

SUPPLEMENTAL MATERIAL

Blohmke et al., <http://www.jem.org/cgi/content/full/jem.20151025/DC1>

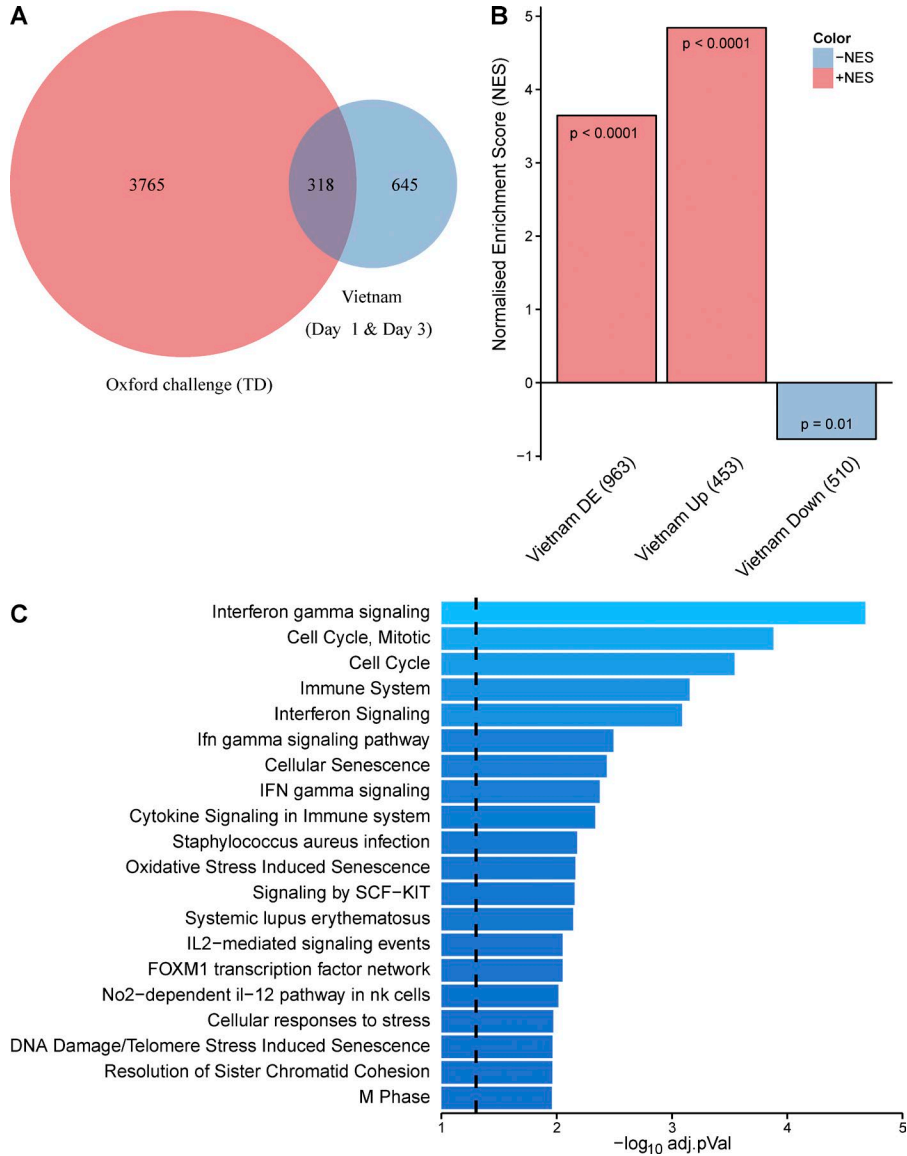


Figure S1. **Transcriptional data in typhoid patients from Vietnam.** (A) Venn diagram illustrating the overlap of differentially expressed genes (FC, ± 1.25 ; false discovery rate, < 0.05) in patients with acute typhoid in Vietnam (day 1 or 3 after hospitalization) and participants at TD in the challenge study. (B) GSEA of custom gene sets from the Vietnam study (1,000 iterations, BH p-value). (C) 20 most significantly overrepresented pathway based on significantly expressed genes (FC, ± 1.5 , $P < 0.01$) in samples derived from typhoid confirmed patients in Vietnam at day 1 or 3 after admission to hospital.

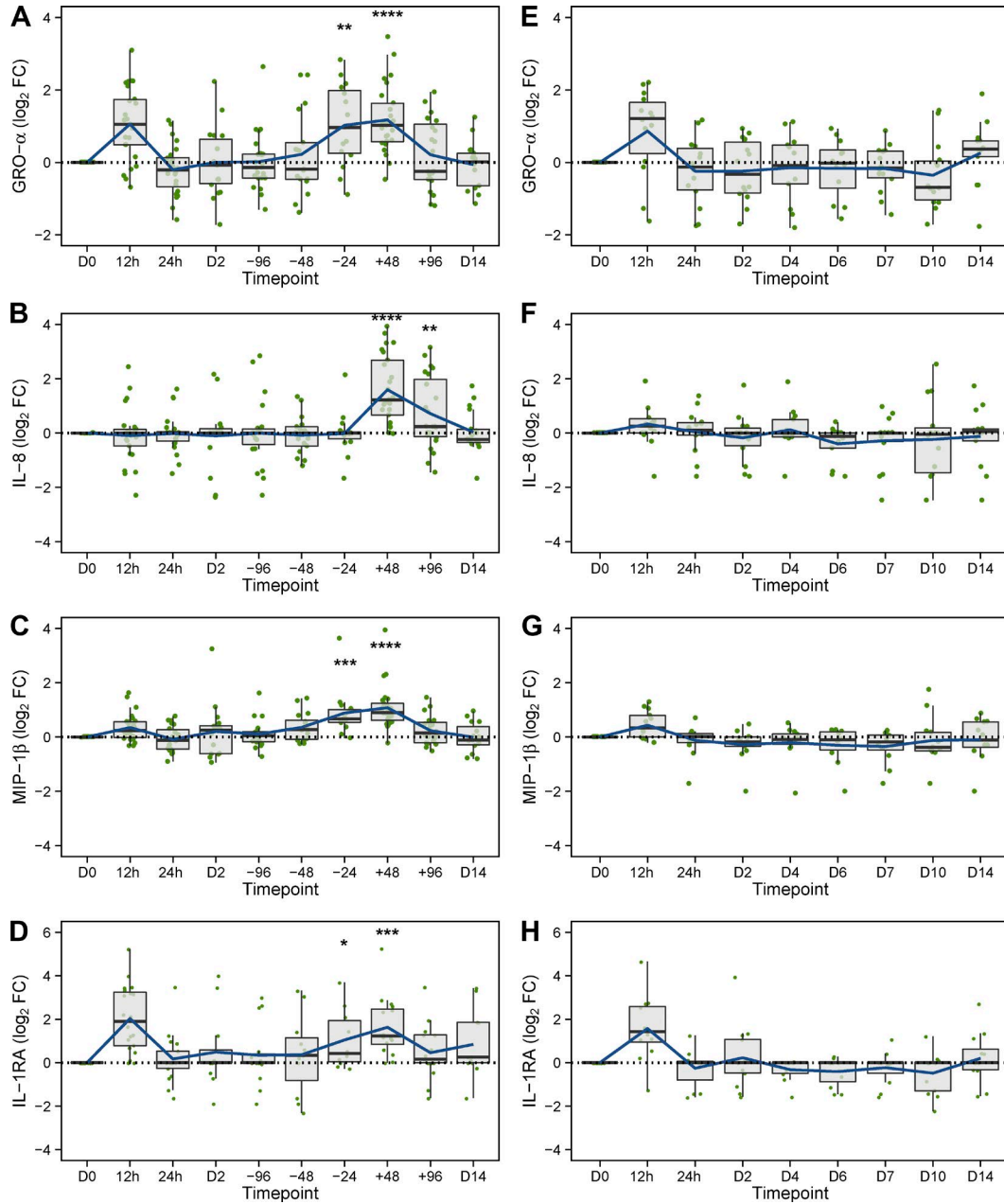


Figure S2. **Longitudinal cytokine levels after challenge.** Cytokine induction [\log_2 FC/D0] in participants diagnosed with acute typhoid ($n = 25$; A-D) and those who stayed well ($n = 16$; E-H). Data are median with 25th/75th percentile, and blue line depicts the mean. Each sample was run in duplicates. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$ using one-sample Student's t test.

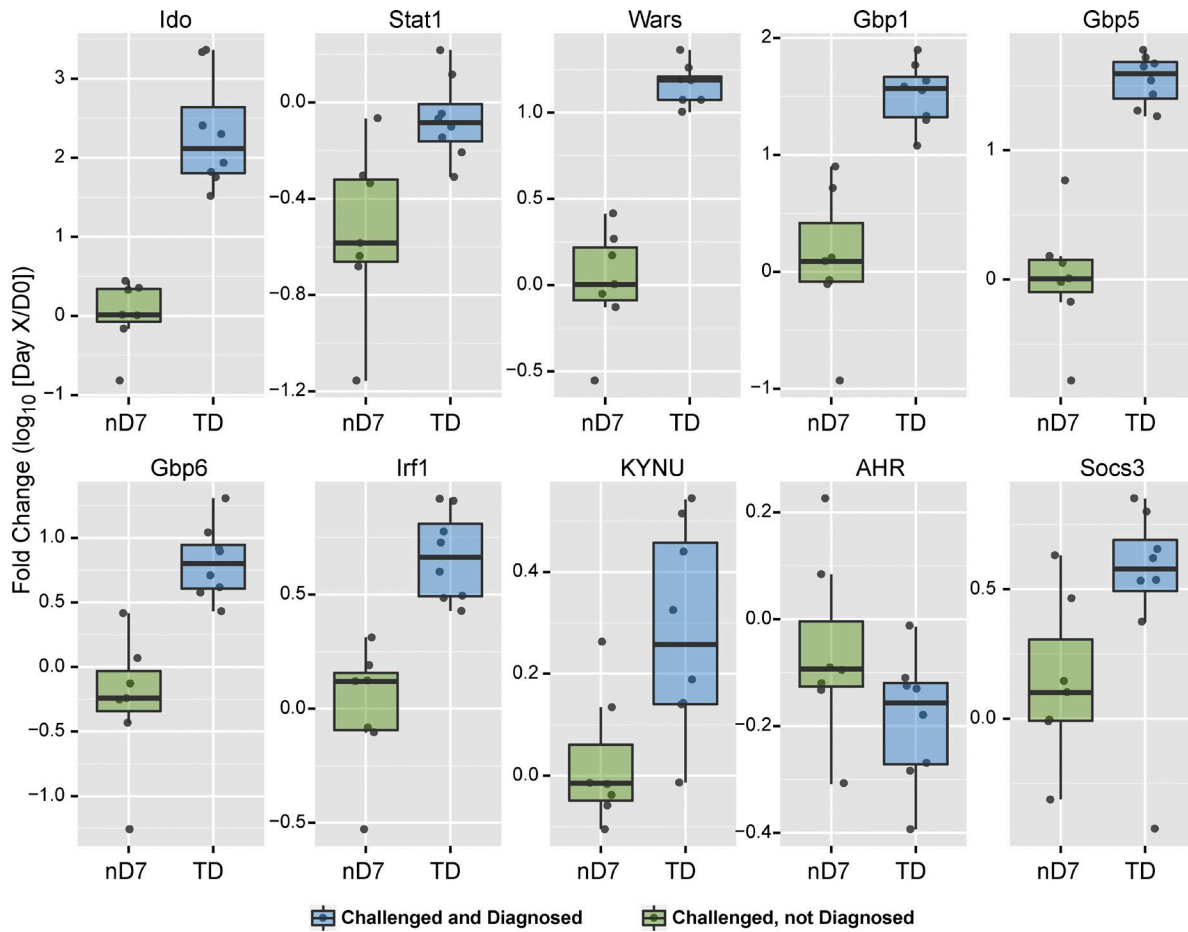


Figure S3. Q-PCR validation of key genes. Gene expression (log₂ FC) compared with baseline samples was determined for those who were diagnosed (TD; $n = 8$; blue) and those who stayed well (nD7; $n = 7$; green).

Table S1. Differentially expressed genes after challenge

Time Point	FC > 1.5; BH < 0.05
nD7	335
TD-24	2,970
TD	2,301
TD+24	3,450

Table S2. Analytes measured by multiplex cytokine assay

Time ^a	Time ^b	Time ^c	Analytes
D0 0 h	D0 0 h	D0 0 h	
D0 12 h	D0 12 h	D0 12 h	
D1 24 h	D1 24 h	D1 24 h	
D2	D2	-	
TD-96 h	-	-	EGF, Flt-3L, Fractalkine (CX3CL1), GRO α , IFN γ , IL-1 α , IL-1RA, IL-2, IL-6, IL-8, IL-10, IL-12p40, IL-17, IP-10, MIP-1 β , sCD40L, TNF α , VEGF
TD-48 h	D4	-	
TD-24 h	D6	-	
TD+48 h	D7	-	
TD+96 h	D10	-	
D14	D14	-	

^aParticipants who were diagnosed with acute typhoid fever.

^bParticipants who stayed well throughout the challenge period.

^cParticipants who ingested 120 ml NaHCO₃ solution only.

Table S3. Summary statistics of cytokine measurements of all 18 analytes

Analyte	Participants diagnosed with typhoid fever			Participants not reaching diagnosis criteria			Category
	Median V1 0 h ¹	Median V1 12 h [range]	Median TD+48 h [range]	Median V1 0 h	Median V1 12 h [range]	Median V1 12 h [range]	
sCD40L	252.83	3447.76 [109.91–10000]	271.05 [103.21–10000]	262.36	4013.005 [48.32–10000]	269.205 [2.55–1113.93]	Proinflammatory
Fractalkine	32.91	92.715 [11.35–287.47]	50.18 [11.35–161.66]	27.285	68.82 [11.35–307.56]	11.35 [11.35–160]	Chemoattractant
VEGF	31.97	102.9 [13.15–3189.95]	89.99 [13.15–3312.65]	13.15	102.9 [13.15–229.83]	13.15 [13.15–167.35]	Growth Factor
EGF	4.575	22.62 [1.6–78.5]	–	6.23	24.295 [1.6–79.19]	1.6 [1.6–45.04]	Growth Factor
IL-1RA	11.405	42.83 [4.15–187.47]	31.86 [4.15–158.64]	11.17	28.09 [4.15–232.35]	9.355 [4.15–151.69]	Antiinflammatory
GRO	272.88	615.96 [197.61–1251.84]	575.24 [276.1–1406.48]	278.82	585.745 [110.57–1498.13]	263.77 [130.23–539.59]	Chemoattractant
IL-8	4.53	5.01 [1.6–85.87]	14.47 [1.6–106.54]	4.865	6 [1.6–74.6]	2.67 [1.6–84.62]	Chemoattractant
MIP-1β	24.775	36.28 [1.6–89.5]	49.61 [24.46–134.17]	23.98	32.105 [11.44–89.15]	23.94 [1.6–84.88]	Chemoattractant
TNFα	8.75	9.7 [1.6–27.29]	31.23 [9.53–55.67]	7.76	9.135 [4.45–22.64]	8.535 [3.94–16.6]	Proinflammatory
IFNγ	1.6	4.25 [1.6–29.87]	148.62 [7.17–472.68]	1.6	2.825 [1.6–25.67]	1.6 [1.6–5.42]	Proinflammatory
IP-10	326.22	319.69 [106.62–1221.91]	4388.32 [513.98–9157.9]	559.22	349.865 [227.88–1201.6]	398.37 [263.65–1513.52]	Chemoattractant
IL-6	1.6	1.6 [1.6–13.04]	4.86 [1.6–22.1]	1.6	2.19 [1.6–34.6]	1.6 [1.6–30.34]	Proinflammatory
IL-12 p40	3.85	3.85 [3.85–120.97]	3.85 [3.85–126.1]	8.665	32.845 [3.85–577.59]	3.85 [3.85–550.04]	Proinflammatory
IL-1α	4.7	4.7 [4.7–110.5]	4.7 [4.7–116.75]	4.7	4.7 [4.7–278.73]	4.7 [4.7–253]	Proinflammatory
IL-2	1.6	1.6 [1.6–7.73]	1.6 [1.6–7.48]	1.6	1.6 [1.6–37.93]	1.6 [1.6–38.56]	Proinflammatory
IL-10	1.6	1.6 [1.6–18.45]	3.69 [1.6–21.17]	1.6	3.74 [1.6–59.34]	1.6 [1.6–57.88]	Proinflammatory
IL-17	1.6	4.13 [1.6–150.39]	1.6 [1.6–153.21]	1.6	2.67 [1.6–51.55]	1.6 [1.6–62.99]	Proinflammatory
Flt-3L	8.155	20.15 [2.7–62.39]	10.705 [2.7–54.9]	6.76	6.45 [2.7–99.44]	6.64 [2.7–129.9]	Proinflammatory

All concentrations are in pictograms/milliliters.

Table S4. Significant cytokine induction at 12 h after challenge

Analyte	P-value
IL12	0.1994
EGF	0.0001
Flt3L	0.3252
Fractalkine	<0.0001
GRO	<0.0001
IFN γ	0.0580
IL1 α	0.5200
IL1RA	<0.0001
IL2	0.4265
IL6	0.2053
IL8	0.9103
IL10	0.4469
IL17	0.3936
IP10	0.1396
MIP1 β	0.0888
sCD40L	<0.0001
TNF	0.0740
VEGF	0.0075

Table S5. Linear regression model for dose-dependency of 12 h cytokine increase from baseline^a

Analyte	Coefficient	P-value	95% CI	<i>n</i>
EGF	0.60	<0.0001	0.34–0.85	35
Flt3L	0.34	0.0257	0.06–0.63	33
Fractalkine	0.21	0.0679	–0.01–0.44	34
GRO	0.16	0.0499	0.01–0.32	39
IFN γ	0.32	0.0002	0.17–0.47	38
IL1RA	0.52	<0.0001	0.32–0.72	32
IL2	0.11	0.0773	–0.01–0.23	39
IL6	0.19	0.0084	0.06–0.33	38
IL10	–0.03	0.6670	–0.16–0.1	39
IL12	0.33	0.0069	0.1–0.55	37
IL17	0.19	0.0317	0.02–0.35	38
IP10	0.01	0.7324	–0.07–0.1	39
MIP1 β	0.19	<0.0001	0.11–0.27	37
sCD40L	0.67	0.0008	0.31–1.02	38
TNF α	0.16	0.0002	0.09–0.24	38
VEGF	0.30	0.0210	0.06–0.54	35
IL8	–0.06	0.4993	–0.24–0.12	39
IL1 α	0.15	0.0371	0.01–0.29	38

^aBaseline cytokine concentrations were included as covariates.

Table S6. Logistic regression model with typhoid diagnosis as dependent variable (binary)^a

Analyte	OR	P-value	95% CI	n
EGF	0.81	0.83	0.11–5.73	35
Flt3L	1.07	0.94	0.18–6.47	33
Fractalkine	6.73	0.13	0.55–81.83	34
GRO	1.56	0.74	0.11–22.4	39
IFN γ	17.55	0.16	0.34–910.51	38
IL1RA	1.31	0.84	0.09–19.53	32
IL2	0.32	0.54	0.01–12.27	39
IL6	0.30	0.47	0.01–7.94	38
IL10	0.19	0.33	0.01–5.37	39
IL12	0.67	0.71	0.08–5.69	37
IL17	0.39	0.50	0.03–6.05	38
IP10	99.56	0.17	0.14–68524.92	39
MIP1 β	0.10	0.46	0–44.57	37
sCD40L	0.66	0.54	0.18–2.45	38
TNF α	20.54	0.35	0.04–11815.39	38
VEGF	1.25	0.83	0.17–8.98	35
IL8	0.14	0.15	0.01–2.03	39
IL1 α	0.04	0.07	0–1.37	38

^aBaseline cytokine concentrations and challenge dose were included as covariates.

Table S7. Cox proportionate hazard model with time to diagnosis as dependent variable^a

Analyte	Hazard ratio	P-value	95% CI	n
EGF	0.99	0.99	0.33–2.97	35
Flt3L	1.14	0.81	0.38–3.41	33
Fractalkine	3.21	0.12	0.73–14.01	34
GRO	1.36	0.73	0.23–7.93	39
IFN γ	6.35	0.05	0.99–40.55	38
IL1RA	1.24	0.79	0.24–6.35	32
IL2	0.68	0.74	0.06–6.76	39
IL6	0.66	0.70	0.08–5.33	38
IL10	0.45	0.45	0.05–3.64	39
IL12	1.15	0.83	0.33–3.88	37
IL17	0.49	0.43	0.08–2.89	38
IP10	4.83	0.40	0.12–181.63	39
MIP1 β	0.29	0.51	0.007–11.74	37
sCD40L	0.91	0.80	0.42–1.92	38
TNF	10.77	0.19	0.3–385.41	38
VEGF	1.18	0.79	0.35–3.87	35
IL8	0.47	0.29	0.11–1.93	39
IL1 α	0.07	0.11	0.003–1.78	38

^aBaseline cytokine concentrations and challenge dose were included as covariates.

Table S8. Probes used for qPCR validation

TaqMan Probe	Gene	Species
Hs00984148_m1	<i>IDO1</i>	Human
Hs00188259_m1	<i>WARS</i>	Human
Hs00977005_m1	<i>GBP1</i>	Human
Hs00369472_m1	<i>GBP5</i>	Human
Hs01584201_m1	<i>GBP6</i>	Human
Hs00971960_m1	<i>IRF1</i>	Human
Hs00169233_m1	<i>AHR</i>	Human
Hs01013996_m1	<i>STAT1</i>	Human
Hs02330328_s1	<i>SOCS3</i>	Human
Hs01114099_m1	<i>KYNU</i>	Human
Hs02758991_g1	<i>GAPDH</i>	Human
Mm00492590_m1	<i>IDO1</i>	Mouse
Mm00457097_m1	<i>WARS</i>	Mouse
Mm01288580_m1	<i>IRF1</i>	Mouse
Mm00439531_m1	<i>STAT1</i>	Mouse
Mm00551012_m1	<i>KYNU</i>	Mouse
Mm99999915_g1	<i>GAPDH</i>	Mouse