

## Supplementary Materials

*Article*

# Tissue Inhibitor of Metalloproteinases-1 Interacts with CD74 to Promote AKT Signaling, Monocyte Recruitment Responses, and Vascular Smooth Muscle Cell Proliferation

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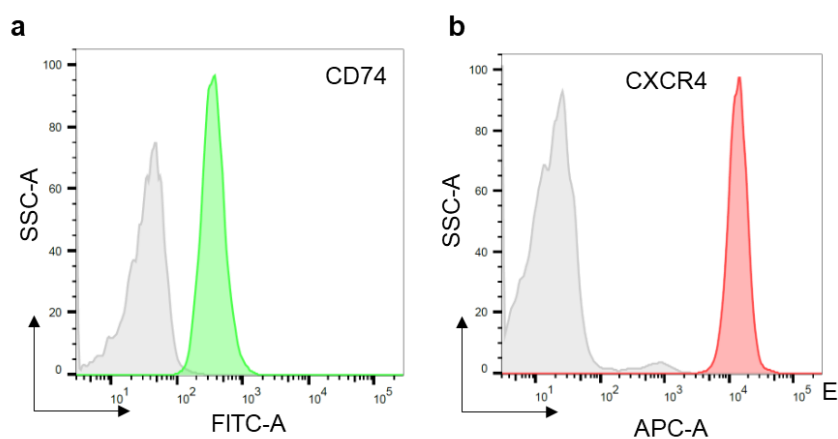
**Supplementary Table S1:** Quantification of the 38 positive spots obtained from the phosphorylation kinase array in response to TIMP-1 over time.

Target	Phosphorylation Site	10 min	30 min	60 min
$\beta$ -catenin	--	102	93	116
STAT-2	Y689	104	102	97
p38 $\alpha$	T180/Y182	100	86	82
JNK 1/2/3	T183/Y185, T221/Y223	98	108	102
Fgr	Y412	102	101	100
CREB	S133	165	158	32
STAT 5a/b	Y694/Y699	92	88	83
PDGF R $\beta$	Y751	106	95	106
Lck	Y394	103	98	105
GSK-3 $\alpha/\beta$	S21/S9	24	23	135
EGF R	Y1086	100	96	98
WNK1	T60	21	46	21
PLC- $\gamma$ 1	Y783	104	92	97
Lyn	Y397	105	101	99
GSK-3 $\beta$	S9	62	58	68
eNOS	S1177	237	95	101
Yes	Y426	102	95	93
Src	Y419	98	117	91
MSK 1/2	S376/S360	96	102	104
HSP27	S78/S82	128	127	99
ERK 1/2	T202/Y204, T185/Y187	81	114	77
STAT6	Y641	124	106	91
STAT1	Y701	102	98	97
PYK2	Y402	129	100	95
p70 S6 kinase	T389	111	96	100
p53	S15	111	103	101
Chk-2	T68	106	100	106
Akt 1/2/3	T308	121	99	102
HSP60	--	104	101	284
STAT3	Y705	108	97	99
RSK 1/2	S221/S227	108	99	104
p70 s6 kinase	T421/S424	100	117	100
c-Jun	S63	107	93	105
Akt 1/2/3	S473	114	447	107
p53	S392	113	127	99
PRAS40	T246	108	117	121
RSK 1/2/3	S380/S386/S377	96	95	127
STAT3	S727	106	111	140

**Supplementary Table S2:** SpotON prediction of active residues in the TIMP-1/CXCR4 interaction.

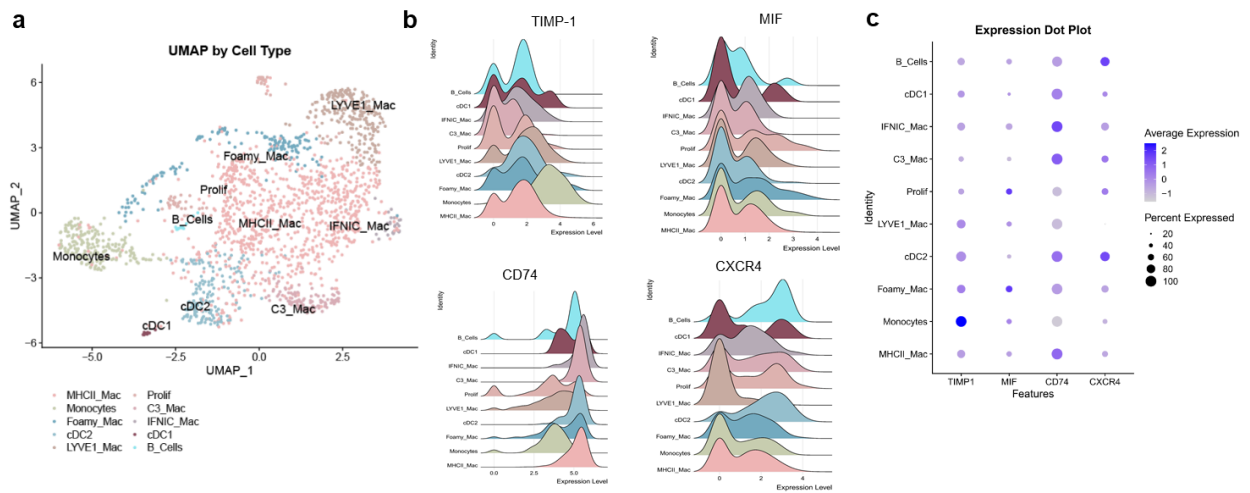
TIMP-1				CXCR4			
Null Spots		Hot Spots		Null Spots		Hot Spots	
PDB Nr.	AA	PDB Nr.	AA	PDB Nr.	AA	PDB Nr.	AA
1	CYS	none		35	ASN	none	
2	THR			151	LEU		
3	CYS			179	GLU		
4	VAL			180	ALA		
5	PRO			181	ASP		
6	PRO			182	ASP		
7	HIS			183	ARG		
10	THR			184	TYR		
14	ASN			185	ILE		
33	THR			187	ASP		
34	LEU			188	ARG		
36	GLN			190	TYR		
64	PRO			191	PRO		
65	ALA			192	ASN		
66	MET			193	ASP		
67	GLU			194	LEU		
68	SER			195	TRP		
69	VAL			196	VAL		
70	CYS			197	VAL		
73	PHE			201	PHE		
88	LYS			265	ILE		
90	GLN			266	LEU		
97	THR			267	LEU		
99	CYS			268	GLU		
129	VAL			269	ILE		
130	PHE			271	LYS		
131	PRO			273	GLY		
132	CYS			274	CYS		
133	LEU			275	GLU		
134	SER			277	GLU		
150	GLN			278	ASN		
151	LEU			281	HIS		
153	GLN						
154	GLY						
155	SER						

# Supplementary Figure S1



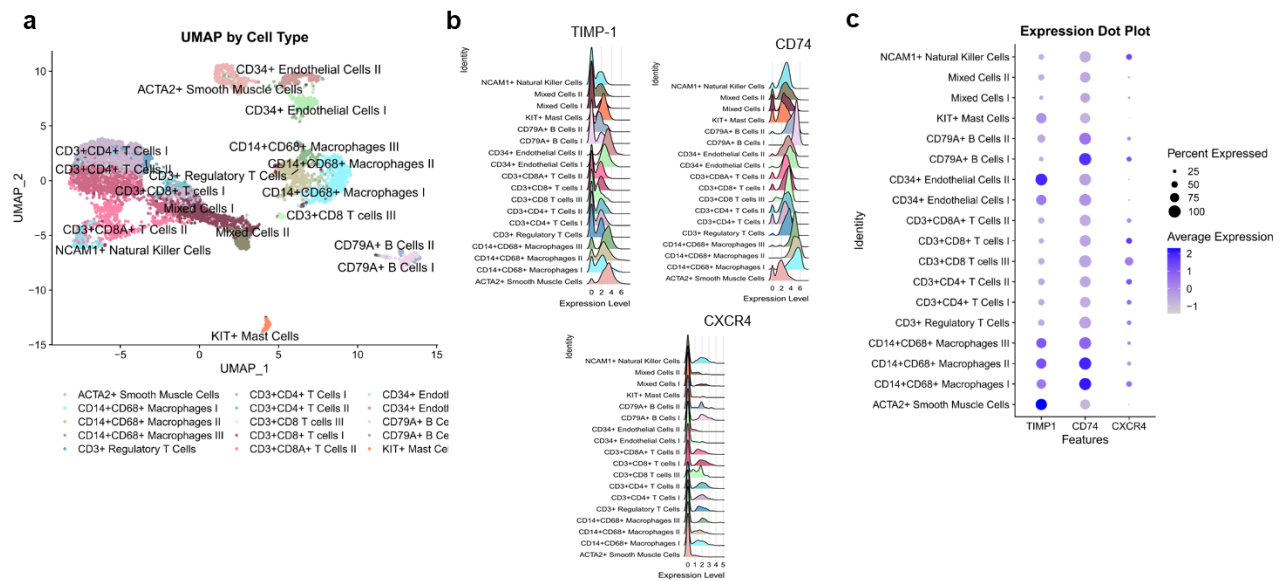
**Figure S1:** CXCR4 and CD74 are expressed in the human monocytic THP-1 cells. Cell surface expression of CD74 and CXCR4 in THP-1 cells was monitored by flow cytometry using FITC-conjugated anti-CD74 (a) (green) and APC-conjugated anti-CXCR4 (b) (red) or the corresponding APC-or FITC-conjugated IgG isotype controls (grey). Quantification of the obtained measurements was performed using FlowJo software.

## Supplementary Figure S2



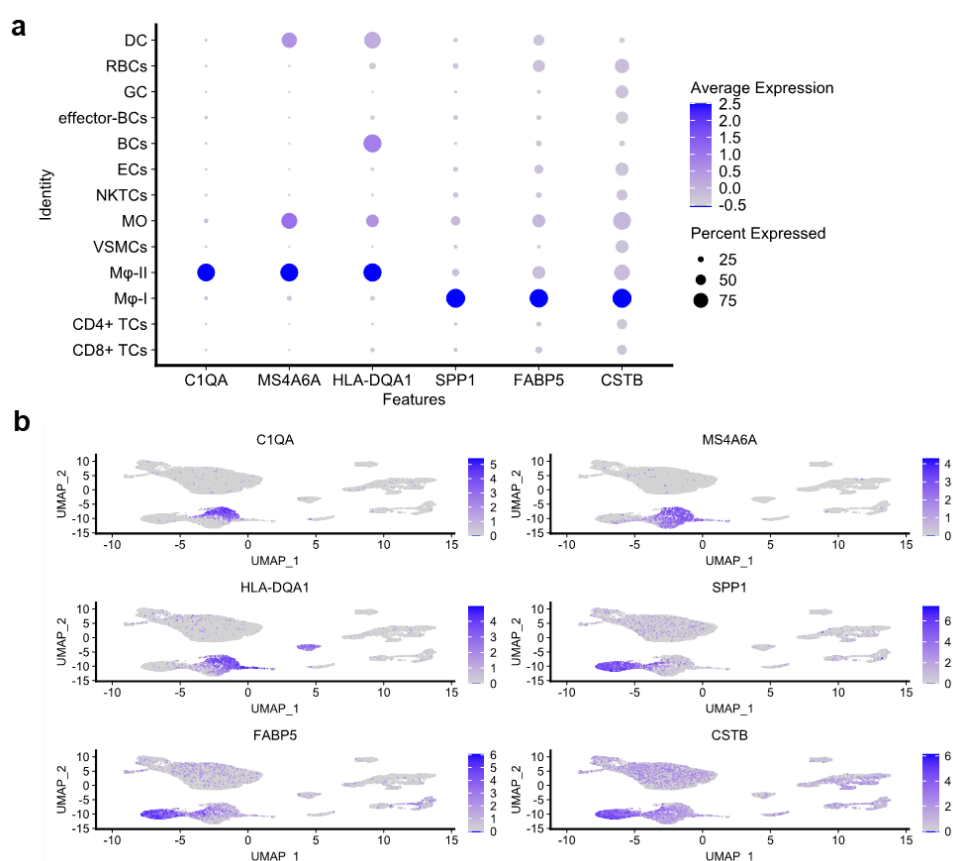
**Figure S2:** Re-analysis of three human atherosclerotic scRNA-seq datasets from two independent studies. Data were integrated by Zernecke et. al. and re-analyzed to examine the expression of TIMP-1, CD74, MIF, and CXCR4 in different single-cell populations using PlaqView [34,37]. **(a)** Uniform Manifold Ap-proximation and Projection of the integrated dataset with ten clusters identified by Louvain clustering. **(b)** Violin plots, and **(c)** dot plot of the genes of interest in the different cell populations found in the integrated scRNA-seq dataset. Xcr1<sup>+</sup> Type1 classical DCs (cDC1), classical Cd209a<sup>+</sup> DCs (cDC2), Macrophages (Mac), C3<sup>+</sup> Macrophages (C3\_Mac), and proliferating *TUBB*<sup>+</sup>, *H2AFZ*<sup>+</sup>, and *STMN1*<sup>+</sup> cells (Prolif.)

### Supplementary Figure S3



**Figure S3:** Re-analysis of the scRNA-seq dataset of 38 human CEA patients undergoing CEA [36]. Data were reanalyzed to examine the expression of TIMP-1, CD74, and CXCR4 in different single-cell populations using PlaqView [34,36]. However, MIF was not found in this dataset. (a) Uniform Manifold Approximation and Projection of the integrated dataset with 18 clusters identified by Louvain clustering. (b) Violin plots, and (c) dot plot of the genes of interest in the different cell populations found in the integrated scRNA-seq dataset.

Supplementary Figure S4



**Figure S4:** Highly expressed marker genes in the Mφ-I/II population of the atherosclerotic core in the Alsaigh et al. scRNAseq dataset defining these populations [6]. **(a)** Dot plot visualization of the expression of *C1QA*, *MS4A6A*, and *HLA-DQA1* in Mφ-II macrophages, and *SPP1*, *FABP5*, and *CSTB* in Mφ-I macrophages. **(b)** Feature plot depiction of the spatial expression of Mφ-I/II marker genes in the UMAP, including B cells (BCs), T cells (TCs), natural killer T cells (NKTCs), red blood cells (RBCs), vascular smooth muscle cells (VSMCs), endothelial cells (ECs), granulocytes (GC), dendritic cells (DC), monocytes (MO), and macrophages (Mφ/MΦ).