Silencing of IFN-stimulated gene transcription is regulated by histone H1 and its chaperone TAF-I

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Supplementary Figure Legends

Supplementary Figure S1. TAF-I negatively regulates ISG transcription. (A) Expression level of TAF-I in TAF-I KD cells. HeLa S3 cells were transfected with siRNA specific for TAF-I (siTAF-I, lane 4) or negative control siRNA (siNC, lanes 1-3). Cell lysates were subjected to western blot analysis using anti-TAF-I and anti-β-actin antibodies. (B) Effects of TAF-I KD in ISG transcription. Total RNA was prepared from siNC- and siTAF-I-transfected cells treated with or without IFN-β for 3 hours, and subjected to qRT-PCR using specific primer sets for each ISG mRNAs and GAPDH mRNA. The amount of ISG mRNA was normalized as a relative amount of GAPDH mRNA. Error bars represents standard deviation (n ≥ 3). *P < 0.05, **P < 0.001 by two-tail paired Student’s t-test (n = 8). (C) Effect of TAF-I KD in time-dependent manner. HeLa S3 cells were transfected with EGFP siRNA expressing vector used as a control (siCont) or with TAF-I siRNA expressing vector (siTAF-I). Total RNA was prepared from siCont- and siTAF-I-transfected cells treated with or without IFN-β for indicated periods, and subjected to qRT-PCR using specific primer sets for ISG56 mRNAs, IFITM1 mRNA, and GAPDH mRNA. The amount of ISG mRNA was normalized as a relative amount of GAPDH mRNA. Error bars represents standard deviation (n ≥ 3). The amount of ISG mRNA in TAF-I KD cells relative to that of siCont cells was shown in right panel of (C).
Supplementary Figure S2.  TAF-I regulates the amounts of transcription factors and histone H1 on ISG promoters.  (A) Promoter binding of the transcriptional factors in TAF-I KD cells.  siCont- and siTAF-I-transfected cells were treated with or without IFN-β for indicated periods, and cell lysates were subjected to ChIP assays using antibodies specific for STAT1 (left), STAT2 (middle), and Pol II (right) followed by qRT-PCR using specific primer sets for the ISG56 and the IFITM1 promoters.  The amount of DNA co-immunoprecipitated with each antibody was shown as % of input.  Error bars represents standard deviation (n ≥ 3).  *P < 0.01, **P < 0.001 by two-tail paired Student’s t-test (n = 6).  (B) Decrease of histone H1 levels on ISG promoters in TAF-I KD cells.  Cells were prepared as shown in (A), and cell lysates were subjected to ChIP assays using antibodies specific for histone H3 (H3), acetylated histone H3 (H3K9/14Ac), and histone H1.2 (H1) followed by qRT-PCR using specific primer sets for the ISG56 and the IFITM1 promoters.  The amount of DNA co-immunoprecipitated with each antibody was shown as % of input.  Error bars represents standard deviation (n ≥ 3).  *P < 0.05, **P < 0.01, ***P < 0.001 by two-tail paired Student’s t-test (n = 6).

Supplementary Figure S3.  Histone H1.2 negatively regulates ISG transcription.  (A) Expression level of histone H1.2 in histone H1 KD cells.  HeLa S3 cells were transfected with siRNA specific for histone H1.2 (siH1.2, lane 4) or negative control siRNA (siNC, lanes 1-3).  Cell lysates were subjected to western blot analysis using anti-histone H1.2 and anti-β-actin antibodies.  (B) Effects of histone H1.2 KD on ISG transcription.  Total RNA was prepared from siNC- and siH1.2-transfected cells treated with or without IFN-β for 3 hours and subjected
to qRT-PCR using specific primer sets for each ISG mRNA and GAPDH mRNA. The amount of ISG mRNA was normalized as a relative amount of GAPDH mRNA. Error bars represents standard deviation (n ≥ 3). *P < 0.05, **P < 0.01, ***P < 0.001 by two-tail paired Student’s t-test (n = 6).

Supplementary Figure S4. Involvement of TAF-I and histone H1 in the chromatin structure of ISG promoter regions. The MNase protection assay for ISG promoter regions in TAF-I KD and H1 KD cells was carried out. MNase-digested DNA was prepared as shown in Figure 5A and subjected to qRT-PCR using specific primer sets for ISG56, IFITM1, and GAPDH promoter regions. The amounts of ISG promoter region DNAs were normalized by the amount of the GAPDH promoter region DNA, and shown as a relative amount to that from IFN-untreated siCont cells. Error bars represents standard deviation (n ≥ 3). *P < 0.001, **P < 0.0001 by two-tail paired Student’s t-test (n = 9).

Supplementary Figure S5. Dissociation of TAF-I from ISG promoters. (A) Effects of TSA treatment on binding of histone H1 to ISG promoters. HeLa S3 cells were treated without (lane 1) or with 0.1 (lane 2) or 1 μM (lane 3) of TSA for 1 hour and subjected to ChIP assays using antibodies specific for histone H3 (H3), acetylated histone H3 (H3K9/14Ac), and histone H1 (H1) followed by qRT-PCR using specific primer sets for ISG56 and IFITM1 promoters. The amount of DNA co-immunoprecipitated with each antibody was shown as % of input. Error bars represents standard deviation (n ≥ 3). *P < 0.001, **P < 0.0001 by two-tail paired Student’s t-test (n = 6). The amount of H3K9/14Ac relative to that of H3 is shown in
H3K9/14Ac/H3 (4th panel).  (B) Effect of TSA treatment on binding of TAF-I to *ISG* promoters.  HeLa S3 cells were transfected with TAF-I siRNA expressing vectors (lanes 1-4) together with empty vector (lane 4) or Flag-tagged TAF-Iα expressing vector (lanes 1-3), and were treated without (lane 1) or with 0.1 (lane 2) or 1 µM (lane 3) of TSA for 1 hour and subjected to ChIP assays using the agarose-conjugated antibody against Flag followed by qRT-PCR using specific primer sets for the *ISG56* and the *IFITM1* promoters.  The amount of DNA co-immunoprecipitated with antibody was shown as % of input.  Error bars represents standard deviation (n ≥ 3).  *P < 0.001 by two-tail paired Student’s *t*-test (n = 6).

**Supplementary Figure S6.** TAF-I and histone H1 negatively regulate *IL8* gene transcription.  (A) Effects of TAF-I KD and histone H1 KD in *IL8* transcription.  Total RNA was prepared from siCont- (lanes 1 and 4), siTAF-I- (lanes 2 and 5) and siH1- (lanes 3 and 6) transfected cells and subjected to qRT-PCR using specific primer sets for *IL8* mRNAs, *IκBα* mRNA, and *GAPDH* mRNA.  The amount of each mRNA was normalized as a relative amount of *GAPDH* mRNA.  Error bars represents standard deviation (n ≥ 3).  *P < 0.01, **P < 0.0001 by two-tail paired Student’s *t*-test (n = 4).  (B) Decrease of the histone H1 level on the *IL8* promoter in TAF-I KD cells.  Cell lysates prepared from siCont- and siTAF-I-transfected cells were subjected to ChIP assays using an antibody specific for histone H1.2 (H1) followed by qRT-PCR using specific primer sets for the *IL8* and *IκBα* promoters.  The amount of DNA co-immunoprecipitated with each antibody was shown as % of input.  Error bars represents standard deviation (n ≥ 3).  *P < 0.0001 by two-tail paired Student’s *t*-test (n = 4).
Kadota, S. et al. Supplementary Figure S1

A

Protein amounts

\( \frac{1}{4} \) 1/2 1 1

\( \beta \)-Actin

TAF-I

B

\si{siNC} \si{TAF-I}

Relative mRNA amounts

ISG56

\( * \)

\( ** \)

IFN (3 h)

- +

1 2 3 4

ISG54

ISG15

C

Relative amounts of ISG mRNA (log_{10})

\si{siCont} \si{TAF-I}

The amounts of ISG mRNA relative for GAPDH mRNA

ISG56

IFITM1

IFN (h)

- 1 3 6

- 1 3 6
Kadota, S. et al. Supplementary Figure S2

A

![Bar charts showing the expression levels of STAT1, STAT2, and Pol II for ISG56 and IFITM1 promoters with siCont and siTAF-I treatments over time (IFN h).](image)

B

![Bar charts showing the expression levels of H3, H3K9/14Ac, and H1 for ISG56 and IFITM1 promoters with siCont and siTAF-I treatments over time (IFN h).](image)
Kadota, S. et al. Supplementary Figure S3

A

siH1.2
Protein amounts 1/4 1/2 1 1
H1.2
β-Actin 1 2 3 4

B

siNC siH1.2

Relative mRNA amounts

0 0.5 1 1.5 2 2.5 3

ISG56
ISG56
ISG15
ISG15

0 0.5 1 1.5 2 2.5 3

ISG54
ISG54
IFITM1
IFITM1

0 0.5 1 1.5 2 2.5 3

IFN (3 h) - + - + - +
Kadota, S. et al. Supplementary Figure S4

Relative ISG pro. amounts (Normalized with GAPDH pro.)

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siCont, siTAF-I, siH1
Kadota, S. et al. Supplementary Figure S6

A

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Relative amounts

**

B

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% of input

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