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Interferon inducible X-linked gene *CXorf21* may contribute to sexual dimorphism in Systemic Lupus Erythematosus

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23 Abstract

24 Systemic lupus erythematosus (SLE) is an autoimmune disease, characterised by increased 25 expression of type I interferon (IFN) regulated genes and a striking sex imbalance towards females. Through combined genetic, in silico, in vitro, and ex vivo approaches, we define 26 27 *CXorf21*, a gene of hitherto unknown function, which escapes X-chromosome inactivation, as 28 a candidate underlying the Xp21.2 SLE association. We demonstrate that CXorf21 is an IFN-29 response gene and that the sexual dimorphism in expression is magnified by immunological 30 challenge. Fine-mapping reveals a single haplotype as a potential causal cis-eQTL for CXorf21. We propose that expression is amplified through modification of promoter and 3'-UTR 31 32 chromatin interactions. Finally, we show that the CXORF21 protein colocalises with TLR7, a 33 pathway implicated in SLE pathogenesis. Our study reveals modulation in gene expression 34 affected by the combination of two hallmarks of SLE: *CXorf21* expression increases in a both 35 an IFN-inducible and sex-specific manner.

37 Introduction

38 Females have a clear immunological advantage over males, with reduced susceptibility to 39 infection at an early age and a superior ability to produce antibodies and serum IgM following immune challenge^{1,2}. The immunological gain in females is thought to contribute to the striking 40 41 sexual dimorphism observed in human autoimmune disease - where over 80% of sufferers are female³ - and corroborates the hypothesis that genetic risk to autoimmunity is an evolutionary 42 consequence of positive selection for favourable immune responses to infection⁴. Systemic 43 lupus erythematosus (SLE), an autoimmune disease characterised by anti-nuclear 44 45 autoantibodies and a type I interferon (IFN) signature, displays one of the most striking female-46 biased imbalances (9:1) in disease prevalence. Although the underlying mechanism has yet to 47 be fully elucidated, a prominent role of X chromosome dosage is supported by the karyotypic 48 risks for SLE. Males with Klinefelter's syndrome (47, XXY) have a 14-fold increased prevalence of SLE compared to 46, XY males, which approximates to the prevalence seen in 49 46, XX females⁵. Furthermore, whereas 45, XO females have lower risk⁶, SLE prevalence in 50 51 47, XXX females is ~2.5 times higher than in 46, XX females⁷. Indeed, mammalian X 52 chromosomes, for which males are hemizygous, are enriched for immune-related genes⁸.

53

X chromosome inactivation (XCI) is a unique mammalian dosage-compensation mechanism which equalises expression of X-linked genes between sexes⁹. This random process results in either the paternally or maternally inherited X chromosome becoming inactivated (Xi) through enriched heterochromatic modifications, which promotes gene silencing to leave one transcriptionally active X chromosome (Xa) in females¹⁰. However, an estimated 15% of Xlinked genes, preferentially found on the Xp arm, escape XCI and thus display expression from both chromosomes, although typically expression is still lower from the Xi compared with

Xa¹¹. A further 10% of X-linked genes display variable expression from the Xi – an observation 61 which itself is variable between both individuals and cell types, and throughout development 62 and ageing¹². It is these XCI-escaping genes, through their partial or complete lack of dosage 63 compensation, that are thought to contribute to genetic sexual dimorphism and phenotypic 64 differences in X-chromosome aneuploidies¹³. Furthermore, the relaxation of Xi silencing in 65 female mammals includes increases in the expression of several immunity-related genes¹⁴. 66 How genes that escape XCI contribute to sexually dimorphic diseases has not been thoroughly 67 68 studied.

A SLE association at the Xp21.2 locus (rs887369; $P_{META} = 5.26 \times 10^{-10}$; OR = 1.15) was 69 recently identified in a European GWAS and replication study¹⁵. Intriguingly, this locus is 70 71 encoded outside the pseudo autosomal region (PAR) and the lead SNP (a synonymous variant) 72 resides in the final exon of *CXorf21*, a protein-coding gene of unknown function. *CXorf21* has 73 been shown to escape XCI in lymphoblastoid cell lines (LCLs), and is one of only 14 X-linked 74 genes that is differentially expressed between Klinefelter's syndrome (47, XXY) and 46, XY 75 males in LCLs^{16,17}. A recent whole-blood gene expression study also identified *CXorf21* as one of seven genes upregulated in female SLE patients displaying disease flare relative to those 76 with infection¹⁸. 77

78

Despite the stark karyotypic risk, there remains a lack of understanding of the contribution of the X chromosome to SLE, which is a leading cause of death in females aged under 34 years of age¹⁹. Here we describe fine-mapping and characterisation of the association at Xp21.2 through complementary genetic, *in silico, in vitro* and *ex vivo* approaches using both existing and newly generated data (all methods are summarised as a flow chart in Supplementary Fig. 1). We demonstrate that the candidate gene, *CXorf21*, is an IFN-responder with both cell-type specific and sexually dimorphic expression amplified by cellular activation. Additionally, we provide 4

86	evidence at	the p	orotei	n-level	of C	XORF21	co-lo	calisa	tion wi	th TLR7; a ge	ene causatively
87	linked to S	SLE	and	which	also	evades	XCI.	Our	study	demonstrates	IFN-inducible
88	magnificatio	on of s	sexua	l dimor	phic g	gene expi	ression	contr	ibuting	to SLE risk.	

90 **Results**

91 Genetic refinement of the Xp21.2 SLE susceptibly locus

92 The source of all cohorts used within this manuscript along with the analyses performed are presented as a flow diagram in Supplementary Fig. 1. The UK10K-1000 Genomes Project 93 Phase 3 reference panel²⁰ was firstly used to impute the Xp21.2 locus of the Bentham and 94 Morris et al SLE GWAS (10,995 individuals of European ancestry)¹⁵. Logistic regression 95 96 revealed a synonymous variant, rs887369 (MAF = 0.24), to be the most significantly associated SNP ($P = 3.34 \times 10^{-7}$; OR = 1.43, 95% C.I = 1.23-1.66; Fig. 1a) and conditional analysis upon 97 rs887369 showed no evidence of independent association signals (Fig. 1b). Haplotype analyses 98 99 revealed that rs887369 tags a single, 1kb haplotype block comprising five near-perfectly correlated SNPs mapping to the 3' region of CXorf21 (Fig. 1c)- encoding a small, 301-amino 100 acid protein of unknown function. SNPs rs2529517 (distal) and rs887369 (proximal) define the 101 boundaries of the associated haplotype, which map downstream of the 3'-UTR of *CXorf21*, and 102 103 to the gene's third and final exon respectively (Fig. 1c). Three of the five associated SNPs are 104 transcribed from CXorf21, with rs887369 effecting a synonymous change (V209) and both 105 rs2532873 and rs2710402 residing in the 3'-UTR. The remaining two SNPs, rs2429517 and 106 rs2429518, are located in the downstream intergenic region of CXorf21. The associated haplotype is distinctly separated from neighbouring haplotypes by high recombination (D' <107 0.6, $r^2 < 0.2$) and accordingly, the risk haplotype itself represents the only observed association 108 with SLE ($\chi^2 = 29.87$, $P = 4.63 \times 10^{-8}$; Chi-Square test; Fig. 1d). 109

110

111 *CXorf21* is known to escape XCI¹⁶. We performed a statistical test on the association with
112 rs887369 to see if a model that assumed the SNP was in an area that escaped inactivation fitted
113 better than a model assuming full inactivation. A likelihood ratio test to fit both association 6

models failed to reject the model of full inactivation (P = 0.78). Therefore, from our 114 115 case/control data we have no evidence against the hypothesis that this association lies in an 116 area of full inactivation. To extend these analyses, we determined the odds ratios of the risk 117 alleles in females and males separately. We observed a higher odds ratio for females 118 homozygous for the rs887369 [C] risk allele with respect to homozygous for [A] non-risk (OR 119 = 1.58, 95% C.I. 1.29 - 1.93) compared to the males (OR = 1.46, 95% C.I. = 1.10 - 1.92), who are hemizygous for the risk or non-risk alleles. The higher odds ratio in females is likely to 120 121 reflect a gene dosage effect secondary to some degree of loss of X inactivation.

122

123 The risk haplotype increases expression of *CXorf21* in LCLs

124 As no protein-altering variants were identified through fine-mapping, we sought to establish 125 whether the SLE risk alleles at CXorf21 colocalised with cis-eQTLs for gene transcription. 126 Non-random inactivation of the X chromosome (skewing) and variability in the degree of 127 silencing of gene expression from the inactivated X in females complicates the identification 128 of X chromosome eQTLs in females. Therefore, to study *cis*-eQTLs at the *CXorf21* locus, we 129 employed two complementary methods of assessing the influence of the risk haplotype, tagged 130 by rs887369, on the expression of genes within the Xp21.2 region: 1) using the hemizygosity 131 of males to isolate the allelic effects; 2) removing females exhibiting strong evidence of 132 extreme skewed XCI to reduce the variability in the degree of skewing of X-chromosome 133 expression.

134

135 The associated haplotype, tagged by rs887369 [C], correlated with increased expression of 136 *CXorf21* in LCLs from male samples in the Geuvadis RNA-Seq dataset ($\beta = 1.56$, P = 1.94 x 137 10⁻⁰³; linear-regression; Fig. 2a). The expression of neighbouring genes *GK* and *TAB3* showed

138 no significant association with rs887369 (P = 0.7 and P = 0.09, respectively, linear-regression, 139 Fig. 2a). Many variants may act as *cis*-eQTLs, however it is important to note that rs887369 140 was the most significantly associated *cis*-eQTL for *CXorf21* (Fig. 2b) and the remaining *cis*-141 genes (the *MAGEB* family and *NR0B1*; +/-1Mb from rs887369) were not expressed in LCLs 142 (RPKM < 1).

143 The allelic effect on *CXorf21* expression was only nominally significant when performing the *cis*-eQTL analyses in female individuals from Geuvadis RNA-Seq dataset in LCLs (P = 0.02; 144 linear-regression; Supplementary Fig. 2a). In order to investigate *cis*-eQTL effects at rs887369 145 146 in females, we interrogated an additional RNA-Seq gene expression dataset in LCLs constructed exclusively from female donors from the TwinsUK cohort²¹. This dataset was 147 148 selected for analysis as it had been previously analysed for skewing of X chromosome 149 inactivation using allele specific expression (ASE) of the Xist silencing lncRNA (manuscript 150 in preparation). In order to study potential cis-eQTLs at the CXorf21 locus, we removed 151 individuals showing marked skewing, in whom the Xist ASE showed that one parental X 152 chromosome contributed less than 20% of the Xist expression. In this subset of 412 non-skewed 153 individuals, we observed a statistically significant increase of *CXorf21* expression with respect to the rs887369 [C] risk allele in females ($P = 7.00 \times 10^{-03}$; linear-regression; Fig. 2c). 154

155

We validated this effect *in vitro* by qPCR of independent LCL samples selected from the HapMap Project on the basis of their genotype at rs887369. In these cells a 1.9-fold increase of *CXorf21* mRNA was detected between rs887369 homozygous risk and non-risk females (P= 4.1 x 10⁻⁵; *t*-test ;Supplementary Fig. 2b). Following validation of the anti-CXORF21 antibody (Supplementary Fig. 3), the observed increase in expression by the risk allele was found to persist at protein-level (β = 0.49, P = 2.88 x 10⁻⁵; Fig. 2d; raw data are shown in Supplementary Fig. 2c). 163

164 **Risk variants increase** *CXorf21* **expression upon activation**

165 We expanded our analysis and interrogated a genotype-expression cohort from a range of 166 human primary ex vivo immune cells. When assessing male samples only, we found that the associated haplotype, tagged by rs887369, was a significant cis-eQTL for CXorf21 in both 167 Lipopolysaccharide (LPS) stimulated ($P = 1.08 \times 10^{-03}$) and IFN- γ -stimulated ($P = 1.10 \times 10^{-103}$) 168 ³; linear-regression) monocytes (Fig. 2e). The [C] risk allele once again correlated with 169 170 increased CXorf21 expression. Interestingly, no statistically significant cis-eQTL associations 171 were observed in the unstimulated experiments: B cells, NK cells, neutrophils, and monocytes, 172 which suggests an activation-state specificity of the *cis*-eQTL. When the same analysis was 173 performed in the female samples of the same cohort, no significant cis-eQTLs were detected 174 in any of the cell types (Supplementary Fig. 2d).

175

176 Epigenetic fine-mapping of the Xp21.2 associated haplotype

Using the Roadmap Epigenomes Project²² (twelve different histone marks across 127 cell and 177 178 tissue types), we used chromatin fine-mapping to functionally prioritize the five SNPs carried 179 on the 1Kb associated haplotype. The associated SNPs localised only to a single histone 180 modification, H3K36me3, across five cell types: blood mononuclear cells, peripheral blood B 181 cells, monocytes, neutrophils, and the lymphoblastoid cell line GM12878. Analysis of the 182 signal value distribution of H3K36me3, designating regions of active transcription, across 183 these cell types revealed that rs887369 localised to the binding site summit of H3K36me3 184 whilst the remaining four SNPs on the haplotype localised to the tails of the signal distribution 185 (Fig. 3a). The greatest enrichment of H3K36me3 across the entire CXorf21 gene locus was concentrated to +/-100bp of rs887369 in monocytes ($P = 6.1 \times 10^{-14}$; MACS2) and neutrophils 186 9

187 ($P = 2.0 \ge 10^{-17}$; MACS2; Fig. 3b). The rs887369 SNP also localised to the binding site summit 188 of H3K36me3 in primary B cells, LCLs, and blood mononuclear cells, with significant, albeit 189 weaker enrichment.

190

191 As verification, we performed the same analysis using ChIP-Seq experiments (n=612) from the venous blood portion of the Blueprint Epigenetics consortium²³ (8 modifications across 24 192 unique cell types from 83 donors). Only 22 ChIP-Seq experiments presented evidence of 193 194 overlap with the SLE-associated haplotype, and strikingly, all of these intersections were again 195 for the H3K36me3 chromatin modification. No other histone modifications intersected this 196 region. All five SNPs on the 1kb SLE-associated haplotype were found to overlap with 197 H3K36me3 in monocytes, B cells and neutrophils – corroborating the Roadmap Epigenomics 198 data. We were unable to make robust conclusions on differential H3K36me3 signal between 199 the sexes as the sample sizes per cell-type were too small (Supplementary Fig. 4, 200 Supplementary Table 1).

201

Lastly, the associated SNPs in the 3'-UTR of *CXorf21* showed no evidence of disrupting a microRNA binding site after interrogation using miRDB²⁴.

204

205 The risk haplotype interacts with the promoter of *CXorf21*

We sought to investigate a conceivable molecular mechanism whereby the SLE-associated haplotype at the 3' end of *CXorf21* modulates expression through alteration of chromosome interactions. The promoter capture Hi-C dataset curated by the CHiCP resource²⁵ was interrogated. This resource comprises Hi-C data from 17 primary immune cell types taken from healthy donors. Three of the five SNPs (rs887369, rs2710402, and rs2532873) on the 211 associated haplotype, which are closest to CXorf21, reside within the chrX:30576528-30582605 target region. Across all primary immune cell types tested, the target region was 212 213 found to interact with four baits (Fig. 3c): the promoter region of CXorf21 (chrX:30595248-214 30603761); the promoter of GK; and two intronic antisense RNAs of TAB3 (TAB3-AS1 and 215 TAB3-AS2). Significant bait-target region interactions (CHiCAGO score \geq 5) were detected 216 exclusively in neutrophils (Fig. 3d), where the *CXorf21* promoter bait interaction presented the 217 greatest strength of interaction with the risk haplotype target region (6.09). Strong but subthreshold interactions ($3 \le CHiCAGO$ score < 5) were also detected for the risk haplotype 218 219 target and the CXorf21 promoter bait region in monocytes (3.72) and naïve B cells (3.15). The 220 strength of the interaction score between the risk haplotype target region and the *CXorf21* 221 promoter was found to correlate strongly with the signal strength of epigenetic marks (from 222 ENCODE²⁶) indicative of active gene-expression (H3K4me3 and H3K27ac) for matched cell types (Fig. 3e). These findings suggest that the 3'-promoter interaction of CXorf21 is more 223 224 pronounced in the cell types in which CXorf21 is expressed, and the interaction itself is involved in regulation of expression. In fact, by assessing the transcription factor landscape at 225 226 the CXorf21 locus, we found significant binding events of RNA polymerase II (POLR2A) at 227 the 3' SLE-associated region in immune cell types only; corroborating our hypothesis that the 228 observed chromatin looping is necessary for transcriptional regulation (Supplementary Fig. 5). 229

230 Sexual dimorphic expression is magnified upon activation

GTEx RNA-Seq data²⁷ across 45 different cell/tissue types confirmed that there is significant sexual dimorphic expression of *CXorf21* in both LCLs and thyroid tissue (LCLs: 1.78-fold greater in females, $P = 1.10 \times 10^{-5}$, thyroid: 1.33-fold greater, $P = 2.65 \times 10^{-3}$ following Bonferroni multiple testing correction; *t*-test; Supplementary Fig. 6a and Supplementary Table 235 2). Neighbouring genes GK and TAB3 were equally expressed in both sexes, in both LCLs and in the cell types in which both genes are most expressed, suggesting escape from XCI at this 236 237 locus is restricted to CXorf21. Using HapMap LCLs selected on the basis of their genotype at 238 haplotype-tagging rs887369, we employed the validated anti-CXORF21 antibody 239 (Supplementary Fig. 3) to quantify protein abundance by western blot. When we examined cell 240 lines that all carried at least one risk haplotype, we confirmed that protein expression was 241 higher in females (Supplementary Fig. 6b): females harboured 3.6 times more CXORF21 than 242 males (P = 0.006; *t*-test). These findings imply that the slight variation in *CXorf21* mRNA 243 results in an amplified effect on overall protein abundance. To ensure these results were not a 244 consequence of monoallelic expression of *CXorf21* in pauciclonal LCLs, we assayed *CXorf21* 245 expression from microarray experiments across a range of primary ex vivo immune cells and 246 found, as with other XCI escaping genes, the effect size of *CXorf21* expression between sexes 247 was cell-type specific¹¹. In resting B cells, NK cells, neutrophils, and monocytes, no significant 248 difference in transcript abundance of *CXorf21* between sexes was observed (Supplementary Fig. 6c, Supplementary Table 3). However, though we see global increase of CXorf21 249 250 expression in both sexes, a striking sexual dimorphic responses to LPS- or IFN-γ-stimulation in monocytes was observed (Fig. 4; $P_{\text{LPS}} = 1.41 \times 10^{-12}$; $P_{\text{IFN-}\gamma} = 9.29 \times 10^{-8}$; *t*-test). Transcript 251 252 abundance of CXorf21 in monocytes is therefore greatest in females under immune-stimulated 253 conditions.

254

255 *CXorf21* is a likely interferon response gene

Given the marked increase of *CXorf21* expression in stimulated immune cells (including LCLs which exhibit a partially activated phenotype²⁸) and the observed up-regulation of IFNregulated genes in SLE^{29} , we investigated whether *CXorf21* is an interferon response gene by 259 profiling gene expression using in-house microarray data in primary ex vivo B cells taken from healthy females (n=49 in total, of which n=32 were treated with IFN- α). We observed *CXorf21* 260 is one of eighteen X chromosome genes (including TLR7, IL13RA1, and ELF4) which were 261 up-regulated in response to IFN- α stimulation (fold-change: 2.41; $P = 6.0 \times 10^{-9}$; ANOVA; 262 263 Fig. 5a). No other Xp21.1 gene was modulated by IFN- α . We profiled the epigenetic landscape surrounding the *CXorf21* locus in ENCODE data and detected significant and localised binding 264 265 events of NF-κB, STAT1, STAT2, STAT3, IRF4, and IRF3 at the immediate promoter region 266 of CXorf21 in LCLs (Fig. 5b). We also identified a single interferon-stimulated response 267 element (ISRE) +25bp upstream of the *CXorf21* transcription start site (TSS). This sequence 268 motif and the array of interferon regulatory factors was not detected in any of the promoters of 269 other genes within the Xp21.2 locus (Fig. 5b).

270

271 Functional characterisation of the Xp21.2 SLE rick locus

Eight genes are encoded at the Xp21.2 SLE risk locus (rs887369; $P = 3.34 \times 10^{-7}$; OR = 1.43): four Melanoma Antigen B (MAGEB) family genes (*MAGEB1-4*), *NR0B1* encoding the DAX1 nuclear receptor, *GK* (glycerol kinase), *TAB3* (TGF-beta activated kinase 1 and MAP3K7 binding protein 3), and *CXorf21* (Fig. 1a; Supplementary Table 4). None of these eight genes had reported associations with immune-related phenotypes in human or mouse.

277

CXorf21 is the only gene in the locus with a discrete immune-specific mRNA expression
profile; being most highly expressed in the spleen, appendix, bone marrow, and lymph nodes
(Protein Atlas; Supplementary Fig. 7, GTEx and FANTOM5 validation in Supplementary Fig.
8). This suggests the mechanism by which the SLE-risk haplotype is affecting disease risk is
through candidate gene *CXorf21*. To refine this analysis in terms of cellular expression, we

283 used data from Blueprint Epigenome (RNA-sequencing) and BioGPS (microarray) to show 284 that within immune cell types, the expression of *CXorf21* is largely restricted to monocytes, neutrophils, and B cells (Supplementary Fig. 9). We corroborated these findings in RoadMap 285 286 Epigenomics data and found a striking chromatin landscape around the transcription start site 287 of CXorf21, indicative of epigenetic silencing in non-immune cell types (Supplementary Fig. 288 10). The expression profile of *CXorf21* at protein-level was largely consistent with the mRNA 289 data; though CXORF21 protein was found to be in equal abundance in certain secondary 290 immune tissue such as the bowel and skin (Supplementary Fig. 11).

291

292 RNA-Seq co-expression analysis across a range of human cell and tissue-types was undertaken using the COEXPRES algorithm³⁰. The results indicate that *GPR65* (G-couple protein receptor 293 65) tops the ranking, whose protein product is important in lysosomal function³¹. Examination 294 295 of the top 100 ranked genes revealed the expression signature of CXorf21 correlated with the 296 Toll-like receptor (TLR) signalling pathway including TLR7, TLR6, PIK3CG, and PIK3CD (Supplementary Table 5^{30}), of which *TLR7* was highest ranked. The correlation between the 297 expression of the two X-linked genes, CXorf21 and TLR7, was replicated in TwinsUK RNA-298 Seq data²¹ from LCLs from non-skewed females (n= 271; $\rho = 0.38$; $P = 6 \times 10^{-11}$). 299

300

In order to gain further insight into the potential function of CXORF21, we utilized highthroughput affinity-purification mass spectrometry data from BioPlex³² and revealed a high confidence (quantitative score: 0.999) protein-protein interaction between CXORF21 and SLC15A4, encoded by the SLE susceptibility gene *SLC15A4* (rs1059312; P_{META} =1.48x10⁻¹³; OR=1.17)¹⁵. *SLC15A4* is an immune-restricted lysosomal amino-acid transporter required for TLR7- and TLR9-mediated type I IFN production in dendritic cells and B cells in lupus³³. 307 Interestingly, in the BioPlex data, CXORF21 was also found to interact with itself, suggesting308 probable oligomerization of this protein.

309

310 Protein level correlates with disease activity in females

311 In a modest cohort ($n_{cases} = 19$; $n_{controls} = 13$) we did not observe a statistically significant 312 difference in CXORF21 protein abundance between female case and controls in CD14+ 313 monocytes or CD19+ B cells (Supplementary Fig. 12). However, we observed an age-314 dependent correlation between CXORF21 and SLE Disease Activity Index 315 (SLEDAI). CXORF21 protein abundance is positively correlated with SLEDAI in SLE females < 35 years of age (CXORF21 ~ SLEDAI * Stratified Age) in both CD14+ monocytes 316 and CD19+ B cells (Supplementary Fig. 13). A likelihood ratio test (LRT) rejected the model 317 318 of SLEDAI as a single variable (upper panels Supplementary Fig. 13) in favour of an 319 interaction model in monocytes (LRT P = 0.0002) and B cells (LRT P = 0.0006). The rejection 320 of the single variable models are also supported by BIC ($\Delta BIC_{monocytes} = 8.1$; $\Delta BIC_{Bcells} =$ 5.9). We observed a significant interaction term (SLEDAI * Stratified Age) in monocytes (P =321 322 0.002), though the interaction term in B cells did not pass multiple testing correction (P =323 0.011; lower panels Supplementary Fig. 13).

324

325 CXORF21 protein may act within endosomal pathway

326 CXORF21 is a small protein of ~34kDa as identified by Western Blot. Very little of the
327 secondary/tertiary protein structure of CXORF21 could be accurately determined by the Phyre
328 bioinformatics prediction tool³⁴. Thus, to gain insight into the protein's function we sought to
329 determine its cellular location in *ex vivo* cells from healthy females and the GM12878
330 lymphoblastoid cell line. We undertook multispectral imaging flow cytometry (MIFC) with a

range of labels for different organelles. The results demonstrated minimal co-localisation of 331 332 CXORF21 with nuclear, Golgi or lysosomal markers in ex vivo PBMCs, and this was not 333 affected by IFN stimulation (Supplementary Fig. 14 and 15). In view of these negative findings 334 and the data showing co-expression of *CXorf21* with components of the Toll-like receptor signalling pathway (Supplementary Table 6), we utilised the increased resolution of structured 335 336 illumination microscopy (SIM) to determine whether there was any evidence for colocalisation of CXORF21 with TLR7. Representative images for the staining in resting and stimulated ex 337 338 vivo B cells are shown (Fig. 6a through 6d). We quantified the correlation between signals 339 obtained from CXORF21 with TLR7 staining (Fig. 6e) and determined the colocalisation of 340 the two staining signals in B cells using Mander's co-efficient (see Methods). These analyses 341 were undertaken in both resting B cells and stimulated B cells (B cell receptor cross-linking 342 and CD40) and in each case with and without exposure to IFN- α . We conclude that there is 343 weak colocalisation between TLR7 and CXORF21 in ex vivo B cells (Pearson correlation 0.3 $< \rho < 0.4$). No significant differences in colocalisation between CXORF21 and TLR7 were 344 345 observed after IFN-α treatment of resting or IgM/CD40 stimulated B cells.

346

As the endosomal intracellular pathway interacts with the autophagy pathway (which has also 347 been implicated in SLE pathogenesis)³⁵ we sought to determine whether CXORF21 colocalised 348 349 with autophagosomes, once more utilising SIM. Using LC3 as a marker of the autophagosome, 350 representative results of the joint staining (LC3 and CXORF21) are shown for Ig/CD40 351 stimulated B cells (Fig. 7a) with exposure to the inhibitor of autophagic flux, bafilomycin (Fig. 352 7b), and Ig/TLR7/8 stimulated B cells (Fig. 7c) with bafilomycin (Fig. 7d). The results from 353 multiple cells are summarised in Fig. 7e and 7f, which show no colocalisation between LC3 354 and CXORF21 in bafilomycin-treated ex vivo B cells when stimulated with Ig/CD40 or

355	Ig/TLR7/8. Assaying CXORF21 protein abundance by western blot in starved LCL (see
356	methods) indicates that the amount of CXORF21 is not altered by the addition of bafilomycin
357	and hence it is unlikely that CXORF21 is an autophagy substrate (Fig. 7g; left panel). The blot
358	shows some elevation of sequestome 1 (p62), an autophagosome cargo protein, following
359	exposure to bafilomycin, which would be expected (Fig. 7g; right panel).

361 Discussion

The underrepresentation of genetic associations on the X chromosome in autoimmune disease 362 363 is highly paradoxical given the prominent sex bias towards females and the increased density of immune related genes compared to the autosomes. This is partly due to the paucity of sex 364 chromosome data in genome-wide studies: only 33% of GWAS report sex chromosome data³⁶. 365 366 We sought to functionally investigate the undefined SLE susceptibility locus Xp21.2 from our own GWAS dataset (rs887369; $P = 3.34 \times 10^{-7}$; OR = 1.43). Our investigation defines *CXorf21* 367 368 - encoding a protein of hitherto unknown function – as the candidate gene and demonstrates 369 its expression is upregulated through by a number of distinct factors: chromosome X dosage 370 and loss of XCI, the risk haplotype (tagged by rs887369), and cellular activation by interferon 371 (summarised in Fig. 8). Our study supports the hypothesis that altered expression of X-linked genes contributes to the sexual dimorphism in autoimmunity¹⁴ and provides some preliminary 372 evidence for the role of CXorf21 in SLE, although this topic clearly warrants further 373 374 investigation.

375

To date, six X-encoded SLE susceptibility loci have been identified, and four have been shown to harbour genes that escape XCI (*TLR7*, *TMEM187*, *IRAK1*, and *CXorf21*). Of these, *CXorf21* is the most robustly escaping; possessing evidence of escape in ~80% of individuals in contrast to the remaining genes that exhibit escape in $<30\%^{16}$. We show that escape from XCI is highly localised to *CXorf21* across a +/-1Mb window.

381

The Xp21.2 locus is not as strongly associated with SLE in individuals of non-European ancestry, although an association has been reported in Koreans³⁷. This is partially explained by the marked disparity in allele frequency of risk allele rs887369 [major allele: C] between 385 populations (1000Genomes: 0.76 in Europeans, 0.92 in Africans, 0.95 in Asians). The lower minor allele frequency in non-Europeans may clearly impact on power, especially as non-386 387 European GWAS have been of smaller sample size. The fraction of individuals who exhibit 388 XCI of *CXorf21* is reported to be diminished in individuals of African descent (relative to those of European descent¹⁶); however, lower allele frequencies of transcribed polymorphisms and 389 390 limited samples sizes impede power. Whether allele frequency of rs887369 and reduced XCI 391 escape are correlated or whether variation at rs887369 itself is causal to a degree of escape 392 poses an interesting line of enquiry. Furthermore, the reduced level of escape in non-Europeans 393 may mean the effect size will limit the power of this locus to be detected.

394

395 CXorf21 has a discrete expression pattern in immune cells, both adaptive and innate, with the 396 greatest expression of *CXorf21* found in monocytes and neutrophils, primary B cells and LCLs. 397 It appears to be epigenetically inert in non-immune cell types, suggesting the regulatory 398 mechanisms driving expression of *CXorf21* are not present in non-immune cell types. These 399 data align with the observation that other candidate genes of SLE and their accompanying 400 causal variants exhibit a discrete expression signature and *cis*-regulatory landscape that is 401 largely restricted to immune cell subsets; particularly B cells (including B-lymphoblastoid cell 402 lines), T cells, and monocytes $^{15,38-40}$.

403

404 We demonstrate that *CXorf21* expression is upregulated in LPS and IFN- γ -stimulated 405 monocytes, and in IFN- α -stimulated B cells, with the magnitude of increase greater in females 406 leading to significant sexually dimorphic expression levels. We have also identified binding 407 sites of respective transcription factors from these signalling cascades: IRF3, NF- κ B and 408 STAT1-3 at the immediate promoter of *CXorf21* suggesting *CXorf21* transcription could be a 409 primary response gene of the TLR4 (IRF3) and IFN (STATs) signalling pathways. However, 19 410 *CXorf21* expression decreases following acute (2h) LPS-stimulation, suggesting *CXorf21* is in 411 fact a late response gene induced by secondary activation of the TLR4-induced type I IFN feed 412 forward loop⁴¹. Indeed, late response genes are characterised by STAT binding sites and 413 ISRE⁴¹, which we also identify in the *CXorf21* promoter. Sex differences in the LPS-induced 414 monocyte response have been previously reported, whereby females have heightened 415 activation and cytokine release compared with males, although the underlying mechanism has 416 yet to be delineated^{42,43}.

417

rs887369 tags a short 1kb haplotype comprising five perfectly correlated SNPs. The haplotype
is an eQTL for *CXorf21*, with the risk allele increasing the gene's expression; we hypothesise
that a self-regulatory mechanism involving modification of H3K36me3 state and chromatin
looping affects RNA polymerase II within the gene promoter (Fig. 8).

422

423 The expression of *CXorf21* transcript has previously been shown to be the most accurate delineator of disease flare from infection in SLE patients¹⁸. Interestingly, this previous study 424 425 was conducted in largely non-European patients, suggesting the role of *CXorf21* is not limited 426 to individuals of European ancestry. Further supporting our hypothesis that CXorf21 is an IFN-427 inducible gene, the genes with dysregulated expression at exome-wide significant expression changes identified in this study are enriched for IFN-inducible genes¹⁸. We observed an age-428 dependent correlation between CXORF21 expression and disease activity using flow 429 430 cytometry in a modest cohort, with CXORF21 protein abundance positively correlating with 431 SLEDAI in patients <35 years of age. These data warrant further investigation and suggest age-432 stratified analysis in disease cohorts could be illuminating.

433

434 The CXORF21 protein has no known function and the primary amino acid sequence gives no 435 clear clues in this regard. In an attempt to provide some insight into the function of CXORF21, 436 we conducted a number of imaging studies to investigate its intracellular location. These 437 studies showed that CXORF21 is present in both the nucleus and cytoplasm. Interestingly, we 438 show that there was some colocalisation of CXORF21 with TLR7 in B cells. This intracellular 439 toll-like receptor was selected for imaging as it is known to play a role in nucleic acid sensing in SLE and our analyses revealed some degree of co-expression of TLR7 and CXorf21 at the 440 RNA level. Intracellular toll-like receptors operate in a complex system involving the 441 endosomal and lysosomal compartments⁴⁴. However, the precise points at which CXORF21 442 443 and TLR7 may interact within these compartments is not clear on the basis of our data, but 444 further exploration of this question should reveal more insights into the function of CXORF21 445 and how it promotes systemic autoimmunity.

446

The female-biased sex imbalance of autoimmune diseases is not understood. Our study, which characterises the SLE association at *CXorf21*, an IFN-inducible gene which escapes XCI, adds support to the hypothesis that sex bias in immune function has a genetic basis and provides an underlying immunological mechanism that underpins the sexual dimorphism in SLE.

452 Methods

453 European SLE GWAS data

Genotype data from 10,995 individuals of matched European ancestry (4,036 SLE cases, 6,959
controls) genotyped on the Illumina HumanOmni1 BeadChip from the Bentham and Morris et
al¹⁵ study were imputed as outlined below. These data had undergone quality control and PCA
as described¹⁵.

458

459 Imputation

The European SLE GWAS¹⁵, Fairfax *et al*^{45,46}, and Naranbhai *et al*⁴⁷ cohorts were imputed using UK10K-1000GP3 merged reference panel across the X:30077468-31077846 1Mb region, plus a 2Mb buffer region (GRCh37 assembly). A full imputation without pre-phasing was conducted using IMPUTE2 to increase the accuracy of imputed genotype calls^{48,49}. Imputed genotypes were filtered using an info score (IMPUTE2) threshold of 0.5. The most likely genotype from IMPUTE2 was taken if its probability was > 0.5. If the probability fell below this threshold, it was set as missing.

467

468 Allelic and haplotype association fine-mapping

Imputed data from the European SLE GWAS were filtered to include variants with MAF > 0.01 and HWE > 1 x 10⁻⁴, and minimum genotype rate > 90%. SNPTEST 2.5.2⁵⁰ was used to test for additive models of allelic associations across the X:30077468-31077846 1Mb region, fitting a logistic regression model (including the first four covariates from the original GWAS¹⁵) with equal effect size between males and females^{50,51}. Independent signals were assessed by including the genotype for the rs887369 SNP as a covariate using the SNPTEST 475 algorithm. Association plots were generated using LocusZoom⁵². Haplotype association 476 analysis and LD calculations between SNPs were performed using Haploview 4.2^{53} 477 (implementing X-chromosome analysis) using the entire GWAS of 10,995 individuals. 478 Specifically, haplotype blocks across a 100Kb region anchored on rs887369 were defined by 479 the confidence internals algorithm⁵⁴ and haplotype association testing performed by a Chi-480 Square test using marker thresholds of MAF > 0.01 and HWE > 1 x 10⁻⁴, and minimum 481 genotype rate > 90% (657 SNPs in total).

482

483 We fitted two models for association in SNPTEST. The inactivation model is the default in 484 SNPTEST's newmlmethod with male genotypes coded as 0/1 and females coded as 0/0.5/1485 and one shared estimated effect (log odds ratio). In the escape model we used SNPTEST with 486 the stratify_on option which fits separate effects for males and females. In both models, we fit 487 a different intercept for males and females (using sex as a covariate in the inactivation model) 488 and so the two models only differ by one parameter (being the differing log odds ratio). A 489 likelihood ratio test (LRT) on one degree of freedom was performed in R (using the likelihood 490 values output by SNPTEST), where the escape model was tested against the simpler inactivation model. A statistically significant result (based on the p-values form the LRT) 491 492 would therefore reject the inactivation model.

493

494 Genotype data for *ex vivo* cell eQTL cohorts

495 X chromosome SNPs of the *Fairfax et al*^{45,46} and *Naranbhai et al*⁴⁷ cohorts with an Illumina 496 GenCall score of <0.7 and called on both X and Y were removed. PLINK v1.9⁵⁵ was used to 497 remove samples that failed sex check assignments. Following separation of male and females, 498 SNPs were removed if: HWE < 1×10^{-4} , MAF < 0.01, and SNP missingness > 10%. Individuals 499 were removed if >10% of SNPs were missing. Coordinates were converted from hg18 to hg19 500 using the UCSC liftOver application⁵⁶.

501

502 Genotype-expression cohorts and *cis*-eQTL analysis

503 Gene-level RNA-Seq data from LCLs were downloaded from ArrayExpress (Geuvadis; EGEUV-1)⁵⁷ and genotypes (X:30077468-31077846) of these individuals containing SNPs 504 (MAF > 0.05) were taken from the 1000 Genomes Project Phase III⁵⁸. Expression data of 505 purified *ex vivo* primary immune cells were obtained from *Fairfax et al*^{45,46} and *Naranbhai et* 506 507 al^{47} . Details are described in the respective articles. Data include resting B-cells, natural killer cells, and monocytes⁴⁵; IFN- γ stimulated monocytes after 24h, LPS stimulated monocytes after 508 2h, LPS stimulated monocytes after 24h⁴⁶; and resting neutrophils⁴⁷. In all instances, *cis*-eOTL 509 510 association analysis (1Mb of rs887369) was performed against expression residuals using the linear-model of the MatrixeQTL R package⁵⁹ including the number of PCs described in the 511 512 respective articles.

513

The TwinsUK RNA-Seq eQTL cohort profiled in LCLs²¹ was used for *cis*-eQTL association 514 515 analysis in non-skewed females (n=412). Individuals were firstly assessed for skewed X-516 chromosome inactivation patterns using allele-specific expression of Xist to estimate the 517 proportion of X inactivation from each parental X chromosome. Individuals were removed if the allele-specific expression of XIST-linked SNPs was <0.2 or >0.8, these parameters were 518 519 chosen on the basis of precedence⁶⁰⁻⁶². *cis*-eQTL analysis in the twins was performed as above 520 against exon-count residuals corrected for probabilistic estimation of expression residuals (PEER) factors and family relatedness⁶³. 521

523 Differential expression analysis of *CXorf21* between males and females using GTEx RNA-Seq 524 (TMP) data across the 45 cell/tissue types where expression data were available for both sexes 525 was performed using an unpaired *t*-test between males and females after grouping by cell/tissue 526 type. Associations passing the Bonferroni adjusted *P*-value cut-off of $P_{BF} < 0.05$ were deemed 527 significant.

528

529 Cell culture

LCLs were obtained from Coriell Biorepository and cultured in suspension at 5% CO₂, 37°C
in RPMI 1640 medium supplemented with 2mM L-glutamine, 15% foetal bovine serum, 100
units/ml penicillin and 100 µg/ml streptomycin. Cells were seeded every two days to a
concentration of 300,000 viable cells/ml. Individuals used in functional assays were of
European descent (GM12878, HG01702, HG01786, HG01746, HG0111, HG01628,
HG00254, HG12878, HG01501, HG01507, HG01504, HG00269, HG00232).

536

537 qPCR

Total RNA was extracted with the RNeasy Mini Kit (QIAGEN) according to manufacturer's 538 539 instructions. cDNA synthesised with the cDNA Synthesis Kit (Thermo Scientific) and quantified using the NanoDrop 2000 spectrophotometer. qPCR reactions were performed using 540 541 the TaqMan® Universal PCR Master Mix and Universal ProbeLibrary System Technology 542 (UPL) from Roche. Primers were purchased from Sigma and reactions performed using the 543 Applied Biosystems 7500 and subsequent analysis with SDS 2.3. CXorf21 F: 544 GGATGTTTGACACAGACTTCAAA, R: CCGGATCAGATGAGCAGATT, UPL #65. ACTB F: AGAGCTACGAGCTGCCTGAC, R: CGTGGATGCCACAGGACT, UPL #9. 545 546 Relative abundance and fold change was calculated using the $\Delta \Delta Ct$ method. 25

548 Verification of anti-CXORF21 antibody by gene-knockdown

Gene-knockdown of CXorf21 in LCLs (GM12878) was performed by siRNA using the 549 550 Nucleofector II Device (Lonza) and Amaxa Cell Line Nucleofector Kit V. Two days before transfection, cells were seeded to a concentration of 0.5×10^6 cells/ml. In duplicate, 2×10^6 cells 551 552 were spun at 100g for 10mins and re-suspended in 100µl supplemented transfection solution 553 and 20pmol Silencer Select Pre-Designed & Validated siRNA (Thermo Fisher Scientific) 554 against *CXorf21* (#4392420). The Silencer Select Negative Control No. 1 siRNA (#4390843) 555 was used as a non-targeting negative control at the same concentration. Cell/siRNA 556 suspensions were transferred to a Nucleofector cuvette and electroporated using the X-001 557 programme. Samples were cultured in 1.5ml medium in a 12-well plate and harvested 48 h 558 post-transfection.

559

560 Immunoblot

561 Cell lysates were prepared in RIPA buffer (Sigma-Aldrich) and run on a SDS polyacrylamide 562 gel for electrophoresis. Protein was transferred onto a nitrocellulose membrane and blocked in 5% milk-PBS solution. The rabbit polyclonal anti-CXORF21 antibody (Atlas Antibodies; 563 564 HPA001185) was used at a concentration of 1:1,000 and the secondary polyclonal swine anti-565 rabbit immunoglobulins/HRP (Dako; P0217) at 1:1,000. Membranes were stripped by RestoreTM Western Blot Stripping Buffer (Thermo Fisher) and re-probed with mouse 566 567 monoclonal β-Actin antibody (Santa Cruz Biotechnology; sc-47778) at 1:4,000 and anti-mouse IgG HRP conjugate (Promega: W4028) at 1:5,000 or secondary goat anti-mouse IgG HRP 568 569 conjugate (Invitrogen; A16078) at 1:10,000. ImageJ was used to calculate the density of the 570 bands relative to the loading control. Rabbit anti-SQSTM1/p62 (Cell Signalling, 5114) was 26

used at a concentration of 1:1,000 and detected with secondary goat anti-rabbit IgG HRP
conjugate (Invitrogen; A16110) at 1:10,000. Raw blots are presented in accompyning Source
Data file.

574

575 **Epigenetic fine-mapping**

SNPs in X:30077468-31077846 were downloaded from the 1000 Genomes Project Phase III⁵⁸. 576 577 Epigenetic data across all available cell types (n=127) in NarrowPeak format were obtained from the NIH Roadmap Epigenomics Project²². Peaks were filtered for genome-wide 578 579 significance using an FDR threshold of 0.01, and peak widths harmonised to 2kb in length 580 centred on the peak summit. SNPs were reported as being localised to an epigenetic mark if 581 they overlapped the 2kb region. The signal value of the epigenetic mark was reported for the 582 exact coordinate of the SNP using the signal track of the mark in bigWig format visualized 583 using IGV v2.3.80⁶⁴.

584

NarrowPeak files of ChIP-Seq experiments (H3K4me3, H3K27ac, H3K4me1, H3K36me3, 585 586 H3K27me3, H3K9me3, H3K9/14ac, H2A.Zac) were downloaded from the Blueprint 587 Epigenome Project ftp site 588 (http://ftp.ebi.ac.uk/pub/databases/blueprint/data/homo_sapiens/GRCh38/). Only non-589 diseased cell-types from venous blood were selected for analysis (24 unique cell-types). Using 590 the GRCh38 genomic positions of the 5 SNPs carried on the associated haplotype, intersection 591 was performed against the genome-wide binding sites of the selected Blueprint ChIP-Seq 592 experiments as per the Roadmap Epigenomics project (above). Fold-enrichment of the peaks 593 that overlapped the associated haplotype were compared by unpaired *t*-test between males and 594 female samples for H3K36me3 across different cell-types.

595

596 **Promoter capture Hi-C chromatin interaction data**

597 Chromatin interaction data across a 17 primary immune cell-types was assessed using Capture 598 Hi-C Plotter (CHiCP; www.chicp.org)²⁵. The study focuses on autoimmune susceptibility loci 599 from GWAS and ImmunoChip integrating promoter capture Hi-C datasets from three separate 500 studies^{65–67}. The bait to target coordinates and interaction scores were extracted from CHiCP 601 manually. Scores were defined by the CHiCAGO algorithm⁶⁸, where scores \geq 5 were 602 considered as significant interactions.

603

604 **B-cell isolation and cell stimulation**

605 CD19⁺ B cells from healthy female subjects (n=49) were isolated by negative selection using 606 the Dynabeads Untouched Human B Cells Kit (Invitrogen). 1.5-3x10⁶ cells/ml *ex vivo* B cells 607 were cultured in RPMI 1640 medium, supplemented with 20% FCS, 2mM L-glutamine and 608 100 U/mL penicillin/streptomycin. B cells from 32 of the 49 subjects were incubated with or 609 without IFN- α 2b (1000 U/ml; PBL Assay Science) at 37°C and 5% CO₂. Cells were harvested 610 after 6 h or 20 h as indicated.

611

For immunostaining, human B Cell Isolation Kit II (Miltenyi Biotec). 1×10^6 cells/ml *ex vivo* B cells were cultured in RPMI 1640 medium, supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine and 100 U/mL penicillin/streptomycin. B cells were stimulated with 10 µg/ml F(ab')2 Fragment Anti-Human IgG+IgM (Jackson ImmunoResearch) and either 0.1 µg/ml CD40L with 0.1 µg/ml Enhancer (Enzo) or 5 µg/ml resiquimod (Sigma). B cells were incubated with or without 10 nM bafilomycin A1 (Sigma) for 3 h before harvesting and with or without 1000 U/ml IFN-α 2b (PBL Assay Science) at 37°C and 5% CO₂. Cells were
harvested after 20 h or 27 h as indicated.

620

621 *Ex vivo* **B cell RNA extraction and array hybridization**

RNA was isolated using the RNeasy Mini kit (Qiagen) according to the manufacturer's instructions and integrity assessed using the Agilent 2100 Bioanalyzer (Agilent) with the RNA 6000 Pico Kit (RIN < 8 excluded). cDNA was synthesised from 50ng of RNA using the High Capacity RNA-to-cDNA Kit (Applied Biosystems). Each sample was hybridised to Affymetrix Human Exon 1.0 ST arrays and expression data were obtained by fluorescence-based detection using the GeneChip Scanner 3000 7G (Affymetrix). Signal intensities were quantified and stored as CEL files.</p>

629

630 **Quality control of exon array**

631 Quality control was carried out using the probe-set and transcript cluster annotation release 632 33.1 (GRCh37 build). Probe and probe-set filters were applied to the data as recommended⁶⁹. 633 All probe sets targeting RefSeq-annotated RNA transcripts were included. Probes containing 634 polymorphisms (MAF>0.01) from 1000Genomes were removed. Cross-hybridizing probes and probe sets containing less than three probes were also excluded. Detection above 635 636 background noise (DABG) was calculated for all CEL files and probe-sets were filtered using 637 Affymetrix Power Tools. Probe sets with DABG *P*>0.01 in 50% of resting or IFN-α stimulated samples were removed. Probes and probe-sets that failed QC filters were removed from the 638 639 data using Affymetrix Power Tools.

Intensity signals were normalized at exon-level and log₂-transformed using the robust multiarray average algorithm in the Affymetrix Expression Console software (build 1.2.1.20). Array hybridization quality was verified using Affymetrix Expression Console according to the recommendations of the Affymetrix Quality Assessment of Exon and Gene Arrays White Paper. All arrays showed high hybridization quality and a normal distribution of probe intensity signals.

PCA was performed using Partek GS version 6.6 (Partek Incorporated) and sample outliers removed. Duplicate data for one monozygotic twin pair was processed in both batches to be used as technical replicates and sibling data from the same twin pair within each batch were used as biological replicates. Correlation between replicates was assessed using a Spearman correlation test in R. All replicates showed high correlation ($r^2 > 0.89$). A total of 81 samples from 49 individual twins were included in the analyses.

653

654 Exon array data normalization and analysis

Probe sets were summarized to generate gene-level data by calculating the winsorized mean 655 (10% and 90%) using Partek GS. Batch effects were accounted for using the sva ComBat 656 function⁷⁰. Differential gene expression was calculated using Partek GS with a mixed-model 657 658 analysis of variance (ANOVA) as follows: $Y = \mu$ + treatment + individual ID + twin ID + PC1 659 + PC3 + error. The fitted ANOVA model regressed expression levels at each gene (Y) on fixed-660 effect terms (treatment, explained by PC2) and on random-effect terms denoting individual ID, 661 family structure and zygosity (twin ID) and PCs explaining most of the data variability (PC1 662 and PC3).

664 SLE Patients and Healthy Controls

Female patients meeting the American College of Rheumatology (ACR) criteria for the 665 definition of SLE active disease⁷¹ were recruited from Louise Coote Lupus unit, Guy's Hospital 666 (n=19), following informed consent and with ethical approval (Research Ethics Committee; 667 REC 12/LO/1273 and REC 07/H0718/49) and SLE Disease Activity Index (SLEDAI) scores 668 were calculated⁷². The investigator was blinded to SLEDAI scores during measurment of 669 CXORF21 protein abundance. Healthy female controls were recruited from the TwinsUK 670 671 Bioresource. The TwinsUK study is approved by the research ethics committee at St Thomas 672 Hospital, London. Volunteers gave informed consent and signed an approved consent form 673 prior to participation. Volunteers were supplied with an appropriate detailed information sheet 674 regarding the research project and procedure by post prior to attendance.

675

676 **PBMC isolation**

677 20ml of whole blood in EDTA anti-coagulant was taken from female volunteers (SLE or 678 healthy controls). Peripheral blood mononuclear cells (PBMCs) were separated from whole 679 blood using Histopaque-1077 Hybri-Max (Sigma-Aldrich) density centrifugation and plated at 680 $2x10^6$ cells/ml in RPMI 1640-medium (Gibco) supplemented with 10% foetal calf serum 681 (FCS), 2mM L-glutamine and 100 U/mL penicillin/streptomycin (all from Invitrogen).

682

683 Flow Cytometry

PBMCs were first incubated with Human TrueStain FcX (5ul; BioLegend) to block Fc
receptors, before cell-surface staining with 1µl anti-human CD14 PerCP-Cy5.5 (eBioscience;

686 45-0149-42) and 1μl anti-human CD19 PE (eBioscience;12-0198-42) for 20min on ice. Cells

687 were fixed with 200ul 1X stabilising fixative (BD biosciences) and then permeabilized in 0.1%31

688 Triton X-100 (Sigma-Aldrich). Fc blocker was again added before intracellular staining of 0.1µg rabbit polyclonal anti-human CXORF21 (Atlas antibodies; HPA001185) or 0.1µg rabbit 689 monoclonal IgG isotype control (Abcam; ab172730), as appropriate, for 60min on ice. 690 691 Following washing, cells were incubated with secondary goat anti-rabbit-Alexa Fluor 488 (Abcam; ab150077) antibody at 1:2000. Cells were washed and resuspended in 250µl PBS for 692 analysis on BD FACSCantoTM II cytometer (BD Biosciences) using BD FACSDiva software 693 694 (version 8.0.1; BD Biosciences). Compensation was performed using compensation beads (BD 695 Biosciences), and cytometer settings were standardised using Cytometer Setup and Tracking 696 Beads (BD Biosciences). Following data acquisition, FlowJo v.10.1 software was used to 697 calculate the Median fluorescent intensity (MFI). An unpaired Student's t-test was used for 698 case-control analyses. Logistic regression models were fitted for CXORF21 abundance as a 699 function of SLEDAI, and as a function of SLEDAI stratified by age (under/over 35 years of 700 age) with an interaction term. The models were compared using a likelihood ratio test (LRT; 701 d.f. = 5) and BIC using R. Multiple testing was corrected using Bonferroni correction. 702 Preliminary results showed no expression of CXORF21 on cell surface.

703

704 ImageStream Analysis

Multispectral imaging flow cytometry (MIFC) was performed on an ImageStreamX (Amnis) instrument. Golgi colocalisation: 2×10^6 cells were fixed with 200ul 1X stabilising fixative (BD biosciences) and then permeabilized in 0.1% Triton X-100 (Sigma-Aldrich). Fc blocker was added before intracellular staining with 0.1µg rabbit polyclonal anti-CXORF21 antibody (Atlas antibodies; HPA001185) and secondary goat anti-rabbit Alexa Fluor 488 (Abcam; ab150077) at 1:2000. Cells were then incubated for 60min on ice with 0.1µg anti-GM130-Alexa Fluor 647 (Abcam; ab195303). Lysosomal and nuclear colocalisation: 2×10^6 cells were 712 incubated at 37°C for 15mins in 1X Assay Buffer and 0.1µl Lyso-ID Red Detection Reagent 713 and 0.1ul Hoechst 33342 Nuclear Stain (Lyso-ID Red Detection Kit; Enzo; anti-ENZ-51005-714 0100). Cells were then fixed with 200ul 1X stabilising fixative (BD biosciences) and 715 permeabilized in 0.1% Triton X-100 (Sigma-Aldrich). Fc blocker was added before 716 intracellular staining with 0.1µg rabbit polyclonal anti-CXORF21 antibody (Atlas antibodies; 717 HPA001185) and secondary goat anti-rabbit Alexa Fluor 488 (Abcam; ab150077) at 1:2000. Cells were resuspended in 60µl PBS. Up to 100,000 images were acquired per sample. Cells 718 719 were gated on aspect ratio to include only singlets, and the gradient root-mean-square feature 720 to include focused cells. Using the co-localisation mask on the IDEAS software (Amnis), we 721 calculated the overlap of CXORF21 and organelle markers for cellular localisation.

722

723 Immunostaining of autophagic LC3-II and CXORF21

724 1x10⁶ cells/ml LCL were starved in EBSS with or without 10 nM or 100 nM bafilomycin A1 725 (Sigma) for 3 h at 37°C and 5% CO₂ before harvesting, when starvation was required. For LC3 726 staining, the cells were selectively permeabilized with 0.05% saponin prior to fixation. Cells 727 were fixed in 4% formaldehyde for 20 min at room temperature, then permeabilized with 0.1% Triton X-100 and 2% goat serum (both Sigma-Aldrich) in PBS for 30 min on ice. After 728 729 overnight incubation in 5% goat serum, cells were Fc receptor blocked (Human TruStain FcX, 730 Biolegend) and incubated with 2 µg/ml rabbit anti-human CXORF21 (Atlas antibodies; 731 HPA001185) and either 2 µg/ml mouse anti-human TLR7 (Novus Biologicals, NBP2-27332) 732 or 40 µg/ml mouse anti-human LC3 (MBL, M152-3) in 5% goat serum for 1 h on ice. Following washing, cells were stained with goat anti-rabbit Alexa Fluor 488 (Abcam; 733 ab150077) and goat anti-mouse Alexa Fluor 594 (Abcam; ab150116), both at 1:2000, in 5% 734 735 goat serum for 30 min on ice. Cells were washed and mounted in ProLongTM Gold Antifade 736 Mountant containing DAPI (Invitrogen). 33

737

738 Imaging and Analysis

739 Imaging was performed at the Nikon Imaging Centre at King's College London. Z stacks were 740 acquired at 0.12 µm step size on an Eclipse Ti-2 Inverted microscope with Vt-iSIM scan head and Hamamatsu Flash4.0 sCMOS camera using a 100x oil immersion objective. Laser settings, 741 742 image capture and Richardson-Lucy deconvolution were managed in NIS-Elements. Images 743 were further processed and Pearson's correlation coefficient and Mander's colocalisation coefficient were calculated using the Colocalization Studio plugin⁷³ in Icy software. Maximum 744 745 intensity projections are shown for better visualisation. A one-way ANOVA with Tukey multiple comparison correction was performed to test for statistical significance in GraphPad 746 747 Prism v7.04.

748

749 Data availability

750 Summary statistics on 10,995 individuals of matched European ancestry (4,036 SLE cases, 751 6,959 controls) genotyped on the Illumina HumanOmni1 BeadChip are available at http://insidegen.com/insidegen-LUPUS-data.html. TwinsUK RNASeq data are deposited in 752 European Genome-Phenome Archive (EGAS00001000805). The UK10K (REL-2012-06-02) 753 plus 1000 Genomes Project Phase3 data (release 20131101.v5) merged reference panel 754 755 (UK10K-1000GP3) was accessed through the European Genome-phenome Archive 756 (EGAD00001000776). All other data are contained within the article and its supplementary 757 information or upon reasonable request from the corresponding author. The source data 758 underlying Figures 2d, 6e-f, 7e-g, and Supplementary Figures 2b, 3, 6b, 12 and 13 are provided 759 as a Source Data file.

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777 Author Contributions

778 C.A.O. performed expression experiments, analysed gene expression and epigenetic data, 779 performed genetic analysis, wrote the manuscript; A.L.R. performed gene expression and Image Stream experiments, performed genetic analysis, analysed data, wrote the manuscript; 780 S.K.V. performed super resolution microscopy experiments, analysed data; C.S.T.D. 781 782 performed gene expression experiments in B cells and analysed these data; S.K.V. and 783 C.S.T.D. contributed equally; C.T.B. performed microscopy experiments, analysed data; 784 A.J.C. designed the microscopy experiments and analysed these data; S.L. performed 785 CXORF21 expression studies in SLE patients; S.D. analysed CXORF21 protein expression; L.C. analysed gene expression data; D.L.M. analysed genetic data; L.J. validated the anti-786 787 CXORF21 antibody; L.B. performed gene expression studies in resting and IFN-stimulated B 788 cells; A.Z. and K.S.S. analysed X chromosome skewing and expression of CXorf21 in 789 TwinsUK; M.M.A.F. designed and supervised the B cell expression studies; D.S.C.G. designed 790 and supervised the study; T.J.V. designed and supervised the study and contributed to writing 791 the manuscript.

793 Competing Interests

The authors declare no competing interests .

796 **References**

- 1. Spolarics, Z. The X-Files of inflammation: Cellular Mosaicism of X-linked
- polymorphic genes and the female advantage in the host response to injury and
- 799 infection. *Shock* **27**, 597–604 (2007).
- 800 2. Gezon, H. M., Rogers, K. D., Yee, R. B. & Hatch, T. F. Excess risk of staphylococcal
 801 infection and disease in newborn males 1. 84, 314–328 (2000).
- 802 3. Libert, C., Dejager, L. & Pinheiro, I. The X chromosome in immune functions: when a
 803 chromosome makes the difference. *Nat. Rev. Immunol.* 10, 594–604 (2010).
- 4. Ramos, P. S. *et al.* A comprehensive analysis of shared loci between systemic lupus
- 805 erythematosus (SLE) and sixteen autoimmune diseases reveals limited genetic overlap.
- 806 *PLoS Genet.* **7**, e1002406 (2011).
- 5. Scofield, R. H. *et al.* Klinefelter's syndrome (47,XXY) in male systemic lupus

808 erythematosus patients: support for the notion of a gene-dose effect from the X

- 809 chromosome. *Arthritis Rheum.* **58**, 2511–7 (2008).
- 810 6. Cooney, C. M. *et al.* 46,X,del(X)(q13) Turner's syndrome women with systemic lupus
 811 erythematosus in a pedigree multiplex for SLE. *Genes Immun.* 10, 478–81 (2009).
- 812 7. Liu, K. *et al.* X Chromosome Dose and Sex Bias in Autoimmune Diseases: Increased
- 813 47,XXX in Systemic Lupus Erythematosus and Sjögren's Syndrome. *Arthritis Rheum*.
- **68,** 1290–1300 (2016).
- 815 8. Ross, M. T. *et al.* The DNA sequence of the human X chromosome. *Nature* 434, 325–
 816 337 (2005).
- 817 9. Lyon, M. F. Gene Action in the X-chromosome of the Mouse. *Nature* 190, 372–373
 818 (1961).
- 819 10. Payer, B. & Lee, J. T. X Chromosome Dosage Compensation: How Mammals Keep

- 820 the Balance. Annu. Rev. Genet. 42, 733–772 (2008).
- 11. Carrel, L. & Willard, H. F. X-inactivation profile reveals extensive variability in Xlinked gene expression in females. *Nature* 434, 400–404 (2005).
- 823 12. Carrel, L., Cottle, A. A., Goglin, K. C. & Willard, H. F. A first-generation X-
- 824 inactivation profile of the human X chromosome. *Proc. Natl. Acad. Sci. U. S. A.* 96,
- 825 14440–4 (1999).
- 826 13. Lyon, M. F. *X-chromosome Inactivation and Disease. Encyclopedia of Life Sciences*827 (2007). doi:10.1038/npg.els.0005480
- 828 14. Wang, J. et al. Unusual maintenance of X chromosome inactivation predisposes
- female lymphocytes for increased expression from the inactive X. *Proc. Natl. Acad.*
- 830 *Sci.* **113**, E2029–E2038 (2016).
- Bentham, J. et al. Genetic association analyses implicate aberrant regulation of innate
 and adaptive immunity genes in the pathogenesis of systemic lupus erythematosus.
- 833 *Nature Genetics* **47**, (2015).
- 834 16. Zhang, Y. et al. Genes That Escape X-Inactivation in Humans Have High Intraspecific
- 835 Variability in Expression, Are Associated with Mental Impairment but Are Not Slow
 836 Evolving. *Mol. Biol. Evol.* **30**, 2588–2601 (2013).
- 837 17. Vawter, M. P., Harvey, P. D. & DeLisi, L. E. Dysregulation of X-linked gene
- 838 expression in Klinefelter's syndrome and association with verbal cognition. Am. J.

839 *Med. Genet. Part B Neuropsychiatr. Genet.* **144**, 728–734 (2007).

- 840 18. Mackay, M. *et al.* Molecular signatures in systemic lupus erythematosus: distinction
- between disease flare and infection. *Lupus Sci. Med.* **3**, e000159 (2016).
- 842 19. Yen, E. Y. & Singh, R. R. Brief Report: Lupus—An Unrecognized Leading Cause of
- 843 Death in Young Females: A Population-Based Study Using Nationwide Death
- 844 Certificates, 2000–2015. *Arthritis Rheumatol.* **70**, 1251–1255 (2018).
 - 40

- 845 20. Walter, K. *et al.* The UK10K project identifies rare variants in health and disease.
 846 *Nature* 526, 82–90 (2015).
- 847 21. Alfonso Buil, Andrew Anand Brown, Tuuli Lappalainen, Ana Viñuela, Matthew N
- 848 Davies, Hou-Feng Zheng, J Brent Richards, Daniel Glass, Kerrin S Small, Richard
- 849Durbin, T. D. S. & E. T. D. Gene-gene and gene-environment interactions detected by
- transcriptome sequence analysis in twins. *Nat. Genet.* **47**, 88–91 (2015).
- 851 22. Roadmap Epigenomics Consortium *et al.* Integrative analysis of 111 reference human
 852 epigenomes. *Nature* 518, 317–330 (2015).
- 853 23. Martens, J. H. A. & Stunnenberg, H. G. BLUEPRINT: Mapping human blood cell
 854 epigenomes. *Haematologica* 98, 1487–1489 (2013).
- Wong, N. & Wang, X. miRDB: An online resource for microRNA target prediction
 and functional annotations. *Nucleic Acids Res.* 43, D146–D152 (2015).
- 857 25. Schofield, E. C. et al. CHiCP: A web-based tool for the integrative and interactive
- visualization of promoter capture Hi-C datasets. *Bioinformatics* **32**, 2511–2513 (2016).
- 859 26. The ENCODE Project Consortium *et al.* An integrated encyclopedia of DNA elements
- 860 in the human genome. *Nature* **489**, 57–74 (2012).
- 861 27. The GTEx Consortium *et al.* The Genotype-Tissue Expression (GTEx) project. *Nat.*
- 862 *Genet.* **45**, 580–585 (2013).
- 863 28. Hussain, T. & Mulherkar, R. Lymphoblastoid Cell lines: a Continuous in Vitro Source
 864 of Cells to Study Carcinogen Sensitivity and DNA Repair. *Int. J. Mol. Cell. Med.* 1,
- 865 75–87 (2012).
- 866 29. Bezalel, S., Guri, K. M., Elbirt, D., Asher, I. & Sthoeger, Z. M. Type I interferon
- signature in systemic lupus erythematosus. Isr. Med. Assoc. J. 16, 246–9 (2014).
- 868 30. Okamura, Y. et al. COXPRESdb in 2015 : coexpression database for animal species by
- BONA-microarray and RNAseq-based expression data with multiple quality assessment
 41

- 870 systems. **43**, 82–86 (2015).
- 871 31. Lassen, K. G. *et al.* Genetic Coding Variant in GPR65 Alters Lysosomal pH and Links
 872 Lysosomal Dysfunction with Colitis Risk. *Immunity* 44, 1392–1405 (2016).
- 873 32. Huttlin, E. L. *et al.* The BioPlex Network: A Systematic Exploration of the Human
 874 Interactome. *Cell* 162, 425–440 (2015).
- 875 33. Kobayashi, T. *et al.* The histidine transporter SLC15A4 coordinates mTOR-dependent
 876 inflammatory responses and pathogenic antibody production. *Immunity* 41, 375–388
 877 (2014).
- Kelley, L. A. & Sternberg, M. J. E. Protein structure prediction on the Web: a case
 study using the Phyre server. *Nat. Protoc.* 4, 363–371 (2009).
- 880 35. Clarke, A. J. et al. Autophagy is activated in systemic lupus erythematosus and
- required for plasmablast development. Ann. Rheum. Dis. 1–9 (2014).
- doi:10.1136/annrheumdis-2013-204343
- 883 36. Wise, A. L., Gyi, L. & Manolio, T. A. EXclusion: Toward integrating the X
- chromosome in genome-wide association analyses. *Am. J. Hum. Genet.* 92, 643–647
 (2013).
- 886 37. Kwon, K.-S., Cho, H.-Y. & Chung, Y.-J. Recapitulation of Candidate Systemic Lupus
 887 Erythematosus-Associated Variants in Koreans. *Genomics Inform.* 14, 85 (2016).
- 888 38. Farh, K. K. *et al.* Genetic and epigenetic fine mapping of causal autoimmune disease
 889 variants. *Nature* 518, 337–343 (2015).
- 890 39. Trynka, G. *et al.* Chromatin marks identify critical cell types for fine mapping
 891 complex trait variants. *Nat. Genet.* 45, 124–30 (2013).
- 40. Odhams, C. A. et al. Mapping eQTLs with RNA-Seq Reveals Novel Susceptibility
- 893 Genes, Non-Coding RNAs, and Alternative-Splicing Events in Systemic Lupus

894 Erythematosus. *Hum. Mol. Genet.* **0**, ddw417 (2017).

895	41.	Escoubet-Lozach, L. et al. Mechanisms establishingTLR4-responsive activation states
896		of inflammatory response genes. PLoS Genet. 7, (2011).
897	42.	Jiang, W., Zhang, L., Lang, R., Li, Z. & Gilkeson, G. Sex differences in monocyte
898		activation in Systemic Lupus Erythematosus (SLE). PLoS One 9, 1–17 (2014).
899	43.	Suurmond, J. et al. Repeated Fc??RI triggering reveals modified mast cell function
900		related to chronic allergic responses in tissue. J. Allergy Clin. Immunol. 138, 869–880
901		(2016).
902	44.	Blasius, A. L. & Beutler, B. Intracellular Toll-like Receptors. Immunity 32, 305–315
903		(2010).
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910 Figure Legends

911 Figure 1: Genetic refinement of the Xp21.2 (rs887369) SLE suseptability locus.

912 (A) Association plot of the 1Mb region (X:30,077,846-31,077,845) of SLE-associated region Xp21.2 following genotype imputation to the level of UK10K-1000G Phase III and association 913 914 testing as described (n = 10,995 individuals of European ancestry). rs887369 is shown as the most significantly associated lead SNP. Genetic association plots were generated using 915 916 LocusZoom. (B) Association plot of the 1Mb region following conditional analysis on lead 917 SNP rs887369. (C) Haplotype construction and visualisation of the Xp21.2 SLE susceptibility 918 locus conducted in Haploview 4.2. The top panel shows the structure of the three blocks and 919 haplotypes surrounding the lead SNP rs887369 (highlighted in red, block B, SNP #15). Blocks 920 are separated by regions of high recombination as specified by D' and r^2 . The frequency of 921 each haplotype is denoted. The middle panel presents the colour-coded haplotypes and 922 individual SNPs by their genomic coordinates around *CXorf21*. The bottom panel shows the LD structure and pair-wise correlation (r^2) of SNPs, and length of each block. (**D**) Right table: 923 924 case-control association analysis of each haplotype using Haploview 4.2.

925

Figure 2: eQTL association analysis of SLE associated risk SNP rs887369 in immune cell types.

(A) *Cis*-eQTL analysis of rs887369 using male samples of the Geuvadis RNA-Seq expression
cohort profiled in LCLs. The MAGEB family of genes and *NR0B1* were not expressed in LCLs
(RPKM < 1). Allele [C] of rs887369 tags the risk haplotype. The number underneath each box-
plot represents the mean of the group and the number underneath the x-axis refers to the number
of inviduals in each group. (B) *Cis*-eQTL analysis performed for all SNPs in *cis* (+/-1Mb) to

933 rs887369 against CXorf21 expression using the males of the Geuvadis cohort. The coordinate 934 of each SNP is plotted on the x-axis and the $-\log_{10}(P)$ value of association on the y-axis; 935 rs887369 is highlighted as the best eQTL. (C) cis-eQTL analysis of rs887369 against CXorf21 936 expression in LCLs from the TwinsUK cohort using only females who exhibit non-skewed patterns of X-chromosome inactivation (see methods). (D) Relative protein abundance of 937 938 CXORF21 in LCLs from females stratified on genotype at the rs887369 SNP. Relative 939 abundance normalized against beta-actin loading control. Source data are provided in the 940 Source Data file (E) Cis-eQTL analysis of rs887369 using the microarray data from the Fairfax et al^{45,46} and Naranbhai et al⁴⁷ cohorts in primary ex vivo immune cell types (see Methods). 941 942 The remaining *cis*-genes did not pass quality control. Box-plots show minimum (Q1-1.5*IQR), 25th percentile (Q1), Median, 75th percentile (Q3), and maximum Q3+1.5*IQR. 943

944

945 Figure 3: Functional priorization of causal variants at the Xp21.2 SLE suceptibility locus. 946 The five SNPs carried on the risk haplotype attributed to SLE susceptibility and modulation of 947 *CXorf21* gene expression were epigenetically fine-mapped using chromatin data from the 948 Roadmap Epigenomes Project (twelve different marks across 127 cell/tissue types). (A) The 949 five SNPs localised to significant H3K36me3 modification sites in five immune cell types. The 950 heatmap shows the fold-enrichment of H3K36me3 between cell-types across SNP positions. 951 (B) Signal tracks of H3K36me3 in primary monocytes (blue) and primary neutrophils (red) 952 from peripheral blood across the CXorf21 susceptibility locus. Only rs887369 localises to the 953 binding site summit of H3K36me3 in these two cell types. (C) Promoter-capture Hi-C 954 interaction of the rs887369 target locus (chrX :30576528-30582605) with four bait loci across 955 17 primary immune cell types from healthy human donors (Note that the majority of the 956 samples are pooled from multiple donors making it impossible to deconvoloute the sex and genotypes of the individuals). Interaction #1 is the interaction between the association target region (at the 3' end of *CXorf21*) and the *CXorf21* promoter region. (**D**) Heatmap of strength of interaction (CHiCAGO score) of the four interactions across immune cell types. (**E**) Correlation of interaction score for interaction #1 (3' of *CXorf21* and *CXorf21* promoter) with Roadmap Epigenomes Project chromatin marks found at the *CXorf21* promoter across different immune cell types. Higher interactions are correlated with greater enrichment of active chromatin marks suggesting the interaction to regulate gene expression is cell-type specific.

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Figure 4: Expression of *CXorf21* in primary *ex vivo* cells stratified on sex and cellular activation.

967 *CXorf21* expression data in resting and stimulated monocytes from healthy individuals of 968 European ancestry derived from the Fairfax *et al* studies (see Methods; n = 322 biologically 969 independent samples). Samples were separated based on sex and activiation condition: 970 following stimulation with interferon gamma (IFN- γ) or with lipopolysaccharide (LPS) and 971 harvested after 24 hours. For each group, the mean is reported in the corners and the effect size 972 (Cohen's d) is reported along the corresponding three-dimenstional regression plane. Plots were 973 constructed using plot3D for R. Source data are provided as a Source Data file.

974

975 Figure 5: CXorf21 as an interferon response gene

976 (A) Differential gene expression of X chromosome genes in response to IFN- α stimulation 977 (harvested after 6 h) in primary *ex vivo* B cells from healthy females of European ancestry (in-978 house data). Genes highlighted in red are significantly differentially expressed (q < 0.01; 979 absolute fold-change > 2). (**B**) Epigenetic landscape of *CXorf21* using ENCODE transcription factor binding data in LCLs (GM12878 cell line). All five transcription factors have genomewide significant binding sites at the *CXorf21* promoter. Heat colour is a function of signal
strength (fold-change over input).

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984 Figure 6: Super resolution microscopy of CXORF21 and TLR7

985 Structured Illumination Microscopy data showing colocalisation of TLR7 and CXORF21 in ex 986 vivo B cells. Representative results on individual cells are shown in panels A through D with 987 TLR7 staining in the first column, CXORF21 in the second column, DAPI nuclear staining in 988 column three, and in the fourth column all three stains are merged: TLR7 (magenta), CXORF21 989 (green) and DAPI (blue). The B cells are under different conditions in the panels: (A) resting, 990 (B) resting and IFN-α treated (1,000U/ml), (C) Ig/CD40 stimulated, and (D) Ig/CD40 991 stimulated and IFN-α treated *ex vivo* B cells at 20 hours. Maximum intensity projections are 992 shown. Scale bar in white on bottom left hand corner is $2 \mu m$. (E) Plot showing the correlation 993 co-efficients (p) between TLR7 and CXORF21 staining of multiple B cells quantified using 994 the results from Z-stack images from individual cells (represented as open circles). From left 995 to right: unstimulated cells (n=84), cells stimulated with IFN- α (n=60), B cells stimulated with 996 Ig/CD40 (n=32), B cells stimulated with Ig/CD40 and IFN-a (n=22). The horizontal bar 997 represents the mean correlation co-efficient (μ^{ρ}) and the bars above and below this denote the 998 standard deviation of the distribution. (F) Mander's colocalisation coefficient (M2) between 999 TLR7 and CXORF21 are shown from Z-stack images from single B cells (represented as open 1000 circles). From left to right: unstimulated cells (n=84), cells stimulated with IFN- α (n=60), B 1001 cells stimulated with Ig/CD40 (n=32), B cells stimulated with Ig/CD40 and IFN- α (n=22). The horizontal bar represents the mean colocalisation co-efficient (μ^{M2}) and the bars above and 1002

below this denote the standard deviation of the distribution. Source data are provided as aSource Data file.

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1006 Figure 7: CXORF21 and the autophagosome

1007 Structured Illumination Microscopy data showing colocalisation of LC3 and CXORF21 in ex 1008 vivo B cells. Representative results on individual cells are shown in panels A through D with 1009 LC3 staining in the first column, CXORF21 in the second column, DAPI nuclear staining in 1010 the third column, and in the fourth column all three stains are merged: LC3 (magenta), 1011 CXORF21 (green) and DAPI (blue). In panel (A) B cells were Ig/CD40 stimulated, (B) 1012 Ig/CD40 stimulated and bafilomycin-treated, (C) Ig and TLR7/8 stimulated, and (D) Ig and 1013 TLR7/8 stimulated and bafilomycin-treated after 27 hours. Maximum intensity projections are 1014 shown. Scale bar in white on bottom left hand corner is $2 \mu m$. (E) Plot showing the correlation 1015 co-efficients (p) between LC3 and CXORF21 staining quantified using the results from Z-stack 1016 images, individual B cells are represented as open circles. From left to right: Ig/CD40 1017 stimulated cells (n=17), Ig/CD40 stimulated cells in the presence of 10nM bafilomycin (n=22), 1018 B cells stimulated with Ig and resignimod (n=21), B cells stimulated with Ig and resignimod 1019 in the presence of 10nM bafilomycin (n=32). The horizontal bar represents the mean 1020 correlation co-efficient (μ^{ρ}) and the bars above and below this horizontal bar denote the 1021 standard deviation of the distribution. (F) Mander's colocalisation coefficient (M2) between 1022 LC3 and CXORF21 are shown from Z-stack images, individual B cells are represented as open 1023 circles. From left to right: Ig/CD40 stimulated cells (n=17), Ig/CD40 stimulated cells in the 1024 presence of 10nM bafilomycin (n=23), B cells stimulated with Ig and resignimod (n=21), B 1025 cells stimulated with Ig and resignimod in the presence of 10nM bafilomycin (n=32). The horizontal bar represents the mean colocalisation co-efficient (μ^{M2}) and the bars above and 1026

1027 below this denote the standard deviation of the distribution. (G) Western blot analysis of 1028 protein extract from starved LCL, in the left-hand blot CXORF21 is quantified in the absence 1029 of bafilomycin and after 10nM and 100nM treatment. The amount of CXORF21 was quantified 1030 by densitometry and the relative abundance shown against a beta actin control, using the 1031 unstimulated conditions as a reference point. In the right-hand blot sequestosome 1 (p62) is 1032 quantified in the absence of bafilomycin and after 10nM and 100nM treatment. The amount of 1033 Sequestosome-1 was quantified by densitometry and the relative abundance shown against a 1034 beta actin control, using the unstimulated conditions as a reference point. Source data are 1035 provided as a Source Data file.

1036

Figure 8: Summary of factors influencing expression of *CXorf21* at RNA and protein level.

1039 We summarise five factors increasing the cellular abundance of *CXorf21* either at RNA level 1040 or protein level across a range of immune cell types. These are: (1) genetic variation at SLE 1041 susceptibility haplotype - tagged by SNP rs887369 - where the risk haplotype [C] may drive 1042 up-regulation by modulation of chromatin interaction and/or modification of H3K36me3 state; 1043 (2) female sex, in which escape from X-inactivation results in an increased amount of transcript 1044 and protein in females; (3) X chromosome aneuploidy; (4) type I and type II interferons, and 1045 LPS, increase the expression of CXorf21 in ex vivo B cells and monocytes; (5) ancestry -1046 potentially linked to the minor allele frequency of rs887369 - in which higher levels of CXorf21 1047 transcript is observed in LCLs derived from donors with European ancestry. We hypothesise 1048 that elevation of CXORF21 is a risk factor for developing SLE and that this is may be mediated 1049 through it's role in the endosomal pathway. Figure generated by C.A.O.

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1051 Methods References

- Fairfax, B. P. *et al.* Genetics of gene expression in primary immune cells identifies cell
 type–specific master regulators and roles of HLA alleles. *Nat. Genet.* 44, 502–510
 (2012).
- 46. Fairfax, B. P. *et al.* Innate immune activity conditions the effect of regulatory variants
 upon monocyte gene expression. *Science* 343, 1246949 (2014).
- 1057 47. Naranbhai, V. *et al.* Genomic modulators of gene expression in human neutrophils.
 1058 *Nat. Commun.* 6, 7545 (2015).
- Howie, B., Fuchsberger, C., Stephens, M., Marchini, J. & Abecasis, G. R. Fast and
 accurate genotype imputation in genome-wide association studies through pre-phasing. *Nat. Genet.* 44, 955–959 (2012).
- 1062 49. Roshyara, N. R. *et al.* Comparing performance of modern genotype imputation
 1063 methods in different ethnicities. *Sci. Rep.* 6, 34386 (2016).
- 1064 50. Marchini, J. & Howie, B. Genotype imputation for genome-wide association studies.
 1065 *Nat. Rev. Genet.* 11, 499–511 (2010).
- 1066 51. Marchini, J., Howie, B. N., Myers, S., McVean, G. & Donnelly, P. A new multipoint
 1067 method for genome-wide association studies by imputation of genotypes. *Nat. Genet.*1068 **39**, 906–13 (2007).
- 1069 52. Pruim, R. J. *et al.* LocusZoom: Regional visualization of genome-wide association
 1070 scan results. *Bioinformatics* 26, 2336–2337 (2010).
- 1071 53. Barrett, J. C., Fry, B., Maller, J. & Daly, M. J. Haploview: Analysis and visualization
 50

- 1072 of LD and haplotype maps. *Bioinformatics* **21**, 263–265 (2005).
- 1073 54. Gabriel SB *et al.* The structure of haplotype blocks in the human genome. *Science (80-*1074 .). **296**, 2225–2229 (2002).
- 1075 55. Chang, C. C. *et al.* Second-generation PLINK: rising to the challenge of larger and
 1076 richer datasets. 1–16 (2014). doi:10.1186/s13742-015-0047-8
- 1077 56. Kent, W. J., Sugnet, C. W., Furey, T. S. & Roskin, K. M. The Human Genome
 1078 Browser at UCSC W. *J. Med. Chem.* 19, 1228–31 (2002).
- 1079 57. Lappalainen, T. *et al.* Transcriptome and genome sequencing uncovers functional
 1080 variation in humans. *Nature* 501, 506–11 (2013).
- 1081 58. Auton, A. *et al.* A global reference for human genetic variation. *Nature* 526, 68–74
 1082 (2015).
- 1083 59. Shabalin, A. A. Matrix eQTL: ultra fast eQTL analysis via large matrix operations.
- 1084 *Bioinformatics* **28**, 1353–1358 (2012).
- 1085 60. Naumova, A. K. *et al.* Heritability of X chromosome-inactivation phenotype in a large
 1086 family. *Am. J. Hum. Genet.* 58, 1111–1119 (1996).
- 1087 61. Amos-Landgraf, J. M. et al. X Chromosome–Inactivation Patterns of 1,005
- 1088 Phenotypically Unaffected Females. Am. J. Hum. Genet. 79, 493–499 (2006).
- Kristiansen, M. *et al.* Twin study of genetic and aging effects on X chromosome
 inactivation. *Eur. J. Hum. Genet.* 13, 599–606 (2005).
- 1091 63. Stegle, O., Parts, L., Piipari, M., Winn, J. & Durbin, R. Using probabilistic estimation

1092		of expression residuals (PEER) to obtain increased power and interpretability of gene
1093		expression analyses. Nat. Protoc. 7, 500–507 (2012).
1094	64.	Thorvaldsdottir, H., Robinson, J. T. & Mesirov, J. P. Integrative Genomics Viewer
1095		(IGV): high-performance genomics data visualization and exploration. Brief.
1096		Bioinform. 14, 178–192 (2013).
1097	65.	Javierre, B. M. et al. Lineage-Specific Genome Architecture Links Enhancers and
1098		Non-coding Disease Variants to Target Gene Promoters. Cell 167, 1369–1384.e19
1099		(2016).
1100	66.	Mifsud, B. et al. Sup Mapping long-range promoter contacts in human cells with high-
1101		resolution capture Hi-C. Nat. Genet. 47, 598–606 (2015).
1102	67.	Martin, P. et al. Capture Hi-C reveals novel candidate genes and complex long-range
1103		interactions with related autoimmune risk loci. Nat. Commun. 6, 1–17 (2015).
1104	68.	Cairns, J. et al. CHiCAGO: Robust Detection of DNA Looping Interactions in Capture
1105		Hi-C data. Genome Biol. 17, 28068 (2016).
1106	69.	Lockstone, H. E. Exon array data analysis using Affymetrix power tools and R
1107		statistical software. Brief. Bioinform. 12, 634–644 (2011).
1108	70.	Leek, J. T., Johnson, W. E., Parker, H. S., Jaffe, A. E. & Storey, J. D. The SVA
1109		package for removing batch effects and other unwanted variation in high-throughput
1110		experiments. Bioinformatics 28, 882-883 (2012).
1111	71.	Tan, E. M. et al. The 1982 revised criteria for the classification of systemic lupus
1112		erythrematosus. Arthritis Rheum. 25, 1271–1277 (1982).
	52	

1113	72.	Bombardier, C., Gladman, D. D., Urowitz, M. B., Caron, D. & Chang, C. H.
1114		Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee
1115		on Prognosis Studies in SLE. Arthritis Rheum. 35, 630-640 (1992).
1116	73.	Lagache, T., Sauvonnet, N., Danglot, L. & Olivo-Marin, J. C. Statistical analysis of
1117		molecule colocalization in bioimaging. Cytom. Part A 87, 568–579 (2015).
1118		