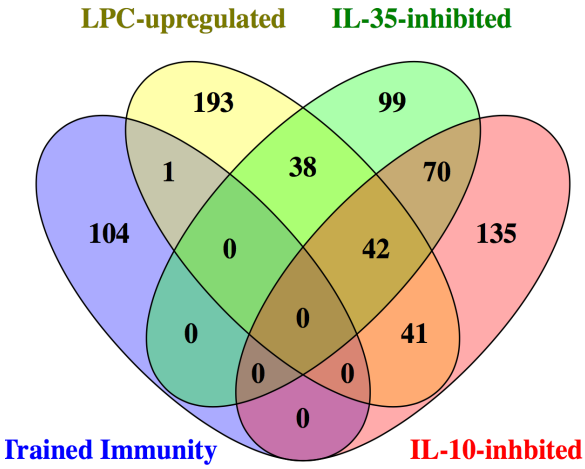
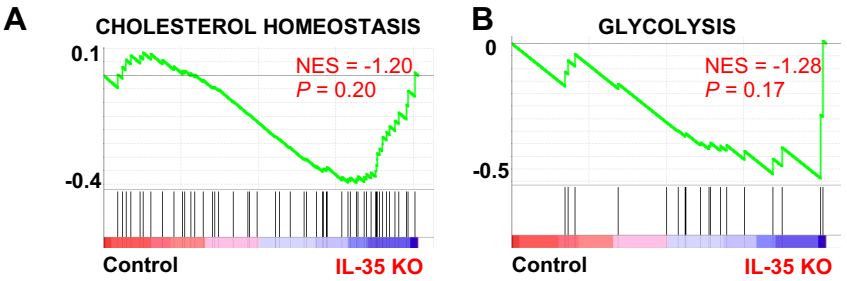
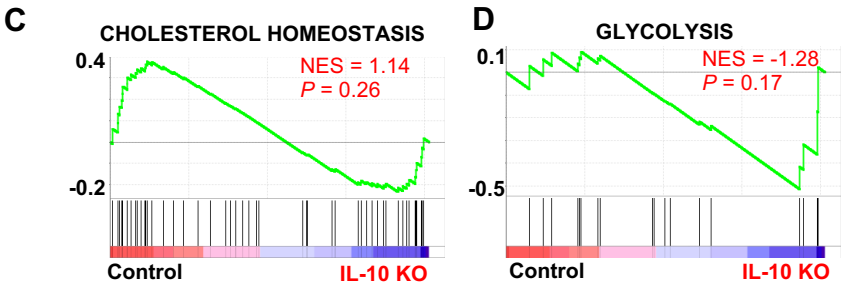
Supplemental Figure 1



Supplemental Figure 1. No overlap is found between β-glucan-induced trained immunity response (innate immune memory) in human monocytes and IL-35/IL-10-inhibited genes in human aortic endothelial cells (HAEC). RNA-Seq data GSE58310 (n=4 in each group) downloaded from NIH-NCBI-Gene Expression Omnibus (GEO) were analyzed to investigate the relationship between genes that are significantly upregulated by β-glucan (trained immunity genes) for more than 2 folds in human monocytes (PMID: 25258085), and LPC-upregulated genes, IL-35-inhibited genes, and IL-10-inhibited genes (by more than 1.4-fold in the three groups) in HAEC.

Supplemental Figure 2





E

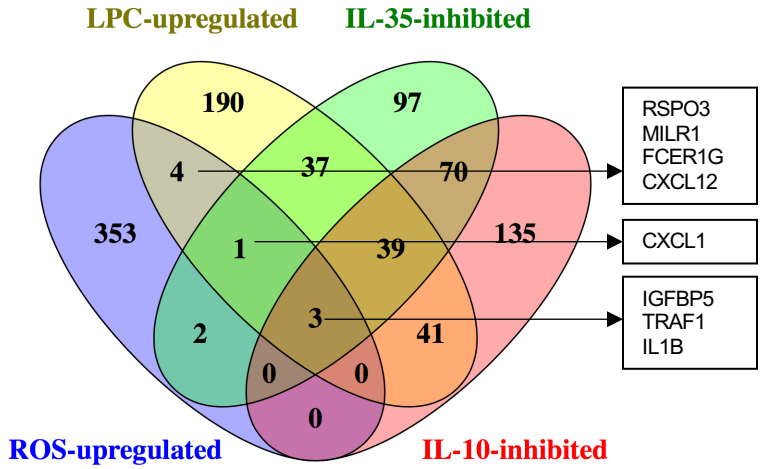
Gene	Category	IL-35 KO /Control	IL-10 KO /Control
Eno3	Glycolysis	1.16	1.36
Mvk	Mevalonate	0.91	0.91
Mvd	Mevalonate	1.01	1.11
Dhcr7	Mevalonate	1.10	0.53
Scd1	Mevalonate	1.05	1.00
Scd2	Mevalonate	0.83	0.89
Acat2	Acetyl-CoA	0.75	0.84
Actb	House-keeping	1.02	1.07

F

Gene	Category	IL-35 /Control
Eno3	Glycolysis	1.08
Mvk	Mevalonate	1.02
Mvd	Mevalonate	1.06
Dhcr7	Mevalonate	1.00
Scd1	Mevalonate	0.91
Scd2	Mevalonate	1.06
Acat2	Acetyl-CoA	0.88
Actb	House-keeping	0.95

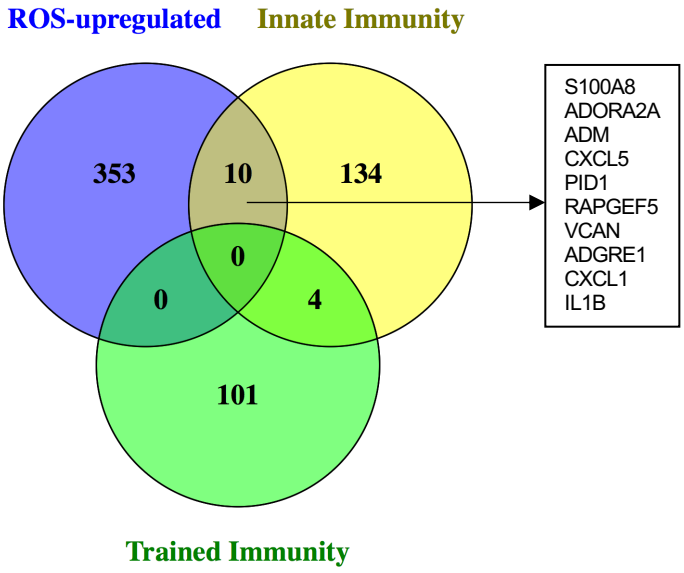
Supplemental Figure 2. IL-35 and IL-10 do not affect trained immunity signatures in T cells. RNA-Seq data GSE24210 (A to E, n =2~3 in each group) downloaded from Gene Expression Omnibus (GEO) were analyzed to investigate the effects of regulatory T cell (Treg)-specific deficiency of IL-35/IL-10 on murine T cells. RNA-Seq data GSE127735 (F, n=3 in each group) downloaded from GEO were analyzed to investigate the effects of IL-35 treatment on murine T cells (F). A&B. GSEA plots showing that glycolysis and cholesterol homeostasis pathways are not affected in T cells upon Treg-specific deficiency of IL-35. C&D. GSEA plots showing that glycolysis and cholesterol homeostasis pathways are not changed in T cells upon Treg-specific deficiency of IL-10. E. Representative gene expression changes in T cells upon Treg-specific deficiency of IL-35/IL-10. F. Representative gene expression changes in T cells upon IL-35 treatment. NES, normalized enrichment score. FDR, false discovery rate.

Supplemental Figure 3



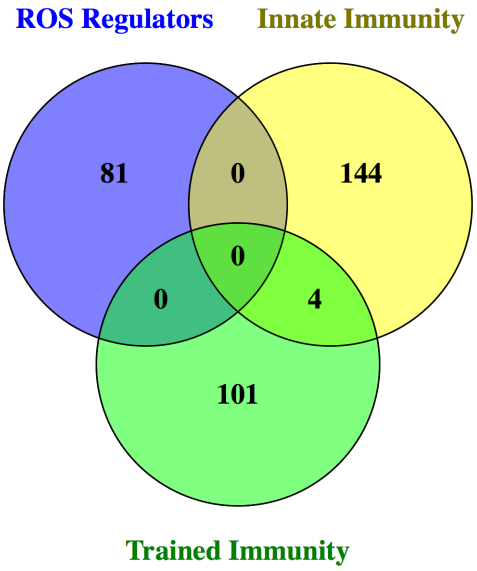
Supplemental Figure 3. Reactive oxygen species (ROS) partially mediate the inhibitory effects of IL-35 and IL-10 in LPC-treated human aortic endothelial cells (HAEC). RNA-Seq data GSE7810 (n=3 in each group) downloaded from Gene Expression Omnibus (GEO) were analyzed to examine the relationship between genes that are significantly upregulated in Nrf2 knockout cells (ROS-upregulated genes) and LPC-upregulated genes, IL-35-inhibited genes, and IL-10-inhibited genes (by more than 1.4-fold in the three groups) in HAEC.

Supplemental Figure 4



Supplemental Figure 4. Reactive oxygen species (ROS) partially mediate LPS-induced innate immunity response, while ROS do not have any effect on β-glucan-induced trained immunity response in human monocytes. RNA-Seq data GSE7810 (n=3 in each group) and GSE58310 (n=4 in each group) downloaded from Gene Expression Omnibus (GEO) were analyzed to investigate the relationship between genes that are significantly upregulated in Nrf2 knockout cells (ROS-upregulated genes) and genes that are significantly upregulated by lipopolysaccharide (LPS, innate immunity genes) or β-glucan (trained immunity genes) for more than 2 folds in human monocytes.

Supplemental Figure 5



Supplemental Figure 5. LPS-induced innate immunity response/ β-glucan-induced trained immunity response does not alter reactive oxygen species (ROS) regulator gene expressions. RNA-Seq data GSE58310 (n=4 in each group) downloaded from Gene Expression Omnibus (GEO) were analyzed to investigate the relationship between ROS regulators and genes that are significantly upregulated by lipopolysaccharide (LPS, innate immunity genes) or β-glucan (trained immunity genes) for more than 2 folds in human monocytes.

Supplemental Table 1. IL-35 and IL-10 block LPC-induced endothelial activation. HAEC were preincubated with 10 ng/ml of IL-35 and IL-10 for 1 hour. After stimulation of LPC (10μM) for 18 hours, RNAs were collected for Human EC Biology PCR array (QIAGEN) analysis. Pooled samples (n=3) in each group.

Gene	LPC /Control	LPC+IL-35 /Control	LPC+IL-10 /Control
SELE	2.22	3.10	2.39
CCL2	2.11	1.88	2.15
PLAT	1.81	1.34	1.49
VCAM1	1.80	0.94	1.14
IL1B	1.74	0.93	1.23
ICAM1	1.61	1.29	1.62
TIMP1	1.45	1.50	1.37
TNFSF10	1.37	1.35	1.49
TYMP	1.33	1.36	0.99
ITGAV	1.29	1.52	1.53
SERPINE1	1.29	1.52	1.52
VEGFA	1.29	1.07	1.05
IL7	1.28	1.00	1.37
EDN1	1.22	1.21	1.33
TEK	1.22	1.39	1.40
THBD	1.22	1.40	1.28
CDH5	1.21	1.41	1.41
OCLN	1.20	1.40	1.49
ITGB1	1.20	1.22	1.31
CFLAR	1.19	1.26	1.25
ENG	1.19	0.90	0.67
PTGIS	1.17	0.73	0.85
ADAM17	1.17	1.17	1.17
ANXA5	1.16	1.19	1.20
HIF1A	1.16	1.19	1.28
CAV1	1.15	1.19	1.25
TFPI	1.12	1.15	1.20
BAX	1.11	1.23	1.21
ITGA5	1.10	1.29	1.25
CASP3	1.09	1.15	1.10
PTGS2	1.08	1.26	1.23
BCL2L1	1.08	1.40	1.29
SPHK1	1.07	1.15	1.19
KIT	1.07	1.17	1.09
FGF2	1.06	1.25	1.24
KDR	1.06	1.26	1.41
PGF	1.06	1.09	1.15
PTK2	1.04	1.29	1.28
IL6	1.02	1.02	1.01
VWF	1.01	1.12	1.13
ITGB3	1.01	1.04	0.91
F2R	1.01	0.97	0.95
PLAU	1.00	1.08	1.07
FLT1	1.00	1.22	1.28
F3	1.00	0.87	0.93
ACE	0.99	1.12	1.18
PROCR	0.99	1.13	0.98
THBS1	0.97	1.17	1.16
COL18A1	0.97	1.01	0.96
SOD1	0.96	0.90	0.93
FAS	0.96	0.98	1.08
BCL2	0.94	1.14	1.22
MMP2	0.92	0.96	0.77
TGFB1	0.92	1.14	0.92
PECAM1	0.91	0.89	0.93
CASP1	0.91	0.84	0.46
NOS3	0.89	1.30	1.22
HMOX1	0.89	0.89	1.08
FN1	0.86	1.18	1.21
SELL	0.85	1.23	0.99
MMP1	0.73	0.70	0.88

Supplemental Table 2. IL-35 specifically induces IL-35 receptor subunit IL35RB1 in HAEC. HAEC were preincubated with 10 ng/ml of IL-35 and IL-10 for 1 hour. After stimulation of LPC (10μM) for 18 hours, RNAs were collected for RNA-Seq analysis. Pooled samples (n=3) in each group.

Gene	Receptor for	LPC/ Control	LPC+IL-35/ LPC	LPC+IL-10/ LPC
IL35RB1	IL-35	-∞	∞	1.00
IL27RA	IL-35	0.93	1.00	0.99
IL6ST	IL-35	0.95	0.97	0.95
IL10RA	IL-10	0.97	-∞	0.99
IL10RB	IL-10	1.04	1.04	0.95

Supplemental Table 3. IL-35 and IL-10 do not change acetyl-CoA generating enzymes in HAEC. HAEC were preincubated with 10 ng/ml of Il-35 and IL-10 for 1 hour. After stimulation of LPC (10μM) for 18 hours, RNAs were collected for RNA-Seq analysis. Genes that are changed by more than 1.5 folds are highlighted in red. Pooled samples (n=3) in each group.

Gene	LPC /Control	LPC+IL-35 /LPC	LPC+IL-10 /LPC
ACAA2	1.05	1.01	1.06
ACAD10	0.93	0.88	1.02
ACAD11	1.14	1.12	0.98
ACAD8	0.95	1.06	0.99
ACAD9	1.07	1.10	1.11
ACADM	1.01	1.01	1.04
ACADS	0.88	1.18	1.15
ACADSB	1.01	0.79	0.80
ACADVL	1.23	1.01	1.02
ACAT1	1.26	0.73	0.73
ACAT2	1.56	0.84	1.27
ACLY	1.21	1.03	1.03
ACO1	1.09	0.99	1.02
ACSS1	1.07	1.08	0.89
ACSS2	1.10	1.09	1.18
ADH1B	1.00	1.00	1.00
ALDH1A1	1.00	0.97	0.97
ALDH1A2	1.04	1.16	0.98
ALDH1A3	0.94	1.01	1.07
ALDH1B1	1.06	1.12	1.15
ALDH1L2	440.00	0.95	0.92
ALDH2	1.03	1.09	1.16
BCAT2	1.16	1.02	0.98
BCKDHA	1.01	1.04	1.00
BCKDHB	0.97	0.99	1.18
BCKDK	1.04	1.04	0.94
BDH1	0.93	1.04	0.84
ECH1	1.32	0.89	0.98
GLS	1.05	0.96	0.94
GLUD1	1.06	0.98	1.04
GOT1	1.00	1.09	1.10
HADH	0.87	1.09	1.00
IDH1	1.23	1.03	0.98
OXCT1	0.95	1.03	1.04
PDHA1	1.14	1.00	1.03
PDHB	1.06	1.04	1.08

Supplemental Table 4. List of 84 ROS regulators.

Gene Symbol	Gene name
GPX1	Glutathione peroxidase 1
GPX2	Glutathione peroxidase 2
GPX3	Glutathione peroxidase 3
GPX4	Glutathione peroxidase 4
GPX5	Glutathione peroxidase 5
GPX6	Glutathione peroxidase 6
GPX7	Glutathione peroxidase 7
GSTK1	glutathione S-transferase kappa 1
EHD2	EH-domain containing 2
PRDX1	Peroxiredoxin 1
PRDX2	Peroxiredoxin 2
PRDX3	Peroxiredoxin 3
PRDX4	Peroxiredoxin 4
PRDX5	Peroxiredoxin 5
PRDX6	Peroxiredoxin 6
AASS	Aminoadipate-semialdehyde synthase
APC	Adenomatous polyposis coli
CAT	Catalase
CTSB	Cathepsin B
DUOX1	Dual oxidase 1
EPX	Eosinophil peroxidase
KIF9	Kinesin family member 9
LPO	Lactoperoxidase
MPO	Myeloperoxidase
PRDX6-RS1	Peroxiredoxin 6 related sequence 1
PTGS1	Prostaglandin-endoperoxide synthase 1
PTGS2	Prostaglandin-endoperoxide synthase 2
RAG2	Recombination activating gene 2
SERPINB1B	Serine (or cysteine) peptidase inhibitor,clade B, member 1b
SLC41A3	Solute carrier family 41, member 3
TMOD1	Tropomodulin 1
TPO	Thyroid peroxidase
GSR	Glutathione reductase
NXN	Nucleoredoxin
SRXN1	Sulfiredoxin 1 homolog
TXNRD1	Thioredoxin reductase 1
TXNRD2	Thioredoxin reductase 2
TXNRD3	Thioredoxin reductase 3
SOD1	Superoxide dismutase 1
SOD2	Superoxide dismutase 2
SOD3	Superoxide dismutase 3
CCS	Copper chaperone for superoxide dismutase
CYBA	Cytochrome b-245, alpha polypeptide
NCF2	Neutrophil cytosolic factor 2
NOS2	Nitric oxide synthase 2
NOX1	NADPH oxidase 1
NOX4	NADPH oxidase 4
NOXA1	NADPH oxidase activator 1
NOXO1	NADPH oxidase organizer 1
RECQL4	RecQ protein-like 4
SCD1	Stearoyl-coenzyme A desaturase 1
FMO2	Flavin containing monooxygenase 2
IL19	Interleukin 19
IL22	Interleukin 22
ALS2	Amyotrophic lateral sclerosis 2
APOE	Apolipoprotein E
ERCC2	Excision repair cross-complementing rodent repair deficiency, complementation group 2
ERCC6	Excision repair cross-complementing rodent repair deficiency, complementation group 6
GAB1	GRB2-associated binding protein 1
IDH1	Isocitrate dehydrogenase 1
MPP4	Membrane protein, palmitoylated 4
NQO1	NAD(P)H dehydrogenase, quinone 1
NUDT15	Nudix (nucleoside diphosphate linked moiety X)-type motif 15
PARK7	Parkinson disease 7
PPP1R15B	Protein phosphatase 1, regulatory subunit 15B
PRNP	Prion protein
PSMB5	Proteasome subunit, beta type, 5
TXNIP	Thioredoxin interacting protein
UCP3	Uncoupling protein 3
XPA	Xeroderma pigmentosum, complementation group A
AQR	Aquarius homolog
ATR	Ataxia telangiectasia and Rad3 related
CYGB	Cytoglobin
DNM2	Dynamin 2
FANCC	Fanconi anemia, complementation group C
HBQ1	Hemoglobin, theta 1
IFT172	Intraflagellar transport 172 homolog
MB	Myoglobin
NGB	Neuroglobin
SLC38A1	Solute carrier family 38, member 1
VIM	Vimentin