Wash with 1 ml PBS, spin down 1300pm 8min 800 ul of LiveDead mix (1:2500) @RT for 15min Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min

Add CD161 biotin antibody for 10 minutes at RT

Add HotStain mix, incubate @ 37C for 30min Wash 2 ml 5% PBS-FBS 1400 rpm, 6min Add ColdStain mix, incubate @ 4C for 30min Add 300-500 ul 1x RBC Lysis for 3 minutes Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

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Marker Close (BA-T-S) Vial Lot # Stim!!! Puring 2 L/D RT RT for 10 and 30min 2 and 20min 2 and 20mi							1		1011	S. Parker					αβ T ce	αρ ι cell SFC panel	
Color Colo	Filte	r Fluorochrome	Marker	Clone	Vial Lot #					Stain min		SoldStain 30min		4*C for	RBC Lyse, then Fix/Perm		2
CORD (FMS-1/28) 1.2 2.4 1.2 2.4 0.7 1.4 0.8 0.8 0.8 0.8 0.8 0.8 0.8 0.0 <	UVZ		CD62L	(DREG-56)					3)	40		@4C					
CD69 [FF50] FF50 FF50	3		CD8	(RPA-TR)								1.2	2.4				
CRAS (1651) (1651) (1651) (1671) (1671) (1782) <td>6/0</td> <td></td> <td>69Q)</td> <td>(FN50)</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>100</td> <td></td> <td>0.7</td> <td>1.4</td> <td></td> <td></td> <td></td> <td></td>	6/0		69Q)	(FN50)						100		0.7	1.4				
CD3 (KS) <t< td=""><td>UV1</td><td></td><td>CCR4</td><td>(161)</td><td>)</td><td></td><td>-</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>80</td><td>16</td></t<>	UV1		CCR4	(161))		-									80	16
CD38 (FRAGS1) (MASE2) (MASE2) (MASE2) (MASE3) <t< td=""><td>UV11</td><td></td><td>V52</td><td>(86)</td><td></td><td></td><td>-</td><td></td><td>-</td><td></td><td>3,6</td><td></td><td></td><td></td><td>7.1</td><td></td><td>2</td></t<>	UV11		V52	(86)			-		-		3,6				7.1		2
CONTINUE	UV14		CD38						7			0.5	1			0.1	0.0
Vay 7.2 (MASE 2) COLOR (MASE 2) COLO	UV16		CD4	(SK3)								0.5	1				7.0
CD19 (M5E2) 1.5 3 1.5 3 street CD25RA (H619) 1.5 3 1.5 3 1.5 3 CXCRS (H619) 1.5 3 1.5 3 1.5 1.	V1		Va7.2	(0)(0)			1		ij	5	3			f		2.0	10
CD150 (HB19] 1.5 3 Street CD45RA (HE100) 1.5 3 Street CXCKS (125D4) 2.5 5 0.5 1 \$4.4 CCKR (13C10) 1.2 2.4 1.5 1.5 1.5 FONP3 (SK7) 1.0 2.4 1.5 1.5 1.5 CDB (MAZ61) 1.0 2.4 1.5 1.5 1.5 PD1 (HAZ61) 1.0 2.4 1.5 1.5 1.5 CCR7 (MAZ61) 1.0 2.4 1.5 3 1.5 1.5 PD2 (MAZ61) 1.0 2.4 1.0 2 1.5 </td <td>73</td> <td></td> <td>CD14</td> <td>(MSE2)</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>1.2</td> <td>2.4</td> <td></td> <td></td> <td></td> <td>1.1</td>	73		CD14	(MSE2)								1.2	2.4				1.1
CD055RAM (H13.00)	33		CD19	(HIB19)			1		1			1.5	3				
BV510 CD45RA (Hi100) CD45RA (Hi100) CD45RA (Hi100) CD45RA (Hi100) CD52D4) CD52D4) CD52D4 CD52	75	BV480	CD161	(REA631)	1				-			1.5	m	Stran			
CXCRS (1252b4) CXCRS (1252b4) CXCRS (1252b4) CXCRS (1252b4) CXCRS CXCRS (11A9) CXCRS	77		CD45RA	(Hi100)			-		i		2			188			
COS (3CLO)	V10		CXCRE	(175704)			+					0.5	1				
COS (3C10) COS	V11			(34777)			1		2		5			1.0			
Five (E27)	V13	BV711	ICOS	(3010)			1										
CCR6 (11A9) 1.2 2.4 1.5 1.5 CDS (KA-25H) 4 1.2 2.4 8 EDZ (IM-425H) 4 1.5 2.4 0.5 PDZ (IM-425H) 4 1.2 2.4 1.5 PDZ (IM-425H) 4 1.2 2.4 1.2 CCR7 (IM-425H) 4 1.2 2.4 1.2 LOS (IM-425H) 4 1.2	V14	BV750	IFNV	(B27)			+			1	1	1.2	2.4				
FoxP3 FoxP3 L. 2.4 L. 2.4 B. 2.4 <td>V15</td> <td>BV786</td> <td>CCR6</td> <td>(11A9)</td> <td></td> <td></td> <td>1</td> <td></td> <td></td> <td>P</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>1.5</td> <td>~</td>	V15	BV786	CCR6	(11A9)			1			P						1.5	~
CD26 (IVA-26.1) CD26 (IVA-26.1) CD26 (IVA-26.1) CD26 (IVA-26.1) CD25 (IVA-26.1) CD25 (IVA-26.1) CD25 (IVA-26.1) CD25 (IVA-26.1) CD25 (IVA-26.1) CD27 (IVA-26.1) IVA-26.1	B2	Alexa Fluor 488	FoxP3		9 -		-		T		4.7						
CD26 (M-A261)	B3	Spark Blue 550	CD3	(SK7)		1										ന	9
PDQ EH12.2H7	B9	PerCP-Cy5.5	CD26	(M-A261)			-					1.2	2.4			0.5	
TNFa	YG1	PE	169	(EH12.2H7			-	1		- 1						1.5	
CD25 (NA.4251) 1.2 2.4 1.2 1.2 CCR7 CCR7 CL43) 6.8 1.6 1.6 1.2 CD38 (A.1) 1.5 3 1.2 2.4 0.5 1.6 1.2 CD27 (M.01- (P)T 1.5 3 1.2 2.4 0.5 1.6 1.2 1.2 CD27 (O323) 1.5 3 1.5 3 1.5 3 Antibody Total 1.5 Antibody Total 1.5 3 1.5 3 Pippette draw volume 19.5 Brilliant Stain 2.18 43.6 27.6 55.2 2.1 4.1	YG3	PE-Dazzle594		(MAB11)	1		-					1.0	2				
HA-DR (1243)	765	PE-Cv5	CD25	(M-A251)												1.2	2.4
14-5-PR (1243)	VG8	cFluor-BYG750							1.7	2.	4.						i
CCR7 (A1) (A2) (A3) (A3) (A3) (A3) (A3) (A4) (A3) (A4) <th< td=""><td>469</td><td></td><td>HLA-DR</td><td>(L243)</td><td></td><td></td><td>-</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></th<>	469		HLA-DR	(L243)			-										
CD39 (A1) (A2) (A3) (A3) (A4A3) (A3) (A12500)	/G10		CCR7				-					0.8	1.6				I
1.2 (MQ1- 1.5 3 2.4			CD39	(A1)			+					0.5	1				T
1.5 3 1.5 3 1.5 3 1.5 3 1.5 3 1.5 3 1.5 3 1.5 3 1.5 3 1.5 3 1.5 3 1.5 3 1.5 3 1.5 3 1.5 3 1.5 3 1.5 3 1.5 1.			11.2	(MO1-			-		1.2		4						T
L/D			CD107a	(H44A3)		-										13	20
CD27 (0323) 1.5 3 Antibody Total 1.5 Antibody Total 1.09 21.8 13.8 27.6 10.5 Pippette draw volume 19.5 Primaths draw 21.8 43.6 27.6 55.2 21	R6		0/1			-	-	1000								7:7	1.1
Antibody Total 1.5 Antibody Total 10.9 21.8 13.8 27.6 10.5 Pippette draw volume 19.5 princate draw colume 19.5 princate draw column	R7		CD27	(0323)			77.7	20005									I
Antibody Total 1.5 Antibody Total 10.9 21.8 13.8 27.6 10.5 R10 Media 19.0 Brilliant Stain 21.8 43.6 27.6 55.2 21 Pippette draw volume 19.5 pinneste draw 22.1 21 21 21	R8			(2)			-					1.5	3				T
R10 Media 19.0 Brilliant Stain 21.8 43.6 27.6 55.2 21 21 21 21 21 21 21	NCTA			Antibody	Total	1.5	Antik	body Total	10.0		-	000	, 10				
19.5 Pinnette draw 22.0 27.0 55.2 21	HICK	INED CONTROLS !!!		R10 Me	edia	19.0	Brilli	ant Stain	21.8	1		13.8	9.17			10.5	21
			-	Pippette drav	-	19.5	Pin	went affent	0.1.2	1000		0./7	7.55			21	42

Second Perm Wash: 2 ml NuclearWash 1500 rpm 6 min

2 ml NuclearWash 1500 rpm 6 min

First PermWash:

(vortex every 10 minutes)

1 ml Nuclear FixPerm, incubate @ 4C for 30min Add Streptavidin Mix, incubate @ 4C for 15min Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

Add Intracellular Stain, incubate @ RT for 40min First PermWash: 2 ml Nuclear/Wash 1500 rpm 6 min Second Perm Wash: 2 ml Nuclear/Wash 1500 rpm 6 min

Cap tubes, wrap rack in foil, store at 4*C

Resuspend in 30 ul 0.4% PFA-PBS

285cs + 2 cmst

PREE 1103 WATERED J. 11:43 / 11:20 Shoop @ 11:15 -> 11:30 Fire 11:45-735 05 12

7:38 can Ses 80,4 7:47 cm Anti hecles gan do-m

7.04 an Abscells ort 7.00 cm Saydus sain Note: Icos + Italy southerds.

528 AM 37854 9.20 Am 20mber

8:33 Hb aix done.

Inha ce 12:50pm 503 dow e 12,5%

1:34 pm 370/spin 1:43 Enc 1 wast 96:43 Samples Had work @ 10:23 (+5)

16.30 cold Samples -> 11:co Art

860H - 4000 12m1 = 13.6my = 13-KH 750 30001 1.5ml x 2x3 - w 5 £35 500

660/1 RBC 430

Icos ... stin?

11645 + 1409 Association 03.12 86 (1 ~ 13011 sis

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