*Htt*	1.3827	0.1999
Atg16l2	0.6814	0.1183	*Ifna2*	3.2394	0.1465
Atg3	0.9550	0.5922	*Ifna4*	0.7880	0.7053
Atg4a	0.9854	0.8892	*Ifng*	1.5530	0.4369
Atg4b	1.0360	0.7856	*Igf1*	0.3101	0.0165*
Atg4c	0.9648	0.9314	*Ins2*	5.2764	0.1492
Atg4d	1.3478	0.2289	*Irgm1*	1.2157	0.2766
Atg5	0.9574	0.5945	*Map1lc3a*	1.1146	0.5115
Atg7	1.6319	0.2775	*Map1lc3b*	1.2992	0.4870
Atg9a	1.3275	0.1002	*Mapk14*	0.9353	0.6870
Atg9b	0.8905	0.7252	*Mapk8*	1.2647	0.1940
Bad	1.2932	0.1303	*Nfkb1*	1.0089	0.9857
Bak1	1.0390	0.6151	*Pik3c3*	1.1535	0.5071
Bax	1.0339	0.9429	*Pik3cg*	0.9688	0.7322
Bcl2	1.7044	0.1853	*Pik3r4*	1.3459	0.9618
Bcl2l1	3.3439	0.0588	*Prkaa1*	0.7302	0.3425
Becn1	0.9032	0.5829	*Prkaa2*	0.9305	0.6784
Bid	0.7720	0.2678	*Pten*	0.8283	0.5124
Bnip3	0.9914	0.9489	*Rab24*	0.7872	0.4023
Casp3	0.8289	0.1707	*Rb1*	0.9608	0.9697
Casp8	0.6919	0.1486	*Rgs19*	0.9889	0.8695
Cdkn1b	0.9686	0.7584	*Rps6kb1*	0.7753	0.3211
Cdkn2a	0.9088	0.7580	*Snca*	0.4336	0.0100*
Cln3	0.8707	0.0575	*Sqstm1*	1.3463	0.2083
Ctsb	1.5891	0.0308*	*Tgfb1*	0.8467	0.4608
Ctss	0.4664	0.0284*	*Tgm2*	3.8451	0.0180*
Cxcr4	0.8204	0.5598	*Fam176a*	19.9364	0.0262*
Dapk1	1.5385	0.3538	*Tmem74*	0.3874	0.1114
1200002N14Rik	0.8517	0.6134	*Tmem77*	1.1523	0.5108
Eif2ak3	0.9380	0.8452	*Tnf*	0.7842	0.5562
Eif4g1	0.9820	0.8100	*Tnfsf10*	1.5466	0.0393*
Esr1	1.2045	0.3704	*Trp53*	1.1342	0.6115
Fadd	3.8551	0.5196	*Trp73*	1.2480	0.4568
Fas	1.7253	0.9694	*Ulk1*	1.1252	0.2987
Gaa	2.5281	0.0011*	*Ulk2*	1.0931	0.3732
Gabarap	1.6585	0.0091*	*Uvrag*	0.9826	0.9947

Supplementary Table 3 | Autophagy-related gene expression profile in old HSCs. Full data set obtained from the SABiosciences autophagy PCR arrays (n = 3). Results are expressed as log2 fold expression compared to levels measured in young HSCs (set to 0). * denote statistically significant *P* values.