

Adhesion of enteropathogenic, enterotoxigenic and commensal *Escherichia coli* to the Major
Zymogen Granule Membrane Glycoprotein 2

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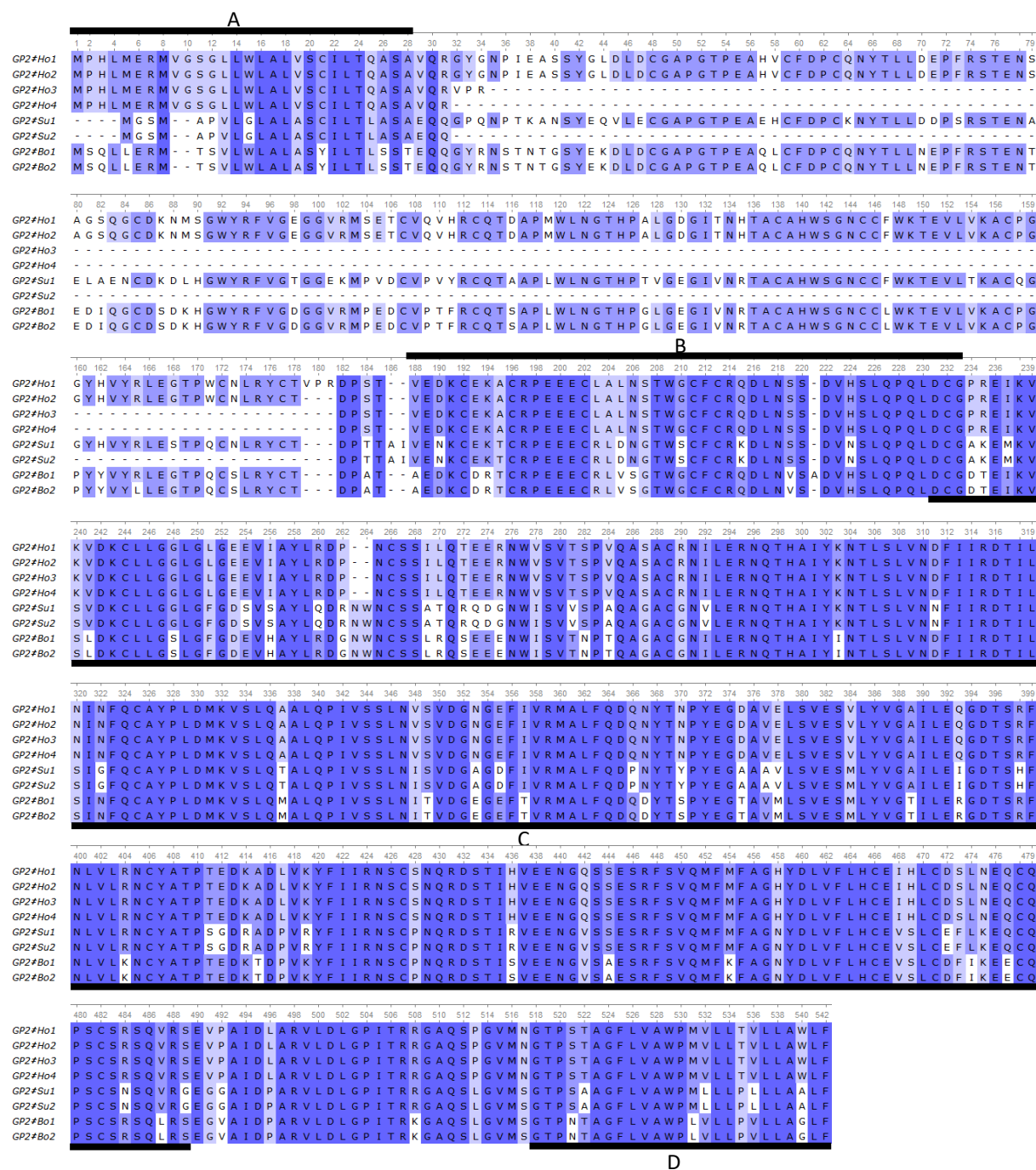
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28 **Key words**

29 major zymogen granule membrane glycoprotein 2 (GP2), *Escherichia coli*, FimH, receptor,
30 adhesion, intestinal cell lines



32

33 Figure S1. GP2 amino acid sequences from humans, pigs and cattle. The human and porcine

34 sequences can be found in the GenBank under the following numbers GP2#Ho1:

35 NP001007241, GP2#Ho2: NP001493, GP2#Ho3: NP001007242, GP2#Ho4: NP001007243,

36 GP2#Su1: KU665995 and GP2#Su2: KU665994. Particularly high matches occur in the

marked areas. A: N-terminal signal peptide, B: EGF-like domain, C: ZP domain, D: C-terminal GPI anchor.

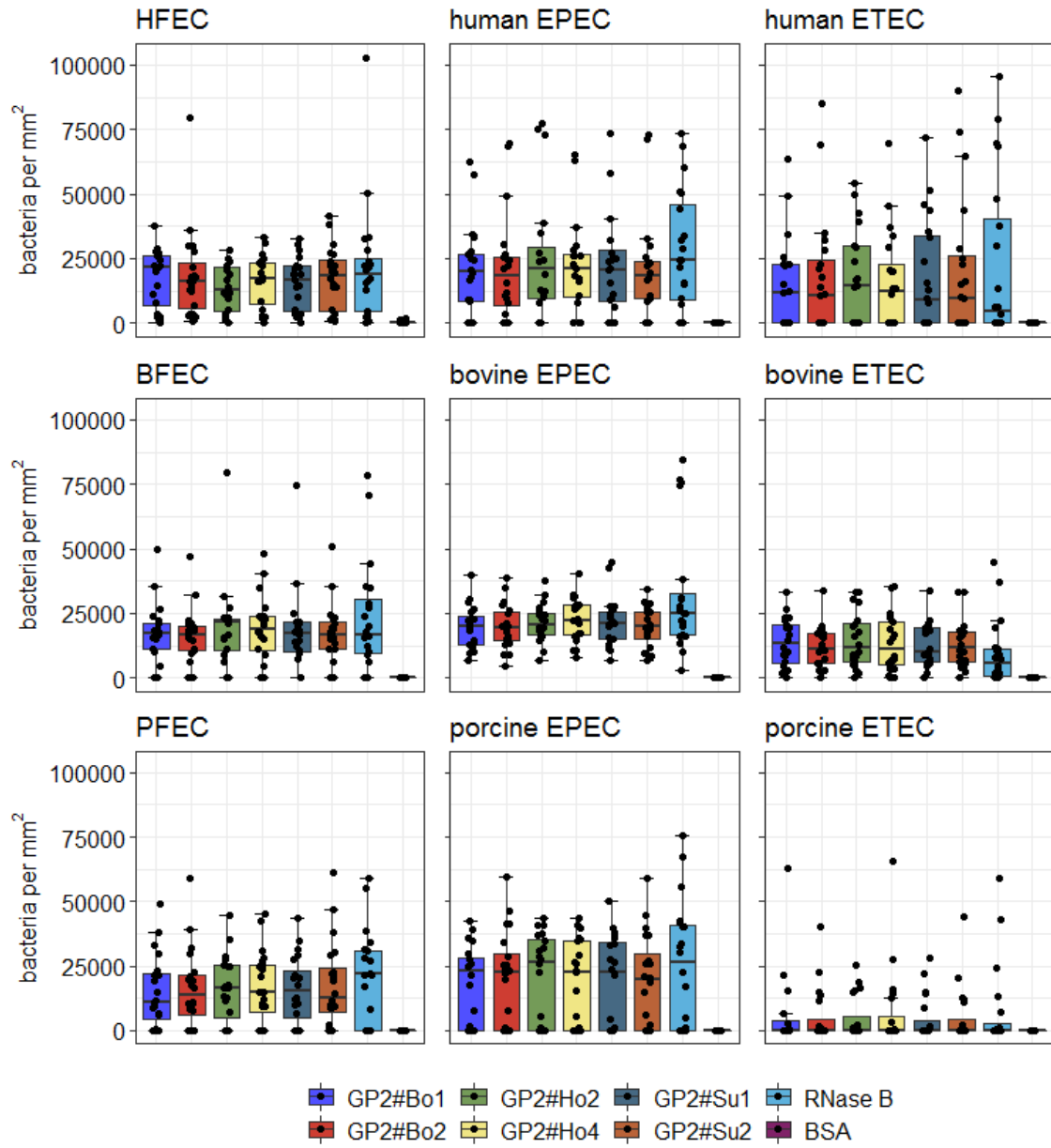


Figure S2. Binding of different *E. coli* pathotypes of human, bovine, and porcine origin to six GP2 isoforms, RNase B and BSA (n=20). RNase B was used as standard positive control glycoprotein, while BSA was used as negative control protein. The experiment was performed three times in duplicates. GP2#Bo1: bovine GP2 isoform 1, GP2#Bo2: bovine GP2 isoform 2,

GP2#Ho2: human GP2 isoform 2, GP2#Ho4: human GP2 isoform 4, GP2#Su1: porcine GP2
isoform 1, GP2#Su2: porcine GP2 isoform 2.

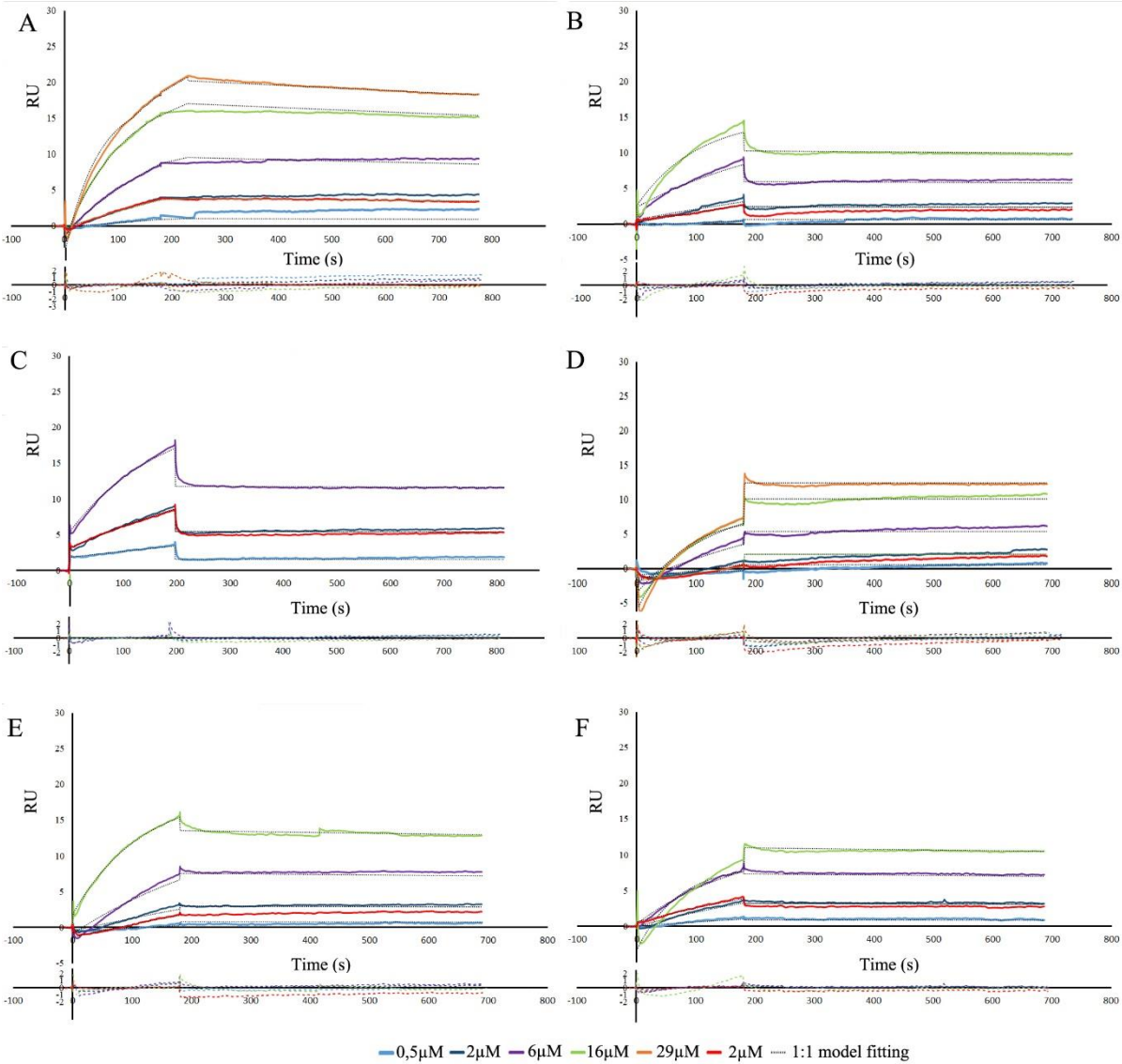
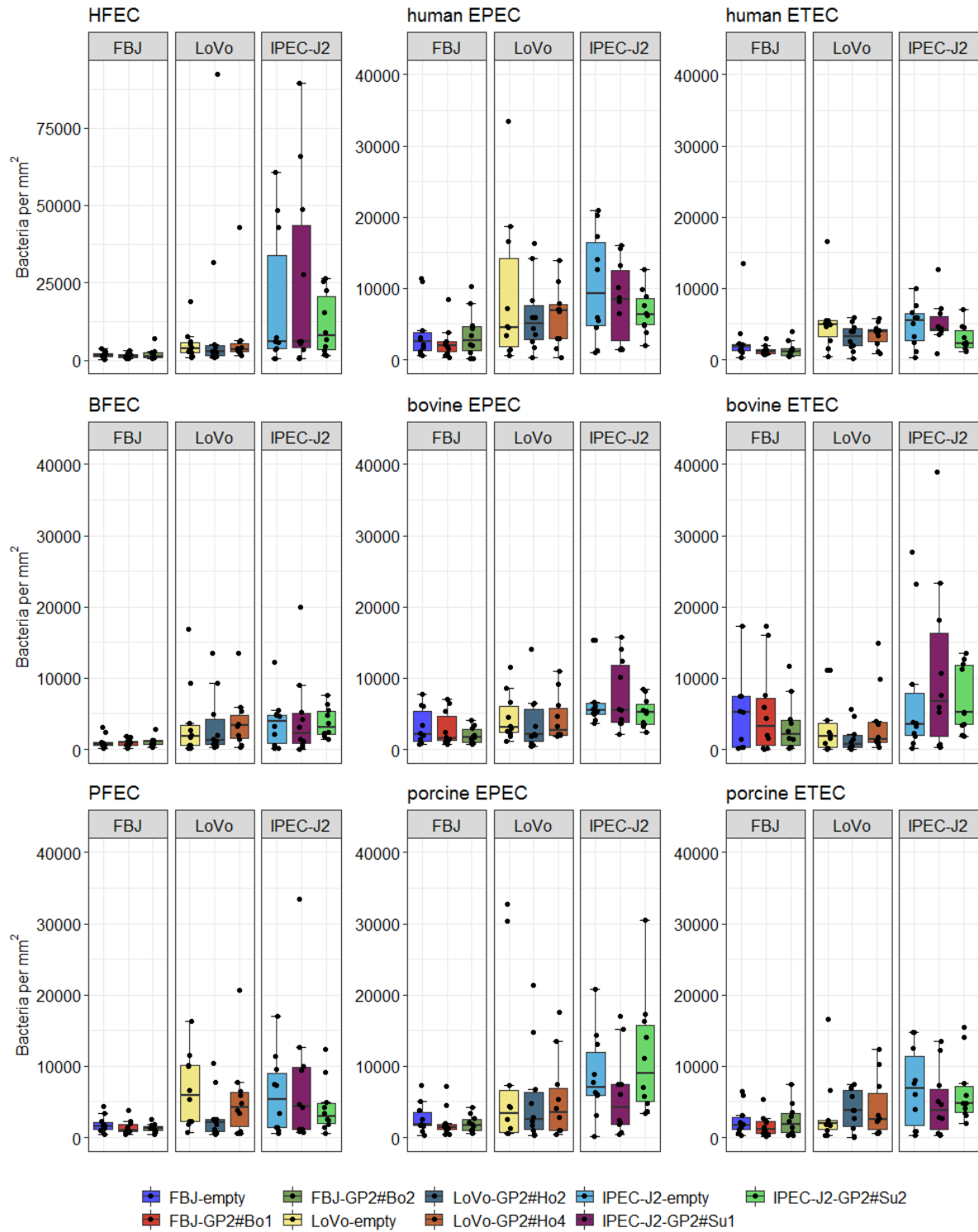


Figure S3. Interaction of various GP2 isoforms (A: GP2#Bo1; B: GP2#Bo2; C: GP2#Ho2; D: GP2#Ho4; E: GP2#Su1; F: GP2#Su2) with immobilized FimH variant 9 analysed with an SPR. Lines represent different concentrations of the analyte (0.5, 2.0, 6.0, 16, 29 μM) in PBS-P+ buffer. Binding data were collected at a flow rate of 30 μl/minutes. Below each sensorgram residual plots are presented, defining differences between experimental data and fitted kinetic model.



55

56 Figure S4. Adhesion of *E. coli* from different pathogeny and origin to cell lines (n = 10).

57 HFEC: human commensal *E. coli*, BFEC: bovine commensal *E. coli*, PFEC: porcine

58 commensal *E. coli*, FBJ/LoVo/IPEC-J2-empty: no GP2 expression on FBJ/LoVo/IPEC-J2

59 cell lines, FBJ-GP2#Bo1/Bo2: bovine GP2 isoform 1/2 expressed on FBJ cell line, LoVo-

GP2#Ho2/Ho4: human GP2 isoform 2/4 expressed on LoVo cell line, IPEC-J2-

GP2#Su1/Su2: porcine GP2 isoform ½ expressed on IPEC-J2 cell line.

Table S1. *E. coli* isolate name (number), pathotype, host origin, FimH variant type, and binding capability to six different GP2 isoforms

- ... no binding, o ... low binding, + ... medium binding, ++ ... high binding

Number	Pathotype	Host origin	FimH variant	Binding to GP2 isoforms					
				GP2 #Bo1	GP2 #Bo2	GP2 #Ho2	GP2 #Ho4	GP2 #Su1	GP2 #Su2
91	commensal	porcine	1	+	+	+	+	+	+
5547	commensal	human	1	o	o	o	o	o	o
5682	ETEC	human	1	-	-	-	-	-	-
5689	ETEC	human	1	-	-	-	-	-	-
5690	ETEC	human	1	-	-	-	-	-	-
5691	ETEC	human	1	-	-	-	-	-	-
5998	EPEC	porcine	1	o	-	-	o	-	o
6586	commensal	bovine	1	+	+	+	+	+	+
6595	commensal	bovine	1	-	-	-	-	-	-
7501	EPEC	bovine	1	+	+	+	+	+	+
7503	EPEC	bovine	1	+	+	+	+	+	+
7506	EPEC	bovine	1	+	+	+	+	+	+
7507	EPEC	bovine	1	+	+	+	+	+	+
7509	EPEC	bovine	1	+	+	+	+	+	+
7514	EPEC	bovine	1	+	+	+	+	+	+
7516	EPEC	bovine	1	o	o	+	+	+	o
7528	EPEC	bovine	1	+	+	+	+	+	o
7551	ETEC	Porcine	1	-	-	-	-	-	-
122	commensal	porcine	2	-	-	-	-	-	-
136	commensal	porcine	2	+	+	+	+	+	+
4297	commensal	porcine	2	+	+	+	+	+	+
4318	commensal	porcine	2	+	+	+	+	+	+
4320	commensal	porcine	2	+	+	+	+	+	+
4401	commensal	porcine	2	+	+	+	++	+	++
4445	commensal	porcine	2	+	+	+	+	+	o
4732	commensal	porcine	2	+	+	+	+	+	+

5200	ETEC	human	2	+	+	+	+	++	+
5201	ETEC	human	2	+	+	++	+	++	++
5204	ETEC	human	2	+	+	+	+	++	++
5205	ETEC	human	2	+	++	+	++	+	++
5263	EPEC	human	2	++	++	++	++	++	++
5268	EPEC	human	2	++	++	++	++	++	++
5281	EPEC	human	2	o	o	o	+	o	+
5282	EPEC	human	2	+	o	+	+	o	o
5285	EPEC	human	2	o	o	+	+	o	+
5289	EPEC	human	2	+	+	+	+	+	+
5527	commensal	human	2	+	+	+	+	+	+
5529	commensal	human	2	+	+	+	+	+	+
5536	commensal	human	2	+	+	+	+	+	+
5543	commensal	human	2	+	+	+	+	+	+
5631	EPEC	human	2	+	+	+	+	+	+
5637	ETEC	human	2	++	++	++	++	++	++
5638	ETEC	human	2	-	-	-	-	-	-
5652	EPEC	human	2	-	-	-	-	-	-
5655	EPEC	human	2	-	-	-	-	-	-
5680	ETEC	human	2	++	+	++	+	+	+
5692	ETEC	human	2	-	-	-	-	-	-
5981	EPEC	porcine	2	+	++	+	+	++	++
5982	EPEC	porcine	2	+	+	++	+	+	+
5983	EPEC	porcine	2	+	+	+	+	+	+
5984	EPEC	porcine	2	+	++	+	+	+	++
5985	EPEC	porcine	2	o	o	o	o	o	o
5988	EPEC	porcine	2	+	+	++	++	+	+
5989	EPEC	porcine	2	-	-	-	-	-	-
5990	EPEC	porcine	2	+	+	+	+	+	+
5991	EPEC	porcine	2	+	+	+	+	+	+
5992	EPEC	porcine	2	-	-	-	-	-	-
5996	EPEC	porcine	2	-	-	-	-	-	-
5997	EPEC	porcine	2	+	+	+	+	+	+
5999	EPEC	porcine	2	+	+	+	+	+	+
6573	commensal	bovine	2	o	o	o	o	o	o
6576	commensal	bovine	2	++	++	++	++	++	++
6583	commensal	bovine	2	+	+	o	+	+	+
6587	commensal	bovine	2	+	+	+	++	+	+
6588	commensal	bovine	2	+	+	+	+	+	+

6589	commensal	bovine	2	+	+	+	+	+	+
6590	commensal	bovine	2	+	+	+	+	+	+
6592	commensal	bovine	2	+	+	+	+	+	+
6593	commensal	bovine	2	+	+	+	+	+	+
6594	commensal	bovine	2	+	+	+	+	+	+
6597	commensal	bovine	2	+	+	+	+	+	+
6602	commensal	bovine	2	+	+	+	+	+	+
6604	commensal	bovine	2	+	+	+	+	+	+
7500	EPEC	bovine	2	+	+	+	+	++	+
7505	EPEC	bovine	2	+	+	+	+	+	+
7510	EPEC	bovine	2	+	+	+	++	++	+
7524	EPEC	bovine	2	+	+	+	+	+	+
7527	EPEC	bovine	2	+	o	+	+	+	o
7533	EPEC	bovine	2	+	+	+	+	+	+
7546	ETEC	porcine	2	++	+	+	++	+	+
7549	ETEC	porcine	2	-	-	-	-	-	-
7550	ETEC	porcine	2	o	+	+	+	o	+
7554	ETEC	porcine	2	+	+	+	+	+	+
148	commensal	porcine	3	o	o	+	+	o	o
4298	commensal	porcine	3	o	+	+	+	+	+
5206	ETEC	human	3	+	+	+	+	o	+
5288	ETEC	human	3	+	+	+	+	+	+
5532	commensal	human	3	o	o	o	o	o	o
4136	commensal	porcine	4	+	+	+	+	+	+
4374	commensal	porcine	4	++	++	++	++	++	++
5211	EPEC	human	4	o	+	+	o	+	o
5267	EPEC	human	4	+	+	+	+	+	+
5993	EPEC	porcine	4	-	-	-	-	-	-
6584	commensal	bovine	4	-	-	-	-	-	-
7535	ETEC	porcine	4	-	-	o	-	o	-
7553	ETEC	porcine	4	+	+	+	+	+	+
4305	commensal	porcine	5	-	-	-	-	-	-
5202	ETEC	human	5	-	-	-	-	-	-
4310	commensal	porcine	6	-	-	-	-	-	o
4418	commensal	porcine	6	+	+	+	+	+	+
5980	EPEC	porcine	6	-	-	-	-	-	-
6596	commensal	bovine	6	+	+	+	+	+	+
7552	ETEC	porcine	6	o	o	o	o	o	o
5207	EPEC	human	7	+	++	++	+	++	+

5283	EPEC	human	7	-	-	-	-	-	-
5284	EPEC	human	7	-	-	-	-	-	-
5995	EPEC	porcine	7	+	+	+	+	+	+
7519	EPEC	bovine	7	+	+	+	+	+	+
5208	EPEC	human	8	+	+	+	+	+	+
5209	EPEC	human	8	+	+	+	+	++	+
5540	commensal	human	8	+	+	o	+	o	+
5542	commensal	human	8	o	o	o	o	o	o
5545	commensal	human	8	+	+	+	+	+	+
5549	commensal	human	8	+	+	+	+	+	+
5553	commensal	human	8	+	+	+	+	+	+
5554	commensal	human	8	+	+	+	+	+	+
5651	EPEC	human	8	+	+	+	+	+	+
5654	EPEC	human	8	+	+	+	+	+	+
5287	ETEC	human	9	+	+	+	+	+	+
5695	ETEC	human	9	+	+	+	+	+	o
5696	ETEC	human	9	+	+	+	+	o	o
7496	ETEC	bovine	9	o	+	o	o	o	o
7504	ETEC	bovine	9	o	o	o	-	o	o
7508	ETEC	bovine	9	+	+	+	+	+	+
7511	ETEC	bovine	9	o	+	o	o	o	+
7512	ETEC	bovine	9	-	-	-	-	-	-
7515	ETEC	bovine	9	o	o	o	o	o	o
7517	ETEC	bovine	9	+	+	+	+	+	+
7518	ETEC	bovine	9	o	o	o	-	+	o
7520	ETEC	bovine	9	+	+	o	+	+	+
7521	ETEC	bovine	9	+	o	o	o	o	+
7522	ETEC	bovine	9	+	+	+	+	+	+
7523	ETEC	bovine	9	o	o	o	o	o	o
7525	ETEC	bovine	9	+	+	+	+	o	+
7526	ETEC	bovine	9	o	o	+	o	o	o
7529	ETEC	bovine	9	+	+	+	+	+	o
7534	ETEC	bovine	9	+	+	+	+	+	+
7543	ETEC	porcine	9	-	-	-	-	-	-
5538	commensal	human	10	o	o	o	o	o	o
5986	EPEC	porcine	11	+	++	++	+	+	+
5987	EPEC	porcine	11	++	++	+	++	+	+
6580	commensal	bovine	12	+	o	+	o	o	+
6598	commensal	bovine	12	-	-	-	-	-	-

7498	EPEC	bovine	13	+	+	+	+	+	+
7502	EPEC	bovine	13	o	o	+	o	o	o
7530	EPEC	bovine	13	+	+	+	+	+	+
7531	EPEC	bovine	13	+	+	+	+	+	+
7499	EPEC	bovine	14	+	+	+	+	+	+
7513	EPEC	bovine	14	o	o	o	o	o	o
7532	EPEC	bovine	14	+	+	+	+	+	+
7555	EPEC	bovine	15	+	+	+	+	+	+
5539	commensal	human	16	+	+	+	+	+	+
7536	EPEC	porcine	17	+	++	+	+	+	++
7497	EPEC	bovine	18	+	+	+	+	+	+
5541	commensal	human	19	+	+	+	+	+	+
5537	commensal	human	20	+	+	+	+	+	+
5531	commensal	human	21	+	++	+	+	+	++
5212	EPEC	human	22	+	+	+	+	+	+
5210	EPEC	human	23	+	+	+	+	+	+
4781	commensal	porcine	24	-	-	-	-	-	-
6605	commensal	bovine	25	+	+	+	+	+	+
5994	EPEC	porcine	26	-	-	-	-	-	-
5548	commensal	human	27	+	+	+	+	+	+
5528	commensal	human	28	-	-	-	-	-	-
4315	commensal	porcine	29	o	+	+	o	+	+
4127	commensal	porcine	30	-	-	-	-	-	-
4444	commensal	porcine	-	o	o	o	o	o	o
5203	EPEC	human	-	-	-	-	-	-	-
5551	commensal	human	-	o	o	o	o	o	o
5686	EPEC	human	-	-	-	-	-	-	-
7537	EPEC	porcine	-	-	-	-	-	-	-
7538	EPEC	porcine	-	-	-	-	-	-	-
7539	EPEC	porcine	-	-	-	-	-	-	-
7540	EPEC	porcine	-	-	-	-	-	-	-
7541	EPEC	porcine	-	-	-	-	-	-	-
7542	EPEC	porcine	-	-	-	-	-	-	-
7544	EPEC	porcine	-	-	-	-	-	-	-
7545	EPEC	porcine	-	-	-	-	-	-	-
7547	EPEC	porcine	-	-	-	-	-	-	-
7548	EPEC	porcine	-	-	-	-	-	-	-

68 Table S2. Relationship between FimH Variant and *fimH* alleles. FimH Variant number was
69 assigned in the course of this study based on amino acid substitutions in FimH precursor (full
70 mature FimH with leader peptide). A unique FimH Variant can be product of multiple *fimH*
71 alleles, with the latter's nomenclature derived from CH typer
72 (<https://cge.cbs.dtu.dk/services/chtyper/>) and Enterobase Database
73 (<http://enterobase.warwick.ac.uk/species/ecoli>) and used for prediction of a sequence type
74 (ST, see Table S2).

FimH Variant	<i>fimH</i> allele(s)
1	54
2	25, 31, 32, 35, 38, 61, 65, 82, 86, 87, 97, 289, 305, 331, 1566, 1569, 1570
3	27, 1567
4	24
5	23
6	54
7	90, 677
8	2, 9, 14, 88, 115
9	54
10	41
11	30
12	54
13	237
14	440
15	319
16	76
17	158
18	446
19	1568
20	22
21	56
22	57
23	12
24	1259
25	69
26	555
29	398
30	1183

76 Table S3. Association of *fimH* alleles found in this study with *E. coli* clonal groups and
77 lineages. This association was determined by analyzing available MLST and CH typing data
78 from multiple databases (unpublished data).

<i>fimH</i> allele	Most prevalent ST	Phylogroup
2	ST127	B2
9	ST73	B2
12	ST73	B2
14	ST14	B2
22	ST131	B2
23	CC10 (multiple ST)	A
24	promiscuous: CC10 (ST43, ST34, ST752 etc.), CC88 (ST410 etc.), CC58 (ST443 etc.)	A, C, B1
25	promiscuous: CC58 (ST17 etc.), ST655	B1, C
27	promiscuous: CC10 (ST10 etc.), ST69, ST95, ST405, ST648	A, D, B2, D, F
30	ST131; less frequent: ST73 (like Nissle1917), ST95, CC10 (ST34 etc.)	B2, B2, B2, A
31	promiscuous among B1 STs: CC58 (ST145, ST40), ST1196, ST453, ST278	B1
32	CC58 (ST58, ST155, ST55, ST162, ST442)	B1
35	ST23, ST448, ST75, ST359	C, B1, A, B1
38	ST158, ST297	B1
41	promiscuous: ST131, ST95, CC10 (ST10, ST48 etc.), ST59	B2, B2, A, F
54	CC10 (ST10, ST48 etc.)	A
56	ST405	D
57	CC1283 (ST15, ST1283)	B2
61	ST224	B1
65	ST38	D
69	ST216	A
76	ST998	B2
82	ST11	E
86	ST16	B1
87	ST196	B1
88	ST1092	B2
90	CC28 (ST28, ST121)	B2
97	ST117	F
115	CC625 (ST969, ST625)	B2
158	CC910 (ST910, ST2952)	C
237	promiscuous: ST2368, ST120 etc.	A, B1
289	promiscuous among B1 STs: ST86, ST711, ST5378 etc.	B1
305	ST607	A
319	ST337	B1
331	ST32	E
398	ST542	A
400	promiscuous among A STs: ST48, ST10, ST4247 etc.	A
440	ST21	B1

446	ST33	B1
555	ST206	B1
677	ST3053	B2
1135	data not available	.
1183	ST7875	unassigned
1252	ST5395	B1
1259	data not available	.
1566	data not available	.
1567	data not available	.
1568	data not available	.
1569	data not available	.
1570	data not available	.