Supplementary Materials

Autophagy in endothelial cells regulates their haematopoiesis-supporting ability

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Supplementary methods

Real-time quantitative polymerase chain reaction (qRT-PCR)

The relative mRNA levels of *Beclin-1* (forward primer: 5′-CTGGACACTCAGCTCAACGTCA-3′; reverse primer: 5′-CTCTAGTGCCAGCTCCTTTAGC-3′), *JAG1* (forward primer: 5′-TGCTACAACCGTGCCAGTGACT-3′; reverse primer: 5′-TCAGGTGTGTCGTTGGAAGCCA-3′), *CSF-1* (forward primer: 5′-TGAGACACCTCTCCAGTTGCTG-3′; reverse primer: 5′-GCAATCAGGCTTGGTCACCACA-3′), *CSF-2* (forward primer: 5′-GGAGCATGTGAATGCCATCCAG-3′; reverse primer: 5′-CTGGAGGTCAAACATTTCTGAGAT-3′), *CSF-3* (forward primer: 5′-TCCAGGAGAAGCTGGTGAGTGA-3′; reverse primer: 5′-

CGCTATGGAGTTGGCTCAAGCA-3′), *ETS1* (forward primer: 5′-GAGTCAACCCAGCCTATCCAGA-3′; reverse primer: 5′-GAGCGTCTGATAGGACTCTGTG-3′), *IL-7* (forward primer: 5′-GACAGCATGAAAGAAATTGGTAGC-3′; reverse primer: 5′-CAACTTGCGAGCAGCACGGAAT-3′), *DLL1* (forward primer: 5′-TGCCTGGATGTGATGAGCAGCA-3′; reverse primer: 5′-ACAGCCTGGATAGCGGATACAC-3′), *THPO* (forward primer: 5′-CCAGAGGTTCACCCTTTGCCTA-3′; reverse primer: 5′-CCAGAATGTCCTGTGCCTTGGT-3′), *CXCL-12* (forward primer: 5′-CTCCAAACTGTGCCCTTCAGA-3′; reverse primer: 5′-CTCCAAACTGTGCCCTTCAGA-3′), *VEGFR2* (forward primer: 5′-GGAACCTCACTATCCGCAGAGT-3′; reverse primer: 5′-CCAAGTTCGTCTTTTCCTGGGC-3′) and *E-selectin* (forward primer: 5′-TGTTTGGCACTGTGTGCAAG-3′; reverse primer: 5′-TGGGAGCTTCACAGGTAGGT-3′) between Beclin-1 knockdown group and control in HUVECs (N=6) were analyzed. Normalized levels of the *Beclin-1, CSF-1, CSF-2, CSF-3, JAG1, ETS1, IL-7* , *DLL1*, *THPO*, *CXCL-12*, *VEGFR2* and *E-selectin* ratios in the qRT-PCR assays were evaluated through comparisons with the *Actin beta* (ACTB) levels (forward primer: 5′-GATCATTGCTCCTCCTGAGC-3′; reverse primer: 5′-CGTCATACTCCTGCTTGCTG-3′).

Supplementary Figures

Figure S1

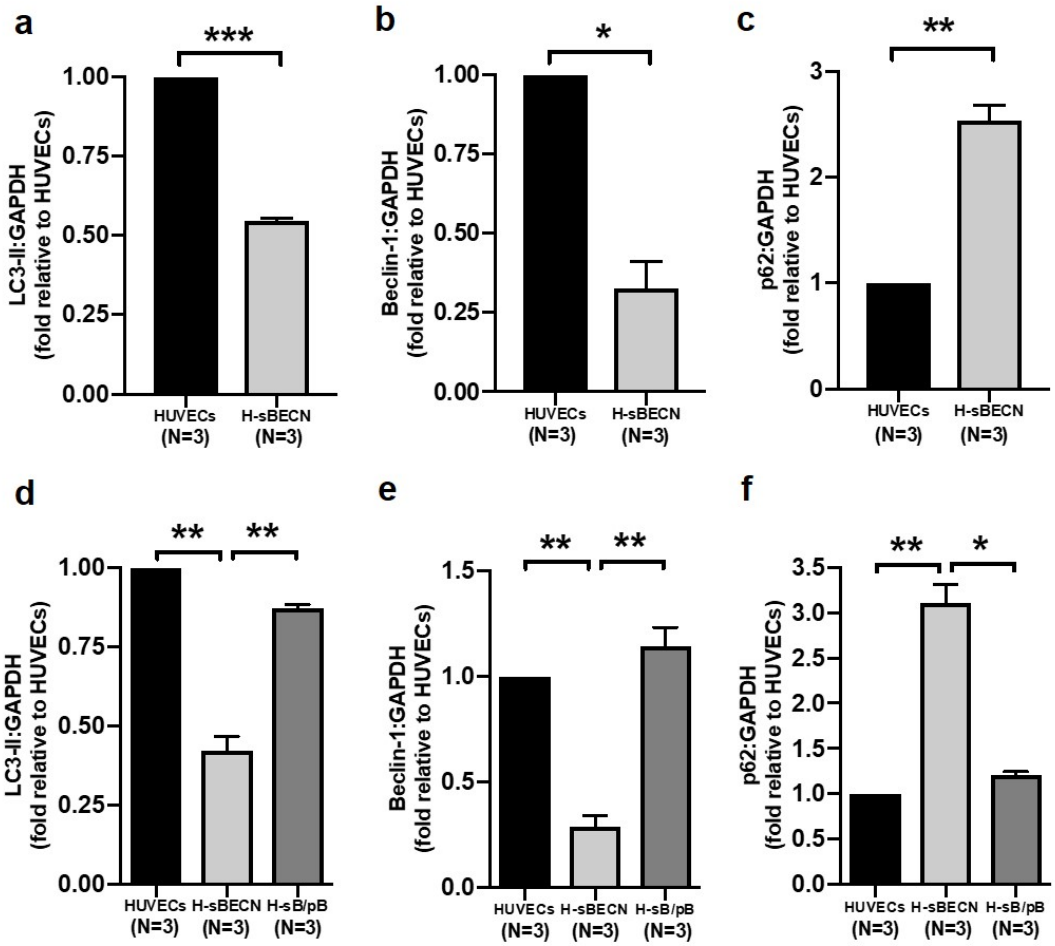

Fig.S1: The quantitative analysis of western blots of intracellular LC3-II, Beclin-1 and p62. (a) The quantitative analysis of LC3-II in the Beclin-1 knockdown group (H-sBECN) and the control group. **(b)** The quantitative analysis of Beclin-1 in the Beclin-1 knockdown group (H-sBECN) and the control group. **(c)** The quantitative analysis of p62 after Beclin-1 knockdown (H-sBECN) and overexpression (H-sB/pB) in HUVECs in the Beclin-1 knockdown group (H-sBECN) and the control group. **(d)** The quantitative analysis of LC3-II after

Beclin-1 knockdown (H-sBECN) and overexpression (H-sB/pB) in HUVECs and the control group. **(e)** The quantitative analysis of Beclin-1 after Beclin-1 knockdown (H-sBECN) and overexpression (H-sB/pB) in HUVECs and the control group. **(f)** The quantitative analysis of p62 after Beclin-1 knockdown (H-sBECN) and overexpression (H-sB/pB) in HUVECs and the control group.Wilcoxon's test for paired data was used to identify drug effects. All *P*-values <0.05 were considered significant and provided in the figure. **P*<0.05, ***P*<0.005, ****P*<0.0005.

Figure S2

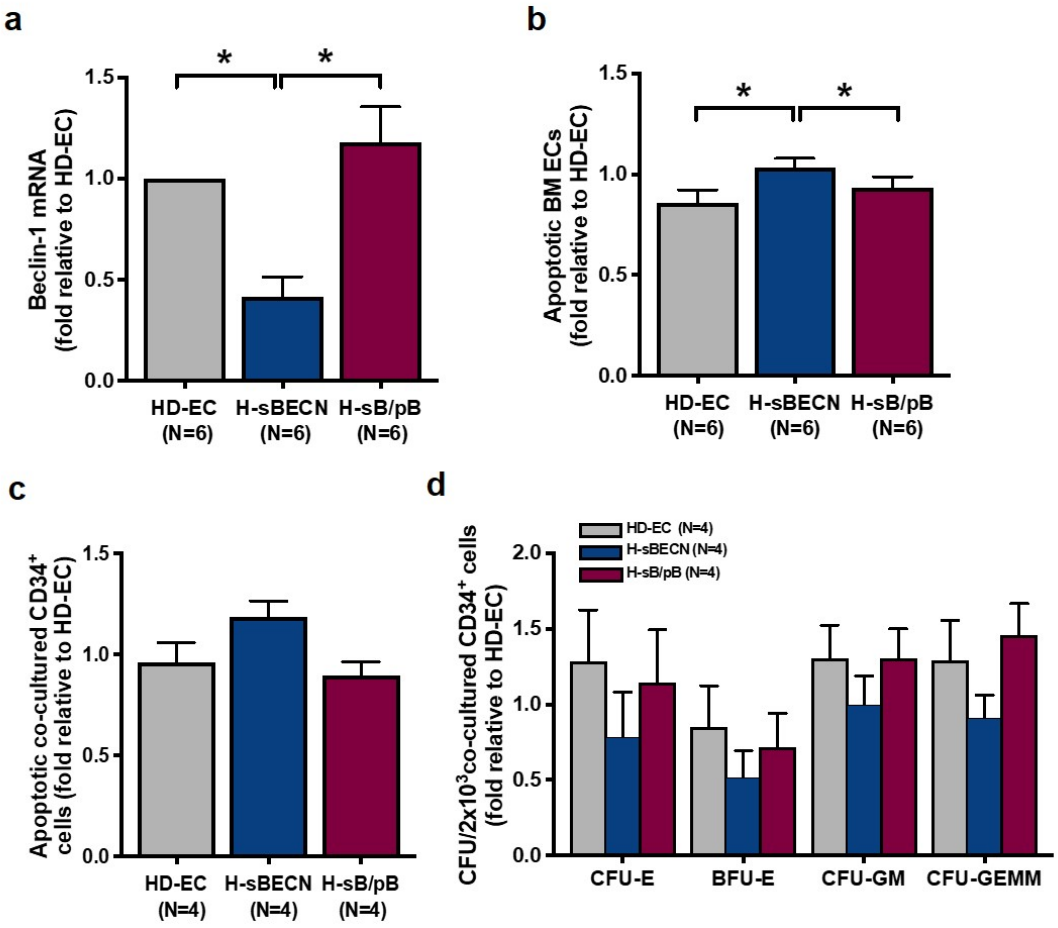

Fig.S2: The impaired ability of primary bone marrow(BM) endothelial cells(ECs) derived from healthy donors was restored by activating autophagy via Beclin-1 upregulation. (a) *Beclin-1* mRNA levels were analysed by qRT-PCR after Beclin-1 knockdown (H-sBECN) and overexpression (H-sB/pB) in primary BM ECs derived from healthy donors(HD-EC). **(b)** The apoptosis rates of primary BM ECs in the H-sBECN, H-sB/pB and HD-EC groups were analysed by flow cytometry. **(c)** The apoptosis rates of BM $CD34^+$ cells and **(d)** the CFU plating efficiency of BM $CD34^+$ cells from healthy

donors were analysed after 7 days of coculture with primary BM ECs in the H-sBECN, H-sB/pB and HD-EC groups. Wilcoxon's test for paired data was used to identify drug effects. All *P*-values <0.05 were considered significant and are provided in the figure. **P*<0.05, ***P*<0.005, ****P*<0.0005.

Figure S3

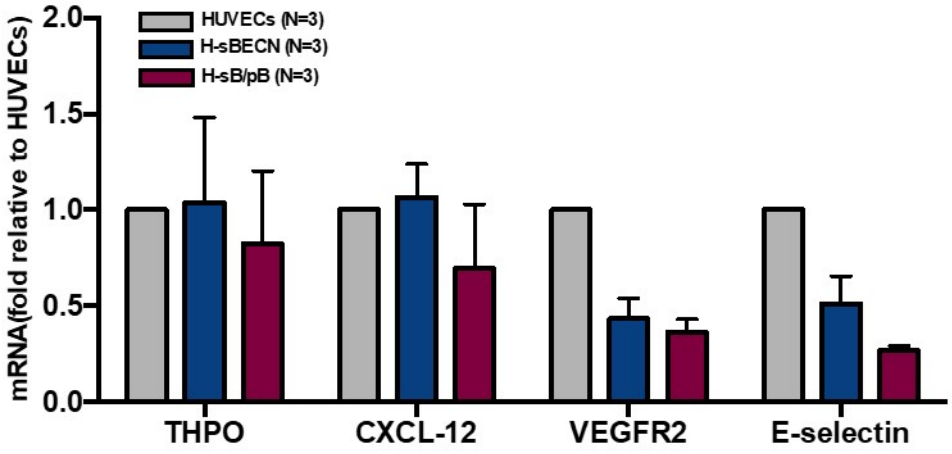

Fig.S3: Haematopoiesis-regulating genes, which were not modified by Beclin-1 knockdown or Beclin-1 overexpression in HUVECs. The relative mRNA levels of *THPO*, *CXCL-12*, *VEGFR2* and *E-selectin* in the H-sBECN, H-sB/pB and control groups were determined using qRT-PCR.

Supplementary Table 1. Characteristics of allo-HSCT patients with PGF

Characteristics	PGF* (N=40)	GGF* (N=40)	*P*-Value**
BM evaluated time (post-HSCT days)	72(26-280)	70(24-282)	0.90
Blood cell count			
Median WBC ($\times 10^9$/L) (range)	2.04(0.10-5.10)	4.95(2.50-9.80)	<0.0001
Median ANC ($\times 10^9$/L) (range)	1.20(0.00-3.30)	3.60(1.50-8.90)	<0.0001
Median Hb (g/L) (range)	77(50-124)	118(80-157)	<0.0001
Median PLT ($\times 10^9$/L) (range)	43(6-121)	120(50-215)	<0.0001
Age at HSCT (years, median, range)	36(18-61)	36(18-57)	0.82
Gender (male/female)	26/14	23/17	0.65
Underlying disease			1.00
AML	18	25	
ALL	14	11	
MDS	8	4	
Status at HSCT			0.67
Standard-risk	27	32	
High-risk	13	8	
Source of stem cell			1.00
BM and PB	40	40	
Transplanted total nucleated cell dose ($\times 10^8$/ kg, median, range)	7.69(3.82-12.37)	7.89(3.98-12.52)	0.78
Transplanted $CD34^+$ cell dose ($\times 10^6$/ kg, median, range)	2.69(0.64-6.14)	2.79(0.76-5.69)	0.74
Donor match			1.00
HLA-identical sibling donor	0	0	
HLA-partially matched related donor	40	40	
Sex mismatch			0.54
Female to male	9	6	
Female to female	4	3	
Male to female	9	13	
Male to male	18	18	
ABO mismatch			0.35
No	27	17	
Minor	2	5	
Major	11	18	
Pre-HSCT cycles of chemotherapy	4 (0-7)	4(0-7)	0.67
Conditioning			1.00
BU/CY	0	0	
BU/CY+ATG	40	40	

History of CMV reactivation	29	23	0.10
Onset of CMV reactivation (days, median, range)	23(9-89)	19(7-75)	0.54
CMV reactivation treated with ganciclovir	12	12	1.00
History of GvHD	18	21	0.82
Onset of aGvHD (days, median, range)	14(2-73)	10(2-72)	0.90

***** Group matching criteria included age at HSCT (±1years), pre-HSCT cycles of chemotherapy (±1cycle), disease status at HSCT and BM microenvironment evaluated time after HSCT (±5 days). For each case, one GGF control was randomly selected from the same cohort at which the PGF occurred ("risk-set sampling").

*** *** The continuous variables were compared using the Mann-Whitney U-test, and the differences in frequency between the 2 groups were compared using the chi-square test. The criterion for statistical significance was $P<0.05$.

Abbreviations: allo-HSCT indicates allogeneic hematopoietic stem cell transplantation; aGvHD, acute graft-versus-host disease; PGF, poor graft function; GGF, good graft function; BM, bone marrow; PB, peripheral blood; WBC, white blood cell; ANC, absolute neutrophil cell; Hb, hemoglobin; PLT, platelet; AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; MDS, myelodysplastic syndrome; HLA, human leukocyte antigen; CMV, cytomegalovirus.