Table S1. Plasmids used in this study.

Plasmid name	Relevant characteristics ^a	Source
pET-41a(+)	Protein expression vector; Km ^r	Novagen
pET-41a(+)::TEV	Protein expression vector with modified protease cleavage site; Km ^r	This study
pET-32a (+)	Protein expression vector; Ap ^r	Novagen
pET-32a (+)::TEV	Protein expression vector with modified protease cleavage site; Ap ^r	This study
pBirACm	pACYC184 with inducible biotin ligase; Cm ^r	Avidity
pET-41a::TEV:: <i>speC_{WT}</i> ::biotin	speC inserted into Ncol and BamHI of pET-41a with a C-terminal biotin tag; Km ^r	This study
pET-41a::TEV:: <i>speC_{Y15A}</i>	$speC_{Y15A}$ inserted into $Ncol$ and $BamHI$ sites of pET-41a; Km^r	[34]
pET-41a::TEV:: <i>speC_{D203A}</i>	$speC_{D203A}$ inserted into $Ncol$ and $BamHI$ sites of pET-41a; Km^r	[35]
pET-41a::TEV:: <i>speC_{Y15A/ D203A}</i>	$speC_{ m Y15A/D203A}$ inserted into $\it Ncol$ and $\it Bam$ HI sites of pET-41a; $\it Km^r$	This study
pUC57:: <i>scFv5T4</i> ::biotin	Codon optimized scFv5T4 sequence with <i>Ncol</i> and <i>Hin</i> dIII sites; C-terminal biotin tag; Ap ^r	GenScript
pET-32a:: <i>scFv5T4</i> :: <i>mRFP1</i>	scFv5T4 from pUC57::scFv5T4 inserted into Ncol and HindIII sites of pET-32a; mRFP1 inserted into EcoRI and HindIII sites replacing biotin tag of scFv5T4; Ap ^r	This study
pET-32a::TEV:: <i>scFv5T4</i> ::biotin tag	scFv5T4 from pUC57::scFv5T4 inserted into Ncol and HindIII sites of pET-32a; Ap ^r	This study
pET-32a::TEV:: <i>scFv5T4</i> :: <i>speC_{D203A}</i> ::biotin	$scFv5T4$ from pUC57:: $scFv5T4$ inserted into $Ncol$ and $HindIII$ sites of pET-32a; translationally fused with $speC_{D203A}$ with an engineered gly-gly-pro (GGP) linker; C-terminal biotin tag; Ap^r	This study
pET-32a::TEV:: <i>scFv5T4</i> :: <i>speC_{Y15A/D203A}::biotin</i>	scFv5T4 from pUC57::scFv5T4 inserted into Ncol and HindIII sites of pET-32a; translationally fused with speC _{Y15A/D203A} with an engineered gly-gly-pro (GGP) linker; C-terminal biotin tag; Ap ^r	This study
pCMV6-XL5	Mammalian expression vector; Ap ^r	Origene
pCMV6-XL5:: <i>egfp</i>	egfp from pEGFP-N1 inserted into Sacl and Xbal sites of pCMV6-XL5; Ap ^r	This study
pCMV6-XL5:: <i>5t4</i>	5T4 inserted into $\it Eco$ RI and $\it Sal$ I sites of pCMV6-XL5; $\it Ap^{r}$	Origene
pEGFP-N1	Mammalian expression vector with N-terminal EGFP tag; Km ^r	Clontech
pEGFP-N1:: <i>5t4</i>	5t4 from pCMV6-XL5::5t4 inserted into <i>Eco</i> RI and <i>Bam</i> HI sites of pEGFP-N1; Km ^r	This study

^a Kn^r, Kanamycin resistance; Ap^r, Ampicillin resistance; Cm^r, Chloramphenicol resistance