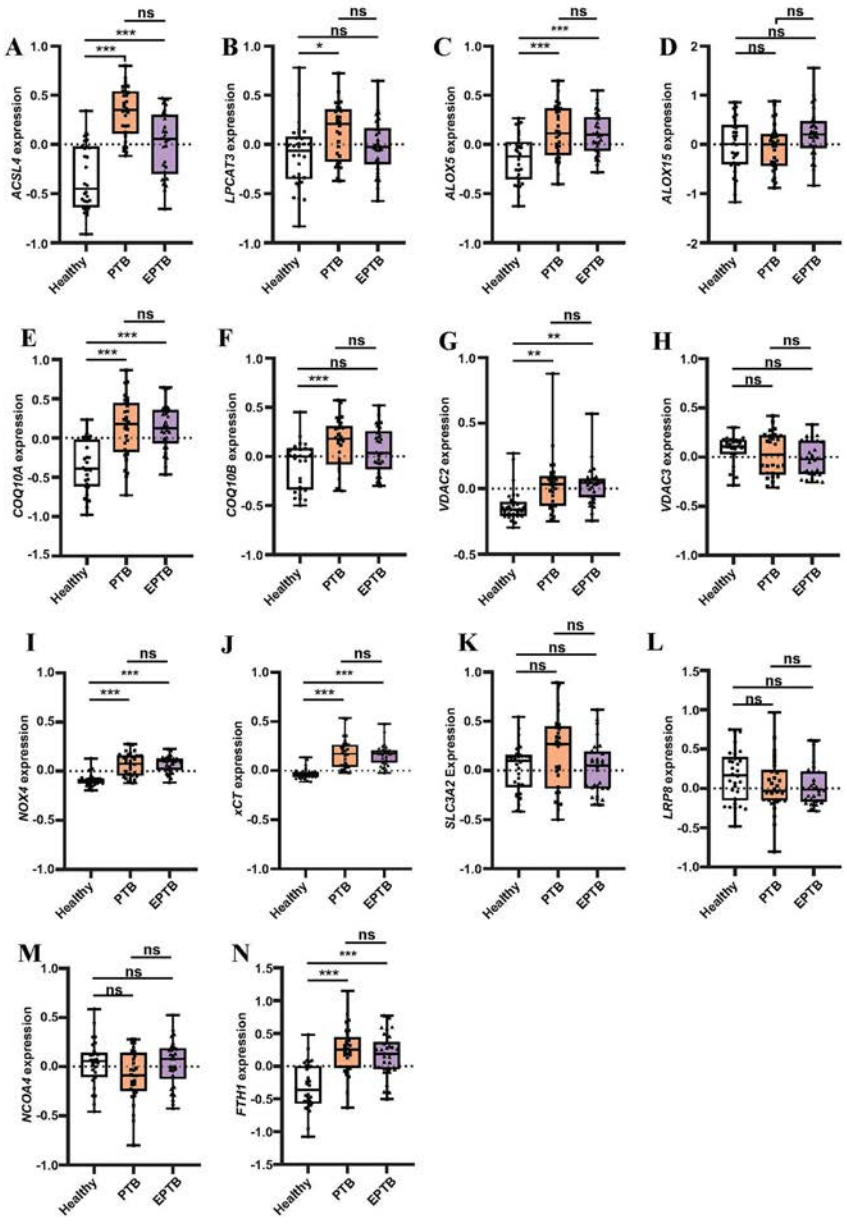
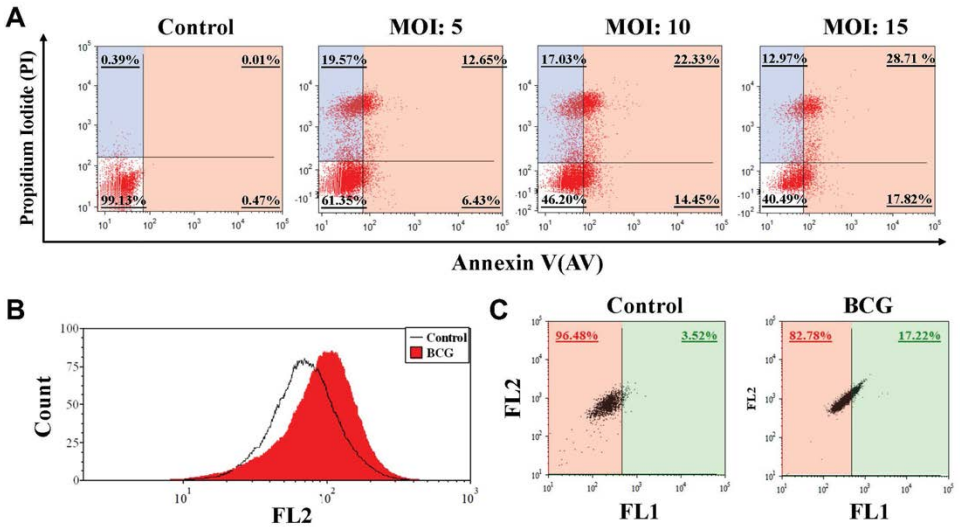
Supplementary Material

1 Supplementary Figures

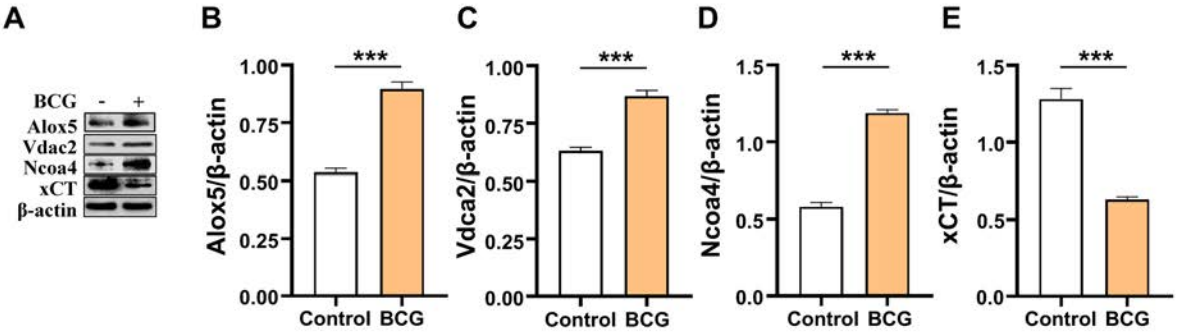


Supplementary Figure S1. Differentiate expression of genes involved in ferroptosis in peripheral blood of TB patients. Peripheral blood from healthy individuals (N=30), patients with pulmonary TB (PTB, N=30), and patients with extra-pulmonary TB (EPTB, N=30) were collected for transcriptome analysis using GEO database GSE83456 (a transcriptome sequencing dataset of peripheral blood from TB patients). (A-O) The differentiate expression of transcript of the *ACSL4* (A), *LPCAT3* (B), *ALOX5*

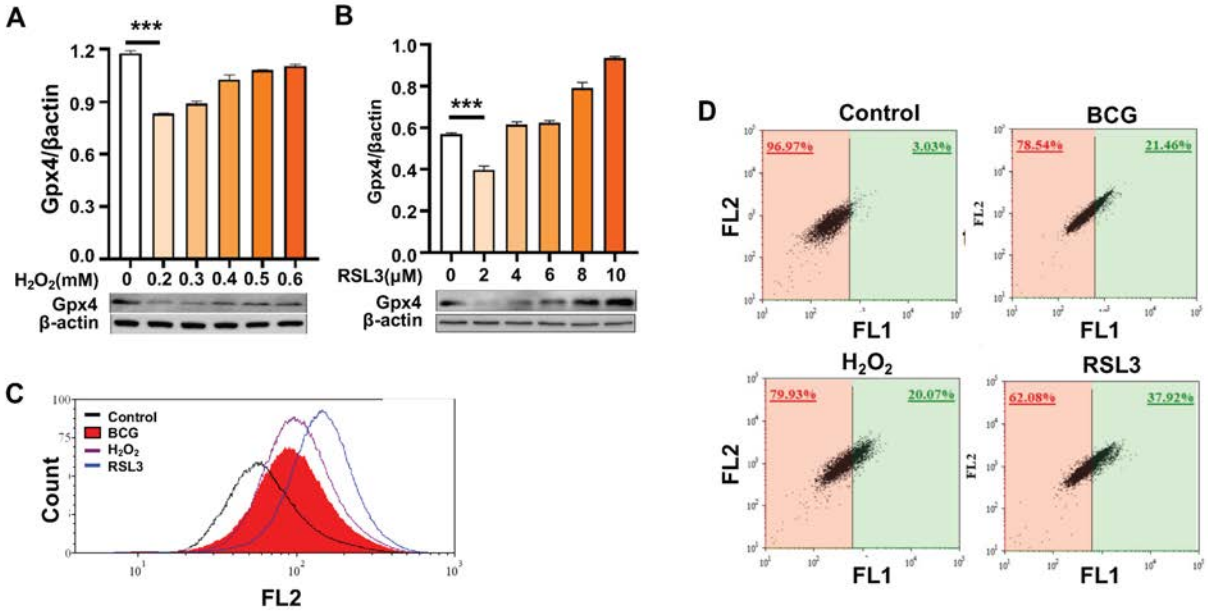
(C), *ALOX15* (D), *COQ10A* (E), *COQ10B* (F), *VDAC2* (G), *VDAC3* (H), *NOX4* (I), *xCT* (J), *SLC3A2* (K), *TFRC* (L), *NCOA4* (M), *FTH1* (N), and *LRP8* (O) in the peripheral blood of TB patients. An increased expression of transcripts of *ACSL4*, *LPCAT3*, *ALOX5*, *COQ10A*, *VDAC2*, *NOX4*, *xCT* and *FTH1* genes was found in peripheral blood of both patients with PTB and EPTB, while an increased *LPCAT3* and *COQ10B* transcripts were only observed in PTB patients, as compared with the healthy individuals. *TFRC* transcript was decreased in peripheral blood of EPTB patients compared with the healthy individuals. There was no statistical significance in differentiate expression of transcripts *ALOX15*, *VDAC3*, *SLC3A2*, *NCOA4* and *LRP8* genes. Data obtained from three independent experiments was processed using GraphPad Prism 8.0.1 software and ImageJ 1.52.a. Unpaired t test was used to analyze the differential changes of the two groups. Data represented mean ± SD, significant differences were indicated with asterisks (**, P＜0.01； ***, P＜0.001) .



Supplementary Figure S2. BCG induces necrosis and apoptosis in macrophages. (A) Representative quadrant plots of cell necrosis and apoptosis of RAW264,7 cells at 24 h post the infection of BCG at MOI of 5, 10 and 15, as determined by using Annexin V/PI in flow cytometry. (B)Histogram represented ROS level produced by treated RAW264.7 cells at MOI of 5 for 24 hours in flow cytometry assay. (C) The infection of BCG induced lipid peroxidation in RAW264.7 cells at MOI of 5 for 24 h as determined by BODIPY 581/591 C11 assays. Upon oxidation, its excitation of Red/590 nm shifts to 510nm (Green). The ratio of Green/Red cells in the BCG-infected cells was 17.22%, while the uninfected cells was 3.52%. One-way ANOVA was used to analyze the differences between groups.

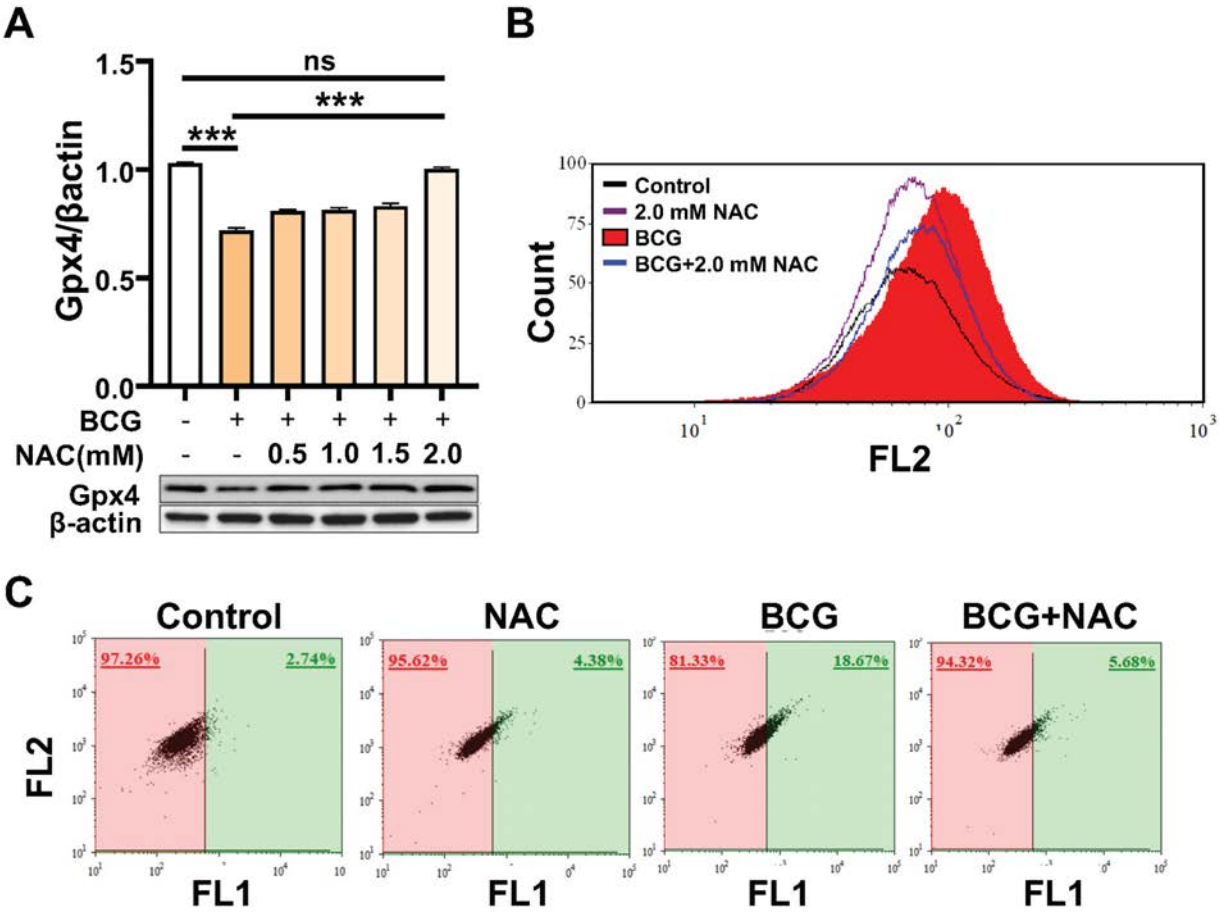


Supplementary Figure S3. BCG-induced macrophage ferroptosis. RAW264.7 cells were infected with BCG at MOI of 5 for 24 h prior to being harvest for immunoblotting analysis. (A) Representative blots of immunoblotting assay for indicated proteins of RAW264.7 cells. (B-E) Semi-quantitative analysis of blots of Alox5 (B), Vdca2 (C), Ncoa4 (D) and xCT (E) proteins of RAW264.7 cells showed in A. Data obtained from three independent experiments was processed using GraphPad Prism 8.0.1 software and ImageJ 1.52.a. Unpaired t test was used to analyze the differential changes of the two groups. Data represented as mean ± SD from three independent experiments (***, $p < 0.001$; $n = 3$).



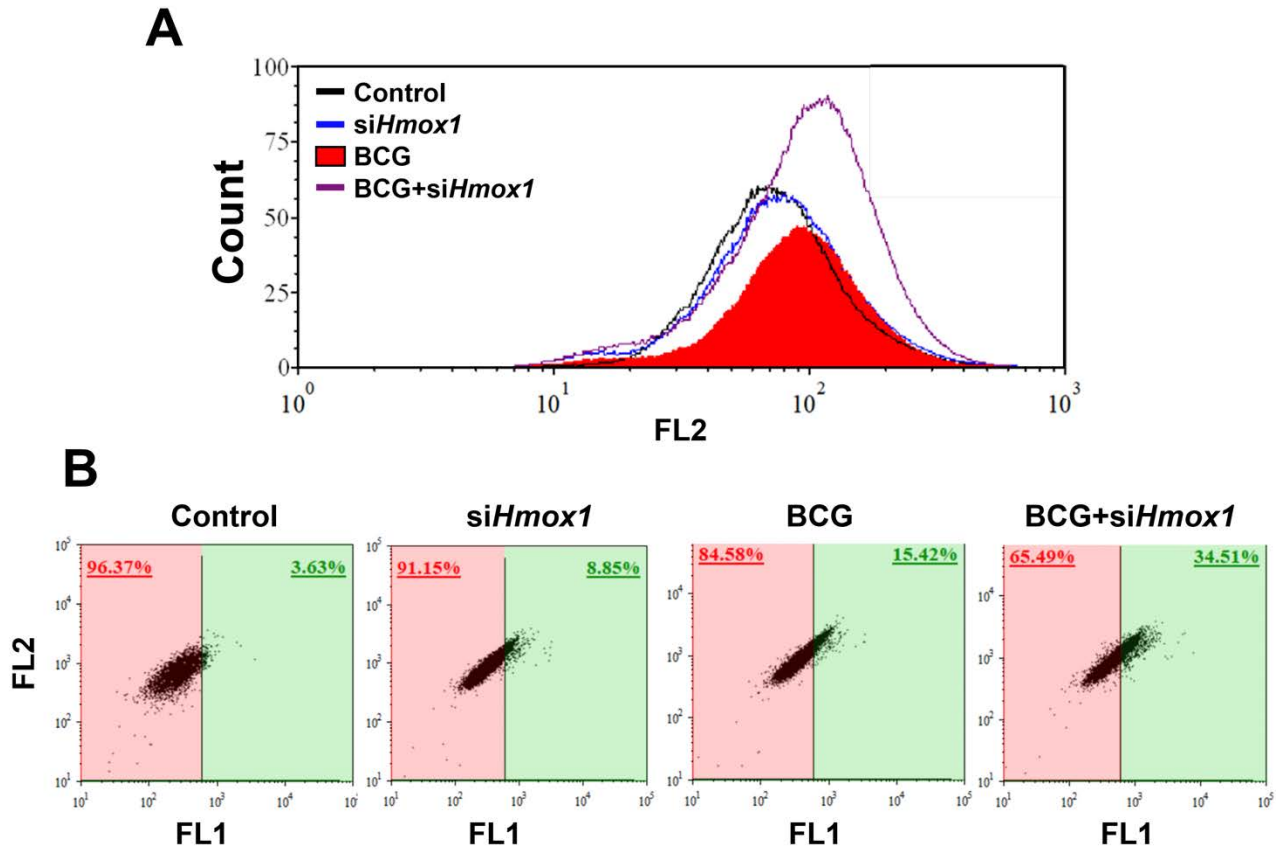
Supplementary Figure S4. H_2O_2 induces ferroptosis in RAW264.7 macrophages. RAW264.7 macrophages were treated with different concentrations of H_2O_2 or RSL3 to determine the optimal concentration that induce cell ferroptosis accessed by the inhibition of Gpx4 expression and flow cytometry. (A) Representative blots of Gpx4 of RAW264.7 cells treated with H_2O_2 at concentrations of 0, 0.2, 0.3, 0.4, 0.5 and 0.6 mM. The most inhibitory expression of Gpx4 was observed in cells treated with 0.2 mM of H_2O_2. (A) Representative blots of Gpx4 of RAW264.7 cells treated with H_2O_2

at concentrations of 0, 2, 4, 6, 8 and 10 mM. The most inhibitory expression of GPX4 was observed in cells treated with 2 mM of RSL3. (C-D) Histogram represented ROS level produced by treated RAW264.7 cells in flow cytometry assay (C) and Dot plot represented lipid peroxidation (D) of RAW264.7 macrophages in response to BCG infection (MOI 5) (top right), H_2O_2 (0.2 mM) (bottom left) and ferroptosis agonist RSL3 (2.0 μM) (bottom right), a major participant of the Fenton response at 24 h post treatments, as determined by flow cytometry and BODIPY 581/591 C11 assays respectively. Data obtained from three independent experiments was processed using GraphPad Prism 8.0.1 software and ImageJ 1.52.a. One-way ANOVA and Tukey's multiple comparisons test was used to analyze the differential changes of the three groups. Data represented mean ± SD from three independent experiments, significant differences are indicated with asterisks (***, $P < 0.001$).



Supplementary Figure S5. ROS scavenger NAC reduces BCG-induced macrophage ferroptosis. RAW264.7 macrophages were preincubated in medium containing NAC at a concentration of 0.5, 1.0, 1.5 or 2.0mM for 1 h prior to being infected with BCG at MOI of 5 for 24 hours. (A) Representative blots (bottom panel) and semi-quantitative analysis (top panel graph) of Gpx4 proteins of RAW264.7

cells showed that NAC was able to recover the BCG-inhibited expression of Gpx4 in a dose-dependent manner. (B-C) Histogram represented ROS level (B) and lipid peroxidation (C) of BCG-infected RAW264.7 macrophages that were pretreated with 2.0 mM of NAC, as determined by flow cytometry and BODIPY 581/591 C11 assays respectively. Data obtained from three independent experiments was processed using GraphPad Prism 8.0.1 software and ImageJ 1.52.a. One-way ANOVA and Tukey's multiple comparisons test was used to analyze the differential changes of the three groups. Data represented mean ± SD from three independent experiments, significant differences are indicated with asterisks (***, $P < 0.001$).



Supplementary Figure S6. Knockdown of *Hmox1* increases BCG-induced macrophage ferroptosis. RAW264.7 cells were transfected with si*Hmox1* to murine *Hmox1* gene, followed by being infected with BCG, prior to accessing the intracellular ROS production and lipid peroxidation by cytometry analysis at 24 h post infection. (A-B) Histogram represented ROS level (A) and Dot plot represented lipid peroxidation (B) of BCG-infected RAW264.7 macrophages that were transfected with si*Hmox1*, as determined by flow cytometry and BODIPY 581/591 C11 assays respectively.

2 Supplementary Tables

Supplementary Table S1: List of antibodies used

Antibody	Host	Reactivity	Mw (Kd)	Vendor	Cat. #
GPX4	Rabbit	mouse	17	Abclonal	A11243
FSP1	Rabbit	mouse	41	Proteintech	20886-1-AP
Hmox1	Rabbit	mouse	28-33	Proteintech	10701-1-AP
ALOX5	Rabbit	mouse	65	Abclonal	A6864
VDAC2	Rabbit	mouse	32	Proteintech	11663-1-AP
NCOA4	Rabbit	mouse	70	Abclonal	A5695
xCT	Rabbit	mouse	40-45	Proteintech	26864-1-AP
β-actin	Rabbit	mouse	42	Proteintech	20536-1-AP
β-Tublin	Mouse	mouse	50	Abmart	M30109
HRP-conjugated Affinipure Goat Anti-Rabbit IgG(H+L)	Goat	rabbit	NA	Proteintech	SA00001-2
Anti-rabbit IgG Fab2 Alexa Fluor（R）555 Molecular	Goat	rabbit	NA	Cell Signaling	4413S

Supplementary Table S2: Demographics of individuals of transcriptome analysis

Group	Sample ID	gender	ethnicity	age	disease state	Source name
Healthy	GSM2203617	M	Black	35	HC	Blood
Healthy	GSM2203604	F	Black	28	HC	Blood
Healthy	GSM2203598	M	Black	49	HC	Blood
Healthy	GSM2203619	F	White	22	HC	Blood
Healthy	GSM2203624	M	White	34	HC	Blood
Healthy	GSM2203629	M	ISC	28	HC	Blood
Healthy	GSM2203603	F	White	52	HC	Blood
Healthy	GSM2203640	F	Other	20	HC	Blood
Healthy	GSM2203633	M	White	54	HC	Blood
Healthy	GSM2203618	F	ISC	35	HC	Blood
Healthy	GSM2203608	F	Black	46	HC	Blood
Healthy	GSM2203599	M	White	23	HC	Blood
Healthy	GSM2203630	M	Black	24	HC	Blood
Healthy	GSM2203605	F	Black	36	HC	Blood
Healthy	GSM2203597	F	Black	47	HC	Blood
Healthy	GSM2203638	M	ISC	29	HC	Blood

Healthy	GSM2203639	F	White	68	HC	Blood
Healthy	GSM2203616	M	Black	64	HC	Blood
Healthy	GSM2203622	M	White	38	HC	Blood
Healthy	GSM2203628	F	ISC	23	HC	Blood
Healthy	GSM2203643	M	ISC	45	HC	Blood
Healthy	GSM2203625	M	White	42	HC	Blood
Healthy	GSM2203642	M	Black	32	HC	Blood
Healthy	GSM2203600	F	Other	49	HC	Blood
Healthy	GSM2203627	F	White	33	HC	Blood
Healthy	GSM2203602	M	White	35	HC	Blood
Healthy	GSM2203613	M	White	30	HC	Blood
Healthy	GSM2203626	F	ISC	34	HC	Blood
Healthy	GSM2203641	M	White	31	HC	Blood
Healthy	GSM2203647	F	White	29	HC	Blood
PTB	GSM2203663	F	Other	34	PTB	Blood
PTB	GSM2203671	F	Other	34	PTB	Blood
PTB	GSM2203656	F	ISC	27	PTB	Blood
PTB	GSM2203692	F	White	25	PTB	Blood
PTB	GSM2203666	M	Other	41	PTB	Blood
PTB	GSM2203685	M	White	32	PTB	Blood
PTB	GSM2203650	M	White	62	PTB	Blood
PTB	GSM2203659	F	Black	31	PTB	Blood
PTB	GSM2203689	M	White	40	PTB	Blood
PTB	GSM2203677	M	Other	25	PTB	Blood
PTB	GSM2203653	M	ISC	22	PTB	Blood
PTB	GSM2203667	M	White	53	PTB	Blood
PTB	GSM2203678	F	ISC	67	PTB	Blood
PTB	GSM2203669	F	White	54	PTB	Blood
PTB	GSM2203672	M	Black	43	PTB	Blood
PTB	GSM2203658	F	Black	22	PTB	Blood
PTB	GSM2203670	M	ISC	38	PTB	Blood
PTB	GSM2203651	M	ISC	38	PTB	Blood
PTB	GSM2203691	M	White	44	PTB	Blood
PTB	GSM2203684	F	ISC	24	PTB	Blood
PTB	GSM2203662	M	ISC	24	PTB	Blood
PTB	GSM2203654	F	ISC	26	PTB	Blood
PTB	GSM2203649	M	Black	42	PTB	Blood

PTB	GSM2203674	F	White	45	PTB	Blood
PTB	GSM2203676	M	White	82	PTB	Blood
PTB	GSM2203675	M	ISC	24	PTB	Blood
PTB	GSM2203687	M	ISC	21	PTB	Blood
PTB	GSM2203690	M	ISC	30	PTB	Blood
PTB	GSM2203683	M	Other	26	PTB	Blood
PTB	GSM2203686	M	Black	27	PTB	Blood
EPTB	GSM2203563	M	ISC	33	EPTB	Blood
EPTB	GSM2203550	M	ISC	33	EPTB	Blood
EPTB	GSM2203567	M	ISC	29	EPTB	Blood
EPTB	GSM2203547	M	Black	48	EPTB	Blood
EPTB	GSM2203564	M	ISC	29	EPTB	Blood
EPTB	GSM2203543	M	ISC	27	EPTB	Blood
EPTB	GSM2203583	M	Other	50	EPTB	Blood
EPTB	GSM2203548	M	White	25	EPTB	Blood
EPTB	GSM2203584	M	White	30	EPTB	Blood
EPTB	GSM2203585	F	Other	47	EPTB	Blood
EPTB	GSM2203540	M	ISC	21	EPTB	Blood
EPTB	GSM2203545	M	ISC	47	EPTB	Blood
EPTB	GSM2203568	M	ISC	51	EPTB	Blood
EPTB	GSM2203554	M	ISC	30	EPTB	Blood
EPTB	GSM2203562	F	Black	23	EPTB	Blood
EPTB	GSM2203542	F	Black	23	EPTB	Blood
EPTB	GSM2203557	M	White	29	EPTB	Blood
EPTB	GSM2203560	F	ISC	28	EPTB	Blood
EPTB	GSM2203556	M	ISC	34	EPTB	Blood
EPTB	GSM2203579	F	Other	41	EPTB	Blood
EPTB	GSM2203553	F	White	20	EPTB	Blood
EPTB	GSM2203576	F	Black	35	EPTB	Blood
EPTB	GSM2203551	M	ISC	34	EPTB	Blood
EPTB	GSM2203544	F	ISC	18	EPTB	Blood
EPTB	GSM2203571	F	White	47	EPTB	Blood
EPTB	GSM2203575	F	Black	51	EPTB	Blood
EPTB	GSM2203573	F	Other	52	EPTB	Blood
EPTB	GSM2203586	M	ISC	48	EPTB	Blood
EPTB	GSM2203580	F	White	30	EPTB	Blood

EPTB	GSM2203549	M	ISC	34	EPTB	Blood