Enterococcus faecalis promotes innate immune suppression and polymicrobial catheter-associated urinary tract infection

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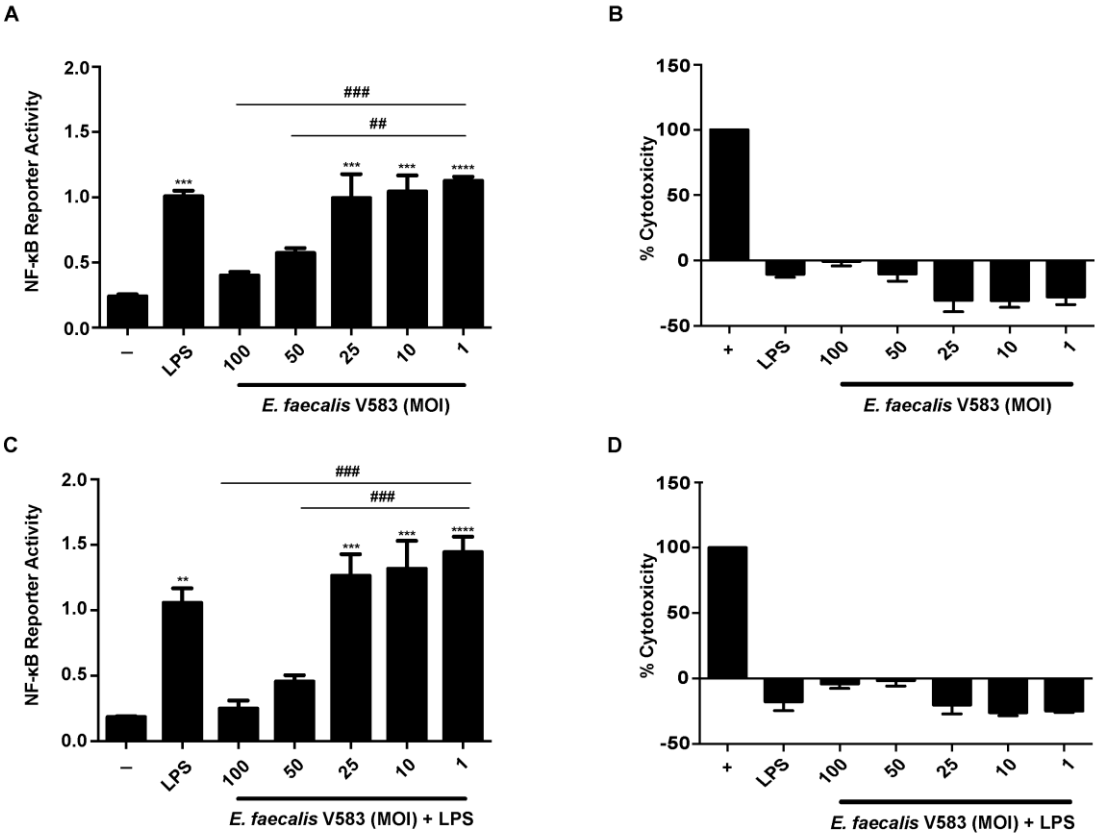

Fig. S1. *E. faecalis* vancomycin-resistant strain V583 prevents NF-κB activation in mouse macrophages.

Mouse RAW 267.4 macrophages were infected with either live *E. faecalis* alone, or treated concurrently with LPS (100 ng/ml) at the specified MOI for 6 hours prior to measurement of NF-κB-driven SEAP reporter activity and percentage cytotoxicity (LDH activity). **(A)** NF-κB-driven SEAP reporter activity and **(B)** LDH activity of RAW 264.7 macrophages infected by *E. faecalis* alone. **(C)** NF-κB-driven SEAP reporter activity and **(D)** LDH activity in the presence of *E. faecalis* and LPS. NF-κB-driven

SEAP reporter assays: exposure to media alone (-) represents background NF-κB reporter activity and stimulation with LPS represents positive controls for reporter activity. LDH assays: Triton-X treatment served as a positive control (+) for cell death. Data are combined from 3 independent experiments; mean values are graphed and error bars represent standard error of the mean (SEM). Statistical analysis was performed using the one-way ANOVA test followed with post-hoc Tukey's multiple comparison test between all conditions where $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$ as compared to media alone (-) controls; and where $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.001$ among all of the MOIs as compared to MOI 100.

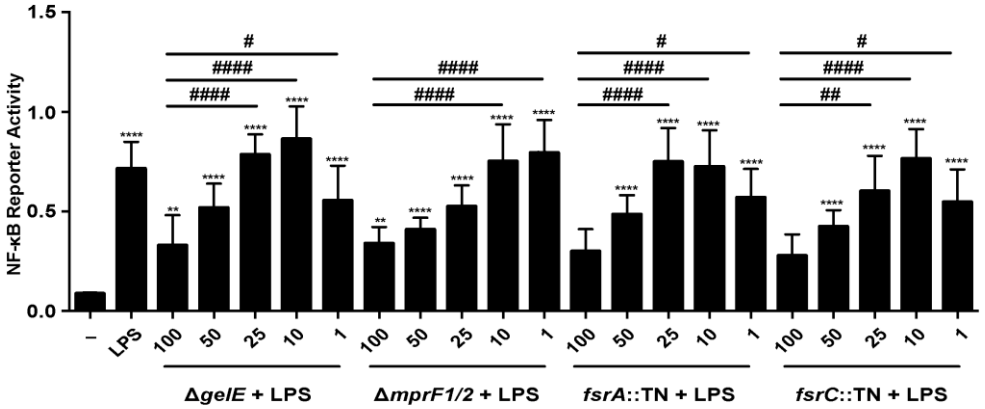

Fig. S2. *E. faecalis gelE, mprF1/2 and fsrAC* are not involved in NF-κB immune suppression in mouse macrophages.

Mouse RAW 267.4 macrophages were infected with live *E. faecalis* mutant strains concurrently with LPS (100 ng/ml) at the specified MOI for 6 hours prior to measurement of NF-κB-driven SEAP reporter activity. In NF-κB-driven SEAP reporter assays, exposure to media alone (-) represents background NF-κB reporter activity and stimulation with LPS represents the positive control for reporter activity. Data are combined from 3 independent experiments; mean values are graphed and error bars represent standard error of the mean (SEM). Statistical analysis was performed using the one-way ANOVA test followed with post-hoc Tukey's multiple comparison test between all conditions where $^{**}P<0.01$ and $^{****}P<0.0001$ as compared to media alone (-) controls; and where $^{\#}P<0.05$, $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.001$ among all of the MOIs as compared to MOI 100.

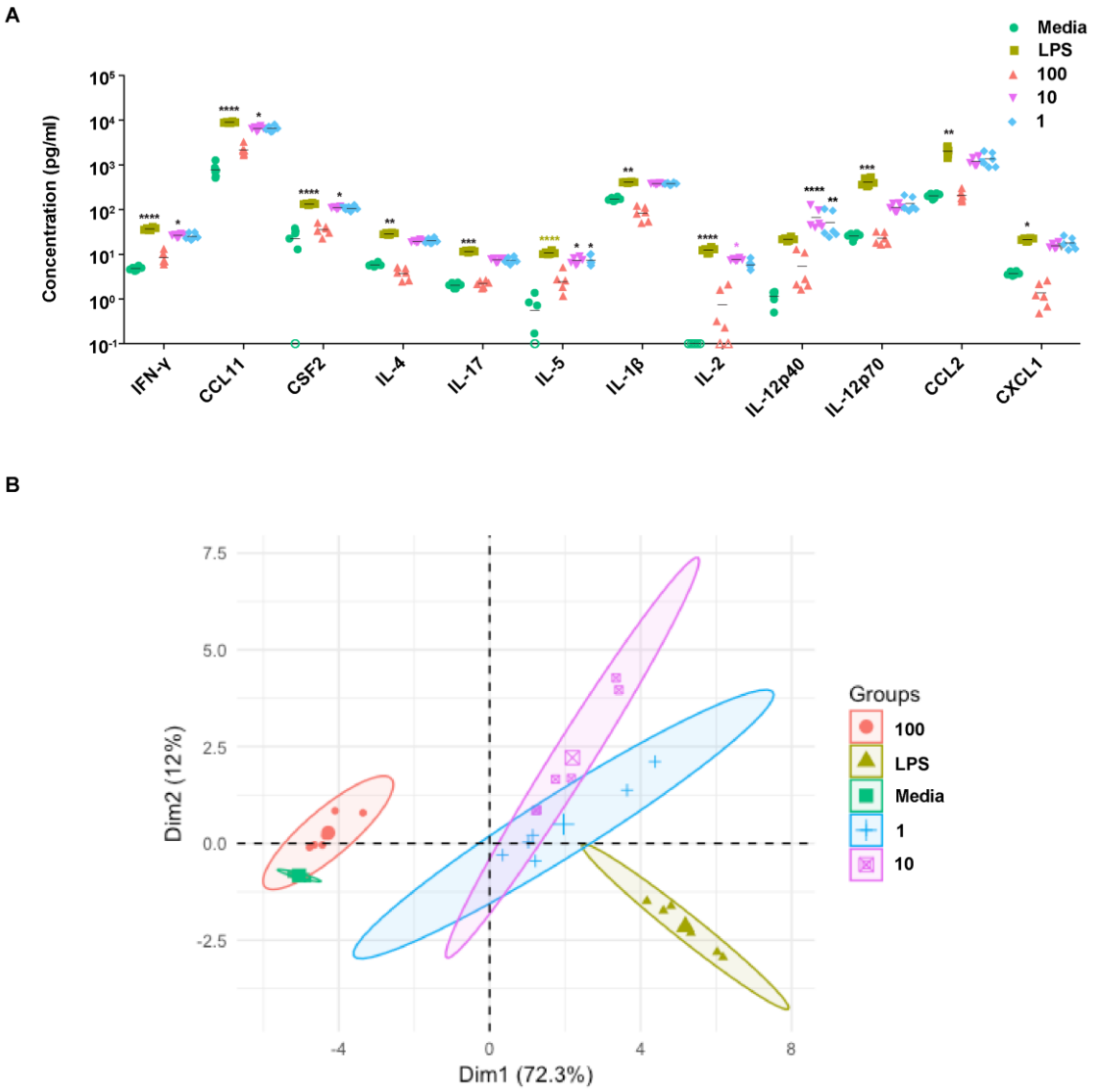

Fig. S3. *E. faecalis* prevents NF-κB driven cytokine and chemokine expression. Mouse RAW 267.4 macrophages were stimulated with live *E. faecalis*, and **(A)** the absolute concentrations in pg/ml of cytokines from filtered supernatants collected 6 hpi at various conditions are shown. Horizontal bars represent the mean value for each condition. Statistical significance was determined by the Kruskal-Wallis test where **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001 as compared to media alone control. Open symbols represent data points that are zero and plotted on the X-axis for representation. **(B)** Principal component analysis (PCA) of cytokines and

chemokines from filtered supernatants collected at 6 hpi from different conditions. Ellipses represent the distribution of each condition. The two dimensions measured explained a total of 84.3% of variability of all analytes measured.

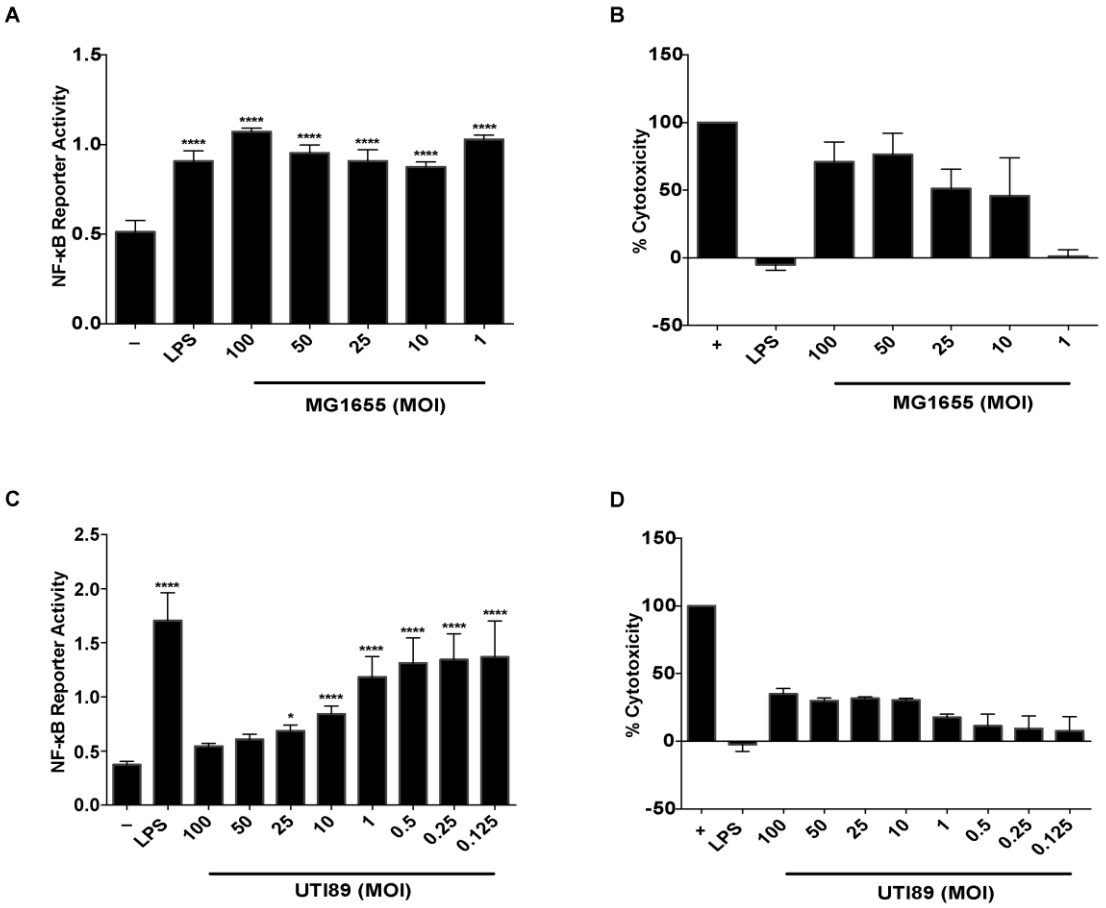

Fig. S4. *E. coli* activates NF-κB at low MOI.

Mouse RAW 264.7 macrophages were stimulated with live *E. coli* K12 strain MG1655 or UTI89 for 6 hours. **(A)** NF-κB-driven SEAP reporter activity and **(B)** LDH activity of RAW 264.7 macrophages infected by MG1655 alone. **(C)** NF-κB-driven SEAP reporter activity and **(D)** LDH activity of RAW 264.7 macrophages infected by UTI89 alone. NF-κB-driven SEAP reporter assays: exposure to media alone (-) represents background NF-κB reporter activity and stimulation with LPS represents positive controls for reporter activity. LDH assays: Triton-X treatment served as a positive control (+) for cell death. Data are combined from 3 independent experiments; mean values are graphed and error bars represent standard error of the mean (SEM). Statistical analysis was performed by the one-way ANOVA test

with Tukey's Multiple Comparison Test where *P<0.05, ****P<0.0001, as compared to media (-) control

Supplementary Table 1: Top ranked 55 differentially expressed transcripts

Entrez	*refseq*	*symbol*	*baseMean*	*log2FoldChange*	*lfcSE*	*stat*	*pvalue*	*padj*
15945	NM_021274	Cxcl10	65.55	-2.21	0.30	-7.27	3.72E-13	3.86E-09
219132	NM_199015	Phf11d	189.06	-0.98	0.16	-5.99	2.09E-09	1.08E-05
24110	NM_011909	Usp18	221.32	-1.19	0.20	-5.89	3.76E-09	1.30E-05
15951	NM_008329	Ifi204	485.17	-1.17	0.21	-5.66	1.48E-08	3.84E-05
100038882	NM_015783	Isg15	503.79	-1.34	0.24	-5.48	4.30E-08	8.56E-05
236312	NM_175026	Pyhin1	117.51	-1.49	0.27	-5.45	4.95E-08	8.56E-05
11687	NM_009660	Alox15	75.21	-2.28	0.43	-5.35	9.03E-08	1.34E-04
19039	NM_011150	Lgals3bp	4444.36	-0.72	0.14	-5.25	1.55E-07	2.01E-04
226695	NM_172648	Ifi205	173.86	-1.69	0.33	-5.16	2.47E-07	2.85E-04
226691	NM_001204910	AI607873	389.99	-1.10	0.22	-5.11	3.16E-07	3.26E-04
55932	NM_018734	Gbp3	356.87	-1.13	0.22	-5.10	3.46E-07	3.26E-04
16688	NM_010669	Krt6b	30.61	-2.40	0.49	-4.95	7.54E-07	6.52E-04
100040462	NM_001170853	Mndal	247.18	-1.03	0.21	-4.90	9.48E-07	7.57E-04
231655	NM_145209	Oasl1	123.10	-1.23	0.25	-4.87	1.13E-06	8.39E-04
209086	NM_010156	Samd9l	438.19	-0.65	0.13	-4.82	1.47E-06	1.01E-03
11801	NM_009690	Cd5l	13.37	-2.45	0.52	-4.75	2.06E-06	1.34E-03
210808	NM_172488	Lacc1	117.05	-0.86	0.18	-4.71	2.51E-06	1.54E-03
64380	NM_029499	Ms4a4c	268.69	-1.40	0.30	-4.66	3.09E-06	1.78E-03
12263	NM_013484	C2	640.30	-0.89	0.19	-4.65	3.32E-06	1.81E-03
67138	NM_025992	Herc6	168.03	-0.75	0.16	-4.59	4.48E-06	2.32E-03
17858	NM_013606	Mx2	49.65	-1.38	0.30	-4.53	5.77E-06	2.85E-03
667370	NM_001005858	Ifit3b	58.01	-1.67	0.37	-4.51	6.34E-06	2.98E-03
15959	NM_010501	Ifit3	302.12	-1.73	0.38	-4.51	6.60E-06	2.98E-03
99899	NM_133871	Ifi44	23.39	-1.56	0.35	-4.49	7.02E-06	3.04E-03
15957	NM_008331	Ifit1	81.73	-1.53	0.35	-4.38	1.20E-05	4.96E-03
80861	NM_030150	Dhx58	243.06	-0.94	0.22	-4.35	1.34E-05	5.34E-03
67775	NM_023386	Rtp4	572.00	-0.96	0.22	-4.34	1.40E-05	5.39E-03
381308	NM_001033450	Mnda	108.42	-1.20	0.28	-4.30	1.70E-05	6.29E-03
276950	NM_181545	Slfn8	67.92	-0.99	0.23	-4.28	1.89E-05	6.78E-03
58203	NM_021394	Zbp1	132.37	-0.98	0.23	-4.26	2.04E-05	7.05E-03
20293	NM_011331	Ccl12	98.27	-1.47	0.35	-4.23	2.32E-05	7.75E-03
232413	NM_177686	Clec12a	98.09	-1.08	0.26	-4.22	2.45E-05	7.93E-03
14938	NM_010370	Gzma	25.78	-1.82	0.43	-4.21	2.58E-05	8.10E-03
75750	NM_029415	Slc10a6	111.46	-1.25	0.30	-4.18	2.95E-05	9.01E-03
230793	NM_146155	Ahdc1	452.17	0.49	0.12	4.16	3.23E-05	9.58E-03
327959	NM_001037713	Xaf1	281.48	-0.92	0.22	-4.12	3.76E-05	1.08E-02
69550	NM_198095	Bst2	1465.05	-0.91	0.22	-4.11	3.96E-05	1.08E-02
20556	NM_011408	Slfn2	1014.92	-0.82	0.20	-4.11	3.97E-05	1.08E-02
231507	NM_139198	Plac8	3113.92	-0.63	0.15	-4.08	4.48E-05	1.19E-02
76933	NM_029803	Ifi27l2a	2431.94	-0.97	0.24	-4.07	4.68E-05	1.21E-02
234311	NM_001081215	Ddx60	56.71	-1.12	0.28	-4.05	5.07E-05	1.28E-02
170743	NM_133211	Tlr7	65.45	-1.21	0.30	-4.03	5.53E-05	1.37E-02
20684	NM_013673	Sp100	390.96	-0.75	0.19	-4.01	6.01E-05	1.45E-02
16145	NM_018738	Igtp	335.77	-0.75	0.19	-3.94	8.18E-05	1.89E-02
80285	NM_030253	Parp9	495.20	-0.55	0.14	-3.94	8.20E-05	1.89E-02
14469	NM_010260	Gbp2	733.54	-0.76	0.19	-3.92	8.78E-05	1.98E-02
23962	NM_011854	Oasl2	443.20	-0.85	0.22	-3.88	1.07E-04	2.35E-02
18619	NM_001002927	Penk	379.03	-0.89	0.23	-3.86	1.15E-04	2.48E-02
16153	NM_010548	Il10	11.90	-1.59	0.41	-3.83	1.27E-04	2.69E-02
16500	NM_008420	Kcnb1	75.40	0.84	0.22	3.79	1.53E-04	3.11E-02
100041546	NM_001099217	Ly6c2	134.86	-1.22	0.32	-3.78	1.55E-04	3.11E-02
631323	NM_001135115	Gm12250	45.25	-1.17	0.31	-3.78	1.56E-04	3.11E-02
23961	NM_001083925	Oas1b	53.98	-0.93	0.25	-3.76	1.70E-04	3.33E-02
71724	NM_023617	Aox3	62.06	-1.23	0.33	-3.70	2.15E-04	4.14E-02
68404	NM_153529	Nrn1	151.87	-0.81	0.22	-3.68	2.29E-04	4.32E-02

Table S1. Top ranked 55 differentially expressed transcripts.

Supplementary Table 2: Summary of Gene Ontology enrichment analysis for genes showing decreased mRNA levels in mixed infection vs. E.coli only infection

GOid	*Top 50 genes*				*Top 60 genes*				*Top 70 genes*				*GO Term*
	N	*O*	*E*	P_{adj} *(-log* $_{10}$*)*	*N*	*O*	*E*	P_{adj} *(-log* $_{10}$*)*	*N*	*O*	*E*	P_{adj} *(-log* $_{10}$*)*	
GO:0043207	19	0.45	0.05	11.76	21	0.45	0.05	13.05	22	0.42	0.05	13.14	response to external biotic stimulus
GO:0051707	19	0.45	0.05	11.76	21	0.45	0.05	13.05	22	0.42	0.05	13.14	response to other organism
GO:0045087	17	0.40	0.03	11.60	18	0.38	0.03	11.89	20	0.38	0.03	13.45	innate immune response
GO:0034097	17	0.40	0.04	11.35	17	0.36	0.04	10.39	17	0.33	0.04	9.56	response to cytokine
GO:0009607	19	0.45	0.05	11.34	21	0.45	0.05	12.58	22	0.42	0.05	12.65	response to biotic stimulus
GO:0002252	15	0.36	0.04	7.49	16	0.34	0.04	7.74	17	0.33	0.04	8.01	immune effector process
GO:0050776	9	0.21	0.04	2.50	9	0.19	0.04	2.09	10	0.19	0.04	2.53	regulation of immune response
GO:0031347	9	0.21	0.04	2.37	9	0.19	0.04	1.96	9	0.17	0.04	1.60	regulation of defense response

Notes:

GOid: unique Gene Ontology Consortium term identifier

N: number of genes of interest annotated to the term

O: observed frequency of annotation to term in gene set of interest

E: expected frequency of annotation to this term

P_{adj} (-log $_{10}$): P value from Fisher's Exact Test (adjusted for number of terms tested)

Term: Gene Ontology Biological Process term

Table S2. Summary of Gene Ontology enrichment analysis for genes showing decreased mRNA levels in mixed infection as compared to *E. coli* only infection.