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III. 研究成果の刊行物・別刷

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Long Noncoding RNA NEAT1-Dependent SFPQ Relocation from Promoter Region to Paraspeckle Mediates IL8 Expression upon Immune Stimuli

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SUMMARY

Although thousands of long noncoding RNAs (lncRNAs) are localized in the nucleus, only a few dozen have been functionally characterized. Here we show that nuclear enriched abundant transcript 1 (NEAT1), an essential lncRNA for the formation of nuclear body paraspeckles, is induced by influenza virus and herpes simplex virus infection as well as by Toll-like receptor3-p38 pathway-triggered poly I:C stimulation, resulting in excess formation of paraspeckles. We found that NEAT1 facilitates the expression of antiviral genes including cytokines such as interleukin-8 (IL8). We found that splicing factor proline/glutamine-rich (SFPQ), a NEAT1-binding paraspeckle protein, is a repressor of *IL8* transcription, and that NEAT1 induction relocates SFPQ from the *IL8* promoter to the paraspeckles, leading to transcriptional activation of *IL8*. Together, our data show that NEAT1 plays an important role in the innate immune response through the transcriptional regulation of antiviral genes by the stimulus-responsive cooperative action of NEAT1 and SFPQ.

INTRODUCTION

Whole-transcriptome analyses have revealed that a new class of non-protein-coding transcripts, designated as long noncoding RNAs (lncRNAs), is transcribed from a large proportion of the mammalian genome (Carninci et al., 2005; Guttman et al., 2009; Kapranov et al., 2007). There is increasing evidence of lncRNA involvement in diverse biological processes (Chen and Carmichael, 2010; Gupta et al., 2010; Ponting et al., 2009; Yoon et al., 2013). Moreover, a large number of lncRNAs is induced by extracellular stimuli, suggesting that lncRNAs participate in stress responses (Mizutani et al., 2012; Tani et al., 2012). In addition, because lncRNAs are also implicated in many human diseases (Huarte and Rinn, 2010; Wang and Chang, 2011), understanding the precise molecular mechanisms by which lncRNAs function could prove important for developing new strategies for early diagnosis and molecular therapy. In particular, there are several emerging hypotheses on lncRNA involvement in infectious diseases (Scaria and Pasha, 2012). However, a mechanistic understanding of the role of lncRNAs in infection is limited. Hence, the functions of lncRNAs in host antiviral response have remained unclear.

The mammalian nucleus is highly organized and contains distinct structural components comprising approximately ten types of nuclear bodies, including speckles and paraspeckles, which are thought to be involved in gene regulation (Mao et al., 2011). Some of these nuclear bodies contain specific lncRNAs that regulate nuclear body function (Kapranov et al., 2007; Prasanth and Spector, 2007). Recent reports have suggested that crosstalk between architectural features of nuclear bodies and lncRNAs contributes to the precise control of gene expression. For example, speckles contain the metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), a lncRNA involved in regulating the expression of several specific genes (Bernard et al., 2010; Miyagawa et al., 2012; Tano et al., 2010; Yang et al., 2011). Paraspeckles contain another lncRNA, NEAT1, which is an essential architectural component of paraspeckle

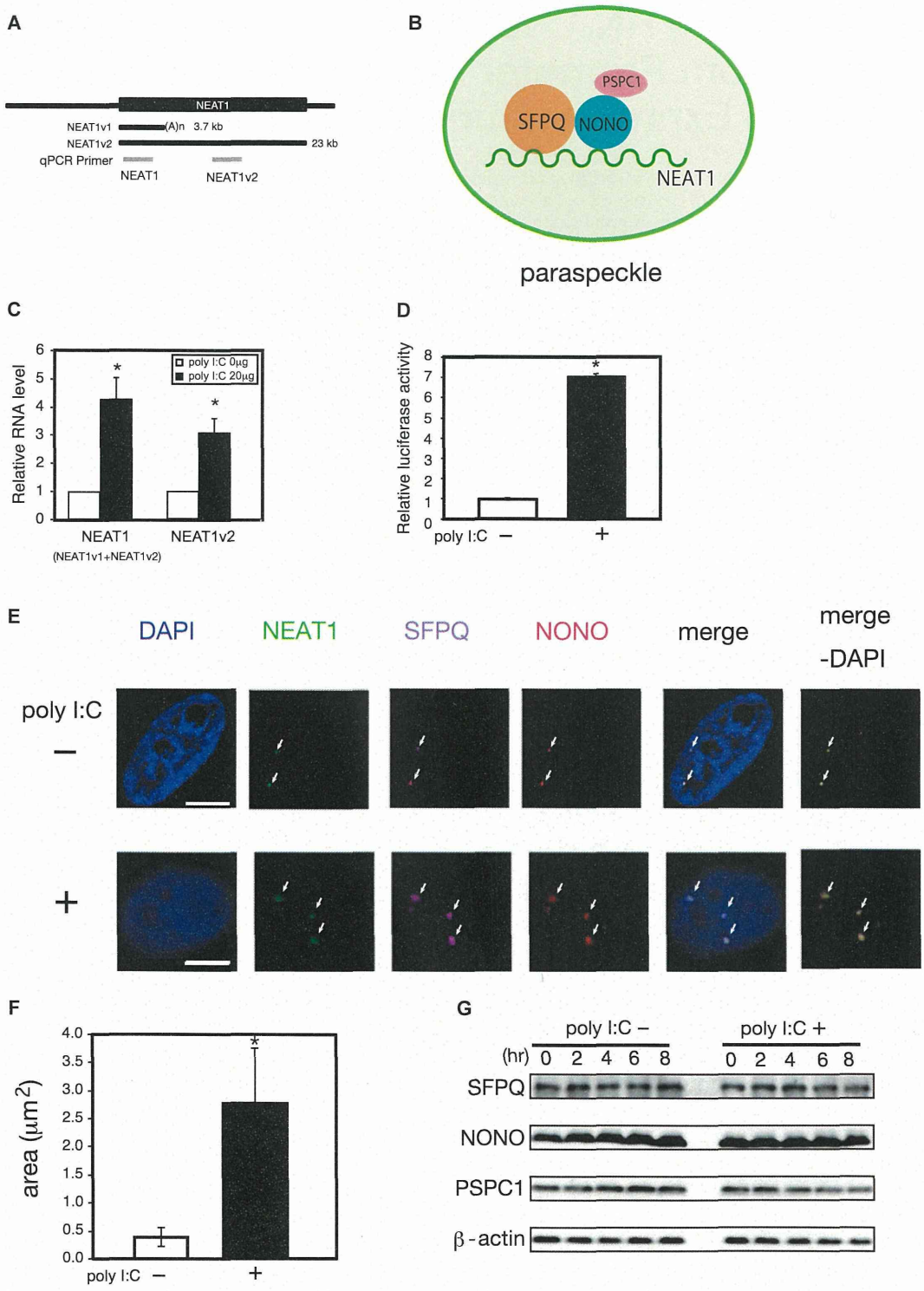

Figure 1. NEAT1 Induction and Excessive Formation of Paraspeckles by Poly I:C

(A) NEAT1 isoforms are shown schematically. The fragment positions amplified by the RT-qPCR primers are shown below.

(B) Entities of paraspeckles are shown schematically.

(legend continued on next page)

structure (Chen and Carmichael, 2009; Clemson et al., 2009; Sasaki et al., 2009; Sunwoo et al., 2009). The *NEAT1* gene (Figure 1A) produces two isoforms, 3.7 kb NEAT1v1 and 23 kb NEAT1v2 (Hutchinson et al., 2007). The effect of NEAT1v2 on the formation of paraspeckles is stronger than that of NEAT1v1 (Naganuma et al., 2012; Sasaki et al., 2009). Paraspeckles have been proposed to control several biological processes, including stress response and cellular differentiation, through control of the nuclear retention of mRNAs containing inverted repeats that form double-stranded RNA regions subject to adenosine-to-inosine editing (Fox and Lamond, 2010; Nakagawa and Hirose, 2012). Paraspeckles contain several protein factors: NONO/p54nrb, SFPQ/PSF, PSPC1, RBM14, and CPSF6 (Fox and Lamond, 2010). Among these, SFPQ and NONO form the heterodimer (Peng et al., 2002), which binds directly to NEAT1 (Sasaki et al., 2009) (Figure 1B). Several studies have demonstrated that SFPQ represses the transcription of several genes through direct promoter binding (Iacobazzi et al., 2005; Song et al., 2004; Urban et al., 2000). Recently, 35 proteins were added into the list of paraspeckle proteins (Naganuma and Hirose, 2013). Several of paraspeckle proteins are likely to be the factors involved in transcriptional control, suggesting that paraspeckles may integrate tightly coupled transcription and posttranscriptional events.

The innate immune response is crucial in the host cellular response to viral infection. Several pathogen-associated molecular pattern recognition receptors, such as the Toll-like receptors, sense the presence of viral molecules and trigger a robust program of gene expression involving the production of antiviral inflammatory cytokines, chemokines, and interferons through numerous transcriptional and posttranscriptional strategies (Arpaia and Barton, 2011; Rathinam and Fitzgerald, 2011; Thompson et al., 2011). For example, poly I:C, a double-stranded RNA (dsRNA)-mimicking immunostimulant that simulates viral infections, activates the TLR3-mediated signaling pathway, and consequently induces a set of antiviral genes (Kawai and Akira, 2010). To achieve the proper immune response, the transcriptional induction of immune response genes is highly coordinated by activators and repressors. For instance, the interleukin-8 (*IL8*) promoter is repressed by the binding of three factors in unstimulated cells (Hoffmann et al., 2002): NF-κB-repressing factor (NRF), octamer-1 (OCT-1), and deacetylation of histone proteins by histone deacetylase-1. When the cells are stimulated, NF-κB and C/EBP bind to the *IL8* promoter; C/EBP displaces OCT-1, whereas NRF switches its function to act as a coactivator. Recruitment of CREB-binding protein/p300 hyperacetylates the histones and remodels the chromatin, resulting in transcriptional activation of *IL8* gene. Although nuclear lncRNAs represent a large class of transcriptional units, the interplay between transcription factors and nuclear lncRNA to control gene expression during immune response remains to be elucidated.

RESULTS

Poly I:C Induces NEAT1 and Large Paraspeckle Formation

A previous study showed that NEAT1 is an inducible lncRNA in mice brains infected with Japanese encephalitis or rabies viruses, although it is unclear whether NEAT1 induction is a consequence of direct effect of viral infection to neural cells (Saha et al., 2006). This observation provided the rationale for the current study, which examined the relevance of NEAT1 in cellular response to viral infection. We therefore initially examined the expression levels of NEAT1 in response to transfection with poly I:C, a double-stranded RNA (dsRNA). As shown in Figure 1A, one primer set recognizes both NEAT1v1 and NEAT1v2 (total NEAT1), while the other recognizes only NEAT1v2. Expression levels of total NEAT1 and NEAT1v2 in HeLa cells and A549 cells were increased by poly I:C, but not by poly I or poly C alone (Figure 1C and Figures S1A–S1C). Treatment of the cells with either IFN-α or IFN-β induced 2′5′-OAS, an interferon response gene, but not NEAT1v2 (Figure S1D), ruling out the possibility of an indirect effect by which IFNs induced by poly I:C lead the expression of NEAT1. To examine whether upregulation of NEAT1 RNA levels by poly I:C stimulation was controlled by transcriptional regulation, we analyzed luciferase reporter activity in HeLa TO cells transfected with a luciferase reporter gene linked to a *NEAT1* promoter and found that poly I:C treatment enhanced the luciferase reporter activity (Figure 1D and Figure S1E). Next, we investigated the signaling pathway that activates transcription of the *NEAT1* gene by poly I:C stimulation. Because TLR3 is known as an intracellular sensor for dsRNAs such as poly I:C (Kawai and Akira, 2010), we tested the involvement of TLR3 in the poly I:C-induced transcriptional activation of the *NEAT1* gene. Knockdown of TLR3 reduced the levels of poly I:C-mediated NEAT1 induction compared with control cells (Figure S1H). We further examined other dsRNA sensors. We found that depletion of MDA-5, but not RIG-I, affected poly I:C-induced NEAT1 expression (Figures S1I and S1J). The effect of MDA-5 depletion for the reduction in poly I:C-induced NEAT1 expression was weaker than that for the reduction in TLR3, suggesting that TLR3 is the major receptor for inducing NEAT1 in response to poly I:C. TLR3-mediated signaling is branched to either the

(C) Total NEAT1 and NEAT1v2 levels of HeLa TO cells with or without poly I:C stimulation were quantified by RT-qPCR. The GAPDH mRNA level was used as the normalizing control. Values represent the mean ± SD (*$p < 0.01$, Student's t test).

(D) The luciferase reporter activity of HeLa TO cells transfected with a luciferase reporter gene harboring the *NEAT1* promoter was measured in the presence or absence of poly I:C. The activity of cotransfected pCMV-RL (Promega) was used as normalizing control. Values represent the mean ± SD (*$p < 0.01$, Student's t test).

(E) HeLa TO cells were transfected with and without poly I:C, followed by FISH staining and immunostaining. NEAT1 (green), SFPQ (magenta), NONO (red), and nuclei stained with DAPI (blue) are shown.

(F) The mean size of NEAT1 control cell foci (white bar; n = 50) and that of cells transfected with poly I:C (black bar; n = 50) was determined by FISH. Values represent the mean ± SD (*$p < 0.01$, Student's t test).

(G) The protein levels of paraspeckle proteins SFPQ, NONO, and PSPC1 were analyzed by western blotting at the indicated time points posttransfection with poly I:C. β-actin was used as the loading control.

p38 or JNK pathways (Arpaia and Barton, 2011). Pretreatment with ML3403, a p38 inhibitor, but not SP600125, a JNK inhibitor, abolished poly I:C-induced NEAT1 induction (Figure S1K). As expected, poly I:C-induced phosphorylation of p38 and JNK was eliminated by ML3403 and SP600125, respectively (Figures S1L and S1M). In contrast, NF-κB was not required for poly I:C-induced NEAT1 induction (Figures S1N and S1O). These results suggest that poly I:C leads to the transcriptional activation of the *NEAT1* gene mainly through the TLR3-p38 pathway.

Previous reports have shown that NEAT1 is an essential core component for the formation of paraspeckles (Chen and Carmichael, 2009; Clemson et al., 2009; Sasaki et al., 2009; Sunwoo et al., 2009). Corresponding with previous observation, paraspeckle proteins were dispersed to the nucleoplasm in the absence of NEAT1 (Figure S1Q). Because overexpression of NEAT1 results in the excess formation of paraspeckles (Clemson et al., 2009), we hypothesized that poly I:C stimulation induces this process. Combination staining of NEAT1 and of paraspeckle proteins SFPQ, NONO, and PSPC1 showed that poly I:C treatment resulted in excess paraspeckle formation in HeLa cells (Figures 1E and 1F and Figure S1P). Western blot analysis revealed that expression levels of paraspeckle proteins SFPQ and NONO remained unaltered throughout poly I:C stimulation (Figure 1G). In the absence of NEAT1, poly I:C stimulation did not induce the formation of paraspeckles (Figure S1Q). Fluorescence recovery after photobleaching (FRAP) analysis showed that the kinetics of paraspeckle-associated SFPQ in poly I:C-stimulated cells ($t_{1/2}$ = 7.08 s) was similar to that in naive cells ($t_{1/2}$ = 6.75 s) (Figures S1R and S1S; Movies S1 and S2), suggesting that the molecular quality of SFPQ was not changed by poly I:C stimulation. These findings suggest that poly I:C stimulation relocates paraspeckle proteins from the nucleoplasm to NEAT1, consequently inducing the excess formation of paraspeckles.

Identification of NEAT1-Regulated Antiviral Genes

We investigated whether NEAT1 induction followed by excess formation of paraspeckles was involved in poly I:C-inducible gene expression (Figures 2A and 2B). Microarray analysis revealed 1,232 poly I:C-inducible genes in HeLa TO cells. The induction of 259 of these poly I:C-inducible genes was abolished by NEAT1 knockdown (Figure 2B). We also identified 113 genes that were upregulated by solo overexpression of mNeat1v2 (Figure 2B). To eliminate false positives, we selected the 85 genes that form the overlap between these two groups of genes (Figure 2B). Interestingly, many antiviral factors, such as IL8 and CCL5, and virus sensors, such as RIG-I and MDA5, were identified in this group of 85 NEAT1-regulated genes, suggesting that NEAT1 is involved in the regulation of antiviral gene expression response. A gene ontology analysis using these data supported this idea (Tables S1 and S2). RT-qPCR experiments confirmed the NEAT1-dependent expression of genes involved in antiviral function, such as *IL8* and *CCL5* (Figure 2C and Table S3). To clarify whether NEAT1v2 is necessary for IL8 mRNA induction and excess paraspeckle formation, we specifically silenced NEAT1v2 using specific siRNAs (Figures S2A, S2B, and S2E) and found that NEAT1v2 depletion eliminated the induction of IL8 mRNA and excess formation of paraspeckles in response to poly I:C treatment (Figures S2C and S2D). mNeat1v2 is more active than mNeat1v1 in the formation of paraspeckles (Figure S2F). Corresponding to these findings, the effect of mNeat1v2 on gene induction was greater than that of mNeat1v1 (Figure 2E). The induction of IL8 mRNA and the size of the paraspeckles were correlated with the levels of mNeat1v2 overexpression (Figures S2G–S2I). Interestingly, NEAT1 knockdown affected the time point at which peak levels of IL8 mRNA induction were observed following poly I:C treatment (Figure 2D; 5 hr poststimulation in control cells; 3 hr poststimulation in NEAT1 knockdown cells), suggesting that NEAT1 also affects the kinetics of IL8 mRNA induction. The expression of IFN-β, a non-NEAT1-regulated gene, was not affected by the expression level of NEAT1 (Figures 2C and 2E).

Cooperative Action of NEAT1 and SFPQ Regulates *IL8* Transcription

Since paraspeckles contain many transcriptional regulators, such as SFPQ and NONO, it is reasonable to assume that excess formation of paraspeckles would affect the expression of a subset of genes. From this viewpoint, we assumed that certain paraspeckle proteins should regulate the expression of NEAT1-regulated genes such as *IL8*. As expected, we found that the expression of IL8, but not IFN-β, was increased in both SFPQ and NONO knockdown cells, but not in PSPC1 knockdown cells (Figure 3A and Figures S3A and S3L). Both SFPQ and NONO knockdown increased the promoter activity of the *IL8* gene, but not that of the *IFNB1* gene (Figure 3B and Figure S3B). In contrast, promoter activities of the *IL8* and *IFNB1* genes were unchanged in PSPC1-depleted cells (Figure 3B). Bioinformatics analysis revealed that the SFPQ-binding motif is located just 3′ downstream of the TATA box of the human *IL8* promoter and is evolutionarily conserved in the corresponding position of primate *IL8* genes (Figure 3C, Figures S3C–S3E, and Table S3). These findings suggest that SFPQ represses the *IL8* promoter. Notably, the SFPQ-binding motif was predicted with statistical significance in the promoter region of the majority of NEAT1-regulated genes (p value: 0.0015) (Table S3). We then employed a chromatin immunoprecipitation (ChIP) experiment to examine whether SFPQ binds the *IL8* promoter in vivo and is released upon poly I:C stimulation. ChIP experiment showed that SFPQ bound the SFPQ-binding motif of *IL8* gene in naive cells (Figure 3D). Conversely, SFPQ did not bind the SFPQ-binding motif when stimulated by poly I:C (Figure 3D). We also showed that binding of SFPQ to this motif decreased in cells transfected with mNeat1v2 expression plasmid (Figure 3E). We detected concomitantly increased binding of NEAT1v2 to SFPQ in response to poly I:C (Figure 3F). Consistent with this observation, the concentrations of SFPQ and NONO within enlarged paraspeckles were increased upon poly I:C treatment (Figures S3F and S3G). We performed kinetic and dose-response analyses of SFPQ binding to NEAT1v2 after poly I:C exposure. The data showed correlations among poly I:C stimulation, SFPQ binding to NEAT1v2, and IL8 mRNA induction (Figures S3H and S3I). These results suggest that SFPQ binds the SFPQ-binding motif of the *IL8* gene, thereby repressing *IL8* transcription in naive cells, and that poly I:C treatment relocates SFPQ from the *IL8* gene to NEAT1, resulting in the formation of excess paraspeckles, which in turn leads to the transcriptional

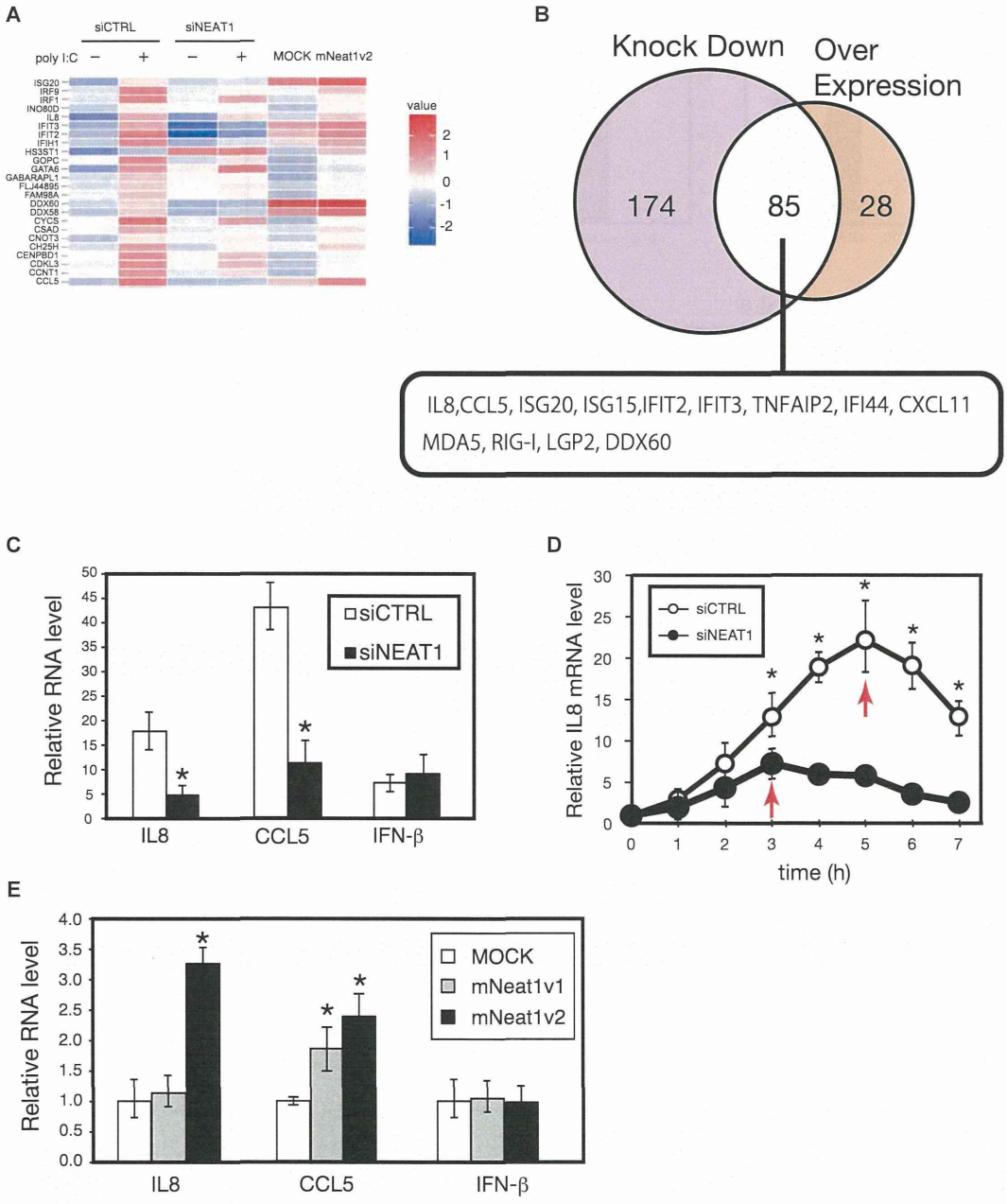

Figure 2. NEAT1-Regulated Genes

(A) Heat map image of microarray analysis of gene expression in the control cells with and without poly I:C stimulation, NEAT1 knockdown cells with and without poly I:C stimulation, and cells transfected with mock plasmid or mNeat1v2 expression plasmid alone.

(B) Venn diagram of genes with altered gene expression as identified by microarray analysis. Left circle contains the 259 poly I:C-induced genes whose induction was abolished by NEAT1 knockdown. Right circle contains the 113 genes induced by solo overexpression of mNeat1v2. Representative genes found in the overlap between these two groups are shown below.

(C) The relative mRNA levels of IL8, CCL5, and IFN-β in the poly I:C-treated cells and the nontreated cells as determined by RT-qPCR analysis. Values represent the mean ± SD (*$p < 0.01$, Student's t test).

(D) Induction kinetics of IL8 mRNA in control cells or NEAT1 knockdown cells after poly I:C stimulation.

(E) Relative mRNA levels of IL8, CCL5, and IFN-β in cells transfected with pCMV-mNeat1v1 or pCMV-mNeat1v2 compared with cells that have undergone mock transfection, as determined by RT-qPCR analysis. Values represent the mean ± SD (*$p < 0.01$, Student's t test).

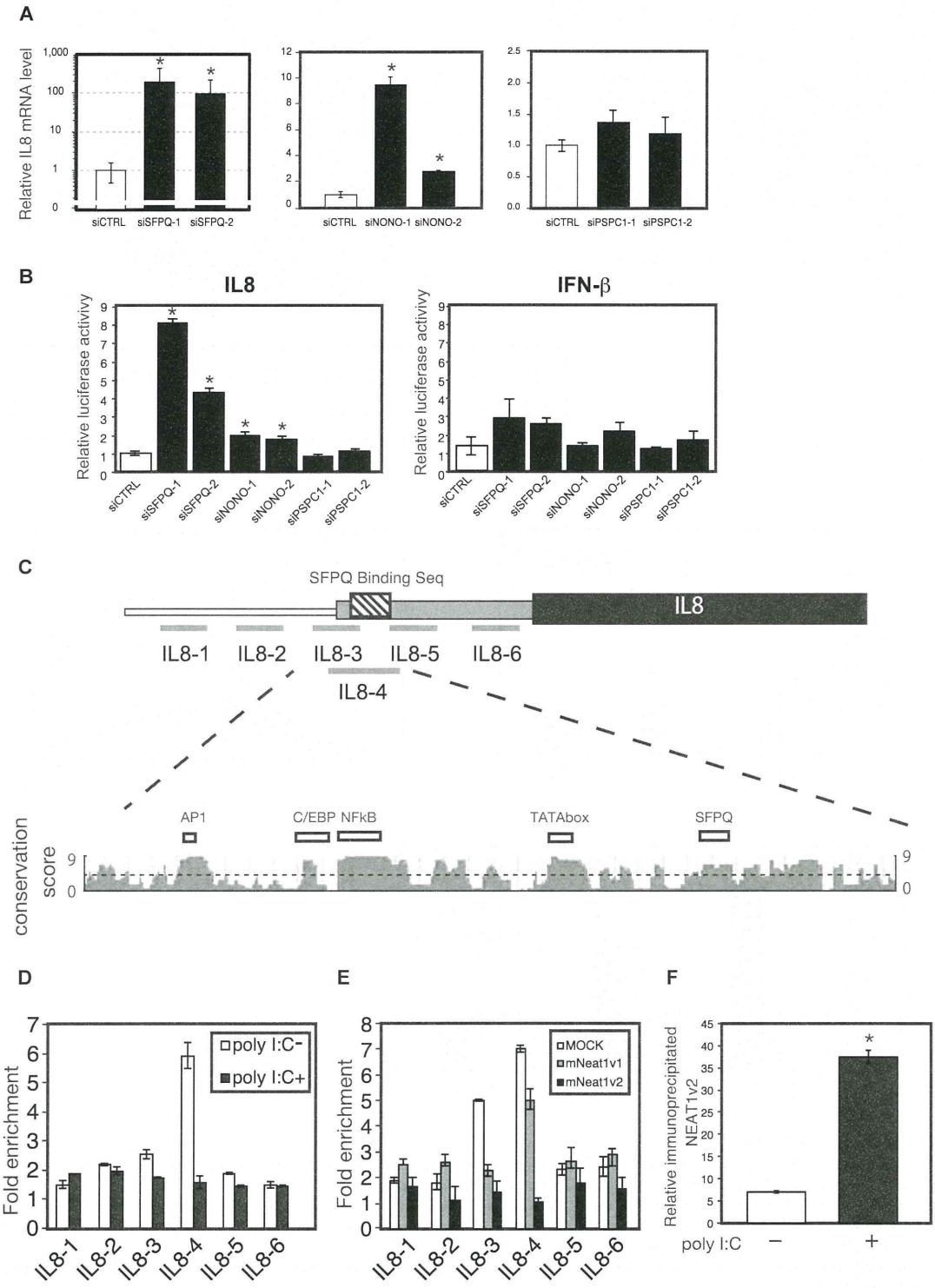

Figure 3. SFPQ-Mediated Transcriptional Repression of the *IL8* Gene and NEAT1-Mediated SFPQ Relocation

(A) IL8 mRNA levels of HeLa TO cells treated with various siRNAs as indicated. Values represent the mean ± SD (*$p < 0.01$, Student's t test).

(B) Luciferase reporter activities driven by the *IL8* promoter or the *INFB1* promoter were determined in cells transfected with the indicated siRNAs. Values represent the mean ± SD (*$p < 0.01$, Student's t test).

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activation of *IL8*. In addition to IL8, we showed that SFPQ bound to gene promoters containing the predicted SFPQ binding motif and that SFPQ binding was reduced by poly I:C stimulation (Table S3). Next, we examined whether NEAT1 is an upstream negative regulator of SFPQ in the repression of the *IL8* gene expression. IL8 mRNA induction by SFPQ depletion was not cancelled by NEAT1 depletion (see poly I:C− condition in Figure S3J). We then examined whether the NEAT1 silencing-mediated re-repression of the IL8 gene would be cancelled by SFPQ depletion. The results showed that poly I:C-induced IL8 mRNA induction was abrogated by NEAT1 knockdown and that the NEAT1 silencing-dependent rerepression of *IL8* gene induction was cancelled by SFPQ knockdown (Figure S3J). These data support the idea in which NEAT1 plays a role as an upstream negative regulator of SFPQ in the repression of the *IL8* gene expression.

To further test our model, we generated SFPQ mutants (ΔRRM1 and ΔRRM2) that retained their ability to bind to the IL8 promoter, but were unable to bind to NEAT1v2 (Figures 4A–4C). These mutants were able to suppress the IL8 expression in response to elevated levels of mNeat1v2 (Figure 4D). Moreover, the repression activity of the mutant SFPQs was stronger than that of wild-type SFPQ (Figure 4D). Experiments using systematically constructed plasmids expressing parts of NEAT1 indicated that an approximately 15 kb portion of NEAT1 was required for IL8 mRNA induction (Figure 4E). Simultaneously, RNA immunoprecipitation (RIP) results in capturing endogenous SFPQ showed that the 15 kb portion of NEAT1 bound to SFPQ in vivo.

Since SFPQ forms a heterodimer with NONO, we examined the contribution of NONO to the function of SFPQ binding to the IL8 promoter. We found that NONO depletion reduced the binding of SFPQ to the IL8 promoter region (Figure S3K). NONO depletion did not affect the expression level of SFPQ (Figure S3L), thereby ruling out the possibility that the reduced SFPQ binding to the IL8 promoter was caused by a reduced amount of SFPQ in response to NONO depletion. These results suggest that NONO affects the binding activity of SFPQ to the IL8 promoter region. In vitro binding assays showed that SFPQ/NONO had a specific binding affinity for an IL8 promoter DNA containing the SFPQ binding motif (Figure S3M). In addition, total RNA isolated from control cells expressing excess amount of NEAT1v2 abrogated SFPQ/NONO-IL8 promoter complex, but that from control cells expressing normal amount of NEAT1 did not (Figure S3N). The destruction of SFPQ/NONO-IL8 promoter complex by total RNA isolated from the cells depleted in NEAT1 was weaker than that by total RNA from the control cells (Figure S3N).

Involvement of NEAT1 in Immune Response upon Viral Infection

We next infected culture cells with either influenza virus, herpes simplex virus 1 (HSV-1), or measles virus (MV) to examine the biological significance of our earlier observations. Influenza virus is recognized by TLR3 in host cells, leading to an immune response that includes *IL8* induction (Guillot et al., 2005). Viral-derived dsRNA produced during the HSV-1 replication cycle also triggers an immune response through TLR3 stimulation (Lafaille et al., 2012; Zhang et al., 2007). Conversely, MV infection is not sensed by TLR3 (Berghäll et al., 2006). As expected, influenza virus and HSV-1, but not MV, induced NEAT1v2 expression (Figure 5A). Corresponding with this, HSV-1 infection induced excess formation of paraspeckles without altering the levels of paraspeckle proteins (Figures 5B and 5C and Figure S5A). HSV-1 also induced NEAT1 and the excess formation of paraspeckles in immune cells (Figures S5B and S5C). As expected, MV did not cause excess formation of paraspeckles (Figures S5D and S5F). Influenza virus infection induced excess formation of paraspeckles, even though the paraspeckles were slightly diffuse (Figures S5E and S5G). The *IL8* induction by influenza virus infection was decreased by NEAT1 knockdown (Figures 5D and 5E). In addition, mice infected with influenza virus or HSV-1 induced mNeat1v2 (Figure S5H). These data suggest that the regulation and function of NEAT1 in response to virus infection is evolutionarily conserved.

Cytokines secreted from the cells in response to pathogen infections are known to induce osteoclast differentiation of mouse bone marrow cells (Koide et al., 2010), so bone marrow differentiation can be used to assess the activation of the innate immune response. We therefore carried out the ex vivo experiment using mouse bone marrow cells to get further evidence of involvement of NEAT1 in innate immune response. The supernatant of virus-infected cells with NEAT1 depletion proved less potent for the activation of mouse bone marrow cells than that of virus-infected cells without NEAT1 depletion (Figure 5F). As expected from this result, mouse bone marrow cells showed more activation in response to the supernatant of cells ectopically expressing mNeat1v2 than to the supernatant of control cells (Figure 5F). To strengthen this observation, we performed a similar experiment using immune cells. The chemotaxis of dimethylsulfoxide differentiated HL-60 (DMSO-HL60) cells, neutrophil-like cells, were more activated by the supernatant of virus-infected cells without NEAT1 depletion than by that of virus-infected cells depleted in NEAT1 (Figure 5G). DMSO-HL60 cells showed more activation in response to the supernatant of cells ectopically expressing mNeat1v2 than to the supernatant of control

(C) Schematic of the 5′ region of the *IL8* gene. Fine line, medium gray line, thick black line, and hatched line box indicate the promoter region, 5′ UTR, ORF, and SFPQ binding sequence, respectively. Gray lines below the *IL8* gene indicate the regions amplified by the qPCR primer sets for chromatin immunoprecipitation (ChIP) analysis. The panel at the bottom of the figure shows the conservation score of sequences along the 5′ region of the *IL8* gene. Transcriptional regulatory elements along this region are indicated.

(D) HeLa TO cells stimulated with (black bar) and without (white bar) poly I:C were subjected to ChIP of the 5′ region of the *IL8* gene. Error bars indicate the errors of two replicates.

(E) ChIP of the 5′ region of the *IL8* gene of HeLa TO cells transfected with either mock vector (white bar), pCMV-mNeat1v1 (gray bar), or pCMV-mNeat1v2 (black bar). Error bars indicate the errors of two replicates.

(F) The amount of NEAT1v2 coimmunoprecipitated with endogenous SFPQ was determined by RT-qPCR analysis. The relative amount of NEAT1v2 isolated by anti-SFPQ antibody was normalized to that isolated by the control IgG. Black and white bars indicate the relative amount of NEAT1v2 isolated from cells treated with or without poly I:C, respectively. Values represent the mean ± SD (*$p < 0.01$, Student's t test).