Figure S1.

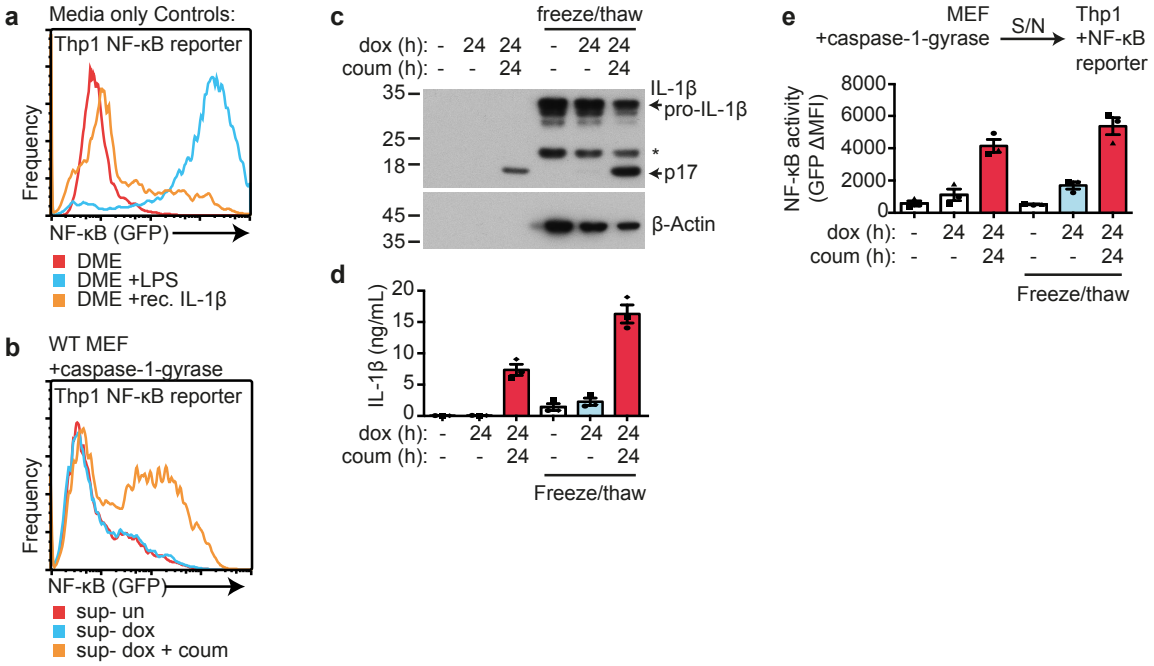
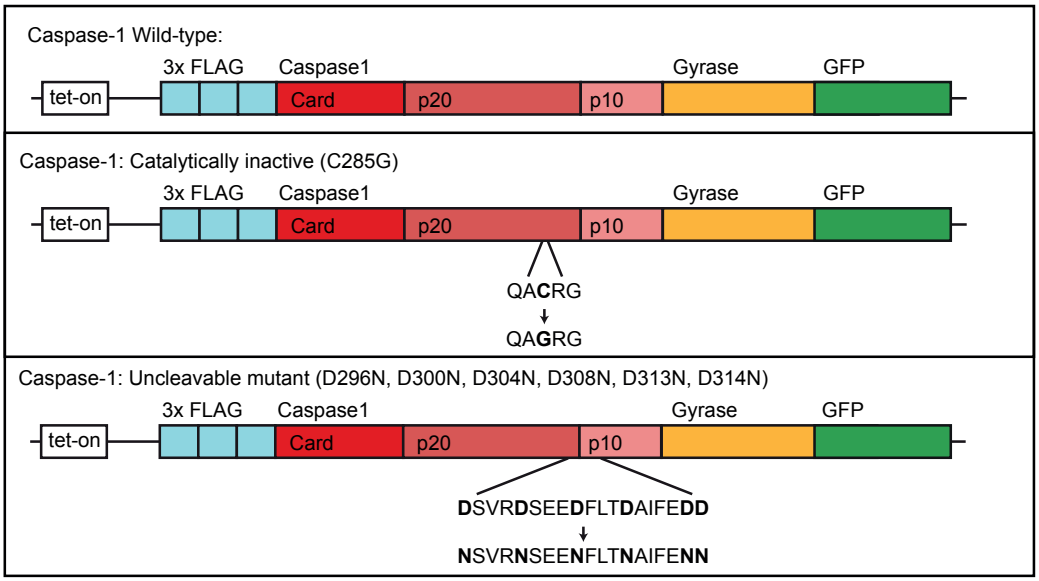


Figure S1. Dimerization of caspase-1-gyrase induces the secretion of bioactive IL-1β and activates NF-κB signalling. Related to Figure 2.

(**a and b**) IL-1β secreted from MEFs with dimerized caspase-1-gyrase is biologically active. Thp1 NF-κB reporter cells were treated with or without (a) 100 ng/mL LPS or recombinant bioactive 10 ng/mL IL-1β for 24 h. (b) Alternatively, MEFs bearing pro-IL-1β and caspase-1-gyrase constructs were treated with 1 μg/mL doxycycline and 700 nM coumermycin for 24 h, and supernatants were subsequently transferred onto Thp1 cells bearing an NF-κB GFP-reporter construct. Representative histograms of GFP expression indicating NF-κB activation. Histograms are representative of 3 independent experiments for (a) and (b), data from (b) are quantified in Figure 2c.
(**c, d and e**) NF-κB is induced in response to cleaved but not pro-IL-1β from MEFs. Cells with pro-IL-1β and caspase-1-gyrase were treated with 1 μg/mL doxycycline and 700 nM coumermycin for the indicated times in duplicate. One duplicate was frozen and thawed 3 times for efficient cellular lysis. Supernatants were analyzed by (c) Western blot for indicated proteins and (d) IL-1β ELISA. (e) Supernatant was also transferred onto Thp1 cells bearing an NF-κB GFP-reporter construct, and after a 24 h incubation GFP expression, indicating NF-κB activation, was quantified as the change in MFI relative to Thp1 cells in media alone. n = 3 independent experiments. Error bars represent the SEM in all graphs. Asterisks denote non-specific bands in Western blots.

Figure S2.

a



b

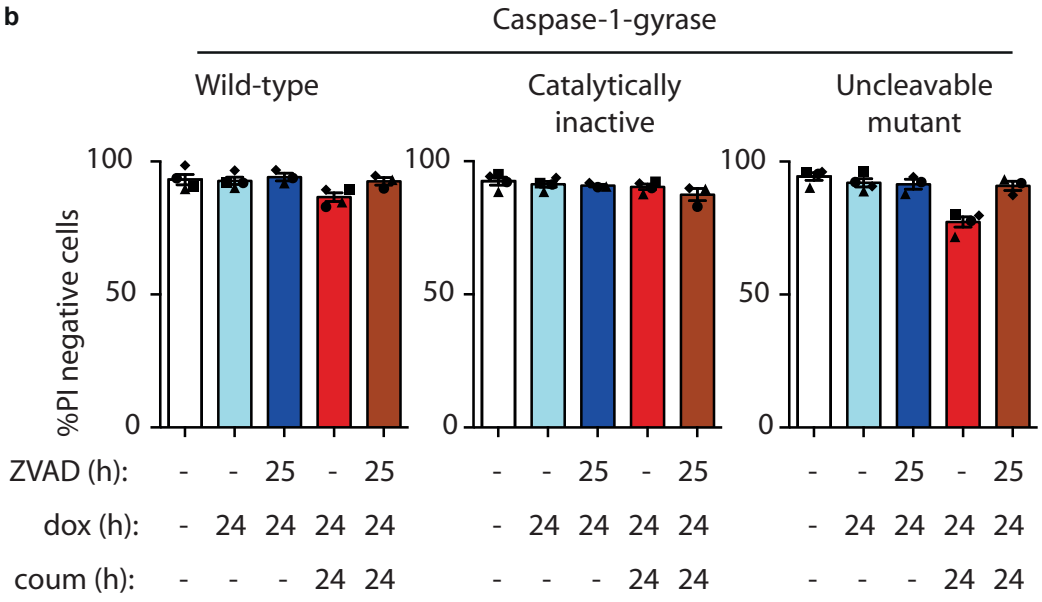


Figure S2. WT and catalytically inactive caspase-1-gyrase do not induce cell death, while the uncleavable mutant induces a small amount of cell death. Related to Figure 2.

(a) Schematic of doxycycline inducible caspase-1-gyrase fusion proteins with mutations to either the catalytic cysteine (C285), or 6 aspartic acid residues between the p10 and p20 regions of caspase-1 (D296N, D300N, D304N, D308N, D313N, D314N).
(b) Dimerization of uncleavable caspase-1-gyrase, but not the wild-type or catalytically inactive version, induces a small amount of cell death. MEFs stably infected with one of three caspase-1-gyrase vectors and pro-IL-1β were treated with 1 μg/mL doxycycline, 700 nM coumermycin and 25 μM Z-VAD-fmk. Cell viability was measured by PI uptake and assessed by flow cytometry. n = 3-4 independent experiments. Error bars represent the SEM.

Figure S3.

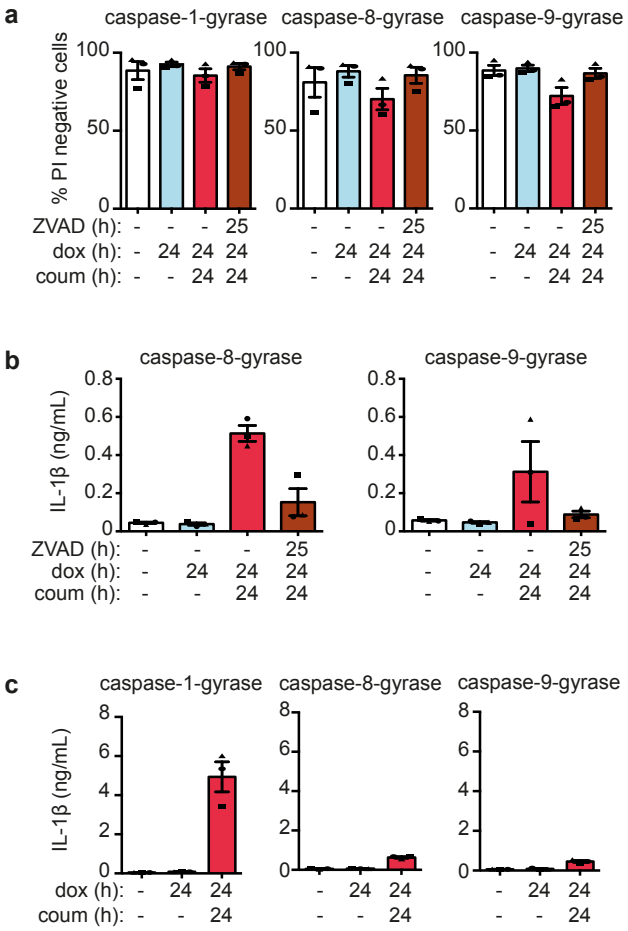


Figure S3. Caspase-1-gyrase, caspase-8-gyrase and caspase-9-gyrase activity can be inhibited by the pan-caspase inhibitor ZVAD-fmk. Related to Figure 4.

(**a and b**) Cell death and secretion of IL-1β activated by caspase dimerization can be inhibited by ZVAD-fmk. MEFs containing pro-IL-β and either caspase-1-gyrase, caspase-8-gyrase or caspase-9-gyrase constructs were treated with 1 µg/mL doxycycline, 700 nM coumermycin and 25 µM Z-VAD-FMK for the indicated times. (a) Cell viability was measured by PI uptake and flow cytometry. (b) IL-1β released into the supernatant was measured by ELISA. n = 3 independent experiments. (**c**) Supernatants used to treat NF-κB GFP-reporter Thp1 cells in Figure 4d were analyzed for IL-1β levels by ELISA. n = 3 independent experiments.
Data for caspase-1, -8 and -9 were collected in parallel but are presented in 3 separate graphs. Error bars represent the SEM in all graphs.

Figure S4.

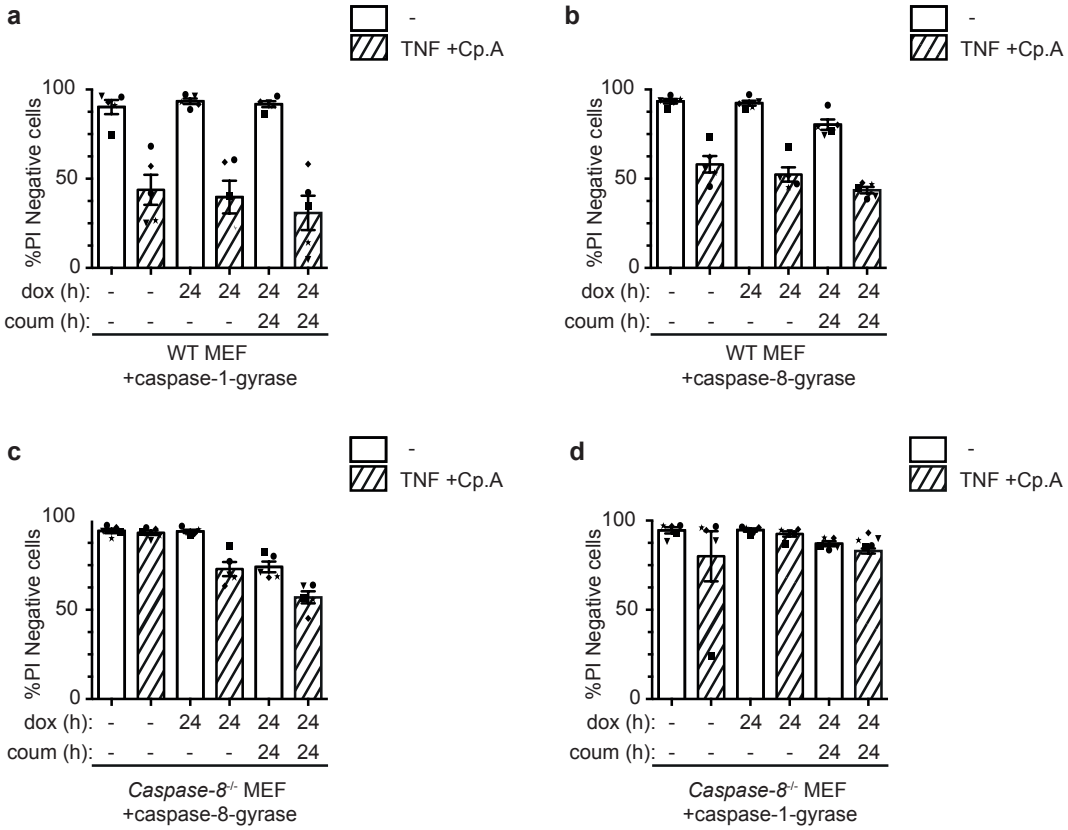


Figure S4. Caspase-8-gyrase, but not caspase-1-gyrase, can reconstitute caspase-8 mediated cell death in caspase-8-deficient MEFs. Related to Figure 5.

(a, b, c and d) MEFs containing pro-IL-1β and either (a and d) caspase-1-gyrase or (b and c) caspase-8-gyrase vectors were treated with 1 μg/mL doxycycline and 700 nM coumermycin, and 100 ng/mL TNF and 1 μM Cp.A as indicated by cross hatched columns. Loss of cell viability was measured by PI uptake and flow cytometry. n = 5 independent experiments. Error bars represent the SEM in all graphs.

Figure S5.

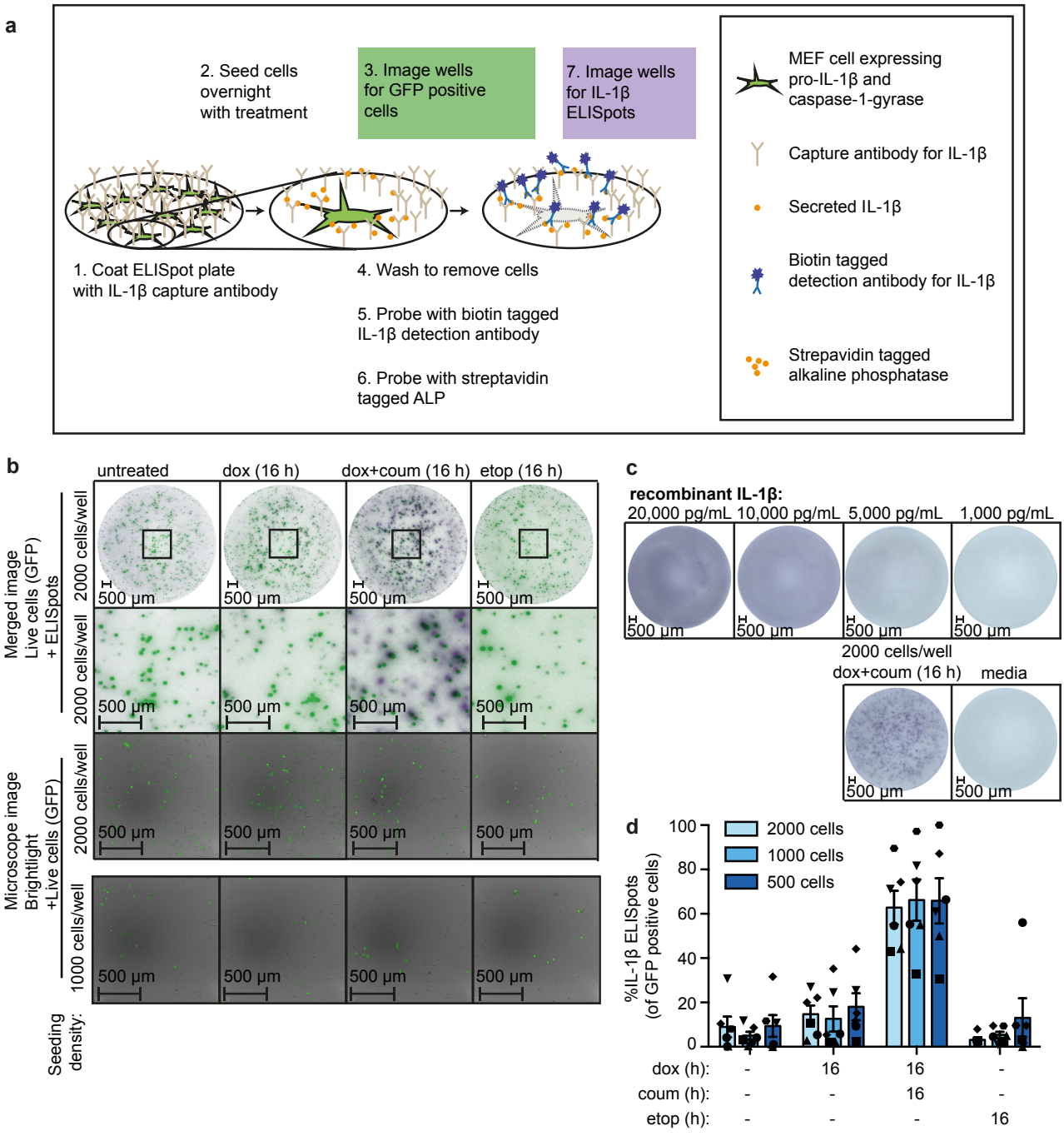


Figure S5. Caspase-1 dimerization, but not etoposide treatment or exogenous IL-1β, causes the formation of ELISpots. Related to Figure 6.

(a) Schematic of the experimental set up of ELISpot experiments presented in Figure 6b, c and d.
(b) Cells seeded at low confluency produce spatially distinct IL-1β ELISpots. Comparison of duplicate wells seeded at the same time for ELISpot or on tissue culture coated plates for microscopy. GFP positive cells and IL-1β ELISpot images were acquired and quantified using the AID ELISpot software. Microscopy images were acquired using the Opera Phenix, taken at 10x with 2x2 binning. The brightness and contrast of images were adjusted using Fiji (ImageJ) and Adobe Illustrator in a linear fashion, with settings applied equally to every image. GFP images were inverted in the merged image and pseudo-colouring applied using Adobe Illustrator to allow overlay of ELISpots and GFP positive cells.
(c) Exogenous IL-1β is detectable as diffuse staining by ELISpot. Recombinant IL-1β in media, at indicated concentrations, media alone, or MEF cells containing pro-IL-1β and caspase-1-gyrase constructs stimulated with 1 µg/mL doxycycline and 700 nM coumermycin, were visualized after 16 h for IL-1β ELISpots (see Fig S5a). Representative images of triplicate wells.
(d) Dimerized caspase-1-gyrase induces IL-1β secretion from 60-70% of GFP-positive cells. Analysis of IL-1β ELISpot results represented as a ratio to GFP positive cells, representing live cells, from the same well. GFP and ELISpot images were acquired and quantified using an AID ELISpot Reader. n = 6 independent experiments. Error bars represent the SEM.