Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Ann Rheum Dis. Author manuscript; available in PMC 2024 April 01.

Published in final edited form as:

Ann Rheum Dis. 2023 April ; 82(4): 483–495. doi:10.1136/ard-2022-223284.

Syntenin-1-mediated arthritogenicity is advanced by reprogramming RA metabolic macrophages and Th1 cells

Anja Meyer[1,2], **Ryan Sienes**[2], **Wes Nijim**[2], **Brian Zanotti**[3], **Sadiq Umar**[1,2], **Michael V. Volin**[3], **Katrien van Raemdonck**[1,2], **Myles J. Lewis**[4,5], **Costantino Pitzalis**[4], **Shiva Arami**[2], **Mina Al-Awqati**[1,2], **Huan Chang**[1,2], **Pim Jetanalin**[1,2], **Georg Schett**[6,7], **Nadera Sweiss**[2], **Shiva Shahrara**[1,2,*]

[1]Jesse Brown VA Medical Center, Chicago, IL, USA.

[2]Department of Medicine, Division of Rheumatology, The University of Illinois at Chicago; IL, USA.

[3]Department of Microbiology and Immunology, Midwestern University; Downers Grove, IL, USA.

[4]Centre for Experimental Medicine and Rheumatology, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London; London, United Kingdom.

[5]Centre for Translational Bioinformatics, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London; London, United Kingdom.

[6]Department of Internal Medicine 3, Rheumatology and Immunology Friedrich-Alexander University Erlangen-Nürnberg and Universitätsklinikum Erlangen, Erlangen, Germany.

[7]Deutsches Zentrum für Immuntherapie, Friedrich-Alexander University Erlangen-Nürnberg and Universitätsklinikum Erlangen, Erlangen, Germany.

Abstract

Objectives: Syntenin-1, a novel endogenous ligand, was discovered to be enriched in rheumatoid arthritis (RA) specimens compared to osteoarthritis synovial fluid (SF) and normal synovial tissue (ST). However, the cellular origin, immunoregulation, and molecular mechanism of Syntenin-1 are undescribed in RA.

*Corresponding author. shahrara@uic.edu.

Author contributions:
Conceptualization: AM, SS
Methodology: AM, RS, WN, BZ, MV, ML, CP, SS
Investigation: AM, RS, WN, BZ, SU, MV, KVR, ML, CP, SA, MAA, HC, PJ, NS, SS
Visualization: AM, BZ, MV, SS
Funding acquisition: SS
Project administration: AM, SS
Supervision: AM, SS
Writing – original draft: AM, SS
Writing – review & editing: AM, RS, WN, BZ, SU, MV, KVR, ML, CP, SA, MAA, HC, PJ, GS, NS, SS

Competing interests: Authors declare that they have no competing interests.

Data and materials availability: All data are available in the main text or supplementary materials. RNAseq data are available under: https://peac.hpc.qmul.ac.uk/.

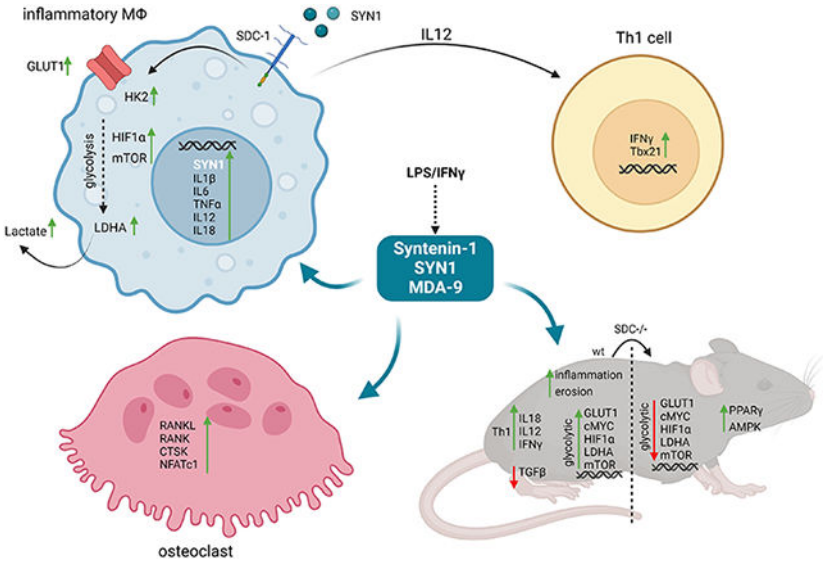
Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Methods: RA patient myeloid and lymphoid cells, as well as preclinical models, were utilized to investigate the impact of Syntenin-1/Syndecan-1 on the inflammatory and metabolic landscape.

Results: Syntenin-1 and Syndecan-1 (SDC-1) co-localize on RA ST macrophages (MΦs) and endothelial cells. Intriguingly, blood Syntenin-1 and ST SDC-1 transcriptome are linked to CCP, ESR, ST thickness, and bone erosion. Metabolic $CD14^{+}CD86^{+}GLUT1^{+}$MΦs reprogrammed by Syntenin-1 exhibit a wide range of proinflammatory IRFs, monokines, and glycolytic factors, along with reduced oxidative intermediates that are downregulated by blockade of SDC-1, glucose uptake, and/or mTOR signaling. Inversely, IL-5R and PDZ1 inhibition are ineffective on RA MΦs-reprogrammed by Syntenin-1. In Syntenin-1-induced arthritis, $F4/80^{+}iNOS^{+}RAPTOR^{+}$MΦs represent glycolytic RA MΦs, by amplifying the inflammatory and glycolytic networks. Those networks are abrogated in SDC-$1^{-/-}$ animals, while joint pro-repair monokines are unaffected and the oxidative metabolites are moderately replenished. In RA cells and/or preclinical model, Syntenin-1-induced arthritogenicity is dependent on mTOR-activated MΦ remodeling and its ability to cross-regulate Th1 cells via IL-12 and IL-18 induction. Moreover, RA and joint myeloid cells exposed to Syntenin-1 are primed to transform into osteoclasts via SDC-1 ligation and RANK, CTSK, and NFATc1 transcriptional upregulation.

Conclusion: The Syntenin-1/SDC-1 pathway plays a critical role in the inflammatory and metabolic landscape of RA through glycolytic MΦ and Th1 cell cross-regulation.

Graphical abstract.



Reprogramming of RA inflammatory macrophages, Th1 cells, and osteoclast formation by Syntenin-1 is recapitulated in the murine arthritis model. LPS/IFNγ stimulation in RA MΦs upregulates Syntenin-1 and SDC-1 expression. Ligation of Syntenin-1 to SDC-1 remodels RA naïve cells into glycolytic MΦs by mTOR signaling. Syntenin-1-induced arthritogenicity in RA and arthritic models can cross-regulate MΦs and Th1 cells thereby advancing osteoclastogenesis.

Keywords

Rheumatoid Arthritis; inflammation; metabolic macrophages; Th1 cells; syntenin-1; syndecan-1

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

INTRODUCTION

Melanoma differentiation-associated gene-9 (MDA-9) or Syntenin-1 is an adaptor-like molecule that was identified as a binding partner of syndecan-1 (SDC-1) [1]. Syntenin-1 was first cloned in Dr. Fisher's lab and described as a tumorigenic factor in melanoma cell lines in response to IFNβ and mezerein [2]. In cancer patients, SDC-1 and IL-5R regulate the function of Syntenin-1 through their interaction with the protein-interaction domain, PDZ2, however, they do not bind to the PDZ1 domain [3]. These findings highlight that PDZ2 is the primary active domain of Syntenin-1/SDC-1 signaling. Additionally, the PDZ2 domain of Syntenin-1 interacts with proto-oncogene tyrosine-protein kinase (SRC) and focal adhesion kinase (FAK), and pharmacological or genetic dysregulation of SRC nullifies Syntenin-1-mediated growth in the human melanoma metastasis model *in vivo* [4, 5]. Distinctly, the PDZ1 domain functions as a docking site for TGFβ [6], EGFR [7], and IGF1R [8] in cancer cells exposed to Syntenin-1. Extending these observations, PDZ1 inhibitors can specifically target Syntenin-1 and EGFR interaction in preclinical models of glioblastoma multiforme [9].

Earlier studies have documented that Syntenin-1 expands tumor-cell migration, invasion, and metastasis; conversely, this function is impaired in knockout mice [10]. Corroborating these findings, patients with melanoma liver metastases exhibit Syntenin-1 overexpression compared to non-metastasizing counterparts [11, 12]. Expanding these findings, activation of SRC and MAPK by Syntenin-1 is responsible for NF-κB signaling that advances melanoma cell migration and invasion in part through MMP2/MMP9 modulation [4, 5]. On the contrary, others found that Syntenin-1 disrupted IL-1- and LPS-induced NF-κB activation and IL-8 transcription in 293 cells [13]. These investigators further demonstrated that in IL-1-stimulated cells, Syntenin-1 interacts with TRAF6 by displacing the IRAK1 association [13]. Additionally, others have shown that SDC-1 deficient mice have a greater susceptibility to colitis-associated tumorigenesis [14] as well as an increase in corneal angiogenesis [15] and skin inflammation [16] compared to wild-type mice.

Interestingly, although both PDZ1 and PDZ2 are postulated to be involved in signaling directed by Syntenin-1, more recent evidence reveals that PDZ2 is indispensable for SRC and NF-κB activation in human melanoma cells [5]. Nonetheless, Syntenin-1-activated signaling pathways or interacting partners are cell-type-specific. In keeping with this concept, Syntenin-1 amplifies PI3K/AKT or STAT3 signaling to exacerbate metastasis in small cell lung cancer[17] or prostate cancer [8] respectively rather than NF-κB activation.

Intriguingly, in the lung metastatic model, the inflammatory imprint of Syntenin-1 is expanded by monokines that promote Th17 cell polarization [10]. While the significance of Syntenin-1 is well described in cancer its cellular origin, immunoregulation, and molecular mechanism are completely unknown in rheumatoid arthritis (RA). Uniquely we found that Syntenin-1 is highly enriched in RA compared to normal (NL) synovial tissue (ST), where it colocalizes with SDC-1 on the $CD14^+$ macrophages (MΦs). Consistently, RNAseq analysis exhibits that Syntenin-1 and SDC-1 transcriptome is closely linked to $CD68^+$ MΦ frequency in RA STs. Intriguingly, when RA myeloid cells were stimulated with a common instigator of Syntenin-1/SDC-1 cascade, while SDC-1 Ab uniquely reduces LPS/IFNγ-enhanced

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

CCL5 transcription, treatment with IL6Rab, TNFαi, and Jaki (Tofacitinib) was ineffective in this process. Since our data indicate that the ligation of Syntenin-1 to SDC-1 may function through novel strategies that do not overlap with current RA biologics, this pathway was further explored.

We uncovered that Syntenin-1 reprograms naïve cells into inflammatory RA MΦs that express a broad range of interferon transcription factors (IRF1/7/8/9) and monokines (IL-1β, TNF-α, IL-6, IL-8, and CCL2) that are exclusively impaired by an SDC-1 antibody but not by blockade of IL-5R or PDZ1 pathways. In parallel with its inflammatory phenotype, metabolic reprogramming of RA $CD14^+CD86^+GLUT1^+$MΦs by Syntenin-1 is heavily dependent on glucose uptake and mTOR signaling. Concurrently, secretion of IL-12 from Syntenin-1-polarized RA $CD14^+CD86^+GLUT1^+$MΦs, rewires $Tbx21^+$ Th1 cells via mTOR activation. Further, Syntenin-1-induced arthritogenicity *in vivo* was dependent on $F4/80^+iNOS^+$MΦs and their cross-regulation of joint Th1 cells through IL-12 and IL-18 induction. Substantiating these findings, $SDC\text{-}1^{-/-}$ mice dysregulated Syntenin-1-mediated arthritis by rebalancing oxidative metabolites AMPK and PPARγ over glycolytic intermediates, GLUT1, HIF1α, and mTOR as well as counteracting a wide range of joint monokines and their inflammatory amplifier, IFNγ. In short, we have discovered an endogenous ligand, Syntenin-1, and characterized its cellular source and mechanism of function for the first time in RA. Our novel finding indicates that the Syntenin-1/SDC-1 pathway plays an important role in the inflammatory and metabolic landscape of RA through MΦ and T effector cell crosstalk. Given that the Syntenin-1/SDC-1 pathway can differentially modulate RA inflammatory response compared to biotherapies, characterization of this cascade may represent a new treatment target for nonresponsive patients.

RESULTS

Syntenin-1 protein levels are enriched in RA compared to OA synovial fluid and its expression in RA synovial tissue or circulation is linked to MΦ markers and clinical manifestation

Through RNAseq analysis, we discovered that blood Syntenin-1 transcriptome is linked to cyclic citrullinated peptide (CCP) antibodies [18], hence its expression and immunoregulation were characterized in RA relative to osteoarthritis (OA) and normal (NL) counterparts. We found that Syntenin-1 mRNA and protein levels are significantly amplified in RA compared to OA synovial fluid (Fig. 1A-B). Morphological studies exhibit that in RA, the lining and sub-lining cells, as well as blood vessels, are the primary sources of Syntenin-1 release relative to OA and NL STs (Fig. 1C-D). Immunofluorescence staining authenticated that Syntenin-1 and SDC-1 are expressed on RA ST $CD14^+$MΦs (Fig. 1E). In parallel, Syntenin-1 ($p=0.003$, $r=0.33$) and SDC-1 ($p=0.013$, $r=0.28$) transcript levels were linked to the number of $CD68^+$MΦs quantified by histology in RA synovial tissues (Fig. 1F-G). Interestingly, $CD14^+CD16^-$ myeloid cells ($p\leq0.001$) and SDC-1 transcript expression ($p\leq0.001$) were associated with RA ultrasound-guided synovial tissue thickness (Fig. 1H-I). However, synovium syntenin-1 transcript expression was not associated with RA ultrasound-guided synovial tissue thickness. Consistent with the importance of Syntenin-1 and SDC-1

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

in RA pathophysiology, circulating Syntenin-1 and/or synovial tissue SDC-1 transcription levels are accompanied by clinical parameters such as the cyclic citrullinated peptide (CCP with blood Syntenin-1) and erythrocyte sedimentation rate (ESR with synovial SDC-1) (Fig. 1J-K). Notably, the classical inflammatory mediators, LPS/IFNγ, mutually upregulate the expression of Syntenin-1 and SDC-1 in human myeloid cells (Fig. 1L). However, in myeloid cells, SDC-1 protein levels are also distinctly escalated by IL-1β and IL-6 exposure (Fig. 1L). We reveal that LPS/IFNγ-induced CCL5 transcription is uniquely suppressed by SDC-1 antibody (SDCab) in contrast to IL-6R Ab, TNFi, or Jaki (Tofacitinib) therapy (Fig. 1M). Overall, the data indicate that Syntenin-1 and SDC-1 overexpression in RA macrophages is associated with clinical features which can uniquely modulate RA pathology in comparison to current biotherapies.

Syntenin-1 ligation to SDC-1 advances RA MΦ inflammatory imprint independently of IL-5R or PDZ1 function

To elucidate the significance of Syntenin-1/SDC-1 in RA pathogenesis, their signaling pathways and inflammatory profile were characterized in MΦs. At the onset of these studies, Syntenin-1 was carefully titrated in RA MΦs, and the effective dose was based on its TNFα induction (Fig. S1A). Human monocytes exposed to Syntenin-1 showed phosphorylation of SRC, protein kinase B (AKT), Signal Transducer And Activator Of Transcription-1 (STAT1), and c-Jun N-terminal kinase (JNK) pathways as well as degradation of IκB (Fig. 2A). However, STAT3, p38, and ERK signaling were unchanged in myeloid cells stimulated by Syntenin-1 (Fig. 2A). In RA MΦs reconfigured by Syntenin-1, the wide range of activated signaling pathways, was in concert with expansion of inflammatory interferon transcription factors, IRF1, IRF7, IRF8, and IRF9 as well as monokines, IL-1β, TNF-α, IL-6, IL-8, and CCL2 (>7x fold) (Fig. 2B to E). On the contrary, the pro-repair transcription factors, IRF3 and IRF4, were uninvolved in Syntenin-1-differentiated RA MΦs (Fig. 2B). Remarkably, while elevated levels of TNF-α, and CCL2 were intercepted by SDC-1 Ab therapy, use of IL-5R or PDZ1 inhibitor (i) was ineffective in this process (Fig. 2F to H). Further, while TLR2 was exclusively amplified in Syntenin-1-polarized RA MΦs, TLR4/5/7/8 transcription remained unchanged (Fig. 2I). In contrary to IL-5R Ab or PDZ1i therapy in RA MΦs, transcriptional regulation of the pro-repair factors by Syntenin-1 was accentuated via SDC-1 Ab (Fig. 2J-K). Taken together, an extensive array of signaling pathways, transcription factors, and monokines are involved in the remodeling of RA MΦs through Syntenin-1/SDC-1 leading to a misbalanced pro-inflammatory over the pro-repair network.

Syntenin-1 and SDC-1 escalate RA MΦ metabolic reprogramming

We and others have shown that in the inflammatory landscape of RA, MΦs can be restrained by glucose uptake inhibition [19-22]. Hence experiments were conducted to determine whether the Syntenin-1-potentiated inflammatory phenotype in RA MΦs is influenced by glycolytic rewiring. In parallel to amplifying inflammatory responses, RA MΦs differentiated by Syntenin-1 display elevated GLUT1, HK2, HIF1α, RAPTOR, and PKM2 expression (Fig. 3A to C, Fig. S1E to J). Additionally, higher protein expression of LDHA and lactate in Syntenin-1-reprogrammed RA MΦs was supported by ATP being mainly generated through glycolysis (↑%glycoATP) over oxidative phosphorylation

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

(↓%mitoATP) (Fig. 3A, 3D to G). We showed that Syntenin-1 reconfigures naïve cells into RA $CD14^+CD86^+GLUT1^+$MΦs through SDC-1 binding, while blockade of IL-5R or PDZ1 was inconsequential on this function (Fig. 3H). Particularly, the differentiation of glycolytic $CD14^+CD86^+GLUT1^+$ MΦs by Syntenin-1 and its ability to promote inflammatory monokines, including CCL2, was dependent on mTOR1 activation, however, this mechanism of action was independent of HIF1α signaling (Fig. 3I-J). Despite the inefficacy of Syntenin-1 on $CD14^+CD206^+GLUT1^+$MΦs frequency (Fig. S1K), downregulation of oxidative intermediate (AMPK) and enzymes (ACO2, OGDH, SDH2, FH, MDH) in these RA myeloid cells were reversed by SDC-1 Ab (Fig. 3K to M, Fig. S1M). In short, the inflammatory imprint of Syntenin-1-differentiated RA MΦs is interconnected to its metabolic activity through SDC-1 ligation and RAPTOR/mTOR signaling.

RA MΦs remodeled by Syntenin-1 promote Th1 and Th17 cell differentiation

Next, the impact of Syntenin-1 was characterized on T effector cell differentiation in RA patients. RA peripheral blood mononuclear cells (PBMCs) exposed to Syntenin-1 displayed a strong Th1 profile by transcriptionally upregulating Tbx21/T-bet, IFNγ, IL-18, and IL-12 (Fig. 4A to F). Flow cytometry analysis validated that, similar to LPS and IL-12, Syntenin-1 polarizes RA naïve cells into the Th1 subtype (Fig. 4G-H). While IL-18 was undetected in the conditioned media generated from Syntenin-1-activated Th1 cells, detection of IL-12 validated that it is predominately responsible for the polarization of $Tbx21^+IFN\gamma^+$Th1 cells by Syntenin-1. We found that anti-IL-12 Ab therapy could impair Syntenin-1-mediated Th1 cell polarization in RA PBMCs but was ineffective in T cell culture alone (Fig. 4I-J). In parallel, the presence of myeloid cells in RA PBMCs can further expand Syntenin-1-induced Th1 differentiation compared to T cell culture (Fig. 4I-J). Like Th1 cells, RA PBMCs exposed to Syntenin-1 were differentiated into Th17 cells, in part through glucose uptake and mTOR signaling which was independent of the HIF1α pathway (Fig. 4K-L). Altogether, our results suggest that activation of glycolysis via mTOR is responsible for Syntenin-1-instigated metabolic RA MΦ reconfiguration and its cross-regulation of Th1 and Th17 cell development.

SDC-1 deficient mice are resistant to Syntenin-1-mediated arthritis

To evaluate the arthritogenic potential of Syntenin-1, adenovirus (ad) expressing Syntenin-1 was intra-articularly injected into wild-type (WT) mice compared to Ad-Control (ctrl). Local injection and expression of Syntenin-1 progressively increased ankle circumference up to day 12, subsequently, joint swelling plateaued until day 15 when mice were sacrificed (Fig. 5A, Fig. S2A). In line with our observations in RA cells, Syntenin-1-induced arthritis was attenuated in SDC-$1^{-/-}$ mice [23] (Fig. 5A-B). Consistently, joint lining thickness, inflammation, and bone erosion, advanced in WT by Syntenin-1-induced arthritis, were dysregulated in SDC-$1^{-/-}$ mice (Fig. 5B-C). While $F4/80^+iNOS^+$MΦs were responsible for the arthritogenicity escalated by Syntenin-1 and the pathology was disrupted in SDC-$1^{-/-}$ relative to WT animals, $F4/80^+Arginase^+$MΦs were unaffected in this process (Fig. 5D-E). Local Syntenin-1 expression represented RA MΦ differentiation by displaying a diverse expansion of inflammatory IRF1, IRF5, IRF7, IRF8, and IRF9 (up to a 15-fold increase) as well as monokines, IL-6, IL-1β, TNF-α, CCL2, CCL5, and CXCL2 (up to 50-fold

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

increase), which were diminished in SDC-1$^{-/-}$ mice (Fig. 5F-G). Inversely, the joint pro-repair mediators, IRF3, IRF4, and TGFβ were suppressed by ectopic Syntenin-1 expression (Fig. 5F-H). However distinct from SDC-1 neutralizing Ab, SDC-1$^{-/-}$ mice were unable to replenish joint IL-10 or TGFβ transcription in Syntenin-1 arthritic animals (Fig. 5H). Collectively, the data reveal that SDC-1 deficiency reverses the arthritic F4/80^{+}iNOS^{+}MΦ reprogramming by Syntenin-1, without influencing the pro-repair F4/80^{+}Arginase^{+}MΦ imprint.

Syntenin-1-induced arthritis is manipulated by the joint hypermetabolic activity

To further characterize arthritis promoted by Syntenin-1, joint immunometabolism was investigated in naïve compared to arthritic mice. We found that GLUT1, HK2, mTOR/p70, and LDHA protein expression were elevated in Syntenin-1 arthritic joints harvested at day 15 compared to non-arthritic counterparts (Fig. 6A). Further, overexpression of these glycolytic metabolites was authenticated by transcriptome and morphological analysis in WT mice locally expressing Syntenin-1 compared to SDC-1$^{-/-}$ or non-arthritic animals. Particularly, GLUT1, HIF1α, cMYC, LDHA, and mTOR/p70 expression increased in Syntenin-1-induced arthritis, was subsided in SDC-1$^{-/-}$ compared to WT mice (Fig. 6B to G). In contrast, the downregulation of oxidative regulators, PPARγ and AMPK, by Syntenin-1 local expression in WT relative to non-arthritic mice was reversed in SDC-1$^{-/-}$ animals (Fig. 6H-I). Overall, SDC-1 dysregulation mitigates Syntenin-1-mediated arthritogenicity by mainly normalizing the inflammatory and glycolytic networks and narrowly restoring the pro-repair or oxidative profile.

Arthritis potentiated by Syntenin-1 is influenced by CD3^{+} T cell migration and Th1 cell polarization

Given that RA PBMCs, exposed to Syntenin-1, were polarized into Th1 and Th17 cells, we asked whether T cells play a critical role in Syntenin-1-mediated arthritis. Intriguingly, local expression of Syntenin-1 attracts CD3^{+} T cells into the arthritic WT joints which are significantly restrained in SDC-1$^{-/-}$ mice (Fig. 7A-B). Despite transcription of Th1 signature genes, IFNγ, IL-18, and IL-12 being highly elevated in WT Syntenin-1-arthritic mice and impaired in SDC-1$^{-/-}$animals, Th17 cell polarization was unaffected (Fig. 7C to E). Taken together, the RA and preclinical data emphasize that Syntenin-1 skews T naïve cell reprogramming towards Th1 cells.

Syntenin-1 remodels RA preosteoclasts and arthritic joint cells into mature osteoclasts through an overlapping mechanism

Circulating Syntenin-1 ($p=0.00033$, $r=0.48$) and synovial tissue SDC-1 ($p=0.0093$, $r=0.31$) transcript levels are linked to bone erosion evaluated in radiographic images of RA hands and feet by Sharp score (Fig. 7F-G). We showed that Syntenin-1 cultivates RA monocytes into mature osteoclasts in part by activating the expression of several osteoclastic mediators, RANK, cathepsin K (CTSK), and NFATc1 (Fig. 7H). Correspondingly, the frequency of TRAP^{+} osteoclasts and transcription of RANK, CTSK, and NFATc1 were elevated in WT compared to SDC-1$^{-/-}$ mice induced with Syntenin-1-mediated arthritis (Fig. 7I to O). Altogether, the data underlines the significance of Syntenin-1/SDC-1 in transforming RA and murine precursor cells into mature osteoclasts by similar osteoclastic mediators.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

DISCUSSION

We have discovered a novel endogenous regulator, Syntenin-1, that is released from classically differentiated inflammatory MΦs. Transcriptome and morphological analysis exhibited that Syntenin-1 and its pathogenic receptor, SDC-1 are co-expressed on RA synovial tissue $CD14^{+}CD68^{+}$MΦs. Concurrently, Syntenin-1 and/or SDC-1 expression in RA blood or synovial tissue is selectively linked to CCP levels, ESR, ultrasound-detected synovial tissue thickness, and bone erosion. Syntenin-1 advances RA $CD14^{+}CD86^{+}GLUT1^{+}$MΦ reprogramming that displays dysregulated oxidative intermediates together with an extensive range of inflammatory IRFs, monokines, and glycolytic factors, that are counteracted by blockade of SDC-1, glucose uptake, and/or mTOR signaling. Recapitulating RA mechanism of function, IL-12 and/or IL-18 transcriptional upregulation in Syntenin-1 arthritic joints reconfigures the infiltrated T cells into Th1 cells. While in WT mice, Syntenin-1-triggered inflammatory, glycolytic, and erosive networks are abrogated in SDC-$1^{-/-}$ animals, joint pro-repair monokines are unchanged and the oxidative metabolites are modestly replenished. Collectively, our study highlights that targeting the Syntenin-1/SDC-1 pathway may introduce a unique strategy for deregulating RA metabolic misfunction.

Syntenin-1 is expressed in metastatic tumor cells in melanoma [4, 11], breast and lung cancer [17, 24] as well as in glioma cells [25] regulating disease expansion in part by cell membrane motility [3]. Nonetheless, the cellular expression, immunomodulation, and pathobiology of Syntenin-1 are undescribed in RA patients and preclinical models. In this study, we reveal that Syntenin-1 is overexpressed in RA specimens compared to OA synovial fluid or OA and NL synovial tissue, particularly, in MΦs and endothelial cells. Interestingly, a recent study has shown that Syntenin-1 protein levels were amplified in exosomes isolated from RA synovial fluid with higher disease activity compared to less severe counterparts [26]. Others have exhibited that MΦ-derived exosomes contain Syntenin-1 [27], indicating that synovial fluid Syntenin-1 may be released from MΦ microvesicles. Concurrently, Syntenin-1 together with SDC-1 interact with proteins responsible for exosome biogenesis to rearrange the extracellular vesicle cargo [28, 29].

Interestingly, in RA MΦs, SDC-1 controls the phosphorylation of the co-receptor, M-CSFR, when exposed to IL-34 [30]. Consequently, SDC-1 is indispensable for IL-34-mediated arthritis by influencing M34 MΦ and osteoclast differentiation in part via joint hypermetabolic activity instigated by HIF1α and cMYC [30, 31]. Distinct from cancer cells, in RA MΦs, the binding partner of Syntenin-1 is restricted to SDC-1 since IL-5R is exclusively expressed in B cells, basophils, and eosinophils [32-34] and its blockade does not impact the function of Syntenin-1.

While stimulation with LPS/IFNγ mutually upregulates Syntenin-1 and SDC-1 protein levels, SDC-1 is uniquely modulated by IL-1β and IL-6 activation in human myeloid cells. Similarly, in SV40-immortalized melanoma cells, Syntenin-1 levels are highly responsive to IFNγ stimulation [2]. Inversely, SDC-1 is differentially regulated by TGF-β and bFGF in various cell types [35]. Moreover, soluble SDC-1 detected in RA sera as a result of MMP-9-mediated shedding was inconsequential in a longitudinal study performed pre- and

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

post-anti-TNFα therapy for 6 weeks [36]. Hence, Syntenin-1 and SDC-1 mechanism of action was characterized in RA MΦs.

Myeloid cells exposed to Syntenin-1 display activated SRC, AKT, STAT1, NF-κB, and JNK signaling. While AKT, STAT1, and JNK signaling pathways are unique to myeloid cells stimulated by Syntenin-1, activation of SRC, p38 MAPK, and NF-κB by Syntenin-1 are also required for human melanoma cell motility and invasion [5, 11]. In Syntenin-1-differentiated RA MΦs or arthritic joints, the pronounced inflammatory landscape was developed by overexpression of IRF1/7/8/9 and IL-1β, IL-6, TNF, CCL2 or CCL5, CXCL2 over the pro-repair profile exhibited as IRF3/IRF4 and TGFβ. Moreover, the dominance of the inflammatory network in RA MΦs or arthritic joints fostered by Syntenin-1 was accompanied by robust Th1 cell differentiation and glycolytic hyperactivity. Particularly, RA $CD14^+CD86^+GLUT1^+$MΦs rewired by Syntenin-1 generate their ATP mainly through glycolysis (%↑glycoATP) over mitochondrial oxidative phosphorylation (%↓mitoATP) which results in GLUT1, HK2, HIF1α, RAPTOR, LDHA escalation, and lactate secretion.

Distinct from SDC-1 blockade, IL-5R Ab or PDZ1i therapy was ineffective in the Syntenin-1-escalated inflammatory landscape in RA MΦs. This is in part due to the lack of IL-5R expression in RA MΦs, despite the cell-type-specific interaction of Syntenin-1 with IL-5 and IL-5R in eosinophil differentiation [37] and mucosal IgA production in B cells [38]. The inability of PDZ1i to nullify Syntenin-1-instigated RA MΦ reprogramming is also inconsistent with the involvement of PDZ1 in advancing IL-1β secretion from myeloid cells in breast cancer [39]. Corroborating these findings, SDC-1 interaction with Syntenin-1 is uniquely facilitated through PDZ2 connection[40, 41].

Substantiating our data in RA pathology, $SDC\text{-}1^{-/-}$ mice were resistant to arthritis developed by local Syntenin-1 expression through restriction of $F4/80^+iNOS^+$ MΦs and $CD3^+$ T cell infiltration. It was noted that the joint MΦs inflammatory (IL-6, IL-1β, TNF, CCL2, CCL5, CXCL2, IL-12, and IL-18) and metabolic (GLUT1, HIFα, RAPTOR/mTOR, and LDHA) landscapes were counteracted in $SDC\text{-}1^{-/-}$ relative to WT mice induced with Syntenin-1-mediated arthritis. However, the oxidative profile was modestly attenuated by joint AMPK and PPARγ upregulation in $SDC\text{-}1^{-/-}$ relative to WT animals ectopically expressing Syntenin-1. Extending these findings, CIA joint inflammation, metabolic activity, bone erosion, and vascularization were attenuated in $SDC\text{-}1^{-/-}$ compared to WT mice [42].

It was shown that T cell recruitment was amplified by Syntenin-1-mediated arthritis and was diminished in melanoma metastasis in $Syntenin\text{-}1^{-/-}$ mice [10]. Syntenin-1 was capable of differentiating RA PBMCs into Th1 and Th17 cells. On the contrary, although Th1 cells were detected in Syntenin-1 arthritic mice via IL-12 and IL-18 induction, joint Th17 cells were unaffected in this process. IL-12 blockade diminished Th1 cell polarization amplified by Syntenin-1 in RA PBMCs compared to T cells alone; further highlighting its significance in MΦ and T cell cross-regulation. Remarkably, RA $CD14^+CD86^+GLUT1^+$MΦs, Th1, and Th17 cells reprogramming by Syntenin-1 were dysregulated by inhibition of mTOR signaling and glucose uptake but not HIF1α dysregulation. Two mTOR subunits, namely RAPTOR and RICTOR are involved in the rewiring of pro-inflammatory and pro-repair/regulatory MΦs and T cells respectively [43-47]. Classical MΦ polarization via mTOR has

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

shown to be dependent on AKT, NF-κB, and JNK pathways [43], hence activation of these cascades in Syntenin-1-stimulated myeloid cells may be linked to mTOR/RAPTOR signaling. Similarly, in classical and Syntenin-1-differentiated MΦs the metabolic activity is reciprocally expanded via GLUT1, LDHA, and lactate which can be impaired by 2-DG therapy [21]. In CIA, SDC-1 deficiency can markedly suppress joint GLUT1 and mTOR hyperactivation observed in WT animals [42]. Others have shown that mTOR/RAPTOR activity plays a critical role in Th17 trans-differentiation into a Th1-like subset [48]. Previous studies also demonstrate that mTOR deficiency compromises Th1 and Th17 cell differentiation by restraining inflammatory monokines, IL-12, IL-6, and IL-1β [21, 49]. Our results underline that mTOR-potentiated glycolysis is accountable for Syntenin-1 cross-regulation of metabolic MΦs and Th1 cells (Fig. 8).

Recapitulating the connection between Syntenin-1/SDC-1 cascade and radiographic bone erosion, osteoclast formation in Syntenin-1 exposed RA cells and arthritic joints are cultivated through RANK, CTSK, and NFATc1 induction which is contingent on the SDC-1 function. In short, dysregulation of the Syntenin-1/SDC-1 signaling may provide a novel therapeutic strategy for RA patients that have a robust innate and adaptive activation and do not respond to current biologics.

MATERIALS AND METHODS

Materials and Methods are described in the online supplementary material.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

The authors would like to thank Balaji B. Ganesh, Director of the Flow Cytometry Core at the University of Illinois at Chicago, Adel Burgos, Pei-Yu Wu for their excellent scientific advice, and the Fluorescent Immunohistochemistry Midwestern University Core Facility, Downers Grove, IL for analyzing the fluorescent stainings. Schematic figures were illustrated by biorender.com.

Funding:

This work was supported in part by awards from the Department of Veteran's Affairs MERIT Award BX002286, the National Institutes of Health NIH R01 AI167155, NIH R41 AI147697, the Innovative Research Award from the Rheumatology Research Foundation (RRF).

REFERENCES

1. Das SK, Sarkar D, Emdad L, Fisher PB. MDA-9/Syntenin: An emerging global molecular target regulating cancer invasion and metastasis. Adv Cancer Res 2019; 144:137–191. [PubMed: 31349898]
2. Lin JJ, Jiang H, Fisher PB. Melanoma differentiation associated gene-9, mda-9, is a human gamma interferon responsive gene. Gene 1998; 207:105–110. [PubMed: 9511750]
3. Shimada T, Yasuda S, Sugiura H, Yamagata K. Syntenin: PDZ Protein Regulating Signaling Pathways and Cellular Functions. Int J Mol Sci 2019; 20.
4. Boukerche H, Su ZZ, Prevot C, Sarkar D, Fisher PB. mda-9/Syntenin promotes metastasis in human melanoma cells by activating c-Src. Proc Natl Acad Sci U S A 2008; 105:15914–15919. [PubMed: 18832467]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

5. Boukerche H, Aissaoui H, Prevost C, et al. Src kinase activation is mandatory for MDA-9/syntenin-mediated activation of nuclear factor-kappaB. Oncogene 2010; 29:3054–3066. [PubMed: 20228839]
6. Menezes ME, Shen XN, Das SK, Emdad L, Sarkar D, Fisher PB. MDA-9/Syntenin (SDCBP) modulates small GTPases RhoA and Cdc42 via transforming growth factor beta1 to enhance epithelial-mesenchymal transition in breast cancer. Oncotarget 2016; 7:80175–80189. [PubMed: 27863394]
7. Dasgupta S, Menezes ME, Das SK, et al. Novel role of MDA-9/syntenin in regulating urothelial cell proliferation by modulating EGFR signaling. Clin Cancer Res 2013; 19:4621–4633. [PubMed: 23873690]
8. Das SK, Pradhan AK, Bhoopathi P, et al. The MDA-9/Syntenin/IGF1R/STAT3 Axis Directs Prostate Cancer Invasion. Cancer Res 2018; 78:2852–2863. [PubMed: 29572229]
9. Das SK, Sarkar D, Cavenee WK, Emdad L, Fisher PB. Rethinking Glioblastoma Therapy: MDA-9/Syntenin Targeted Small Molecule. ACS Chem Neurosci 2019; 10:1121–1123. [PubMed: 30681320]
10. Das SK, Guo C, Pradhan AK, et al. Knockout of MDA-9/Syntenin (SDCBP) expression in the microenvironment dampens tumor-supporting inflammation and inhibits melanoma metastasis. Oncotarget 2016; 7:46848–46861. [PubMed: 27341128]
11. Boukerche H, Su ZZ, Emdad L, et al. mda-9/Syntenin: a positive regulator of melanoma metastasis. Cancer Res 2005; 65:10901–10911. [PubMed: 16322237]
12. Boukerche H, Su ZZ, Emdad L, Sarkar D, Fisher PB. mda-9/Syntenin regulates the metastatic phenotype in human melanoma cells by activating nuclear factor-kappaB. Cancer Res 2007; 67:1812–1822. [PubMed: 17308124]
13. Chen F, Du Y, Zhang Z, et al. Syntenin negatively regulates TRAF6-mediated IL-1R/TLR4 signaling. Cell Signal 2008; 20:666–674. [PubMed: 18234474]
14. Binder Gallimidi A, Nussbaum G, Hermano E, et al. Syndecan-1 deficiency promotes tumor growth in a murine model of colitis-induced colon carcinoma. PLoS One 2017; 12:e0174343. [PubMed: 28350804]
15. Gotte M, Joussen AM, Klein C, et al. Role of syndecan-1 in leukocyte-endothelial interactions in the ocular vasculature. Invest Ophthalmol Vis Sci 2002; 43:1135–1141. [PubMed: 11923257]
16. Jaiswal AK, Sadasivam M, Archer NK, et al. Syndecan-1 Regulates Psoriasiform Dermatitis by Controlling Homeostasis of IL-17-Producing gammadelta T Cells. J Immunol 2018; 201:1651–1661. [PubMed: 30045969]
17. Kim WY, Jang JY, Jeon YK, Chung DH, Kim YG, Kim CW. Syntenin increases the invasiveness of small cell lung cancer cells by activating p38, AKT, focal adhesion kinase and SP1. Exp Mol Med 2014; 46:e90. [PubMed: 24722482]
18. Lewis MJ, Barnes MR, Blighe K, et al. Molecular Portraits of Early Rheumatoid Arthritis Identify Clinical and Treatment Response Phenotypes. Cell reports 2019; 28:2455–2470 e2455. [PubMed: 31461658]
19. Van Raemdonck K, Umar S, Palasiewicz K, et al. Metabolic reprogramming of macrophages instigates CCL21-induced arthritis. Immunol Cell Biol 2022; 100:127–135. [PubMed: 34779007]
20. Umar S, Palasiewicz K, Volin MV, et al. IRAK4 inhibitor mitigates joint inflammation by rebalancing metabolism malfunction in RA macrophages and fibroblasts. Life Sci 2021:120114. [PubMed: 34732329]
21. Umar S, Palasiewicz K, Volin MV, et al. Metabolic regulation of RA macrophages is distinct from RA fibroblasts and blockade of glycolysis alleviates inflammatory phenotype in both cell types. Cell Mol Life Sci 2021.
22. McGarry T, Hanlon MM, Marzaioli V, et al. Rheumatoid arthritis CD14(+) monocytes display metabolic and inflammatory dysfunction, a phenotype that precedes clinical manifestation of disease. Clin Transl Immunology 2021; 10:e1237. [PubMed: 33510894]
23. Alexander CM, Reichsman F, Hinkes MT, et al. Syndecan-1 is required for Wnt-1-induced mammary tumorigenesis in mice. Nat Genet 2000; 25:329–332. [PubMed: 10888884]
24. Koo TH, Lee JJ, Kim EM, Kim KW, Kim HD, Lee JH. Syntenin is overexpressed and promotes cell migration in metastatic human breast and gastric cancer cell lines. Oncogene 2002; 21:4080–4088. [PubMed: 12037664]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

25. Li Q, Li X, Guo Z, et al. MicroRNA-574-5p was pivotal for TLR9 signaling enhanced tumor progression via down-regulating checkpoint suppressor 1 in human lung cancer. PLoS One 2012; 7:e48278. [PubMed: 23133627]
26. Foers AD, Dagley LF, Chatfield S, et al. Proteomic analysis of extracellular vesicles reveals an immunogenic cargo in rheumatoid arthritis synovial fluid. Clin Transl Immunology 2020; 9:e1185.
27. Garin J, Diez R, Kieffer S, et al. The phagosome proteome: insight into phagosome functions. J Cell Biol 2001; 152:165–180. [PubMed: 11149929]
28. Baietti MF, Zhang Z, Mortier E, et al. Syndecan-syntenin-ALIX regulates the biogenesis of exosomes. Nat Cell Biol 2012; 14:677–685. [PubMed: 22660413]
29. Zimmermann P, Zhang Z, Degeest G, et al. Syndecan recycling [corrected] is controlled by syntenin-PIP2 interaction and Arf6. Dev Cell 2005; 9:377–388. [PubMed: 16139226]
30. Van Raemdonck K, Umar S, Palasiewicz K, et al. Interleukin-34 Reprograms Glycolytic and Osteoclastic Rheumatoid Arthritis Macrophages via Syndecan 1 and Macrophage Colony-Stimulating Factor Receptor. Arthritis Rheumatol 2021; 73:2003–2014. [PubMed: 33982895]
31. Umar S, Palasiewicz K, Van Raemdonck K, et al. IRAK4 inhibition: a promising strategy for treating RA joint inflammation and bone erosion. Cell Mol Immunol 2021; 18:2199–2210. [PubMed: 32415262]
32. Takatsu K. Interleukin-5 and IL-5 receptor in health and diseases. Proc Jpn Acad Ser B Phys Biol Sci 2011; 87:463–485.
33. Denburg JA, Sehmi R, Upham J. Regulation of IL-5 receptor on eosinophil progenitors in allergic inflammation: role of retinoic acid. Int Arch Allergy Immunol 2001; 124:246–248. [PubMed: 11306982]
34. Kabashima K, Nakashima C, Nonomura Y, et al. Biomarkers for evaluation of mast cell and basophil activation. Immunol Rev 2018; 282:114–120. [PubMed: 29431209]
35. Cizmeci-Smith G, Carey DJ. Thrombin stimulates syndecan-1 promotor activity and expression of a form of syndecan-1 that binds antithrombin III in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 1997; 17:2609–2616. [PubMed: 9409233]
36. Deyab G, Reine TM, Vuong TT, et al. Antirheumatic treatment is associated with reduced serum Syndecan-1 in Rheumatoid Arthritis. PLoS One 2021; 16:e0253247. [PubMed: 34242246]
37. Beekman JM, Verhagen LP, Geijsen N, Coffer PJ. Regulation of myelopoiesis through syntenin-mediated modulation of IL-5 receptor output. Blood 2009; 114:3917–3927. [PubMed: 19654410]
38. Moon BG, Takaki S, Miyake K, Takatsu K. The role of IL-5 for mature B-1 cells in homeostatic proliferation, cell survival, and Ig production. J Immunol 2004; 172:6020–6029. [PubMed: 15128785]
39. Pradhan AK, Maji S, Bhoopathi P, et al. Pharmacological inhibition of MDA-9/Syntenin blocks breast cancer metastasis through suppression of IL-1beta. Proc Natl Acad Sci U S A 2021; 118.
40. Koroll M, Rathjen FG, Volkmer H. The neural cell recognition molecule neurofascin interacts with syntenin-1 but not with syntenin-2, both of which reveal self-associating activity. J Biol Chem 2001; 276:10646–10654. [PubMed: 11152476]
41. Kang BS, Cooper DR, Devedjiev Y, Derewenda U, Derewenda ZS. Molecular roots of degenerate specificity in syntenin's PDZ2 domain: reassessment of the PDZ recognition paradigm. Structure 2003; 11:845–853. [PubMed: 12842047]
42. Meyer A, Sienes R, Zanotti B, et al. Dysregulation of IL-34 ligation to SDC-1 mitigates collagen-induced arthritis. Cell Mol Immunol 2022; 19:1070–1072. [PubMed: 35676327]
43. Covarrubias AJ, Aksoylar HI, Horng T. Control of macrophage metabolism and activation by mTOR and Akt signaling. Semin Immunol 2015; 27:286–296. [PubMed: 26360589]
44. Cheng SC, Quintin J, Cramer RA, et al. mTOR- and HIF-1alpha-mediated aerobic glycolysis as metabolic basis for trained immunity. Science 2014; 345:1250684. [PubMed: 25258083]
45. Kelly B, O'Neill LA. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. Cell Res 2015; 25:771–784. [PubMed: 26045163]
46. Corcoran SE, O'Neill LA. HIF1alpha and metabolic reprogramming in inflammation. J Clin Invest 2016; 126:3699–3707. [PubMed: 27571407]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

47. Huang H, Long L, Zhou P, Chapman NM, Chi H. mTOR signaling at the crossroads of environmental signals and T-cell fate decisions. Immunol Rev 2020; 295:15–38. [PubMed: 32212344]

48. Karmaus PWF, Chen X, Lim SA, et al. Metabolic heterogeneity underlies reciprocal fates of TH17 cell stemness and plasticity. Nature 2019; 565:101–105. [PubMed: 30568299]

49. Delgoffe GM, Kole TP, Zheng Y, et al. The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. Immunity 2009; 30:832–844. [PubMed: 19538929]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

What is already known on this topic – *summarize the state of scientific knowledge on this subject before you did your study and why this study needed to be done*

We introduce an innovative concept by unmasking an unidentified endogenous ligand, Syntenin1, whose cellular origin, immunoregulation, and molecular mechanism are uncharacterized in RA and its malfunctioning metabolic machinery will provide powerful therapeutic targets.

What this study adds – *summarize what we now know as a result of this study that we did not know before*

The instigators and the mechanism by which glycolytic RA macrophages cross-regulate Th1 cells are unclear. Our novel finding documents that the Syntenin-1/SDC-1 pathway plays an important role in the inflammatory and metabolic landscape of RA through macrophage and T effector cell crosstalk.

How this study might affect research, practice or policy – *summarize the implications of this study*

Since the expression of Syntenin-1/SDC-1 is unaffected by biotherapies in RA circulating cells, this pathway may represent a new treatment target for nonresponsive patients.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

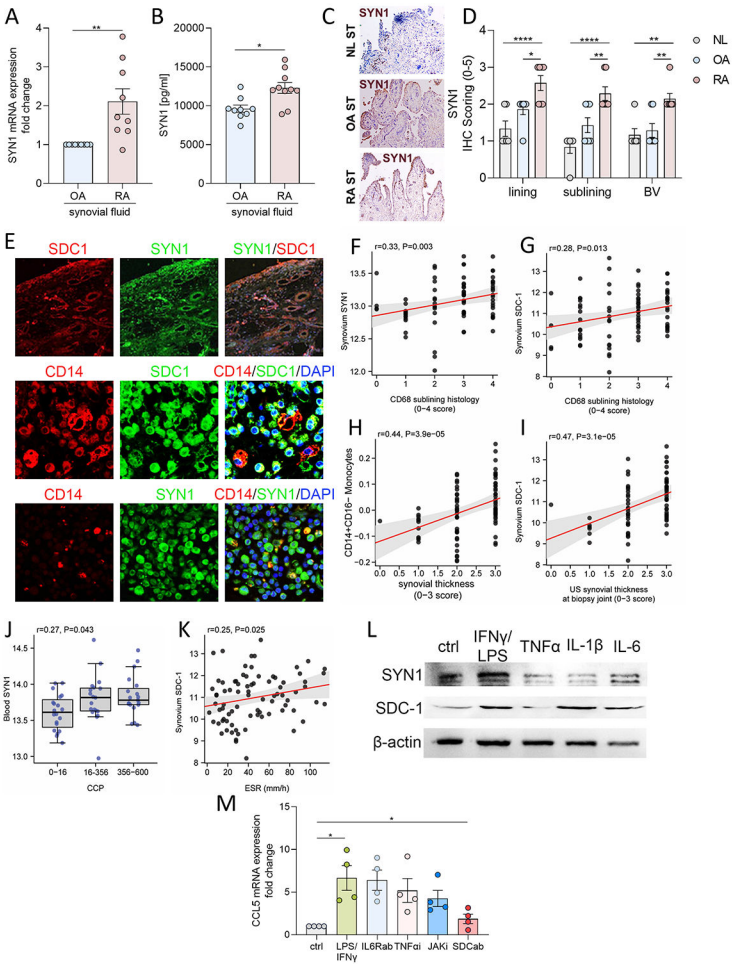


Figure 1. Syntenin-1 and SDC-1 expression is linked to RA clinical manifestation and is mutually enhanced by LPS/IFNγ stimulation in MΦs.

(**A** and **B**) Syntenin-1 transcription (A) and protein levels (B) were quantified in OA (n=9) and RA (n=9-10) synovial fluids by qRT-PCR or ELISA. (**C** and **D**) Synovial tissues from NL (n=6), OA (n=7), or RA (n=7) individuals were used to determine Syntenin-1 presentation (C) and its relative expression was scored in the lining, sublining, and blood vessels (BV) (on a 0-5 scale) (D). (**E**) RA STs were fluorescently stained to authenticate the colocalization of SDC-1 with Syntenin-1 and their expression on $CD14^+$ cells in presence of DAPI. (**F** and **G**) Relative expression of Syntenin-1 (F) or SDC-1 (G) was determined by RNAseq (n=87)[18] in RA synovial tissue biopsies and linked to the number of $CD68^+$ MΦs quantified by histology scoring (score 0-4). (**H** to **K**) $CD14^+CD16^-$ myeloid cells (H) or SDC-1 transcript levels (I) were evaluated by RNAseq (n=90)[18] and correlated with RA ultrasound-guided synovial tissue thickness (score 0-3). Blood Syntenin-1 (J, n=67) or synovial tissue SDC-1 (K, n=87) transcript level was quantified by RNAseq [18] and linked to CCP or ESR. (**L**) Human myeloid cells were stimulated with IFNγ and LPS, TNFα, IL-1β, and IL-6 (all 100 ng/ml), and expression of Syntenin-1 and SDC-1 was analyzed by western blot. β-actin served as a loading control. (**M**) RA MΦs were untreated or treated with LPS/IFNγ (100 ng/ml) with or without TNFαi, IL6R Ab, Jaki (Tofacitinib; all 10 μg/ml) or SDC-1 antibody (SDCab, 1:100), and transcription levels of CCL5 were determined by qRT-

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

PCR (n=4). Data are presented as mean ± SEM; significant differences were determined by the Mann-Whitney test, 2way-ANOVA, or one-way ANOVA. For RNAseq data, spearman rank correlation was utilized. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

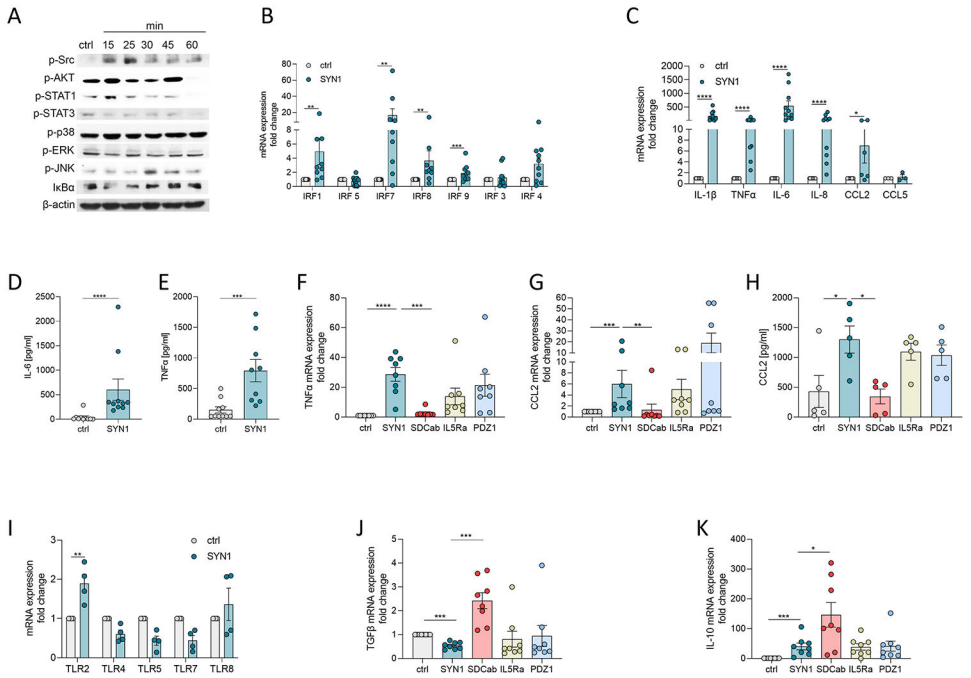


Figure 2. Ligation of Syntenin-1 to SDC-1 expands RA MΦ inflammatory profile independent of IL 5R or PDZ1 function.

(**A**) Human myeloid cells were treated with Syntenin-1 (SYN1; 1000 ng/ml) for 0-60 mins and phosphorylation of Src, AKT, STAT1, STAT3, p38, ERK, and JNK, and degradation of IκBα was determined by western blot analysis and β-actin served as a loading control. (**B** to **E**) RA MΦs were treated with PBS (ctrl) or Syntenin-1 (1000 ng/ml) for 6h or 24h. Transcription of IRFs (**B**) and the inflammatory monokines (**C**) was assessed by qRT-PCR (n=3-10), and protein levels of IL-6 (D) or TNFα (E) were determined in the conditioned media by ELISA (n=10). (**F** to **K**) RA MΦs were treated with PBS or Syntenin-1 (1000 ng/ml) in the presence or absence of SDC-1 Ab (SDCab; 1:100), IL-5R Ab (IL5Ra; 2 μg/ml), or PDZ1i (PDZ1; 10 μM) for 6h or 24h before quantifying TNFα (**F**), CCL2 (**G**), TLRs (**I**), TGFβ (**J**), or IL-10 (**K**) mRNA levels by qRT-PCR or CCL2 protein levels (**H**) by ELISA (n=4-8). Data are presented as mean ± SEM; significant differences were determined by the Mann-Whitney test, 2way- ANOVA, or one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

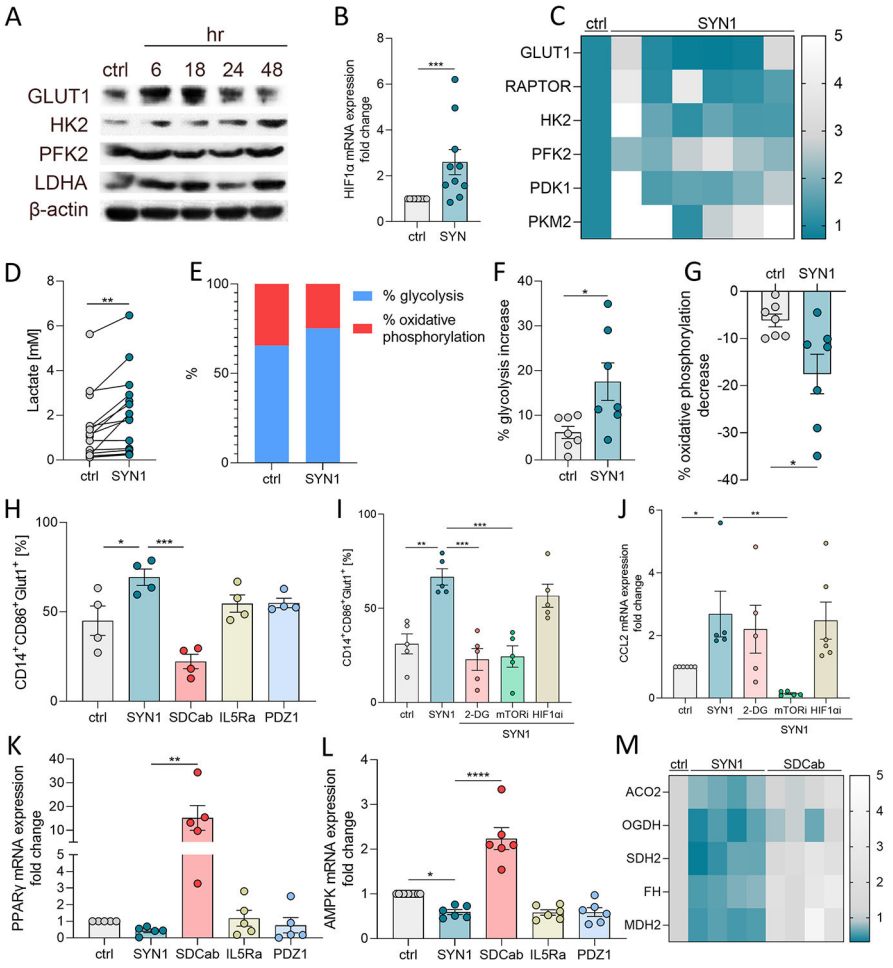


Figure 3. RA MΦ metabolic reprogramming is potentiated by activation of the Syntenin-1/SDC-1 pathway.

(**A**) RA MΦs were untreated (ctrl) or treated with Syntenin-1 (SYN1; 1000 ng/ml) for 6h to 48h before determining the expression of GLUT1, HK2, PFK2, and LDHA by western blot analysis. β-actin served as a loading control. (**B** and **C**) RA MΦs were treated with PBS (ctrl) or Syntenin-1 (1000 ng/ml) for 6h and transcription levels of HIF1α (n=10) (B), GLUT1, RAPTOR, HK2, PFK2, PDK1, PKM2 (C) were quantified by qRT-PCR (n=6). (**D**) RA monocyte-differentiated MΦs were treated with PBS or Syntenin-1 (1000 ng/ml) for 24h before measuring lactate protein levels colorimetrically (n=16). (**E** to **G**) RA monocyte-differentiated MΦs (2×10^5 cells/well) were treated with PBS or Syntenin-1 (1000 ng/ml) and % glycolysis increase (E and F) and % oxidative phosphorylation decrease (E and G) were calculated by Seahorse XF Real-Time ATP Rate Assay Kit (n=7). (**H** to **M**) RA monocyte-differentiated MΦs were treated with PBS or Syntenin-1 (1000 ng/ml) in the presence or absence of SDC-1 Ab (SDCab; 1:100) and/or IL-5R Ab (IL5Ra; 2 μg/ml), or PDZ1i (PDZ1; 10 μM) (H and K to L), and 2-DG (5 mM), mTORi (1 μM), or HIF1αi (2 μM) (I and J). (**H** and **I**) After 24h impact of treatment was determined on $CD14^+CD86^+GLUT1^+$ frequency by flow cytometry (n=4-5). (**J** to **M**) mRNA expression of CCL2 (J), PPARγ (K), and AMPK (L), or oxidative enzymes (M) was determined after 6h by qRT-PCR (n=4-6). Data are presented as mean ± SEM; significant differences were

determined by Mann-Whitney test, students-*t*-test, 2way-ANOVA, or one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

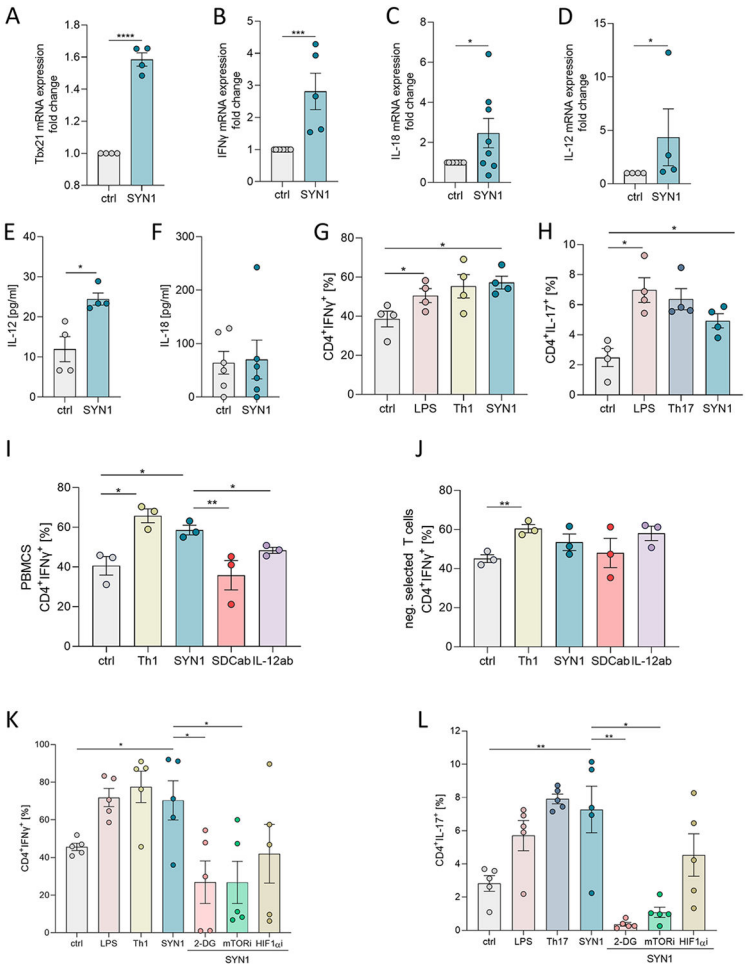


Figure 4. Syntenin-1 enhances Th1 and Th17 cell differentiation via IL-12 and/or IL-18 induction.

(**A** to **F**) RA PBMCs were treated with PBS (ctrl) or Syntenin-1 (SYN1; 1000 ng/ml) for 6h (qRT-PCR) or 24h (protein). Transcriptional regulation of Tbx21(A), IFN**γ** (B), IL-18 (C), and IL-12 (D) was assessed by qRT-PCR (n=4-8). (**E** and **F**) Protein secretion of IL-12 (E) and IL-18 (F) was determined by ELISA (n=4-6). (**G** to **J**) RA PBMCs (G to I) or negatively selected T cells (J) were supplemented with anti-CD3 and anti-CD28 (both 0.25 μg/ml) and were untreated (ctrl) or stimulated with LPS (100 ng/ml, +control), IL-12 (10 ng/ml, Th1 cells), IL-1β, IL-6, and TGF-β (20 ng/ml and 4 ng/ml, respectively, Th17 cells) or Syntenin-1 (1000 ng/ml) in the presence of absence of SDC-1 and IL-12 antibody (SDCab, IL-12ab) for 72h prior to determining the number of $CD4^+IFN\gamma^+$ T cells (G, I and J) or $CD4^+IL\text{-}17^+$ T cells by flow cytometry (H) (n=3-4). (**K** and **L**) RA PBMCs were cultured with anti-CD3 and anti-CD28 (both 0.25 μg/ml) and were untreated (ctrl) or stimulated with LPS (100 ng/ml, +control), IL-12 (10 ng/ml, Th1 cells) or Syntenin-1 (1000 ng/ml) alone or in combination with 2-DG (5 mM), mTORi (1 μM), and HIF1αi (2 μM) for 72h before determining the number of $CD4^+IFN\gamma^+$ T cells (K) or $CD4^+IL\text{-}17^+$ T cells (L) by flow cytometry (n=5). Data are presented as mean ± SEM; significant differences were determined by the Mann-Whitney test or one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

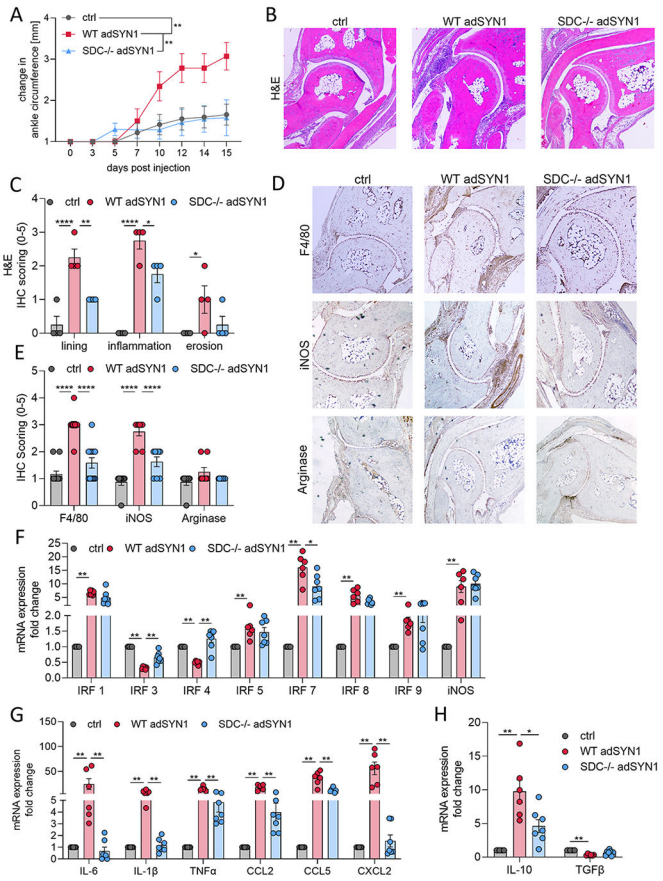


Figure 5. Local expression of Syntenin-1 advances arthritis in WT but not in SDC-1$^{-/-}$ mice.

(A to **H)** Wildtype (WT) and SDC-1$^{-/-}$ C57BL/6 (SDC−/−) mice were injected intra-articularly with adctrl (ctrl) or adSYN1 (3×10^{10} viral particles/ankle) on days 0, 7, and 14 and joint circumference (A) was monitored over 15 days (n=10 mice/group). On day 15, mice were sacrificed, and ankles were either used for histological analysis or qRT-PCR. (**B** and **C**) Sections from non-arthritic WT ctrl and WT or SDC$^{-/-}$ mice injected with adSYN1 were stained for H&E (B) and scored on a 0-5 scale for synovial lining thickness, inflammation, and bone erosion (C) (n=4). (**D** and **E**) Ankle sections from non-arthritic WT ctrl and WT or SDC$^{-/-}$ mice injected with adSYN1 were stained for the macrophage markers F4/80, iNOS, and arginase 1 (D) and subsequently scored on a 0-5 scale (E) (n=8-12). (**F** to **H**) Ankles from non-arthritic WT ctrl and WT or SDC$^{-/-}$ mice injected with adSYN1 were homogenized and transcription levels of IRFs and iNOS (F), inflammatory cytokines (G), or pro-repair factors (H) were quantified by qRT-PCR (n=6-7). Data are presented as mean ± SEM; significant differences were determined by one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

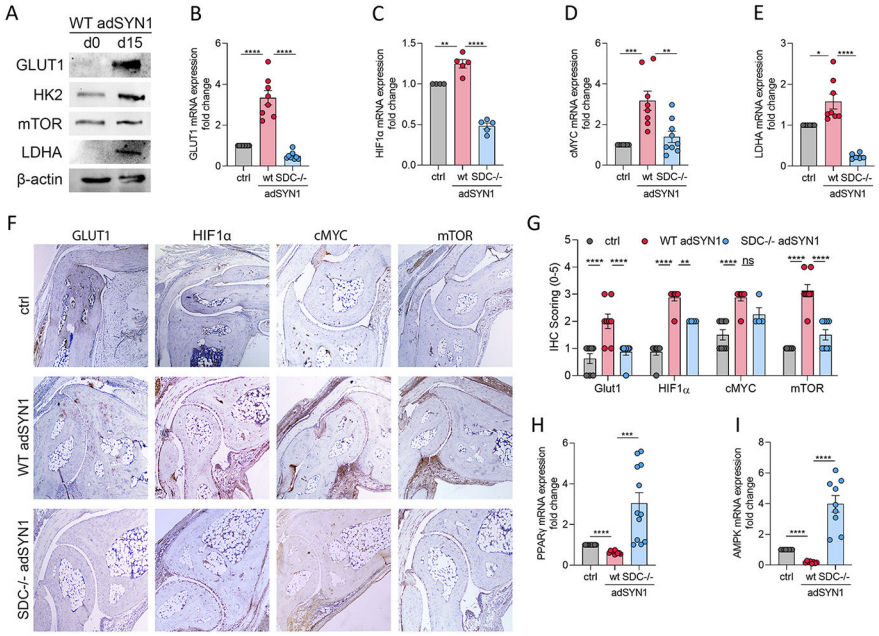


Figure 6. Syntenin-1 arthritic mice display hypermetabolic activity in wild-type mice which was mitigated in SDC-1$^{-/-}$ animals.

(**A** to **I**) WT and SDC$^{-/-}$ mice were injected intra-articularly with adctrl (ctrl) or adSYN1 (3×10^{10} viral particles/ankle) on days 0, 7, and 14. (**A**) Ankles from non-arthritic WT ctrl (day 0) and WT adSYN1 mice (day 15) were homogenized and expression of glycolytic proteins, GLUT1, HK2, mTOR/p70, and LDHA was determined by western blot analysis and β-actin served as a loading control. (**B** to **E**) Ankles from non-arthritic WT ctrl and WT or SDC$^{-/-}$ mice injected with adSYN1 were homogenized and transcriptional regulation of the glycolytic factors GLUT1 (B), HIF1α (C), cMYC (D), and LDHA (E) was determined by qRT-PCR (n=5-9). (**F** and **G**) Ankles from non-arthritic WT ctrl and WT or SDC$^{-/-}$ mice injected with adSYN1 were stained for GLUT1, HIF1α, cMYC, and mTOR/p70 (F) and their staining was scored on a 0-5 scale (G) (n=8). (**H** and **I**) mRNA levels of PPARγ (H) and AMPK (I) were quantified in joints from non-arthritic WT ctrl and WT adSYN1 or SDC$^{-/-}$ adSYN1 mice by qRT-PCR (n=8-12). Data are presented as mean ± SEM; significant differences were determined by one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

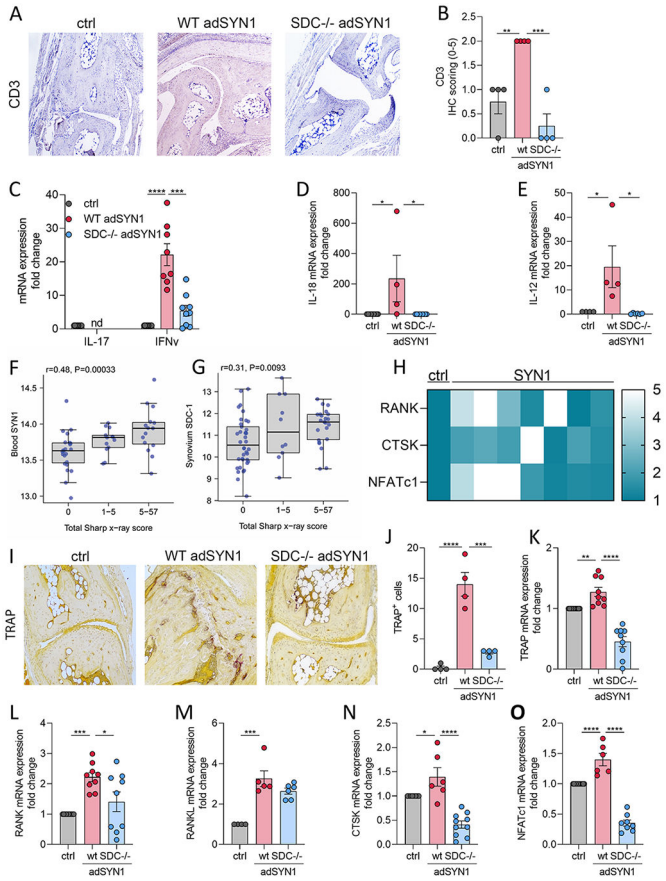


Figure 7. RA preosteoclasts and arthritic joint cells are transformed into mature osteoclasts by Syntenin-1.

(**A** to **B**) WT and SDC$^{-/-}$ mice were injected intra-articularly with adctrl (ctrl) or adSYN1 (3×10^{10} viral particles/ankle) on days 0, 7, and 14. Ankles from non-arthritic WT ctrl and WT or SDC$^{-/-}$ mice injected with adSYN1 were stained for T cell marker CD3 (A) and scored on a 0-5 scale (B) (n=4). (**C** to **E**) Ankles from non-arthritic WT ctrl and WT or SDC$^{-/-}$ mice injected with adSYN1 were homogenized and transcription levels of IL-17 and IFN**γ** (C), IL-18 (D), and IL-12 (E) were quantified by qRT-PCR (n=4-10). (**F** and **G**) The transcript levels of blood Syntenin-1 (F, n=67) or synovial tissue SDC-1 (G, n=87) determined by RNAseq [18] were correlated against bone erosion as determined by radiographic images of hands and feet by Sharp/van der Heijde score. (**H**) In presence of M-CSF and RANKL (10 ng/ml each, suboptimal condition) RA monocytes were differentiated into preosteoclasts for 7 days, stimulated with PBS (ctrl) or Syntenin-1 (1000ng/ml) for 6h and osteoclastic factors were assessed by qRT-PCR (H) (n=7). (**I** and **J**) Ankles from non-arthritic WT ctrl and WT or SDC$^{-/-}$ mice injected with adSYN1 were stained for TRAP (I) and TRAP^{+} cells (**J**) and were quantified at x100 magnification (n=4). (**K** to **O**) Ankles from non-arthritic WT ctrl and WT or SDC$^{-/-}$ mice injected with adSYN1 were homogenized and analyzed for transcriptional regulation of TRAP (K), RANK (L), RANKL (M), CTSK (N), and NFATc1 (O) by qRT-PCR (n=4-12). Data are presented as mean ± SEM; significant differences were determined by one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

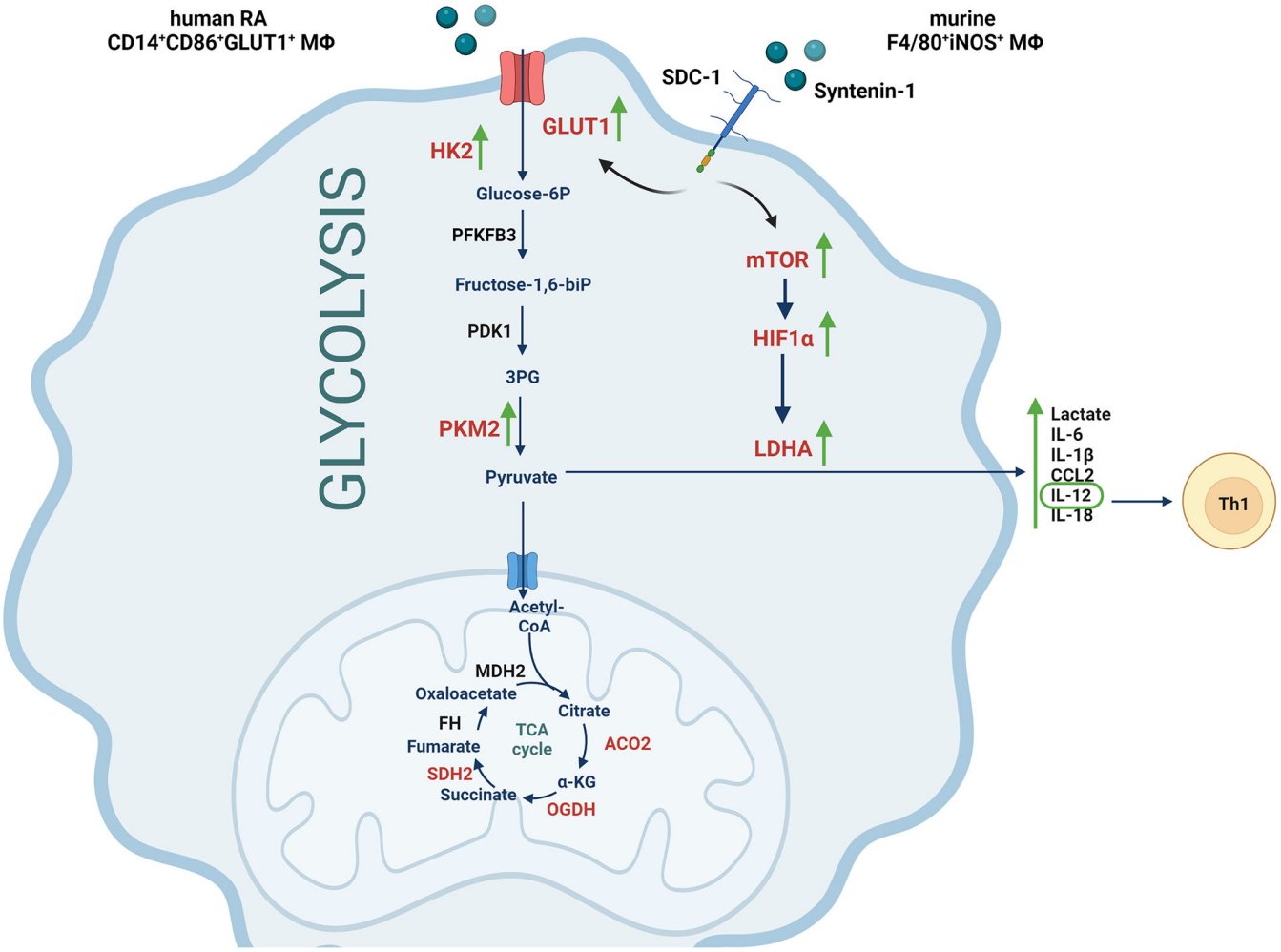


Figure 8. Syntenin-1 advances glycolytic reprogramming in RA CD14^{+}CD86^{+}GLUT1^{+}MΦs and murine F4/80^{+}iNOS^{+}MΦs.

Syntenin-1 reconfigures naive cells into metabolic RA CD14^{+}CD86^{+}GLUT1^{+}MΦs that display a broad array of glycolytic factors together with impaired oxidative intermediates through SDC-1 ligation, glucose uptake, and/or mTOR signaling. In Syntenin-1-induced arthritis, F4/80^{+}iNOS^{+}MΦs recapitulate glycolytic RA myeloid cell mechanism of function, by expanding the inflammatory and glycolytic imprints which are dysregulated in SDC-1$^{-/-}$ animals. Both in RA cells and/or experimental models, mTOR-driven MΦ glycolytic reprogramming and their crosstalk with Th1 cells via IL-12 escalation are responsible for Syntenin-1-induced arthritogenicity.