

UV-sensitive syndrome protein UVSSA recruits USP7 to regulate transcription-coupled repair

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Transcription-coupled nucleotide-excision repair (TC-NER) is a subpathway of NER that efficiently removes the highly toxic RNA polymerase II blocking lesions in DNA. Defective TC-NER gives rise to the human disorders Cockayne syndrome and UV-sensitive syndrome (UV^S)¹. NER initiating factors are known to be regulated by ubiquitination². Using a SILAC-based proteomic approach, we identified UVSSA (formerly known as KIAA1530) as part of a UV-induced ubiquitinated protein complex. Knockdown of UVSSA resulted in TC-NER deficiency. UVSSA was found to be the causative gene for UV^S, an unresolved NER deficiency disorder³. The UVSSA protein interacts with elongating RNA polymerase II, localizes specifically to UV-induced lesions, resides in chromatin-associated TC-NER complexes and is implicated in stabilizing the TC-NER master organizing protein ERCC6 (also known as CSB) by delivering the deubiquitinating enzyme USP7 to TC-NER complexes. Together, these findings indicate that UVSSA-USP7-mediated stabilization of ERCC6 represents a critical regulatory mechanism of TC-NER in restoring gene expression.

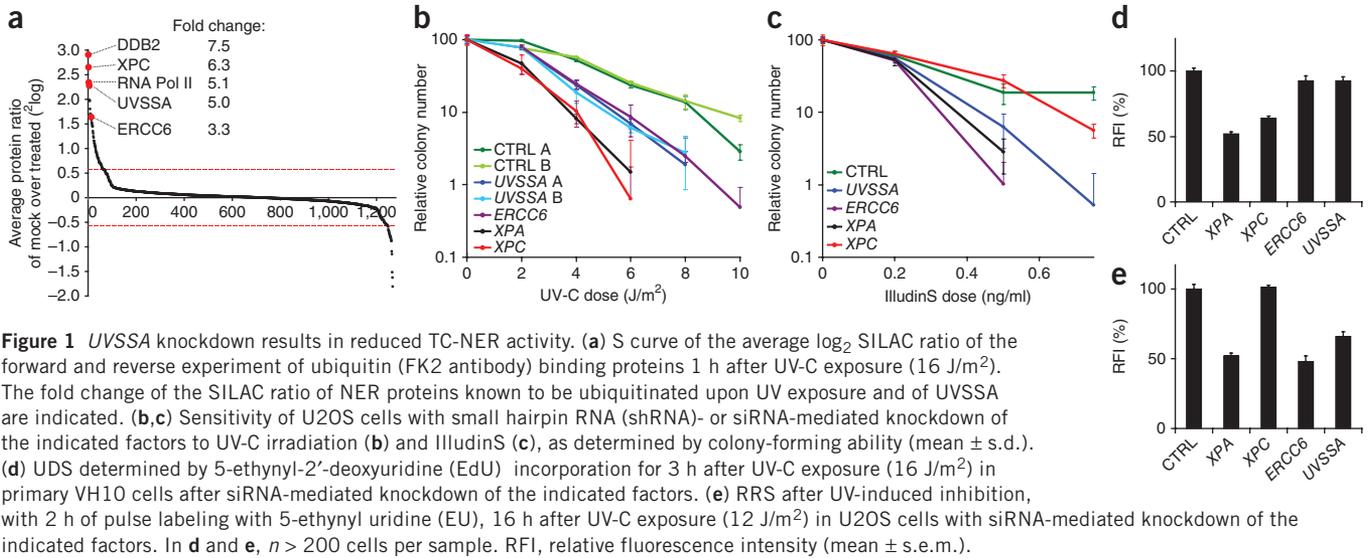
Nucleotide-excision repair removes a wide range of DNA damage, including UV-induced lesions. Inherited NER defects lead to extreme cancer proneness (xeroderma pigmentosum) or dramatic premature aging (Cockayne syndrome), showing the clinical impact of NER⁴. NER is initiated by two damage recognition pathways: global genome NER (GG-NER) and transcription-coupled NER (TC-NER). DNA helix-distorting injuries located throughout the genome are repaired by GG-NER to avoid replication-induced mutations and resultant cancer. TC-NER targets transcription-blocking lesions to enable recovery of arrested transcription, thereby preventing damage-induced apoptosis and resultant aging. In addition, it was shown that TC-NER is also important in overcoming UV-induced transcription-associated mutations⁵.

NER is regulated in response to UV irradiation by ubiquitination² of the process-initiating factors xeroderma pigmentosum group C (XPC)⁶ and DNA damage-binding protein 2 (DDB2)⁷ in GG-NER and Cockayne syndrome group B (CSB, also known as

ERCC6) and the largest subunit of RNA polymerase II (RNA Pol II) in TC-NER^{8–10}. However, how the entire pathway is controlled via ubiquitination remains enigmatic. To characterize the UV-induced ubiquitination network, we performed an unbiased proteomic analysis of differentially ubiquitinated protein complexes after UV irradiation. Using a ubiquitin-binding (FK2) resin and selecting for mono- and polyubiquitinated proteins¹¹, we achieved a strong and specific enrichment of endogenously ubiquitinated protein complexes (**Supplementary Fig. 1a**). With stable isotope labeling by amino acids in cell culture (SILAC)-based proteomics, we compared UV-treated and mock-treated cells and identified 50 upregulated and 13 down-regulated proteins that were changed (by >1.5-fold) in response to UV (**Fig. 1a**). The prominent presence of four known ubiquitin-regulated NER factors, DDB2, XPC, RNA Pol II and ERCC6 (**Fig. 1a**)^{6–9}, at the very top of the list of enriched proteins shows the validity of our approach. The first unknown candidate was UVSSA (UV-stimulated scaffold protein A, encoded by a predicted gene *KIAA1530* (ref. 12)), which has been renamed, with support from the Human Gene Nomenclature Committee (HGNC) (**Fig. 1a**). UVSSA is a highly conserved 709 amino acid protein predicted to contain two conserved yet poorly characterized domains (**Supplementary Fig. 1b,c**): an N-terminal Vps27-Hrs-STAM (VHS) domain¹³ and a C-terminal DUF2043 domain¹⁴. To verify UVSSA ubiquitination, we coexpressed HA-tagged ubiquitin (HA-Ub) and Flag-tagged UVSSA (UVSSA-Flag) in U2OS cells and identified monoubiquitinated UVSSA after both HA-Ub or Flag-UVSSA immunoprecipitation (**Supplementary Fig. 1d,e**). Unexpectedly, UVSSA ubiquitination did not increase in response to UV. As this screen was performed under non-denaturing conditions, it is possible that UVSSA was isolated as part of a UV-induced ubiquitinated protein complex and that it is implicated in the UV DNA damage response (DDR) independent of its own ubiquitination status. To test this possibility, we employed RNA interference to deplete UVSSA in NER-proficient U2OS cells (**Supplementary Fig. 2a**) and observed clear UV hypersensitivity, similar to that achieved with knockdown of known NER factors (**Fig. 1b**). In addition, UVSSA knockdown resulted in IlludinS hypersensitivity (**Fig. 1c**), indicative of a role of UVSSA in TC-NER but not in GG-NER, as this drug specifically sensitizes

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TC-NER-deficient cells¹⁵. The specific role of UVSSA in TC-NER was further shown, as knockdown resulted in a clear reduction of recovery of RNA synthesis (RRS) after UV irradiation, a measure that is specific for TC-NER¹⁶, whereas no effect was found on UV-induced DNA repair synthesis (UDS), which is mainly a measure of GG-NER efficiency¹⁶ (Fig. 1d,e and Supplementary Fig. 2b). Together, these data show that UVSSA is specifically implicated in TC-NER.

The specific TC-NER deficiency after UVSSA knockdown strongly parallels the cellular TC-NER defect of individuals with UV-sensitive syndrome (UV^SS)^{3,17}. UV^SS is a rare autosomal recessive disorder characterized by photosensitivity and mild freckling but, in contrast to Cockayne syndrome, occurs without neurological abnormalities. UV^SS is genetically heterogeneous, which is caused by mutations in either *ERCC6*, *ERCC8* (CSA) or the previously unknown *UVSSA* causal gene^{3,17–19}. To test whether UVSSA was the causative gene of UV^SS, we expressed differently tagged UVSSA proteins (Supplementary Fig. 2c) in the UV^SS group A (UV^SS-A) cell line TA-24 (ref. 17). UVSSA expression restored UV and IlludinS sensitivity (Fig. 2a,b) and RRS deficiency (Fig. 2c,d) to wild-type levels. In addition, immunoblot analysis showed that the UVSSA protein was absent in UV^SS-A cells relative to GG-NER-deficient (XP-C) or TC-NER-deficient (CS-B) cells (Fig. 2e). Together with mutational analysis of UVSSA in accompanying papers by Zhang *et al.*²⁰ and Nakazawa *et al.*²¹, our data show that UVSSA is the causative gene in UV-sensitive syndrome and also establish that the encoded protein is a new TC-NER factor.

To investigate the *in vivo* role of UVSSA, we measured the dynamic association of UVSSA with NER in living cells. Green fluorescent protein (GFP)-tagged UVSSA, which was shown to be biologically active (Fig. 2a–d), accumulated at local UV-C DNA damage (LUD). LUD was either induced by a 266 nm UV-C laser²² (Fig. 3a,b) or by irradiation with a 254 nm UV-C lamp through a microporous filter²³ (Supplementary Fig. 3a,b). Recruitment kinetics of GFP-UVSSA to LUD were strikingly similar to those of the TC-NER factor ERCC6 but were markedly different from the assembly rate of the GG-NER factors DDB2 and XPC (Fig. 3a,b). Inhibition of transcription by α -amanitin substantially attenuated the association of GFP-UVSSA with LUD (Fig. 3a,b), consistent with a role of UVSSA in TC-NER. UVSSA still accumulated at LUD in XP-C, XP-A, CS-A and CS-B cells (Fig. 3c), indicating that UVSSA accumulates early in the TC-NER process. Our ubiquitinome analysis, performed under non-denaturing conditions, showed that UVSSA was enriched to the same extent as RNA Pol II (fivefold; Fig. 1a), which is known to be polyubiquitinated in response to UV^S. This might suggest that UVSSA is copurified as part of an RNA Pol II-containing protein complex, such as lesion-stalled elongating RNA Pol II. To test this possibility, we compared the mobility of GFP-UVSSA by measuring fluorescence recovery after photobleaching (FRAP) in the presence of different transcription inhibitors. The DNA-intercalating drug actinomycin D, which strongly immobilizes GFP-RNA Pol II as a result of stalling at intercalating molecules²⁴, also resulted in immobilization of GFP-UVSSA (Fig. 3d), as was the case

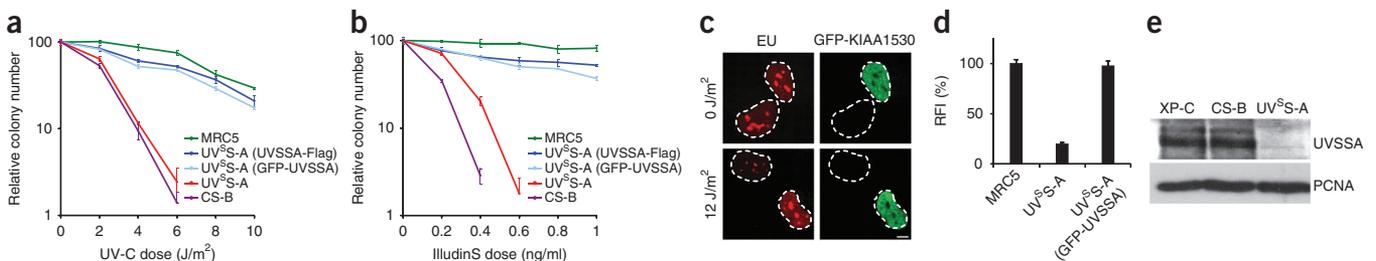


Figure 2 UVSSA expression rescues TC-NER deficiency in UV^SS-A (TA-24) cells. (a,b) Colony survival of NER-proficient MRC5 cells, TC-NER-deficient CS-B (CS1AN) cells and the cell lines from subjects with UV^SS-A TA-24 after UV-C irradiation (a) and IlludinS treatment (b). (c) *In situ* transcription in UV^SS-A cells with and without expression of GFP-UVSSA, before and 16 h after UV-C exposure (12 J/m²), as measured by EU pulse labeling. Scale bar, 7 μ m. (d) RRS activity of MRC5, UV^SS-A and GFP-UVSSA-expressing UV^SS-A cells with 2 h of pulse labeling with EU, 16 h after UV-C exposure (12 J/m²) ($n > 200$ cells, mean \pm s.e.m.). RFI, relative fluorescence intensity. (e) Immunoblot analysis of UVSSA in chromatin fractions of XP-C (XP4PA), CS-B (CS1AN) and UV^SS-A (TA-24) cells. PCNA staining was used as loading control.

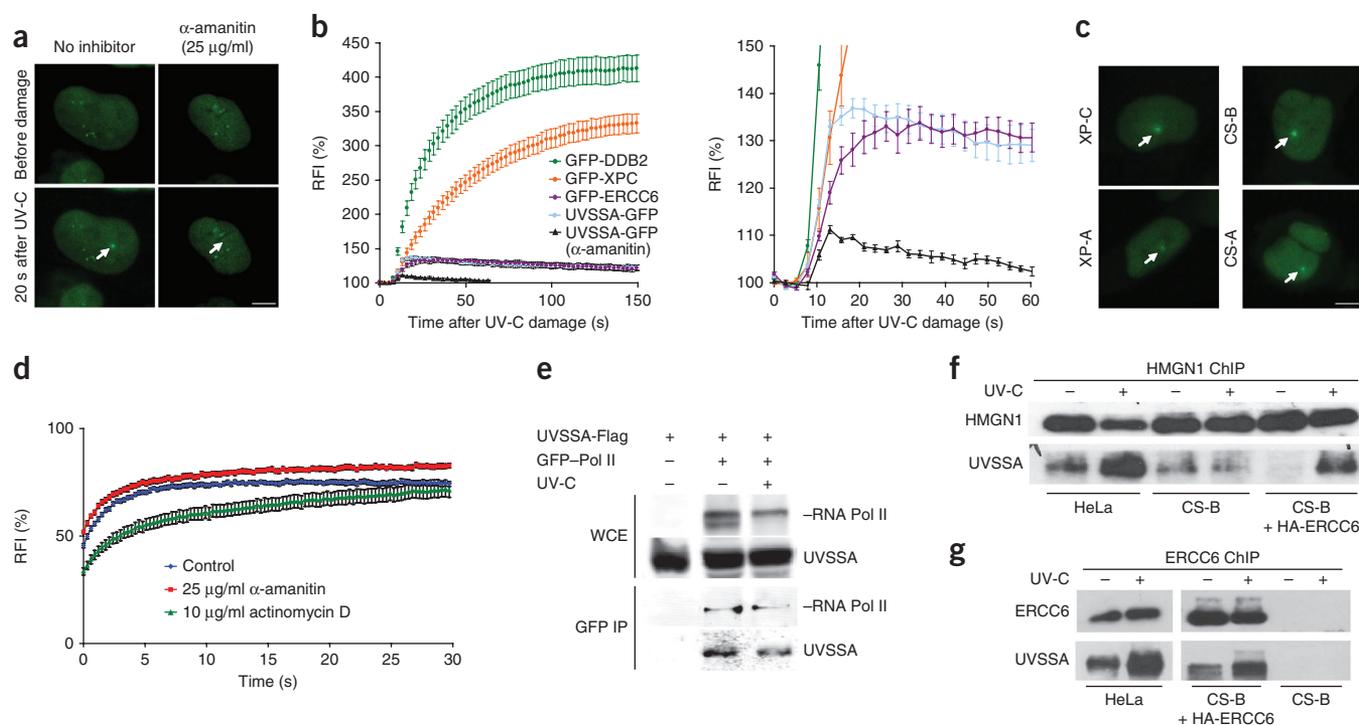


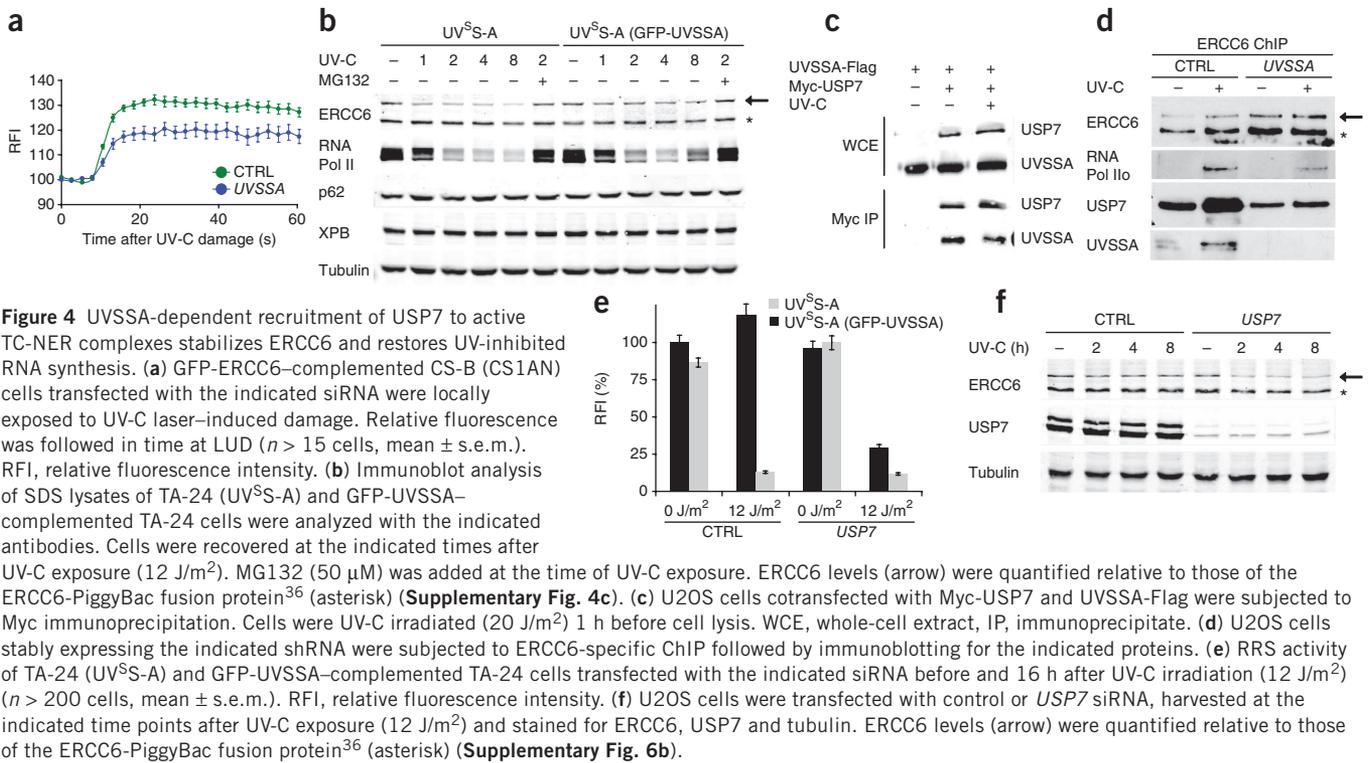
Figure 3 UVSSA is recruited to active TC-NER sites in a UV damage- and transcription-dependent manner. **(a)** LUD infliction (arrows) in MRC5 cells stably expressing UVSSA-GFP. Right, cells were pretreated with 25 $\mu\text{g/ml}$ α -amanitin for 16 h. Scale bar, 7 μm . **(b)** GFP fluorescence intensity of the indicated proteins at LUD relative to pre-damage intensity were recorded over time using live-cell confocal imaging ($n > 10$ cells, mean \pm s.e.m.). Right, magnification of the early time points. **(c)** XPC-C (XP4PA), XPC-A (XP2OS), CS-B (CS1AN) and CS-A (CS3BE) cells stably expressing GFP-tagged UVSSA were exposed to UV-C (266 nm) laser-induced damage (arrows). Scale bar, 7 μm . **(d)** Graph of FRAP results in which the relative fluorescence recovery after bleaching is plotted against the time after UVSSA-expressing MRC5 cells were untreated or treated with 25 $\mu\text{g/ml}$ α -amanitin (16 h) or 10 $\mu\text{g/ml}$ actinomycin D (1 h). RFI, relative fluorescence intensity. **(e)** U2OS cells cotransfected with GFP-RNA Pol II and UVSSA-Flag were subjected to GFP immunoprecipitation. Cells were UV-C irradiated (60 J/m^2) 1 h before cell lysis. WCE, whole-cell extract; IP, immunoprecipitate. **(f, g)** ChIP analysis of *in vivo* cross-linked HeLa, CS-B (CS1AN) or HA-ERCC6-complemented CS-B cells, which were subjected to HMGN1 **(f)** or ERCC6 **(g)** ChIP 1 h after mock or UV-C treatment (20 J/m^2). Immunoblot analysis of the coimmunoprecipitated proteins was performed with the indicated antibodies.

with GFP-ERCC6 (ref. 25). In contrast, α -amanitin released RNA Pol II from DNA, which also resulted in increased mobility of UVSSA. The opposing effects by these transcription inhibitors on GFP-UVSSA mobility in living cells cannot be caused by an indirect UVSSA interaction with RNA Pol II via ERCC6 (ref. 25), as similar effects were found in CS-B cells (**Supplementary Fig. 3c**).

Because these findings argue for an interaction of UVSSA with the protein complex containing the elongating form of RNA Pol II (RNA Pol IIo), we performed coimmunoprecipitation experiments. Indeed, UVSSA-Flag coprecipitated with GFP-RNA Pol II in U2OS cells in equal amounts before or after UV damage (**Fig. 3e**). In addition, using a specialized chromatin immunoprecipitation (ChIP) procedure aimed at revealing endogenous TC-NER interacting proteins²⁶, we found that chromatin-bound RNA Pol IIo also interacts with UVSSA in a UV-independent manner (**Supplementary Fig. 3d**) in UVSSA-expressing UV^S-A and CS-B cells. This UV-independent UVSSA-RNA Pol II interaction explains the enrichment of UVSSA in our ubiquitin screen after UV irradiation (**Fig. 1a**), as this protein will be copurified by virtue of the enhanced ubiquitination of RNA Pol IIo in response to UV⁸, thereby explaining the similar SILAC ratio for both proteins. Together, these data suggest that UVSSA dynamically interacts with active transcription complexes.

To examine whether UVSSA is also present in active, chromatin-bound TC-NER complexes via its interaction with RNA Pol IIo, we performed ChIP experiments with antibodies against HMGN1, a chromatin remodeler that is enriched in lesion-stalled TC-NER complexes²⁶. This analysis revealed a clear UV-dependent interaction

of endogenous UVSSA with active, chromatin-bound TC-NER complexes in TC-NER-proficient cells (**Fig. 3f**). The UVSSA-HMGN1 interaction was absent in CS-B cells, consistent with the notion that HMGN1 is not recruited to TC-NER complexes in the absence of ERCC6 (ref. 26), showing that UVSSA only resides in HMGN1 complexes that are active in TC-NER. Using antibodies to ERCC6 for ChIP, we confirmed the UV-induced enrichment of UVSSA in active TC-NER complexes upon UV damage (**Fig. 3g**). Because UVSSA is targeted to active TC-NER complexes and is essential for efficient transcription restart after UV damage¹⁷, we tested whether UVSSA is involved in the recruitment of the TC-NER master organizer ERCC6 to sites of UV damage. Knockdown of UVSSA attenuated the assembly of GFP-ERCC6 on LUD (**Fig. 4a**), whereas it did not affect XPC (GG-NER) accumulation (**Supplementary Fig. 4a**). In addition, we noted that with UVSSA depletion in cells stably expressing GFP-ERCC6 (ref. 25), the total level of GFP-ERCC6 fluorescence was decreased (**Supplementary Fig. 4b**). ERCC6 protein levels are controlled by the ubiquitin-proteasome system after UV damage⁹. To test whether UVSSA has an effect on ERCC6 stability, we quantified endogenous ERCC6 levels in UV^S-A and GFP-UVSSA-complemented UV^S-A cells at different time points after UV irradiation (**Fig. 4b** and **Supplementary Fig. 4c**). In UV^S-A cells, we observed a clear proteasome- and UV-dependent decrease in ERCC6 levels compared to UVSSA-complemented UV^S-A cells. The levels of RNA Pol II were equally reduced in both cell lines⁸; however, RNA Pol II reappeared 8 h after UV damage only in the complemented UV^S-A cells.



Whether this RNA Pol II recovery is a direct effect of UVSSA or an indirect consequence of rescued TC-NER remains to be determined.

In order to examine how UVSSA influences ERCC6 protein stability, we immunoprecipitated GFP-UVSSA and analyzed UVSSA-interacting proteins by mass spectrometry. Of note, we identified the deubiquitinating enzyme ubiquitin carboxyl-terminal hydrolase 7 (USP7, also called HAUSP) that is known to have various functions in DDR^{27–30}, as one of the most prominent interacting partners of UVSSA (Supplementary Fig. 4d). This interaction was confirmed by coimmunoprecipitation of UVSSA-Flag with Myc-USP7 (Fig. 4c). Moreover, we showed that endogenous USP7 resides in chromatin immunoprecipitated TC-NER complexes in a UV- and UVSSA-dependent manner (Fig. 4d and Supplementary Fig. 5a,b). Like the UVSSA–RNA Pol II interaction, the UVSSA-mediated USP7–RNA Pol II interaction is not increased after UV damage (Supplementary Fig. 5c). This suggests that USP7 is recruited to TC-NER complexes via its interaction with UVSSA. As UVSSA is missing in UV^S-A cells, decreased ERCC6 stability after UV could be explained by the absence of USP7 deubiquitinating activity in the TC-NER complex. Indeed, depletion of *USP7* by RNA interference (Supplementary Fig. 6a) caused a similar defect in RRS (Fig. 4e) and in reduction of ERCC6 levels (Fig. 4f and Supplementary Fig. 6b–d) after UV irradiation as UVSSA deficiency. These data suggest that USP7, recruited by UVSSA, has an important role in TC-NER. Additionally, the absence of further sensitization to the RRS defect by combined interference with UVSSA and *USP7* expression (Fig. 4e) suggests that these genes are epistatic.

In summary, within our mass spectrometry analysis of the UV-induced ubiquitinome, we identified an uncharacterized protein (UVSSA), which was highly enriched upon UV irradiation in immunopurified ubiquitinated protein complexes. Functional analysis indicated that UVSSA is a new factor implicated in TC-NER and, of note, is the causative gene in the unresolved UV^S-A NER disorder. Two accompanying studies aimed at finding the genetic defect in UV^S-A also identified *UVSSA* as the gene mutated in individuals with

UV^S-A^{20,21}. Their further functional analysis is consistent with our observation that UVSSA has an important role in TC-NER and is part of the protein complex containing the elongating form of RNA Pol II. Our data argue for a UV-independent UVSSA–RNA Pol IIo interaction. In contrast to GFP-UVSSA, we were not able to observe RNA Pol IIo accumulation at LUD with current technology. The transient or low-affinity interaction between UVSSA and RNA Pol IIo might be stabilized upon UV irradiation, explaining the UV dependency observed in native immunoprecipitations²⁰ and the UV independence of the fixed interactions by cross-linking in ChIP shown here.

We propose a model (Supplementary Fig. 6f) in which one of the key functions of UVSSA in TC-NER is to protect ERCC6 against UV-induced degradation, by targeting the deubiquitinating enzyme USP7 to lesion-stalled RNA Pol II complexes. Of note, overexpression of ERCC6 in UV^S-A cells did not correct the TC-NER defect (Supplementary Fig. 6e), suggesting that the reduced levels of ERCC6 observed in UV^S-A cells is not sufficient to explain the UV^S phenotype. This suggests that deubiquitination of ERCC6 by UVSSA is an essential step for proper TC-NER, either to provide an increased time frame for this key assembly factor to orchestrate TC-NER complex formation or to inhibit a ubiquitin-mediated functional change in ERCC6. In cells with UVSSA knockdown, reduced interaction was observed between RNA Pol IIo and ERCC6 (Fig. 4d). Whether this reduced interaction is a direct consequence or is independent of ERCC6 ubiquitination remains to be elucidated. Either way, our data show the importance of the delicate ERCC6 ubiquitination equilibrium during TC-NER by counteracting TC-NER-specific E3 ligases^{9,31}. USP7 has multiple roles in the DNA damage response^{27–30}, and its pleiotropic activity is even broader, targeting tumor suppressors, immune responders, viral proteins and epigenetic modulators³². An important role for UVSSA might be to deliver the deubiquitinating USP7 enzyme to TC-NER, thereby providing substrate specificity to this pleiotropic deubiquitinase. The implication of UVSSA in ubiquitination of the elongating form of RNA

Pol II after UV damage²¹ seems to imply that UVSSA has functions in TC-NER beyond being a specific shuttle protein for USP7.

The causative genes for the two TC-NER defective disorders Cockayne syndrome and UV^SS (ERCC6, ERCC8 and UVSSA) are all cofactors of lesion-stalled RNA Pol II. However, the phenotypes of these two TC-NER deficiencies are strikingly different; Cockayne syndrome is characterized by severe neurological and developmental abnormalities in conjunction with UV sensitivity, whereas individuals with UV^SS mainly exhibit sun sensitivity, without any clear additional complications¹. It has been proposed that this phenotypic difference is caused by the distinct abilities of affected individuals to process certain oxidative lesions: Cockayne syndrome cells are sensitive for oxidative base damage, whereas UV^SS cells are not^{1,19,33–35}. This suggests that UVSSA and the subsequent deubiquitination of ERCC6 by USP7 are crucial for the execution of the complete NER process, but these seem to not be essential in processing oxidative damage in transcribed strands. Of course, the possibility is not excluded that additional functions of the Cockayne syndrome proteins also contribute to the severe Cockayne syndrome phenotype. The discovery of two new TC-NER factors, UVSSA and USP7, which are both implicated in regulating TC-NER activity, represents an important advance toward further elucidation of the transcription-coupled repair process.

METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/naturegenetics/>.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

D.H.W.D. and J.A.A.D. performed the mass spectrometry analyses, A.R. performed UDS and RRS experiments, A.C.v.d.H. performed immunoprecipitation experiments, C.L. provided technical assistance, and M.F. designed and, together with A.L., performed ChIP experiments. J.H.J.H. provided support and advice, and helped write the manuscript. P.S. and J.A.M. performed experiments and generated reagents. W.V. and J.A.M. designed the study and supervised the project. W.V., J.A.M. and P.S. wrote the manuscript. All authors discussed the results and commented on the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Cell lines and culture. For SILAC labeling, HeLa cells were cultured for 2 weeks in DMEM without lysine, arginine or leucine (AthenaES) supplemented with antibiotics, 10% dialyzed FCS (Invitrogen) and 105 $\mu\text{g/ml}$ leucine (Sigma) and either 73 $\mu\text{g/ml}$ light [$^{12}\text{C}_6$]-lysine and 42 $\mu\text{g/ml}$ [$^{12}\text{C}_6$, $^{14}\text{N}_4$]-arginine (Sigma) or with heavy [$^{13}\text{C}_6$]-lysine and [$^{13}\text{C}_6$, $^{15}\text{N}_4$]-arginine (Cambridge Isotope Laboratories) at 37 °C and 5% CO_2 .

U2OS, VH10 and the SV40-immortalized MRC5, TA-24 (UVSS-A), XP2OS (XP-A), XP4PA (XP-C), XP4PA (expressing GFP-XPC), CS3BE (CS-A), CS1AN (CS-B) and CS1AN (expressing GFP-ERCC6) cells were cultured in a 1:1 ratio of DMEM and Ham's F10 (Invitrogen) containing 10% FCS and antibiotics at 37 °C and 5% CO_2 . Medium was supplemented with hygromycin (25 $\mu\text{g/ml}$) for MRC5 (expressing UVSSA-GFP), TA-24 (expressing GFP-UVSSA) and TA-24 (expressing UVSSA-Flag) cells.

Cells were treated with 25 mg/ml α -amanitin (16 h), 10 mg/ml actinomycin D (1 h) or 50 mM MG132. DNA damage was inflicted by 24-h exposure to IlludinS¹⁵ or by UV-C irradiation (254 nm; TUV Lamp, Phillips).

Plasmid constructs. UVSSA cDNA was PCR amplified from a cDNA clone (BC110331) using the primers listed in **Supplementary Table 1**. The PCR product was cloned into pCR-Blunt-II-TOPO (Invitrogen) and transferred into pENTR1A-GFP-N2 (Addgene, plasmid 19364) and pENTR1A-GFP-C1 (Addgene, plasmid 17396) using EcoRI-SacII sites or into pENTR4 (Addgene, plasmid 17424) using EcoRI-NotI sites³⁷. Recombination into the pLenti CMV Hygro destination vector (Addgene, plasmid 17454)³⁷ was performed with the Gateway LR Clonase II Enzyme Mix (Invitrogen).

The GFP-RNA Pol II and Myc-USP7 constructs have been previously described^{38,39}. DNA transfections were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Third-generation lentiviruses were made in HEK293T cells.

RNA interference. Sequences for siRNA oligonucleotides (Thermo Fisher Scientific) are shown in **Supplementary Table 2**. siRNA transfections were performed using RNAiMax (Invitrogen) or Hiperfect (Qiagen), according to the manufacturer's instructions. shRNAs from the MISSION shRNA library (Sigma) were used to make third-generation lentiviruses. Target sequences are provided in **Supplementary Table 2**.

Quantitative RT-PCR. Total RNA was isolated from siRNA-transfected or shRNA-transduced U2OS cells using the RNeasy mini kit (Qiagen). cDNA was synthesized using random hexamer primers and SuperScript II Reverse Transcriptase (Invitrogen).

UVSSA and GAPDH expression levels were analyzed using qRT-PCR with the TaqMan Gene Expression Assay, using a Bio-Rad CFX96 device. UVSSA expression levels were normalized to GAPDH expression.

Antibodies. For immunoprecipitation, we used mouse monoclonal antibodies to monoubiquitinated and polyubiquitinated conjugates (FK2, BML-PW8810, Biomol) and Myc (9E10, sc-40, Santa Cruz Biotechnology) and FLAG-M2 antibody conjugated to agarose beads (Sigma), HA antibody conjugated to agarose beads (Sigma) and GFP antibody conjugated to agarose beads (ChromoTek). For ChIP, we used mouse monoclonal antibody to RNA Pol II (H5, Babco) and rabbit polyclonal antibodies to HMGN1 (Ab5212, Abcam) and ERCC6 (H-300, sc-25370, Santa Cruz Biotechnology). For protein blots, we used monoclonal antibodies to GFP (Roche and Santa Cruz Biotechnology), CPD (TDM-2, MBL International), HA (3F10, Roche), RNA Pol II (8wG16, Ab5095, Abcam), XPA (Abcam) and p62 (3C9, kindly provided by J.M. Egly (Institut de Génétique et de Biologie Moléculaire et Cellulaire)). The polyclonal antibodies used were to Flag (F7425, Sigma), ubiquitin (Z0458, Dako), RNA Pol II (Ab5095, Abcam), Myc (9E10, sc-40, Santa Cruz Biotechnology), USP7 (A300-033A, Bethyl), ERCC6 (E-18, sc-10459, Santa Cruz Biotechnology), p89/XPB (S-19, sc-293, Santa Cruz Biotechnology), HMGN1 (Ab5212, Abcam), KIAA1530 (UVSSA) (NBPI-32598, Novus, and P-12, sc-138374, Santa Cruz Biotechnology) and XPC. Odyssey-compatible secondary antibodies were from LI-COR, and horseradish peroxidase (HRP)-conjugated secondary antibodies were from Dako (to mouse and goat), Southern Biotech (to rabbit) and Sigma (to IgM).

Isolation of ubiquitinated protein complexes. SILAC-labeled HeLa cells were UV irradiated (16 J/m^2) or mock treated. Label swapping was performed to validate the biological findings, aid contaminant removal and exclude possible SILAC-derived differences. One hour after UV cells were washed twice in ice-cold PBS and harvested in RIPA buffer (PBS containing 1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS) supplemented with 15 μM MG132 (Biomol), 10 mM N-Ethylmaleimide (Sigma), 20 μM PR-619 (LifeSensors) and Complete protease inhibitor cocktail (Roche). Lysates were centrifuged at 16,000g at 4 °C for 15 min. Cleared UV- and mock-treated lysates were combined in a 1:1 ratio based on protein concentrations and added to the anti-ubiquitin resin (100 μl slurry per 5.3 mg of lysate) for 4 h at 4 °C. The anti-ubiquitin resin was prepared by incubating 100 μl of 50% ProtG slurry (Pierce) with 87.5 μg of FK2 antibody (Biomol) for 40 min at room temperature, and four washes were performed with RIPA buffer. Protein complexes were eluted (after four washes with RIPA of lysate-bound resin) in two bead volumes of 4% SDS by shaking for 10 min at 1,000 rpm in an Eppendorf Thermomixer. The eluted sample was concentrated over 30-kDa spin columns (Millipore), supplemented with 2 \times Laemmli buffer and loaded onto 4–15% SDS-PAGE gradient gels (Jule).

Mass spectrometry. SDS-PAGE gel lanes were cut into 2-mm slices and subjected to in-gel reduction with dithiothreitol, alkylation with iodoacetamide (98%; D4, Cambridge Isotope Laboratories) and digested with trypsin (sequencing grade; Promega), as described previously⁴⁰. Nanoflow liquid chromatography tandem mass spectrometry (LC-MS/MS) was performed on an 1100 series capillary liquid chromatography system (Agilent Technologies) coupled to an LTQ-Orbitrap XL mass spectrometer (Thermo Scientific) operating in positive mode. Peptide mixtures were trapped on a ReproSil C18 reversed phase column (Dr Maisch; 1.5 $\text{cm} \times 100 \mu\text{m}$) at a rate of 8 $\mu\text{l/min}$. Peptides were separated on a ReproSil-C18 reversed-phase column (Dr Maisch; 15 $\text{cm} \times 50 \mu\text{m}$) using a linear gradient of 0–80% acetonitrile (in 0.1% formic acid) during 170 min at a rate of 200 nl/min using a splitter. The elution was directly sprayed into the electrospray ionization (ESI) source of the mass spectrometer. Spectra were acquired in continuum mode; fragmentation of the peptides was performed in data-dependent mode.

Raw mass spectrometry data were analyzed with MaxQuant software (version 1.1.1.25)⁴¹. A false discovery rate of 0.01 for proteins and peptides and a minimum peptide length of 6 amino acids were set. The Andromeda search engine⁴² was used to search the tandem mass spectrometry (MS/MS) spectra against the International Protein Index (IPI) human database (release 3.68). A maximum of two missed cleavages and mass tolerance of 0.6 Da was allowed. Further modifications were cysteine carbamidomethylation-2D (fixed), proline methionine oxidation and lysine ubiquitination (variable). MaxQuant automatically quantified SILAC peptides and proteins. From the 2,586 identified proteins in the forward and reverse experiments, we removed 116 known contaminants and reverse hits, 411 proteins that had less than 2 quantitation events, 441 proteins that were only found in the forward or reverse experiment, 71 hits with opposite ratios (>1.25-fold) in the label-swap experiment and 311 proteins with background levels (<1.25-fold) in one of the label-swap experiments. The remaining 1,236 proteins were plotted on the S curve (**Fig. 1a**).

For the identification of UVSSA interactors, the software package Scaffold (version 3_00_03, Proteome Software) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted at >95.0% probability with the Peptide Prophet algorithm⁴³. Protein identifications assigned by the Protein Prophet algorithm^{38,44} were accepted at >95.0% probability.

Clonogenic survival assay. Cells from indicated cell lines were seeded in triplicate in 6-well plates (400 cells/well) and treated with UV-C or IlludinS¹⁵ (24-h exposure) 1 d after seeding. After 1 week, colonies were fixed and stained in 50% methanol, 7% acetic acid and 0.1% Coomassie blue.

Recovery of RNA synthesis and unscheduled DNA synthesis after UV irradiation. Fluorescence-based RRS and UDS were performed as described¹⁶. In short, for RRS, the cell lines were UV irradiated with 12 J/m^2 of UV-C. Transcription levels were determined 16 h after UV irradiation by 2 h of incubation with ethynyluridine (EU) incorporation. For UDS, VH10 cells

were UV irradiated with 16 J/m² of UV-C 48 h after siRNA transfection. UDS was determined by 3 h of incubation with 5-ethynyl,2'-deoxyuridine (EdU) incorporation. RRS and UDS were quantified by determining fluorescence intensities for >200 cells with ImageJ software of images obtained with a Zeiss LSM700. The cells displayed in **Figure 2c** were stained with antibody to GFP (Abcam) after the RRS procedure to visualize GFP signal.

Coimmunoprecipitation. Cells expressing Flag-, Myc-, HA- and/or GFP-tagged proteins were treated 24 h after transfection with the indicated UV-C doses and/or with 50 μM MG132 and lysed in RIPA buffer supplemented with Complete protease inhibitor cocktail (Roche) after 1 h of recovery. Lysates were incubated on ice for 10 min and were centrifuged at 16,000g for 15 min at 4 °C. The supernatant was incubated for 4 h at 4 °C with antibody-conjugated agarose beads. Beads were washed four times with RIPA buffer and eluted with one bead volume of 2× Laemmli SDS sample buffer at 95 °C.

In vivo cross-linking and ChIP. The procedure for ChIP has been described previously²⁶. Briefly, cells were mock treated or exposed to UV-C light (20 J/m²) and left to recover for 1 h at 37 °C and then cross-linked *in vivo* by 1% formaldehyde at 4 °C. Cross-linked cells were lysed, and purified chromatin was sheared with the Bioruptor Sonicator (Diagenode) using cycles of 30 s on and 60 s off. ChIP was performed on chromatin fragments of 200–600 bp. Reversal of the cross-linking and elution of the precipitated proteins was performed by extended boiling in Laemmli SDS sample buffer, and eluted proteins were analyzed by protein blotting²⁶.

Live-cell confocal laser-scanning microscopy and immunofluorescence. Confocal laser-scanning microscopy images were obtained with a Leica SP5 confocal microscope using a 100× quartz objective. Kinetic studies of GFP-

tagged UVSSA, ERCC6, DDB2 and XPC accumulation were performed using UV-C (266 nm) laser irradiation, as described previously²². FRAP experiments were performed as described previously⁴⁵. All FRAP data (average of >15 cells) were normalized to the average fluorescence before bleaching after removal of the background signal.

Immunofluorescence experiments were executed as described⁴⁶, with or without pre-extraction by a 20-s treatment with 0.5% Triton X-100 before fixation. GFP-tagged proteins were visualized by staining with an antibody to GFP.

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