Supporting information

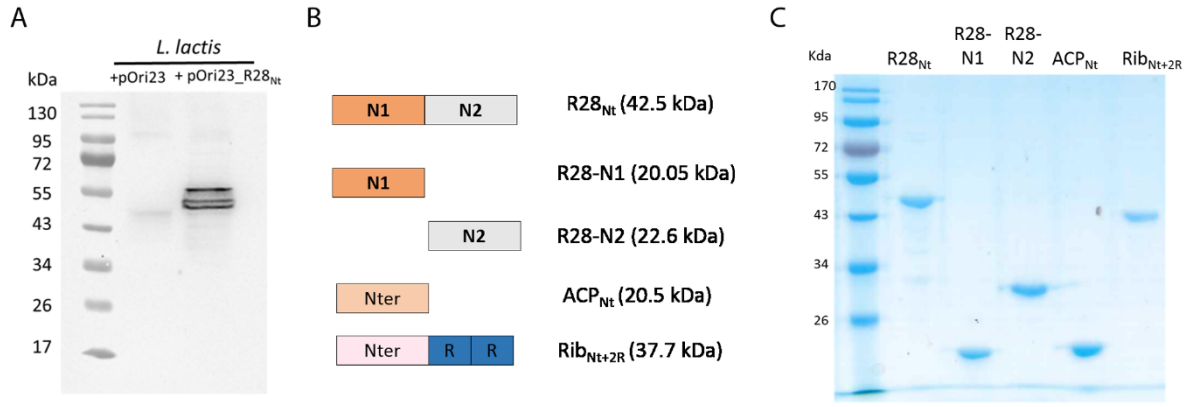

Figure S1. Schematic representation of constructed peptides used in this study, and their expression in *L. lactis* or *E. coli*. *A,* Western blot with anti-R28$_{Nt}$ antiserum of cell wall extracts of *L. lactis* expressing R28$_{Nt}$, + pOri_R28$_{Nt}$; or not, +pOri. *B,* schematic representation of the different peptides used in the study. Color code corresponds to Fig. 1*A*. *C,* Coomassie blue staining of an acrylamide gel with 1 µg of each peptide loaded.

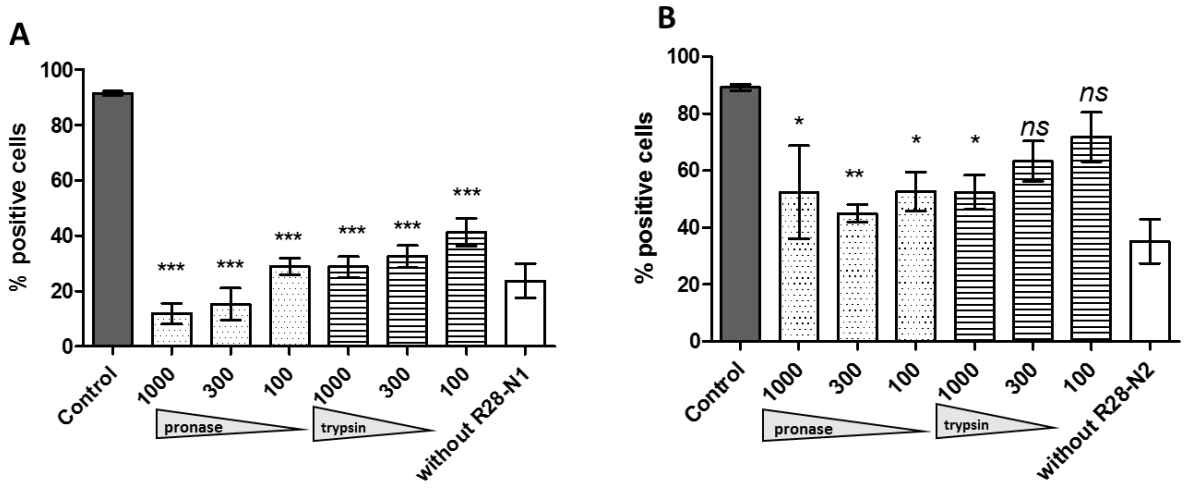

Figure S2. Effect of different treatments on the binding of *A*, R28-N1 and *B*, R28-N2 to HEC-1-A cells. Treatments were applied to HEC-1-A cells in suspension prior to incubation with purified R28-N1 and R28-N2; bound peptides were immunolabelled and cells were analyzed by flow cytometry. Statistical analysis was performed against the untreated condition Error bars correspond to SEM of three independent experiments. Two-Way ANOVA: *, $p<0.05$; **, $p<0.005$; ***, $p<0.001$; ns, not significant.

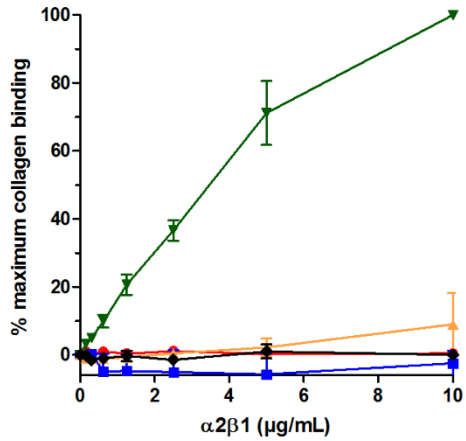

Figure S3. R28Nt, R28-N1 and R28-N2 do not bind the α2β1 integrin. Assessment of integrin α2β1 binding to R28$_{Nt}$, red; R28-N1, blue; R28-N2, orange; Type I collagen, green; BSA, black. The binding is expressed as percentage of that of the collagen at 10μg/mL integrin.

Table S1. Strains and plasmids used in this study

Strains or plasmid	Relevant properties	Source or reference
Escherichia coli		
BL21 λDE3	F$^-$ *ompT gal (dcm) (lon) hsdSB (*rB$^-$ mB$^-$) *endA1 hsdR17*(rK$^-$mK$^+$)	(1)
Streptococcus pyogenes		
M28PF1	*emm28* source of DNA for R28$_{Nt,}$ R28-N1, R28-N2 amplifications	(2)
ΔR28	M28PF1 lacking the *Spy1336* coding for R28	This study
Streptococcus agalactiae		
A909	serotype Ia, sequence type 7 , used for amplification of the gene of ACP	(3)
BM110	serotype III, sequence type 17, used for the amplification of the gene encoding Rib	(4)
Lactococcus lactis		
MG1363	Lac$^-$ Prt$^-$; NCDO 712 derivative	(5)
CCH2022	MG1363+ pOri23	This study
CCH2023	MG1363+ pOri23_R28$_{Nt}$	This study
Plasmids		
pG+host5	Erm; ColE1 replicon, thermosensitive derivative of pGK12; MCS pBluescript	(6)
pG1_R28	500 pb upstream (R28F1-R28R1) and 500 bp downstream (R28F2-R28R2) sequence of *Spy1336* cloned in pG+host5	This study
pET2818	Amp, oriR pBR322, T7 promoter, His-Tag coding sequence, pET28/16 derivative.	S. Mesnage (Pers. com.)
pET2818_R28$_{Nt}$	pET2818 with the sequence encoding the R28$_{Nt}$, without signal peptide. For the production of the peptide in *E. coli* with a His Tag	This study
pET2818_R28.N1	Ibidem with the sequence encoding R28-N1	This study
pET2818_R28.N2	Ibidem with the sequence encoding R28-N2	This study
pET2818_ACP	Ibidem with the sequence encoding ACP	This study
pOri23	Erm; *ermB,* ori ColE1, thermosensitive derivative of pIL253, P23 promoter of *L. lactis* MG1363	(7)
pOri23_R28$_{Nt}$	High level of expression of R28$_{Nt}$ at the cell wall of Gram-positive bacteria	This study

Table S2. Primers used in this study for cloning and protein purifications

Primer Name	Sequence*
R28-F1	CGACTCTAGA**GGATCC**GCCTGTGAGAGACGATCATA
R28-R1	ATCATTCTTATCGGCCCGTTGTTTCGTCTGTGAAG
R28-F2	GCCGATAAGAATGATCCAGC
R28-R2	CCATGATTAC**GAATTC**GCCTTAACTCGTATTCCTTT
R28_F	**CCATGG**CTACAATTCCAGGGAGTGC
R28_R	**GGATCC**ACCTTTTGGTTCGTTGCTATC
R28_N1_F	**CCATGG**GGTCTACAATTCC
R28_N1_R	**GGATCC**TGGTAGCGATACAACTAA
R28_N2_F	**CCATGG**ATAAAATTAAGTATTCGCC
R28_N2_R	**GGATCC**TTTTGGTTCGTTG
ACP_F	**CCATGG**GGTCAATAGTTGCTGCATCTACAAT
ACP_R	**GGATCC**TACTGACAATACTAACAATTTCTC

* restriction enzyme sites used for cloning are highlighted in bold

Supporting information references:

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Supporting information file

The raw data of hits found by mass-spectrometry analysis of the co-immunoprecipitations are available in the supporting information file. In green and yellow are highlighted hits that are significantly enriched in the presence of $R28_{Nt}$ in all three independent experiments. In yellow are the surface exposed membrane proteins that correspond to Table 1 hits. In pink are highlighted the samples in which the given protein was not detected. CT-1, CT-2, CT-3 and R28-1, R28-2, R28-3, samples eluted from the control zones and $R28_{Nt}$ co-immunoprecipitated zones of experiments 1, 2, 3, respectively