Elevated levels of Ube2g1 in hematopoietic stem cells lead to segmental aging of the hematopoietic system

Julian Niemann,[1] Tanja Schuster,[1] Vadim Sakk,[1] Karin Soller,[1] Andreas Brown,[1] Sebastian Wiese,[2] Karina Eiwen,[1] Markus Hoenicka,[3] Andreas Liebold,[3] Moritz Oltmanns,[4] Heiko Reichel,[4] Medhanie A. Mulaw[5] and Hartmut Geiger[1]

[1]Institute of Molecular Medicine, Ulm University; [2]Core Unit Mass Spectrometry and Proteomics, Ulm University; [3]Department of Cardiothoracic and Vascular Surgery, University Hospital; [4]Department of Orthopaedic Surgery, University Hospital and [5]Single-Cell Sequencing Unit, Ulm University, Ulm, Germany

Correspondence: J. Niemann
julian.niemann@uni-ulm.de

H. Geiger
hartmut.geiger@uni-ulm.de

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Online Supplementary Appendix

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[1]Institute of Molecular Medicine, Ulm University, Germany,
[2]Core Unit Mass Spectrometry and Proteomics, Ulm University, Germany,
[3]Department of Cardiothoracic and Vascular Surgery, University Hospital, 89081 Ulm, Germany,
[4]Department of Orthopaedic Surgery, University Hospital, 89081, Ulm, Germany.
[5]Single-Cell Sequencing Unit, Ulm University, Germany

Supplementary Methods

RT-PCR

TaqMan technology (Thermo Fisher Scientific) was used to determine the level of expression of genes. Quantification was done using the $\Delta\Delta C_T$ method.

Immunoprecipitation and Western Blotting

Cell lysates were prepared using NP-40 lysis buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1% IGEPAL CA-630). For IP, antibodies were crosslinked to Dynabeads A/G (Thermo Fisher Scientific) using BS3 and incubated with lysates overnight at 4°C. For western blotting, lysates were separated by SDS-PAGE, transferred to PVDF membranes, and probed with antibodies (*Online Supplementary Table 1*).

T-cell activation assay

The CD8 T-cell Isolation kit (Miltenyi) and the Dynabeads Mouse T-cell CD3/CD28 Activator Kit (Thermo Fisher) were used to isolate and activate $CD8^+$ T-cells from single cell splenocytes of young and aged C57/BL6J wildtype mice. T-cells were activated and transduced overnight in TexMACS (MOI: 30; TexMACS with 50 U/mL rhIL-2) before culture for up to 48 hours until analysis.

Supplementary Figure Legends

Online Supplementary Figure S1: Characterisation of Ube2g1-C90S-OE HSC in spleen and bone marrow of transplanted mice

A) Schematic of sequencing of plasmids after site directed mutagenesis (SDM) for Ube2g1-C90S in genetically engineered NIH/3T3s cells. **B)** Level of GFP in NIH/3T3 cells stably transduced with Control, Ube2g1-OE and Ube2g1^{C90S}-OE, determined by Western Blot. The factor below the panel indicates the normalisation factor based on total protein quantification. Arrows indicate expressed Ube2g1-OE-GFP fusion protein (~50 kDa) and GFP (~30 kDa). **C)** Level of Ube2g1 in NIH/3T3 cells stably transduced with Control, Ube2g1-OE and Ube2g1^{C90S}-OE, determined by Western Blot. The factor below the panel indicates the normalisation factor based on total protein quantification. Left panel shows a low and right panel a high luminescence exposure. Arrows indicate expressed Ube2g1-OE-GFP fusion protein (~50 kDa) and endogenous Ube2g1 (~20 kDa). **D)** Relative (normalised to young control) intensity of Ube2g1 in young control, Ube2g1-OE and Ube2g1-C90S-OE transduced murine HSC (N = 3, biological repeats equal to an average of 308/274/237 (young control/OE/C90S) cells per replicate). **E)** Percentage of donor-derived (GFP$^+$) T-cells (CD3e$^+$), B-cells (CD19$^+$) and myeloid cells (Gr-1$^+$/CD11$^+$) in the spleen 24 weeks post-transplantation (N = 5-6). **F)** Ratio of the percentage of donor-derived (GFP$^+$) myeloid over lymphoid cells in the spleen 24 weeks post-transplantation (N = 5-6). **G)** Percentage of donor-derived (GFP$^+$) T-cells, B-cells and myeloid cells in the BM 24 weeks post-transplantation (N = 5-6). **H)** Percentage of donor-derived (GFP$^+$) HSC in the BM 24 weeks post-transplantation (N = 5-6). **I)** Percentage of donor-derived (GFP$^+$) myeloid progenitors MEP (Lin$^-$, c-Kit$^+$, CD34^{low}, CD16/32$^-$), CMP (Lin$^-$, c-Kit$^+$, CD34$^+$, CD16/32$^-$) and GMP (Lin$^-$, c-Kit$^+$, CD16/32$^+$) in the BM 24 weeks post-transplantation (N = 5-6). Data show individual biological replicates as mean ± SD. Data were assessed by One-Way ANOVA or Kruskal-Wallis test depending on normality (D-I). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Online Supplementary Figure S2: Expression of degradation specific ubiquitin is increased in aged murine HSC and aged human HSPC

A) Schematic of the mutagenesis screen performed using a SFß91- based retroviral insertion to identify genes relevant for clonal selection of Lin-negative bone marrow (BM) cells. Upstream of the insertion site (blue arrow) Ube2g1 was identified to show increased expression in the clonally selected BM cells. **B)** Representative IF-pictures from young (12-16 weeks [wks], WT) and aged (>80 wks, AcRFP$^+$) murine hematopoietic stem cells (HSC) stained for Ubiquitin (yellow) and DAPI (blue), Scalebar = 5µm. **C)** Relative (log2 transformed) intensity of Ubiquitin in young and old murine HSC (N = 7-10, biological

repeats equal to an average of 79/143 (young/old) cells per replicate). **D)** Representative IF-pictures of young and old murine HSC stained for K48-Ubiquitin (green) and DAPI, Scalebar = 5µm. **E)** Relative (log2 transformed) intensity of K48-Ubiquitin in young and old murine HSC (N = 7-10, biological repeats equal to an average of 79/143 (young/old) cells per replicate). **F)** Representative IF-pictures of human $CD34^+$ HSPC from young (25-33 years donors; Median = 32y) and old (55-80 years; Median = 71y) donors stained for Ubiquitin (yellow) and DAPI (blue), Scalebar = 5µm. **G)** Relative (log2 transformed) intensity of Ubiquitin in young and old human $CD34^+$ HSPC (N = 6, three young and six old biological repeats equal to an average of 199/178 (young/old) cells per replicate). **H)** Representative pictures of human HSPC from young and old donors stained for K48-Ubiquitin (green) and DAPI, Scalebar = 5µm. **I)** Relative (log2 transformed) intensity of K48-Ubiquitin in young and old human $CD34^+$ HSPC (N = 5, four young old biological repeats equal to an average of 191/166 (young/old) cells per replicate). Data show log2 transformed total intensity of individual replicates with each replicate as mean ± SEM. Data were assessed by a One sample t test against a value of 0 for young control (C, E, G, I). **p < 0.01.

Online Supplementary Figure S3: Elevated levels of Ube2g1 result in segmental premature aging of peripheral hematopoiesis

A) Relative (log2 transformed) gene expression of Ube2g1 two days post-transduction in HSPC derived from young (12-16 weeks [wks], WT) and aged (>80 wks, $AcRFP^+$) mice (N = 5-8). **B)** Relative (normalised to young control) intensity of Ube2g1 in young control and Ube2g1-OE transduced murine HSC (N = 3, biological repeats equal to an average of 308/274 (young control/OE) cells per replicate). **C)** Level of GFP in NIH/3T3 cells stably transduced with Control or Ube2g1-OE fusion protein, determined by Western Blot. The number below the panel indicates the normalisation factor based on total protein quantification. An asterisk (*) indicates Ube2g1-OE-GFP fusion protein (~50 kDa) and a hashtag (#) GFP (~30 kDa). **D)** Level of Ube2g1 protein in NIH/3T3 cells stably transduced with Control or Ube2g1-OE, determined by Western Blot. The factor below the panel indicates the normalisation factor based on total protein quantification. Left panel shows a data from a short and right panel a longer exposure of the membrane. An asterisk (*) indicates Ube2g1-OE-GFP fusion protein (~50 kDa) and a hashtag (#) endogenous Ube2g1 (~20 kDa). **E)** Percentage of donor-derived (GFP^+) T-cells ($CD3e^+$), B-cells ($CD19^+$) and myeloid cells ($Gr\text{-}1^+/CD11^+$) in the spleen 24 weeks post-transplantation (N = 17-19). **F)** Ratio of the percentage of donor-derived (GFP^+) myeloid over lymphoid cells in the spleen 24 weeks post-transplantation (N = 16-19). **G)** Percentage of donor-derived (GFP^+) T-cells, B-cells and myeloid cells in the BM 24 weeks post-transplantation (N = 17-19). **H)** Ratio of the percentage of donor-derived (GFP^+) myeloid over lymphoid cells in the BM 24 weeks

post-transplantation (N = 16-17). **I)** Percentage of donor-derived (GFP^+) HSC ($CD34^{low}$, Lin^-, $Sca1^+$, $c\text{-}Kit^+$) in the BM 24 weeks post-transplantation (N = 17-19). **J)** Percentage of donor-derived (GFP^+) myeloid progenitors MEP (Lin^-, $c\text{-}Kit^+$, $CD34^{low}$, $CD16/32^-$), CMP (Lin^-, $c\text{-}Kit^+$, $CD34^+$, $CD16/32^-$) and GMP (Lin^-, $c\text{-}Kit^+$, $CD16/32^+$) in the BM 24 weeks post-transplantation (N = 17-19). **K)** Percentage of donor-derived (GFP^+) naïve and memory $CD4^+$ T-cells in the spleen 24 weeks post-transplantation (N = 11-12). Data show individual biological replicates from four independent experiment repeats as mean ± SD. Data were assessed by One-Way ANOVA or Kruskal-Wallis test depending on normality (A, B, E-K). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Online Supplementary Figure S4: Reduced levels of Ube2g1 result in segmental rejuvenation of aged HSC

A) Relative (log2 transformed) gene expression of Ube2g1 of a mixed HSC population two days post- transduction in cells derived from young (12-16 weeks [wks], WT) and aged (>80 wks, $AcRFP^+$) mice (N = 4-7). **B)** Level of Ube2g1 in NIH/3T3 cells stably transduced with Scrambled (Control) or Ube2g1-KD, determined by Western Blot. The factor below the panel indicates the normalisation factor based on total protein quantification. Arrow indicates endogenous Ube2g1 (~20 kDa). **C)** Relative (normalised to old control) intensity of Ube2g1 in old control and Ube2g1-KD transduced murine HSC (N = 4, biological repeats equal to an average of 770/582 (old control/KD) cells per replicate). **D)** Schematic of the experimental set-up of the competitive transplantation experiment, using sorted HSC from young (12-16 wks, WT) and aged (>80 wks, $AcRFP^+$) donor mice ($CD45.2^+$). HSC were genetically engineered and transplanted alongside 5 $x10^4$ $CD45.1^+$ BM cells into lethally irradiated recipient mice ($CD45.1^+$). **E)** Ratio of the percentage of donor-derived (GFP^+) myeloid over lymphoid cells in PB 24 weeks post-transplantation (N = 7-9). **F)** Percentage of donor-derived (GFP^+) T-cells ($CD3e^+$), B-cells ($CD19^+$) and myeloid cells ($Gr\text{-}1^+/CD11^+$) in PB 24 weeks post-transplantation (N = 9-11). **G)** Percentage of donor-derived (GFP^+) naïve, CM and EM $CD8^+$ T-cells in the spleen 24 weeks post-transplantation (N = 12-13). **H)** Ratio of the percentage of donor-derived (GFP^+) myeloid over lymphoid cells in the spleen 24 weeks post- transplantation (N = 12-13). **I)** Percentage of donor-derived (GFP^+) T-cells, B-cells and myeloid cells in the BM 24 weeks post-transplantation (N = 11-13). **J)** Percentage of donor-derived (GFP^+) HSC ($CD34^{low}$, Lin^-, $Sca1^+$, $c\text{-}Kit^+$) in the BM 24 weeks post-transplantation (N = 12-13). Data show individual biological replicates from two independent experiment repeats as mean ± SD. Data were assessed by One-Way ANOVA or Kruskal-Wallis test depending on normality (A, C, E-J). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Online Supplementary Figure S5: PLA specificity controls and validation of Uba52 and Creb3 as Ube2g1 interactor

A) Ube2g1 levels for immunoprecipitation (IP) processed samples of control and Ube2g1-OE NIH/3T3 cells, determined by Western Blot. IP was performed based on anti-IgG or -GFP antibody. Samples are grouped as input, IgG Control IP and anti-GFP IP. Highlighted are endogenous Ube2g1 as well as Ube2g1-GFP fusion protein. **B)** Uba52 levels for immunoprecipitation (IP) processed samples of control and Ube2g1-OE NIH/3T3 cells, determined by Western Blot. IP was performed based on anti-IgG or -GFP antibody. Samples are grouped as input, IgG Control IP and anti-GFP IP. Highlighted are endogenous Uba as well as a likely Ube2g1-Uba52 protein complex. **C)** Representative IF-pictures from young and old control and young Ube2g1-OE transduced murine HSC stained for Hsf1 (orange), GFP (green) and DAPI (blue), Scalebar = 5µm. **D)** Relative (log2 transformed) intensity of Hsf1 in young and old control and young Ube2g1-OE transduced murine HSC (N = 5, biological repeats equal to an average of 116/130 and 171/150 (young control/OE, old control/OE) cells per replicate). **E)** Proximity Ligation assay (PLA) controls without primary antibody (No 1^{st}-Ab) as well as an IgG-Rb control instead of Ube2g1 in a PLA with Shp2 at the same concentration. All controls were performed in old control cells to acknowledge the potential influence of GFP counterstaining. For better comparability of values, three young control samples Ube2g1-Shp2 PLA were added to the graph for better visualisation. **F)** Representative IF-pictures from old control, Ube2g1-OE and Ube2g1-C90S-OE transduced murine HSC showing PLA signal (Ube2g1-Uba52) with counterstaining for GFP and DAPI, White arrows indicate PLA interactions (dots), Scalebar = 5µm. **G)** Percentage of cells with 0, 1, 2, 3, 4 or ≤ 5 dots (N = 3-5, biological repeats equal to an average of 907/947/492 (old control/OE/C90S) cells per replicate). **H)** PLA signal (Ube2g1-Uba52) per cell normalised to young control (N = 3-5, biological repeats equal to an average of 907/947/492 (old control/OE/C90S) cells per replicate). **I)** Representative IF-pictures from old control and Ube2g1-OE transduced murine HSC showing PLA signal (Ube2g1-Creb3) with counterstaining for GFP and DAPI, White arrows indicate PLA interactions (dots), Scalebar = 5µm. **J)** Percentage of cells with 0, 1, 2, 3, 4 or ≤ 5 dots (N = 3, biological repeats equal to an average of 625/629 (old control/OE) cells per replicate). **K)** PLA signal (Ube2g1-Creb3) per cell normalised to young control (N = 3, biological repeats equal to an average of 625/629 (old control/OE) cells per replicate). Data show log2 transformed total intensity of individual biological replicates with each replicate as mean ± SD. Data were assessed by a One sample t test against a value of 0 for control samples or a non-parametric Mann-Whitney test (D, H, K). *$p < 0.05$, **$p < 0.01$.

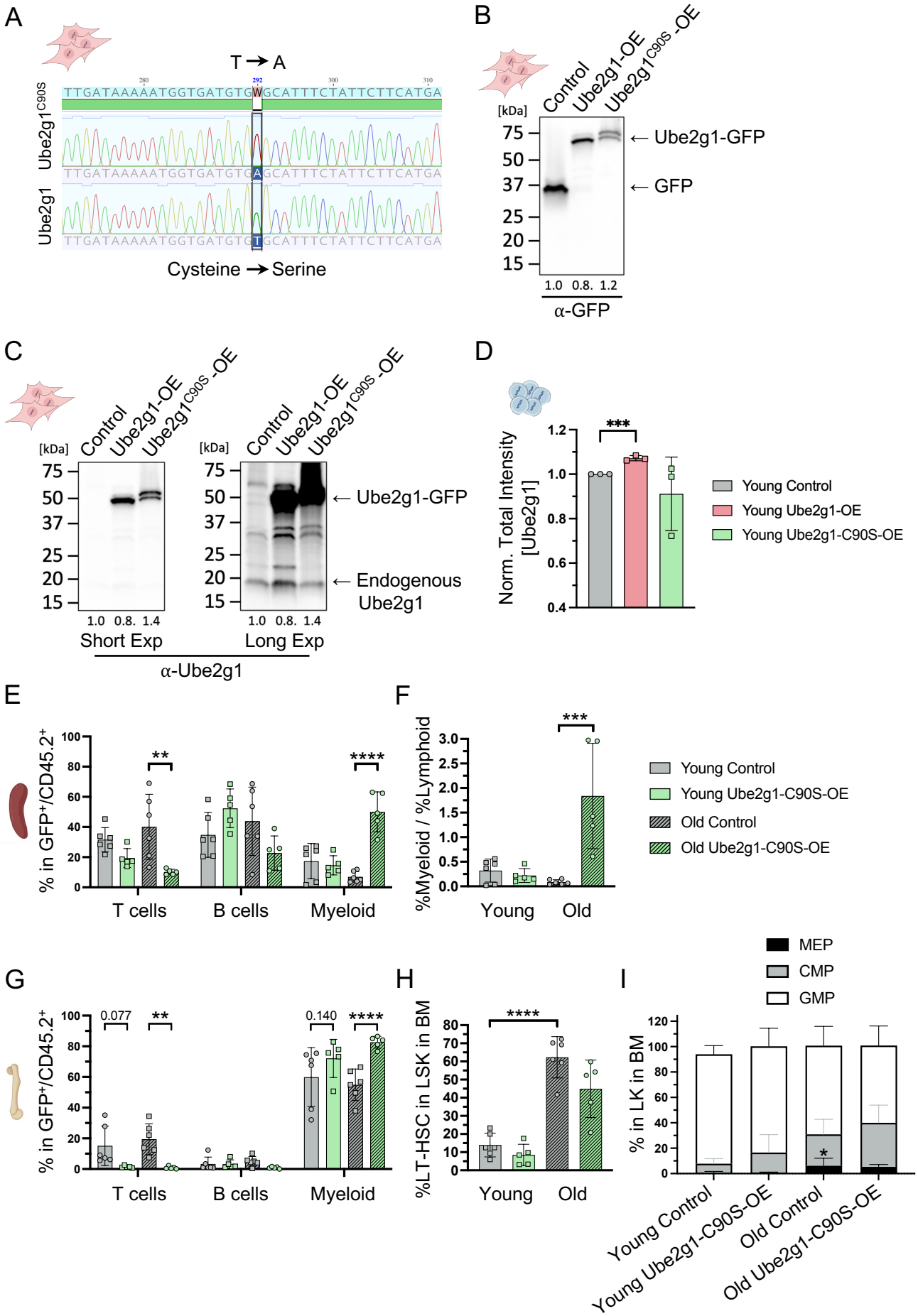
Online Supplementary Figure S6: Multicolour staining confirmation and analysis of Shp2 and pTyr after knockdown and Ube2g1 mutation

A) Representative IF-pictures from young (12-16 weeks [wks], WT) murine HSC single colour stained either without primary antibody or with an antibody against GFP (green), Hsf1 (orange) or pTyr (red) followed by a mix of all corresponding secondary antibodies, Scalebar = 5µm. **B)** Total intensity of phosphorylated tyrosine (pTyr) in old control and Ube2g1-KD HSC (N = 418/182 (old control/OE) from two biological replicates). **C)** Total intensity of Shp2 in old control and Ube2g1-KD HSC (N = 218/337 (old control/OE) from two biological replicates). **D)** Total intensity of phosphorylated tyrosine (pTyr) in young control, Ube2g1-OE, Ube2g1-C90S-OE and old control HSC (N = 82/93/79/208 cells (young control/OE/C90S/old control) from one biological replicate)). **E)** Total intensity of Shp2 in young control, Ube2g1-OE, Ube2g1-C90S-OE and old control HSC (N = 104/71/60/119 cells (young control/OE/C90S/old control) from one biological replicate)). Data were assessed by non-parametric Mann-Whitney test (B-E). **p < 0.01, ****p < 0.0001.

Online Supplementary Figure S7: Young Ube2g1-OE T-cells show increased exhaustion and decreased TCR activation

A) Counts of donor-derived (GFP^+) total live DN and DP cells in the thymus 24 weeks post-transplantation (N = 11-12). **B)** Counts of donor-derived (GFP^+) total live DN1-4 cells in the thymus 24 weeks post-transplantation (N = 11-12) **C)** Percentage of donor-derived (GFP^+) Tim-3 and/or PD-1 exhaustion markers on $CD4^+$ T-cells in the spleen 24 weeks post-transplantation (N = 11-12). **D)** Schematic of the experimental setup for $CD8^+$ T-cell isolation, transduction and activation using MACS based isolation, CD3/CD28 activation beads and flow cytometry along with IF to analyse the cells. **E)** Percentage of $CD8^+$ T-cells in $CD3^+/GFP^+$ cells. $T_{0/24h/48}$ represent measurements after isolation, 24 hours and 48 hours post-transduction/activation (n = 4 biological replicates). **F)** Percentage of $CD69^+$ T-cells in $CD8^+/CD3^+/GFP^+$ cells. $T_{0/24h/48}$ represent measurements after isolation, 24 hours and 48 hours post-transduction/activation (N = 4 biological replicates). **G)** Total intensity of Ube2g1 in young control, Ube2g1-OE and old control HSC 48 hours post-activation/transduction (N = 147/140/147 cells (young control/OE/old control) from one biological replicate)). **H)** Total intensity of phosphorylated tyrosine (pTyr) in young control, Ube2g1-OE and old control HSC 48 hours post-activation/transduction (N = 145/148/148 cells (young control/OE/old control) from one biological replicate)). **I)** Total intensity of phosphorylated Shp2 (pY542) in young control, Ube2g1-OE and old control HSC 48 hours post-activation/transduction (N = 146/148/148 cells (young control/OE/old control) from one biological replicate)). Data show individual biological replicates from two/four (A-C; E, F) independent experiment repeats as mean ± SD. Data were assessed by One-Way ANOVA or Kruskal-Wallis (A-C) and non-

parametric Mann-Whitney or unpaired student's t test (E-I) depending on normality. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.



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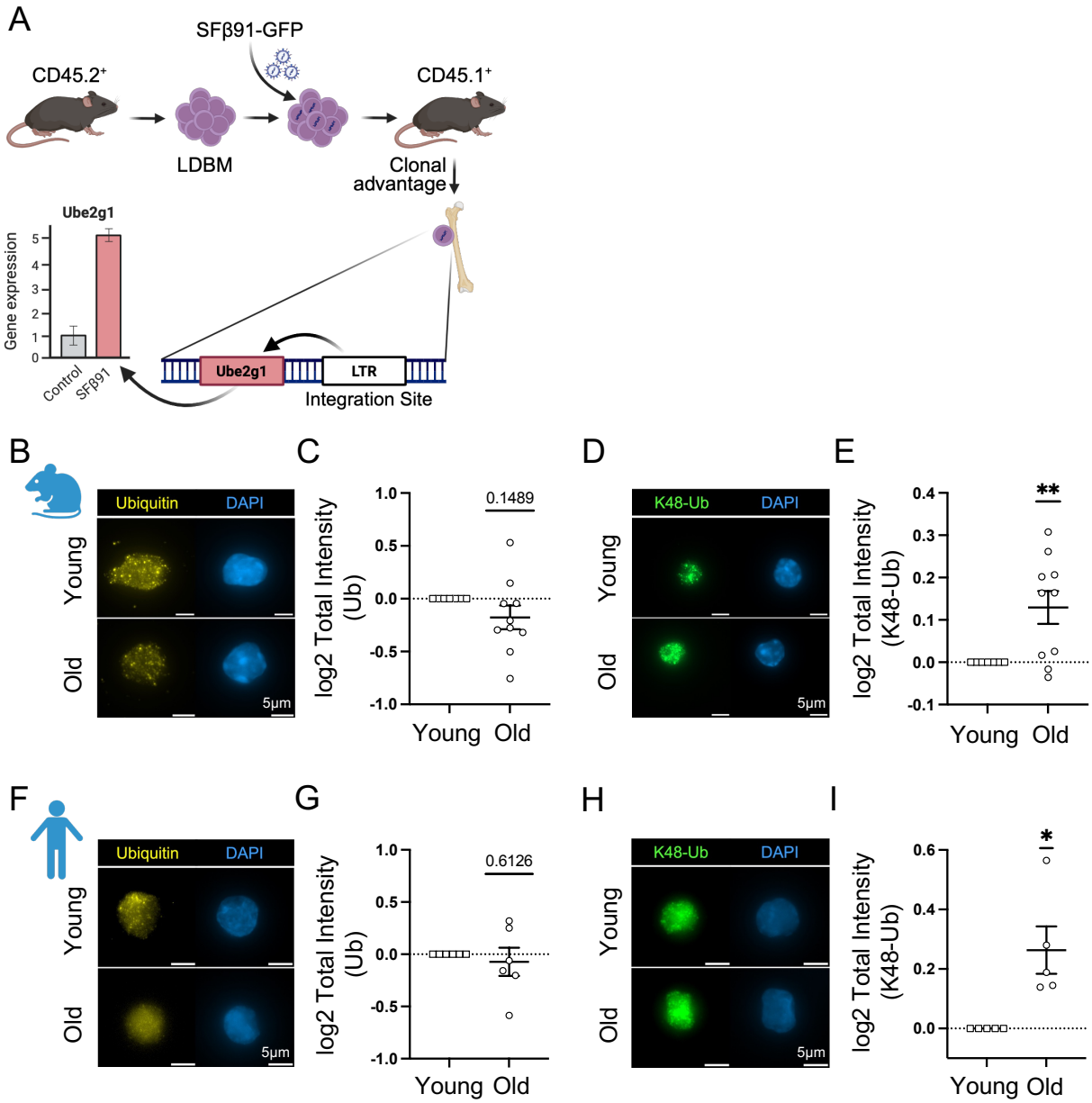
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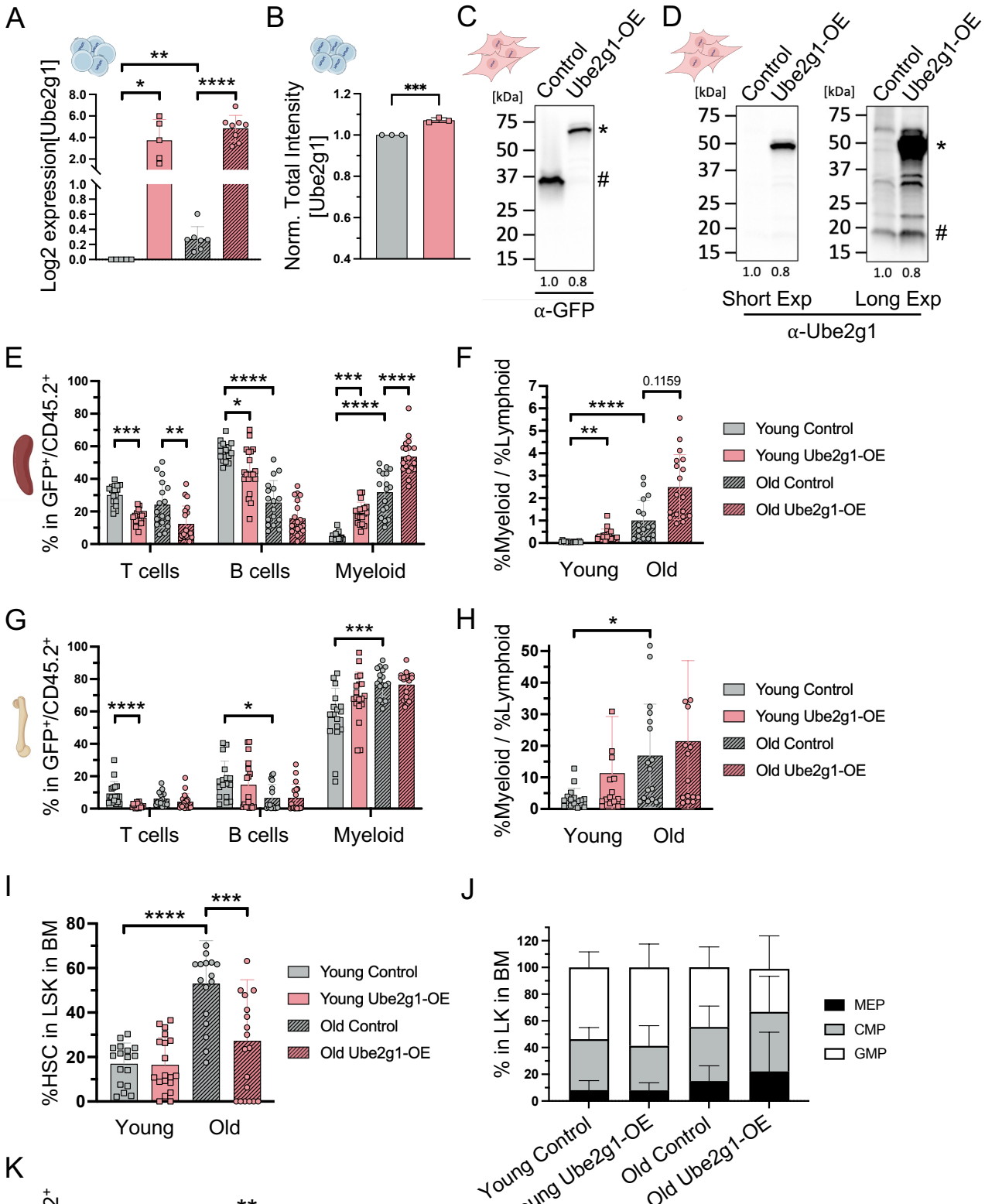

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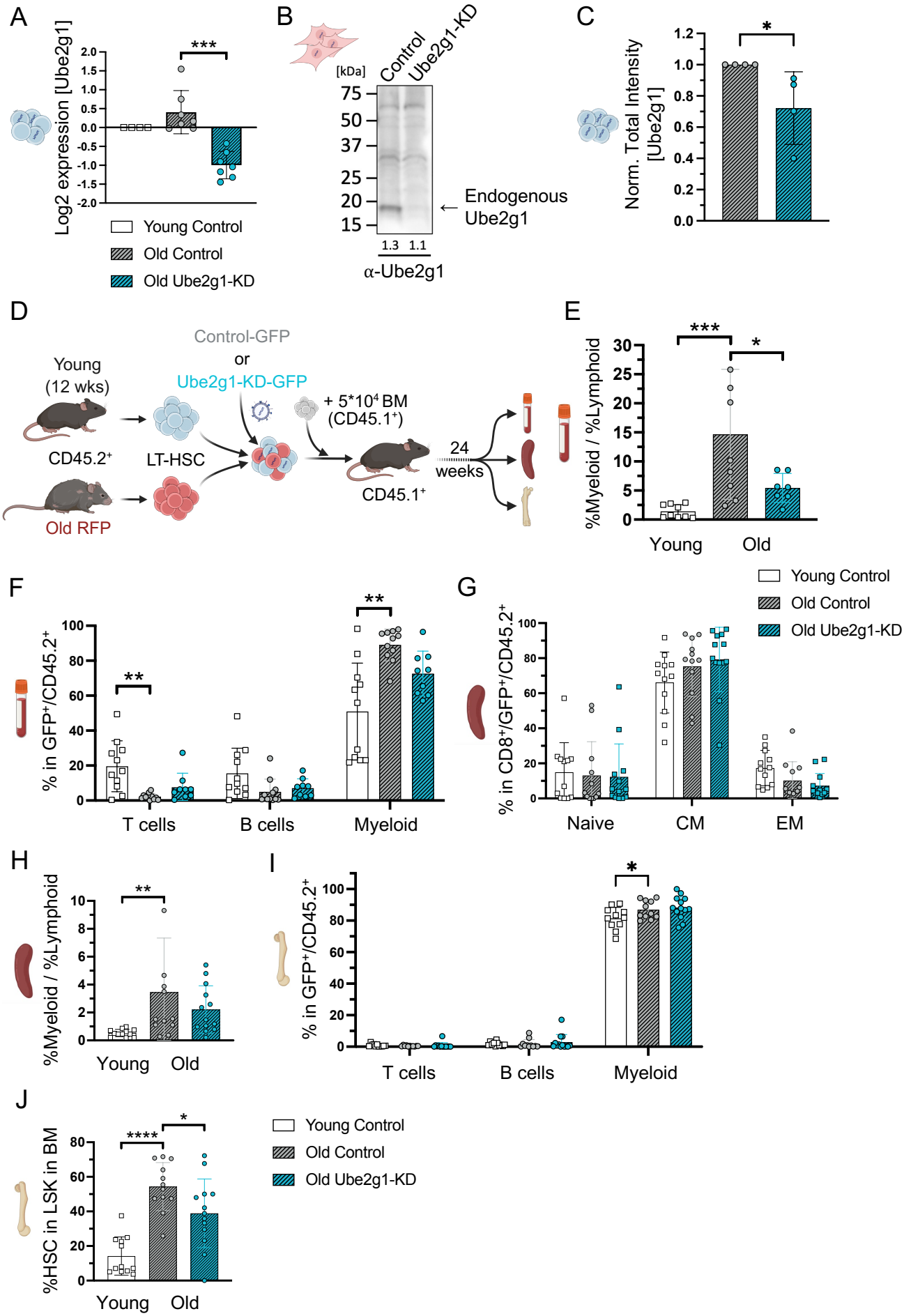

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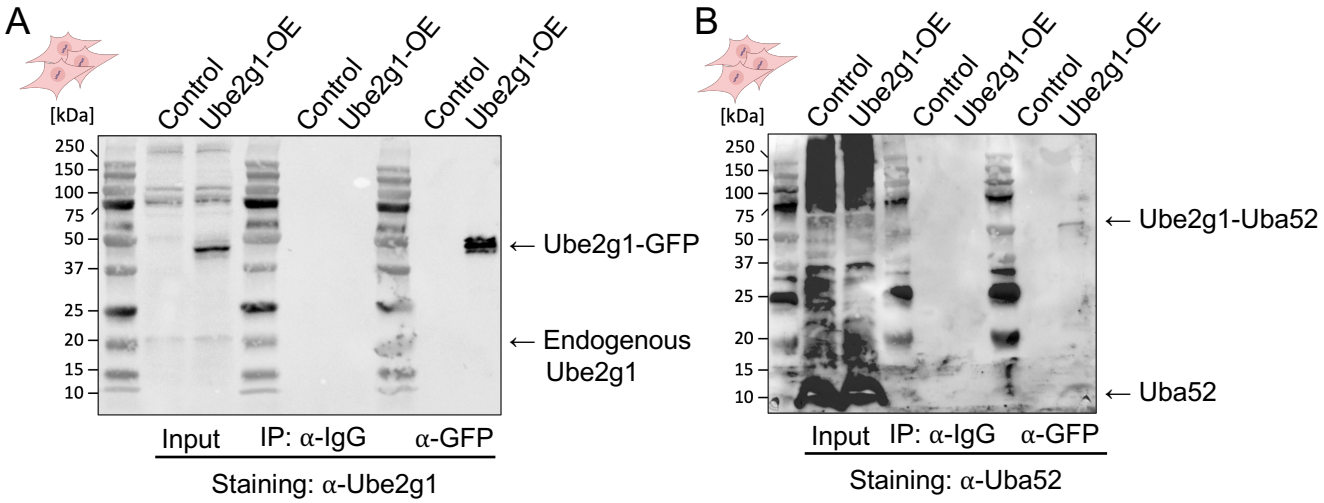

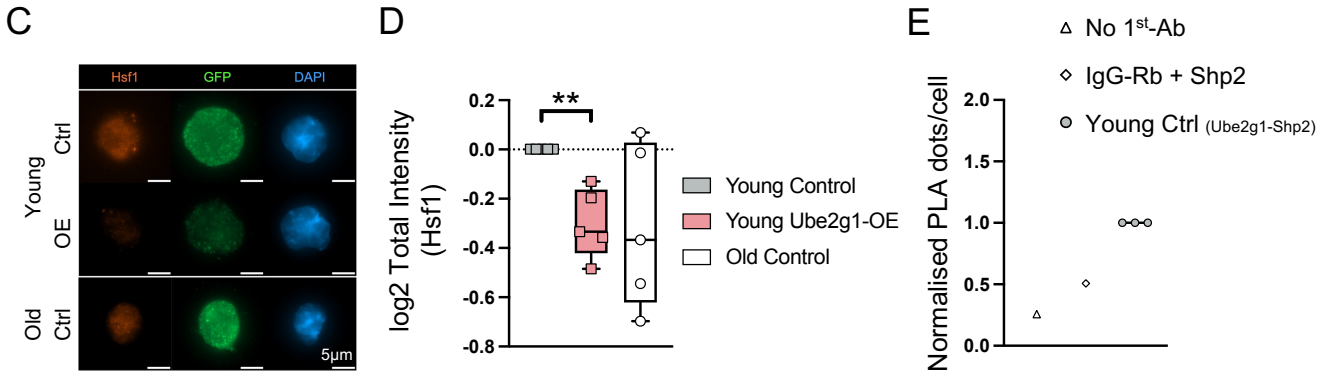

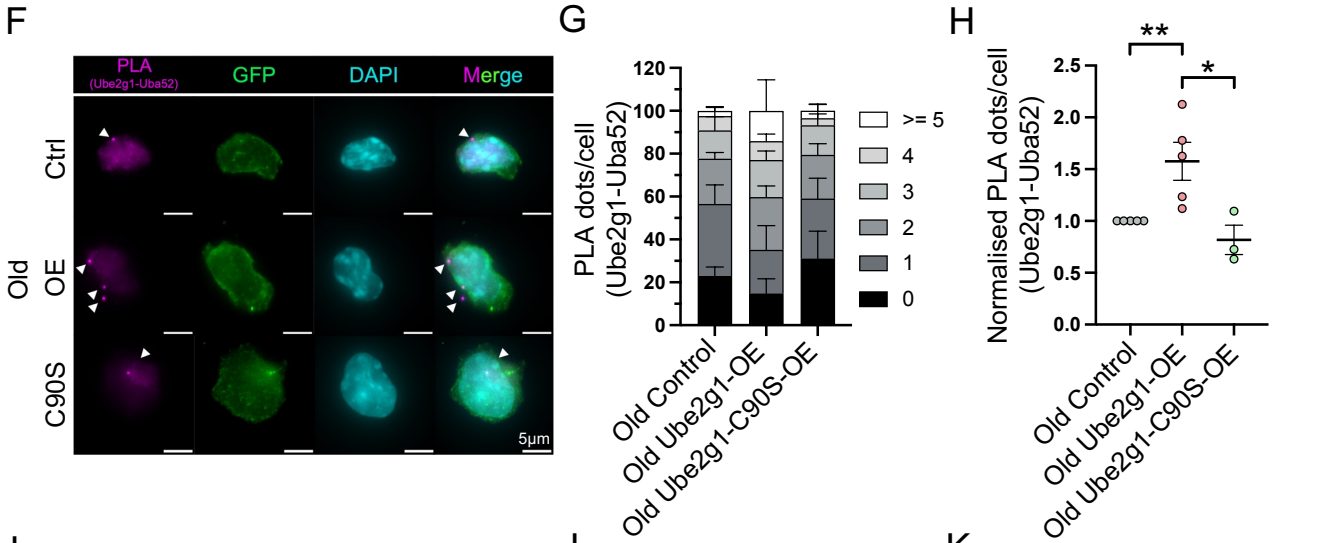

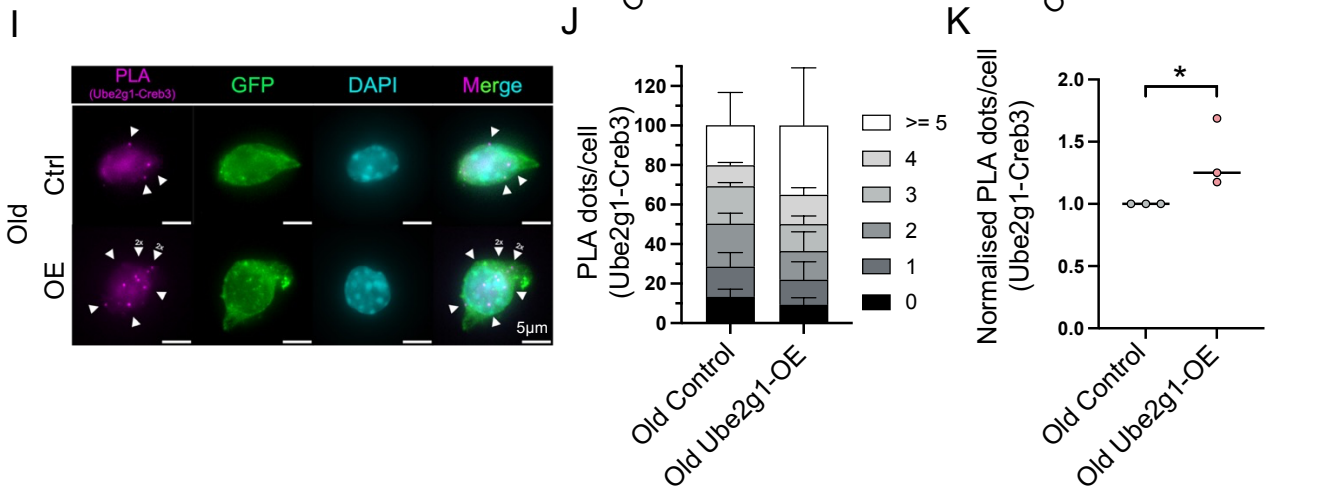

Online Supplementary Figure S5

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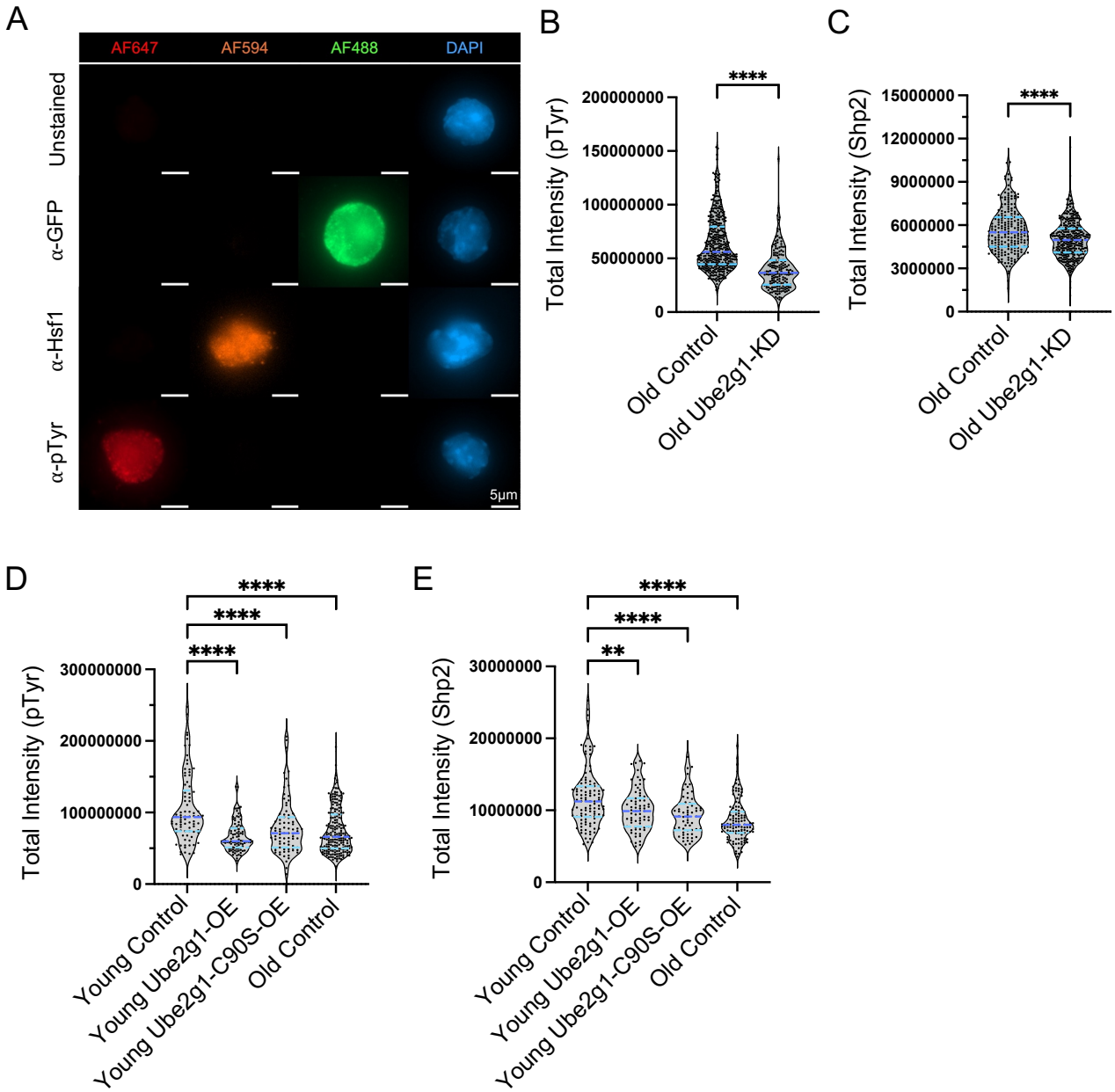
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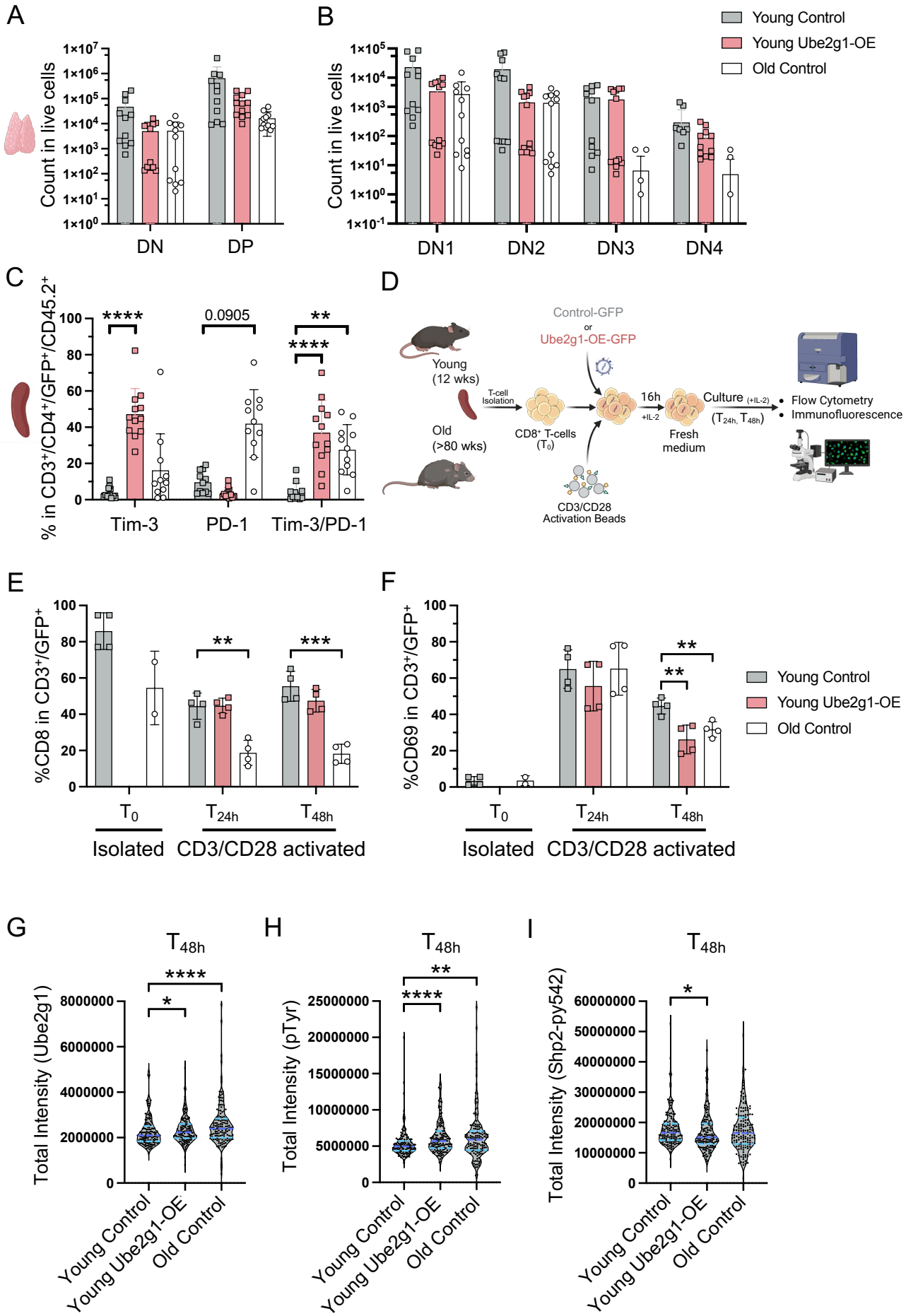

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Supplementary Table 1: Antibody Information

Flow Cytometry (FC)			
Antibody	**Conjugate**	**Vendor**	**Identifier**
CD8α	Pacific Blue	BD Biosciences	558106
CD16/32	APC-Cy7		560541
Streptavidin	eF450	eBioscience	48-4317-82
c-Kit	AlexaFluor 700		56-1172-82
Sca-1	PE-Cy7		25-5981-82
CD34	FITC		11-0341-85
CD34	APC		50-0341-82
Flt3	PE		12-1351-83
Ly6C/G (Gr-1)	eF450		48-5931-82
CD11b	AlexaFluor 700		56-0112-82
CD19	APC		17-0193-82
CD3ε	PE-Cy7		25-0031-82
	AlexaFluor 700	BioLegend	100216
CD44	PE-Cy7		103030
CD366 (Tim-3)	BrilliantViolet 605		119721
CD279 (PD-1)	PE/Dazzle 594		109115
CD4	APC	Thermo Fisher Scientific	17-0042-83
CD62L	PerCp-Cy5.5		45-0621-82
CD25	PerCp-Cy5.5		45-0251-80
CD45.2	APC-Vio770	Miltenyi Biotec	130-119-128

Immunofluorescence (IF)			
Antibody	**Species**	**Vendor**	**Identifier**
Ube2g1	Rabbit	Abcam	ab101371
K48-Ubiquitin	Rabbit		ab140601
GFP	Goat		ab6673
pTyr	Mouse		ab10321
Hsf1	Rabbit	Cell Signaling	4356S
Ubiquitin	Mouse	Thermo Fisher Scientific	PA-10023
Shp2	Mouse		MA5-36980
Shp2-pY542	Rabbit		44-554G
α-Mouse	AlexaFluor 647	Jackson ImmunoResearch	715-605-150
	AlexaFluor 594		715-585-150
	AlexaFluor 488		715-545-150
α-Rabbit	AlexaFluor 647		711-605-152
	AlexaFluor 594		711-585-152
	AlexaFluor 488		711-545-152
α-Goat	AlexaFluor 488		705-545-147

Western Blotting (WB)			
Antibody	**Species**	**Vendor**	**Identifier**
GFP	Goat	Abcam	ab6673
Ube2g1	Rabbit	Thermo Fisher Scientific	PA5-62212
Uba52	Rabbit	Abcam	ab109227
IgG	Goat	R&D Systems	AB-108-C
α-Rabbit	HRP	SouthernBiotech	4030-05
α-Goat	HRP	SouthernBiotech	6460-05

Supplementary Table 2: Human Donor Information

Group	Donor ID	Age [years]	Sex
Young Group Median age: 32.5 years Females: 25%	Young_1	25	Male
	Young_2	33	Male
	Young_3	33	Female
	Young_4	32	Male
Old Group Median age: 71.0 years Females: 33%	Old_1	67	Female
	Old_2	55	Male
	Old_3	80	Male
	Old_4	63	Male
	Old_5	75	Female
	Old_6	72	Male
	Old_7	67	Male
	Old_8	79	Male
	Old_9	71	Female

Supplementary Table 3: Data from Figure 3D

GO Term	Description	p-value	FDR	Count	Genes
GO:0001932	regulation of protein phosphorylation	2,21E-05	1,16E-01	20	Cdkn1c, Btc, Bmp4, Timp3, Gas6, Pdgfd, Plc1, Tnfaip3, Ocln, Smpd3, Fzd5, Plcb1, Pde5a, Tlr1, Cdk5r1, Ankrd6, Ksr1, Slc11a1, Gpnmb, Camkk1
GO:0045860	positive regulation of protein kinase activity	2,42E-04	6,35E-01	11	Fzd5, Btc, Pde5a, Bmp4, Tlr1, Cdk5r1, Gas6, Ksr1, Slc11a1, Pdgfd, Camkk1
GO:0042325	regulation of phosphorylation	2,68E-04	4,69E-01	20	Cdkn1c, Btc, Bmp4, Timp3, Gas6, Pdgfd, Plc1, Tnfaip3, Ocln, Smpd3, Fzd5, Plcb1, Pde5a, Tlr1, Cdk5r1, Ankrd6, Ksr1, Slc11a1, Gpnmb, Camkk1
GO:0051347	positive regulation of transferase activity	2,76E-04	3,63E-01	11	Fzd5, Btc, Pde5a, Bmp4, Serinc5, Tlr1, Cdk5r1, Gas6, Ksr1, Slc11a1, Pdgfd
GO:0007167	enzyme linked receptor protein signaling pathway	3,32E-04	3,49E-01	10	Smpd3, Soga1, Btc, Plcb1, Bmp4, Megf8, Gucy2g, Cdk5r1, Gas6, Pdgfd
GO:0055085	transmembrane transport	3,98E-04	3,48E-01	26	Trpc1, Sort1, Scn11a, Ga6, Slc16a4, Slc6a19, Slc9a8, Hcn1, Slc23a3, Itpr1, Slc11a1, Rhd, Tmco3, Slc20a2, Slc39a12, Slc27a4, Slc39a13, Scn2a1, Slc30a10, Slc35f1, Slc7a2, Hpn, Alg10b, Abcc9, Alc25a30, Fkbp1b
GO:0045859	regulation of protein kinase activity	4,22E-04	3,17E-01	13	Cdkn1c, Btc, Bmp4, Gas6, Pdgfd, Tnfaip3, Fzd5, Pde5a, Tlr1, Cdk5r1, Ksr1, Slc11a1, Camkk1
GO:0032417	positive regulation of sodium:proton antiporter activity	4,44E-04	2,91E-01	2	Plcb1, Tesc
GO:0032415	regulation of sodium:proton antiporter activity	4,44E-04	2,59E-01	2	Plcb1, Tesc
GO:0001934	positive regulation of protein phosphorylation	4,86E-04	2,55E-01	13	Btc, Bmp4, Gas6, Pdgfd, Fzd5, Plcb1, Pde5a, Tlr1, Cdk5r1, Ksr1, Slc11a1, Ankrd6, Gpnmb
GO:0002682	regulation of immune system process	5,15E-04	2,46E-01	18	Cd276, Spta1, Bmp4, Gas6, Zbtb16, Spon2, Pdgfd, Tnfaip3, Smpd3, Fzd5, C1rl, Plcb1, Ddx60, Pde5a, Tesc, Tlr1, Slc11a1, Gpnmb
GO:1903555	regulation of tumor necrosis factor superfamily cytokine production	6,20E-04	2,71E-01	6	Fzd5, Tlr1, Gas6, Spon2, Tnfaip3, Gpnmb
GO:0032680	regulation of tumor necrosis factor production	6,20E-04	2,50E-01	6	Fzd5, Tlr1, Gas6, Spon2, Tnfaip3, Gpnmb
GO:0071229	cellular response to acid chemical	6,67E-04	2,50E-01	3	Plcb1, Tesc, Pdgfd
GO:0033674	positive regulation of kinase activity	6,69E-04	2,34E-01	11	Fzd5, Btc, Pde5a, Bmp4, Tlr1, Cdk5r1, Gas6, Ksr1, Slc11a1, Pdgfd, Camkk1
GO:0002691	regulation of cellular extravasation	9,62E-04	3,16E-01	2	Plcb1, Pdgfd
GO:2000437	regulation of monocyte extravasation	9,62E-04	2,97E-01	2	Plcb1, Pdgfd

Supplementary Table 4: Data from Figure 3E

Gene	log2ratio[YUvsYC]	-log10[pval_YU_vs_YC]
Ppm1j	8,352310604	3,05E-08
Eml6	7,71988933	2,25E-05
Ocln	6,200369776	0,000194095
Pdgfd	6,052335111	0,00047957
Trem1	-8,025885335	0,000957357
Bmp4	5,365593736	0,003397018
Cdk5r1	5,547283848	0,004245703
Hrh2	6,337566369	0,004527515
Trem3	-8,526148984	0,004642915
Tnfaip3	6,701040475	0,005705899
Hectd2	5,756077353	0,006327258
Plcl1	4,534163271	0,009474752
Gas6	4,744492158	0,012017658
Socs7	2,574613829	0,013011665
Fcgr2b	-5,91947358	0,017270032
Il11	4,293118372	0,017448841
Prkci	2,041038002	0,017461162
Ptpn11	1,379582604	0,021868096
Cebpa	-5,222329074	0,023681109
Hspb8	3,825562144	0,02407896
Prkcb	3,800626251	0,027301982
Irf8	-4,789833405	0,0276582
Zap70	2,450950392	0,033369023
Rhoc	3,737242818	0,03356817
Il4	-3,643321404	0,038134857
Ccr2	-6,631600505	0,040740731

Supplementary Table 5: Data from Figure 3G

Protein IDs	Protein names	Gene name	Mol. weight [kDa]	LOG2 (Ube2g1/ctr)
Bait protein				
P62254;D6RES1	Ubiquitin-conjugating enzyme E2 G1;Ubiquitin-conjugating e	Ube2g1	19,509	8,387606404
Significantly enriched proteins (SigB)				
A0A0A6YW67;E9Q9J0;E9Q5F6;Q5SX22	Ubiquitin-60S ribosomal protein L40;Ubiquitin;60S ribosoma	Uba52	8,7279	5,688760512
E0CYW8		Creb3	5,5726	6,660076799
Q8C8I3;E9PUG7;A0A0R4J298;Q61211	Eukaryotic translation initiation factor 2D	Eif2d	50,089	3,842720958
Q922H1	Protein arginine N-methyltransferase 3	Prmt3	59,902	4,34686287
Q9ES97	Reticulon-3	Rtn3	103,88	4,240909003
G5E902;Q8VEM8	Phosphate carrier protein, mitochondrial	Slc25a3	39,736	4,240909003
P56873	Sjoegren syndrome/scleroderma autoantigen 1 homolog	Sssca1	21,336	4,240909003
Q99KF1	Transmembrane emp24 domain-containing protein 9	Tmed9	27,127	4,240909003
Slightly enriched proteins (LOG2 (Ube2g1/ctr)>2)				
Q61696;P17879	Heat shock 70 kDa protein 1A;Heat shock 70 kDa protein 1B	Hspa1a	70,078	3,268238584
P16627	Heat shock 70 kDa protein 1-like	Hspa1l	70,636	2,311084
P17156	Heat shock-related 70 kDa protein 2	Hspa2	69,641	2,815526742
Q3U2G2;Q61316	Heat shock 70 kDa protein 4	Hspa4	94,208	2,440637893
A0A0N4SUH8;Q9QZ23	NFU1 iron-sulfur cluster scaffold homolog, mitochondrial	Nfu1	28,654	3,291648515
Q9DBU3	Serine/threonine-protein kinase RIO3	Riok3	58,704	4,014575907
P61982	14-3-3 protein gamma;14-3-3 protein gamma, N-terminally p	Ywhag	28,302	2,352143664
A0A1W2P6R9;A0A0R4J211;G5E825;Q9	Rho guanine nucleotide exchange factor 25	Arhgef25	64,116	2,03048796
Q9CQQ7;A0A0G2JGX3	ATP synthase F(0) complex subunit B1, mitochondrial	Atp5f1	28,948	2,567060961
A0A0R4IZW8;O88456;A0A0R4J1C2	Calpain small subunit 1	Capns1	28,406	2,179152842
Q62426	Cystatin-B	Cstb	11,045	2,777159627
Q8BH95	Enoyl-CoA hydratase, mitochondrial	Echs1	31,474	3,736075522
Q9DCM0	Persulfide dioxygenase ETHE1, mitochondrial	Ethe1	27,738	3,313081915
P19157;P46425	Glutathione S-transferase P 1;Glutathione S-transferase P 2	Gstp1	23,609	3,462575024
P00493	Hypoxanthine-guanine phosphoribosyltransferase	Hprt1	24,57	2,893240508
F6RJV6;Q9JJK2	LanC-like protein 2	Lancl2	49,742	3,042036104
A2A813;Q99LX0;A2A815;A2A817;A2A81	Protein DJ-1	Park7	18,474	2,307930944
Q8K183	Pyridoxal kinase	Pdxk	35,015	2,198238424
A0A1W2P6Q4;P12382	ATP-dependent 6-phosphofructokinase, liver type	Pfkl	7,088	2,225597656
P20108	Thioredoxin-dependent peroxide reductase, mitochondrial	Prdx3	28,127	3,772640024
P49722	Proteasome subunit alpha type-2	Psma2	25,926	3,8081003
P35235	Tyrosine-protein phosphatase non-receptor type 11	Ptpn11	68,46	2,075190725
Q8K2B3	Succinate dehydrogenase [ubiquinone] flavoprotein subunit,	Sdha	72,585	2,490640739
Q9CQA3	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, n	Sdhb	31,814	2,448896515
Q9QZD8	Mitochondrial dicarboxylate carrier	Slc25a10	31,715	2,753724829
A2AQU8;Q9D975	Sulfiredoxin;Sulfiredoxin-1	Srxn1	16,063	3,610604168
A0A1B0GR11;Q93092	Transaldolase	Taldo1	42,151	3,286025997
P17751;H7BXC3	Triosephosphate isomerase	Tpi1	32,191	3,035519396
D3Z6I8;E9Q7Q3;A0A0R4J1P2;P21107;I	Tropomyosin alpha-3 chain	Tpm3	28,723	2,425450645
P68510	14-3-3 protein eta	Ywhah	28,211	3,529074473