Supplementary Material

Endoplasmic reticulum selective autophagy alleviates anthracycline-induced cardiotoxicity

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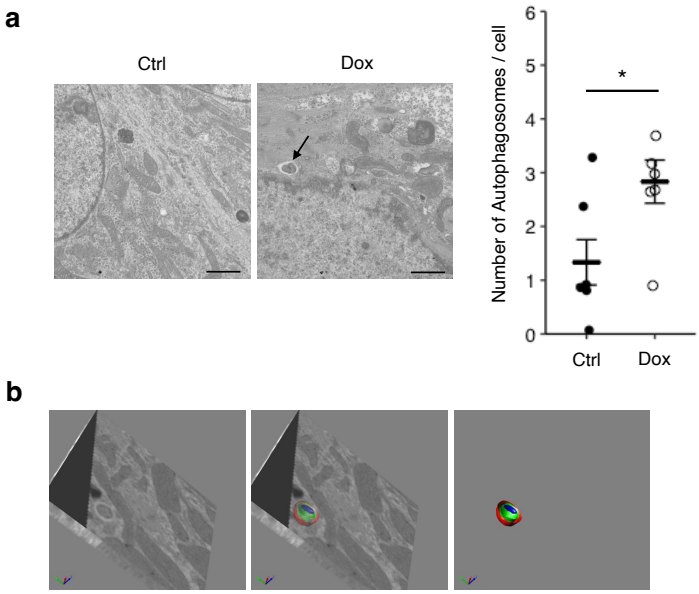
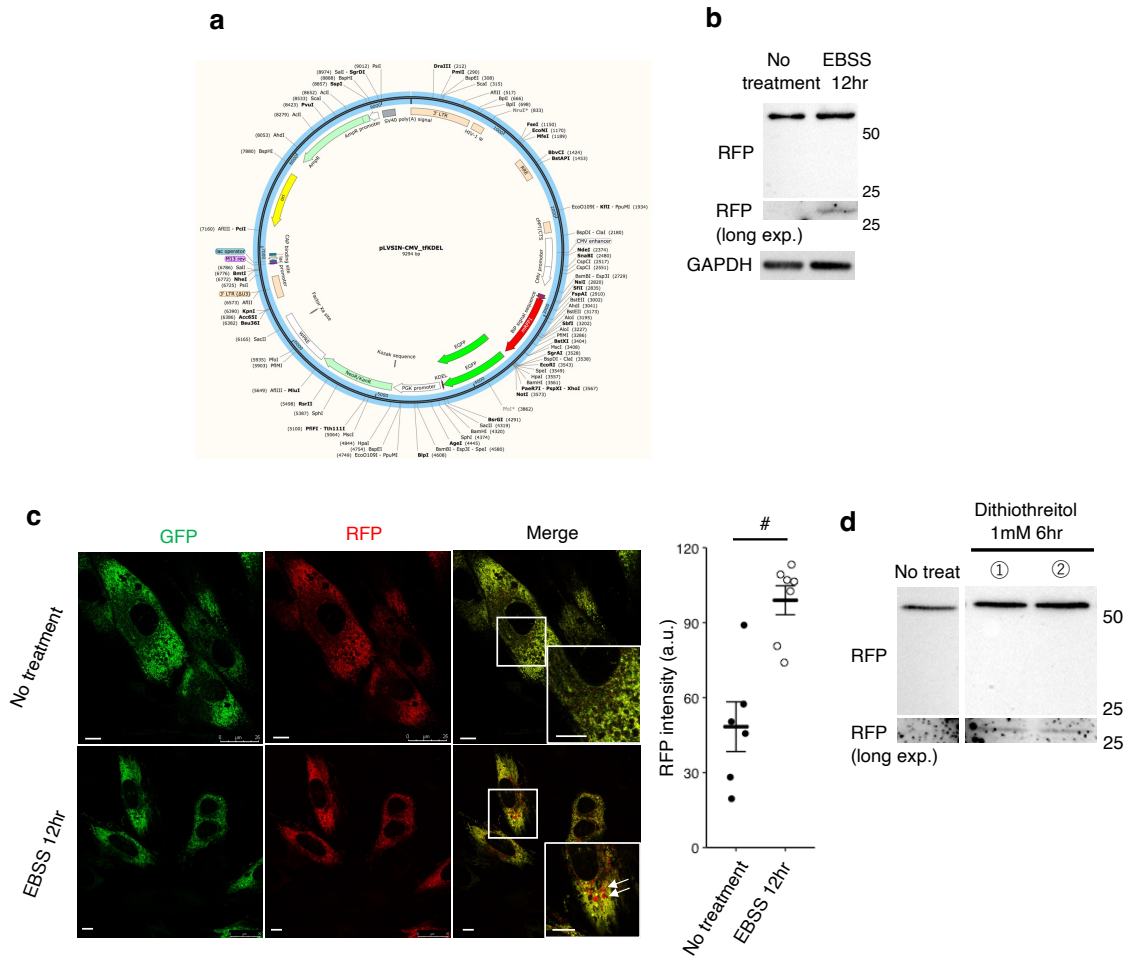


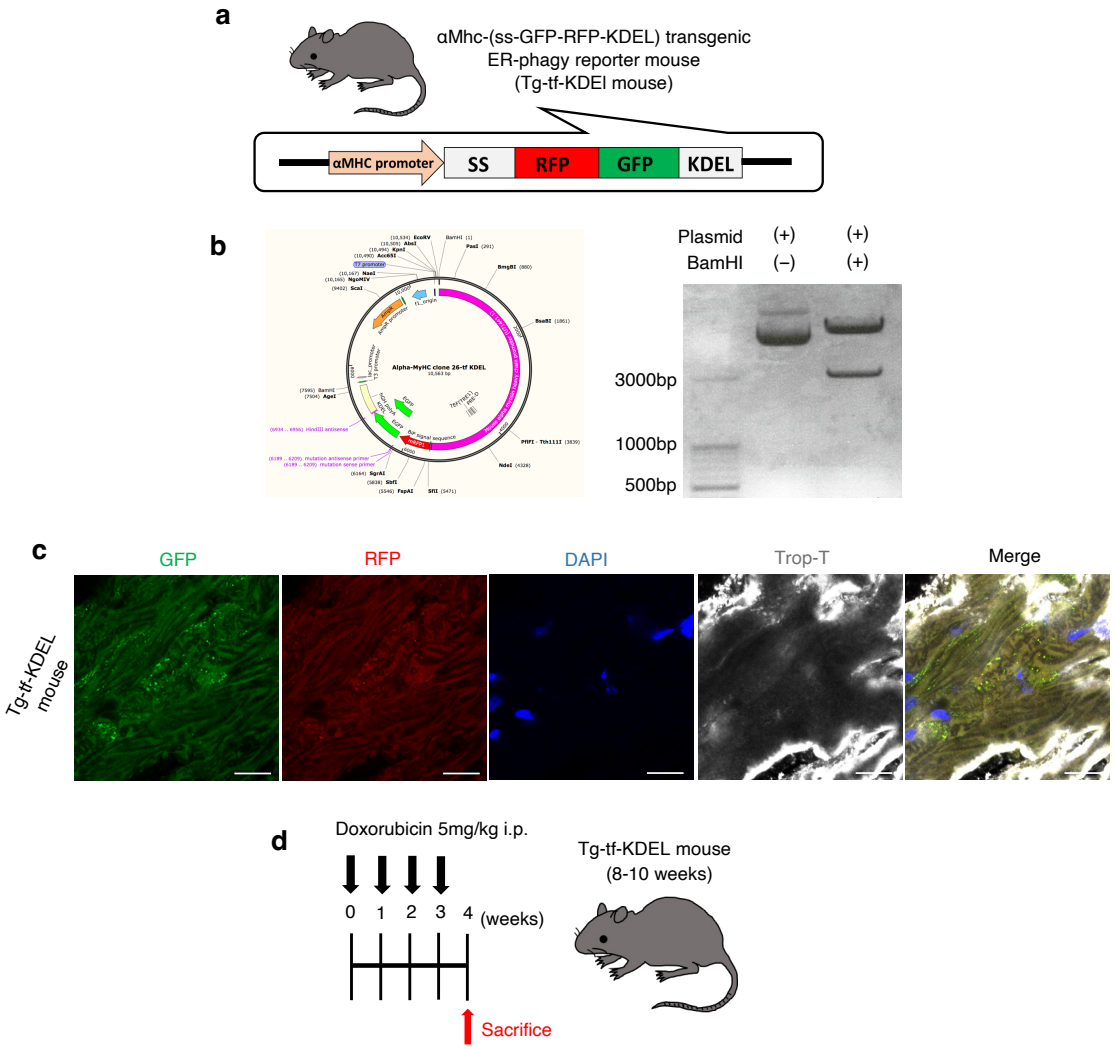
Supplementary Fig. S1 Identification of ER-sequestering autophagosome on electron microscopic analyses

a Representative images of neonatal rat ventricular myocytes, which were captured by a transmission electron microscope, are shown. The numbers of autophagosomes (arrow) per cell were counted and compared between Doxorubicin-treated and non-treated cells. The scale bars represent 1 μm. ($n = 6$, * $p < 0.05$) **b** Scanning electron microscope images from ten consecutive sections, which captured an ER-containing autophagosome (as shown in Fig. 1D), were reconstructed to make a three-dimensional (3D) image. Besides the original 3D image (left), the outer and inner membranes of the autophagosome and ER fragments inside the autophagosome are colored as red, green and blue, respectively (middle). These colored structures are also shown without the EM phase (right).

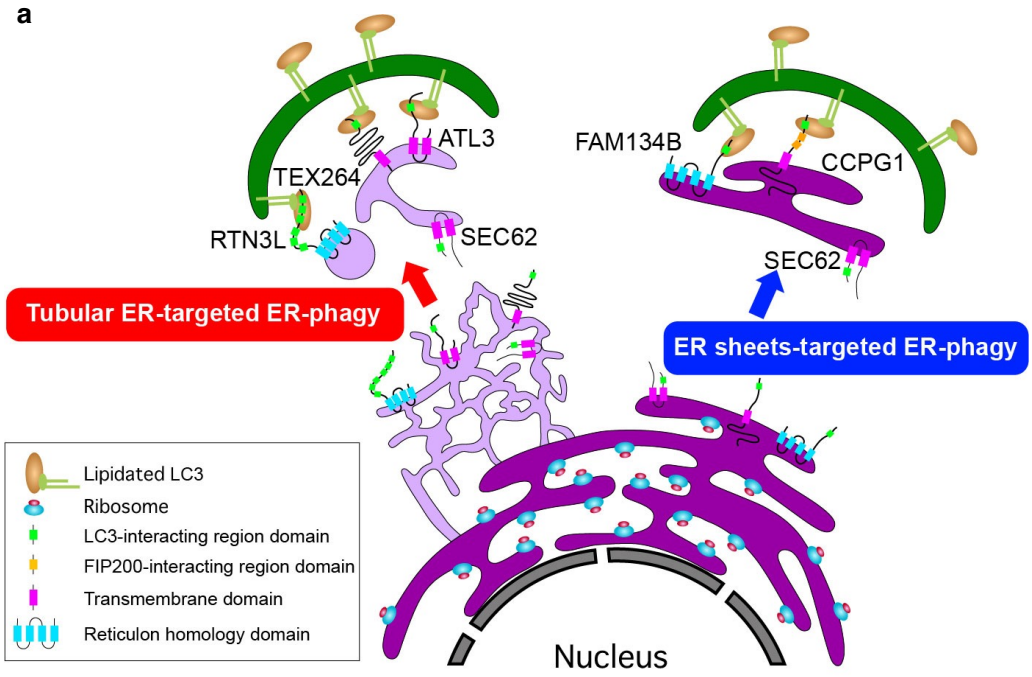


Supplementary Fig.2 Additional information for preparing tf-H9c2 cells

a The map of the plasmid used to generate lentivirus containing the ss-RFP-GFP-KDEL sequence. **b** Lysates of tf-H9c2 cells were immunoblotted for anti-RFP antibodies. Cells were lysed after the indicated treatment. **c** The same tf-H9c2 cells prepared in Supplementary Fig. 2c were visualized on a fluorescence microscope to detect digested RFP signals (arrows). Single RFP intensities were calculated from the obtained images. The scale bar represents 10 μm. (n = 6, # $p < 0.01$) **d** Lysates of tf-H9c2 cells, treated with or without dithiothreitol at 1 mM for 6 h, were immunoblotted for anti-RFP antibodies.

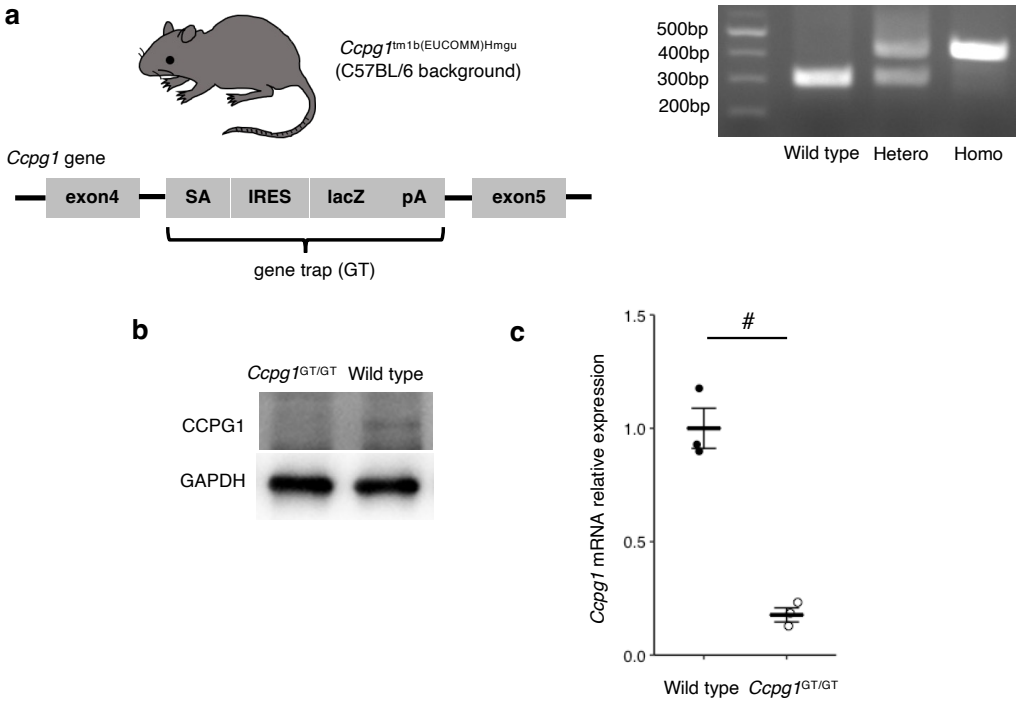


Supplementary Fig. 3 Supplemental information regarding the experiments with Tg-tf-KDEL mice **a** The scheme of C57BL/6 Transgenic-mouse harboring the sequence for cardiomyocyte-specific ER-phagy reporter (Tg-tf-KDEL mouse). **b** The sequence map of the plasmid used to generate the Tg-tf-KDEL mouse. Restriction enzyme digestion confirmed the insertion of the ss-RFP-GFP-KDEL sequence into the plasmid. **c** Representative immunohistopathological images of Tg-tf-KDEL heart. The image shows that RFP and GFP signals overlap with Troponin-T staining. **d** The protocol to induce the Dox cardiomyopathy model. 5 mg/kg of Dox or saline was injected intraperitoneally for four times every week and euthanized a week after the last injection.



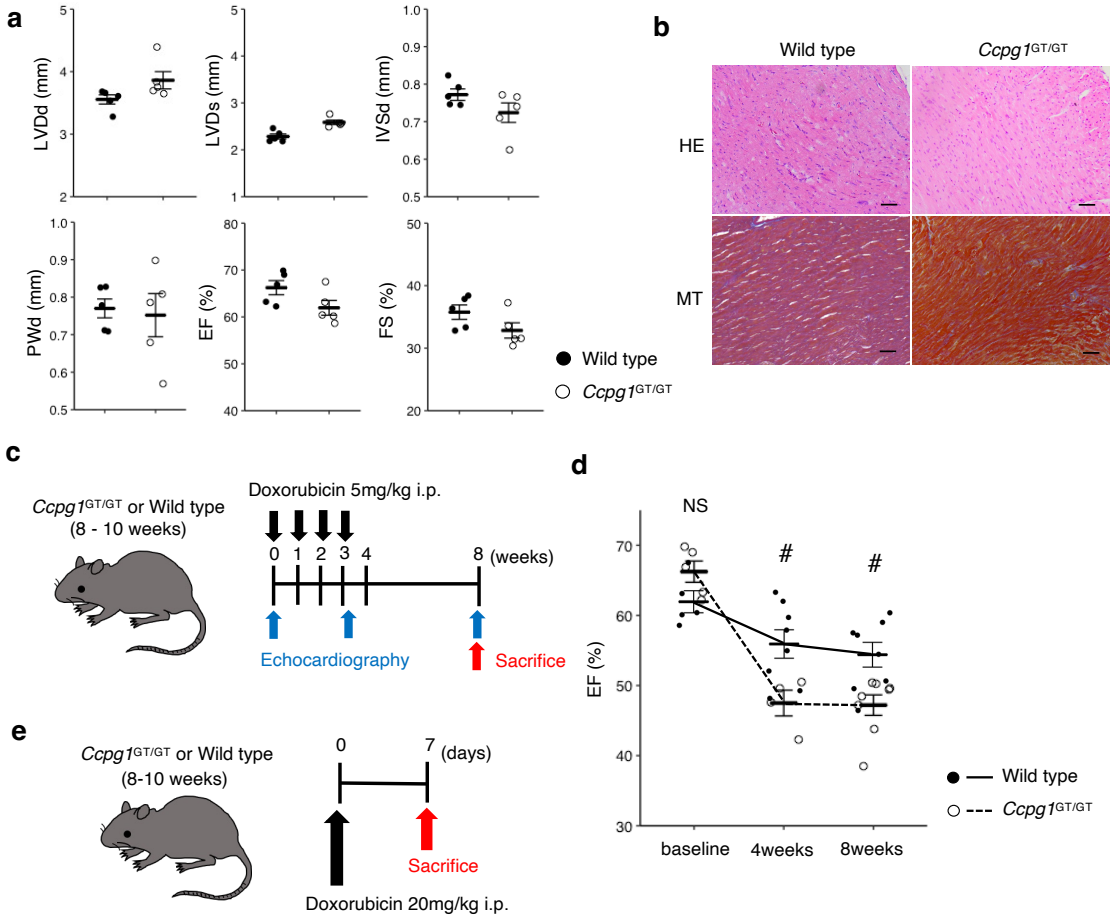
Supplementary Fig. 4 Schematic representation of ER-phagy regulators

a TEX264, FAM134B, ATL3, SEC62 and CCPG1 are reported as ER-phagy receptors to regulate the activity of ER-phagy. The location and the conceptual morphology of each protein are shown in the schema.



Supplementary Fig. 5 Development of CCPG1-hypomorphic (*Ccpg1*$^{GT/GT}$) mice

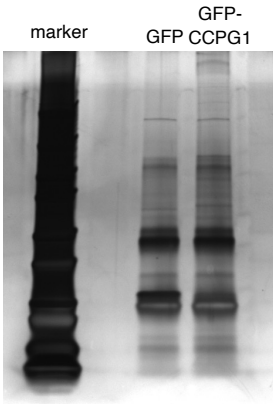
a The scheme of a target construct expressed in the *Ccpg1*$^{GT/GT}$ mouse. The *Ccpg1* mutant gene harboring this construct results in a gene trap at the *LacZ* sequence between exons 4 and 5. The pattern of genotyping PCR results is shown using the primers listed in Supplementary Table 2. SA, splice acceptor; IRES, internal ribosome entry site; pA, poly A; Hetero, heterozygote; Homo, homozygote. **b** Lysates of homogenized hearts from *Ccpg1*$^{GT/GT}$ and wild-type mice were immunoblotted for anti-CCPG1 and anti-GAPDH antibodies. **c** Transcripts were collected from heart tissue homogenates of wild-type and *Ccpg1*$^{GT/GT}$ mice, and the gene expression of *Ccpg1* (normalized to *Gapdh*) was compared. (n = 3, # $p < 0.01$)



Supplementary Fig. 6 Assessment of cardiac phenotypes in *Ccpg1*$^{GT/GT}$ mouse experiments

a The baseline echocardiographic measurements were compared between wild type and *Ccpg1*$^{GT/GT}$ mice (n = 5). **b** Representative images of HE and MT stained sections of baseline wild type and *Ccpg1*$^{GT/GT}$ hearts are shown. The scale bar represents 50 μm. **c** Scheme of the protocol. 5 mg/kg of doxorubicin was injected intraperitoneally for four times every week and euthanized 5 weeks after the last injection. Echocardiography was performed on the day of the initial Dox injection and 4 and 8 weeks, respectively, after the Dox treatment was started. **d** The transitions of EF along the time course are shown. The values of EF were compared between both groups at baseline, 4 and 8 weeks, respectively, after the Dox treatment. (# $p < 0.05$) **e** Scheme of the high-dose Dox protocol. A total of 20 mg/kg of Dox was injected intraperitoneally once and euthanized a week after the injection. LVDd, left ventricular end-diastolic diameter; LVDs, left ventricular end-systolic diameter; IVSd, intraventricular septum end-diastolic diameter; PWd, posterior wall end-diastolic diameter; EF, Ejection fraction; FS, Fraction shortening

a



b

Protein name	Protein symbol
RB1-inducible coiled-coil protein 1	RB1CC1
Serine/threonine-protein kinase TBK1	TBK1
Serine/threonine-protein kinase ULK1	ULK1
Autophagy-related protein 13	ATG13
Autophagy-related protein 101	ATG101
Kelch-like ECH-associated protein 1	KEAP1
Translocation protein SEC62	SEC62
Next to BRCA1 gene 1 protein	NBR1
Autophagy-related protein 2 homolog B	ATG2B
Beclin-1	BECN1

Supplementary Fig. 7 Additional data of the co-immunoprecipitation analyses for identifying CCPG1-interactors

a An image of silver-stained gel is shown. Eluted samples from immunoprecipitation experiments using GFP antibody-attached magnetic beads were subjected to SDS-PAGE and stained. **b** The list of selective autophagy-related proteins identified as possible interactors with CCPG1 by the proteomic analyses.

Supplementary Video 1. The 3D-reconstructed appearance of autophagosome containing ER fragments
The video shows the overall appearance of the 3D-reconstructed image of ER-containing autophagosome, shown in Supplementary Figure. 1A.

Supplementary Video 2. Live imaging of ER-phagy in H9c2 cells
After 24 h of treatment with Dox (1 μM), live tf-H9c2 cells were visualized with fluorescence microscopy. Time-lapse videos were acquired by capturing consecutive images every 3 seconds for 1 h. Each video was edited, and the representative one was shown. Arrows in the video show the transition of an autophagosome containing ER fragments (yellow punctum) to an autolysosome (red punctum), suggesting the moment of ER-phagy.