**Supplementary Sequence. S1\_ Nucleotide sequences of coding region for yeast expression plasmids**

**Target-AID2S**

* Truncated PmCDA1 with point mutation (30-150, W122E, W139Q)

ATGTCGCATAGATGCTACGTTCTCTTTGAATTAAAACGACGGGGTGAACGTAGAGCGTGTTTTTGGGGCTATGCTGTGAATAAACCACAGAGCGGGACAGAACGTGGCATTCACGCCGAAATCTTTAGCATTAGAAAAGTCGAAGAATACCTGCGCGACAACCCCGGACAATTCACGATAAATTGGTACTCATCCTGGAGTCCTTGTGCAGATTGCGCTGAAAAGATCTTAGAATGGTATAACCAGGAGCTGCGGGGGAACGGCCACACTTTGAAAATCGAAGCTTGCAAACTCTATTACGAGAAAAATGCGAGGAATCAAATTGGGCTGCAAAACCTCAGAGATAACGGGGTTGGGTTGAATGTA

* GS Linker

GGCGGAGGTGGCAGTGGCGGGGGTGGCTCGGGTGGGGGAGGCAGTGGCGGAGGTGGCAGTGGCGGGGGTGGCTCGGGTGGGGGAGGATCC

* nSpCas9 (D10A) plus SV40 NLS

ATGGACAAGAAGTACTCCATTGGGCTCGCTATCGGCACAAACAGCGTCGGTTGGGCCGTCATTACGGACGAGTACAAGGTGCCGAGCAAAAAATTCAAAGTTCTGGGCAATACCGATCGCCACAGCATAAAGAAGAACCTCATTGGCGCCCTCCTGTTCGACTCCGGGGAGACGGCCGAAGCCACGCGGCTCAAAAGAACAGCACGGCGCAGATATACCCGCAGAAAGAATCGGATCTGCTACCTGCAGGAGATCTTTAGTAATGAGATGGCTAAGGTGGATGACTCTTTCTTCCATAGGCTGGAGGAGTCCTTTTTGGTGGAGGAGGATAAAAAGCACGAGCGCCACCCAATCTTTGGCAATATCGTGGACGAGGTGGCGTACCATGAAAAGTACCCAACCATATATCATCTGAGGAAGAAGCTTGTAGACAGTACTGATAAGGCTGACTTGCGGTTGATCTATCTCGCGCTGGCGCATATGATCAAATTTCGGGGACACTTCCTCATCGAGGGGGACCTGAACCCAGACAACAGCGATGTCGACAAACTCTTTATCCAACTGGTTCAGACTTACAATCAGCTTTTCGAAGAGAACCCGATCAACGCATCCGGAGTTGACGCCAAAGCAATCCTGAGCGCTAGGCTGTCCAAATCCCGGCGGCTCGAAAACCTCATCGCACAGCTCCCTGGGGAGAAGAAGAACGGCCTGTTTGGTAATCTTATCGCCCTGTCACTCGGGCTGACCCCCAACTTTAAATCTAACTTCGACCTGGCCGAAGATGCCAAGCTTCAACTGAGCAAAGACACCTACGATGATGATCTCGACAATCTGCTGGCCCAGATCGGCGACCAGTACGCAGACCTTTTTTTGGCGGCAAAGAACCTGTCAGACGCCATTCTGCTGAGTGATATTCTGCGAGTGAACACGGAGATCACCAAAGCTCCGCTGAGCGCTAGTATGATCAAGCGCTATGATGAGCACCACCAAGACTTGACTTTGCTGAAGGCCCTTGTCAGACAGCAACTGCCTGAGAAGTACAAGGAAATTTTCTTCGATCAGTCTAAAAATGGCTACGCCGGATACATTGACGGCGGAGCAAGCCAGGAGGAATTTTACAAATTTATTAAGCCCATCTTGGAAAAAATGGACGGCACCGAGGAGCTGCTGGTAAAGCTTAACAGAGAAGATCTGTTGCGCAAACAGCGCACTTTCGACAATGGAAGCATCCCCCACCAGATTCACCTGGGCGAACTGCACGCTATCCTCAGGCGGCAAGAGGATTTCTACCCCTTTTTGAAAGATAACAGGGAAAAGATTGAGAAAATCCTCACATTTCGGATACCCTACTATGTAGGCCCCCTCGCCCGGGGAAATTCCAGATTCGCGTGGATGACTCGCAAATCAGAAGAGACCATCACTCCCTGGAACTTCGAGGAAGTCGTGGATAAGGGGGCCTCTGCCCAGTCCTTCATCGAAAGGATGACTAACTTTGATAAAAATCTGCCTAACGAAAAGGTGCTTCCTAAACACTCTCTGCTGTACGAGTACTTCACAGTTTATAACGAGCTCACCAAGGTCAAATACGTCACAGAAGGGATGAGAAAGCCAGCATTCCTGTCTGGAGAGCAGAAGAAAGCTATCGTGGACCTCCTCTTCAAGACGAACCGGAAAGTTACCGTGAAACAGCTCAAAGAAGACTATTTCAAAAAGATTGAATGTTTCGACTCTGTTGAAATCAGCGGAGTGGAGGATCGCTTCAACGCATCCCTGGGAACGTATCACGATCTCCTGAAAATCATTAAAGACAAGGACTTCCTGGACAATGAGGAGAACGAGGACATTCTTGAGGACATTGTCCTCACCCTTACGTTGTTTGAAGATAGGGAGATGATTGAAGAACGCTTGAAAACTTACGCTCATCTCTTCGACGACAAAGTCATGAAACAGCTCAAGAGGCGCCGATATACAGGATGGGGGCGGCTGTCAAGAAAACTGATCAATGGGATCCGAGACAAGCAGAGTGGAAAGACAATCCTGGATTTTCTTAAGTCCGATGGATTTGCCAACCGGAACTTCATGCAGTTGATCCATGATGACTCTCTCACCTTTAAGGAGGACATCCAGAAAGCACAAGTTTCTGGCCAGGGGGACAGTCTTCACGAGCACATCGCTAATCTTGCAGGTAGCCCAGCTATCAAAAAGGGAATACTGCAGACCGTTAAGGTCGTGGATGAACTCGTCAAAGTAATGGGAAGGCATAAGCCCGAGAATATCGTTATCGAGATGGCCCGAGAGAACCAAACTACCCAGAAGGGACAGAAGAACAGTAGGGAAAGGATGAAGAGGATTGAAGAGGGTATAAAAGAACTGGGGTCCCAAATCCTTAAGGAACACCCAGTTGAAAACACCCAGCTTCAGAATGAGAAGCTCTACCTGTACTACCTGCAGAACGGCAGGGACATGTACGTGGATCAGGAACTGGACATCAATCGGCTCTCCGACTACGACGTGGATCATATCGTGCCCCAGTCTTTTCTCAAAGATGATTCTATTGATAATAAAGTGTTGACAAGATCCGATAAAAATAGAGGGAAGAGTGATAACGTCCCCTCAGAAGAAGTTGTCAAGAAAATGAAAAATTATTGGCGGCAGCTGCTGAACGCCAAACTGATCACACAACGGAAGTTCGATAATCTGACTAAGGCTGAACGAGGTGGCCTGTCTGAGTTGGATAAAGCCGGCTTCATCAAAAGGCAGCTTGTTGAGACACGCCAGATCACCAAGCACGTGGCCCAAATTCTCGATTCACGCATGAACACCAAGTACGATGAAAATGACAAACTGATTCGAGAGGTGAAAGTTATTACTCTGAAGTCTAAGCTGGTCTCAGATTTCAGAAAGGACTTTCAGTTTTATAAGGTGAGAGAGATCAACAATTACCACCATGCGCATGATGCCTACCTGAATGCAGTGGTAGGCACTGCACTTATCAAAAAATATCCCAAGCTTGAATCTGAATTTGTTTACGGAGACTATAAAGTGTACGATGTTAGGAAAATGATCGCAAAGTCTGAGCAGGAAATAGGCAAGGCCACCGCTAAGTACTTCTTTTACAGCAATATTATGAATTTTTTCAAGACCGAGATTACACTGGCCAATGGAGAGATTCGGAAGCGACCACTTATCGAAACAAACGGAGAAACAGGAGAAATCGTGTGGGACAAGGGTAGGGATTTCGCGACAGTCCGGAAGGTCCTGTCCATGCCGCAGGTGAACATCGTTAAAAAGACCGAAGTACAGACCGGAGGCTTCTCCAAGGAAAGTATCCTCCCGAAAAGGAACAGCGACAAGCTGATCGCACGCAAAAAAGATTGGGACCCCAAGAAATACGGCGGATTCGATTCTCCTACAGTCGCTTACAGTGTACTGGTTGTGGCCAAAGTGGAGAAAGGGAAGTCTAAAAAACTCAAAAGCGTCAAGGAACTGCTGGGCATCACAATCATGGAGCGATCAAGCTTCGAAAAAAACCCCATCGACTTTCTCGAGGCGAAAGGATATAAAGAGGTCAAAAAAGACCTCATCATTAAGCTTCCCAAGTACTCTCTCTTTGAGCTTGAAAACGGCCGGAAACGAATGCTCGCTAGTGCGGGCGAGCTGCAGAAAGGTAACGAGCTGGCACTGCCCTCTAAATACGTTAATTTCTTGTATCTGGCCAGCCACTATGAAAAGCTCAAAGGGTCTCCCGAAGATAATGAGCAGAAGCAGCTGTTCGTGGAACAACACAAACACTACCTTGATGAGATCATCGAGCAAATAAGCGAATTCTCCAAAAGAGTGATCCTCGCCGACGCTAACCTCGATAAGGTGCTTTCTGCTTACAATAAGCACAGGGATAAGCCCATCAGGGAGCAGGCAGAAAACATTATCCACTTGTTTACTCTGACCAACTTGGGCGCGCCTGCAGCCTTCAAGTACTTCGACACCACCATAGACAGAAAGCGGTACACCTCTACAAAGGAGGTCCTGGACGCCACACTGATTCATCAGTCAATTACGGGGCTCTATGAAACAAGAATCGACCTCTCTCAGCTCGGTGGAGACAGCAGGGCTGACCCCAAGAAGAAGAGGAAGGTGGGTACC

* UGI

ATGACCAACCTTTCCGACATCATAGAGAAGGAAACAGGCAAACAGTTGGTCATCCAAGAGTCGATACTCATGCTTCCTGAAGAAGTTGAGGAGGTCATTGGGAATAAGCCGGAAAGTGACATTCTCGTACACACTGCGTATGATGAGAGCACCGATGAGAACGTGATGCTGCTCACGTCAGATGCCCCAGAGTACAAACCCTGGGCTCTGGTGATTCAGGACTCTAATGGAGAGAACAAGATCAAGATGCTA

**Target-AID3S**

* nSpCas9 (D10A, 1-1054)

ATGGACAAGAAGTACTCCATTGGGCTCGCTATCGGCACAAACAGCGTCGGTTGGGCCGTCATTACGGACGAGTACAAGGTGCCGAGCAAAAAATTCAAAGTTCTGGGCAATACCGATCGCCACAGCATAAAGAAGAACCTCATTGGCGCCCTCCTGTTCGACTCCGGGGAGACGGCCGAAGCCACGCGGCTCAAAAGAACAGCACGGCGCAGATATACCCGCAGAAAGAATCGGATCTGCTACCTGCAGGAGATCTTTAGTAATGAGATGGCTAAGGTGGATGACTCTTTCTTCCATAGGCTGGAGGAGTCCTTTTTGGTGGAGGAGGATAAAAAGCACGAGCGCCACCCAATCTTTGGCAATATCGTGGACGAGGTGGCGTACCATGAAAAGTACCCAACCATATATCATCTGAGGAAGAAGCTTGTAGACAGTACTGATAAGGCTGACTTGCGGTTGATCTATCTCGCGCTGGCGCATATGATCAAATTTCGGGGACACTTCCTCATCGAGGGGGACCTGAACCCAGACAACAGCGATGTCGACAAACTCTTTATCCAACTGGTTCAGACTTACAATCAGCTTTTCGAAGAGAACCCGATCAACGCATCCGGAGTTGACGCCAAAGCAATCCTGAGCGCTAGGCTGTCCAAATCCCGGCGGCTCGAAAACCTCATCGCACAGCTCCCTGGGGAGAAGAAGAACGGCCTGTTTGGTAATCTTATCGCCCTGTCACTCGGGCTGACCCCCAACTTTAAATCTAACTTCGACCTGGCCGAAGATGCCAAGCTTCAACTGAGCAAAGACACCTACGATGATGATCTCGACAATCTGCTGGCCCAGATCGGCGACCAGTACGCAGACCTTTTTTTGGCGGCAAAGAACCTGTCAGACGCCATTCTGCTGAGTGATATTCTGCGAGTGAACACGGAGATCACCAAAGCTCCGCTGAGCGCTAGTATGATCAAGCGCTATGATGAGCACCACCAAGACTTGACTTTGCTGAAGGCCCTTGTCAGACAGCAACTGCCTGAGAAGTACAAGGAAATTTTCTTCGATCAGTCTAAAAATGGCTACGCCGGATACATTGACGGCGGAGCAAGCCAGGAGGAATTTTACAAATTTATTAAGCCCATCTTGGAAAAAATGGACGGCACCGAGGAGCTGCTGGTAAAGCTTAACAGAGAAGATCTGTTGCGCAAACAGCGCACTTTCGACAATGGAAGCATCCCCCACCAGATTCACCTGGGCGAACTGCACGCTATCCTCAGGCGGCAAGAGGATTTCTACCCCTTTTTGAAAGATAACAGGGAAAAGATTGAGAAAATCCTCACATTTCGGATACCCTACTATGTAGGCCCCCTCGCCCGGGGAAATTCCAGATTCGCGTGGATGACTCGCAAATCAGAAGAGACCATCACTCCCTGGAACTTCGAGGAAGTCGTGGATAAGGGGGCCTCTGCCCAGTCCTTCATCGAAAGGATGACTAACTTTGATAAAAATCTGCCTAACGAAAAGGTGCTTCCTAAACACTCTCTGCTGTACGAGTACTTCACAGTTTATAACGAGCTCACCAAGGTCAAATACGTCACAGAAGGGATGAGAAAGCCAGCATTCCTGTCTGGAGAGCAGAAGAAAGCTATCGTGGACCTCCTCTTCAAGACGAACCGGAAAGTTACCGTGAAACAGCTCAAAGAAGACTATTTCAAAAAGATTGAATGTTTCGACTCTGTTGAAATCAGCGGAGTGGAGGATCGCTTCAACGCATCCCTGGGAACGTATCACGATCTCCTGAAAATCATTAAAGACAAGGACTTCCTGGACAATGAGGAGAACGAGGACATTCTTGAGGACATTGTCCTCACCCTTACGTTGTTTGAAGATAGGGAGATGATTGAAGAACGCTTGAAAACTTACGCTCATCTCTTCGACGACAAAGTCATGAAACAGCTCAAGAGGCGCCGATATACAGGATGGGGGCGGCTGTCAAGAAAACTGATCAATGGGATCCGAGACAAGCAGAGTGGAAAGACAATCCTGGATTTTCTTAAGTCCGATGGATTTGCCAACCGGAACTTCATGCAGTTGATCCATGATGACTCTCTCACCTTTAAGGAGGACATCCAGAAAGCACAAGTTTCTGGCCAGGGGGACAGTCTTCACGAGCACATCGCTAATCTTGCAGGTAGCCCAGCTATCAAAAAGGGAATACTGCAGACCGTTAAGGTCGTGGATGAACTCGTCAAAGTAATGGGAAGGCATAAGCCCGAGAATATCGTTATCGAGATGGCCCGAGAGAACCAAACTACCCAGAAGGGACAGAAGAACAGTAGGGAAAGGATGAAGAGGATTGAAGAGGGTATAAAAGAACTGGGGTCCCAAATCCTTAAGGAACACCCAGTTGAAAACACCCAGCTTCAGAATGAGAAGCTCTACCTGTACTACCTGCAGAACGGCAGGGACATGTACGTGGATCAGGAACTGGACATCAATCGGCTCTCCGACTACGACGTGGATCATATCGTGCCCCAGTCTTTTCTCAAAGATGATTCTATTGATAATAAAGTGTTGACAAGATCCGATAAAAATAGAGGGAAGAGTGATAACGTCCCCTCAGAAGAAGTTGTCAAGAAAATGAAAAATTATTGGCGGCAGCTGCTGAACGCCAAACTGATCACACAACGGAAGTTCGATAATCTGACTAAGGCTGAACGAGGTGGCCTGTCTGAGTTGGATAAAGCCGGCTTCATCAAAAGGCAGCTTGTTGAGACACGCCAGATCACCAAGCACGTGGCCCAAATTCTCGATTCACGCATGAACACCAAGTACGATGAAAATGACAAACTGATTCGAGAGGTGAAAGTTATTACTCTGAAGTCTAAGCTGGTCTCAGATTTCAGAAAGGACTTTCAGTTTTATAAGGTGAGAGAGATCAACAATTACCACCATGCGCATGATGCCTACCTGAATGCAGTGGTAGGCACTGCACTTATCAAAAAATATCCCAAGCTTGAATCTGAATTTGTTTACGGAGACTATAAAGTGTACGATGTTAGGAAAATGATCGCAAAGTCTGAGCAGGAAATAGGCAAGGCCACCGCTAAGTACTTCTTTTACAGCAATATTATGAATTTTTTCAAGACCGAGATTACACTGGCCAAT

* Truncated PmCDA1 with point mutation (30-150, W122E, W139Q)

TCGCATAGATGCTACGTTCTCTTTGAATTAAAACGACGGGGTGAACGTAGAGCGTGTTTTTGGGGCTATGCTGTGAATAAACCACAGAGCGGGACAGAACGTGGCATTCACGCCGAAATCTTTAGCATTAGAAAAGTCGAAGAATACCTGCGCGACAACCCCGGACAATTCACGATAAATTGGTACTCATCCTGGAGTCCTTGTGCAGATTGCGCTGAAAAGATCTTAGAATGGTATAACCAGGAGCTGCGGGGGAACGGCCACACTTTGAAAATCGAAGCTTGCAAACTCTATTACGAGAAAAATGCGAGGAATCAAATTGGGCTGCAAAACCTCAGAGATAACGGGGTTGGGTTGAATGTA

* GS Linker

GGCGGAGGCGGTAGCGACGTCGGTGGAGGTTCA

* nSpCas9 (D10A, 1055-1368)

GGAGAGATTCGGAAGCGACCACTTATCGAAACAAACGGAGAAACAGGAGAAATCGTGTGGGACAAGGGTAGGGATTTCGCGACAGTCCGGAAGGTCCTGTCCATGCCGCAGGTGAACATCGTTAAAAAGACCGAAGTACAGACCGGAGGCTTCTCCAAGGAAAGTATCCTCCCGAAAAGGAACAGCGACAAGCTGATCGCACGCAAAAAAGATTGGGACCCCAAGAAATACGGCGGATTCGATTCTCCTACAGTCGCTTACAGTGTACTGGTTGTGGCCAAAGTGGAGAAAGGGAAGTCTAAAAAACTCAAAAGCGTCAAGGAACTGCTGGGCATCACAATCATGGAGCGATCAAGCTTCGAAAAAAACCCCATCGACTTTCTCGAGGCGAAAGGATATAAAGAGGTCAAAAAAGACCTCATCATTAAGCTTCCCAAGTACTCTCTCTTTGAGCTTGAAAACGGCCGGAAACGAATGCTCGCTAGTGCGGGCGAGCTGCAGAAAGGTAACGAGCTGGCACTGCCCTCTAAATACGTTAATTTCTTGTATCTGGCCAGCCACTATGAAAAGCTCAAAGGGTCTCCCGAAGATAATGAGCAGAAGCAGCTGTTCGTGGAACAACACAAACACTACCTTGATGAGATCATCGAGCAAATAAGCGAATTCTCCAAAAGAGTGATCCTCGCCGACGCTAACCTCGATAAGGTGCTTTCTGCTTACAATAAGCACAGGGATAAGCCCATCAGGGAGCAGGCAGAAAACATTATCCACTTGTTTACTCTGACCAACTTGGGCGCGCCTGCAGCCTTCAAGTACTTCGACACCACCATAGACAGAAAGCGGTACACCTCTACAAAGGAGGTCCTGGACGCCACACTGATTCATCAGTCAATTACGGGGCTCTATGAAACAAGAATCGACCTCTCTCAGCTCGGTGGAGACAGCAGGGCTGACCCCAAGAAGAAGAGGAAGGTGGGTACC

* UGI

ATGACCAACCTTTCCGACATCATAGAGAAGGAAACAGGCAAACAGTTGGTCATCCAAGAGTCGATACTCATGCTTCCTGAAGAAGTTGAGGAGGTCATTGGGAATAAGCCGGAAAGTGACATTCTCGTACACACTGCGTATGATGAGAGCACCGATGAGAACGTGATGCTGCTCACGTCAGATGCCCCAGAGTACAAACCCTGGGCTCTGGTGATTCAGGACTCTAATGGAGAGAACAAGATCAAGATGCTA

**Supplementary Sequence. S2\_ Nucleotide sequences of coding region of SpCas9 related variants for mammalian expression**

**Target-AID2S**

* NLS unit

ATGAAACGGACAGCCGACGGAAGCGAGTTCGAGTCACCAAAGAAGAAGCGGAAAGTC

* Truncated PmCDA1 with point mutation (30-150, W122E, W139Q)

TCACACCGCTGCTACGTGCTGTTTGAGTTGAAGCGAAGGGGCGAAAGAAGGGCTTGCTTTTGGGGCTATGCCGTCAACAAGCCCCAAAGTGGCACCGAGAGAGGAATACACGCTGAGATATTCAGTATCCGAAAGGTGGAAGAGTATCTTCGGGATAATCCTGGGCAGTTTACGATCAACTGGTATTCCAGCTGGAGTCCTTGCGCTGATTGTGCCGAGAAAATTCTGGAATGGTATAATCAGGAACTTCGGGGAAACGGGCACACATTGAAAATCGAAGCCTGCAAGCTGTACTACGAGAAGAATGCCCGGAACCAGATAGGACTCCAGAATCTGAGGGACAATGGTGTAGGCCTGAACGTG

* GS Linker

GGTGGAGGTGGCAGTGATGTCGGCGGAGGCTCC

* nSpCas9 (D10A) plus linker

GACAAGAAGTACAGCATCGGCCTGGCCATCGGCACCAACTCTGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAATTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGAGCCCTGCTGTTCGACAGCGGCGAAACAGCCGAGGCCACCCGGCTGAAGAGAACCGCCAGAAGAAGATACACCAGACGGAAGAACCGGATCTGCTATCTGCAAGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTGGAAGAGTCCTTCCTGGTGGAAGAGGATAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGAGAAAGAAACTGGTGGACAGCACCGACAAGGCCGACCTGCGGCTGATCTATCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAAAACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAGCAGACGGCTGGAAAATCTGATCGCCCAGCTGCCCGGCGAGAAGAAGAATGGCCTGTTCGGAAACCTGATTGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGATGCCAAACTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTTCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGAAATCACCAAGGCCCCCCTGAGCGCCTCTATGATCAAGAGATACGACGAGCACCACCAGGACCTGACCCTGCTGAAAGCTCTCGTGCGGCAGCAGCTGCCTGAGAAGTACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTACAAGTTCATCAAGCCCATCCTGGAAAAGATGGACGGCACCGAGGAACTGCTCGTGAAGCTGAACAGAGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGAGAGCTGCACGCCATTCTGCGGCGGCAGGAAGATTTTTACCCATTCCTGAAGGACAACCGGGAAAAGATCGAGAAGATCCTGACCTTCCGCATTCCCTACTACGTGGGCCCTCTGGCCAGGGGAAACAGCAGATTCGCCTGGATGACCAGAAAGAGCGAGGAAACCATCACCCCCTGGAACTTCGAGGAAGTGGTGGACAAGGGCGCTTCCGCCCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTATAACGAGCTGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTTCCTGAGCGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAATCGAGTGCTTCGACTCCGTGGAAATCTCCGGCGTGGAAGATCGGTTCAACGCCTCCCTGGGCACATACCACGATCTGCTGAAAATTATCAAGGACAAGGACTTCCTGGACAATGAGGAAAACGAGGACATTCTGGAAGATATCGTGCTGACCCTGACACTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACCTATGCCCACCTGTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGATACACCGGCTGGGGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACAATCCTGGATTTCCTGAAGTCCGACGGCTTCGCCAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTTAAAGAGGACATCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATAGCCTGCACGAGCACATTGCCAATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCCTGCAGACAGTGAAGGTGGTGGACGAGCTCGTGAAAGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACCCAGAAGGGACAGAAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGGCGGGATATGTACGTGGACCAGGAACTGGACATCAACCGGCTGTCCGACTACGATGTGGACCATATCGTGCCTCAGAGCTTTCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACCGGGGCAAGAGCGACAACGTGCCCTCCGAAGAGGTCGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATTACCCAGAGAAAGTTCGACAATCTGACCAAGGCCGAGAGAGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGAGACAGCTGGTGGAAACCCGGCAGATCACAAAGCACGTGGCACAGATCCTGGACTCCCGGATGAACACTAAGTACGACGAGAATGACAAGCTGATCCGGGAAGTGAAAGTGATCACCCTGAAGTCCAAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGTTTTACAAAGTGCGCGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTCGTGGGAACCGCCCTGATCAAAAAGTACCCTAAGCTGGAAAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAAATCGGCAAGGCTACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGATCCGGAAGCGGCCTCTGATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGGATAAGGGCCGGGATTTTGCCACCGTGCGGAAAGTGCTGAGCATGCCCCAAGTGAATATCGTGAAAAAGACCGAGGTGCAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCCAAGAGGAACAGCGATAAGCTGATCGCCAGAAAGAAGGACTGGGACCCTAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTATTCTGTGCTGGTGGTGGCCAAAGTGGAAAAGGGCAAGTCCAAGAAACTGAAGAGTGTGAAAGAGCTGCTGGGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAATCCCATCGACTTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCAAGCTGCCTAAGTACTCCCTGTTCGAGCTGGAAAACGGCCGGAAGAGAATGCTGGCCTCTGCCGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCCTCCAAATATGTGAACTTCCTGTACCTGGCCAGCCACTATGAGAAGCTGAAGGGCTCCCCCGAGGATAATGAGCAGAAACAGCTGTTTGTGGAACAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATCCTGGCCGACGCTAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCACCGGGATAAGCCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTGACCAATCTGGGAGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGGAAGAGGTACACCAGCACCAAAGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACACGGATCGACCTGTCTCAGCTGGGAGGTGACAGCGGCGGGAGCGGCGGGAGCGGGGGGAGC

* 2xUGI plus SV40 NLS

ACTAATCTGAGCGACATCATTGAGAAGGAGACTGGGAAACAGCTGGTCATTCAGGAGTCCATCCTGATGCTGCCTGAGGAGGTGGAGGAAGTGATCGGCAACAAGCCAGAGTCTGACATCCTGGTGCACACCGCCTACGACGAGTCCACAGATGAGAATGTGATGCTGCTGACCTCTGACGCCCCCGAGTATAAGCCTTGGGCCCTGGTCATCCAGGATTCTAACGGCGAGAATAAGATCAAGATGCTGAGCGGAGGATCCGGAGGATCTGGAGGCAGCACCAACCTGTCTGACATCATCGAGAAGGAGACAGGCAAGCAGCTGGTCATCCAGGAGAGCATCCTGATGCTGCCCGAAGAAGTCGAAGAAGTGATCGGAAACAAGCCTGAGAGCGATATCCTGGTCCATACCGCCTACGACGAGAGTACCGACGAAAATGTGATGCTGCTGACATCCGACGCCCCAGAGTATAAGCCCTGGGCTCTGGTCATCCAGGATTCCAACGGAGAGAACAAAATCAAAATGCTGTCTGGCGGCTCAAAAAGAACCGCCGACGGCAGCGAATTCGAGCCCAAGAAGAAGAGGAAAGTC

**Target-AID3S**

* NLS unit

ATGAAACGGACAGCCGACGGAAGCGAGTTCGAGTCACCAAAGAAGAAGCGGAAAGTC

* nSpCas9 (D10A, 1-1054)

GACAAGAAGTACAGCATCGGCCTGGCCATCGGCACCAACTCTGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAATTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGAGCCCTGCTGTTCGACAGCGGCGAAACAGCCGAGGCCACCCGGCTGAAGAGAACCGCCAGAAGAAGATACACCAGACGGAAGAACCGGATCTGCTATCTGCAAGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTGGAAGAGTCCTTCCTGGTGGAAGAGGATAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGAGAAAGAAACTGGTGGACAGCACCGACAAGGCCGACCTGCGGCTGATCTATCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAAAACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAGCAGACGGCTGGAAAATCTGATCGCCCAGCTGCCCGGCGAGAAGAAGAATGGCCTGTTCGGAAACCTGATTGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGATGCCAAACTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTTCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGAAATCACCAAGGCCCCCCTGAGCGCCTCTATGATCAAGAGATACGACGAGCACCACCAGGACCTGACCCTGCTGAAAGCTCTCGTGCGGCAGCAGCTGCCTGAGAAGTACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTACAAGTTCATCAAGCCCATCCTGGAAAAGATGGACGGCACCGAGGAACTGCTCGTGAAGCTGAACAGAGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGAGAGCTGCACGCCATTCTGCGGCGGCAGGAAGATTTTTACCCATTCCTGAAGGACAACCGGGAAAAGATCGAGAAGATCCTGACCTTCCGCATTCCCTACTACGTGGGCCCTCTGGCCAGGGGAAACAGCAGATTCGCCTGGATGACCAGAAAGAGCGAGGAAACCATCACCCCCTGGAACTTCGAGGAAGTGGTGGACAAGGGCGCTTCCGCCCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTATAACGAGCTGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTTCCTGAGCGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAATCGAGTGCTTCGACTCCGTGGAAATCTCCGGCGTGGAAGATCGGTTCAACGCCTCCCTGGGCACATACCACGATCTGCTGAAAATTATCAAGGACAAGGACTTCCTGGACAATGAGGAAAACGAGGACATTCTGGAAGATATCGTGCTGACCCTGACACTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACCTATGCCCACCTGTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGATACACCGGCTGGGGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACAATCCTGGATTTCCTGAAGTCCGACGGCTTCGCCAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTTAAAGAGGACATCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATAGCCTGCACGAGCACATTGCCAATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCCTGCAGACAGTGAAGGTGGTGGACGAGCTCGTGAAAGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACCCAGAAGGGACAGAAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGGCGGGATATGTACGTGGACCAGGAACTGGACATCAACCGGCTGTCCGACTACGATGTGGACCATATCGTGCCTCAGAGCTTTCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACCGGGGCAAGAGCGACAACGTGCCCTCCGAAGAGGTCGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATTACCCAGAGAAAGTTCGACAATCTGACCAAGGCCGAGAGAGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGAGACAGCTGGTGGAAACCCGGCAGATCACAAAGCACGTGGCACAGATCCTGGACTCCCGGATGAACACTAAGTACGACGAGAATGACAAGCTGATCCGGGAAGTGAAAGTGATCACCCTGAAGTCCAAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGTTTTACAAAGTGCGCGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTCGTGGGAACCGCCCTGATCAAAAAGTACCCTAAGCTGGAAAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAAATCGGCAAGGCTACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTTTTCAAGACCGAGATTACCCTGGCCAAC

* Truncated PmCDA1 with point mutation (30-150, W122E, W139Q)

TCACACCGCTGCTACGTGCTGTTTGAGTTGAAGCGAAGGGGCGAAAGAAGGGCTTGCTTTTGGGGCTATGCCGTCAACAAGCCCCAAAGTGGCACCGAGAGAGGAATACACGCTGAGATATTCAGTATCCGAAAGGTGGAAGAGTATCTTCGGGATAATCCTGGGCAGTTTACGATCAACTGGTATTCCAGCTGGAGTCCTTGCGCTGATTGTGCCGAGAAAATTCTGGAATGGTATAATCAGGAACTTCGGGGAAACGGGCACACATTGAAAATCGAAGCCTGCAAGCTGTACTACGAGAAGAATGCCCGGAACCAGATAGGACTCCAGAATCTGAGGGACAATGGTGTAGGCCTGAACGTG

* GS Linker

GGTGGAGGTGGCAGTGATGTCGGCGGAGGCTCC

* nSpCas9 (D10A, 1055-1368) plus linker

GGCGAGATCCGGAAGCGGCCTCTGATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGGATAAGGGCCGGGATTTTGCCACCGTGCGGAAAGTGCTGAGCATGCCCCAAGTGAATATCGTGAAAAAGACCGAGGTGCAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCCAAGAGGAACAGCGATAAGCTGATCGCCAGAAAGAAGGACTGGGACCCTAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTATTCTGTGCTGGTGGTGGCCAAAGTGGAAAAGGGCAAGTCCAAGAAACTGAAGAGTGTGAAAGAGCTGCTGGGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAATCCCATCGACTTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCAAGCTGCCTAAGTACTCCCTGTTCGAGCTGGAAAACGGCCGGAAGAGAATGCTGGCCTCTGCCGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCCTCCAAATATGTGAACTTCCTGTACCTGGCCAGCCACTATGAGAAGCTGAAGGGCTCCCCCGAGGATAATGAGCAGAAACAGCTGTTTGTGGAACAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATCCTGGCCGACGCTAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCACCGGGATAAGCCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTGACCAATCTGGGAGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGGAAGAGGTACACCAGCACCAAAGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACACGGATCGACCTGTCTCAGCTGGGAGGTGACAGCGGCGGGAGCGGCGGGAGCGGGGGGAGC

* 2xUGI plus SV40 NLS

ACTAATCTGAGCGACATCATTGAGAAGGAGACTGGGAAACAGCTGGTCATTCAGGAGTCCATCCTGATGCTGCCTGAGGAGGTGGAGGAAGTGATCGGCAACAAGCCAGAGTCTGACATCCTGGTGCACACCGCCTACGACGAGTCCACAGATGAGAATGTGATGCTGCTGACCTCTGACGCCCCCGAGTATAAGCCTTGGGCCCTGGTCATCCAGGATTCTAACGGCGAGAATAAGATCAAGATGCTGAGCGGAGGATCCGGAGGATCTGGAGGCAGCACCAACCTGTCTGACATCATCGAGAAGGAGACAGGCAAGCAGCTGGTCATCCAGGAGAGCATCCTGATGCTGCCCGAAGAAGTCGAAGAAGTGATCGGAAACAAGCCTGAGAGCGATATCCTGGTCCATACCGCCTACGACGAGAGTACCGACGAAAATGTGATGCTGCTGACATCCGACGCCCCAGAGTATAAGCCCTGGGCTCTGGTCATCCAGGATTCCAACGGAGAGAACAAAATCAAAATGCTGTCTGGCGGCTCAAAAAGAACCGCCGACGGCAGCGAATTCGAGCCCAAGAAGAAGAGGAAAGTC

**Supplementary Sequence. S3\_ Nucleotide sequences SaCas9 related variants for mammalian expression**

**SaAID**

* CMV promotor

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCT

* NLS unit

ATGGCACCGAAGAAGAAGCGTAAAGTCGGAATCCACGGAGTTCCTGCGGCA

* nSaCas9 (D10A)

AAGCGAAATTACATTCTGGGGCTGGCCATTGGCATTACATCAGTGGGCTATGGCATCATTGACTACGAGACAAGGGACGTGATCGACGCCGGCGTGAGACTGTTCAAGGAGGCCAACGTGGAGAACAATGAGGGCCGGAGATCCAAGAGGGGAGCAAGGCGCCTGAAGCGGAGAAGGCGCCACAGAATCCAGAGAGTGAAGAAGCTGCTGTTCGATTACAACCTGCTGACCGACCACTCCGAGCTGTCTGGCATCAATCCTTATGAGGCCAGAGTGAAGGGCCTGTCCCAGAAGCTGTCTGAGGAGGAGTTTAGCGCCGCCCTGCTGCACCTGGCAAAGAGGAGAGGCGTGCACAACGTGAATGAGGTGGAGGAGGACACCGGCAACGAGCTGTCCACAAAGGAGCAGATCAGCCGCAATTCCAAGGCCCTGGAGGAGAAGTATGTGGCCGAGCTGCAGCTGGAGCGGCTGAAGAAGGATGGCGAGGTGAGGGGCTCCATCAATCGCTTCAAGACCTCTGACTACGTGAAGGAGGCCAAGCAGCTGCTGAAGGTGCAGAAGGCCTACCACCAGCTGGATCAGTCCTTTATCGATACATATATCGACCTGCTGGAGACAAGGCGCACATACTATGAGGGACCAGGAGAGGGCTCTCCCTTCGGCTGGAAGGACATCAAGGAGTGGTACGAGATGCTGATGGGCCACTGCACCTATTTTCCAGAGGAGCTGAGAAGCGTGAAGTACGCCTATAACGCCGATCTGTACAACGCCCTGAATGACCTGAACAACCTGGTCATCACCAGGGATGAGAACGAGAAGCTGGAGTACTATGAGAAGTTCCAGATCATCGAGAACGTGTTCAAGCAGAAGAAGAAGCCTACACTGAAGCAGATCGCCAAGGAGATCCTGGTGAACGAGGAGGACATCAAGGGCTACCGCGTGACCTCCACAGGCAAGCCAGAGTTCACCAATCTGAAGGTGTATCACGATATCAAGGACATCACAGCCCGGAAGGAGATCATCGAGAACGCCGAGCTGCTGGATCAGATCGCCAAGATCCTGACCATCTATCAGAGCTCCGAGGACATCCAGGAGGAGCTGACCAACCTGAATAGCGAGCTGACACAGGAGGAGATCGAGCAGATCAGCAATCTGAAGGGCTACACCGGCACACACAACCTGAGCCTGAAGGCCATCAATCTGATCCTGGATGAGCTGTGGCACACAAACGACAATCAGATCGCCATCTTTAACCGGCTGAAGCTGGTGCCAAAGAAGGTGGACCTGTCCCAGCAGAAGGAGATCCCAACCACACTGGTGGACGATTTCATCCTGTCTCCCGTGGTGAAGCGGAGCTTCATCCAGAGCATCAAAGTGATCAACGCCATCATCAAGAAGTACGGCCTGCCCAATGATATCATCATCGAGCTGGCCAGGGAGAAGAACTCCAAGGACGCCCAGAAGATGATCAATGAGATGCAGAAGAGGAACCGCCAGACCAATGAGCGGATCGAGGAGATCATCAGAACCACAGGCAAGGAGAACGCCAAGTACCTGATCGAGAAGATCAAGCTGCACGATATGCAGGAGGGCAAGTGTCTGTATTCTCTGGAGGCCATCCCTCTGGAGGACCTGCTGAACAATCCATTCAACTACGAGGTGGATCACATCATCCCCCGGAGCGTGAGCTTCGACAATTCTTTTAACAATAAGGTGCTGGTGAAGCAGGAGGAGAACAGCAAGAAGGGCAATAGGACCCCTTTCCAGTACCTGTCTAGCTCCGATTCTAAGATCAGCTACGAGACATTCAAGAAGCACATCCTGAATCTGGCCAAGGGCAAGGGCCGCATCAGCAAGACCAAGAAGGAGTACCTGCTGGAGGAGCGGGACATCAACAGATTCTCCGTGCAGAAGGACTTCATCAACCGGAATCTGGTGGACACCAGATACGCCACACGCGGCCTGATGAATCTGCTGCGGTCTTATTTCAGAGTGAACAATCTGGATGTGAAGGTGAAGAGCATCAACGGCGGCTTCACCTCCTTTCTGCGGAGAAAGTGGAAGTTTAAGAAGGAGCGCAACAAGGGCTATAAGCACCACGCCGAGGATGCCCTGATCATCGCCAATGCCGACTTCATCTTTAAGGAGTGGAAGAAGCTGGACAAGGCCAAGAAAGTGATGGAGAACCAGATGTTCGAGGAGAAGCAGGCCGAGAGCATGCCCGAGATCGAGACAGAGCAGGAGTACAAGGAGATTTTCATCACACCTCACCAGATCAAGCACATCAAGGACTTCAAGGACTACAAGTATTCTCACAGGGTGGATAAGAAGCCCAACCGCGAGCTGATCAATGACACCCTGTATAGCACACGGAAGGACGATAAGGGCAATACCCTGATCGTGAACAATCTGAACGGCCTGTACGACAAGGATAATGACAAGCTGAAGAAGCTGATCAACAAGTCTCCCGAGAAGCTGCTGATGTACCACCACGATCCTCAGACATATCAGAAGCTGAAGCTGATCATGGAGCAGTACGGCGACGAGAAGAACCCACTGTATAAGTACTATGAGGAGACAGGCAACTACCTGACAAAGTATAGCAAGAAGGATAATGGCCCCGTGATCAAGAAGATCAAGTACTATGGCAACAAGCTGAATGCCCACCTGGACATCACCGACGATTACCCTAACTCTCGCAATAAGGTGGTGAAGCTGAGCCTGAAGCCATACCGGTTCGACGTGTACCTGGACAACGGCGTGTATAAGTTTGTGACAGTGAAGAATCTGGATGTGATCAAGAAGGAGAACTACTATGAGGTGAACAGCAAGTGCTACGAGGAGGCCAAGAAGCTGAAGAAGATCAGCAACCAGGCCGAGTTCATCGCCTCTTTTTACAACAATGACCTGATCAAGATCAATGGCGAGCTGTATAGAGTGATCGGCGTGAACAATGATCTGCTGAACAGAATCGAAGTGAATATGATCGACATCACCTACAGGGAGTATCTGGAGAACATGAATGATAAGAGGCCCCCTCGCATCATCAAGACCATCGCCTCTAAGACACAGAGCATCAAGAAGTACAGCACAGACATCCTGGGGAACCTGTATGAAGTCAAGAGCAAGAAACATCCTCAGATTATCAAGAAAGGC

* SV40 NLS plus GS linker

CCCAAGAAGAAGAGGAAGGTGGGTGGAGGAGGTACCGGCGGTGGAGGCTCA

* Dead SH3 domain

GCAGAATACGTACGAGCTCTGTTTGACTTCAATGGGAATGACGAGGAGGATCTCCCCTTTAAGAAGGGCGATATTCTCCGCATCAGAGATAAGCCCGAAGAACAATGGTGGAATGCCGAGGATAGCGAAGGGAAAAGGGGCATGATTCTGGTGCCATATGTGGAGAAATATTCCGGT

* 3xFlag domain

GACTACAAAGACCATGATGGGGATTACAAAGACCACGACATCGACTACAAAGACGACGACGATAAATCAGGG

* PmCDA1 plus SV40 NLS

ATGACAGACGCCGAGTACGTGCGCATTCATGAGAAACTGGATATTTACACCTTCAAGAAGCAGTTCTTCAACAACAAGAAATCTGTGTCACACCGCTGCTACGTGCTGTTTGAGTTGAAGCGAAGGGGCGAAAGAAGGGCTTGCTTTTGGGGCTATGCCGTCAACAAGCCCCAAAGTGGCACCGAGAGAGGAATACACGCTGAGATATTCAGTATCCGAAAGGTGGAAGAGTATCTTCGGGATAATCCTGGGCAGTTTACGATCAACTGGTATTCCAGCTGGAGTCCTTGCGCTGATTGTGCCGAGAAAATTCTGGAATGGTATAATCAGGAACTTCGGGGAAACGGGCACACATTGAAAATCTGGGCCTGCAAGCTGTACTACGAGAAGAATGCCCGGAACCAGATAGGACTCTGGAATCTGAGGGACAATGGTGTAGGCCTGAACGTGATGGTTTCCGAGCACTATCAGTGTTGTCGGAAGATTTTCATCCAAAGCTCTCATAACCAGCTCAATGAAAACCGCTGGTTGGAGAAAACACTGAAACGTGCGGAGAAGCGGAGATCCGAGCTGAGCATCATGATCCAGGTCAAGATTCTGCATACCACTAAGTCTCCAGCCGTTGGTCCCAAGAAGAAAAGAAAAGTCGGTACC

* UGI

ATGACCAACCTTTCCGACATCATAGAGAAGGAAACAGGCAAACAGTTGGTCATCCAAGAGTCGATACTCATGCTTCCTGAAGAAGTTGAGGAGGTCATTGGGAATAAGCCGGAAAGTGACATTCTCGTACACACTGCGTATGATGAGAGCACCGATGAGAACGTGATGCTGCTCACGTCAGATGCCCCAGAGTACAAACCCTGGGCTCTGGTGATTCAGGACTCTAATGGAGAGAACAAGATCAAGATGCTA

* SV40 poly(A) signal

AACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTA

* H1 promotor

AATTCGAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCgggccCAGTGTCACTAGGCGGGAACACCCAGCGCGCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGACAGGGGAGTGGCGCCCTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAATCACCATAAACGTGAAATGTCTTTGGATTTGGGAATCTTATAAGTTCTGTATGAGGACCACAGATCCCC

* SaCas9 gRNA scaffold

GTTTTAGTACTCTGGAAACAGAATCTACTAAAACAAGGCAAAATGCCGTGTTTATCTCGTCAACTTGTTGGCGAGA

* iRFP670

ATGGCGCGTAAGGTCGATCTCACCTCCTGCGATCGCGAGCCGATCCACATCCCCGGCAGCATTCAGCCGTGCGGCTGCCTGCTAGCCTGCGACGCGCAGGCGGTGCGGATCACGCGCATTACGGAAAATGCCGGCGCGTTCTTTGGACGCGAAACTCCGCGGGTCGGTGAGCTACTCGCCGATTACTTCGGCGAGACCGAAGCCCATGCGCTGCGCAACGCACTGGCGCAGTCCTCCGATCCAAAGCGACCGGCGCTGATCTTCGGTTGGCGCGACGGCCTGACCGGCCGCACCTTCGACATCTCACTGCATCGCCATGACGGTACATCGATCATCGAGTTCGAGCCTGCGGCGGCCGAACAGGCCGACAATCCGCTGCGGCTGACGCGGCAGATCATCGCGCGCACCAAAGAACTGAAGTCGCTCGAAGAGATGGCCGCACGGGTGCCGCGCTATCTGCAGGCGATGCTCGGCTATCACCGCGTGATGTTGTACCGCTTCGCGGACGACGGCTCCGGGATGGTGATCGGCGAGGCGAAGCGCAGCGACCTCGAGAGCTTTCTCGGTCAGCACTTTCCGGCGTCGCTGGTCCCGCAGCAGGCGCGGCTACTGTACTTGAAGAACGCGATCCGCGTGGTCTCGGATTCGCGCGGCATCAGCAGCCGGATCGTGCCCGAGCACGACGCCTCCGGCGCCGCGCTCGATCTGTCGTTCGCGCACCTGCGCAGCATCTCGCCCTGCCATCTCGAATTTCTGCGGAACATGGGCGTCAGCGCCTCGATGTCGCTGTCGATCATCATTGACGGCACGCTATGGGGATTGATCATCTGTCATCATTACGAGCCGCGTGCCGTGCCGATGGCGCAGCGCGTCGCGGCCGAAATGTTCGCCGACTTCTTATCGCTGCACTTCACCGCCGCCCACCACCAACGCTAA

**SaAID-3S**

* ScpI promotor

GTACTTATATAAGGGGGTGGGGGCGCGTTCGTCCTCAGTCGCGATCGAACACTCGAGCCGAGCAGACGTGCCTACGGACCG

* NLS unit

ATGGCACCGAAGAAGAAGCGTAAAGTCGGAATCCACGGAGTTCCTGCGGCA

* nSaCas9 (D10A, 1-615)

AAGCGAAATTACATTCTGGGGCTGGCCATTGGCATTACATCAGTGGGCTATGGCATCATTGACTACGAGACAAGGGACGTGATCGACGCCGGCGTGAGACTGTTCAAGGAGGCCAACGTGGAGAACAATGAGGGCCGGAGATCCAAGAGGGGAGCAAGGCGCCTGAAGCGGAGAAGGCGCCACAGAATCCAGAGAGTGAAGAAGCTGCTGTTCGATTACAACCTGCTGACCGACCACTCCGAGCTGTCTGGCATCAATCCTTATGAGGCCAGAGTGAAGGGCCTGTCCCAGAAGCTGTCTGAGGAGGAGTTTAGCGCCGCCCTGCTGCACCTGGCAAAGAGGAGAGGCGTGCACAACGTGAATGAGGTGGAGGAGGACACCGGCAACGAGCTGTCCACAAAGGAGCAGATCAGCCGCAATTCCAAGGCCCTGGAGGAGAAGTATGTGGCCGAGCTGCAGCTGGAGCGGCTGAAGAAGGATGGCGAGGTGAGGGGCTCCATCAATCGCTTCAAGACCTCTGACTACGTGAAGGAGGCCAAGCAGCTGCTGAAGGTGCAGAAGGCCTACCACCAGCTGGATCAGTCCTTTATCGATACATATATCGACCTGCTGGAGACAAGGCGCACATACTATGAGGGACCAGGAGAGGGCTCTCCCTTCGGCTGGAAGGACATCAAGGAGTGGTACGAGATGCTGATGGGCCACTGCACCTATTTTCCAGAGGAGCTGAGAAGCGTGAAGTACGCCTATAACGCCGATCTGTACAACGCCCTGAATGACCTGAACAACCTGGTCATCACCAGGGATGAGAACGAGAAGCTGGAGTACTATGAGAAGTTCCAGATCATCGAGAACGTGTTCAAGCAGAAGAAGAAGCCTACACTGAAGCAGATCGCCAAGGAGATCCTGGTGAACGAGGAGGACATCAAGGGCTACCGCGTGACCTCCACAGGCAAGCCAGAGTTCACCAATCTGAAGGTGTATCACGATATCAAGGACATCACAGCCCGGAAGGAGATCATCGAGAACGCCGAGCTGCTGGATCAGATCGCCAAGATCCTGACCATCTATCAGAGCTCCGAGGACATCCAGGAGGAGCTGACCAACCTGAATAGCGAGCTGACACAGGAGGAGATCGAGCAGATCAGCAATCTGAAGGGCTACACCGGCACACACAACCTGAGCCTGAAGGCCATCAATCTGATCCTGGATGAGCTGTGGCACACAAACGACAATCAGATCGCCATCTTTAACCGGCTGAAGCTGGTGCCAAAGAAGGTGGACCTGTCCCAGCAGAAGGAGATCCCAACCACACTGGTGGACGATTTCATCCTGTCTCCCGTGGTGAAGCGGAGCTTCATCCAGAGCATCAAAGTGATCAACGCCATCATCAAGAAGTACGGCCTGCCCAATGATATCATCATCGAGCTGGCCAGGGAGAAGAACTCCAAGGACGCCCAGAAGATGATCAATGAGATGCAGAAGAGGAACCGCCAGACCAATGAGCGGATCGAGGAGATCATCAGAACCACAGGCAAGGAGAACGCCAAGTACCTGATCGAGAAGATCAAGCTGCACGATATGCAGGAGGGCAAGTGTCTGTATTCTCTGGAGGCCATCCCTCTGGAGGACCTGCTGAACAATCCATTCAACTACGAGGTGGATCACATCATCCCCCGGAGCGTGAGCTTCGACAATTCTTTTAACAATAAGGTGCTGGTGAAGCAGGAGGAGAACAGCAAGAAGGGCAATAGGACCCCTTTCCAGTACCTGTCTAGCTCCGATTCTAAGATCAGCTACGAGACATTCAAGAAGCACATCCTGAATCTGGCCAAGGGCAAG

* Truncated PmCDA1 with point mutation (30-150, W122E, W139Q)

TCACACCGCTGCTACGTGCTGTTTGAGTTGAAGCGAAGGGGCGAAAGAAGGGCTTGCTTTTGGGGCTATGCCGTCAACAAGCCCCAAAGTGGCACCGAGAGAGGAATACACGCTGAGATATTCAGTATCCGAAAGGTGGAAGAGTATCTTCGGGATAATCCTGGGCAGTTTACGATCAACTGGTATTCCAGCTGGAGTCCTTGCGCTGATTGTGCCGAGAAAATTCTGGAATGGTATAATCAGGAACTTCGGGGAAACGGGCACACATTGAAAATCGAAGCCTGCAAGCTGTACTACGAGAAGAATGCCCGGAACCAGATAGGACTCCAGAATCTGAGGGACAATGGTGTAGGCCTGAACGTG

* GS Linker

GGTGGAGGTGGCAGTGATGTCGGCGGAGGCTCC

* nSaCas9 (D10A, 616-1053) plus SV40 NLS

GGCCGCATCAGCAAGACCAAGAAGGAGTACCTGCTGGAGGAGCGGGACATCAACAGATTCTCCGTGCAGAAGGACTTCATCAACCGGAATCTGGTGGACACCAGATACGCCACACGCGGCCTGATGAATCTGCTGCGGTCTTATTTCAGAGTGAACAATCTGGATGTGAAGGTGAAGAGTATCAACGGCGGCTTCACCTCCTTTCTGCGGAGAAAGTGGAAGTTTAAGAAGGAGCGCAACAAGGGCTATAAGCACCACGCCGAGGATGCCCTGATCATCGCCAATGCCGACTTCATCTTTAAGGAGTGGAAGAAGCTGGACAAGGCCAAGAAAGTGATGGAGAACCAGATGTTCGAGGAGAAGCAGGCCGAGAGCATGCCCGAGATCGAGACAGAGCAGGAGTACAAGGAGATTTTCATCACACCTCACCAGATCAAGCACATCAAGGACTTCAAGGACTACAAGTATTCTCACAGGGTGGATAAGAAGCCCAACCGCGAGCTGATCAATGACACCCTGTATAGCACACGGAAGGACGATAAGGGCAATACCCTGATCGTGAACAATCTGAACGGCCTGTACGACAAGGATAATGACAAGCTGAAGAAGCTGATCAACAAGTCTCCCGAGAAGCTGCTGATGTACCACCACGATCCTCAGACATATCAGAAGCTGAAGCTGATCATGGAGCAGTACGGCGACGAGAAGAACCCACTGTATAAGTACTATGAGGAGACAGGCAACTACCTGACAAAGTATAGCAAGAAGGATAATGGCCCCGTGATCAAGAAGATCAAGTACTATGGCAACAAGCTGAATGCCCACCTGGACATCACCGACGATTACCCTAACTCTCGCAATAAGGTGGTGAAGCTGAGCCTGAAGCCATACCGGTTCGACGTGTACCTGGACAACGGCGTGTATAAGTTTGTGACAGTGAAGAATCTGGATGTGATCAAGAAGGAGAACTACTATGAGGTGAACAGCAAGTGCTACGAGGAGGCCAAGAAGCTGAAGAAGATCAGCAACCAGGCCGAGTTCATCGCCTCTTTTTACAACAATGACCTGATCAAGATCAATGGCGAGCTGTATAGAGTGATCGGCGTGAACAATGATCTGCTGAACAGAATCGAAGTGAATATGATCGACATCACCTACAGGGAGTATCTGGAGAACATGAATGATAAGAGGCCCCCTCGCATCATCAAGACCATCGCCTCTAAGACACAGAGCATCAAGAAGTACAGCACAGACATCCTGGGGAACCTGTATGAAGTCAAGAGCAAGAAACATCCTCAGATTATCAAGAAAGGCGGTGGAGGTCCCAAGAAGAAAAGAAAAGTCGGTACC

* UGI

ATGACCAACCTTTCCGACATCATAGAGAAGGAAACAGGCAAACAGTTGGTCATCCAAGAGTCGATACTCATGCTTCCTGAAGAAGTTGAGGAGGTCATTGGGAATAAGCCGGAAAGTGACATTCTCGTACACACTGCGTATGATGAGAGCACCGATGAGAACGTGATGCTGCTCACGTCAGATGCCCCAGAGTACAAACCCTGGGCTCTGGTGATTCAGGACTCTAATGGAGAGAACAAGATCAAGATGCTA

* Ploy-A tail

AATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGTTTTTTGTGTG

* H1 promotor

AATTCGAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCgggccCAGTGTCACTAGGCGGGAACACCCAGCGCGCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGACAGGGGAGTGGCGCCCTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAATCACCATAAACGTGAAATGTCTTTGGATTTGGGAATCTTATAAGTTCTGTATGAGGACCACAGATCCCC

* SaCas9 gRNA scaffold

GTTTTAGTACTCTGGAAACAGAATCTACTAAAACAAGGCAAAATGCCGTGTTTATCTCGTCAACTTGTTGGCGAGA

* iRFP670

ATGGCGCGTAAGGTCGATCTCACCTCCTGCGATCGCGAGCCGATCCACATCCCCGGCAGCATTCAGCCGTGCGGCTGCCTGCTAGCCTGCGACGCGCAGGCGGTGCGGATCACGCGCATTACGGAAAATGCCGGCGCGTTCTTTGGACGCGAAACTCCGCGGGTCGGTGAGCTACTCGCCGATTACTTCGGCGAGACCGAAGCCCATGCGCTGCGCAACGCACTGGCGCAGTCCTCCGATCCAAAGCGACCGGCGCTGATCTTCGGTTGGCGCGACGGCCTGACCGGCCGCACCTTCGACATCTCACTGCATCGCCATGACGGTACATCGATCATCGAGTTCGAGCCTGCGGCGGCCGAACAGGCCGACAATCCGCTGCGGCTGACGCGGCAGATCATCGCGCGCACCAAAGAACTGAAGTCGCTCGAAGAGATGGCCGCACGGGTGCCGCGCTATCTGCAGGCGATGCTCGGCTATCACCGCGTGATGTTGTACCGCTTCGCGGACGACGGCTCCGGGATGGTGATCGGCGAGGCGAAGCGCAGCGACCTCGAGAGCTTTCTCGGTCAGCACTTTCCGGCGTCGCTGGTCCCGCAGCAGGCGCGGCTACTGTACTTGAAGAACGCGATCCGCGTGGTCTCGGATTCGCGCGGCATCAGCAGCCGGATCGTGCCCGAGCACGACGCCTCCGGCGCCGCGCTCGATCTGTCGTTCGCGCACCTGCGCAGCATCTCGCCCTGCCATCTCGAATTTCTGCGGAACATGGGCGTCAGCGCCTCGATGTCGCTGTCGATCATCATTGACGGCACGCTATGGGGATTGATCATCTGTCATCATTACGAGCCGCGTGCCGTGCCGATGGCGCAGCGCGTCGCGGCCGAAATGTTCGCCGACTTCTTATCGCTGCACTTCACCGCCGCCCACCACCAACGCTAA

**Supplementary Table. S1\_ CAN1 target sequence**

|  |  |  |  |
| --- | --- | --- | --- |
| Site name | gRNA | Protospacer (CAN1) | PAM |
| CAN1 | Site 1 | GCCCAGTTGGATTCCGTTAT | TGG |
| Site 2 | CTCCAATAACGGAATCCAAC | TGG |
| Site 3 | TTGGCCAAGTCATTCAATTT | TGG |

**Supplementary Table. S2\_Target protospacers and amplicons used in this study conducting with mammalian cell expression**

* SpCas9 genomic loci:

|  |  |  |  |
| --- | --- | --- | --- |
| Site name | Protospacer | PAM | Amplicon |
| HEK2 | GAACACAAAGCATAGACTGC | GGG | ATGAATGGATTCCTTGGAAACAATGATAACAAGACCTGGCTGAGCTAACTGTGACAGCATGTGGTAATTTTCCAGCCCGCTGGCCCTGTAAAGGAAACTGGAACACAAAGCATAGACTGCGGGGCGGGCCAGCCTGAATAGCTGCAAACAAGTGCAGAATATCTGATGATGTCATACGCACAGTTTGACAGATGGGGCTGGACAATTTTTCCCCCTTTAA |
| HEK3 | GGCCCAGACTGAGCACGTGA | TGG | CGCCCATGCAATTAGTCTATTTCTGCTGCAAGTAAGCATGCATTTGTAGGCTTGATGCTTTTTTTCTGCTTCTCCAGCCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAGCCCTGCTTCCTCCAGAGGGCGTCGCAGGACAGCTTTTCCTAGACAGGGGCTAGTATGTGCAGCTCCTGCACCGGGATACTGGT |
| RNF2 | GTCATCTTAGTCATTACCTG | AGG | ACGTCTCATATGCCCCTTGGCAGTCATCTTAGTCATTACCTGAGGTGTTCGTTGTAACTCATATAAACTGAGTTCCCATGTTTTGCTTAATGGTTGAGTTCCGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAGAGAGAAAAACAATTTTAGTATTTGGAAGGGAAGTGCTATGGTCTGAATGTATGTGTCCCACCAAAATTCCTACGT |
| VEGFA | CCCCCTCCACCCCGCCTC | CGG | CTCCGCTCGGCTCGGCTTCCCCCGCGCGGACCACGGCTCCTCCGAAGCGAGAACAGCCCAGAAGTTGGACGAAAAGTTTCAGTGCGACGCCGCGAGCCCCGACCCCCTCCACCCCGCCTCCGGGCGCGGGCTCCGGCCCCTGCCCGCGGCTCGCCGCCGCGTCCACTGTCCGCCGCCGGCCGGGGAGGAGGTGGTAGCTGGGGCTGGGGGCGGTGTCTGT |

* SaCas9 genomic loci:

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| --- | --- | --- | --- |
| Site name | Protospacer | PAM | Amplicon |
| SaCas9 site 1 | GTGGTAGACAGCATGTGTCCTA | AAGGGT | ATGCCCCTTTGCCTCAAGTCTGGTTATTTTAGGGGGATGCTAGGTTGCTTTGGGTCTACCTTACTGAGAAAATGGCCCCAGGTCATTGTCATGTCCAGTTGTGGTAGACAGCATGTGTCCTAAAGGGTATATTCACATGCATGTGCAAAAATACAGGGGTCCTTCTAACCCTATCACAGAGAAGCAGGAGACTGCAGGATGAGTCAAGATGCTAAGTGATGA |
| SaCas9 site 2 | ATTTACAGCCTGGCCTTTGGGG | TCGGGT | CCTCAGACCAGGGTGCGGTGGGAGATCTGGTTTCCGGAAGACGGAATGGGGAGAAGGGCAGGTTCCCCGAGGCGCCCAGACACCCAATCCTCCCGGTGACATTTACAGCCTGGCCTTTGGGGTCGGGTCAACGCTAGGCTGGCAGGGGAAGGGCGGGGCCGTGAGGTGAGCCGGCGCTGCAGGAAGGGGCCACCACCAGAGGGGCCATTTTGCGGTGGAAAT |
| SaCas9 site 3 | GTGTCAGGTAATGTGCTAAACA | GAGAGT | GTGGCATCCAGAGACATGGTTTCTTATCTCCTTAAGTGTTCAGCTGCTTTTCTTTCATTTATTCCACATATAATTACTATAATTGCTAAACATTTATTTAGTGTCAGGTAATGTGCTAAACAGAGAGTTACTGCTCAGACATGTAATAATAATAAATAACACATCAAATAACCATACCATTTTAAGCTGTAGTATTATGAAGGGAAATCTGGAGCAAAGAGA |
| SaCas9 site 4 | GGTGGAGGAGGGTGCATGGGGT | CAGAAT | TTTTGCTTATCCAGAAAAGGGAGTGATTGCTTCCAGGGGCCTCAGGGGAATAAATCATAGAATCCTGGACAAGGTTTGAAGGACAGGTAGGATTTGGGTGGGTGGAGGAGGGTGCATGGGGTCAGAATTGTAACCGAAAACTCATTCCAGGTGGATAGAGAAAATTTCTAGTGTTGTTGTTTTTAAACTATTTGGGGGACTGGCACAGACCCTTTTTGAATA |
| SaCas9 site 5 | TCTGCTTCTCCAGCCCTGGC | CTGGGT | GATGAGAGAAGCCTGGAGACAGGGATCCCAGGGAAACGCCCATGCAATTAGTCTATTTCTGCTGCAAGTAAGCATGCATTTGTAGGCTTGATGCTTTTTTTCTGCTTCTCCAGCCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAGCCCTGCTTCCTCCAGAGGGCGTCGCAGGACAGCTTTTCCTAGACAGG |
| SaCas9 site 6 (FANCF) | GATGTTCCAATCAGTACGCA | GAGAGT | GGCTTTTAAGTTGCCCAGAGTCAAGGAACACGGATAAAGACGCTGGGAGATTGACATGCATTTCGACCAATAGCATTGCAGAGAGGCGTATCATTTCGCGGATGTTCCAATCAGTACGCAGAGAGTCGCCGTCTCCAAGGTGAAAGCGGAAGTAGGGCCTTCGCGCACCTCATGGAATCCCTTCTGCAGCACCTGGATCGCTTTTCCGAGCTTCTGGCGG |
| SaCas9 site 7 (VEGFA) | GCCCGCCCTCCCCCGCCCGG | CCGGGT | TGGGAAAAGTTTTGGGGTGGATTGCTGCGGGGACCCCCCCTCCCTGCTGGGCCACCTGCGCCGCGCCAACCCCGCCCGTCCCCGCTCGCGTCCCGCTCGGTGCCCGCCCTCCCCCGCCCGGCCGGGTGCGCGCGGCGCGGAGCCGATTACATCAGCCCGGGCCTGGCCGGCCGCGTGTTCCCGGAGCCTCGGCTGCCCGAATGGGGAGCCCAGAGTGGCGAG |

* SpCas9 gRNA dependent off-target genomic loci:

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| --- | --- | --- | --- |
| Site name | Protospacer | PAM | Amplicon |
| HEK2\_site1 | GAACACAATGCATAGATTGC | CGG | TCTGTAACTAGAACAATGGGTTCAAAAACAAACAGAGAAGACAAGTAAATTAATAAGTACTGTAGTCCAGATAATCAGAGTGTGGAGAGTGAGTAAGCCAGAACACAATGCATAGATTGCCGGTAAATAGGTTTAGATTCATCCATTTTTAAAAAATGGTGTGGGAGCATTAAATATGTATATAGTAGATATGGAAAAATGATTCTCATAATAACTGACA |
| HEK2\_site2 | AAACATAAAGCATAGACTGC | AAA | TAGCTCAGGGAAGGAGCAGTGAGTTTGGGCACTTGTGACAGAATAGTGGGACTATGCCAGAGATACACAGGAGGAGGTGGTACCTTCTAGCTCCCCCTCAAAACATAAAGCATAGACTGCAAAGTACTCCCAAGCAGGCTGAATAACACTCGAGTACCAAAAAAATACACTCTGAAACAATACCAATAATTTTTTTAAAAATTATATATACCATGATAGT |
| VEGFA\_site1 | GACCCCCCCCACCCCGCCCC | CGG | CTTTTTAAGGTTTCCCAATTTCATCTTCAGAACAGTTAGAATCTACTGACTAATTCTGAGCAGAGGAATATGTGACATGAGGAGATTTGCATTTCCAAAAGACCCCCCCCACCCCGCCCCCGGCTGCTGTTGCAGAGGACAAGTTGAGGAGGGCAGAGAGGATGTGGAGAGGACAGCAGGGAGTACTGCAGCTGTCCAAGTCAGAGATTGTAGTGGCTCT |
| VEGFA\_site2 | GCCCCCACCCACCCCGCCTC | TGG | GAGCCCTCCTCTCTTGCTTCTTGCTCTGACCTTGTTTGTTATTCCACAGAGAAAACTTTCAAAAACAGCCAGAAGAGAACATCCACGAGCTCCCACCACTGCCCCCACCCACCCCGCCTCTGGCCCATGTATTCTGCTTTCTCTTGTTTTAATCTTTCCCTCTCAACAAAGAGCAACTTCTTCCTTCTAACTGCTCAGCCGAAAAGCCTTGGAGTCACCC |
| VEGFA\_site3 | CGCCCTCCCCACCCCGCCTC | CGG | TCTTGCCTGTCACGCAGACGCCCCCATCAAGCCCACCGCCCACCCCCAACCCCCGTGCGGGCAGCGGCTCCTCGGTGCTCTGGTCCGACGCCCCTTCTGTCGCCCTCCCCACCCCGCCTCCGGCGGACAGAGCTGGGCGGGAGCTGGGACTGGTACCCCCTCCCCACCTCGGAGACACCCTTAACTCCAGGCTGCGATCCTCGTGCCGGGGCCCCGCCGT |
| VEGFA\_site4 | CTCCCCACCCACCCCGCCTC | AGG | CTCTGCTTCATGTTGTTGGCAGGAGGCCCCTCCTTCTCATCCTTGTATCAGCTGCCTTCTCATCACAAGATGACTATGTCCCTCTGGGCCCCATCCTCCCCTCCCCACCCACCCCGCCTCAGGCTTGAAGAGGAAAGAAGAGCGCAGGGCCAAAAAAACTTTGTCCTTTAGAAAGGATCTACCTTTTAGGCTGAGAATGGCACTCTCCCCAGAAACATCC |

**Supplementary Table. S3\_ Primer list used in this study**

|  |  |  |  |
| --- | --- | --- | --- |
| Name | Sequence (5'-3') | Target | Experiment |
| HEK2 1st-1 | TCCCACGTATTGCACTGCCATT | HEK2 gene | 1st round PCR for deep-sequence |
| HEK2 1st-2 | CCCCTCAGCATTCAGCCACTAATA | HEK2 gene | 1st round PCR for deep-sequence |
| HEK2 2nd-1 | TCTTTCCCTACACGACGCTCTTCCGATCTCGGAATGAATGGATTCCTTG | HEK2 gene | 2nd round PCR for deep-sequence |
| HEK2 2nd-2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCCCCATCTGTCAAACT | HEK2 gene | 2nd round PCR for deep-sequence |
| HEK3 1st-1 | AGCTTGGCATGAGAAACCTTGGAG | HEK3 gene | 1st round PCR for deep-sequence |
| HEK3 1st-2 | TCTGGGTGCCCTGAGATCTTTT | HEK3 gene | 1st round PCR for deep-sequence |
| HEK3 2nd-1 | TCTTTCCCTACACGACGCTCTTCCGATCTGGGAAACGCCCATGCAATTAGTCT | HEK3 gene | 2nd round PCR for deep-sequence |
| HEK3 2nd-2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGCACATACTAGCCCCTGTCT | HEK3 gene | 2nd round PCR for deep-sequence |
| RNF2 1st-1 | GTCTGTAAAGTCCATGGTTGGTGC | RNF2 gene | 1st round PCR for deep-sequence |
| RNF2 1st-2 | CTCCCAAAGTCCCCACCTCTTAAT | RNF2 gene | 1st round PCR for deep-sequence |
| RNF2 2nd-1 | TCTTTCCCTACACGACGCTCTTCCGATCTCCAACATACAGAAGTCAGGAATGC | RNF2 gene | 2nd round PCR for deep-sequence |
| RNF2 2nd-2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTCTCAGGCTGTGCAGACAA | RNF2 gene | 2nd round PCR for deep-sequence |
| VEGFA 1st-1 | AAGGCTCCAATGCACCCAAGA | VEGFA gene | 1st round PCR for deep-sequence |
| VEGFA 1st-2 | CGCTCGGTGCTGGAATTTGATA | VEGFA gene | 1st round PCR for deep-sequence |
| VEGFA 2nd-1 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCCTCCGAAGCGAGAACA | VEGFA gene | 2nd round PCR for deep-sequence |
| VEGFA 2nd-2 | TCTTTCCCTACACGACGCTCTTCCGATCTACGGACAGACAGACAGACAC | VEGFA gene | 2nd round PCR for deep-sequence |
| SaCas9 site\_1 1st-1 | GTGGAGTGCTCTGTGTTTGTCT | SaCas9 site\_1 R-loop off-target | 1st round PCR for deep-sequence |
| SaCas9 site\_1 1st-2 | TACCTGTGCACCTAAGGCATCA | SaCas9 site\_1 R-loop off-target | 1st round PCR for deep-sequence |
| SaCas9 site\_1 2nd-1 | TCTTTCCCTACACGACGCTCTTCCGATCTTACCAACATACATGCCCCTTTGCC | SaCas9 site\_1 R-loop off-target | 2nd round PCR for deep-sequence |
| SaCas9 site\_1 2nd-2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTGACTCATCCTGCAGTCTCCT | SaCas9 site\_1 R-loop off-target | 2nd round PCR for deep-sequence |
| SaCas9 site\_2 1st-1 | GATTTGCCAAGTTGCCTGTCCTTC | SaCas9 site\_2 R-loop off-target | 1st round PCR for deep-sequence |
| SaCas9 site\_2 1st-2 | TGGGTTTATGGAGGTCCTCTTGTG | SaCas9 site\_2 R-loop off-target | 1st round PCR for deep-sequence |
| SaCas9 site\_2 2nd-1 | TCTTTCCCTACACGACGCTCTTCCGATCTTGCGGTGGGAGATCTGGTTTC | SaCas9 site\_2 R-loop off-target | 2nd round PCR for deep-sequence |
| SaCas9 site\_2 2nd-2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGACATTTCCACCGCAAAATGGC | SaCas9 site\_2 R-loop off-target | 2nd round PCR for deep-sequence |
| SaCas9 site\_3 1st-1 | AGCTGTCCAGAGGTGTTAAGTGCC | SaCas9 site\_3 R-loop off-target | 1st round PCR for deep-sequence |
| SaCas9 site\_3 1st-2 | TTCACTGTGTTAGCCAGGATGGTC | SaCas9 site\_3 R-loop off-target | 1st round PCR for deep-sequence |
| SaCas9 site\_3 2nd-1 | TCTTTCCCTACACGACGCTCTTCCGATCTTGGCATCCAGAGACATGGTTTC | SaCas9 site\_3 R-loop off-target | 2nd round PCR for deep-sequence |
| SaCas9 site\_3 2nd-2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTTTGCTCCAGATTTCCCTTCA | SaCas9 site\_3 R-loop off-target | 2nd round PCR for deep-sequence |
| SaCas9 site\_4 1st-1 | ACAACCTGTCCGCAAGGGAAAA | SaCas9 site\_4 R-loop off-target | 1st round PCR for deep-sequence |
| SaCas9 site\_4 1st-2 | AGTAGGGGTGAGGGAACAAACCA | SaCas9 site\_4 R-loop off-target | 1st round PCR for deep-sequence |
| SaCas9 site\_4 2nd-1 | TCTTTCCCTACACGACGCTCTTCCGATCTAGGGGCCTCAGGGGAATAAATCAT | SaCas9 site\_4 R-loop off-target | 2nd round PCR for deep-sequence |
| SaCas9 site\_4 2nd-2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGGATTCGACAGAAATGTGAGCC | SaCas9 site\_4 R-loop off-target | 2nd round PCR for deep-sequence |
| SaCas9 site\_5 1st-1 | AGCTTGGCATGAGAAACCTTGGAG | SaCas9 site\_5 R-loop off-target | 1st round PCR for deep-sequence |
| SaCas9 site\_5 1st-2 | TCTGGGTGCCCTGAGATCTTTT | SaCas9 site\_5 R-loop off-target | 1st round PCR for deep-sequence |
| SaCas9 site\_5 2nd-1 | TCTTTCCCTACACGACGCTCTTCCGATCTGGGAAACGCCCATGCAATTAGTCT | SaCas9 site\_5 R-loop off-target | 2nd round PCR for deep-sequence |
| SaCas9 site\_5 2nd-2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGCACATACTAGCCCCTGTCT | SaCas9 site\_5 R-loop off-target | 2nd round PCR for deep-sequence |
| SaCas9 site\_6 1st-1 | CATCTCGCACGTGGTTCCGGAA | SaCas9 site\_6 R-loop off-target | 1st round PCR for deep-sequence |
| SaCas9 site\_6 1st-2 | CGACCAAAGCGCCGATGGATGTG | SaCas9 site\_6 R-loop off-target | 1st round PCR for deep-sequence |
| SaCas9 site\_6 2nd-1 | TCTTTCCCTACACGACGCTCTTCCGATCTGGCTTTTAAGTTGCCCAGAG | SaCas9 site\_6 R-loop off-target | 2nd round PCR for deep-sequence |
| SaCas9 site\_6 2nd-2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGCCAGAAGCTCGGAAAA | SaCas9 site\_6 R-loop off-target | 2nd round PCR for deep-sequence |
| SaCas9 site\_7 1st-1 | ACTGCCACTCGGTCTCTTCA | SaCas9 site\_7 R-loop off-target | 1st round PCR for deep-sequence |
| SaCas9 site\_7 1st-2 | AGGGGTAGGGATGGTTCTCT | SaCas9 site\_7 R-loop off-target | 1st round PCR for deep-sequence |
| SaCas9 site\_7 2nd-1 | TCTTTCCCTACACGACGCTCTTCCGATCTGGGAAAAGTTTTGGGGTGGA | SaCas9 site\_7 R-loop off-target | 2nd round PCR for deep-sequence |
| SaCas9 site\_7 2nd-2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCGCCACTCTGGGCTCCCCATT | SaCas9 site\_7 R-loop off-target | 2nd round PCR for deep-sequence |
| HEK2\_off\_site-1 1st-1 | GCAGAAGTCGTACTATGCAAGC | HEK2 gene off-target 1 | 1st round PCR for deep-sequence |
| HEK2\_off\_site-1 1st-2 | TGCATGGGCACATGTGTTTG | HEK2 gene off-target 1 | 1st round PCR for deep-sequence |
| HEK2\_off\_site-1 2nd-1 | TCTTTCCCTACACGACGCTCTTCCGATCTGACAGATCTGTAACTAGAACAATGGG | HEK2 gene off-target 1 | 2nd round PCR for deep-sequence |
| HEK2\_off\_site-1 2nd-2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATATCTACTATATACATATTTAATGCTCCC | HEK2 gene off-target 1 | 2nd round PCR for deep-sequence |
| HEK2\_off\_site-2 1st-1 | AGAGGCCTTCCAAGCCTGAAAATG | HEK2 gene off-target 2 | 1st round PCR for deep-sequence |
| HEK2\_off\_site-2 1st-2 | GCATTAATCCATATTTGTATCACCCTCC | HEK2 gene off-target 2 | 1st round PCR for deep-sequence |
| HEK2\_off\_site-2 2nd-1 | TCTTTCCCTACACGACGCTCTTCCGATCTAGCAGTGAGTTTGGGCACTTGT | HEK2 gene off-target 2 | 2nd round PCR for deep-sequence |
| HEK2\_off\_site-2 2nd-2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTATGATTTCTAACTATCATGG | HEK2 gene off-target 2 | 2nd round PCR for deep-sequence |
| VEGFA\_off\_site-1 1st-1 | AAAACAGTGCGGTGGAGAAAGG | VEGFA gene off-target 1 | 1st round PCR for deep-sequence |
| VEGFA\_off\_site-1 1st-2 | CAGAGGGAGAATCAAGCTGTACAC | VEGFA gene off-target 1 | 1st round PCR for deep-sequence |
| VEGFA\_off\_site-1 2nd-1 | TCTTTCCCTACACGACGCTCTTCCGATCTAAGGTTTCCCAATTTCATCTTCAGAACAG | VEGFA gene off-target 1 | 2nd round PCR for deep-sequence |
| VEGFA\_off\_site-1 2nd-2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTCTGACTTGGACAGCTGCAGTA | VEGFA gene off-target 1 | 2nd round PCR for deep-sequence |
| VEGFA\_off\_site-2 1st-1 | ACAGCACCATGGTTAGACTGAGGA | VEGFA gene off-target 2 | 1st round PCR for deep-sequence |
| VEGFA\_off\_site-2 1st-2 | CAACAGGATTTGCTGAGGGATTGC | VEGFA gene off-target 2 | 1st round PCR for deep-sequence |
| VEGFA\_off\_site-2 2nd-1 | TCTTTCCCTACACGACGCTCTTCCGATCTTTCTCCCTACTCAACCCCGAGC | VEGFA gene off-target 2 | 2nd round PCR for deep-sequence |
| VEGFA\_off\_site-2 2nd-2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTCGGCTGAGCAGTTAGAAGGAAG | VEGFA gene off-target 2 | 2nd round PCR for deep-sequence |
| VEGFA\_off\_site-3 1st-1 | TGTGGCTGCCCCGCTTATTAAA | VEGFA gene off-target 3 | 1st round PCR for deep-sequence |
| VEGFA\_off\_site-3 1st-2 | CCGCCTGTGAGCTATTTCCACATT | VEGFA gene off-target 3 | 1st round PCR for deep-sequence |
| VEGFA\_off\_site-3 2nd-1 | TCTTTCCCTACACGACGCTCTTCCGATCTTCCTGGCCACCTCTTGCCTGTCA | VEGFA gene off-target 3 | 2nd round PCR for deep-sequence |
| VEGFA\_off\_site-3 2nd-2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGGATCGCAGCCTGGAGTTAAG | VEGFA gene off-target 3 | 2nd round PCR for deep-sequence |
| VEGFA\_off\_site-4 1st-1 | ATCACACCCTTTGGGGATCTTTGG | VEGFA gene off-target 4 | 1st round PCR for deep-sequence |
| VEGFA\_off\_site-4 1st-2 | AAAGGCTTCCTTGAGGCTGAAGTC | VEGFA gene off-target 4 | 1st round PCR for deep-sequence |
| VEGFA\_off\_site-4 2nd-1 | TCTTTCCCTACACGACGCTCTTCCGATCTTGCTTTCCAGCTCTGCTTCATGT | VEGFA gene off-target 4 | 2nd round PCR for deep-sequence |
| VEGFA\_off\_site-4 2nd-2 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGAGTGCCATTCTCAGCCTAAA | VEGFA gene off-target 4 | 2nd round PCR for deep-sequence |