Figure S1. iNam promotes necroptosis. MEF cells were incubated in the presence of iNam (20 mM) for 8 hours and then exposed to hTNF (1 ng/ml) for 16 hours before monitoring cell survival. MEF cells were also incubated with GSK'843 (R3i; 3 μM), a specific RIPK3 inhibitor, for 1 hour before exposure to hTNF (1 ng/ml) to further confirm that TNF plus iNam induces necroptosis (n=4). Data represent mean + S.D. of four independent experiments (*P<0.05).

Figure S2. Cambinol protects from TNF-induced cell death. A typical flow cytometry profile of L929 cells treated as previously described in figure 2. Dying/dead cells are characterized by the accumulation of the cell impermeable, propidium iodide fluorescent marker and by a reduction in size as estimated by a reduction in forward scattering (FSC).

Figure S3. Additional pharmacological properties of cambinol. (a) Cambinol and necrostatin-1 display additive effects. Cell survival in response to recombinant hTNF-induced necroptosis was assessed after 16 hours in L929 cells previously incubated in the presence of cambinol (12.5 μM), necrostatin-1 (0.1 μM) and a combination of both compounds (n=4). Data represent mean + S.D. of four independent experiments (*P<0.05).

(b) Cambinol sensitizes to ionomycin-induced cell death. Cell survival in response to ionomycin (20 μM) was assessed 16 hours after stimulation in L929 cells in the presence of cambinol (100 μM) and necrostatin-1 (20 μM) (n=4). Data represent mean + S.D. of four independent experiments (*P<0.05). **(c)** Cambinol treatment leads to accumulation of acetylated forms of tubulin. L929 cells were incubated in the presence of the indicated doses of cambinol for 6 hours, and expression of total and acetylated forms of tubulin assessed by western blot using appropriate antibodies. In keeping with the well-described role for SIRT2 in controlling tubulin acetylated status, cambinol treated cells displayed increased expression of Ac-tubulin, confirming that cambinol, at the doses used thorough this study, acts as a bona fide sirtuin-inhibitor (n=3). Data represent mean + S.D. of four independent experiments (*P<0.05).

Figure S4. SIRT2 and SIRT5 knockout L929 are protected from necroptosis. L929 cells were invalidated for SIRT2 and SIRT5 using the CRISPR/Cas9 technology. **(a, b)** In a first round of experiments, **(a)** L929 cells were tested for efficacy and specificity of each CRISPR/Cas9 vector as illustrated using western blot analysis (n=2). **(b)** L929 were also assessed for cell survival in response to recombinant hTNF (200 pg/ml) 16 hours post-treatment (n=4). **(c, d, e)** In a second round of experiments, two sublines of L929 invalidated for SIRT2 were generated and **(c)** tested for efficacy by western blot analysis (n=2). **(d)** The two L929 cells sublines were again assessed for cell survival in response to recombinant hTNF (200 pg/ml), **(e)** or anti-hFAS (CH-11; 50-150 ng/ml) 16 hours post-treatment (n=4) Data represent mean + S.D. of four independent experiments (*P<0.05).