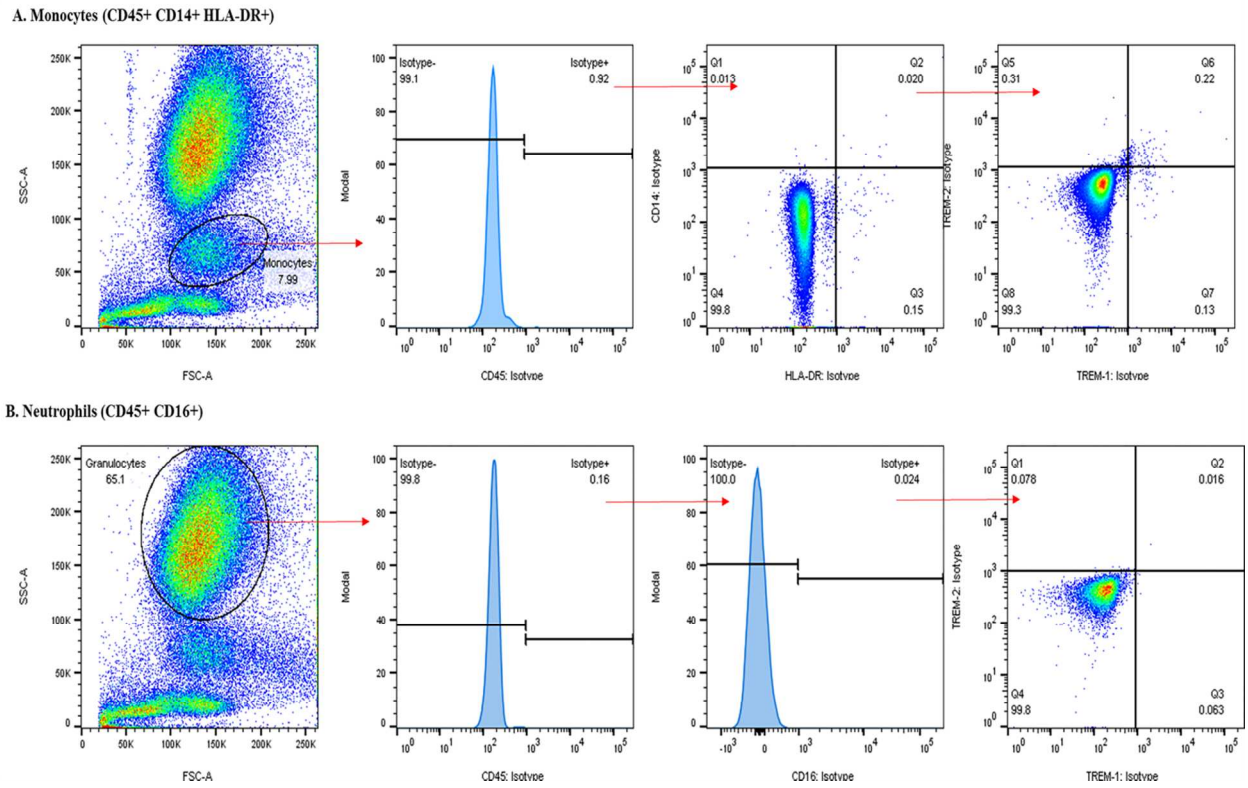

Figure S1 Isotype control for TREM-1 and TREM-2 expression in monocytes and neutrophils of the study subjects. Cell phenotype was performed using anti-human antibodies and isotypes for monocytes (CD45+, CD14+, HLA-DR+) represented in figure A and neutrophils (CD45+, CD16+) in figure B. A) The representative figures for isotype control for monocytes gating; live monocytes were gated from FSC/SSC gating. Then CD45+ were gated using the basal level of CD45 Isotype and analyzed for CD14 and HLA-DR expression with respective CD14 and HLA-DR Isotypes. The double positive populations (CD14+ HLA-DR+) were further gated for expression of TREM-1+ and TREM-2+ using the basal level of TREM-1 and TREM-2 Isotypes. B) The representative figures for Isotype control for neutrophils gating; live granulocytes were gated from FSC/SSC gating. Then CD45+ were gated using the basal level of CD45 Isotype and analyzed for CD16 expression using the basal level of CD16 Isotype. CD16+ populations were further gated for expression of TREM-1 and TREM-2 using the basal level of TREM-1 and TREM-2 Isotypes.

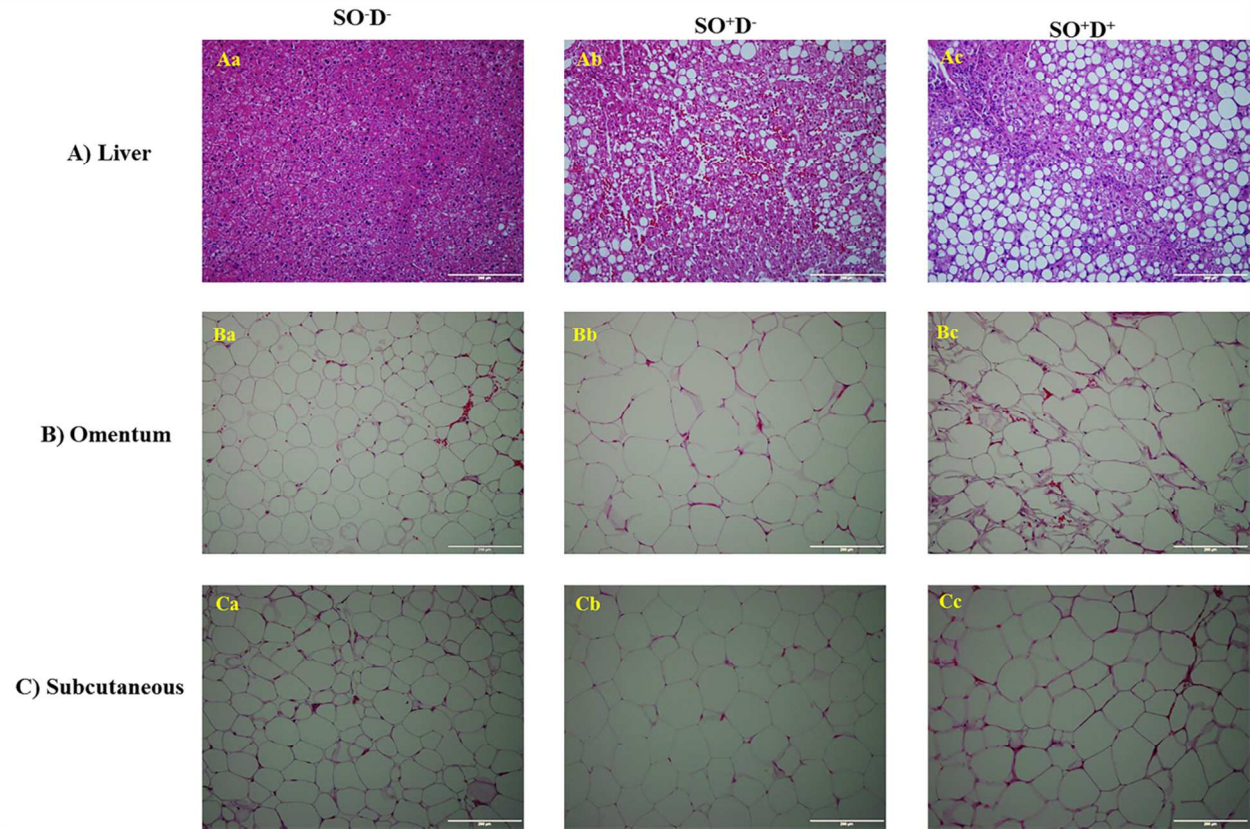

Figure S2 Hematoxylin and Eosin staining for fatty liver grading and inflammation. H & E in biopsy samples of SO^-D^-, SO^+D^- and SO^+D^+ groups respectively in liver (images Aa, Ab & Ac), omental fat (images Ba, Bb & Bc) and subcutaneous fat (images Ca, Cb & Cc). Liver biopsy samples showed steatosis in SO^+D^- and fibrosis and cirrhosis in SO^+D^+ groups. Size of the adipocyte was larger in subjects with obesity (images Bb, Bc, Cb, & Cc) and inflammation was increased in SO^+D^+ (images Bc & Cc) compared to SO^-D^- group (N= 5 SO^-D^-; 7 SO^+D^-; 15 SO^+D^+). Abbreviations: SO^-D^- = subjects without obesity and diabetes; SO^+D^- = subjects with obesity but not diabetes; SO^+D^+ = subjects with obesity and diabetes.

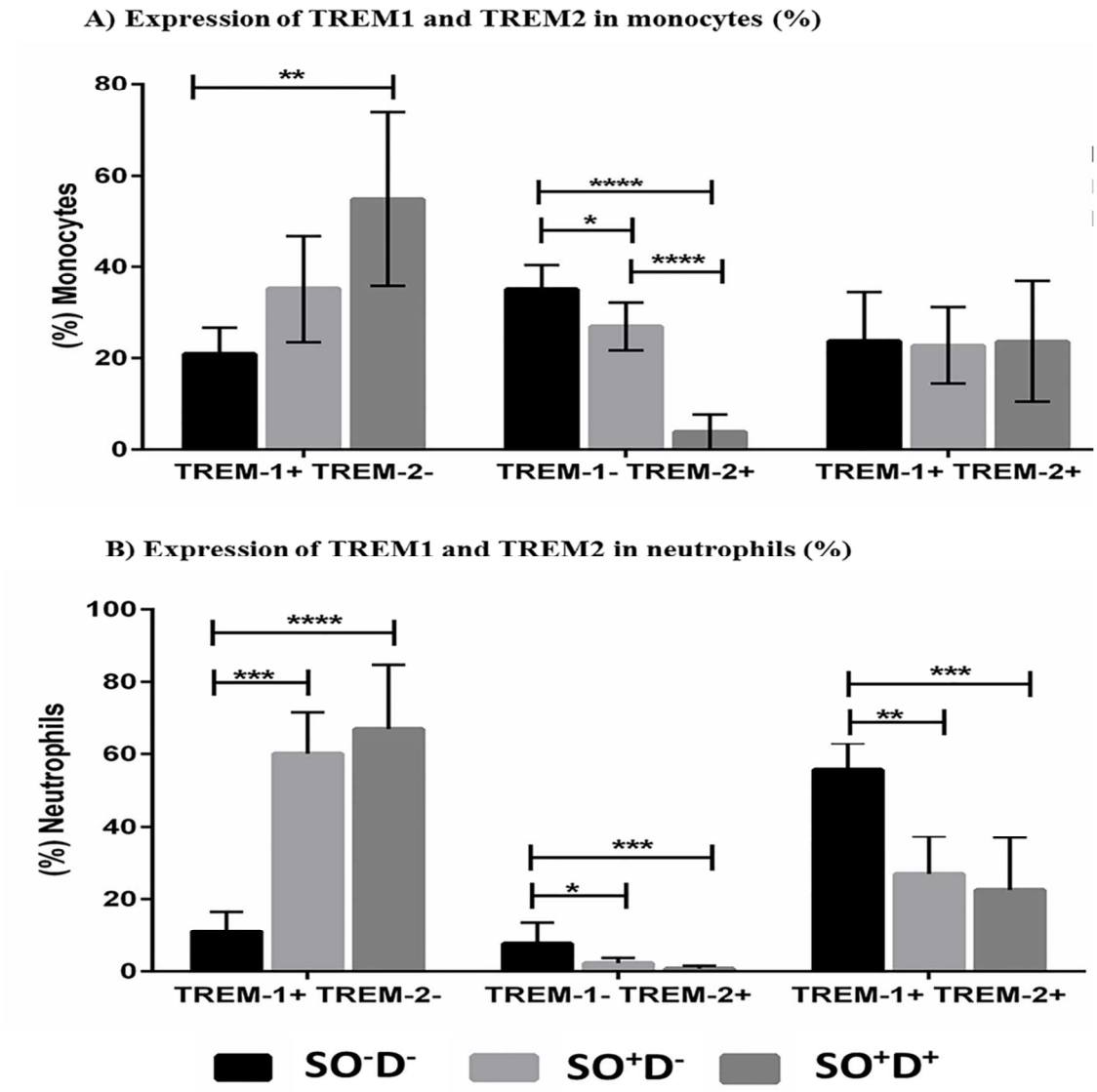

Figure S3 TREM-1 and TREM-2 expression in monocytes and neutrophils of the study subjects. The protein expression of TREM-1 and TREM-2 in monocytes and neutrophils were compared between SO^-D^-, SO^+D^- and SO^+D^+ groups using One-way ANOVA for continuous variables. A) The expression of $TREM1^+$ $TREM2^-$, $TREM1^-$ $TREM2^+$ and $TREM1^+$ $TREM2^+$ levels in monocytes (%). Data are shown as mean ± SD (N= 5 SO^-D^-; 7 SO^+D^-; 15 SO^+D^+); A) The expression of $TREM1^+$ $TREM2^-$, $TREM1^-$ $TREM2^+$ and $TREM1^+$ $TREM2^+$ levels in neutrophils (%). Data are shown as mean ± SD (N= 5 SO^-D^-; 7 SO^+D^-; 15 SO^+D^+); *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. Abbreviations: SO^-D^- = subjects without obesity and diabetes; SO^+D^- = subjects with obesity but not diabetes; SO^+D^+ = subjects with obesity and diabetes.

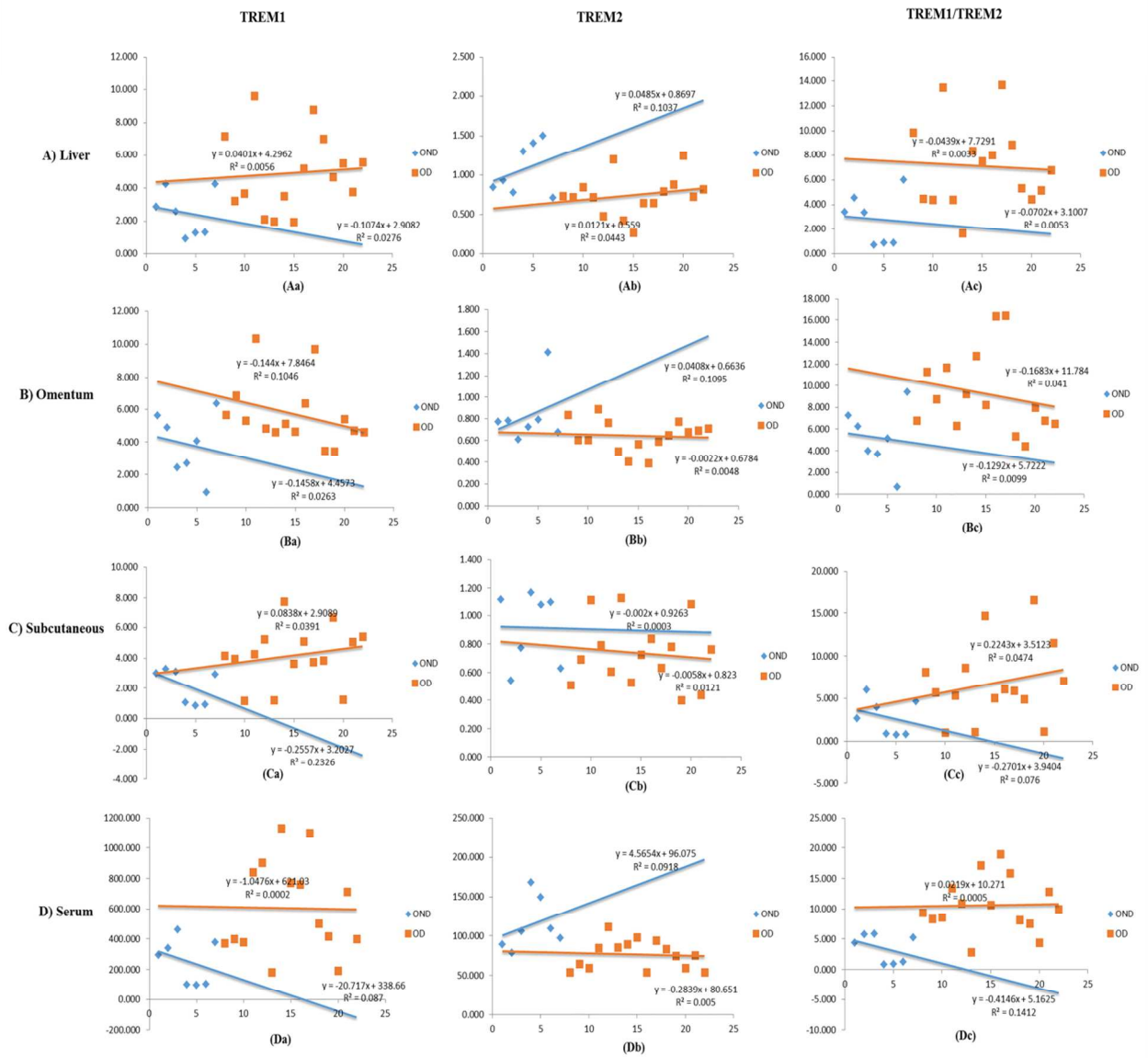

Figure S4 Correlation of TREM-1, TREM-2 and TREM-1/TREM-2 ratio in liver, omentum and subcutaneous and serum samples between $SO^{+}D^{-}$ and $SO^{+}D^{+}$ groups. A) Correlation analysis in Liver biopsy sample (Aa) TREM-1; (Ab) TREM-2; (Ac) TREM-1/TREM-2 ratio. B) Correlation analysis in Omentum biopsy sample (Ba) TREM-1; (Bb) TREM-2; (Bc) TREM-1/TREM-2 ratio. C) Correlation analysis in Subcutaneous biopsy sample (Ca) TREM-1; (Cb) TREM-2; (Cc) TREM-1/TREM-2 ratio. D) Correlation analysis in serum (Da) TREM-1; (Db) TREM-2; (Dc) TREM-1/TREM-2 ratio. Abbreviations: $SO^{+}D^{-}$ = subjects with obesity but not diabetes; $SO^{+}D^{+}$ = subjects with obesity and diabetes.

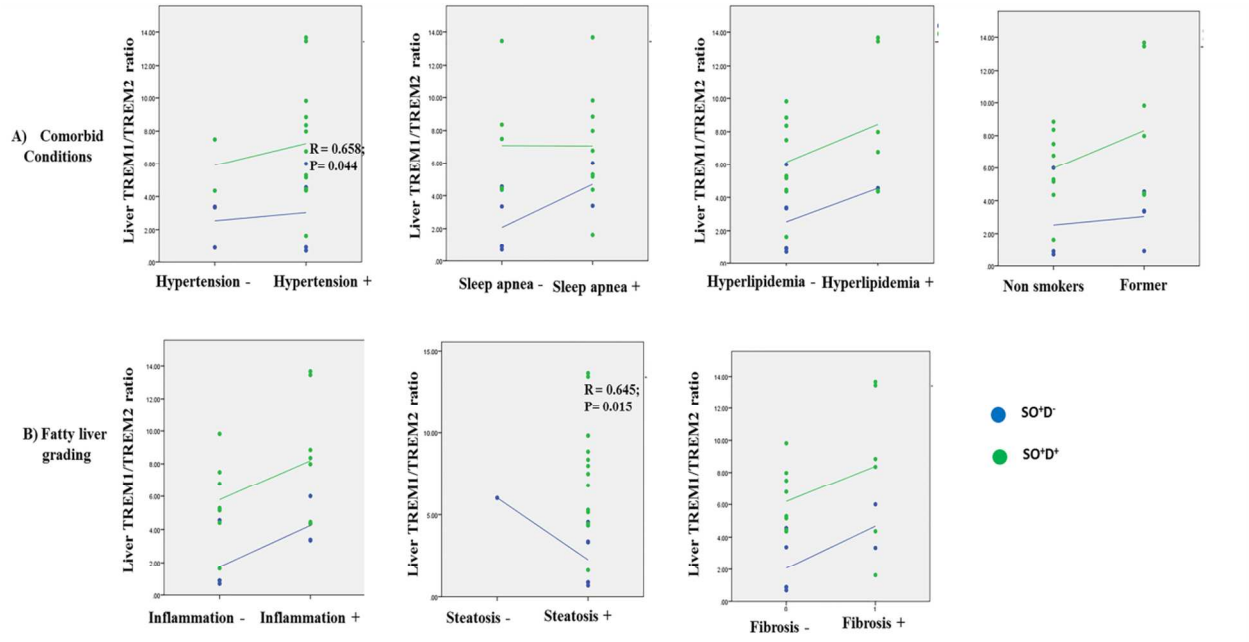

Figure S5 Graphical representation of the analysis of the liver TREM-1/TREM-2 ratio in association with co-morbid conditions (hypertension, hyperlipidemia, sleep apnea, and smoking) and fatty liver grading (inflammation, steatosis, and fibrosis) in liver biopsy samples (Table 3). Subject's with strong correlation between liver TREM-1/TREM-2 ratio and co-morbid conditions or fatty liver grading were analyzed among SO^+D^- and SO^+D^+ groups. Analysis showed strong association of the increased TREM-1/TREM-2 ratio in subjects with SO^+D^+**.** A) Association between liver TREM-1/TREM-2 ratio with comorbid conditions; B) Association between liver TREM-1/TREM-2 ratio with fatty liver grading. Abbreviations: SO^-D^- = subjects without obesity and diabetes; SO^+D^- = subjects with obesity but not diabetes; SO^+D^+ = subjects with obesity and diabetes.

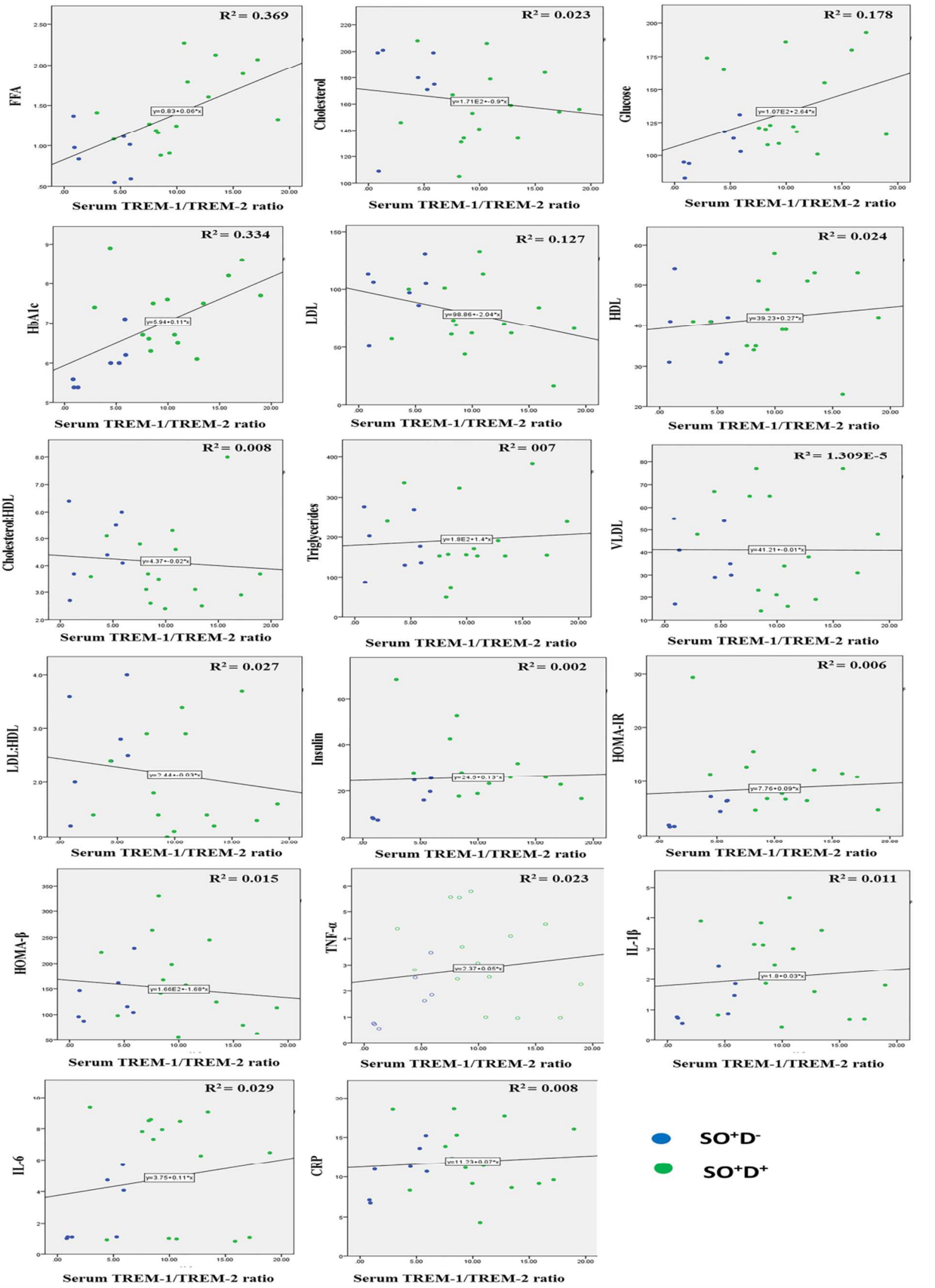
FFA
R² = 0.369
y=0.83+0.06*x
Cholesterol
R² = 0.023
y=1.71E2+-0.9*x
Glucose
R² = 0.178
y=1.07E2+2.64*x
HbA1c
R² = 0.334
y=5.94+0.11*x
LDL
R² = 0.127
y=98.86+-2.04*x
HDL
R² = 0.024
y=39.23+0.27*x
Cholesterol:HDL
R² = 0.008
y=4.37+-0.02*x
Triglycerides
R² = 007
y=1.8E2+1.4*x
VLDL
R² = 1.309E-5
y=41.21+-0.01*x
LDL:HDL
R² = 0.027
Insulin
R² = 0.002
y=24.3+0.13*x
HOMA-IR
R² = 0.006
y=7.76+0.09*x
HOMA-β
R² = 0.015
y=1.66E2+-1.68*x
TNF-α
R² = 0.023
y=2.37+0.05*x
IL-1β
R² = 0.011
y=1.8+0.03*x
IL-6
R² = 0.029
y=3.75+0.11*x
CRP
R² = 0.008
Serum TREM-1/TREM-2 ratio
SO+D-
SO+D+

Figure S6 Correlation of sTREM-1/sTERM2 ratio and biochemical profile of study subjects. SPSS scatter blot was used to analyze the association between sTREM-1/sTREM-2 ratio and biochemical profiles among SO^+D^- and SO^+D^+ groups. Abbreviations: SO^+D^- = subjects with obesity but not diabetes; SO^+D^+ = subjects with obesity and diabetes.

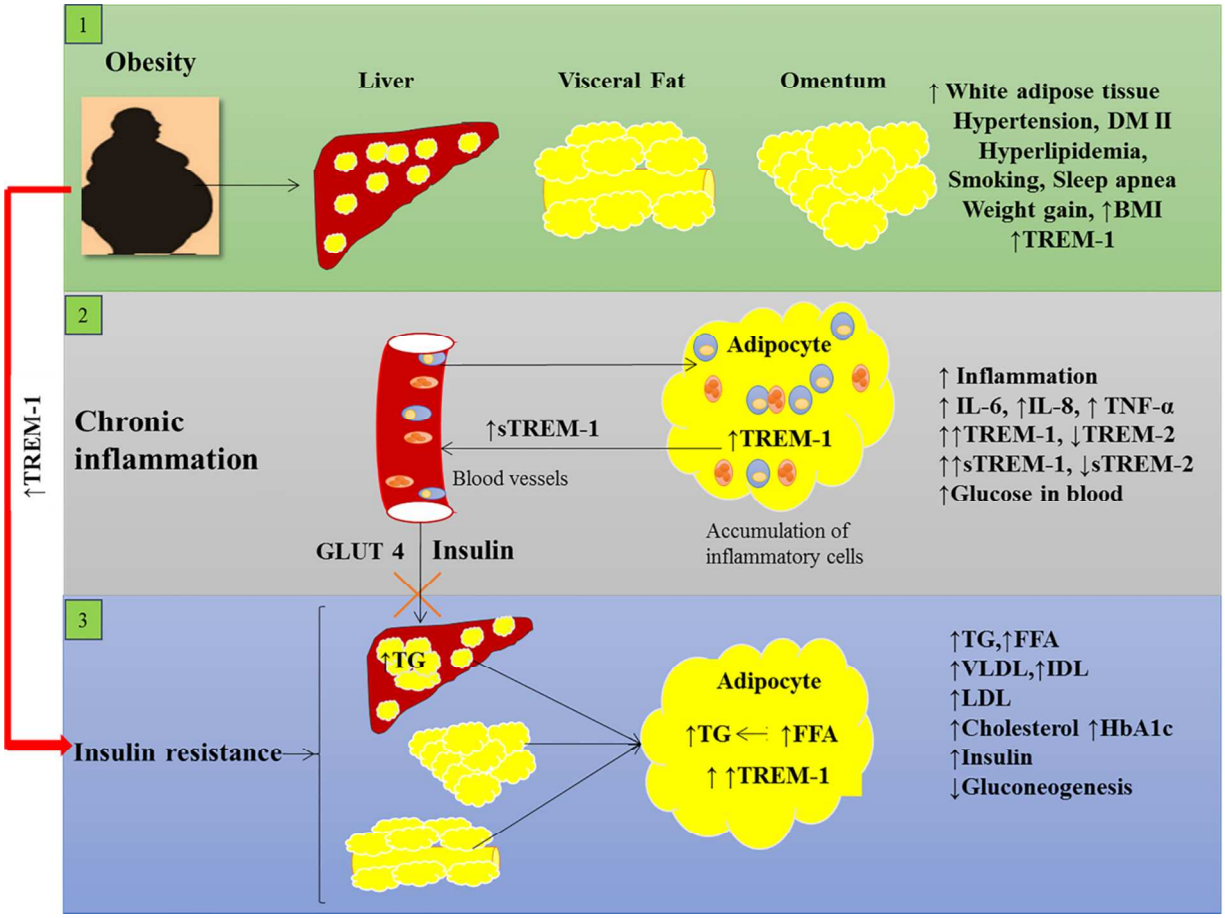

Figure S7 Model of TREM-1 induction in obesity and insulin resistance. 1- Obesity results in deposition of white adipose tissue in liver, omentum and subcutaneous space, and results in metabolic syndrome with increased parameters like hypertension, hyperlipidemia, hyperinsulinemia and hyperglycemia. Obesity leads to obstructive sleep apnea, weight gain and increase in BMI. 2- Chronic inflammation in white adipose tissue leads to increased TREM-1 via secretion of proinflammatory cytokines like IL-6 and TNF-alpha, and decreased TREM-2 expression. Chronic inflammation further increases the levels of TREM-1. Detachment of TREM-1 protein from the cell surface increases sTREM-1. sTREM-1 increases more with chronic inflammation in $SO^{+}D^{+}$ group. 3- Over expression of TREM-1 leads to insulin resistance in obesity. Insulin resistance decreases the glucose transport from blood to liver resulting in further increase in the FFA, TG, glucose and lipoprotein levels by decreasing lipolysis and gluconeogenesis.

Table S1 Biochemical profile of subjects with obesity

Biochemical Analysis	SO^+D^- (7)	SO^+D^+ (15)	P value
Cholesterol (mg/dl)	176.28 ± 29.79	157.13 ± 28.23	NS
Triglycerides (mg/dl)	182.85 ± 66.62	195.4 ± 93.52	NS
FFA (μM/ml)	0.92 ± 0.26	1.48 ± 0.44**	P=0.007
HDL (mg/dl)	38.14 ± 7.45	42.6 ± 9.26	NS
LDL (mg/dl)	98.42 ± 23.23	74.13 ± 28.67	NS
VLDL (mg/dl)	39.14 ± 12.15	42.86 ± 22.65	NS
Cholesterol: HDL	4.81 ± 1.20	3.92 ± 1.46	NS
LDL: HDL	2.72 ± 0.86	1.97 ± 0.88	NS
CRP (mg/l)	10.80 ± 3.10	12.28 ± 4.26	NS
HbA1c (%)	5.95 ± 0.54	7.29 ± 0.83 **	P=0.001
Glucose (mg/dl)	105.28 ± 15.20	139.4 ± 32.14*	P=0.016
Insulin (μIU/ml)	15.73 ± 7.24	30.17 ± 14.06*	P=0.020
HOMA-IR	4.27 ± 2.27	10.52 ± 6.06*	P=0.017
HOMA-β	134.39 ± 46.04	160.74 ± 78.95	NS
TNF-α (pg/ml)	1.75 ± 0.95	4.19 ± 2.19*	P=0.014
IL-1β (pg/ml)	1.24 ± 0.70	2.38 ± 1.35	NS
IL-6	2.23 ± 1.61	5.63 ± 3.52*	P=0.036

Biochemical profile comparison was done between SO^+D^- and SO^+D^+ using student t-test for continuous variables. All data are presented as mean values ± SD, (normal physiological levels), *p* values for significance. Abbreviations: SO^-D^- = subjects without obesity and diabetes; SO^+D^- = subjects with obesity but not diabetes; SO^+D^+ = subjects with obesity and diabetes; NS = not significant; FFA = free fatty acids; HDL= high density lipoprotein; LDL = low density lipoprotein; VLDL = very low density lipoprotein; CRP = C reactive protein; HbA1c = glycosylated hemoglobin; HOMA-IR = homeostatic model assessment (HOMA)-Insulin resistance (IR). *$p<0.05$, **$p<0.01$.

Table S2 The criteria used for fatty liver grading and inflammation

Inflammation (0–4-point scale)	Hepato-steatosis (0–3-point scale)	Fibrosis (0–4-point scale)
0=No inflammation	0 = no steotosis	0 = no fibrosis
1 = minimal	Grade I = 0-33%	1 = portal fibrosis
2 = mild	Grade II = 33-66%	2 = periportal fibrosis
3 = moderate	Grade III = 66-100%	3 = septal fibrosis
4 = severe		4 = cirrhosis

Tissue specimens were fixed in 4% formalin and was transversely sectioned at 2mm and embedded in paraffin. Thin sections (5μm) were cut using a microtome and stained with hematoxylin and eosin (H&E) following manufacturer's standard protocol (Newcomer/supply). The H&E stained biopsy samples of liver was reviewed blindly by a board certified pathologists for fatty liver grading and inflammation.

Table S3 The fluorescent conjugated antibody panel used for determination of TREM-1 and TREM-2 in monocytes and neutrophils

Antibodies	Fluorescent conjugated	Dilution used
CD45	FITC; e-bioscience 11-9459-42	1:400
CD16	APC cyanine7; BD bioscience 557831	1:200
CD14	PE cyanine5; BD bioscience 555408	1:800
HLA-DR	perCP cyanin5.5; Biolegend 307630	1:200
TREM-1	PE; Biolegend 314906)	1:100
TREM-2	APC; R & D FAB17291A	1:20

Table S4 Primers used in this study for real time PCR

Gene name	Forward primer (5'-3')	Reverse primer (5'- 3')	PCR cycling conditions
TREM-1	AGT TAC AGC CCA AAA CAT GC	CAG CCC CCA CAA GAG AAT TA	Initial denaturation 5 min at 95^0C, 40 cycles of denaturation 30s at 95^0C, 30s at 55-60^0C (according to the primer annealing temperatures) and extension 30s at 72^0C followed by melting curve analysis
TREM-2	ACA GAA GCC AGG GAC ACA TC	CCT CCC ATC ATC TTC CTT CA	
GAPDH	GGT GAA GGT CGG AGT CAA CGG ATT TGG TCG	GGA TCT CGC TCC TGG AAG ATG GTG ATG GG	
TNF-α	ACC CTC AAC CTC TTC TGG CTC AAA	AAT CCC AGG TTT CGA AGT GGT GGT	
IL-1β	ATG GAC AAG CTG AGG AAG ATG	CCC ATG TGT CGA AGA AGA TAG G	
IL-6,	ATA GGA CTG GAG ATG TCT GAG G	GCT TGT GGA GAA GGA GTT CAT AG	
IL-5	GAG ACT CTG AGG ATT CCT GTT C	GAC TCT CCA GTG TGC CTA TTC	
CRP	GCT CCC TAT CTG GAG GAT AGT T	GTC TCA CTC CCA AAG TCC ATA AC	
CD64	CAG CTC TAC ACA GTG GTT TCT C	ACC TCT CTG GCA CCT GTA TT	

Table S5 Grading of Fatty liver in subjects with obesity

Fatty liver grading	Subjects with obesity (22)		
	SO^+D^- (7)	SO^+D^+ (15)	P value
(i) Inflammation 11/22 (50%)	3 (42.9%)	8 (53.3%)	NS
No inflammation	--	--	
Minimal	--	--	
Mild	2 (28.5%)	6 (40%)	
Moderate	1 (14.2%)	2 (13.3%)	
Severe	--	--	
(ii) Hepatosteatosis 21/22 (95.4%)	6 (85.7%)	15 (100%)	NS
0%	--	--	
0-33%	6 (85.7%)	9 (60%)	
33-66%	--	--	
66-100%	--	6 (40%)	
(iii) Fibrosis 8/22 (36.3%)	2 (28.5%)	6 (40%)	NS
Portal fibrosis	2 (28.5%)	3 (20%)	
Periportal fibrosis	--	1 (6.66%)	
Septal fibrosis	--	1 (6.66%)	
Cirrhosis	--	1 (6.66%)	

Fatty liver grading was compared between SO^+D^- and SO^+D^+ using Fisher's exact test or Pearson's χ^2 for categorical variables. Inflammation in liver biopsy was categorized with no inflammation, minimal, mild, moderate and severe inflammation; hepatosteatosis was categorized as 0%, 0-33%, 33-66% and 66-100%; and fibrosis was categorized as portal, periportal and septal fibrosis and cirrhosis for classification. Data show number of subjects in respective group (% subjects). Abbreviations: SO^+D^- = subjects with obesity but not diabetes; SO^+D^+ = subjects with obesity and diabetes.

Table S6 Expression of TNF-α, IL-6, IL-1β, CRP, IL-5, and CD64 in subjects with obesity compared to controls.

Expression in subjects with obesity (22)			SO^+D^- (7)	SO^+D^+ (15)	Correlation (R); P value
TNF-α	Liver	17 (77.27%)	4 (57.14%)	13 (86.66%)	
	Omentum	21 (95.45%)	6 (85.7%)	15 (100%)	
	Subcutaneous	15 (68.18%)	4 (57.14%)	11 (73.3%)	
	Serum	16 (72.72%)	4 (57.14%)	12 (80%)	NS
IL-6	Liver	14 (63.63%)	4 (57.14%)	10 (66.7%)	
	Omentum	18 (81.18%)	5 (71.4%)	13 (86.66%)	
	Subcutaneous	16 (72.72%)	3 (42.85%)	13 (86.66%)	NS
	Serum	13 (59.09%)	3 (42.85%)	10 (66.7%)	
IL-1β	Liver	14 (63.63%)	3 (42.85%)	11 (73.3%)	
	Omentum	17 (77.27%)	5 (71.4%)	12 (80%)	
	Subcutaneous	16 (72.72%)	3 (42.85%)	13 (86.66%)	
	Serum	14 (63.63%)	3 (42.85%)	11 (73.3%)	NS
CRP	Liver	18 (81.18%)	5 (71.4%)	13 (86.66%)	
	Omentum	21 (95.45)	6 (85.7%)	15 (100%)	
	Subcutaneous	16 (72.72%)	4 (57.14%)	12 (80%)	
	Serum	18 (81.18%)	5 (71.4%)	13 (86.66%)	NS
IL-5	Liver	12 (54.54%)	3 (42.85%)	9 (60%)	
	Omentum	17 (77.27%)	5 (71.4%)	12 (80%)	
	Subcutaneous	12 (54.54%)	3 (42.85%)	9 (60%)	NS
CD64	Liver	17 (77.27%)	4 (57.14%)	13 (86.66%)	
	Omentum	20 (90.9%)	5 (71.4%)	15 (100%)	
	Subcutaneous	14 (63.63%)	5 (71.4%)	9 (60%)	NS

Higher number of subjects with overexpression of TNF-α, IL-6, IL-1β, CRP, IL-5 and CD64 were analyzed between SO^+D^- and SO^+D^+ using Fisher's exact test or Pearson's χ^2 for categorical variables. Increased in folds of target gene expressions were regarded as over expression, likewise decrease in folds as under-expression. Data show number of subjects having higher values of these compared to SO^-D^- group. Values show number of subjects (% subjects of total), not significant (NS). Abbreviations: SO^-D^- = subjects without obesity and diabetes; SO^+D^- = subjects with obesity but not diabetes; SO^+D^+ = subjects with obesity and diabetes.