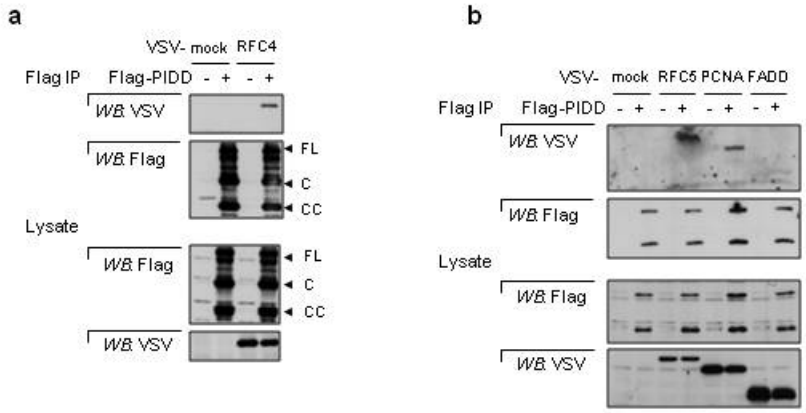
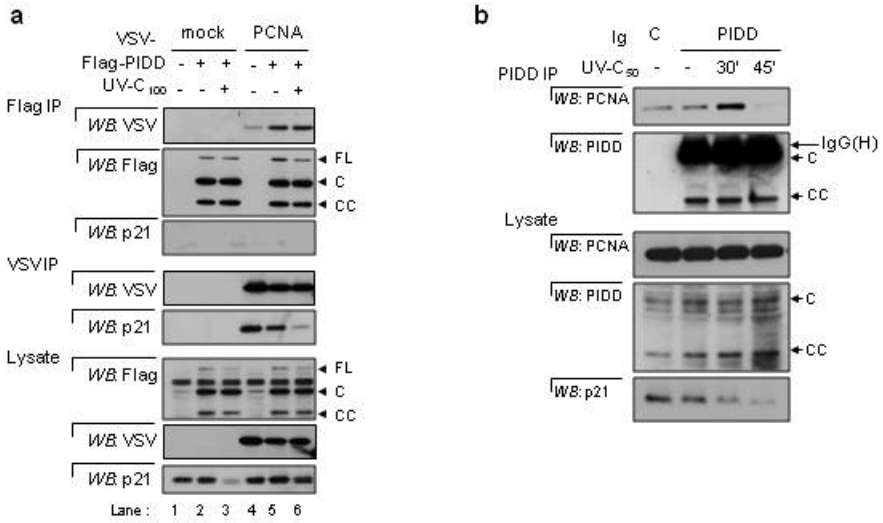
## SUPPLEMENTAL DATA

Supplementary Figure 1



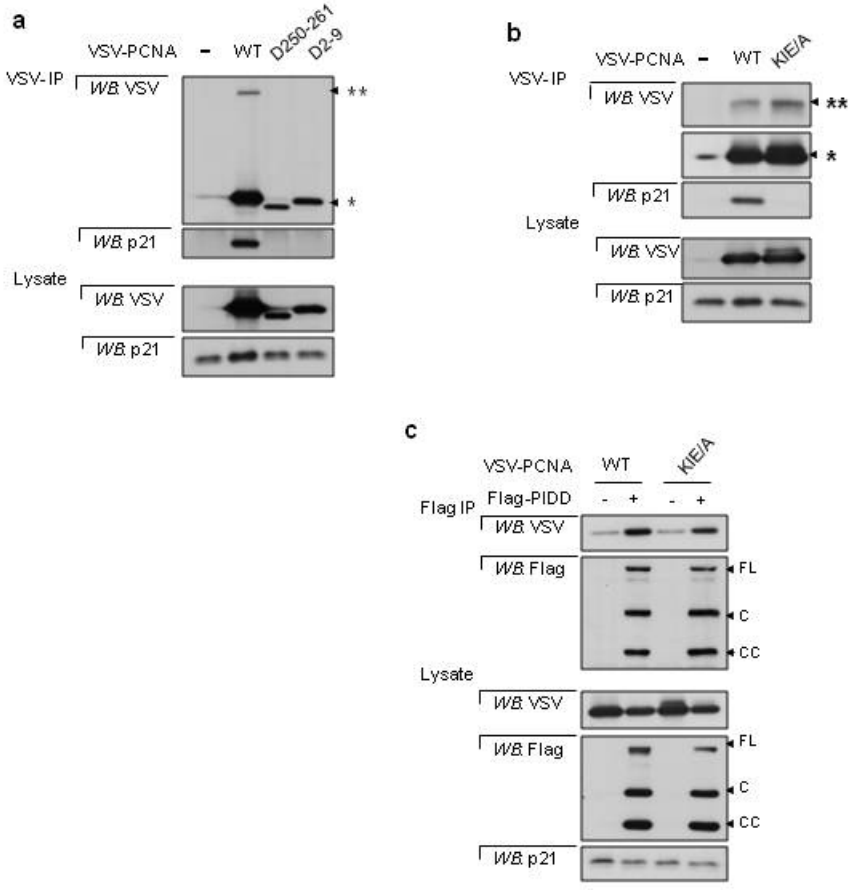

**Suppl Figure 1. Related to Figure 1 (a-b) PIDD interacts with RFC4**. Different VSV-tagged constructs were cotransfected in 293T cells with Flag-tagged PIDD and tested for interaction by co-immunoprecipitation as described in 'experimental procedures'.

Supplementary Figure 2



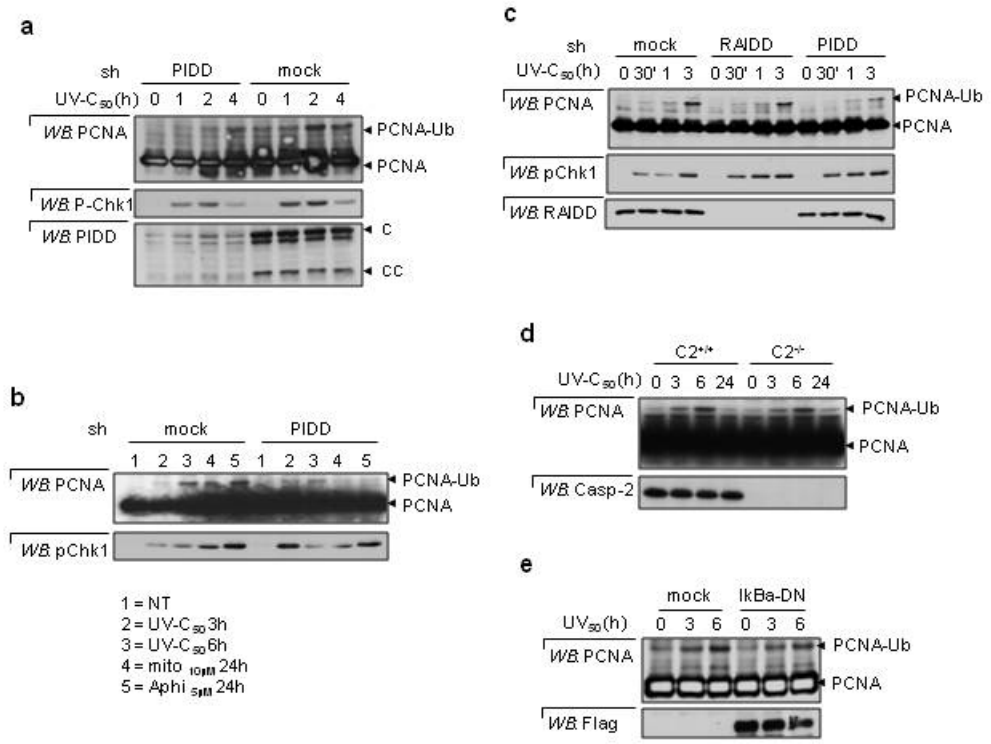

**Suppl Figure 2. Related to figure 2 (a)** VSV-tagged PCNA or a control vector was cotransfected in 293T cells with Flag-tagged PIDD and tested for interaction (after 100 J/m$^2$ UV-C irradiation where indicated). Endogenous p21 recruitment was analyzed in both VSV and Flag-IP. **(b)** 293T cells were subjected to different times of 50 J/m$^2$ UV-C irradiation. Endogenous PIDD was immunoprecipitated and endogenous PCNA interaction was analyzed (C = Ig Control, IgG(H) = IgG Heavy Chains).

Supplementary Figure 3



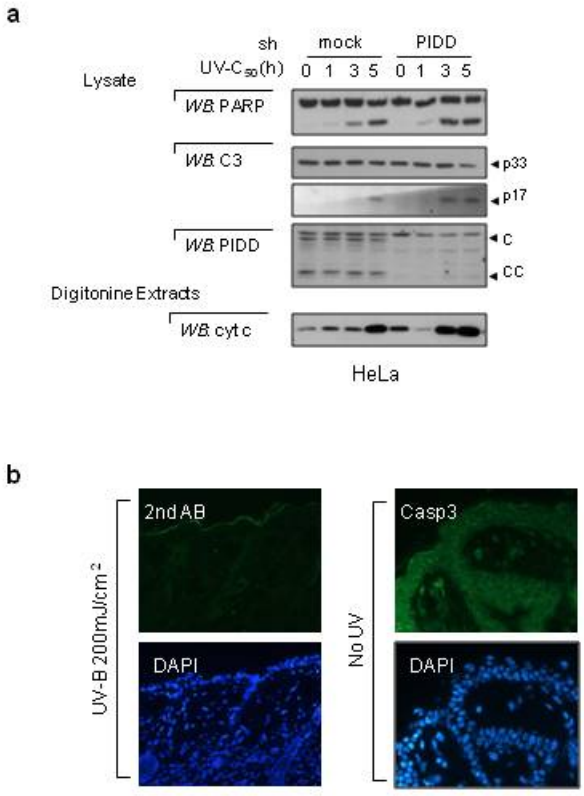

**Suppl Figure 3. Related to figure 3 (a)** 293T cells were transfected with differents constructs of VSV-tagged PCNA. **(b)** 293T cells were transfected with wt or KIE/A mutant of VSV-tagged PCNA. **(c)** 293T cells were cotransfected with wt or KIE/A mutant of VSV-tagged PCNA and Flag-tagged PIDD where indicated. *=PCNA monomers, **=PCNA trimers). Where indicated, endogenous p21 interaction with PCNA was analysed by anti-VSV IP, endogenous PCNA interaction with FLAG-tagged PIDD was analysed after anti-Flag IP.

## Supplementary Figure 4



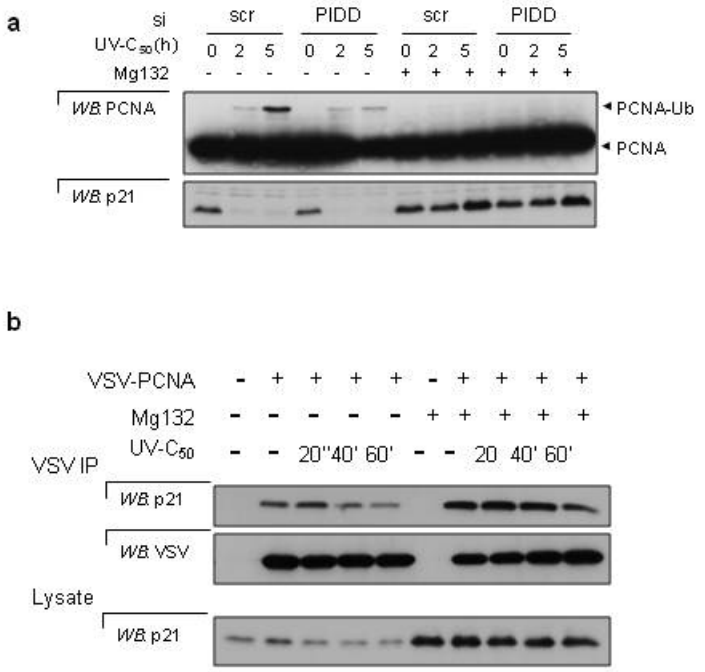

**Suppl Figure 4. Related to figure 4. PIDD deficiency impairs PCNA monoubiquitination induced by UV-irradiation (a-b)** Jurkat or 293T cell lines are stably knockdown for PIDD and subjected to different DNA damage treatment (HU = Hydroxyurea, mito = mitomycine C, Aphi = Aphidicolin) for different time. **(c-e)** Cells were subjected to different times of 50 J/m$^2$ UV-C irradiation and different proteins were analyzed by western blotting. **(c)** HeLa cells stably knocked-down for PIDD, RAIDD or corresponding control cells. **(d)** 3T3 immortalized caspase-2$^{+/+}$ or caspase-2$^{-/-}$ MEF cells. **(e)** Hela cells transfected with Flag-tag version of the dominant-negative form of IkBa or with a control vector.

Supplementary Figure 5



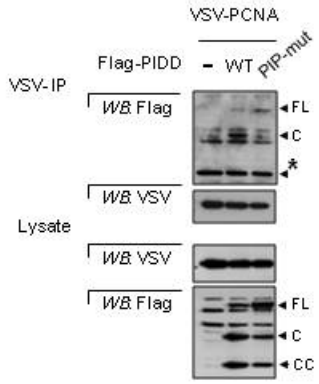

**Suppl Figure 5. Related to figure 6 (a) PIDD deficiency sensitizes cells to UV induced apoptosis.** HeLa cells stably knockdown for PIDD or the control cells were subjected to different times of 50 J/m$^2$ UV-C irradiation. PARP and caspase-3 cleavage as well as cytochrome c release were analysed by western-blotting. **(b)** Control of staining specificity in figure 6c.

Supplementary Figure 6




**Suppl Figure 6. Related to discussion (a)** HeLa cells are transfected with control or PIDD siRNA for 48h and subjected where indicated to a 1h pretreatment of 10$\mu$M MG132 before harvesting at different times post-UV-C irradiation. Monoubiquitination of PCNA and p21 degradation were analysed by western-blotting. **(b)** 293T cells were transfected with VSV-tagged PCNA and subjected where indicated to a 1h pretreatment of 10$\mu$M MG132 and harvested as in (a). Endogenous p21 interaction with PCNA was analysed by anti-VSV IP, and p21 degradation was detected in total extracts.

Supplementary Figure 7




**Suppl Figure 7. Related to discussion**. VSV-tagged PCNA was cotransfected in 293T cells with Flag-tagged PIDD WT or mutated in the PIP identified region and tested for interaction by co-immunoprecipitation as described in 'experimental procedures'. *=unspecific band

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

**Cell culture.** Jurkat T cells were grown in RPMI (Life Technologies) and 3T3 immortalized MEF Casp2$^{+/+}$ or Casp2$^{-/-}$ were grown in DMEM + Glutamax (Life Technologies) containing 50$\mu M$ β-mercaptoethanol. All media were supplemented with 10% fetal calf serum and 100 U/ml penicillin, 100 □g/ml streptomycin.

**Mice.** Mice were housed according to the Swiss Federal and Cantonal Veterinary Office laws and guidelines. PIDD deficient mice (Manzl et al., 2009) were backcrossed 4 to 5 times to C57BL/6-J OlaHSd (Harlan, Holland), and all experiments were performed using 8 to 12 week-old littermate animals. For acute irradiation, mice shaved on their back the previous day were exposed to a single irradiation of 200 mJ/cm$^2$ of UV-B. 24h after irradiation, the mice were killed by cervical dislocation followed by carotid section and bleeding.

**Expression vectors.** pCR3-PIDD-Flag, pMSCV-PIDD-Flag as well as all mutant versions of PIDD have been described previously (Tinel et al., 2007). All PIDD constructs are C-terminally Flag-tagged. XPG expression vector was kindly provided by Dr I. Dunand-Sauthier (University of Geneva Medical School, Switzerland) (Dunand-Sauthier *et al*., 2005, *JBC* 280, 7030-7037). pCR3-VSV expression vector for PCNA, RFC5, RFC4 and FADD were obtained by RT-PCR on mRNA from 293T cells using oligonucleotides containing EcoR1 (for oligos sens) and Not1 (for antisens) and cloned in the N-terminus-VSV-pCR3 vector. Oligonucleotides used are the following:

PCNA sense : 5' ggaattcTTCGAGGCGCGCCTGGTCCAGGGC 3'
antisens : 5' cgcggccgcAGATCCTTCTTCATCCTCGATCTTGG 3'
RFC5 sense : 5' ggaattcGAGACCTCAGCACTCAAGCAGCAG 3'
antisense : 5' cgcggccgcGGCCTCTGCAACAATCAGCTC 3'
RFC4 sense : 5' ggaattcCAAGCATTTCTTAAAGGTACATCC 3'
antisense : 5' cgcggccgcACAATTCTGAGATAACTGCTGC 3'
FADD sense : 5' ggaattcGACCCGTTCCTGGTGCTGCTG 3'
antisense : 5' cgcggccgcGGACGCTTCGGAGGTAGTAG 3'

**PCNA and PIDD mutagenesis**

Deletion mutants of PCNA Δ9 (Δ1-9), Δ250 (Δ250-263), ΔC-ter (1-120) and ΔN-ter (130-261) were obtained by PCR on the pCR3-VSV-PCNA parental vector with the relevant oligonucleotides (described below) and cloned EcoR1/Not1 in the N-terminus-VSV-pCR3 vector.

PCNA Δ9 sense : 5' ggaattcATCCTCAAGAAGGTGTTGGAGG 3'
antisense : 5' cgcggccgcAGATCCTTCTTCATCCTCGATCTTGG 3'

PCNA Δ250 sense : 5' ggaattcTTCGAGGCGCGCCTGGTCCAGGGC 3'
antisense : 5' cgcggccgcGTATTTTAAGTGTCCCATATCCGC 3'

PCNA ΔC-ter sense : 5' ggaattcTTCGAGGCGCGCCTGGTCCAGGGC 3'
antisense : 5' cgcggccgcATCCATCAACTTCATTTCATAGTC 3'

PCNA ΔN-ter sense : 5' ggaattcCAGGAGTACAGCTGTGTAGTAAAG 3'
antisense : 5' cgcggccgcAGATCCTTCTTCATCCTCGATCTTGG 3'

To generate the KIE/A PCNA mutant (K-I-E in position 254-255-256 changed in AAA), and the PIP-mutant of PIDD (QxxV in position 391-394 changed in AxxA), point mutations in pCR3-VSV-PCNA or pCR3-PIDD-Flag respectively were introduced with the « QuickChange site directed mutagenesis » kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions. Briefly, fourty ng of parental vectors were used with the relevant oligonucleotides (KIE/A PCNA mutant: reverse primer : 5' CTACTTGGCTCCC**GC**G**GC**CG**C**GGATGAAGAAGGATCT 3' , forward primer : 5' AGATCCTTCTTCATCC**GC**G**GC**C**GC**GGGAGCCAAGTAG 3'; (PIP-PIDD mutant: reverse primer : 5' GGGGTGGCCTTCGCGCAGGATGCGGGGCTGTGG 3' , forward primer : 5' CCACAGCCCCGCATCCTGCGCGAAGGCCACCCC 3') and PCR was performed for 18 cycles. The mutation was confirmed by sequencing.

**SiRNA and shRNA sequences**

Control (Allstar scramble siRNA) and PIDD siRNA were provided from Qiagen.

PIDD target sequence: 5' CAGACTGTTCCTGACCTCAGA 3'

Lentiviral vector for shRNA expression were provided from Openbiosystems. (PIDD shRNA: NM_145886 TRCN0000166358, RAIDD shRNA NM_003805 TRCN0000107205). In all experiments, the control used is the pLKO empty vector.

**Identification of PIDD-binding Partners by mass-spectrometry**

**Cytoplasmic and nuclear fractionations**. Around $10^6$ 293T cells stably expressing Flag-PIDD or control cells (obtained by retroviral infection as described in experimental procedures) were harvested and washed two times with PBS and then two times with PBS 10mM sodium butyrate. Cells were lysed in 2ml lysis buffer containing 0.1% Triton X-100, 250mM sucrose, 10mM Tris pH 7.4, 10mM sodium butyrate, 4mM $MgCl_2$ and complete protease inhibitor (PI, Roche). Cells were gently disrupted in a loose Dounce homogenizer (25 strokes) and the resulting lysate was centrifugated at 4500rpm at 4°C, 10 min. The supernatant corresponding to the cytoplasmic fraction was cleared by centrifugation at 13'000rpm at 4°C, 10 min. The pellet was resuspended in 4ml of buffer C containing 250mM sucrose, 10mM Tris pH 7.4, 10mM sodium butyrate, 4mM $MgCl_2$, and PI, put on a 30% sucrose cushion (30% sucrose in buffer C) and centrifuged at 3300rpm, 5 min at 4°C (swing-out rotor, brake set to minimum). The pellet containing the nuclei was resuspended in 1ml buffer N containing 0.5% NP-40, 10% glycerol, 420mM NaCl, 20mM Hepes pH 7.9, 1.5mM $MgCl_2$, 1mM DTT and PI. Nuclear extracts were incubated on a rotating wheel at 4°C for 30 min, homogenized in a loose Dounce homogenizer (20 strokes) and clarified by centrifugation at 13'000rpm 4°C for 20 min. Nuclear fractions were diluted before immunoprecipitations with buffer C without salt to obtain a 150mM NaCl concentration. Tris pH 7.4 and NaCl were added to cytoplasmic fractions to obtain a 50mM and 150mM concentration respectively before immunoprecipitations (described in Experimental procedures).

**Fractionation, digestion and analysis by mass spectrometry**

Eluted proteins from IP were separated on a 10% mini SDS-polyacrylamide gel and stained with Coomassie blue. Entire gel lanes were excised into 20 equal regions from top to bottom and digested with trypsin (Promega) as described (1,2). Data-dependent LC-MS/MS analysis of extracted peptide mixtures after digestion was carried out on a hybrid linear trap LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific) interfaced

to an Agilent 1100 nanocapillary HPLC equipped with a 75um internal diameter C18 reversed-phase column. Collections of tandem mass spectra for database searching were generated from raw data and searched using Mascot (Matrix Science, London, UK; version 2.1.0) against the release 11.0 of the UNIPROT database, (SWISSPROT + TrEMBL, www.expasy.org) restricted to human taxonomy. The software Scaffold (version Scaffold-01_06_03, Proteome Software Inc.) was used to validate MS/MS based peptide (minimum 95% probability (4)) and protein (min 99 % probability (3)) identifications, perform dataset alignment and subtraction as well as parsimony analysis to discriminate homologous hits. In an additional filtering step, only proteins identified with at least 3 spectra were retained for further analysis.

## SUPPLEMENTAL REFERENCES


1. Shevchenko, A.; Wilm, M.; Vorm, O.; Mann, M., Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. Anal Chem 1996, 68, (5), 850-8.

2. Wilm, M.; Shevchenko, A.; Houthaeve, T.; Breit, S.; Schweigerer, L.; Fotsis, T.; Mann, M., Femtomole sequencing of proteins from polyacrylamide gels by nano-electrospray mass spectrometry. Nature 1996, 379, (6564), 466-9.

3. Nesvizhskii, A. I.; Keller, A.; Kolker, E.; Aebersold, R., A statistical model for identifying proteins by tandem mass spectrometry. Anal Chem 2003, 75, (17), 4646-58.

4. Keller, A.; Nesvizhskii, A. I.; Kolker, E.; Aebersold, R., Empirical statistical model to estimate the accuracy of peptide identifications made by MS/MS and database search. Anal Chem 2002, 74, (20), 5383-92.