SUPPORTING INFORMATION

Batf2/Irf1 induces inflammatory responses in classically activated macrophages, LPS and mycobacterial infection

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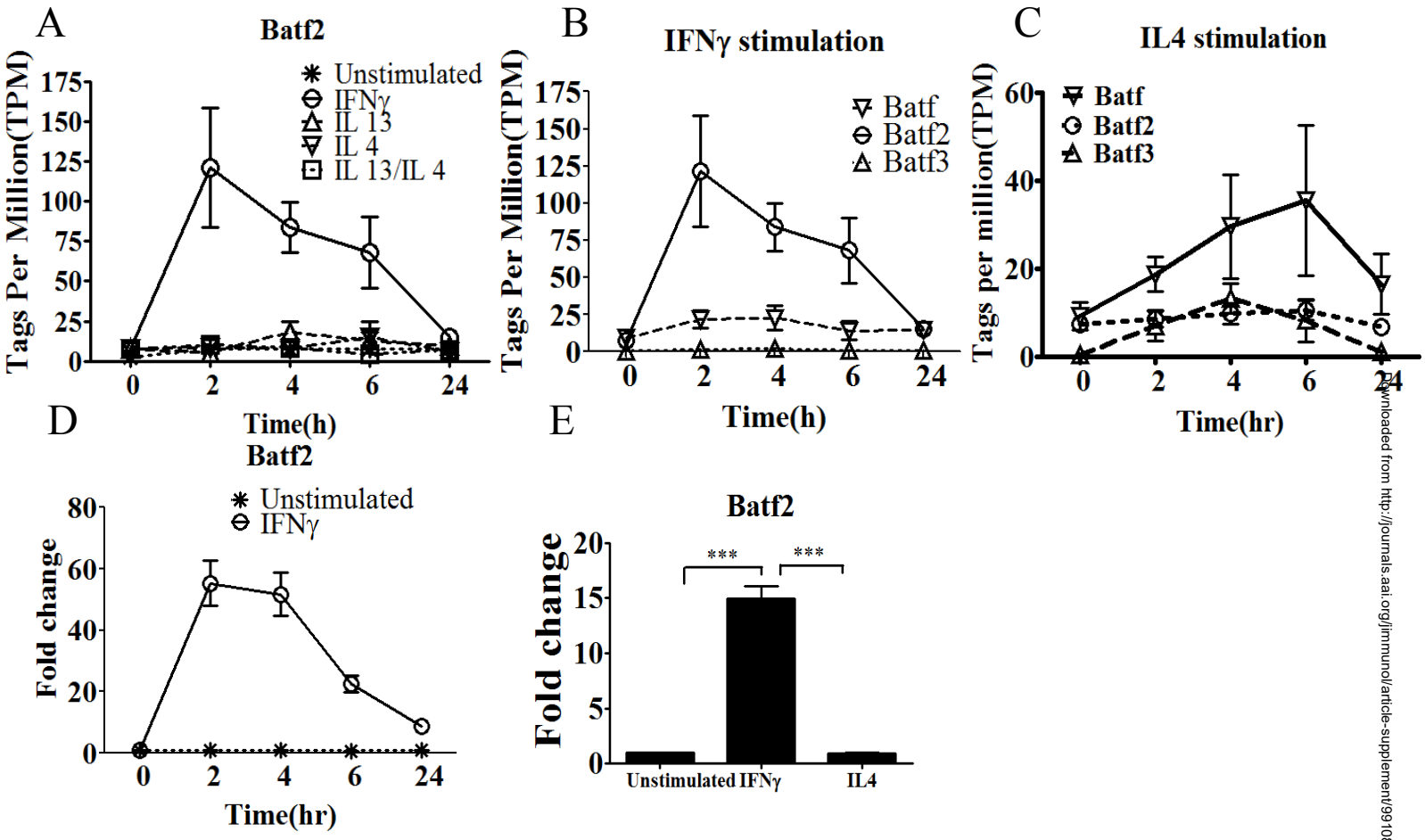

Figure. S1. Confirmation of *Batf2* induction in IFNγ-stimulated classically activated macrophages using CAGE analysis and qRT-PCR**.** RNA was prepared from un-stimulated control and stimulated with IFNγ, IL-4, IL-13, IL-4/IL-13 across five time points (0, 2, 4, 6 and 24 hr) for CAGE analysis. The CAGE analysis was performed as published previously. Three independent biological replicates were used for the CAGE analysis and the average was plotted. Expression intensity of each gene is represented by Tags per million (TPM). (A) *Batf2* expression in IFNγ, IL-4, IL-13, IL-4/IL-13-stimulated cells was plotted with un-stimulated control. (B) Expression of BATF family genes was plotted in IFNγ-stimulated cells. (C) Expression of BATF family genes was plotted in IL-4-stimulated cells. In (A) – (C), the data plotted as mean +/- SEM. (D) The relative expression level of *Batf2* in IFNγ-stimulated cells was plotted in comparison with un-stimulated control by using qRT-PCR data. (E) The expression of Batf2 was further confirmed in thioglycollate-elicited peritoneal macrophages following IFNγ and IL-4 stimulation by using quantitative RT-PCR at 4 hours. In (D)-(E), Gapdh was used as internal control. The data was plotted as mean +/- SEM. Three independent biological experiments were used.

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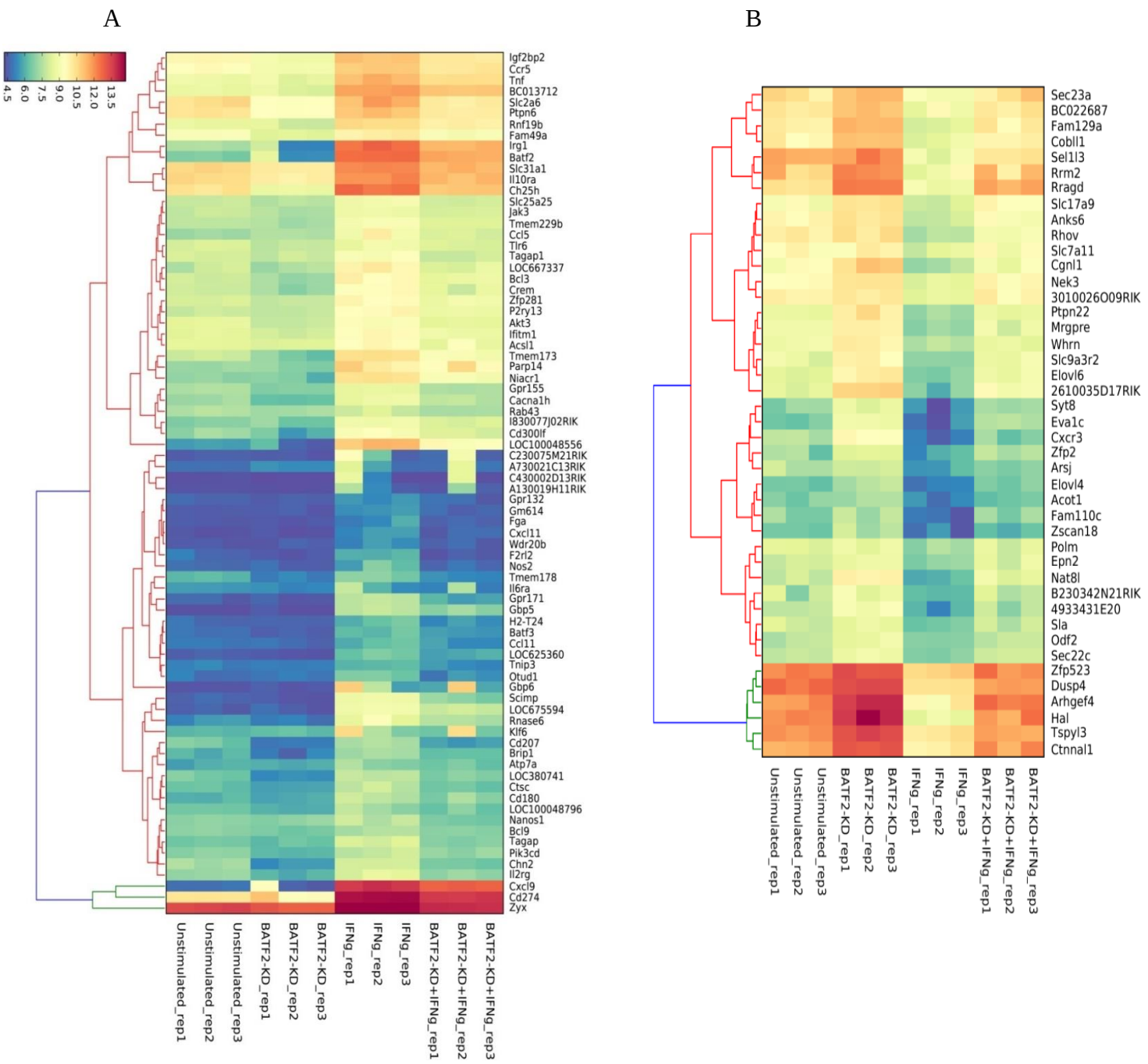

Figure. S2. Microarray analysis of Batf2-regulated genes from IFNγ-stimulated macrophages at 4 hours. (A) Heat map of Batf2-regulated genes, Out of 670 IFNγ-induced genes, 78 genes (11.6%) were down-regulated by *Batf2* knockdown, which were defined as *Batf2* positively regulated genes. ($P < 0.001$). Three independent experiments were performed. (B) Heat map of Batf2-negatively regulated genes. Out of 458 IFNγ-down regulated genes, 43 genes, down-regulated by IFNγ stimulation and up-regulated by IFNγ stimulation and Batf2-knockdown, were defined as Batf2-negatively regulated genes. These genes are significantly selected (P <0.001).

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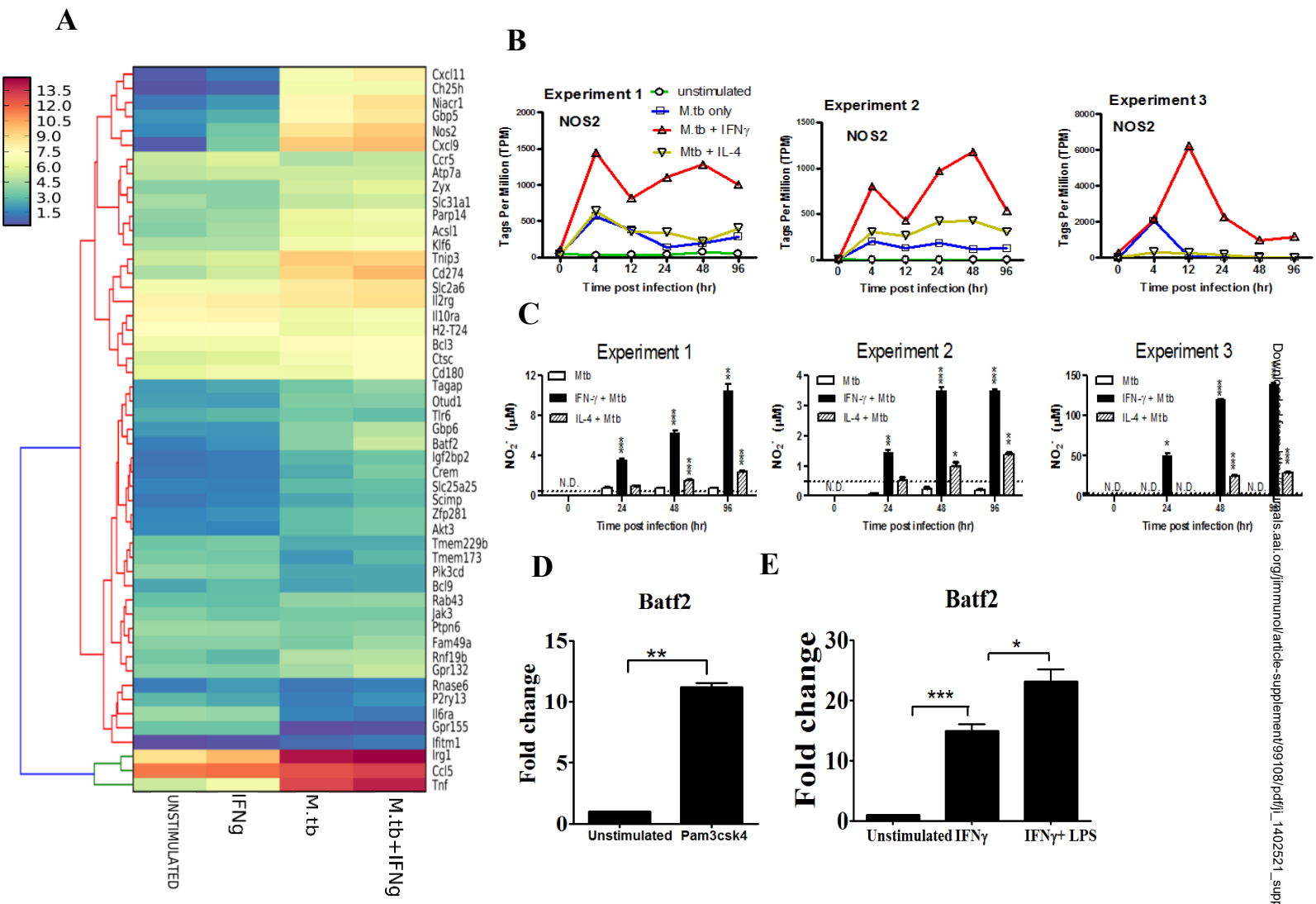

Figure. S3. *Mtb* regulates most of the Batf2 perturbed genes. (A) BMDMs were stimulated with IFN-γ (100 Units/well), IL-4 (100 Units/well) or left untreated for 24 hours. At 24 hours cells were then infected with HN878 strain of *M. tuberculosis* (MOI:5) and RNA was collected at 0, 4, 12, 24, 48 and 96 hours post infection for CAGE analysis. Tags Per Million (TPM) values from the time course (0, 4, 12, 24, 48 and 96 hours p.i) was recorded for each treatment. Differential expression analysis was performed and compared with unstimulated BMDM cells. 78 genes significantly perturbed by Batf2 knockdown following IFNγ stimulation, shown in Figure S3*A*, among them 51 gene was induced by *Mtb* infection and IFNγ stimulation at 4 hrs were shown in these heatmap using cage analysis. Three independent biological experiments were performed. (B) *Mtb*-induced *Nos2* expression with or without cytokine stimulation. Each biological experiment was shown separately. (C) Measurement of nitrite ($NO2^-$) in culture medium by Griess reagent assay. Each biological experiment was shown separately. (D) The relative expression level of *Batf2* in Pam3csk4-stimulated BMDM cells was plotted in comparison with un-stimulated control by using qRT-PCR data at 4 hours. (E) The expression of Batf2 was further confirmed in thioglycollate-elicited peritoneal macrophages following IFNγ, IFNγ/LPS stimulation by using quantitative RT-PCR at 4 hours. In (D) and (E), Gapdh was used as internal control. The data was plotted as mean +/- SEM. Three independent biological experiments were used.

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Table S1: Enrichment of Irf1 transcription factor binding site in Batf2 regulated gene

Gene ID(s)	Chr	Start	End	Strand	Nearest TSS	TFBS Start	TFBS End	TFBS Sequence
Acsl1	8	47556395	47621405	+	47576328	47576162	47576173	CAAAATGAAAAC
Tnip3	6	65540392	65584034	+	65540392	65540087	65540098	GAAAGTCACACC
Batf2	19	6140983	6172476	+	6164439	6160815	6160826	AAAAGCAAAACC
Ccl5	11	83339280	83344020	-	83344020	83344167	83344178	AAAACTGAAATC
Zfp281	1	138521478	1.39E+08	+	138521478	138520886	1.39E+08	CAAAGGGAAACC
Rab43	6	87738847	87762158	-	87761772	87760799	87760810	GAAACCGAAAAC
Irg1	14	103446194	1.03E+08	+	103446194	103442993	1.03E+08	AAAAGTGAACCT
Crem	18	3266046	3337746	-	3299557	3301393	3301404	GATACTGAAACC
Cd274	19	29441945	29462585	+	29441945	29440927	29440938	CATAATGAAACC
Gbp5	3	142159864	1.42E+08	+	142159906	142163390	1.42E+08	AAAAATAAAACC
Tmem178	17	81343972	81401156	+	81343972	81342115	81342126	ATAAATGAAACC
Gm614		98456715	98459331	-	98459331	98460069	98460080	GAAATCGAAACT
Tmem173	18	35893332	35900208	-	35900208	35904407	35904418	AAAAACTAAACC
Il6ra	3	89673246	89717084	-	89717084	89715310	89715321	GGAAGTGAAAGC
Pik3cd	4	149023277	1.49E+08	-	149049295	149045849	1.49E+08	AAAAAGGAAACC
Atp7a		103222615	1.03E+08	+	103222661	103225030	1.03E+08	GAAAATGAAAAT
Ccr5	9	124036282	1.24E+08	+	124036282	124035225	1.24E+08	CAAAGAGAAAGC
Fam49a	12	12268945	12383165	+	12268945	12273692	12273703	TAAAATGAAACT
Bcl3	7	20393811	20408119	-	20401345	20401521	20401532	GAAAACCAAACT
Akt3	1	178950204	1.79E+08	-	179188334	179185732	1.79E+08	GAAAGCTAAACT
Ptpn6	6	124670725	1.25E+08	-	124688732	124691209	1.25E+08	GAAAGCAAAACA
Bcl9	3	97007585	97032769	-	97017932	97015377	97015388	CAAACCGAAAAC
Cxcl9	5	92750357	92757105	-	92757105	92761533	92761544	GAAATCGAAACT
Rnf19b	4	128735515	1.29E+08	+	128736058	128737414	1.29E+08	GAAAGTGAAAGC
Tmem229b	12	80062782	80108614	-	80081765	80081850	80081861	GAAACAGAAACC
Klf6	13	5860735	5869639	+	5860735	5861559	5861570	CAAAACAAAACC
Tnf	17	35336326	35338952	-	35338952	35341950	35341961	AAAAATCAAAGC

Table S1.An enrichment analysis of transcription factor binding motif (oPOSSUM program) was performed. In the table among the 27 out of 79 Batf2 regulated genes that have Irf1 binding site are enlisted.

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