

Biochemical and structural bioinformatics studies of fungal CutA nucleotidyltransferases explain their unusual specificity towards CTP and increased tendency for cytidine incorporation at the 3'-terminal positions of synthesized tails

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SUPPLEMENTAL MATERIAL

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SUPPLEMENTAL FIGURE

AnCutA	ATGGTGACCGTGACCGATGGCAACGATCGCCTGAGCAGCCGCCCGGGCGAACAGAGCCCG
AnCutA_opt	ATGGTCACCGTCACCGATGGTAACGACCGCCTGTCCAGCCGCCCGGGCGAACAAAGTCCG
protein	M V T V T D G N D R L S S R P G E Q S P
AnCutA	ATTGCGAGCGCGAAACTGAGCACCATGCCGTGGCGCAGCAGCAACGAAGAAAGCAGCGAA
AnCutA_opt	ATTGCCTCAGCCAAACTGTCCACGATGCCGTGGCGTAGCTCTAACGAAGAAAGTTCGGAA
protein	I A S A K L S T M P W R S S N E E S S E
AnCutA	ACCCCGAACCGCCCGGCGCTGACCAGCCAGCAGAGCAACAGCCTGCCGAGCACCCTGAT
AnCutA_opt	ACCCCGAATCGCCCGGCACTGACGTACAGCAATCGAACAGCCTGCCGTGACCCCGTAT
protein	T P N R P A L T S Q Q S N S L P S T P Y
AnCutA	CAGCATGCGCGCAAAATTAGCTTTTCATAGCCGAGCCCGAGCCCGCGCCATGGCAGCACC
AnCutA_opt	CAGCATGCTCGTAAAATTAGCTTTTCACTCTCGTAGTCCGTCCCCGCGTCATGGTTCAACG
protein	Q H A R K I S F H S R S P S P R H G S T
AnCutA	AGCCCGCGCAGCACCCATAGCGAAAGCACCCATATTCCGCGAGCGCGCGCAAACAGTTT
AnCutA_opt	TCGCCGCGTAGCACCCATAGCGAATCTACGCACATCCCGCCGTCTGCACGCAAACAGTTC
protein	S P R S T H S E S T H I P P S A R K Q F
AnCutA	ACCGGCTGCAAATATGAAACCGCGATGAGCTTTTTTCGCCGCGCATGCCGTATAGCATT
AnCutA_opt	ACCGGCTGCAAGTATGAAACCGGCTATGTCAATTTTTCCGTGCGCGTATGCCGTACTCGATT
protein	T G C K Y E T A M S F F R R R M P Y S I
AnCutA	GGCGCGGATGTGCTGCCGGAAGAAAAAGAAGGCCTGAAACAGCGCCTGCGCCCGGAAGAA
AnCutA_opt	GGTGCGGATGTGCTGCCGGAAGAAAAAGAAGGCCTGAAGCAGCGTCTGCGCCCGGAAGAA
protein	G A D V L P E E K E G L K Q R L R P E E
AnCutA	GAACGCAAACGACCGCGGATATGCTGGAAGTGTATGAACGCCTGCTGCCGAGCGCGGAA
AnCutA_opt	GAACGTAAACGACCGCGGATATGCTGGAAGTTTATGAACGCCTGCTGCCGAGCGCGGAA
protein	E R K L T A D M L E V Y E R L L P S A E
AnCutA	AGCGAAAGCCGCCCGCCGCGCTGGTGCGCAAACGGAAGATCTGTTTAACCGCCAGTGG
AnCutA_opt	AGTGAATCCCGCGTCCGCTGCTGGTCCGTAAACGGAAGACCTGTTTAACCGCCAGTGG
protein	S E S R R R R L V R K L E D L F N R Q W
AnCutA	CCGGGCTGCGATATTAAAGTGCATGTGTTTGGCAGCAGCGGCAACAACTGTGCAGCAGC
AnCutA_opt	CCGGGTGCGACATTAAAGTGCATGTTTTCGGTTCATCGGGCAATAAGCTGTGTAGCTCT
protein	P G C D I K V H V F G S S G N K L C S S
AnCutA	GATAGCGATGTGGATATTTGCATTACCACCACCTGCAAAGAACTGGAACATGTGTGCCTG
AnCutA_opt	GACAGTGATGTGGACATTTGCATCACCACGACCTGTAAAGAACTGGAACATGTTTGCCTG
protein	D S D V D I C I T T T C K E L E H V C L
AnCutA	CTGGCGGATGTGCTGGCGAAAAACGGCATGGAACGCGTGGTGTGCATTAGCCATGCGAAA
AnCutA_opt	CTGGCGGATGTCTGGCCAAGAACGGCATGGAACGTGTGGTTTGTATCTCTCACGCGAAA
protein	L A D V L A K N G M E R V V C I S H A K
AnCutA	GTGCCGATTGTGAAAAATTGGGATCCGGAACGCGCCTGGCGTGCGATATGAACGTGAAC
AnCutA_opt	GTGCCGATTGTTAAGATCTGGGATCCGGAACGCGCCTGGCGTGTGACATGAACGTGAAC
protein	V P I V K I W D P E L R L A C D M N V N

AnCutA	AACACCATGGCGCTGGAAAACACCCGCATGGTGCGCACCTATGTGGAAATTGATGAACGC
AnCutA_opt	AATACCATGGCCCTGGAAAATACCCGTATGGTGCGCACGTATGTTGAAATTGATGAACGT
protein	N T M A L E N T R M V R T Y V E I D E R
AnCutA	GTGCGCCCGCTGGCGATGATTATTAAACATTGGACCAAACGCCGATCTCTGAACGATGCG
AnCutA_opt	GTTCGCCCGCTGGCGATGATTATCAAACATTGGACCAAGCGCCGTATCCTGAACGACGCC
protein	V R P L A M I I K H W T K R R I L N D A
AnCutA	GGCCTGGGCGGCACCCTGAGCAGCTATACCTGGATTGCGCTGATTATTAACTTTCTGCAG
AnCutA_opt	GGTCTGGGCGGTACCCTGAGTTCCTACACGTGGATTGCGCTGATTATCAATTTTCTGCAG
protein	G L G G T L S S Y T W I C L I I N F L Q
AnCutA	ACCCGCGAACC GCCGATTCTGCCGAGCCTGCAGGCGCGCCGCATAAAAACGCCGTGACC
AnCutA_opt	ACCCGTGAACCGCCGATCCTGCCGAGCCTGCAAGCACGTCCGCACAAAAAGCGCCTGACG
protein	T R E P P I L P S L Q A R P H K K R L T
AnCutA	GCGGATGGCCTGGTGTGCAGCTTTGATGATGATCTGGATAGCCTGGTGGGCTATGGCAAA
AnCutA_opt	GCCGATGGCCTGGTCTGTAGTTTCGATGACGATCTGGACTCCCTGGTGGGTTATGGCAAA
protein	A D G L V C S F D D D L D S L V G Y G K
AnCutA	CAGAACAAACAGAGCCTGGGCGAACTGTTTTTTCAGTTTTTTCGCTATTATGGCCATGAA
AnCutA_opt	CAGAATAAGCAATCTCTGGGTGAACTGTTTTTCCAGTTTTTCCGCTATTACGGCCATGAA
protein	Q N K Q S L G E L F F Q F F R Y Y G H E
AnCutA	CTGGATTTTGAAAAATATGTGATTAGCGTGCGCGAAGGCCGCTGATTAGCAAAGAAGGC
AnCutA_opt	CTGGATTTCGAAAAGTACGTCATTAGCGTGCGTGAAGGTCGCCTGATCTCTAAAGAAGGT
protein	L D F E K Y V I S V R E G R L I S K E G
AnCutA	AAAGGCTGGCATCTGCTGCAGAACAACCGCCTGTGCGTGGAAGAACCGTTTAAACACCATT
AnCutA_opt	AAAGGTTGGCACCTGCTGCAGAACAATCGTCTGTGCGTGGAAGAACCGTTTAAACACCATT
protein	K G W H L L Q N N R L C V E E P F N T I
AnCutA	CGCAACCTGGGCAACACCGCGGATGATACCAGCTTTCGCGGCGTG CATATGGAAC TGCGC
AnCutA_opt	CGCAACCTGGGTAATACGGCGGACGATACCAGTTTCCGTGGCGTTCATATGGAAC TGCGC
protein	R N L G N T A D D T S F R G V H M E L R
AnCutA	CGCGCGTTTAAAGCGCTGAGCGATGGCAACTTTGAACTGTGCTGCGAACAGTATGAATAT
AnCutA_opt	CGTGCAATTTAAAGCTCTGTCCGATGGCAATTTGAACTGTGCTGTGAACAGTATGAATAC
protein	R A F K A L S D G N F E L C C E Q Y E Y
AnCutA	CCGCCGGAAGCGGAACGCAGCTGGGAACGCCCGCGCCGAGCCGCGCCCGGTGGTGACC
AnCutA_opt	CCGCCGGAAGCAGAACGTTTCATGGGAACGCCCGGCTCCGCAACCGCGCCCGGTCTGTGACG
protein	P P E A E R S W E R P A P Q P R P V V T
AnCutA	ACCGCGCCGCCCGGAGCCGCGGCGGCGCGGCGCGGCGCGGCCGCGCGGCCCATAGCAAC
AnCutA_opt	ACCGCACCGCCCGCGTCCCGTGGCGGTGCGGCGGCGGCGGCGGCGGCCCATAGTAAC
protein	T A P P P S R G G R G G G R G G R H S N
AnCutA	CAGTATAGCCGCGGCGGCCATAGCGGCGGCGCCGCGAGCAGCAACGCGGCGAACA AAAAC
AnCutA_opt	CAGTATTCGCGTGGTGCCACAGTGGTGGCCGCGGTTTCATCGAATGCGGCCAACAAAAT
protein	Q Y S R G G H S G G R R S S N A A N K N

AnCutA	CATTTTCGCCAGGCGAACAACGGCATGAGCGCGAGCGAACTGAGCCTGCAGGCGCAGCAT
AnCutA_opt	CATTTTCGCCAGGCGAACAATGGCATGTACAGCCTCGGAACTGAGCCTGCAGGCGCAACAC
protein	H F R Q A N N G M S A S E L S L Q A Q H
AnCutA	GCGCAGTATCTGCTGCATGATCATCTGTATCAGCAGATTGAGATTCTGCAGGCGCAGGAA
AnCutA_opt	GCCCAGTATCTGCTGCATGATCACCTGTACCAGCAAATTCAAATCCTGCAGGCACAAGAA
protein	A Q Y L L H D H L Y Q Q I Q I L Q A Q E
AnCutA	CAGGAACTGCGCCTGCAGCTGCAGAACCAGGCGCTGATTACCGGCCGCCCGCCGCCGGTG
AnCutA_opt	CAGGAACTGCGTCTGCAACTGCAGAACCAAGCTCTGATTACCGGTCTCCGCCGCCGGTT
protein	Q E L R L Q L Q N Q A L I T G R P P P V
AnCutA	CTGATTGCCAGCCGTTTATTTCAGTTTCCGGTGCCGCGCAGGAAACCAGCGCGATGAT
AnCutA_opt	CTGATTGCTCAGCCGTTTATCCAATTCCCGGTCCCGGCACAGGAAACCAGCGGTGACGAT
protein	L I R Q P F I Q F P V P A Q E T S G D D
AnCutA	AGCAGCCGCGAGCCGCGAGCGCGAGCCGCGAGCGCGGTGAACCATCAGCCGATTAGCGTG
AnCutA_opt	AGCTCTCGTAGCCGCTCTGGTAGTCGTTCCGGCGCGGTGAATCATCAGCCGATCAGTGTG
protein	S S R S R S G S R S G A V N H Q P I S V
AnCutA	CCGAGCCGCCAGAACGTGTATTATAACAGCACCTATCTGCCGGTGGCGGTGCCGGGCGGC
AnCutA_opt	CCGTCCCGCCAAAACGTTTATTACAATTCAACCTATCTGCCGGTTCGAGTGCCGGGTGGC
protein	P S R Q N V Y Y N S T Y L P V A V P G G
AnCutA	CCGGGCGACAAACCAACCCGCCGAGCCCGAGCGCGGCGGCGGCGATGCCGGATCTGCGC
AnCutA_opt	CCGGGTTCGAACACGAATCCGCCGTCACCGTCGGCAGCTGCAGCAATGCCGGATCTGCGC
protein	P G S N T N P P S P S A A A A M P D L R
AnCutA	CGAACCCGCGCCGCGAGCAGCGTGCGGAACGGCAGCCGAACGGCAGCCTGCGCGCGCAT
AnCutA_opt	CGTAACCCGCGCCGTAGTTCCGTGGCCAACGGTTACCGAATGGCTCGCTGCGTGACAT
protein	R N P R R S S V A N G S P N G S L R A H
AnCutA	AGCCAGCCGGCGCGCCCGGTGAACAGCCTGCCGAACCTTTGCGCCGCTGTATAGCATTAGC
AnCutA_opt	AGTCAGCCGGCACGTCCGGTTAACTCCCTGCCGAATTTGCGCCGCTGTACAGCATTCTCT
protein	S Q P A R P V N S L P N F A P L Y S I S
AnCutA	CAGCCGACCGAAACCAGCCAGAGCAGCAAACAGCGCCATCCGAGCGGCGGAGCGATGAT
AnCutA_opt	CAACCGACGGAACCCAGCCAGTCATCGAAACAACGCCACCCGTCAGGTGCCTCGGACGAT
protein	Q P T E T S Q S S K Q R H P S G A S D D
AnCutA	AGCCTGAGCGAAGAAGATGGCACCAGCATGCCGAACAGCCTGCCGAACCCGACCCGTGTTT
AnCutA_opt	AGTCTGTCCGAAGAAGATGGCACCAGCATGCCGAACCTCTCTGCCGAATCGTACGCTGTTT
protein	S L S E E D G T S M P N S L P N R T L F
AnCutA	ATTGATGAAGCGCGCAGCAACGATTATATGACCTATTATTTTGGCGGCAGCCCGCAGCTG
AnCutA_opt	ATCGATGAAGCGCGCAGTAACGACTATATGACCTATTACTTCGGTGGCTCCCCGCAGCTG
protein	I D E A R S N D Y M T Y Y F G G S P Q L
AnCutA	CATGCGTATCATCAGAACCGCATGCTGAGCCCGATGAGCGCGAGCCTGGGCCTGGCGCTG
AnCutA_opt	CATGCATACCACAAAATGCTATGTGTCTCCGATGTCAGCATCGTGGGTCTGGCACTG
protein	H A Y H Q N A M L S P M S A S L G L A L

AnCutA	CCGAACGGCGGCATTGTGCCGTGGGTGGGCAACCCGAGGAATATATGAGCGCGGCGCCG
AnCutA_opt	CCGAACGGTGGCATTGTTCCGTGGGTCCGCAATCCGAGGAATATATGAGTGCAGTCCG
protein	P N G G I V P W V G N P Q E Y M S A A P
AnCutA	GGCGCGGAAAGCTATGGCCATAGCTTTGATAACGGCAGCATTACCAGCCGAAAAGCGGC
AnCutA_opt	GGTGCAGGAAAGTTACGGCCATTCTTTGATAATGGCAGCATCACCTCTCCGAAAAGTGGC
protein	G A E S Y G H S F D N G S I T S P K S G
AnCutA	CAGAGCCAGCAGCGCATGGGCAGCCGACCGCGGCGAGCAGCGATCGCGGCGGCCCGCTG
AnCutA_opt	CAGTCCCAGCAACGTATGGGTAGCCGTACGGCAGCAAGCTCTGATCGTGGTGGCCCGCTG
protein	Q S Q Q R M G S R T A A S S D R G G P L
AnCutA	ATTGTGGATGGCAGCGTGCCGCCGAGCGAACAGCGCATGAGCATTCCGGCGGATCATTAT
AnCutA_opt	ATTGTGACGGTTCAGTGCCGCCGTCGGAACAGCGCATGAGCATCCCGGCAGATCATTAT
protein	I V D G S V P P S E Q R M S I P A D H Y
AnCutA	GAACATTATGGCCTGATGAGCCATTGCCCGAGCAACAGCGATGATCAGAACACCGGATACC
AnCutA_opt	GAACACTACGGCCTGATGTCTCACTGTCCGAGCAACTCTGACGATCAGAATACGGATACC
protein	E H Y G L M S H C P S N S D D Q N T D T
AnCutA	CCGCGAGCGTGAGCGATAGCTTTAGCCAGGATTTTCAGGATAGCAGCAGCGTGGAATTT
AnCutA_opt	CCGGCCAGTGTCTCCGACTCATTTTCGCGAGGATTTCCAAGACAGTTCCTCAGTGGAATTT
protein	P A S V S D S F S Q D F Q D S S S V E I
AnCutA	GATCCGAGCCCGTTTTTTAGCCGCCAGGGCTTTGATAGCCATGCGATGGGCGGCCCGCGC
AnCutA_opt	GATCCGAGCCCGTTTTTCTCTCGTCAGGGTTTTGATAGCCATGCAATGGGTGGCCCGGCT
protein	D P S P F F S R Q G F D S H A M G G P A
AnCutA	GATGTGTTTGTGAACGGCCATAGCGGCAAAACAGAGCCTGCTGAGCAGCCGCTGCAGGGC
AnCutA_opt	GACGTTTTCTGTCACGGTCAAGTGGCAAAACAGTCCCTGCTGTCGAGCCGCTGCAAGGT
protein	D V F V N G H S G K Q S L L S S R L Q G
AnCutA	CTGCATCTGAGCAACGGCGAAAAAGTGGATATTGCGAGCAAAAGCGCGCAGGATAAACCG
AnCutA_opt	CTGCATCTGTCTAATGGCGAAAAAGTGGATATTGCAAGCAAGTCTGCTCAGGACAAACCG
protein	L H L S N G E K V D I A S K S A Q D K P
AnCutA	AAAGCGGTGGTGCCGCATAACGATGCGGTGGAACGCGAAGCGCCGCGCGGCGAAAAACCAT
AnCutA_opt	AAGGCAGTTGTCCCGCACACGATGCTGTGGAACGTGAAGCACCGCGCGCTAAAAATCAT
protein	K A V V P H N D A V E R E A P R A K N H
AnCutA	CATGCGGTGGATAAAGGCCCGAGCGCGAGCAGCTTTAGCCATGCGGCGGATAGCCATATT
AnCutA_opt	CACGCGGTTGATAAGGGCCCGAGCGCTCTAGTTTTTCTCATGCAGCTGACTCACACATC
protein	H A V D K G P S A S S F S H A A D S H I
AnCutA	GCGAGCCCGAACGGCGCCGCCGCCATCATGGCAGCGATACCAGCGAAAAAGTGAACGGC
AnCutA_opt	GCATCGCCGAACGGTGCCTGCGCCATCACGGCTCAGATACCTCGGAAAAAGTTAACGGT
protein	A S P N G R R R H H G S D T S E K V N G
AnCutA	ACCGCGCATAAAGGCAAAACCGCGCAACCAGACCAGCAGCACCGCGAGCAACGATCAGAAA
AnCutA_opt	ACGGCACATAAAGGCAAGCCGCGTAATCAGACGTCTCAACCGCAAGCAACGATCAAAAA
protein	T A H K G K P R N Q T S S T A S N D Q K

AnCutA
AnCutA_opt
protein
GAACGCAACGCGGGCGGCCCGCGCAAACCGAACGCGGCGGCCTGACCCCGCAGGATAGC
GAACGTAATGCGGGTGGCCCCGCTAAGCCGAATGCAGCAGGTCTGACCCCGCAGGACAGC
***** ** ***** ***** ** ***** ** ** ***** ***** ***
E R N A G G P R K P N A A G L T P Q D S

AnCutA
AnCutA_opt
protein
AACCATAGCCATGCGAACAGCGGCTGGCAGACCACCAAACGCAAAAAACAAAAAACAAC
AACCATTCTCACGCGAATTCAGGCTGGCAAACGACCAAGCGTAAGAACAAAAAGAACAAC
***** ** ***** ***** ** ***** ** ** ***** *****
N H S H A N S G W Q T T K R K N K K N N

AnCutA
AnCutA_opt
protein
AAACTGTTTAGCGATCATCGCCATGGCCTGCAGAACGGCGCGGAACCGCTGCCGATTGAT
AAGCTGTTCTCGGATCATCGCCACGGTCTGCAGAATGGCGCCGAACCGCTGCCGATTGAC
** ***** ***** ** ***** ***** *****
K L F S D H R H G L Q N G A E P L P I D

AnCutA
AnCutA_opt
protein
GAAAGCCTGCGCAAAGGCGGC
GAAAGCCTGCGCAAAGGTGGC
***** ***** ***
E S L R K G G

TtCutA
TtCutA_opt
protein
ATGGATAGCCAGCCGCGACCGCGGAAAGCAAACATCATTATCAGAGCTATCCGTGGCCG
ATGGACTCGCAACCGGCAACCGCAGAATCAAAGCATCACTACCAATCATATCCGTGGCCG
***** ** ***** ***** ** ***** ** ** ***** *****
M D S Q P A T A E S K H H Y Q S Y P W P

TtCutA
TtCutA_opt
protein
GGCGCGCGAGCGCGGGCACCACCAGCCGCGTGAAACCGGCGCCGGTGCCGGAAGCAGC
GGCGCTCCGTCAGCAGGCACCACCAGCCGCGTGAAACCGGCGCCGGTCCGGAAGCTCT
***** ** ***** ***** ***** ***** *****
G A P S A G T T S R V K P A P V P E S S

TtCutA
TtCutA_opt
protein
CCGGGCTATTTTCCGGCGCAGCTGGATCTGCTGCCGATTCTGTTTCCGCAGCAGCTGTAT
CCGGGTATTTTCCGGCCCGAGCTGGATCTGCTGCCGATTCTGTTTCCGCAGCAACTGTAT
***** ***** ***** ***** ***** *****
P G Y F P A Q L D L L P I L F P Q Q L Y

TtCutA
TtCutA_opt
protein
CAGGATAAACTGCTGCAGTATAACAACTGGTGAGCGCGGGCCGCGGCGGCGGCGGCGGC
CAAGACAAGCTGCTGCAGTACAACAACTGGTCAGCGCAGGTCGTGGCGGTGGCGGTGGC
** ** ***** ***** ***** ** ** ***** ***** ***
Q D K L L Q Y N K L V S A G R G G G G

TtCutA
TtCutA_opt
protein
CTGCAGACCCCGCCGATCCGCCGTGCTGAGCAGCCCGCAGAGCAAAAGCAGCACCCGC
CTGCAGACGCCCGCCGACCCGCCGTTCTGAGTTCCTCCCGCAGAGTAAATCATCGACCCGC
***** ***** ***** ***** ***** *****
L Q T P P H P P V L S S P Q S K S S T R

TtCutA
TtCutA_opt
protein
AGCCGCAGCAGCAGCAAAGTGAGCAACAAAGATAACAGCGCGCAGGCGAAAAGCGCGCCG
TCCCGTAGCTCTAGTAAGGTGTCTAACAAAGATAATTCGCCCCAAGCAAAAAGCGCGCCG
** ** ***** ***** ***** ***** *****
S R S S S K V S N K D N S A Q A K S A P

TtCutA
TtCutA_opt
protein
CAGAGCTGCCATGGCAACAAACCGCGCAAAAACAGCGAACCGGCGGAACGCGCGCTGATG
CAGTCTTGCCATGGTAACAAGCCGCGCAAAAATTCAGAACCGGCAGAACGCGCTCTGATG
*** ***** ***** ***** ***** *****
Q S C H G N K P A K N S E P A E R A L M

TtCutA
TtCutA_opt
protein
AAAAGCGGCAAAGAACCGAGCAGCAAAATGAGCGCGAAAAACCGAGCGAACAGGCGGTG
AAGTCGGGCAAAGAACCGTCCTCAAAAATGTCAGCAAAAAGCCGTCGGAACAGGCTGTC
** ***** * ***** ** ***** ** *****
K S G K E P S S K M S A K K P S E Q A V

TtCutA	GATGGCGATGCGGCGGTGCGCCAGCCGCCGGCGAGCCGCCAGGGCGCGCCGGCGACCGCG
TtCutA_opt	GATGGTGACGCGGCGGTGCGTCAACCGCCGGCATCACGTCAGGGTGCTCCGGCAACCGCC
protein	D G D A A V R Q P P A S R Q G A P A T A
TtCutA	ACCCAGAGCAGCAGCGTGCCGAGCACCCCGCATCAGCATCCGCGCAAATTTAGCTTTGAA
TtCutA_opt	ACGCAATCGAGCTCTGTTCCGTCGACCCCGCATCAGCACCCGCGCAAATTTTCCTTCGAA
protein	T Q S S S V P S T P H Q H P R K F S F E
TtCutA	AGCCGCGAACAGAGCCCGGGCGGCGCAGAACCATAGCCGCGCAGCGGTATAGCGAA
TtCutA_opt	TCAGTGAAACAAAGTCCGGGTGCAGCTCAGAACCATTCGCCGCGCAGCGCTATTCTGAA
protein	S R E Q S P G A A Q N H S P R S A Y S E
TtCutA	ACCAACGGCAACGTGCCGAGCCTGCGCCCGCTGCCGCCGCGCTGGGCGGCTGCCGCTTT
TtCutA_opt	ACCAACGGTAATGTGCCGAGCCTGCGTCCGCTGCCGCCGCGTCTGGGTGGCTGTCGCTTC
protein	T N G N V P S L R P L P P R L G G C R F
TtCutA	GAAACCGGATTCGGTTTAGCCGCCGCGCATGCCGTATAGCCTGGGCACCGATAAACTG
TtCutA_opt	GAAACGGCAATTCCGTTTAGCCGTGCGCGTATGCCGTACTCTCTGGGCACCGATAAGCTG
protein	E T A I P F S R R R M P Y S L G T D K L
TtCutA	GAAAAAGTGGATCCGGATAAAATTAAAAGCAAACCTGAGCGAAGATGTGGAACGCAAACCTG
TtCutA_opt	GAAAAAGTGGATCCGGACAAAATCAAGAGTAAACCTGCCGAAGACGTTGAACGTAAACCTG
protein	E K V D P D K I K S K L S E D V E R K L
TtCutA	GAAACCGATATGCGCGAACTGTATGATGCGCTGCTGCCGACCGAAGCGATTGAAGTGAAC
TtCutA_opt	GAAACGGATATGCGTGAACCTGTATGACCGCTGCTGCCGACCGAAGCGATTGAAGTTAAC
protein	E T D M R E L Y D R L L P T E A I E V N
TtCutA	CGCCGCGAACTGGTGAGCAAACCTGGAACGCCTGTTTAAACACCGAATGGCCGGGCCATGAT
TtCutA_opt	CGCCGTGAACCTGGTCAGCAAACCTGGAACGCCTGTTC AATACCGAATGGCCGGGTATGAT
protein	R R E L V S K L E R L F N T E W P G H D
TtCutA	ATTCGCGTGCACTGTGTTGGCAGCAGCGGCAACCTGCTGTGTCAGCGATGATAGCGATGTG
TtCutA_opt	ATCCGTGTTACCTGTTTGGTAGTTCGGCAATCTGCTGTGCTCAGATGACTCGGATGTT
protein	I R V H L F G S S G N L L C S D D S D V
TtCutA	GATATTTGCATTACCACCCCGTGGCGCGAACTGGAAAGCGTGTGCATGATTGCGGAACTG
TtCutA_opt	GACATTTGTATCACCACGCCGTGGCGCGAACTGGAAAGCGTGTGCATGATTGCAGAACTG
protein	D I C I T T P W R E L E S V C M I A E L
TtCutA	CTGGATCGCCATGGCATGGAAAAAGTGGTGTGCGTGAGCAGCGCGAAAGTGCCGATTGTG
TtCutA_opt	CTGGATCGTCATGGCATGGAAAAAGTGGTTTGTGTGTCATCGGCGAAGGTGCCGATTGTT
protein	L D R H G M E K V V C V S S A K V P I V
TtCutA	AAAATTTGGGATCCGGAACCTGAAACTGGCGTGCGATATGAACGTGAACAACACCCCTGGCG
TtCutA_opt	AAAATCTGGGATCCGGAACCTGAAACTGGCATGCGACATGAACGTTAACAATACCCCTGGCT
protein	K I W D P E L K L A C D M N V N N T L A
TtCutA	CTGGAAAAACACCCGCATGGTGCACCTATGTGAGCATTGATGATCGCGTGCGCCCGCTG
TtCutA_opt	CTGGAAAAATACCCGCATGGTCCGTACGTATGTGAGCATTGATGACCGCGTGCCTCCGCTG
protein	L E N T R M V R T Y V S I D D R V R P L

TtCutA GCGATGATTATTAAATATTGGACCCGCCGCCGCGTGGTGAACGATGCGGCGTTTGGCGGC
TtCutA_opt GCGATGATTATCAAATACTGGACCCGCCGTCGCGTCGTGAACGATGCAGCCTTCGGTGGC

protein A M I I K Y W T R R R V V N D A A F G G

TtCutA ACCCTGAGCAGCTATACCTGGATTTGCATGATTATTGCGTTTCTGCAGCTGCGCGATCCG
TtCutA_opt ACCCTGAGCTCTTACACGTGGATCTGTATGATTATCGCATTTCTGCAGCTGCGTGATCCG

protein T L S S Y T W I C M I I A F L Q L R D P

TtCutA CCGGTGCTGCCGGCGCTGCATCAGCAGCATGATCTGAAACTGGTGAACAGGATGGCGCG
TtCutA_opt CCGGTCTTGCCGGCTCTGCATCAGCAACACGACCTGAAGCTGGTGAACAGGATGGTGCG

protein P V L P A L H Q Q H D L K L V K Q D G A

TtCutA CTGAGCGATTTTGGCGATGATATTCGAAACTGCGCGGCTTTGGCGCGAAAAACAAAGAT
TtCutA_opt CTGTCTGACTTCGCCGATGACATTCGAAACTGCGTGGTTTTGGCGCGAAGAATAAAGAT

protein L S D F A D D I P K L R G F G A K N K D

TtCutA AGCCTGGCGGTGCTGCTGTTTCAGTTTTTTCGCTTTTATGCGCATGAATTTGATTATGAT
TtCutA_opt AGTCTGGCCGTGCTGCTGTTTCAGTTTTTTCGCTTCTATGCACACGAATTTGATTATGAC
**
protein S L A V L L F Q F F R F Y A H E F D Y D

TtCutA AAATATACCCTGAGCATTCGCATGGGCACCCTGCTGACCAAAGCGGAAAAAACTGGCAG
TtCutA_opt AAATACACCCTGAGCATCCGTATGGGCACCCTGCTGACGAAGGCAGAAAAAGAACTGGCAG

protein K Y T L S I R M G T L L T K A E K N W Q

TtCutA TATCTGGTGAACAACGCGCTGTGCGTGGAAGAACCGTTTAACGATGGCCGCAACCTGGGC
TtCutA_opt TACCTGGTGAACAATGCTCTGTGCGTTGAAGAACCGTTTAATGATGGTGCACACCTGGGC
**
protein Y L V N N A L C V E E P F N D G R N L G

TtCutA AACACCGCGATGAAACAGCTTTTCGCGCCTGCATATGGAACGCGCCGCGCGTTTGAT
TtCutA_opt AATACCGCCGACGAAACGAGCTTCCGTGGTCTGCATATGGAACGCGTCGCGCATTTGAT
**
protein N T A D E T S F R G L H M E L R R A F D

TtCutA CTGATTGCGGAAGGCAAACTGGAAGAATGCTGCGAACAGTATGTGTTCCGAAAGAAGAA
TtCutA_opt CTGATTGCTGAAGGCAAACTGGAAGAATGCTGTGAACAGTATGTGTTCCGAAAGAAGAA

protein L I A E G K L E E C C E Q Y V F P K E E

TtCutA GAACGCGTGTGGCAGAAACCGCCGCCGCCGCCGCGCCGATTCCTGCTGCGCAGCAGCAGC
TtCutA_opt GAACGCGTTTGGCAGAAACCGCCGCCGCCGCCGCCGCGTCCGATCCTGCTGCGCAGTTCCCTCA

protein E R V W Q K P P P A P R P I L L R S S S

TtCutA CAGCAGCATGGCAACAGCGGCAACCGCCTGCGCGGCGGCGGCTTTGCGGCAACCGCCAG
TtCutA_opt CAGCAACACGGTAACAGCGGCAATCGCCTGCGTGGTGGCGGTTTCCGCGGTAACCGTCAG

protein Q Q H G N S G N R L R G G G F R G N R Q

TtCutA TTTCATCGCAACGCGGCGCGGGGCCGCCGAGCAGCAGCAGCGTGACCTATGATCCGAAC
TtCutA_opt TTTCATCGTAATGGCGGTGCAGGTGCTGCTCGAGCTCTAGTGTACGTATGATCCGAAC

protein F H R N G G A G R R S S S S V T Y D P N

TtCutA AGCGTGTATAGCCAGGCGGGCATGCCGCCGACCATGAGCCCGCAGGATCTGCTGTGGTAT
TtCutA_opt AGCGTGTACTCTCAAGCCGGTATGCCGCCGACCATGAGCCCGCAGGATCTGCTGTGGTAT

protein S V Y S Q A G M P P T M S P Q D L L W Y

TtCutA CAGGCGCAGAGCCCGCAGCTGAGCGTGCCGCGAGGAAATTCTGACCACCAGCCTGAACGCG
TtCutA_opt CAAGCTCAGAGTCCGCAACTGTCCGTGCCGCGAGGAAATTCTGACCACGTCACTGAACGCG
** ** ***** ** *****
protein Q A Q S P Q L S V P Q E I L T T S L N A

TtCutA CTGGCGGCGCAGGAAAGCATGCGCTTTCAGCTGTATACCCAGCTGAACCAGCAGCAGGTG
TtCutA_opt CTGGCAGCTCAGGAATCGATGCGCTTTCAGCTGTACACCCAGCTGAATCAGCAACAGGTT
***** ** ***** ***** ***** *****
protein L A A Q E S M R F Q L Y T Q L N Q Q Q V

TtCutA CTGGCGCATGCGCAGCGCGTGCGAGCGGGCGCGAGCACCACCGATCGCAGCCGCACCAAC
TtCutA_opt CTGGCACACGCTCAGCGTGTCCAACAGGGCGCCAGCACCACGGATCGCTCACGTACGAAC
***** ** ** ***** ** * ***** ***** ** * **
protein L A H A Q R V Q Q G A S T T D R S R T N

TtCutA AGCCTGGATAACCCGCGCTGACCGCGCCGCTGCGCCCGGAAGTATGTATGGCATTGGC
TtCutA_opt TCAGCTGGACAATCCGCGCTGACCGCACCGGTTCTGCCGAAGTATGTACGGTATTGGC
***** ** ***** ***** ** *****
protein S L D N P P L T A P V R P E L M Y G I G

TtCutA TTTCCGATTTCAGCATGCGCCGTATTTTCATCCGGGCTTTACCACCTATCCGAGCAGCCCG
TtCutA_opt TTCCCGATCCAGCATGCGCCGTATTTTCACCCGGGTTTACCACGTACCCGTCCTCACCG
** ***** ***** ***** ***** ** ***** * **
protein F P I Q H A P Y F H P G F T T Y P S S P

TtCutA GCGACCGCGCGCCGCCGAGCAACGCCAGCCGGAATTTTCGCCGAGCCTGCATCGCACC
TtCutA_opt GCAACCGCAGCACCGCCGAGCAACGCCAGCCGGAATTTTCGTCGCTCTCTGCATCGTACC
** ***** ** ***** ***** *****
protein A T A A P P S N G Q P E F R R S L H R T

TtCutA GCGGCGGCGAGCGATAGCGGCGTGAGCCCGGCGAGCGGCGGCTGCGCAGCCAGAGCCAG
TtCutA_opt GCAGCTGCGTCAGATTGCGGTGTGAGTCCGGGTTCGGGTGCACTGCGTAGCCAATCTCAG
** ** *** ** ***** ***** ** ** ***** *****
protein A A A S D S G V S P G S G A L R S Q S Q

TtCutA CCGGCGAGCCGCACCACCCTGCCGCCGATAGCATGGCGGTGTATAGCACCAGCAGCCAG
TtCutA_opt CCGGCTTCTCGTACCACGCTGCCGCCGATAGCATGGCGGTTTATTCTACCTCGAGCCAG
***** ** ***** ***** *****
protein P A S R T T L P P H S M A V Y S T S S Q

TtCutA AGCCATACCGGCATTGGCGCGAGCCAGAGCCGCCAGGTGCATGATGTGGCGGTGCCGAGC
TtCutA_opt AGTCACACGGGTATCGGCGCGAGTCAATCCCGCCAGGTGCATGATGTGCCGTCCCGAGC
** ** ** ** * ***** ** ***** *****
protein S H T G I G A S Q S R Q V H D V A V P S

TtCutA TTTATGCCGATGAACGCGGCGATCTGGGCTTTGATGATGTGCCGACAAACCGATTAGC
TtCutA_opt TTCATGCCGATGAACGTGGTGACCTGGGCTTTGATGACGTTCCGACAAACCGATTTC
** ***** ** * ***** *****
protein F M P D E R G D L G F D D V P T K P I S

TtCutA GATAGCCCGCCGAAAACGATGGCCCGCGCTTTATTGGCTATTATGTGAACGCCATAGC
TtCutA_opt GATTCGCCCGCCGAAAATGACGGTCCGCGCTTCATCGGCTATTACGTGAACGGTCACAGC
*** ***** ** * ***** ** ***** ** *
protein D S P P E N D G P R F I G Y Y V N G H S

TtCutA GGCAGCAGCCGCCCGGCGAACGGCTTTCGAGCGTGGGCGTGGCGAGCGCGGAAAGCAGC
TtCutA_opt GGCTCTAGTCGTCCGGCCAATGGTTTTCGTCGCGTGGGCGTGGCGTACGCCGAATCTCTCA
*** ** ** ***** ** * ***** ***** ** *
protein G S S R P A N G F P S V G V A S A E S S

TtCutA CAGAGCGGCCAGGGCGGCGGCCCGCCCGCTGAGCACCGATCAGCTGCCGCGAGCGGTG
TtCutA_opt CAATCTGGTCAGGGCGGTGGCCGTCCGCGTCTGAGTACGGATCACTGCCGCGAGTCCGTG
** ** ***** ***** ***** ** ***** *****
protein Q S G Q G G G R R R L S T D Q L P Q S V

TtCutA CTGGATCGCCGCATGAAACGCACCAGCCGAGCCCGATGGGCCATGCGCGCGCG
TtCutA_opt CTGGACCGCCGTATGAAACGCACCAGCCGTTCTCCGAGTCCGATGGGTATGCACGTGCT

protein L D R R M K R T S R S P S P M G H A R A

TtCutA CTGAGCGTGGGCGTGAACAGCGCGCCGCTGCTGAGCGCGCCGTTTGCAGAGCAACGGC
TtCutA_opt CTGTCCGGTCGGCGTGAACAGCGCACCGCTGCTGTCTGCACCGTTTGCACAGTCAAACGGC
*** **
protein L S V G V N S A P L L S A P F A Q S N G

TtCutA AAAGTCCGAGCAAACCGCTGGTGGTGAACGGCACCGCGCGGAAACCGCGAGCACCCCG
TtCutA_opt AAGCTGCCGTCAAACCGCTGGTGTGTAATGGTACCGCACCGGAAACCGCAAGCACGCCG
** *****
protein K L P S K P L V V N G T A P K T A S T P

TtCutA GGCAGCGGCCGCCCCACCGTGGTGACCGAAAGCCTGGTGAGCGATGATAGCCTGCATGAT
TtCutA_opt GGTCTGGTTCGTCCGACCGTGGTTACGGAATCACTGGTGTCCGATGACAGCCTGCATGAT
** **
protein G S G R P T V V T E S L V S D D S L H D

TtCutA AACGCGCTGCATATTCATCAGGGCGTGAGCCCGAGCAGCAGCTGGCCGGAACAGCAGCCG
TtCutA_opt AATGCCCTGCATATTCACAGGGGTGTTTCCCGTCGAGCTCTTGCCCGGAACAACAGCCG
** *
protein N A L H I H Q G V S P S S S W P E Q Q P

TtCutA ACCCGCCGAGCAGCGCGCGGAAAGCGTGGCGACCCGCGTGCCGGAACGCCCGGTGATT
TtCutA_opt ACGCCGCGAGTTCGCGAGCAGAAAGCGTGGCAACCCGCTGTCCCGGAACGTCCGGTTATC
** *****
protein T P P S S A A E S V A T R V P E R P V I

TtCutA GTGAACGGCAGCAGCACCAACCGCAGCCCGAGCGCGACCGTGCCGAACCTGCTGGATAACC
TtCutA_opt GTCAACGGCTCATCGACCAATCGCTCCCCGTGAGCCACGGTTCGGAACCTGCTGGATAACC
** *****
protein V N G S S T N R S P S A T V P N L L D T

TtCutA AGCTTTCAGCAGCGCGTGGCGATGGGCGCGGCCTGCATCTGCCGTATAGCGCGGTGGTG
TtCutA_opt AGCTTCCAACAGCGTGTGCGAATGGGTGCTGGCCTGCACCTGCCGTATAGTGGGTGCTG

protein S F Q Q R V A M G A G L H L P Y S A V V

TtCutA GGCGATCCGAACGGCCTGGCGGGCTTTAGCCGCGCGCATACCCGCCAGCAGCTGGCGCCG
TtCutA_opt GGTGATCCGAATGGTCTGGCGGGCTTTCCCGCGCCATACGCGTCAACAGCTGGCACCG
** *****
protein G D P N G L A G F S R A H T R Q Q L A P

TtCutA CTGGATCTGGCGACCGGCGATTATGCGGCGCATCAGGATCTGCCGATCTGAGCCCGGTG
TtCutA_opt CTGGATCTGGCTACCGGCGACTATGCTGCGCATCAGGATCTGCCGACCTGAGTCCGGTC

protein L D L A T G D Y A A H Q D L P H L S P V

TtCutA TATGAACATCGCACCCCGAGCCCGACCGTGCCGCGCAAATTTGAACCGAGCCCGATTAGC
TtCutA_opt TACGAACACCGTACCCCGTCCCCGACGGTGCCGCGTAAGTTTGAACCGTCTCCGATTAGT
** *****
protein Y E H R T P S P T V P R K F E P S P I S

TtCutA CAGCTGAGCGGCGCGGGCGGAAAGATTTTCGCGAGCGATTTTAGCCCGAAAGCGACCCAG
TtCutA_opt CAGCTGTCCGGTGGGCGGCCAAAGATTTTCGCTCAGACTTCTCGCCGAAGGCAACCCAG

protein Q L S G A G A K D F R S D F S P K A T Q

TtCutA AAAGCGCCGAGCAGCCGAGCCCGAACGGCGGCGCGCCGACCCGCCAGCAGGATAGCGCG
TtCutA_opt AAAGCTCCGAGCTCTCGTAGCCGAATGGTGGCGCACCGACGCGTCAACAGGATTCTGCA

protein K A P S S R S P N G G A P T R Q Q D S A

TtCutA	ACCAGCCGCAGCCCGGCGCAGGATCAGCGCATTAACGGCCTGGCGCGCGAAAAACGGCCAT
TtCutA_opt	ACCTCGCGTAGCCCGGCACAAGACCAGCGTATCAACGGTCTGGCGCGCGAAAAATGGCCAT
	*** ** ***** ** ** ***** ** ***** ***** ***** *****
protein	T S R S P A Q D Q R I N G L A R E N G H
 TtCutA	 GTGCGCGCGGCGAAAAAGCCAGACCGAAAGCTTTAGCGGCTGGCAGAAAACCAAACCCGC
TtCutA_opt	GTGCGTGCCGCAAAAAGTCAAACCGAATCTTTCAGTGGTTGGCAGAAGACCAAAACGCGC
	***** ** ** ***** ** ***** ** ** ** ***** ***** ***** **
protein	V R A A K S Q T E S F S G W Q K T K T R
 TtCutA	 AAAAAAGGCGGCGCGGCGGCGGCGGAACTGAAACAGAGCAGCGCGGCGAACGGCTTTCCG
TtCutA_opt	AAAAAGGGTGGCGCTGCAGCAGCAGAACTGAAACAAAGTTCCGCTGCGAACGGCTTTCCG
	***** ** ***** ** ** ** ***** ** *** ***** ***** *****
protein	K K G G A A A A E L K Q S S A A N G F P
 TtCutA	 CAGAGCGAACCGCCGCCGAAAAACGAAGCGGATCGCAAAGGCGGC
TtCutA_opt	CAGAGCGAACCGCCGCCGAAGAATGAAGCGGATCGTAAAGGTGGC
	***** ***** ** ***** ***** **
protein	Q S E P P P K N E A D R K G G

SUPPLEMENTAL FIGURE 1. Alignments of the native (AnCutA/TtCutA) and recoded (AnCutA_opt/TtCutA_opt) open reading frames corresponding to AnCutA and TtCutA proteins. Nucleotides unchanged during recoding are marked with asterisks. Amino acid sequences of the proteins are shown in bold.

SUPPLEMENTAL TABLES

SUPPLEMENTAL TABLE 1. Frequency of nucleotide incorporation into the tails synthesized by full-length wild-type AnCutA and TtCutA depending on the identity of 1 or 2 preceding nucleotides at the primer's end. Higher and lower frequencies, as compared to the average values (marked in grey), which are discussed in the main text, are indicated with green and red colors, respectively.

Primer's end	AnCutA								TtCutA							
	ss22-A ₄				ss22-U ₄				ss22-A ₄				ss22-U ₄			
	A	G	C	U	A	G	C	U	A	G	C	U	A	G	C	U
Average frequency of nucleotide incorporation [%]																
*	30.9	0.5	52.8	15.9	20.2	1.0	65.7	13.1	48.3	0.5	43.5	7.6	36.7	1.1	56.3	5.9
Frequency of nucleotide incorporation depending on <u>1</u> preceding nucleotide																
*A	42.2	0.3	47.0	10.5	28.0	0.6	63.0	8.4	58.5	0.4	36.1	4.9	45.9	0.7	50.1	3.3
*G	28.0	4.0	50.3	17.7	18.6	1.5	68.3	11.6	39.4	2.2	45.8	12.5	30.9	2.8	59.8	6.5
*C	25.1	0.6	52.5	21.8	22.6	1.4	55.7	20.3	39.5	0.7	48.6	11.3	33.7	1.3	56.9	8.0
*U	14.6	0.4	76.6	8.4	11.1	0.7	80.8	7.4	18.4	0.5	76.0	5.1	19.2	0.9	75.4	4.5
Frequency of nucleotide incorporation depending on <u>2</u> preceding nucleotides																
*AA	48.9	0.3	39.4	11.5	31.3	0.4	61.8	6.5	60.4	0.4	33.9	5.3	47.9	0.7	48.5	2.9
*AG	28.7	2.9	53.4	15.1	15.7	3.1	73.1	8.1	42.5	1.4	43.8	12.2	30.7	2.7	59.7	6.9
*AC	25.9	0.3	56.0	17.8	18.6	0.6	65.5	15.4	39.2	0.5	50.9	9.4	29.9	0.9	62.6	6.6
*AU	20.3	0.2	71.5	7.9	11.4	0.3	83.9	4.4	21.2	0.4	73.7	4.7	19.2	0.8	76.6	3.4
*GA	26.5	2.4	62.7	8.4	18.1	2.0	72.7	7.2	55.2	1.5	36.8	6.5	53.4	0.9	42.4	3.4
*GG	19.2	32.7	9.6	38.5	34.9	14.0	41.9	9.3	23.2	27.0	29.5	20.4	27.2	6.6	57.9	8.3
*GC	24.5	0.8	48.4	26.3	20.6	1.6	56.4	21.4	37.7	1.5	47.8	13.0	41.0	1.5	52.9	4.6
*GU	14.7	6.1	63.2	16.0	15.8	3.7	66.6	13.9	20.5	3.7	68.3	7.4	24.2	3.0	67.1	5.7
*CA	32.1	0.3	59.6	8.1	28.8	0.7	61.5	9.0	53.2	0.4	42.4	4.1	44.0	0.8	51.8	3.4
*CG	26.7	1.4	55.6	16.3	24.2	1.1	62.7	12.0	37.2	1.3	49.7	11.8	31.6	2.6	60.2	5.6
*CC	25.1	0.6	49.5	24.8	17.7	1.5	53.7	27.0	39.1	0.7	47.7	12.4	29.2	1.6	60.4	8.7
*CU	12.1	0.4	79.4	8.1	10.5	0.7	81.3	7.5	16.7	0.4	77.7	5.2	18.9	0.9	75.4	4.8
*UA	31.4	0.4	58.2	10.0	23.6	0.6	67.5	8.3	51.6	0.4	42.7	5.3	47.6	0.7	47.5	4.2
*UG	25.6	11.0	43.9	19.5	15.0	1.9	68.4	14.8	33.0	7.3	43.2	16.5	31.1	3.4	58.3	7.2
*UC	22.9	0.9	55.1	21.2	22.9	1.8	53.9	21.5	40.6	0.9	44.8	13.7	44.1	1.5	42.9	11.5
*UU	13.9	0.8	76.6	8.8	11.7	1.0	78.4	8.9	17.8	0.9	75.2	6.1	17.2	1.0	77.3	4.5

SUPPLEMENTAL TABLE 2. Plasmids used in this study.

Plasmid	Source or genotype
pET28M N-6xHis-SUMOTag	(Lebreton et al, 2008; Malakhov et al, 2004)
pET28 C-6xHisTag	pET-28b(+) from Novagen
pUC57-AnCutA	this study; recoded AnCutA ORF synthesized by Genscript
pUC57-TtCutA	this study; recoded TtCutA ORF synthesized by Genscript
pCC1	[pET28 C-6xHisTag] AnCutA ^{WT} , full-length
pCC2	[pET28 C-6xHisTag] AnCutA ^{D203A/D205A} (DADA), full-length
pCC3	[pET28M N-6xHis-SUMOTag] AnCutA _{tr} ^{WT}
pCC4	[pET28 C-6xHisTag] TtCutA ^{WT} , full-length
pCC5	[pET28M N-6xHis-SUMOTag] TtCutA _{tr1} ^{WT}
pCC6	[pET28 C-6xHisTag] TtCutA _{tr2} ^{WT}
pCC7	[pET28 C-6xHisTag] TtCutA _{tr3} ^{WT}
pCC8	[pET28 C-6xHisTag] TtCutA _{tr3} ^{R557A} (PAP1)
pCC9	[pET28 C-6xHisTag] TtCutA _{tr3} ^{R557Q} PAP2 (PAP2)
pCC10	[pET28 C-6xHisTag] TtCutA _{tr3} ^{E551D/N554E/R557A} (PAP3)
pCC11	[pET28 C-6xHisTag] TtCutA _{tr3} ^{E551T/R557A/L559S} (PAP4)
pCC12	[pET28 C-6xHisTag] TtCutA _{tr3} ^{R557N/N561R} (PAP5)
pCC13	[pET28 C-6xHisTag] TtCutA _{tr3} ^{E551D/R557N/N561R} (PAP6)
pCC14	[pET28 C-6xHisTag] TtCutA _{tr3} ^{R557H} (PUP1)
pCC15	[pET28 C-6xHisTag] TtCutA _{tr3} ^{E551D/R557H} (PUP2)
pCC16	[pET28 C-6xHisTag] TtCutA _{tr3} ^{E551D/N554E/R557H} (PUP3)
pCC17	[pET28M N-6xHis-SUMOTag] Cid1 ^{WT}
pCC18	[pET28M N-6xHis-SUMOTag] Cid1 ^{D330E/E333N/H336R} (PCP)

SUPPLEMENTAL TABLE 3. Oligonucleotides used in this study.

Oligonucleotide	Sequence (5'-3')	Purpose
<i>Oligonucleotides for cloning and site-directed mutagenesis</i>		
278F	ttttgtttaactttaagaaggagatatata ccATGG TCACCGTCA CCGATGG	cloning of AnCutA WT ORF into pET28 C-6xHisTag
278R	atctcagtggtggtggtggtggtg ctcgag GCCACCTTTGCGC AGGCTTTC	cloning of AnCutA WT ORF into pET28 C-6xHisTag
280F	ttttgtttaactttaagaaggagatatata ccATGG ACTCGCAAC CGGCAAC	cloning of TtCutA WT ORF into pET28 C-6xHisTag
280R	atctcagtggtggtggtggtggtg ctcgag GCCACCTTTACGA TCCGCTTC	cloning of TtCutA WT ORF into pET28 C-6xHisTag
CUCUmutF	CTCTGAC agcgct GTGGCCATTTCATCACCACGACCTGTAAA GAAGTGG	site-directed mutagenesis (AnCutA D203A D205A)
CUCUmutR	TGCAAATGGCCAC agcgct GTTCAGAGCTACACAGCTTATTGCC CGATGAACC	site-directed mutagenesis (AnCutA D203A D205A)
278sumoF	gaagtctaccaggaacaaaccggt ggatcc AGCACCCATAGCG AATCTAC	cloning of AnCutA_tr into pET28M N-6xHis-SUMOTag
278sumo2	tcagtgggtggtggtggtggtg ctcgag ttAACGGGACGGCGGC GGTGCGG	cloning of AnCutA_tr into pET28M N-6xHis-SUMOTag
280sumoF	gaagtctaccaggaacaaaccggt ggatcc AGCGCCTATTCTG AAACCAAC	cloning of TtCutA_tr1 into pET28M N-6xHis-SUMOTag
280sumo2	tcagtgggtggtggtggtggtg ctcgag ttAGCGCAGCAGGATC GGACGCG	cloning of TtCutA_tr1 into pET28M N-6xHis-SUMOTag
12.280 S_F	ttttgtttaactttaagaaggagatatata ccatg AGCGCCTATT CTGAAACC	cloning of TtCutA_tr2 into pET28 C-6xHisTag
12.280 sl_R	atctcagtggtggtggtggtggtg ctcgag GCGACCATCATTA AACGGTTC	cloning of TtCutA_tr2 into pET28 C-6xHisTag
280sIIIF	ttttgtttaactttaagaaggagatatata ccatg AAGCCGGCCA AAAATTC	cloning of TtCutA_tr3 into pET28 C-6xHisTag
280sIIIR	atctcagtggtggtggtggtggtg ctcgag CAGCGTGGTACGA GAAGCCG	cloning of TtCutA_tr3 into pET28 C-6xHisTag
280PAP1F	GTTTAATGATGGT GCCAAcctagg CAATACCGCCGACGAAACG	site-directed mutagenesis TtCutA WT→PAP1 (R557A)
280PAP1R	CGGCGGTATTG cctagg TTGGCACCATCATTAACGGTTCTTC	site-directed mutagenesis TtCutA WT→PAP1 (R557A)
280PAP2F	GTTTAATGATGGT CAGAAcctagg CAATACCGCCGACGAAACG	site-directed mutagenesis TtCutA WT→PAP2 (R557Q)
280PAP2R	CGGCGGTATTG cctagg TTCTGACCATCATTAACGGTTCTTC	site-directed mutagenesis TtCutA WT→PAP2 (R557Q)
280PAP3F	GCGTTGAAGACCCGTTT GAGG ATGGTGCCAAcct agg CAATAC CGCCGAC	Site-directed mutagenesis TtCutA PAP1→PAP3 (E551D N554E R557A)
280PAP3R	ATTGcc gagg TTGGCACCATC CTCAA ACGG GTCTT CAACGCAC AGAGCATTG	site-directed mutagenesis TtCutA PAP1→PAP3 (E551D N554E R557A)
280PAP4F	TTGAAACACCGTTTAATGATGGTGCCAAc agcgg CAATACCGC CGACGAAAC	site-directed mutagenesis TtCutA PAP1→PAP4 (E551T R557A L559S)
280PAP4R	TTGcc gctg TTGGCACCATCATTAACGGT GT TTCAACGCACA GAGCATTGTTC	site-directed mutagenesis TtCutA PAP1→PAP4 (E551T R557A L559S)
280PAP5F	TTTAATGATGGT AACAAcctagg CAGGACCGCCGACGAAACGA GCTTCC	site-directed mutagenesis TtCutA WT→PAP5 (R557N N561R)

280PAP5R	TTTCGTCGGCGGTCTGcctaggTTGTTACCATCATTAACGG TTCTTC	site-directed mutagenesis TtCutA WT→PAP5 (R557N N561R)
280PAP6F	TTTAATGATGGTAACAAcct tggCAGGACCGCCGACGAAACGA GCTTCC	site-directed mutagenesis TtCutA PUP2→PAP6 (E551D R557N N561R)
280PAP6R	TTTCGTCGGCGGTCTGccaggTTGTTACCATCATTAACGG ATCTTC	site-directed mutagenesis TtCutA PUP2→PAP6 (E551D R557N N561R)
280PUP1F	GTTTAATGATGGTCACAAcctaggCAATACCGCCGACGAAACG	site-directed mutagenesis TtCutA WT→PUP1 (R557H)
280PUP1R	GCGGTATTGcctaggTTGTGACCATCATTAACGGTTCTTC	site-directed mutagenesis TtCutA WT→PUP1 (R557H)
280PUP2F	GCGTTGAAGATCCGTTTAATGATGGTCACAAcctaggCAATAC CGCCGACGAAAC	site-directed mutagenesis TtCutA WT→PUP2 (E551D R557H)
280PUP2R	GCGGTATTGcctaggTTGTGACCATCATTAACGGATCTTCAA CGCACAGAGCATTG	site-directed mutagenesis TtCutA WT→PUP2 (E551D R557H)
280PUP3F	AGATCCGTTTGAAGATGGTCACAAcct cggCAATACCGCCGAC GAAACG	site-directed mutagenesis TtCutA PUP2→PUP3 (E551D N554E R557H)
280PUP3R	CGGTATTGccgaggTTGTGACCATCTTCAAACGGATCTTCAAC GCACA	site-directed mutagenesis TtCutA PUP2→PUP3 (E551D N554E R557H)
Cid1SumoF	tgattgaagtctaccaggaacaaaccggtggatccATGAACAT TTCTTCTGCACAA	cloning of Cid1 WT ORF into pET28M N-6xHis- SUMOtag
Cid1SumoR	cggatctcagtggtggtggtggtggtgctcgagtcaCTCAGAA TTGTCACCATCGG	cloning of Cid1 WT ORF into pET28M N-6xHis- SUMOtag
Cid1PCPF	GCGATTGAAgagcccTTCAATATTTACGTAATGTGGGTAGGA CAGTTAG	site-directed mutagenesis Cid1 WT→PCP (D330E E333N H336R)
Cid1PCPR	TACCCACATTACGTGAAATATTGAagggtcTTCAATCGCAAG AATATAC	site-directed mutagenesis Cid1 WT→PCP (D330E E333N H336R)
Sequencing primers		
T7promoter	TAATACGACTCACTATAGGG	Sequencing of inserts in pET28 C-6xHisTag
SumoF	TCATACTGTCAAAGACAGGG	Sequencing of inserts in pET28M N-6xHis-SUMOtag
T7terminator	TGCTAGTTATTGCTCAGCGG	Sequencing of inserts in pET28 C-6xHisTag and pET28M N-6xHis-SUMOtag
278 seq 1	GAACAATACCATGGCCCTGG	Sequencing of AnCutA
278 seq 2	AGTAACCAGTATTCCCGTGG	Sequencing of AnCutA
12_107_s1	ACATTTGCATCACCACGACC	Sequencing of AnCutA
12_107_s2	AACACCATTGCAACCTGGG	Sequencing of AnCutA
12_107_s3	CAATTCAACCTATCTGCCGG	Sequencing of AnCutA
12_107_s4	GCTGATTGTGACGGTTTTCAG	Sequencing of AnCutA
280seq5	CAGCATTCTTCCAGTTTGCC	Sequencing of TtCutA
12_109_s1	GCAGCGCCTATTCTGAAACC	Sequencing of TtCutA
12_109_s2	TATCAAATACTGGACCCGCC	Sequencing of TtCutA
12_109_s3	CCGCGGTAACCGTCAGTTTC	Sequencing of TtCutA
12_109_s4	GAGCTTCATGCCGGATGAAC	Sequencing of TtCutA

<i>RNA oligonucleotides</i>		
5'-FAM-ss17-A ₃₅	FAM-r (CCCCACCACCAUCACUUAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAA)	RNA substrate used in biochemical assays
5'-FAM-ss17-A ₃₁ U ₄	FAM-r (CCCCACCACCAUCACUUAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAUUUU)	RNA substrate used in biochemical assays
5'-FAM-ss17-A ₃₁ C ₄	FAM-r (CCCCACCACCAUCACUUAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAACCCC)	RNA substrate used in biochemical assays
5'-FAM-ss22-A ₄	FAM-r (CGACUGGAGCACGAGGACACUGAAAA)	RNA substrate used in biochemical assays
5'-FAM-ss22-U ₄	FAM-r (CGACUGGAGCACGAGGACACUGUUUU)	RNA substrate used in biochemical assays
5'-FAM-ss22-C ₄	FAM-r (CGACUGGAGCACGAGGACACUGCCCC)	RNA substrate used in biochemical assays
<i>Oligonucleotides used in the 3'-RACE-seq procedure</i>		
RA3	TGGAATTCTCGGGTGCCAAGG	Adapter oligonucleotide
RTP	<u>GCCTTGGCACCCGAGAATTCCA</u>	Reverse transcription primer
RA5 _{ss22}	G TTCAGAGTTCTACAGTCCGACGATC CGACTGGAGCACGAGGA CACTG	First-round PCR primer (forward)
RP1	AATGATACGGCGACCACCGAGATCTACACGTT CAGAGTTCTAC AGTCCGA	Second-round PCR primer (forward)
RPI_X	CAAGCAGAAGACGGCATACGAGATNNNNNNGTGACTGGAGTT <u>CCTTGGCACCCGAGAATTCCA</u>	PCR primer with RNA-seq index (reverse)

SUPPLEMENTAL TABLE 4. Summary of protein purification strategies.

CutA variant	Epitope	Purification scale	SUMO cleavage + desalting + second nickel column	Buffering agent	[NaCl]	Additional purification	Figure
An FL WT An FL DADA Tt FL WT	C-6xHisTag	Small	-	Na-phosphate pH=8.0	300 mM	-	Figs. 2-4
An_tr WT Tt_tr1 WT	N-6xHis-SUMOTag	Large	Yes	Na-phosphate pH=4.5	300 mM	Gel filtration (Superdex S200) 20 mM Na-phosphate; 150 mM NaCl	Fig. 5
Tt_tr3 WT	C-6xHisTag	Large	-	Na-phosphate pH=4.5	300 mM	Gel filtration (Superdex S200) 20 mM Na-phosphate; 150 mM NaCl	Fig. 5
An_tr WT Tt_tr1 WT	N-6xHis-SUMOTag	Small	No	Na-phosphate pH=4.5	300 mM	-	Fig. 6
An FL WT Tt FL WT Tt_tr3 WT	C-6xHisTag	Small	-	Na-phosphate pH=4.5	300 mM	-	Fig. 6
Tt_tr3 (WT, PAP1-PAP6, PUP1-PUP3)	C-6xHisTag	Large	-	Na-phosphate pH=4.5	300 mM	Gel filtration (S200) 20 mM Na-phosphate; 150 mM NaCl	Figs. 8-13
Cid1 (WT, PCP)	N-6xHis-SUMOTag	Large	Yes	Tris-HCl pH=8.0	150 mM	Gel filtration (S200) 20 mM Tris-HCl 150 mM NaCl	Fig. 14