DNA damage regulates senescence-associated extracellular vesicle release via the ceramide pathway to prevent excessive inflammatory responses.

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This supplementary information file includes:

Supplementary Figure 1 to 4

**Figure S1.** DNA damage enhanced small EV release from ATG5 knockout mouse embryonic fibroblasts (MEFs) compared with their release in wild-type MEFs. (A–C) Murine primary MEFs derived from wild type (WT) or ATG5−/− mice were rendered senescent by 20Gy ionizing radiation (IR). After 10 days, these cells were subjected to western blotting using antibodies shown right (A), RT-qPCR analysis of SASP-factor gene expression (B) or to NanoSight analysis (NTA) for quantitative measurement of isolated small EV particles (C). For all graphs, error bars indicate mean ± standard
deviation (s.d.) of triplicate measurements. \( P \) values was calculated by unpaired two-tailed Student’s \( t \)-test (**\( P < 0.01 \), ***\( P < 0.001 \), N.S. (not significant)).

**Figure S2.** Activation of the autophagy pathway prevents inflammatory gene expression in senescent cells. (A,B) Pre-senescent TIG-3 cells were rendered senescent by ectopic expression of oncogenic ras (+HRasV12). These cells were treated with 50 nM bafilomycin A1 (BafA1) or 10 \( \mu \)M rapamycin (Rap) for 24 h and then subjected to western blotting using antibodies shown right (A) or RT-qPCR analysis of IFN-\( \beta \) and CXCL10 gene expression (B). For all graphs, error bars indicate mean \( \pm \) standard deviation (s.d.) of triplicate measurements. \( P \) values was calculated by unpaired two-tailed Student’s \( t \)-test (**\( P < 0.001 \)).

**Figure S3.** Inhibiting the small EV pathway provokes inflammasome activation and apoptosis in Bacillus Calmette–Guérin (BCG)-infected cells. (A, B) THP-1 cells were treated with 100nM PMA for 7 days and transfected with indicated siRNA oligos followed by infection with Bacillus Calmette–Guérin (BCG). These cells were subjected to western blotting using antibodies shown right (A) and to NanoSight analysis (NTA) for quantitative measurement of isolated small EV particles (B). For all graphs, error bars indicate mean \( \pm \) standard deviation (s.d.) of triplicate measurements. \( P \) values was calculated by unpaired two-tailed Student’s \( t \)-test (**\( P < 0.001 \)).
Figure S4. Inhibiting both autophagy and small EV biogenesis promotes apoptotic cell death following Bacillus Calmette-Guérin (BCG) infection. (A–C) THP-1 cells were treated with 100 nM PMA for 7 days and followed by infection with Bacillus Calmette-Guérin (BCG) with or without 5 mM 3-MA for 9 h. These cells were subjected to quantitative measurement of isolated bacterial DNA from cells using qPCR (A), NanoSight analysis (NTA) for quantitative measurement of isolated small EV particles (B) or to apoptotic cell death analysis (C). For all graphs, error bars indicate mean ± standard deviation (s.d.) of triplicate measurements. P values was calculated by unpaired two-tailed Student’s t-test (***P < 0.001).