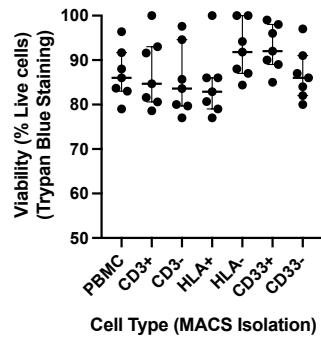


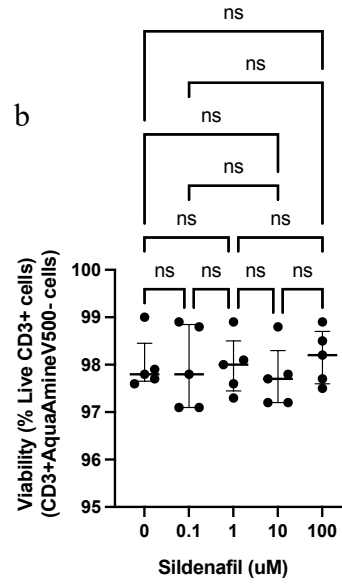
# Appendices

## Appendix 1. Cell Viability

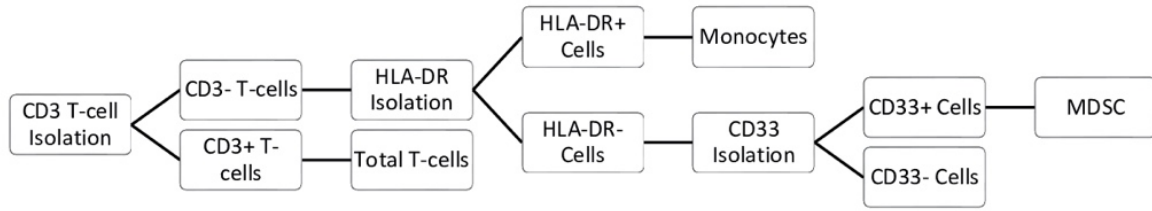
a



b



## Appendix 2. MACS Isolation Flow Diagram



### Appendix 3. Co-culture Plate Layout

H2O									
<i>Untreated</i>	M-aT	Mo-aT			M	Mo	Sil		
	aT				T		Sil		
<i>Sildenafil</i>	M-aT	Mo-aT	<i>Unstimulated</i>						
	aT		M	Mo					
			MT	MoT					
			T						
H2O									

Key:

M: MDSC

Mo: Control Monocyte

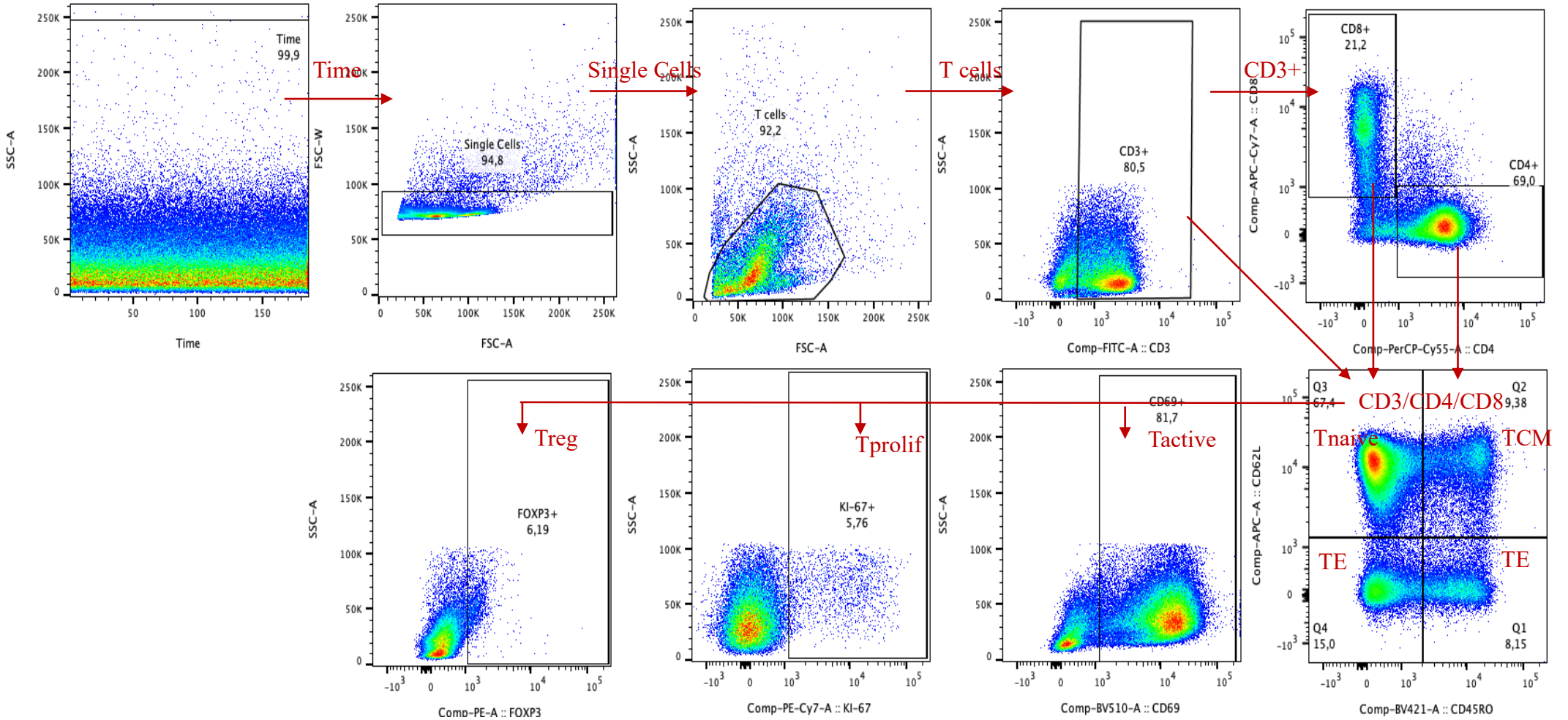
T: Unstimulated T cell. aT: Stimulated T cell (anti-CD2CD3CD28 beads, Miltenyi T cell Activation Kit)

#### Appendix 4. Sildenafil dilution

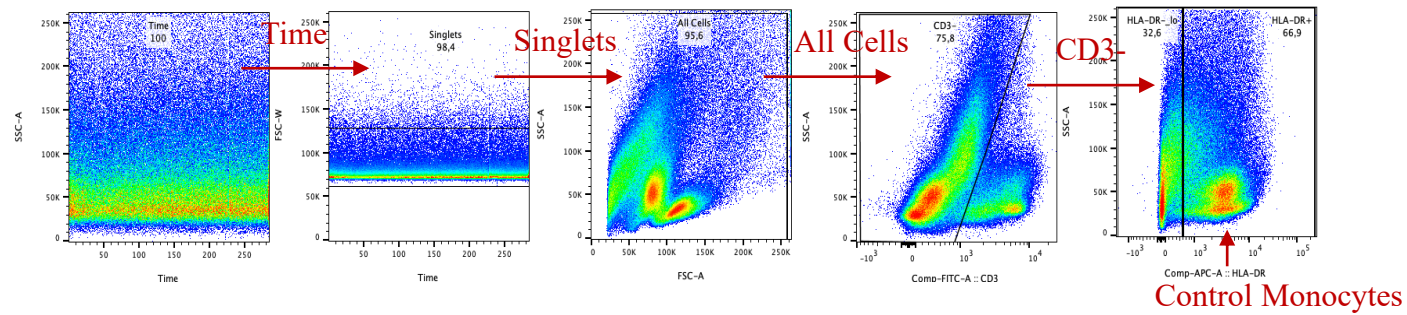
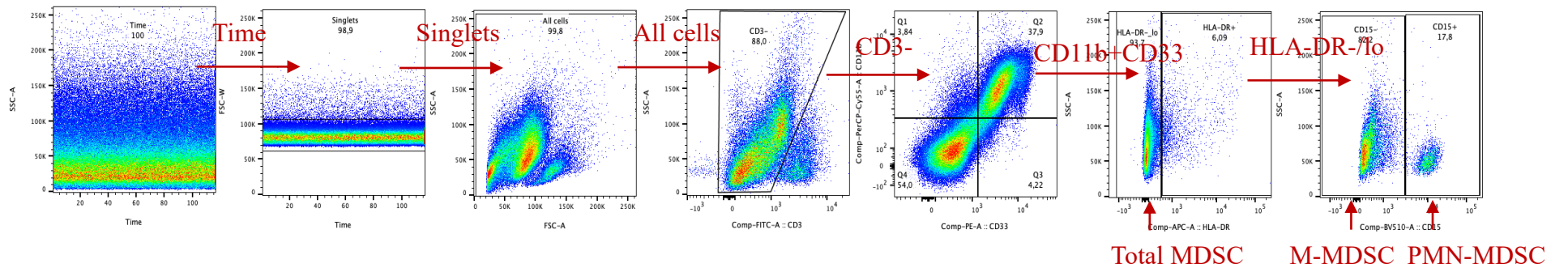
- 1) Sildenafil (Merck) 5mg/ml Stock.
- 2) Dilute stock (powder) in 1ml DMSO.
- 3) Prepared 100 aliquots of 10ul (5mg/ml stock), stored at -20°C
- 4) Add 90uL RPMI to an aliquot (0.5 mg/mL)
- 5) [Sildenafil]
  - 0.5mg/mL = 500 000 ug/L
  - $500\ 000\ \text{ug/L} / 666.7\ (\text{Mw}) = 749.99\ \mu\text{M}$  (concentration in 100 uL)
  - X 10 aliquots of Sildenafil = 750uM in 1mL RPMI
  - 750 uM in 1mL = 100 uM in 7.5 mL
- 6) Add 6.5 mL RPMI to reach 7.5 mL [100uM Sildenafil]
- 7) Serial dilution from 100 uM to 0.1 uM
  - 750 uL from 100uM stock, into 6.75 mL RPMI = 10uM Sildenafil Stock in 7.5 mL
  - 750 uL from 10uM stock, into 6.75 mL RPMI = 1uM Sildenafil Stock in 7.5 mL
  - 750 uL from 1uM stock, into 6.75 mL RPMI = 0.1 uM Sildenafil Stock in 7.5 mL



# Appendix 5a. Gating Strategy (T Cell Panel)



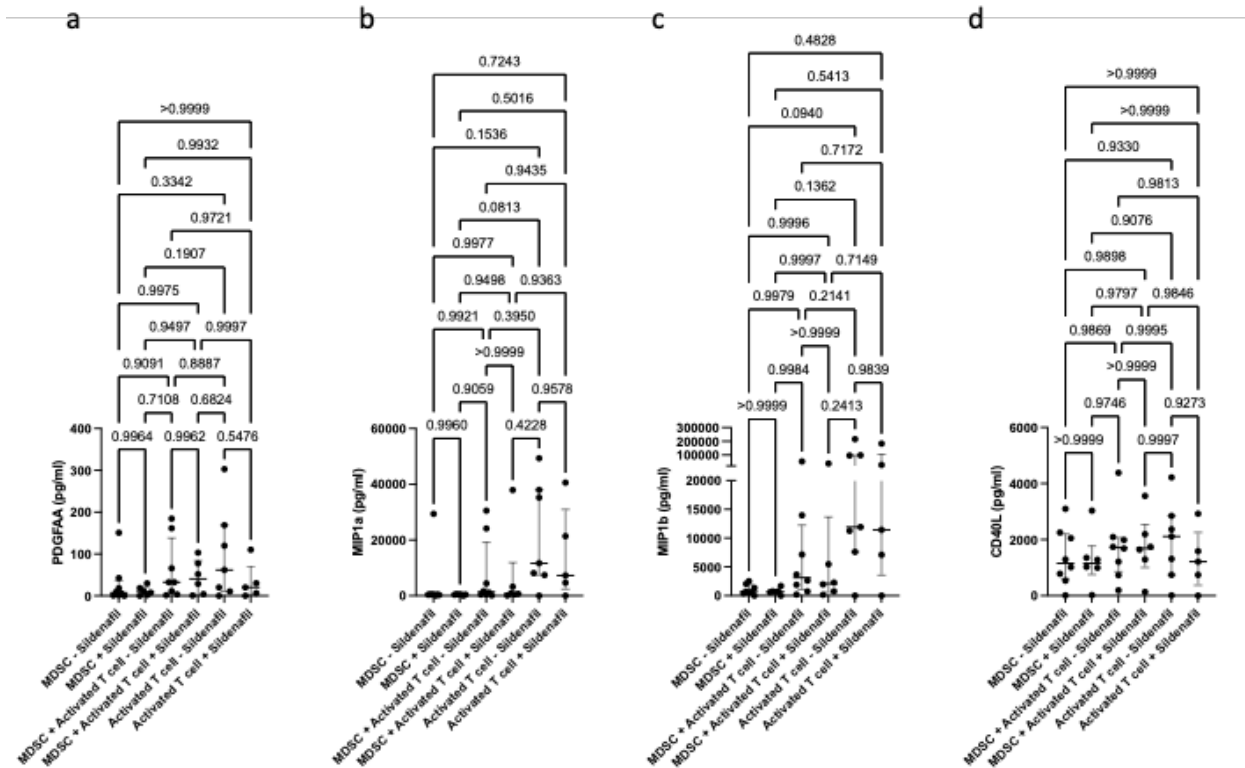
## Appendix 5b. Gating Strategy (MDSC Panel)





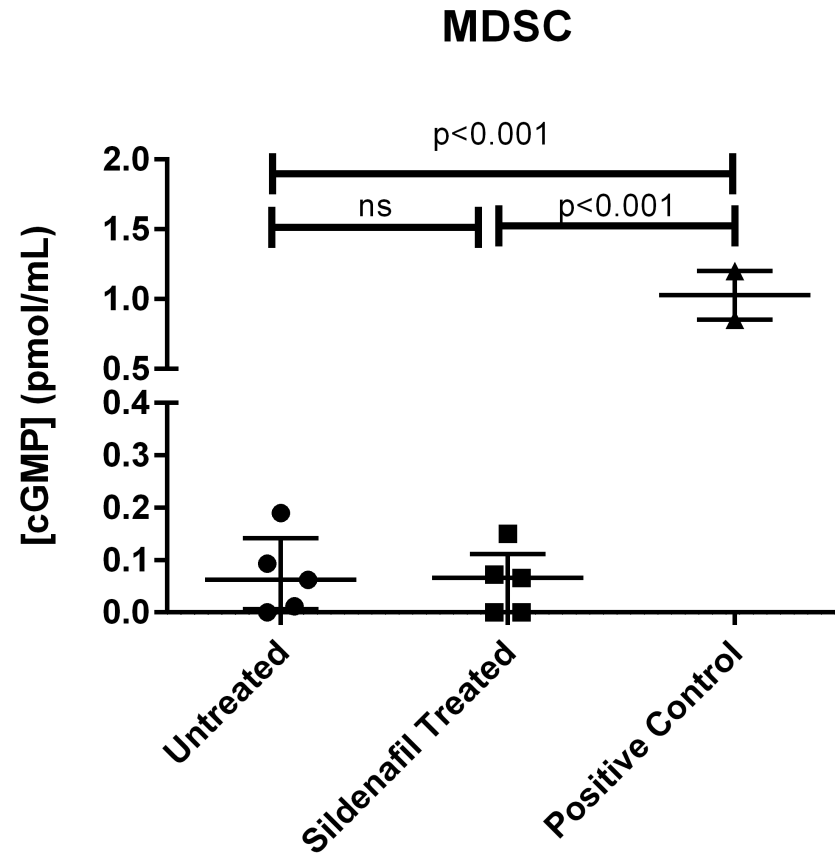


## Supplementary Figure 2:



Supplementary Figure 2: Sildenafil supplementation does not affect T cell or MDSC cytokine production (PDGFAA, MIP1a, MIP1b, CD40L). Analyte concentration (pg/ml) was measured for various culture combinations (MDSC only, MDSC-T cell coculture, and T cell only), under differing stimulation combinations (Unstimulated and anti-CD3CD28 bead Activation) with or without Sildenafil treatment, by multiplex analysis. Plots show analyte concentration in culture supernatant. Mixed model analysis, One-way ANOVA with Tukey post-hoc correction Median with interquartile range indicated on plots.  $P < 0.05$  was considered significant.

Supplementary Figure 3:



Supplementary Figure 3: 10uM Sildenafil treatment does not increase cGMP concentration within MDSC. cGMP concentration (pmol/ml) was measured in culture supernatant of MDSC treated with 10uM Sildenafil for 48 hours. As positive control, cGMP was measured in culture supernatant of PBMC that were stimulated with 5ug/ml PHA for 12 hours. One-way ANOVA with Tukey post-hoc correction. Median with interquartile range indicated on plots.  $P < 0.05$  was considered significant.