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Supplemental information

FEZ1 phosphorylation regulates HSPA8 localization and interferon-stimulated gene expression

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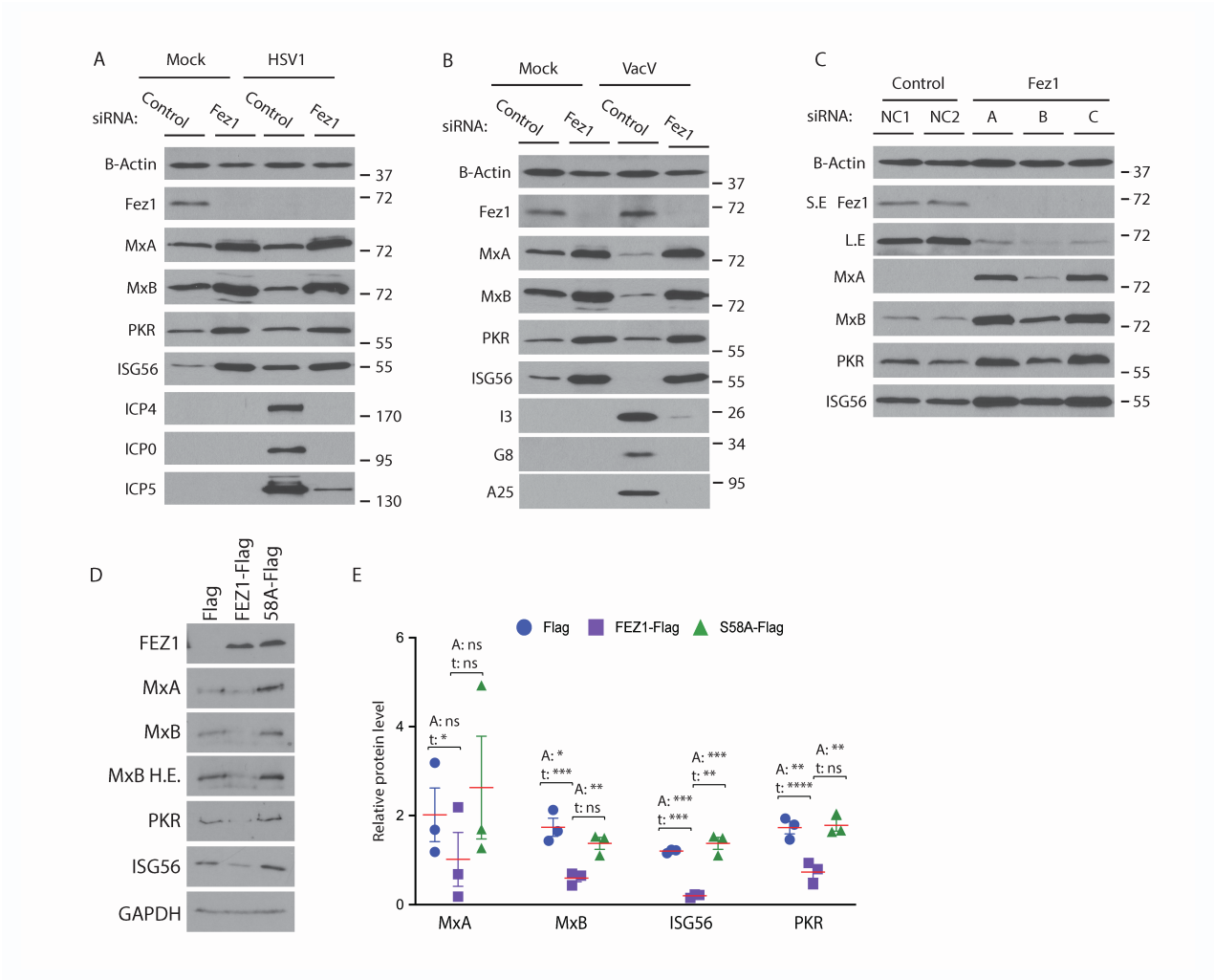

Figure S1. FEZ1 regulates ISG expression in primary normal human dermal fibroblasts, Related to Figure 1. **(A-B)** NHDF cells transfected with negative control or FEZ1 siRNA for 48 hours (h) and were then mock infected or infected with HSV-1 (**A**) or VacV (**B**) at MOI 5 for 30h. Expression of ISGs (MxA, MxB, PKR and ISG56) and viral proteins (HSV1: ICP4, ICP0, ICP5 and VacV: I3, G8, A25) in total cell lysates were analyzed by WB. **(C)** WB analysis showing an increase in ISG levels in lysates from NHDFs treated with 3 independent FEZ1 specific siRNAs (A, B and C), but not with control siRNAs (NC1 and NC2). Results in A-C are representative of 3 biological replicates. **(D)** WB analysis showing effects of Flag-tagged FEZ1 (FEZ1-Flag) or FEZ1-S58A (S58A-Flag) on expression of ISGs in NHDF cells. **(E)** Quantification of ISG levels relative to HSPA8 in control, FEZ1-Flag, or FEZ1-S58A-Flag expressing CHME3 cells from **D.** Data is presented as the ratio to the difference between control and treatment groups. Statistical significance is presented as "A" when it was calculated for all groups using one-way ANOVA followed by Dunnet post-hoc test or as "t" if student's t-test was applied to compare pairwisely Flag or FEZ1-S58A-Flag with FEZ1-Flag. (n=3 blots; red line = mean; bars = SD).

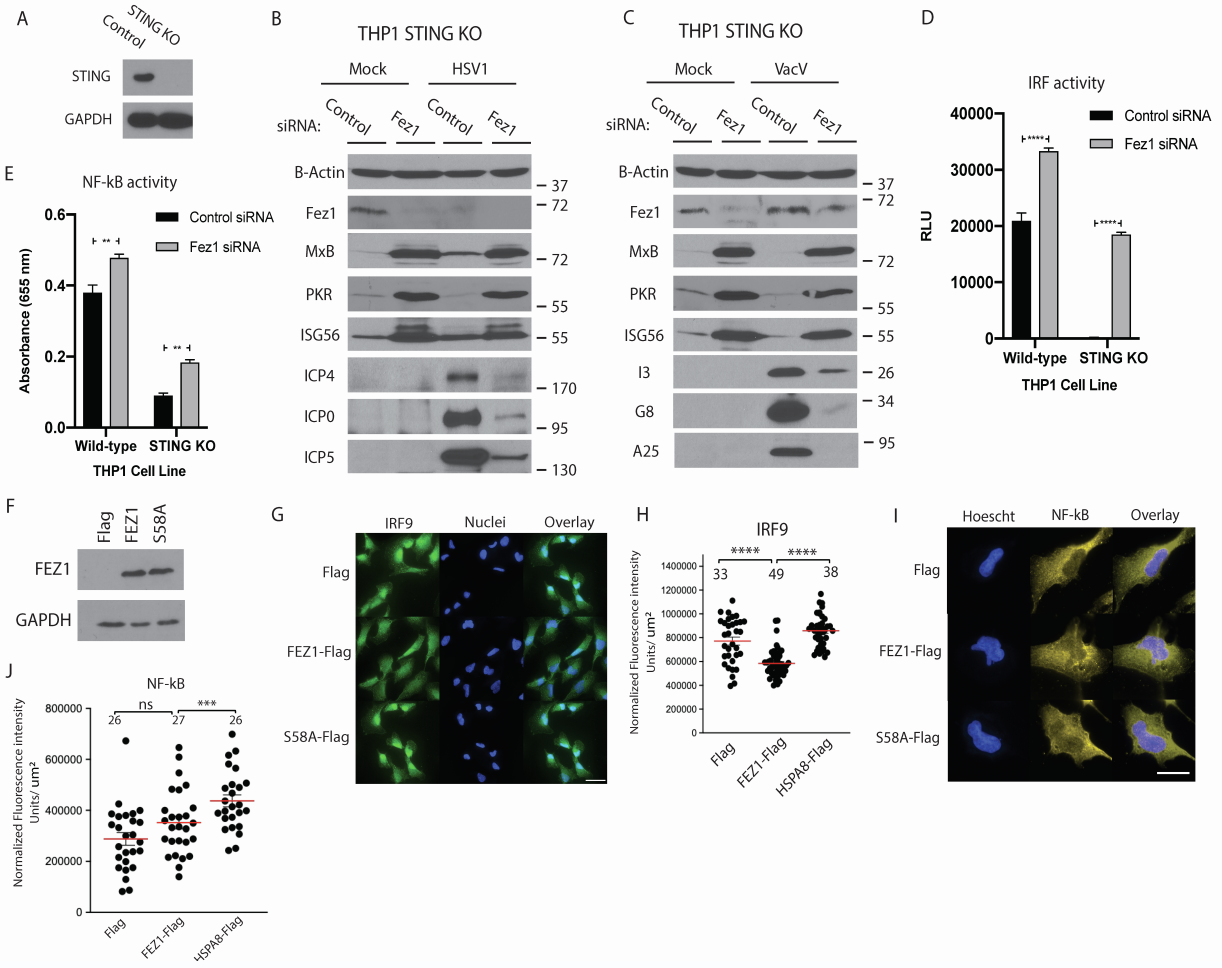

Figure S2. FEZ1 regulates IRF and NF-κB activation in a STING-independent manner, Related to Figure 1 and 2. (A) WB analysis of lysates from control or STING KO THP1 cells confirming STING KO in cells used in B-C. **(B-C)** Differentiated THP1-Dual™ STING KO cells were transfected with negative control or FEZ1 siRNA for 48 h and were then mock infected or infected with HSV-1 **(B)** or VacV **(C)** at MOI 5 for 42 h. Expression of ISGs (MxB, PKR and ISG56) and viral proteins (HSV1: ICP4, ICP0, ICP5 and VacV: I3, G8, A25) were detected in total cell lysates by WB analysis. **(D-E)** Culture media from differentiated THP1-Dual or THP1-Dual STING KO cells treated with control or FEZ1 specific siRNAs were analyzed for secretion of reporters of IRF (**D**) or NF-κB **(E)** activity. 2-way ANOVA followed by Sidak's multiple comparison test was used to calculate statistical significance. All results are derived from or representative of 3 biological replicates. **(F-G)** FEZ1 regulates IRF9 localization in NHDFs. NHDFs expressing Flag, FEZ1-Flag or S58A-FEZ1-Flag (**F**) were stained for IRF9 or NF-κB. Representative images and quantification of effects on IRF9 localization (**G** and **H**, respectively) or NF-κB localization (**I** and **J**, respectively). Number of cells analysed is shown; red line = mean; bars = SD. One-way ANOVA was used with Tukey (**H**) and Dunnett (**J**) post-hoc tests to calculate statistical significance.

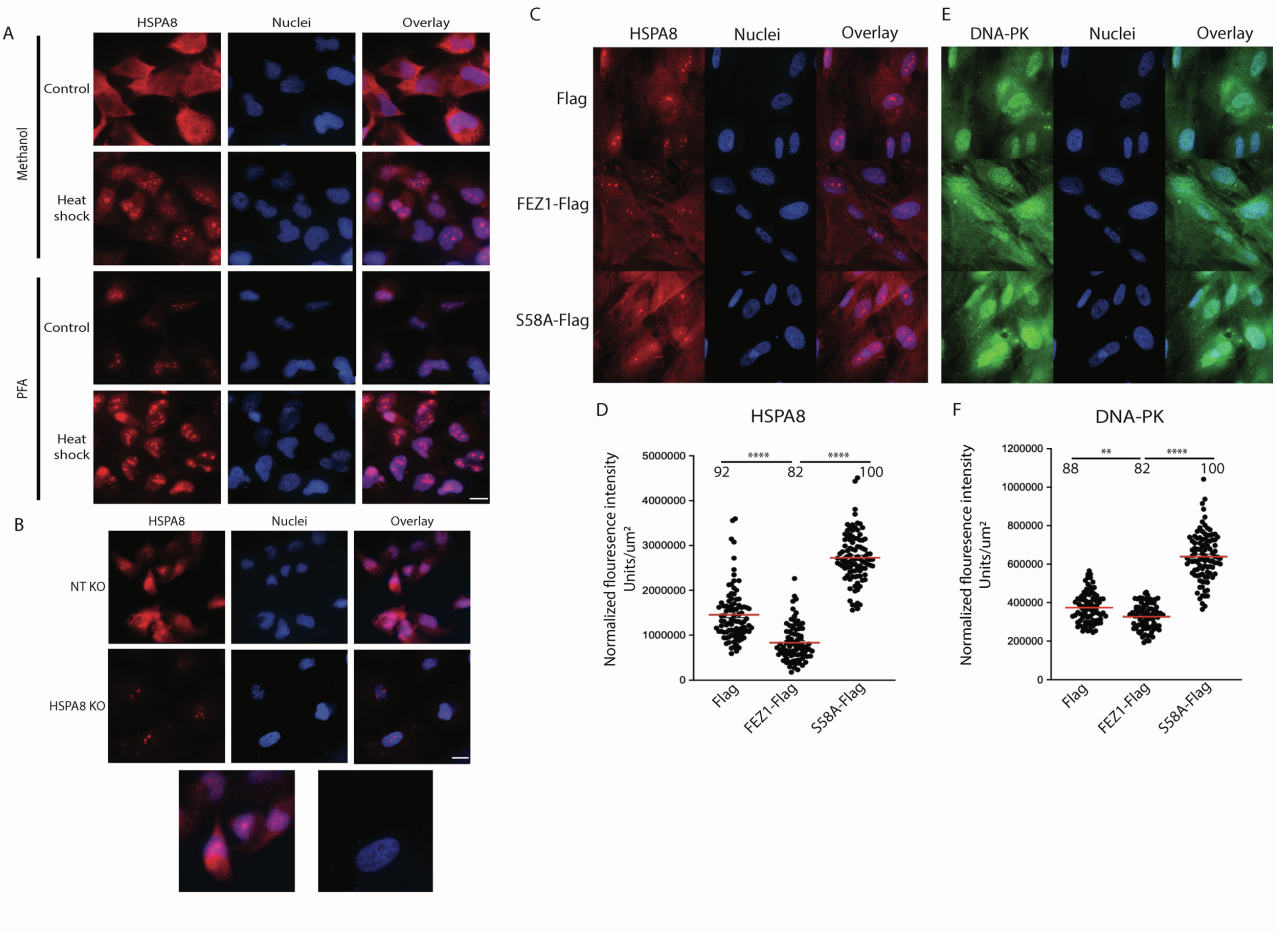

Figure S3. Validation of HSPA8 staining and effects of S58A-FEZ1 in NHDFs, Related to Figure 3. (A) Representative IF images in CHME3 cells incubated at 37 ºC (Control) or 42 ºC (Heat shock) for 2 h, fixed with either methanol or PFA and stained for HSPA8. Nuclei were stained with Hoechst (blue). Results are representative of 3 experimental replicates. Note that PFA causes artifactual localization or staining of HSPA8 in the nucleus irrespective of temperature. As such, methanol fixation was used in all experiments assessing HSPA8 localization. Scale bar is 10 µm. (**B**) Confirmation of the specificity of HSPA8 staining using HSPA8 KO CHME3 pools fixed with Methanol. Scale bar is 10 µm. **(C** and **E)** Representative IF images showing intracellular distribution of HSPA8 (**C**) or DNA-PK (E) in NHDFs stably expressing Flag control, or FEZ1-Flag or S58A-Flag. Nuclei were stained with Hoechst. Scale bar is 10 µm. Quantitative analysis of HSPA8 (**D**) or DNA-PK (**F**) staining in nuclei of NHDF cells shown in **C** or **E**, respectively. Number of cells analyzed is indicated; red line = mean; bars = SD. One-way ANOVA followed by Tukey post-hoc test was used to calculate statistical significance in **D** and **F**.

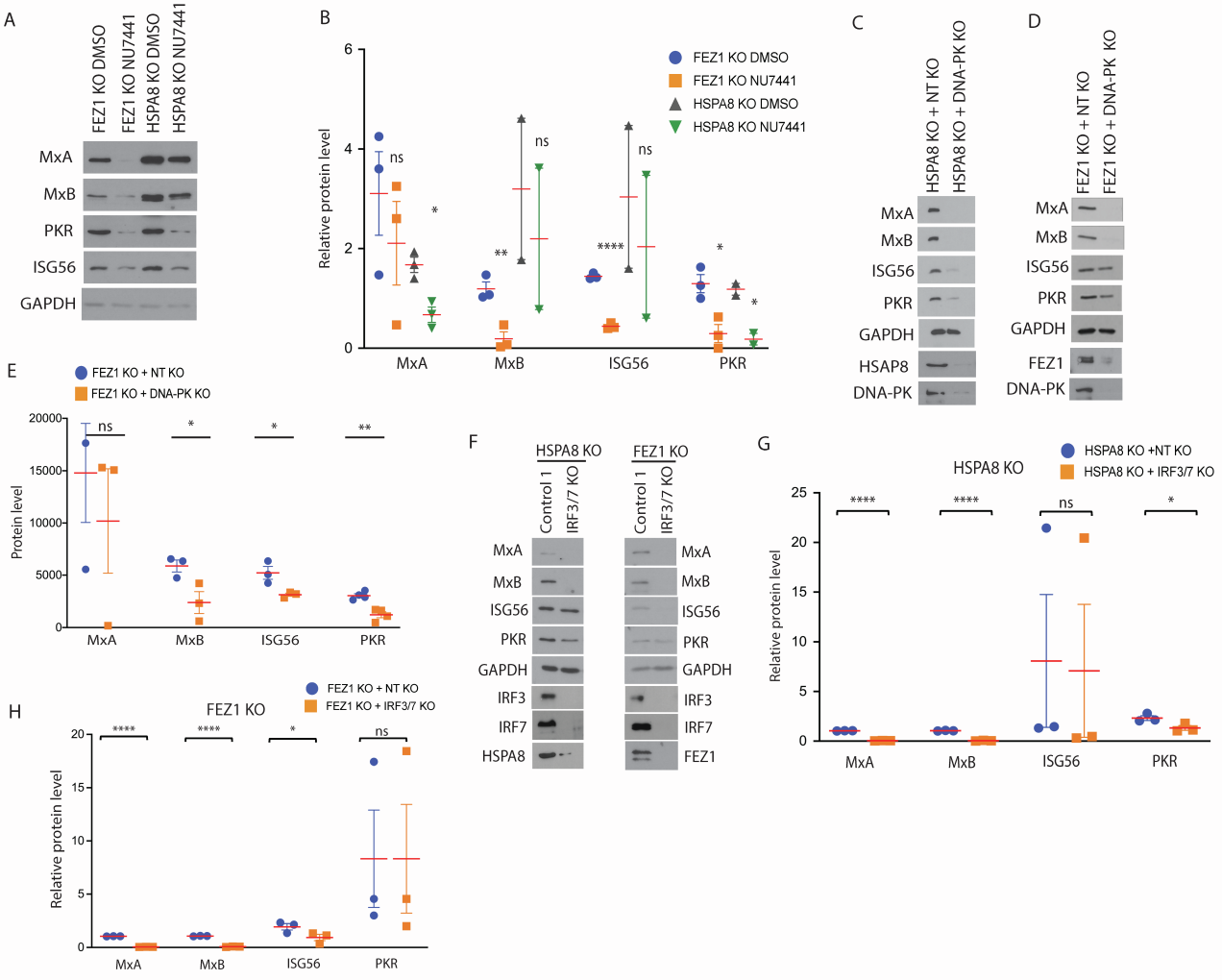

Figure S4. ISG expression in FEZ1 or HSPA8 KO cells requires DNA-PK and IRF3/7, Related to Figure 4. **(A-B)** FEZ1 or HSPA8 KO CHME3 cells were treated with the DNA-PK inhibitor, NU7441. Representative WB analysis of ISG expression (**A**) and quantification of effects of NU7441 (**B**). Data is presented as the ratio to the difference between control and treatment groups. n = 3, red line = mean; bars = SD. **(C)** Effect of DNA-PK KO on ISG expression in HSPA8 KO CHME3 cells. **(D-E)** Effect of DNA-PK KO on ISG expression in FEZ1 KO CHME3 cells. Representative WB is shown (**D**) and quantification of effects of DNA-PK KO on each ISG tested (**E**). Data is presented as the ratio to the difference between control and treatment groups. n = 3, red line = mean; bars = SD. **(F-H)** Effect of IRF3/7 KO on ISG expression in HSPA8 or FEZ1 KO CHME3 cells. Representative WB analysis of changes in ISG expression (**F**) and quantification in effects of IRF3/7 KO on each ISG tested, HSPA8 (**G**) or FEZ1 (**H**). Data is presented as the ratio to the difference between control and treatment groups. n = 3, red line = mean; bars = SD. A Student's *t*-test was used in **B, E, G** and **H** to calculate statistical significance.

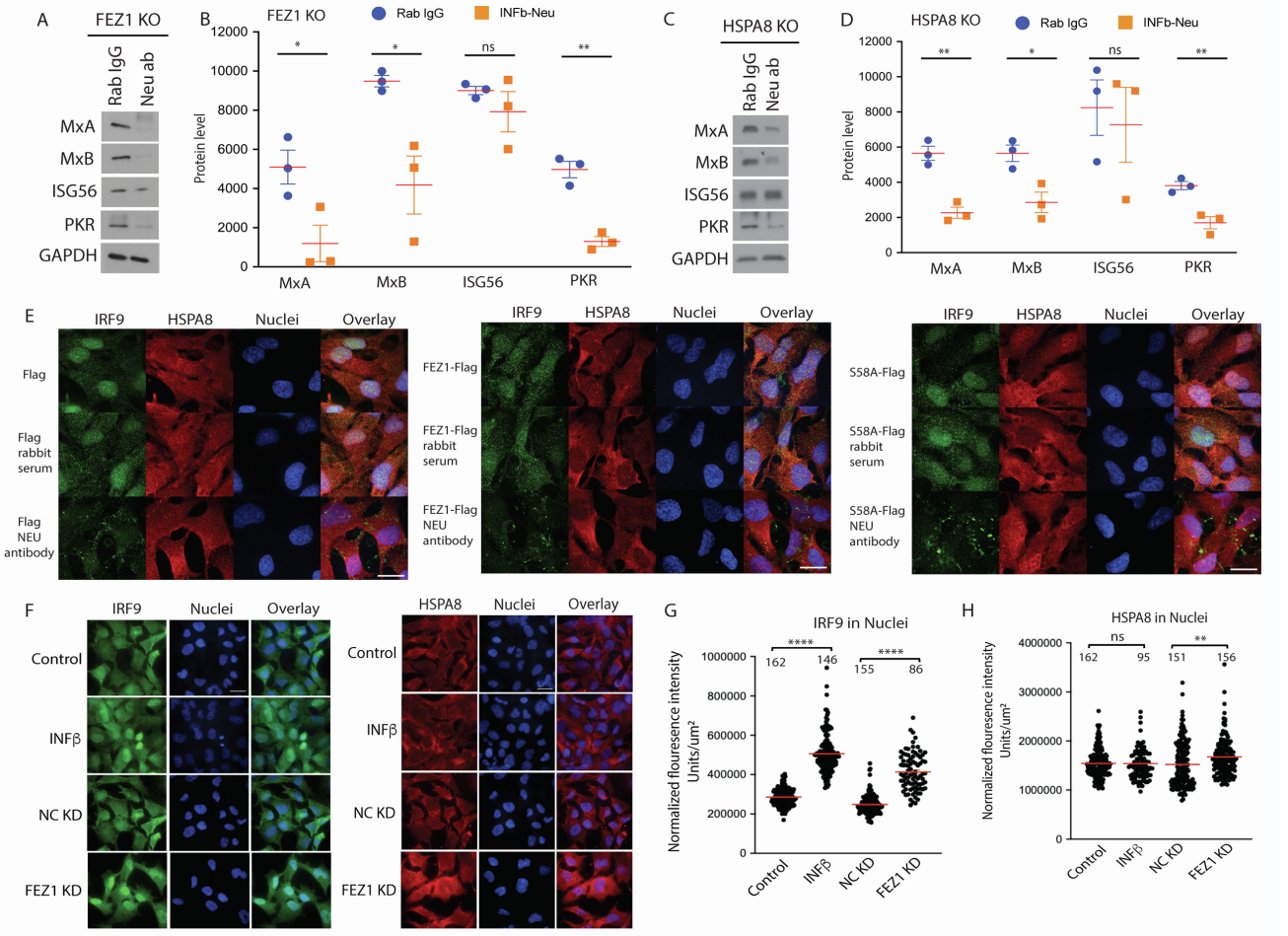

Figure S5. The differing contributions of IFN to ISG expression and IRF9 versus HSPA8 localization, Related to Figure 4. **(A-B)** Effect of IFNβ neutralizing antibody on ISG expression in FEZ1 KO CHME3 cells. Representative WB is shown (**A**) and quantification of effects of IFNβ neutralization on each ISG tested (**B**). n = 3, red line = mean; bars = SD. **(C-D)** Effect of IFNβ neutralizing antibody on ISG expression in HSPA8 KO CHME3 cells. Representative WB is shown (**C**) and quantification of effects of IFNβ neutralization on each ISG tested (**D**). n = 3, red line = mean; bars = SD. **(E)** Effect of IFNβ neutralizing antibody on IRF9 or HSPA8 localization in Flag, FEZ1-Flag or S58A-FEZ1-Flag expressing CHME3 cells. IRF9 translocation is reduced by IFN neutralization but large aggregates formed by neutralizing antibody taken up by cells complicates quantification of effects. **(F-H)** Effect of IFNβ treatment on IRF9 or HSPA8 localization in CHME3 cells compared with CHME3 cells treated with non-targeting control (NC) or FEZ1 siRNAs. Representative images of effects observed (**F**) and quantification of effects of IFNβ versus FEZ1 depletion on the levels of IRF9 or HSPA8 in the nucleus (**G**) are shown. Number of cells analyzed is indicated, red line = mean; bars = SD. *t*-test was used in **B**, **D**, **G** and **H** to calculate statistical significance.

Table S1. Related to STAR Methods. gRNA sequence information.

Gene target	Guide Number	Sequence	Catalog Number (Dharmacon)
tracrRNA	n/a	n/a	U-002005-50
Non-targeting	4	n/a	U-007504-20
HSPA8	1	CATACCTGAATAAGCACACC	CM-017609-01
HSPA8	2	TGGTGGTATTACGCTTGATG	CM-017609-02
HSPA8	3	AAACGTGCTCATCTTTGACC	CM-017609-03
HSPA8	4	ACGCTCGCCTTCATAAACCT	CM-017609-04
FEZ1	1	TCCGGGTCCTCCGAGCAGGA	CM-013010-01
FEZ1	2	ATCTGTAACTGGTTCTTCAC	CM-013010-02
FEZ1	3	TTCATTTACGAGGTCCTCCA	CM-013010-03
FEZ1	4	TTCTCAAGCTCGGAGAGGGA	CM-013010-04
PRKDC	1	ACTGTCTGCCAAATATGACA	CM-005030-01
PRKDC	2	TATGTCTTCCCTGTCATATT	CM-005030-02
PRKDC	3	TACGTTCCTGCACTGCAGGT	CM-005030-03
PRKDC	4	TACCTGCAGTGCAGGAACGT	CM-005030-04
PRKDC	5	GTGATTTCCACTCAGCAAGG	CM-005030-05
IRF3	1	TACCCGGGCCATTTCTACCA	CM-006875-01
IRF3	2	ACCAAGGCCCTGAGGCACGT	CM-006875-02
IRF3	3	GATCTGATTACCTTCACGGA	CM-006875-03
IRF3	4	CCCAGATCTGATTACCTTCA	CM-006875-04
IRF3	5	GCACCAACAGCCGCTTCAGT	CM-006875-05
IRF7	1	CCAGGTAGATGGTATAGCGT	CM-011810-01
IRF7	2	TCATTAGACTGGGTTCTAGG	CM-011810-02
IRF7	3	CTGTGCCGAGTGCACCTAGA	CM-011810-03
IRF7	4	TAGGTGCACTCGGCACAGCC	CM-011810-04
IRF7	5	AGCTGGTGGAATTCCGGGCA	CM-011810-05

Table S2. Related to STAR Methods. Oligonucleotide information.

Silencer® Negative Control #1 siRNA	Thermo Fisher Scientific	cat #AM4635
Silencer® Negative Control #2 siRNA	Thermo Fisher Scientific	cat #AM4637
FEZ1-A siRNA	Thermo Fisher Scientific	ID# 15759
FEZ1-B siRNA	Thermo Fisher Scientific	ID# 45012
FEZ1-C siRNA	Thermo Fisher Scientific	ID# 45101
tracrRNA	Dharmacon	U-002005-50
Non-targeting gRNA	Dharmacon	U-007504-20
HSPA8 #1 CATACCTGAATAAGCACACC	Dharmacon	CM-017609-01
HSPA8 #2 TGGTGGTATTACGCTTGATG	Dharmacon	CM-017609-02
HSPA8 #3 AAACGTGCTCATCTTTGACC	Dharmacon	CM-017609-03
HSPA8 #4 ACGCTCGCCTTCATAAACCT	Dharmacon	CM-017609-04
FEZ1 #1 TCCGGGTCCTCCGAGCAGGA	Dharmacon	CM-013010-01
FEZ1 #2 ATCTGTAACTGGTTCTTCAC	Dharmacon	CM-013010-02
FEZ1 #3 TTCATTTACGAGGTCCTCCA	Dharmacon	CM-013010-03
FEZ1 #4 TTCTCAAGCTCGGAGAGGGA	Dharmacon	CM-013010-04
IRF3 #1 TACCCGGGCCATTTCTACCA	Dharmacon	CM-006875-01
IRF3 #2 ACCAAGGCCCTGAGGCACGT	Dharmacon	CM-006875-02
IRF3 #3 GATCTGATTACCTTCACGGA	Dharmacon	CM-006875-03
IRF3 #4 CCCAGATCTGATTACCTTCA	Dharmacon	CM-006875-04
IRF3 #5 GCACCAACAGCCGCTTCAGT	Dharmacon	CM-006875-05
IRF7 #1 CCAGGTAGATGGTATAGCGT	Dharmacon	CM-011810-01
IRF7 #2 TCATTAGACTGGGTTCTAGG	Dharmacon	CM-011810-02
IRF7 #3 CTGTGCCGAGTGCACCTAGA	Dharmacon	CM-011810-03
IRF7 #4 TAGGTGCACTCGGCACAGCC	Dharmacon	CM-011810-04
IRF7 #5 AGCTGGTGGAATTCCGGGCA	Dharmacon	CM-011810-05
PRKDC #1 ACTGTCTGCCAAATATGACA	Dharmacon	CM-005030-01
PRKDC #2 TATGTCTTCCCTGTCATATT	Dharmacon	CM-005030-02
PRKDC #3 TACGTTCCTGCACTGCAGGT	Dharmacon	CM-005030-03
PRKDC #4 TACCTGCAGTGCAGGAACGT	Dharmacon	CM-005030-04
PRKDC #5 GTGATTTCCACTCAGCAAGG	Dharmacon	CM-005030-05
eGFP-SbfI forward primer: TAACTATCCTGCAGGTATGGTGAGCAAGGGCGAG	This paper	N/A
eGFP-NotI reverse primer: ATAAGAATGCGGCCGCCTTGTACAGCTCGTCCATGCC	This paper	N/A
NotI-HSPA8 forward primer: ATAAGAATGCGGCCGCCATGTCCAAGGGA	This paper	N/A
HSPA8-EcoRI reverse primer: CGGAATTCATCAACCTCTTCAATGGT	This paper	N/A
HSPA8-FLAG-EcoRI reverse primer: CGGAATTCCTTGTCGTCATCGTCTTTGTAGTCATCAACCTCTTCAATGGT	This paper	N/A