

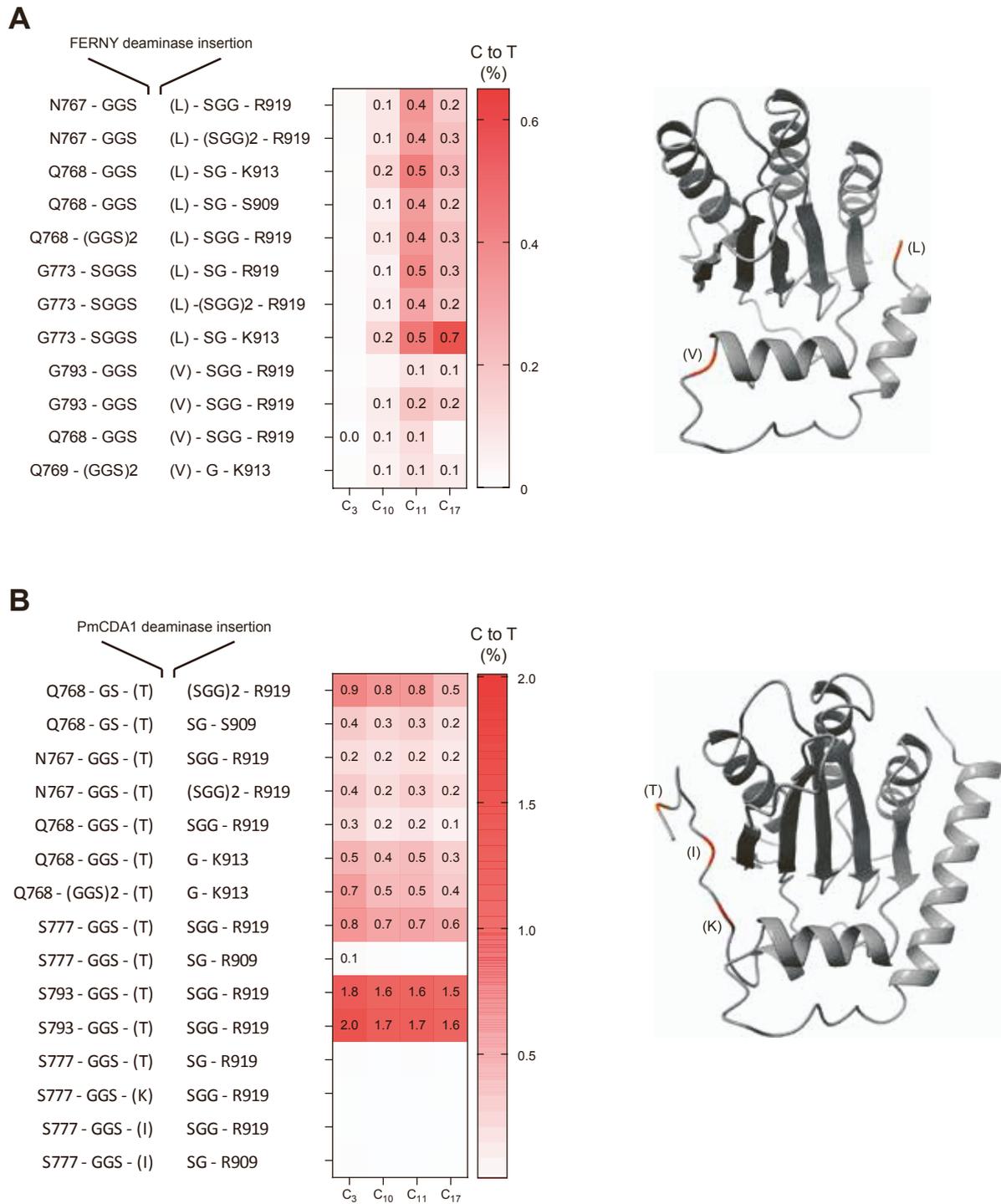
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## **Supplemental information**

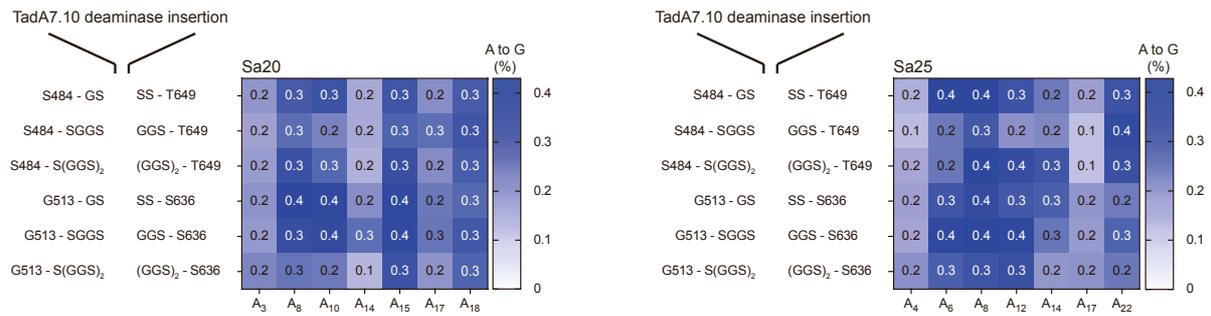
**Replacing the *SpCas9* HNH domain  
by deaminases generates compact base  
editors with an alternative targeting scope**

**Lukas Villiger, Lukas Schmidheini, Nicolas Mathis, Tanja Rothgangl, Kim Marquart, and Gerald Schwank**

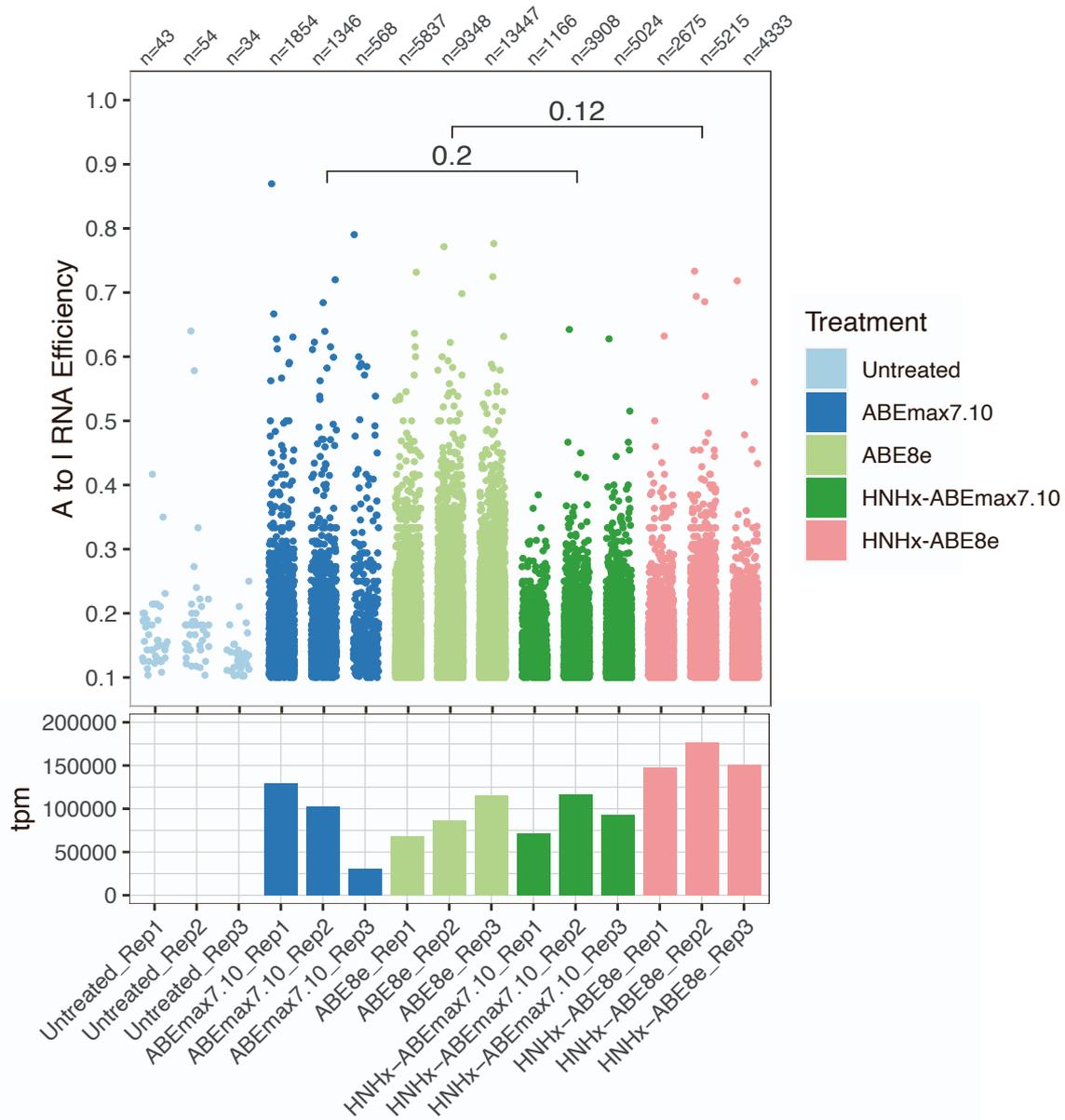




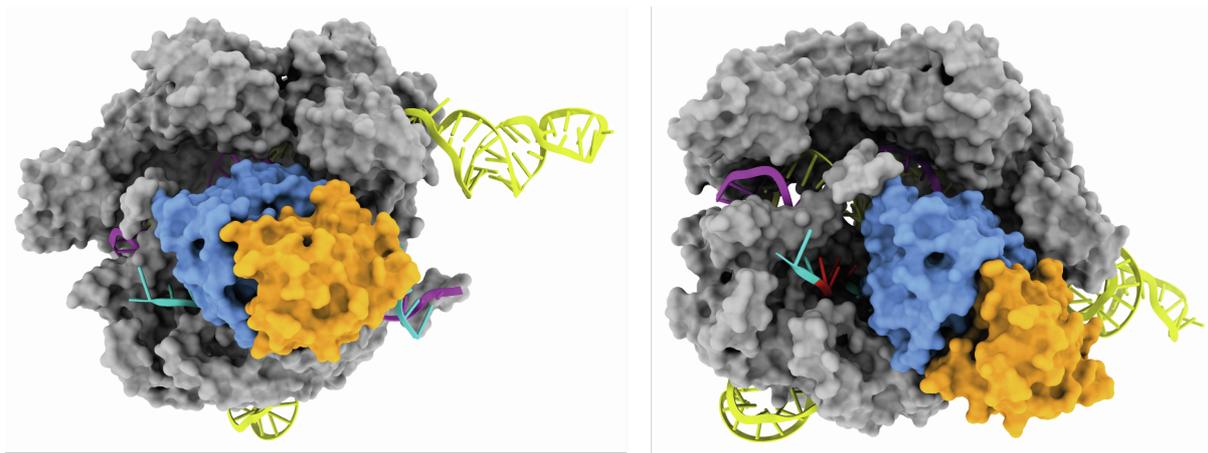
**Figure S2. Linker optimization for FERNY and PmCDA1 integration (A, B)** Heat map depicting different flanking amino acids of Cas9 and linkers to incorporate the FERNY (A) and PmCDA1 (B) deaminases in place of the HNH domain. The FERNY and PmCDA1 deaminase reading frames are listed in the Supplementary Information. Linkers that join Cas9 and deaminase at different amino acid positions within the deaminase are indicated in brackets.



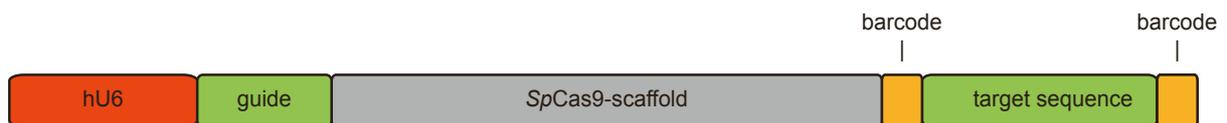
**Figure S3. *Sa*KKH-HNHx-ABEmax7.10 constructs** Heat map depicting editing efficiencies with *Sa*KKH-HNHx-ABEmax7.10 constructs, where different linkers were used to incorporate the TadA deaminase in place of the HNH domain of *Sa*Cas9-KKH. Editing efficiencies of different adenine bases within the protospacer region on endogenous loci Sa20 (left) and Sa25 (right) in HEK293T cells. Numbering starts with PAM-distal nucleotides. Values represent mean of two independent biological replicates performed on separate days  $\pm$  s.d. The TadA deaminase reading frame is listed in the Supplementary Information.



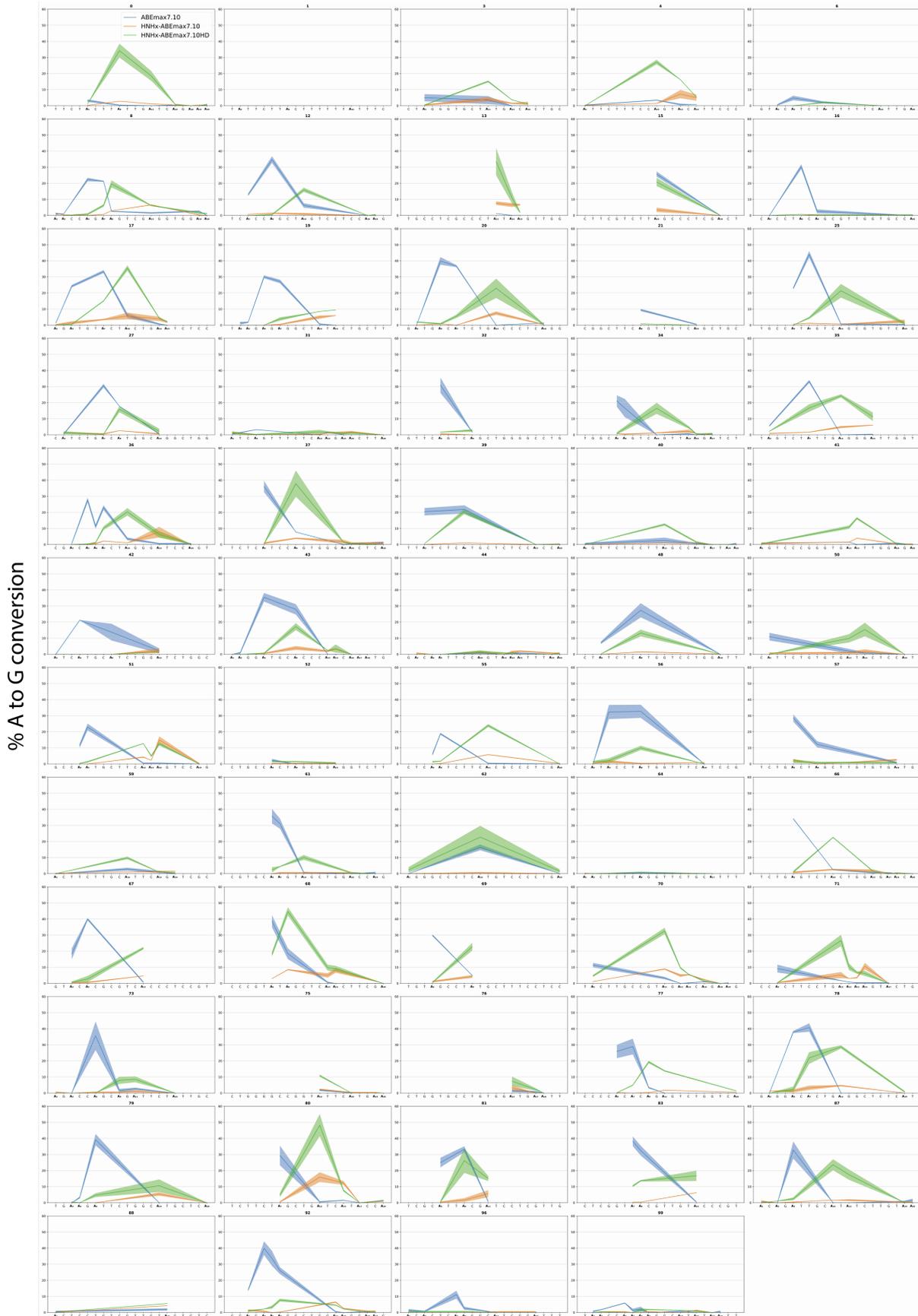
**Figure S4. Transcriptome-wide RNA off-target deamination** RNA-seq analysis of transcriptome-wide A to I RNA off-target editing after transfection of ABEmax7.10, ABE8e, HNHx-ABEmax7.10 and HNHx-ABE8e constructs. Indicated p-Values represent comparison of means of three independent biological replicates using two-tailed unpaired t-test.



**Figure S5. Different viewing angles of a hypothetical HNHx-ABE variant with a dimerized TadA** Subunits are colored as follows: Cas9  $\Delta$ HNH, grey; TadA linked to Cas9, blue; TadA from another molecule dimerizes to Cas9-bound TadA for functional deamination, orange.

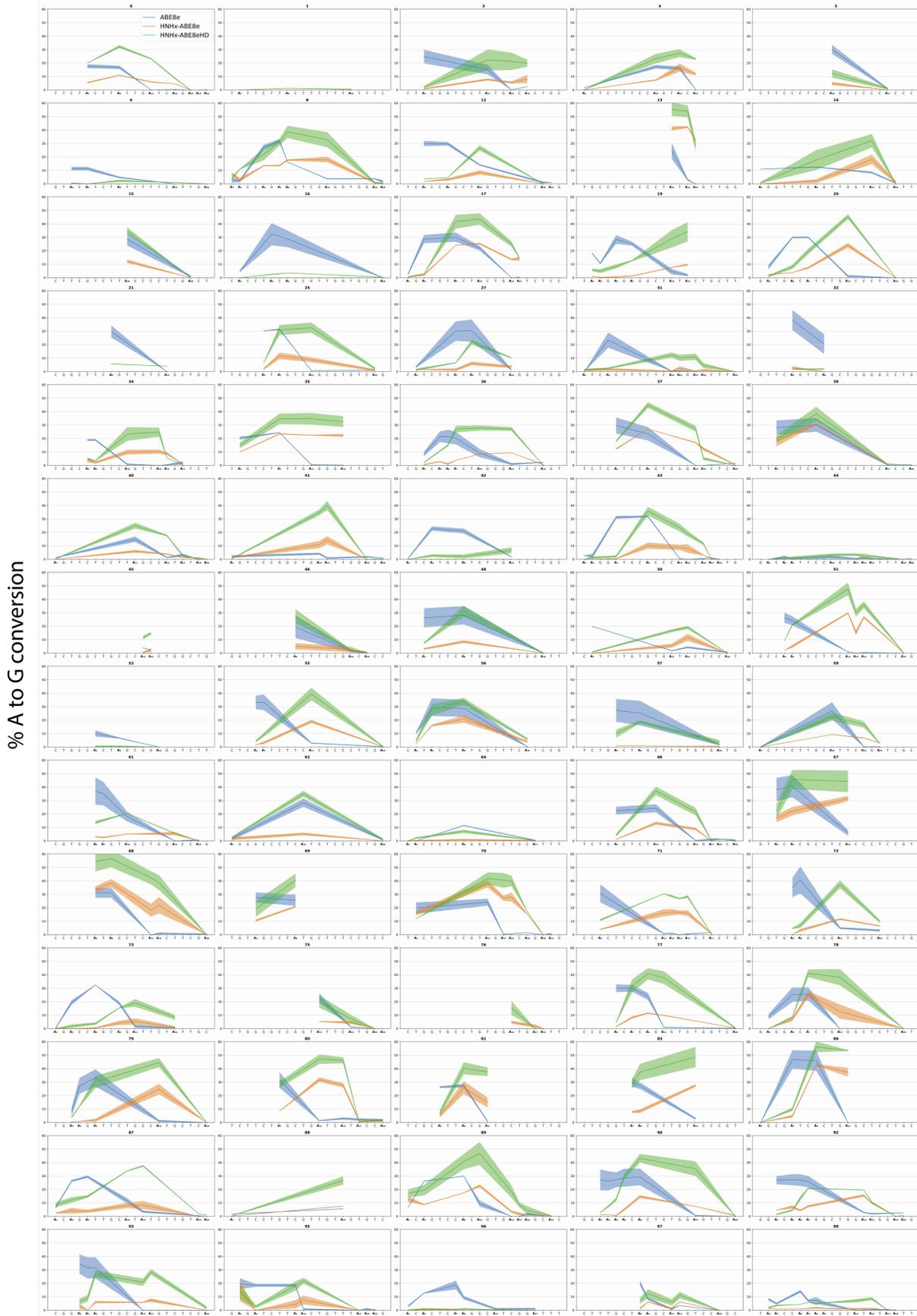


**Figure S6. Schematic organisation of a self-targeting construct** Each self-targeting construct consists of a human U6 promoter (red), a constant *SpCas9* scaffold (grey), a matching guide- and target sequence (green) and unique barcodes (orange). Sequences used are listed in Supplementary Table 2.



**Figure S7. A to G conversions using Tada7.10-based constructs on self-targeting loci** High throughput sequencing data compares editing efficiencies of ABEmax7.10, HNHx-

ABEmax7.10 and HNHx-ABEmax7.10HD on 59 self-targeting loci in HEK293T cells. Solid line represents mean of two independent biological replicates performed on separate days, with error band indicating  $\pm$  s.d.



**Figure S8. A to G conversions using TadA8e-based constructs on self-targeting loci. High throughput sequencing data compares editing efficiencies of ABE8e, HNHx-ABE8e and**

HNHx-ABE8eHD on 70 self-targeting loci in HEK293T cells. Solid line represents mean of two independent biological replicates performed on separate days, with error band indicating  $\pm$  s.d.

## PRIMER and gBLOCK SEQUENCES

See Supplemental tables 1 & 2.

## AMINO ACID SEQUENCES OF BASE EDITOR CONSTRUCTS

### HNHx-ABEmax7.10

MKRTADGSEFEFPKKKRKVGSDDKKYSIGLAIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLI  
GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSSFHRLSEESFLVEEDKKHERH  
PIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVKL  
FIQLVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIALSGLTPNF  
KSNFDLAEDAQLQLSKDQYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS  
MIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEE  
LLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLA  
RGNRSFAWMTRKSEETITPWNFEVVDKGSASQSFIERMTNFDKNLPNEKVLPHKSLLYEYFTVYNEL  
TKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLG  
TYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWG  
RLSRKLINGIRDKQSGKTILDFLKSDGFANRFMQLIHDDSLTFKEDIQKAQVSGQGDLSHEHIANLA  
GSPAIIKKGILQTVKVVDELVKVMGRHKPENIVIAMARENQTTQKGQKNSRERMKRIEEGIKELGSGGS  
SEVEFSHEYWMRHALTLAKRARDEREVPVGAIVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLV  
MQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGIL  
ADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGRQLVETRQITKHVAQILD SRMNTKYDENDKLIREV  
KVI TLKSKLVSDFRKDFQFYK VREINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKM  
IAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL SM  
PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKL  
KSVKELLGITIMERSSEFKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNE  
LALPSKYVNFLYLASHYEKLGKSPEDNEQKQLFVEQHKHYLDEIIIEQISEFSKRVI LADANLDKVL SA  
Y NKHRDKPIREQAENI IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSI TGLYETRID  
LSQLGGDSGGSKRTADGSEFEFPKKKRKV

Nuclear localization signal, SpCas9, Linker (Cas9), Linker (Gly-Gly-Ser, Ser-Gly-Gly), TadA (adenosine deaminase, 7.10)

## HNHx-ABE8e

MKRTADGSEFE<sup>S</sup>PKKKR<sup>KV</sup>GS<sup>D</sup>DKKYSIGLAIGTNSVGVAVITDEYKVPSKKFKVLGNTDRHSIKKNLI  
GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERH  
PIFGNIVDEVAYHEKYPTIYHLRKKLVSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVVKL  
FIQLVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIASLGLTPNF  
KSNFDLAEDAQLQSKDQYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS  
MIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEE  
LLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPIYYVGPLA  
RGNSRFAMTRKSEETITPWNFEEVVDK<sup>G</sup>ASAQSFIERMTNFDKNLPNEKVL<sup>PKH</sup>SLLYEYFTVYNEL  
TKVKYVTEGMRKPAFLS<sup>G</sup>EQKAI<sup>V</sup>DLLFKTNRKVT<sup>V</sup>KQLKEDYFKKIECFDSVEISGVEDRFNASLG  
TYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWG  
RLSRKLINGIRDKQSGKTILD<sup>F</sup>LKSDGFANRNF<sup>M</sup>QLIHDDSLTFKEDIQKAQVSGQGD<sup>S</sup>LHEHIANLA  
GSPA<sup>I</sup>KKGILQTVK<sup>V</sup>DEL<sup>V</sup>KVMGRHKPENIV<sup>I</sup>EMARENQTTQK<sup>G</sup>QKNSRERM<sup>K</sup>RIEEGIKEL<sup>G</sup>SGGS  
<sup>S</sup>EV<sup>E</sup>FSHE<sup>Y</sup>W<sup>M</sup>RHAL<sup>T</sup>LAKRARDERE<sup>V</sup>PV<sup>G</sup>AVL<sup>V</sup>LN<sup>N</sup>RVIGEG<sup>W</sup>NRAIGLHDPTAHAEIMALRQ<sup>G</sup>GLV  
MQNYRLIDATLYVT<sup>F</sup>EPCVMCAGAMI<sup>H</sup>SRIGRVV<sup>F</sup>GVRNSK<sup>R</sup>GAA<sup>G</sup>SLMN<sup>V</sup>LNYPGMNHRVEITEGIL  
ADECAALLCDFYRMP<sup>R</sup>QVFNAQ<sup>K</sup>KAQSSTN<sup>S</sup>GG<sup>R</sup>QLVETRQITKHVAQILD<sup>S</sup>RMNTKYDENDKLI<sup>R</sup>EV  
KVITL<sup>K</sup>SKLVSDFRKDFQFYK<sup>V</sup>REINNYHHAHDAYLNAV<sup>V</sup>GTALIKKY<sup>P</sup>KLESEFVYGDYK<sup>V</sup>YDVRKM  
IAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRK<sup>R</sup>PLIETNGETGEIVWDKGRDFATVRK<sup>V</sup>LSM  
PQVNIVKKTEVQ<sup>T</sup>GGFSKESILPKRNSDKLIARK<sup>K</sup>DWD<sup>P</sup>KKYGGFDSPTVAYSVLV<sup>V</sup>AKVEK<sup>G</sup>KSKKL  
KSVKELLGITIMERS<sup>S</sup>FEKNPIDFLEAKGYKEV<sup>K</sup>DLIIKLPK<sup>Y</sup>SLFELENGRKRMLASAGELQ<sup>K</sup>GNE  
LALPSKYVNF<sup>L</sup>YLASHYEKLK<sup>G</sup>SPEDNEQ<sup>K</sup>QLFVEQHKHYLDEII<sup>E</sup>QISEFSKRVILADANLDK<sup>V</sup>LSA  
YNKHRDKPIREQAENIIHLFTLTNLGAPAA<sup>F</sup>KYFDTTIDR<sup>K</sup>RYTSTKEVLDATLIHQ<sup>S</sup>ITGLYETR<sup>I</sup>D  
LSQLGGDSGG<sup>S</sup>KRTADGSEFE<sup>S</sup>PKKKR<sup>KV</sup>

Nuclear localization signal, SpCas9, Linker (Cas9), Linker (Gly-Gly-Ser, Ser-Gly-Gly), TadA (adenosine deaminase, 8e)

## HNHx-ABEmax7.10HD

MKRTADGSEFE<sup>S</sup>PKKKR<sup>KV</sup>GS<sup>D</sup>DKKYSIGLAIGTNSVGVAVITDEYKVPSKFKVLGNTDRHSIKKNLI  
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PIFGNIVDEVAYHEKYPTIYHLRKKLVSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVVKL  
FIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIALSGLTPNF  
KSNFDLAEDAQLSKDQTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS  
MIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEE  
LLVKNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLA  
RGNSRFAMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPHKSLLYEYFTVYNEL  
TKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLG  
TYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWG  
RLSRKLINGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLA  
GSPAIKKGI<sup>LQ</sup>TVKVVDELVKVMGRHKPENIVIEMARENQ<sup>TTQKGQKNSRERMKRIEEGIKELGSGGS</sup>  
<sup>SEVEFS</sup>HEYWMRHALTLAKRARDEREVPVGVAVLVLN<sup>NRVIGEGWNRAIGLHDPTAHAEIMALRQGGLV</sup>  
<sup>MQNYRL</sup>IDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGIL  
ADECAALLCYFFRMPRQVFNAQKKAQSSTD<sup>SGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFS</sup>  
<sup>HEYWMRHALTLAKRARDEREVPVGVAVLVLN<sup>NRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRL</sup></sup>  
<sup>IDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAA</sup>  
<sup>LLCYFFRMPRQVFNAQKKAQSSTD</sup>SGGRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLK  
SKLVSDFRKDFQFYK<sup>VREINNYHHAHDAYLNAVVG</sup>TALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQ  
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL<sup>SMPQVNIV</sup>  
KKTEVQTGGFSKESILPKRNSDKLIAR<sup>KKDWDPKKYGGFDSPTVAYSVLV</sup>VAKVEK<sup>GKSKKLKSVKEL</sup>  
LGITIMERS<sup>SFEKNPIDFLEAKGYKEVKKDLIIKLPKYS</sup>LFELENGRKRMLASAGELQKGNELALPSK  
YVNFLYLASHYEKLK<sup>GSPEDNEQQLFVEQHKHYLDEII</sup>EQISEFSKRVI<sup>LADANLDKVL</sup>SAYNKHRD  
KPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDR<sup>KRYTSTKEVLDATLIHQ</sup>SITGLYETRIDLSQLGG  
DSGGS<sup>KRTADGSEFE</sup>PKKKR<sup>KV</sup>

Nuclear localization signal, SpCas9, Linker (Cas9), Linker (Gly-Gly-Ser, Ser-Gly-Gly), TadA (adenosine deaminase, 7.10), Linker, TadA (WT)

## HNHx-ABE8eHD

MKRTADGSEFE<sup>S</sup>PKKKR<sup>KV</sup>GS<sup>D</sup>KKYSIGLAIGTNSVGWAVITDEYKVPSK<sup>F</sup>K<sup>F</sup>V<sup>L</sup>GN<sup>T</sup>DRHSIK<sup>K</sup>N<sup>L</sup>I  
GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSF<sup>F</sup>HRLEESFLVEEDKKHERH  
PIFGNIVDEVAYHEKYPTIYHLRKKLV<sup>D</sup>STDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDV<sup>D</sup>KL  
FIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLI<sup>A</sup>LSLGLTPNF  
KSNFDLAEDAQLQLSKD<sup>T</sup>YDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS  
MIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEE  
LLV<sup>K</sup>LNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY<sup>P</sup>FLKDNREKIEKILTFRIPYYVGPLA  
RGNSRFAMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVL<sup>P</sup>KHSLLYEYFTVYNEL  
TKVKYVTEGMRKPAFLSGEQKKAIVD<sup>L</sup>L<sup>L</sup>FKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLG  
TYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWG  
RLSRKLINGIRDKQSGKTILD<sup>F</sup>LKSDGFANRNF<sup>M</sup>QLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLA  
GSPA<sup>I</sup>KKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIE<sup>E</sup>G<sup>I</sup>KELGSGGS  
SEVEFSHEYWMRHALTLAKRARDEREVPV<sup>G</sup>AVLVLN<sup>N</sup>RVIGEGWNRAIGLHDPTAHAEIMALRQGG<sup>L</sup>V  
MQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNSKRGAA<sup>G</sup>SLMNVLNYPGMNHRVEITEGIL  
ADECAALLCDFYRMPRQVFNAQKKAQSSTNSGGSSGGSSGSETPGTSESATPESSGGSSGGSSSEVEFS  
HEYWMRHALTLAKRARDEREVPV<sup>G</sup>AVLVLN<sup>N</sup>RVIGEGWNRAIGLHDPTAHAEIMALRQGG<sup>L</sup>VMQNYRL  
IDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAA  
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SKLVSDFRKDFQFYK<sup>V</sup>REINNYHHAHDAYLNAVVG<sup>T</sup>ALIKKYPKLESEFVYGDYK<sup>V</sup>YDVRKMIAKSEQ  
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL<sup>S</sup>MPQVNIV  
KKTEVQTGGFSKESILPKRNSDKLIAR<sup>K</sup>KDWD<sup>P</sup>KKYGGFDSPTVAYSVLV<sup>V</sup>AKVEK<sup>G</sup>SK<sup>K</sup>L<sup>S</sup>V<sup>K</sup>EL  
LGITIMERS<sup>S</sup>FEKNPIDFLEAKGYKEV<sup>K</sup>DLI<sup>I</sup>KLPK<sup>Y</sup>SLFELENGRKRMLASAGELQKGNELALPSK  
YVNF<sup>L</sup>YLASHYEK<sup>L</sup>KGSPEDNEQQLFVEQHKHYLDEIEEQISEFSKR<sup>V</sup>ILADANLDK<sup>V</sup>LSAYNKHRD  
KPIREQAENIIHLFTLTNLGAPAAF<sup>K</sup>YFDTTIDR<sup>K</sup>RYTSTKEVLDATLIHQ<sup>S</sup>ITGLYETRIDL<sup>S</sup>QLGG  
DSGGSKRTADGSEFE<sup>S</sup>PKKKR<sup>KV</sup>

Nuclear localization signal, SpCas9, Linker (Cas9), Linker (Gly-Gly-Ser, Ser-Gly-Gly), TadA (adenosine deaminase, 7.10), Linker, TadA (WT)

## HNHx - PmCDA1

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PIFGNIVDEVAYHEKYPTIYHLRKKLVSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKL  
FIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIALLSLGLTPNF  
KSNFDLAEDAQLSKDQTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS  
MIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEE  
LLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPIYYVGPLA  
RGNSRFAMTRKSEETITPWNFEEVVDKGASQSFIERMTNFDKNLPNEKVLPHKSLLYEYFTVYNEL  
TKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLG  
TYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWG  
RLSRKLINGIRDKQSGKTILDFLKSDFANRNFQMQLIHDDSLTFKEDIQKAQVSGQGDLSHEHIANLA  
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EYVRIHEKLDIYTFKKQFSNNKKSVMHRCYVLFELKRRGERRACFWGYAVNKPQSGTERGIHAEI FSI  
RKVEEYLRDNPQQTINWYSSWSPCADCAEKILEWYNQELRGNHTLKIWVCKLYYEKNARNQIGLWN  
LRDNGVGLNVMVSEHYQCCRKIFIQSSHNQLNENRWLEKTLKRAEKRRSELSIMFQVKILHTTKSPAV  
SGGSGGRQLVETRQITKHVAQILD SRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVINN  
YHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVDVRKMIKSEQEIGKATAKYFFYSNIMNFFKT  
EITLANGEIRKRELIETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNS  
DKLIARKKDWDPKKYGGFDSPTVAYSVLVAKVEK GKSKKLSVKELLGITIMERSSEKPNPIDFLEA  
KGYKEVKDLIIKLPKYSLFELENGRKRLASAGELQKGNELALPSKYVNFYLYLASHYEKLGSPEDN  
EQKQLFVEQHKHYLDEIEQISEFSKRVI LADANLDKVL SAYNKHRDKPIREQAENI IHLFTLTNLGA  
PAAFKYFDTTIDRKRYTSTKEVLDATLIHQ SITGLYETRIDLSQLGGDSGGSGGSGGSTNLSDIIEKE  
TGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKPWALVIQDSNGEN  
KIKMLSGGSGGSGGSTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDEN  
VMLLTSDAPEYKPWALVIQDSNGENKIKMLSGGSKRTADGSEFEFPPKPKKRV

Nuclear localization signal, SpCas9, Linker (Cas9), Linker (Ser-Gly-Gly-Ser-Gly-Gly), PmCDA1, UGI

**FERNY (=evolved APOBEC) Variant**

MKRTADGSEFFESPKKKRKVGSDDKKYSIGLAIGTNSVGVAVITDEYKVPSKKFKVLGNTDRHSIKKNLI  
GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERH  
PIFGNIVDEVAYHEKYPTIYHLRKKLVSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVKL  
FIQLVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIASLGLTPNF  
KSNFDLAEDAQLQSKDQYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS  
MIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEE  
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RGNSRFAMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPHKSLLYEYFTVYNEL  
TKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLG  
TYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWG  
RLSRKLINGIRDKQSGKTILDFLKSDGFANRFMQLIHDDSLTFKEDIQKAQVSGQGDLSHEHIANLA  
GSPAIKKGIQTVKVVDELVKVMGRHKPENIVIAMARENQTTQKSGSGSGSFERNYDPRELRKETYLL  
YEIKWKGSKGLWRHWCQNNRTQHAEVYFLENIFNARRFNPSTHCSITWYLSWSPCAECSQKIVDFLKE  
HPNVNLEIYVARLYYPENERNRQGLRDLVNSGVTIRIMDLDPYNYCWKTFVSDQGGDEYWPGHFAPW  
IKQYSLKLSGKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDF  
QFYKVINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGDYKVDVRKMIKSEQEI GKATAYFF  
YSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFS  
KESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVAKVEKKGSKKLSVKELLGITIMERSSE  
EKNPIDFLEAGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHY  
EKLKGS PEDNEQQLFVEQHKHYLDEII EQISEFSKRVILADANLDKVL SAYNKHRDKPIREQAENII  
HLFTLTNLGAPAAFYFDTTIDRKRYTSTKEVL DATLIHQSI TGLYETRIDLSQLGGDSGGSGSGGS  
TNLSDIIEKETGKQLVIQESILMLPEEVEEVI GNKPESDILVHTAYDESTDENVMLLTSDAPEYKPWA  
LVIQDSNGENKIKMLSGSGSGSGSTNLSDIIEKETGKQLVIQESILMLPEEVEEVI GNKPESDILVH  
TAYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIKMLSGGSKRTADGSEFFESPKKKRKV

Nuclear localization signal, SpCas9, Linker (Cas9), Linker (Ser-Gly-  
Gly-Ser-Gly-Ser, Ser-Gly), FERNY (=evolved APOBEC), UGI

**ABEmax7.10 PI1**

MKRTADGSEFESPKKKRKVGSDDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI  
GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERH  
PIFGNIVDEVAYHEKYPTIYHLRKKLVSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKL  
FIQLVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIALSGLTPNF  
KSNFDLAEDAQLQSKDQTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS  
MIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEE  
LLVKNLREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPIYYVGPLA  
RGNSRFAMTRKSEETITPWNFEEVVDKGAQSFIERMTNFDKNLPNEKVLPHKSHLLYEYFTVYNEL  
TKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLG  
TYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWG  
RLSRKLINGIRDKQSGKTILDFLKSDFANRNFQMQLIHDDSLTFKEDIQKAQVSGQGDLSHEHIANLA  
GSPAIIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGKELGSQIL  
KEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNR  
GKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVA  
QILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVVREINNYHHAHDAYLNAVVGTAIIKK  
YPKLESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGE  
TGEIVWDKGRDFATVRKVLSPQVNIIVKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDS  
PTVAYSVLVAKVEKGGKSKLKSVEKLLGITIMERSSEFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLE  
ELENKRKRLASAGELQKGNELALPSKYVNFYLYLASHYEKLGGSGGSGSGSGSGGSSEVEFSHEYWM  
RHALTLAKRARDEREVPVGAVVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATL  
YVTFEPCVMCAGAMIHSRIGRVVFGVNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYF  
FRMPRQVFNAQKKAQSSTDGGSGSGSGSGSGSPEDNEQKQLFVEQHKHYLDEIIIEQISEFSKRVIL  
ADANLDKVL SAYNKHRDKPIREQAENI IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ  
SITGLYETRIDLSQLGGDSGGSKRTADGSEFEPKKKRKV

Nuclear localization signal, SpCas9, Linker (Gly-Gly-Ser), TadA  
(adenosine deaminase, 7.10)

**ABEmax7.10 PI2**

MKRTADGSEFESPKKKRKVGSDDKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI  
GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERH  
PIFGNIVDEVAYHEKYPTIYHLRKKLVSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKL  
FIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIALSGLTPNF  
KSNFDLAEDAQLQSKDQYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS  
MIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEE  
LLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPIYYVGPLA  
RGNSRFAMTRKSEETITPWNFEEVVDKGAQSFIERMTNFDKNLPNEKVLPHKSLLYEYFTVYNEL  
TKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLG  
TYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWG  
RLSRKLINGIRDKQSGKTILDFLKSDFANRNFQMQLIHDDSLTFKEDIQKAQVSGQGDLSHEHIANLA  
GSPAIAKKGILQTVKVVDELVKVMGRHKPENIVIEARENQTTQKGQKNSRERMKRIEEGKELGSQIL  
KEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNR  
GKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVA  
QILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTAIIKK  
YPKLESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGE  
TGEIVWDKGRDFATVRKVLSPQVNIIVKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDS  
PTVAYSVLVAKVEKGSKLLKSVKELLGITIMERSSEFKNPIDFLEAKGYKEVKKDLIIKLPKYSLE  
ELENKRMLASAGELQKGNELALPSKYVNFYLYLASHYEKLGGSSGGSSEVEFSSHEYWMRHALTL  
AKRARDEREVPVAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEP  
CVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNRVEITEGILADECAALLCYFFRMPRQ  
VFNAQKKAQSSTDGGSGSGSGSGSPEDNEQKQLFVEQHKHYLDEIIIEQISEFSKRVILADANLDKVL  
SAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETR  
IDLSQLGGDSSGGSKRTADGSEFEPKKKRKV

Nuclear localization signal, SpCas9, Linker (Gly-Gly-Ser), TadA  
(adenosine deaminase, 7.10)

**ABEmax7.10 PI3**

MKRTADGSEFESPKKKRKVGSDDKYSIGLAIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLI  
GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERH  
PIFGNIVDEVAYHEKYPTIYHLRKKLVSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKL  
FIQLVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIASLGLTPNF  
KSNFDLAEDAQLQSKDQTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS  
MIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEE  
LLVKNLREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPIYYVGPLA  
RGNSRFAMTRKSEETITPWNFEEVVDKGASQSFIERMTNFDKNLPNEKVLPHKSLLYEYFTVYNEL  
TKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLG  
TYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWG  
RLSRKLINGIRDKQSGKTILDFLKSDFANRNFQMQLIHDDSLTFKEDIQKAQVSGQGDLSHEHIANLA  
GSPAIIKQILQTVKVVDELVKVMGRHKPENIVIEARENQTTQKGQKNSRERMKRIEEGKELGSQIL  
KEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNR  
GKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVA  
QILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTAIIKK  
YPKLESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGE  
TGEIVWDKGRDFATVRKVLSPQVNIIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDS  
PTVAYSVLVAKVEKGSKLLKSVKELLGITIMERSSEFKNPIDFLEAKGYKEVKKDLIIKLPKYSLE  
ELENKRKRLASAGELQKGNELALPSKYVNFYLYLASHYEKLGGSSGGSSEVEFSSHEYWMRHALTL  
AKRARDEREVPVAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEP  
CVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNRHVEITEGILADECAALLCYFFRMPRQ  
VFNAQKKAQSSTDGGSGSGSSPEDNEQKQLFVEQHKHYLDEIEEQISEFSKRVILADANLDKVL  
SAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSI  
TGLYETRIDLSQLGGDSGGSKRTADGSEFEPKKRKV

Nuclear localization signal, SpCas9, Linker (Gly-Gly-Ser), TadA  
(adenosine deaminase, 7.10)

**SaKKH-HNHx-ABEmax7.10 (S484)**

MKRTADGSEFFESPKKKRKVGSKRNYILGLAIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGR  
SKRGARRLKRRRRHRIQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAKRR  
GVHNVNEVEEDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKTSDYVKEAKQLL  
KVQKAYHQLDQSFIDTYIDLLETRRYYEGPGEPSFGWKDIKEWYEMLMGHCTYFPEELRSVKYAYN  
ADLYNALNDLNNLVITRDENEKLEYEYKQI IENVFKQKKKPTLKQIAKEILVNEEDIKGYRVTSTGK  
PEFTNLKVYHDIKDITARKEI IENAELLDQIAKILTIYQSSEDIQEELTNLNSELTQEEIEQISNLKG  
YTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKLVPKKVDLSQQKEIPTTLVDDFILSPVVKRSFIQ  
SIKVINAI IKKYGLPNDII IELAREKNSGSSEVEFSHEYWMRHALTLAKRARDEREVPGAVLVLNNR  
VIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVR  
NAKTGAAGSLMDVLHYPGMNRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDSSTRYATR  
GLMNLRSYFRVNNLDVKVKS INGGFTSFLRRKWKFKKERNKGYKHAEDALI IANADFI FKEWKKLD  
KAKKVMENQMFEEKQAESMPEIETEQEYKEIFITPHQIKHIKDFKDYKYSHRVDKKPNRKLINDTLYS  
TRKDDKGNTLIVNNLNGLYDKDNDKLLKLINKSPEKLLMYHHPQTYQKLKLIMEQYGDEKNPLYKYY  
EETGNYLTKYSKKNPVIKIKYGNKLNALDITDDYPNSRNKVVKLSLKPYPFDVYLDNGVYKVV  
TVKNLDVIKKENYEVNSKCYEEAKKLLKISNQAEFIASFYKNDLIKINGELYRVIQVNNDLLNRIEV  
NMIDITYREYLENMNDKRPPHI IKTIAKTQSIKKYSTDILGNLYEVKSKKHPQIIKKGSGGSKRTAD  
GSEFEPKKKRKV

Nuclear localization signal, SaKKH, Linker (Gly-Ser, Ser-Ser), TadA  
(adenosine deaminase, 7.10)

**SaKKH-HNHx-ABEmax7.10 (G513)**

MKRTADGSEFFESPKKKRKVGSKRNYILGLAIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGR  
SKRGARRLKRRRRHRIQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAKRR  
GVHNVNEVEEDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRSINRFKTSDYVKEAKQLL  
KVQKAYHQLDQSFIDTYIDLLETRRYYEGPGEKSPFGWKDIKEWYEMLMGHCTYFPEELRSVKYAYN  
ADLYNALNDLNNLVITRDENEKLEYEYKQI IENVFKQKKKPTLKQIAKEILVNEEDIKGYRVTSTGK  
PEFTNLKVYHDIKDITARKEI IENAELLDQIAKILTIYQSSEDIQEELTNLNSELTQEEIEQISNLKG  
YTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKLVPKKVDLSQQKEIPTTLVDDFILSPVKRSFIQ  
SIKVINAI IKKYGLPNDII IELAREKNSKDAQKMINEMQKRNRQTNERIEEII RTTGSSSEVEFSHEY  
WMRHALTLAKRARDEREVPGAVLVLNRRVIGEGWNRAIGLHDPTAHAEIMALRQGGGLVMQNYRLIDA  
TLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNRVEITEGILADECAALLC  
YFFRMPRQVFNAQKKAQSSTDSSSVQKDFINRNLVDTRYATRGLMNLRSYFRVNNLDVKVKSINGGF  
TSFLRRKWKFKKERNKGYKHAEDALI IANADFIKWKKLDKAKKVMENQMFEKQAESMPEIETEQ  
EYKEIFITPHQIKHIKDFKDYKYSRVDKKNRKLINDTLYSTRKDDKGNTLIVNNLNGLYDKDNDKL  
KKLINKSPEKLLMYHHDPPQTYQKLLIMEQYGDENPLYKYEETGNLYLTKYSKKDNGPVIKKIKYYG  
NKLNAHLDITDDYPNSRNKVVKLSLKPFRFDVYLDNGVYKFTVKNLDVIKENYEVNSKCYEEAKK  
LKKISNQAEFIASFYKNDLIKINGELYRVIGVNDLLNRIEVMIDITYREYLENMNDKRPPIIKTI  
ASKTQSIKKYSTDILGNLYEVKSKKHPQIIKKGSGGSKRTADGSEFFEPKKKRKV

Nuclear localization signal, SaKKH, Linker (Gly-Ser, Ser-Ser), TadA  
(adenosine deaminase, 7.10)