Supplementary material

Figure S1. Alignment of the sequences of *Edwardsiella tarda* Ivy homologues. Dots denote gaps introduced for maximum matching. Numbers in brackets indicate overall sequence identities between CsEBI3 and the compared sequences. The consensus residues are in black; the residues that are ≥75% identical among the aligned sequences are in grey. The three residues that were subjected to mutagenesis analysis are boxed. The GenBank accession numbers of the aligned sequences are as follows: *Edwardsiella ictaluri*, ACR68365.1; *Serratia marcescens*, YP_007345734.1; *Serratia proteamaculans*, YP_001479800.1; *Aeromonas hydrophila*, YP_857437.1; *Yersinia pestis*, WP_002227858; *Yersinia pseudotuberculosis*, YP_001873342.1; *Yersinia enterocolitica*, YP_006007815.1; *Burkholderia pseudomallei*, YP_002895840.1; *Escherichia coli*, BAL37520.1.

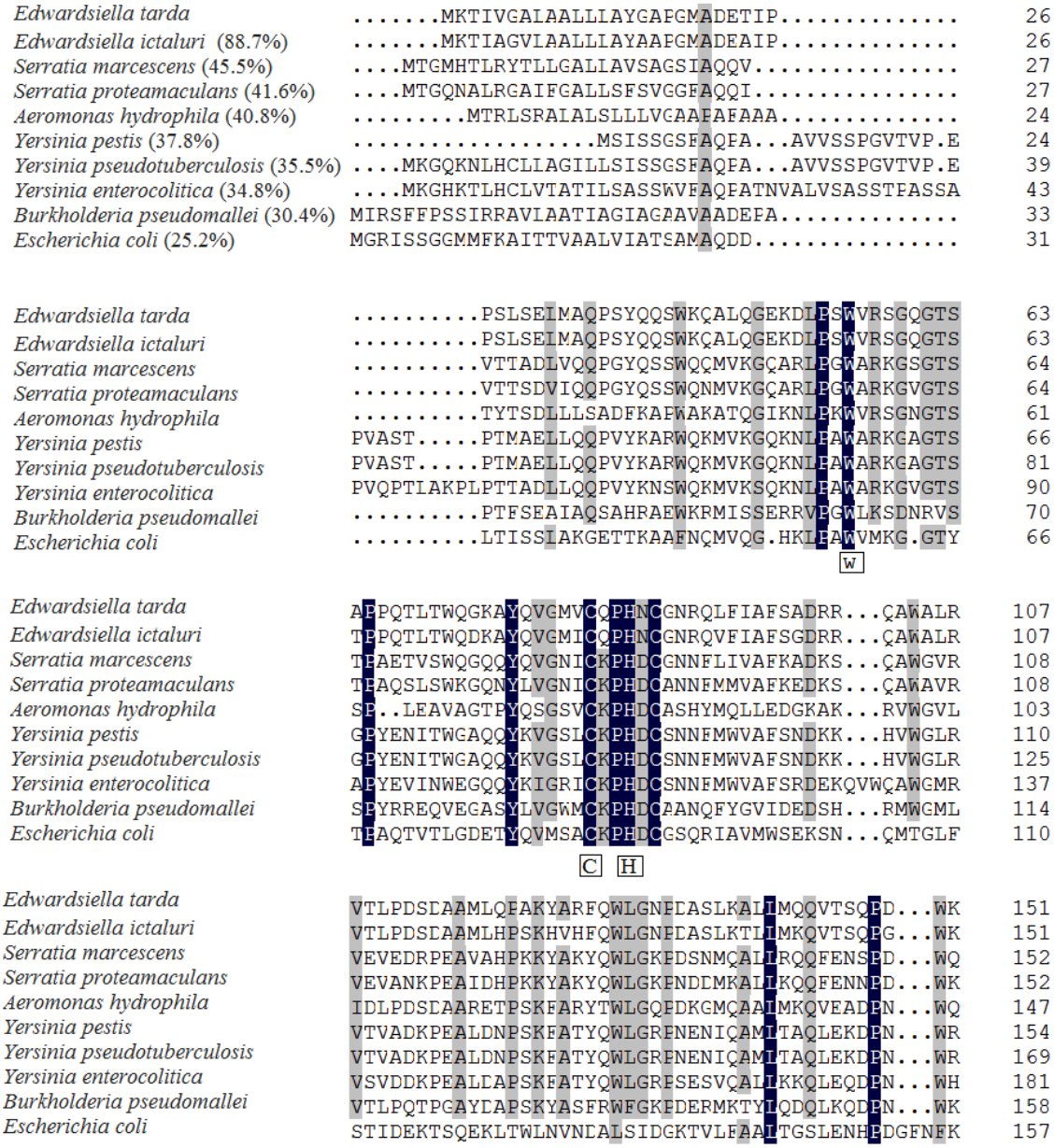
Edwardsiella tarda .....MKTIVGALAALLIAYGAPGMADETIP..... 26
Edwardsiella ictaluri (88.7%) .....MKTIAGVLAALLIAYAAPGMADEAIP..... 26
Serratia marcescens (45.5%) ....MTGMHTLRYTLLGALLAVSAGSIAQQV..... 27
Serratia proteamaculans (41.6%) ....MTGQNALRGAIFGALLSFSVGGFAQQI..... 27
Aeromonas hydrophila (40.8%) .....MTRLSRALALSLLLVGAAFAFAAA..... 24
Yersinia pestis (37.8%) .....MSISSGSFAQFA...AVVSSPGVTVP.E 24
Yersinia pseudotuberculosis (35.5%) ....MKGQKNLHCLIAGILLSISSGSFAQFA...AVVSSPGVTVP.E 39
Yersinia enterocolitica (34.8%) ....MKGHKTLHCLVTATILSASSWVFAQFATNVALVSASSTFASSA 43
Burkholderia pseudomallei (30.4%) MIRSFFPSSIRRAVLAATIAGIAGAAVAADEFA..... 33
Escherichia coli (25.2%) MGRISSGGMMFKAITTVAALVIATSAMAQDD..... 31
Edwardsiella tarda .....PSLSEIMAQPSYQQSWKQALQGEKDLPSWVRSGQGTS 63
Edwardsiella ictaluri .....PSLSEIMAQPSYQQSWKQALQGEKDLPSWVRSGQGTS 63
Serratia marcescens .....VTTADLVQQPGYQSSWQQMVKGQARLPGWARKGSGTS 64
Serratia proteamaculans .....VTTSDVIQQPGYQSSWQNMVKGQARLPGWARKGVGTS 64
Aeromonas hydrophila .....TYTSDLLLSADFKAPWAKATQGIKNLPKWVRSGNGTS 61
Yersinia pestis PVAST.....PTMAELLQQPVYKARWQKMVKGQKNLPAWARKGAGTS 66
Yersinia pseudotuberculosis PVAST.....PTMAELLQQPVYKARWQKMVKGQKNLPAWARKGAGTS 81
Yersinia enterocolitica PVQPTLAKPLPTTADLLQQPVYKNSWQKMVKSQKNLPAWARKGVGTS 90
Burkholderia pseudomallei .....PTFSEAIAQSAHRAEWKRMISSERRVPGWLKSDNRVS 70
Escherichia coli .....LTISSLAKGETTKAAFNQMVQG.HKLPAWVMKG.GTY 66
W
Edwardsiella tarda APPQTLTWQGKAYQVGMVCQPHNCGNRQLFIAFSADRR...QAWALR 107
Edwardsiella ictaluri TPPQTLTWQDKAYQVGMICQPHNCGNRQVFIAFSGDRR...QAWALR 107
Serratia marcescens TPAETVSWQGQQYQVGNICKPHDCGNNFLIVAFKADKS...QAWGVR 108
Serratia proteamaculans TPAQSLSWKGQNYLVGNICKPHDCANNFMMVAFKEDKS...QAWAVR 108
Aeromonas hydrophila SP..LEAVAGTPYQSGSVCKPHDCASHYMQLLEDGKAK...RVWGVL 103
Yersinia pestis GPYENITWGAQQYKVGSLCKPHDCSNNFMWVAFSNDKK...HVWGLR 110
Yersinia pseudotuberculosis GPYENITWGAQQYKVGSLCKPHDCSNNFMWVAFSNDKK...HVWGLR 125
Yersinia enterocolitica APYEVINWEGQQYKIGRICKPHDCSNNFMWVAFSRDEKQVWQAWGMR 137
Burkholderia pseudomallei SPYRREQVEGASYLVGWMCKPHDCAANQFYGVIDEDSH...RMWGML 114
Escherichia coli TPAQTVTLGDETYQVMSACKPHDCGSQRIAVMWSEKSN...QMTGLF 110
C H
Edwardsiella tarda VTLPDSDAAMLQPAKYARFQWLGNPDASLKALLMQQVTSQPD...WK 151
Edwardsiella ictaluri VTLPDSDAAMLHPSKHVHFQWLGNPDASLKTLLMKQVTSQPG...WK 151
Serratia marcescens VEVEDRPEAVAHPKKYAKYQWLGKPDSNMQALLRQQFENSPD...WQ 152
Serratia proteamaculans VEVANKPEAIDHPKKYAKYQWLGKPNDDMKALLKQQFENNPD...WK 152
Aeromonas hydrophila IDLPDSDAARETPSKFARYTWLGQPDKGMQAALMKQVEADPN...WQ 147
Yersinia pestis VTVADKPEALDNPSKFATYQWLGRPNENIQAMLTAQLEKDPN...WR 154
Yersinia pseudotuberculosis VTVADKPEALDNPSKFATYQWLGRPNENIQAMLTAQLEKDPN...WR 169
Yersinia enterocolitica VSVDDKPEALDAPSKFATYQWLGRPSESVQALLKKQLEQDPN...WH 181
Burkholderia pseudomallei VTLPQTPGAYDAPSKYASFRWFGKPDERMKTYLQDQLKQDPN...WK 158
Escherichia coli STIDEKTSQEKLTWLNVNDALSIDGKTVLFAALTGSLENHPDGFNFK 157

Figure S2. SDS-PAGE analysis of rIvy and its mutants. Purified rIvy, rIvyW55A, rIvyC82S, and rIvyH85D (lanes 2 to 5 respectively) were analyzed by SDS-PAGE and viewed after staining with Coomassie blue. Lane 1, protein markers.

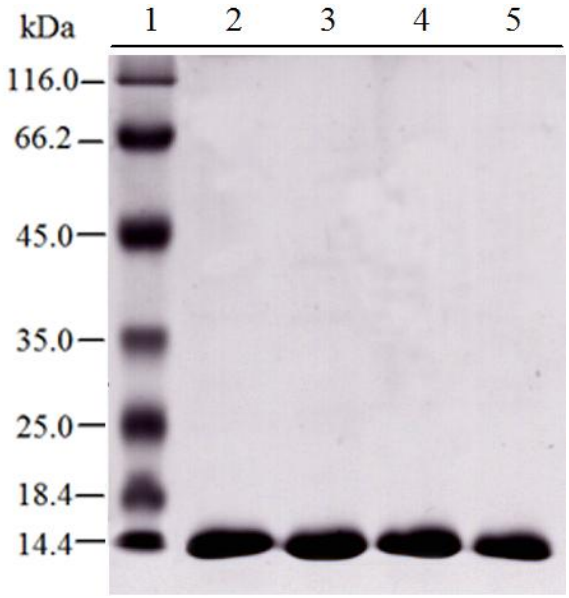

Table S1. Data of one representative experimental repeat of Figure 6.

Experimental Repeat 2

Blood (CFU/ml)

		TX01	TXivy	TXivy+rIvy	TXivy+rIvyC82S
Fish 1	plate 1	7670	680	5880	1220
	plate 2	6430	480	7120	1890
	plate 3	5630	920	7600	900
	Mean	6576.667	693.3333	6866.666667	1336.666667
Fish 2	plate 1	2770	390	6340	2990
	plate 2	3760	110	3210	4210
	plate 3	4170	540	6740	400
	Mean	3566.667	346.6667	5430	2533.333333
Fish 3	plate 1	3830	890	3210	1320
	plate 2	5110	1470	2070	890
	plate 3	3430	1350	1190	1150
	Mean	4123.333	1236.667	2156.666667	1120
Fish 4	plate 1	780	750	11200	230
	plate 2	1220	620	8810	100
	plate 3	1790	1050	10600	250
	Mean	1263.333	806.6667	10203.33333	193.3333333
Fish 5	plate 1	12300	670	6570	540
	plate 2	10800	350	8200	280
	plate 3	9810	690	8740	150
	Mean	10970	570	7836.666667	323.3333333
Mean of five fish		5300	730.6667	6498.666667	1101.333333

Kidney (CFU/g)

		TX01	TXivy	TXivy+rIvy	TXivy+rIvyC82S
Fish 1	plate 1	24439.66	7826.087	21864.95177	9142.857143
	plate 2	21077.59	5296.443	23183.27974	13500
	plate 3	34482.76	7391.304	25401.92926	8142.857143
	Mean	26666.67	6837.945	23483.38692	10261.90476
Fish 2	plate 1	15411.26	12403.43	31448.76325	4885.496183
	plate 2	20216.45	5751.073	15229.68198	8435.114504

Fish 2	plate 3	20519.48	8626.609	18409.89399	3129.770992
	Mean	18715.73	8927.039	21696.11307	5483.46056
Fish 3	plate 1	20156.25	7877.358	24782.6087	2977.777778
	plate 2	21927.08	5801.887	26576.08696	2488.888889
	plate 3	10885.42	3396.226	54891.30435	5822.222222
	Mean	17656.25	5691.824	35416.66667	3762.962963
Fish 4	plate 1	27966.8	7959.184	15357.14286	6326.530612
	plate 2	34066.39	10255.1	17103.1746	10728.86297
	plate 3	35020.75	2755.102	17936.50794	9154.51895
	Mean	32351.31	6989.796	16798.9418	8736.637512
Fish 5	plate 1	19334.81	11551.72	48015.87302	14662.37942
	plate 2	15232.82	6250	35317.46032	19967.84566
	plate 3	30177.38	13965.52	38809.52381	20257.23473
	Mean	21581.67	10589.08	40714.28571	18295.81994
Mean of five fish		23394.33	7807.137	27621.87884	9308.157147

Liver (CFU/g)

		TX01	**TXivy**	**TXivy+rIvy**	**TXivy+rIvyC82S**
Fish 1	plate 1	5966.736	611.8547	8069.105691	2300.319489
	plate 2	6569.647	898.6616	9491.869919	2843.450479
	plate 3	9771.31	1051.625	8048.780488	750.798722
	Mean	7435.897	854.0472	8536.585366	1964.85623
Fish 2	plate 1	4005.525	804.1958	5525.81262	4107.883817
	plate 2	4917.127	472.028	3193.116635	2116.182573
	plate 3	4033.149	734.2657	4990.439771	3132.780083
	Mean	4318.6	670.1632	4569.789675	3118.948824
Fish 3	plate 1	5536.398	1482.301	4620.253165	2675.276753
	plate 2	4904.215	929.2035	2215.189873	1642.066421
	plate 3	5536.398	1725.664	7468.35443	2011.070111
	Mean	5325.67	1379.056	4767.932489	2109.471095
Fish 4	plate 1	7973.485	1423.895	3121.495327	1070.28754
	plate 2	4640.152	916.5303	1495.327103	1421.72524
	plate 3	8522.727	1423.895	2485.981308	1102.236422
	Mean	7045.455	1254.774	2367.601246	1198.083067

	plate 1	7987.013	5214.844	12248.62888	499.2199688
	plate 2	10584.42	6093.75	16819.0128	234.0093604
	plate 3	9069.264	1718.75	12248.62888	265.2106084
Fish 5	Mean	9213.564	4342.448	13772.09019	332.8133125
Mean of five fish		6667.837	1700.098	6802.799793	1744.834506

Spleen (CFU/g)

		TX01	**TXivy**	**TXivy+rIvy**	**TXivy+rIvyC82S**
	plate 1	69146.34	19565.22	95357.14286	51780.82192
Fish 1	plate 2	52560.98	44347.83	111071.4286	13972.60274
	plate 3	125365.9	9565.217	166785.7143	30547.94521
	Mean	82357.72	24492.75	124404.7619	32100.45662
	plate 1	103939.4	47142.86	202058.8235	42678.57143
Fish 2	plate 2	56666.67	15952.38	270588.2353	30535.71429
	plate 3	43030.3	30714.29	164411.7647	43214.28571
	Mean	67878.79	31269.84	212352.9412	38809.52381
	plate 1	211250	50952.38	119423.0769	45211.26761
Fish 3	plate 2	145312.5	42380.95	87692.30769	56478.87324
	plate 3	180312.5	26984.13	148076.9231	22112.67606
	Mean	178958.3	40105.82	118397.4359	41267.60563
	plate 1	89807.69	26046.51	193442.623	25769.23077
Fish 4	plate 2	100192.3	38837.21	255737.7049	36346.15385
	plate 3	99423.08	23720.93	87704.91803	27500
	Mean	96474.36	29534.88	178961.7486	29871.79487
	plate 1	249253.7	31964.29	76666.66667	71111.11111
Fish 5	plate 2	344776.1	46964.29	106666.6667	49444.44444
	plate 3	262238.8	12857.14	89375	49166.66667
	Mean	285422.9	30595.24	90902.77778	56574.07407
Mean of five fish		142218.4	31199.71	145003.9331	39724.691