# Supporting Information

## Kristensen et al. 10.1073/pnas.1220071110

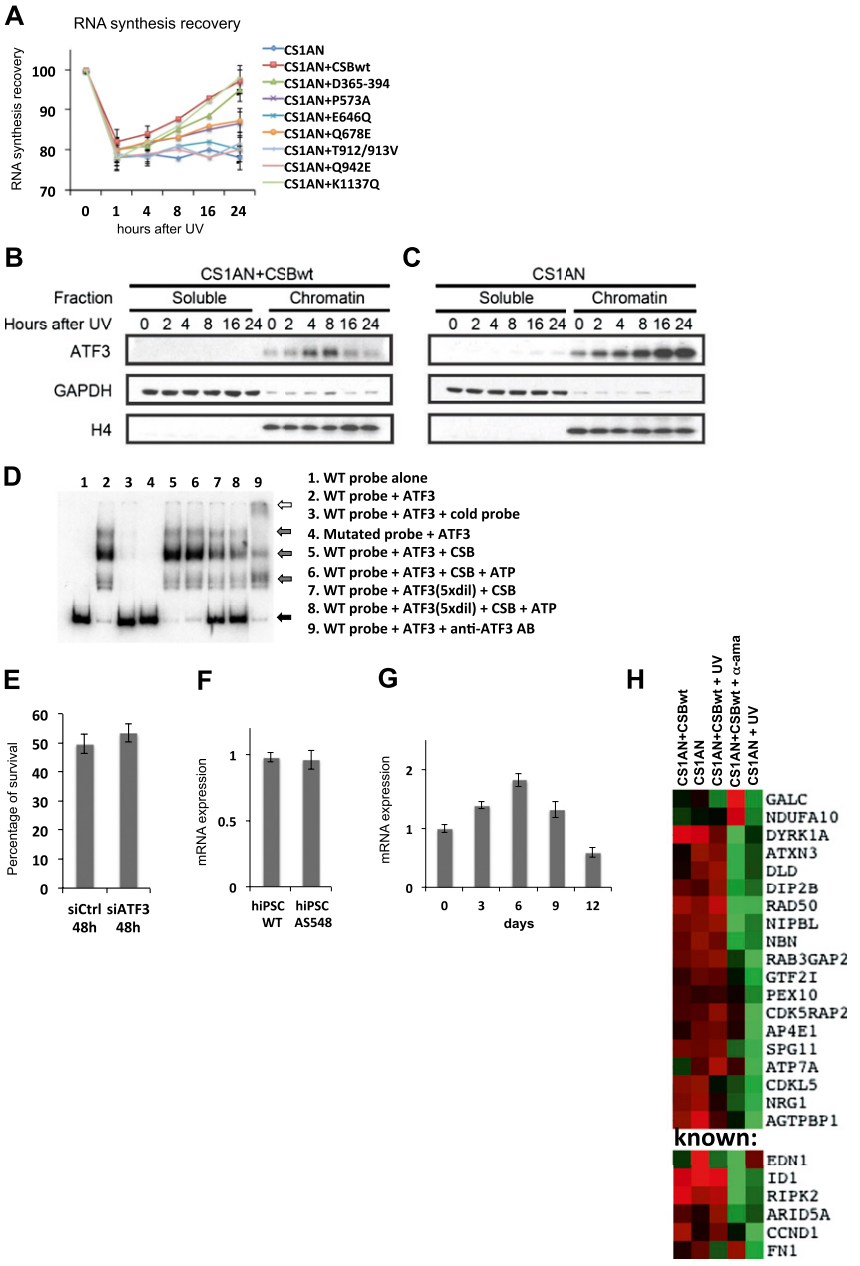


**Fig. S1.** (*A*) Recovery of RNA synthesis after UV irradiation. After prelabeling with $^{14}$C-thymidine (0.02 μCi/mL) for 2 d, unirradiated or UV-irradiated (10 J/m$^2$) cells were pulse-labeled for 30 min with $^3$H-uridine at different incubation times after irradiation, and the acid-insoluble radioactivity was determined. (*B* and *C*) Western blot analysis for activating transcription factor 3 (ATF3), GAPDH, and histone 4 (H4) in both chromatin-soluble and chromatin-bound protein fractions in UV-irradiated CS1AN+CSBwt and CS1AN cell lines. (*D*) EMSA assay showing specific ATF3 binding to the part of the CDK5 regulatory subunit associated protein2 (*CDK5RAP2*) promoter containing the ATF/cAMP response element (CRE)-binding site. The black arrow indicates the unbound probe, gray arrows indicate specific ATF3–probe complexes, and the white arrow indicates super shift with anti-ATF3 antibody. CSB, Cockayne syndrome type B. (*E*) Survival of CS1AN cells transfected with siCtrl and siATF3 48 h after UV treatment. Graphs represent the average of three independent experiments. siATF3, siRNA against ATF3; siCtrl, control siRNA without a target. (*F* and *G*) The ATF3 mRNA level during differentiation of WT and AS548 human induced pluripotent stem cells (hiPSCs) after 48 h of treatment with retinoic acid (*F*) or directed neural differentiation of wild-type hiPSCs (*G*). (*H*) Analyzing a pattern of expression in CS1AN+CSBwt and CS1AN cells 24 h after UV treatment, we found 334 genes that were down-regulated in the CS1AN cell line (values <0.5, $P < 0.001$) upon UV treatment and that contained ATF3-binding cites. The heat map histogram represents the expression level of some of these genes (19 newly identified and six known targets), which were selected on the basis of their relevance to neural degeneration, retardation, and other Cockayne syndrome phenotypes (Tables S2 and S3) and were reanalyzed via quantitative RT-PCR. The gene expressions are depicted in a two-color heat map. The results are presented as fold recruitment, which represents the ratio of the value obtained at each time point relative to that of the untreated cells at time $t = 0$. Each point represents the average of three real-time PCR reactions in three independent experiments.

**Table S1. Abbreviations, full names, and GenBank accession numbers of genes named in the text and figures**

| Abbreviation | Full name | Accession no. |
|---|---|---|
| *DHFR* | Dihydrofolate reductase | NM_000791 |
| *GADD45* | Growth arrest and DNA damage-inducible protein 45 | NM_001009199 |
| *CDKN1A* | Cyclin-Dependent Kinase Inhibitor 1A (p21, Cip1) | NM_000389 |
| *ID1* | Inhibitor of DNA binding 1 protein | NM_002165 |
| *CCND1* | Cyclin D1 | NM_053056 |
| *JUN* | Jun proto-oncogene | NM_002228 |
| *JUNB* | Jun B proto-oncogene | NM_002229 |
| *IER2* | Immediate early response 2 | NM_004907 |
| *IER3* | Immediate early response 3 | NM_003897 |
| *ATF3* | Activating transcription factor 3 | NM_001030287 |
| *FOS* | FBJ murine osteosarcoma viral oncogene homolog | NM_005252 |
| *FOSB* | FBJ murine osteosarcoma viral oncogene homolog B | NM_006732 |
| *EGR1* | Early growth response 1 | NM_001964 |
| *EGR2* | Early growth response 2 | NM_000399 |
| *EGR3* | Early growth response 3 | NM_004430 |
| *NIPBL* | Nipped-B homolog (*Drosophila*) | NM_133433 |
| *GALC* | Galactosylceramidase | NM_000153 |
| *NDUFA10* | NADH dehydrogenase 1 alpha subcomplex, 10, 42kDa | NM_004544 |
| *DYRK1A* | Dual-specificity tyrosine-phosphorylation kinase 1A | NM_001396 |
| *ATXN3* | Ataxin 3 | NM_004993 |
| *DLD* | Dihydrolipoamide dehydrogenase | NM_000108 |
| *DIP2B* | DIP2 disco-interacting protein 2 homolog B | NM_173602 |
| *RAD50* | RAD50 homolog (*S. cerevisiae*) | NM_005732 |
| *NBN* | Nibrin | NM_002485 |
| *RAB3GAP2* | RAB3 GTPase activating protein subunit 2 | NM_012414 |
| *GTF2i* | General transcription factor IIi | NM_032999 |
| *PEX10* | Peroxisomal biogenesis factor 10 | NM_153818 |
| *CDK5RAP2* | CDK5 regulatory subunit associated protein 2 | NM_018249 |
| *AP4E1* | Adaptor-related protein complex 4, epsilon 1 subunit | NM_007347 |
| *SPG11* | Spastic paraplegia 11 (autosomal recessive) | NM_025137 |
| *ATP7A* | ATPase, Cu++ transporting, alpha polypeptide | NM_000052 |
| *CDKL5* | Cyclin-dependent kinase-like 5 | NM_003159 |
| *NRG1* | Neuregulin 1 | NM_013956 |
| *AGTPBP1* | ATP/GTP binding protein 1 | NM_015239 |
| *EDN1* | Endothelin 1 | NM_001955 |
| *RIPK2* | Receptor-interacting serine-threonine kinase 2 | NM_003821 |
| *ARID5A* | AT rich interactive domain 5A (MRF1-like) | NM_212481 |
| *FN1* | Fibronectin 1 | NM_212482 |

**Table S2. Alignment of gene-expression data to ATF3 CRE/ATF global occupation for representative previously described and newly identified ATF3 target genes**

| | | Cell line | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Gene symbol | Peaks | 549_1 | 549_2 | 562_1 | 562_2 | MG12878_1 | MG12878_2 | HepG2_1 | HepG2_2 | hESc_1 | hESc_2 |
| *GALC* | 4 | | | 101 | 145 | | | 137 | | | 141 |
| *NDUFA10* | 7 | 0 | | 61 | 38 | −17 | | −12 | −44 | 8 | |
| *DYRK1A* | 8 | | | −979 | −941 | −866 | −862 | −959 | −965 | −962 | −879 |
| *ATXN3* | 7 | | | −118 | −93 | −96 | | −114 | −121 | −128 | −109 |
| *DLD* | 4 | 27 | 25 | −36 | | | 1,150 | | | | |
| *DIP2B* | 5 | | 122 | −39 | | | | 55 | −26 | −51 | |
| *RAD50* | 12 | 43 | 1 | −11 | 35 | 23 | −1,901,949 | 7 | 33 | −4 | 3 |
| *NIPBL* | 2 | | | −132 | | | 4,127 | | | | |
| *NBN* | 3 | 258 | 158 | 262 | | | | | | | |
| *RAB3GAP2* | 8 | | 136 | 57 | −4 | −58 | | −32 | −51 | −3 | −21 |
| *GTF2i* | 8 | 3,334,693 | 721 | | | | | | −145 | | |
| *PEX10* | 8 | | | −1,472, −483,539 | 0 | | | 59 | 45 | 55 | 4 |
| *CDK5RAP2* | 4 | −67 | −172 | −33 | | | −4,070 | | | | |
| *AP4E1* | 8 | −88 | −120 | −44 | −74 | | | −61 | −18 | 0 | −118 |
| *SPG11* | 5 | −19 | −95 | −63 | | | | | −70 | −32 | |
| *ATP7A* | 3 | | | −193 | | | | −192 | | −184 | |
| *CDKL5* | 1 | −3,060 | | | | | | | | | |
| *NRG1* | 4 | −225,349,968 | 3,349 | | | | | | | | |
| *AGTPBP1* | 1 | | | | | | −1,433 | | | | |
| *EDN1* | 3 | −16 | −38, −775 | | | | | | | | |
| *ID1* | 3 | −976,267 | | | | | −881 | | | | |
| *RIPK2* | 1 | | | | | | −35 | | | | |
| *ARID5A* | 2 | | | −118 | | | −4,506 | | | | |
| *CCND1* | 8 | −2030,32 | 215,624,203,092,343,178 | | | | | | | | |
| *FN1* | 1 | | | | | | | | | | −382 |

The table shows the sum of ATF3-binding peaks from all ATF3 ChIP-Seq experiments. The numbers indicate the position of the Transcription Start Site (TSS) of each gene (*Materials and Methods*).

**Table S3. Alignment of gene expression data to ATF3 CRE/ATF global occupation: Relation of selected ATF3 target genes and respective OMIM inherited disease phenotypes to CSB clinical features**

| | | | CSB clinical features* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Gene symbol | OMIM database no. | Syndrome | A | B | C | D | E | F | G |
| *GALC* | 245200/ | Globoid cell leukodystrophy | + | | | | | | |
| *NDUFA10* | 256000/ | Leigh syndrome | +/− | | | | | | |
| *DYRK1A* | 614104/ | Mental retardation, autosomal dominant 7 | | + | + | | | | |
| *ATXN3* | 109150/ | Machado–Joseph disease | | | | | | | |
| *DLD* | 256000/ | Leigh synndrome | | | | | | | |
| *DIP2B* | 136630/ | Mental retardation, Fra12a type | | + | | | | | + |
| *RAD50* | 613078/ | Nijmegen breakage syndrome-like disorder | | | + | | | | + |
| *NIPBL* | 122470/ | Cornelia de Lange syndrome 1 | | + | | | | + | + |
| *NBN* | 251260/ | Nijmegen breakage syndrome | | + | | | | | + |
| *RAB3GAP2* | 212720/614225 | Martsolf syndrome/Warburg micro syndrome 2 | | + | | + | | | |
| *GTF2i* | 194050/ | Williams–Beuren syndrome | | + | | | + | + | |
| *PEX10* | 202370/214100 | Peroxisome biogenesis disorder 1a | + | + | | | | | |
| *CDK5RAP2* | 604804/ | Microcephaly 3, primary, autosomal recessive | | | + | | | | |
| *AP4E1* | 613744/ | Spastic paraplegia 51, autosomal recessive | | + | + | | | | |
| *SPG11* | 604360/ | Spastic paraplegia 11, autosomal recessive | | + | | | | | |
| *ATP7A* | 309400/304150 | Menkes disease/occipital horn syndrome | | | | | | | |
| *CDKL5* | 105830/312750 | Angelman syndrome/Rett syndrome | | + | + | | | | |
| *NRG1* | 603013/ | Schizophrenia 6 | | | | | | | |
| *AGTPBP1* | *606830/ | Purkinje cell degeneration phenotype | | | | | | | |
| *ERCC6* | 133540/ | Cockayne syndrome, type b | + | + | + | + | + | + | + |

*CSB clinical features: A, hypomyelination/demyelination; B, mental retardation; C, microcephaly; D, cataracts; E, dental abnormalities; F, facial abnormalities; G, growth retardation; OMIM, Online Mendelian Inheritance in Man database (National Center for Biotechnology Information). +/−, clinical phenotype was observed within the patient having defect in the genes listed at the extreme left of the table.