Supplemental Figure 1. (**A**) Anti-CD11c antibody-targeted zwiterionic liposomes containing siRNA fail to silence gene expression. Total ascites from mice bearing advanced ovarian tumors were incubated with optimized amounts of anti-CD11c-coated DPPC: DSPE-PEG: DSPE-PEG carboxylic (4:0.25:0.05 molar ratio) liposomes containing or not PD-L1-specific siRNA, and the mRNA levels of PD-L1 were analyzed by Real-Time RT-PCR 48 hours later. (**B**) Treatment of mice bearing aggressive ID8-*Defb29/Vegf-A* ovarian tumors with anti-CD11c antibody-targeted zwiterionic liposomes containing PD-L1-specific siRNA did not induce a significant increase in survival, compared to untreated mice or mice receiving empty liposomes.

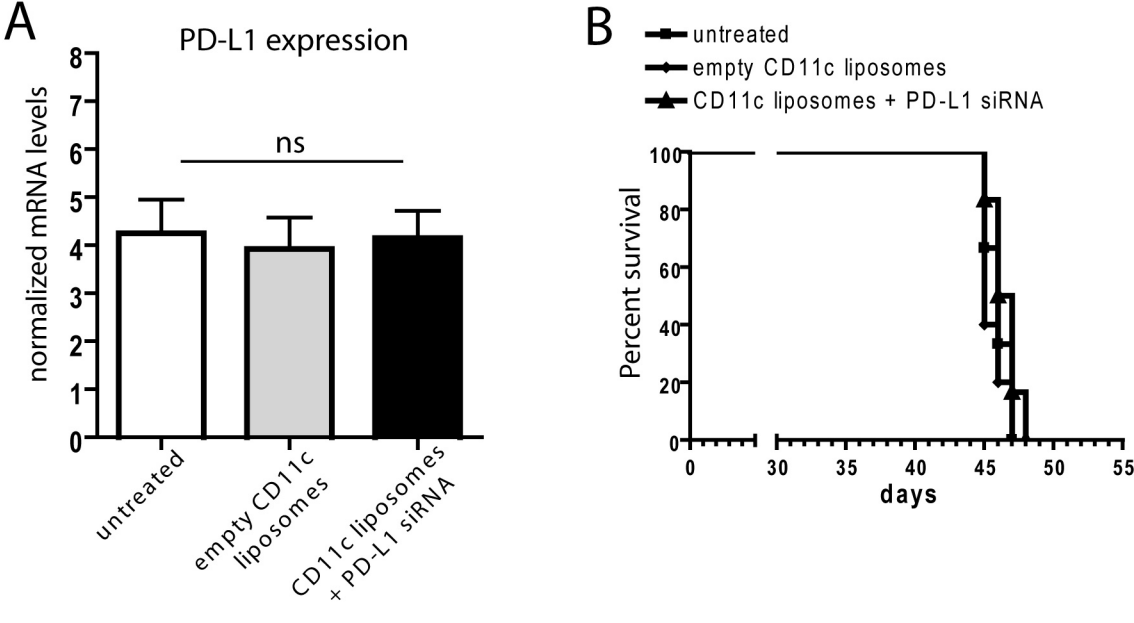
Supplemental Figure 2. (**A**) Preferential uptake of siRNA-PEI nanocomplexes by peritoneal tumor DCs in the absence of targeting motifs. Transgenic ITGAX mice, which express the green fluorescent protein (GFP) under control of the CD11c promoter, were challenged with ID8-*Defb29/Vegf-A* ovarian tumors and three weeks later mice were left untreated or intraperitoneally injected with rhodamine-labeled non-targeting siRNA-PEI nanoparticles. 24 hours post-injection, peritoneal wash samples (10 mL) were collected and analyzed by FACS to determine the cell types that engulfed the nanoparticles *in situ*. Note that siRNA-PEI nanoparticles are exclusively internalized by $CD45^{+}GFP^{+}(CD11c^{+})$ peritoneal tumor-associated DCs. Identical results were obtained when samples were stained with anti-CD11c antibodies (Figure 1, B and C). (**B**) Representative immunofluorescence microscopy demonstrating co-localization (white arrows) of rhodamine-labeled nanocomplexes and the DC marker MHC-II in histological sections of ovarian tumors from mice injected with nanoparticles. Blue indicates nuclei. (**C**) Representative fluorescence microscopy showing engulfment of rhodamine-labeled nanocomplexes (white arrows) by splenic DCs (GFP^{+}) in histological sections of ITGAX mice bearing ovarian tumors 24 hours post nanoparticle injection. (**D**) Representative FACS analysis of nanoparticle engulfment by splenic DCs over time in tumor-bearing mice after a single intraperitoneal injection with rhodamine-labeled nanocomplexes. Data are representative of three independent experiments.

Supplemental Figure 3. (**A**) Engulfment of PEI-based nanocomplexes containing gene- (PD-L1) specific siRNA also induces maturation of tumor DCs *in vivo*. Filled histograms represent CD80 and MHC expression levels by tumor DCs that did not engulf nanoparticles. Open histograms indicate expression on tumor DCs that internalized the nanoparticles *in situ* three days post-injection. (**B**) PEI-based nanocomplexes induce upregulation of CD80 on tumor DCs via a MyD88-dependent mechanism. $5x10^6$ Ficoll-enriched leukocytes isolated from the ascites of wild-type (WT) or MyD88-deficient (-/-) mice bearing aggressive ID8-*Defb29/Vegf-A* ovarian tumors for three weeks were incubated in 5 mL of RPMI and stimulated *ex vivo* with PEI (0.2 μmol diluted in 100μL of 5% glucose) or siRNA-PEI nanocomplexes (12 μg non-targeting siRNA complexed with 0.2 μmol PEI in 100 μL of 5% glucose) and the induction of surface CD80 on $CD11c^+MHC\text{-}II^+$ tumor DCs was determined by FACS 24 hours later. Dotted histograms represent basal CD80 expression by unstimulated cells. Data are representative of three independent experiments. (**C**) CD11b expression by human $CD45^+DEC205^+CD3^-CD14^-CD20^-$ solid tumor DCs from six patients with stage III ovarian cancer. (**D**) PEI-based nanocomplexes induce CD80 upregulation on immature human monocyte-derived DCs. Monocyte-derived DCs were generated by incubating magnetically purified $CD14^+$ monocytes from aphaeresis samples for 7 days with Granulocyte–macrophage colony stimulating factor (20 ng/ml, Peprotech) and IL-4 (50 ng/ml, R&D). Then, DCs were stimulated with PEI or non-targeting siRNA-PEI as described above and surface levels of CD80 were determined by FACS after 24 hours. Filled histograms represent staining with isotype control antibodies. Dotted histograms indicate basal CD80 expression by unstimulated cells. Data are representative of five independent samples.

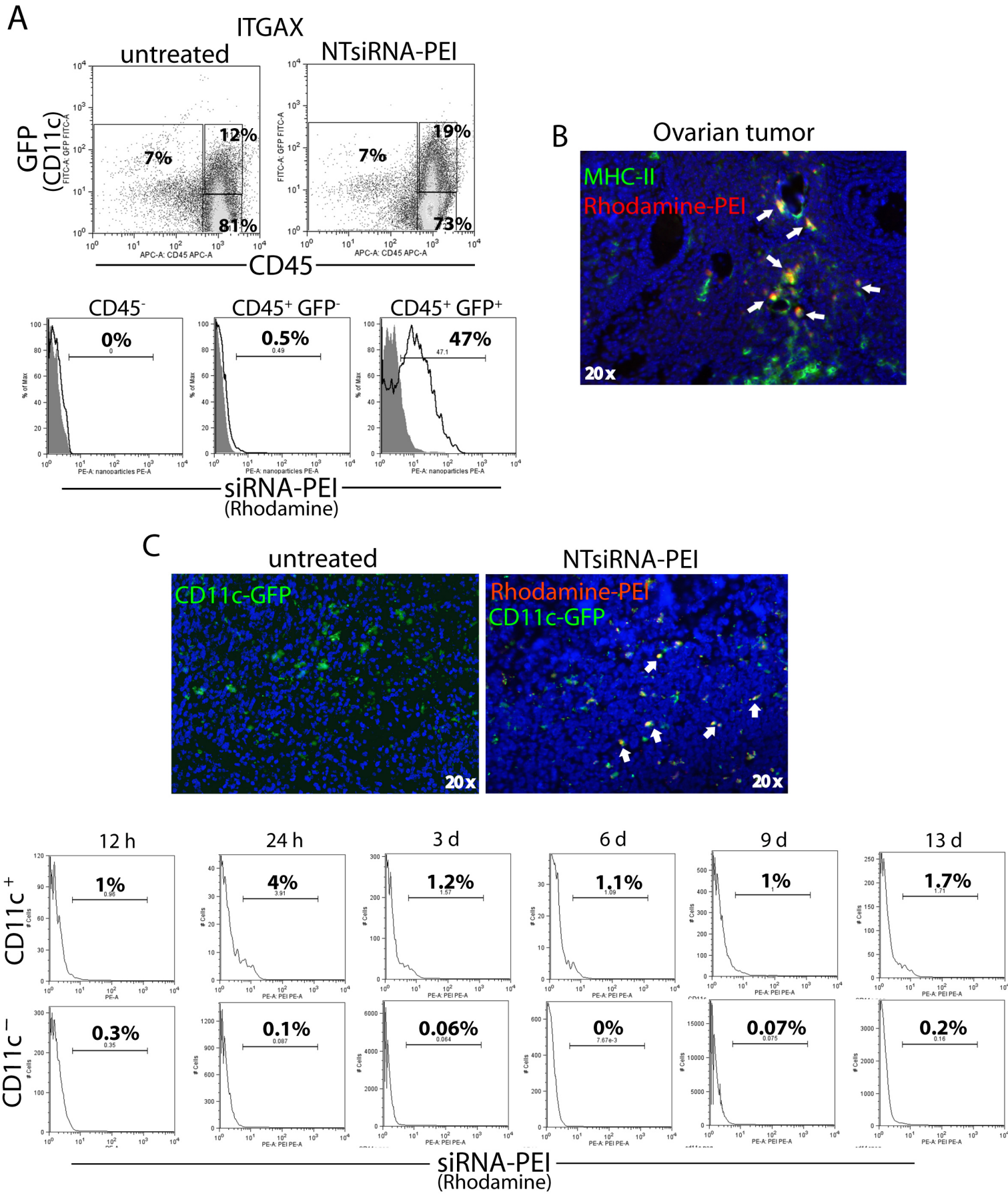
Supplemental Figure 4. (**A**) PEI alone does not stimulate TLR2, TLR3 or TLR7. HEK293 cells were individually cotransfected with each murine TLR and an NFkB-dependent luciferase reporter plasmid, and 30 hours later cells were stimulated with increasing amounts of PEI or positive control agonists, as described in the Methods. Data are shown as the fold increase in luciferase activity compared to cotransfected unstimulated cells, and are representative of two independent experiments. (**B**) PEI-mediated KC induction *in vivo* is not caused by contamination of PEI with bacterial flagellin or other protein source. Wild-type mice were intraperitoneally injected with 200 μl of 5% glucose, proteinase K (20 μg incubated at 55°C overnight and diluted in 200 μl of 5% glucose), or 0.9 μmol of PEI pretreated overnight at 55°C with proteinase K at 1 mg/ml, and serum levels of KC were analyzed by ELISA two hours later. Data are representative of two independent experiments. $^{**}P<0.01$, compared with either glucose or proteinase K alone. (**C**) PEI or non-targeting siRNA-PEI does not induce secretion of IL-1β. Wild-type (WT) mice were intraperitoneally injected with 200 μl of 5% glucose, PEI alone (0.9 μmol of "in vivo-jetPEI" diluted in 200 μl of Glucose 5%) or NTsiRNA-PEI (50 μg non-targeting siRNA complexed with "in vivo-jetPEI" at N/P 6) and serum levels of IL-1β were analyzed by ELISA two hours post-injection.

Supplemental Figure 5. (A) Representative FACS analysis of PD-L1 expression by cells in the peritoneal cavity of ovarian cancer-bearing mice. Tumor ($CD45^-$) cells do not express surface PD-L1. (**B**) Representative FACS analysis of *in vivo* PD-L1 silencing using PEI-based siRNA nanoparticles. Mice bearing ID8-*Defb29/Vegf-A* ovarian tumors for three weeks were intraperitoneally injected with rhodamine-labeled non-targeting siRNA-PEI (filled histogram) or PD-L1-specific siRNA-PEI nanoparticles (open histogram) and three days later the surface levels of PD-L1 on peritoneal tumor DCs that engulfed the nanoparticles (rhodamine-stained) were determined by flow cytometry. MFI, mean fluorescence intensity of PD-L1 staining.

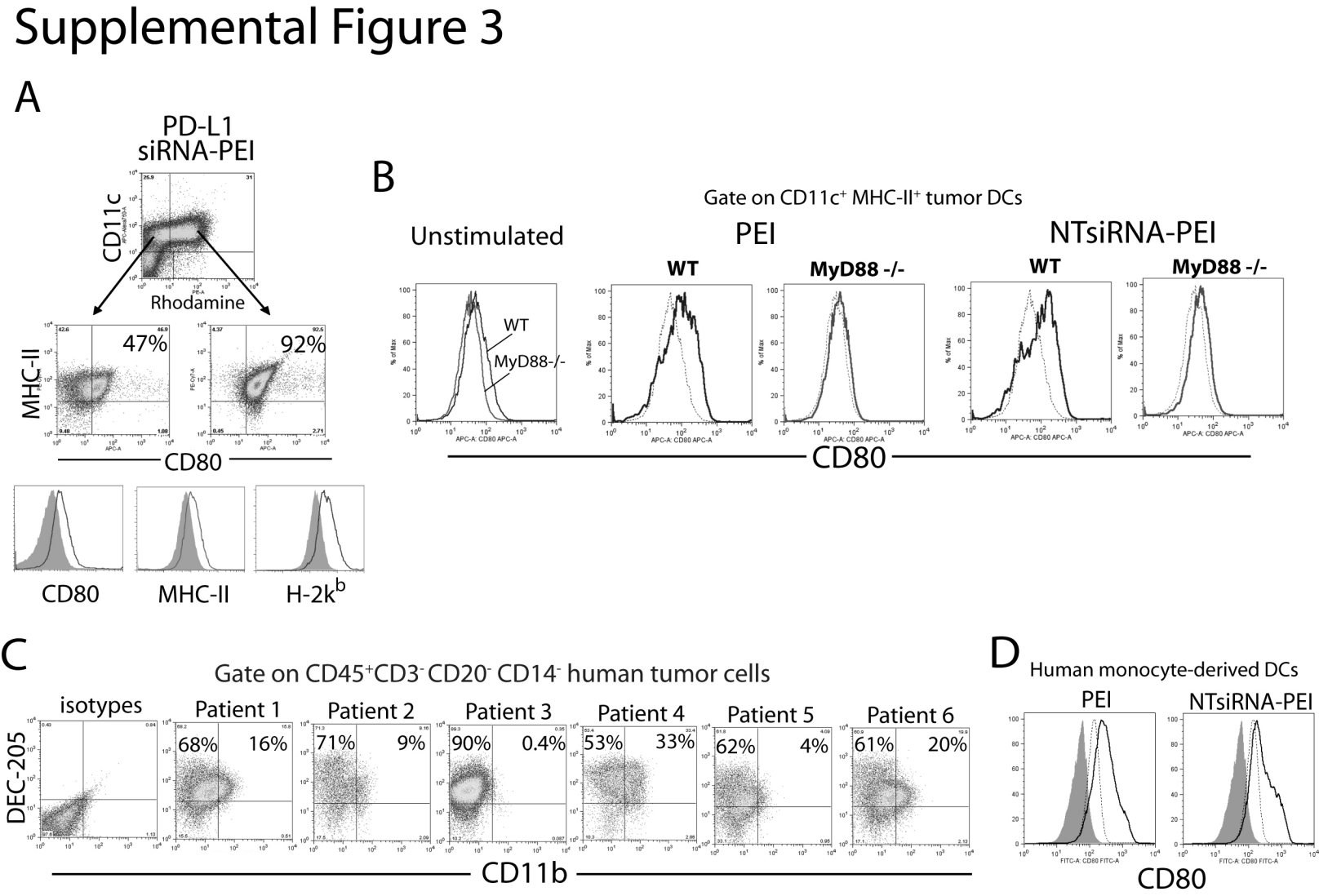
Supplemental Figure 1



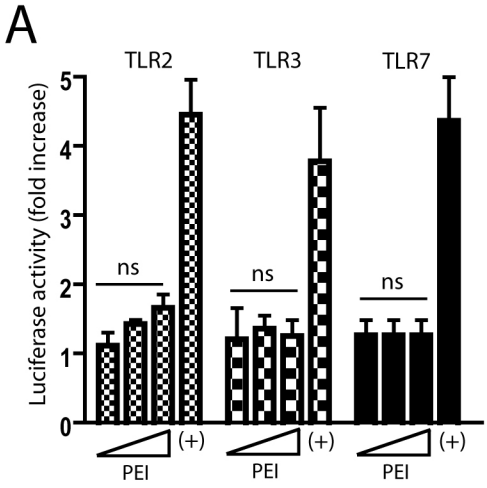
Supplemental Figure 2

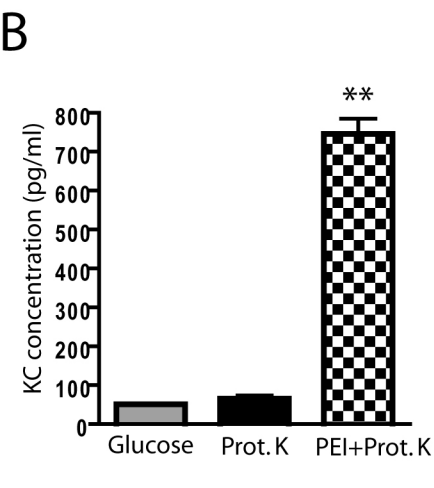


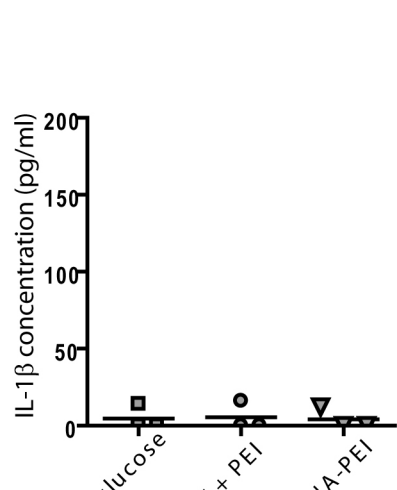
Supplemental Figure 3



Supplemental Figure 4







Supplemental Figure 5