

SUPPLEMENTARY FILE SUPPORTING:

Title: Plasma membrane perforation by GSDME during apoptosis-driven secondary necrosis.

In Cellular and Molecular Life Sciences

Author information

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Table S1 sgRNA sequences, PCR and sequencing primers used for *Gsdme* CRISPR-Cas9 gene editing

Sequence	Forward Sequence (5'→3')	Reverse complement (5'→3')
Guide sequence	TCCCAATAGCCCCGCTCTTA	TAAGAGCGGGGCTATTGGGA
Primers	GCATTCAATACATGGTTTTTGG	TAATCACCCCTAGGCTCTGG

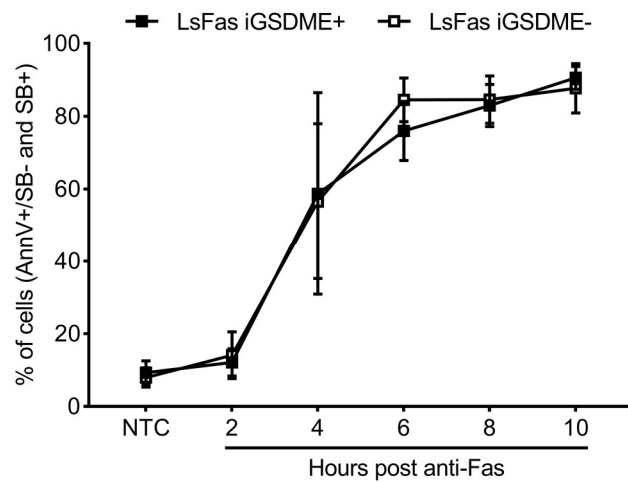


Fig. S1 Total amount of cell death, represented by the sum of the AnnV+/SB- and SB+ cells, in L929sAhFas cells with (L929sAhFas iGSDME+) or without (L929sAhFas iGSDME-) doxycycline-induced GSDME expression when treated with anti-Fas. AnnV, Annexin V; LsFas, L929sAhFas; NTC, non-treatment control; SB, SYTOX Blue

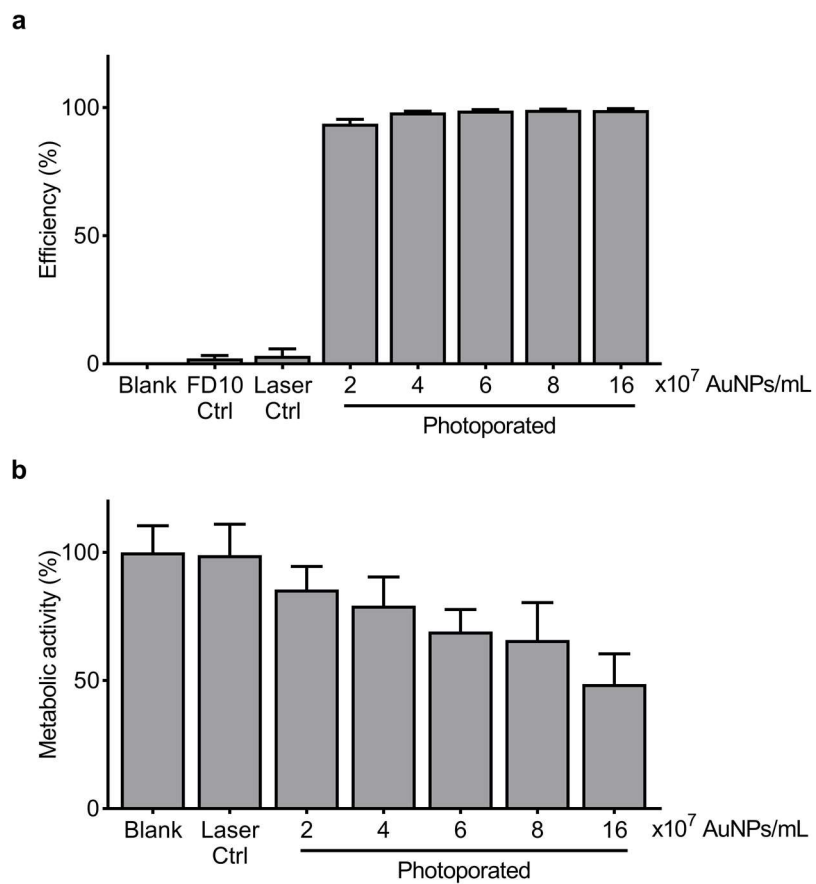


Fig. S2 Optimization of AuNP concentrations using a fixed laser fluence of 1.6 J/cm² in L929sAhFas cells. **a** Delivery efficiency of FITC-labeled dextran of 10 kDa (FD10) in function of increasing AuNP concentrations. **b** Metabolic activity in function of increasing AuNP concentrations. AuNPs, gold nanoparticles; Ctrl, control

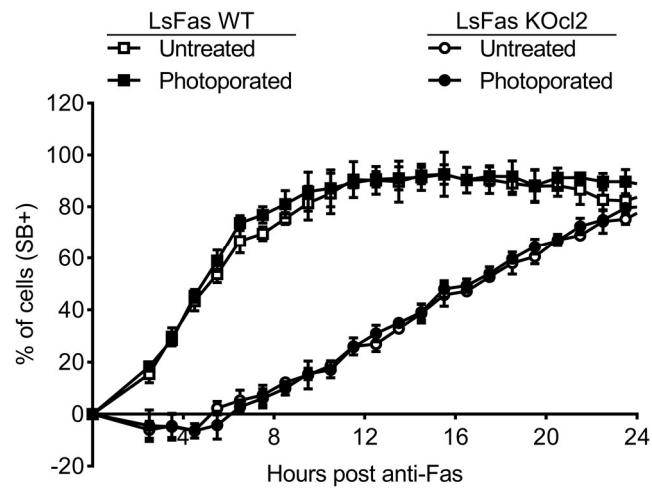


Fig. S3 Cell death kinetics, as determined by the SB staining, in untreated and photoporated cells, both in *Gsdme* WT and *Gsdme* KOcl2 L929sAhFas cells in function of anti-Fas treatment.

KO, knockout; LsFas, L929sAhFas; SB, SYTOX Blue; WT, wild-type

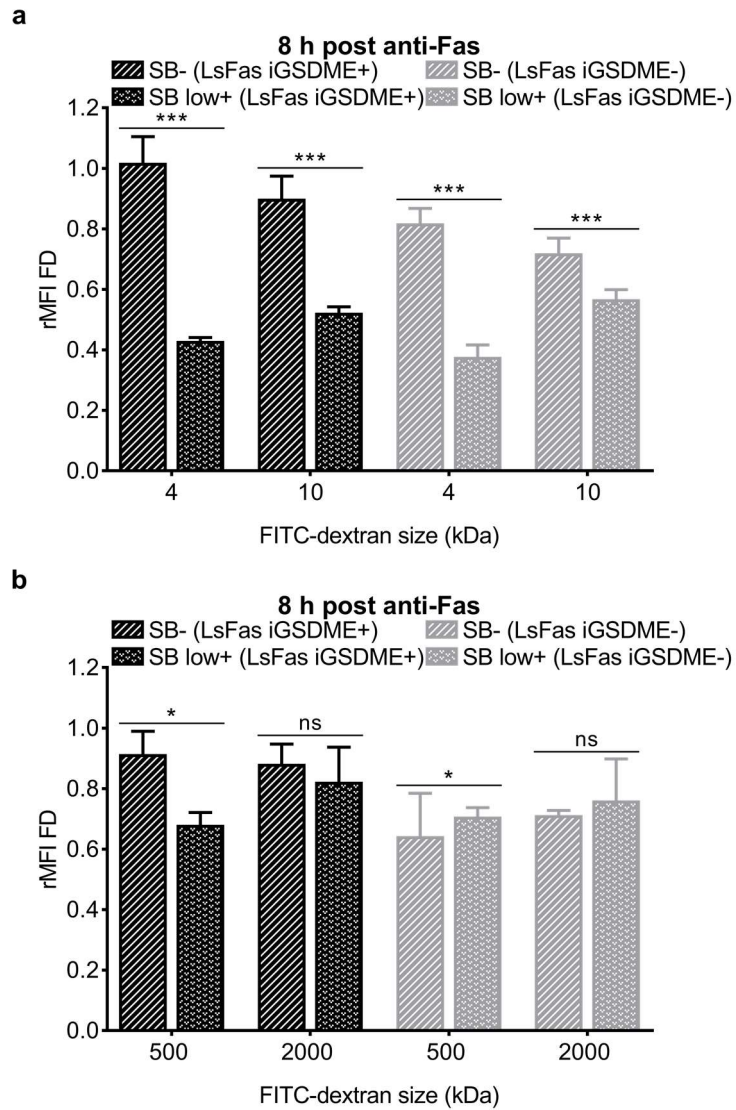


Fig. S4 Comparison of the relative mean fluorescence intensity (rMFI, relative to the untreated SB- population) for different sizes of FITC-labeled dextrans, between the SB- and SB low+ population after 8 h of anti-Fas treatment. **a** FITC-labeled dextran 4 kDa (FD4) and 10 kDa (FD10). **b** FITC-labeled dextran 500 kDa (FD500) and 2000 kDa (FD2000). FD, FITC-labeled dextran; LsFas, L929sAhFas; SB, SYTOX Blue