

**Table S1: Summary of statistically significant infection kinetic differences for wild type compared to  $\Delta cesT$  during real-time analysis of effector-TEM-1 translocation.** Statistical analyses were conducted with GraphPad Prism (6.0) using the unpaired t-test method to compare mean and standard deviation at individual time points. The range of time (min) over which the comparison was statistically significant ( $P < 0.01$ ) is listed.

Effector-TEM-1 construct	** ( $P < 0.01$ )
Tir	t = 30-100 min
EspF	n.s.
NleA	t = 90-100 min
NleH1	t = 50-100 min
NleH2	t = 60-100 min

**Table S2: Summary of statistically significant differences observed during real-time analysis of effector translocation for CesT phosphosite variants.** Statistical analyses were conducted with GraphPad Prism (6.0) using the unpaired t-test method to compare mean and standard deviation at individual time points. The range of time (min) over which the comparison was statistically significant is listed for two P values. No statistically significant differences were observed for EspF or Tir translocation in context of CesT phosphosite mutations. Refer to **Fig 4A in main manuscript** for comparative analysis of wild type and  $\Delta cesT$  translocation data.

Group	CesT phosphosite variants:	** ( $P < 0.01$ )	* ( $P < 0.05$ )
<i>nleA::nleA-blaM</i>	WT vs. Y152F	t = 90-100 min	t = 80-100 min
<i>nleA::nleA-blaM</i>	WT vs. Y153F	t = 90-100 min	t = 80-100 min
<i>nleA::nleA-blaM</i>	WT vs. Y152F, Y153F	t = 80-100 min	t = 70-100 min
<i>nleA::nleA-blaM</i>	Y153F vs. Y152F	t = 70-100 min	t = 60-100 min
<i>nleA::nleA-blaM</i>	Y153F vs. Y152F, Y153F	t = 60-100 min	t = 60-100 min
<i>nleH1::nleH1-blaM</i>	WT vs. Y152F	t = 70-100 min	t = 70-100 min
<i>nleH1::nleH1-blaM</i>	WT vs. Y152F, Y153F	t = 80-100 min	t = 70-100 min
<i>nleH1::nleH1-blaM</i>	Y153F vs. Y152F	t = 70-100 min	t = 70-100 min
<i>nleH1::nleH1-blaM</i>	Y153F vs. Y152F, Y153F	t = 80-100 min	t = 70-100 min
<i>nleH2::nleH2-blaM</i>	WT vs. Y152F	t = 90-100 min	t = 90-100 min
<i>nleH2::nleH2-blaM</i>	WT vs. Y152F, Y153F	t = 80-100 min	t = 80-100 min
<i>nleH2::nleH2-blaM</i>	Y153F vs. Y152F	t = 90-100 min	t = 90-100 min
<i>nleH2::nleH2-blaM</i>	Y153F vs. Y152F, Y153F	t = 80-100 min	t = 80-100 min