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Supplementary information

Translation-dependent degradation of *cas12* mRNA triggered by an anti-CRISPR

In the format provided by the authors and unedited

Fig. 1a

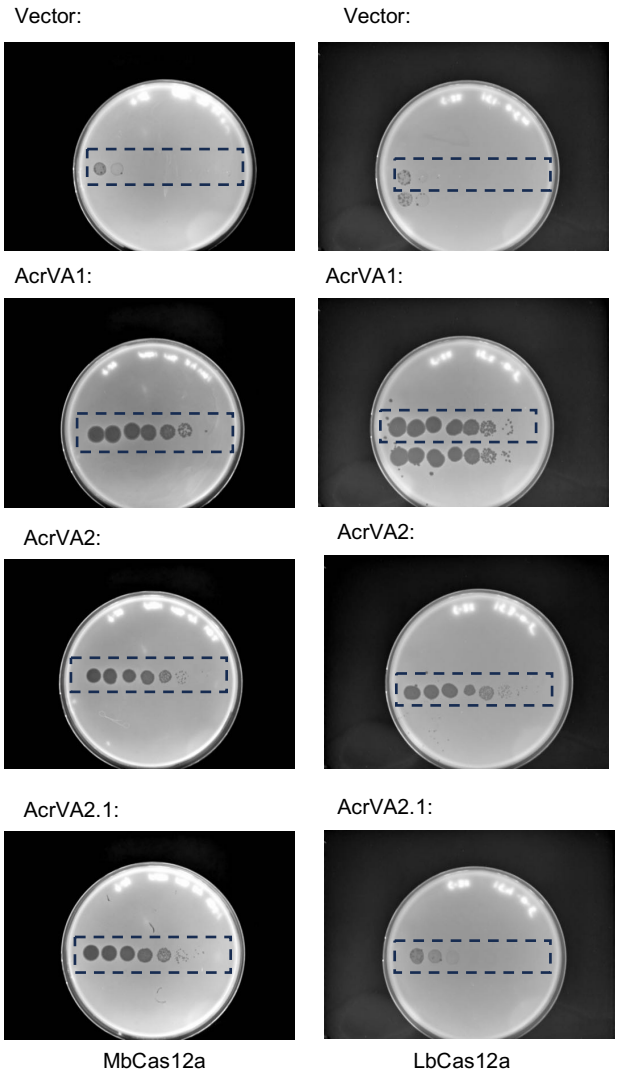


Fig. 1b

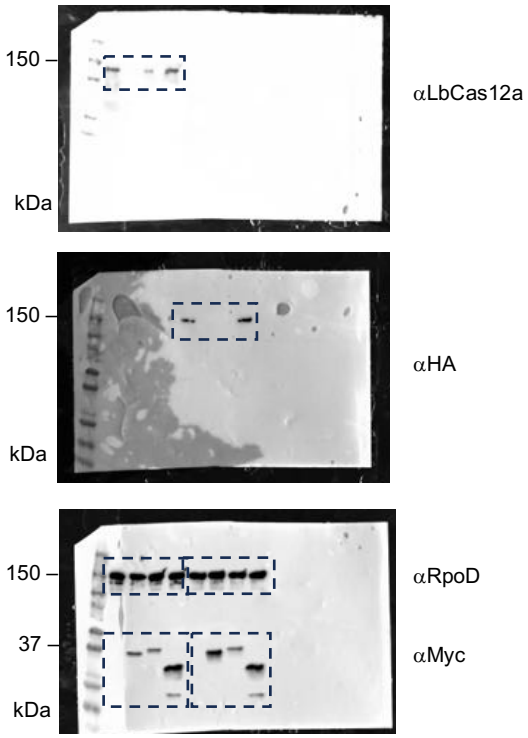


Fig. 2c

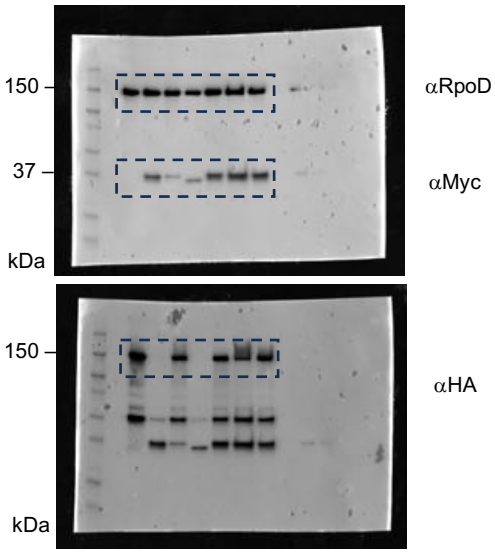


Fig. 2d

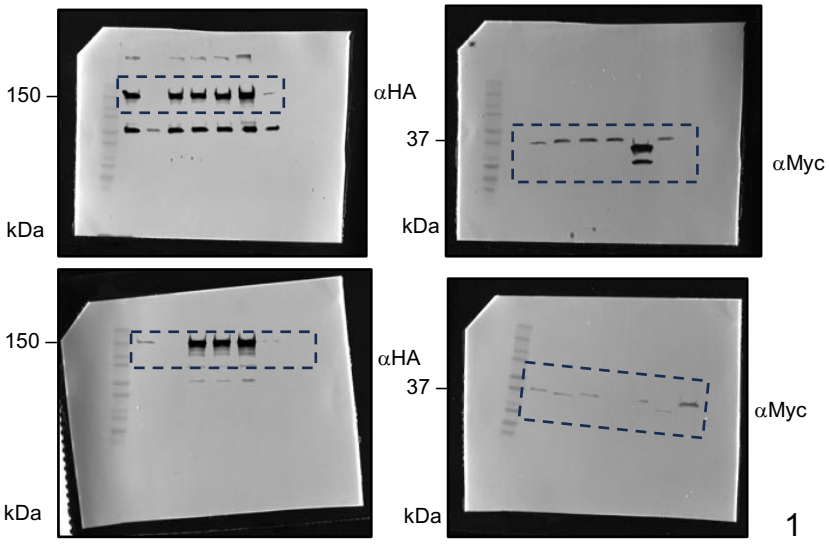


Fig. 2e

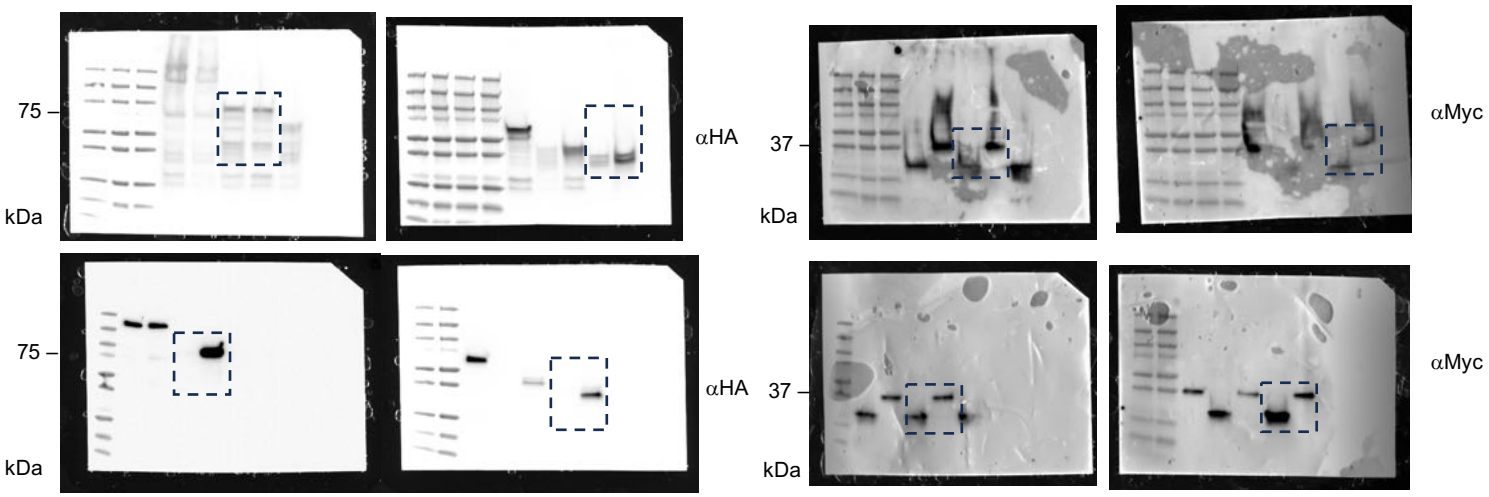


Fig. 2f

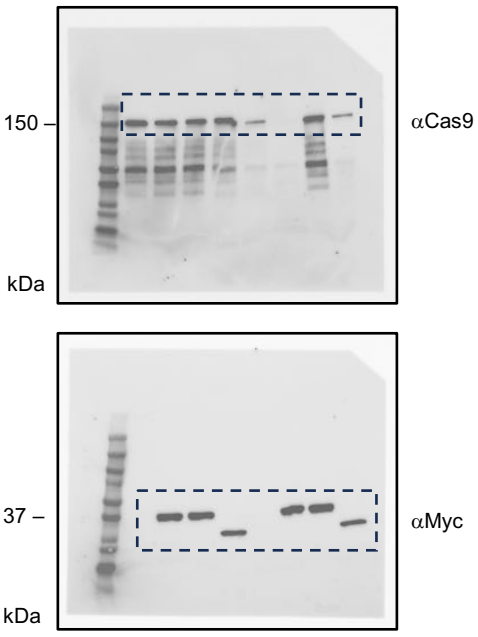


Fig. 2g

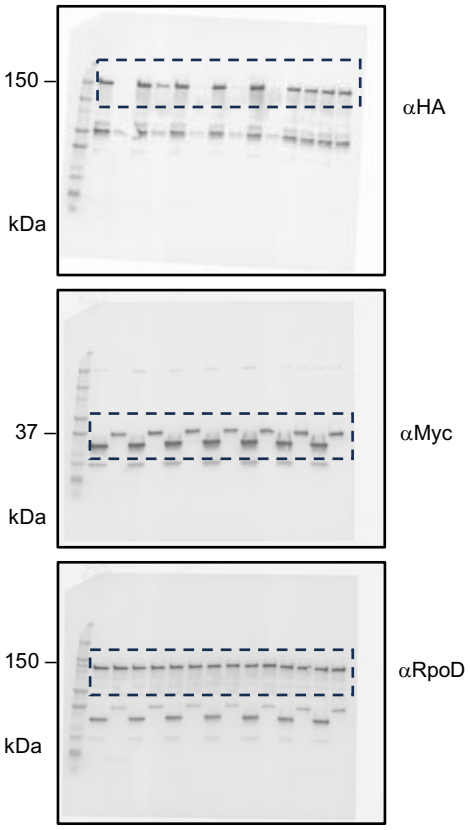


Fig. 2j

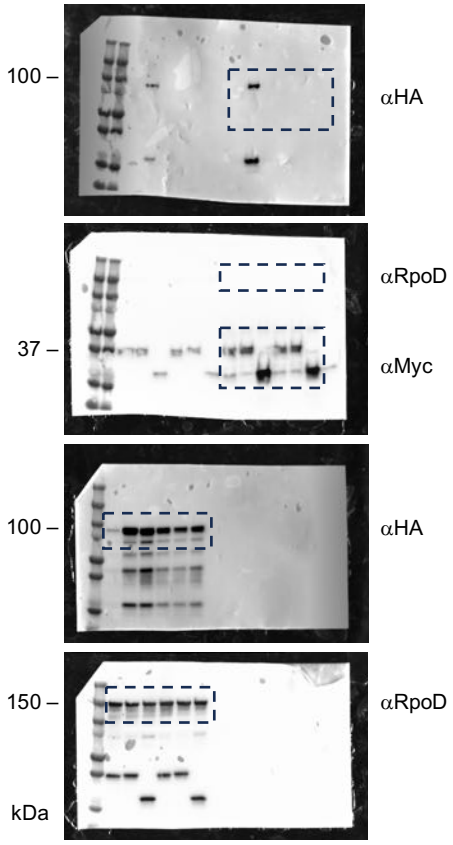


Fig. 2h

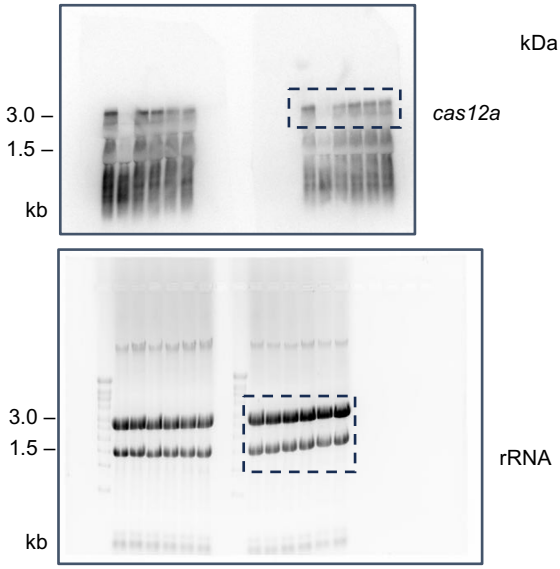


Fig. 2k

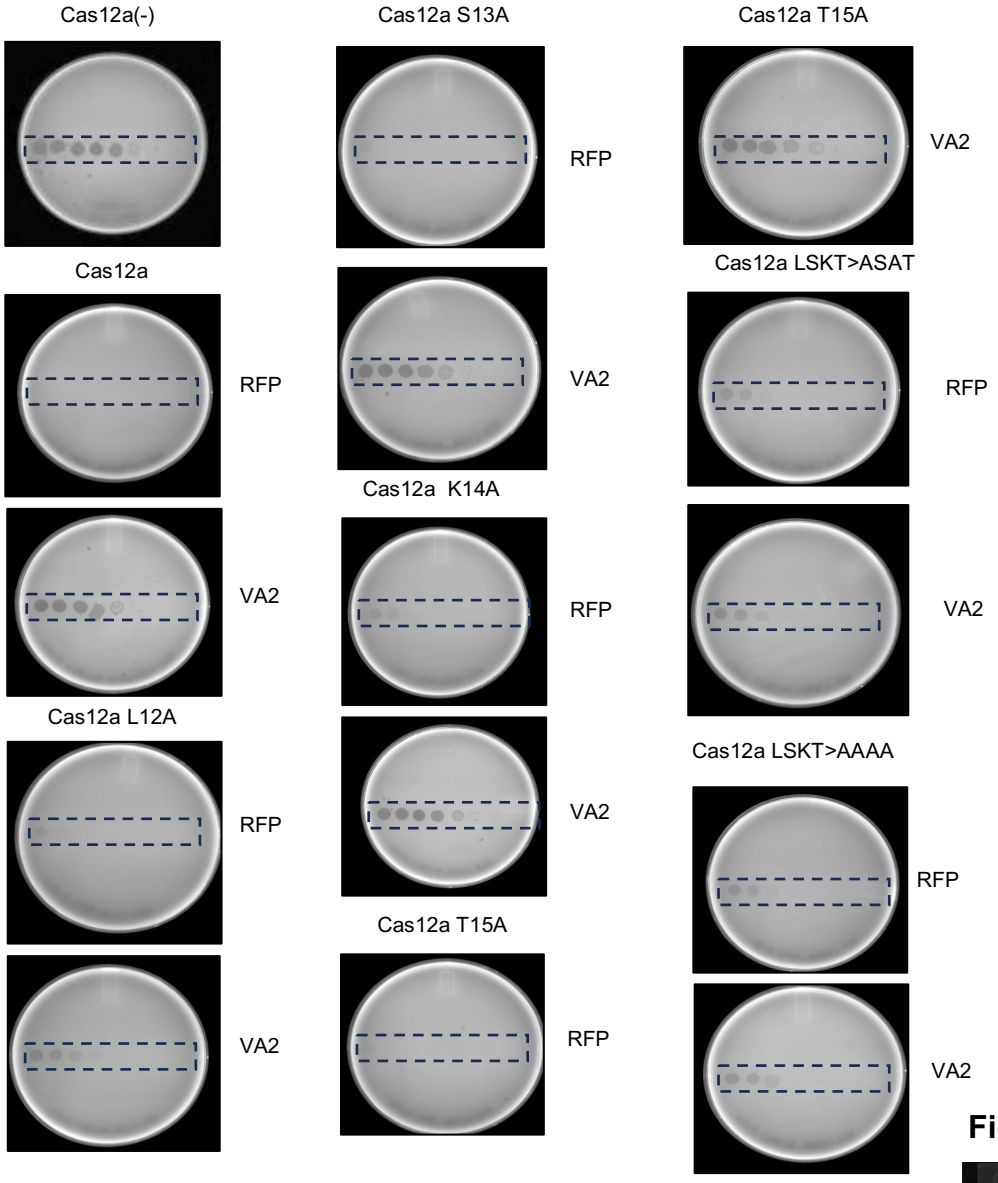


Fig. 3a

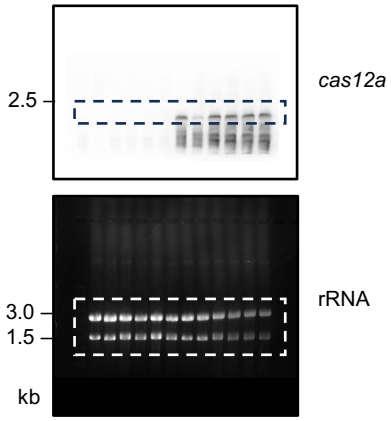


Fig. 3b

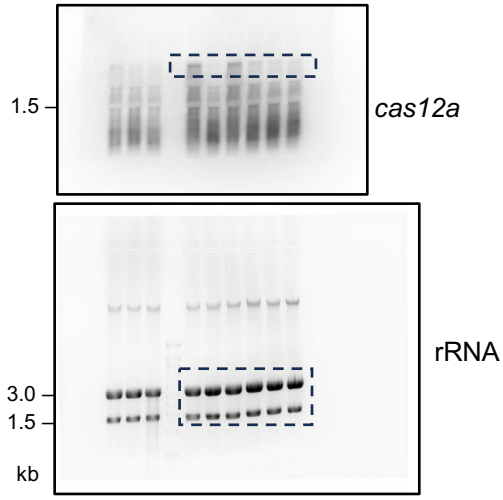


Fig. 3c

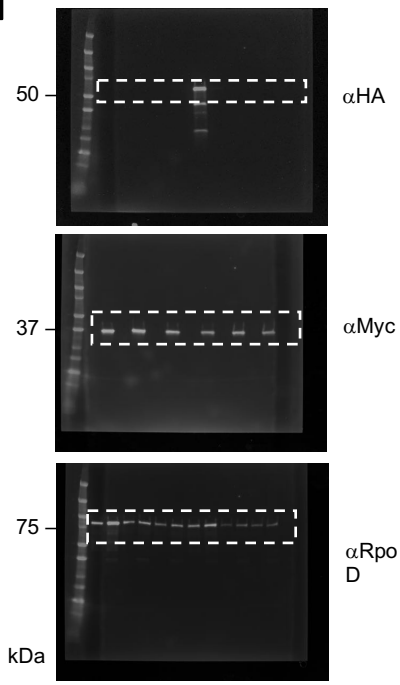


Fig. 3d

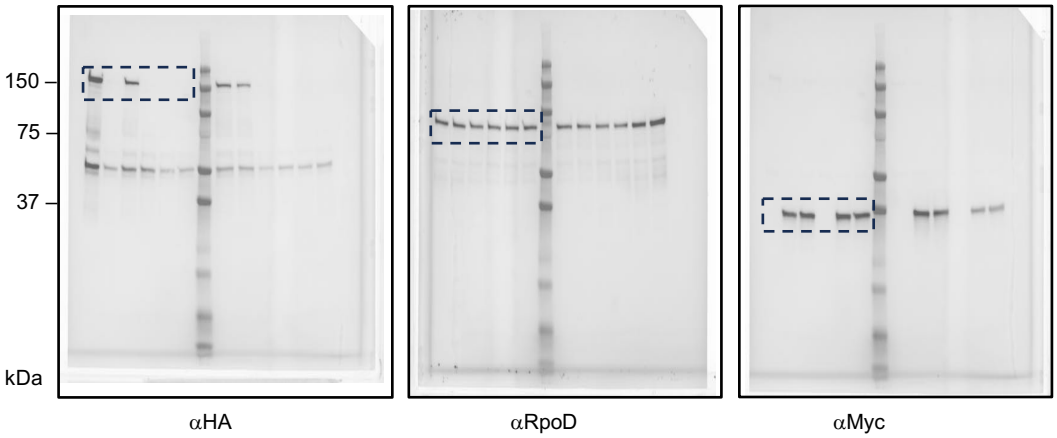
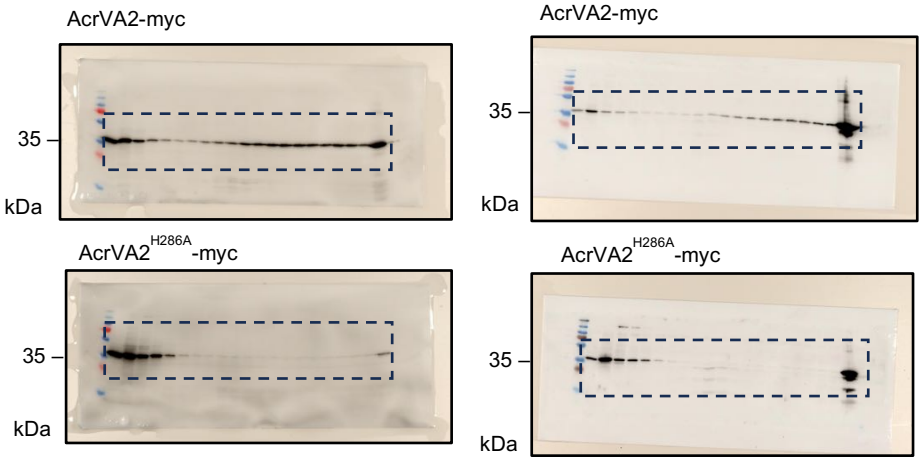
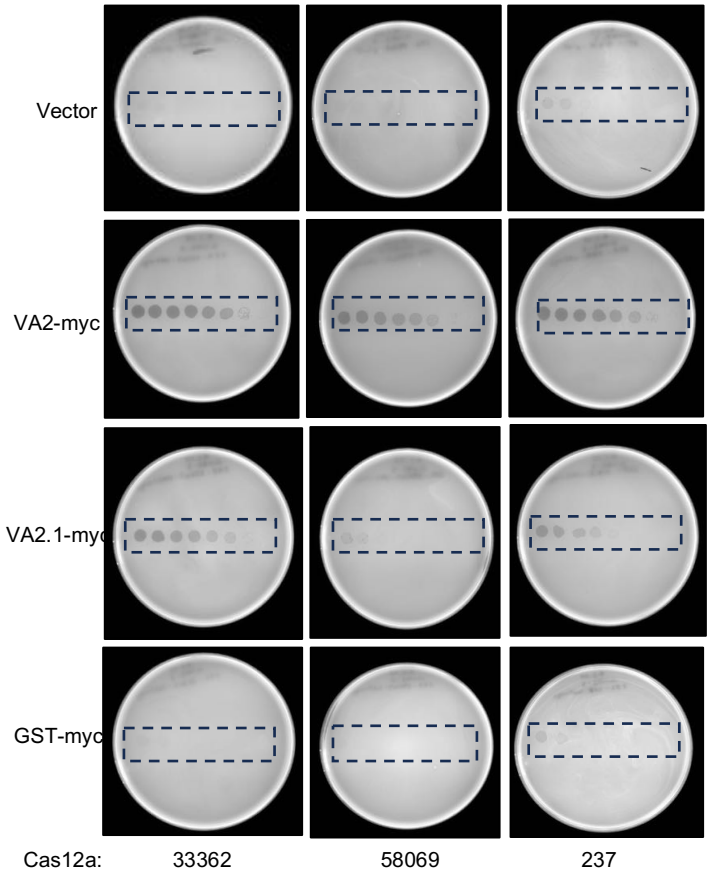


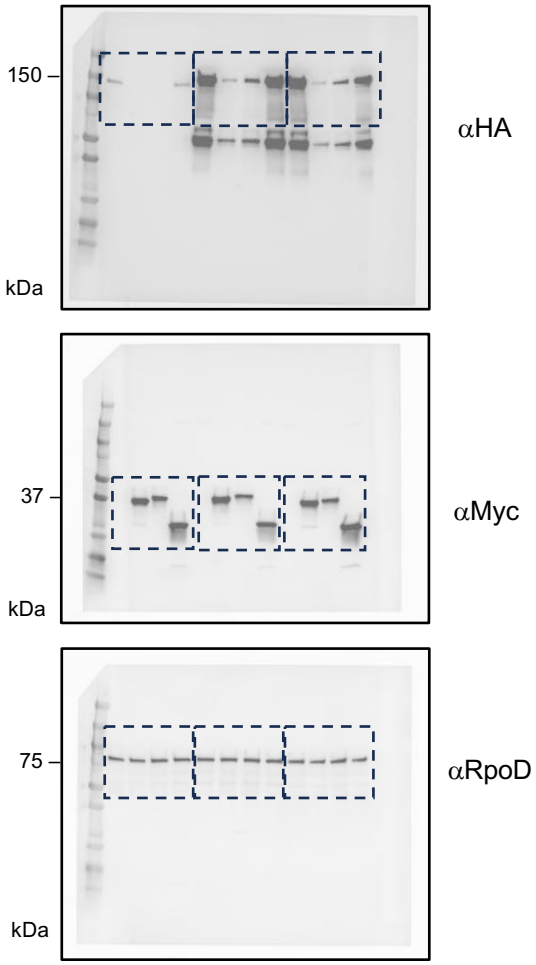
Fig. 3e



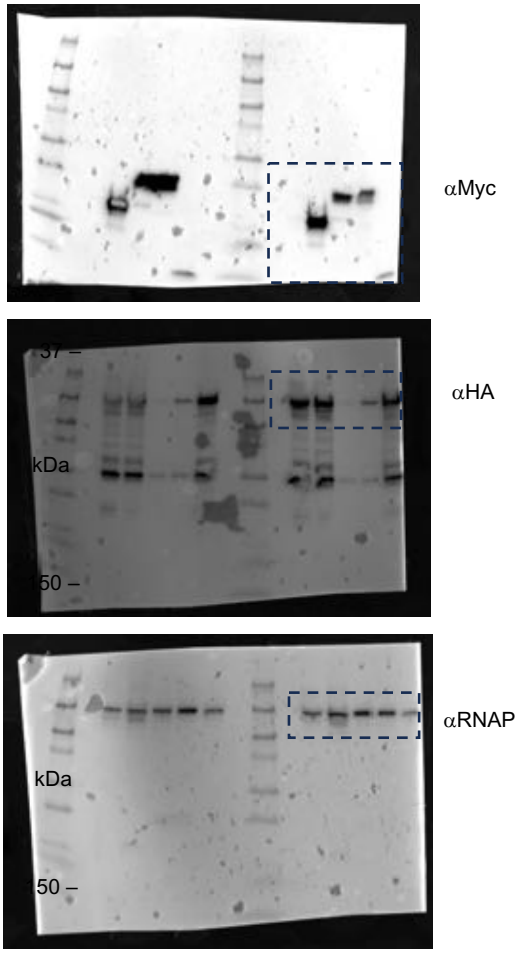
Extended Data Fig. 1a



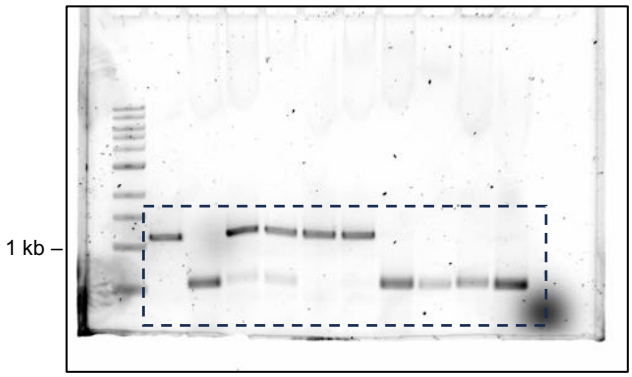
Extended Data Fig. 1b



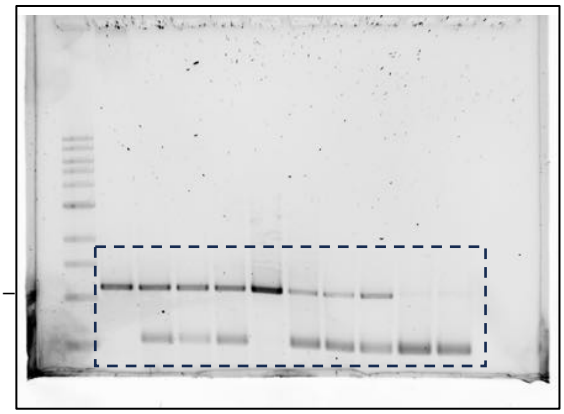
Extended Data Fig. 1d



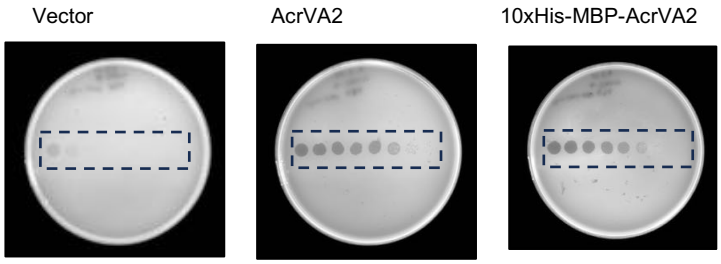
Extended Data Fig. 3a



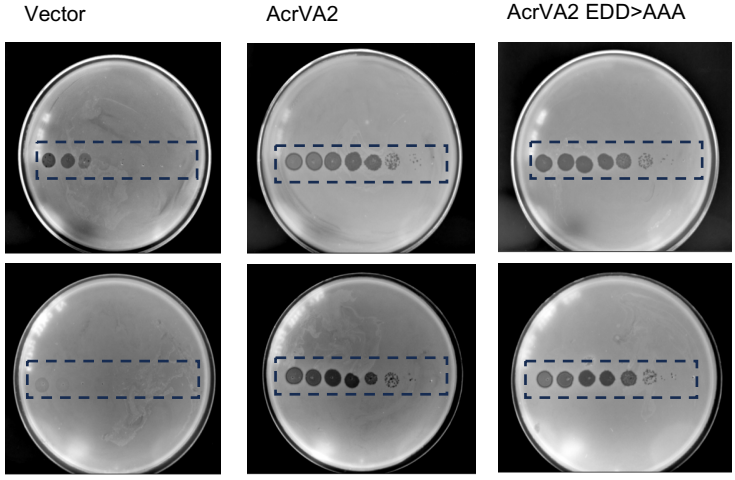
Extended Data Fig. 3b



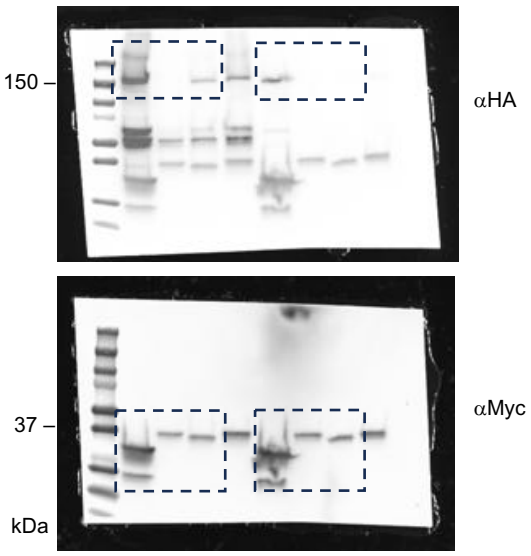
Extended Data Fig. 3c



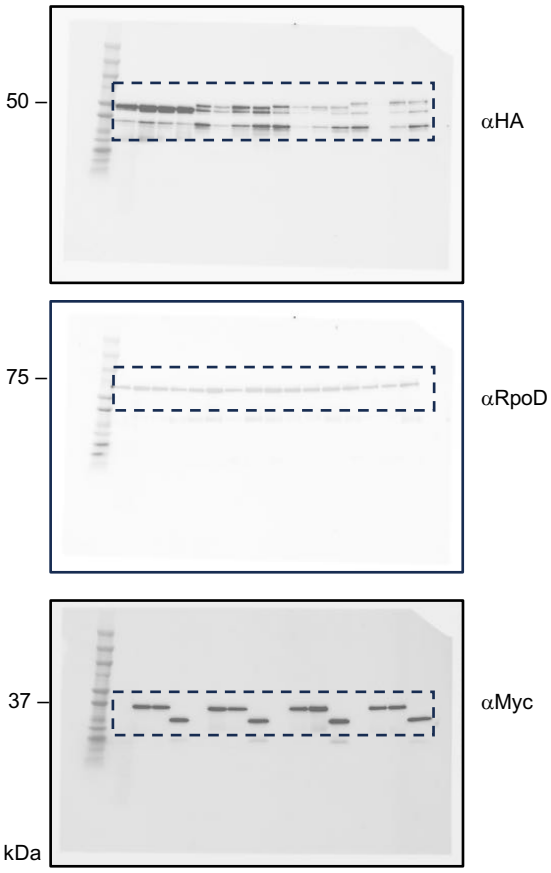
Extended Data Fig. 3d



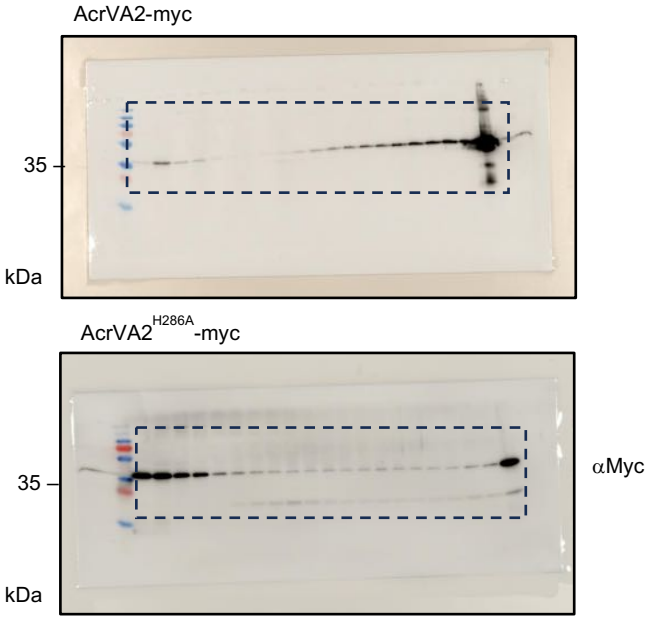
Extended Data Fig. 3e



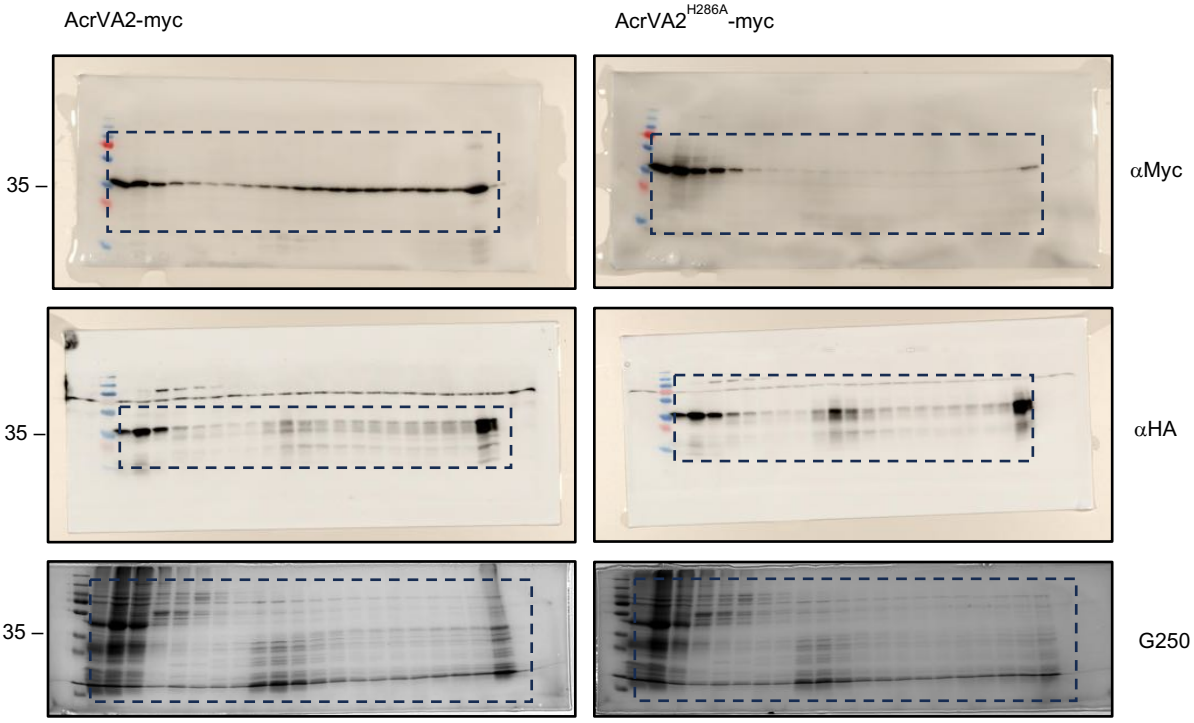
Extended Data Fig. 4c



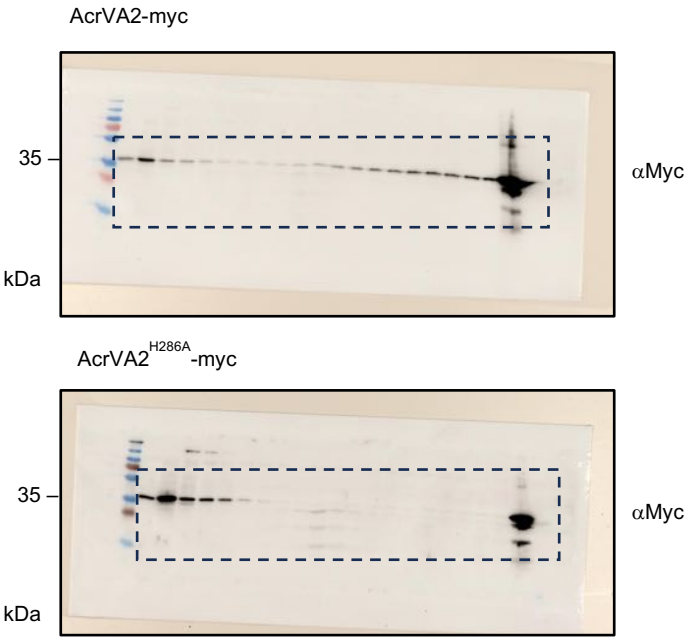
Extended Data Fig. 7b



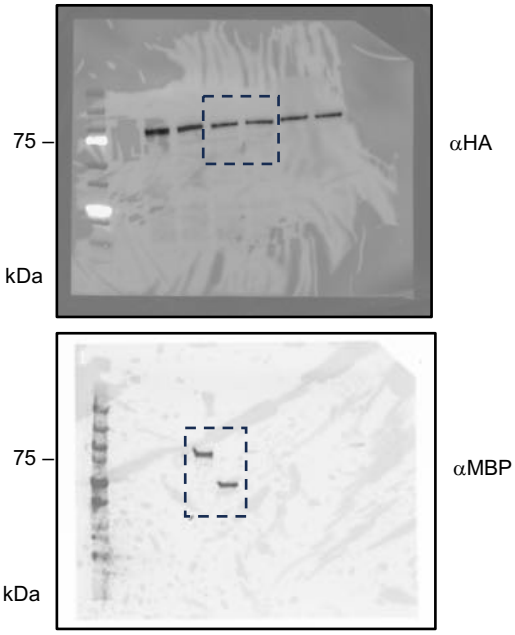
Extended Data Fig. 7a



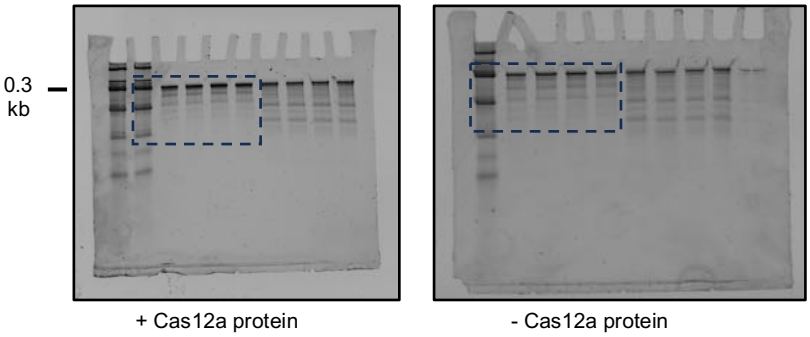
Extended Data Fig. 7c



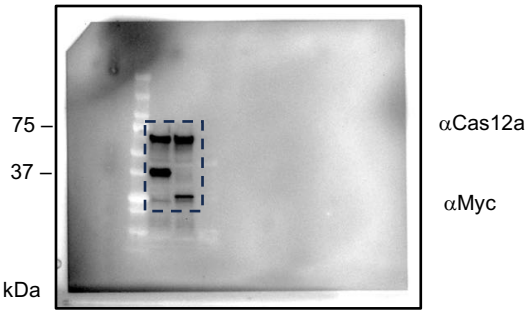
Extended Data Fig. 8b



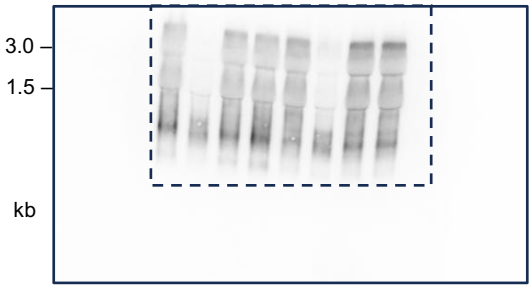
Extended Data Fig. 8a

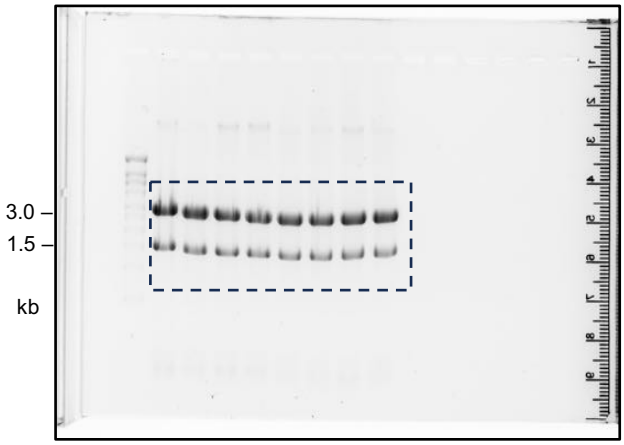


Extended Data Fig. 8c

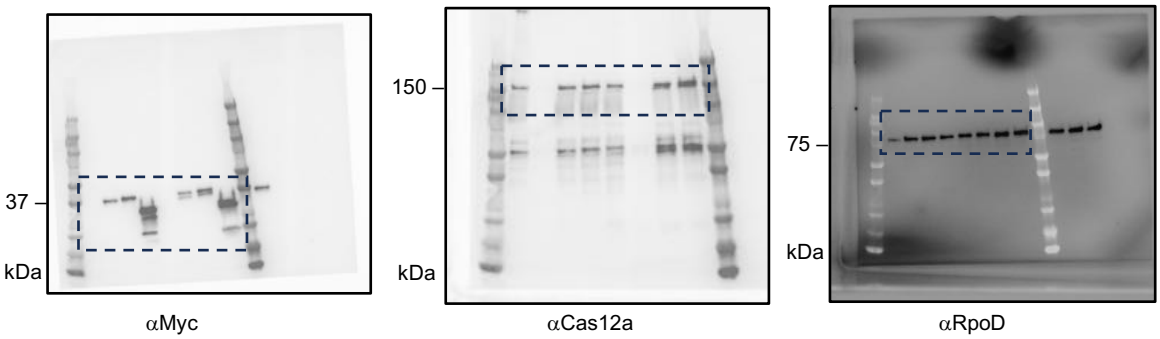


Extended Data Fig. 9a

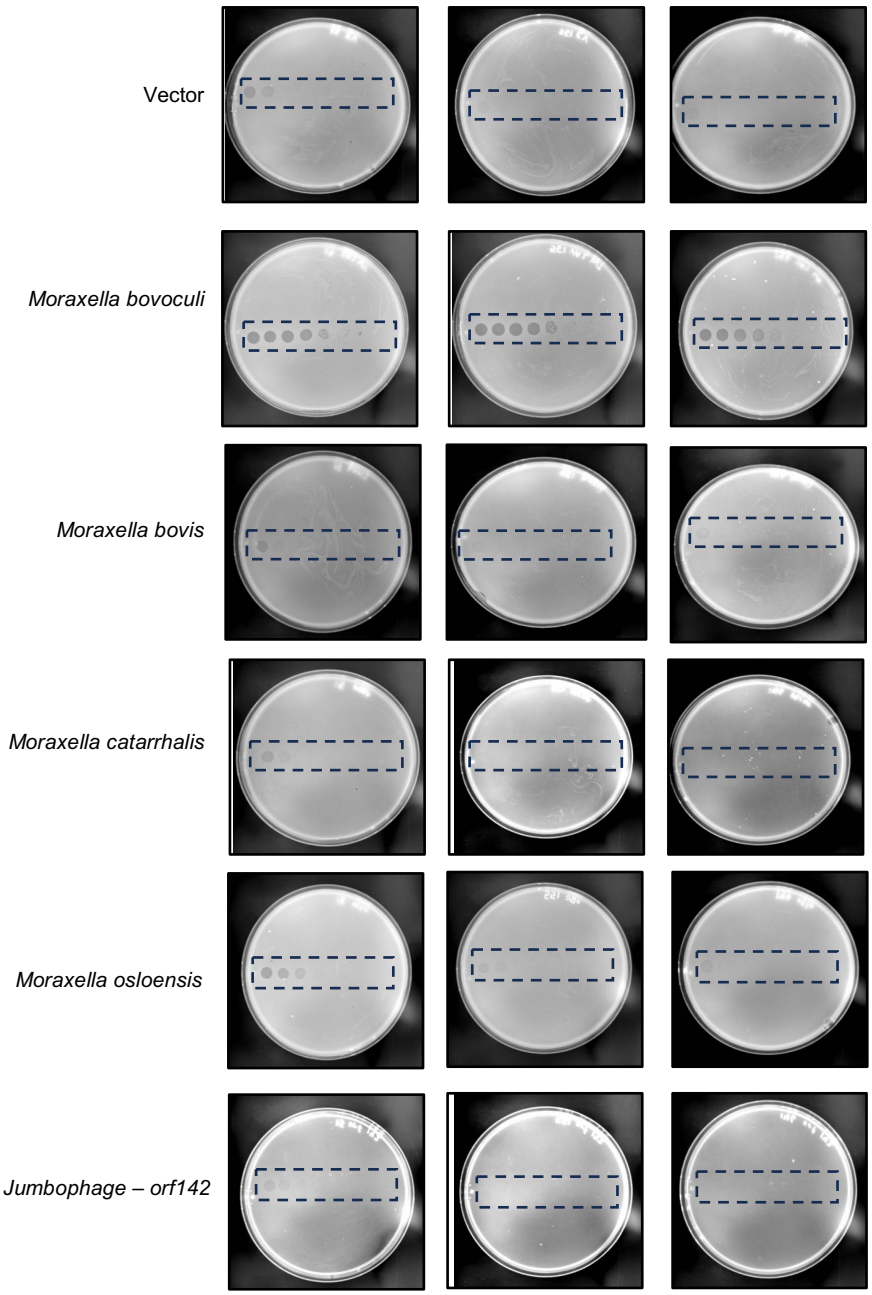


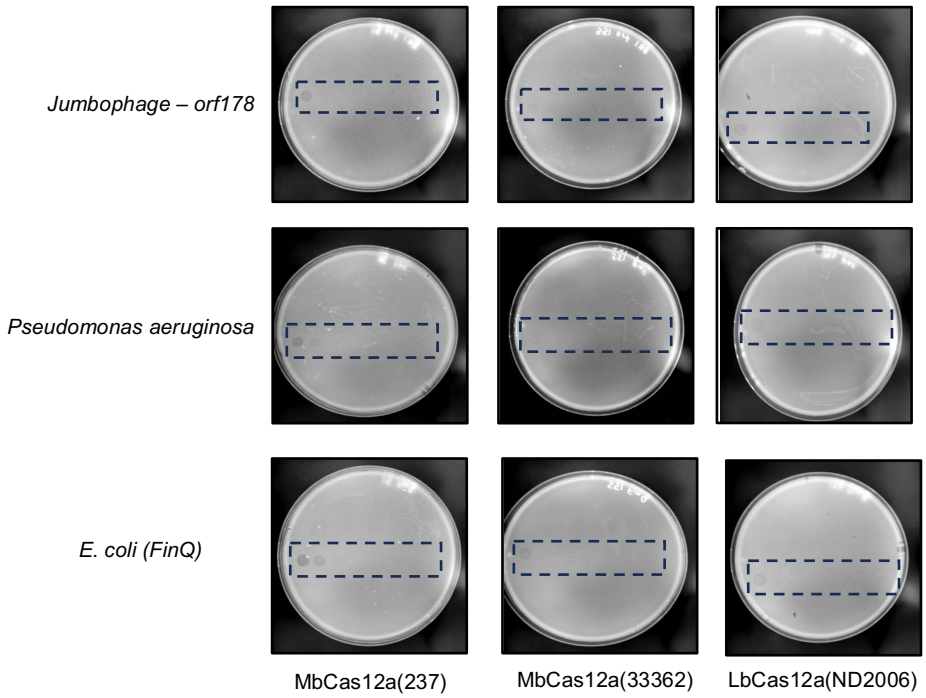


Extended Data Fig. 9b



Extended Data Fig. 10





Supplementary Figure 1. Uncropped images used in main figures and extended data. Black dash boxes indicate areas cropped for the main figure.

Supplementary Table 1. Strains used in this study.

Figure 1a

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})cRNA24 pHERD30T
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA1-Myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})cRNA24 pHERD30T-AcrVA2-Myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2.1-Myc

PAO1 tn7::(P_{lac})LbCas12a attB::(P_{ara})crRNA24 pHERD30T
PAO1 tn7::(P_{lac})LbCas12a attB::(P_{ara})crRNA24 pHERD30T-AcrVA1-myc
PAO1 tn7::(P_{lac})LbCas12a attB::(P_{ara})crRNA24 pHERD30T-AcrVA2-myc
PAO1 tn7::(P_{lac})LbCas12a attB::(P_{ara})crRNA24 pHERD30T-AcrvA2.1-myc

Figure 1b,c

PAO1 tn7::(P_{ara})MbCas12a(237)^{native}-3xHA pHERD20T
PAO1 tn7::(P_{ara})MbCas12a(237)^{native}-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{native}-3xHA pHERD20T-AcrVA2.1-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{native}-3xHA pHERD20T-RFP-myc

PAO1 tn7::(P_{lac})LbCas12a attB::(P_{ara})crRNA24 pHERD30T-myc
PAO1 tn7::(P_{lac})LbCas12a attB::(P_{ara})crRNA24 pHERD30T-AcrVA2-myc
PAO1 tn7::(P_{lac})LbCas12a attB::(P_{ara})crRNA24 pHERD30T-AcrVA2.1-myc
PAO1 tn7::(P_{lac})LbCas12a attB::(P_{ara})crRNA24 pHERD30T-RFP-myc

Figure 1d

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrIIA4-Myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})cRNA24 pHERD30T-AcrVA2-Myc

Figure 2a

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA pHERD30T
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB(P_{ara})::crRNA24 pHERD30T
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB(P_{ara})::crRNA24 pHERD30T-AcrVA2
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB(P_{ara})::crRNA24 pHERD30T-AcrVA2^{W79A}
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB(P_{ara})::crRNA24 pHERD30T-AcrVA2^{D89A}
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB(P_{ara})::crRNA24 pHERD30T-AcrVA2^{C213A}
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB(P_{ara})::crRNA24 pHERD30T-AcrVA2^{D218A}
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB(P_{ara})::crRNA24 pHERD30T-AcrVA2^{H286A}
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB(P_{ara})::crRNA24 pHERD30T-AcrVA2^{R288A}
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB(P_{ara})::crRNA24 pHERD30T-AcrVA2^{H291A}

PAO1 tn7::(P_{lac})LbCas12a(ND2006) pHERD30T
PAO1 tn7::(P_{lac})LbCas12a(ND2006) attB::(P_{ara})crRNA24 pHERD30T
PAO1 tn7::(P_{lac})LbCas12a(ND2006) attB::(P_{ara})crRNA24 pHERD30T-AcrVA2
PAO1 tn7::(P_{lac})LbCas12a(ND2006) attB::(P_{ara})crRNA24 pHERD30T-AcrVA2^{W79A}
PAO1 tn7::(P_{lac})LbCas12a(ND2006) attB::(P_{ara})crRNA24 pHERD30T-AcrVA2^{D89A}
PAO1 tn7::(P_{lac})LbCas12a(ND2006) attB::(P_{ara})crRNA24 pHERD30T-AcrVA2^{C213A}
PAO1 tn7::(P_{lac})LbCas12a(ND2006) attB::(P_{ara})crRNA24 pHERD30T-AcrVA2^{D218A}
PAO1 tn7::(P_{lac})LbCas12a(ND2006) attB::(P_{ara})crRNA24 pHERD30T-AcrVA2^{H286A}
PAO1 tn7::(P_{lac})LbCas12a(ND2006) attB::(P_{ara})crRNA24 pHERD30T-AcrVA2^{R288A}
PAO1 tn7::(P_{lac})LbCas12a(ND2006) attB::(P_{ara})crRNA24 pHERD30T-AcrVA2^{H291A}

Figure 2c
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2^{W79A}-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2^{D89A}-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2^{H286A}-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2^{R288A}-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2^{H291A}-myc

Figure 2d
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2^{W79A}-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2^{D89A}-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2^{H286A}-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2^{R288A}-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2^{H291A}-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-RFP-myc

Figure 2e
PAO1 tn7::(P_{ara})MbCas12a(237)^{560aa}-RFP-3xHA pHERD20T-GST-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{560aa}-RFP-3xHA pHERD20T-AcrVA2^{H286A}-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{30aa}-RFP-3xHA pHERD20T-GST-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{30aa}-RFP-3xHA pHERD20T-AcrVA2^{H286A}-myc

Figure 2f
PAO1 tn7::(P_{lac})SpCas9-3xHA pHERD20T
PAO1 tn7::(P_{lac})SpCas9-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{lac})SpCas9-3xHA pHERD20T-AcrVA2 H286A-myc
PAO1 tn7::(P_{lac})SpCas9-3xHA pHERD20T-GST-myc
PAO1 tn7::(P_{lac})MbCas12a(237)^{30aa}-SpCas9-3xHA pHERD20T
PAO1 tn7::(P_{lac})MbCas12a(237)^{30aa}-SpCas9-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{lac})MbCas12a(237)^{30aa}-SpCas9-3xHA pHERD20T-AcrVA2 H286A-myc
PAO1 tn7::(P_{lac})MbCas12a(237)^{30aa}-SpCas9-3xHA pHERD20T-GST-myc

Figure 2g
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)^{L12A}-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)^{L12A}-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)^{S13A}-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)^{S13A}-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)^{K14A}-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)^{K14A}-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)^{T15A}-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)^{T15A}-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)$^{LSKT>ASAT}$-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)$^{LSKT>ASAT}$-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)$^{LSKT>AAAA}$-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)$^{LSKT>AAAA}$-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24

Figure 2h
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA pHERD20T
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA pHERD20T-AcrVA2^{H286A}-myc
PAO1 tn7::(P_{ara})MbCas12a(237)$^{LSKT>AAAA}$-3xHA pHERD20T
PAO1 tn7::(P_{ara})MbCas12a(237)$^{LSKT>AAAA}$-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(237)$^{LSKT>AAAA}$-3xHA pHERD20T-AcrVA2^{H286A}-myc

Figure 2i
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA pHERD20T-RFP-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(237)$^{LSKT>AAAA}$-3xHA pHERD20T-RFP-myc
PAO1 tn7::(P_{ara})MbCas12a(237)$^{LSKT>AAAA}$-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(237)$^{silent\ mutation}$-3xHA pHERD20T-RFP-myc
PAO1 tn7::(P_{ara})MbCas12a(237)$^{silent\ mutation}$-3xHA pHERD20T-AcrVA2-myc

Figure 2j
PAO1 tn7::(P_{ara})MbCas12a(237)^{560aa}-RFP-3xHA pHERD20T-GST-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{560aa}-RFP-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{560aa}-RFP-3xHA pHERD20T-AcrVA2^{H286A}-myc
PAO1 tn7::(P_{ara})MbCas12a(237)LSKT>AAAA^{560aa}-RFP-3xHA pHERD20T-GST-myc
PAO1 tn7::(P_{ara})MbCas12a(237)LSKT>AAAA^{560aa}-RFP-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(237)LSKT>AAAA^{560aa}-RFP-3xHA pHERD20T-AcrVA2^{H286A}-myc

Figure 2k
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)^{L12A}-3xHA attB::(Para)RFP-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)^{L12A}-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)^{S13A}-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)^{S13A}-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)^{K14A}-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)^{K14A}-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)^{T15A}-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)^{T15A}-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)$^{LSKT>ASAT}$-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)$^{LSKT>ASAT}$-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)$^{LSKT>AAAA}$-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)$^{LSKT>AAAA}$-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24

Figure 2l
PAO1 pHERD20T-crRNA24
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA pHERD20T-crRNA24

PAO1 tn7::(P_{ara})MbCas12a(237)$^{LSKT>AAAA}$-3xHA pHERD20T-crRNA24

Figure 3a,b

MG1655 attTn7::(P_{lac})MbCas12a(237)^{299aa}-RFP-3xHA pHERD20T

MG1655 attTn7::(P_{lac})MbCas12a(237)^{299aa}-RFP-3xHA pHERD20T-AcrVA2-myc

Figure 3c,d

PAO1 attTn7::(P_{ara})MbCas12a(237)-3xHA pHERD20T

PAO1 attTn7::(P_{ara})MbCas12a(237)-3xHA pHERD20T-AcrVA2-myc

PAO1 attTn7::(P_{ara})MbCas12a(237)-3xHA pHERD20T-AcrVA2^{H286A}-myc

PAO1 attTn7::(P_{ara})MbCas12a(237)$^{\Delta AUG}$-3xHA pHERD20T

PAO1 attTn7::(P_{ara})MbCas12a(237)$^{\Delta AUG}$-3xHA pHERD20T-AcrVA2-myc

PAO1 attTn7::(P_{ara})MbCas12a(237)$^{\Delta AUG}$-3xHA pHERD20T-AcrVA2^{H286A}-myc

Figure 3e

MG1655 attTn7::(P_{ara})3xHA-MbCas12a(237)^{299aa}-SecM pHERD20T-AcrVA2-myc

MG1655 attTn7::(P_{ara})3xHA-MbCas12a(237)^{299aa}-SecM pHERD20T-AcrVA2^{H286A}-myc

Extended Data Figure 1a,b

PAO1 attTn7:(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA pHERD20T

PAO1 attTn7:(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA pHERD20T-AcrVA2-myc

PAO1 attTn7:(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA pHERD20T-AcrVA2.1-myc

PAO1 attTn7:(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA pHERD20T-GST-myc

PAO1 attTn7:(P_{ara})MbCas12a(58069)-3xHA attB::(P_{ara})crRNA pHERD20T

PAO1 attTn7:(P_{ara})MbCas12a(58069)-3xHA attB::(P_{ara})crRNA pHERD20T-AcrVA2-myc

PAO1 attTn7:(P_{ara})MbCas12a(58069)-3xHA attB::(P_{ara})crRNA pHERD20T-AcrVA2.1-myc

PAO1 attTn7:(P_{ara})MbCas12a(58069)-3xHA attB::(P_{ara})crRNA pHERD20T-GST-myc

PAO1 attTn7:(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA pHERD20T

PAO1 attTn7:(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA pHERD20T-AcrVA2-myc

PAO1 attTn7:(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA pHERD20T-AcrVA2.1-myc

PAO1 attTn7:(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA pHERD20T-GST-myc

Extended Data Figure 1c,d

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA1-Myc

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-acrVA2-Myc

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2.1-Myc

Extended Data Figure 2a

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})cRNA24 pHERD30T-AcrVA2-Myc

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})cRNA24 pHERD30T-AcrVA2^{H286A}-Myc

Extended Data Figure 2b

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})cRNA24 pHERD30T-AcrVA2-Myc

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA1-Myc

Extended Data Figure 2c

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2.1-Myc

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrIIA4-Myc

Extended Data Figure 2d

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2.1-Myc

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA1-Myc

Extended Data Figure 2e

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrIIA4-Myc

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})cRNA24 pHERD30T-AcrVA2-Myc

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2.1-Myc

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA1-Myc

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})cRNA24 pHERD30T-AcrVA2^{H286A}-Myc

Extended Data Figure 3c

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})cRNA24 pHERD30T
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})cRNA24 pHERD30T-10xHis-MBP-TEV-AcrVA2-Myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2-Myc

Extended Data Figure 3d

PAO1 tn7::(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA24 pHERD30T
PAO1 tn7::(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2$^{E89A/D129A/D195A}$-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2$^{E89A/D129A/D195A}$-myc

Extended Data Figure 3e

PAO1 tn7::(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA24 pHERD30T-RFP-myc
PAO1 tn7::(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2$^{E89A/D129A/D195A}$-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-RFP-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA24 pHERD30T-AcrVA2$^{E89A/D129A/D195A}$-myc

Extended Data Figure 4a

PAO1 tn7::(P_{ara})MbCas12a(237)-RFP-3xHA pHERD20T-AcrIIA4-myc
PAO1 tn7::(P_{ara})MbCas12a(237)-RFP-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{560aa}-RFP-3xHA pHERD20T-AcrIIA4-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{560aa}-RFP-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{299aa}-RFP-3xHA pHERD20T-AcrIIA4-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{299aa}-RFP-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{100aa}-RFP-3xHA pHERD20T-AcrIIA4-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{100aa}-RFP-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{30aa}-RFP-3xHA pHERD20T-AcrIIA4-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{30aa}-RFP-3xHA pHERD20T-AcrVA2-myc

Extended Data Figure 4b

PAO1 tn7::(P_{lac})SpCas9-3xHA pHERD20T
PAO1 tn7::(P_{lac})SpCas9-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{lac})MbCas12a(237)^{30aa}-SpCas9-3xHA pHERD20T
PAO1 tn7::(P_{lac})MbCas12a(237)^{30aa}-SpCas9-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{lac})MbCas12a(237)^{100aa}-SpCas9-3xHA pHERD20T
PAO1 tn7::(P_{lac})MbCas12a(237)^{100aa}-SpCas9-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{lac})MbCas12a(237)^{299aa}-SpCas9-3xHA pHERD20T
PAO1 tn7::(P_{lac})MbCas12a(237)^{299aa}-SpCas9-3xHA pHERD20T-AcrVA2-myc

Extended Data Figure 4c

PAO1 tn7::(P_{ara})AsCas12f-3xHA pHERD20T
PAO1 tn7::(P_{ara})AsCas12f-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{ara})AsCas12f-3xHA pHERD20T-AcrVA2^{H286A}-myc
PAO1 tn7::(P_{ara})AsCas12f-3xHA pHERD20T-GST-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{30aa}-AsCas12f-3xHA pHERD20T
PAO1 tn7::(P_{ara})MbCas12a(237)^{30aa}-AsCas12f-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{30aa}-AsCas12f-3xHA pHERD20T-AcrVA2^{H286A}-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{30aa}-AsCas12f-3xHA pHERD20T-GST-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{46aa}-AsCas12f-3xHA pHERD20T
PAO1 tn7::(P_{ara})MbCas12a(237)^{46aa}-AsCas12f-3xHA pHERD20T-AcrVA2-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{46aa}-AsCas12f-3xHA pHERD20T-AcrVA2^{H286A}-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{46aa}-AsCas12f-3xHA pHERD20T-GST-myc
PAO1 tn7::(P_{ara})MbCas12a(237)^{100aa}-AsCas12f-3xHA pHERD20T
PAO1 tn7::(P_{ara})MbCas12a(237)^{100aa}-AsCas12f-3xHA pHERD20T-AcrVA2-myc

PAO1 tn7::(P_{ara})MbCas12a(237)^{100aa}-AsCas12f-3xHA pHERD20T-AcrVA2^{H286A}-myc

PAO1 tn7::(P_{ara})MbCas12a(237)^{100aa}-AsCas12f-3xHA pHERD20T-GST-myc

Extended Data Figure 5a

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA pHERD20T-IIA4-myc

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA pHERD20T-VA2-myc

PAO1 tn7::(P_{ara})MbCas12a(237)$^{\Delta 1\text{-}30aa}$-3xHA pHERD30T-IIA4-myc

PAO1 tn7::(P_{ara})MbCas12a(237)$^{\Delta 1\text{-}30aa}$-3xHA pHERD30T-VA2-myc

Extended Data Figure 5b

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24

PAO1 tn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24

PAO1 tn7::(P_{ara})MbCas12a(237)^{L12A}-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24

PAO1 tn7::(P_{ara})MbCas12a(237)^{L12A}-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24

PAO1 tn7::(P_{ara})MbCas12a(237)^{S13A}-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24

PAO1 tn7::(P_{ara})MbCas12a(237)^{S13A}-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24

PAO1 tn7::(P_{ara})MbCas12a(237)^{K14A}-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24

PAO1 tn7::(P_{ara})MbCas12a(237)^{K14A}-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24

PAO1 tn7::(P_{ara})MbCas12a(237)^{T15A}-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24

PAO1 tn7::(P_{ara})MbCas12a(237)^{T15A}-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24

PAO1 tn7::(P_{ara})MbCas12a(237)$^{LSKT>ASAT}$-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24

PAO1 tn7::(P_{ara})MbCas12a(237)$^{LSKT>ASAT}$-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24

PAO1 tn7::(P_{ara})MbCas12a(237)$^{LSKT>AAAA}$-3xHA attB::(P_{ara})RFP-myc pHERD20T-crRNA24

PAO1 tn7::(P_{ara})MbCas12a(237)$^{LSKT>AAAA}$-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-crRNA24

Extended Data Figure 7a

MG1655 attTn7::(P_{ara})3xHA-MbCas12a(299aa)-SecM pHERD20T-AcrVA2-myc

MG1655 attTn7::(P_{ara})3xHA-MbCas12a(299aa)-SecM pHERD20T-AcrVA2^{H286A}-myc

Extended Data Figure 7b

MG1655 attTn7::(P_{ara})3xHA-MbCas12a(299aa)$^{LSKT>AAAA}$-SecM pHERD20T-AcrVA2-myc

MG1655 attTn7::(P_{ara})3xHA-MbCas12a(299aa)$^{LSKT>AAAA}$-SecM pHERD20T-AcrVA2^{H286A}-myc

Extended Data Figure 7c

MG1655 attTn7::(P_{ara})3xHA-SpyCas9(299aa)-SecM pHERD20T-AcrVA2-myc

MG1655 attTn7::(P_{ara})3xHA-SpyCas9(299aa)-SecM pHERD20T-AcrVA2^{H286A}-myc

Extended Data Figure 9a,b

PAO1 attTn7::(P_{ara})MbCas12a(237)-3xHA attB::CTX2 pHERD20T

PAO1 attTn7::(P_{ara})MbCas12a(237)-3xHA attB::CTX2 pHERD20T-Dip

PAO1 attTn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T

PAO1 attTn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-Dip

PAO1 attTn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})AcrVA2^{H286A}-myc pHERD20T

PAO1 attTn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})AcrVA2^{H286A}-myc pHERD20T-Dip

PAO1 attTn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})RFP-myc pHERD20T

PAO1 attTn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})RFP-myc pHERD20T-Dip

Extended Data Figure 9c

PAO1 attTn7::(P_{ara})MbCas12a(237)-3xHA attB::CTX2 pHERD20T

PAO1 attTn7::(P_{ara})MbCas12a(237)-3xHA attB::CTX2 pHERD20T-Dip

Extended Data Figure 9d

PAO1 attTn7::(P_{ara})MbCas12a(237)-3xHA attB::CTX2 pHERD20T

PAO1 attTn7::(P_{ara})MbCas12a(237)-3xHA attB::CTX2 pHERD20T-Dip

PAO1 attTn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T

PAO1 attTn7::(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})AcrVA2-myc pHERD20T-Dip

Extended Data Figure 10

PAO1 attTn7:(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA pHERD30T

PAO1 attTn7:(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA pHERD30T-AcrVA2(*M. bovoculi*)

PAO1 attTn7:(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA pHERD30T-AcrVA2(*M. bovis*)
PAO1 attTn7:(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA pHERD30T-AcrVA2(*M. catarrhalis*)
PAO1 attTn7:(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA pHERD30T-AcrVA2(*M. osloensis*)
PAO1 attTn7:(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA pHERD30T-AcrVA2(orf142)
PAO1 attTn7:(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA pHERD30T-AcrVA2(orf178)
PAO1 attTn7:(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA pHERD30T-AcrVA2(*P. aeruginosa*)
PAO1 attTn7:(P_{ara})MbCas12a(237)-3xHA attB::(P_{ara})crRNA pHERD30T-FinQ(*E.coli*)
PAO1 attTn7:(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA pHERD30T
PAO1 attTn7:(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA pHERD30T-AcrVA2(*M. bovoculi*)
PAO1 attTn7:(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA pHERD30T-AcrVA2(*M. bovis*)
PAO1 attTn7:(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA pHERD30T-AcrVA2(*M. catarrhalis*)
PAO1 attTn7:(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA pHERD30T-AcrVA2(*M. osloensis*)
PAO1 attTn7:(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA pHERD30T-AcrVA2(orf142)
PAO1 attTn7:(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA pHERD30T-AcrVA2(orf178)
PAO1 attTn7:(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA pHERD30T-AcrVA2(*P. aeruginosa*)
PAO1 attTn7:(P_{ara})MbCas12a(33362)-3xHA attB::(P_{ara})crRNA pHERD30T-FinQ(*E.coli*)
PAO1 attTn7:(P_{lac})LbCas12a(ND2006) attB::(P_{ara})crRNA pHERD30T
PAO1 attTn7:(P_{lac})LbCas12a(ND2006)attB::(P_{ara})crRNA pHERD30T-AcrVA2(*M. bovoculi*)
PAO1 attTn7:(P_{lac})LbCas12a(ND2006) attB::(P_{ara})crRNA pHERD30T-AcrVA2(*M. bovis*)
PAO1 attTn7:(P_{lac})LbCas12a(ND2006) attB::(P_{ara})crRNA pHERD30T-AcrVA2(*M. catarrhalis*)
PAO1 attTn7:(P_{lac})LbCas12a(ND2006) attB::(P_{ara})crRNA pHERD30T-AcrVA2(*M. osloensis*)
PAO1 attTn7:(P_{lac})LbCas12a(ND2006) attB::(P_{ara})crRNA pHERD30T-AcrVA2(orf142)
PAO1 attTn7:(P_{lac})LbCas12a(ND2006) attB::(P_{ara})crRNA pHERD30T-AcrVA2(orf178)
PAO1 attTn7:(P_{lac})LbCas12a(ND2006) attB::(P_{ara})crRNA pHERD30T-AcrVA2(*P. aeruginosa*)
PAO1 attTn7:(P_{lac})LbCas12a(ND2006) attB::(P_{ara})crRNA pHERD30T-FinQ(*E.coli*)

Supplementary Table 2. Sequences used for protein alignment.

>AcrVA2
MHHTIARMNAFNKAFANAKDCYKKMQAWHLLNKPKHAFFPMQNTPALDNGLAALYELRGGKEDAHILSILS
RLYLYGAWRNTLGIYQLDEEIIKDCKELPDDTPTSIFLNLPDWCVYVDISSAQIATFDDGVAKHIKGFWAIYDIV
EMNGINHDVLDFVVDTDTDDNVYVPQPFILSSGQSVAEVLDYGASLFDDDTSNTLIKGLLPYLLWLCVAEPDI
TYKGLPVSREELTRPKHSINKKTGAFVTPSEPFIYQIGERLGSEVRRYQSIIDGEQKRNRPHTKRPHIRRGHW
HGYWQGTGQAKEFRVRWQPAVFVNSGRVSS

>AcrVA2.1
MHHTIARMNAFNKAFGNAKDCYKKMQAWHLNNKPKHIFSPLQNTLSLNEGLAALYELHGGKEDEHILSILCC
LYLYGTWRNTLGIYQLDEEIIKDCKELPDDTPTSIFLNLPDWCVYVDISSAKIATIDGGVAKHIKGFWAIYDNIE
MHGVNHDVLNFIIDTDTDNNIYVPQSLILSSEMSVAESLDYGLTLFGYDESNELVKGMLPYLLWLCVAEPDITH
KGLPVSREELTKPKHGINKKTGAFVTPSEPFIYQIGERLGGEVRRYQSLIDDEKNQNRHHTKRPHIRRGHWH
GYWQGTGQAKEFKVRWQPAVFVNSGV

>FinQ
MEMSQLKQPIFLKKIKKVINTIPGLEEQIFACRNKKRSDNPLLFIDRKDEERILMSRLQSQQKNEELASKLESLFHGNELSSPHSI
LCFIYWRYTKKIYRLSEDIISDVANTYVDNIPAQILKELPSWSIYVSAENLHTILPTSYPIHGFFFYPFLDNNGNIIRLFIIDDLKQSQ
GTTGLKEKDVDVVNNIIRIKDSREGLLDPRKMECIDGEVVVTVNEKLKDFRDREFNLLNAQISMVLYICSQINDIKEKNQFKRS
EKHKKHVHTHHELPAQNIREWDVGIRMGQAIRQYRQAEPTGKERTTIGSKRPHIRRGHWHTYWTGSKKPELAHERKPRLI
WLPPVPVNLEDVNKLPVVITPIDK

>bjp_ig2103_scaffold_170.fasta.genes.faa|orf152
MGITDHRARQHLIAAGKLYPGAWRVLDGMRQERGKSLPDWSEWCYLPLAASYAVVSGGGDNRVPPHLAGDVGRLGALA
AWRPTQGIYRFDPTLYAALTDTPLSGDLPCEVLHHLPEWCVYIETPGYEWMGSELYGFYAHLECDPEANREELRLLMDSEAA
LAPIPIHLGPWPLAEAVARAIDVSRTYGAALGLPVPDGIAAATSEAISPLLSLLLYLCADESEIGDGQARPTMPRPKRTKQGWR

LFPADKPAQWDVGVRLGAALRRAYHQQETGRQDIDPQTGRMRPRAHVRCAHWHTYRHGEGRTLTRLRWLPPIPVNIDD
PDAMPSTVRPTK*

>S2_005_002_R2_scaffold_27|orf142
MSTDVDNFFREGLDVYENNLNKGFFERWVTARKGKIPTHFISPNAIPVDPDHNAYFKSKMEIGSDYKTFLSLGYGWRYGKT
TIKASAGLIELLSQTPASDLIPASWLDAIPGWTTFIPLRDEPDAPGVFFGLRKYGEQKVLLVGSAWDEMYRISSYDLQDEEDGF
VSFKFPEWFLKDTSKEDIELTKSHLNAILFLCTLIPHQPAAVKVPKRITGSKKVTYAMSPRKHNVINLESAVDKYLKEYDTDTRT
LVLNQRKAHMRRAHWRTYWTGPRNGEQVKVLRWIPPTFVKGFVAE*

>S2_005_003_R2_scaffold_22.fasta.genes.faa|orf248
MSTDVDNFFREGLDVYENNLNKGFFERWVTARKGKIPTHFISPNAIPVDPDHNAYFKSKMEIGSDYKTFLSLGYGWRYGKT
TIKASAGLIELLSQTPASDLIPASWLDAIPGWTTFIPLRDEPDAPGVFFGLRKYGEQKVLLVGSAWDEMYRISSYDLQDEEDGF
VSFKFPEWFLKDTSKEDIELTKSHLNAILFLCTLIPHQPAAVKVPKRITGSKKVTYAMSPRKHNVINLESAVDKYLKEYDTDTRT
LVLNQRKAHMRRAHWRTYWTGPRNGEQVKVLRWIPPTFVKGFVAE*

>S2_009_000_R2_scaffold_143.fasta.genes.faa|orf85
MNKSTPQTFLDLFLKDVKPFLSMFDRGLDAYFLKCKGKPGAFGVSLHQYLKWLNPAAKAPEDASRLSIVISGYLHYFWQLSK
PNVVFEPTLVAHLADSEIPENLPAEILQRLPYWCQWVTLPIHLASKDNTMSLDFDGAFVGYTKIEHRPALILVAPFVSSDDSIR
TNGFTPSATSYVYLDEPVNHLSFMKNSHLLNVSDEMPESFFQELMFNVYHYLTKIVCCVMYICSQQDALYSEGHQQPKPQR
LGKSYRITPPKTDRMITVGSEMTKIIKDFEDEVESCTRAFNGRRPHLRKAHYHHFWTGPKVGPRKLVCKWLPPSIVRGTVVE
Q*

>S2_012_000_R2_scaffold_26.fasta.genes.faa|orf178
MNKSTPQTFLDLFLKDVKPFLSMFDRGLDAYFLKCKGKPGAFGVSLHQYLKWLNPAAKAPEDASRLSIVISGYLHYFWQLSK
PNVVFEPTLVAHLADSEIPENLPAEILQRLPYWCQWVTLPIHLASKDNTMSLDFDGAFVGYTKIEHRPALILVAPFVSSDDSIR
TNGFTPSATSYVYLDEPVNHLSFMKNSHLLNVSDEMPESFFQELMFNVYHYLTKIVCCVMYICSQQDALYSEGHQQPKPQR
LGKSYRITPPKTDRMITVGSEMTKIIKDFEDEVESCTRAFNGRRPHLRKAHYHHFWTGPKVGPRKLVCKWLPPSIVRGTVVE
Q*