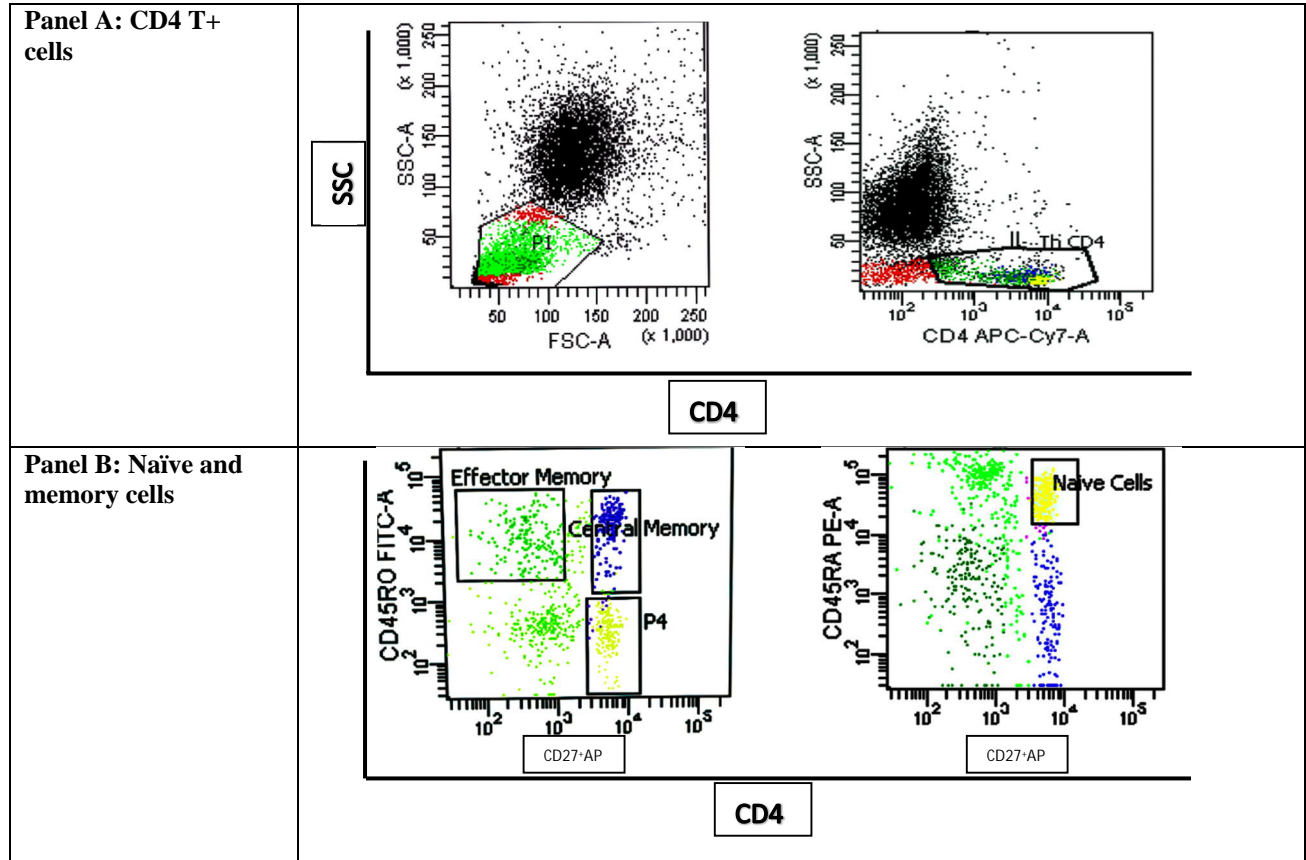**Supplementary tables and figures**

Fig S1. Gating

Figure S1. **Flow cytometry quantification of CD4+ T cells in the PMBC following the gating strategy.**

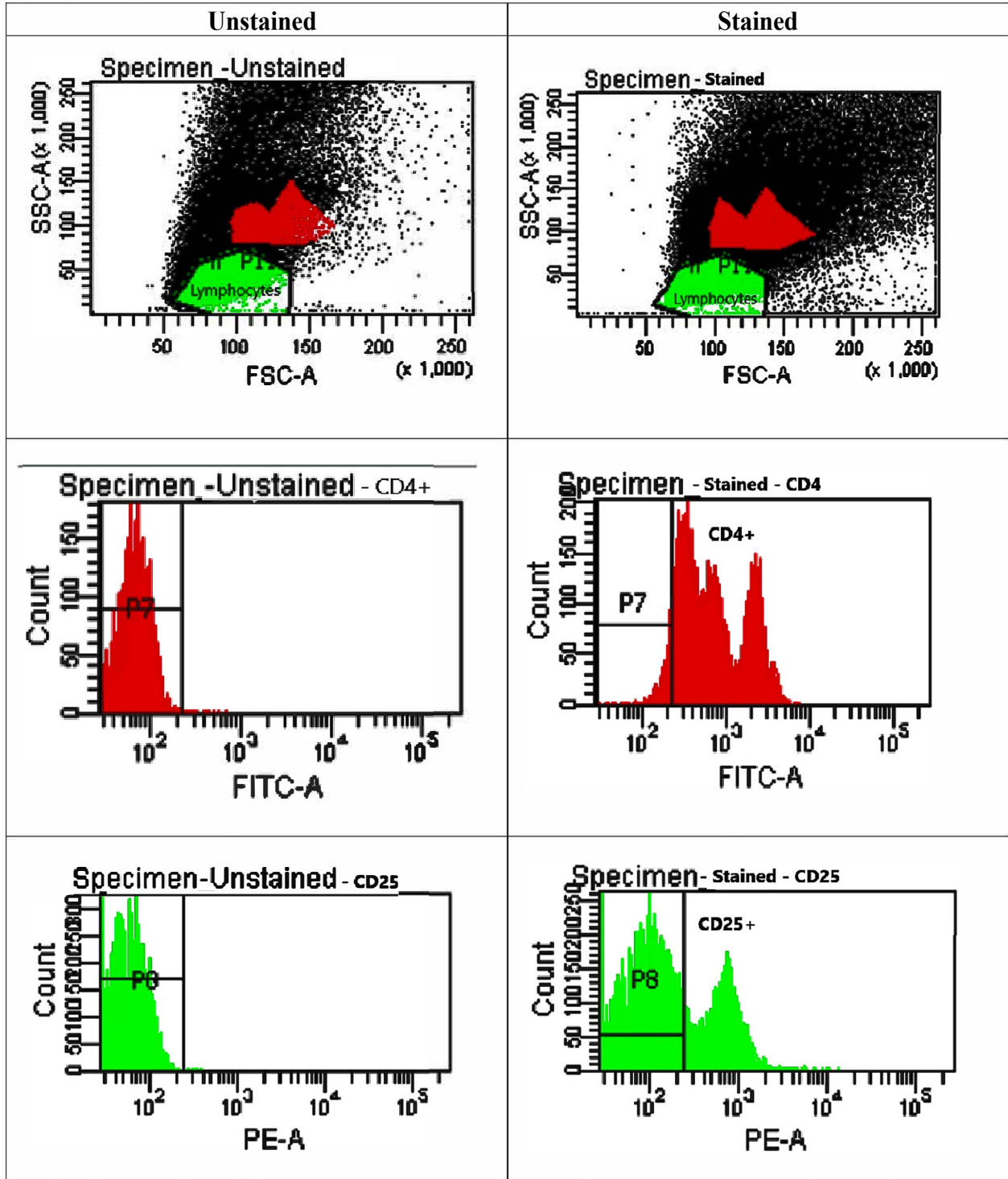| Panel A: CD4 T+ cells	
Panel B: Naïve and memory cells	

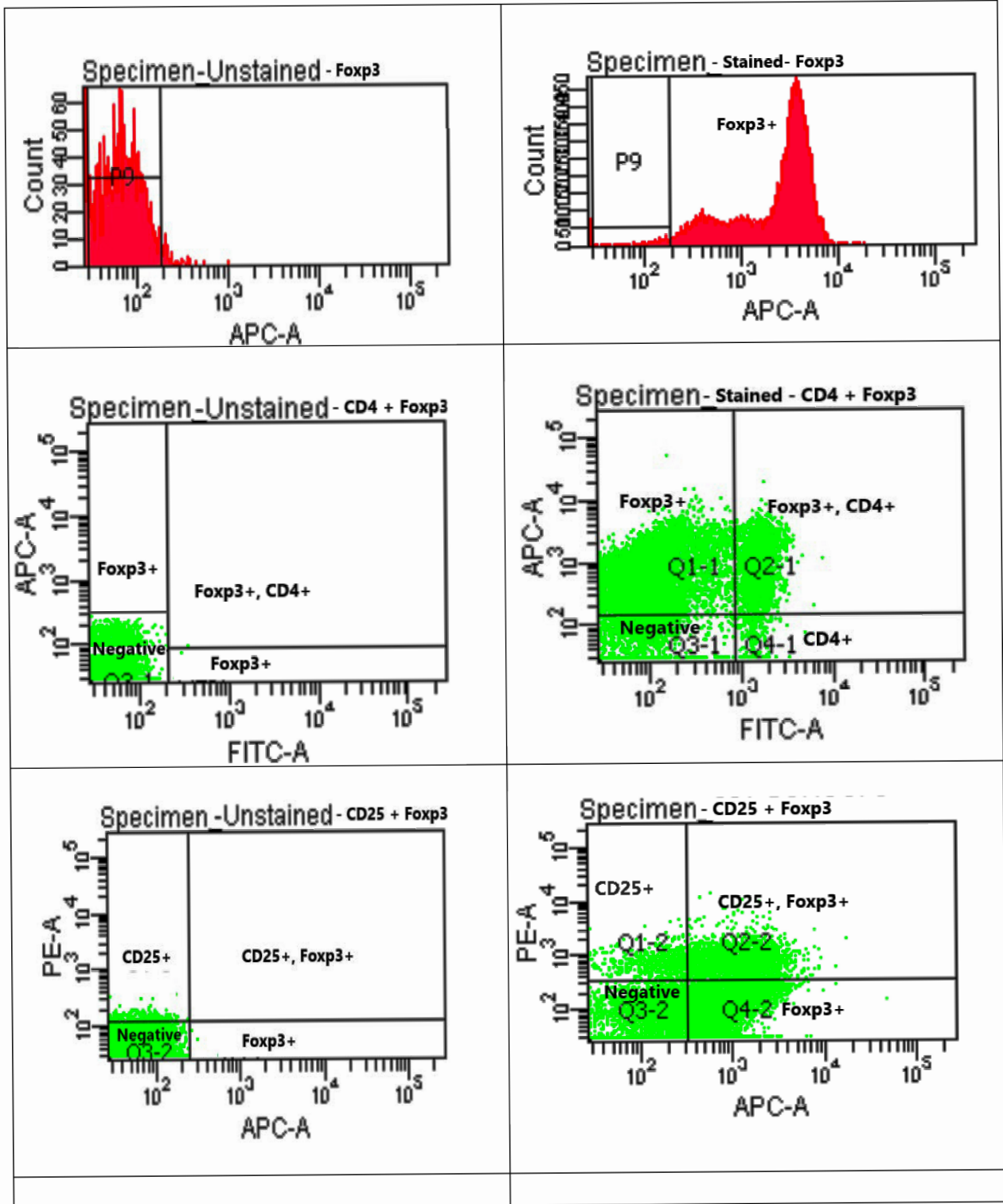Fig S1 displays the gating of CD4+ T cells from PBMC.

Panel A, CD4+ cells were first gated for T- cells CD4+. **Panel B**, CD4+ cells, are followed by gating of the intersection of both cell populations for CD45RO+ and CD27+ for central memory "CM" cells and CD45RO+ and CD27- for effector memory "EM" cells and CD45RA+ and CD27+ for naive cells.

Fig S2. Gating of T reg cells with unstained and stained Foxp3/ isotype control

B

Specimen-Unstained - Foxp3

Count

P9

APC-A

Specimen - Stained - Foxp3

Count

P9

Foxp3+

APC-A

Specimen-Unstained - CD4 + Foxp3

APC-A

Foxp3+

Foxp3+, CD4+

Negative

Q3-1

Foxp3+

FITC-A

Specimen - Stained - CD4 + Foxp3

APC-A

Foxp3+

Foxp3+, CD4+

Q1-1   Q2-1

Negative

Q3-1   Q4-1   CD4+

FITC-A

Specimen-Unstained - CD25 + Foxp3

PE-A

CD25+

CD25+, Foxp3+

Negative
Q3-2

Foxp3+

APC-A

Specimen - CD25 + Foxp3

PE-A

CD25+

CD25+, Foxp3+

Q1-2   Q2-2
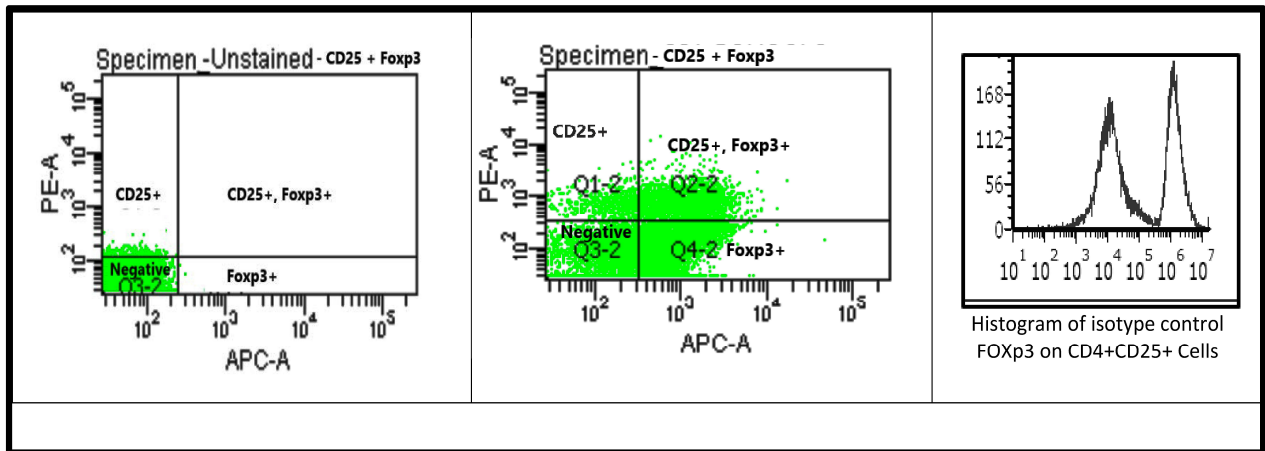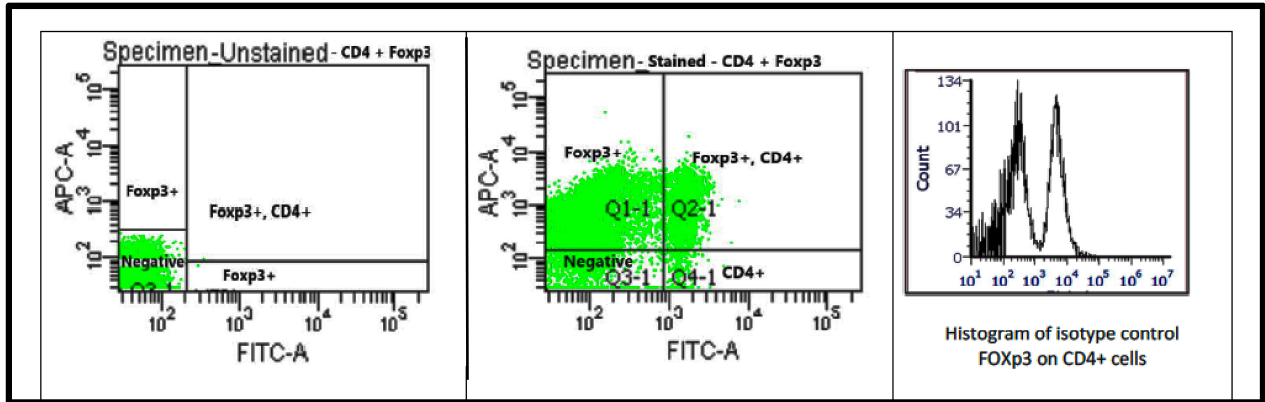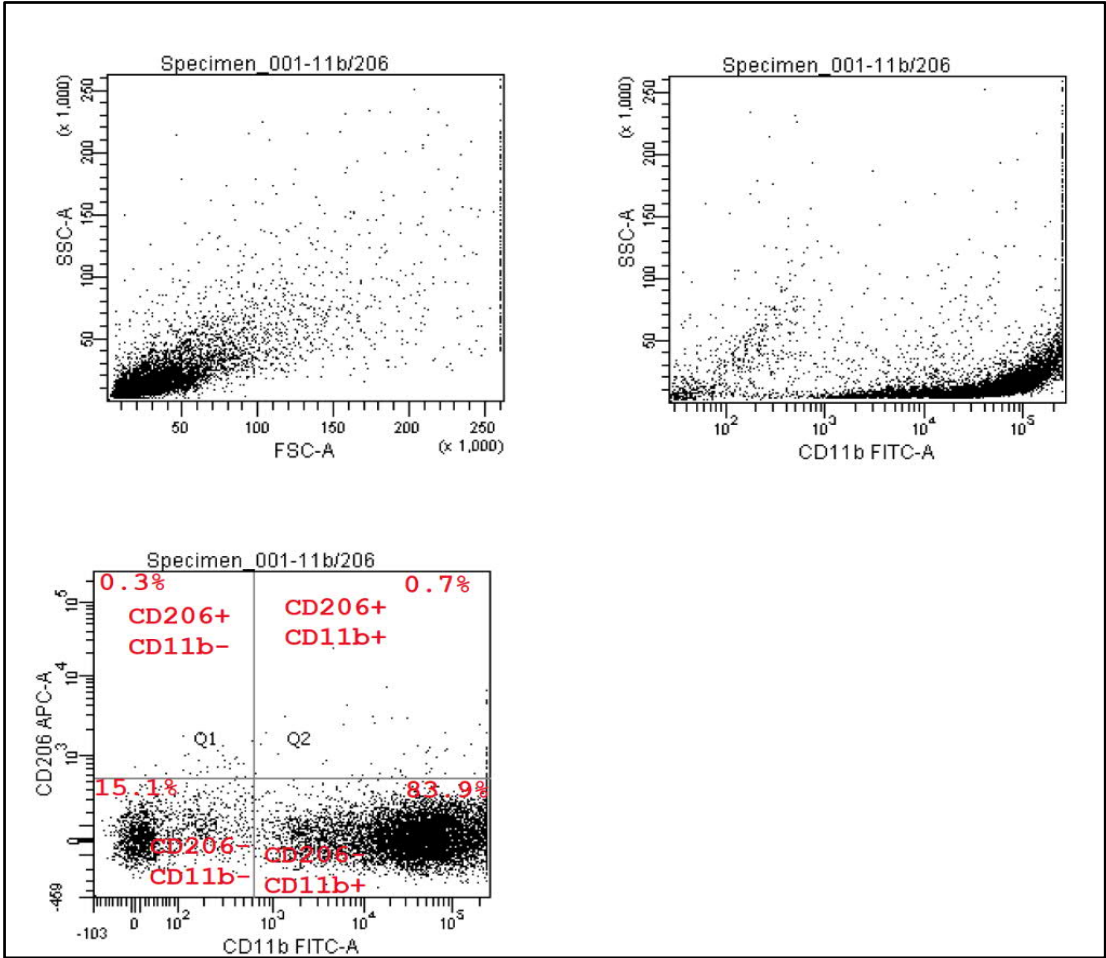
Negative
Q3-2   Q4-2   Foxp3+

APC-A

Fig S2. Displays the gating of CD4+De25+FoxP3+ T cells of unstained/ stained with histogram depicting isotype control for FoxP3 (A-C).

Fig S3. Flow cytometry gating for VAT macrophages (CD206 and CD11b)

**Fig S3** displays the gating of CD206+/CD11b+ macrophage cells from visceral adipose tissue.

Fig S4. Comparison of T reg cells in healthy control subjects versus obese subjects after and before surgery
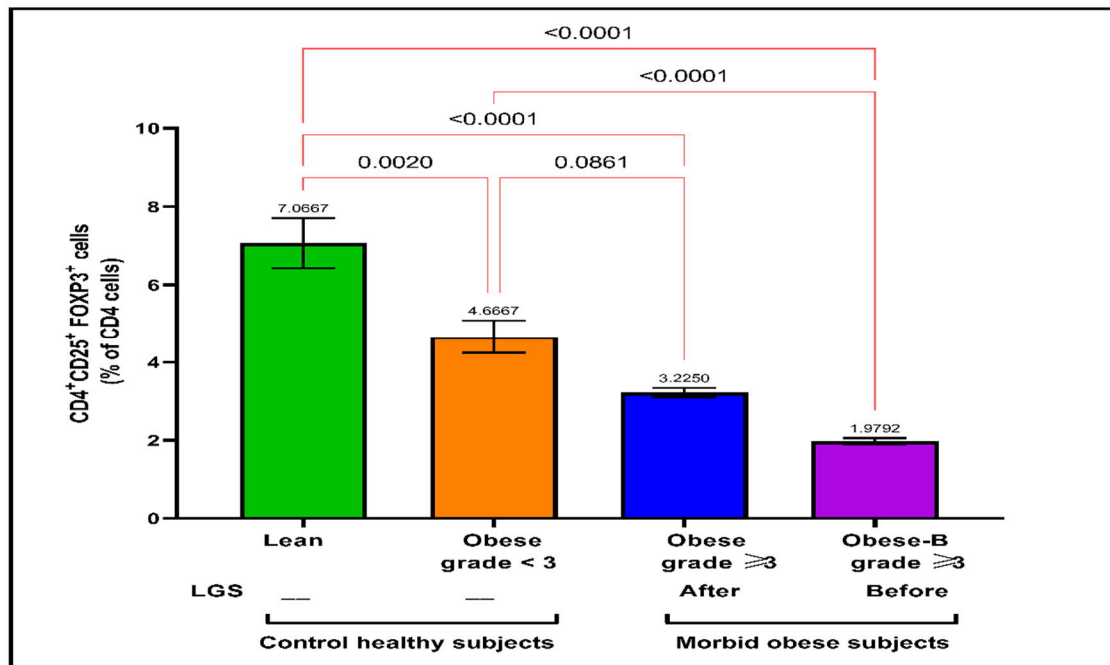
Bars represent mean and SD of T Reg cells in healthy subjects (lean, obese with grade less than 3), and obese with grade 3 or above before (B) and after (A) LGS. Two-tailed p value is significant at ≤ 0.05.

Fig S5. Naive and TEM cells in diabetic and non-diabetic obese usbjects before and after LGS.
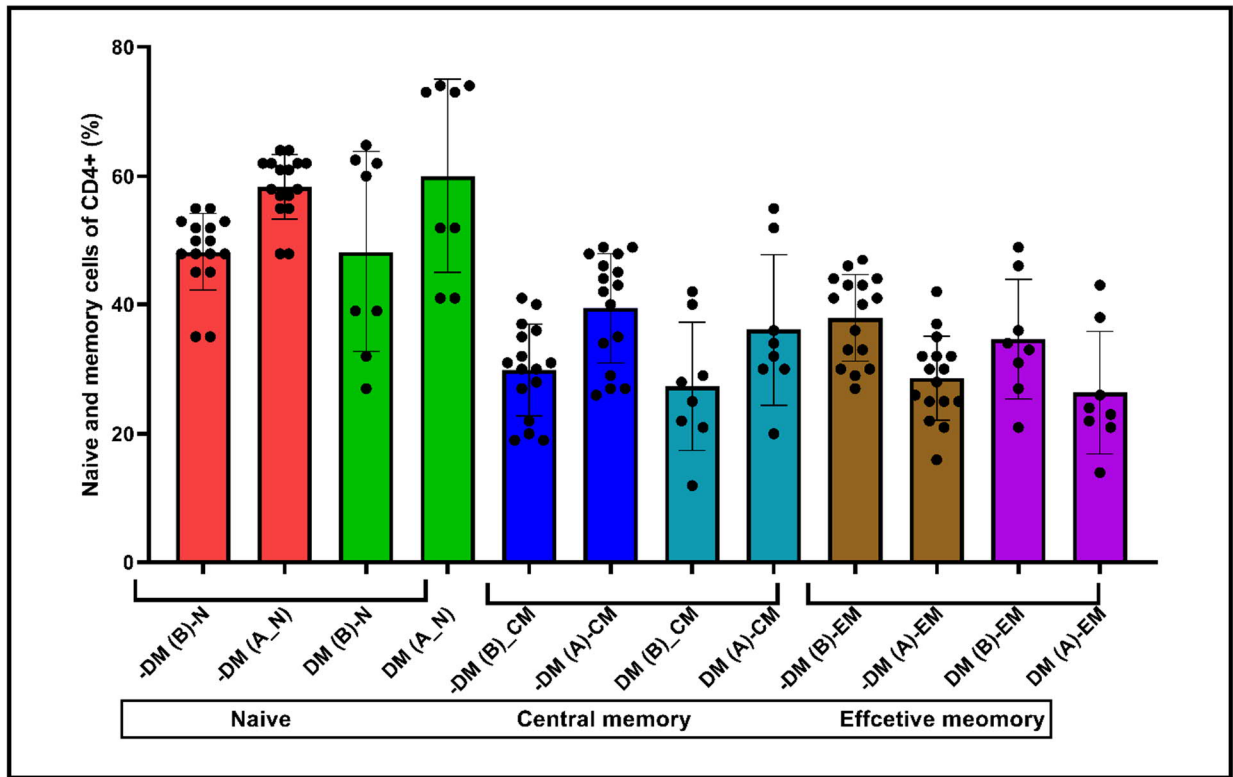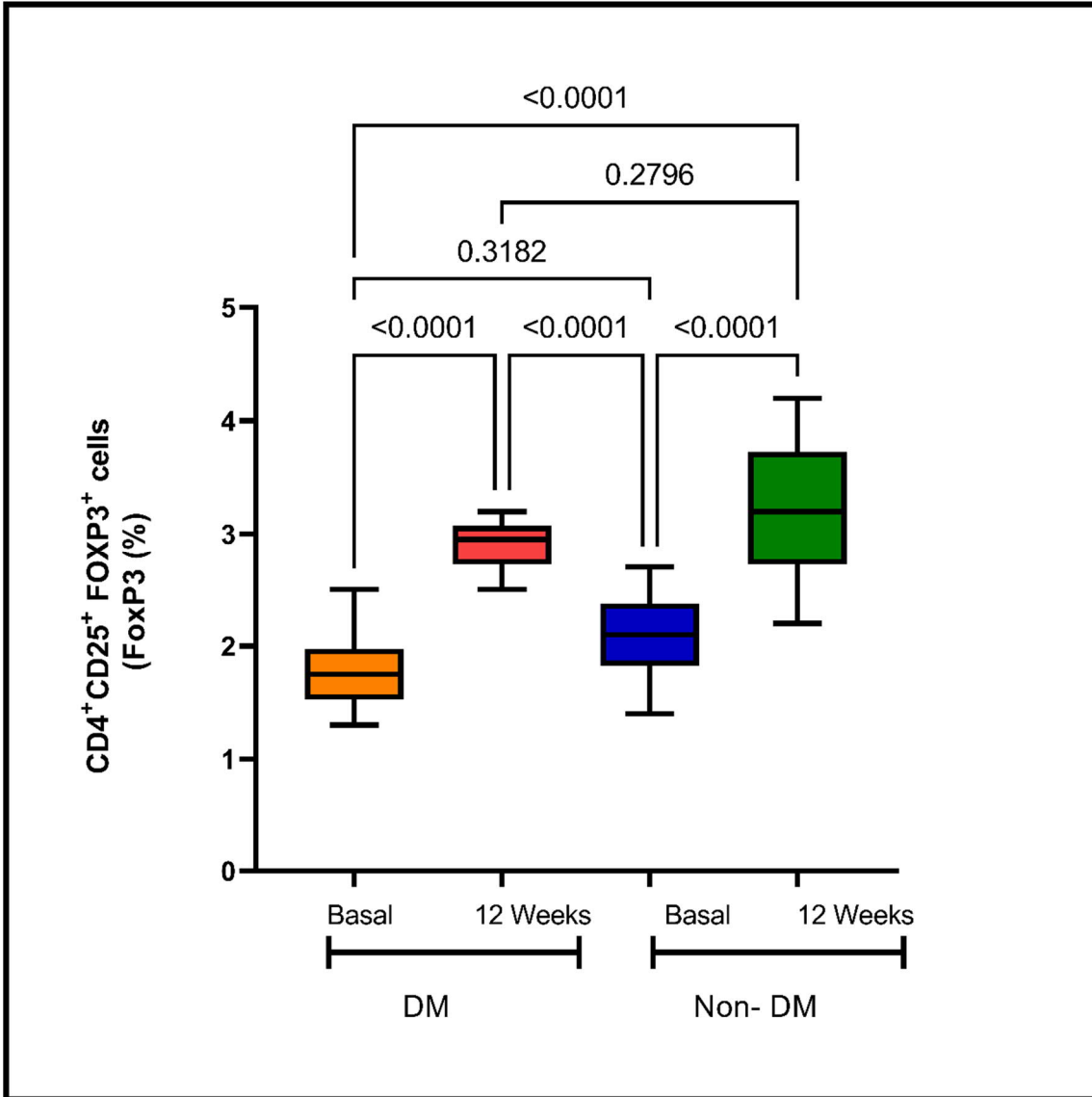

Fig S5. Figure diplays the mean and SD of Naive and TEM cells in healthy subjects (lean, obese with grade less than 3), and obese with grade 3 or above before (B) and after (A) LGS. Two-tailed p value is significant at ≤ 0.05.

Figure S6. Impact of DM on Treg cells at baseline and after LGS in Class 111 obese subjects.

Fig S6. Bars represent mean with minimum and maximum of T reg cells in diabetic and non-diabetic obese usbjects at basal (before) and after after 12 weeks of LGS surgery. P values in numbers were indicated between different group comparison. Two-tailed p value is significant at $\leq$ 0.05.

Figure S 7. Impact of Insulin Resistance on Treg cells at baseline and after LGS in Class 111 obese subjects.
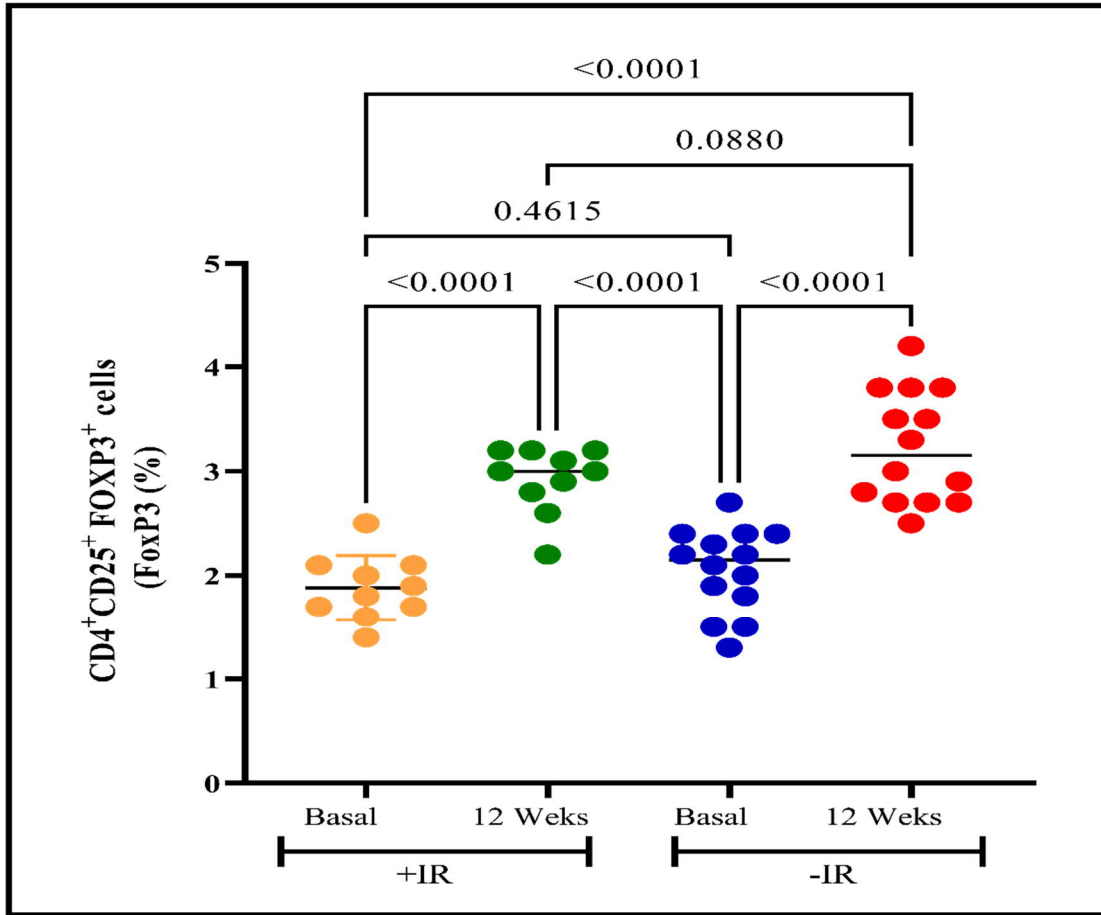
Scatter dot Plot represent means and SD of T reg cells (%) in Class 111 obese subjects before surgery (basal) and after LGS (12 weeks) with/without insulin resistance. P values in numbers were indicated between different group comparison. Two-tailed p value is significant at ≤ 0.05.

Figure S8. Correlations between CRP and MCP-1 after LGS with TEM and Naive cells

A
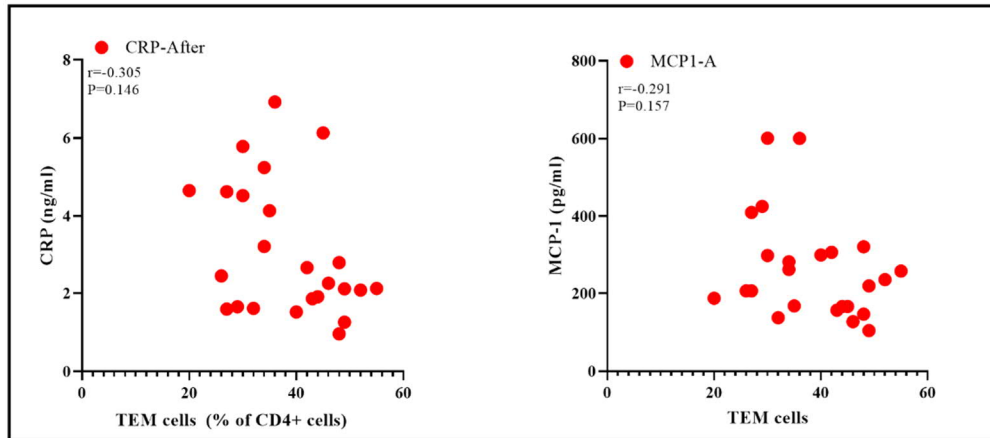
B
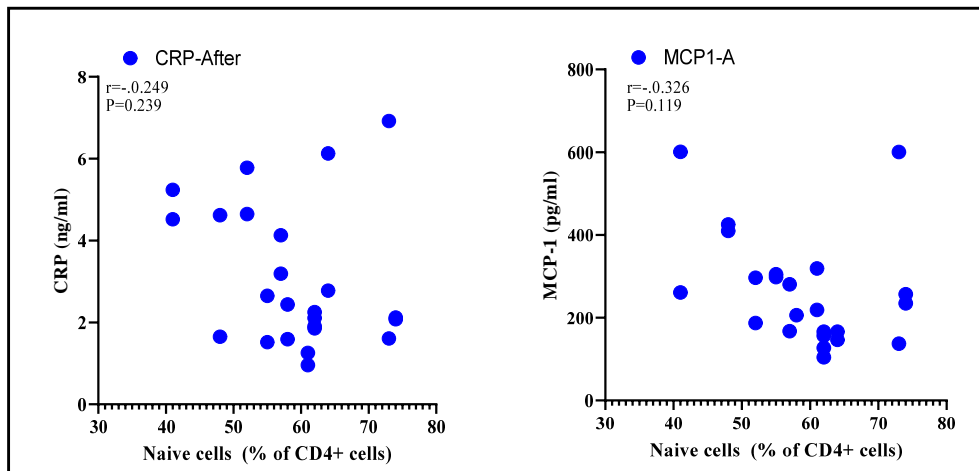
Figure S8. Spearman's Correlations (r)between effector memory naïve(A) , and naïve CD4+ T cells(B) count in morbidly obese subjects with CRP, and MCP-1 after LGS .*Two tailed p* value is significant ≤0.05.

Supplementary tables

Table S1 shows the CD4-T cell type and markers used for identification**.**

CD4+ T cell	CD marker
naïve CD4 T cells -1	CD45RA+and CD27+
central memory T cells	CD45RO+and CD27+
effector/peripheral memory	CD45RO+and CD27-
terminally differentiated T cells	CD45RA+and CD27-
T regulatory cells	CD4+CD25+FoxP3+

Supplementary Table 2. Fluorochrome-antibody combinations used for flow cytometry experiments.

Human	Fluorochrome	Clone	Company
CD4	APC-cy7	SK3	BD Biosciences (San Jose, CA, USA)
CD25	APC	2A3	BD Biosciences (San Jose, CA, USA)
CD27	APC	L128	BD Biosciences (San Jose, CA, USA)
CD45RA	PE	5H9	BD Biosciences (San Jose, CA, USA)
CD45RO	FITC	UCHL1	BD Biosciences (San Jose, CA, USA)
FoxP3	PE	PCH101	E Bioscience (San Diego, CA, USA)
CD206	APC	19.2	BD Biosciences (San Jose, CA, USA)
CD11b	FITC	ICRF44	BD Biosciences (San Jose, CA, USA)
CD3	PerCP	SK7	Biosciences (San Jose, CA, USA)
Mouse IgG1 kappa Isotype Control	PE	P3.6.2.8.1	E Bioscience (San Diego, CA, USA)

Table S3. Correlations coefficient (r)between Treg CD4+ (before and after LGS), CD4+T
subpopulations, and metabolic, adipokines, and inflammatory and cytokines at baseline.

Variable	Treg -B	Naïve CD4	TEM CD4	CM CD4	Treg -A
BMI	0.217	0.133	-0.032	-0.053	0.196
T. Cholesterol	-0.129	-0.367	-0.103	-0.171	0.102
Triglycerides	0.022	0.342	-0.054	-0.081	0.146
HbA1C	-0.175	-0.155	-0.257	-0.165	-0.224
IR-HOMA	-0.154	-0.242	-0.397	-0.338	-0.015
Insulin sensitivity	0.152	0.075	0.152	0.197	0.146
CRP	0.091	-0.256	-0.261*	-0.133	0.143
IL-10	0.262	0.307*	0.199	0.241	-0.065
Leptin	-0.458*	0.278	0.291	0.269	-0.137
Adiponectin	0.256	-0.157	0.027	-0.012	0.225
IL-6	-0.013	0.312	0.283	0.209	0.088
TNF-α	-0.266	0.052	0.046	0.064	0.130
MCP-1	0.191	-0.128	-0.195*	-0.217	0.225

Spearman's correlation coefficient [r] is presented. * P value is significant with a cut off value of ≤0.05
(two-tailed).

Table S4 impact of diabetes on CD4+ subpopulations(A) , inflammatory, cytokine, chemokine and adipokine markers (B) at baseline and after 12 weeks of LGS.

A- Impact on CD4+ subpopulations

Variables (%)	Basal state (24)	3 months (24)
	Before LGS	After LGS
Treg		
DM (-)	2.08 (0.34)	3.200 (0.54)
DM(+)	1.79 (0.36)	2.90 (0.22)
P value	0.318	0.279
Naive		
DM (-)	48.35(6.017)	58.38(4.978)
DM(+)	48.29 (15.54)	59.00 (15.02)
P value	0.990	0.997
CM		
DM (-)	29.88 (7.09)	39.50 (8.53)
DM(+)	27.38 (9.91)	36.13(11.74)
P value	0.915	0.818
EM		
DM (-)	37.94(6.70)	28.63(6.51)
DM(+)	34.63(9.24)	26.38(9.49)
P value	0.745	0.902

Data are presented of CD4+ subpopulations count expressed in percentage of total CD4+ cells.

P value is significant between before with (DM+) and without diabetes (DM-) and after LGS with (DM+) and without diabetes(DM-). Two-tailed *p* value is significant at ≤0.05.

B- <u>Impact on inflammatory, cytokine, chemokine and adipokine markers at baseline and after 12 weeks of LGS.</u>

Variables	Basal state (24) Before LGS	3 months (24) After LGS
CRP		
DM (-)	3.82 (0.50)	2.56 (0.39)
DM(+)	5.96(0.71)	4.11 (0.55)
P value	0.022	0.033
IL-6		
DM (-)	1.61(0.68)	1.27(0.48)
DM(+)	1.30 (1.09)	1.05 (0.63)
P value	0.450	0.758
TNF-alpha		
DM (-)	3.54(1.47)	2.85(1.03)
DM(+)	3.25(1.50)	2.70(1.49)
P value	0.542	0.903
MCP-1		
DM (-)	249.38(87.77)	231.38(97.70)
DM(+)	366.22(172.71)	321.89(175.86)
P value	0.098	0.270
IL-10		
DM (-)	0.62(0.31)	0.81(0.35)
DM(+)	0.47(0.19)	0.61(0.23)
P value	0.230	0.186
Leptin		
DM (-)	28.15(7.56)	17.50(5.92)
DM(+)	28.87 (6.30)	18.66(6.40)
P value	0.970	0.452
Adiponectin		
DM (-)	6.07(1.24)	11.54(2.77)
DM(+)	5.32(2.35)	10.76(2.64)
P value	0.782	0.759

Data are presented as mean (SD). P value is significant between before with (DM+) and without diabetes (DM-) and after LGS with (DM+) and without diabetes(DM-). Two-tailed p value is significant at ≤0.05.

Two-tailed p value is significant at ≤0.05.