Supplemental Figure 1
PG545 is a more potent activator of NK cells in vivo NK than CpG or Poly(I:C). Mice were injected with CpG (100 µg, i.p.), Poly(I:C) (200 µg, i.p.) or PG545 (20 mg/kg, s.c.) and after two days, splenic NK cells (NKp46+CD3−) were FACS analyzed for CD69, Ki-67, and IFN-γ expression. (A) Representative FACS analysis and gating are shown (A). The mean percentage ± SEM of CD69+, Ki-67+ or IFN-γ+ NK cells among total splenic NK cells is shown (n=3 mice per group). *p<0.05.
Supplemental Figure 2
Assessment of NK and T cell activation in tumor-bearing mice. BALB/c mice were injected intravenously with $2 \times 10^5$ luciferase expressing A20 cells and followed until tumor burden reached $10^5$ photons/second. Mice were then treated with cyclophosphamide (CP) alone (50 mg/kg, i.p., single dose), PG545 (20 mg/kg, s.c., once-weekly dosing), PG545+CP, or PBS (vehicle). A) Splenic NK cell numbers were quantified by FACS. B-E) NK and T cells were analyzed for IFN-$\gamma$ and CD107a expression by FACS. F) Frozen section of liver at 13 days following start of therapy were assessed for co-localization of NK cells (NKp46+, red) and A20 B-cell lymphoma (CD45R+, brown) by immunohistochemistry.
Supplemental Figure 3
Efficiency of NK cell depletion in vivo. BALB/c and C57BL/6 mice were NK depleted by twice-weekly injections of anti-asialo-GM1 (α-GM1) and anti-NK1.1 antibody (PK136) beginning 4 days prior to inoculation with 2 x 10^5 A20-luciferase and 1 x 10^5 EL4-luciferase lymphoma cells, respectively. NK depletion was determined at two weeks following tumor injection for BALB/c mice (A-B) and C57BL/6 mice (C-D). N=4-5 mice per group. *p<0.05.
Supplemental Figure 4
PG545 suppresses type I IFN production by DCs in response to CpG ODN. BMDCs from WT or TLR9⁻/⁻ C57BL/6 mice were cultured in triplicate against CpG ODN at the specified concentrations, with or without PG545 (5 µg/mL). Eighteen hours later, cell culture supernatants were assayed for IFN-α (A) and IFN-β (B) by ELISA. *p<0.05.
DCs are critical for PG545-mediated NK activation in vivo. (A-B) WT and CD11c-DTR mice (n=3 mice per group) were treated with diphtheria toxin (+DT, 12 ng/gm) or without DT (-DT) and depletion of CD11c+ cells was determined two days later by FACS. Representative FACS plots of WT and CD11c-DTR mice (A); Mean number of CD11c+ splenocytes (SC) ± SEM from CD11c-DTR mice (B). (C-D) NK cells (NK1.1+CD3-) from WT and CD11c-DTR mice treated with DT two days prior to treatment with PG545 (20 mg/kg) or PBS were tested for CD69, Ki-67, and IFN-γ expression by FACS. Representative FACS analysis are shown (C) and the mean percentage ± SEM of CD69+, Ki-67+ or IFN-γ+ NK cells among total splenic NK cells is shown (D) (n=3 mice per group). *p<0.05.
Supplemental Figure 6
Lysosome function is critical for PG545-mediated augmentation of CpG induced IL-6 production. BMDCs were co-cultured with (+) or without (-) CpG (0.3 µg/mL), PG545 (5 µg/mL), LPS (100 ng/mL) or CQ (20 µM) for 18 h and culture supernatants were tested for IL-6 by ELISA. Conditions were performed in triplicate. Mean ± SEM for each condition are shown. *p<0.05 compared with no treatment group.