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Supplemental information

Contrasting behavior between the three human monocyte subsets in dengue pathophysiology

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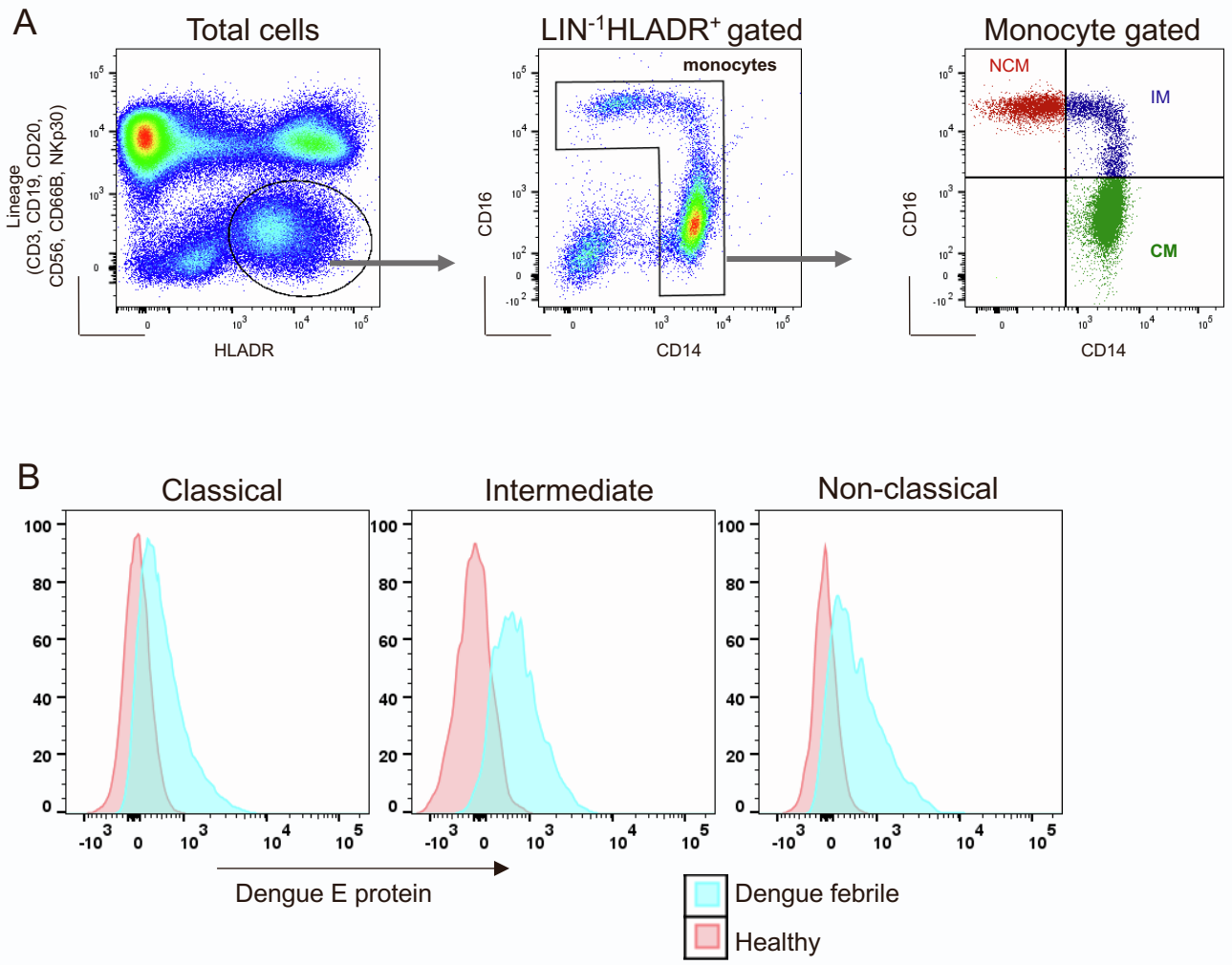


Figure S1. Gating strategy used for identification of the CM, IM, and NCM subsets. Related to STAR Methods, Figure 1 and TableS1.

(A) Freshly stained live peripheral blood mononuclear cells (PBMC) were first excluded for T/B cells, NK cells, and neutrophils by gating out CD3, CD19, CD20, CD56, CD66b, and NKp30 positive cells and then gated on HLA-DR positive population. From this population, the CD14+CD16low classical monocytes (CM), the CD14+CD16+ intermediate monocytes (IM), and the CD14lowCD16+ non-classical monocytes (NCM) were identified.

(B) Histogram shows the flow cytometry staining for Dengue E protein showing the infection in the three monocyte subsets from one of the patient's samples is shown in Figure 2 A, where red represents healthy, and blue represents the dengue subject.

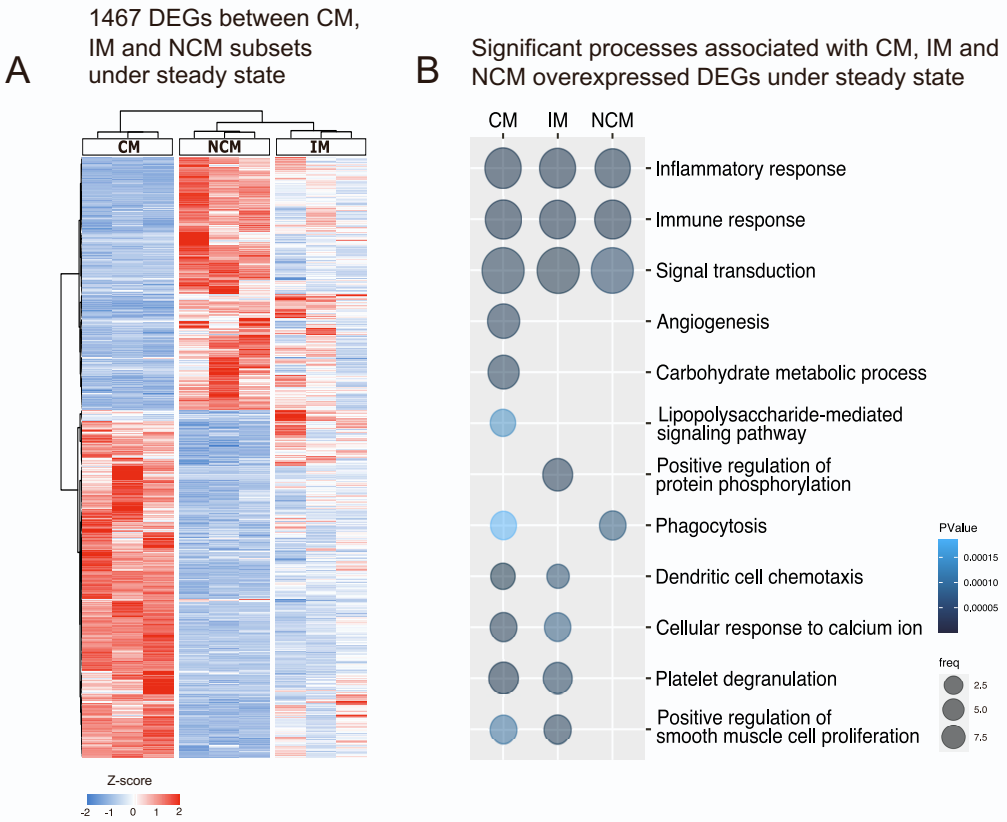


Figure S2. Comparison of the DEGs between the CM, IM, and NCM subsets from healthy children and the significant biological processes associated with these DEGs. Related to Figure 1, Table S2A and Table S2B.

(A) Heatmap showing clustering and relative expression of 1467 significantly differentially expressed genes (DEGs) deduced from CM versus IM; CM versus NCM; and NCM versus IM comparisons in healthy children. Z-score of normalized counts was used for plotting the heatmap. Ward.D2 method was used for clustering. Gradient of high to low gene expression is indicated from red to blue color. (For significance: adjusted P-value < 0.05 and P-value < 0.01; for differential expression: log2 fold change >= 1 or =<-1).

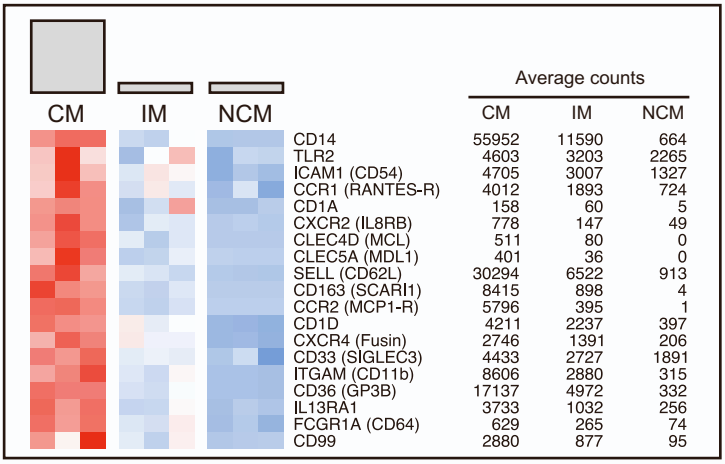
(B) Significant biological processes associated with overexpressed genes in CM, IM, and NCM subsets from healthy children.

A

Gene expression profiles for select genes for which flow cytometric staining data available from literature

Expression pattern expected by flow cytometric staining

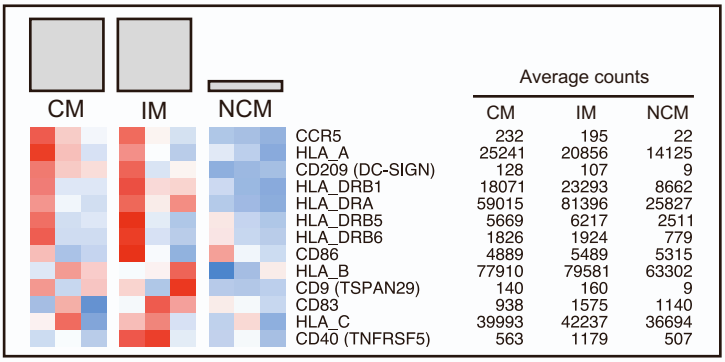
Expression pattern found by RNA seq in our study



	Average counts		
	CM	IM	NCM
CD14	55952	11590	664
TLR2	4603	3203	2265
ICAM1 (CD54)	4705	3007	1327
CCR1 (RANTES-R)	4012	1893	724
CD1A	158	60	5
CXCR2 (IL8RB)	778	147	49
CLEC4D (MCL)	511	80	0
CLEC5A (MDL1)	401	36	0
SELL (CD62L)	30294	6522	913
CD163 (SCARI1)	8415	898	4
CCR2 (MCP1-R)	5796	395	1
CD1D	4211	2237	397
CXCR4 (Fusin)	2746	1391	206
CD33 (SIGLEC3)	4433	2727	1891
ITGAM (CD11b)	8606	2880	315
CD36 (GP3B)	17137	4972	332
IL13RA1	3733	1032	256
FCGR1A (CD64)	629	265	74
CD99	2880	877	95

Expression pattern expected by flow cytometric staining

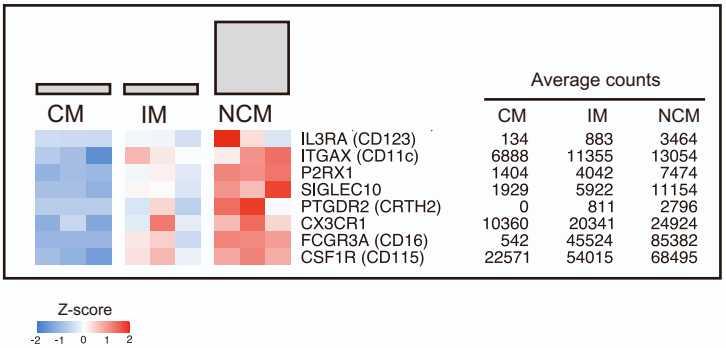
Expression pattern by found RNA seq in our study



	Average counts		
	CM	IM	NCM
CCR5	232	195	22
HLA_A	25241	20856	14125
CD209 (DC-SIGN)	128	107	9
HLA_DRB1	18071	23293	8662
HLA_DRA	59015	81396	25827
HLA_DRB5	5669	6217	2511
HLA_DRB6	1826	1924	779
CD86	4889	5489	5315
HLA_B	77910	79581	63302
CD9 (TSPAN29)	140	160	9
CD83	938	1575	1140
HLA_C	39993	42237	36694
CD40 (TNFRSF5)	563	1179	507

Expression pattern expected by flow cytometric staining

Expression pattern found by RNA seq in our study

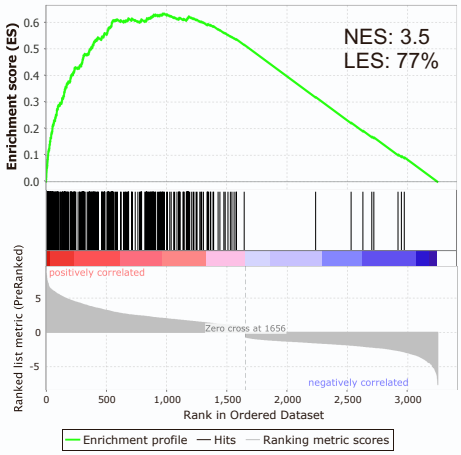


	Average counts		
	CM	IM	NCM
IL3RA (CD123)	134	883	3464
ITGAX (CD11c)	6888	11355	13054
P2RX1	1404	4042	7474
SIGLEC10	1929	5922	11154
PTGDR2 (CRTH2)	0	811	2796
CX3CR1	10360	20341	24924
FCGR3A (CD16)	542	45524	85382
CSF1R (CD115)	22571	54015	68495

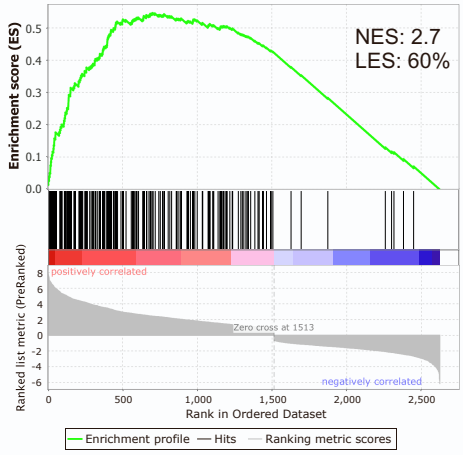
B

Enrichment based comparisons for each monocyte subset between the significant differential genes reported in Monaco et al. (2019) with top 500 overexpressed genes from our study.

CM



IM



NCM

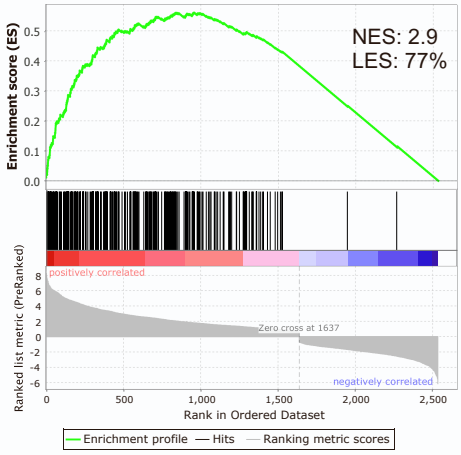


Figure S3. Comparison of steady-state gene expression profile reported in this study with select genes confirmed by flow cytometry in literature and an external dataset for healthy adults for CM, IM, and NCM. Related to Figure 1.

(A) The bars above the heatmap show the expected pattern as per the literature, and the heatmap below shows the expression of genes in this data. Z-score of normalized counts was were used for plotting the heatmap. A gradient of high to low gene expression is indicated from red to blue.

(B) GSEA plots showing the comparison of the top 500 significant overexpressed genes in CM, IM, and NCM of healthy children from this study with published single-cell RNA seq dataset (GSE107011, (Monaco et al., 2019)) of significant differentially expressed genes in CM, IM, and NCM of healthy adults.

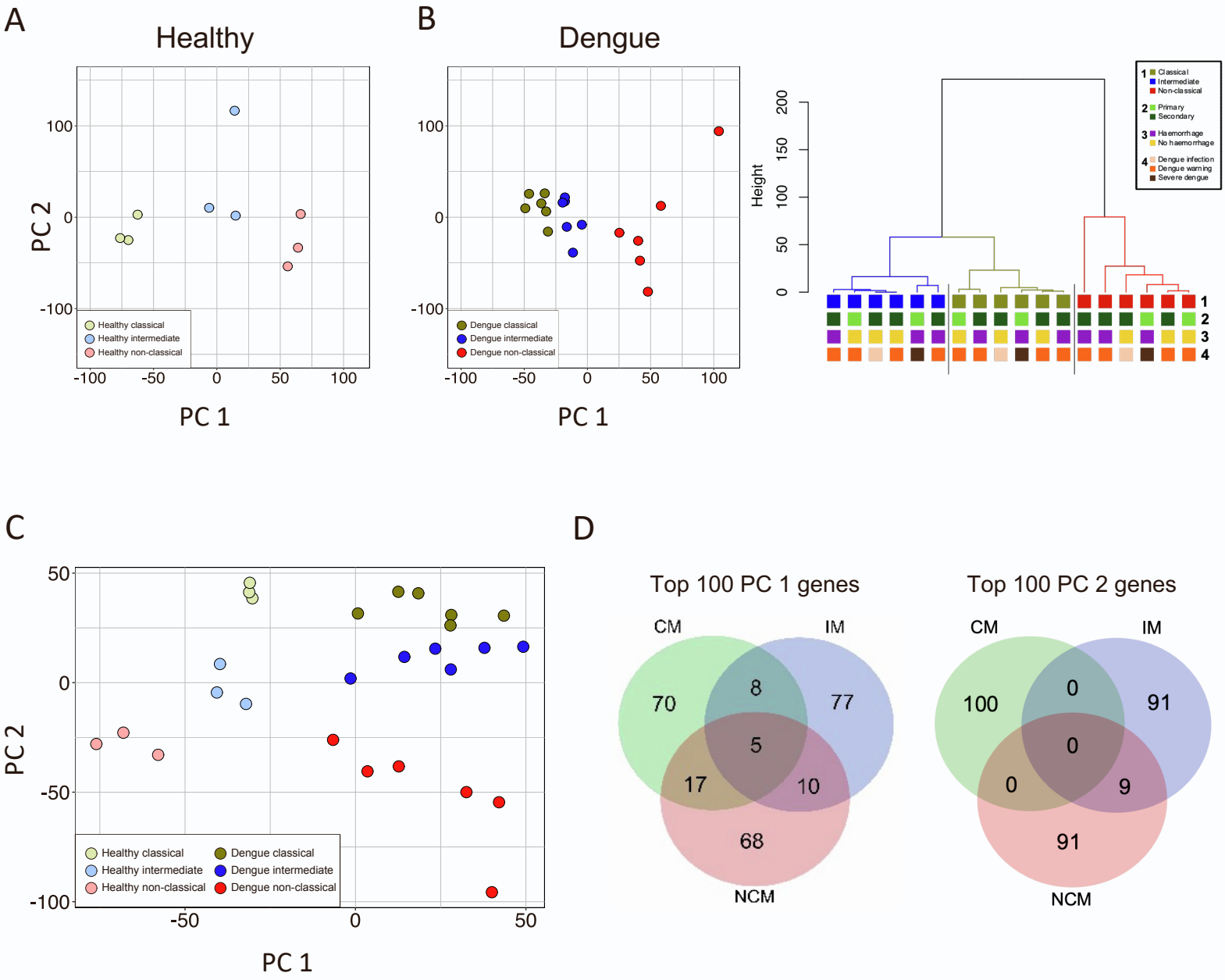


Figure S4. Donor-to-donor variation among healthy children and dengue patients. Related to Figure 1B and Table S3A.

(A) PCA score plot obtained from normalized counts of all genes from CM, IM, and NCM subsets of healthy children (n= 3). Genes with no count in all the samples were excluded.
(B) PCA score plot obtained from normalized counts of all genes from CM, IM, and NCM subsets of dengue subjects (n= 6). Genes with no count in all the samples were excluded. Unsupervised cluster analysis using ward.D2 method for 6 dengue subjects as shown in the respective PCA plot, further highlighting the subset clusters (1), type of infection (2), hemorrhage status (3), and disease severity (4).
(C) Combined PCA score plot of healthy children and dengue patients obtained from all normalized genes from CM, IM and NCM subsets after excluding genes with no count in all the samples.
(D) Venn diagrams representing the overlap of genes among the subsets, mentioned in the top 100 PC1 and PC2 gene list that separate dengue and healthy subjects in different monocyte subsets.

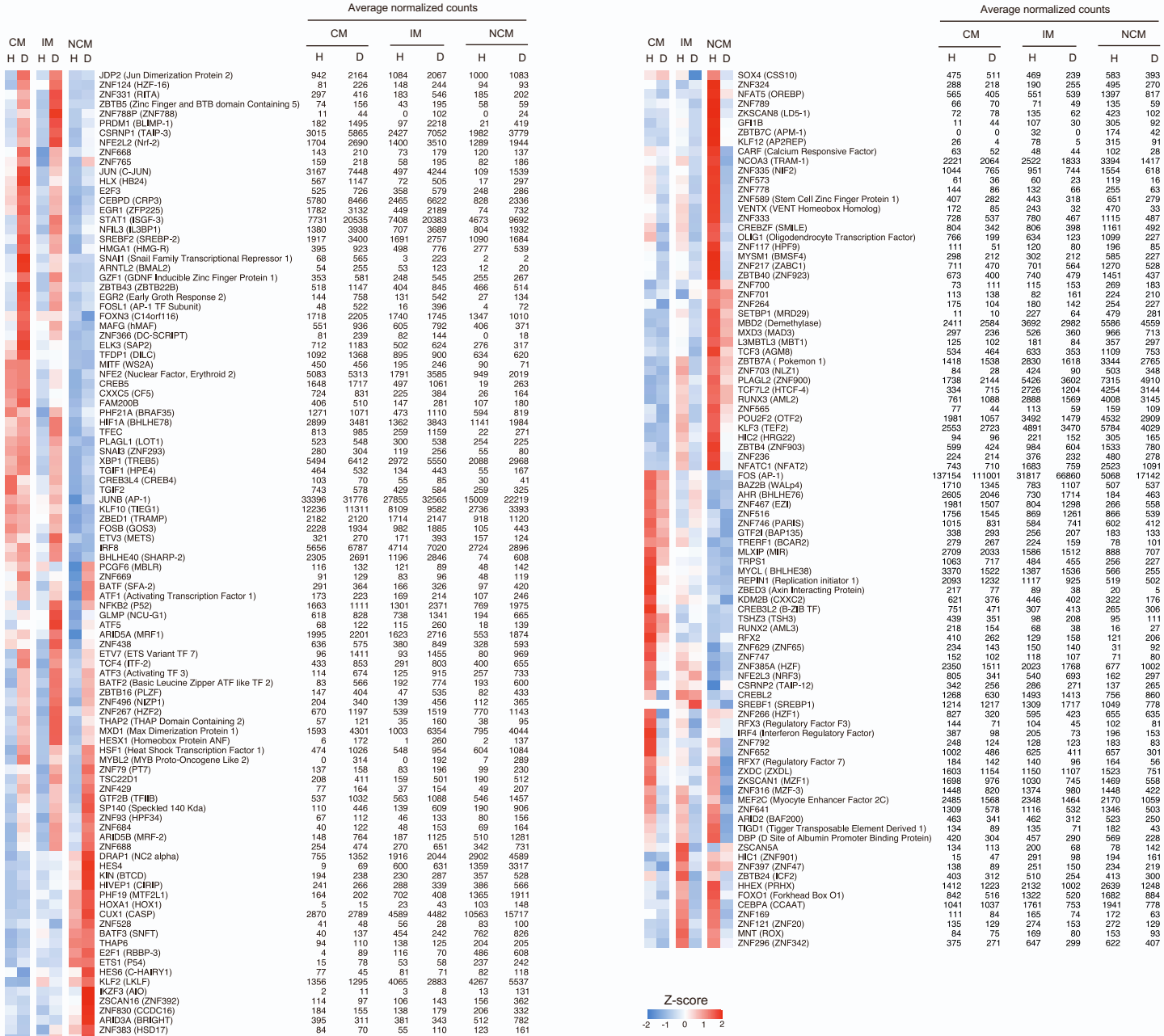


Figure S5. Heat map and expression reads of transcription factors (TFs) differed between the CM, IM, and NCM subsets or between the dengue versus steady state in one or more subsets. Related to Figure 1D.

Left, heat maps of the indicated TFs in CM, IM, and NCM subsets from Healthy individuals (H, n=3) and Dengue patients (D, n=6). Z-score of normalized counts was used for plotting the heatmap. A gradient of high to low gene expression, is indicated from red to blue color. Right, normalized average counts were indicated.

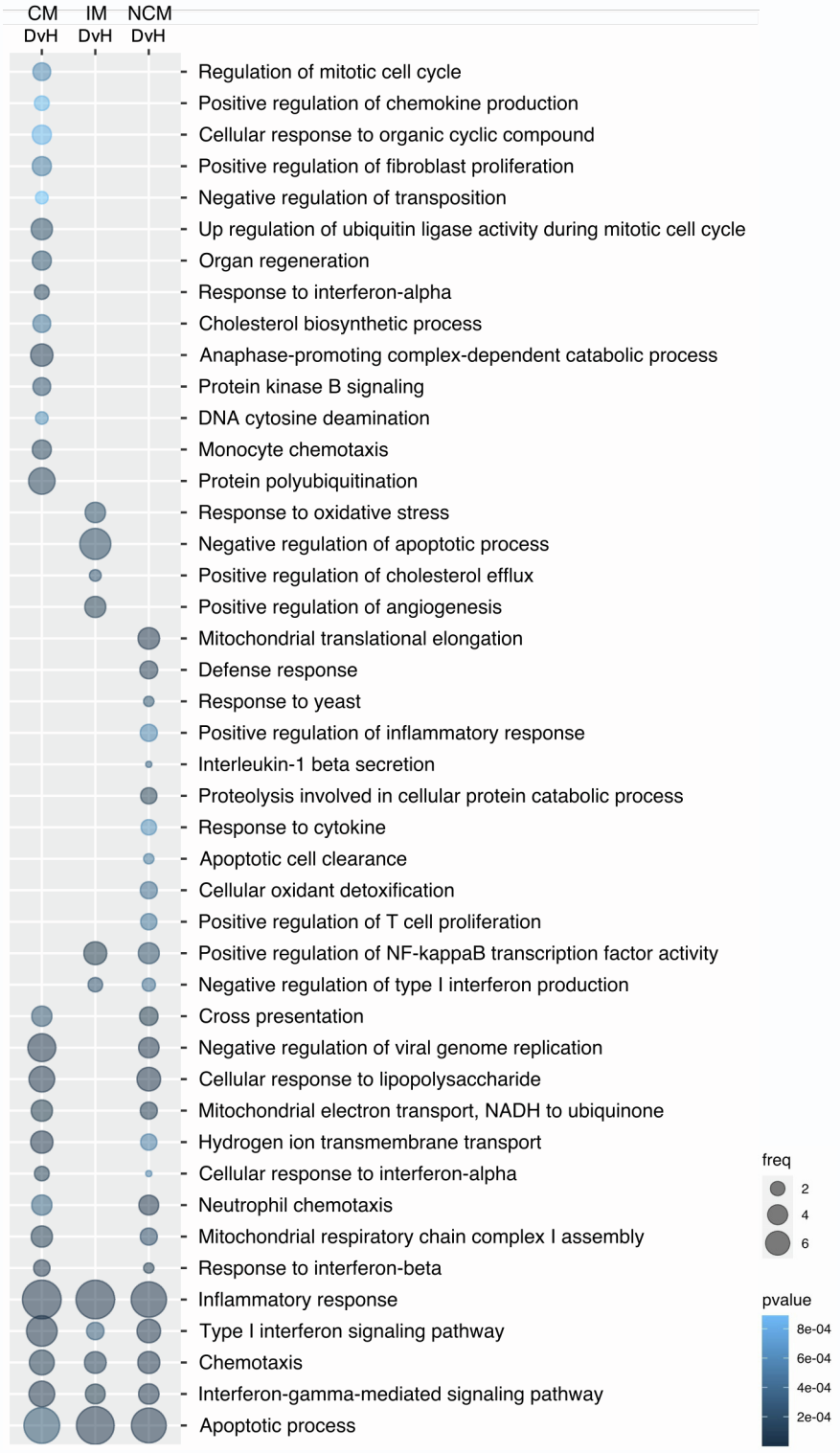


Figure S6. Significant biological processes associated with the CM, IM, and NCM overexpressed DEGs in dengue patients as compared to steady-state. Related to Figure 1C and Table S4.

Significant biological processes with adjusted P-value < 0.05 are shown. The size of each bubble represents the frequency of genes for each process, and color represents the adjusted P-value.

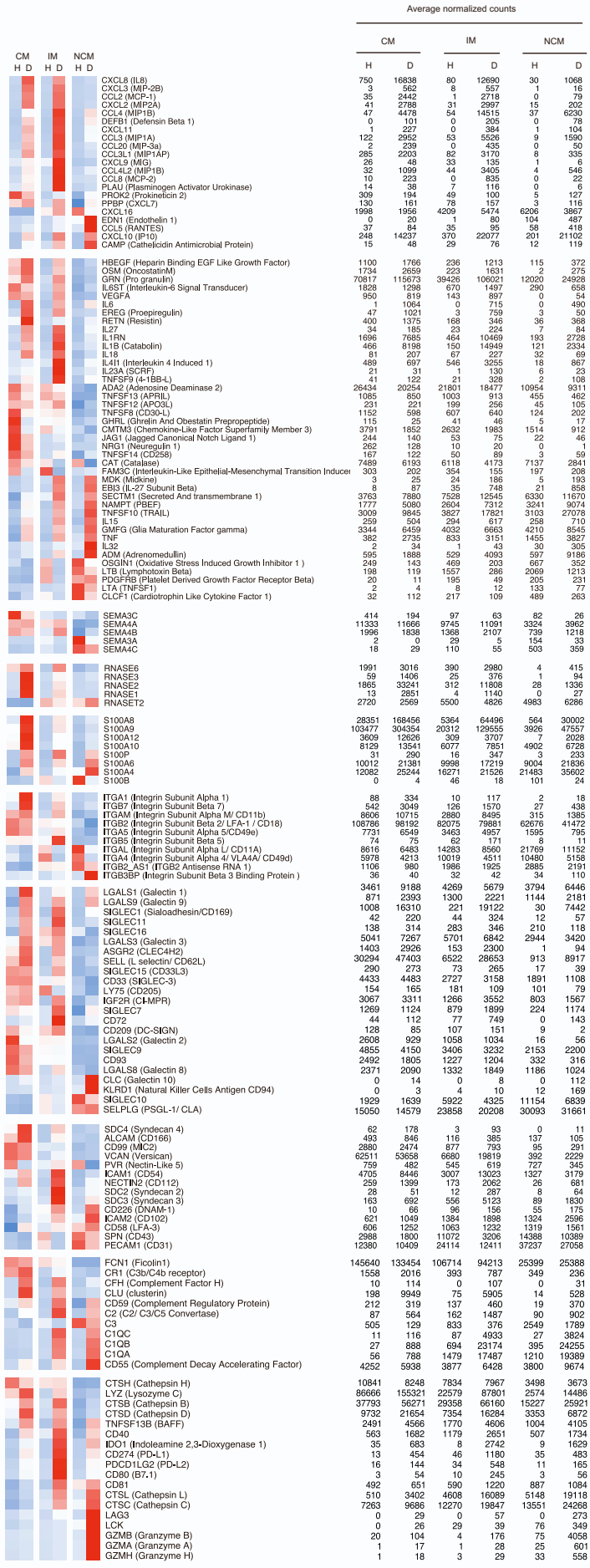

Average normalized counts
CM
IM
NCM
H D
Chemokines
Cytokines
Semaphorins & RNAses
S100-proteins
Integrins
Lectins & selectins
Other cell adhesion molecules
Complement molecules
Other effector molecules
CXCL8 (IL8) 750 16838 80 12690 30 1068
CXCL3 (MIP-2B) 3 562 8 557 1 16
CCL2 (MCP-1) 35 2442 1 2718 0 79
CXCL2 (MIP2A) 41 2788 31 2997 15 202
CCL4 (MIP1B) 47 4478 54 14515 37 6230
DEFB1 (Defensin Beta 1) 0 101 0 205 0 78
CXCL11 1 227 0 384 1 104
CCL3 (MIP1A) 122 2952 53 5526 9 1590
CCL20 (MIP-3a) 2 239 0 435 0 50
CCL3L1 (MIP1AP) 285 2203 82 3170 8 335
CCL9 (MIG) 26 48 33 135 1 6
CCL4L2 (MIP1B) 32 1099 44 3405 4 546
CCL8 (MCP-2) 10 223 0 835 0 22
PLAU (Plasminogen Activator Urokinase) 14 38 7 116 0 6
PROK2 (Prokineticin 2) 309 194 49 100 5 127
PPBP (CXCL7) 130 161 78 157 3 116
CXCL16 1998 1956 4209 5474 6206 3867
EDN1 (Endothelin 1) 0 20 1 80 104 487
CCL5 (RANTES) 37 84 35 95 58 418
CXCL10 (IP10) 248 14237 370 22077 201 21102
CAMP (Cathelicidin Antimicrobial Protein) 15 48 29 76 12 119
HBEGF (Heparin Binding EGF Like Growth Factor) 1100 1766 236 1213 115 372
OSM (OncostatinM) 1734 2659 223 1631 2 275
GRN (Pro granulin) 70817 115673 39426 106021 12020 24928
IL6ST (Interleukin-6 Signal Transducer) 1828 1298 670 1497 290 658
VEGFA 950 819 143 897 0 54
IL6 1 1064 0 715 0 490
EREG (Proepiregulin) 47 1021 3 759 3 50
RETN (Resistin) 400 1375 168 346 36 368
IL27 34 185 23 224 7 84
IL1RN 1696 7685 464 10469 193 2728
IL1B (Catabolin) 466 8198 150 14949 121 2334
IL18 81 207 67 227 32 69
IL4I1 (Interleukin 4 Induced 1) 489 697 546 3255 18 867
IL23A (SCRF) 21 31 1 130 6 23
TNFSF9 (4-1BB-L) 41 122 21 328 2 108
ADA2 (Adenosine Deaminase 2) 26434 20254 21801 18477 10954 9311
TNFSF13 (APRIL) 1085 850 1003 913 455 462
TNFSF12 (APO3L) 231 221 199 256 45 105
TNFSF8 (CD30-L) 1152 598 607 640 124 202
GHRL (Ghrelin And Obestatin Prepropeptide) 115 25 41 46 5 17
CMTM3 (Chemokine-Like Factor Superfamily Member 3) 3791 1852 2632 1983 1514 912
TNFSF8 (CD30-L) 244 140 53 75 22 46
NRG1 (Neuregulin 1) 262 128 10 20 0 1
TNFSF14 (CD258) 167 122 50 89 3 59
CAT (Catalase) 7489 6193 6118 4173 7137 2841
FAM3C (Interleukin-Like Epithelial-Mesenchymal Transition Inducer 303 202 354 155 197 208
MDK (Midkine) 3 25 24 186 5 193
EBI3 (IL-27 Subunit Beta) 8 87 35 748 21 858
SECTM1 (Secreted And transmembrane 1) 3763 7880 7528 12545 6330 11670
NAMPT (PBEF) 1777 5080 2604 7312 3241 9074
TNFSF10 (TRAIL) 3009 9845 3827 17821 3103 27078
IL15 259 504 294 617 258 710
GMFG (Glia Maturation Factor gamma) 3344 6459 4032 6663 4210 8545
TNF 382 2735 833 3151 1455 3827
IL32 2 34 1 43 30 305
ADM (Adrenomedullin) 595 1888 529 4093 597 9186
OSGIN1 (Oxidative Stress Induced Growth Inhibitor 1) 249 143 469 203 667 352
LTB (Lymphotoxin Beta) 198 119 1557 286 2069 1213
PDGFRB (Platelet Derived Growth Factor Receptor Beta) 20 11 195 49 205 231
LTA (TNFSF1) 2 4 8 12 133 77
CLCF1 (Cardiotrophin Like Cytokine Factor 1) 32 112 217 109 489 263
SEMA3C 414 194 97 63 82 26
SEMA4A 11333 11666 9745 11091 3324 3962
SEMA4B 1996 1838 1368 2107 739 1218
SEMA3A 2 0 29 5 154 33
SEMA4C 18 29 110 55 503 359
RNASE6 1991 3016 390 2980 4 415
RNASE3 59 1406 25 376 1 94
RNASE2 1865 33241 312 11808 28 1336
RNASE1 13 2851 4 1140 0 27
RNASET2 2720 2569 5500 4826 4983 6286
S100A8 28351 168456 5364 64496 564 30002
S100A9 103477 304354 20312 129555 3926 47557
S100A12 3609 12626 309 3707 7 2028
S100A10 8129 13541 6077 7851 4902 6728
S100P 31 290 16 347 3 233
S100A6 10012 21381 9998 17219 9004 21236
S100A4 12082 25244 16271 21526 21483 35602
S100B 0 4 46 18 101 24
ITGA1 (Integrin Subunit Alpha 1) 88 334 10 117 2 18
ITGB7 (Integrin Subunit Beta 7) 542 3049 126 1570 27 438
ITGAM (Integrin Subunit Alpha M/ CD11b) 8606 10715 2880 8495 315 1385
ITGB2 (Integrin Subunit Beta 2/ LFA-1 / CD18) 108796 98192 82075 77881 62676 41472
ITGA5 (Integrin Subunit Alpha 5/CD49e) 7731 6549 3463 4957 1595 795
ITGB5 (Integrin Subunit Beta 5) 74 75 62 171 8 11
ITGAL (Integrin Subunit Alpha L/ CD11A) 8616 6483 14283 8560 21769 11152
ITGA4 (Integrin Subunit Alpha 4/ VLA4A/ CD49d) 5978 4213 10019 4511 10480 5158
ITGB2_AS1 (ITGB2 Antisense RNA 1) 1106 980 1986 1925 2885 2191
ITGB3BP (Integrin Subunit Beta 3 Binding Protein) 36 40 32 42 34 110
LGALS1 (Galectin 1) 3461 9188 4269 5679 3794 6446
LGALS9 (Galectin 9) 871 2393 1300 2221 1144 2181
SIGLEC1 (Sialoadhesin/CD169) 1008 16310 221 19122 30 7442
SIGLEC11 42 220 44 324 12 57
SIGLEC16 138 314 283 346 210 118
LGALS3 (Galectin 3) 5041 7267 5701 6842 2944 3420
ASGR2 (CLEC4H2) 1403 2926 153 2300 1 94
SELL (L selectin/ CD62L) 30294 47403 6522 28653 913 8917
SIGLEC15 (CD33L3) 290 273 73 265 17 39
CD33 (SIGLEC-3) 4433 4483 2727 3158 1891 1108
LY75 (CD205) 154 165 181 109 101 79
IGF2R (CI-MPR) 3067 3311 1266 3552 803 1567
SIGLEC7 1269 1124 879 1899 224 1174
CD72 44 112 77 749 0 143
CD209 (DC-SIGN) 128 85 107 151 9 2
LGALS2 (Galectin 2) 2608 929 1058 1034 16 56
SIGLEC9 4855 4150 3406 3232 2153 2200
CD93 2492 1805 1227 1204 332 316
LGALS8 (Galectin 8) 2371 2090 1332 1849 1186 1024
CLC (Galectin 10) 0 14 0 8 0 112
KLRD1 (Natural Killer Cells Antigen CD94) 0 3 4 10 12 169
SIGLEC10 1929 1639 5922 4325 11154 6839
SELPLG (PSGL-1/ CLA) 15050 14579 23858 20208 30093 31661
SDC4 (Syndecan 4) 62 178 3 93 0 11
ALCAM (CD166) 493 846 116 385 137 105
CD99 (MIC2) 2880 2474 877 793 95 291
VCAN (Versican) 62511 53658 6680 19819 392 2229
PVR (Nectin-Like 5) 759 482 545 619 727 345
ICAM1 (CD54) 4705 8446 3007 13023 1327 3179
NECTIN2 (CD112) 259 1399 173 2062 26 681
SDC2 (Syndecan 2) 28 51 12 287 8 64
SDC3 (Syndecan 3) 163 692 556 5123 89 1830
CD226 (DNAM-1) 10 66 96 156 55 175
ICAM2 (CD102) 621 1049 1384 1898 1324 2596
CD58 (LFA-3) 606 1252 1063 1232 1319 1561
SPN (CD43) 2988 1800 11072 3206 14388 10389
PECAM1 (CD31) 12380 10409 24114 12411 37237 27058
FCN1 (Ficolin1) 145640 133454 106714 94213 25399 25388
CR1 (C3b/C4b receptor) 1558 2016 393 787 349 236
CFH (Complement Factor H) 10 114 0 107 0 31
CLU (clusterin) 198 9949 75 5905 14 528
CD59 (Complement Regulatory Protein) 212 319 137 460 19 370
C2 (C2/ C3/C5 Convertase) 87 564 162 1487 90 902
C3 505 129 833 376 2549 1789
C1QC 11 116 87 4933 27 3824
C1QB 27 888 694 23174 395 24255
C1QA 56 788 1479 17487 1210 19389
CD55 (Complement Decay Accelerating Factor) 4252 5938 3877 6428 3800 9674
CTSH (Cathepsin H) 10841 8248 7834 7967 3498 3673
LYZ (Lysozyme C) 86666 155321 22579 87801 2574 14486
CTSB (Cathepsin B) 37793 56271 29358 66160 15227 25921
CTSD (Cathepsin D) 9732 21654 7354 16284 3353 6872
TNFSF13B (BAFF) 2491 4566 1770 4606 1004 4105
CD40 563 1682 1179 2651 507 1734
IDO1 (Indoleamine 2,3-Dioxygenase 1) 35 683 8 2742 9 1629
CD274 (PD-L1) 13 454 46 1180 35 483
PDCD1LG2 (PD-L2) 16 144 34 548 11 165
CD80 (B7.1) 3 54 10 245 3 56
CD81 492 651 590 1220 887 1084
CTSL (Cathepsin L) 510 3402 4608 16089 5148 19118
CTSC (Cathepsin C) 7263 9686 12270 19847 13551 24268
LAG3 0 29 0 57 0 273
LCK 0 26 29 39 76 349
GZMB (Granzyme B) 20 104 4 176 75 4058
GZMA (Granzyme A) 1 17 1 28 25 601
GZMH (Granzyme H) 1 18 3 29 33 558
Average normalized counts
CM
IM
NCM
H D
Extra cellular matrix interacting proteins
Antigen presentation and processing
Scavenger receptors
FC-gamma receptors
Chemokine receptors
Pattern recognition receptors
ADAMDEC1 (Disintegrin Protease) 56 95 42 141 2 16
TGM2 (Transglutaminase 2) 144 1060 558 1554 37 276
MMP19 (Matrix Metallopeptidase 19) 84 115 7 103 1 10
HPSE (Heparanase) 1401 1868 527 2008 85 388
LAMC1 (Laminin B2 Chain) 74 158 0 188 0 22
SERPING1 723 12348 430 25292 251 14251
NTNG2 (Netrin G2) 84 184 36 303 10 174
AGRN (Agrin) 20 119 7 164 4 56
LGALS3BP (M2BP) 1581 22826 1167 29556 459 10306
COL9A3 (Collagen Alpha-3(IX) Chain) 23 174 4 198 5 96
HAPLN3 (Hyaluronan And Proteoglycan Link Protein 3) 48 884 30 1065 9 197
IGFBP7 (Angiomodulin) 563 1253 459 793 254 563
SERPINB1 3816 5625 2709 5343 1728 4034
PTX3 (Pentraxin 3) 243 977 256 803 124 542
SERPINF1 (EPC-1) 100 280 21 101 16 86
EMILIN1 (Elastin Microfibril Interfacer 1) 68 241 42 145 45 33
ADAMTS2 (Procollagen N-Endopeptidase) 2 482 0 290 0 8
F3 (Coagulation Factor III, Tissue Factor) 5 202 1 170 0 7
THBS1 (Thrombospondin 1) 4484 10585 159 7473 18 1298
F12 (Coagulation Factor XII) 56 137 19 94 34 31
ANXA4 (Annexin A4) 1223 3294 1742 2760 2594 2535
LAMB2 (Laminin S) 574 117 83 84 0 6
WNT5B 180 22 43 39 0 18
ATRN (Attractin) 1634 586 987 422 559 185
FBN2 (Fibrillin 2) 612 433 234 230 112 141
ADAM19 (MLTNB) 579 268 54 114 0 26
LRRC32 (LERN2) 387 245 157 208 169 124
MEGF9 (EGFL5) 7460 6163 2156 3505 1209 2147
F13A1 (Coagulation factor XIIIa) 4747 4619 672 1116 11 92
ANXA1 (AnnexinA1) 15907 18288 5791 10907 4227 7983
CRISPLD1 (CRISP11) 2920 3508 240 2092 6 169
ZP3 (ZPC) 121 193 23 88 17 7
ANXA6 (Annexin A6) 3114 4377 1155 2358 802 747
PKM (Pyruvate Kinase M1/2) 25948 32490 14618 17958 9745 7312
COL8A2 (Collagen type VIII alpha 2) 322 690 224 298 1 27
SPARC (Osteonectin) 356 582 191 260 0 91
FN1 (Fibronectin 1) 263 636 90 319 3 1
SERPINB8 (CAP20) 2020 1881 1108 1544 554 1046
EMILIN2 (FOAP-10) 12392 11120 9287 10277 6105 7442
THBS3 (Thrombospondin 3) 162 174 108 147 53 66
TGFBI 16486 11110 11670 11476 10750 4134
NID1 (Nidogen 1) 2104 1470 2110 1341 1180 548
APOE (Apolipoprotein E) 4 6 0 140 1 7
CST3 (Cystatin 3) 20438 21230 30788 24878 15339 19399
PLSCR1 2091 6251 1908 6840 881 7178
KAZALD1 (Kazal Type Serine Peptidase Inhibitor Domain 1) 5 52 4 99 5 109
TIMP1 4051 9137 8092 17424 5447 23971
SPON2 (Spondin 2) 22 63 21 53 53 475
SLPI (Antileukoproteinase) 2 49 7 43 15 161
ELANE (Neutrophill Elastase) 137 226 34 98 2 239
DST (Dystonin) 302 92 394 109 438 122
DLG1 (SAP 97) 339 274 283 267 402 168
FBLN1 (Fibulin 1) 0 0 2 0 150 55
FIBCD1 0 0 23 0 245 49
PHOSPHO1 (Phosphoethanolamine) 63 37 450 105 762 152
CDH13 (Cadherin 13) 11 11 59 25 98 107
MMP17 (Matrix Metallopeptidase 17) 997 1035 1135 742 1563 1594
C17orf58 62 116 150 155 159 220
RELL2 (C5orf16) 114 213 292 278 284 278
CSTB (Cystatin B) 1985 3002 3234 3084 4230 3661
HLA_A 25241 38931 20856 47217 14125 38763
MICB (MHC Class I Polypeptide-Related Sequence B) 378 791 239 846 323 700
TAP1 (Antigen Peptide Transporter 1) 6248 16084 5778 18106 3877 11591
PSME2 4012 9841 7681 8967 4380 8249
MICA (MHC Class I Polypeptide-Related Sequence A) 103 149 70 186 116 197
TAP2 (Antigen Peptide Transporter 2) 161 314 220 364 148 427
B2M (Beta-2-Microglobulin) 97285 209219 130494 238195 105771 230314
RFXANK (ANKRA1) 356 613 357 646 273 671
HSPA6 707 852 799 1718 640 849
RELB 1358 1380 992 2109 604 1577
LGMN (Legumain) 50 167 167 1659 33 186
HLA_DPA1 22064 17421 50988 39837 27587 26833
HLA_DRB1 18071 15558 23293 24093 8662 12282
CD74 (HLA-DR-Gamma) 137235 119404 194199 177245 77172 86111
HLA_DRA 59015 45562 81396 79769 25827 29732
HLA_DMA 7199 5440 7088 7001 1879 2198
HLA_DMB 5956 4329 5263 5937 1348 1479
CD1D 4211 3655 2237 3703 397 1218
TAPBP (Tapasin) 16728 17216 15989 14473 10060 8418
ECPAS 1735 1478 1034 1010 960 645
HSPA1 12179 10516 9426 7638 5622 7017
CD1A 158 90 60 65 5 8
HFE 340 192 216 180 97 61
CIITA 3841 2150 2571 2529 2025 567
CANX (Calnexin) 12269 7033 8051 4870 5415 3713
CD1C 431 104 317 173 7 24
HLA_DOA 728 138 1554 258 237 124
HSPA1A 1644 521 1532 684 972 752
HLA_E 42851 51493 61761 75026 86433 85386
UBR1 403 464 480 465 651 304
IFI30 758 385 1029 546 1249 852
HSP90AB1 6559 5439 8737 3953 8708 4930
STAB1 (Stabilin 1) 4530 7671 1682 5754 19 403
CD36 (SCARB3) 17137 18201 4972 10551 332 2803
CD14 55952 55740 11590 45069 664 5629
SCARF1 1223 1175 274 1043 11 108
CD163 (SCARI1) 8415 9019 898 12610 4 513
CD209 (DC-SIGN) 128 85 107 151 9 2
SCARB1 (CLA-1) 1077 621 719 473 69 98
MARCO (SCARA2) 863 1880 1260 4004 208 1233
MSR1 (SCARA1) 37 429 151 1892 78 1144
FCGR1A (CD64) 629 2973 265 3011 74 1361
FCER1A (FC Epsilon Receptor Ia) 413 21 24 7 0 25
FCAR (CD89) 2010 1918 612 1814 227 418
FCGR2A (CD32A) 7801 5839 5913 7843 4107 5649
FCGRT (Neonatal Fc receptor) 14053 10756 11047 13065 5431 6933
FCGBP (IgG Fc binding protein) 23 5 0 9 116 3
FCGR3B (CD16B) 11 133 237 4923 615 11281
FCGR2B (CD32B) 148 37 153 144 88 371
FCMR (Fc Fragment Of IgM Receptor) 8 24 66 42 17 182
FCGR3A (CD16A) 542 3443 45524 33446 85382 86107
FCRL1 (IFGP1) 0 5 50 27 33 59
PLXND1 (Plexin D1) 2800 1359 1437 1097 1239 283
LTB4R (Leukotriene B4 receptor) 1902 1884 523 1662 235 392
PTAFR (Platelet Activating Factor Receptor) 11802 8237 3791 8301 851 1411
PTGDR2 (Prostaglandin D2 Receptor 2) 0 5 811 51 2796 430
C5AR1 (Complement C5a Receptor 1) 4114 6272 4358 10234 5736 8452
CCR5 232 222 195 823 22 247
CMKLR1 (Chimerin Receptor) 499 1736 1419 5156 361 2681
CCRL2 169 346 169 1065 153 537
FPR2 (Formyl Peptide Receptor 2) 1827 5497 1762 7522 1171 5877
FPR1 (Formyl Peptide Receptor 1) 16343 23275 6356 27018 2212 14943
PLAUR (Plasminogen Activator, Urokinase Receptor) 5467 9256 3575 12058 3143 5493
CCR1 4012 6783 1893 8573 724 5339
CXCR2 (IL-8RB) 778 453 147 780 49 1084
CXCR4 2746 2220 1391 3973 206 2756
C5AR2 (Complement Component 5a Receptor 2) 135 172 277 266 473 412
CXCR1 (IL-8RA) 49 49 5 163 0 537
PLXNA2 (Plexin A2) 211 160 214 407 376 1009
CYSLTR2 (Cysteinyl Leukotriene Receptor 2) 214 73 300 219 215 569
CX3CR1 (Fractalkine Receptor) 10360 3511 20341 11633 24924 44246
CLEC2B 224 400 475 695 565 2168
DHX58 537 2282 509 3190 630 2249
DDX58 843 3128 738 3128 710 3663
IFIH1 934 2625 746 4158 857 3820
TLR4 5313 5130 3863 6702 2735 5485
CLEC4A 1267 829 814 1176 457 714
CLEC10A 544 341 361 893 1 90
TLR6 315 213 276 399 115 249
CLEC4D 511 641 80 530 0 128
CLEC6A 124 224 5 182 0 13
CLEC5A 401 699 36 455 0 17
CLEC4E 1018 1169 152 1633 1 323
NLRP3 3728 6594 1399 7861 360 1947
TLR2 4603 5434 3203 6162 2265 3455
CLEC11A 184 200 97 174 28 60
NOD2 3309 2981 2191 2784 1413 1713
NLRP12 3200 3046 827 1389 479 299
CLEC4G 256 165 129 96 14 21
TLR5 1484 986 726 1004 430 430
PGLYRP2 26 26 131 30 246 204
NLRP6 10 15 165 103 814 694
CLEC4F 7 6 1017 114 1466 825
NLRP1 8972 3644 5491 4208 9548 3617
Z-score
-2 -1 0 1 2

Figure S7. Side-by-side comparison of gene expression between the CM, IM, and NCM subsets and dengue (D) versus steady-state (H) in each subset for the indicated immunological categories. Related to Figure 2-6.

Left, heat maps of the indicated genes in the CM, IM, and NCM subsets from Healthy individuals (H, n=3) and Dengue patients (D, n=6). Z-scores of normalized counts were used for plotting the heatmap. The gradient of high to low gene expression is indicated from red to blue color. Right, average normalized counts were indicated. Shown is an expression for chemokines, cytokines, semaphorins and RNases, s100 proteins, integrins, lectins and selectins, other cell adhesion molecules, complement molecules, another effector molecule, extracellular matrix interacting proteins, antigen presentation and processing, scavenger receptors, FC-gamma receptors, chemokine receptors, and pattern recognition receptors.

Table S1. Characteristics of the individuals from whom the three monocyte subsets were sorted. Related to Figure 1A.

Subject *	Dengue Status **	Days post onset of symptoms	Age (in years)	Primary/ Secondary infection *** (P/S)	Disease severity **** (DI/ DW/ SD)	Hemorrhage ***** (Y/N)	Frequencies of monocyte subsets******			Percent infected cells*******		
							CM	IM	NCM	CM	IM	NCM
1	Dengue Confirmed	3	13	S	DI	N	62.7	30.3	3.94	ND*	ND	ND
2	Dengue Confirmed	4	13	P	SD	Y	64.2	35	0.79	ND	ND	ND
3	Dengue Confirmed	3	9	S	DW	N	65.6	21.4	12.5	ND	ND	ND
4	Dengue Confirmed	5	13	P	DW	N	63.4	29.5	7.09	67.9	73.1	56.5
5	Dengue Confirmed	3	14	S	DW	Y	79.6	19.2	1.14	27.2	47.7	32.8
6	Dengue Confirmed	6	7	S	DW	Y	55.4	40.6	3.93	0.13	0.27	0.67
7	Healthy	NA	2	NA	NA	NA	72.6	10.5	16.9	NA*	NA	NA
8	Healthy	NA	2	NA	NA	NA	70.4	16	13.6	NA	NA	NA
9	Healthy	NA	10	NA	NA	NA	82.3	5.7	12	NA	NA	NA

** All were children age between 5 to 15 years.*

*** Dengue confirmation was by febrile fever (2-6 days post onset of symptoms) and positive for dengue NS1 and or PCR and or IgM.*

****Primary and Secondary is defined by the WHO criteria,* primary dengue infections were those that had an IgM/IgG ratio >1.2 and secondary infections were those that had IgM/IgG ratio <1.2

*****Disease severity is classified based on WHO, 2009 criteria. DI- Dengue Illness, DW- Dengue with Warning Sign and SD- Severe Dengue*

****** By WHO 1997 classification, patients who are classified as DF don't have hemorrhage and those patients who are classified as DHF have hemorrhage.*

******** Frequencies within the monocyte gated population as shown in gating strategy in Figure S1.*

Table S5. Characteristics of dengue infected patients. (n=40). Related to Figure 7.

Age in years Mean (range)	10.9 (5-15)
Day of fever Mean (range)	4.3 (2-7)
Male/Female	22/18
Severity	
Dengue Illness (DI)	14
Dengue with warning Sign (DW)	16
Severe Dengue (SD)	10