# Appendix

## STAT1 is required to establish but not maintain interferon-γ-induced transcriptional memory

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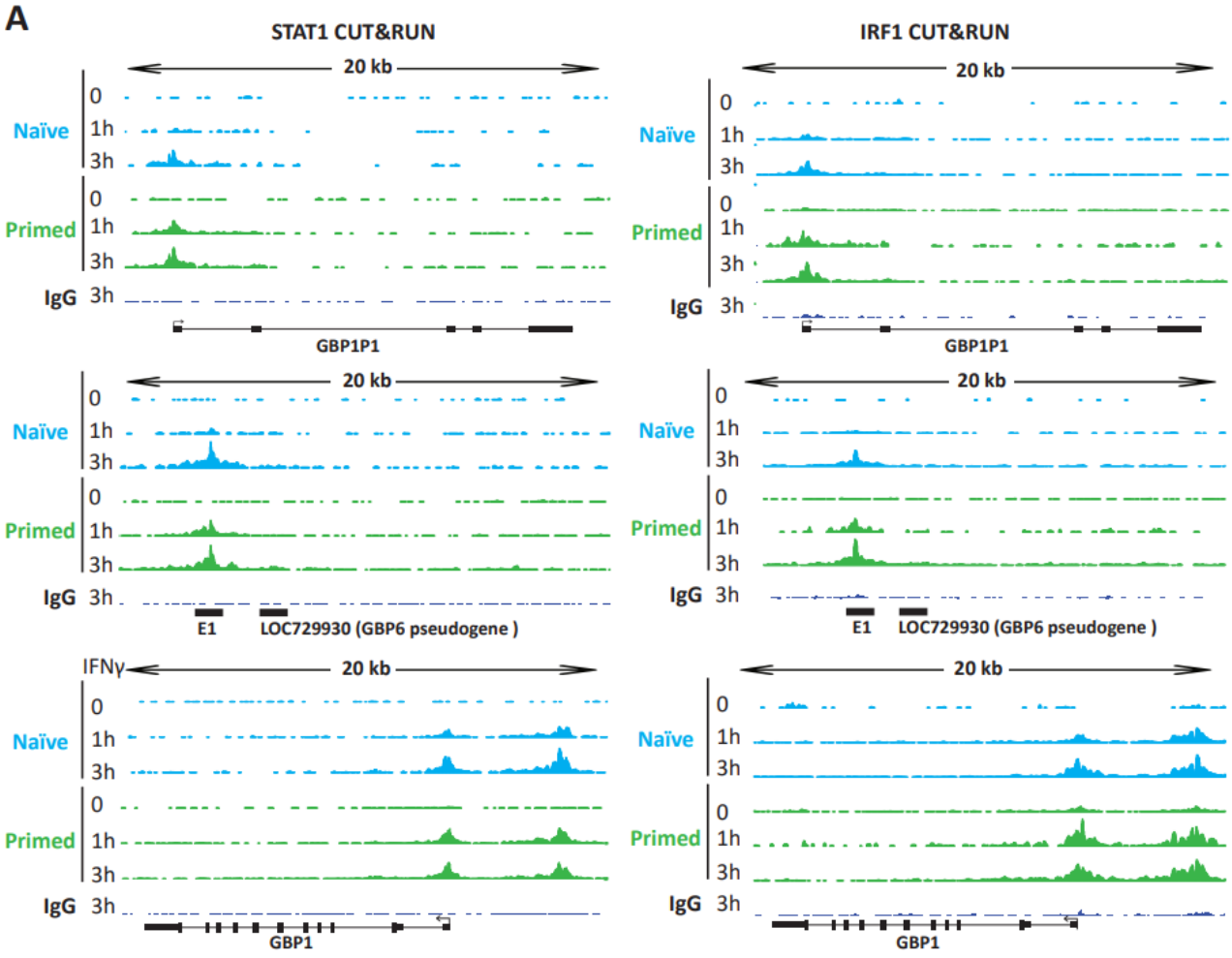
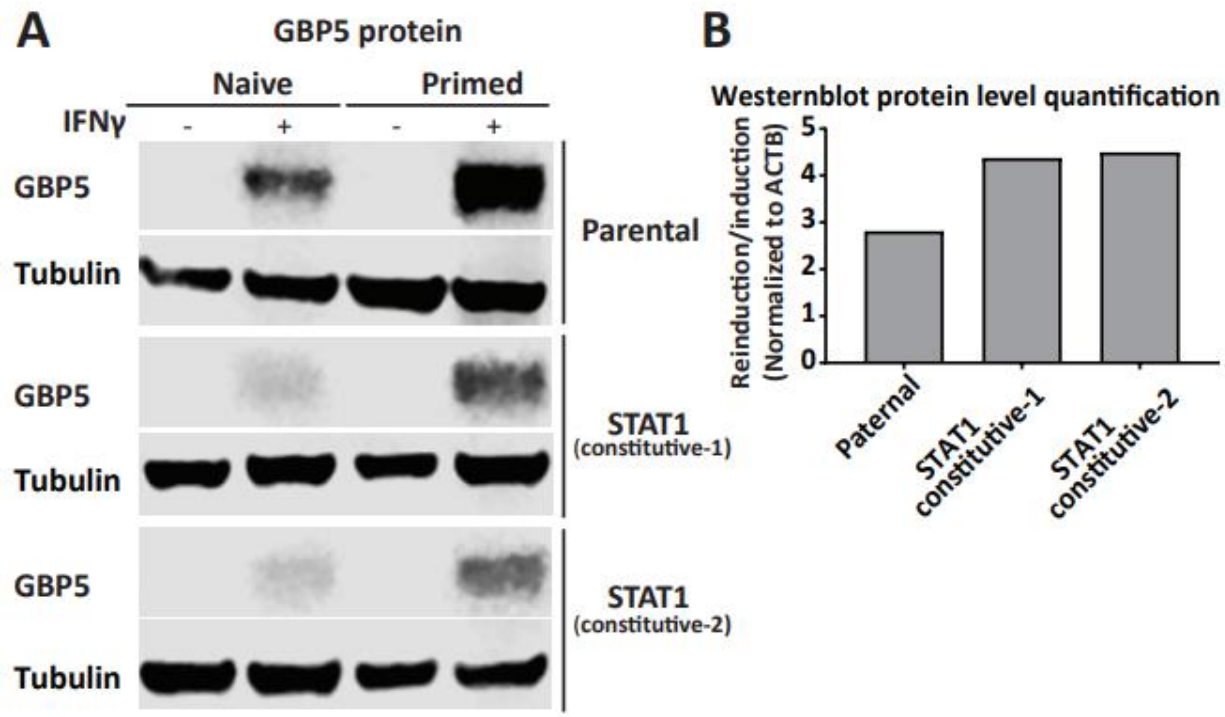



**B** **list of overlap genes in Figure 3G, that showed both faster STAT1 and IRF1 recruitment**

| | Ensembl | Gene name | description |
|---|---|---|---|
| 1 | ENSG00000225492 | GBP1P1 | guanylate binding protein 1 pseudogene |
| 2 | ENSG00000154451 | GBP5 | guanylate binding protein 5 (see Figure 3) |
| 3 | ENSG00000162654 | GBP4 | guanylate binding protein 4 (see Figure 3) |
| 4 | ENSG00000237568 | nan | LncRNA inside GBP5 gene |
| 5 | ENSG00000238081 | LOC729930 | GBP6 pseudogene, transcription factor binding site is found 2.4 kbp upstream of this site |
| 6 | ENSG00000284734 | GBP4 (antisense) | novel transcript, antisense to GBP4 (see Figure 3) |
| 7 | ENSG00000117228 | GBP1 | guanylate binding protein 1 |
| 8 | ENSG00000269588 | LGALS13 | lectin, galactoside-binding, soluble, 13 (LGALS13) pseudogene |
| 9 | ENSG00000253838 | nan | lncRNA inside IDO1 |
| 10 | ENSG00000262151 | CIITA (antisense) | novel transcript, antisense to CIITA |
| 11 | ENSG00000226025 | LGALS17A | galectin 14 pseudogene |
| 12 | ENSG00000131203 | IDO1 | indoleamine 2,3-dioxygenase 1 |

**Appendix Figure S1. Accelerated STAT1 and IRF1 binding in primed genes (A)** Representation of processed CUT&RUN data for STAT1 and IRF1 occupancy at the selected GBP genes loci (GBP1P1, LOC729930 and GBP1). **(B)** List of loci showing enhanced STAT1 and IRF1 binding in primed cells as in Figure 3G (−3kb of TSS and +3kb of TTS with a minimum of 1.5-fold difference between primed and naïve upon 1 hour of IFNγ treatment).




**Appendix Figure S2 .** Replicates of experiment show in Figure 5D. Two independent clones of STAT1 rescue cells and their parental control were subjected to IFNγ induction and reinduction regime as outlined in Figure 1A. **(A)** Cell extracts were processed for western blotting and probed for GBP5 expression before and after induction and reinduction as indicated in Figure 1A. α-Tubulin (Tubulin) was used as a loading control. Note that GBP5 and Tubulin blot for STAT1 (constitutive-1) are as in Figure 5D, STAT1 (constitutive). **(B)** Western blot signal quantification of blot in 6A. Data are shown as fold change of reinduction to induction.

**Appendix Table S1.** list of gRNAs and primes used in this study.

| **CRISPRa gRNA** | |
|---|---|
| **Primer name #** | **sequence(5'->3')** |
| F_GBP1_SAM_gRNA1 | CACCGAATTAGTGGAGTGTGCCAG |
| F_GBP1_SAM_gRNA2 | CACCGAAATCTTTAAACCCTCCCAC |
| F_GBP1_SAM_gRNA3 | CACCGTAGAACATGAGTACAACACA |
| R_GBP1_SAM_gRNA1 | AAACCTGGCACACTCCACTAATTC |
| R_GBP1_SAM_gRNA2 | AAACGTGGGAGGGTTTAAAGATTTC |
| R_GBP1_SAM_gRNA3 | AAACTGTGTTGTACTCATGTTCTAC |
| F_ASCL1_gRNA1 | CACCGCGGGAGAAAGGAACGGGAGG |
| R_ASCL1_gRNA1 | AAACCCTCCCGTTCCTTTCTCCCGC |
| **CRISPR/Cas9** | |
| **Primer name #** | **sequence(5'->3')** |
| IRF1B_F | CACCGCATGGCTGGGACATCAACA |
| IRF9B_F | CACCGAGGGCTCAGCAACATCCATG |
| STAT1B_F | CACCGAGAACACGAGACCAATGGTG |
| STAT2B-F | CACCGAAGAATAGCATGGTAGCCT |
| STAT3A-F | CACCGCTACAGTGACAGCTTCCCAA |
| STAT5BA-F | CACCGTGCGGCATTATTTATCCCAG |
| ContorlA-F | CACCGTATTACTGATATTGGTGGG |
| ControlB-F | CACCGTTCCGCGTTACATAACTTA |
| IRF1B_R | AAACTGTTGATGTCCCAGCCATGC |
| IRF9B_R | AAACCATGGATGTTGCTGAGCCCTC |
| STAT1B_R | AAACCACCATTGGTCTCGTGTTCTC |
| STAT2B-R | AAACAGGCTACCATGCTATTCTTC |
| STAT3A-R | AAACTTGGGAAGCTGTCACTGTAGC |
| STAT5BA-R | AAACCTGGGATAAATAATGCCGCAC |
| ContorlA-R | AAACCCCACCAATATCAGTAATAC |
| ControlB-R | AAACTAAGTTATGTAACGCGGAAC |
| **qPCR** | |
| Primer name # | sequence(5'->3') |
| GBP1_qPCR_F | GTGGAACGTGTGAAAGCTGA |
| GBP1_qPCR_R | CAACTGGACCCTGTCGTTCT |
| GBP5_trancC_F | TTCAATTTGCCCCGTCTGTG |
| GBP5_trancC_R | AGGCAGTGTTTCAAGTTGGG |
| b-actin Forward | AACTGGAACGGTGAAGGTGACAGC |
| b-actin Reverse | TGGCTTTTAGGATGGCAAGGGAC |