Supplemental Data

Supplemental Materials and Methods

Subject recruitment

Ugandan index cases with culture-confirmed pulmonary TB were recruited between 2002-2012 along with a total of 2,585 household contacts as part of the Kawempe Community Health Study (Kampala, Uganda) when serial TST testing was performed over 2 years(*8*). A subset of these household contacts were successfully re-contacted 8-10 years after initial enrollment to complete IGRA testing (x3) using Quantiferon-Gold In-tube (QFT-GIT, Qiagen) and a repeat TST using the Mantoux method (0.1 mL containing 5 tuberculin units, Tubersol, Connaught Laboratories) where a positive TST reflected an induration of $\geq$ 10mm(*7*). For these investigations, HIV-negative subjects were defined as RSTRs if they met 'definite' criteria where all 5 available testing measurements from the initial and re-tracing studies (2 TST and 3 IGRA) were negative whereas LTBI subjects had concordant positive tests in each study(*7*). During the retracing study, PBMCs were collected by Ficoll gradient, cryopreserved and shipped to Seattle, WA along with whole blood collected in PAXgene Blood RNA tubes (Qiagen).

The Highly Exposed TB Uninfected (HETU) study was a prospective, longitudinal study of HIV-negative gold miners that were screened between August 2015 and December 2016 as part of their annual occupational health center (OHC) medical evaluation in North West Province, South Africa. Subjects that had worked in the mining industry for $\geq$ 15 years, were aged 33-60, had no history of TB nor previous treatment (including isoniazid preventative therapy), had no active TB symptoms, and no serious medical conditions including silicosis were eligible. Subjects underwent sputum collection to exclude culture-positive cases, followed by Quantiferon-TB Gold In-tube (QFT-GIT, Qiagen) and TST (Matoux method, 5 tuberculin units

of PPD, Statens Serum Institute, Copenhagen, Denmark) evaluations at baseline. These gold miner subjects were defined as RSTRs if they had concordant negative TST (<5mm induration) and negative QFT-GIT (as defined by manufacturer) testing at enrollment. Additionally, most subjects (23 of 26 RSTR subjects in the monocyte analysis and 19 of 22 RSTR subjects in the whole blood analysis) were also evaluated after 12 months with repeat TST and QFT-GIT testing. LTBI subjects had concordant positive testing at enrollment, some of which had 12-month follow-up testing (8 of 29 LTBI subjects in monocyte analysis and 8 of 24 subjects in whole blood analysis). Among subjects with follow-up testing, we further excluded any subjects with TST/IGRA conversions or reversions. At enrollment, PBMCs were collected and isolated by Ficoll gradient, cryopreserved, and shipped to Seattle, WA along with whole blood frozen in PAXgene RNA tubes (Qiagen).

Generation of NLRP3, NLRC4 and ULK1 gene-edited U937 lines

U937 cells (ATCC# CLR1593) with disruptions in *NLRP3* and *NLRC4* were generated using CRISPR/Cas9 by lentivirus delivery of guide RNAs and selection in Puromycin (manuscript in preparation) as previously described for *ULK1*(*83*). The following 20-mer guide RNA sequences, excluding the genomic protospacer adjacent motif sequence (underlined), were cloned into pRRL-Cas9-Puro (gift of Dan Stetson) using sense/anti-sense oligos with appropriate flanking sequences: NLRP3, *GAAGAAGACGTACACCGCGGTGG*; NLRC4, *GGACCAACACCATCACCGCGTGG*; ULK1, *GGACGCCTCCATGCTCAGCGTGG*. Edited U937 cellular pools were screened by Sanger sequencing of PCR amplicons spanning each editing site using deconvolution software (ICE Analysis, www.synthego.com; TIDE version 2.0.1, Desktop Genetics). Inflammasome knockouts were additionally phenotypically screened

by quantitating IL1β secretion by ELISA after Nigericin (to stimulate NLRP3) or *B.thailandensis* needle protein (to stimulate NLRC4, Supplemental 7B) stimulations.

Gene set enrichment analysis

To identify enrichment of biologic pathways or macrophage activation states with the RSTR or LTBI clinical phenotypes, we used the Correlation Adjusted Mean Rank gene set test (CAMERA) implemented in limma R package version 3.42.0(*30*), which is a competitive gene set test that is similar to GSEA(*31*), but less susceptible to inter-gene correlations. We performed separate analyses for each of three lists of gene sets. The first analysis combined gene sets available at MSigDB(*32*), derived from prior transcriptional profiling in cells with genetic or chemical perturbations (C2) or from immunologic signatures (C7), totaling 9547 gene sets (MsigDB 6.2, date accessed August 2, 2019) where gene sets with <5 genes were excluded. The second analysis queried MSigDB C5 gene sets (n=5914), which are collated by GO term (MsigDB 6.2, date accessed August 2, 2019). In a final analysis to specifically look for enrichment of activated macrophage states, we derived gene sets from WGCNA transcriptional modules identified by Xue et al (Modules 1-49)(*33*). These WGNCA modules (each containing 27-884 genes) were computed after stimulation of healthy donor primary macrophages with one of 29 conditions including cytokines (IFNβ, IFNγ, TNFα, IL4, IL10, IL13), TLR ligands (LPS, PAM3, PGE2, polyI:C), glucocorticoids, free fatty acids (lauric acid, linoleic acid, oleic acid, palmitic acid, stauric acid), lipoprotein particles (HDL), combinations of these stimuli or unstimulated conditions. Nominal *P* values were calculated for the degree of enrichment for individual gene sets using CAMERA, which were then adjusted (FDR) to account for simultaneous comparisons.

Supplemental figure legends

Supplemental Figure 1: Comparison of Ugandan household contact RSTR and LTBI whole blood transcriptional profiles. RNA was isolated from whole blood of Ugandan household contacts of tuberculosis cases (31 RSTR versus 28 LTBI) and analyzed by RNA sequencing. Deconvolution of transcriptomic data (immunoStates) compares predicted cell frequencies. *P* values were calculated by one-sided *t*-test.

Supplemental Figure 2: Comparison of South African gold miner RSTR and LTBI whole blood transcriptional profiles. RNA was isolated from whole blood of South African goldminers (16 RSTR versus 24 LTBI) and analyzed by RNA sequencing. Deconvolution of transcriptomic data (immunoStates) compares predicted cell frequencies. *P* values were calculated by one-sided *t*-test.

Supplemental Figure 3: Comparison of cell population frequencies in RSTR and LTBI donor whole blood. Peripheral blood mononuclear cells were fixed and stained with surface markers before cell population frequencies were quantitated by flow cytometry. Mean cell population frequencies in RSTR and LTBI were compared by the Mann-Whitney test (*P* value).

Supplemental Figure 4: Multidimensional scaling of transcriptomes by available epidemiologic and demographic data in Uganda. Global effects of clinical phenotype (RSTR versus LTBI), age, body mass index (BMI), BCG status, exposure risk score, gender and family relatedness on monocyte transcription in Uganda was assessed by multidimensional scaling (MDS).

Supplemental Figure 5: Multidimensional scaling of transcriptomes by available epidemiologic and demographic data in South Africa. Global effects of clinical phenotype (RSTR versus LTBI), age, body mass index (BMI), BCG status, occupation, housing arrangement, country of origin, ethnicity, and current employment or cumulative exposure within gold mines (i.e. employment 'underground') on monocyte transcription in South Africa were assessed by multidimensional scaling (MDS).

Supplemental Figure 6: Gene sets that enrich among RSTR phenotypes across Uganda and South Africa cohorts suggest differential monocyte activation states related to free fatty acid stimuli. To identify macrophage activation states that correlate with the gene sets enriched among RSTR monocyte transcriptomes, heat maps were constructed using the primary WGNCA data by Xue et al(*33*). Each macrophage module that was enriched among RSTR donor monocytes in Uganda and South Africa (FDR <0.0001) are represented as rows. The Pearson correlation coefficients of each stimulus and each Xue WGNA module eigengene are indicated (colors) along with the degree of significance of that correlation. **P* <0.05, ***P* <0.01, ****P* <0.001. LA, lauric acid; PA, palmitic acid; SA, stauric acid; LiA, linoleic acid; OA, oleic acid; HDL, high-density lipoprotein; IFNb/g, interferon-β/γ; upLPS, ultrapure lipopolysaccharide; P3C, Pam3CSK4; PGE2, prostaglandin E2; IC, immune complex; GC, dexamethasone; sLPS, standard LPS; TPP, TNF+prostaglandin E2+Pam3CSK4.

Supplemental Figure 7: Palmitic acid and oleic acid are minimally toxic to MDMs at doses ≤ 200 μM. Cytotoxicity was measured hourly in healthy monocyte derived macrophages after

exposure to oleic acid (OA), palmitic acid (PA) or controls matched for BSA vehicle content over 72 hours. Disruption of membrane integrity due to treatment toxicity was quantified as the number of cells positive for nuclear fluorescent stain (Sytox+, green) divided by the number of total cells seeded in control wells (Syto+, not shown). Bright field and fluorescent overlay after treatment with 200 μM FFA for 72 hours is shown along with peak cytotoxicity calculations at indicated doses **(A)** and findings were consistent over two independent experiments. Despite cytotoxicity, PA (400 mM) further potentiated intracellular Mtb growth in monocyte-derived macrophages **(B)** that were infected with Mtb-lux and treated as described in Figure 4. Data were fit using a simple linear model and a dose-dependent treatment effect of PA was significant (****$P<0.0001$).

Table S1: Epidemiologic and demographic characteristics of Ugandan donors: whole blood transcriptional analysis

	RSTR	LTBI	p-value[A]
No. subjects included	31	28	
Age at enrollment, median (IQR)	13 (6)	10 (7)	0.25
Age at re-tracing, median (IQR)	21 (5)	20 (6)	0.53
Sex, % male (n/N)	48 (15/31)	43 (12/28)	0.67
BMI (median, IQR)	20.8 (6.8)	21.4 (4.3)	0.63
% HIV+	0	0	
% BCG scar (n/N)	76 (19/25)	73 (16/22)	0.95
Exposure score at enrollment[B]			
Pediatric risk score, mean (SD)	6.0 (0.9)	6.3 (1.0)	0.28
Adult risk score, mean (SD)	6.6 (1.4)	7.9 (1.5)	0.10
Relatedness within phenotype[C]			
Mean 3° & closer relations per person (SD)	0.65 (1.31)	0.46 (1.04)	0.64
Mean 1° & closer relations per person (SD)	0.55 (1.1)	0.46 (1.04)	0.71

[A] Statistical comparisons were made using Pearson Chi-square (categorical variables) or two-sample Wilcoxon rank-sum (Mann-Whitney) tests (continuous variables).

[B] Exposure scores were calculated according to patient age at initial enrollment (<15 years with the Pediatric risk score, ≥ 15 years with the Adult Exposure Risk Score).

[C] Relatedness of subjects within each phenotype were compared by averaging the number of 1st degree relationships and 3rd degree-or-closer relationships. BMI, body mass index; IQR, interquartile range; n/N, (number subjects counted/number of subjects with available data); SD, standard deviation.

Table S2

Epidemiologic and demographic characteristics of South African donors: whole blood transcriptional analysis

	RSTR	LTBI	p-value[A]
No. subjects included	22	24	-
Age at enrollment, median (IQR)	47 (12)	48.5 (5)	0.60
Sex, % male	100	100	1.00
BMI, median (IQR)	27.8 (4.1)	30.6 (5.8)	0.23
Yrs worked underground, median (IQR)	21 (11)	24 (11.5)	0.28
Ethnicity			0.003
% Black/African (n/N)	68.2 (15/22)	100.0 (24/24)	
% White/European (n/N)	27.3 (6/22)	0.0 (0/24)	
% Other (n/N)	4.6 (1/22)	0.0 (0/24)	
% BCG Scar (n/N)	71 (15/21)	71 (17/24)	0.97

[A] Statistical comparisons were made using Pearson Chi-square or Fisher's exact test (categorical variables) or two-sample Wilcoxon rank-sum (Mann-Whitney) tests. BMI, body mass index; IQR, interquartile range; n/N, (number subjects counted/number of subjects with available data).

Table S3: Differentially expressed genes in whole blood, Uganda

Data file: Supplemental Tables 3_4_7_8_9_10_11_12. xls

Fold-change (log 2) reflects relative expression of the indicated genes in RSTR (positive) versus LTBI (negative) phenotypes in Ugandan whole blood PAXgene RNA blood donors. *P* values are adjusted for multiple comparisons by false discovery rate (adj.P.Val).

Table S4: Differentially expressed genes in whole blood, South Africa

Data file: Supplemental Tables 3_4_7_8_9_10_11_12. xls

Fold-change (log 2) reflects relative expression of the indicated genes in RSTR (positive) versus LTBI (negative) phenotypes in South African whole blood PAXgene RNA blood donors. *P* values are adjusted for multiple comparisons by false discovery rate (adj.P.Val).

Table S5
Epidemiologic and demographic characteristics of Ugandan donors in monocyte transcriptional analysis

	RSTR	LTBI	p-value[A]
No. subjects included	38	40	
Age at enrollment, median (IQR)	14 (7)	10.5 (7)	0.33
Age at re-tracing, median (IQR)	21 (9)	21.5 (8)	0.79
Sex, % male (n/N)	52.6 (20/38)	62.5 (25/40)	0.38
BMI (median, IQR)	21.1 (5.5)	22.0 (5.0)	0.28
% HIV+	0	0	
% BCG scar (n/N)	74.2 (23/31)	72.7 (24/33)	0.99
Exposure score at enrollment [B]			
Pediatric risk score, mean (SD)	6.14 (0.99)	6.32 (0.86)	0.33
Adult risk score, mean (SD)	6.63 (1.45)	6.83 (1.40)	0.63
Relatedness within phenotype [C]			
Mean 3° & closer relations per person (SD)	0.37 (0.88)	0.33 (0.89)	0.73
Mean 1° & closer relations per person (SD)	0.32 (0.74)	0.33 (0.89)	0.80

[A] Statistical comparisons were made using Pearson Chi-square (categorical variables) or two-sample Wilcoxon rank-sum (Mann-Whitney) tests (continuous variables).

[B] Exposure scores were calculated according to patient age at initial enrollment (<15 years with the Pediatric risk score, ≥ 15 years with the Adult Exposure Risk Score).

[C] Relatedness of subjects within each phenotype were compared by averaging the number of 1st degree relationships and 3rd degree-or-closer relationships. BMI, body mass index; IQR, interquartile range; n/N, (number subjects counted/number of subjects with available data); SD, standard deviation.

Table S6
Epidemiologic and demographic characteristics of South African donors in monocyte transcriptional analysis

	RSTR	LTBI	p-value [A]
No. subjects included	26	29	-
Age at enrollment, median (IQR)	50 (9)	49 (5)	0.99
Sex (% male)	100	100	1.00
BMI, median (IQR)	27.6 (5.2)	31.0 (5)	0.09
Yrs worked underground, median (IQR)	21.5 (10)	26 (11)	0.75
Ethnicity			0.003
% Black/African (n/N)	73.1 (19/26)	100.0 (29/29)	
% White/European (n/N)	23.1 (6/26)	0.0 (0/29)	
% Other (n/N)	3.9 (1/26)	0.0 (0/29)	
% BCG Scar (n/N)	68 (17/25)	69 (20/29)	0.94

[A] Statistical comparisons were made using Pearson Chi-square or Fisher's exact test (categorical variables) or two-sample Wilcoxon rank-sum (Mann-Whitney) tests. BMI, body mass index; IQR, interquartile range; n/N, (number subjects counted/number of subjects with available data).

Table S7: Differentially expressed genes in monocytes, Uganda

Data file: Supplemental Tables 3_4_7_8_9_10_11_12. xls

Fold-change (log 2) reflects relative expression of the indicated genes in RSTR (positive) versus LTBI (negative) phenotypes in Ugandan monocyte donors. *P* values are adjusted for multiple comparisons by false discovery rate (adj.P.Val).

Table S8: Differentially expressed genes in monocytes, South Africa

Data file: Supplemental Tables 3_4_7_8_9_10_11_12. xls

Fold-change (log 2) reflects relative expression of the indicated genes in RSTR (positive) versus LTBI (negative) phenotypes in South African monocyte donors. *P* values are adjusted for multiple comparisons by false discovery rate (adj.P.Val).

Table S9: CAMERA gene set enrichment (RSTR vs. LTBI), Overlap between Uganda and SA, MSigDB collections C2+C7

Data file: Supplemental Tables 3_4_7_8_9_10_11_12. xls

CAMERA enrichment analysis of monocyte transcriptomes using the Molecular Signature Database (MSigDB) curated collections C2 (canonical pathways, chemical and genetic perturbations) and C7 (immunologic signatures) excluding gene sets with <5 genes. Enrichment direction indicates the majority of genes within that gene set have increased expression among RSTR phenotypes (Up) or LTBI phenotypes (Down). Gene set categories were manually assigned to carbon metabolism, antiviral or Type 1 interferon (T1IFN) responses, comparison of different cell types/populations, or other.

Table S10: Table S10: CAMERA gene set enrichment (RSTR vs. LTBI), MSigDB C5 collection (GO terms)

Data file: Supplemental Tables 3_4_7_8_9_10_11_12. xls

CAMERA enrichment analysis of monocyte transcriptomes using the Molecular Signature Database (MSigDB) curated collections C5 (GO, gene ontology terms) excluding gene sets with <5 genes. Enrichment direction indicates the majority of genes within that gene set have increased expression among RSTR phenotypes (Up) or LTBI phenotypes (Down).

Table S11: CAMERA gene set enrichment (RSTR vs. LTBI) in Uganda monocytes , Xue macrophage activation modules

Data file: Supplemental Tables 3_4_7_8_9_10_11_12. xls

CAMERA enrichment analysis of Uganda monocyte transcriptomes using the 49 gene sets derived from transcriptional modules from Xue et al (33). These modules represent weighted gene co-expression network analysis modules of primary human macrophages treated in one of 29 different conditions (listed in Supplemental Figure 6). Enrichment direction indicates the majority of genes within that gene set have increased expression among RSTR phenotypes (Up) or LTBI phenotypes (Down).

Table S12: CAMERA gene set enrichment (RSTR vs. LTBI) in South Africa monocytes, Xue macrophage activation modules

Data file: Supplemental Tables 3_4_7_8_9_10_11_12. xls

CAMERA enrichment analysis of South Africa monocyte transcriptomes using the 49 gene sets derived from transcriptional modules from Xue et al (33). These modules represent weighted gene co-expression network analysis modules of primary human macrophages treated in one of 29 different conditions (listed in Supplemental Figure 6). Enrichment direction indicates the majority of genes within that gene set have increased expression among RSTR phenotypes (Up) or LTBI phenotypes (Down).

Table S13:

PRKAG2 polymorphism associations with RSTR (case) versus LTBI (control) by genetic model

	Dominant model		Recessive model	
SNP	OR[A] (95% CI)	p-value	OR[B](95% CI)	p-value
rs1860746	2.26 (1.20 – 4.24)	1.15E-02	5.89 (1.15 – 30.18)	3.35E-02
rs56145758	3.28 (1.43 – 7.54)	5.04E-03	15.88 (0.77 – 325.43)	7.27E-02
rs115310513	2.98 (1.33 – 6.69)	7.96E-03	18.51 (1.53 – 223.75)	2.17E-02
rs114166988	2.56 (1.37 – 4.78)	3.13E-03	13.18 (2.76 – 63.04)	1.24E-03
rs10480299	2.88 (1.61 – 5.17)	3.86E-04	7.27 (2.59 – 20.39)	1.64E-04
rs10480300	2.42 (1.33 – 4.4)	3.97E-03	9.07 (2.44 – 33.75)	1.00E-03
rs10224002	2.03 (1.11 – 3.72)	2.17E-02	3.27 (1.54 – 6.95)	2.07E-03

[A] OR, odds ratio, of being a case (RSTR) compared to control (LTBI) using dominant (AA vs. Aa + aa) and [B] recessive (AA + Aa vs. aa) genetic models.

Table S14:

Linkage disequilibrium of *PRKAG2* polymorphisms associated with RSTR phenotype.

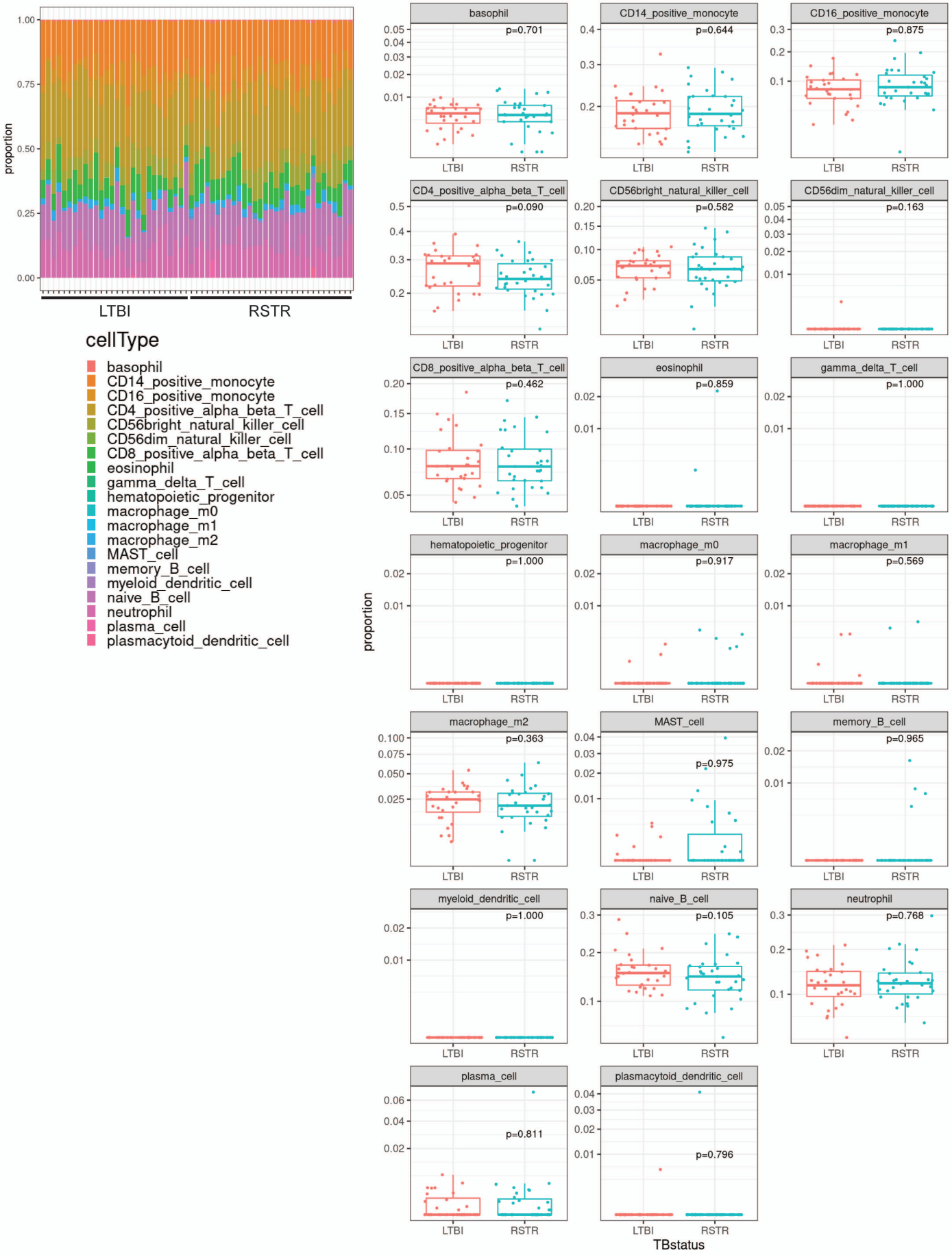
SNP	rs1860746	rs56145758	rs115310513	rs114166988	rs10480299	rs10480300	rs10224002
rs1860746	1						
rs56145758	0.00	1					
rs115310513	0.01	0.07	1				
rs114166988	0.03	0.09	0.39	1			
rs10480299	0.02	0.02	0.13	0.34	1		
rs10480300	0.03	0.06	0.24	0.59	0.59	1	
rs10224002	0.00	0.15	0.08	0.16	0.12	0.27	1

R^2 values for pairwise comparisons among each *PRKAG2* SNP associated with the RSTR phenotype.a

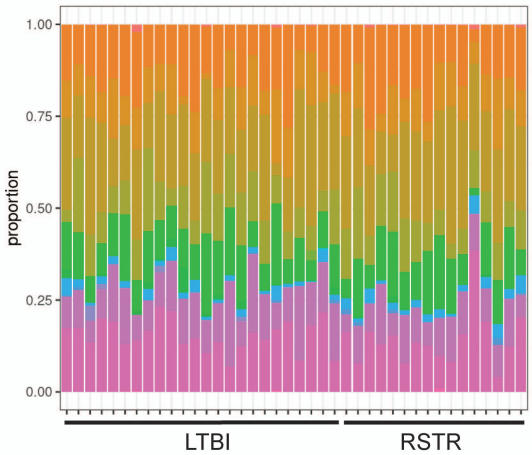
Table S15:
Antibodies used in this study

Specificity	Fluorophore	Clone	Supplier
CD56	BV510	NCAM16.2	BD Horizon
CD3	ECD	UCHT1	Beckman Coulter
CD4	Alexa 400	RPA-T4	eBiosciences
CD8	PerCP Cy5.5	SK1	BD Biosciences
CD11c	APC	Bu15	BioLegend
CD14	BUV395	M5E2	BD OptiBuild
CD16	BV421	3G8	BD Horizon
CD19	PE Cy7	HIB19	BD Pharmigen
CD45	FITC	HI30	BD Pharmigen
CD66	PE	ASL-32	BioLegend

Supplemental Figure 1



Supplemental Figure 2



cellType

- basophil
- CD14_positive_monocyte
- CD16_positive_monocyte
- CD4_positive_alpha_beta_T_cell
- CD56bright_natural_killer_cell
- CD56dim_natural_killer_cell
- CD8_positive_alpha_beta_T_cell
- eosinophil
- gamma_delta_T_cell
- hematopoietic_progenitor
- macrophage_m0
- macrophage_m1
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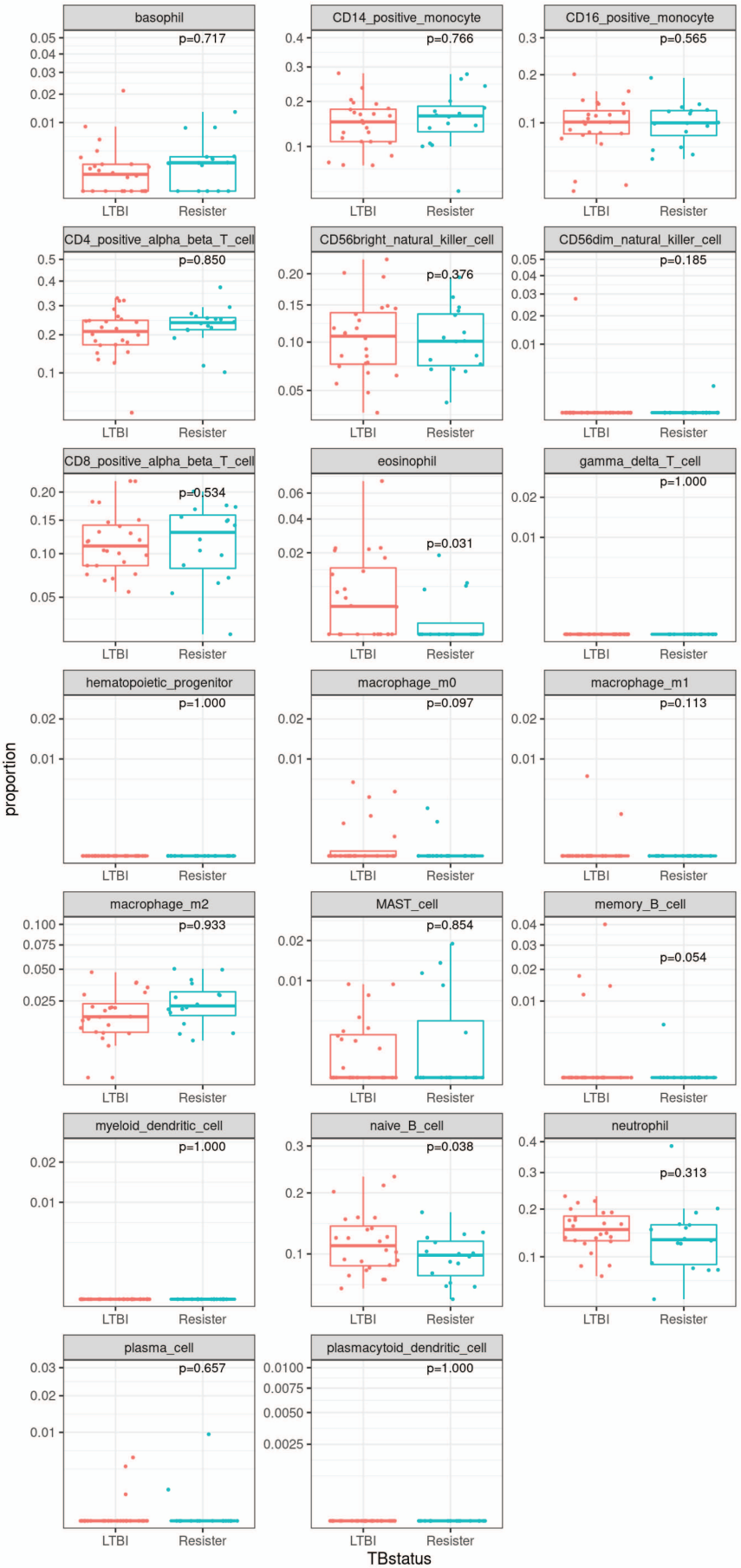
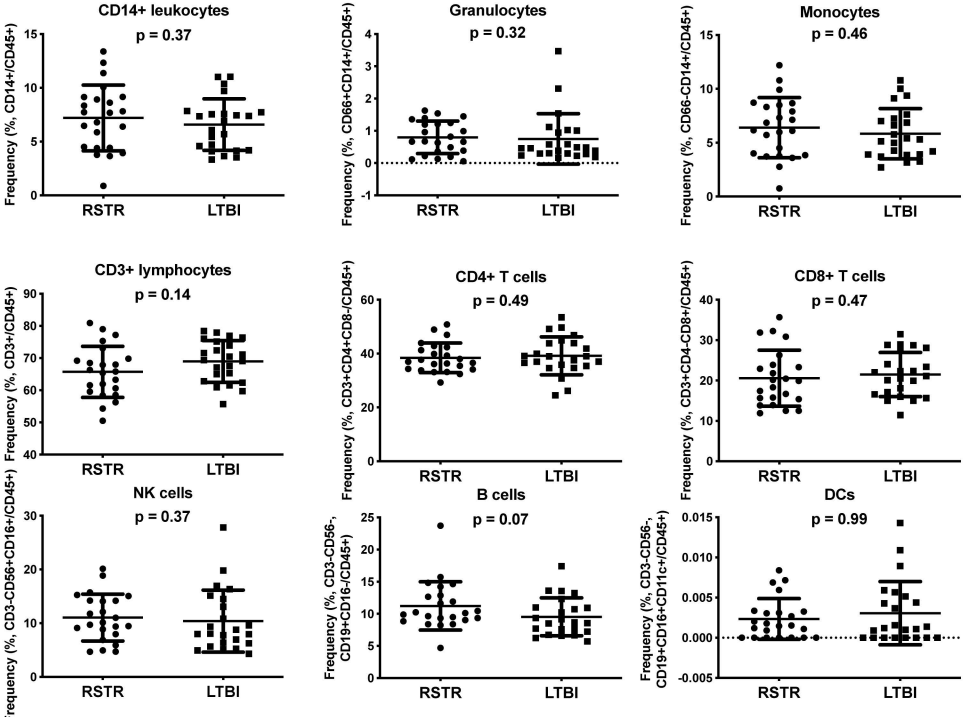
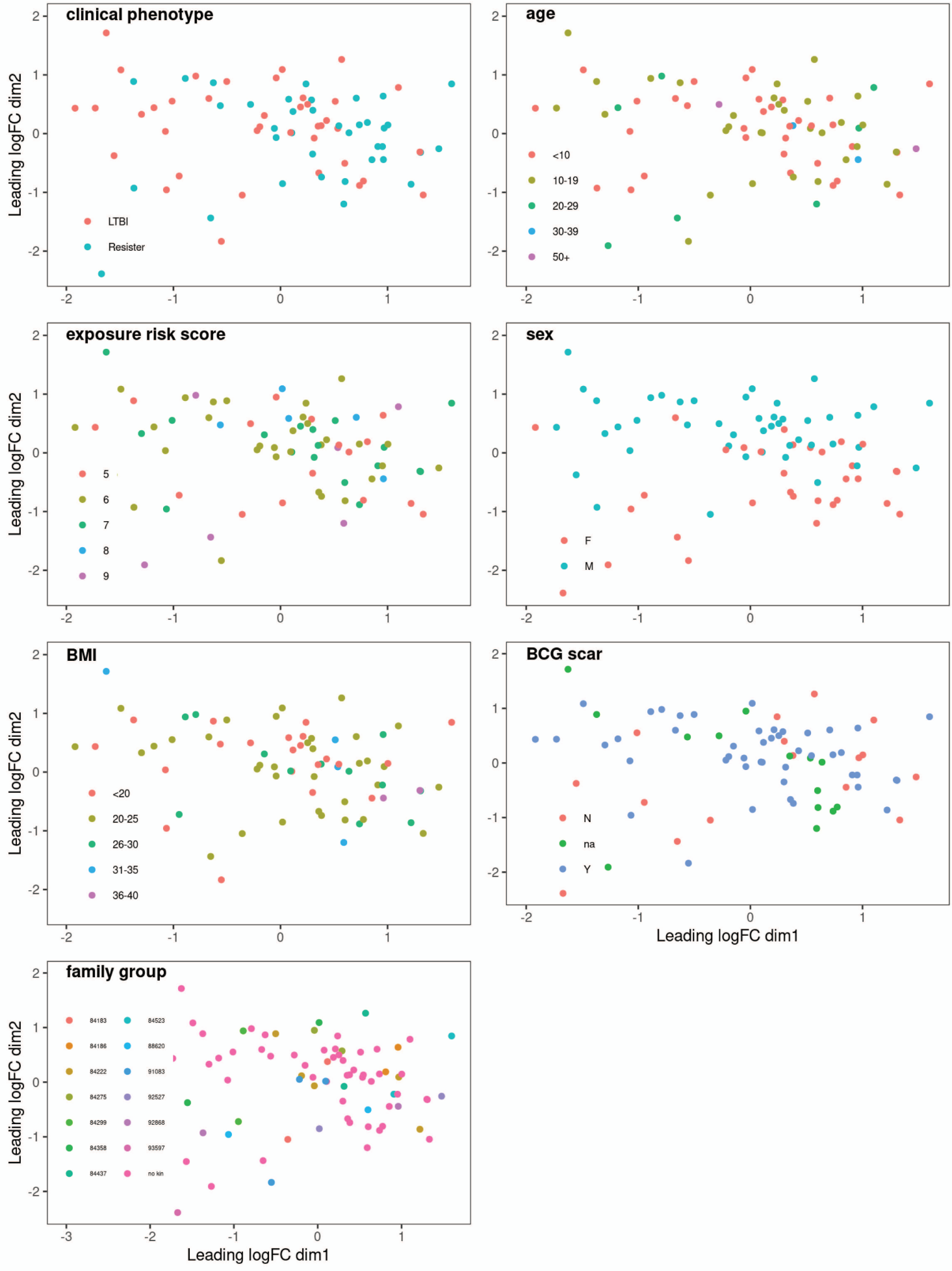
- plasma_cell
- plasmacytoid_dendritic_cell



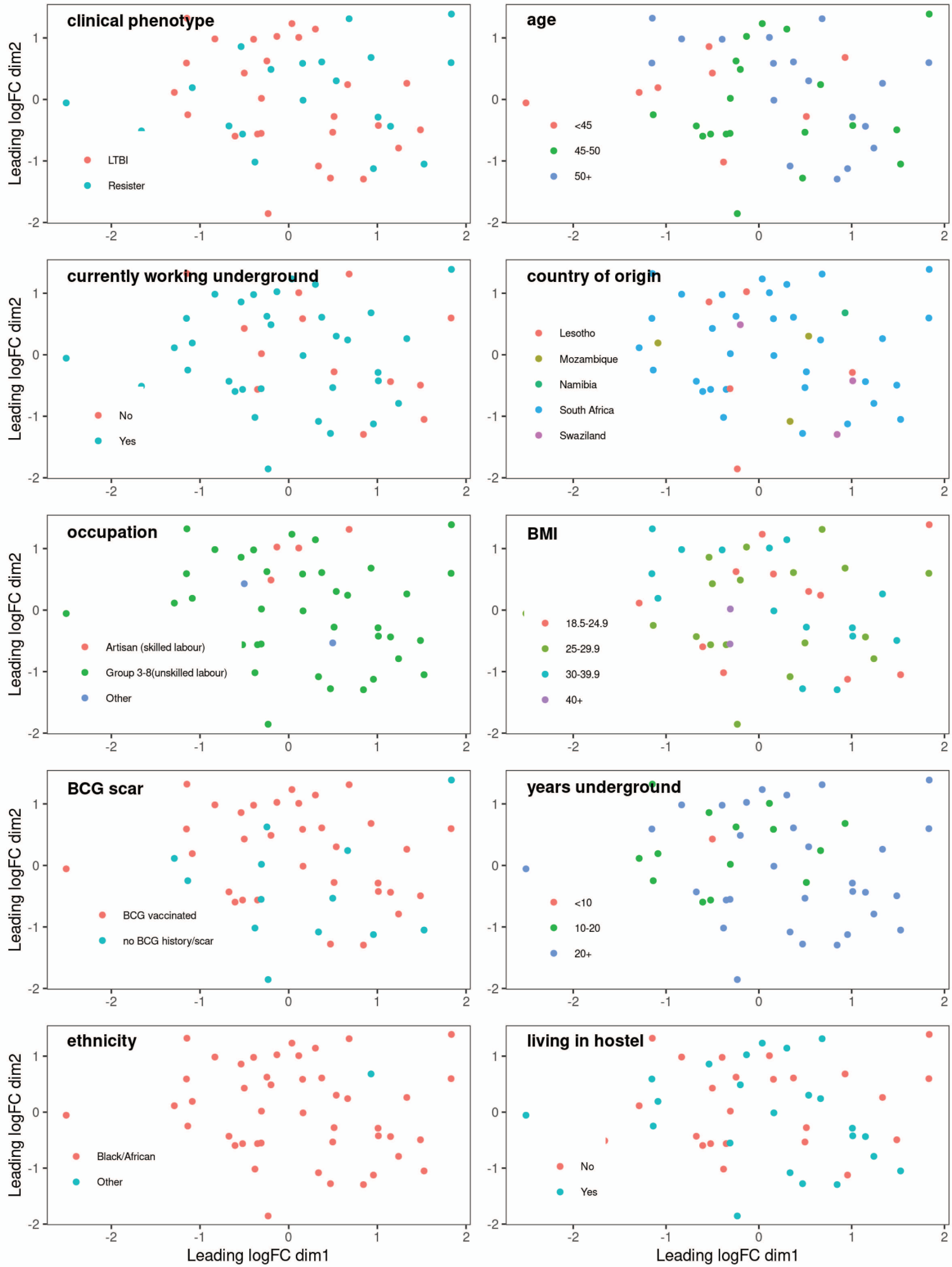
Supplemental Figure 3



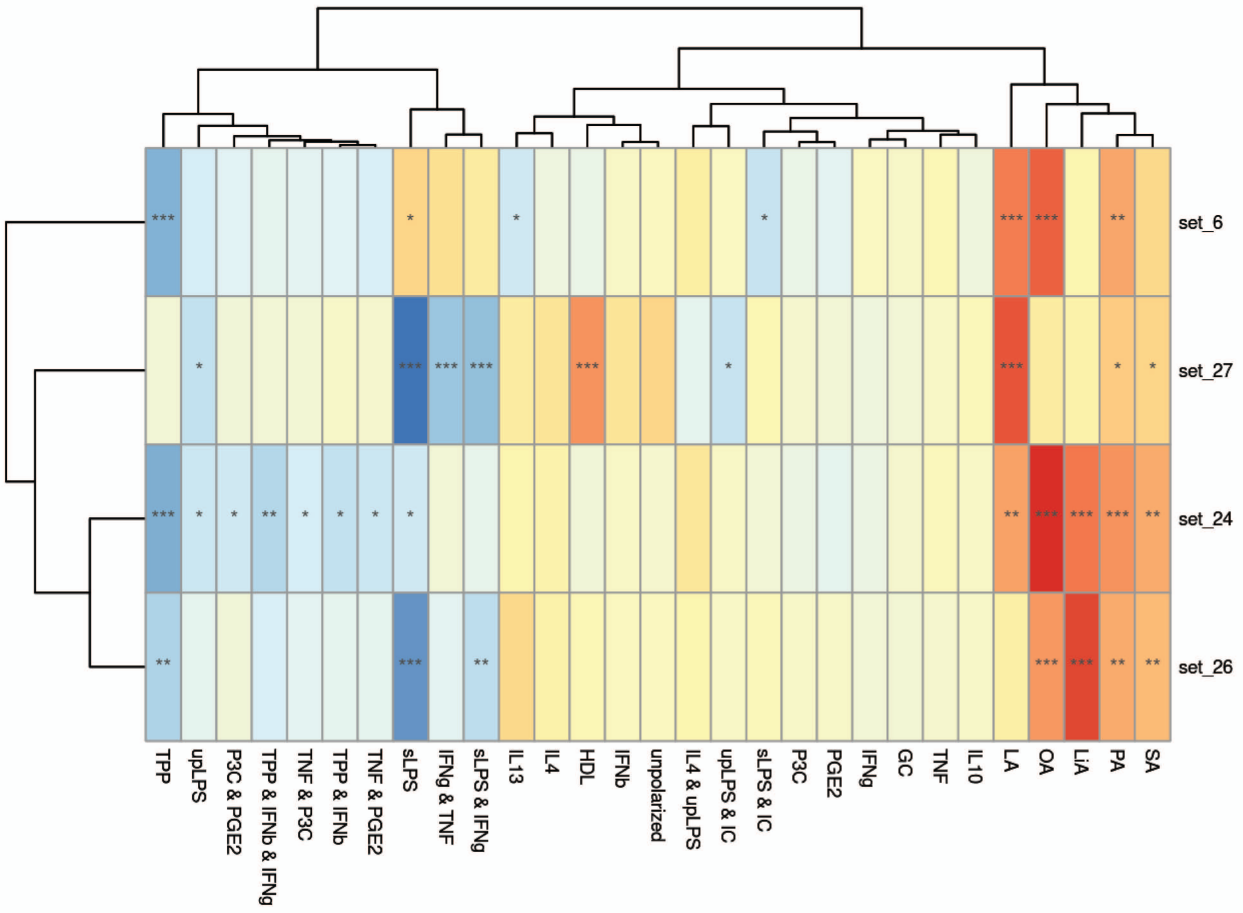
Supplemental Figure 4



Supplemental Figure 5

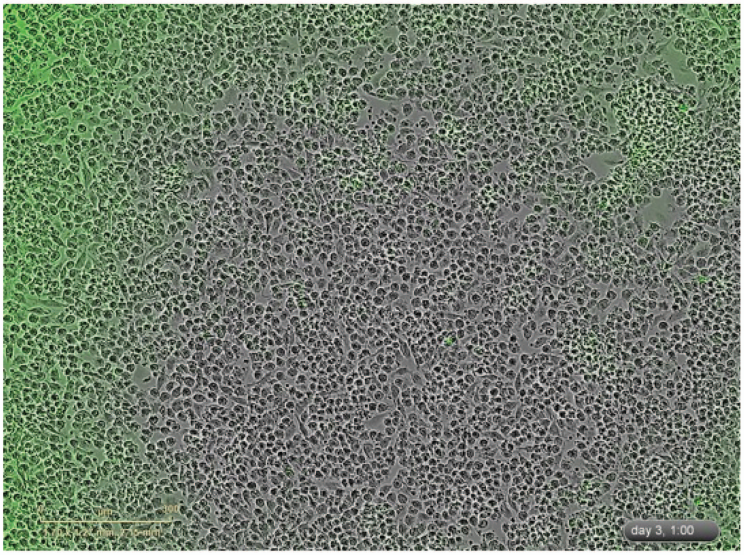


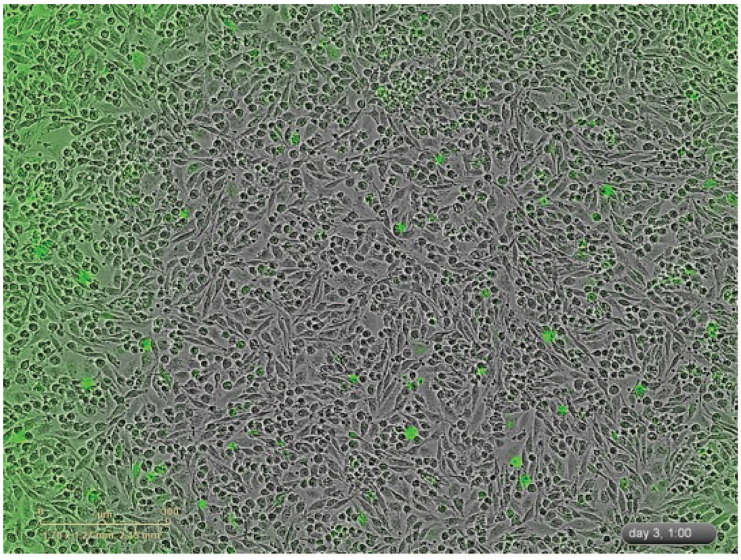
Supplemental Figure 6



Supplemental Figure 7

A)





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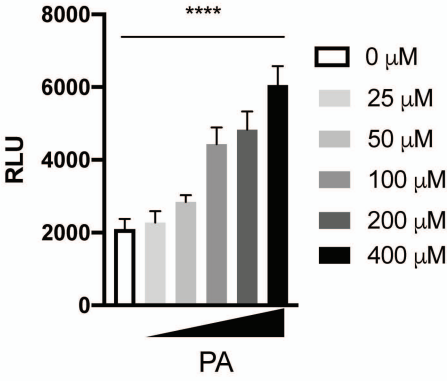

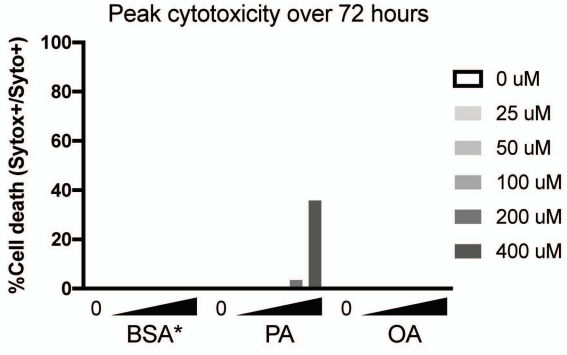

Peak Cytotoxicity over 72 hours (%)

	0	25	50	100	200	400	μM
BSA	0.28	0.21	0.27	0.22	0.19	0.24	
PA	0.28	0.17	0.15	0.25	3.46	35.8	
OA	0.28	0.19	0.14	0.13	0.14	0.16	