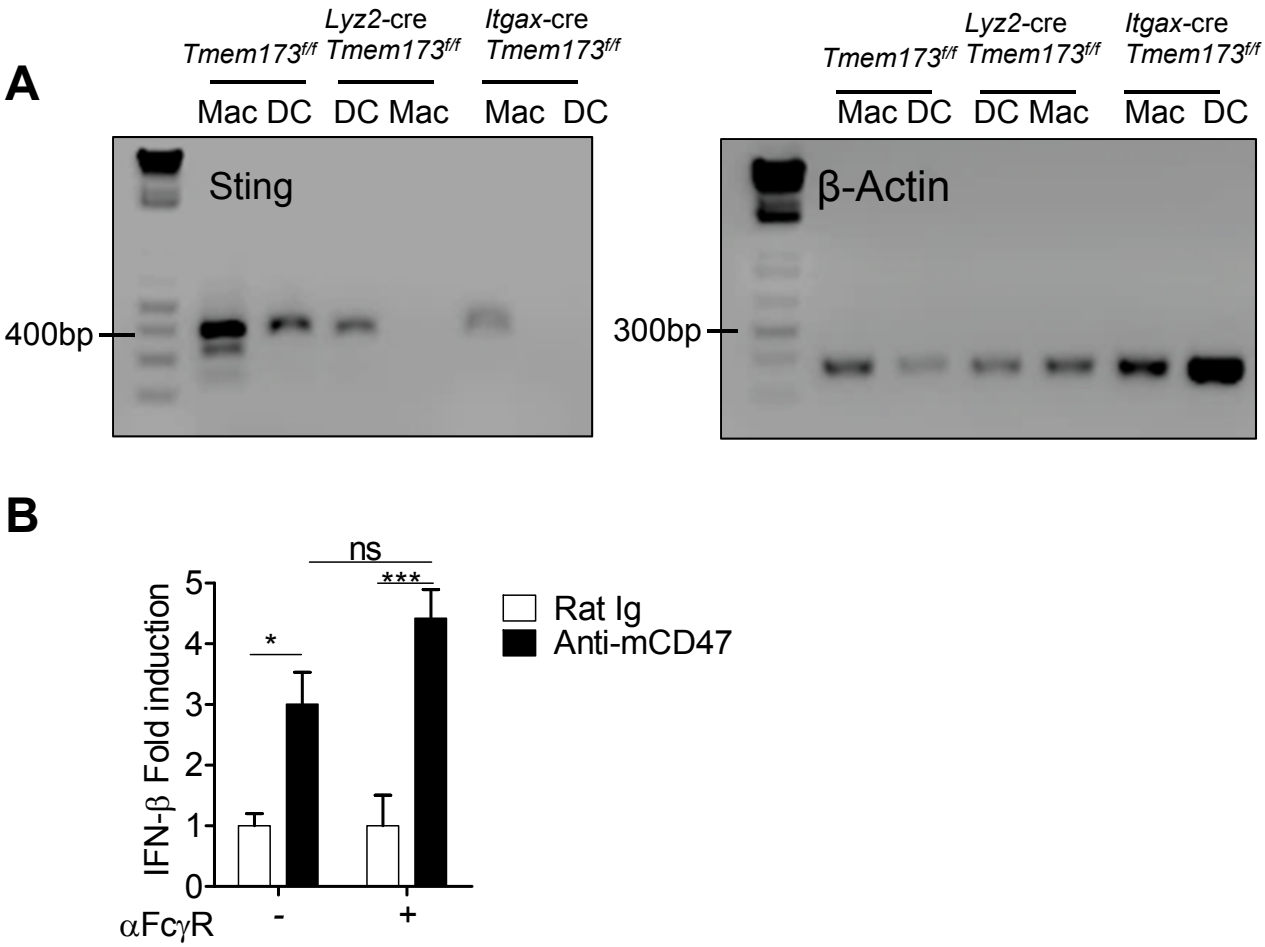

Figure S1, related to Figure 1.STING expression in *Lys2*-cre*;Tmem173$^{f/f}$* and *Itgax*-cre*;Tmem173$^{f/f}$* mice.

(A) The single-cell suspensions from spleens were sorted into $CD11c^{-}CD11b^{+}Ly6c^{-}F4/80^{+}$ (macrophages, Mac) and $CD11c^{+}MHCII^{+}F4/80^{-}$ (DCs) populations. Sting transcript was done in cDNA made from indicated cells from *Tmem173$^{f/f}$, Lyz2*-cre*;Tmem173$^{f/f}$* and *Itgax*-cre*;Tmem173$^{f/f}$* mice. β-Actin was used as the intrasample reference gene.

(B) BMDCs isolated from wild-type mice were cultured with MC38 tumor cells in the presence of rat Ig or anti-CD47 mAb along with FcγR blocking antibody (2.4G2) for 16 hours. Subsequently, purified $CD11c^{+}$ cells were evaluated for IFN-β transcripts by real-time PCR assay. Data are representatives of two independent experiments and presented as mean ± SEM. * $p< 0.05$, *** $p< 0.001$ (Two-tailed student's t test).

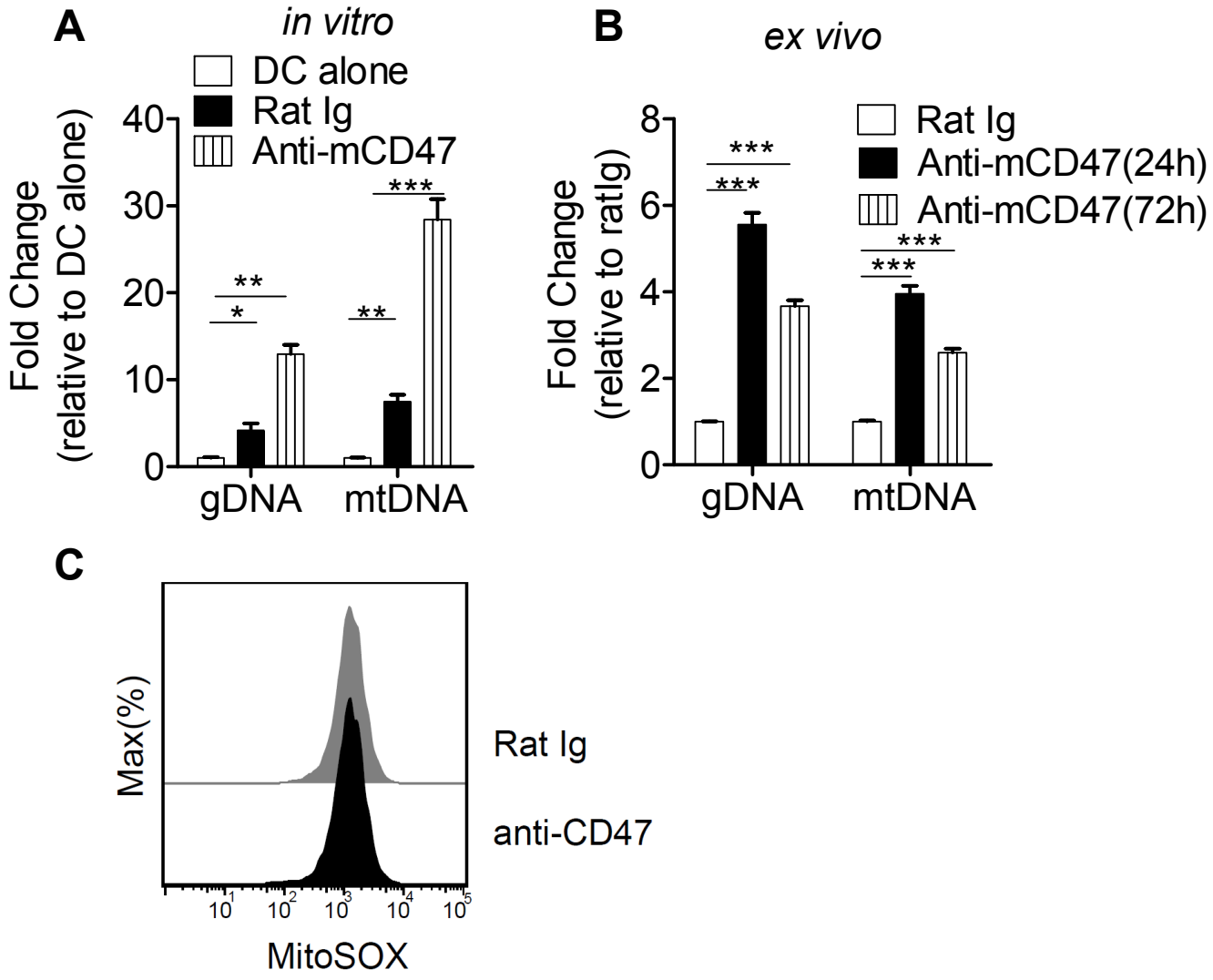

Figure S2, related to Figure 2.Tumor derived DNA is increased inside the cytosol of DCs in response to CD47 blockade.

(A) BMDCs were co-cultured with MC38 in the presence of rat Ig or anti-CD47 mAb for four hours. $CD11c^+$ DCs were purified and treated with digitonin. Cytosolic DNA was extracted and quantitated via qPCR using the primer specific for genomic (*Polg1*) or mitochondrial DNA(*nd1*). Representative data are reported as mean fold change ± SEM after normalizing to DC alone group.

(B) C57BL/6 mice were injected s.c. with 1×10^6 MC38 cells and treated i.t. with 50 μg of anti-CD47 mAb or isotype control rat Ig on day 12. 24 to 72 hours after anti-CD47 mAb or rat Ig treatment, the $CD45^+CD11c^+CD11b^{-/+}Ly6C^-F4/80^-$ DCs were sorted from the tumor by flow cytometry. Cytosolic levels of mtDNA and gDNA were quantitated by real-time PCR assay. Representative data are reported as mean fold change ± SEM after normalizing to ratIg group.

(C) BMDCs isolated from wild-type mice were cultured with MC38 tumor cells in the presence of rat Ig or anti-CD47 mAb for 2 hours. Mitochondiral stress in DC was evaluated by mitoSOX. Data are representatives of two independent experiments.

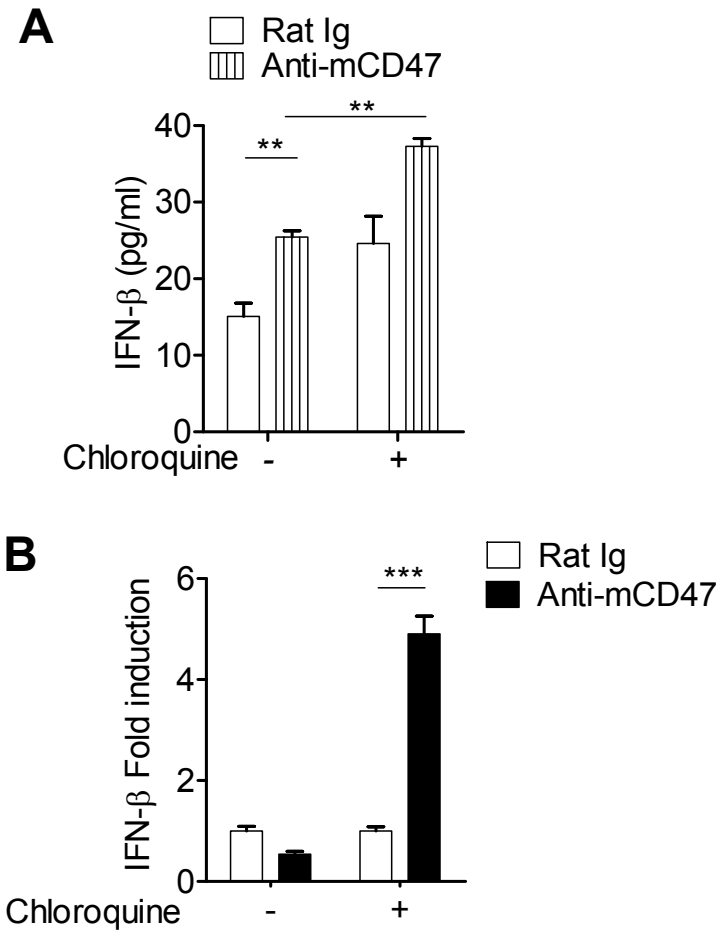

Figure S3, related to Figure 4. Inhibition of phagosomal acidification in DC and macrophage enhances the production of IFNβ after CD47 blockade.
(A)BMDCs were treated with 5μM chloroquine and cultured with MC38 in the presence of anti-CD47 or rat Ig for eight hours. ELISA assays for IFN-β level were performed. Data are representatives of two independent experiments and presented as mean ± SEM. ** p< 0.01 (Two-tailed student's t test).
(B) Bone marrow-derived macrophages (BMDM) were treated with 5μM chloroquine and cultured with MC38 in the presence of anti-CD47 or rat Ig for eight hours. Subsequently, purified macrophages were evaluated for IFN-β transcripts by real-time PCR assay. Data are representatives of two independent experiments and presented as mean ± SEM. *** p< 0.001 (Two-tailed student's t test).

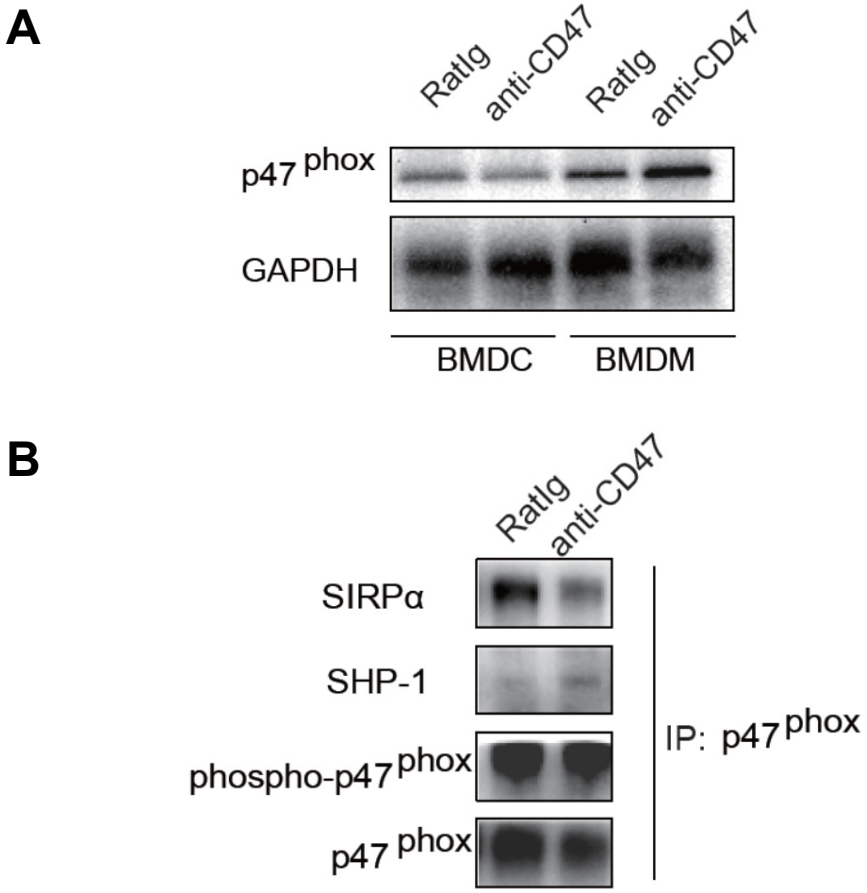

Figure S4, related to Figure 5. CD47 blockade does not modulate the activation of NOX2 in BMDMs.

(A) BMDC or BMDMs were co-cultured with MC38 in the presence of rat Ig or anti-CD47 mAb for four hours. $CD11c^+$ DCs or $CD11b^+$ macrophages were sorted by FACS. Immunoblot analysis of basal p47phox and GAPDH was shown.

(B) BMDMs were co-cultured with MC38 in the presence of rat Ig or anti-CD47 mAb for four hours. $CD11b^+$ macrophages were sorted by FACS and immunoprecipitated with antibody to p47phox. Immunoblot analysis of tyrosine-phosphorylated proteins (p-Tyr) of p47phox, or total p47phox, SIRPα and SHP-1 was shown.

Data are representatives of two independent experiments.

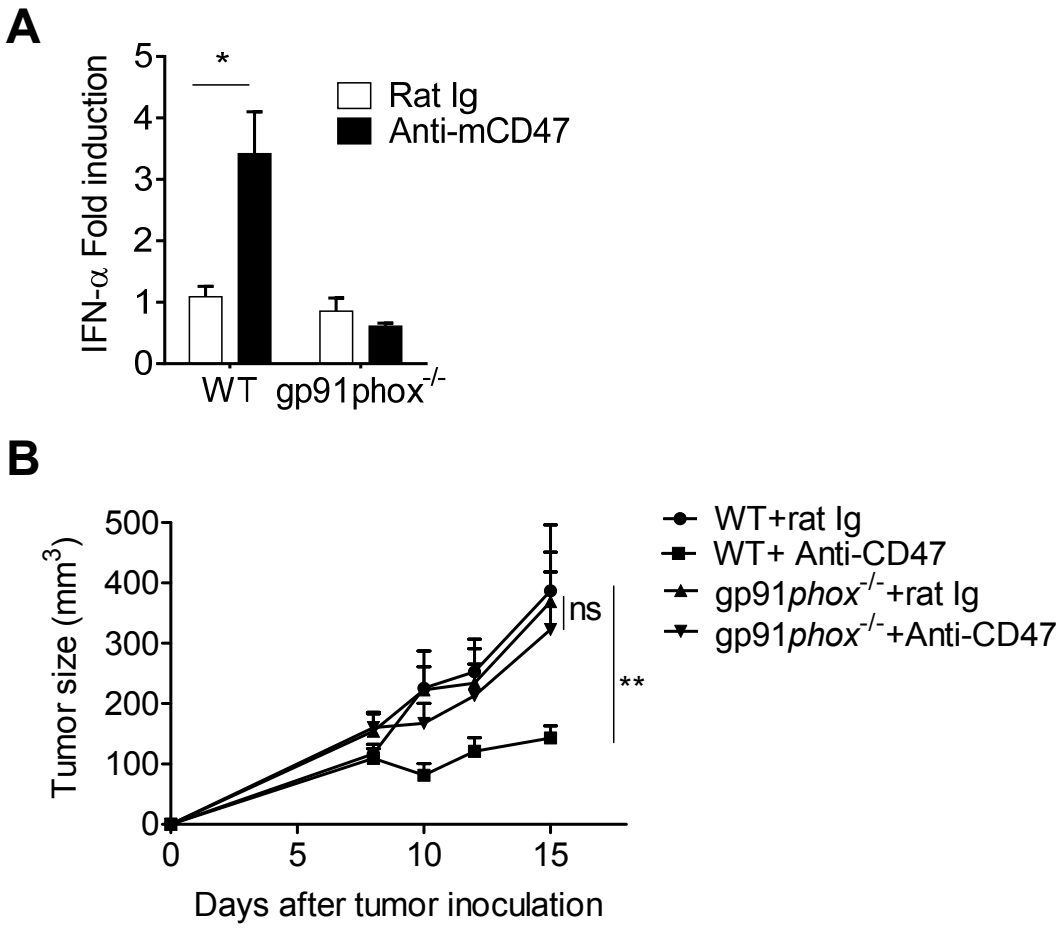

Figure S5, related to Figure 5.NOX2 is required for antitumor response after anti-CD47 treatment.

(A)BMDCs isolated from wild-type (WT) mice or gp91*Phox*$^{-/-}$ (NOX2) mice were cultured with MC38 tumor cells in the presence of rat Ig or anti-CD47 mAb for 8 hours. Subsequently, purified CD11c^{+} cells were evaluated for IFN-α transcripts by real-time PCR assay.

(B) WT mice or gp91*Phox*$^{-/-}$ mice (n = 5 mice/group) were injected s.c. with 1x10^{6} MC38 cells and treated i.t. with 50 μg of anti-CD47 or rat Ig on days 8 and 10. Tumor growth is shown.

Data are representatives of two independent experiments and presented as mean ± SEM. * $p< 0.05$, ** $p< 0.01$ (Two-tailed student's t test).

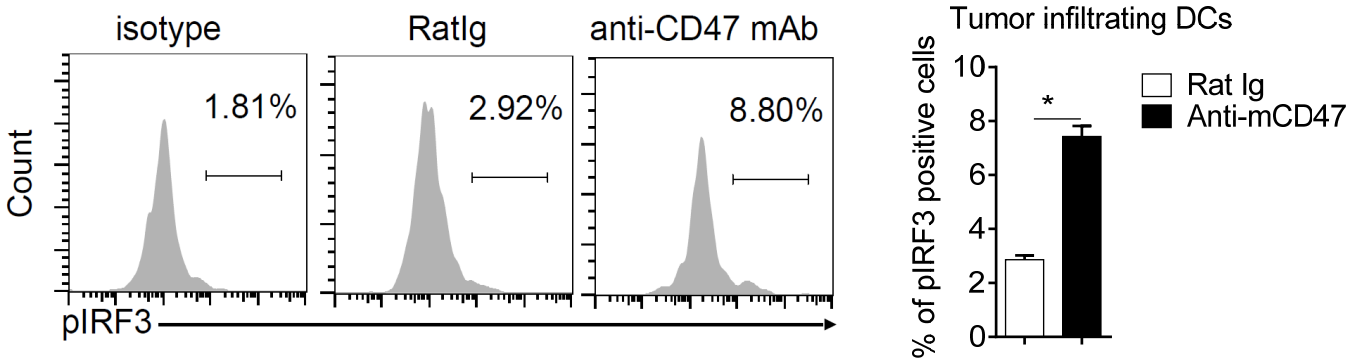

Figure S6, related to Figure 7. IRF3 signaling in DCs is activated by anti-CD47 mAb treatment.

C57BL/6 mice were injected s.c. with 1 × 10^6 MC38 cells and treated i.t. with 50 μg of anti-CD47 mAb or isotype control rat Ig on day 12. 24 hours after treatment, tumors were harvested and digested. Tumor-infiltrating cells were fixed, permeabilized, and stained with CD11c, CD45 and anti-phospho-IRF3 antibody. Data were analyzed by FlowJo software. Phospho-IRF3 positive DCs were quantitated as shown in the panels on the right. Data are represented as mean ± SEM. *p< 0.05 (Student's t test). Data shown is representative of two independent experiments.

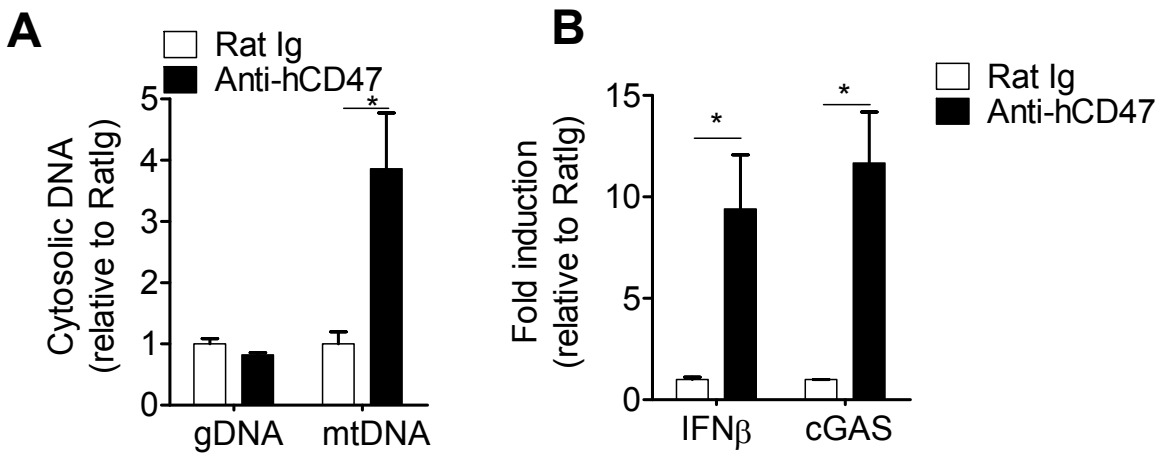

Figure S7, related to Figure 7. CD47 blockade promotes the accumulation of tumor-derived mtDNA in the cytosol of human primary DCs and enhances the production of type I IFN.

(A) Human DCs were co-cultured with HCT116 in the presence of rat Ig or anti-human CD47 mAb (B6H12) for eight hours. CD11c+ DCs were purified and treated with digitonin. Cytosolic DNA was extracted and quantitated via qPCR using the primer specific for genomic or mitochondrial DNA. Representative data are reported as relative to ratIg after normalizing to the copy number of gDNA obtained from the whole-cell extract.

(B) Human DCs were co-cultured with HCT116 in the presence of rat Ig or anti-human CD47 mAb (B6H12) for eight hours. CD11c+ DCs were purified and mRNA levels IFN-β and cGAS were quantified by real-time PCR assay. Representative data are reported as mean copy numbers ± SEM after intrasample normalization to the levels of reference gene β-Actin.

Data are representatives of two independent experiments and presented as mean ± SEM. * $p < 0.05$ (Two-tailed student's t test).