

SUPPLEMENTAL MATERIALS

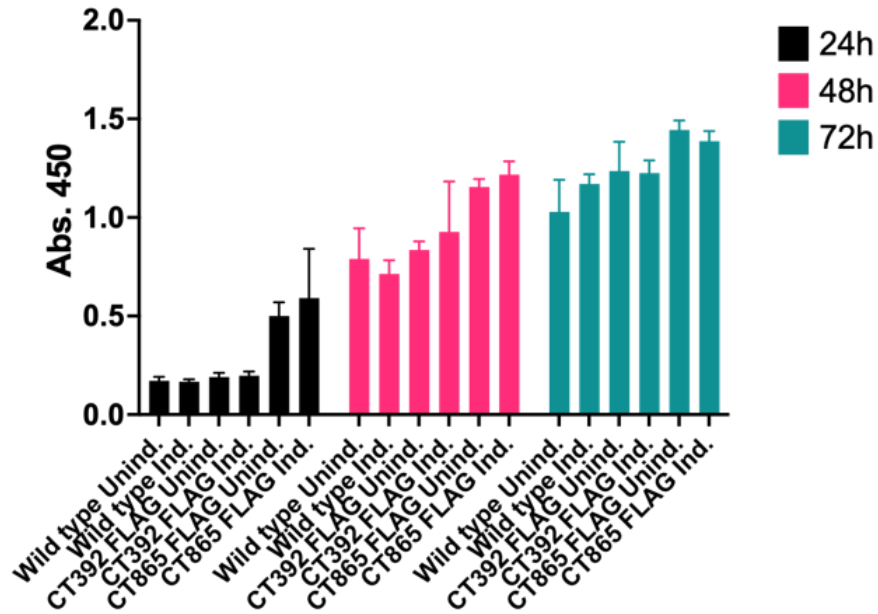


Fig S1. Overexpression of CT392 FLAG does not affect cytotoxicity. HeLa cells were infected with chlamydia overexpressing effector-FLAG constructs in either uninduced or 10ng/ul anhydrotetracycline inducing conditions for 24, 48, or 72h. Lactate dehydrogenase release was measured using a Tecan plate reader. Error bars are SD. Results are representative of 3 replicates.

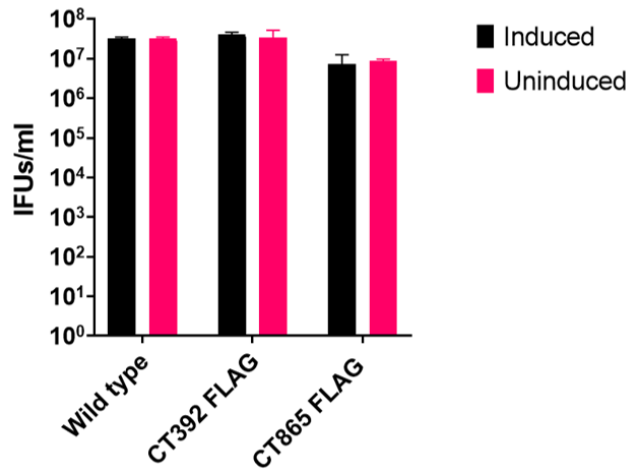


Fig S2. Overexpression of CT392 FLAG does not result in a difference in growth at 48hpi. HeLa cells were infected with chlamydia overexpressing effector-FLAG constructs uninduced or induced with 10ng/ul anhydrous tetracycline. IFUs/ml was calculated at 48hpi. Error bars are SD. Results are representative of 3 replicates.

Table S1. Primers used in this study.

Name	Sequence	Uses
Secretion by <i>Yersinia pseudotuberculosis</i> T3SS		
NPT F	ATTGAACAAGATGGATTGCACGCAGGTTCTCC	Cloning into pFlag-ctc
NPT R	CTTGTAGTCGACAGAGAAGAAGAACTCGTCAAGAAGGCGATAG	Cloning into pFlag-ctc
CTL037 NPT SOE F	CACAGGAGATATCATATGATAGATCCTCTTAAGCTTTT	Expression in YP as Flag fusion
CTL037 NPT SOE R	GGAGAACCTGCGTGCAATCCATCTTGTTCAATAGAGAATGAGGCAACATTTTAC	Expression in YP as Flag fusion
CTL052 NPT SOE F	CACAGGAGATATCATATGGAAGAGGCGGAAAAGC	Expression in YP as Flag fusion
CTL052 NPT SOE R	GGAGAACCTGCGTGCAATCCATCTTGTTCAATCGAGTCTAACTCTAAGGACTCT	Expression in YP as Flag fusion
CTL060 NPT SOE F	CACAGGAGATATCATATGCAAGTCCTAGCAAGTTTATT	Expression in YP as Flag fusion
CTL060 NPT SOE R	GGAGAACCTGCGTGCAATCCATCTTGTTCAATGCCTTCTTAAGAGCGCTGAAAA	Expression in YP as Flag fusion
CTL065 NPT SOE F	CACAGGAGATATCATATGCTATTAGATTCTCGTTCCCTAC	Expression in YP as Flag fusion
CTL065 NPT SOE R	GGAGAACCTGCGTGCAATCCATCTTGTTCAATAGGGATCGCCTCCAACATA	Expression in YP as Flag fusion
CTL105 NPT SOE F	CACAGGAGATATCATATGCAACTCACCTACAAGC	Expression in YP as Flag fusion
CTL105 NPT SOE R	GGAGAACCTGCGTGCAATCCATCTTGTTCAATACTTTTAGCCTCTCTAGGGACAT	Expression in YP as Flag fusion
CTL113 NPT SOE F	CACAGGAGATATCATATGAATATTTATAGATTCATCTC	Expression in YP as Flag fusion
CTL113 NPT SOE R	GGAGAACCTGCGTGCAATCCATCTTGTTCAATCGCCTTGCGAACGTCTGC	Expression in YP as Flag fusion
CTL137 NPT SOE F	CACAGGAGATATCATATGGATATTCCAGAACAGGGCTC	Expression in YP as Flag fusion
CTL137 NPT SOE R	GGAGAACCTGCGTGCAATCCATCTTGTTCAATTGCTGAGATCTCTGAAGAAGA	Expression in YP as Flag fusion
CTL307 NPT SOE F	CACAGGAGATATCATATGAATAAAAAAGAACGAAT	Expression in YP as Flag fusion
CTL307 NPT SOE R	GGAGAACCTGCGTGCAATCCATCTTGTTCAATACTCAGCTTAGTGAATGAAGAGA	Expression in YP as Flag fusion
CTL386 NPT SOE F	CACAGGAGATATCATATGAAGCGTTCTCCATGGTATAA	Expression in YP as Flag fusion
CTL386 NPT SOE R	GGAGAACCTGCGTGCAATCCATCTTGTTCAATAAGAAACAGATATTTCCCGATT	Expression in YP as Flag fusion
CTL404 NPT SOE F	CACAGGAGATATCATATGAAAAAGTTTGTACTTTCT	Expression in YP as Flag fusion
CTL404 NPT SOE R	GAGAACCTGCGTGCAATCCATCTTGTTCAATTCCTTATCATTGATAGTAATGT	Expression in YP as Flag fusion
CTL604 NPT SOE F	CACAGGAGATATCATATGGGACTATCTCGTCTAGCCTT	Expression in YP as Flag fusion
CTL604 NPT SOE R	GGAGAACCTGCGTGCAATCCATCTTGTTCAATTGAGGGACTGATTTTGGC	Expression in YP as Flag fusion
CTL614 NPT SOE F	CACAGGAGATATCATATGAGAAAAGCATTCTTGTTTGG	Expression in YP as Flag fusion
CTL614 NPT SOE R	GGAGAACCTGCGTGCAATCCATCTTGTTCAATAACTGCTGAAATGAATGTC	Expression in YP as Flag fusion
CTL648 NPT SOE F	CACAGGAGATATCATATGTCTTCTATAACAAGGAACA	Expression in YP as Flag fusion
CTL648 NPT SOE R	GGAGAACCTGCGTGCAATCCATCTTGTTCAATAGCAGGGAAAGAAATAGTC	Expression in YP as Flag fusion
CTL720 NPT SOE F	CACAGGAGATATCATATGAGTCAAAAATAAGAACTCTGC	Expression in YP as Flag fusion
CTL720 NPT SOE R	GGAGAACCTGCGTGCAATCCATCTTGTTCAATGTAATCCCACATTTTCTTAATGA	Expression in YP as Flag fusion
CTL765 NPT SOE F	CACAGGAGATATCATATGTGGCTTTGGTCTTTAGTTA	Expression in YP as Flag fusion

CTL765 NPT SOE R	GGAGAACCTGCGTGCAATCCATCTTGTTC AATAAACAGCTGAAGCATGGAACG	Expression in YP as Flag fusion
CTL799 NPT SOE F	CACAGGAGATATCATATGGGTAGATACAGAAGAGT	Expression in YP as Flag fusion
CTL799 NPT SOE R	GGAGAACCTGCGTGCAATCCATCTTGTTC AATTCTGAGCCGTAGTCCCCTG	Expression in YP as Flag fusion
CTL847 NPT SOE F	CACAGGAGATATCATATGACGACGAAACCCAAA ACTCT	Expression in YP as Flag fusion
CTL847 NPT SOE R	GGAGAACCTGCGTGCAATCCATCTTGTTC AATATTTTGAAATTCTCTAAACG	Expression in YP as Flag fusion
CTL891 NPT SOE F	CACAGGAGATATCATATGGAAAAGAATTATTATGC	Expression in YP as Flag fusion
CTL891 NPT SOE R	GGAGAACCTGCGTGCAATCCATCTTGTTC AATGTAATCCGGCAAGAAACATCC	Expression in YP as Flag fusion
CyaA and BlaM Tag Construction		
CyaA KpnI F	CCGGTACCATGCAGCAATCGCATCAGGCTGG	Cloning CyaA into pBomb4-tet-mcherry
CyaA Sall R	CCGTGCGACTCAGTCGACATAGCCGGAATCCTG	Cloning CyaA into pBomb4-tet-mcherry
BlaM KpnI F	CCGGTACCATGAGTATTCAACATTTCCGTG	Cloning BlaM into pBomb4-tet-mcherry
BlaM Sall R	CCACCGGTTTACCAATGCTTAATCAGTGAG	Cloning BlaM into pBomb4-tet-mcherry
Novel Candidates For CYA		
CT131 NotI F	CCGCGGCCGCATGAAGCGTTCTCCATGGTATAA	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT131 KpnI R	CCGGTACCTTCTGTTTCAGAAACTTCCCAC	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT149 NotI F	CCGCGGCCGCATGAAAAAGTTTGCTACTTTCCT	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT149 KpnI R	CCGGTACCAAGATTTGATTGGAATGCGTTA	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT360 NotI F	CCGCGGCCGCATGAAAAAGTTTGCTACTTTCCT	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT360 KpnI R	CCGGTACCTTTCTGCTCTCTCTCACAA	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT392 NotI F	CCGCGGCCGCATGTCTTCTATACAAGGAAC	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT392 KpnI R	CCGGTACCAAATCCTCTATCATCATCGG	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT460 NotI F	CCGCGGCCGCATGAGTCAAATAAGAACTCTGC	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT460 KpnI R	CCGGTACCTTTAATGATGTGTGAGAAACCA	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT503 NotI F	CCGCGGCCGCATGTGGCTTTGGTCTTTAGTTA	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT503 KpnI R	CCGGTACCAACCTCTTCTTTGCTAA	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT537 NotI F	CCGCGGCCGCATGGGTAGATACAGAAGAG	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT537 KpnI R	CCGGTACCATCGTTCTCCATAAAAAAAGC	Expression in CT as CyaA, BlaM or GSK-FLAG fusion

CT584 NotI F	CCGCGGCCGCATGACGACGAAACCCAAAAC	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT584 KpnI R	CCGGTACCCACAGATTTTCGTTAATTCTTCAA	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT627 NotI F	CCGCGGCCGCATGGAAAAGAATTATTATGC	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT627 KpnI R	CCGGTACCTGCTGGTTGGTTTTCTTGTTCCTT	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT668 NotI F	CCGCGGCCGCATGATAGATCCTCTTAAGCTTTT	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT668 KpnI R	CCGGTACCACCTAACTGGGTTGTCATAATCG	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT683 NotI F	CCGCGGCCGCATGGAAGAGCGGAAAAG	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT683 KpnI R	CCGGTACCAAATAATGTCTCCTTTTTACTCA	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT691 NotI F	CCGCGGCCGCATGCAAGTCCTAGCAAGTTTATT	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT691 KpnI R	CCGGTACCTTTCTCTTCTAGCGTCATACTCA	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT736 NotI F	CCGCGGCCGCATGCAACTCACCTCACAAG	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT736 KpnI R	CCGGTACCGTCTTTTTTCATATGTGCC	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT744 NotI F	CCGCGGCCGCATGAATATTTATAGATTCATCTCTGGAAG	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT744 KpnI R	CCGGTACCCCATGAGGCATCTGCTAATAG	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT768 NotI F	CCGCGGCCGCATGGATATTCCAGAACAGGGCTC	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
CT768 KpnI R	CCGGTACCAACAACTGCTGTATTGTCTTT	Expression in CT as CyaA, BlaM or GSK-FLAG fusion
	Ectopic Expression and Yeast Toxicity of Novel T3S Effectors	
CT392 KpnI F	CCGGTACCATGTCTTCTATACAAGGAACA	Expression in HeLa
CT392 KpnI +1 F	CCGGTACCAATGTCTTCTATACAAGGAACA	Expression in <i>S. cerevisiae</i>
CT392 XhoI R	CCCTCGAGAAATCCTCTATCATCATCGG	Expression in HeLa or <i>S. cerevisiae</i>
CT460 KpnI F	CCGGTACCATGAGTCAAAATAAGAACTCTGC	Expression in HeLa
CT460 KpnI +1 F	CCGGTACCAATGAGTCAAAATAAGAACTCTGC	Expression in <i>S. cerevisiae</i>
CT460 XhoI R	CCCTCGAGTTTAATGATGTGTTGAGAAACCA	Expression in HeLa or <i>S. cerevisiae</i>
CT537 KpnI F	CCGGTACCATGGGTAGATACAGAAGAG	Expression in HeLa
CT537 KpnI +1 F	CCGGTACCAATGGGTAGATACAGAAGAG	Expression in <i>S. cerevisiae</i>
CT537 XhoI R	CCCTCGAGATCGTTCTCCATAAAAAAGC	Expression in HeLa or <i>S. cerevisiae</i>
CT627 KpnI F	CCGGTACCATGGAAGAATTATTATGC	Expression in HeLa

CT627 KpnI +1 F	CCGGTACCAATGGAAAAGAATTATTATGC	Expression in <i>S. cerevisiae</i>
CT627 XhoI R	CCCTCGAGTGCTGGTTGGTTTTCTTGTTCTT	Expression in HeLa or <i>S. cerevisiae</i>
CT691 KpnI F	CCGGTACCATGCAAGTCCTAGCAAGTTTATT	Expression in HeLa
CT691 KpnI +1 F	CCGGTACCAATGCAAGTCCTAGCAAGTTTATT	Expression in <i>S. cerevisiae</i>
CT691 XhoI R	CCCTCGAGTTTCTCTTCTAGCGTCATACTCA	Expression in HeLa or <i>S. cerevisiae</i>
CT736 KpnI F	CCGGTACCATGCAACTCACCTCACAAG	Expression in HeLa
CT736 KpnI +1 F	CCGGTACCAATGCAACTCACCTCACAAGC	Expression in <i>S. cerevisiae</i>
CT736 XhoI R	CCCTCGAGGTCTTTTTCATATGTGCCCATCA	Expression in HeLa or <i>S. cerevisiae</i>
Yeast Toxicity Truncations of CT392		
CT392 KpnI +1 F	CCGGTACCAATGTCTTCTATACAAGGAACA	Expression in <i>S. cerevisiae</i>
CT392 -20aa C-term XhoI R	CCCTCGAGAAATCCTCTATCATCATCGG	Expression in <i>S. cerevisiae</i>
CT392 -40aa C-term XhoI R	CCCTCGAGAGGGTCTTCCACAGAAGGACTAG	Expression in <i>S. cerevisiae</i>
CT392 -80aa C-term XhoI R	CCCTCGAGTGGACGCATGTAATAGTCTGTGT	Expression in <i>S. cerevisiae</i>
CT392 -100aa C-term XhoI R	CCCTCGAGAAAGAAACGCAAGATCTGGTAGT	Expression in <i>S. cerevisiae</i>
CT392 -20aa N-term KpnI +1 F	CCGGTACCAATGGAGGGGATGAATCCCGAAG	Expression in <i>S. cerevisiae</i>
CT392 -40aa N-term KpnI +1 F	CCGGTACCAATGGGGAAGAGTACCTCTGCA	Expression in <i>S. cerevisiae</i>
CT392 -60aa N-term KpnI +1 F	CCGGTACCAATGGCAATGGCATCTTCTCC	Expression in <i>S. cerevisiae</i>
CT392 -80aa N-term KpnI +1 F	CCGGTACCAATGATCGCGGATGTAATGGAGAA	Expression in <i>S. cerevisiae</i>
CT392 -100aa N-term KpnI +1 F	CCGGTACCAATGGCTCTACTGCAAGCGATTTC	Expression in <i>S. cerevisiae</i>
CT392 -200aa N-term KpnI +1 F	CCGGTACCATAGCGATACGGTTCTAGCTCGC	Expression in <i>S. cerevisiae</i>
CT392 XhoI R	CCCTCGAGAAATCCTCTATCATCATCGG	Expression in <i>S. cerevisiae</i>