Figure S1. Supplement related to Figure 1.

(A) The presence of the EV-markers CD81, CD63, CD9 and phosphatidylserine on purified particles isolated by ultracentrifugation from HEK293 and B-16V cell supernatants was verified using bead-assisted flow cytometry. The data is representative of three independent experiments. Specific antibodies were either directly labelled (-) or probed with a secondary fluorochrome-labelled antibody (+).
(B) Representative size distribution histograms obtained by NTA analysis of EVs isolated from HEK293 and B-16V cell supernatants either non-treated or treated with 100nM doxo or 100nM LBH for 16 hours. NTA confirmed that the purified EVs with or without treatment corresponded to small particles with a mean diameter of about 100-150nm, which are referred to as exosomes by many authors. The data are representative of three independent experiments.

(C) Analysis of BAG6 expression by bead-assisted flow cytometry and immunoblotting with indicated antibodies of HEK293 EVs purified by serial ultracentrifugation at 10k x g (larger EVs up to 1000µm) and 100k x g (exosome-like sized EVs) showing that BAG6 is predominantly associated with 100k x g fraction. The experiment was performed two independent times.

(D) Free flow electrophoresis (FFE) was applied to isolate EVs from HEK293 wild-type cells (WT) and BAG6 overexpressing cells (+BAG6) since the purification of EVs using ultracentrifugation cannot fully exclude the co-precipitation of contaminating soluble proteins. The plot shows the protein concentration by BCA assay and particle concentration by NTA analysis of the separated fractions. EVs associated with BAG6 and with the EV-markers HSP70 and CD81 were specifically detected by bead-assisted flow cytometry in the EV-containing fraction 8, but were absent in the soluble protein-rich fractions 25-33.

(E) Flow cytometric analysis of 7AAD and AnnexinV stainings of HEK293 and B-16V cells either non-treated, treated with 100nM doxo or with 100 nM LBH for 16h to ensure viability of the releasing cells (% AnnexinV/7AAD negative cells is depicted). Viability was checked regularly and data represent two independent experiments.

NT, non-treated; iso, isotype control antibody; 10k, ultracentrifugation fraction at 10k x g; 100k, ultracentrifugation fraction at 100k x g; WT, wild-type; kDa, kilodalton; doxo, doxorubicin; LBH, LBH-589/Panobinostat.
A

B AG6KO clones

kDa WT 3 5 8
170
130
40

BAG6
Actin

BAG6KO
nuc3
Wildtype
CTGGAGCATCTTCTTGGGACGGGCTCTGCTCAGCAACCTCTGTTGACCCCTGCTCTG

BAG6KO
nuc5
Wildtype
TCCGTCATGAAACAAGAGCCCGGAGGCCAGCAGGAGCCAAGGGTGCGGACCCACCACATGA

BAG6KO
nuc8
Wildtype
TCCGTCATGAAACAAGAGCCCGGAGGCCAGGAGCCAAGGGTGCGGACCCACCACATGA

C

B-16V EVs: Hypoxia

NTA

EVs release (x 10^6 particles/cell count)

Normoxia Hypoxia

WT BAG6KO

*** *

BAG6 ELISA

Rel. BAG6 expression (ng BAG6/particle)

NT Hypoxia Heat Shock Hypoxia

WT BAG6KO

* *
D  EVs RNAseq analysis

E  Functional classification of EVs RNAs

F  WCL BAG6KO/WT (log2 FC)

G  kDa

WT  BAG6KO

ANNEXIN A1
CHMP2B
VPS4B
VTA1
Melanosome-like WT-EVs

| Gene names | EV proteome | Cellular proteome |
|------------|-------------|------------------|
|            | log2 FC BAG6KO/WT | q-value | log2 FC BAG6KO/WT | q-value |
| Cnp        | -1.86       | 0.00E+00 | N/A         | 1.00E+00 |
| Atp6v0a1   | -0.52       | 2.15E-02 | -0.21       | 4.10E-01 |
| Atp1b3     | -1.10       | 9.66E-03 | -0.48       | 8.69E-01 |
| Sec22b     | -0.54       | 6.35E-02 | 0.78        | 2.19E-01 |
| Calu       | -1.76       | 0.00E+00 | -0.68       | 1.67E-03 |
| Mreg       | N/A         | 7.85E-01 | N/A         | 8.42E-01 |
| Ppib       | -0.92       | 6.32E-03 | -0.20       | 1.73E-01 |
| Pdia3      | -0.41       | 1.01E-01 | 0.00        | 9.96E-01 |
| Slc3a2     | -0.92       | 0.00E+00 | -0.10       | 6.93E-01 |
| Trpv2      | N/A         | 6.71E-01 | 0.00        | 1.00E+00 |
| Ywhab      | -0.68       | 3.46E-02 | -0.09       | 7.84E-01 |
| Ywhae      | -0.55       | 3.02E-03 | -0.03       | 8.79E-01 |
| Atp6v1b2   | -0.39       | 5.80E-02 | -0.58       | 4.65E-03 |
| Rab27a     | -1.00       | 2.31E-03 | -1.03       | 2.51E-03 |
| Anxa2      | -0.84       | 3.55E-02 | -0.58       | 2.59E-03 |
| Gna13      | -0.39       | 5.63E-02 | -0.20       | 5.73E-01 |
| Oct        | -1.68       | 0.00E+00 | -1.55       | 0.00E+00 |
| Gpnmb      | -1.48       | 3.00E-03 | -0.78       | 1.98E-03 |
| Lamp1      | -1.66       | 0.00E+00 | -0.48       | 3.00E-02 |
| Myo5a      | -1.12       | 0.00E+00 | -0.54       | 6.62E-03 |
| Myo7a      | -0.70       | 1.14E-02 | -0.89       | 0.00E+00 |
| Ncstin     | -0.62       | 6.42E-02 | -1.22       | 0.00E+00 |
| Pdia4      | -1.13       | 2.47E-02 | -0.41       | 1.07E-02 |
| Pdia6      | -0.34       | 1.21E-01 | -0.49       | 7.40E-03 |
| Snd1       | -0.28       | 1.23E-01 | -0.45       | 2.72E-03 |
| Sytl2      | -1.00       | 0.00E+00 | -0.68       | 3.49E-03 |
| Tyr        | N/A         | 8.57E-01 | N/A         | 8.84E-01 |
Exosome-like BAG6KO-EVs

| Gene names | log2 FC BAG6KO/WT | q-value | log2 FC BAG6KO/WT | q-value |
|------------|-------------------|---------|-------------------|---------|
| Chmp2a     | 0.958249          | 2.07E-02| 0.0050621         | 9.99E-01|
| Chmp2b     | 1.17935           | 2.35E-03| N/A               | 8.25E-01|
| Chmp4b     | 1.0868            | 2.12E-03| 0.363785          | 9.07E-02|
| Chmp5      | 1.33184           | 1.22E-02| -0.057766         | 8.78E-01|
| Tsg101     | 1.03054           | 2.61E-03| -0.280121         | 1.01E-01|
| Vps28      | 0.742254          | 4.10E-02| -0.159735         | 4.48E-01|
| Vps36      | 0.910931          | 0.00E+00| -0.158687         | 4.01E-01|
| Vps37b     | 1.21613           | 4.04E-03| -0.360706         | 2.71E-01|
| Vps37c     | 0.784838          | 1.34E-02| -0.75221          | 4.49E-02|
| Vps4b      | 0.812873          | 4.98E-03| -0.0346775        | 9.10E-01|
| Vta1       | 0.755167          | 8.95E-03| 0.466124          | 7.30E-02|
| Vps4a      | 0.941038          | 1.28E-02| -1.07789          | 2.04E-03|
| Ifitm3     | 2.56832           | 0.00E+00| 0                  | 1.00E+00|
| Sh3g1      | 0.573079          | 4.20E-02| -0.962532         | 0.00E+00|
| Snf8       | 0.841376          | 9.95E-03| -0.10839          | 7.72E-01|
| Sec31a     | 0.810083          | 0.00E+00| 0.195366          | 1.66E-01|
| Appl2      | 0.510428          | 6.90E-02| 0.252258          | 1.45E-01|
| Bst2       | 0.729434          | 3.89E-02| 0                  | 1.00E+00|
| Grb2       | 0.575356          | 5.65E-02| 0.415249          | 3.73E-02|
| Mvb12a     | 0.92278           | 1.42E-03| -0.839358         | 2.54E-03|
| Mvb12b     | 1.0013            | 4.11E-02| 0                  | 1.00E+00|
| Sort1      | 0.948903          | 9.21E-03| -0.025821         | 9.48E-01|
| Eea1       | 1.32199           | 8.42E-03| 0.419401          | 5.76E-02|
| Lrp1       | 0.952581          | 2.27E-03| 1.40089           | 8.82E-04|
| Argef2     | 0.426093          | 8.55E-02| -0.12036          | 3.22E-01|
| Cdc68      | 2.96219           | 5.34E-03| N/A               | 9.91E-01|
| Ehd3       | 0.965844          | 2.19E-02| 0.308753          | 9.66E-02|
| Ecm29;A1314180 | 0.578709     | 9.99E-03| 0.347452          | 5.71E-03|
| Flot1      | 0.961083          | 1.31E-02| 0.773437          | 1.08E-02|
| Grb2       | 0.575356          | 5.65E-02| 0.415249          | 3.73E-02|
| Pacsin2    | 1.25372           | 1.39E-03| 0.597606          | 6.54E-03|
| Stx7       | 0.925775          | 1.09E-02| 0.640253          | 4.73E-03|
| Snx2       | 0.179757          | 1.74E-01| 0.0277119         | 8.93E-01|

Figure S2. Supplement related to Figure 2.

(A) Immunoblot analysis of CRISPR-generated BAG6KO B-16V cell clones (numbered 3, 5 and 8) probing for BAG6 and actin as a loading control. Immunoblot analysis for the loss of the BAG6 protein was performed in regular intervals during cell propagation and after thawing of cells.

(B) Sequencing analysis of three selected BAG6KO clones aligning the mutant sequence with the sequence obtained for the WT cell line. Sequencing was performed on a PCR product of 537 bp length cloned into the pUC18 plasmid and sequenced with both forward and reverse (M13-F20 and M13R) sequencing primer.

(C) Left graph: NTA analysis of 48h EV release from B-16V WT and BAG6KO cells under normoxic and hypoxic (1% O2) conditions. Bar graphs represent mean ± SEM of three independent experiments. Right graph: Quantification of EV-associated BAG6 by ELISA (normalized to EV vesicle count) purified by ultracentrifugation at 100k x g from
B-16V cells that were either non-treated or cultured under hypoxic conditions (1% O2).
2h EV release collections from B-16V WT cells after heat-shock for 40 min at 42° were
used as a positive control and B-16V BAG6KO EVs served as a negative control. Bar
graphs represent mean ±SEM of three independent experiments.
(D) RNAseq data-based waterfall plot showing the log2 fold change of up- and
downregulated transcripts in EVs isolated from hypoxia-stressed BAG6KO B-16V
cells compared to WT cells. RNAseq was performed using EVs of three independent
EV purifications from each WT and BAG6KO B-16V cells.
(E) Overview of functional groups given as percentages of the total dataset based on
significantly up- and downregulated RNAs.
(F) Plot showing the log2 change BAG6KO over WT proteins detected by mass
spectrometry in EVs compared to their respective cells (whole cell lysate, WCL). Cut-off
lines are indicated and proteins that are not significantly (FDR ≤ 0.5) deregulated in
BAG6KO cells compared to WT cells, but which show at least 2-fold change difference
in EV expression are highlighted in red.
(G) Western blotting of B-16V WT-EVs and BAG6KO-EVs (18 µg protein per lane) to
validate differential expression of ANNEXIN A1, CHMP2B, VPS4B and VTA1 quantified
by mass-spec analysis. Antibodies: ANNEXIN A1 (ab2114486, Abcam); CHMP2B
(ab157208, Abcam); VPS4B (ab224736; Abcam); (VTA1 PA556605, Thermo Fischer
Scientific).
(H) Lists of proteins detected by mass spectrometry clustering into melanosome-like (WT)
EVs and exosome-like (BAG6KO) EVs and comparison to their respective cellular
levels. WT, wild-typeM; kDa, kilodalton; NT, non-treated; n.d., not detected.

Other supplement files related to Figure 2:
Table S1. Transcriptomics of WT- and BAG6KO-EVs
Table S2. Proteomics of WT and BAG6KO B-16V EVs and cells
Figure S3. Supplement related to Figure 3.

(A) Bar graph representing the log2 fold change of mRNAs upregulated in lungs of mice treated with WT-EVs compared to both lungs of PBS control-treated mice (WT/PBS) and lungs of BAG6KO-EV-treated mice (BAG6KO/WT). The EV treatment plan of this experiment is provided in Figure 3A.

(B) Determination of protein content in WT- compared to BAG6KO-EVs isolated by ultracentrifugation and used for in vivo treatment experiments. Protein concentration was determined by nanodrop2000 and normalized to the particle concentration determined by NTA analysis. Graphs represent mean ± SEM of measuring 6 independent experimental samples.

(C) qRT-PCR analysis validating the indicated hits identified by RNAseq of lungs from mice treated according to the treatment plan provided in Figure 3A (n=3 mice per group). Bar graphs represent mean ± SEM.

(D) Immunohistochemistry using specific antibodies against S100a8, ELANE and MPO of lung tissue from mice that were treated with B-16V WT-EVs, BAG6KO-EVs or PBS according to the treatment plan presented in Figure 3A. Stainings represent the lung tissue of one out of three mice per group.

(E) Representative gating strategy of myeloid cells in the bone marrow of mice educated with EVs or PBS as a control. Dead cells and erythrocytes remaining after ACK lysis were excluded by staining with Zombie viability dye and Ter119 antibody, respectively.
CD11b, Ly6C and Ly6G staining was done to delineate macrophages (CD11b+Ly6C–Ly6G–), monocytes (CD11b+Ly6C+/lowLy6G–) and neutrophils (CD11b+Ly6C+Ly6G+).

(F) Wound healing scratch assay of 24h using B-16V cells incubated with EVs isolated from either WT B-16V cells, BAG6KO B-16V cells or BAG6KO B-16V cells transfected with TIMP3. PBS was used as a control. Bar graphs represent mean ±SEM of four independent experiments.

(G) qRT-PCR analysis of indicated M1 macrophage markers after 7 days of in vitro macrophage differentiation (+M-CSF) of mouse bone marrow-derived monocytes in the absence or presence of WT-EVs, BAG6KO-EVs or BAG6KO-EVs derived from TIMP3 transfected BAG6KO B-16V cells. Bar graphs represent mean ±SEM of two technical replicates and results are representative of three independent experiments. Representative microscopic images of macrophages after 7 days are shown.

EVs, extracellular vesicles; WT, wild-type; SSC, side scatter; OE, overexpression.

Other supplement files related to Figure 3:
Table S3A,B. Transcriptomics of lungs from mice treated with either WT-EVs, BAG6KO-EVs or PBS.
Figure S4. Supplement related to Figure 4.

(A) Immunoblot analysis of BAG6 and p53 in HEK293 BAG6KO cell lysates immunoprecipitated with anti-BAG6, anti-p53 or anti-p300 specific antibodies after treatment with 1 µM doxo for 1h or left untreated. Immunoprecipitation with either mouse (ms) or rabbit (rb) IgG isotype controls was performed as control and HEK293 WT cell lysate was loaded as a control for antibody staining of the membrane.

(B) Immunoblot analysis of p53 in p53KO-HCT116 cells either mock-transfected or re-transfected with p53 WT or p53 acetylation mutant (+p53K373R) after treatment with...
100 nM doxo or left non-treated. The blot is representative of three independent experiments.

(C) Analysis of the EV release by NTA from WT or p53 siRNA knock down (kd) HEK293 cells that were either non-treated or treated with 100 nM doxorubicin or LBH for 16h. Immunoblot for p53 and actin as a loading control is shown.

(D) Immunoblot analysis of p53 in WT and BAG6KO HEK293 and B-16V cells either non-treated (-) or treated with the indicated concentrations of doxo (+) for 1h. One representative experiment out of three experiments is shown.

EVs, extracellular vesicles; WT, wild-type; kDa, kilodalton; doxo, doxorubicin; IP, immunoprecipitation; ms, mouse; rb, rabbit; IgG, immunoglobulin G.
**Figure S5. Supplement related to Figure 5.**

(A) Immunoblot analysis of HEK293 BAG6KO cells transfected with full-length BAG6 (+WT BAG6) or with a N-terminal deleted BAG6 mutant (+nucBAG6), detected by using a myc-tag specific antibody. Probing for GAPDH was done as a loading control.

The blot represents one out of three independent experiments.

(B) Immunofluorescence microscopic analysis of HEK293 cells transfected with either full length BAG6 (+WT BAG6) or N-terminal deleted BAG6 mutant (+nucBAG6).

Transfected BAG6 was visualized by a GFP-tag, the nucleus and cell membrane were visualized by staining with DAPI and PKH, respectively, and merged images are shown. Scale bar: 2 µm.

(C) The CRISP paint method was used to fuse a GFP-tag to the endogenous BAG6 gene (C-terminus) and the cellular localization of the BAG6-GFP protein was monitored in living cells using an inverse spinning disc microscope (Zeiss) with 5% CO\textsubscript{2}/37°C. A 8-chamber slide and a 40x objective with a NA of 1.1 (0.333µm/pixel) were applied.

Cells were either left non-treated or incubated with 1µM doxo and pictures were taken at the indicated time points.

(D) Schematic illustrating the impact of BAG6 expression and subcellular localization on the EV release.

WT, wild-type; kDa, kilodalton; DAPI, 4′,6-Diamidino-2-phenylindol; GFP, green fluorescent protein.

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**Figure S6. Supplement related to Figure 6.**

(A) Schematic representation of BAG6 protein domains highlighting its PT/SAP motif in analogy to the corresponding element found in HIV gag binding to the TSG101 UEV domain. UBL, ubiquitin-like domain; NLS, nuclear localization signal; BAG, Bcl-2-associated athanogene domain; UEV, Ubiquitin E2 variant domain; HRS, hepatocyte growth factor-regulated tyrosine kinase substrate; NKp30, Natural cytotoxicity receptor 30; Hsp70, Heat shock protein 70.
Figure S7. Supplement related to Figure 7.

(A) Test of qRT-PCR assay robustness using cDNA made from RNA isolated out of melanoma patient plasma EVs. The graph depicts a dilution series of EV cDNA using RNA isolated from three plasma samples (mean ± SEM) and experiments in Figure 7B were performed at equal concentrations, either undiluted or diluted 1:2.

(B) Schematic representation of the BAG6R786* mutant protein.

(C) Immunoblot analysis of BAG6R786* mutant protein transfected into WT or BAG6KO HEK293 cells using a BAG6 N-terminus-specific antibody which also detects the endogenous full length BAG6 protein (WT BAG6). GAPDH was probed for as a loading control. # indicates weak non-specific bands. The blot is representative for two independent experiments.

EVs, extracellular vesicles; SEM, Standard error of the mean; UBL, ubiquitin-like domain; NLS, nuclear localization signal; BAG, Bcl-2-associated athanogene domain; HRS, hepatocyte growth factor-regulated tyrosine kinase substrate; NKp30, Natural cytotoxicity receptor 30; Hsp70, Heat shock protein 70; WT, wild-type; kDa, kilodalton; BAG6R786, BAG6 mutant with stop codon at Arginin position 786.
Figure S8. Supplement related to the discussion.

(A) Cre mRNA detection by PCR of EVs isolated from WT and BAG6KO cells stably transfected with pcDNA3.1 CMV-CFP-Ubc-Cre-zipcode-zeo plasmid (kindly provided by Jacco van Rheenen, Utrecht, Netherlands). cDNA from cellular RNA and cDNA of EVs from Cre-negative cells was used as a positive and negative control, respectively. H2O served as a technical negative control.

(B) qRT-PCR analysis comparing the Cre mRNA levels detected in WT versus BAG6KO B-16V EVs. Bar graphs represent the mean ± SEM of Ct values obtained using three independent EV samples. Equal amounts of cDNA were used in each run.

(C) Flow cytometric analysis of GFP converted Cd11b+ Ly6C+ Ly6G+ neutrophils by flow cytometry of cells isolated from the lung obtained from tumour-burden mTmG reporter mice i.v. transplanted with WT or BAG6KO Cre-expressing B-16V cells or treated with PBS.
Figure S9. Summary model
See discussion for explanation
Extended Materials and Methods

Cell culture and culture conditions
HEK293 (ACC-305) and B-16V (ACC-370) cell lines were maintained according to the DSMZ guidelines in DMEM and RPMI medium, respectively. HCT116 cell lines, kindly provided by Annette Paschen, Essen, Germany, were maintained in McCoy medium. All media were supplemented with 0.5% Penicillin-Streptomycin and 10% fetal bovine serum. Cell lines were kept under standard culturing conditions with 5% CO₂, 37°C or under hypoxic conditions at 1% oxygen saturation and 5% CO₂, 37°C. Cell lines were regularly tested for mycoplasma contamination.

Mice
C57BL/6J mice were purchased from Charles River and all experiments were approved by the state authorities of North Rhine-Westphalia (number 84-02.04.2013.A.073). For EV injections (Figure 3A-B), two male and one female mice between 8-12 weeks of age were used. For EV education experiments (Figure 3C-E), experiments were performed with female mice starting at an age of 10 weeks.

p53-proficient and deficient murine CLL models were derived from the established Eµ:Tcl1a-driven CLL model [1]. In brief, Eµ:Tcl1a mice were crossed with Cd19:Cre [2] and Tp53fl/wt mice [3] to generate Eµ:Tcl1a; Cd19:Cre; Tp53fl/fl-compound mutant mice [4].

Human samples
Plasma were obtained with informed written consent by the patients and approval by the local ethics committee of the University Hospital Essen [ref. no. 11-4715].

CRISPR/CAS9-mediated knockout cell line generation
Generation of HEK293 BAG6KO and CBP/p300 double KO were described [5]. Knockout of BAG6 in B-16V cells was performed using the Zhang Lab’s reagents [6, 7] according to their protocol. Briefly, target sequences for sgRNA were designed using the Zhang Lab design tool (available at http://crispr.mit.edu/). Cells were transfected with px330 (nuclease approach) expression plasmid (Addgene #42230) and screened for protein loss by Immunoblotting. In experiments using WT or BAG6KO B-16V cells, three BAG6KO clones were pooled and compared to three isolated WT clones.

In vivo treatment experiments and sample preparation from experimental mice
8-12 weeks old C57BL/6J mice, housed and fed under pathogen-free conditions, were injected with EVs or cells intravenously into the tail vein using 27 gauge (EVs) or 26 gauge (cells) needles according to the time treatment schedule provided in Figure 2A and 2D. Blood sampling was done by tail vein scratching. For flow cytometric analysis of cells from EV-treated and/or B-16V metastasis-bearing C57BL/6J mice, single cell suspensions of spleen, blood and bone marrow were generated. Cut spleen pieces and bone marrow-flushed cells were passed through a 100 µm mesh and single cell suspensions as well as blood were incubated in erythrocyte lysing buffer (ACK) for 5 minutes and washed in PBS before antibody staining for flow cytometry. Dissected mouse lungs were either frozen in optimal-cutting-temperature compound (OCT Tissue Tec) on dry ice and stored at -80°C for immunohistochemistry or directly taken up in RNA stabilization solution and frozen at -20°C until further processed. For RNA isolation, lung tissue was homogenized using the gentleMACS™dissociator according to the manufacturer’s instructions (M-tubes, MACS Program RNA_02) and the RNeasy mini kit according to the manufacturer’s instructions. On-column DNase digestion was performed.
Murine splenocytes from Eµ:Tcl1a;Cd19:Cre mice were purified and cultured as described [8]. The minimum purity of CD5+CD19+ B cells from mice was 85%. Purified splenocytes were cultured for 24 h in Panserin 411S media (Pan-Biotech, Germany) and treated with 100 nM doxorubicin for 16 h. Supernatants were collected and exosome purification was performed by sequential ultracentrifugation. Viability of cells was confirmed (>90%).

**Cell treatment**

Cell lines were treated for 16 h with 100 nM doxorubicin or 100nM LBH, respectively. For short term (5–120 min) DNA damage induction, cells were treated with 1 or 10 µM doxorubicin (as stated in the figure legends).

**Plasmids and transfection**

pcDNA3.1 wild-type BAG6 (BAT3) and nucBAG6 are described [9]. pcDNA3.1 BAG6R786* was generated by cloning the stop codon TGA at position 2337 of the full-length BAG6 sequence introduced by site-directed mutagenesis using QuickChange II Site-Directed Mutagenesis Kit and the expression plasmid pcDNA3.1 wild-type BAG6 as a template. HRS expression vector pCS2 was obtained from Addgene (#29685) and pcDNA3.1 for p53 was kindly provided by Dr. Pattingre (INSERM, France). Transfection was performed using Lipofectamine 2000 or jetprime according to the manufacturer’s instructions.

**Antibodies**

All antibodies used are listed in Supplementary Table S4.

**Flow cytometry**

Fluorescence-activated cell sorter (FACS) was performed on a FACSCalibur or Gallios. Cells were stained with either directly-labelled antibodies, PE- or DyLight649-labelled goat anti-mouse or donkey anti-rabbit secondary antibodies as indicated in the figure legends. Flow cytometry was used to assess cell death (7AAD/Annexin V staining), EV expression levels of CD9, CD81, CD82, CD63, BAG6, phosphatidylserine, and acetylation of H3K18, H3K9 or p53K373 using specific antibodies. EVs were bound to polystyrene beads prior to flow cytometry as previously described [10]. Flow cytometric analysis of intracellular proteins was performed after fixation of cells with 4% formaldehyde in PBS for 12 minutes at 37°C, cooled down on ice for 1 minute and subsequently permeabilised with methanol for 30 minutes on ice prior to antibody staining. Single cell suspensions of mouse spleen, bone marrow and blood cells were stained with Zombie Aqua™ and Ter119 to exclude dead cells and remaining erythrocytes, respectively, and specific antibodies against CD11b, Ly6C and Ly6G were used to differentiate between the different myeloid cell populations. A minimum of 1,000 events was counted in FACS measurements. FACS data were analyzed with FlowJo software.

**In vitro expression assay**

*In vitro* expression experiments were performed using cell-free protein expression kit based on Leishmania tarentolae. Recombinant proteins were obtained by cloning in pLEXSY-invitro vector.

**Immunoprecipitation**

Cell lysates or *in vitro* expressed proteins were precipitated using specific antibodies against BAG6, p53, CBP/p300, HRS, ubiquitin or acetyl-lysine. A minimum of 1 µg of antibody were used for 100 µg of total protein and Protein A magnetic beads were used for pull-down.

**Yeast Two Hybrid analysis**
To confirm BAG6 interaction with the ESCRT protein TSG101, we used Gold Yeast two hybrid system. BAG6 and TSG101 were cloned into pGBT9 and pGADT7 expression vectors. Transformation, mating and binding analysis was done according to the manufacturer’s instructions.

**Microscopy**

**Immunofluorescence**

HEK293 cells were transfected with pcDNA3.1 FL-BAG6 or CT-BAG6 using lipofectamine according to the manufacturer’s instructions. 24h later cells were stained with PKH26 for membrane labelling and seeded onto glass slides. 3h later, cells were either left untreated or treated with 1 µM Doxo for 1h before fixation using 70% MeOH and blocking using PBS/10% FBS. The transfected BAG6 was detected by staining with an anti-myc antibody and subsequent visualisation by anti-mouse-FITC. Images were acquired with a Leica DMi8 inverse microscope (Leica TSC SP8).

**Immunohistochemistry**

Frozen tissue blocks containing mouse lungs were sectioned in slices of 7 µm and slices were stained with DAPI to visualize cell nuclei and with specific antibodies against MPO, ELANE and S100a8 visualized by incubation with biotinylated secondary antibody and subsequent HRP-labelled streptavidin using KPL Histomark® Biotin Streptavin-HRP Systems Kit and ACE (red) substrate kit according to the manufacturer’s instructions. Sections were finally stained with hematoxylin before embedment with Aquatex. Images were acquired with a Leica Type DM1000 LED.

Microscopy images were analysed with Leica Application Suite X 3.1.5.16308 and Imaris 8.3.1 software (3D reconstruction and cropping).

**ELISA**

The mouse monoclonal 3E4 (raised against the BAG6 N-terminus) and the BAG6-specific chicken polyclonal 13pp2 antibodies (own lab) were used to detect BAG6 in a sandwich ELISA procedure as previously described(10).

**EV preparation**

EVs were collected in cell culture for either 24 or 48h in either EV-depleted medium (overnight centrifugation at 100,000 x g) or protein-free CD293 medium. For quantification, EVs were purified by sequential centrifugation. The centrifugation protocol included consecutive pre-centrifugation steps at 300 x g (10 min), 2,000 x g (10 min) and 3,500 x g (20 min) for clearance of cells and cellular debris before ultracentrifugation at 10,000 x g (60 min) and/or 100,000 x g (90 min) using SW 41 Ti rotor or Type 45Ti) for at least two times with intermediate resuspension in PBS or HBSS and ultra-centrifugation at the respective g force using TLA-45 rotor in the last centrifugation. EVs were resuspended in PBS or HBSS. The amount of EV protein was quantified by Nanodrop 1000 and/or using BCA assay. The number of particles was determined by Nanoparticle Tracking Analysis. In experiments analyzing EV release, we have seeded equal numbers of cells and the measure of EVs released per cell is based on the end of the conditioning period to also account for cell growth during this period. RNA from EVs was isolated using the RNeasy Mini Kit according to the manufacturer’s protocol recommended for processing animal cell lysates. The viability of EV-releasing cells was regularly checked by both trypan blue staining and microscopic analysis as well as by 7AAD/Annexin5 staining and flow cytometry.
Free flow electrophoresis (FFE)

Conditioned CD293 medium from either BAG6-transfected or non-transfected HEK293 cells was sent to FFE Service GmbH (Feldkirchen, Germany) for analysis on a FFE NextGen to fractionate vesicles from soluble proteins. Samples were thawed and 100 ml were concentrated to 4-7 ml by tangential flow ultrafiltration using a Microkros hollow fiber module (Spectrum Lab, C02-E300-05-N) at room temperature. Concentrates were then subjected to continuous Zone Electrophoresis (ZE) with 3 min separation time at 1000 V in a horizontal chamber position (500 mm x 100 mm, 0.2 mm gap). Fractions of typically 200 µL were collected in black flat bottom 96-well plates (Greiner microloan Fluotrac 200) or protein low bind polypropylene deep well plates with 2 mL per well and read in a microplate reader (Tecan M200) equipped for UV-VIS and fluorescence spectroscopy. A mixture of dyes of different isoelectric points (pI) in the range of pI 4-10 was used in system suitability testing and read at 420 nm, 517 nm and 595 nm. Proteins were detected by top count fluorescence at 280 nm (excitation 350 nm, emission 10 nm bandwidth) and a photomultiplier gain setting of 80. pH measurements on microplate were performed with a robotic system equipped with a pH-meter and electrode. Fractions obtained by FFE were analysed for protein concentrations BCA assay and for particle concentration by NTA. Particle-containing fractions were analysed by bead-assisted flow cytometry for the presence of BAG6 and the vesicle markers CD81 and HSP70.

Quantitative RT-PCR

RNA from EVs or lung tissue was reverse transcribed with RevertAid First Strand cDNA Synthesis Kit using oligo-d(T) primers and/or random primers. Quantitative PCR measurements were performed on a 7500 real-time PCR system using SYBR Green. Initial heat inactivation was 95°C for 15 min and 40 cycles of 15 sec at 94°C, 30 sec at 56°C, and 30 sec at 72°C were performed followed by melting curve analysis. All primers used are listed in supplement Table S5.

Western Blot

Cells or EVs were lysed in buffer (50 mM Tris-HCl pH 8, 150 mM NaCl, 0.5% Triton X-100, 0.5% protease inhibitors). Cytoplasmic extraction of lysates was performed using soft lysis conditions for 15 minutes with Triton X-100 lysis buffer followed by sequential centrifugation. Protein concentration was measured with a BCA protein assay. 10 to 30 µg were loaded onto sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels for blotting using standard methods. Blots were incubated with primary antibody BAG6, p53, ALIX, CD81, HRS, flotillin-1, and p300 and TSG101. The western blots were developed using X-ray films or by detection with a CCD camera. For quantification, rolling-ball background subtraction was applied and band intensity was quantified using GelQuantNET.

Scratch assay

B-16V WT cells were seeded onto 24-well plates and scratch wounds were made using a 200 µl pipette tip 24h post seeding when cells reached around 80% confluency. After wounding, cells were washed two times with PBS and cells were incubated in RPMI and 10 µg of EVs from B-16V WT cells, BAG6KO cells or BAG6KO cells transiently transfected with Timp-3 plasmid using lipofectamine or PBS as a control. Microscopic pictures were taken at 5x magnification of at least 3 different spots along the scratch immediately after wounding and 24h later. The experiments were performed in 3 technical replicates and repeated 4 times in independent biological replicates.
**Macrophage differentiation and polarization**

Bone marrow cells were seeded in 12 well plates at 10^6 cells/well and monocytes were allowed to adhere in the presence of 20 ng/ml M-CSF in RPMI medium. After 24h, cells in suspension were removed and adherent cells were washed thoroughly before cells were incubated in RPMI medium supplemented with M-CSF (control) or additionally with 10 µg/ml EVs isolated from either WT, BAG6KO or BAG6KO cells transiently transfected with Timp-3 plasmid using lipofectamine. Medium was refreshed every second day until day 7 where cells were lysed in the plate and processed for RNA isolation. qRT-PCR was performed using primers against macrophage M1 markers IL-12 (IL-12 for ACGCAGCACTTCAGAATCAC and IL-12 rev CGCAGAGTCTCGCCATTATG); Nos2 (Nos2 for GGCAGCCTGTGAGACCTTT and Nos2 rev TTGGAAGTGAAGCGTTTCG); and Cxcl10 (Cxcl-10 for GACGGTCCGCTGCAACTG and Cxcl-10 rev CTTCCCTATGGCCCTCATTCT).

Three independent experiments were performed.

**Mass Spectrometry**

Samples were obtained by ultracentrifugation of CD293 supernatants of B-16V WT or BAG6KO cells or by harvesting the respective cells cultured under hypoxic conditions for 48h. 10 µg EV protein or cell lysate were lysed in 6M Urea/2M thiourea in Hepes buffer additionally aided by freezing at -80°C and thawing. Proteins were then linearised using 1:10 volume of 100 mM DTT for 30 minutes at RT and alkylated using 1:10 volume of 550 mM for 30 min and RT. Proteins were Lys-C digested for 2h at RT and afterwards solution was diluted to 1M urea using 50 mM ABC buffer for trypsin digestion overnight at RT (both digests at 1 to 100 enzyme to protein ratio). The reaction was stopped by acidification using 5% concentrated nitric acid/0.2% trifluoroacetic acid before peptides were desalted on a C18 Stage tip. All samples were analyzed at the CECAD proteomics facility (Cologne, Germany) on a Q-Exactive Plus (Thermo Scientific) mass spectrometer that was coupled to an EASY nLC 1000 UPLC (Thermo Scientific). Briefly, peptides were loaded with solvent A (0.1% formic acid in water) onto an in-house packed analytical column (50 cm × 75 µm I.D., filled with 2.7 µm Poroshell EC120 C18, Agilent). Peptides were chromatographically separated at a constant flow rate of 250 nL/min using linear gradient (solvent B 0.1% formic acid in 80% acetonitrile) over 150 min gradients.

**Mass spectrometry bioinformatics and statistical analysis**

All mass spectrometric raw data were processed with Maxquant using default parameters. Briefly, MS2 spectra were searched against the Uniprot MOUSE database, including a list of common contaminants. False discovery rates on protein and PSM level were estimated by the target-decoy approach to 1% (Protein FDR) and 1% (PSM FDR), respectively. The minimal peptide length was set to 7 amino acids and carbamidomethylation at cysteine residues was considered as a fixed modification. Oxidation (M) was included as variable modification. The match-between runs option was enabled. LFQ quantification was enabled using default settings. Downstream data processing was conducted within the Perseus computational platform. Briefly, protein groups flagged as „reverse“, „potential contaminant“ or „only identified by site“ were removed from the data. LFQ data were log2 transformed. Statistical analysis of differentially regulated proteins was performed using a two-sided t-test (fudge factor s0 was adjusted to 0.1). Resulting p values were corrected for multiple testing using a permutation-based FDR approach.
RNAseq
RNA quality was assessed using the Experion RNA StdSens Analysis Kit. RNA-seq libraries were prepared from total RNA using the TruSeq Stranded mRNA LT kit according to the manufacturer’s instructions. Quality of sequencing libraries was controlled on a Bioanalyzer 2100 using the Agilent High Sensitivity DNA Kit. Pooled sequencing libraries were quantified with digital PCR (QuartStudio 3D) and sequenced on the HiSeq 1500 Illumina platform in Rapid-Run mode with 50 base single reads. RNAseq was performed from RNA isolated using the RNeasy mini kit with the Illumina Truseq mRNA kit v2 on an Illumina Hiseq 1500 according to the manufacturer’s instructions.

RNA-Seq Bioinformatic Analysis
Raw transcriptome reads were aligned to the mouse genome from Ensembl 89 [11] using STAR version 2.4.1a [12]. Gene expression was quantified on gene models that included only protein coding transcripts for protein coding genes using custom python scripts. Differential genes were called using edgeR [13] at a threshold of FDR <= 0.05; |log2FC| >= 1 and counts per million >= 3.

Statistical analysis
Statistical analyses were performed using Graphpad Prism software. Data are presented as mean ± SEM values of at least three biologically independent experiments or one representative experiment as indicated. *, ** and *** indicates P < 0.05, P< 0.001 and P<0.001. Two-tailed, unpaired Student’s t-tests were performed to analyze the significance of mean values between two variables, if not otherwise stated. Statistical analysis of EVs education animal experiments was performed using non-parametric Kruskal-Wallis test (mean ranks compared with WT-EVs group) and Dunn’s multiple comparisons test. Data are shown as mean ± SEM (n ≥ 8). An unpaired Welch’s t-test was performed to analyze TCGA data to account for unequal sample sizes.

Data deposition
The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD010677. RNAseq data of mouse lungs were deposited at ArrayExpress accession E-MTAB-7119. RNAseq data of B-16V EVs were deposited at ArrayExpress accession E-MTAB-7119.

Table S4. List of Resources

| Reagent or Resource | Source | Identifier |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| BAG6 (monoclonal mouse, 3E4; raised against N-term) | Own lab | Pogge von Strandmann, Simhadri et al., 2007 |
| BAG6 (polyclonal chicken, 13pp2; raised against recombinant N-term) | Own lab | |
| Alexa Fluor700 rat anti-Cd11b (M1/70) | Biolegend, San Diego, USA | Cat# 101222 |
| PerCP-Cy5.5 anti-Ly6C (HK1.4) | Biolegend, San Diego, USA | Cat# 128011 |
| Brilliant violet 421 rat anti-Ly6G (1A8) | Biolegend, San Diego, USA | Cat# 127627 |
| Fictr anti-Ter119 (Ly-76) | Biolegend, San Diego, USA | Cat# 116205 |
| Antibody/Tag | Supplier | Location | Cat# |
|-------------|----------|----------|------|
| Rabbit anti-p53 | Cell signaling, Danvers, Massachusetts, USA | 9282S |
| Rabbit anti-p53K373 (EP356(2)AY) | Abcam, Cambridge, UK | 62376 |
| Rabbit anti-p300 (C-20) | SantaCruz Biotech | sc-585 |
| Mouse anti-beta actin (AC-15) | Sigma, Kawasaki, Japan | A5441 |
| Rabbit anti-gapdh HRP (D16H11) | Cell signaling, Danvers, Massachusetts, USA | 8884 |
| Rabbit anti-HRS | Bethyl Lab Inc, Montgomery, Texas, USA | A300-989A |
| Rabbit anti-TSG101 | Abcam, Cambridge, UK | ab30871 |
| Mouse anti-ALIX (3A9) | Biolegend, San Diego, USA | 349501 |
| Mouse anti-flotillin-1 (RUO) | BD Biosciences, Franklin Lakes, New Jersey, USA | 610821 |
| Mouse anti-human CD81 (TAPA-1) | Biolegend, San Diego, USA | 349501 |
| Mouse anti-CD63 | Biolegend, San Diego, USA | 353014 |
| PE-anti mouse CD63 (NVG-2) | Biolegend, San Diego, USA | 143903 |
| Mouse anti-CD9 (HI9a) | Biolegend, San Diego, USA | 312102 |
| Alexa Fluor647 anti-mouse CD9 | Biolegend, San Diego, USA | 124809 |
| Annexin V – PE | Biolegend, San Diego, USA | 640908 |
| Mouse anti-HSP70 | SantaCruz Biotech, TX, USA | sc-66048 |
| Mouse anti-Adam10 (EPR5622) | Abcam, Cambridge, UK | ab124695 |
| Anti-myc tag | Biolegend, San Diego, USA | 626802 |
| Goat anti-MPO | R&D systems, Minneapolis, Minnesota, USA | AF3667-SP |
| Rabbit anti- S100a8 | Sigma, Kawasaki, Japan | HPA024372 |
| Rabbit anti-Elane | Sigma, Kawasaki, Japan | HPA066836 |
| PE-anti mouse IgG1 | Biolegend, San Diego, USA | 406607 |
| LEAF™ Purified Mouse IgG1, κ Isotype Ctrl | Biolegend, San Diego, USA | 400153 |
| FITC Goat anti-mouse IgG (minimal x-reactivity) Antibody | Biolegend, San Diego, USA | 405305 |
| Dylight™649 donkey anti-rabbit IgG | Biolegend, San Diego, USA | 405312 |

**Biological samples**

| Samples | Supplier | Location | Cat# |
|---------|----------|----------|------|
| Plasma from melanoma patients | Annette Paschen, Essen | N/A |

**Chemicals**

| Chemical | Supplier | Location | Cat# |
|----------|----------|----------|------|
| Doxorubicin | Sigma, Kawasaki, Japan | D2975000 |
| Panobinostat (LBH589) | SantaCruz Biotech, Dallas, Texas, USA | sc-208148 |
| Aquatex | Merck, Darmstadt, Germany | 1085620050 |

**Medium and Solutions**

| Medium | Supplier | Location | Cat# |
|--------|----------|----------|------|
| RPMI | Life technologies (Gibco), Carlsbad, California, USA | 11875093 |
| DMEM | Life technologies (Gibco), Carlsbad, California, USA | 10567014 |
| Product Name          | Manufacturer                             | Catalog Number |
|----------------------|-------------------------------------------|---------------|
| McCoy’s              | Life technologies (Gibco), Carlsbad, California, USA | Cat# 16600082 |
| Panserin 411S        | Pan-Biotech, Aidenbach, Bayer, Germany     | Cat# P04-71411S |
| CD293                | Life technologies (Invitrogen), Carlsbad, California, USA | Cat# 11913-019 |
| PBS                  | Thermo Fisher Scientific, Waltham, Massachusetts, USA | Cat# 14190-169 |
| HBSS                 | Thermo Fisher Scientific, Waltham, Massachusetts, USA | Cat# 14025092 |

### Plasmids

| Plasmid                           | Manufacturer                             | Catalog Number |
|-----------------------------------|-------------------------------------------|---------------|
| pcDNA™3.1 (+) Mammalian Expression Vector | Life technologies (Invitrogen), Carlsbad, California, USA | Cat# V79020 |
| pcDNA3.1 FL-BAG6 and CT-BAG6      | own lab                                   | Pogge von Strandmann, Simhadri et al., 2007 |
| pcDNA3.1 BAG6R786*                | Own lab                                   | This paper    |
| pCS2-HRS                          | Addgene, Cambridge, Massachusetts, USA    | Cat# 29685    |
| pcDNA3.1-p53                      | kindly provided by Dr. Pattingre, INSERM, France | Sebti, Prébois et al., 2014 |
| pcDNA 3.1-p53 K372-373R           |                                          |               |

### Commercial kits

| Kit Name                                | Manufacturer                             | Catalog Number |
|-----------------------------------------|-------------------------------------------|---------------|
| RNeasy mini Kit                         | Qiagen, Venlo, Netherlands                | Cat# 74104    |
| RNase-free DNase Set                    | Qiagen, Venlo, Netherlands                | Cat# 79254    |
| Pierce™ protein A magnetic beads         | Thermo Fisher Scientific, Waltham, Massachusetts, USA | Cat# 88845 |
| Lexsy in vitro translation Kit          | Jena Bioscience, Jena, Thüringen, Germany | Cat# EGE-2010-15 |
| Matchmaker® Gold Yeast two hybrid system | BD Clontech, USA                          | Cat# 630489   |
| ExoRNeasy Serum/Plasma Midi Kit        | Qiagen, Venlo, Netherlands                | Cat# 77044    |
| Polystrene beads for EV flow cytometry  | Polysciences, Inc, Warrington, Pennsylvania, USA | Cat# 17135-5 |
| Zombie Green™ Fixable Viability Kit     | Biolegend, San Diego, USA                | Cat# 423111   |
| Hs_TP53_7 FlexiTube siRNA              | Qiagen, Venlo, Netherlands                | Cat# S102623747 |
| Pierce™ BCA protein assay Kit          | Thermo Fisher Scientific, Waltham, Massachusetts, USA | Cat# 23225 |
| KPL Histomark® Biotin Streptavin- HRP Systems | SerCare, Milford, Massachusetts, USA       | Cat# 71-00-38 |
| AEC (red) Substrate Kit                | Life technologies (Invitrogen),           | Cat# 002007   |
| **QuickChange II Site-Directed Mutagenesis Kit** | Carlsbad, California, USA | Agilent Technology, Santa Clara, California, USA | Cat# 200524 |
| --- | --- | --- | --- |
| Jetprime | peqLab, Polyplus transfections | Cat# 13-114-07 |
| Lipofectamine® 2000 Transfection Reagent | Thermo Fisher Scientific Waltham, Massachusetts, USA | Cat# 11668027 |
| RevertAid First Strand cDNA Synthesis Kit | Thermo Fisher Scientific Waltham, Massachusetts, USA | Cat# K1622 |
| SYBR® Green JumpStart™ Taq ReadyMix™ | Sigma, Kawasaki, Japan | Cat# S4438-100RXN |
| Experion RNA StdSens Analysis Kit | BioRad, Hercules, California, USA | Cat# 7007104 |
| TruSeq Stranded mRNA LT kit | Illumina, San Diego, California, USA | Cat# RS-122-2101, RS-122-2102 |
| Agilent High Sensitivity DNA Kit | Agilent, Santa Clara, California, USA | Cat# 5067-4626 |

**Experimental models: Cell lines**

| HEK293 | DSMZ, Braunschweig, Germany | This paper Sauer, Schuldner et al., 2017 | Cat# ACC 635 |
| HEK293 BAG6KO | DSMZ, Braunschweig, Germany | This paper Sauer, Schuldner et al., 2017 |
| HEK293 CBP/p300KO | DSMZ, Braunschweig, Germany | This paper Sauer, Schuldner et al., 2017 |
| B-16V | DSMZ, Braunschweig, Niedersachsen, Germany | This paper |
| B-16V BAG6KO clones | DSMZ, Braunschweig, Niedersachsen, Germany | This paper |
| HCT116 and HCT116 p53KO | Annette Paschen, University Hospital Essen, Germany |

**Experimental models: Organisms/Strains**

| C57BL/6J | Charles River, Wilmington, Massachusetts, USA |
| Eµ:Tcl1a; Cd19:Cre; Tp53f/f | Rickert, Rajewsky et al. 1995 Jonkers, Meuwissen et al. 2001 Knittel, Rehkmper et al., 2017 |

**Deposited Data**

| Melanoma BAG6 expression data & survival data | The Cancer Genome Atlas, TCGA, National Cancer Institute, National Human Genome Research Institute | https://www.proteina tlas.org/ENSG00000 204463- BAG6/pathology/tiss ue |

**Microscopes and instruments**

| Immunohistochemistry microscope | Leica Typ DM1000 LED, Leica Microsystems, Wetzlar, Germany |
| Equipment Type                                      | Model/Details                                                                 | Manufacturer/Location                                      |
|-----------------------------------------------------|-------------------------------------------------------------------------------|------------------------------------------------------------|
| Immunefluorescence microscope                      | Leica Dmi8, Leica Microsystems, Wetzlar, Germany                             |                                                            |
| Flow cytometer                                     | Gallios (Beckman Coulter, Brea, California, USA)                               | Gallios (Beckman Coulter, Brea, California, USA)           |
|                                                     | FACSCalibur (Becton Dickinson, Franklin Lakes, New Jersey, USA)                | Gallios (Beckman Coulter, Brea, California, USA)           |
| real-time PCR system                               | 7500 real-time PCR system, Life Technologies, Carlsbad, California, USA       |                                                            |
| CCD camera                                          | Intas ChemoCam Imager ECL, Type HR 16-3200, Intas Science Imaging Instruments GmbH, Göttingen, Niedersachsen, Germany | Intas ChemoCam Imager ECL, Type HR 16-3200, Intas Science Imaging Instruments GmbH, Göttingen, Niedersachsen, Germany |
| Nanodrop                                            | Nanodrop1000, Thermo Fisher Scientific, Waltham, Massachusetts, USA           |                                                            |
| Nanoparticle tracking analysis                      | NS300, Malvern Instruments, Malvern, UK                                       |                                                            |
| Software and Algorithms                            |                                                                               |                                                            |
| Graphpad Prism 6.0 software                         | GraphPad Software, Inc.                                                       | http://www.graphpad.com/scientific-software/prism/         |
| FlowJo V10                                          | FlowJo LLC                                                                    | www.flowjo.com                                            |
| RNAseq                                              | STAR version 2.4.1a                                                           |                                                            |
| Functional annotation analysis                      | DAVID Bioinformatics Resources 6.8                                             | https://david.ncifcrf.gov/                                 |
| Protein interaction analysis                        | Search tool for the Retrieval of Interacting Genes/Proteins (String) database v9.0 | http://www.string-db.org/                                  |
| Immunoblot imaging software                         | Chemostar, Intas, Ahmedabad, Indien                                            |                                                            |
| NTA analysis software                               | NanoSight NS300 Software NTA 3.1 Build 3.1.46, Malvern Panalytical, Malvern, UK |                                                            |
| Immunohistochemistry and fluorescence microscopy software | Leica Application Suite X (LAS X) and Imaris 8.3.1 software                    |                                                            |
| CRISPR sgRNA design Zhang lab                       | Cong, Ran et al. 2013, Ran, Hsu et al. 2013                                   | http://crispr.mit.edu/                                     |
| Mass spectrometry raw data                          | Maxquant, version 1.5.3.8                                                      |                                                            |

**Software and Algorithms**

**Graphpad Prism 6.0 software**
GraphPad Software, Inc.

**FlowJo V10**
FlowJo LLC

**RNaseq**
STAR version 2.4.1a

**Functional annotation analysis**
DAVID Bioinformatics Resources 6.8

**Protein interaction analysis**
Search tool for the Retrieval of Interacting Genes/Proteins (String) database v9.0

**Immunoblot imaging software**
Chemostar, Intas, Ahmedabad, Indien

**NTA analysis software**
NanoSight NS300 Software NTA 3.1 Build 3.1.46, Malvern Panalytical, Malvern, UK

**Immunohistochemistry and fluorescence microscopy software**
Leica Application Suite X (LAS X) and Imaris 8.3.1 software

**CRISPR sgRNA design Zhang lab**
Cong, Ran et al. 2013, Ran, Hsu et al. 2013

**Mass spectrometry raw data**
Maxquant, version 1.5.3.8
## Table S5. List of oligonucleotides

| Oligonucleotide | Sequence 5'-3' |
|-----------------|----------------|
| **sgRNA for CRISPR** |                |
| sgRNA sequences targeting murine BAG6 | GCTTGAGGACCCCGGCCC and GGGCCGGGTCTTACAAGC |
| **Primer for qRT-PCR** |                |
| actin-for | ACACTGTGGCCCATCTACGAGG |
| actin-rev | AGGGGCGGAGCTGTGACACT |
| Murine NFkBia-for | CTCAACTTCCAGAACACCTGCA |
| Murine NFkBia –rev | GAGGCTCAAGATCACAGGCA |
| Murine Eno2-for | ACTCCGACCTCAGTTCG |
| Murine Eno2-rev | CTCTGGCCCAAGTGCGATG |
| Murine Murine HDAC7-for | CCTGAGATGGCTGCTACAAACC |
| Murine Murine HDAC7-rev | GAGGAATCTCCAAGGTGCTC |
| Murine Rgs16-for | TCAGTGAGGAGACCTGGAGT |
| Murine Rgs16-rev | TCACCTCTTGGAGGCTTCG |
| Murine Efemp2-for | CGAGCCTGATGACAGGAGA |
| Murine Efemp2-rev | CGACTCATCTATGTTCCACAC |
| Murine Nes-for | AACAGATGGAAAGGCCTG |
| Murine Nes-rev | AGGGCAGATCTTGGAGGTCG |
| Murine Ptpn3-for | AGATCGCCGTCTGGTGTCT |
| Murine Ptpn3-rev | AGGGCCAGTTTCCTCAGGT |
| Murine s100a9-for | TCGGCTTTGACAGAGTGCAA |
| Murine s100a9-rev | GCCCCAGCTTCACAGAAGTAT |
| Murine Elane-for | CAGCAGGACCCACTGAGAAG |
| Murine Elane-rev | TTGTGCAAGATGCTGGGAGG |
| Murine MPO-for | CTGTTAGCAGAGCTGGGAC |
| Murine MPO-rev | GGCGCCATAAGTGCAAGCCA |
| Murine Prtn3-for | TTCTGCGGCGCATAACCAT |
| Murine Prtn3-rev | GCACATCCCGAGATGACGAA |
| Murine Mmp8-for | AAACGTTACAGAGCTACTCAG |
| Murine Mmp8-rev | ATTTGGCTTCCGTCACAT |
| Murine Ctnnal1-for | CAGAATGGCCTGCTGGAGG |
| Murine Ctnnal1-rev | CACTCTCATCCGGGTCAAC |
| Human Ctnnal1-for | TCAGATGGAAATAACCGGATGCGT |
| Human Ctnnal1-rev | TGTTCCAGAGATCTGACCCCA |
Site-directed mutagenesis primer

BAG6R786* for
CGGCTCCAGCCCCAGCTGTGATCCTTCTTCCACCAGCAC
BAG6R786* rev
GTGCTGGTGGAAGAAGGATCACAGCTGGGGCTGGAGCCG

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