

**Table S3.** Genes of lineage 4 with average  $dn/ds$  values ( $\omega$ )  $\geq 0.25$  and/or site-specific positive selection detected by CodeML (model M2a vs. model M1a). Genes are ordered according to  $\omega$ . Genes with  $\omega < 0.25$ , but with sites under positive selection are indicated by gray color. *virB* T4SS, *bep*, and *trw* T4SS genes are indicated by orange color. Orthologous genes present in genomes of other lineages are shown. Likelihood-ratio-test (LRT) results of the CodeML selection analysis are given for comparison of model M2a vs. M1a and model M8 vs. M7.

Gene	Product	Length (aa) <sup>d</sup>	Orthologs in other genomes <sup>a</sup>				$\omega$	CodeML results <sup>b</sup>	
			<i>Brucella abortus</i>	<i>B. bacilliformis</i>	<i>B. schoenbuchensis</i>	<i>B. clarridgeiae</i>		M1a vs M2a	M7 vs M8
BQ12460	conserved protein of unknown function	203	no	no	no	no	0.9 ±0.08	6.18*	7.35*
BQ04750	conserved protein of unknown function	134	no	no	no	BARCL_1038	0.84 ±0.09	4.26	5.16
BQ03120	conserved protein of unknown function	135	BruAb1_0406	BARBAKC583_0375	BARSC_20121	BARCL_1059	0.57 ±0.08	2.13	3.60
BQ10680	VirB T4SS effector protein, BepF	555	no	no	no	BARCL_0069 <sup>c</sup>	0.51 ±0.02	7.26*	18.84**
BQ01050	conserved protein of unknown function	83	no	BARBAKC583_1287	BARSC_10492	BARCL_0125	0.5 ±0.24	0.00	0.00
BQ09660	conserved protein of unknown function	228	no	no	no	BARCL_0279	0.49 ±0.11	1.72	3.54
BQ12470	Trw T4SS protein, TrwN	189	no	no	no	no	0.48 ±0.1	13.35**	16.79**
BQ11430	conserved protein of unknown function	62	no	BARBAKC583_0195	BARSC_10207	BARCL_1199	0.46 ±0.18	2.47	3.88
BQ03700	conserved exported protein of unknown function	111	no	no	no	no	0.45 ±0.1	0.00	0.07
BQ12560	Trw T4SS protein, TrwL5	102	no	no	no	no	0.43 ±0.18	7.42*	8.76*
BQ13300	conserved exported protein of unknown function	129	no	no	no	no	0.41 ±0.13	1.20	2.10
BQ04170	TonB protein	248	BruAb1_0622	BARBAKC583_0461	BARSC_20204	BARCL_0416	0.41 ±0.1	10.25**	15.14**
BQ12930	Chorismate mutase	110	BruAb1_1804	no	BARSC_10329	BARCL_1321	0.4 ±0.09	2.55	3.34
BQ11180	conserved protein of unknown function	83	BruAb1_0275	BARBAKC583_0213	no	BARCL_1184	0.4 ±0.18	2.45	4.83
BQ10970	conserved protein of unknown function	67	no	BARBAKC583_0251	BARSC_10148	BARCL_1160	0.4 ±0.16	0.00	0.00
BQ09320	conserved exported protein of unknown function	99	BruAb1_1503	BARBAKC583_0985	no	BARCL_0336	0.39 ±0.37	0.00	0.00
BQ12610	Trw T4SS protein, TrwH1	47	no	no	no	no	0.37 ±0.09	5.66	6.75*
BQ04650	conserved exported protein of unknown function	87	no	BARBAKC583_0511	BARSC_20256	no	0.37 ±0.14	0.00	0.12
BQ12600	Trw T4SS protein, TrwL1	281	no	no	no	no	0.36 ±0.05	0.00	0.00
BQ11050	conserved protein of unknown function	148	BruAb1_1640	BARBAKC583_0248	BARSC_10151	BARCL_1164	0.35 ±0.13	0.17	0.63
BQ06010	conserved protein of unknown function	103	no	no	BARSC_40129	BARCL_0768	0.35 ±0.14	1.27	0.00
BQ11480	conserved protein of unknown function	290	no	BARBAKC583_0188	BARSC_10210	BARCL_1202	0.35 ±0.08	6.81*	12.38**
BQ00140	conserved exported protein of unknown function	213	BruAb1_0151	BARBAKC583_1370	BARSC_10400	BARCL_0015	0.35 ±0.05	0.15	1.95
BQ03410	conserved exported protein of unknown function	205	BruAb1_1507	BARBAKC583_0987	BARSC_40414	no	0.35 ±0.16	0.00	3.56
BQ09010	conserved protein of unknown function	176	no	no	no	BARCL_1013	0.35 ±0.09	2.67	3.54
BQ07710	conserved protein of unknown function	819	no	BARBAKC583_0748	BARSC_40172	BARCL_0670	0.35 ±0.05	11.50**	16.37**
BQ01360	conserved protein of unknown function	73	no	no	no	no	0.34 ±0.13	0.00	0.29
BQ10580	VirB T4SS protein, VirB7	100	no	no	no	BARCL_0075 <sup>c</sup>	0.34 ±0.08	1.13	2.54
BQ10660	VirB T4SS effector protein, BepE	414	no	no	no	BARCL_0069 <sup>c</sup>	0.33 ±0.07	0.00	0.87
BQ06720	conserved exported protein of unknown function	109	no	no	no	no	0.33 ±0.08	0.00	1.27
BQ11620	conserved protein of unknown function	70	no	BARCK0786	no	no	0.32 ±0.06	0.00	0.00
BQ11600	conserved exported protein of unknown function	74	no	BARBAKC583_1056 <sup>c</sup>	BARSC_20032 <sup>c</sup>	no	0.32 ±0.19	0.89	2.96
BQ08690	conserved protein of unknown function	60	BruAb1_1401	BARBAKC583_0931	BARSC_40323	BARCL_0978	0.32 ±0.27	5.19	5.20
BQ01620	conserved exported protein of unknown function	353	BruAb2_0057	no	no	no	0.32 ±0.05	0.00	0.00
BQ10630	VirB T4SS effector protein, BepA	272	no	no	no	BARCL_0069 <sup>c</sup>	0.32 ±0.09	0.00	2.47

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			<i>Brucella abortus</i>	<i>B. bacilliformis</i>	<i>B. schoenbuchensis</i>	<i>B. clarridgeiae</i>		M1a vs M2a	M7 vs M8
BQ02210	conserved exported protein of unknown function	134	no	no	no	no	0.31 ±0.08	4.14	7.18*
BQ12190	conserved protein of unknown function	93	no	no	BARSC_10278	BARCL_1261	0.31 ±0.14	0.00	0.56
BQ11040	conserved protein of unknown function	114	no	no	no	BARCL_1163	0.31 ±0.08	0.00	0.00
BQ11100	conserved protein of unknown function	231	no	no	BARSC_10167	BARCL_1175	0.31 ±0.07	4.52	9.25**
BQ13000	conserved protein of unknown function	389	BruAb1_1862	BARBAKC583_0063	BARSC_10335	BARCL_1327	0.31 ±0.06	1.57	4.80
BQ13100	Thiol 3-disulfide interchange protein	226	BruAb1_1956	BARBAKC583_0053	BARSC_10347	BARCL_1329	0.3 ±0.06	0.13	0.70
BQ10410	conserved exported protein of unknown function	792	no	BARBAKC583_1132 <sup>c</sup>	BARSC_10091	BARCL_0476 <sup>c</sup>	0.3 ±0.07	0.00	14.87**
BQ05360	conserved protein of unknown function	584	no	no	BARSC_20378	BARCL_0831	0.3 ±0.06	0.00	0.95
BQ12260	ATP synthase delta chain	194	BruAb1_1782	BARBAKC583_0111	BARSC_10285	BARCL_1274	0.3 ±0.07	0.13	1.18
BQ05570	conserved protein of unknown function	164	no	BARBAKC583_0647	BARSC_20400	BARCL_0810	0.3 ±0.13	22.22**	25.47**
BQ07410	conserved protein of unknown function	157	no	BARBAKC583_0854	BARSC_40141	BARCL_0699	0.29 ±0.05	5.81	13.24**
BQ03690	conserved exported protein of unknown function	118	no	no	no	no	0.29 ±0.08	1.17	1.65
BQ04440	colicin V production protein	189	BruAb1_0470	BARBAKC583_0487	BARSC_20233	BARCL_0541	0.29 ±0.04	0.22	2.08
BQ10530	VirB T4SS protein, VirB2	104	no	no	no	BARCL_0080 <sup>c</sup>	0.29 ±0.09	0.00	0.00
BQ02150	ribosomal-protein-alanine acetyltransferase	160	BruAb1_2125	BARBAKC583_1242	BARSC_10535	BARCL_0171	0.29 ±0.07	0.00	0.00
BQ13290	conserved exported protein of unknown function	127	BruAb1_0462	BARBAKC583_0036	BARSC_10358	BARCL_1361	0.28 ±0.06	1.23	3.51
BQ10840	orotidine 5'-phosphate decarboxylase	280	no	no	no	no	0.28 ±0.27	0.00	2.83
BQ01520	filament-A precursor	424	BruAb1_1817	BARBAKC583_0323	BARSC_20069	BARCL_1107	0.28 ±0.04	0.00	0.65
BQ08360	conserved protein of unknown function	184	BruAb1_0955	BARBAKC583_0653	BARSC_40292	BARCL_0949	0.28 ±0.03	5.59	7.19*
BQ10650	VirB T4SS effector protein, BepC	532	no	no	no	BARCL_0069 <sup>c</sup>	0.28 ±0.02	0.00	5.30
BQ03680	conserved exported protein of unknown function	117	no	no	no	no	0.28 ±0.05	0.00	2.18
BQ00120	conserved exported protein of unknown function	89	BruAb1_0148	BARBAKC583_1372	BARSC_10398	BARCL_0013	0.28 ±0.11	2.10	4.03
BQ05220	lysophospholipase L2	300	BruAb2_0046	no	no	BARCL_0847	0.28 ±0.08	0.00	1.49
BQ13310	Na <sup>+</sup> /H <sup>+</sup> antiporter	133	BruAb2_0717	BARBAKC583_0035	BARSC_10359	no	0.27 ±0.05	0.85	2.19
BQ12110	conserved exported protein of unknown function	480	BruAb1_1746	BARBAKC583_0127	BARSC_10269	BARCL_1252	0.27 ±0.05	0.09	2.72
BQ03350	conserved protein of unknown function	85	BruAb1_1516	BARBAKC583_0993	BARSC_40420	BARCL_0327	0.27 ±0.13	3.11	4.24
BQ03940	Cytochrome c maturation protein E, Heme chaperone ccmE	159	BruAb1_0627	BARBAKC583_0431	BARSC_20177	BARCL_0388	0.27 ±0.06	0.00	0.00
BQ07900	conserved exported protein of unknown function	181	no	BARBAKC583_0732	BARSC_40244	no	0.27 ±0.06	0.00	0.28
BQ02450	conserved exported protein of unknown function	160	BruAb2_0198	no	BARSC_10563	BARCL_0200	0.27 ±0.05	1.81	3.65
BQ02710	predicted membrane protein	212	BruAb1_1363	BARBAKC583_1194	BARSC_10008	BARCL_0216	0.27 ±0.06	1.08	2.68
BQ05460	conserved protein of unknown function	183	BruAb1_0790	BARBAKC583_0636	BARSC_20388	BARCL_0821	0.27 ±0.08	0.00	0.02
BQ04740	sec-independent protein translocase	103	BruAb1_0894	no	no	BARCL_1039	0.27 ±0.07	0.54	1.59
BQ03900	conserved protein of unknown function	77	BruAb1_0622	BARBAKC583_0427	BARSC_20172	BARCL_0383	0.26 ±0.12	0.00	0.00
BQ11060	Phage tail protein	260	no	BARBAKC583_0238	BARSC_10163	BARCL_1171	0.26 ±0.04	0.00	0.00
BQ10290	autotransporter	1753	no	no	no	no	0.26 ±0.08	202.48**	254.68**
BQ12220	ATP synthase epsilon chain	138	BruAb1_1778	BARBAKC583_0115	BARSC_10281	BARCL_1270	0.26 ±0.07	0.00	0.70
BQ12590	Trw T4SS protein, TrwJ1	240	no	no	no	no	0.26 ±0.05	13.80**	20.03**
BQ10850	conserved membrane protein of unknown function	357	no	no	no	no	0.26 ±0.06	0.00	1.24
BQ00960	conserved membrane protein of unknown function	163	BruAb1_2048	BARBAKC583_1295	BARSC_10481	BARCL_0115	0.26 ±0.05	1.63	4.89
BQ11450	conserved protein of unknown function	129	no	BARBAKC583_0193	BARSC_10208	BARCL_1200	0.26 ±0.1	0.00	2.28
BQ02420	Hemin binding protein, HbpA	267	BruAb1_0115 <sup>c</sup>	BARBAKC583_1214 <sup>c</sup>	BARSC_10560 <sup>c</sup>	BARCL_0196 <sup>c</sup>	0.25 ±0.03	19.27**	24.17**
BQ12970	conserved protein of unknown function	327	no	no	no	no	0.25 ±0.05	0.00	0.00
BQ10280	autotransporter	799	no	BARBAKC583_1109	BARSC_10011	BARCL_1020	0.25 ±0.06	0.00	14.65**
BQ10380	Inducible Bartonella autotransporter	759	no	BARBAKC583_1132 <sup>c</sup>	BARSC_10092	BARCL_0475 <sup>c</sup>	0.25 ±0.04	0.00	0.00
BQ02810	conserved protein of unknown function	447	no	BARBAKC583_0286	BARSC_20039	BARCL_0226	0.25 ±0.03	0.16	2.96
BQ11830	TolA protein	381	BruAb1_1683	BARBAKC583_0145	BARSC_10253	BARCL_1236	0.25 ±0.03	0.00	5.10
BQ04010	Hemin binding protein, HbpD	270	BruAb1_0115 <sup>c</sup>	BARBAKC583_1214 <sup>c</sup>	BARSC_40297 <sup>c</sup>	BARCL_0196 <sup>c</sup>	0.25 ±0.05	17.49**	26.87**
BQ09930	conserved membrane protein of unknown function	361	no	no	BARSC_40486	BARCL_1127	0.25 ±0.06	0.00	0.00
BQ10560	VirB T4SS protein, VirB5	146	no	no	no	BARCL_0077 <sup>c</sup>	0.25 ±0.07	1.08	2.57
BQ02680	conserved exported protein of unknown function	106	no	no	no	BARCL_0220	0.25 ±	1.52	3.58
BQ08430	Hemin-binding protein E	204	no	no	BARSCv2_40484	BARCLv2_0197	0.23 ± 0.12	46.4**	53.52**
BQ01540	RNA pyrophosphohydrolase	173	BruAb1_1815	BARBAKC583_0325	BARSCv2_20071	BARCLv2_1105	0.2 ± 0.02	14.5**	16.8**

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			<i>Brucella abortus</i>	<i>B. bacilliformis</i>	<i>B. schoenbuchensis</i>	<i>B. clarridgeiae</i>		M1a vs M2a	M7 vs M8
BQ12670	TrwE protein	370	BruAb2_0060	no	no	no	0.19 ± 0.01	11.7**	16.6**
BQ03870	conserved protein of unknown function	179	BruAb1_0602	no	no	no	0.16 ± 0.05	6.8*	11.52**
BQ03470	4-hydroxybenzoate octaprenyltransferase	334	BruAb1_0438	BARBAKC583_0393	BARSCv2_20139	BARCLv2_0423	0.13 ± 0.02	6.97*	9.53**
BQ02550	Tetraacyldisaccharide 4'-kinase (Lipid A 4'-kinase)	339	BruAb2_0212	BARBAKC583_1203	BARSCv2_10573	BARCLv2_0210	0.11 ± 0.02	8.65*	22.41**
BQ11750	cell division protein FtsH	709	BruAb1_1676	BARBAKC583_0168	BARSCv2_10228	BARCLv2_1216	0.11 ± 0.01	8.45*	10.66**
BQ13340	multicomponent K <sup>+</sup> :H <sup>+</sup> antiporter subunit D	540	BruAb2_0720	BARBAKC583_0032	BARSCv2_10362	no	0.1 ± 0.01	7.37*	16.28**
BQ11850	tolQ protein	237	BruAb1_1685	BARBAKC583_0147	BARSCv2_10251	BARCLv2_1234	0.1 ± 0.03	9.38**	13.72**
BQ09260	aspartate aminotransferase A	400	BruAb1_1488	BARBAKC583_0979	BARSCv2_40405	BARCLv2_0344	0.09 ± 0.01	6.7*	9.56**
BQ13010	Probable O-sialoglycoprotein endopeptidase	364	BruAb1_1865	BARBAKC583_0062	BARSCv2_10336	BARCLv2_1338	0.08 ± 0.01	6.57*	13.09**
BQ10230	homoserine dehydrogenase	442	BruAb2_0263	BARBAKC583_1106	BARSCv2_40508	BARCLv2_0496	0.08 ± 0.02	6.67*	14.13**
BQ05680	NADH dehydrogenase I, E subunit	216	BruAb1_0820	BARBAKC583_0782	BARSCv2_40094	BARCLv2_0720	0.06 ± 0.03	30.19**	45.73**
BQ10090	Ferredoxin--NADP reductase (FNR) (Protein X)	252	BruAb1_0357	BARBAKC583_1092	BARSCv2_40496	BARCLv2_1141	0.03 ± 0.01	9.41**	16.38**

<sup>a</sup>for ortholog assignment the “Phylo Profile Synteny” tool of MaGe [58] was used (parameters: Bidirectional Blast Hit, 30 % identities, 0.6 for minLrap and maxLrap), automated ortholog assignments were manually curated.

<sup>b</sup>LRT statistics for model M2a vs. model M1a and model M8 vs. model M7, values indicating significant positive selection are depicted by asterisks (\*: p <0.05, \*\*: p <0.01).

<sup>c</sup>more than one orthologous gene is present in the corresponding genome, no clear ortholog assignment possible.

<sup>d</sup>aa means analyzed gene length in amino acids.