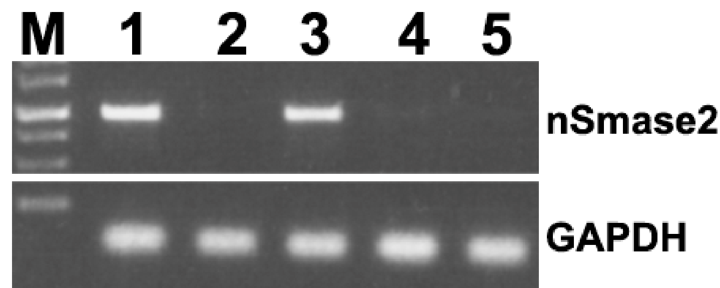
Supplement

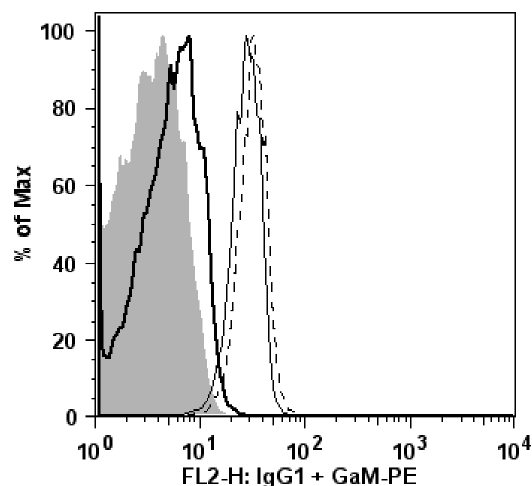
1. **Supplementary Figures**

Supplementary Figure 1



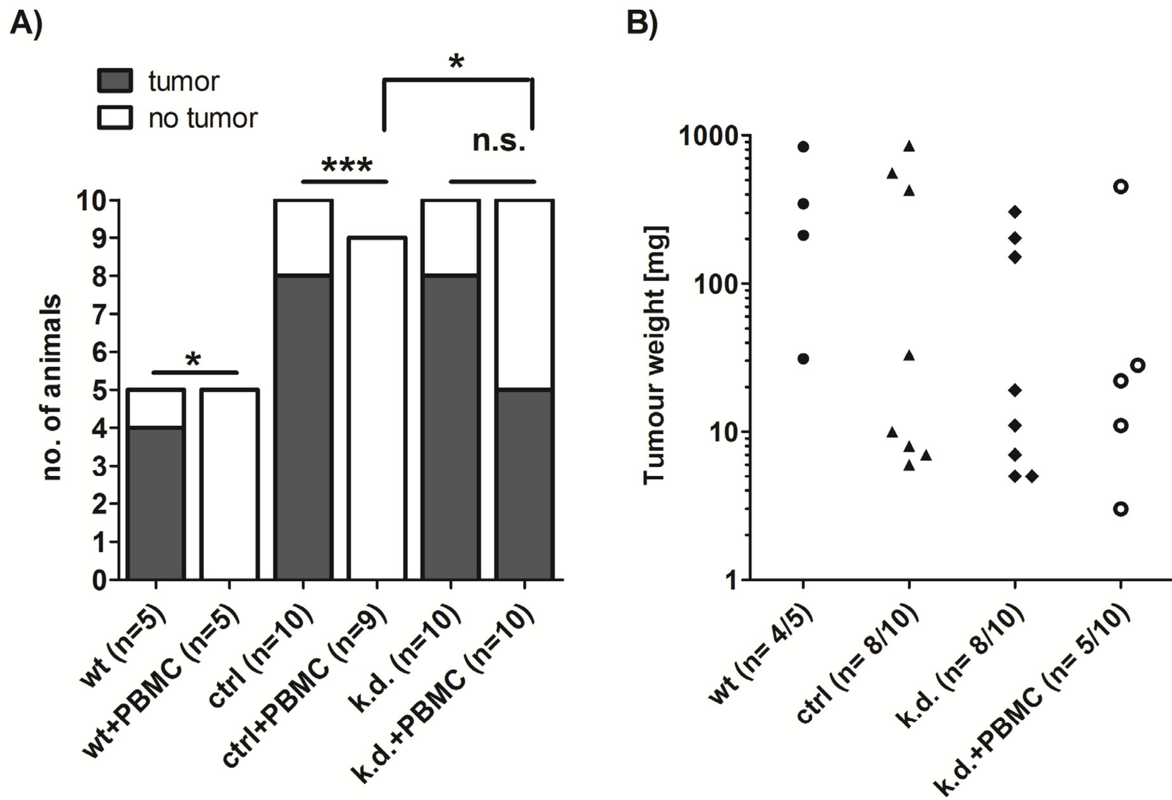
Supplementary Figure 1: RT-PCR for mRNA from MCF7 cells with nSmase2 overexpression.
Lanes: M = marker, 1 = nSmase2 overexpressing cells, 2 = wildtype, 3 = nSmase2 overexpression plus control siRNA, 4 and 5 = nSmase2 overexpression plus siRNAs 1 and 2, respectively. The data indicated overexpression of nSmase2 and its abrogation by specific siRNA co-transfection (upper panel), while the GAPDH loading control indicates roughly equal amounts of loaded cDNA (lower panel).

Supplementary Figure 2



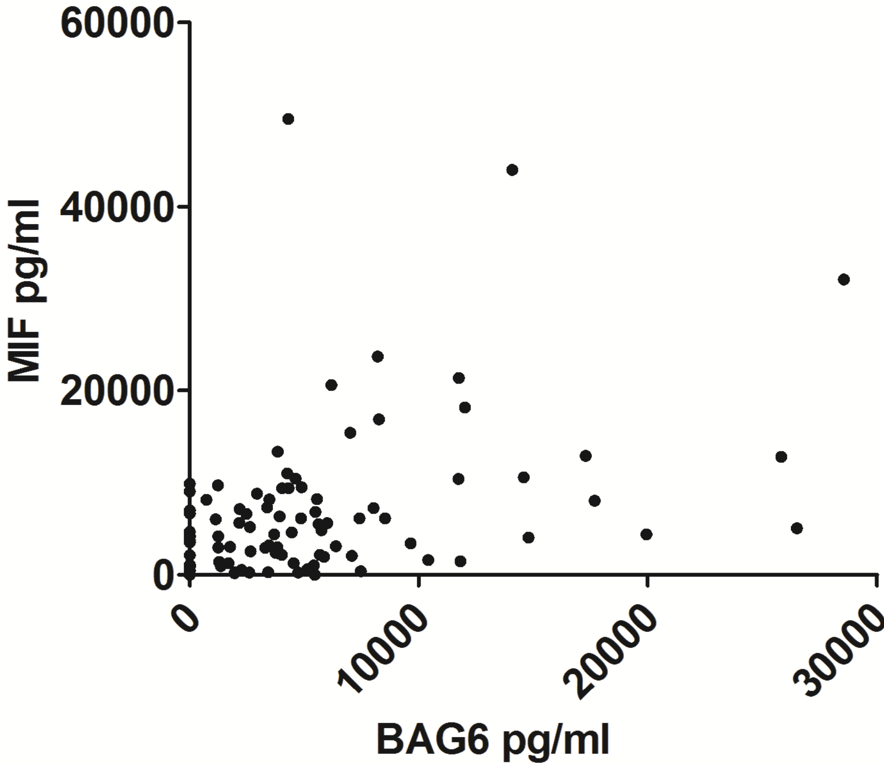
Supplementary Figure 2: BAG6 knock down in cells resulted in absence of BAG6 on their exosomes.
Flow cytometry for BAG6 on the surface of exosomes from wildtype, mock-shRNA and BAG6 knock down shRNA-transduced cells revealed the absence of BAG6 on exosomes from BAG6 knock down cells. The grey, shaded area represents the staining with IgG control of wildtype exosomes, while the lines represent BAG6 staining from wildtype cell-derived exosomes (dashed thin line), mock-shRNA-transduced exosomes (solid thin line) or BAG6 knock down exosomes (solid thick line). The data indicate absence of BAG6 in exosomes of BAG6 knock down cells.
One representative experiment of at least three independent experiments is shown.

Supplementary Figure 3



Supplementary Figure 3:
The incidence of tumors and their weights in the xenograft model.
A) The results of the tumor incidence for each treatment group in detail at the final day of the observational period. The 293T wildtype (wt) and control shRNA transduced cells (ctrl) formed tumors that were readily eliminated by PBMCs. However, the tumors formed by BAG6 knock down 293T cells (k.d.) were able to escape PBMC anti-tumor surveillance and formed in 5 of 10 animals tumors. In the group ctrl plus PBMCs, one animal died of unknown cause three days prior to the end of the observation period but had no tumor until then (not included). Groups were compared with a two-sided Fisher's exact test for significant differences. Results are indicated as $p \leq 0.05$ with one asterisk, $p \leq 0.01$ with two asterisks, and $p \leq 0.001$ with three asterisks. **B)** The dissected tumors were weighed and plotted on a logarithmic scale. A statistical comparison did not indicate any significant difference (Mann Whitney U-test).

Supplementary Figure 4



Pearson r	0.3822
95% confidence interval	0.1935 to 0.5436
P value (two-tailed)	0.0002
P value summary	***
Is the correlation significant? (alpha=0.05)	Yes
R squared	0.1461

Supplementary Figure 4:
Correlation between macrophage migration inhibitory factor (MIF) and BAG6 in the plasma of CLL patients.
More than 90 samples could be charted and there is a highly significant correlation ($p<0.001$) between these two parameters, as shown by the statistics below the graph.

Supplement: Methods and reagents

Supplementary Table 1: Reagents and Chemicals

Abbreviations: **LT** life technologies (Darmstadt, Germany),**PAA** (Linz, Austria), **PBT** Pierce Biotechnology / Thermo Scientific (Rockford, IL, USA), **Ro** Roche Diagnostics GmbH (Mannheim Germany), **WM** Whatman GmbH (Dassel, Germany)

Reagent /Chemical /Kit	Application	Vendor
Iscove's Modified Dulbecco's Medium (IMDM)	Cell culture	LT
Dulbecco's Modified Eagle Medium (DMEM)	Cell culture	LT
RPMI1640	Cell culture	LT
Fetal bovine serum (FBS) Gold	Cell culture	PAA
Penicillin/Streptomycin, 100x	Cell culture	PAA
LSM1077 Lymphocyte separation medium	PBMC purification	PAA
Lipofectamin2000	Transient transfection	LT
Complete protease inhibitors	Cell lysis buffer, WB	Ro
BCA Protein Assay Kit	Protein quantification	PBT
ProTran nitrocellulose membrane	WB	WM
enhanced luminescence detection kit	WB	PBT
1-Step Ultra TMB-ELISA	ELISA	PBT

Supplementary Table 2: Antibodies used in Western Blot, ELISA and Flowcytometry
Abbreviations of chromophores or functional groups:
APC allophycocyanin, **FITC** fluorescein isothiocyanate, **HRP** horseradish peroxidase, **PE** phycoerythrin,
Abbreviations of vendors:
abcam (Cambridge, UK), **BA** Bamomab GmbH (Gräfeling, Germany) **BC** Beckman Coulter **BL** BioLegend (San Diego, CA, USA), **DN** Dianova (Hamburg, Germany), **JIT** Jackson ImmunoResearch Laboratories (West Grove, PA, USA), **S-A** Sigma-Aldrich (Munich, Germany)

Antibody	Application	Dilution	Vendor	Catalogue no.
CD9	WB / FACS	1:200 / 1:100	BL	312102
CD63	WB	1:200	BL	312002
CD81	WB	1:200	BL	349502
Hsp70	WB	1:200	BL	648002
BAG6 (3E4)	WB / FACS/ ELISA	1:2000/1:100/1:1000	own lab	-
Actin	WB	1:4000	S-A	A1978
anti-Mouse-HRP	WB	1:10000	DN/JIT	115-035-003
anti-rabbit-HRP	WB/ELISA	1:10000	DN/JIT	111-035-003
ULBP-1	FACS	1:50	BA	AUMO2
ULBP-2	ELISA/FACS	2 ug/ml / 1:50	BA	BUMO1
ULBP-3	FACS	1:50	BA	CUMO3
MICA	ELISA	4 ug/ml	BA	AMO1
Annexin V-PE	FACS	1:50	BD	556422
CD3-APC	FACS	1:50	BL	300412
CD16-FITC	FACS	1:50	BL	302006
CD56-PE	FACS	1:50	BD	345810
CD69-FITC	FACS	1:50	BL	310904
NKG2D-FITC	FACS	1:50	abcam	Ab35035
NKp30-PE	FACS	1:50	BL	325208
NKp46-PE	FACS	1:50	BL	331908
goat-anti-mouse-PE	FACS	1:50	BL	405307
Isotype control IgG1-PE	FACS	1:50	BL	400114
NKp30-Fc NKG2D-Fc	FACS	1:20	R&D	1849-NK-025 1299NK-050
Cy3 anti-human Fc	FACS	1:50	DN	709-165-098

ELISA

The following ELISA kits were purchased from R&D Systems (Wiesbaden, Germany) and used to measure plasma samples: DuoSet Human MICA (Cat. DY1300), DuoSet Human MICB (Cat. DY1599), DuoSet Human MIF (Cat. DY289) and DuoSet Human ULBP2 (Cat. DY1298). In a pilot experiment, standards were diluted either in PBS or human plasma samples. Interestingly, the higher the concentration of plasma (0, 25 or 50%), the lower were the absorbance values at 450 nm wave length (data not shown). To avoid this bias, we ran all plasma samples once and chose 3-5 samples with absorbance close to or equal to the blank, pooled them, and used them as dilution solution for the standards and blank in the ELISA measurements.

For the MICA ELISA, we used the monoclonal mouse anti-human MICA antibody AMO1as capture AB and for the ULBP2 ELISA we used the monoclonal mouse anti-human ULBP2 antibody BUMO1 as capture antibody. Otherwise we adhered to the manufacturer's instructions and used 1-Step Ultra TMB-ELISA and 30% H_2SO_4 for the final step. Absorbance at 450 nm wavelength was measured in the photospectrloscopic µQuant plate reader (Bio-Tek, Turku, Finland).

Animal study

The mice were all females, aged 4-5 weeks upon delivery and housed on campus in a specialized rodent facility with 12h/12h light/dark cycle, controlled relative humidity (50% +/- 10%) and temperature (20C +/- 1) with five animals per cage. Animals received autoclaved or sterilized water, food, bedding and enrichment. Animals were acclimatized for two weeks prior to study start and eventually randomized for one of the six treatment groups:

1) wt 293T cells and PBS control injection (n=5)
2) wt 293T cells and PBMC injection (n=5)
3) ctrl 293T cells and PBS control injection (n=10)
4) ctrl 293T cells and PBMC injection (n=10)
5) BAG6 k.d. 293T cells and PBS control injection (n=10)
6) BAG6 k.d. 293T cells and PBMC injection (n=10)

Per animal, 3 x 10^6 293T cells were resuspended in 150 µl PBS and injected subcutaneously into the right flank. One day later animals received 3 x 10^6 PBMCs or PBS intraperitoneal and this day was considered as experimental day 1. Tumor growth was monitored three times a week by a trained person, who was blinded to the treatments, for 43 days. On the last study day, all animals were dissected and checked for macroscopic, hitherto latent tumors at the injection site. During the study time the animals were weighed as an additional means of health monitoring beside visual inspections before tumor measurements. One animal of group four died of unknown cause three days prior to the end of the study and was excluded from evaluation (there was no visible or palpable tumor in this animal).

Reverse transcription polymerase chain reaction (RT-PCR)

mRNA from MCF7 cells was purified, reverse transcribed and used in RT-PCR to confirm overexpression and knock down of nSmase2.

The PCR program was 2 min for 95°C, 30 cycles of 94°C for 30sec 58°C for 30 sec and 72°C for 2 min, followed by 10 min at 72°C final extension and pause at 4°C. Primer sequences were **SMPD3for** 5'-GCTCCTCTGACGACAAGCTG-3' and **SMPD3rev** 5'-TGTCCAGCAGAGTACCGATG-3', resulting in a PCR product of 114 bp length.