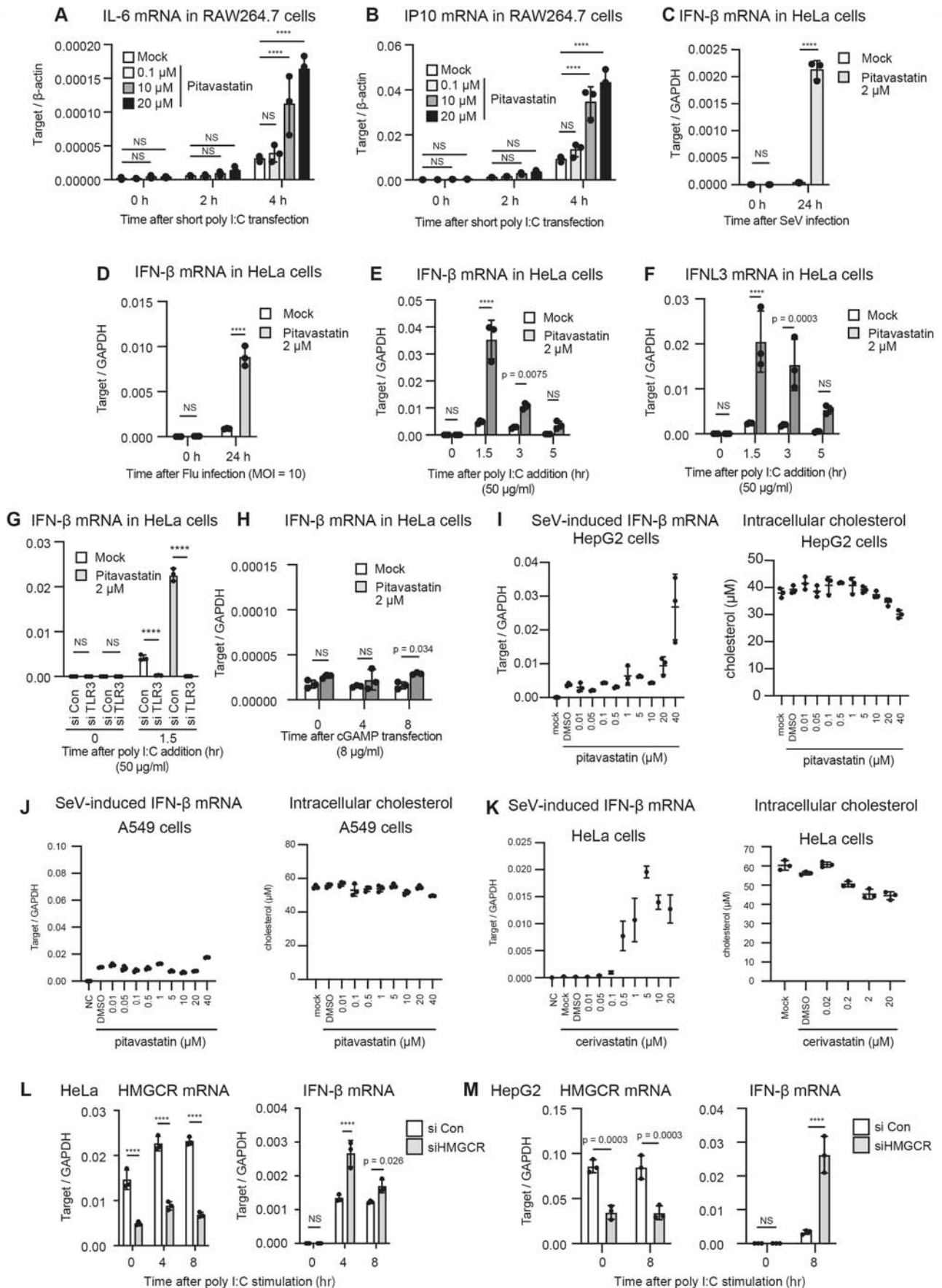


Expanded View Figures

Figure EV1. Effects of statins on HeLa cells.

(A–H) HeLa cells were treated with pitavastatin or DMSO (mock) for 24 h. Subsequently, they were stimulated by either transfecting 200 ng/mL of short poly I:C (A, B), infection with SeV (C) at MOI = 10 or Flu at MOI = 10 (D), adding poly I:C (50 µg/mL) to cell culture medium (E–G), or transfecting cGAMP (8 µg/mL) (H). siRNAs for negative control (si Con) and TLR3 (si TLR3) were transfected into HeLa cells for 2 days and then stimulated by adding poly I:C (50 µg/mL) to the cell culture medium (G). The expression of each gene was determined by RT-qPCR and normalized to that of GAPDH ($n = 3$, (A–C); technical replicates, (D–H); biological replicates). (I–K) HepG2 (I), A549 (J), and HeLa (K) cells were treated with pitavastatin (I, J), cerivastatin (K), or solvent (DMSO) for 24 h, and the cellular cholesterol levels were measured. Statin-pretreated cells were infected with SeV for 24 h, and then the expression of IFN- β mRNA was determined by RT-qPCR and normalized to that of GAPDH ($n = 3$ biological replicates). (L, M) HeLa (L) and HepG2 (M) cells were transfected with HMGCR or control siRNA. Twenty-four hours after siRNA transfection, the cells were stimulated by short poly I:C transfection (200 ng/mL) for the indicated time period. The mRNA expression of each gene was determined using RT-qPCR and normalized to that of GAPDH ($n = 3$ biological replicates). Data Information: in (A–M), Data are represented as mean $\pm$ SD ($n = 3$, **** $p < 0.0001$, one-way ANOVA (I–K), two-way ANOVA (A–H), NS: not significant).



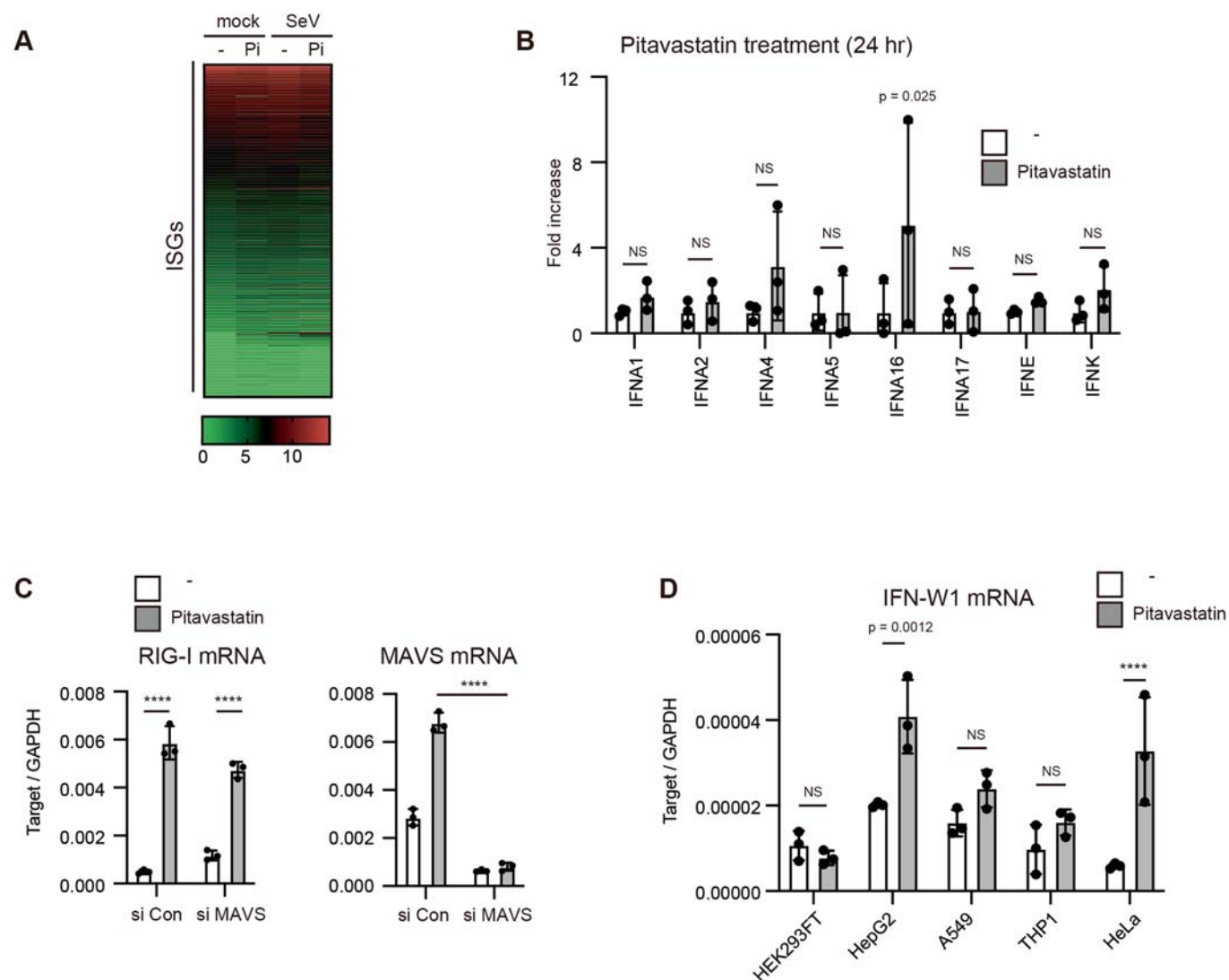


Figure EV2. Expression of non-canonical type I IFNs after pitavastatin treatment.

(A) Heatmap of interferon-stimulated genes (ISGs) from the microarray analysis presented in Fig. 2. ISGs were selected by searching for interferon-stimulated genes encoding proteins in the GeneCards database (<https://www.genecards.org>). (B) HeLa cells were treated with pitavastatin for 24 h. The expression of each gene was determined by RT-qPCR and normalized to that of β -actin. The fold increase was calculated by dividing the normalized expression level of a pitavastatin-treated sample by a pitavastatin-untreated sample value ($n = 3$, biological replicates). (C) HeLa cells were transfected with siRNA for MAVS (si MAVS) and negative control (si Con) for 72 h. Cells were then treated with pitavastatin for 48 h. The expression of RIG-I and MAVS were determined by RT-qPCR and normalized to GAPDH ($n = 3$, biological replicates). (D) HEK293, HepG2, A549, THP-1, and HeLa cells were treated with pitavastatin for 24 h, and IFN- ω expression was determined by RT-qPCR and normalized to that of GAPDH ($n = 3$, technical replicates). Data Information: In (B–D), data are represented as mean $\pm$ SD (**** $p < 0.0001$, two-way ANOVA, NS: not significant).

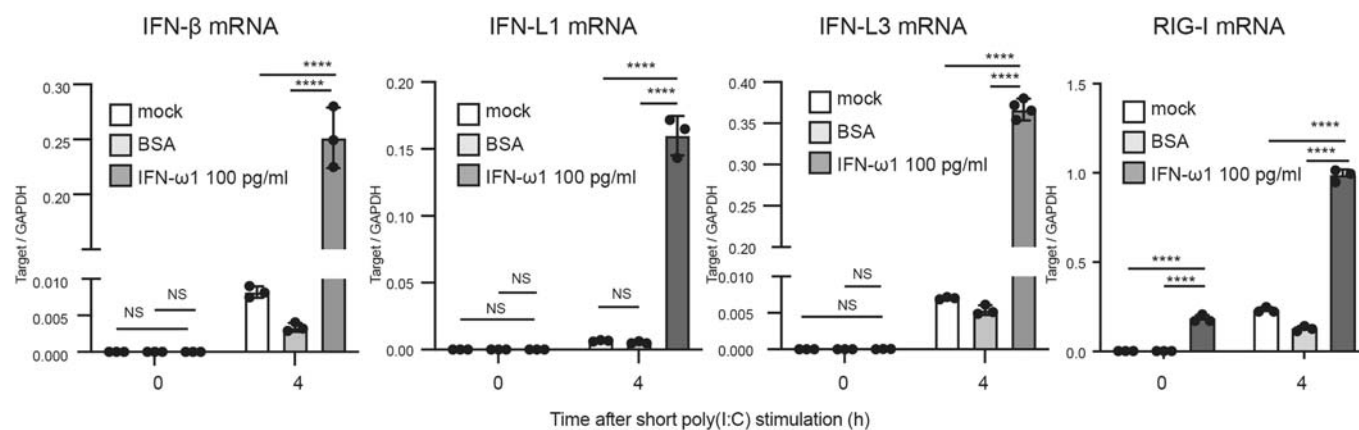
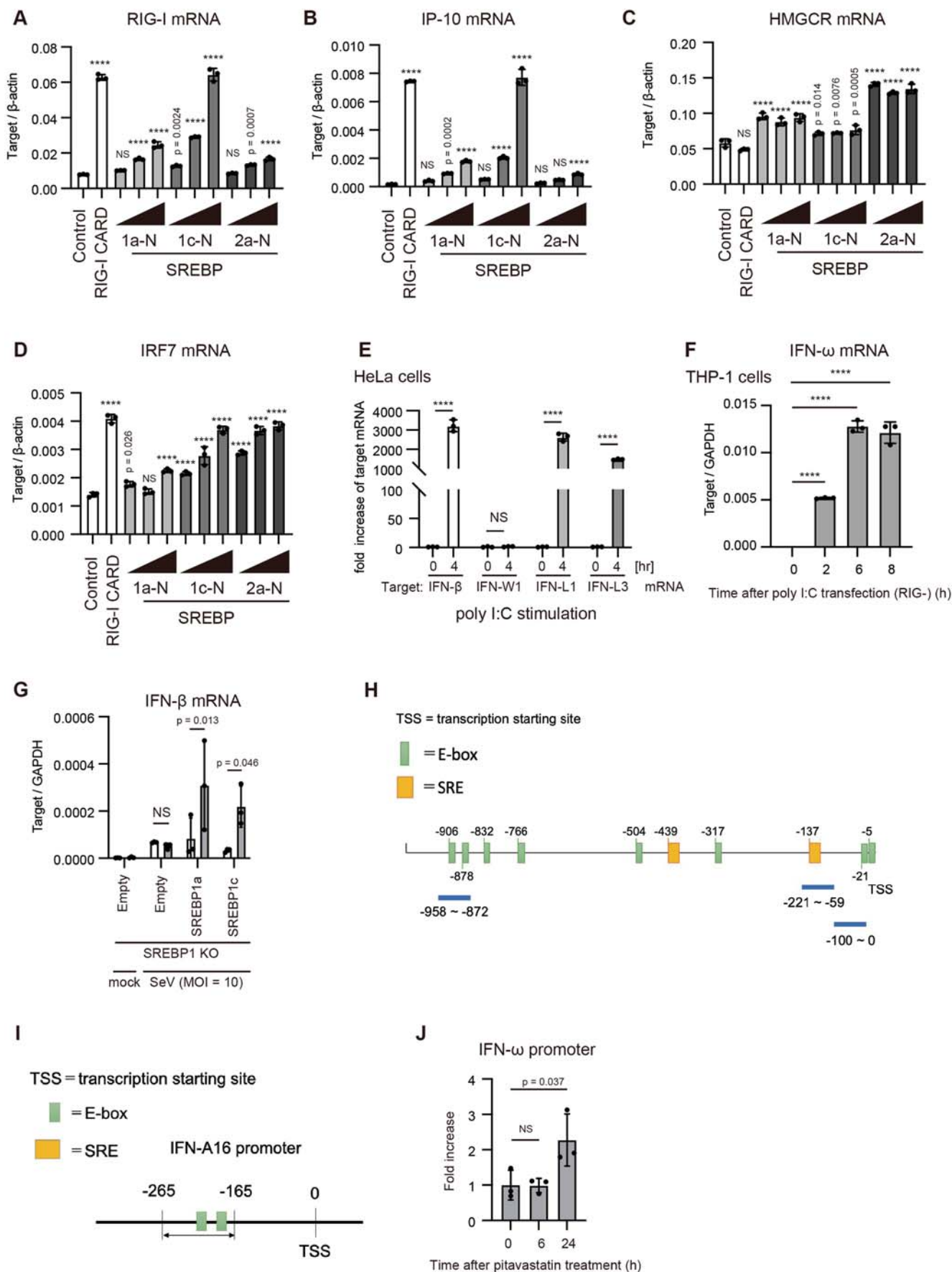


Figure EV3. Effect of IFN-ω on the RIG-I-dependent innate immune response.

HeLa cells were treated with 100 pg/mL of recombinant IFN-ω protein for 24 h, and stimulated with short poly I:C (200 ng/mL) transfection. The expression of each gene was determined by RT-qPCR and normalized to that of GAPDH. Data Information: Data are represented as mean ± SD ($n = 3$, biological replicates, **** $p < 0.0001$, two-way ANOVA, NS: not significant).



◀ **Figure EV4. The role of SREBPs in type I IFN responses.**

(A–D) HEK293 cells were transfected with 200 ng/mL of RIG-I CARD expressing vectors or 200, 1000, or 2000 ng/mL of SREBP1a-N, 1c-N, or 2a-N expressing vectors in a 24-well plate. The total amount of DNA was adjusted to the same amount by adding an empty vector. Twenty-four hours after transfection, the expression of each gene was determined by RT-qPCR and normalized to that of β -actin. Each *p*-value represents the comparison with the control ($n = 3$, technical replicates). (E) HeLa cells were stimulated via transfection with 200 ng/mL of short poly I:C, and the mRNA expression of each gene was determined by RT-qPCR. The fold increase was calculated by dividing mRNA expression at each time point by that at 0 h ($n = 3$, biological replicates). (F) THP1 cells were stimulated via transfection with short poly I:C, and the expression of IFN- ω mRNA was determined by RT-qPCR and normalized to that of GAPDH ($n = 3$, technical replicates). (G) SREBP1 KO HeLa cells were transfected with empty vector (Empty) or full-length SREBP1a or SREBP1c. Twenty-four hours after transfection, cells were treated with pitavastatin for 24 h, and subsequently infected with SeV or mock for 24 h. The expression of IFN- β mRNA was measured by RT-qPCR and normalized to GAPDH ($n = 3$, biological replicates). (H, I) Promoter regions of human IFN- ω and IFN- $\alpha 16$. The E-box and SRE motifs are shown in the green and yellow boxes, respectively. Numbers represent the positions of the nucleotides from the start of the ORF. (J) The IFN- ω reporter plasmid was transfected into HeLa cells with *Renilla* luciferase plasmid (internal control). Twenty-four hours after transfection, cells were treated with pitavastatin (2 μ M) at indicated h. Cell lysates were prepared, and the luciferase activities were measured and normalized to *Renilla* luciferase activities ($n = 3$, biological replicates). Data Information: In (A–G, J), data are represented as mean $\pm$ SD (**** $p < 0.0001$, one-way ANOVA (A–D, J), two-way ANOVA (E, G)).

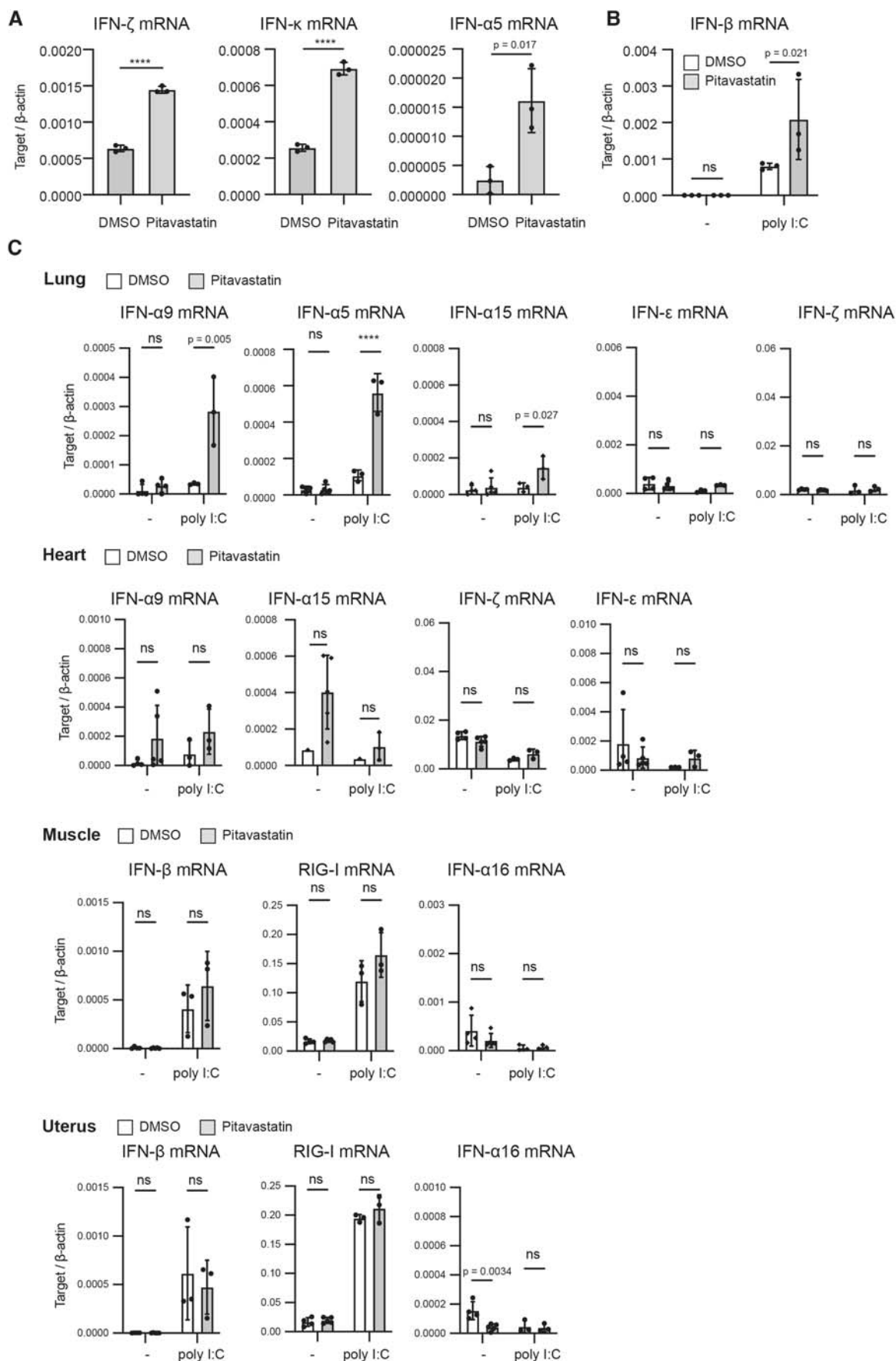


Figure EV5. Tissue-specific effects of pitavastatin on type I IFN responses.

(A) BMDMs of mice were treated with pitavastatin (10 μ M) for 24 h, and the expression of noncanonical type I IFNs was measured by RT-qPCR normalized to β -actin. (B) BMDMs treated with pitavastatin (10 μ M) for 24 h were stimulated with transfection of poly I:C (200 ng/ml) for 4 h. The expression of IFN- β mRNA was measured by RT-qPCR normalized to β -actin ($n = 3$, biological replicates). (C) Pitavastatin or solvent (DMSO) was intraperitoneally injected into mice every day for three days, followed by intraperitoneal injection of poly I:C (200 μ g/head) or solvent (DMSO). Twenty-four hours after poly I:C injection, the lung, heart, muscle, and uterus were isolated. The total RNAs were extracted from each tissue. The expression of each gene was determined by RT-qPCR and normalized to that of β -actin ($n = 3$ –6 mice for each group). Data Information: In (A, C), data are represented as mean $\pm$ SD. Statistical significance was determined using t-test (A) and two-way ANOVA (B, C). **** $p < 0.0001$. ns: not significant.