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Supplementary Materials for

The probacterial effect of type I interferon signaling requires its own negative regulator USP18

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at immunology.sciencemag.org/cgi/content/full/3/27/eaau2125/DC1)

Figure S1

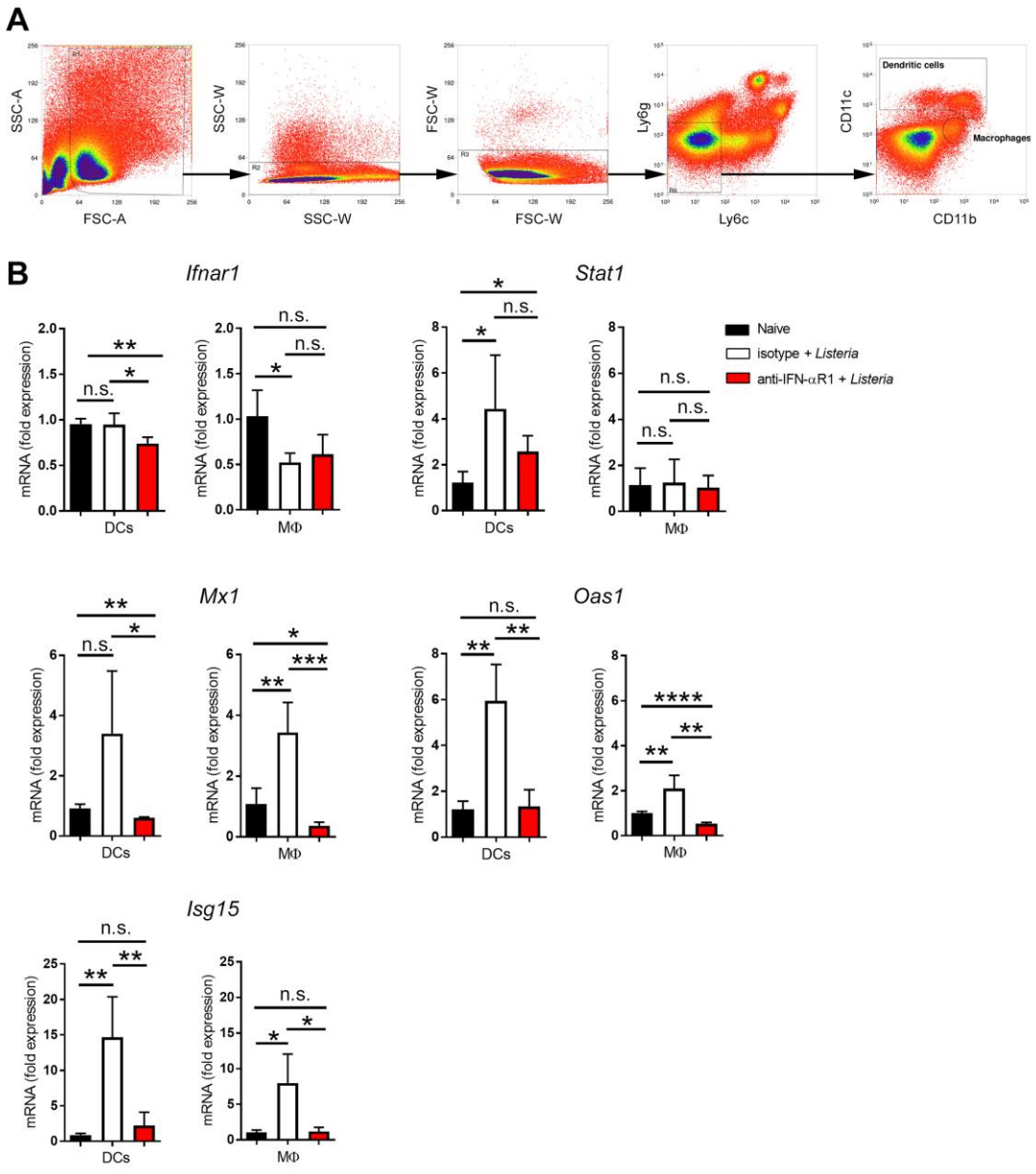

Fig. S1. ISG levels in DCs and MΦ after *L.m.* infection.
C57BL/6 wild-type mice were treated with 1 mg of anti-IFN-αR1 or isotype control antibody. Next day, mice were infected with 4000 CFU *Listeria monocytogenes* (*L.m.*) for 24 hours. DCs and macrophages were sorted by FACS (A) and indicated genes were measured by qRT-PCR (B) (n = 4). Statistical significance was determined by Student`s t-test (B). n.s., not significant; *P < 0.05; **P < 0.01; ***P < 0.001 and ****P < 0.0001. Data are representative of at least two independent experiments.

Figure S2

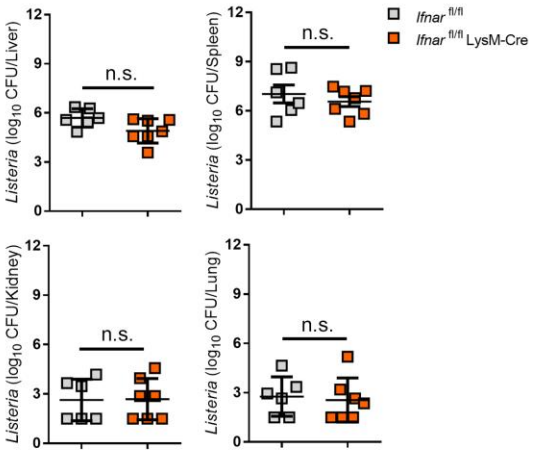

Fig. S2. The role of IFNAR on MΦ during *L.m.* infection.
Ifnar$^{fl/fl}$ LysM-Cre mice and littermate controls were infected with 4000 CFU *Listeria monocytogenes* (*L.m.)*. After 4 days, bacterial titers were measured in the indicated organs (n = 6-7). Statistical significance was determined by Student`s t-test. n.s., not significant. Data are pooled from two independent experiments.

Figure S3

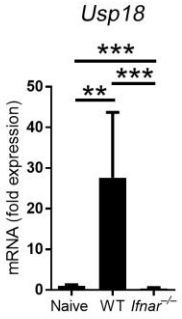

Fig. S3. *Usp18* expression after *L.m.* infection.
C57BL/6 wild-type and *Ifnar* $^{-/-}$ mice were infected with 4000 CFU. After 24 hours, *Usp18* expression was measured in splenocytes by qRT-PCR (n = 6-7). Statistical significance was determined by Student`s t-test. **P < 0.01. and ***P < 0.001. Data are pooled from two independent experiments.

Figure S4

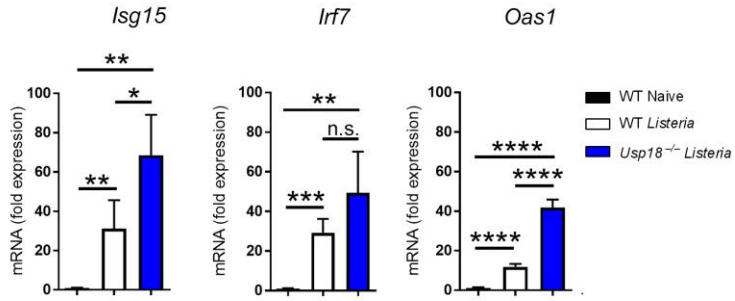

Fig. S4. ISG levels in WT and *Usp18*$^{-/-}$ after *L.m.* infection.
WT or *Usp*18 $^{-/-}$ mice were infected with 4000 CFU *L.m.* After 24 hours, indicated genes were measured by qRT-PCR in the spleen (n = 3-4). Statistical significance was set at the level of $P < 0.05$ and was determined by Student`s t-test. n.s., not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ and ****$P < 0.0001$.

Figure S5

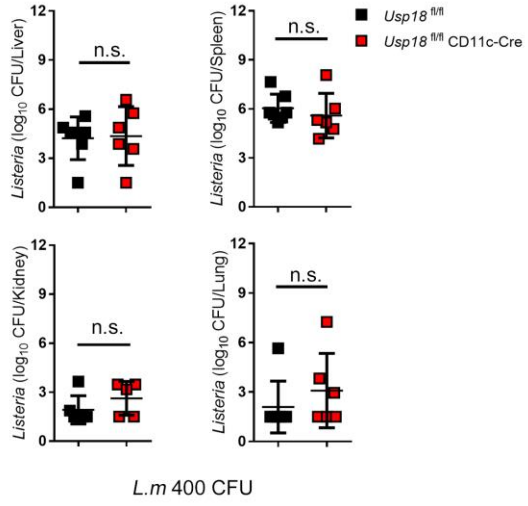

Fig. S5. The role of USP18 in DCs after low dose (400 CFU) of *L.m.*
Usp18$^{fl/fl}$ CD11c-Cre mice and littermate controls were infected with 400 CFU *L.m.* After 4 days, bacterial titers were measured in the indicated organs (n = 6-7). Statistical significance was determined by Student`s t-test. n.s., not significant. Data are pooled from two independent experiments.

Figure S6

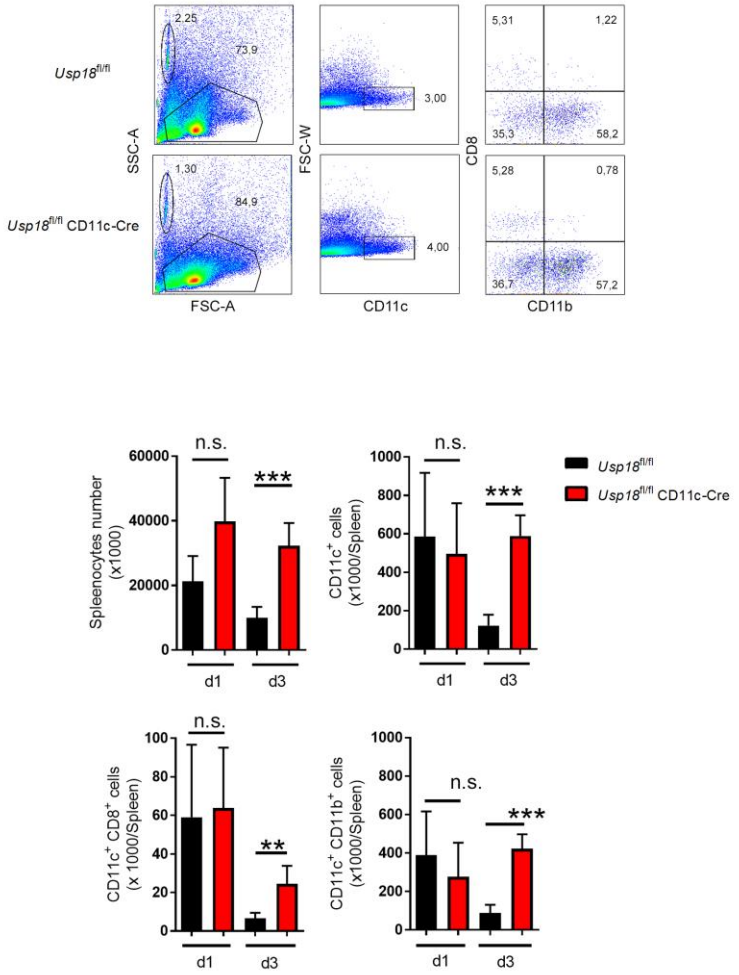

Fig. S6. DC numbers in *Usp18*$^{fl/fl}$ CD11c-Cre mice after *L.m.* infection.
Usp18$^{fl/fl}$ CD11c-Cre mice and littermate controls were infected with 4000 CFU *L.m.* Number of splenocytes and dendritic cells were measured in spleens by FACS on indicated days (n = 4-6). Statistical significance was was determined by Student`s t-test. n.s., not significant; **P < 0.01. and ***P < 0.001. Data are representative of at least two independent experiments.

Figure S7

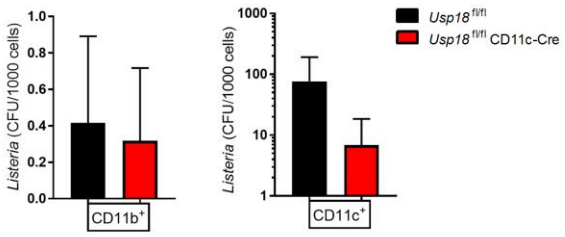

Fig. S7. Bacterial titers in sorted macrophages and DCs after *L.m.* infection.
Usp18$^{fl/fl}$ CD11c-Cre mice and littermate controls were infected with 7000 CFU *Listeria monocytogenes (L.m.)*. After 2 days, splenic CD11b^{+} and CD11c^{+} cells were sorted by FACS, lysed and bacterial titers were measured (n = 4).

Figure S8

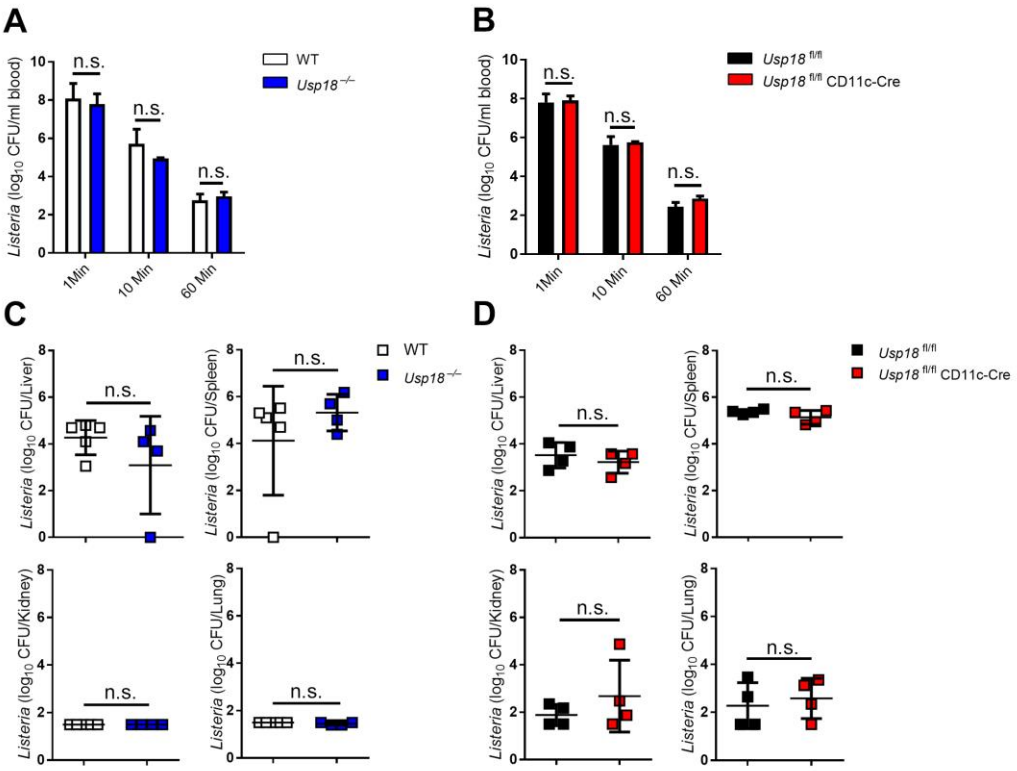

Fig. S8. The role of USP18 in the uptake and early replication of *L.m.*
(A) WT or *Usp18* $^{-/-}$ mice were infected with 1 x 10^9 CFU *Listeria monocytogenes (L.m.).* Bacterial titers were measured in blood at the indicated time points (n = 3). Data are representative of at least two independent experiments. (B) *Usp18*$^{fl/fl}$ CD11c-Cre mice and littermate controls were infected with 1 x 10^9 CFU *L.m..* Bacterial titers were measured in blood at indicated time points (n = 3). Data are representative of at least two independent experiments. (C-D) WT and *Usp18* $^{-/-}$ mice (C) or *Usp18*$^{fl/fl}$ CD11c-Cre mice and littermate controls (D) were infected with 4000 CFU *L.m..* After 24 hours, bacterial titers were measured in indicated organs (n = 4). Data are representative of at least two independent experiments. Statistical significance was determined by Student`s t-test (A-D). n.s., not significant.

Figure S9

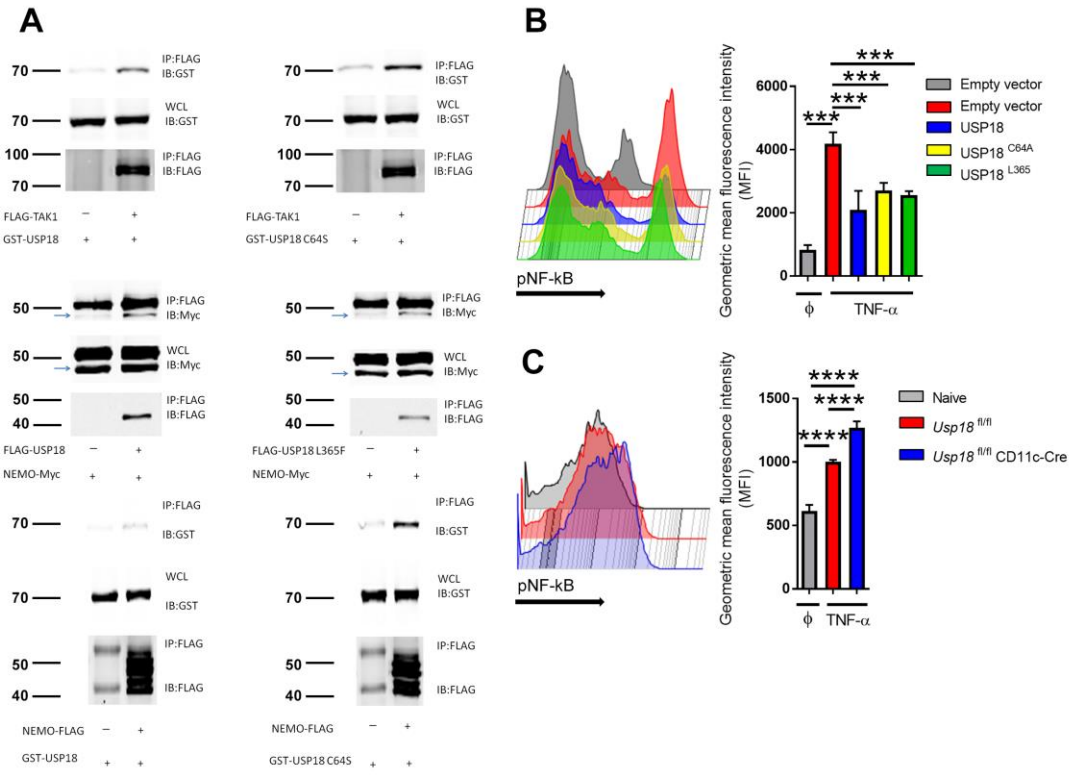

Fig. S9. The role of USP18 in inhibiting TNF-α signaling.
(A) Immunoblot (IB) analysis of whole cell lysates (WCL) or immunoprecipitates (IP) derived from 293T cells 24 hours after co-transfection with plasmids encoding GST-USP18, and NEMO-FLAG, or FLAG-TAK1. Data are representative of at least two independent experiments. (B) HeLa cells were transfected with empty vector, USP18, USP18^{C64A} or USP18^{L365F} plasmid. Cells were treated with 100 ng of recombinant human TNF- α for 20 minutes. P-NF-κb p65 was measured by FACS (n = 6). Data are representative of at least two independent experiments. (C) Bone marrow-derived dendritic cells from *Usp18*$^{fl/fl}$ CD11c-Cre mice and littermate controls were treated with 25 ng of recombinant mouse TNF-α for 15 minutes or left untreated. P-NF-κb p65 was measured by FACS (n = 4). Data are representative of at least two independent experiments. Statistical significance was determined by Student`s t-test (B-C). ***P < 0.001 and ****P < 0.0001.

Figure S10

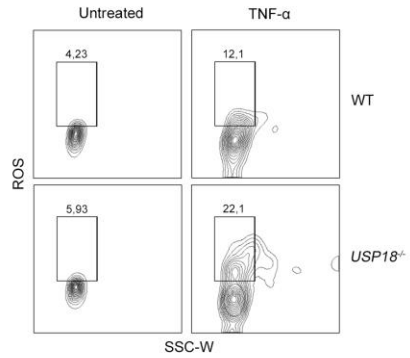

Fig. S10. USP18 inhibits ROS production.
Usp18 $^{-/-}$ and control mice were treated with 50ng TNF-α for 30 minutes or left untreated. Reactive oxygen species (ROS) was measured in DCs (n = 7-9).

Figure S11

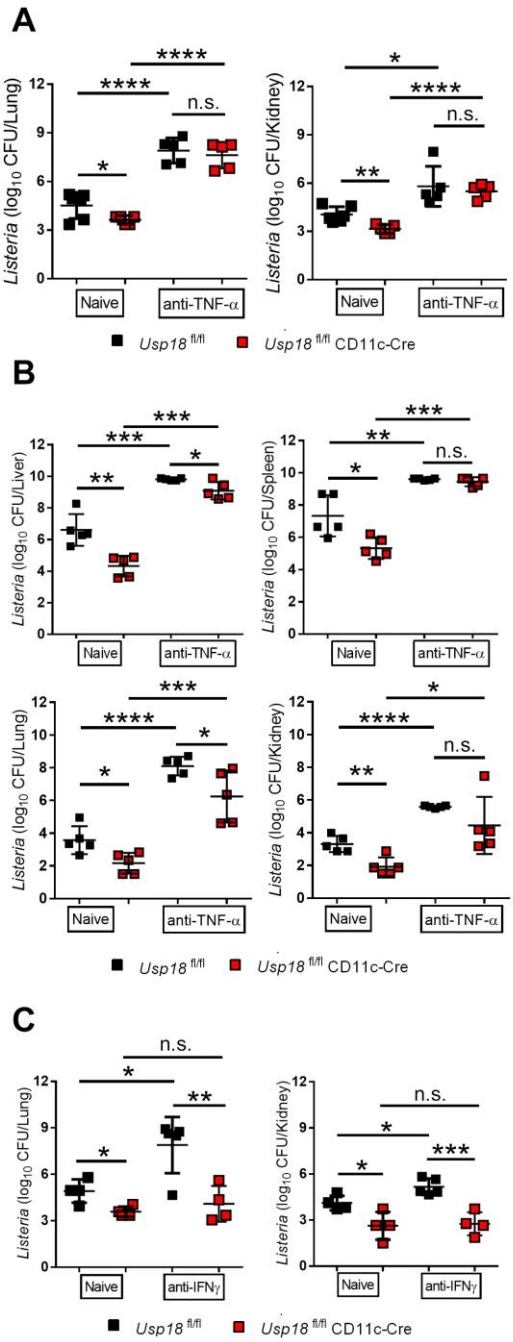

Fig. S11. USP18 inhibits antibacterial effect of TNF-α but not IFN-γ.
(A-B) *Usp18*$^{fl/fl}$ CD11c-Cre mice and littermate controls were treated intraperitoneally with 500 µg (A) or 50 µg (B) of anti-TNF-α antibody on day -1, 1 and 2 or left untreated. Next day, mice were infected with 4000 CFU *L.m.*. After 4 days, titers of bacteria were measured in the indicated organs (n = 5). Data are representative of at least two independent experiments. (C) *Usp18*$^{fl/fl}$ CD11c-Cre mice and littermate controls were treated with 250 µg of anti-IFN-γ antibody on day -1,1 and 2 or left untreated. Next day, mice were infected with 4000 CFU *L.m.* After 4 days, titers of bacteria were measured in the indicated organs (n = 4-5). Data are representative of at least two independent experiments. Statistical significance was determined by Student`s t-test (A-C) n.s., not significant; *P < 0.05; **P < 0.01; ***P < 0.001 and ****P < 0.0001.

Figure S12

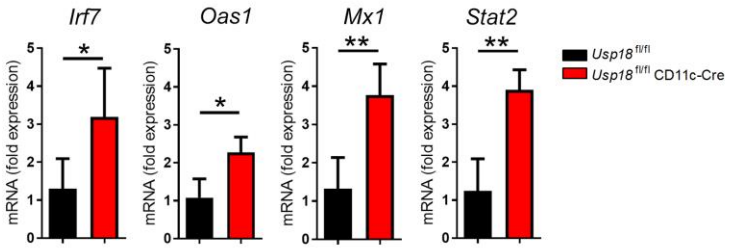

Fig. S12. ISG levels in sorted DCs after LCMV infection.
Usp18$^{fl/fl}$ CD11c-Cre mice and littermate controls were infected with 2 x 10^6 PFU LCMV-WE. After 2 days, DCs were sorted by FACS and indicated genes were measured by qRT-PCR. Relative quantities (RQs) were determined with the equation $RQ = 2^{-ddCt}$ (n = 4). Statistical significance was determined by Student`s t-test. *P < 0.05; **P < 0.01.

Table S1. Mutation sites of murine and human USP18 and the consequences on the functional domains.

Species	Mutation site	Interferon signaling	Isopeptidase activity
Human	C64	not affected	inhibited
Mouse	C61	not affected	inhibited
Human	L365	increased	inhibited
Mouse	L361	increased	inhibited