Supplementary Materials

Supplementary Methods

Supplementary Figure 1. Duvelisib-FCR Trial, study timeline and patient characteristics

Supplementary Figure 2. CyTOF Panel

Supplementary Figure 3. T Cell Subtypes, with relevant CyTOF panel markers

Supplementary Figure 4. CD4 T cell counts over time in patients treated with duvelisib FCR

Supplementary Figure 5. Cluster-defining markers of c9 and c38

Supplementary Figure 6. Decrease in Naïve CD4, Total CD4, and CD4:CD8 Ratio in Patients With and Without Toxicity

Supplementary Figure 7. Cluster-defining markers of c24, c34, and c4

Supplementary Figure 8. Increase in Activated CD8 T cells and Decrease in Naïve CD8s identified in Patients with Toxicity

Supplementary Figure 9. Changes in Tregs and Th17s with dFCR Treatment as shown with Mass Cytometry

Supplementary Figure 10. Cytokines evaluated that showed no significant differences between Tox and No Tox patient groups

Supplementary Methods

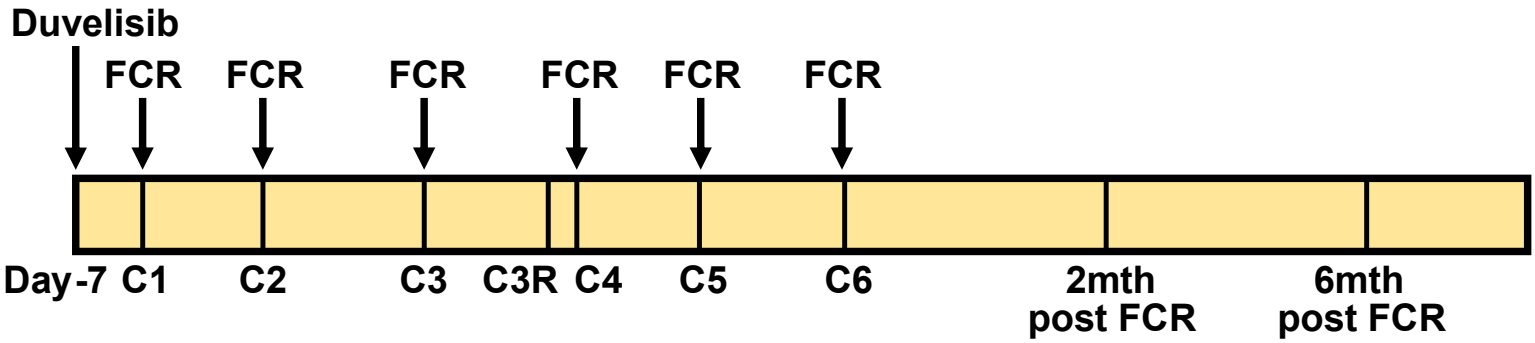
Luminex multiplex cytokine detection assay

For multiplex cytokine analysis of serum, samples were collected from 26 patients (12 patients without toxicity and 14 patients with toxicity at timepoints screen, C1D1, C3R and 6 months post FCR; Supplementary Figure 1) and frozen directly at -80 °C. Luminex assays were conducted using 20 µL of plasma sample and standards were run in each experiment. The 35 cytokine panel included: TNFα, IL-18, IL-1α, IL-1β, IL-1RA, IL-10, IL-33, IL-23, IL-22, IL-6, IL-21, IL-8, Tweak, MCP-1, IFNγ, MIP-1α, GM-CSF, Trem-1, GROα, ENA-78, IL-17A, PDGF-AA, PDGF-BB, MCP-3, MIG, MDC, Flt3L, IL-15, IP-10, IL-2, IL-4, IL-5, IL-13, MIP-1β and TGFβ. The plate was read and analyzed on a FLEXMAP 3D instrument (Luminex Corporation, Austin, Texas). Toxicity status by sampling time interactions were estimated and tested using generalized linear mixed models without correction for multiple testing; all tests are two-sided.

BH3 Profiling

Primary cells were suspended in MEB2P buffer (150 mM mannitol, 10 mM HEPES-KOH pH 7.5, 150 mM KCl, 1 mM EGTA, 1 mM EDTA, 0.1% BSA, 5 mM succinate, 0.25% poloxamer 188) prior to analysis. Single cell suspensions were added to 384-well plates and incubated for 60 minutes with BH3-only peptides (BIM and PUMA) in the presence of 0.002% digitonin (for permeabilization of cells). After fixation with 4% paraformaldehyde and neutralization with N2 buffer (1.7 M Tris, 1.25 M glycine pH 9.1), cells were stained overnight with a cocktail of anti–cytochrome c–Alexa Fluor 488, anti–CD19-PE/Cy7, anti–CD5-PE, and Hoechst 33342. Prepared plates were analyzed using a BD FACS Fortessa. Flow cytometry data were analyzed using FACS Diva version 8.0.1 (BD Pharmingen). Cytochrome c (cyto c) release was used to assess the degree of mitochondrial outer membrane permeabilization in response to each BH3 peptide, which was normalized relative to cyto c release with DMSO (0% loss, negative control) and the ion-channel forming peptide alamethicin (100% loss, positive control). Individual analyses were performed in duplicate for all drug treatment conditions.

Supplementary Figure 1: Duvelisib-FCR Trial, study timeline and patient characteristics



Patient Characteristics

	Luminex	CYTOF
Number, n	26	16
Median Age (IQR), years	55 (50-58)	56 (55-59)
Men, n (%)	19 (73)	12 (75)
Women, n (%)	7 (27)	4 (25)
ECOG, performance status, n (%)		
0	11 (42)	9 (56)
1	15 (58)	7 (44)
Rai stage, n (%)		
0	6 (23)	4 (25)
1	8 (31)	6 (38)
2	2 (7.5)	1 (6)
3	2 (7.5)	0 (0)
4	8 (31)	5 (31)
Median WBC (IQR), × 10^9 /L	109 (41-182)	116 (44-168)
Median Haemoglobin (IQR), g/dL	12 (10-13)	12 (11-12)
Median haematocrit (IQR), %	35 (32-39)	35 (33-40)
Median platelets (IQR), × 10^9 /L	120 (90-156)	109 (83-143)
Median BM involvement (IQR), %	80 (72-90)	80 (68-90)
Median IgM (IQR), mg/dL	25 (25-33)	25 (25-32)
Median IgG (IQR), mg/dL	675 (508-931)	647 (538-900)
Median IgA (IQR), mg/dL	71 (52-90)	77 (58-114)
Median CD4 (IQR), /µL	1233 (819-2149)	1612 (883-2284)
Median β_2-microglobulin (IQR), mg/L	5 (4-6)	5 (3-6)
***IGHV* unmutated**	16/26 (62)	9/16 (56)
ZAP-70 positive	16/25 (64)	7/15 (47)
del(17p)	2/25 (8)	1/16 (6)
del(11q)	7/25 (28)	4/16 (25)
del(13q)	11/23 (48)	5/14 (36)
Complex Karyotype	3/26 (12)	2/16 (13)
Normal FISH	7/23 (30)	4/16 (25)
Trisomy 12	5/25 (20)	3/15 (20)
6q detected	3/15 (20)	1/9 (11)
T(14:18)	1/16 (6)	1/9 (11)
***TP53* mutation**	1/26 (4)	0/16 (0)
***NOTCH1* mutation**	1/23 (4)	1/14 (7)
***MYD88* mutation**	1/19 (4)	5/16 (31)

Supplementary Figure 2: CyTOF panel

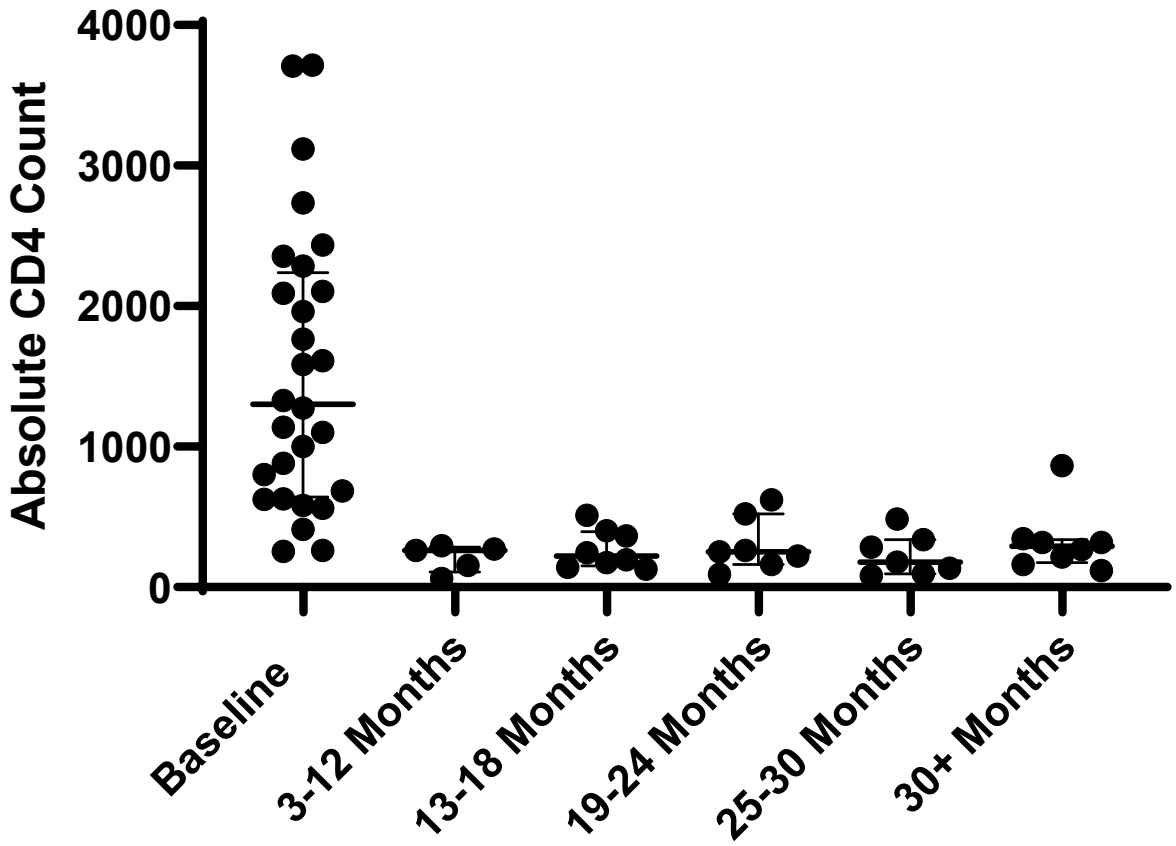
Marker	Clone	Metal	Dilution	Intracellular?	Vendor
CD20	2H7	113In			BioLegend
CD3	UCHT1	115In			BioLegend
CD196 (CCR6)	G034E3	141Pr			BioLegend
CD45RA	HI100	142Nd			BioLegend
CD134 (OX40)	OX-86	143Nd			BioLegend
CD39	A1	144Nd			BioLegend
CD16	3G8	145Nd			BioLegend
CD8α	RPA T8	146Nd			BioLegend
CD45RO	UCHL1	147Sm			BioLegend
CD183 (CXCR3)	G025H7	148Nd			BioLegend
CD25 (IL-2R)	M-A251	149Sm			BioLegend
CCR4	L291H4	150Nd			BioLegend
CD279 (PD-1)	EH12.2H7	151Eu			BioLegend
CTLA-4	L3D10	152Sm			BioLegend
GranzymeB	GB11	153Eu	1:400	Yes	BioLegend
CD185 (CXCR5)	J252D4	154Sm			BioLegend
CD4	RPA T4	155Gd			BioLegend
CD73	AD2	156Gd			BioLegend
Tim-3	F38-2E2	157Gd			BioLegend
T-bet	4B10	158Gd	1:50	Yes	BioLegend
CD137/4-1BB	4B4-1	159Tb			BioLegend
CD278/ICOS	C398.4A	160Gd			BioLegend
Galectin-9	GAL-9	161Dy		Yes	BioLegend
CD11α	HI111	162Dy			BioLegend
CCR5	J418F1	163Dy			BioLegend
CD161	HP-3G10	164Dy			BioLegend
FoxP3	PCH101	165Ho		Yes	eBioscience
CCR9	9B1	166Er			BioLegend
CD146	SHM-57	167Er			BioLegend
Helios	22F6	168Er	1:200	Yes	BioLegend
CD95/Fas	DX2	169Tm	1:200		BioLegend
CD197 (CCR7)	G043H7	170Er			BioLegend
CD127	eBioRDR5	171Yb			eBioscience
CD38	HIT2	172Yb			BioLegend
TIGIT	MBSA43	173Yb			eBioscience
HLA-DR	L243	174Yb	1:200		BioLegend
CD357 (GITR)	621	175Lu			BioLegend
RORγT	AFKJS-9	176Yb	1:50	Yes	eBioscience
CD45	HI30	209Bi			BioLegend

Supplementary Figure 3: T Cell subtypes, with relevant CyTOF panel markers

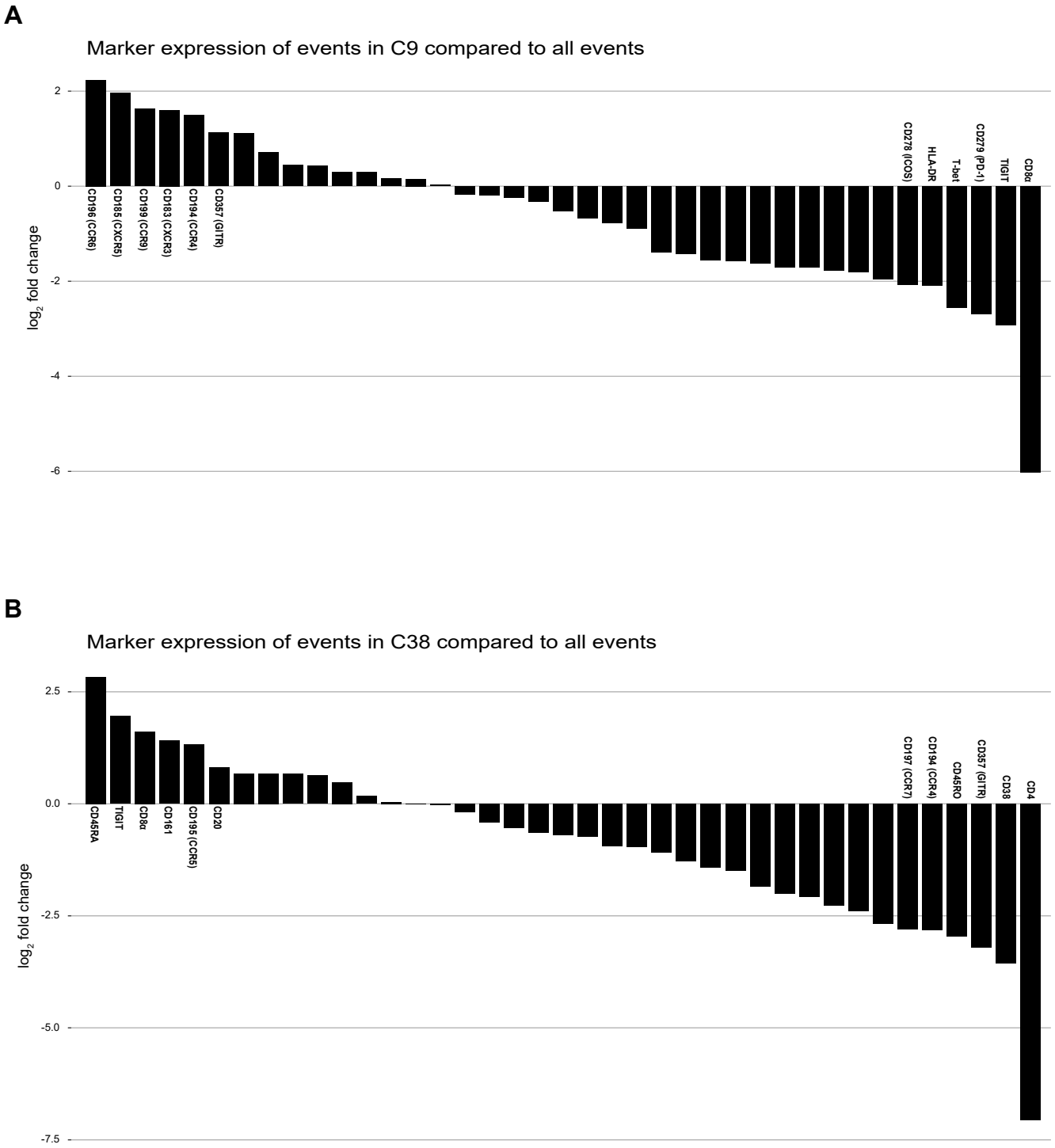
Naïve	Tregs	Th17	Th1	Th2	Th22	Tfr	Tfh	T_{CM}	T_{EM}	Checkpoint
CD4	CD4	CD4	CD4	CD4	CD4	CD4	CD4	CD4	CD4	PD-1
CD8	CD25	CCR4	CXCR3	CCR4	CCR4	FoxP3	PD-1	CD8	CD8	Tim-3
CD45RA	$CD127^{lo}$	CCR6	CCR5		CCR6	PD-1	CXCR5	CCR7	CD45RO	ICOS
$CD45RO^{-}$	FoxP3	RORγT	T-bet			CXCR5		CD45RO		CTLA-4
CCR7	Helios	CD146								
	CD39	CD161								

Supplementary Figure 4: CD4 T cell counts over time in patients treated with duvelisib FCR

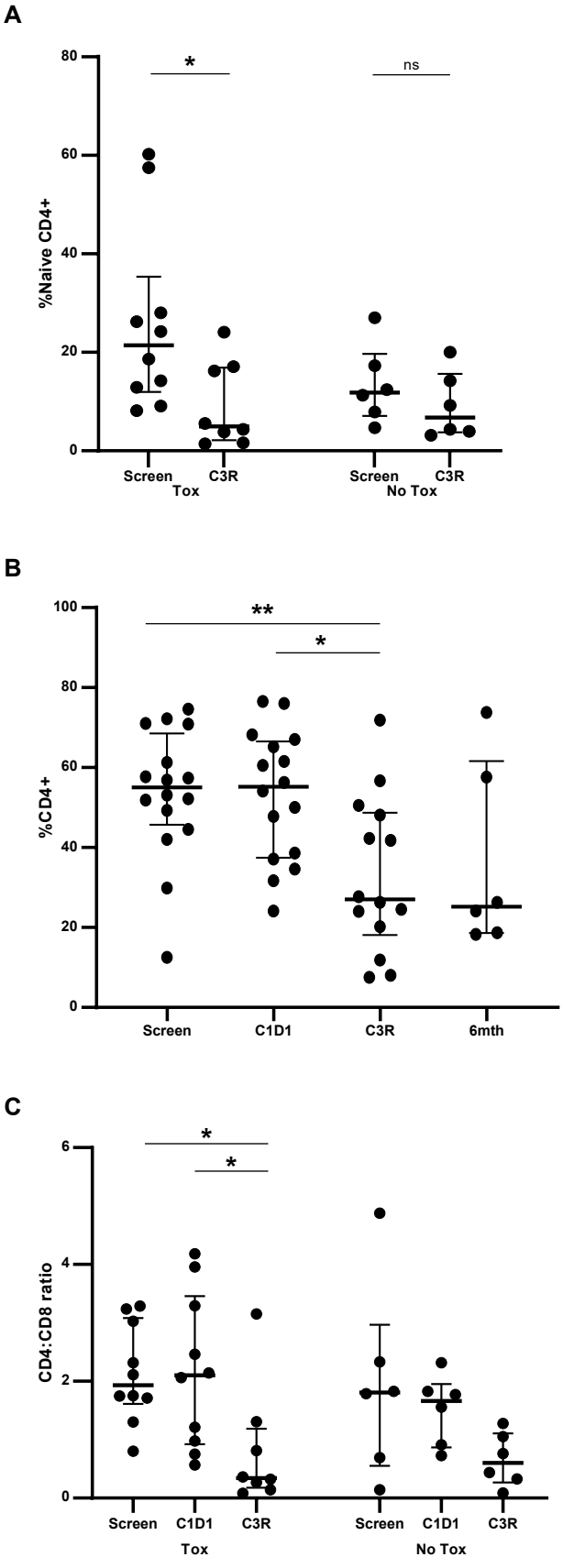
	Baseline	3-12 Months	13-18 Months	19-24 Months	25-30 Months	30+ Months
Median	1301	263	220	252	179	293.33
Q1 (25th percentile)	671.25	154	166.25	191	114	201
Q3 (75th percentile)	2149	273	374.5	390.5	311.5	323.75
Total Range	255-3714	61-294	128-512	91-623	82-486	117-866



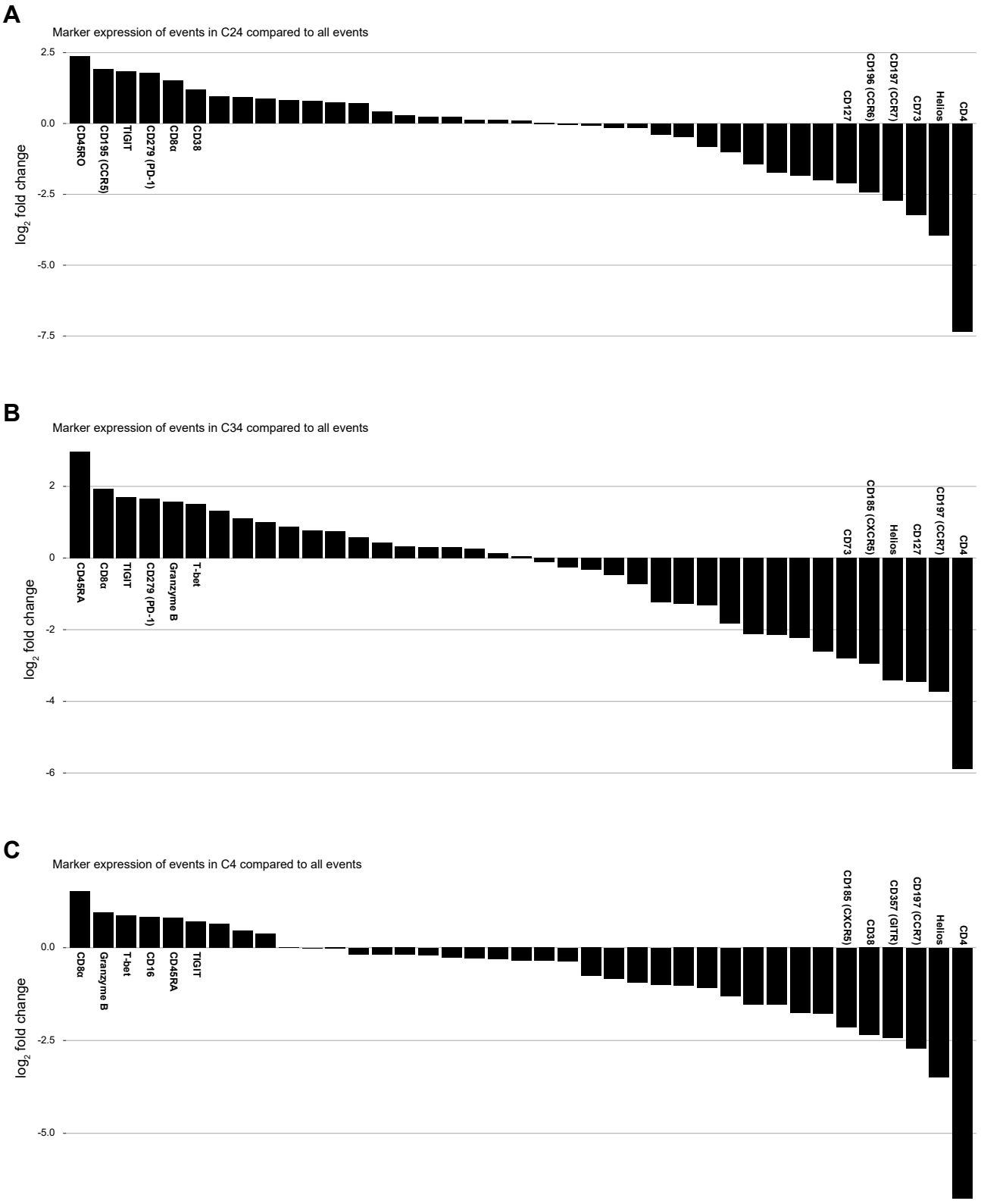
Supplementary Figure 5: Cluster-defining markers of c9 and c38



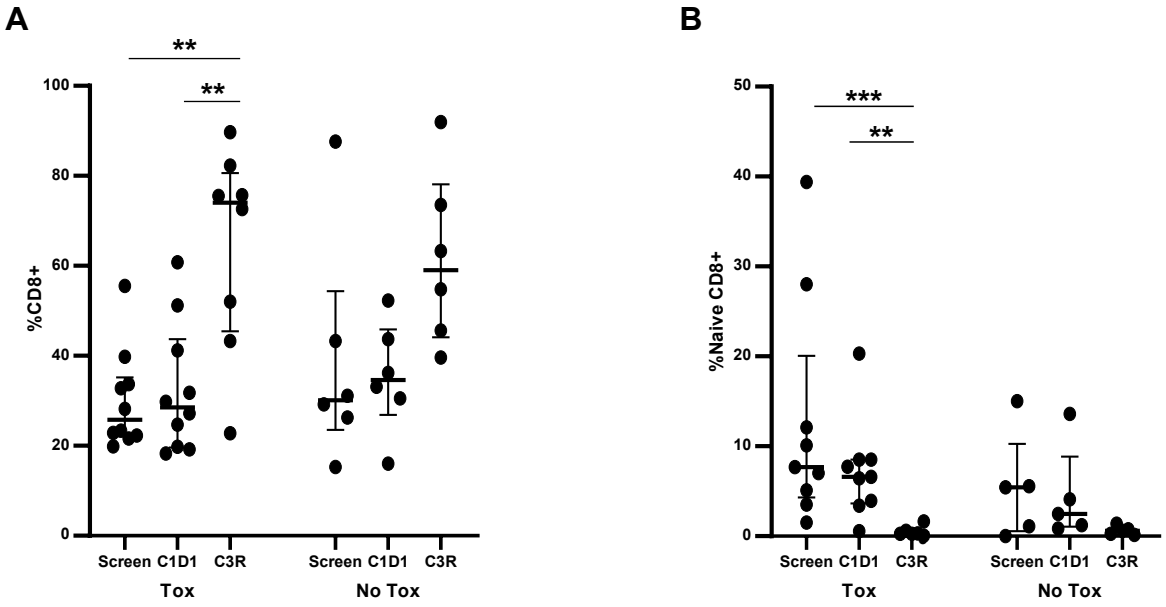
Supplementary Figure 6: Decrease in Naïve CD4, Total CD4, and CD4:CD8 Ratio in Patients With and Without Toxicity **A.** Change in naïve CD4s comparing tox and no tox patients between screen and C3R. **B.** Change in total CD4 T cells across all samples over 6 months. **C.** CD4:CD8 ratio across all samples over three months, comparing tox and no tox patients. ns = not significant; * p≤ to 0.05; ** p≤ to 0.01



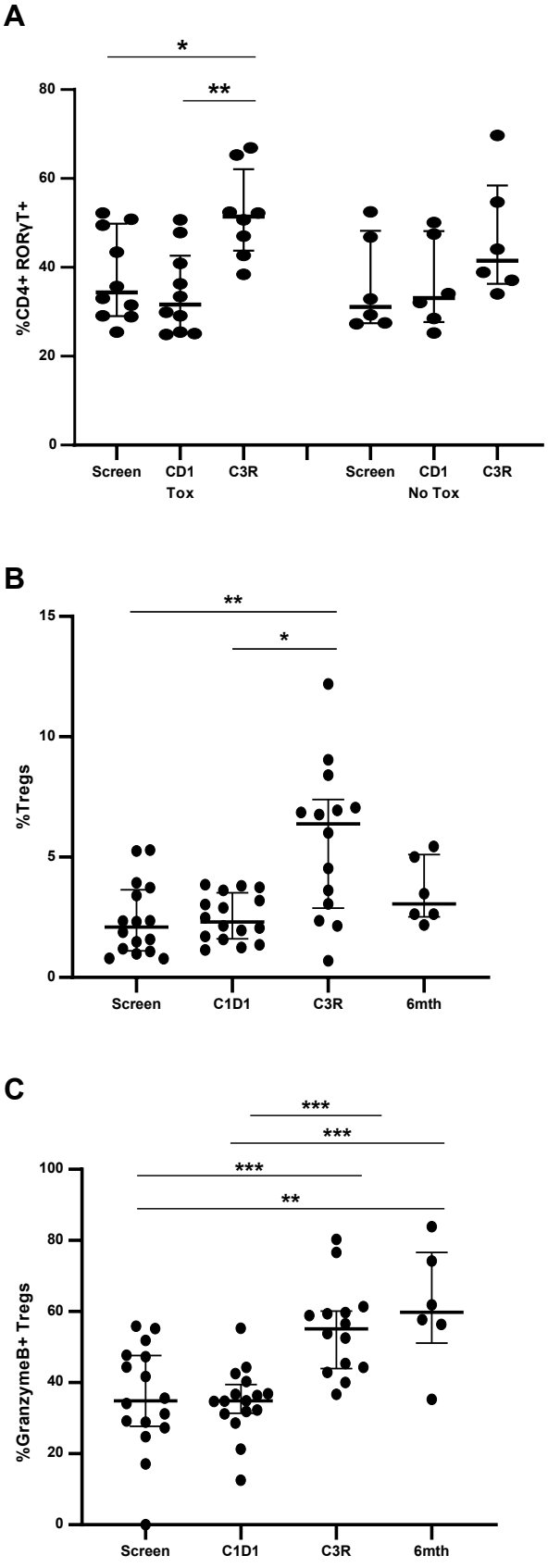
Supplementary Figure 7: Cluster-defining markers of c24, c34, and c4



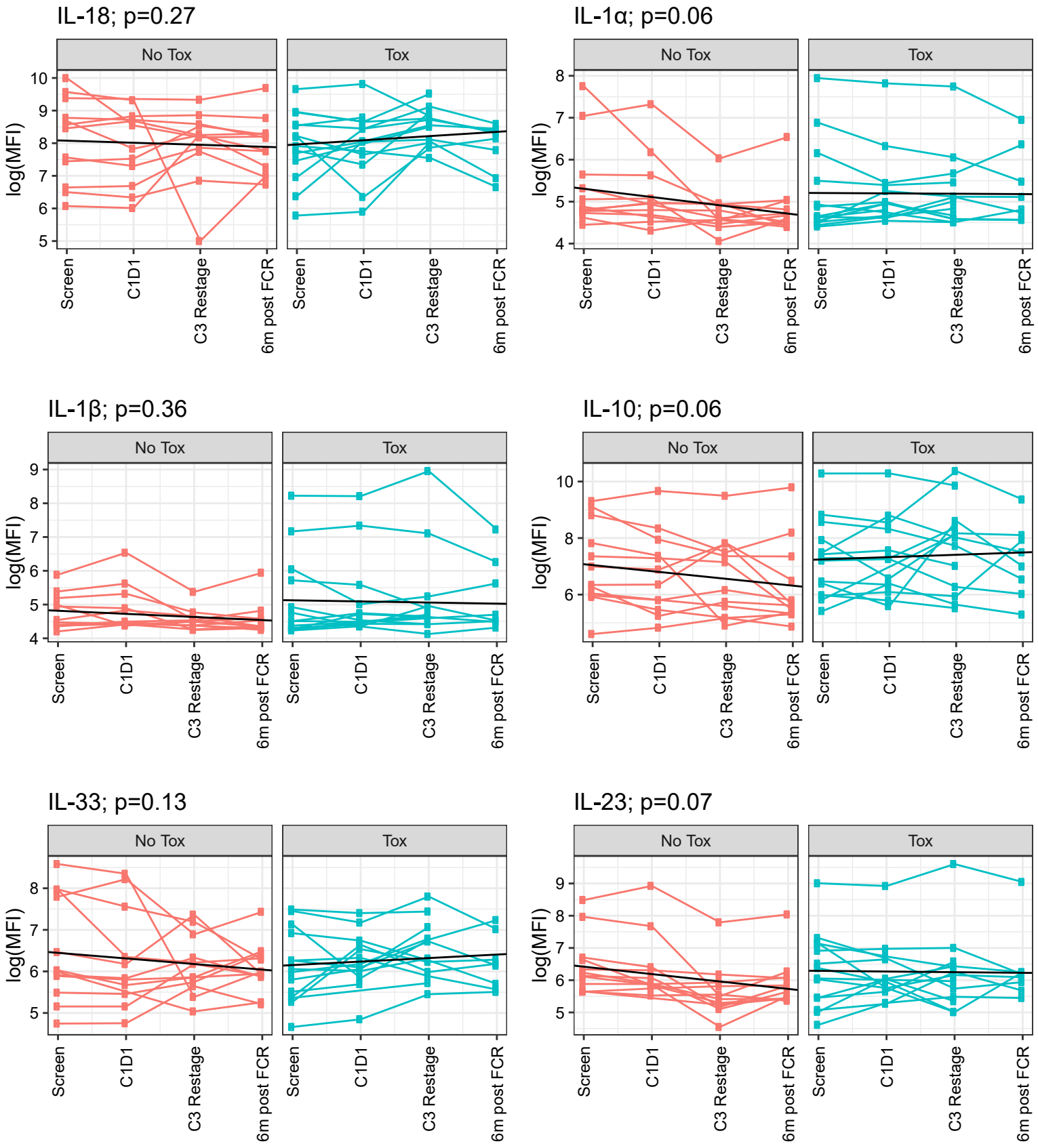
Supplementary Figure 8: Increase in Activated CD8 T cells and Decrease in Naïve CD8s identified in Patients with Toxicity. **A.** Change in total CD8s comparing patients with and without toxicity between screen and C3R. **B.** Change in naïve CD8s across all patients, divided by whether they developed toxicity or not. ** p≤ to 0.01; *** p≤ to 0.001.



Supplementary Figure 9: Changes in Tregs and Th17s with dFCR Treatment as shown with Mass Cytometry **A.** Change in CD4+ RORγT+ cells over time by occurrence of toxicity. **B.** %Tregs over time in all patients. **C.** %Granzyme B+ Tregs between screen and C6, in all patients. * p≤ to 0.05; ** p≤ to 0.01; *** p≤ to 0.001.



Supplementary Figure 10: Cytokines evaluated that showed no significant differences between Tox and No Tox patient groups



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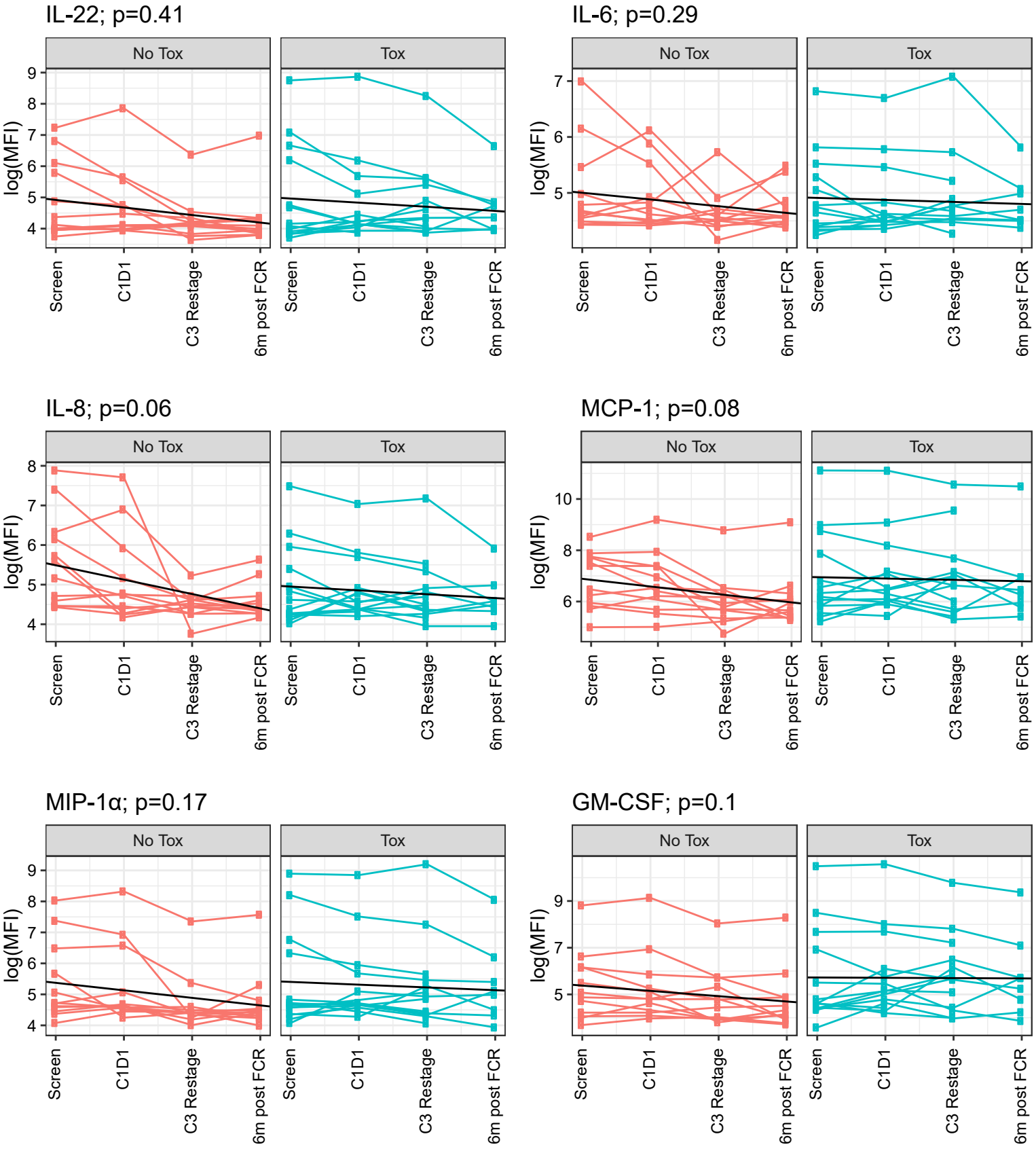

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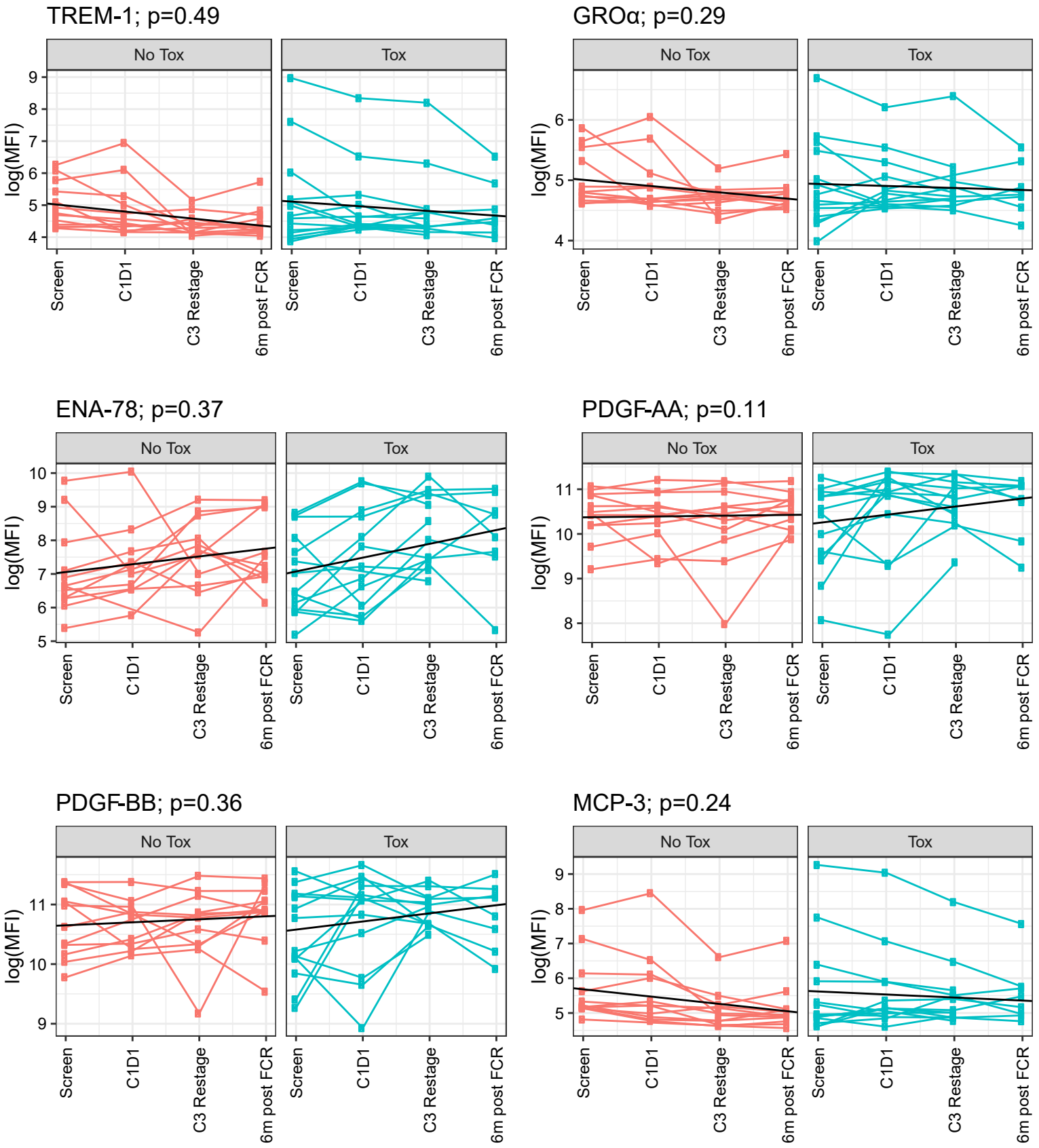

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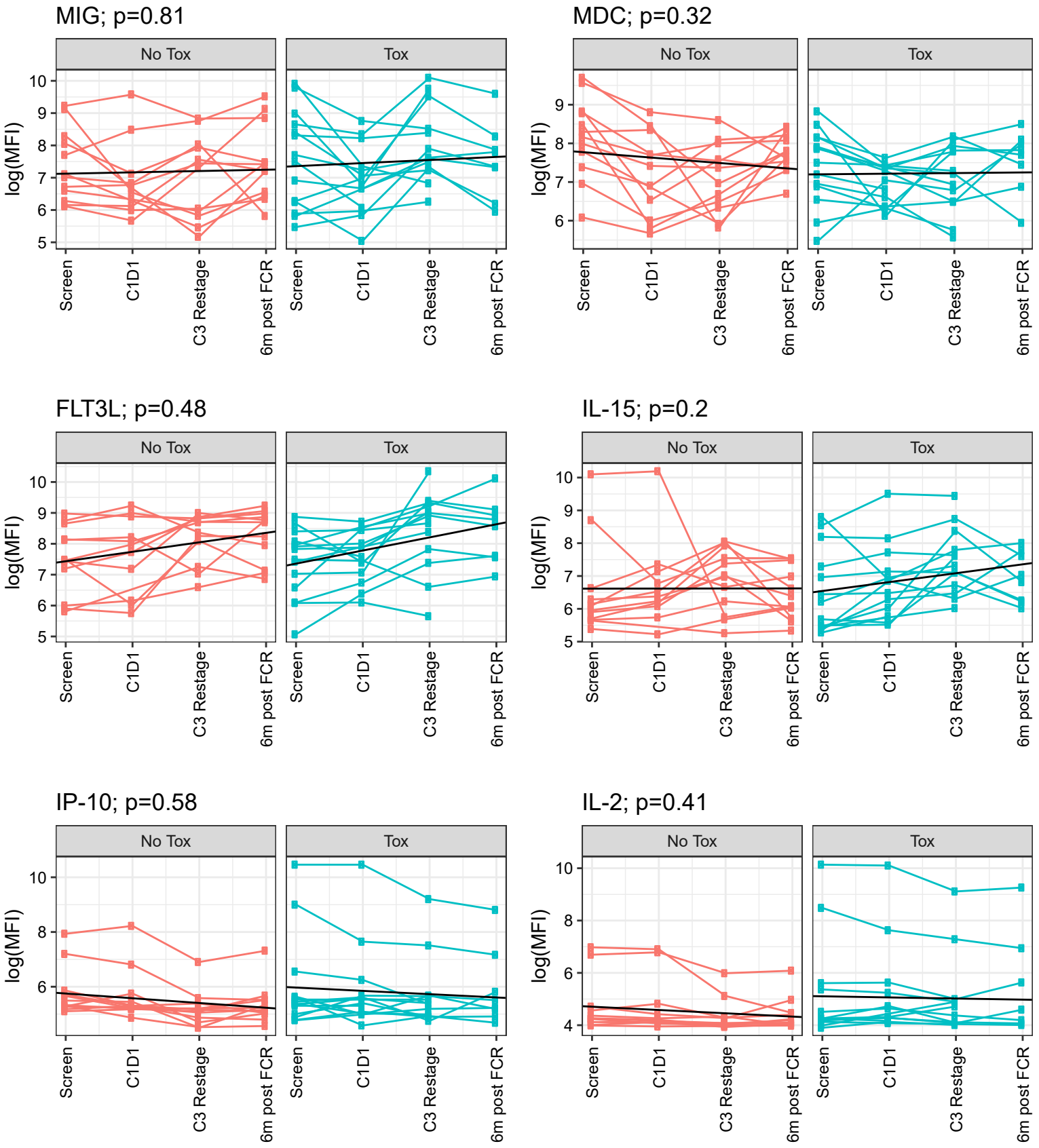

Supplementary Figure 10 (continued…)