Gene	SE-1 (μM)	Ct	$\Delta\Delta Ct^1$	$\Delta\Delta Ct^2$	Average∆∆Ct	RQ $(2^{-\Delta\Delta Ct})$
gapA	0	26.70		0	0	1
gapA	20	27.33		1	1	0.5
gapA	40	26.76		-0.69	-0.69	0.62
rrsA	0	18.91	0		0	1
rrsA	20	18.55	-1.0		-1.0	2
rrsA	40	18.29	0.69		0.69	1.62
virB	0	22.00	0	0	0	1
virB	20	23.26	0.63	1.63	1.13	0.46
virB	40	23.18	1.12	1.81	1.47	0.36
icsA	0	27.70	0	0	0	1
icsA	20	27.88	-0.46	0.54	0.04	0.97
icsA	40	28.59	0.82	1.52	1.17	0.44
icsB	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
ipaB	0	24.32	0	0	0	1
ipaB	20	25.57	0.62	1.61	1.11	0.46
ipaB	40	26.23	1.85	2.54	2.20	0.22

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

 $^{1}\Delta\Delta$ Ct values after normalizing data to *gapA*

² $\Delta\Delta$ Ct values after normalizing data to *rrsA*

 $\Delta\Delta Ct = (Ct, Target - Ct, Internal Control)$ SE-1 - (Ct, Target - Ct, 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 μ 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 μ M 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 $ipgD^{-}$ (SME4331) in the absence (filled square) or presence of SE-1 (20 μ M, open squares; 40 μ M, filled circles). Results are the average of three replicates, error bars represent the standard error of the mean.

	10	20	30	40	50	60
RhaS			·	MTVLHSVDFFP	SGNASVAIE	PRLPQAD
RhaR	MAFCNNANLLN	/FVRHIANNOLF	SLAEVATVAH	OLKLLKDDFFA	SDOOAVAVAI	DRYPODV
VirF				MM	DMGHKNKID	IKVRLHN
				•		•
						· ·
	70	80	9.0	100	110	120
	70	00	90	100	110	120
Dh e C						
Rhas	FPEHHHDFHEI	TVEHGTGIHVE	NGQPYTTTGG	TVCFVRDHDRH.		
RnaR	FAEHTHDFCEL	/ I VWRGNGLHVI	NDRPYRTTRG		SYASVNDLV	LQNIIY-
VirF'	YIILYAKRCSMI	VSSGNETLTIE	EGQIAFIERN	IQINVSIKKSD	SINPFEIIS	DRNLL-
	• • • • • • • • • • • • • • • • • • • •		··· * ·	:	: : 3	* . :
	130	140	150	160	170	180
RhaS	SPDRFQFLAGL	IQLLPQELD <mark>G</mark> QY	PSHWRVNHSV	LQQVRQLVAQM	EQQ <mark>E</mark> GENDLI	P <mark>ST</mark> ASRE
RhaR	CPERLKLNLDWO)GAIPGFNASAG	OPHWRL <mark>G</mark> SMG	MAOAROVIGOL	EHESSOHVPI	FANEMAE
VirF	ISTIRIN	- IEPTYSFOH <mark>S</mark> YS	EEKRGLNKKT	FLUSEEEVSTD	I.FKSTKEMPI	FGKRKTY
		•	• •	• • •	• •	
		• •	• • • •			••
	190	200	210	220	230	240
	150	200	210	220	230	240
DhaC						
Rilas						
RhaR		CHRYTSDSLPP1	SETLLDKLI	TRLAASLKSPF	ALDKFCDEA	SCSERVL
VirF'	SLACLLSAVSD	EALYTS-ISIA	SSLSFSDQIR	KIVEKNIE <mark>K</mark> RW.	RLSDISNNL	NLSEIAV
	* * : .	•	.* : : :	: :.	::	• * ••
	250	260	270	280	290	300
RhaS	HRQL <mark>KQQTGLT</mark> H	PORY <mark>LNRL</mark> RLMK	ARHLLRHSEA	SVTDIAYRCGF	SDSNHFSTLI	FRREFNW
RhaR	RQQF <mark>RQ</mark> QTGMT]	INQY <mark>L</mark> RQV <mark>R</mark> VCH	AQYLLQHSRL	LI <mark>SD</mark> ISTEC <mark>G</mark> F	EDSNYFSVVI	TRETGM
VirF	RKRLESEK-LTE	OOILLDIRMHH	AAKLLLNSOS	YINDVSRLIGI	SSPSYFIRKI	FNEYYGI
		. * .*. :	* ** *	. * * .	*	*
	310					
	510					
Dh e C						
Rhas	SPRDIRQGRDGE	ГLQ				
кпак	TPSQWRHLNSQF	(D -				
VirF	T <mark>PKK</mark> FYLYHKKE	? 				
	:*					

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 "-".