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Supplemental Information

A Ubiquitin-Binding Domain in Cockayne Syndrome B Required for Transcription-Coupled Nucleotide Excision Repair

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Supplemental Experimental Procedures

Plasmids

CSB was PCR amplified from human cDNA and cloned into the mammalian expression vector pIRES-puro (Clontech) with a C-terminal myc-tag (CSB^{WT}). Site-directed mutagenesis (Stratagene) was used to generate CSB^{GG}**.** To construct CSB^{del}, CSB coding sequence except for 819 base pairs from the 3' end was PCR amplified and cloned into pIRES-puro vector, as above. To generate the CSB-Rad23 UBA fusion ($CSB^{Rad23UBA}$), the UBA2 domain from yeast Rad23 (amino acids 341-398) was PCR amplified using genomic DNA from *S. cerevisiae* (W303), and cloned in-frame with the CSB UBD deletion construct. To generate YFP-CSB fusion gene, CSB and the mutants mentioned above were cloned downstream of YFP cDNA in pEYFP-C1 (Clontech). Details are available on request.

To construct GST-UBD fusion proteins, sequence corresponding to amino acid 1220-1493 of CSB was PCR amplified and cloned into the pGEX6P-1 vector (GE Healthcare) in-frame with GST. Leucine 1427 and 1428 of UBD^{WT} were then mutated as above to generate UBD^{GG}. For expressing recombinant GST-

ubiquitin, the human ubiquitin gene was PCR amplified and cloned into pGEX6P-1. Isoleucine 44 was mutated to alanine by site-directed mutagenesis as above to create GST–Ub^{I44A}. GST-tagged proteins were expressed in *E. coli* (BL21) and purified on glutathione Sepharose (GE Healthcare) by standard techniques.

Cell Lines and Cell Cultures

Cells were maintained in DMEM supplemented with antibiotics and 10% fetal bovine serum (Gibco) in a 5% CO_2, 95% air at 37°C. Transient transfection into HEK293 cells using Lipofectamine 2000 was performed as per manufacturer's instruction (Invitrogen). To generate stable cell lines expressing CSB and mutants, CSB-deficient human fibroblast (CS1AN-Sv (Mayne et al., 1986)) were transfected using Lipo-Taxi transfection reagent as per manufacturer's instruction (Stratagene). pIRES-puro vector based constructs were selected with puromycin (0.25 µg/ml), and pEYFP-C1 based constructs with G418 (400 µg/ml). Stably expressing clones were characterized for protein expression by Western blot using anti-CSB rabbit polyclonal antibody (Bethyl Laboratories).

Supplemental Figures

Figure S1, related to Figure 1. Multiple sequence alignment of CSB's UBD from different mammalian species (http://smart.emblheidelberg.de). Conserved residues are highlighted. Arrows above indicate the important proline and leucine residues. See main text for details.

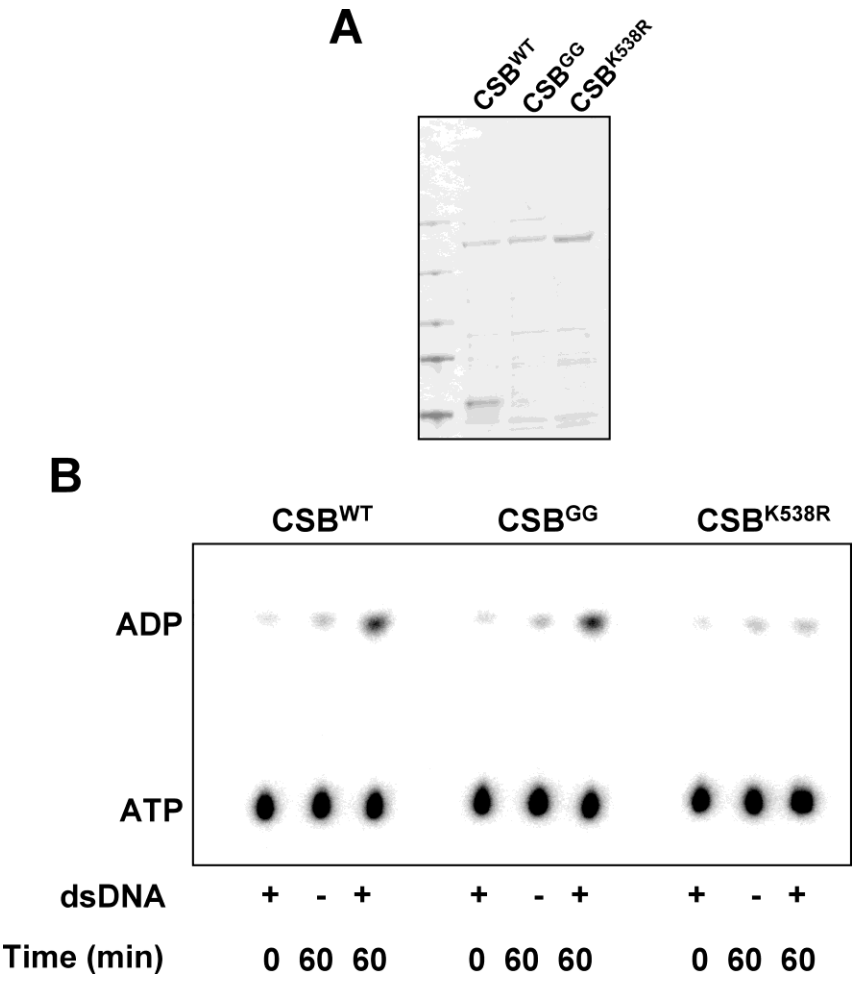
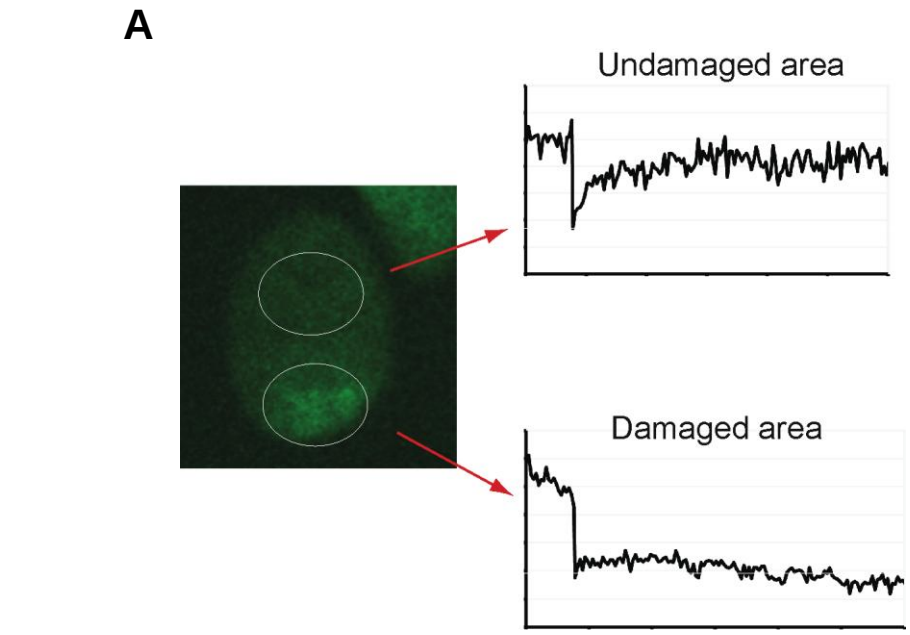


Figure S2, related to Figure 2. (A) Different versions of Flag-tagged CSB were cloned into the yeast GAL-expression plasmid pYes2 (Invitrogen), and the expressed protein purified by one-step purification via M2-agarose affinity chromatography (Sigma). **(B)** DNA-dependent ATPase activity of the purified CSB proteins, measured as generation of α-P^{32}-ADP from α-P^{32}-ATP. Since the proteins were not purified to homogeneity, CSB^{K538R} was an important negative control. It has a mutation in the invariant lysine residue in the NTP-binding motif and thus cannot hydrolyze ATP (Citterio et al., 1998).



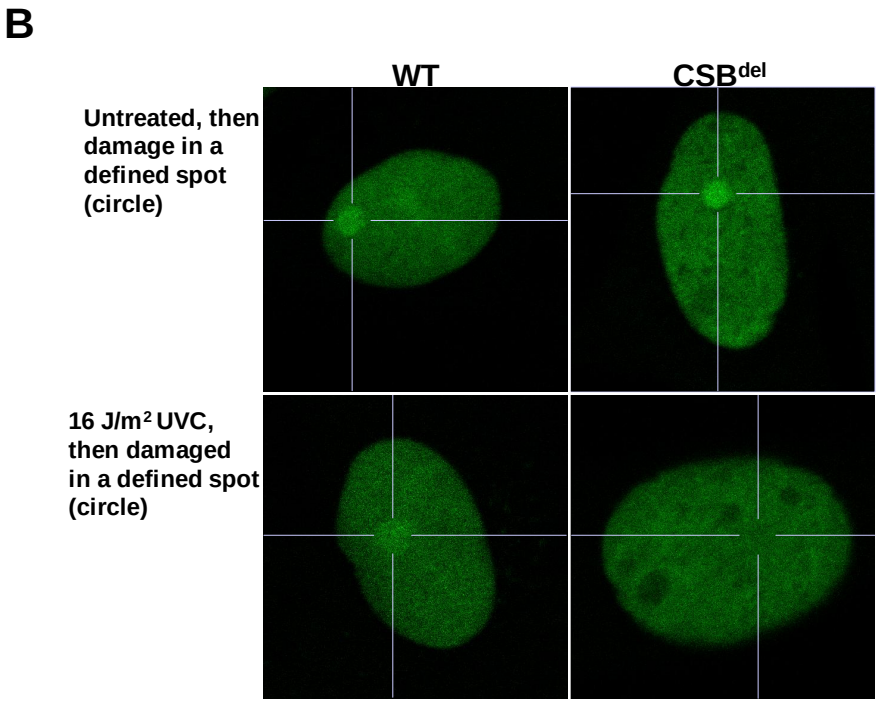


Figure S3, related to Figure 4. (A) In order to confirm that CSB^{del} was immobilized specifically in the damaged area, a UVC-laser was used to locally induce a damaged region in YFP-CSB^{del} cells (see (Dinant et al., 2007) for details). CSB^{del} mobility into the undamaged (upper right) and damaged (lower right) sub-nuclear regions was then measured by FRAP analyses, as previously

described (Mari et al., 2006). This indicated that the reduction in CSB^{del}'s mobility was restricted to UV-damaged regions of the nucleus, suggesting that the mutation specifically affects the ability of CSB to leave sites of DNA damage. This was further supported by the experiments in **(B)**, in which wild type (left panels) or CSB^{del} cells (right panels) were damaged in a defined area by multiphoton laser in the absence (upper panels), or presence (lower panels), of previous general UV-irradiation.

References

Citterio, E., Rademakers, S., van der Horst, G.T., van Gool, A.J., Hoeijmakers, J.H., and Vermeulen, W. (1998). Biochemical and biological characterization of wild-type and ATPase- deficient Cockayne syndrome B repair protein. J Biol Chem *273*, 11844-11851.

Dinant, C., de Jager, M., Essers, J., van Cappellen, W.A., Kanaar, R., Houtsmuller, A.B., and Vermeulen, W. (2007). Activation of multiple DNA repair pathways by sub-nuclear damage induction methods. J Cell Sci *120*, 2731-2740.

Mari, P.O., Florea, B.I., Persengiev, S.P., Verkaik, N.S., Bruggenwirth, H.T., Modesti, M., Giglia-Mari, G., Bezstarosti, K., Demmers, J.A., Luider, T.M.*, et al.* (2006). Dynamic assembly of end-joining complexes requires interaction between Ku70/80 and XRCC4. Proc Natl Acad Sci U S A *103*, 18597-18602.

van den Boom, V., Citterio, E., Hoogstraten, D., Zotter, A., Egly, J.M., van Cappellen, W.A., Hoeijmakers, J.H., Houtsmuller, A.B., and Vermeulen, W. (2004). DNA damage stabilizes interaction of CSB with the transcription elongation machinery. J Cell Biol *166*, 27-36.