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Supplementary Information

Interferon Stimulation Creates Chromatin Marks And Establishes Transcriptional Memory

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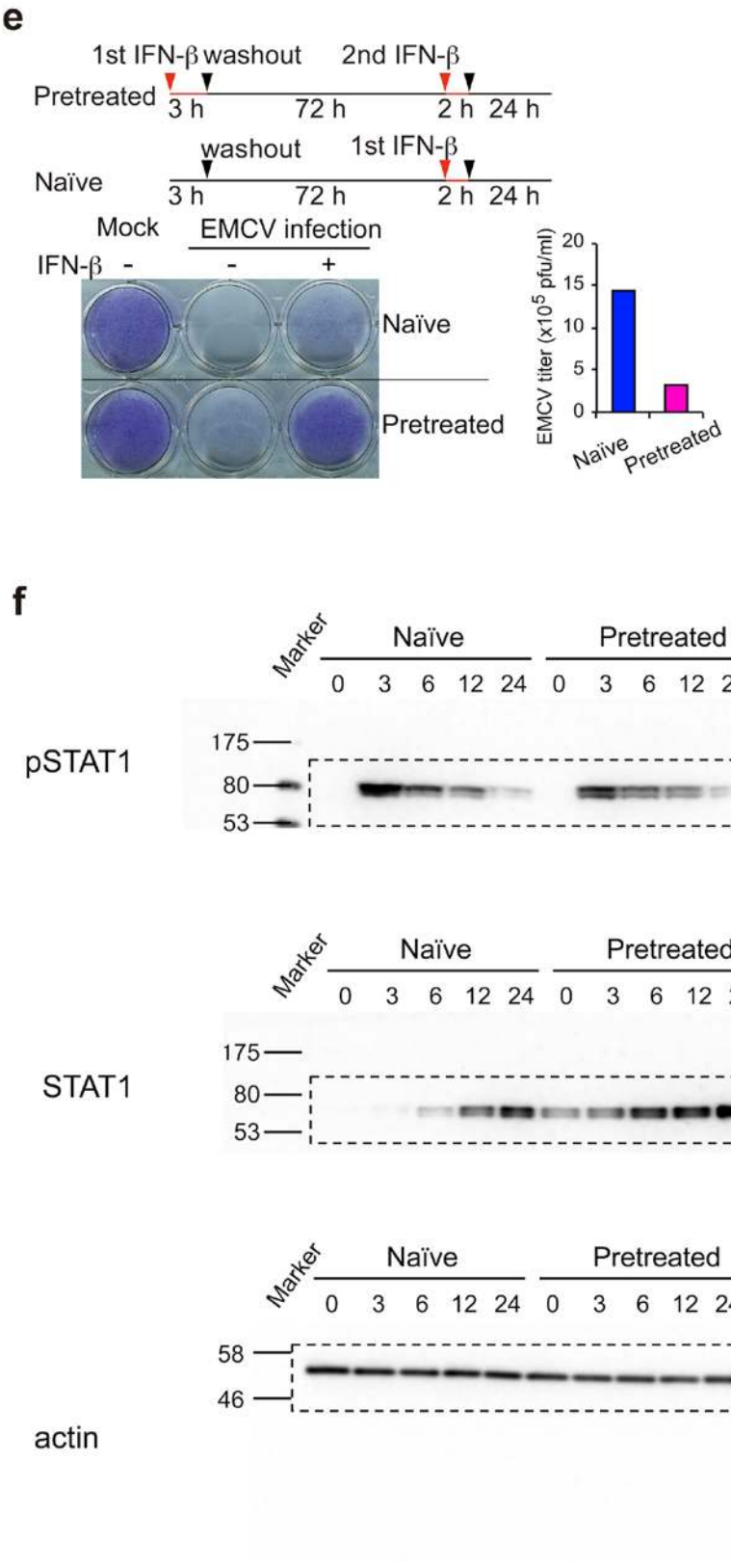
Supplementary Fig. 1 (a-d)

(a) Naïve and pretreated cells were stimulated with IFN as in Fig. 1a. mRNA expression of indicated ISGs were detected by qRT-PCR, normalized by Gapdh, and expressed as fold induction. Values represent the average of three experiments ± S.D.

(b) Top: experimental design. Naïve and pretreated cells were left without IFN for 24 h to 96 h and stimulated with IFN for 3h. ISG mRNAs were detected as above.

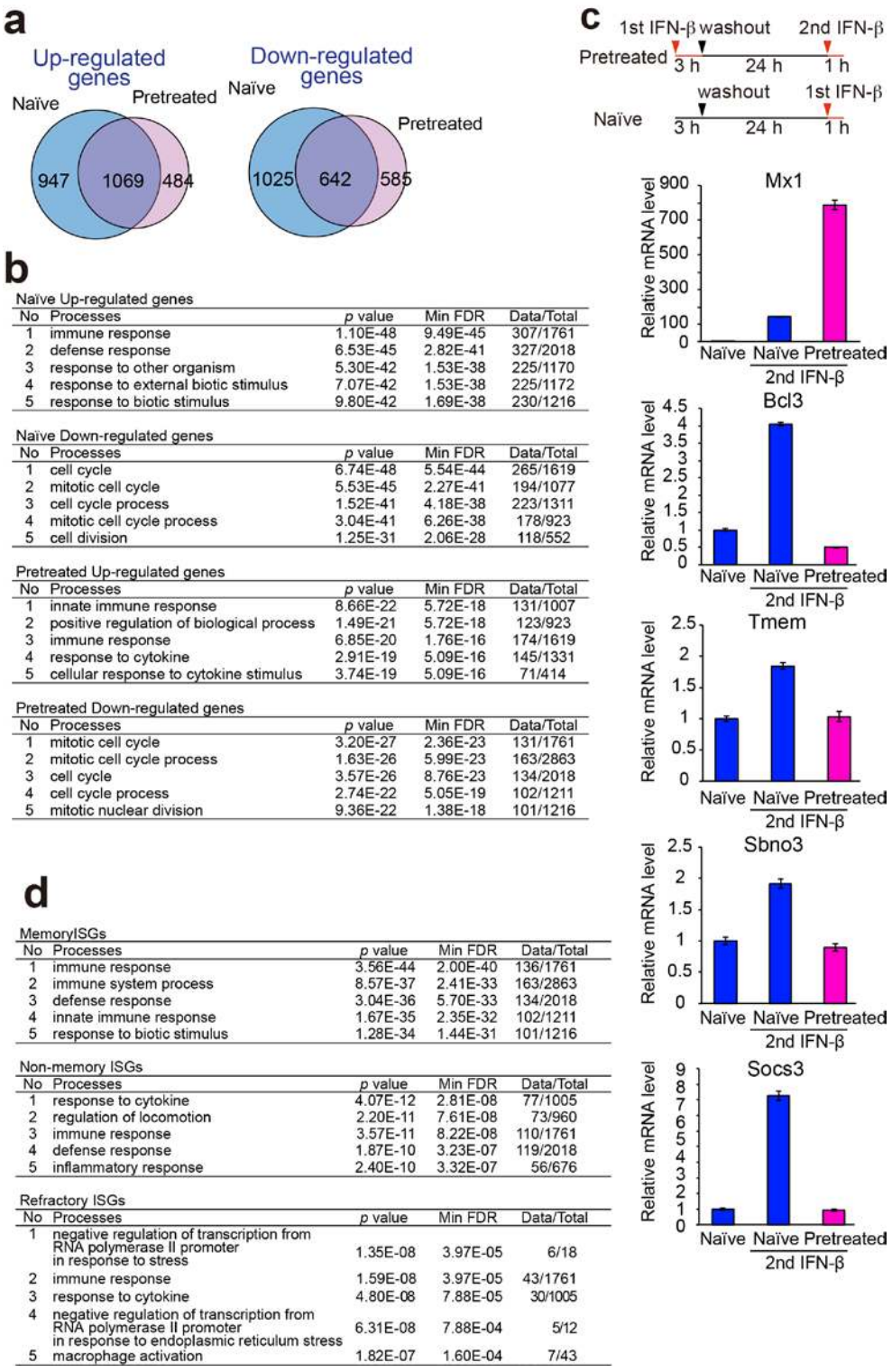
(c) Naïve and pretreated cells were stimulated with IFN for indicated times (h) as in Fig. 1a. Nascent ISG RNAs were detected by qRT-PCR using primers covering an exon-intron boundary[1]**.**

(d) NIH3T3 cells were stimulated with IFN for 24 h and followed by 48 h incubation without IFN. Naïve and pretreated cells were stimulated with IFN for indicated times (h) and mRNA expression of indicated ISGs were detected by qRT-PCR, normailzed by Gapdh. Values represent the average of three experiments ± S.D.



Supplementary Fig. 1 (e,f)

(e) Top: experimental design. Naïve and pretreated cells were treated with IFN for 2 h and then infected with EMCV (moi=10) for 24 h. Cell survival was assessed by crystal violet staining (left) and EMCV viral yields in the culture supernatants were determined by plaque assay (right).
(f) Uncropped images for western blots in Fig. 1g. Dotted box marks the borders of the cropped images for each protein. Marker: molecular weight marker (kDa).



b

Naïve Up-regulated genes

No	Processes	*p* value	Min FDR	Data/Total
1	immune response	1.10E-48	9.49E-45	307/1761
2	defense response	6.53E-45	2.82E-41	327/2018
3	response to other organism	5.30E-42	1.53E-38	225/1170
4	response to external biotic stimulus	7.07E-42	1.53E-38	225/1172
5	response to biotic stimulus	9.80E-42	1.69E-38	230/1216

Naïve Down-regulated genes

No	Processes	*p* value	Min FDR	Data/Total
1	cell cycle	6.74E-48	5.54E-44	265/1619
2	mitotic cell cycle	5.53E-45	2.27E-41	194/1077
3	cell cycle process	1.52E-41	4.18E-38	223/1311
4	mitotic cell cycle process	3.04E-41	6.26E-38	178/923
5	cell division	1.25E-31	2.06E-28	118/552

Pretreated Up-regulated genes

No	Processes	*p* value	Min FDR	Data/Total
1	innate immune response	8.66E-22	5.72E-18	131/1007
2	positive regulation of biological process	1.49E-21	5.72E-18	123/923
3	immune response	6.85E-20	1.76E-16	174/1619
4	response to cytokine	2.91E-19	5.09E-16	145/1331
5	cellular response to cytokine stimulus	3.74E-19	5.09E-16	71/414

Pretreated Down-regulated genes

No	Processes	*p* value	Min FDR	Data/Total
1	mitotic cell cycle	3.20E-27	2.36E-23	131/1761
2	mitotic cell cycle process	1.63E-26	5.99E-23	163/2863
3	cell cycle	3.57E-26	8.76E-23	134/2018
4	cell cycle process	2.74E-22	5.05E-19	102/1211
5	mitotic nuclear division	9.36E-22	1.38E-18	101/1216

d

MemoryISGs

No	Processes	*p* value	Min FDR	Data/Total
1	immune response	3.56E-44	2.00E-40	136/1761
2	immune system process	8.57E-37	2.41E-33	163/2863
3	defense response	3.04E-36	5.70E-33	134/2018
4	innate immune response	1.67E-35	2.35E-32	102/1211
5	response to biotic stimulus	1.28E-34	1.44E-31	101/1216

Non-memory ISGs

No	Processes	*p* value	Min FDR	Data/Total
1	response to cytokine	4.07E-12	2.81E-08	77/1005
2	regulation of locomotion	2.20E-11	7.61E-08	73/960
3	immune response	3.57E-11	8.22E-08	110/1761
4	defense response	1.87E-10	3.23E-07	119/2018
5	inflammatory response	2.40E-10	3.32E-07	56/676

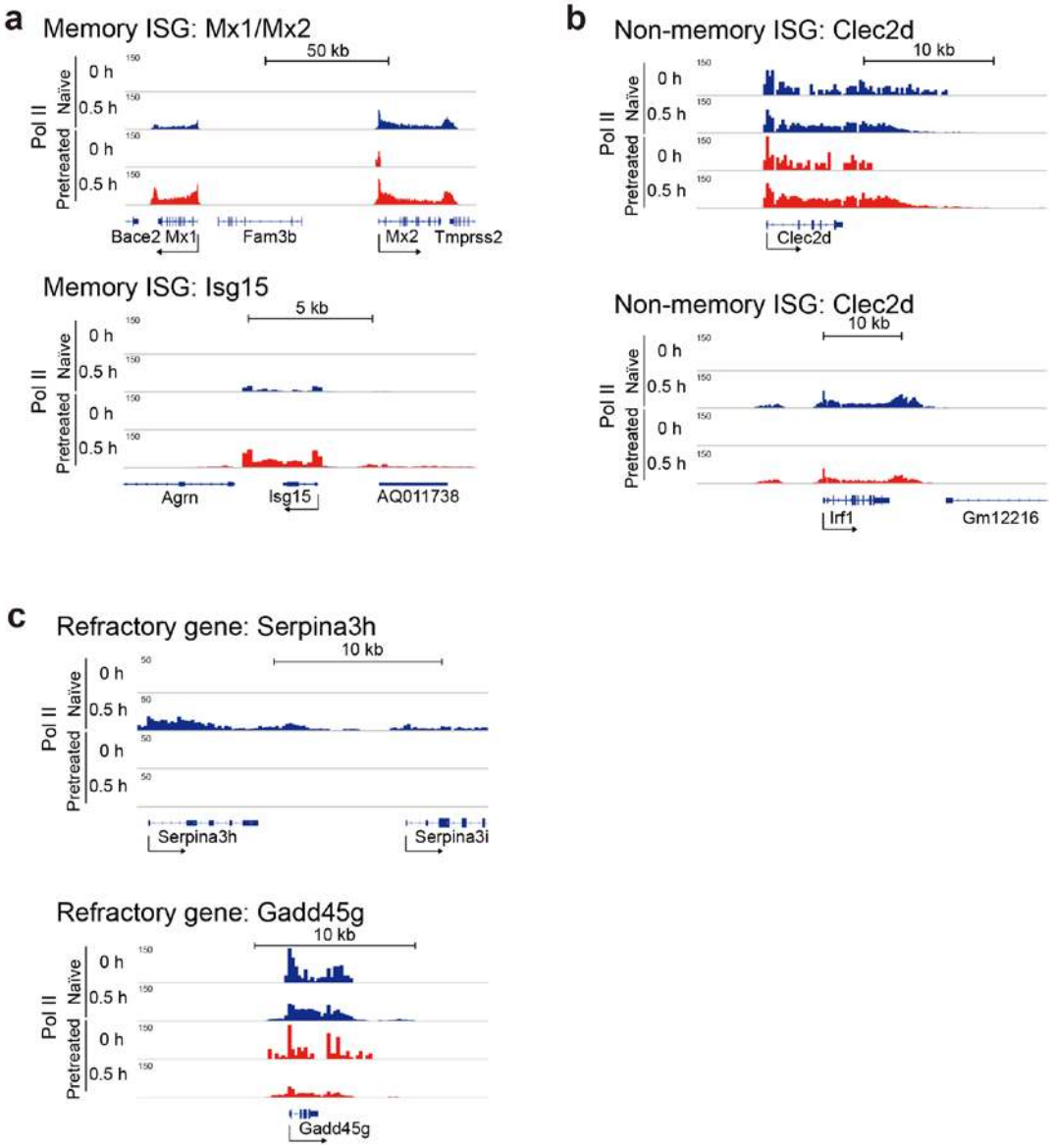
Refractory ISGs

No	Processes	*p* value	Min FDR	Data/Total
1	negative regulation of transcription from RNA polymerase II promoter in response to stress	1.35E-08	3.97E-05	6/18
2	immune response	1.59E-08	3.97E-05	43/1761
3	response to cytokine	4.80E-08	7.88E-05	30/1005
4	negative regulation of transcription from RNA polymerase II promoter in response to endoplasmic reticulum stress	6.31E-08	7.88E-04	5/12
5	macrophage activation	1.82E-07	1.60E-04	7/43

Supplementary Fig. 2

(a) Venn diagrams depicting the number of genes upregulated (left) or downregulated by IFNβ (cut off for upregulated genes, > 2.0, p< 0.05, downregulated genes <2.0, p<0.05, at least one time point between 1, 2, 4, and 6 h over 0 h).

(b) GO annotation of genes upregulated or downregulated by IFN stimulation in naïve and pretreated cells. Analysis was performed with the MetaCore program at https://portal.genego.com/. The tables show the list of the most significant processes in upregulated or downregulated genes found in naive or pretreated cells.
(c) Top, left: experimental design. mRNA expression of refractory ISGs identified by RNA-seq (Bcl3, Tmem, Sbno3, Socs3) was tested by qRT-PCR for independently prepared samples. The values represent the average of three experiments ± S.D.
(d) GO analysis was performed for memory, non-memory and refractory ISGs. The tables show the list of the most significant processes in the three types of ISGs.

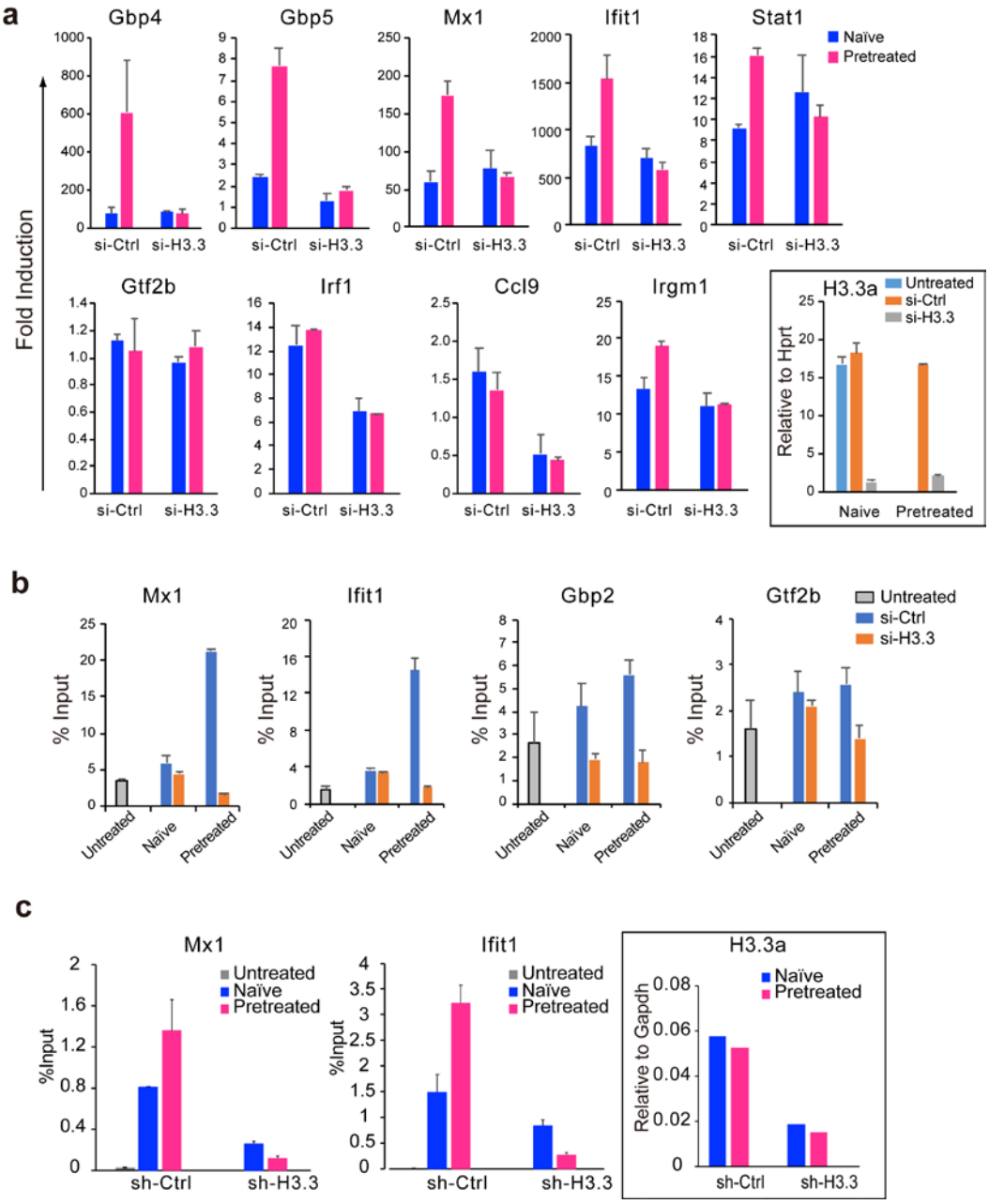


Supplementary Fig. 3

(a) IGV depiction of distribution of Pol II over the indicated memory ISGs (Mx1, Mx2, and Isg15) in naive (blue) and pretreated (red) cells.

(b) IGV data for distribution of Pol II over the non-memory ISGs (Clec2d and Irf1) in naïve (blue) and pretreated (red) cells.

(c) IGV data for Pol II over the refractory genes (Serpina3h and Gadd45g) in naive (blue) and pretreated (red) cells.



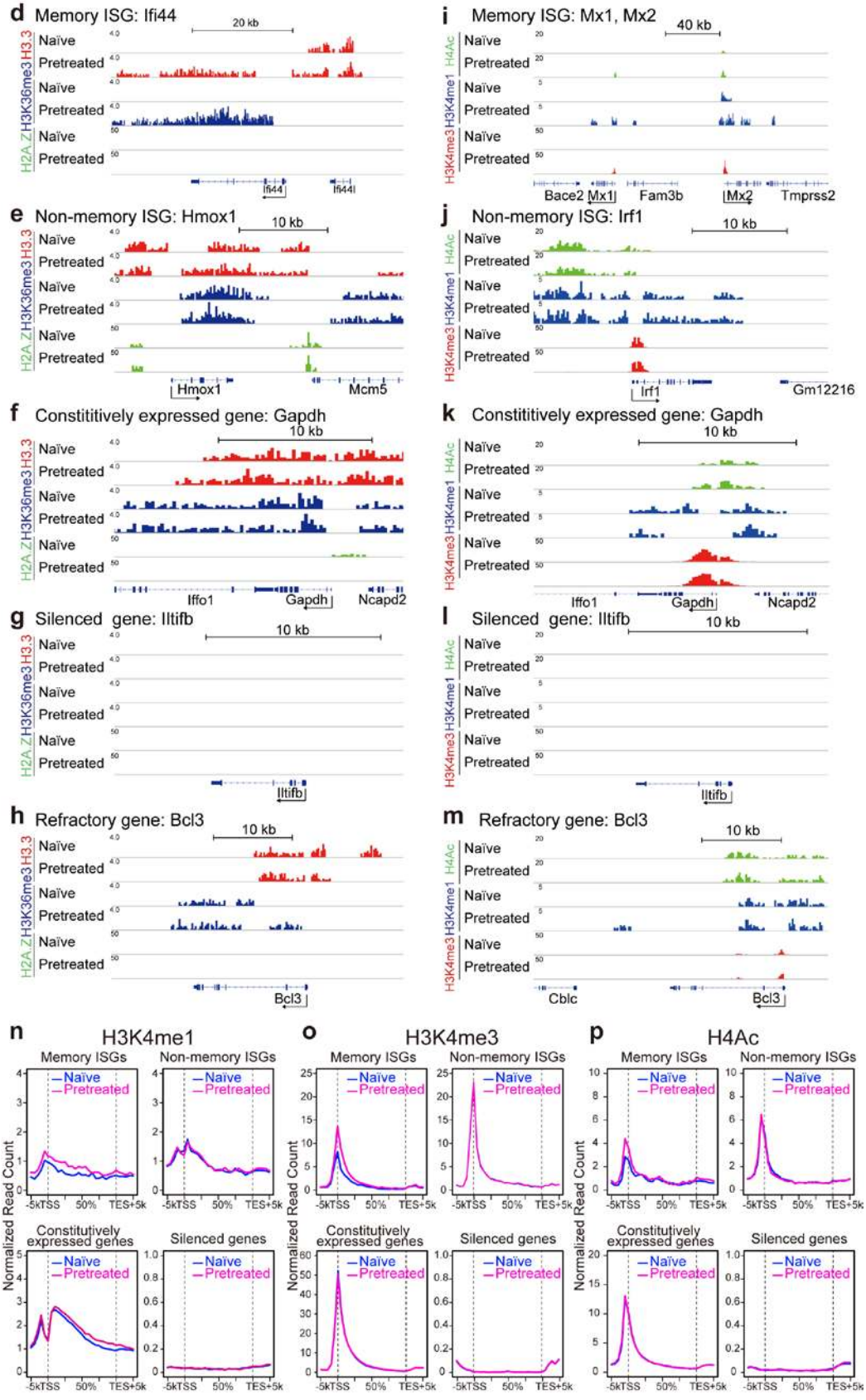
Supplementary Fig. 4(a-c)

(a) Naïve MEF cells were transfected with 50 nM of H3.3 specific, or control siRNA using Lipofectamine 2000 (Invitrogen) for 48 h prior to IFN stimulation. Pretreated MEFs (3h IFN pretreatment) were incubated with control or H3.3 specific siRNA during IFN washout as above for 48 h. siRNA treated cells were stimulated by IFN for 1h. Levels of mRNA induction were measured by qRT-PCR for indicated ISGs. Values (expressed as fold induction relative to untreated cells) represents the average of triplicate samples

from a representative experiments +/- S.D. The effect of siRNA was tested in three independently performed experiments, which gave very similar results.

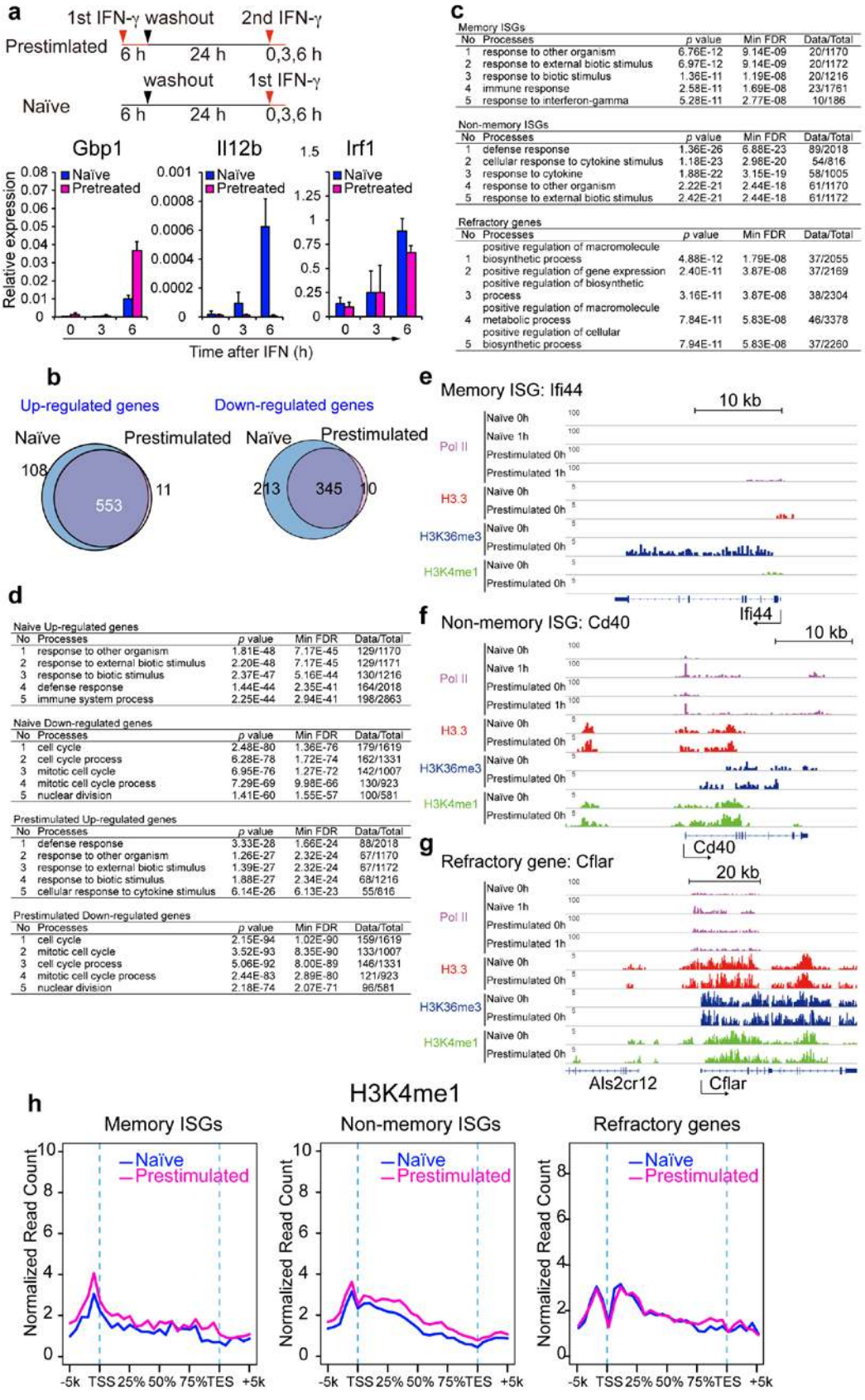
(b) Naïve and pretreated cells were incubated with siRNA exactly as above, and chromatin was prepared 30 min after IFN stimulation. qChIP was performed for RNA Pol II binding at/near the TSS of indicated ISGs. We used the antibody as used in Figure 4 (a, d) and Figure 5 (e). Data represents the average of three independently precipitated samples +/- S.D. **(c)**

(c) Naïve and pretreated cells (3 h pretreated and 48 h interval time) of control- or H3.3-knocked down MEFs via shRNAs, were re-stimulated with IFN for 30 min and Pol II-qChIP assays were performed to detect binding of Pol II at the TSS/promoter region of indicated memory ISGs. Data expressed as % of input. The data represent the mean ± S.D. of triplicate samples. Representative data from one of two independent experiments are shown. Knockdown efficiency by shRNAs determined by RT-qPCR was shown in right panel.



Supplementary Fig. 4(d-p)

(d-h) IGV images of the distribution of H3.3 (red), H3K36me3 (blue), and H2A.Z (green) over the memory ISG (Ifi44, d), non-memory ISG (Hmox1, e), constitutively expressed gene (Gapdh, f), silenced gene (Iltifb, g), and refractory ISG (Bcl3, h).
(i-m) IGV images of the distribution of H4ac (red), H3K4me1 (blue), and H3K4me3 (green) over indicated memory ISGs (Mx1 and Mx2, i), non-memory ISG (Irf1, j), constitutively expressed gene (Gapdh, k), silenced gene (Iltifb, l), and refractory gene (Bcl3, m).
(n-p) Genome-wide distribution of H3K4me1 (n), H3K4me3 (o), and H4ac (p) over indicated memory ISGs, non-memory ISGs (top panels), constitutively expressed genes, or silent genes (bottom panels).



Supplementary Fig. 5

(a) Experimental design (top). Naïve and pretreated macrophages were stimulated with IFNγ for 3 h and 6 h. ISG mRNA expression was detected by qRT-PCR and normalized by Gapdh. Gbp1, Il12b, Irf1 are representative of memory, refractory and non-memory ISG, respectively. The values represent the average of three assays ± SD.
(b) Venn diagrams depicting the number of genes upregulated (left) or downregulated by IFNγ (cut off for upregulated genes, > 2.0, $p < 0.05$, downregulated genes <2.0, $p<0.05$). Some ISGs showing expression above untreated levels at time 0 in prestimulated macrophages were removed from further analysis.
(c) GO analysis of genes upregulated or downregulated by IFNγstimulation in naïve and pretreated macrophages carried out with MetaCore program. The tables represent the list of the most significant processes.
(d) GO analysis of memory, non-memory, and refractory ISGs identified in macrophages (Fig 6C). The tables represent the list of the most significant processes for three types of ISGs in macrophages.
(e-g) IGV data for the distribution of Pol II (magenta), H3.3 (red), H3K36me3 (blue), and H3K4me1 (green) over the memory ISG (Ifi44, e), non-memory ISG (Cd40, f), refractory gene (Cflar, g).
(h) Genome-wide distribution of H3K4me1 in naïve (blue) and pretreated (magenta) macrophages over memory ISGs (left), non-memory ISGs (middle), and refractory genes (right).

References

[1]Patel, M.C. *et al.* BRD4 coordinates recruitment of pause release factor P-TEFb and the pausing complex NELF/DSIF to regulate transcription elongation of interferon-stimulated genes. *Molecular and cellular biology* **33**, 2497-2507 (2013).