Figure S1.

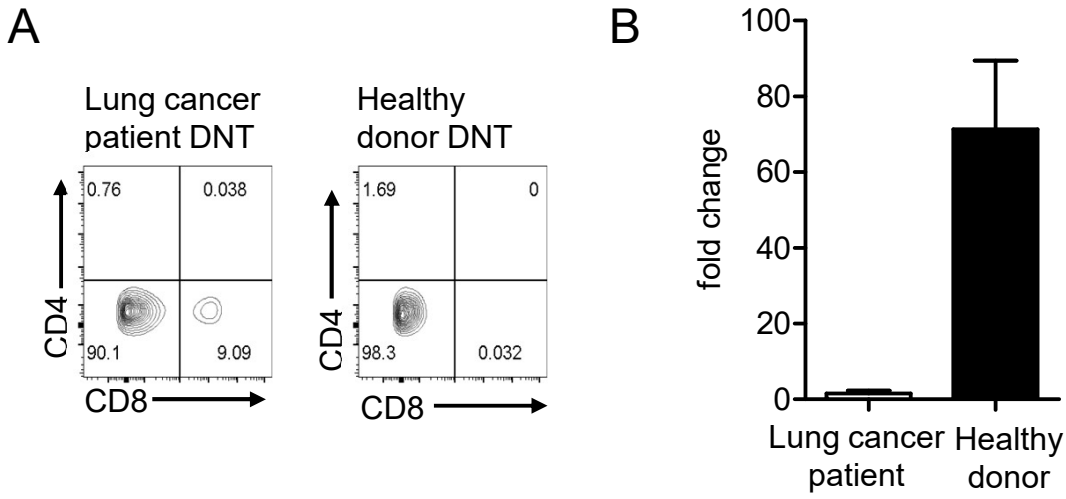

Figure S1. Purity and yield of lung cancer patient- and healthy donor-derived DNT cells. Lung cancer patient-derived DNT cells (n=4) or healthy donor-derived DNT cells (n=5) were expanded as described in the Materials and Methods and analyzed for their phenotype **(A)** and yield **(B)** 14 days after expansion.

Figure S2.

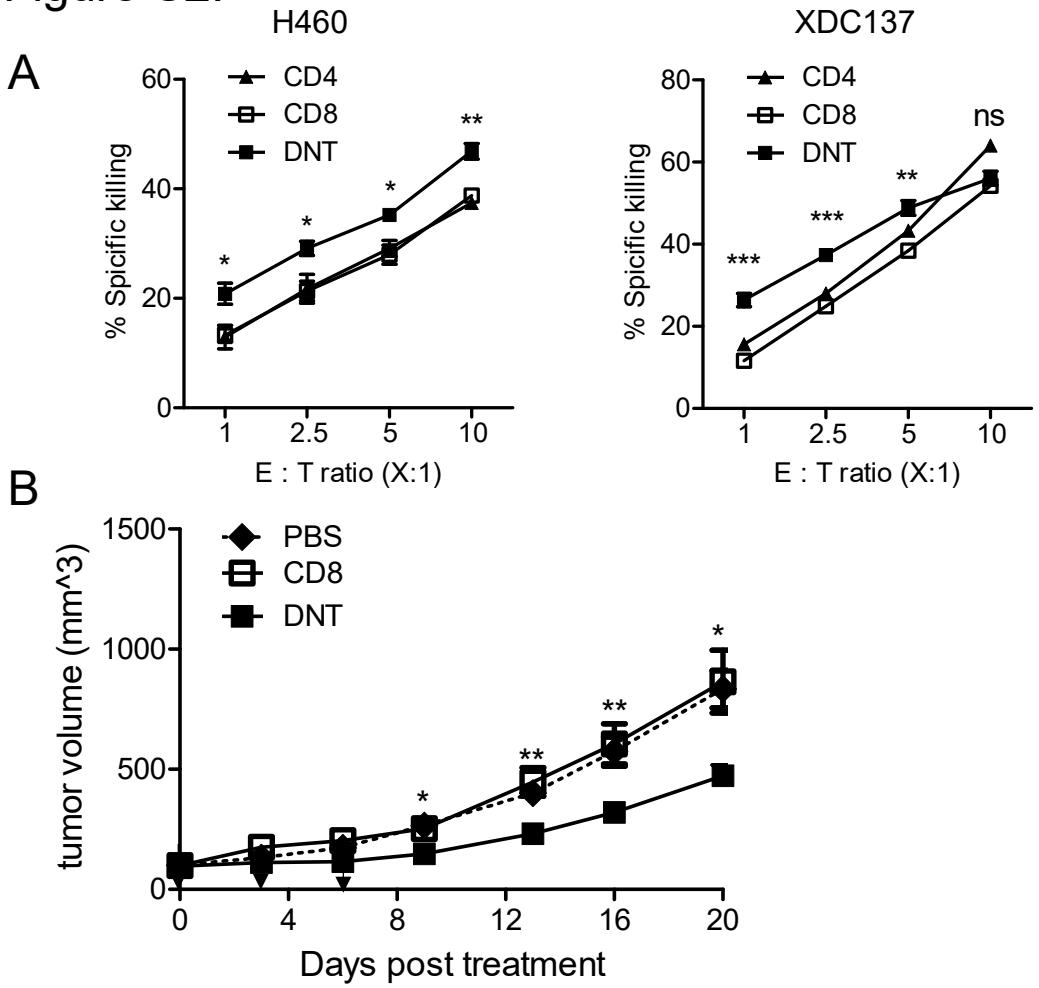

Figure S2. A. Healthy donor derived DNT, CD4 and CD8 T cells were co-cultured with indicated lung cancer cell lines at various T cell to tumor cell ratios for 12-14hr. % specific killing of target cells is shown. The results represent 3 independent experiments from 3 different donors, each with triplicate cultures. Results shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 indicate the differences in the cytotoxicity of DNT versus CD8 T cells at indicated E:T ratios by two-tailed unpaired t-test. **B**. NSG mice were inoculated subcutaneously with NCI-H460 in 50% Matrigel solution and grown to ~100mm3. After tumors were established, tumor bearing mice were randomized into groups and treated with peritumoral injection of IL-2 with or without DNT or CD8 T cells from the same donor on day 0, 3 and 6. Tumor volume was measured at indicated time points. Results represent one of two independent experiments, each had 5 mice per treatment group. Results shown as mean ± SEM. *p<0.05, **p<0.01 show the difference in tumor volume at indicated time-points between DNT and CD8 treated groups by two-tailed unpaired student t-test.

Figure S3.

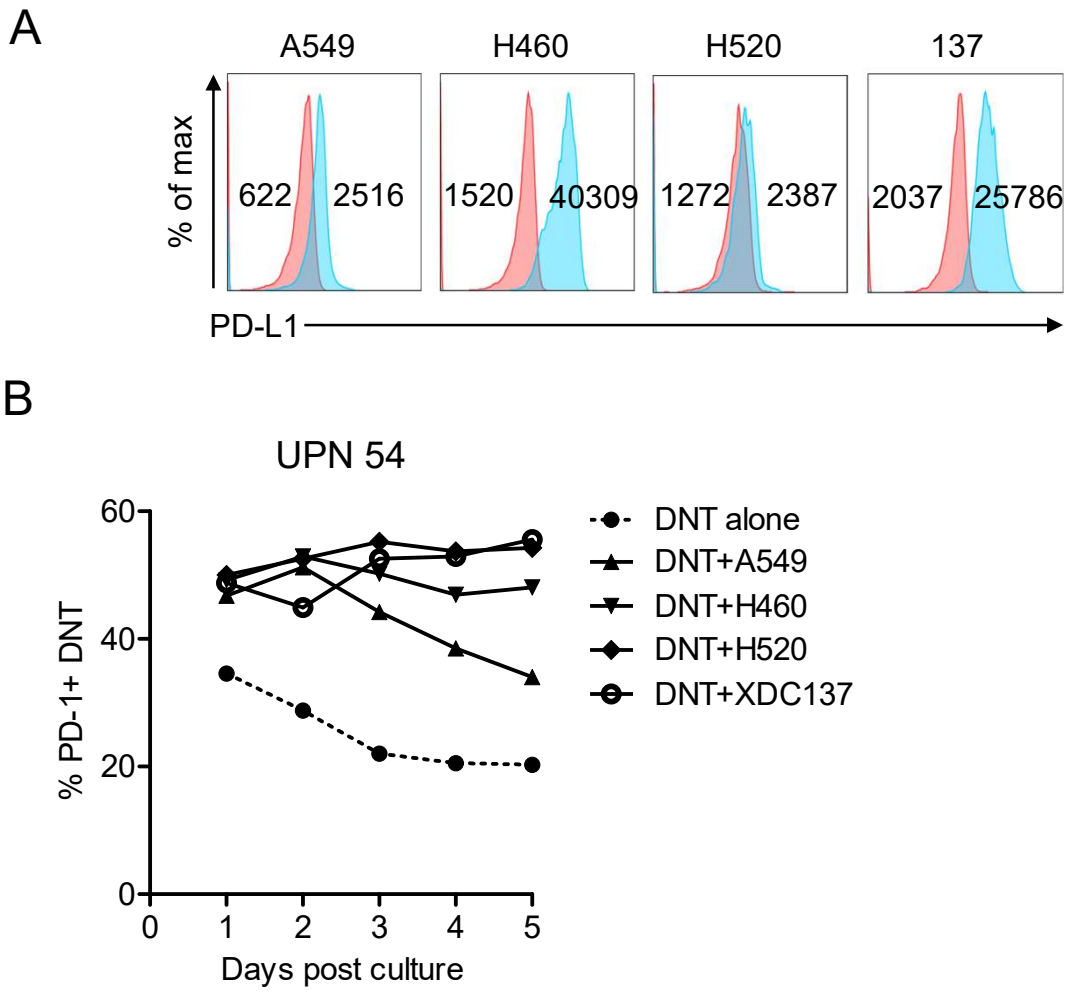

Figure S3. A. The expression of PD-L1 on NSCLC cell lines. NSCLC cell lines were stained with either anti-human PD-L1 (blue histograms) or control (red histograms), numbers represent MFI. **B.** Time course of PD-1 expression on expanded DNT cells, cultured alone or with various NSCLC cell lines for varying time points. Results represent the data obtained from 2 different donors.

Figure S4.

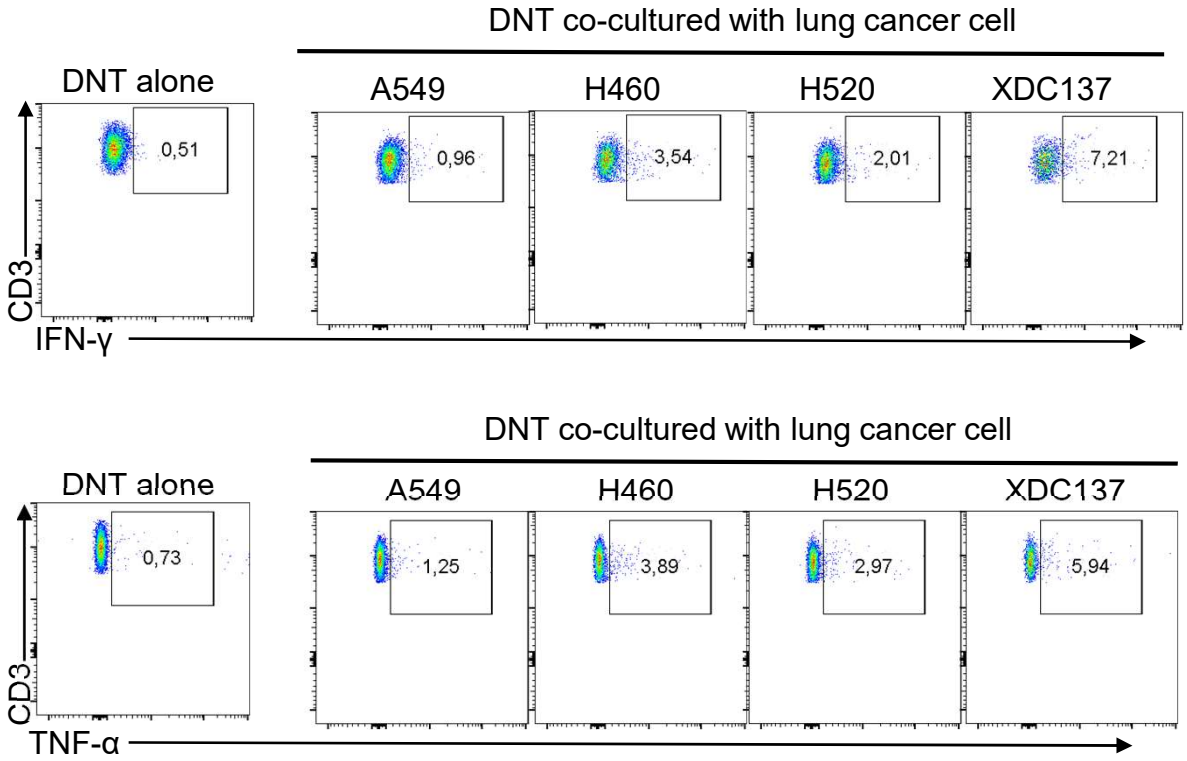

Figure S4. Intracellular cytokine analysis of DNT cells, either cultured alone or with various NSCLC cell lines. Expanded DNT cells were culture alone or co-cultured with indicated NSCLC cell lines for 48hr and prior to analysis of indicated intracellular cytokines. % INF-γ and TNF-α positive DNT cells are shown.

Figure S5.

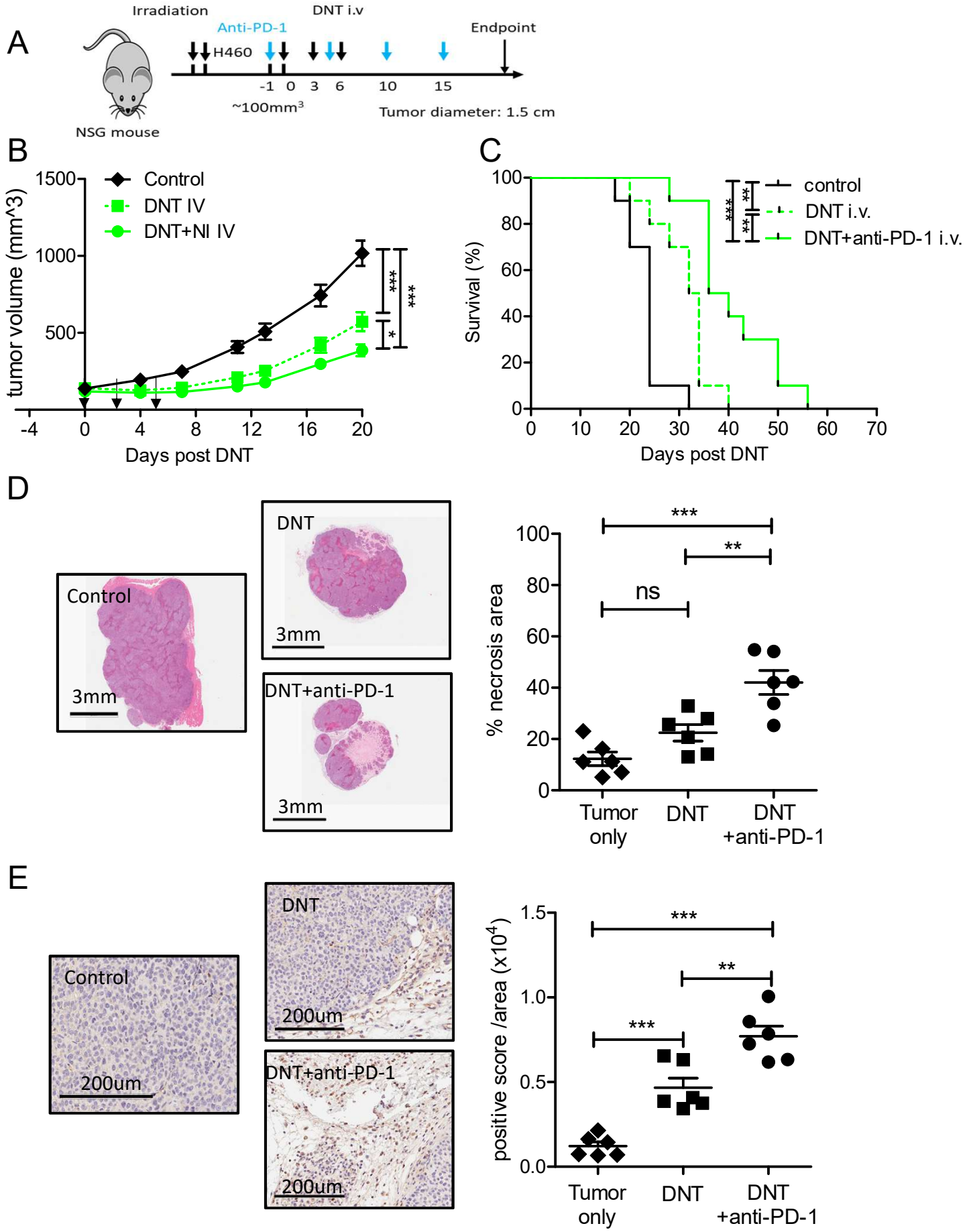
A
Irradiation
Anti-PD-1
DNT i.v
Endpoint
H460
-1 0 3 6 10 15
~100mm3
Tumor diameter: 1.5 cm
NSG mouse
B
Control
DNT IV
DNT+NI IV
tumor volume (mm^3)
Days post DNT
C
control
DNT i.v.
DNT+anti-PD-1 i.v.
Survival (%)
Days post DNT
D
Control
DNT
DNT+anti-PD-1
3mm
% necrosis area
ns
Tumor only
DNT
DNT +anti-PD-1
E
Control
DNT
DNT+anti-PD-1
200um
positive score /area (x10^4)
Tumor only
DNT
DNT +anti-PD-1

Figure S5. Anti-PD-1 antibody enhances efficacy of DNT cell mediated anti-tumor response and DNT cell tumor infiltration in late stage tumor xenograft model. NSG mice were inoculated subcutaneously with NCI-H460 in 50% Matrigel solution and tumors were allowed to grow to ~100mm^3. After tumors were established, tumor bearing mice were randomized and received intravenous injection of IL-2 (control treatment) or DNT cell plus IL-2 on days 0, 3 and 6. Additionally, some mice received PBS or anti-PD-1 antibody (10mg/kg repeated every 5 days i.p., starting one day prior to 1st DNT cell infusion to the end of the experiment). **A.** Schematic diagram of the treatment protocol of NCI-H460 xenograft model. **B.** Tumor volume were measured at indicated time points (n=10 for each group). **C.** Humane end point survival of treated mice (n=10 for each group). **D**. Representative H&E staining of xenografts from indicated treatment groups 9 days post DNT cell infusion and percent necrotic area in tumors from indicated treatment groups calculated by histological analysis. **E.** Immunohistochemical analysis of CD3$^+$ human T cells in tumor xenografts 9 days post DNT cell infusion. Representative staining and analysis of tumor infiltrating DNT cells in indicated treatment groups. Results shown as mean ± SEM, from untreated, DNT (n=6) or DNT plus anti-PD1 treatment groups (n=6). Results shown are representative of 2 separate experiments. **p<0.01, ***p<0.001, by two-tailed unpaired t-test **(B**), by log-rank test **(C)** or by one-way ANOVA **(D and E).**

Figure S6.

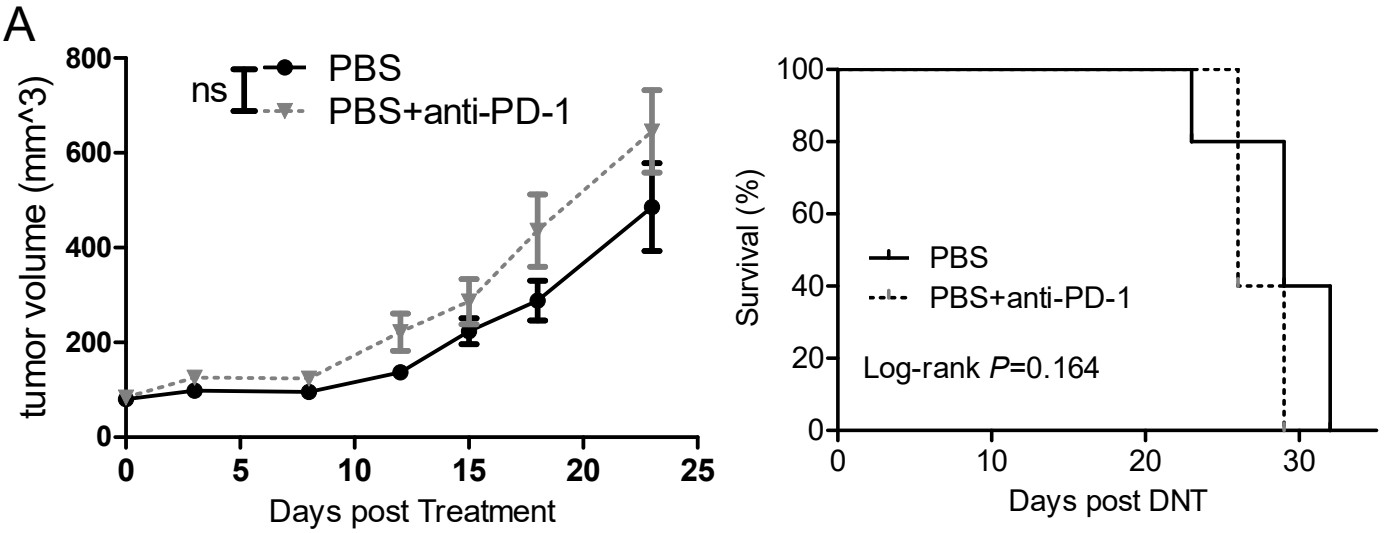

Figure S6. **Treatment with anti-PD-1 alone has no effect on NCI-H460 xenograft growth or mouse survival. A.** NSG mice were inoculated subcutaneously with NCI-H460 in 50% Matrigel solution and grown to ~100mm^3, and treated with 10mg/kg anti-PD-1 or PBS i.p. every 5 days to the end of the experiment. Tumor volume and recipient survival were monitored (n=5 for each group).

Figure S7.

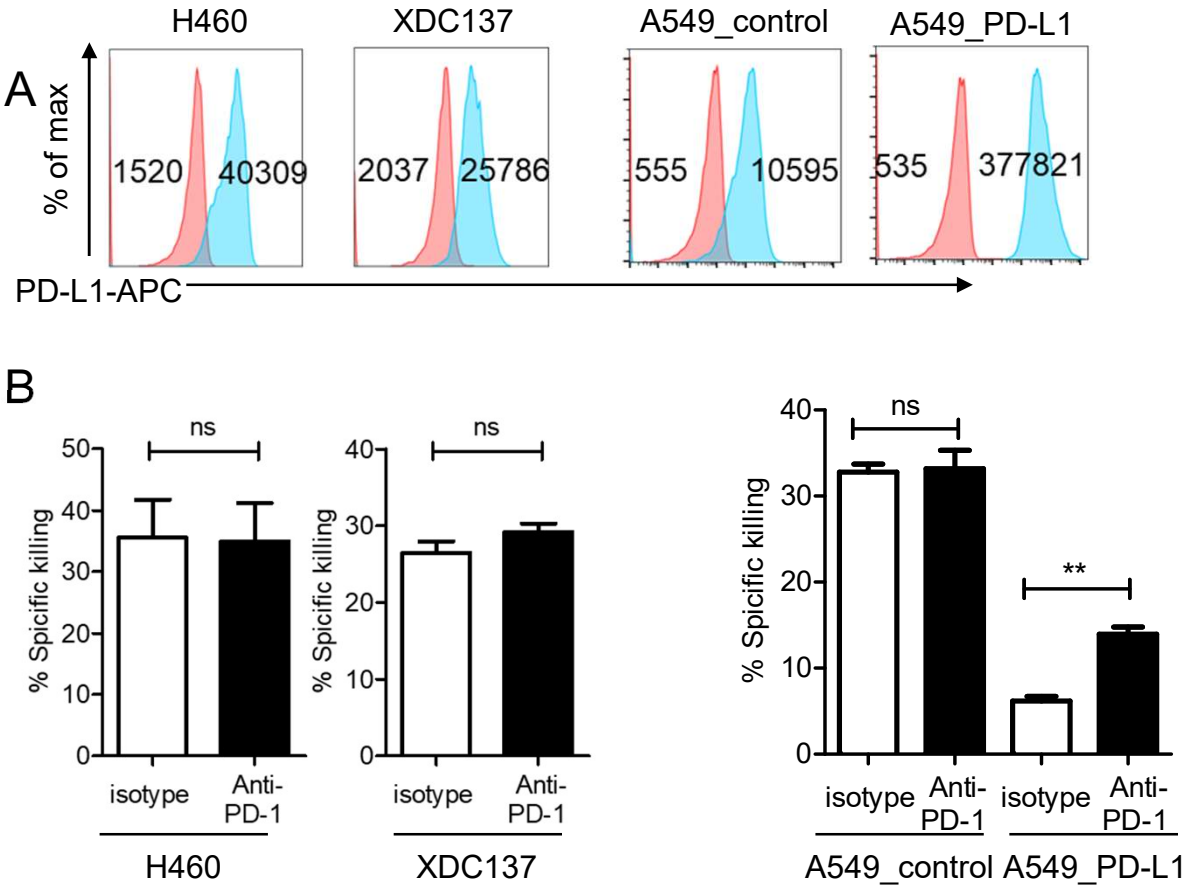

Figure S7. A. The expression of PDL1 on NSCLC cell line H460, XDC137, A549 control vector and A549 PD-L1 overexpressing cell line. NSCLC cell lines were stained with either anti human PD-L1(blue histograms) or control (red histograms), values represent MFI. **B.** DNT cells were co-cultured with indicated lung cancer cell lines at a 5 to 1 DNT cell to tumor cell ratio. % specific killing of target cells is shown. Results shown as mean ± SEM. The results represent 3 independent experiments each with triplicate cultures. **p<0.01, by two-tailed unpaired t-test.