SUPPLEMENTAL MATERIAL

EXPANDED MATERIALS AND METHODS

Data Availability

All data, methods and essential research materials used for research and analysis have been provided.

Study subjects

Two different cohorts of healthy subjects were recruited through La Jolla Institute's Clinical core. First, a screening cohort, which included participants enrolled in the Normal Blood Donation Program (NBDP donor age and gender details in Table S3). The donors were pre-screened to confirm the absence of any significant systemic disease or viral infections including hepatitis B or C, and HIV. Ethical approval for the study was provided by the Institutional Review Boards at La Jolla Institute for Immunology (VD-057). For the second validation cohort of healthy adult donors (demographics in Table S10), donor blood samples were also sent to clinical labs at UCSD Center for Advanced Laboratory Medicine (CALM) for evaluating Lp(a), lipids, metabolites, hsCRP and HbA1c, on the day of sample collection (Table S12). None of the donors had COVID 19 (was an exclusion criteria) or any other ongoing infection. They had no known conditions of cancer, diabetes, heart or kidney or liver disease. Donors were neither pregnant nor nursing. The study was approved by the Institutional Review Boards at La Jolla Institute for Immunology (IB-248-0821) and at University of California, San Diego (IRB 190053). Written informed consents were obtained from all participants enrolled in either cohort.

For the clinical Coronary Assessment in Virginia (CAVA) cohort, we obtained cryopreserved peripheral blood mononuclear cells from 18 patients (Table S13) undergoing standard cardiac

catheterization at the Cardiac Catheterization laboratory at the University of Virginia Health System, Charlottesville, Virginia, USA. Written informed consents were obtained from all participants before enrollment. Blood samples were collected prior to cardiac catheterization. The study was approved by the Human Institutional Review Board (IRB No. 15328) at the University of Virginia. In this study, Quantitative Coronary Angiography (QCA) was performed using automatic edge detection from an end-diastolic frame, which was selected for each lesion, based on demonstration of the most severe stenosis with minimal foreshortening and branch overlap. The minimum lumen diameter, reference diameter, percent diameter stenosis, and stenosis length were calculated by blinded, experienced investigators who assessed disease severity based on the Gensini score[48]. Briefly, each artery segment was assigned a score of 0-32 based on the percent stenosis. For each segment, this score was multiplied by 0.5-5, depending on the location of the stenosis. Scores for all segments are then added together to given a final score of angiographic disease burden. Score adjustment for collateral was not performed for this study.

HLA binding predictions and experimental validation

The peptide binding groove of HLA class II molecules generally accommodates a 9-residue stretch of amino acids, with specificity being conferred by distinct pockets in the binding region. The peptide-binding pocket of HLA class II molecules is open, and the presence of 2-3 additional residues flanking the nonameric core at both the N- and C-termini facilitates stable peptide binding. Accordingly, it is common to probe class II binding and CD4 T cell reactivity utilizing 15-mer sequences (9-mer core plus 3 aa overhangs on each side).

Overall, there are 4,549 possible 15-mer sequences spanning the entire human APOB protein (UniProtKB P04114, 4,563 amino acid length). For our *in silico* screening, we removed the 15-

mers that overlapped by more than 10 residues because these sequences redundantly span the same nonameric core. The remaining 911 15-mer peptides were scored for their ability to bind a reference set of 27 frequent and representative HLA DP, DQ and DR alleles[30] using MHC-II binding prediction tools available in the Immune Epitope Database (www.iedb.org). For each peptide, allele-specific consensus median percentile ranks were generated from all algorithms queried by the IEDB tool. A peptide with a median percentile score <20 was considered a binder to a specific allele[30], and for each peptide the number of alleles predicted to be bound was tabulated. Using this criterion, 887 sequences were predicted to bind at least one HLA-II allele. We avoided peptides with narrow HLA restriction because our goal was to develop broadly applicable T cells assays that can screen APOB-specific responses in diverse donor populations. Therefore, we selected a set of 30 peptides predicted to bind ≥20 alleles (i.e., ≥75% of reference set).

Next, the capacity of these 30 peptides to bind 28 common HLA alleles (27 from the reference set plus DPB1*03:01) was determined experimentally using classical competition assays based on the inhibition of binding of high affinity radiolabeled ligands to purified MHC molecules[31]. Under the conditions utilized, where [radiolabeled peptide] is < [MHC], and [MHC] < [inhibitor peptide], the measured IC50 values, defined as the concentrations of inhibitor peptide that inhibits the binding of the radiolabeled ligand by 50%, are reasonable estimates of true K_d values. Binding to a specific allele was defined as IC50 <1000 nM. Based on this assay, the top 20 most promiscuous binders (those that bound >50% of the tested alleles), were finally selected for screening in our restimulation assays.

Peptides

Human APOB-derived peptides were synthesized as crude material (>70% purity) on a 5mg

scale by TC Peptide Lab (San Diego). Each peptide was resuspended in dimethyl sulfoxide (DMSO, Sigma Aldrich) and combined in equal proportions to prepare the $APOB_{20}$ peptide pool. A subset of the peptides was synthesized as purified material (>95% purity by reversed-phase HPLC, confirmed by mass spectrometry) on a 2mg scale by TC Peptide Lab (San Diego). CEFX-II (PM-CEFX-3), a positive control pool of 68 known MHC class-II restricted peptides from different infectious agents, the negative control pool of 92 15-mer peptides spanning the human actin alpha protein (PM-ACTS), and custom-made APOB peptides (P2, P4, P5, P11, P12, P17) were purchased from JPT Peptide Technologies (Berlin, Germany).

Scrambled versions of APOB epitopes were generated using the tool at https://www.bioinformatics.org/sms2/shuffle_protein.html. Both original and scrambled peptides were synthesized by TC Peptide Lab (San Diego).

HLA Typing

Genomic DNA was isolated from donor PBMCs using REPLI-g DNA midi kit (QIAGEN). HLA typing with Illumina next generation sequencing was done using services provided by an ASHI-accredited laboratory at the Institute for Immunology & Infectious Diseases, Murdoch University, Western Australia). Class I genes – HLA A, B, C and Class II genes DPB1, DQA1, DQB1, DRB1, DRB3, DRB4 and DRB5, were resolved using exon specific targeted PCR amplification of genomic DNA. Filtered reads were passed through a proprietary algorithm, IIID Allele caller, and mapped using the ASHI-accredited IIID HLA Analysis suite and the latest human HLA allele reference sequences from ImMunoGeneTics (IMGT) HLA database.

Isolation of Peripheral blood mononuclear cells (PBMCs) and cell culture

Venous blood samples were collected in blood bags or tubes coated with anti-coagulant (sodium

heparin or K2-EDTA). For clinical tests, samples were collected either in Lithium heparin tubes (for comprehensive metabolic panel, lipid panel, Lp(a), hsCRP) or K2-EDTA tubes (for HbA1c). PBMCs were isolated from whole blood by Ficoll-Paque Plus (Millipore Sigma) density-gradient centrifugation. Briefly, tubes were centrifuged at 800xg for 15 min at room temperature with brakes off. Top layer of plasma was removed and an equivalent amount of serum-free cell culture medium was added and resuspended thoroughly. Diluted blood (seven parts) was then gently layered on Ficoll-paque plus (three parts) and centrifuged at 800xg for 30 min at room temperature with brakes off. The layer of cells at the interface was carefully harvested and washed twice with medium. Cells were counted using a hemocytometer and viability was evaluated using Trypan Blue dye exclusion method. For cryopreservation in liquid nitrogen, cells were re-suspended in CryoStor® CS10 (Stemcell), a serum-free and animal component-free cryopreservation medium containing 10% DMSO. All i*n vitro* cultures and re-stimulation regimes were carried out in serum-free cell culture medium (TexMACS™, Miltenyi Biotec) supplemented with 1% penicillin/streptomycin (Thermo Fisher Scientific) and cultured at 37°C with 5% CO_2.

Expansion of antigen-specific T cells and Enzyme-linked immunospot (ELISpot) assay

For *in vitro* expansion, PBMCs were plated at a density of 2×10^6 cells/ml in 24-well plates and were cultured in the presence of $APOB_{20}$ and CEFX-II peptide pools. 10 U/ml human IL-2 (Invitrogen) was added to the media at Days 4, 7 and 10. On Day 14, cells were washed and re-plated in 96-well ELISpot plates (Millipore) coated with 5 μg/ml mouse anti-human IFNγ (clone 1-D1K) antibody (Mabtech). PBMCs were re-stimulated with cognate or irrelevant peptide pools (for crossover stimulation experiments) or with individual APOB-derived peptides (for deconvolution and epitope mapping). Unstimulated and PHA-L stimulated sets served as

negative and positive controls, respectively. $APOB_{20}$ (20 peptides) and CEFX-II (68 peptides) pools were used at peptide concentrations 5μg/ml and 1.5μg/ml, respectively. Individual APOB peptides were used at 20μg/ml final concentrations. $APOB_6$ pool of six dominant epitopes was used at 10μg/ml concentration. Positive control Phytohemagglutinin-L (PHA-L, eBioscience) was used at 1X concentration. After 24h incubation at 37^0C, plates were washed six times with PBS containing 0.05% Tween 20 (Millipore Sigma). Plates were then incubated with 1 μg/mlmouse anti-human IFNγ (clone 7-B6-1) biotinylated antibody (Mabtech) in PBS containing 0.5% BSA (Millipore Sigma), for 2h at room temperature. Plates were again washed six times with PBS/0.05% Tween 20. Plates were then incubated with VECTASTAIN® Elite ABC-HRP Kit, Peroxidase (Vector Laboratories) in PBS/0.1% Tween 20 for 1h at room temperature. Plates were washed six times with Ultrapure distilled water (Invitrogen). Plates were incubated with 3-amino-9-ethylcarbazole (Millipore Sigma) tablets dissolved in N,N-Dimethylformamide (Millipore Sigma), acetate buffer and Hydrogen peroxide solution (Millipore Sigma) for 10 min at room temperature to develop secreted cytokine spots. Plates were dried and wells were imaged and spot-forming cells (SFCs) were quantified using the Zeiss KS or AID (Autoimmun Diagnostika) ELISpot readers.

In experiments involving HLA blockade, HLA-DR (clone L243), DP (clone B7/21), DQ (clone SVPL3) or pan HLA class I (W6/32) monoclonal antibodies (kindly provided by Dr. Alessandro Sette, LJI) were added at 10 μg/ml concentration, 30 min prior to restimulation with peptides.

In vitro expansion and intracellular cytokine staining (ICS) assay

PBMCs were plated at a density of 2×10^6 cells/ml in 24-well plates and were cultured with desired peptides or peptide pools. 10U/ml IL-2 was added at Days 4, 7 and 10. After 14 days of

in vitro expansion in the presence of individual epitopes or peptide pools, PBMCs were harvested, washed and re-plated in U-bottom 96-well plates. PBMCs at $1x10^6$ cells per conditions were re-stimulated with desired sets of cognate or irrelevant pools and peptides. $APOB_{20}$ (20 peptides) and CEFX-II (68 peptides) pools were used at peptide concentrations 5µg/ml and 1.5µg/ml, respectively. Individual APOB peptides were used at 20µg/ml. After 2h, protein transport inhibitor cocktail (eBioscience) was added at 1X concentration and incubated for an additional period of 4h. After the 6h stimulation period, cells were washed with FACS buffer (PBS w/o Ca/Mg, 2% FBS) and resuspended in staining master mix containing anti-human Fc-Block (Biolegend), fixable viability dye and antibodies against T cell and non-T cell (Dump) surface markers (antibody details in Table S4). Viability dye at 1:1000 dilution and antibodies at 1:200 dilutions were used. Cells were stained for 30 min on ice. For intracellular cytokine detection, cells were fixed (eBioscience™ IC Fixation Buffer) for 30min at room temperature. Fixed cells were washed, permeabilized and stained in 1X Perm buffer solution (eBioscience™ Permeabilization Buffer (10X). Cells were stained for CD40L and T helper cytokines for 45 min at room temperature (antibody details in Table S4). Antibodies against intracellular markers were used at a final dilution of 1:50. Single color-stained beads (UltraComp eBeads™, Invitrogen) were used for compensation. Data was acquired on a BD LSR II flow cytometer and analyzed with FlowJo software. Events collected per sample ranged between 500,000-2,000,000 events. Data from unstimulated samples were used as background controls to set the gates for CD40L and cytokines.

Activation-induced marker (AIM) assay

In *ex vivo* AIM assay, $1x10^6$ PBMCs per condition were plated in flat-bottomed 96-well plates and cultured for 24h in the presence of the indicated peptide pools. To account for differences in

the number of peptides in each pool, peptides in the $APOB_{20}$ pool (20 peptides) were used at 10μg/ml, CEFX-II pool (68 peptides) at 3μg/ml and Actin pool (92 peptides) at 2μg/ml, so that total concentration of the pools remain comparable. $APOB_6$ pool of dominant epitopes ("pos peps") and the pool of poorly immunogenic APOB peptides ("neg peps") were each used at 20μg/ml per peptide. To improve surface detection of CD40L, a CD40 blocking antibody (Novus Biologicals) was added to each well 15 min before stimulation at a final concentration of 1μg/ml. In experiments with HLA-II blocking, purified mouse Anti-human HLA-DR, DP, DQ (clone Tu39, BD Biosciences) was added to each well 30 min before stimulation at a final concentration of 20μg/ml. At the end of the stimulation period, cells were washed with FACS buffer (PBS w/o Ca/Mg, 2% FBS) and resuspended in a staining master mix containing anti-human Fc-Block (Biolegend), fixable viability dye and antibodies against T cell and non-T cell (Dump) surface markers, activation markers and memory markers (antibody details in Table S4). Viability dye was used at 1:1000 dilution, antibodies were used at a final dilution of 1:100 (activation markers) and 1:200 (other extracellular markers). Cells were stained for 60 min on ice. Single color-stained beads (UltraComp eBeads™, Invitrogen) were used for compensation. Data was acquired on a BD LSR II flow cytometer and analyzed with FlowJo software. Events collected per sample ranged between 500,000-2,000,000 events. Data from unstimulated samples were used as background controls to set the gates for activation markers.

RATE analysis

The RATE (Restrictor Analysis Tool for Epitopes) tool (http://iedb-rate.liai.org/) was used to computationally infer HLA restriction of the twenty APOB peptides based on observed responses (Figure 3) in HLA typed donors[39, 40]. RATE creates contingency tables between donor alleles and responder frequencies and estimates Relative Frequency (RF) and Odds ratio to

determine associations between the expression of a specific allele in a donor and the occurrence of a positive immune response in that donor. An RF > 1 indicates a positive association between an allele and a response. RF is calculated according to the formula:

$RF = [A^+R^+/(A^+R^+ + A^+R^-)] / [(A^+R^+ + A^-R^+)/\text{Total donors}]$

where,

A^+R^+ = Number of subjects who expressed a specific allele and gave a positive immune response to the specific peptide

A^-R^- = Number of subjects who did not express the specific allele and did not give a positive immune response to the specific peptide

A^-R^+ = Number of subjects who did not express the specific allele but gave a positive immune response to the specific peptide

A^+R^- = Number of subjects who expressed the specific allele but did not give a positive immune response to the specific peptide

Statistical significance of an association is determined using the Fisher's exact test.

Single HLA-II transfected cell lines

RM3 (derived from human B lymphocyte cell line Raji) cells transfected with either DPB1*05:01 or DRB3*02:02 HLA-II alleles[41] (kindly provided by Dr. Alessandro Sette, LJI) were used to examine the immunogenicity of APOB epitopes in the context of defined peptide-HLA-II binder, non-binder scenarios. In preparation for the assay, donor PBMCs expressing the HLA-II allele under study were expanded with the binder or the non-binder APOB peptide at 20μg/ml concentration (intermittent 10U/ml IL-2 feeding at days 4, 7 and 10). The single HLA-

II transfected cell lines were maintained in culture. At day 14 of the PBMC expansion protocol, each transfected cell line was plated at $2x10^5$ cells/well in a flat-bottom 96-well plate and pulsed with 20μg/ml individual peptide for 1h at 37^0C. Cells without any added peptide served as "no pep" background control. Cells were washed four times in PBS to remove free unbound peptides. Peptide-expanded PBMCs, at $2x10^5$/well, were plated in 96-well ELISpot plates (Millipore) coated with mouse anti-human IFNγ (clone 1-D1K) antibody (Mabtech). Peptide-pulsed and control "no pep" cell lines were added at $5x10^4$ cells/well. No exogenous peptides were added to the wells containing PBMCs and cell lines. Separate unstimulated and cognate peptide-stimulated (20μg/ml) PBMC alone sets served as negative and positive controls, respectively. After 24h incubation, secreted IFNγ cytokine was detected using mouse anti-human IFNγ (clone 7-B6-1) biotinylated antibody (Mabtech). Wells were imaged using the AID (Autoimmun Diagnostika) ELISpot reader.

Cell isolation and T cell receptor sequencing

For isolation of APOB epitope-specific $CD4^+$T cells, PBMCs were expanded with the $APOB_6$ pool (10μg/ml per peptide). On Day 14, cultured cells were washed and re-stimulated with $APOB_6$ (10μg/ml) for 24h (AIM assay protocol). 1μg/ml of CD40 blocking antibody was added 15 min before stimulation. At the end of the stimulation period, cells were washed with FACS buffer (PBS w/o Ca/Mg, 2% FBS). Day 14 ($APOB_6$ expanded and restimulated) and Day 0 (*ex vivo* samples with no *in vitro* stimulation) PBMC samples from six donors were resuspended in a staining master mix containing anti-human Fc-Block (Biolegend), fixable viability dye and antibodies against T cell and non-T cell (Dump) surface markers, activation markers and memory markers (antibody details in Table S4). Viability dye was used at 1:1000 dilution, antibodies were used at a final dilution of 1:100 (activation markers) and 1:200 (other

extracellular markers). Cells were stained for 60 min on ice. AIM^+ $CD4^+$T cells were identified in a serial gating scheme where expressions of four combinations of CD40L, CD69, CD25, 4-1BB and OX-40 activation markers were sequentially assessed. Gates were set based on unstimulated and PHA-L stimulated negative and positive controls, respectively. Antigen-specific AIM^+ and non-specific control AIM^- $CD4^+$T cells from Day 14 samples and naïve ($CD45RA^+CCR7^+$), central memory ($CD45RA^-CCR7^+$, TCM) and effector memory ($CD45RA^-$ $CCR7^-$, TEM) $CD4^+$T cells from Day 0 PBMCs were sorted into HEPES-containing buffer (PBS with 2% FBS and 0.025 M HEPES) using BD FACSAria flow cytometry sorters. A combination of single color-stained cells and beads (UltraComp eBeads™, Invitrogen) were used for compensation. Acquired data was analyzed with FlowJo software. Naïve and memory T cell gates (Day 0 samples) were set using CD45RA and CCR7 FMO sets. Unstimulated samples were used as background controls to set the gates for activation marker expression (Day 14 samples).

Genomic DNA was extracted from sorted cells using QIAamp DNA Micro Kit (QIAGEN) and sent to Adaptive Biotechnologies for survey-level TCRβ sequencing (depth ~60,000 reads) using their established immunoSEQ platform[44]. Briefly, highly variable CDR3 regions on somatically rearranged human TCRβ chains were amplified with a two-step multiplex PCR approach using optimized set of primers that target the VDJ region spanning each unique CDR3B. A synthetic repertoire containing all possible V/J templates is used as a built-in control to quantify and correct PCR amplification biases. Processing of raw Illumina sequence reads, filtering, demultiplexing, clustering and mapping of CDR3 sequences and annotation of VDJ genes using IMGT database sequences were performed at Adaptive Biotechnologies and made available for download and analysis. Details of input DNA and total (productive templates) and unique

(productive rearrangements) TCR counts for Day 0 (Naïve, TCM, TEM) and Day14 APOB-AIM^{+} and AIM^{-} CD4^{+}T subsets in all donors are provided in Table S7. TCR repertoire characteristics and individual rearrangements were analyzed using immunoSEQ Analyzer 3.0. All TCR rearrangements in Additional File 3.

Proliferation assay

10 million PBMCs were resuspended in 1ml PBS buffer. Cells were labelled with 5-chloromethylfluorescein diacetate (CellTrace™ CFSE Cell Proliferation Kit, Invitrogen) at a working concentration of 1μM. Cells were thoroughly mixed with CFSE and incubated at 37^{0}C for 15 min, with intermittent mixing. Cells were washed twice with PBS containing 20% FBS. Labelled cells were plated in a 48-well plate with 1 million cells per well. PBMCs were stimulated with either the $APOB_6$ pool of dominant epitopes ("pos peps") or with the pool of poorly immunogenic APOB peptides ("neg peps"), both used at 10μg/ml per peptide. Unstimulated sets were used as negative controls to subtract background. A set of PBMCs stimulated with soluble CD3/CD28 (ImmunoCult™ Human CD3/CD28 T Cell Activator) was used as a positive control. After 5 days, media was replenished with fresh culture medium. On day 10, cells were harvested, washed and stained with viability dye, non-T cell markers (Dump) and T cell markers (antibody details in Table S4). Viability dye at 1:1000 dilution and antibodies at 1:200 dilution was used. A combination of single color CFSE-stained cells and single color-stained beads (UltraComp eBeads™, Invitrogen) were used for compensation. Data was acquired on a BD LSR II flow cytometer and analyzed with FlowJo software. "No CFSE" set was used as control to set the gates in flow cytometry.

Cytometric Bead Array

$2x10^6$ PBMCs per condition were plated in flat-bottomed 96-well plates and stimulated with "pos pep" or "neg pep" APOB peptides (20μg/ml) or with CEFX-II (3μg/ml per peptide). After 24h of incubation, plates were centrifuged at 500 x g for 5 min at 4°C. Supernatants were collected and frozen at −80 °C until further analysis with Human Th1/Th2/Th17 cytokine kit (BD Biosciences, Catalog No. 560484), performed according to manufacturer's instructions.

Statistical analysis

Data analysis and statistical comparisons were done using GraphPad Prism Version 9.3.1 and R version 4.0.1. All statistical tests, sample sizes and axes descriptions of graphs are detailed in the legends of respective figures.

Illustrations

Images in the graphical abstract are based on graphics from Servier Medical Art.

ONLINE FIGURES S1-S9

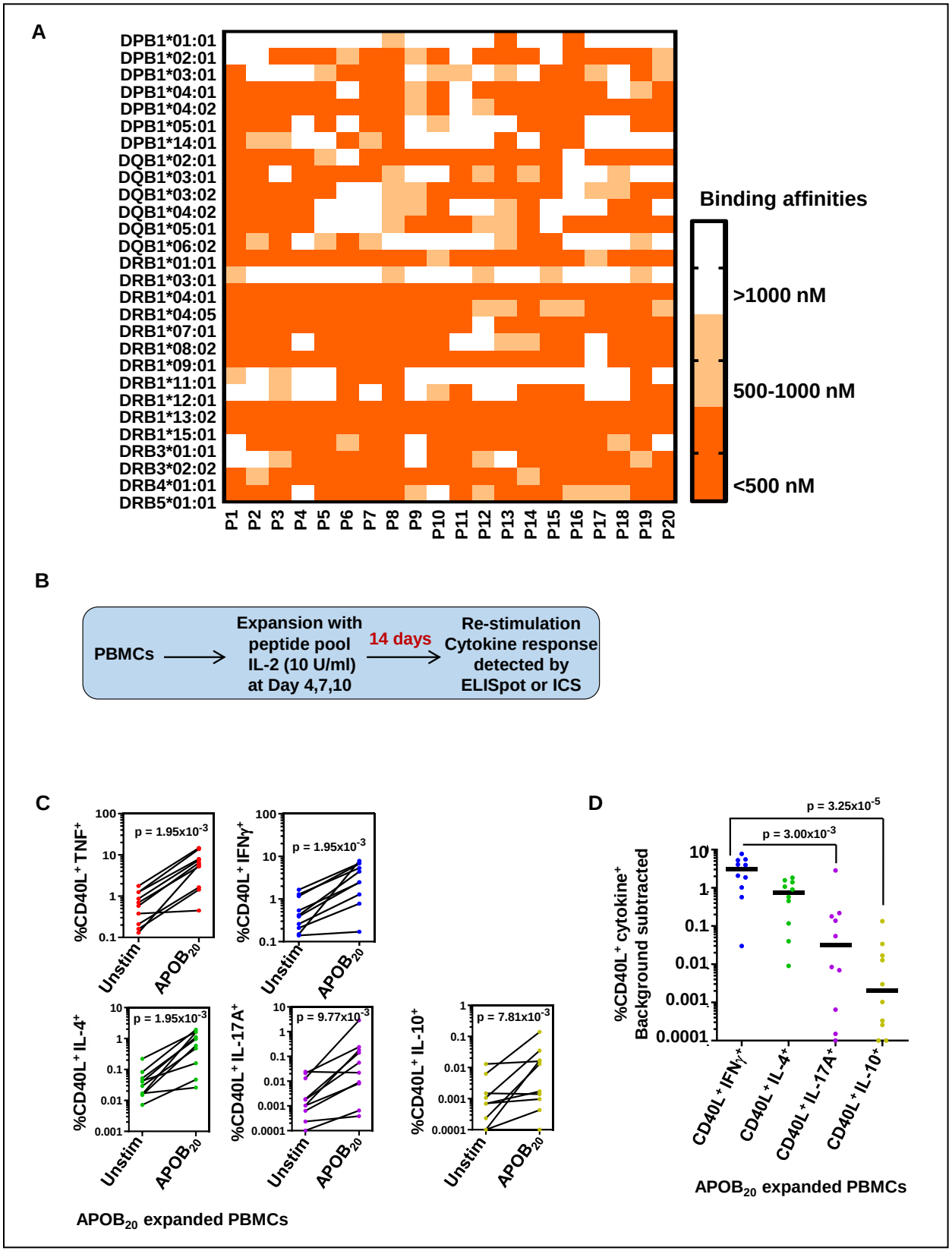


Figure S1: MHC-II binding of $APOB_{20}$ peptides and cytokine analysis in expansion-based re-stimulation assays. A) Binding affinities (nM), as assessed in a competition binding assay, of *in silico* predicted peptides to a reference set of most common human HLA Class II alleles. Non-binding: affinities >1000 nM. **B)** Schematic workflow for antigen-dependent expansion of PBMCs and subsequent re-stimulation-based detection of cytokine producing antigen-specific T cells. **C) and D)** PBMCs from ten independent donors were expanded with $APOB_{20}$ pool and cytokine responses in 6h $APOB_{20}$ restimulated and in unstimulated control cells were assessed using FACS-based ICS assay. Cytokine producing cells were gated on singlets, live, $dump^-$, $CD3^+$, $CD8^-$, $CD4^+$, $CD40L^+$ T cells. **C)** $\%CD40L^+cytokine^+CD4^+$ T cells in paired sets of $APOB_{20}$-restimulated and unstimulated PBMCs. Th cytokines TNF, IFNγ, IL-4, IL-17A and IL-10 responses are shown. **D)** Median frequencies of $APOB_{20}$-induced Th1 (IFNγ), Th2 (IL-4), Th17 (IL-17A), Treg or Tr1 (IL-10) production in $CD40L^+CD4^+$ activated T cells. Y-axis (**C and D**) log10 transformed (data points with 0 or negative values collapsed onto the minimum value on the scale). Colored symbols represent data from individual donors. Pairwise statistical comparisons **(C)** were performed with the Wilcoxon test. Statistical tests across multiple cytokine responses (**D**) were performed using Kruskal-Wallis test with Dunn's multiple comparison testing.

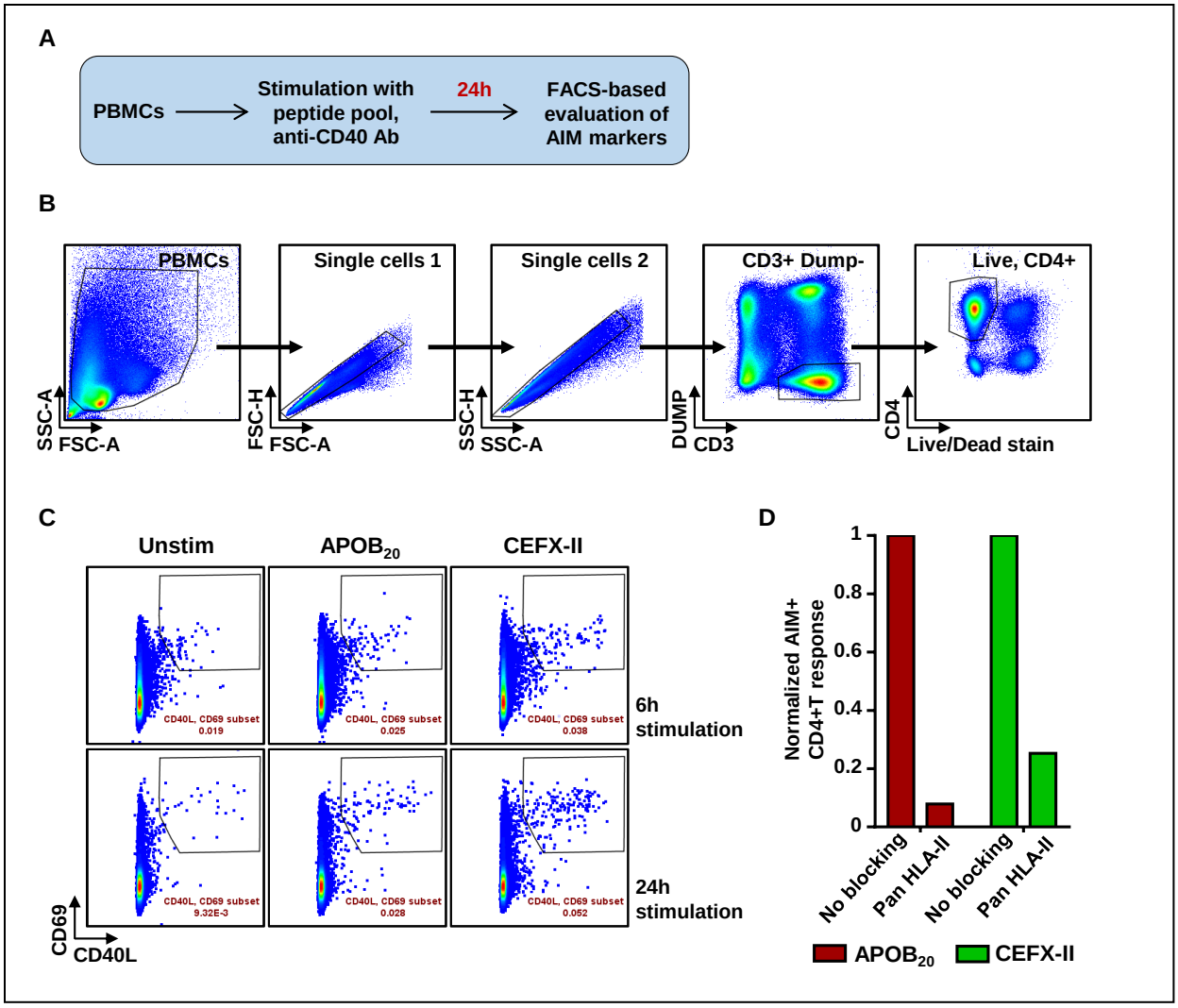
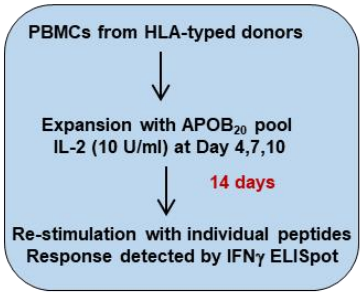


Figure S2: Workflow for AIM assay. A) Schematic workflow for the 24h AIM assay. **B)** Example of flow cytometry gating strategy to designate $CD4^+$T cells for AIM marker evaluation in *ex vivo* stimulated PBMCs. **C)** Representative FACS plots showing $\%CD40L^+CD69^+$ (AIM^+) $CD4^+$ T cells after 6h and 24h of stimulation with $APOB_{20}$ or CEFX-II pools. D) Normalized values of background subtracted $\%CD40L^+CD69^+$ (AIM^+) $CD4^+$ T cells in PBMCs stimulated under conditions of no HLA-II block and pan HLA-II blocking.

A



B

Peptide	Original Sequence	Scrambled Sequence
2	TLTAFGFASADLIEI	ATDIETSAFGIFALL
4	VEFVTNMGIIIPDFA	GFEIIDIVAMNPTFV
5	VGSKLIVAMSSWLQK	QSKSWGIVKMLSVAL
11	LIINWLQEALSSASL	AINSLEAQLLSSLWI
12	LEVLNFDFQANAQLS	VANALNEFLQSLQFD
17	ILFSYFQDLVITLPF	QFTLVYLPFFDLSII

C

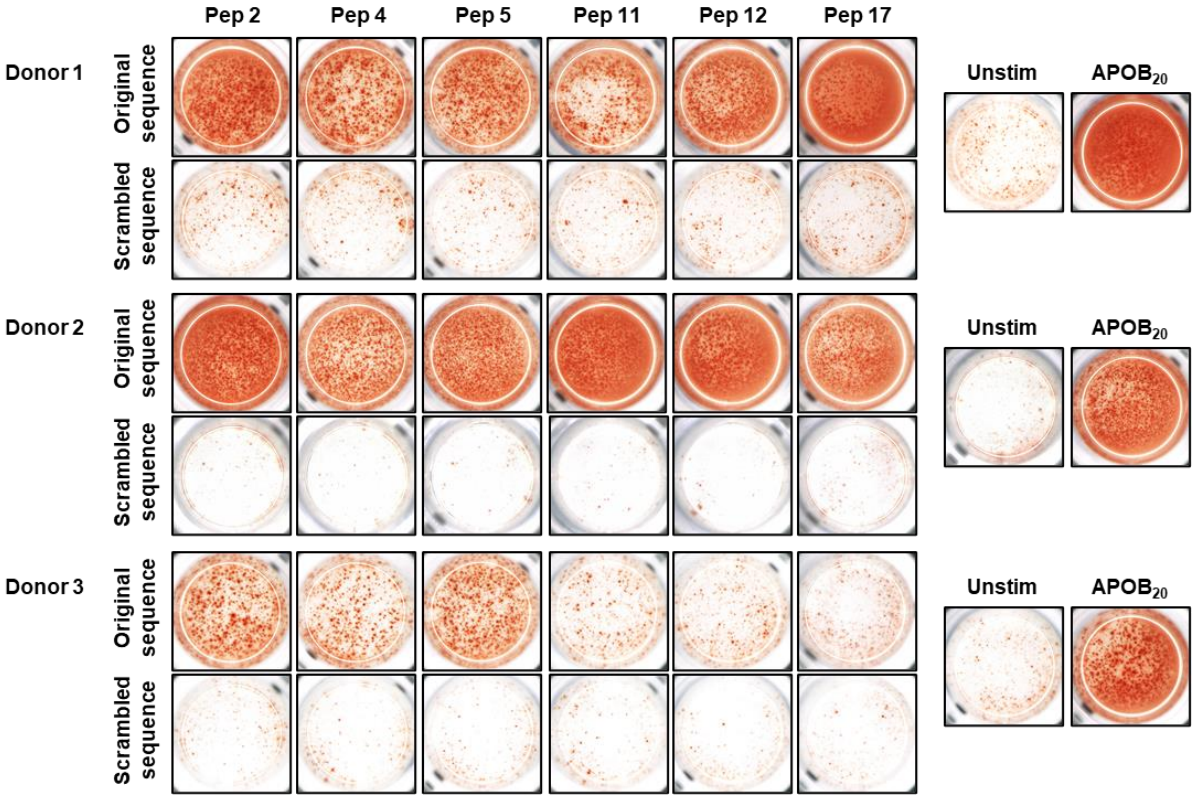


Figure S3: Deconvolution and IFNγ responses to APOB epitopes using original and scrambled peptide sequences. A) Schematic workflow for deconvolution of responses from $APOB_{20}$ pool to individual peptides. **B)** Amino acid sequences of original and scrambled versions of the six dominant APOB peptides. **C)** Representative ELISpot wells showing IFNγ responses in PBMCs restimulated with either the original or the scrambled APOB peptides (left). Unstimulated negative controls and $APOB_{20}$ pool-stimulated positive control PBMCs are shown (right).

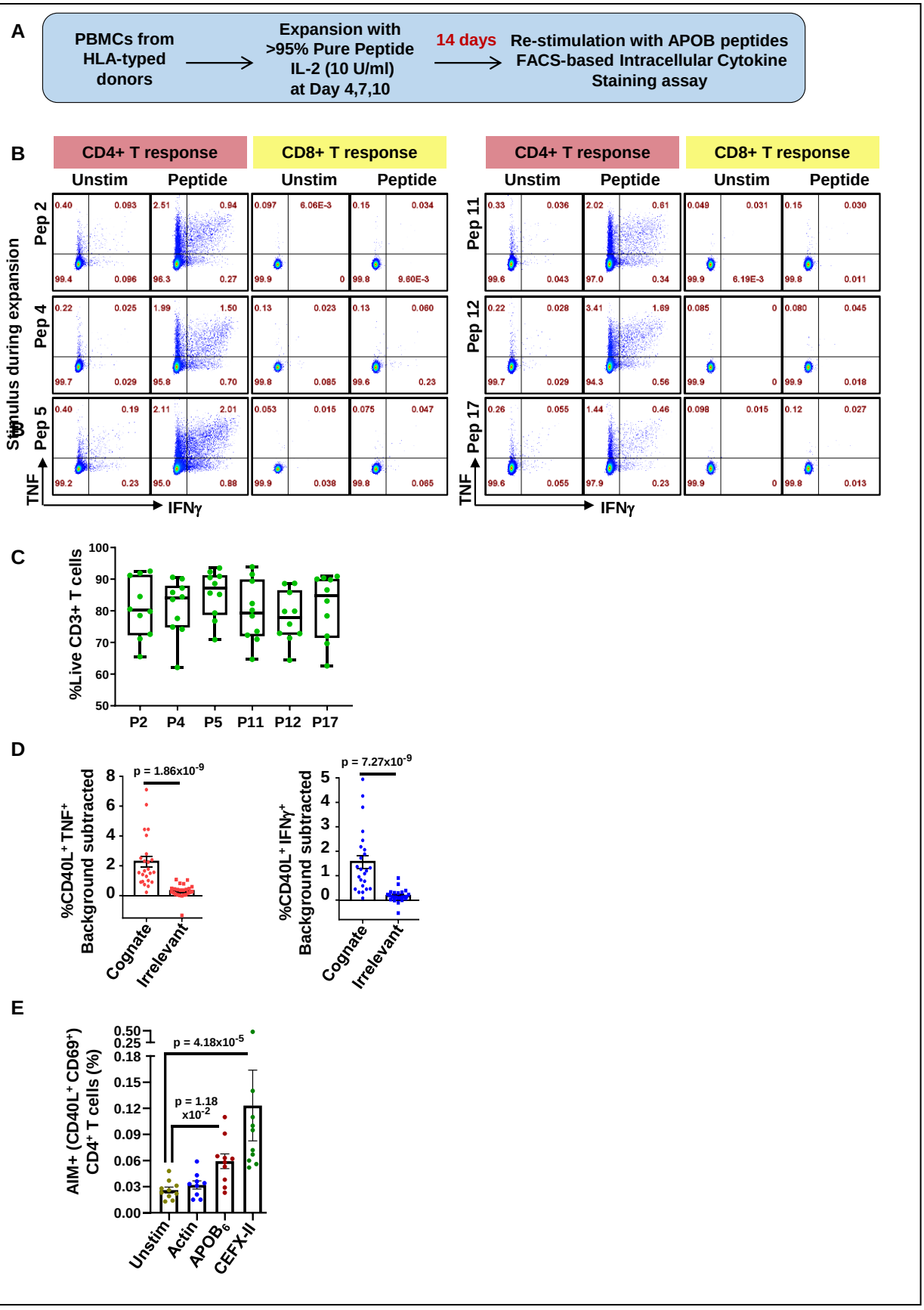
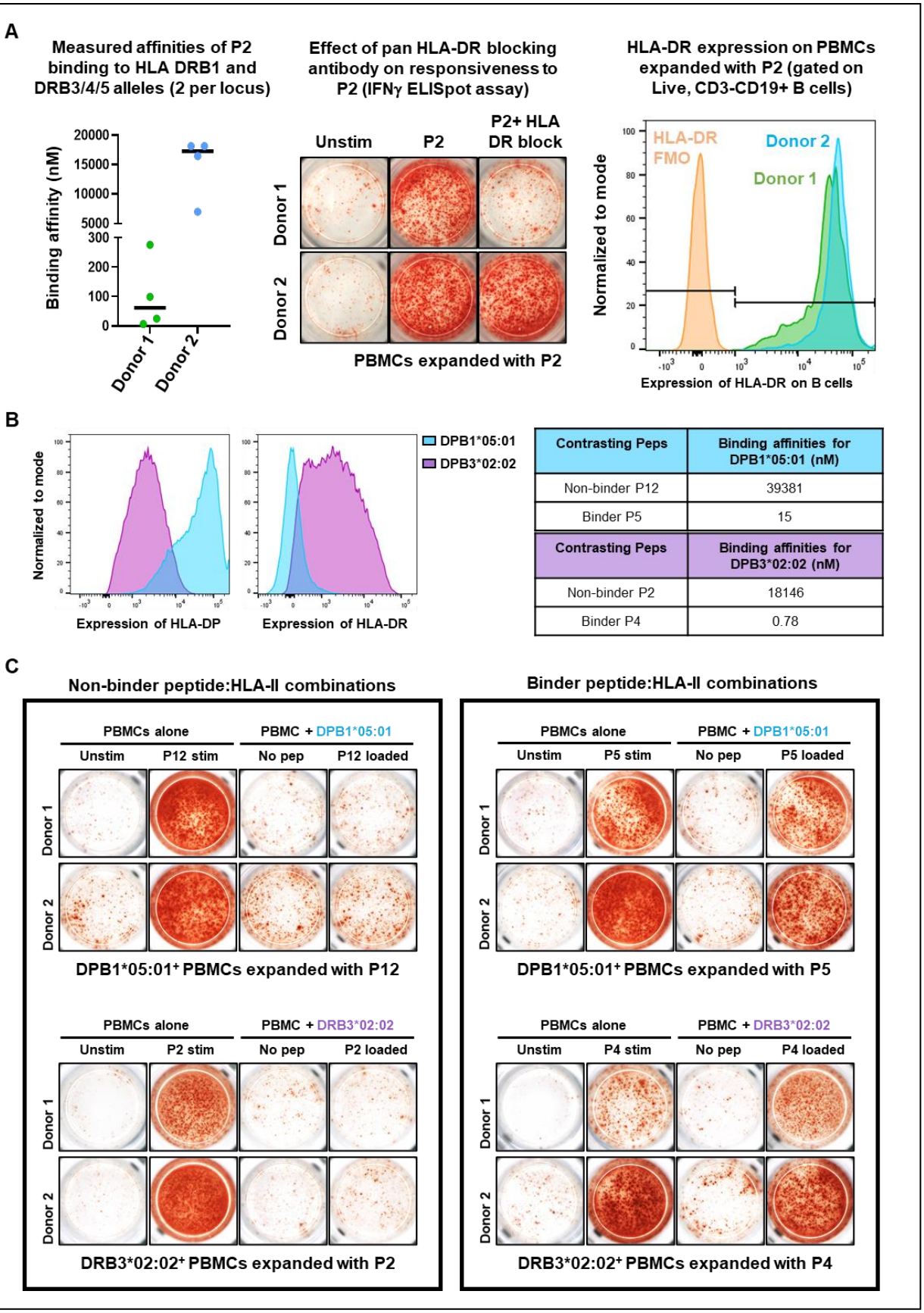

A
PBMCs from HLA-typed donors
Expansion with >95% Pure Peptide IL-2 (10 U/ml) at Day 4,7,10
14 days
Re-stimulation with APOB peptides FACS-based Intracellular Cytokine Staining assay
B
CD4+ T response
CD8+ T response
Unstim
Peptide
Stimulus during expansion
Pep 2
Pep 4
Pep 5
Pep 11
Pep 12
Pep 17
TNF
IFNγ
C
%Live CD3+ T cells
P2
P4
P5
P11
P12
P17
D
%CD40L+ TNF+ Background subtracted
p = 1.86x10-9
Cognate
Irrelevant
%CD40L+ IFNγ+ Background subtracted
p = 7.27x10-9
E
AIM+ (CD40L+ CD69+) CD4+ T cells (%)
p = 4.18x10-5
p = 1.18 x10-2
Unstim
Actin
APOB6
CEFX-II

Figure S4: Validation of responses to dominant epitopes using flow cytometry-based assays. **A)** Schematic workflow for expansion and subsequent re-stimulation with individual peptides. Cytokine responses were evaluated using flow cytometry-based ICS assay. **B)** Cytokine producing cells were gated on singlets, live, dump$^-$, CD3$^+$, CD4$^+$ or CD8$^+$ T cells. Representative FACS plot showing %TNF$^+$IFNγ$^+$, %TNF$^+$IFNγ$^-$ and %TNF$^-$IFNγ$^+$ populations among CD4$^+$ and CD8$^+$ T cell subsets in unstimulated and individual peptide stimulated PBMCs. **C)** Box plots showing median frequencies of Live CD3$^+$ T cells present in PBMCs expanded and re-stimulated with individual epitopes. Live cells were gated on CD3$^+$ T cells that stained negative for the fixable live/dead dye. Ten independent donors. **D)** Each epitope-specific expanded PBMC set was re-stimulated with the cognate peptide or an irrelevant negative control peptide. Cytokine producing cells were gated on singlets, live, dump$^-$, CD3$^+$, CD8$^-$, CD4$^+$, CD40L$^+$ T cells. Background (responses in unstimulated set) subtracted frequencies of %CD40L$^+$cytokine$^+$CD4$^+$T cells in cognate and irrelevant peptide stimulated sets are shown for TNF and IFNγ. Combined data from four independent donors, with six independent peptide-expanded sets per donor. **E)** %CD40L$^+$CD69$^+$ (AIM$^+$) CD4$^+$ T cells in unstimulated and Actin (JPT) or $APOB_6$ (JPT) or CEFX-II (JPT) pool stimulated PBMCs. CD4$^+$ T cells were gated on singlets, live, dump$^-$, CD3$^+$ cells. Ten independent donors. Colored symbols represent data from individual donors. Bars represent mean values with standard error of mean (SEM) shown. Statistical tests between mean responses to cognate and irrelevant peptide (**D**) were done using Mann-Whitney test. Average responses to different peptide pools were compared to unstimulated controls **(E)** using the Kruskal-Wallis test with Dunn's multiple comparison testing.



Contrasting Peps	Binding affinities for DPB1*05:01 (nM)
Non-binder P12	39381
Binder P5	15

Contrasting Peps	Binding affinities for DPB3*02:02 (nM)
Non-binder P2	18146
Binder P4	0.78

Figure S5: Determining associations between donor HLA-II expression, peptide-HLA binding affinities and immunogenicity of APOB epitopes. A) PBMCs from two donors were expanded with APOB peptide P2. Left panel shows the binding affinities of donor HLA-DR alleles (at loci DRB1 and DRB3/4/5) for P2, as determined in our competition binding assay. Middle panel shows IFNγ responses in PBMCs expanded and restimulated with P2, either alone or in the presence of a pan-HLA-DR blocking antibody. Right panel is a FACS plot showing surface expression of HLA-DR on live $CD3^-$ $CD19^+$ B cells, the primary antigen presenting cells in PBMCs harvested after P2-dependent expansion. HLA-DR FMO (fluorescence minus one) was used as background control. **B)** Left panel shows FACS plots for surface **e**xpression of HLA-DP and HLA-DR molecules on single HLA-II transfected B cell lines expressing either HLA DPB1*05:01 (blue) or HLA DRB3*02:02 (purple) alleles. Right panel shows binding affinities of two sets of contrasting peptides for these HLA-II alleles. **C)** Immunogenicity of non-binder (left) and binder (right) peptide-HLA-II combinations was assessed in IFNγ ELISpot assay in donors that expressed the allele under study (upper panel: DPB1*05:01, bottom panel: DRB3*02:02). Peptide-expanded PBMCs were incubated with single HLA-II transfected cell lines that were pre-loaded with the cognate peptide. No peptide loaded cell lines were used as background controls. Unstimulated and exogenous cognate peptide treated PBMC alone sets served as negative and positive controls, respectively.

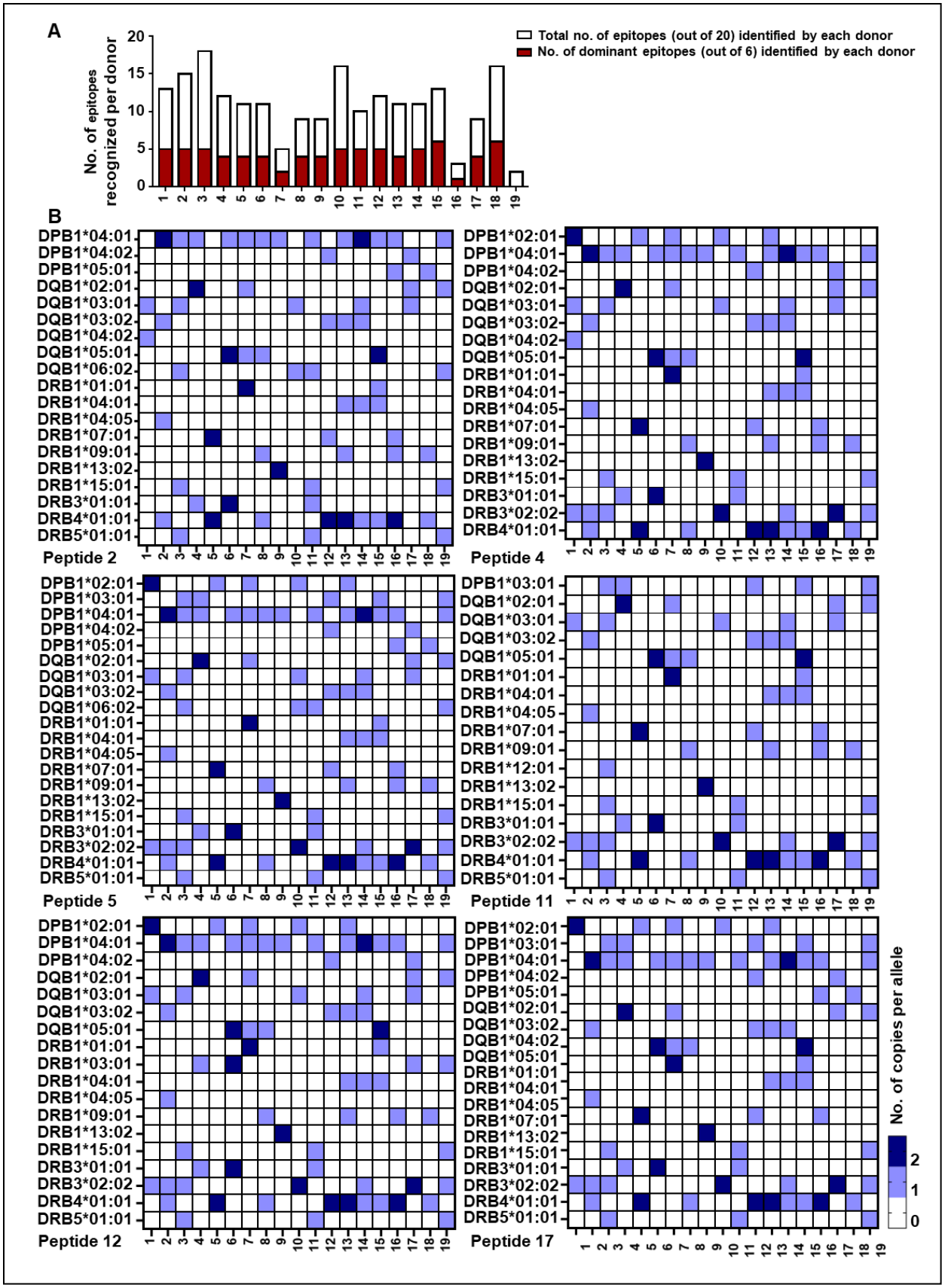

A
No. of epitopes recognized per donor
Total no. of epitopes (out of 20) identified by each donor
No. of dominant epitopes (out of 6) identified by each donor
B
Peptide 2
Peptide 4
Peptide 5
Peptide 11
Peptide 12
Peptide 17
No. of copies per allele

Figure S6: Immunogenicity of dominant APOB epitopes and expression of binder HLA-II alleles examined in individual donors. A) Breadth of response denoted by the total number of epitopes identified by each donor. Red shading in the bars represent numbers of dominant epitopes (P2, 4, 5, 11, 12 or 17) recognized per donor. **B)** Peptide-specific heatmaps showing expressions of binder alleles (rows) in HLA-typed donor (columns) samples examined in the epitope-specific deconvolution assay (Figure 3). White, light purple and dark blue colored boxes denote 0, 1 or 2 copies of each allele, respectively. Nineteen independent donors.

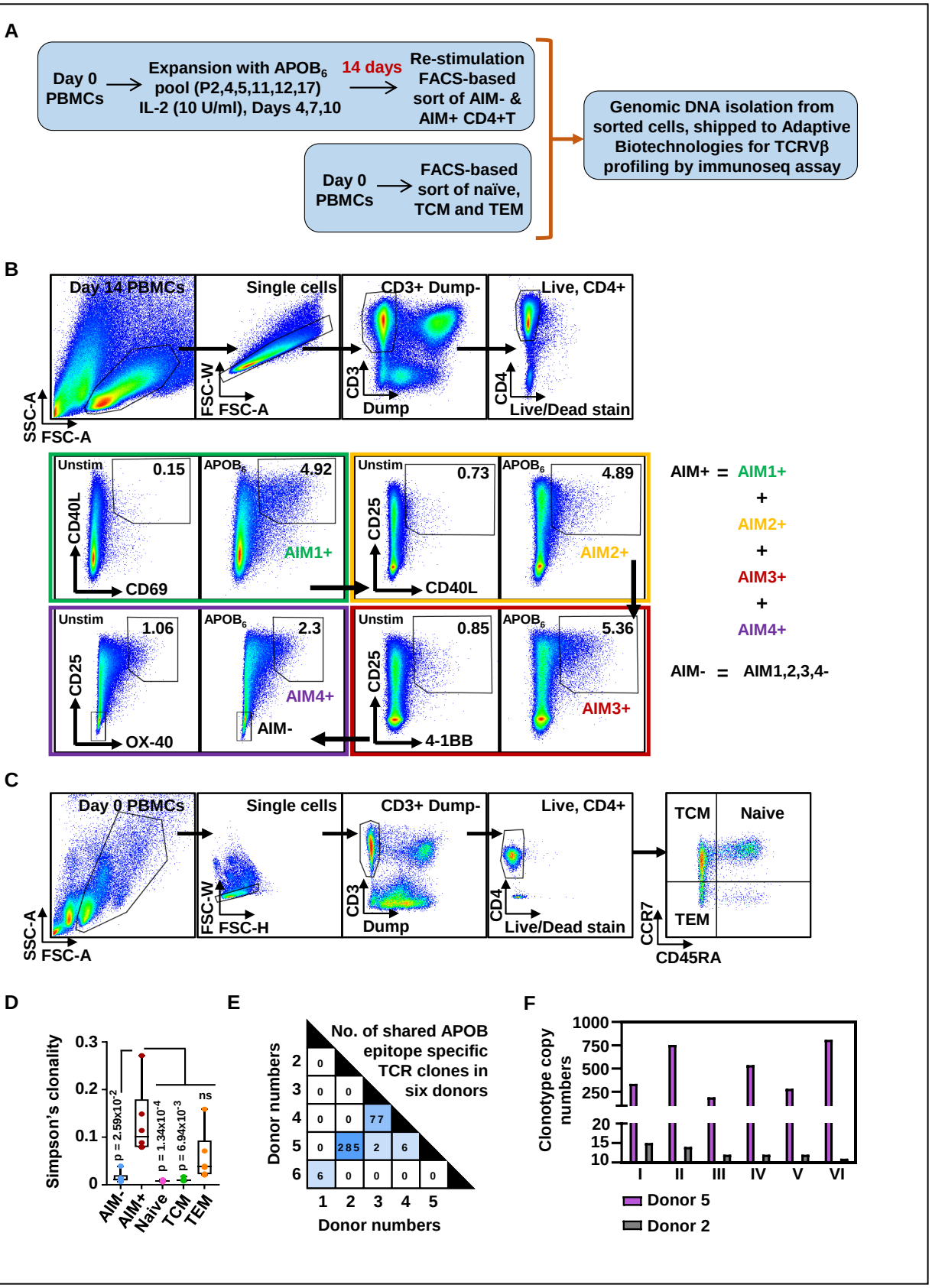

A
Day 0 PBMCs
Expansion with APOB6 pool (P2,4,5,11,12,17) IL-2 (10 U/ml), Days 4,7,10
14 days
Re-stimulation FACS-based sort of AIM- & AIM+ CD4+T
Day 0 PBMCs
FACS-based sort of naïve, TCM and TEM
Genomic DNA isolation from sorted cells, shipped to Adaptive Biotechnologies for TCRVβ profiling by immunoseq assay
B
Day 14 PBMCs
Single cells
CD3+ Dump-
Live, CD4+
SSC-A
FSC-A
FSC-W
CD3
Dump
CD4
Live/Dead stain
Unstim
0.15
APOB6
4.92
CD40L
CD69
AIM1+
Unstim
0.73
APOB6
4.89
CD25
CD40L
AIM2+
Unstim
1.06
APOB6
2.3
CD25
OX-40
AIM4+
AIM-
Unstim
0.85
APOB6
5.36
CD25
4-1BB
AIM3+
AIM+ = AIM1+ + AIM2+ + AIM3+ + AIM4+
AIM- = AIM1,2,3,4-
C
Day 0 PBMCs
Single cells
CD3+ Dump-
Live, CD4+
TCM
Naive
TEM
SSC-A
FSC-A
FSC-W
FSC-H
CD3
Dump
CD4
Live/Dead stain
CCR7
CD45RA
D
Simpson's clonality
0.3
0.2
0.1
0
p = 2.59x10-2
p = 1.34x10-4
p = 6.94x10-3
ns
AIM-
AIM+
Naive
TCM
TEM
E
No. of shared APOB epitope specific TCR clones in six donors
Donor numbers
2
3
4
5
6
0
0 0
0 0 77
0 285 2 6
6 0 0 0 0
1 2 3 4 5
Donor numbers
F
Clonotype copy numbers
1000
750
500
250
20
15
10
I
II
III
IV
V
VI
Donor 5
Donor 2

Figure S7: Schematic, gating strategy and analysis of $APOB_6$-specific TCRβ repertoire. A) Schematic workflow for cell isolation and TCR sequencing. **B)** Example of flow cytometry gating strategy to isolate activated AIM^+ and control AIM^- $CD4^+$ T cells from PBMCs that were expanded and restimulated for 24h with $APOB_6$ pool (P2,4,5,11,12,17). $APOB_6$ expanded but unstimulated sets were used to set the gates for activation marker expression. **C)** Example of flow cytometry gating strategy to isolate naïve, central memory (TCM) and effector memory (TEM) $CD4^+$ T cells from Day 0 samples. $CD4^+$ T cells (in B and C) were gated on singlets, live, $dump^-$, $CD3^+$ cells. **D)** Simpson's clonality measured for productive TCRs from AIM^-, AIM^+, naïve, TCM and TEM $CD4^+$T subsets. Six independent donors. Horizontal bars in box-plots represent median values and whiskers denote min to max data distribution. **E)** Matrix showing Venn diagram analysis of productive TCR clonotypes shared amongst AIM^+ $CD4^+$T cells from six donor PBMCs. **F)** Copy numbers of six productive AIM^+ TCR clonotypes that are shared between donors 2 and 5 and are present in >10 copies in both donors. Colored symbols represent data from individual donors. Statistical comparison of clonality across all subsets (**D**) was done using Kruskal-Wallis test with Dunn's multiple comparison testing.

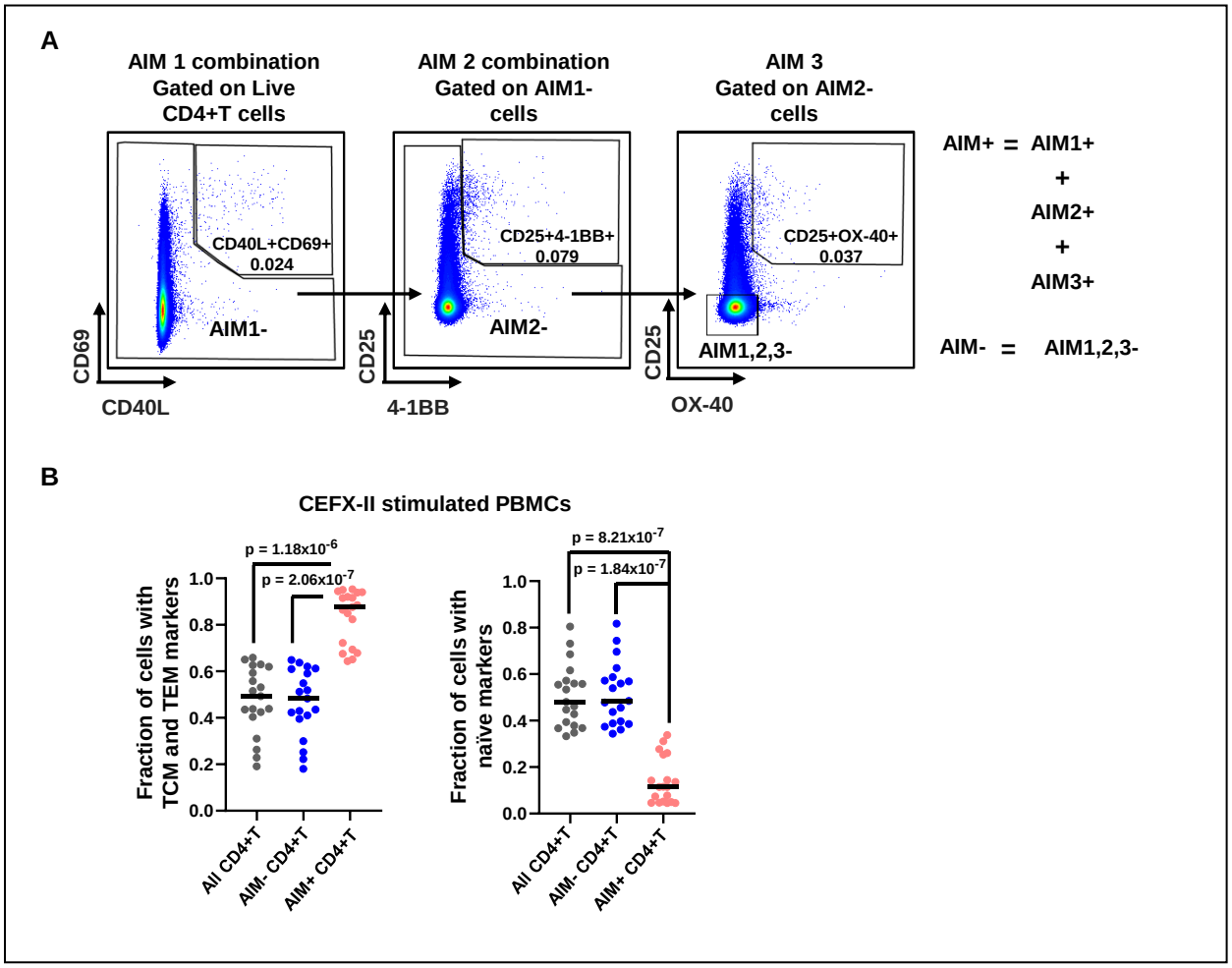


Figure S8: Sequential gating strategy to assess expression of multiple combinations of T cell activation-induced markers (AIM) on peptide stimulated CD4$^+$ T cells. A) Representative FACS plot showing $APOB_6$-induced %AIM$^+$CD4$^+$ T cells gated using sequential gating scheme in a 24h AIM assay. CD4$^+$ T cells were gated on singlets, live, dump$^-$, CD3$^+$ cells. Gates for activation markers were set using unstimulated negative controls. Left, %CD69$^+$CD40L$^+$ (AIM1), gated on CD4$^+$T; middle, %CD25$^+$4-1BB$^+$ (AIM2), gated on AIM1$^-$CD4$^+$T; right, %CD25$^+$OX-40$^+$ (AIM3), gated on AIM2$^-$CD4$^+$T. AIM$^-$ cells did not express any combination of the analyzed AIM markers (AIM1,2,3$^-$). **B)** Median frequencies of naïve and memory (TCM+TEM) fractions within AIM$^+$, AIM$^-$ and all CD4$^+$T subsets in CEFX-II stimulated

PBMCs. Nineteen independent donors. Colored symbols represent data from individual donors. Statistical comparisons across different subsets (**B**) were performed using Kruskal-Wallis test with Dunn's multiple comparison testing.

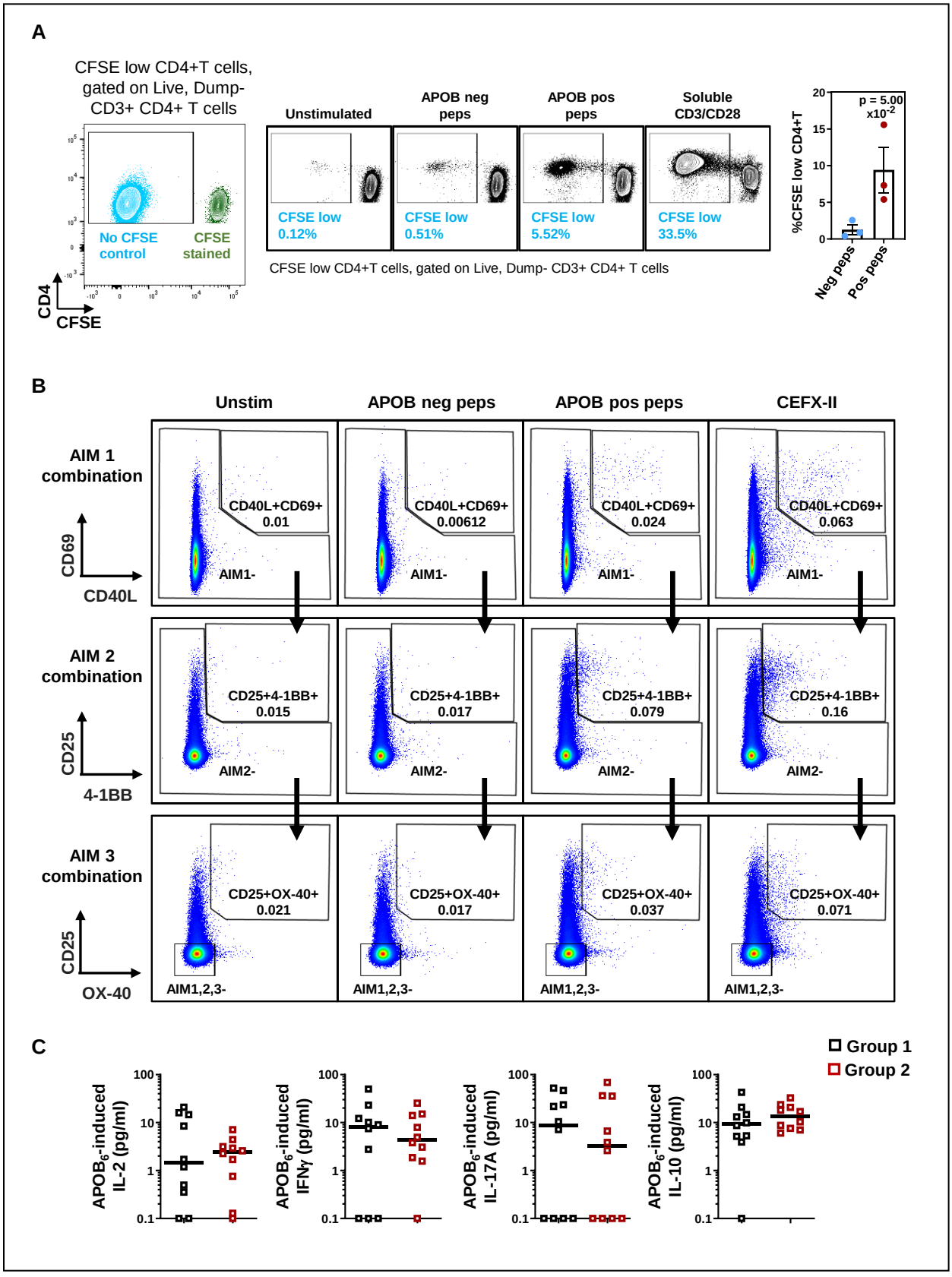

A
CFSE low CD4+T cells, gated on Live, Dump- CD3+ CD4+ T cells
No CFSE control
CFSE stained
CD4
CFSE
Unstimulated
APOB neg peps
APOB pos peps
Soluble CD3/CD28
CFSE low 0.12%
CFSE low 0.51%
CFSE low 5.52%
CFSE low 33.5%
CFSE low CD4+T cells, gated on Live, Dump- CD3+ CD4+ T cells
%CFSE low CD4+T
p = 5.00 x10-2
Neg peps
Pos peps
B
Unstim
APOB neg peps
APOB pos peps
CEFX-II
AIM 1 combination
CD69
CD40L
CD40L+CD69+ 0.01
CD40L+CD69+ 0.00612
CD40L+CD69+ 0.024
CD40L+CD69+ 0.063
AIM1-
AIM 2 combination
CD25
4-1BB
CD25+4-1BB+ 0.015
CD25+4-1BB+ 0.017
CD25+4-1BB+ 0.079
CD25+4-1BB+ 0.16
AIM2-
AIM 3 combination
CD25
OX-40
CD25+OX-40+ 0.021
CD25+OX-40+ 0.017
CD25+OX-40+ 0.037
CD25+OX-40+ 0.071
AIM1,2,3-
C
Group 1
Group 2
APOB6-induced IL-2 (pg/ml)
APOB6-induced IFNγ (pg/ml)
APOB6-induced IL-17A (pg/ml)
APOB6-induced IL-10 (pg/ml)
100
10
1
0.1

Figure S9: Dominant APOB epitopes trigger stronger $CD4^+$T activation and proliferation, as compared to APOB peptides with low reactivity. A) Proliferation of $CD4^+$T cells in response to APOB "neg" and "pos" peps were compared in a CFSE dilution assay. Left, gates were set on CFSE low $CD4^+$T cells using unstimulated controls with and without CFSE staining. Middle, representative FACS plots showing frequencies of CFSE low $CD4^+$T cells in "neg" and "pos" peps stimulated PBMCs. Unstimulated negative controls and soluble CD3/CD28 stimulated positive controls are shown. Right, %CFSE low $CD4^+$T cells (mean with SEM shown). Three independent donors. $CD4^+$ T cells were gated on singlets, live, $dump^-$, $CD3^+$ cells. **B)** Representative FACS plots showing frequencies of $\%AIM^+$ $CD4^+$ T cells (AIM1, 2 and 3 in the serial gating scheme). PBMCs were stimulated for 24h with "neg" or "pos" APOB peptide pools or with CEFX-II pool. $CD4^+$ T cells were gated on singlets, live, $dump^-$, $CD3^+$ cells. **C)** Median levels (pg/ml) of IL-2, IFNγ, IL-17A and IL-10 secreted in response to 24h stimulation with the $APOB_6$ pool. Y-axis (**C**) log10 transformed and data points with 0 or negative values collapsed onto the minimum value on the scale. Groups 1 and 2 each had ten independent donors. Colored symbols represent data from individual donors. Statistical comparison of responses between two APOB pools (**A**) and between two donor groups (**C**) were performed using Mann-Whitney test.

ONLINE TABLES S1-S13

Table S1 - Average phenotypic frequencies of the HLA-II alleles in IEDB's reference set

Locus	Allele	Phenotype frequency
DPB1	DPB1*01:01	16.0
	DPB1*02:01	17.5
	DPB1*04:01	36.2
	DPB1*04:02	41.6
	DPB1*05:01	21.7
	DPB1*14:01	7.4
	Total	94.5
DQB1	DQB1*02:01	11.3
	DQB1*03:01	35.1
	DQB1*03:02	19
	DQB1*04:02	12.8
	DQB1*05:01	14.6
	DQB1*06:02	14.6
	Total	81.6
DRB1	DRB1*01:01	5.4
	DRB1*03:01	13.7
	DRB1*04:01	4.6
	DRB1*04:05	6.2
	DRB1*07:01	13.5
	DRB1*08:02	4.9
	DRB1*09:01	6.2
	DRB1*11:01	11.8
	DRB1*12:01	3.9
	DRB1*13:02	7.7
	DRB1*15:01	12.2
	Total	71.1
DRB3/4/5	DRB3*01:01	26.1
	DRB3*02:02	34.3
	DRB4*01:01	41.8
	DRB5*01:01	16.0
	Total	87.7

Table adapted from Greenbaum *et.al.*, Immunogenetic, 2011.

Table S2 – Details of the twenty APOB peptides used in the study

Peptide Number	Sequence	Start site	No. of HLA-II alleles bound
1	DKRLAAYLMLMRSPS	556	23
2	TLTAFGFASADLIEI	676	21
3	FLHYIFMENAFELPT	826	24
4	VEFVTNMGIIIPDFA	881	18
5	VGSKLIVAMSSWLQK	1226	21
6	IKHIYAISSAALSAS	1836	20
7	HFSNVFRSVMAPFTM	1891	22
8	QLYSKFLLKAEPLAF	1926	27
9	LSQLQTYMIQFDQYI	2171	17
10	HVKHFVINLIGDFEV	2316	21
11	LIINWLQEALSSASL	2491	17
12	LEVLNFDFQANAQLS	2801	18
13	SLFFSAQPFEITAST	3036	23
14	GKIDFLNNYALFLSP	3066	23
15	RGLKLATALSLSNKF	3391	23
16	YKKLRTSSFALNLPT	3771	21
17	ILFSYFQDLVITLPF	4241	17
18	KFTYLINYIQDEINT	4321	19
19	QIHQYIMALREEYFD	4376	25
20	KIVSLIKNLLVALKD	4406	20

Table S3 – Re-stimulation protocols used to detect APOB-specific $CD4^+$T responses

Assay	Restimulation regime	PBMCs per well	Advantage	Disadvantage
Activation Induced Marker (AIM) assay (Flow cytometry Based)	24h stimulation (*ex vivo* or after expansion) in the presence of anti-CD40 antibody to prevent internalization of CD40L	1,000,000 – 2,000,000	Sensitive detection of all antigen-specific T cells irrespective of cytokine-secreting potential, allows isolation of Ag-specific live cells for sequencing	Not suitable for high throughput epitope screening
ELISpot	14 days expansion 20-24h restimulation	100,000 – 200,000	Ideal for high throughput screening of peptides	Limited information (1-2 cytokines)
Intra cellular staining (ICS) (Flow cytometry Based)	14 days expansion 6h restimulation, last 4h with Protein transport inhibitor	1,000,000	Sensitive and multiparametric.	Viable cells of interest not retrievable, not suitable for high-throughput epitope screening

Table S4 – Details of antibodies used in flow cytometry

Group	Antibody	Fluorochrome	Clone	Company	Catalog
Dump channel	Anti-CD8	APC-Cy7	RPA-T8	Biolegend	#301015
	Anti-CD14	APC-Cy7	M5E2	Biolegend	#301819
	Anti-CD16	APC-Cy7	3G8	Biolegend	#302017
	Anti-CD19	APC-Cy7	HIB19	Biolegend	#302217
	Anti-CD41	APC-Cy7	HIP8	Biolegend	#303715
	Anti-CD56	APC-Cy7	HCD56	Biolegend	#318331
T cell markers	Anti-CD3	PerCp-Cy5.5	UCHT1	Biolegend	#300429
	Anti-CD4	Pacific Blue	RPA-T4	Biolegend	#300524
	Anti-CD8	AF700	RPA-T8	Biolegend	#301027
Activation markers	Anti-CD40L	PE	24-31	Thermo Fisher	#12-1548-42
	Anti-CD69	BV650	FN50	Biolegend	#310933
	Anti-CD25	PE-Cy7	BC96	Biolegend	#302611
	Anti-OX-40	APC	Ber-ACT35	Biolegend	#350007
	Anti-4-1BB	BV605	4B4-1	Biolegend	#309821
Memory markers	Anti-CCR7	FITC	REA108	Miltenyi	#130-117-812
	Anti-CD45RA	AF700	HI100	Biolegend	#304119
Cytokines (in ICS)	Anti-TNF	BV650	Mab11	Biolegend	#502937
	Anti-IFNγ	PE-Cy7	4S.B3	Biolegend	#502527
	Anti-IL-4	PE-Dazzle594	MP4-25D2	Biolegend	#500831
	Anti-IL-17A	APC	eBio64DEC17	eBioscience	#17-7179-42
	Anti-IL-10	AF488	JES3-9D7	Biolegend	#501413
Activation (in ICS)	Anti-CD40L	PE	TRAP1	BD	#555700
B cell marker	Anti-CD19	APC-Cy7	HIB19	Biolegend	#302217
HLA-II	Anti-HLA-DR	BV785	L243	Biolegend	#307641
	Anti-HLA-DP	PE	B7/21	BD	#566825
Live/Dead	Fixable Ghost viability dye	BV510	N/A	Tonbo Biosciences	#13-0870-T100

Table S5 – Summary of demographic characteristics of healthy subjects in screening cohort

	AIM assay	**ELISpot assay**	**Peptide-specific ICS assay**	**TCRβ Immunoseq**
Age (years)	22-46 (median = 27, IQR = 12.5)	22-43 (median = 27, IQR = 12)	23-43 (median = 26, IQR = 8)	24-37 (median = 25, IQR = 2)
Male	38.1% (8/21)	36.8% (7/19)	20% (2/10)	33.33% (2/6)
Female	61.9% (13/21)	63.2% (12/19)	80% (8/10)	66.67% (4/6)

Table S6 – HLA Class II alleles of donors in the screening cohort

Donors used in ELISpot-based deconvolution assay										
1	DPB1* 02:01	DPB1* 02:01	DQA1* 04:01	DQA1* 05:01	DQB1* 03:01	DQB1* 04:02	DRB1* 08:11	DRB1* 11:01	DRB3* 02:02	n/a
2	DPB1* 04:01	DPB1* 04:01	DQA1* 01:03	DQA1* 03:01	DQB1* 03:02	DQB1* 06:03	DRB1* 04:05	DRB1* 13:01	DRB3* 02:02	DRB4* 01:01
3	DPB1* 03:01	DPB1* 04:01	DQA1* 01:02	DQA1* 05:01	DQB1* 03:01	DQB1* 06:02	DRB1* 12:01	DRB1* 15:01	DRB3* 02:02	DRB5* 01:01
4	DPB1* 03:01	DPB1* 04:01	DQA1* 04:01	DQA1* 05:01	DQB1* 02:01	DQB1* 02:01	DRB1* 03:01	DRB1* 08:01	DRB3* 01:01	n/a
5	DPB1* 02:01	DPB1* 17:01	DQA1* 01:02	DQA1* 02:01	DQB1* 06:04	DQB1* 06:04	DRB1* 07:01	DRB1* 07:01	DRB4* 01:01	DRB4* 01:01
6	DPB1* 01:01	DPB1* 04:01	DQA1* 01:01	DQA1* 01:01	DQB1* 05:01	DQB1* 05:01	DRB1* 03:01	DRB1* 03:01	DRB3* 01:01	DRB3* 01:01
7	DPB1* 02:01	DPB1* 04:01	DQA1* 05:01	DQA1* 05:01	DQB1* 02:01	DQB1* 05:01	DRB1* 01:01	DRB1* 01:01	n/a	n/a
8	DPB1* 04:01	DPB1* 11:01	DQA1* 01:01	DQA1* 03:01	DQB1* 03:03	DQB1* 05:01	DRB1* 09:01	DRB1* 10:01	DRB4* 01:01	n/a
9	DPB1* 04:01	DPB1* 17:01	DQA1* 01:02	DQA1* 01:02	DQB1* 06:04	DQB1* 06:04	DRB1* 13:02	DRB1* 13:02	DRB3* 03:01	DRB3* 03:01
10	DPB1* 02:01	DPB1* 18:01	DQA1* 01:02	DQA1* 05:01	DQB1* 03:01	DQB1* 06:02	DRB1* 11:01	DRB1* 11:02	DRB3* 02:02	DRB3* 02:02
11	DPB1* 04:01	DPB1* 13:01	DQA1* 01:02	DQA1* 01:03	DQB1* 06:02	DQB1* 06:03	DRB1* 13:01	DRB1* 15:01	DRB3* 01:01	DRB5* 01:01
12	DPB1* 03:01	DPB1* 04:02	DQA1* 02:01	DQA1* 03:01	DQB1* 02:02	DQB1* 03:02	DRB1* 04:03	DRB1* 07:01	DRB4* 01:01	DRB4* 01:01
13	DPB1* 02:01	DPB1* 04:01	DQA1* 03:01	DQA1* 03:01	DQB1* 03:02	DQB1* 03:03	DRB1* 04:01	DRB1* 09:01	DRB4* 01:01	DRB4* 01:01
14	DPB1* 04:01	DPB1* 04:01	DQA1* 03:01	DQA1* 05:01	DQB1* 03:01	DQB1* 03:02	DRB1* 04:01	DRB1* 11:01	DRB3* 02:02	DRB4* 01:01
15	DPB1* 03:01	DPB1* 04:01	DQA1* 01:01	DQA1* 03:01	DQB1* 05:01	DQB1* 05:01	DRB1* 01:01	DRB1* 04:01	DRB4* 01:01	n/a
16	DPB1* 04:01	DPB1* 05:01	DQA1* 02:01	DQA1* 03:01	DQB1* 02:02	DQB1* 03:03	DRB1* 07:01	DRB1* 09:01	DRB4* 01:01	DRB4* 01:01
17	DPB1* 02:02	DPB1* 04:02	DQA1* 05:01	DQA1* 05:01	DQB1* 02:01	DQB1* 03:01	DRB1* 03:01	DRB1* 11:02	DRB3* 02:02	DRB3* 02:02
18	DPB1* 05:01	DPB1* 13:01	DQA1* 01:03	DQA1* 03:01	DQB1* 03:03	DQB1* 06:01	DRB1* 08:03	DRB1* 09:01	DRB4* 01:01	n/a
19	DPB1* 03:01	DPB1* 04:01	DQA1* 01:02	DQA1* 05:01	DQB1* 02:01	DQB1* 06:02	DRB1* 03:01	DRB1* 15:01	DRB3* 02:02	DRB5* 01:01
Donors used in Peptide-specific ICS assay										
1	DPB1* 03:01	DPB1* 04:01	DQA1* 04:01	DQA1* 05:01	DQB1* 02:01	DQB1* 02:01	DRB1* 03:01	DRB1* 08:01	DRB3* 01:01	n/a
2	DPB1* 04:01	DPB1* 11:01	DQA1* 01:01	DQA1* 03:01	DQB1* 03:03	DQB1* 05:01	DRB1* 09:01	DRB1* 10:01	DRB4* 01:01	n/a
3	DPB1* 04:01	DPB1* 13:01	DQA1* 01:02	DQA1* 01:03	DQB1* 06:02	DQB1* 06:03	DRB1* 13:01	DRB1* 15:01	DRB3* 01:01	DRB5* 01:01
4	DPB1* 02:01	DPB1* 04:01	DQA1* 03:01	DQA1* 03:01	DQB1* 03:02	DQB1* 03:03	DRB1* 04:01	DRB1* 09:01	DRB4* 01:01	DRB4* 01:01
5	DPB1* 04:01	DPB1* 04:01	DQA1* 03:01	DQA1* 05:01	DQB1* 03:01	DQB1* 03:02	DRB1* 04:01	DRB1* 11:01	DRB3* 02:02	DRB4* 01:01

6	DPB1* 03:01	DPB1* 04:01	DQA1* 01:01	DQA1* 03:01	DQB1* 05:01	DQB1* 05:01	DRB1* 01:01	DRB1* 04:01	DRB4* 01:01	n/a
7	DPB1* 04:01	DPB1* 05:01	DQA1* 02:01	DQA1* 03:01	DQB1* 02:02	DQB1* 03:03	DRB1* 07:01	DRB1* 09:01	DRB4* 01:01	DRB4* 01:01
8	DPB1* 03:01	DPB1* 04:01	DQA1* 01:02	DQA1* 03:01	DQB1* 03:03	DQB1* 06:02	DRB1* 09:01	DRB1* 15:01	DRB4* 01:01	DRB5* 01:01
9	DPB1* 04:01	DPB1* 13:01	DQA1* 01:01	DQA1* 01:01	DQB1* 05:01	DQB1* 05:01	DRB1* 01:01	DRB1* 01:01	n/a	n/a
10	DPB1* 02:01	DPB1* 02:01	DQA1* 01:02	DQA1* 05:01	DQB1* 03:01	DQB1* 06:02	DRB1* 11:04	DRB1* 15:01	DRB3* 02:02	DRB5* 01:01
Donors used in Immunoseq-based TCR profiling experiment										
1	DPB1* 04:01	DPB1* 11:01	DQA1* 01:01	DQA1* 03:01	DQB1* 03:03	DQB1* 05:01	DRB1* 09:01	DRB1* 10:01	DRB4* 01:01	n/a
2	DPB1* 04:01	DPB1* 13:01	DQA1* 01:02	DQA1* 01:03	DQB1* 06:02	DQB1* 06:03	DRB1* 13:01	DRB1* 15:01	DRB3* 01:01	DRB5* 01:01
3	DPB1* 04:01	DPB1* 04:01	DQA1* 03:01	DQA1* 05:01	DQB1* 03:01	DQB1* 03:02	DRB1* 04:01	DRB1* 11:01	DRB3* 02:02	DRB4* 01:01
4	DPB1* 04:01	DPB1* 05:01	DQA1* 02:01	DQA1* 03:01	DQB1* 02:02	DQB1* 03:03	DRB1* 07:01	DRB1* 09:01	DRB4* 01:01	DRB4* 01:01
5	DPB1* 02:02	DPB1* 04:02	DQA1* 05:01	DQA1* 05:01	DQB1* 02:01	DQB1* 03:01	DRB1* 03:01	DRB1* 11:02	DRB3* 02:02	DRB3* 02:02
6	DPB1* 03:01	DPB1* 04:01	DQA1* 01:02	DQA1* 03:01	DQB1* 03:03	DQB1* 06:02	DRB1* 09:01	DRB1* 15:01	DRB4* 01:01	DRB5* 01:01

Table S7 – Sample details for Day 0 and Day 14 APOB stimulated sets

Day 14_AIM (-)	Input amount (ng)	Total productive templates	Unique rearrangements
Donor 1	400.388	49956	46895
Donor 2	72.412	7206	6731
Donor 3	179.588	21770	16762
Donor 4	221.588	25928	18974
Donor 5	400.332	48987	41250
Donor 6	392.484	45364	33615
Day 14_AIM (+)			
Donor 1	202.76	21697	2082
Donor 2	189.724	18591	3215
Donor 3	179.588	16700	1492
Donor 4	357.724	25796	1741
Donor 5	327.312	31354	2944
Donor 6	400.652	34927	2926
Day 0_Naive			
Donor 1	217.24	30844	29599
Donor 2	108.62	14606	14177
Donor 3	146.276	20152	19594
Donor 4	196.964	25982	25351
Donor 5	107.172	13665	13204
Donor 6	107.172	9506	9224
Day 0_TCM			
Donor 1	215.792	21331	18637
Donor 2	118.76	15076	13188
Donor 3	102.828	13933	11867
Donor 4	127.448	14966	12432
Donor 5	114.412	14668	12681
Donor 6	91.24	11027	9306
Day 0_TEM			
Donor 1	37.656	2457	1853
Donor 2	69.516	5889	4506
Donor 3	47.792	6218	4245
Donor 4	82.552	7937	3325
Donor 5	28.964	2824	1935
Donor 6	39.104	4514	2855

Table S8 – Details of top 10 TCR rearrangements detected in AIM^+ $CD4^+$T cells from individual donors.

Donor 1

Bio-identity (CDR3 AA, resolved VJ genes)	No. of clone-specific productive templates		FDR adjusted p values Fisher's exact test	Log_{10} Odds Ratio
	AIM^- $CD4^+$ T (Total 49956)	AIM^+ $CD4^+$ T (Total 21697)		
CASSTTGLAAYNEQFF +TCRBV19-01+TCRBJ02-01	13	519	$1.74x10^{-247}$	1.97
CASSFVDSPESYEQYF +TCRBV12-X+TCRBJ02-07	11	486	$1.94x10^{-233}$	2.01
CASSLTRIPEQYF +TCRBV28-01+TCRBJ02-07	136	459	$1.63x10^{-123}$	0.90
CSARVTGGGAHTGELFF +TCRBV20-X+TCRBJ02-02	2	422	$1.39x10^{-215}$	2.69
CASSLALGTGDTEAFF +TCRBV05-01+TCRBJ01-01	1	410	$1.55x10^{-211}$	2.98
CASSLAGTGTNEQFF +TCRBV05-01+TCRBJ02-01	1	396	$2.89x10^{-204}$	2.97
CASRGQRDTGELFF +TCRBV19-01+TCRBJ02-02	2	367	$4.90x10^{-187}$	2.63
CASSIRPNDEQFF +TCRBV09-01+TCRBJ02-01	1	366	$1.34x10^{-188}$	2.93
CASSYSSGGGNEQFF +TCRBV06-05+TCRBJ02-01	2	348	$3.75x10^{-177}$	2.61
CASRTIGQGSFNYGYTF +TCRBV27-01+TCRBJ01-02	1	257	$5.35x10^{-132}$	2.78

Donor 2

Bio-identity (CDR3 AA, resolved VJ genes)	No. of clone-specific productive templates		FDR adjusted p values Fisher's exact test	Log_{10} Odds Ratio
	AIM^- $CD4^+$ T (Total 7206)	AIM^+ $CD4^+$ T (Total 18591)		
CASSSLTPEAYGYTF +TCRBV28-01+TCRBJ01-02	2	796	$1.69x10^{-111}$	2.21
CSAMSPSGTGELFF +TCRBV20-X+TCRBJ02-02	0	549	$9.47x10^{-80}$	12.19
CASSDDLAANVLTF +TCRBV13-01+TCRBJ02-06	4	411	$8.59x10^{-53}$	1.61
CASSPRAGGAVREQYF +TCRBV05-08+TCRBJ02-07	0	375	$1.81x10^{-54}$	12.03
CAWSVGGPGANSPLHF +TCRBV30-01+TCRBJ01-06	10	369	$6.38x10^{-40}$	1.16
CASSKVNEKLFF +TCRBV03-01/03-02+TCRBJ01-04	9	361	$6.85x10^{-40}$	1.19
CASSLGGRSNQPQHF +TCRBV05-01+TCRBJ01-05	0	359	$3.53x10^{-52}$	12.01
CSAREPHDTSTDTQYF +TCRBV20-X+TCRBJ02-03	0	338	$3.77x10^{-49}$	11.98
CSASRTYDNQPQHF +TCRBV20-01+TCRBJ01-05	0	326	$2.004x10^{-47}$	11.96
CASSWDRTKFGNQPQHF +TCRBV19-01+TCRBJ01-05	1	268	$3.32x10^{-37}$	2.02

Donor 3

Bio-identity (CDR3 AA, resolved VJ genes)	No. of clone-specific productive templates		FDR adjusted p values Fisher's exact test	Log_{10} Odds Ratio
	AIM^- $CD4^+$ T (Total 21770)	AIM^+ $CD4^+$ T (Total 16700)		
CSARADGQGYGYTF +TCRBV20-X+TCRBJ01-02	26	996	$<2.22x10^{-308}$	1.73
CASSQEVGGDHGYTF +TCRBV04-03+TCRBJ01-02	4	759	$4.73x10^{-270}$	2.41
CASSSSGSNYNEQFF +TCRBV03-01/03-02+TCRBJ02-01	52	597	$1.63x10^{-154}$	1.19
CASIRPGLNYGYTF +TCRBV06-05+TCRBJ01-02	48	436	$6.78x10^{-105}$	1.08
CASSPADREETQYF +TCRBV18-01+TCRBJ02-05	56	410	$6.06x10^{-91}$	0.99
CSARWYNEQFF +TCRBV20-01+TCRBJ02-01	7	349	$1.78x10^{-115}$	1.82
CATSEPGPLNQPQHF +TCRBV24-01+TCRBJ01-05	11	348	$7.96x10^{-110}$	1.62
CASSPLLYSGNTIYF +TCRBV07-02+TCRBJ01-03	0	347	$3.98x10^{-127}$	12.09
CASRTTTGFLSPLHF +TCRBV27-01+TCRBJ01-06	5	273	$3.31x10^{-91}$	1.86
CASSLPRDRDYGYTF +TCRBV06-02+TCRBJ01-02	1	269	$1.91x10^{-96}$	2.55

Donor 4

Bio-identity (CDR3 AA, resolved VJ genes)	No. of clone-specific productive templates		FDR adjusted p values Fisher's exact test	Log_{10} Odds Ratio
	AIM^- $CD4^+$ T (Total 25928)	AIM^+ $CD4^+$ T (Total 25796)		
CASSELGLNTDTQYF +TCRBV06-01+TCRBJ02-03	190	2236	$<2.22x10^{-308}$	1.11
CASSAMVEQPQHF +TCRBV06-X+TCRBJ01-05	503	1709	$1.58x10^{-160}$	0.55
CASSEMGLNTDTQYF +TCRBV06-01+TCRBJ02-03	601	1601	$5.93x10^{-110}$	0.45
CASSPRGAVNTEAFF +TCRBV07-09+TCRBJ01-01	130	950	$3.33x10^{-158}$	0.88
CASSELGTRPNEQFF +TCRBV10-01+TCRBJ02-01	1	558	$9.31x10^{-168}$	2.76
CASRKQGLGYTEAFF +TCRBV02-01+TCRBJ01-01	2	544	$2.28x10^{-161}$	2.45
CASSYRQTNQPQHF +TCRBV06-02/06-03+TCRBJ01-05	0	509	$2.75x10^{-155}$	12.14
CSVLQHEQYF +TCRBV29-01+TCRBJ02-07	153	466	$9.39x10^{-39}$	0.49
CASSPQPSTDTQYF +TCRBV07-08+TCRBJ02-03	11	431	$2.35x10^{-113}$	1.60
CASSVRDYSPLHF +TCRBV07-08+TCRBJ01-06	6	390	$3.93x10^{-108}$	1.82

Donor 5

Bio-identity (CDR3 AA, resolved VJ genes)	No. of clone-specific productive templates		FDR adjusted p values Fisher's exact test	Log_{10} Odds Ratio
	AIM^- $CD4^+$ T (Total 48987)	AIM^+ $CD4^+$ T (Total 31354)		
CASSYGGGPPDTQYF +TCRBV06-06+TCRBJ02-03	6	810	$4.24x10^{-320}$	2.34
CASSQTALYSGNTIYF +TCRBV19-01+TCRBJ01-03	0	777	$8.25x10^{-320}$	12.08
CATALAGGHEQYF +TCRBV06-05+TCRBJ02-07	3	753	$1.07x10^{-302}$	2.60
CSAREGALDNSPLHF +TCRBV20-X+TCRBJ01-06	4	537	$5.0006x10^{-212}$	2.33
CATSDFGKEGDEKLFF +TCRBV24-01+TCRBJ01-04	2	518	$3.10x10^{-208}$	2.61
CASSFTYDEQFF +TCRBV07-09+TCRBJ02-01	16	457	$5.71x10^{-162}$	1.66
CASSPGREPGNTIYF +TCRBV07-09+TCRBJ01-03	179	422	$2.52x10^{-54}$	0.57
CASTATESSPLHF +TCRBV12-03/12-04+TCRBJ01-06	0	415	$1.45x10^{-170}$	11.80
CASRKTGSLYGYTF +TCRBV27-01+TCRBJ01-02	0	411	$6.13x10^{-169}$	11.80
CASSLRTGIINEQFF +TCRBV05-01+TCRBJ02-01	0	383	$1.64x10^{-157}$	11.77

Donor 6

Bio-identity (CDR3 AA, resolved VJ genes)	No. of templates out of all productive templates		FDR adjusted p values Fisher's exact test	Log_{10} Odds Ratio
	AIM^- $CD4^+$ T (Total 45364)	AIM^+ $CD4^+$ T (Total 34927)		
CASSIPQGSGSPLHF +TCRBV05-06+TCRBJ01-06	63	9283	$<2.22x10^{-308}$	2.41
CASSLERGAYGYTF +TCRBV07-02+TCRBJ01-02	2	963	$<2.22x10^{-308}$	2.81
CATSQGVGSGANVLTF +TCRBV15-01+TCRBJ02-06	5	649	$2.52x10^{-225}$	2.24
CSATGGTNEKLFF +TCRBV20-X+TCRBJ01-04	17	500	$9.66x10^{-155}$	1.59
CASRETGGAGELFF +TCRBV06-05+TCRBJ02-02	1	393	$1.05x10^{-140}$	2.71
CASSISLALIYEQYF +TCRBV19-01+TCRBJ02-07	4	371	$3.85x10^{-127}$	2.09
CASSLYAGQNTEAFF +TCRBV07-02+TCRBJ01-01	2	344	$5.41x10^{-121}$	2.35
CASSPDFSGANVLTF +TCRBV05-06+TCRBJ02-06	3	339	$2.19x10^{-117}$	2.17
CASSQTGQGYNEQFF +TCRBV23-01+TCRBJ02-01	13	308	$8.39x10^{-93}$	1.49
CATSEGGRGRGPGELFF +TCRBV24-01+TCRBJ02-02	1	292	$1.84x10^{-103}$	2.58

Table S9 – Comparison of productive frequencies of $APOB_6$ and CEFX-II top 10 TCR rearrangements in $APOB_6$ vs CEFX-II stimulated AIM^+ $CD4^+$T cells from individual donors.

Top 10 APOB clones in Donor 1		
TCR rank	Frequency among all APOB AIM+ productive TCR templates	Frequency among all CEFX-II AIM+ productive TCR templates
APOB_1	0.024	0
APOB_2	0.022	0
APOB_3	0.021	0
APOB_4	0.02	0
APOB_5	0.019	0
APOB_6	0.018	0
APOB_7	0.017	0
APOB_8	0.017	0
APOB_9	0.016	0
APOB_10	0.012	0
Top 10 CEFX-II clones in Donor 1		
TCR rank	Frequency among all APOB AIM+ productive TCR templates	Frequency among all CEFX-II AIM+ productive TCR templates
CEFX-II_1	0	0.05
CEFX-II_2	0	0.04
CEFX-II_3	0	0.02
CEFX-II_4	0	0.018
CEFX-II_5	0	0.018
CEFX-II_6	0	0.015
CEFX-II_7	0	0.015
CEFX-II_8	0	0.015
CEFX-II_9	0	0.013
CEFX-II_10	0	0.013
Top 10 APOB clones in Donor 2		
TCR rank	Frequency among all APOB AIM+ productive TCR templates	Frequency among all CEFX-II AIM+ productive TCR templates
APOB_1	0.043	0
APOB_2	0.03	0
APOB_3	0.022	0.0013
APOB_4	0.02	0
APOB_5	0.02	0
APOB_6	0.0194	0
APOB_7	0.0193	0
APOB_8	0.018	0.03
APOB_9	0.0175	0
APOB_10	0.0144	0

Top 10 CEFX-II clones in Donor 2		
TCR rank	Frequency among all APOB AIM+ productive TCR templates	Frequency among all CEFX-II AIM+ productive TCR templates
CEFX-II_1	0	0.052
CEFX-II_2	0.018	0.03
CEFX-II_3	0	0.029
CEFX-II_4	0	0.0265
CEFX-II_5	0.0136	0.024
CEFX-II_6	0	0.021
CEFX-II_7	0	0.0204
CEFX-II_8	0	0.018
CEFX-II_9	0	0.018
CEFX-II_10	0	0.017
Top 10 APOB clones in Donor 3		
TCR rank	Frequency among all APOB AIM+ productive TCR templates	Frequency among all CEFX-II AIM+ productive TCR templates
APOB_1	0.06	0
APOB_2	0.045	0
APOB_3	0.036	0
APOB_4	0.026	0
APOB_5	0.025	0
APOB_6	0.021	0
APOB_7	0.021	0
APOB_8	0.021	0
APOB_9	0.016	0
APOB_10	0.016	0
Top 10 CEFX-II clones in Donor 3		
TCR rank	Frequency among all APOB AIM+ productive TCR templates	Frequency among all CEFX-II AIM+ productive TCR templates
CEFX-II_1	0	0.06
CEFX-II_2	0	0.054
CEFX-II_3	0	0.045
CEFX-II_4	0	0.036
CEFX-II_5	0	0.028
CEFX-II_6	0	0.021
CEFX-II_7	0	0.017
CEFX-II_8	0	0.015
CEFX-II_9	0	0.013
CEFX-II_10	0	0.011
Top 10 APOB clones in Donor 4		
TCR rank	Frequency among all APOB AIM+ productive TCR templates	Frequency among all CEFX-II AIM+ productive TCR templates
APOB_1	0.087	0
APOB_2	0.066	0.007

APOB_3	0.062	0
APOB_4	0.037	0
APOB_5	0.022	0
APOB_6	0.021	0
APOB_7	0.02	0
APOB_8	0.018	0.002
APOB_9	0.017	0
APOB_10	0.015	0
Top 10 CEFX-II clones in Donor 4		
TCR rank	Frequency among all APOB AIM+ productive TCR templates	Frequency among all CEFX-II AIM+ productive TCR templates
CEFX-II_1	0	0.14
CEFX-II_2	0	0.10
CEFX-II_3	0	0.043
CEFX-II_4	0	0.037
CEFX-II_5	0	0.03
CEFX-II_6	0	0.027
CEFX-II_7	0	0.026
CEFX-II_8	0	0.016
CEFX-II_9	0	0.016
CEFX-II_10	0	0.0154
Top 10 APOB clones in Donor 5		
TCR rank	Frequency among all APOB AIM+ productive TCR templates	Frequency among all CEFX-II AIM+ productive TCR templates
APOB_1	0.026	0
APOB_2	0.025	0
APOB_3	0.024	0
APOB_4	0.017	0
APOB_5	0.0165	0
APOB_6	0.015	0
APOB_7	0.0135	0.0006
APOB_8	0.0132	0
APOB_9	0.0131	0
APOB_10	0.0122	0.019
Top 10 CEFX-II clones in Donor 5		
TCR rank	Frequency among all APOB AIM+ productive TCR templates	Frequency among all CEFX-II AIM+ productive TCR templates
CEFX-II_1	0	0.06
CEFX-II_2	0	0.05
CEFX-II_3	0	0.046
CEFX-II_4	0	0.042
CEFX-II_5	0	0.033
CEFX-II_6	0	0.027
CEFX-II_7	0.012	0.019

CEFX-II_8	0	0.019
CEFX-II_9	0	0.019
CEFX-II_10	0	0.018

Table S10 – Demographic details of healthy donors in the validation cohort

Donor ID	Ethnicity	Race	Gender	Age (yrs)
3865	Hispanic/Latino	Unknown	F	27
3922	Not Hispanic/Latino	Asian	F	35
3992	Not Hispanic/Latino	White	F	66
4010	Hispanic/Latino	White	M	38
4022	Hispanic/Latino	White	F	62
4101	Hispanic/Latino	Unknown	F	25
4108	Not Hispanic/Latino	White	M	29
4109	Hispanic/Latino	White	M	52
4111	Not Hispanic/Latino	White	M	37
4121	Not Hispanic/Latino	White	F	38
4142	Not Hispanic/Latino	White	M	25
4148	Not Hispanic/Latino	White	M	61
4158	Not Hispanic/Latino	Asian	M	21
4161	Not Hispanic/Latino	More	M	27
4164	Not Hispanic/Latino	White	F	23
4166	Hispanic/Latino	Unknown	M	28
4173	Unknown	White	F	58
4181	Hispanic/Latino	More	M	20
4186	Not Hispanic/Latino	Asian	F	20
4187	Not Hispanic/Latino	More	F	20

“More” represents donors from more than one race.

“Unknown” represents donors who declined to report.

Table S11 – HLA-II alleles of donors in the validation cohort

3865	DPB1* 04:01	DPB1* 04:02	DQA1* 01:10	DQA1* 05:01	DQB1* 03:01	DQB1* 06:03	DRB1* 13:01	DRB1* 13:05	DRB3* 01:01	DRB3* 02:02
3922	DPB1* 05:01	DPB1* 15:01	DQA1* 01:03	DQA1* 05:01	DQB1* 03:01	DQB1* 06:03	DRB1* 12:01	DRB1* 13:01	DRB3* 01:01	DRB3* 02:02
3992	DPB1* 04:01	DPB1* 04:01	DQA1* 01:03	DQA1* 05:01	DQB1* 03:01	DQB1* 06:03	DRB1* 13:01	DRB1* 13:03	DRB3* 01:01	DRB3* 01:01
4010	DPB1* 04:01	DPB1* 17:01	DQA1* 02:01	DQA1* 05:01	DQB1* 02:02	DQB1* 03:01	DRB1* 07:01	DRB1* 11:04	DRB3* 02:02	DRB4* 01:01
4022	DPB1* 03:01	DPB1* 06:01	DQA1* 02:01	DQA1* 03:01	DQB1* 02:02	DQB1* 03:02	DRB1* 04:04	DRB1* 07:01	DRB4* 01:01	DRB4* 01:01
4101	DPB1* 04:01	DPB1* 04:02	DQA1* 01:01	DQA1* 01:02	DQB1* 05:01	DQB1* 06:02	DRB1* 01:01	DRB1* 15:01	DRB5* 01:01	n/a
4108	DPB1* 02:01	DPB1* 04:01	DQA1* 01:02	DQA1* 02:01	DQB1* 02:02	DQB1* 06:02	DRB1* 07:05	DRB1* 15:01	DRB4* 01:01	DRB5* 01:01
4109	DPB1* 04:02	DPB1* 04:02	DQA1* 03:01	DQA1* 05:01	DQB1* 03:01	DQB1* 03:02	DRB1* 04:07	DRB1* 16:02	DRB4* 01:01	DRB5* 02:02
4111	DPB1* 03:01	DPB1* 04:01	DQA1* 04:01	DQA1* 05:01	DQB1* 03:01	DQB1* 04:02	DRB1* 08:01	DRB1* 12:01	DRB3* 02:02	n/a
4121	DPB1* 02:01	DPB1* 03:01	DQA1* 01:02	DQA1* 01:02	DQB1* 02:02	DQB1* 06:02	DRB1* 07:01	DRB1* 13:02	DRB3* 03:01	DRB4* 01:01
4142	DPB1* 04:01	DPB1* 04:01	DQA1* 02:01	DQA1* 03:01	DQB1* 02:02	DQB1* 03:02	DRB1* 04:01	DRB1* 07:01	DRB4* 01:01	DRB4* 01:01
4148	DPB1* 03:01	DPB1* 04:01	DQA1* 01:01	DQA1* 02:01	DQB1* 02:02	DQB1* 05:03	DRB1* 07:01	DRB1* 14:01	DRB3* 02:02	DRB4* 01:01
4158	DPB1* 05:01	DPB1* 05:01	DQA1* 03:01	DQA1* 06:01	DQB1* 03:01	DQB1* 03:03	DRB1* 09:01	DRB1* 12:02	DRB3* 01:01	DRB4* 01:01
4161	DPB1* 02:01	DPB1* 04:01	DQA1* 03:01	DQA1* 03:01	DQB1* 03:01	DQB1* 04:02	DRB1* 04:01	DRB1* 04:10	DRB4* 01:01	DRB4* 01:01
4164	DPB1* 04:01	DPB1* 04:02	DQA1* 01:01	DQA1* 01:02	DQB1* 05:01	DQB1* 06:02	DRB1* 01:01	DRB1* 15:01	DRB5* 01:01	n/a
4166	DPB1* 04:01	DPB1* 04:01	DQA1* 01:03	DQA1* 04:01	DQB1* 04:02	DQB1* 06:03	DRB1* 08:01	DRB1* 13:01	DRB3* 01:01	n/a
4173	DPB1* 03:01	DPB1* 04:01	DQA1* 01:01	DQA1* 02:01	DQB1* 02:02	DQB1* 05:03	DRB1* 07:01	DRB1* 14:16	DRB3* 02:02	DRB4* 01:01
4181	DPB1* 02:01	DPB1* 04:02	DQA1* 03:01	DQA1* 04:01	DQB1* 03:02	DQB1* 04:02	DRB1* 04:04	DRB1* 08:02	DRB4* 01:01	n/a
4186	DPB1* 05:01	DPB1* 13:01	DQA1* 01:01	DQA1* 02:01	DQB1* 02:02	DQB1* 05:01	DRB1* 01:01	DRB1* 07:01	DRB4* 01:01	n/a
4187	DPB1* 03:01	DPB1* 05:01	DQA1* 03:01	DQA1* 06:01	DQB1* 03:01	DQB1* 03:02	DRB1* 04:04	DRB1* 12:02	DRB3* 03:01	DRB4* 01:01

Table S12 – Details of clinical parameters measured in blood samples from donors in the validation cohort

Lipoprotein(a), lipid profile, hsCRP, HbA1c								
Donor	**Lp(a)**	**TC**	**HDL-c**	**LDL-c**	**Non-HDL-c**	**TG**	**hsCRP**	**HbA1c**
3865	< 6	147	77	60	70	51	0.7	5
3922	24	165	46	101	119	91	0.7	5.2
3992	10	218	81	120	137	83	1.6	5
4010	86	207	34	143	173	149	1.4	5.5
4022	11	167	57	86	110	118	0.4	5
4101	24	178	48	115	130	74	4.5	5.1
4108	22	163	51	60	112	261	< 0.3	4.9
4109	<6	183	22	Invalid	161	1033	1.9	6.4
4111	< 6	167	58	96	109	64	0.3	5
4121	< 6	161	63	90	98	41	< 0.3	4.9
4142	114	163	36	89	127	191	0.9	5
4148	<6	244	45	174	199	127	0.8	5.6
4158	< 6	153	59	62	94	158	<0.3	5.2
4161	<6	243	75	152	168	82	<0.3	5
4164	< 6	152	74	68	78	52	1	4.8
4166	17	187	40	128	147	93	0.8	5.3
4173	6	233	65	123	168	227	0.8	5.6
4181	< 6	225	29	Invalid	196	571	1.1	4.8
4186	121	186	70	93	116	117	0.6	5.2
4187	8	161	70	82	91	45	< 0.3	5.1

Lp(a), lipoprotein(a), mg/dL; TC, total cholesterol, mg/dL; HDL-c, high-density lipoprotein cholesterol, mg/dL; LDL-c, low-density lipoprotein cholesterol (calculated), mg/dL; non-HDL-c, total cholesterol minus high-density lipoprotein cholesterol, mg/dL; TG, triglyceride, mg/dL; hsCRP, high sensitivity CRP, mg/L; HbA1c, Hemoglobin A1c, %. LDL-c could not be calculated when TG >400mg/L.

Glucose and electrolytes							
Donor	**Glucose**	**Sodium**	**Potassium**	**Chloride**	**Bicarbonate**	**AGAP**	**Calcium**
3865	95	138	3.9	104	24	10	9
3922	94	135	3.8	101	24	10	8.9
3992	46	139	3.6	99	26	14	9.6
4010	78	145	5.4	103	28	14	9.4
4022	89	139	4.4	101	27	11	9.4
4101	102	139	3.9	100	26	13	10.2
4108	76	146	4	102	29	15	9.9
4109	138	143	4.6	102	29	12	9.5
4111	65	141	3.7	104	25	12	8.8
4121	74	143	3.6	106	25	12	9.4
4142	57	139	3.6	100	25	14	9.8
4148	95	141	4.1	102	27	12	10.2
4158	87	140	4.6	103	28	9	9.8
4161	82	140	3.6	100	27	13	9.5
4164	38	141	4.1	102	28	11	10
4166	89	143	4.2	105	27	11	9.6
4173	71	139	3.9	99	26	14	9.8
4181	79	142	4.8	101	27	14	10
4186	93	142	4.3	103	29	10	10.1
4187	92	143	4.1	105	27	11	9.5

Glucose, mg/dL; Sodium mmol/L; Potassium mmol/L; Chloride mmol/L; Bicarbonate mmol/L; AGAP, Anion Gap mmol/L; Calcium, mg/dL.

Liver and kidney function tests									
Donor	**Tot Prt**	**Albumin**	**Bilirubin**	**AST**	**ALT**	**ALP**	**BUN**	**Creatinine**	**eGFR**
3865	7	4.4	0.23	16	10	90	17	0.78	> 60
3922	6.7	4.5	0.29	11	8	48	10	0.5	> 60
3992	8.1	4.8	0.29	53	58	47	8	0.56	>60
4010	7.6	4.6	0.21	39	43	61	16	1.06	>60
4022	7	4.7	0.27	19	16	88	14	0.69	>60
4101	7.7	5.2	0.22	16	16	113	17	0.76	> 60
4108	7.6	5.2	0.48	20	10	62	12	1.16	56
4109	6.8	4.2	0.33	22	24	74	21	1.05	>60
4111	6.9	4.4	0.17	31	16	76	11	0.74	> 60
4121	6.8	4.6	0.56	12	8	31	9	0.77	> 60
4142	7.1	4.7	0.27	30	38	85	15	0.98	>60
4148	7.3	4.7	0.52	31	43	68	12	0.93	>60
4158	7.4	4.8	1.24	26	21	75	10	0.99	>60
4161	7.7	5.3	0.52	21	20	74	10	0.93	>60
4164	7.3	5	0.54	16	11	80	7	0.83	> 60
4166	7.1	5	0.48	24	29	79	15	0.86	> 60
4173	7.5	4.7	0.38	17	16	110	13	0.67	>60
4181	8.4	5.1	0.4	65	68	101	9	0.84	> 60
4186	7.6	4.8	0.47	14	10	99	14	0.67	> 60
4187	7.2	4.9	1.21	14	10	57	10	0.85	> 60

Tot Prt, Total protein, g/dL; Albumin, g/dL; Bilirubin total, mg/dL; AST, Aspartate aminotransferase, U/L; ALT, Alanine transaminase, U/L; ALP, Alkaline phosphatase, U/L; BUN, blood urea nitrogen, mg/dL; Creatinine, mg/dL; eGFR, estimated glomerular filtration rate based on Creatinine, mL/min.

Table S13 – Characteristics of CAD patients in the CAVA clinical cohort

	Variable Count [%] or Mean [± SD]		
	CAD low severity (n=9)	**CAD high severity (n=9)**	**p-value**
Demographics			
Age (years)	62 [± 6.09]	61 [± 6.43]	0.63
Sex (Male)	9 (100%)	9 (100%)	ns
Race (Caucasian)	7 (77.8%)	9 (100%)	0.13
Ethnicity (Non-Hispanic)	9 (100%)	9 (100%)	ns
Diabetes (Yes)	5 (55.6%)	5 (55.6%)	ns
Current smoker (Yes)	2 (22.2%)	3 (33.3%)	0.60
Former smoker (Yes)	4 (44.4%)	4 (44.4%)	0.82
BMI	34.1 [±9.18]	32.9 [± 6.81]	0.99
BP Systolic	139 [± 22.20]	129 [± 11.36]	0.28
BP Diastolic	77 [± 8.63]	79 [± 16.11]	0.73
Medications			
Statins (Yes)	5 (55.6%)	7 (77.8%)	0.32
Diuretics (Yes)	1 (11.1%)	1 (11.1%)	ns
Beta Blockers (Yes)	3 (33.3%)	7 (77.8%)	0.06
Calcium Channel Blockers (Yes)	1 (11.1%)	0 (0%)	0.30
ACE (Yes)	1 (11.1%)	3 (33.3%)	0.26
ATR (Yes)	0 (0%)	0 (0%)	ns
NSAID (Yes)	7 (77.8%)	8 (88.9%)	0.53
Lab values			
Creatinine (mg/dL)	0.96 [± 0.25]	0.88 [± 0.12]	0.57
hs-CRP (mg/L)	2.86 [± 1.97]	2.23 [± 1.68]	0.54
Total Cholesterol (mg/dL)	150 [± 42.94]	149 [± 47.22]	0.97
Triglyceride (mg/dL)	112 [± 61.83]	150 [± 88.40]	0.54
HDL Cholesterol (mg/dL)	43 [± 8.62]	35 [± 7.84]	0.05
Non-HDL Cholesterol (mg/dL)	107 [± 46.45]	114 [± 42.57]	0.76
LDL Cholesterol (mg/dL)	88 [± 39.01]	89 [± 44.35]	0.86
Glucose (mg/dL)	106 [± 13.40]	115 [± 29.91]	0.97
A1c (%)	6.13 [± 0.95]	6.82 [± 1.72]	0.41
Disease severity			
Gensini Scores	10.6 [± 8.3]	70.9 [± 28.7]	0.003

Statistical comparisons between low and high severity groups of patients were done using the Mann-Whitney test for continuous variables and Chi-square test for categorical variables. The two non-Caucasian subjects in the low severity group are both African-American. Former smoker applies only to those who are not current smokers.

Online legends for data sets in Additional files 1-3

Additional file 1: Analysis of sequence homology between APOB peptides and known epitopes in IEDB database using BLAST and custom Python scripts (PEPMatch) that performed peptide searches allowing for many amino acid residue substitutions

Additional file 2: Analysis report from RATE (Restrictor Analysis Tool for Epitopes) analysis of each dominant APOB peptide/HLA-II allele combination derived from the deconvolution assay in Figure 3.

Additional file 3: Details of TCRVβ CDR3 rearrangements, translated amino acid sequence, resolved VDJ genes, template copy numbers and productive frequencies of all productive TCR rearrangements identified in the Immunosequencing experiment in Figure 4.