Figure legends for supplementary figures

Fig. S1. Phenotyping of human B and T cells after GM-CSF and IL-4 treatment before immunization.

Seven days after GM-CSF and IL-4 plasmid injection, splenocytes from seven mice per group were stained for human CD45, CD19 plus the indicated B cell markers, or CD45, CD3 plus the indicated T cell markers. (A) Staining profiles of CD19 versus CD40, IgM, CD80, CD5, CD20, HLA-DR, CD10, CD86 or CD27 gating on $CD45^+$ human cells non-treated and cytokine-treated mice. (B) Comparison of mean fluorescent intensity of CD40 and CD5 of human $CD45^+CD19^+$ cells between non-treated and cytokine-treated mice. The values represent mean ± SEM of seven mice per group. (C) Staining profiles of CD3 versus CD25, CD45RA, CD40L, HLA-DR or CD4 gating on $CD45^+$ human cells from non-treated and cytokine-treated mice. (D) Comparison of percentages of $CD45RA^+$ and $CD45RA^-$ cells within the human $CD45^+CD3^+$ cells between non-treated and cytokine-treated mice. The values represent mean ± SEM of seven mice per group. (E, F) Staining profiles of CD4 and CD8 versus CD25, CD40L and HLA-DR gating on $CD4^+$ (E) and $CD8^+$ (F) human T cells, respectively. Ctrl: vector plasmid treated mice. Representative data from one of seven mice per group are shown. The numbers indicate percentages of cells in the gated quadrants. *$P<0.05$.

Fig. S2. GM-CSF and IL-4 treated humanized develop all human immunoglobulin and phenotyping of human B and T cells after immunization.

Humanized mice were injected with vector (Ctrl) or GM-CSF plus IL-4 plasmids and then immunized with TT three times. Sera and splenocytes were analyzed 2 weeks after the third immunization.

(A) Sera from GM-CSF and IL-4 treated humice two weeks after the third immunization with TT were collected and assayed for human immunoglobulin isotypes: IgA, IgE, IgD, IgM and IgG subtypes: IgG1, IgG2, IgG3, and IgG4 by human immunoglobulin protein array Average with standard means are shown for four mice per group.

(B) Splenocytes were stained for human CD45 (hCD45) versus mouse CD45.1 (mCD45). Shown are representative plots of human CD45 versus mouse CD45.1 between mice injected with vector control plasmid or GM-CSF and IL-4 plasmids.

(C) Splenocytes were analyzed for human CD45, CD19 plus the indicated markers. Shown are plots of CD19 versus CD40, IgM, CD86, CD5, CD20, HLA-DR, CD10, CD80 or CD27 gating on $CD45^+$ human cells between mice injected with vector control plasmid or GM-CSF and IL-4 plasmids.

(D) Splenocytes were analyzed for human CD45, CD3 plus the indicated markers. Shown are plots of CD3 versus CD25, CD45RA, CD40L, HLA-DR and CD4 gating on $CD45^+$ human cells between mice injected with vector control plasmid or GM-CSF and IL-4 plasmids.

(E) Splenocytes were analyzed for human CD45, CD4 or CD8 plus the indicated markers. Shown are plots of CD4 and CD8 versus CD25, CD40L and HLA-DR gating on $CD4^+$ and $CD8^+$ human T cells, respectively.

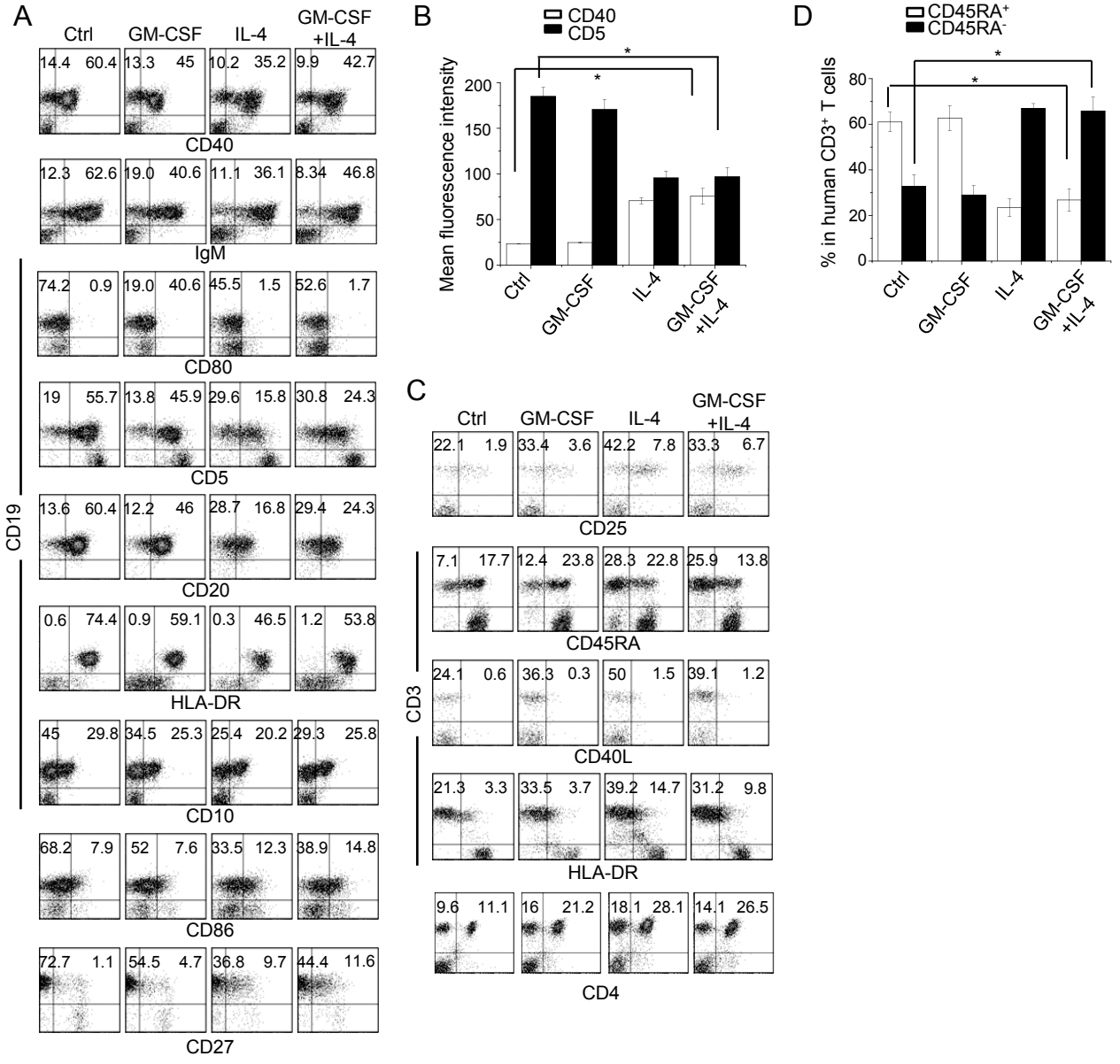
The numbers indicate percentages of cells in the gated quadrants.

Fig. S3. Comparison of B and T cell numbers in non-treated and cytokine-treated mice without immunization.

Humanized mice were injected with vector (Ctrl), IL-4, GM-CSF, or GM-CSF plus IL-4 plasmids and analyzed for various cell types 9 weeks later. Single cell suspensions of spleens were counted and analyzed by flow cytometry for human CD45 plus CD19 or CD3, or CD3, CD8 and CD4. The numbers of human cells were calculated by multiplying the total cell numbers with the frequency the specific cell type. Values represent mean ± SEM of five mice per group. Shown are total human $CD45^+$ leukocytes, $CD19^+$ B cells, $CD3^+$ T cells, $CD4^+$ T cells, and $CD8^+$ T cells in the spleens of various humanized mice. *$P<0.05$.

Fig. S4. Generation of NP-KLH specific human antibody responses.

Humanized mice were injected with vector plasmid (ctrl) or GM-CSF and IL-4 plasmids and immunized three times with NP-KLH at three weeks apart. Sera were collected two weeks after the third immunization and assayed for human, total IgG (A), total IgM (B) and NP-KLH specific IgG (C) by ELISA. The numbers indicate the average concentrations of antibodies ($n = 7$). *$P<0.05$.



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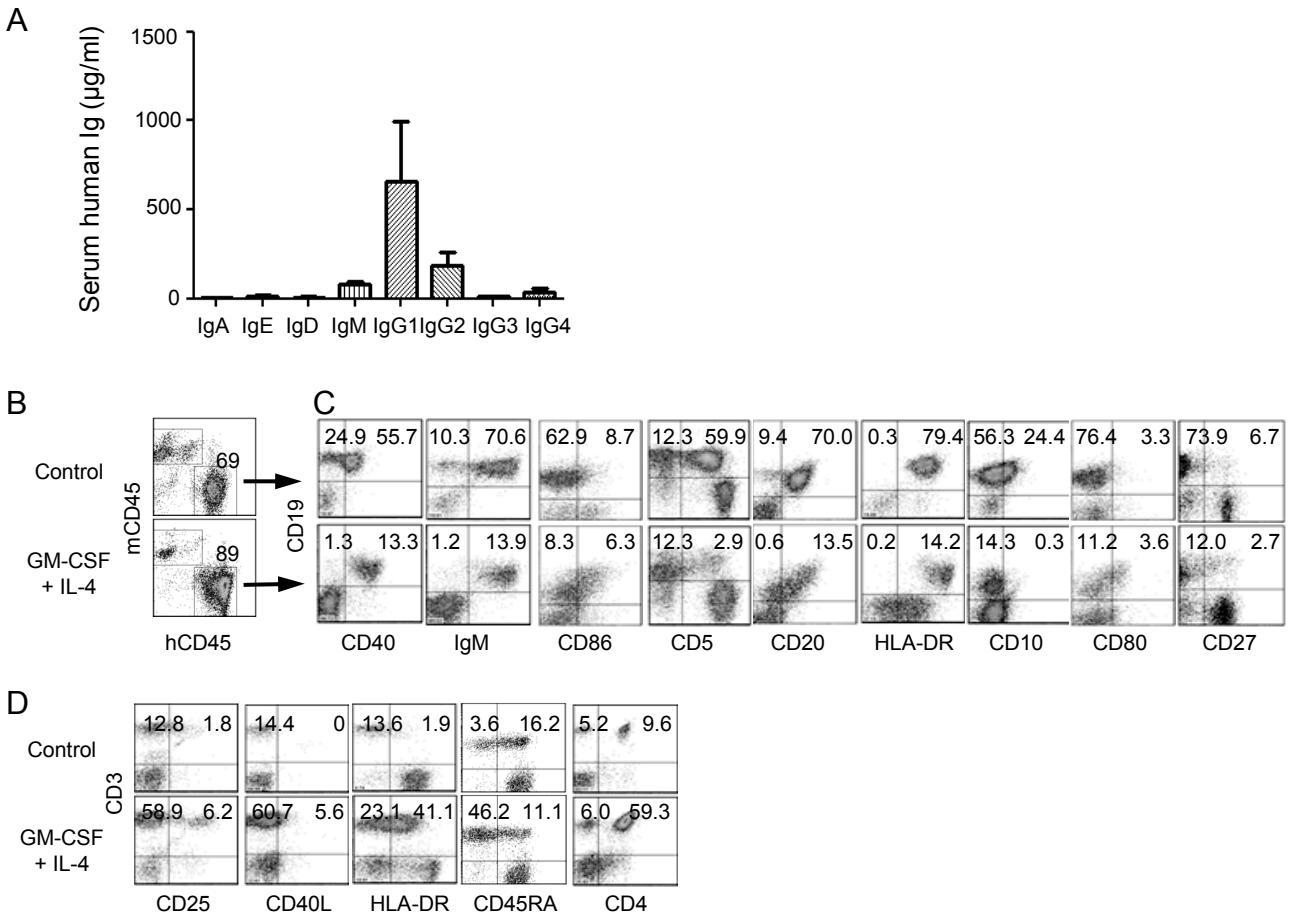

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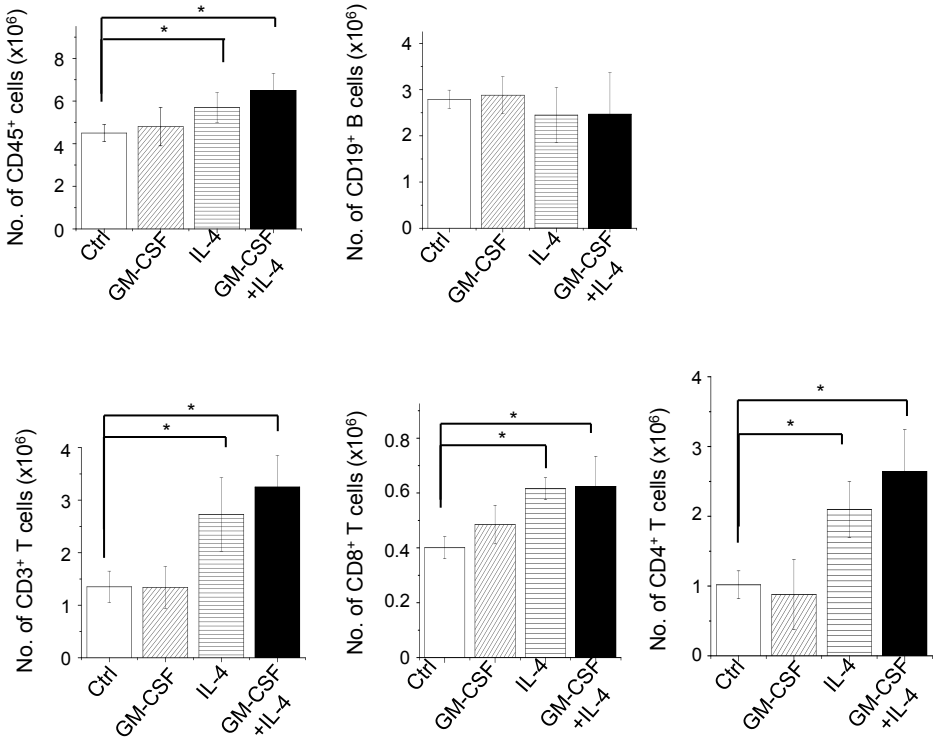

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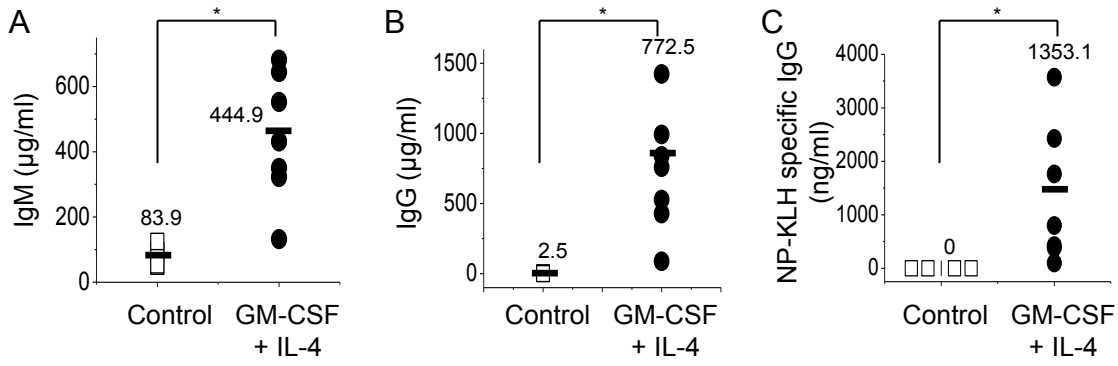

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