**Figure S1**

**(A and B)** IMR90 cells were transfected with plasmids pUC and pBSK, as indicated. Lysates were harvested at 24h post infection or transfection and DNA binding measured by anti-HIRA ChIP assay (anti-HA as negative control) using primers binding different regions throughout the input DNA. Data are mean +/- SD (error bars) (n=3 biological repeats). $p<0.05$ for all regions as compared to control IgG (HA). PCR primer pairs in Supplementary Table S3.

**Figure S2**

**(A)** IMR90 cells were transfected with plasmid pcDNA3. 24 hours post-transfection level of secreted IFN-β was measured using ELISA. Data are mean +/- SD (error bars) (n=3 biological repeats). $p<0.05$ comparing mock vs pcDNA3 transfection. **(B)** IMR90 cells were transfected with pcDNA3, media was harvested 24 hours post-transfection and applied to un-transfected cells for another 24 hours. Cells were fluorescent stained with antibodies to HIRA and PML. **(C)** Quantitation of cells with HIRA localized to PML bodies from (B). Data are mean +/- SEM from three independent experiments, $p<0.05$ as compared to mock treated. **(D)** IMR90 cells were infected with lentivirus encoding SV40 T antigen and western blotted with indicated antibodies. Mock-uninfected lysate was used as control. **(E)** Cells from D were treated with 2000U/ml of IFN-β and fluorescent stained with antibodies to HIRA and PML. **(F)** Quantitation of cells with HIRA localized to PML bodies from (E). Data are mean +/- SEM from three independent experiments, $p<0.05$ as compared to mock treated.

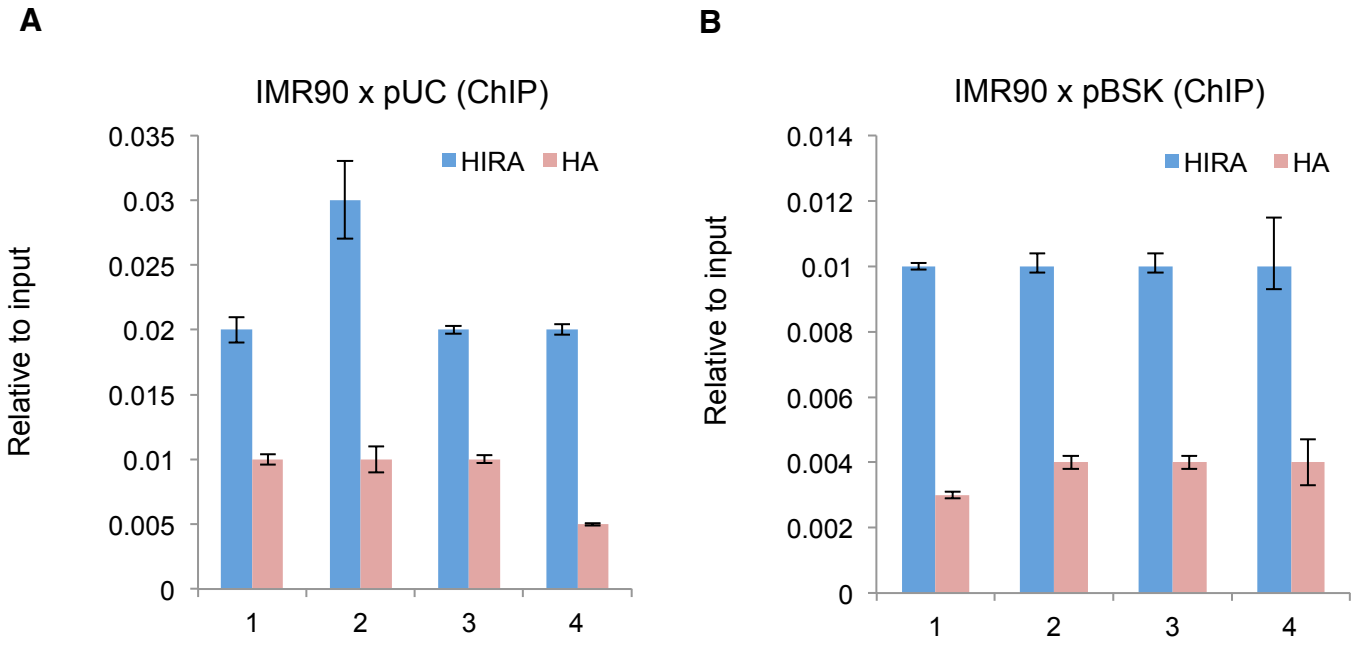
**Figure S3**

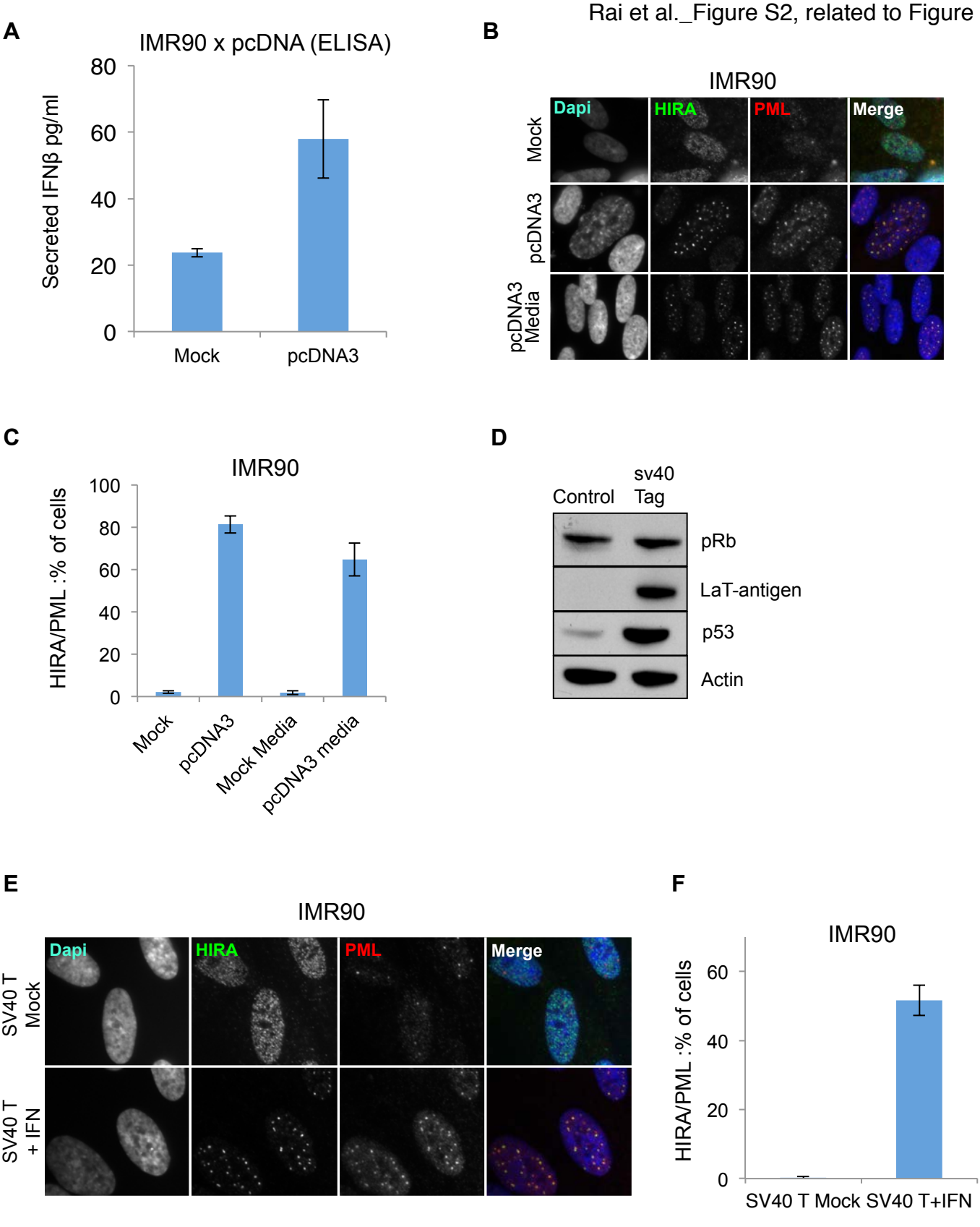
**(A)** Venn diagram showing the overlap between genes that change in expression (FDR <= 5%) between 0h-6h of IFN-b (red circle) and between 6h-24h of IFN-b (blue circle) in shLuc IMR90 cells. **(B)** As B, however also showing the overlap with canonical IFN-β target genes (green circle) (https://interferome-v1.erc.monash.edu.au/). The empirical p value for the overlap between 0h-6h IFN-b and IFN-β target genes was <0.001, and between 6h-24h IFN-b and IFN-β target genes was <0.001 **(C)** Representative UCSC plot of the IFITM1 gene. Showing expression by RNA-seq (top four tracks) and HIRA enrichment by ChIP-seq (bottom two tracks). The Y-axis represents library normalized read count for each track. For gene track, intron is shown as thin horizontal line, exons as thick lines (5' and 3' UTR intermediate thickness). 0hr (untreated) and 24hr post-treatment with IFN-β. **(D)** Scatter plot comparing the change in promoter (TSS+/-1kb) HIRA enrichment with change in gene expression after IFN-β treatment for 24h. HIRA ChIP-seq signals have been normalised to input control. Showing only those genes from the grey highlighted section in Figure 3B (expression fold >= 2.5 and HIRA fold >=1). **(E)** Venn diagram showing the overlap between canonically up regulated IFN-β target genes (red circle, IFN-β Up) and the genes within the highlighted section of Figure 3B (blue circle, HIRA+, Exp+). The 14 genes in the overlap are ISG15, IFI44, IFI6, IFIT2, IFIT3, IFIT1, IFIT5, OAS1, OAS2, OASL, BST2, NMI, STAT1, MX2. The empirical p value for the overlap is <0.001 the fold enrichment is 33.17. **(F)** Representative UCSC plot of the HIRA gene. Showing gene expression by RNA-seq in control (shLuc) and HIRA knockdown (shHIRA). The Y-axis represents library normalized read count for each track. For gene track, introns are shown as thin horizontal line, exons as thick lines. **(G)** IMR90 cells were infected with a lentivirus-encoded shRNA to HIRA

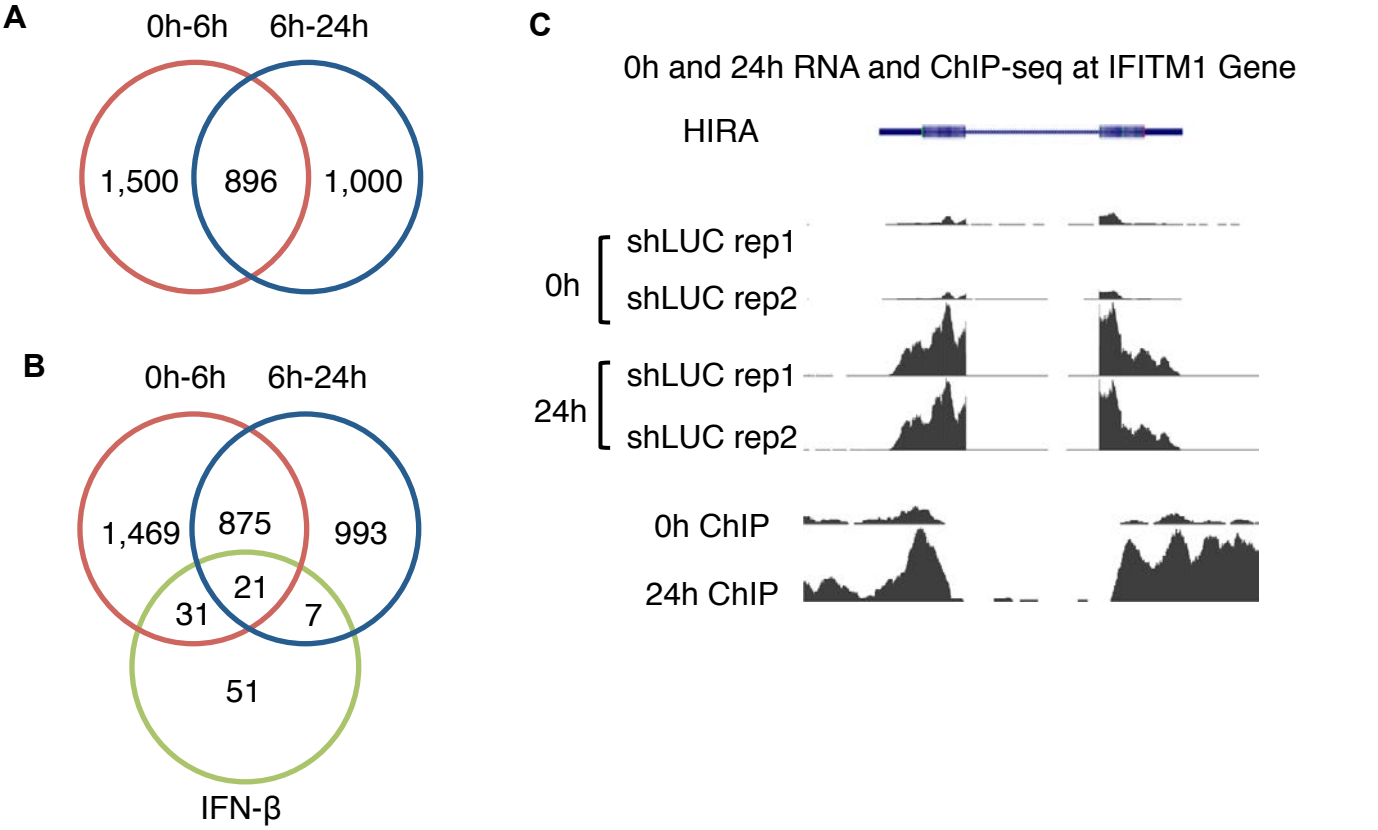
(shHIRA) or control (shLuc) and stable cells were generated by drug selection with puromycin. Cells were treated with 2000U/ml of IFN-β and fluorescent stained with antibodies to PML and H3. **(H)** Quantitation of cells with H3 localized to PML bodies from (E). Data are mean +/- SEM from three independent experiments, $p<0.05$ comparing increase in H3 foci in shLuc vs increase in H3 foci in shHIRA after IFN-β. **(I)** Principal component analysis (PCA) of shLuc and shHIRA RNA-seq samples at 0h (red), 6h (green) and 24h (blue) after IFN-β treatment. The plot was generated based on expression of each known coding gene by FPKM. **(J)** Bar chart showing the proportion of genes that change in expression (FDR <= 5%) in shLuc between 0h-6h IFN-β or in shLuc between 6h-24h IFN-β, that have a concordant expression profile (i.e. same direction of expression change) upon HIRA knock down.

**Figure S4**

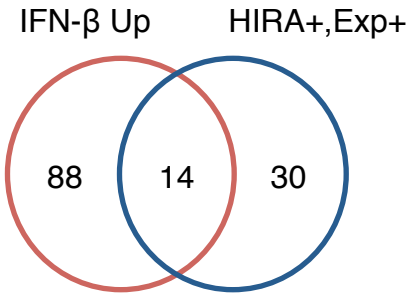
Control (*CAGG-Cre-ER*, WT, +tamoxifen) or Hira-deficient (*CAGG-Cre-ER*, *Hirafl/fl*, +tamoxifen) mice were infected with MCMV and liver harvested four days later. Plaque forming units (PFU) of MCMV recovered from liver samples of WT or *Hira*$^{-/-}$ mice were calculated as PFU/gram of tissue. Significance was assessed using the Mann Whitney-U test (n=9 for WT mice and n=18 for *Hira*$^{-/-}$mice).

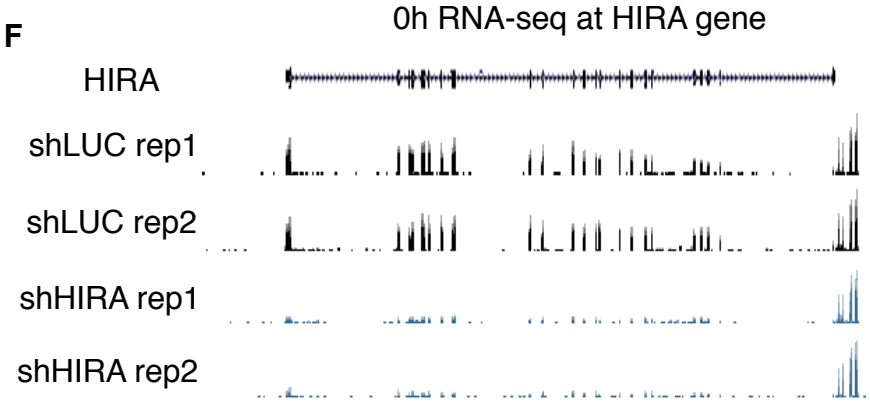

A
IMR90 x pUC (ChIP)
Relative to input
HIRA
HA
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
1
2
3
4
B
IMR90 x pBSK (ChIP)
Relative to input
HIRA
HA
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
1
2
3
4

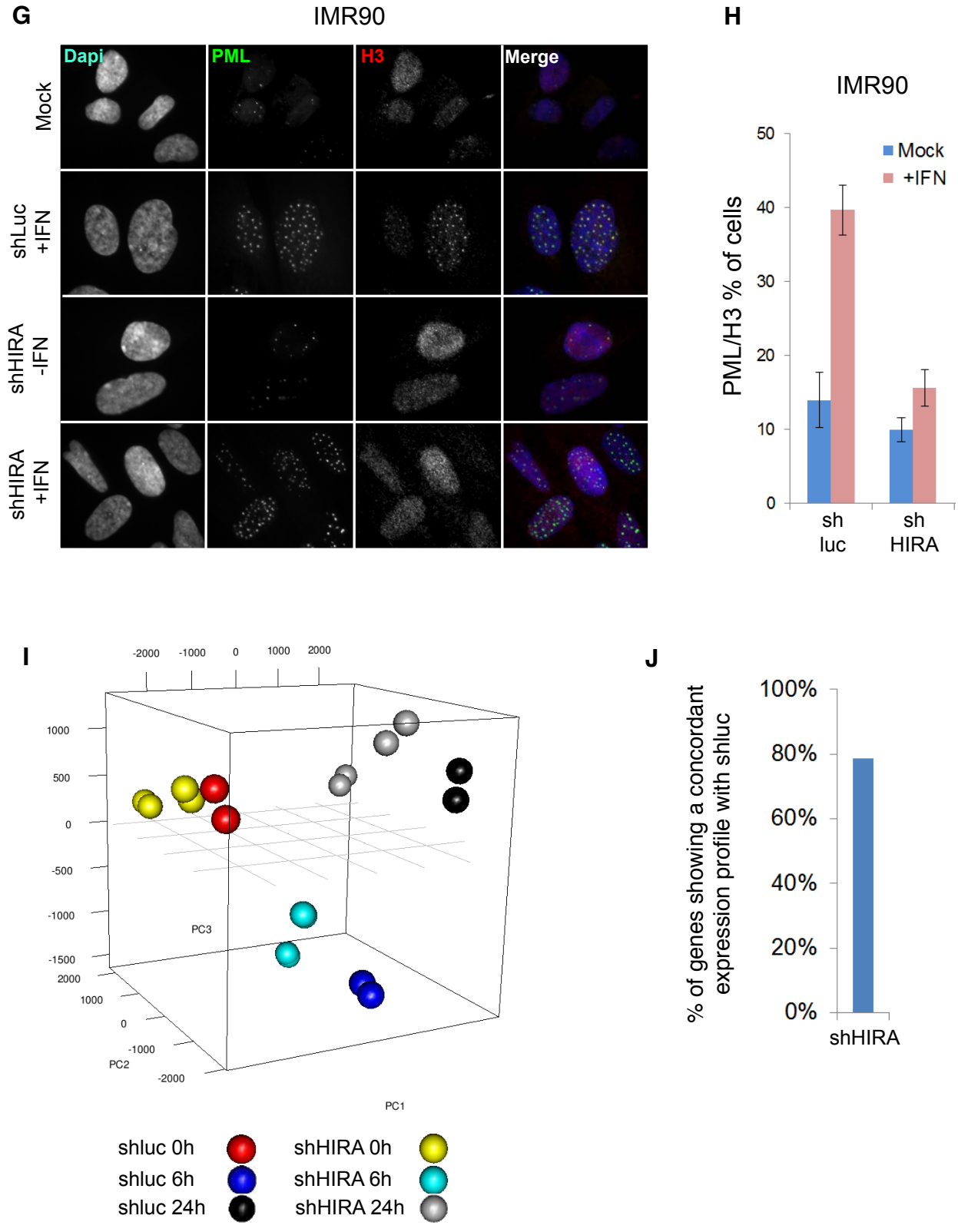

A
IMR90 x pcDNA (ELISA)
Secreted IFNβ pg/ml
80
60
40
20
0
Mock
pcDNA3
B
IMR90
Dapi
HIRA
PML
Merge
Mock
pcDNA3
pcDNA3 Media
C
IMR90
HIRA/PML :% of cells
100
80
60
40
20
0
Mock
pcDNA3
Mock Media
pcDNA3 media
D
sv40 Tag
Control
pRb
LaT-antigen
p53
Actin
E
IMR90
Dapi
HIRA
PML
Merge
SV40 T Mock
SV40 T + IFN
F
IMR90
HIRA/PML :% of cells
60
40
20
0
SV40 T Mock
SV40 T+IFN

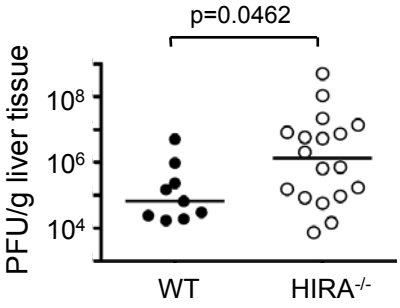

A
0h-6h
6h-24h
1,500
896
1,000
B
0h-6h
6h-24h
1,469
875
993
21
31
7
51
IFN-β
C
0h and 24h RNA and ChIP-seq at IFITM1 Gene
HIRA
0h
shLUC rep1
shLUC rep2
24h
shLUC rep1
shLUC rep2
0h ChIP
24h ChIP


D
Log2 fold difference in expression (24hr/0hr)
Log2 fold difference in HIRA ChIP (24hr/0hr)
0
2
4
6
8
10
12
14
0
1
2
3
4
5


E
IFN-β Up
HIRA+,Exp+
88
14
30


F
0h RNA-seq at HIRA gene
HIRA
shLUC rep1
shLUC rep2
shHIRA rep1
shHIRA rep2


G
IMR90
Dapi
PML
H3
Merge
Mock
shLuc +IFN
shHIRA -IFN
shHIRA +IFN
H
IMR90
PML/H3 % of cells
Mock
+IFN
sh luc
sh HIRA
I
PC1
PC2
PC3
shluc 0h
shluc 6h
shluc 24h
shHIRA 0h
shHIRA 6h
shHIRA 24h
J
% of genes showing a concordant expression profile with shluc
shHIRA


p=0.0462
PFU/g liver tissue
$10^8$
$10^6$
$10^4$
WT
HIRA$^{-/-}$

## 1. Supplemental Tables and Data

### Table S1. RNA sequencing

| Sample | Timepoint | Replicate | Raw reads | Aligned reads (% of raw reads) | Non-duplicate reads (% of aligned reads) |
|---|---|---|---|---|---|
| shLUC | 0 | 1 | 37,243,231 | 35,362,419 (94.95%) | 30,803,490 (87.11%) |
| shLUC | 0 | 2 | 40,109,120 | 38,330,377 (95.57%) | 33,057,866 (86.24%) |
| shLUC | 6 | 1 | 39,568,364 | 37,921,837 (95.84%) | 32,549,338 (85.83%) |
| shLUC | 6 | 2 | 41,879,610 | 40,271,287 (96.16%) | 34,454,279 (85.56%) |
| shLUC | 24 | 1 | 75,034,469 | 71,852,202 (95.76%) | 61,435,186 (85.50%) |
| shLUC | 24 | 2 | 85,794,160 | 81,961,651 (95.53%) | 70,046,554 (85.46%) |
| shHIRA1 | 0 | 1 | 40,970,155 | 39,232,567 (95.76%) | 32,264,776 (82.24%) |
| shHIRA1 | 0 | 2 | 38,309,633 | 36,583,400 (95.49%) | 29,777,717 (81.40%) |
| shHIRA1 | 6 | 1 | 41,063,476 | 39,295,686 (95.69%) | 32,116,387 (81.73%) |
| shHIRA1 | 6 | 2 | 40,300,138 | 38,550,705 (95.66%) | 31,409,101 (81.47%) |
| shHIRA1 | 24 | 1 | 39,603,935 | 37,738,917 (95.29%) | 30,637,502 (81.18%) |
| shHIRA1 | 24 | 2 | 78,388,480 | 74,748,607 (95.36%) | 60,495,638 (80.93%) |

### Table S2. ChIP sequencing

| Sample | Raw reads | Aligned reads (% of raw reads) | Uniquely Aligned Reads (% of Aligned Reads) | Duplicate Reads (% of Uniquely Aligned Reads) |
|---|---|---|---|---|
| HIRA IFN- | 58,875,273 | 56,415,129 (95.82%) | 41,774,709 (74.05%) | 2,800,932 (6.70%) |
| HIRA IFN+ | 69,045,926 | 65,592,946 (95.00%) | 47,064,100 (71.75%) | 3,053,906 (6.49%) |
| HIRA IFN- Input | 72,935,989 | 71,742,243 (98.36%) | 54,554,023 (76.04%) | 2,006,335 (3.68%) |
| HIRA IFN+ Input | 32,549,655 | 32,038,804 (98.43%) | 24,308,580 (75.87%) | 854,717 (3.52%) |

### Table S3. primer sequences

| | |
|---|---|
| pCDNA3F1 | AACGCCAATAGGGACTTTCC |
| pCDNA3R1 | GGGCGTACTTGGCATATGAT |
| pCDNA3F2 | TAGTTGCCAGCCATCTGTTG |
| pCDNA3R2 | GCGATGCAATTTCCTCATTT |
| pCDNA3F3 | GGCGGTAATACGGTTATCCA |
| pCDNA3R3 | TTTTTGTGATGCTCGTCAGG |
| pCDNA3F4 | TTGCCGGGAAGCTAGAGTAA |
| pCDNA3R4 | AAGCCATACCAAACGACGAG |
| pUC F1 | GGTGTGAAATACCGCACAGA |
| pUC R1 | CTGGCGTAATAGCGAAGAGG |
| pUC F2 | GGCGCTTTCTCATAGCTCAC |
| pUC R2 | GTCTTACCGGGTTGGACTCA |
| pUC F3 | TTTGTTTGCAAGCAGCAGAT |
| pUC R3 | CGTGAGTTTTCGTTCCACTG |
| pUC F4 | TTGCCGGGAAGCTAGAGTAA |
| pUC R4 | AAGCCATACCAAACGACGAG |
| pBSK F1 | AATTTCCATTCGCCATTCAG |
| pBSK R1 | AACGTCGTGACTGGGAAAAC |
| pBSK F2 | GGCGGTAATACGGTTATCCA |
| pBSK R2 | TTTTTGTGATGCTCGTCAGG |

| | |
|---|---|
| pBSK F3 | GGCGCTTTCTCATAGCTCAC |
| pBSK R3 | GTCTTACCGGGTTGGACTCA |
| pBSK F4 | AAGTTGGCCGCAGTGTTATC |
| pBSK R4 | CGCCGCATACACTATTCTCA |
| HSV F2 | ACTTAATCAGGTTGTTGCCG |
| HSV R2 | GAAGTTGTGGACTGGGAAGG |
| HSV F3 | CTGGACGATAAGTCGGTGGAA |
| HSV F3 | AGTGTGCTCGTCGTCTCCTGT |
| HSV F4 | ATTAGCGTAAGGATGATGGT |
| HSV R4 | AGACCTCAACGTGCTGTACT |
| HSV F5 | CGCCGGTGTGTGATGATTT |
| HSV R5 | TTTATACCCGGGCCCCAT |
| HSV F6 | AGATCTGCGGCACGCTGTTG |
| HSV R6 | CAGCTGCTTCATCCCCGTGG |