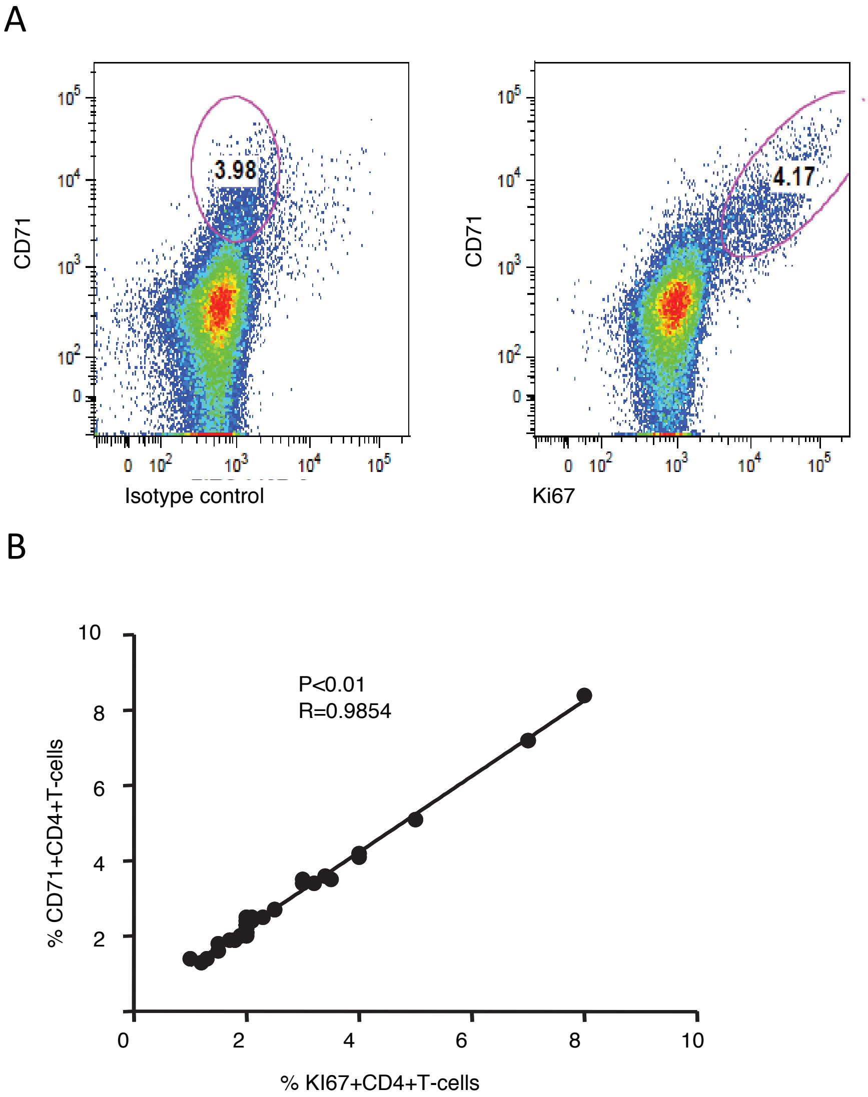
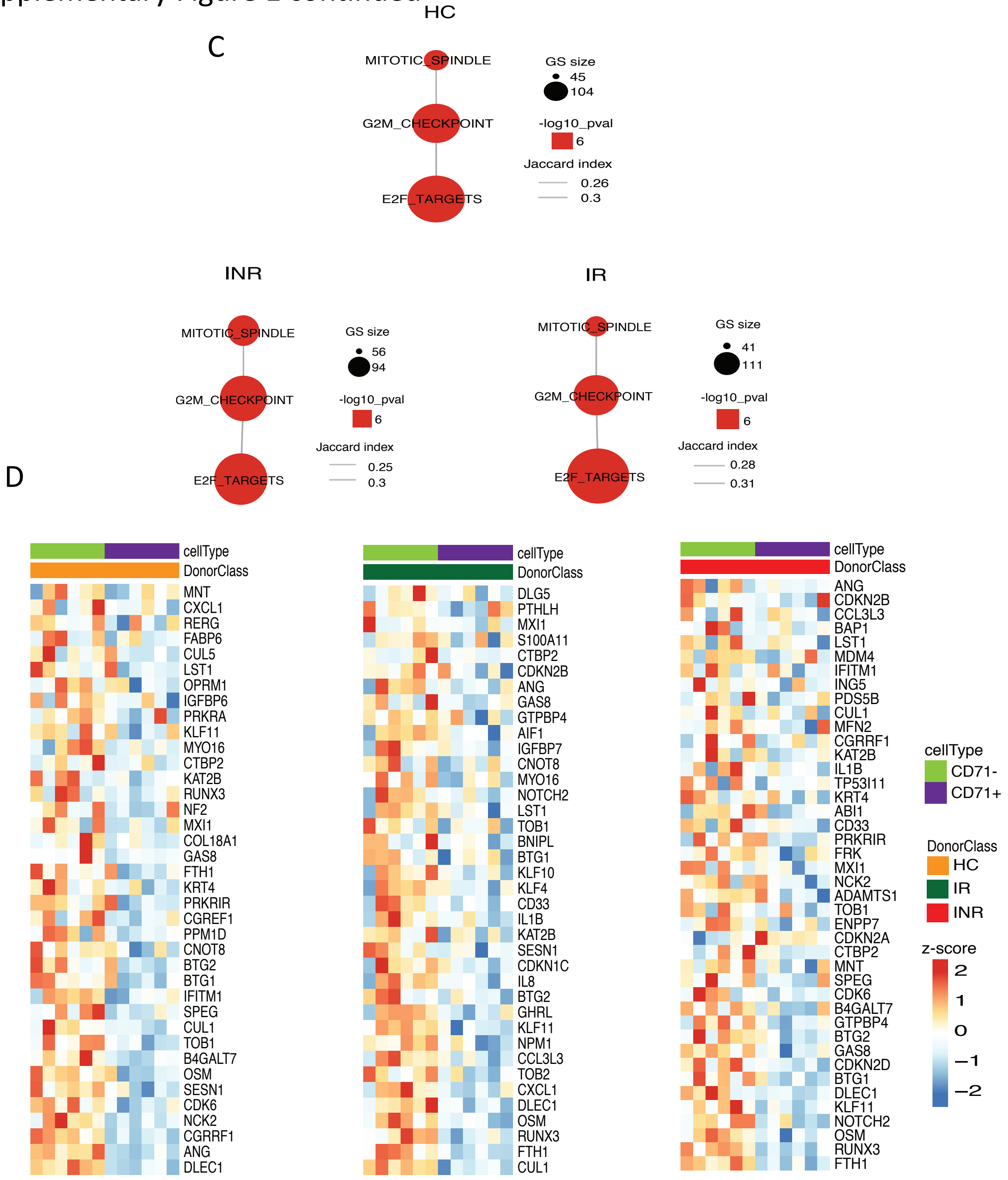
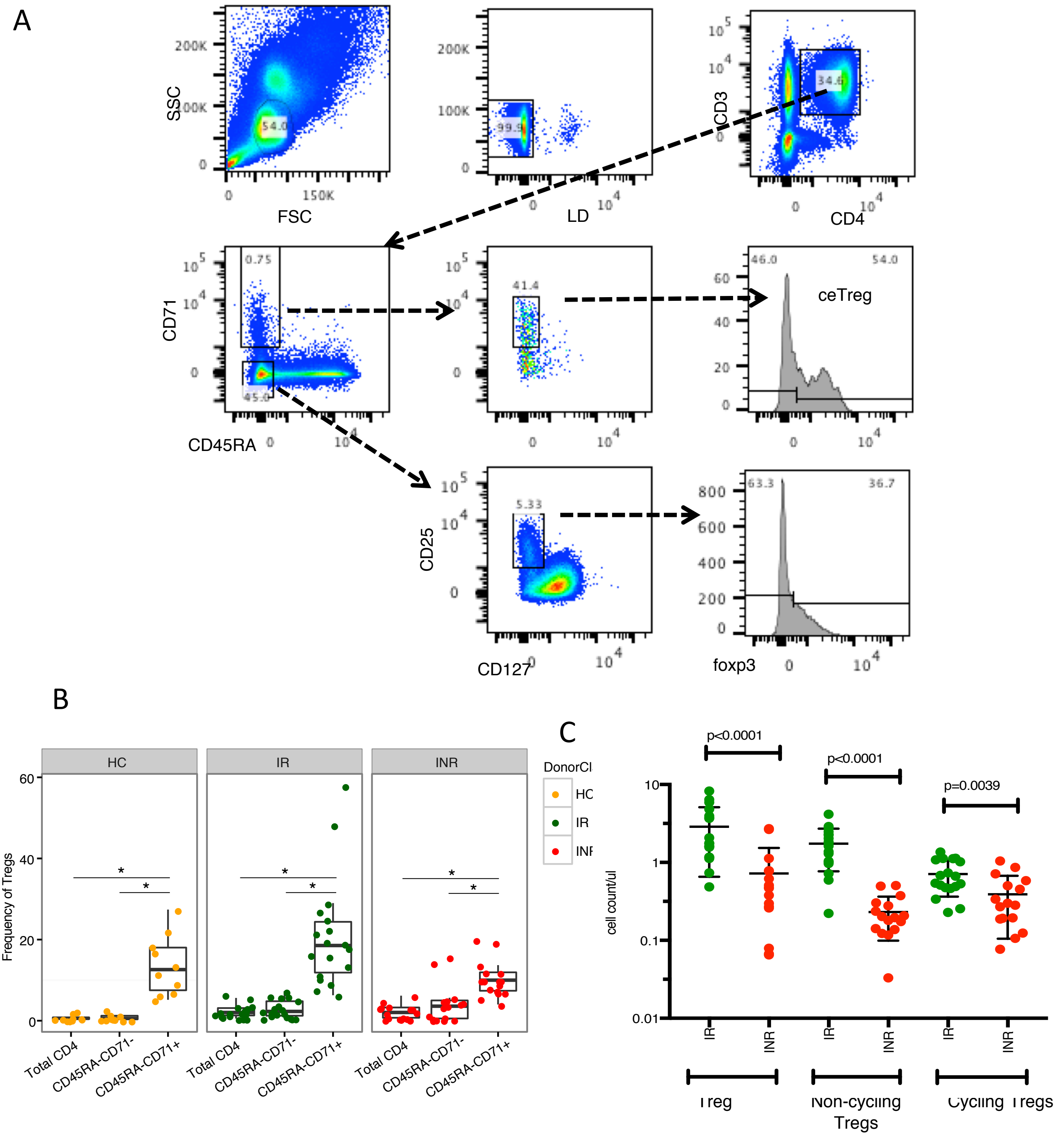
Supplementary Figure 1



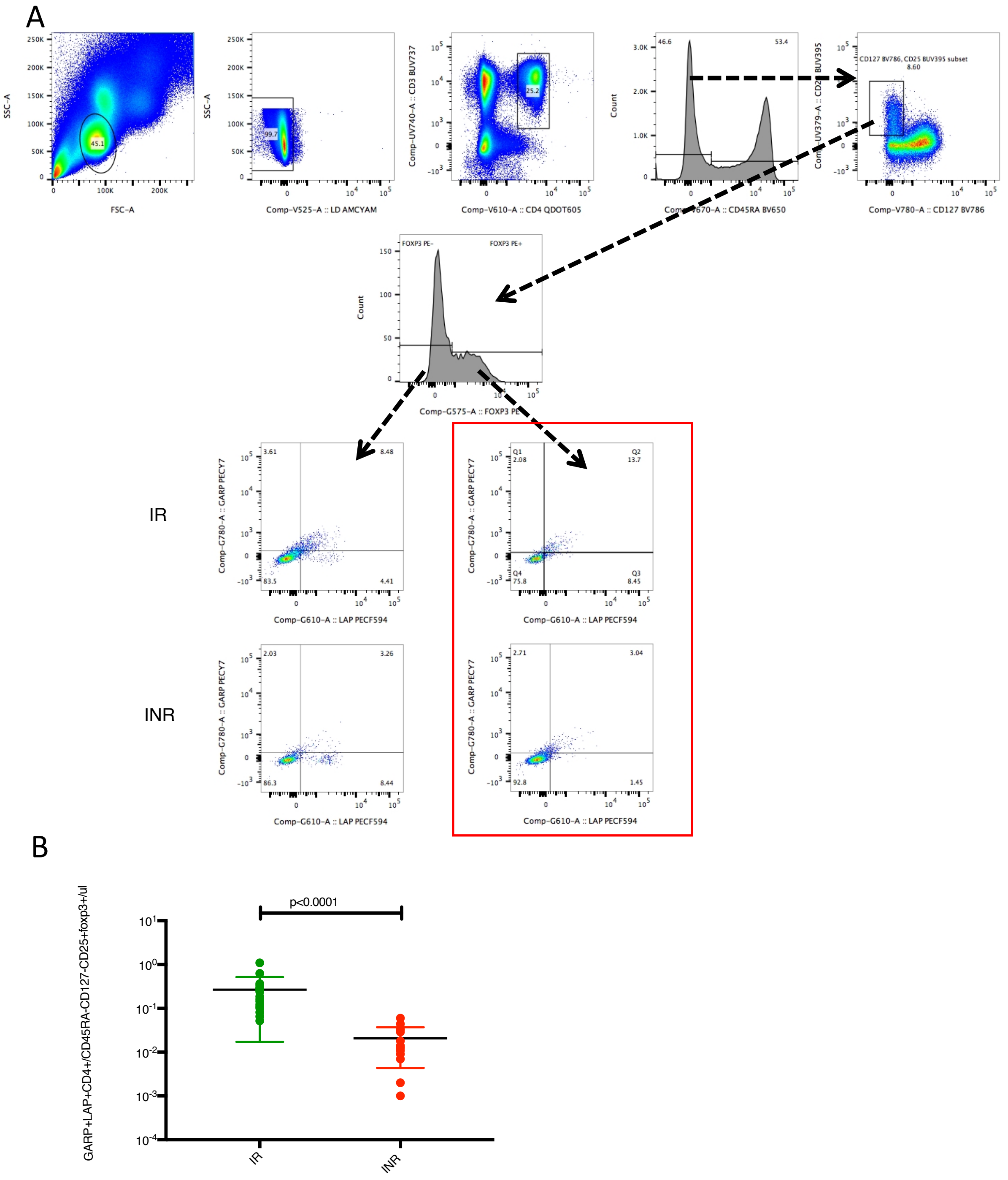
Supplementary Figure 1 continued



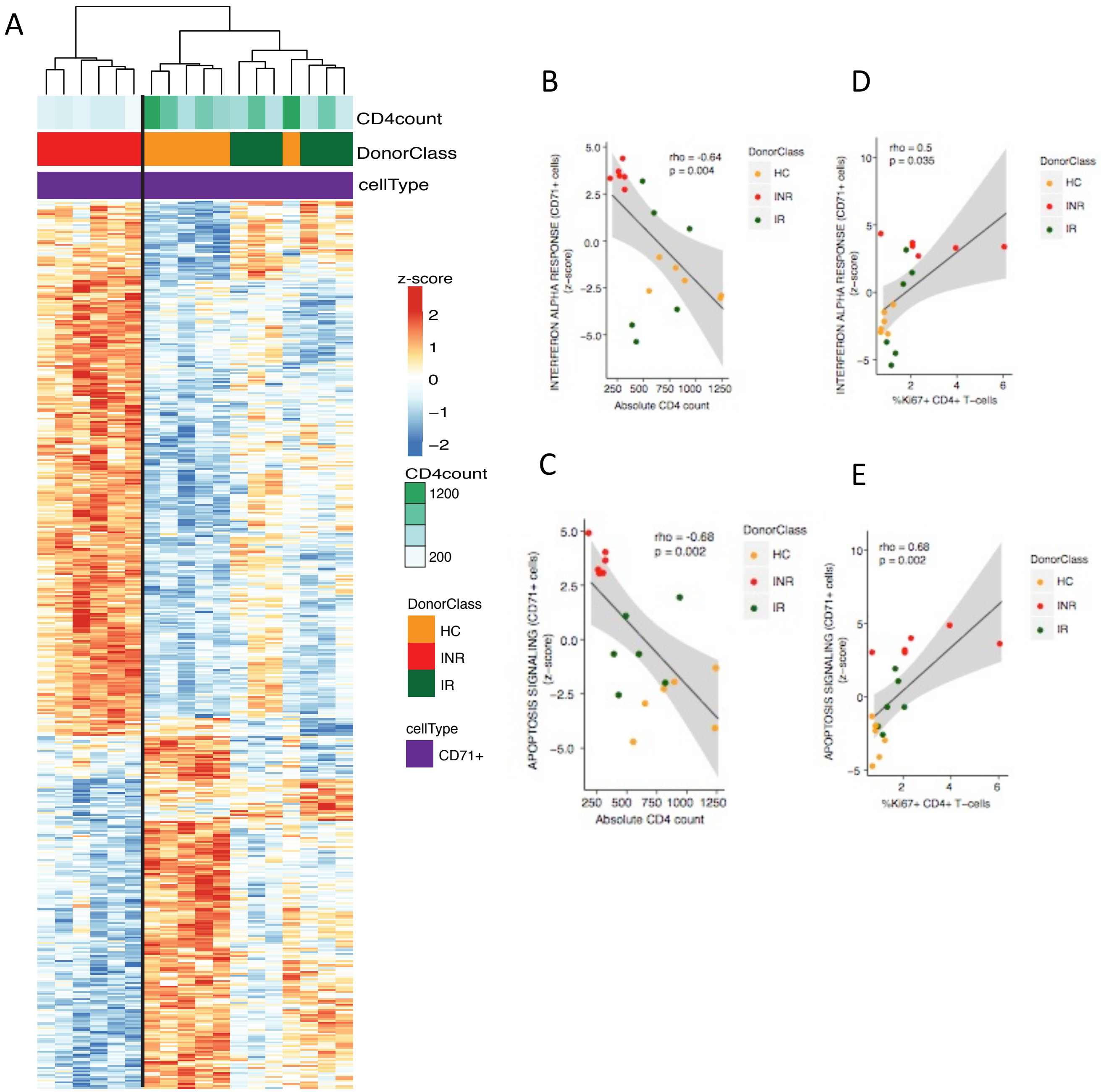
Supplementary Figure 2



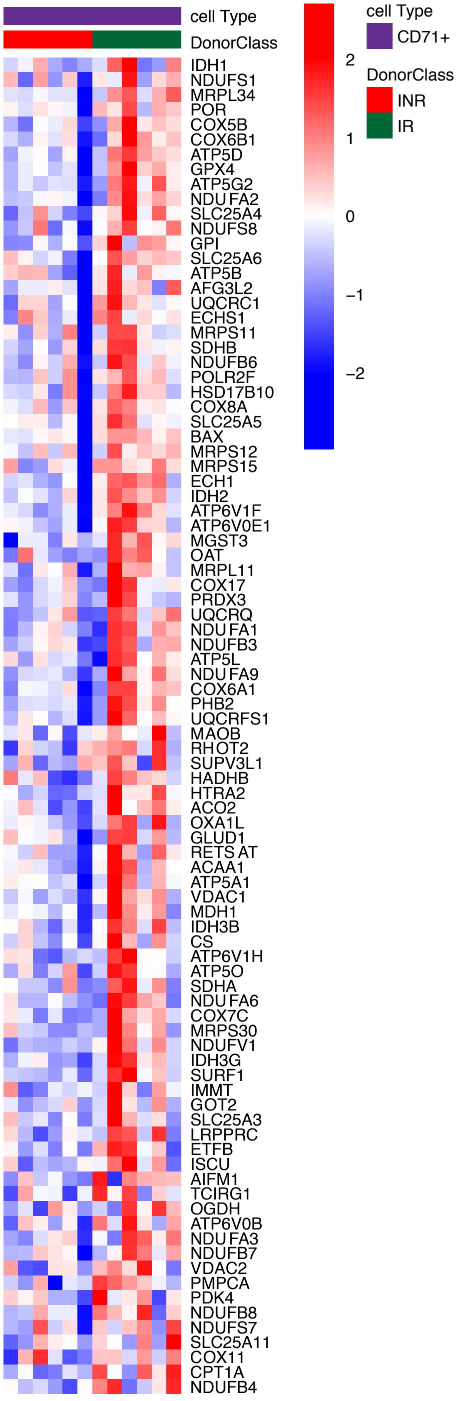
Supplementary Figure 3



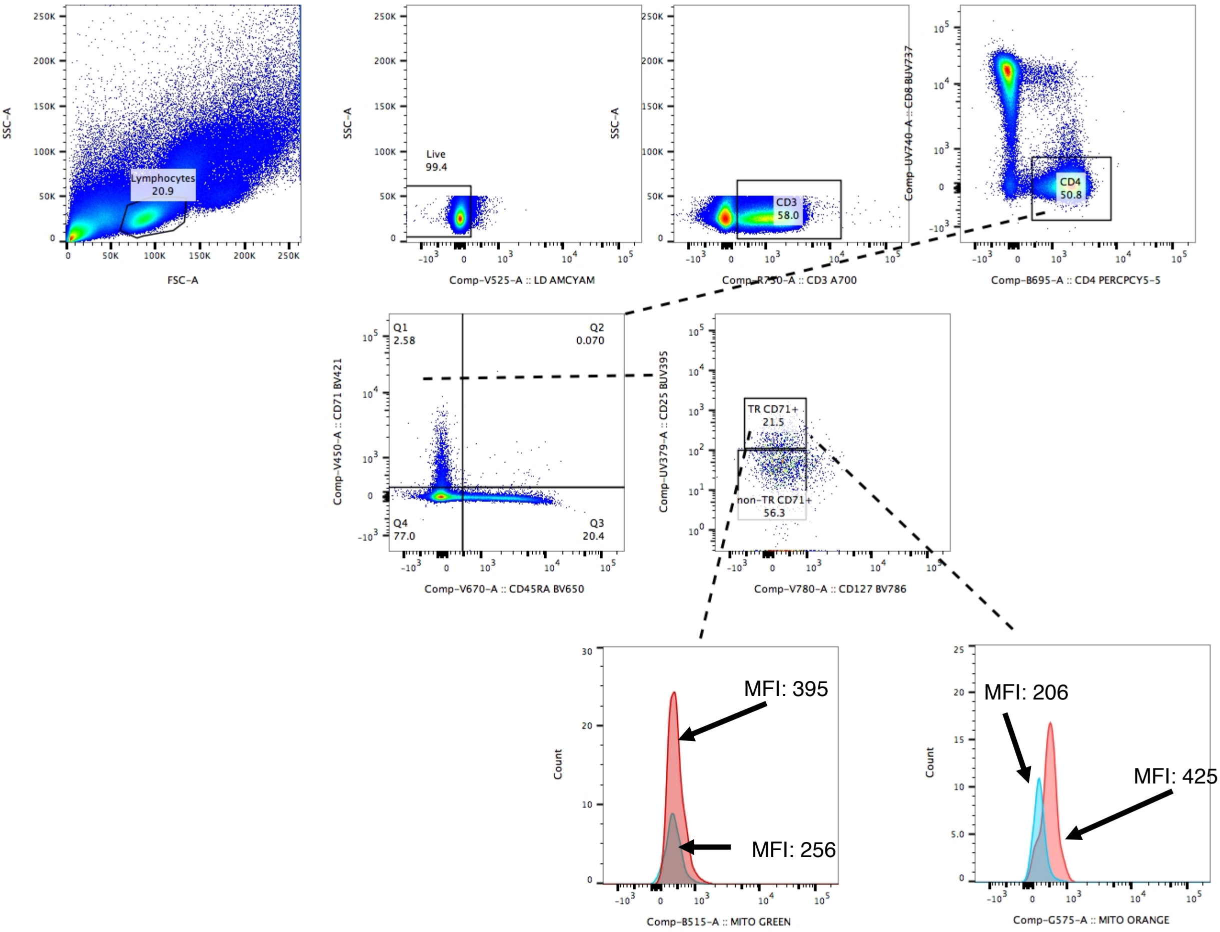
Supplementary Figure 4



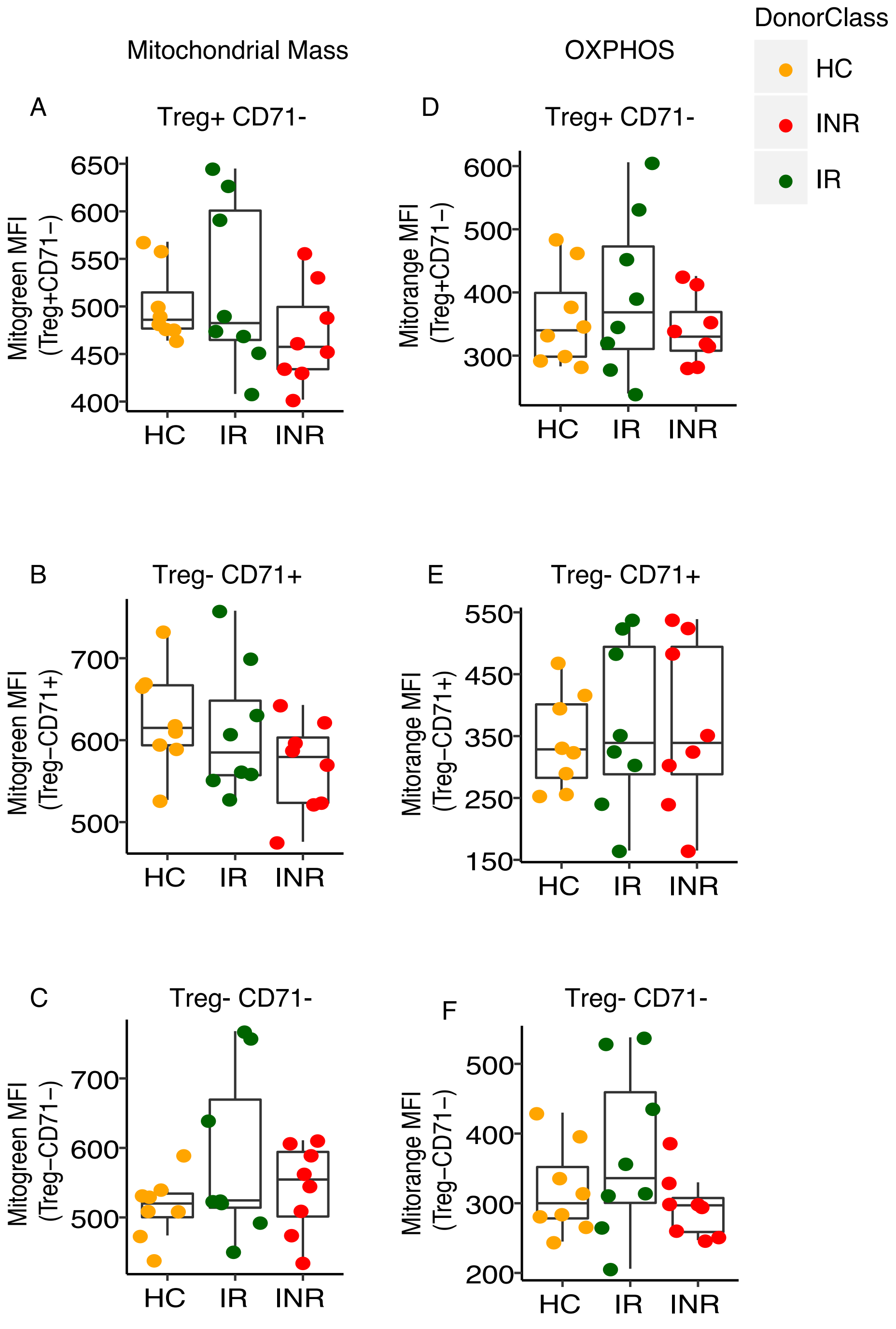
Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 1

A) Virtually all CD4+Ki67+T-cells are also CD71+. **B)** The proportions of CD71+ and Ki67+ cells among CD3+CD4+T-cells are tightly correlated in HIV-1 infected subjects (10 IR, 10 INR) (r=0.9854, $p<0.01$, Spearman rank test, Cleveland cohort subjects). **C)** Modules of cell cycle pathways (identified by the enrichment map strategy – see online methods) enriched ($p<0.05$) among genes differentially expressed between cycling (CD45RA-CD71+) and non-cycling (CD45RA-CD71-) memory CD4+T-cells in each subject group. The nodes represent the pathways and the edges represent the percentage of genes (at least 25%) overlapping between pathways (jaccard index). The size of the node represents the number of genes in the gene set. **D)** Heatmaps of the enrichment ($p<0.05$) of genes involved in negative regulation of cell proliferation showing down-regulation in the cycling memory cells in all donor classes. C and D derived from Russian cohort subjects.

Supplementary Figure 2

A) Gating strategy to evaluate expression of CD25, FOXP3 and CD127 among live CD4+T-cells and CD71- or CD71+ CD45RA- CD3+CD4+ T-cells. **B)** Box plot showing Treg frequencies (y-axis) in total, non-cycling memory (CD45RA-CD71-), and cycling memoryCD45RA-CD71+CD4+T-cells in 9 HC (orange), 18 IR (green), and 16 INR (red) Cleveland cohort subjects. Frequencies of FOXP3+ cells in CD71- and CD71+ CD45RA- are reported. **C)** Absolute Treg count (CD4+CD127-CD25+Foxp3+) in total CD4 T lymphocytes, in non-cycling and cycling memory CD4 T cells among IR (n=18) and INR (n=16). P-values were determined by Wilcoxon rank sum test (* - $p<0.05$). Data represent Mean ± SD in S2B and S2C

Supplementary Figure 3

A) Gating strategy to evaluate the expression of GARP/LAP in CD25+CD127-FOXP3+ cells among CD45RA-CD3+CD4+T-cells. The frequencies in the red quadrant are reported in Figure 2F. **B)** Absolute numbers of GARP+LAP+ effector regulatory T cells (CD45RA-CD127-CD25+Foxp3+) among IR (n=18) and INR (n=16) subjects.

Supplementary Figure 4

A) Heatmap of the genes differentiating the three subject groups (Russian cohort) among cycling memory (CD4+CD45RA-CD71+) cells at $p<0.05$, with the hierarchical clustering showing that the HCs and IRs show similar gene expression profiles and are different from the INRs. B**/C)** Spearman correlation between the expression (z-score determined by SLEA) of the IFN-α response pathway and apoptosis signaling in the cycling memory cells (y-axis), and the absolute CD4 cell counts. **D/E)** Spearman correlation between expression of IFN-α response pathway genes and apoptosis signaling in the cycling memory cells (y-axis), and the frequency of Ki67+ cells among CD4+T-cells.

Supplementary Figure 5.

OXPHOS pathway in cycling memory CD71+CD4+T-cells in INRs (n=6) compared to OXPHOS pathway in cycling memory cells of IRs (n=6)

Supplementary Figure 6

FACS analysis strategy to monitor the MFI of MitoTracker® green and MitoTracker® orange fluorescence in cycling memory (CD71+CD45RA-) CD4+T-cells that are CD25+CD127-. Red and blue histograms designate samples of IR and INR subjects. As the MitoTrackers® cannot be used on fixed cells, FOXP3 staining was omitted in this assay.

Supplementary Figure 7

Mitochondrial mass (**A/B/C**) and OXPHOS (**D/E/F**) estimates in Treg+CD71-(CD25+CD127-CD71-), Treg-CD71+ (CD25-CD127-CD71+) and Treg-CD71- (CD25+CD127-CD71-) CD45RA-CD4+T-cells. P-values were determined by Wilcoxon rank sum test. (Cleveland cohort subjects n= 8 HCs, 8 IRs, and 8 INR). Data represent Mean ± SD.

Supplementary Table 1: Russian cohort patient characteristics

Subjects	n	Gender	Median Age (Min-Max)	Median nadir CD4 (min-max)	Median CD4 count (min-max)	Median years ART duration (Min-Max)
Controls	20	8F12M	32 (25-54)	NA	1071 (573-2021)	NA
IR	21	17F4M	37 (30-57)	165 (18-200)	582 (400-840)	4 (2-5)
INR	16	12F4M	35 (26-46)	95 (8-170)	262 (74-340)	4 (2-6)

Supplementary Table 2: Russian cohort subjects from whom transcriptomic analysis were performed

Patient ID	Gender (0-m; 1-f)	Age	Viral load	CD4 nadir (cell/ul)	CD4 T cell count (cell/ul)	ART Therapy	ART duration (yr)
Control							
5.1	0	35	-		1255	NA	-
5.2	1	26	-		915	NA	-
5.7	0	35	-		828	NA	-
5.1	1	28	-		573	NA	-
5.11	1	26	-		672	NA	-
5.13	1	54	-		1263	NA	-
IR							
3.1	0	50	<50	140	450	CBV ATV/r	3
3.13	1	25	<50	170	410	CBV LPV/r	3
3.14	1	42	<50		840	CBV EFV	5
3.16	1	32	<50	180	620	ABC/3TC NVP	4
3.17	1	34	76	180	960	ABC/3TC LPV/r	6
3.19	1	34	<50	180	510	ABC 3TC ATV/r	2
INR							
4.7	1	35	<50	130	280	ABC ddI LPV/r	6
4.8	1	33	<50	150	290	3TC FAZT LPV/r	2
4.9	1	32	74	110	320	ABC/3TC LPV/r	4
4.14	0	36	<50	12	340	3TC FAZT ETR	4
4.15	0	39	53	8	340	CBV FPV/r	2
4.16	1	41	<50	19	200	ABC/3TC LPV/r	3

Supplementary Table 3: Cleveland cohort patient characteristics

Subjects	n	Gender	Median Age (Min-Max)	Median nadir CD4 count (min-max)	Median CD4 count (min-max)	Median years ART duration (Min-Max)
Controls	10	10F	46 (31-53)	NA	ND	NA
IR	20	14M6F	50 (30-61)	211.5 (0-531)	887 (492-1296)	8 (3-21)
INR	16	13M3F	51 (30-68)	41 (2-288)	277 (151-368)	8.8 (3-21)

Supplementary Table 5

NAME	NES	P-value	LEADING_edge
HALLMARK_OXIDATIVE_PHOSPHORYLATION	2.08	0	VDAC1,ETFB,IDH3G,ISCU,OXA1L,ECH1,NDUFV1,ACAA1,IDH3B,PDHB,ATP5G2,MDH1,COX4I1,ATP6V1F,SDHB,MRPS30,SLC25A3,SURF1,LRPPRC,ATP5A1,MAOB,ATP6V0E1,PHB2,RETSAT,GOT2,SLC25A6,HTRA2,COX8A,GPX4,IDH2,SLC25A5,UQCRFS1,MRPS15,SLC25A4,COX17,ATP5D,NDUFA9,ATP6V1H,HSD17B10,NNT,GLUD1,CASP7,COX7C,COX6A1,RHOT2,BAX,PMPCA,UQCRH,ACO2,COX5B,HSPA9,GPI,ATP5O,UQCRC1,MRPL34,ECHS1,MRPS12,NDUFS1,NDUFA6,SLC25A12,NDUFA2,POLR2F,NDUFS8,SUPV3L1,ATP5L,ATP5B,PRDX3,HADHB,ETFA,POR,MRPS11,IMMT,ATP5H,NDUFB3,TIMM13,MDH2,TIMM9,ACADVL,IDH3A,ATP5F1,UQCR11,ATP6V1E1,LDHA,AFG3L2,OPA1,MGST3,COX6B1,AIFM1,ATP6V1D,NDUFA1,ETFDH,TOMM70A,SDHA,NDUFS7,TCIRG1,NDUFB4,COX5A,OAT,NDUFB5,ATP6V1C1,COX11
MARSON_FOXP3_TARGETS_UP	1.91	0	VIM,S100A10,PSMB8,MYH9,STK10,PTPLAD1,LRRC8C,RCSD1,CD2,DUSP4,S100A4,CORO1A,ARHGAP9,MAPRE2,NCF4,CELF2,RAC2,CD81,OSBPL9,NRIP1,PLIN2,TNFRSF4
REGULATORY T CELL SIGNATURE	1.64	0	S100A10,DGKA,SELL,PSMA4,LEF1,MYC,STAM,RORA,RBMS1,S100A4,MCM5,EEF1A1,CD3G,CNOT2,MAPK9,SMAD7,NINJ2,FKBP5,PIM1,CD81,NOSIP,LY6E,CTLA4,LGALS1,CD3D,IL7R,GBP2,PKP3,PTTG1,TOP2A,TGFBR3,THY1,IL2RB,TXN
HALLMARK_TGF_BETA_SIGNALING	1.67	0.00358	PPP1CA,HDAC1,FKBP1A,TGIF1,JUNB,NCOR2,SMAD7,RHOA,CDH1,TRIM33,SKI,CTNNB1,LEFTY2,SMAD1,FNTA
HALLMARK_INTERFERON_ALPHA_RESPONSE	-1.4	0.0169	IL4R,CASP8,NMI,TDRD7,IFITM1,IFIT3,PRIC285,PNPT1,PSMB9,IFI44,IRF2,LPAR6,SP110,FTSJD2,BST2,IFI27,IFIT2,NCOA7,EPSTI1,FAM125A,IL15,EIF2AK2,SAMD9,PARP14,RSAD2,IFIH1,PARP9

Supplementary Table 7

V1	V2	spearman correlation coefficient	p-value
FreqTregs	REGULATORY_TCELL_SIGNATURE	0.8266254	1.63E-05
FreqTregs	OXIDATIVE_PHOSPHORYLATION	0.8121775	4.97E-05
FreqTregs	FOXP3_UPREGULATED_TARGETS	0.8410733	2.20E-16
FreqTregs	TGF_BETA_SIGNALING	0.8885449	2.20E-16
FreqTregs	INTERFERON_ALPHA_RESPONSE	-0.8245614	2.01E-05
FreqTregs	CD4	0.545173	1.93E-02
Ki67	REGULATORY_TCELL_SIGNATURE	-0.5954592	1.05E-02
Ki67	OXIDATIVE_PHOSPHORYLATION	-0.5128999	3.14E-02
Ki67	FOXP3_UPREGULATED_TARGETS	-0.5232198	2.77E-02
Ki67	INTERFERON_ALPHA_RESPONSE	0.5479876	2.03E-02
CD4	Ki67	-0.7733543	2.83E-12
Ki67	FreqTregs	-0.5149639	0.03061
APOPTOSIS	REGULATORY_TCELL_SIGNATURE	-0.65	0.0046
APOPTOSIS	TGF_BETA_SIGNALING	-0.525	0.027
APOPTOSIS	INTERFERON_ALPHA_RESPONSE	0.77296	0.0002579
APOPTOSIS	CD4	-0.68	0.0018
APOPTOSIS	FreqTregs	-0.5975	0.01
APOPTOSIS	Ki67	0.6821465	0.002415
INTERFERON_ALPHA_RESPONSE	CD4	-0.64	0.004