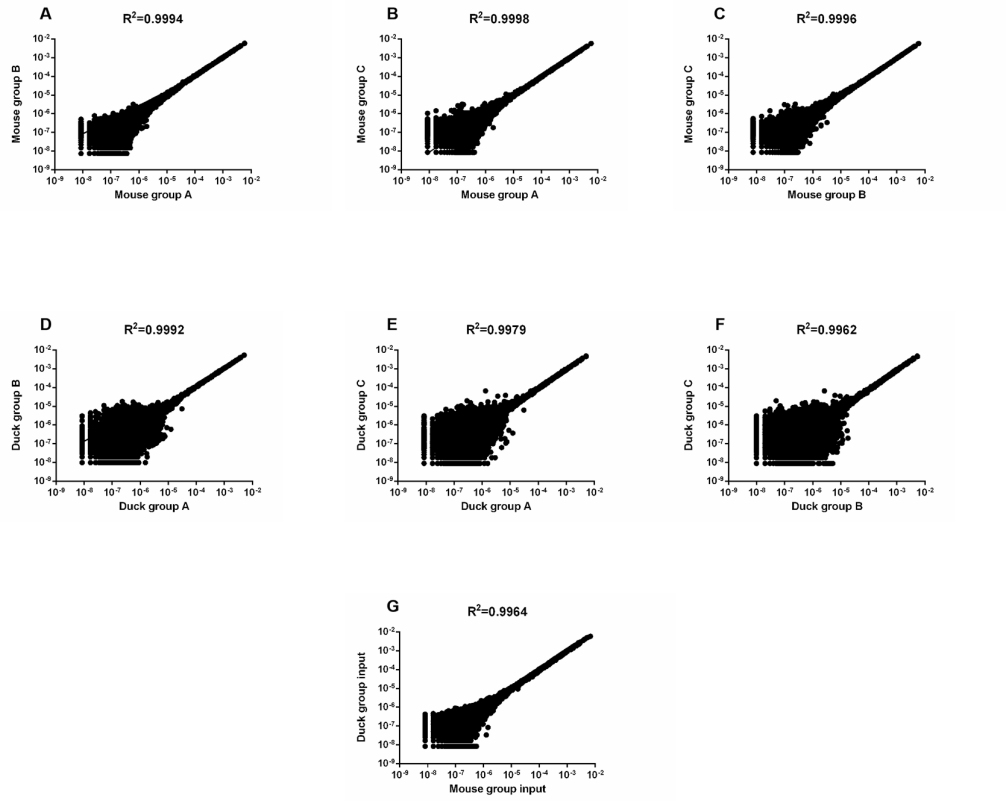

Figure S1. Correlation among biological replicates of the TraDIS experiments. The frequency of each mutant in the TraDIS library is shown for the input samples between two models and the three biological replicates of the output samples. The non-parametric Spearman correlation coefficient (R) is shown in each case.

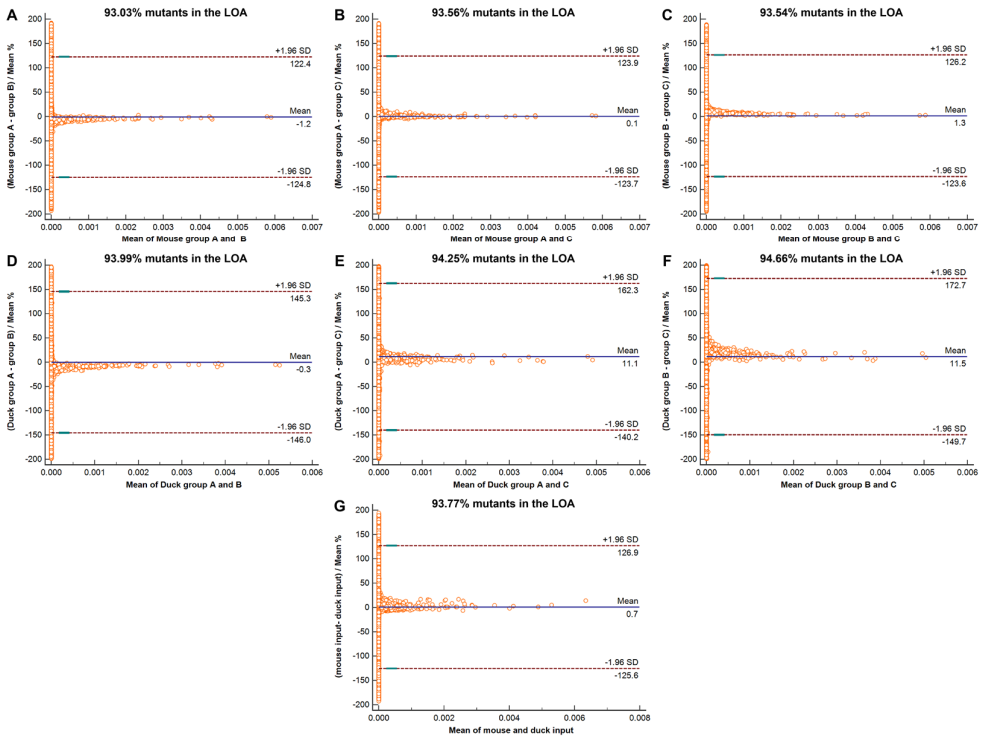

Figure S2. Agreement analysis among biological replicates of the TraDIS analyses.

The frequency of each mutant in the TraDIS library is shown for the input samples between two models and the three biological replicates of the output samples. The limits of agreement (LOA) is shown in each case.

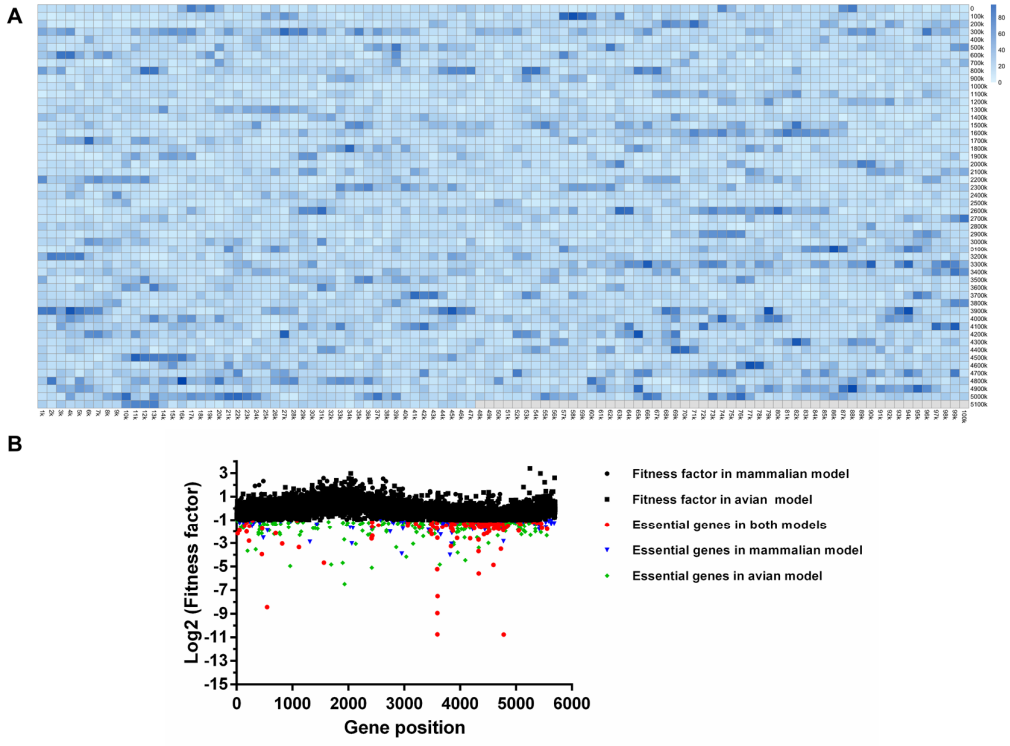

Figure S3. Overview of the ExPEC XM insertion sites and essential genes. (A) The heat map shows the distribution of the insertion sites. (B) Color-coding data dots (genes) by their essentiality.

Table S3 Bacterial strains and plasmids

Strain	Description	Source
ExPEC		
ExPEC XM	Bacteremia isolate, wild-type	(1)
APEC O1	Bacteremia isolate, wild-type	(2)
APEC DE471	Bacteremia isolate, wild-type	This study
CFT 073	Bacteremia isolate, wild-type	(3)
RS 218	Bacteremia isolate, wild-type	(4)
NMEC 38	Bacteremia isolate, wild-type	(5)
rml	ExPEC XM (Δ*CXG97_RS12075 - CXG97_RS12130)*	This study
neu	ExPEC XM Δ*neuSECABD (CXG97_RS17935-CXG97_RS17960)*	This study
mprA	ExPEC XM Δ*mprA (CXG97_RS16045)*	This study
nhaA	ExPEC XM Δ*nhaA (CXG97_RS00095)*	This study
yga	ExPEC XM Δ*ygaYZH (CXG97_RS16030 - CXG97_RS16040)*	This study
sanA	ExPEC XM Δ*sanA (CXG97_RS12690)*	This study
T6SS2	ExPEC XM (Δ*CXG97_RS01185-CXG97_RS01265)*	This study
rnf	ExPEC XM Δ*rnfABCDEG-nth (CXG97_RS09505 - CXG97_RS09535)*	This study
bio	ExPEC XM Δ*bioABCDF (CXG97_RS04640 - CXG97_RS04660)*	This study
cre	ExPEC XM Δ*creABCD (CXG97_RS26720 - CXG97_RS26735)*	This study
Plasmid		
pUTmini-Tn5km2	Constructing TraDIS library	(6)

pKD46	Lambda red recombineering	(7)
pKD3	Template for cat	(7)
pKD4	Template for kan	(7)
pCP20	Eliminating antibiotic resistance gene	(7)
pGEN-MCS	Vector for complementation	(8)

Table S4 Oligonucleotide primers used in this study

Primer	Sequence
TraDIS-F	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGCACTTG TGTATAAGAGTCAG
TraDIS-R	CAAGCAGAAGACGGCATACGAG
⊿rml-F	AAGCAGCGTTACGTGATCTTTCCCTGCTATATAAGGTCAATTATATTATTGTTAATGCGTGTGTAG GCTGGAGCTGCTTCGA
⊿rml-R	TTAATCAAATCTCGAGCAGTCTATTTCACAGTATGCTCTCTGGCTATATGGAATAAAAAACATATG AATATCCTCCTTAG
rml-F	CAGCGTTACGTGATCTTTCC
rml-R	ACGGCAGTGAAGATTCGTAG
⊿neu-F	GGAACACAACAAACTGCCAACATAATATATATTAAATTTCAAGTCAATATCTTTTGAATTGTGTAG GCTGGAGCTGCTTCGA
⊿neu-R	AAGACCTATAGTGGTTACATTCCAATATTATGCCTTGGAAATATTTAACTGAGACATATCCATATG AATATCCTCCTTAG
neu-F	GTGAGCGCGTCATTTATGTG
neu-R	TTGGCTGGTGGATTTCAAGG
⊿mprA-F	ATTTACTTTATTTATCACTGTCGTTACTATATCGGCTGAAATTAATGAGGTCATACCCAAGTGTAG GCTGGAGCTGCTTCGA
⊿mprA-R	TGCGATGCTGGCCAGTCATTTTTTCTTTTATAAATCTGGATTTTTGAGCGAGATGACGCGCATATG AATATCCTCCTTAG
mprA-F	ACACGCAGCATTATCATCCC
mprA-R	GCGCTCATATTGTTCTCCAC

⊿nhaA-F	CGATGATTCGTGCGGGGTAAAATCGTGAAAACGATCTATTCACCTGAAGAGAAATAAAAAGTGT AGGCTGGAGCTGCTTCGA
⊿nhaA-R	TTTCTCTCCCTGATAACAATGAAAAGGGAGCCGTTTATGGCTCCCCAGTACATCGTCCTGCATAT GAATATCCTCCTTAG
nhaA-F	TCTCGCTGATGGCGCAATTC
nhaA-R	CTGCCCGGTAATGGTTTGTG
⊿yga-F	ACATCCGCTATTATTGATTTCCAGCTTAATCATCACCTGATGAACAAAAATAATGACTAAGTGTAG GCTGGAGCTGCTTCGA
⊿yga-R	ATAGTAACGACAGTGATAAATAAAGTAAATGTATTGTTTTAGAAAAATGATTCTTGTGGGCATATG AATATCCTCCTTAG
yga-F	GCTGGCGCGTCTTATCATAC
yga-R	TGCAAAGACGGGTCAGAAGG
⊿sanA-F	TGCGCAAAACCAGCGGGTAAAGTAGCCTGATGGAAATTTTCCTTAGATCGAGTCTCCTGCGTGT AGGCTGGAGCTGCTTCGA
⊿sanA-R	AACAAAATATCGGGATAAGGAAAAAAACGGCAACGACAAAAAACTGCTGTACATCCATAACAT ATGAATATCCTCCTTAG
sanA-F	AACCAGCGGGTAAAGTAGCC
sanA-R	TTCGCTCGCCAGACATACAC
⊿rnf-F	CTGTTGTCGCCTGCTCTGGATTAACGGATAATAGGCGGCTTTTTTATTTCAGGCCGAAAAGTGTA GGCTGGAGCTGCTTCGA
⊿rnf-R	ATGTTTTAAAAGAGGATAAAGAAAGGTTATGAATGGGGTAATCGGTGTTACCCCTGATCTCATAT GAATATCCTCCTTAG
rnf-F	GTCGCCTGCTCTGGATTAAC
rnf-R	GCCACAGCATCGTGATCTTG

△*cre*-F	CAATATGTTATTTACCGTGACGAACTAATTGCTCGTGTAATAGATAAAAATGGTAACGATGTGTAG GCTGGAGCTGCTTCGA
△*cre*-R	ATCGGCTTTACCACCGTCAATAAAAACGGCGCTTTTTAGCGCCGTTTTTATTTTTCTACCCATATG AATATCCTCCTTAG
cre-F	AACAACGAGCACCTGACATC
cre-R	GGTGAAGGTTATCGCTTCTG
△*bio*-F	ATGAACCCTCCTTTCTTGTTTGCAGAAAGTGTAGCCAGAAACCCTCACGCTGACTTCCCGGTGT AGGCTGGAGCTGCTTCGA
△*bio*-R	GTTAAATTGCAGTCAATCGGAGACGCGATCTCGCTCACAATTTAACCAAGCACAGGATGACATA TGAATATCCTCCTTAG
bio-F	GAAACCCTCACGCTGACTTC
bio-R	CCTGGGCAATTCCCACATTC
△*T6SS2*-F	TAATGTTAAATTGCCTTTTTAAAATATAACAATAATGCAGATGAAAGACTCCCTGGTAACGTGTA GGCTGGAGCTGCTTCGA
△*T6SS2*-R	CACAAATGGTGATTCACAGGCGTATAAAGCAAATACAATCACCATGTTTTATATCCTGCACATATG AATATCCTCCTTAG
T6SS2-F	CAGCCTTGATGTGGCAGAAC
T6SS2-R	GGGCATGAGCACTACCTGTC
C△mprA-F	CCGGAATTCGTTTCGTGCCCACACTGGTC
C△mprA-R	CGCGGATCCCGCGTTAGCTCATCGCTTCG
C△nhaA-F	CCGGAATTCTGTCAAAGAGCGCGGTGTGG
C△nhaA-R	CGCGGATCCTCGTCCTGTCAAACTGATGG
pGEN-F	GGCACTTGCTCACGCTCTG

pGEN-R	GTGGTCACGCTTTTCGTTGG

1. Ma J, Bao Y, Sun M, Dong W, Pan Z, Zhang W, Lu C, Yao H. 2014. Two functional type VI secretion systems in avian pathogenic Escherichia coli are involved in different pathogenic pathways. Infect Immun doi:https://doi.org/10.1128/iai.01769-14:IAI.
2. Johnson TJ, Kariyawasam S, Wannemuehler Y, Mangiamele P, Johnson SJ, Doetkott C, Skyberg JA, Lynne AM, Johnson JR, Nolan LK. 2007. The genome sequence of avian pathogenic Escherichia coli strain O1: K1: H7 shares strong similarities with human extraintestinal pathogenic E. coli genomes. J Bacteriol 189:3228-3236 doi:https://doi.org/10.1128/jb.00537-07.
3. Mobley H, Green D, Trifillis A, Johnson D, Chippendale G, Lockatell C, Jones B, Warren J. 1990. Pyelonephritogenic Escherichia coli and killing of cultured human renal proximal tubular epithelial cells: role of hemolysin in some strains. Infect Immun 58:1281-1289
4. Teng C-H, Cai M, Shin S, Xie Y, Kim K-J, Khan NA, Di Cello F, Kim KS. 2005. Escherichia coli K1 RS218 interacts with human brain microvascular endothelial cells via type 1 fimbria bacteria in the fimbriated state. Infect Immun 73:2923-2931 doi:https://doi.org/10.1128/iai.73.5.2923-2931.2005.
5. Tivendale KA, Logue CM, Kariyawasam S, Jordan D, Hussein A, Li G, Wannemuehler Y, Nolan LK. 2010. Avian-pathogenic Escherichia coli strains are similar to neonatal meningitis E. coli strains and are able to cause meningitis in the rat model of human disease. Infect Immun 78:3412-3419 doi:https://doi.org/10.1128/iai.00347-10.

6. Li G, Laturnus C, Ewers C, Wieler LH. 2005. Identification of genes required for avian Escherichia coli septicemia by signature-tagged mutagenesis. Infect Immun 73:2818-2827 doi: https://doi.org/10.1128/iai.73.5.2818-2827.2005

7. Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proceedings of the National Academy of Sciences 97:6640-6645 doi:https://doi.org/10.1073/pnas.120163297.

8. Jiang F, An C, Bao Y, Zhao X, Jernigan RL, Lithio A, Nettleton D, Li L, Wurtele ES, Nolan LK. 2015. ArcA Controls Metabolism, Chemotaxis and Motility Contributing to the Pathogenicity of Avian Pathogenic E. coli. Infect Immun doi:https://doi.org/10.1128/iai.00312-15:IAI.