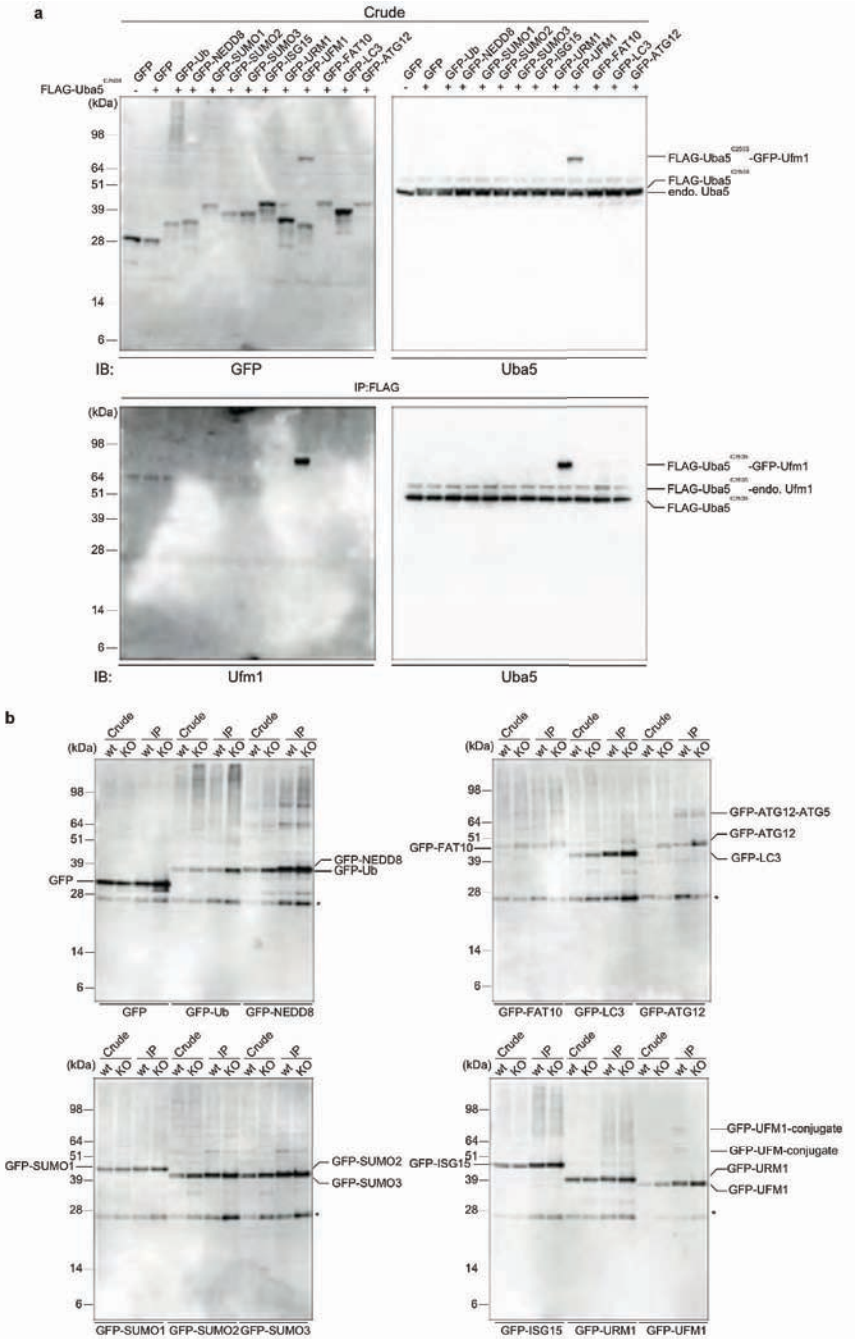
Supplementary information

The Ufm1-activating enzyme Uba5 is indispensable for erythroid differentiation in mice

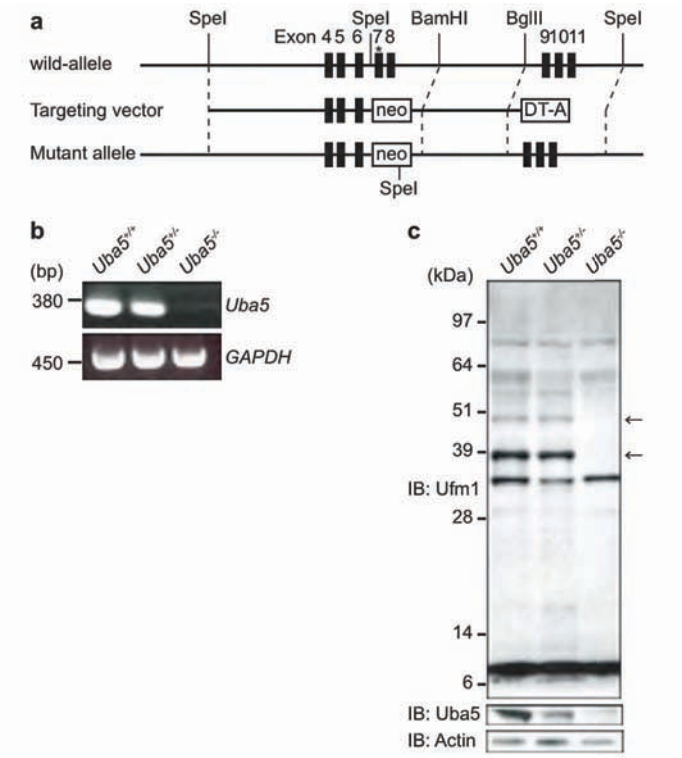
Kanako Tatsumi[1, 2], Harumi Yamamoto-Mukai[2], Ritsuko Shimizu[3], Satoshi Waguri[4], Yu-Shin Sou[1], Ayako Sakamoto[1], Choji Taya[5], Hiroshi Shitara[5], Takahiko Hara[6], Chin Ha Chung[7], Keiji Tanaka[1], Masayuki Yamamoto[2] and Masaaki Komatsu[1, 8]

[1]Protein Metabolism Project, [5]Laboratory of Mouse Models for Human Heritable Diseases, [6]Stem Cell Project, Tokyo Metropolitan Institute of Medical Science, Setagaya-ku, Tokyo 156-8506, Japan
[2]Department of Medical Biochemistry, [3]Department of Molecular Hematology, Tohoku University Graduate School of Medicine, Aoba-ku, Sendai 980-8575, Japan
[4]Department of Anatomy and Histology, Fukushima Medical University School of Medicine, 1 Hikarigaoka, Fukushima 960-1295, Japan
[7]School of Biological Science, Seoul National University, Seoul 151-742, Korea
[8]PRESTO, Japan Science and Technology Corporation, Kawaguchi 332-0012, Japan

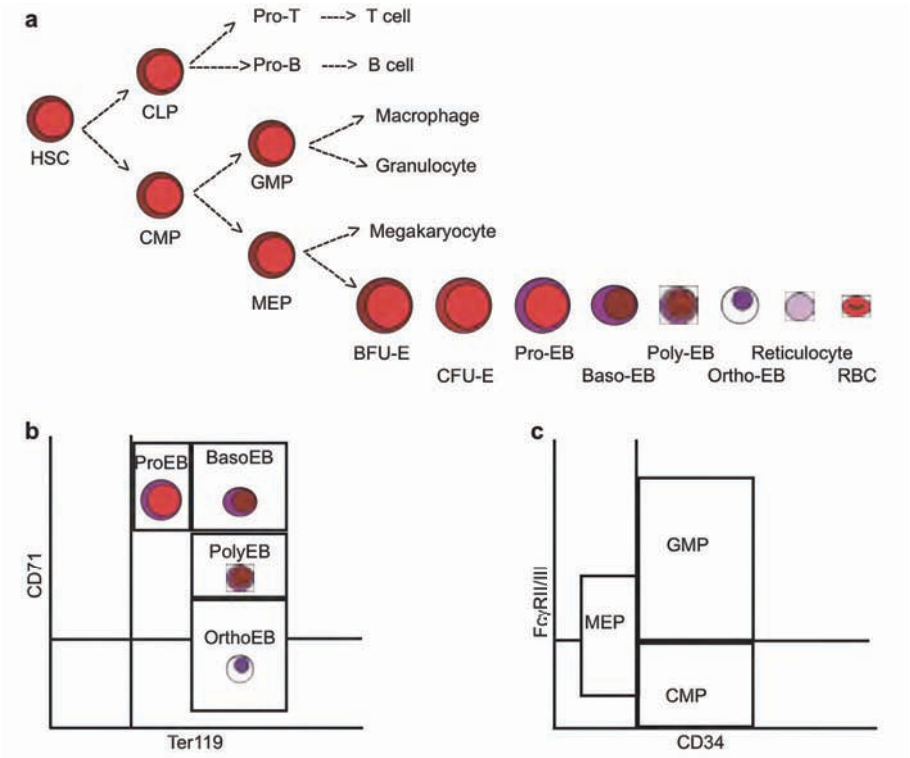
Supplementary figures



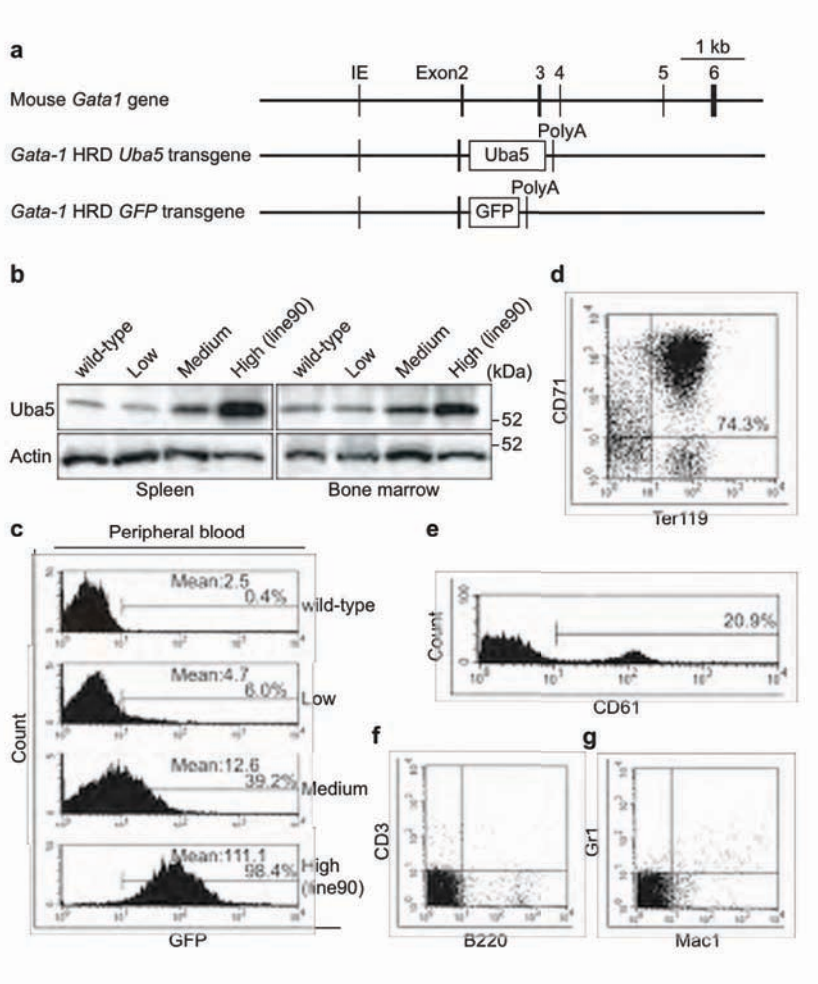
Supplementary Figure S1. Uba5 activates Ufm1 but not other ubiquitin-like proteins (UBLs). (**a**) Immunoblot analysis. FLAG-Uba5^{C250S} together with each GFP-UBL were expressed in HEK293T cells. The cell lysates (top panels) and immunoprecipitants by anti-FLAG antibody (bottom panels) were subjected to SDS-PAGE, followed by immunoblot analysis with the indicated antibodies. (**b**) Immunoblot analysis of UBL-conjugations in *Uba5*-knockout mouse embryonic fibroblasts (MEFs). GFP-tagged UBLs were expressed in wild-type or *Uba5*-deficient MEFs. The cell lysates and immunoprecipitants by anti-GFP antibody were subjected to SDS-PAGE followed by immunoblot analysis with anti-GFP antibody. Asterisks: irrelevant bands.



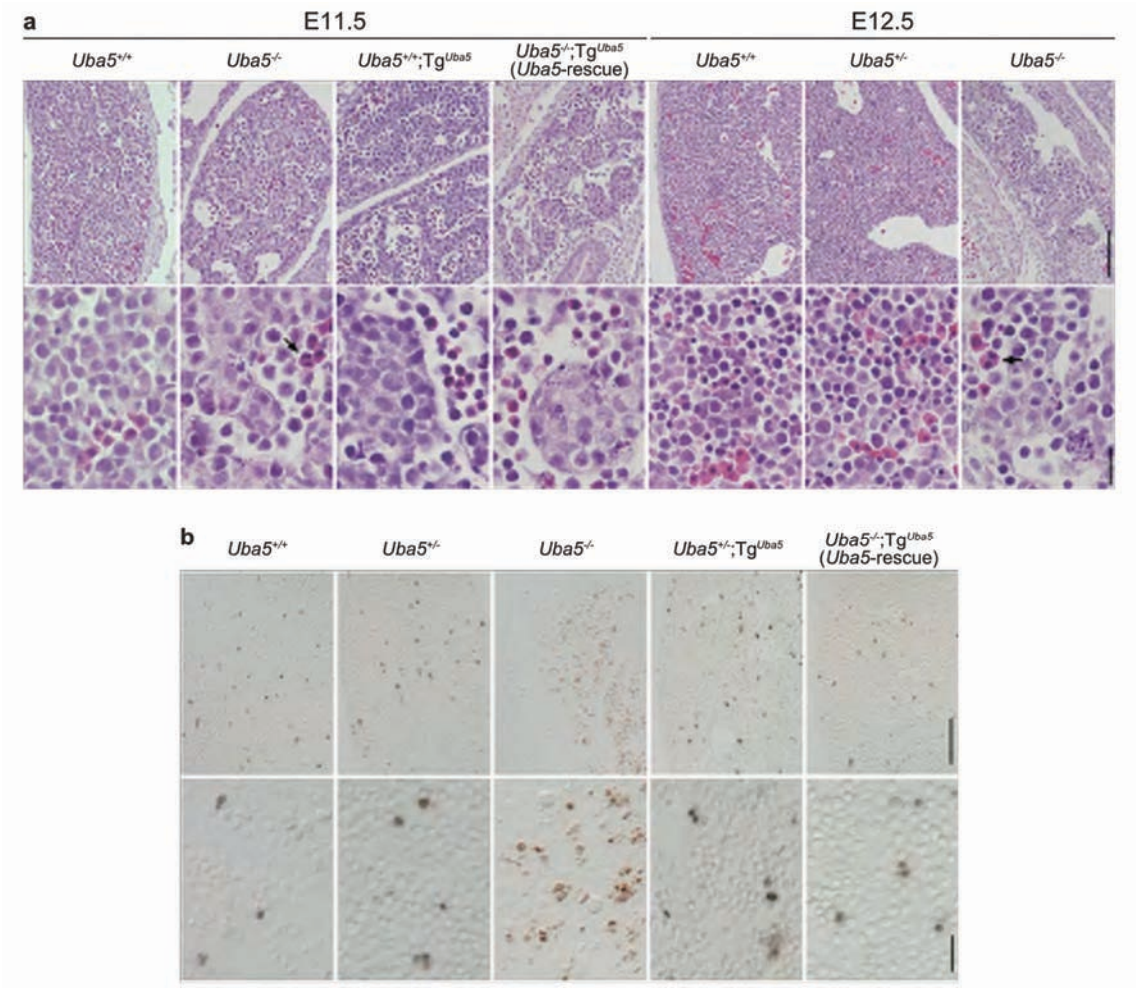
Supplementary Figure S2. Generation of *Uba5* knockout mice. (**a**) Schematic representation of the targeting vector and the targeted allele of the *Uba5* gene. The coding exons numbered in accordance with the initiation site as exon 1 are depicted by black boxes. Asterisk: the essential cysteine residue on exon 7. SpeI, SpeI sites; BamHI, BamHI site; BglII, BglII site; neo, neomycin resistance gene cassette; DT-A, diphtheria toxin gene. (**b**) Expression of Uba5 transcript. Total RNA was extracted from each genotyped MEF. The Uba5 transcript level was detected by RT-PCR. (**c**) Immunoblot analysis of Uba5 in MEFs. The lysates were immunoblotted with anti-Ufm1, anti-Uba5 and anti-actin antibodies. Arrows: presumptive target proteins for ufmylation (e.g. 39- and 51-kDa bands).



Supplementary Figure S3. Schemas for erythroid differentiation and FACS analyses. (**a**) Schematic diagram of erythropoiesis from haematopoietic stem cells (HSCs). CMP, common myeloid progenitor; MEP, megakaryocyte/erythroid progenitor; BFU-E, erythroid burst-forming unit; CFU-E, erythroid colony-forming unit; Pro-EB, proerythroblast; Baso-EB, basophilic erythroblast; Poly-EB: polychromatic erythroblast; Ortho-EB, orthochromatic erythroblast; RBC, red blood cell; CLP, common lymphoid progenitor; GMP, granulocyte macrophage progenitor. (**b**) Schematic diagram of Pro-EB ($Ter119^{med}CD71^{high}$), Baso-EB ($Ter119^{high}CD71^{high}$), Poly-EB ($Ter119^{high}CD71^{med}$) and Ortho-EB ($Ter119^{high}CD71^{low}$) populations by FACS analysis. (**c**) Schematic diagram of CMP ($CD34^{high}Fc\gamma RII/III^{low}$), MEP ($CD34^{low}Fc\gamma RII/III^{low}$) and GMP ($CD34^{high}Fc\gamma RII/III^{high}$) populations by FACS analysis.



Supplementary Figure S4. Generation of mouse lines expressing *Uba5* in erythroid lineage. (**a**) Genomic structure of the mouse *Gata-1* gene and construction of the *Gata-1*-HRD-*Uba5* and *Gata-1*-HRD-*GFP* transgenes. The coding exons numbered in accordance with the initiation site (IE) as exon 1 are depicted by black boxes. (**b**) Expression of Uba5 in the transgenic lines. Whole cell lysates were prepared from the spleen and bone marrow of 4-week-old transgenic mice and subsequently subjected to immunoblot analysis with an anti-Uba5 antibody. An anti-actin antibody was used as an internal control. (**c**) Expression of GFP in the transgenic lines. GFP-positive peripheral blood samples were analysed by FACS. Percent values denote the proportion of GFP-positive cells in peripheral blood of 4-week-old transgenic mice. (**d**, **e**, **f** and **g**) GFP-positive peripheral blood cells were sub-fractionated into erythroid cells (**d**), CD61-expressing cells representing megakaryocytes (**e**), CD3- and B220-expressing cells representing lymphoid cells (**f**), and Gr1- and Mac1-expressing cells representing myeloid cells (**g**).



Supplementary Figure S5. Morphological comparison of the different mouse genotypes. (**a**) Haematoxylin & eosin-stained sections of livers from mice with the indicated genotype at E11.5 and E12.5. Arrows: abnormal multinucleated erythrocytes. Bars: top panel, 100 μm; bottom panel, 20 μm. (**b**) TUNEL staining of livers from mice with the indicated genotype at E12.5. Bars: top panel, 100 μm; bottom panel, 20 μm.

Supplementary table

Supplementary Table S1. List of E1s and their corresponding UBLs and E2s in humans.

E1 enzyme	UBL	E2 enzyme
UBA1	UBIQUITIN	Many
UBA2-AOS1	SUMO1, 2, 3	UBC9
UBA3-ULA1	NEDD8	UBC12
UBA4	URM1	Unknown
UBA5	UFM1	UFC1
UBA6	UBIQUITIN, FAT10	USE1
UBE1L	ISG15	UBCH8
ATG7	ATG12, LC3	ATG10, ATG3