

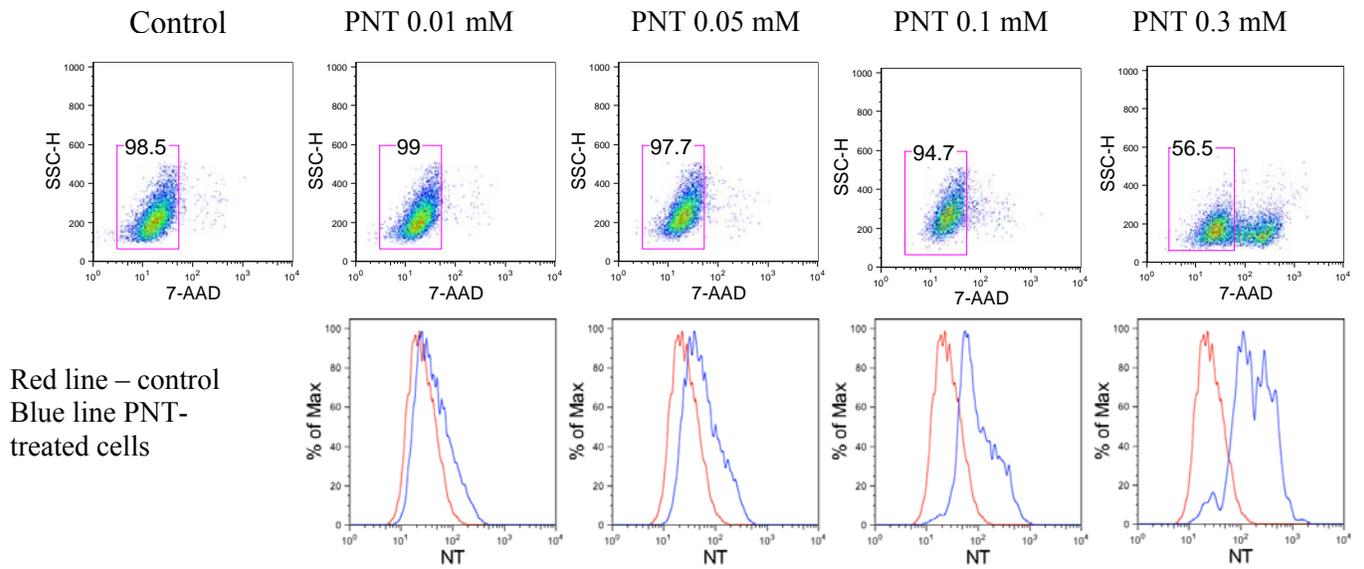
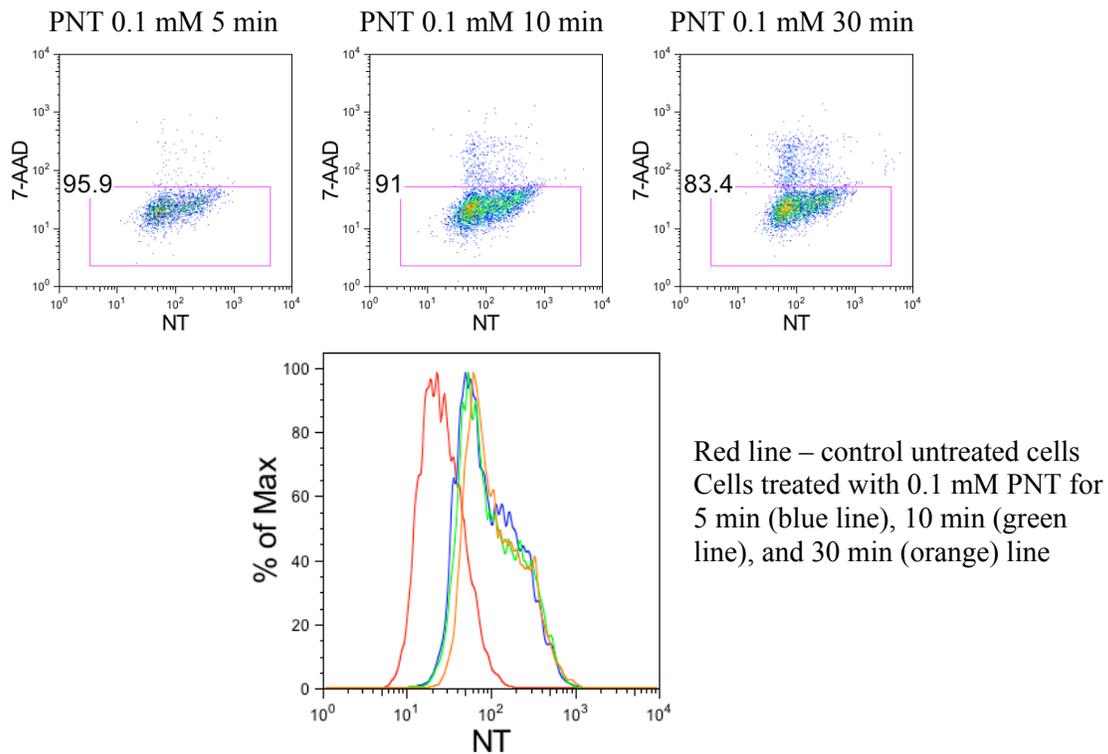
A**B**

Figure S1. Nitration on tumor cells by peroxynitrite (PNT) is concentration dependent. EL-4 tumor cells were incubated with PNT at different concentrations (0.01mM to 0.3mM) for 10 min (A), or at 0.1mM for 5, 10 and 30 min (B). After the treatment cells were washed and NT levels were detected by stained with anti-NT antibodies. The proportions of dead cells were evaluated by staining with 7-AAD. Data are representative of three experiments.

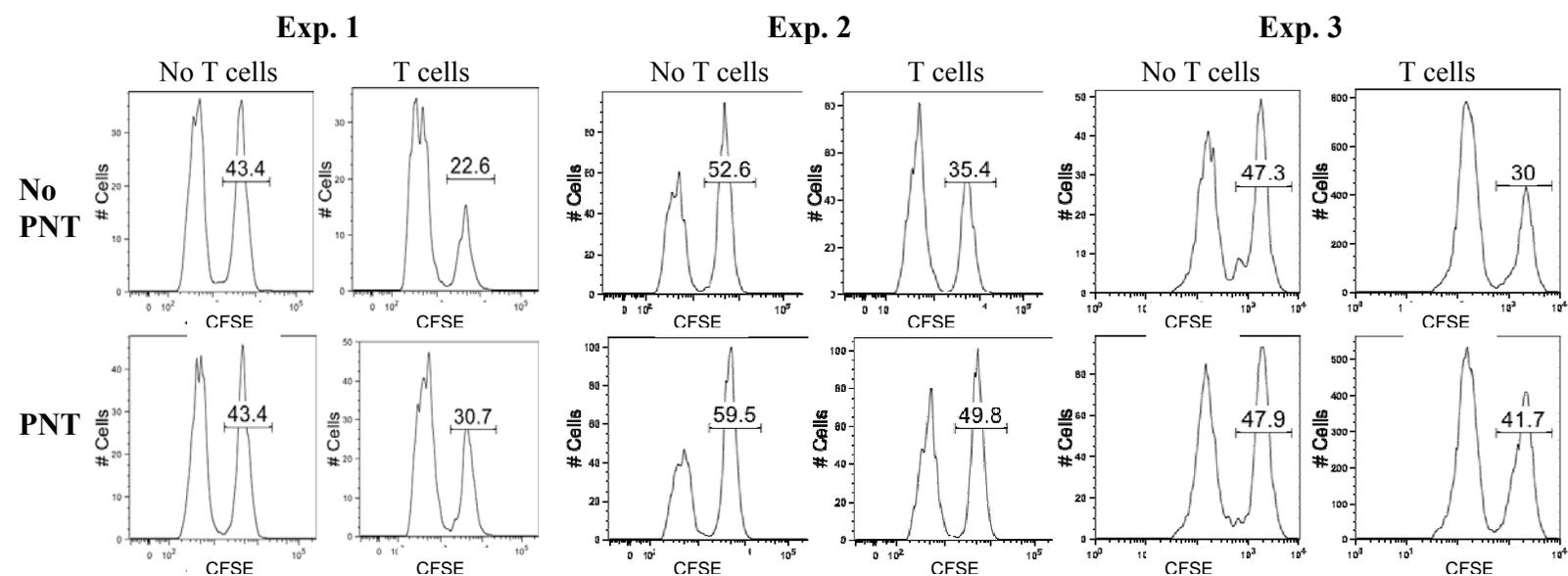


Figure S2. PNT decreased the sensitivity of B16.F10 tumor cells to pmel-1 CTLs. B16.F10 and LLC (control) target cells were incubated with 100 ng/ml IFN- γ 48 hr (to up-regulate MHC class I expression). After that time B16.F10 and LLC cells were labeled with high and low concentrations of CFSE respectively. Target cells were treated with PNT (0.1 mM) for 10 min, washed and incubated with pmel-1 CTLs at 1:10 ratio for 5 hr. The results of three independent experiments are shown.

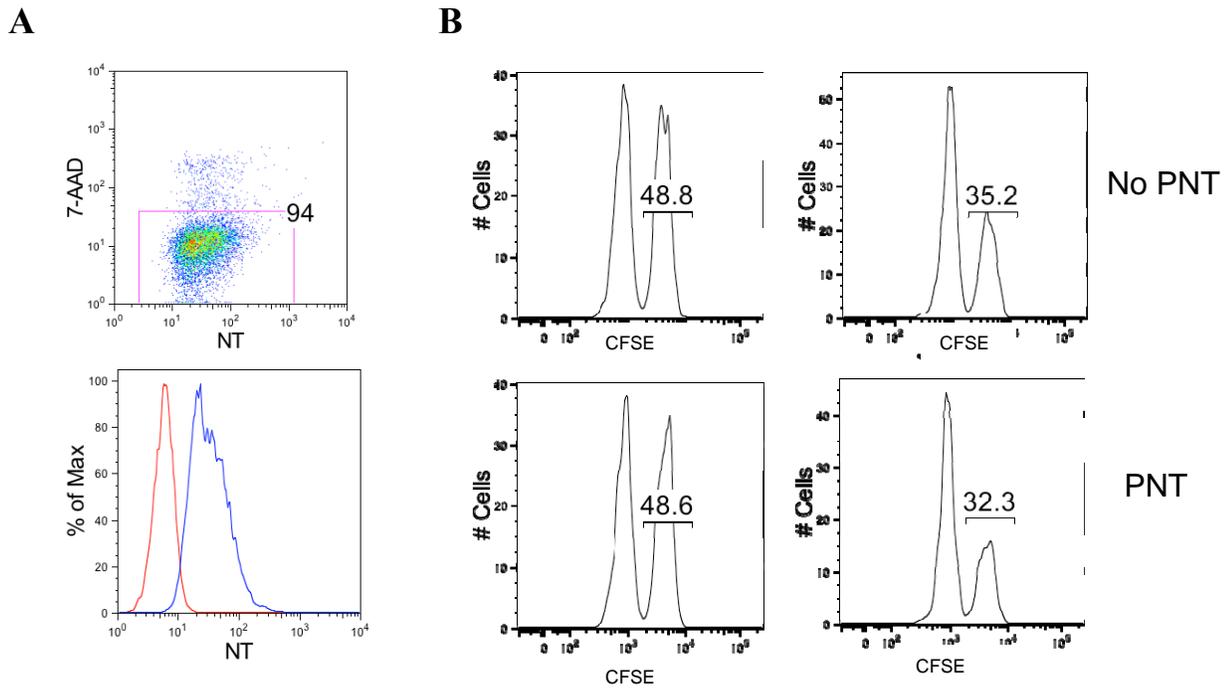


Figure S3. Effect of PNT on NK specific killing. NK cells were isolated from splenocytes of naïve mice. Splenocytes were stimulated with mIL-2 (5 ng/ml) for 18 hr and passed through nylon wool. NK cells were then enriched on a four-step discontinuous Percoll gradient. The enriched NK cells from 45% Percoll were further isolated by anti-NK1.1 and magnetic beads on MiniMACS columns (Miltenyi Biotec). EL-4 (control) and NK sensitive YAC-1 target cells were labeled with low and high doses of CFSE respectively and pre-treated for 10 min with 0.1 mM PNT. Cells then were washed and incubated with isolated NK cells at 1:10 ratio for 5 hr. **A.** The viability and NT levels in YAC cells treated with PNT. Red line – NT staining in untreated YAC cells, blue line – PNT treated cells. Isotype control in all experiments was less than 10^1 (not shown). **B.** Staining of target cells in the presence of NK cells (Targets : effectors ratio 1:10). Three experiments with the same results were performed.

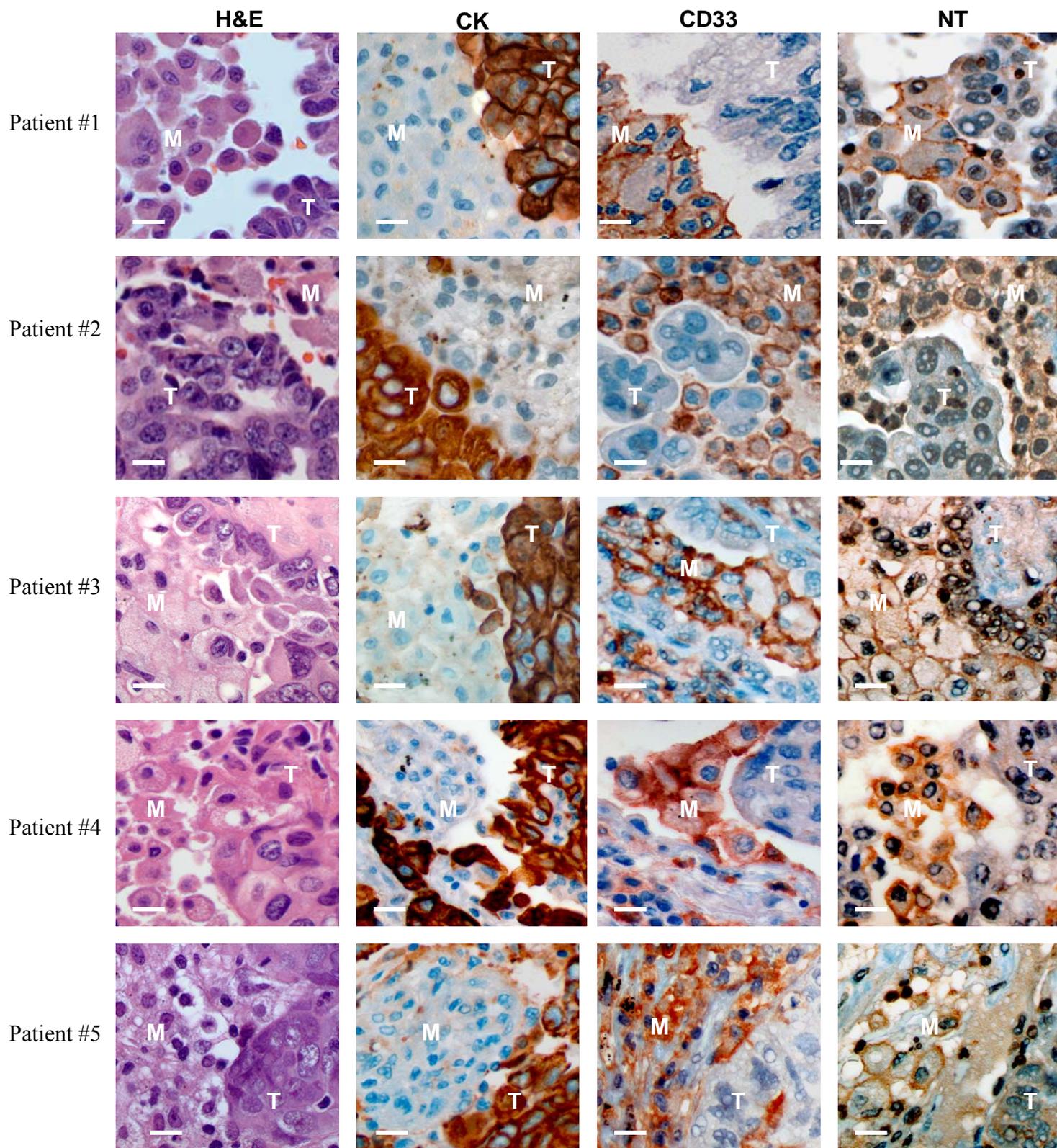


Figure S4. NT expressions on myeloid cells in cancer patients. Samples of tumor tissues from 5 patients diagnosed with non-small cell lung cancer were evaluated. Staining with hematoxylin-eosin (H&E), anti-cytokeratin antibody (detecting tumor cells) (brown), anti-CD33 antibody (detecting myeloid cells) (brown), and anti-NT antibody (brown). Bars = 10 μ m. T – designate tumor areas, M – myeloid cells.

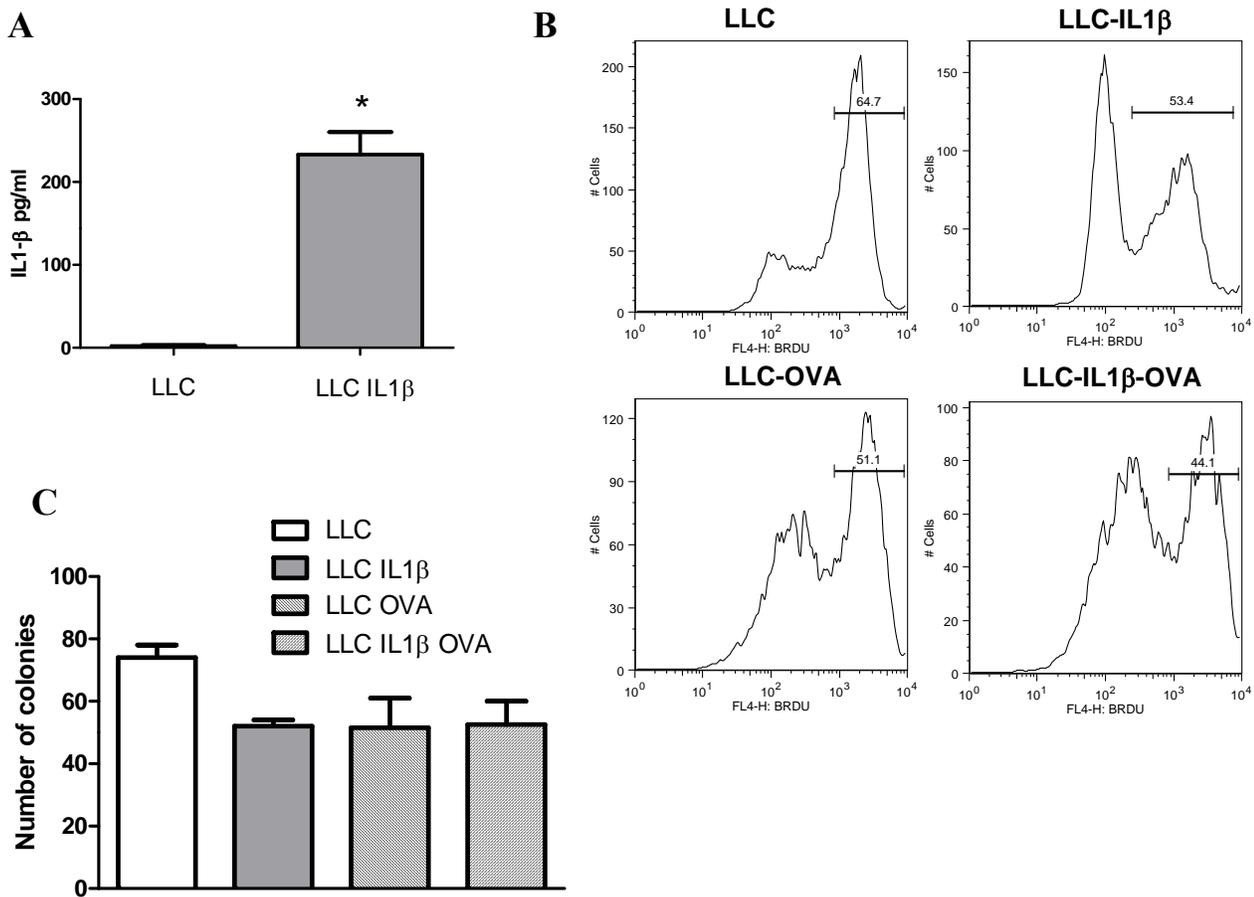


Figure S5. Effect of IL-1 β overexpression on LLC tumor cells. **A.** LLC or LLC-IL-1 β cells (5×10^5 /ml) were cultured overnight and the cytokine production in supernatants was analyzed by ELISA in triplicates. **B.** Cultured cells were incubated with BrdU (10 μ M, BD Pharmingen) for 5 hr and BrdU positive cells were evaluated by flow cytometry using BrdU-specific antibody. Typical result of one experiment out of three performed is shown. **C.** In clonogenic assays, 1000 cells of each cell line were cultured in duplicates in semi-soluble medium for 5 days. The number of colonies was counted. Data are Mean \pm SEM from 4 experiments.

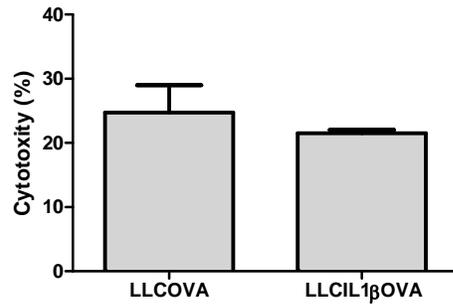


Figure S6. LLC-OVA and LLC-IL-1 β -OVA cells have similar sensitivity to OT-1 CTLs. LLC-OVA and LLC-IL1- β -OVA cells were used as targets in CTL assay. The ratio of effectors to targets was 100 to 1. Tumor cells were cultured for 48 hr with purified mouse IFN γ (100 ng/ml) to enhance MHC class I expressions. Data are mean \pm SEM, from three performed experiments.

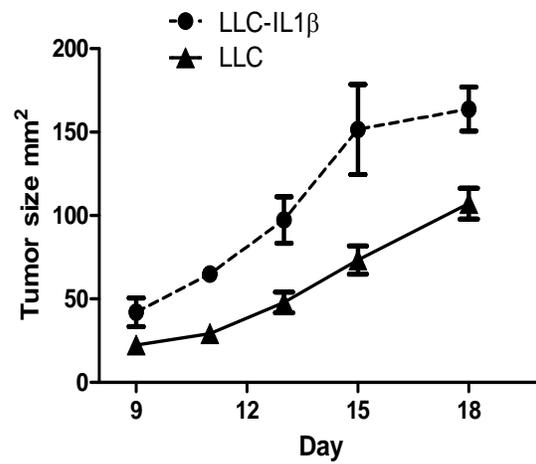


Figure S7. LLC-IL-1 β tumors grow faster than LLC primary tumors. LLC or LLC-IL-1 β cells (5×10^5) were s.c. injected to C57BL6 mice on day 0 and tumors were measured every 2-3 days. There were 3-5 mice each group.

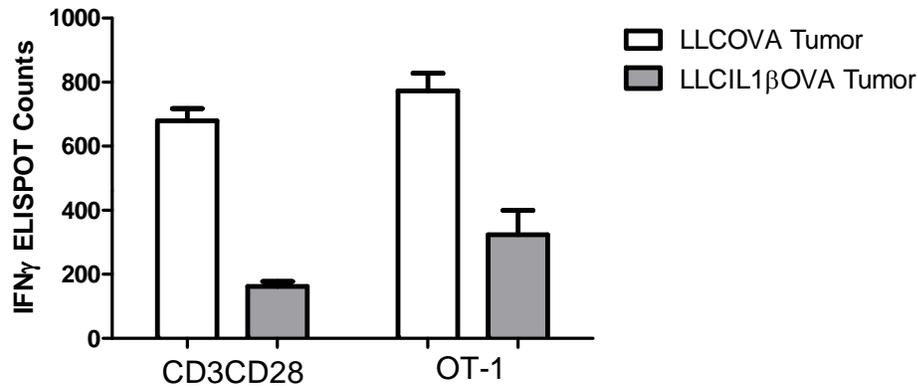
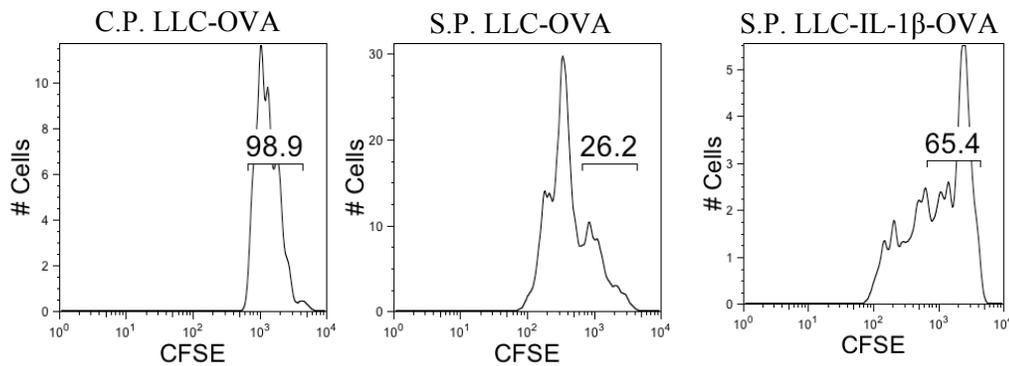
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Figure S8. Suppression of OT-1 T cell activities in LLC-IL-1 β -OVA mice after partial reconstitution of myeloid compartment after TBI. LLC-OVA and LLC-IL-1 β -OVA tumors were established as described in **Fig. 6**. All mice received TBI and bone marrow transplant on day 0. Activated OT-1 T cells were transferred to the treatment groups not on day 1 as in Fig. 6 but on day 8. Five days later, T cells were isolated from tumors, mixed at 1:1 ratio with irradiated syngenic control splenocytes and stimulated with either control or specific peptides, or anti-CD3/CD28 antibodies. **A.** IFN γ production was measured in ELISPOT assay. The number of spots per 5×10^4 T cells was calculated. Each experiment was performed in triplicates and included 3 mice. Cumulative Mean \pm SEM are shown. **B.** The proliferation of T cells isolated from tumors was determined by labeling of T cells with CFSE followed by stimulating with specific (S.P.) or control (C.P.) peptides in the presence of irradiated naïve splenocytes. The experiments were performed twice with similar results.