Supplementary Methods

Cell isolation and cultures: Briefly, bone marrow cells were flushed from the femurs of 8- to 12-week-old female C57BL/6 mice and cultured in 150 x 15-mm CytoOne petri dishes (USA Scientific, Ocala, FL) at $1x10^7$ in 20ml of RPMI supplemented with 10% FCS, 4mM L-glutamine, 10 U/ml penicillin, 100 μg/ml streptomycin, 0.5 mM 2-ME, 20ng/ml GM-CSF. On day 4, 20ml of fresh complete RPMI containing 20ng/ml GM-CSF was added to each culture. After 7 days of culture, the loosely adherent cells were harvested and purified using anti-CD11c magnetic microbeads (Miltenyi Biotec Ltd., Auburn, CA) and the autoMACS (Miltenyi Biotec).

Human peripheral blood mononuclear cells (PBMCs) were isolated from the blood of healthy donor volunteers. PBMCs were isolated according to a protocol obtained from Current Protocols in Immunology. Briefly, whole heparinized blood was centrifuged (2000 rpm, 20ºC, 30 min) using a Ficoll Hypaque density gradient. The mononuclear layer was removed from the gradient and washed in complete RPMI and used as either responders in human MLR or monocytes were cultured at $1x10^6$ cells/ml in complete RPMI containing 800 U/ml human recombinant GM-CSF and 500 U/ml human recombinant IL-4. On day 5, fresh complete RPMI containing 100 U/ml TNF-α as well as 800 U/ml human recombinant GM-CSF and 500 U/ml human recombinant IL-4 was added to the culture. Cells were harvested on day 9 to 10 of incubation. All human recombinant cytokines were purchased from Peprotech (Rocky Hill, NJ).

The DC cell line, JAWSII, was established from bone marrow cells of a p53-knockout C57BL/6 mouse, and was purchased from the American Type Culture Collection (CRL-11904; ATCC, Manassas, VA). Cells were grown in a CO_2 incubator at 37ºC and 5% CO_2 in complete culture medium consisting of α-MEM with 10% FCS, 4mM L-glutamine, 10U/ml penicillin and 100μg/ml streptomycin, 0.5mM 2-ME, 1mM sodium pyruvate, and 5ng/ml murine GM-CSF. Cultures were maintained by transferring nonadherent cells to a centrifuge tube and treating attached cells with 0.25% trypsin-0.03% EDTA

(Gibco) at 37ºC for 5 min after rinsing culture flask with pre-warmed PBS. The two populations were pooled together, washed once (1000rpm, 10min) and distributed into new culture flasks

Cytokine Detection: Isolated BMDC and human moDCs were seeded on 60mm culture dishes at $3x10^6$ cells/well and a 24-well plate at $2x10^5$ cells/well, respectively. Pretreated cells were cultured with DMSO (Sigma), MLN4924 (Active Biochem), dexamethasone (APP Pharmaceuticals), or bortezomib (Fisher Scientific) at the indicated dosages for 2 hours. Cells were then stimulated with *E. coli*-derived LPS (Invivogen), synthetic Pam3CSK4 (Invivogen), PGN (Invivogen), or CD40L (Shenandoah Biotechnology) at the indicated concentrations for a concurrent 4 hours. Supernatants were subsequently collected and stored at -20ºC until analysis. TNFα and IL-6 ELISA kits (mouse and human) were purchased from R&D Systems and performed as per the manufacturers' instructions and read at 450nm by a SpectraMax microplate reader (Molecular Devices, Sunnyvale, CA).

Quantitative PCR: Using 1μg of each RNA template, cDNA was synthesized using SuperScript VILO (Invitrogen; Carlsbad, CA). qPCR primers for murine GAPDH (Forward: CCACAGTCCATGCCATCACTGC; Reverse: GCCCAAGATGCCCTTCAGTGGG), TNF α (Forward: CGACGTGGAGGCAGAAGAGGC; Reverse: CGTGGGCTACAGGCTTGTCACTC), IL-6 (Forward: CGGAGAGGAGACTTCACAGAGG; Reverse: GCAAATTTCCTGATTATATCCAG), Rbx2 (Forward: GGACGTTGAGTGCGATACCT; Reverse: TGCCGATTCTTTGGACTACC), βTrCP (Forward: TCTGCACCTGCGCTTCAATA; Reverse: GAGGGTGATGTCAGTTGGGG), or Cul-5 (Forward: CAACGGAACTCCCAGATGCT; Reverse: GCTGCAAGCGTCCAATCAAA).

Flow Cytometry: To analyze DC surface phenotype, DCs were incubated in the presence or absence of MLN4924 or the vehicle (DMSO, Sigma). Cells were then harvested and stained with CD11c-conjugated APC (Clone: N418) and one of the following per triplicate group: Annexin V (BD Biosciences), CD80 (Clone: 16-10A1), CD86 (Clone: GL-1), MHCII-I-A^b (Clone: AF6-120.1), PD-L1 (Clone: MIH5), PD-L2

(Clone: TY25). All flow cytometry Abs were purchased from eBiosciences. Stained cells were then analyzed with an Accuri C6 Flow Cytometer (BD Biosciences).

Western Blot and Subcellular Fractionation: BMDCs were seeded in 60mm dishes with $3x10^6$ cells per dish. Pretreated cells were cultured with DMSO (Sigma), MLN4924 (Active Biochem), or dexamethasone (APP Pharmaceuticals) at the indicated dosages for 2 hours followed by concurrent stimulation with *E. coli*-derived LPS (100ng) for an additional 4 hours. Following treatment and stimulation, whole cell lysates where obtained and protein concentrations determined with Pierce BCA Protein Assay (Thermo Scientific). Equal amounts of protein were separated by SDS-PAGE gel (120V, 1.5h) and subsequently transferred to nitrocellulose membrane (20V, 1h). The following antibodies were used to analyze the membranes: Nedd8 (19E3), α-tubulin (11H10), NF-**κ**B p65 (D14E12), Lamin A/C (4C11), pIκBα (5A5), IκBα (L35A5), pERK (D13.14.4E), ERK (137F5) were purchased from Cell Signaling (Danvers, MA). Cullin1 (EPR3103Y) and LDH (EP1566Y) were purchased from abcam (Cambridge, MA). Secondary antibodies conjugated to HRP (Jackson ImmunoResearch) were used to detect primary antibodies. Densitometric analysis performed by ImageJ software. Subcellular fractionation was performed on $3x10^6$ BMDC seeded in 60mm dishes. Cells were pretreated in the presence or absence of vehicle, MLN4924, or dexamethasone for 2 hours followed by concurrent LPS stimulation for 30 minutes. The cytoplasmic and nuclear fractions were isolated using the Nuclear Extract Kit (Active Motif) per the manufacturers instructions. The extracts where then analyzed via western blot.

Mixed Lymphoctye Reaction (MLR): Splenic T cells ($2x10^5$/well) were magnetically separated from WT-B6 or WT-BALB/c mice by autoMACS using CD90.2 microbeads and subsequently cultured with irradiated (30 Gy) WT-B6 DC at 40:1 ($5x10^3$/well) and 100:1 ($2x10^3$/well) for 72 hours and 96 hours. Human MLRs were performed by co-culture of PBMCs ($1x10^5$/well) and moDCs at 1:1($1x10^5$) and 10:1 ($1x10^4$) ratios for 96 hours and 120 hours. Incorporation of ^{3}H-thymidine (1μCi/well) by proliferating T cells or PBMCs during the final 6 hours of culture was measured by a TopCount (PerkinElmer).

Genetic Knockdown: JAWS II cells were seeded into a 24-well plate ($2.5x10^5$/well) overnight at 37ºC. Cells were transfected with siRNA (3μg) using Oligofectamine Reagent (Invitrogen) as per the manufacturers instructions. Cells were incubated with transfection medium and siRNA for 24 hours before stimulation with LPS for 4 hours. RNA was extracted from cell pellets using RNeasy Mini Kit (Qiagen) and analyzed via qPCR. Rbx1, Rbx2, βTrCP, and Cul-5 siRNA were obtained from Ambion (Carlsbad, CA)

Confocal Microscopy: BMDC were seeded onto Corning glass cover slips ($1x10^5$ cells/slip) (Fisher Scientific) overnight at 37ºC. Pretreated cells were cultured with DMSO (Sigma) or MLN4924 (Active Biochem) at the indicated dosages for 2 hours followed by concurrent stimulation with *E. coli*-derived LPS for an additional 1 hour. Cover slips were then washed, fixed with 4% paraformaldehyde for 20 minutes, and subsequently permeabilized with 0.3% Triton X. Cells were stained with NF-**κ**B p65 (D14E12) primary (1:500) and Alexa Fluor 488 (Molecular Probes) secondary (1:1000), DAPI (Invitrogen), and Alexa Fluor 555 Phalloidin (1:500). Coverslips were then mounted using ProLong Gold Antifade Reagent (Molecular Probes) and Z-stack images were acquired using a Nikon A-1 confocal microscope (Mellville, NY) using an oil immersion 60X objective with a numerical aperture = 1.4 and imported into NIS-Elements Software (Nikon). Excitation lasers 405nm, 488nm, and 561nm were used. Microscope laser exposure and settings were obtained using appropriate isotype controls and were retained for each experimental group.

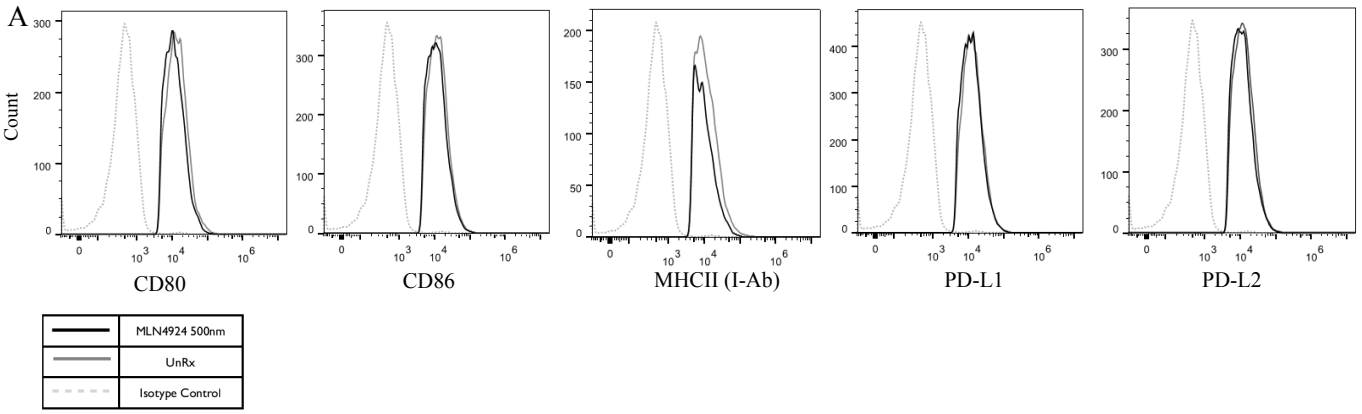
Supplementary Figure Legends

Figure S1. Inhibition of neddylation does not alter phenotype or cell viability in BMDC. (A) Flow cytometric analysis of cell surface phenotypic markers on BMDC. (B) Cell viability of BMDC cultured in the presence or absence of vehicle or MLN4924 for 24 hours. One representative experiment of three is shown. ** $P<0.01$

Figure S2. Description of in vivo transplant scheme. Abb recipient mice, which lack expression of MHC II, were lethally irradiated (10 Gy) and transplanted with vehicle or MLN4924 treated WT B6 BMDC ($10x10^6$) in 2 doses separated by 24 hours, on d-1 and d0. $CD90.2^+$ T cells ($2x10^6$) were transferred on d0 to Abb recipients. The spleens of recipient mice were analyzed for T cell activation following sacrifice on day 6.

Figure S3. T cell proliferation and Th1 lineage cytokines are decreased following stimulation with α-CD3 and α-CD28 functional Ab. Balb/c T cells were stimulated with α-CD3 and α-CD28 functional Abs in the presence or absence of MLN4924. After 42h incubation, ^3H (1μCi/well) was added for and additional 6 hours followed by (A) determination of proliferating T cells. Supernatants were removed prior to ^3H stimulation and analyzed for (B) IFNγ via ELISA.

Figure S1



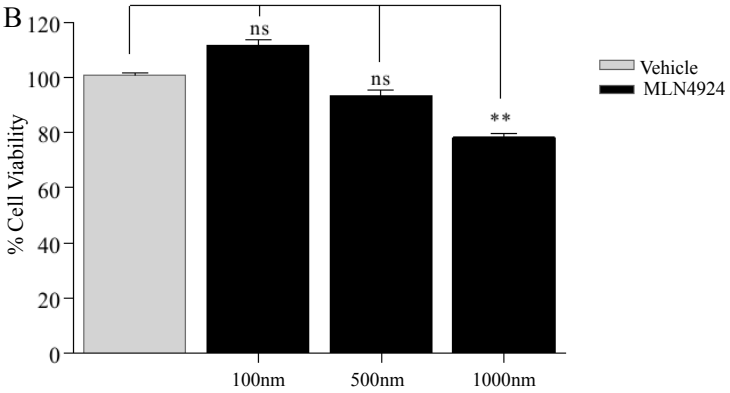


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Figure S2

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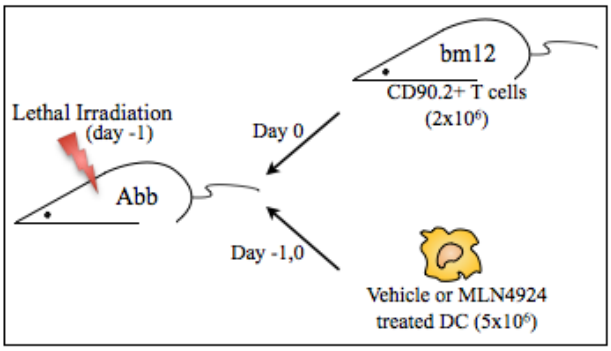

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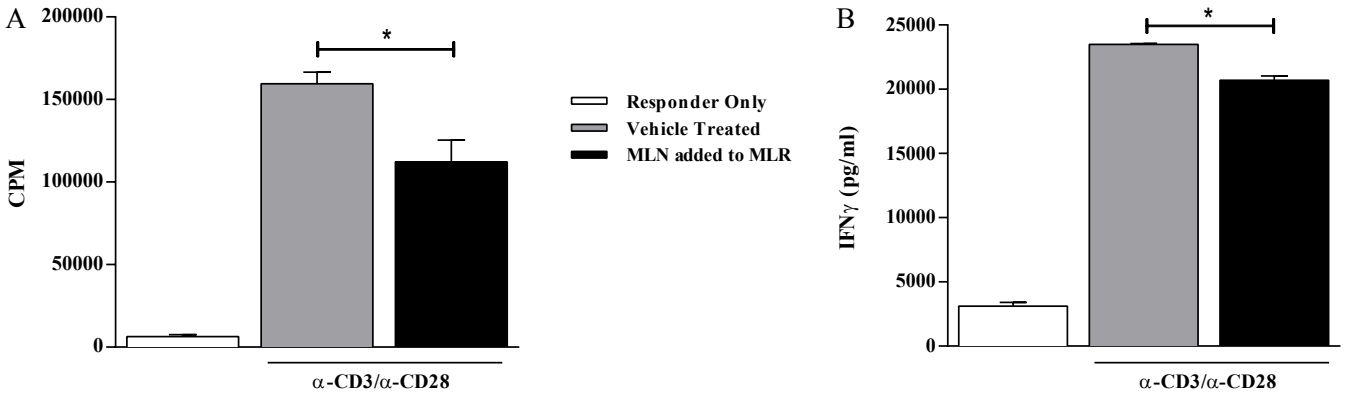
Figure S3



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