ONLINE SUPPLEMENTAL MATERIAL

ONLINE SUPPLEMENTAL METHODS

qRT-PCR

RNA from hCMEC/D3 were extracted using the Nucleospin RNA (Macherey-Nagel) and quantified using the NanoDrop ND-100 (ThermoFisher). cDNA synthesis was performed from 1 µg of RNA using oligo-dT (ThermoFisher) and SuperScript IV RT (ThermoFisher). RT-qPCR was performed using Power SYBR Green PCR system (ThermoFisher) and specific primers (listed in Supplemental Table 3) on the LightCycler 480 Real-Time PCR System (Roche). Results were normalized using 18S as the housekeeping gene and expressed as mean fold change relative to uninfected condition ± SEM. Statistical analysis : One-Way Anova, with NS, non-significant; *, $p < 0.05$.

ONLINE SUPPLEMENTAL TABLES AND FIGURES

TABLE S1: Bacterial strains used in this study

Strains	**Characteristics**	**Reference**
BM110	CC17 isolate from neonate blood culture (serotype III)	(15)
NEM316	CC23 isolated from neonate blood culture (serotype III)	(15)
Δ*srr2*	BM110 deleted in *srr2* gene	(6)
Δ*srr2*/pSrr2	BM110 Δ*srr2* complemented with pAT28-BR_{Srr2}	(16)
Δ*srr2*/pempty	BM110 Δ*srr2* complemented with pAT28 empty vector	(16)
BM110-GFP		This study

TABLE S2: Plasmids used in this study

Plasmids	Vectors	insert	reference
pET-BR2	pET2818	BR_{Srr2}	(16)
pET-BR2-RGD*	pET2818	BR_{Srr2} RGD:AAA	This study
pET-BR2-SDV*	pET2818	BR_{Srr2} SDV:AAA	This study
pET-BR2-RGD*-SDV*	pET2818	BR_{Srr2} RGA:AAA;SDV:AAA	This study
pET-BR2-FSVKI*	pET2818	BR_{Srr2} FSVKI:FAAAI	This study
pET-BR2-ETYVI*	pET2818	BR_{Srr2} ETYVI:AAYAI	This study
pGU2664		GFP	Sullivan et al.

SUPPLEMENTARY REFERENCE

Sullivan MJ, and Ulett GC. Stable Expression of Modified Green Fluorescent Protein in Group B Streptococci To Enable Visualization in Experimental Systems. *Appl Environ Microbiol.* 2018;84(18).

TABLE S3: Primers used in this study

Primers	Sequence (5'-3')
RGD:AAA-F	cactattgattctgtgaatgcagctgctacttttgaaattaagttatc
RGD:AAA-R	gataacttaatttcaaaagtagcagctgcattcacagaatcaatagtg
SDV:AAA-F	gatgatgatgggatccagcattagcagctgcatatcccgatccagatgagtaagt
SDV:AAA-R	acttactcatctggatcgggatatgcagctgctaatgctggatcccatcatcatc
FSVKI-FAAAI-F	acagttcaaaacacaaaagaagatgttcttttgcggcagcaataaaggatcaagaggctacaatt aaagaaac
FSVKI-FAAAI-R	gtttctttaattgtagcctcttgatccttattgctgccgcaaaaagaacatcttcttttgtgttttgaactgt
ETYVI-AAYAI-F	gggggcagtttattgcatcaaatgcagcgtatgcaattgttgtagaaacaccatttac
ETYVI-AAYAI-R	gtaaatggtgtttctacaacaattgcatacgctgcatttgatgcaataaactgccccc
q-PCR-α5-F	aatcttattaccccgagtacc
q-PCR-α5-R	tatcctaggtagctgtcatc
q-PCR-β3-F	ctccggccagatgattc
q-PCR-β3-R	tcctccatggagtaagacag

Mutagenized motifs of interest are indicated in red letters.

TABLE S4: Antibodies used in this study

target	clone	supplier	use
Anti-Srr2		Laboratory stock (16)	Dot-Blot/inhibition
Anti-Srr1		S. Dramsi	Dot-Blot
Anti-GBS	PA1-7250	Thermofisher	Counter-staining
Anti-ZO1	339100	Thermofisher	IF
Anti-ICAM1	Ab2213	Abcam	ELISA
Anti-β1	AIIB2/P5D2	DSHB	ELISA (human integrin)
Anti-αvβ3	LM609	Millipore	ELISA (human integrin)
Anti-β1	Mab1997	Chemicon international	ELISA (mouse integrin)
Anti-β3	MAB41182	R&D	ELISA (mouse integrin)
Anti-α5	Ab150361	Abcam	IF, WB, IHC
Anti-β3	sc-19671-L	Santa Cruz	IF
Anti-β3	4702	Cell signalling	WB
Anti-β3	13166	Cell signalling	IHC
Anti-α5	MFR5 5H10-27	BD pharmingen	Blocking *in vivo*
Isotype contro	16-4321-85	Invitrogen	Control blocking *in vivo*
Anti-CD31		BD pharmingen	IHC
Anti-TTR		Abcam	IHC

The AIIB2 and P5D2 antibodies were developed by Damsky, C.H. and Wayner, E.A., respectively and obtained from the Developmental Studies Hybridoma Bank (DSHB), created by the NICHD of the NIH and maintained at The University of Iowa, Department of Biology, Iowa City, IA 52242.

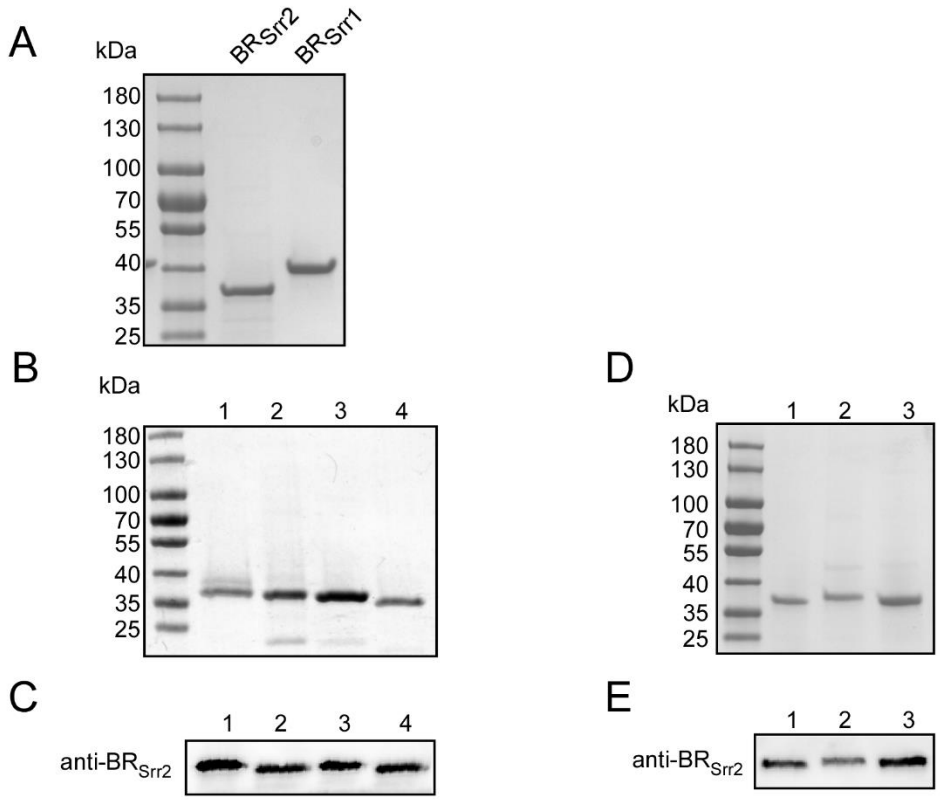


Figure S1: Purification of BR_{Srr2} and BR_{Srr1} forms. (A) BR_{Srr1} and BR_{srr2} were purified and analyzed by Coomassie staining. (B and C) RGD and/or SDV motifs were mutagenized. Resulting proteins were purified and analyzed by (B) Coomassie staining (C) or Western Blot analysis using an anti-Srr2 antibody; lane 1, Native BR_{Srr2}; 2, RGD:AAA; 3, SDV:AAA; 4, RGD:AAA + SDV:AAA. (D and E) FSVKI or ETYVI motifs were mutagenized. Resulting proteins were purified and analyzed by (D) Coomassie staining (E) or Western Blot analysis using an anti-Srr2 antibody; lane 1, Native BR_{Srr2}; 2, FSVKI:FAAAI; 3, ETYVI:AAYVI.

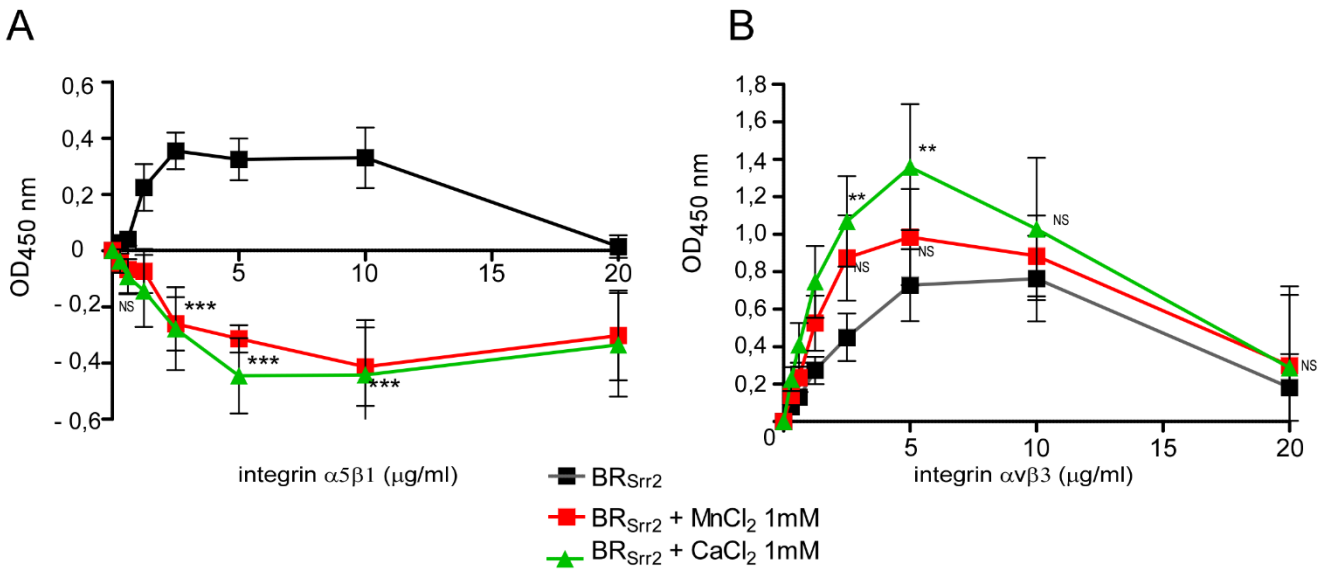


Figure S2: Influence of divalent cations on the interaction of BR_{Srr2} with α5β1 or αvβ3 integrins. Interaction of BR_{Srr2} with (A) α5β1 or (B) αvβ3 integrin was assessed by ELISA in the absence of divalent cations (black) or in the presence of 1mM of $CaCl_2$ (green) or 1mM of $MnCl_2$ (red). Results were normalized to the negative control (BSA). Error bars *correspond* to SEM of three independent experiments. Two-Way ANOVA statistical analysis between divalent cations treatments or absence of treatment was performed with NS, non-significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure S3

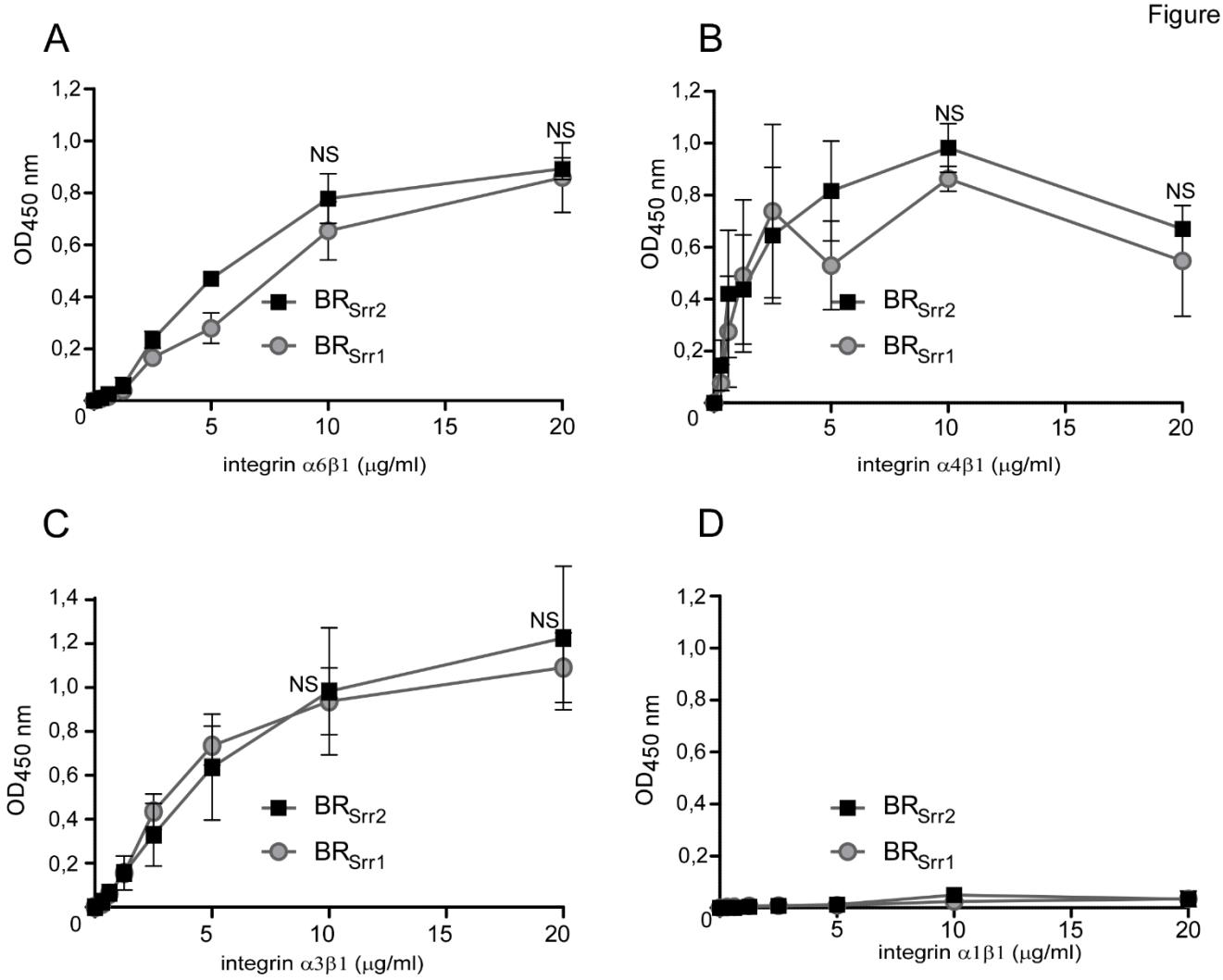
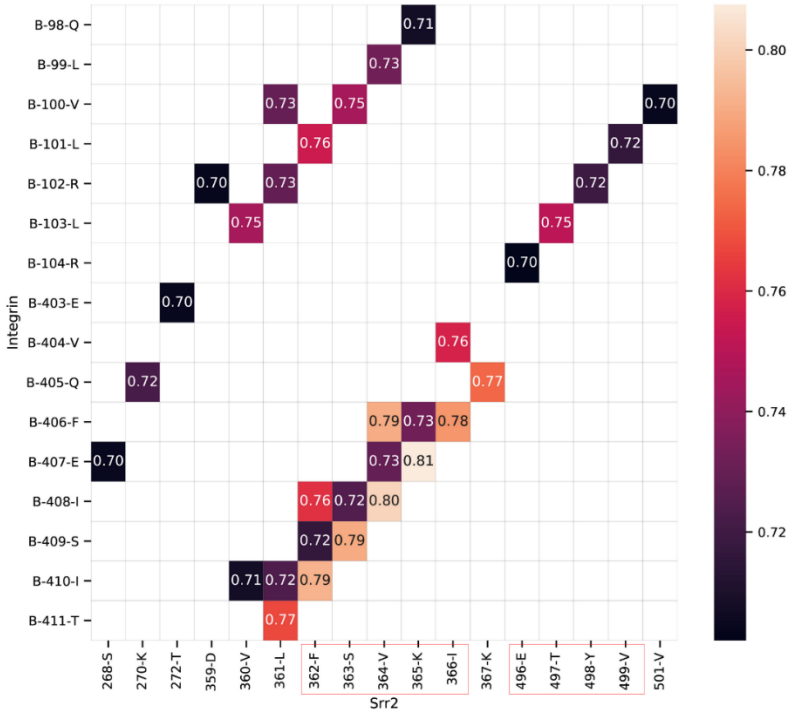


Figure S3: BR_{Srr1} and BR_{Srr2} recognize other integrins. Interaction of BR_{Srr2} (black) and BR_{Srr1} (grey) with integrin (A) α6β1, (B) α4β1, (C) α3β1, or (D) α1β1 was assessed by ELISA. Results were normalized to negative control (BSA). Error bars correspond to SEM of three independent experiments.

A



B

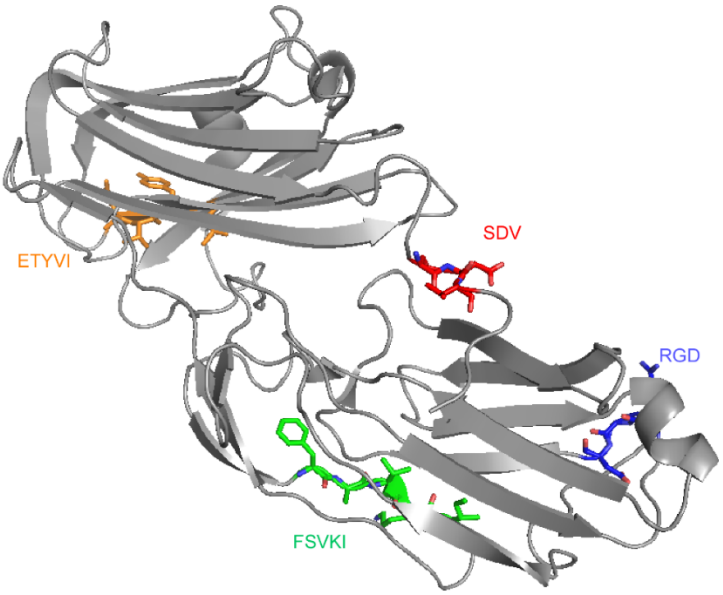


Figure S4: α5β1 integrin binding motif on BR_{Srr2}. (A) *In silico* predicted contacts between BR_{Srr2} (PDB code: 4MBR) and α5β1Integrin (PDB code: 4WJK) using the RaptorX Protein Complex Contact Prediction server (http://raptorx.uchicago.edu/ComplexContact/). Residues of BR_{Srr2} and α5β1 integrin predicted as involved in the interactions are given on the x and y-axis, respectively. The probability values were thresholded at 0.7 to highlight relevant contact clusters. (B) Structure of BR_{Srr2} showing that both αvβ3 binding motifs: RGD (blue) and SDV (red) are localized on the same side of BR_{Srr2}, while α5β1 binding motif, FSVKI (green) is located on the opposite side.

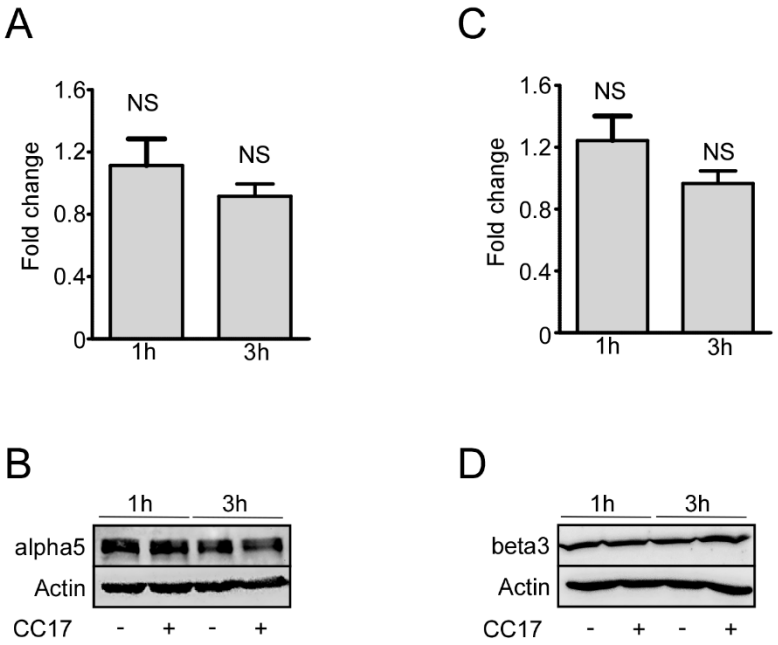


Figure S5: Infection of hCMEC/D3 by CC17 strain does not affect integrin expression. (A and C) q-RT-PCR data analyzing fold change of (A) α5 subunit integrin or (C) β3 subunit integrin following hCMEC/D3 infection with CC17 strain for 1 or 3 h. Results are expressed as mean ± SEM of at least 3 independent experiments in duplicate. Statistical analysis: One-Way ANOVA with NS, non-significant. (**B and D**) Changes in protein levels assessed by Western Blot analysis of (B) α5 or (D) β3 integrin subunit, in hCMEC/D3 after 1 or 3 h of infection with CC17 strain.

Figure S6

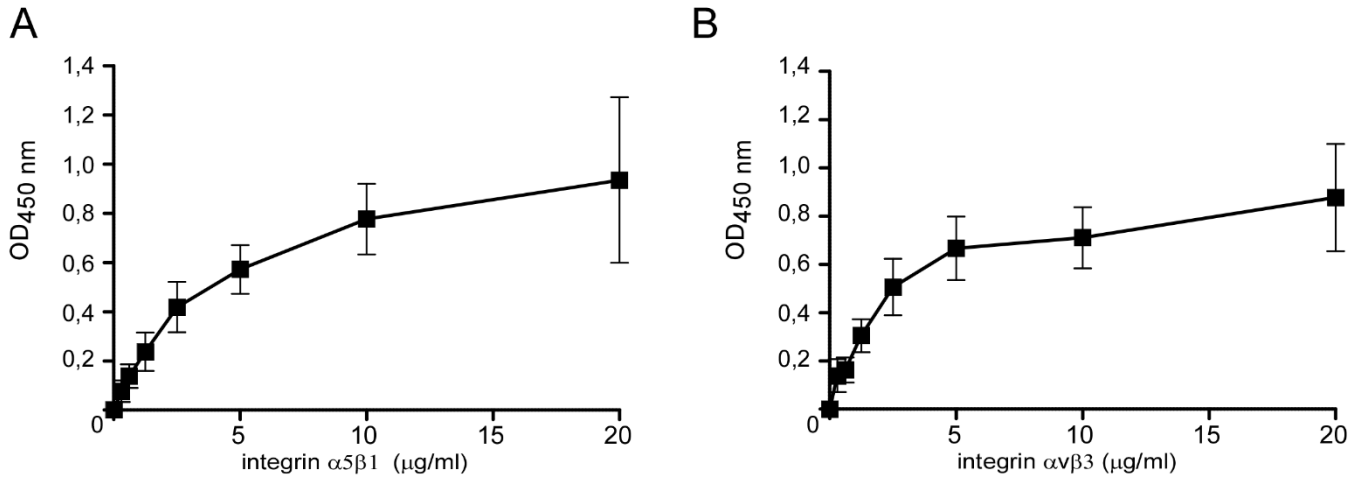


Figure S6: BR_{Srr2} recognize α5β1 and αvβ3 mouse integrins. Interaction of BR_{Srr2} with mouse (A) α5β1, or (B) αvβ3 integrins were assessed by ELISA. Results were normalized to negative control (BSA). Error bars *correspond* to SEM of three to five independent experiments.