

Supplementary Information for:

**Deconvolution of Heterogeneous Wound Tissue Samples into Relative Macrophage Phenotype Composition via Models based on Gene Expression**

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**Supplemental Table 1.** Primers used for RT-PCR analysis

Gene	Forward	Reverse
APOL1	TTACCAACTCACACGAGGCATT	CTCCACCTGTTCACCGCTTT
NOD2	CACCGTCTGGAATAAGGGTACT	TTCATACTGGCTGACGAAACC
ASPHD2	CCGAGGACTGATTGTCTGACC	CAGTACCACACGAAGAGGACC
CLEC4D	CTGATACCTTCGGTTATTGCTGT	GCACTCCTGTGCCTCTCTTAC
CCL19	CCAGCCTCACATCACTCACACCT TGC	TGTGGTGAACACTACAGCAGGCACC C
XAF1	GAGCATGCAGAAGTCCTCGCT	CCTGTTCACTGCGACAGACATCT
ALOX15	GGGCAAGGAGACAGAACTCAA	CAGCGGTAACAAGGGAACCT
CTNNAL1	GGGACTGGAGATCAAACTCG	GTCCTACACGCTGAATTGCTT
RAMP1	GAGACGCTGTGGGTGACTG	TCGGCTACTCTGGACTCCTG
CCL26	CTGGACCTGGGTGCGAAGC	TGGATGGGTACAGACTTTCTTGCC
PLCB1	AGCTCTCAGAACAAGCCTCCAAC A	ATCATCGTCGTCGTCACCTTCCGT
WNT5B	AGATCGTGGACCAGTACATCTG	TTACGGAACCCATCTACATTCTG
CLEC4G	GCCATGGACACCACCAGGTACAG C	GACTCAGCAGTTGTGCCTTTTCTC

**Supplementary Table 2.** All M1 and M2 genes found to be significantly DE between all three datasets included in the analysis.

M1	M2
WARS	ALOX15
STAT1	CCL26
TNFAIP2	CD180
SLAMF7	CD1C
CD274	CD1E
SAMD9L	CD200R1
XRN1	CLEC4A
TRIM22	CLEC4G
GBP1	CTNNAL1
PARP9	DACT1
GBP5	DNASE1L3
MX1	ESPNL
TNFSF13B	F13A1

DDX60	FAM198A
SERPING1	FCER2
TNFAIP6	GALNT18
RSAD2	GGTA1P
PSTPIP2	HOMER2
XAF1	PLCB1
DDX58	PLXDC1
MYO1G	PPP1R14A
IFIT2	RAMP1
APOL3	SPINT2
APOL1	SYT6
IFI35	TNFRSF11A
TIFA	WNT5B
GBP2	
IDO1	
IFIT5	
TYMP	
NOD2	
HESX1	
GBP4	
PTGS2	
IFI44	
EPSTI1	
TNFSF10	
IFIH1	
PDE4B	
IFIT3	
SP140	
C1S	
IFITM3	
IL15	
OASL	
IL15RA	
IRF1	
IRF7	
ISG15	
FAM26F	
GCH1	
CRISPLD2	
LIMK2	
PLEKHG3	

RTP4	
NAMPT	
CLEC4E	
OAS2	
OAS3	
BATF2	
RARRES3	
ASPHD2	
CXCL1	
PSMB9	
CD38	
C5orf56	
GRIN3A	
RHOH	
IFI44L	
KLHDC7B	
APOBEC3B	
ISG20	
C1R	
SNX10	
SOD2	
AIM2	
IFI27	
STX11	
TAP1	
EMR1	
USP30-AS1	
IFITM1	
ANKRD22	
IL31RA	
C4B	
CLEC4D	
ETV7	
LAG3	
VAMP5	
UBD	
CXCL10	
CCL19	

**Supplementary Table 3.** List of genes determined to be associated with additional cell types present in the wound environment. This is not an exhaustive list of all cell types expressing these genes.

<b>M1</b>	<b>Cell type</b>	<b>Source</b>	<b>M2</b>	<b>Cell type</b>	<b>Source</b>
WARS	Endothelial cells, B and T lymphocytes	GeneCards	CD180	B lymphocytes, natural killer cells, monocytes	GeneCards
STAT1	Fibroblasts	Human Protein Atlas	CD1C	B and T lymphocytes	GeneCards
TNFAIP2	Epidermal cells, fibroblasts, melanocytes	Human Protein Atlas	CD1E	Dendritic cells, T cells	GeneCards
SLAMF7	Natural killer cells, activated B cells, monocytes	GeneCards	CD200R1	T cells, B cells, neutrophils	GeneCards
CD274	Activated T and B cells, dendritic cells, keratinocytes, and monocytes	GeneCards	CLEC4A	Monocytes, neutrophils, B cells	GeneCards
SAMD9L	Fibroblasts, keratinocytes, epidermal cells, dendritic cells, melanocytes	Human Protein Atlas	DACT1	Myocytes, T cells, B Cells	GeneCards
XRN1	Fibroblasts, keratinocytes, epidermal cells, dendritic cells, melanocytes	Human Protein Atlas	DNASE1L3	Epidermal cells	Human Protein Atlas
TRIM22	Fibroblasts, keratinocytes, epidermal cells, dendritic cells, melanocytes	Human Protein Atlas	ESPNL	Epidermal cells	Human Protein Atlas
GBP1	Fibroblasts	Human Protein Atlas	F13A1	Fibroblasts	Human Protein Atlas
PARP9	B-cells, natural killer cells, and lymphocytes	GeneCards	FAM198A	Fibroblasts, epidermal cells, melanocytes	Human Protein Atlas
GBP5	B and T lymphocytes, natural killer cells	GeneCards	FCER2	B cells, melanocytes	Human Protein Atlas, GeneCards
MX1	Fibroblasts, keratinocytes, epidermal cells	Human Protein Atlas	GALNT18	Fibroblasts, keratinocytes, epidermal cells, dendritic cells	Human Protein Atlas
TNFSF13B	Fibroblasts, keratinocytes, epidermal cells, dendritic cells, melanocytes	Human Protein Atlas	GGTA1P	Pseudogene	GeneCards
DDX60	Fibroblasts, keratinocytes, epidermal cells,	Human Protein Atlas	HOMER2	Fibroblasts, keratinocytes, epidermal cells,	Human Protein Atlas

	dendritic cells, melanocytes			dendritic cells	
SERPING1	Epidermal cells	Human Protein Atlas	PPP1R14A	Melanocytes	Human Protein Atlas
TNFAIP6	Fibroblasts	Human Protein Atlas	SPINT2	Keratinocytes, epidermal cells	Human Protein Atlas
RSAD2	Epidermal cells	Human Protein Atlas	SYT6	Fibroblasts, keratinocytes, dendritic cells, melanocytes	Human Protein Atlas
PSTPIP2	Fibroblasts, keratinocytes, epidermal cells, dendritic cells, melanocytes	Human Protein Atlas	TNFRSF11 A	Endothelial cells	GeneCards
DDX58	Fibroblasts, keratinocytes, epidermal cells, dendritic cells	Human Protein Atlas			
MYO1G	Dendritic cells, neutrophils, B and T lymphocytes	GeneCards			
IFIT2	B lymphocytes and T cells	GeneCards			
APOL3	B lymphocytes and T cells, endothelial cells	GeneCards			
IFI35	Monocytes, neutrophils	GeneCards			
TIFA	Fibroblasts, epidermal cells, keratinocytes	Human Protein Atlas			
GBP2	Keratinocytes, dendritic cells, epidermal cells	Human Protein Atlas			
IDO1	Fibroblasts, keratinocytes, epidermal cells, dendritic cells, melanocytes	Human Protein Atlas			
IFIT5	T cells, B lymphocytes	GeneCards			
TYMP	Keratinocytes, epidermal cells	Human Protein Atlas			
HESX1	Fibroblasts, keratinocytes, epidermal cells, dendritic cells, melanocytes	Human Protein Atlas			
GBP4	Keratinocytes, epidermal cells	Human Protein Atlas			
PTGS2	Fibroblasts, keratinocytes, dendritic cells, melanocytes	Human Protein Atlas			
IFI44	Natural killer cells, endothelial cells, T	GeneCards			

	cells				
EPSTI1	Keratinocytes, epidermal cells, melanocytes	Human Protein Atlas			
TNFSF10	Fibroblasts	Human Protein Atlas			
IFIH1	Fibroblasts, keratinocytes, epidermal cells, dendritic cells, melanocytes	Human Protein Atlas			
PDE4B	Keratinocytes, epidermal cells	Human Protein Atlas			
IFIT3	Fibroblasts, melanocytes	Human Protein Atlas			
SP140	B cells, endothelial cells, myocytes	GeneCards			
C1S	Monocytes	GeneCards			
IFITM3	Fibroblasts, melanocytes, epidermal cells	Human Protein Atlas			
IL15	Epidermal cells	Human Protein Atlas			
OASL	Epidermal cells	Human Protein Atlas			
IL15RA	Fibroblasts, keratinocytes, epidermal cells, dendritic cells, melanocytes	Human Protein Atlas			
IRF1	Fibroblasts, keratinocytes, epidermal cells	Human Protein Atlas			
IRF7	Keratinocytes, dendritic cells, keratinocytes	Human Protein Atlas			
ISG15	Keratinocytes, epidermal cells, dendritic cells, melanocytes	Human Protein Atlas			
FAM26F	Fibroblasts	Human Protein Atlas			
GCH1	Keratinocytes, epidermal cells, dendritic cells, melanocytes	Human Protein Atlas			
CRISPLD2	Keratinocytes	Human Protein Atlas			
LIMK2	Fibroblasts, keratinocytes, epidermal cells, dendritic cells, melanocytes	Human Protein Atlas			
PLEKHG3	T cells, keratinocytes	Li, J., et al.			

		(2015). <i>The Journal of dermatology</i> , 42 (9), 874-880.			
RTP4	Fibroblasts, keratinocytes, epidermal cells, dendritic cells, melanocytes	Human Protein Atlas			
NAMPT	Neutrophils, B cells, natural killer cells, T cells	GeneCards			
CLEC4E	Fibroblasts	Human Protein Atlas			
OAS2	Fibroblasts, keratinocytes, epidermal cells, dendritic cells, melanocytes	Human Protein Atlas			
OAS3	Keratinocytes, melanocytes, epidermal cells	Human Protein Atlas			
BATF2	Keratinocytes, melanocytes, epidermal cells	Human Protein Atlas			
RARRES3	Keratinocytes	Hsu, T. H., et al. (2015). <i>Molecular &amp; Cellular Oncology</i> , 2(4)e 999512.			
PSMB9	Keratinocytes, epidermal cells	Human Protein Atlas			
CD38	B and T lymphocytes, natural killer cells	GeneCards			
C5orf56	Non-coding	GeneCards			
GRIN3A	White blood cells	GeneCards			
RHOH	B cells, T cells	GeneCards			
IFI44L	T cells	GeneCards			
KLHDC7B	Whole blood, white blood cells	GeneCards			
APOBEC3 B	B lymphocytes, mesenchymal stem cells	GeneCards			
ISG20	Fibroblasts, dendritic cells	GeneCards			
C1R	Fibroblasts	Human Protein Atlas			
SNX10	Melanocytes, epidermal cells	Human Protein Atlas			
SOD2	Fibroblasts, keratinocytes, epidermal cells, melanocytes	Human Protein Atlas			

AIM2	Keratinocytes, epidermal cells, dendritic cells, melanocytes	Human Protein Atlas			
IFI27	Epidermal cells	Human Protein Atlas			
STX11	Fibroblasts, epidermal cells	Human Protein Atlas			
TAP1	Fibroblasts, keratinocytes, epidermal cells, dendritic cells, melanocytes	Human Protein Atlas			
EMR1	Eosinophils	Hamann, J., et al. (2007). <i>European journal of immunology</i> , 37 (10) 2797-2802.			
USP30-AS1	Non-coding	GeneCards			
IFITM1	Natural killer cells, T cells	Human Protein Atlas			
ANKRD22	Peripheral blood mononuclear cells, T cells	Collares, C. V., et al. (2013). <i>BMC research notes</i> , 6(1), 1.			
IL31RA	Epithelial cells, blood cells	GeneCards			
C4B	B cells, natural killer cells	GeneCards			
LAG3	Natural killer cells, T cells	GeneCards			
VAMP5	B cells, T cells, Natural killer cells	GeneCards			
UBD	Keratinocytes	Human Protein Atlas			
CXCL10	Keratinocytes	Flier, J., et al. (2001). <i>The Journal of pathology</i> , 194 (4), 398-405.			



**Supplementary Table 4.** Log<sub>2</sub>FC of gene expression between baseline and activated cell types for the M1 and M2 markers. The following datasets were used in this analysis: GSE30355 – Human keratinocytes response to injury, GSE21648 – Dermal fibroblasts, GSE7890 – Gene expression in keloid fibroblasts, GSE3037 – Neutrophils from sepsis response to LPS, GSE38396 – Endothelial cell response to type 2 diabetes, GSE39596 – T cell activation transcriptome. Genes in bold font were eliminated from the list of M1 and M2 markers. N/A indicates not included in study.

Gene	Keratinocyte	Dermal fibroblast	Keloid Fibroblast	Neutrophil	Endothelial Cell	T-cell	B-cell
APOL1	0.147	0.706	0.229	-0.132	0.095	-0.116	-0.071
CLEC4D	0.110	N/A	-0.020	N/A	-0.080	-0.143	N/A
<b>CXCL1</b>	-6.09	-4.89	<b>3.59</b>	-2.147	-0.208	0.144	0.323
<b>ETV7</b>	<b>3.3</b>	0.106	-0.0246	-0.108	0.126	-0.243	0.078
ASPHD2	1.29	N/A	-0.060	0.208	-0.329	-1.039	N/A
CCL19	-0.35	-1.18	0.076	0.052	0.254	-0.096	0.149
XAF1	1.09	0.367	0.277	0.131	-0.431	0.174	0.901
NOD2	0.663	-0.021	-0.021	0.342	0.814	-0.254	-0.126
CCL26	-0.281	0.556	0.252	N/A	0.220	-0.106	N/A
CLEC4G	0.001	N/A	-0.056	N/A	-0.264	-0.051	N/A
CTNNAL1	-0.647	-0.639	-0.281	-0.097	0.499	-1.238	-1.35
ALOX15	-0.001	-0.902	-0.054	0.025	0.054	0.073	0.162
PLCB1	-0.795	-0.033	0.774	-0.176	-0.207	0.241	0.118
<b>PLXDC1</b>	<b>1.66</b>	1.06	-0.035	-0.033	-0.605	<b>2.294</b>	0.262
RAMP1	0.224	-0.437	-0.311	-0.056	0.244	-0.108	0.175
WNT5B	0.172	-1.18	0.179	-0.048	-0.334	0.057	0.191

**Supplementary Table 5.** Log<sub>2</sub>FC of gene expression between macrophages and dendritic cells from data available from GSE33273 consisting of macrophage and dendritic cell samples activated from primary human monocytes.

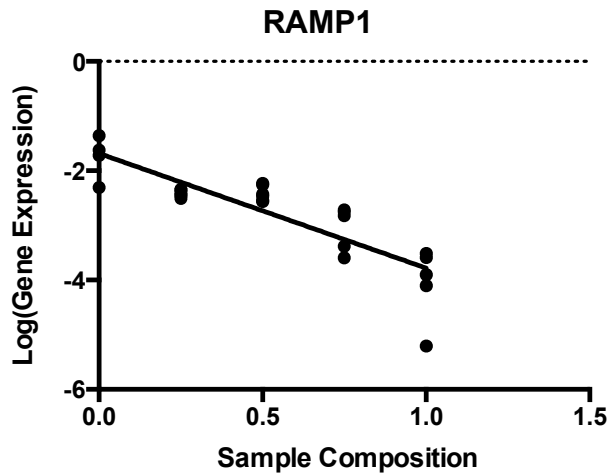
Gene	Log <sub>2</sub> FC	FDR corrected p-val	M2 Gene	Log <sub>2</sub> FC	FDR corrected p-val
XAF1	4.48	0.011	CCL26	4.74	0.056
CCL19	8.39	0.024	CLEC4G	5.05	0.084
APOL1	3.66	0.022	ALOX15	2.07	0.501
ASPHD2	2.51	0.155	PLCB1	0.59	0.676
CLEC4D	2.51	0.398	RAMP1	5.84	0.024
NOD2	-0.21	0.97	WNT5B	0.79	0.97

**Supplementary Table 6. Expression of final genes in macrophages isolated from tissues.**

Expression is coded as Y if any probe mapping to the gene has a mean expression in the described samples that is higher than the first quartile (25%) of expression across all samples present in the dataset. It is coded as N otherwise.

Dataset	Description	XAF1	CCL19	ASPHD2	CCL26	CLEC4G	ALOX15	PLCB1	RAMP1	WNT5B
GSE54350	Adipose macrophages from obese patients	Y	Y	Y	Y	Y	Y	Y	N	Y
GSE40885	Alveolar macrophages exposed to LPS	Y	Y	Y	Y	Y	Y	Y	Y	Y
GSE8823	Alveolar macrophages from smokers	Y	Y	Y	Y	Y	N	Y	Y	Y
GSE8823	Alveolar macrophages from non smokers	Y	Y	Y	Y	N	N	Y	Y	Y
GSE42495	Macrophages from T cell/histiocyte rich B cell lymphoma patients	Y	Y	Y	Y	Y	Y	Y	Y	Y
GSE13896	Alveolar macrophages from non smokers	Y	Y	Y	Y	N	N	Y	Y	Y
GSE13896	Alveolar macrophages from smokers	Y	Y	Y	Y	N	N	Y	Y	Y

## Supplementary figures



**Supplementary Figure 1.** An example of a linear regression fit to gene expression as a function of sample composition for one of the included genes (RAMP1). In order to calculate a correlation coefficient for each gene and sample composition, the sum of the squares of the distance between each point and the line of best fit is compared to the null hypothesis that a flat horizontal line best fits the data, and the resulting ratio of the sum of square of distances from each point to the line of best fit and the horizontal line is subtracted from 1 to represent the  $R^2$  value, and the square root represents the correlation coefficient.