Supplementary Figure legends

Supplementary Figure 1.

Experimental workflow.

Supplementary Figure 2.

IRF9 binding to promoters.

a) Verification of mIRF9 antibody by site-directed ChIP. IFNβ-stimulated binding of IRF9 to the ISRE sequences of Mx2 was analyzed using BMDMs of WT and Irf9$^{-/-}$ (IRF9-/-) mice. Cells were treated with 250 IU/ml of IFNβ for 1.5h. Data represent mean and SEM values of three independent experiments. *P*-values were calculated using the paired ratio *t*-test (*$P \leq 0.05$; **$P \leq 0.01$, ***$P \leq 0.001$).

b) Browser tracks showing complexes assigned as STAT-IRF9 in IFNγ treated wild type BMDMs. Input, STAT2, IRF9 (scale 0-200). STAT1 (scale 0-150).

Supplementary Figure 3.

Experimental system for BioID.

a) Kinetics of STAT1, STAT2 and IRF9 synthesis in Raw 264.7 macrophages and wild type BMDMs treated with 250 IU/ml as indicated. Whole-cell extracts were tested in western blot for STAT1 phosphorylation at Y701 and of STAT2 at Y689 as well as total STAT1, STAT2, IRF9 and GAPDH levels. The blots are representative of three independent experiments. b) Irf9-/- mouse embryonic fibroblasts (MEFs) were transiently transfected with the indicated expression vectors, including constitutively active IRF7-M15. One day after transfection, RNA was isolated and *Mx2* expression determined by qPCR. c) Myc-BirA*-IRF9 transgenic Raw 264.7 were treated with increasing amounts of doxycycline (dox) (0,2µg/ml, 0,4µg/ml, 0,6µg/ml, 0,8µg/ml, 1mg/ml) and 50µM biotin. Whole-cell extracts were collected and tested in western blot for levels of IRF9, MYC, and GAPDH. Biotinylated IRF9 was visualized via HRP-coupled streptavidin. d) Stat2-/- MEFs were transiently transfected with the indicated

expression vectors, including constitutively active IRF7-M15. One day after transfection, RNA was isolated and *Mx2* expression was determined by qPCR. e) Myc-BirA*-STAT2 transgenic Raw 264.7 macrophages were treated with increasing amounts of doxycycline as in f) and 50µM biotin. Whole-cell extracts were collected and tested in western blot for levels of STAT2, MYC, and GAPDH. Biotinylated STAT2 was visualized via HRP-coupled streptavidin.

Supplementary Figure 4.

IRF9 and STAT2 interactome

a), b) IRF9 or STAT2 interactors, either pre-associated or after IFN-β or IFN-γ treatment, were identified by streptavidin affinity purification and mass spectrometry. Proteins that displayed a threefold enrichment (in two biological replicates) in the samples of interest compared to the ligase control were counted as hits and are shown in the heatmaps. c) Multi-scatter plot of LFQ-Intensities, displaying Pearson-correlation of each biological replicate.

Supplementary table 1.

Genes bound by STAT1, STAT2-IRF9 and ISGF3 transcription factor complexes.

Supplementary table 2.

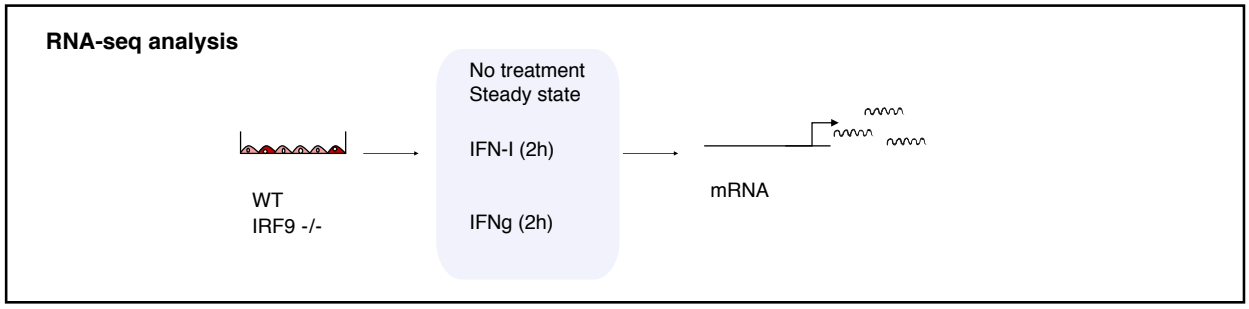
PRM analysis, mass spectrometry analysis and list of interactors.

Supplementary table 3.

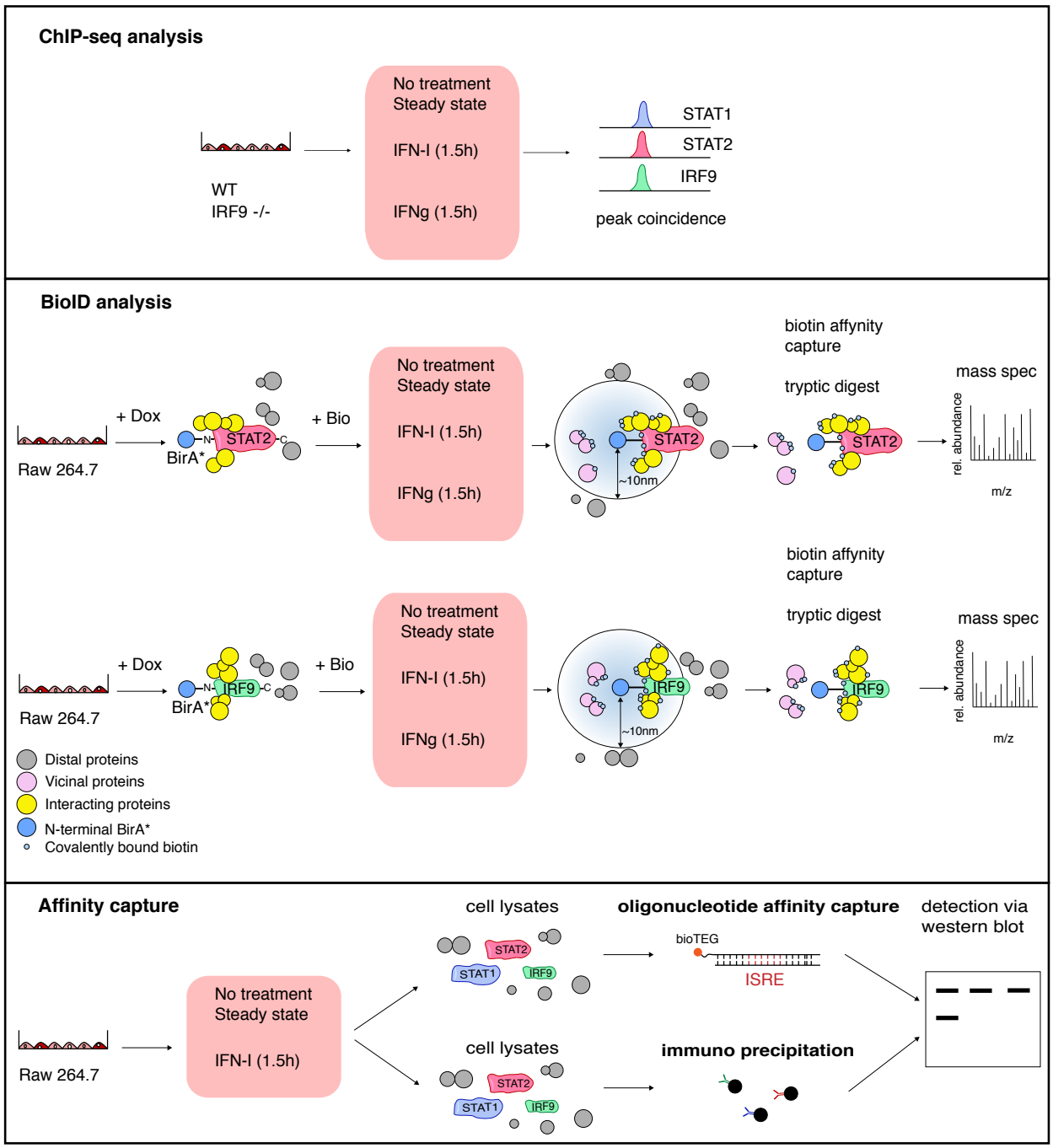
RT-qPCR and ChIP primer sequences.

Supplementary Figure 1

1) Expression analysis

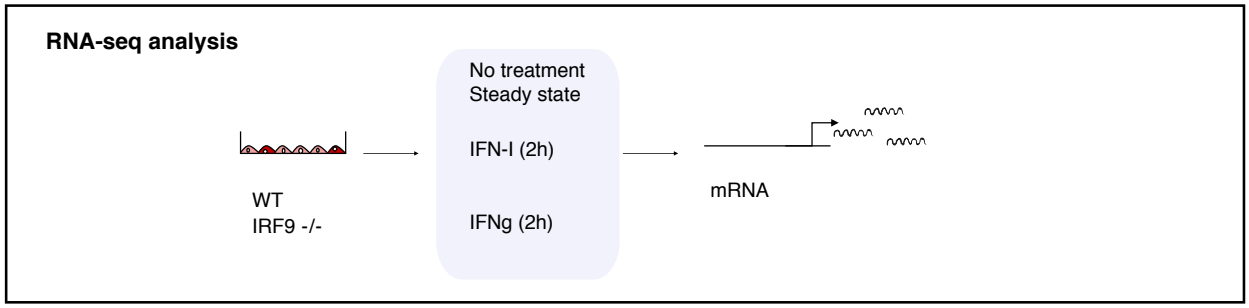


2) Transcription factor complexes

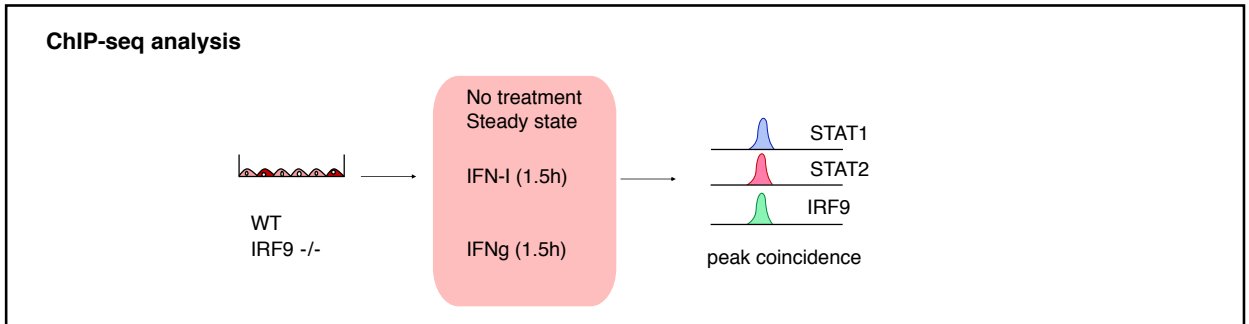


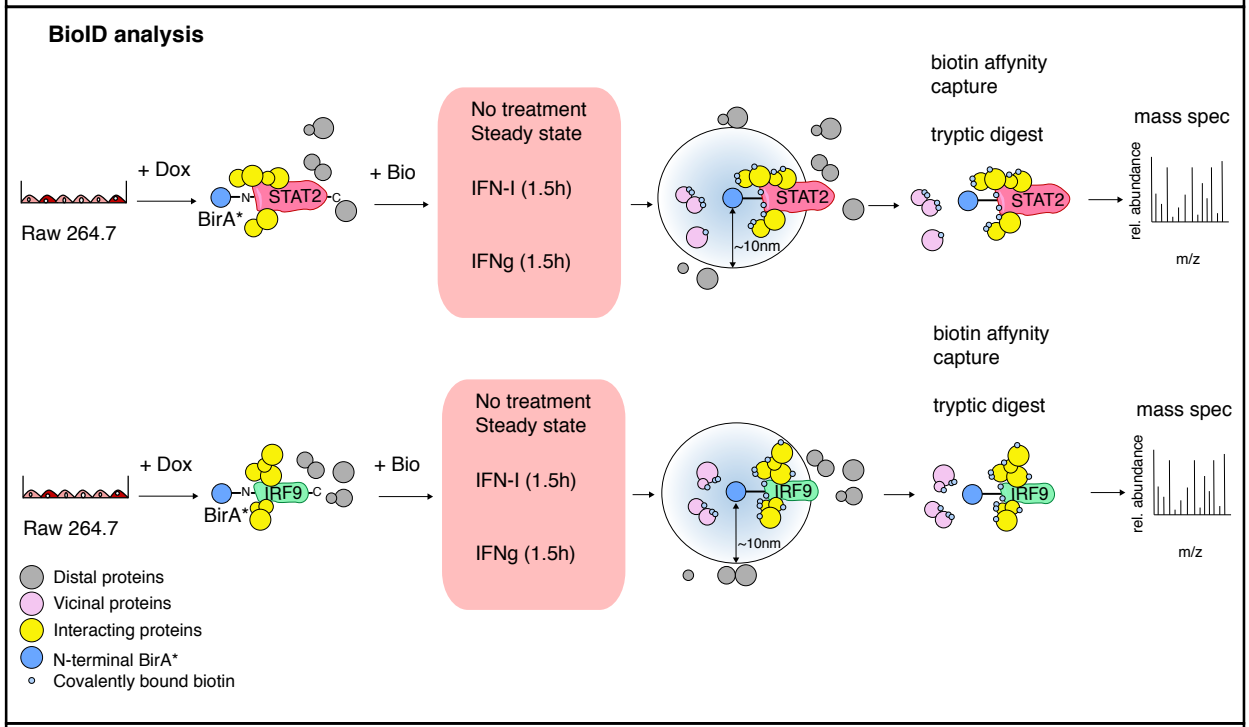
Supplementary Figure 1

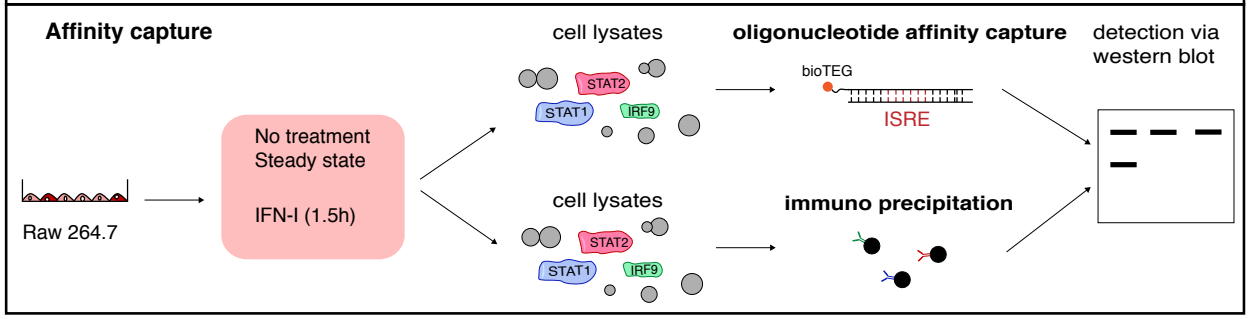
1) Expression analysis



2) Transcription factor complexes

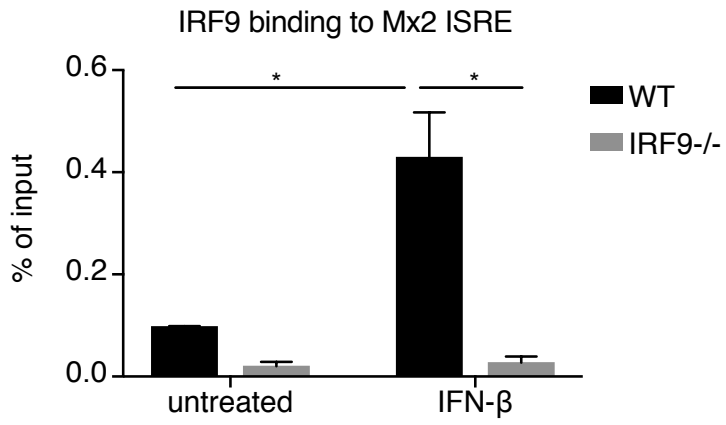




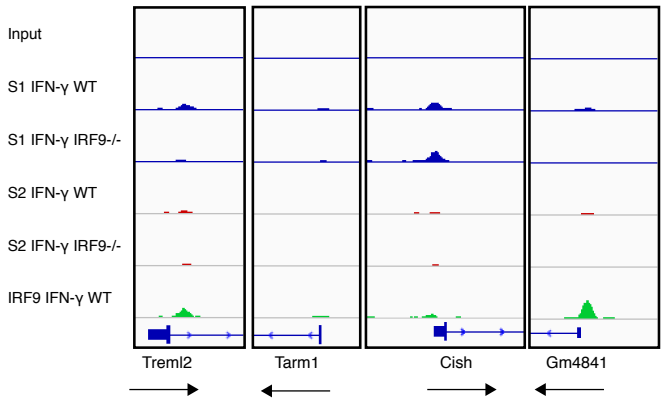


Supplementary Figure 2

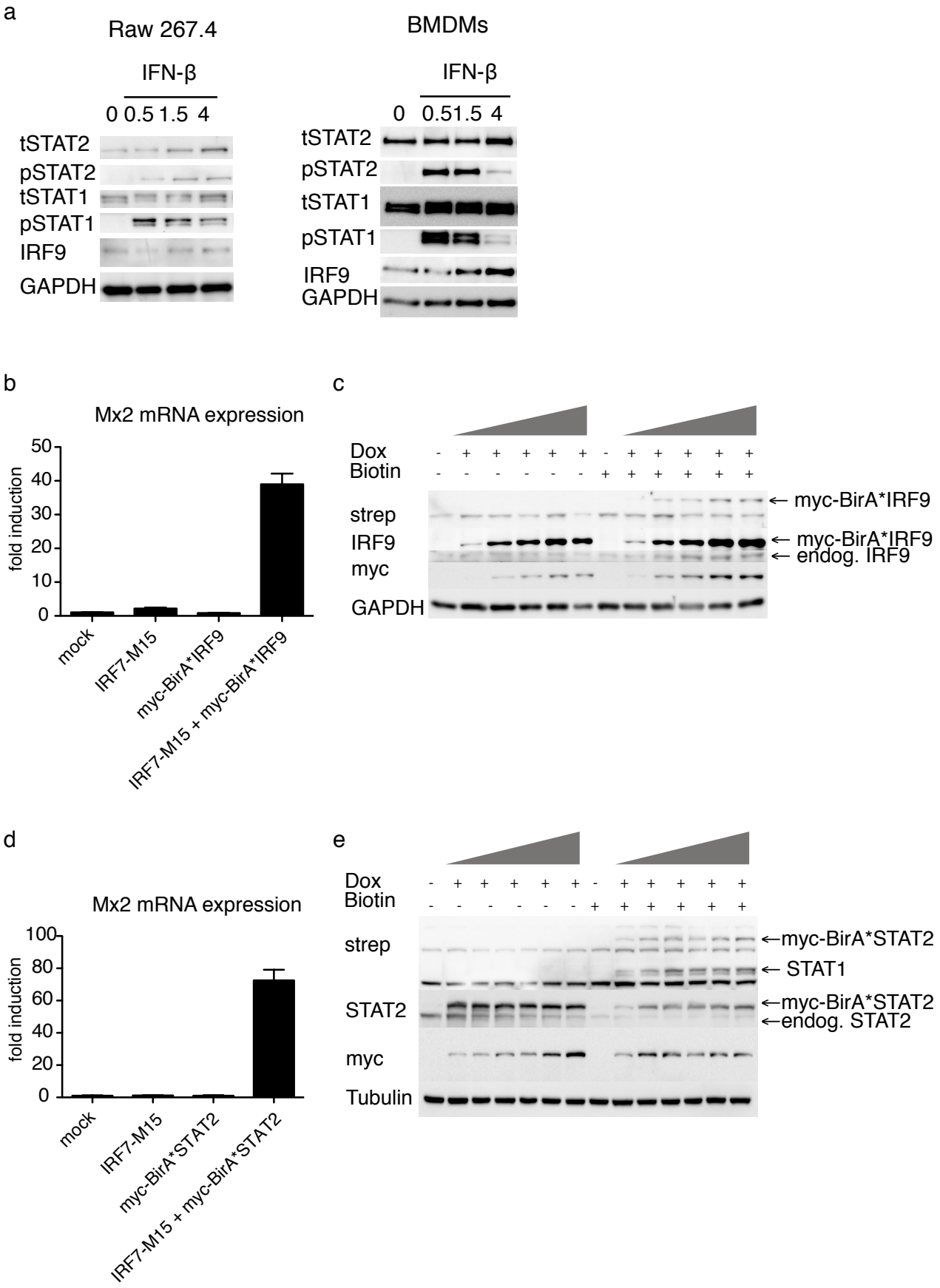
a



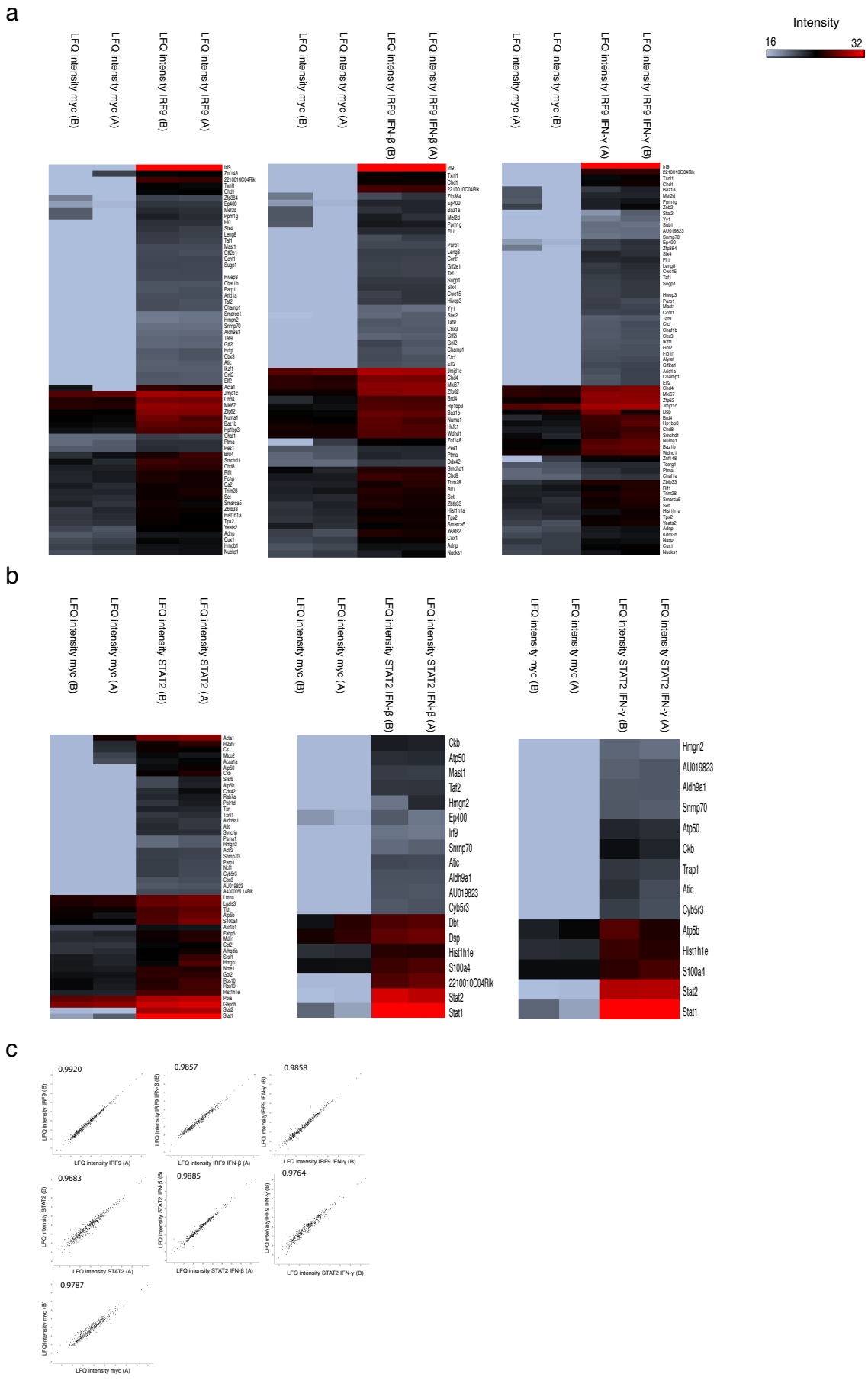
b



Supplementary Figure 3



Supplementary Figure 4



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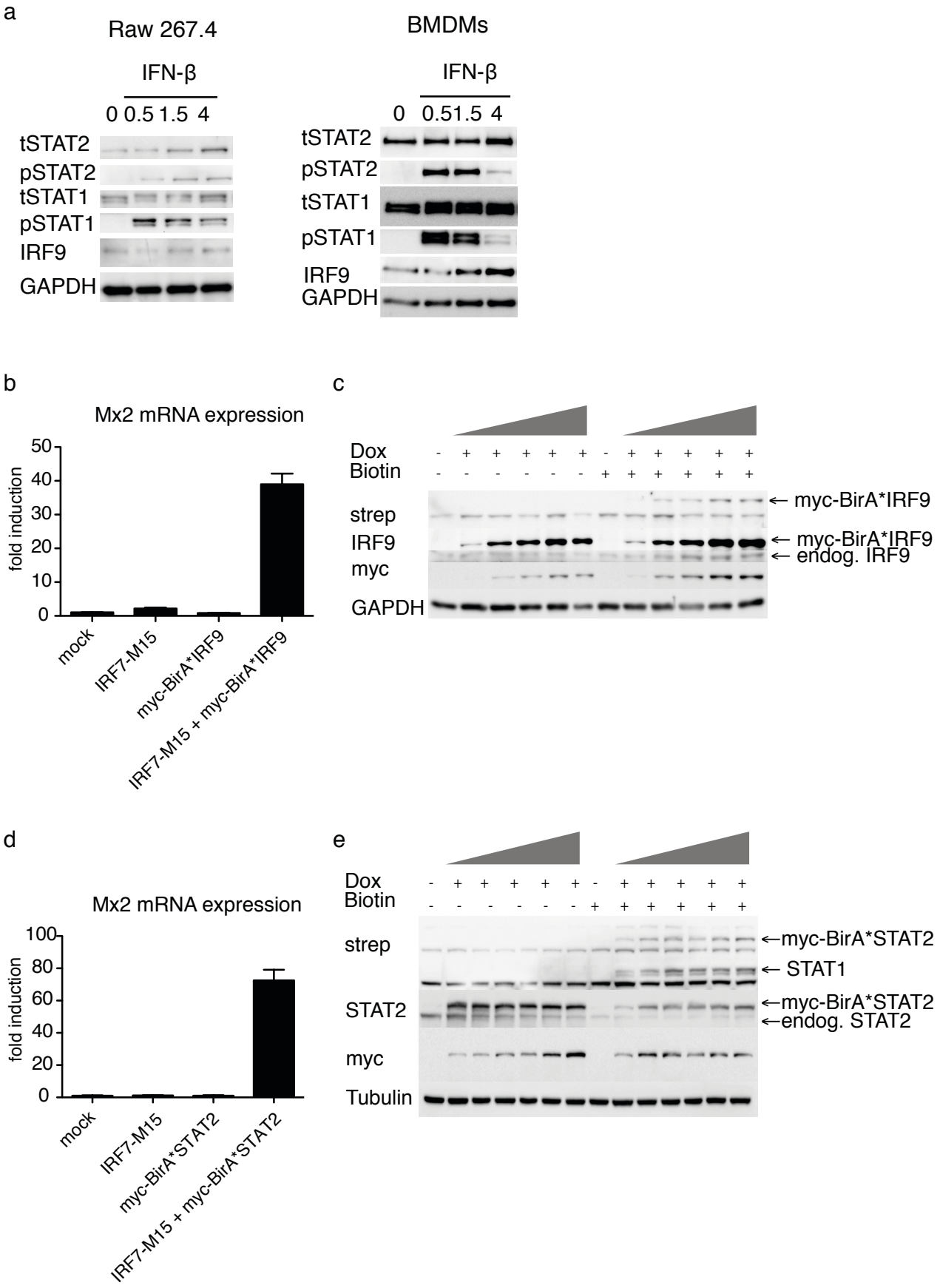
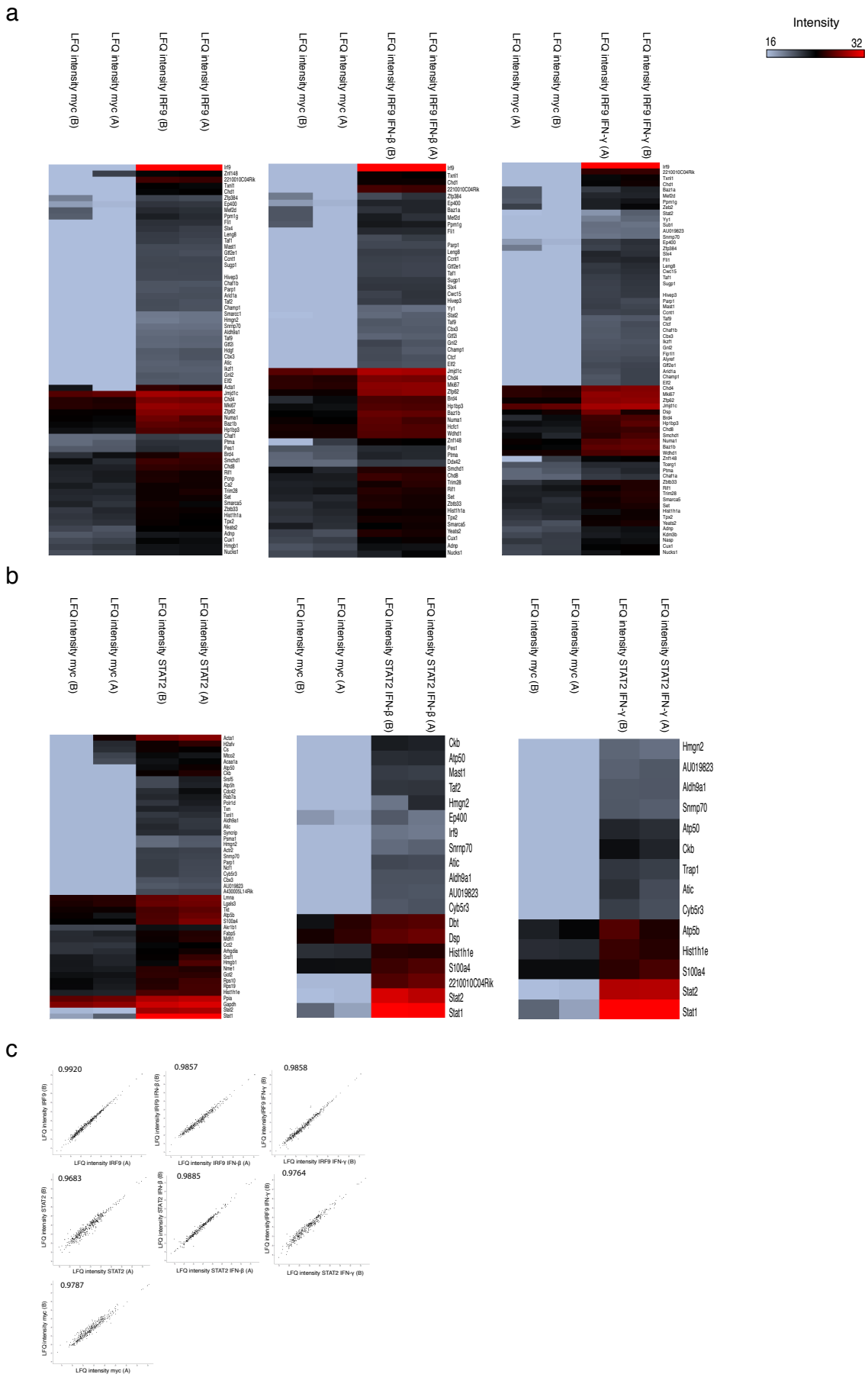


Supplementary Figure 4



Supplementary table 3

Primers qPCR		
Gene name	Forward 5‘-3‘	Reverse 5‘-3‘
Irf1	CCGAAGACCTTATGAAGCTCTTTG	GCAAGTATCCCTTGCCATCG
Irf8	GGCTGCATGAGCGAAGTTC	CTCCTCTTGGTCATACCCATGTA
Usp18	TGCCTCGGAGTGCAGAAGA	CGTGATCTGGTCCTTAGTCAGG
Irf7	ATTTCGGTCGTAGGGATCTGG	GCACAGCGGAAGTTGGTCT
Oas1a	GGGTCATGTTAATACTTCCAGCA	CAATGGCTTCCCCAGCTTCT
Gapdh	CATGGCCTTCCGTGTTCCTA	GCGGCACGTCAGATCCA
Primers ChIP qPCR		
Gene name	Forward 5‘-3‘	Reverse 5‘-3‘
Mx2 ISRE	CTTCTGCCCAGAATCAGGC	AGTTTCACTTTCATTTCTCTGGTTC