

## **Supplemental Information for:**

### **The two-component signaling system VraSR<sub>SS</sub> is critical for multidrug resistance and full virulence in *Streptococcus suis* serotype 2**

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**Supplemental Table S1. Calculations of LD<sub>50</sub> on the WT and  $\Delta$ vraSR<sub>SS</sub> strains for zebrafish**

Dose of challenge CFU	Number of death/total	
	ZY05719	$\Delta$ vraSR <sub>SS</sub>
$5.0 \times 10^7$	15/15	11/15
$5.0 \times 10^6$	13/15	5/15
$5.0 \times 10^5$	12/15	5/15
$5.0 \times 10^4$	1/15	3/15
LD <sub>50</sub>	$3.09 \times 10^5$ CFU	$6.84 \times 10^6$ CFU

**Supplemental Table S2. Differentially expressed genes in the  $\Delta$ *vraSR<sub>SS</sub>* strain versus WT strain**

Gene/locus name	Function or description	log2 Fold Change	p-value	q-value
<i>ZY05719_00865</i>	molecular chaperone GroES	-2.4719	6.86E-85	4.07E-83
<i>ZY05719_00870</i>	molecular chaperone GroEL	-1.997	2.82E-139	2.51E-137
<i>ZY05719_01205</i>	PTS system trehalose-specific transporter subunits IIBCA	-2.3568	0.017399	0.035302
<i>ZY05719_01910</i>	4-alpha-glucanotransferase	-2.8234	0.020566	0.040633
<i>ZY05719_01915</i>	maltose phosphorylase	-2.941	0.0083811	0.018798
<i>ZY05719_01925</i>	endonuclease	-3.2354	0.01209	0.025703
<i>ZY05719_01930</i>	PTS system glucose/maltose-specific transporter subunit IIBCA	-4.0443	0.0019246	0.0051754
<i>yvqF<sub>SS</sub></i>	membrane protein	-1.9191	2.38E-43	6.57E-42
<i>ZY05719_02090</i>	peptidylprolyl isomerase	-3.147	3.02E-258	3.48E-256
<i>ZY05719_02095</i>	RNA-binding protein	-3.1654	6.38E-117	5.21E-115
<i>ZY05719_02235</i>	PTS fructose transporter subunit IID	-1.9845	1.94E-05	7.94E-05
<i>cps2A</i>	LytR family transcriptional regulator	-2.4865	2.04E-64	8.33E-63
<i>cps2B</i>	capsular biosynthesis protein CpsC	-2.5359	1.71E-74	8.15E-73
<i>cps2C</i>	tyrosine protein kinase	-2.5519	4.79E-51	1.54E-49
<i>cps2D</i>	tyrosine protein phosphatase	-2.6023	2.69E-38	6.75E-37
<i>cps2E</i>	galactosyl transferase	-2.4008	5.48E-23	9.02E-22
<i>cps2F</i>	glycosyl transferase	-2.4365	1.76E-27	3.44E-26
<i>cps2G</i>	glycosyl transferase family 1	-2.4692	4.36E-22	6.83E-21
<i>cps2H</i>	glycosyl transferase	-2.451	1.49E-16	1.84E-15
<i>cps2I</i>	polymerase	-2.5556	8.07E-22	1.23E-20
<i>cps2J</i>	glycosyl transferase	-2.429	2.44E-25	4.46E-24
<i>cps2K</i>	glycosyl transferase	-2.4795	2.00E-67	8.71E-66
<i>ZY05719_02805</i>	membrane protein	-2.4584	2.47E-142	2.30E-140

ZY05719_02830	lipooligosaccharide sialyltransferase	-2.4233	1.97E-80	9.87E-79
ZY05719_02835	capsid assembly protein	-2.3994	1.19E-91	7.50E-90
<i>neuB</i>	N-acetyl neuramic acid synthetase NeuB	-2.1127	5.54E-101	3.87E-99
<i>neuC</i>	UDP-N-acetylglucosamine 2-epimerase	-2.122	6.34E-151	6.54E-149
<i>neuD</i>	NeuD protein	-2.153	3.10E-145	3.03E-143
<i>neuA</i>	acylneuraminate cytidyltransferase	-2.1608	3.41E-46	1.01E-44
ZY05719_02860	transposase	-2.1879	1.97E-35	4.53E-34
ZY05719_02865	transposase	-2.4367	3.19E-15	3.53E-14
ZY05719_02870	transposase	-1.7393	0.008582	0.019204
ZY05719_02880	transposase	-1.8402	0.0003175	0.0010377
ZY05719_03085	glucosamine-6-phosphate deaminase	-1.7509	2.80E-104	2.03E-102
ZY05719_03350	phosphopantothenate--cysteine ligase	-1.6782	1.40E-84	8.05E-83
ZY05719_03965	PTS fructose transporter subunit IIC	-2.0035	0.0004108	0.001308
ZY05719_04825	hypothetical protein	-3.7858	3.07E-274	3.76E-272
ZY05719_06720	iron transporter FeoA	-1.9556	1.24E-16	1.53E-15
ZY05719_07065	6-phospho-beta-glucosidase	-2.234	3.52E-12	3.25E-11
ZY05719_07070	PTS beta-glucoside transporter subunit IIABC	-2.3212	1.33E-19	1.86E-18
ZY05719_07075	transcription antiterminator BglG	-2.4408	7.75E-17	9.91E-16
ZY05719_07740	multidrug ABC transporter ATPase	-1.7944	1.37E-58	5.07E-57
ZY05719_09640	carbonate dehydratase	-1.7899	2.53E-99	1.71E-97
ZY05719_09685	membrane protein	-1.8771	4.12E-81	2.12E-79
ZY05719_09775	PTS system ascorbate-specific transporter subunit IIB	-1.7488	0.0004965	0.001543
ZY05719_10085	4-alpha-glucanotransferase	-2.1776	0.021202	0.041638
ZY05719_10090	sugar ABC transporter substrate-binding protein	-2.3805	0.0094868	0.020942
ZY05719_10095	sugar ABC transporter permease	-2.7956	0.0059016	0.013855
ZY05719_10100	sugar ABC transporter permease	-2.7684	0.0053412	0.012754

ZY05719_10105	maltodextrose utilization protein MalA	-1.9918	0.012738	0.026875
ZY05719_10110	LacI family transcriptional regulator	-1.9682	0.012214	0.025938
ZY05719_10115	alpha-amylase	-2.0705	0.0037	0.0092904
ZY05719_00300	amino acid transporter	2.0608	1.46E-06	7.50E-06
ZY05719_00310	membrane protein	2.1684	0.0017557	0.004768
ZY05719_00315	multidrug ABC transporter ATPase	1.7575	1.11E-05	4.83E-05
ZY05719_00320	membrane protein	1.8094	3.96E-05	0.0001543
ZY05719_00700	hypothetical protein	2.024	0.002147	0.0057117
ZY05719_00720	zinc-binding protein	1.7036	2.90E-84	1.62E-82
ZY05719_00770	competence protein CglA	1.8436	9.85E-05	0.0003598
ZY05719_01650	adhesion protein	2.4821	3.92E-60	1.51E-58
ZY05719_01655	histidine triad protein	3.4093	6.67E-93	4.35E-91
ZY05719_04345	integrase	2.42	6.15E-129	5.23E-127
ZY05719_04350	hypothetical protein	4.8682	2.21E-116	1.73E-114
ZY05719_04390	hypothetical protein	7.6572	1.61E-194	1.75E-192
ZY05719_04425	ATPase AAA	1.759	1.10E-26	2.14E-25
ZY05719_04440	hypothetical protein	3.9844	0.014971	0.030856
ZY05719_04445	DNA-binding protein	2.1871	0.024965	0.048064
ZY05719_04450	DNA-binding protein	1.6697	2.10E-13	2.20E-12
ZY05719_04460	conjugal transfer protein	2.2911	2.93E-05	0.0001161
ZY05719_04465	peptidase P60	1.8754	0.00344	0.0086909
ZY05719_04470	membrane protein	2.2684	4.07E-05	0.0001582
ZY05719_04475	ATP/GTP-binding protein	1.8812	3.66E-08	2.32E-07
ZY05719_04485	antirestriction protein ArdA	3.8565	0.0021101	0.0056289
ZY05719_04500	cell division protein FtsK	2.5251	2.11E-06	1.05E-05

ZY05719_04545	chemotaxis protein	2.4807	0.0063012	0.014705
ZY05719_04555	DNA primase	1.7658	2.20E-05	8.97E-05
ZY05719_04600	lantibiotic-modifying protein	1.7234	8.70E-17	1.10E-15
ZY05719_04670	hypothetical protein	1.666	5.26E-15	5.72E-14
ZY05719_04685	glucan-binding protein	2.1056	6.59E-19	8.97E-18
ZY05719_04705	ATPase AAA	2.1849	2.83E-11	2.39E-10
ZY05719_04715	conjugal transfer protein TrbL	2.087	0.0003308	0.0010722
ZY05719_04725	conjugal transfer protein TraG	3.1796	8.83E-11	7.11E-10
ZY05719_04730	hypothetical protein	2.3217	0.0078248	0.017692
ZY05719_04735	CAAX protease	2.5037	0.0001938	0.0006599
ZY05719_04750	hypothetical protein	1.8276	0.002348	0.0061544
ZY05719_04765	modification methylase	2.1982	1.79E-05	7.35E-05
ZY05719_04775	replication initiator protein	2.4023	0.000227	0.0007651
ZY05719_04935	amino acid ABC transporter ATP-binding protein	1.9674	1.78E-20	2.62E-19
ZY05719_05060	hypothetical protein	1.7674	0.018181	0.036548
ZY05719_05765	aconitate hydratase	1.8571	1.63E-41	4.36E-40
ZY05719_05870	transposase	1.6207	9.07E-59	3.41E-57
ZY05719_05880	hypothetical protein	1.6288	2.65E-20	3.88E-19
ZY05719_05900	XRE family transcriptional regulator	2.71	0.0011353	0.0032357
ZY05719_05905	hypothetical protein	1.6998	3.39E-37	8.31E-36
ZY05719_06500	ABC transporter	1.8692	9.09E-62	3.56E-60
ZY05719_06505	glutamine ABC transporter substrate-binding protein	1.7082	1.06E-67	4.70E-66
ZY05719_06510	glutamine ABC transporter permease	1.638	7.14E-55	2.41E-53
ZY05719_07220	hypothetical protein	1.8961	0.0017442	0.0047563
ZY05719_07485	histidine triad protein	2.1649	3.61E-106	2.72E-104
ZY05719_07650	transposase	3.8633	5.34E-58	1.94E-56

<i>ZY05719_09070</i>	sugar ABC transporter permease	1.6614	0.018166	0.036548
<i>ZY05719_09220</i>	translation factor (SUA5)	1.671	2.57E-67	1.09E-65
<i>ZY05719_09225</i>	isopropylmalate isomerase	1.6656	1.01E-45	2.94E-44
<i>ZY05719_09620</i>	MFS transporter	3.4855	4.13E-278	5.39E-276
<i>ZY05719_09625</i>	membrane protein	1.9987	2.20E-91	1.34E-89
<i>ZY05719_09630</i>	DNA-binding protein	1.8095	1.57E-62	6.27E-61

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**Supplemental Table S3. Bacterial strains and plasmids used in this study**

Strains or plasmids	Description <sup>a</sup>	Source or reference
Bacterial strains		
ZY05719	The SS2 strain was isolated from a diseased pig	Our laboratory
$\Delta cps$	Deletion mutant of the CPS synthesis cluster with ZY05719 background	Our laboratory
$\Delta vraSR_{SS}$	Deletion mutant of <i>vraSR_{SS}</i> with ZY05719 background	This study
$C\Delta vraSR_{SS}$	$\Delta vraSR_{SS}$ with the vector pSET2- <i>vraSR_{SS}</i> , Spc <sup>r</sup>	This study
$\Delta yvqF_{SS}$	Deletion mutant of <i>yvqF_{SS}</i> with ZY05719 background	This study
$C\Delta yvqF_{SS}$	$\Delta yvqF_{SS}$ with the vector pSET2- <i>yvqF_{SS}</i> , Spc <sup>r</sup>	This study
DH5 $\alpha$	Cloning host for maintaining the recombinant plasmids	Invitrogen
BL21 (DE3)	Host for expressing the recombinant proteins	Invitrogen
Plasmids		
pSET4S	Temperature-sensitive suicide vector for <i>S. suis</i> mutagenesis, Spc <sup>r</sup>	(1)
pSET4S- <i>vraSR_{SS}</i>	Derived from pSET4S used to knock out <i>vraSR_{SS}</i> in ZY05719, Spc <sup>r</sup>	This study
pSET4S- <i>yvqF_{SS}</i>	Derived from pSET4S used to knock out <i>yvqF_{SS}</i> in ZY05719, Spc <sup>r</sup>	This study
pSET2	<i>E. coli</i> - <i>S. suis</i> shuttle vector for <i>S. suis</i> complementation, Spc <sup>r</sup>	(2)
pSET2- <i>vraSR_{SS}</i>	pSET2 containing the promoter followed by the full-length <i>vraSR_{SS}</i> ORF, Spc <sup>r</sup>	This study
pSET2- <i>yvqF_{SS}</i>	pSET2 containing the promoter followed by the full-length <i>yvqF_{SS}</i> ORF, Spc <sup>r</sup>	This study
pET28a (+)	His-tag expressing vector, Kan <sup>r</sup>	Invitrogen
pET28a- <i>vraR_{SS}</i>	pET28a inserted in-frame with the <i>vraR_{SS}</i> gene for expressing VraR <sub>SS</sub> , Kan <sup>r</sup>	This study

<sup>a</sup>Spc<sup>r</sup>, spectinomycin resistant; Kan<sup>r</sup>, kanamycin resistant.



**Supplemental Table S4. Primers used in this study**

<b>Primers</b>	<b>Primers sequence (5'-3')</b>
For Deletion	
Del-vraSR-Upstream-F	CTGATCGTCGAC TTACGATGGCAGCCTTTGAT
Del-vraSR-Upstream-R	CTATCCTTTGGT CACAAAAACAAT AAGACAGGC
Del-vraSR-Downstream-F	ATTGTTTTTGTG ACCAAAGGATAG TGGAATTA
Del-vraSR-Downstream-R	GAGTCAGAATTC TTCAACAACCTTAGCAACAC
Del-yvqF-Upstream-F	CTGATCGTCGAC AGGAAAAGAATTGATTATGCC
Del-yvqF-Upstream-R	ATCTCCCAGTAT TACCACATCAAA GGCTGCCAT
Del-yvqF-Downstream-F	TTTGATGTGGTA ATA CTGGGAGAT GTGGAGGTG
Del-yvqF-Downstream-R	GAGTCAGAATTC TCAATTAGTTTCGGTAACTCC
For identification	
vraSR-identification-F	GCCCAAAGAGACCTACGCATCC
vraSR-identification-R	CTTCCTCGCCATCACTAGCTTC
yvqF-identification-F	CTAGGAAGAAAAGTCAACAGT
yvqF-identification-R	GGACTATTGACCGCTATACTT
For complementation	
vraSR complementation-F	AACTGCAG GAAAATCAAAATTATCCGTTGTC
vraSR complementation-R	CGGGATCC TATCTGAGTTTTGCATCCATATA
yvqF complementation-F	AACTGCAG ACCCAATGTCATTAAGTTATC
yvqF complementation-R	CGGGATCC AAAAGTGGGAGGGTGGAAGCA
For VraR <sub>SS</sub> expression	

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VraR 28a-F	CGGGATCC ATGAATACGATTCGAGTGATGCTG
VraR 28a-R	CGGAATTC CTATTGGTCCTCTTGCGCCACCAAAC

For checking

*yvqF<sub>ss</sub>/vraS<sub>ss</sub>/vraR<sub>ss</sub>* operon

yvqFvraS-F	GTAATTCGTAAAACGTTTGGGA
yvqFvraS-R	AACTTCGTCCGCATAACCTGA
vraSvraR-F	CATTTGGATGTTTACCTCTTG
vraSvraR-R	GCGCTTGCGGCCATTCCTTCA

For EMSA

vra EMSA-F	CCAATGTCATTAAGTTATCTA
vra EMSA-R	GGGCTATAAACTAAATATTG
cps EMSA-F	ATTATGACCAAGAAGTTGGTG
cps EMSA-R	TATTCAAAGCCATCCACCTTA
neu EMSA-F	AAGAATTGACCATCACTGCCA
neu EMSA-R	ATTTCTTTCGAATAGTCTCAG
16s EMSA-F	CGAGCGCAGGCGGTTTGATAA
16s EMSA-R	CTCATCGTTTACGGCGTGGAC

For quantitative real-time reverse transcription-PCR

yvqF qPCR-F	TGATGTGGTAGCCAATGAGG
yvqF qPCR-R	GAGATAAGGAGCTGACCAGTAAA
vraS qPCR-F	CAGGTTATGCGGACGAAGTTA
vraS qPCR-R	GCGTGAGGTTTCTGACCTTAT
vraR qPCR-F	CCAAGATGACTGGCGTAGAA

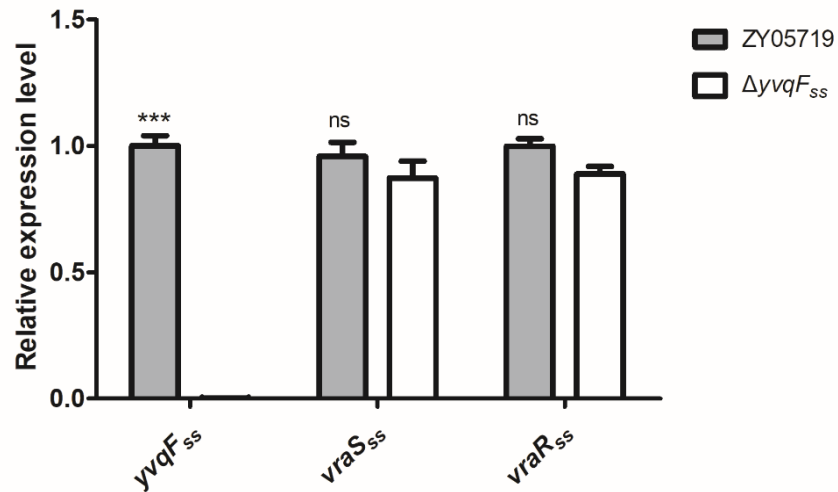
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vraR qPCR-R	GCTTCCAGCACAGGATAGAT
00865 qPCR-F	CAGTTGGTGGCTTTGTCCTA
00865 qPCR-R	CTCCGTTCAAGGTCCGAATC
01910 qPCR-F	ATGCGGAGTTCATGGCTATC
01910 qPCR-R	TGCCAATTCTAAGCGGTACTT
02090 qPCR-F	GTGTTTCGTTGGGATGCTATTTT
02090 qPCR-R	TTTCAGGTTTCAGGCCTAGTT
02775(cpsF) qPCR-F	CTTGTCGGATTGGTCCTTTCA
02775(cpsF) qPCR-R	CAATACTGTTCGGACGGGATAAC
02840(neuB) qPCR-F	TAGCACAGATATGCCCGTTTAT
02840(neuB) qPCR-R	ACAGCCATAACCAGTTGAAAGA
07070 qPCR-F	GGCCTACTCAGCTTCACAAA
07070 qPCR-R	GAAGTGGATCACCTGAAGATAACC
01650 qPCR-F	ACTCAACACACAGCCTTCTC
01650 qPCR-R	CAACTGTCTAGGACTTGGTTCTT
04460 qPCR-F	GGATTTTCAGATATGGACGGTAGAG
04460 qPCR-R	CAGAGTGGACGGTCTTTGTATT
04600 qPCR-F	GTGGGATTTGCTCATGGATTTAC
04600 qPCR-R	ACCATCCCGAGCTAGGATATAA
09625 qPCR-F	GTCGCTTGTCTTACTCTGTCTT
09625 qPCR-R	GCTGTATCGCGTCCCTTATC
<i>parC</i> qPCR-R	TGGAGATGCACGGAAACAA
<i>parC</i> qPCR-R	CTCGATGTCAGCCAAGAGATAG

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Underlined are restriction cutting sites;



**Supplemental Figure S1 Expression levels of *yvqF<sub>ss</sub>*, *vraS<sub>ss</sub>*, and *vraR<sub>ss</sub>* in strains ZY05719 and  $\Delta yvqF_{ss}$  were measured by qRT-PCR.** qRT-PCR expression values are means plus standard deviations (error bars) from at least three independent experiments. The unpaired two-tailed Student's *t* test were used for statistical analysis (ns,  $P > 0.05$ ; \*\*\*,  $P < 0.001$ ).

1. Takamatsu D, Osaki M, Sekizaki T. 2001. Thermosensitive suicide vectors for gene replacement in *Streptococcus suis*. *Plasmid* 46:140-8.
2. Takamatsu D, Osaki M, Sekizaki T. 2001. Construction and characterization of *Streptococcus suis*-*Escherichia coli* shuttle cloning vectors. *Plasmid* 45:101-13.