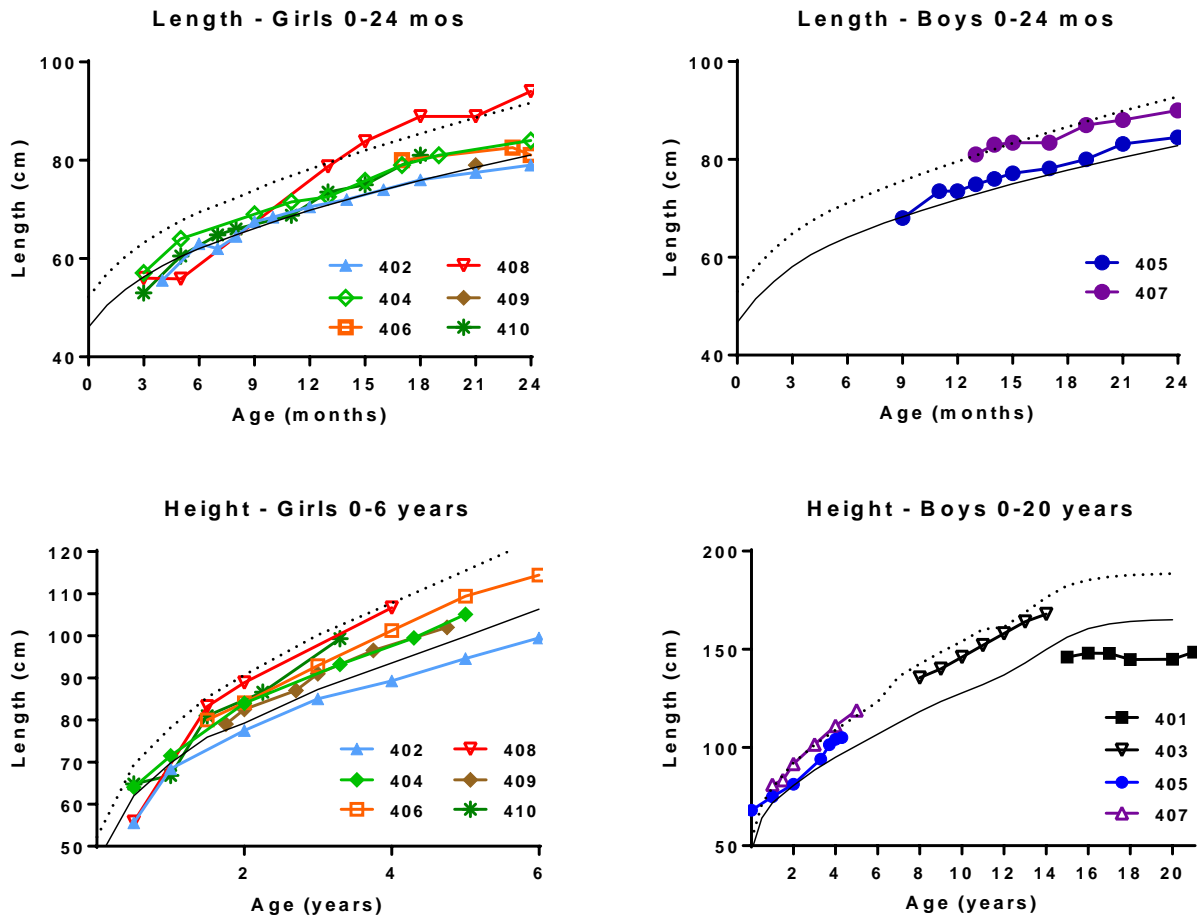


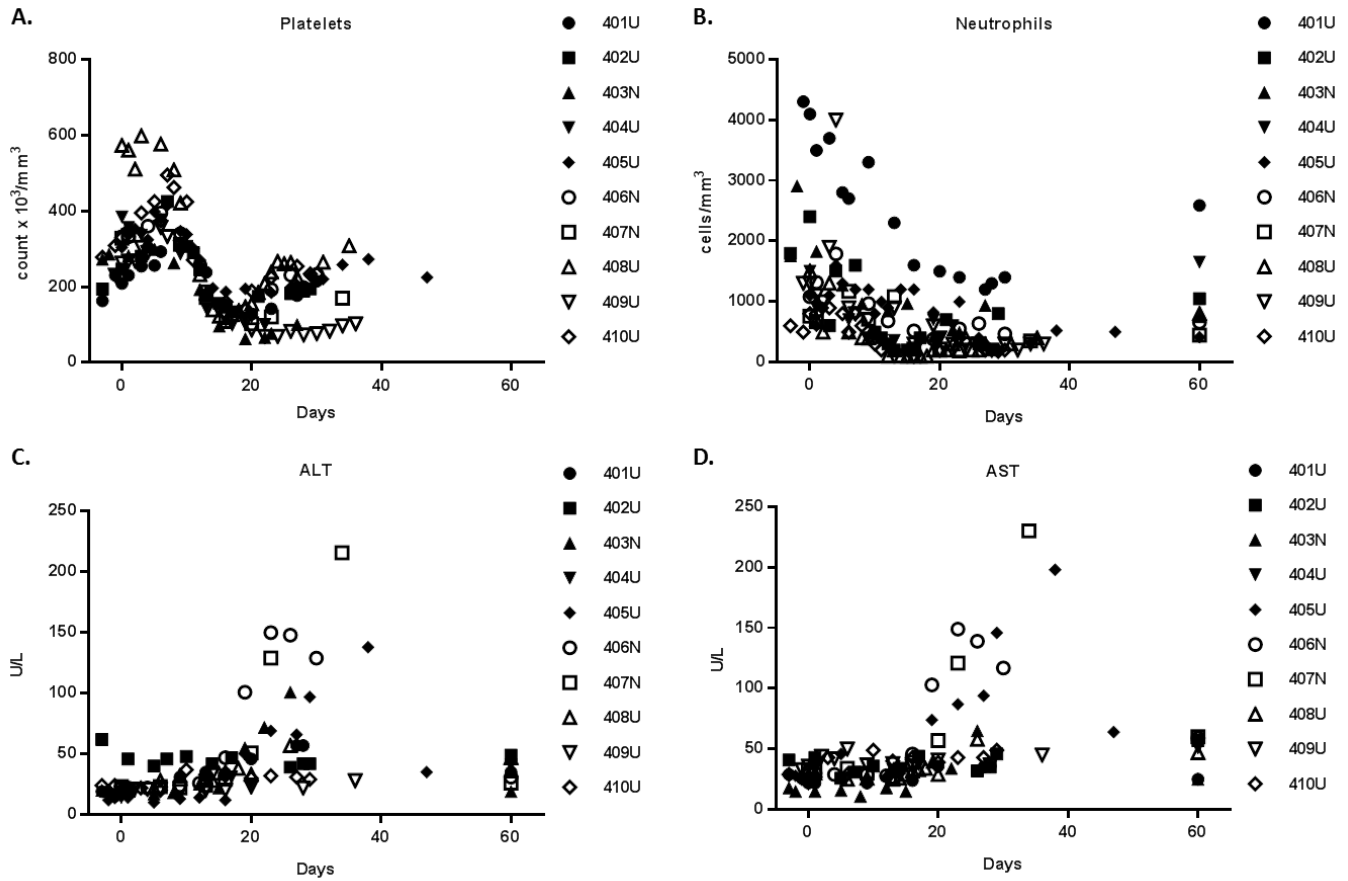
*Supplemental Materials*

## Supplemental Figure 1



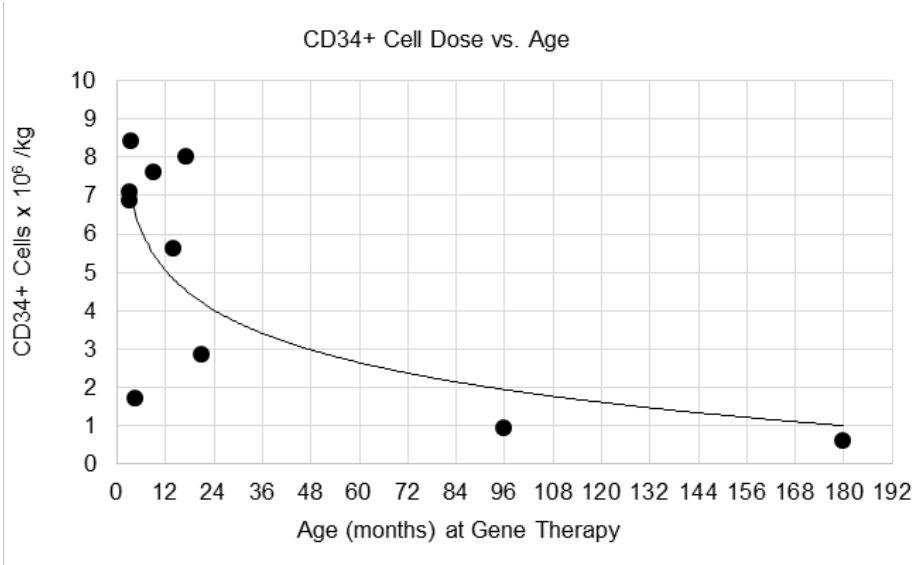
Growth Charts. Subject's length and height (cm) was recorded at each follow-up visit and plotted according to gender and age. Lower solid black line represents the 5<sup>th</sup> percentile of normal and upper dotted black line represents the 95<sup>th</sup> percentile of normal.

## Supplemental Figure 2



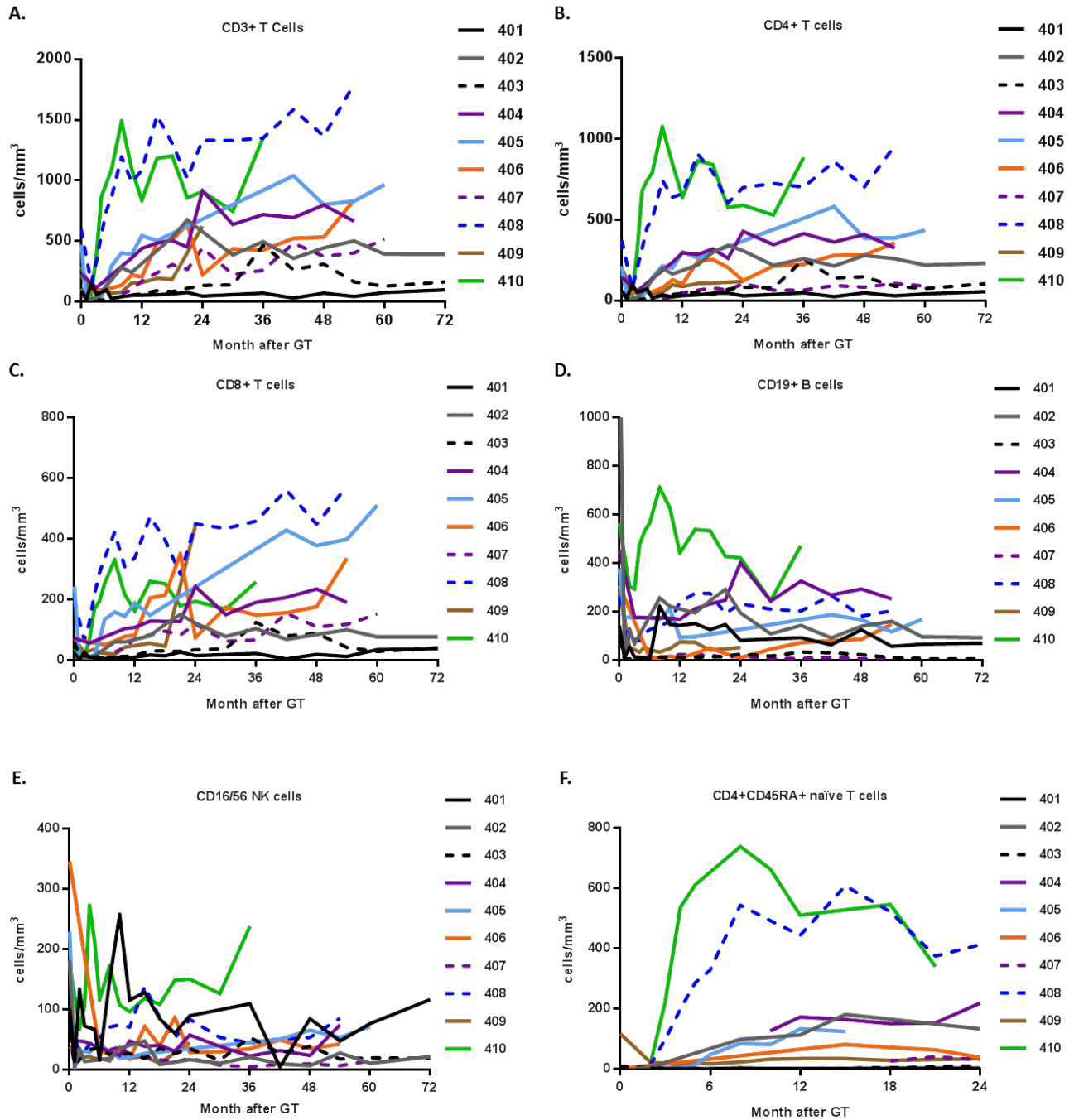
Toxicity after busulfan administration shows effect of RIC on cell counts and liver enzymes. **A.** Platelets. **B.** Neutrophils. **C.** Alanine aminotransferase. **D.** Aspartate aminotransferase.

**Supplemental Figure 3**



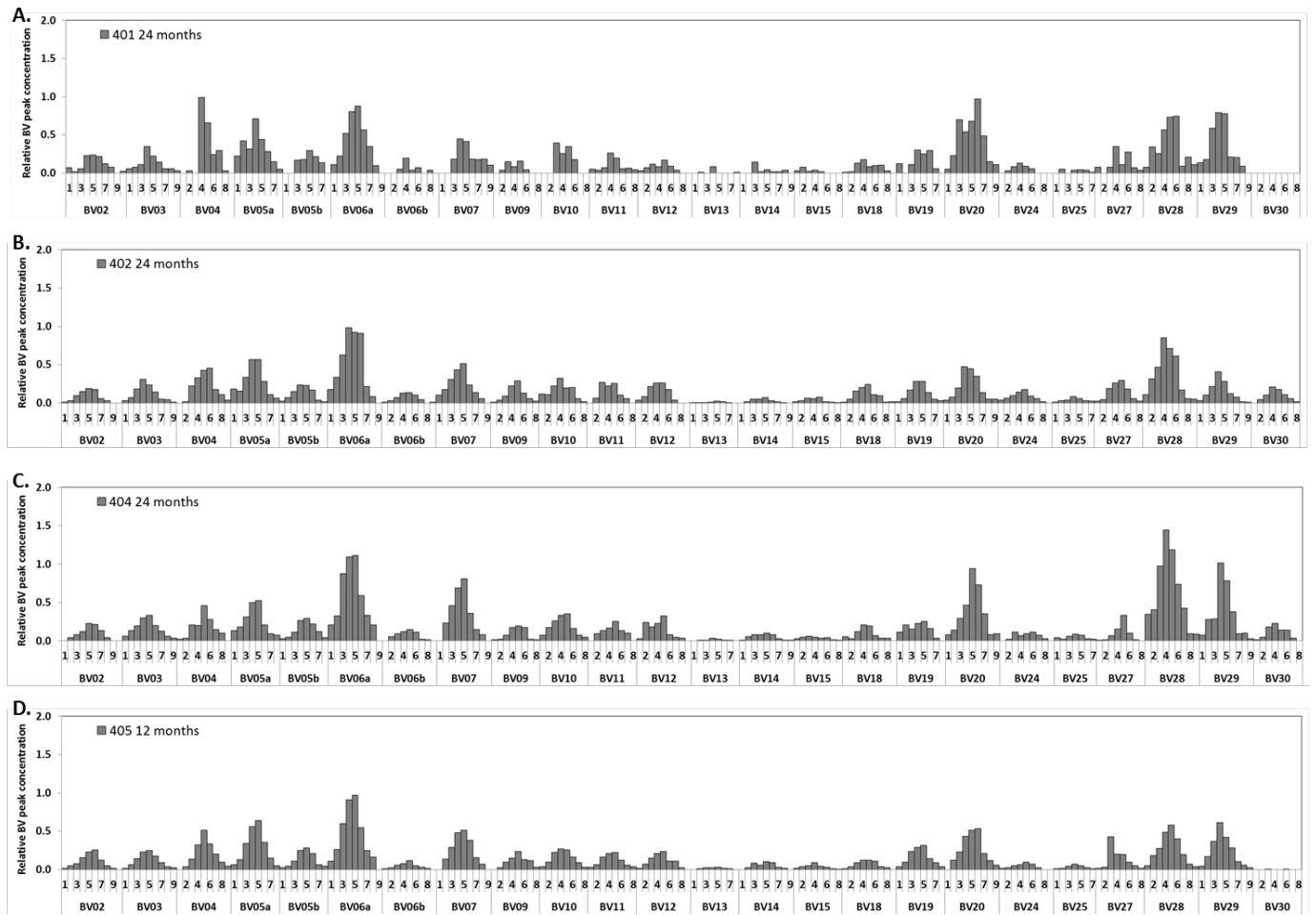
Comparison of CD34+ cell dose received to subject age (months) at time of GT.

## Supplemental Figure 4



Cell counts after gene therapy through subjects' last recorded follow-up. **A.** CD3+ pan T cells. **B.** CD4+ T cells. **C.** CD8+ T cells. **D.** CD19+ B cells. **E.** CD16/56+ NK cells. **F.** CD4/CD45RA+ Naïve T cells

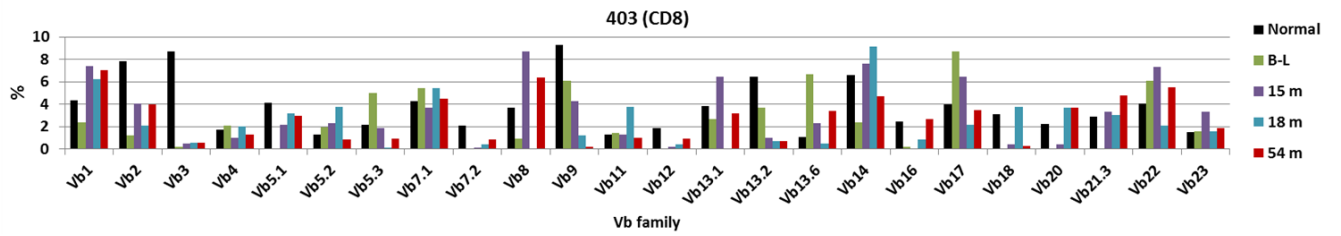
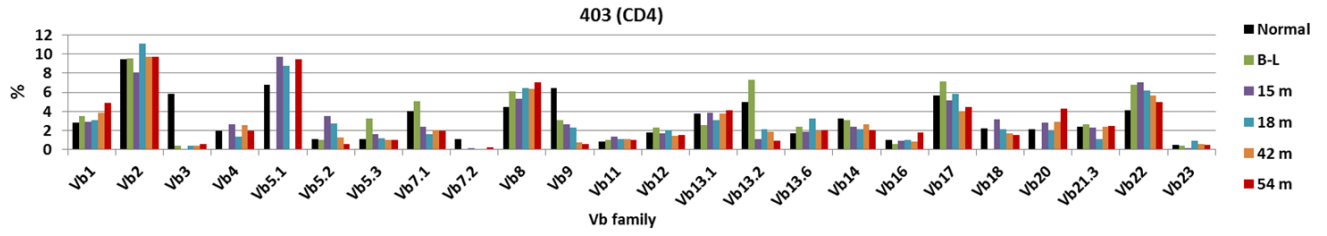
## Supplemental Figure 5



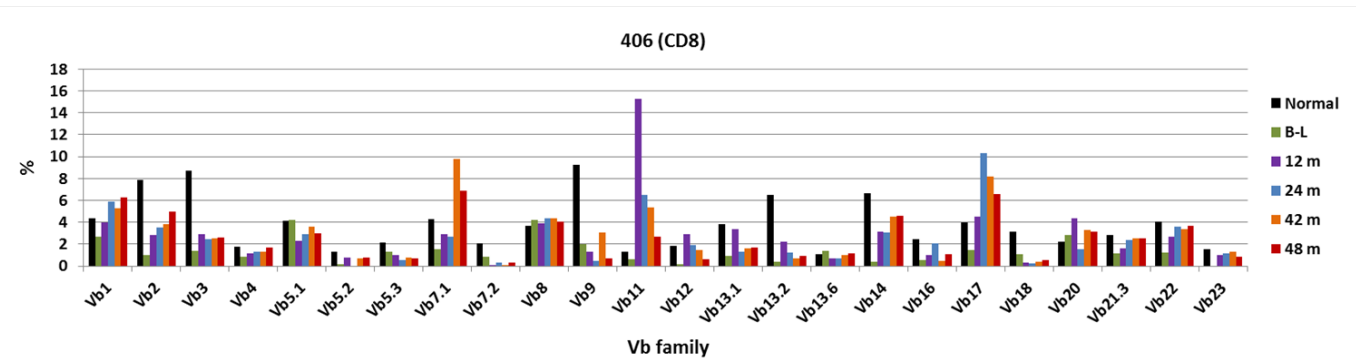
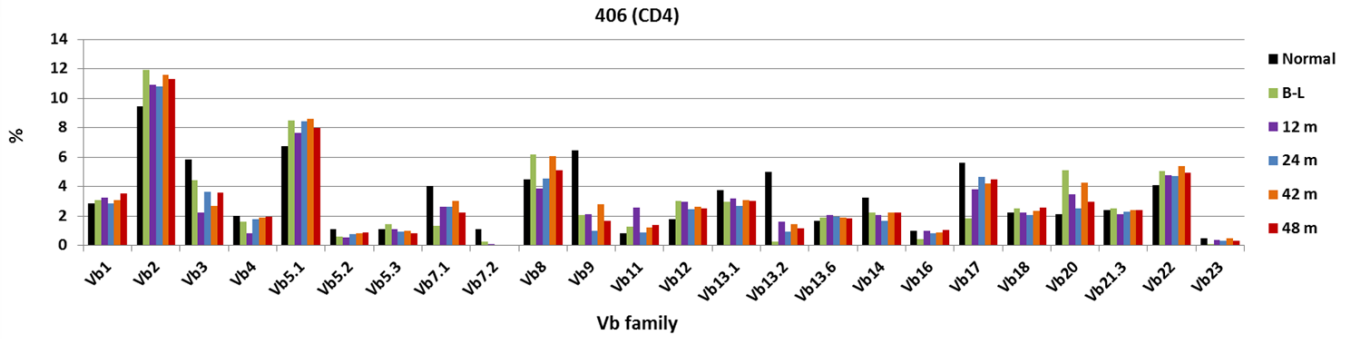
T cell receptor (TCR) Vbeta spectratypes at indicated times after GT. **A.** Subject 401, 24 months. **B.** Subject 402, 24 months. **C.** Subject 404, 24 months. **D.** Subject 405, 12 months.

# Supplemental Figure 6

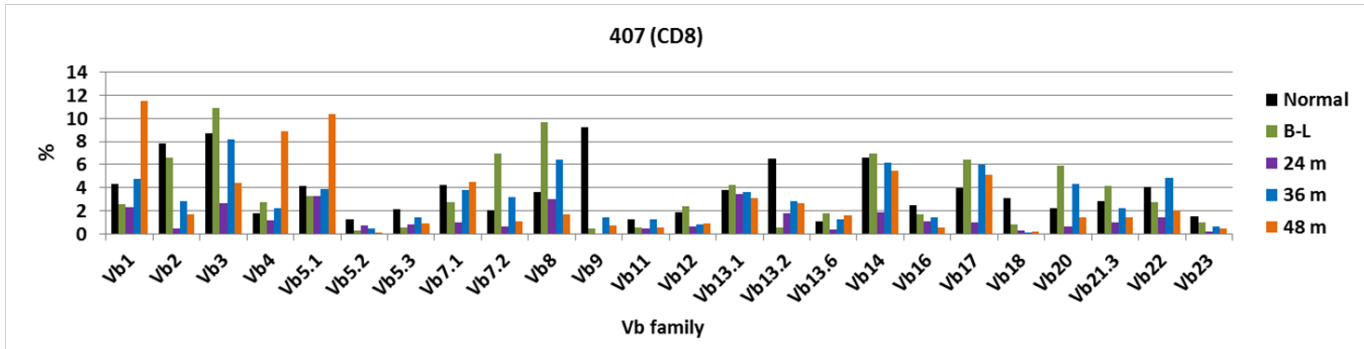
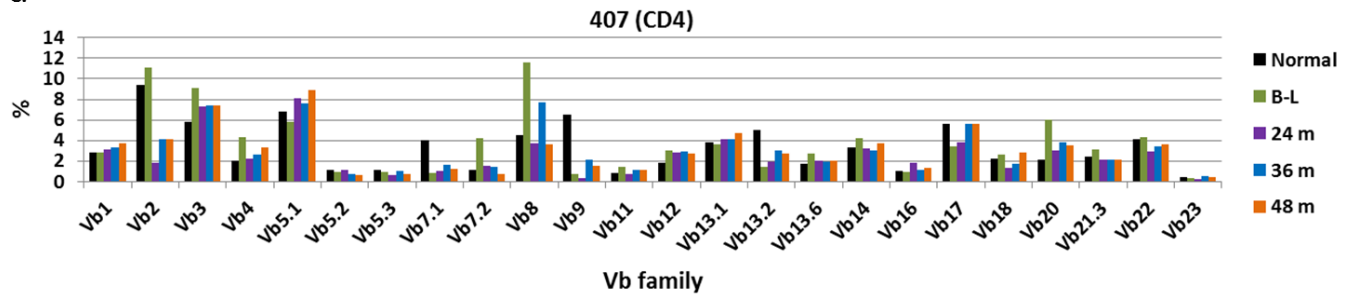
A.



B.



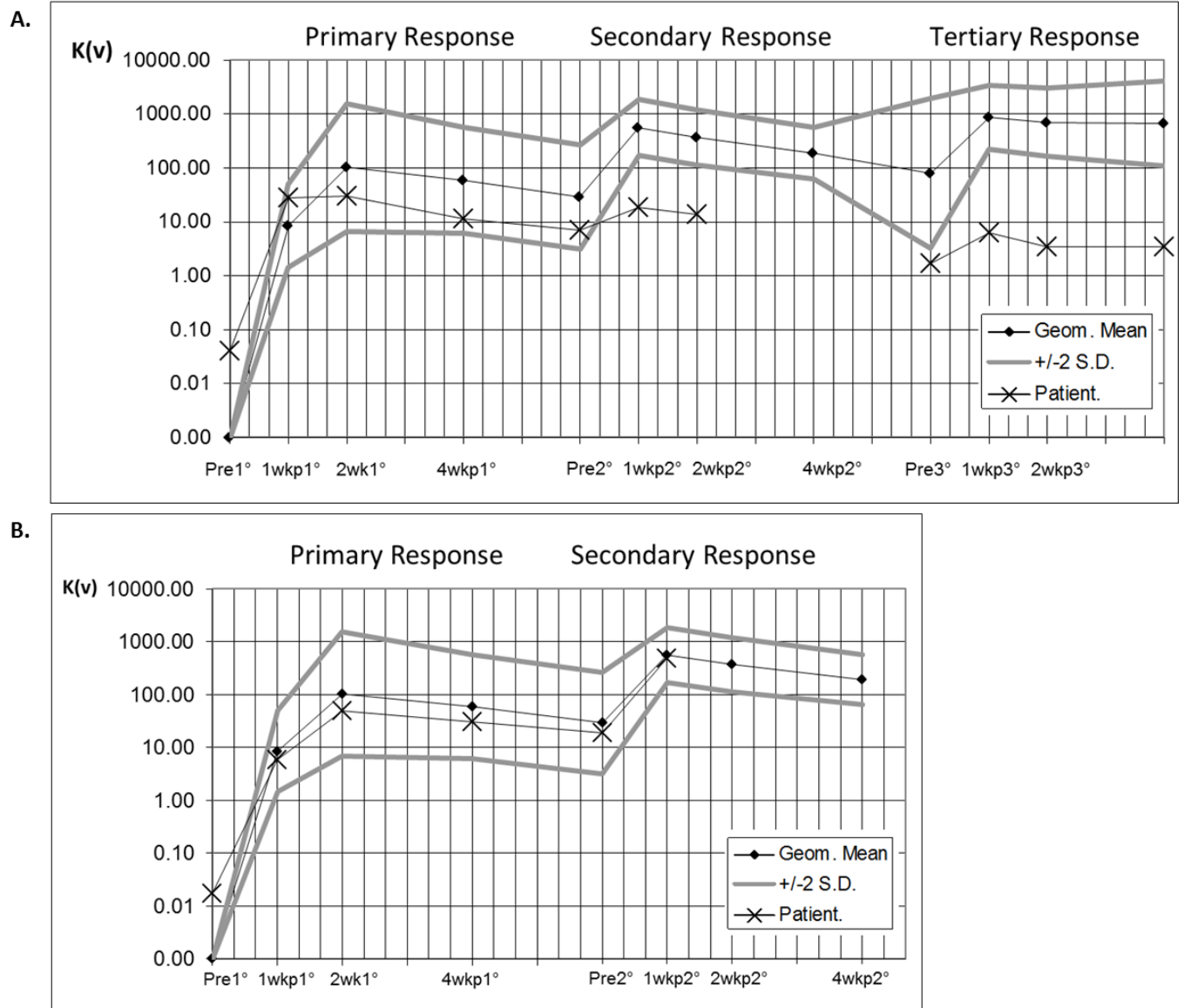
C.



TCR Vbeta families sorted by CD4+ T cells (top panels) and CD8+ T cells (bottom panels) measured at indicated times after gene therapy. **A.** Subject 403. **B.** Subject 406. **C.** Subject 407.

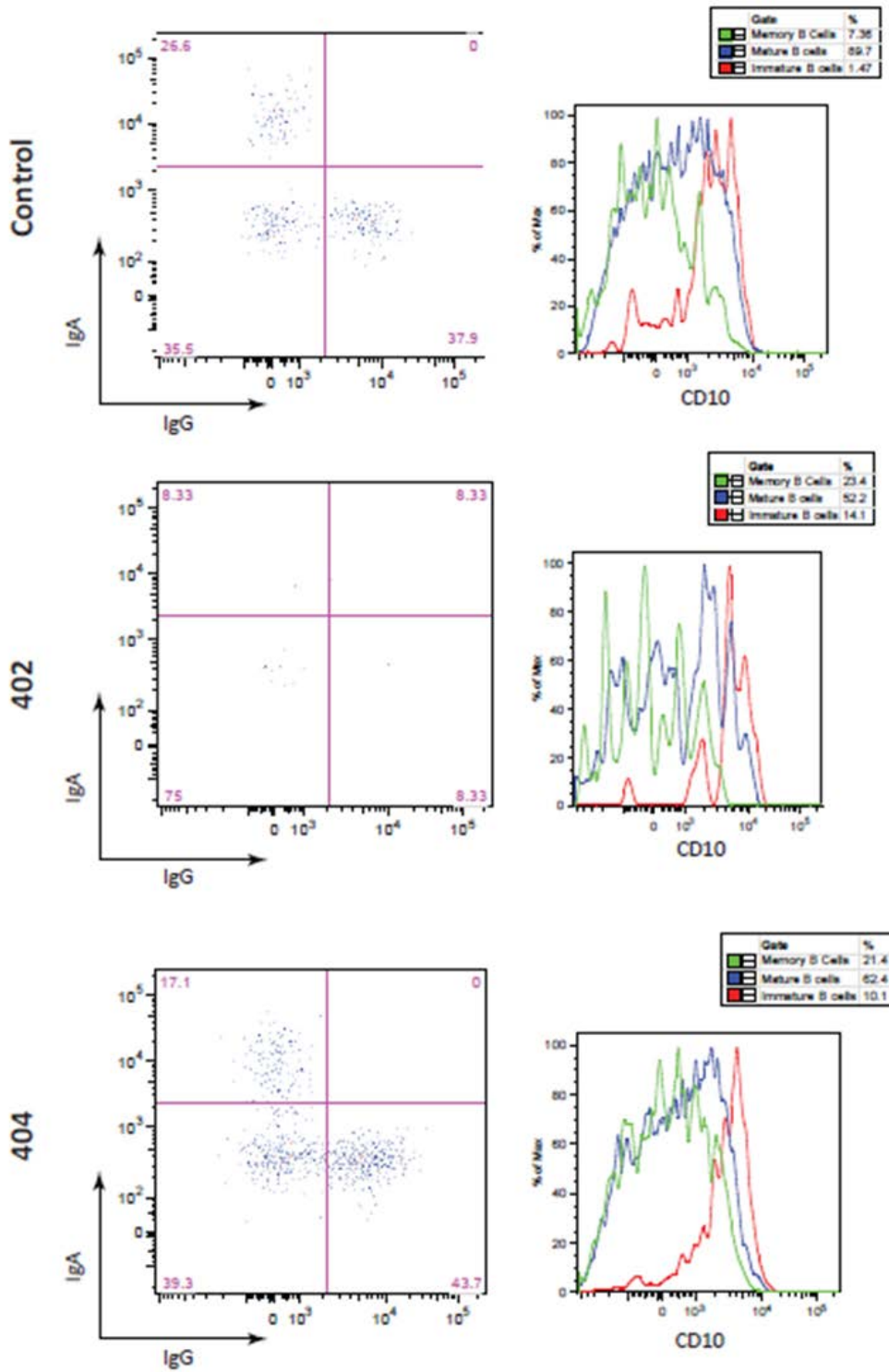


### Supplemental Figure 7



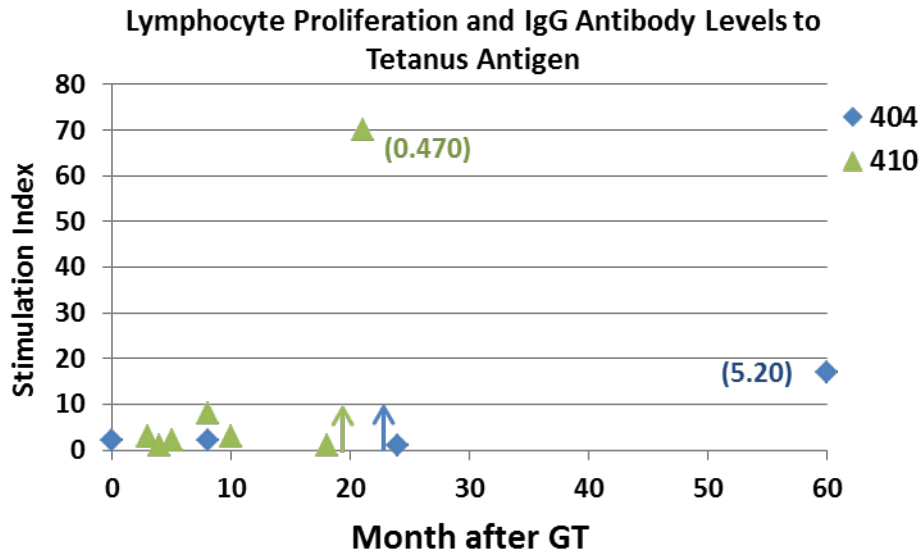
Immune response to bacteriophage  $\phi\chi 174$ . **A.** Subject 402 (Primary: 20 months, Secondary: 21 months, Tertiary: 31 months after GT). **B.** Subject 404 (Primary: 17 months, Secondary: 18 months after GT).

Supplemental Figure 8



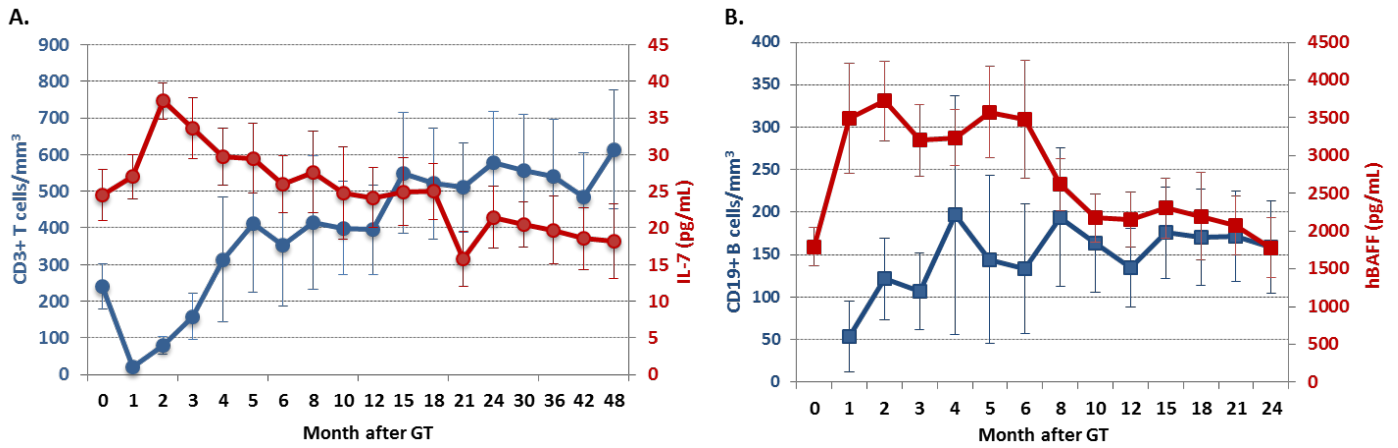
B cell studies. **Top Panel:** normal control, **Middle Panel:** Subject 402 (67 months after GT), **Bottom Panel:** Subject 404 (53 months after GT).

### Supplemental Figure 9



Lymphocyte proliferation and IgG antibody levels to tetanus antigen in subjects 404 and 410. Arrows indicate the time of vaccination with Tetanus (DTaP). Numbers in parenthesis indicate the Tetanus IgG antibody level measured (in IU/mL, normal >0.15) after vaccination and within 1 year of the tetanus proliferation response shown.

## Supplemental Figure 10



Comparison of lymphocyte counts and cytokine levels. **A.** Serum IL-7 levels (secondary Y axis) compared to CD3+ T cell counts (primary Y axis) at the indicated times after GT. **B.** Serum BAFF levels (secondary Y axis) compared to CD19+ B cell counts (primary Y axis) at the indicated times after GT. Error bars represent the standard error of the mean.

Supplemental Tables

**Table S1 Infectious Complications Requiring Initial or Prolonged Hospitalization after GT**

<b>Subject</b>	<b>Event</b>	<b>Time after Transplant</b>	<b>Days in Hospital</b>	<b>Treatment</b>
<b>402</b>	line infection with coagulase negative <i>Staphylococcus sp</i>	2 days	N/A <sup>A</sup>	antibiotics
<b>404</b>	Urinary tract infection/fever with <i>E. coli</i>	4 months	4	antibiotics
<b>406</b>	fever	3 months	5	antibiotics
<b>406</b>	abscess	4 months	5	antibiotics
<b>407</b>	RSV infection	5 months	8	Ribaviran
<b>410</b>	Rash with fever	20 months	5	antibiotics

<sup>A</sup>Already in hospital for GT procedure

## *Supplemental Methods*

T cell receptor (TCR) Vbeta spectratyping was performed as described (1) using RNA extracted from cryopreserved PBMC samples.

The TCR V-beta repertoire was measured using the IOTest© Beta Mark TCR V beta Repertoire Kit (Beckman Coulter, Inc., Brea, CA), which provides a quantitative determination of the TCR V-beta repertoire on T lymphocytes. Whole blood was stained with both CD4 and CD8 reagents and the TCR V-beta antibody cocktail, and the sub-populations were analyzed on these lymphocyte subsets. Data was acquired on a BD FACSCalibur™ (BD Biosciences, San Jose, CA) and analyzed in FlowJo© (FlowJo, LLC, Ashland, OR).

Immunological Challenge with bacteriophage  $\phi\chi$ 174 was performed as described (2).

B cell studies were performed as described (3).

Serum IL-7 and B cell activating factor of the TNF family (BAFF) levels were measured using commercially available ELISA kits (Quantikine® HS Human IL-7 and Human BAFF/BLyS/TNFSF13B ELISA Kits, R&D Systems, Minneapolis, MN) on samples that had been collected at the specified time points, frozen in aliquots and stored at -80° C. At the time of analysis, sera were thawed and assayed according to the manufacturer's instructions.

### *Supplemental References*

1. Balamurugan, A., Ng, H.L., and Yang, O.O. 2010. Rapid T cell receptor delineation reveals clonal expansion limitation of the magnitude of the HIV-1-specific CD8+ T cell response. *J Immunol* 185:5935-5942.
2. Ochs, H.D., Buckley, R.H., Kobayashi, R.H., Kobayashi, A.L., Sorensen, R.U., Douglas, S.D., Hamilton, B.L., and Hershfield, M.S. 1992. Antibody responses to bacteriophage phi X174 in patients with adenosine deaminase deficiency. *Blood* 80:1163-1171.
3. Meyer-Bahlburg, A., Andrews, S.F., Yu, K.O., Porcelli, S.A., and Rawlings, D.J. 2008. Characterization of a late transitional B cell population highly sensitive to BAFF-mediated homeostatic proliferation. *J Exp Med* 205:155-168.