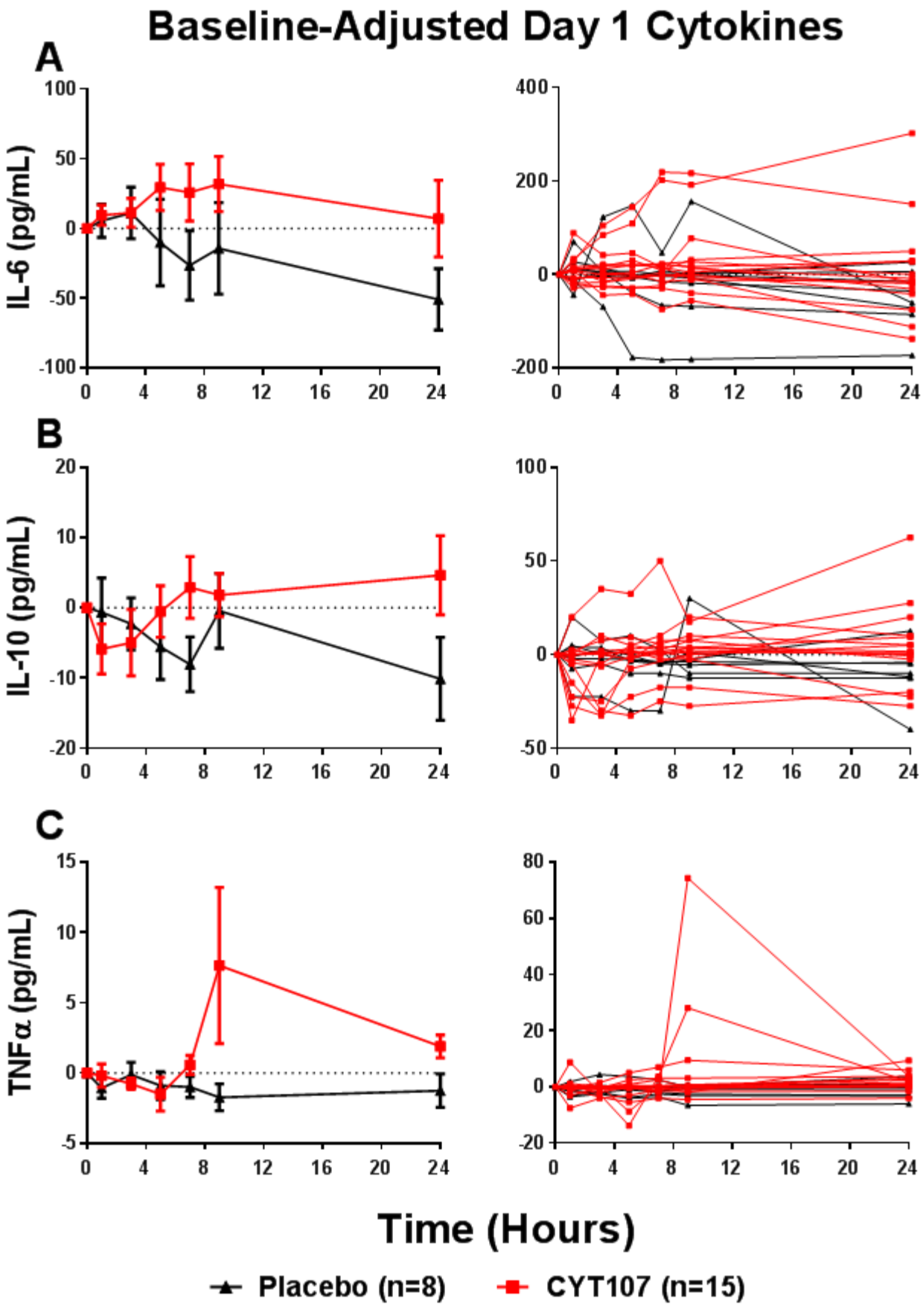
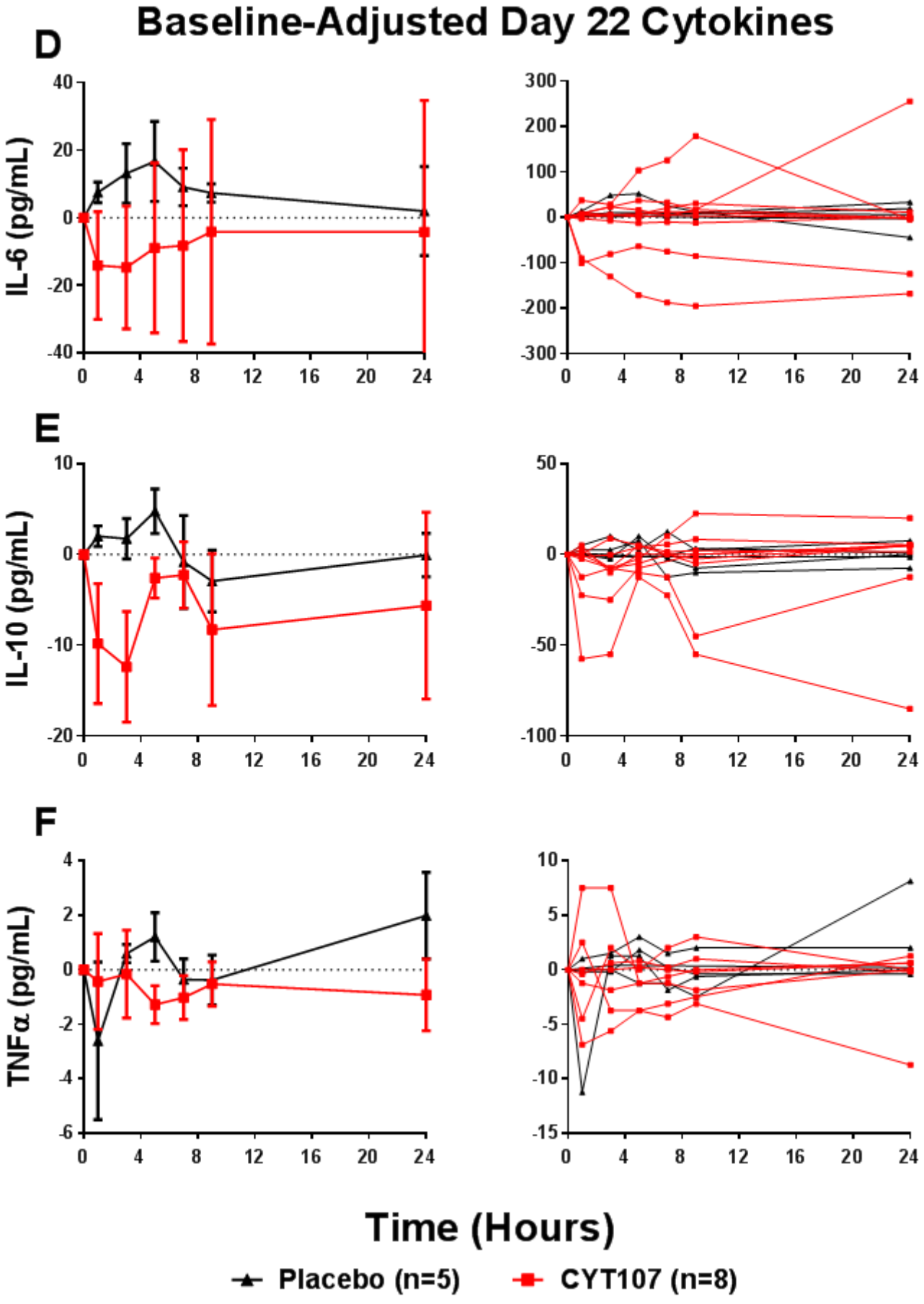
Supplemental Figures

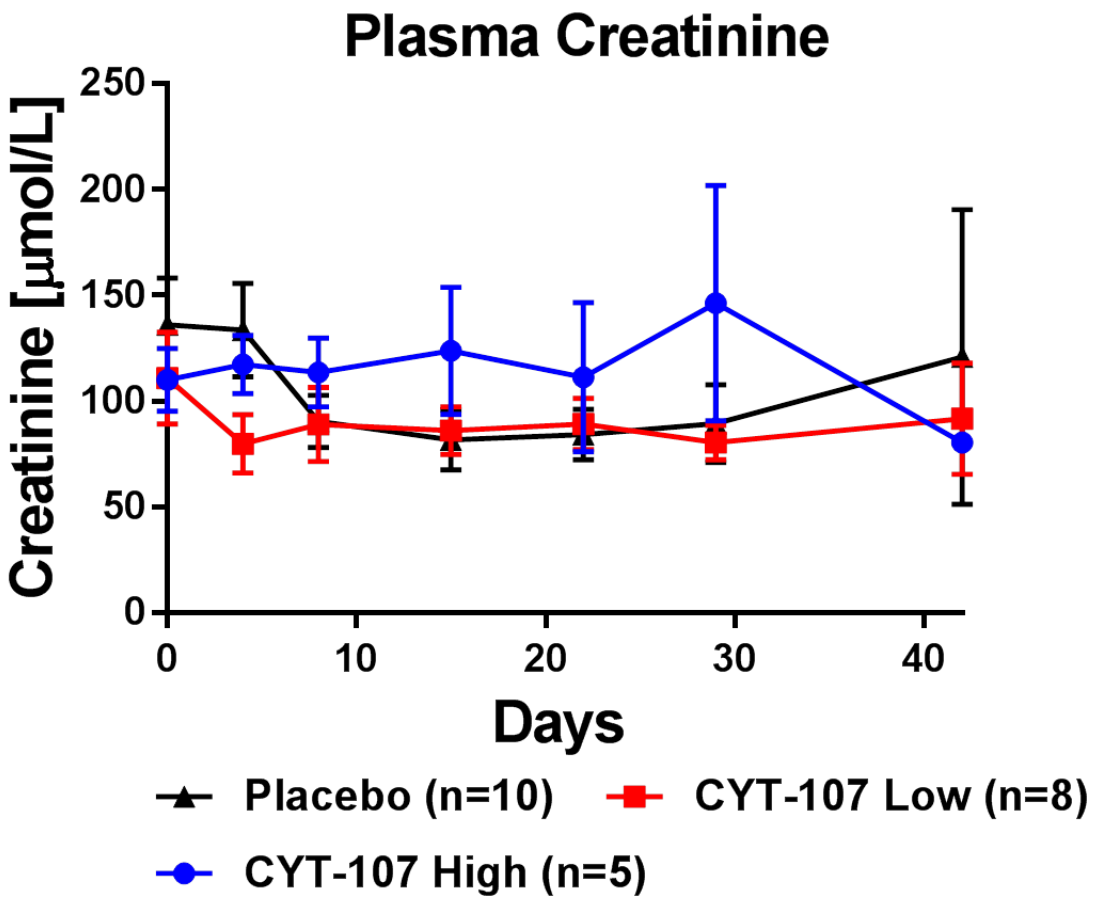
Supplemental Fig. 1



Supplemental Fig. 1

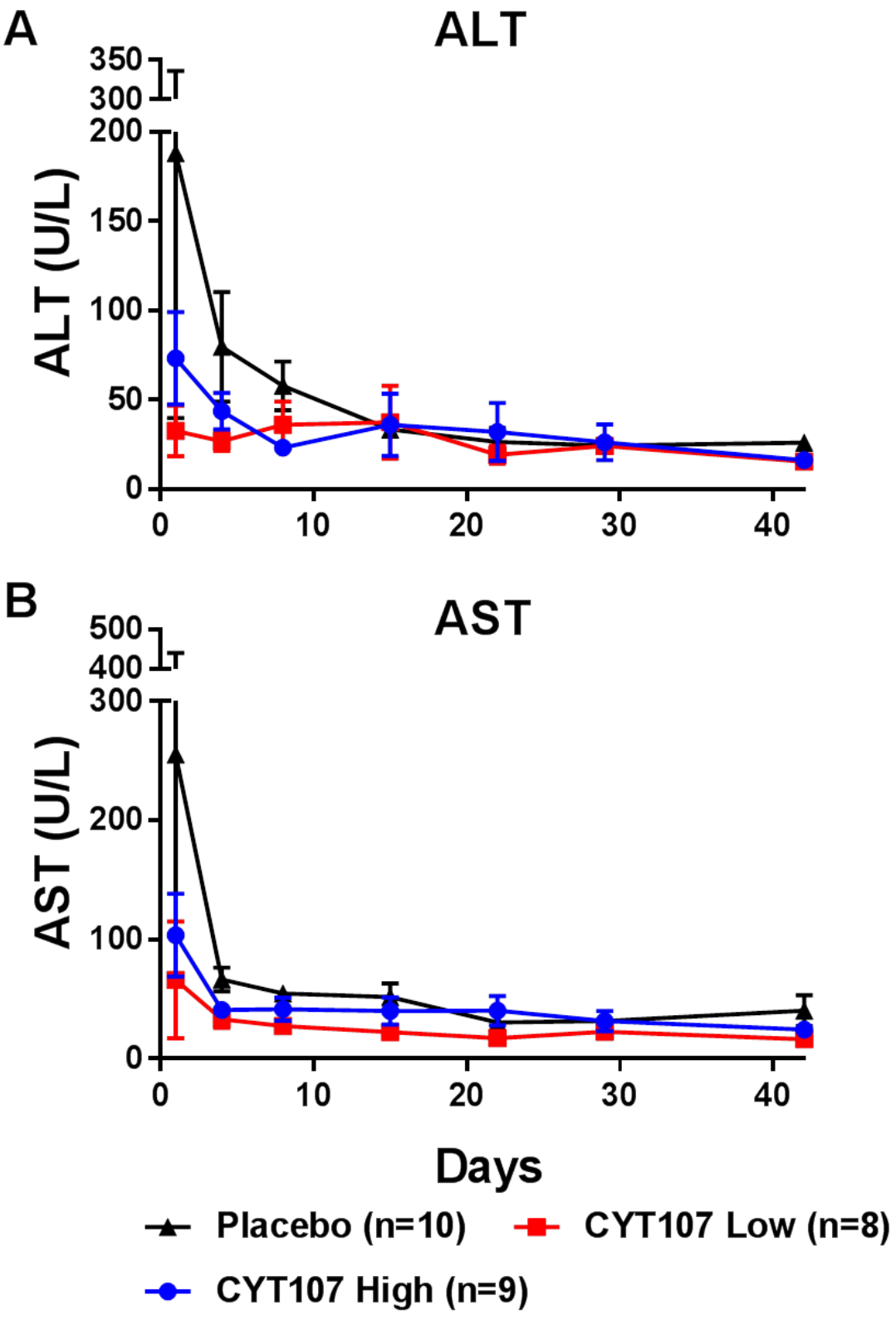


Supplemental Figure 2



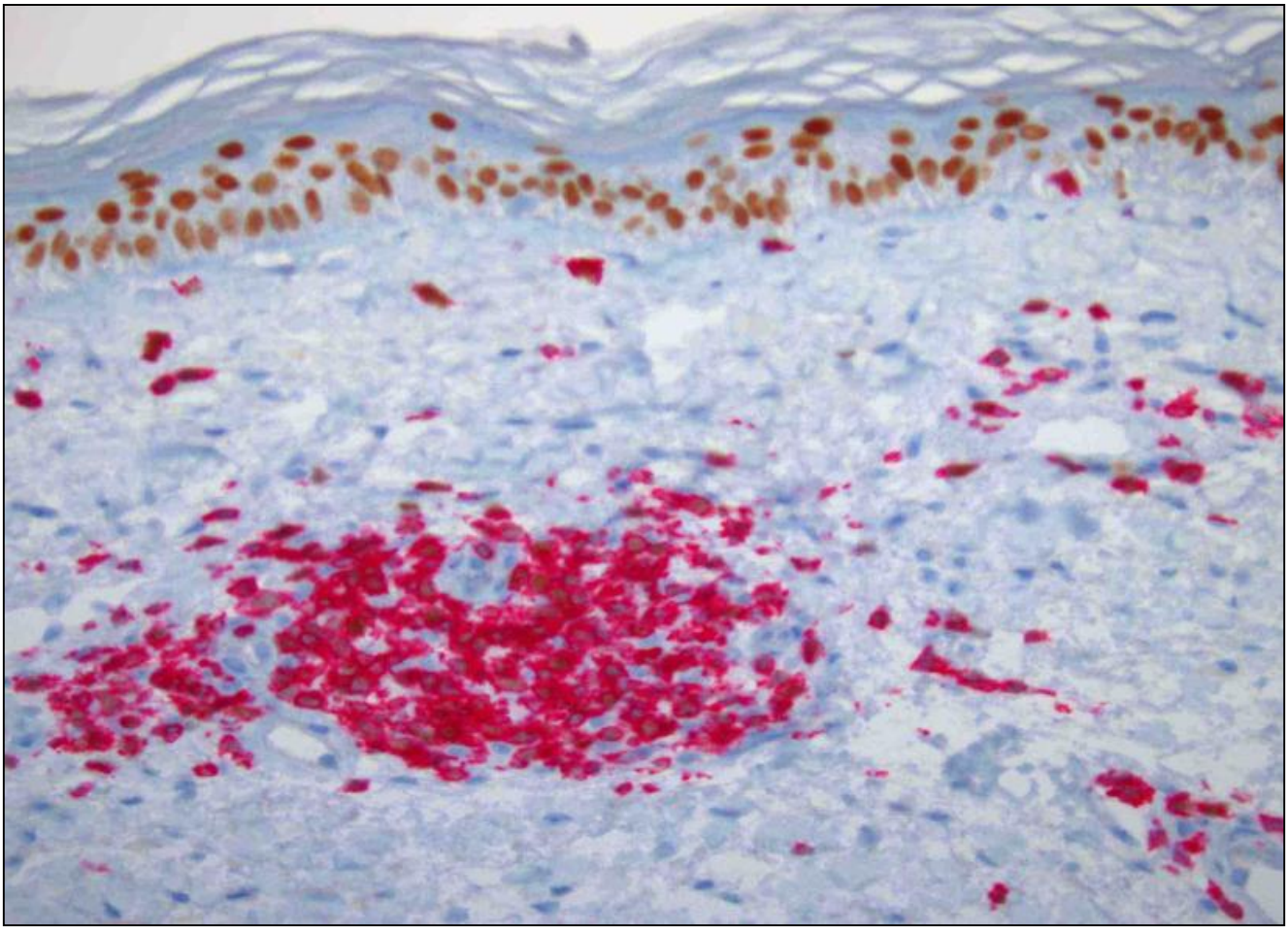
* Patients who were on renal replacement therapy were not included because of the impact of dialysis on serum creatinine.

Supplemental Figure 3. Liver Function Tests

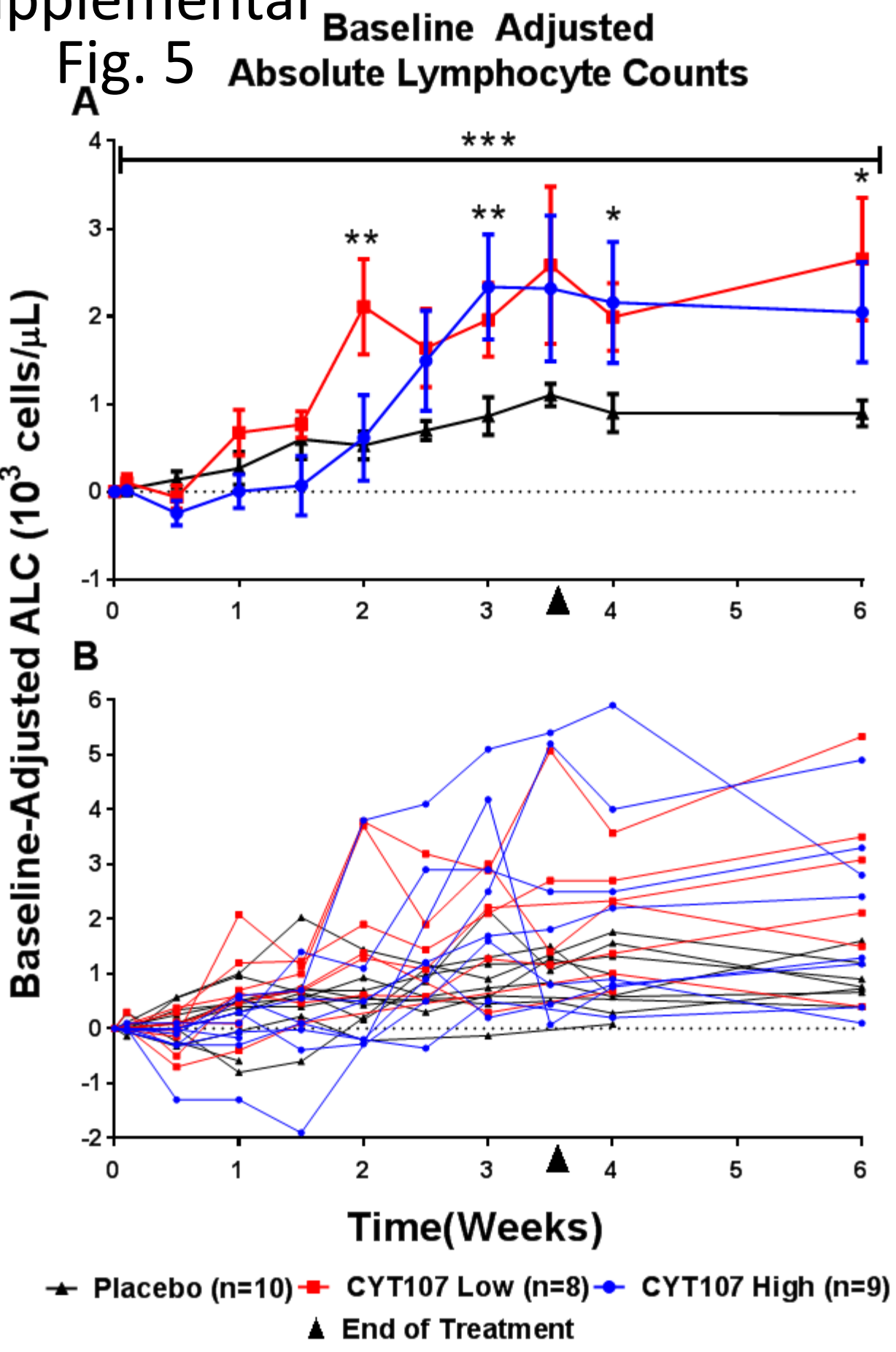


Supplemental Figure 4.

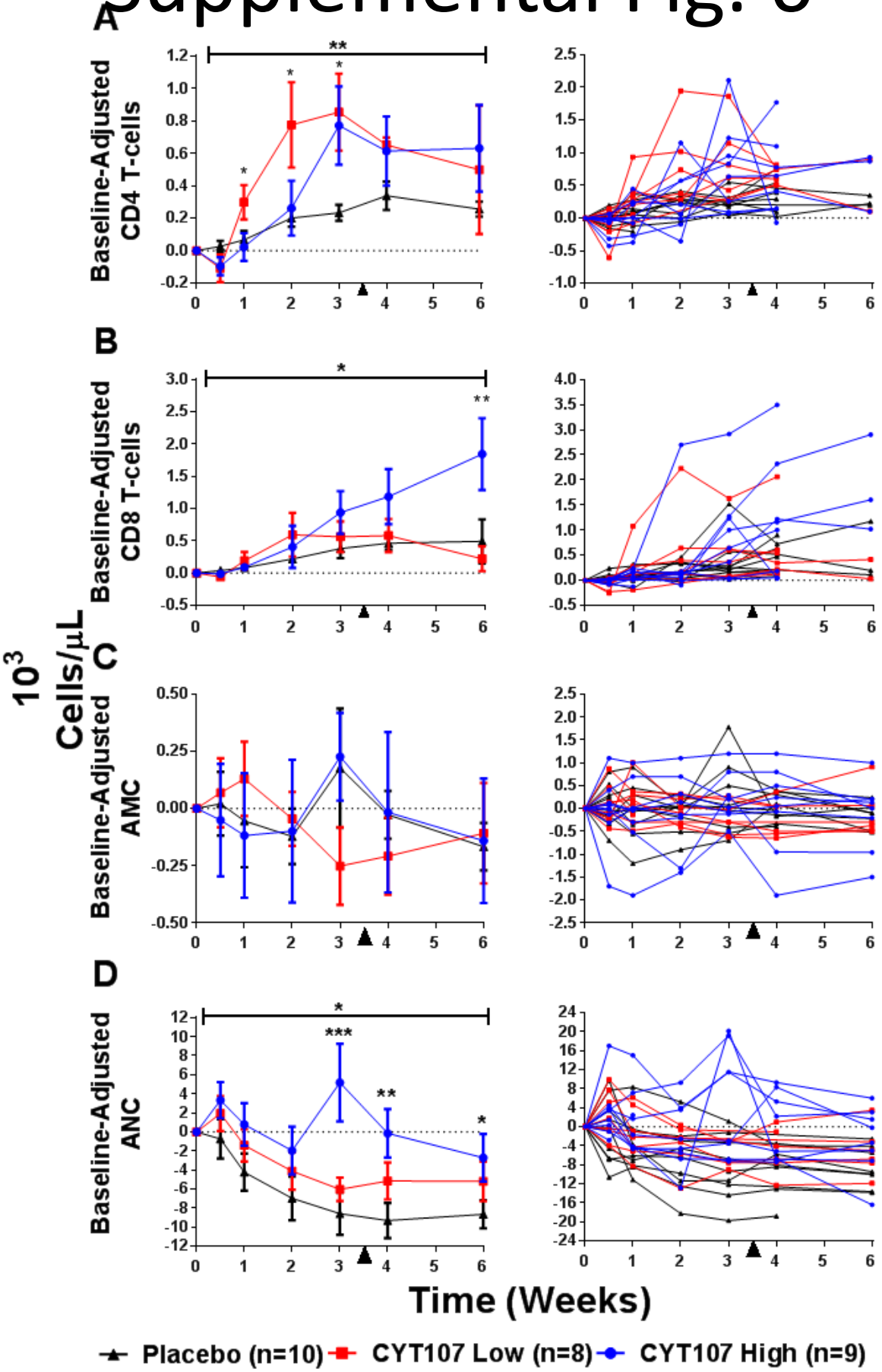
Immunohistochemistry of Skin Biopsy



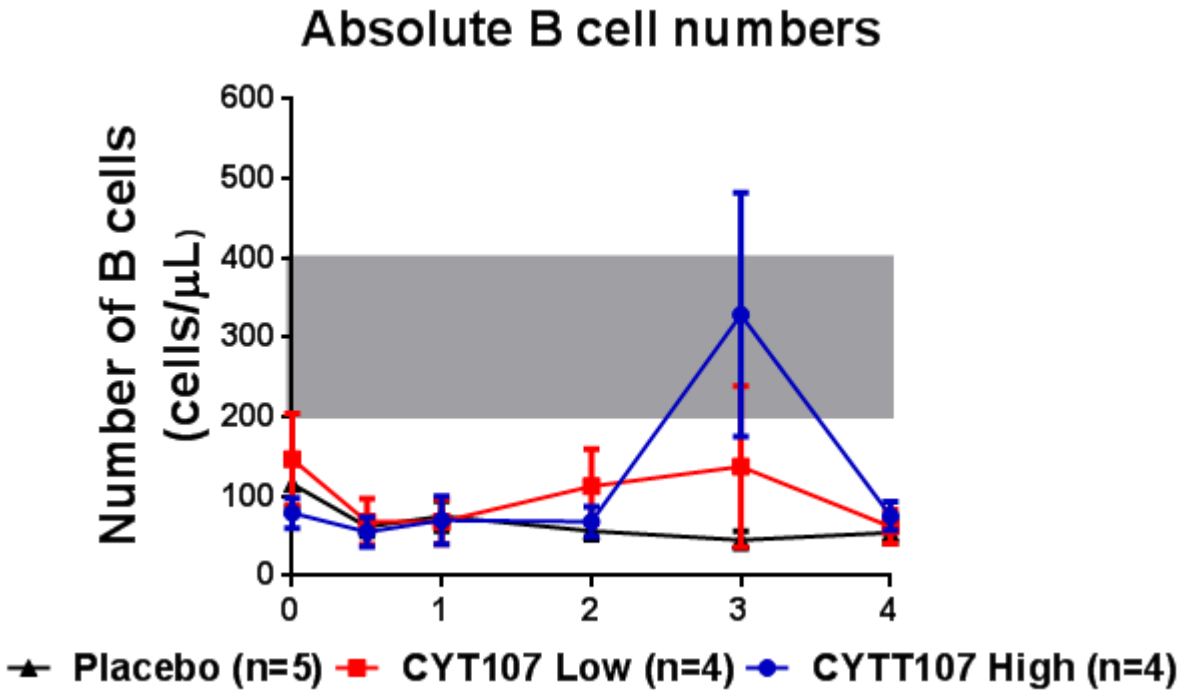
Supplemental Fig. 5



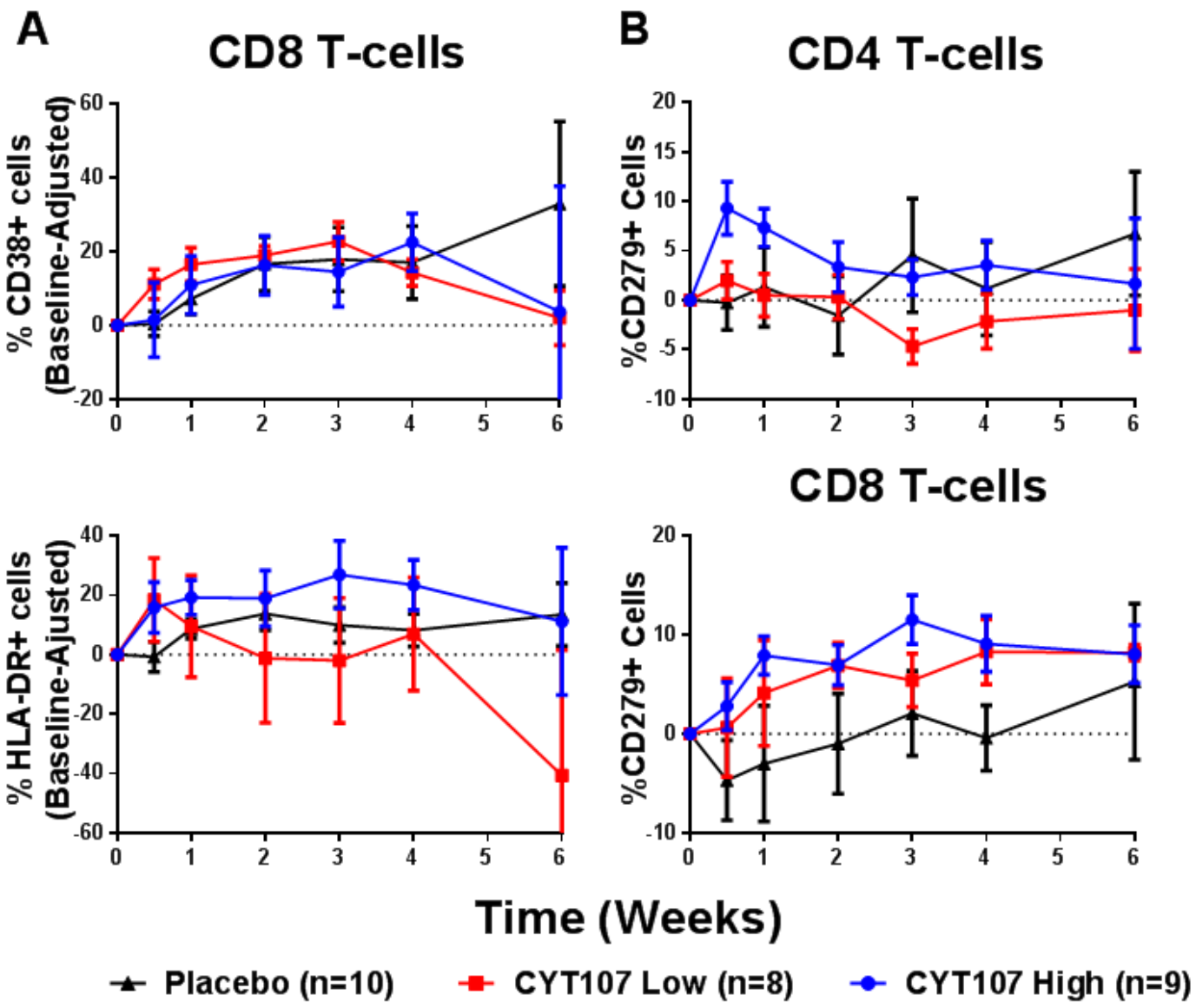
Supplemental Fig. 6



Supplemental Figure 7.

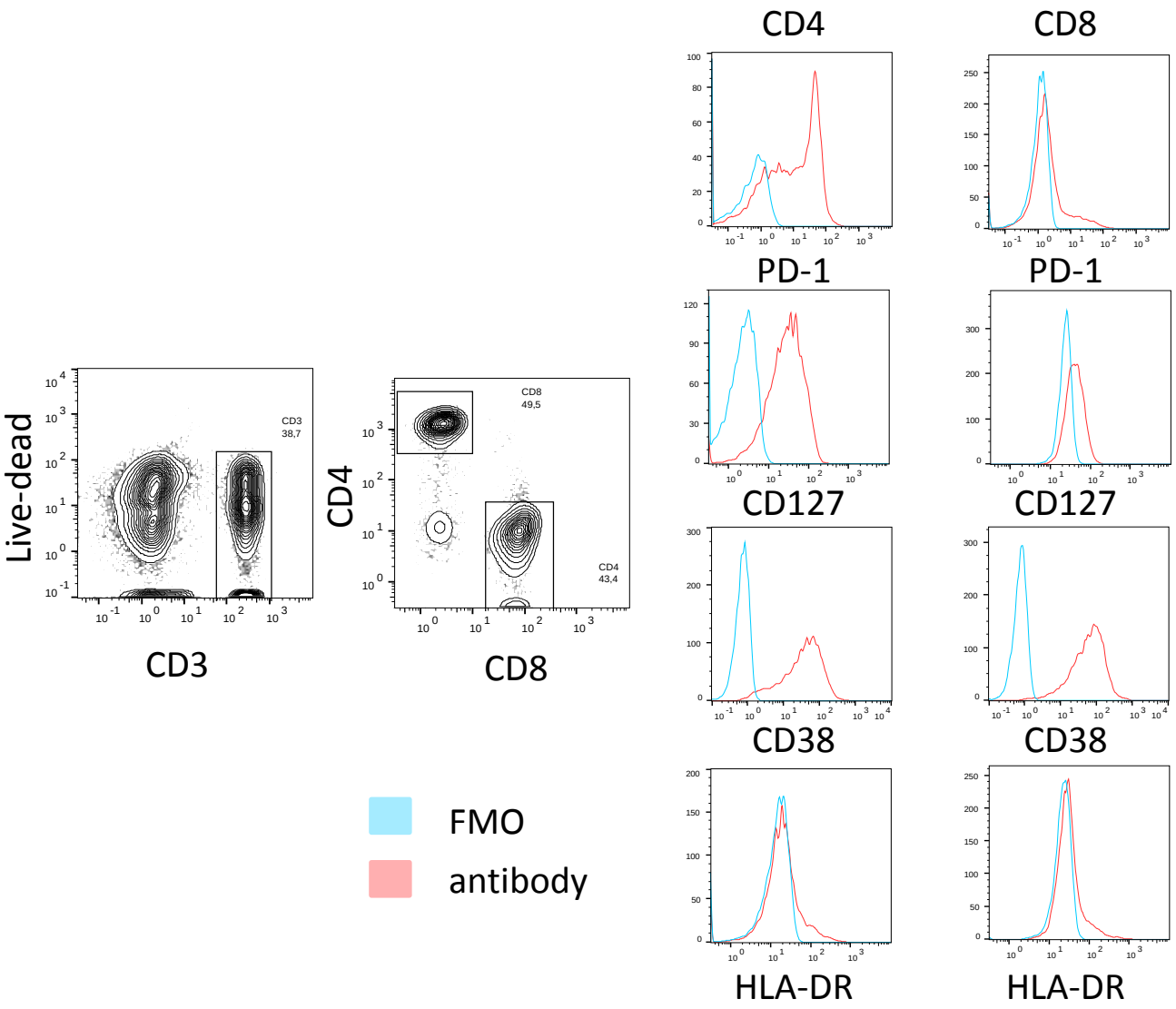


Supplemental Figure 8.

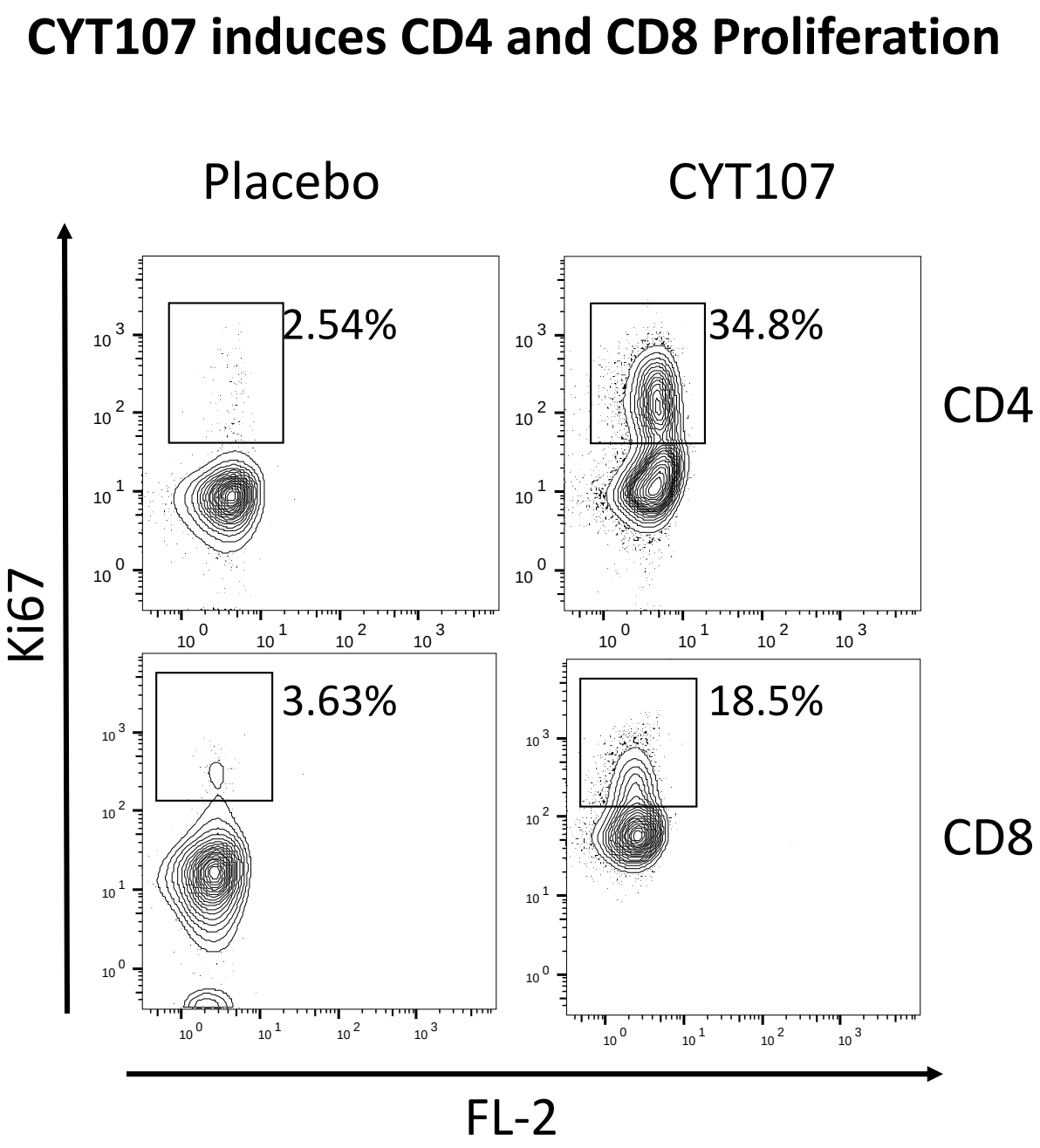


Supplemental Figure 9

Flow Cytometry Gating Strategy for Cell Surface Markers



Supplemental Figure 10



Supplemental Figures

Supplemental Figure 1. Effect of CYT107 on circulating cytokines. The effect of CYT107 versus placebo on the pro-inflammatory cytokines IL-6 (**A**, **D**) and TNF-α (**C**, **F**) and the anti-inflammatory cytokine IL-10 (**B**, **E**) are presented as the averaged values for the two treatment groups (placebo and CYT107, left hand panels) and for each individual patient (right hand panels). Cytokines were measured just prior to dosing of CYT107 or placebo and at 1, 3, 5, 7, 9, and 24 hours after dosing on the initial day of treatment (**A**, **B**, **C**; 15 patients received CYT107, and 7 received placebo). Cytokines were measured again on Day 22 after repeat dosing of CYT107 or placebo (**D**, **E**, **F**; 8 patients received CYT107, and 5 received placebo) . Baseline-adjustment reflects the difference between the post dosing value (i.e. 1 hour, 3 hour, etc) and the predosing value (i.e. time point 0). Samples from patients at the Vanderbilt and Lyons sites were unavailable for analysis. Day 22 samples were not collected from patients whose treatment was stopped prior to dosing on that day. Values depicted for the groups are mean ± SEM.

Supplemental Figure. 2. Serial creatinine values were not different in placebo and CYT107 patients. The potential impact of CYT107 on renal function was assessed by serial creatinine determinations. Note that the creatinine did not increase or vary in the different treatment groups. Patients who were on renal replacement therapy (dialysis) were not included because their serum creatinine values are not indicative of renal function as they are impacted by dialysis. Values reported are mean ± SEM.

Supplemental Figure. 3. Serial aspartate aminotransferase (AST) and alanine transaminase (ALT) were not different in placebo and CYT107 patients. The potential impact of CYT107 on liver function was assessed by serial quantitation of AST and ALT. Note that there no differences in AST or ALT in the low or high frequency regimen CYT107 vs placebo treated patients. Values reported are mean ± SEM.

Supplemental Figure 4. CYT107 caused rash formation with increased infiltration of $CD3^+$ T cells. Injection site reactions are common with CYT107 administration by the subcutaneous or intramuscular method. Six patients developed a grade 2-3 skin reaction and 2 patients had biopsies by the Dermatology Consult service with immunohistochemical (IHC) staining. IHC staining was positive for CD3+ T cells. All rashes resolved following completion of CYT107 therapy.

Supplemental Figure 5. Baseline adjusted absolute lymphocyte counts. (**A**) The effect of CYT107 on the baseline adjusted absolute lymphocyte counts (ALC) for the 3 treatment groups are presented. For baseline adjustment, the starting ALCs for each patient are set at zero and the change in the ALC in cells/μL is recorded. CYT107 low frequency group (red color) was greater than placebo-treated at days 15** and 42*, and CYT107 high frequency group (blue color) was greater than placebo-treated at Days 22** and 29*. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical tests were conducted using a Wald-type multiple-degree-of-freedom method as described in the method section. Dark arrowhead represents the last day of treatment with CYT107. Values reported are mean ± SEM. N = 10, 8, and 9 for placebo, low frequency CYT107, and high frequency CYT107 treated patients respectively. (**B**) The CYT107 treatment

effect on baseline adjusted absolute lymphocyte counts is displayed for each individual patient over the entire duration of the study. Dark arrowhead represents last day of treatment with CYT107. N = 10, 8, and 9 for placebo, low frequency CYT107, and high frequency CYT107 treated patients respectively. Baseline-adjustment reflects the difference between study day values and the pre-dosing values

Supplemental Figure 6. Baseline adjusted CD4, CD8, absolute monocyte, and absolute neutrophil counts. (A) Compared to placebo treatment (black color), CYT107 low frequency regimen (red color) significantly increased CD4 T cells at days 8, 15, and 42 as well as having an overall treatment effect. Asterisks (*) are as in Figure 3. (B) CYT107 high frequency regimen (blue color) was associated with increased CD8 T cell counts at day 42. Asterisks (*) are as in Figure 3 (C) Compared to placebo, neither high nor low frequency dosing regimens of CYT107 had an effect on the absolute monocyte cell counts (AMCs). (**D**) Compared to placebo (black color), there was an overall treatment effect on ANC. Further there was an effect of high frequency CYT107 to increase ANCs at week 3, 4, and 6. At week 6 there was also a very modest but significant effect to increase ANC in patients receiving low frequency CYT107. Asterisks (*) are as in Figure 5. Values reported are mean ± SEM. N = 10, 8, and 9 for placebo, low frequency CYT107, and high frequency CYT107 treated patients respectively. The left hand graphs show results of the average values for each of the 3 treatment groups while the right hand graphs show results for each individual patient. Baseline-adjustment reflects the difference between study day values and the pre-dosing values.

Supplemental Figure 7. Effect of CYT107 on absolute B cell counts. Absolute number of B cells in circulating peripheral blood from septic patients treated with placebo (black color), low frequency CYT107 (red color), or high frequency CYT107 (blue color). Other than a small transient blip at day 21 in the high frequency group only, CYT107 had no consistent effect to increase B cell numbers. N = 5, 4, and 4 for the number of patients in the placebo treated, low frequency, and high frequency CYT107 groups respectively. Grey shaded area represents the range for normal B cell counts in healthy individuals.

Supplemental Figure 8. CYT107 did not increase expression of activation markers in CD8 T cells or programmed cell death 1 (PD-1; CD279) in T cells. (4A) There was no evidence of increased expression of activation markers CD 38 or HLA-DR on the surface of CD8 T cells in patients treated with either high or low frequency dosing regimens of CYT-107. *(4B*) PD-1 is a marker of T cell exhaustion. Neither high nor low frequency dosing regimens for CYT107 had any impact on expression on CD4 or CD8 T cells.

Supplemental Figure 9. Flow cytometry gating strategy for cell surface markers. -Flow events were initially gated for CD3 positivity. CD3+ cells were then analyzed for CD4 and CD8 positivity, yielding a CD3+/CD4+ population and a CD3+CD8+ population. These populations were used as a parent population for determining % positivity for PD-1, CD127, CD38, and HLA-DR. The gating control was a modified Fluorescence Minus One (FMO) staining tube which included Live/Dead Yellow, CD3, CD4, and CD8 antibodies. All antibodies were titrated and the modified FMO was validated against true FMOs for each marker.

Supplemental Figure 10. Representative flow cytometry histogram showing effect of CYT107 to increase cell proliferation. Blood samples were obtained from patients on day 8 following initiation of therapy with CYT107 or placebo. Cells were stained for CD4 or CD8 followed by labeling with Ki67, a marker of cell proliferation. Note the marked increase in the percentage of CD4 and CD8 T cells in CYT107 vs placebo treated patients.

SUPPLEMENTARY MATERIAL AND METHODS

Inclusion criteria: Inclusion criteria were as follows: patients must be between the ages of 18 and 80, have two or more criteria for systemic inflammatory response syndrome (SIRS), have a clinically or microbiologically suspected infection, a SOFA score ≥2 at 48-120 hours after admission to the ICU, require vasopressor treatment, an absolute lymphocyte count ≤900 cells/μL within 24 hours of a signed informed consent from the patient or legal authorized representative, and a predicted length of stay in the ICU of two weeks after starting drug therapy treatment. The criteria for defining septic shock were defined by the 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference (38).

Exclusion criteria: Exclusion criteria were as follows: patients currently undergoing chemo- or radiotherapy because of cancer, current or a history of hematologic malignancy or lymphoma, cardiopulmonary resuscitation within 4 weeks, history of or current evidence of an autoimmune disorder, organ transplant recipient, HIV/AIDS, hepatitis B or hepatitis C; receiving corticosteroids at a dose greater than the equivalent of 300 mgs of hydrocortisone per day, receiving biologics that block or inhibit TNF-α or other cytokines (See supplemental methods for expanded description of inclusion and exclusion criteria).

Detailed Inclusion Criteria:

1) Patients of age ≥ 18 yrs to 80 yrs

2) Patients with persistent suspected sepsis as defined by a consensus conference (Reference #38) at 48-120 hrs after admission a

3) Two or more criteria for the systemic inflammatory response syndrome (SIRS) (see reference for SIRS criteria) and a clinically or microbiologically suspected infection.

4) At least one organ failure as defined by a SOFA score of ≥2 at any time point during the 48-120 hrs after admission to the ICU

5) Requirement of vasopressor treatment as follows: i) epinephrine or norepinephrine at ≥ 0.05 μg/kg/min ideal body weight; ii) vasopressin, or iii) dopamine at ≥ 4-5 μg/kg/min ideal body weight, continuously for 4 hrs or more, provided that at least 20 ml/kg of ideal body weight of crystalloid or an equivalent volume of colloid was administered during the 24-hour interval surrounding the start of vasopressor treatment, to maintain systolic pressure ≥ 90 mmHg or a mean arterial pressure ≥ 60 mmHg at any time point during their sepsis course preceding enrollment into the CYT107 study.

6) Lymphopenia with an absolute lymphocyte count ≤ 900 cells/μL at either the day of consent or the day prior to consent during their ICU stay. The level of 900 lymphocyte/ μL was

chosen because it is below the lower limit of normal for most hospital laboratories (Mayo Clinic Medical Laboratories) and because of studies showing that patients with absolute lymphocyte counts approximating this level of lymphopenia have higher mortality (5).

7) Predicted length of stay in the ICU of up to two weeks after starting drug therapy treatment in the trial (ICU may also include a close medical ward on the same study site where the patient will remain under medical control of the Investigator).
8) Ability to obtain a signed informed consent from patient or LAR consent.

Detailed Exclusion Criteria:

1) Cancer with current chemotherapy or radiotherapy and/or .receipt of chemotherapy or radiotherapy within the last 6 weeks. All patients with current, or history of, hematologic malignancy (including, but not limited to, ALL, AML, CLL, CML, etc.) or lymphoma will be excluded, regardless of receipt of recent chemotherapy.

2) Cardiopulmonary resuscitation within the previous 4 weeks without objective evidence of full neurologic recovery) or patients who have minimal chance of survival and are not expected to live > 3-5 days as defined by an APACHE II score of ≥ 35 at time of consideration for study eligibility

3) Patients with a history of or who currently have evidence of autoimmune disease including for example: myasthenia gravis, Guillain Barre syndrome, systemic lupus erythematosis, multiple sclerosis, scleroderma, ulcerative colitis, Crohn's disease, autoimmune hepatitis, Wegener's etc.

4) Patients who have received solid organ transplant or bone marrow transplant

5) Patients with active or a history of acute or chronic lymphocytic leukemia

6) AIDS-defining illness (category C) diagnosed within the last 12 months prior to study entry

7) History of splenectomy

8) Any hematologic disease associated with hypersplenism, such as thalassemia, hereditary spherocytosis, Gaucher's Disease, and autoimmune hemolytic anemia

9) Pregnant or lactating women

10) Participation in another investigational interventional study within the last 6 months prior to study entry, with the exception of studies aimed at testing sedation products belonging to standard of care such as Propofol, Dexmedetomidine, Midazolam.

11) Patients receiving immunosuppressive drugs, e.g., TNF-alpha inhibitors, for rheumatoid arthritis, inflammatory bowel disease or any other reason, or systemic corticosteroids other than hydrocortisone at a dose of ≤ 300 mg/day

12) Patients receiving concurrent immunotherapy or biologic agents including: growth factors, cytokines and interleukins, (other than the study medication); for example IL-2,growth factors, interferons, HIV vaccines, immunosuppressive drugs, hydroxyurea, immunoglobulins, adoptive cell therapy

13) Prisoners

Study Deviations: IL-7 administration was discontinued on 4 patients due to a grade 2-3 rash formation at injection site (see Results section on patient safety). Study enrollment was halted at 27 patients out of a planned enrollment of 30 patients because of requirement for additional drug stability testing that was due at this time.

Thirty three patients were enrolled into the study but only 27 patients were dosed with drugs (Fig. 1). Three patients who were enrolled were not dosed because of marked clinical deterioration with likely impending demise occurring between the time of enrollment and the time of planned administration of drug (CYT107 or placebo). Two patients who consented were not dosed because of rapid clinical improvement within 24-48 hrs with resolution of signs of sepsis and SOFA scores <2. One patient who was consented was not dosed because of discovery of an exclusion criterion which had been missed earlier.

Immunogenicity for detection of antibodies to CYT107

Results obtained from the analysis of clinical samples were within the limits of acceptance defined in the bioanalytical protocol and therefore validated the detection of the anti-CYT107 antibodies in the human plasma samples. Immunogenicity studies for detection of antibodies against the recombinant human CYT107 employed in this trial were performed at days 0, 11, 22, and 60 for all seventeen CYT107-treated patients and were negative for antibody detection.

Since no antibodies were detected on day 60 in any patient, further testing at days 120 and 365 were not indicated and were not conducted.

CYT107 preparation and drug dosing: A recombinant human IL-7 preparation (CYT107) that is expressed in Chinese Hamster Ovary cell line and which has been extensively used in other clinical trials was employed (30, 31). Patients were randomized by allocation to a permuted-block randomization (block size of 3 and allocation ratio of 1:1:1, stratified by country: US and France), and dosed using three different administration protocols: CYT107 high frequency, CYT107 low frequency, and placebo. CYT107 or placebo was administered by intramuscular injection. If the patient's platelet count was less than 35,000 per mm^3 or the international normalized ratio greater than 3.5 seconds, the drug was administered by subcutaneous injection. All patients were injected twice per week for 4 weeks. All CYT107 patients received 10 µg/kg of CYT107 twice in the first week, followed by: the same dosage, twice per week for the high frequency regimen, and once per week followed by an injection of placebo (NaCl 0.9%) midweek for the low frequency. The control, or placebo group were given placebo (NaCl 0.9%) twice per week. On site, all study staff but the pharmacist were blinded to randomization group.

Patients were allocated to CYT107 high frequency, low frequency or placebo (NaCl 0.9%) according to a randomization table prepared by the statistician. CYT107 is a clear solution fully similar to saline. Patient drug treatments were prepared by hospital pharmacists based on the randomization table provided by the statistician. Blinded prefilled syringes were delivered to the ICU for administration. The statistician, local pharmacist and the central laboratory in charge of PK / immunogenicity analysis were the only unblinded entities.

Blood Samples were drawn for flow cytometric analysis prior to the first dose (Day 0), following dosing on days 4, 8, 15, and 22, and then on days 29 and 42. PK samples were drawn on the initial dosing day and on day 22: The first sample was obtained immediately prior to dosing, and subsequent samples were obtained 1, 3, 5, 7, 9, and 24 hours after dosing. Immunogenicity samples were collected prior to initial dosing (Day 0), and then on days 11, 22, and 60, with plans to draw on days 180 and 365 if any of the initial 4 samples showed positive results.

Flow Cytometry: Flow Cytometric analysis was performed as previously described (28, 46). Briefly, blinded laboratory staff stained whole blood with one of two cocktails, and then lysed using Red Blood Cell Lysis Buffer (BioLegend). Cells stained with cocktails examining only surface markers were then fixed in 1% Paraformaldehyde, while cells to be stained for intracellular markers were first surface stained, then permeabilized with eBioscience Fixation/Permeabilization reagent (Affymetrix), and then stained for intracellular markers (Ki67). Acquisitions were analyzed with FlowJo v 10.2(FlowJo, LLC). Stains used were CD3, CD4, CD8, CD279, HLADR, CD127, CD38, and Ki-67 (BioLegend), and Live/Dead Yellow (ThermoFisher). Values derived from FlowJo were plotted in Prism Graphpad (Graphpad

Software). Molecules of equivalent soluble fluorochrome (MESF) were calculated for CD279, CD127, and Ki-67 (See below).

Number of CD4 and CD8 T-cells: CD4 and CD8 numbers were calculated using flow cytometric results. First, the lymphocytes were gated on FSC x SSC, and then the percentage of CD3+CD4+ cells and CD3+CD8+ cells were determined (Supplemental Figure 5 provides gating strategy). The frequency of parent percentages were multiplied by Absolute Lymphocyte Count and the product was used as the number of CD4 and CD8 T-cells respectively.

Molecules of equivalent soluble fluorochrome (MESF): MESF was calculated by obtaining population's GMFI and plotting it against values derived from Quantum PE (monocyte HLA-DR, CD279) or Quantum APC (Ki-67, CD127) beads (Bangs Lab) in Microsoft Excel.

Quantitation of circulating cytokines IL-6, IL-10, and TNF-α via ELISA: Cytokine kits for TNF-α, IL6, and IL-10 were obtained from Biolegend and R&D Systems – see Supplemental Materials and Methods. Assays were performed exactly as per the manufacturer's instructions and as previously described (46).

Pharmacokinetic (PK) and immunogenicity analysis: PK and immunogenicity testing were carried out by Eurofins ADME BIOANALYSES after validation of both methods.

For Pharmacokinetic analysis CYT107 (CYT107) was measured in EDTA-3K plasma samples using a validated DIACLONE ELISA kit (851.680.020). The method includes a capture CYT107 antibody and a biotinylated detection antibody. The results obtained during the validation studies are reported in an Eurofins full bioanalytical report.

The pharmacokinetic parameters were calculated with the KINETICA program (Version 4.3 - Thermo Electron Corporation - Philadelphia - USA). A model independent method (non-compartmental analysis) was used.

For immunogenicity testing, anti-CYT107 antibodies were detected in heparin-Li plasma samples. Briefly the ability of plasma samples to bind CYT107 are compared with a positive anti-rh-CYT107 IgG (Preprotech) in a preliminary screening test aimed at detecting anti-CYT107 binding antibodies. Positive samples from this screening assay are then spiked with CYT107 to displace and detect the specificity of the binding activity. Only positive samples from this confirmatory assay contain anti-CYT107 antibodies and should be used for immunogenicity assessment.

Statistical Analysis: Comparisons across treatment groups at baseline were performed using the Kruskal-Wallis test and Fisher exact test. Each outcome variable (e.g., absolute lymphocyte count) was baseline adjusted by subtracting the corresponding baseline value. Baseline adjusted outcomes were then analyzed using linear mixed-effects regression, adjusting for study day (as a categorical variable), treatment group, and the interaction between study day and treatment

group. A random intercept indexed by study subject was used to account for within-subject correlation among longitudinal measurements. The error variance was allowed to vary by study day to account for heteroscedasticity. The combined treatment effect across all study days was tested against the null hypothesis that the mean outcome is identical among groups at each study day. If the combined treatment effect across all study days met the statistical significance threshold (0.05), a series of similar null hypotheses were used to assess the combined treatment effect at each specific study day. These null hypotheses were tested using a Wald-type multiple degree-of-freedom test. All tests were evaluated at the 0.05 level, thus ensuring a type-I error rate of 5%. This method ensures that the family of comparisons across study days are adjusted such that the type-I error rate for the overall assessment of effectiveness of CYT107 on each outcome is preserved at 5%.. No additional adjustments were made to control the type-I error rate across different outcomes. Differences in means between treatment groups at each time point were summarized using point estimates and the associated Wald-type 95% confidence intervals. In order to estimate the average difference in mean absolute lymphocyte count across the two CYT107 groups, relative to placebo, a secondary regression analysis was implemented by combining low and high frequency CYT107 groups. Normal quantile-quantile plots of Pearson residuals and fitted-versus-residual plots were examined to asses for deviations from residual normality or homoscedasticity, respectively. No transformations of baseline adjusted outcomes were necessary. All statistical analyses were performed using R version 3.3.1 (http://www.r-project.org; last accessed 2017-10-01). All figures show values plotted as mean +/- SEM.

Supplemental Table 1 Flow CytometryAntibodies

Antibody	Vender	Clone	Catalog #
CD3-PerCP/Cy5.5	BioLegend	UCHT1	300430
CD4-FITC	BioLegend	RPA-T4	300538
CD8-APC/Cy7	BioLegend	SK1	344714
CD279 (PD1)-PE	BioLegend	EH12.2H7	329906
CD127(IL-7R)-APC	BioLegend	A019D5	351316
Ki67-APC	BioLegend	ki67	350514
HLA-DR-PE/Cy7	BioLegend	G46-6	307616
CD38-BV421	BioLegend	HB7	356618

Other stains:

Item	**Vender**	**Catalog #**
Leve/Dead Yellow	ThermoFisher	L34959

CYTOKINE QUANTITATION

Quantitation of circulating cytokines IL-6, IL-10, and TNF-α via ELISA – US patient samples: Cytokine kits for TNF-α, IL6, and IL-10 were obtained from Biolegend (San Diego, CA 9212; catalog numbers respectively: 430204, 430504, 430604). The expected minimum detectable concentration of TNF-α and IL-10 is 2 pg/ml; 4 pg/ml for IL-6. 100 µl aliquots of plasma were used for TNF-α and IL-10 assays; 50 µl aliquot for IL-6. ELISA method was according to the procedure described in the kit: overnight incubation at 4^{o} of capture antibody; wash, block 1 hour room temperature; wash, incubate 2 hours with biotinylated detection antibody; wash, 1 hour incubation with avidin-HRP; wash, 15-30 minute incubation with TMB; stop with 1M sulfuric acid; read absorbance at 450 nm and 570 nm using *µQuant* plate reader (Bio-Tek Instruments, Inc., Winooski, VT).

Quantitation of circulating cytokines IL-6, IL-10, and TNF-α via ELISA – French samples: Cytokine kits for TNF-a, IL6, and IL-10 were obtained from R&D Systems Europe (catalog numbers respectively: DY206-05, DY217B-05, DY210-05). 100 □l aliquots of plasma were used for TNF-a, IL-10 and IL-6. ELISA method was according to the procedure described in the kit: overnight incubation at RT of capture antibody; wash, block 1 hour at room temperature; wash, incubate 2 hours with sample; wash, incubate 2 hours with biotinylated detection antibody; wash, 20 minutes incubation with Streptavidin-HRP; wash, 20 minutes incubation with Substrate solution SIGMAFAST OPD (Merck, Germany); stop with 1M sulfuric acid; read absorbance at 450 nm and 570 nm using Multiskan^{TM} FC plate reader (Thermo Fischer Scientific).