

**Table S3: Details of the Competitive Index data.**

Gene	Mutant	Locus	ABACUS (pool of 933 mutants)		Competition with wt (single mutant)		
			ratio spleen / input	p-value	CI	standard error	p-value
STM0731	$\Delta$ STM0731::kan	Spleen	<b>0.18</b>	<b>2.E-4</b>			
STM0732	<sup>1</sup> $\Delta$ STM0732::kan	Spleen*			0.68	0.06	<b>5.E-4</b>
		Liver*			0.55	0.07	7.E-2
		Cecum*			0.74	0.10	<b>7.E-3</b>
STM0857	$\Delta$ STM0857::kan	Spleen	<b>0.47</b>	<b>8.E-3</b>	1.28	0.03	<b>2.E-3</b>
		Liver			1.37	0.04	<b>1.E-2</b>
		Cecum			1.08	0.02	<b>7.E-3</b>
STM1131	<sup>2</sup> $\Delta$ STM1131::kan	Spleen*	<b>0.44</b>	<b>1.E-4</b>	0.0008	0.78	<b>2.E-2</b>
		Liver*			0.002	0.25	<b>5.E-4</b>
		Cecum*			0.007	0.78	6.E-2
	$\Delta$ STM1131::FRT	Spleen			<b>0.37</b>	0.06	<b>2.E-4</b>
		Liver			0.58	0.07	<b>3.E-3</b>
		Cecum			<b>0.32</b>	0.40	2.E-1
STM1760	$\Delta$ STM1760::kan	Spleen*	<b>0.19</b>	<b>1.E-6</b>	<b>0.35</b>	0.17	<b>4.E-2</b>
		Liver*			0.59	0.22	3.E-1
		Cecum*			<b>0.46</b>	0.24	2.E-1
STM2120	$\Delta$ STM2120::kan	Spleen	<b>0.22</b>	<b>9.E-12</b>	<b>0.09</b>	0.06	<b>1.E-4</b>
		Liver			<b>0.07</b>	0.08	<b>2.E-4</b>
		Cecum			<b>0.04</b>	0.25	<b>6.E-3</b>
STM2215	$\Delta$ STM2215::kan	Spleen*	<b>0.12</b>	<b>2.E-8</b>	<b>0.01</b>	0.14	<b>1.E-4</b>
		Liver*			<b>0.01</b>	0.14	<b>1.E-4</b>
		Cecum*			<b>0.02</b>	0.22	<b>1.E-3</b>
STM2303	$\Delta$ STM2303::kan	Spleen	<b>0.08</b>	<b>6.E-12</b>	<b>0.12</b>	0.08	<b>3.E-4</b>
		Liver			<b>0.27</b>	0.14	<b>2.E-2</b>
		Cecum			<b>0.20</b>	0.16	<b>2.E-2</b>
	$\Delta$ STM2303::FRT	Spleen			<b>0.22</b>	0.09	<b>1.E-3</b>
		Liver			<b>0.32</b>	0.24	9.E-2
		Cecum			1.42	0.22	5.E-1
	$\Delta$ STM2303::FRT pWSK29	Spleen			<b>0.23</b>	0.04	<b>1.E-4</b>
		Liver			<b>0.40</b>	0.09	<b>1.E-2</b>
		Cecum			0.52	0.08	<b>2.E-2</b>
	$\Delta$ STM2303::FRT pWSK29::STM2303	Spleen			1.51	0.04	<b>3.E-2</b>
		Liver			2.18	0.04	<b>2.E-3</b>
		Cecum			4.76	0.14	<b>8.E-3</b>
STM2639	$\Delta$ STM2639::kan	Spleen*	<b>0.15</b>	<b>5.E-8</b>	1.22	0.04	5.E-1
		Liver*			1.31	0.05	3.E-1
		Cecum*			1.20	0.25	9.E-1
STM3120	$\Delta$ STM3120::kan	Spleen*	<b>0.19</b>	<b>1.E-6</b>	<b>0.13</b>	0.15	<b>1.E-2</b>
		Liver*			<b>0.28</b>	0.04	<b>9.E-4</b>
		Cecum*			<b>0.35</b>	0.09	<b>2.E-2</b>
	$\Delta$ STM3120::FRT	Spleen			<b>0.22</b>	0.04	<b>&lt;1.E-7</b>
		Liver			<b>0.17</b>	0.10	<b>7.E-4</b>

		Cecum			<b>0.06</b>	0.25	<b>6.E-3</b>
STM3121	$\Delta STM3121::kan$	Spleen	<b>0.38</b>	<b>1.E-5</b>	<b>0.31</b>	0.11	<b>4.E-3</b>
		Liver			<b>0.28</b>	0.06	<b>2.E-4</b>
		Cecum			<b>0.27</b>	0.26	9.E-2
	$\Delta STM3121::FRT$	Spleen			<b>0.07</b>	0.07	<b>8.E-6</b>
		Liver			<b>0.08</b>	0.05	<b>2.E-6</b>
		Cecum			<b>0.03</b>	0.10	<b>2.E-5</b>
	$\Delta STM3121::FRT$ pWSK29	Spleen			<b>0.14</b>	0.08	<b>1.E-4</b>
		Liver			<b>0.22</b>	0.19	<b>1.E-2</b>
		Cecum			<b>0.25</b>	0.32	9.E-2
	$\Delta STM3121::FRT$ pWSK29:: <i>STM3121</i>	Spleen			3.75	0.09	<b>1.E-2</b>
		Liver			4.83	0.15	<b>3.E-2</b>
		Cecum			5.32	0.33	2.E-1
<i>leuX</i>	$\Delta leuX::kan$	Spleen	<b>0.05</b>	<b>6.E-12</b>	<b>0.0001</b>	0.14	<b>&lt;1.E-7</b>
		Liver			<b>0.0002</b>	0.14	<b>&lt;1.E-7</b>
		Cecum					
	$\Delta leuX::FRT$	Spleen			<b>0.01</b>	0.12	<b>&lt;1.E-7</b>
		Liver			<b>0.01</b>	0.16	<b>&lt;1.E-7</b>
		Cecum			<b>0.05</b>	0.29	<b>6.E-3</b>
<i>istR</i>	$\Delta istR::kan$	Spleen	<b>0.41</b>	<b>5.E-6</b>	<b>0.34</b>	0.12	<b>3.E-2</b>
		Liver			<b>0.24</b>	0.22	7.E-2
		Cecum					
	$\Delta istR::FRT$	Spleen			<b>0.42</b>	0.09	<b>1.E-2</b>
		Liver			<b>0.41</b>	0.09	<b>1.E-2</b>
		Cecum			<b>0.03</b>	0.40	<b>2.E-2</b>
<i>sroA</i>	$\Delta sroA::kan$	Spleen	<b>0.13</b>	<b>2.E-10</b>	<b>0.81</b>	0.04	<b>5.E-3</b>
		Liver			1.34	0.05	6.E-1
		Cecum					
	$\Delta sroA::FRT$	Spleen			<b>0.24</b>	0.04	<b>&lt;1.E-7</b>
		Liver			<b>0.21</b>	0.05	<b>4.E-3</b>
		Cecum			<b>0.09</b>	0.25	<b>4.E-3</b>
<i>oxyS</i>	$\Delta oxyS::kan$	Spleen	<b>2.30</b>	<b>6.E-6</b>	1.67	0.04	<b>3.E-3</b>
		Liver			1.23	0.17	7.E-1
		Cecum					
	$\Delta oxyS::FRT$	Spleen			1.74	0.06	<b>3.E-3</b>
		Liver			1.34	0.07	<b>2.E-2</b>
		Cecum			0.68	0.29	8.E-1
<i>rybB</i>	$\Delta rybB::kan$	Spleen	<b>0.50</b>	<b>6.E-4</b>	1.79	0.09	2.E-2
		Liver			1.24	0.14	4.E-1
		Cecum					
<i>sraA</i>	$\Delta sraA::kan$	Spleen	<b>0.35</b>	<b>1.E-7</b>	1.03	0.02	<b>1.E-2</b>
		Liver			0.63	0.06	<b>4.E-3</b>
		Cecum					

Red indicates either  $P < 0.05$  or a ratio of greater than two-fold for mutants with reduced fitness in systemic infection. Blue indicates the same thresholds for mutants with increased fitness after selection.

, \*Indicates mice were euthanized later than 24 but prior to 48 hours in CI experiments.

<sup>1</sup> overlapping mutant with STM0731

<sup>2</sup> $\Delta STM1131::kan$ , the P22 transduced version of this mutant used in CI experiments, was discovered to have rough LPS.  $\Delta STM1131::FRT$  was generated by re-transduction of our original red-swap mutant, and does not have rough LPS (data not shown).