

## Supplementary Figure Legends

### Supplementary Figure 1:

(A) Time-dependent PAR formation by PARP1 in the absence or presence of DNA as revealed by western blot. (B) Time-dependent PAR formation by PARP1 in the absence or presence of DNA as revealed by vacuum slot blot. (C) PAR formation by PARP1, PARP2 and PARP3. (D) PAR formation by PARP1, chimera PARP1-2 and chimera PARP1-3. (E) PAR formation of chimera PARP2-1, chimera PARP3-1 and PARPs-1. PARP1 served as control (ctr.). Molecular size markers in kDa and the border between stacking and separating gel (asterisk) are indicated.

### Supplementary Figure 2:

(A) Overlay of the crystal structures available for the catalytic domains of chicken PARP1 (PDB: 1A26), mouse PARP2 (PDB: 1GS0) and human PARP3 (PDB: 2PA9). PDB files were obtained from RCSB PDB ([www.rcsb.org/pdb/home/home.do](http://www.rcsb.org/pdb/home/home.do)), the alignment was performed using the Magic Fit function of the Swiss-PDBViewer with the catalytic domain of chicken PARP1 as template. Yellow: PARP1, blue: PARP2, green: PARP3. (B) Sequence alignment of the conserved carboxyl-termini of PARP1, PARP2 and PARP3. The alignment was performed using Clustal2W ([www.ebi.ac.uk/Tools/clustalw2/index.html](http://www.ebi.ac.uk/Tools/clustalw2/index.html)). The conserved residues VDP at position 533 to 535 in hPARP1 used to generate the different PARP family chimera are marked.

### Supplementary Figure 3:

(A) PARP1 E988K and PARP1 M890V/D899N are enzymatically inactive as revealed by western blot. (B) The stimulation of PARP2-1 by fragment 1-373 is resistant to high salt conditions (left panel). The stimulation of PARP2-1 by fragment 1-373 is not due to a PAR carrier effect of fragment 1-373 (middle panel). \* 1-373 was added after the reaction. The stimulation of PARP1 373-1014 by fragment 1-373 is resistant to high salt conditions (right panel). Molecular size markers in kDa and the border between stacking and separating gel (asterisk) are indicated.

### Supplementary Figure 4:

(A) PAR formation after long incubation of PARP2-1, PARP3-1, PARPs-1 and PARP 656-1014 in the absence or presence of DNA and PARP1 fragment 1-373 as revealed by western blot (60 minutes incubation, upper panel), coomassie staining (60

minutes incubation, middle panel) or auto-modification as detected by autoradiography (5 minutes incubation, lower panel). Molecular size markers in kDa and the border between stacking and separating gel (asterisk) are indicated. (B) PAR formation after 5 minutes incubation by PARP family members and PARP chimera in the absence or presence of PARP1 fragment 1-373 as revealed by silver staining. Equal molar amounts of proteins were used. (C) Time course of PAR formation by PARP2, PARP3, PARP1-2 and PARP1-3 in the absence or presence of fragment 1-373. Reactions were performed in triplicates, error bars represent standard deviations.

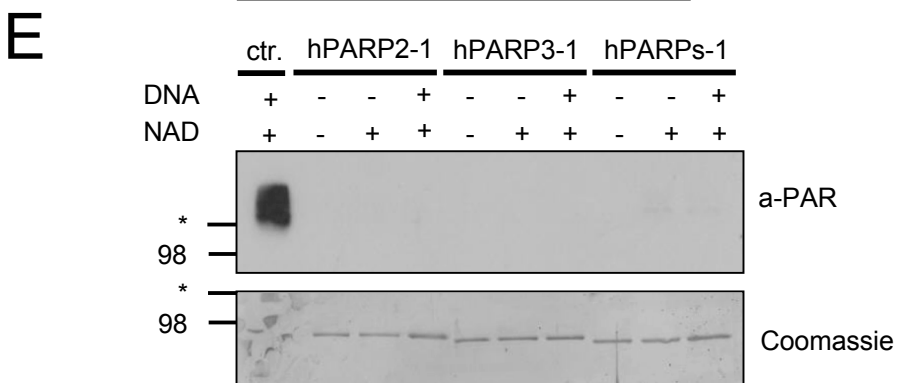
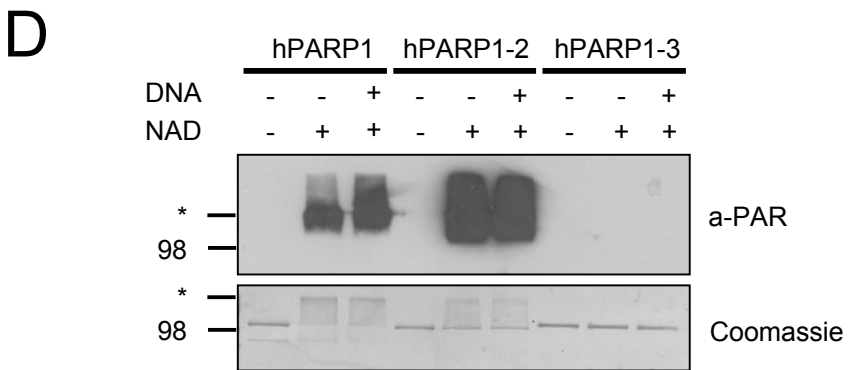
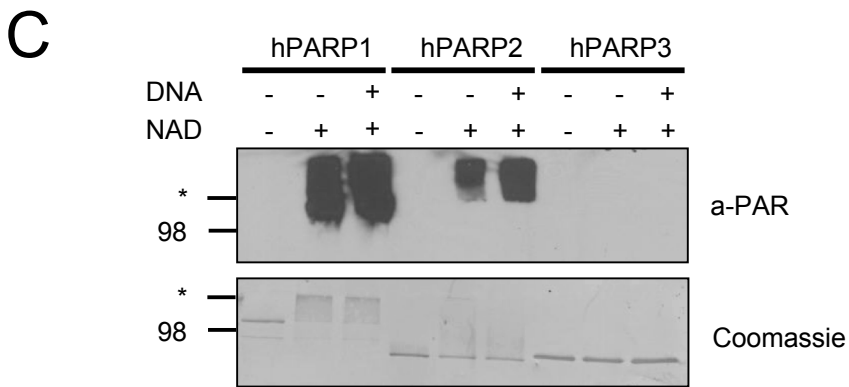
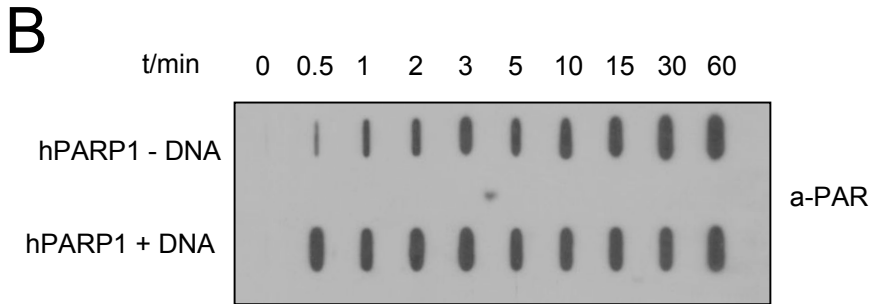
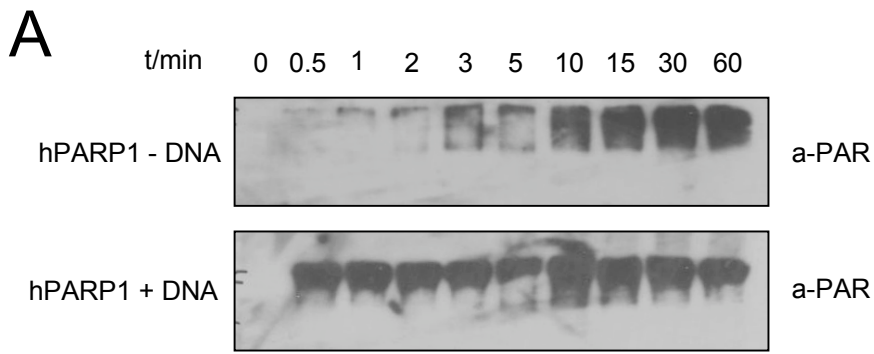
### **Supplementary Figure 5:**

(A) PAR formation by the PARP1 mutants  $\Delta$ BRCT and  $\Delta$ BRCT/E. (B) PAR formation by the PARP1 mutant  $\Delta$ Ac. (C) Trans-poly(ADP-ribosylation) of different PARP1 fragments by full-length PARP1. 1, aa 1-214; 2, aa 215-373; 3, aa 373-525; 4, aa 525-656; 5, aa 656-1014. (D) Stability of the PARP1-ADP-ribose linkage under different pH conditions. Auto-modified PARP1 was incubated for 30 minutes at 60°C (top) or 30°C (bottom) under the indicated conditions and subsequently subjected to SDS-PAGE and detection by autoradiography. Tris-HCl pH 8.0 served as control. (E) Auto-modification of PARP1 mutants. \* PARP inhibitor 3-aminobenzamide (3-AB) was added after the reaction. A long exposure was required to detect the shown levels of auto-modification. Molecular size markers in kDa and the border between stacking and separating gel (asterisk) are indicated.

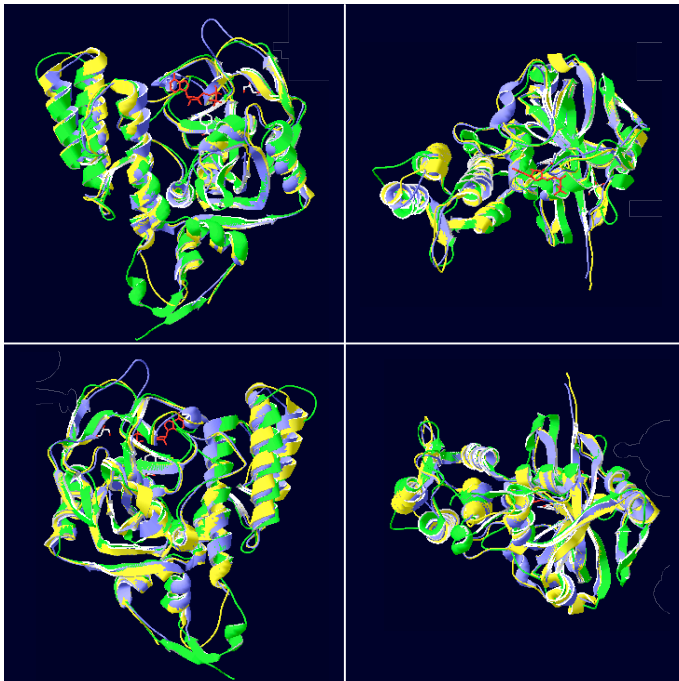
### **Supplementary Figure 6:**

(A) Model for the sequential activation and regulation of chimera PARP2-1 by the PARP1 DBD bound to double strand break mimicking DNA. The DNA-dependent interaction between the PARP1 DBD and the PARP1 WGR/CAT induces a state of high substrate affinity and high turnover rate. Subsequently, acceptor amino acids most likely situated in the amino-terminal region of chimera PARP2-1 and in the PARP1 DBD are poly(ADP-ribosylated). (B) Scheme depicting PAR acceptor lysine residues in chimera PARP2-1 in accordance with our previous finding, that lysines 36 and 37 of mPARP2 are targets for auto-ADP-ribosylation (1).

1. Haenni, S.S., Hassa, P.O., Altmeyer, M., Fey, M., Imhof, R. and Hottiger, M.O. (2008) Identification of lysines 36 and 37 of PARP-2 as targets for acetylation and auto-ADP-ribosylation. *Int J Biochem Cell Biol*.



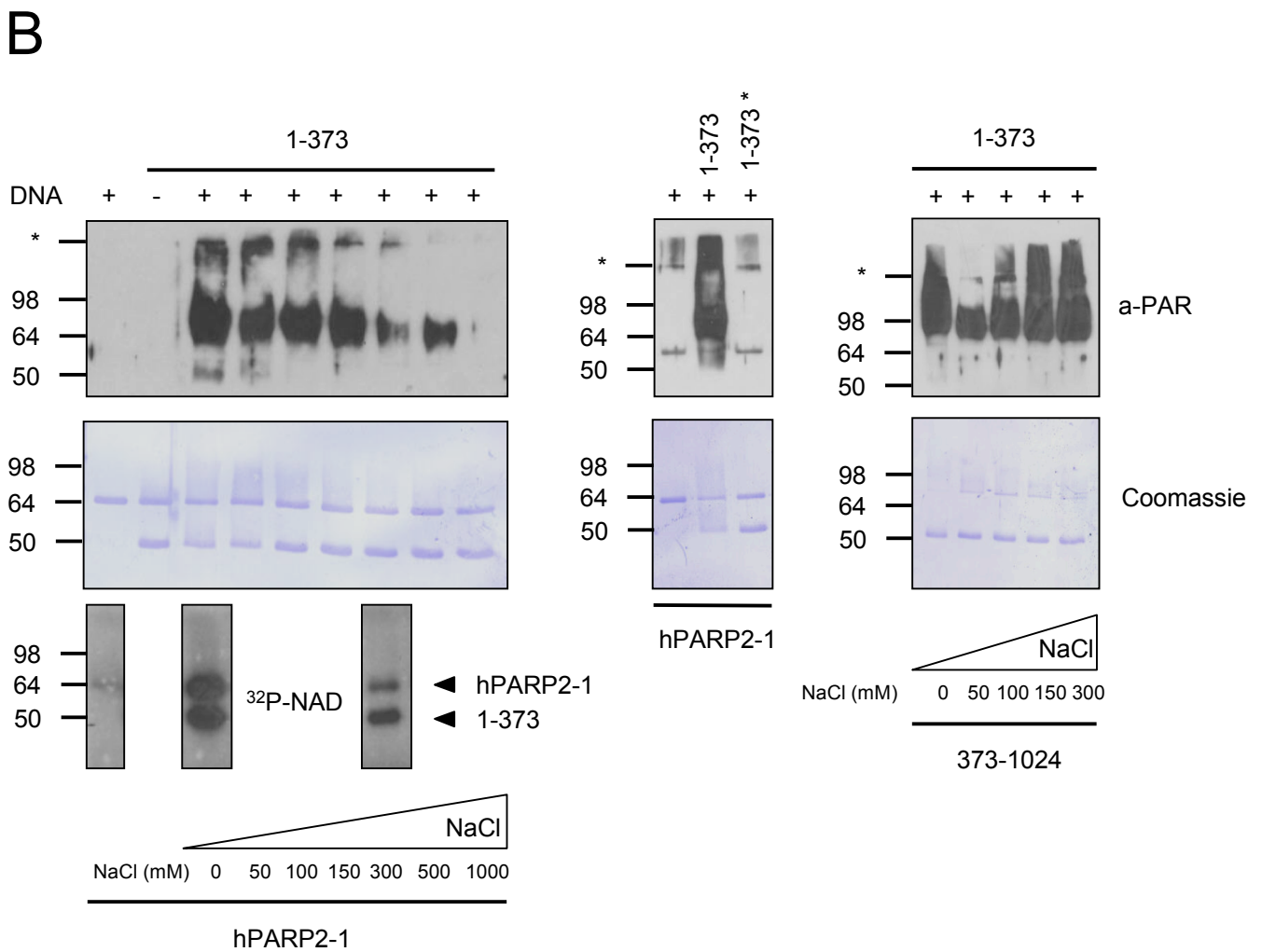
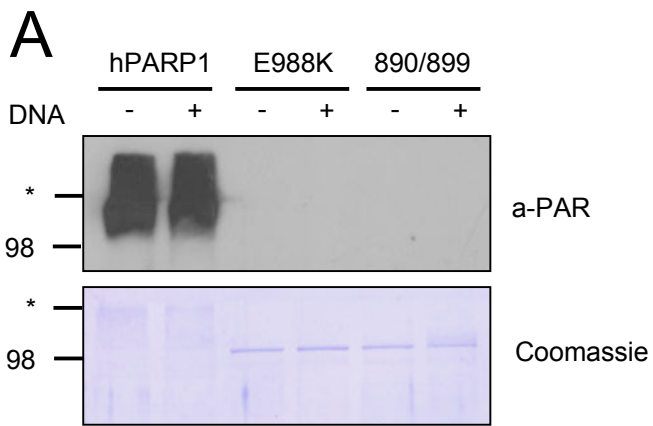
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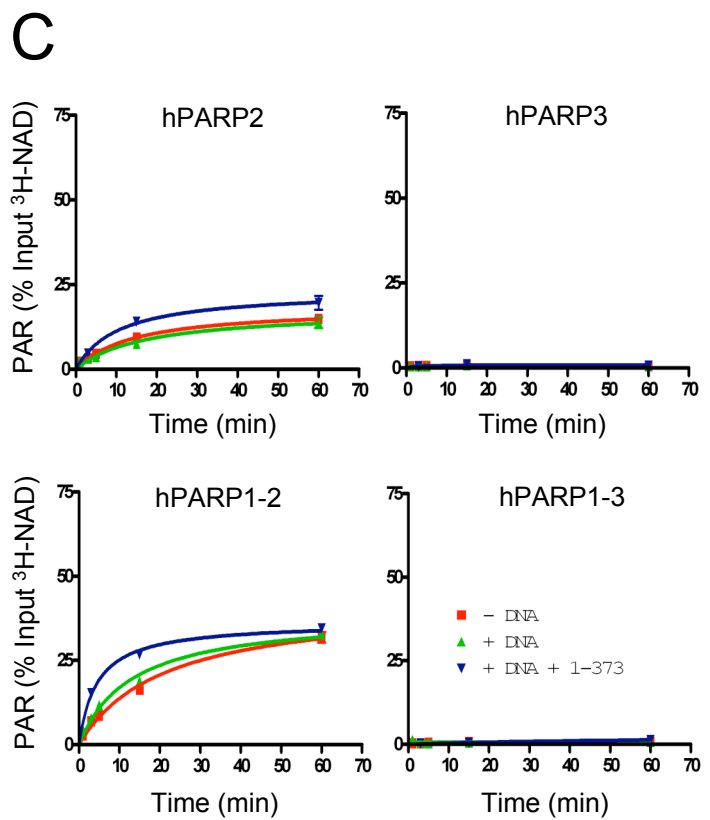
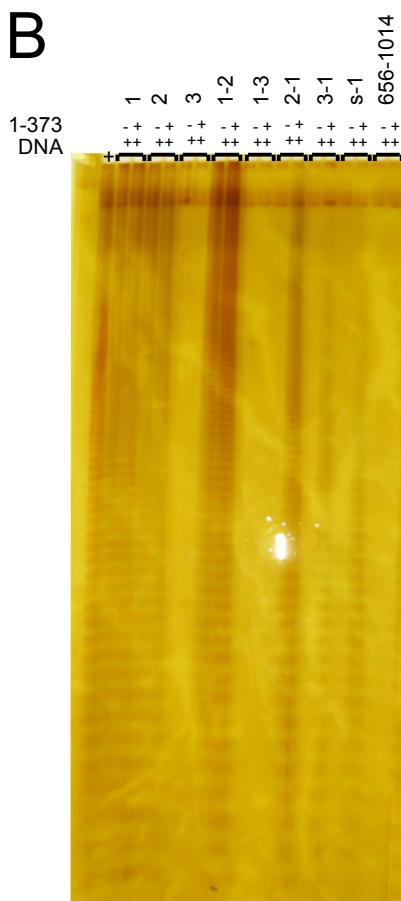
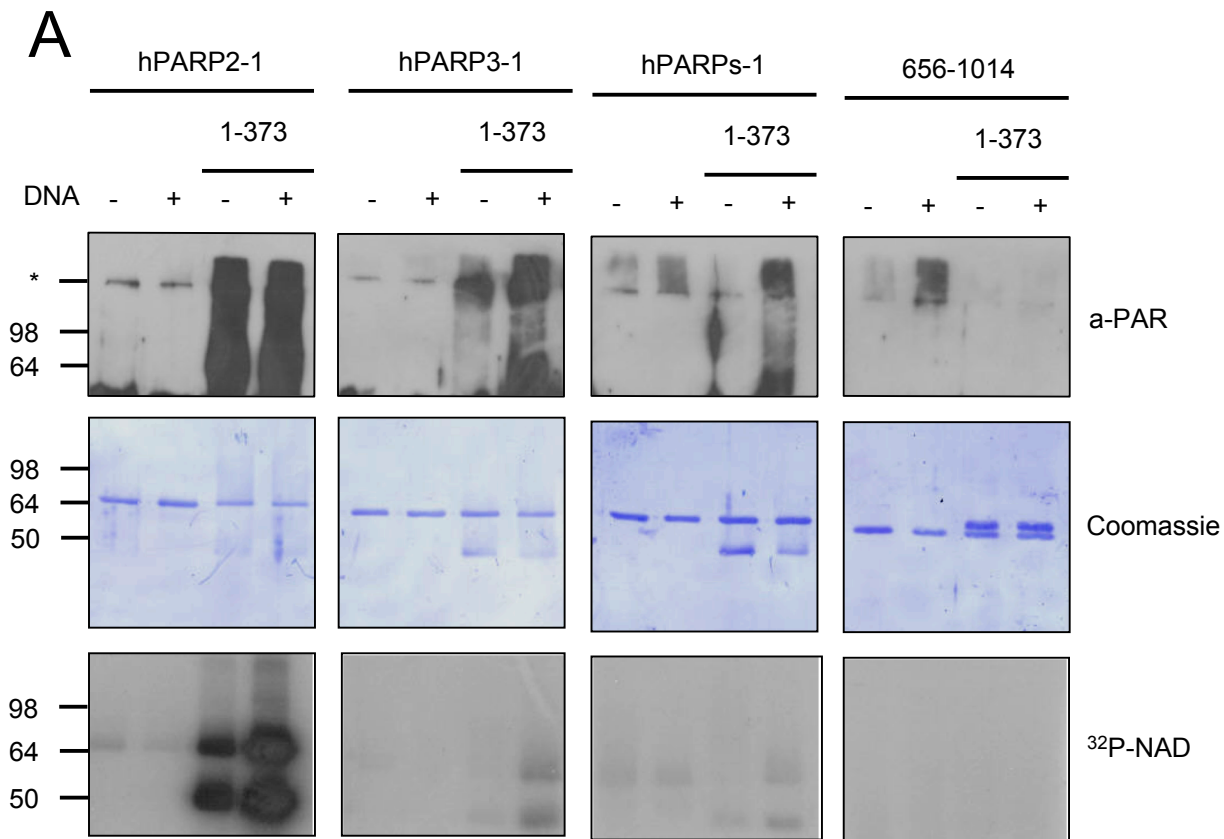


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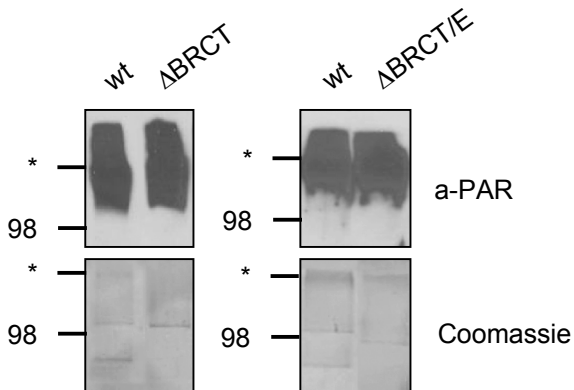
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hPARP3  -----GRQAGREEDPFRSTAEALKAIPA EKRIIRVDPTCPL 54
          . . : : * : :
hPARP1  EH-SAHVLEKGGKVFSA TLGLVDIVKGTNSYKQLLEDDKENRYWIFRSWGRVGTVIGS 598
hPARP2  KVGKAHVYCEGNDVDVMLNQTLNFNNKYLIQLLEDDAQRNFVSWMRWGRVGMKGQH 147
hPARP3  SSNPGTQVYED---YNCTLNQTNIE NNNKFYIQLLQD-SNRFFTCWNRWGRVGEVQ- 109
          . . : . * . : : . . : * : * : * : : : * : : : * : : :
hPARP1  NKLEQMPKSDAIEHFMKLYE EKTGNAWH SKN-FTKYPKKFYPLEIDYG---QDEEAVK 654
hPARP2  SLVACSGNLNKAKEIFQKKFLDKTKNNWEDREKFEKVPKGYDMLQMDYATNTQDEEETK 207
hPARP3  SKINHTRLEDAKKDFEKKFREKT KNNWAERDHFVSHPGKYTLIEVQAEDEAQEAVVKVD 169
          . : : * : * : : * * * . : : * . * * : : : : * : .
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hPARP2  EESLKSPLK--PESQLDLRVQELIKLICNVQAMEEMMEMKYNTKKAPLGKLTVAQIKAG 265
hPARP3  RGPVTRVTKRVQPCSLDPATQKLITNIFSKEMFKNTMALMDLDVKKMPLGKLSKQOIARG 229
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hPARP2  YQSLKKIEDCIR-AGQHGRALMEACNEFYTRIPHDFGLRTPPLIRTOKELSEKIQLLEAL 324
hPARP3  FEALEALEEALKGPTDGGQSLEELSSHFYTVIPHNFHGSQPPPINSPELLQAKDMLLVL 289
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hPARP2  GDIEIAIKLVKTELQ-----SPEHPLDQHYRNLHCALRPLDHESYEFKVISQYLQSTHAP 379
hPARP3  ADIELAQALQAVSEQEKTVEEVP HPLDRDYQLLKQQLLDGSAPEYKVIQTYLEQTGSN 349
          * * : * : . * * * . * : : : * : * : * : * : * : * :
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hPARP2  THSDYTMTLLDLFEVEKDGEKEAFR--EDLHNRMLLWHGSRMSNWV GILSHGLRIAPPEA 437
hPARP3  HR---CPTLQHIWKVNQEGEEDRFQAHSKLGNRKL LWHGTNMAVVAAILTSGLRIMP--- 403
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hPARP1  PVTGYMFGKGIYFADMVSKSANYCHTSQG--DPIGLILLGEVALGNMYELKHASHISK-L 941
hPARP2  PITGYMFGKGIYFADMSSKSANYCFASRL--KNTG LLLLSEVALGQCNELLEANPKA EGL 495
hPARP3  -HSGGRVQKGIYFASENSKAGYVIGMKCAH HVGYMFLGEVALGREHHINTDNP SLKSP 462
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hPARP2  LQGKHS TKGLGKMAPSS--AHFVTLNGSTVPLGPASDTGILNPDGYTLN YNEYIVYNPNQ 553
hPARP3  PPGFDSVIARGHTEPDPTQDTELELDGQQVVVPGQVPVPCPEFSSSTFSQSEYLIYQESQ 522
          * . * . * : * . : * * * * : . : : : * * : : * : * : *
hPARP1  VNLKYLKLLKFNFKTSLW 1014
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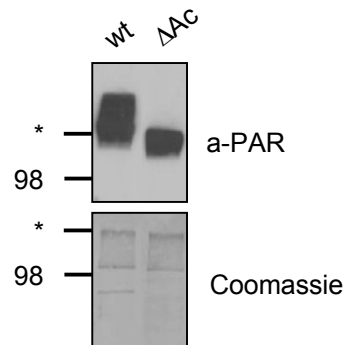




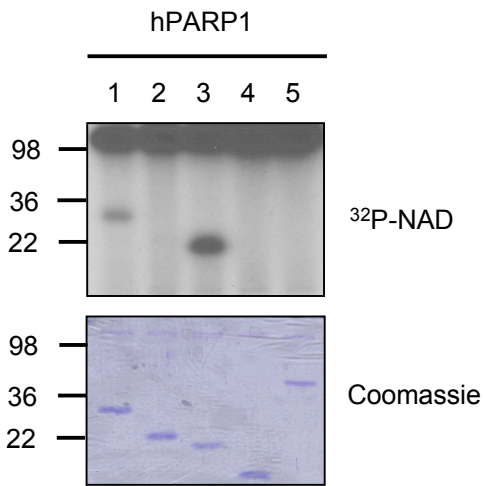
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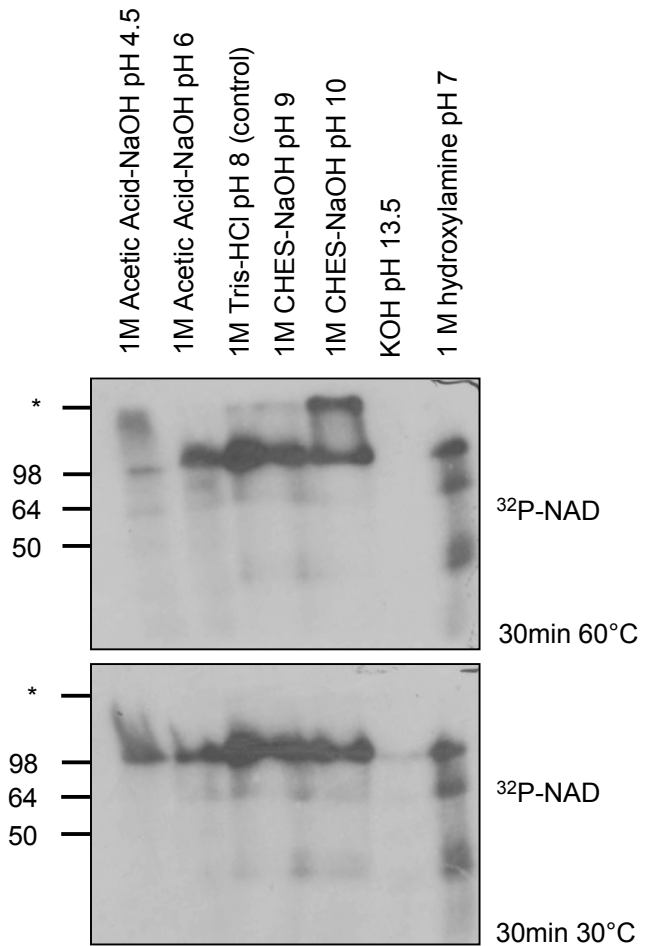
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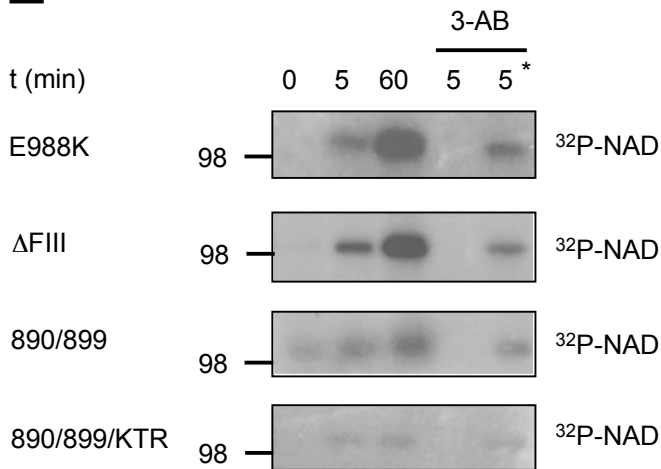
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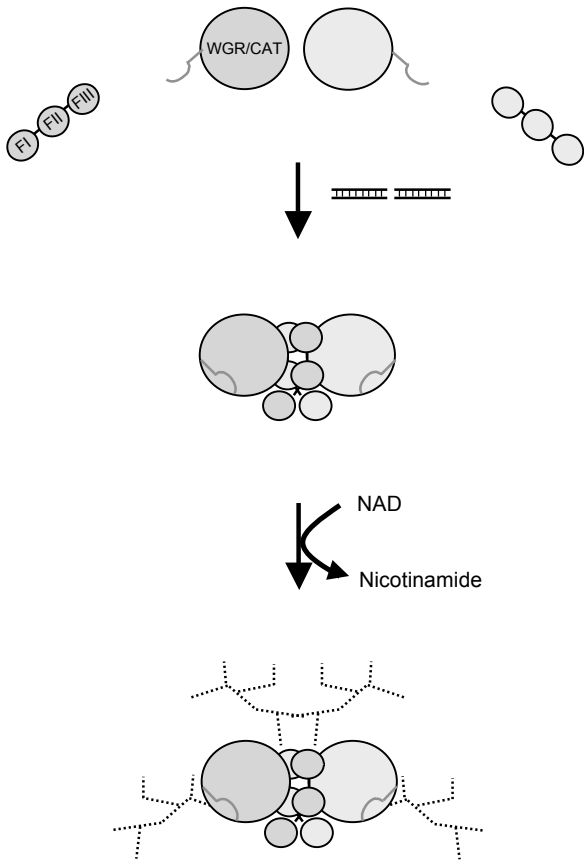
**D**



**E**



A



B

