Supplementary Information

hCAF1/CNOT7 regulates interferon signaling by targeting STAT1

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Supplementary Information on Materials and Methods

Microarray analysis

Total RNA from biological triplicates of hCAF1^{kd} and mock cells was isolated, processed and hybridized using Affymetrix HuEx arrays. HuEx arrays contain probes targeting >1 million exons from well-annotated or computationally predicted genes. In this study, we focused on genes with known cDNAs. For each gene, the average intensity of exonic probes was calculated in each sample from four independent experiments. Human Exon 1.0 ST Array dataset analysis and visualization were made using EASANA® (GenoSplice technology, www.genosplice.com) based on GenoSplice's FAST DB® annotations.

Pre-treatment data

Exon Array data were normalized using quantile normalization. Background correction was made using the antigenomic probes and probe selection was made as described previously [1] Only probes targeting annotated exons from FAST DB® transcripts were selected in order to focus on well-annotated genes whose mRNA sequences are available in public databases [2, 3]. Poor-quality probes (*e.g.*, probes classified as "cross-hybridizing" by Affymetrix) and probes with too low signal intensity compared to antigenomic background probes with the same GC content were removed from the analysis. Only probes with a DABG p value ≤0.05 in at least half of the arrays were considered for statistical analysis.[1]

Selection of regulated genes

Only genes expressed in at least one compared condition were analyzed. Genes were considered expressed if the Log_2 gene signal intensity was ≥6.0 and the DABG p value ≤0.05 for at least half of the gene probes. We performed a paired Student's *t*-test to compare gene intensities in the different biological replicates. Genes were considered significantly regulated when fold-change was ≥1.5 and p-value ≤0.05.

Hierarchical Clustering

The distance between the gene signal in a given sample and the corresponding average signal in the 6 samples was calculated for each regulated gene. Corresponding values were displayed and clustered with Me V4.6.2 software from The Institute of Genome Research using Euclidean distance and complete linkage clustering.

Supplementary References

1 de la Grange P, Gratadou L, Delord M, Dutertre M, Auboeuf D (2010) Splicing factor and exon profiling across human tissues. *Nucleic Acids Res* **38**:2825-2838

2 de la Grange P, Dutertre M, Martin N, Auboeuf D (2005) FAST DB: a website resource for the study of the expression regulation of human gene products. *Nucleic Acids Res* **33**:4276-4284

3. de la Grange P, Dutertre M, Correa M, Auboeuf D (2007) A new advance in alternative splicing databases: from catalogue to detailed analysis of regulation of expression and function of human alternative splicing variants. *BMC Bioinformatics* **8**:180

Sequences of the synthetic oligonucleotides used for real-Time PCR:

Gene	Forward	Reverse
IFI27	5’ ACGGAATTAACCCGAGCAG 3’	5’ GCCACAACTCCTCCAATCAC 3’
IFITM1	5’ ATGTCGTCTGGTCCCTGTTC 3’	5’ CAGGATGAATCCAATGGTCA 3’
IRF9	5’ CAAGGCCTGGGCAATATTTA 3’	5’ ACTGTGCTGTCGCTTTGATG 3’
STAT1	5’ CACGCACACAAAAGTGATGA 3’	5’AGAGGTCGTCTCGAGGTCAA 3’
PLSCR1	5’ GGTTTACTTTGCAGCGGAAG 3’	5’ TGGTACACCAGGAGGAGCTT 3’
CAF1	5’TGAAGAGATGAAGAAAATTCGTCAAG 3’	5’ CTTGCAACCACACCTGGAAAC 3’
SULF1	5’ CAGACAGCCTGTGAACAACC 3’	5’ GCCGTTGACTCTTTCTTTGG 3’

HERC6	5’ GTTCCTGCATGTGGTTTCCT 3’	5’ CTTGCAAACTCTTCCCCAAC 3’
IFI6	5’ CTCGGAGAGCTCGGACAG 3’	5’ CATTCAGGATCGCAGACCAG 3’
CSTA	5’ TTTGGTTCCAGCATCCTGTC 3’	5’ TGCACAGCTTCCAATTTTCC 3’
SOCS1	5’ GGAAGGAGCTCAGGTAGTC 3’	5’ AGCTTCGACTGCCTCTT 3’
IRF1	5’ AAAAGGAGCCAGATCCCAAGA 3’	5’ CATCCGGTACACTCGCACAG 3’
TAP1	5’ AGGTACTGCTCTCCATCTAC 3’	5’ AGTGTAAGGGAGTCAACAGA 3’
36B4	5’ GTGTTCGACAATGGCAGCAT 3’	5’ GACACCCTCCAGGAAGCGA 3’

Sequences of the synthetic oligonucleotides used for ChIP and REHA analyses:

Region	**Forward**	**Reverse**
Gapdh 3’ region	5’ ATGGTTGCCACTGGGGATCT 3’	5’ TGCCAAAGCCTAGGGGAAGA 3’
Ifi27 promoter	5’ TCTTCTGGACTGCGCATGAG 3’	5’ GAAGCACTGCCCCTTGACAC 3'
Ifi27 last exon	5’ TCTCCGGATTGACCAAGTTCA 3’	5’ CAGGGAGCTAGTAGAACCTCGC 3’
Ifitm1 promoter	5’ TTAGCCTTCAGCCTTTCCTCC 3’	5’ TAGCCAGGGACCAATGAGGT 3’
Stat1 promoter	5’ CGCCAGGGAGAAACCGTTGGG 3’	5’ GGCTTTGCGCGGTGCAGTTT 3’

Supplementary Tables

Supplementary Table 1. DNA array analysis of hCAF1^{kd} cells versus mock cells.

Gene symbol	Intensity CTRL	Intensity siCAF1	Regulation	Fold-Change	P-value
IFI27	6,37	10,85	up	22,34	5,60E-03
BST2	5,55	8,74	up	9,13	6,00E-04
OAS2	5,44	8,23	up	6,90	6,06E-03
IFI6	9,25	11,89	up	6,26	1,74E-03
IFITM1	7,85	10,25	up	5,25	4,16E-03
IFI44L	4,82	7,01	up	4,55	1,15E-02
ISG15	7,75	9,82	up	4,20	1,39E-02
LGALS3BP	8,74	10,51	up	3,41	2,44E-03
HERC6	6,14	7,88	up	3,33	2,80E-02
OAS1	7,69	9,22	up	2,88	2,57E-02
OAS3	7,69	9,20	up	2,85	7,80E-03
STAT1	8,67	10,03	up	2,57	2,38E-02
IFI35	7,83	9,19	up	2,56	4,07E-02
CMPK2	5,76	7,10	up	2,53	3,25E-02
PSMB8	6,35	7,59	up	2,36	3,91E-02
IFITM3	10,33	11,56	up	2,34	7,02E-03
PSMB9	7,43	8,55	up	2,18	3,95E-02
UBE2L6	9,05	10,17	up	2,18	1,25E-02
PARP12	7,98	8,93	up	1,94	2,28E-02
CLDN1	6,74	7,68	up	1,92	1,65E-02
AKR1C3	8,56	9,49	up	1,91	6,94E-03
IFITM2	10,38	11,30	up	1,89	4,62E-02
USP18	8,13	9,03	up	1,86	1,77E-02
IFIT5	5,98	6,87	up	1,85	3,83E-02
PLSCR1	7,07	7,96	up	1,85	4,62E-03
RDX	7,34	8,22	up	1,84	1,17E-02
HLA-C	9,67	10,52	up	1,80	7,64E-03
DTX3L	7,70	8,51	up	1,75	2,93E-02
SLC7A11	6,87	7,67	up	1,74	3,99E-02
HLA-F	10,12	10,90	up	1,71	4,75E-02
HLA-B	9,33	10,09	up	1,69	1,33E-02
SP110	6,67	7,41	up	1,67	2,76E-02
CNN2	8,39	9,09	up	1,63	2,80E-02
TAP1	8,11	8,80	up	1,62	3,69E-02
IGFBP3	8,49	9,12	up	1,55	2,45E-02
PPM1K	6,91	7,52	up	1,53	6,00E-04
CNOT7	8,56	6,80	down	3,38	2,01E-03
SLC14A1	8,20	7,55	down	1,57	4,58E-02

S100A16	10,20	9,57	down	1,55	1,93E-02
CEACAM6	7,89	7,34	down	1,47	2,83E-02
MGST2	7,81	7,29	down	1,44	2,21E-02
BMPR1B	7,52	7,01	down	1,43	2,21E-02
CCNE1	8,24	7,74	down	1,41	4,26E-02
CKMT1A	9,49	8,99	down	1,41	4,82E-04

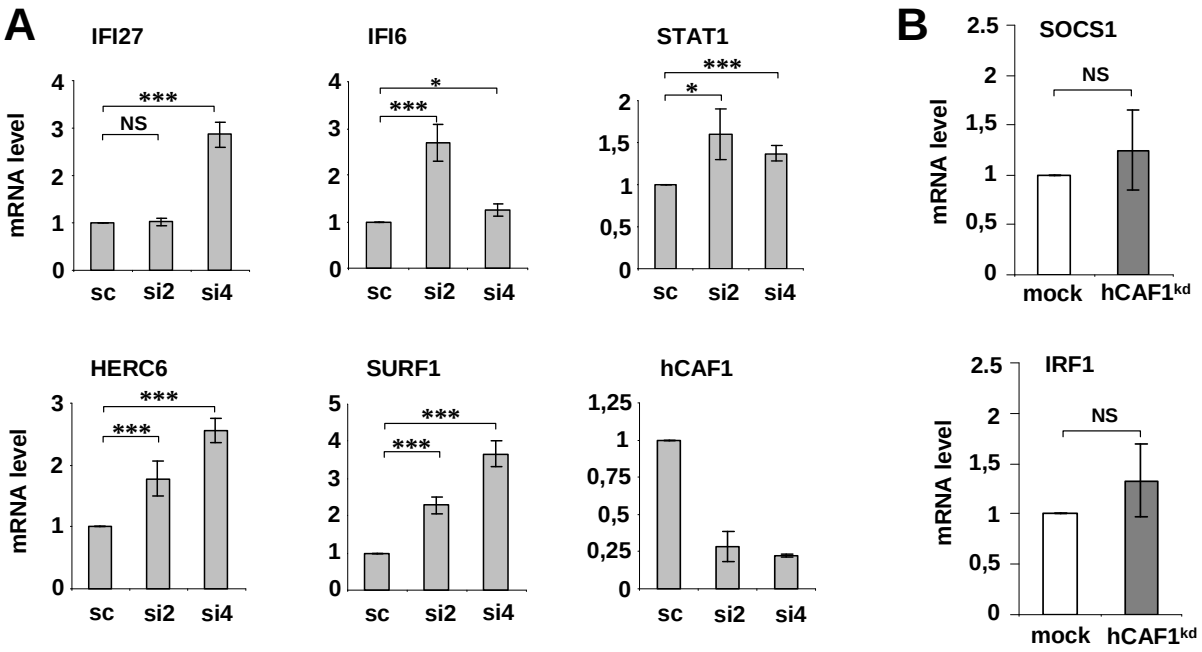
Supplementary Table 2 . Functional analysis of putative hCAF1 target genes using PANTHER (www.pantherdb.org)

FUNCTIONNAL ANALYSIS USING PANTHER (www.pantherdb.org)

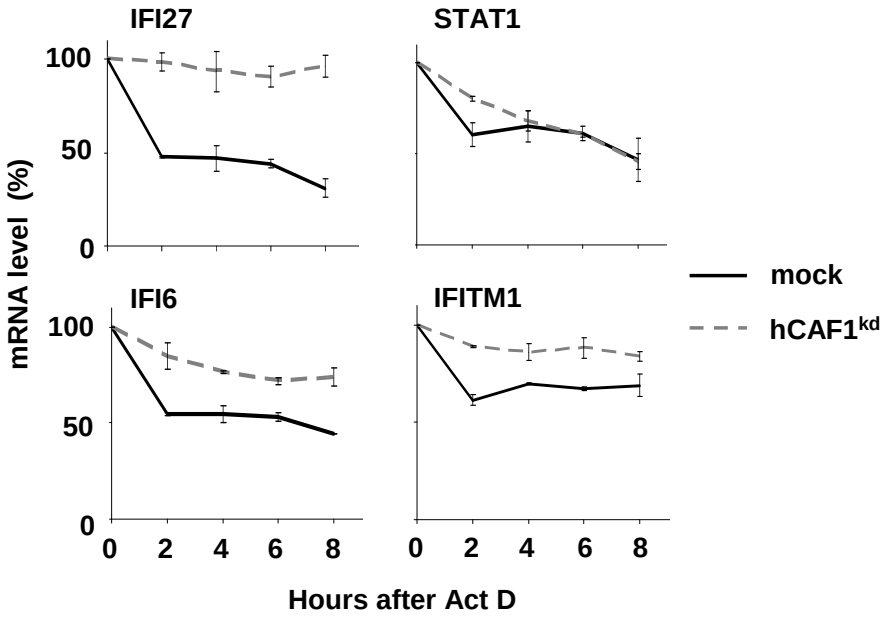
Biological Process	Nb genes in the Reference list	Nb regulated genes	Expected nb genes	P-value
Immunity and defense	1318	12	1,40	3,13E-09
Interferon-mediated immunity	63	5	0,07	7,20E-09
Other transport	61	2	0,06	1,94E-03
Cholesterol metabolism	68	2	0,07	2,40E-03
Extracellular transport and import	89	2	0,09	4,06E-03
Transport	1306	5	1,39	1,12E-02
Steroid metabolism	183	2	0,19	1,61E-02
T-cell mediated immunity	194	2	0,21	1,80E-02
Other metabolism	559	3	0,59	2,10E-02
MHCI-mediated immunity	22	1	0,02	2,31E-02
Other intracellular signaling cascade	225	2	0,24	2,37E-02
Lipid, fatty acid and steroid metabolism	770	3	0,82	4,73E-02
Amino acid transport	47	1	0,05	4,87E-02
Molecular Function	**Nb genes in the Reference list**	**Nb regulated genes**	**Expected nb genes**	**P-value**
Nucleotidyltransferase	70	3	0,07	5,81E-05

Synthetase	96	3	0,10	1,47E-04
Defense/immunity protein	369	4	0,39	5,96E-04
ATP-binding cassette (ABC) transporter	46	2	0,05	1,11E-03
Synthase and synthetase	213	3	0,23	1,48E-03
Transporter	648	4	0,69	4,63E-03
Transfer/carrier protein	327	3	0,35	4,94E-03
Other transporter	334	3	0,35	5,23E-03
Nucleic acid binding	2850	7	3,03	2,60E-02
Amino acid transporter	31	1	0,03	3,24E-02
Tight junction	32	1	0,03	3,34E-02
Other cytoskeletal proteins	40	1	0,04	4,16E-02
Major histocompatibility complex antigen	46	1	0,05	4,77E-02

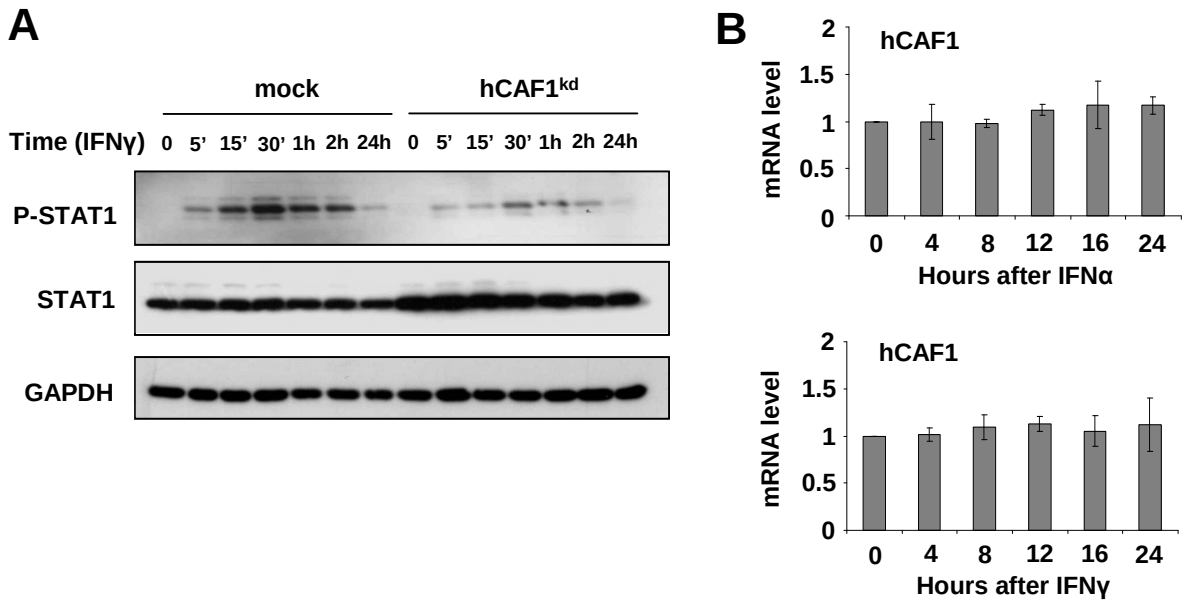
Supplementary Figures



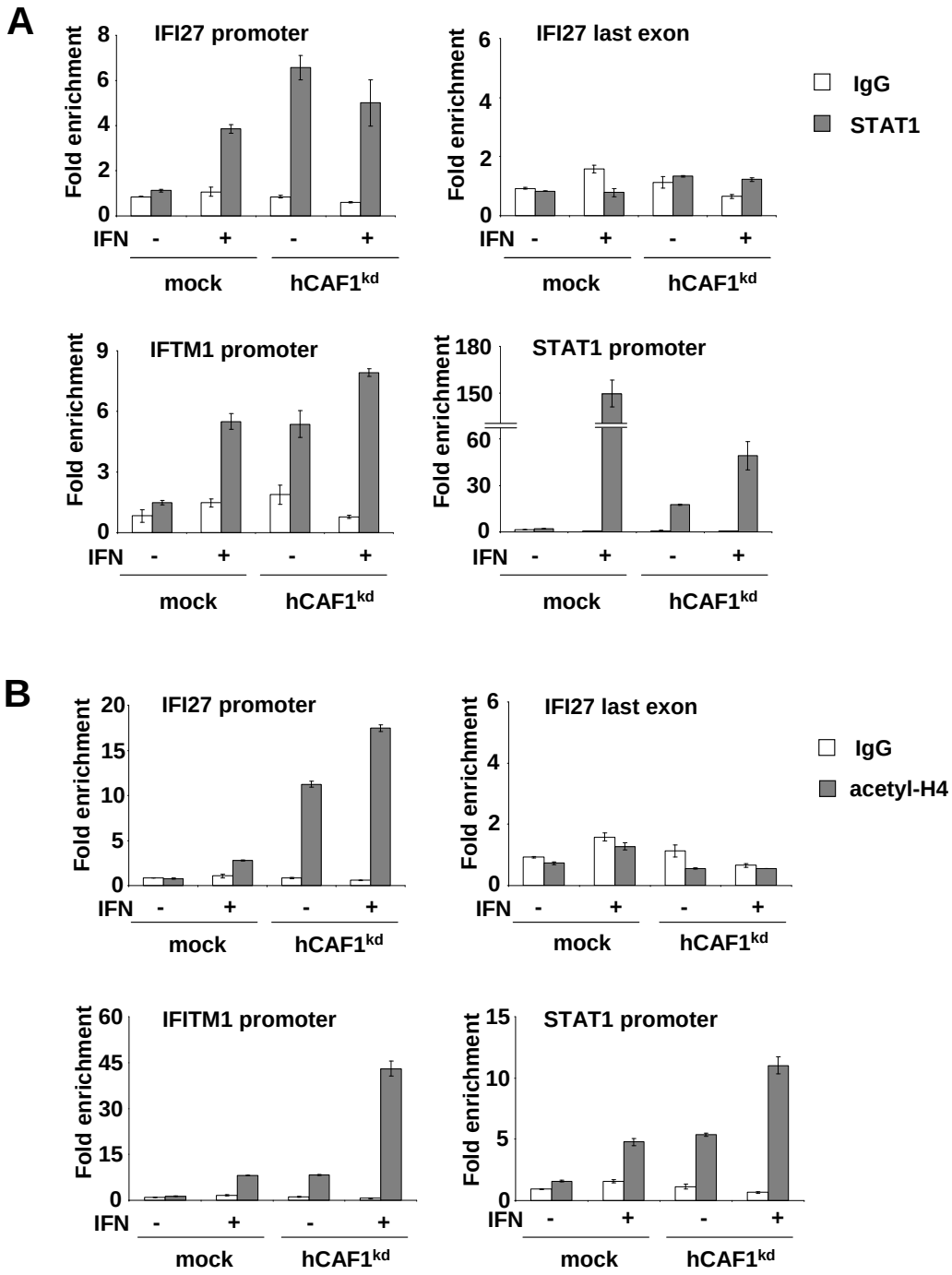
Supplementary Figure 1. (A) MCF7 cells were transfected with control siRNA (sc) or with two alternative siRNAs against hCAF1. 72h after transfection, the cells were harvested and total RNA extracts were prepared. SYBR green real-time RT-PCR analysis was performed with primers specific for the transcripts of the indicated genes. Gene expression levels were normalized to internal controls 36B4 and shown as expression levels relative to expression levels in (sc) control cells (arbitrarily set to 1). (B) Expression of two Interferon-inducible genes, SOCS and IRF1, in control MCF7 cells (mock) and hCAF1 knock-down cells ($hCAF1^{kd}$) was measured by SYBR green real-time RT-PCR analysis. mRNA levels were determined as previously described in (A). Error bars represent standard deviation of the mean of three independent experiments. *P*-values represent unpaired one-tailed *t*-test significance values. (*) $P < 0.05$; (***) $P < 0.005$; (NS) Non significant.



Supplementary Figure 2. **Determination of the stability of hCAF1 regulated genes**. (A) Mock and hCAF1^{kd} cells were treated with Actinomycin D and total mRNA was isolated at 0, 2, 4, 6 and 8 h after treatment. mRNA levels of the indicated genes were determined by SYBR green real-time RT-PCR. Results were plotted as a function of time from drug addiction. Results are expressed as mean values of at least three independent experiments. Standard deviations are shown.

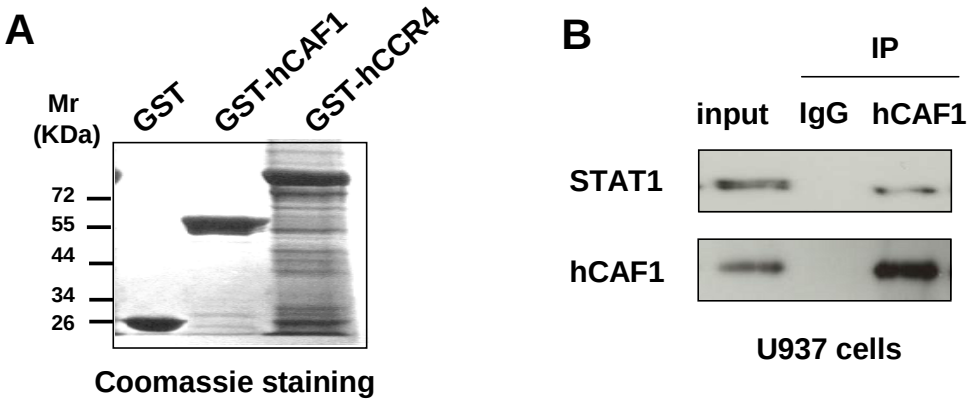


Supplementary Figure 3. (A) STAT1 activation in hCAF1 depleted cells. After IFN γ stimulation for the indicated times, the level of tyrosine 701 phosphorylation of STAT1 was measured in hCAF1^{kd} and control mock cells. Total protein extracts were prepared and analyzed by Western blot using the indicated antibodies. (B) Expression of hCAF1 gene upon interferon stimulation. Transcript level of hCAF1 was measured in MCF7 cells using RT-qPCR as described in Supplementary Figure 1 after IFNα (upper panel) or IFNγ (lower panel) stimulation for the indicated times. The experiments were performed in triplicate, expressed as mean values of three independent experiments. Standard deviations are shown.

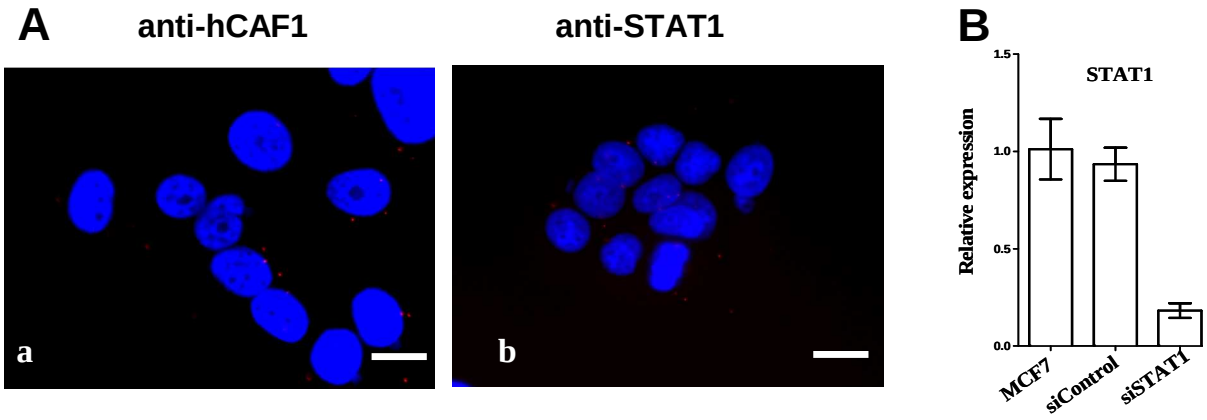


Supplementary Figure 4. Recruitment of STAT1 at a subset of STAT1 target promoters in hCAF1 knock-down cells treated or not with 5 ng/mL of Interferon γ for 1h. ChIP assays were performed using antibodies anti–STAT1 (A) and anti-acetyl H4 (B). Enriched DNA fragments were quantified by Q-PCR using specific primers for the indicated promoters with respect to the input DNA and normalized to a reference locus (3' downstream region of the *GAPDH* gene). Rabbit IgGs were used as a negative control. The illustrated experiments were

performed in triplicate, expressed as mean values and are representative of at least three independent experiments. Standard deviations are shown.

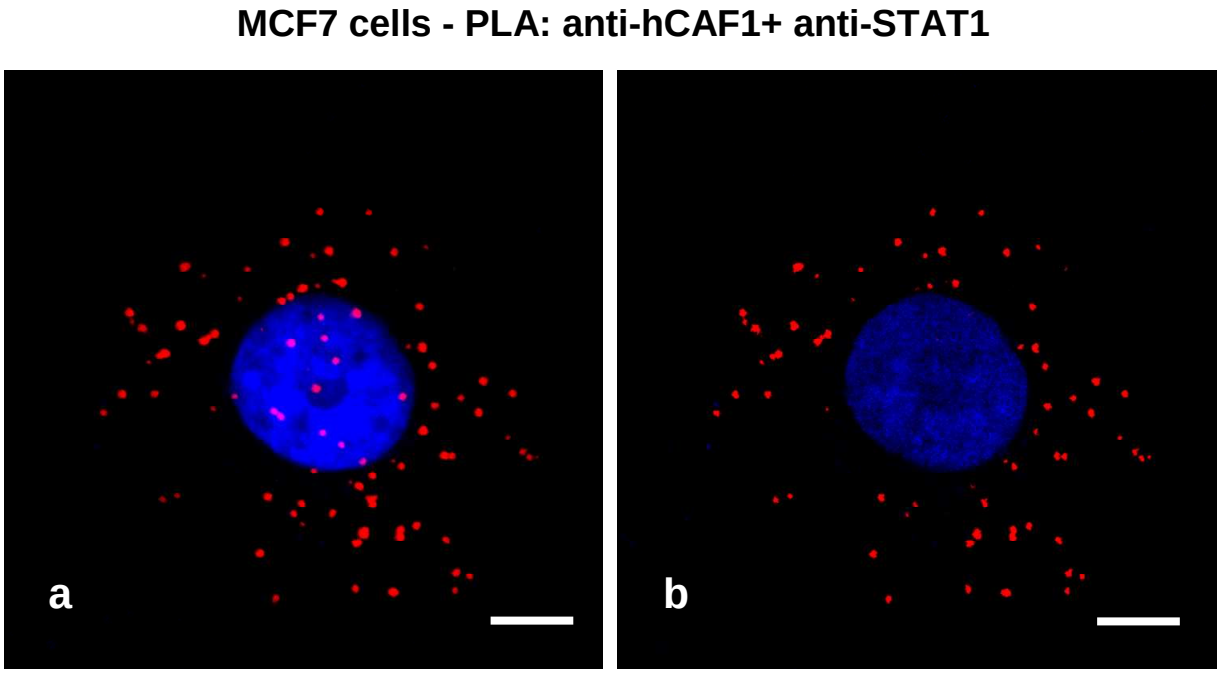


Supplementary Figure 5. (A) Comassie blue staining of 5 µg of GST alone, GST–hCAF1 and GST-hCCR4 used in GST pull-down analysis. (B) Interaction of endogenous hCAF1 and STAT1 in U937 cells. Extracts from U937 cells were immunoprecipitated (IP) with either normal rabbit IgG or anti-hCAF1 antibodies. Immunoprecipitates were then analyzed by immunoblotting with anti-STAT1 antibodies.

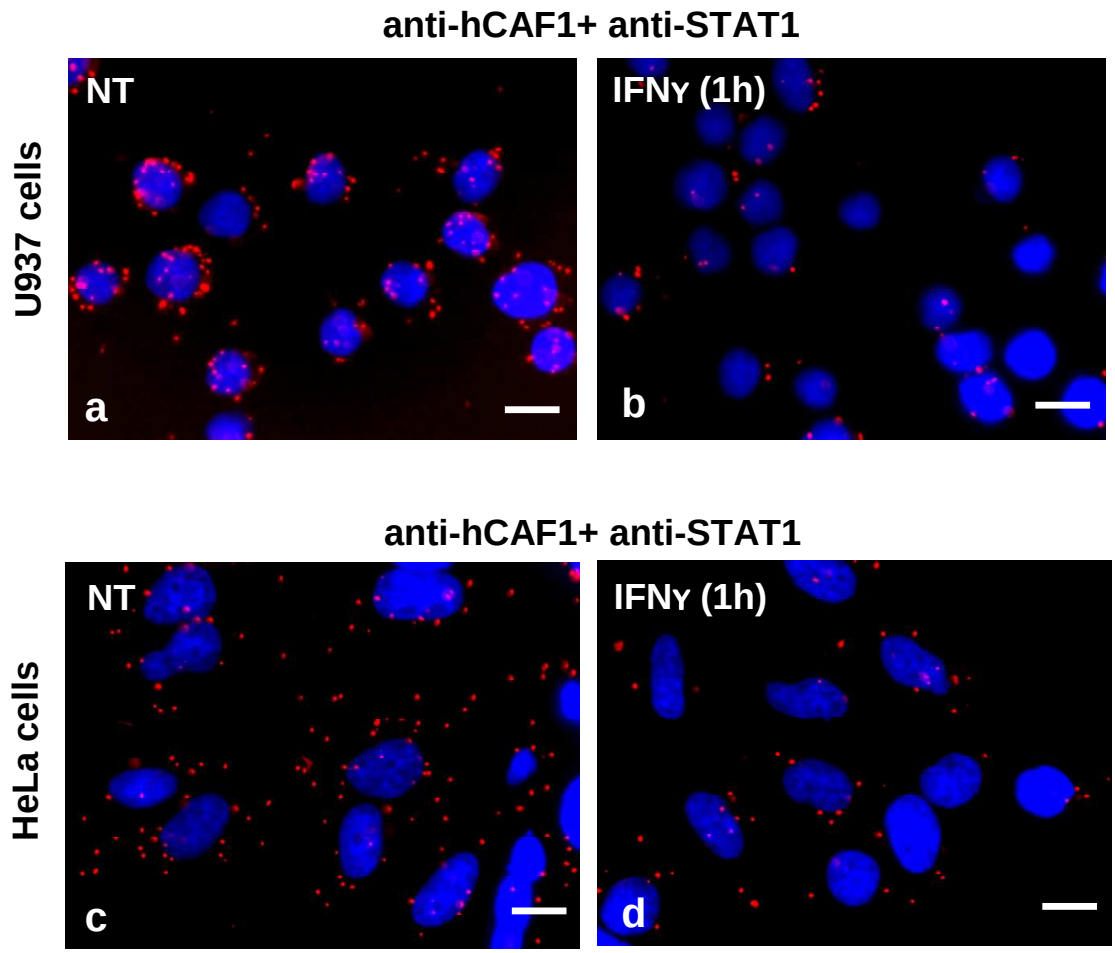


Supplementary Figure 6. (A) PLA control on untreated MCF7 cells using anti-hCAF1 (a) or anti-STAT1 (b) alone. MCF7 cells were grown on coverslips in 12-well plates. Proximity Ligation Assay (PLA) was performed using anti-hCAF1 or anti-STAT1 alone according to the manufacturer's instructions. Nuclei were counter-stained with DAPI (blue). View: ×63. Scale bars = 20 µm. (B) MCF7 cells were transfected with control siRNA (sc) or with siRNAs against STAT1. 72h after transfection, the cells were harvested and total RNA was prepared

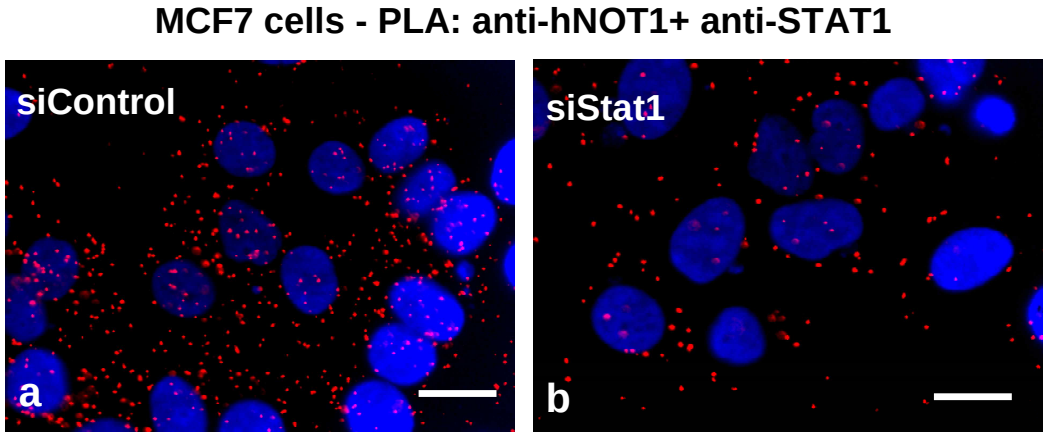
to test STAT1 knockdown efficiency. SYBR green real-time RT-PCR analysis was performed for detection of transcript levels of STAT1. Results were normalized using 36B4 mRNA level as an internal control.



Supplementary Figure 7. Confocal microscopy after Proximity Ligation Assay (PLA) using anti-hCAF1 and anti-STAT1 on untreated MCF7 cells was used to localize the hCAF1/STAT1 interaction. (a) *z*-stack projection of 20 confocal sections from the basal to the apical cell side. (b) Single confocal section into the nucleus of the cell. Nuclei were counter-stained with DAPI (blue). View: ×63. Scale bars = 10 μm.



Supplementary Figure 8. STAT1 and hCAF1 colocalize in the cytoplasm of the unstimulated cells. (a) U937 cells were cytospined and (c) HeLa cells were grown on coverslips and then (b and d) treated with 5 ng/mL of Interferon γ for 1h. Proximity Ligation Assay (PLA) was used to detect the cellular co-localization of endogenous hCAF1 and STAT1 using anti-hCAF1 and anti-STAT1 on (a and c) untreated cells and (b and d) cells treated with 5 ng/mL of Interferon γ for 1h. Nuclei were counter-stained with DAPI (blue). View: ×63. Scale bars = 20 μm.



Supplementary Figure 9. STAT1 and hNOT1 colocalize in the cytoplasm of the unstimulated cells. MCF7 cells were grown on coverslips and Proximity Ligation Assay (PLA) was used to detect the cellular co-localization of endogenous hNOT1 and STAT1. PLA was performed by using anti-hNOT1 and anti-STAT1 on MCF7 cells transfected with a) control siRNA or with b) siRNA against STAT1. Nuclei were counter-stained with DAPI (blue). View: ×63. Scale bars = 20 μm.