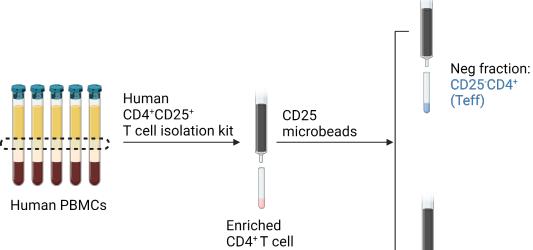
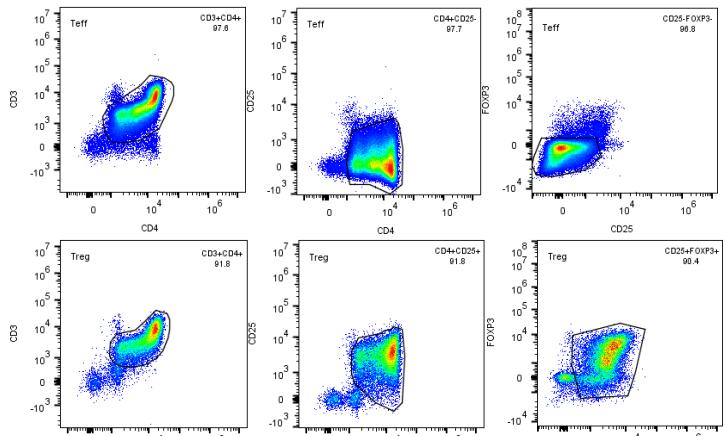


Fig. S1

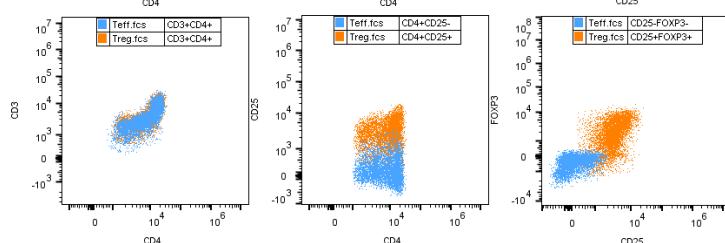
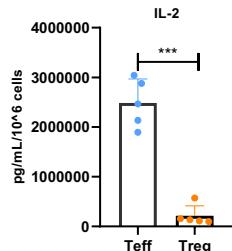
A



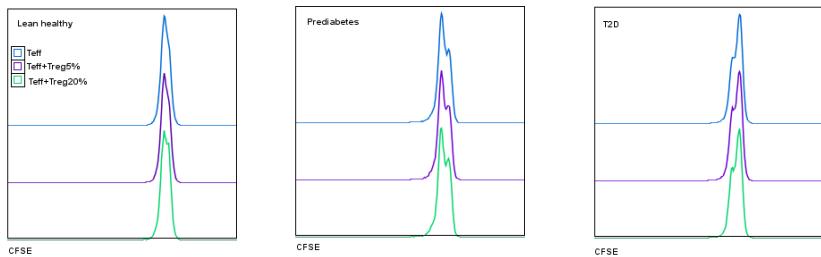
B



C



D



E

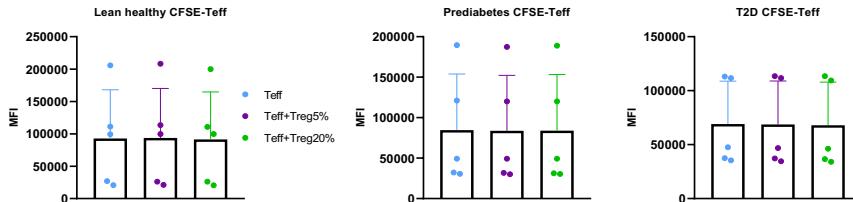


Figure S1 Cell purification and proliferation validations.

- A. Two-step isolation of human Teff (CD25⁻CD4⁺) and Treg (CD25⁺CD4⁺).
- B. Isolated resting human Teff (CD25⁻CD4⁺) and Treg (CD25⁺CD4⁺) cells were stained with CD3 BV605, CD4 FITC, CD25 APC and FOXP3 PE antibodies and analyzed for purity in flow cytometry.
- C. Concentrations of IL-2 in cell culture supernatant of 40h αCD3/CD28 stimulated Teff and Treg from lean healthy (N=5) subjects. Data shown are mean±SD. Paired *t* test showed differences at *** *P* <0.005.
- D. Histograms of CFSE dilution to indicate cell proliferation following stimulation with αCD3/CD28 for 40h. Live cells (counterstained with Zombie NIR dye) were analyzed by flow cytometry. Lack of cell proliferation was reflected by undetectable dye dilution.
- E. Mean fluorescence intensity (MFI) quantification of CFSE signals from panel D. Data shown are mean±SD, N=5.

Fig. S2

Treg vs Teff + Treg20%

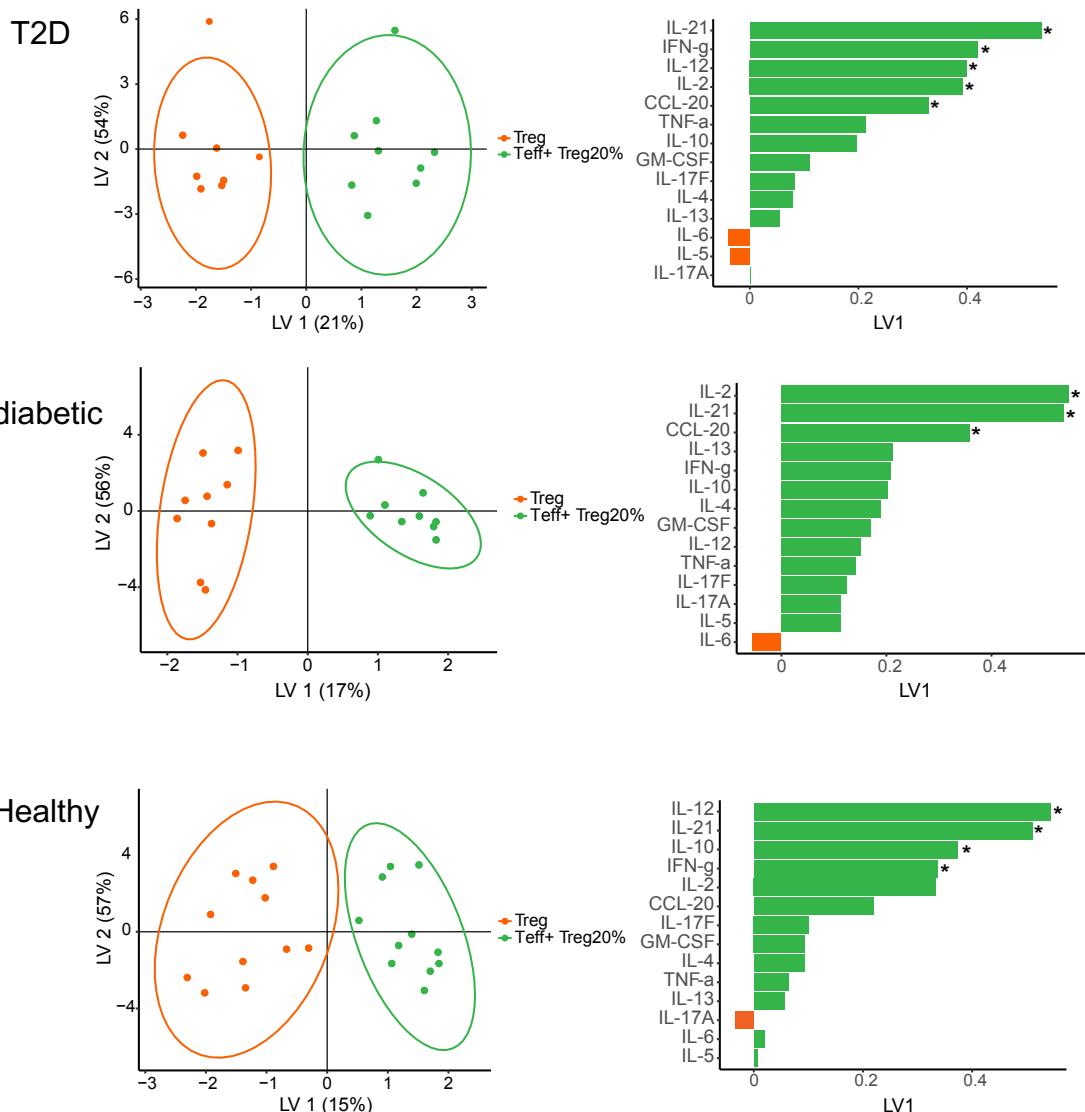


Figure S2: Cytokines that differentiate Treff:Treg co-culture profiles from Treg profiles are independent of disease. PLSDA analysis of cytokines in supernatants from Tregs (orange) or Teff+Tregs 20% (green) from subject groups as indicated. Left panels show distribution in the first two dimensions of a mathematical combination of all cytokines measured with each dot representing compendium outcomes (“inflammation”) from one sample. Right panels rank cytokines for importance (top=more important; bottom=less important) in differentiating combinatorial inflammatory profiles from the groups indicated, with color fill indicating the culture group that contributed that cytokine. * indicates statistical differences ($P<0.05$) in post hoc analysis of ranked cytokines. N=9-11.

Fig. S3

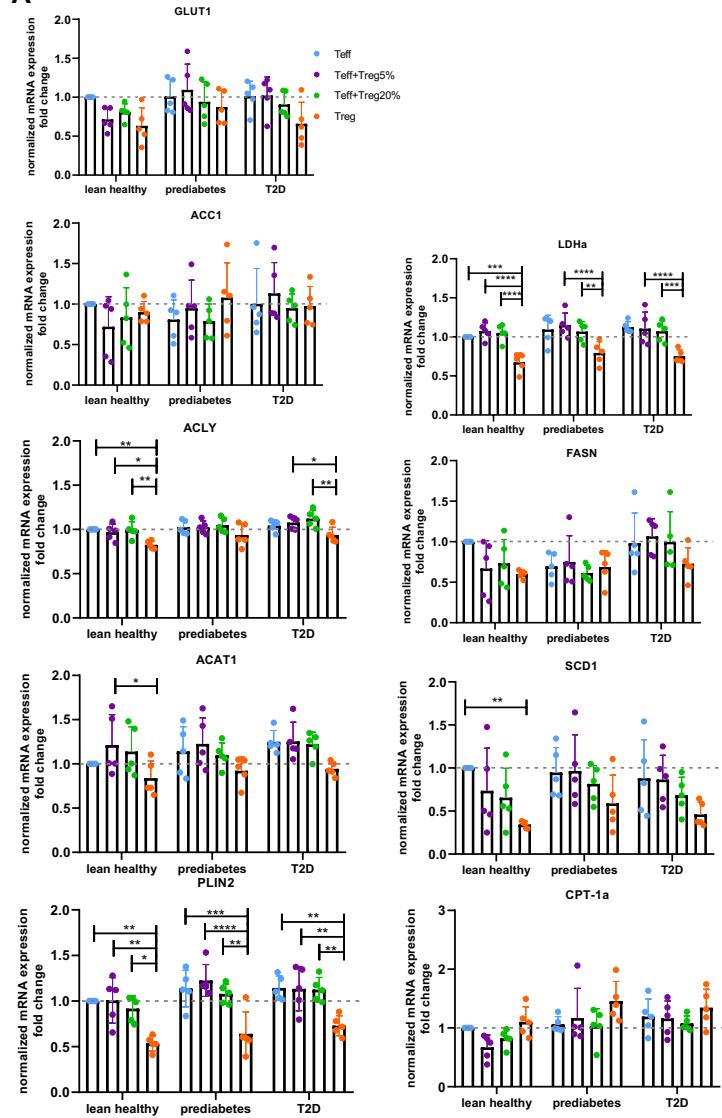
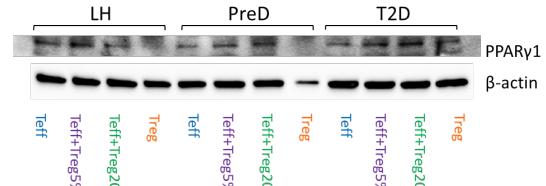
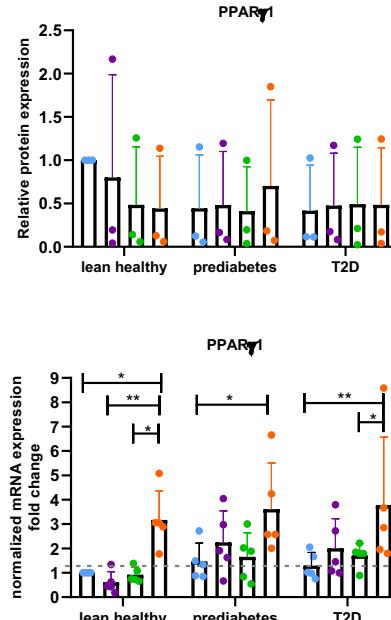
A**B****C**

Figure S3 Expression of multiple metabolic markers have cell type- but not disease-determined differences.

- A. mRNA expression of the indicated genes expressed by stimulated Teff, Tregs, or co-cultures as indicated. N=5 independent samples for each clinical group.
- B. PPAR γ 1 protein in N=3 samples/group as described in panel A. One representative western blot is at top with western quantification in the bottom panel.
- C. PPAR γ 1 mRNA in N=5 samples/group as described in panel A.

Data were shown as Mean \pm SD, analyzed by Two-way ANOVA. * $P<0.05$, ** $P<0.01$, *** $P<0.005$, **** $P<0.001$.

	Lean Healthy	Prediabetes	Type 2 Diabetes
Age	57±8	59±11	54±12
BMI (kg/m ²)	21.96±2.02	31.15±4.65 ****	33.80±4.50 ****
HbA1c (%)	5.2±0.2 (n=7)	5.6±0.4 (n=14) ###	7.5±1.6 *** (n=14)
HbA1c (mmol/mol)	33±3 (n=7)	38±4 (n=14) ###	58±18 *** (n=14)
FPG (mg/dL)	89±4 (n=7)	105±13 (n=14) ###	151±34 **** (n=5)
Total Cholesterol (mg/dL)	195±42 (n=7)	181±27 (n=14) #	140±48 * (n=12)
HDL (mg/dL)	61±6 (n=7)	58±14 (n=14) ##	41±10 ** (n=12)
LDL (mg/dL)	113±40 (n=7)	102±18 (n=14)	74±37 * (n=12)
Triglycerides (mg/dL)	69±17 (n=7)	115±50 (n=14)	164±88 ** (n=12)
Systolic Blood Pressure (mmHg)	112±6 (n=7)	134±11 (n=14) \$\$\$	125±11 * (n=12)
Diastolic Blood Pressure (mmHg)	70±12 (n=7)	83±8 (n=14) \$	80±9 (n=12)
N (Female)	14 (F=9)	15 (F=12)	20 (F=7)

Table S1. Data are shown as Mean ± SD, analyzed by One-way ANOVA with multiple comparisons.

* P<0.05, ** P<0.01, *** P<0.005, **** P<0.001, type 2 diabetes compared with lean healthy subjects. # P<0.05, ## P<0.01, ### P<0.005, prediabetes compared with type 2 diabetes subjects. \$ P<0.05, \$\$\$ P<0.005, prediabetes compared with lean healthy subjects. FPG: fasting plasma glucose, F: female.

Table S2

Target	Full name	Function	Gene name	Gene ID (NCBI)	Primer bank ID	Forward Primer Sequence	Reverse Primer Sequence
CPT-1a	Carnitine-palmitate transferase 1a	FA oxidation	PPARGC1A	10891	116284374c2	GCTTCTGGGTGG ACTCAAGT	GAGGGCAATCCGTC TTCATCC
PPAR γ 1	Peroxisome proliferator-activated receptor γ	stimulate lipid uptake and adipogenesis by fat cells	PPARG	5468	116284372c1	GGGATCAGCTCC GTGGATCT	TGCACTTGGTACTC TTGAAGTT
GLUT1	Glucose transporter 1 (also SLC2A1)	This gene encodes a major glucose transporter in the mammalian blood-brain barrier. The encoded protein is found primarily in the cell membrane and on the cell surface, where it can also function as a receptor for human T-cell leukemia virus (HTLV) I and II.	SLC2A1	6513	166795298c2	TCTGGCATCAACG CTGTCTTC	CGATACCGGAGCCA ATGGT
FASN	fatty acid synthase	FA synthesis	FASN	2194	41872630c1	AAGGACCTGTCTA GGTTTGATGC	TGGCTTCATAGGTGA CTTCCA
ACC1	Acetyl-CoA carboxylase	FA synthesis	ACACA (ACC1)	31	38679973c1	ATGTCTGGCTTGC ACCTAGTA	CCCCAAAGCGAGTA ACAAATTCT
SCD1	stearoyl-CoA desaturase	enzyme for FA biosynthesis, primarily oleic acid	SCD1	6319	53759150c1	TCTAGCTCCTATA CCACCACCA	TCGTCTCCAACTTAT CTCCTCC
ACLY	ATP-citrate lyase	Converts citrate to acetyl-CoA to link carbohydrate metabolism through a citrate intermediate, with fatty acid biosynthesis, which consumes acetyl-CoA	ACLY	47	38569422c2	ATCGGTTCAAGTA TGCTCGGG	GACCAAGTTTCCAC GACGTT
LDHA	lactate dehydrogenase A	Catalyzes interconversion of lactate to pyruvate as it interconverts NAD $^+$ to NADH	LDHA	3939	260099724c1	ATGGCAACTCTAA AGGATCAGC	CCAACCCCACAACT GTAATCT
ACAT1	acetyl-CoA acetyltransferase 1	Mitochondrial enzyme that catalyzes the reversible formation of acetoacetyl-CoA from two molecules of acetyl-CoA.	ACAT1	38	223890189c1	AAGGCAGGCAGTAACATCAGTTAGCCCCG TTGGGTG TCTTTAC	
PLIN2	perilipin 2	Coats intracellular lipid storage droplets; a marker of lipid accumulation in diverse cell types and diseases.	PLIN2	123	327199305c3	TTGCAGTTGCCAA TACCTATGC	CCAGTCACAGTAGTC GTCACA

Table S3: Summary of mixed models for all cytokines

Response	Cell P-value	Group P-value	Interaction P-value
Log-transformed normalized IL.17F	2e-06	0.282036	
Log-transformed normalized GM.CSF	0.000756	0.023104	
Log-transformed normalized CCL.20	<0.00001	0.203504	
Log-transformed normalized IL.12	0.004858	0.088778	
Log-transformed normalized IL.13	0.064615	0.148338	
Log-transformed normalized IL.17A	<0.00001	0.088729	
Log-transformed normalized IL.21			0.022862
Log-transformed normalized IL.4	3e-06	0.038177	
Log-transformed normalized IL.5			0.026999
Log-transformed normalized IL.2	<0.00001	0.736857	
Log-transformed normalized IL.10			0.043958
Log-transformed normalized IFN.G	0.000642	0.198864	
Log-transformed normalized IL.6	1e-06	0.134613	
Log-transformed normalized TNF.a	0.014269	0.061071	

Cell column p value compares amongst Teff, Treg, Teff + Treg5% and Teff +Treg 20%

Group column p value compares amongst lean/healthy, prediabetes and type 2 diabetes

Missing values are P>0.5 and are omitted to simplify table