SUPPLEMENTAL MATERIAL

Splicing machinery is impaired in Rheumatoid Arthritis, associated with disease activity and modulated by Anti-TNF therapy

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Patients and Methods

Blood sample collection, assessment of clinical and biological parameters, and B-mode ultrasound IMT measurements.

Peripheral blood leukocyte subsets including monocytes, neutrophils and lymphocytes were isolated from the peripheral blood of RA patients and HD, using Ficoll Hypaque gradient and specific cell separation commercial kits (Miltenyi Biotec) and an autoMACS Pro Separator (Miltenyi Biotech, Bergisch Gladbach, Germany) as previously described.14

In a subset of patients from the first RA cohort, mononuclear cells were also collected from both, synovial fluid and peripheral blood by Ficoll Hypaque gradient to perform matching studies.

In the second cohort, comprising RA patients treated with anti-TNF, blood samples were obtained before and after 3 and 6 months of treatment. To avoid blood composition changes promoted by diet and circadian rhythms, samples were always collected in the early hours of the morning and after a fasting period of 8 hours.

Radiological involvement was assessed by evaluating the presence and number of eroded joints, joint space narrowing, soft tissue swelling, or joint effusion among other manifestations, such as osteoporosis or the presence of subcutaneous rheumatoid nodules.
B-Mode Ultrasound IMT Measurements

RA patients and controls underwent B-mode ultrasound imaging for carotid intima media thickness (CIMT) measurements as previously described. B-mode ultrasound imaging of the carotid arteries was performed by using Toshiba equipment (Aplio platform) equipped with 7–10 MHz broadband linear array transducers. Patients were examined in the supine position, with the head turned 45° contralateral to the side of scanning. Three carotid arterial segments were assessed: the common carotid (1 cm proximal to the bulb), the carotid bulb (between the dilatation and flow divider), and the internal carotid (1 cm distal to the flow divider). Of each segment, the near and the far walls of the left and right carotid artery segments were imaged at 2 different angles. The maximum distance of the intima-media thickness, defined as “maximum IMT” was calculated for each view. The plaque was defined as a focal structure that encroached into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value or demonstrated a thickness >1.5 mm as measured from the media-adventitia interface to the intima-lumen interface.

Analysis of splicing machinery components by qPCR dynamic array based on microfluidic technology

Total RNA from monocytes, neutrophils and lymphocytes was isolated using TRIzol (Bioline, Memphis, TX, USA) followed by a DNAse treatment (RQ1DNase, Promega; Wisconsin, USA) ensuing manufacturer's instructions. RNA purity and concentration were evaluated using Nanodrop spectrophotometer (Thermo Scientific, Waltham, MA, USA). 1000 ng of total RNA were retrotranscribed using NZY Reverse Transcriptase kit (NZYTech, Lisboa, Portugal) using random hexamer primers. A 48.48 Dynamic Array (Fluidigm, San Francisco, CA, USA) was used to assess the expression of 45 selected transcripts of the major and minor spliceosome and associated splicing factors as previously reported.

Fluidigm (South San Francisco, CA, USA) dynamic arrays use microfluidics [an “integrated fluidic circuit” (IFC) connected to reagent input wells] and high-resolution imaging to perform qPCR with fluorescence detection in nanoliter reaction volumes. This microfluidics system allows more rapid screening of large sample sets and
consumption of substantially lower amounts of PCR reagents, while avoiding the reaction-compatibility requirements of multiplex systems that combine several target detection assays in 1 reaction (J Biomol Tech. 2016 Jul; 27(2): 75–83. doi: 10.7171/jbt.16-2702-003).

The panel of splicing machinery components was selected on the basis of two main criteria: 1) the relevance of the given spliceosome components in the splicing process [such as the components of the spliceosome core (SNRNP70, RNU2, RNU4, RNU5, SNRNP200, U2AF1, U2AF2, SF3B1, TCERG1, PRPF40A, PRPF8, RBM22, RNU11, RNU12, RNU4ATAC, RNU6ATAC)], and 2) its demonstrated role in autoimmune, rheumatic or inflammatory diseases (NOVA1, PTBP1, RAVER1, RBM45, SFPQ, SND1, SNW1, SRSF1, SRSF2, SRSF5, TIA1).

In the present study, a 48.48 Dynamic Array based on microfluidic technology (Fluidigm, San Francisco, CA, USA) was implemented, to determine the expression of 48 transcripts in 48 samples, simultaneously. Specific primers for human transcripts including components of the major (n=13) and minor spliceosome (n=4), associated SFs (n=28) and three housekeeping genes were specifically designed with the Primer3 software and StepOne™ Real-Time PCR System software v2.3 (Applied Biosystems, Foster City, CA, USA). Following manufacturer’s instructions, 12.5ng of cDNA of each sample were pre-amplified using 1µL of PreAmp Master Mix (Fluidigm) and 0.5µL of all primers mix (500nM) in a T100 Thermal-cycler (BioRad, Hercules, CA, USA), using the following program: 1) 2 min at 95ºC; 2) 15 sec at 94ºC and 4 min at 60ºC (14 cycles). Then, samples were treated with 2µL of 4U/µL Exonuclease I solution (New England BioLabs, Ipswich, MA, USA) following manufacturer’s instructions. Then, samples were diluted with 18µL of TE Buffer (Thermo Scientific), and 2.7µL were mixed with 3µL of EvaGreen Supermix (Bio-Rad) and 0.3µL of DNA Binding Dye Sample Loading Reagent (Fluidigm). Primers were diluted to 5µM with 2X Assay Loading Reagent (Fluidigm). Control line fluid was charged in the chip and Prime script program was run into the IFC controller MX (Fluidigm). Finally, 5µL of each primer and 5µL of each sample were pipetted into their respective inlets on the chip and the Load Mix script in the IFC controller software was run. After this program, the qPCR was run in the Biomark System (Fluidigm) following the thermal cycling program: 1) 95ºC for 1min; 2) 35 cycles of denaturing (95ºC for 5sec) and annealing/extension (60ºC for 20sec); and 3) a last
cycle where final PCR products were subjected to graded temperature-dependent
dissociation (60°C to 95°C, increasing 1°C/3 sec).
Data were processed with Real-Time PCR Analysis Software 3.0 (Fluidigm).
Additionally, total RNA from PAXgene tubes containing whole blood samples, obtained
from the second RA cohort involving patients treated with TNFi for six months, was
purified by using the PAXgene Blood RNA kit (PreAnalytiX, Hombrechtikon, Switzerland).
Validation of altered spliceosome components in RA patients treated with TNFi and in
HD treated with purified ACPA-IgG from RA patients or with non-immune IgG from HD
was performed by quantitative real time PCR (qPCR) using LightCycler 480 (Applied
Biosystems, Foster City, CA, USA) thermocycler and above-mentioned primers.

**Bioplex assay of the inflammatory profile in plasma of RA patients**
Secreted levels of 27 cytokines/chemokines/adhesion molecules in plasma of RA
patients were determined using a 27-plex panel in a multiplex bead-based assay
system (Bio-Plex multiplex immunoassays, Bio Rad; CA, USA). The assay was performed
according to the manufacturer’s protocol using Bio-Plex 200 system based on Luminex
technology. Briefly, plasma samples were transferred to magnetic beads and incubated
for 1 h at room temperature. After incubation, a series of steps including plate wash,
antibody and streptavidin incubation before exposure of plate were performed.
Finally, the samples were acquired and quantified using Bio-plex 200 (Luminex 200,
USA). Cytokine concentrations were determined from standard curves prepared on
each plate and expressed as pg/ml using the Bio-Plex ManagerTM software (Bio-Rad,
Hercules, CA, USA).

**Analysis of an external RNAseq dataset to validate gene expression and
identify splicing variants.**
RNA-seq data of an external cohort of 44 patients (E-MTAB-6141) was analyzed as
validation cohort to explore gene expression and splicing profile. This dataset included
matching data of whole blood cells and synovial biopsies.

**Gene expression and splicing variants analysis.**
Raw paired-end FASTQ files were trimmed using Trim Galore and quality check was
assessed using Fast QC and normalized using Salmon and the last release (v34) of
human GENCODE transcriptome. The relative abundance of transcripts in transcripts per million (TPM) generated by Salmon were used as input for SUPPA2 software to perform the calculation of relative abundances of the splicing events as Percent Spliced In Index (PSI or Ψ). For differential expression analyses and to compare splicing variants considering high or low expression, gene abundances were imported to R and summarized to gene-level using Tximeta and normalized and quantified using DESeq2. In each comparison, samples were classified according to gene expression using third and first quartiles samples. PSI and TPM values for the low and high expression groups were used with SUPPA2 to perform the differential splicing analysis with local events, then splicing differences using delta PSI (ΔΨ) were calculated. The difference in average PSI from each group with adjusted, and p value < 0.05 were considered significant. The PSI values were used to calculate the relative frequency of each splicing event per sample [Relative Frequency (event i) = (Σ PSI (event i))/(Σ PSI (total events))] and estimate the splicing event composition per sample. The comparisons between high and low groups were tested by t test with significance cutoff at p < 0.05. Classification of splicing event profiling was established into 7 types of events according to their splicing pattern: alternative 3’ splice site, alternative 5’ splice site, alternative first exon, alternative last exon, mutually exclusive exons, retained intron and skipped exon.

Gene Ontology (GO) terms enrichment analyses were performed using DAVID online software v6.8, (Huang Da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009;4(1):44-57. doi: 10.1038/nprot.2008.211. Huang Da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009;37(1):1-13. doi: 10.1093/nar/gkn923) using differentially expressed genes and genes with differentially spliced events.

**In vitro studies**

1. **Effects of anti-citrullinated protein antibodies on HD-leukocytes subsets.**

IgGs from serum of 5 RA patients with high titers of anti-citrullinated protein antibodies (ACPAs) and negative for RF were isolated using HiTrap protein-G-HP columns (GE Healthcare, Chicago, IL, USA). IgG-ACPAS were subsequently purified using CCP affinity columns as previously described (Barbarroja N et al., ATVB 2014). Briefly, Streptavidin agarose columns (Thermo Fisher Scientific, Waltham, MA, USA) were coupled with biotinylated CCP peptides.
following the manufacturer’s recommendations (EZ-Link Sulfo-NHS-LC-LC-Biotin, Thermo Fisher Scientific). A cyclic-CCP peptide was synthesized according to the sequence previously designed by Schellekens et al. (Arthritis Rheum 2000; 43:155–63) for the clinical test of ACPAS (Immunostep, Salamanca, Spain): HQCHQESTXGRSRGRCGRSGS, where X refers to citrulline. The IgGs-containing ACPAs from RA serum were incubated with the CCP affinity column 6 hours at room temperature. Afterward, the column was washed with PBS to obtain the flow through with IgGs depleted in ACPAS. Finally, IgG-ACPAS were eluted with 0.1M Glycine-hydrogen chloride (HCL) pH2.5 and neutralized with 2M Tris. The activity of both, IgG-ACP and flow-through (IgGs depleted in ACPAS) was confirmed by ELISA (Immunoscan CCPlus, SVAR Life Science, Sweden).

Then, 500,000 cells/well from HD peripheral blood leukocytes subsets were treated with purified IgG-ACPAS and flow-through (IgG depleted in ACPAS) (10 µg/ml), either for 6 h (neutrophils) or for 24 h (monocytes and lymphocytes), and effects on SM components and inflammatory cytokines were assessed.

To evaluate the effects of FcR (Fc receptor) blockage, monocytes, lymphocytes and neutrophils were preincubated with FcR Blocking Reagent (Miltenty) for 15 minutes at 4 °C before the treatment with purified IgG-ACPAS and flow-through (IgG depleted in ACPAS), as above described.

To further validate the specificity of ACPAS, parallel experiments were carried out using human monoclonal ACPAS (anticitrullinated fibrinogen immunoglobulin, clone 1F11, 10 µg/ml) (MQR 2.101-100, Modique, Molenweg, The Netherlands) and synthetic human IgG (Jackson ImmunoResearch Laboratories, Inc, Newmarket, Suffolk, UK).

Lastly,

2) Effects of inflammatory cytokines on the expression of SME in HD leukocytes subsets

500,000 cells/well from HD peripheral blood leukocytes subsets were treated with purified TNFa, IL6, and CCL2 (20 ng/ml each) either for 6 h (neutrophils) or for 24 h (monocytes and lymphocytes), and effects on SM components were assessed by RT-PCR.

3) Transfection studies

Peripheral blood leukocytes subsets from 4 RA patients were used for transfection studies. Briefly, 500,000 cells/well were transiently overexpressed using Lipofectamine 2000 (Life Technologies) with KHDRBS1 (OHu20035, Genscript, Leiden, The Netherlands) or SNRNP70 (OHu21864, Genscript) vectors for 24h, being the empty pCDNA3.1+ (mock, Life Technologies, Grand Island, NY, USA) used as negative control, as previously described.32
Then, effectiveness of transfections was assessed by RT-PCR and Western blot assays. A 27-plex panel of secreted inflammatory molecules was assessed in RA-lymphocytes; adhesion was evaluated on RA-monocytes after 24 h using Vybrant cell adhesion kit (Molecular Probes Inc, Leiden, The Netherlands); nucleosomes and neutrophil elastase were assessed in the supernatant of RA-neutrophils using the ELISAPLUS kit for detection of human cell death (Roche Diagnostics) and the Human Elastase PMN Sandwich ELISA Kit (Abcam, Cambridge, UK), respectively.

4) Effects of supernatants from transfected lymphocytes on functional activity of synovial fibroblasts.

For isolation of synovial fibroblasts (SF), synovial fluid was aspirated from joints of 10 patients with established and active RA. Fluid was collected in heparinized syringes, then centrifuged at 1200 rpm for 15 min. The resulting cell pellet was resuspended in 7 ml of growth medium [DMEM / F12 (1:1) HAM with 15% heat inactivated fetal bovine serum (FBS), 2mM l-glutamine, 1% penicillin/streptomycin solution (Sigma)], supplemented with fibroblast growth factor beta (FGFb, 1 ng/ml) and plated in 25 ml tissue culture flasks. Cultures were incubated at 37°C with 5% CO₂ for 24 to 48 h, after which medium was aspirated and cultures were washed with phosphate buffered saline (PBS) to remove nonadherent cells. Growth medium was replaced every 3 to 4 days. After 10 to 14 days, adherent cells were removed from flasks by trypsinization, washed, and transferred to 75 ml tissue culture flasks in fresh growth medium. Purified SF were passaged (split 1:3) when they reached confluence, generally at 10 to 14 days. Passages 2 through 6 were used for experiments.

Cultured SF were grown either in 96-well culture plates (5,000 cells/well) or 24-well culture plates (500,000 cells/well), and subsequently stimulated for 24 h with the supernatant obtained from transfection cultures (diluted 1:1 with growth medium).

Then, SF proliferation and migration were measured using resazurin and wound-healing assays, respectively, as previously reported (Jiménez-Vacas JM, Herrero-Aguayo V, Gómez-Gómez E, et al. Spliceosome component SF3B1 as novel prognostic biomarker and therapeutic target for prostate cancer. Transl Res 2019;212:89-103. doi: 10.1016/j.trsl.2019.07.001). Gene expression of inflammatory mediators was evaluated by RT-PCR.

Identification of the citrullinome in PBMCs by LC-MS/MS

Sample preparation and LC-MS/MS analysis
Equal amounts of each sample pool (approximately 10µg) were reduced with 10 mM dithiothreitol for 1 h at 37 °C, and subsequently alkylated with 50 mM iodoacetamide for 45 minutes at room temperature in the dark. Samples were digested with sequencing grade modified trypsin (Promega) at 1:40 enzyme-to-substrate ratio. After 16 h of digestion at 37 °C, samples were acidified with 10% trifluoroacetic acid to ~pH 3. The digested peptides were desalted using in-house made stage tips (3M Empore SPE-C18 disk, 47 mm, Sigma Aldrich) and finally dried under speed-vacuum (Thermo, USA). The dried eluates were re-constituted in water with 2% acetonitrile (ACN) and 0.1% FA for direct LC-MS. The peptide mixture (200ng) was loaded in a nanoElute (Bruker Daltonics) nano-flow LC coupled to a high-resolution TIMS-QTOF (timsTOF Pro, Bruker Daltonics) with a CaptiveSpray ion source (Bruker Daltonics). Liquid chromatography was performed at 50 °C and with a constant flow of 500 nL/min on a reversed-phase column (15 cm * 75 m i.d.) with a pulled emitter tip, packed with 1.9 m C18-coated porous silica beads (Dr. Maisch, Ammerbuch-Entringen, Germany). Chromatographic separation was carried out using a linear gradient of 5-35% buffer B (100% ACN and 0.1% FA) over 60 min. After ESI ionization, peptides were analyzed in data-dependent mode with Parallel Accumulation–Serial Fragmentation (PASEF) enabled.

Data analysis
Mass spectrometry raw files were processed with PEAKS Studio 10.6 build 20201221 (Bioinformatics solutions Inc.). The MS/MS spectra were matched to in silico derived fragment mass values of tryptic peptides against the UniProtKB/Swiss-Prot human database (release 2021_02). Search parameters were: Parent Mass Error Tolerance: 15.0 ppm; Fragment Mass Error Tolerance: 0.05 Da; Enzyme: Trypsin; Fixed Modifications: Carbamidomethylation; Variable Modifications: Acetylation (Protein N-term), Deamidation (NQ), Oxidation (M), Acetylation (N-term) and Citrullination (R); Max Variable PTM Per Peptide: 3. Matches were filtered for 1% FDR at peptide level. For PTMs quantification, citrullinated peptides with AScore >20 (p value < 0.01) (PMID: 16964243) were considered.
Quantification of citrullination was calculated as the sum of intensities of all redundant identifications of a peptide in a certain citrullination state, divided by the sum of intensities of all identifications of the same peptide, independently of modifications.

**Statistical analysis**

Data were expressed as mean ± SEM or median ± IQR according to data distribution, evaluated using Kolmogorov-Smirnov test. Thus, Student’s t test or Mann-Whitney rank sum test were used to assess statistical differences in unpaired data, and paired t tests and Wilcoxon matched-pairs signed rank tests for paired data. The chi-square test was used to associate qualitative variables. Correlations were evaluated by Spearman’s correlation test. In addition, for adjusting the p-values towards multiple hypothesis testing, a Benjamini Hochberg-based false discovery rate (FDR) was applied (REF). Statistically significant differences were considered at p-value<0.05 and FDR<0.15 (Y. Benjamini and Y. Hochberg, *Controlling the False Discovery Rate-a Practical and Powerful Approach to Multiple Testing*, J. Roy. Stat. Soc. B Stat. Methodol., 1995, 57, 289-300).

Logistic regression models were calculated using the formula

\[ P = \frac{1}{1+e^{-(\theta_0 + \theta_1 x_1 + \ldots + \theta_k x_k)}} \]

being \( \theta_i \) the coefficients of the parameters. Receiver operating characteristic (ROC) curves were performed to evaluate the specificity and sensibility of the different diagnostic or discriminating models. Statistical analysis of each ROC curve was performed by evaluating the area under the curve (AUC) of each model and comparing them with the reference line.

Data analyses were performed using SPSS 24.0 (IBM, Chicago, IL, USA) and GraphPad Prism 8.0 (GraphPad Software, La Jolla, CA).

**SUPPLEMENTAL FIGURES**

**Supplemental Figure 1.** Spliceosome components are dysregulated in a coordinated way. Heat map of correlation between the signature of 8 spliceosome components commonly altered in neutrophils, monocytes and lymphocytes. Positive and negative correlations are displayed in red or blue respectively accordingly to the Spearman correlation coefficient. Lym, lymphocytes; Mon, monocytes; Neu, neutrophils.

**Supplemental Figure 2.** RA patients exhibit an altered circulating pro-inflammatory profile. Plasmatic levels of a panel of 27 molecules including cytokines, chemokines and adhesion molecules were analysed by using a multiplex bead-based assay system.
(A) Heat map showing the differential expression of those molecules between RA and HD are shown. Blue and red colours represent downregulated and upregulated pro-inflammatory molecules respectively. (B) Violin plots indicating the expression levels of differentially expressed pro-inflammatory molecules in plasma from RA and HD. *p<0.05, **p<0.01, ***p<0.001. RA, Rheumatoid Arthritis; HD, Healthy Donors.

Supplemental Figure 3. Differential expression of SME in whole blood cells and synovial biopsies. Heatmap showing unsupervised hierarchical clustering of expression-pattern change of splicing machinery and splicing factors. Normalized transcripts have been scaled by autoscaling method to perform the heatmap.

Supplemental Figure 4.- The SME expression pattern is associated with differential splice events, splice variants, and gene pathways. A. Volcano-plots where the ΔΨ of total events calculated is plotted against the −log10 (p-value) of the Fisher’s Exact Test to assay differentially expressed splicing variants between high and low SME expression groups of samples. B. Relative frequency of each alternative splicing event between low and high SME expression groups. C. UpSet plot for all transcript variants generated. Strips show the number of differentially spliced transcripts significantly different between low and high SME expression. Dots and lines represent specific transcripts. The histogram represents the number of differentially spliced transcripts.

Supplemental Figure 5.- Enrichment plots according to differential splicing variants. Gene Ontology (GO) terms enrichment analyses were performed using DAVID online software, using genes with differentially spliced events.

Supplemental Figure 6.- Enrichment plots according to differential expressed genes. A. Gene Ontology (GO) terms enrichment analyses were performed using DAVID online software, using differentially expressed genes. B. Volcano-plots where the log2 (Fold Change) of total differentially expressed genes is plotted against the −log10 (p-value) of the Fisher’s Exact Test to assay differentially expressed genes between high and low SME expression groups of samples.

Supplemental Figure 7. Anti-TNF therapy for six months reverse the altered spliceosome signature of whole blood along with the inflammatory and clinical profile in RA patients. (A) Table showing clinical and serological characteristics of 25 responders RA patients before and after 6 months of TNFi therapy. (B) Heat map showing levels of circulating inflammatory molecules in plasma of RA patients before and after 6 months of TNFi therapy. Levels of inflammatory molecules are expressed as log 2 and normalized to time 0 (T0), before therapy in responders RA patients. (C) Violin plots representing the expression distribution of the 8 spliceosome components in whole blood before and after 6 months of TNFi therapy in responders’ RA patients. *p < 0.05, **p < 0.01. T0, time before TNFi therapy; T6, time 6 months after TNFi therapy; R, responders RA patients.

Supplemental Figure 8. In vitro treatment of healthy leukocytes in the presence of FcR blockers prevents the alterations of SME induced by ACPAs. Monocytes (A, D), lymphocytes (B, E) and neutrophils (C, F) from healthy donors were preincubated with
FcR Blocking Reagent (Miltenty) for 15 minutes at 4 °C before the treatment with 10 ug/ml of either, IgG-ACPA purified from RA patients through CCP-affinity column chromatography [IgG-ACPAS (+)] or the flow through depleted in Ig-ACPAS [IgG-ACPAS(-)] for 24 in monocytes and lymphocytes and 6 h in neutrophils. Spliceosome components (A, B, C) and inflammatory molecules (D, E, F) were analysed by RT-PCR. Data from 5 independent experiments carried out in triplicate are shown. *p < 0.05, **p < 0.01, IgG, immunoglobulin G; ACPAs, Anti-citrullinated protein antibodies.

Supplemental Figure 9. In vitro treatment of healthy leukocytes with monoclonal ACPAs modify the expression of the spliceosome signature along with their associated inflammatory profile. Monocytes (A, D), lymphocytes (B, E) and neutrophils (C, F) from healthy donors were treated with 10 ug/ml of a monoclonal IgG-ACPA (human monoclonal anticitrullinated fibrinogen immunoglobulin, clone 1F11) and a commercial IgG control (human IgG Jackson ImmunoResearch Laboratories, Inc, Newmarket, Suffolk, UK) for 24 in monocytes and lymphocytes and 6 h in neutrophils. Spliceosome components (A, B, C) and inflammatory molecules (D, E, F) were analysed by RT-PCR. Data from 5 independent experiments carried out in triplicate are shown. *p < 0.05, **p < 0.01, IgG, immunoglobulin G; ACPAs, Anti-citrullinated protein antibodies.

Supplemental Figure 10. Citrullination status of PBMCs from RA patients associated to the SME alteration. A) Diagram showing the experimental design. RA patients were selected based on their opposite patterns of SME alteration analyzed previously by RT-PCR. Cell lysates from PBMC of 10 RA patients belonging to the first (5 patients) and third tercile (5 patients) mainly characterized by high (“mild SME alteration”) and low (“severe SME alteration”) levels of SME respectively were pooled and the global citrullination status was assessed by Liquid Chromatography with tandem mass spectrometry (LC-MC/MS). B) Levels of the 8 SME in PBMC from RA patients selected by using RT-PCR. C) Quantification of the global citrullination status in each pooled sample from PBMCs of RA patients. Mass spectrometry raw files were processed with PEAKS Studio 10.6 and total label-free quantification intensities (LFQ) were obtained. D) Citrullination status of well-stablished proteins as autoantigens of ACPAS in PBMCs from RA patients with differential SME alteration status. *p<0.05

Supplemental Figure 11. Effect of Cytokines in the expression of splicing machinery elements (SME) from healthy leukocytes. Three cytokines with a key role in the pathogenesis of RA were added to monocytes, lymphocytes and neutrophils (10 ng/ml) purified from healthy donors. After 6 hours of treatment in neutrophils and 24 hours in monocytes and lymphocytes, SME levels were analyzed by RT-PCR. Data from 5 independent experiments carried out in triplicate are shown. *p<0.05

Supplemental Figure 12. Impact of the modulation of the lymphocyte-splicing machinery elements in synovial fibroblast phenotype. A) Schematic representation of the experimental design. Supernatant from RA lymphocyte transfected with mock, KHDRBS1 and SNRNP70 were added to synovial fibroblast (SF) and its impact was
evaluated through functional assays after 24 hours of culture. B) Migration capacity of SF was evaluated through wound-healing assay. C) Proliferation rate of SF was studied by using resazurin-based fluorescent dye based assay. D) Activation status of SF was analyzed through RT-PCR where genes related to cytokine and chemokine activity and collagen fibrin organization were assessed. Data from 5 independent experiments carried out in triplicate are shown. Data from 4 independent experiments carried out in triplicate are shown. * p<0.05

Supplemental Figure 1
Supplemental Figure 2
Supplemental Figure 3
Supplemental Figure 4
Supplemental Figure 5
Supplemental Figure 7

**A**

| Clinical parameters | Before TNFα | After TNFα | p    |
|---------------------|-------------|------------|------|
| Female/male, n/n    | 23/6        | -          | -    |
| Age, y              | 53 ± 11     | -          | -    |
| Evolution time, y   | 10 ± 6      | -          | -    |
| Swollen joints (n)  | 4.7 ± 3.9   | 0.8 ± 1.1  | 0.001|
| Tender joints (n)   | 8.5 ± 6.4   | 2.9 ± 0.5  | <0.001|
| DAS28               | 4.8 ± 1.1   | 2.9 ± 1.3  | <0.001|
| VAS                 | 60.9 ± 27.0 | 32.1 ± 26.4| <0.001|
| HAQ                 | 1.4 ± 0.8   | 1.3 ± 0.7  | 0.065|

**B**

![Graph showing TNFα, FGF, IL-1, IL-6, IL-8, IP-10, MCP-1, MIP-1α, TNFα, VEGF levels over time (T0, T6).](image)

**C**

Supplemental Figure 7
Supplemental Figure 8
Supplemental Figure 9

A. Monocytes-Splicing machinery components

B. Lymphocytes-Splicing machinery components

C. Neutrophils-Splicing machinery components

D. Monocytes-Inflammation

E. Lymphocytes-Inflammation

F. Neutrophils-Inflammation
Supplemental Figure 10
Supplemental Figure 11

Monocytes: Splicing machinery components

Lymphocytes: Splicing machinery components

Neutrophils: Splicing machinery components
Supplemental Figure 12
### SUPPLEMENTAL TABLES

Supplemental Table 1. List of primer sequences for inflammatory and selected splicing machinery components mRNAs

| GENE   | SEQUENCE          | Forward         | Reverse         |
|--------|-------------------|-----------------|-----------------|
| GAPDH  | TGTAGTTGAGGTCAATGAAGGG | ACATGCTAGACACCATG |
| IL1B   | CAGATTCTTTTCTTGAGGC | GCAACAAAGTGGTCTTC | |
| IL2    | CACTAAGTCTTGCACTTGTC | CTAAATGTGACATCCTGG | |
| IL6    | AAGATTCCAAAGATGTAGCC | ACATGTCTCTTTCTCGG | |
| IL8    | TACTCCAAACCTTTCCACC | CTCAGCCCTCTTCAAAAC | |
| IL12A  | ATGAGAGTTGCTTAAATTCC | CATAAAAGAGGTCTTTCTGGAG | |
| IL17A  | GTATGAGAAAAATCCAGCCC | TGGTTACGATGAAAGCTTG | |
| IFNg   | GGTAGCTAGCTGAAATGTC | TTTTCGCTTCCCTAGTG |
| IP10   | AAAGCGATGCAAGGAAAGAAG | TCATTGGTCACCCTTATG | |
| MCP1   | CTAAGAGAATACACCAGCAG | CTAGGGGAAAATAGTGGCTG | |
| RANTES | ATCTGCTCTCTCTTCCATTC | AAGAGTTGACCTGACTCGG | |
| TF     | GGTAGAAATACGCTTCTGAC | ATTCAACATCTGAGTG | |
| TNF    | CTAGCCCTCTCTCTCTCC | AGAAGATGATCTGACTGCC | |
| VEGFA  | GACCAAGGAAAGATAGAGCAAG | ATACGGCTCAGAGCTTATAC | |
| SNRNP70| TCTCTGCGGAGAGTGAAT | GCTTCCGCACGGTACTCT | |
| SNRNP200| GGGTCTGCTCTCTTTGG | CTTCTCTGCTCTGCTCTTCT | |
| U2AF2  | CTGGCTGAGGGCCAAT | TACTGCATGGAGTGCTG | |
| RN34ATAC| GTTGGCGTACTGCATTGA | CAAAATTTGCCAAAATAA | |
| RBM17  | CAAAGGCCCCAGGAGCAA | TACATGCGGGTCTGCTG | |
| RBM3   | AAGAGTCTGGGAGGAGG | TTGAACAGGGCAGCCTCAC | |
| KHDBRS1| GAGGAGTGGTCTGATACCTGTC | CACCACTGCTTCTGCAAGTC | |
| SRSF10 | CTGACCTCTCCCTTCAAGAG | CCGTCCAAATCCACTTTC | |
### Supplemental Table 2. Citrullinated proteins in PBMCs from Rheumatoid Arthritis patients identified by mass spectrometry

| Citrullinated Protein | Protein Description | Protein Position | Modified Peptide Sequence |
|-----------------------|---------------------|------------------|---------------------------|
| Q9Y490|TLN1_HUMAN          | Talin-1 OS=Homo sapiens OX=9606 GN=TLN1 PE=1 SV=3 | R181 | LNWLDHGRTLrEQGVEEHETL |
|                      |                     | Talin-1 OS=Homo sapiens OX=9606 GN=TLN1 PE=1 SV=3 | R207 | FFYSDDQNVDSrDPVQLNLLYY |
|                      |                     | Talin-1 OS=Homo sapiens OX=9606 GN=TLN1 PE=1 SV=3 | R454 | HGSVALPAMrSGASGPENFQ |
|                      |                     | Talin-1 OS=Homo sapiens OX=9606 GN=TLN1 PE=1 SV=3 | R1222 | ALRAVGDASKrLSDSLPPST |
|                      |                     | Talin-1 OS=Homo sapiens OX=9606 GN=TLN1 PE=1 SV=3 | R1241 | STGTFQEASrLNEAAAGLNQ |
|                      |                     | Talin-1 OS=Homo sapiens OX=9606 GN=TLN1 PE=1 SV=3 | R1523 | SARTTNPTAKrQFQVQSAKEVA |
|                      |                     | Talin-1 OS=Homo sapiens OX=9606 GN=TLN1 PE=1 SV=3 | R1618 | ESAGGLIQTArALAVNPRDPP |
|                      |                     | Talin-1 OS=Homo sapiens OX=9606 GN=TLN1 PE=1 SV=3 | R2197 | AKAVAAGNQrQEDVIAATNL |
|                      |                     | Talin-1 OS=Homo sapiens OX=9606 GN=TLN1 PE=1 SV=3 | R2210 | DVIATANLSrAIADMLRACK |
|                      |                     | Talin-1 OS=Homo sapiens OX=9606 GN=TLN1 PE=1 SV=3 | R2368 | ALVKAASAAQrELVAQKVG |
|                      |                     | Talin-1 OS=Homo sapiens OX=9606 GN=TLN1 PE=1 SV=3 | R2538 | QYYKFLPSElrDEH****** |
| P21333|FLNA_HUMAN          | Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=4 | R7 | ****MSSSHrSAGQSAAGAAP |
|                      |                     | Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=4 | R24 | GAAPGGGVDTrDAEMPATEK |
|                      |                     | Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=4 | R301 | IEPTGNMVKrAEFTVETRSA |
|                      |                     | Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=4 | R678 | QDFHPDRVKArGPGLEKTGVA |
|                      |                     | Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=4 | R828 | AEADIFDFIrNDNDTFTVKY |
|                      |                     | Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=4 | R1032 | PGLGADNSVVrFPLPREEPE |
|                      |                     | Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=4 | R2003 | REEPCLLKrrNNGHVGIFVP |
|                      |                     | Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=4 | R2288 | PSKAEISFEdrKDGS CGVAV |
|                      |                     | Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=4 | R2391 | TEIDQDKrAVrrFrPRENGV |
|                      |                     | Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=4 | R2395 | QDKYAVRFrPENGVYLIDV |

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| Protein          | UniProt ID | OS                 | GN     | PE  | SV  | Amino Acid Sequence          |
|------------------|------------|--------------------|--------|-----|-----|------------------------------|
| Filamin-A        | P6079      | Homo sapiens       | FLNA   | 1   | 4   | R2484 MDCQECPEGYrVTYTPMAPEGS |
| Actin cytoplasmic 1 | P60709    | Homo sapiens       | ACTB   | 1   | 1   | R62  SYVGDEAQSKrGILTLKYPIE   |
| Actin cytoplasmic 1 | P60709    | Homo sapiens       | ACTB   | 1   | 1   | R177 EGYALPHAILrDLAGRDLTD    |
| Actin cytoplasmic 1 | P60709    | Homo sapiens       | ACTB   | 1   | 1   | R312 GTTMYPGIArDLMQKEITALAP |
| Actin cytoplasmic 2 | P60709    | Homo sapiens       | ACTG1  | 1   | 1   | R62  SYVGDEAQSKrGILTLKYPIE   |
| Actin cytoplasmic 2 | P60709    | Homo sapiens       | ACTG1  | 1   | 1   | R177 EGYALPHAILrDLAGRDLTD    |
| Actin cytoplasmic 2 | P60709    | Homo sapiens       | ACTG1  | 1   | 1   | R312 GTTMYPGIArDLMQKEITALAP |
| Myosin-9         | P35579     | Homo sapiens       | MYH9   | 1   | 4   | R159 HLEGKIEQAQrWIDNPTVDDR   |
| Myosin-9         | P35579     | Homo sapiens       | MYH9   | 1   | 4   | R1923 EVSSLKNKLRgDLPFVPRR    |
| Actin alpha cardiac muscle 1 | P68032 | Homo sapiens       | ACTC   | 1   | 1   | R64  SYVGDEAQSKrGILTLKYPIE   |
| Vinculin         | P18206     | Homo sapiens       | VCL    | 1   | 4   | R502 HLEGKIEQAQrWIDNPTVDDR   |
| Vinculin         | P18206     | Homo sapiens       | VCL    | 1   | 4   | R538 RLANVMMGPIrQDCLAKCDRV   |
| Thrombospondin-1 | P07996     | Homo sapiens       | THBS1  | 1   | 2   | R20  LFLMHCNGTrNPESGDGSNV    |
| Thrombospondin-1 | P07996     | Homo sapiens       | THBS1  | 1   | 2   | R479 MNGKPCEGAErETKACKKDAC   |
| Pyruvate kinase PKM | P14618   | Homo sapiens       | PKM    | 1   | 4   | R279 SKIENHEGVRrFDEIlEASDG   |
| Pyruvate kinase PKM | P14618   | Homo sapiens       | PKM    | 1   | 4   | R294 LEASDGIMVArGDLGIEIPAE  |
| Pyruvate kinase PKM | P14618   | Homo sapiens       | PKM    | 1   | 4   | R376 AKGDYPLEAVrMQHLIAEAE    |
| Vimentin         | P08670     | Homo sapiens       | VIM    | 1   | 4   | R196 REKLQEMLQrEAAENTLQSF    |
| Vimentin         | P08670     | Homo sapiens       | VIM    | 1   | 4   | R273 PDLLTAALrDqvQYESVAAKN   |
| Vimentin         | P08670     | Homo sapiens       | VIM    | 1   | 4   | R304 KFLDLSAAnrNNDLRAQAKQ    |
| Vimentin         | P08670     | Homo sapiens       | VIM    | 1   | 4   | R381 NMKEAMRHLrEYQDLDNVMK    |
| Vimentin         | P08670     | Homo sapiens       | VIM    | 1   | 4   | R410 YRKLLEGEErISLPNTFS      |
| Integrin beta-3  | P05106     | Homo sapiens       | ITGB3  | 1   | 2   | R93  PVSEARrLrDPLSKDGSOS     |
| Lactotransferrin | P02788     | Homo sapiens       | LTFR   | 1   | 6   | R462 EGYLAARVrSDDSLSLTNSV    |
| Alpha-enolase     | P06733     | Homo sapiens       | ENO1   | 1   | 2   | R429 SKAKFARNFrNPLAK*****    |
| Accession | Description                  | OS     | OX   | GN     | PE   | SV  | Sequence  |
|-----------|------------------------------|--------|------|--------|------|------|-----------|
| P12814|ACTN1_HUMAN | Alpha-actinin-1 | Homo sapiens | 9606 | ACTN1 | 1 | 2 | R232 |
|         | | | | | | | | LDAEDIVGTArPDEKAIMTYV |
| P07437|TBBS5_HUMAN | Tubulin beta chain | Homo sapiens | 9606 | TUBB | 1 | 2 | R2 |
|         | | | | | | | | **********MrEIVHIQAGQC |
| P68371|TBB4B_HUMAN | Tubulin beta-4B chain | Homo sapiens | 9606 | TUBB4B | 1 | 2 | R2 |
|         | | | | | | | | **********MrEIVHLQAGQC |
| P02768|ALBU_HUMAN | Albumin | Homo sapiens | 9606 | ALB | 1 | 2 | R281 |
|         | | | | | | | | HGDLLECADDrADLAKYICEN |
| Q6S8J3|POTEE_HUMAN | POTE ankyrin domain family member E | Homo sapiens | 9606 | POTEE | 2 | 3 | R762 |
|         | | | | | | | | SYVGKEAQSkrGILTLYKPM |
| O75083|WDR1_HUMAN | WD repeat-containing protein 1 | Homo sapiens | 9606 | WDR1 | 1 | 4 | R470 |
|         | | | | | | | | VAIGGVDGNrLYSILGTLK |
| P00558|PGK1_HUMAN | Phosphoglycerate kinase 1 | Homo sapiens | 9606 | PGK1 | 1 | 3 | R66 |
|         | | | | | | | | KSVVLMSSHGrPDGVPMF |
| P06396|GELS_HUMAN | Gelsolin | Homo sapiens | 9606 | GSN | 1 | 2 | R623 |
|         | | | | | | | | AETKGAQELLrVLRAQPVQVA |
| P04075|ALDOA_HUMAN | Fructose-bisphosphate aldolase A | Homo sapiens | 9606 | ALDOA | 1 | 2 | R201 |
|         | | | | | | | | ILPDGDHDLKrCQYVTEKVL |
| P11021|BIP_HUMAN  | Endoplasmic reticulum chaperone BiP | Homo sapiens | 9606 | HSPAS | 1 | 2 | R49 |
|         | | | | | | | | YSCGVFKKnGrVEIANDQGN |
|         | | | | | | | | VEIANDQGNrTPSYVAFTP |

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| Gene Name                        | Organism       | Protein Name                        | Peptide Sequence                     | Start | End  |
|---------------------------------|----------------|-------------------------------------|--------------------------------------|-------|------|
| BiP (HSPA5)                     | Homo sapiens   | Endoplasmic reticulum chaperone BiP | SYVAFTPEGERLIGDAAKNQL                  | R74   |      |
| BiP (HSPA5)                     | Homo sapiens   | Endoplasmic reticulum chaperone BiP | FDLTGIPPAPrGVPQIEVTFE                  | R492  |      |
| Hemoglobin beta (HBB)           | Homo sapiens   | Hemoglobin subunit beta             | VDEVGGEALGrLLVVYPWTQR                 | R31   |      |
| Moesin (MSN)                    | Homo sapiens   | Moesin                              | MGNELYMRRrKPDTIEVQQM                   | R295  |      |
| Coagulation factor XIII A chain | Homo sapiens   | Coagulation factor XIII A chain     | LIASMSSDSlrHVYGLDVQI                   | R716  |      |
| Myeloperoxidase (MPO)           | Homo sapiens   | Myeloperoxidase                     | RTITGMCNNRrSPTLGSNRA                  | R184  |      |
| Lamrin-B1 (LMNB1)               | Homo sapiens   | Lamrin-B1                           | ATPVPRRMRGsrAGGPTTPLSP                 | R14   |      |
| Adenylyl cyclase-associated 1   | Homo sapiens   | Adenylyl cyclase-associated 1       | LVERLERAVGrLEAHSHTSDM                  | R17   |      |
| Tubulin beta-2B chain (TUBB2B) | Homo sapiens   | Tubulin beta-2B chain               | ***********MrEIVHIQAGQC                 | R2    |      |
| Tubulin beta-2B chain (TUBB2B) | Homo sapiens   | Tubulin beta-2B chain               | ELVDSVLDVvrKECESCDCLQ                  | R121  |      |
| Fibrinogen beta chain (FGB)     | Homo sapiens   | Fibrinogen beta chain               | ENGGWTVIQNrQDGSVDFGRK                  | R285  |      |
| Fermitin family homolog 3      | Homo sapiens   | Fermitin family homolog 3           | EDPEAESVTLeVTGESHIGGV                  | R35   |      |
| 60 kDa heat shock protein       | Homo sapiens   | 60 kDa heat shock protein mitochondrial | IVLGGGCALLrCIPALDSLTP           | R446  |      |
| Accession | Description | Source | Peptide Sequence |
|-----------|-------------|--------|-----------------|
| P62937| Peptidyl-prolyl cis-trans isomerase A | Homo sapiens | DKVPKTAENFrALSTGEKGFG |
| P06576| ATP synthase subunit beta | Homo sapiens | LATDMGTMQErIITTKKSIT |
| P69905| Hemoglobin subunit alpha | Homo sapiens | AGEYGAEEALrMFSLFPTTK |
| A5A3E0| POTE ankyrin domain family member F | Homo sapiens | SYVGKEAQSKrGILTLKYPME |
| P07900| Heat shock protein HSP 90-alpha | Homo sapiens | MERIMKAQALrDNSTMGYMAA |
| P25705| ATP synthase subunit alpha | Homo sapiens | APGIIPRISvEPMQTGIKAV |
| P08238| Heat shock protein HSP 90-beta | Homo sapiens | STYGWTANMErIMKAQALRDN |
| P31146| Coronin-1A | Homo sapiens | RCEPIAMTVPrKSDLFQEDLY |
| Q13509| Tubulin beta-3 chain | Homo sapiens | ELVDSDLVVRKECENCDCQLQ |
| Accession | Description | OS | GN | PE | SV | Sequence |
|-----------|-------------|----|----|----|----|----------|
| P22626    | Heterogeneous nuclear ribonucleoproteins A2/B1 | Homo sapiens | HNRNPA2B1 | 1 | 2 | R200 RQEMQEVSrSGRGGNFGFG |
|           | Heterogeneous nuclear ribonucleoproteins A2/B1 | Homo sapiens | HNRNPA2B1 | 1 | 2 | R203 MQEVQSRSGrGGNFGFGDSR |
|           | Heterogeneous nuclear ribonucleoproteins A2/B1 | Homo sapiens | HNRNPA2B1 | 1 | 2 | R228 NFGPSPNFrGGSGYSGSr |
| P08133    | Annexin A6 | Homo sapiens | ANXA6 | 1 | 3 | R358 VARVELKGTvrPANDFPAD |
| P68104    | Elongation factor 1-alpha 1 | Homo sapiens | EEF1A1 | 1 | 1 | R423 ESFDYPPGrFAVHDRMRQT |
| Q03252    | Lamin-B2 | Homo sapiens | LMB2 | 1 | 4 | R28 AATMATPLPrAGGPATPLSp |
| P52272    | Heterogeneous nuclear ribonucleoprotein M | Homo sapiens | HNRNPM | 1 | 3 | R410 GIERMGPGrLGGAGMERMG |
|           | Heterogeneous nuclear ribonucleoprotein M | Homo sapiens | HNRNPM | 1 | 3 | R429 MGAGLGHGMDvVGSEIERMGL |
|           | Heterogeneous nuclear ribonucleoprotein M | Homo sapiens | HNRNPM | 1 | 3 | R443 EIERMGLVMDvMGSVERMG |
| P63104    | 14-3-3 protein zeta/delta | Homo sapiens | YWHAZ | 1 | 1 | R18 QKAKLAEQArYDDMAACMK |
| P60174    | Triosephosphate isomerase | Homo sapiens | TPI1 | 1 | 4 | R53 APPTAYIDFArQKLDPKIAV |
| P60660    | Myosin light polypeptide 6 | Homo sapiens | MYL6 | 1 | 2 | R110 GNGTVMAEirHVLVTLEK |
| Q00610    | Clathrin heavy chain 1 | Homo sapiens | CLTC | 1 | 5 | R1620 VDKLDAEStLrKEEQQATETQ |
| Q93084    | Sarcoplasmic/endoplasmic reticulum calcium ATPase 3 | Homo sapiens | ATP2A3 | 1 | 2 | R164 AVGKVPADLrLEIKSTTL |
| P22314    | Ubiquitin-like modifier-activating enzyme 1 | Homo sapiens | UBA1 | 1 | 3 | R239 PGVVTCLDEArHGFESGDFV |

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Ubiquitin-like modifier-activating enzyme 1 OS=Homo sapiens OX=9606 GN=UBA1 PE=1 SV=3

P52566|GDIR2_HUMAN
Rho GDP-dissociation inhibitor 2 OS=Homo sapiens OX=9606 GN=ARHGDB PE=1 SV=3

P61978|HNRPK_HUMAN
Heterogeneous nuclear ribonucleoprotein K OS=Homo sapiens OX=9606 GN=HNRNPK PE=1 SV=1

P08567|PLEK_HUMAN
Pleckstrin OS=Homo sapiens OX=9606 GN=PLEK PE=1 SV=3

P37802|TAGL2_HUMAN
Transgelin-2 OS=Homo sapiens OX=9606 GN=TAGLN2 PE=1 SV=3

P61158|ARP3_HUMAN
Actin-related protein 3 OS=Homo sapiens OX=9606 GN=ACTR3 PE=1 SV=3

P31946|1433B_HUMAN
14-3-3 protein beta/alpha OS=Homo sapiens OX=9606 GN=YWHAB PE=1 SV=3

P08311|CATG_HUMAN
Cathepsin G OS=Homo sapiens OX=9606 GN=CTSG PE=1 SV=2

P02671|FIBA_HUMAN
Fibrinogen alpha chain OS=Homo sapiens OX=9606 GN=FGA PE=1 SV=2

P15311|EZRI_HUMAN
Ezrin OS=Homo sapiens OX=9606 GN=EZR PE=1 SV=4

Q9Y4G6|TLN2_HUMAN
Talin-2 OS=Homo sapiens OX=9606 GN=TLN2 PE=1 SV=4

P26599|PTBP1_HUMAN
Polypyrimidine tract-binding protein 1 OS=Homo sapiens OX=9606 GN=PTBP1 PE=1 SV=1

P02545|LMNA_HUMAN
Prelamin-A/C OS=Homo sapiens OX=9606 GN=LMNA PE=1 SV=1

P41218|MND4A_HUMAN
Myeloid cell nuclear differentiation antigen OS=Homo sapiens OX=9606 GN=MND4A PE=1 SV=1

P23527|H2B1Q_HUMAN
Histone H2B type 1-O OS=Homo sapiens OX=9606 GN=H2BC17 PE=1 SV=3

AENYDIPSADrHKSdlAKG
ATFMVGYSYGrPEEFLTPV
MEEEQAFKRSrNTDEMVELR
WLVSNQSVRrQEGLMIASSL
VTSVESNSGrKSEEELFEI
********MANrGPAYGLSREV
QAGMTGYGmrPQL********
********MAGrLPACVDCGT
QAKLAEQAerYDDMAAAMKA
VTLGAHNIrQrENTQHHTAR
DMPQMRMLErPFGNEITRGG
GIGTDGFRrHPDAEAFFDT
MGNHLEYMrRrKPDTIESQQM
FFYSDQNVDrDPVQLNLYV
AKAVAAAGCrQEDVIIATNL
QNIYNACCTrIIFSKLTSN
KLRLDELSArERDTSRRLLA
TQAQRQVDDRrNVPQNPDVT
HYNKRSTITSrEIQTAVRLL
| Accession | Gene Symbol | Description | Species | Genomic Position | Protein | Peptide | Score |
|-----------|-------------|-------------|---------|------------------|---------|---------|-------|
| Q99879|H2B1M_HUMAN | Histone H2B type 1-M | Homo sapiens | 9606:G=H2BC14 | 1 | R93 | HYNKRSTITSrEIQTAVRLLL |
| Q93079|H2B1H_HUMAN | Histone H2B type 1-H | Homo sapiens | 9606:G=H2BC9 | 1 | R93 | HYNKRSTITSrEIQTAVRLLL |
| P33778|H2B1B_HUMAN | Histone H2B type 1-B | Homo sapiens | 9606:G=H2BC3 | 1 | R93 | HYNKRSTITSrEIQTAVRLLL |
| P06899|H2B1J_HUMAN | Histone H2B type 1-J | Homo sapiens | 9606:G=H2BC11 | 1 | R93 | HYNKRSTITSrEIQTAVRLLL |
| P58876|H2B1D_HUMAN | Histone H2B type 1-D | Homo sapiens | 9606:G=H2BC5 | 1 | R93 | HYNKRSTITSrEIQTAVRLLL |
| Q99877|H2B1N_HUMAN | Histone H2B type 1-N | Homo sapiens | 9606:G=H2BC15 | 1 | R93 | HYNKRSTITSrEIQTAVRLLL |
| O60814|H2B1K_HUMAN | Histone H2B type 1-K | Homo sapiens | 9606:G=H2BC12 | 1 | R93 | HYNKRSTITSrEIQTAVRLLL |
| P09382|LEG1_HUMAN | Galectin-1 | Homo sapiens | 9606:G=LGALS1 | 1 | R21 | NLKPGECRLrGEVAPDAKSF |
| Q16181|SEPT7_HUMAN | Septin-7 | Homo sapiens | 9606:G=SEPTIN7 | 1 | R425 | QRILEQQNSSrTLEKNKKKGK |
| P61626|LYSC_HUMAN | Lysozyme C | Homo sapiens | 9606:G=LYZ | 1 | R68 | TRATYNAGDrSTDYGIFQIN |
| P18669|PGAM1_HUMAN | Phosphoglycerate mutase 1 | Homo sapiens | 9606:G=PGAM1 | 1 | R162 | SCESLKDITArALPFWEENIEIV |
| Q9UGI8|TES_HUMAN | Testin | Homo sapiens | 9606:G=TES | 1 | R254 | KEGDPAIYArAGYDLKWHPA |
| P47756|CAPZB_HUMAN | F-actin-capping protein subunit beta | Homo sapiens | 9606:G=CAPZB | 1 | R15 | QLDCALDLMrLPPQIEKKNL |
| P62820|RAB1A_HUMAN | Ras-related protein Rab-1A | Homo sapiens | 9606:G=RAB1A | 1 | R72 | LQIWDTAGQErFRTITSSYYR |
| P52209|6PGD_HUMAN | 6-phosphogluconate dehydrogenase decarboxylating | Homo sapiens | 9606:G=6PGD | 1 | R136 | SGVSNGEEGArYGPSLMPGGN |
| Q13576|IQGA2_HUMAN | Ras GTPase-activating-like protein IQGAP2 | Homo sapiens | 9606:G=IQGAP2 | 1 | R1342 | EVDHATDMVSrAMIDSRTPEE |
| P19971|TYPH_HUMAN | Thymidine phosphorylase | Homo sapiens | 9606:G=TYMP | 1 | R265 | LVVGASGLrVAAALTAMDK |
| P61981 | 1433G_HUMAN | SV=2 | 14-3-3 protein gamma OS=Homo sapiens OX=9606 GN=YWHAG PE=1 | R19 | QKARLAEQAErYDDMAAMKN |
| P61160 | ARP2_HUMAN | SV=2 | Actin-related protein 2 OS=Homo sapiens OX=9606 GN=ACTR2 PE=1 | R80 | VNYPMENGIrNWDDMKHLWD |
| Q13418 | ILK_HUMAN | SV=2 | Integrin-linked protein kinase OS=Homo sapiens OX=9606 GN=ILK PE=1 | R56 | RSAVVEMLMrGARINVMNRG |
| Q9H0U4 | RAB1B_HUMAN | SV=1 | Ras-related protein Rab-1B OS=Homo sapiens OX=9606 GN=RAB1B PE=1 | R69 | LQIWDTAGQErFRTITSSYR |
| P09972 | ALDOC_HUMAN | SV=2 | Fructose-bisphosphate aldolase C OS=Homo sapiens OX=9606 GN=ALDOC PE=1 SV=2 | R201 | ILPDGDHDLKrCQYVTEKVL |
| Q15233 | NONO_HUMAN | SV=4 | Non-POU domain-containing octamer-binding protein OS=Homo sapiens OX=9606 GN=NONO PE=1 SV=4 | R202 | IVEFSGPAArKALDRCEGS |
| Q13011 | ECH1_HUMAN | SV=2 | Delta(3 5)-Delta(2 4)-dienoyl-CoA isomerase mitochondrial OS=Homo sapiens OX=9606 GN=ECH1 PE=1 SV=2 | R59 | EAPDHSYEsLrVTSAOQKHVLH |
| P04843 | RPN1_HUMAN | SV=1 | Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 OS=Homo sapiens OX=9606 GN=RPN1 PE=1 SV=1 | R65 | LAHLGGGSTSrATSFLLALE |
| P21281 | VATB2_HUMAN | SV=3 | V-type proton ATPase subunit B brain isoform OS=Homo sapiens OX=9606 GN=ATP6V1B2 PE=1 SV=3 | R185 | SADVGMNSIArGQXPIFSAA |
| P21281 | VATB2_HUMAN | SV=3 | V-type proton ATPase subunit B brain isoform OS=Homo sapiens OX=9606 GN=ATP6V1B2 PE=1 SV=3 | R471 | NFIAQGPYEnrTVFETLDIGW |
| P61106 | RAB14_HUMAN | SV=4 | Ras-related protein Rab-14 OS=Homo sapiens OX=9606 GN=RAB14 PE=1 | R72 | LQIWDTAGQErFRAVTRYYS |
| P27797 | CALR_HUMAN | SV=1 | Calreticulin OS=Homo sapiens OX=9606 GN=CALR PE=1 | R162 | GKNVLrINKDrCKDDEFTLY |
|        |        | SV=1 | Calreticulin OS=Homo sapiens OX=9606 GN=CALR PE=1 | R222 | DASKPEDWDErAKIDDDTSDK |
| Accession  | Description                                           | Sequence                                                                 |
|------------|--------------------------------------------------------|--------------------------------------------------------------------------|
| Q96AE4     | Far upstream element-binding protein 1 OS=Human sapiens GN=FUBP1 PE=1 SV=3 | R271 FREVRNEYGSrIGGNEGIDVP                                               |
| P35241     | Radixin OS=Human sapiens GN=RDX PE=1 SV=1              | R295 MGNHELYMRRrPKDTEIVQVM                                               |
| P14543     | Nidogen-1 OS=Human sapiens GN=NID1 PE=1 SV=3           | R1017 LHGGEPTTLrQDLGSPEGIA                                               |
| P00491     | Purine nucleoside phosphorylase OS=Human sapiens GN=PNP PE=1 SV=2 | R229 MSTVPEVlVARHCGLRFGFS                                               |
| P02787     | Serotransferrin OS=Human sapiens GN=TF PE=1 SV=3       | R696 TSSLEACTFrRP**********                                              |
| P84243     | Histone H3.3 OS=Human sapiens GN=H3-3A PE=1 SV=2      | R43  TGGVKKPnHRYrPGTVLREIR                                              |
| P59998     | Actin-related protein 2/3 complex subunit 4 OS=Human sapiens GN=ARPC4 PE=1 SV=3 | R71  VLEIGSINSVeVSIAVKQADE                                               |
| O00194     | Ras-related protein Rab-27B OS=Human sapiens GN=RAB27B PE=1 SV=4 | R80  LQLWDTAQGErFRSLTTAFFR                                              |
| P52565     | Rho GDP-dissociation inhibitor 1 OS=Human sapiens GN=ARHGDIa PE=1 SV=3 | R172  VEEAPKGMLArGSYSIKSRFT                                             |
| P35908     | Keratin type II cytoskeletal 2 epidermal OS=Human sapiens GN=KRT2 PE=1 SV=2 | R430  VQDAIADAEnrGEHALKDARN                                             |
| P05141     | ADP/ATP translocase 2 OS=Human sapiens GN=SLC25A5 PE=1 SV=7 | R259  IMYTGLDCWrrKIARDEGGKA                                              |
| Q9NRW1     | Ras-related protein Rab-6B OS=Human sapiens GN=RAB6B PE=1 SV=1 | R74  LQLWDTAQGErFRSLIPSYIR                                              |
| Q96KP4     | Cytosolic non-specific dipeptidase OS=Human sapiens GN=CNDP2 PE=1 SV=2 | R453  GAHSQNEKLrTNYIEGTKML                                              |
P46459|NSF_HUMAN  Vesicle-fusing ATPase OS=Homo sapiens OX=9606 GN=NSF PE=1 SV=3  R67  PGSIASFPLQrKWAGLSIGQE
Vesicle-fusing ATPase OS=Homo sapiens OX=9606 GN=NSF PE=1 SV=3  R533  LLVQQTKNSDrTPLVSLLEG

P20340|RAB6A_HUMAN  Ras-related protein Rab-6A OS=Homo sapiens OX=9606 GN=RAB6A PE=1 SV=3  R74  LQLWDTAGQErFRSLIPSYIR

P62491|RB11A_HUMAN  Ras-related protein Rab-11A OS=Homo sapiens OX=9606 GN=RAB11A PE=1 SV=3  R33  GKSNNLRSFTrNEFNLESKST

P49748|ACADV_HUMAN  Very long-chain specific acyl-CoA dehydrogenase mitochondrial OS=Homo sapiens OX=9606 GN=ACADVL PE=1 SV=1  R229  PSSGDAASlrTSAPSPCGK

P14222|PERF_HUMAN  Perforin-1 OS=Homo sapiens OX=9606 GN=PRF1 PE=1 SV=1  R177  YSFSRTDTVEcRFYSHVHVT
Q13126|MTAP_HUMAN  S-methyl-5'-thioadenosine phosphorylase OS=Homo sapiens OX=9606 GN=MTAP PE=1 SV=2  R133  SFYDSHSCArGVCHIPMAEP

P09486|SPRC_HUMAN  SPARC OS=Homo sapiens OX=9606 GN=SPARC PE=1 SV=1  R205  RVKIHENEKreLEAGDHVPEL
P61026|RAB10_HUMAN  Ras-related protein Rab-10 OS=Homo sapiens OX=9606 GN=RAB10 PE=1 SV=1  R70  LQIWDTAGQErFHTITSSYR

O14983|AT2A1_HUMAN  Sarcoplasmic/endoplasmic reticulum calcium ATPase 1 OS=Homo sapiens OX=9606 GN=ATP2A1 PE=1 SV=1  R164  AVGDKVPADrlLAIkSTTLR

P54577|SYYC_HUMAN  Tyrosine--tRNA ligase cytoplasmic OS=Homo sapiens OX=9606 GN=YARS1 PE=1 SV=4  R207  KYPALGYSKrVHLNMNPVPG

P49821|NDUV1_HUMAN  NADH dehydrogenase [ubiquinone] flavoprotein 1 mitochondrial OS=Homo sapiens OX=9606 GN=NDUFV1 PE=1 SV=4  R256  ETVAVSPTCrRGGTWFAGFG

Q92930|RAB8B_HUMAN  Ras-related protein Rab-8B OS=Homo sapiens OX=9606 GN=RAB8B PE=1 SV=2  R69  LQIWDTAGQErFRTITTYR

O43488|ARK72_HUMAN  Aflatoxin B1 aldehyde reductase member 2 OS=Homo sapiens OX=9606 GN=AKR7A2 PE=1 SV=3  R61  MDAPASAAMArAFLERGHTEL

O14974|MYPT1_HUMAN  Protein phosphatase 1 regulatory subunit 12A OS=Homo sapiens OX=9606 GN=PPP1R12A PE=1 SV=1  R31  ETDLEPPVVKrQTKVFKDDG
| Accession | Protein Name | Organism | Gene ID | Peptide | Sequence |
|-----------|--------------|----------|---------|---------|----------|
| P51159    | Ras-related protein Rab-27A | Homo sapiens | RAB27A | R80 | LQLWDTAGQErFRSLTATTFR |
| P25325    | 3-mercaptopyruvate sulfurtransferase OS | Homo sapiens | MPST | R137 | LLDGGLRHWLrQNLPLSSGKS |
| P30038    | Delta-1-pyrroline-5-carboxylate dehydrogenase mitochondrial OS | Homo sapiens | ALDH4A1 | R338 | DVESVSGTlrSAFEYGGQKC |
| Q8NF50    | Deducator of cytokinesis protein 8 OS | Homo sapiens | DOCK8 | R1358 | KVSTQVLQKSrDVKARLEELAL |
| Q14166    | Tubulin--tyrosine ligase-like protein 12 OS | Homo sapiens | TTL12 | R461 | KYIESPVFLrEDVGKVKFDI |
| P62753    | 40S ribosomal protein S6 OS | Homo sapiens | RPS6 | R51 | LGEEWKGYYVrlSGGNDQKGF |
| Q15286    | Ras-related protein Rab-35 OS | Homo sapiens | RAB35 | R69 | LQIWDTAGQErFTITSTYYR |
| Q02878    | 60S ribosomal protein L6 OS | Homo sapiens | RPL6 | R105 | PVGGDKNGGTrVVKLRMPRY |
| P25098    | Beta-adrenergic receptor kinase 1 OS | Homo sapiens | GRK2 | R454 | AQQEVKESPFlrSLDWQMVFLQ |
| Q14558    | Phosphoribosyl pyrophosphate synthase-associated protein 1 OS | Homo sapiens | PRPSAP1 | R295 | THGILSAEAPrLIEESSVDEV |
| O95716    | Ras-related protein Rab-3D OS | Homo sapiens | RAB3D | R83 | LQIWDTAGQErYRTITTAAYR |
| P11217    | Glycogen phosphorylase muscle form OS | Homo sapiens | PYGM | R17 | DQEKRKQISVglAGVENVTE |
| P61018    | Ras-related protein Rab-4B OS | Homo sapiens | RAB4B | R69 | LQIWDTAGQErFRSVTRSSYYR |
| Q96E17    | Ras-related protein Rab-3C OS | Homo sapiens | RAB3C | R91 | LQIWDTAGQErYRTITTAAYR |
| Accession | Description |
|-----------|-------------|
| Q96AX2 | Ras-related protein Rab-37 |
| Q9Y2Z0 | Protein SGT1 homolog |
| Q96GD0 | Pyridoxal phosphate phosphatase |
| Q13501 | Sequestosome-1 |
| Q86YS6 | Ras-related protein Rab-43 |
| P14174 | Macrophage migration inhibitory factor |
| P20337 | Ras-related protein Rab-3B |
| Q7Z6P3 | Ras-related protein Rab-44 |
| Q9BWS9 | Chitinase domain-containing protein 1 |
| Q16658 | Fascin |
| P02749 | Beta-2-glycoprotein 1 |
| Q0VD83 | Apolipoprotein B receptor |
| P35250 | Replication factor C subunit 2 |
| Q9HBH5 | Retinol dehydrogenase 14 |
| P07478 | Trypsin-2 |
| Q8TBH0 | Arrestin domain-containing protein 2 |

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| Accession  | Description                                                                 | Start MSA | End MSA | Sequence                      |
|-----------|------------------------------------------------------------------------------|-----------|---------|-------------------------------|
| Q8WY91    | Peroxynitrite isomerase (THAP4)                                               | 156       | 1655    | QAALQGEATPrAAQEAASQEQ        |
| Q8IVF5    | T-lymphoma invasion and metastasis-inducing protein 2 (TIAM2)                | 1655      | 1764    | DRGTLKAIrHQSLDSQSEN           |
| Q92618    | Zinc finger protein 516 (ZN516)                                              | 1086      | 1195    | WGVSGPGLrHRGTLRTQARPGL        |
| P98160    | Basement membrane-specific heparan sulfate proteoglycan core protein (PGM)  | 2655      | 2764    | QTDLNCVVARQPAIITWYK           |
| Q86SX3    | Tubulin epsilon and delta complex protein 1 (TEDC1)                          | 257       | 2655    | HSFCPTGMrPTFWDNLWLC           |
| A3KMH1    | Von Willebrand factor A domain-containing protein 8 (VWA8)                   | 1259      | 1368    | SLTVLVDLEGrTHTISLPINL         |
| Q14993    | Collagen alpha-1(XIX) chain (COL1A1)                                         | 27        | 38      | LLPASTSVTrDKTEESCPIL         |
| Q6ZRP7    | Sulfhydryl oxidase 2 (QSOX2)                                                 | 109       | 1195    | PTWRAgDvrDWASAIRVAA          |
| A6N79     | Coiled-coil domain-containing protein 69 (CDDC69)                            | 271       | 379     | LQEKEELLYrVLGANASPAF         |
| O15409    | Forkhead box protein P2 (FOXP2)                                               | 382       | 490     | ALDDRSTQQCrVQMQVQQLE         |
GRAPHICAL ABSTRACT

IMPAIRED SPICING MACHINERY IS DIRECTLY ASSOCIATED WITH THE PATHOGENESIS OF RHEUMATOID ARTHRITIS

Study cohorts (n=129)
- 29 HDs
- 72 RA

RA mouse model (n=6)
- 38 RA
  - Before TNFi
  - After 3 & 6 months TNFi

In vitro stimulation of healthy leukocytes with ACPAs

In vitro transfections of RA leukocytes
- 3 Control mice
- 3 K/BxN mice

MOLECULAR ANALYSIS
- Splicing Machinery
- Peripheral Blood (Leukocyte subtypes)
  - Lymphocytes
  - Monocytes
  - Neutrophils
- Synovial Fluid (PBMCs)

CLINICAL PROFILE
- Clinical/serological parameters
  - DAS28 score
  - Radiological involvement
- Atheroma plaques
- Rheumatoid Factor
- Anti-CCPs antibodies
- CRP / ESR

A NEW PLAYER ON THE BOARD?

RA Leukocytes transfected with Spliceosome components

HD Leukocytes treatment with IgG-ACPA +/ FcR blocking

MOLECULAR ANALYSIS
- HD Leukocyte Subsets
  - Synovial Fibroblast Function
- Leukocyte Functional Assays
- Inflammatory cytokines
- 8 Spliceosome components

MOLECULAR ANALYSIS
- RA Leukocyte Subsets

MOLECULAR ANALYSIS
- Ankle joints
- Identification of altered spliceosome components

Supplemental material placed on this supplemental material which has been supplied by the author(s)