Supplementary information

Newcastle disease virus suppresses antigen presentation via inhibiting IL-12 expression in dendritic cells

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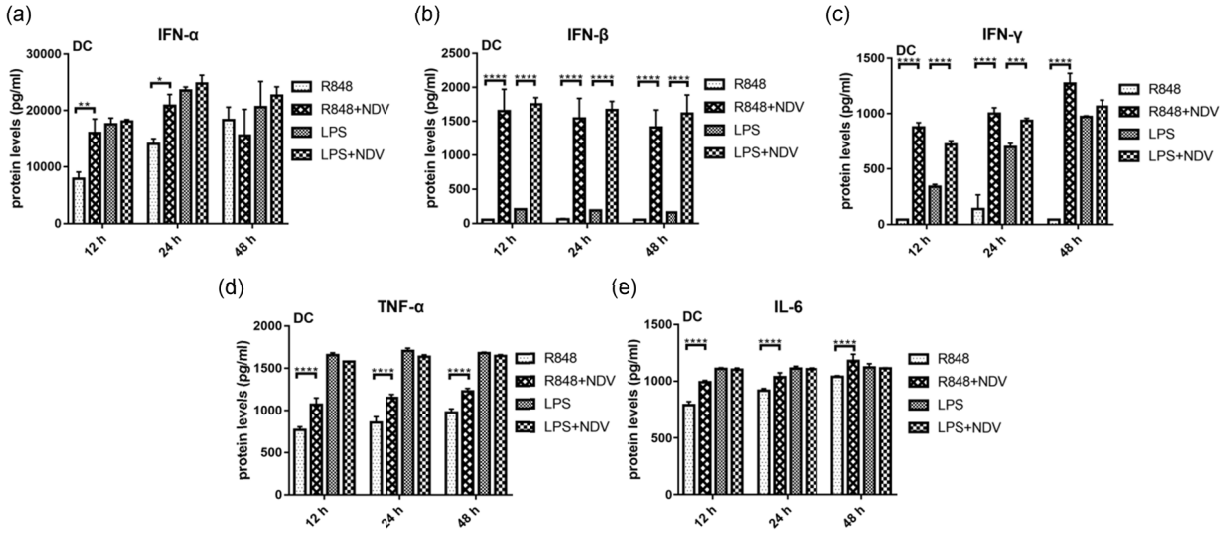

Fig. S1 Proinflammatory cytokine responses in treated DCs. DCs isolated from bone marrow of mice were pretreated with NDV at an MOI of 3 for 12 h and stimulated with LPS for 48 h. Then, supernatants of treated DCs were harvested to measure secretion of IFN-α (a), IFN-β (b), IFN-γ (c), TNF-α (d), and IL-6 (e) by ELISA. Data are expressed as mean±SD of triplicate experiments. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$. DCs: dendritic cells; NDV: Newcastle disease virus; MOI: multiplicity of infection; LPS: lipopolysaccharide; IFN: interferon; TNF-α: tumor necrosis factor-α; IL: interleukin; ELISA: enzyme-linked immunosorbent assay; SD: standard deviation.

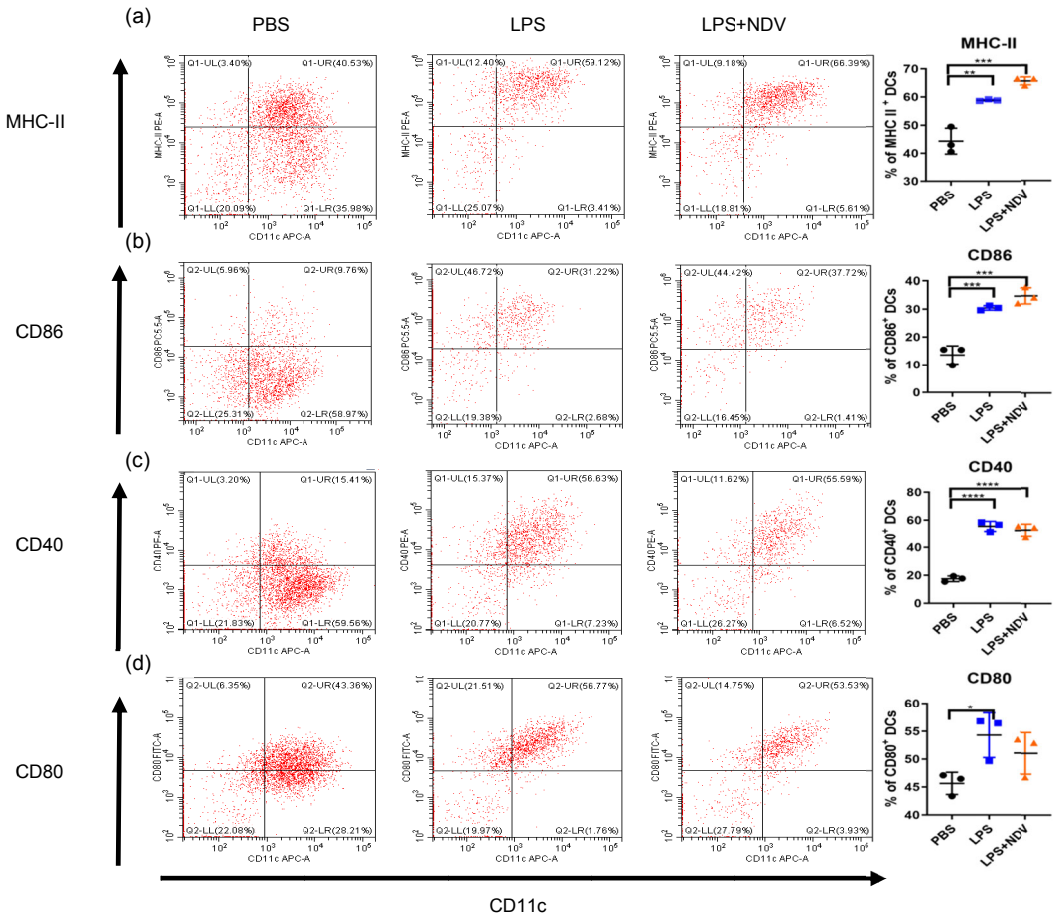

Fig. S2 Surface molecular marker expression in treated DCs. DCs were pretreated with NDV at an MOI of 3 for 12 h and stimulated with LPS for 48 h. Then, flow cytometry was performed to analyze the proportions of MHC-II (a), CD86 (b), CD40 (c), and CD80 (d) expressing DCs. DCs: dendritic cells; NDV: Newcastle disease virus; MOI: multiplicity of infection; LPS: lipopolysaccharide; MHC-II: major histocompatibility complex class II; CD: cluster of differentiation.

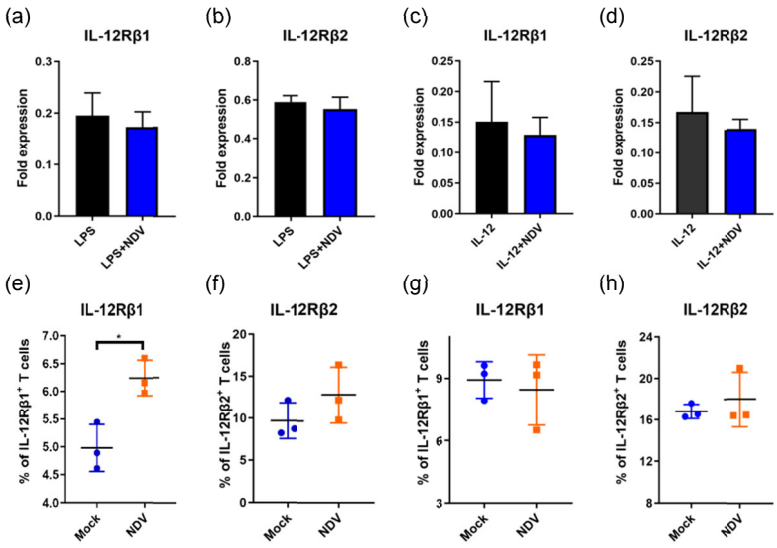

Fig. S3 Expression of IL-12 receptors in treated DCs and T cells. (a, b) DCs were pretreated with NDV at an MOI of 3 for 12 h and stimulated with LPS for 48 h. Transcriptional levels of IL-12Rβ1 and IL-12Rβ2 in DCs were evaluated via qRT-PCR. (c, d) T cells were pretreated with NDV at an MOI of 3 for 12 h and stimulated with 200 ng/mL IL-12 for 48 h. Transcriptional levels of IL-12Rβ1 and IL-12Rβ2 in T cells were evaluated via qRT-PCR. (e, f) Ratios of IL-12Rβ1- and IL-12Rβ2-expressing T cells were detected by flow cytometry. (g, h) DCs were pretreated with NDV at an MOI of 3 and stimulated with LPS for 48 h, and then treated DCs were co-cultured with splenic lymphocytes for 48 h. IL-12Rβ1- and IL-12Rβ2-expressing T cells in co-cultures were measured through flow cytometry. Data are expressed as mean±SD of triplicate experiments. $^{*}P<0.05$. IL: interleukin; DCs: dendritic cells; NDV: Newcastle disease virus; MOI: multiplicity of infection; LPS: lipopolysaccharide; qRT-PCR: quantitative real-time polymerase chain reaction; SD: standard deviation.

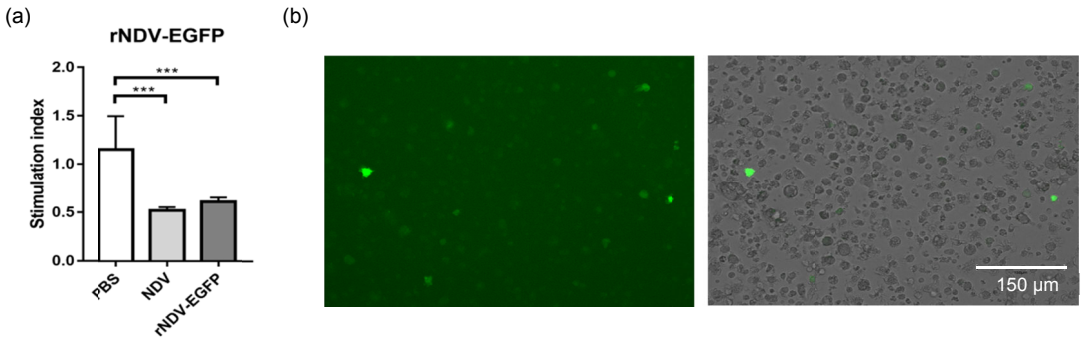

Fig. S4 Inhibitory effect of rNDV-EGFP. (a) DCs were treated with rNDV-EGFP/LaSota parental strain at an MOI of 3 and then co-cultured with T cells for 48 h. Proliferation of T cells was measured using MLR assay. (b) DCs were infected with rNDV-EGFP and the expression of green fluorescent was observed under a fluorescence microscope (20×). Data are expressed as mean±SD of triplicate experiments. $^{***}P<0.001$. NDV: Newcastle disease virus; rNDV: recombinant NDV; EGFP: enhanced green fluorescent protein; DCs: dendritic cells; MOI: multiplicity of infection; MLR: mixed leukocyte reaction; PBS: phosphate-buffered saline; SD: standard deviation.

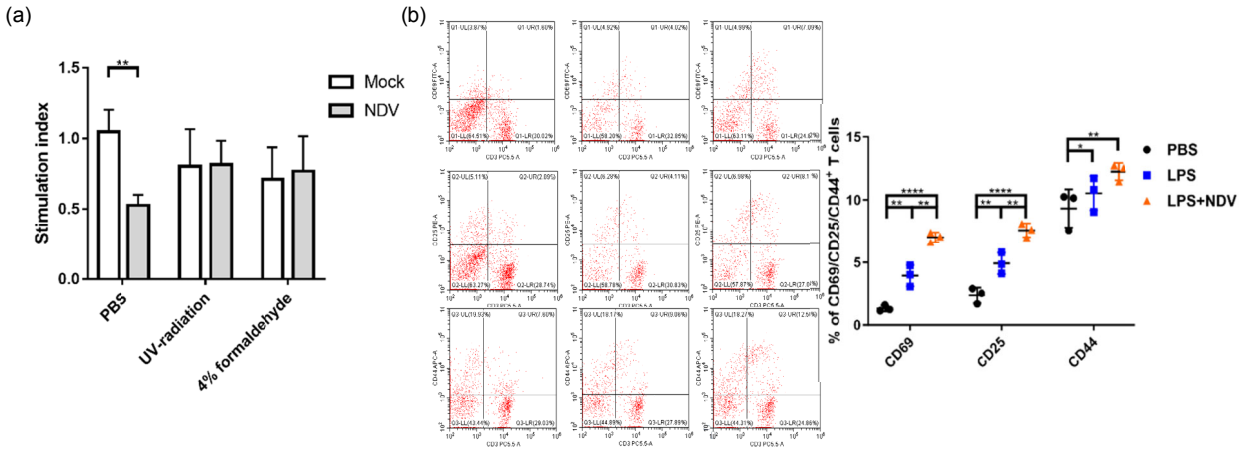

Fig. S5 Identification of immunological synapse on DCs and activation of T cells in co-cultures. (a) DCs were pretreated with NDV at an MOI of 3 for 12 h and stimulated with LPS. After 48 h, DCs were treated with mock (PBS), UV-radiation or 4% formaldehyde (maintaining immunological synapse) for 30 min, and then co-cultured with T cells for 48 h. Proliferation of T cells was measured using MLR assay. (b) DCs were pretreated with NDV for 12 h and then stimulated with LPS. After 48 h, DCs were co-cultured with T cells and proportions of $CD69^+$, $CD25^+$, and $CD44^+$ T cells (activated T cells) were detected via flow cytometry. Data are expressed as mean±SD of triplicate experiments. $^*P<0.05$, $^{**}P<0.01$, $^{****}P<0.0001$. DCs: dendritic cells; NDV: Newcastle disease virus; MOI: multiplicity of infection; LPS: lipopolysaccharide; PBS: phosphate-buffered saline; UV: ultraviolet; MLR: mixed leukocyte reaction; CD: cluster of differentiation; SD: standard deviation.

Table S1 qRT-PCR primers used for detection

Primers	Primer sequence	Gene
GAPDH F	5'-CATGGCCTTCCGTGTTCCT-3'	Mouse *GAPDH*
GAPDH R	5'-TCAGGGGCTACTCTCAGCTC-3'	
IL-12p35 F	5'-GGACCAAACCAGCACAT-3'	Mouse *IL-12p35*
IL-12p35 R	5'-CGCAGAGTCTCGCCATTA-3'	
IL-12p40 F	5'-TGAACTGGCGTTGGAAG-3'	Mouse *IL-12p40*
IL-12p40 R	5'-GAAGTAGGAATGGGGAGTG-3'	
IL-12Rβ1 F	5'-ACTGGAATGTGTCTGAAG-3'	Mouse *IL-12Rβ1*
IL-12Rβ1 R	5'-CGTATCTGGATCTCTTGG-3'	
IL-12Rβ2 F	5'-CCTCAATGGTATAGCAGAAC-3'	Mouse *IL-12Rβ2*
IL-12Rβ2 R	5'-TAGCCTTGGAATCCTTGG-3'	
NDV F	5'-AGTGATGTGCTCGGACCTTC-3'	*NP* gene of NDV
NDV R	5'-CCTGAGGAGAGGCATTTGCTA-3'	
IFN-γ F	5'-GCTGTTACTGCCACGGCACAGT-3'	Mouse *IFN-γ*
IFN-γ R	5'-CACCATCCTTTTGCCAGTTCCTCC-3'	
TNF-α F	5'-CCCTCCTGGCCAACGGCATG-3'	Mouse *TNF-α*
TNF-α F	5'-TCGGGGCAGCCTTGTCCCTT-3'	

qRT-PCR: quantitative real-time polymerase chain reaction; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; IL: interleukin; NDV: newcastle disease virus ; IFN-γ: interferon-γ; TNF-α: tumor necrosis factor-α; F: forward; R: reverse.