Supplementary methods

^{3}H thymidine incorporation assay

Lymphocytes were harvested as described in "BMDC generation, lymphocytes and CD4 cells collection and MLR". Lymphocytes were seed in 96 well round-bottom plate, then stimulated with plate-bound anti-mouse CD3e (0.5μg/mL) and CD28 (1μg/mL) for 72 hours. 0.5μCi ^{3}H-thymidine per well was added 16 hours prior to measurement of incorporated thymidine. Cells were then transferred to glass-fiber filter. Subsequently, Betaplate Scintillation liquid was added and the radioactivity was measured using a 1450 MicroB counter.

In vivo effects of TNP-470 on T cell distribution

Mice were administrated with TNP-470 (20mg/kg) every two days for 10 days. Spleens and lymph nodes were harvested and lymphocytes were collected respectively. Percentages of CD4 and CD8 cells in spleen and lymph node were determined by flow cytometry analysis.

Antigen binding assay

BMDCs on day5 were collected as described in "BMDC generation, lymphocyte and CD4 cells collection and MLR", and incubated with FITC-dextran (Invitrogen) at 37 ℃ for 30 min and at 4 ℃ for 30 min (as negative control). Cells were then washed with ice-cold PBS twice and stained with APC-conjugated CD11c. Antigen-binding capacities were detected by quantifying the FITC fluorescence intensity from CD11c$^+$ cells.

Human DC differentiation

To promote DC differentiation, human monocytic cell line THP-1 was differentiated into DCs with cytokine cocktail as described [1]. Briefly, THP-1 cells were resuspended at concentration of $2X10^5$ cells/ml, then rhIL-4 (100 ng/mL) and rhGM-CSF (100ng/mL) were added and cultured for 5 days to induce DC differentiation. TNP-470 and vehicle control DMSO were added on day0. Fresh medium with TNP-470/DMSO and fresh cytokine were supplemented every two days. To induce DC maturation, differentiated DCs were harvested and stimulated with LPS (1μg/mL) for 6hrs. Gene expression of IL-12 and IL-10 were determined by RT-qPCR.

Immune responses against B16 melanoma

To compare the potencies of immune responses induced by TNP-DC vaccine, mice were immunized with tumor lysate-pulsed TNP-DC vaccine, vehicle DC vaccine and class I MHC restricted epitope peptide Trp-2 -pulsed TNP-DC vaccine, vehicle DC vaccine on day0, 7 and 14. Lymphocytes were harvested on day21 and stimulated with tumor lysate for 24, 48 and 72 hrs ex vivo. Culture supernatant was collected to detect IFN-Y secretion.

Colony formation assay

Bone marrow progenitor cells were collected from the femur and tibia of C57BL mice. After incubation with ACK lysis buffer at 37℃ for 10 min to remove red blood cells, bone marrow progenitor cells were washed with PBS twice,

then mixed with methylcellulose based medium (R&D system) with vehicle/TNP-470 (5nM) and cultured in 35mm dish for 15 days. To evaluate the capacities of cell differentiation, numbers of colonies formed on the matrix were quantified under microscope on day 12 and 15.

Cell apoptosis assay

Bone marrow progenitor cells as described in "Colony formation assay" were incubated with GM-CSF/IL-4 with and without 5nM TNP-470 for 5 days. Cells were then stained with AnnexV/PI and apoptotic cells were counted by $AnnexV^{+}PI^{+}$ cells.

Table S1. Primer sequences for RT-qPCR and ChIP assay

Figure S1. In vivo effect of TNP-470 on T lymphocytes. To determine the in vivo effects of TNP-470 on T lymphocytes, TNP-470 (20mg/kg, every two days) was administrated on mice for 10 days. Lymphocytes were then harvested from spleens and lymph nodes respectively. Percentages of CD4 and CD8 cells in spleen and lymph node were measured by flow cytometry analysis. Data presented as mean ± SD (n=6). N.S. indicated no significant difference.

Figure S2. Phenotypic analyses of TNP-470/vehicle -treated DCs. (**A**) Expression of co-stimulatory molecules on BMDCs. TNP-470/vehicle -treated BMDCs on day5 were collected as immature DCs (iDCs) and stimulated with LPS (200 ng/mL) for 24hr as mature DCs (mDCs). Cells were stained with CD86-FITC /CD83-PE/ CD80-FITC, then expression of costimulatory molecules were measured by flow cytometric analyses. Representative images from three independent studies were showed and the number in the graph represented the mean fluorescence intensity (M.F.I) of the corresponding fluorophores. (**B**) Antigen-binding assay. TNP-470/vehicle -treated BMDCs on day 5 were incubated with FITC-dextran at 37 ℃ for 30 min, then washed with PBS and stained with CD11c antibody. FITC-dextran-incorporated DCs were quantified by $FITC^{+}CD11c^{+}$ cells. Data presented as mean ± SEM (n=2) $^{***}P<0.001$ Vehicle DCs versus TNP-470 DCs.

Figure S3. Shift of IL-12/IL-10 balance of human DCs by TNP-470 upon LPS stimulation. Human monocytic cell line THP-1 was differentiated into dendritic cells under the cytokine cocktail (rhIL4: 100ng/mL; rGM-CSF:100ng/mL) and treated with TNP-470 (5 and 10nM) and vehicle control for 5 days. Differentiated human DCs were stimulated with LPS (1µg, 6hr) to evaluate gene expression of IL-12 and IL-10. Fold-of-change in expression of IL-12 (**A**) and IL-10 (**B**) when normalized with GAPDH. Data presented as mean ± SEM. $^{*}P<0.05$ IL-12: DCs treated with 10nM TNP-470 versus control; $^{**}P<0.01$ IL-10: DCs treated with 5nM/10nM TNP-470 versus control.

Figure S4. Solid tumor development in prophylactic setting of murine B16 melanoma vaccination model. Mice were vaccinated with vehicle PBS, DC without tumor lysate pulsing, tumor lysate-pulsed DC vaccine, TNP-470 -treated DC vaccine. Tumor volume of solid B16 melanoma developed on mice from day10 to day26 (end-point). Data presented as mean ±SD (n=6) * $P<0.05$, vehicle DC vaccine versus PBS solvent control; $^{**}P<0.01$, TNP-470 -treated DC vaccine versus PBS solvent control.

Figure S5. Comparison of immune responses between tumor lysate and Trp2 peptide pulsed DCs upon TNP-470/vehicle pre-treatment. Mice were immunized with tumor lysate-pulsed TNP/CTR-DC vaccine and Trp-2 peptide-pulsed TNP/CTR-DC vaccine. (**A**) IFN-Y secretion from lymphocytes in different groups upon ex vivo stimulation of tumor lysate (50µg/mL) for 24, 48 and 72hrs. Data presented as mean ±SD (n=3). *P<0.05 and ***P<0.001 TNP-DC vaccine versus vehicle DC vaccine (tumor lysate/Trp-2 -pulsed). (**B**) Fold of IFN-Y induction when compared with vehicle DC vaccine counterparts. IFN-Y secretion in tumor lysate/ trp-2 peptide -pulsed vehicle DC vaccine groups were normalized to 1. Fold-of-change of TNP-DC vaccines when compared with corresponding vehicle DC vaccines was showed. Data presented as mean ±SD (n=3). $^{*}P<0.05$ and $^{**}P<0.01$ tumor lysate-pulsed TNP-DC vaccine versus Trp2 -pulsed TNP-DC vaccine. (**C**) Tumor-specific cytolytic activity. Lymphocytes from mice in

different groups were collected and stimulated with tumor lysate (50µg/mL) for 72hr ex vivo, then harvested as effector cells (E), and B16-F10 cells were used as target cells (T). Specific lysis was detected by LDH assay at E:T ratio = 100:1. Data presented as mean ±SD (n=3). $^{**}p<0.01$ tumor lysate-pulsed TNP-DC vaccine versus CTR-DC vaccine. $^{***}P<0.001$ trp2 peptide-pulsed TNP-DC vaccine versus CTR-DC vaccine.

Figure S6. TNP-470 treatment does not affect HSC colony formation and cell apoptosis. (**A**) Colony formation assay for bone marrow progenitor cells in the presence of TNP-470/vehicle. To evaluate the differentiation capacities of bone marrow progenitor cells upon treatment. Progenitor cells were mixed with methylcellulose base medium with vehicle/TNP-470 (5nM) for 15 days. Numbers of colonies formed on the matrix medium on day 12 and day 15 were showed. Data presented as mean ± SEM from two independent studies. (**B**) AnnexV/PI staining of bone marrow progenitors on day3 and BMDCs on day5. Representative images from two independent studies were showed (left panel) and percentage of apoptotic cells ($AnnexV^{+}PI^{+}$) were summarized (right panel). Data presented as mean ± SEM (n=2).

Table S1. Primer sequences for RT-qPCR and ChIP assay.

	Forward primer (5' to 3')	**Reverse primer (5' to 3')**
IL-12p40 (qPCR)	5' GGAAGCACGGCAGCAGAATA 3'	5' AACTTGAGGGAGAAGTAGGAATGG 3'
IL-10 (qPCR)	5' GGTTGCCAAGCCTTATCGGA 3'	5' ACCTGCTCCACTGCCTTGCT 3'
p52 (qPCR)	5' GATCTCCCGAATGGACAAGA 3'	5' AACCGAACCTCAATGTCGTC 3'
p65 (qPCR)	5'ACTGCCGAGCTCAAGATCTGCC GAGTAAAC 3'	5'GGAGGAGTCCGGAACACAATG GCCACTTGCCG 3'
c-rel (qPCR)	5' CCCATTGTTTCTAACCCAAT 3'	5' TCTCCTCCCCTGACACTTCC 3'
relB (qPCR)	5' TGATCCACATGGAATCGAGA 3'	5' CAGGAAGGGATATGGAAGCA 3'
GAPDH(qPCR)	5' GTGTTCCTACCCCCAATGTG 3'	5' TGAAGTCGCAGGAGACAACC 3'
IL-12p40 NFKB binding site (ChIP)	5' AGTATCTCTGCCTCCTTCCTT 3'	5' GCAACACTGAAAACTAGTGTC 3'
IL-12p40 (human)	5'CCAAGAACTTGCAGCTGAAG 3'	5' TGGGTCTATTCCGTTGTGTC 3'
IL-10 (human)	5' GGTTGCCAAGCCTTGTCTGA 3'	5' AGGGAGTTCACATGCGCCT 3'

Figure S1

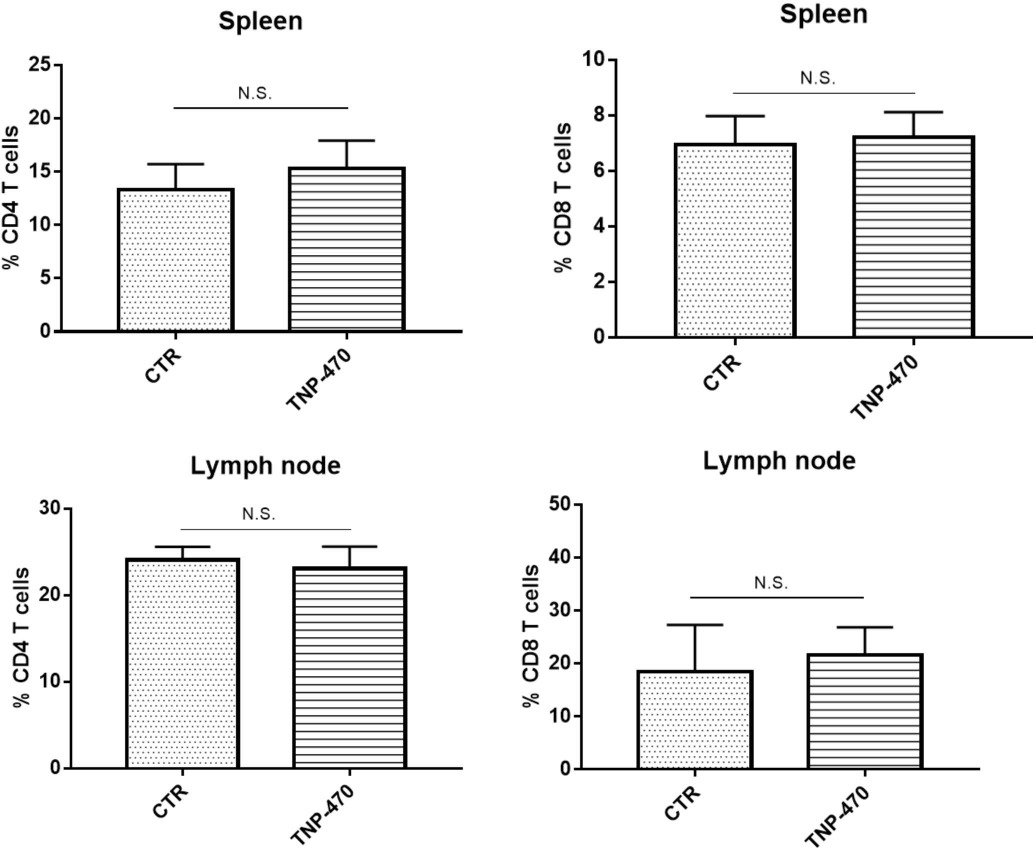
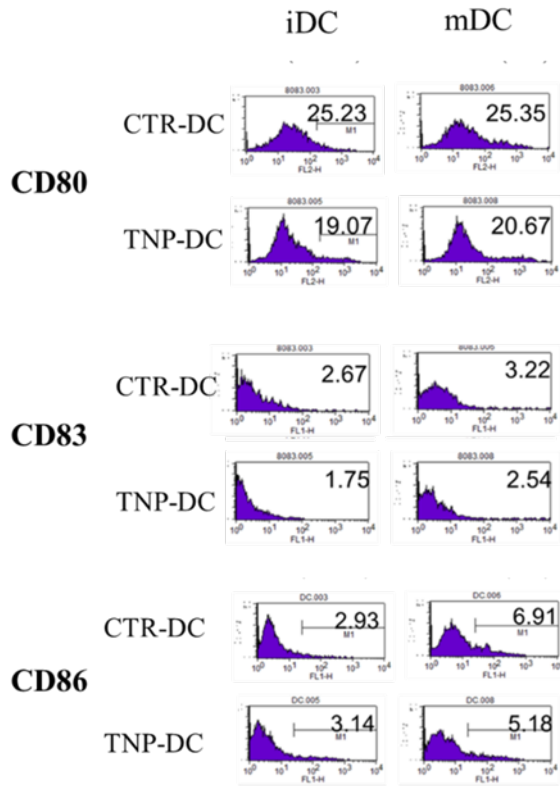


Figure S2



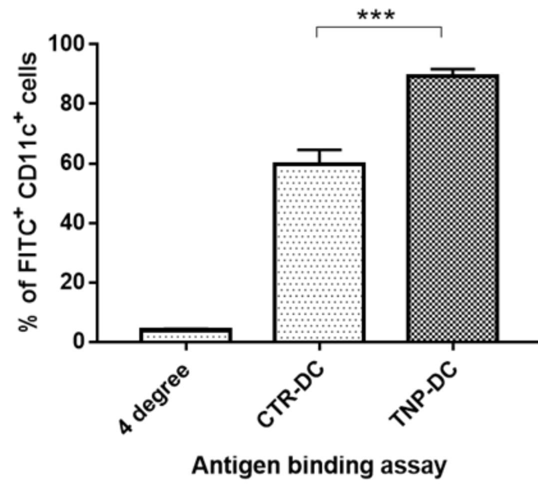
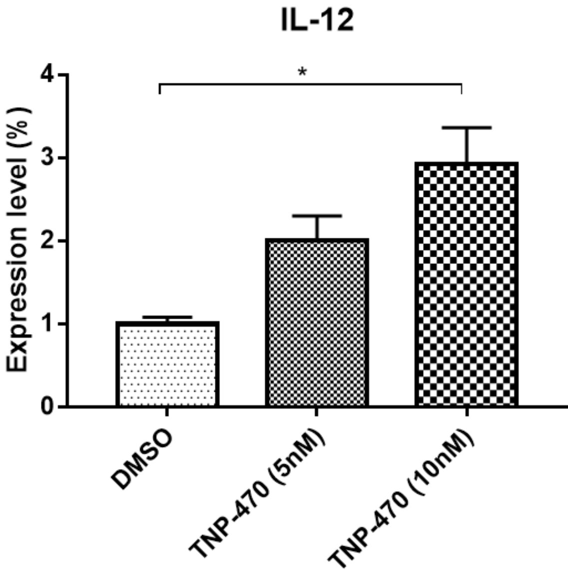


Figure S3

A



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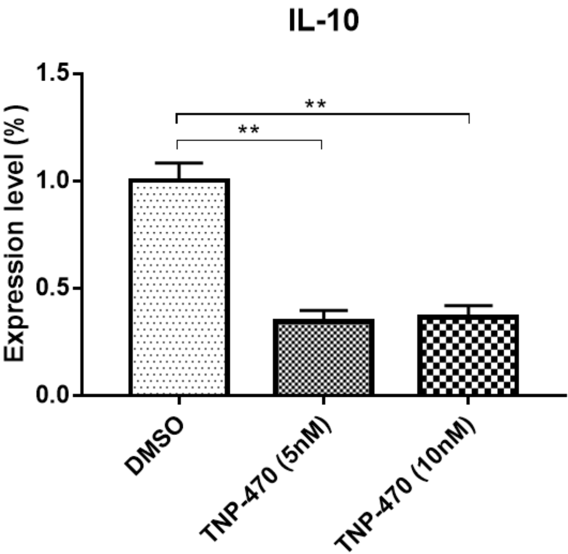

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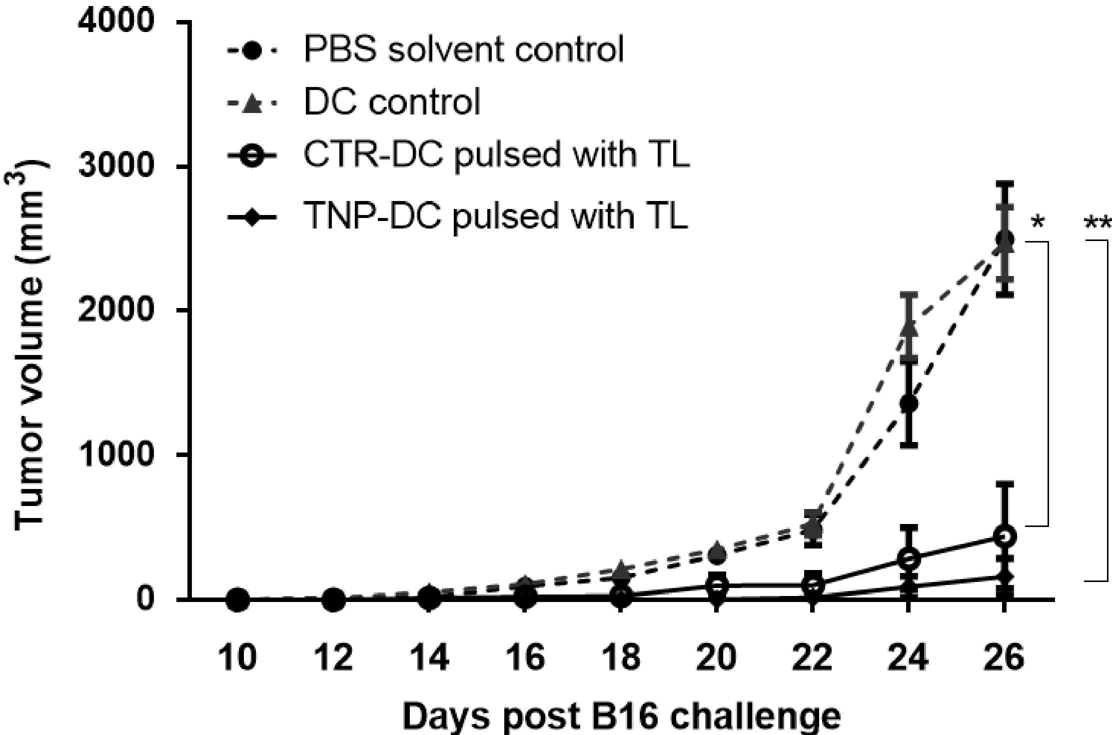
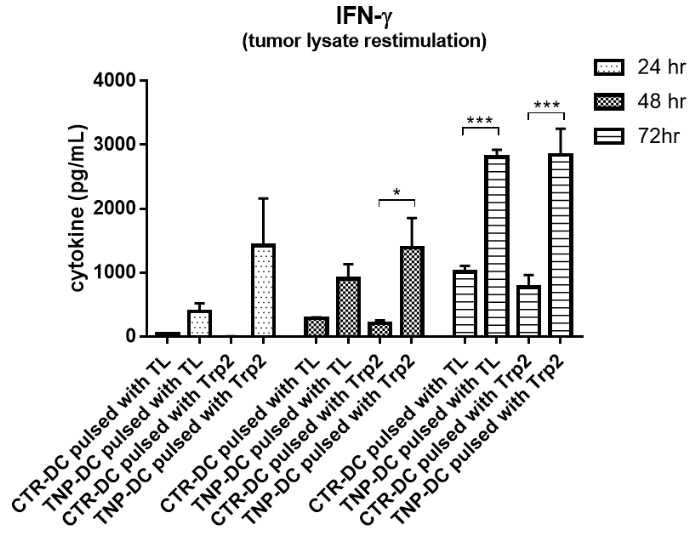


Figure S5

A



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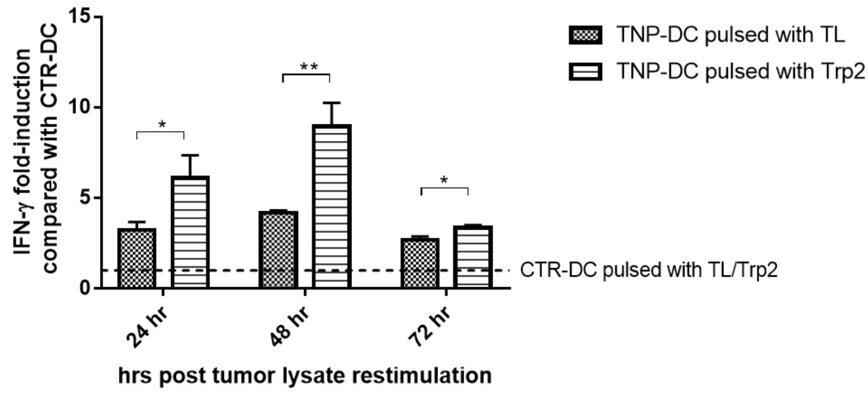

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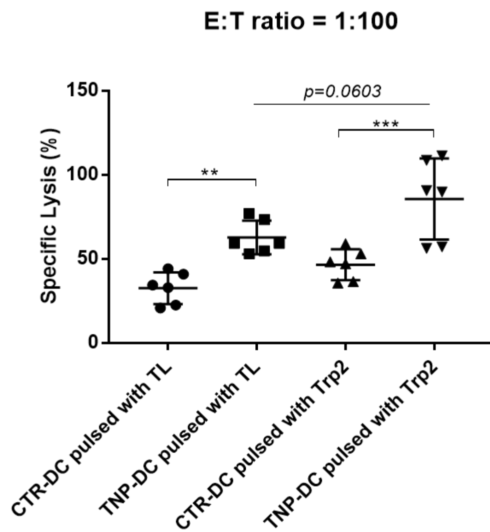
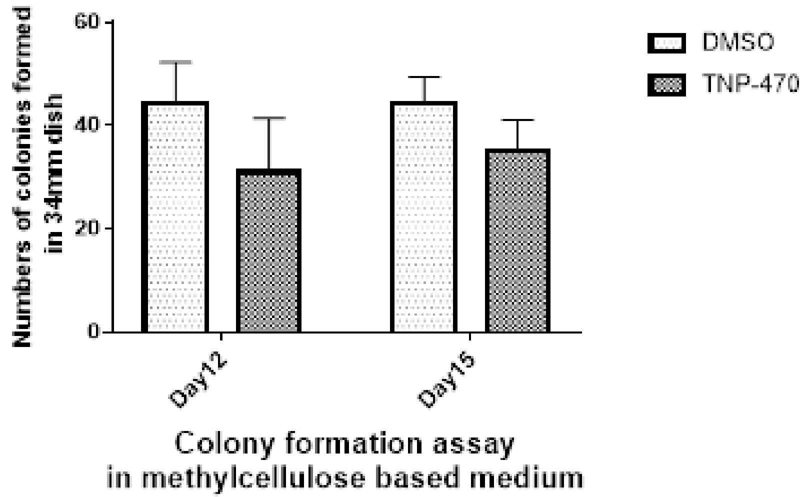


Figure S6

A



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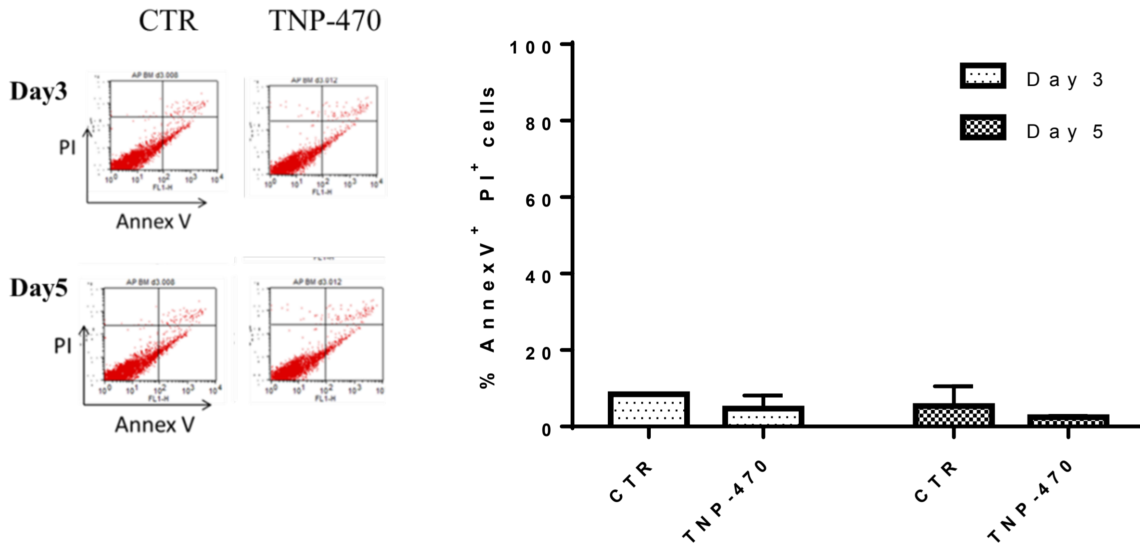

Reference for supplementary methods

1. Berges C, Naujokat C, Tinapp S, et al. A cell line model for the differentiation of human dendritic cells. *Biochemical and biophysical research communications.* 2005;333(3):896-907.