The autoimmune disease risk allele rs6897932 modulates monocyte IL7R surface and soluble receptor levels in a context-specific manner

Hussein Al-Mossawi[2], Evelyn Lau[3], Sara Danielli[1], Nicole Yager[2], Jelle de Wit[2], Seiko Makino[3], James Gilchrist[3], Wanseon Lee[3], Isar Nassiri[1], Elise A Mahe[1], Laila Rizvi[2], Jane Cheeseman[4], Matt Neville[4], Julian C Knight[3¶], Paul Bowness[2¶], Benjamin P Fairfax[1¶*]

¶ joint contribution, *corresponding author

[1]Department of Oncology, Weatherall Institute of Molecular Medicine; [2]Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences; [3]Nuffield Department of Medicine, Wellcome Trust Centre for Human Genetics; [4]Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, United Kingdom.

Abstract

IL-7 is a key factor in T-cell immunity and *IL7R* polymorphisms are implicated in autoimmune pathogenesis. We previously reported an expression quantitative trait locus (eQTL) at rs931555, 5' to *IL7R,* associated with stimulated monocyte IL7R mRNA expression. Unlike in T-cells, a role for IL7R in monocyte biology is poorly described. Here we detail replication and characterization of this eQTL at protein level across cell subsets and conditions in a separate cohort. We find rs6897932, a non-synonymous IL7R polymorphism associated with susceptibility to Multiple Sclerosis, Ankylosing Spondylitis and Primary Biliary Cirrhosis, is the

key determinant of monocyte IL7R surface expression and soluble IL7R (sIL7R) and functions in a context-specific manner. Monocyte surface IL7R is markedly induced by LPS and TNF stimulation under the genotypic regulation of rs6897932, whereas no effect of this allele was observed on $CD4^+$, $CD8^+$ or $CD56^+$ cell surface IL7R or in unstimulated monocytes. LPS-induced monocyte release of sIL7R was strongly associated with both rs6897932 genotype and expression of the splicing factor gene DDX39A. After induction of IL7R expression, human monocytes display a robust and pleiotropic transcriptional response to exogenous IL-7. Monocytes from the synovial fluid of patients with Spondyloarthritis were similarly found to express high levels of surface IL7R. These data demonstrate that disease-associated genetic variants in the IL7R gene critically impact monocyte IL7R and sIL7R expression following innate immune stimulation, suggesting a previously unappreciated key role for monocytes in IL-7 pathway biology and IL7R-associated diseases.

Introduction

Elucidating the cell types and context in which disease risk loci have functional effects can provide novel insights into disease aetiology and pathogenic mechanisms. Previous expression quantitative trait locus (eQTL) analysis of monocytes stimulated with the Toll-like receptor 4 (TLR4) agonist lipopolysaccharide (LPS) or with interferon gamma found over 40% of all genes with eQTL to only be detectable in the induced state[1]. One such eQTL that mapped to rs931555 (chromosome 5p13.2, intronic to *SPEF2* and *cis* to *IL7R*) was associated with IL7R expression (encoding the alpha chain of the IL-7 receptor

IL7R, CD127) upon exposure to LPS. rs931555 is in linkage disequilibrium (LD) with rs6897932 (r2=0.75, D'=0.87), a non-synonymous polymorphism in *IL7R* associated with differential splicing of the 6th exon of the gene[2]. The resultant splice variant is lacking the trans-membrane domain of the receptor and is instead translated to form a non-membrane bound soluble receptor[2]. In European populations the major allele at rs6897932 is a risk allele for Multiple Sclerosis (MS)[2–4], Primary Biliary Cirrhosis[5] and is a proxy for rs11742270, a risk allele for Ankylosing Spondylitis (AS)[6]. IL7R heterodimerizes with the common gamma chain (CD132) to form a functional receptor for the cytokine IL-7 and is essential in T-cell development, with mutations in man associated with a severe combined immunodeficiency phenotype[7]. Whilst unstimulated monocytes have very low IL7R mRNA expression, the transcript is markedly induced in monocytes after prolonged stimulation[1,8]. Interestingly, increased numbers of IL7R^{+} macrophages have also been reported in the blood and synovium of Rheumatoid Arthritis patients[9]. Given the large effect size of the eQTL to this gene after LPS stimulation, and the high LD with a disease-associated polymorphism, in this paper we aimed to ascertain the degree to which monocyte surface IL7R could be detected upon induction of expression across a population of healthy individuals whilst systematically characterizing the functional consequences of genetic variation at this locus. Considering the key role of IL7R in T-cell development and maturation, we performed parallel experiments in isolated monocytes under the same conditions as our previous eQTL analysis, as well as across mixed cell populations using flow cytometry. This enabled simultaneous analysis of quantitative genetic effects on IL7R surface expression across cell subsets.

Here we report that monocytes from healthy individuals robustly express surface protein IL7R after exposure to innate immune activators, and that this is strongly associated with

genetic variation at rs6897932. Moreover, we observe no consistent effect of this allele on surface expression of IL7R across lymphoid subsets from the same samples. Stimulated monocytes similarly release high levels of non-membrane bound IL7R, with the amount correspondingly associated with both rs6897932 allele and expression of the splicing factor gene DDX39A. We describe the monocyte transcriptional responses to exogenous IL-7 after induction of IL7R. Finally we demonstrate IL7R^{+} monocytes are comparatively enriched in the synovial fluid of patients with active Spondyloarthritis. These data demonstrate that genetic variation in the IL7R gene impacts IL-7 biology specifically in the setting of inflammation and suggest a hitherto unappreciated myeloid role in the functional mechanism of disease risk variants.

Results

Monocyte activation induces expression of surface IL7R

Both peripheral blood mononuclear cells (PBMCs) and CD14^{+} bead-purified monocytes from healthy volunteers were incubated for 24 hours in the presence or absence of LPS and surface IL7R was characterized with flow cytometry (Supplementary Figure 1). Percentage IL7R^{+} within isolated monocytes was lower than that within PBMCs (Median 0.7 vs. 1.8%, IQR_{mono} 0.31 -0.83%, IQR_{PBMC} 1.02-3.64, n_{PBMC}=157, n_{mono}=84, P=<2.2^{-16} - Wilcoxon test). In both preparations treatment with LPS led to a pronounced induction of surface IL7R (Figure 1a-b), demonstrating that LPS induction of IL7R mRNA is accompanied by readily detectable surface receptor expression. LPS elicits release of multiple cytokines from monocytes in PBMCs, including IL4 and IL6, which can reduce T-cell surface IL7R[10]. In keeping with this, we observed CD4^{+}, CD8^{+} T-cells and CD56^{+} NK cell surface IL7R to markedly fall upon stimulation (Figure 1c, Supplementary Figure 3). There was a large degree of inter-individual variation in IL7R response to LPS,

particularly in monocytes, and there was little correlation between cell types (Figure 1d), indicative of cell-type specific regulation. Notably, whilst within-cell type correlation of IL7R^{+} between treated and untreated lymphoid cells was high, correlation between percentage IL7R^{+} CD14 cells between untreated and LPS treated states was poor (CD14^{+} within PBMC culture: r=0.20, p=0.03; purified monocytes: r=0.05, p=0.66). These effects are thus highly context- and cell-type specific, with significant implications for any investigation of genotypic effects and for understanding function in health and activated states.

Monocyte IL7R expression is modulated by TNF

To test whether monocyte IL7R induction was specific to LPS we explored the effect of the TLR1:2 agonist Pam3CysK4 and the TLR7 agonist Imiquimod in six individuals. Whilst Pam3CysK4 was able to induce monocyte IL7R expression across all samples, the response to Imiquimod was variable and non-significant (Figure 2a). Since TLR agonists elicit the release of multiple cytokines we reasoned that late IL7R induction might relate to the magnitude of earlier (autocrine) cytokine response. We therefore explored the relationship between genes induced at 2 hours and expression of IL7R at 24 hours following LPS for monocytes from 228 individuals[1]. We noted 382/15421 probes (FDR<0.05) where the 2 hour expression was correlated with 24 hour IL7R (Supplementary table 1). Within the top 10 associated genes we found both tumour necrosis factor (*TNF*) and chemokine ligand 5 (*CCL5*) were strongly associated (*TNF*: r=0.34, P adj.=2.9^{-4}; CCL5: r=0.36, P adj.=1.1^{-4}; Figure 2b). Given the key role for anti-TNF therapies in the treatment of many autoimmune conditions, we sought to explore whether released TNF may drive monocyte IL7R induction in an autocrine manner by incorporating the anti-TNF monoclonal antibody Infliximab in the media in addition to LPS. This led to a significant reduction in IL7R^{+} monocytes at the end of the experiment

(n=69, mean -8.1%, 95 CI: -5.7:-10.5%, $P=5.6^{-9}$, figure 2c). Finally, we repeated the experiment in a further 78 individuals, incubating cells with TNF (10ng/ml) for 24 hours as opposed to LPS. This showed that, akin to LPS, TNF alone robustly induces the expression of surface IL7R in monocytes (Figure 3d), whilst it concomitantly reduces T-cell IL7R surface positivity.

rs6897932 specifically regulates monocyte cell surface IL7R levels

In our previous eQTL analysis we reported the most significant association for *IL7R* mRNA expression after exposure to LPS for 24h with rs931555 ($P=2.1^{-26}$), which was also detectable after 2h LPS (Figure 3a). Interrogating this data further, we find rs6897932 is associated with *IL7R* expression ($P=8.6^{-14}$) reflecting the degree of LD between these two loci. Conditional analysis controlling for rs931555 resolves the rs6897932 association however (P=0.92, Supplementary Figure 4). By contrast, controlling for rs6897932 leaves a residual effect of rs931555 ($P=7.6^{-11}$), suggesting that rs931555 likely marks the functional haplotype at the mRNA level. Upon completion of the flow-cytometry experiments, we genome-wide genotyped the cohort using the Illumina OmniExpress array. Genotypes were further imputed using the UK10K dataset and association with surface expression in purified monocytes post LPS stimulation was analysed (n=93).

We found that the most significant locally associated SNP with surface expression of IL7R in purified monocytes post LPS was rs6897932, with no significant association in the unstimulated samples (Figure 3b,3c). In monocytes within LPS stimulated PBMC

cultures the genetic association was weaker, although again a significant association with rs6897932 was apparent (Figure 3d). As noted, the surface expression of IL7R in PBMC cultures was higher than in purified monocytes. We also observed a weak effect of rs6897932 in unstimulated PBMCs (Supplementary Figure 5), suggesting that carriage of this allele not only modulates surface expression of monocyte IL7R upon robust stimuli but may also sensitise monocyte IL7R expression to the levels of other cytokines. Of note, we were unable to observe a genetic association between surface IL7R and rs6897932 in either $CD4^+$ or $CD8^+$ T-cells, in the basal (Supplementary Figure 5) or LPS/TNF-stimulated state. We had reasoned that if genetic regulation of IL7R expression in T-cells was context dependent then an alternative stimulant to TNF might be required. To explore this we subjected a subset of samples (n=44) to T-cell-specific stimulation with CD3/CD28. This reduced T cell IL7R surface levels whilst inducing robust monocyte IL7R expression, without evidence of a genetic association (Supplementary figure 6). Finally we explored the association between rs6897932 genotype and monocyte surface IL7R after TNF alone, again finding an association, with the disease risk major allele being associated with reduced surface IL7R ($P<0.0007$, Figure 3e). In this experiment a weak association for $CD4^+$ T-cell IL7R expression was also noted, although after taking into account the number of tests performed this was not significant. These data demonstrate this rs6897932 has a previously unrecognized role in regulating the surface expression of monocyte IL7R after exposure to inflammatory mediators LPS and TNF.

Monocyte derived sIL7R is regulated by rs6897932 carriage and associated with DDX39A expression

The reported role of rs6897932 in determining splicing of a soluble form of IL7R in PBMCs[2] led us to investigate whether stimulated monocytes produce sIL7R and if this is

under allelic control. We performed an ELISA for sIL7R on supernatant from randomly selected monocyte samples treated with LPS for 24 hours from our previous eQTL study (n=99). We observe a strong association with rs6897932 and sIL7R, whilst notably there was no association with rs931555 if rs6897932 allele was not considered. When controlling for rs6897932 however we observed a weak but significant effect of rs931555 allele (P=0.018) on soluble protein, consistent with the lead eQTL SNP having a synergistic effect with rs6897932 to modulate soluble receptor (Figure 4a).

It has recently been reported that rs2523506, at the 6p21 gene *DDX39B*, is in epistasis to rs6897932 and that allelic combinations of these two loci further increase the risk of multiple sclerosis[11]. *DDX39B* encodes an RNA helicase that forms a component of the spliceosome[12] . Risk polymorphisms in this gene were associated with increased sIL7R in rs6897932 risk allele carriers[11]. In light of these findings, we proceeded to explore both genetic and transcriptional associations between *DDX39B* and sIL7R in our cohort. Given the low frequency of the minor allele at rs2523506 we were likely underpowered to replicate an epistatic effect and we did not observe a significant interaction between these two alleles on soluble protein levels (Supplementary Figure 7). For all samples with ELISA-measured sIL7R we had previously measured gene expression using microarrays and this enabled us to explore associations between mRNA expression and sIL7R. In support of the relative independence of soluble protein and total transcript we see no association between total IL7R transcript and sIL7R (Figure 4b). In monocytes we did not find an association between DDX39B expression (at either 2h or 24h of LPS) and sIL7R. In support of the postulated role of the spliceosome on sIL7R levels however, we did note a significant correlation between expression of the DDX39B paralogue DDX39A after 24h LPS and sIL7R at this time, with increased expression of DDX39A

being associated with reduced sIL7R (2h LPS mRNA vs. sIL7R rho=-0.31, P=0.01; 24h LPS mRNA vs. sIL7R rho=-0.463,P=6.2^{-6}, FDR<0.05, Figure 4c, Supplementary Table 2). This is in keeping with the directional effect of DDX39B reported in transfected HeLa cells[11]. Total IL7R transcript and DDX39A expression were not correlated (Supplementary Figure 7), supporting a specific role for DDX39A in sIL7R splicing as opposed to expression.

Monocyte surface IL7R is sensitive to IL-7 and stimulation activates multiple transcriptional pathways

Although our data demonstrate evidence for monocyte IL7R expression, translation and surface trafficking in the context of inflammatory ligands, whether $IL7R^{+}$ monocytes are sensitive to IL-7 is unclear. To explore this, we co-incubated fresh PBMCs with LPS in the presence of recombinant human IL-7 and performed flow cytometry for a subset of individuals. This demonstrated that, as previously reported for T-cells[10], co-incubation with IL-7 led to a marked reduction in monocyte surface IL-7 expression, indicating sensitivity of monocyte IL7R to free IL-7 (Figure 5a). We next explored the transcriptional profile of IL7R monocytes following exposure to IL-7. Monocytes were stimulated with LPS for 22 hours to induce IL7R and then media was supplemented with IL-7 for a further two hours. RNA sequencing was performed on these cells, as well as cells treated with LPS alone from the same individuals. Pairwise differential expression analysis performed using the DESEQ2 package[14] demonstrated that this relatively short exposure to IL-7 elicited marked transcriptional activity - with 3240/16186 transcripts tested being differentially expressed (FDR<0.05, Supplementary table 3 and Figure 5b). 1714 transcripts were suppressed, including marked downregulation of the genes encoding transcription factor TCF7 which is required for immature thymocyte survival, the non-coding RNA MALAT1 (Figure 5c) and the cell-surface marker encoding CD6. IL-

7 has been demonstrated to be capable of inducing the cytokines LTA and LTB which are crucial for the formation of embryological peripheral lymph nodes[15] and we find these cytokines are in the top 10 of 1526 IL-7 induced transcripts, a list headed by the transcriptional activator MYB.

Gene ontology analysis of suppressed genes did not identify any significantly enriched biological pathways, whereas analysis of induced genes was consistent with the known anti-apoptotic effects of IL-7 with significant enrichment of genes involved in translation, ribosomal small subunit biogenesis, and DNA repair (Figure 5d). Also of relevance was upregulation of (TAP-dependent) peptide-antigen presentation indicating a potentially enhanced presenting capacity of stimulated monocytes. Disease ontology analysis of all IL-7 differentially expressed genes showed very significant enrichment for neurodegenerative pathways as well as response to E.Coli infection (Figure 5e), demonstrating the potential disease relevance of our findings.

IL7R+ monocytes are detectable in synovial fluid of Spondyloarthritis patients

Although our data clearly show the ability of monocytes to induce and express surface IL7R in response to stimulation, the physiological and pathological significance of these novel observations remains uncertain. To explore the relevance of these cells in an inflammatory disease state, we performed pairwise flow cytometry from blood and synovial fluid in 4 patients with spondyloarthropathies (2 AS, 2 PsA). We found that, whereas IL7R^{+} monocytes were infrequent within PBMCs from these individuals, they were readily observed in the synovial fluid from actively inflamed joints, comprising 12-35% of the monocyte population (Figure 5f,g). This localizes IL7R positive monocytes to sites of active inflammation in disease states.

Discussion

The degree to which eQTL correspond to subsequent protein quantitative trait loci is variable[15] and relatively poorly characterised for primary immune tissues. The IL7R eQTL was a particularly intriguing finding from our previous study given the large effect size (r^2=0.30), the LD with a disease associated locus, and the fact that IL7R has not previously been associated with monocyte function. Here we find the disease-associated polymorphism rs6897932 is most significantly associated with IL7R surface expression in isolated monocytes post LPS. As per the eQTL data, the surface receptor QTL is only observed in the stimulated state and has the same directional effect in both isolated monocyte cultures (as per the original eQTL analysis) and in whole PBMC cultures, reinforcing the reproducibility of these findings. The anti-TNF monoclonal antibody infliximab reduces LPS induction of IL7R. This both implicates autocrine release of TNF, and suggests that our observations may be of pathogenic and clinical significance, since anti-TNF therapy plays a key role in the disease management of the spondyloarthritides. We further confirm that TNF alone can elicit monocyte IL7R expression, an observation previously made in rheumatoid arthritis blood and synovial macrophages[9]. In doing so we again replicate the allelic effect of rs6897932 on monocyte surface IL7R in a separate cohort upon treatment with TNF. Monocytes act as primary secretors of TNF during acute inflammatory events and so this observation of monocyte-specific feedback resulting in expression of IL7R is of particular interest given the accumulating evidence for myeloid role in many autoimmune disease states.[16]

The observation of increased numbers of $IL7R^+$ monocytes in unstimulated PBMCs (compared to cultured purified monocytes) demonstrates that monocyte IL7R

expression is influenced by other cell subsets. This is supported by increased IL7R^{+} monocyte expression following specific T-cell stimulation, and the previous observation that IL-7 treatment of T-cells can elicit monocyte TNF expression[17], demonstrates complex cellular cross-talk in the IL-7 pathway.

Soluble IL7R is thought to potentiate IL-7 signaling[18] and likely plays an important role in IL-7 biology and disease associations. We find activated monocytes produce considerable amounts of sIL7R in a rs6897932-delineated manner suggesting that, in the context of innate immune activity, monocytes may significantly contribute to the local pool of sIL7R. Although we have limited power to determine epistatic effects, analysis of individuals heterozygous for rs6897932 demonstrates an additional significant allelic effect of rs931555 (in the direction of the previously identified eQTL) on the regulation of sIL7R. It would thus appear that rs6897932 is the primary determinant of sIL7R release from monocytes and is also key in determining surface expression. In keeping with a recently described role for the spliceosome component DDX39B in IL7R splicing[11], we observed a highly significant correlation between expression of the DDX39B paralogue gene, DDX39A, and sIL7R levels, with increasing expression of DDX39A associated with reduced sIL7R. The absence of association to DDX39B may reflect cell type specific mechanisms and may reflect a divergence from the steady state induced by stimulation.

We are unable to observe a strong genetic effect of rs6897932 on surface expression of IL7R in either CD4+ or CD8+ T-cells in both the resting or stimulated states. Given we did not measure T-cell derived soluble IL7R we cannot exclude an

allelic effect of rs6897932 on this, but it is notable that previous associations of this allele on soluble IL7R mRNA were from mixed cell populations[18]. Furthermore, there is an absence of an eQTL to IL7R in the GTEX data at either rs931555 or rs6897932 and an eQTL is not observed in $CD4^+$ T-cells[19]. Whereas soluble IL7R levels are consistently demonstrated to be raised in autoimmune conditions – notably MS[18], specific attempts to assay surface IL7R in T-cell subsets have not demonstrated an allelic association with rs6897932[20–22]. Our data is thus in keeping with a predominant effect of this allele on both transcript and surface protein expression in activated monocytes.

There are a number of questions this study raises – namely, despite prolonged LPS treatment, only a limited subset of monocytes become positive for IL7R (never greater than 60% of total). Further studies using single cell sequencing should help delineate whether the $IL7R^+$ fraction represents one or more distinct monocyte subsets. These approaches will be especially pertinent in the characterization of IL7R monocytes after exposure to IL-7. IL7R can also dimerize with the thymic stromal lymphopoietin receptor[23,24], however monocyte expression of this is very low and it is not induced by LPS[1], thus we focused in this study to investigate the role of IL-7 on monocyte biology. It will be interesting to assess whether $IL7R^+$ monocytes in areas of inflammation have transcriptional profiles akin to those stimulated by IL-7 as this would support *in vivo* monocyte IL-7 responses. The overlap with neurodegeneration pathways (which may bear relevance to MS), and the predicted enhanced antigen presenting capacity of such cells are similarly intriguing aspects for further work. Moreover, whilst we demonstrated TNF induces IL7R expression, the degree of this response tended to be less than to that of LPS. This was unlikely to be a dosage effect, as higher concentrations of TNF led to cell death (not shown), but instead suggests a role for other co-released cytokines or the

TLR4 pathway directly. Finally, whilst we did not observe an effect of this allele on other cell subsets, our analysis was of crude $CD4^+$ and $CD8^+$ fractions, and thus allelic modulation of surface IL7R in smaller subsets cannot be discounted.

Previous analyses have shown an association between rs6897932 and $CD8^+$ T-cell count[6], an observation reinforced by analysis of UK biobank datasets where rs6897932 is associated with lymphocyte percentage[25]. Given sIL7R can potentiate T-cell signalling and survival[18], it is highly plausible that monocyte-derived sIL7R may provide one source of this, specifically in the context of inflammation. The other key aspect of this allele being that the protective allele leads to both reduced soluble IL7R, whilst simultaneously increasing surface expression. Given the vital role played by bioavailability of IL-7 in T-cell populations, it may be envisaged that monocyte surface IL7R can further act as a sink for free IL-7[26] – whether such cells have separate immunomodulatory potential warrants further investigation. Interestingly, in the UK biobank both rs931555 and rs6897932 are far more strongly associated with eosinophil count and percentage than that of lymphocytes (http://geneatlas.roslin.ed.ac.uk) further implicating a previously unrecognized myeloid role for this allele.

In summary, our study provides robust evidence supporting a role for the disease associated polymorphism rs6897932 in specifically modulating monocyte surface and sIL7R in the context of inflammation. We further demonstrate that IL7R-expressing monocytes are highly responsive to IL-7 and are readily observed in the joints of patients with spondyloarthropathies. Thus monocytes have a previously unappreciated and potentially pathogenic role in IL-7 biology.

Figure legends:

Fig. 1 LPS induces profound monocyte IL7R cell surface expression

1 a) Representative flow cytometry plots from live gated positively selected CD14 monocytes from one individual where cells were incubated for 24h alone (top panel) or in presence of LPS (bottom panels) from same individual

1 b) Violin plot demonstrating significant induction of IL7R+ monocytes in across paired cultures of positively selected monocytes (Monocyte IL7R+ post LPS- median:23.8, min:6.5, max:55.8, IQR:15.6-34.9%; n=84, paired T-test)

1c) Comparative effects of LPS on IL7R+ counts across CD14+ monocytes, CD4+ and CD8+ T-cells and CD56+ NK cells from the same PBMC cultures, untreated or with LPS for 24h (PBMC IL7R+ post LPS- median:29.9%, min:5.9, max:66.40, IQR:22.3-40.0; n=157, paired T-test)

1d) Pearson correlation analyses were performed between indicated cells and treatments on number of IL7R+ cells. Effect sizes are indicated for where correlation

adjusted P <0.05.

Fig. 2 LPS induced monocyte IL7R cell surface expression is mediated significantly by autocrine TNF production

2 a) Comparative induction of IL7R+ monocytes from 6 individuals with monocytes treated for 24h with either the TLR7 agonist Imiquimod, TLR1/2 agonist Pam3CysK4 or TLR4 agonist LPS (one way ANOVA).

b) Expression of TNF at 2h LPS assayed versus expression of IL7R from monocytes from same individuals at 24h LPS.

c) Comparative incubation of monocytes alone (n=69), with LPS or with LPS + anti-TNF monoclonal antibody (Infliximab). Incubation with TNF antagonist significantly reduces LPS induced IL7R+ monocyte counts (paired t-test).

d) Violin plots of responses across cell types of PBMCs treated with TNF alone leads to significant induction of IL7R+ monocytes and significantly reduces CD4 and CD8 T-cell IL7R+ positivity (n=78, paired T-test).

Fig. 3 rs6897932 specifically regulates monocyte cell surface IL7R levels

3 a) Microarray data demonstrating eQTL to IL7R noted at rs931555 after both 2 and 24 hour treatment with LPS (2h LPS: n= 261, 24h LPS n= 322)

b) Local association plot for monocyte surface IL7R after exposure to LPS from positively selected monocytes demonstrates peak association to rs6897932 (n=84)

c) Batch corrected log values for surface IL7R from positively selected monocytes demonstrating significant effect of rs6897932 carriage after stimulation in comparison to

baseline monocytes (n=103, ANOVA for linear fit model)

d) Surface IL7R in PBMCs across cell subsets after exposure to LPS. (Note a significant effect of rs6897932 on surface IL7R is only observed in CD14+ monocytes, ANOVA for linear fit model)

e) As per d) but PBMCs treated with TNF for 24h (n=62)

Fig. 4 Monocyte derived soluble IL7R is regulated by rs6897932 carriage and DDX39A expression

4 a) Elisa of soluble IL7R performed on supernatants of monocytes treated with LPS for 24h (n=99). Samples were randomly chosen from original eQTL dataset and genotypes revealed *post-hoc* – a significant association was observed at rs6897932 with a weak additional effect at rs931555 (linear model)

b) Correlation analysis of soluble IL7R (x axis) and IL7R mRNA from same samples at 24h post LPS treatment is poor, indicative of predominant post-transcriptional regulation for soluble IL7R

c) Correlation analysis was performed between 15421 microarray probes and soluble IL7R (y-axis). This identified DDX39A expression to be significantly anti-correlated with soluble IL7R levels, supporting evidence for its regulation of soluble IL7R

Fig. 5 Monocyte surface IL7R is functional, activating multiple transcriptional pathways. Surface IL7R-expressing monocytes are present in joints of patients with Spondyloarthritis.

5 a) Violin plot demonstrating monocytes from PBMC cultures in the untreated state, after exposure to LPS and after exposure to LPS with recombinant IL-7. IL-7 leads to

marked downregulation of IL7R on LPS stimulated monocytes, indicative sensitivity to exogenous cytokine (paired T-test).

b) Volcano plot of mRNA from RNAseq experiments of 8 paired monocyte samples either treated with LPS alone for 24h or with LPS with additional IL-7 added for the last 2h of culture. Treatment leads to widespread differential transcript expression with the most significant 20 transcripts labelled.

c) Results from gene ontology biological pathway analysis was performed on IL-7 induced genes with all significant pathways labelled

d) Results of disease ontology pathway analysis – all terms with Z-score > 3 were significant after correction for multiple testing (FDR<0.05)

e) Example boxplots of genes differentially regulated by recombinant IL-7 in monocytes (linear model)

f) Representative flow cytometry of IL7R+ cells gated on CD14 from a patient with AS i) top panel – PBMC derived monocytes ii) isotype control iii) synovial fluid mononuclear cells (SFMC) from inflamed joint from same individual

g) Results from 4 patients demonstrating comparative monocyte IL7R+ in PBMC and SFMC

Supplementary figures

S1 IL7R MFI correlates with percentage IL7R positive monocytes

S2 Correlation between baseline and induced expression of IL7R across cell types after exposure to LPS

S3 Cross cellular correlation of IL7R+ between individuals

S4 Association plots for ILR in LPS stimulated monocytes

S5 Surface IL7R by rs6897932 allele in untreated PBMC cultures

S6 Induction of monocyte IL7R+ by T-cell specific stimulation with CD3.CD28 beads

S7 Exploration of epistatic effect of rs2523506 and rs6897932

Supplementary tables:

T1 Genes where expression at 2h LPS is correlated with 24h LPS IL7R

T2 Genes correlated with soluble IL7R at 24h LPS

T3 Genes differentially regulated by IL-7

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Materials and Methods

Study participants

Peripheral blood was obtained from genotyped individuals recruited via the Oxford biobank (www.oxfordbiobank.org.uk) with full ethical approval (REC: 06/Q1605/55) and written informed consent. Genotype was blinded at the time of study and only revealed at the end of recruitment. Peripheral blood and synovial fluid samples from were recruited with informed consent from patients with inflammatory arthritis attending the Oxford University Hospitals NHS Foundation Trust (Ethics reference number 06/Q1606/139).

Cell isolation and stimulation

Whole blood was collected into EDTA tubes (BD vacutainer system) and peripheral blood mononuclear cells were obtained by density centrifugation (Ficoll Paque). CD14 cell isolation was carried out by positive selection (Miltenyi) according to the manufacturer's instructions. Cells were rested overnight (16h) at 37°C, 5% CO2 in 5ml non-adherent polypropylene cell-culture tubes (BD Biosciences) prior to stimulation assays. Cells were stimulated for 24 hours with 20ng/ml LPS (Invivogen), 100 ng/ml pam 3cysk4 (manufacturer), 100ng/ml imiquimod (manufacturer), 20ng/ml TNF (manufacturer), 10ng/ml IL-7 (Peprotech) and CD2/3/28 beads (Miltenyi) at a ratio of 1

bead to 2 cells. In all experiments an unstimulated incubator control was included. TNF blockade was achieved with 5µg/ml of infliximab (Remicade, Janssen).

Flow cytometry

Staining antibodies and dye clones, dilutions and manufacturer shown in supplementary table 1. Cells were stained in phosphate buffered saline containing 1% fetal calf serum on ice and in the dark for 20 minutes, then fixed in 1.6% paraformaldehyde. All samples included fixable amine reactive viability dye and isotype control for IL-7R. Flow cytometry was performed on a BD Fortessa calibrated daily with calibration and tracking beads from BD Biosciences. Data was analysed using FlowJo software (Treestar®).

ELISA

Cell supernatants from stimulated cells were collected and sIL7R quantified as previously described[11]. Briefly, 96-well plates were coated overnight with 1 µg/ml mouse anti-human CD127 mAb (R&D Systems). Plates were blocked with 5% BSA for 1 hour, washed, and cell supernatants added for 2 hours. Bound sIL7R was detected with 12.5 ng/ml biotinylated goat anti-human CD127 polyclonal Ab (R&D Systems) for 1 h, followed by a 30 min incubation with streptavidin-HRP and a 15 min incubation with TMB peroxidase substrate (Thermo Fisher). The reaction was stopped with H_2SO_4 and plates read at 450 nm. A standard curve was generated using recombinant human CD127 Fc chimera protein (R&D Systems).

RNA extraction

The AllPrep DNA/RNA/miRNA kit (Qiagen) was used for RNA extraction. Cells were spun down and re-suspended in 350µl of RLTplus buffer and transferred to 2ml tubes. Samples were then stored at -80°C for batched RNA extraction. Homogenization of the

sample was carried out using the QIAshredder (Qiagen). DNase I was used during the extraction protocol to minimise DNA contamination. RNA was eluted into 35µl of RNase-free water. The RNA amount was quantified by qubit and the RNA samples stored at -80°C for storage until ready for sequencing.

RNA sequencing

RNA sequencing was carried out at the Wellcome Trust Centre for Human Genetics core facility. RNA underwent quality control testing using a bioanalyser (RNA 6000 Nano kit, Agilent) followed by cDNA library preparation. Paired end sequencing was performed at 100 base pairs on each side of the DNA fragment on the HiSeq 4000 platform. 47-69 million reads were sequenced per sample (mean=56.5 million) with samples multiplexed to 8 samples per lane.

Analytical Methods

Bioinformatic analysis of RNA sequencing samples was carried out using validated packages in R. Reads were mapped to human genome reference sequence GRCh38 with HISAT[27]. Gene counts were retrieved using HTseq-count[28] and the Ensembl gene annotation. DESeq2[13] was used for differential expression analysis using a pairwise model where individual was coded for. Pathway and network analysis was performed using the R package XGR[29]. All statistical analysis was performed using base R. Batch correction was peformed by incorporating batch within linear regression of logit transformed cell counts. Plots were created using the ggplot2 package[30] and association plots using LocusZoom[31]

Author contributions:

HAM & BPF conceived the project; cell purification and stimulation was performed by EL, BPF, SM and SD; flow experiments were performed by HAM with assistance from JW & SD; samples were procured using the Oxford Biobank organized by JG, JC & MN; NY, EM & LR performed ELISA analysis, BPF, WL & IN performed statistical and bioinformatic analysis. BPF drafted the manuscript and figures with subsequent contributions from all authors. BPF, PB & JCK jointly supervised the project.

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Antibody Target	**Fluorochrome**	**Clone**	**Supplier**	**Dilution**
CD3	AF700	UCHT1	BioLegend	1:50
CD4	FITC	RPA-T4	BioLegend	1:50
CD8α	BV510	RPA-T8	BioLegend	1:50
CD14	PE	TÜK4	Miltenyi	1:100
CD19	PerCP/Cy5.5	SJ25C1	BioLegend	1:50
CD56	PE/Cy7	5.1H11	BioLegend	1:50
CD127 (IL-7Rα)	BV605	A019D5	BioLegend	2:50
IgG1 isotype	BV605	MOPC-21	Biolegend	2:50
Fixable viability dye	eFluor780	N/A	eBiosciences	1:250

Figure 1

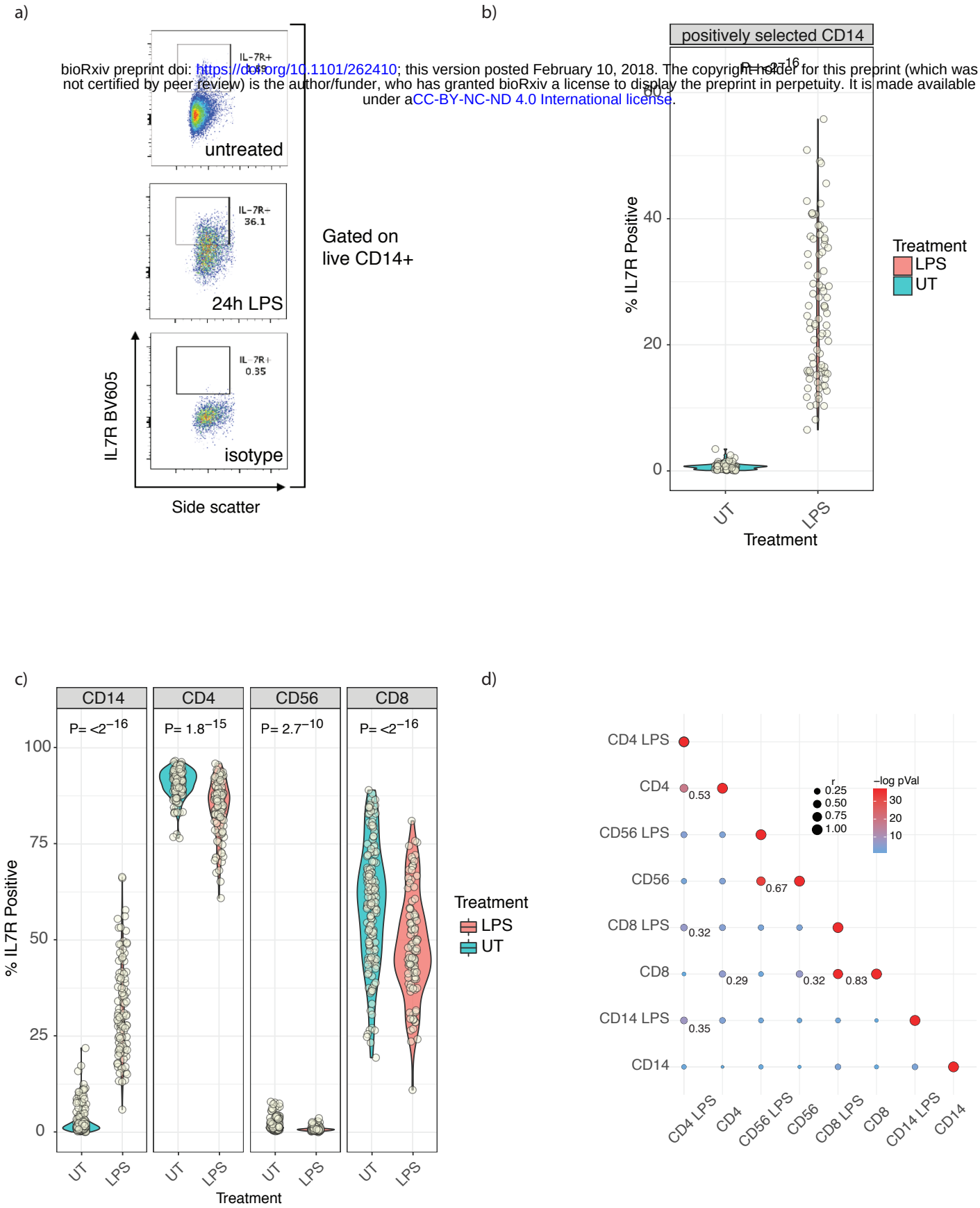


Figure 2

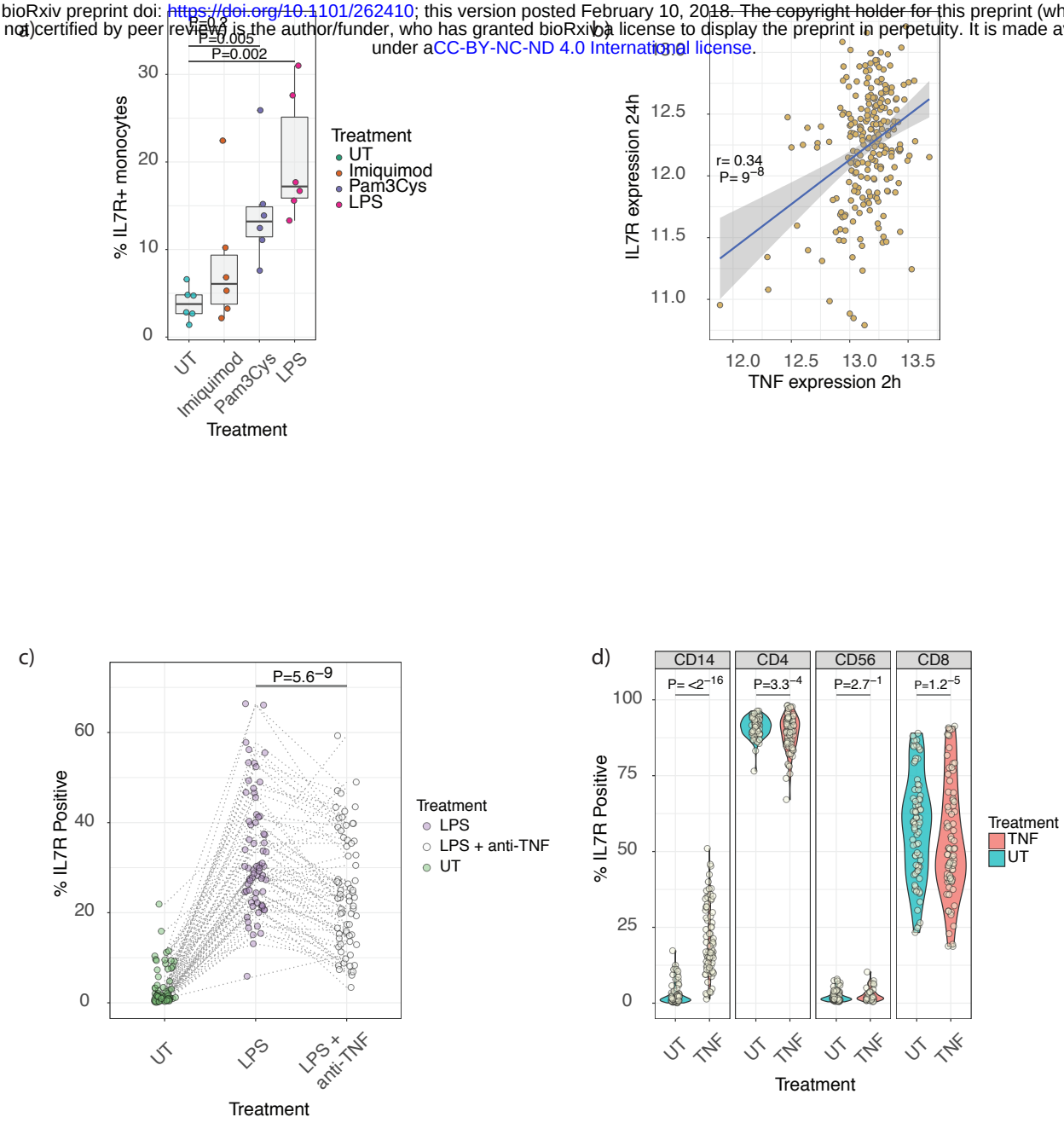


Figure 3

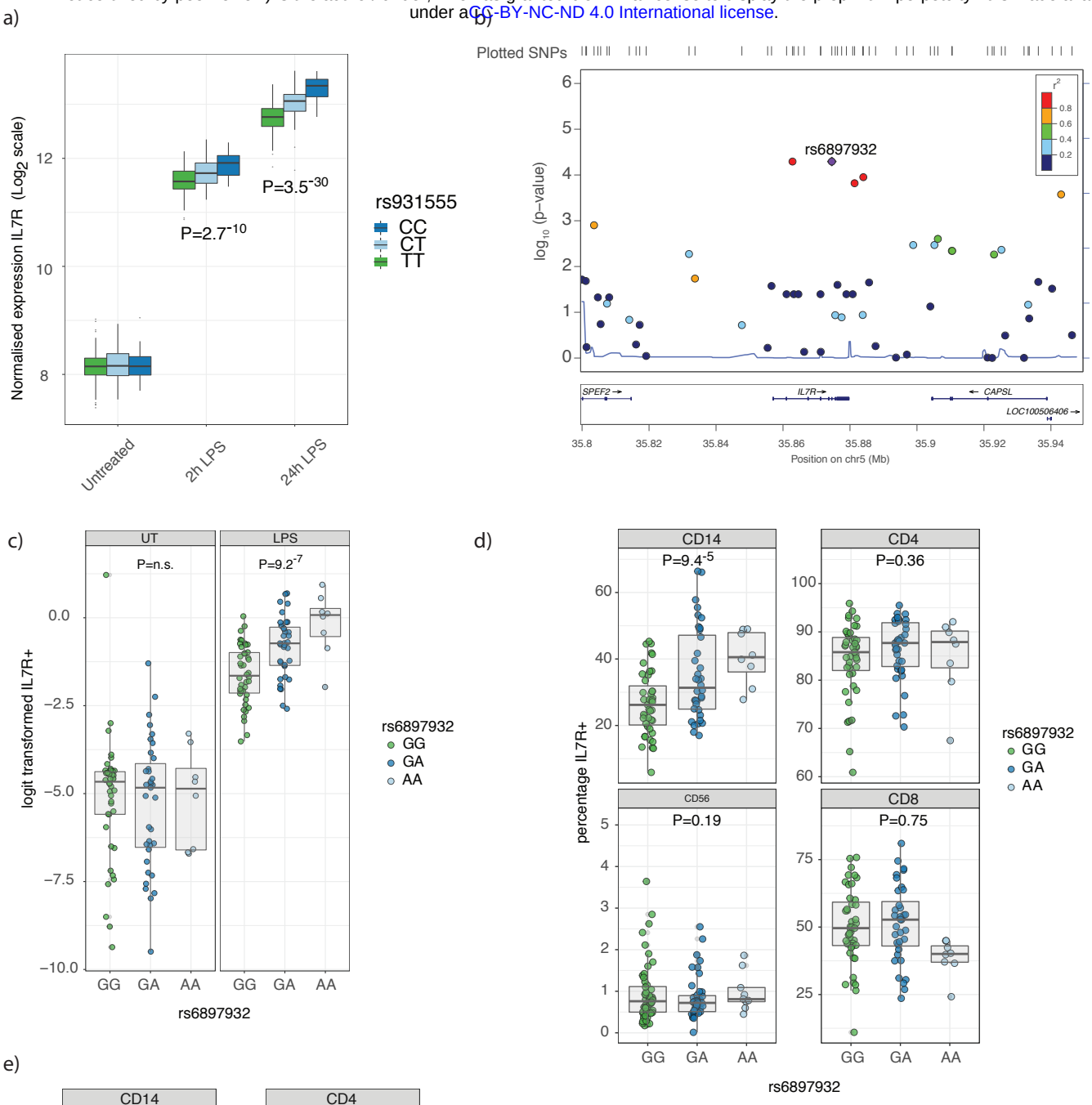


Figure 4

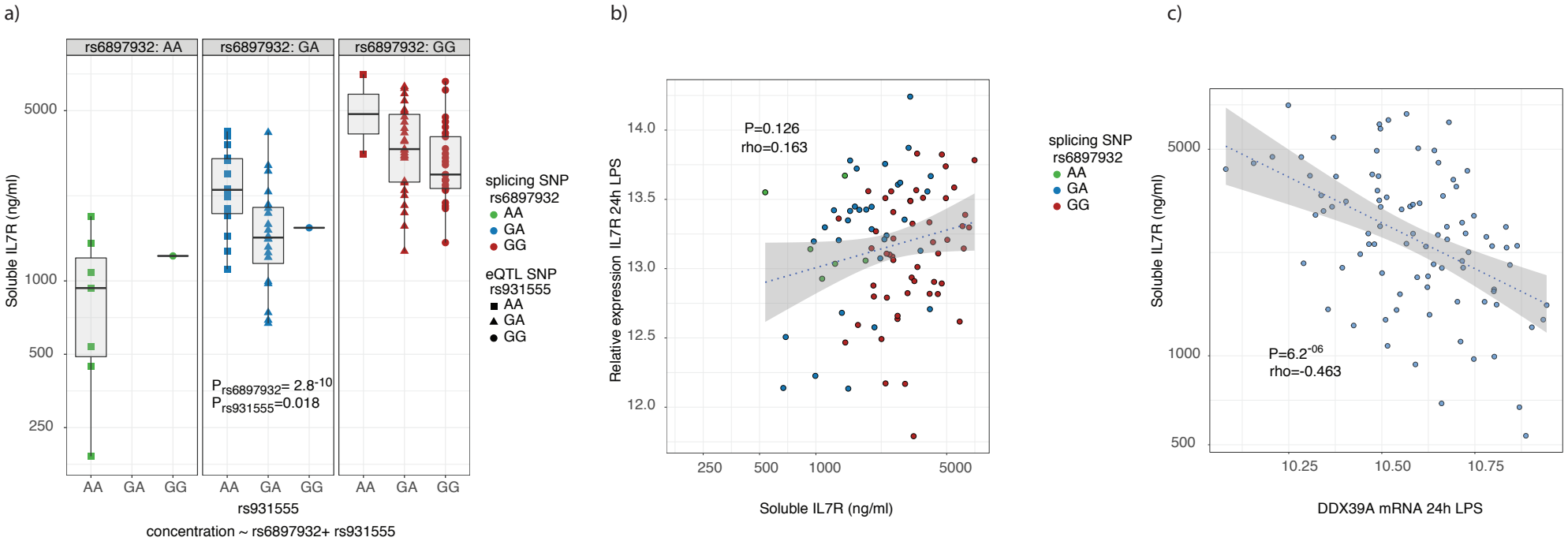
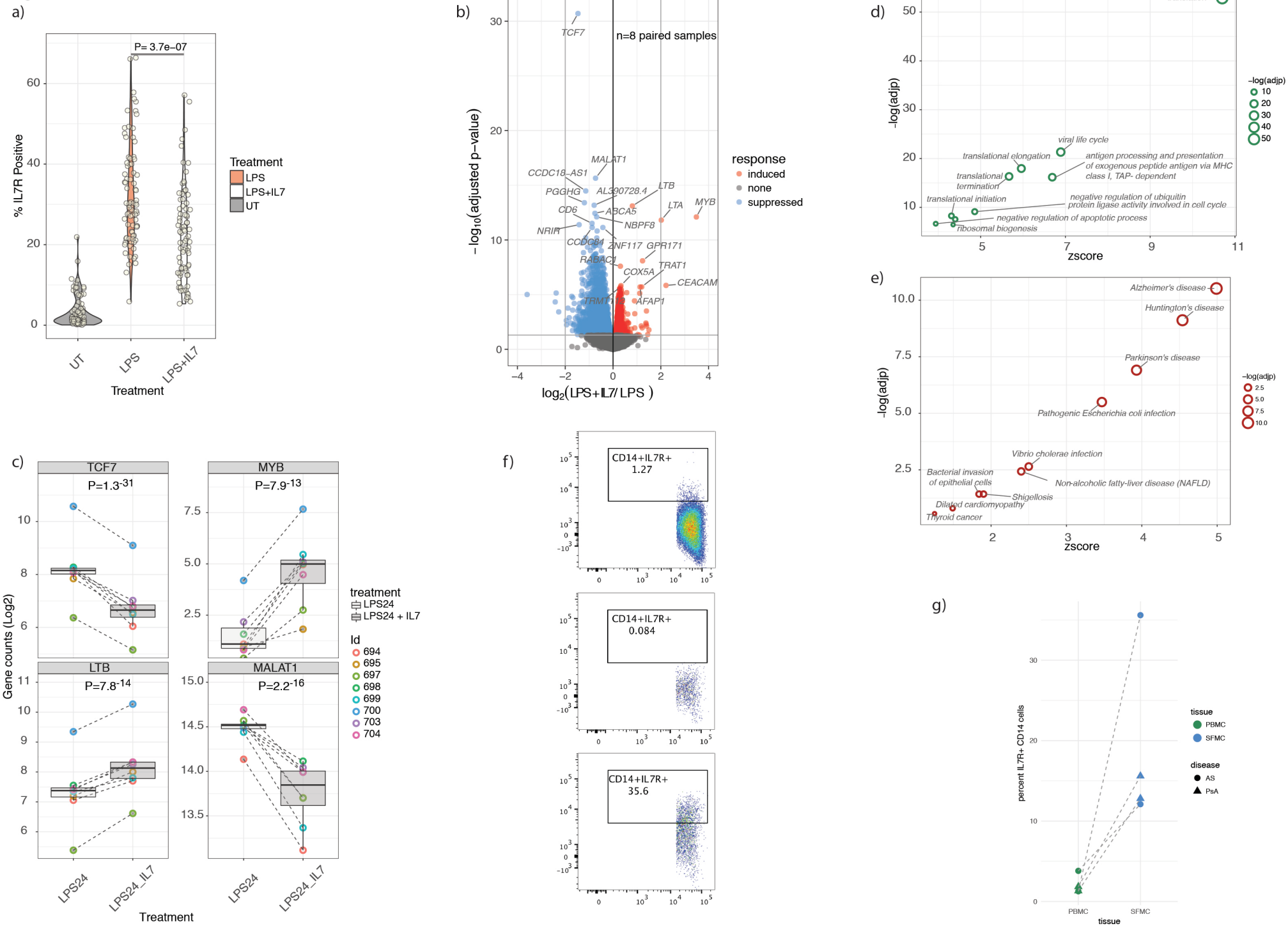
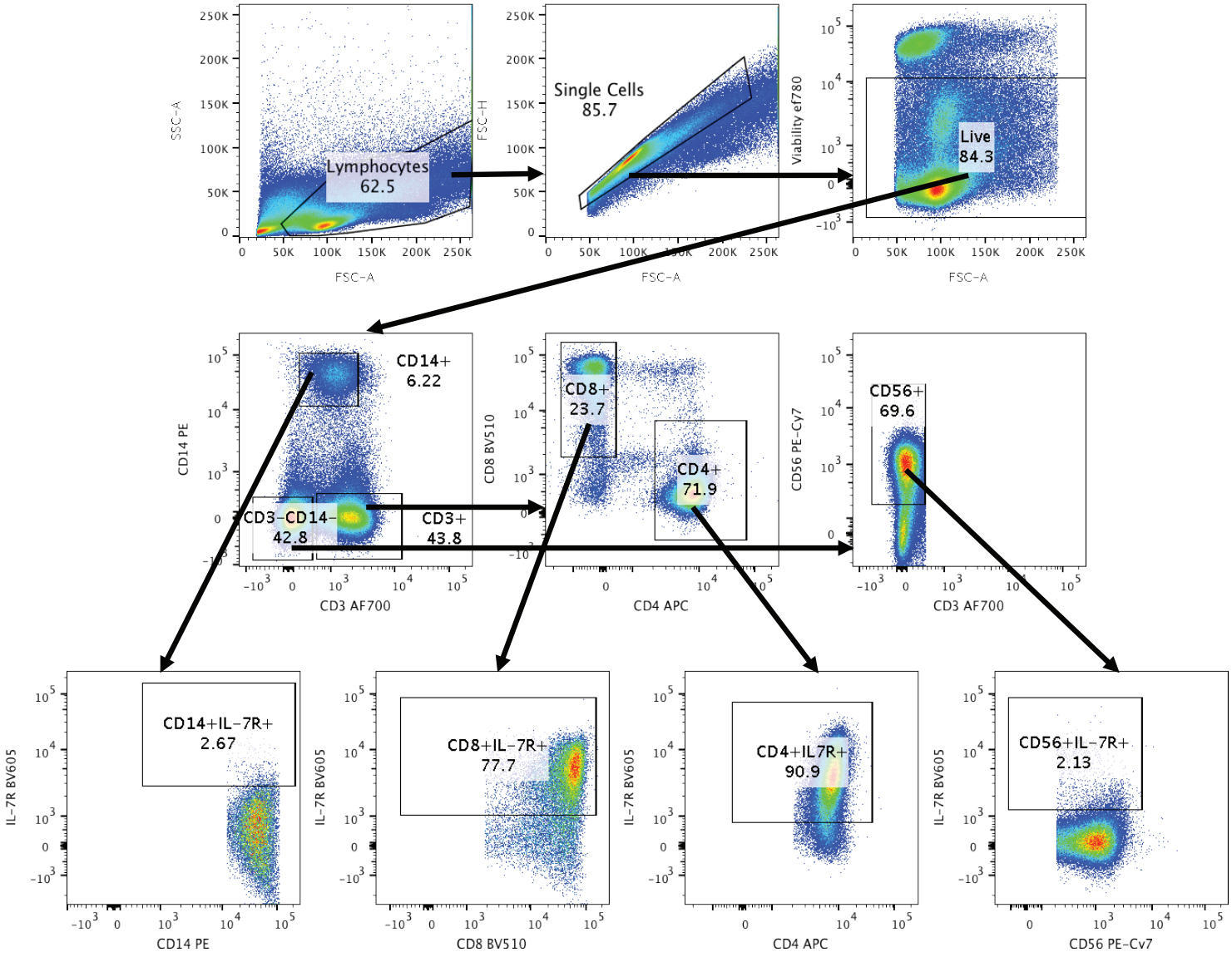

a)
rs6897932: AA
rs6897932: GA
rs6897932: GG
Soluble IL7R (ng/ml)
5000
1000
500
250
AA GA GG AA GA GG AA GA GG
rs931555
$P_{rs6897932}$= 2.8^{-10}
$P_{rs931555}$=0.018
concentration ~ rs6897932+ rs931555
splicing SNP
rs6897932
AA
GA
GG
eQTL SNP
rs931555
AA
GA
GG
b)
Relative expression IL7R 24h LPS
14.0
13.5
13.0
12.5
12.0
P=0.126
rho=0.163
250 500 1000 5000
Soluble IL7R (ng/ml)
splicing SNP
rs6897932
AA
GA
GG
c)
Soluble IL7R (ng/ml)
5000
1000
500
P=6.2^{-06}
rho=-0.463
10.25 10.50 10.75
DDX39A mRNA 24h LPS

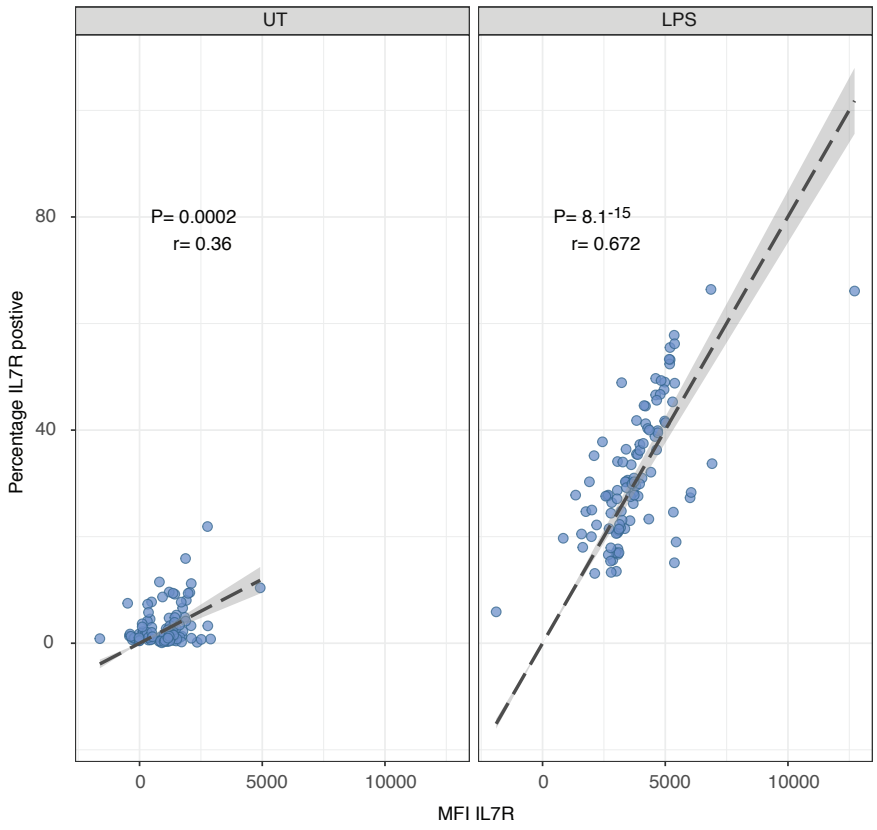
Figure 5


a)
P= 3.7e−07
% IL7R Positive
Treatment
LPS
LPS+IL7
UT
b)
n=8 paired samples
TCF7
MALAT1
CCDC18−AS1
PGGHG
AL390728.4
LTB
LTA
MYB
CD6
ABCA5
NBPF8
NRIP1
CCDC64
ZNF117
GPR171
RABAC1
TRAT1
COX5A
CEACAM1
TRMT112
AFAP1
response
induced
none
suppressed
log2(LPS+IL7/ LPS)
d)
translation
viral life cycle
translational elongation
antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP- dependent
translational termination
translational initiation
negative regulation of ubiquitin protein ligase activity involved in cell cycle
negative regulation of apoptotic process
ribosomal biogenesis
−log(adjp)
zscore
e)
Alzheimer's disease
Huntington's disease
Parkinson's disease
Pathogenic Escherichia coli infection
Vibrio cholerae infection
Bacterial invasion of epithelial cells
Non-alcoholic fatty-liver disease (NAFLD)
Shigellosis
Dilated cardiomyopathy
Thyroid cancer
c)
TCF7
P=1.3-31
MYB
P=7.9-13
LTB
P=7.8-14
MALAT1
P=2.2-16
Gene counts (Log2)
LPS24
LPS24_IL7
Treatment
treatment
LPS24
LPS24 + IL7
Id
694
695
697
698
699
700
703
704
f)
CD14+IL7R+
1.27
CD14+IL7R+
0.084
CD14+IL7R+
35.6
g)
percent IL7R+ CD14 cells
tissue
PBMC
SFMC
disease
AS
PsA



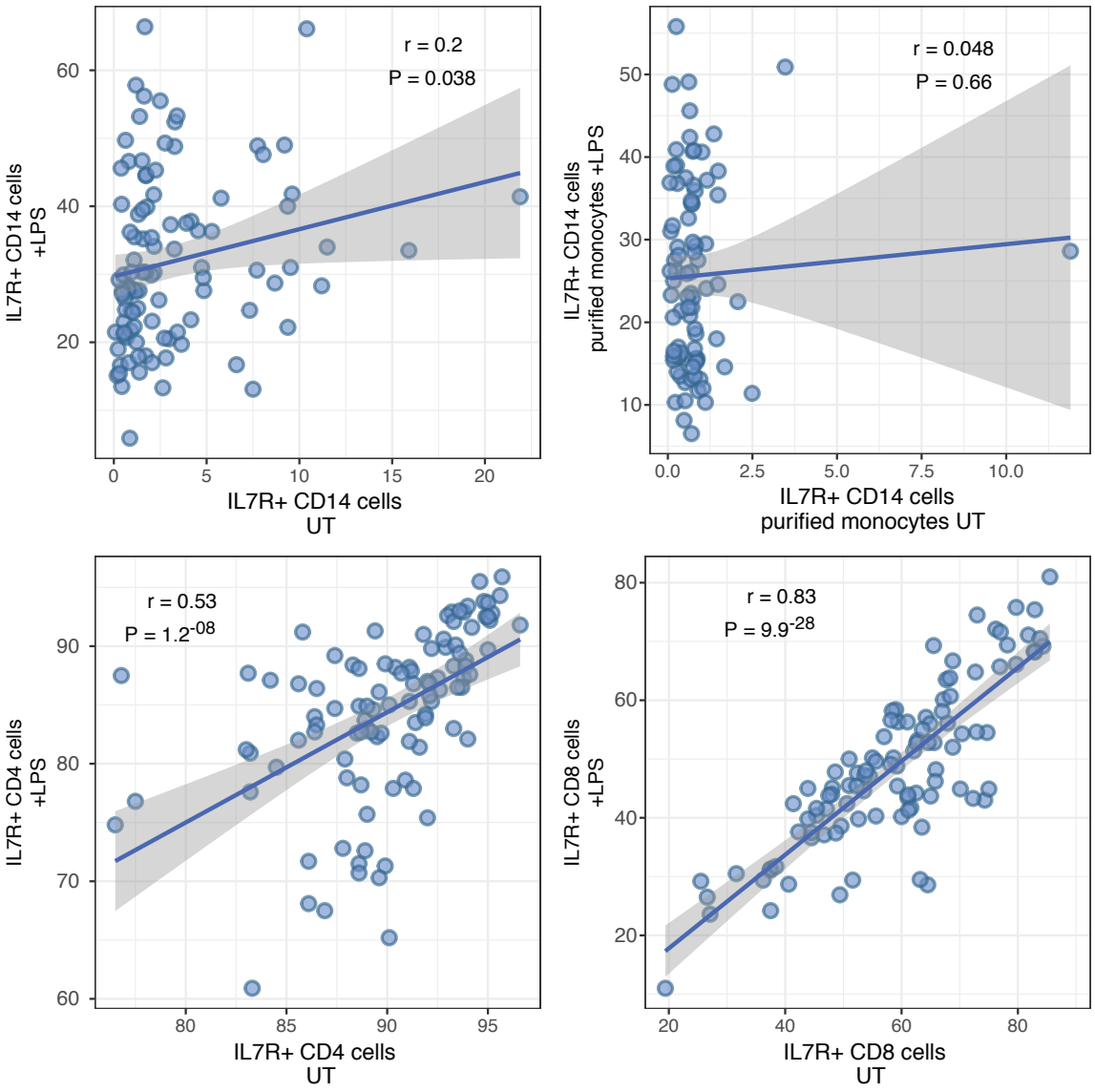
Supplementary Figure 1

Gating strategy for determining cell populations assessed across the cohort - here shown are unstimulated PBMCs illustrating the relative absence of IL7R + monocytes



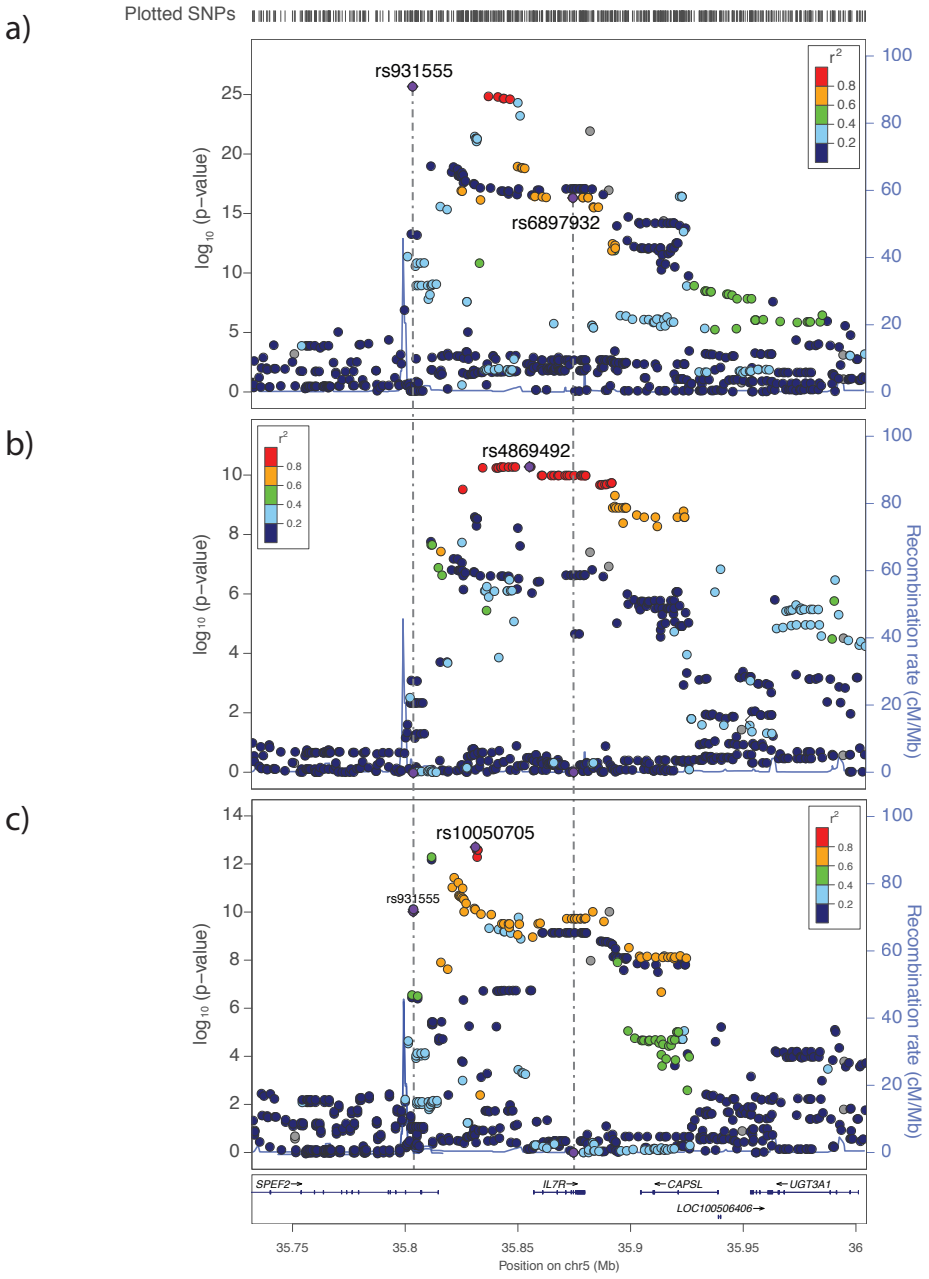
Supplementary Figure 2

Background corrected MFI for IL7R versus percentage of IL7R+ monocytes



Supplementary Figure 3

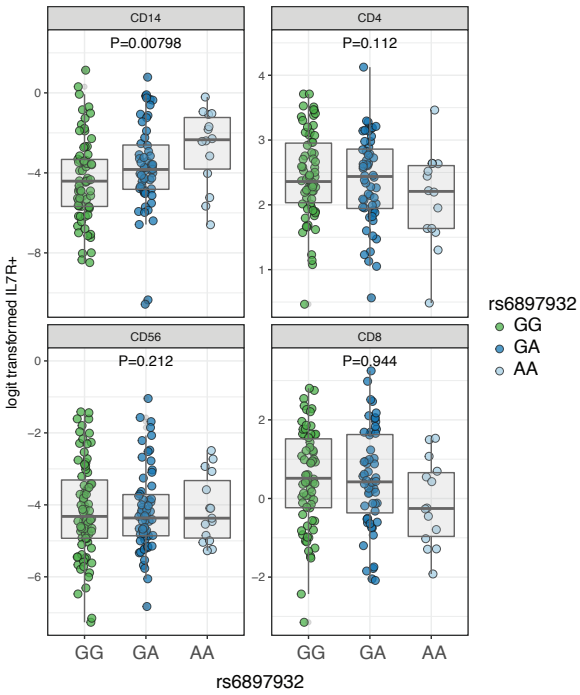
Whereas there is a strong correlation between baseline and treated IL7R+ CD4+ and CD8+ T-cells, there is little association between untreated IL7R+ monocytes and the percentage induced by treatment.



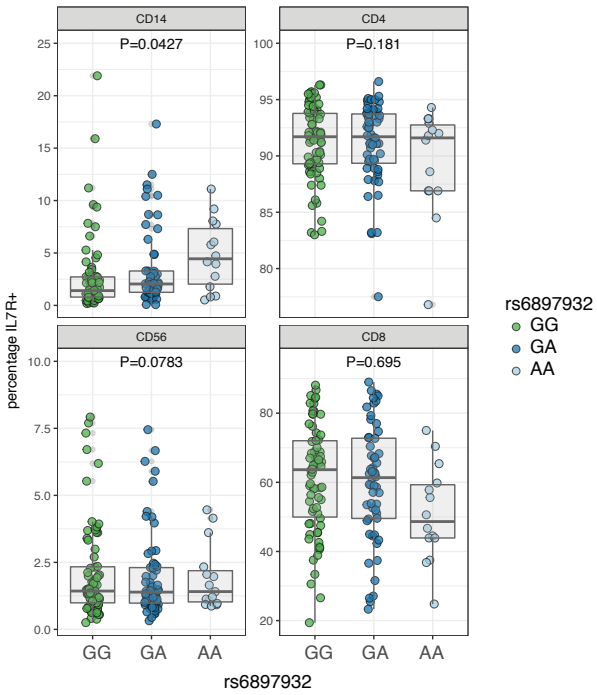
Supplementary Figure 4

a) Peak eQTL for IL7R after LPS maps to rs931555, b) controlling for rs931555 resolves association at rs6897932, c) controlling for rs6897932 carriage leaves residual associtaion at rs931555

S5a

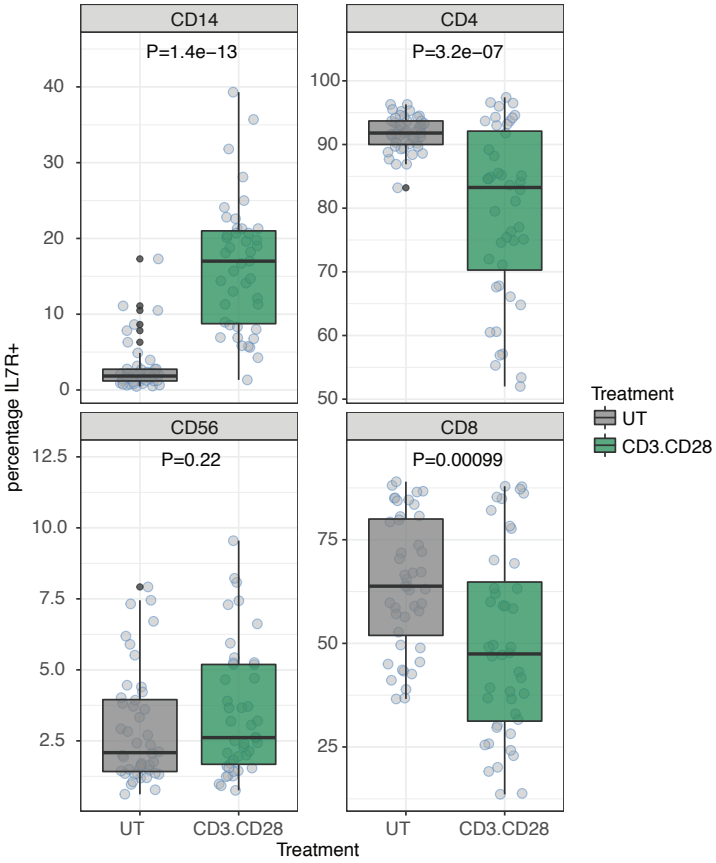


S5b



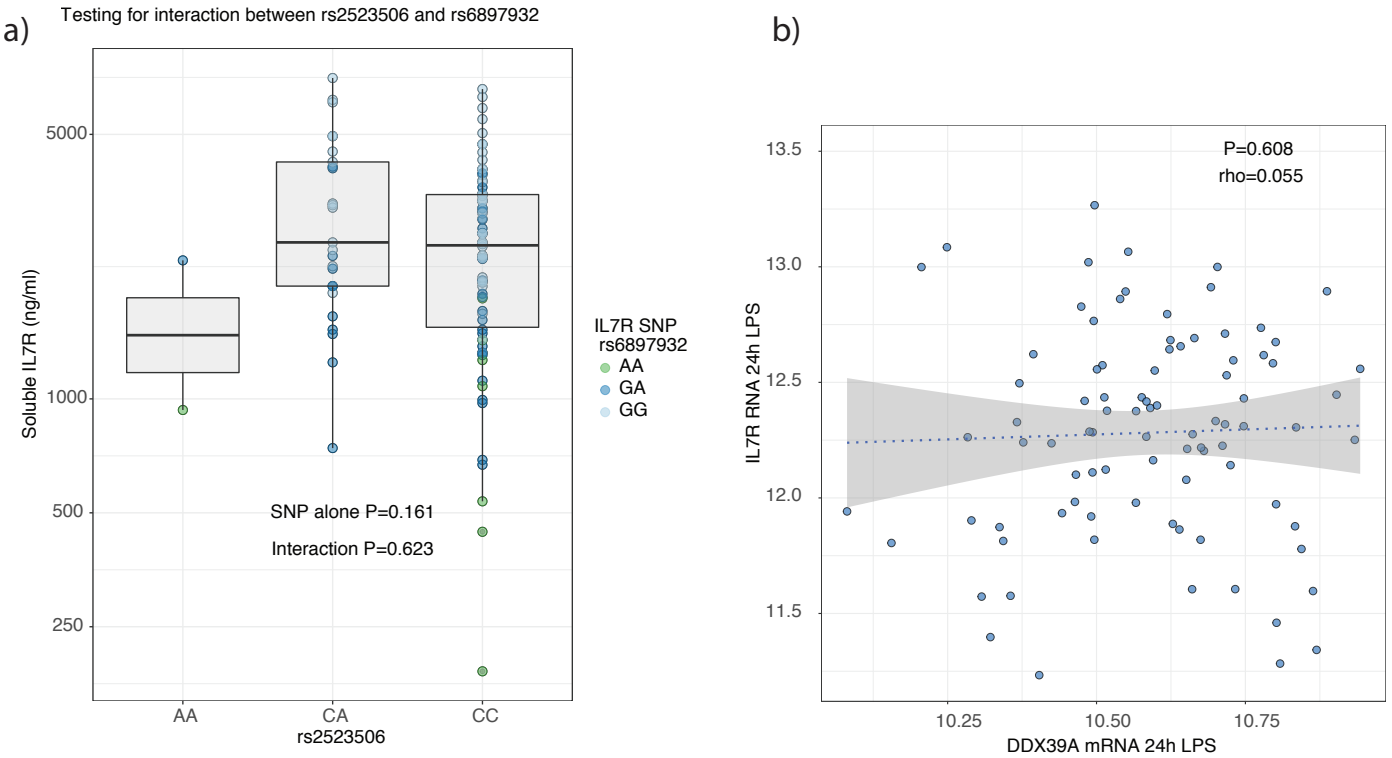
Supplementary Figure 5

Baseline untreated surface IL7R staining by genotype from PBMC cultures a) batch corrected log values, b) raw data; both showing significant effect of rs68979342 carriage on monocytes surface IL7R levels



Supplementary Figure 6

PBMC cultures were treated with CD3.CD38 beads for 24h and IL7R positivity measured with flow cytometry. Significant induction of CD14 IL7R+ was noted upon T-cell specific stimulation indicating cross-talk between cell types. Experiments performed in n=44 subjects over 4 batches. A genetic effect of rs6897932 was not observed in any cell type (P>0.05), potentially reflecting small sample size and large batch variation in this experiment.



Supplementary Figure 7

a) No significant effect of rs2523506 on monocyte derived soluble IL7R was detected. A significant interaction between rs2523506 and rs6897932 was not observed
b) There was no relationship between DDX39A expression and IL7R expression