Supplemental digital content 1.

Supplemental methods

Intracellular cytokine staining

Two million thawed PBMC were stimulated overnight at 37°C by five HIV-1 peptide pools covering the Gag, Pol and Nef proteins (15mer peptides overlapping by 11 amino acids, with: 1 pool Gag p17, 1 pool Gag p24, 1 pool Gag p2/p6/p7, 1 pool Pol and 1 pool Nef). Control conditions included unstimulated cells (negative control) and cells stimulated with a CEF peptide pool, CMV lysate and SEB (Staphylococcus Enterotoxin B; Sigma Aldrich, St Louis, MO, USA), respectively, as positive controls. PBMC were incubated in the presence of 1 µg/ml of anti-CD28 and anti-CD49d, respectively (BD Biosciences, San Jose, CA, USA), and 10 µg/ml of brefeldin A (Sigma Aldrich, St Louis, MO, USA). After incubation, cells were washed with phosphate-buffered saline (PBS) and stained with fluorochrome-conjugated monoclonal antibodies (anti-CD3 APC, anti-CD4 Pacific Blue, and anti-CD8 APC-H7; all from BD Biosciences, San Jose, CA, USA) and an amine-reactive dye to exclude dead cells (LIVE/DEAD Aqua, Invitrogen, Life Technologies, Carlsbad, CA, USA) for 15 minutes at room temperature. Following cell surface staining, PBMC were washed with wash buffer (PBS - 0.5% bovine serum albumin), fixed with 4% paraformaldehyde for 10 minutes at +4°C, and permeabilized with Perm II Buffer (BD Biosciences, San Jose, CA, USA) for 10 minutes at room temperature. PBMC were then stained with anti-IFN-γ FITC, -TNF-α PE-Cy7 and -IL-2 PE (all BD Biosciences, San Jose, CA, USA) for 30 minutes at room temperature. Following staining, PBMC were washed, re-suspended in PBS and stored at 4°C until analysis. Data were acquired with a LSRII flow cytometer (Becton

Dickinson, Franklin Lakes, NJ, USA). At least 100000 events gated on $CD3^+$ were collected and analyzed with DIVA software, version 6.1.2 (Becton Dickinson, Franklin Lakes, NJ, USA).

Multiplex cytokine measurements

After collection of the supernatants (as described in the text of the manuscript), median fluorescence intensity for each sample was measured with the Bio-Plex 200 system™ (Bio-Rad, Marnes-la-Coquette, France). The Bio-Plex Manager software (version 6.0), incorporating a weighted five-parameter logistic curve-fitting method, was used to calculate cytokine concentrations.

Microarray analysis

Gene transcription in PBMC was assessed after 6- and 24-hour stimulations, respectively, in the same samples as in the cytokine multiplex assay (samples from 12 vaccine recipients at W0 and W14). As in the cytokine multiplex assay, PBMC were stimulated with either i) HIV-LIPO-5; or ii) a pool of 15-mer Gag peptides included in HIV-LIPO-5 (Gag+); iii) a pool of 15-mer Gag peptides not included in HIV-LIPO-5 (Gag-); or iv) no stimulating peptide (unstimulated control). There were 192 post-stimulation samples in total (8 different stimulation conditions per volunteer and time point).

RNA were purified using RNeasy Plus Micro Kit (Qiagen, France) and quantified using a ND-8000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) before being checked for integrity on the 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA). In-vitro transcription was generated from 150 ng of RNA using

Ambion Illumina TotalPrep RNA Amplification Kits (Applied Biosystems/Ambion, Life Technologies, Carlsbad, CA, USA). cRNA was hybridized onto Illumina Human HT-12v4 Expression BeadChips (Illumina, San Diego, CA, USA), following the manufacturer's protocol. The arrays included over 47000 probes targeting approximately 36400 genes, including splice variants and non-annotated gene candidates.

Twenty-nine post-stimulation samples were not analyzed due to RNA Integrity Number (RIN) below 6. Two additional post-stimulation samples were excluded because they did not reach hybridization quality criteria (insufficient number of detected genes and low signal relative to other samples).

Statistical analyses

Analyses of the ICS data were based on the background-subtracted percentages of cytokine-positive cells. Negative values after background-subtraction were censored at zero in graphical representations only. Wilcoxon rank-sum tests were performed on the uncensored distribution to compare the vaccine and the placebo groups after vaccination (W14), using an adaptive false discovery rate (FDR) [1] to adjust for test multiplicity.

Statistical analyses of Luminex data were performed on fluorescence intensity (FI) and concentration (pg/ml). When adequate, data were log-transformed before performing Wilcoxon signed rank tests to ensure a symmetrical distribution of the difference between the two time points. Analyses of FI rather than concentration were considered the primary analyses, since conversion to concentration resulted in a loss of power (increase of data variability, reduction of number of observations). To account for

dependency among statistical tests of different cytokines, we used a dependent false discovery rate [2]. Given the conservative nature of this multiplicity adjustment procedure, dependent FDR-adjusted p values <0.10 were considered statistically significant.

For the analysis of gene transcription, data of the analyzable post-stimulation samples were pre-processed using normal-exponential convolution models, quantile normalization and log2-transformation (neqc function of Limma package with default offset) [3-6]. We first performed a principal variance components analysis to describe sources of variability in the data and to determine the portion of variability due to batch effects [7], and then corrected the data for a chip effect with the non-parametric ComBat method [8], before proceeding to the selection procedure and further analysis as described in the test of the manuscript.

References

1. Benjamini Y, Hochberg Y. On the adaptive control of the false discovery rate in multiple testing with independent statistics. *J Ed Behav Stat* 2000,**25**:60-83.
2. Benjamini Y, Yekutieli D. The Control of the False Discovery Rate in Multiple Testing under Dependency. *Ann Stat* 2001,**29**:1165-1188.
3. Bolstad BM, Irizarry RA, Astrand M, Speed TP. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics* 2003,**19**:185.
4. Shi W, Oshlack A, Smyth GK. Optimizing the noise versus bias trade-off for Illumina whole genome expression BeadChips. *Nucleic Acids Res* 2010,**38**:e204.

5. Smyth GK. Limma: linear models for microarray data. In: *Bioinformatics and computational biology solutions using R and Bioconductor*. Edited by Gentleman R, Carey V, Huber W, Irizarry R, Dudoit S. New York: Springer; 2005. pp. 397-420.

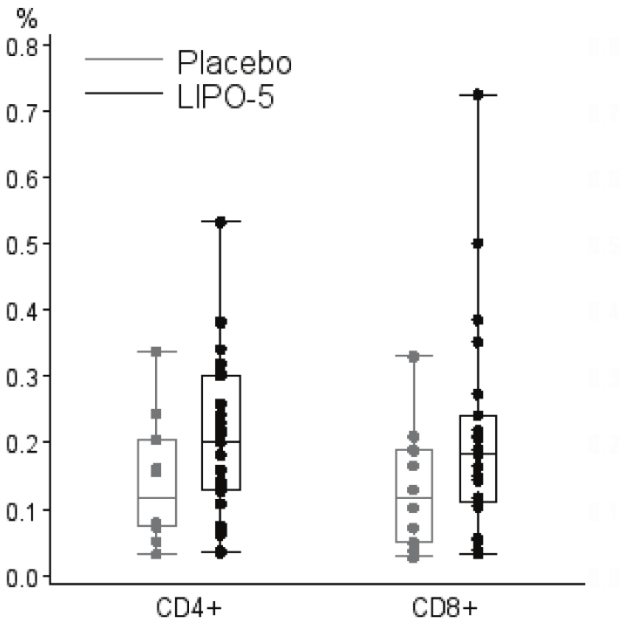
6. Xie Y, Wang X, Story M. Statistical methods of background correction for Illumina BeadArray data. *Bioinformatics* 2009,**25**:751-757.

7. Li J, Bushel PR, Chu TM, Wolfinger RD. Principal Variance Components Analysis: Estimating Batch Effects in Microarray Gene Expression Data. In: *Batch Effects and Noise in Microarray Experiments: Source and solutions*. Edited by Scherer A. Chichester, UK: John Wiley & Sons Ltd; 2009. pp. 141-154.

8. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics* 2007,**8**:118-127.

Supplemental digital content 2.

Figure. Cytokine positive CD4+ and CD8+ T-cells in healthy volunteers vaccinated by HIV-LIPO-5 or placebo.



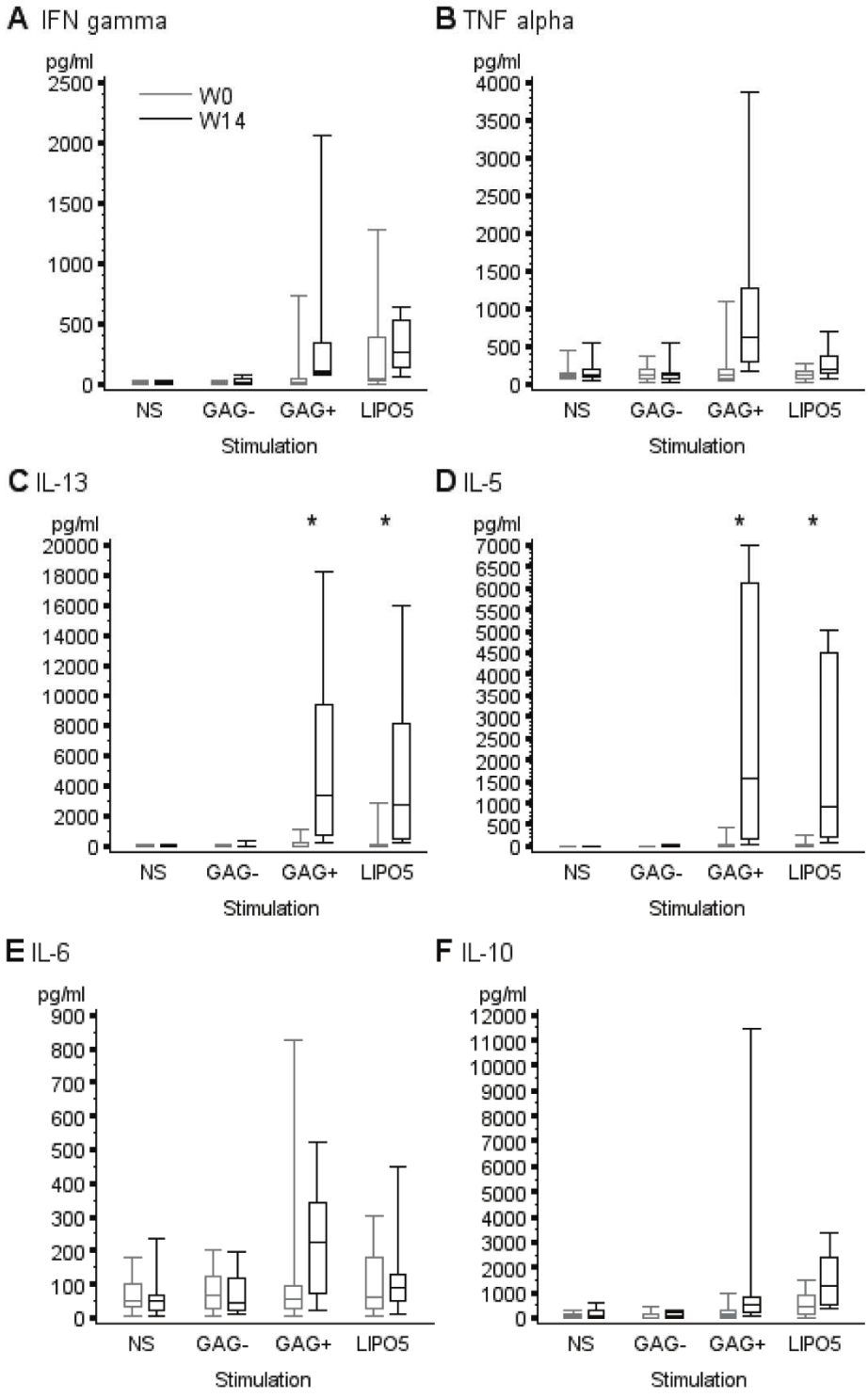
Background-subtracted percentages of CD4+ and CD8+ T-cells, respectively, positive for any cytokine (among IFN-γ, IL-2, TNF-α) measured by intracellular cytokine staining in PBMC from healthy volunteer after vaccination by HIV-LIPO-5 or placebo (W14). Stimulation of PBMC by peptide pools belonging to the HIV-LIPO-5 vaccine. Percentage of positive cells summed over the different peptide pools.

Number of analysed samples: placebo n = 10; HIV-LIPO-5 n = 27.

Boxplots with minimum, 25th percentile, median, 75th percentile and maximum

Supplemental digital content 3.

Figure. Cytokines concentrations in supernatant of stimulated PBMC from volunteers before and after HIV-LIPO-5 vaccination.



* dependent FDR-adjusted p value <0.10.

Cytokine concentration measured by Luminex in supernatant from PBMC from 12 healthy volunteers before (W0) and after (W14) HIV-LIPO-5 vaccination. PBMC stimulated in the following conditions: NS: no stimulation (negative control); GAG-: pool of 15-mers Gag peptides not included in the HIV-LIPO-5 vaccine; GAG+: pool of 15-mers Gag peptides included in the HIV-LIPO-5 vaccine; LIPO5: HIV-LIPO-5. Boxplots with minimum, 25^{th} percentile, median, 75^{th} percentile and maximum.

A: IFN-γ. **B:** TNF-α. **C:** IL-13. **D:** IL-5. **E:** IL-6. **F:** IL-10.

Supplemental digital content 4.

Table. Number of significant probes in PBMC from volunteers before and after HIV-LIPO-5 vaccination.

Incubation duration		Before vaccination (W0)			After vaccination (W14)		
		n (pairs)	p (probes)	# probes	n (pairs)	p (probes)	# probes
6 hours		10	21283		9	20433	
	Significant			**77**			**1223**
	Significant + |FC| >1.5			**44**			**248**
	Significant + |FC| >2			**14**			**34**
24 hours		7	20148		10	21217	
	Significant			**22**			**1415**
	Significant + |FC| >1.5			**22**			**444**
	Significant + |FC| >2			**14**			**100**

Gene expression measured in PBMC from 12 healthy volunteers before (W0) and after (W14) HIV-LIPO-5 vaccination. Two different stimulation durations of PBMC (6 and 24 hours) by HIV-LIPO-5. Gene expression in stimulated PBMC compared to unstimulated PBMC.

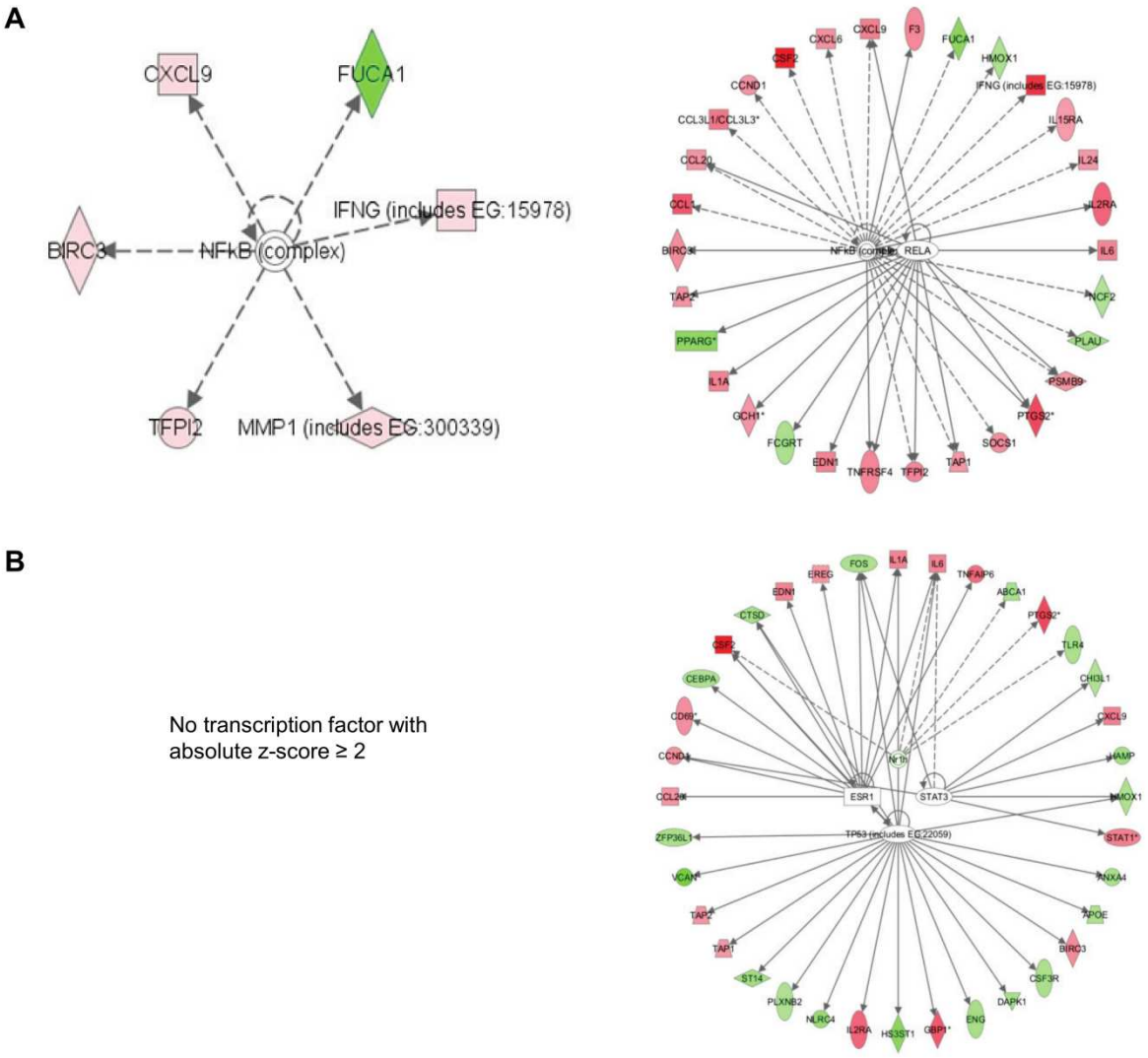
probes: Number of significant probes for each probe expression comparison.

Pre-processed data, corrected for a chip effect, detection p-value selection, paired empirical Bayes moderated t-statistics, adaptive FDR-adjusted p values <0.05, N = 161 post-stimulation samples.

FC: fold change. n: Number of pairs of samples used. p: Number of tested probes.

Supplemental digital content 7.

Figure. Canonical pathway analysis of transcription factors in PBMC from vaccinated volunteers after HIV-LIPO-5 stimulation.



Prediction of transcription factors that may be responsible for gene expression changes observed in PBMC from healthy volunteers vaccinated by HIV-LIPO-5 (W14). Six- (left panel) and 24-hour stimulation (right panels) of PBMC by HIV-LIPO-5. Modulated genes in stimulated PBMC compared to unstimulated PBMC. Pre-processed data, corrected for a chip effect, detection p-value selection, paired empirical Bayes moderated t-statistics, adaptive FDR-adjusted p values <0.05 and fold change |FC| >1.5. Pathway analyses performed with IPA software (Ingenuity).

A: Left panel: activated transcription factor (z-score ≥2) after 6-hour stimulation: NF-kB (z-score = 2.22). Right panel: activated transcription factors (z-score ≥2) after 24-hour stimulation: NF-kB (z-score = 4.25) and RELA (z-score = 2.15).

B: Left panel: inhibited transcription factors (z-score ≤-2) after 6-hour stimulation: none. Right panel: inhibited transcription factors (z-score ≤-2) after 24-hour stimulation: STAT3 (z-score = -2.03), ESR1 (z-score = -2.17), Nr1h (z-score = -2.28) and TP53 (z-score = -2.48).

Supplemental digital content 5.

Table. Top biological functions of modulated genes in vaccinated volunteers after 24-hour stimulation by HIV-LIPO-5.

Functions annotation	z-score	N	Molecules
cell movement of vascular smooth muscle cells	3,3	6	CCL1, CSF2, IFNG (includes EG:15978), IL1A, IL6, PTGS2
differentiation of antigen presenting cells	3,1	10	C1QC, CSF1R, CSF2, IFNG (includes EG:15978), IL1A, IL6, LAIR1, PPARG, TLR4, UBD
activation of mononuclear leukocytes	3,0	8	CD163, CSF2, CTLA4, IFNG (includes EG:15978), IL2RA, IL6, PECAM12RA, IL6, PECAM1, SOCS2
cell movement of endothelial cells	2,9	12	APOE, BMP6, CCL1, CD36, CD9, CSF2, EDN1, ENG, PTGS2, THBS1, TIMP2 (includes G:21858), THBS1,
differentiation of mononuclear leukocytes	2,8	10	CEBPA, CSF1R, CSF2, IFNG (includes EG:15978), IL2RA, IL6, LAIR1, PPARG, TLR4, TNFRSF4
differentiation of leukocytes	2,8	14	C1QC, CEBPA, CSF1R, CSF2, CSF3R, IFNG (includes EG:15978), IL1A, IL2RA, IL6, LAIR1, PPARG,
proliferation of lymphocytes	2,7	19	AIF1 (includes EG:11629), APOE, CD33, CD36, CSF2, CTLA4, EBI3, HMOX1, IDO1, IFNG (includes
development of leukocytes	2,6	6	CEBPA, CSF2, IFNG (includes EG:15978), IL2RA, IL6, TNFRSF4
synthesis of eicosanoid	2,5	9	APOE, COTL1, CSF2, EDN1, IFNG (includes EG:15978), IL1A, IL6, LTA4H, PTGS2
differentiation of dendritic cells	2,5	6	CSF2, IFNG (includes EG:15978), IL1A, LAIR1, TLR4, UBD
migration of endothelial cells	2,5	10	APOE, BMP6, CD9, CSF2, EDN1, ENG, PTGS2, THBS1, TIMP2 (includes EG:21858), VASH1
differentiation of phagocytes	2,5	11	C1QC, CEBPA, CSF1R, CSF2, IFNG (includes EG:15978), IL1A, IL6, LAIR1, PPARG, TLR4, UBD
proliferation of T lymphocytes	2,5	18	AIF1 (includes EG:11629), APOE, CD33, CD36, CSF2, CTLA4, EBI3, HMOX1, IDO1, IFNG (includes
proliferation of mononuclear leukocytes	2,5	20	AIF1 (includes EG:11629), APOE, CD33, CD36, CSF1R, CSF2, CTLA4, EBI3, HMOX1, IDO1, IFNG
differentiation of blood cells	2,4	15	C1QC, CEBPA, CSF1R, CSF2, CSF3R, HIST1H4A (includes others), IFNG (includes EG:15978), IL1A,
synthesis of prostaglandin	2,4	6	APOE, EDN1, IFNG (includes EG:15978), IL1A, IL6, PTGS2
cell movement of smooth muscle cells	2,3	9	APOE, CCL1, CSF2, IFNG (includes EG:15978), IL1A, IL6, PLAU, PTGS2, THBS1
mobilization of Ca2+	2,3	14	C3AR1, CCL1, CCR1, CCR2, CD33, CMKLR1, CSF2, CXCL9, EDN1, IFNG (includes EG:15978), LAIR1,
proliferation of immune cells	2,2	22	AIF1 (includes G:11629), APOE, CCL20, CD33, CD36, CSF1R, CSF2, CSF3R, CTLA4, EBI3, HMOX1, IDO1, IFNG (includes EG:15978), IL2RA, IL6, LAIR1, LILRB2, MNDA, PECAM1, THBS1, TNFRSF4,
binding of polysaccharide	2,2	5	IFNG (includes EG:15978), SULF2, THBS1, TLR4, TNFAIP6

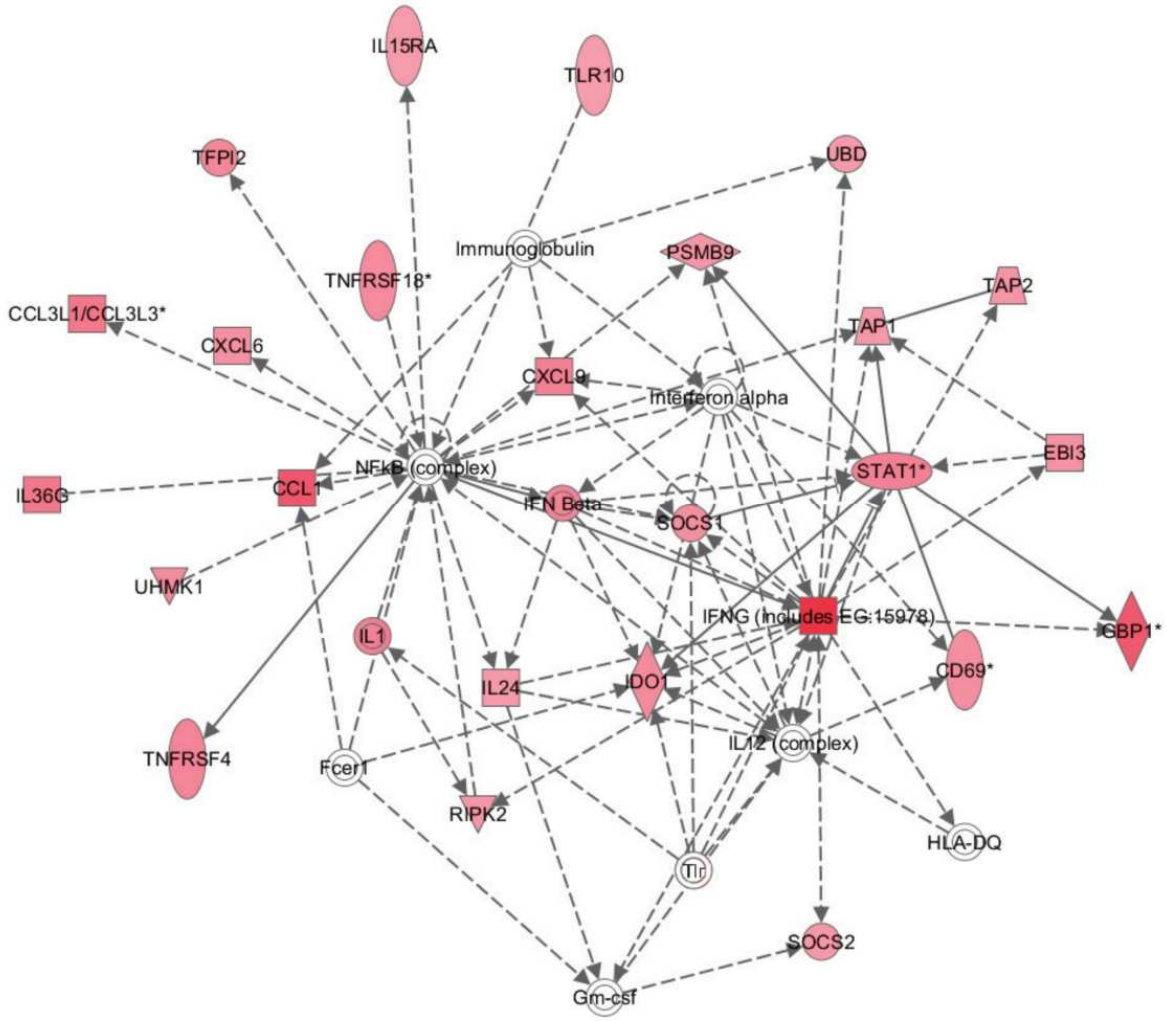
Functions annotation	z-score	N	Molecules
synthesis of fatty acid	2,2	12	ABCA1, APOC1, APOE, COTL1, CSF2, EDN1, IFNG (includes EG:15978), IL1A, IL6, LTA4H, NR1H3,
chemotaxis of endothelial cells	2,2	4	CCL1, CD36, PTGS2, THBS1
development of blood cells	2,2	7	CEBPA, CSF1R, CSF2, IFNG (includes EG:15978), IL2RA, IL6, TNFRSF4
activation of blood cells	2,2	12	APOE, CD163, CSF2, CTLA4, EDN1, IFNG (includes EG:15978), IL2RA, IL6, PECAM1, SOCS2, THBS1,
chemotaxis of leukocytes	2,1	18	AIF1 (includes G:11629), CCDC88A, CCL1, CCL14, CCL20, CCL3L1/CCL3L3, CCR1, CCR2, CMKLR1,
quantity of hematopoietic progenitor cells	2,1	4	CD9, CSF2, IFNG (includes EG:15978), IL1A
inflammatory response	2,1	27	AIF1 (includes EG:11629), 3AR1, CCDC88A, CCL1, CCL14, CCL20, CCL3L1/CCL3L3, CCR1, CCR2, CMKLR1, CSF1R, CSF2, CXCL6, CXCL9, CYBB, FOS, IFNG (includes EG:15978), IL6, LYZ, PLA2G7,
maturation of cells	2,1	10	CCND1, CSF2, IFNG (includes EG:15978), IL6, NR4A2, PPARG, RNASE1, STAT1, THBS1, TLR4
activation of leukocytes	2,1	11	CD163, CSF2, CTLA4, EDN1, IFNG (includes EG:15978), IL2RA, IL6, PECAM1, SOCS2, THBS1, TLR4
stimulation of cells	2,0	6	IFNG (includes EG:15978), IL1A, IL6, LILRB2, THBS1, TLR4
chemotaxis of phagocytes	2,0	16	AIF1 (includes EG:11629), CCDC88A, CCL1, CCL14, CCL20, CCL3L1/CCL3L3, CCR1, CCR2, CMKLR1,
migration of neutrophils	-2,0	5	CCR1, CSF2, IL6, PECAM1, PLAU

Selection of the most important function annotation for gene expression pattern in PBMC from healthy volunteers vaccinated by HIV-LIPO-5 (W14). PBMC stimulated for 24 hours with HIV-LIPO-5 compared to unstimulated PBMC. Pre-processed data, corrected for a chip effect, detection p-value selection, paired empirical Bayes moderated t-statistics, adaptive FDR-adjusted p values <0.05 and fold change $|FC| > 1.5$.

The z-score predicts the direction of change for the function. An absolute z-score ≥ 2 is considered significant. A function is increased if the z-score is ≥ 2 and decreased if the z-score is ≤ -2.

Supplemental digital content 6.

Figure. Functional network of up-regulated genes in PBMC from vaccinated volunteers after 24-hour HIV-LIPO-5 stimulation.



Top functional network for genes that are preferentially up-regulated in PBMC from healthy volunteers vaccinated by HIV-LIPO-5 (W14). PBMC stimulated for 24 hours with HIV-LIPO-5 compared to unstimulated PBMC.

Pre-processed data, corrected for a chip effect, detection p-value selection, paired empirical Bayes moderated t-statistics, adaptive FDR-adjusted p values <0.05 and fold change |FC| >1.5.

Solid line: genes that interact directly. Broken lines: genes that interact indirectly (broken lines). Functionally, the network corresponds to cellular function and maintenance, hematological system development and function and inflammatory response. Shades of red are used to display the level of expression for each gene in the stimulated PBMC compared to unstimulated PBMC.