Supplemental Materials for:

Cell-free DNA Profiling Informs All Major Complications of Hematopoietic Cell Transplantation

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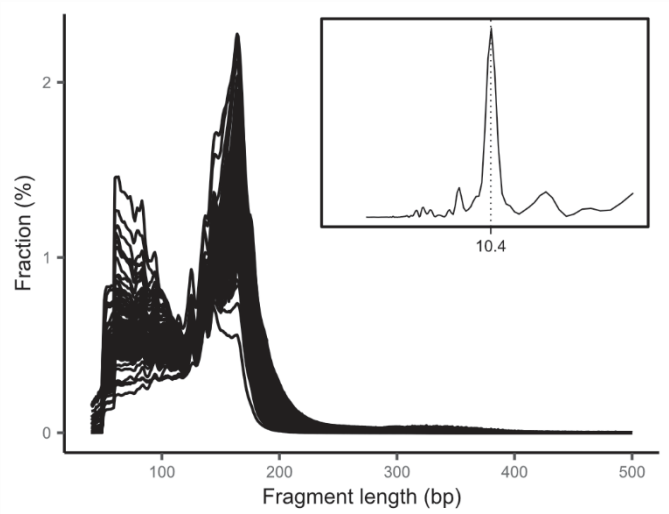
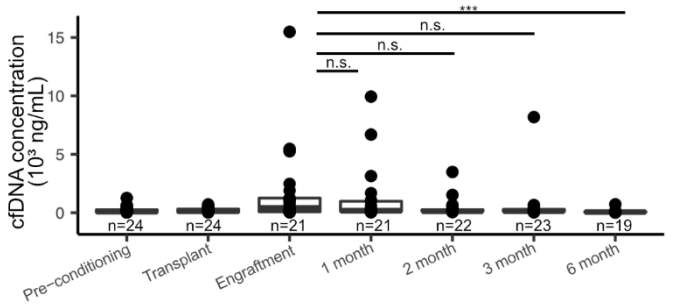
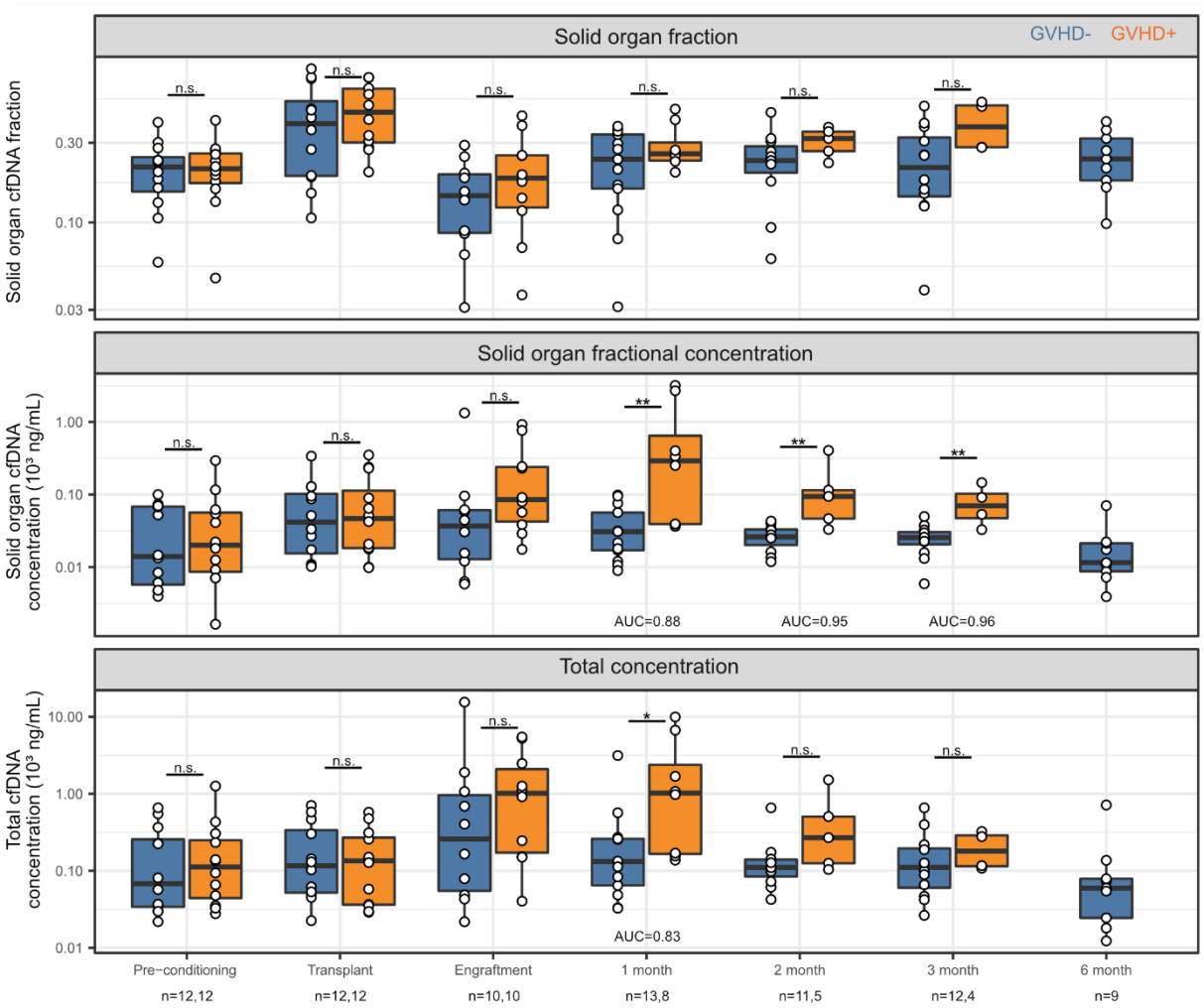


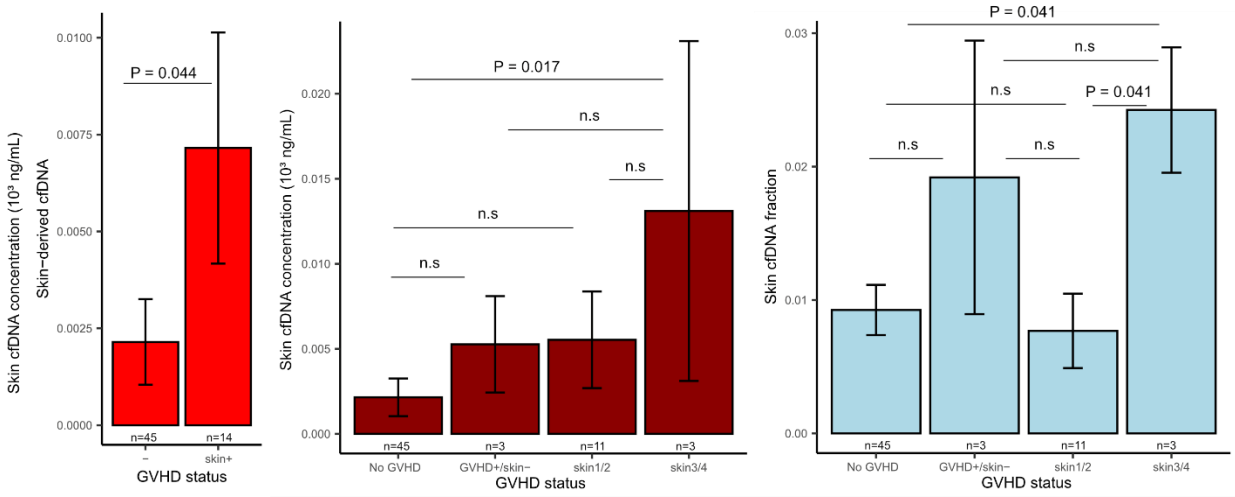
Supplementary figure 1. Fragment length profiles of 170 cfDNA samples after bisulfite treatment. Inset: Fourier analysis reveals a 10.4 bp periodicity in the fragment length profiles of bisulfite treated cfDNA.



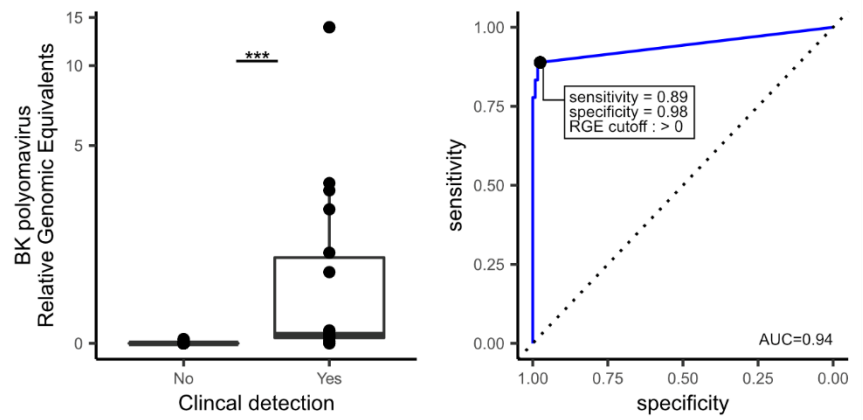
Supplementary figure 2. Total cfDNA concentration by timepoint.



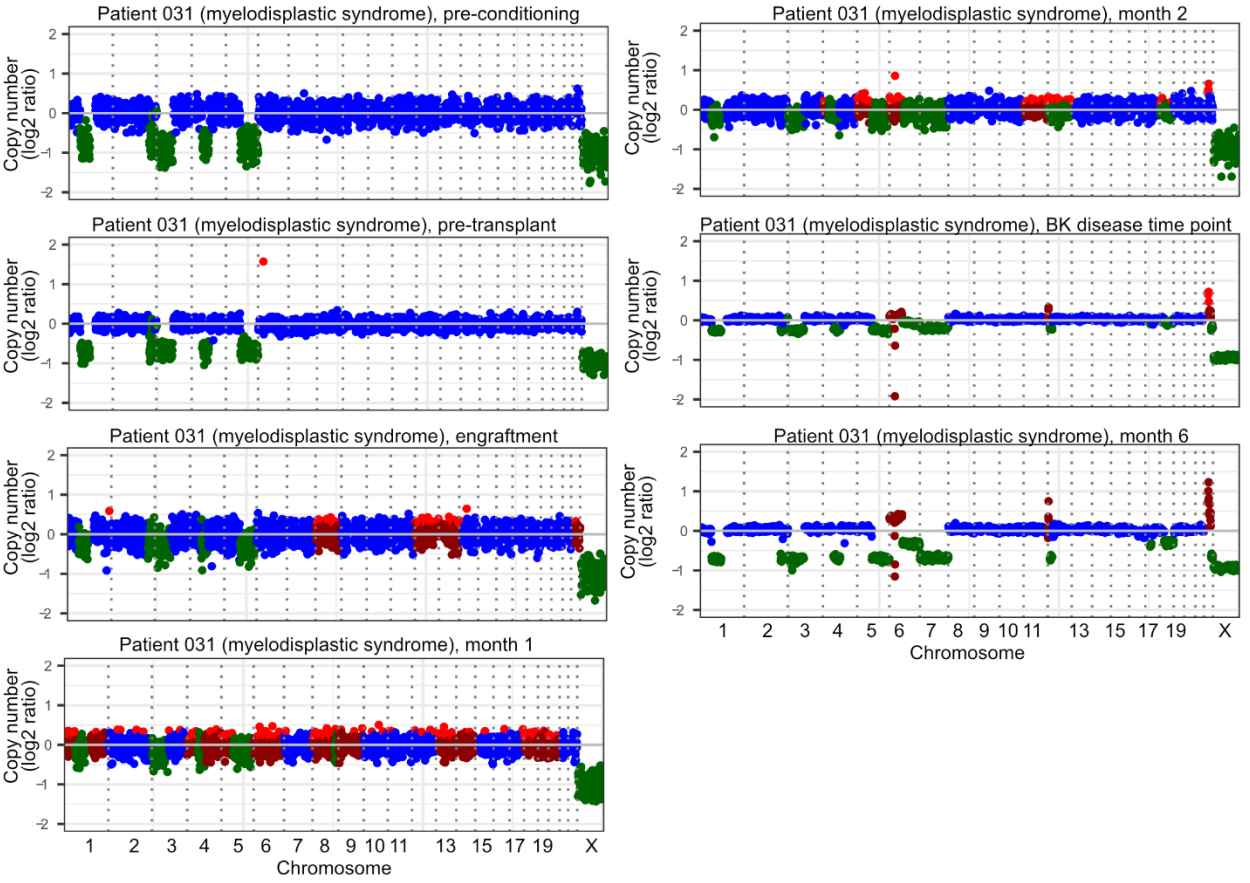
Supplementary figure 3. Tissue of origin and cell-free DNA concentrations to identify graft-versus-host disease. Top: Solid organ fraction. Middle: Solid organ fractional concentration. Bottom: Total concentration. Statistical tests for significance were performed with a two-sided Wilcoxon test. Receiving operating characteristic areas under the curve (AUCs) are shown for comparisons with a significant p-value. *: p-value < 0.05; **: p-value < 0.01.



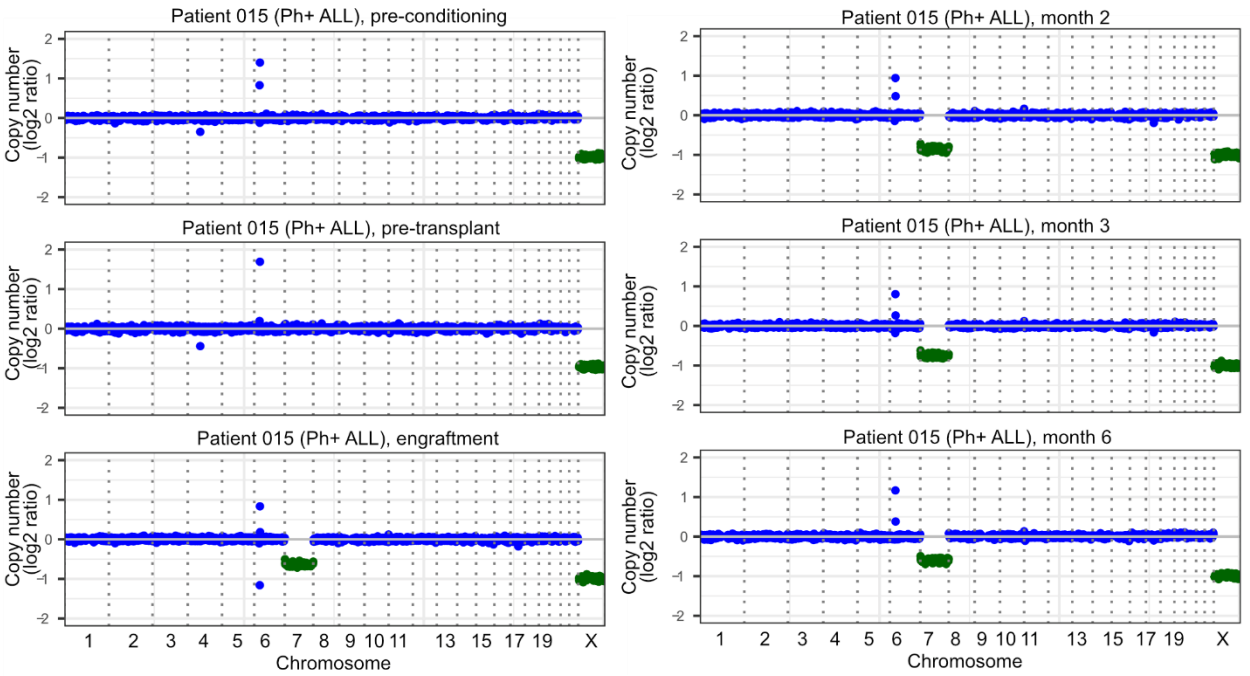
Supplementary figure 4. Skin-derived cfDNA in patients with GVHD. Left: skin cfDNA concentration by status (GVHD-, skin+). Middle: skin cfDNA concentration in 4 groups of patients (No GVHD, GVHD+/skin-, low-grade skin GVHD (grades 1 or 2), high grade skin GVHD (grades 3 or 4). Right: skin cfDNA proportion in 4 patient groups. Error bars represent standard error of the mean.



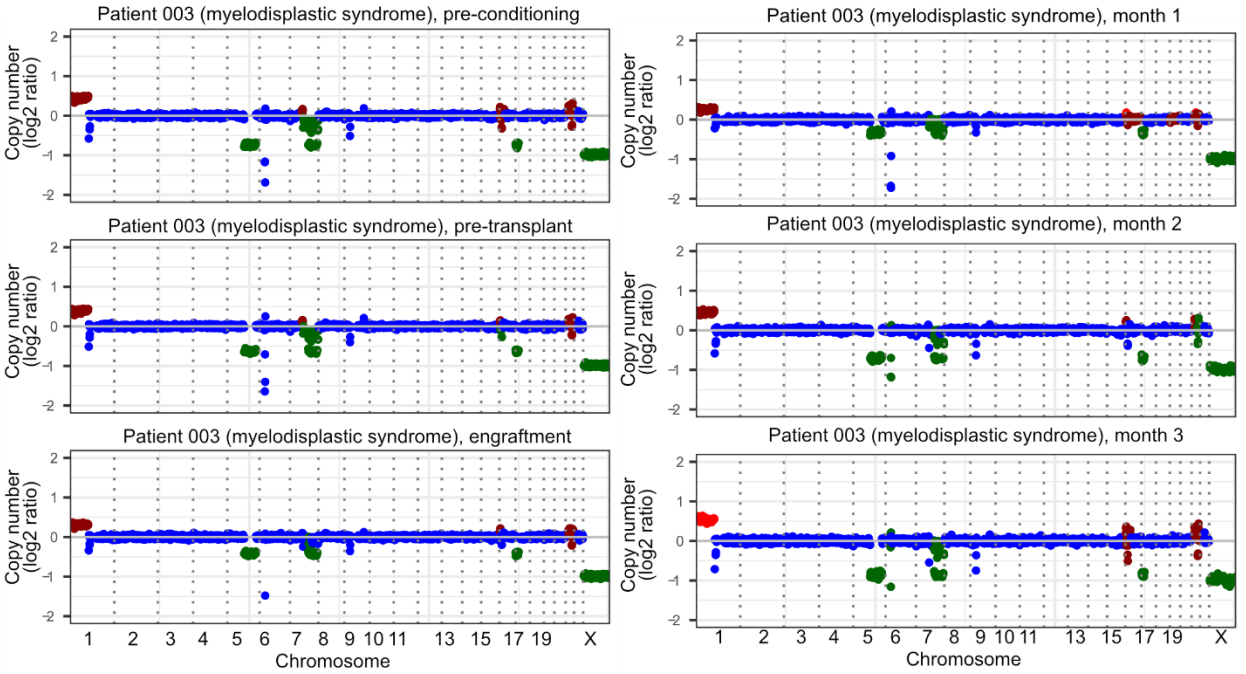
Supplementary figure 5. Comparative analysis between clinical detection and cell-free DNA detection of BK polyomavirus. Left: Boxplot comparison between the BK polyomavirus relative genomic abundance (RGE) in samples with clinical detection of BK in the blood and in samples without. Right: receiving operating characteristic curve analyzing the ability of BK RGE to identify BK polyomavirus in the blood. Note: at a cutoff of RGE > 0, there are 3 false positives and 2 false negatives identified. ***: p-value < 0.001.



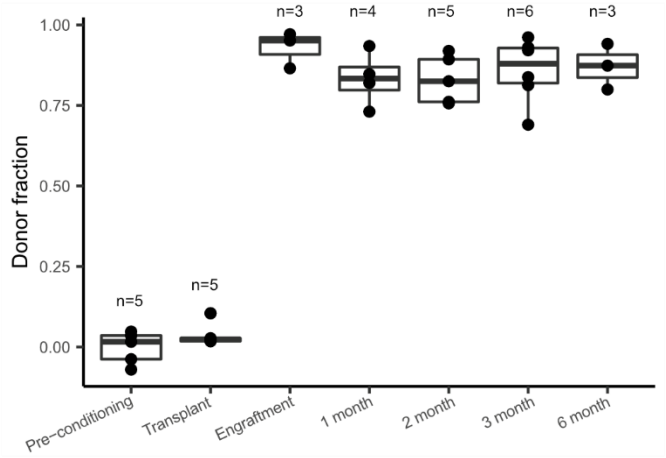
Supplementary figure 6. Copy number profiles for patient 031.



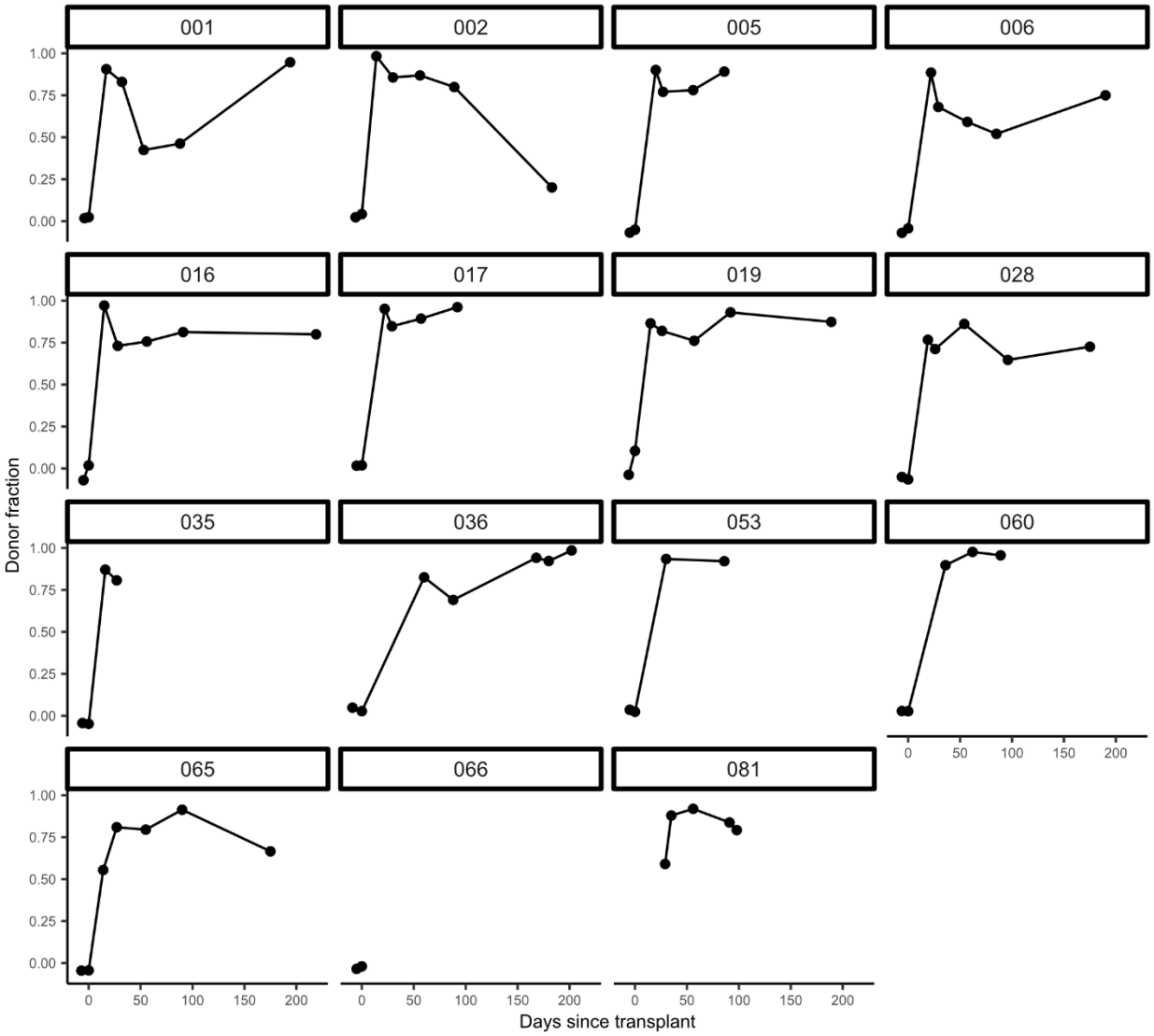
Supplementary figure 7. Copy number profiles for patient 015.



Supplementary figure 8. Copy number profiles for patient 003.



Supplementary figure 9. Donor fraction in sex-mismatched patients who did not suffer from GVHD, relapse, or loss of graft during the first 6 months of their transplant.



Supplementary figure 10. Donor fractions in sex-mismatched hematopoietic cell transplant patients by day since transplant. Plot titles refer to patient IDs.

Supplementary table 1. Clinical information

Age at enrollment -- median (range) (years)	60 (20-73)	**Relation to donor -- no (%)**	
Female sex -- no (%)	11 (41%)	Unrelated	21 (78%)
Race / Ethnicity -- no (%)		Related	6 (22%)
Caucasian	25 (93%)	**Recipient CMV status -- no (%)**	
American Indian/Alaskan Native	1 (4%)	**R+**	14 (52%)
Asian	1 (4%)	**R-**	13 (48%)
Reason for hematopoietic cell transplant -- no (%)[1]		**GVHD status -- no (%)[2]**	
Acute myeloid leukemia	7 (26%)	Overall grade I	6 (22%)
Myelodysplastic syndrome	5 (19%)	Overall grade II	4 (15%)
Acute lymphocytic leukemia	5 (19%)	Overall grade III	2 (7%)
T-Cell lymphoma	3 (11%)	Overall grade IV	5 (19%)
Mantle cell lymphoma	2 (7%)	Skin staging I	3 (11%)
Aplastic anemia	2 (7%)	Skin staging II	3 (11%)
Chronic myelomonocytic leukemia	1 (4%)	Skin staging III	2 (7%)
Chronic lymphocytic leukemia	1 (4%)	Skin staging IV	3 (11%)
Myelofibrosis	1 (4%)	Liver staging I	3 (11%)
Paroxysmal nocturnal hemoglobinuria	1 (4%)	Liver staging II	0 (0%)
Cutaneous lymphoma	1 (4%)	Liver staging III	1 (4%)
Source of HCT -- no (%)		Liver staging IV	0 (0%)
Peripheral blood	19 (70%)	Gut staging I	1 (4%)
Bone marrow	5 (19%)	Gut staging II	1 (4%)
Umbilical cord	3 (11%)	Gut staging III	1 (4%)
HLA matching -- no (%)		Gut staging IV	2 (7%)
Match	17 (63%)	**Conditioning regimen -- no (%)**	
Mismatch	6 (22%)	Reduced intensity	25 (93%)
Haploidentical	4 (15%)	Myeloablative	2 (7%)
Conditioning regimen -- no (%)		**Other characteristics -- no (%)**	
Busulfan, Fludarabine	14 (52%)	Mortality	3 (11%)
Cyclophosphamide, Fludarabine, total body irradiation (TBI)	4 (15%)	Previous HCT	0 (0%)
Fludarabine, Melphalan	2 (7%)	Time to GVHD onset (median ± std)	71 ± 55 days
Cyclophosphamide, Fludarabine, TBI, anti-thymocyte globulin	2 (7%)	T-cell depletion	0 (0%)
Busulfan, Fludarabine, Venetoclax	1 (4%)	**GVHD treatment – no (%)**	
Busulfan, Fludarabine, Thiotepa	1 (4%)	Glucocorticoids	9 (33%)
Fludarabine, anti-thymocyte globulin, Melphalan	1 (4%)	ruxolitinib, glucocorticoids	2 (7%)
Cyclophosphamide, TBI	1 (4%)	Sirolimus, ruxolitinib, glucocorticoids	1 (4%)

Cyclophosphamide, Fludarabine, TBI	1 (4%)	Tacrolimus, glucocorticoids	1 (4%)
GVHD prophylaxis -- no (%)		Mycophenolate mofetil, tacrolimus, glucocorticoids	1 (4%)
Methotrexate, Tacrolimus, Sirolimus	9 (33%)		
Methotrexate, Tacrolimus	9 (33%)		
Mycophenolate mofetil, Tacrolimus, Post-transplant cyclophosphamide	6 (22%)		
Tacrolimus, Sirolimus	2 (7%)		
Mycophenolate mofetil, Tacrolimus	1 (4%)		

1. Two individuals received an HCT for two blood disorders
2. Three individuals had two separate incidences of GVHD

Supplementary table 2. Oligonucleotides comprising nucleic acid control.

	Sequence (5'-3')
oligo1	TTTAACGCATAAACATGCGTTTTGGGTAGTGTTTTTTGGAAACACAGATCCGTGCGCACACCTGGTGGAG
oligo2	ATAAACATGCGTTTTGGGTAGTGTTTTTTGGAAACACAGATCCGTGCGCACACCT
oligo3	GCGTTTTGGGTAGTGTTTTTTGGAAACACAGATCCGTGCG
oligo4	GGTAGTGTTTTTTGGAAACACAGAT