Supplemental Table 1. Clinical data for prospective study patients.

Age	Disease	Donor	Conditioning	GVHD Prophylaxis	Development of skin GVHD
59	AML	MURD	Myeloablative Flu/Bu	Tac + MTX	Stage III (began 126 days post-tx)
44	AML	MRD	Myeloablative Flu/Bu	Tac + MTX	None
69	MDS	MURD	Non-myeloablative Flu/Bu	Tac + Siro + MTX	Stage II (began 180 days post-tx)

AML - acute myelogenous leukemia, MDS - myelodysplastic syndrome, MURD – matched unrelated donor, MRD – matched related donor, Flu – fludarabine, Bu – busulfan, Tac – tacrolimus, MTX – methotrexate, Siro – sirolimus, tx – transplant

Supplemental Table 2. Retrospective patient cohort (skin and gut) clinical parameters.

Clinical parameters	Skin Cohort	Gut Cohort
No. of patients	26	15
Age, median (range)	45.5 (19-64)	51 (16-66)
Conditioning		
Myeloablative, n (%)	17 (65.4)	9 (60)
Cy + TBI, n	15	0
Cy + Bu, n	1	9
Cy + TBI + ATG, n	1	0
Non-myeloablative, n (%)	9 (34.6)	6 (40)
Flu + Bu, n	8	2
Flu + Mel, n	1	0
Flu + Cy, n	0	3
Flu + Treo, n	0	1
MHC allele match/mismatch		
Matched, n (%)	17 (65.4)	12 (80)
Mismatched, n (%)	9 (34.6)	3 (20)
Stem cell source		
PBSCT, n (%)	22 (84.6)	12 (80)
BMSCT, n (%)	4 (15.4)	3 (20)
GVHD prophylaxis		
Tac + MTX, n	13	0
Tac + Siro, n	6	0
Tac + Siro + MTX, n	3	0
MMF + Siro, n	3	0
Tac + MTX + Bortezemib, n	1	0
MTX + Cy, n	0	14
MTX + Siro, n	0	1
GVHD following DLI, n	1	0
Underlying diagnosis (n)	AML (6), MDS/MPD/MF (5), ALL (3), CML (3), CMML (1), CLL (3), Follicular lymphoma (3), Mantle cell lymphoma (1), Aplastic anemia (1)	AML (6), MDS (1), Hodgkin's lymphoma (1), CMML (3), ALL (2), DLBCL (1), Follicular lymphoma (1)

Cy – cyclophosphamide, TBI – total body irradiation, Bu – busulfan, ATG – anti-thymocyte globulin, Flu – fludarabine, Mel – melphalan, Treo – treosulfan, PB – peripheral blood, BM – bone marrow, Tac – tacrolimus, MTX – methotrexate, Siro – sirolimus, MMF – mycophenylate mofetil AML - acute myelogenous leukemia, MDS/MPD/MF - myelodysplastic syndrome/myeloproliferative disease/myelofibrosis, ALL – acute lymphocytic leukemia, CML – chronic myelogenous leukemia, CLL – chronic lymphocytic leukemia, CMML – chronic myelomonocytic leukemia, DLBCL – diffuse large B cell lymphoma

Supplemental Table 3. Anti-cancer medications administered prior to allogeneic transplant in retrospective skin cohort.

Age[A]	Disease[B]	Cancer Medications Prior to Allogeneic Transplant[C]	% Host T Cells During aGVHD
57	CLL	Fludarabine, Cyclophosphamide, Rituximab, Mycophenolic acid	50
60	CLL	Chlorambucil, Fludarabine, Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Corticosteroids, Methotrexate, Cytarabine, Alemtuzumab	13
46	AML	Cytarabine, Idarubicin	95
47	CML	Cytarabine, Daunorubicin, Imatinib	29
32	CML	Hydroxyurea, Imatinib	39
27	Aplastic Anemia	None	100
23	CML	L-asparaginase, Cyclophosphamide, Cytarabine, Corticosteroids, Vincristine, Doxorubicin, Methotrexate, 6-Mercaptopurine, Imatinib	37
45	ALL	Cyclophosphamide, Doxorubicin, Vincristine, Corticosteroids, Methotrexate, Cytarabine, Mitoxantrone, Imatinib, Dasatinib	38
33	AML	Cytarabine, Daunorubicin, Clofarabine	52
21	ALL	L-asparaginase, Cyclophosphamide, Cytarabine, Vincristine, Thioguanine, Corticosteroids, Methotrexate, 6-Mercaptopurine, Daunorubicin	10
44	CMML	None	4
52	MDS (arising out of Hodgkin's)	Doxorubicin, Bleomycin, Vinblastine, Dacarbazine, Ifosfamide, Carboplatin, Etoposide, Cyclophosphamide, Gemcitabine, Vinorelbine, Chlorambucil, Procarbazine, Corticosteroids, Rituximab, anti-CD25 monoclonal antibody, Prior autologous transplant (Cyclophosphamide, Carmustine, Etoposide)	65
57	MF	Anagrelide, Hydroxyurea	59
19	AML	Hydroxyurea, Daunorubicin, Cytarabine, Etoposide	63
45	CLL	Fludarabine, Rituximab, Pentostatin, Cyclophosphamide, Corticosteroids, Cytarabine, Cisplatin, Gemcitabine, Vinorelbine, Doxorubicin, Alemtuzumab	11
41	MDS	Azacitidine	43
59	FL	Rituximab, Cyclophosphamide, Vincristine, Doxorubicin, Corticosteroids, Mitumprotimut-T/GM-CSF trial, Fludarabine, Mitoxantrone, Ibritumomab, Ifosfamide, Carboplatin, Etoposide, Prior autologous transplant (Cyclophosphamide, Carmustine, Etoposide)	58
64	MDS	None	51
53	MCL	Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Corticosteroids, Bortezomib	72
54	FL	Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Corticosteroids, Fludarabine, Ifosfamide, Carboplatin, Etoposide, Ibritumomab	78
41	AML	Anthracycline, Cytarabine	14
38	MDS/MPD	Decitabine	58
57	FL	Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Corticosteroids, Bendamustine, Ifosfamide, Carboplatin, Etoposide	50
46	AML	Cytarabine, Daunorubicin, Etoposide	41
54	AML	Idarubicin, Cytarabine, Mitoxantrone, Etoposide, Lenalidomide	3
24	ALL	Cyclophosphamide, Doxorubicin, Vincristine, Corticosteroids, Methotrexate, Cytarabine, Mitoxantrone	20

[A]Age at time of transplant

[B]AML - acute myelogenous leukemia, MDS/MPD/MF - myelodysplastic syndrome/myeloproliferative disease/myelofibrosis, ALL – acute lymphocytic leukemia, CML – chronic myelogenous leukemia, CLL – chronic lymphocytic leukemia, CMML – chronic myelomonocytic leukemia, FL – follicular lymphoma; MCL – mantle cell lymphoma

[C]Treatments are not necessarily in temporal order and some treatments were repeated one or more times.

Supplemental Table 4. Anti-cancer medications administered prior to allogeneic transplant in retrospective gut cohort.

Age[A]	**Disease[B]**	**Cancer Medications Prior to Allogeneic Transplant[C]**	**% Host T cells During aGVHD**
58	AML	Cytarabine, Daunorubicin	15
21	ALL	Vincristine, Doxorubicin, Methotrexate, Cyclophosphamide, 6-mercaptopurine	28
66	MDS	None	7
16	HL	Vincristine, Doxorubicin, Etoposide	16
46	CMML	None	22
65	CMML	Azacitidine	9
35	AML	Cytarabine, Daunorubucin	6
24	AML	Cytarabine, Etoposide, Amsacrine	8
45	AML	Cytarabin, Daunorubicin, Idarubucin	73
61	AML	Cytarabine, Amsacrine, Idarubicine	41
50	FL	Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Etoposide, Fludarabine	13
42	DLBCL	Radiotherapy, Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Etoposide	10
61	ALL	Vincristine, Doxorubicin, Methotrexate, Cyclophosphamide, 6-mercaptopurine	87
60	CMML	Daunorubicin, Cytarabine, Fludarabine, Idarubicine	37
55	AML	Daunorubicin, Cytarabine	17

[A]Age at time of transplant

[B]AML - acute myelogenous leukemia, MDS - myelodysplastic syndrome, ALL – acute lymphocytic leukemia, CLL – chronic lymphocytic leukemia, CMML – chronic myelomonocytic leukemia, HL – Hodgkin's lymphoma, FL – follicular lymphoma, DLBCL – diffuse large B cell lymphoma

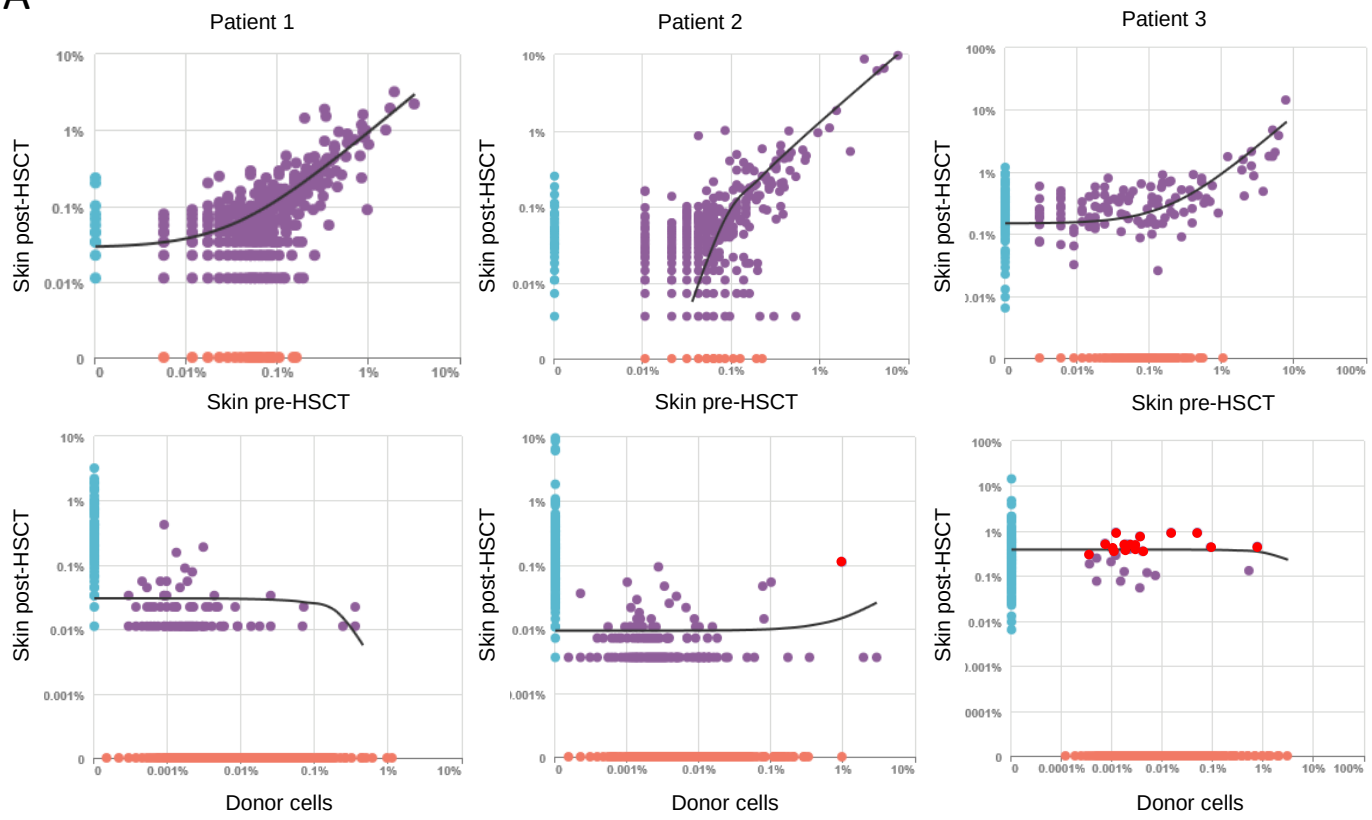
[C]Treatments are not necessarily in temporal order and some treatments were repeated one or more times.

Supplemental Table 5. UK prospective patient cohort clinical parameters.

Clinical parameters	**Skin Cohort**
No. of patients	34
Age, median (range)	52 (25-68)
Conditioning	
Myeloablative, n (%)	12 (35)
TBI + Mel + Etop or Cy, n	7
Cy + Bu, n	5
Non-myeloablative, n (%)	22 (65)
Flu + Mel, n	22
BEAM, n	1
MHC allele match/mismatch	
Matched, n (%)	34 (100)
Mismatched, n (%)	0 (0)
Stem cell source	
PBSCT, n (%)	33 (97)
BMSCT, n (%)	1 (3)
GVHD prophylaxis	
Alemtuzumab + Cy, n	27
MTX + Cy, n	7

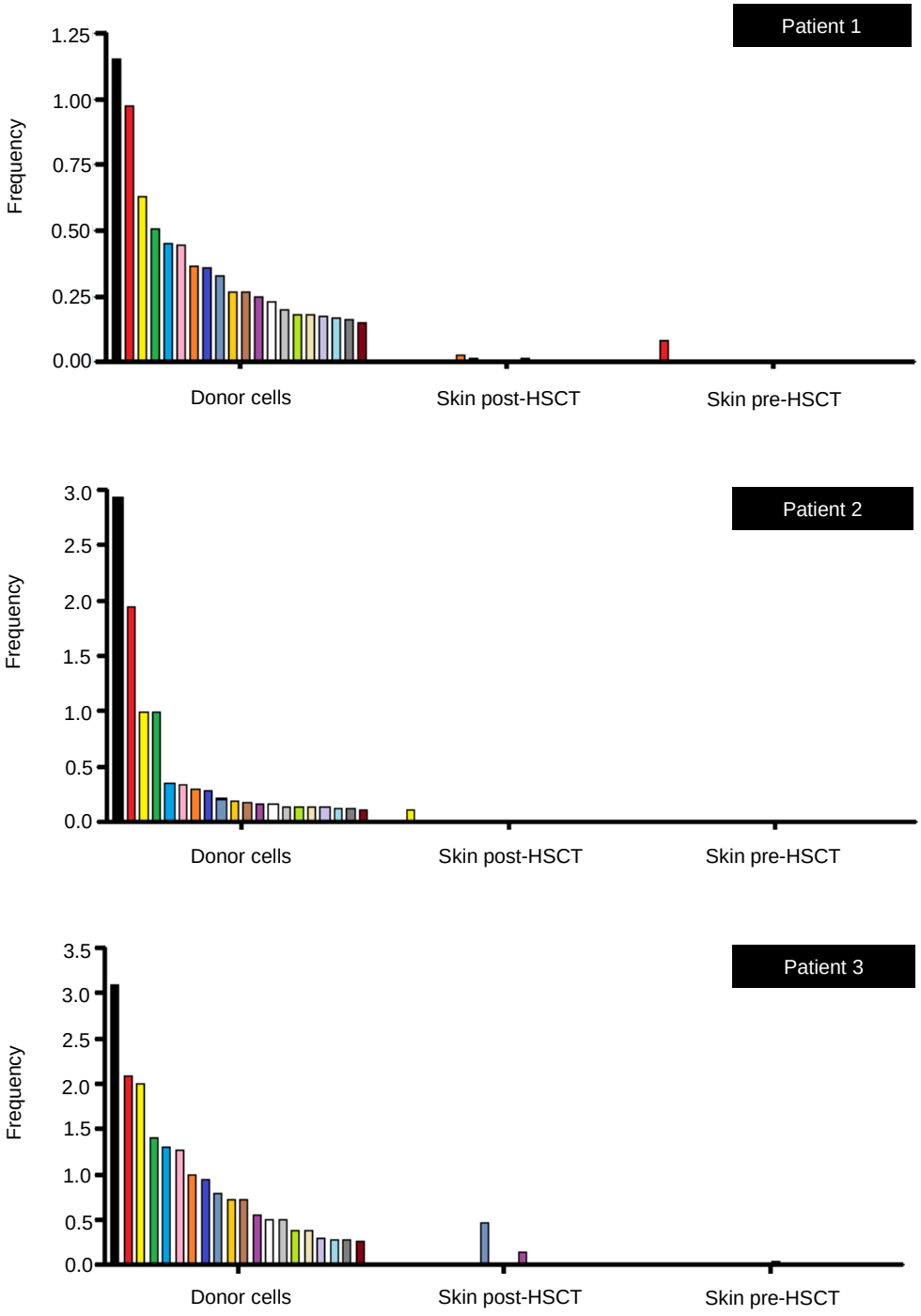
TBI – total body irradiation, Cy – cyclophosphamide, Bu – busulfan, Flu – fludarabine, Mel – melphalan, Etop – etoposide, PB – peripheral blood, BM – bone marrow, MTX – methotrexate, BEAM – BCNU + etoposide + Ara-C + melphalan

A



Supplemental Figure 1A

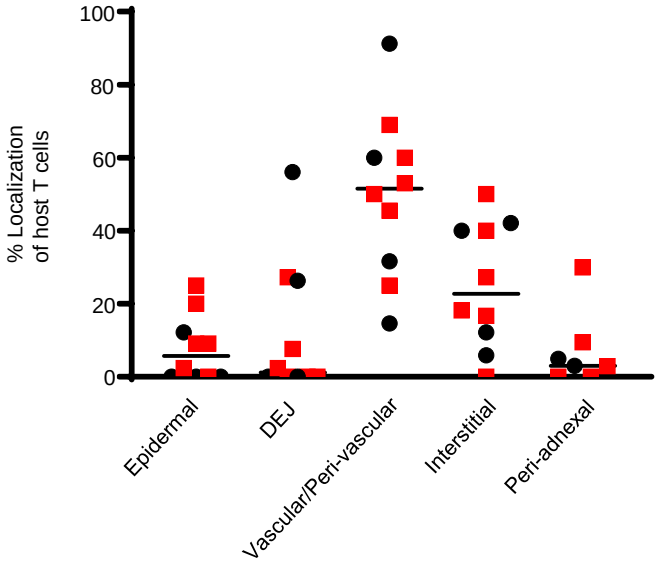
B



Supplemental Figure 1B

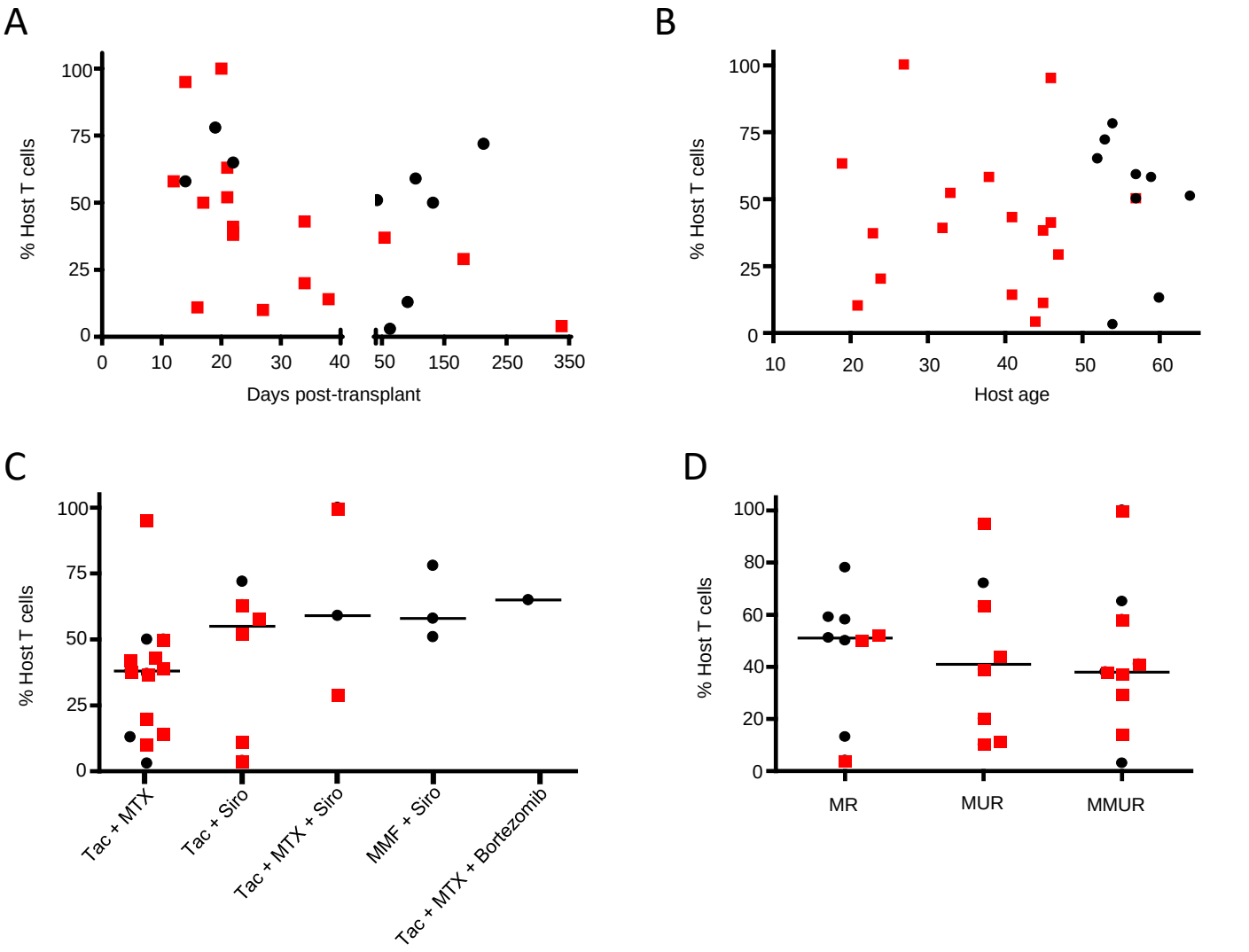
Supplemental Figure 1. Host skin T cells post-HSCT originate from skin pre-HSCT not donor infusion product.

(A) Dot plots showing unique T cell clones (skin post-HSCT = teal dots, donor cell or host skin pre-HSCT = orange dots) and shared T cell clones (purple or red dots) between host skin post-HSCT vs host skin pre-HSCT (top row) or host skin post-HSCT vs donor infusion product "Donor cells" (bottom row). Of the 100 most frequent T cell clones in host skin post-HSCT, those derived from donor infusion cells are shown as red circles. Axes are clonal frequency. Line of regression on log transformed scale, skin post-HSCT vs skin pre-HSCT r^2 patient 1-0.6464, patient 2-0.8740, patient 3-0.5867; skin post-HSCT vs donor cells r^2 patient 1-0.0041, patient 2-0.0142, patient 3-0.0012. **(B)** Bar graph for each of 3 patients showing the top 20 T cell clones in donor infusion product "Donor cells" and whether those same clones were present, and if so at what frequency, in host skin pre-HSCT or host skin post-HSCT. Each individual clone is color coded.



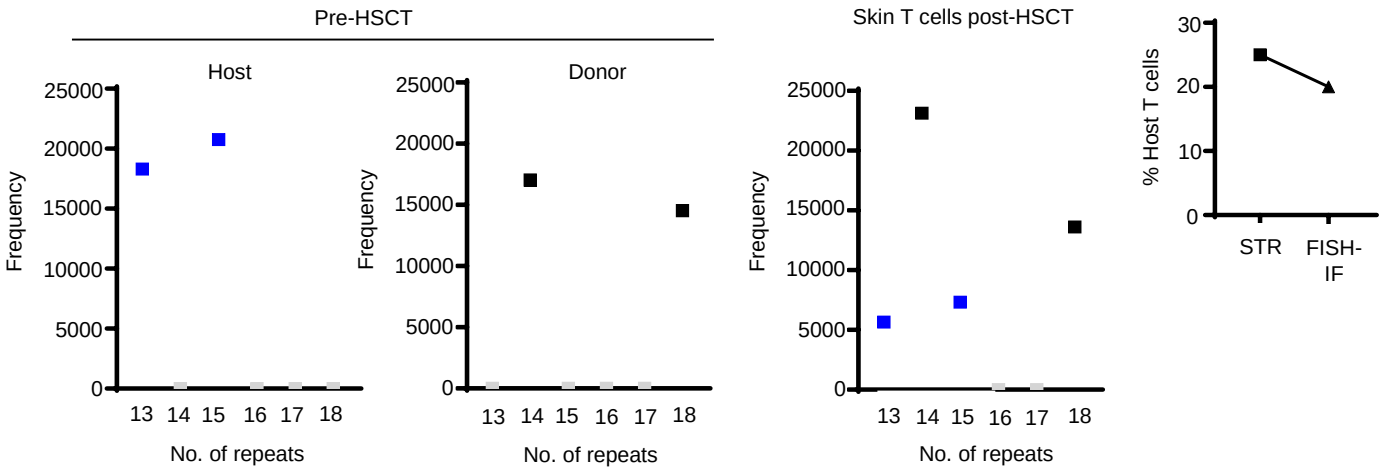
Supplemental Figure 2

Supplemental Figure 2. Host T cells are present throughout skin compartments during acute GVHD. Distribution of host T cells as a percentage of all localizable host T cells in skin of a representative subset of patients, $n = 10$ (including a range of clinical severity and both myeloablative and non-myeloablative patients). Adnexal structures were only available for analysis in 7 of 10 cases. Myeloablative – red squares; non-myeloablative – black circles. Black lines – median. DEJ-dermal-epidermal junction.



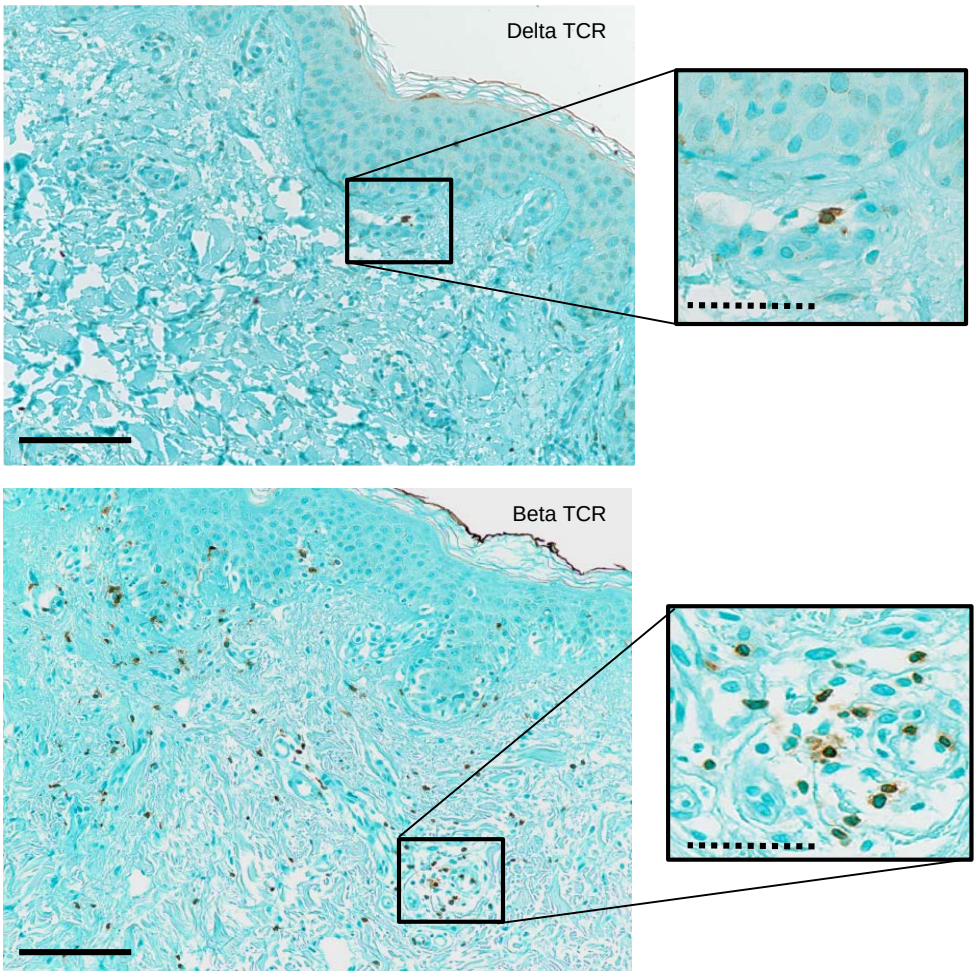
Supplemental Figure 3

Supplemental Figure 3. Host T cells in skin during acute GVHD relative to clinical parameters. (A) Percent host T cell chimerism, determined by FISH-IF, during acute GVHD as a function of time post-HSCT. Linear regression myeloablative slope -0.1384, $P = 0.09$; non-myeloablative slope 0.01133, $P = 0.94$. **(B)** Percent host T cells in skin determined by FISH-IF as a function of host age at time of transplant. Linear regression myeloablative slope -0.1302, $P = 0.84$; non-myeloablative slope -1.619, $P = 0.52$. **(C)** Percent host T cells in skin determined by FISH-IF as a function of GVHD prophylaxis. One-way Kruskal-Wallis test, excluding Tac+MTX+bortezomib since $n = 1$, $P = 0.13$. **(D)** Percent host T cells in skin determined by FISH-IF as a function of donor type - matched related (MR), matched unrelated (MUR), or mismatch unrelated (MMUR). One-way Kruskal-Wallis test, $P = 0.84$. Myeloablative – red squares; non-myeloablative – black circles. Red squares – myeloablative; Black circles – non-myeloablative. Black lines – median. $n = 26$.



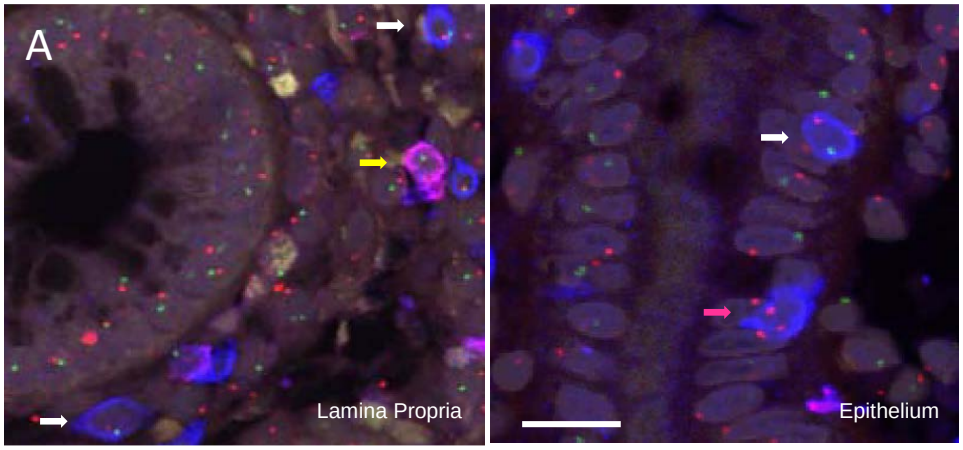
Supplemental Figure 4

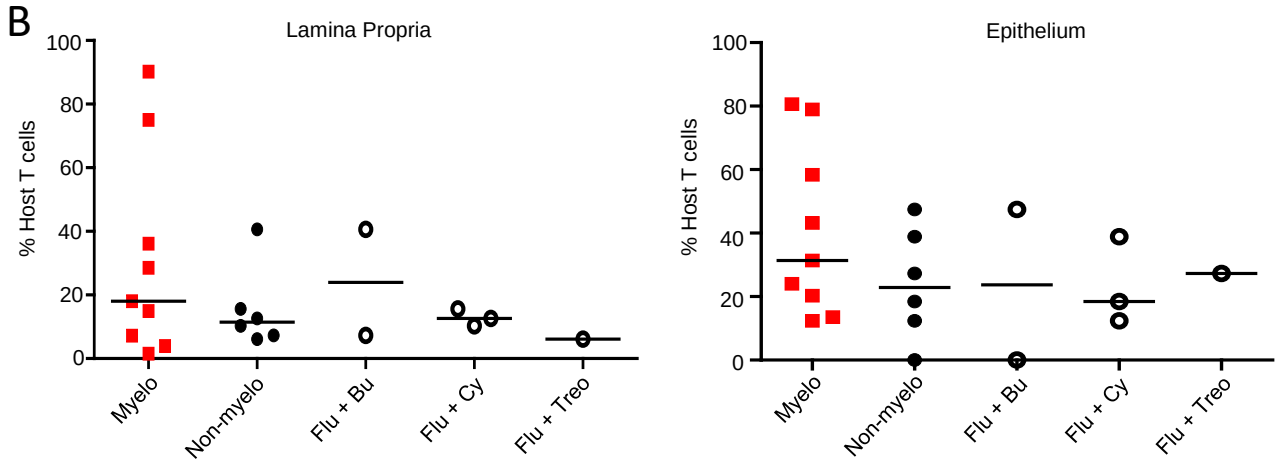
Supplemental Figure 4. Short tandem repeat analysis supports chimerism calculated from FISH-IF. STR analysis at the D18S51 locus of host pre-HSCT, blue (one allele 13 repeats, one allele 15 repeats) and donor pre-HSCT, black (one allele 14 repeats, one allele 18 repeats) then of host skin T cells during acute GVHD with 4 alleles present – 2 donor, black; 2 host, blue – at a 3:1 frequency. Inset shows comparison of percentage host T cells of the same patient by STR and FISH-IF analyses. Analysis performed on freshest patient specimen; older FFPE samples were too degraded for sufficient genomic material for analysis.

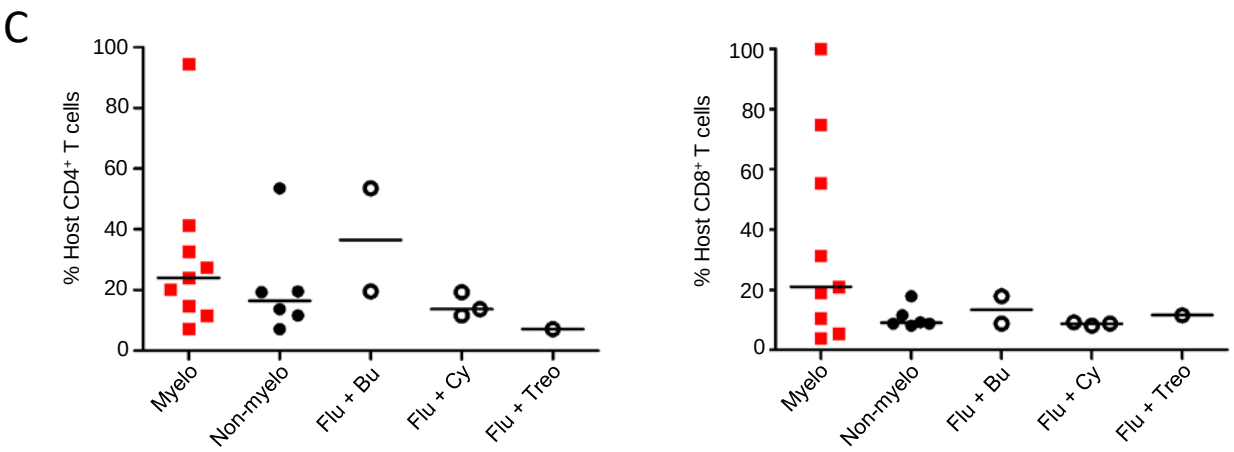


Supplemental Figure 5

Supplemental Figure 5. Host T cells in skin during acute GVHD are αβ T cells not γδ T cells. Skin samples from five patients were labelled via immunohistochemistry using antibodies against delta TCR or beta TCR. Representative images from one patient are shown. Staining was developed using DAB (brown) and counterstained with methyl green. Black bars: 100 μm. Black dashed bars: 50 μm.

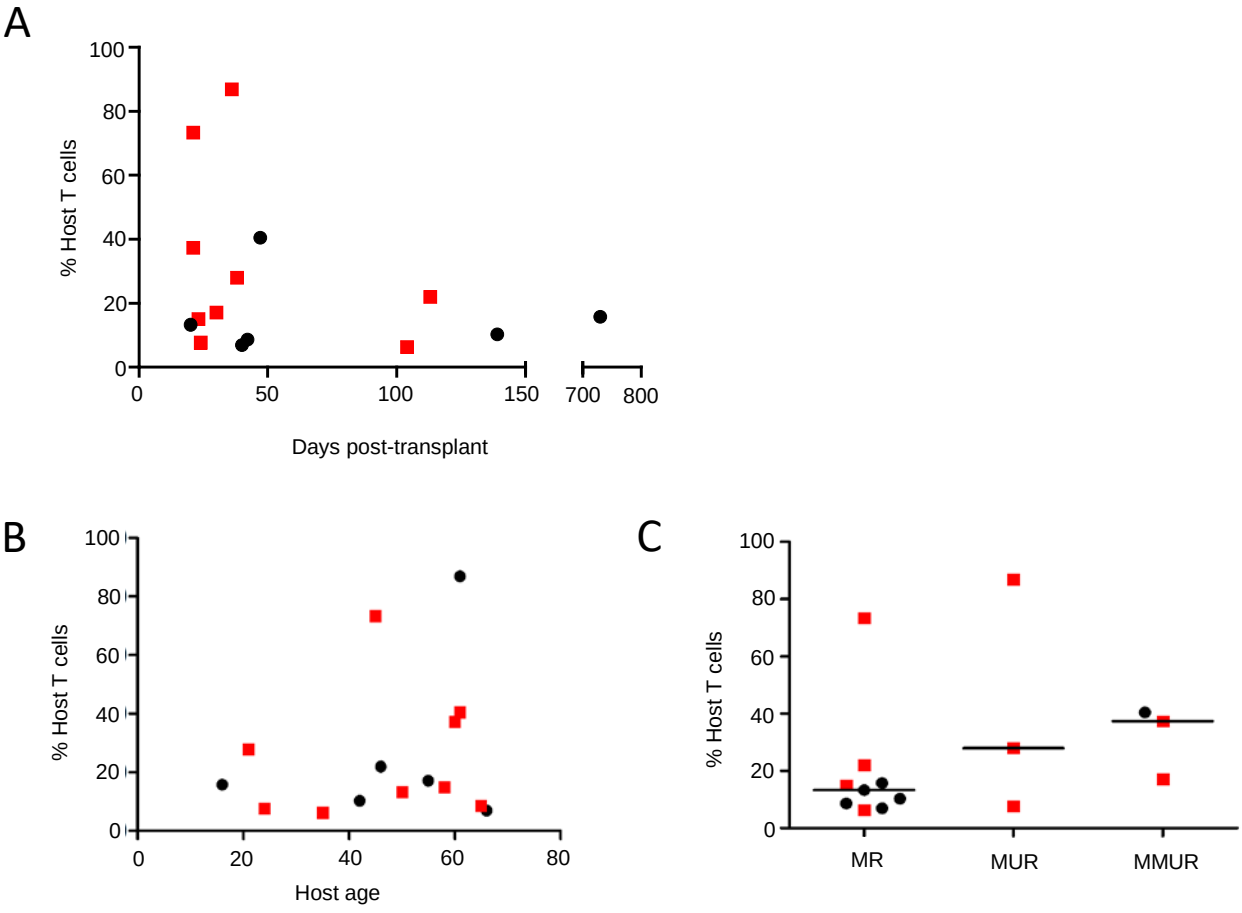






Supplemental Figure 6

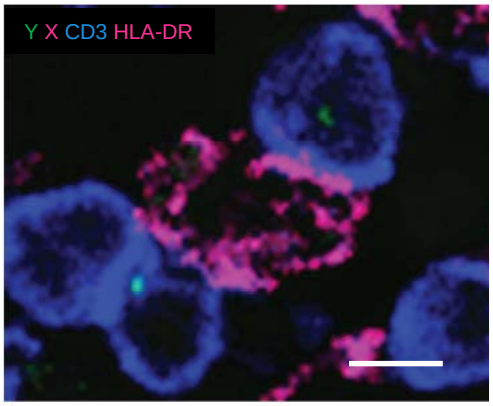
Supplemental Figure 6. Host T cells in colon during acute GVHD are present in both epithelium and lamina propria and are both $CD8^+$ and $CD8^-$ ($CD4^+$) T cell subsets. (A) Example FISH-IF from FFPE colon during acute GVHD showing host T cells within lamina propria and epithelium. X chromosome, red; Y chromosome, green; CD3, blue; CD8, red; Hoechst nuclear stain, grey. White bar: 20 μm. Blue staining indicates CD4 T cells ($CD3^+CD8^-$) whereas pink staining (mixed blue and red) indicates $CD3^+CD8^+$ T cells. Pink arrow points to donor (XX) T cell, yellow arrow points to host (Y) $CD8^+CD3^+$ T cell, and white arrows point to host (Y) CD4 T cell ($CD3^+CD8^-$). **(B)** Percent host T cell chimerism in acute GVHD in lamina propria and epithelium. **(C)** Percent host CD4 and CD8 T cell chimerism in acute gut GVHD. Red squares - myeloablative conditioning; Solid black circles - non-myeloablative conditioning; Open black circles - breakdown of non-myeloablative regimens. Black lines – median. CD4 T cells: myeloablative, median 24% range 7-95%; non-myeloablative, median 17% range 7-54%, $P = 0.33$, Mann-Whitney, two-tailed. CD8 T cells: myeloablative, median 21% range 4-100%; non-myeloablative, median 9.0% range 8-18%, $P = 0.15$, Mann-Whitney, two-tailed. $n = 15$ for all experiments.



Supplemental Figure 7

Supplemental Figure 7. Host T cells in colon during acute GVHD relative to clinical parameters. (A) Percent host gut T cell chimerism determined by FISH-IF during acute GVHD as a function of time post-HSCT. Linear regression slope -0.10, $P = 0.54$. **(B)** Percent host T cells as a function of host age. Linear regression slope 0.3195, $P = 0.44$ and **(C)** type of donor - matched related (MR), matched unrelated (MUR), or mismatch unrelated (MMUR). One-way Kruskal-Wallis test, $P = 0.1$. Red squares – myeloablative; Black circles – non-myeloablative. Black lines – median.

A



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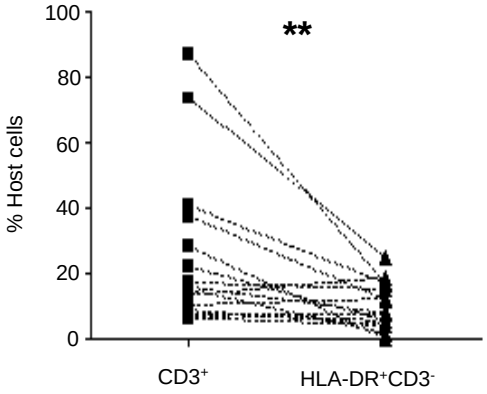
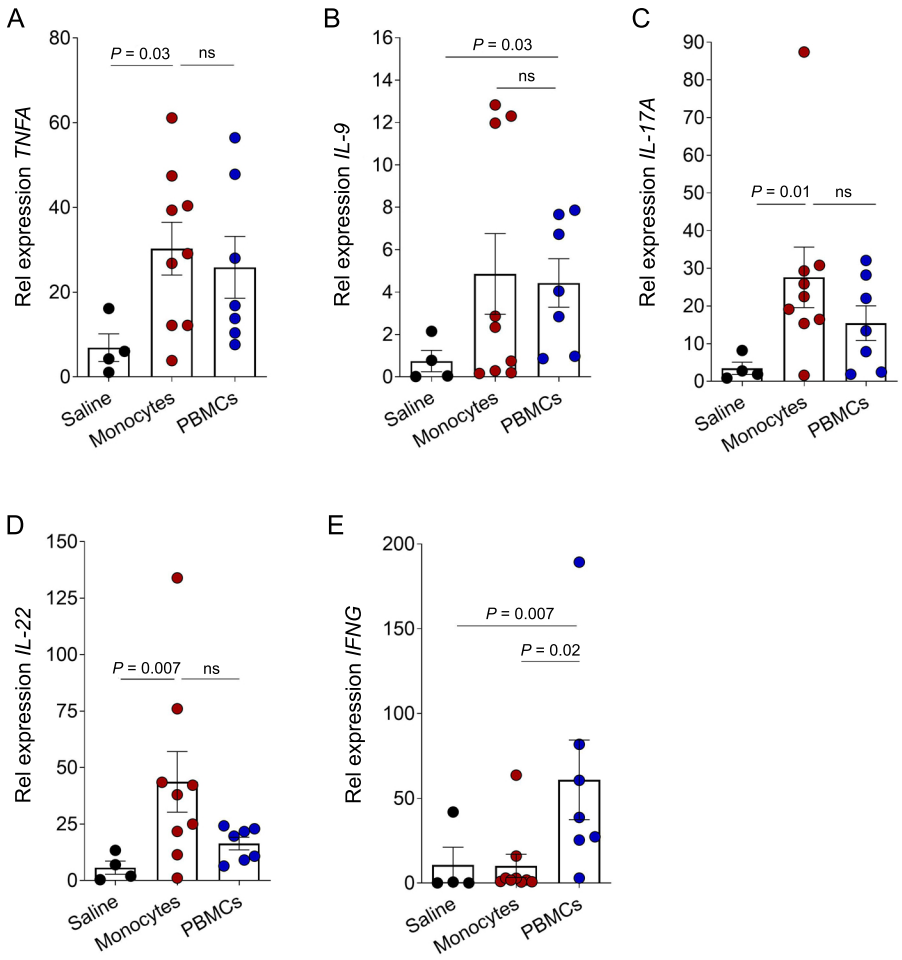


Supplemental Figure 8

Supplemental Figure 8. Donor APC are in close proximity to host T cells in gut acute GVHD. (A) Example FISH-IF staining for X chromosome and HLA-DR, red; Y chromosome, green; CD3, blue; Hoechst nuclear stain, grey. White bar: 20 μm. **(B)** Percent host $CD3^+$ T cells and APC ($HLA\text{-}DR^+CD3^-$) within each gut acute GVHD sample, by FISH-IF. Wilcoxon signed rank test, paired, two-tailed. ** $P = 0.004$.



Supplemental Figure 9

Supplemental Figure 9. Host skin T cells induce GVHD-like dermatitis with similar cytokine profile as donor T cell mediated disease in human skin grafted mice. Inflammatory cytokine production in skin grafts of *TNFA* **(A)**, *IL-9* **(B)**, *IL-17A* **(C)**, and *IL-22* **(D)** was similar in monocyte- and PBMC-injected mice. Only *IFNG* transcript **(E)** was lower in monocyte-injected mice compared to PBMC-injected mice. Analysis was performed on saline-injected (n = 4), monocyte-injected (n = 9) and PBMC-injected (n = 7) mice. Mean and SEM (error bars) are shown. One-way Kruskal–Wallis test with Dunn's post-test was used for comparing multiple independent groups. ns, not significant.