Investigations into SCAMP5, a candidate lupus risk gene expressed in plasmacytoid dendritic cells

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ABSTRACT

Objective We have investigated the molecular function of SCAMP5, a candidate lupus risk gene for SLE exclusively expressed in plasmacytoid dendritic cells (pDCs) among peripheral leukocytes.

Methods We tested the independence of the association in SCAMP5 with SLE by performing conditional analyses. We profiled the expression pattern of SCAMP5 among circulating leukocytes at the transcript and protein levels. Using lentiviral vectors, we localised the subcellular distribution of SCAMP5 alongside the interferon secretory pathway. We analysed pDCs for the expression of SCAMP5 and interferon production capacity by SCAMP5 genotype. Finally, we examined pDC-specific SCAMP5 isoforms by total RNAseq analysis and examined for genotype-associated quantitative differences therein.

Results A conditional analysis revealed evidence of an independent genetic association of SCAMP5 with SLE. Among circulating leukocytes, SCAMP5 is uniquely expressed in pDCs at the transcript and protein levels, with main presence in the Golgi apparatus and minor presence at the cell periphery. In live cells, SCAMP5 displayed dynamic Golgi-cell surface trafficking and localised with the interferon secretory pathway. SCAMP5 did not differ in expression levels in pDCs between genotyped donors; however, a transient interferon secretory defect was noted in pDCs from donors carrying the risk genotype.

Conclusions SCAMP5 constitutes a novel SLE risk gene on the basis of genomic data and expression in a cell type widely implicated in SLE pathogenesis. While we could not find evidence of quantitative expression differences in SCAMP5 between genotyped donors, SCAMP5 remains an attractive gene to explore given its highly restricted expression pattern and colocalisation with interferon secretion.

INTRODUCTION

Plasmacytoid dendritic cells (pDCs) are rare innate immune cells which express the pattern recognition receptors toll-like receptors 7 and 9 (TLR7 and TLR9), which together with their constitutive expression of the type I interferon (IFN-I) inducer interferon regulatory factor 7 (IRF7) are poised to rapidly respond to viral infection.1 Secretion of massive amounts of IFN-I, particularly IFN-α, is characteristic of pDCs, and downstream signalling in response to IFN drives induction of an antiviral programme in host cells, an ‘IFN signature’ that is constitutively activated in the blood of many patients with SLE.2

A role of pDCs in the pathogenesis of SLE has also been suggested by their presence in cutaneous lupus lesions and in lupus nephritis.3 4 Furthermore, antibody-mediated depletion of pDCs has demonstrated therapeutic efficacy in human trials.5 From the genomic standpoint, several SLE risk genes are enriched for expression in pDCs, including TLR7, IRF7, IRF8 and DNASE1L3.6 7

Key messages

What is already known about this subject?

- SLE is associated with overactivity of the type I interferon pathway, and plasmacytoid dendritic cells (pDCs) are major producers of type I interferon in this disease.

What does this study add?

- A polymorphism on chromosome 15q24 associates with SLE by virtue of a novel candidate SLE risk gene, SCAMP5.
- We confirmed that among circulating leukocytes, SCAMP5 is uniquely expressed in pDCs at the transcript and protein levels, with main presence in the Golgi apparatus and minor presence at the cell periphery.
- We detected a transient interferon secretory defect in pDCs from donors homozygous for the SLE risk genotype, providing a potential link between a functional consequence of the genetic risk variant and the SLE risk association.

How might this impact on clinical practice or future developments?

- Molecular dissection of heritable traits in type I interferon secretion may unveil novel opportunities for drug development.
pDC production of IFN-γ is potentially a major driver of the IFN signature. IFN-γ signalling has been shown to enhance antigen presentation, promote B cell differentiation and suppress regulatory T cells—processes central to the development of SLE.

In their recent meta-analysis of genomewide association studies (GWAS) of SLE, Bentham et al. reported an association at single-nucleotide polymorphism (SNP) rs2289583, with an OR of 1.19 and p value of 6×10⁻¹⁵. rs2289583 occurs in intron 6 of SCAMP5, a member of the secretory carrier membrane protein (SCAMP) family. The SCAMPs are tetraspan membrane proteins with cytoplasmic N-terminal and C-terminal and both luminal and extraluminal peptide loops connecting the transmembrane domains along the surface of intracellular trafficking vesicles (online supplemental figure S1). SCAMPs 1–3 are ubiquitously expressed, whereas SCAMPs 4–5, lacking the characteristic N-terminal NPF repeats of the other SCAMPs, display a more limited expression pattern, largely restricted to the brain. This expression profile of SCAMP5 in the central nervous system appears to be conserved among mammalian species.

Outside of the brain, we noted a strong signal for SCAMP5 expression in pDCs. Given the multiplicity of evidence for the role played by pDCs in SLE, and the association of this risk locus with the disease, we sought to determine whether SCAMP5 contributed to SLE by modulating a key function of pDC biology: IFN-γ secretion.

METHODS
See online supplemental material.

RESULTS
SCAMP5 associates independently with SLE on chr15q24
A recent SLE GWAS meta-analysis reported on susceptibility loci concentrated in genes involved in innate and adaptive immune function. This study coupled analyses of their respective discovery and replication cohorts with a previous SLE GWAS from our group. Among the associations was the polymorphism rs2289583. CSK was annotated as the likely gene explaining the association signal, citing prior work from our group establishing a link between CSK and SLE in this genomic region. However, inspection of SNP associations across this region reveals that the lead SNP from this analysis lies within the SCAMP5 gene, ~155 kb telomeric to CSK. This raised the question as to whether CSK drives the association signal at rs2289583. We examined the primary data in this region, with individual-level data on 4036 SLE cases and 6959 controls from the main GWAS of the aforementioned study. As shown in figure 1A (left panel), two SNPs associated with SLE risk achieve genome-wide significance in the region: rs2289583 in the last intron of SCAMP5 (OR=1.2, p=3.4×10⁻⁶) and rs1378940 in the first intron of CSK (OR=1.17, p=2.8×10⁻⁶). Our group previously identified B cell phenotypes associated with a lupus risk variant in the CSK gene (rs34933034). The rs34933034 SNP was not genotyped in the Bentham et al. data set. In fact, this variant in CSK is poorly imputed by other variants in the region—the lead SNP in CSK in this analysis (rs1378940) exhibits an r²=0.34 with rs34933034. Moreover, the signal at rs2289583 is unlikely to reflect the effects of our previously identified association at CSK, as the linkage disequilibrium (LD) between these two SNPs is also quite modest (r²=0.2 between rs34933034 and rs2289583).

Therefore, we carried out formal conditional analysis to seek evidence for SCAMP5 as a secondary or alternative driver of the SLE risk association in the CSK/SCAMP5 region. On conditioning on the associated SNP within SCAMP5, the signal at rs1378940 within CSK is substantially reduced (figure 1A, middle panel). In contrast, the association signal in SCAMP5 (rs2289583 OR=1.2, p=3.4×10⁻⁶) was only partially reduced following conditioning on the lead risk-association SNP in CSK (p=1.66×10⁻³) (figure 1A, right panel), indicating that the signal at SCAMP5 is independent of the association signals observed within CSK itself.

SCAMP5 is uniquely expressed in pDCs among peripheral blood leucocytes
Having established that SCAMP5 may represent a novel SLE risk gene, we sought to probe the functional biology underlying this association. SCAMP5 is reported to be expressed uniquely by pDCs among peripheral blood leucocytes in humans (BioGPS and Human Protein Atlas; online supplemental figure S2). To confirm this, we sorted leucocyte subsets from four healthy donors and examined SCAMP5 expression by reverse transcription (RT)-PCR. SCAMP5 transcript was detected in pDCs from all donors and was absent in their B, T and NK cells, as well as monocytes and conventional dendritic cells (cDCs) (figure 1B, left panel). To determine whether SCAMP5 is expressed at the protein level in pDCs, we resolved lysates from several leucocyte subsets and detected SCAMP5 protein using the polyclonal antibody (pAb) against SCAMP5 employed by the Human Protein Atlas. We found that SCAMP5 is indeed expressed at the protein level only in pDCs (figure 1B, middle panel). To agnostically determine whether any other (unsorted) peripheral blood leucocyte subset expressed SCAMP5, RBC-depleted whole blood was stained intracellularly for SCAMP5 using the same pAb. Only pDCs stained for SCAMP5, confirming the unique expression of this protein in the circulation (figure 1B, right panel).

To localise expression of SCAMP5 within pDCs, fixed and permeabilised pDCs were stained for SCAMP5 and visualised by immunofluorescence. Colocalisation was evident with the Golgi marker golgin-97, with additional evidence of staining for SCAMP5 at the cell periphery (figure 1C).

The novelty of SCAMP5 as an SLE risk gene, coupled with the striking expression pattern restricted to pDCs, a cell type highly invoked in SLE pathogenesis, prompted us to further explore a role of SCAMP5 in modulating pDC function.
SCAMP5 traffics between the Golgi stack and cell surface and colocalises with clathrin and the IFN-I export pathway

The SCAMP protein family remains understudied. To develop tools to enable a more complete understanding of SCAMP5 in particular, several SCAMP5 constructs were generated and cloned into lentiviral vectors. To localise and track SCAMP5 in live cells, a SCAMP5-mKate2 fusion protein was designed. SCAMP5-mKate2 was transduced...
Localisation of SCAMP5-mKate2 in HEK cells. SCAMP5-mKate2 localised mainly to the Golgi body, with puncta observed at the cell periphery (figure 2). This distribution matched the SCAMP5 localisation patterns observed by immunofluorescence on endogenously expressed SCAMP5 in primary pDCs (figure 1C). On time-series imaging, SCAMP5 was highly motile in the cell, with both anterograde and retrograde trajectories observed between the Golgi and the plasma membrane (figure 2 and online supplemental videos 1–4).

To probe for a possible interaction between SCAMP5 and the IFN-α trafficking pathway, the fluorescent protein mVenus was generated as a soluble expression construct, designated ‘cytosolic/c-mVenus’ or with the signal peptide from human IFN-α at the N-terminus (designated ‘IFNsp-mVenus’). Both c-mVenus and IFNsp-mVenus were cloned downstream of a doxycycline-inducible promoter. Expression of c-mVenus in HEK cells revealed a fluorescence pattern that was diffusely present in the cytoplasm, as expected, including in lamellipodia. IFNsp-mVenus displayed a more restricted fluorescence pattern, highlighting the endoplasmic reticulum (ER) throughout the cell (figure 3A and online supplemental videos 5; 6). Both c-mVenus and IFNsp-mVenus were induced in a doxycycline dose-responsive manner. IFNsp-mVenus was detected in the culture media and cell lysates, whereas expression of c-mVenus was only detectable in cell lysates (figure 3B), demonstrating that IFNsp-mVenus was actively secreted. Coexpression of IFNsp-mVenus with SCAMP5-mKate2 demonstrates abundant colocalisation of SCAMP5 and IFNsp-mVenus within the Golgi (figure 3C and online supplemental video 7). Moreover, IFNsp-mVenus, SCAMP5 and the clathrin light chain could be observed within the same vesicle (figure 3D). As clathrin is involved in both endocytic and exocytic pathways, we hypothesised that SCAMP5 is an integral component of the trafficking machinery in cells in which it is expressed and is positioned to participate in IFN-I secretion mechanisms.

Quantitative expression analysis on SCAMP5 in pDCs from genotyped donors and assays of pDC IFN-I output

We next sought to investigate the effect SCAMP5 genotypes may have on SCAMP5 expression in pDCs and to determine a potential impact on pDC function. To this end, we obtained fresh blood from homozygous carriers of the risk haplotype and non-risk haplotype tagged by rs2289583 from the Genotype and Phenotype Registry, a resource previously used into human embryonic kidney (HEK) cells. SCAMP5-mKate2 localised mainly to the Golgi body, with puncta observed at the cell periphery (figure 2).
Genetics

in genotype-phenotype investigations in autoimmune disease (online supplemental tables S1 and S2). 19–21

For expression quantitative trait locus (eQTL) analysis, quantitative RT-PCR for SCAMP5 and the neighbouring genes, CSK, COX5A, RPP25 and PPCDC, was performed. No significant differences in mRNA levels were seen for SCAMP5, COX5A, RPP25 or PPCDC (figure 4A and online supplemental figure S3).

Nominal evidence of an eQTL effect was observed for CSK, with elevated CSK transcript in the risk haplotype when normalised to 18s rRNA or IRF7 (online supplemental figure S3). To explore whether the risk haplotype polymorphisms might exert their effect at the level of translation, SCAMP5 protein was measured in pDC lysates. Median SCAMP5 protein levels were increased in the risk haplotype when normalised to

Figure 3 SCAMP5 co-traffics with the IFN-α secretory pathway. Soluble c-mVenus and IFNsp-mVenus were transduced into HEK cells under the regulation of a Tet-On system. (A) Induction using doxycycline revealed that c-mVenus was diffusely expressed in the cytoplasm, whereas IFNsp-mVenus exhibited a lacy pattern characteristic of the ER. Insets represent brightfield images. (B) c-mVenus and IFNsp-mVenus were expressed in a doxycycline dose-responsive manner as determined by western blotting using an anti-GFP antibody. c-mVenus was restricted to intracellular expression, whereas IFNsp-mVenus was detected in cell lysates as well as the culture media. (C) Coexpression of IFNsp-mVenus and SCAMP5-mKate2 reveals colocalisation of SCAMP5 with the IFN-α secretory pathway. (D) SCAMP5 colocalises with IFNsp-mVenus and clathrin in the Golgi apparatus as well as in the vesicles on the cell surface. Magnification of a single vesicle (white box) reveals SCAMP5 and clathrin coating the surface of the vesicle, with IFNsp-mVenus occupying the lumen. (A and C) 63× with oil immersion; (D) 100× with oil immersion. NucBlue delineates the nucleus. See online supplemental material for fluorescent protein designs. Correlate with online supplemental videos 5–7. BF, brightfield; ER, endoplasmic reticulum; GFP, green-fluorescent protein; HEK, human embryonic kidney; IFN, interferon.
As some risk variants only manifest endophenotypic effects upon cell stimulation (response eQTL, reQTL), we next assessed for a phenotypic difference between genotypes at the functional level. Culture media from pDCs stimulated with influenza H1N1, imiquimod or ODN-2216 for 4 and 24 hours were assayed for pan-subtype IFN-α. When comparing total results from donors for either haplotype, IFN output trended towards lower values for pDCs from the risk group (figure 5A). This difference reached statistical significance only for influenza virus stimulation at the earlier time point.

To determine whether the expression of SCAMP5 in pDCs was influenced by stimulation with influenza virus in a genotype-dependent manner, potentially explaining the kinetic differences in IFN-α production seen above, we assayed for SCAMP5 expression and observed that SCAMP5 is downregulated on stimulation with influenza virus by 4 hours, with no significant differences by genotype (figure 5B). Moreover, there were no consistent differences in IFN-α transcript induction in this condition between the genotypes (figure 5B).

Taken together, these findings suggest, but do not prove, that the SCAMP5 risk haplotype may be related to a delay in IFN protein production or secretion in the early period after viral infection.

Identification of novel SCAMP5 splice variants in pDCs

To address the possibility that the SCAMP5 risk haplotype influences pDC-specific SCAMP5 isoform expression not detected by our PCR and protein assays, we recalled an

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**Figure 4** Comparative SCAMP5 expression in pDCs from donors homozygous for the risk or non-risk haplotypes about rs2289583. (A) Quantitative RT-PCR for SCAMP5 transcript from pDCs normalised individually to a highly expressed housekeeping gene (18s rRNA), a moderately expressed housekeeping gene (GAPDH) and a pDC-specific housekeeping gene (IRF7, to account for small differences in pDC purity). (B) Quantitative determination of SCAMP5 protein-level expression by western blotting normalising individually to either actin or golgin-97. Comparisons between genotypes by Mann-Whitney test. ns, not significant; pDCs, plasmacytoid dendritic cells; RT-PCR, reverse transcription PCR.

**Figure 5** Comparative regulation of IFN-α and SCAMP5 in pDCs from donors homozygous for the risk or non-risk haplotypes about rs2289583 in response to various stimuli. (A) Isolated pDCs were stimulated individually using influenza virus H1N1, imiquimod or ODN-2216 for 4 and 24 hours on separate stimulation plates per time point. Culture media were assayed for IFN-α using a pan-subtype ELISA. Each data point represents biological triplicates of 3000 pDCs per condition. Lines connect paired non-risk/risk pDCs collected and assayed on the same day. (B) Quantitative RT-PCR for SCAMP5, IFNA2, IFNA10 and IFNB1 from pDCs cultured for 4 hours with influenza H1N1. Data were normalised to GUSB expression and compared with paired samples from unstimulated (unstim) wells at 4 hours by the ΔΔCt method. IFN, interferon; ns, not significant; pDCs, plasmacytoid dendritic cells; RT-PCR, reverse transcription PCR.
DISCUSSION

We have investigated a novel candidate SLE risk gene, SCAMP5, located on chromosome 15q24. An intronic variant in CSK, ~155 kb distant from SCAMP5, was previously reported to be associated with SLE by our group and others.\(^\text{11,16}\) However, as shown in figure 1, a more detailed analysis of this region in a larger data set has suggested the presence of an independent genetic effect located within the SCAMP5 gene.

SCAMP5 is a member of the SCAMP gene family and is specifically expressed in neuronal cells of the central nervous system. Interestingly, SCAMP5 is present in the neurohypophysis, derived from neuroectoderm, and is absent in the adenohypophysis, derived from Rathke’s pouch, implying tight ontogenic control over SCAMP5 expression.\(^\text{13}\) In cells of the immune system, we found that SCAMP5 is uniquely and highly expressed in pDCs, a rare cell type with the capacity to produce large amounts of IFN-I. Given the importance of IFN-I in SLE, and the large body of literature attributing pDCs as a major source of IFN-I in SLE, we pursued further analysis of SCAMP5 function in pDCs.

SCAMP5 is highly conserved. Rare copy number variations in SCAMP5 can lead to severe neurological phenotypes in humans and mice, while coding-variant variants in SCAMP5 are extremely rare.\(^\text{25-26}\) Therefore, we expected that a pDC-specific regulatory variant acting in pDCs underlies the association with SLE. In neuronal cells, the SCAMP family of proteins has been implicated in various roles in intracellular vesicular trafficking, including cargo sorting, endocytosis, and exocytosis. SCAMP5 was shown to be necessary for the sustained release of neurotransmitter-preloaded synaptic vesicles in response to tonic stimulation. In the absence of SCAMP5, membrane recycling pathways were disrupted, leading to the depletion of the synaptic vesicle reserve pool and neurons refractory to further stimulation.\(^\text{28}\) Elsewhere, SCAMP5 has been reported to facilitate the export of signal-peptide containing cytokines, whereas cytokines exported via Golgi-independent pathways were unaffected by SCAMP5 expression.\(^\text{29}\) Taken together, a highly specialised role for SCAMP5 in protein trafficking/membrane recycling seems likely.

pDCs are noteworthy for their capacity to secrete tremendous amounts of protein, including all members of the IFN-I family (with the exception of IFN-α). This undoubtedly requires efficient mechanisms for protein packaging and subsequent export. IFN-I is secreted along the classical pathway: a signal sequence directs nascent polypeptide chains to the ER, where the protein is translated directly into the ER lumen. Proteins destined for export are then transported to the Golgi apparatus, where sequential protein processing and sorting take place en route to the cell surface via protein-loaded vesicles.\(^\text{30}\) Using IFNsp-mVenus as a traceable surrogate for IFN-I secretion, we have observed that the signal peptide from IFN-α indeed directs protein synthesis to the ER, with later presence in the Golgi. We have also observed that SCAMP5 localises predominantly to the Golgi, with puncta present on the cell surface which traffic to the Golgi in a bidirectional fashion. With these observations, we hypothesised that SCAMP5 licenses the secretary capacity of pDCs by continuous replenishment of Golgi membrane lost to exocytosis on the cell surface. While it stands to reason that any protein destined for secretion via the Golgi may be affected by this process, we focused on IFN-α secondary to its association with SLE and its relative abundance versus other pDC-derived cytokines.

To test our hypotheses, we isolated pDCs to high purity from female donors of childbearing age homozygous for risk or non-risk haplotypes about rs2289583. pDCs from these donors were used in expression-level and functional assays. SCAMP5 expression was similar between the genotypes in unperturbed pDCs, ruling out a baseline eQTL. At the protein level, SCAMP5 had a median higher expression in the risk group when normalised to a Golgi membrane protein loading control; however, the magnitude of this difference and the limited number of samples precluded statistical significance. The possibility that SCAMP5 is differentially regulated between the genotypes upon stimulation was not supported by our reQTL analysis. Finally, whether a novel SCAMP5 isoform exists in pDCs and explains the association with SLE was explored using RNAseq. Novel putative isoforms of SCAMP5 in pDCs were detected; however, they did not differ in expression levels between the genotypes.

The expression of neighbouring genes to either side of SCAMP5 was likewise similar, with the exception of CSK, which exhibits a minor (<1.24-fold) yet statistically significant increase in expression in the risk group. This may reflect the small residual LD between rs2289583 and the previously identified independent CSK risk haplotype, which has been associated with increased CSK expression in B cells. However, we note that CSK expression in pDCs is low in comparison with B cells. Thus, to the extent that we can identify pDC phenotypes regulated by rs2289583, SCAMP5 remained a leading candidate gene to explore.

Functionally, pDCs from risk donors produce less IFN-α on average compared with non-risk, when stimulated with imiquimod, ODN-2216 or influenza virus at 4 or 24 hours of stimulation. These differences were more pronounced with imiquimod and influenza virus—both TL7 agonists—and at the earlier time point. These data run counter to the expectation that SLE risk variants in...
the IFN pathway increase IFN production. With the exception of SLE variants in STAT4 which increase sensitivity to signalling through the IFN-α/β receptor, resulting in decreased serum IFN activity,\(^3\) most IFN-related SLE risk variants indeed confer a greater predilection for IFN production.\(^3\) Statistically significant differences in these studies usually involve dozens or hundreds of representatives of each genotype, and IFN presence is often inferred through biological activity assays rather than direct IFN measurements as performed here.

Our observations raise several possibilities. The IFN production defect is more pronounced at 4 hours than at 24 hours, suggesting that a kinetic trait in IFN export may be at play. Rapid production of IFN is critical for antiviral defence. As viral prodromes often precede onset of SLE symptoms, it may be the case that enhanced susceptibility to viral infection through slower IFN secretion may induce SLE in an otherwise susceptible host. Alternatively, the observed IFN secretion defect may represent an incidental finding unrelated to SCAMP5’s role in pDCs. That SCAMP5 colocalises with the IFN-α secretory pathway does not necessarily indicate an interaction between the two. CRISPR knockout studies in primary pDCs may be necessary for establishing a definitive link between SCAMP5 and IFN secretion. We have successfully used CRISPR to knock out BDCA4 expression in primary human pDCs (online supplemental figure S5); however, knockout of SCAMP5 has proven elusive, possibly due to longer half-life of this protein in these short-lived cells.

Separately, we have investigated human pDC responses to influenza virus by secretome profiling using liquid chromatography tandem mass spectrometry and at the single pDC level using single cell RNAseq (Ghanem MH et al, 2021, manuscript in preparation). There, we observed that SCAMP5 is widely expressed in pDCs immediately ex vivo, but is downregulated upon stimulation, implying a role of SCAMP5 that is distinct from ongoing IFN export. Moreover, we observed evidence for granzyme B release from these cells via a mechanism consistent with degranulation rather than the classical secretory pathway. A role of SCAMP5 in this process would bear more similarity to synaptic vesicle release from neuronal cells where the function of SCAMP5 is more thoroughly described. These possibilities are being addressed by ongoing investigation. This work marks the first description of a role of SCAMP5 in pDC biology and IFN secretion; much remains to be explored.

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Contributors MHG, SJK, KRS and PKG conceived and designed the study. MHG, HV and LNC collected the experimental data. MHG and KRS analysed the experimental data. AS performed the RNAseq analysis. WL performed the statistical genetics analysis. MHG, KRS and PKG wrote the manuscript. PKG was the principal investigator on the study and serves as the guarantor of this work. All authors revised and approved the final manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The Genotype and Phenotype (GaP) Registry at the Feinstein Institutes for Medical Research provided de-identified blood from donors consented under an IRB-approved protocol (IRB# 09-081). The Committee for Participant Protection at the Feinstein Institutes for Medical Research approved this study (TA0307.5.73). The GaP is a subprotocol of the Tissue Donation Program (TDP) at Northwell Health and a national resource for genotype–phenotype studies (https://www.feinsteininstitute.org/robert-sboa-center-for-genomics-and-human-genetics/gap-registry/).

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Supplementary materials for:

Investigations into SCAMP5, a candidate lupus risk gene expressed in plasmacytoid dendritic cells

By Ghanem et al.
**Materials and Methods**

**Human blood donors**

The Genotype and Phenotype (GaP) Registry at The Feinstein Institutes for Medical Research provided de-identified blood from donors consented under an IRB-approved protocol (IRB# 09-081). The Committee for Participant Protection at The Feinstein Institutes for Medical Research approved this study (TAP0307.5.73). The GaP is a sub-protocol of the Tissue Donation Program (TDP) at Northwell Health and a national resource for genotype-phenotype studies (https://www.feinsteininstitute.org/robert-sboas-center-for-genomics-and-human-genetics/gap-registry/). For experiments comparing genotypes, subjects were selected on the basis of genotype from the Global Screening Array (GSA).

**Patient and Public Involvement**

Patients in our studies are actively interested in advancing knowledge of autoimmunity, even when they participate as controls in protocols such as the GaP. Thus, a Community Advisory Board comprised of diverse (ethnicity, age and gender) community members and study participants are consulted quarterly, and their input is integrated into the GaP study. Patients have not been involved in the design of the experimental work. There were no interventions in this study. The results will be communicated via publication and newsletters to participants.

**FACS analysis and cell sorting**

Fresh whole blood was drawn from healthy donors into sodium-heparin vacutainers (BD). PBMCs were extracted over Ficoll-Paque (GE Healthcare) using standard methods. PBMCs were then labeled as follows for cell sorting: CD19, CD3, CD4, CD8, CD56, CD14, CD16, CD304, CD303, CD11c, HLA-DR. Lymphocytes were sorted by gating on the lymphocyte population on light scatter: B cells (CD19+CD3-CD56-); CD4 T cells (CD3+CD4+); CD8 T cells (CD3+CD8+); NK cells (CD56+CD3-CD19-). Monocytes were sorted by gating on the monocyte population on light scatter: CD14 classical monocytes (CD14+CD16-); CD16 non-classical monocytes (CD16+CD14-). Dendritic cells were sorted by gating on the lineage negative population (CD19-CD3-CD56-CD16-): pDCs (CD304+CD303+); cDCs (HLA-DR+CD11c+). Cells were sorted into microfuge tubes on the BD FACSAria SORP cell sorter. Sorted cells were immediately pelleted by centrifugation and frozen dry at -80°C until RNA extraction for RT-PCR or lysate preparation for western blotting.

For intracellular staining of SCAMP5 and flow cytometric analysis, PBMCs were surface stained for CD304 (BDCA-4) followed by fixation and permeabilization using BD Cytofix/CytoPerm. Cells were then kept in BD Perm/Wash and stained for SCAMP5 using a rabbit polyclonal antibody (PAS-61269, ThermoFisher Scientific) followed by staining using anti-rabbit AF488. Cells were acquired using the BD Fortessa flow cytometer.
**mRNA Expression**

RNA was prepared from frozen cell pellets using the Qiagen RNeasy Micro Plus kit (Qiagen 74034). RNA was immediately reverse transcribed using Superscript IV VILO master mix at 50°C for 10 minutes (Invitrogen 11756050), or the SuperScript IV VILO Master Mix 'No RT' Control, using the Applied Biosystems Veriti thermal cycler. cDNA was then diluted in TE buffer and stored at -20°C pending further analysis. qPCR was performed using Taqman assays (ThermoFisher Scientific) and Taqman Gene Expression Master Mix (Applied Biosystems 4369016). qPCR reactions were performed on the Applied Biosystems Viia7 thermal cycler.

**Protein expression studies**

Lysates were prepared by resuspending cell pellets in 1X CHAPS lysis buffer supplemented with 1X HALT protease inhibitor (Thermo Scientific 78429). Lysates were incubated on ice for 30 minutes with periodic vortexing. LDS sample buffer was added to a final concentration of 1X and β-mercaptoethanol to a final concentration of 5%. Lysates were then directly loaded into a 10% Bis-Tris gel without boiling, as heating of lysates was found to aggregate SCAMP5 protein. Lysates were resolved at 200V for 45 minutes using 1X Novex BOLT running buffer. Gels were then equilibrated in 1X Novex Transfer buffer/20% methanol, followed by transfer onto 0.45uM PVDF membranes at 100V/350mA for 25 minutes. Blots were blocked for 60 minutes at room temperature using Licor Odyssey Blocking Buffer diluted 1:1 in PBS. Primary anti-SCAMP5 rabbit polyclonal antibody (PA5-61269) was diluted in staining buffer (Licor blocking buffer diluted 1:5 in PBS) at a dilution of 1:1,000 overnight at 4°C. After 3 washes in PBS Tween-20, secondary anti-rabbit HRP was added at a dilution of 1:10,000 in staining buffer for 2 hours at room temperature. After another 2 rounds of washing in PBS-T and a final wash in PBS, the blot was developed using chemiluminescent substrate and acquired using the BioRad Chemidoc Imager. The blot was reprobed for actin (Ab clone AC-15) as described for SCAMP5.

**Confocal microscopy of pDCs**

For confocal analysis of SCAMP5 in pDCs, pDCs were isolated from PBMCs by immunomagnetic negative selection. Freshly isolated pDCs were cytopspun onto charged glass slides. Cells were fixed and permeabilized by immersion in BD Cytofix/Cytoperm, and simultaneously stained for SCAMP5 (rabbit polyclonal antibody PA5-61269) and Golgin-97 (ThermoFisher A-21270). A pre-immune rabbit polyclonal antibody was used as a negative control for SCAMP5 staining, (no staining was observed, data not shown). Cells were washed in BD Perm/Wash and stained using anti-rabbit AF488 and anti-mouse AF647. No. 1 coverslips were mounted using Prolong Antifade Glass with NucBlue and sealed using clear nail polish. After curing at 4°C for 30 minutes, images were acquired using the Zeiss LSM 880 confocal microscope.

**Genomic analysis of the SLE association polymorphism rs2289583**

Phased haplotypes were built from genotypes of rs2472300, rs6495122 and rs2289583 for 6,959 controls and 4,036 lupus cases (totaling 10,995 subjects and 21,990 haplotypes). Plink binary files were converted to a vcf file with plink2. Variants were phased using beagle 5.0 (v2018) using the hg37 genetic map. P values and odds ratios were calculated by comparing counts of each haplotype to the sum of other haplotypes.
Linkage disequilibrium (LD) statistics between SNPs were obtained using the ‘LDpair Tool’ on LD Link (https://ldlink.nci.nih.gov/?tab=ldpair) (Machiela and Chanock, 2015). LD statistics were derived from the European population.

Conditional analysis was carried out by multi-variable logistic regression, with the SNP to be conditioned as one of the independent variables, and phenotype being the dependent variable. The coefficients and the corresponding p-values for other SNPs, being other independent variables, are the association results conditional on the SNP to be conditioned.

**Phenotyping of GaP Subjects on the basis of SCAMP5 risk haplotype**

The GaP Registry is a cohort of ~7,500 (and counting) genotyped healthy individuals who have agreed to recall on the basis of their genotype. The GaP has been previously utilized for the study of endophenotypes regulated by various autoimmune risk variants and is available to the scientific community. To assay for pDC phenotypes related to genotype at rs2289583, we chose from among this cohort. As the relationship of the causal polymorphism to rs2289583 has not yet been established, we sought to enrich for likely carriers of the causal allele by constructing SLE risk haplotypes inclusive of rs2289583. rs6495122 and rs2472300 are the next most significantly associated SNPs in the region and together along with rs2289583 they denote a risk haplotype.

Female subjects of childbearing age (here stipulated 18-40) homozygous for the risk or non-risk haplotypes were contacted for recall (subject characteristics in Table S2). 60CC whole blood was drawn from each donor into sodium-heparin vacutainers. Subjects were drawn in pairs, with one homozygous risk and one homozygous non-risk donor per day. Altogether, 9 risk:non-risk pairs were collected, with an additional 3 non-risk:non-risk pairs, over 12 discontinuous days of sampling.

Whole blood lysates were genotyped to confirm the fidelity of the recalled subject genotypes. Taqman genotyping was performed for rs2289583 on crude whole blood lysates: Frozen aliquots of whole blood were thawed and prepared for PCR using DNA Extract All Reagents Kit (Applied Biosystems 4403319). PCR was performed using a custom Taqman genotyping assay (assay ID: C__15881999_10) and TaqPath ProAMP Master Mix (Applied Biosystems A30865), with the VIC probe detecting the minor allele (A=risk) and the FAM probe detecting the major allele (C=non-risk). Genotypes were called on the Viia7 Real-Time PCR System (Applied Biosystems).

PBMCs were extracted over Ficoll-Paque using established methods. A small aliquot of PBMCs was kept for determination of baseline pDC frequency. pDCs were isolated using Miltenyi Biotec Human Plasmacytoid Dendritic Cell Isolation Kit II (130-097-415) in accordance with the manufacturer’s protocol. A small aliquot of isolated pDCs was used for purity determination by staining for BDCA4 and BDCA2 (Figure S6).

For pDC stimulation cultures, 3,000 pDCs were cultured per well of a round-bottom 96-well plate using Advanced RPMI (Gibco 12633012) with 5% FCS. Triplicate cultures were established per condition: no stimulation, imiquimod 4μg/mL (Invivogen), ODN-2216 2μM (Invivogen), or influenza virus 0.4HAU/mL (A/PR/8/34 H1N1, Advanced Biotechnologies Inc.).
RNA preparation, cDNA synthesis, qPCR, and western blotting on pDC pellets were performed as described above. IFN-α ELISAs were performed using MABTECH 3425-1H-6 in accordance with the manufacturer’s protocol. QTL and ELISA experiments were performed in a single batch after all GaP donors had been collected.

Taqmas assays for eQTL experiments are as follows:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Assay ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCAMP5</td>
<td>Hs01547727_m1</td>
</tr>
<tr>
<td>PPCDC</td>
<td>Hs00222418_m1</td>
</tr>
<tr>
<td>RPP25</td>
<td>Hs00706565_s1</td>
</tr>
<tr>
<td>CSK</td>
<td>Hs01062581_m1</td>
</tr>
<tr>
<td>COX5A</td>
<td>Hs00362067_m1</td>
</tr>
<tr>
<td>18S rRNA</td>
<td>Hs03003631_g1</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Hs02786624_g1</td>
</tr>
<tr>
<td>IRF7</td>
<td>Hs01014809_g1</td>
</tr>
<tr>
<td>POLR2A</td>
<td>Hs00172187_m1</td>
</tr>
<tr>
<td>IFNB1</td>
<td>Hs01077958_s1</td>
</tr>
<tr>
<td>IFNA2</td>
<td>Hs00265051_s1</td>
</tr>
<tr>
<td>IFNA10</td>
<td>Hs03406429_gH</td>
</tr>
<tr>
<td>GUSB</td>
<td>Hs00939627_m1</td>
</tr>
</tbody>
</table>

Taqman assays for intron-inclusion transcripts were custom generated by ThermoFisher and are described in the table below:

<table>
<thead>
<tr>
<th>Intron interval name</th>
<th>qPCR assay ID</th>
<th>Chromosome 15 (Hg38)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start position</td>
<td>End position</td>
</tr>
<tr>
<td>1-1</td>
<td>7,499,567</td>
<td>74,995,966</td>
</tr>
<tr>
<td>1-2</td>
<td>7,499,632</td>
<td>74,997,233</td>
</tr>
<tr>
<td>1-3</td>
<td>7,499,731</td>
<td>75,007,587</td>
</tr>
<tr>
<td>1-4</td>
<td>7,500,772</td>
<td>75,009,890</td>
</tr>
<tr>
<td>1-5</td>
<td>7,500,994</td>
<td>75,011,657</td>
</tr>
<tr>
<td>1-6</td>
<td>APEPXWT</td>
<td>75,011,791</td>
</tr>
<tr>
<td>2-1</td>
<td>7,501,184</td>
<td>75,012,676</td>
</tr>
<tr>
<td>3-1</td>
<td>APFVTGP</td>
<td>75,016,592</td>
</tr>
<tr>
<td>4-1</td>
<td>APGZK2M</td>
<td>75,017,652</td>
</tr>
<tr>
<td>5-1</td>
<td>7,501,797</td>
<td>75,018,393</td>
</tr>
<tr>
<td>6-1</td>
<td>7,501,853</td>
<td>75,018,788</td>
</tr>
</tbody>
</table>
Design of lentiviral expression constructs

SCAMP5-mKate2, c-mVenus, IFNsp-mVenus and mTagBFP2-clathrin were ordered as gene blocks from IDT. SCAMP5-mKate2 was cloned into the lentiviral backbone vector pLJM1-eGFP (Addgene #19319) using NheI and BstBI in place of eGFP. c-mVenus and IFNsp-mVenus were first cloned into the Gateway entry vector pENTR4 using NcoI and XhoI followed by recombination into the lentiviral Gateway destination vector pLenti CMVtight Puro DEST (Addgene #26430) using LR Clonase II. A lentiviral backbone vector expressing rtTA3 was obtained separately (Addgene #26429).

Lentiviral vectors were produced by co-transfection of lentiviral backbone vectors along with a lentiviral packaging mix (Invitrogen A43237) carrying the Rev, VSV-G and Gag/Pol genes using Lipofectamine 3000 into HEK 293T cells. Lentiviral media was collected 72 hours post-transfection, cleared through 0.22μM syringe filters and stored at -80°C.

Transduction of HEK-293T cells with lentiviral particles

For SCAMP5-mKate2 expression, SCAMP5-mKate2 lentiviral particles were transduced into HEK-293T cells. Cells expressing mKate2 were sorted on the BD FACSAria and maintained in culture. For c-mVenus and IFNsp-mVenus expression, the respective lentiviral particles were co-transduced with rtTA3 into HEK-293T cells previously transduced with SCAMP5-mKate2, to generate cells constitutively expressing SCAMP5-mKate2 and either mVenus or IFNsp-mVenus in a doxycycline inducible system.

Live-cell confocal microscopy

HEK-293T cells expressing the desired transgenes were seeded onto 35mm poly-D-lysine coated dishes with No. 1 coverslip center pieces. Prior to imaging, the media was aspirated and replaced with FluoroBrite DMEM Media containing 10% FBS, with or without NucBlue for counterstaining of nuclei. Images were acquired on a Zeiss LSM880 Confocal microscope.

pDC total RNAseq

pDCs were isolated from 4 homozygous risk and 5 homozygous non-risk donors as described above. Yield and purities are presented in the table below. pDC pellets were immediately stored at -80°C. RNA extraction was performed using Qiagen RNeasy Micro Plus kit (Qiagen 74034). Sequencing libraries were prepared with normalized inputs using TruSeq RNA Single Indexes Set A (Illumina Cat. # 20020492). Paired end sequencing reactions were performed on the Illumina NextSeq 500 sequencer using NextSeq 500/550 High Output Kit v2.5 (150 Cycles) (Illumina Cat. #20024907). FastQC was used to insure there was no adapter contamination and reads had acceptable quality scores. Intronic retention was quantified using iREAD to the Gencode grrch38 reference assembly.
<table>
<thead>
<tr>
<th>Subject Genotype</th>
<th>pDC yield</th>
<th>pDC Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-risk / Risk</td>
<td>#</td>
<td>%Percent</td>
</tr>
<tr>
<td>3538</td>
<td>435,000</td>
<td>95.8</td>
</tr>
<tr>
<td>4752</td>
<td>61,272</td>
<td>67.4</td>
</tr>
<tr>
<td>5050</td>
<td>569,000</td>
<td>96.1</td>
</tr>
<tr>
<td>7406</td>
<td>217,000</td>
<td>94.1</td>
</tr>
<tr>
<td>2727</td>
<td>132,000</td>
<td>95.3</td>
</tr>
<tr>
<td>8640</td>
<td>567,000</td>
<td>96.5</td>
</tr>
<tr>
<td>8461</td>
<td>256,200</td>
<td>84.2</td>
</tr>
<tr>
<td>4855</td>
<td>229,000</td>
<td>81.2</td>
</tr>
<tr>
<td>8123</td>
<td>374,900</td>
<td>84.2</td>
</tr>
</tbody>
</table>

**Data and Statistical Analyses**

Unless otherwise indicated, raw data was tabulated in Microsoft Excel. ELISA standard curve interpolation, graph generation and statistical analyses were performed using GraphPad Prism. Flow cytometry data was analyzed using FlowJo. Confocal images were analyzed using ZenBlue. In all figures, numerical values are indicated for p-values <0.2, all other p-values denoted as *not significant.*
**Fusion protein constructs**

**Scamp5-mKate2 protein sequence:**

```
MAEKVNNFPFPLPKPCFYQDFEADIPPQHLSLTKRLYYLWMLNSVTLAVNLVGCLAWLIGGGGATNFGLAFLWLILFTPCSYVCWF
RPIYKAKFDSFFSFAFFFTMAAQLVIIIAVQVIPIGWGCWGIATSSFFGTNIGSAVM/MIPTV/MTFVAVFIALSMVHKFNYRGSGG
SFSAQEEWTTGAKPNHPQAAQNAAMAAGQAAMNPQTQSYATPNYSTNEMGGGPGGGGSVELIKENMMKLYMEG
TVNHHFKCTSEGEGKPYEGTQTMRIKAVEGGLPFAFDILATSFGMYSKTFINHTQGIPDFFKQSFPEGFTWERTTTYEDGGVLTATQ
DTSLQDGCLIFNYLGVNFSNGPVMQKKTGLGWEASTETLYPADGGEGRADMLKLVGGHGLCNKTTYRSKKPAKNLMPGVYY
VDARRLERIKEADKETYVEQHEVAVARYCDLP3SKGLHR
```

**SCAMP5 sequence**

**GI linker**

**mKate2 sequence**

**mVenus protein sequences:**

**Sequence of mVenus with human IFN-α leader peptide [human codon-optimized]:**

```
MALSFSMLAVLVSYSICLSGCDLPVSKGEELFTGVPIVPELDGVNGHKFSVSGEGDATYKLKLCTTGKLPVPWPTLVTTLG
YGLQCFAVYPDHMKQDHDFFKSAMPEGVQERTIKFDGNYKTRAEVKFEGDTLVNRIELKIDFKEGDNGLHGKLEYNYNSHVYITA
DKQKNGIKANFKIRHNIEDGGVQLADHYQQTIGDGPVLLPDHLYYSQQSKLSDKPNEKRDMVLMLEFVTAAGITLGMDELYK
```

**Sequence of untagged mVenus:**

```
MVSKGEELFTGVPIVPELDGVNGHKFSVSGEGDATYKLKLCTTGKLPVPWPTLVTTLGYYGLQCFARYPDHMKQDHDFSASA
MPEGVQERTIFKDKGNYKTRAEVKFEGDTLVNRIELKIDFKEDGDNLHGKLEYNYNSHVYITADKQKNGIKANFKIRHNIEDGGVQ
LADHYQQTPIGDGPVLLPDHLYYSQQSKLSDKPNEKRDMVLMLEFVTAAGITLGMDELYK
```

**IFN-α leader peptide**

**mVenus**

**mTagBFP2-Clathrin protein sequence:**

```
MVSKGEELKIKENMMKLYMEGTVNDHNFKCTSEGEGKPYEGTQTMRIKAVEGGLPFAFDILATSLFYSGSKTFINHTQGIPDFFKQSFPP
EGFTWERTTTYEDDDGVTATQDTSLQDGCLIFNYLGVNFSNGPVMQKKTGLGWEASTETLYPADGGEGRADMLKLVGGHGLCNKTTYRSKKPAKNLMPGVYY
VDARRLERIKEADKETYVEQHEVAVARYCDLP3SKGLHR
```

**mTagBFP2 sequence**

**Linker peptide**

**Clathrin light chain B isoform A**
Supplementary figures

**Figure S1.** Schematic of SCAMPS topology. Generated using BioRender.
Figure S2.

A) **SCAMP5** expression across human tissues determined using microarray. (BioGPS)

[http://biogps.org/#goto=genereport&id=192683](http://biogps.org/#goto=genereport&id=192683)

B) **SCAMP5** expression across circulating human leukocyte subsets as determined by RNA-seq. (Human Protein Atlas)

[https://www.proteinatlas.org/ENSG00000198794-SCAMP5/blood](https://www.proteinatlas.org/ENSG00000198794-SCAMP5/blood)
Figure S3. Comparative expression in pDCs of genes neighboring SCAMP5 from donors homozygous for the risk or non-risk haplotypes about rs2289583. Quantitative RT-PCR for CSK, COX5A, RPP25 and PPCDC transcript from pDCs normalized individually to 18s rRNA, GAPDH and IRF7. Comparisons between genotypes by Mann-Whitney test. ns= not significant.
**Figure S4. Expression of SCAMP5 intron sequences.**  
A) A schematic diagram of the SCAMP5 gene (ENST00000425597) shows the location of the reported expressed intronic segments, and the identifier assigned to each.  
B) RNAseq FPKM values of SCAMP5 intronic segments.  
C) Relative expression of the three mostly highly expressed SCAMP5 intron segments, relative to total expression of SCAMP5 (as measured by quantitative RT-PCR assay spanning the exon 2 to exon 3 boundaries of intron 2 of the SCAMP5 gene). The ‘No RT’ control cDNAs were also included and no amplification was observed for any assay within the 40 cycles tested, indicating that the intron expression measured here was from mRNA and not amplification of contaminating genomic DNA. Each data point represents one subject (for qRT-PCR each data point represents the mean of technical triplicates). Bars show the median expression values.
Figure S5. BDCA4 KO using CRISPR in primary human pDCs. pDCs were isolated to near purity and electroporated with Cas9 particles complexed with non-targeting or BDCA4 gRNA A or B. Cells were cultured for 72hrs and stained for viability and BDCA4 expression prior to acquisition by flow cytometry.
**Figure S6.** Representative pre- and post-isolation purity of pDCs from recalled GaP subjects.
### Table S1. Construction of an SLE risk haplotype about rs2289583.

<table>
<thead>
<tr>
<th></th>
<th>Number of subjects</th>
<th>Frequency</th>
<th>P value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>SLE</td>
<td>Controls</td>
<td>SLE</td>
</tr>
<tr>
<td><strong>Non-risk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G C C</td>
<td>6,324</td>
<td>3,404</td>
<td>45.4%</td>
<td>42.2%</td>
</tr>
<tr>
<td>A A A</td>
<td>2,508</td>
<td>1,645</td>
<td>18.0%</td>
<td>20.4%</td>
</tr>
<tr>
<td>G A C</td>
<td>1,953</td>
<td>1,137</td>
<td>14.0%</td>
<td>14.1%</td>
</tr>
<tr>
<td>A A C</td>
<td>1,286</td>
<td>780</td>
<td>9.2%</td>
<td>9.7%</td>
</tr>
<tr>
<td>G C A</td>
<td>957</td>
<td>548</td>
<td>6.9%</td>
<td>6.8%</td>
</tr>
<tr>
<td>G A A</td>
<td>545</td>
<td>355</td>
<td>3.9%</td>
<td>4.4%</td>
</tr>
<tr>
<td>A C C</td>
<td>300</td>
<td>174</td>
<td>2.2%</td>
<td>2.2%</td>
</tr>
<tr>
<td>A C A</td>
<td>45</td>
<td>29</td>
<td>0.3%</td>
<td>0.4%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>13,918</td>
<td>8,072</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table S2. Characteristics of GaP subjects recalled for pDC isolation.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex Assigned at Birth</th>
<th>Race</th>
<th>Medical Conditions</th>
<th>SCAMP5 Haplotype</th>
<th>pDC Frequency Pre-Enrichment</th>
<th>pDC yield</th>
<th>pDC purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>3333</td>
<td>Female</td>
<td>Caucasian</td>
<td>None</td>
<td>Non-Risk</td>
<td>0.60%</td>
<td>4.55E+05</td>
<td>97.00%</td>
</tr>
<tr>
<td>3538</td>
<td>Female</td>
<td>Caucasian</td>
<td>Hypothyroidism</td>
<td>Non-Risk</td>
<td>0.40%</td>
<td>3.18E+05</td>
<td>97.70%</td>
</tr>
<tr>
<td>4315</td>
<td>Female</td>
<td>Caucasian</td>
<td>None</td>
<td>Non-Risk</td>
<td>0.44%</td>
<td>2.18E+05</td>
<td>95.10%</td>
</tr>
<tr>
<td>4752</td>
<td>Female</td>
<td>Caucasian</td>
<td>Allergies</td>
<td>Non-Risk</td>
<td>0.11%</td>
<td>3.55E+04</td>
<td>86.10%</td>
</tr>
<tr>
<td>4855</td>
<td>Female</td>
<td>Caucasian</td>
<td>Eczema</td>
<td>Non-Risk</td>
<td>0.39%</td>
<td>2.35E+05</td>
<td>96.50%</td>
</tr>
<tr>
<td>4892</td>
<td>Female</td>
<td>Caucasian</td>
<td>Allergies, Asthma</td>
<td>Non-Risk</td>
<td>0.36%</td>
<td>8.50E+04</td>
<td>97.40%</td>
</tr>
<tr>
<td>5121</td>
<td>Female</td>
<td>Caucasian</td>
<td>Allergies</td>
<td>Non-Risk</td>
<td>0.75%</td>
<td>4.43E+05</td>
<td>95.00%</td>
</tr>
<tr>
<td>5135</td>
<td>Female</td>
<td>Caucasian</td>
<td>Psoriasis</td>
<td>Non-Risk</td>
<td>0.73%</td>
<td>2.10E+05</td>
<td>98.20%</td>
</tr>
<tr>
<td>5444</td>
<td>Female</td>
<td>Caucasian</td>
<td>Allergies</td>
<td>Non-Risk</td>
<td>0.44%</td>
<td>1.45E+05</td>
<td>95.80%</td>
</tr>
<tr>
<td>6845</td>
<td>Female</td>
<td>Caucasian</td>
<td>None</td>
<td>Non-Risk</td>
<td>0.64%</td>
<td>2.56E+05</td>
<td>98.60%</td>
</tr>
<tr>
<td>7101</td>
<td>Female</td>
<td>Caucasian</td>
<td>None</td>
<td>Non-Risk</td>
<td>0.78%</td>
<td>3.00E+05</td>
<td>92.70%</td>
</tr>
<tr>
<td>7153</td>
<td>Female</td>
<td>Caucasian</td>
<td>None</td>
<td>Non-Risk</td>
<td>0.13%</td>
<td>1.56E+05</td>
<td>93.20%</td>
</tr>
<tr>
<td>7170</td>
<td>Female</td>
<td>Caucasian</td>
<td>Allergies, Asthma, Arthritis</td>
<td>Non-Risk</td>
<td>0.41%</td>
<td>1.20E+05</td>
<td>94.50%</td>
</tr>
<tr>
<td>7644</td>
<td>Female</td>
<td>Caucasian</td>
<td>Arthritis, Diabetes Type 1, Psoriasis</td>
<td>Non-Risk</td>
<td>1.94%</td>
<td>5.85E+05</td>
<td>98.60%</td>
</tr>
<tr>
<td>7738</td>
<td>Female</td>
<td>Caucasian</td>
<td>Allergies, Hypothyroidism</td>
<td>Non-Risk</td>
<td>0.53%</td>
<td>1.60E+05</td>
<td>97.70%</td>
</tr>
<tr>
<td>2727</td>
<td>Female</td>
<td>Caucasian</td>
<td>None</td>
<td>Risk</td>
<td>0.25%</td>
<td>1.13E+05</td>
<td>93.70%</td>
</tr>
<tr>
<td>3425</td>
<td>Female</td>
<td>Caucasian</td>
<td>None</td>
<td>Risk</td>
<td>0.74%</td>
<td>4.20E+05</td>
<td>97.80%</td>
</tr>
<tr>
<td>5050</td>
<td>Female</td>
<td>Caucasian</td>
<td>Allergies</td>
<td>Risk</td>
<td>0.62%</td>
<td>3.13E+05</td>
<td>98.80%</td>
</tr>
<tr>
<td>5181</td>
<td>Female</td>
<td>Caucasian</td>
<td>None</td>
<td>Risk</td>
<td>0.63%</td>
<td>2.32E+05</td>
<td>97.10%</td>
</tr>
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<td>Female</td>
<td>Caucasian</td>
<td>None</td>
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<td>2.19E+05</td>
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<td>Celiac Disease</td>
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<td>9.18E+04</td>
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Videos 1-4. Intracellular trafficking of SCAMP5-bearing vesicles in transduced HEK cells.

Videos 5-7. Time-lapse imaging of c-mVenus, IFNsp-mVenus and SCAMP5 in transduced HEK cells.