Supplementary Methods

Production of DuoBody® antibodies

JNJ-67571244 DuoBody® antibody was generated by controlled antigen binding arm exchange from 2 parental antibodies: 1) an anti-CD33 mAb that was derived from OmniMouse™ hybridoma technology (transgenic mouse) immunized with extracellular domain CD33, and 2) an anti-CD3ε mAb that was derived from OmniRat™ hybridoma technology (transgenic rat) immunized with CD3ε DNA vaccines. Following isolation and manipulation, the resulting genes expressing human monoclonal antibodies against CD33 and CD3 were transfected into separate Chinese Hamster Overy (CHO) cell lines for production, purification and formulation.

CD33 antigen density quantification

Quantum™ Simply Cellular (QSC) microspheres were used according to manufacturer's instructions to correlate geometric mean fluorescence intensity (gMFI) with CD33 antigen expression as molecules/cell. The microspheres and cells were stained for 30 minutes at RT with the appropriate concentrations of the same anti-CD33 antibody (clone P67.6, R&D systems, catalog# FAB1137P for human samples and clone AC104.3E3, Biolegend, catalog# 130-091-732 for cyno samples) or mouse IgG1-PE isotype control. CD33 surface antigen density was calculated by first correcting for non-specific binding by subtracting the number of bound isotype molecules from the number of bound CD33 antibody molecules on each cell line. CD33 positivity was established as > 0 receptors per cell post correction of non-specific binding. Source of cells included cell lines (MOLM-13, KG-1, Kasumi-1, SKNO-1, OCI-AML3, CARNAVAL, and KG-1ΔCD33), frozen bone marrow and apheresis samples from 49 newly diagnosed or disease relapsed AML patients (obtained from the University of Pennsylvania) or fresh whole blood from 5 healthy human or cyno donors.

Binding Assay

All cells (HEK-293F parental, HEK-293F line overexpressing cyno CD33, MOLM-13, KG-1, Kasumi-1, OCI-AML3, CARNAVAL, and KG-1ΔCD33) were centrifuged, washed twice with Dulbecco's phosphate-buffered saline (DPBS) and 1×10^4 cells were added to the center of each well of a 96-well U-bottom plate along with fragment crystallizable (Fc) block (human IgG1

fragment) which was added at 2 mg/mL for 10 minutes. JNJ-67571244 and the null arm controls (nullxCD3, CD33xnull, nullxnull) were tested. Serially diluted 40× concentrations of all test antibodies (final concentrations from 533.3 nM to 53 fM, in 10-fold increments) were added to the appropriate wells. A single replicate was plated per test condition. Plates were incubated in the dark at 37°C with 5% CO_2 for 4 hours. The cells were then washed with DPBS and binding of the bispecific antibody was detected by staining with mouse anti-human IgG4 (Southern Biotech, clone HP6025, catalog# 9200-09) and LIVE/DEAD (L/D; Invitrogen, catalog# L34976) for 30 minutes. Finally, cells were washed, resuspended in stain buffer, and analyzed on the FACSCanto II flow cytometer (BD Biosciences). Geometric mean fluorescence intensity (gMFI) was plotted in Prism version 7 (GraphPad). The X-axis was log transformed and a 4-parameter non-linear curve fit was applied.

T Cell Redirection Assays

The ability of JNJ-67571244 and the control antibodies to activate T cells and induce T cell-mediated killing of CD33-expressing cells was assessed in a T cell redirection assay. Readouts included T cell activation, cytotoxicity, and measurement of cytokines in the supernatants. The assays described below differed in the source of the effector T cells, such as purified from healthy donors (obtained from BioIVT (Westport, NY), within whole blood of healthy donors or AML patients (obtained from Dx Biosamples (San Diego, CA) and University of Pennsylvania). The target cells included tumor cell lines, AML blasts from whole blood of patients, or immune cells that express CD33 endogenously. The whole blood assays were considered more physiologically relevant as they contained all immune cell populations and were expected to contain sCD33, similar to the situation in AML patients. All T cell redirection assays were performed for 48 hours.

The bispecific antibodies have mutations in the Fc region that suppress binding to Fc receptors, but binding may not be fully prevented (Vafa et al., 2014). Some of the cells in the assays could express Fc receptors, which could recruit binding of the test antibodies via their Fc region, and not by the variable regions that bind to either CD33 or CD3. To prevent this, Fc block was added to the assay.

a. T Cell Redirection Using Purified T Cells with Tumor Cell Lines as Targets

Tumor cell lines (MOLM-13, KG-1, Kasumi-1, OCI-AML3, CARNAVAL, and KG-1ΔCD33) were labelled with carboxyfluorescein succinimidyl ester (CFSE) at 1×10^7 cells per mL of CFSE

for 8 minutes at room temperature (RT). Equal volumes (100 µL) of purified T cells (n=6 donors) and labeled tumor cells were combined in wells of a 96-well plate with 2 mg/mL Fc block at an E:T ratio of 5:1. The test antibodies, including JNJ-67571244, nullxCD3, and CD33xnull, were serially diluted to 40× final concentrations and added to the appropriate wells (final test concentrations 533.3 nM to 53 fM, in 10-fold increments). A single well was plated for each test condition. All plates were incubated at 37°C with 5% CO_2 for 48 hours.

Clarified supernatant (60 to 80 µL) was first transferred to a 96-well U-bottom plate and frozen at -80°C until analyzed. The cells were then washed with DPBS and stained for T cell activation (CD25 upregulation; anti-CD25 antibody from Biolegend, clone BC96, catalog# 302606) and for cytotoxicity (staining with L/D) for 30 minutes. Finally, cells were washed and resuspended in stain buffer. Data was acquired on a FACSCanto II and recorded events were limited to 1×10^4 tumor cells. The percentage of either dead tumor cells or $CD25^+$ cells was graphed using Prism and analyzed with a 4-parameter non-linear regression curve fit. For statistical analysis, four-parameter logistic mixed-effects regression model was performed in R software (version 3.4.2) with post-hoc estimation of donor-specific EC20, EC50, and EC90 estimates.

b. T Cell Redirection Assay Using Healthy Human or Cyno Whole Blood

Serially diluted 40× concentrations of JNJ-67571244 (final concentrations from 533.3 nM to 0.53 fM, in 3-fold increments) were added to healthy human or cyno blood. One well was plated per test condition. All plates were incubated at 37°C, 5% CO_2 for 48 hours.

Cells were prepared for flow cytometry and antibodies was added directly to each well of whole blood and incubated for 30 minutes at RT in the dark. The following antibodies were used for staining: anti-CD3 (Biolegend, clone SP34-2, catalog# 552852), anti-CD177 (Biolegend, clone MEM-166, catalog#315808), anti-CD14 (Biolegend, clone M5E2, catalog# 301814), anti-CD33 (Miltenyi Biotech, clone AC104.3E3, catalog# 130-091-732), anti-CD45 (BD Biosciences, clone D058-1283, catalog# 557803). Cells were then centrifuged and lysed with 200 µL of multi-species RBC lysis buffer for four rounds. Cells were washed once with DPBS and stained with L/D near-IR for 15 minutes. Each well was washed and resuspended in stain buffer before the cells were acquired on a FACSCanto II. The percentages of dead $CD33^+$ $CD14^+$ monocytes, dead $CD33^+$ $CD177^+$ neutrophils or $CD25^+$ Tcells were graphed using Prism and analyzed with a non-linear curve fit. EC50 values were estimated using donor-specific least square four-parameter logistic

regression (GraphPad Prism, version 7). Cell acquisition on the FACSCanto II was limited to 1×10^4 tumor cells. The percentage of either dead tumor cells or $CD25^+$ cells was graphed using Prism and analyzed with a non-linear curve fit. Statistical analysis was performed using a four-parameter logistic mixed-effects regression model with post-hoc estimation of donor-specific EC20, EC50, and EC90 estimates in R software (version 3.4.2).

c. T Cell Redirection Assay Using Primary AML Whole Blood

Primary AML whole blood (n=6 donors; in heparin tubes) was diluted with an equal volume of complete medium and plated at 200 µL/well to a 96-well U-bottom plate. Each well was treated with 5 µL of JNJ-67571244 or the nullxCD3 control antibody (at 40× final concentration) and incubated at 37°C, 5% CO_2 for 48 hours (final concentrations 267 nM to 1 pM or 300 nM to 0.03 pM, depending on the experiment). Cell-surface-staining antibodies were added directly to each well of whole blood and incubated for 30 minutes at RT, protected from light. The following antibodies were used for staining: anti-CD4 (Biolegend, clone SK3, catalog# 344608), anti-CD8 (Biolegend, clone SK1, catalog# 344710), anti-CD33 (Biolegend, clone HIM3-4, catalog# 303304), anti-CD45 (BioLegend, clone HI30, catalog# 304029) and L/D Near IR. Cells were then lysed with 200 µL of RBC lysis buffer, which was repeated four more times and stained with L/D near-IR (diluted in DPBS) for 15 minutes. Pellets were washed, centrifuged, and resuspended in 150 µL stain buffer before acquisition on a FACSCanto II, where collection was limited by sample volume, ie, 90 µL.

T cell activation was assessed by CD25 expression on $CD4^+CD8^+$ T cells. $CD33^+$ AML blasts were identified by gating on the $CD33^+$ $CD45^{low}$ SSC^{low} population and the following equation was used to calculate cytotoxicity of $CD33^+$ blasts:

$$\frac{\%CD33^+ \text{ in untreated control} - \%CD33^+ \text{ in treated sample}}{\%CD33^+ \text{ in untreated sample}}$$

The percentages of CD33 cytotoxicity or $CD25^+$ cells and subsequent EC values were graphed using Prism and analyzed with a non-linear regression curve fit. Statistical analysis to estimate EC50 values was performed using donor-specific least square four-parameter logistic regression (GraphPad Prism, version 7).

d. T Cell Redirection Assay Using Monocytes with Different CD33 SNP Genotypes

T cell redirection assays were performed to assess the correlation between the CD33 SNP rs12459419 genotype and the in vitro killing potential of JNJ-67571244. Monocytes from 25 healthy donors were used for DNA extraction and cytotoxicity assays. Approximately 1×10^7 monocytes were lysed in 600 µL of RLT plus buffer and stored at -80°C for genotyping analysis. Detection of SNP rs12459419 was accomplished using a commercially available Taqman genotyping assay according to the manufacturer's instructions. Input DNA (20 ng) was combined with a 2× Taqman MasterMix, 40× Primer/Probe Mix, and nuclease-free water in a 20 µL reaction on a Quantstudio 12k-flex instrument (ThermoFisher Scientific). The following cycling parameters were used: 50°C for 2 minutes, 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds each, and 60°C for 1 minute. Genotype calling was performed using the instrument's default parameters for Taqman genotyping assays. p values were calculated by GraphPad Prism using the Welch's unequal variance t-test.

The remaining monocytes were then labelled with CFSE as described above and combined with purified matched T cells in the 96-well plate at an E:T of 5. JNJ-67571244 was diluted to a final concentration of 0.27 nM. All plates were incubated at 37°C with 5% CO_2 for 48 hours. Cells were then stained with L/D near-IR for 30 minutes. Data was acquired on a FACSCanto II and recorded events were limited to 1×10^4 tumor cells. Monocyte cell death was assessed and analyzed in Prism. Specific cytotoxicity was calculated as the percentage of monocyte killing by JNJ-67571244 at 0.27 nM (a concentration at which maximum killing was reached) minus the percentage of monocyte killing at baseline (defined as the amount of monocyte death in DPBS-treated wells).

e. Cytokine Analysis

Supernatants from the in vitro T cell redirection assays were analyzed using the ProInflammatory Panel I V-Plex, which measures human interferon (IFN)-γ, interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and tumor necrosis factor (TNF)-α. Supernatants were thawed on wet ice, centrifuged at 500× gravity for 5 minutes at 4°C, then placed on ice. 1:3 and 1:50 dilutions of the samples were then prepared. The V-Plex assay plates were pre-washed per manufacturer's protocol. Briefly, plates were washed three times with 150 µL of wash buffer (prepared by adding 15 mL of 20× MSD wash buffer in 285 mL of water). A standard curve was prepared by serial dilution of the provided calibrator, reconstituted in 1.0 mL of MSD Diluent 2. A 50 µL aliquot of

each sample or standard was added directly to pre-washed plates. Plates were then sealed and incubated overnight at 4°C. Plates were washed three times and 25 µL of the detection antibody mix was added to each well. Plates were then sealed and incubated with gentle mixing at RT. Plates were again washed three times prior to adding 150 µL of the read buffer. Assay plates were read on the SECTOR Imager 6000. Statistical analysis on this data set was performed using four-parameter logistic mixed-effects regression model with post-hoc estimation of donor-specific EC20, EC50, and EC90 estimates.

In vivo rodent studies

a. Tumor Xenografts in NSG Mice

For all studies, female NSG mice (Jackson Laboratory) were used at approximately 5 to 8 weeks of age. All animals were allowed to acclimate and recover from any shipping-related stress for a minimum of 5 days prior to experimental use. Autoclaved water and irradiated food (Laboratory Autoclavable Rodent Diet 5010, Lab Diet) were provided ad libitum, and the animals were maintained on a 12 hour light and dark cycle. Cages, bedding, and water bottles were autoclaved before use and changed weekly. All experiments were carried out in accordance with The Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Janssen R&D, Spring House, PA.

To humanize the NSG mice, human pan-T cells were activated and expanded in vitro using the T-cell activation and expansion kit and grown in medium containing interleukin (IL)-2 at a concentration of 0.1 µg/µL, starting 3 days after thaw. On the day of engraftment into mice, beads were removed from the T cells using magnets and cells were resuspended in serum-free medium for an IP injection of 2×10^7 cells in 0.2 mL per mouse. In all studies, T-cell-engrafted mice were given fragment crystallizable (Fc) block at 0.2 mg/mouse IP and human immune globulin infusion at 10 mg/mouse IP at least half an hour prior to antibody dosing, to compensate for the low immunoglobulin environment in the NSG mouse.

To generate KG-1 xenografts, 5×10^6 cells were injected SC in the right flank on Day 0. On Day 13, mice were engrafted with human T cells, and they were randomized into groups of 10 by tumor volume. Treatment was initiated the next day with IP dosing twice a week of JNJ-67571244 (0.1, 0.5, or 1 mg/kg) or nullxCD3 control (1 mg/kg) for a total of 12 doses. To generate systemic

luciferase labeled MOLM-13 disease, 1×10^5 MOLM 13-luc cells were injected IV in the tail vein on Day 0. Animals were randomized into groups of 10 on Day 6 by bioluminescence intensity (photons/second/cm2/steradian [p/s/cm2/sr]). Beginning on Day 7, mice were treated IP twice a week with JNJ-67571244 at 0.005, 0.05, or 0.5 mg/kg or nullxCD3 control (0.5 mg/kg) for a total of 13 doses. To assess T cell infiltration, flushed bone marrow was harvested for flow analysis and whole femurs were collected for IHC staining on Days 12, 15, and 19.

b. Data Analysis

Tumor volume, percent body weight change and BLI were graphed using GraphPad Prism (version 7). Whole body BLI (sum of ventral and dorsal ROIs) was measured using IVIS imager with Living Image® software (PerkinElmer, version 4.7). Percent TGI was defined as the difference between mean tumor burden of the treated and control groups, corrected for initial tumor burden. For survival assessment, results were plotted as the percentage survival against study days. Median survival was determined using Kaplan-Meier survival analysis evaluated by log-rank test (GraphPad Prism). Percent ILS was defined as the difference between median survival of the treated versus control group. Statistical significance for efficacy on KG-1 xenografts was calculated using the linear mixed-effects analysis (R software version 3.4.2) for pairwise treatment comparisons to the control group. Statistical significance of treatment effects on BLI were calculated using logarithmic transformed (base 10) BLI values using cross-sectional non-parametric Kruskal-Wallis test, followed by non-parametric post-hoc Dunnett`s test on Day 13.

c. Histology

IHC staining was performed on formalin fixed paraffin embedded FFPE tissues following bone decalcification. Rabbit monoclonal anti-human-CD8 antibody (Ventana Medical Systems, 5 µg/mL) was used for primary detection and secondary detection was achieved using UMap horseradish peroxidase-labeled anti-rabbit secondary antibody and the ChromoMap diaminobenzidine detection kit (Ventana Medical Systems) on the Discovery XT Autostainer (Ventana Medical Systems). CD33 IHC staining was executed using the Bond Polymer Refine Detection Kit (Leica Bond) on an autostainer (Leica Bond) at RT. Sections were incubated with anti-human CD33 (Leica Bond). Secondary detection was achieved using HRP-labeled anti-rabbit polymer, followed by diaminobenzidine. Slides were counterstained with hematoxylin.

d. Ex Vivo Flow Cytometry

Flushed bone marrow was dissociated into single-cell suspensions and subjected to RBC lysis. Approximately 2×10^5 to 4×10^5 cells per sample were stained with CD3/V500 (BD Biosciences, clone UCHT1, catalog# 561416) and CD45/Pacific Blue (BioLegend, clone HI30, catalog# 304029) in stain buffer, followed by L/D staining. Samples were acquired on a FACSCanto flow cytometer (BD Biosciences) and analyzed using FlowJo 9.7.7 software (TreeStar).

Immunocapture Mass Spectrometry for levels for sCD33

To determine the physiological C2-domain sCD33 levels in normal human serum and AML donors, an immunocapture coupled mass spectrometry assay was developed. JNJ-67571244 was added to all calibrators, QCs and samples. After incubation, JNJ-67571244 was immobilized on protein A magnetic resin and washed thoroughly. After on-resin reduction, alkylation and digestion, the calibrators, QCs and samples were loaded onto the instrument. 2D orthogonal reverse phase - reverse phase chromatography was used to isolate the signature peptide (FAGAGVTTER). The signature peptide was monitored via Sciex 6500+ triple quadripole mass spectrometer in positive ion mode. C2-domain sCD33 concentrations at or above 3 ng/mL (0.11 nM) were considered reportable. Soluble C2-domain CD33 concentrations in healthy and AML donor samples were reportable for 5 of the 10 samples and 6 out of 10 samples tested, respectively.

Hydrogen/Deuterium Exchange Mass Spectrometry

For H/D exchange, the procedures used to analyze the Fab perturbation were similar to that described previously (Hamuro *et al.,* J. Biomol. Techniques 14:171–182, 2003; Horn *et al.,* Biochemistry 45:8488-8498, 2006) with some modifications.

a. Pepsin/protease XIII digestion and LC-MS

5.77 µg of native or 7.4 µg deglycosylated CD33 ECD in 130 µL of control buffer (50 mM phosphate, 100 mM sodium chloride at pH 7.4) was denatured by adding 130 µL of 4 M guanidine hydrochloride, 0.85 M TCEP buffer (final pH was 2.5) and incubating the mixture for 3 min at 10 °C. Then, the mixture was subjected to on-column pepsin/protease XIII digestion using an in-house packed pepsin/protease XIII (w/w, 1:1) column (2.1 x 30 mm). The resultant peptides were analyzed using an UPLC-MS system comprised of a Waters Acquity UPLC coupled to a Q ExactiveTM Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo). The peptides were

separated on a 50 x 1 mm C8 column with a 16.5 min gradient from 2-34% solvent B (0.2% formic acid in acetonitrile). Solvent A was 0.2% formic acid in water. The injection valve and pepsin/protease XIII column and their related connecting tubings were inside a cooling box maintained at 15 °C or 20 °C for native and deglycosylated antigen, respectively. The second switching valve, C8 column and their related connecting stainless steel tubings were inside another chilled circulating box maintained at -6 °C. Peptide identification was done through searching MS/MS data against the CD33 sequence with Mascot. The mass tolerance for the precursor and product ions were 7 ppm and 0.02 Da, respectively.

b. Deglycosylation treatment

CD33 µg was deglycosylated by incubation with 10 µL of Rapid PNGase F at 37ºC for 5h.

c. HDX MS for deglycosylated CD33 with and without the presence of mAb C33B904

12 µL of CD33 (7.4 µg) or 12 µL of CD33 & mAb JNJ-66610479 or JNJ-66586351 mixture (7.4 µg: 19.73 µg) was incubated with 118 µL deuterium oxide labeling buffer (50mM sodium phosphate, 100 mM sodium chloride at pD 7.4) for 0 s, 10 s, 60 s, 600 s or 3600 s at 10°C. Hydrogen/deuterium exchange was quenched by adding 130 µL of 4 M guanidine hydrochloride, 0.85 M TCEP buffer (final pH was 2.5). Subsequently, the quenched samples were subjected to on column pepsin/protease XIII digestion and LC-MS analysis as described above. The mass spectra were recorded in MS only mode. 95.6% sequence coverage was achieved for deglycosylated CD33.

Raw MS data was processed using HDX WorkBench, software for the analysis of H/D exchange MS data (*J. Am. Soc. Mass Spectrom.* **2012**, *23 (9)*, 1512-1521). The deuterium levels were calculated using the average mass difference between the deuterated peptide and its native form (t_0).

Cynomologous monkey studies

The PK and PD properties of JNJ-67571244 were evaluated in naive cynomolgus monkeys at Charles River Laboratories (CRL, Reno, NV).

In the single dose PK/PD study, cynomolgus monkeys were treated with a single IV dose of control (vehicle), 0.05, 0.2, or 1 mg/kg of JNJ-67571244. Blood samples were collected by venipuncture

via the femoral vein pre-study and at selected time points up to 28 days after dosing for analysis of hematology, PK, and PD endpoints including T lymphocyte activation and cytokines. All procedures were approved by the IACUC and were performed in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal welfare.

a. Bioanalytical Methods

PK: Serum samples were analyzed for total JNJ-67571244 concentrations using a validated electrochemiluminescence-based immunoassay (ECLIA). A biotin-conjugated mAb specific to the CD3 arm of JNJ-67571244 (in-house) was used as the capturing reagent and a Sulfo-Tagged anti-human IgG mAb (R10Z8E9, Abcam, Cambridge, MA) was used as the detection reagent. The lowest quantifiable concentration in a sample is 0.01 µg/mL. Non-compartmental analysis was conducted to estimate the pharmacokinetic parameters using Phoenix™ (Pharsight Corporation, a Certara™ Company, Mountain View, CA).

T Cell Activation: Whole blood samples were used to analyze T-cell activation using a flow cytometric assay. The samples were transferred to the laboratory and stored at ambient temperature upon receipt. Sample analysis was initiated within 5 hours of sample collection. T-cell activation was measured by the percentage of CD25+ cells within the population of CD45+/CD4+ and CD45+/CD159-/CD8+ T-lymphocytes.

Cytokine analysis: Blood (1.0 mL) was collected the cyno monkey study once pretreatment and on Day 1 at 2 and 24 hours postdose, and processed to plasma. The plasma samples were analyzed at CRL using a using a validated analytical method (AP.I-001489.IM.06.) for cytokines TNF-α, IFN-γ, IL-2, IL-6, MCP-1, and IL-10.

Supplementary Figure Titles and Legends

Supplementary Figure 1: CD33 is expressed on normal monocytes and neutrophils

(A) CD33 antigen density was assessed on monocyte, neutrophil, and lymphocyte populations from five normal human whole blood donors. Means ± SD are graphed. (B) CD33 antigen density was assessed on various hematologic cell lines.

Supplementary Figure 2: JNJ-67571244 binds to the C2 domain and mediates cytotoxicity of primary samples regardless of their SNP 12459419 genotype status

(A) T cell-mediated cytotoxicity and activation (CD25 upregulation on $CD3^+$ T cells) assays using CD33xCD3 or CD123xCD3 bispecific antibodies in $CD33^+$ KG-1 and $CD33^-$ KG1ΔCD33 cell lines.

(B) T cells alone were incubated with increasing concentrations of JNJ-67571244 for 48 hours and T cell activation (CD25 upregulation on $CD3^+$ T cells) was measured by flow cytometry. Mean ± SD is graphed.

Supplementary Figure 3: JNJ-67571244 mediates cytokine responses in T cell-mediated assays with Kasumi-1 cells

Kasumi-1 cell supernatants for JNJ-67571244-treated groups from six different donors were analyzed for cytokine levels. Mean ± SD is graphed.

Supplementary Figure 4: JNJ-67571244 mediates anti-tumor responses in a disseminated murine AML model

T cell-engrafted NSG mice bearing disseminated MOLM-13Luc cells were i.p. dosed with JNJ-67571244 at 0.005, 0.05, and 0.5 mg/kg (denoted by bar below X-axis). Bioluminescence was measured twice weekly and the results presented as the mean bioluminescence ± SEM for each group (statistical significance denoted by asterisks).

Supplementary Figure 5: CD33 is expressed in cynomolgus monkey immune subsets

Whole blood from six normal healthy cynomolgus monkey donors was stained with a monoclonal antibody to CD33 and analyzed by flow cytometry. Mean ± SD is graphed.

Supplementary Figure 6: JNJ-67571244 mediates cytotoxicity of healthy CD33$^+$ cyno monocytes and neutrophils along with activating cyno T cells

Ex vivo assessment of JNJ-67571244-mediated cytotoxicity in healthy cynomolgus monkey whole blood (n=6). Percent of cytotoxicity of CD33$^+$ monocytes and neutrophils as well as T cell activation (CD25 upregulation on CD3$^+$ T cells) using JNJ-67571244 and nullxCD3 bispecific antibodies are shown. Mean ± SEM is graphed.

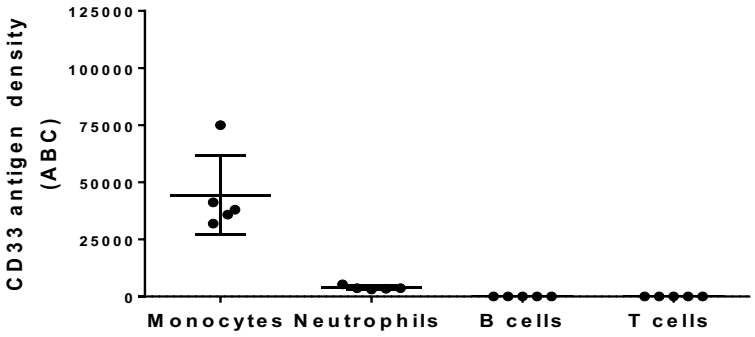
Supplementary Figure 7: JNJ-67571244 mediates cytotoxicity of healthy human CD33$^+$ monocytes and neutrophils along with activating T cells

Ex vivo assessment of JNJ-67571244-mediated cytotoxicity in healthy human whole blood (n=6). Percent of cytotoxicity of CD33$^+$ monocytes and neutrophils as well as T cell activation (CD25 upregulation on CD3$^+$ T cells) using JNJ-67571244 and nullxCD3 bispecific antibodies are shown. Mean ± SEM is graphed.

Supplementary Figure 8: Cytokine release following JNJ-67571244 dosing in cyno monkeys

Mean (SD) cytokine levels in cyno monkeys following a single IV dose of JNJ-67571244. (A) IFNγ, (B) IL-10, (C) IL-2, (D) IL-6, (E) MCP, (F) TNFα. All below LLOQ values were treated as ½ of LLOQ for plotting and mean calculation purposes.

A

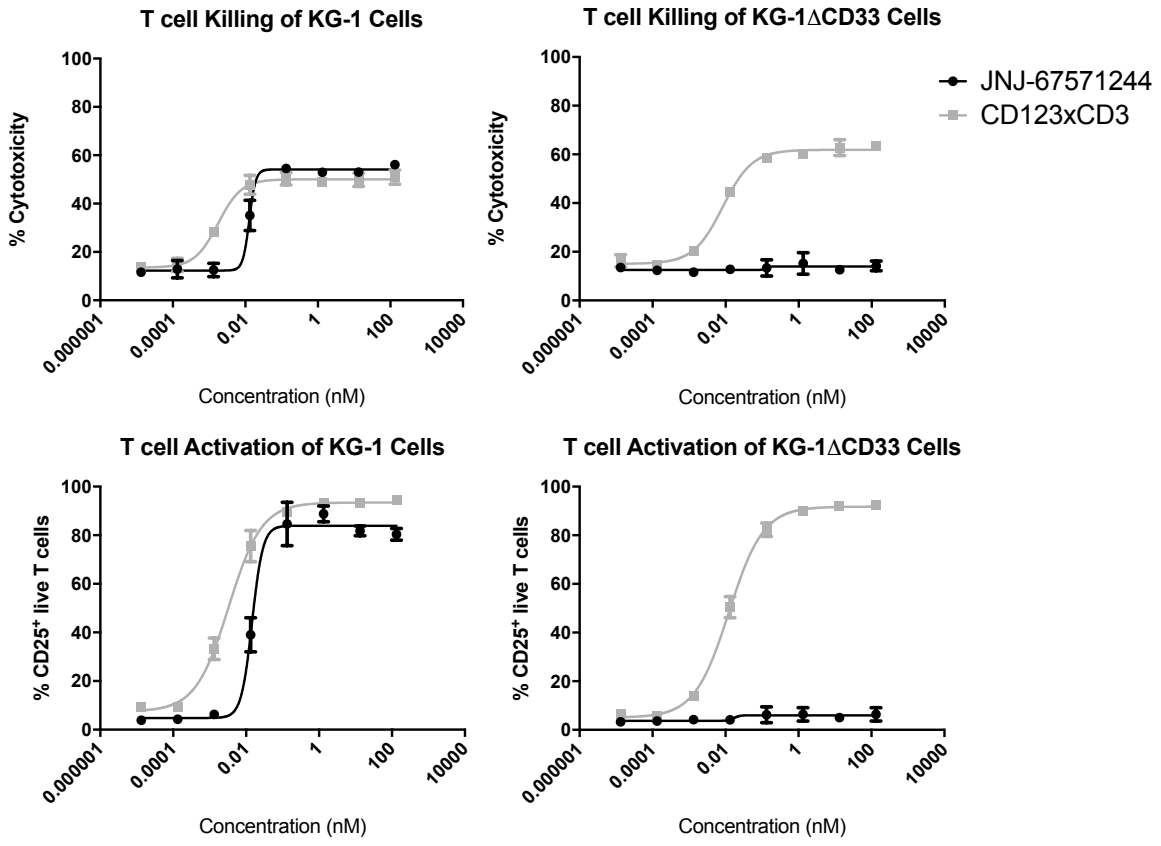


B

Cell line	Receptor density CD33
KG-1	122,599
MOLM-13	185,752
SKNO-1	26,371
Kasumi-1	11,273
OCI-AML3	5,395
CARNAVAL	0
KG1ΔCD33	0

Supplementary Figure 1

A



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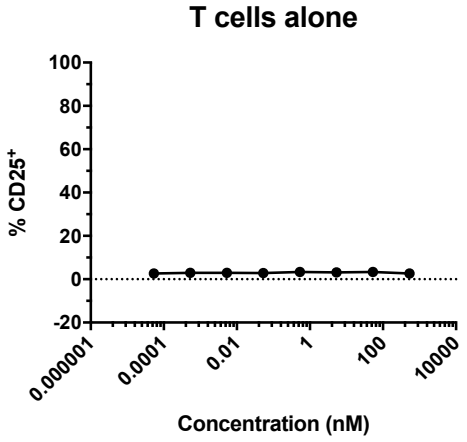

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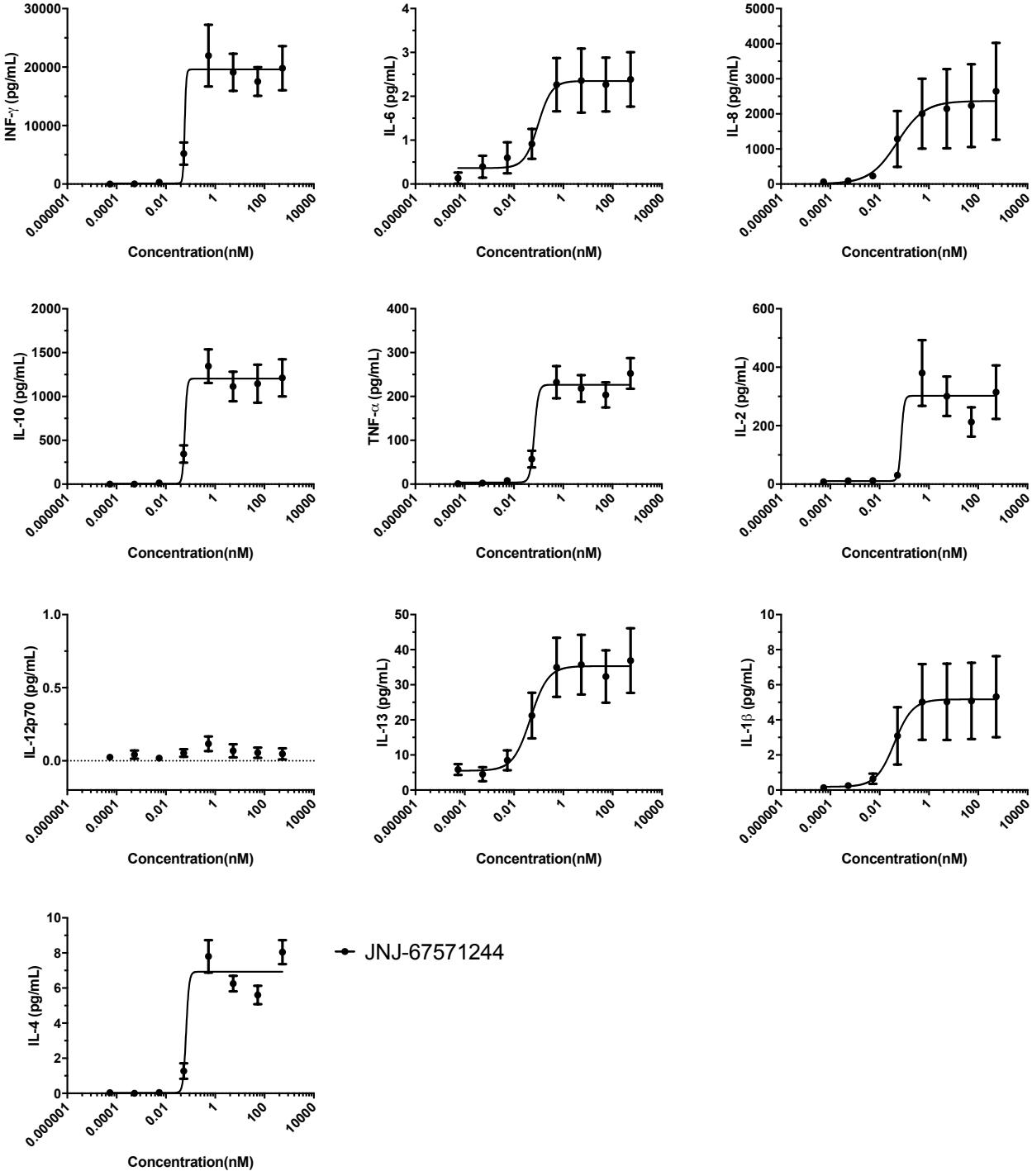

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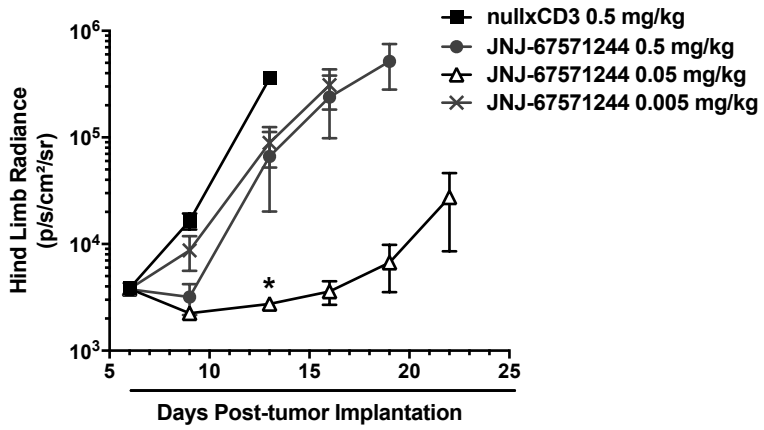

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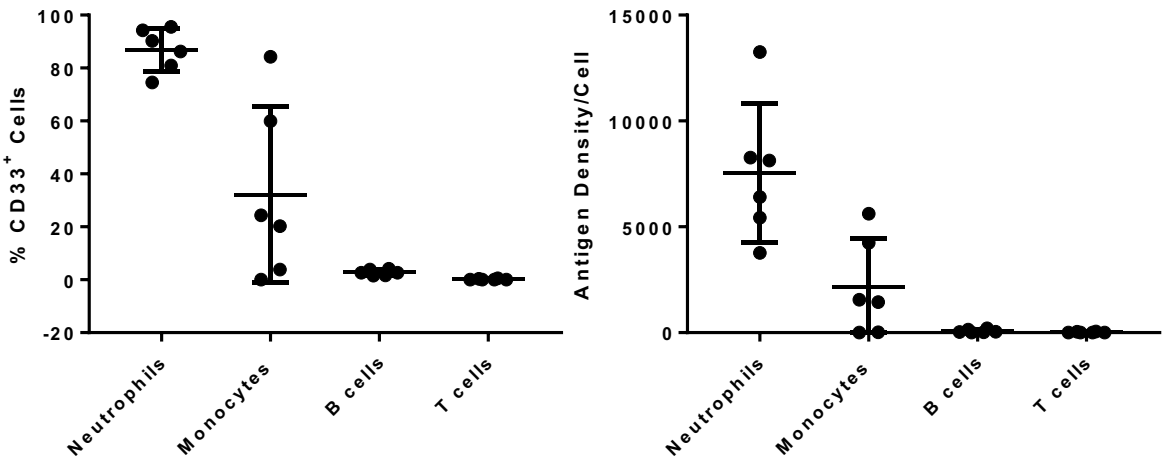
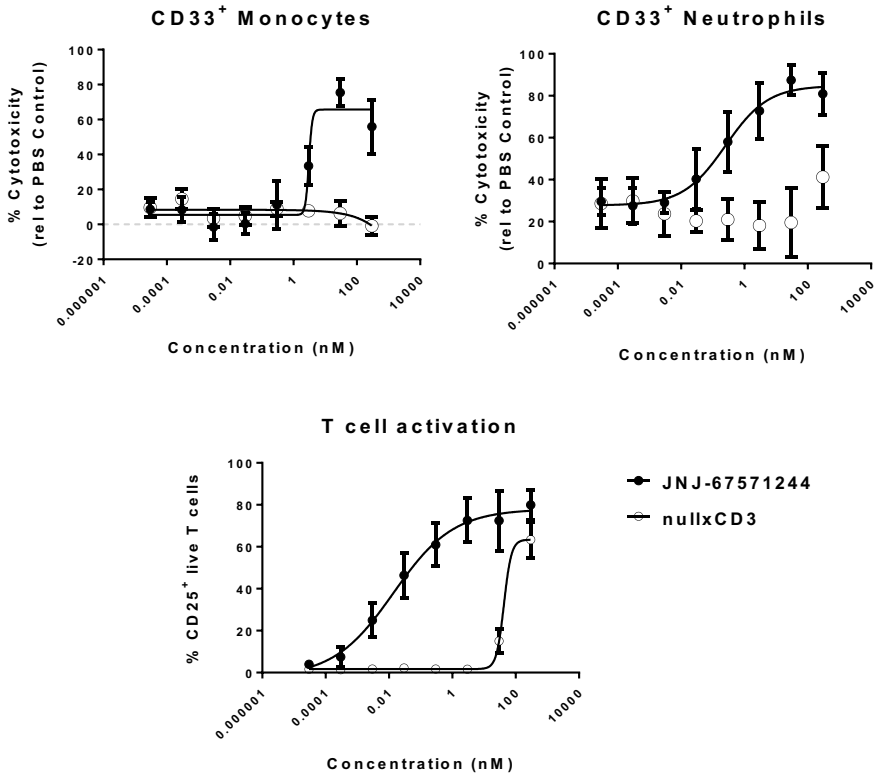
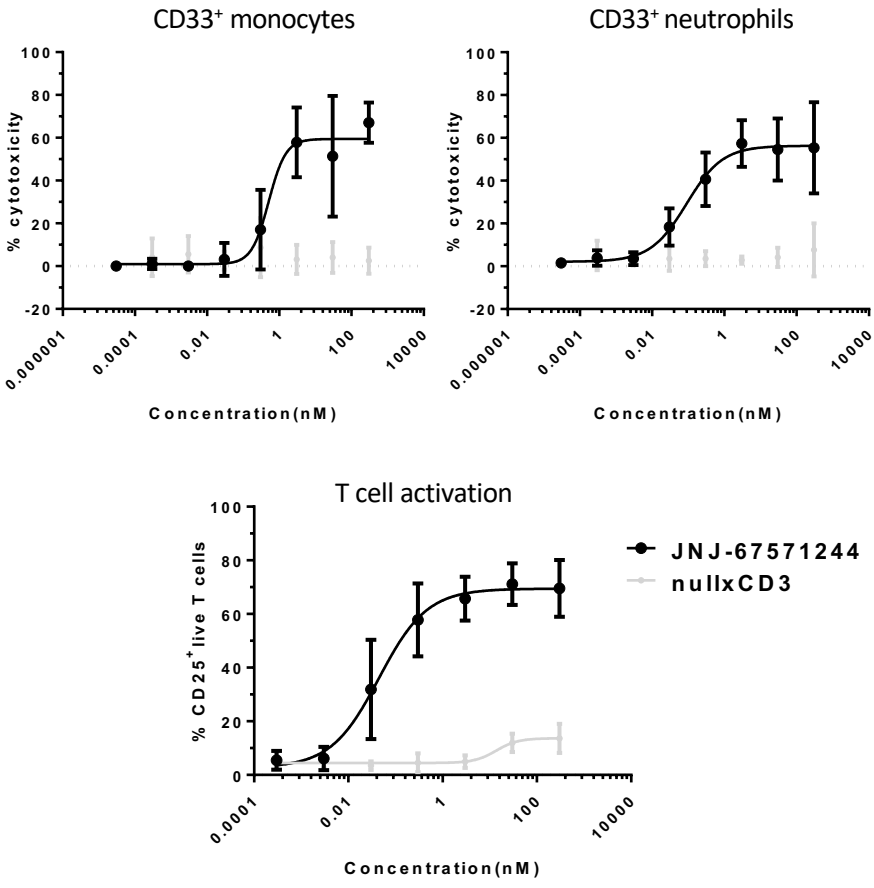


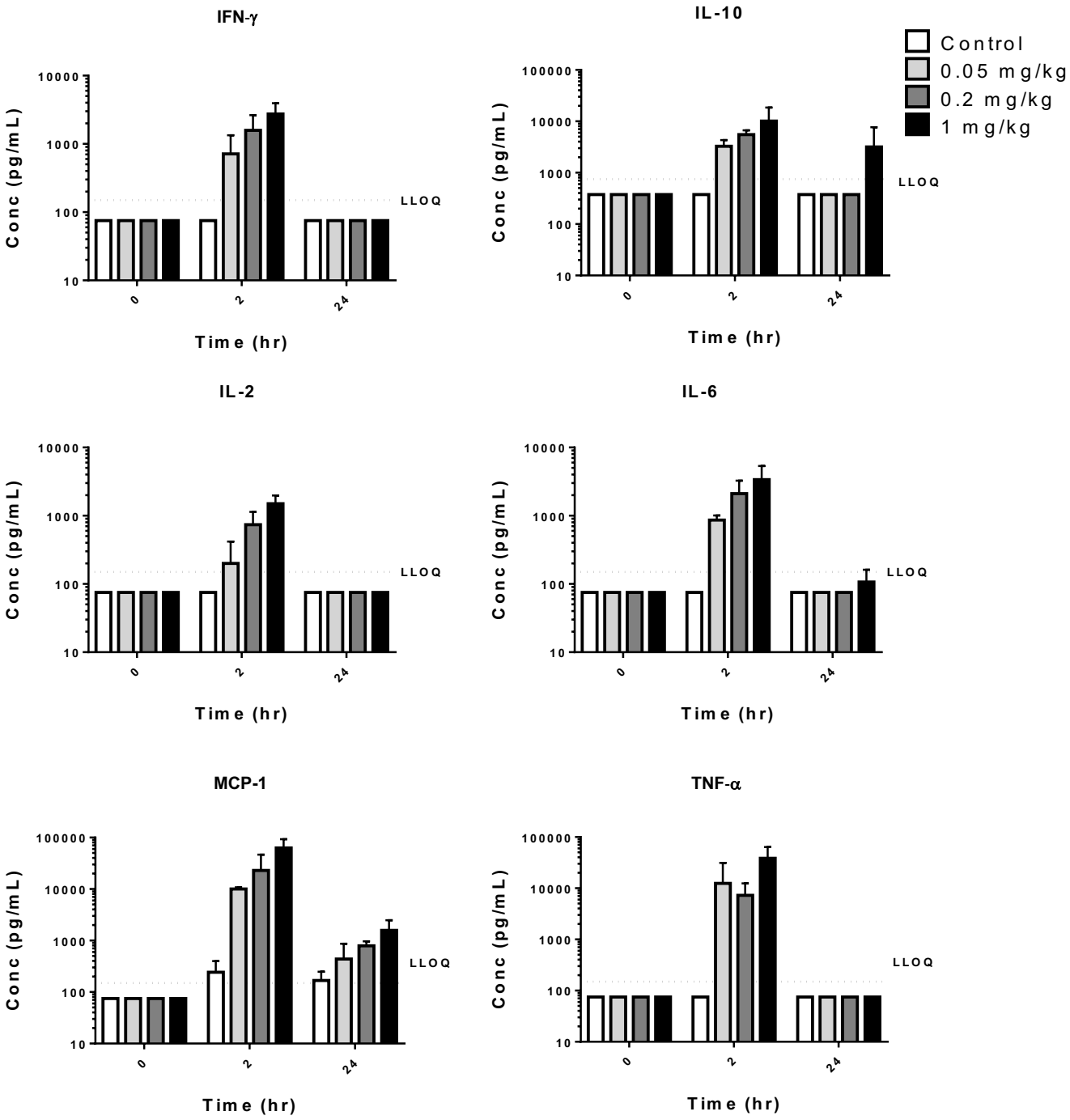
Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8