

**Supplementary Table 1.** Analysis of example real-time PCR gene expression data.

---

<b>Gene</b>	<b>SE-1 (<math>\mu\text{M}</math>)</b>	<b>Ct</b>	<b><math>\Delta\Delta\text{Ct}^1</math></b>	<b><math>\Delta\Delta\text{Ct}^2</math></b>	<b>Average<math>\Delta\Delta\text{Ct}</math></b>	<b>RQ (<math>2^{-\Delta\Delta\text{Ct}}</math>)</b>
<i>gapA</i>	0	26.70		0	0	1
<i>gapA</i>	20	27.33		1	1	0.5
<i>gapA</i>	40	26.76		-0.69	-0.69	0.62
<i>rrsA</i>	0	18.91	0		0	1
<i>rrsA</i>	20	18.55	-1.0		-1.0	2
<i>rrsA</i>	40	18.29	0.69		0.69	1.62
<i>virB</i>	0	22.00	0	0	0	1
<i>virB</i>	20	23.26	0.63	1.63	1.13	0.46
<i>virB</i>	40	23.18	1.12	1.81	1.47	0.36
<i>icsA</i>	0	27.70	0	0	0	1
<i>icsA</i>	20	27.88	-0.46	0.54	0.04	0.97
<i>icsA</i>	40	28.59	0.82	1.52	1.17	0.44
<i>icsB</i>	0	26.17	0	0	0	1
<i>icsB</i>	20	26.25	-0.56	0.44	-0.06	1.04
<i>icsB</i>	40	27.04	0.80	1.49	1.14	0.45
<i>ipaB</i>	0	24.32	0	0	0	1
<i>ipaB</i>	20	25.57	0.62	1.61	1.11	0.46
<i>ipaB</i>	40	26.23	1.85	2.54	2.20	0.22

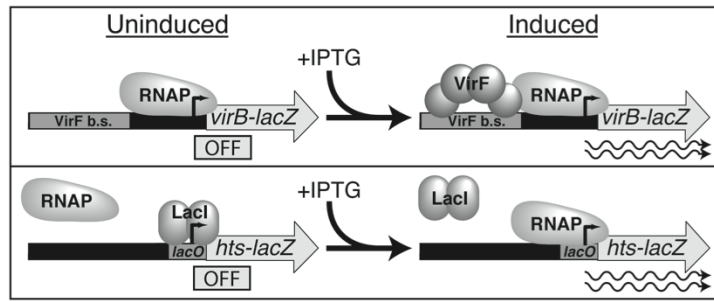
---

<sup>1</sup> $\Delta\Delta C_t$  values after normalizing data to *gapA*

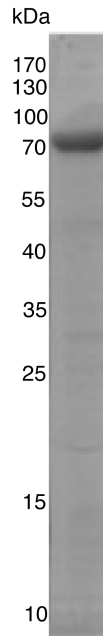
<sup>2</sup> $\Delta\Delta C_t$  values after normalizing data to *rrsA*

$$\Delta\Delta C_t = (C_{t,Target} - C_{t,Internal Control})_{SE-1} - (C_{t,Target} - C_{t, Internal Control})_{no SE-1}$$

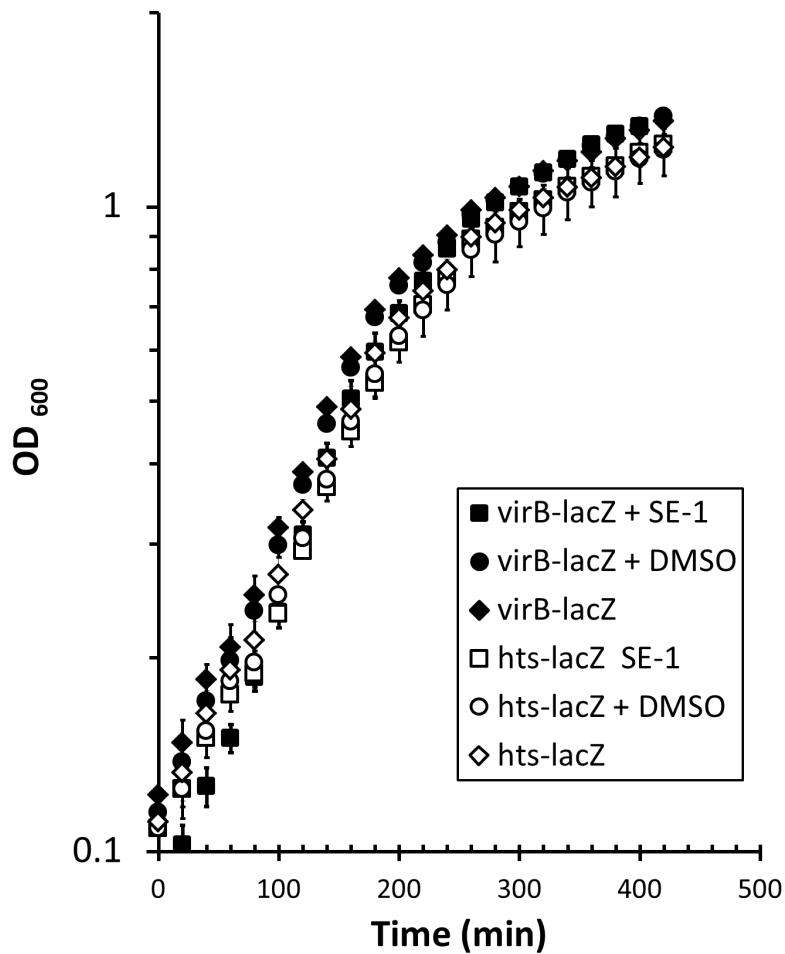
The values in the table are for a single replicate. The RQ values from three such replicates were averaged to get the average gene expression levels and to calculate the standard error.



**Supplementary Fig. 1. Reporter fusions used for *in vivo* assays.** VirF-activated *virB-lacZ* fusion (Top) and LacI-repressed *hts-lacZ* control fusion (Bottom), each shown in their uninduced (-) IPTG state (left) and their induced (+) IPTG state (right). Gray rectangles: VirF and LacI (*lacO*) binding sites. Gray arrows: *lacZ* gene expressed from *virB* or synthetic *hts* promoter. Thick black/gray lines: Promoter region DNA. Right angle black arrows: Transcription start sites. Rounded gray shapes: RNAP, VirF or LacI proteins. Wavy lines: active transcription in the presence of IPTG.

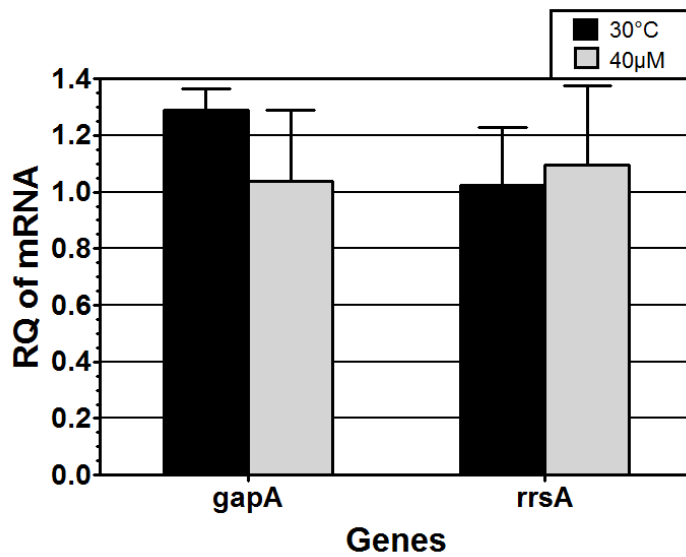


**Supplementary Fig. 2. MBP-VirF protein after purification using amylose affinity chromatography.** The image represents a single lane of a 12% SDS-PAGE gel stained with Coomassie Brilliant Blue. Numbers to the left of the gel indicate the positions of protein standards with the indicated molecular masses. MBP-VirF protein has a predicted molecular mass of 73 kDa.

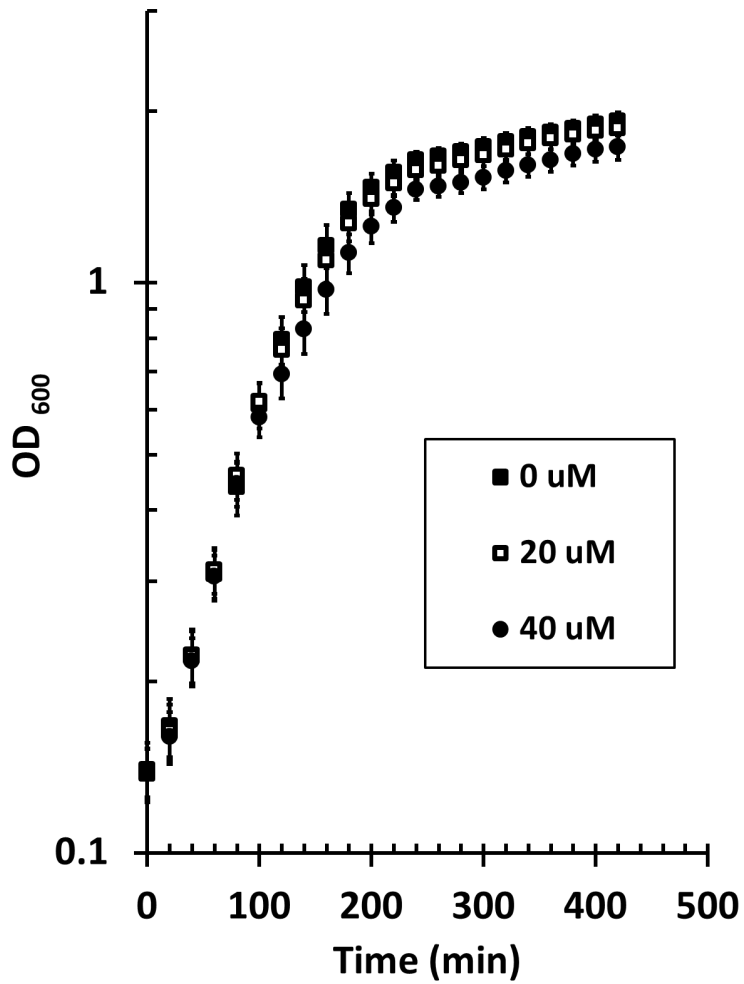


**Supplementary Fig. 3. Growth curves of *E. coli* strains in the absence or presence of SE-1.**

Strains carrying *virB-lacZ* and VirF expressed from pHG165 (SME4382, squares) or *hts-lacZ* and LacI expressed from pHG165 (SME3359, circles) grown in the absence (-) or presence (+) of SE-1 (44  $\mu$ M), or the absence or presence of DMSO (0.3%). Results are the average of three replicates, error bars represent the standard error of the mean.

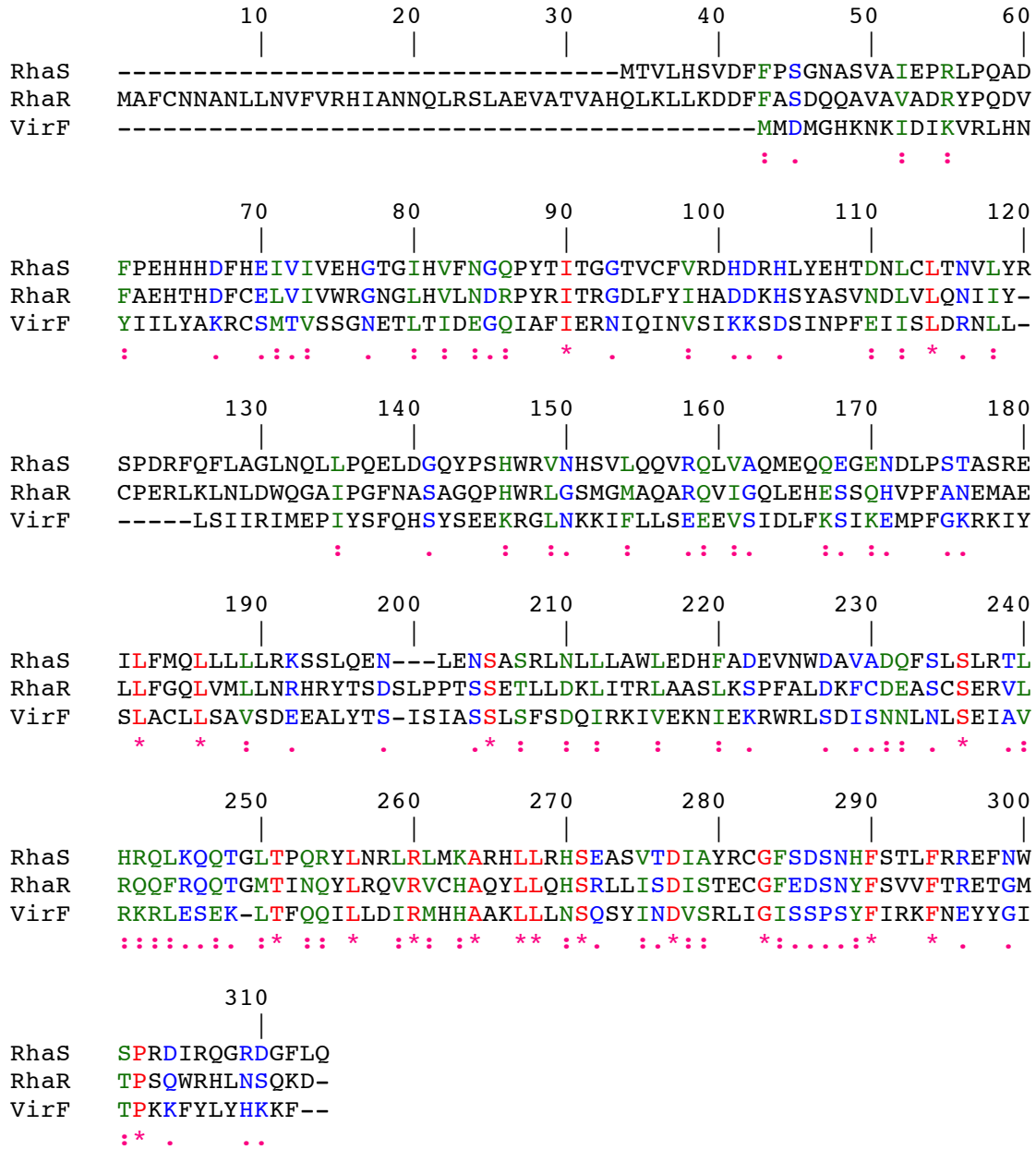


**Supplementary Fig. 4. Relative quantification of mRNA levels of the constitutively expressed genes *gapA* and *rrsA* of *Shigella*.** Levels of *gapA* were normalized to the *rrsA* values, and *vice versa*. Cultures were grown at 30°C (Black bars) or at 37°C with 40 μM SE-1 (Grey bars). Values are relative to the mRNA levels of *gapA* (for *gapA*) or *rrsA* (for *rrsA*) for *Shigella* grown at 37°C with no SE-1, which were set to one. Error bars represent the standard error of the mean calculated from three independent replicates.



**Supplementary Fig. 5. Growth curves of *Shigella* in the absence or presence of SE-1.**

*Shigella* strain carrying *ipgD*<sup>-</sup> (SME4331) in the absence (filled square) or presence of SE-1 (20  $\mu$ M, open squares; 40  $\mu$ M, filled circles). Results are the average of three replicates, error bars represent the standard error of the mean.



**Supplementary Fig. 6. Alignment of RhaS, RhaR and VirF protein sequences.** Sequences were aligned using ClustalW. Identical positions are red and marked “\*”; strongly similar positions are green and marked “:”; weakly similar positions are blue and marked “.”; different positions are black and unmarked. Gaps in the alignment are marked “-”.