Supplementary figure legends

Figure S1. MLKL translocates to the nuclei in response to TBZ treatment. Immunocytochemical analysis of the content of MLKL in nuclei isolated from untreated HT29 cells and from cells treated for 4 h with TBZ. Bars, 10 μm.

Figure S2. Nuclear translocation of MLKL occurs independently of cell death. Assessment of the impact of the reducing agent N-acetylcysteine (NAC; 5 mM) and of the free radical-scavenging agent butylated hydoxyanisole (BHA, 30 μM) on the extent of nuclear accumulation of MLKL, and on death of mouse L929 cells treated with TBZ. Cell death was quantified by the LDH-release assay. Mean ± SD of tetraplicates are shown.

Figure S3. RIPK1 translocates to the nucleus in response to TBZ treatment. Immunocytochemical analysis of HT29 cells transiently expressing RIPK1 that was fused at its N-terminus to the streptavidin-binding peptide (SBP−hRIPK1). Cells were immunostained with Cy2-conjugated streptavidin (green) and with an antibody to lamin (red). Bars, 10 μm.

Figure S4. RIPK3 translocates to the nucleus in response to TBZ treatment. Immunocytochemical analysis of MEFs constitutively expressing mRIPK3 (mouse RIPK3) that was fused at its N-terminus to the SBP (SBP−mRIPK3). Cells were immunostained with Cy2-conjugated streptavidin (green) and with an antibody to lamin (red). Bars, 10 μm. Both necroptosis and nuclear translocation of MLKL, RIPK1 and RIPK3 are induced by TBZ much more rapidly in MEFs than in HT29 cells. The immunocytochemical analysis of the MEFs was therefore done at an earlier time point after TBZ application (2 h, compared to 4 h in Figure S3 where HT29 cells were examined).

Figure S5. The lower spontaneous death mediated by the MLKL(1−180) mutant than that mediated by the MLKL S358D and MLKL K230M/Q356A mutants cannot be accounted for by lower expression or lesser oligomerization of MLKL(1−180). To induce the three mutants, 4-hydroxytamoxifen (4OHT) was applied at different concentrations for 6 h (upper and middle panels) or for 6 h and 12 h (lower panel). MLKL expression was analyzed by western blotting under either reducing (upper panel) or non-reducing (middle panel) conditions. Arrows and the brackets at the right side of the middle panel point to the monomeric and oligomeric forms of MLKL, respectively. Cell viability at the times indicated on the bottom panel was determined in cultures expressing the specified MLKL mutants using the 4OHT concentrations indicated by red and yellow arrows in the upper and middle panels.