SUPPLEMENTAL MATERIAL

Supplemental Methods:

Human heart valve tissue collection

Human aortic valve (AV) and mitral valve (MV) leaflets were obtained from removal of failing hearts during transplantation (non-disease valves/ND) and from valve replacement surgeries (RHVD: Universidade Federal de Minas Gerais CAAE protocol #: 32715214.9.0000.5149; CAVD: Brigham and Women's Hospital IRB protocol #:2011P001703). Our patient cohort show a predominance of RHVD in women (Supplementary Table I), and therefore our specimens are derived predominantly from female cases. Fresh tissue was immediately transferred from the operating room in high-glucose Dulbecco's modified Eagle's medium (DMEM, Gibco 10569010) on ice. Radial tip-to-base segments from the middle portion of all AV and MV leaflets were transferred to microcentrifuge tubes and frozen at -80°C. Remaining heart valve leaflet tissues were frozen in Optimum Cutting Temperature compound (OCT, Sakura Finetek, USA) for histological analysis. A total of 46 specimens were collected including: 7 non-disease aortic valves (NDAV), 10 non-disease mitral valve (NDMV), 4 rheumatic aortic valve disease (RAVD), 20 rheumatic mitral valve disease (RMVD) and, 5 calcific aortic valve disease (CAVD).

Proteomics sample preparation

Tissue samples were then pulverized in liquid nitrogen and re-suspended on ice in RIPA buffer (Thermo Scientific Pierce, USA) with protease inhibitor (Roche, cOmplete ULTRA tablets) and phosphatase inhibitor (Roche, PhosSTOP tablets), and vortexed for 30 seconds. To obtain peptides for mass spectrometry, whole-tissue samples were sonicated after RIPA lysis for 4 x 15 seconds (Branson Sonifier 450). Protein precipitation

was performed by methanol-chloroform and proteolysis by trypsin/RapiGest (Promega Gold Grade, V5280/Waters RapiGest SF). 15 µg of precipitated protein per sample was used for proteolysis. Tryptic peptides were desalted with Oasis HLB 1 cc/10 mg cartridges (Waters) at 37°C overnight and dried with a tabletop speed vacuum (SPD1010, Thermo Scientific, USA), then re-suspended in 40 µl of 5% mass spectrometry grade acetonitrile (Thermo Fisher Scientific, USA) and 0.5% formic acid (Sigma-Aldrich, USA).

Mass spectrometry:

Peptides were analyzed using the Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Fisher Scientific, USA) fronted with an Easy-Spray ion source and coupled to an Easy-nLC1000 HPLC pump (Thermo Fisher Scientific, USA). The peptides were separated using a dual column set-up: An Acclaim PepMap RSLC C18 trap column, 75 µm X 20 mm; and an EASY-Spray LC heated (45^{o}C) column, 75 µm X 250 mm (Thermo Fisher Scientific). The gradient flow rate was 300 nl/min from 5 to 21% solvent B (acetonitrile/0.1% formic acid) for 75 minutes, 21 to 30 % Solvent B for 15 minutes, followed by 10 minutes of a 'jigsaw wash', alternating between 5 and 95 % Solvent B. Solvent A was 0.1% formic acid. The instrument was set to 120 K resolution, and the top N precursor ions in a 3 second cycle time (within a scan range of 375-1500 m/z; isolation window, 1.6 m/z; ion trap scan rate, normal) were subjected to collision induced dissociation (collision energy 30%) for peptide sequencing (or MS/MS). Dynamic exclusion was enabled (60 seconds).

Mass spectrometric data analysis:

The MS/MS spectra, from NDAV (n=6), NDMV (n=6), RAVD (n=4), RMVD (n=9) and CAVD (n=5), were queried against the human UniProt database (downloaded on August 01, 2018;155,133 entries); using the HT-SEQUEST search algorithm, via the Proteome Discoverer (PD) Package (version 2.2, Thermo Fisher Scientific), USA. Methionine oxidation and n-terminal acetylation were set as a variable modification, and carbamidomethylation of cysteine was set as a fixed modification. The enzyme was set to trypsin (full), with a maximum of 4 missed cleavages, using a 10-ppm precursor tolerance window and a 0.02 Da fragment tolerance window. Peptides were filtered based on a 1% FDR based on the reverse database (decoy) results. In order to quantify peptide precursors detected in the MS1 but not sequenced from sample to sample, we enabled the 'Feature Mapper' node. Chromatographic alignment was done with a maximum retention time (RT) shift of 10 minutes and a mass tolerance of 10 ppm. Feature linking and mapping settings were, RT tolerance minimum of 0 minutes, mass tolerance of 10 ppm and signal-to-noise minimum of five. Precursor peptide abundances were based on their chromatographic intensities and total peptide amount was used for normalization. Peptides assigned to a given protein group, and not present in any other protein group, were considered as unique. Consequently, each protein group is represented by a single master protein (PD Grouping feature). We used unique and razor peptides per protein for quantification and filtered for proteins with 2 or more unique peptides. The total of 30 valve sample datasets comprised a single PD output file, that resulted in non-overlapping/undetected proteins, or missing values, in some samples (see below).

Proteomics statistical analysis:

The quantified proteins were exported from via the Proteome Discoverer (PD) Package (version 2.2, Thermo Fisher Scientific, USA) and median-normalized (per

sample), to scale the proteins and assign “0.001” to missing values, using in-house scripts written in Python v3.4. The data were then analyzed using Qlucore Omics Explorer statistical software (Qlucore, v3.6, Sweden). Thresholding of protein intensities was performed at level = 0.001, then intensities were log2-transformed for subsequent statistical analysis. Significantly differentially enriched proteins were calculated using a two-group comparison ($q<0.1$).

Proteomics bioinformatic analysis

Regulatory gene networks involving transcription factor (TF) and gene interactions are important to understand which TFs regulates the expression of the differentially expressed proteins in the omics datasets[1]. Using network centrality score we can identify the hub genes/proteins that might play an important regulatory role in disease pathophysiology. We used NetworkAnalyst (https://www.networkanalyst.ca)[2], a web-based tool to create a TF-gene interaction network among the 213 RHVD altered proteins along and its first neighbors using Encode database. To avoid “hairball effect” and better visualization we restricted the network to zero order interactions. In the PPI network the most important hub genes were identified based on the two widely used network centrality scores[3]: node degree, a simple topological index, corresponding to the number of nodes connected to a given node in the PPI and betweenness centrality, also known as the network “bottleneck” measures number of shortest paths going through the node and therefore critical to the information flow in the network. The nodes with highest betweenness and degree are supposed to be the hub genes in the PPI networks. Since ProTα, the top candidate protein prioritized in our proteomic and network analysis can affect the function of estrogen receptor alpha (ERα), a TF, we created transcription factor-gene interaction network and used the list in PPI to understand the enriched pathways associated. Using cytoscape (https://cytoscape.org)[4] tool we created a

pathway enrichment network with pathways as the source nodes and its associated genes as the target nodes. We highlighted the estrogen signaling pathway and genes associated with it in blue color in the network.

Histology

Leaflet thickness measurements and immunohistochemistry

Heart valve leaflets (NDAV, NDMV, RAVD and RMVD) embedded in OCT were cut at 7 µm thickness. Cryosections were stained with hematoxylin and eosin for overall morphology and leaflet thickness measurements. MV leaflet thickness was measured ten times per donor in areas free of chordal attachment; all values were averaged and presented in mm. For immunohistochemistry and immunofluorescence, cryosections were fixed in -20°C acetone (Fisher Scientific, USA), blocked with 0.3% hydrogen peroxidase (Fisher Scientific, USA) and incubated with Protein Block Serum-Free (Dako, USA). Primary antibodies anti-prothymosin alpha - ProTα (human, 1:200, PAS71580, Thermo Fisher Scientific, USA), anti-CD4 (human, 1:30, M7310, Dako, USA), anti-CD8 (human, 1:80, M0707, Dako, USA) and CD68 (human, 1:500, M0814, Dako, USA), were diluted in 5% normal horse serum (Vector Laboratories, CA, USA). Sections were incubated with primary antibodies for 90 minutes at room temperature, and then incubated with biotinylated goat anti-mouse and anti-rabbit secondary antibodies (Dako, LSAB Kit, USA). The streptavidin peroxidase method (Dako, LSAB Kit, USA) was performed for each staining, and the reaction was visualized with a 3-amino-9-ethylcarbazol substrate (AEC Substrate Chromogen, Dako). Sections were counterstained with Gill's No. 3 Hematoxylin (Sigma-Aldrich, USA). For quantification of CD4, CD8 and CD68, AEC-positive cells (red reaction product) and total nuclei (blue) per high-power field (400x magnification) were counted and expressed as AEC-positive

cells/nuclei for each marker. Ten fields were quantified for each section. Because ProTα is secreted protein, the results were reported as percentage of ProTα-positive area by multiplying AEC-positive area per 100 and then divided by leaflet total area. Two-group comparisons between non-disease valves and disease valves were made using an unpaired *t* test.

Multi-labeled immunofluorescence

Immunofluorescence labeling was performed using anti-ProTα (rabbit, 1:200, Thermo Fisher Scientific, USA), anti-CD45 (rat, 1:100, Invitrogen, USA), anti-α-SMA (mouse, 1:150, Dako, USA), anti-granzyme B (rat, 1:200, Thermo Fisher Scientific, USA), anti-perforin (mouse, 1:100, Thermo Fisher Scientific, USA) anti-vimentin (mouse, 1:300, Thermo Fisher Scientific, USA). Sections were fixed in -20°C acetone (Thermo Fisher Scientific, USA) and blocked with PBS containing 3% bovine serum albumin (Sigma-Aldrich, MO, USA) in 0.1% Tween (Research Products International, USA). All primary antibodies were incubated overnight at 4°C. Secondary conjugated antibodies were Alexa Fluor anti-rabbit 647, Alexa Fluor anti-rabbit 488, Alexa Fluor anti-rabbit 594 (1:500, Thermo Fisher Scientific, USA). Sections were counterstained with 4',6-diamidino-2-phenylindole - DAPI (Fisher Scientific, USA). Images were analyzed using the imaging software NIS-Elements AR (Advanced Research) 3.1 (Nikon Instruments, Melville, USA).

Multiparameter immunophenotyping by flow cytometry

Purification of peripheral blood mononuclear cells

For the purification of peripheral blood mononuclear cells (PBMC), heparinized blood was applied over a Ficoll-Hypaque (GE Healthcare Life Sciences, USA) gradient, centrifuged at 600g for 40 minutes, at room temperature, and PBMC were collected at the interface between the plasma and Ficoll-Hypaque. Cells were washed 3 times by centrifugation with Dulbecco's Phosphate Buffered Saline - DPBS (Gibco, USA) and resuspended in Dulbecco's Modified Eagle Medium – DMEM phenol red free (Thermo Fisher Scientific, USA) supplemented with antibiotic (penicillin 200 U/ml and streptomycin 0.1 mg/ml; Sigma, USA) and 1 mM L-glutamine (Sigma, USA) and 5% of human charcoal stripped serum (Innovative research, USA) to a concentration of 10^7 cells/ml. Frozen PBMCs were purchased from Lonza (USA).

Ex vivo

For the *ex vivo* assays, 100ul of Brilliant buffer solution (BD, USA) containing a combination of monoclonal antibodies was used to access the expression of ProTα and ERα in peripheral mononuclear cells from health donors and RHVD patients. Anti-CD4 (PerCP/Cyanine5.5, Biolegend, USA) anti-CD8 (APCCy7, Biolegend, USA), anti-CD14 (PECy7, Biolegend, USA), were added to peripheral mononuclear cell suspensions ($1x10^6$ cells/mL) for 20 minutes at 4°C. Zoombie Aqua fixable viability kit was used to access cell viability (BV510, Biolegend, USA). Samples were washed in flow cytometry staining buffer (FACSbuffer, eBioscience, USA) and fixed by 10 minutes with a 2% paraformaldehyde solution (Thermo Fisher Scientific, USA). After washing with FACSbuffer, cells were permeabilized for 45min with fixation/permeabilization solution (eBioscience, USA) and proceeded to the intracellular staining. Samples were incubated with anti-ProTα (APC, LSBio, USA) and anti-ERα (PE, Abcam, USA) for 45 minutes at room temperature, washed twice with FACSbuffer and read in a BD LSR II Flow

Cytometer. 50,000 cells/sample were collected. FACS data were analyzed using FlowJo (FlowJo, USA).

In vitro

PBMCs isolated from whole heparinized blood were plated at 1 x 10^6 cells/well in 96 well cell culture round bottom plates (Corning,USA) with DMEM phenol red free (Thermo Fisher Scientific, USA) supplemented with antibiotic (penicillin 200 U/ml and streptomycin 0.1 mg/ml; Sigma, USA) and 1 mM L-glutamine (Sigma, USA) and 5% of human charcoal stripped serum (Innovative research, USA) only (control cultures – CTRL), in the presence 10nM of estradiol (E_2) (Sigma, USA), in the presence of 100mM of ProTα (Enzo Life Sciences, USA), or in the combination of the both 10nM of E_2 and ProTα for 20 hours. Brefeldin A (1 µg/mL, Thermo Fisher Scientific, USA was added for last 4 h of culture to prevent cell secretion.

After a total of 24 hours of culture, cell cultures were centrifuged at 600g for 8 minutes, at 4°C. 100ul of Brilliant buffer solution (BD, USA) containing monoclonal antibodies specific for human leukocyte cell-surface markers were added to the cells, including anti-CD8 (APCCy7, Biolegend, USA), anti-CD4 (PerCP/Cyanine5.5, Biolegend, USA) to identify T-cells subpopulations. Anti-CD49a/VLA-1 (FITC, Biolegend, USA), anti-CD49b/VLA-2 (PerCP/Cyanine5.5, Biolegend, USA), anti-FasL (PECy7, Biolegend, USA) were used to access phenotypic characteristics of T-cells subpopulations. Zoombie Aqua fixable viability kit was used to access cell viability (BV510, Biolegend, USA). Samples were washed in flow cytometry staining buffer (FACSbuffer, eBioscience, USA) and fixed by 10 minutes with a 2% paraformaldehyde solution (Thermo Fisher Scientific, USA). After washing with FACSbuffer, cells were permeabilized for 45 minutes with fixation/permeabilization solution (eBioscience, USA) and proceeded to the intracellular staining. Samples were incubated with anti-granzyme

B (BV421, Biolegend, USA), anti-perforin (APC, Biolegend, USA), anti-ERα (PE, Abcam, USA), anti-FOXP3 (Pacific blue, Biolegend, USA), anti-RORγt (Thermo Fisher Scientific, USA USA), anti-T-bet (FITC, Biolegend, USA) for 45 minutes at room temperature, washed twice with FACSbuffer and read in a BD LSR II Flow Cytometer. 50,000 cells/sample were collected. FACS data were analyzed using FlowJo (FlowJo, USA). Paired T test was used to compare unstimulated versus stimulated cultures. p values ≤0.05 were considered statistically significant.

***In vitro* - CD8$^+$ T-cells estrogen receptor alpha blocking assay**

CD8$^+$ T-cells were purified from frozen PBMCs (Lonza, USA) using Dynabeads™ FlowComp™ Human CD8 Kit according to the manufacturer's instructions (Thermo Fisher Scientific, USA). For each sample, two cell suspensions were obtained: CD8 depleted (CD8$^-$) cells and CD8 purified (CD8$^+$) cells. CD8$^+$ cell suspensions were treated with 2 uM and 4 uM of 1,3-Bis(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy)phenol]-1H-pyrazole dihydrochloride (MPP, Sigma, USA), a highly selective ERα antagonist for a period of 2 hours (37°C, 5% CO2). CD8+ cell without stimulus were used as controls. Simultaneously, CD8- cell suspensions were also incubated with DMEM phenol red free (Thermo Fisher Scientific, USA) supplemented with antibiotic (penicillin 200 U/ml and streptomycin 0.1 mg/ml; Sigma, USA) and 1 mM L-glutamine (Sigma, USA) and 5% of human charcoal stripped serum (Innovative research, USA). After 2 hours of incubation, CD8$^+$ cell suspensions were washed twice with DMEM phenol red free (Thermo Fisher Scientific, USA). Then, CD8 cell suspensions were reintroduced to the CD8$^+$ cells: control, 2 uM and 4 uM. Ultimately, cell suspensions were plated at 1 x 10^6 cells/well in 96 well cell culture round bottom plates (Corning,USA) with DMEM phenol red free (Thermo Fisher Scientific, USA) supplemented with antibiotic (penicillin 200 U/ml and streptomycin 0.1 mg/ml; Sigma, USA) and 1 mM L-glutamine

(Sigma, USA) and 5% of human charcoal stripped serum (Innovative research, USA) only or in the presence of 100 mM of ProTα (Enzo Life Sciences, USA) for 20 hours (37°C, 5% CO2). Brefeldin A (1 µg/mL, Thermo Fisher Scientific, USA was added for last 4h of culture to prevent cell secretion. Immunophenotyping by multiparameter flow cytometry was performed as described above. Comparisons between non-stimulated (CTRL) and stimulated cell (ProTα) cultures and between non-MPP treated and MPP-treated were made using a two-way ANOVA and Tukey's as post-hoc test. Statistical significance is indicated in each graph.

ELISA

Plasma levels of ProTα were determined using a sandwich ELISA kit according to the manufacturer's instructions (LSBio, USA, cat#LS-F7929). ProTα concentrations were calculated using a four-parameter logistic (4-PL) curve on Spectramax®i3X (Molecular Devices, USA). Two-group comparisons between healthy individuals and RHVD patients were made using a paired *t* test.

Valve interstitial cells (VICs) isolation and culture

Human aortic valve leaflets were obtained from aortic valve replacement surgeries for severe aortic stenosis and valvular interstitial cells were isolated using sequential collagenase digestions. Both sides of the leaflet were scratched by razor blade to remove the endothelial cells. After cutting into 1–2 mm cubes, tissue pieces were digested using 1 mg/mL collagenase (Sigma-Aldrich,USA) in DMEM (Thermo Fisher Scientific, Waltham, MA) for 1 hour at 37°C and gently mixing every 20 minutes. The cells isolated in the first digestion potentially included endothelial cells, which were washed out by DMEM and discarded. Next, tissue pieces were further digest using 1

mg/mL collagenase for 3 hours and isolated VICs were collected by centrifugation and plated in 75 cm^2 culture flasks. Isolated VICs were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin (Corning, USA) until cells were >90% confluent (~7days). After this period, cells were then trypsinized and plated at a density 3.0×10^4 cells/0.9 cm^2 (1 well of a 48-well plate) with DMEM phenol red free (Thermo Fisher Scientific, USA) supplemented with antibiotic (penicillin 200 U/ml and streptomycin 0.1 mg/ml; Sigma, USA) and 1 mM L-glutamine (Sigma, USA) and 5% of human charcoal stripped serum (Innovative research, USA) only (control cultures – CTRL) and in the presence of 100 mM of ProTα (Enzo Life Sciences, USA). Cultured VICs were harvested and placed in TRIzol reagent (Thermo Fisher Scientific, USA) to isolate RNA.

Real-time quantitative polymerase chain reaction (qPCR)

cDNA was generated from the non-stimulated and ProTα stimulated VICs RNAs using Quanta qScript cDNA Synthesis Kit (Quantabio, USA). Briefly, 0.2 mL chloroform was added to each sample and homogenized. After a 2-minute incubation at room temperature (RT), samples were centrifuged at 12,000 x g, 4°C for 15 minutes using an Eppendorf 5430R microcentrifuge. The aqueous (upper) phase of each sample was then transferred to a new 1.5 mL tube. Next, 0.5 mL of isopropanol and 1.5 µL of GlycoBlue™ coprecipitant (Invitrogen, USA) were added to each sample, followed by sample homogenization, incubation at RT for 10 minutes, and centrifugation at 12,000 x g, 4°C for 10 minutes. After discarding the supernatant, RNA pellets were washed using 75% ethanol, mixed using a vortex, and centrifuged at 7,500 x g, 4°C for 5 minutes. The supernatant was discarded, RNA pellets were air dried for 10 minutes, and resuspended in 10 µL RNase-free water.

RNA concentration was quantified using the NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, USA). Next, cDNA was prepared using

200ng of RNA per sample with 5X EasyQuick RT MasterMix (CoWin Biosciences, CW2019M) per manufacturer's protocol. In brief, 200 ng RNA was transferred to a clean 8-strip 200 uL tube and volume was brought to 16 µL total using RNase-free water. Then, 4 µL 5X EasyQuick RT MasterMix was added to each sample, mixed thoroughly, and centrifuged briefly to ensure collection of all solution at the bottom of the tube. Reverse-transcription performed using a PCR Thermal Cycler T100™ (Bio-Rad Laboratories, USA) set at 37°C for 15 minutes, followed by incubation at 85°C for 5 minutes. Prepared cDNA diluted 1:4 using RNase-free water and stored at -20°C. PerfeCTa® SYBR® Green FastMix®, ROX (Quantabio, USA) was used for quantitative real-time qPCR analysis with the 7900HT Fast Real-Time PCR System (Applied Biosystems, USA) following the manufacturer's instructions. Gene-specific primers were used to detect human HLA-A, HLA-B, HLA-C. Forward and reverse primers sequences are shown in the table below. Samples were normalized to endogenous human HPRT. Fold changes were calculated by ΔΔCt method. Significance was determined by student's two-tailed t-test, $p<0.05$.

Gene	Forward Sequence (5'-3')	Reverse Sequence (5'-3')
HLA-A	AAAAGGAGGGAGTTACACTCAGG	CAGCCGTACATGCTCTGGA
HLA-B	CAGTTCGTGAGGTTCGACAG	CAGCCGTACATGCTCTGGA
HLA-C	CCATGAGGTATTTGTGGACCG	TCTCGGACTCTCGTCGTCG

Identification of cross-reactive epitopes using *in silico* approaches

The Clustal Omega Server Multiple Sequence Alignment (https://www.ebi.ac.uk/Tools/msa/clustalo/) was used to verify the similarities between human type 1 collagen protein sequences (https://www.uniprot.org/uniprot/P02452 and https://www.uniprot.org/uniprot/A0A0S2Z3H5) and *S. pyogenes* (serotype M1) "collagen-like surface protein" (https://www.uniprot.org/proteomes/UP000000750). Protein sequences were obtained from UniProt. Next, homologous amino acids regions

between human and *S. pyogenes* proteins were used to predict CD8 T-cells epitopes. For that, initially we used TepiTool from (IEDB Analysis Resource- http://tools.iedb.org/tepitool/) to search within the identified homologue regions, sequences of nine amino acids (9-mer) which can bind to human leukocyte antigen class I (HLA-I). Identified 9-mer's sequences were then used to predict the ability to bind to a specific HLA-I molecule. The most frequent A & B alleles were used for that analysis (http://tools.iedb.org/mhci/). Then, we used T cell class I pMHC immunogenicity predictor tool (http://tools.iedb.org/immunogenicity/) to predicts the relative ability of a peptide/MHC complex to elicit an immune response. Additionally, we checked the immunogeneicity of the corresponding (homologue) 9-mer on *S. pyogenes* sequences were also immunogenic. Immunogenic epitopes (class I immunogenicity score >0) were selected for peptide docking.

HLA-I peptide docking

The shortlisted conserved regions in the collagen after homology analysis were explored for their ability to act as T-cell epitopes. Peptides from these homologous regions between human and *S. pyogenes* proteins were subjected to HLA-I binding analysis using Tepitool (http://tools.iedb.org/mhci/)[5]. Nonameric peptides generated from the conserved regions, which were predicted as potential binders for HLA-I alleles, were selected for further analysis. Peptide-HLA docking was performed using HPEPDOCK (http://huanglab.phys.hust.edu.cn/hpepdock/), an online server which utilizes a fast rigid-body peptide–protein docking based on a hierarchical algorithm, predicting interactions between receptor protein and ligand[6]. For protein-peptide docking the crystal structures of HLA-A*0301 (PDB ID: 6J1W), HLA-B*4001 (PDB ID: 6IEX) and HLA-B*0701 (PDB ID: 5EO0) were retrieved from Protein Data Bank database (http://www.rcsb.org/pdb/)[7]. The resident peptide in the crystal structure was stripped off using Chimera tool

(https://chimeratool.com/)[8] to make the binding groove of HLA-I alleles available for docking the bacterial collagen peptide and its human homologue.

Expanded results

RHVD has higher prevalence in females than in males.

Over the last decade, 679 patients were referred for management of RHVD at the Universidade Federal de Minas Gerais (UFMG, Brazil). The main clinical characteristics of this population are presented in Table 1. Mean age was 47 ± 12 years, with predominance of female (85%). RAVD was associated with RMVD in 43% of patients; rheumatic tricuspid valve disease was detected in 6%. All patients presented with rheumatic mitral stenosis with valve area of 1.1 ± 0.4 cm^2. At baseline, mitral valvuloplasty had previously been performed in 223 patients (33%), including either percutaneous or surgical intervention. Atrial fibrillation was more frequent in male without difference in stroke prevalence.

Supplemental table II: Clinical characteristics of rheumatic heart valve disease population stratified according to sex

		Overall (n=679)	Female (n=576)	Male (n=103)	p value
Age, years		47 (38-55)	47 (16-83)	49 (14-77)	0.274
NYHA functional class	I/II	431 (64)	357 (62)	74 (72)	0.042
	III/IV	248 (36)	219 (38)	29 (28)	
Stroke		131 (19)	115 (20)	16 (16)	0.303
Prior MV intervention		223 (33)	198 (34)	25 (25)	0.047
Atrial fibrillation		211 (31)	165 (29)	46 (45)	0.002
Pattern of valve involvement					
Isolated mitral valve*		344 (50.7)	306 (53)	38 (37)	0.022
Mitral and aortic valves		294 (43.3)	235 (40)	59 (57)	
Mitral, aortic and tricuspid valves		32 (4.7)	27 (5)	5 (5)	
Mitral and tricuspid valves		9 (1.3)	8 (2)	1 (1)	

Data are expressed as median (interquartile range-IQR) or the absolute numbers (percentage). NYHA, New York Heart Association

* Mitral stenosis was detected in all patients.

Supplemental Figures:

Supplemental Figure I

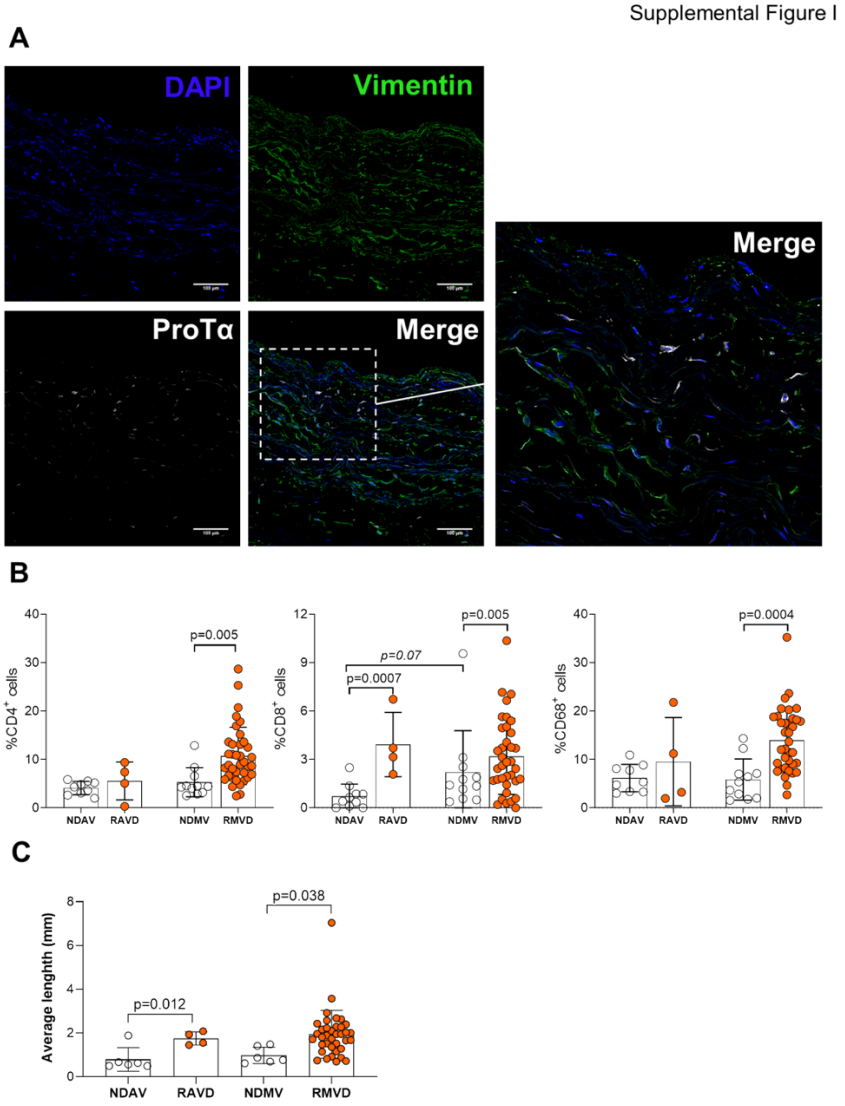
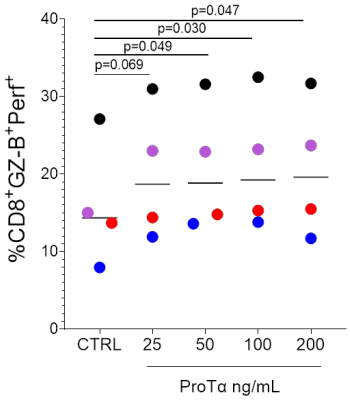


Figure I: RHVD histological features. A. Representative multi-labeled immunofluorescence staining for vimentin (green) and ProTα (white) in RMVD. DAPI depicted nuclei. Scale bars = 100 µm. **B,** Histological evaluation of frequency of T-cells ($CD4^+$ and $CD8^+$) and macrophages ($CD68^+$) in non-disease aortic and mitral valves (NDAV, n=7 and NDMV, n=10) and rheumatic aortic and mitral valve disease (RAVD, n=4 and RMVD n=20). **C,** Leaflet thickness measurements. Two-group comparisons between non-disease valves and disease valves were made using an unpaired *t* test.

Supplemental Figure II



	% CD8+GzB+Perf+				
Donor ID	CTRL	25ng/mL	50ng/mL	100ng/mL	200ng/mL
D1	13.7	14.4	14.8	15.3	15.5
D2	7.96	11.9	13.6	13.8	11.7
D3	15.0	23.0	22.9	23.2	23.7
D4	27.1	31.0	31.6	32.5	31.7

Figure II: CD8⁺ cytotoxicity induced by ProTα is dose dependent. Evaluation of the frequency of $CD8^+GzB^+Perf^+$ cells in peripheral blood mononuclear cells stimulated with 25, 50, 100 and 200 ng/mL of recombinant ProTα. Non-stimulated cells were used as controls (CTRL). Each color represents a donor (n=4). Results can be visualized in the graph (left) and in the table (right). Two-group comparisons between non-stimulated and stimulated cell cultures were made using a paired *t* test. Statistical significance is indicated in the graph.

Supplemental Figure III

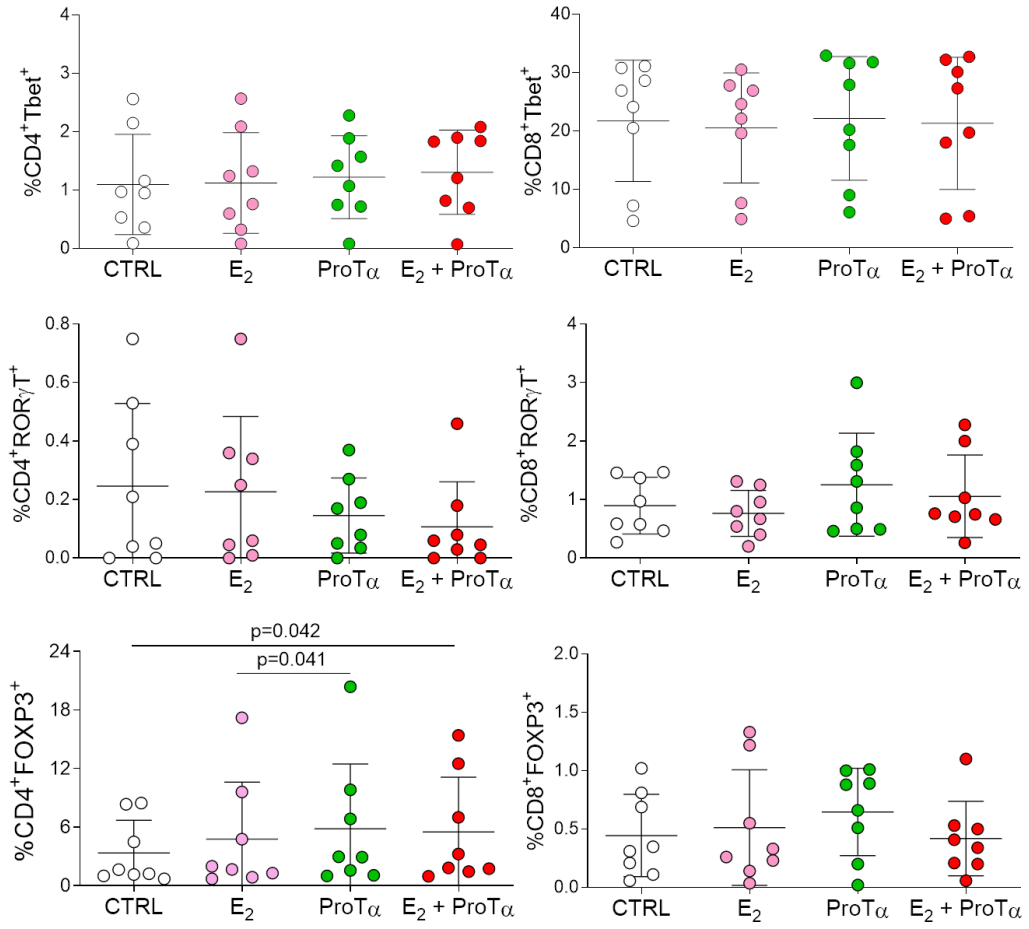


Figure III: Effects of recombinant ProTα on CD4⁺ and CD8⁺ T-cells. Frequency of $CD4^+$ and $CD8^+$ T-cells expressing transcription factors associated with pro-inflammatory T-cell phenotype (T-bet and RORγT) and regulatory phenotype (FOXP3) in $CD4^+$ or $CD8^+$. Control cultures (non-stimulated, CTRL) are represented by white dots, ProTα stimulated cultures are represented by green dots, estradiol stimulated cultures (E_2) are represented by pink dots, combination of ProTα and E_2 (ProTα+E_2) stimulated cultures are represented by red dots. Bar graphs show the mean of values in each group (n=8) and standard deviation. Two-group comparisons between non-stimulate and stimulated cell cultures were made using a paired *t* test. Statistical significance is indicated when is present.