Figure legends for supplementary figures

Figure 1: Mapping of PIDD-fragment interaction.

(A) Detection of endogenous PIDD. Western blot analysis of cell extracts of Jurkat T and Ramos B cell lines probed with a monoclonal anti-PIDD antibody recognizing the death domain of PIDD. (B) HEK293T cells were transfected with siRNA targeting PIDD for 36 h and analyzed for efficiency by Western blotting. (C) Anti-VSV immunoprecipitation was carried out on lysates from HEK293T cells stably expressing VSV-PIDDΔLRR-Flag (PIDDΔ1-285) and the eluates were then analyzed for Flag-tagged fragments. (D) Immunoprecipitation using AL249, anti-PIDD (148-174), was carried out on lysates from HEK293T cells stably expressing C-terminally tagged PIDD, PIDDΔID (PIDDΔ555-777), or PIDDΔDD (PIDDΔ778-910) and the eluates were analyzed for the presence of Flag-tagged fragments. (E) Analysis of the cell extracts from the cell lines used in (C) and (D).

Figure 2: In vitro processing of PIDD.

In vitro processing of inactive F445H mutant by the nucleophile NH_2OH. Expression of the Flag-tagged PIDD mutants was induced in HEK293Trex during 5h. The various proteins were purified on an anti-Flag affinity column. Immunoprecipitates were eluted using Flag peptides. The various purified PIDD mutants were then incubated with NH_2OH in the presence or absence of denaturing SDS. PIDD cleavage was analyzed by Western blotting using the monoclonal anti-PIDD antibody.

Figure3: PIDD-N does not act as a dominant interfering on PIDD functions.

(A) HEK293T cells were transfected with the indicated constructs and the lysates were analyzed by western-blot. caspase-2 activation as monitored by the appearance of the p19 fragment. (B) HEK293T cells were transiently transfected with PIDD-N or empty vector and subsequently treated with 40μM etoposide for the indicated times. NF-κB activation was monitored by western-blot.

Figure 4: PIDD-CC generation is required for caspase-2 activation.

(A) VSV-RAIDD and different Flag-PIDD constructs were coexpressed in HEK293T cells as indicated. PIDD fragments associated with RAIDD in RAIDD immunoprecipitates were analyzed by Western blotting. (B) PIDD expression was induced in HEK293Trex cells stably expressing the indicated FL or fragments of PIDD by doxycycline for the indicated times. PIDD was immunoprecipitated and recruitment of

RAIDD and caspase-2 analyzed. (C) PIDD-CC mediated caspase-2 activation: requirement of the DD and intermediate domain. HEK293T cells stably expressing Flag-tagged PIDD fragments were monitored for caspase-2 cleavage by Western blot.

Figure 5: PIDD-C is essential for PIDD nuclear translocation.
PIDD-C or PIDD-CC expression was induced in HEK293Trex cells stably expressing the indicated FL or fragments of PIDD by doxycycline (doxy) for the indicated times and analysed for their capacity to translocate to the nucleus. While PIDD-C showed a nuclear localisation upon genotoxic stress (camptothecin, CPT) or treatment with the nuclear export inhibitor Ratjadone C, PIDD-CC was found exclusively in the cytosol. Confocal images showing nuclear (blue, DRAQ5) or PIDD (green, Alexa 488) staining.

Figure 6: Analysis of PIDD auto-processing (cleavage) mutants for their capacity to induce NF-κB activation.
The different mutants were stably expressed in HEK293T cells and tested for their NF-κB activating potential by following the phosphorylation of IκB upon exposure to 40 μM etoposide for the indicated time.

Figure 7: PIDD-N levels are not affected by cycloheximide treatment.
HEK293T cells stably expressing PIDD were treated for 0, 8 or 16 h with CHX (10μg/ml) in presence or absence of MG-132 (10μM) for the indicated times. The presence of PIDD-N was then assessed. *: unspecific band.