Supplemental Figure 1. Control afucosylated antibodies (R347aFuc) do not bind pre-B ALL cells or mediate internalization. Mean fluorescence intensity of internalized R347aFuc (stripped, red) as compared to the fraction representing the level of all, non- and internalized mAb (unstripped, blue) measured by FACS in (A-D) pre-B ALL cell lines and (E-H) blasts from four patients. Pre B-ALL cells were incubated with 1 µg of Medi-551 Alexa488 for indicated times, followed by acid stripping and/or fixation with 2% PFA. Bars represent standard deviations (SD).
Supplemental Figure 2. Medi-551 does not induce decrease in cell viability of pre-B ALL cell lines and patient derived blasts. (A-B) Potential anti-proliferative effect of Medi-551 and R347aFuc antibodies was measured by MTS assay. Pre-B ALL cells were incubated at 37°C with indicated concentrations of antibody for 72 hrs at 37°C. MTS dye was added four hours prior to the end of incubation, followed by absorbance measurement at 490 nm. Experiment was performed in triplicate and data are presented as mean ± SD. (C-D) The viability of primary patient derived blasts was estimated after 24 hrs incubation with Medi-551 and R347aFuc antibodies using the same colorimetric MTS assay. Data represent mean ± SD for triplicate samples. (E) Medi-551 treatment does not alter levels of Akt phosphorylation in 697 cells.
Supplemental Figure 3. Control afucosylated antibodies (R347aFuc) do not mediate *in vitro* ADCC activity against pre-B ALL cell lines and patient derived blasts. ADCC was measured by LDH assay. Target pre-B ALL cells were incubated with R347aFuc for 1 hr at 37°C, then washed and co-incubated for 4 hr with IL2-stimulated primary NK cells as effectors (T:E ratio 1:3). (A-D) Comparison of antibody-mediated cytotoxicity using four pre-B ALL cell lines and (E-H) blasts from six patients (Table 2) as targets and NK cells from donors with determined FcyRIIIA-158 polymorphism status. Measurements were performed in triplicate and data are presented as mean ± SD.
Supplemental Figure 4. Despite low cytotoxicity, immunological synapses can form between antibody-bound pre-B ALL and NK cells from donors homozygous for FcγRIIIA-158F/F. Pre-B ALL cells were preincubated with Medi-551^Alexa594^ (1 μg/ml, 1 hr) and co-incubated with IL2-stimulated primary NK FcγRIIIA-158F/F cells at T:E ratio 1:3. NK cells were stained with SP-DiOC18. Live cell imaging was performed in imaging medium (IMDM phenol free, 5% FBS) on a Zeiss LSM510 META confocal microscope equipped with 63x water objective heater. Formation of immune synapses between target and effector cells, and concentration of Medi-551 at synapse, was observed for these NK cells with both (A) 697 cells and (B) patient-derived blasts (1 hr).
Supplemental Figure 5. Medi-551 recruits primary mouse monocyte-derived macrophages to mediate phagocytosis of human ALL target cells. (A) 697 cells were stained with Cell Trace Violet followed by incubation with Medi-551 (1 hr). Cells were then co-incubated with primary mouse monocyte-derived macrophages (MΦ) at T:E = 1:3 for 4 hrs. After fixation with 2% PFA, cells were visualized on a Zeiss LSM510 META laser-scanning confocal microscope (63x/1.4 lens) and Zen 2009 Software. In this micrograph, a Medi-551-coated human 697 target cell (blue) is engulfed by a murine MΦ cell. (B) Live cell imaging captures murine macrophage binding and phagocytosis of leukemia cell; experiment was performed in RPMI 1640 phenol free medium, 2% FBS. Target 697 cells were pre-incubated with 1 μg/ml Medi-551Alexa488 for 1 hr and primary mouse MΦ were stained with Orange Cell Mask. Cells were co-incubated at a T:E ratio of 1:3 for up to 60 min while imaging. Murine macrophages were seen to form “synapse-like” structures with antibody-bound pre-B ALL cells. (C) The kinetics of target cell binding (“synapses”) and internalization by MΦ. 697 cells were coated with Medi-551Alexa488 (1 μg/ml; 1 hr) and nucleoli of these cells were stained with Hoechst 33342. Next, 697 cells were co-incubated with primary mouse MΦ at T:E ratio 1:3 for indicated intervals. Cells were fixed with 2% PFA and analyzed by confocal microscopy.
Supplemental Figure 5. R347aFuc incubation does not support in vitro ADCP activity against pre-B ALL cell lines and patient derived blasts. Pre-B ALL cells were stained with Violet Trace followed by incubation with R347aFuc isotype control for 1 hr. Washed cells were co-incubated with primary human monocyte derived macrophages (MΦ) at T:E = 1:3 for 4 hrs. After fixation with 2% PFA, cells were enumerated on a Zeiss LSM510 META laser-scanning confocal microscope. Percent phagocytosis was determined; at least 300 target cells were scored per condition. (A-D) Summary of phagocytosis results using four cell lines (697, Nalm6, MHH-Call3, RS4;11) and patient-derived blasts (E-H) as a function of FcγRIIIA-158 polymorphism on donor macrophages. Data represent mean ± SD for triplicate samples.