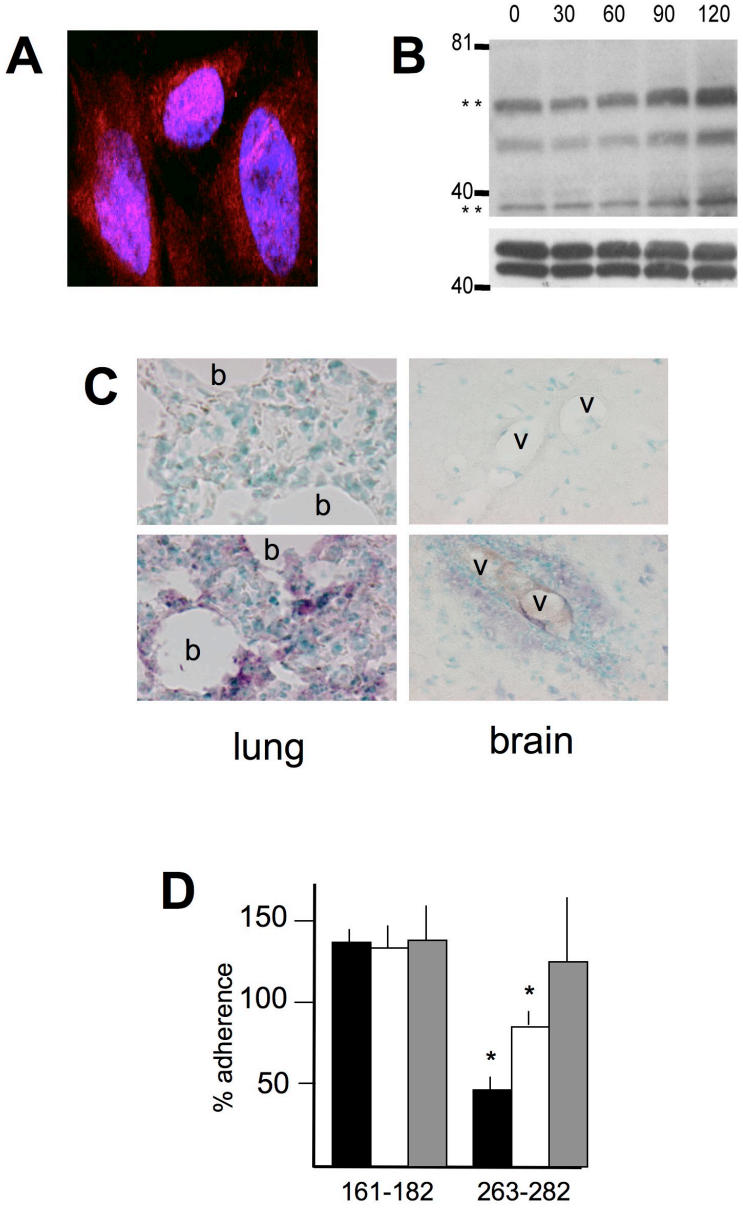
Figure S1


A
B
0
30
60
90
120
81
40
40
C
b
b
b
b
v
v
v
v
lung
brain
D
% adherence
150
100
50
161-182
263-282

A) Surface distribution of LR on non-permeabilized human cerebral endothelial cells as shown by anti-LR (red); nuclei are stained with DAPI (blue); magnification 40X. Identical results were obtained with $rBCEC_6$ cells. **B)** Upregulation of LR was tested in $rBCEC_6$ cells treated with TNFα for 0 to 120 min. Western blot of lysates shows progressively increased expression of LR (**37 and 67 kDa). Lower panel shows p42/44 MAPK loading control. Molecular weight markers are indicated**. C)** Immunohistochemical staining for LR (purple) in lungs and brain of mice challenged with PBS (top) or pneumococcus (bottom) at 48 h. b = bronchi; v= vessel; magnification 40x **D)** Inhibition of adherence of FITC-labeled pneumococcus (black), *H. influenzae* (white) and meningococcus (grey) to human cerebral endothelial cells by LR peptides 161-182 or 263-282. A scrambled peptide 263-282 was used as negative control to set 100% binding. 100% (cfu/ml) = Sp: $2x10^5$; Hi: $3x10^4$; Nm: $1x10^4$ (mean±SD, n=3); * $p<0.05$ compared to scrambled peptide controls for each bacteria: Student's *t*-test.

Figure S2

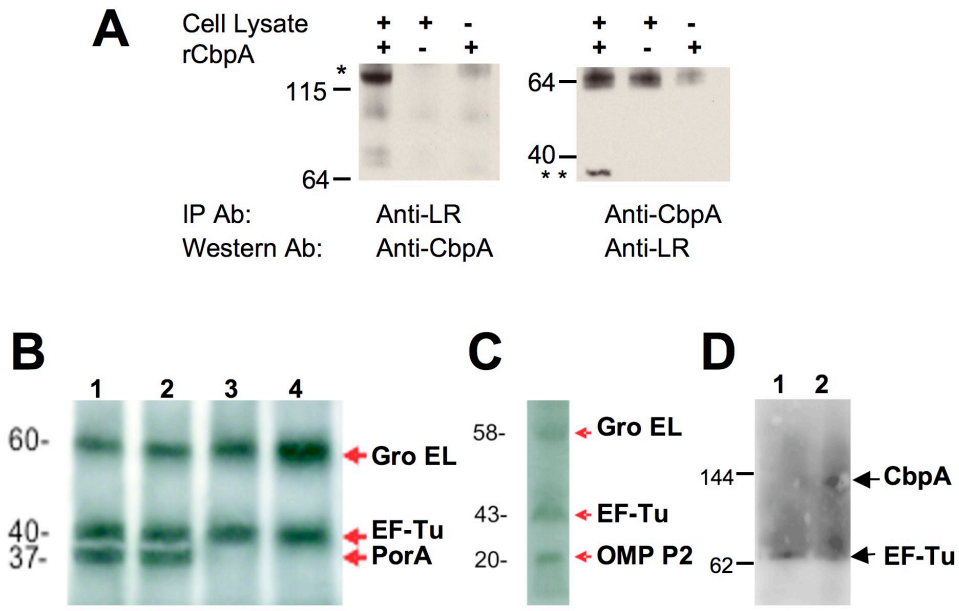


Figure S2. LR-binding ligands in bacteria.
A) Co-immunoprecipitation from lysate of TNFα activated rBCEC$_6$ cerebral endothelial cells mixed with rCbpA by antibody to LR or CbpA. * CbpA band; ** LR bands. Whole cells of meningococcus MC58 **(B),** *Haemophilus* Rd **(C),** or pneumococcus T4 **(D)** were biotin-labeled with cross-linked LR and labeled proteins were detected after separation by SDS-PAGE and identified by MALDI-TOF. For meningococcus, proteins of 37kDa, 40kDa, 60kDa and a large protein that did not enter the gel (not visible in this figure) were identified as PorA, EF-Tu, GroEL and PilQ, respectively (B, lane 1). When cells of isogenic *pilQ*$^-$ (B, lane 2), *porA*$^-$ (B, lane 3) or *porA*$^-$/*pilQ*$^-$ double mutants (B, lane 4) were tested, similar profiles were observed except that the band corresponding to PorA was absent from the *porA*$^-$ and *porA*$^-$, *pilQ*$^-$ double mutants and the band corresponding to PilQ was absent from the *pilQ*$^-$ and *porA*$^-$/*pilQ*$^-$ double mutants. Proteins detected in wild type *Haemophilus* extracts (C) were identified as GroEL, EF-Tu and outer membrane protein (OMP) P2. Proteins detected in wild type pneumococcus extracts (D) were identified as EF-Tu and CbpA. For all 3 bacteria, LR binding to EF-Tu or GroEL was deemed an artifact since binding persisted upon deletion of the adhesins (B, lane 4; D, lane 1) or when wild type cells were probed with labeled KLH (not shown). Testing of E. coli K12 as a negative control by the same methods yielded only EF-Tu (not shown).

Figure S3

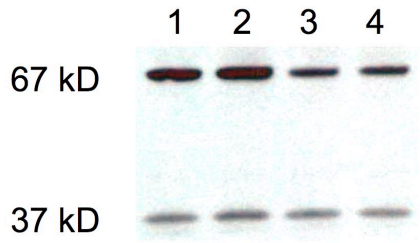


Figure S3. siRNA silencing of expression of LR on rBCEC$_6$ cells.
Western blot of two LR forms of indicated size: Lane 1: non-tranfected control. Lane 2: MapK siRNA transfected control. Lanes 3 and 4: LR siRNA transfected. Lanes 1,2,3 are 48 hour post-transfection; Lane 4 is 72 hours post-transfection. Lanes 3 and 4, 67 kD bands are 39 and 41% reduced from Lane 1 by densitometry.

Figure S4

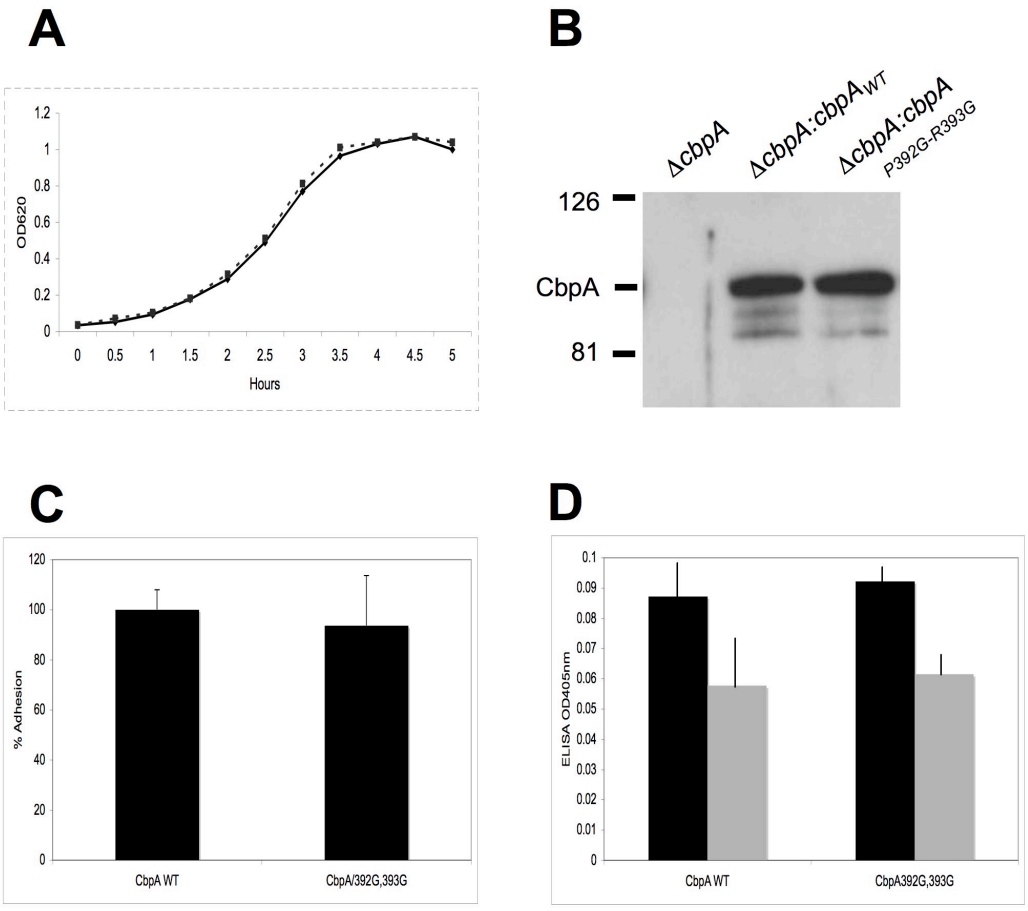


Figure S4. Characterization of Δ*cbpA* pneumococcal mutants expressing either CbpA wild type (WT) or CbpA$_{P392G-R393G}$ *in trans.*
A) Growth kinetics: WT= solid line, double mutant= dashed line; **B)** Expression of CbpA as determined by Western blot analysis of whole cell lysates probed with 1:1000 anti-CbpA polyclonal antibody. **C)** Binding to A549 epithelial cells; **D**) Binding of Factor H to bacteria expressing the indicated CbpA as measured by ELISA; plates coated with bacteria, exposed to Factor H and probed with anti-Factor H antibody at 1:100 (black) or 1:1000 (grey); * indicates $p<0.01$ compared to wild-type value; Student's *t*-test.

Supplementary Table S1
Adherence of clinical isolates to rLR in ELISA format†

Species/No	ID	Origin	Serotype	OD $_{405nm}$	Grade
Sp 1	T4	New York	4	0.13	++
Sp 2*	D39	New York	2	0.05	-
Sp 3*	A66.1	CDC	3	0.05	-
Sp 4	Tupelo	Mississippi	14	0.10	++
Sp 5	E239	CDC	19F	0.09	+
Sp 6	SPA105	Texas	Non-vaccine	0.09	+
Sp 7	SPA106	Tennessee	Non-vaccine	0.08	+
Sp 8	6308	ATCC	8	0.07	+
Sp 9	LB877	Tennessee	6	0.12	++
Sp 10	LB1719	Tennessee	6	0.10	++
Sp 11	LB1700	Tennessee	23	0.06	+
Sp 12	LB6131	Tennessee	6	0.07	+
Sp 13	CO1002	Texas	Non-vaccine	0.13	++
Sp 14	CO1401	Texas	6	0.11	++
Sp 15	CO1602	Texas	6	0.11	++
Sp 16	CO1802	Texas	6	0.10	++
Sp 17	CO2201	Texas	19	0.09	+
Sp 18	CO2301	Texas	6	0.12	++
Sp 19	CO3302	Texas	19	0.08	+
Sp 20	CO3303	Texas	6	0.10	++
Nm 1	MC58	Type strain	B	0.40	+++
Nm 2	4677	DDR	B	0.53	++++
Nm 3	4699	Norway	B	0.62	++++
Nm 4	4681	DDR	B	0.33	+++
Nm 5	4675	Denmark	B	0.60	++++
Nm 6	1001	USA	B	1.07	+++++
Nm 7	4708	China	B	0.44	+++
Nm 8	4706	Chile	B	0.45	+++
Nm 9	4687	Norway	B	1.16	+++++
Nm 10	6418	Cuba	B	0.65	++++
Nm 11	4683	Norway	B	0.52	++++
Nm 12	1054	Finland	A	0.54	++++
Nm 13	4323	Israel	C	0.62	++++
Nm 14	4665	Netherlands	B	0.67	++++
Nm 15	6114	New Zealand	C	0.66	++++
Nm 16	6412	Iceland	B	0.50	+++
Nm 17	4667	Netherlands	B	0.51	++++
Nm 18	6423	Chile	B	0.24	+++
Nm 19	4710	USSR	B	0.33	+++
Nm 20	4674	Netherlands	B	0.52	++++
Nm 21	4680	DDR	B	0.08	+
Nm 21	3906	China	A	0.43	+++

Nm 22	3842	Norway	B	0.0	-
Nm 23	4684	Norway	B	0.69	++++
Nm 24	4664	Netherlands	B	0.24	+++
Nm 25	6416	Greece	B	0.18	++
Nm 26	6424	Netherlands	B	0.12	++
Nm 27	3667	Sudan	A	0.14	++
Nm 28	4323	Israel	C	0.10	+
Nm 29	1269	Burkina	A	0.03	+
Nm 30	4682	Norway	B	0.05	+
Nm 31	4662	Netherlands	B	0.08	+
Nm 32	4678	DDR	B	0.02	+
Nm 33	6426	Netherlands	B	0.04	+
Nm 34	3524	Chad	A	0.06	+
Nm 35	1392	Greece	A	0.11	++
Nm 36	4765	Brazil	C	0.10	+
Nm 37	1092	W. Germany	A	0.09	+
Nm 38	4701	Norway	B	0.10	+
Nm 39	4695	Norway	B	0.01	+
Nm 40	1035	Pakistan	A	0.07	+
Nm 41	6419	Austria	B	0.00	-
Nm 42	4671	Netherlands	B	0.03	+
Nm 43	4756	India	A	0.49	+++
Nm 44	4262	USA	B	0.04	+
Nm 45	4672	Netherlands	B	0.05	+
Nm 46	6421	Iceland	B	0.07	+
Nm 47	6420	Greece	B	0.39	+++
Nm 48	4688	Norway	B	0.23	+++
Nm 49	1073	Canada	A	0.52	++++
Nm 50	5035	China	A	0.55	++++
Nm 51	4239	Norway	C	0.44	+++
Nm 52	4242	Italy	C	0.32	+++
Nm 53	4686	Norway	B	0.26	+++
Nm 54	5515	Saudi Arabia	A	0.78	++++
Nm 55	6426	New Zealand	B	0.96	+++++
Nm 56	1275	Niger	A	0.29	+++
Nm 57	6415	England	C	0.38	+++
Nm 58	4696	Norway	B	0.51	++++
Nm 59	6427	Scotland	B	0.40	+++
Nm 60	4709	Switzerland	B	0.68	++++
Nm 61	4181	Mali	C	0.46	+++
Nm 62	5010	Djibouti	A	0.27	+++
Nm 63	4684	Norway	B	0.37	+++
Nm 64	6411	England	B	0.28	+++
Nm 65	5005	Morocco	A	0.66	++++
Nm 66	6413	South Africa	C	0.31	+++
Nm 67	1506	Brazil	A	0.20	++
Nm 68	4631	Scotland	C	0.42	+++

Hi 1	RD	USA	B	0.16	++
Hi 2	3/2	UK	B	0.23	+++
Hi 3	13/2	UK	B	0.22	+++
Hi 4	14/2	UK	B	0.22	+++
Hi 5	22/2	UK	B	0.27	+++
Hi 6	29/2	UK	B	0.18	++
Hi 7	13/3	UK	B	0.21	+++
Hi 8	14/3	UK	B	0.18	++
Hi 9	15/3	UK	B	0.21	+++
Hi 10	23/3	UK	B	0.27	+++
Hi 11	20/4	UK	B	0.25	+++
Hi 12	152/4	UK	B	0.26	+++
Hi 13	155/4	UK	B	0.31	+++
Hi 14	2/7	UK	B	0.31	+++
Hi 15	13/7	UK	B	0.24	+++
Hi 16	101/7	UK	B	0.21	+++
Hi 17	2/9	UK	B	0.22	+++
Hi 18	3/9	UK	B	0.16	++
Hi 19	10/9	UK	B	0.20	++
Hi 20	13/9	UK	B	0.17	++
Hi 21	18/9	UK	B	0.22	+++
Hi 22	22/9	UK	B	0.14	++
Hi 23	35/9	UK	B	0.21	+++
Hi 24	49/9	UK	B	0.18	++
Hi 25	50/9	UK	B	0.22	+++
Hi 26	2/10	UK	B	0.16	++
Hi 27	101/10	UK	B	0.13	++
Hi 28	2/13	UK	B	0.21	+++
Hi 29	16/13	UK	B	0.26	+++
Hi 30	27/13	UK	B	0.18	++
Hi 31	31/16	UK	B	0.18	++
Hi 32	9/17	UK	B	0.16	++
Hi 33	18/10	UK	B	0.22	+++
Hi 34	31/10	UK	B	0.27	+++
Hi 35	36/17	UK	B	0.31	+++
Hi 36	1/23	UK	B	0.19	++
Hi 37	8/23	UK	B	0.14	++
Hi 38	42/43	UK	B	0.26	+++
Hi 39	47/23	UK	B	0.12	++

† For ELISA assays, plates were coated with 0.5 µg of rLR and blocked with 100 µg/ml BSA. As a negative control, BSA without rLR was used. OD values shown are adjusted to subtract background binding to BSA without rLR.

* Express truncated CbpA

Non-vaccine: positive with antibody to pool of serotypes 29, 34, 35, 42 & 47