Extended data

Maternal Dendritic Cells Influence Fetal Allograft Response and Tolerance

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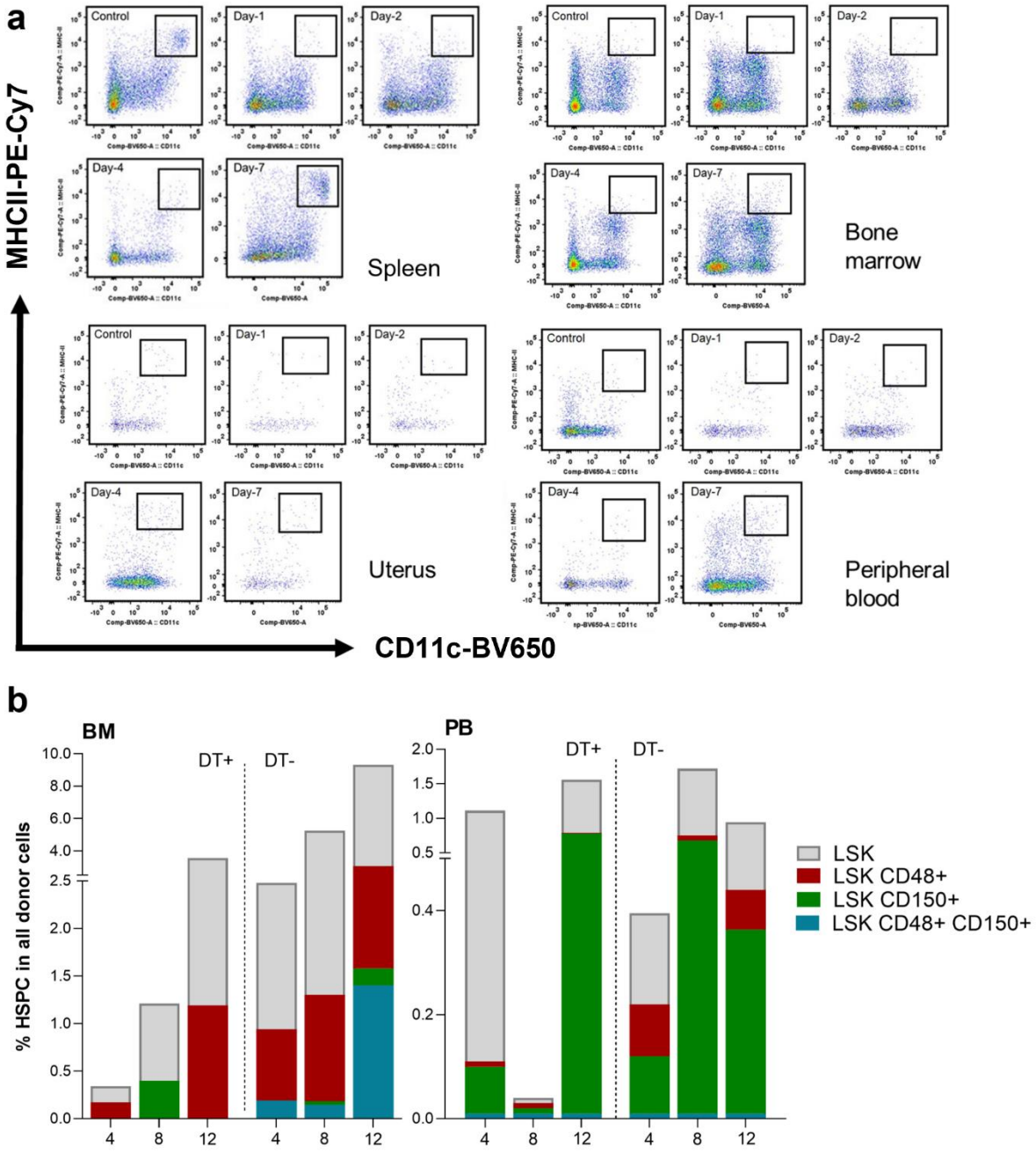
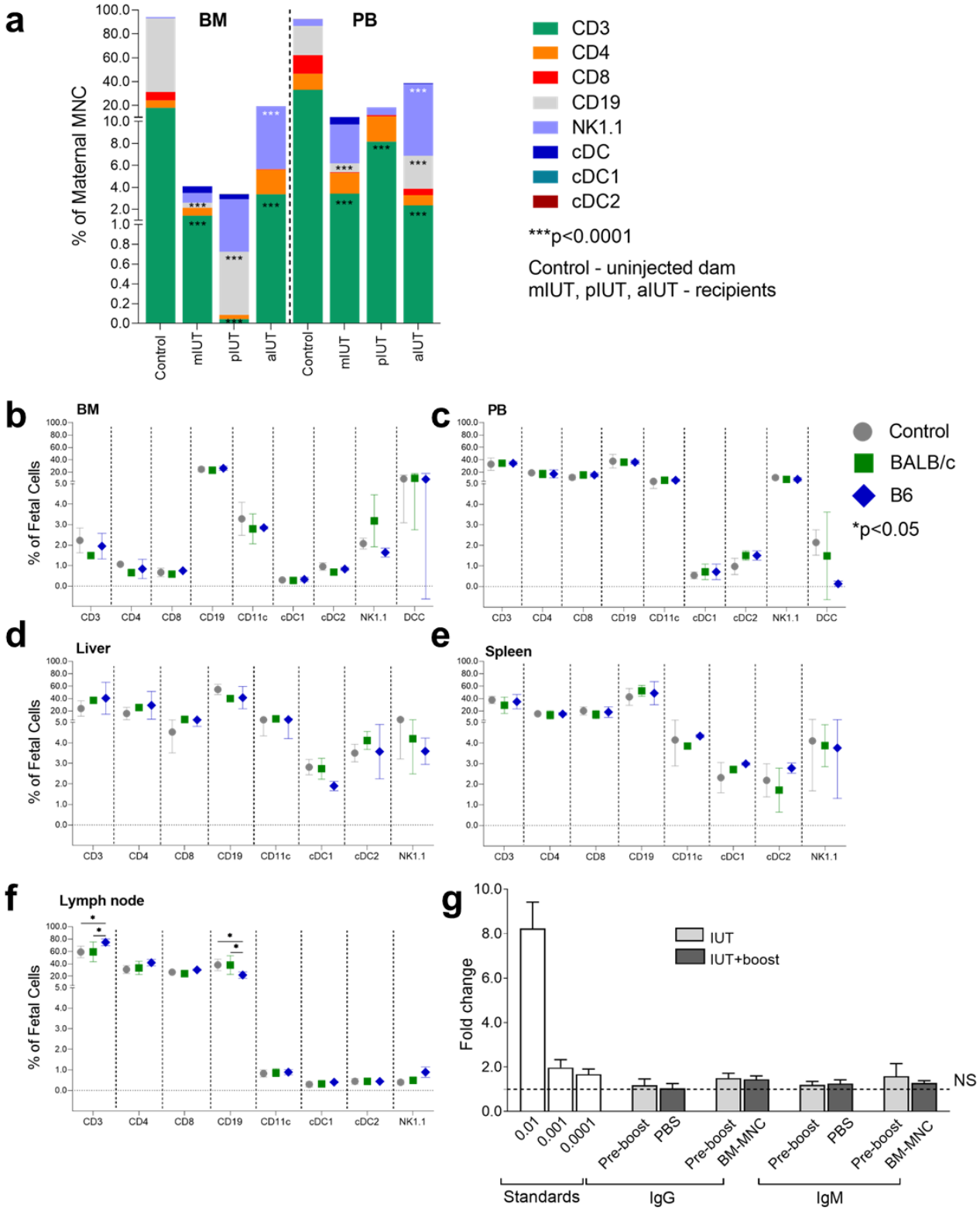
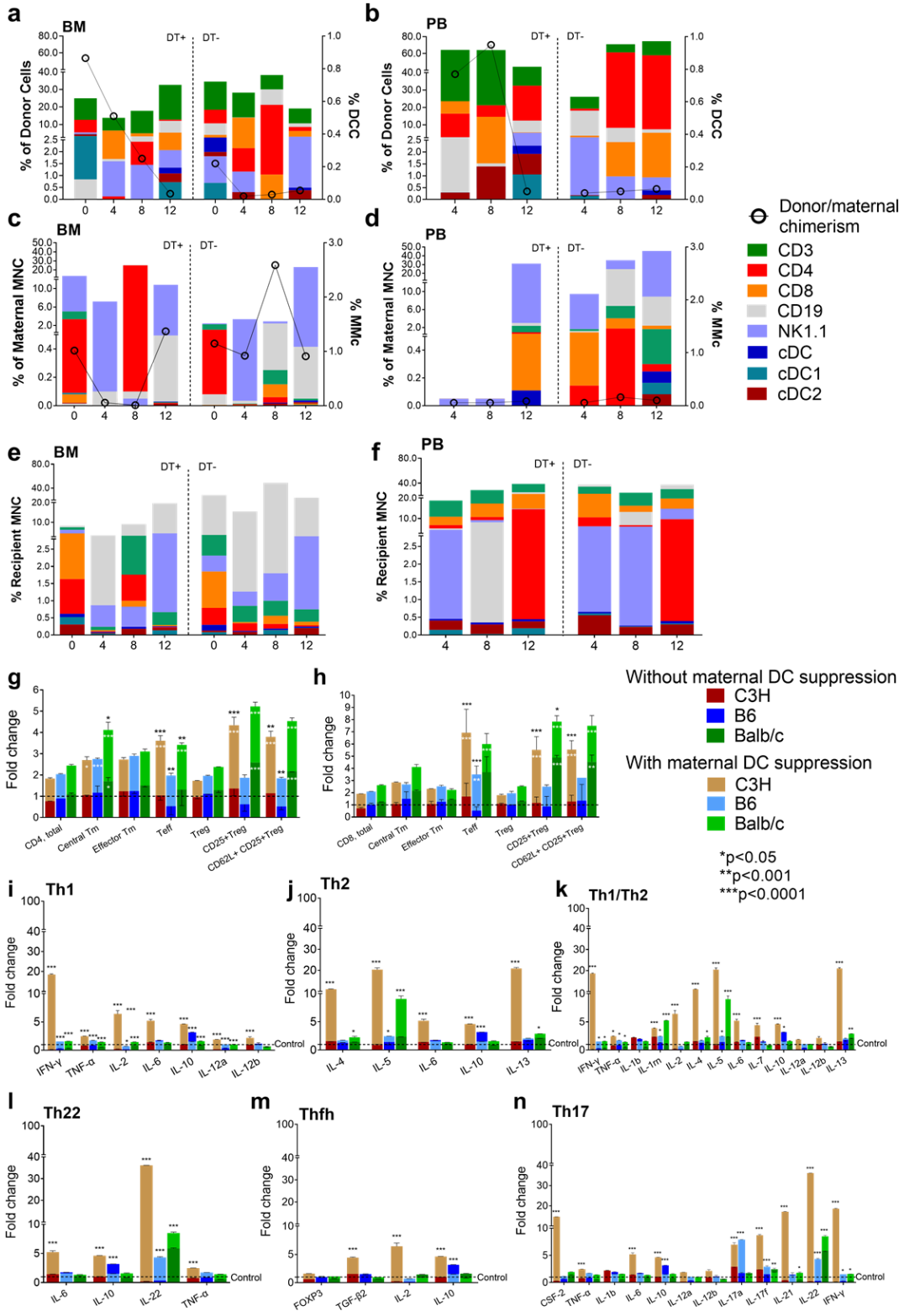


Extended data Figure S1. Diphtheria toxoid transiently suppresses conventional dendritic cells in various organs, and engrafted donor cells maintain haemopoetic stem and progenitor markers. Conventional dendritic cells identified as CD11c+ and MHC-II+ were observed to be suppressed for up to one week in multiple organs (**a**). Engrafted donor cells harvested from recipient bone marrow (DT+ n=11, DT- n=9) and peripheral blood (DT+ n=8, DT- n=8) at all timepoints maintained markers of haemopoiesis (**b**): Lin- Sca1+c-Kit+ (LSK), LSK CD48+ and CD48+ CD150+ (haemopoietic progenitors), and LSK 150+ (long-term repopulation).



Extended data Figure S2. Intrauterine transplantation elicits active and selective maternal cell trafficking and does not produce donor-specific antibodies. Maternal cells recovered from recipient BM and PB had lower levels of all immune cells after any IUT, particularly CD3 and CD19 (black*), compared to the immune profile of non-IUT dams (controls), except for NK1.1 being higher after aIUT than controls (white*), demonstrating an active process (**a**). DT+pIUT recipients showed only increased CD3 and reduced CD19 within lymph nodes following B6 boost (**b-f**, n=7). Donor-specific IgG and IgM were not detected following IUT with/without boost (**g**). Data represent mean ± SEM, analysed by two-way ANOVA with Tukey's multiple comparisons test.



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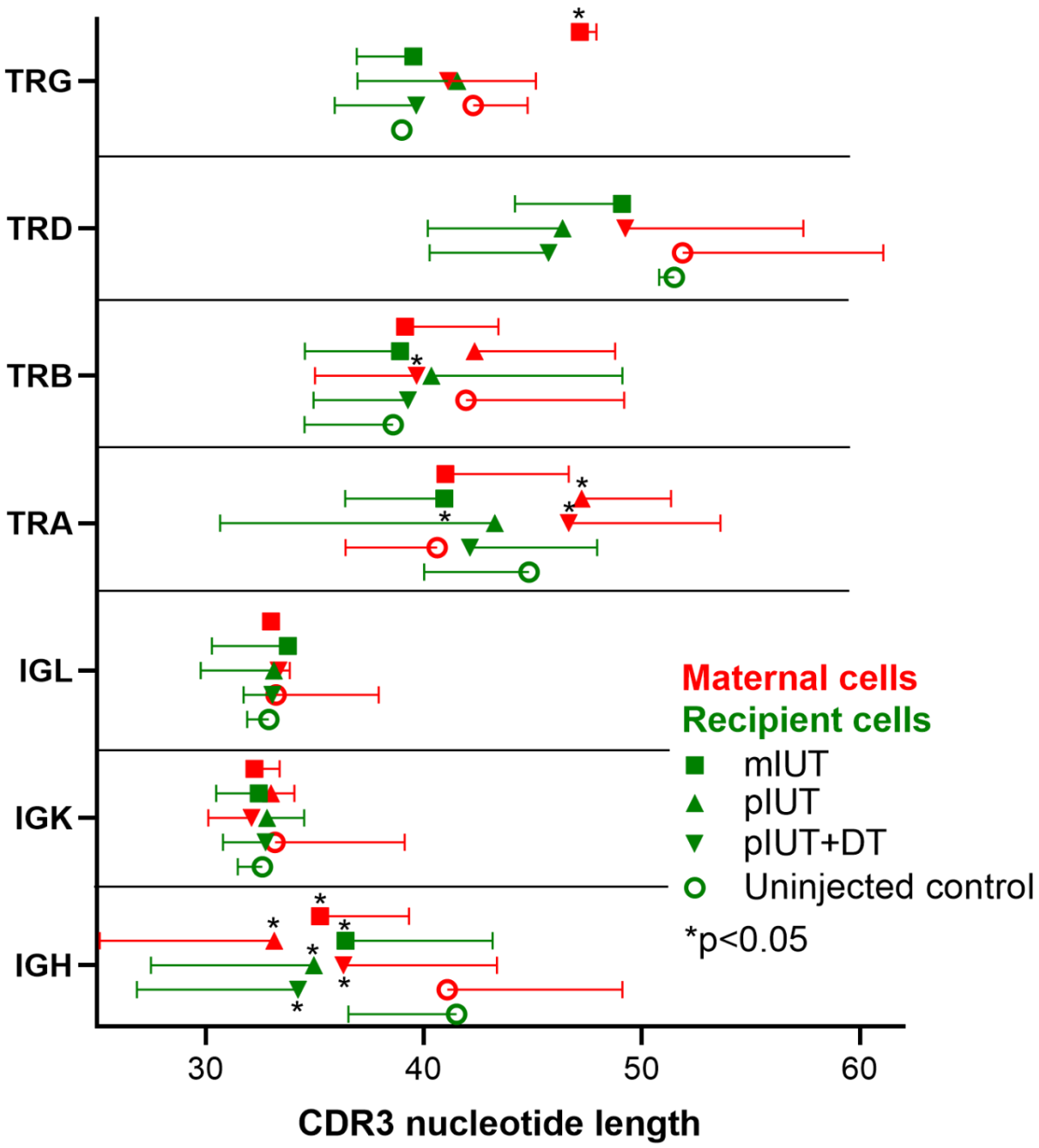
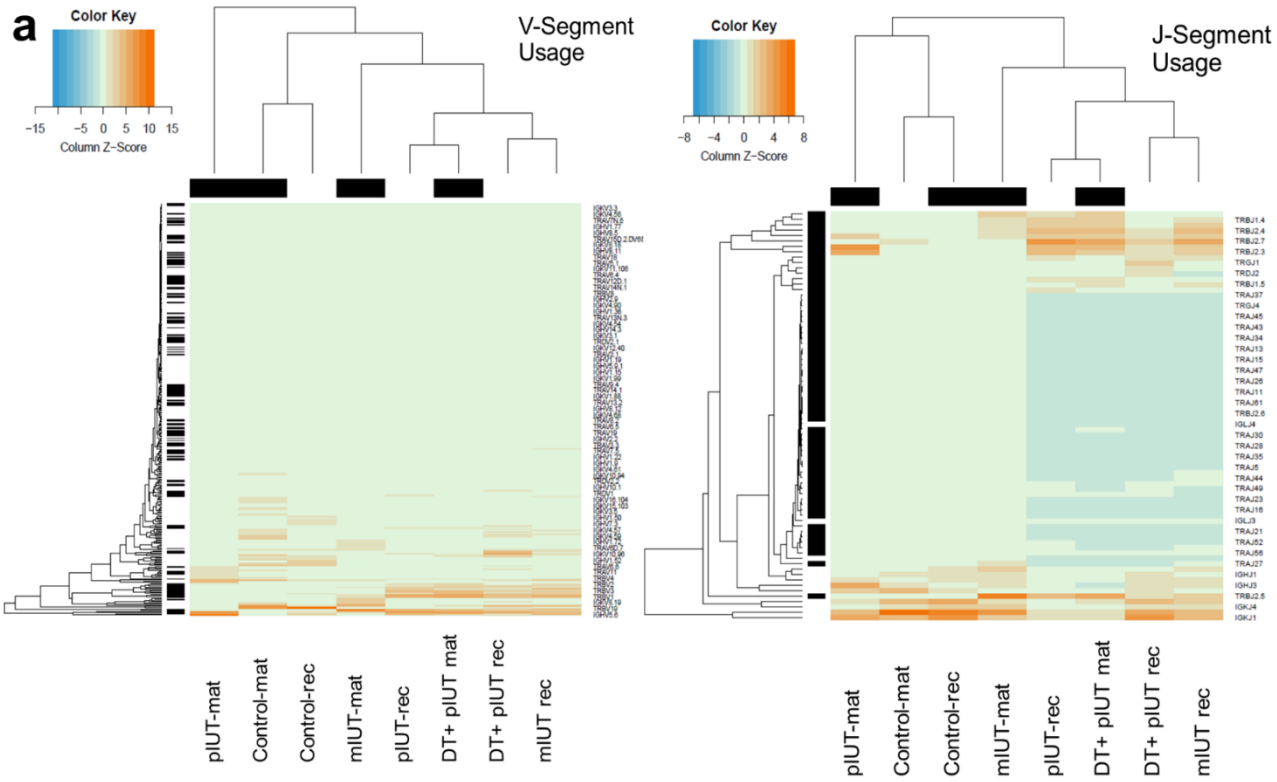
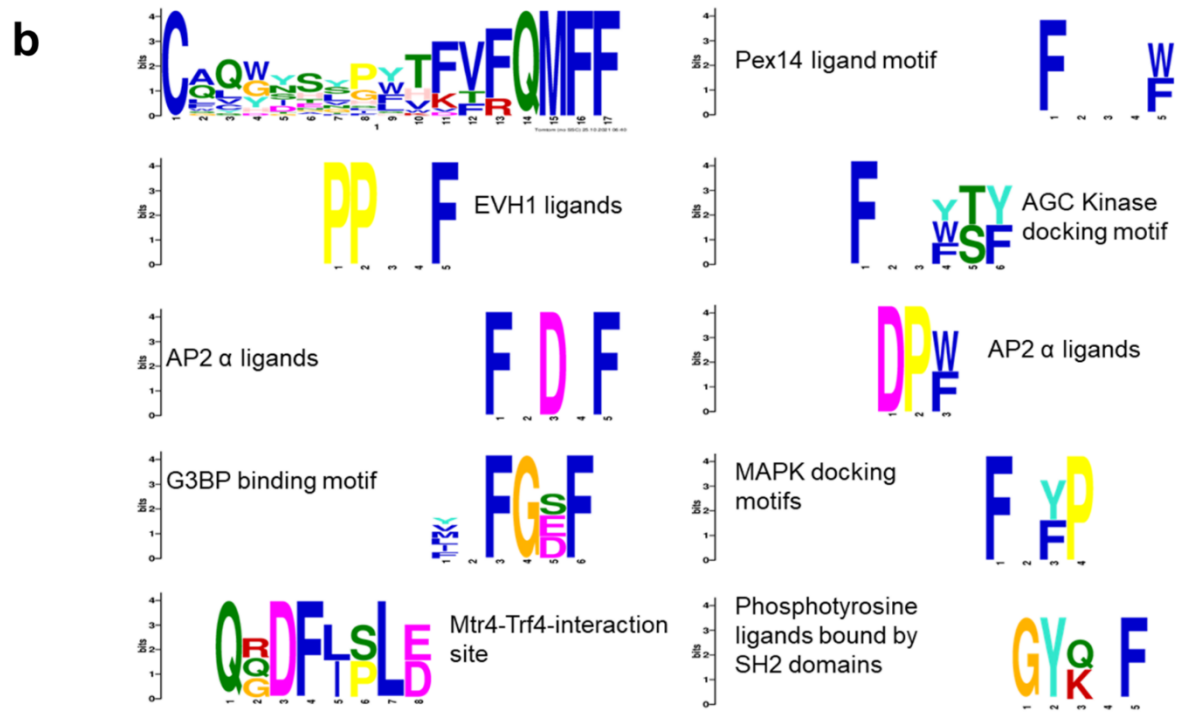
37 **Extended data Figure S3. Immune profiles of engrafted**
38 **donor, trafficked maternal and recipient cells, T cell profile**
39 **and cytokine expression following allogenic cell**
40 **transplantation.** Donor microchimerism <1% was observed in
41 BM and PB (**a,b**). No differences in donor, maternal (**c,d**) or
42 recipient (**e,f**) immune cell profiles were observed in DT+ and
43 DT-. With DT+, CD4+ central and effector memory (Tm), CD4+
44 and CD8+ effector (Teff) and regulatory (Treg) T cells
45 increased above DT- (black*) and uninjected controls (white*)
46 on exposure to all donor cells (**g,h**), and increased all cytokines
47 except FOXP3 with C3H exposure (**i-n**). Data represent mean
48 ± SEM, analysed by two-way ANOVA with Tukey's multiple
49 comparisons test.



Extended data Figure S4. TCR and BCR clonotypes show CDR3 nucleotide length changes in response to IUT. Increased CDR3 nucleotide sequence lengths were observed in maternal TRG (from mIUT), TRA (pIUT, DT+ pIUT), and shorter lengths in maternal TRB (DT+pIUT), recipient TRA (mIUT), and maternal and recipient IGH (any IUT), compared to uninjected control pups. Mean ± SD are compared against controls, analysed with paired t-test.





Extended data Figure S5. CDR3 V-segment and J-segment analyses and functionality. Similarities in variable V-segment and joining J-segment usage were observed between pIUT, DT+ pIUT and mIUT recipient-derived clonotypes and DT+ pIUT maternal-derived clonotypes, with upregulated IGH, IGK, TRA, TRB V- and J-segments, and TRG and TRD J-segments; TRA J-segments were downregulated (**a**). CDR3 amino acid sequences of the top 20 shared clonotypes demonstrated motif enrichment (listed in **b**) corresponding to Ras GTPase activating proteins, mitogen-activated protein kinases (MAPK) substrates, and signal transduction molecules, among others

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77 **Extended data Table S1**: List of abbreviations.

aIUT	Allogenic donor cells (C3H) used in IUT
ANOVA	Analysis of variance
B6	C57BL/6 black mice
BCR	B-cell receptor
BM	Bone marrow
Breg	Regulatory B cells
C3H	C3H/HeNTac
cDC	Conventional dendritic cell
CDR3	Complementarity-determining region 3
Central Tm	Central memory T cells (CD44+ CD62L+)
DC	Dendritic cells
DCC	Donor cell chimerism / Donor cell engraftment
DCC^{BM}	Donor cell chimerism / Donor cell engraftment in BM
DCC^{PB}	Donor cell chimerism / Donor cell engraftment in PB
DSA	Donor-specific antibodies
DT	Diphtheria toxin
DT+	Diphtheria toxin injected
DT-	Saline injected
DTR	Diphtheria toxin receptor
E-13	Embryonic day 13
E-14	Embryonic day 14
Effector Tm	Effector memory T cells (CD44+)
F1	Filial 1 generation
FACS	Fluorescence activated cell sorting
FBS	Fetal bovine serum
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase/housekeeping gene
HSC	dehydrogenase/housekeeping gene
IGH	Immunoglobulin Heavy chain
IGK	Immunoglobulin Heavy *Kappa*
IGL	Immunoglobulin Heavy *Lambda*
IUT	Intrauterine hematopoietic cell transplantation
LSK	Lin^{-} $Sca1^{+}$ $c\text{-}Kit^{+}$
MACS	Magnetic activated cell sorting
MAPK	Mitogen-activated protein kinases
MHC	Major histocompatibility complex
mIUT	Maternal donor cells used in IUT
MLR	Mixed lymphocyte reactivity assay
MMc	Maternal microchimerism / trafficked maternal cells
MNC	Mononuclear cells
NK	Natural killer cells

PB	Peripheral blood
qPCR	Quantitative Polymerase chain reaction
pIUT	Paternal donor cells used in IUT
SEM	Standard error mean
TCR	T-cell receptor
Teff	Effector T cells (CD25+)
TRA	T-cell receptor - alpha
TRB	T-cell receptor - Beta
TRD	T-cell receptor - Delta
Treg	Regulatory T cells (FOXP3+)
TRG	T-cell receptor - Gamma
VDJ	variable (V), diversity (D), and joining (J) gene segment

Extended data: Table S2: Antibody conjugates used for flow cytometry. Each antibody was validated with respective positive control as per manufacturer's instructions.

Antibodies	Clone	Fluorescence	Catalogue #	Company
B220	RA3-6B2	APC	103212	Biolegend
CD117 (c-kit)	2B8	BV605	563146	BD Biosciences
CD11b	M1/70	Alexa Fluor 700	557960	BD Biosciences
CD11c	N418	BV650	117339	Biolegend
CD127	SB/199	BV510	563353	BD Biosciences
CD150	TC15-12F12.2	BV650	115932	Biolegend
CD172a	P84	Alexa Fluor 700	144021	Biolegend
CD19	6D5	FITC	115506	Biolegend
CD25	PC61	BV650	564021	BD Biosciences
CD3	17A2	BV421	564008	BD Biosciences
CD4	GK1.5	Alexa Fluor 700	100430	Biolegend
CD44	IM7	FITC	561859	BD Biosciences
CD45.1	A20	APC	110714	Biolegend
CD45.2	104	PE	109808	Biolegend
CD62L	MEL-14	BV605	563252	BD Biosciences
CD8a	53-6.7	BV605	563152	BD Biosciences
FC block	2.4G2		553142	BD Biosciences
Fixable viability stain 620		PE-CF594	564996	BD Biosciences
FOXP3	R16-715	PerCP Cy5.5	563902	BD Biosciences
GR1	RB6-8C5	PE-Cy7	25593182	Thermofisher Scientific
H-2Kb	AF6-88.5	PerCP-Cy5.5	562831	BD Biosciences
H-2Kd	SF1-1.11	BV510	742432	BD Biosciences
H2K-k	36-7-5	BUV395	750340	BD Biosciences
IgG	Poly4053	APC	405308	Biolegend
IgM	RMM-1	BV421	406517	Biolegend
MHC-II	M5/114.15.2	PE-Cy7	25532182	Thermofisher Scientific
NK1.1	PK136	APC-Cy7	560618	BD Biosciences
Sca1	D7	PerCP	108121	Biolegend
TER119	TER-119	APC-Cy7	560509	BD Biosciences
XCR1	ZET	APC-Cy7	148223	Biolegend

Extended data: Table S3: List of forward and reverse primers used for cytokine gene expression qPCR

Acronym/ Gene ID	Strand	Primer Pairs	Amplicon (bp)
IL-1β	Forward	5'-AGTTGACGGACCCCAAAAGAT-3'	90
	Reverse	5'-GTGCTGCTGCGAGATTTGAA-3'	
IL-1rn	Forward	5'-CCACCACCAGCTTTGAGTCA-3'	70
	Reverse	5'-GGACGGTCAGCCTCTAGTGTTG-3'	
IL-2	Forward	5'-CCTGAGCAGGATGGAGAATTACA-3'	132
	Reverse	5'-TGCCGCAGAGGTCCAAGT-3'	
IL-4	Forward	5'-ACCCCCAGCTAGTTGTCATCCT-3'	70
	Reverse	5'-TTGTCGCATCCGTGGATATG-3'	
IL-5	Forward	5'-CCATGAGCACAGTGGTGAAAGA-3'	80
	Reverse	5'-CCTCATCGTCTCATTGCTTGTC-3'	
IL-6	Forward	5'-CCAACAGACCTGTCTATACCACTTCA-3'	70
	Reverse	5'-TCCACGATTTCCCAGAGAACA-3'	
IL-7	Forward	5'-GTGCTGCTCGCAAGTTGAAG-3'	93
	Reverse	5'-GTGTTTGTGTGCCTTGTGATACTG-3'	
IL-10	Forward	5'-GCCAGTACAGCCGGGAAGA-3'	83
	Reverse	5'-GAAGGCAGTCCGCAGCTCTA-3'	
IL-12a	Forward	5'-ATCAACGCAGCACTTCAGAATC-3'	113
	Reverse	5'-CGCAGAGTCTCGCCATTATG-3'	
IL-12b	Forward	5'-GACTCTCGGGCAGTGACATGT-3'	70
	Reverse	5'-AGTCCCTTTGGTCCAGTGTGA-3'	
IL-13	Forward	5'-TTCCCTGACCAACATCTCCAA-3'	70
	Reverse	5'-GTTACAGAGGCCATGCAATATCC-3'	
IL-17a	Forward	5'-ACCGCAATGAAGACCCTGAT-3'	84
	Reverse	5'-TTCCCTCCGCATTGACACA-3'	
IL-17f	Forward	5'-CTTGCAGAAGGCTGGGAACT-3'	79
	Reverse	5'-CTGGTTTTGGTTGAAGATTCGAA-3'	
IL-21	Forward	5'-TGAAAATGACTTGGATCCTGAACTT-3'	111
	Reverse	5'-GGGTTTGATGGCTTGAGTTTG-3'	
IL-22	Forward	5'-CATCGTCAACCGCACCTTTA-3'	70
	Reverse	5'-GAGCCGGACATCTGTGTTGTT-3'	
CSF-2	Forward	5'-GTCACGTTGAATGAAGAGGTAGAAGT-3'	70
	Reverse	5'-TCTGCACACATGTTAGCTTCTTGA-3'	
FOXP3	Forward	5'-CACAACCTGAGCCTGCACAA-3'	78
	Reverse	5'-CTCAAATTCATCTACGGTCCACACT-3'	
IFN-γ	Forward	5'-CTGGAGGAACTGGCAAAAGG-3'	103
	Reverse	5'-GATGGCCTGATTGTCTTTCAAGA-3'	
TGF-β2	Forward	5'-CTGCCTTCGCCCTCTTTACA-3'	94
	Reverse	5'-CCCAGCACAGAAGTTAGCATTG-3'	
TNF-α	Forward	5'-GCCTCTTCTCATTCCTGCTTGT-3'	71
	Reverse	5'-ACCGATCACCCCGAAGTTC-3'	
GAPDH	Forward	5'-GAGTGTTTCCTCGTCCCGTAGA-3'	75
	Reverse	5'-TGACCAGGCGCCCAATAC-3'	