

Supplementary Information

Detection of clinically relevant immune checkpoint markers by multicolor flow cytometry

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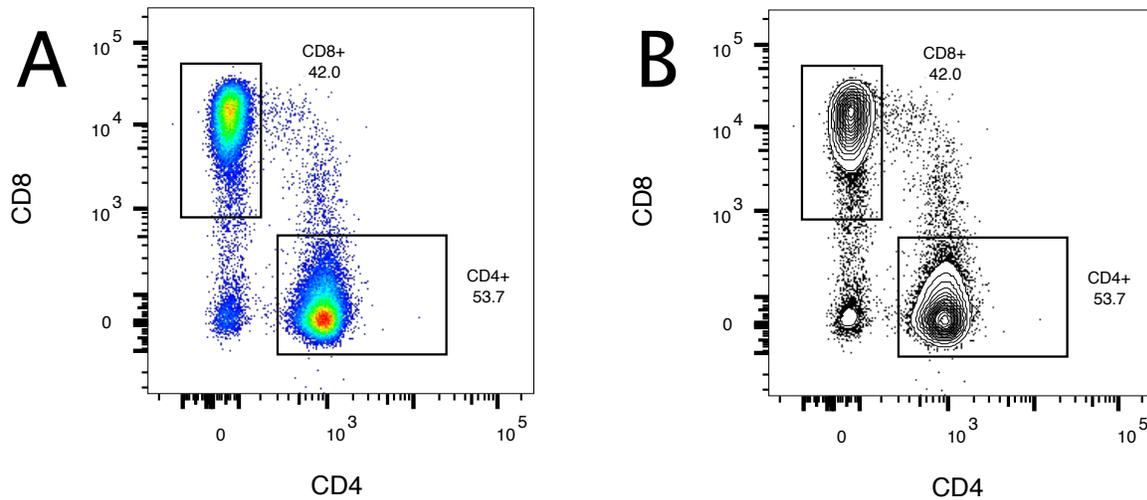


Figure S1 Example gating of CD4+ vs CD8+ cells using contour plots (**A**) and pseudocolor dot plots (**B**) on Donor C FMO1. Gated on Lymphocytes/singlets/live cells/CD3+. Figure generated using FlowJo.

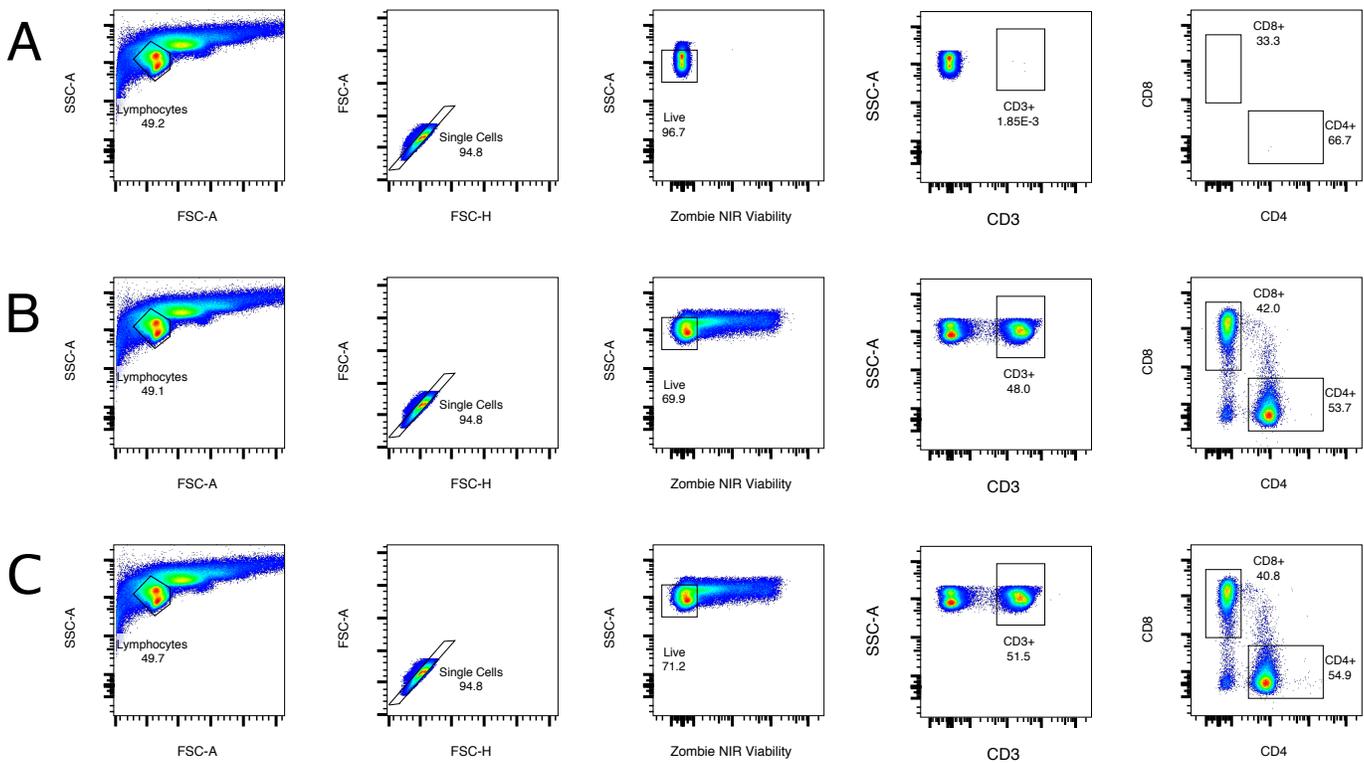


Figure S2 Dot plots of unstained (**A**), FMO1 (**B**), and FMO2 (**C**) controls in lineage gating. Figure generated using FlowJo.

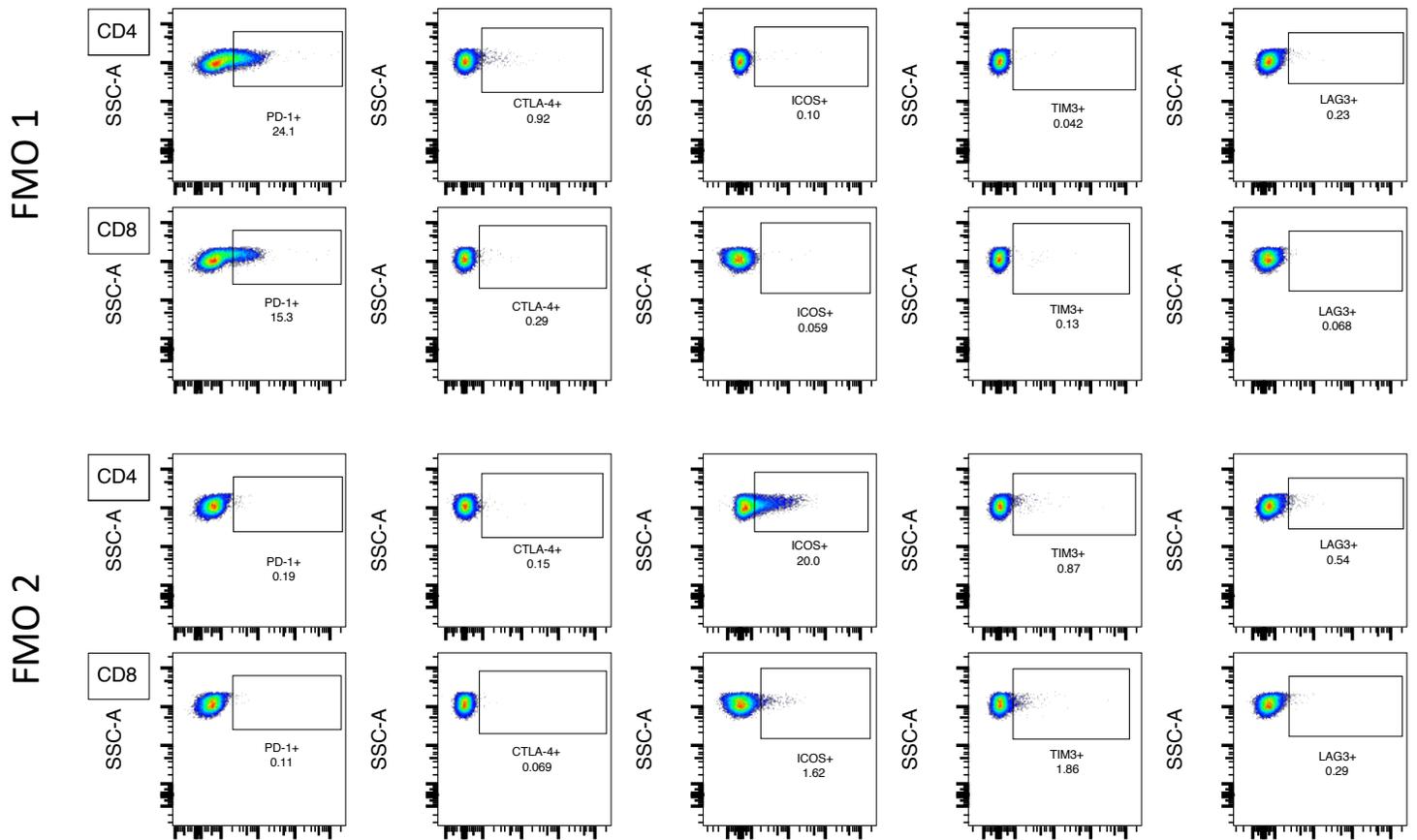


Figure S3 Dot plots of FMO1 and FMO2 checkpoint marker expression. Figure generated using FlowJo.

	PE-Cy7-A	FITC-A	APC-A	BV 510-A	PE-A	BV 650-A	BV 421-A	PerCP-Cy5-5-A	Zombie NIR-A
PE-Cy7-A	1.00	0.00	0.00	0.00	0.05	0.00	0.00	0.05	0.13
FITC-A	0.00	1.00	0.00	0.03	0.00	0.00	0.00	0.02	0.00
APC-A	0.00	0.00	1.00	0.00	0.00	0.03	0.00	0.01	0.03
BV 510-A	0.00	0.00	0.00	1.00	0.00	0.12	0.06	0.00	0.00
PE-A	0.01	0.00	0.00	0.00	1.00	0.02	0.00	0.21	0.00
BV 650-A	0.00	0.00	0.20	0.01	0.01	1.00	0.04	0.04	0.01
BV 421-A	0.00	0.00	0.00	0.19	0.00	0.00	1.00	0.00	0.00
PerCP-Cy5-5-A	0.01	0.00	0.14	0.00	0.02	0.16	0.00	1.00	0.03
Zombie NIR-A	0.04	0.00	0.01	0.00	0.00	0.00	0.00	0.03	1.00

Table S1 FlowJo compensation matrix for fluorophores on antibody panel.