

Supplementary Table S1. Oligonucleotides used in this study

Purpose/vector	Oligonucleotide name and sequence
Mutagenesis	
pAA256 (<i>tyeA</i> _{Δ19-59})	ptyeAd20-59a, 5'-AAG GCT CGA GGG ATA TAG ACT CGG TGA-3' (<i>XhoI</i>) and ptyeAd20-59b, 5'-GCG CTT GTC AAC CAG TGC-3' ptyeAd20-59c, 5'-GCA CTG GTT GAC AAG CGC TTT AGC GAT GAG GAG CAA C-3' and ptyeAd20-59d, 5'-AAG GTC TAG AGC GCT AAC CAC AAT GTC A-3' (<i>XbaI</i>)
pSF019 ($\Delta yopN$, <i>tyeA</i>)	pyopNa1, 5'-AAA GTC GAC ACC TAC AAT GCC ATG ACG-3' (<i>Sall</i>) and pyopNtyeAb, 5'-AGA TAG GTT ATG AAG CGT CG-3' pyopNtyeAc, 5'-ACG CTT CAT AAC CTA TCT TTT AGC GAT GAG GAG CAA CGG-3' and ptyeAd, 5'-AAA TCT AGA GAA CGC GCT AAC CAC-3' (<i>XbaI</i>)
pAA251 (<i>yopN</i> _{Yps→Yen})	pyopNa2, 5'-AAA GTC GAC CTG CGG CTT GCG TGA TG-3' (<i>Sall</i>) and pyopN-23b, 5'-TAT ACC CTC TGA AAA AAA TTG CC-3' pyopN-23c, 5'-TTT CAG AGG GTA TAA CTA ATG GCG T-3' and ptyeAd (<i>XbaI</i>)
pAA242 (<i>yopN</i> _{278(F+1)} <i>tyeA</i>)	pyopNa2 (<i>Sall</i>) and pyopN278+1b, 5'-CCA AAT CCT TTA ACT TGG TCA-3' pyopN278+1c, 5'-CCA AGT TAA AGG ATT TGG CAA TTT TTT TCA GA-3' and ptyeAd (<i>XbaI</i>)
pAA243 (<i>yopN</i> _{278(F+1)} , <i>SDtyeA</i>)	pyopNa2 (<i>Sall</i>) and ptyeA-SDb, 5'-ACG TTG AAA AAA ATT GCC AAA TCC TTT AAC TTG GTC-3' ptyeA-SDc, 5'-GGA TTT GGC AAT TTT TTT CAA CGT GTA AAA CTA ATG GCG T-3' and ptyeAd (<i>XbaI</i>)
pAA247 (<i>yopN</i> _{287(F+1)} <i>tyeA</i>)	pyopNa2 (<i>Sall</i>) and pyopN287+1b, 5'-ACG CCA TAG TTT TAC CCT CTG-3' pyopN287+1c, 5'-CAG AGG GTA AAA CTA TGG CGT ACG ACC TTT CTG-3' and ptyeAd (<i>XbaI</i>)
pAA248 (<i>yopN</i> _{287(F+1)} , <i>SDtyeA</i>)	pyopNa2 (<i>Sall</i>) and ptyeA-SDb2, 5'-CCA TAG TTT TGC CTT CGG AAA AAA ATT GCC AAA ATC C-3' ptyeA-SDc2, 5'-CCG AAG GCA AAA CTA TGG CGT ACG ACC TTT CTG-3' and ptyeAd (<i>XbaI</i>)
pAA252 (<i>yopN</i> _{F278F})	pyopNa2 (<i>Sall</i>) and pyopN-24b, 5'-GAA TCC TTT AAC TTG GTC AC-3' pyopN-24c, 5'-ATT CTG GCA ATT TTT TTC AGA GG-3' and ptyeAd (<i>XbaI</i>)
pAA253 (<i>yopN</i> _{W279F})	pyopNa2 (<i>Sall</i>) and pyopN-26b, 5'-GAA AAA TCC TTT AAC TTG GTC-3' pyopN-26c, 5'-GAT TTT TCC AAT TTT TTT CAG AGG-3' and ptyeAd (<i>XbaI</i>)
pAA254 (<i>yopN</i> _{Δ278F})	pyopNa2 (<i>Sall</i>) and pyopN-28b, 5'-TCC TTT AAC TTG GTC ACT C-3' pyopN-28c, 5'-GTT AAA GGA TGG CAA TTT TTT TCA G-3' and ptyeAd (<i>XbaI</i>)

pAA255 (*yopN*_{Δ279W})

pyopNa2 (*Sall*) and pyopN-28b

pyopN-29c, 5'-AAG GAT TTC AAT TTT TTT CAG AGG-3' and ptyeAd (*XbaI*)

pUA066 (YPTB0523 polar mutation)

pFpNQ066, 5'-TAT CGA TAC CGT CGA CTA TTC ACC CTG GCC CTA ACG C-3' (*Sall*) and pRpNQ066, 5'-ATG CGG TAC CTC TAG AAG TGC ACC GCC CAC GAG CAG T-3' (*XbaI*)

Ectopic expression

pAA269 (pYopN⁺, TyeA⁺) and
pAA271 (pYopN_{278(F+1),SD}TyeA⁺)

pYopN-bla_for, 5'-ACGTGGATCCTCTCAGGGAGTAGTTATGACG-3' (*BamHI*) and
YopN-TyeA_rev, 5'-ACGTGAAATTCAGATCCTGGCAGAAATGG-3' (*EcoRI*)

pAA304 (pYopN⁺, TyeA-Flag[®]),
pAA305 (pYopN_{Yps→Yen}⁺, TyeA-
Flag[®]), pAA306

(pYopN_{278(F+1)}TyeA-Flag[®]),
pAA307 (pYopN_{278(F+1),SD}TyeA-
Flag[®]), pAA308

(pYopN_{287(F+1)}TyeA-Flag[®]),
pAA309 (pYopN_{287(F+1),SD}TyeA-
Flag[®])

pYopN-bla_for (*BamHI*) and pYopN-TyeAflag_rev, 5'-
ACGTGAAATTCCTACTTGTCATCGTCGTCCTTGTAGTCATCCAACCTCAATTC
TTCC-3' (*EcoRI*)
