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Supplementary Information

# Design of a Human Rhinovirus-14 3C Protease-Inducible Caspase-3

Hanna J. Wagner* and Wilfried Weber*

*Correspondence: hanna.wagner@biologie-uni-freiburg.de
wilfried.weber@biologie.uni-freiburg.de

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**Table S1.** Plasmids used in this study.

| Plasmid | Description | Reference |
|---|---|---|
| pHJW1 | $P_{T7}$-Casp3-TCS-$His_6$<br>Bacterial expression vector for the production of human Caspase-3 fused to a C-terminal linker region containing a TEV cleavage site (TCS) and a hexa histidine-tag ($His_6$). | [1,2] |
| pHJW4 | $P_{T7}$-$His_6$-3CPRO<br>Bacterial expression vector for the production of His-tagged HRV14 3C protease. | [1,2] |
| pHJW14 | $P_{T7}$-$His_6$-AviTag-GyrB-GyrB-CS-SNAP25(141-206)-TEV<br>Bacterial expression vector coding for the TEV protease fused to His- and Avi-tagged GyrB domains via a Casp3-cleavable linker and amino acids 141-206 of human SNAP25. | [3] |
| pHJW181 | $P_{T7}$-Casp3(3CS_ins)-TCS-$His_6$<br>Bacterial expression vector encoding the Casp3 construct 3CS_ins (3C protease cleavage site inserted at position 175).<br>Amino acid sequence: prodomain, p17, 3CS, p12, TEV cleavable linker, His-tag<br>MMENTENSVDSKSIKNLEPKIIHGSESMDSGISLDNSYKMDYPEMGLCIIINNKNFHKSTGMTSRSGTDVDAAN<br>LRETFRNLKYEVRNKNDLTREEIVELMRDVSKEDHSKRSSFVCVLLSHGEEGIIFGTNGPVDLKKITNFFRGDRC<br>RSLTGKPKLFIIQACRGTELDCGIETLEVLFQGPSGVDDDMACHKIPVEADFLYAYSTAPGYYSWRNSKDGSWF<br>IQSLCAMLKQYADKLEFMHILTRVNRKVATEFESFSFDATFHAKKQIPCIVSMLTKELYFYSGGGSGGGGENLY<br>FQSGGGPAGEASSIPNREGKPIPNPLLGLGSTRTGEFHHHHHHH | [1,2] |
| pHJW187 | $P_{T7}$- Δpro-Casp3(3CS_subs)-TCS-$His_6$<br>Bacterial expression vector encoding the Casp3 construct 3CS_subs -pro (residues 170 – 177 substituted by the 3C protease cleavage site).<br>The plasmid sequences were amplified from pHJW181 using oligonucleotides oHJW435 and oHJW436 (PCR 1), oHJW5 and oHJW437 (PCR 2), oHJW6 and oHJW205 (PCR 3). All three fragments were assembled by Gibson cloning [4].<br>Amino acid sequence: p17, 3CS, p12, TEV cleavable linker, His-tag<br>MSGISLDNSYKMDYPEMGLCIIINNKNFHKSTGMTSRSGTDVDAANLRETFRNLKYEVRNKNDLTREEIVELM<br>RDVSKEDHSKRSSFVCVLLSHGEEGIIFGTNGPVDLKKITNFFRGDRCRSLTGKPKLFIIQACRGTELDLEVLFQG<br>PVDDDMACHKIPVEADFLYAYSTAPGYYSWRNSKDGSWFIQSLCAMLKQYADKLEFMHILTRVNRKVATEFE<br>SFSFDATFHAKKQIPCIVSMLTKELYFYSGGGSGGGGENLYFQSGGGPAGEASSIPNREGKPIPNPLLGLGSTRT<br>GEFHHHHHHH | This work |
| pHJW188 | $P_{T7}$-Δpro-Casp3(3CS_ins)-TCS-$His_6$<br>Bacterial expression vector for the production of the Casp3 construct 3CS_ins with deleted prodomain.<br>Plasmid fragments were amplified from pHJW181 using oligonucleotides oHJW5 and oHJW435 (PCR 1), and oHJW6 and oHJW205 (PCR 2), and assembled by Gibson cloning.<br>Amino acid sequence: p17, 3CS, p12, TEV cleavable linker, His-tag<br>MSGISLDNSYKMDYPEMGLCIIINNKNFHKSTGMTSRSGTDVDAANLRETFRNLKYEVRNKNDLTREEIVELM<br>RDVSKEDHSKRSSFVCVLLSHGEEGIIFGTNGPVDLKKITNFFRGDRCRSLTGKPKLFIIQACRGTELDCGIETLE<br>VLFQGPSGVDDDMACHKIPVEADFLYAYSTAPGYYSWRNSKDGSWFIQSLCAMLKQYADKLEFMHILTRVNR | This work |

| Plasmid | Description | Reference |
|---|---|---|
| | KVATEFESFSFDATFHAKKQIPCIVSMLTKELYFYSGGGSGGGGENLYFQSGGGPAGEASSIPNREGKPIPNPLL GLGSTRTGEFHHHHHHH | |
| pHJW189 | $P_{T7}$-Casp3(3CS_ins-D169E)-TCS-$His_6$<br>Bacterial expression vector encoding the Casp3 construct 3CS_ins with D169E mutation. The mutation D169E was inserted into 3CS_ins by site-directed mutagenesis of pHJW181 using oligonucleotides oHJW438 and oHJW439. | This work |
| pHJW190 | $P_{T7}$-Casp3(3CS_ins-D192E)-TCS-$His_6$<br>Bacterial expression vector encoding the Casp3 construct 3CS_ins with D192E mutation. The mutation D192E was inserted into 3CS_ins by site-directed mutagenesis of pHJW181 using oligonucleotides oHJW440 and oHJW441. | This work |
| pHJW191 | $P_{T7}$-Casp3(3CS_ins-D179-181E)-TCS-$His_6$<br>Bacterial expression vector coding for the Casp3 construct 3CS_ins with safety catch mutation (D179-181E).<br>The plasmid sequences were amplified from pHJW181 using oligonucleotides oHJW6 and oHJW442 (PCR 1), oHJW5 and oHJW443 (PCR 2). The resulting DNA fragments were assembled by Gibson cloning. | This work |
| pHJW192 | $P_{T7}$-Casp3(3CS_ins-D179-181E, D192E)-TCS-$His_6$<br>Bacterial expression vector coding for the mutant Casp3 construct 3CS_ins with safety catch (D179-181E) and D192E mutation.<br>The plasmid sequences were amplified from pHJW181 using oligonucleotides oHJW6 and oHJW444 (PCR 1), oHJW5 and oHJW445 (PCR 2). The resulting DNA fragments were assembled by Gibson cloning. | This work |
| pHJW193 | $P_{T7}$-Δpro-Casp3(mCherry-3CS_subs)-TCS-$His_6$<br>Bacterial expression vector for the production of Casp3 with deleted prodomain and incorporated mCherry; amino acids 170 – 177 of Casp3 were substituted by 3CS-mCherry-3CS.<br>The plasmid sequence was amplified from pHJW2 (Ref.: [1,2]) using oligonucleotides oHJW446 and oHJW447 (PCR 1), and from pHJW181 using oHJW435 and oHJW436 (PCR 2), oHJW5 and oHJW437 (PCR 3), and oHJW6 and oHJW205 (PCR 4). PCR fragments 1 and 2 were assembled by fusion PCR using oligonucleotides oHJW435 and oHJW447. The resulting fragment was assembled with PCR 3 and 4 by Gibson cloning.<br>Amino acid sequence: p17, 3CS, mCherry, p12, TEV cleavable linker, His-tag<br>MSGISLDNSYKMDYPEMGLCIIINNKNFHKSTGMTSRSGTDVDAANLRETFRNLKYEVRNKNDLTREEIVELM RDVSKEDHSKRSSFVCVLLSHGEEGIIFGTNGPVDLKKITNFFRGDRCRSLTGKPKLFIIQACRGTELDLEVLFQG PGGGSMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAY VKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWE ASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERA EGRHSTGGMDELYKSGGGLEVLFQGPVDDDMACHKIPVEADFLYAYSTAPGYYSWRNSKDGSWFIQSLCAM LKQYADKLEFMHILTRVNRKVATEFESFSFDATFHAKKQIPCIVSMLTKELYFYSGGGSGGGGENLYFQSGGGP AGEASSIPNREGKPIPNPLLGLGSTRTGEFHHHHHHH | This work |

| Plasmid | Description | Reference |
|---|---|---|
| pHJW272 | $P_{T7}$-Δpro-Casp3(3CS_ins-D192E)-TCS-$His_6$<br>Bacterial expression vector encoding the Casp3 construct 3CS_ins with D192E mutation and deleted prodomain.<br>The plasmid sequence was amplified from pHJW190 using oligonucleotides oHJW5 and oHJW435 (PCR 1), and oHJW6 and oHJW205 (PCR 2). The resulting DNA fragments were assembled by Gibson cloning. | This work |
| pHJW273 | $P_{T7}$-Δpro-Casp3(3CS_ins-D169E)-TCS-$His_6$<br>Bacterial expression vector for the production of the Casp3 construct 3CS_ins with D169E mutation and deleted prodomain.<br>The plasmid sequence was amplified from pHJW189 using oligonucleotides oHJW5 and oHJW435 (PCR 1), and oHJW6 and oHJW205 (PCR 2). The resulting DNA fragments were assembled by Gibson cloning. | This work |
| pHJW274 | $P_{T7}$-Casp3(mCherry-3CS_ins)-TCS-$His_6$<br>Bacterial expression vector for the production of Casp3 with deleted prodomain and incorporated mCherry; aspartate-175 was exchanged by 3CS-mCherry-3CS.<br>The plasmid sequence was amplified from pHJW2 (Ref.: [1,2]) using oligonucleotides oHJW447 and oHJW537 (PCR 1), and from pHJW1 using oHJW5 and oHJW538 (PCR 2), and oHJW6 and oHJW424 (PCR 3). The resulting fragments were assembled by Gibson cloning.<br>Amino acid sequence: p17, 3CS, mCherry, p12, TEV cleavable linker, His-tag<br>MMENTENSVDSKSIKNLEPKIIHGSESMDSGISLDNSYKMDYPEMGLCIIINNKNFHKSTGMTSRSGTDVDAAN<br>LRETFRNLKYEVRNKNDLTREEIVELMRDVSKEDHSKRSSFVCVLLSHGEEGIIFGTNGPVDLKKITNFFRGDRC<br>RSLTGKPKLFIIQACRGTELDCGIETLEVLFQGPGGGSMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGT<br>QTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSL<br>QDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAK<br>KPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYKSGGGLEVLFQGPSGVDDDMACHKIP<br>VEADFLYAYSTAPGYYSWRNSKDGSWFIQSLCAMLKQYADKLEFMHILTRVNRKVATEFESFSFDATFHAKKQ<br>IPCIVSMLTKELYFYSGGGSGGGGENLYFQSGGGPAGEASSIPNREGKPIPNPLLGLGSTRTGEFHHHHHHH | This work |

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**Table S2.** Oligonucleotides used in this study. Annealing sequences are underlined.

| Oligo | Sequence (5′ → 3′) | Reference |
|---|---|---|
| oHJW1 | CTTGATCCGGCTGCTAACAAAG | [1,2] |
| oHJW5 | CTTTGATCTTTTCTACGGGGTCTG | [1,2] |
| oHJW6 | GCGTCAGACCCCGTAGAAAAG | [1,2] |
| oHJW205 | CTCCTTCTTAAAGTTAAACAAAATTATTTCTAGAGG | [1,2] |
| oHJW424 | TGTCTCAATGCCACAGTCCAG | This work |
| oHJW435 | GTTTAACTTTAAGAAGGAGATATACATATGTCTGGAATATCCCTGGACAACAG | This work |
| oHJW436 | CCTGGAACAGAACTTCCAGGTCCAGTTCTGTACCACGGC | This work |
| oHJW437 | GGAAGTTCTGTTCCAGGGGCCCGTTGATGATGACATGGCGTGTC | This work |
| oHJW438 | GTGGTACAGAACTGGAGTGTGGCATTGAGACACTG | This work |
| oHJW439 | CAGTGTCTCAATGCCACACTCCAGTTCTGTACCAC | This work |
| oHJW440 | CATAAAATACCAGTGGAGGCCGAGTTCTTGTATGCATACTC | This work |
| oHJW441 | GAGTATGCATACAAGAACTCGGCCTCCACTGGTATTTTATG | This work |
| oHJW442 | CACGCCATCTCTTCTTCAACACCACTGGGCCC | This work |
| oHJW443 | GAAGAAGAGATGGCGTGTCATAAAATACCAGTG | This work |
| oHJW444 | CTCGGCCTCCACTGGTATTTTATGACACGCCATCTCTTCTTCAACACCACTGGGCCC | This work |
| oHJW445 | CATAAAATACCAGTGGAGGCCGAGTTCTTGTATGCATACTCCACAGCAC | This work |
| oHJW446 | CCTGGAAGTTCTGTTCCAGGGGCCCGGCGGCGGCAGCATGGCCATCATCAAGGAGTTC | This work |
| oHJW447 | GGGCCCCTGGAACAGAACTTCCAGGCCGCCGCCAGACTTGTACAGCTCGTCCATGC | This work |
| oHJW537 | GGACTGTGGCATTGAGACACTGGAAGTTCTGTTCCAGGGGCCCGGCGGCGGCAGCATGGCCATCATCAAGGAGTTC | This work |
| oHJW538 | GTTCTGTTCCAGGGGCCCAGTGGTGTTGATGATGACATGG | This work |

## References


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