

Supplemental Methods

Design of modified sgRNA

We modified sgRNA sequences by adding RNA binding sites to a previous design shown below (Chen et al. 2013). (Annotations: crRNA targeting sequence, **crRNA sequence**, **tracrRNA sequence**, linker sequence – lower case, **MS2 stem-loop**, **termination sequences**)

5'NNNNNNNNNNNNNNNNNNNNNGTTTAAGAGCTATGCTGGAAACAGCATAGCAAGTTTAAATAAGGC TAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGC**TTTTTTT** 3'

2×MBS sgRNA

5'NNNNNNNNNNNNNNNNNNNNNGTTTAAGAGCTATGCTGGAAACAGCATAGCAAGTTTAAATAAGGC TAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCgggagcACATGAGG**ATCACCCATGT**gccacg agcgACATGAGG**ATCACCCATGT**cgctcgtgttccc**TTTTTTT** 3'

12×MBS sgRNA: Design 1

5'NNNNNNNNNNNNNNNNNNNNNGTTTGAGAGCTATGCTGGAAACAGCATAGCAAGTTTAAATAAGGC TAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCtcttCGTACACC**ATCAGGGTACG**tctCAGACACC**ATCAGGGTCTG**tctGGTACAGC**ATCAGCGTACC**tctCGTACAGG**ATCACCCGTACG**tctGCAGCAGG**ATCACCGCTGC**tctCGAGCACG**ATCACGGCTCG**tctGACTCAGC**ATCAGCGAGTC**tctGCTAGAGC**ATCAGCC**TAGCtctCCTTGACC**ATCAGGCAAGG**tctGTCGTAGT**ATCAACACGAC**tctACATGAGG**ATCACCCATGT**tctGCACGAGC**ATCAGCCGTGC****TTTTTTT** 3'

12×MBS sgRNA: Design 2

5'NNNNNNNNNNNNNNNNNNNNNGTTTGAGAGCTATGCTGGAAACAGCATAGCAAGTTTAAATAAGGC TAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCgggagcACATGAGG**ATCACCCATGT**gccacg agcgACATGAGG**ATCACCCATGT**cgctcgtgttcccttCGTAGAGG**ATCACCCCTACG**tctACATGAGG**ATCACCC**ATGTtctGGTAGAGG**ATCACCC**TACCtctCGTAGAGG**ATCACCC**TACGtctGCAGGAGG**ATCACCC**CTGCtctCGAGGAGG**ATCACCC**CTCGtctGACTGAGG**ATCACCC**AGTCtctGCTAGAGG**ATCACCC**TAGCtctCCTTGA**GGATCACCCA**AGGtctGTCGAGG**ATCACCC**CGAC**TTTTTTT** 3'

12×MBS sgRNA: Design 3

5'NNNNNNNNNNNNNNNNNNNNNGTTTGAGAGCTAGGCCgggagcACATGAGG**ATCACCCATGT**gccacgag cgACATGAGG**ATCACCCATGT**cgctcgtgttcccttCGTAGAGG**ATCACCC**TACGtctACATGAGG**ATCACCC**ATGTtctGGTAGAGG**ATCACCC**TACCtctCGTAGAGG**ATCACCC**TACGtctGCAGGAGG**ATCACCC**CTGCtctCGAGGAGG**ATCACCC**CTCGtctGACTGAGG**ATCACCC**AGTCtctGCTAGAGG**ATCACCC**TAGCtctCCTTGA**ATCACCCA**AGGGCCTAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGCCAACATGAGG**ATCACCC**ATGTCTGCAGGGCCAAGTGGCACCGAGTCGGTGC**TTTTTTT** 3'

12×MBS sgRNA: Design 4

5'NNNNNNNNNNNNNNNNNNNNNGTTTGAGAGCTAGGCCgggagcACATGAGG**ATCACCCATGT**gccacgag cgACATGAGG**ATCACCCATGT**cgctcgtgttcccttCGTAGAGG**ATCACCC**TACGtctACATGAGG**ATCACCC**ATGTtctGGTAGAGG**ATCACCC**TACCtctCGTAGAGG**ATCACCC**TACGtctGCAGGAGG**ATCACCC**CTGCtctCGAGGAGG**ATCACCC**CTCGtctGACTGAGG**ATCACCC**AGTCtctGCTAGAGG**ATCACCC**TAGCtctCCTTGA**AGGATCACCCA**AGGGCCTAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGCCAACATGAGG**ATCACCC**ATGTCTGCAGGGCCAAGTGGCACCGAGTCGGTGC**TTTTTTT** 3'

ATCACCCAAGGGCCTAGCAAGTTGAAATAAGGCTAGTCCGTTATCAACTTGGCCAACATGAGGATC
ACCCATGTCTGCAGGGCCAAGTGGCACCGAGTCCGGTGC TTTTTT 3'

Design of CRISPR and Fluorescent Proteins

Split fluorescent proteins were designed by using previously developed split-sfGFP designs (Cabantous et al. 2005, Kim et al. 2011) and further engineering NLS, GB1, MCP, and the SunTag system into the CRISPR system.

GFP(1-9)-GB1-NLS (Annotations: NLS, GFP1-9, GB1)

5' ATG ccaagaagaagcgcaaggtgatgagaaaaaggagaagaacttttactggaggtgccaattctattgaattagatgggatgtaatgggcacaaattttcg
tccgtggagagggtgaaggatgctactaattgaaaactctccctaaatftattgcactactgaaaactactgtccgtggccaactgtcactactctgacatg
gtgtcaatgctttcccgtatccggatcacatgaacggcatgacttttcaagagtgccatcccgaagggtatgtacaggaacgcactatatttcaaatgacggga
cctacaagacgctgctgaagtcaagttgaaggatgataccttgaatcgtatcaggtaaagggtattgatttaagaagatggaacattctggacacaaactggag
tacaacttaactacacaaagtatacatcacggcagacaaacaaacaatggaatcaaaactaacaccattcgcacacggtgaagatggtccgtcaactagcag
accattatcaacaaatactcaattggcgatggccctgtcctttaccaggaggggaggttctgggggagggaggtagtgccggtggtggtcaatcgtggtgagggt
cggaccgaagatcaagcttactctgaacggtaaaacctgaaagggtgaaaccaccaccgaagctgttgacgctgtaccgcggaaaaagtttcaaacgtagccta
acgacaacggtgtgacggtgaatgacctacgacgacgctacaaaacctcacggaaccgaaggtggtgtagcgggtggtgtagtcccaagaagaagcgc
aagggtTAA 3'

NLS-dCas9-GFP10-GB1-NLS (Annotations: dCas9 underlined, NLS, GFP10, GB1)

5' ATG gctagcccaaaaagaagaggaaagtgacaaagaatfctatcggactggccatcgggactaatagcgtcgggtggccgtgatcactgacgagtacaa
ggtgccctetaagaagtcaaggtgctcgggaacaccgaccggcattccatcaaaaaatctgacggagctctcctcttfgattcaggggagaccgctgaagcaacc
gcctcaagcggactgctagacggcgttacaccaggagaaacaccgatttftacttcaagagatattcacaacgaaatggcaaaagctcagcagacagcttctccat
aggctggaagaatcattctcgtggaagagataaagacatgaacggcattcctcgtgtaatactgctgacgaggtggtcctatcacgaaataccaaccatcta
ccatctcgcaaaaagctggtgactcaaccgacaagcagacctccgcttactctcctggccctgcccacatgatcaaggtcagaggccacttctgacgagggc
gacctcaactctgacaatagcgtatgataaactgttcatccagctggtgcagacttacaaaccgctcttgaagagaaccccatcaatgcaagcggagctgatgcaa
ggcattctgtagccggctgtcaaaagccgcagacttgaaatctatcgtcagctgccgggtgaaaaaataatgactgtcgggaacctgattgctcttactt
ggcgtgactcccaattcaagttaatttcgacctggcagaggtatccaagctgcaactgtccaaggacacctatgatgacgatctcacaacctctggccagatcggt
gaccaatagccgaccttctctgctgtaagaatcttctgacgccatctctgctgacattctcggctgaaactgaaatcacaaggccctcttcaactcaatga
ttaagcggatgatgagcaccaccagacctgacctgctaaaggcactcgtccggcagcagctccggagaagatcaagaaatcttcttgaccagcaagaatgga
tacccggctacatcagcggaggtgctcccaagaagaatttataagttatcaaacctatcctgagaagatggacggcaccgaagagctcctctgaaactgaatc
ggagagctgctcggaaagcagcgcacttgcacaatgggagcattcccaccagatccatctggggagcttcacgccatcctcggcgaagaggacttctaccct
ttcttaaggacaacagggagaagattgagaaaattctacttccgcacccctactacgtgggaccctcggcagagaaatagccggttctgcttgatgaccagaaat
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tcgtaatcgaacttcatgcaacttatccatgatgattcctgacctttaaaggagacatccagaagcccaaggtctggtgacaagggtgactcactgcacgagcatatcg
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agatggccagggaaaaccagactaccagaagggacagaagaacagcaggggagcggatgaaagaaattgaggaaggattaaaggagctcgggtcacagatcctta
aagagcaccgggtgaaaaccaccagctcagaatgagaagctctatctgtactacctcaaaatgacgcgatattatgtgaccaagaagcttgatatacaacagcctc
cagactacgacgtgacgccatcgtccctcagagctcctcaaacgactcaattgacaataaggtgctgactcgtcagacaagaaccgggaaaagtcagataacgt

gcctcagaggagtcgtgaaaaagatgaaactatftggcgccagctctgaacgcaaagctgactactcagcggaggttcgacaatctactaaggctgagagggg
cggactgagcgaactggacaaagcaggttcattaaacggcaactgtggagactcggcagattactaaacatgctcccaaatcctgactacgcgatgaataccaagt
acgacgaaaacgacaaacttatcccgaggtgaaagtgattaccctgaagccaagctggtcagcgatttcagaaagacatttcaattctacaaagtgcgggagatcaat
aactatcatcatgctcatgacgcatactgaatgccgtggggaaccgccctgatcaagaagttaccaaaagctgaaaagcgaattcgtgtacggagactacaaggctca
cgactgcgcaagatgattgccaaactgagcagagatcgaaaagccaccgcaaaagtacttctctacagcaacatcatgaatttctcaagaccgaaatcccttgc
aacgggtgagatccggaagggccgctcctcgaactaattggggagactggcgaaatcgtgtgggacaaggcgagagatttcgtaccgtgcaaaagtcttcttat
gcctcaagtgaacatcgtgaagaaaaccgaggtgcaaacccggagcttttcttaaggatcaatcctcccaagcgaactccgacaagctcattgcaaggaaagga
ttggaccctaaagaagtagcgcggttcgattaccaactgtgcttattctgctcgtgctgcttaaggtgaaaaagaaaagctaaagagctcaagagcgtgaagg
aactgctgggatcaccattatggagcgcagctccttcgaagaaccattgacttctcgaagccaagggttacaaggaaagcaagaaggaccttatcatcaagctcc
caaaagtatagcctgttcgaactggagaatggcgcggaagcggatgctcgcctccgctggcgaactcagaagggtaatgagctggctcccctcgaagtagtgaattc
ctctaccttcaagccattacgagaagctgaaagggaagcccgaggacaacagcaaaaacactgtttgtggagcagcataagcattatctgacgagatcattgac
agatttccgatttctaaacgcgtcattctcctgatccaactcgaataagctcttagcgcatacaataagcacagagacaacaaatccggagcagcgtgagaat
catccactgttaccctcacaactctgtgccccctgccgattcaagtagctcgcaccaccatcgaccggaacgctataccctccacaaaagaagtgctgacgccac
ctcatccaccagagcatcaccggaacttacgaaactcgattgacctctcacagctcggaggggagtgagggaagctccaagaaaaagcgaaggtaggtagtccctc
gagatgccaagaaaaagcgaagggtggagggcgtgggtctgggggagggcgtagtgccggtggaggtcaatggacctgcccagcaccactactgtccacc
cagaccatccttccaaggacctgaacgacgttggtggtgaggatcagaaggagggcgtagggggccctggttgggagggggaaggttctgctgggggaggg
agcgtggcggggggtctatgatggtggggcgaccgaaggtacaagcttatctgaacggtaaaccttgaaggtgaaaccaccaccgaagctgttgacct
gctaccgcgaaaaagtttcaaacgtagcgaacgacagctgttgacggtagatggacttacgacgactaccaaaccttccaggtaacgaaagggtggtga
gcgggtggtgtagtccaagaaagcgaagggtTAA 3'

MCP-mCherry-GFP(11)-GB1-NLS (Annotations: MCP underlined, mCherry, NLS, GFP11, GB1)

5' ATGGCTTCTAACTTTACTCAGTTCGTTCTCGTCGACAATGGCGGAACTGGCGACGTGACTGTGCCCC
AAGCAACTTCGCTAACGGGATCGCTGAATGGATCAGCTCTAACTCGCGTTCACAGGCTTACAAAGTAA
CCTGTAGCGTTCGTCAGAGCTCTGCGCAGAATCGCAAATACACCATCAAAGTCGAGGTGCCTAAAGGC
GCCTGGCGTTCGTACTTAAATATGGAACATAACCATTCCAATTTTCGCCACGAATTCGACTGCGAGCTT
ATTGTTAAGGCAATGCAAGGTCTCTAAAAGATGGAAACCCGATTCCCTCAGCAATCGCAGCAAACCT
CGCATCTACGCGGATCCACCTGTTGCTACTctcagagatggtgacgaagggcgaggaggataaacatggccatcatcaaggatcatgcg
cttcaaggtgacacatggaggctccgtgaacggccagagctcgatcaggcgagggcgaggcccccctacgagggcaccagaccgcaagctgaagg
tgaccaagggtggccccctgccctcctgggacatctgtcccctagttcatgtagcgtccaaggtctacgtgaagcaccgacatccccgactactgaag
ctgtcttccccgagggctcaagtgaggcgcgtgatgaacttcaggagcggcggtgacctgaccaggaactcctcctcgaggacggcgagtcatctac
aaggtgaagctgcccggaccaactccccctacgacggcccgtaatgcagaagaagacctgggctgggagcctcctcagagcgtgacccgagacggc
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cgcgctacaacgcaacatcaagttgacatcaccctccacaacaggactacaccatctggaacagtagaacgcccagggccaccctccaccggcgcc
atgacgagctgtaaacaccggtatgctagatgcccaagaagaagcgaaggtggttccggcgtggcggaagccctggggcgggagcggtggtctgtgtt
cttctgtagtggcggtcaacatctATGGAGAAGAGAGACCACATGGTGCTGCTAGAGTACGTGACCGCCGACAGAA
TCACAGACGCtCaggagggcgaggttctggtggaaggcgtagtgccggtgagggtcaatcgatggtggaggtcgaccgaagtagtaacgtttac
ctgaacggtaaacctgaaaggtgaaaccaccaccgaagctgttgcgctcctaccggaaaaagtttcaaacagtagcctaaccgacaacgggtgtgac
ggtgaatggacctacgacgctaccaaaaccttaccgtaaccgaaggtggtgtagcgggtggtgtagtccaagaaagcgaagggtTAA 3'

scFv-GCN4-GFP10-GB1-NLS (Annotations: GCN4 italicized, HA-tag underlined, NLS, GFP10, GB1)

5' ATGgccccgacatcgtgatgaccagagccccagcagcctgagcgccagcgtggggcaccgctgaccatcacctgccgcagcagcaccggcgccgtg
accaccagcaactacgccagctgggtgaggagaagcccggcaagctgttcaagggcctgacggcgccaccaacaaccgcccggcggtgccagccgc
ttcagcggcagcctgacggcgacaagccaccctgaccatcagcagcctgcagcccaggacttcgccactacttctgcccctgtgttacagcaaccactgg
gtftcggccagggcacaaggtgagctgaagcgggcgggcgggcagcgggcgggcgggcagcagcggcgggcgggcagcagcggcgggcggcagcag
gtgaagctgctggagagcggcgggcgccctggtgaccccggcgccagcctgaagctgagctgcggctgagcggcttaccctgaccgactacggcgtgaact
gggtgcaccagggccccggcgccctggagtgatcggtgatctggggcgacggcataccgactacaacagcgcctgaaggaccgcttcatcatcag
caaggacaacggcaagaacaccgtgtacctgcagatgagcaaggtgcgacgacgacaccgctgtactactgctgacggcctgttctgactactggggc
cagggcaccctggtgacctgagcagctaccatacgaatgctccagattacgctggtgagggcgagggttctgggggagggaggtagtgccggtggtggtcaggag

gcggcggaagcttggatccaATGcctaagaaaaagcgcaaggtgggagggcgggtggctctggggagggcggtagtggcggtggaggetcaATGGACCTG
CCCGACGACCACTACCTGTCCACCCAGACCATCCTGTCCAAGGACCTGAACGACGTTGGTGGTGGCGG
ATCAGAAGGAGGCGGTAGCGGGGGCCCTGGTTTCGGGAGGGGAAGGTTCTGCTGGGGGAGGGAGCGCT
GGCGGGGGGTCTatcgatggtggaggtcggaccgaagagtacaagcttacctgaacggtaaacctgaaaggtaaacaccaccgaagctgtgacgc
tgctaccgcgaaaaagtttcaaacagtacgtaacgacaacgggtgtgacggtgaatggacctacgacgacgtacaaaacctcacgtaaccgaaggtggt
agcgggtggtactagtccaagaagaagcgcaagggtTAA 3'

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