SUPPLEMENTARY APPENDIX

Long-term serial xenotransplantation of juvenile myelomonocytic leukemia recapitulates human disease in Rag2$^{-/-}\gamma c^{-/-}$ mice

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Supplemental Table 1

Patient	#1	#2	#3	#4	#5
Age at diagnosis (months)	23	26	60	29	44
Sex	Female	Male	Male	Male	Male
Platelet count at diagnosis (x 10^9/L)	137	21	10	60	201
White blood count at diagnosis (x 10^9/L)	54.0	57.0	14.1	13.6	38.2
Fetal hemoglobin at diagnosis (%)	10.3	21.8	9.2	Not done	28.3
Mutation	*PTPN11* c.C215T	*PTPN11* c.G181T	*PTPN11* c.G214A	*KRAS* c.G37T	*NRAS* c.38G>A c.181C>A
Leukemia karyotype	Normal	Normal	Normal	Normal	Normal
Therapy before splenectomy*	None	None	Steroids	None	Thioguanine Cytarabine 2x HSCT

*All patients received splenectomies for clinical indication.

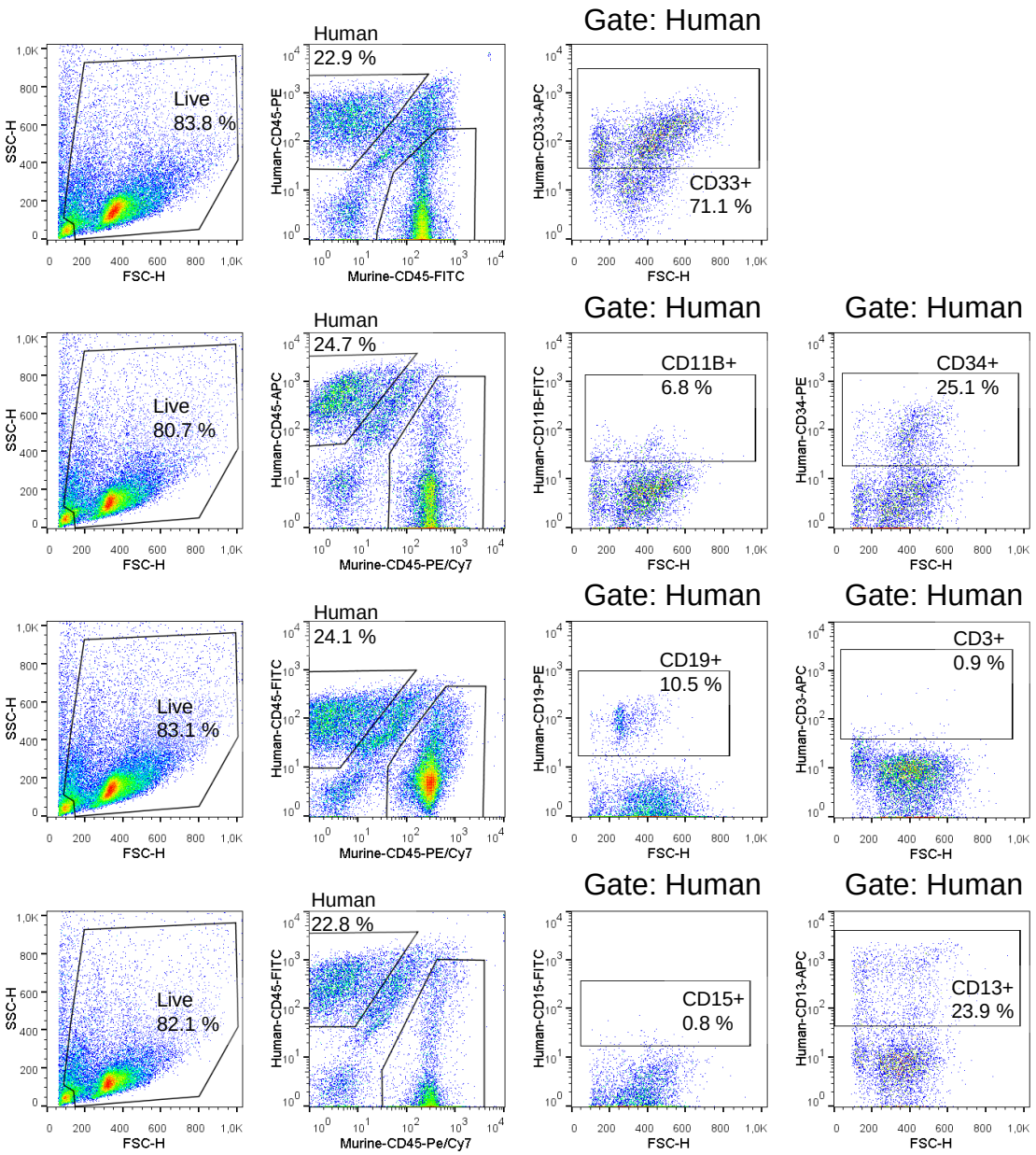
Supplemental Table 1:
Characteristics of JMML patients used as donors for xenotransplantation.

Supplemental Table 2

Antibody	Clone	Company	Method
Human CD45-PE	HI30	BD	Flow cytometry
Human CD33-APC	WM-53	BD	Flow cytometry
Human CD45-FITC	2D1	BD	Flow cytometry
Human CD19-PE	4G7	BD	Flow cytometry
Human CD3-APC	SK7	BD	Flow cytometry
Human CD13-APC	WM 15	BD	Flow cytometry
Human CD15-PE	HI98	BD	Flow cytometry
Murine CD45-FITC	30-F11	BD	Flow cytometry
Murine CD45-PeCy7	30-F11	BD	Flow cytometry
Human CD34-PE	AC136	Miltenyi	Flow cytometry
Human CD45-APC	MEM-28	Immunotools	Flow cytometry
Human CD11b-FITC	ICRF44	eBioscience	Flow cytometry
Human CD45	2B11 + PD7/26	Dako	Immunohistochemistry
Human CD34	QBEnd 10	Dako	Immunohistochemistry
Human CD68	PG-M1	Dako	Immunohistochemistry
Human lysozyme	A0099	Dako	Immunohistochemistry
Murine CD45	30-F11	BD	Immunohistochemistry
Murine Ig	K5005	Dako	Immunohistochemistry
Rat Ig	P0450	Dako	Immunohistochemistry

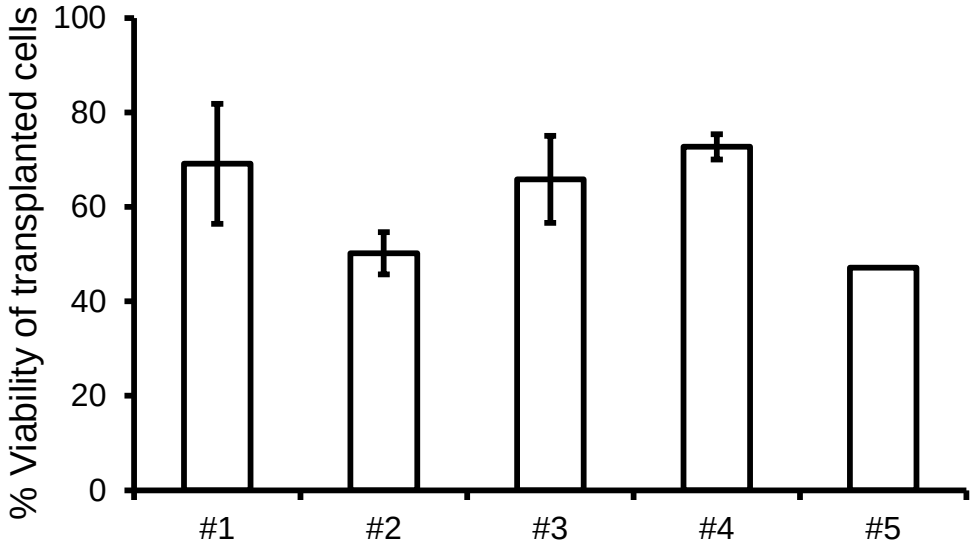
Supplemental Table 2:
Antibodies used for characterization of JMML cell populations in xenotransplanted mice.

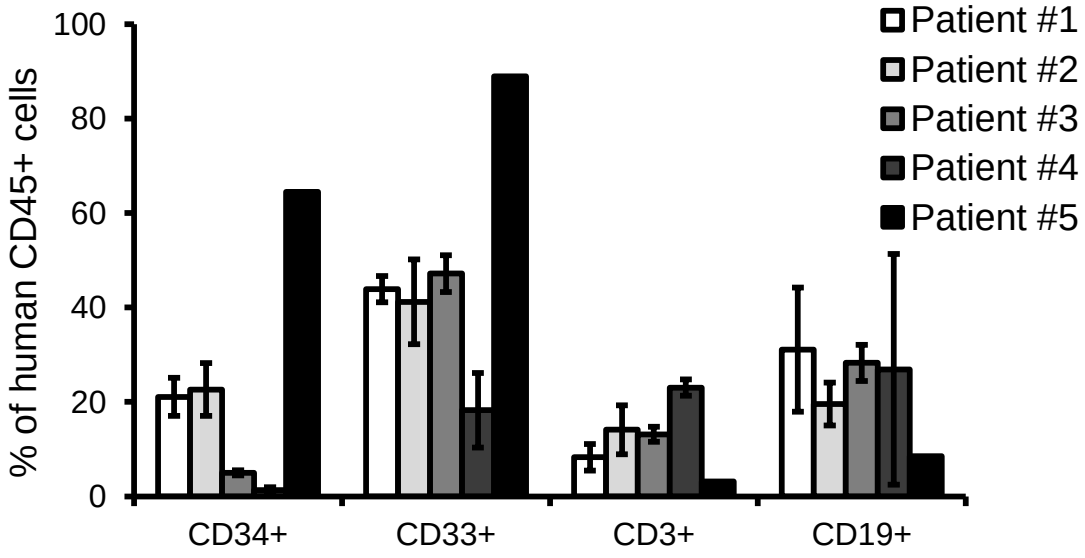
Supplemental Figure 1



Supplemental Figure 1:
Analysis strategy of flow cytometry data.
The gating strategy for the characterization of engrafted cell populations (murine CD45, human CD45, CD33, CD11B, CD34, CD19, CD3, CD15 and CD13) is shown. Exemplary plots show flow cytometry data of murine bone marrow.

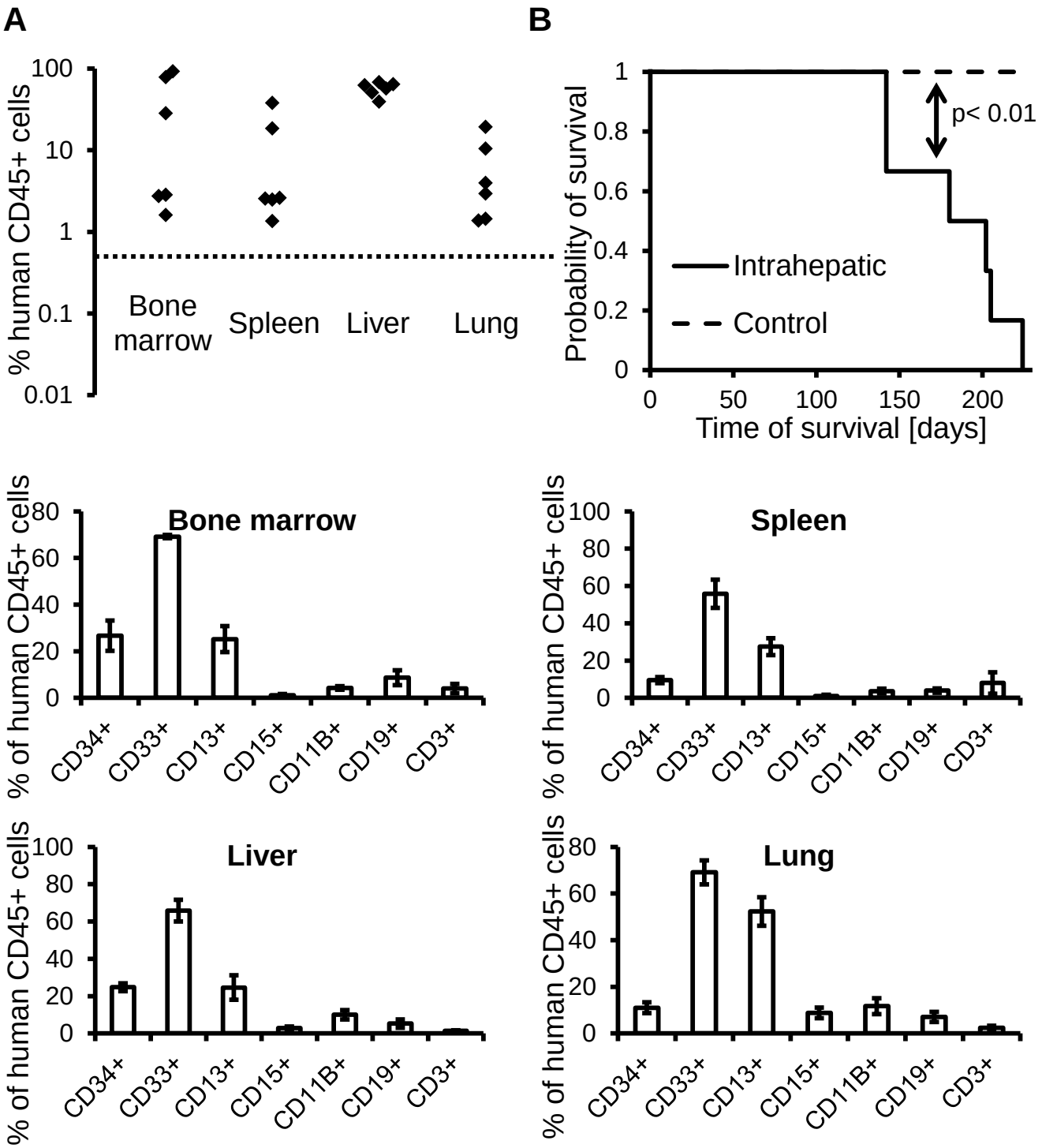
Supplemental Figure 2





Supplemental Figure 2:
Characteristics of xenotransplanted material.
Cell viability upon thawing (upper panel) as well as cell composition (lower panel) of xenotransplanted material is depicted for patients #1 to #5. Xenotransplantation of JMML cells from patient #4 was unsuccessful.

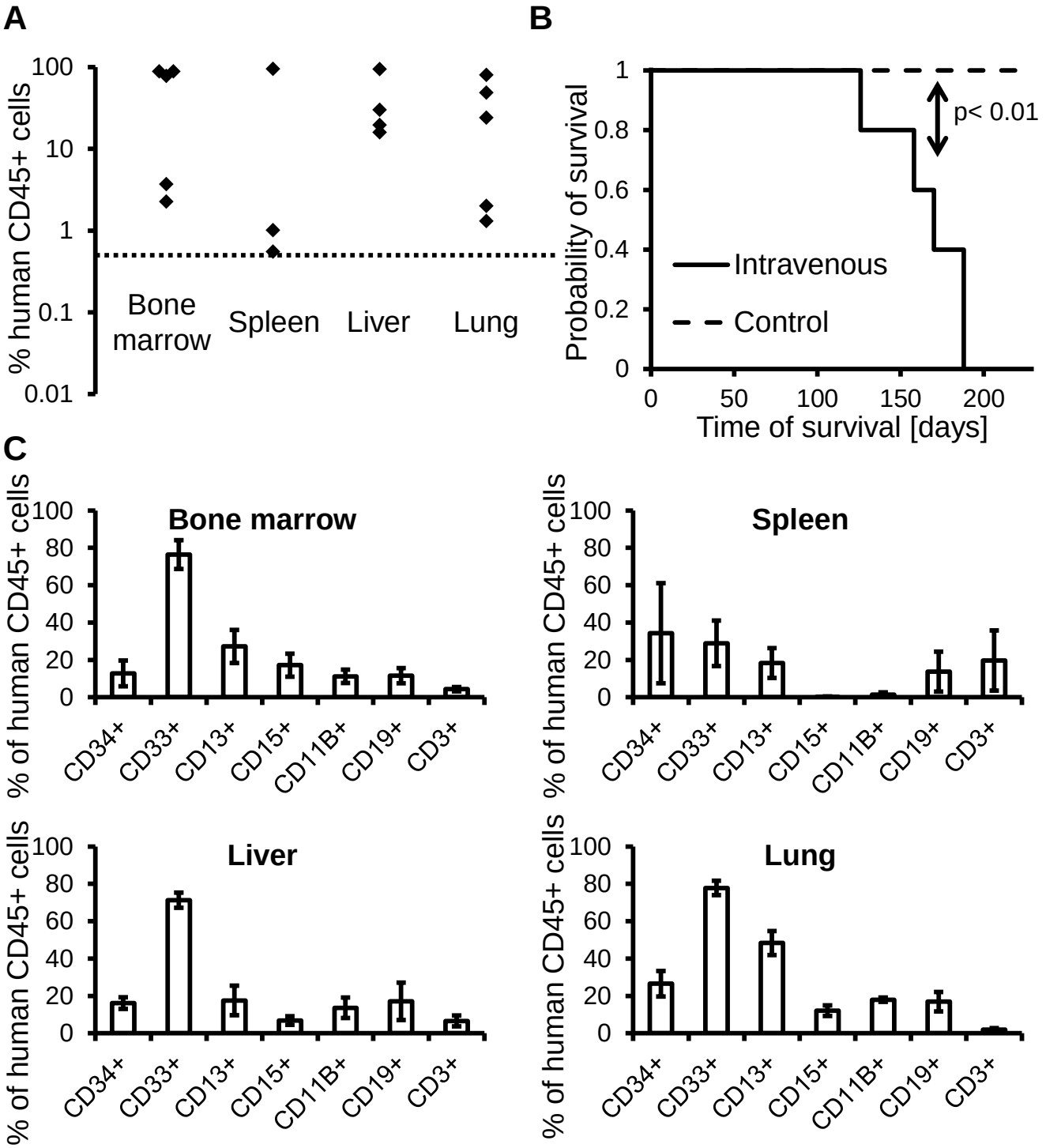
Supplemental Figure 3



Supplemental Figure 3. JMML phenotype in *Rag2$^{-/-}$γc$^{-/-}$* mice after intrahepatic route of xenotransplantation

(A) Intrahepatic transplantation of 1 x 10^6 JMML MNC into newborn mice (black dots, N=6, patient #2) led to successful engraftment. Mice were sacrificed for analysis when critically sick. Human cell engraftment was assessed by flow cytometry of CD45+ cells. The level of human engraftment was defined as proportion of human CD45+ cells within the total population of murine and human CD45+ cells. The dotted line represents the definition of successful engraftment (≥0.5% human CD45+ cells). **(B)** Survival of xenograft mice was reduced (solid line) as compared to the survival of untransplanted control mice (N=5, dashed line) ($p = 0.0005$, Mantel-Cox log-rank test). **(C)** Hematopoietic cells were obtained from indicated organs and cell subpopulations were assessed by flow cytometry with antibodies to human CD45, CD34, CD33, CD13, CD15, CD11B, CD19 and CD3. Bars indicate mean value and standard error.

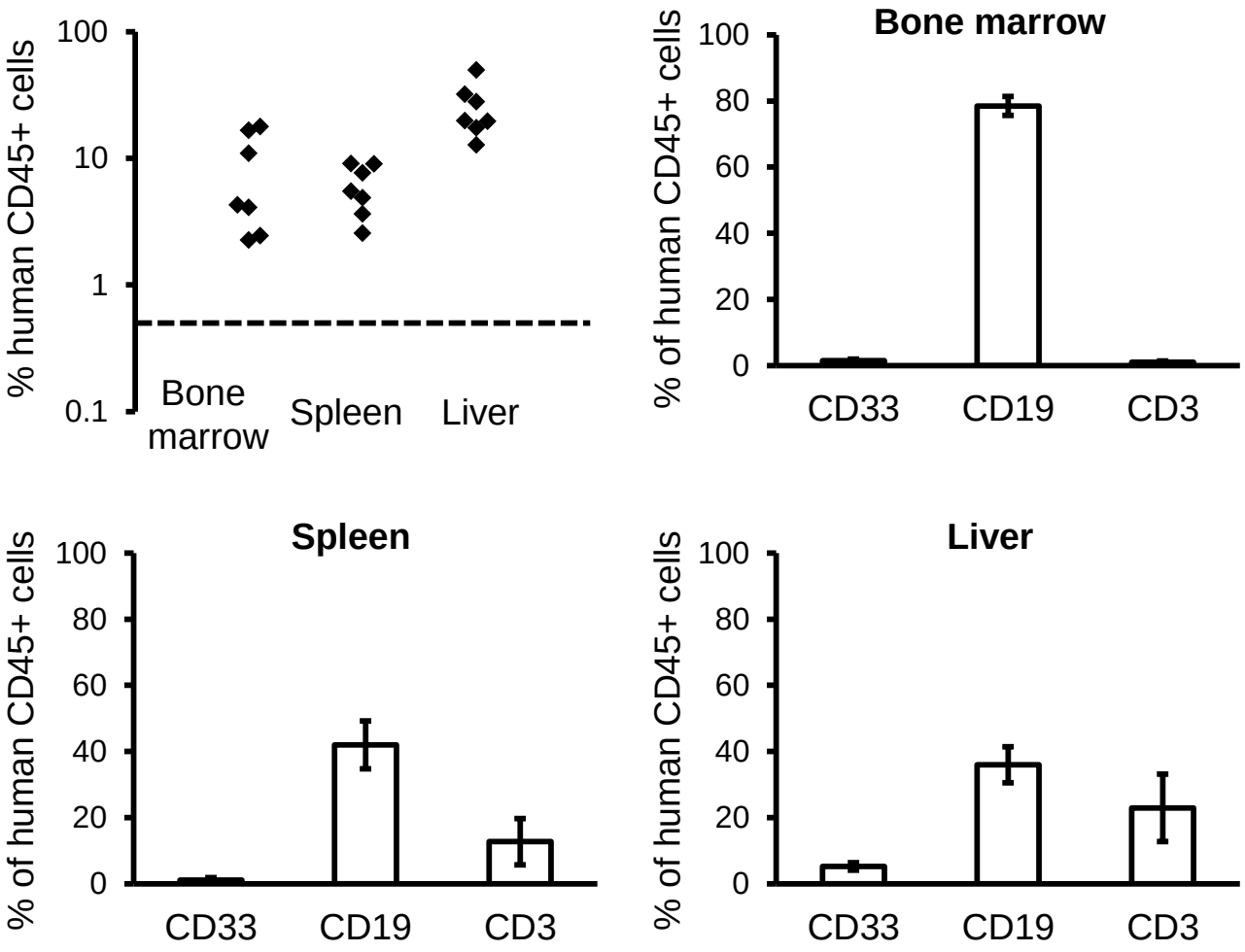
Supplemental Figure 4



Supplemental Figure 4. JMML phenotype in *Rag2$^{-/-}$γc$^{-/-}$* mice after intravenous route of xenotransplantation

(A) Intravenous transplantation of 5 x 10^6 JMML MNC into 5-week-old mice (black dots, N=5, patient #1) led to successful engraftment. Mice were sacrificed for analysis at terminal disease. Human cell engraftment was assessed by flow cytometry of CD45+ cells. The level of human engraftment was defined as proportion of human CD45+ cells within the total population of murine and human CD45+ cells. The dotted line represents the definition of successful engraftment (≥0.5% human CD45+ cells). **(B)** Survival of xenograft mice was reduced (solid line) as compared to the survival of untransplanted control mice (N=5) ($p = 0.0011$, Mantel-Cox log-rank test). **(C)** Hematopoietic cells were obtained from indicated organs and cell subpopulations were assessed by flow cytometry with antibodies to human CD45, CD34, CD33, CD13, CD15, CD11B, CD19 and CD3. Bars indicate mean value and standard error.

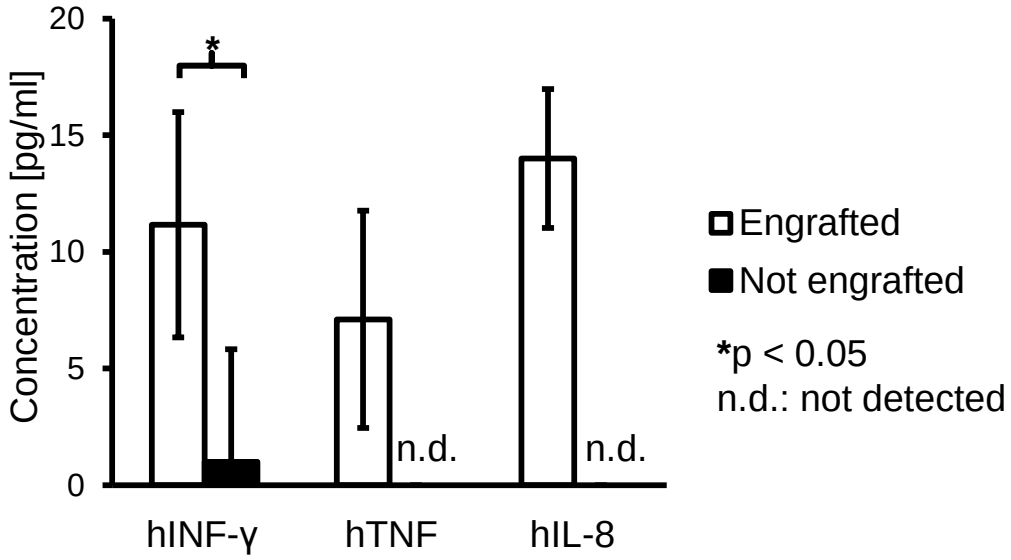
Supplemental Figure 5



Supplemental Figure 5. Xenotransplantation of healthy CD34+ cord blood cells in *Rag2$^{-/-}$γc$^{-/-}$* mice

CD34+ cells from umbilical cord blood of healthy newborns were transplanted into *Rag2$^{-/-}$ γc$^{-/-}$* mice (N=7, 1 x 10^5 cells per mouse). Recipients were analyzed 8 weeks after transplantation. Human cell engraftment was assessed by flow cytometry of CD45+ cells. The level of human engraftment was defined as proportion of human CD45+ cells within the total population of murine and human CD45+ cells. The dotted line represents the definition of successful engraftment (≥0.5% human CD45+ cells). Human cell subpopulations in indicated organs were assessed by flow cytometry with antibodies for human CD45, CD33, CD19 and CD3. Bars indicate mean value and standard error.

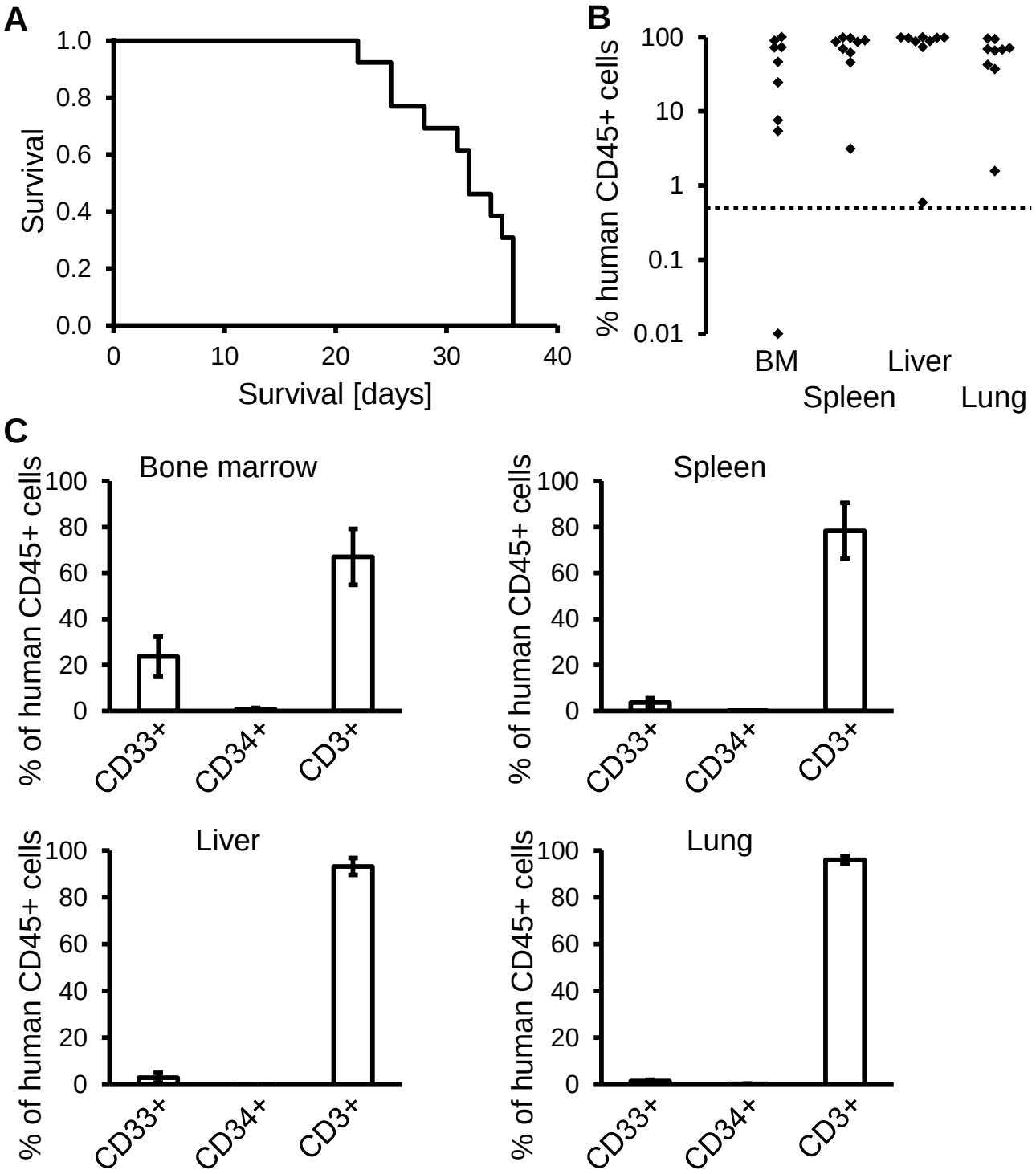
Supplemental Figure 6



Supplemental Figure 6. Human cytokines were detected in blood serum of engrafted mice

Blood serum was analyzed for human cytokine levels in a subset of engrafted (N=34) and not engrafted mice (N=6). Bars indicate mean value and standard error. Human interferon-γ (hINF-γ) was significantly increased over unspecific murine signals (Mann-Whitney test). Human tumor necrosis factor (hTNF) and interleukin-8 (hIL-8) were only detected in engrafted mice.

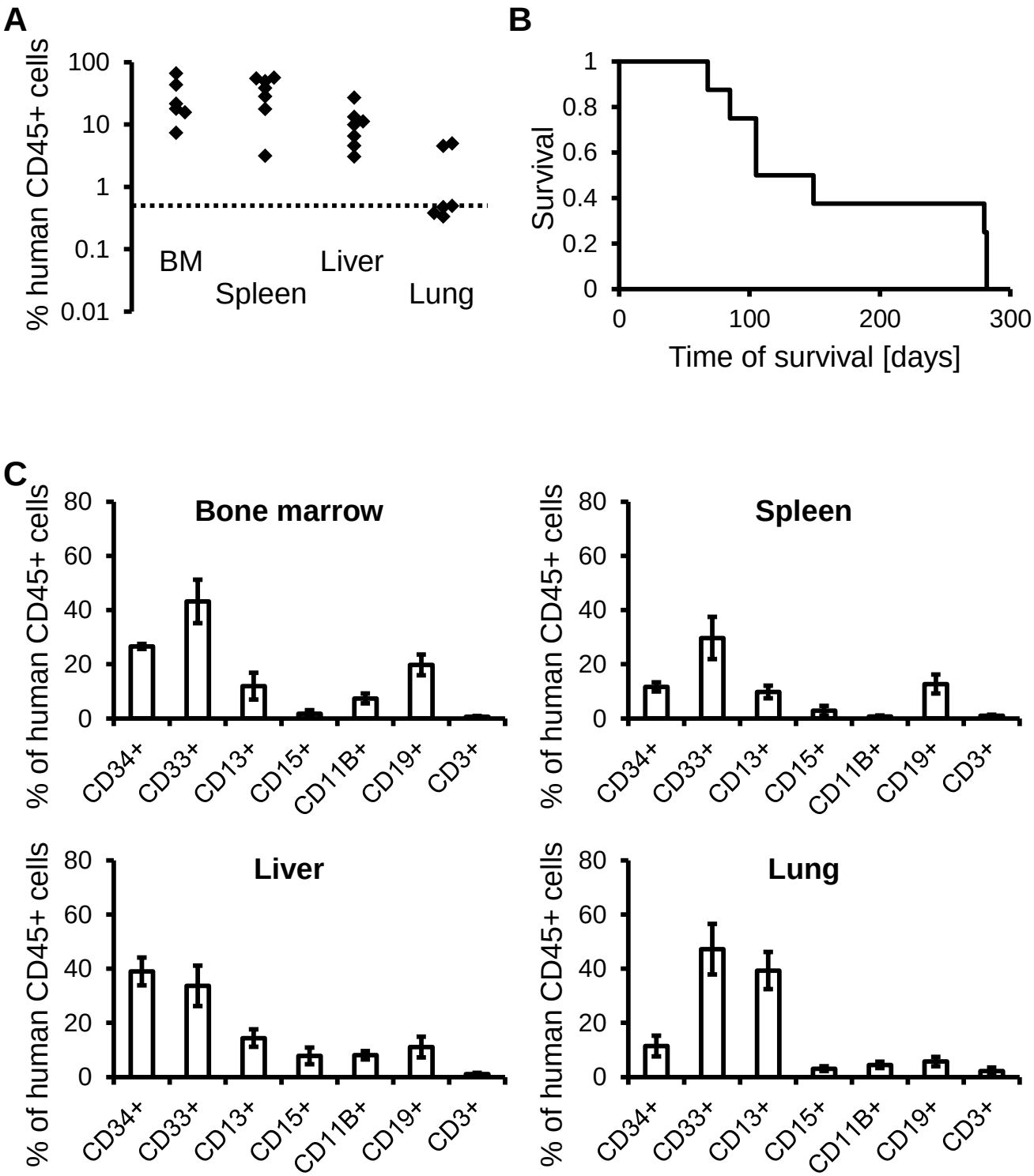
Supplemental Figure 7



Supplemental Figure 7. Graft-versus-host disease caused by co-transplanted, non-leukemic human T cells.

Thirteen newborn Rag2$^{-/-}$γc$^{-/-}$ mice were transplanted with 1x10^{6} spleen MNC from JMML patient #3. **(A)** All animals died within 36 days. **(B)** Human leukocytes were assessed by flow cytometry of CD45+ cells (N=9 mice available for analysis). The level of human engraftment was defined as proportion of human CD45+ cells within the total population of murine and human CD45+ cells. **(C)** Lineage-specific antibodies showed predominance of human CD3+ T cells. Mean value and standard error are shown (N=9).

Supplemental Figure 8



Supplemental Figure 8. Tertiary transplantation of JMML cells.
Eight newborn Rag2$^{-/-}$γc$^{-/-}$ mice were transplanted with BM cells (1 x 10^6 cells per animal) obtained from 4 secondary recipients of JMML cells originating from patient #2. The tertiary recipient animals were sacrificed for analysis when terminally sick. One mouse was unavailable for flow cytometry but successful engraftment was confirmed using DNA sequencing. **(A)** Human cell engraftment was assessed by flow cytometry of CD45+ cells. The level of human engraftment was defined as proportion of human CD45+ cells within the total population of murine and human CD45+ cells. The dotted line represents the definition of successful engraftment (≥0.5% human CD45+ cells). **(B)** Tertiary transplanted JMML xenograft mice survived 68 to 282 days after transplantation. **(C)** Cell subpopulations were assessed by flow cytometry with antibodies for human CD34, CD33, CD13, CD15, CD11B, CD19 and CD3. Bars indicate mean value and standard error.