Giordano et. al. Supplemental Data and Methods.

Figure S1

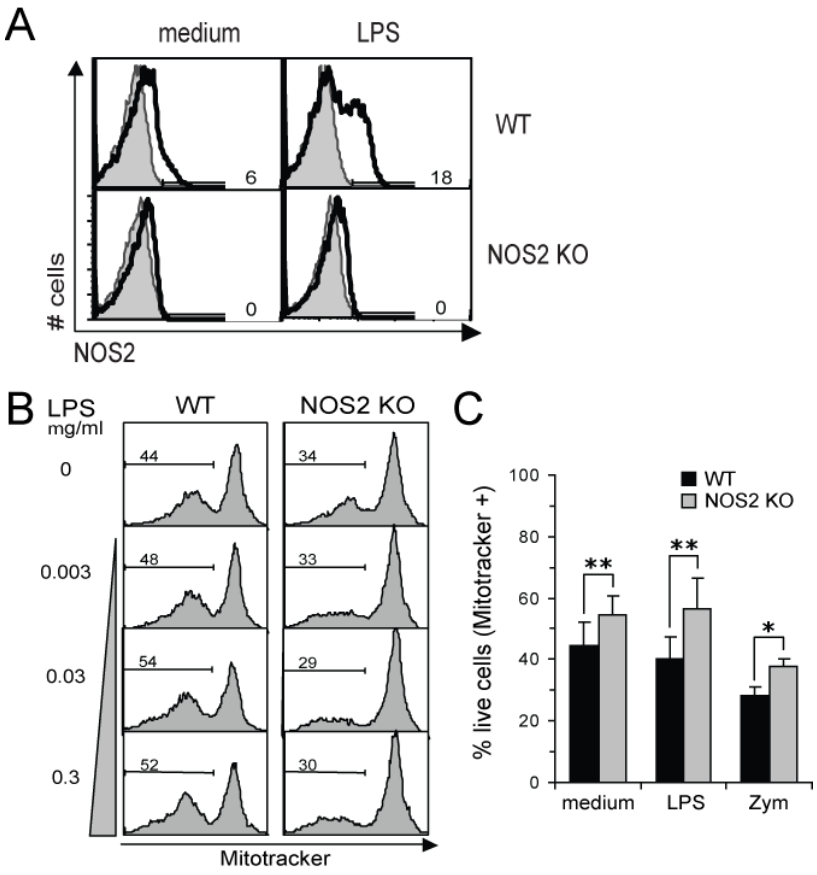

Figure S1. BM-DCs from NOS2 KO mice have increased survival. WT and NOS2 KO BM-DCs were treated 24h with medium, 0.3 μg/ml LPS or 100 μg/ml Zymosan. (A) Cells were stained for NOS2 expression. Plots are gated on $CD11c^{+}$ cells. Percentages of $NOS2^{+}$ cells are shown. Data are representative of >7 independent experiments. (B) Representative flow-cytometric analysis of apoptotic cells detected by mitotracker assay. Percentages of apoptotic cells with ruptured mitochondria are shown. Data are representative of 4 independent experiments. (C) Quantitation of mitotracker analysis from multiple experiments. Live cells were measured as $mitotracker^{bri}$ cells. Graph shows means ± SD; N=7 for medium and LPS and N=3 for Zymosan; $^{*}p<0.05$ $^{**}p<0.01$.

Figure S2

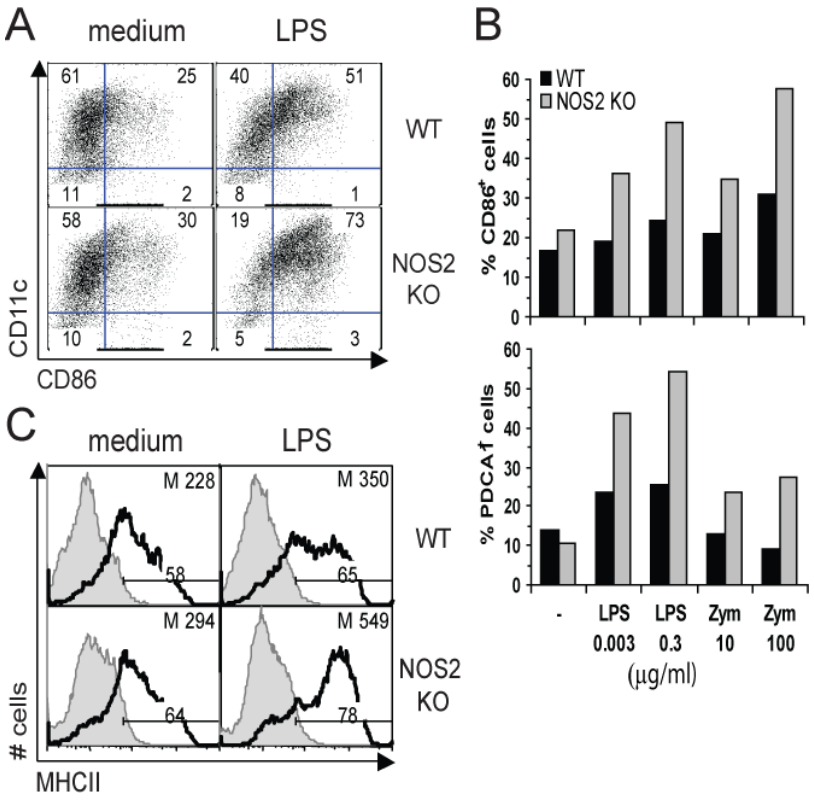

Figure S2. TLR2 and TLR4 stimulation induce more CD86$^+$, PDCA1$^+$ and MHC class II$^+$ DCs in NOS2 KO than in WT BM-DC cultures. WT and NOS2 KO BM-DCs were stimulated for 24h with medium or 0.3 µg/ml LPS (A, C) or the indicated doses of LPS and Zymosan (B). (A) Representative flow cytometric analysis showing the enhanced % of CD11c$^+$CD86$^+$ cells after LPS treatment in NOS2 KO vs. WT DCs. (B) Dose dependent enhancement in CD11c$^+$CD86$^+$ cells (*upper panel*) and CD11c$^+$/PDCA1$^+$ cells (*lower panel*) in NOS2 KO vs. WT DCs is shown. (C) Enhanced % of MHCII$^+$ cells (*gate*) and MHCII mean fluorescence intensity (*M*) (*thick empty histograms*) in NOS2 KO vs. WT DCs after LPS treatment. In (A) and (C) isotype controls are shown as *grey filled histograms.* In (A-C) data are representative of >4 independent experiments.

Figure S3

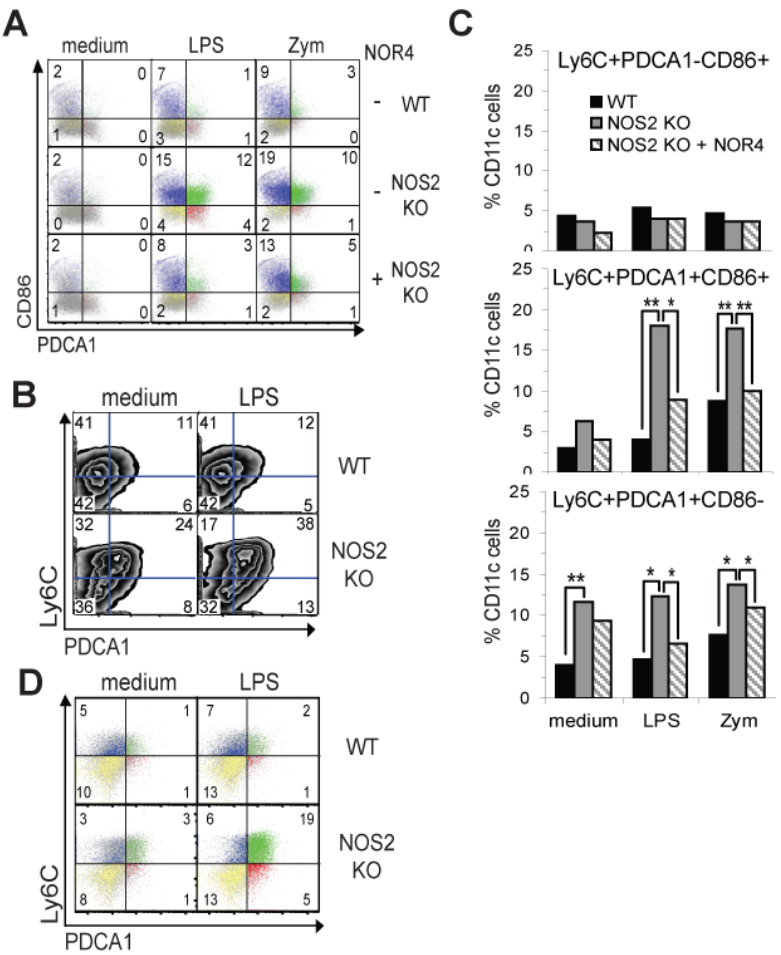

Figure S3. A Ly6C^{hi}PDCA1^{+}CD86^{+} DC subset is up-regulated in NOS2 KO vs. WT BM-DCs. WT and NOS2 KO BM-DCs were stimulated for 14h with medium or 0.3 μg/ml LPS (A-D) and 100 μg/ml Zymosan (A, C). (A, C) NOS2 KO cultures were also treated or not with 100 μM NOR4. Plots are gated on CD11c^{+} cells. (A) Intracellular IL12/23 expressed by the CD86^{+} and PDCA1^{+} DC subsets. Colored dots and % in the plots represent IL-12/23-expressing cells from each DC subset: *blue*, CD86^{+}PDCA1^{-}; *green*, CD86^{+}PDCA1^{+}; *red*, CD86^{-}PDCA1^{+}; *yellow*, CD86^{-}PDCA1^{-}. (B) Enhanced Ly6C^{+}PDCA1^{+}DCs in NOS2 KO cultures. (C) After TLR stimulation the up-regulation of Ly6C^{+}PDCA1^{+}CD86^{+} and Ly6C^{+}PDCA1^{+}CD86^{-} DCs in NOS2 KO cultures is reverted to the WT phenotype by the addition of NOR4. Graph shows means; N=3; $^{*}p<0.05$ $^{**}p<0.01$. (D) LPS stimulation induces enhanced TNFα expression by NOS2 KO Ly6C^{+}PDCA1^{+}DC subset. Colored dots and % in the plots represent TNFα-expressing cells from each DC subset: *blue*, Ly6C^{+}PDCA1^{-}; *green*, Ly6C^{+}PDCA1^{+}; *red*, Ly6C^{-}PDCA1^{+}; *yellow*, Ly6C^{-}PDCA^{-}. In A, B and D, data are representative of ≥3 independent experiments.

Figure S4

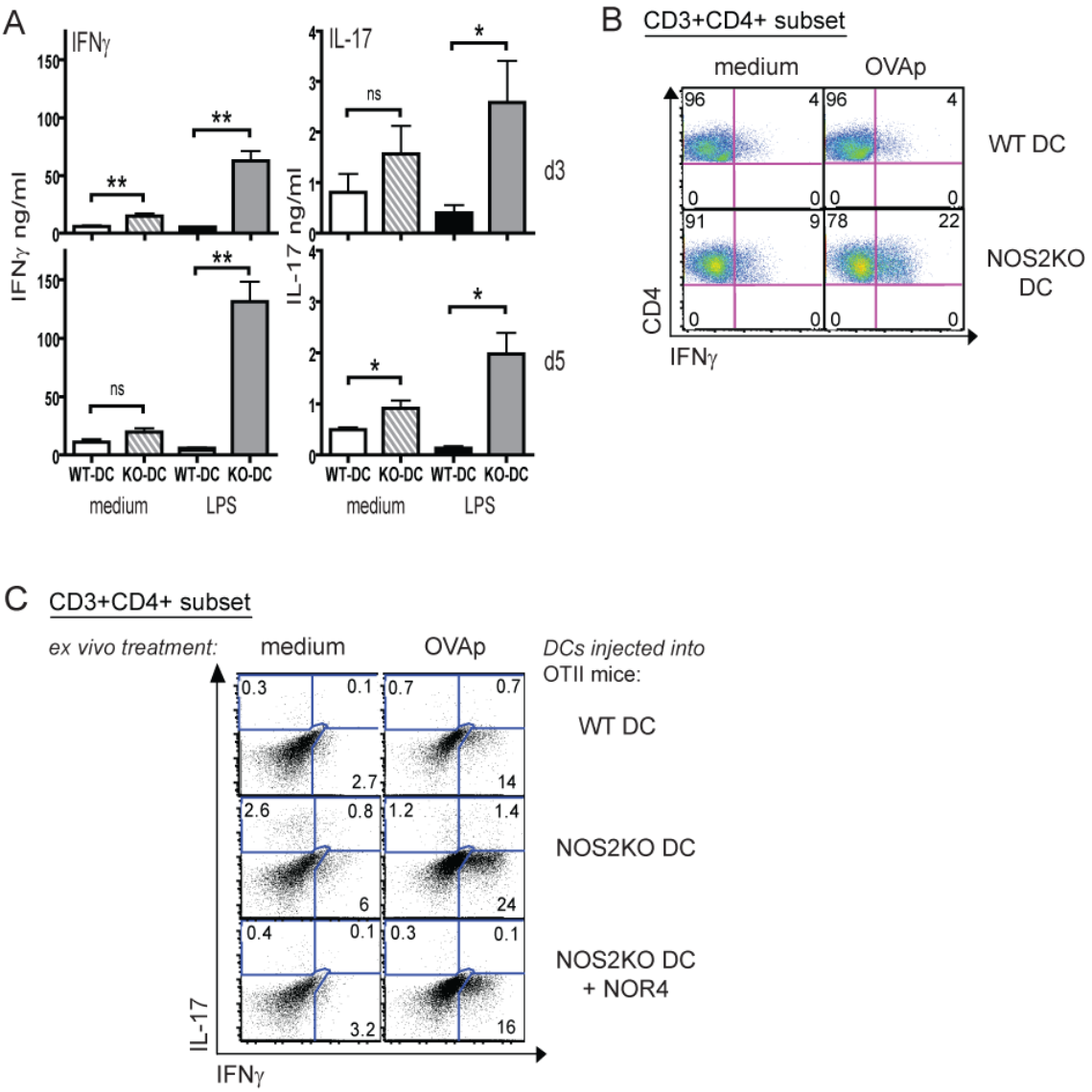

Figure S4. NOS2 KO DCs compared to WT DCs induce CD4$^+$ T cells to produce more IFNγ and IL-17 *in vitro* and *in vivo*. (A, B) NOS2 KO or WT BM-DCs were co-cultured for 5 days with WT CD4$^+$ T cells in medium containing anti-CD3 only or anti-CD3 + LPS. (A) IFNγ and IL17 released in the medium at day 3 and day 5 were measured by ELISA. Data were analyzed with Two-tailed Paired Student's *t*-test and are represented as means ± SD; N=3; **p*< 0.05, ***p*< 0.01. (B) At day 3, IFNγ produced by CD4+ T cells was detected by intracellular staining. Plots are gated on CD3$^+$CD4$^+$ cells. (C) BM-DCs from WT or NOS2 KO mice were treated for 18h with OVAp plus LPS, in absence or presence of 100µM NOR4 for NOS2 KO DCs, then injected i.p. into OTII T cell receptor Tg mice. Spleens were harvested from mice 5 days after injection and splenocytes cultured for 2 days in medium only or in presence of OVAp and restimulated for 5h with Brefeldin A plus PMA/Ionomycin. IFNγ and IL-17 were detected by intracellular staining. Plots are gated on CD3$^+$CD4$^+$ cells. In B and C data are representative of 3 independent experiments.

Supplemental Methods

IFNγ and IL-17 intracellular staining of $CD4^+$ T cells in co-cultures with WT and NOS2 KO BM-DCs (Fig. S4 B), and after *in vivo* priming of OTII mice with WT or NOSKO DCs (Fig S4 C), cells were then stained with PECy5-anti-CD3 (145-2C11) and APC-anti-CD4 (RM4-5) (eBioscience, San Diego, CA, USA), fixed and permeabilized using BD Cytofix/ Cytoperm (BD Bioscience, San Jose, CA, USA), followed by FITC-anti-IFNγ (XMG1.2; eBioscience, San Diego, CA, USA), PE-anti- IL-17 (TC11-18H10; BD Bioscience, San Jose, CA, USA) . Fluorescence acquisition was done on LSRII FACScan analyzer (Becton Dickinson, Franklin Lakes, NJ, USA) using FACSDiva software and data analysis was performed with FlowJo software.