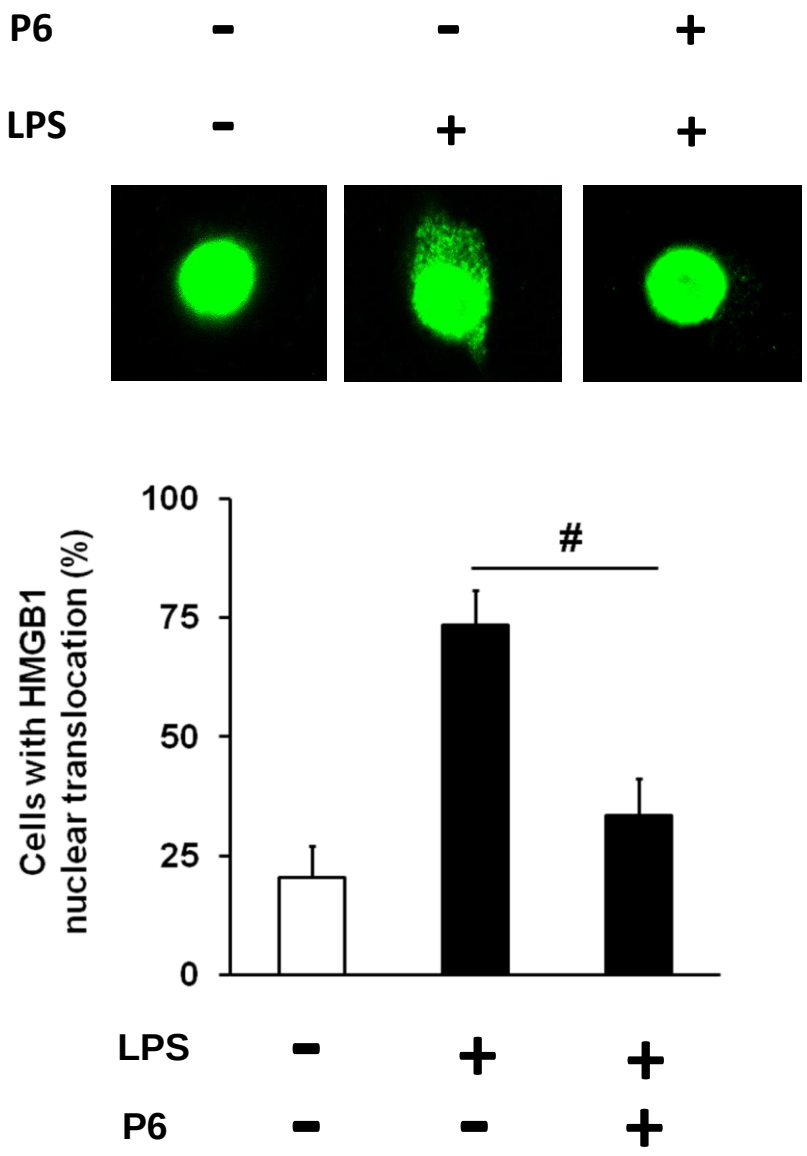
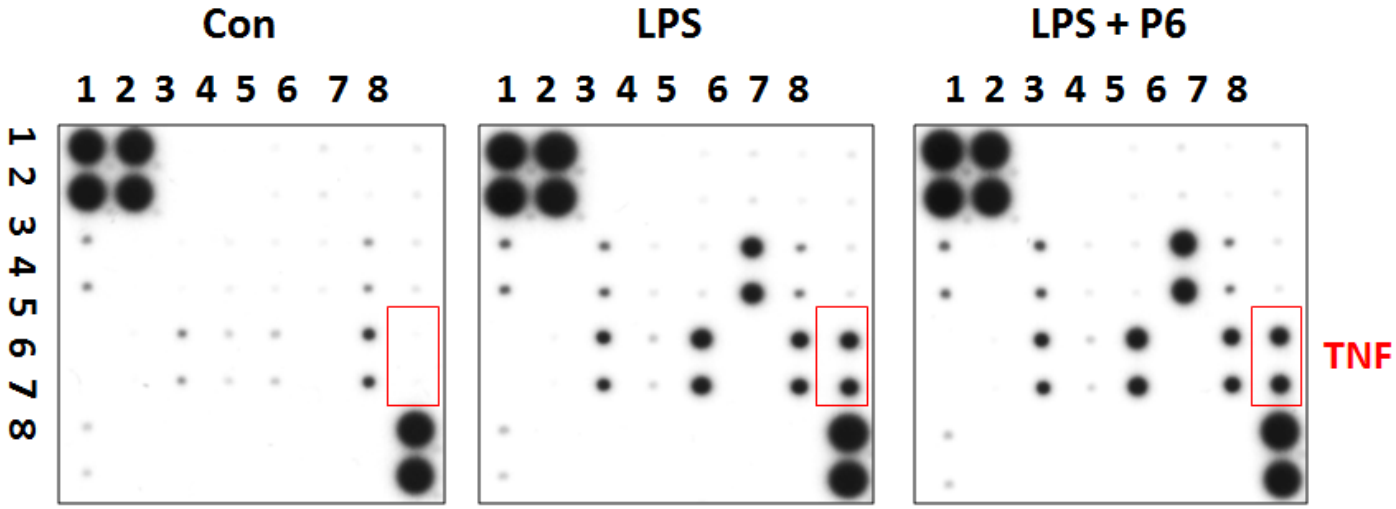
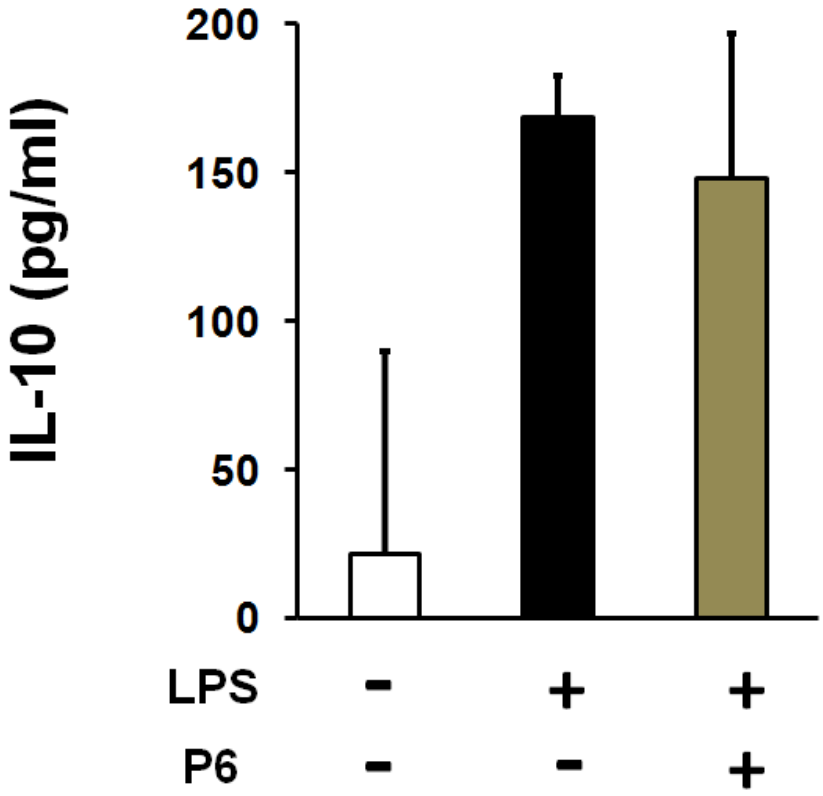


Supplementary Fig. 1. Pharmacological inhibition of the JAK/STAT blocks LPS-induced HMGB1 nuclear translocation. RAW 267.4 cells were stimulated with LPS in the absence or the present of pyridone 6 (P6) for 16h. The localization of cellular HMGB1 was measured by fluorescent immunostaining using confocal microscopy. Shown in the upper panel are representative images of HMGB1 immunostaining; shown in the lower panel are Means ± SEM of two independent experiments. #, $p<0.05$.

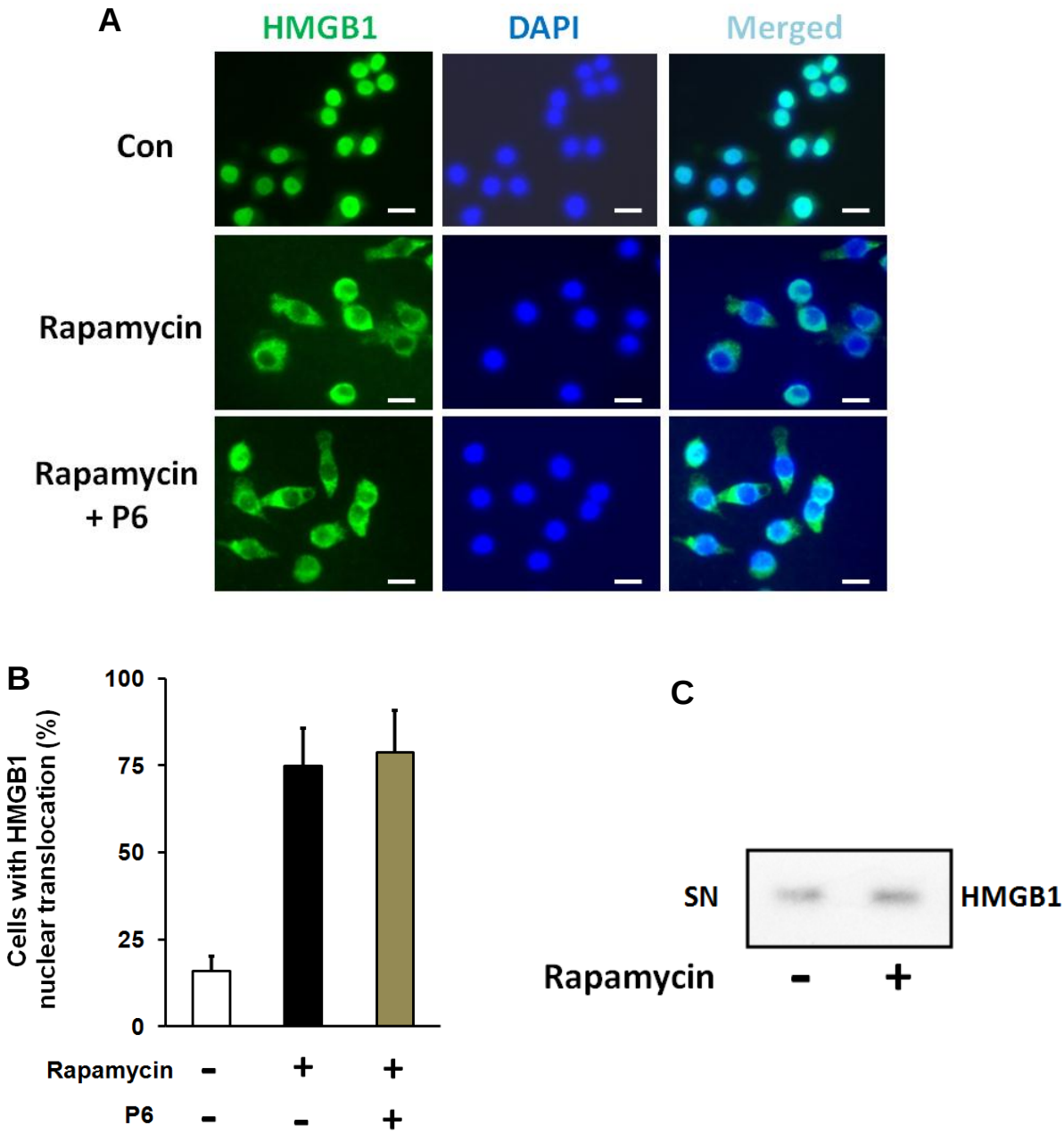


	1	2	3	4	5	6	7	8
1	Pos	Pos	Neg	Neg	GCSF	GM-CSF	IL-2	IL-3
2	Pos	Pos	Neg	Neg	GCSF	GM-CSF	IL-2	IL-3
3	IL-4	IL-5	IL-6	IL-9	IL-10	IL-12 p40p70	IL-12p70	IL-13
4	IL-4	IL-5	IL-6	IL-9	IL-10	IL-12 p40p70	IL-12p70	IL-13
5	IL-17	IFN-γ	MCP-1	MCP-5	RANTES	SCF	sTNFRI	TNF-α
6	IL-17	IFN-γ	MCP-1	MCP-5	RANTES	SCF	sTNFRI	TNF-α
7	Thrombopoietin	VEGF	Blank	Blank	Blank	Blank	Blank	Pos
8	Thrombopoietin	VEGF	Blank	Blank	Blank	Blank	Blank	Pos

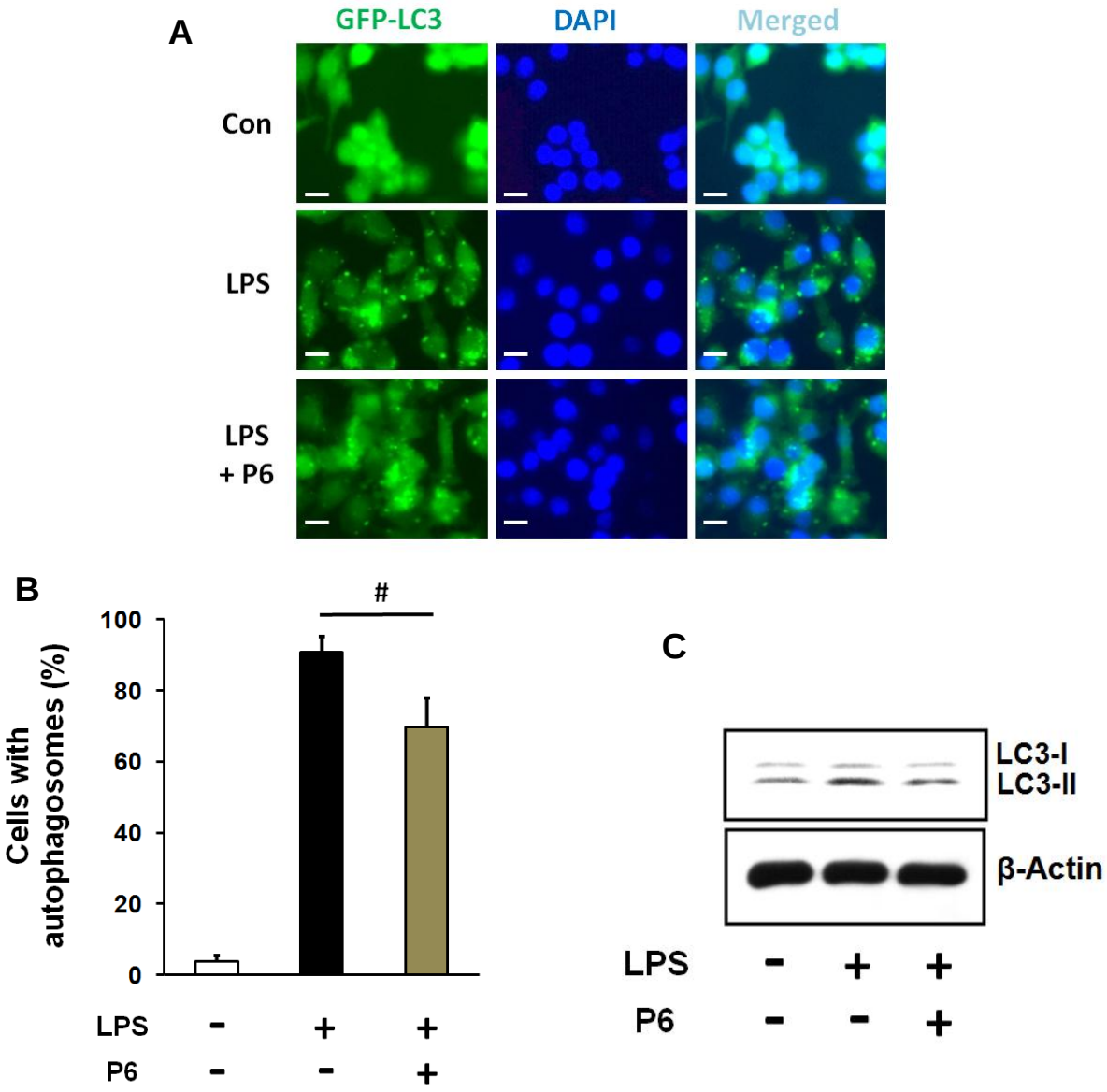
Supplementary Fig 2. Mouse macrophages were stimulated with LPS in the absence or the present of pyridone 6 (P6) for 6 h. The levels of cytokines or chemokines in the culture medium were measured by cytokine antibodies array.



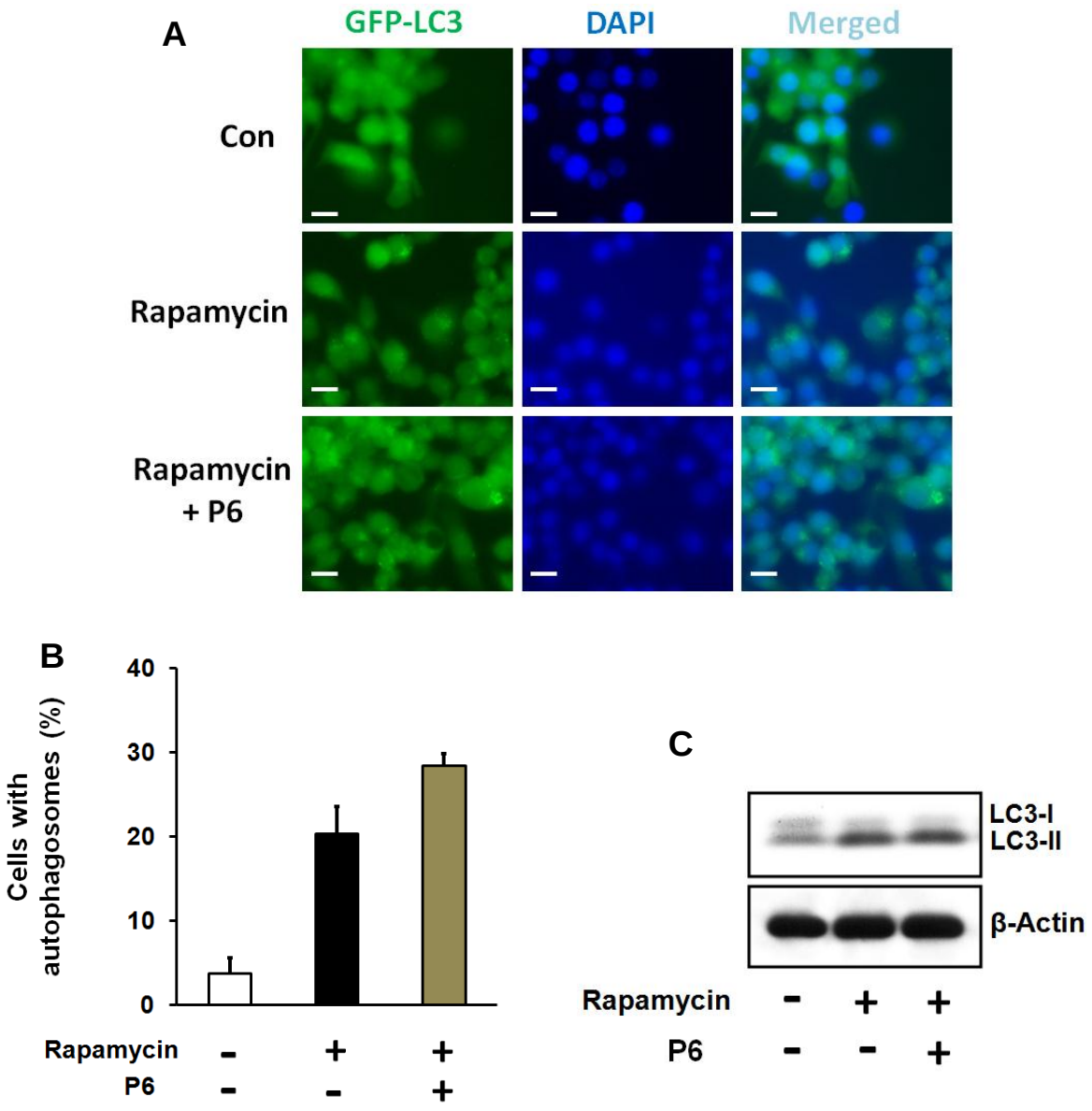
Supplementary Fig. 3. Mouse macrophages were stimulated with LPS in the absence or the present of pyridone 6 (P6) for 6 h. The levels of IL-10 in the culture medium were assessed by ELISA.



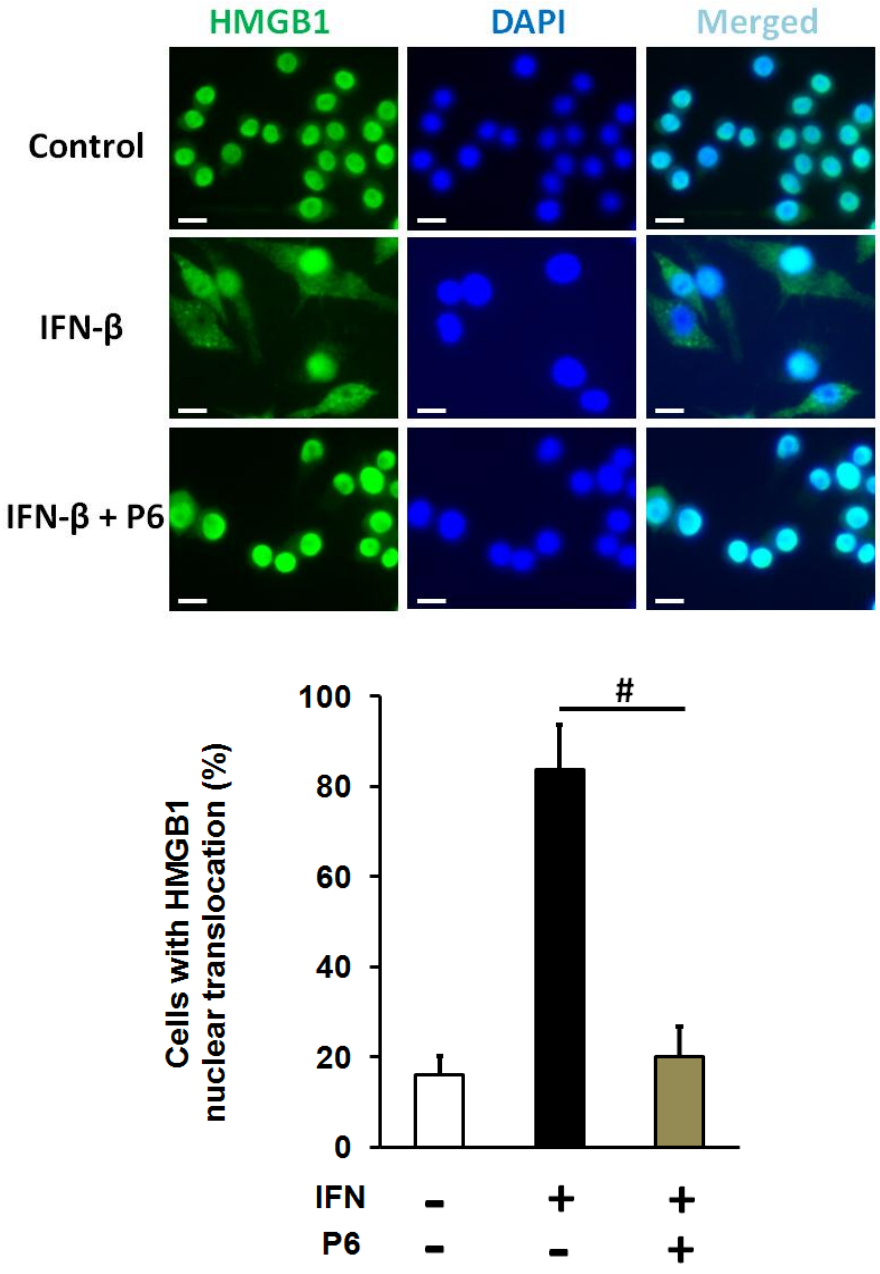
Supplementary Fig. 4. RAW 267.4 cells were incubated with rapamycin (500 nM) for 18 h. (A, B) HMGB1 nuclear translocation was assessed by immunostaining. Shown in (A) are representative images of HMGB1 immunostaining; shown in (B) are Means ± SEM of two independent experiments. Scale bars = 20 μm. (C) Levels of HMGB1 in cell culture medium were measured by western-blot.



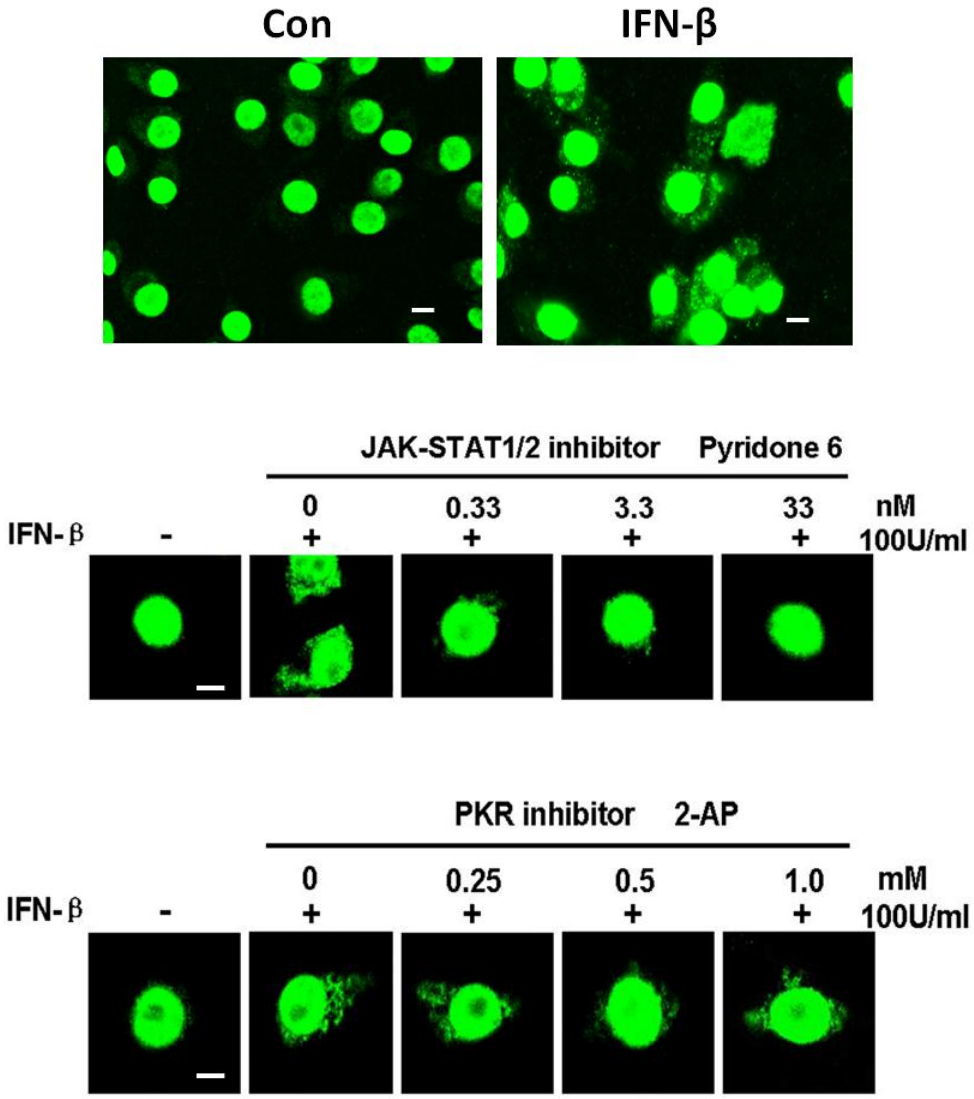
Supplementary Fig. 5. RAW 267.4 cells that stably express GFP-LC3 were stimulated with LPS (1 μg/ml) for 16 h in the presence or the absence of pyridone 6 (P6). (A, B) Quantitation of autophagy was performed based on the percentage of cells with GFP-LC3-positive punctate dots. Scale bars = 20 μm. Shown in (A) are representative images; shown in (B) are Means ± SEM of two independent experiments. (C) RAW 267.4 cells were stimulated with LPS or rapamycin for 16 h in the presence or the absence of P6, and then the cellular levels of LC3B-II were measured by western-blot. #, $p<0.05$.



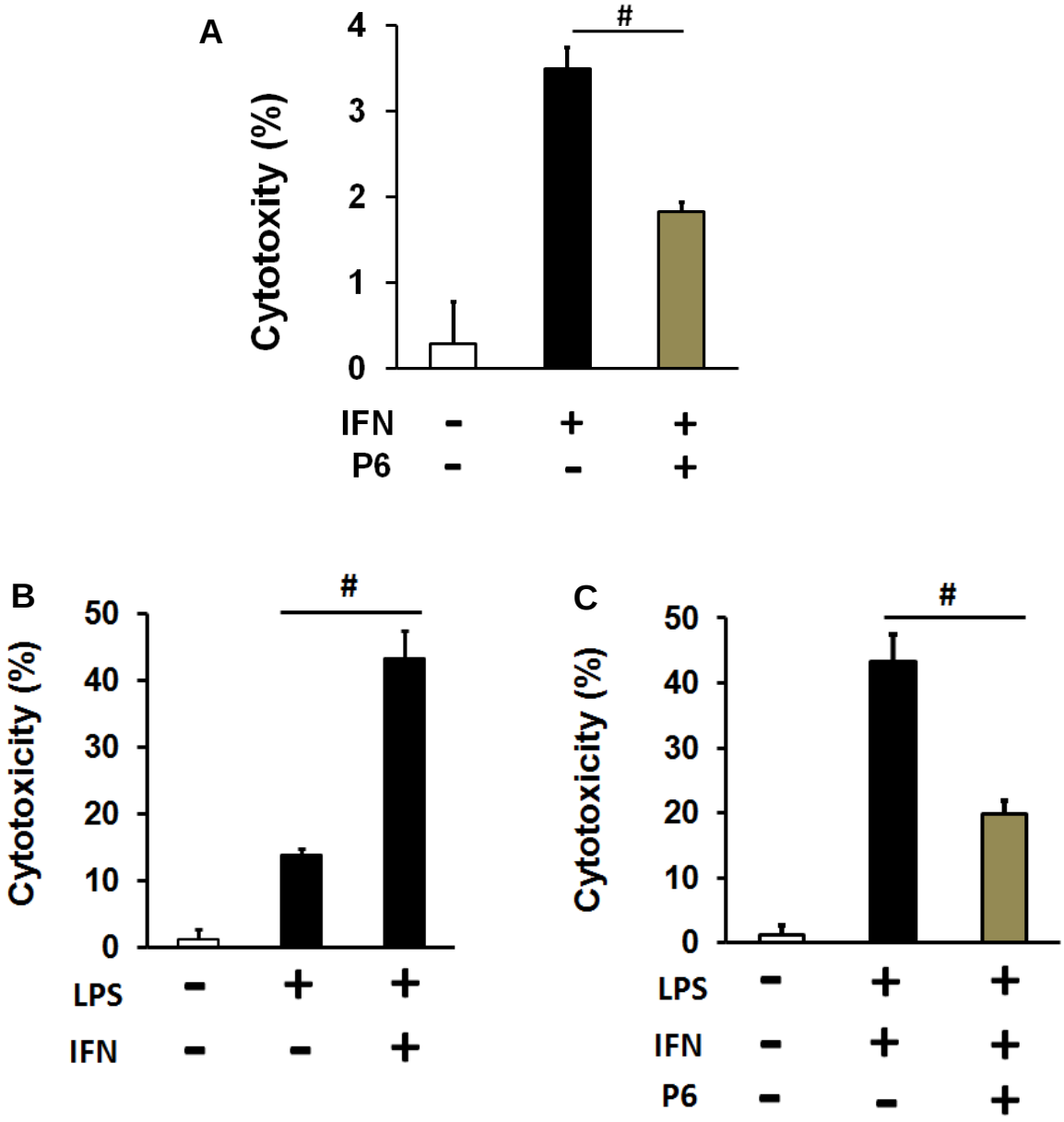
Supplementary Fig. 6. RAW 267.4 cells that stably express GFP-LC3 were stimulated with rapamycin (500 nM) for 16 h in the presence or the absence of pyridone 6 (P6). (A, B) Quantitation of autophagy was performed based on the percentage of cells with GFP-LC3-positive punctate dots. Scale bars = 20 μm. Shown in (A) are representative images; shown in (B) are Means ± SEM of two independent experiments. (C) RAW cells were stimulated with LPS or rapamycin for 16 h in the presence or the absence of P6, and then the cellular levels of LC3B-II were measured by western-blot.



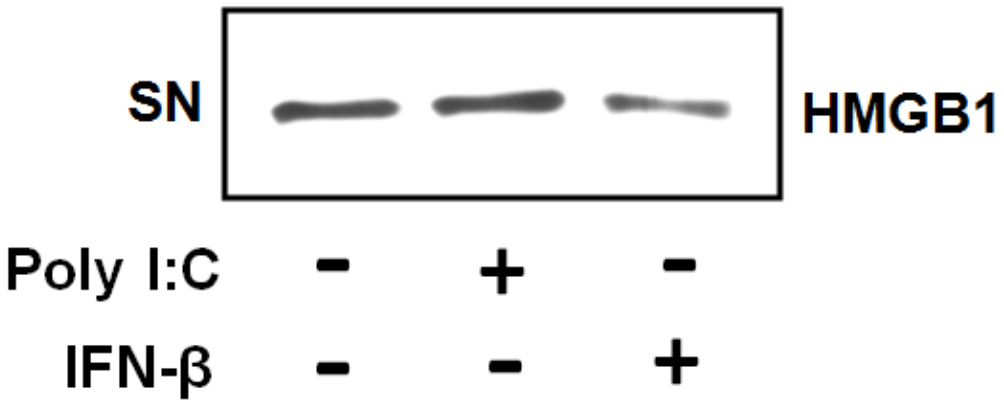
Supplementary Fig. 7. RAW 267.4 cells were stimulated with LPS (1 µg/ml) for 16 h in the presence or the absence of pyridone 6 (P6). HMGB1 nuclear translocation was assessed by immunostaining. Scale bars = 20 µm. Shown in upper panel are representative images of HMGB1 immunostaining; shown in lower panel are Means ± SEM of two independent experiments. #, $p<0.05$.



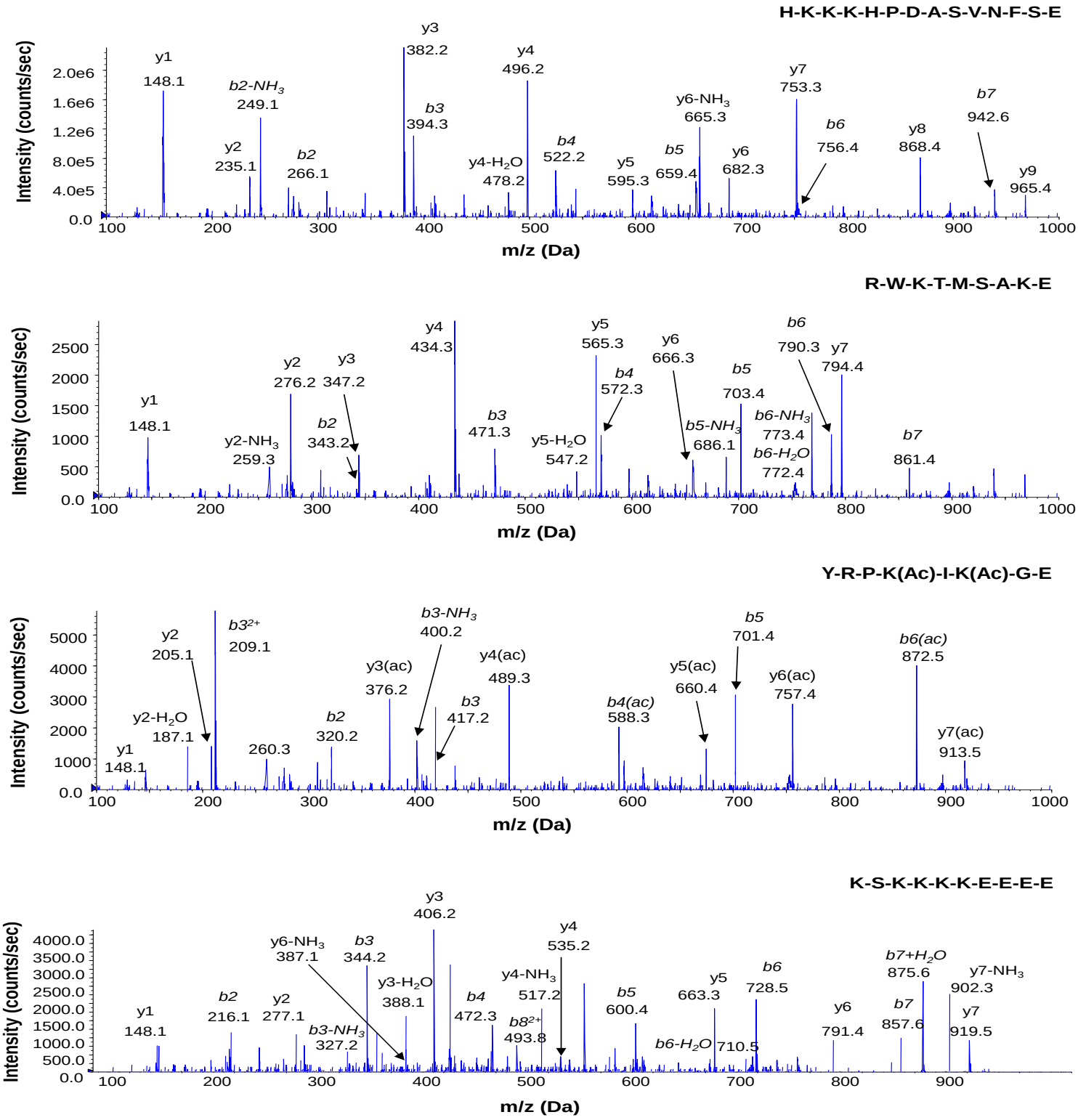
Supplementary Fig. 8. Activation of the JAK/STAT pathway by type 1 IFN induces HMGB1 cytoplasmic accumulation. RAW 267.4 cells were stimulated with IFN-β in the absence or the present of different dose of pyridone 6 or 2-AP for 16h. The localization of cellular HMGB1 was measured by fluorescent immunostaining using confocal microscopy. Scale bars = 10 μm.



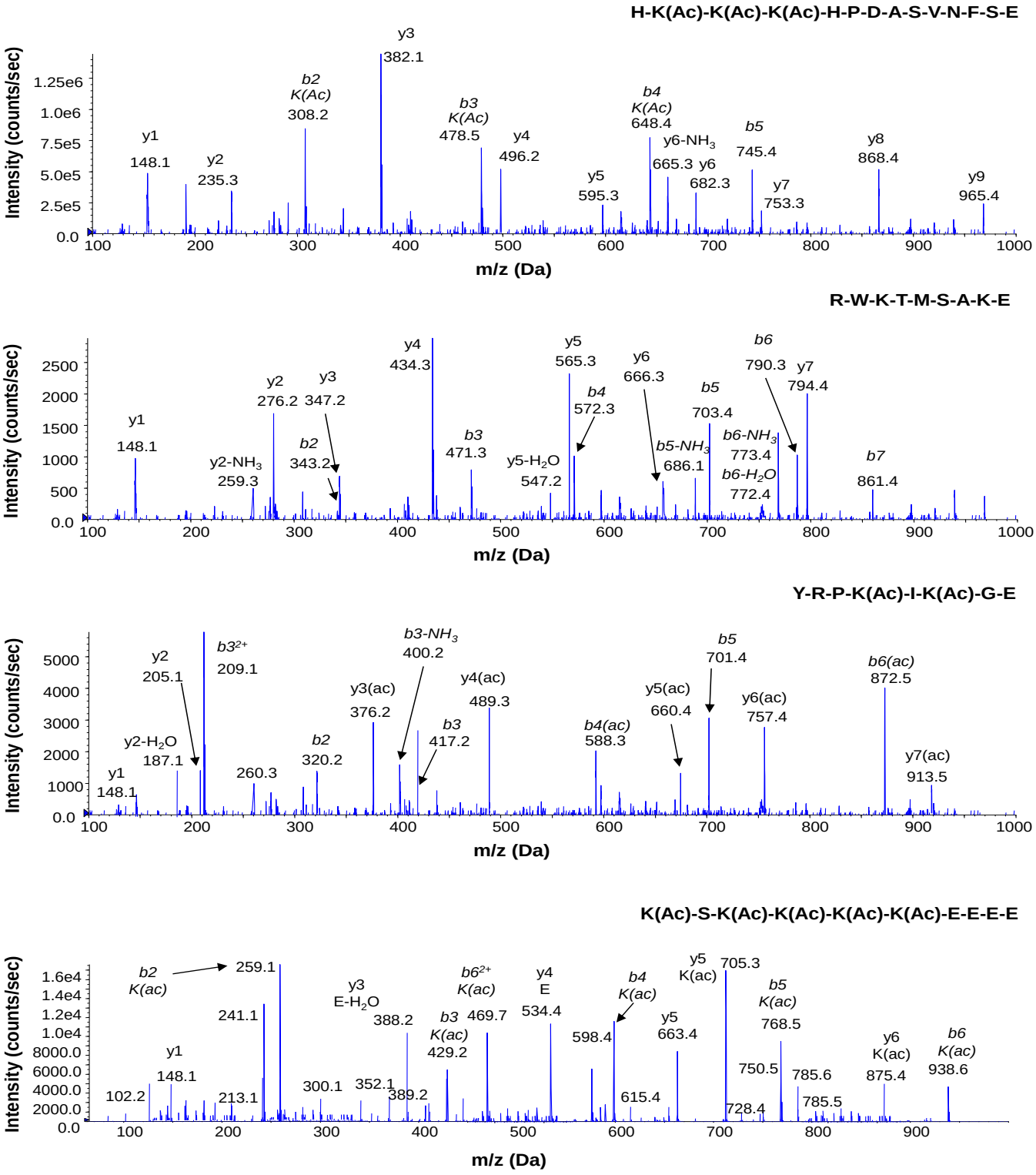
Supplementary Fig. 9. (A) RAW 267.4 cells were stimulated with LPS (1 μg/ml) for 16 h in the presence or the absence of pyridone 6 (P6). (B) Mouse peritoneal macrophages were stimulated with LPS (100 ng/ml) in the absence or the presence of IFN-β (100 U/ml) for 16 h. (C) Mouse peritoneal macrophages were stimulated with LPS (100 ng/ml) and IFN-β (100 U/ml) in the absence or the presence of IFN-β (100 U/ml) for 16 h. Levels of LDH in cell culture medium were measured by LDH assay. Results are Means ± SEM of two independent experiments. #, $p<0.05$.



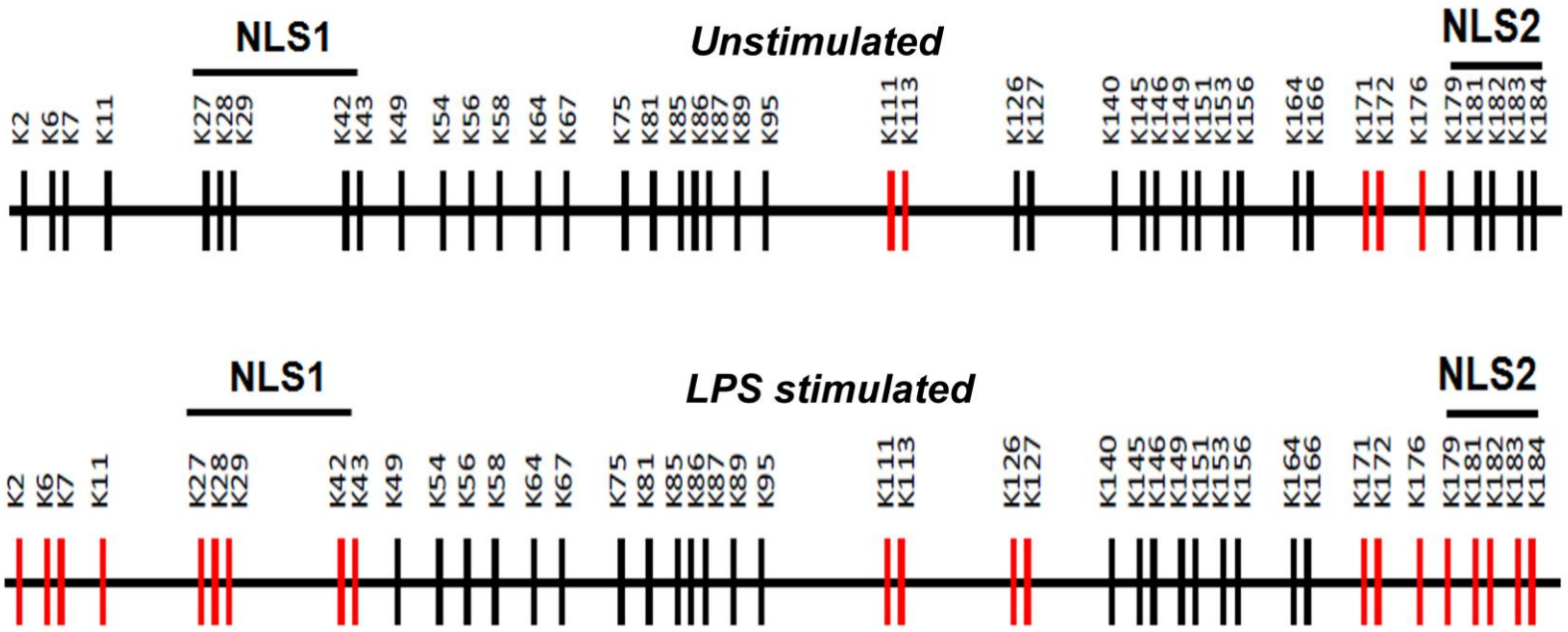
Supplementary Figure 10. Mouse embryonic fibroblasts (MEFs) were stimulated with IFN-β (1000 U/ml) or Poly I:C (10 μg/ml) for 16 h. Levels of HMGB1 in cell culture medium were assessed by western-blot.



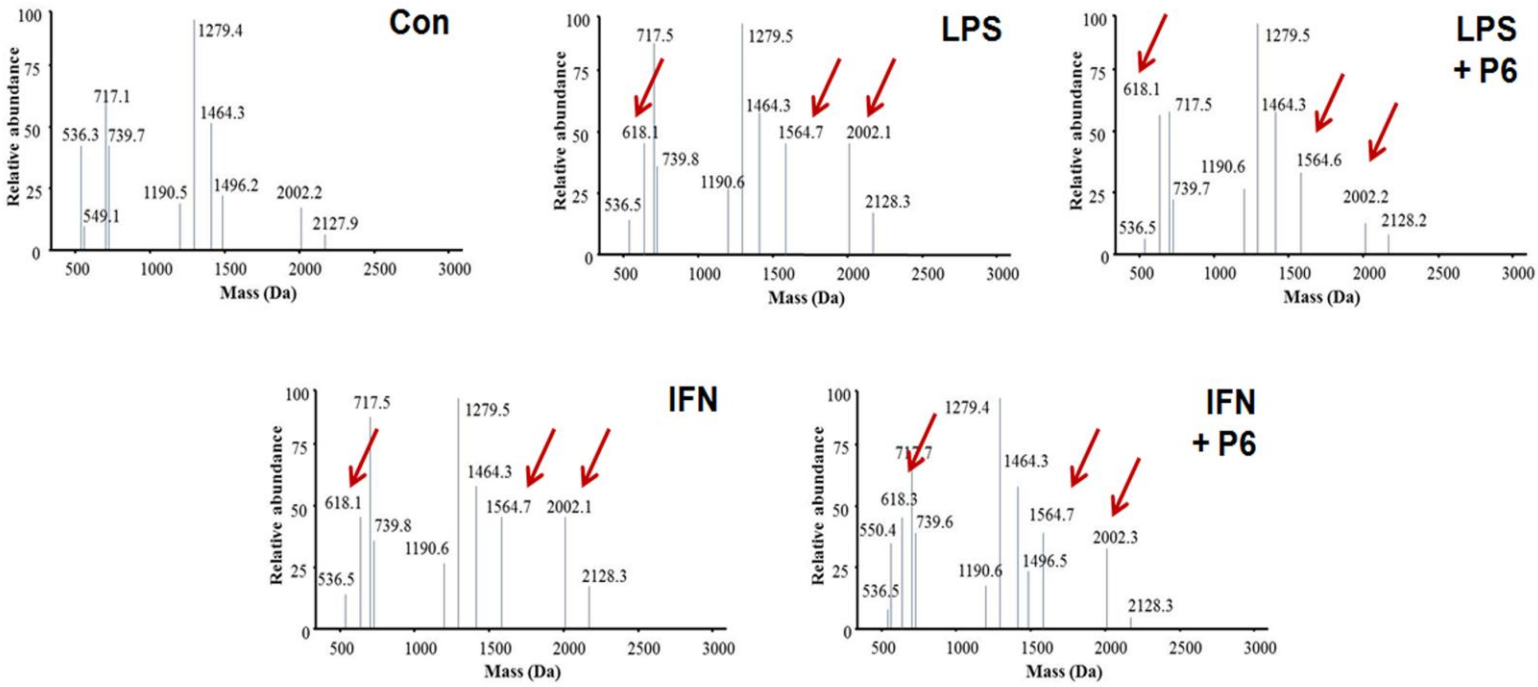
Supplementary Fig. 11. Acetylated lysine residues within HMGB1 protein of unstimulated THP-1 cells was assessed by high resolution liquid chromatography tandem mass spectrometric analysis (LC-MS/MS). Shown in the figure are representative MS traces.



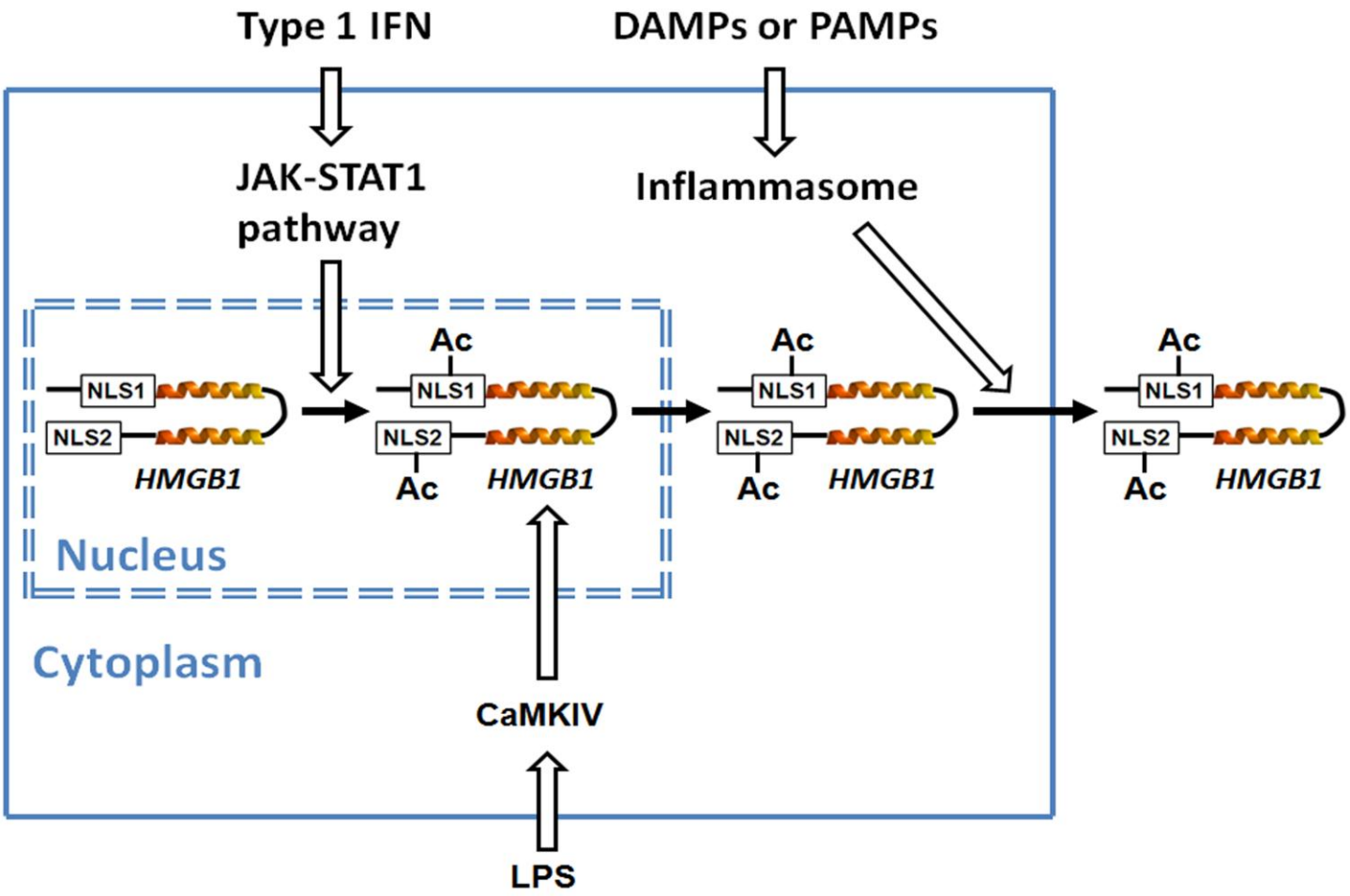
Supplementary Fig. 12. THP-1 cells were stimulated with LPS for 3 h. Acetylated lysine residues within HMGB1 protein was assessed by high resolution liquid chromatography tandem mass spectrometric analysis (LC-MS/MS). Shown in the figure are representative MS traces.



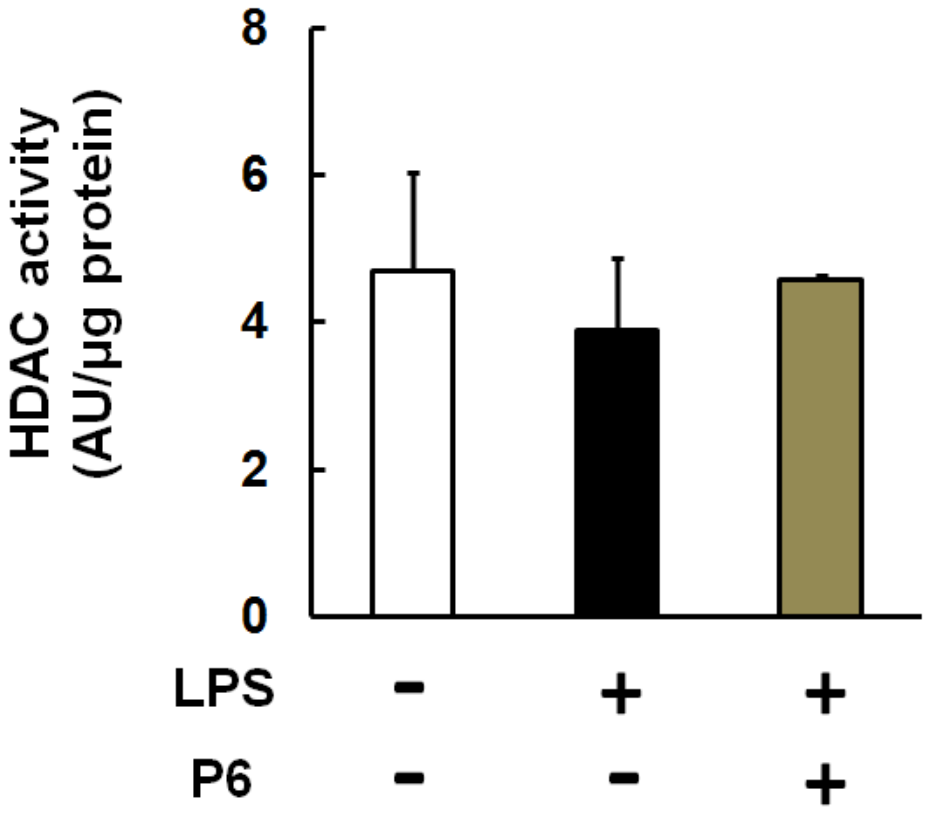
Supplementary Fig. 13. THP-1 cells were stimulated with LPS for 3 h. And the acetylation of intracellular HMGB1 protein was assessed by high resolution liquid chromatography tandem mass spectrometric analysis (LC-MS/MS). Acetylated lysine residues within HMGB1 protein are shown in red.



Supplementary Fig. 14. JAK/STAT1 signaling is dispensable for LPS- or IFN-induced HMGB1 oxidation. Mouse peritoneal macrophages were stimulated with indicated stimuli in the absence or the presence of pyridone 6 (P6) for 6 h. The redox status of intracellular HMGB1 protein was assessed by LC-MS/MS. Shown in the panels are representative MS traces.



Supplementary Fig. 15. The mechanism of HMGB1 release by activated immune cells. There are two important steps for HMGB1 release from activated immune cells during infection. In the first step, LPS induces the expression of type 1 interferon. This is followed by activation of JAK/STAT1 signaling that mediates HMGB1 cytoplasmic accumulation by inducing HMGB1 hyperacetylation at the NLS sites. LPS also activates CaMKIV and promotes HMGB1 cytoplasmic accumulation by inducing HMGB1 serine phosphorylation. In the second step, endogenous danger signals or pathogens induce canonical or non-canonical inflammasome activation, which in turn activates caspase-1 or caspase-11, respectively. This is followed by pyroptosis, which mediates cytoplasmic HMGB1 release into the extracellular space.



Supplementary Fig. 16. Pharmacological inhibition of JAK/STAT signaling by pyridone 6 did not affect histone deacetylase activity. RAW 267.4 cells were stimulated with LPS (1 μg/ml) in the presence or the absence of pyridone 6 (P6) for 6 h. Histone deacetylase activity of cell lysates was determined by histone deacetylase assay kit, and normalized by total protein levels.