Supplemental Materials

Non-classical monocytes (CD14dimCD16+) are associated with carotid intima-media thickness progression for men but not women: The Multi-Ethnic Study of Atherosclerosis

Running Title: Monocyte subtypes and carotid IMT: Sex differences

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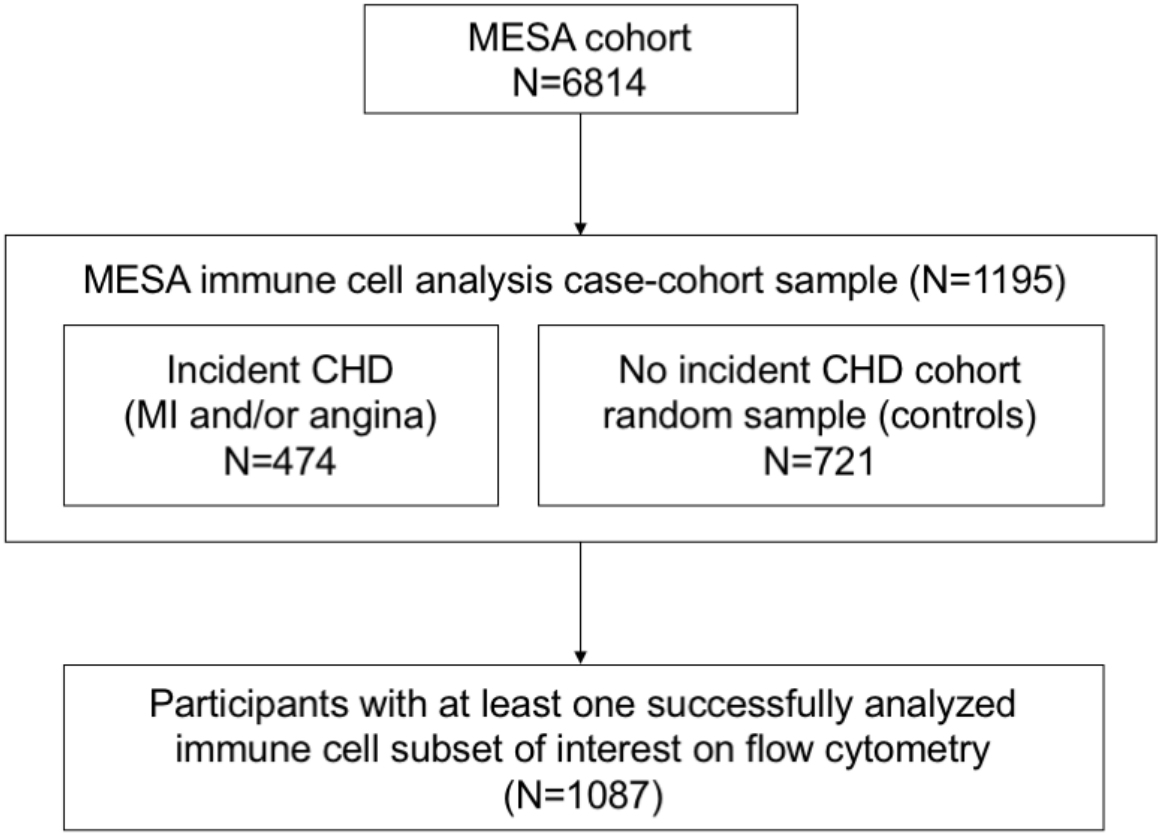
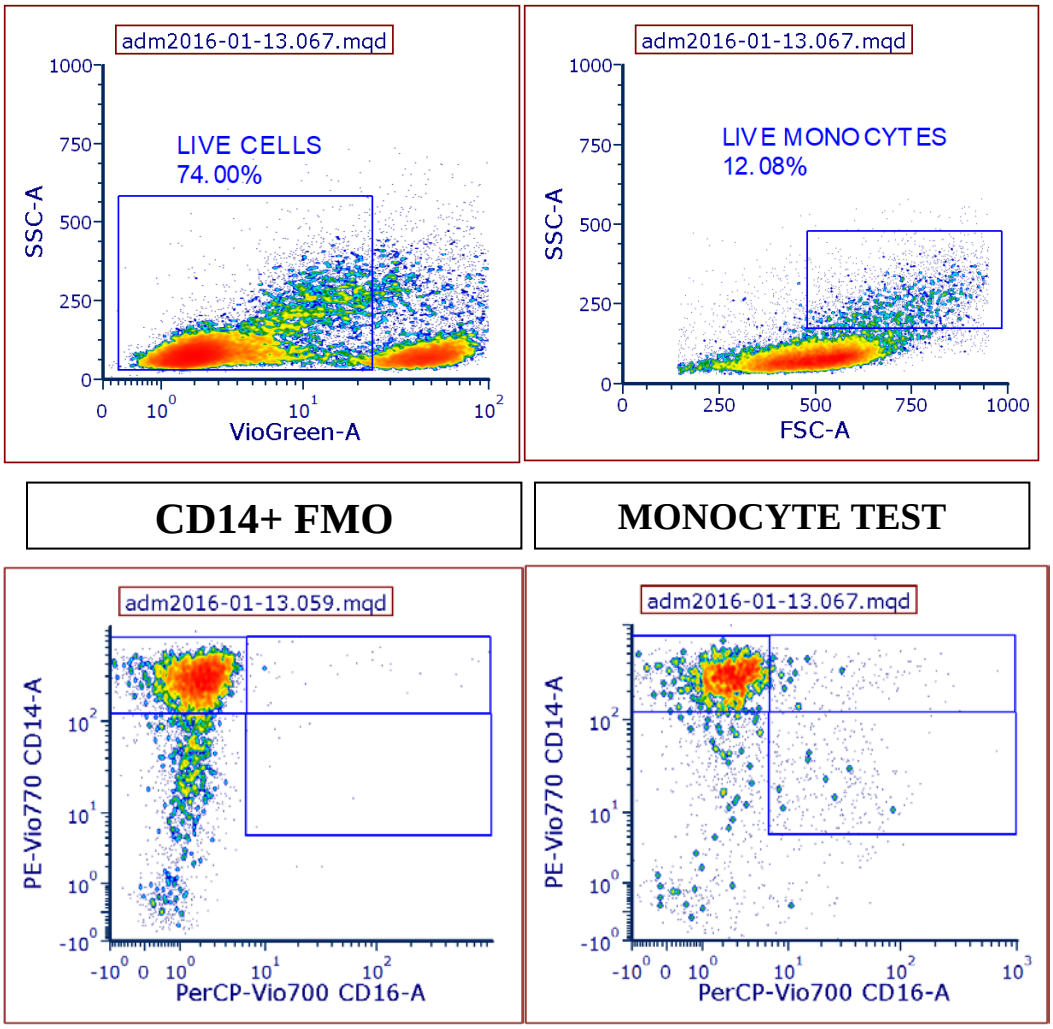
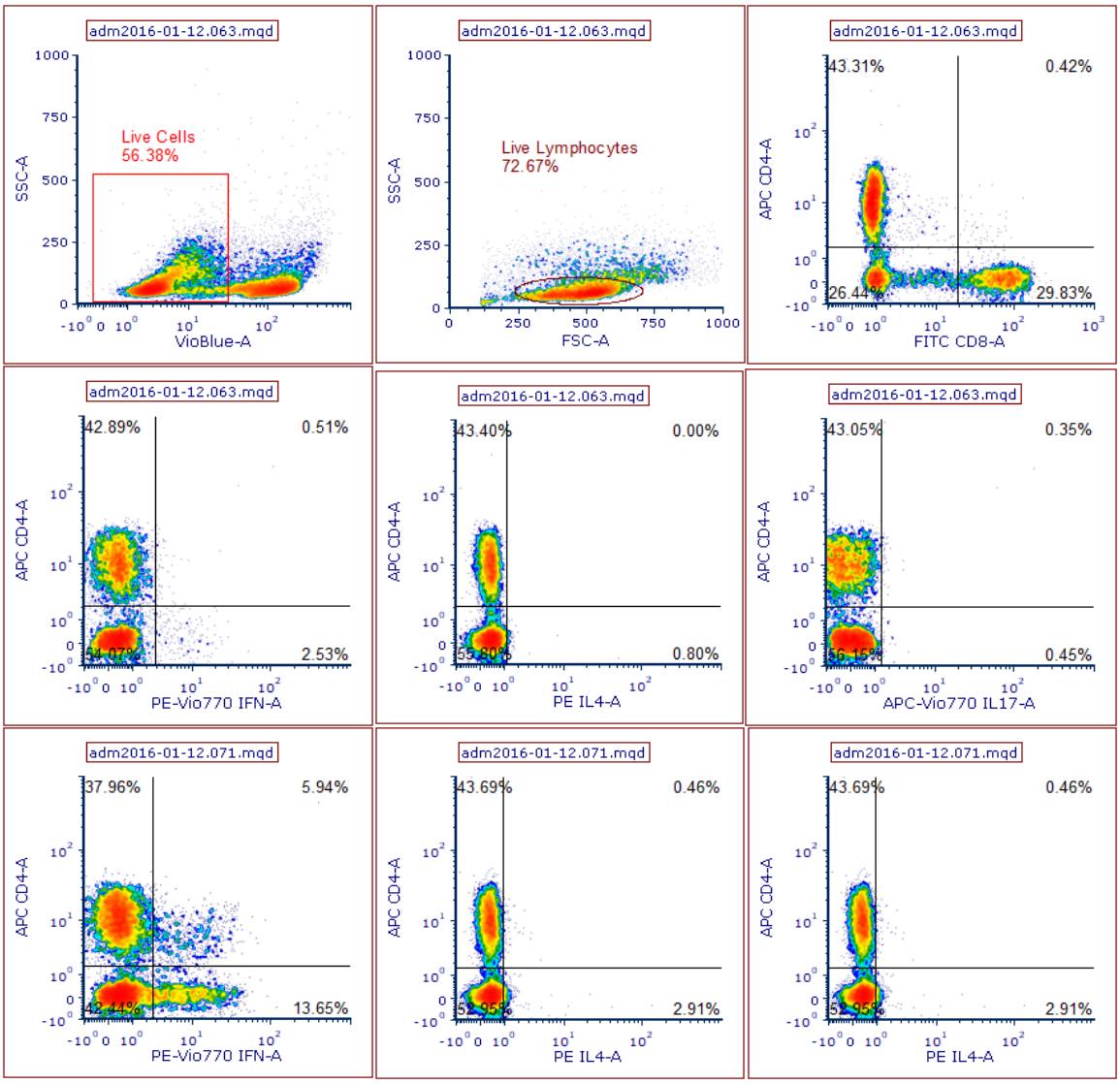
Supplementary Figure I. Case-cohort sample with immune cell subsets analyzed within MESA.



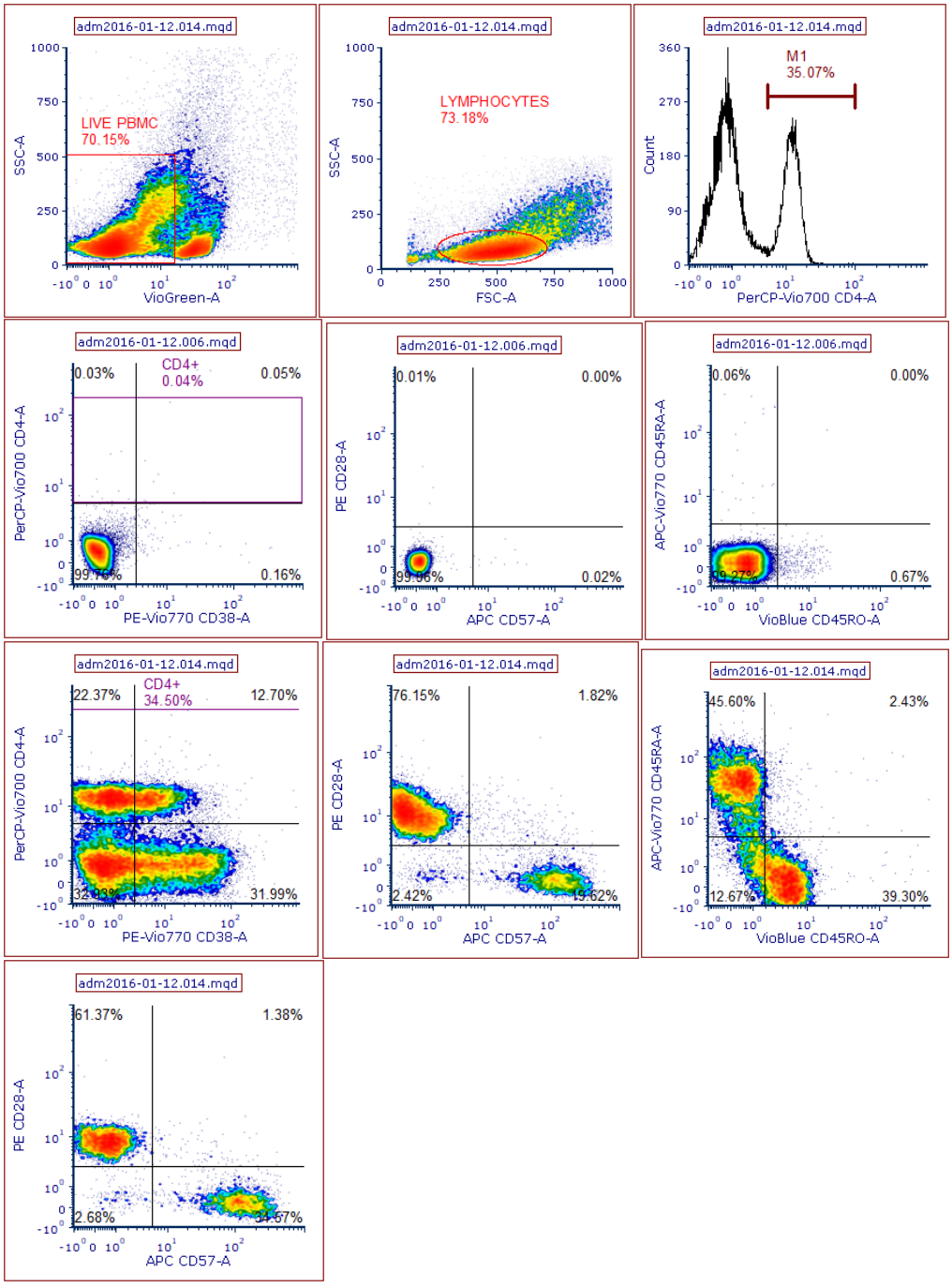
Supplementary Figure II. Monocyte gating strategy: PBMCs are gated on live cells, then on monocytes based on size/shape. Using a CD14+ fluorescence minus one (FMO) approach, the negative CD16 gate was set. Finally, the CD16 Ab was added to the test sample to yield the CD14++CD16- gate, the CD14+CD16+ gate and the CD14dimCD16+ gate. These 3 gates are added to yield the CD14 total and the 3 gates are reported as percentage of this total CD14.



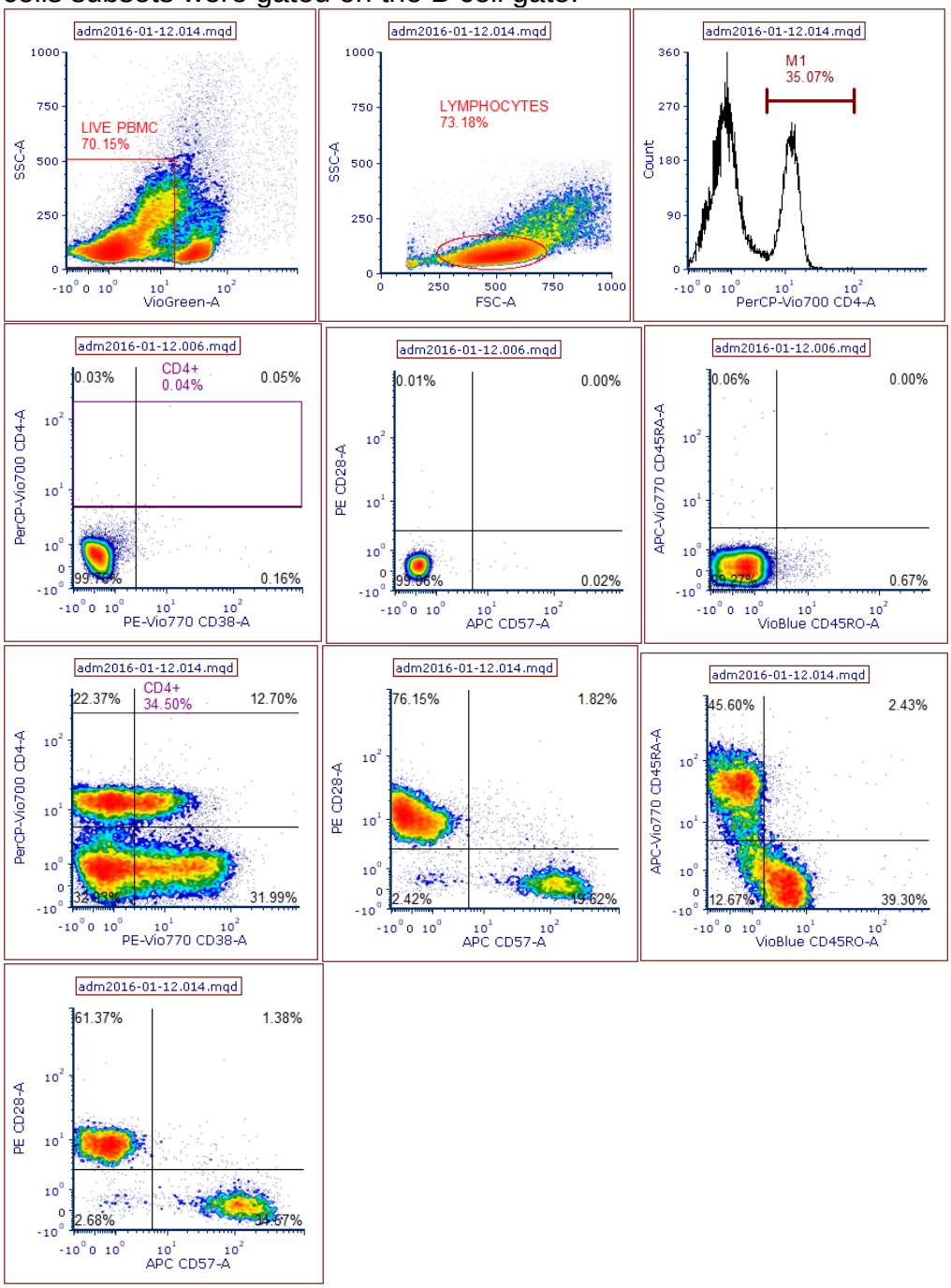
Supplementary Figure III. Gating strategy for Th1/2/17 and Tc1/2/17: PBMCs are gated on live cells, then on lymphocytes based on size and shape (top row). Using a modified FMO approach (middle row), a CD4/CD8 tube with appropriate isotypes was used to set negative gates for IFNg, IL4 and IL17. Finally, the test sample allow for the quantitation of IFNg, IL4 and IL17 produced by the cells. CD8 cells were done identically.



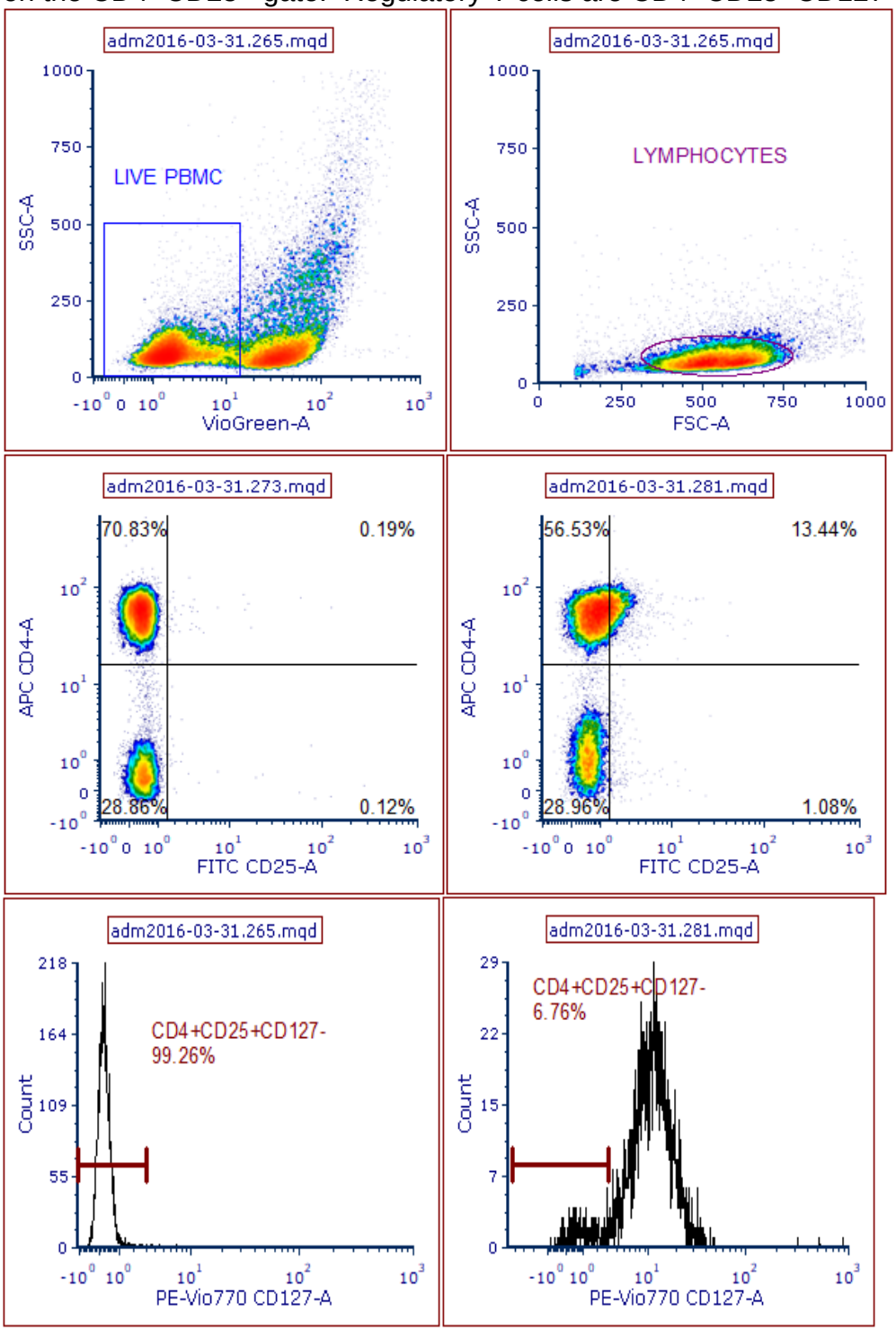
Supplementary Figure IV. Gating Strategy for CD4/CD8 subsets: PBMCs are gated on live cells, then on lymphocytes based on size and shape. Isotype controls were used for negative gate setting (Row 2) and the test sample (3rd row). The 4th row is gated on the CD4+CD45RA+ gate to yield the CD4+ T_{EMRA} cells (CD4+CD45RA+CD28-CD57+). The CD8 samples were done identically except CD8 was substituted for CD4.



Supplementary Figure V. Gating strategy for gamma delta T cells, NK cells, B cell subsets: PBMCs are gated on live cells, then on lymphocytes based on size and shape. Isotype controls were used to set negative gates (2nd row, plots 1 & 3, and 3rd row plot 2). The lymphocyte gate was used to determine CD3+, CD3-, B cells and gamma delta T cells (expressed as %CD3). NK cells were gated on the CD3- gate. B cells subsets were gated on the B cell gate.



Supplementary Figure VI. Regulatory T cell gating strategy: PBMCs were gated on live cells, then gated on lymphocytes, based on size and shape. Isotype controls were used to set negative gates using a CD4+ FMO tube. Histograms (Row 3) were gated on the CD4+CD25+ gate. Regulatory T cells are CD4+CD25+CD127-.



Supplementary Table I. Missing baseline data among MESA participants with immune cells measured (N=1195)

Variable	# (%) missing out of 1195
Age (years)	0
Male, n %	0
White, n %	0
Chinese, n %	0
Black, n %	0
Hispanic, n %	0
SBP (mmHG)	0
DBP (mmHG)	0
Cholesterol (mg/dL)	1 (0.08%)
HDL Cholesterol (mg/dL)	1
BMI (kg/m^2)	0
Diabetes, n %	0
Smoker, n %	0
Alcohol user	200 (16.7%)
Statin user, n %	1
CCB user, n %	1
Diuretic user, n %	1
Beta Blocker user, n %	1
Vasodilator user, n %	1
ACE inhibitor user, n %	1
College education, n %	3 (0.25%)
Intentional Exercise (met-min)	3 (0.25%)
IL-6 (pg/mL)	33 (2.7%)
CMV (EU/mL)	6 (0.50%)
Baseline mean common carotid intima media thickness	17 (1.4%)

Supplementary Table II. Main effects for additional subsets for which interactions were explored.

Variable	Estimate (in mm)	95% CI Lower	95% CI Upper	p-value
th17 as %cd4	0.001076	-0.01130	0.01346	0.8643
CD4+CD28-	-0.00965	-0.02447	0.005168	0.2012
CD14++CD16-	-0.01216	-0.02814	0.003816	0.1353
CD14+CD16++	0.01614	0.000623	0.03166	**0.0415**

Supplementary Table III. Smoking interactions (with current smoking)

Variable	Estimate (in mm)	95% CI Lower	95% CI Upper	p-value
th17 as %cd4	-0.02058	-0.07875	0.03760	0.4870
CD4+CD28-	0.03292	-0.01597	0.08181	0.1863
CD14++CD16-	-0.03145	-0.08848	0.02557	0.2788
CD14+CD16++	-0.02026	-0.07916	0.03864	0.4992

Supplementary Table IV. Male sex interactions

Variable	Estimate (in mm)	95% CI Lower	95% CI Upper	p-value
th17 as %cd4	0.002776	-0.02155	0.02710	0.8225
CD4+CD28-	-0.00430	-0.03486	0.02627	0.7824
CD14++CD16-	-0.05310	-0.08078	-0.02542	**0.0002**
CD14+CD16++	0.03442	0.01154	0.05730	**0.0033**

Supplementary Table V. Sex-stratified analyses of change in mean common carotid artery thickness (micrometers) over 10 years of follow-up [a]

V.A. Males

Cells scaled per SD	Estimate (in mm)	95% CI Lower	95% CI Upper	p-value
CD14++CD16-	-0.03969	-0.06310	-0.01628	0.0010
CD14dimCD16++	0.03578	0.01395	0.05761	0.0014

V.B. Females

Cells scaled per SD	Estimate (in mm)	95% CI Lower	95% CI Upper	p-value
CD14++CD16-	0.007977	-0.01028	0.02623	0.3894
CD14dimCD16++	0.004704	-0.00953	0.01894	0.5149

[a] Linear models adjusted for age, sex, race/ethnicity, current smoking, baseline exercise, body mass index, baseline college education, current diabetes, systolic blood pressure, total cholesterol, HDL cholesterol, and log transformed CMV. Confidence intervals use robust (sandwich) standard error estimates to ensure validity in the presence of weights that are used to account for case-cohort sampling. All estimates are per standard deviation. Complete case data (numbers vary by subset).

Supplementary Table VI. Baseline and follow-up IMT, stratified by sex and CHD status

Sex	N	Variable	Mean	Standard Deviation	Minimum	Maximum
Female	**571**	**IMT**	0.0843180	0.1757412	-1.061	0.882
		E5 IMT (mm)	0.8487273	0.2045589	0.468	1.621
		E1 IMT (mm)	0.7714774	0.2249838	0.415	2.061
Male	**624**	**Δ IMT**	0.1092593	0.1678261	-0.574	1.124
		E5 IMT (mm)	0.9219966	0.2579272	0.429	2.382
		E1 IMT (mm)	0.8153382	0.2367937	0.4340	2.067
CHD status						
No CHD	**721**	**Δ IMT**	0.1014513	0.1385810	-0.574	0.766
		E5 IMT (mm)	0.8532099	0.2044133	0.429	2.294
		E1 IMT (mm)	0.7535743	0.1975299	0.434	2.067
CHD	**474**	**Δ IMT**	0.0897647	0.2242594	-1.061	1.124
		E5 IMT (mm)	0.9558457	0.2792489	0.504	2.382
		E1 IMT (mm)	0.8754914	0.2711684	0.415	2.061

Supplementary Table VII. High blood pressure (median split on adjusted BP) interaction

Variable	Estimate (in mm)	95% CI Lower	95% CI Upper	p-value
th17 as %cd4	-0.00143	-0.02958	0.02673	0.9205
CD4+CD28-	0.008201	-0.01370	0.03010	0.4622
CD14++CD16-	0.002093	-0.00549	0.009678	0.5878
CD14+CD16++	0.01959	-0.00429	0.04347	0.1076

Supplementary Table VIII. Difference in mean common carotid artery thickness (mm) at baseline.[a]

Variable	Estimate (in mm)	95% CI Lower	95% CI Upper	p-value
Group 1				
th1 as %cd4	0.01198	-0.00407	0.02804	0.1431
th17 as %cd4	-0.00942	-0.02365	0.004803	0.1938
CD4+CD38+	-0.00438	-0.01961	0.01086	0.5728
CD8+CD38+	-0.00345	-0.01874	0.01185	0.6583
NK %lymphs	-0.01339	-0.02691	0.000131	0.0523
cd3+gd+	-0.0097	-0.02211	0.002703	0.1251
CD14++CD16-	0.01162	-0.00577	0.02902	0.1899
th2 as %cd4	-0.01083	-0.02805	0.006393	0.2173
CD14dimCD16++	-0.00467	-0.02199	0.01264	0.5963
cd4+cd25+	0.004075	-0.01068	0.01882	0.5877
CD14+CD16+	0.000921	-0.01622	0.01807	0.916
CD4+CD45RA+	-0.01345	-0.02922	0.002328	0.0947
CD8+CD45RA+	-0.01252	-0.02876	0.003723	0.1306
CD4+CD28-	-0.00062	-0.01713	0.0159	0.9417
CD8+CD28-	-0.02929	-0.04609	-0.01248	0.0007
CD4+CD45RO+	0.01697	-0.00012	0.03406	0.0516
CD8+CD45RO+	0.005438	-0.0103	0.02118	0.4979

[a] Linear models adjusted for age, sex, race/ethnicity, current smoking, baseline exercise, body mass index, baseline college education, current diabetes, systolic blood pressure, total cholesterol, HDL cholesterol, baseline antihypertensive use, baseline statin use, and log transformed CMV. Confidence intervals use robust (sandwich) standard error estimates to ensure validity in the presence of weights that are used to account for case-cohort sampling. Pre-specified hypotheses are in red (and highlighted for B&W printers). All estimates are per standard deviation. Complete case data (numbers vary by subset).

Supplementary Table IX. Major Resources.

Antibodies

Phenotype Assay	Target Antigen	Catalog #	Fluorophore	Isotype Control Catalog #
γδ T and Natural Killer cells	CD3	130-094-363	VioBlue	130-094-671
	γδ TCR	130-096-869	PE	130-092-212
	CD56	130-100-676	PE-Vio770	130-104-616
	CD16	130-100-430	PerCP-Vio700	130-098-595
$CD4^+$ and $CD8^+$ subsets (cell stimulation assays)	CD4	130-092-374	APC	130-091-836
	CD8	130-104-519	VioBright FITC	130-104-512
	IFN-γ	130-096-752	PE-Vio770	130-096-654
	IL-4	130-091-647	PE	130-092-212
	IL-17A	130-096-656	APC-Vio770	130-096-653
$CD4^+$ subsets	CD4	130-103-793	PerCP-Vio700	130-097-561
	CD45RA	130-096-604	APC-Vio770	130-096-822
	CD45RO	130-099-044	VioBlue	130-094-671
	CD28	130-092-921	PE	130-092-212
	CD38	130-099-151	PE-Vio770	130-096-638
	CD57	130-092-141	APC	130-093-176
	CD27	130-104-845	VioBright FITC	130-104-513
$CD4^+$ chemokine receptor assays	CD4	130-103-793	PerCP-Vio700	130-097-561
	CD194 (CCR4)	130-103-814	PE-Vio770	130-096-654
	CD196 (CCR6)	130-100-373	APC	130-093-176
	CD183 (CXCR3)	130-106-009	VioBright FITC	130-104-513
	CD195 (CCR5)	130-106-223	PE	130-092-212
$CD8^+$ subsets	CD8	130-097-911	PerCP-Vio700	130-097-563
	CD45RA	130-096-604	APC-Vio770	130-096-822
	CD45RO	130-099-044	VioBlue	130-094-671
	CD28	130-092-921	PE	130-092-212
	CD38	130-099-151	PE-Vio770	130-096-638
	CD57	130-092-141	APC	130-093-176
	CD27	130-104-845	VioBright FITC	130-104-513
B Cell subsets	CD19	130-096-643	APC-Vio770	130-096-653

	CD5	130-096-577	APC	130-092-214
	CD27	130-104-845	VioBright FITC	130-104-513
Monocyte subsets	CD14	130-096-628	PE-Vio770	130-096-638
	CD16	130-100-430	PerCP-Vio700	130-098-595
CD4$^+$ T Regulatory cells	CD4	130-092-374	APC	130-091-836
	CD25	130-104-274	VioBright FITC	130-104-575
	CD127	130-099-719	PE-Vio770	130-096-638
	CD6	130-105-129	APC-Vio770	130-096-653

All antibodies were from Miltenyi Biotec (San Diego, CA). All assays used antibody dilutions as recommended by Miltenyi.