SUPPLEMENTARY

TABLE S1: Bacterial strains used in this study.

Strains	Relevant characteristics	Reference
BM110	serotype III, CC17, bacteremia	(1)
BM110-GFP	BM110 strain carrying pGU2664 plasmid for GFP expression	(1)
Δ*srr2*	BM110 strain deleted in *srr2* gene	(2)
Δ*hvgA*	BM110 strain deleted in *hvgA* gene	(3)
Δ*spb1*	BM110 strain deleted in *spb1* gene	(4)
BM110 Δ*cps*	BM110 strain deleted in *cps*D/E genes (capsular mutant)	(5)
NEM316	serotype III, CC23, EOD bacteremia	(3)
NEM316-GFP	NEM316 strain carrying pGU2664 plasmid for GFP expression	This study
NEM316 Δ*cps*	NEM316 strain deleted in *cps*D/E gene (capsule mutant)	(5)
L. lactis + vector	MG1363 strain of *Lactococcus lactis* subsp. *cremoris* carrying empty pOri232 plasmid	(3)
L. lactis + HvgA	MG1363 strain of *Lactococcus lactis* subsp. *cremoris* carrying pOri232 plasmid for HvgA expression	(3)

References

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3. Tazi A, Disson O, Bellais S, Bouaboud A, Dmytruk N, Dramsi S, Mistou MY, Khun H, Mechler C, Tardieux I, Trieu-Cuot P, Lecuit M, Poyart C. 2010. The surface protein HvgA mediates group B *Streptococcus* hypervirulence and meningeal tropism in neonates. J Exp Med 207:2313-22.
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TABLE S2: GBS clinical isolates used in this study.

Strains	Capsular serotype	Complex clonal (CC)	Type of infection
CNR CCH1569	CPS III	CC17	LOD Meningitis
CNR CCH1570	CPS III	CC17	LOD Meningitis
CNR CCH1571	CPS III	CC17	LOD Meningitis
CNR CCH1573	CPS III	CC17	LOD Bacteremia
CNR CCH1575	CPS III	CC17	LOD Meningitis
CNR CCH1577	CPS III	CC17	EOD Meningitis
CNR CCH1578	CPS III	CC17	LOD Meningitis
CNR CCH1581	CPS III	CC17	EOD Meningitis
CNR CCH1584	CPS III	CC17	LOD Meningitis
CNR CCH1586	CPS III	CC17	LOD Meningitis
CNR CCH1588	CPS III	CC17	LOD Meningitis
CNR CCH1589	CPS III	CC17	LOD Meningitis
CNR CCH1591	CPS III	CC17	LOD Meningitis
CNR CCH1594	CPS III	CC17	LOD Meningitis
CNR CCH1596	CPS III	CC17	LOD Meningitis
CNR CCH1597	CPS III	CC17	LOD Meningitis
CNR CCH1602	CPS III	CC17	EOD Meningitis
CNR CCH0620	CPS IV	CC17	Adult Bacteremia
CNR CCH0755	CPS IV	CC17	Adult Bacteremia
CNR CCH0778	CPS IV	CC17	Adult Bacteremia
CNR CCH0950	CPS IV	CC17	Adult Bacteremia
CNR CCH1030	CPS IV	CC17	Adult Meningitis
CNR CCH1174	CPS IV	CC17	Adult Bacteremia
CNR CCH1239	CPS IV	CC17	Adult Bacteremia
CNR CCH1360	CPS IV	CC17	Adult Meningitis
CNR CCH1472	CPS IV	CC17	EOD pneumonia
CNR CCH1506	CPS IV	CC17	LOD Bacteremia
CNR CCH0050	CPS III	non-CC17	EOD Bacteremia
CNR CCH0107	CPS III	non-CC17	LOD Meningitis
CNR CCH0112	CPS III	non-CC17	EOD Bacteremia
CNR CCH0150	CPS III	non-CC17	LOD Bacteremia
CNR CCH0205	CPS III	non-CC17	LOD Bacteremia
CNR CCH0311	CPS III	non-CC17	EOD Bacteremia
CNR CCH0362	CPS III	non-CC17	LOD Bacteremia
CNR CCH0382	CPS III	non-CC17	Adult osteoarticular infection
CNR CCH0384	CPS III	non-CC17	LOD Bacteremia
CNR CCH0513	CPS III	non-CC17	LOD Meningitis
CNR CCH0700	CPS III	non-CC17	LOD Meningitis
CNR CCH1207	CPS III	non-CC17	LOD Meningitis
CNR CCH1261	CPS III	non-CC17	LOD Bacteremia
CNR CCH1280	CPS III	non-CC17	LOD Meningitis
CNR CCH1319	CPS III	non-CC17	LOD Bacteremia
CNR CCH1393	CPS III	non-CC17	LOD Bacteremia
CNR CCH1396	CPS III	non-CC17	LOD Bacteremia

CNR CCH1490	CPS III	non-CC17	Intra-uterine infection (pregnancy-associated)
CNR CCH1809	CPS III	non-CC17	LOD Bacteremia
CNR CCH1881	CPS III	non-CC17	LOD Meningitis
CNR CCH1585	CPS IV	non-CC17	EOD Bacteremia
CNR CCH0966	CPS IV	non-CC17	EOD Meningitis
CNR CCH1134	CPS IV	non-CC17	Adult Bacteremia
CNR CCH1157	CPS IV	non-CC17	LOD pneumonia
CNR CCH1165	CPS IV	non-CC17	EOD Bacteremia
CNR CCH1274	CPS IV	non-CC17	Adult Bacteremia
CNR CCH1363	CPS IV	non-CC17	LOD Bacteremia
CNR CCH1369	CPS IV	non-CC17	Adult Bacteremia
CNR CCH1403	CPS IV	non-CC17	Adult Bacteremia
CNR CCH1686	CPS IV	non-CC17	LOD Bacteremia
CNR CCH1565	CPS II	non-CC17	EOD Bacteremia
CNR CCH1566	CPS Ia	non-CC17	EOD Meningitis
CNR CCH1567	CPS Ia	non-CC17	EOD Bacteremia
CNR CCH1568	CPS Ia	non-CC17	EOD Bacteremia
CNR CCH1572	CPS Ia	non-CC17	EOD Bacteremia
CNR CCH1576	CPS Ia	non-CC17	EOD Bacteremia
CNR CCH1579	CPS V	non-CC17	EOD Bacteremia
CNR CCH1580	CPS V	non-CC17	LOD Bacteremia
CNR CCH1582	CPS Ib	non-CC17	LOD Meningitis
CNR CCH1583	CPS Ia	non-CC17	LOD Bacteremia

TABLE S3: Inhibitors used in this study.

Chemical and reagents	Target	Concentration
Cytochalasin D	Actin filaments	2 µM
Nocodazole	Microtubules	1 µM
LY29002	Pi 3-kinase	40 µM
PP2	Src kinase	10 µM
Staurosporin	Protein kinase C	500 nM
NSC23766	Rac	100 µM
ZCL278	CDC42	50 µM
Bay61-3606	Syk	1 µM
Y27632	Rock	10 µM
Human FC Block™	FCy receptors	2 µg/ml
Anti CR3 antibody (vim12)	Complement Receptor 3	20 µg/ml
IgG1 mouse antibody	Isotype control	20 µg/ml
Mannan	Lectin receptors	200 µg/ml
Mannose	Lectin receptors	10 mg/ml
Laminarin	Lectin receptors	200 µg/ml
RGD peptide	RGD dependent integrins	50 µM
RGDfV peptide	RGDfV dependent integrins	50 µM
Fucoidan	Scavenger receptors	500 µg/ml
Poly (I)	Scavenger receptors	200 µg/ml
Poly (C)	Inactive analog of Poly(I)	200 µg/ml

TABLE S4: Primers used in this study.

Target	Primers	Sequence (5'-3')	Expected size (bp)
Actin	F-1462	TTCCAAATATGAGATGCGTTGTTA	118
	R-1580	ATGCTATCACCTCCCCTGTG	
VE-cadherin	F-453	GGTCGATGCAGAGACAGGAG	118
	R-571	GAGTCTCCAGGTTTTCGCCA	
SR-A1/MSR1	F-595	GCCAACCTCATGGACACAGA	112
	R-707	CCATGTCCCTGGACTGAGGA	
SR-A6/MARCO	F-1228	TGTGGAGCTGCACCAAGAAT	133
	R-1361	CCACATATGAGCCCGAGGAC	
SR-A3/SCARA3	F-866	CGAGGAGACCCTGACCCTCCAG	126
	R-992	CCCAGGGTGGCCTGGATGTTC	
SR-A4/SCARA4	F-618	ACGCTGGAGAAGTTACAGGC	178
	R-796	TTCTGCAGATTGCCCTGGAG	
SR-A5/SCARA5	F-2386	CCATGCACCAGGCCTCAATA	193
	R-2579	CACTTGACGTTGCCTCTTGC	
SR-B2/CD36	F-1559	CAATTTGCAAAACGGCTGCAG	123
	R-1682	CTTCTCATCACCAATGGTCCC	
SR-B1/SCARB1	F-1520	AGGGGGAGACTCTTCACACA	132
	R-1652	GGCTCCGGATTTGGCAGATG	
SR-D1/CD68	F-1093	GATCCTTCTTGGCCTCCTCG	168
	R-1261	CTTTGAGCCAGTTGCGTGTC	
SR-E1/LOX1	F-740	CGAGGAGCTGTTTATGCGGA	180
	R-920	TGGCACCCAAGTGACAAAGA	
SR-E2/Dectin1	F-830	ACCCATCTCCAAATTGTGTA	113
	R-943	CCACCCTTCCTCTTACATTG	
SR-F1/SCARF1	F-2964	CTCCCTCTGTCCCCCAGGCT	136
	R-3100	GCCAAGCGTGGTGGAGGGCACC	
SR-G1/CXCL16	F-1041	CCACCCTCCCAGTAGGATCA	121
	R-1162	CTGCTTCTGGTTCTCCCCAG	
SR-H1/FEEL1	F-774	CAACCCCTGCTGGCCATCAC	163
	R-937	GTAGAGTTGCTGGGGCAGCC	
SR-H2/FEEL2	F-4194	GCTGTGCCGGCTTCTTTGGC	181
	R-4375	GGTCACAGTGGATGCCGTAC	
SR-I1/CD163	F-310	GCGGGAGAGTGGAAGTGAAA	145
	R-455	ACCTGCACTGGAATTAGCCC	
DC-SIGN/CD209	F-2342	GAATTGTGCCTCTCCTGGCT	142
DC-SIGN/CD209	R-2484	GTGGGCCACCACGATGAATA	
SR-E4/ASGPR	F-155	GGAATCAGACCCTGAGACCC	134
	R-289	TGCAGCTGGGAGTCTTTTCTG	
SR-F2/MEGF10	F-29	AGCAAGTACTTTCCCGGTGC	148
	R-177	CAATCAACGCGGTTAGCGTC	
SR-J1/RAGE	F-1431	GGCCCACAGACAGATCCCAT	140
	R-1571	GGGGGCTCTGGTTGTAGAAG	
SR-L1/LRP1	F-3616	GACTGTGGGGACAACAGTGAC	154

	R-3770	TTGGCGTGTGTCTCATCACT	
SR-L2/LRP2	F-15028	AGCCTCAACTGGGTTTTTGT	129
	R-15157	GTACACATTTAGCCACAGGGC	
SSC4D/SRCRB4D	F-2046	GGACAATGTCAAGTGCCGTGGG	182
	R-2228	GTAGTCATCACAAGAGGAGGGC	
SSC5D	F-1990	CTGGAGAAAACAACCACGAAG	170
	R-2160	GTCAGTGCTGCAGTGGTCTGTG	
CD14	F-352	CTGACACGGTCAAGGCTCTC	114
	R-466	AGTTCCTTGAGGCGGGAGTA	
LY75	F-5058	ACTGATGAGAAACCCGCCAG	127
	R-5185	ACTGATGAGAAACCCGCCAG	
CD207/Langerin	F-963	ATGCCCCATGTGACAAAACG	103
	R-1066	GCGTTGGAGCTCAAAGAGTG	

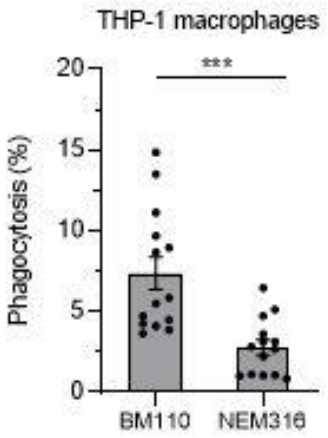

Figure S1: BM110 are more phagocytosed than NEM316 in THP-1 macrophages. Phagocytosis level of BM110 and NEM316 strains was assessed by CFU count after infection at MOI 10 followed by antibiotic treatment to kill extracellular bacteria. Results are expressed as the percentage of phagocytosed bacteria normalized to the initial inoculum.

Statistical analysis: data shown are mean ± SEM of at least four independent experiments. t test was performed with ***, $p < 0.001$.

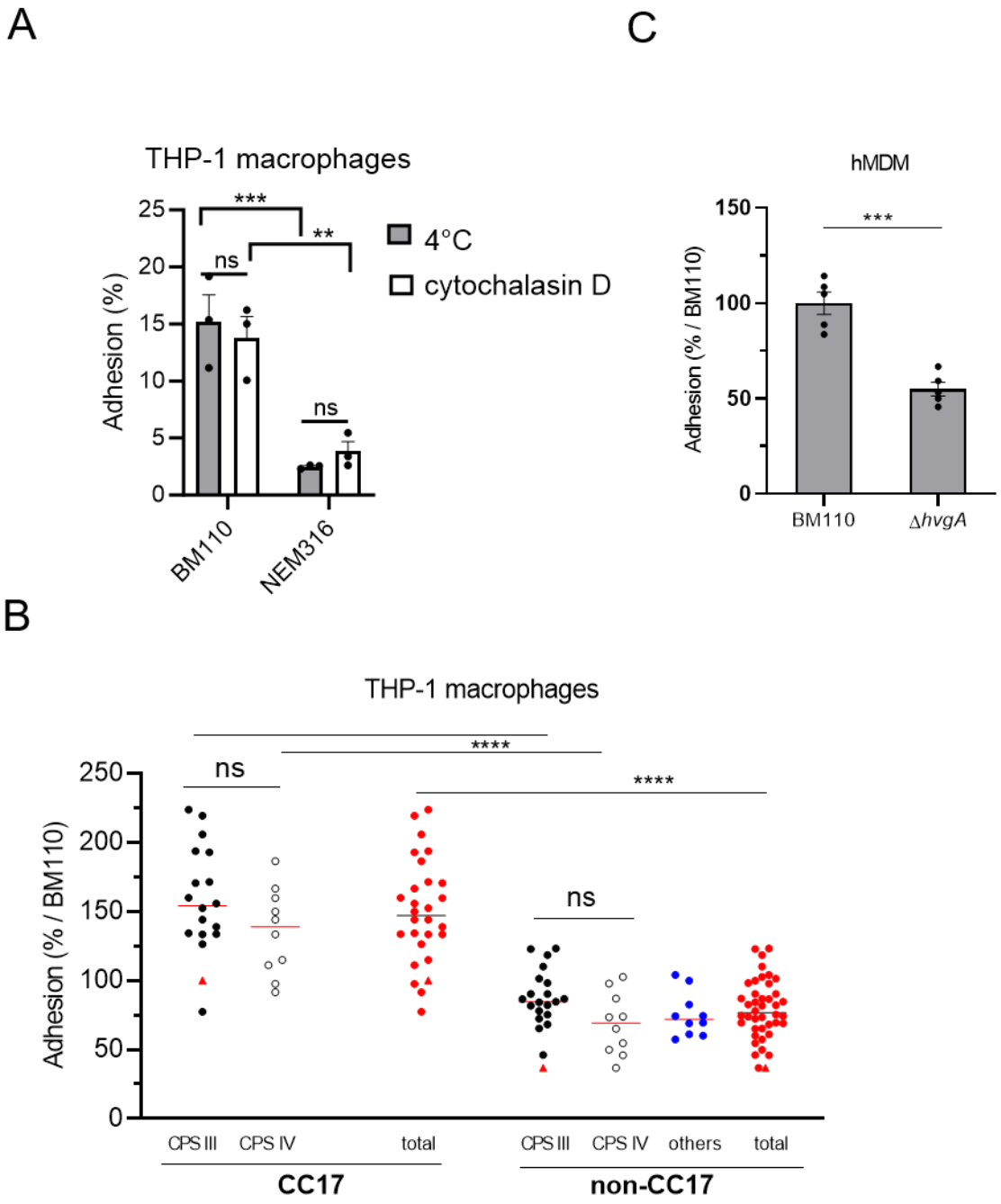

Figure S2: GBS adhesion to phagocytes. (A) Adhesion level of GBS strains was assessed by CFU count after infection at MOI 10 at (A) 37 °C in the presence of cytochalasin D or at (A, B, C) 4°C to avoid bacterial engulfment. Adhesion was assessed in (A, B) THP-1 macrophages or (C) hMDM. (A) Adhesion level of BM110 and NEM316 strains are expressed as the percentage of adherent bacteria normalized to the initial inoculum. (B) Adhesion level of CC17 and non-CC17 GBS clinical isolates from invasive infections, each point representing a clinical strain. Triangles correspond to BM110 strain (CC17) and NEM316 (non-CC17). Results are shown according to capsular type (CPS III, CPS IV, or other CPS) and are expressed as the percentage of adhesion relative to BM110 strain adhesion, with horizontal lines indicating median value. (C) Adhesion level of BM110 and its derivative mutant strain (Δ*hvgA*). Results are expressed as the percentage of adherent bacteria normalized to BM110 strain adhesion.

Statistical analysis: data shown are mean ± SEM of at least three independent experiments. (A, B) Two-way ANOVA or (C) t test, were performed with ns, non-significant; ***, $p < 0.001$; ****, $p < 0.0001$.

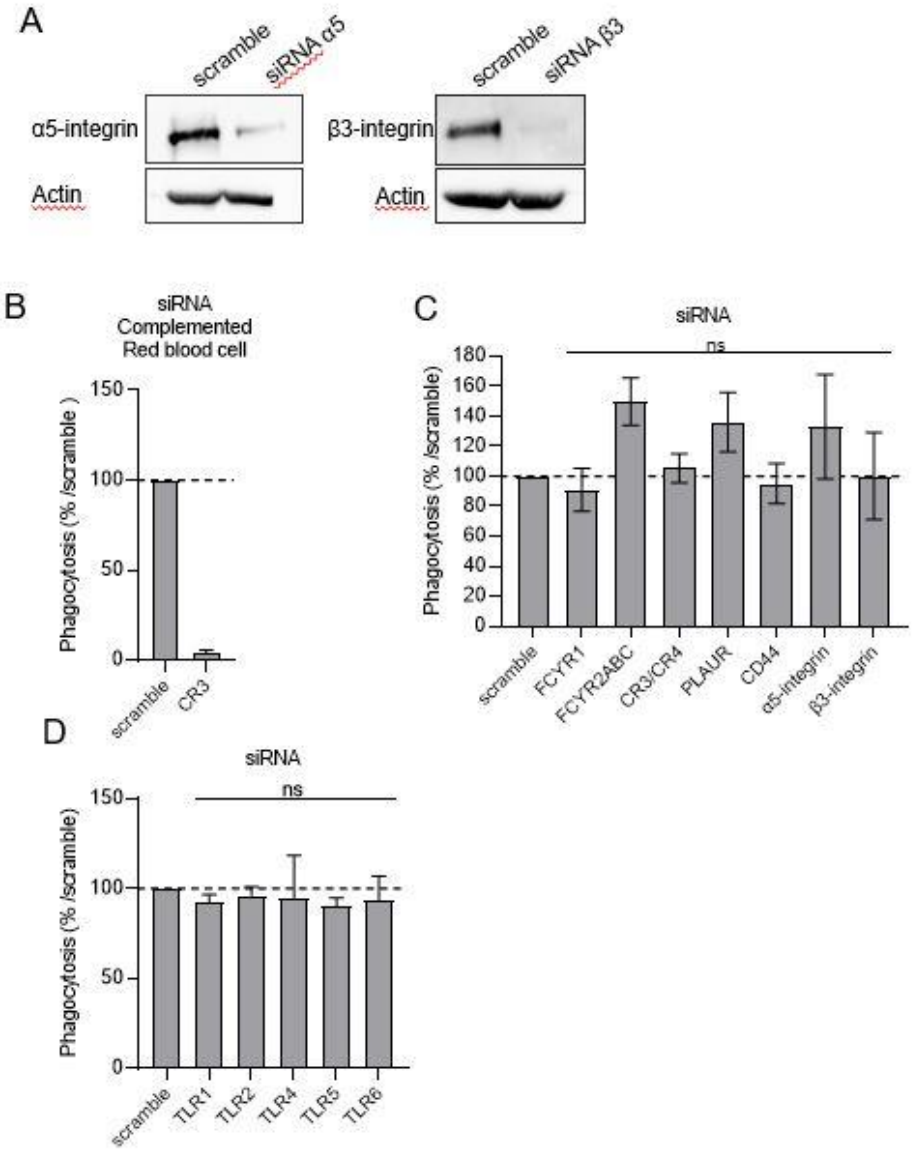

Figure S3: siRNA screen in THP-1 macrophages. (A) Silencing efficiency obtained by siRNA treatment of THP-1 macrophages was evaluated by western blot analysis on two independent targets (integrin α5 and β3). (B) Phagocytosis of complement-opsonized red blood cells was evaluated my fluorescence microscopy in THP-1 treated with scramble siRNA or siRNA targeting complement receptor 3(CR3, *itgaM*). Results are expressed as the percentage of phagocytosed red blood cells normalized to scramble control condition. (C, D) Phagocytosis of BM110 strain was evaluated in THP-1 macrophages treated with (C) siRNA targeting FCγR1; FCγR2A,B,C; Complement Receptor3 and 4 (CR, *itgAM*), PLAUR, SR-K1 (CD44) and integrins α5 and β3 or (D) TLRs receptors. Results are expressed as the percentage of phagocytosis normalised to the scramble control conditions.

Statistical analysis: data shown are mean ± SEM of at least three independent experiments. (C, D) Kruskall Wallis test with Dunn's multiple comparisons tests were performed with ns, non-significant.

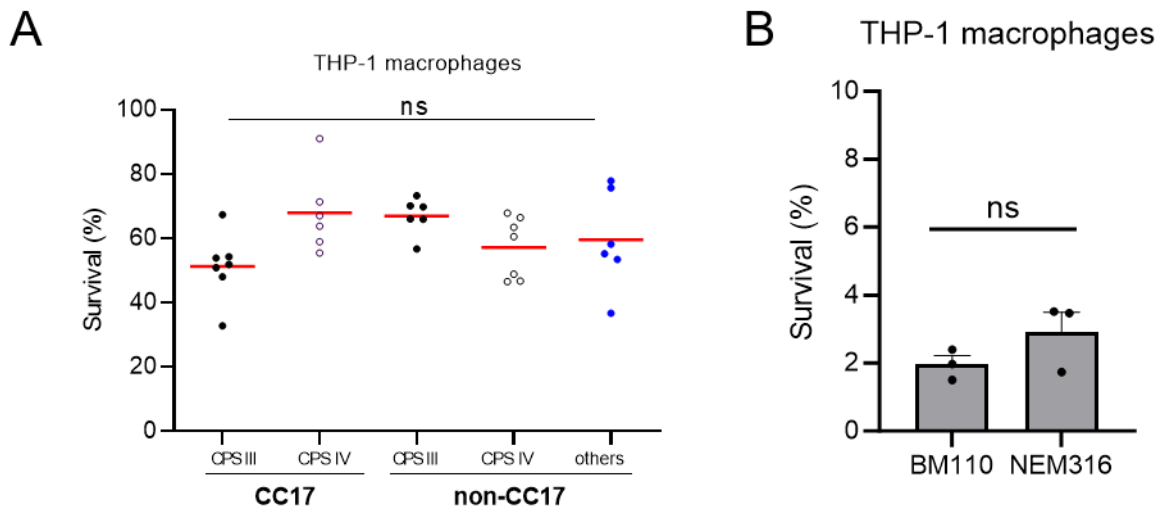

Figure S4: Survival of GBS strains. Survival level of GBS strains in THP-1 macrophages was assessed by CFU count. Results are expressed as the percentage of viable intracellular bacteria normalised to the number of phagocytosed bacteria. (A) Survival level of CC17 and non-CC17 GBS clinical isolates from invasive infections was determined 2.5 h after phagocytosis. Each point represents one clinical strain. Results are shown according to capsular type (CPS III, CPS IV, or other CPS). (B) Survival level of BM110 and NEM316 strains was determined 24 h post-phagocytosis.

Statistical analysis: data shown are mean ± SEM of at least three independent experiments. Two-way ANOVA was performed with ns, non-significant.