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Paradoxical effects of obesity on T cell function during tumor progression and PD-1 checkpoint blockade

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Supplementary Figure 1: Phenotypes of 6-month-old male control and DIO mice. a) Body (subcutaneous and visceral) fat of mice quantified by T1-weighted MRI scans (n=3/group). b) Body weights (n=3/group), serum c) glucose (n=3/group), d) HbA1c (n=3/group), and e) leptin levels (n=3/group). PD-1 expression of f) CD8+ and g) CD4+ T cells in the liver assessed by flow cytometry (n=3/group). Data in this figure are all depicted as mean ±s.e.m., with all individual points shown. One-tailed unpaired Student's t-test p-values shown.



Supplementary Figure 2: Phenotypes of 11-12-month-old male control and DIO mice. a) Serum glucose levels (n=4/group). b) Frequency of CD44+ T cells in the peripheral blood (n=3/group). c) Representative flow plots and frequency of PD-1+ T cell in the peripheral blood (n=3/group). PD-1 expression on peripheral blood d) CD8+ and e) CD4+ T cells (n=3 in control group, n=4 in DIO group). Data in this figure are all depicted as mean ±s.e.m., with all individual points shown. One-tailed unpaired Student's t-test p-values shown. ns=not significant.



Supplementary Figure 3: T cell functions of 11-12-month-old male control and DIO mice. Ki67 expression on a) CD8+ and b) CD4+ T cells in the spleen after ex vivo stimulation (n=3 in control group, n=4 in DIO group). Ki67 expression on c) CD8+ and d) CD4+ T cells in the liver after ex vivo stimulation (n=3/group). e-f) IFN γ and g-h) TNF α production on CD8+ and CD4+ T cells in the liver after ex vivo stimulation (n=3 in control group, n=4 in DIO group). Data in this figure are all depicted as mean ±s.e.m., with all individual points shown. One-tailed unpaired Student's t-test p-values shown.



Supplementary Figure 4: Phenotypes of non-obese vs. obese higher species. a) Body weights of non-obese and obese rhesus macaques (n=3/group). b) Representative flow plots and frequency of CD95+ T cells in the peripheral blood of non-obese and obese rhesus macaques (n=3/group). PD-1 expression on c) CD8+ and d) CD4+ T cells in the peripheral blood of rhesus macaques (n=3/group). Ki67 expression on e) CD8+ and f) CD4+ T cells after ex vivo stimulation of non-obese and obese rhesus macaque PBMCs. g) BMI of non-obese (BMI<30, n=7) and obese (BMI>=30, n=8) human healthy volunteers. Data in this figure are all depicted as mean ±s.e.m., with all individual points shown. One-tailed unpaired Student's t-test p-values shown.



Supplementary Figure 5: Phenotypes of tumor-bearing control and DIO mice. a) Individual tumor volumes of B16 melanoma in 6-month-old control (n=4) and DIO (n=5) C57BL/6 male mice. b) Quantification of tumor burden by PET-CT imaging of 6-month-old B16-F0-tumor-bearing control and DIO (n=6/group) mice 13 d.p.i. c) Representative H&E staining of tumors from 6-month-old control and DIO B16-F0-bearing male mice. d) Representative flow staining of PD-1, Tim3, and Lag3 on tumor infiltrating CD8+ T cells from 6-month-old B16-bearing control and DIO male mice at 16 d.p.i. Frequency of e) T regulatory (FoxP3+CD25+CD4+) cells (n=7/group) and PD-L1+ cells (n=3/group) in the B16-F0 tumors from 6-month-old B16-bearing control and DIO male mice. g) The relative expression of *Cpt1a* in the tumors from 6-month-old B16-bearing control and DIO male mice (n=5/group). h) Body weights of 6-month-old control and DIO female BALB/c mice (n=3/group). b, e-h) Data are depicted as mean ±s.e.m., with all individual points shown. One-tailed unpaired Student's t-test p-values shown. i) Tumor volume of 4T1 inoculated orthotopically in 6-month-old control and DIO female BALB/c mice (n=3/group). Tumor volume of 4T1 tumors in 6-month-old control and DIO female BALB/c mice (n=3/group). Tumor volume of 4T1 tumors in 6-month-old control and DIO female BALB/c mice (n=3/group).



Supplementary Figure 6: Obesity promotes T cell exhaustion systemically during tumor progression. Frequency of PD-1 on CD8+ T cells from the inguinal LN of 6-month-old a) non-tumor-bearing and b) B16-F0-bearing control and DIO mice (n=5/group). Frequency of Eomes on PD-1+CD8+ T cells from c) draining LN and h) spleens of 6-month-old B16-F0 bearing control and DIO mice (n=5/group). Frequency of PD-1 on CD8+ T cells from the d) liver and f) spleen of 6-month-old B16-F0-bearing control and DIO mice (n=5/group). Frequency of T-bet on PD-1+CD8+ T cells from the e) liver (n=4 in control group, n=5 in DIO group) and g) spleen (n=5/group) of 6-month-old B16-F0-bearing control and DIO mice (n=5/group). Frequency of 6-month-old B16-F0-bearing control and DIO mice (n=5/group). Jota in this figure are all depicted as mean ±s.e.m., with all individual points shown. One-tailed unpaired Student's t-test p-values shown. ns=not significant.





Markers

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TGFB Pathway Chemokines EGFR Pathway Lef 1 Myc Elk1 XcI1 Mapk8 Ccl5 Fos Ccl4 Dusp1 Cxcl10 Jun Ccl3 Egf Csf1







Anergy/Exhaustion Effector Markers Senesence Markers Obesity, Diabetes, Insulin-Resistance Gzmk Cd28 Eef1a1 Nfatc2 Prf 1 Sirt1 Satb1 Cdkn1 Gzma Pdcd1 Prdm1 Dusp1 Hk1 Gzmm Raplgap2 Gzmb KIrg1 Lag3 Spp1 Cd28 Ltb Cebpa CD160 lfng

Ltb4r1 Adcy 9

Car2



112 Ctla4 h Hba-a1 Functions Cytokine metabolic process Regulation of chemokine production Negative regulation of interferon-gamma production Regulation of cytokine biosynthetic process Tumor necrosis factor biosynthetic process

Network: Predicted Physical Interactions Co-expression Pathway

Genetic Interactions Co-localization Shared protein domains

Supplementary Figure 7: Differential gene expression and network analysis on memory CD8+ T cells from tumor-bearing control and DIO mice. a) Heat maps showing differentially expressed gene clusters (>1.5 fold) and b) network analysis of up-regulated genes from enriched gene sets (using GeneMANIA) in CD8+CD44+ T cells isolated from the spleens and lymph nodes of 6-month-old control or DIO mice bearing B16-F0 at 16 d.p.i. Data shown as fold change of DIO over control.



Supplementary Figure 8: The correlation of leptin level with immune exhaustion profiles. a) Heat maps showing differentially expressed leptin-associated genes (\geq 1.5-fold difference) in CD44+CD8+ T cells isolated from the spleens and lymph nodes of 6-month-old B16-F0-bearing control and DIO C57BL/6 male mice at 16 d.p.i. (data shown as fold change over controls) (n=3/group). b) Frequency of PD-1 expression on CD8+ T cells in the peripheral blood of low (<10,000 pg/ml, n=13) and high (>10,000 pg/ml, n=8) serum leptin cohorts of human healthy volunteers. c) Body weight (n=4/group) and d) leptin levels (n=2/group) of 19-month-old AL and CR male mice. e) Frequency of Eomes expression on liver PD-1+CD8+ T cells of 19-month-old AL and CR male mice (n=4/group). f) Frequency and g) total number of PD-1+CD8+ splenic T cells in AL and CR mice (n=4/group). h) Frequency and i) total number of Eomes+PD-1+CD8+ splenic T cells in AL and CR mice (n=4/group). Data in this figure are all depicted as mean \pm s.e.m., with all individual points shown. One-tailed unpaired Student's t-test p-values shown.

Supplementary Figure 8



Supplementary Figure 9: The lack of leptin signalling is associated with decreased T cell dysfunction. a) Body weights of age-matched (9-month-old) control and *db/db* male mice (n=3/group). b) Body weights of age-matched control (n=3) and DIO (n=3) mice and weight-matched *ob/ob* (n=2) mice. c) Representative flow plots and d) frequency of PD-1ex-pression on splenic T cells of age-matched control (n=3) and DIO (n=3) mice and weight-matched *ob/ob* (n=2) mice. Data are depicted as mean ±s.e.m., with all individual points shown. b, d) One-way ANOVA with Tukey post-hoc test used to compare groups *p<0.05, **p<0.01, ***p<0.001. e) Schema of adoptive transfer of cells from spleens and LNs of WT and *db/db* mice into NSG mice. The fold change of WT or *db/db* CD8+ T cells transferred into NSG mice versus the number f) in the spleen (n=4 in WT group, n=2 in *db/db* group), and g) in the liver (n=3 in WT group, n=2 in *db/db* group) at 13 days post-transfer. h) Fold change in PD-1 expression on splenic CD8+ T cells of NSG mice prior to versus 13 days post-transfer (n=4 in WT group, n=2 in *db/db* group). a, f-h) Data are depicted as mean ±s.e.m., with all individual points shown. One-tailed unpaired Student's t-test p-values shown. ns=not significant.



Supplementary Figure 10: Leptin effects on T cell function in vitro. a) Frequency of pSTAT3 and b) fold change of PD-1 on splenic CD8+ T cells cultured with α CD3 + rmleptin, α CD3 + rmleptin and STAT3 inhibitor, or medium only for 24 hrs (n=2 technical replicates). c) Frequency of pSTAT3 on splenic CD8+ T cells stimulated with α CD3+ rmleptin for 24 hrs (n=2 technical replicates). d) Total number of sorted naïve (CD45RA+CD45RO-) and memory (CD45RA-CD45RO+) T cells from human PBMCs after stimulation by ConA with or without the addition of rhleptin (n=3 technical replicates). Data are depicted as mean ±s.e.m., with all individual points shown. One-tailed unpaired Student's t-test *p<0.05, **p<0.01.



Supplementary Figure 11: Exogenous leptin promotes T cell exhaustion in vivo. a) Schema of tumor implantation and rmleptin treatment in *ob/ob* mice. b) Body weight of 3-month-old *ob/ob* male mice bearing B16-F0 treated with or without rmleptin. Data are depicted as mean \pm s.e.m. Two-way ANOVA with Tukey post-hoc test used to compare groups. **p<0.01, ****p<0.0001. c) Tumor volumes of 3-month-old ob/ob male mice bearing B16-F0 treated with or without rmleptin. Data are depicted as mean+individual volumes (n=3/group). d) Total number of PD-1+CD8+ T cells and e) representative flow plot and frequency of Ki67+PD-1+CD8+ T cells in the B16-F0 tumors of *ob/ob* mice with and without rmleptin treatment. f) The relative expression of *Eomes* in the tumor of B16-F0-bearing *ob/ob* mice with and without rmleptin treatment. g) Frequency of PD-1+CD8+ T cells and the relative expression of h) *Pdcd1*, i) *HAVCR2*, and j) *Cpt1a* in the livers of B16-F0-bearing *ob/ob* mice with and without rmleptin treatment. dividual points shown. One-tailed unpaired Student's t-test p-values shown.



Supplementary Figure 12: The lack of leptin signaling prevents T cell exhaustion mediated tumor progression in DIO mice. a) Body weights of 5-month-old control and DIO C57BL/6 Rag2-/- male mice (n=4/group). Data are depicted as mean ±s.e.m., with all individual points shown. One-tailed unpaired Student's t-test p-value shown. b) Tumor volumes and c) individual tumor volumes following B16-F0 subcutaneous inoculation in 5-month-old control and DIO Rag2-/- mice with adoptively transferred WT T cells. d) Individual B16-F0 tumor growth in control and DIO Rag2-/- mice after adoptive transfer of T cells from either WT or *db/db* C57BL/6 mice. e) Representative flow plots of PD-1 on tumor infiltrating CD8+ T cells from B16-F0-bearing control and DIO Rag2-/- mice with adoptive transfer of T cells from either WT or *db/db* C57BL/6 mice. f) Representative flow staining and frequency of g) IFNγ and h) TNFα on adoptively transferred WT and *db/db* CD8+ T cells from the tumors of B16-F0-bearing DIO Rag2-/- mice after ex vivo stimulation (n=2/group, 2-3 technical replicates in WT group).



Supplementary Figure 13: αPD-1 therapy in tumor-bearing control and DIO mice. a) Schema of tumor implantation and aPD-1 therapy in control and DIO mice. b) Individual tumor volumes of B16-F0 melanoma in 6-month-old control and DIO C57BL/6 male mice (n=4 in control and DIO rIgG groups, n=5 in control and DIO αPD-1 groups). c) Kaplan-Meier survival curves are shown to compare B16-F0-tumor-bearing control and DIO mice treated with or without αPD-1 (n=10/group). Log-rank (Mantel-Cox) test used to compare groups. d) Tumor volume of 3LL inoculated subcutaneously in 6-month-old control and DIO C57BL/6 male mice treated with or without αPD-1 (n=3 in control rlgG, αPD-1 and DIO rlgG groups, n=4 in DIO αPD-1 group). Tumor volumes are depicted as mean ±s.e.m. Two-way ANOVA with Tukey post-hoc test used to compare groups. e) Individual tumor volumes of 3LL tumors in 6-month-old control and DIO C57BL/6 male mice treated with or without αPD-1. Frequency of f) CD3+ and g) CD3+CD8+ cells in the tumors of B16-F0-bearing control and DIO mice treated with or without α PD-1 (n=6 in DIO rlgG group, n=5 in control rlgG and DIO α PD-1 groups, n=4 in control αPD-1 group). h) The ratio of tumor-infiltrating CD8+ to CD4+ T cells in the tumors of B16-F0-bearing control and DIO mice treated with or without αPD-1 (n=6 in DIO rlgG group, n=5 in control rlgG, αPD-1 and DIO αPD-1 groups). f-h) Data are depicted as mean ±s.e.m., with all individual points shown. One-way ANOVA with Tukey post-hoc test used to compare groups. Frequency of PD-1 expression on i) CD3+ and j) CD8+ T cells in the tumors of DIO mice with and without α PD-1 treatment (n=5/group). Data are depicted as mean ±s.e.m., with all individual points shown. One-tailed unpaired Student's t-test *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.





Supplementary Figure 14: α PD-1 treatment reinvigorates T cell function with less toxicity in tumor-bearing DIO mice. Frequency of a) IFN γ and b) TNF α producing CD8+ splenic T cells from tumor-bearing control and DIO mice after ex vivo stimulation (n=6 in DIO rIgG group, n=5 in control rIgG and α PD-1 groups, n=4 in DIO α PD-1 group). Data are depicted as mean ±s.e.m., with all individual points shown. One-way ANOVA with Tukey post-hoc test used to compare groups. c) Schema of systemic B16-F10 implantation and α PD-1 therapy in 6-month-old control and DIO male mice. The levels of d) ALT, e) IFN γ , f) IL-6 and g) TNF α in the serum of B16-F0-bearing DIO mice which were treated with either α CD40/rhIL-2 or α PD-1. Data are depicted as mean ±s.e.m., with all individual points shown. One-tailed unpaired Student's t-test used to compare groups. *p<0.05, **p<0.01. ns=not significant.





Supplementary Figure 15: Immune exhaustion profiles of cancer patients. Human TCGA data analysis of a) CD274 (PD-L1), b) LEPR, c) CTLA4, and d) FOXP3 expression in melanoma tumors from non-obese (n=86) vs. obese (n=40) patients. The line within each notch box represents the median. The lower and upper boundaries of the box indicate first and third quartiles respectively. The whiskers indicate the minimum and maximum values. P-value was calculated via DESeq2 (Wald-Test) to compare groups.

Age	Sex	BMI				
35	F	18.8				
37	F	18.9				
33	F	20				
32	F	20.8				
40	F	21.3				
27	F	21.3				
31	F	22.2				
58	F	22.3				
38	F	22.5				
29	F	30.2				
28	F	31.4				
37	F	34.8				
60	F	34.8				
35	F	35.2				
53	F	36.2				
41	F	36.9				
27	F	38.1				
54	F	38.5				
52	F	41.6				
27	F	42.6				
43	F	50.6				
BMI<30: Mean age: 38 +/- 10 BMI≥30: Mean age: 44 +/- 13 p=0.46						

Supplementary Table 1. Healthy donor patient characteristics

Supplementary Table 1

Variable	Overall (n=250)	BMI < 30 (n=169)	BMI ≥ 30 (n=81)
BMI Mean (SD)	27.4 (7.3)	23.4 (3.6)	35.7 (6.2)
BMI Range	15 -56.6	15 - 29.9	30.0 - 56.6
Age Mean (SD)	61.7 (13.7)	62.6 (14.6)	59.70 (11.52)
Age Range	23, 91	23, 91	36, 87
Sex M (%) F (%)	114 (45.6) 136 (54.4)	80 (47.3) 89 (52.7)	34 (42.0) 47 (58.0)
Cancer Type Lung (%) Melanoma (%) Ovarian (%) Other (%)	55 (22.0) 45 (18.0) 20 (8.0) 130 (52.0)	44 (26.0) 23 (13.6) 17 (10.1) 85 (50.3)	11 (13.6) 22 (27.2) 3 (3.7) 45 (55.6)
ECOG 0 (%) 1 (%) 2 or More (%)	70 (28.00) 134 (53.6) 46 (18.4)	48 (28.4) 87 (51.5) 34 (20.1)	22 (27.2) 47 (58.0) 12 (14.8)
Prior Therapy 1 (%) 2 (%) 3 or More (%)	44 (17.6) 91 (36.4) 115 (46.0)	24 (14.2) 66 (39.1) 79 (46.7)	20 (24.7) 25 (30.9) 36 (44.4)
irAE (%)	52 (20.8)	32 (18.9)	20 (24.7)
irAE type Colitis Pnuemonitis Thyroid Colitis + Thyroid	23 11 14	13 8 9 2	10 3 5 2

Supplementary Table 2. Patient characteristics and irAE

Supplementary Table 2

Supplementary Table 3. Details of flow cytometry antibodies.

Name	Vendor	Cat#	Fluorochrome	Clone #	Dilution (for 50ul)	Validation	Reference
CD45 CD45	Biolegend Biolegend	103126 103116	PB APC-Cy7	30-F11 30-F11	200 200	https://www.biolegend.com/en-us/products/pacific-blue-anti-mouse-cd45-antibody-3102 https://www.biolegend.com/en-us/products/apc-cy7-anti-mouse-cd45-antibody-2530	Jiang Q, et al. 2008. Blood. 112:2858 Krasteva G, et al. 2011. Proc Natl Acad Sci U S A. 108:9478
CD44	Biolegend	103020	РВ	IM7	100	https://www.biolegend.com/en-us/products/pacific-blue-anti-mouse-human-cd44-antibody-3099	Lee JW, et al. 2006. Nature Immunol. 8:181.
CD4	Biolegend	100550	BV711	RM4-5	200	https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-mouse-cd4-antibody-7925	Shigeta A, et al. 2008. Blood 112:4915.
CD8a	Biolegend	100744	BV605	53-6.7	320	https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd8a-antibody-7636	Ko SY, et al. 2005. J. Immunol. 175:3309.
CD3 TNFα	Biolegend Biolegend	100232 506324	BV785 PE-Cy7	17A2 MP6-XT22	40 80	https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-mouse-cd3-antibody-7953 https://www.biolegend.com/en-us/products/pe-cy7-anti-mouse-tnf-alpha-antibody-5866	Lofano G, et al. 2015. J Immunol. 195: 1617-1627. Lawson BR, <i>et al.</i> 2007. <i>J. Immunol</i> . 178:5366
Ki67 T-bet	Biolegend Biolegend	652420 644804	Alexa Fluor 700 Alexa Fluor 647	16A8 4B10	400 50	https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-ki-67-antibody-10366 https://www.biolegend.com/en-us/products/alexa-fluor-647-anti-t-bet-antibody-5759	Guillaumond F, <i>et al.</i> 2015. <i>PNAS.</i> 112:2473 Feng J,et al. 2017. Nat Commun. 10.1038/s41467-017-01056-8
CD279 (PD-1)	ebioscience/ThermoFisher	11-9981-82	FITC	RMP1-30	50	https://www.thermofisher.com/antibody/product/CD279-PD-1-Antibody-clone-RMP1-30- Monoclonal/11-9981-82 https://www.thermofisher.com/antibody/product/FOXP3-Antibody-clone-FJK-16s-Monoclonal/11-	Starobinets H, et al. 2016. JCl. 1;126(12):4417-4429. doi: 10.1172/JCl85705
FoxP3	ebioscience/ThermoFisher	11-5773-80	FITC	FJK-16s	100	5773-82 https://www.thermofisher.com/antibody/product/EOMFS-Antibody-clone-Dan11mae-Monoclonal/61-	Liu Z, et al. 2017. Nat Commun. 27;8:14754. doi: 10.1038/ncomms14754
Eomes	ebioscience/ThermoFisher	61-4875-82	PE-eFluor 610	Dan11mag	50	4875-82 https://www.thermofisher.com/antibody/product/CD366-TIM3-Antibody-clone-RMT3-23-	Wang H, et al. 2017. Int J Med Sci. 18;14(10):977-983. doi: 10.7150/ijms.20212. eCollection 2017
Tim3	ebioscience/ThermoFisher	12-5870-82	PE	RMT3-23	80	Monoclonal/12-5870-82 https://www.thermofisher.com/antibody/product/CD223-LAG-3-Antibody-clone-eRioC9R7W-C9R7W-	Kim DS, et al. 2017. Nat Commun. 24;8(1):344. doi: 10.1038/s41467-017-00324-x. Di Marco Barros B. et al. 2016. Cell. 22:167(1):203-218.e17. doi: 10.1016/j.cell.2016.08.030. Epub.2016.
CD223 (Lag3)	ebioscience/ThermoFisher	46-2231-82	PerCP-ef710	eBioC9B7W	80	Monoclonal/46-2231-82 https://www.thermofisher.com/antibody/product/CD621-I-Selectin-Antibody-clone-MFI-14-	Sep 15
CD62L	ebioscience/ThermoFisher	25-0621-82	PE-Cy7	MEL-14	160	Monoclonal/25-0621-82 https://www.thermofisher.com/antibody/product/CD274-PD-11-87-H1-Antibody-clone-MIH5-	Deng R, et al, Nat Commun. 17;8(1):978. doi: 10.1038/s41467-017-00880-2
CD274 (PD-L1)(B7-H1)	ebioscience/ThermoFisher	12-5982-83	PE	MIH5	50	Monoclonal/12-5982-82 https://www.thermofisher.com/antibody/nroduct/Phospho-STAT3-Tyr705-Antibody-clone-LUVNKL4-	Tian Y, et al, Immunity. 17;44(5):1204-14. doi: 10.1016/j.immuni.2016.04.008. Epub 2016 May 3.
pSTAT3	ebioscience/ThermoFisher	17-9033-42	APC	LUVNKLA	40	Monoclonal/17-9033-42	
IENv	BD Biosciences	561479	APC-Cv7	XMG1.2	80	http://www.bdbiosciences.com/us/applications/research/t-cell-immunology/th-1-cells/intracellular- markers/cytokines-and-chemokines/mouse/apc-cy7-rat-anti-mouse-ifnxmg12/p/561479	Cherwinski HM, et al. J Exp Med. 1987; 166(5):1229-1244
CD3	BD	558124	PacBlue	SP34-2	167	http://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti- non-human-primate-antibodies/cell-surface-antigens/pacific-blue-mouse-anti-human-cd3-sp34- 2/p/558124	Carter DL, Shieh TM, Blosser RL et al. CD56 identifies monocytes and not natural killer cells in rhesus macaques. Cytometry. 1999; 37(1):41-50.
CD4	BD	562843	BV605	L200	50	http://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti- non-human-primate-antibodies/cell-surface-antigens/bv605-mouse-anti-human-cd4-l200/p/562843	Giorgi JV, Hultin LE, Desrosiers RC. The immunopathogenesis of retroviral diseases: no immunophenotypic alterations in T, B, and NK cell subsets in SIVmac239-challenged rhesus macaques protected by SIV delta nef vaccination. J Med Primatol. 1996; 25(3):186-191.
CD8	BD	557945	AF700	RPA-T8	50	http://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti- human-antibodies/cell-surface-antigens/alexa-fluor-700-mouse-anti-human-cd8-rpa-t8/p/557945	Reimann KA, Waite BC, Lee-Parritz DE, et al. Use of human leukocyte-specific monocional antibodies for clinically immunophenotyping lymphocytes of rhesus monkeys. Cytometry. 1994; 17(1):102-108.
CD95	BD	558814	APC	DX2	50	http://www.bdbiosciences.com/us/applications/research/t-cell-immunology/regulatory-t-cells/surface- markers/human/apc-mouse-anti-human-cd95-dx2/p/558814	Villinger, F., Bostik, P., Mayne, A., King, C. L., Genain, C. P., Weiss, W. R., & Ansari, A. A. (2001). Cloning, sequencing, and homology analysis of nonhuman primate Fas/Fas-ligand and co-stimulatory molecules. <i>Immunogenetics</i> , 53(4), 315-328.
CD28	BD	561368	APC-H7	CD28.2	10	https://www.bdbiosciences.com/us/applications/research/t-cell-immunology/regulatory-t- cells/surface-markers/human/apc-h7-mouse-anti-human-cd28-cd282/p/561368	Page, A., Srinivasan, S., Singh, K., Russell, M., Hamby, K., Deane, T., & Strobert, E. (2012). CD40 blockade combines with CTLA4Ig and sirolimus to produce mixed chimerism in an MHC defined rhesus macaque transplant model. <i>American Journal of Transplantation</i> , <i>1</i> 2 (1), 115-125. Xu, H., Wang, X., Lackner, A., A., & Veazev, R. S. (2014) PD-1HIGH folliariar CD4 Theiner cell subsets
PD-1	Biolegend	329906	PE	EH12.2H7	50	https://www.biolegend.com/en-us/products/pe-anti-human-cd279-pd-1-antibody-4412	residing in lymph node germinal centers correlate with B cell maturation and IgG production in rhesus macaques. Frontiers in immunology, 5, 85.
Ki-67	BD	561165	AF488	B56	50	http://www.bdbiosciences.com/us/applications/research/apoptosis/conjugated-antibodies/alexa- fluor-488-mouse-anti-ki-67-b56/p/561165	Pitcher CJ, Hagen SI, Walker JM, et al. Development and homeostasis of T cell memory in rhesus macaque. J Immunol. 2002; 168(1):29-43. Lugli E., Gattinoni, L., Roberto, A., Mavilio. D., Price, D. A., Restifo, N. P., & Roederer, M. (2013).
Live/Dead	invitrogen	L34966	Aqua	n/a	50	https://www.thermofisher.com/order/catalog/product/L34966	Identification, isolation and in vitro expansion of human and nonhuman primate T stem cell memory cells. <i>Nature protocols</i> , 8(1), 33.