Supplementary Information

Elements that Regulate the DNA Damage Response of Proteins Defective in Cockayne Syndrome

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Table S1. Synthetic Oligonucleotides. Oligonucleotide name and nucleotide sequence are designated.

Oligonucleotide name	Nucleotide sequence
CSA pcDNA Fw	5'-AGCCGGTCTCGAGAGCCGCCATGCTGGGGTTTTTGTCC-3'
CSA pcDNA Rv	5'-TAAAGGTACCATTCATCCTTCTTCATCACTGCTGC-3'
CSA N-GFP Fw	5'-TGAGGACTCGAGATGCTGGGGTTTTTGTCC-3'
CSA N-GFP Rv	5'-TAAAGGTACCATTCATCCTTCTTCATCACTGCTGC-3'
CSA C-GFP Fw	5'-AGCCGGCTCGAGGAGCCGCCATGCTGGGGTTTTTGTCC-3'
CSA C-GFP Rv	5'-TAAAGGTACCATTCATCCTTCTTCATCACTGCTGC-3'
N-CSB Fw	5'-AGTCCTCGAGGAATGCCAAATGAGGGAATC-3'
N-CSB Rv	5'-TCTGGGATCCCTATTAAAGCTTTTTGAACAGAAAACC-3'
M-CSB Fw	5'-AGATCTCGAGGTGATGGAGATGAAGATT-3'
M-CSB Rv	5'-TCTGGGATCCTTATTAACTAGTCAG-3'
C-CSB Fw	5'-TACTCCTCGAGGTCCTGATGCATCCCAGAGCAC-3'
C-CSB Rv	5'-AAGCAAGGATCCTTAGCAGTATTCTGGCTTGAG-3'
N-CSB Fw	5'-AGTCCTCGAGGAATGCCAAATGAGGGAATC-3'
M-CSB Rv	5'-TCTGGGATCCTTATTAACTAGTCAG-3'
M-CSB Fw	5'-AGATCTCGAGGTGATGGAGATGAAGATT-3'
C-CSB Rv	5'-AAGCAAGGATCCTTAGCAGTATTCTGGCTTGAG-3'
CSB K538A Fw	5'-GGCAATTATCTGGATGGTCGCGCCCAATCCCATTTCATCT-3'
CSB K538A Rv	5'-AGATGAAATGGGATTGGGCGCGACCATCCAGATAATTGCC-3'
CSB LL1427,8GG Fw	5'-ATGAAGTTTCTCATCTCCACCCCACCGTCATCGTGTTCTGTGGTGGG-3'
CSB LL1427,8GG Rv	5'-CCCACCACAGAACACGATGACGGTGGGGTGGAGATGAGAAACTTCAT-3'

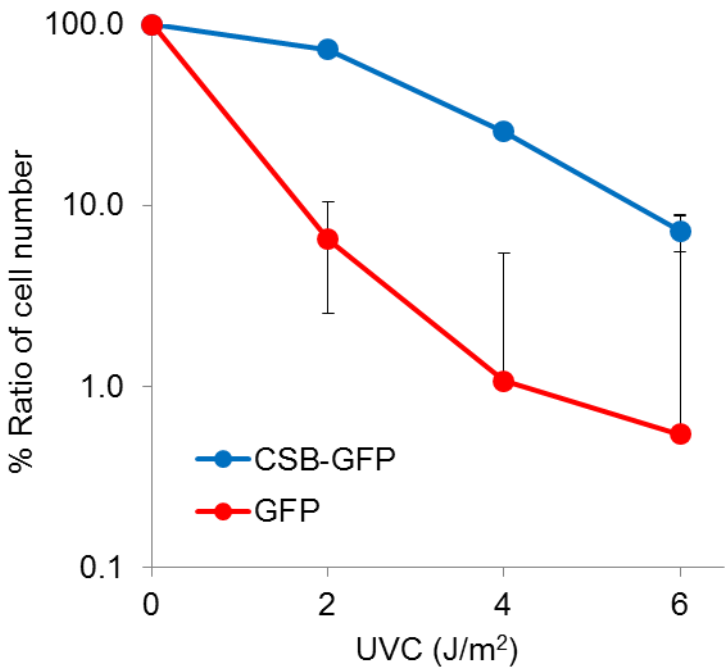
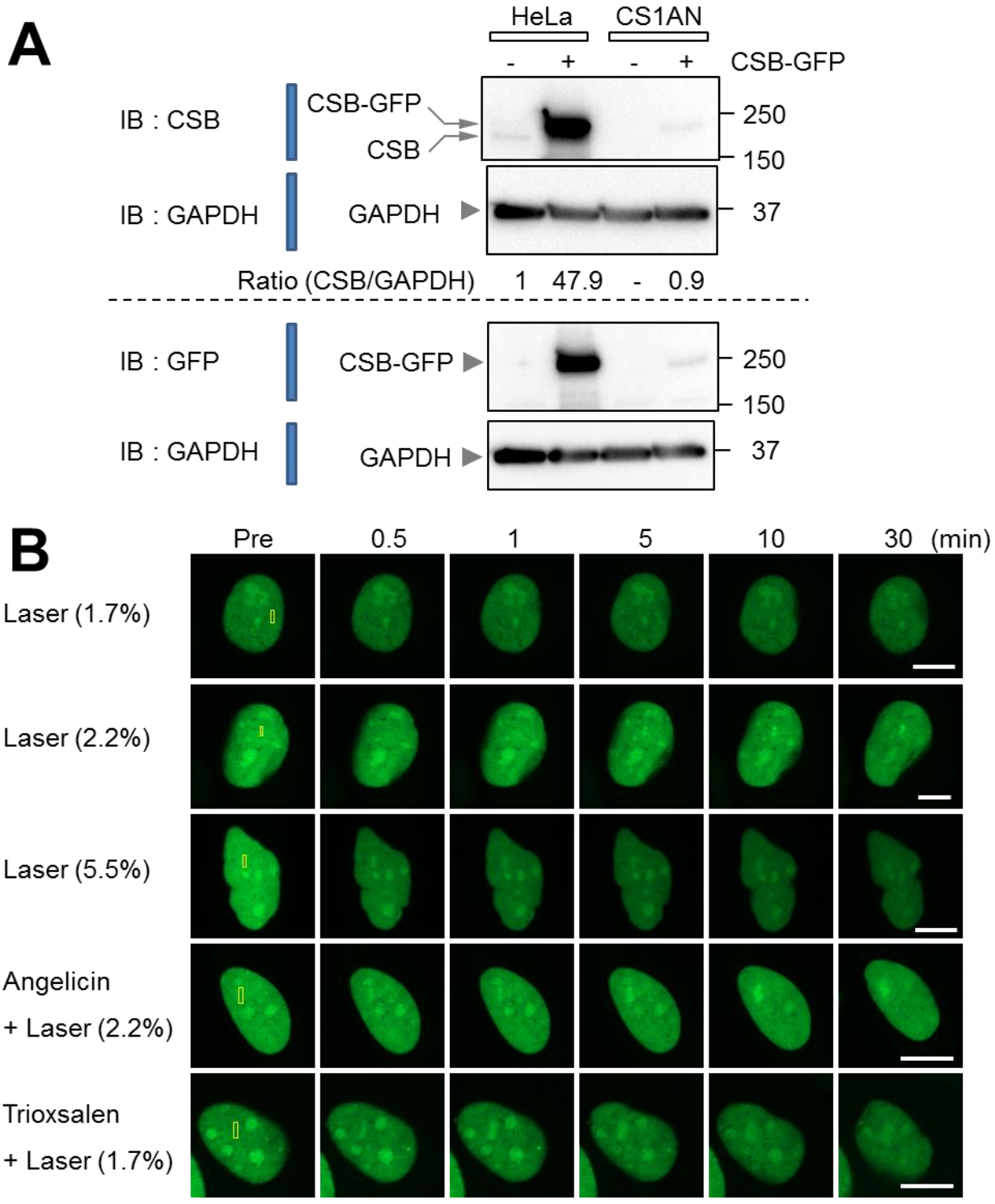


Figure S1. CSB-GFP is functionally active.

UV sensitivity of CSB-deficient CS1AN cell lines after no transfection (parental), or stable integration of pCSB-GFP (N-terminal GFP) or vector (pGFP). Cell viability was determined by hemocytometer 6 days after UVC irradiation (0, 2, 4 or 6 J/m^2). Reported is the ratio of the cell count determined at the indicated UVC dose in comparison to the untreated control sample. Each data point is derived from three independent experiments. Error bars indicate SD.


A
HeLa
CS1AN
- + - +
CSB-GFP
IB : CSB
CSB-GFP
CSB
250
150
IB : GAPDH
GAPDH
37
Ratio (CSB/GAPDH) 1 47.9 - 0.9
IB : GFP
CSB-GFP
250
150
IB : GAPDH
GAPDH
37
B
Pre
0.5
1
5
10
30 (min)
Laser (1.7%)
Laser (2.2%)
Laser (5.5%)
Angelicin
+ Laser (2.2%)
Trioxsalen
+ Laser (1.7%)

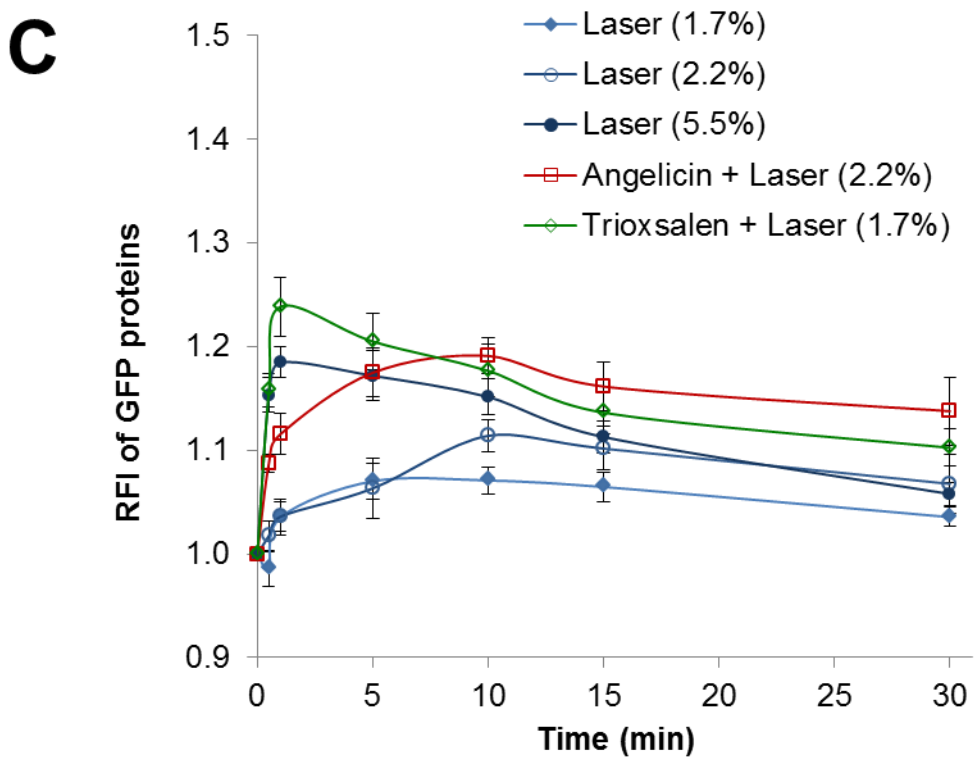


Figure S2. CSB-GFP protein expression and recruitment in CSB-deficient cells.

(A) Expression of CSB protein as determined by western blot analysis of whole cell extracts (25 µg) prepared from HeLa cells transfected with (+) or without (-) pCSB-GFP, or from CSB-GFP stably expressing CS1AN CSB mutant cells ("-" = CS1AN non-transfected cells). Membranes were immunoblotted (IB) with either antibody to CSB (top) or GFP (bottom). The GFP fusion protein and endogenous CSB, as well as the molecular weight standards, are indicated. The relative ratio of GFP-CSB to endogenous CSB, after normalization to the indicated standard (GAPDH), is reported (below; from a representative gel), with HeLa cells being set as 1.
(B) CSB recruitment and retention at localized DNA damage in a stably-complemented CSB-deficient CS1AN cell line. The indicated region (yellow box) was laser irradiated as specified: 1.7%, 2.2% or 5.5% laser, or angelicin + laser (2.2%) or trioxsalen + laser (1.7%). Shown are representative images of unirradiated cells (Pre), and the CSB response at 0.5, 1, 5, 10 and 30 min post-laser irradiation. Bar; 10 µm. Quantified data of at least 10 individual cells are shown in (C).

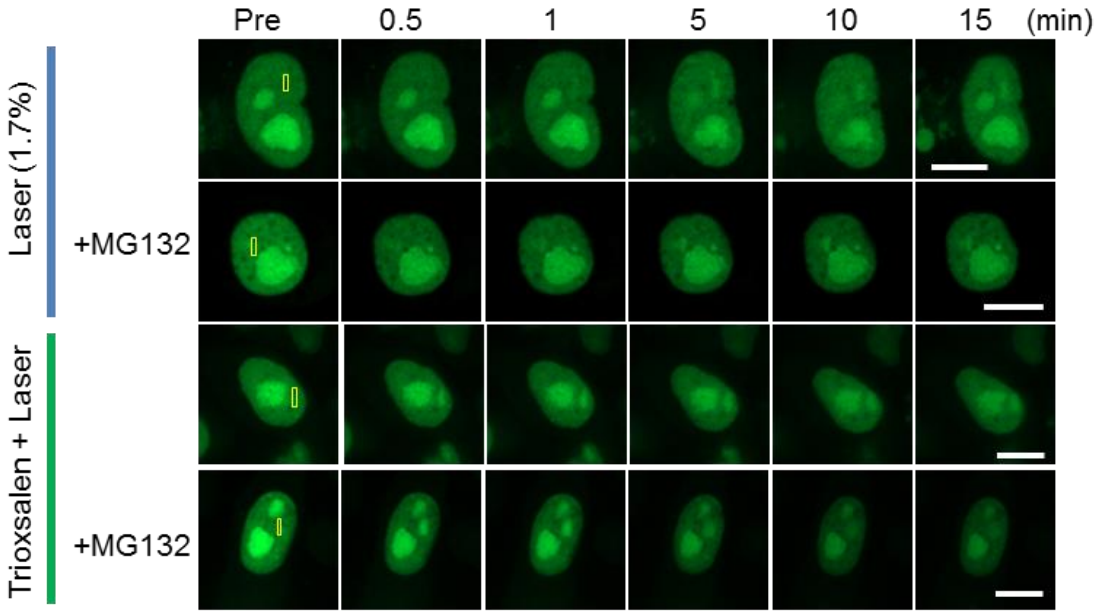


Figure S3. Proteosome inhibitor does not influence the recruitment dynamics of CSB to oxidative DNA damage or ICLs.

Where indicated, cells were grown in the presence of trioxsalen and/or 10 μM MG132. Shown are representative images of unirradiated cells (Pre), and the CSB response at 0.5, 1, 5, 10 and 15 min post-laser irradiation. Bar; 10 μm.

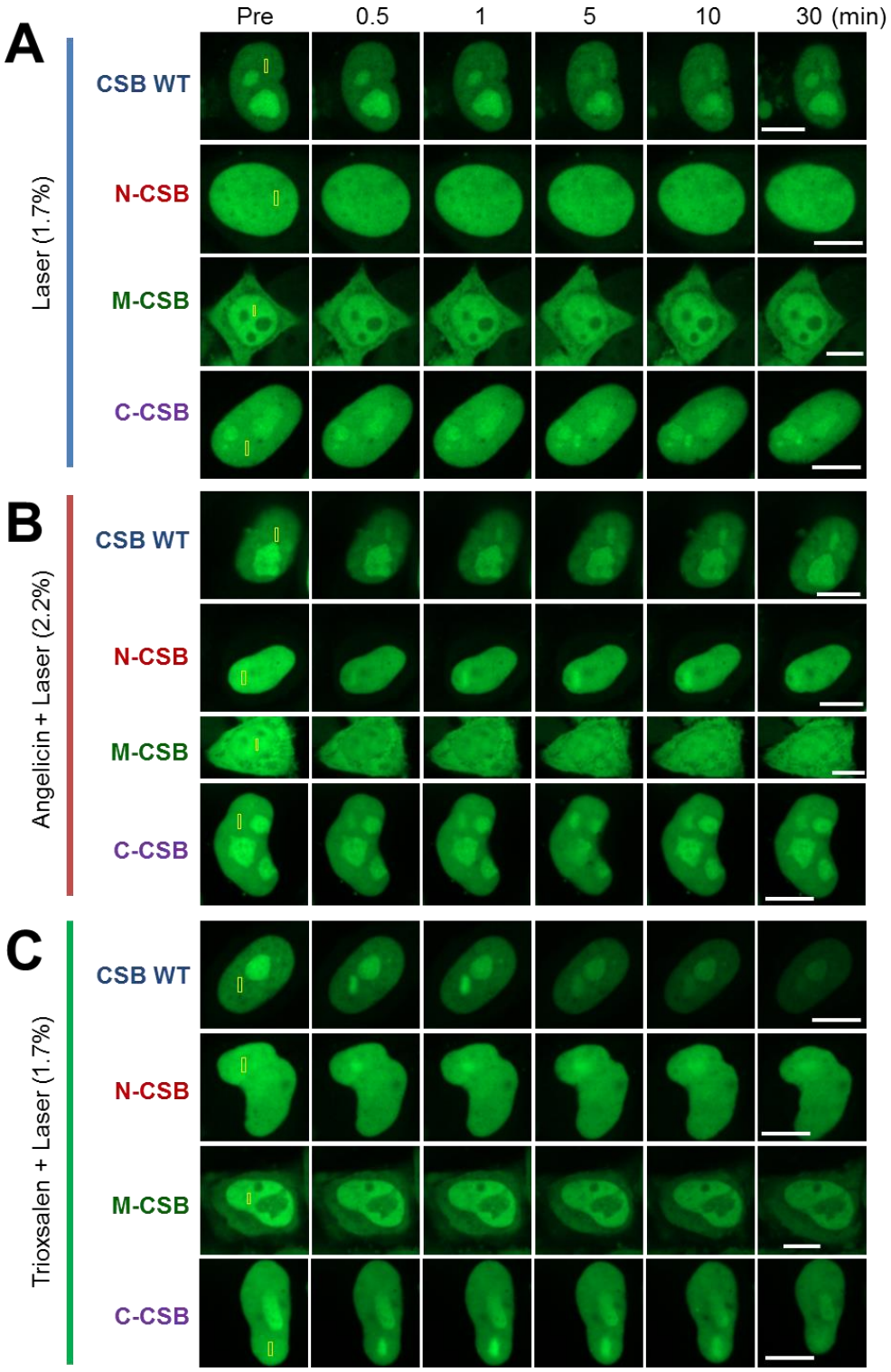


Figure S4. C-terminus of CSB, and to a lesser extent its N-terminus, contains key elements involved in CSB recruitment to DNA damage.

Shown are representative images of unirradiated cells (Pre), and the responses of GFP-tagged full-length CSB or CSB truncated mutants (N-CSB, M-CSB or C-CSB) to localized (A) oxidative damage (1.7% laser alone), (B) monoadducts (angelicin + laser), or (C) ICLs (trioxsalen + laser) at 0.5, 1, 5, 10 and 30 min post-laser irradiation. Bar; 10 µm. Quantified data of the representative images (A-C) is in Figure 5C-E, respectively.

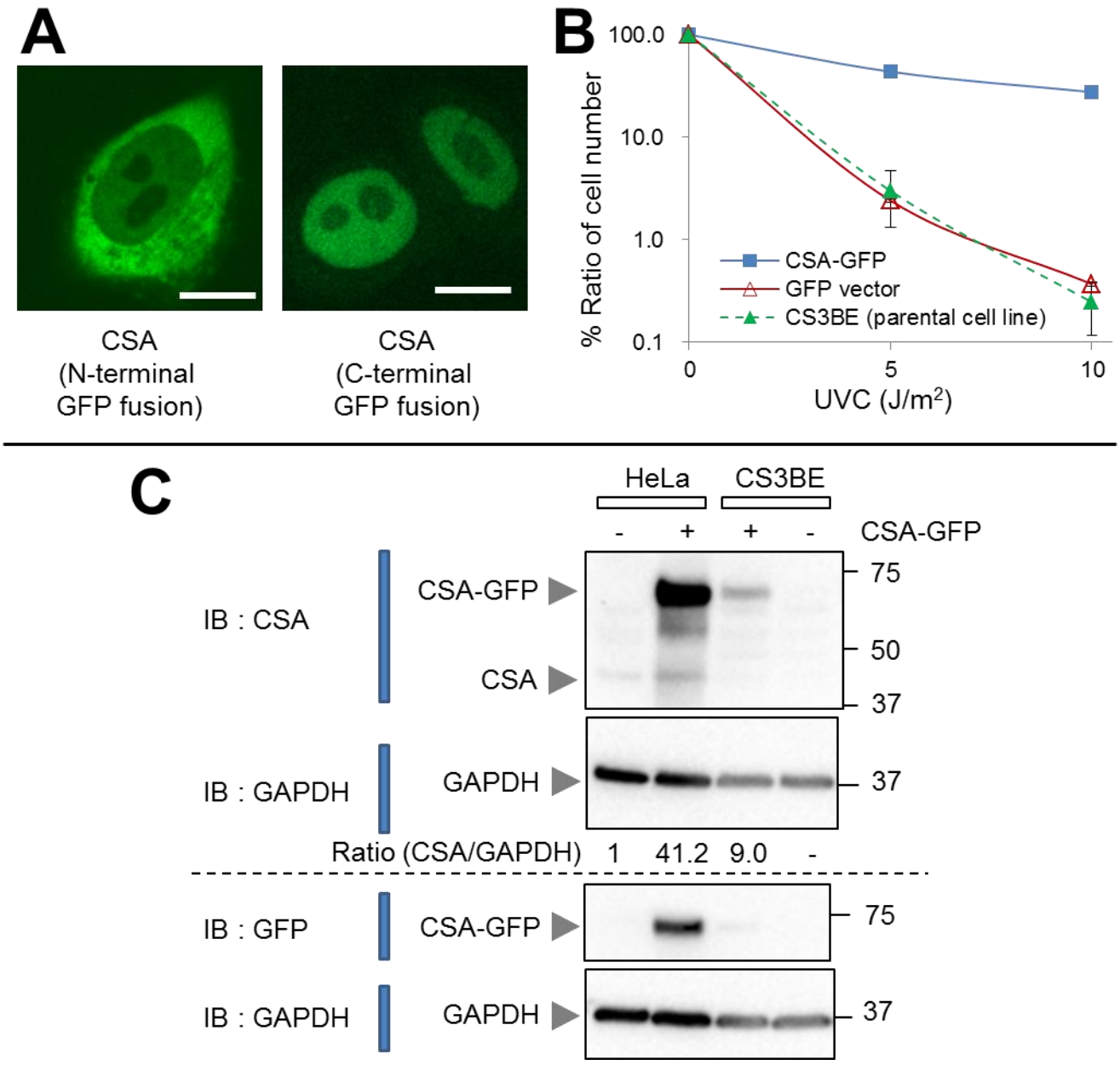


Figure S5. C-terminal GFP-tag CSA is functionally active.

(A) Intracellular distribution of CSA with an N-terminal or C-terminal GFP fusion in HeLa cells. HeLa cells were transfected with pEGFP-CSA (N-terminal GFP) or pCSA-AcGFP (C-terminal GFP), and representative images were captured after a 24 hr incubation. Bar; 10 µm.
(B) UV sensitivity of CSA-deficient CS3BE cell lines after no transfection (parental), or stable integration of pCSA-AcGFP (C-terminal GFP) or vector (pAcGFP). Cell viability was determined by hemocytometer 6 days after UVC irradiation (0, 5, or 10 J/m^2). Reported is the ratio of the cell count determined at the indicated UVC dose in comparison to the untreated control sample. Each data point is derived from three independent experiments. Error bars indicate SD.
(C) Expression of CSA as determined by western blot analysis of whole cell extracts (25 µg) prepared from HeLa cells transfected with (+) or without (-) pCSA-GFP, or from CSA-GFP stably expressing CSA-deficient CS3BE cell line. See Figure S2 legend for further information.