SUPPLEMENTARY DATA

'Adipose recruitment and activation of plasmacytoid dendritic cells fuel metaflammation'

Amrit Raj Ghosh et al.

Supplementary Table S1. Primer oligonucleotide sequences used in real time PCR

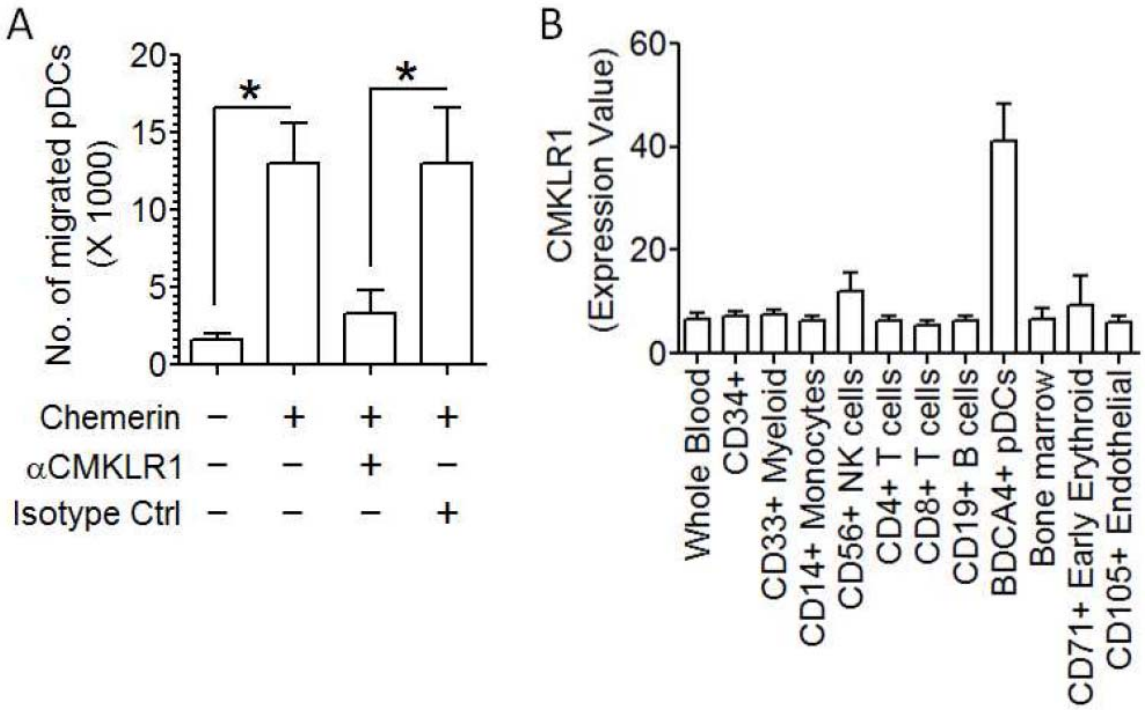
GENES	PRIMER	SEQUENCE
IRF7	FORWARD	ACCTGTAGCCCCCTCTGCCA
	REVERSE	GTGGGGCTGCCCTCTCAGGA
TRIP14	FORWARD	GAGCCCTGGGGCCTTCTCTTC
	REVERSE	GCTCTGGGGGACCTGGCTTTC
MX1	FORWARD	ACTCCTCTGGGAGGGTGGCT
	REVERSE	GCACCTCCTTGGAATGGTGGCT
ISG15	FORWARD	CTACGAGGTCCGGCTGACGC
	REVERSE	GTGGAGGCCCTTAGCTCCGC
TIG2	FORWARD	TGAGGAGCACCAGGAGAC
	REVERSE	TTGGAGAAGGCGAACTGTC
CLEC4C	FORWARD	GACCGAGAGAAAGGACTCTGGTGG
	REVERSE	AGTCCAAGGGGTTGGGCAGC
HMGB1	FORWARD	ACATCCAAAATCTTGATCAGTTA
	REVERSE	AGGACAGACTTTCAAAATGTTT
IRF5	FORWARD	CGGACTGATGTGGAGATGTG
	REVERSE	CTCTCCTTCTTGGCCCAAAT
CCL22	FORWARD	GCAACTGAGGCAGGCCCCTA
	REVERSE	CCTGGAGGAGCCAAGGCCAC
NOS2	FORWARD	AGCTCAACAACAAATTCAGG
	REVERSE	ATCAATGTCATGAGCAAAGG
F13A1	FORWARD	GAGCGCCTGCAGGACCTTGT
	REVERSE	GCCCTCTGCGGACAATCAGC
TLR9	FORWARD	AAATCCCTCATATCCCTGTC
	REVERSE	TTGTAATAACAGTTGCCGTC
18S	FORWARD	GTAACCCGTTGAACCCCATT
	REVERSE	CCATCCAATCGGTAGTAGCG

SUPPLEMENTARY DATA

Supplementary Figure 1. Function and restricted expression of chemerin receptor CMKLR1 plasmacytoid dendritic cells.

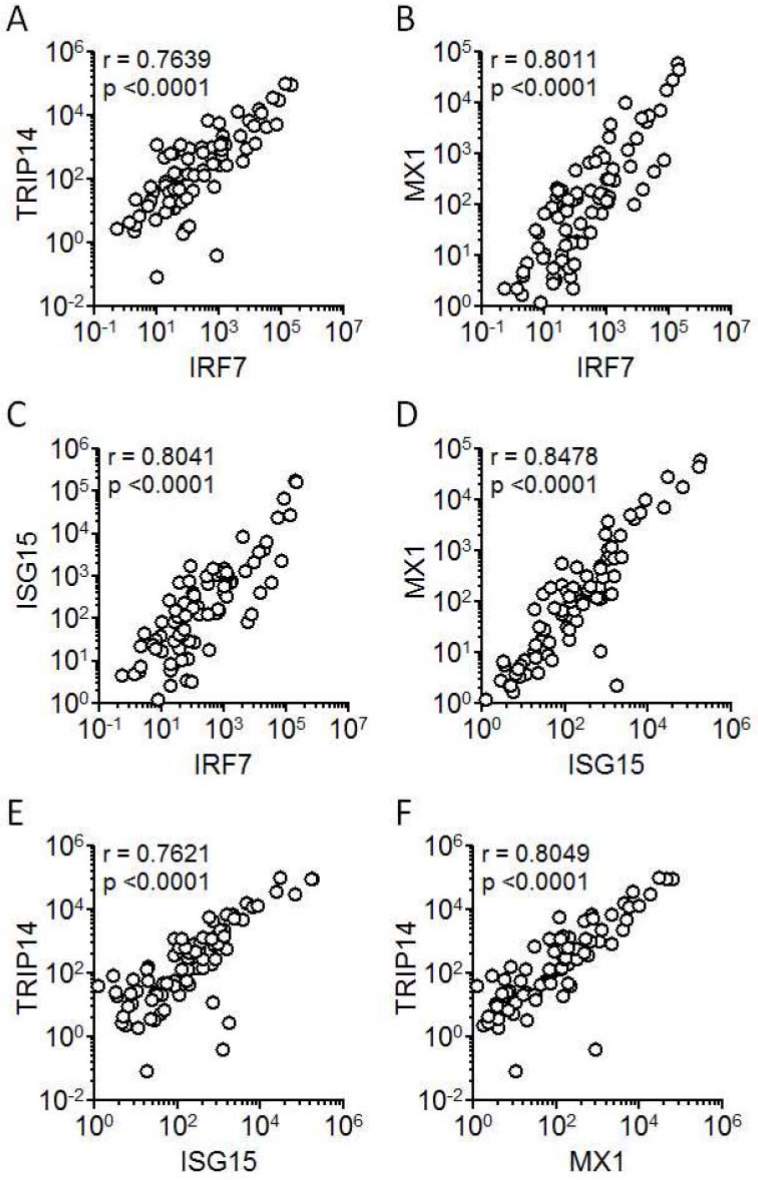
(A) Migration of pDC was assessed in response to human recombinant chemerin in transwells. pDCs were either untreated or pretreated with anti-CMKLR1 or isotype control antibody and the number of migrated pDCs, were compared by performing two tailed paired T test (*$p < 0.05$). Cumulative data of three independent experiments are represented.

(B) Expression data of CMKLR1 on different human immune cell subsets collated from high density oligonucleotide array database GSE1133 (accessed from http://ds.biogps.org/?dataset=GSE1133&gene=1240), showing restricted expression on plasmacytoid dendritic cells. Normalized expression data (mean ± SD) are presented for two different probes targeting CMKLR1 (207652_s_at and 210659_at) each in duplicate.



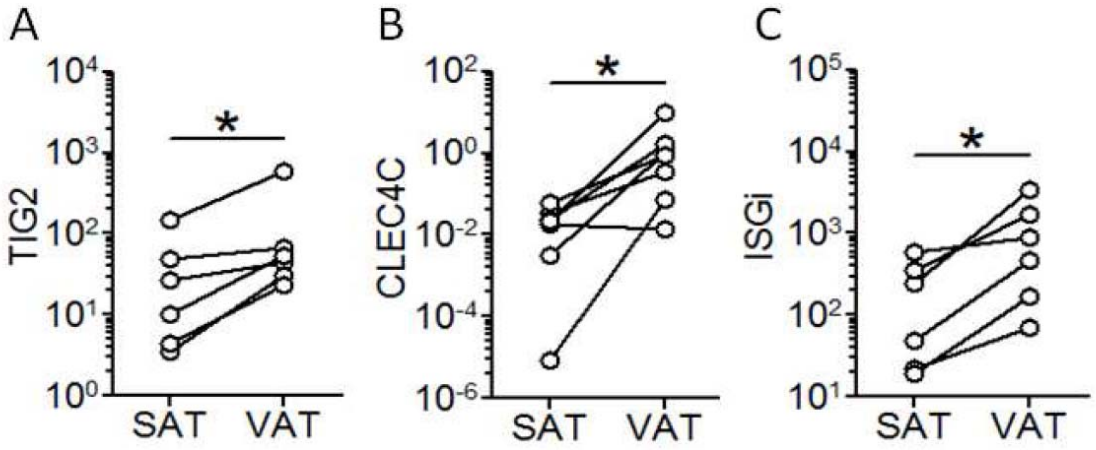
SUPPLEMENTARY DATA

Supplementary Figure 2. Coherent expression of the selected type I IFN signature genes in VAT. (A)-(F) Coherent expression of the four interferon signature genes or ISGs (*IRF7*, *TRIP14*, *MX1* and *ISG15*) in VAT, validating their selection to formulate the interferon signature gene index (ISGi). Correlation of expression was checked with Spearman's ranked correlation (N=83).



SUPPLEMENTARY DATA

Supplementary Figure 3. Comparison of gene expression between subcutaneous and visceral adipose tissue samples.
(A)-(C) Paired samples of subcutaneous adipose tissue (SAT) with VAT samples (N=6) were assessed for expression of TIG2, enrichment of CLEC4C transcript and value of interferon signature gene index. Comparisons were done by performing two tailed paired T test (*$p < 0.05$).

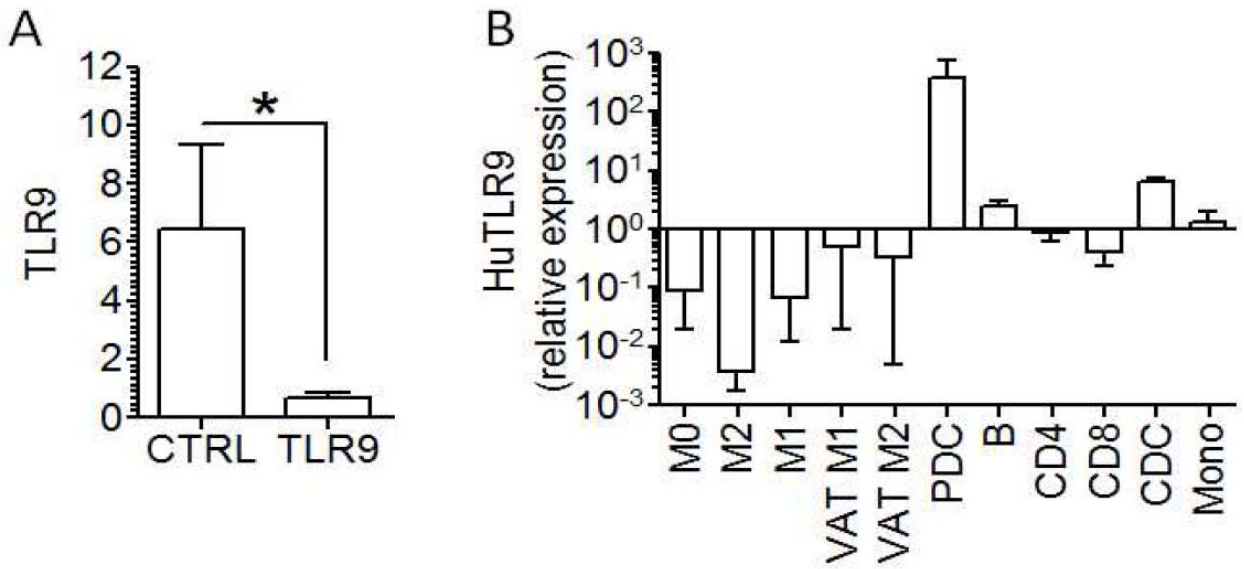


SUPPLEMENTARY DATA

Supplementary Figure 4. TLR9 knock down in pDCs and expression of TLR9 in different immune cell subsets.

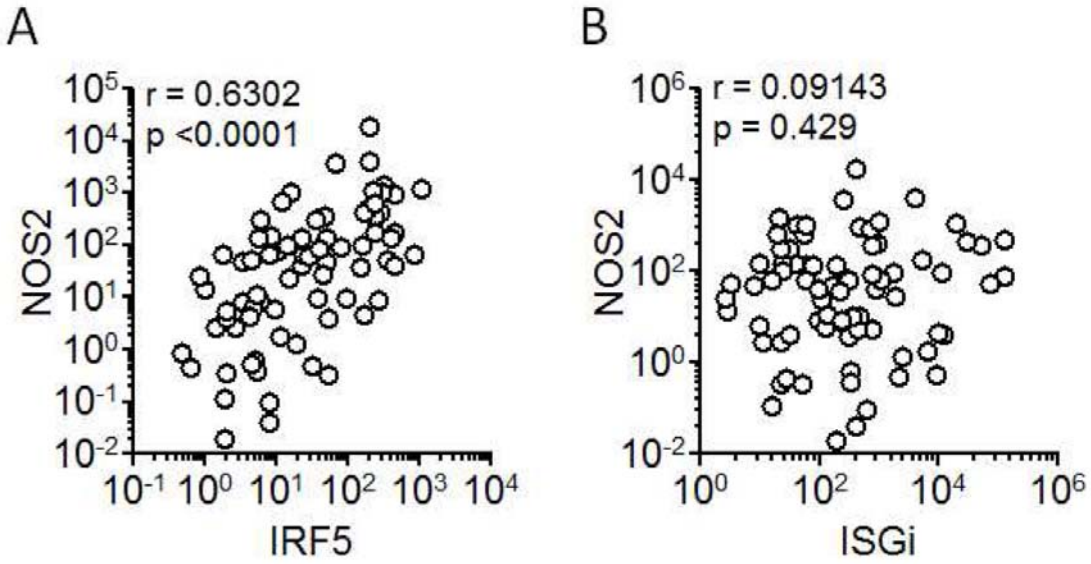
(A) TLR9 expression was knocked down in pDCs using siRNA transfection. Comparison between control and target siRNA transfection was done by paired T test (*$p<0.05$).

(B) Expression of TLR9 was assessed by real time PCR in circulating plasmacytoid DCs (pDC), B cells (B), CD4 T cells (CD4), CD8 T cells (CD8), conventional dendritic cells (cDC), monocytes (Mono), *in vitro* generated M1 and M2 macrophages (M1 and M2) and *ex vivo* isolated M1 and M2 macrophages from visceral adipose tissue of obese individuals (Vat M1 and Vat M2).



SUPPLEMENTARY DATA

Supplementary Figure 5. Coherent expression of the genes selected as signature for M1 macrophages.
(A) Coherent expression of the genes *IRF5* and *NOS2* in VAT. Correlation of expression was checked with Spearman's ranked correlation (N=79, r= 0.6302, p <0.0001).
(B) Relationship between VAT expression of NOS2 and tissue ISGi (N=77, r = 0.99143, p = 0.429).



SUPPLEMENTARY DATA

Supplementary Figure 6. Comparison of group L and group R with respect to body mass index and gene expression
(A) Comparison of body mass index between group L and group R (NS= not significant).
(B)-(D) Comparison between group L and group R for expression of pDC-specific transcript CLEC4C (B), interferon signature gene index (C) and expression of M1 macrophage signature IRF5 (D)