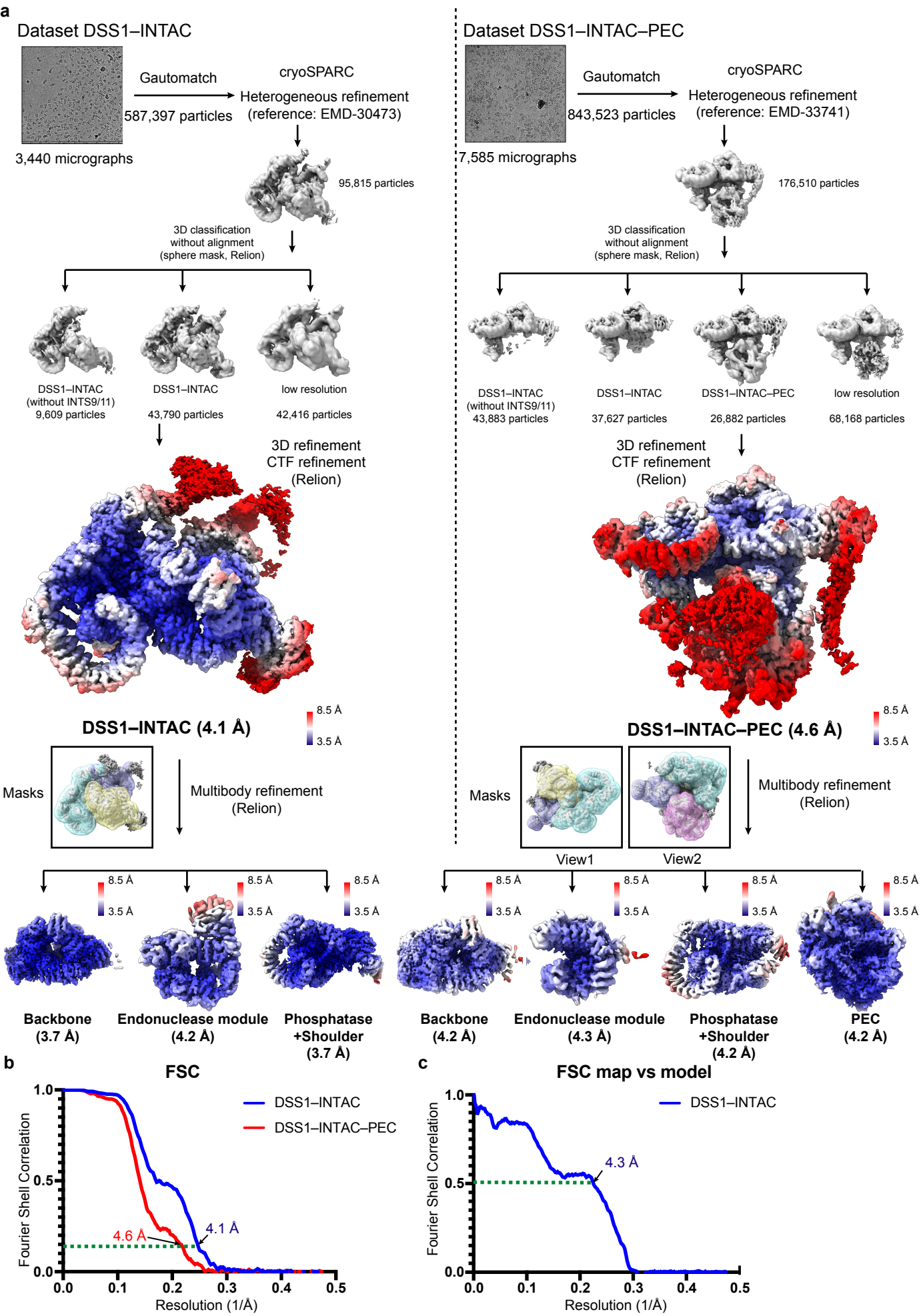
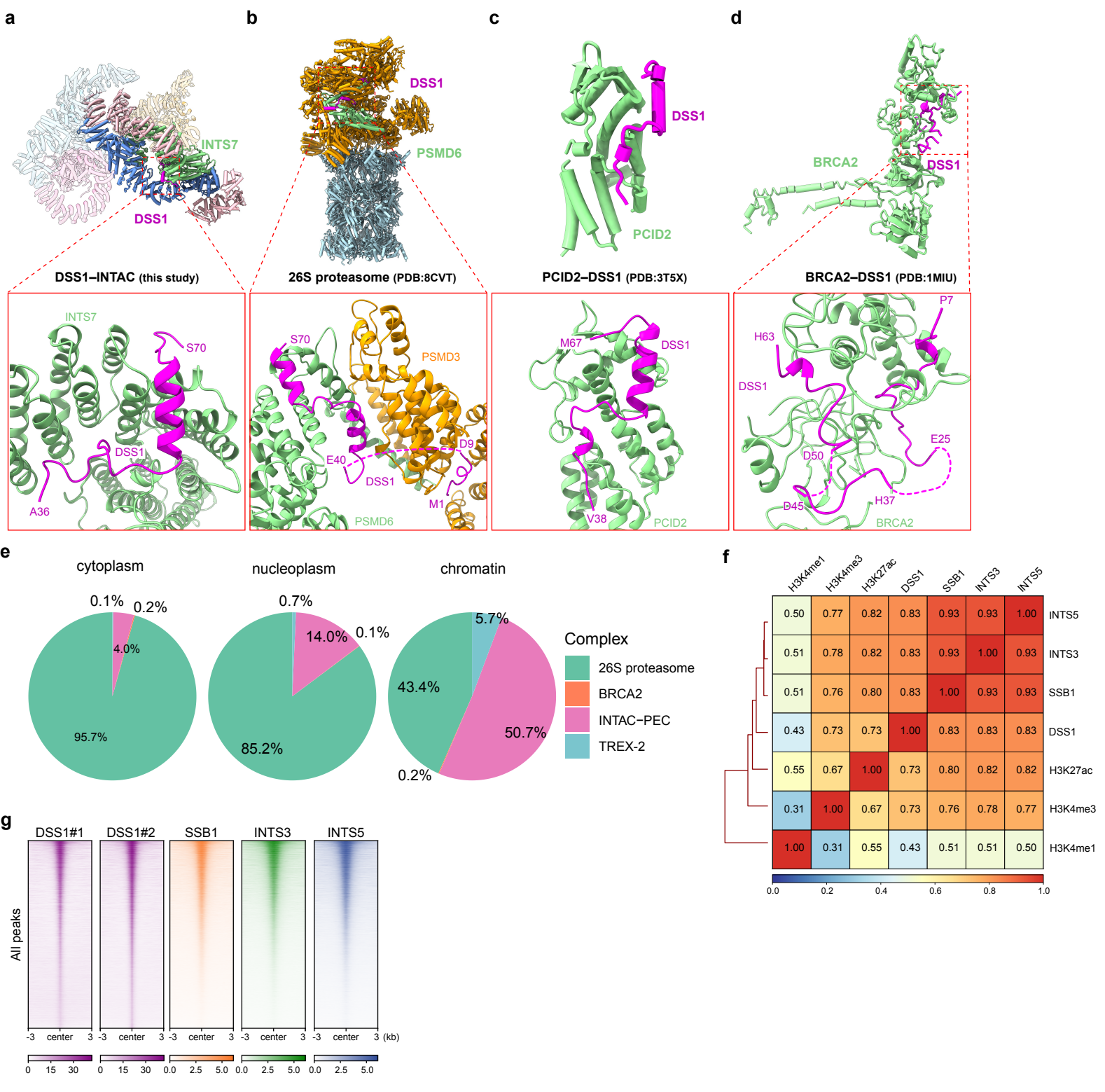
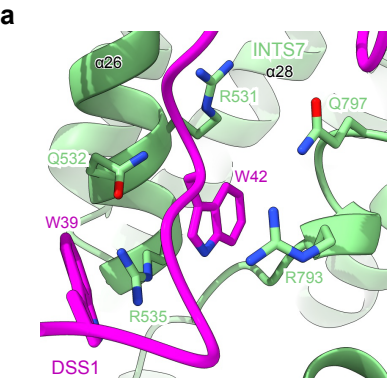
DSS1 protein is required for proper Integrator–PP2A function.

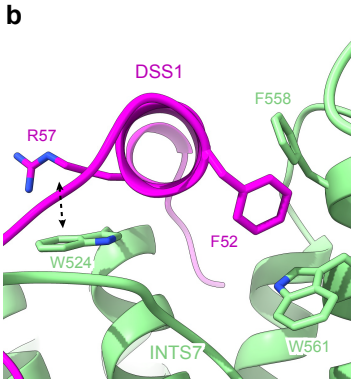

a
Dataset DSS1–INTAC
3,440 micrographs
Gautomatch
587,397 particles
cryoSPARC
Heterogeneous refinement
(reference: EMD-30473)
95,815 particles
3D classification
without alignment
(sphere mask, Relion)
DSS1–INTAC
(without INTS9/11)
9,609 particles
DSS1–INTAC
43,790 particles
low resolution
42,416 particles
3D refinement
CTF refinement
(Relion)
DSS1–INTAC (4.1 Å)
8.5 Å
3.5 Å
Masks
Multibody refinement
(Relion)
Backbone
(3.7 Å)
Endonuclease module
(4.2 Å)
Phosphatase
+Shoulder
(3.7 Å)
Dataset DSS1–INTAC–PEC
7,585 micrographs
Gautomatch
843,523 particles
cryoSPARC
Heterogeneous refinement
(reference: EMD-33741)
176,510 particles
3D classification
without alignment
(sphere mask, Relion)
DSS1–INTAC
(without INTS9/11)
43,883 particles
DSS1–INTAC
37,627 particles
DSS1–INTAC–PEC
26,882 particles
low resolution
68,168 particles
3D refinement
CTF refinement
(Relion)
DSS1–INTAC–PEC (4.6 Å)
Masks
View1
View2
Multibody refinement
(Relion)
Backbone
(4.2 Å)
Endonuclease module
(4.3 Å)
Phosphatase
+Shoulder
(4.2 Å)
PEC
(4.2 Å)
b
FSC
DSS1–INTAC
DSS1–INTAC–PEC
4.6 Å
4.1 Å
Fourier Shell Correlation
Resolution (1/Å)
c
FSC map vs model
DSS1–INTAC
4.3 Å
Fourier Shell Correlation
Resolution (1/Å)

Supplementary Fig. 1. Cryo-EM structure determination of DSS1–INTAC and DSS1–INTAC–PEC. a The sorting schemes of the cryo-EM datasets for the DSS1–INTAC sample (left of the dash line) and DSS1–INTAC–PEC sample (right of the dash line). The masks and software used during data processing are shown on the side. The final DSS1–INTAC and DSS1–INTAC–PEC maps are colored according to their local resolution distribution. The angular distributions of particles are shown as inserts. **b, c** The FSC curves (**b**) of the maps, along with the model-to-map correlation curve (**c**, applicable only to DSS1–INTAC), were calculated using Relion. The resolution was estimated using either the 0.143 or 0.5 cutoff criterion (indicated by the green line).


a
b
c
d
INTS7
DSS1
PSMD6
DSS1
DSS1
PCID2
BRCA2
DSS1
DSS1–INTAC (this study)
26S proteasome (PDB:8CVT)
PCID2–DSS1 (PDB:3T5X)
BRCA2–DSS1 (PDB:1MIU)
INTS7
S70
DSS1
A36
S70
PSMD3
E40
DSS1
D9
M1
PSMD6
M67
DSS1
V38
PCID2
P7
H63
DSS1
E25
D50
D45
H37
BRCA2
e
cytoplasm
nucleoplasm
chromatin
0.1%
0.2%
4.0%
95.7%
0.7%
14.0%
0.1%
85.2%
5.7%
43.4%
50.7%
0.2%
Complex
26S proteasome
BRCA2
INTAC−PEC
TREX-2
f
H3K4me1
H3K4me3
H3K27ac
DSS1
SSB1
INTS3
INTS5
0.50 0.77 0.82 0.83 0.93 0.93 1.00 INTS5
0.51 0.78 0.82 0.83 0.93 1.00 0.93 INTS3
0.51 0.76 0.80 0.83 1.00 0.93 0.93 SSB1
0.43 0.73 0.73 1.00 0.83 0.83 0.83 DSS1
0.55 0.67 1.00 0.73 0.80 0.82 0.82 H3K27ac
0.31 1.00 0.67 0.73 0.76 0.78 0.77 H3K4me3
1.00 0.31 0.55 0.43 0.51 0.51 0.50 H3K4me1
0.0 0.2 0.4 0.6 0.8 1.0
g
DSS1#1
DSS1#2
SSB1
INTS3
INTS5
All peaks
-3 center 3 (kb)
0 15 30
0.0 2.5 5.0

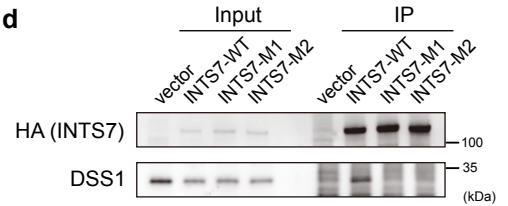
Supplementary Fig. 2. Structural comparison of DSS1-containing complexes and correlation analysis of INTAC subunits including DSS1. **a-d** Overall (top) and close-up views (bottom) of structures of DSS1-containing complexes, including DSS1–INTAC (**a**), 26S proteasome (**b**), TREX-2 components PCID2–DSS1 (**c**), and BRCA2–DSS1 (**d**). The internal missing regions of the DSS1 are connected by dash lines. **e** Pie charts showing the proportion of DSS1 interacting complexes in the cytoplasm, nucleoplasm and chromatin determined by cellular fractionation followed by immunoprecipitation and mass spectrometry analysis. **f** Correlation analysis for the genomic occupancy of DSS1, SSB1, INTS3, INTS5, H3K27ac, H3K4me3, and H3K4me1. The numbers indicate Pearson correlation coefficients. **g** Occupancy of DSS1, SSB1, INTS3, INTS5 over 6 kb regions centered on all peaks of DSS1.

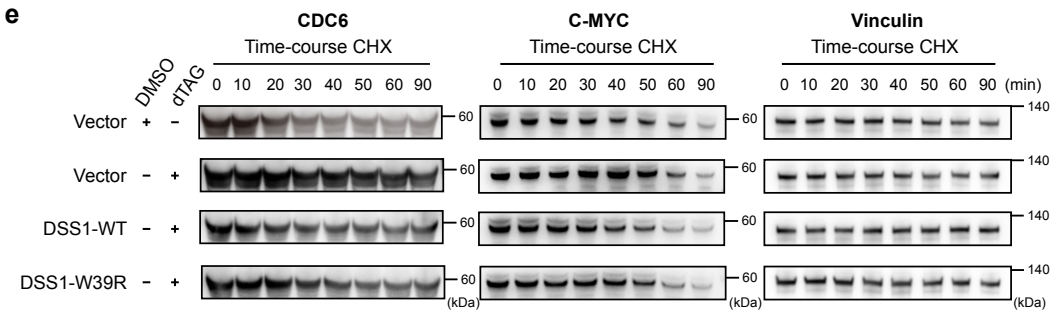


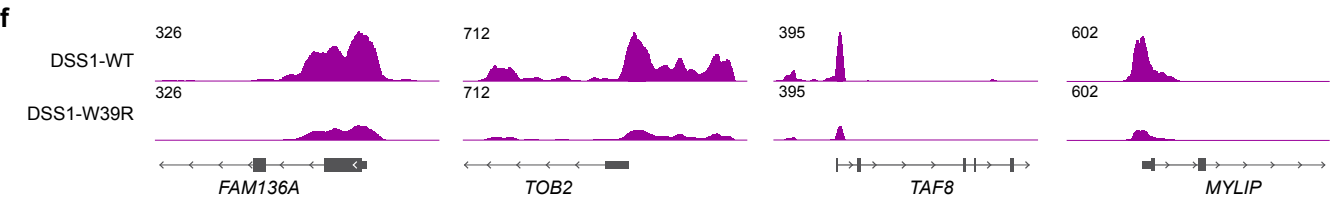


c

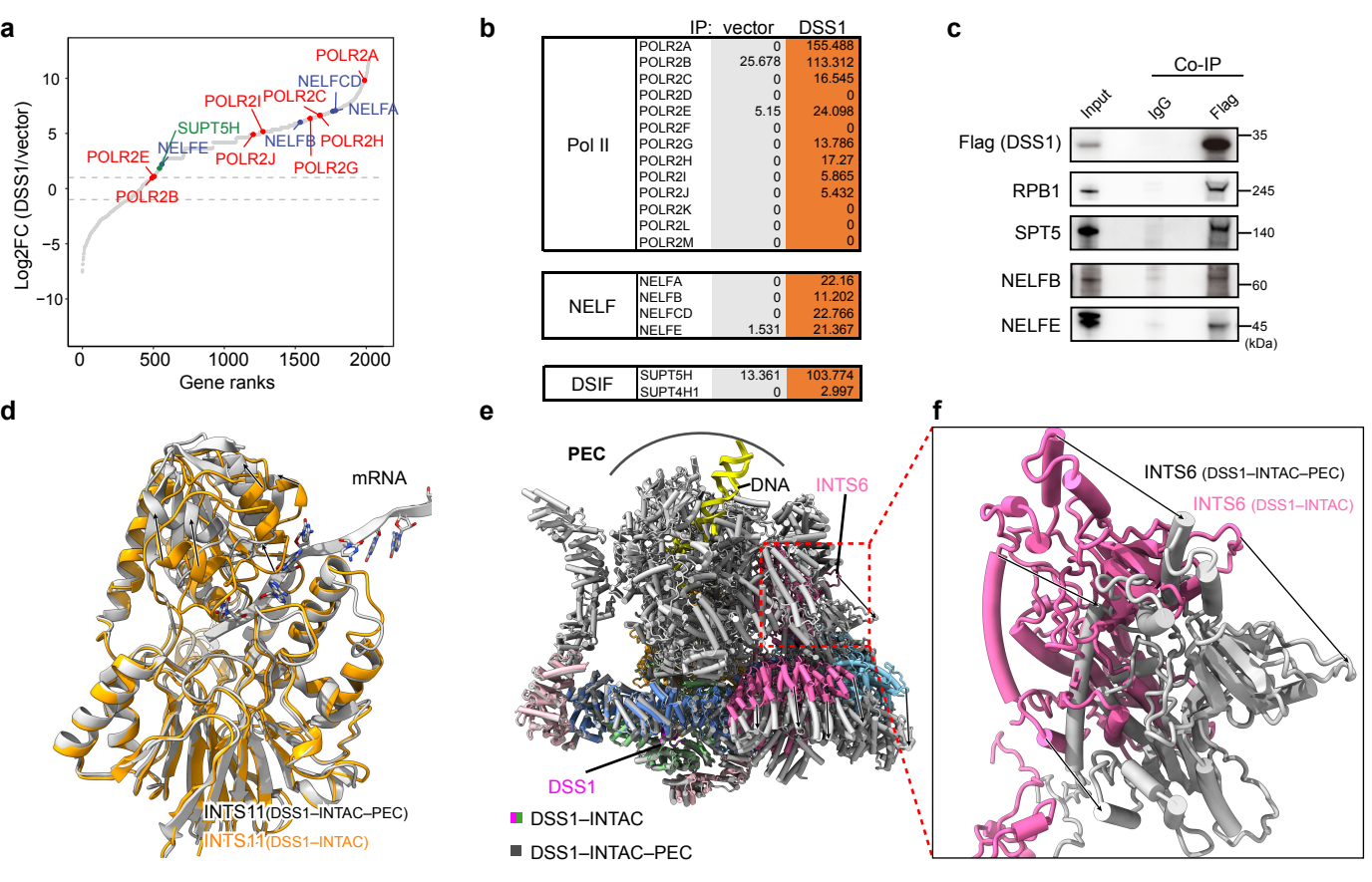
INTS7 mutants	mutation sites
# 1	W524R,F558R,W561R
# 2	R531A,Q532A,R535A,R793A, Q797A,W524R,F558R,W561R



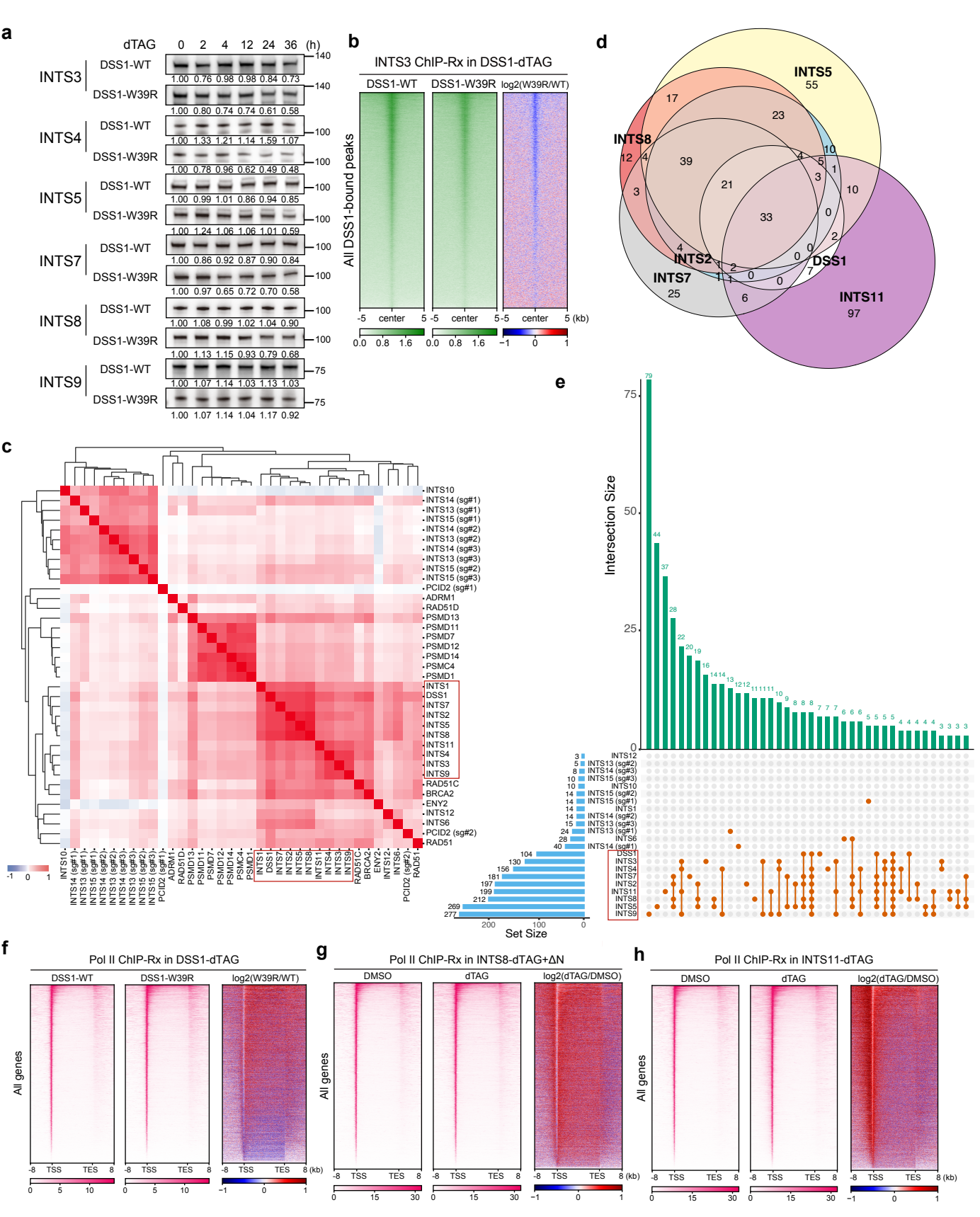




Supplementary Fig. 3. Separation of DSS1's implication in INTAC and proteasome. a, b Close-up views present a detailed interactions between DSS1 (magenta) and INTS7 (green) at two different interfaces. **c** Mutating essential residues of INTS7 for its interaction with DSS1 highlighted in **a** and **b**. **d** Co-IP analysis of HA (INTS7) in cells overexpressing wild-type INTS7 (INTS7-WT), or the two INTS7 mutants shown in **c**. Data represent three biological independent experiments with similar results. Source data are provided as a Source Data File. **e** Time-course cycloheximide (CHX) administration in the dTAG treated DSS1–Flag–dTAG cells with the overexpression of DSS1-WT, DSS1-W39R, or vector. Data represent three biological independent experiments with similar results. Source data are provided as a Source Data File. **f** Representative track examples showing the occupancy of DSS1 in dTAG-treated DSS1–Flag–dTAG cells expressing DSS1-WT or DSS1-W39R.


a
Log2FC (DSS1/vector)
Gene ranks
POLR2A
NELFCD
POLR2I
POLR2C
NELFA
SUPT5H
NELFB
POLR2H
NELFE
POLR2E
POLR2J
POLR2G
POLR2B
b
IP: vector DSS1
Pol II
POLR2A 0 155.488
POLR2B 25.678 113.312
POLR2C 0 16.545
POLR2D 0 0
POLR2E 5.15 24.098
POLR2F 0 0
POLR2G 0 13.786
POLR2H 0 17.27
POLR2I 0 5.865
POLR2J 0 5.432
POLR2K 0 0
POLR2L 0 0
POLR2M 0 0
NELF
NELFA 0 22.16
NELFB 0 11.202
NELFCD 0 22.766
NELFE 1.531 21.367
DSIF
SUPT5H 13.361 103.774
SUPT4H1 0 2.997
c
Co-IP
Input
IgG
Flag
Flag (DSS1)
RPB1
SPT5
NELFB
NELFE
35
245
140
60
45
(kDa)
d
mRNA
INTS11(DSS1–INTAC–PEC)
INTS11(DSS1–INTAC)
e
PEC
DNA
INTS6
DSS1
DSS1–INTAC
DSS1–INTAC–PEC
f
INTS6 (DSS1–INTAC–PEC)
INTS6 (DSS1–INTAC)

Supplementary Fig. 4. DSS1 associates with INTAC in complex with PEC. a Proteins identified by MS after DSS1 purification were ranked by the enrichment in purification of DSS1 versus vector, with PEC components highlighted. Pol II subunits are labeled in red. NELF subunits are labeled in blue. DSIF subunits are labeled in green. **b** The values of posterior error probability (PEP) score showing the abundance of identified PEC components. **c** Co-IP analysis of Flag (DSS1) followed by western blotting in DSS1–Flag–dTAG cells. Data represent three biological independent experiments with similar results. **d** Overlaying comparison of INTS11 from the DSS1–INTAC (orange) and DSS1–INTAC–PEC (gray) structures. **e** Overlaying comparison of INTAC from the DSS1–INTAC (colored) and DSS1–INTAC–PEC (gray) structures. The alignment is based on INTS1, INTS2, and INTS7. Source data are provided as a Source Data File. **f** Close-up view of INTS6 from the DSS1–INTAC (pink) and DSS1–INTAC–PEC (gray) structures.


a
dTAG 0 2 4 12 24 36 (h)
INTS3
DSS1-WT
1.00 0.76 0.98 0.98 0.84 0.73
DSS1-W39R
1.00 0.80 0.74 0.74 0.61 0.58
INTS4
DSS1-WT
1.00 1.33 1.21 1.14 1.59 1.07
DSS1-W39R
1.00 0.78 0.96 0.62 0.49 0.48
INTS5
DSS1-WT
1.00 0.99 1.01 0.86 0.94 0.85
DSS1-W39R
1.00 1.24 1.06 1.06 1.01 0.59
INTS7
DSS1-WT
1.00 0.86 0.92 0.87 0.90 0.84
DSS1-W39R
1.00 0.97 0.65 0.72 0.70 0.58
INTS8
DSS1-WT
1.00 1.08 0.99 1.02 1.04 0.90
DSS1-W39R
1.00 1.13 1.15 0.93 0.79 0.68
INTS9
DSS1-WT
1.00 1.07 1.14 1.03 1.13 1.03
DSS1-W39R
1.00 1.07 1.14 1.04 1.17 0.92
140
140
100
100
100
100
100
100
75
75
b
INTS3 ChIP-Rx in DSS1-dTAG
DSS1-WT
DSS1-W39R
log2(W39R/WT)
All DSS1-bound peaks
-5 center 5
-5 center 5
-5 center 5 (kb)
0.0 0.8 1.6
0.0 0.8 1.6
−1 0 1
d
INTS5
55
INTS8
12
INTS2
25
DSS1
97
INTS7
25
INTS11
97
17
23
4
39
10
5
1
3
21
10
3
33
0
2
4
0
0
2
1
1
1
0
0
7
6
e
Intersection Size
79
44
37
28
22
20
19
16
14
14
13
12
12
11
11
11
11
10
9
8
8
8
7
7
7
6
6
6
5
5
5
5
4
4
4
4
4
3
3
3
3
75
50
25
0
INTS12
INTS13 (sg#2)
INTS14 (sg#3)
INTS15 (sg#3)
INTS10
INTS15 (sg#2)
INTS15 (sg#1)
INTS1
INTS14 (sg#2)
INTS13 (sg#3)
INTS13 (sg#1)
INTS6
INTS14 (sg#1)
DSS1
INTS3
INTS4
INTS7
INTS2
INTS11
INTS8
INTS5
INTS9
3
5
8
10
10
14
14
14
14
15
24
28
40
104
130
156
181
197
199
212
269
277
200
100
0
Set Size
c
INTS10
INTS14 (sg#1)
INTS13 (sg#1)
INTS15 (sg#1)
INTS14 (sg#2)
INTS13 (sg#2)
INTS14 (sg#3)
INTS13 (sg#3)
INTS15 (sg#2)
INTS15 (sg#3)
PCID2 (sg#1)
ADRM1
RAD51D
PSMD13
PSMD11
PSMD7
PSMD12
PSMD14
PSMC4
PSMD1
INTS1
DSS1
INTS7
INTS2
INTS5
INTS8
INTS11
INTS4
INTS3
INTS9
RAD51C
BRCA2
ENY2
INTS12
INTS6
PCID2 (sg#2)
RAD51
-1 0 1
f
Pol II ChIP-Rx in DSS1-dTAG
DSS1-WT
DSS1-W39R
log2(W39R/WT)
All genes
-8 TSS TES 8
-8 TSS TES 8
-8 TSS TES 8 (kb)
0 5 10
0 5 10
−1 0 1
g
Pol II ChIP-Rx in INTS8-dTAG+ΔN
DMSO
dTAG
log2(dTAG/DMSO)
All genes
-8 TSS TES 8
-8 TSS TES 8
-8 TSS TES 8 (kb)
0 15 30
0 15 30
−1 0 1
h
Pol II ChIP-Rx in INTS11-dTAG
DMSO
dTAG
log2(dTAG/DMSO)
All genes
-8 TSS TES 8
-8 TSS TES 8
-8 TSS TES 8 (kb)
0 15 30
0 15 30
−1 0 1

Supplementary Fig. 5. DSS1 is required for INTAC function in transcription. **a** Western blotting of INTAC subunits in DSS1-WT or DSS1-W39R overexpressed DSS1-dTAG cell line with time course treatment of $dTAG^V$-1. Data represent three biological independent experiments with similar results. Source data are provided as a Source Data File. **b** Heatmaps showing ChIP-Rx signals of INTS3 over 10 kb regions centered on all DSS1-bound peaks after endogenous DSS1 degradation in DSS1–Flag–dTAG cells with the overexpressing of DSS1-WT or DSS1-W39R. **c** Perturb-seq correlation analysis of gene expression levels in DSS1 and its interacting complex subunits (INTAC, 26S proteasome, BRCA2, TREX-2) knockout cells. **d** Venn diagram analysis of differentially expressed genes caused by knockdown of INTAC subunits including INTS2, INTS7, INTS8, INTS5, INTS11 and DSS1. **e** UpSet plot displays differentially expressed genes (DEGs) set by depletion of INTAC subunits. Left bars: Number of DEGs by individual INTAC subunits perturbation, Top bars: Size of DEGs intersections across multiple depleted INTAC subunit combinations. **f** Heatmaps showing Pol II ChIP-Rx signals of all genes in DSS1–Flag–dTAG cells overexpressing DSS1-WT or DSS1-W39R. **g, h** Heatmaps showing Pol II ChIP-Rx signals of all genes in INTS8-dTAG+ΔN (**g**) and INTS11-dTAG cells (**h**) treated with DMSO or $dTAG^V$-1.