

### Constructs used in this study

Tev169-xOnu (MegaTev)

MKSGIYQIKNTLNNKVYVGSAKDFEKRWKRHFKDLEKGCHSSIKLQRSFNKHGNVFECSILEEIPYEKDLIIERENFWIKEL  
NSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAKMLKLPDGRKALYSKPGSKNGRWNPETHKFCKCGVRIQTsay  
TCSKCRNGGGSGSGSAYMSRRESINPWILTGFADAQGSFLLIRNSNKRSVGYATELGQICLHIKDKSILENIQSTWKV  
GVIANGDNAVSLRVTRFEDLKVIDHFEKYPLITQKLDYMLFKQAFCVMENKEHLKINGIKELVRIAKLNWGLTDELK  
KAFPEIISKERSLINKNIPNFKWLAGFTSGEGCFFVNLIKSNSKLGVQVQLVFSITQHIKDKNLMNSLITYLGCYIKEKNRS  
EFSWLDVVTKFSDINDKIIPVFQENTLIGVKLEDFEDWCKVAKLIEEKHLTESGLDEIKKIKLNMMNKGRVF

V117F Tev169-xOnu

MKSGIYQIKNTLNNKVYVGSAKDFEKRWKRHFKDLEKGCHSSIKLQRSFNKHGNVFECSILEEIPYEKDLIIERENFWIKEL  
NSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAKMLKLPDGRKALYSKPGSKNGRWNPETHKFCKCGVRIQTsay  
TCSKCRNGGGSGSGSAYMSRRESINPWILTGFADAQGSFLLIRNSNKRSVGYATELGQICLHIKDKSILENIQSTWKV  
GVIANGDNAVSLRVTRFEDLKVIDHFEKYPLITQKLDYMLFKQAFCVMENKEHLKINGIKELVRIAKLNWGLTDELK  
KAFPEIISKERSLINKNIPNFKWLAGFTSGEGCFFVNLIKSNSKLGVQVQLVFSITQHIKDKNLMNSLITYLGCYIKEKNRS  
EFSWLDVVTKFSDINDKIIPVFQENTLIGVKLEDFEDWCKVAKLIEEKHLTESGLDEIKKIKLNMMNKGRVF

S134G Tev169-xOnu

MKSGIYQIKNTLNNKVYVGSAKDFEKRWKRHFKDLEKGCHSSIKLQRSFNKHGNVFECSILEEIPYEKDLIIERENFWIKEL  
NSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAKMLKLPDGRKALYSKPGSKNGRWNPETHKFCKCGVRIQTsay  
TCSKCRNGGGSGSGSAYMSRRESINPWILTGFADAQGSFLLIRNSNKRSVGYATELGQICLHIKDKSILENIQSTWKV  
GVIANGDNAVSLRVTRFEDLKVIDHFEKYPLITQKLDYMLFKQAFCVMENKEHLKINGIKELVRIAKLNWGLTDELK  
KAFPEIISKERSLINKNIPNFKWLAGFTSGEGCFFVNLIKSNSKLGVQVQLVFSITQHIKDKNLMNSLITYLGCYIKEKNRS  
EFSWLDVVTKFSDINDKIIPVFQENTLIGVKLEDFEDWCKVAKLIEEKHLTESGLDEIKKIKLNMMNKGRVF

K135N Tev169-xOnu

MKSGIYQIKNTLNNKVYVGSAKDFEKRWKRHFKDLEKGCHSSIKLQRSFNKHGNVFECSILEEIPYEKDLIIERENFWIKEL  
NSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAKMLKLPDGRKALYSNPGSKNGRWNPETHKFCKCGVRIQTsay  
TCSKCRNGGGSGSGSAYMSRRESINPWILTGFADAQGSFLLIRNSNKRSVGYATELGQICLHIKDKSILENIQSTWKV  
GVIANGDNAVSLRVTRFEDLKVIDHFEKYPLITQKLDYMLFKQAFCVMENKEHLKINGIKELVRIAKLNWGLTDELK  
KAFPEIISKERSLINKNIPNFKWLAGFTSGEGCFFVNLIKSNSKLGVQVQLVFSITQHIKDKNLMNSLITYLGCYIKEKNRS  
EFSWLDVVTKFSDINDKIIPVFQENTLIGVKLEDFEDWCKVAKLIEEKHLTESGLDEIKKIKLNMMNKGRVF

K135R/N140S Tev169-xOnu

MKSGIYQIKNTLNNKVYVGSAKDFEKRWKRHFKDLEKGCHSSIKLQRSFNKHGNVFECSILEEIPYEKDLIIERENFWIKEL  
NSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAKMLKLPDGRKALYSRPGSKSGRWNPETHKFCKCGVRIQTsay  
CSKCRNGGGSGSGSAYMSRRESINPWILTGFADAQGSFLLIRNSNKRSVGYATELGQICLHIKDKSILENIQSTWKVG  
VIANSGDNAVSLRVTRFEDLKVIDHFEKYPLITQKLDYMLFKQAFCVMENKEHLKINGIKELVRIAKLNWGLTDELK  
AFPEIISKERSLINKNIPNFKWLAGFTSGEGCFFVNLIKSNSKLGVQVQLVFSITQHIKDKNLMNSLITYLGCYIKEKNRSE  
FSWLDVVTKFSDINDKIIPVFQENTLIGVKLEDFEDWCKVAKLIEEKHLTESGLDEIKKIKLNMMNKGRVF

V117F/K135N Tev169-xOnu

MKSGIYQIKNTLNNKVYVGSAKDFEKRWKRHFKDLEKGCHSSIKLQRSFNKHGNVFECSILEEIPYEKDLIIERENFWIKEL  
NSKINGYNIADATFGDTCSTHPLKEEIIKKRSETVKAKMLKLPDGRKALYSNPGSKNGRWNPETHKFCKCGVRIQTsay  
TCSKCRNGGGSGSGSAYMSRRESINPWILTGFADAQGSFLLIRNSNKRSVGYATELGQICLHIKDKSILENIQSTWKV  
GVIANGDNAVSLRVTRFEDLKVIDHFEKYPLITQKLDYMLFKQAFCVMENKEHLKINGIKELVRIAKLNWGLTDELK

KAFPEIISKERSLINKNIPNFKWLAGFTSGEGCFFVNLIKSNSKLGVQVQLVFSITQHIKDKNLMNSLITYLGCYIKEKNRS  
EFSWLDVVTKFSDINDKIIPVFQENTLIGVKLEDFEDWCKVAKLIEEKHLTESGLDEIKKIKLNMMNKGRVF

V117F/K135R/N140S Tev169-xOnu

MKSGIYQIKNTLNNKVYVGSAKDFEKWRHKDLEKGCHSSIKLQRSFNKHGNVFECISILEEIPYEKDLIIERENFWIKEL  
NSKINGYNIADATFGDTCTHPLKEEIKKRSETVKAKMLKLGPDRKALYSRPGSKSGRWNPETHFKCKCGVRIQTSAYT  
CSKCRNGGGSGSGSAYMSRRESINPWILTGFADAQGSFLIRNSNKRSVGYATELGFQICLHIKDKSILENIQSTWKVG  
VIANSGDNAVSLRVTRFEDLKVIIIDHFEKYPLITQKLGDYMLFKQACVMENKEHLKINGIKELVRIKAKLNWGLTDELKK  
AFPEIISKERSLINKNIPNFKWLAGFTSGEGCFFVNLIKSNSKLGVQVQLVFSITQHIKDKNLMNSLITYLGCYIKEKNRSE  
FSWLDVVTKFSDINDKIIPVFQENTLIGVKLEDFEDWCKVAKLIEEKHLTESGLDEIKKIKLNMMNKGRVF

xTev169-Onu

MKSGIYQIKNTLNNKVYVGSAKDFEKAWKRHKDLEKGCHSSIKLQRSFNKHGNVFECISILEEIPYEKDLIIERENFWIKEL  
NSKINGYNIADATFGDTCTHPLKEEIKKRSETVKAKMLKLGPDRKALYSRPGSKSGRWNPETHFKCKCGVRIQTSA  
TCSKCRNGGGSGSGSAYMSRRESINPWILTGFADAEGSFLIRNSNKRSVGYATELGFQICLHIKDKSILENIQSTWKVG  
VIANSGDNAVSLRVTRFEDLKVIIIDHFEKYPLITQKLGDYMLFKQACVMENKEHLKINGIKELVRIKAKLNWGLTDELKK  
AFPEIISKERSLINKNIPNFKWLAGFTSGEGCFFVNLIKSNSKLGVQVQLVFSITQHIKDKNLMNSLITYLGCYIKEKNRSE  
FSWLDVVTKFSDINDKIIPVFQENTLIGVKLEDFEDWCKVAKLIEEKHLTESGLDEIKKIKLNMMNKGRVF

TevCas9

MGKSGIYQIKNTLNNKVYVGSRKDFEKAWKRHKDLEKGCHSSIKLQRSFNKHGNVFECISILEEIPYEKDLIIERENFWIKE  
LNSKINGYNIADATFGDTCTHPLKEEIKKRSETVKAKMLKLGPDRKALYSRPGSKSGRWNPETHFKCKCGVRIQTSA  
YTCRKCRNGGGSDKKYSIGLDIGTNSVGWAVITDEYKPSKKFKVLGNTDRHSIKKNLIGALLFDSETAEATRLKRTA  
RRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVSTD  
LRLIYLALAHMIKFRGHFLIEGDLNPDNSVDKLFQLVQTYNQLFEENPINASGVDAKIALSKSRRLENLIAQLPGE  
KKNGLFGNLIALSLGLTPNFSNFDAEDAKLQLSKDTYDDLDNLLAQIGDQYADLFLAAKNLSDAILSDILRVNTEITK  
APLSASMIKRYDEHHQDLTLLKALVRQQLPKYKEIFFDQSNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLV  
KLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNRKIEKILTFRIPYYVGPLARGNSRF  
AWMTRKSEETITPW  
NFEVVVDKGASAQSFIERNMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKK  
AIVDLLFTK  
RKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLKI  
KKDFLDNEENEDEDIVLT  
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R27A xTevCas9

MGKSGIYQIKNTLNNKVYVGSAKDFEKAWKRHKDLEKGCHSSIKLQRSFNKHGNVFECISILEEIPYEKDLIIERENFWIKE  
LNSKINGYNIADATFGDTCTHPLKEEIKKRSETVKAKMLKLGPDRKALYSRPGSKSGRWNPETHFKCKCGVRIQTSA  
YTCRKCRNGGGSDKKYSIGLDIGTNSVGWAVITDEYKPSKKFKVLGNTDRHSIKKNLIGALLFDSETAEATRLKRTA  
RRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVSTD  
LRLIYLALAHMIKFRGHFLIEGDLNPDNSVDKLFQLVQTYNQLFEENPINASGVDAKIALSKSRRLENLIAQLPGE  
KKNGLFGNLIALSLGLTPNFSNFDAEDAKLQLSKDTYDDLDNLLAQIGDQYADLFLAAKNLSDAILSDILRVNTEITK

APLSASMIKRYDEHHQDLTLLKALVRQQQLPEKYKEIFFDQSNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNR  
EDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNRKREKIEKILTFRIPYYVGPLARGNSRFAMTRKSEETITPW  
NFEVVVDKGASAQSFIERMNTFDKNLPNEKVLPHSLLYEYFTVYNELTKVKVYVTEGMRKPAFLSGEQKKAIVDLLFKTN  
RKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLKIICKDFLDNEENEDILEDIVLTTLFEDREMIEERLKTYA  
HLFDDKVMKQLKRRRTGWGRLSRKLINGIRDQSGKTILDFLKSDFANRNFMQLIHDDSLTFKEDIQKAQVSGQGD  
SLHEHIANLAGSPAIIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTQKGQKNSRERMKRIEGIKEGLSQLKE  
HPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDHDIVPQSLKDDSIDNKVLTRSDKNRGKSDNVPSEEVV  
KKMKNYWRQLNAKLITQRKFNDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDLSRMNTKYDENDKLIREVKVI  
TLKSKLVSDFRKDFQFYKVRREINNYHHAHDAYLNAVVGTLAKKYPKLESEFVYGDYKVDVRKMIAKSEQEIGKATAKYF  
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLMPQVNIVKKTEVQTGGFSKESILPKRNSDK  
LIARKKDWDPKYGGFDSPVTAVSVLVAKVEGKSKKLKSVKELLGITMERSSFKNPIDFLEAKGYKEVKKDLIILPKY  
SLFELENGRKRMLASAGELQKGNELALPSKYVNFYLASHYEKLGSSEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILA  
DANLDKVLSAYNKHRDKPIREQAENIIHLFTLNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLHQHSITGLYETRIDSQLG  
GDKRPAATKKAGQAKKK

V117F (V) TevCas9

MGKSGIYQIKNTNNKVVGVSRKDFEKAWKRHFKDLKGCHSSIKLQRSFNKHGNVFECISILEEIPYKDLIIERENFWIKE  
LNSKINGYNIADATFGDTCSTHPLKEEIKKRSETFKAKMLKGPDGRKALYSKPGSKNGRWNPETHKFCCKGVRIQTSAY  
TCSKCRNGGGSGSDKKYSIGLDIGTNSVGWAVITDEYKPSKKFKVLGNTDRHSIKKNLIGALLFDGETAEATRLKRTAR  
RRYTRRKNRICYLQEIFSNEAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADL  
RLIYLALAHMIKFRGHFLIEGDLNPNSDVKLFQLVQTYNQLFEENPINASGVDAKILSARLSKSRRLENLIAQLPGE  
KNGLFGNLIALSGLTPNFKSNFDLAEDAKLQLSKDTYDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKA  
PLSASMIKRYDEHHQDLTLLKALVRQQQLPEKYKEIFFDQSNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNR  
DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNRKREKIEKILTFRIPYYVGPLARGNSRFAMTRKSEETITPW  
FEEVVVDKGASAQSFIERMNTFDKNLPNEKVLPHSLLYEYFTVYNELTKVKVYVTEGMRKPAFLSGEQKKAIVDLLFKTN  
KVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLKIICKDFLDNEENEDILEDIVLTTLFEDREMIEERLKTYA  
LFDDKVMKQLKRRRTGWGRLSRKLINGIRDQSGKTILDFLKSDFANRNFMQLIHDDSLTFKEDIQKAQVSGQGD  
HEHIANLAGSPAIIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTQKGQKNSRERMKRIEGIKEGLSQLKE  
VENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDHDIVPQSLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKK  
MKNYWRQLNAKLITQRKFNDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDLSRMNTKYDENDKLIREVKVITL  
KSKLVSDFRKDFQFYKVRREINNYHHAHDAYLNAVVGTLAKKYPKLESEFVYGDYKVDVRKMIAKSEQEIGKATAKYFFY  
SNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLMPQVNIVKKTEVQTGGFSKESILPKRNSDKLI  
ARKKDWDPKYGGFDSPVTAVSVLVAKVEGKSKKLKSVKELLGITMERSSFKNPIDFLEAKGYKEVKKDLIILPKYSL  
FELENGRKRMLASAGELQKGNELALPSKYVNFYLASHYEKLGSSEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILA  
ANLDKVLSAYNKHRDKPIREQAENIIHLFTLNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLHQHSITGLYETRIDSQLG  
GDKRPAATKKAGQAKKK

V117F/K135N (VK) TevCas9

MGKSGIYQIKNTNNKVVGVSRKDFEKAWKRHFKDLKGCHSSIKLQRSFNKHGNVFECISILEEIPYKDLIIERENFWIKE  
LNSKINGYNIADATFGDTCSTHPLKEEIKKRSETFKAKMLKGPDGRKALYSNPGSKNGRWNPETHKFCCKGVRIQTSAY  
YTC SKCRNGGGSDKKYSIGLDIGTNSVGWAVITDEYKPSKKFKVLGNTDRHSIKKNLIGALLFDGETAEATRLKRTA  
RRYTRRKNRICYLQEIFSNEAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD  
RLIYLALAHMIKFRGHFLIEGDLNPNSDVKLFQLVQTYNQLFEENPINASGVDAKILSARLSKSRRLENLIAQLPGE  
KKNLFGNLIALSGLTPNFKSNFDLAEDAKLQLSKDTYDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKA  
APLSASMIKRYDEHHQDLTLLKALVRQQQLPEKYKEIFFDQSNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNR  
EDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNRKREKIEKILTFRIPYYVGPLARGNSRFAMTRKSEETITPW  
NFEVVVDKGASAQSFIERMNTFDKNLPNEKVLPHSLLYEYFTVYNELTKVKVYVTEGMRKPAFLSGEQKKAIVDLLFKTN

RKTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLIKDKDFLDNEENEDILEDIVLTTLFEDREMIEERLKTYA  
HLFDDKVMKQLKRRRTGWRSLRKLINGIRDQSGKTILDFLKSDFANRNFMQLIHDDSLTFKEDIQKAQVSGQGD  
SLHEHIANLAGSPAIIKKGILQTVKVVDLVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKEGLSQLKE  
HPVENTQLQNEKLYLYLQNNGRDMDYVDQELDINRLSDYDHDHVIPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVV  
KKMKNYWRQQLNAKLITQRKFNDLTKAERGGLELDKAGFIKRQLVETRQITKHVAQILDLSRMNTKYDENDKLIREVKVI  
TLKSKLVSDFRKDFQFYKVRREINNYHHADAYLNAVVTALIKKYPKLESEFVYGDYKVDVRKMIAKSEQEIGKATAKYF  
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDK  
LIARKKDWDPKYGGFDPTVAYSVLVAKVEKGSKKLKSVKELLGITMERSFEKNPIDFLEAKGYKEVKKDLIILPKY  
SLELENGRKMLASAGELQKGNELALPSKYVNFLYASHYEKLKGSPEDNEQKQLFVEQHCKHYLDEIIEQISEFSKRVILA  
DANLDKVLSAYNHRDKPIREQAENIIHLFTLNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLHQHSITGLYETRIDLSQLG  
DKRPAATKKAGQAKKK

V117F/K135R/N140S (VKN) TevCas9

MGKSGIYQIKNTNNKVVGVSRKDFEKAWKRHFKDLEGCHSSIKLQRSFNKHGNVFECISILEEIPYEKDLIIERENFWIKE  
LNSKINGYNIADATFGDTCSTHPLKEEIKKRSETFKAKMLKGPDGRKALYSRPGSKSGRWNPETHFKCKCGVRIQTSAY  
TCSKCRNGGGSGSDKKYSIGLDIGTNSVGWAVITDEYKPSKKFKVLGNTDRHSIKKNLIGALLFDGETAEATRLKRTAR  
RRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADL  
RLIYLALAHMIKFRGHFLIEGDLNPNSDVKLFQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEK  
KNGLFGNLIALSGLTPNFKNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKA  
PLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFKPILEKMDGTEELLVKNRE  
DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNRKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWN  
FEEVVDKGASAQSIERMTNFDKNLPNEKVLPHSLLYEYFTVYNELTKVVKVTEGMRKPAFLSGEQKKAIVDLLFKTNR  
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