

Figure S2. Sequence alignment of the fusion protein (Signal peptide-FedF Nanobody-ProtA) expressed in *Lactococcus lactis*. The highlighted sections show the Usp signal peptide (green), anti-FaeG nanobody sequences (blue) and the ProtA sequences (red). The long and short ProtA repeat (consensus EDNNKPGK) sequences are indicated in italic. The LPETG C-terminal motif, recognized by the sortase enzyme, is shown in bold. Dashes were introduced to align the amino acid sequences.

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pEXP362-1 (NbFed6)      MKKKIISAILMSTVILSAAAPLSGVYAQVQLQESGGGSVQAGGSLRLSCAASGYTSGRDSMGWFRQAPGK
pEXP364-3 (NbFedF7)    MKKKIISAILMSTVILSAAAPLSGVYAQVQLQESGGGSVQAGGSLRLSCTASGYTYRKYCMGWFRQAPGK
pEXP459 (NbFedF9)      MKKKIISAILMSTVILSAAAPLSGVYAQVQLQESGGGSVQAGGSLRLSCTASGYTSSNCMAWFRQVPGK
pEXP461 (NbFedF11)     MKKKIISAILMSTVILSAAAPLSGVYAQVQLQESGGGSVQAGGSLRLSCTASGLPNRIYSMGWYRQAPGK
pEXP463 (NbFedF12)     MKKKIISAILMSTVILSAAAPLSGVYAQVQLQESGGGSVQAGGSLRLSCTASGNT-KSYWMGWFRQAPGK
pEXP363 (NbFedF6)      MKKKIISAILMSTVILSAAAPLSGVYAQVQLQESGGGSVQAGGSLRLSCTASGYTSGRDSMGWFRQAPGK
pEXP365 (NbFedF7)      MKKKIISAILMSTVILSAAAPLSGVYAQVQLQESGGGSVQAGGSLRLSCTASGYTYRKYCMGWFRQAPGK
pEXP447 (NbFedF9)      MKKKIISAILMSTVILSAAAPLSGVYAQVQLQESGGGSVQAGGSLRLSCTASGYTSSNCMAWFRQVPGK
pEXP449 (NbFedF11)     MKKKIISAILMSTVILSAAAPLSGVYAQVQLQESGGGSVQAGGSLRLSCTASGLPNRIYSMGWYRQAPGK
pEXP451 (NbFedF12)     MKKKIISAILMSTVILSAAAPLSGVYAQVQLQESGGGSVQAGGSLRLSCTASGNT-KSYWMGWFRQAPGK
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pEXP362-1 (NbFed6)      EREGVACIDTSGIVNY-ADSVKGRFTISQDSAKKTLYLEMNSLKPEDTALYSCA-TGPFVYGRGCL--GQ
pEXP364-3 (NbFedF7)    EREGVACINSGGGTSYYADSVKGRFTISQDNAKDTVFLRMNSLKPEDTALYSCA-L---SSNSVCPPGHV
pEXP459 (NbFedF9)      EREGVASINTRGGITYYADSVKGRFTISRDNAKNTVSLQMNSLKPEDTATYYCAAVREATYSDNRCVSRS
pEXP461 (NbFedF11)     DRELVSYYIRFDGR-TYYDDSVKGRFTISQDNAKNTVFLQMNSLKPEDTAMVCKIVSSGTGD-----
pEXP463 (NbFedF12)     EREGVAVIAGGGAFTYYAESVKGRFTISQDNTKDTVYLAMNSLKPEDTALYSCA-LRTLNNGGIFLLNSP
pEXP363 (NbFedF6)      EREGVACIDTSGIVNY-ADSVKGRFTISQDSAKKTLYLEMNSLKPEDTALYSCA-TGPFVYGRGCL--GQ
pEXP365 (NbFedF7)      EREGVACINSGGGTSYYADSVKGRFTISQDNAKDTVFLRMNSLKPEDTALYSCA---LSSNSVCPPGHV
pEXP447 (NbFedF9)      EREGVASINTRGGITYYADSVKGRFTISRDNAKNTVSLQMNSLKPEDTATYYCAAVREATYSDNRCVSRS
pEXP449 (NbFedF11)     DRELVSYYIRFDGR-TYYDDSVKGRFTISQDNAKNTVFLQMNSLKPEDTAMVCKIVSSGTGD-----
pEXP451 (NbFedF12)     EREGVAVIAGGGAFTYYAESVKGRFTISQDNTKDTVYLAMNSLKPEDTALYSCA-LRTLNNGGIFLLNSP
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pEXP362-1 (NbFed6)      AFYSYWGQGTQVTVSSGPKEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGKED
pEXP364-3 (NbFedF7)    AWYNDWGQGTQVTVSSGPKEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGKED
pEXP459 (NbFedF9)      YTYDYWGQGTQVTVSSGPKEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGKED
pEXP461 (NbFedF11)     ---DYWGQGTQVTVSSGPKEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGKED
pEXP463 (NbFedF12)     SSFHYWGQGTQVTVSSGPKEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGKED
pEXP363 (NbFedF6)      AFYSYWGQGTQVTVSSGPKEDNNKPGKED-----
pEXP365 (NbFedF7)      AWYNDWGQGTQVTVSSGPKEDNNKPGKED-----
pEXP447 (NbFedF9)      YTYDYWGQGTQVTVSSGPKEDNNKPGKED-----
pEXP449 (NbFedF11)     ---DYWGQGTQVTVSSGPKEDNNKPGKED-----
pEXP451 (NbFedF12)     SSFHYWGQGTQVTVSSGPKEDNNKPGKED-----
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pEXP362-1 (NbFed6)      NKKPGKEDGNKPGKEDNNKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGNKPGKED
pEXP364-3 (NbFedF7)    NKKPGKEDGNKPGKEDNNKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGNKPGKED
pEXP459 (NbFedF9)      NKKPGKEDGNKPGKEDNNKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGNKPGKED
pEXP461 (NbFedF11)     NKKPGKEDGNKPGKEDNNKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGNKPGKED
pEXP463 (NbFedF12)     NKKPGKEDGNKPGKEDNNKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGNKPGKED
pEXP363 (NbFedF6)      -----NGIHVVKPGDVTNDIAKANGTTADKIAAD
pEXP365 (NbFedF7)      -----NGIHVVKPGDVTNDIAKANGTTADKIAAD
pEXP447 (NbFedF9)      -----NGIHVVKPGDVTNDIAKANGTTADKIAAD
pEXP449 (NbFedF11)     -----NGIHVVKPGDVTNDIAKANGTTADKIAAD
pEXP451 (NbFedF12)     -----NGIHVVKPGDVTNDIAKANGTTADKIAAD
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pEXP362-1 (NbFed6)      NKLADKNMIKPGQELVVDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRREI
pEXP364-3 (NbFedF7)    NKLADKNMIKPGQELVVDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRREI
pEXP459 (NbFedF9)      NKLADKNMIKPGQELVVDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRREI
pEXP461 (NbFedF11)     NKLADKNMIKPGQELVVDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRREI
pEXP463 (NbFedF12)     NKLADKNMIKPGQELVVDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRREI
pEXP363 (NbFedF6)      NKLADKNMIKPGQELVVDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRREI
pEXP365 (NbFedF7)      NKLADKNMIKPGQELVVDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRREI
pEXP447 (NbFedF9)      NKLADKNMIKPGQELVVDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRREI
pEXP449 (NbFedF11)     NKLADKNMIKPGQELVVDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRREI
pEXP451 (NbFedF12)     NKLADKNMIKPGQELVVDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRREI
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UspA signal peptide-Nb-ProtA

LPETG sortase motif

Table S1. F18 *L. lactis* agglutination assay. Equal suspension volumes of *L. lactis* strains were mixed with *E. coli* 107/86 (F18⁺) and C95-72 (F4⁺, negative control) on agglutination slide for 5 min. Agglutination was scored on 0 – 5 scale.

<i>L. lactis</i> strain	Agglutination score	
	<i>E. coli</i> 107/86	<i>E. coli</i> C95-72
pEXP362-1 (NbFedF6) L	5	1
pEXP364-3 (NbFedF7) L	5	1
pEXP459 (NbFedF9) L	4	0
pEXP463 (NbFedF12) L	3	1
pEXP461 (NbFedF11) L	1	1
pEXP447 (Nb239) S	2	0
pEXP451 (Nb244) S	3	0
pEXP449 (Nb242) S	1	1
pEXP365 (Nb234) S	3	1
pEXP363 (Nb232) S	2	1

L and S refers to long and short protein A sequences, respectively. Agglutination scores: 5=Rapid uniform agglutination <15 sec; 4=Rapid uniform agglutination 15> <30 sec; 3=Slow uniform agglutination 30sec > <1min; 2=Limited agglutination seen >1 min; 1=Apparent agglutination seen >1 min; 0=No agglutination >3 min.

Table S2. F4 *L. lactis* agglutination assay. Equal volumes of prepared *L. lactis* and *E. coli* strains C544-78, C95-72, C585-80 and 107/8 (control) were applied on the slide and mixed for 5 minutes by rocking. Agglutination was scored on 0 – 5 scale.

Strain	Agglutination score			
	C544-78 (F4ab)	C95-72 (F4ac)	C585-80 (F4ad)	107/86 (F18)
pEXP366 (V2) L	4	5	5	0
pEXP368 (V4) L	4	4	5	0
pEXP441 (V1) L	4	4	5	1
pEXP457 (V4) L	4	3	5	1
pEXP444 (V2) S	3	3	3	1
pEXP445 (V3) S	3	3	3	0
pEXP455 (V1) S	3	3	3	0

L and S refers to long and short anchor domain of protein A respectively. Agglutination scores; 5=Rapid uniform agglutination <15 sec; 4=Rapid uniform agglutination 15> <30 sec; 3=Slow uniform agglutination 30sec > <1min; 2=Limited agglutination seen >1 min; 1=Apparent agglutination seen >1 min; 0=No agglutination >3 min.

Table S3. Effect of heat treatment on viability (CFUs/mL) and agglutination properties of *L. lactis* strains. Anti-FaeG Nb expressing *L. lactis* (pEXP368) was tested against F4⁺ *E. coli* C544-78, anti-FedF Nb expressing *L. lactis* strain (pEXP362) was tested against the F18⁺ *E. coli* 107/86. Agglutination was scored on 0 – 5 scale.

Temperature (°C)	<i>L. lactis</i> strain	Time (mins)	CFU/mL	Agglutination score
50	pEXP368	20	3.8 × 10 ⁸	5
50		40	7.5 × 10 ⁷	5
50		60	4.6 × 10 ⁶	5
50	pEXP362	20	3.1 × 10 ⁷	5
50		40	7.8 × 10 ⁶	5
50		60	2.9 × 10 ⁵	5
55	pEXP368	20	1.8 × 10 ⁵	5
55		40	0	5
55		60	0	5
55	pEXP362	20	4.5 × 10 ³	5
55		40	1.5 × 10 ³	5
55		60	0	5
60	pEXP368	20	0	5
60		40	0	5
60		60	0	5
60	pEXP362	20	0	5
60		40	0	5
60		60	0	5
65	pEXP368	20	0	3
65		40	0	3
65		60	0	3
65	pEXP362	20	0	3
65		40	0	3
65		60	0	3
70	pEXP368	20	0	1
70		40	0	1
70		60	0	1
70	pEXP362	20	0	1
70		40	0	1
70		60	0	1
Not heated	pEXP368		3.3 × 10 ⁸	5
Not heated	pEXP362		6.6 × 10 ⁸	5
DTT treated (5 mM)	pEXP368	20	1.9 × 10 ⁸	0
DTT treated (5 mM)	pEXP362	20	1.0 × 10 ⁸	0

Agglutination scores; 5=Rapid agglutination <15 sec; 4=Rapid agglutination 15> <30 sec; 3=Slow agglutination 30sec > <1min; 2=Limited agglutination seen >1 min; 1=Apparent agglutination seen >1 min; 0=No agglutination >3 min.

Table S4. Average weight of piglets assigned to the 3 experimental groups. The piglets were weighed on days 0, 7 and 14. The average weights were comparable between the three groups. Group 1, Negative control group (not infected); Group 2, Positive group (infected and not treated); Group 3; Test group (infected with F4⁺ ETEC and anti-FaeG Nanobodies on the surface of *L. lactis* added to the meals from day 0 to day 8).

Day	Treatment Group	N	Mean Weight	SE	95%CI	
					Lower	Upper
0	Group 1	5	4.84	0.472	3.892	5.788
	Group 2	4	5.23	0.785	3.649	6.800
	Group 3	8	5.15	0.317	4.513	5.787
7	Group 1	5	5.30	0.524	4.247	6.353
	Group 2	4	5.80	0.820	4.153	7.446
	Group 3	8	5.89	0.342	5.200	6.575
14	Group 1	5	6.06	0.610	4.816	7.264
	Group 2	4	6.48	0.930	4.606	8.344
	Group 3	8	6.59	0.368	5.848	7.237

Table S5. Meant percent weight gain between day 0–14. The mean percent weight gain was comparable between the three groups. Group 1, Negative control group (not infected); Group 2, Positive group (infected and not treated); Group 3; Test group (infected with F4⁺ ETEC and anti-FaeG Nanobodies on the surface of *L. lactis* added to the meals from day 0 to day 8).

Treatment Group	Mean Weight	SD	Min	Max	p50
Group 1	19.64	6.402	9.1	26	21.3
Group 2	19.50	2.096	17.2	22	19.4
Group 3	21.81	4.790	12.7	29.5	21.6
Total	20.63	4.730	9.1	29.5	21.4

Table S6. Mixed effects logistic regression estimates of weight gain in experimental piglets with treatment and experiment days as fixed factors, and animal ID as random factor. Treatment group had no significant association weight gain while experiment day 14 was significant. All interactions between treatment groups and experiment days were not significant.

Variable		Coef.	Std. Err.	p-value	95% CI	
					Lower	Upper
Treatment Group	Group 1	Referent				
	Group 2	.385	.7456728	0.606	-1.076492	1.846492
	Group 3	.31	.6337001	0.625	-.9320293	1.552029
Experiment day	Day 0	Referent				
	Day 7	.46	.1474821	0.002	.1709403	.7490597
	Day 14	1.2	.1474821	<0.001	.9109403	1.48906
Interaction: Treatment/ Experiment Day	Group 1	Referent				
	Group 2/Day 7	.115	.2212232	0.603	-.3185895	.5485895
	Group 2/Day 14	.05	.2212232	0.821	-.3835895	.4835895
	Group 3/Day 7	.2775	.1880036	0.140	-.0909802	.6459802
	Group 3/Day 14	.2375	.1880036	0.206	-.1309802	.6059802
Intercept		4.84	.4971152	<0.001	3.865672	5.814328

Table S7. Mixed effects logistic regression estimates of Log10 CFU/g fecal bacteria shedding by experimental piglets, with treatment and experiment days as fixed factors and animal ID as random factor. Though not significant, estimated fecal bacterial shedding was lower in groups 2 and 3 by 0.24 and 0.28 logs, respectively. Analysis of the interaction between treatment groups and experiment day estimated a significant >1 log increase in fecal bacterial shedding in group 2 on days 5–10 compared to reference group 1 ($p < 0.05$). In group 3, significant increase in fecal bacterial shedding ($p < 0.05$) was observed on days 3–6, >1 log increase was estimated for day 5 only. Group 1, Negative control group (not infected); Group 2, Positive group (infected and not treated); Group 3; Test group (infected with F4⁺ ETEC and anti-FaeG Nanobodies on the surface of *L. lactis* added to the meals from day 0 to day 8).

Variable		Coef.	SE	p-value	95% CI	
					Lower	Upper
Treatment	Group 1	Referent				
	Group 2	-.2448348	.3889586	0.529	-1.00718	.5175099
	Group 3	-.2799788	.3305512	0.397	-.9278473	.3678897
Interaction:		Group 1	Referent			
Treatment/ Experiment Day	Group 2/Day -2	.2318059	.5326869	0.663	-.8122413	1.275853
	Group 2/Day 3	.6112077	.5326869	0.251	-.4328395	1.655255
	Group 2/Day 5	1.485867	.5176017	0.004	.4713859	2.500347
	Group 2/Day 6	1.243328	.5176017	0.016	.2288474	2.257809
	Group 2/Day 7	1.144239	.5176017	0.027	.1297586	2.15872
	Group 2/Day 8	1.135374	.5327436	0.033	.0912154	2.179532
	Group 2/Day 9	.8479532	.5327436	0.111	-.196205	1.892111
	Group 2/Day 10	1.193363	.5327436	0.025	.1492052	2.237522
	Group 2/Day 13	.3191032	.5176017	0.538	-.6953775	1.333584
	Group 3/Day -2	.327863	.457532	0.474	-.5688832	1.224609
	Group 3/Day 3	.8283653	.457532	0.070	-.0683809	1.725111
	Group 3/Day 5	1.055514	.4398768	0.016	.1933716	1.917657
	Group 3/Day 6	.8237689	.4462344	0.065	-.0508344	1.698372
	Group 3/Day 7	.4325005	.4398768	0.325	-.4296423	1.294643
	Group 3/Day 8	.3159568	.4575979	0.490	-.5809186	1.212832
	Group 3/Day 9	.2130587	.4575979	0.642	-.6838168	1.109934
	Group 3/Day 10	.2905302	.4575979	0.525	-.6063452	1.187406
	Group 3/Day 13	.3062692	.4398768	0.486	-.5558735	1.168412
Intercept		6.25745	.2593057	<0.001	5.74922	6.76568

Table S8. Mixed effects logistic regression estimates of immune response in experimental piglets with treatment and experiment days as fixed factors, and animal ID as random factor. While not significant, the analysis predicted a lower and increased immune response in groups 2 and 3, respectively. A similar pattern was observed for experiment days 7 and 14. A significant increase in immune response was estimated for group 3 on day 14. Immune response was measured as OD (optical density) in ELISA method.

Variable		Coef.	SE	p-value	95% CI	
					Lower	Upper
Treatment	Group 1	Referent				
	Group 2	-.0706	.2449453	0.773	-.550684	.409484
	Group 3	.049525	.2081635	0.812	-.358468	.457518
Experiment day	Day 0	Referent				
	Day 7	-.01	.1982159	0.960	-.3984961	.3784961
	Day 14	.0872	.1982159	0.660	-.3012961	.4756961
Interaction: Treatment/ Experiment Day	Group 1	Referent				
	Group 2/Day 7	.0715	.2973239	0.810	-.5112441	.6542441
	Group 2/Day 14	.5493	.2973239	0.065	-.0334441	1.132044
	Group 3/Day 7	.21225	.2526767	0.401	-.2829872	.7074872
	Group 3/Day 14	.884925	.2526767	<0.001	.3896878	1.380162
Intercept		.3726	.1632969	0.023	.052544	.692656