Online Supplemental material

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T-COFFEE, Version_8.93Thu Aug 5 18:09:23 CEST 2010
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  cons          :  77

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  cons          

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  cons          :*     :*:** .* ::     *  * **.:
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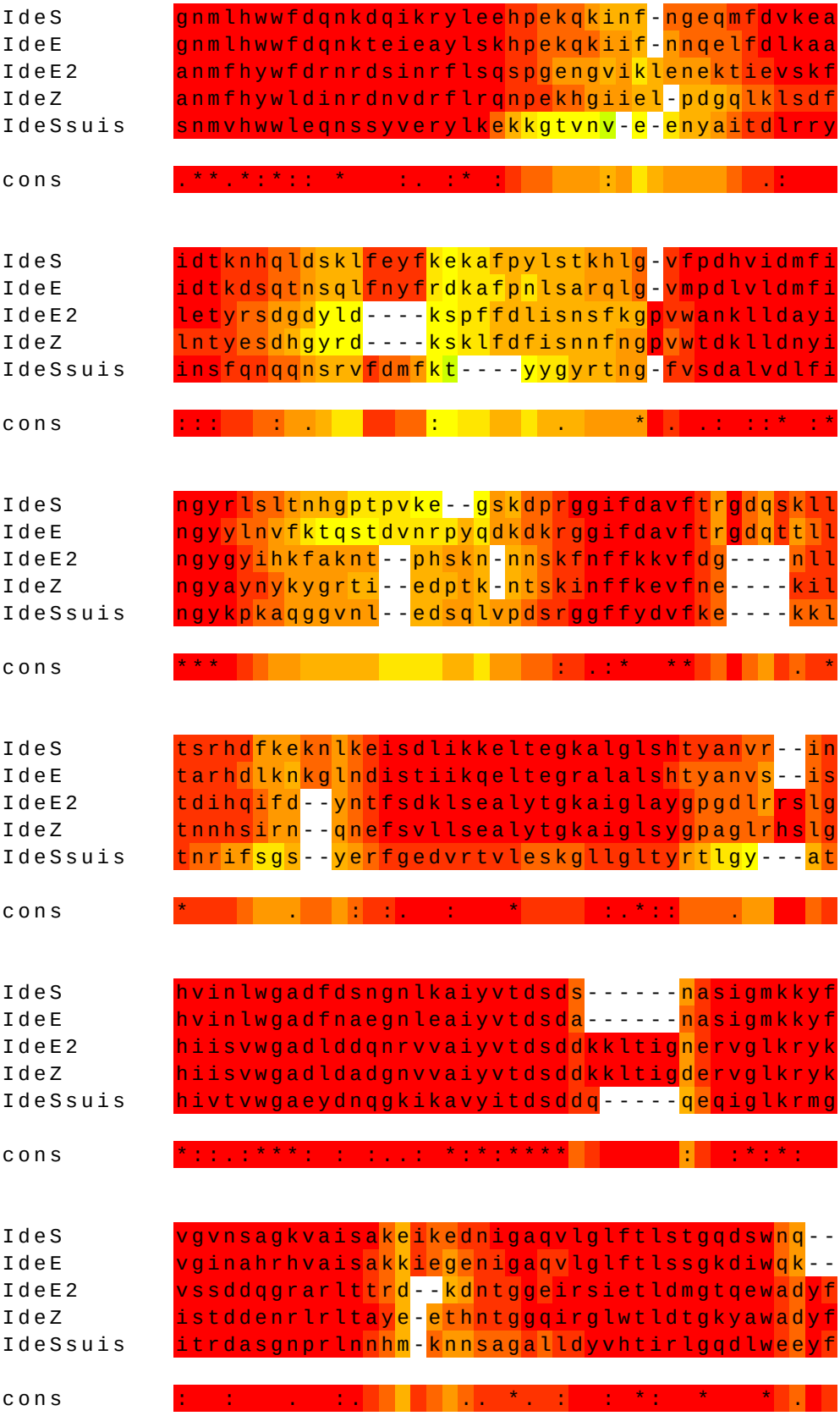
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cons .**.*:*:: * :. :* :
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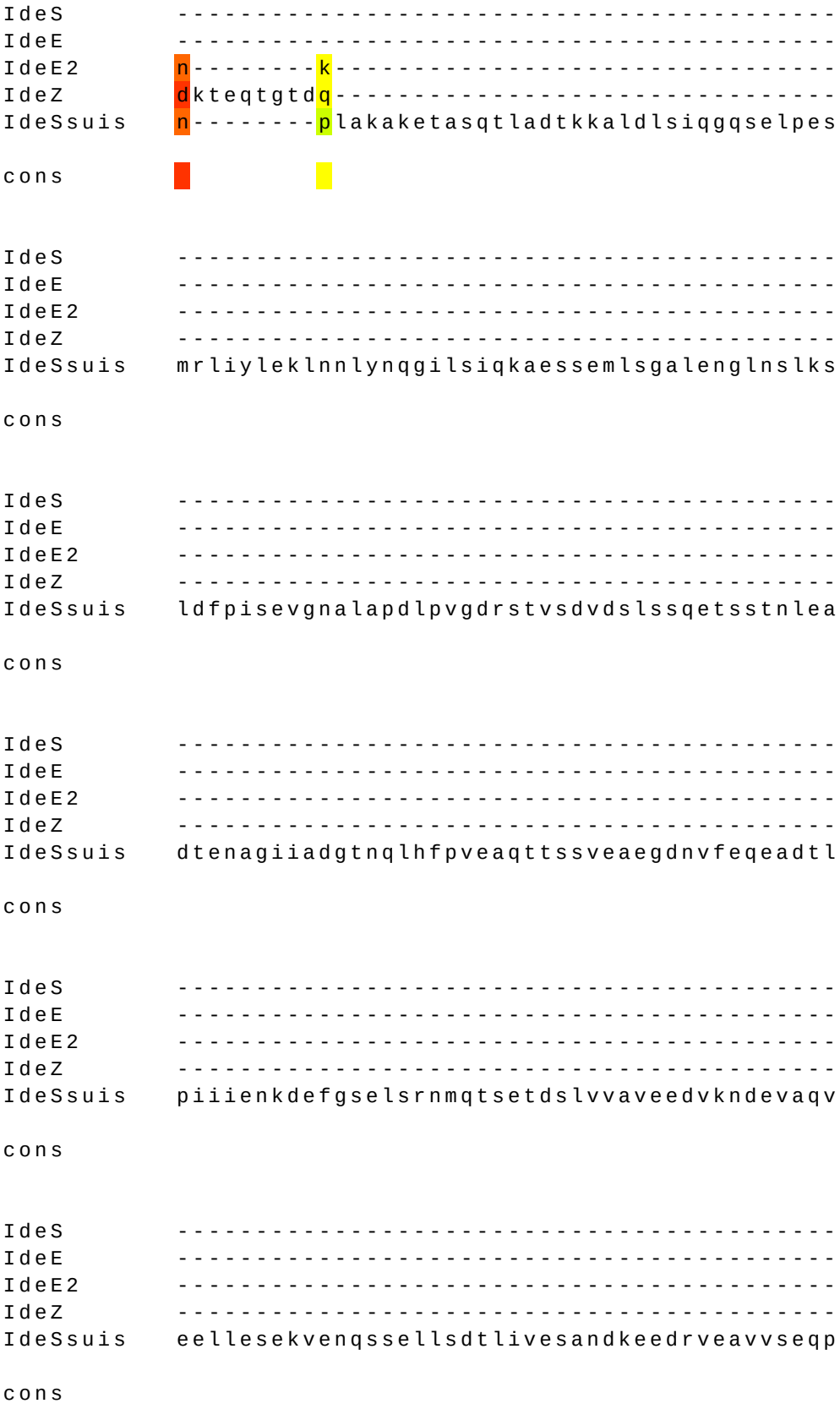
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cons


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cons


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cons
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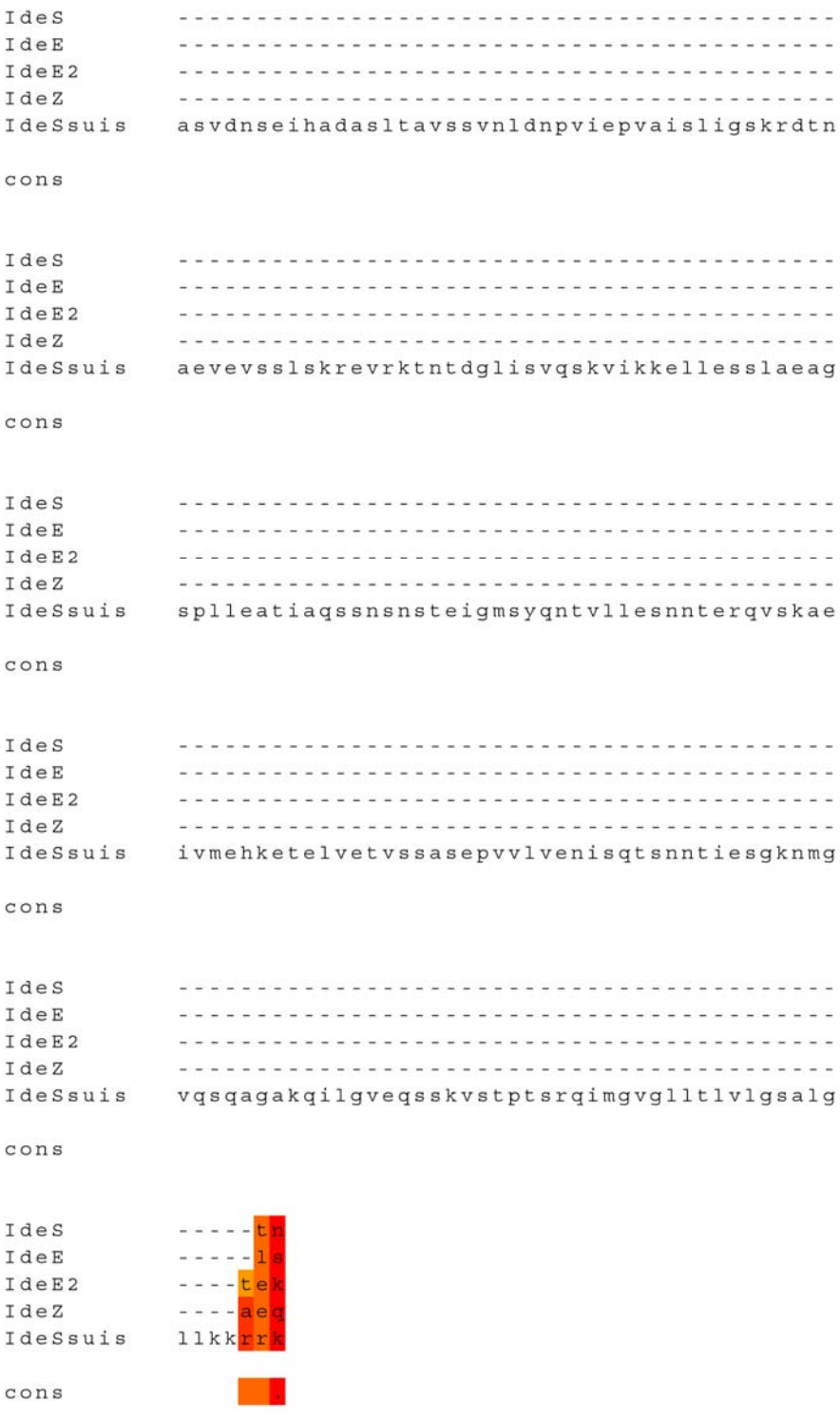

Fig. S1. Multiple sequence alignment (T-coffee) of different members of the IdeS family (accession no.): *S. pyogenes* M1 IdeS (Q7DAM2), *S. equi subsp. equi* IdeE (YP_002746323.1), *S. equi subsp. equi* IdeE2 (ACU12335.1), *S. equi subsp. zooepidemicus* IdeZ (AEJ25107.1) and *S. suis* IdeSsuis (SSU0496).

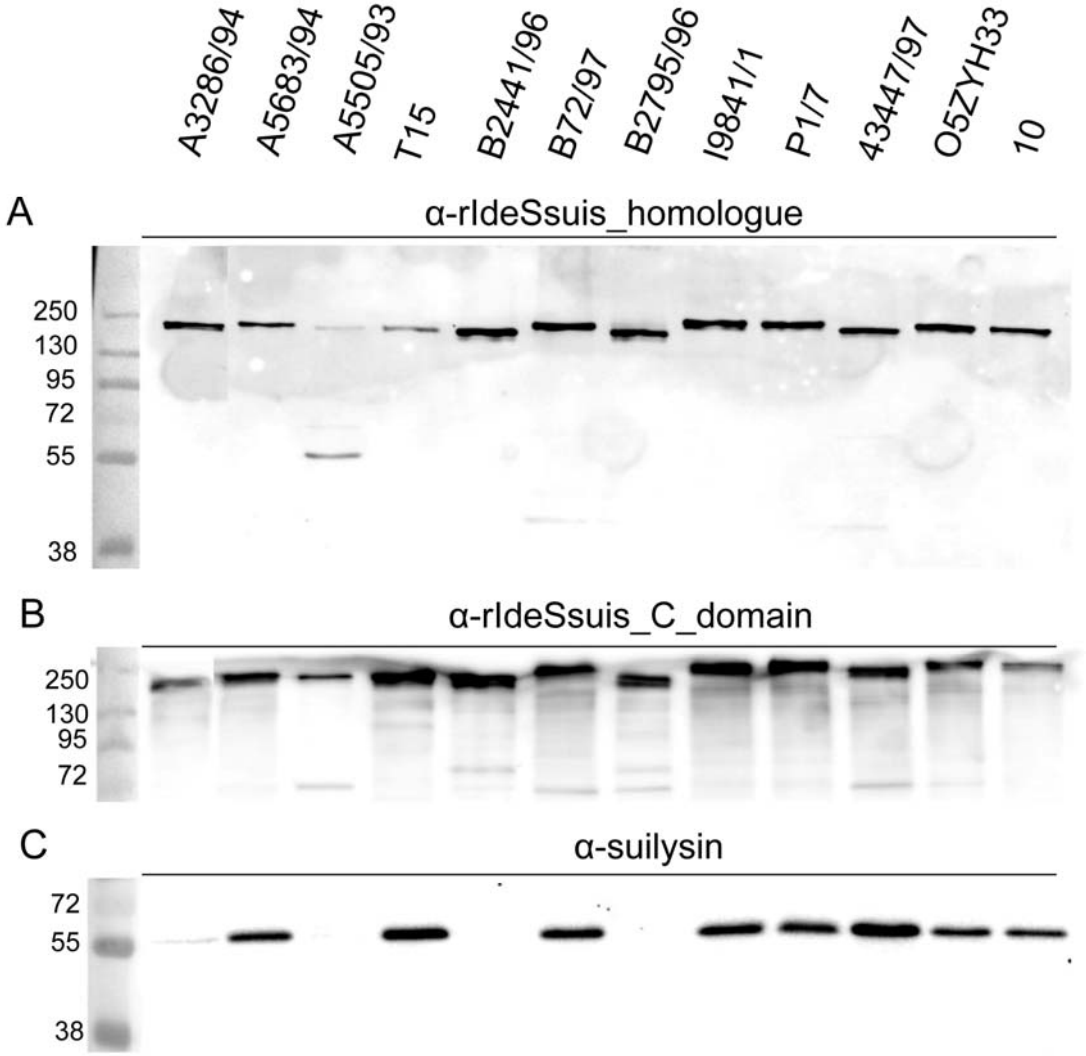

Fig. S2 IdeSsuis is recognized by rIdeSsuis_homologue (A) and rIdeSsuis_C_domain (B) specific antisera. (A-C) Western blot analysis of 24 fold concentrated culture supernatants of different *S. suis* wildtype strains as indicated at the top after separation of proteins in 10% SDS-PAGE under reducing conditions. (C) As control, supernatants were investigated with an antiserum against suilysin. The marker bands are shown on the left side (sizes in kDa).

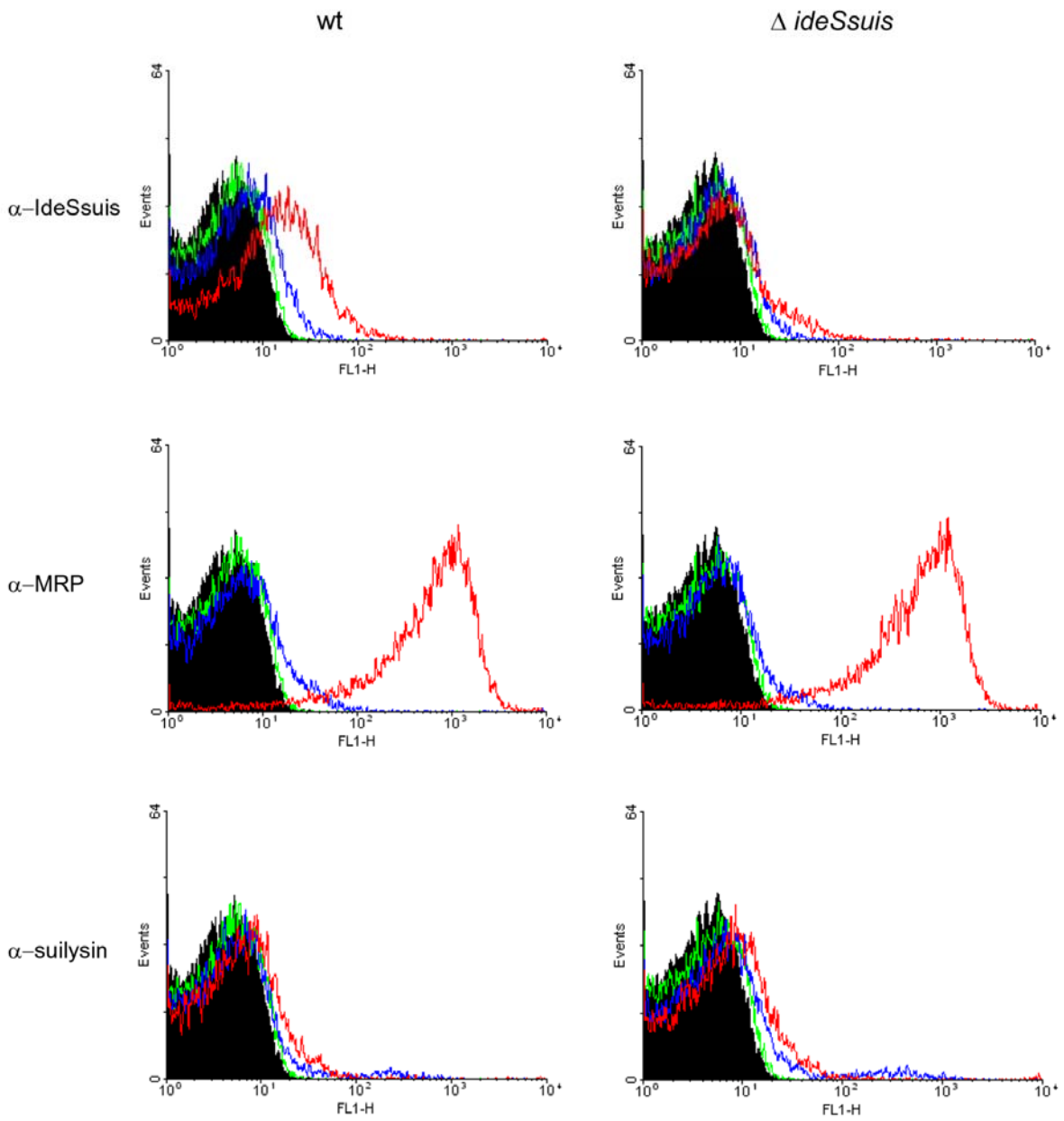

Fig. S3 Detection of IdeSsuis on the surface of *S. suis* wt (strain 10, serotype 2) in flow cytometry (red line in the upper left panel). For comparison, flow cytometry analysis was also conducted with the isogenic mutant 10Δ*ideSsuis* and with α–MRP (a surface-associated protein) and α–suilysin (a secreted cytolysin) as indicated. The red line shows the results for the specific rabbit hyperimmune sera, the green line for the control lacking the first antibody and the blue line for the respective rabbit pre immune sera. The panels show representative results of three independent experiments. Differences between strain 10 and the isogenic mutant 10Δ*ideSsuis* were significant for α–IdeSsuis but not for α–MRP and α–suilysin ($p < 0.05$).

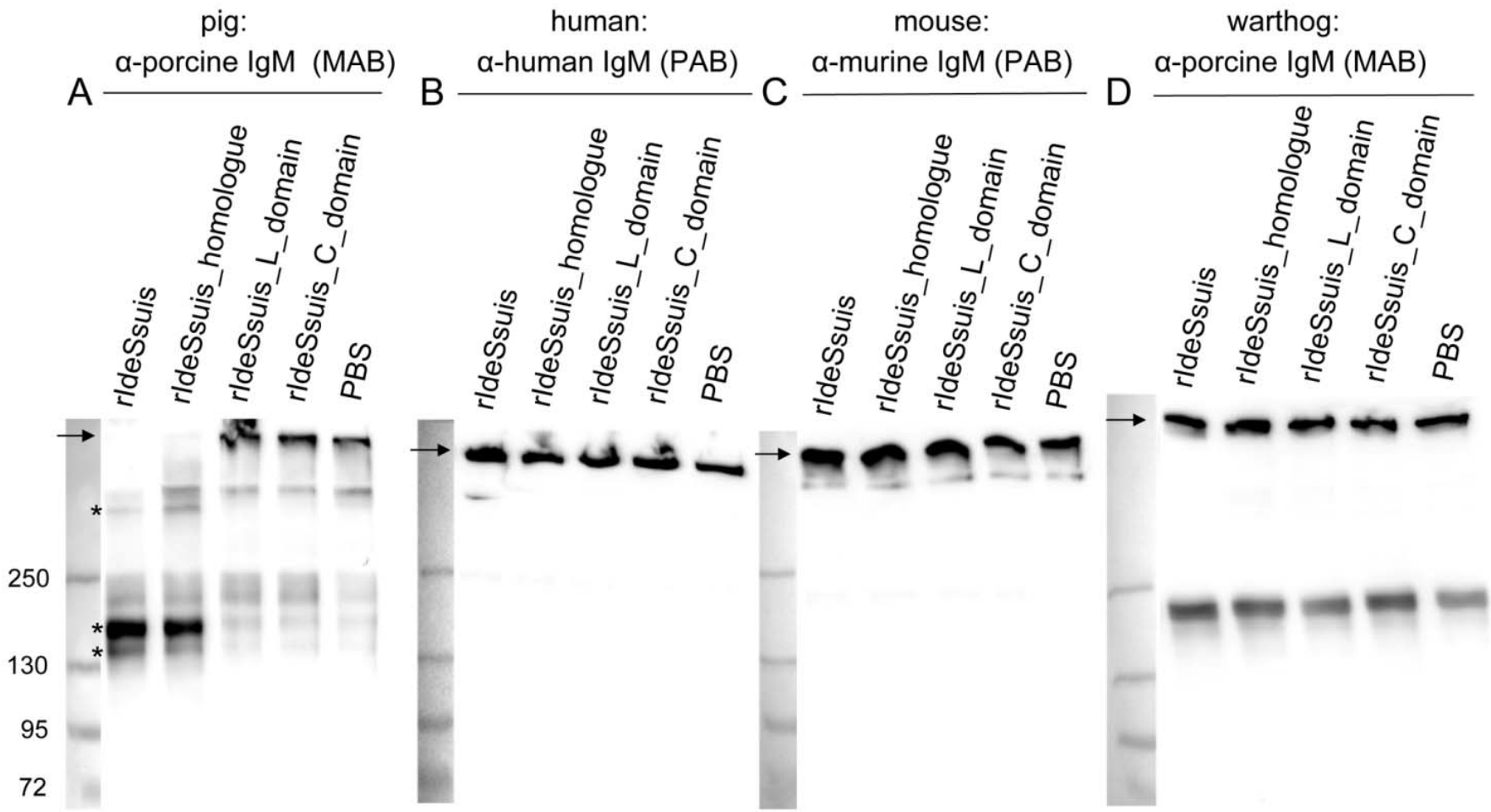

Fig. S4 rIdeSsuis and rIdeSsuis_homologue cleave IgM from pigs (**A**) but not IgM from humans (**B**), mice (**C**) and warthogs (**D**). Anti-IgM Western blot analysis of diluted plasma or serum from the indicated species incubated as indicated with 5 µg/ml of the different rIdeSsuis constructs and as control with PBS. The position of the IgM multimer is marked with an arrow on the left side of the blots and the cleavage products of porcine IgM are indicated by asterisks on the left side. The marker bands are shown on the left side (sizes in kDa).

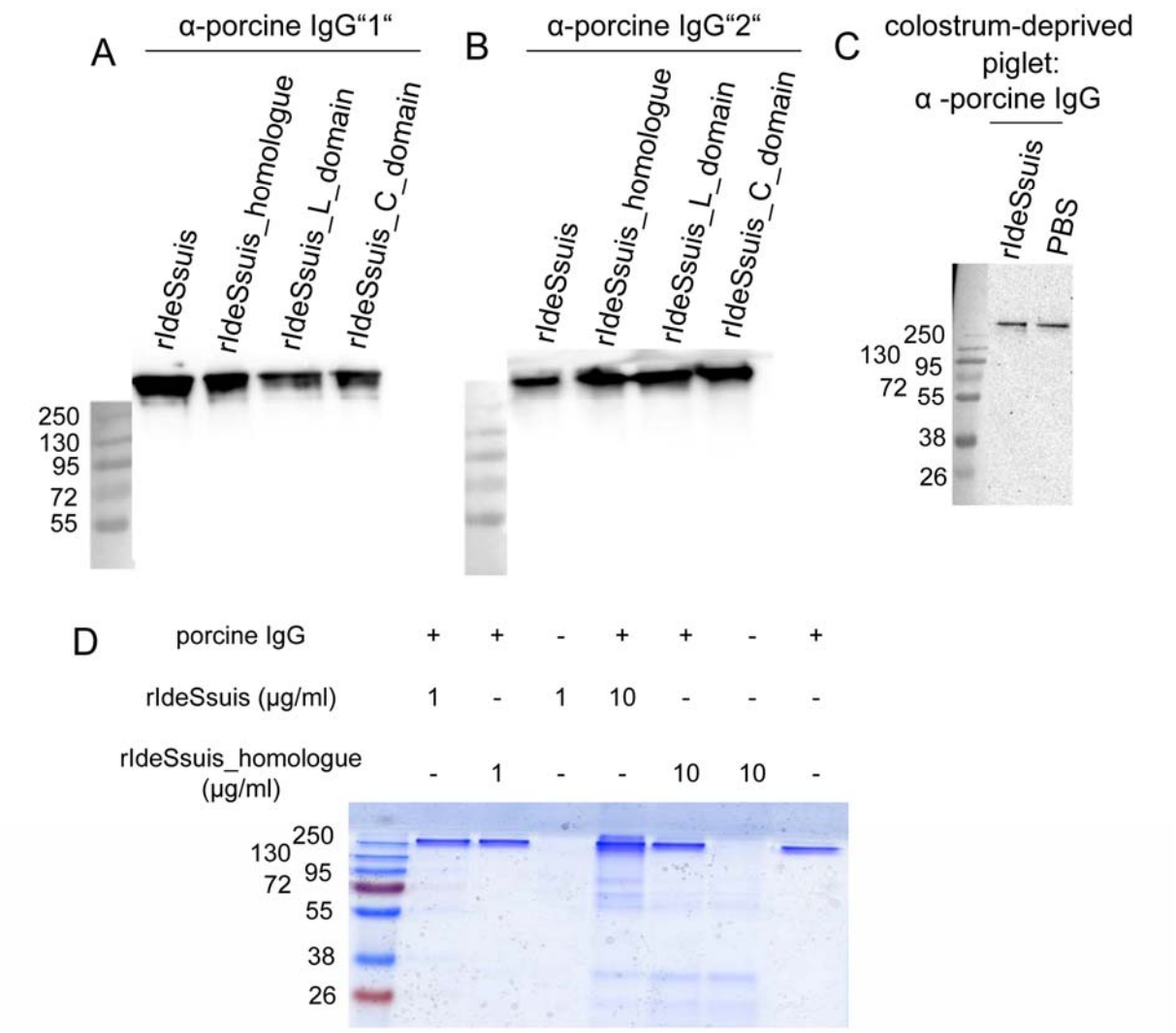

Fig. S5 Recombinant IdeSsuis does not cleave porcine IgG. (A) Anti-IgG”1” and (B) anti-IgG”2” Western blot analysis of diluted porcine plasma incubated with 5 µg/ml of the different rIdeSsuis constructs as indicated. IgG”1” and IgG”2” refer to purified porcine IgGs with different biochemical and biological properties (Serotec, personal communication). However, IgG”1” or IgG”2” do not correspond to the recent definition of porcine IgG subclasses by genomics and transcriptomics (John E. Butler, personal communication). (C) Anti-IgG Western blot analysis (PAB against porcine IgG) of diluted serum from a colostrum-deprived neonatal piglet incubated with 15 µg rIdeSsuis (in 750 µl) for 3 hours or with PBS as control. (D) Coomassie-stained gel of purified porcine IgGs incubated with rIdeSsuis as indicated. The marker bands are shown on the left side (sizes in kDa).

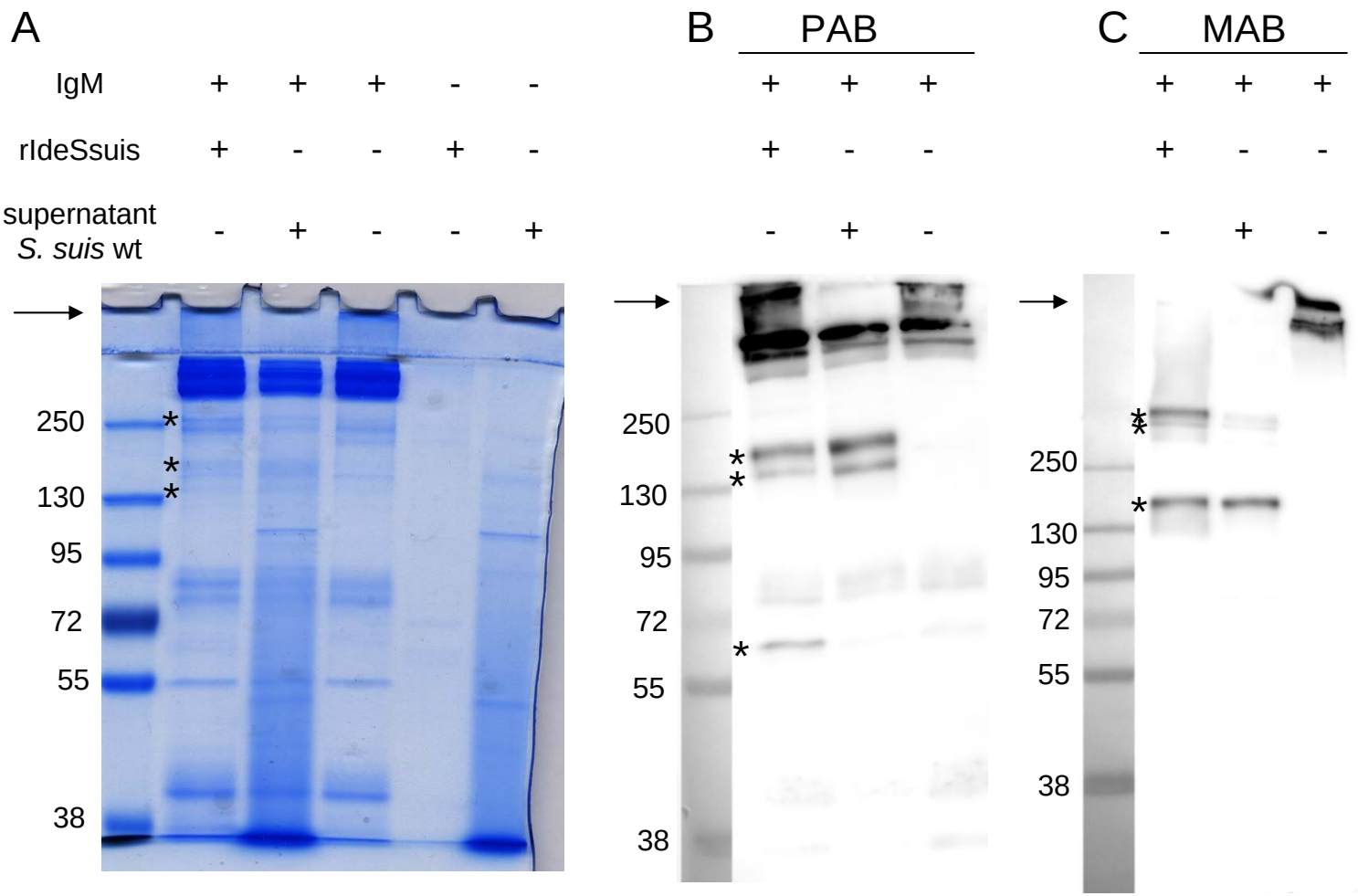

Fig. S6. Cleavage of purified porcine IgM by rIdeSsuis and supernatant of *S. suis* serotype 2 (strain 10, wt). A. Colloidal Coomassie stained 8% gel of purified porcine IgM incubated as indicated with rIdeSsuis or purified 24 fold concentrated supernatant of *S. suis* wt (strain 10). B and C. Western blot analysis of purified IgM cleaved with rIdeSsuis or supernatant of *S. suis* wt as indicated (8% SDS-PAGE). Western blot analysis was conducted with a PAB (B) or MAB (C) against porcine IgM. The position of the porcine IgM multimer is marked with an arrow and the cleavage products of porcine IgM are indicated by asterisks on the left side for the first positive lane. The marker bands are shown on the left side (sizes in kDa).

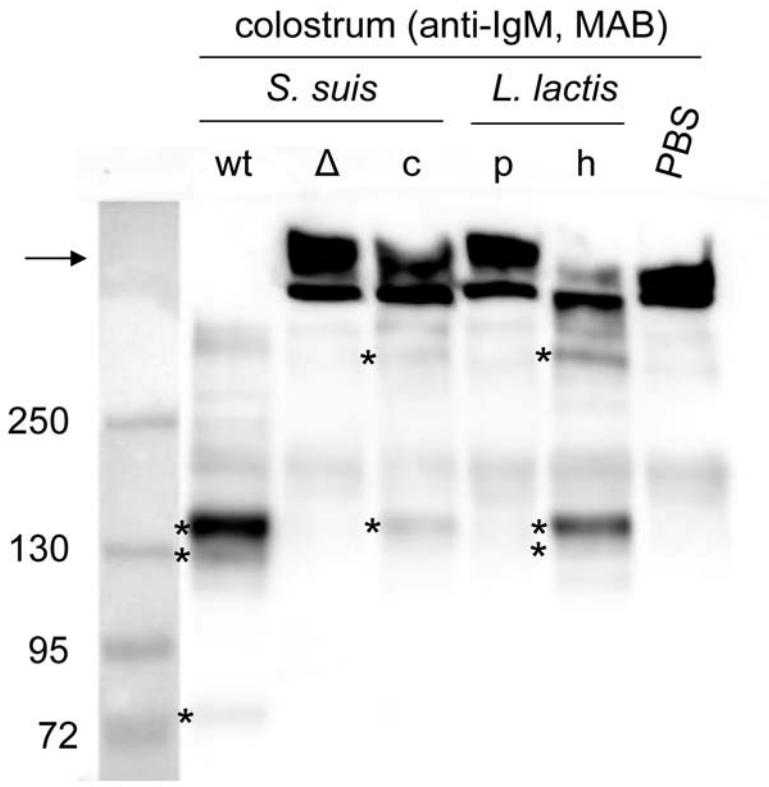

Fig. S7 IdeSsuis in culture supernatants cleaves IgM in colostrum. Anti-IgM Western blot analysis was carried out with diluted colostrum incubated with the 24 fold concentrated supernatants of *S. suis* strain 10 (wt), 10ΔideSsuis (Δ) and 10ΔideSsuis pGA14ideSsuis (c) or with the 6 fold concentrated supernatants of *L. lactis* pOri23 (p) and *L. lactis* pOriideSsuis (h) as well as with PBS as control. The position of the porcine IgM multimer is marked with an arrow and the cleavage products of porcine IgM are indicated by asterisks on the left side. The marker bands are shown on the left side (sizes in kDa).

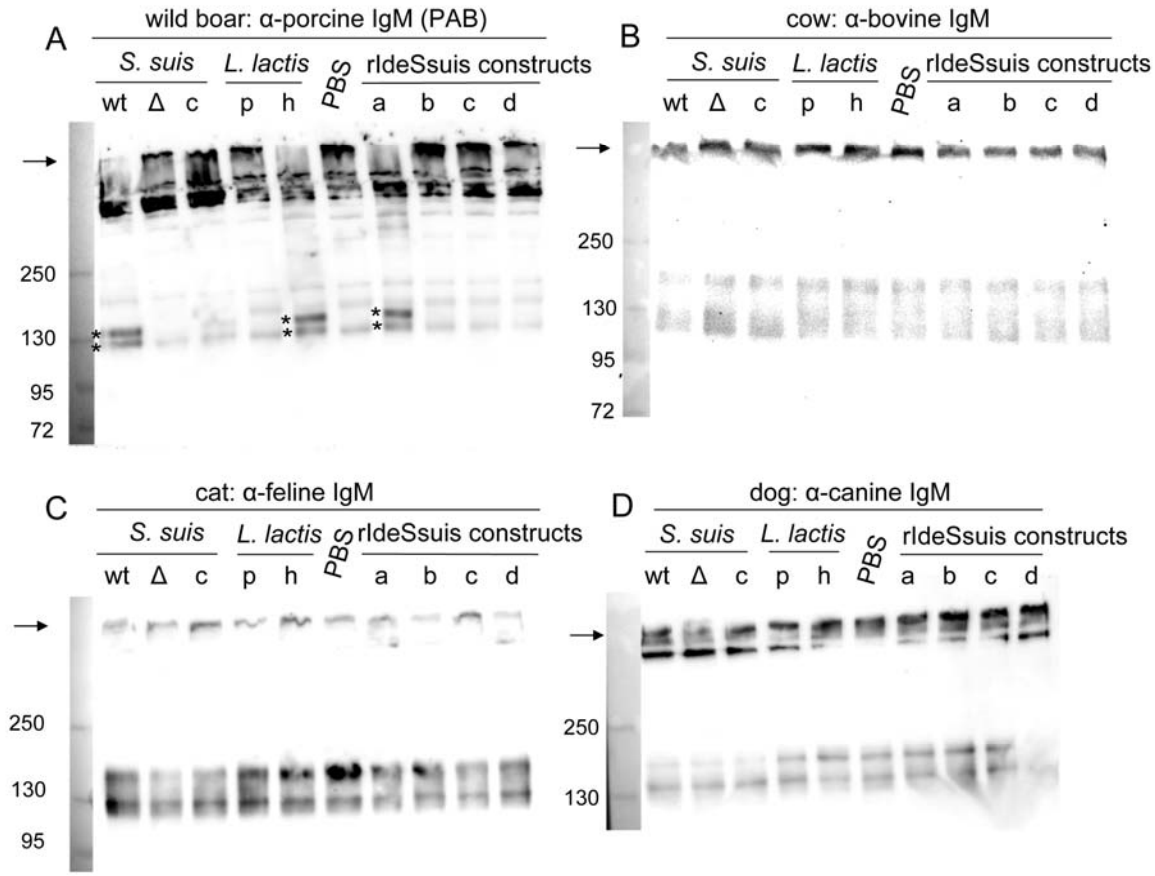

Fig. S8 IdeSsuis in culture supernatants and rIdeSsuis cleave IgM from wild boars (A) but not bovine (B), feline (C) or canine (D) IgM. Anti-IgM Western blot analysis of diluted serum from respective animals incubated with the 24 fold concentrated supernatants of *S. suis* strain 10 (wt), 10ΔideSsuis (Δ) and 10ΔideSsuis pGA14ideSsuis (c) as well as with the 6 fold concentrated supernatants of *L. lactis* pOri23 (p) and *L. lactis* pOriideSsuis (h) and with rIdeSsuis (a), rIdeSsuis_ homologue (b), rIdeSsuis_L_domain (c) and rIdeSsuis_C_domain (d). Detection of IgM was conducted with PABs against IgM of the different species tested (Supplementary Table 2). The final dilution of serum was 1:100 in all samples. The position of the IgM multimers are marked with arrows and the cleavage products of porcine IgM are indicated by asterisks on the left side. The marker bands are shown on the left side (sizes in kDa).

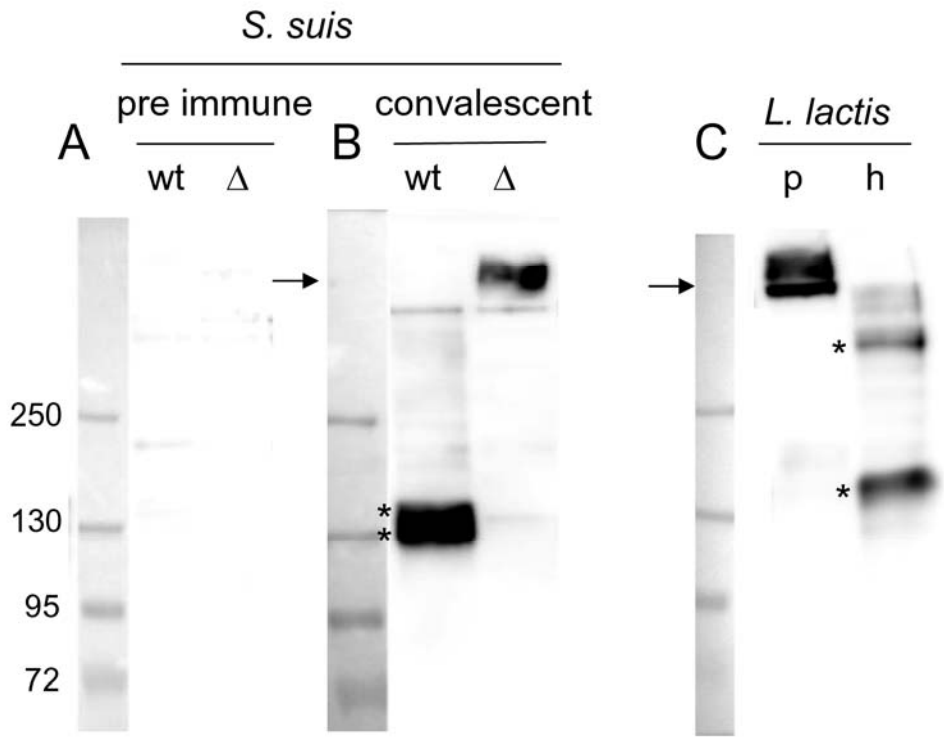

Fig. S9 IdeSsuis cleaves IgM bound to the bacterial surface. (A-C) Anti-IgM Western blot analysis (MAB) of culture supernatants of *S. suis* (A, B) and *L. lactis* (C) after opsonization with pre-immune (A), convalescent (B) and anti *L. lactis* hyper-immune (C) serum, respectively, and subsequent incubation of *S. suis* (A, B) strain 10 (wt) and 10ΔideSsuis (Δ) as well as *L. lactis* (C) pOri23 (p) and pOriideSsuis (h) for 2.5 h. The position of the high molecular weight porcine IgM multimer is marked with an arrow and the cleavage products of porcine IgM are indicated by asterisks. The marker bands are shown on the left side (sizes in kDa).

Table S1. Oligonucleotide primer sequences used in this study.

primer	sequence	position[a]
ideSsuispostSSBamHI	TGCGGATCCAGTTGTTACAGGAGTGAAT	101 to120
ideSsuisterPstI	AACCTGCAGACGATACTTCTTTTACTTA	3420 to 3439
ideSsuis_L_domain_rev	TAACTGCAGCTAAAAATTCGATTTGTCAG	937 to 956
ideSsuis_ hom_rev	GTTCTGCAGGCTTTTGCAAGCGGATTGAA	1282 to 1302
ideSsuisCfor_BamHI	GAAGAGGATCCCAATCCGCTTGCAAAAG	1284 to1300
ideSsuisBamHIforL	ATAGGATCCTAAACGGAGGAAAGATGAAC	- 14 to 6
preProIdeSsuisPstI	CTTCTGCAGTAAAAGACGCATG	- 504 to - 483
postSSideSuisBamHI	ATTGGATCCTGTAACAACTGTATCGGC	94 to 114
preEndideSsuisBamHI	TAAGGGATCCTCAAGACAGATTATGGGAGT	3346 to 3365
postEndideSsuisEcoRI	AACGGAATTCTATACTTCAACTTATTCAC	3926 to 3945
ideSsuismid_for	GAAATGCTTTGGCACCAGAT	1538 to 1557
ideSsuismid_rev2	CAACCGCCTCCACTCTATCT	1983 to 2002
pSET5_for	TACCGGGTTGGACTCAAGAC	
pSET5_rev	GCTTCCTCGCTCACTGACTC	
ideSsuis_SacI_for	CAGGAGCTCTTGGACCTTTTTGTAGTAAT	- 148 to -128
ideSsuis_SacI_rev	GAACGAGCTCGCTTATTTCTATCTAGAATA	3632 to 3651

a. Numbers indicate the location of the oligonucleotide primer with regard to the initiation ATG codon. The gene *ideSsuis* ends at position 3426.

Table S2. Antibodies used in Western blot analysis.

detection of	first antibody				second antibody			
	specificity (manufacturer)	source[a]	conjugation[b]	dilution	specificity (manufacturer)	source[a]	conjugation[b]	dilution
His-tagged proteins	$(His)_n$ (34660, Qiagen, Hilden, Germany)	mouse MAB		1:1000	mouse IgG (GE Healthcare, Freiburg, Germany)	sheep	POD	1:10000
IdeSsuis	rIdeSsuis	rabbit PAB	-	1:1000	rabbit IgG (GE Healthcare)	donkey	POD	1:10000
IdeSsuis	rIdeSsuis_homologue	rabbit PAB	-	1:1000	rabbit IgG (GE Healthcare)	donkey	POD	1:10000
IdeSsuis	rIdeSsuis_C_domain	rabbit PAB	-	1:1000	rabbit IgG (GE Healthcare)	donkey	POD	1:10000
Suilysin	rSuilysin[c]	rabbit PAB	-	1:1000	rabbit IgG (GE Healthcare)	donkey	POD	1:10000
porcine IgM	porcine µ chain (LS-C59959,Lifespan Biosciences, Seattle, USA)	rabbit PAB	POD	1:2000				
porcine IgM	porcine IgM (MCA637, Serotec, Duesseldorf, Germany)	mouse MAB	-	1:250	mouse IgG (GE Healthcare)	sheep	POD	1:10000
porcine IgM	porcine IgM (A100-100A, Bethyl Laboratories, Inc, Montgomery, USA)	goat PAB	-	1:8000	goat IgG (Jackson Laboratories, Bar Harbor, USA)	rabbit	POD	1:10000
porcine IgG	porcine IgG“1“ (MCA635, Serotec)	mouse MAB	-	1:10000	ati-mouse IgG (Dianova, Hamburg, Germany)	sheep	POD	1:5000
porcine IgG	porcine IgG“2“ (MCA636, Serotec)	mouse MAB	-	1:10000	mouse IgG (Dianova)	sheep	POD	1:5000
porcine IgA	porcine IgA, (PA1-84625, Thermo Fisher, Bonn, Germany)	goat PAB	POD	1:6000				
human IgM	human µ chain (A0420, Sigma-Aldrich, Taufkirchen, Germany)	goat PAB	POD	1:20000				

detection of	first antibody				second antibody			
	specificity (manufacturer)	source[a]	conjugation[b]	dilution	specificity (manufacturer)	source[a]	conjugation[b]	dilution
murine IgM	murine µ chain (115-035-075, Dianova)	goat PAB	POD	1:10000				
equine IgM	equine µ chain (A70-114A, Bethyl Laboratories)	goat PAB	-	1:10000	goat IgG (Jackson Laboratories)	rabbit	POD	1:10000
canine IgM	canine IgM (A40116A, Bethyl Laboratories)	goat PAB	-	1:8000	goat IgG (Jackson Laboratories)	rabbit	POD	1:10000
feline IgM	feline IgM (A20-100A, Bethyl Laboratories)	goat PAB	-	1:8000	goat IgG (Jackson Laboratories)	rabbit	POD	1:10000
bovine IgM	bovine IgM (A10-101A, Bethyl Laboratories)	sheep PAB	-	1:8000	sheep biotin (Miltenyi Biotec, Bergisch Gladbach, Germany), biotin[d] (Miltenyi Biotec)	rabbit mouse	biotin, AP	1:5000, 1:10000
warthog IgM	porcine µ chain (LS-C59959, Lifespan Biosciences)	rabbit PAB	POD	1:2000				
warthog IgM	porcine IgM (MCA637, Serotec)	mouse MAB	-	1:250	mouse IgG (GE Healthcare)	sheep	POD	1:10000

a. MAB = monoclonal antibody; PAB = polyclonal antibody
b. POD = peroxidase; AP = alkaline phosphatase
c. hyper-immune serum against suilysin was raised in a previous study (Kock *et al.* 2009).
d. third antibody