Distinct Molecular Signature of Human Skin Langerhans Cells Denotes Critical Differences in Cutaneous Dendritic Cell Immune Regulation

Supplementary Material

Detailed experimental procedures

1. Skin dendritic cell isolation and culture

Following dispase (2U/ml, Gibco, UK) digestion of epidermal sheets, migratory DCs were harvested after 48 hours culture of epidermal and dermal fragments, and immediately cryopreserved in 90% FBS (Invitrogen, UK), 10% DMSO (Sigma, UK). Low density cells were enriched using density gradient centrifugation (Optiprep 1:4.2, Axis Shield, Norway (Polak, 2011)) and purified with magnetic beads according to manufacturer's protocol (epidermal cells: CD1a+, dermal cells: CD11c+, Milenyi Biotec, UK). For pulsing with a nominal CD8+ T cell epitope, DCs were incubated with 100 μM of a HLA-A2 restricted EBV-derived peptide (GLC: GLCTLVAML; Cambridge Peptides, UK) for 18 hours, with TNF-α added at 2 hours, and washed thoroughly before co-culture with T cells. For cross-presentation experiments, DCs were pulsed with 10 μM proGLC (FNNFTVSFWLRVPKVSASHLEGLCTLVAML; Peptide Protein Research, Fareham, UK) for 24 hours and supplemented with TNF-α after 6 hours (Polak *et al.*, 2012).

2. EBV-peptide specific T cell expansion

PBMC from HLA-A2 individuals were isolated by Ficoll-Hypaque density gradient centrifugation and co-cultured with 40 μM EBV peptide for 12 days in complete medium supplemented with 1% sodium pyruvate (Gibco, UK) plus 10% human serum (Sigma,UK). IL-2 (100 IU/ml, Peprotech, UK) was added every 3 days. IL-2 was removed from the culture for 24 hours prior to testing in ELISpot. For proliferation assays CFSE (Invitrogen, UK) labelled EBV-specific T cells were re-stimulated with GLC pulsed or un-pulsed CD1a+LC or CD11c+ DDC for 7 days. Prior to flow cytometric analysis, cells were labelled with CD3-PerCP, CD8-APC antibodies (BD Biosciences UK). IFN-γ release from GLC-specific T cells was assessed with ELISpot assay (Mabtech, Sweden). For ELISpot assays, TNF-α matured and washed EBV peptide pulsed DCs ($1x10^3$ cells) were co-cultured with GLC peptide-specific T cells ($5x10^4$ cells/ per well) for 20 hours as per manufacturer's protocol (Mabtech, Sweden). Spot forming units (sfu) were enumerated with ELISpot 3.5 reader. For caveolin-1 blocking assay, cell cultures were supplemented with Filipin III (Sigma, UK) at pre-titrated concentrations (1 μg/ml and 0.1 μg/ml).

3. Alignment of T0 migratory cells transcriptome to transcriptomes of cells isolated from the human skin by trypsinisation.

To test whether the skin migratory LCs and DDCs recapitulate the transcriptome of cells rapidly isolated from skin using trypsinisation protocol followed by bead purification, we conducted a whole transcriptome analysis of existing expression datasets. To date, four such studies were performed, by Santegoets at al [1], Allen at al [2] Hutter at al [3] and Harman et al [4] (Supplementary Table 2). Data sets for analysis were selected based on the following criteria: (i) cell type studied, (ii) microarray genechip platform (Affymetrix human genome expression arrays).

As outlined in Supplementary Table 3 only datasets from study [1] and [2] were suitable for direct comparison with our data, and only study [1] comprised both LCs and DDCs. Raw data (.cel) files from GSE23618 and LC files from GSE16395 and GSE35340 were downloaded and normalized using RMA algorithm within the Affymetrix package, annotated, and taken forward for further analysis. Due to the pronounced study-related batch effect only LCs and DDCs from GSE23618 were compared directly with our migratory cell dataset, while LCs from the study by Allen and colleagues (GSE16395) and Hutter and coleagues (GSE35340) were assayed for the presence of identified gene signatures.

4. Validation of gene expression differences by qPCR

RNA was extracted from bead-purified CD1a+ LCs and CD11c+DDCs from three independent skin samples stimulated with TNF-α (Miltenyi Biotec, UK, 25 ng/ml) for 0, 2, 8 and 24 hours, using Qiagen pico. The quantity and purity of extracted RNA was assessed using a NanoDrop-2000 spectrophotometer and the RNA sample concentration was adjusted to 50 ng/µl. RNA samples, including RT-negative control, were transcribed to cDNA using NanoScript ^{TM} reverse transcription kit (Primer Design, UK). The RT reaction was carried out in a pre-programmed GeneAmp 9700 Applied Biosystems Thermal Cycler (ABI, USA), in a two-step reaction consisting of 5 min at 60°C followed by 60 min at 42°C. PCR was carried out using the TaqMan gene expression assays for target genes: YWHAZ (HS03044281_g1), CAV1 (Hs00971716_m1), PSMD14 (Hs01113429_m1), CCL18 (Hs00268113_m1). qPCR was performed in a small volume 384 well plate assay, using Applied Biosystems 7900HT Fast Real-Time PCR System. Gene expression levels were normalised to housekeeping gene expression (YWHAZ), and expressed as 2^{-dCT} where dCT = target gene expression level – YWHAZ expression level.

Supplementary Figure Legends

Supplementary Figure 1.

(a-c) Mean RMA normalised expression (± SEM) expression of HLA class II and transactivator (CIITA) (a), HLA class I and β-2 microglobulin (B2M) (b) and key co-stimulatory molecules (c) in 48h migratory human skin derived LCs (black bars) and DDCs (grey bars), n=3 independent skin donors in duplicate. No statistically significant differences detected (paired T test, Bonferroni correction).

Supplementary Figure 2.

The comparative heat maps of expression levels of the genes of interest (GOI), identified as gene signature in migratory cells at T0 (> 1.5 fold difference in log2(x) RMA normalised gene expression levels between the cell types) in migratory (a,c) and trypsinised (b,d) LCs vs DDCs. 100 top GOI up-regulated in LC (a,b) and 100 top GOI up-regulated in DDCs (c,d) are shown. Biological processes identified with gene ontology analysis are indicated for the genes enriched in LCs and DDCs.

Supplementary Figure 3.

(a-c) Mean RMA normalised expression of (a) cytokines (b) chemokines (c) co-stimulatory molecules in in LC (black bars) or DDCs (grey bars) during the time course of stimulation with TNF-α as assessed by microarrays, n=3 independent skin donors.

Supplementary Figure 4.

(a-e) RMA normalised expression of (a) genes involved in metabolism (b) cytoskeleton reorganisation and signalling (c) proteasome subunits (d-e) mitochondria associated genes in in LC (black bars) or DDCs (grey bars) during the time course of stimulation with TNF-α as assessed by microarrays, n=3 independent skin donors.

Table S1.

	LC Total (higher in LC)	DDC Total (higher in DDC)
Migratory cells (time 0)	969	1,648
Probesets up-regulated by TNFa	311 (178)	1,007 (874)
Probesets down-regulation by TNFa	201 (128)	1,058 (985)

Table S1. Number of probesets differentially expressed in LCs and DDCs at time 0 and over the time course of stimulation with TNF-α, (inclusion criteria: 1.5 fold difference in $\log_2(x)$ RMA normalised gene expression levels between the cell types (time 0) or between the maximum RMA normalised gene expression level over the $\log_2(x)$ RMA normalised gene expression level at time 0, (for TNF-α regulated genes exceeding 0.05 of Bayesian Estimation of Temporal Regulation).

Supplementary Table S2. Gene ontology analysis for genes overexpressed in migratory LCs and DDCs before stimulation with TNF-α.

LC	
hsa01040: Biosynthesis of unsaturated fatty acids (6.1801) hsa00280: Valine, leucine and isoleucine degradation (3.7081) hsa04520: Adherens junction (2.8252) hsa00640: Propanoate metabolism (4.2488) hsa00072: Synthesis and degradation of ketone bodies (9.0642) hsa00330: Arginine and proline metabolism (3.0784) hsa00071: Fatty acid metabolism (3.3991) hsa00512: O-Glycan biosynthesis (3.6257)	
Surface receptors	*ACVR1, ACVR1C, C21orf63, CALCRL, CD207, CX3CR1, CXADR, CXCR6, GABBR1 / UBD, GPR125, GPR153, GPR55, GPR64, HTR2B, LDLR, LSR, P2RX5, S1PR1, TGFBR3, TNFRSF11A, TNFRSF11B, TNFRSF8, TREML1*
Regulators of signal transduction	*CHN1, CASP6, AGK, ALPK2, ANXA2, ANXA, APBB1IP, ARHGEF12, ARL2, ASB1, AXIN2, BAIAP2, BMP2K, BMPR2, C2orf72, C6orf170, CABYR, CASP3, CDKN2A, CDKN2B, DBI, DEF8, DEPDC6, ECE1, ENSA, FARP1, FARP2, FER, FKBP1A, FMOD, GATSL3 /TBC1D10A, GNAI1, GNG11, IGFBPL1, INADL, LANCL1, LANCL2,*

	MAP3K6, MNAT1, NXN, PHACTR1, PIK3CG, PPAP2A, PPP1R16B, PPP1R1C, PPP2R3A, PRKAR2B, PRKCZ, PTPRK, PTPRM, RAB23, RABGAP1L, RABL3, RABL5, RAD1, RAP1GDS1, RAPGEF4, RARRES3, RHOF, RHPN2, RINL, RRAD, RSU1, SCUBE2, SOX4, STAT1, TBC1D4, TBCK, THEMIS, THOP1, TIFAB, WEE1, WNT5B, TNFAIP8L3, TRAF5
Regulators of transcription/translation	*ASL, POLR1B, SF3A3, SFPQ, SYNCRIP, TSEN54, PRMT7, ARNT2, BATF, BCL11B, C17orf79, CELF4, ELP3, FHL1, FHL2, GTF2H5, HINT2, HMGN3, HOMEZ, IFT57, IKZF2, KDM5B, LMCD1, LZTFL1, MCM2, MEX3D, MKL2, MTA3, MTERFD3, MXD4, MYEF2, NUFIP1, OCIAD2, PASK, PBXIP1, PELP1, PHB, PHTF1, REXO2, RQCD1, SMARCA1, SMARCE1, SP110, SP140, TAF5L, TAF9B, TCF3, TIGD2, TRIM16, TRIM44, TTF2, ZBED3, ZBTB8A, ZC3HC1, ZDHHC16, ZDHHC16, ZG16B, ZMAT3, ZNF195, ZNF260, ZNF268, ZNF287, ZNF362, ZNF428, ZNF480, ZNF544, ZNF593, ZNF608, ZNF642, ZNF643, ZNF75A, ZNF789, ZNF880, ZNF93, ZNHIT6, ZNRF1, ZSCAN2, CPEB1, CPEB2, DTD1, EBNA1BP2, EIF2AK1, ELAC1, GFM2, HNRNPA3 / HNRNPA3P1, IARS, MARS2, NHP2, NOC3L, NPM1, NUP35, PTRH1, FARS2, CDX2, SMARCAD1*
Effectors	*ABCA10, ABCA3, ABCB10, ABCC4, ABP1, ACACA, ACADVL, ACAT1, ACAT2, ACBD6, ACOT1 / ACOT2, ACOT7, ACOX3, ACTB, ACTR3B, ACTR3C, ADAM12, AGA, AGL, AHI1, ALDH18A1, ALDH1B1, ALG2, AMIGO2, ANK3, ANKRD36 / ANKRD36B, ANKRD55, AP1S1, AP2B1, APITD1, APOA1BP, APOC1, APOC2, APOE, ARL2 /SNX15, ASNS, ATIC, ATOX1, ATP1A4, ATP6V0A2, ATXN10, AVEN, BAG2, BARD1, BCKDHB,*

	BDH2 / NHEDC2, BET1, BID, BLMH, BPHL, C10orf35, C11orf2, C11orf41, C14orf1, C14orf126, C14orf145, C18orf10, C19orf12, C1orf115, C3, C4orf23, C4orf34, C6orf105, C6orf108, CAPN3, CASQ1, CBS, CCL22, CD70, CDC14B, CDCA5, CDH1, CETN2, CFL1, CHI3L1, CHL1, CHST2, CLIC3, CLU, CLUAP1, CNN2, COG5, COTL1, CPSF6, CSRP2, CSTA, CTPS, CTTN, CYB561, CYB5R2, CYBASC3, CYFIP2, CYP2U1, DCTPP1, DDA1, DDHD2, DENND1A, DENND5B, DFFA, DHCR24, DMD, DNAJC10, DNAJC21, DNAJC30, DPP3, DSP, EDEM1, EFNB3, ENO3, EPB41L4A, EPCAM, FAM118A, FAM129A, FAM136A, FAM164A, FAM165B, FAM171B, FAM189A1, FAM69A, FANCF, FBXO2, FBXO25, FCGBP, FDPS, FECH, FGGY, FKBP1B, FLOT2, FN1, FNBP1L, FSCN1, FUT8, GALK2, GALNT1, GALNT11, GALNTL4, GCNT1, GCNT2, GCSH, GJA1, GLS, GNPNAT1, GOT1, GSPT1, HEATR1, HEATR2, HLA-DQA2, HMOX2, HSD17B10, HSD17B8, HSPB11, ICAM4, IGSF3, IMPA2, INPP5J, ITGB1BP1, ITIH1, ITM2C, ITPA, KCNMB1, KDELR3, KIF21A, KIF3A, KIF3C, KIF5C, KIFAP3, LAMB1, LIMA1, LIMCH1, LLGL2, LMNB1, LOR, LSP1, MAP4, MARVELD2, MCC, MCOLN2, MCOLN3, MGMT, MGP, MICAL2, MIPEP, MMAB, MMP13, MOCOS, MOSC2, MRPL1, MRPL11, MRPL24, MRPL48, MRPS28, MRPS33, MRPS35, MSH2, MTHFS, MTMR2, MXRA7, MYH9, MYL6B, MYL9, MYO6, NAA10, NACAD, NAV1, NDUFB7, NEK1, NFU1, NID2, NOP16, NOS1, NQO1, NRCAM, NRXN2,OTUD7B, PALLD, PCCA, PDLIM3, PECR, PEX11B, PFAS, PFDN6, PFKM, PFN1, PGM2L1, PIGN, PLA2G16, PLEK2, PLEKHG1, PLP1, PLS3, PNKD, PNPT1, PON2, PPCDC, PRRT3, PSAT1, PSMC3, PXMP4, QDPR, RAD50,

	RARRES2, RIMS3, S100A13, S100B, SAMM50, SCN4B, SEC11C, SEC22C, SEMA4F, SEPT11, SERF1A / SERF1B, SERPINE2, SETMAR, SH3D19, SH3KBP1, SHMT2, SILV, SLAMF7, SLC24A3, SLC25A4, SLC25A43, SLC25A46, SLC38A9, SLC39A13, SLC7A6, SLC9A2, SNRNP25, SNX4, SNX7, SPAG16, SPOCK2, SPTBN1, SQLE, SRR, SSX2IP, ST8SIA1, STARD9, STOML2, STRBP, SWAP70, SYNPO, SYNPO2, SYT11, TAPBPL, TBCB, TBCEL, THBS2, THEM4, TIAM2, TIMM10, TIMM13, TIMM8B, TJP1, TMED4, TMEM110, TMEM136, TMEM14A, TMEM14B, TMEM150C, TMEM160, TMEM163, TMEM169, TMEM200A, TMEM97, TMSB15B, TOMM34, TP53I3, TPM1, TPM3, TPST1, TRAPPC6A, TRIM32, TRO, TRPC1, SPAN13, TSPAN15, TTPAL, TYR, TYRP1, UBE2QL1, UBFD1, UCHL3, UROS, USP46, VCAM1, VPS41, WDR1

DDC

hsa04060: Cytokine-cytokine receptor interaction (3.0069)

hsa04062: Chemokine signaling pathway (2.2980)

hsa04621: NOD-like receptor signaling pathway (5.0827)

hsa04650: Natural killer cell mediated cytotoxicity (2.2617)

hsa04142: Lysosome (2.3261)

hsa04620: Toll-like receptor signaling pathway (2.4110)

hsa04640: Hematopoietic cell lineage (2.6650)

hsa04610: Complement and coagulation cascades (3.1139)

hsa04670: Leukocyte transendothelial migration (1.6995)

hsa04666: Fc gamma R-mediated phagocytosis (1.9601)

hsa04664: Fc epsilon RI signaling pathway (2.2037)

hsa05322: Systemic lupus erythematosus (1.7362)

hsa03320: PPAR signaling pathway (2.2835)

hsa04520: Adherens junction (1.8603)

hsa05219: Bladder cancer (2.7284)

hsa05014: Amyotrophic lateral sclerosis (ALS) (2.1621) hsa00590: Arachidonic acid metabolism (2.0463) hsa05020: Prion diseases (2.8648) hsa00920: Sulfur metabolism (4.7746)	
Surface receptors	*ACVR1B, ADORA2B, ADORA3, ADRB2, AGTRAP, ASGR1, ASGR2, C3AR1, C5AR1, CD14, CD163, CD163L1, CD180, CD209, CD274, CD300A, CD300C, CD300E, CD300LF, CD302, CD33, CD36, CD44, CD48, CD53, CD68, CD72, CD82, CD93, CLEC10A, CLEC12A, CLEC1A, CLEC2B, CLEC4A, CLEC4D, CLEC4E, CLEC4G, CLEC5A, CLEC7A, CMKLR1, CNRIP1, CRISPLD2, CSF1R, EVI2A, EVI2B, F13A1, FABP4, FCAR, FCER1G, FCGR1A, FCGR2A, FCGR3A, FCGRT, FFAR2, FGFR1, FOLR2, FPR1, FPR2, FPR3, GFRA2, GPNMB, GPR109A / GPR109B, GPR155, GPR34, GPR68, GPR84, HAVCR2, HBEGF, HEBP1, INSR, ITGAL, ITGAM, ITGAX, IVNS1ABP, KIR2DL4 /KIR2DL5A, KLRC1, KLRK, LDLRAD3, LEPR, LGALS8, LILRA2, LILRA3, LILRA6, LILRA6 / LILRB3, LILRB1, LILRB2, LILRB4, LILRB5, LPAR2, LPAR6, LYVE1, MARCO, MRC1 /MRC1L1, MRC1L1, MSR1, NKGC2, OLR1, OSBPL1A, P2RX4, P2RX7, P2RY6, PILRA, PLAUR, PTAFR, PTGER2, PTPRE, PTPRJ, RXRA, S1PR2, SCARA5, SCARB2, SCARF1, SIGLEC1, SIGLEC12, SIGLEC14, SIGLEC7, SIGLEC9, SORL1, STAB1, SUCNR1, THRB, TLR1, TLR2, TLR4, TLR5, TLR8, TREM1, TNFRSF10A, TNFRSF10B, TNFRSF10D, TNFRSF14, TNFRSF21, TNFSF12, TNFSF12-TNFSF13 / TNFSF13, TNFSF15,*

	TREM2, VLDLR
Regulators of signal transduction	*ABCA1, ADA, ADM, AK4, ANG, AREG, ARHGAP18, ARHGAP19, ARHGAP22, ARHGAP24, ARHGAP26, ARHGAP6, ARHGAP9, ARHGEF10L, ATP6AP2, AZI2, C13orf15, C13orf18, C19orf61, CALML4, CAMK2D, CARD16, CASP4, CASP5, CDC42EP3, CISH, CLK1, CRIM1, CYTH4, DAPK1, DDIT4, DNMBP, DOK3, DUSP6, ECM1, EMR2, EPS8, EREG, F3, FES, FGD3, FGD4, FLT1, GDF15, GEM, GIMAP4, GIMAP5, GIMAP8, GNA15, GNG4, GRK5, HCST, HIPK2, HPCAL1, INHBA, INPP4A, INPP5D, IQGAP2, IQSEC1, IRAK3, ITGB2, KITLG, KL, LCP2, LPIN1, MAP2K3, MAP3K5, MAPK13, MAPK14, MCTP1, MERTK, MET, MLKL, MOBKL2B, MOBKL2C, MRAS, NOTCH2, NDRG1, NRP1, OSM, PAG1, PDE2A, PID1, PIK3AP1, PLAT, PLAU, PNP, PPAP2B, PPBP, PPP1R3B, PRKCH, PTP4A3, PTPN6, PYCARD, RAB20, RAB24, RAC2, RALGDS, RASA3, RASA4, RBPJ, RCAN1, RGL1, RHOB, RHOH, RHOQ, RHOU, RIN2, RIPK2, RRAGD, SAMHD1, SASH1, SASH3, SDC2, SDCBP2, SGK1, SH3BP2, SH3BP5, SH3PXD2B, SIK3, SLA, SLAMF1, SOCS3, SPRED1, STAC, SYK, TBC1D2, TESK2, TGM2, THBS1, TIMP2, TK1, TNIK, TNS3, TRAF3IP2, TSPAN4, VSIG4, WSB1*

Transcription regulators	*AHRR, BACH1, BCL11A, BCLAF1, BHLHE41, BMP6, C11orf30, CBFA2T3, CCNL1, CEBPA, CEBPB, CEBPD, CITED2, CREB5, DEDD2, DOT1L, E2F3, EAF1, EGR1, EGR2, EHF, EPAS1, FLI1, FOS, FOSB, FOSL2, H3F3B, HIF1A, HIST1H2BN, HOPX, HOXB2, ING2, IRF2BP2, IRF8, JARID2, KLF2, KLF3, KLF9, LRRFIP1, MAF, MAFB, MAFF, MAMLD1, MDM4, MITF, MXD1, MYC, NR4A1, NR4A2, NR4A3, NUPR1, PER1, PRDM8, RFX8, RUNX1, RUNX2, SAP30, SATB1, SCML1, SMAD3, SNRPG, SSBP2, SSBP3, SSR1, TCF7L2, TFEC, TRERF1, TRIM22, TSC22D1, TSC22D3, TSPYL2, TWIST1, TXNIP, VENTX, ZBTB16, ZC3H11A, ZCCHC14, ZCCHC2, ZCCHC6, ZEB2, ZFP36L2, ZMYND8, ZNF331, ZNF395, ZNF467, ZNF503, ZNF697, ZNRF2, ZXDC, ETS2, ID3, ANKHD1, DMXL2, EIF4G3, RPL37, RPL39L*
Effectors	*ABCC3, ABCG1, ABHD5, ACSL1, ACSL5, ACTN1, ADAMDEC1, ADARB1, ADSSL1, AIF1, AKR1C1, AKR1C2, AKR1C3, ALDH1A1, ALDH3B1, ALOX15B, ALOX5, AMPD3, AMY1A / AMY1B / AMY1C, AMY2B, ANGPTL4, ANKH, ANO6, ANPEP, AOAH, APP, AQP9, ARAP1, ARG2, ARID5B, ARSG, ASAH1, ASRGL1, ATG16L2, ATG5, ATP13A3, ATP1B1, ATP6V0D2, ATP8B4, AVPI1, B3GALNT1, BCAT1, BCL2L14, BCL6, BEST1, BIN1, BNIP3, BNIP3L, BTG1, C1QA, C1QB, C1QC, C6orf192, CA12, CACNA2D3, CACNA2D4, CALCOCO2, CAPN5, CAPN7, CARD6, CASP1, CCL13, CCL17, CCL18, CCL19, CCL2, CCL20, CCL3, CCL3L1 / CCL3L3, CCL4 / CCL4L1 / CCL4L2, CCL4L1 / CCL4L2, CCL7, CCNE2, CCR1, CCR5, CCRL2, CDH26, CDKN2D, CECR1, CES1, CFD, CFLAR, CH25H, CHST15, CIDEB, CLDN23, CLN8, CLPB, CMAH, COLEC12, CPM, CPVL, CRYL1,*

	CSGALNACT2, CST3, CTSB, CTSD, CTSK, CTSL1, CXCL1, CXCL12, CXCL13, CXCL2, CXCL3, CXCL5, CXCL5 / GLYR1, CXCL6, CXCR7, CYBB, CYP27B1, CYP2R1, DAB2, DHRS9, DLL1, DNER, DOCK2, DPEP2, DPYD, DRAM1, DST, EEPD1, EGLN3, EHD1, ELOVL7, EMP1, EPB41L3, ERO1LB, FAH, FAIM3, FBP1, FCN1, FKBP15, FMN1, FMNL2, FTH1, FUCA1, FUT11, FXYD5, FZD2, G0S2, GAA, GAS7, GATM, GBGT1, GBP2, GBP5, GFOD1, GK, GLRX, GLT25D1, GLT25D2, GLUL, GNLY, GNS, GPCPD1, GPX3, GYPC, HERPUD1, HFE, HIP1, HK2, HK3, HNMT, HOMER3, HPSE, HS3ST3B1, HSD11B1, HSPA6, HTRA1, HVCN1, IFITM3, IGSF21, IGSF6, IL10, IL15RA, IL17RA, IL18BP, IL18R1, IL18RAP, IL1A, IL1B, IL1R2, IL1RAP, IL1RL1, IL1RN, IL24, IL2RA, IL3RA, IL6, IL8, IRS2, ITGB8, JAG1, KCNE1, KCNE3, KCNJ15, KCNK13, KCNMA1, KCNN4, KCTD12, LAIR1, LAMB3, LAPTM5, LAT2, LATS2, LGMN, LIPA, NAIP, LPCAT2, LPL, LRP1, LTBP2, LY86, LYZ, MAD1L1, MAOA, MARCH1, MFSD1, MGAT4A, MGST1, MICALL2, MKKS, MMP1, MMP10, MMP12, MMP19, MMP7, MMP8, MMP9, MPEG1, MPHOSPH6, MT1F, MT2A, MYO7A, MYOF, NAMPT, NCEH1, NCF2, NCF4, NDRG1, NDST1, NDUFS2, NEFH, NINJ1, NISCH, NLRC4, NLRP1, NLRP3, NOD2, NPC1, NPL, NRIP3, NT5E, NUP214, P4HA1, PAPSS2, PCTP, PDCD1LG2, PDK1, PDK4, PDPN, PDXK, PFKFB3, PFKFB4, PFN2, PGCP, PGS1, PLA2G4A, PLIN2, PLSCR1, PLTP, PMP22, POR, PSTPIP1, PTGES, PTGS2, PYGL, QKI, QPCT, RNASE4, RNASE6, RNASET2, RNF130, RNF144B, RNF149, RRM2B, S100A12, S100A8, S100A9, SBF2, SCCPDH, SEC24A, SECTM1, SEMA4B, SEMA4D, SERINC2, SERPINA1, SERPINB2,

	SERPINB3 / SERPINB4, SERPINE1, SLC11A1, SLC11A2, SLC12A6, SLC16A10, SLC16A6, SLC1A2, SLC1A3, SLC22A15, SLC22A4, SLC25A37, SLC26A11, SLC2A14 / SLC2A3, SLC30A1, SLC35B4, SLC36A4, SLC37A2, SLC38A6, SLC39A8, SLC7A7, SLC7A8, SLC8A1, SLCO2B1, SLCO4A1, SLITRK4, SMOX, SMPDL3A, SNTB1, SNX10, SNX30, SOD2, SPARC, SPOCK1, SPP1, ST3GAL1, ST8SIA4, STARD13, STC1, STEAP3, STEAP4, STT3B, SULF2, SYPL1, SYTL3, TAGLN, TBXAS1, TCIRG1, TET2, TFPI, TGFBI, THBD, TKTL1, TMPRSS11E, TNF, TNFAIP6, TNNT1, TPCN1, TPST2, TRPM2, TTLL3, TUBA4A, UBAC2, UNC119, UPP1, UTRN, VAMP4, VAV3, VCAN, VEGFA, VNN1, VNN2, VNN3, WIPF1, XCL1 /XCL2, YOD1

Supplementary Table S2. Gene ontology analysis for genes overexpressed in migratory LCs and DDCs before stimulation with TNF-α.

Lists of genes overexpressed in migratory LCs and DDCs (above 1.5 fold difference between the cell types in $\log_2(x)$ RMA normalised gene expression levels) were submitted to gene set enrichment (GSE) analysis was done using ‘‘functional annotation clustering’’ tool, (similarity threshold 0.5, multiple linkage threshold 0.5, EASE:1.0 and Benjamini correction) within DAVID web-based tool and mapped over the KEGG pathway database. Basing on the functional annotation results genes were classified as “receptors” “regulators of signal transduction” “regulators of gene transcription and translation” or “effector”. Gene annotations were confirmed by detailed direct analysis using Gene Expression Atlas (http://www.ebi.ac.uk/gxa/).

Supplementary Table S3.

	Study	Dataset no	LC (no datasets)	CD1a+ DDC (no datasets)	Platform
[1]	Santegoets et al	GSE23618	3	3	Affymetrix HG U133 plus 2
[2]	Allen et al	GSE16395	12	0	Affymetrix HG U133 plus 2
[3]	Hutter et al	GSE35340	3	0	Affymetrix HG U133 plus 2
[4]	Harman et al	GSE32648	2	3	Illumina
		GSE32400	4	4	AGRF Human 8K cDNA Microarray

Supplementary Table S3. Datasets of human skin derived Langerhans cells isolated by trypsinisation available in public domain.

Supplementary Table S4

Pathways overrepresented in trypsinised DDC	**Pathways overrepresented in trypsinised LC**
hsa04060:Cytokine-cytokine receptor interaction	hsa00240:Pyrimidine metabolism
hsa04512:ECM-receptor interaction	hsa00590:Arachidonic acid metabolism
hsa04610:Complement and coagulation cascades	hsa02010:ABC transporters
hsa04640:Hematopoietic cell lineage	hsa04010:MAPK signaling pathway
hsa04630:Jak-STAT signaling pathway	hsa04110:Cell cycle
hsa04062:Chemokine signaling pathway	hsa04514:Cell adhesion molecules (CAMs)
hsa04510:Focal adhesion	hsa04520:Adherens junction
hsa04620:Toll-like receptor signaling pathway	hsa04540:Gap junction
hsa04621:NOD-like receptor signaling pathway	hsa04810:Regulation of actin cytoskeleton
hsa05020:Prion diseases	hsa04310:Wnt signaling pathway
hsa04672:Intestinal immune network for IgA production	
hsa03320:PPAR signaling pathway	GO-Term biological process analysis:
hsa04514:Cell adhesion molecules (CAMs)	fatty acid metabolic process, prostaglandin metabolic process,
hsa04650:Natural killer cell mediated cytotoxicity	carbohydrate metabolism, ubiquitine-mediated proteolysis

hsa05200:Pathways in cancer hsa04623:Cytosolic DNA-sensing pathway hsa04360:Axon guidance hsa05222:Small cell lung cancer hsa00561:Glycerolipid metabolism hsa00010:Glycolysis / Gluconeogenesis hsa03320:PPAR signaling pathway hsa00770:Pantothenate and CoA biosynthesis hsa00230:Purine metabolism	

Supplementary Table S4. DAVID functional gene classification and functional annotation of genes overexpressed in trypsinised LCs and trypsinised DDCs

Gene lists of 742 transcripts (516 genes) preferentially expressed in trypsinised LC, and 1268 transcripts (804 genes) preferentially expressed in DDCs (> 1.5 fold difference in log2(x) RMA normalised gene expression levels between the cell types, GSE23618) were submitted to DAVID functional classification and annotation to identify biological processes overrepresented in each cell type.

Supplementary Table S5.

Genes preferentially regulated in DDCs (ANOVA)*	**Peak of expression [hours]**	**Number of probesets**	**Over-represented processes and cell compartments**	**Examples of genes in the cluster**
Cluster 01 ($p<0.0001$)	0h	760	Immune response Surface receptors Phagocytosis	*ADAM28, CD163, CD180, CD209,CD36, CD274, CLEC1A, CLEC4G, CLEC5A, CLEC7A, CXCL2, CXCR7, FCGR2A, FCGR2B, FCGR3A, IL1R2, IFR2BP, IRF3, IRF4, ITGAM, ITGB2, MSR1, THBS1 (CD141), TLR2, TLR4, TLR5, TNFSF12, TNFSF13*
Cluster 02 ($p=0.0001$)	2h	293	Transcription Immune response Signal transduction regulation	*E2F6, EIF2C2,EIF4A3, EIF4E, EIF5, GPR109A, GPR18, GPRC5A, CREM, RPL28, TNFRSF10D, TNFRSF 12A, TNFSF14, TNFSF15, TNF, IL7, IL1RN, CXCR4, CCL3, CCL4, DYRK-3, RIPK1, MAPK2K3, PPP1R10, PPM1D, ZNF36, ZNF295, ZNF 331, ZNF697, ZNF703*

Cluster 04 ($p<0.0001$)	8h	172	Oxidative stress response Sphingiolipid metabolism, Lysosome activity, Immune response	*AKR1C3, PTGER2, CYP1B1, CYP7B1, SOD2, ITGB8, SNX10, IL1A, IL23A, MT1H*
Cluster 06 ($p<0.0001$)	24h	105	Immune signalling Cytokine secretion Chemokine activity Rearrangement of extracellular matrix Regulation of cell migration	*CYBB, CYP27A1A, CCL1, CCL2, CCL17, CCL18, CCL24,CD44, CD82, CLEC4D, CLEC4E, CXCL1, CXCL6, GPR68, IFNAR1, IL10,IL1B, ITGB3, ITGB8, MMP1, MMP7,MMP9, MMP14, PDPN*
Cluster 07 ($p<0.0001$)	8h	61	Immune response Macromolecule metabolism	*CD109,IFI44, IFIT1, IFIT3, LRP12,MT1G, MT1X, MX1, MX2, PTGER3, TNFSF13B AKR1C1, ADA, OAS1*

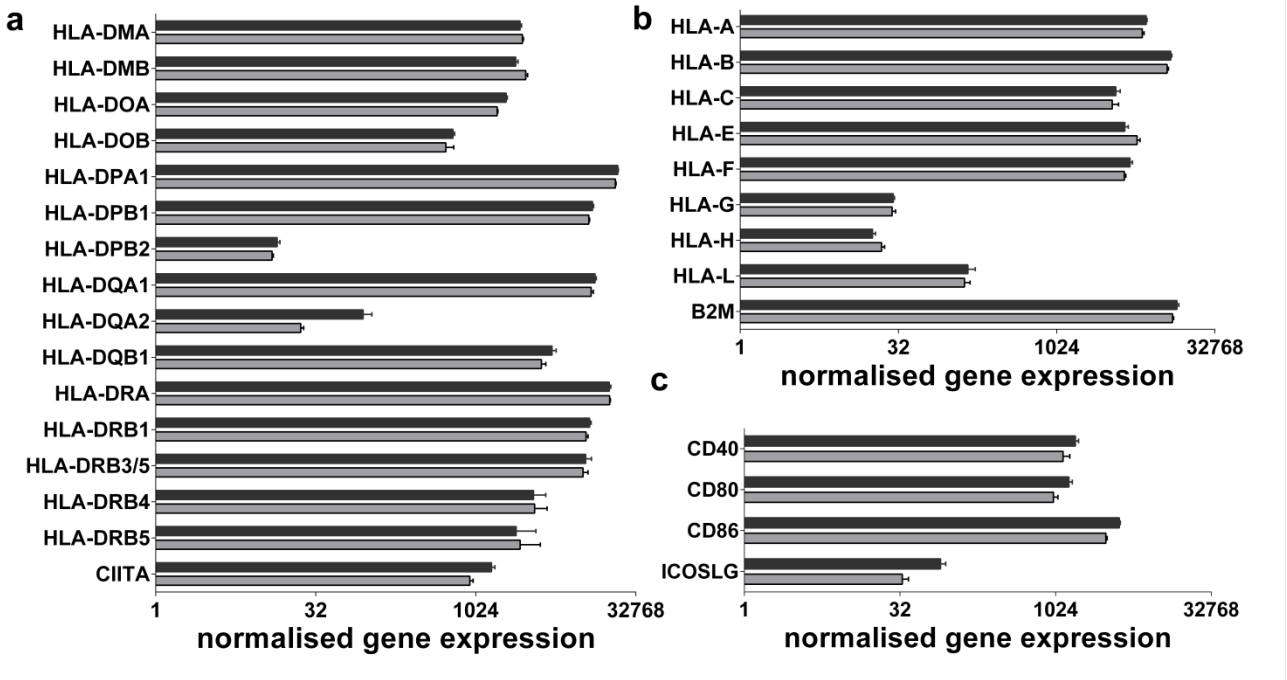
Cluster 08 (p<0.0001)	0h	56	Immune response Surface receptors Ion channel function	*CD14, CD33, DPYD, GIMAP4, IL15RA, LAIR1, TLR8, KCTD12*
Cluster 09 (p<0.005)	8h	32	Cell homeostasis Cellular transport	*ANXA5, GCLC, GCLM, TXNRD1, PTGER3, DDX39, TNPO1*
Genes preferentially regulated in LCs (ANOVA)*				
Cluster 03 (p=0.0001)	24h	208	Mitochondrial activity Ribosomal activity Response to hormones/nutrients, Proteasome assembly and function, Cell	*MRLP11, MRLP15, MRLP23,MRLP24,MRLP27, MRLP3, MRLP48, MRPS28, MRPS35,MRPS7, NDUFA9, NDUFAF4, GLRX3, ACAT1, CYB561, DBI, ENSA, PSMA3, PSMB7, PSMD14, PSME3, SNN, IL15, CCL22, WNT5B*

			cycle	
Cluster 05 (p<0.0001)	T0	105	Organisation of cytoskeleton, Macromolecule metabolism Carbohydrate metabolism Mitochondrial processes	*ACOT7, VCAM-1, DSP, GSN, EPCAM, IDH2, GALC, PC, SCD, OXNAD, TRAF5, SCN4B, SQLE, UBA5, CIITA, PI3CG,*
Cluster 10 (p<0.0001)	2h	27	Regulation of transcription Intracellular transport	*DENND4A, RFX2, RAB11FIP1*
Cluster 12 (p<0.0001)	24h	24	Metal Ion binding, Intracellular transport, ROS scavenging	*PTGER4, SESN3, SNN,SNX11,SOCS1, SYNPO2,TRAF1,*

Cluster 13 ($p<0.0001$)	2h	23	Regulation of transcription Metal ion binding	*KLF7,PDLIM1, RHOV, TBCEL*
Cluster 14 ($p<0.0001$)	24h	21	Endocytosis, Cytoskeleton reorganisation, Signal transduction	*CAV-1, SSPN, SYNPO, PTPRK*,

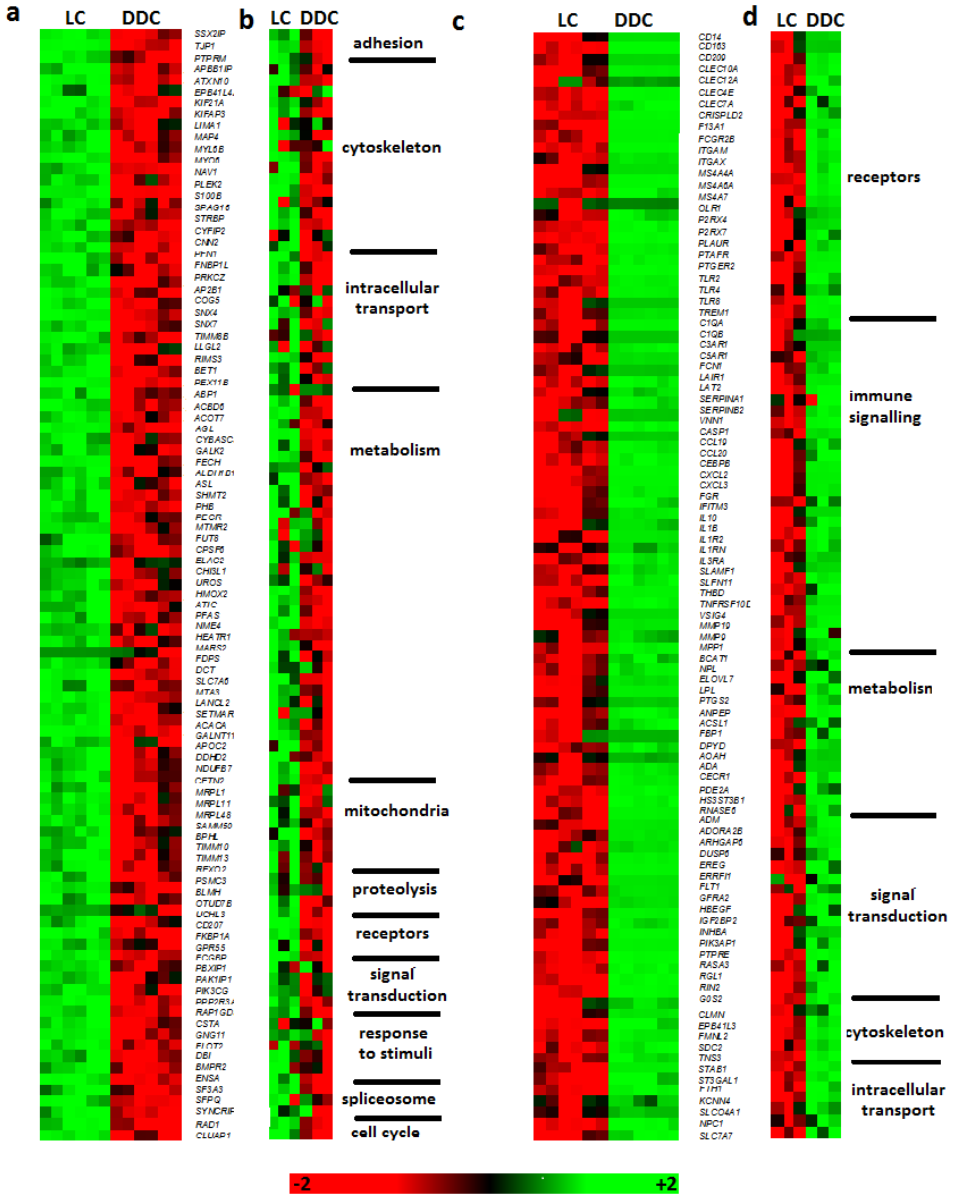
Supplementary Table S5. Cluster analysis of gene expression changes in CD1a+ LCs and CD11c+ DDCs activated with TNF-α over 24h.

Biological processes over-represented in 14 biggest clusters of genes identified with BioLayout *Express*^{3D} network analysis (r=0.85, MCL=1.7, 2,334 probesets differentially regulated by TNF-α, Bayesian Estimation of Temporal Regulation) were categorised using ‘‘functional annotation clustering’’ tool, (similarity threshold 0.5, multiple linkage threshold 0.5, EASE:1.0 and Benjamini correction) from DAVID web-based tool. * p values indicate the statistical difference for LCs and DDCs average gene expression profiles across the time course of stimulation with TNF-α were compared with two-way repeated ANOVA for each cluster separately (Bonferroni correction, GraphPad Prism, USA).



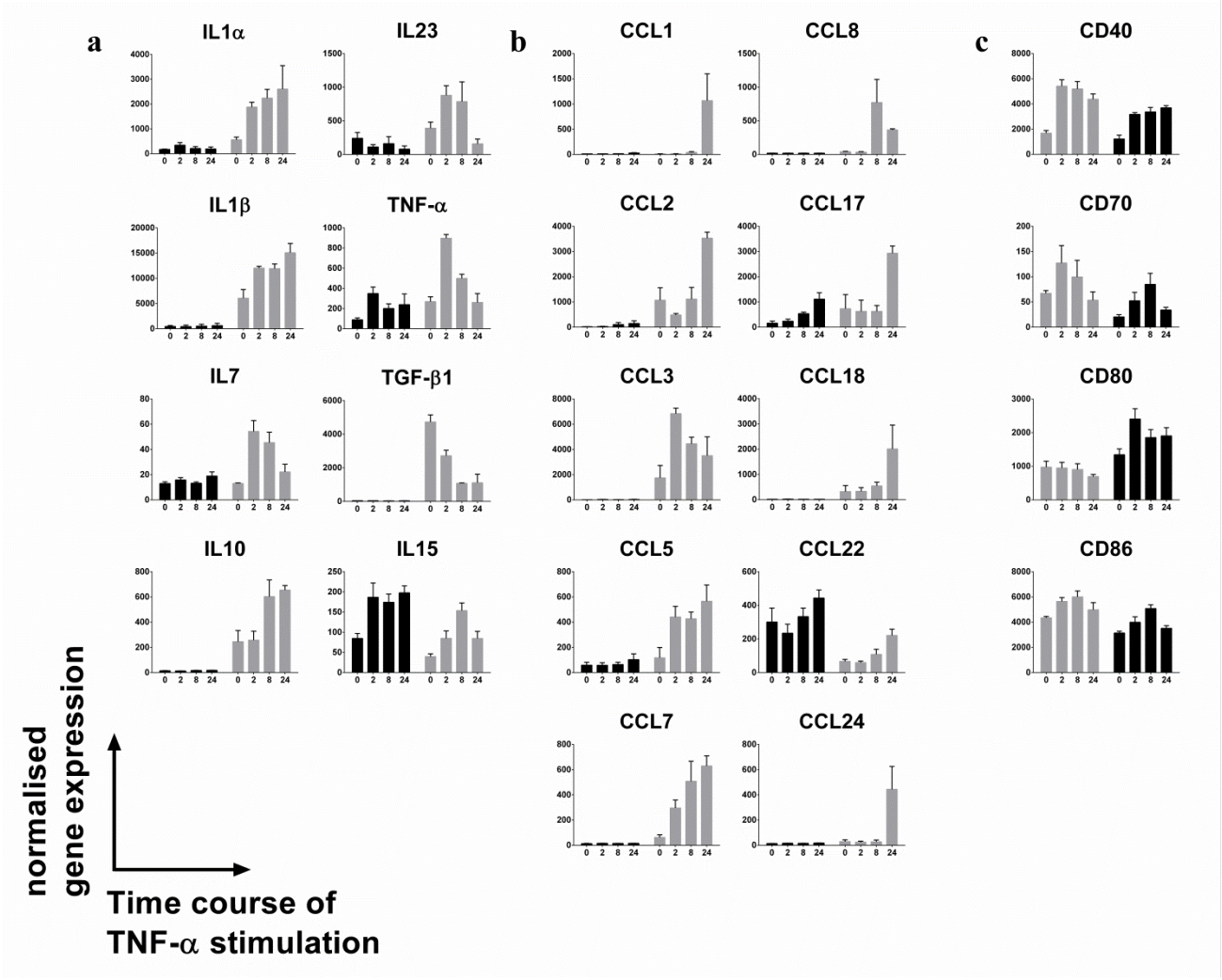
Supplementary Figure 1.

(a-c) Mean RMA normalised expression (± SEM) expression of HLA class II and transactivator (CIITA) (a), HLA class I and β-2 microglobulin (B2M) (b) and key co-stimulatory molecules (c) in migratory human skin derived LCs (black bars) and DDCs (grey bars), n=3 independent skin donors in duplicate. No statistically significant differences detected (paired T test, Bonferroni correction).



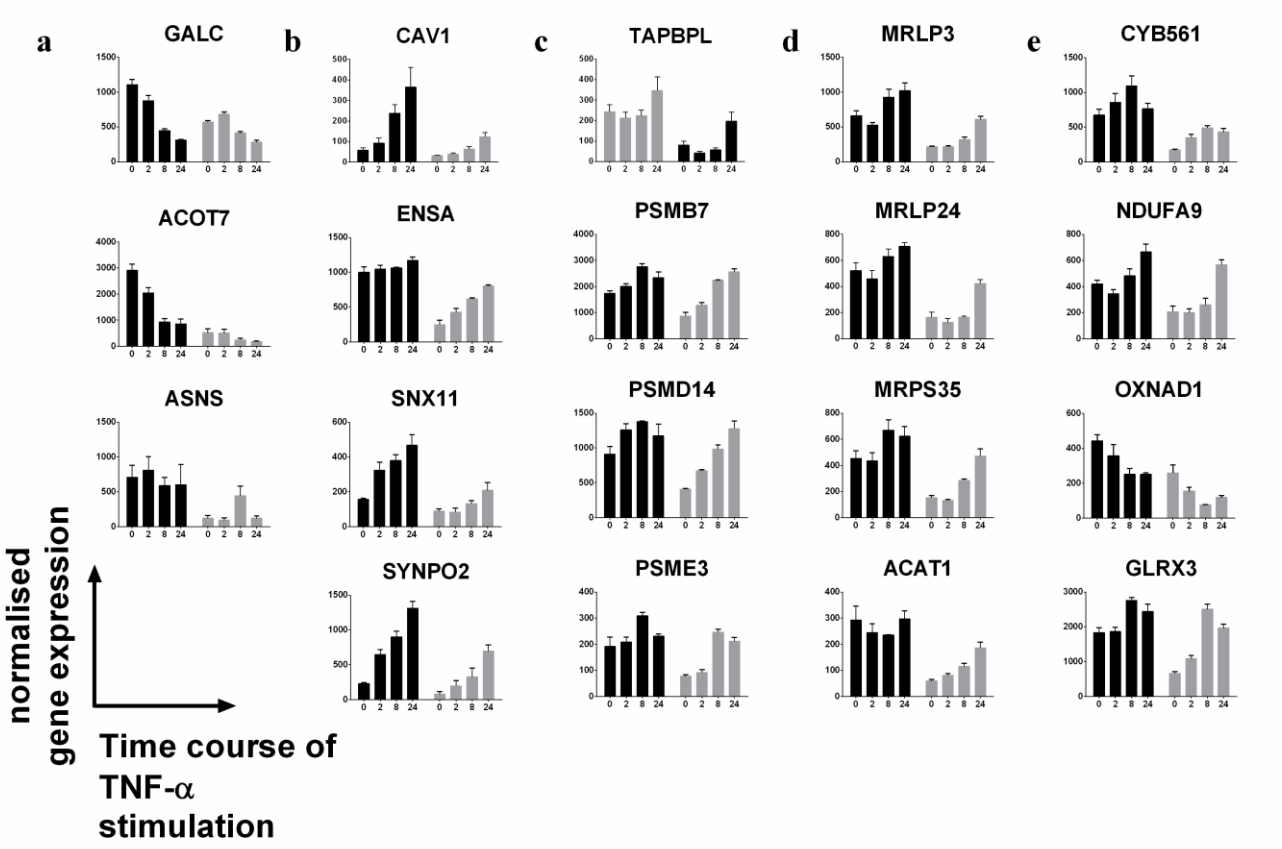
Supplementary Figure 2.

The comparative heat maps of expression levels of the genes of interest (GOI), identified as gene signature in migratory cells at T0 (> 1.5 fold difference in log2(x) RMA normalised gene expression levels between the cell types) in migratory (a,c) and trypsinised (b,d) LCs vs DDCs. 100 top GOI up-regulated in LC (a,b) and 100 top GOI up-regulated in DDCs (c,d) are shown. Biological processes identified with gene ontology analysis are indicated for the genes enriched in LCs and DDCs.



Supplementary Figure 3.

(a-c) Mean RMA normalised expression of (a) cytokines (b) chemokines (c) co-stimulatory molecules in in LCs (black bars) or DDCs (grey bars) during the time course of stimulation with TNF-α as assessed by microarrays, n=3 independent skin donors.



Supplementary Figure 4.

(a-e) RMA normalised expression of (a) genes involved in metabolism (b) cytoskeleton reorganisation and signalling (c) proteasome subunits (d-e) mitochondria associated genes in in LCs (black bars) or DDCs (grey bars) during the time course of stimulation with TNF-α as assessed by microarrays, n=3 independent skin donors.