METHODS

Study population. A large Caucasian multiplex AxSpA family of North European Ancestry from Newfoundland, Canada was identified in a university based rheumatology clinic. All available family members were invited to participate in our study, and were systematically assessed using a standardized SpA protocol by an experienced rheumatologist (PR). Nine individuals satisfied the imaging arm of the new ASAS criteria for AxSpA. This included 6 members with AS indicated by radiographic sacroillitis and three members exhibited normal SI joints on plain radiography with a positive MRI. Extra-articular features included two individuals with iritis, one with Crohn's disease and one with psoriasis. One affected family member also had features of lupus. All family members affected with AxSpA were *HLA-B27* positive. The Spondyloarthritis Consortium of Canada (SPARCC) provided clinical and radiographic data, as well as DNA samples for the replication cohorts. All patients in this study have provided informed consent and ethics (HREA #1999.172) have approved the study.

B-cell lines. Acid citrate dextrose anti-coagulated whole blood was collected from family members. Lymphoblastiod B-cell lines (BCL) were generated by Epstein-Barr transformation of peripheral blood B-cells as previously described. [1]

Nucleic acid extraction. DNA was extracted from EDTA anti-coagulated whole blood using a traditional salting-out method. [2] B-cell line pellets were re-suspended in 0.5ml TRIzol® (Life Technologies) and RNA extracted as per manufacturer's instructions and dissolved in Molecular Biology grade water. RNA samples were then treated with TURBO DNA-*free*™ (Ambion) to remove possible DNA/DNAse contamination.

Quantification was performed using a Nanodrop 2000 (Thermo Scientific). RNA was evaluated using a 2100 Bioanalyzer (Agilent Technologies) and samples with a RIN value greater than 8.5 were used for study.

HLA-B*27 **tesing.** Targeted analysis of the *HLA-B* locus located on 6p21.3 was performed using a commercially available kit (LABType SSO HLA-B locus kit) on a Luminex 100/200 platform.

Exome sequencing. The samples were sequenced targeting whole genome exons with an average coverage of 110x using Illumina HiSeq 2000. The mapping of reads was aligned using Burrows Wheeler Alignment (BWA) version 0.7.10, and the genome analysis toolkit (GATK) version 1.1.28 was used to call variants against the reference genome. To reduce false positive calling, 40% support reads was used as a cut off for alternative allele. Analysis was carried out to detect rare mutations that segregate only within the affected individuals. Annovar (April 2014 version) was used for variant annotation.

Fragment analysis. DNA was amplified using specific primers for *SEC16A* (Forward: 5'CCACCTGGCCAATAACTCTG; Reverse: 5'AGGAAGGGTCCAAATTGAGG) and *MAMDC4* (Forward: 5'GAGGGAAAGAGGCATCCAC; Reverse: 5'ATTTCGGGTGGCAGAGAAG) in a standard touchdown reaction on a GeneAmp PCR System 9700 (Applied Biosystems). PCR product was mixed with HiDi-TM Formamide and LIZ600 standards (Life Technologies), denatured and assayed by capillary

electrophoresis on a 3130XL Genetic Analyzer (Applied Biosystems). Results were analyzed using GeneMapper Software Version 4.0 (Applied Biosystems).

Linkage Analysis. A phased VCF file for the nuclear family (II-1, II-2, & III-2) was obtained using the GATK software (version 3.3). VCFtools (version 0.1.12b) [3] were used to calculate pairwise r^2, D and D' for the genetic variants identified in chromosome 9 from 138,000,000 to 141,000,000 bp (GRCh37) of the nuclear family. This genomic region includes the two novel deletions detected in *SEC16A* and *MAMDC4*. We investigated LD between *SEC16A* and *MAMDC4* in the general population using DistilLD Database [4] and GLIDERS [5].

Quantitative PCR (qPCR). Real-time PCR was performed using TaqMan Gene Expression Assays for *SEC16A* (Hs_00389570_m1) and *GAPDH* (Hs_99999905_m1) from Life Technologies. Samples were tested as per manufacturer's instructions and run on a StepOnePlus (Applied Biosystems). Triplicate samples were analyzed using the comparative threshold cycle ($\Delta\Delta^{CT}$) method and results normalized to *GAPDH*. Statistical analyses were performed by One-way Anova and unpaired t-test with GraphPad Prism v6.04.

Western blot. RIPA lysates were prepared from BCL as previously described. [6] Protein determination was performed using a BCA protein assay kit according to manufacturer's instructions (Thermo Scientific). Protein was subjected to 6% SDS-PAGE electrophoresis at a concentration of 20ug/lane. After transfer to nitrocellulose,

blots were blocked in 5% milk/TBS-Tween (0.15M NaCl, 0.05M Tris pH 7.4, 0.05% Tween 20). The top portion was probed for Sec16A (ab70722, 50ng/ml) and the bottom for tubulin (ab44928, 250ng/ml). Secondary antibodies were horse-radish peroxidase (HRP)-conjugated affiniPure F(ab)2 fragment goat antimouse (GAM) IgG, Fc specific and HRP-conjugated affiniPure F(ab)2 fragment goat anti-rabbit (GAR) IgG, Fc specific antibodies (Jackson Immunoresearch). Bands were detected using Immobilon Western Chemiluminescent Substrate (Millipore) and the ImageQuant LAS 4000 imaging system (GE Healthcare Life Sciences). ImageQuant TL program was used to quantitate the density of the bands. Each sample was expressed as a ratio to tubulin then compared with the proband. Statistical analyses were performed using one-way Anova and unpaired t-test with GraphPad Prism v6.04.

Peptide expression and purification. cDNA (bacteria codon optimized) encoding a 40 amino acid peptide (PLGAGAGSGCAPLEADSGASGALAMFFQGGETENEENLSS) of the Sec16A N-terminus or the complete N-terminus (1-564), plus an enterokinase digestion site and a C-terminal 6-His tag on a fusion protein, was inserted into a PET23d(+) vector (GenScript). The plasmid was transformed into BL21 competent *Escherichia coli* cells for large-scale expression of Sec16A. Cells were grown at 37ºC to an OD of 0.6, induced with 1mM IPTG and harvested after 3h. The 40 amino acid deletion peptide (PLGAGAGSGCAPLEADSGALAMFFQGGETENEENLSS) and the full-length N-terminus void of the amino acids SGA at position 369-371 were prepared in the exact same way. The harvested BL21 cells were lysed using a French pressure cell and solubilized in 8M urea, 100mM Tris-HCl (pH 8.0) and 1mM DTT. The resulting

supernatant was loaded onto an equilibrated Ni^{2+}-nitrilotriacetic acid-agarose column (5ml) and washed with 10mM Tris-HCl (pH 8.0), 100mM sodium chloride and 8M urea. Peptides were eluted using an imidazole gradient and the purified peptide-containing fractions were pooled and desalted. The desalted fusion peptides were diluted and incubated with enterokinase overnight at 30°C. [7] The reaction mixture was purified by a Millipore HiPrep 16/60 sephacryl S-100 HR gel filtration column using a 50mM sodium phosphate buffer (150mM NaCl, pH 7.0). One mL fractions were collected and analyzed by both UV spectroscopy and with Tricine-SDS-PAGE.

Circular dichroism spectroscopy. Circular dichroism (CD) spectra in the far-ultraviolet range were recorded using a Jasco-810 spectrapolarimeter. Equal aliquots of the peptide were dialyzed against 20mM Tris-HCl, 150mM NaCl, pH 8.0, and the absorbance (222nm) of the protein/reagents mixture did not exceed 1.0. The temperature (25°C) was controlled and the scanning speed of the instrument was set at 100nm/min with normal sensitivity. A water-jacketed cell (light path = 0.5mm) was used and spectra were collected between 190 and 260nm. Baselines were established using the appropriate buffers and 30 spectra were collected and averaged for each sample.

Immunofluorescence. Cytospin preparations were prepared from BCL and fixed with acetone. Indirect immunofluorescence was performed as previously described. [8] Primary antibodies were applied for 1hr at RT (anti-SEC16A, Abcam ab70722, or SEC31A, BD 612350, at 2ug/ml in wash buffer). Secondary antibody was applied (Alexa Fluor 488 Goat anti-Rabbit IgG, or Alexa Fluor 555 Goat anti-mouse IgG1, Invitrogen,

1/500) for 1hr at RT. Slides were washed then mounted with Vectashield Hardset Mounting Media with DAPI (Vector Labs). Images were acquired using Zeiss Imager Z1 and Zeiss AxioVision Rel 4.8 software.

References:

1. Mason RD, Bowmer MI, Howley CM, et al. Cross-reactive cytotoxic T lymphocytes against human immunodeficiency virus type 1 protease and gamma interferon-inducible protein 30. J Virol 2005;79:5529-36.

2. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.

3. Danecek P, Auton A, Abecasis G, et al. The variant call format and VCFtools. Bioinformatics. 2011;27:2156-8.

4. Pallejà A, Horn H, Eliasson S and Jensen LJ. DistiLD Database: diseases and traits in linkage disequilibrium blocks. Nucl. Acids Res 2012;40(D1):D1036-D1040.

5. Lawrence R, Day-Williams AG, Mott R, et al. GLIDERS--a web-based search engine for genome-wide linkage disequilibrium between HapMap SNPs. BMC Bioinformatics. 2009;10:367.

6. Mostafa AA, Codner D, Hirasawa K, et al. Activation of ERα signaling differentially modulates IFN-γ induced HLA-class II expression in breast cancer cells. PLoS One 2014;9:e87377.

7. Fonseca P, Light A. The purification and characterization of bovine enterokinase from membrane fragments in the duodenal mucosal fluid. J Biol Chem 1983;258:14516-20.

8. van Zuylen WJ, Doyon P, Clément JF, et al. Proteomic profiling of the TRAF3 interactome network reveals a new role for the ER-to-Golgi transport compartments in innate immunity. PLoS Pathog 2012;8:e1002747.