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Supplemental information

MDC1 maintains active elongation complexes of RNA polymerase II

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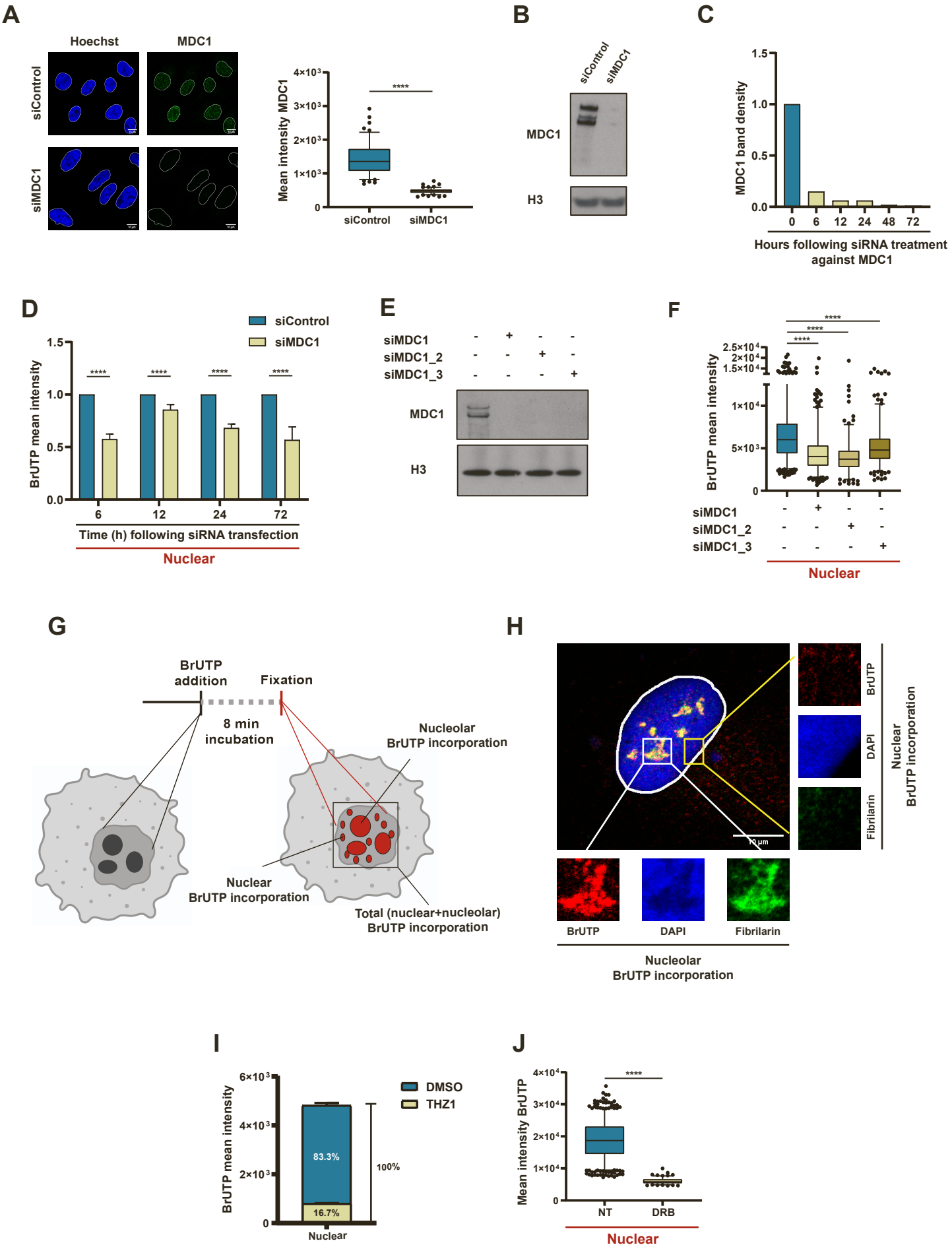

A
Hoechst
MDC1
siControl
siMDC1
Mean intensity MDC1
siControl
siMDC1
B
siControl
siMDC1
MDC1
H3
C
MDC1 band density
Hours following siRNA treatment against MDC1
D
siControl
siMDC1
BrUTP mean intensity
Time (h) following siRNA transfection
Nuclear
E
siMDC1
siMDC1_2
siMDC1_3
MDC1
H3
F
BrUTP mean intensity
siMDC1
siMDC1_2
siMDC1_3
Nuclear
G
BrUTP addition
Fixation
8 min incubation
Nucleolar BrUTP incorporation
Nuclear BrUTP incorporation
Total (nuclear+nucleolar) BrUTP incorporation
H
BrUTP
DAPI
Fibrilarin
Nuclear BrUTP incorporation
Nucleolar BrUTP incorporation
I
BrUTP mean intensity
DMSO
THZ1
83.3%
16.7%
100%
Nuclear
J
Mean intensity BrUTP
NT
DRB
Nuclear

Figure S1. MDC1 facilitates RNA polymerase II activity. Related to figure 1.

(A) Representative images of U2OS cells immunostained for MDC1, upon transfection with the indicated siRNAs (Number of cells: n>100). Individual values have been pooled together and data are represented as mean ± SEM. Scale bar represents 10μm.

(B) Representative immunoblots of whole cell protein extracts (WCE) of U2OS cells transfected with the indicated siRNAs.

(C) Semi-quantification of MDC1 protein abundance following siRNA transfection.

(D) Quantification of nascent RNA synthesis in U2OS cells following siMDC1 transfection for the indicated time points (Number of cells irradiated: n> 150). Individual values have been pooled together and data are represented as mean ± SEM.

(E) Representative immunoblots of whole cell protein extracts (WCE) of U2OS cells transfected with the indicated siRNAs.

(F) Quantification of nascent RNA synthesis in U2OS cells following transfection with the indicated siRNAs (Number of cells: n>150). Individual values have been pooled together and data are represented as mean ± SEM.

(G and H) BrUTP incorporation assay: Schematic representation and representative image of nuclear and nucleolar BrUTP incorporation. Scale bar represents 10μm.

(I) Quantification of nuclear nascent RNA synthesis upon treatment with THZ1 inhibitor (1μM for 1 hour) (Number of cells: n>100). Individual values have been pooled together and data are represented as mean ± SEM.

(J) Quantification of nuclear nascent RNA synthesis upon treatment with DRB inhibitor (100μM for 3.5 hours) (Number of cells: n>100). Individual values have been pooled together and data are represented as mean ± SEM.

****p<0.0001, by unpaired student t-test.

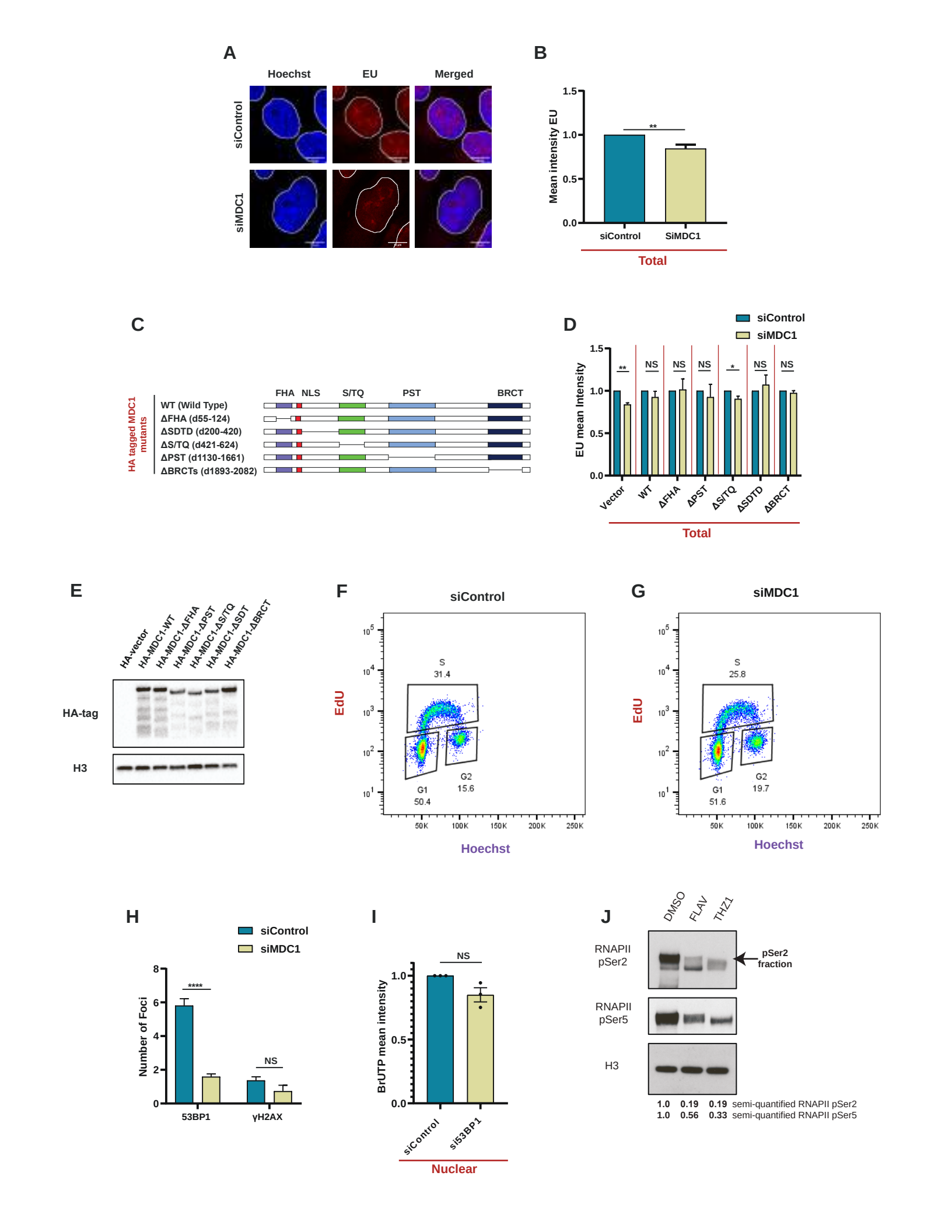

A
Hoechst
EU
Merged
siControl
siMDC1
B
Mean intensity EU
1.5
1.0
0.5
0.0
**
siControl
SiMDC1
Total
C
HA tagged MDC1 mutants
FHA
NLS
S/TQ
PST
BRCT
WT (Wild Type)
ΔFHA (d55-124)
ΔSDTD (d200-420)
ΔS/TQ (d421-624)
ΔPST (d1130-1661)
ΔBRCTs (d1893-2082)
D
siControl
siMDC1
EU mean Intensity
1.5
1.0
0.5
0.0
**
NS
NS
NS
*
NS
NS
Vector
WT
ΔFHA
ΔPST
ΔS/TQ
ΔSDTD
ΔBRCT
Total
E
HA-vector
HA-MDC1-WT
HA-MDC1-ΔFHA
HA-MDC1-ΔPST
HA-MDC1-ΔS/TQ
HA-MDC1-ΔSDT
HA-MDC1-ΔBRCT
HA-tag
H3
F
siControl
EdU
S
31.4
G1
50.4
G2
15.6
50K
100K
150K
200K
250K
Hoechst
G
siMDC1
EdU
S
25.8
G1
51.6
G2
19.7
50K
100K
150K
200K
250K
Hoechst
H
siControl
siMDC1
Number of Foci
8
6
4
2
0
****
NS
53BP1
γH2AX
I
BrUTP mean intensity
1.0
0.5
0.0
NS
siControl
si53BP1
Nuclear
J
DMSO
FLAV
THZ1
RNAPII pSer2
pSer2 fraction
RNAPII pSer5
H3
1.0 0.19 0.19 semi-quantified RNAPII pSer2
1.0 0.56 0.33 semi-quantified RNAPII pSer5

Figure S2. MDC1 facilitates RNA polymerase II activity via its S/TQ domain without affecting genome integrity and cell cycle progression. Related to figure 1.

(A) Representative images of EU incorporation in U2OS, upon transfection with the indicated siRNAs. Scale bar represents 10μm.

(B) Relative quantification of EU incorporation in the whole nucleus of U2OS cells transfected with the indicated siRNAs using QIBC (Number of biological replicates: n=3). Biological replicates have been pooled together and data are represented as mean ± SEM.

(C) Schematic representation of HA-MDC1 deletion mutant plasmids.

(D) Relative quantification of whole nuclear EU incorporation upon transient overexpression of HA-MDC1 mutant constructs in U2OS cells transfected with the indicated siRNAs via QIBC. (Number of biological replicates: n=3. In each biological replicate the numbers of HA-positive cells varied from 50- 80 cells). Biological replicates have been pooled together and data are represented as mean ± SEM.

(E) Representative immunoblots of U2OS cells transfected with the indicated siRNA-resistant HA-MDC1 WT and mutant plasmids. Whole-cell protein extracts (WCE) were analyzed.

(F and G) Cell cycle profile of U2OS cells upon transfection with the indicated siRNAs.

(H) Analysis of DNA damage in U2OS cells upon transfection with the indicated siRNAs. Cells were pre-extracted and the number of 53BP1 and γH2AX foci was quantified via QIBC (Number of cells: n>3000). Individual values have been pooled together and data are represented as mean ± SEM.

(I) Relative quantification of nuclear nascent RNA synthesis in U2OS cells following si53BP1 transfection (Number of cells: n>150). Individual values have been pooled together and data are represented as mean ± SEM.

(J) Representative immunoblots of whole protein extracts and chromatin enriched fraction of the indicative proteins. Relative semi-quantification of band densities was carried out via Fiji (Image J). Arrow indicates pSer2 fraction.

NS, not significant; *p<0.05; **p<0.01; ****p<0.0001, by unpaired student t-test.

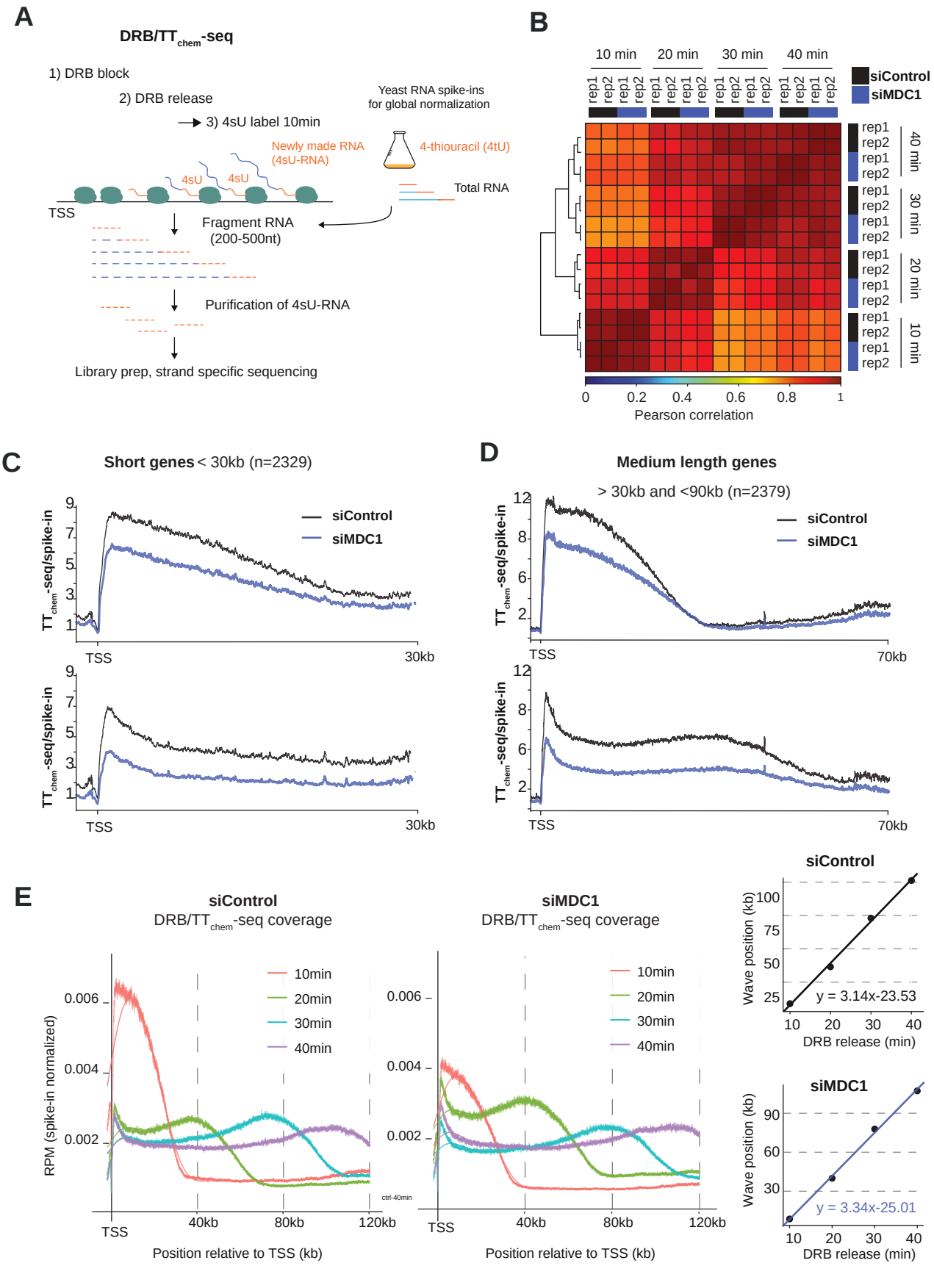

A
DRB/TTchem-seq
1) DRB block
2) DRB release
3) 4sU label 10min
Yeast RNA spike-ins
for global normalization
Newly made RNA
(4sU-RNA)
4-thiouracil (4tU)
4sU
4sU
Total RNA
TSS
Fragment RNA
(200-500nt)
Purification of 4sU-RNA
Library prep, strand specific sequencing
B
10 min
20 min
30 min
40 min
siControl
siMDC1
rep1
rep2
40 min
30 min
20 min
10 min
0
0.2
0.4
0.6
0.8
1
Pearson correlation
C
Short genes < 30kb (n=2329)
siControl
siMDC1
TTchem-seq/spike-in
TSS
30kb
D
Medium length genes
> 30kb and <90kb (n=2379)
TSS
70kb
E
siControl
DRB/TTchem-seq coverage
siMDC1
10min
20min
30min
40min
RPM (spike-in normalized)
0.006
0.004
0.002
TSS
40kb
80kb
120kb
Position relative to TSS (kb)
ctrl-40min
Wave position (kb)
y = 3.14x-23.53
DRB release (min)
y = 3.34x-25.01

Figure S3: MDC1 impacts the engagement of RNAPII elongation complexes. Related to figure 3.

(**A**) Detailed overview of the workflow for DRB/TT_{chem}-seq, including generation of yeast spike-in normalization controls. Early elongation is blocked by the CDK9 inhibitor DRB, which leads to synchronization of all RNAPII complexes close to the TSS. After washing out of DRB, RNAPII complexes start elongating into the gene body and the progression can be tracked by TT_{chem}-seq. Here, nascent RNA is labeled *in vivo* by the addition of 4sU directly to the tissue culture medium. The reaction is stopped by TRIzol, and total RNA is extracted, spiked in with yeast RNA (4tU labelled), and fragmented by controlled base hydrolysis. 4sU residues in the fragmented RNA are biotinylated and used for streptavidin pull-down of 4sU-containing RNA.

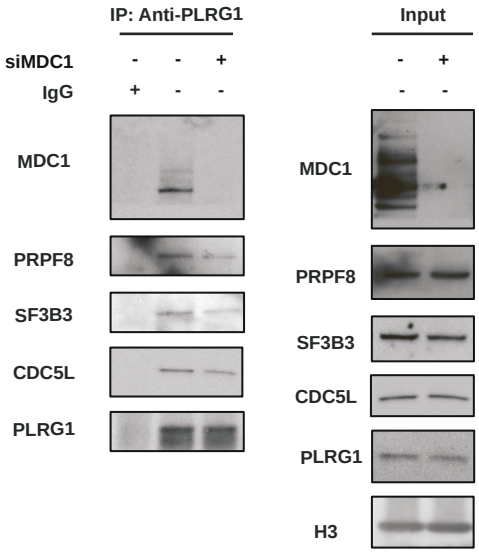
(**B**) Pearson's correlation matrix between each sample (siControl in black and siMDC1 in blue) and replicates calculated based on coverage from all protein-coding genes.

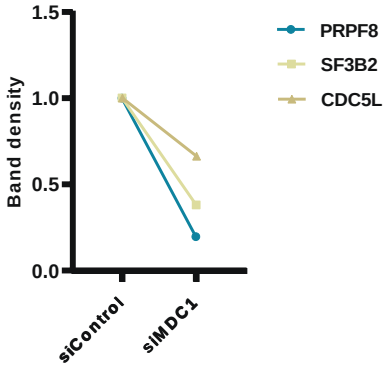
(**C**) Metagene profile of the average coverage normalized over spike-in and anchored at the TSS of non-overlapping protein-encoding genes with size below 30 kb (*n* = 2,329).

(**D**) Metagene profile of normalized over spike-in average coverage anchored at the TSS for non-overlapping protein-encoding genes with size above 30 kb and below 90 kb (*n* = 2379)

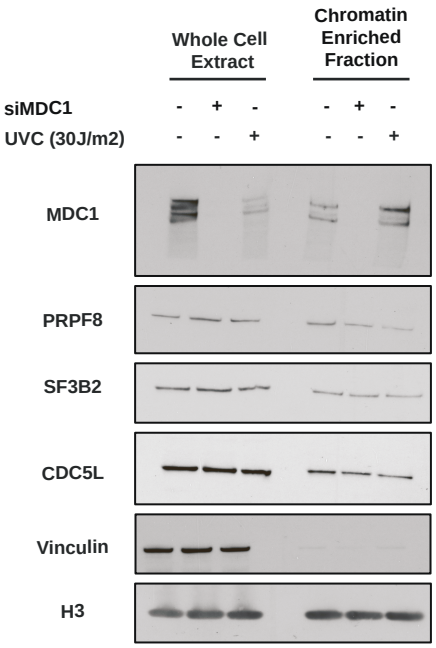
(**E**) DRB/TT_{chem}-seq metagene profiles for siControl (left) and siMDC1 (right) samples of protein-encoding genes between 80 and 300 kb from standard chromosomes (1–22,X,Y) with non-overlapping transcriptional units. The gene ranges were extended around their TSSs (−2 kb to +120 kb); any extensions beyond the limit of the chromosome were dropped (*n* = 3,942). lines are computationally fitted splines. Calculation of RNAPII elongation rates and linear regression equation for siControl and siMDC1 based on metagene profile.

A


IP: Anti-PLRG1
Input
siMDC1
IgG
MDC1
PRPF8
SF3B3
CDC5L
PLRG1
H3


Band density
1.5
1.0
0.5
0.0
siControl
siMDC1
PRPF8
SF3B2
CDC5L

B


Whole Cell Extract
Chromatin Enriched Fraction
siMDC1
UVC (30J/m2)
MDC1
PRPF8
SF3B2
CDC5L
Vinculin
H3

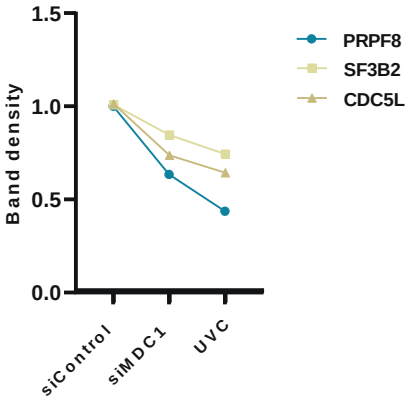
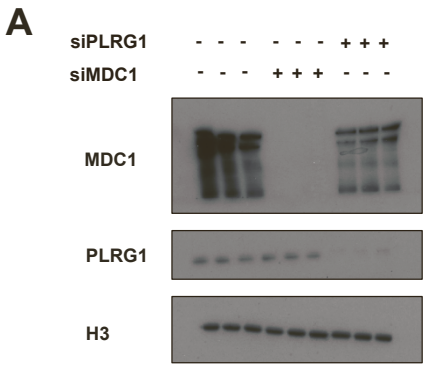
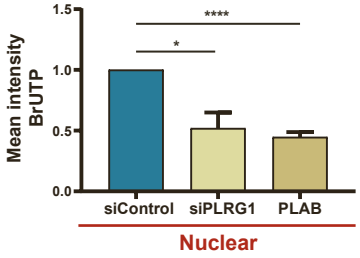

Band density
1.5
1.0
0.5
0.0
siControl
siMDC1
UVC
PRPF8
SF3B2
CDC5L

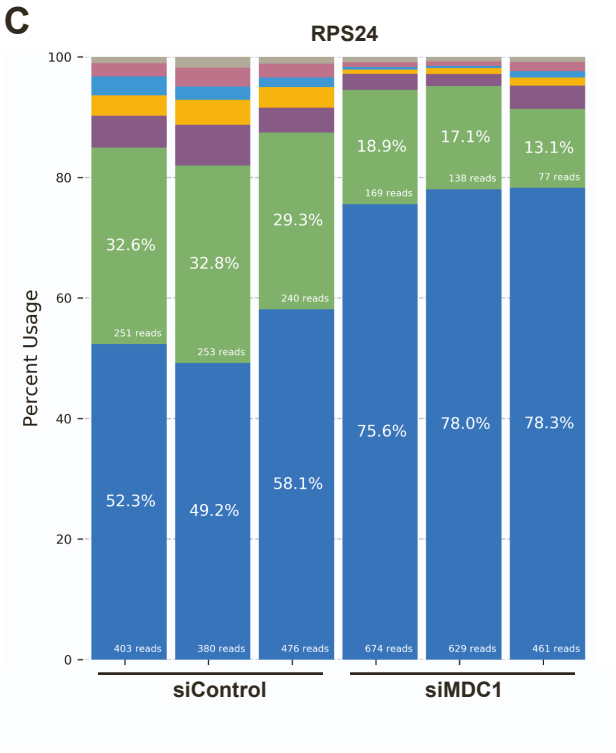
Figure S4: MDC1 knockdown impacts spliceosome assembly. Related to figure 4.

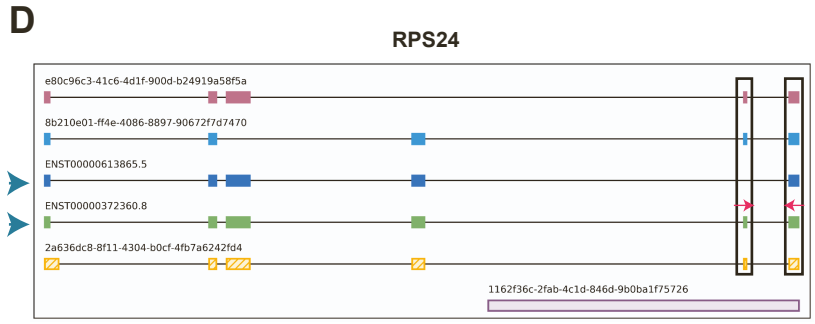
(A) Representative immunoblots of co-immunoprecipitation assay carried out by antibody against PLRG1 indicating its protein-protein interaction with different proteins of the spliceosome. Band densities were semi-quantified and plotted.

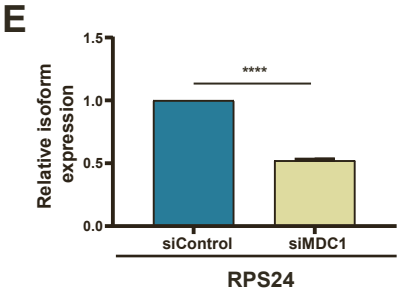
(B) Representative immunoblots of whole protein extracts and chromatin enriched fraction of the indicative proteins upon MDC1 knockdown and UVC irradiation. Band densities were semi-quantified and plotted.

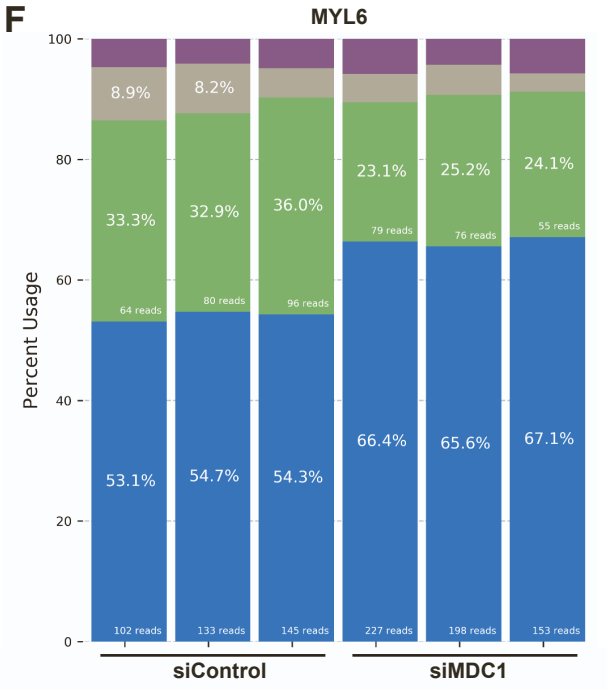

A
siPLRG1 - - - - - - + + +
siMDC1 - - - + + + - - -
MDC1
PLRG1
H3

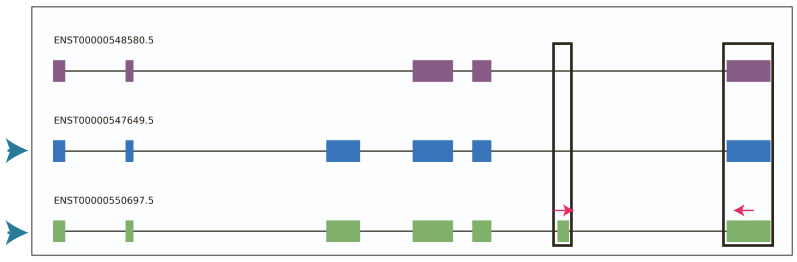

B
Mean intensity BrUTP
1.5
1.0
0.5
0.0
****
*
siControl
siPLRG1
PLAB
Nuclear


C
RPS24
Percent Usage
100
80
60
40
20
0
32.6%
32.8%
29.3%
18.9%
17.1%
13.1%
52.3%
49.2%
58.1%
75.6%
78.0%
78.3%
251 reads
253 reads
240 reads
169 reads
138 reads
77 reads
403 reads
380 reads
476 reads
674 reads
629 reads
461 reads
siControl
siMDC1


D
RPS24
e80c96c3-41c6-4d1f-900d-b24919a58f5a
8b210e01-ff4e-4086-8897-90672f7d7470
ENST00000613865.5
ENST00000372360.8
2a636dc8-8f11-4304-b0cf-4fb7a6242fd4
1162f36c-2fab-4c1d-846d-9b0ba1f75726


E
Relative isoform expression
1.5
1.0
0.5
0.0
****
siControl
siMDC1
RPS24


F
MYL6
Percent Usage
100
80
60
40
20
0
8.9%
8.2%
33.3%
32.9%
36.0%
23.1%
25.2%
24.1%
53.1%
54.7%
54.3%
66.4%
65.6%
67.1%
64 reads
80 reads
96 reads
79 reads
76 reads
55 reads
102 reads
133 reads
145 reads
227 reads
198 reads
153 reads
siControl
siMDC1


G
MYL6
ENST00000548580.5
ENST00000547649.5
ENST00000550697.5

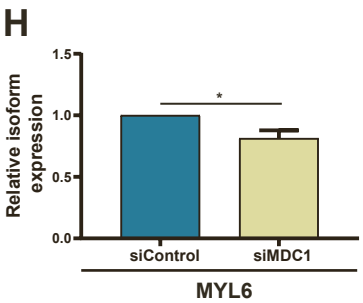

H
Relative isoform expression
1.5
1.0
0.5
0.0
*
siControl
siMDC1
MYL6

Figure S5: MDC1 induces changes in pre-mRNA splicing. Related to figure 5.

(A) U2OS cells were transfected with the indicated siRNAs and whole cell protein extracts (WCE) were immunoblotted with the indicated antibodies.

(B) Relative quantification of nuclear nascent RNA synthesis in U2OS cells following siPLRG1 transfection and treatment with PLAB inhibitor (Number of biological replicates: n=3 biological replicates). Biological replicates have been pooled together and data are represented as mean ± SEM.

(C and D) Isoform usage changes in *RPS24*: Usage of the different isoforms detected in nanopore data with bar plot colors matching the corresponding isoforms. The two arrowheads indicate the two most abundant isoforms. The black brackets show the exon regions amplified with qPCR and the two red arrows indicate the primers designed for this purpose used in S5E (listed in table S1).

(E) qPCR was carried out using primers hybridizing to the chosen exon regions (Number of biological replicates: n=3 biological replicates). Biological replicates have been pooled together and data are represented as mean ± SEM.

(F and G) Isoform usage changes in *MYL6*: Usage of the different isoforms detected in nanopore data with bar plot colors matching the corresponding isoforms. The two arrowheads indicate the two most abundant isoforms. The black brackets show the exon regions amplified with qPCR and the two red arrows indicate the primers designed for this purpose used in S5H (listed in table S1).

(H) qPCR was carried out using primers hybridizing to the chosen exon regions (Number of biological replicates: n=3 biological replicates). Biological replicates have been pooled together and data are represented as mean ± SEM.

*p<0.05; ****p<0.0001, by unpaired student t-test.

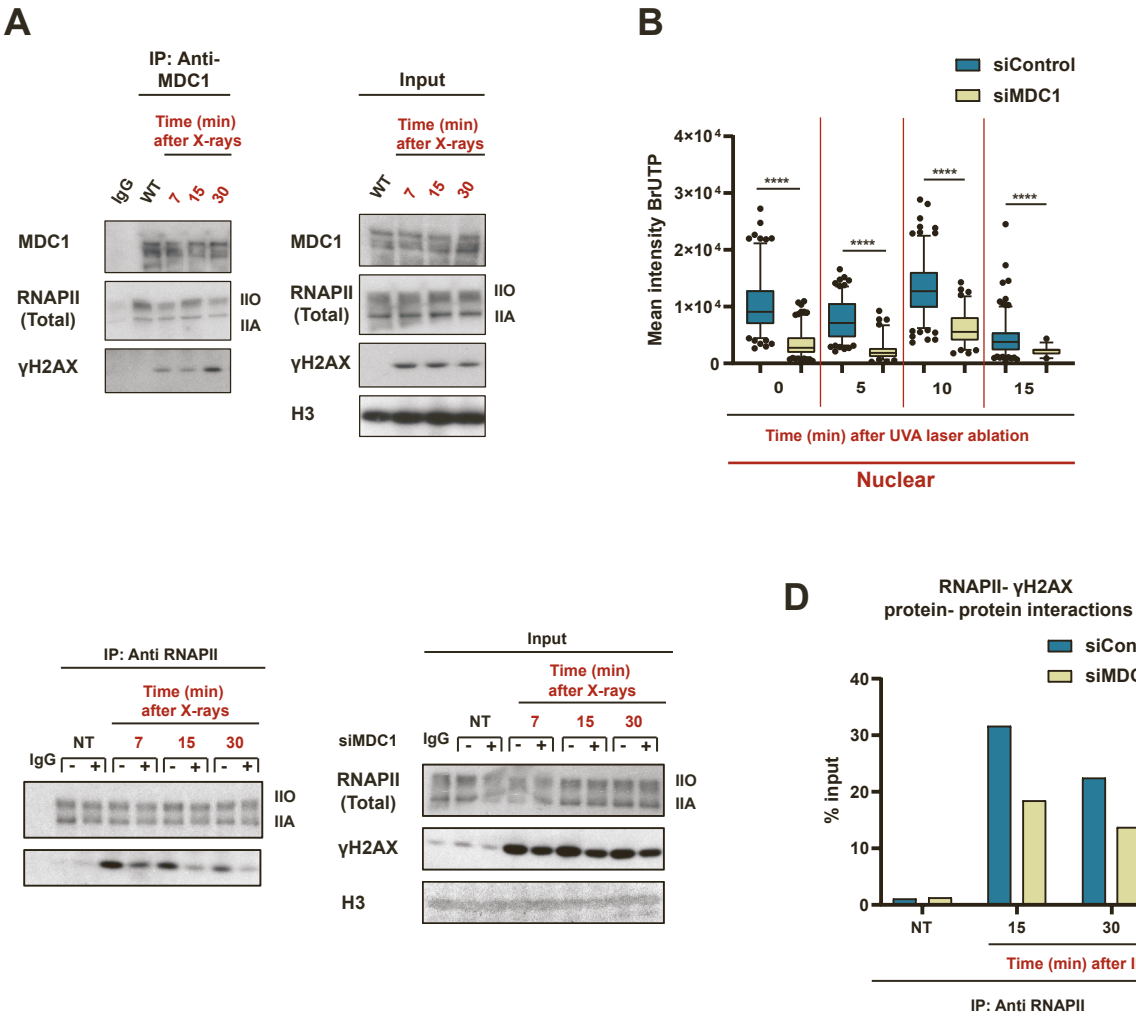

A
IP: Anti-MDC1
Time (min) after X-rays
IgG
WT
7
15
30
MDC1
RNAPII (Total)
IIO
IIA
γH2AX
Input
Time (min) after X-rays
WT
7
15
30
MDC1
RNAPII (Total)
IIO
IIA
γH2AX
H3
B
siControl
siMDC1
Mean intensity BrUTP
4×10⁴
3×10⁴
2×10⁴
1×10⁴
0
****
****
****
****
0
5
10
15
Time (min) after UVA laser ablation
Nuclear
C
IP: Anti RNAPII
Time (min) after X-rays
NT
7
15
30
siMDC1
IgG
- + - + - + - +
RNAPII (Total)
IIO
IIA
γH2AX
Input
Time (min) after X-rays
NT
7
15
30
siMDC1
IgG
- + - + - + - +
RNAPII (Total)
IIO
IIA
γH2AX
H3
D
RNAPII- γH2AX protein- protein interactions
siControl
siMDC1
% input
40
30
20
10
0
NT
15
30
Time (min) after IR
IP: Anti RNAPII
E
Time after UVA laser ablation
5 min
10 min
Mean intensity BrUTP on DSBs
1.5×10⁴
1×10⁴
5×10³
0
****
****
****
NS
**
NS
siControl
siMDC1
DRB (30min)
DRB (4h)
siControl
siMDC1
DRB (30min)
DRB (4h)

Figure S6: MDC1 is required for RNAPII-mediated transcription on DSBs. Related to figures 6 and 7.

(A) Representative immunoblots of protein-protein interaction of MDC1, RNAPII and γH2AX (IIO= phosphorylated RNAPII, IIA= non-phosphorylated RNAPII).

(B) Quantification of nuclear nascent RNA synthesis in U2OS cells following UVA laser ablation (Number of cells irradiated: n> 20). Individual values have been pooled together and data are represented as mean ± SEM.

(C) Representative immunoblots showing protein-protein interactions between RNAPII and γH2AX following X-rays upon transfection with the indicated siRNAs (IIO= phosphorylated RNAPII, IIA= non-phosphorylated RNAPII).

(D) Relative semi-quantification of protein-protein interactions between RNAPII and γH2AX shown in S6C.

(E) Quantification of nascent RNA synthesis on DSB sites based on BrUTP incorporation. U2OS cells were transfected with the indicated siRNAs or incubated with DRB (100μM) for specific periods of time. (Number of irradiated cells: n> 20). Individual values have been pooled together and data are represented as mean ± SEM.

NS, not significant; **p<0.01; ****p<0.0001, by unpaired student t-test.

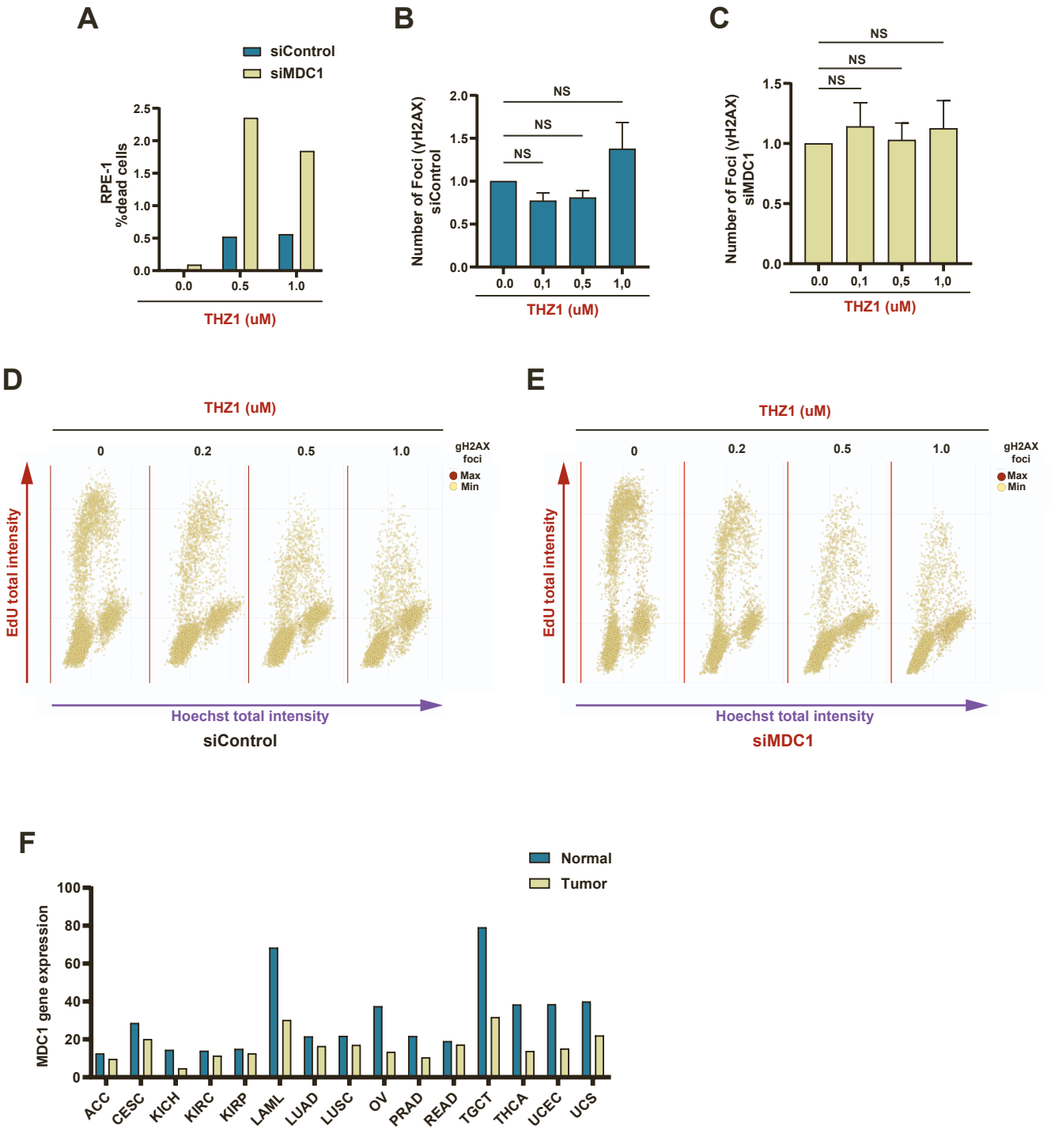

A
siControl
siMDC1
RPE-1
%dead cells
2.5
2.0
1.5
1.0
0.5
0.0
0.0
0.5
1.0
THZ1 (uM)
B
Number of Foci (γH2AX)
siControl
NS
NS
NS
2.0
1.5
1.0
0.5
0.0
0.0
0,1
0,5
1,0
THZ1 (uM)
C
Number of Foci (γH2AX)
siMDC1
NS
NS
NS
1.5
1.0
0.5
0.0
0.0
0,1
0,5
1,0
THZ1 (uM)
D
THZ1 (uM)
0
0.2
0.5
1.0
gH2AX
foci
Max
Min
EdU total intensity
Hoechst total intensity
siControl
E
THZ1 (uM)
0
0.2
0.5
1.0
gH2AX
foci
Max
Min
EdU total intensity
Hoechst total intensity
siMDC1
F
Normal
Tumor
MDC1 gene expression
100
80
60
40
20
0
ACC
CESC
KICH
KIRC
KIRP
LAML
LUAD
LUSC
OV
PRAD
READ
TGCT
THCA
UCEC
UCS

Figure S7: MDC1 depletion sensitizes cells to THZ1. Related to figure 7.

(A) Representative bar plots of % dead cells of RPE-1 cells transfected with the indicated siRNAs upon THZ1 treatment (Experiment has been performed two times).

(B and C) Analysis of DNA damage in U2OS cells upon transfection with the indicated siRNAs followed by treatment with THZ1 inhibitor (48 hours of treatment) based on the quantification of γH2AX foci via QIBC (Number of biological replicates: n= 3). Individual values have been pooled together and data are represented as mean ± SEM.

(D and E) Cell cycle profile of U2OS cells transfected with the indicated siRNAs followed by treatment with THZ1 inhibitor (48 hours of treatment). The number of γH2AX foci was analyzed in each cell cycle phase via QIBC.

(F) MDC1 gene expression levels in several cancer types. Data were acquired from Gepia database.

NS, not significant; by unpaired student t-test.

Table S1. DNA oligos and DNA primers. Related to figures 2, S2, and S5

qPCR Primers		
MYL6 Forward: 5-GGCATGAGGACAGCAATGGT-3	This paper	N/A
MYL6 Reverse: 5-CCCGACAGGATATGCCTCA-3	This paper	N/A
RPS24 Forward: 5-GCAACGAAAGGAACGCAAGA-3	This paper	N/A
RPS24 Reverse: 5-TGTGATCCAATCTCCAGCTCA-3	This paper	N/A
GAPDH Forward: 5-GAAACTGTGGCGTGATGGC-3	Singh J and Padgett R.A., 2009 [S1]	https://pubmed.ncbi.nlm.nih.gov/19820712/
GAPDH Reverse: 5-CACCACTGACACGTTGGCAG-3	Singh J and Padgett R.A., 2009 [S1]	https://pubmed.ncbi.nlm.nih.gov/19820712/
ITPR1 Ex1 Forward: 5-TCTTCGCGGACATGGGATTACC-3	Singh J and Padgett R.A., 2009 [S1]	https://pubmed.ncbi.nlm.nih.gov/19820712/
ITPR1 In1 Reverse: 5-GCATGCACATCCATCAAGATCTCCC-3	Singh J and Padgett R.A., 2009 [S1]	https://pubmed.ncbi.nlm.nih.gov/19820712/
ITPR1 Ex5 Forward: 5-AGTTCTGGAAAGCCGCTAAGCC-3	Singh J and Padgett R.A., 2009 [S1]	https://pubmed.ncbi.nlm.nih.gov/19820712/
ITPR1 In5 Reverse: 5-ACCAAGGCAGCCACTCACTACT-3	Singh J and Padgett R.A., 2009 [S1]	https://pubmed.ncbi.nlm.nih.gov/19820712/
OPA1 Ex 18 Forward: 5-GGAACAGCTCTGAAAGCATTG-3	Singh J and Padgett R.A., 2009 [S1]	https://pubmed.ncbi.nlm.nih.gov/19820712/
OPA1 Ex 19 Forward: 5-GGACAAGCATGCTAAAGGCACACC-3	Singh J and Padgett R.A., 2009 [S1]	https://pubmed.ncbi.nlm.nih.gov/19820712/
OPA1 Ex 19 Reverse: 5-TCGTATGGATGCCAAAGATTGCCAG-3	Singh J and	https://pubmed.ncbi.nlm.nih.gov/19820712/

	Padgett R.A., 2009 [S1]	
***MDC1* sequencing primers**		
MDC1 seq primer #1: 5-CACCCAGGCTATTGACTGG-3	This Paper	N/A
MDC1 seq primer #2: 5-CTACTTCCTCCTCCGAGTCC-3	This Paper	N/A
MDC1 seq primer #3: 5-CTGATGCGGAAGAAGAGAGG-3	This Paper	N/A
MDC1 seq primer #4: 5-CCACACTGTCATCACTGTCC-3	This Paper	N/A
MDC1 seq primer #5: 5-AGAGAACAACATGTGGGTGG-3	This Paper	N/A
MDC1 seq primer #6: 5-TCTGTCCTGTTTCCCCTTGG-3	This Paper	N/A
MDC1 seq primer #7: 5-ATTGCAAGATGCCACCTGC-3	This Paper	N/A
MDC1 seq primer #8: 5-GGAAGGCTGGAGCTCAAGG-3	This Paper	N/A
MDC1 seq primer #9: 5-CTGAGCTCCAGATTTCCACC-3	This Paper	N/A
MDC1 seq primer #10: 5-CGGAAGCCTGTAGCTCAGG-3	This Paper	N/A
MDC1 seq primer #11: 5-CACAGACCAGTCCGTCACC-3	This Paper	N/A
MDC1 seq primer #12: 5-ACTGTCTTCTGGGAGACTTCC-3	This Paper	N/A
MDC1 seq primer #13: 5-TGAGATCTATGTGACCCCTGG-3	This Paper	N/A

Table S2. siRNAs against *MDC1* (siMDC1), *PLRG1* (siPLRG1), and Luciferase gene (siControl). Related to all figures 1-7 and S1-S7

siRNAs		
SiMDC1 sense: 5-UCCAGUGAAUCCUUGAGGU (dTdT)-3	Lou Z., et al., 2003 [S2]	https://pubmed.ncbi.nlm.nih.gov/12607004/
SiMDC1 anti-sense: 3-ACCUCAAGGAUUCACUGGA (dTdT)-5	Lou Z., et al., 2003 [S2]	https://pubmed.ncbi.nlm.nih.gov/12607004/
SiMDC1_2 sense: 5-GUUGUAACUGAAAUCCAGC (dTdT)-3	Eliezer Y., et al., 2014 [S3]	https://pubmed.ncbi.nlm.nih.gov/24509855/
SiMDC1_2 anti-sense: 3-GCUGGAUUUCAGUUACAAC (dTdT)-5	Eliezer Y., et al., 2014 [S3]	https://pubmed.ncbi.nlm.nih.gov/24509855/
SiMDC1_3 sense: 5-AAUCCUGAGACCUCCUAAGGUUU (dTdT)-3	Goldberg M., et al., 2003 [S4]	https://pubmed.ncbi.nlm.nih.gov/12607003/
SiMDC1_3 anti-sense: 3-AAACCUUAGGAGGUCUCAGGAUU (dGdT)-5	Goldberg M., et al., 2003 [S4]	https://pubmed.ncbi.nlm.nih.gov/12607003/

SiPLRG1_1 sense: 5-UCAUAAACAGUACCCUGCCAAUCAA (dTdT)-3	Mu R., et al., 2014 [S5]	https://pubmed.ncbi.nlm.nih.gov/24675469/
SiPLRG1_1 anti-sense: 3-UUGAUUGGCAGGGUACUGUUUAUGA (dAdA)-5	Mu R., et al., 2014 [S5]	https://pubmed.ncbi.nlm.nih.gov/24675469/
SiPLRG1_2 sense: 5-CCACCGUGGAAACUCUACAGGGUUA (dTdT)-3	Eurofins Genomics	N/A
SiPLRG1_2 anti-sense: 3-UAACCCUGUGAGGUUUCCACGGUGG (dTdG)-5	Eurofins Genomics	N/A
SiGL3 (Luciferase) sense: 5-CUUACGCUGAGUACUUCGA (dTdT)-3	Lubas M., et al., 2015 [S6]	https://pubmed.ncbi.nlm.nih.gov/25578728/
SiGL3 (Luciferase) anti-sense: 3-UCGAAGUACUCAGCGUAAG (dTdT)-5	Lubas M., et al., 2015 [S6]	https://pubmed.ncbi.nlm.nih.gov/25578728/

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