

Supplementary information, Data S1 Sequences and material and methods

TALE-Ctrl

1 GTCGACGGATCGGGAGATCTCCCATCCCCTATGGTGCACCTCTCAGTACAATCTGCTCTG
 61 ATGCCGCATAGTTAAGCCAGTATCTGCTCCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGT
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 301 ATATGGAGTTCGCGGTTACATAACTTACGGTAAATGGCCCCGCTGGCTGACCGCCCAACG
 361 ACCCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTT
 421 TCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAG
 481 TGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGGC
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 1921 GGGATTTGGGGTTGCTCTGGAAAACCTATTTGCACCCTGCTGTGCCTTGGAAATGCTAGT
 1981 TGGAGTAATAAATCTCTGGAACAGATTTGGAATCACACGACCTGGATGGAGTGGGACAGA
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5701 CACCAGAGCAAGTAGTGGCTATTGCAAGTAACATCGGTGGCAAACAAGCGCTGGAGAGCA
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5881 ACGCCCCAGCCCTGATCAAGCGGACCAACAGAAGGATTCCCAGAGGACATCACATCGAG
5941 TGGCAGATCACGCGCAAGTGGTCCGCGTGTCTCGGATTCCTCCAGTGTCACTCCCACCCCG
6001 CACAAGCGTTCGATGACGCCATGACTCAATTTGGTATGTGAGACACGGACTGTG CAGC
6061 TCTTTCGTAGAGTCGGTGTACAGAACTCGAGGCCCGCTCGGGCACACTGCCTCCCACCT
6121 CCCAGCGTGGGACAGGATTC TCCAAGCGAGCGGTATGAAACGCGCGAAGCCTTCCACCTA
6181 CGTCAACTCAGACACCTGACCAGGCGAGCCTTCATGCGTTTCGAGACTCGCTGGAGAGGG
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6301 AGAAGAAGAGAAAGGTGGAGGCCAGCGGTTCCGGACGGGCTGACGCATTGGACGATTTTG
6361 ATCTGGATATGCTGGGAAGTGACGCCCTCGATGATTTTGACCTTGACATGCTTGGTTCCG
6421 ATGCCCTTGATGACTTTGACCTCGACATGCTCGGCAGTGACGCCCTTGATGATTTCCGACC
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6961 ACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCG
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 7861 CGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCATC
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 7981 GCAGCTACCAATGCTGATTGTGCCCTGGCTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTT
 8041 CCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGC
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 8161 ATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACA
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 9181 GTTGGTAGCTCTTGTCCGGCAAACAACCACCGCTGAGGTAGCGGTGGTTTTTTTTTTTGC
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 9301 GGGTCTGACGCTCAGTGGAAACGAAAACCTACGTTAAGGGATTTTGGTCATGAGATTATCA
 9361 AAAAGGATCTTCACCTAGATCCTTTTAAATTAATAATGAAGTTTTAAATCAATCTAAAGT
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 9481 GCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCCTGACTCCCCGTCGTGTAGATAACTACG
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 9601 CCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGT
 9661 CCTGCAACTTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGT
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 10141 TCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGA
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 10381 ATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGAC

2622-3276 bp: CMV promoter

3348-4064 bp: TALE N-terminus

4065-5756 bp: TALE repeat array (NI NN NG HD NI NG HD NN HD NG NI NG NG NI HD HD NI)

5757-6290 bp: TALE C-terminus

6291-6332 bp: NLS

6333-6497 bp: VP64

6504-6566 bp: 2A peptide

6567-7274 bp: mCherry

RVD Library Entry Vector

The following sequence was inserted into the TA-cloning site of pMD19-T vector (Takara, Inc.).

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1   TAGCTATACGTCTCATTGACCCCCGAACAGGTTGTAGCCATAGCTTCTAAGTCTTCAGAG
61  ACGCTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTGGCTAGTTAAG
121 CTATCAACAAGTTTGTACAAAAAAGCTGAACGAGAAACGTAAAATGATATAAATATCAAT
181 ATATTTAAATTAGATTTTGCATAAAAAACAGACTACATAATACTGTAAAACACAACATATC
241 CAGTCACTATGAATCAACTACTTAGATGGTATTAGTGACCTGTAGTCGACCGACAGCCTT
301 CCAAATGTTCTTTCGGGTGATGCTGCCAACTTAGTCGACCGACAGCCTTCCAAATGTTCTT
361 CTCAAAACGGAATCGTCGTATCCAGCCTACTCGCTATTGTCTCAATGCCGTATTAAATCA
421 TAAAAAGAAAATAAGAAAAAGAGGTGCGAGCCTCTTTTTTGTGTGACAAAATAAAAAACATC
481 TACCTATTTCATATACGCTAGTGTATAGTCTGAAAATCATCTGCATCAAGAACAATTTTC
541 ACAACTCTTATACTTTTCTCTTACAAGTCGTTTCGGCTTCATCTGGATTTTTCAGCCTCTAT
601 ACTTACTAAACGTGATAAAGTTTCTGTAATTTCTACTGTATCGACCTGCAGACTGGCTGT
661 GTATAAGGGAGCCTGACATTTATATTTCCCGAGAATCAGGTTAATGGCGTTTTTGTATGT
721 CATTTTTCGCGGTGGCTGAGATCAGCCACTTCTTCCCGATAACGGAGACCGGCACACTGG
781 CCATATCGGTGGTCATCATGCGCCAGCTTTCATCCCCGATATGCACCACCGGGTAAAGTT
841 CACGGGAGACTTTTATCTGACAGCAGACGTGCACTGGCCAGGGGGATCACCATCCGTCGCC
901 CGGGCGTGTCAATAATATCACTCTGTACATCCACAAACAGACGATAACGGCTCTCTCTTT
961 TATAGGTGTA AACCTTAAACTGCATTTTACCAGCCCCGTTCCTCGTCAGCAAAAAGAGCCG
1021 TTCATTTCAATAAACCGGGGACCTCAGCCATCCCTTCCCTGATTTTCCGCTTTCCAGCGT
1081 TCGGCACGCAGACGACGGGCTTTCATTTCTGCATGGTTGTGCTTACCAGACCGGAGATATTG
1141 ACATCATATATGCCTTGAGCAACTGATAGCTGTGCTGTCAACTGTCACTGTAATACGCT
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1261 ACCGCAAAAATCAGCGCGCAAATACGCATACTGTTATCTGGCTTTTAGTAAGCCGGATCC
1321 ACGCGGCGTTTACGCCCCCTGCCACTCATCGCAGTACTGTTGTAATTCATTAAGCATT
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1441 ACCTTGTCGCCTTGCGTATAATATTTGCCCATGGTGAAAACGGGGGCGAAGAAGTTGTCC
1501 ATATTGGCCACGTTTAAATCAAACCTGGTGAAACTCACCCAGGGATTGGCTGAGACGAAA
1561 AACATATTCTCAATAAACCCCTTAGGGAAATAGGCCAGGTTTTTACCAGTAACACGCCACA
1621 TCTTGCGAATATATGTGTAGAAACTGCCGAAATCGTCGTGGTATTCACTCCAGAGCGAT
1681 GAAAACGTTTCAGTTTGCATGGAACGGTGTAAACAAGGGTGAACACTATCCCATATC
1741 ACCAGCTCACCGTCTTTTCAATGCCATACGGAATTCGGGATGAGCATTTCATCAGGCGGCA
1801 AGAATGTGAATAAAGCCGGATAAAAACCTTGTGCTTATTTTTCTTTACGGTCTTTAAAAAG
1861 GCCGTAATATCCAGCTGAACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAATGCC
1921 TCAAAAATGTTCTTTACGATGCCATTTGGGATATATCAACGGTGGTATATCCAGTGATTTTT
1981 TTCTCCATTTTAGCTTCCCTTAGCTCCTGAAAATCTCGATAACTCAAAAAATACGCCCGGT
2041 AGTGATCTTATTTTCAATATGGTGAAAGTTGGAACCTCTTACGTGCCGATCAACGTCTCAT
2101 TTTCGCCAAAAGTTGGCCCAGGGCTTCCCGGTATCAACAGGGACACCAGGATTTATTTAT
2161 TCTGCGAAGTGATCTTCCGTACAGGTATTTATTCGGCGCAAAGTGCGTCGGGTGATGCT
2221 GCCAACTTAGTCGACTACAGGTCACTAATACCATCTAAGTAGTTGATTCATAGTGACTGG
2281 ATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTAATATA
2341 TTGATATTTATATCATTTTACGTTTCTCGTTCAGCTTCTTGTACAAAGTGTTGATCTA
2401 GAGGGCCCCGGTTCCGACGTCCTGAAGACAAGGAGGTAAGCAGGCACTGGAACCGTG
2461 CAGCGCCTGCTCCAGTACTGTGTGTCAGGCTCATGGGTGAGACGTATAGCTA

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16-48 bp: N-terminus of the TALE repeats

57-2425 bp: ccdB cassette

2434-2496 bp: C-terminus of the TALE repeats

RVD Library Vector

1 GTCGACGGATCGGGAGATCTCCCGATCCCCATGGTGCACCTCTCAGTACAATCTGCTCTG
61 ATGCCGCATAGTTAAGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGT
121 GCGCGAGCAAAATTTAAGCTACAACAAGGCAAGGCTTGACCCGACAATTGCATGAAGAATC
181 TGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATGTACGGGCCAGATATACGCGTTGAC
241 ATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCAT
301 ATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACG
361 ACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGGACTT
421 TCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAG
481 TGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGC
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841 CTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTT
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961 TCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTGGC
1021 GCCCGAACAGGGACTTGAAAGCGAAAGGGAAACCAGAGGAGCTCTCTCGACGCAGGACTC
1081 GGCTTGTGTAAGCGCGCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACGCCAAAAA
1141 TTTTGACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGTCAGTATTAAGCGGG
1201 GGAGAAATTAGATCGCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAAGAAAAAATATA
1261 AATTAAAACATATAGTATGGGCAAGCAGGGAGCTAGAACGATTCGCAGTTAATCCTGGCC
1321 TGTTAGAAACATCAGAAGGCTGTAGACAATACTGGGACAGCTACAACCATCCCTTCAGA
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2101 GAAAAGAATGAACAAGAATTATTGGAATTAGATAAATGGGCAAGTTTGTGGAATTTGGTTT
2161 AACATAACAAAATTTGGCTGTGGTATATAAAAATTAATCATAATGATAGTAGGAGGTTGGTA
2221 GGTTTAAGAATAGTTTTTGTGCTGACTTTCTATAGTGAATAGAGTTAGGCAGGGATATTCA
2281 CCATTATCGTTTTAGACCCACCTCCCAACCCGAGGGGACCCGACAGGCCCGAAGGAATA
2341 GAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTCGATTAGTGAACGGATCGGCA
2401 CTGCGTGCGCCAATTCCTGCAGACAAATGGCAGTATTCATCCACAATTTTAAAAGAAAAGG
2461 GGGGATTTGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACA
2521 AACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTCGGGTTTTATTACAGGGA
2581 CAGCAGAGATCCAGTTTGGTTAGTACCGGGCCCGCTCTAGACGATGTACGGGCCAGATAT
2641 ACGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTT
2701 CATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGA
2761 CCGCCCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCA
2821 ATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCA
2881 GTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGG
2941 CCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCCTACTTGGCAGTACATC
3001 TACGTATTAGTCATCGCTATTACCATGGTGTATGCGGTTTTGGCAGTACATCAATGGGCGT
3061 GGATAGCGGTTTACTCACGGGGATTTCCAAGTCTCCACCCCATTTGACGTCAATGGGAGT
3121 TTGTTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCATTTG
3181 ACGCAAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCATATAAGCAGAGCTCTCTGGCTA
3241 ACTAGAGAACCCTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACC
3301 CAAGCTGGCTAGCGAAGTTCCATTCTCTAGAAAAGTATAGGAACTTCATGAGGACCAGGC
3361 TGCCATCTCCCCCTGCCCTTCCCCCGCATTTAGCGCTGGGAGCTTTAGCGACCTGCTTA
3421 GGCAGTTTCGACCCAGCTTGTTCACACCAGCCTGTTTACAGCCTGCCTCCCTTCGGAG
3481 CGCACCACACCGAAGCCGCCACCGGCGAGTGGGACGAGGTGCAAAGCGGCCCTGAGGGCAG
3541 CGGACGCTCCTCCGCCAACCATGAGGGTGGCAGTGACAGCAGCTAGGCCCCCTCGGGCAA

3601 AACCTGCACCCAGGAGAAGGGCTGCCAACCCAGCGACGCGAGTCCAGCCGCACAGGTGG
3661 ACCTCAGGACGCTGGGCTACAGCCAGCAACAGCAAGAGAAGATCAAGCCCAAAGTAAGGA
3721 GCACCGTGGCCAGCACCACGAGGCCCTGGTGGGTACGGCTTACCCACGCGCATATCG
3781 TTGCTCTGAGCCAACATCCCGCAGCTCTGGGTACCCTTGGCGTGAAGTATCAGGACATGA
3841 TCGCGGCACTGCCTGAAGCTACACACGAAGCCATAGTGGGCGTGGCAAGCAGTGGAGCG
3901 GTGCCAGAGCGCTTGAGGCACTGTGACGGTGGCTGGCGAGCTGAGGGGACCGCCACTGC
3961 AACTGGACACCGCCAACCTGCTGAAGATCGCCAAGAGGGGAGGCGTGACGGCGGTGGAGG
4021 CCGTGCATGCCCTGGAGGAATGCCCTGACCGGCGGCCCTGAACAGAGACGCTGGCTTAT
4081 CGAAATTAATACGACTCACATATAGGGAGACCCAAGCTGGCTAGTTAAGCTATCAACAAGT
4141 TTGTACAAAAAGCTGAACGAGAAACGTAAAATGATATAAATATCAATATATTAAATTAG
4201 ATTTTGCATAAAAAACAGACTACATAATACTGTA AAAACACAACATATCCAGTCACTATGA
4261 ATCAACTACTTAGATGGTATTAGTGACCTGTAGTTCGACCGACAGCCTTCCAAATGTTCTT
4321 CGGGTGATGCTGCCAACTTAGTTCGACCGACAGCCTTCCAAATGTTCTTCTCAAACGGAAT
4381 CGTCGTATCCAGCCTACTCGCTATTGTCTCAATGCCGTATTAATCATAAAAAAGAAATA
4441 AGAAAAAGAGGTGCGAGCCTCTTTTTTGTGTGACAAAATAAAAAACATCTACCTATTCTATA
4501 TACGCTAGTGTCTATAGTCCTGAAAATCATCTGCATCAAGAACAATTTCACTACTTTATA
4561 CTTTTCTCTTACAAGTCTTCCGGCTTCATCTGGATTTTTCAGCCTCTATACTTACTAAACG
4621 TGATAAAGTTTCTGTAATTTCTACTGTATCGACCTGCAGACTGGCTGTGTATAAGGGAGC
4681 CTGACATTTATATTTCCCCAGAACATCAGGTTAATGGCGTTTTTGATGTCATTTTCGCGGT
4741 GGCTGAGATCAGCCACTTCTTCCCGGATAACGGGAGACCGGCACACTGGCCATATCGGTGG
4801 TCATCATGCGCCAGCTTTCATCCCGATATGCACCACCGGGTAAAGTTCACGGGAGACTT
4861 TATCTGACAGCAGACGTGCACTGGCCAGGGGGATCACCATCCGTCGCCCGGGCGTGTCAA
4921 TAATATCACTCTGTACATCCACAAACAGACGATAACGGCTCTCTCTTTTTATAGGTGTAAA
4981 CCTTAAACTGCATTTACCAGCCCTGTTCTCGTCAGCAAAAGAGCCGTTTCAATTTCAATA
5041 AACCGGGCGACCTCAGCCATCCCTTCCGTATTTCCGCTTTCCAGCGTTCGGCACGCAGA
5101 CGACGGGCTTCACTTGCATGGTGTGCTTACCAGACCGGAGATATTGACATCATATATG
5161 CCTTGAGCAACTGATAGCTGTGCTGTCAACTGTCACTGTAATACGCTGCTTCATAGCAT
5221 ACCTCTTTTTGACATACTTCGGGTATACATATCAGTATATATTCTTATACCGCAAAAATC
5281 AGCGCGCAAATACGCATACTGTTATCTGGCTTTTAGTAAGCCGGATCCACGCGGGCTTTA
5341 CGCCCCCTGCCACTCATCGCAGTACTGTTGTAATTCATTAAGCATTCTGCCGACATGG
5401 AAGCCATCACAAACGGCATGATGAACCTGAATCGCCAGCGGCATCAGCACCTTGTGCGCT
5461 TGCGTATAAATTTGCCCATGGTGA AAACGGGGGCGAAGAAGTTGTCCATATTGGCCACG
5521 TTTAAATCAAACCTGGTGA AACCTCACCAGGGATTGGCTGAGACGAAAAACATTTCTCA
5581 AATAACCTTTTAGGAAATAGGCCAGGTTTTTACCCTAACACGCCACATCTTGCAATAT
5641 ATGTGTAGAACTGCCGAAATCGTCTGGTATTCACTCCAGAGCGATGAAAACGTTTCA
5701 GTTTGCTCATGGAAAACGGTGTAAACAAGGGTGAACACTATCCCATATCACCAGCTCACC
5761 TCTTTCATTGCCATACGGAATTCGGGATGAGCATTCATCAGGCGGCAAGAATGTGAATA
5821 AAGCCGGATAAAACTTGTGCTTATTTTTCTTTACGGTCTTTAAAAAGGCCGTAATATCC
5881 AGCTGAACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAATGCCTCAAATGTTCT
5941 TTACGATGCCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTTCTCCATTTTA
6001 GCTTCCCTAGCTCCTGAAAATCTCGATAACTCAAAAATACGCCCGGTAGTGATCTTATT
6061 TCATTTATGGTGAAGTTGGAACCTCTTACGTGCCGATCAACGTCTCATTTTCGCCAAAAG
6121 TTGGCCAGGGCTTCCCGGTATCAACAGGGACACCAGGATTTATTTATTCTGCGAAGTGA
6181 TCTTCCGTACAGGTATTTATTCGGCGCAAAGTGCCTCGGGTGTGCTGCCAAGTTGATG
6241 GACTACAGTCACTAATACCATCTAAGTAGTTGATTCATAGTGACTGGATATGTTGTGTT
6301 TTACAGTATTATGTAGTCTGTTTTTTTATGCAAAAATCTAATTTAATATATTGATATTTATA
6361 TCATTTTACGTTTCTCGTTCAGCTTTCTTGTACAAAGTGGTTGATCTAGAGGGCCCGCG
6421 TTCGAACGCTCTTAGCATCGTGGCCAGCTGTCTCGGCCCGACCCTGCCCTCGCCGCTCT
6481 GACCAACGACCACCTGGTGGCCCTGGCTTGCCCTCGGGGCGAGCCAGCTCTTGACGCCGT
6541 GAAGAAGGGCTTCCCTACGCCCCAGCCCTGATCAAGCGGACCAACAGAAGGATTTCCCGA
6601 GAGGACATCACATCGAGTGGCAGATCACGCGCAAGTGGTCCGCGTGCTCGGATTTCCA
6661 GTGTCATCCCACCCCGCACAAAGCTTCGATGACGCCATGACTCAATTTGGTATGTGCGAG
6721 ACACGGACTGCTGCAGCTCTTTTCGTAGAGTCCGGTGTACAGAACTCGAGGCCCGCTCGGG
6781 CACACTGCCCTCCCGCTCCAGCGGTGGGACAGGATTTCTCCAAGCGAGCGGTATGAAACG
6841 CGCGAAGCCTTACCCTACGTCAACTCAGACACCTGACCAGGCGAGCCTTCATGCGTTCCG
6901 AGACTCGCTGGAGAGGGATTTGGACGCGCCCTCGCCCATGCATGAAGGGGACCAAACTCG
6961 CGCGTACGATGCCCAAAGAAGAAGGAGGAGGAGGCGGTTCCGGACGGGCTGAC
7021 CGCATTTGACGATTTTGTATCTGGATATGCTGGGAAGTGCAGCCCTCGATGATTTTGACCT
7081 TGACATGCTTGGTTCCGATGCCCTTGATGACTTTGACCTCGACATGCTCGGCAGTGACGC
7141 CCTTGATGATTTTCGACCTGGACATGCTGATTAACCTTAGAGGCAGTGGAGAGGGCAGAGG
7201 AAGTCTGCTAACATGCGGTGACGTCGAGGAGAATCCTGGCCAGTGAGCAAGGGCGAGGA
7261 GGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGT
7321 GAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCTACGAGGGCACCCA

7381 GACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTG
7441 CCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTA
7501 CTTGAAGCTGTCCCTCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGG
7561 CGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGT
7621 GAAGCTGCGCGGCACCAACTTCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGG
7681 CTGGGAGGCCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAA
7741 GCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAA
7801 GGCCAAGAAGCCCGTGCAGCTGCCCGCGCCCTACAACGTCAACATCAAGTTGGACATCAC
7861 CCCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTC
7921 CACCGGCGGCATGGACGAGCTGTACAAGTAACATGTTTAAGGGTTCGGTTCCTACTAGGT
7981 ACAATTTCGATATCAAGCTTATCGATAATCAACCTCTGGATTACAAAATTTGTGAAAGATT
8041 GACTGGTATCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCC
8101 TTTGTATCATGCTATTTGCTTCCCGTATGGCTTTCATTTTCTCCTTGTATAAATCCTG
8161 GTTGTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCAC
8221 TGTGTTTGTCTGACGCAACCCCACTGGTGGGGCATTGCCACCACCTGTGAGCTCCTTTC
8281 CGGGACTTTCGCTTTCCCCCCTCCCTATTGCCACGGCGGAACCTATCGCCGCCTGCCTTG
8341 CCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCGGTGGTGTGTCGGGGAA
8401 ATCATCGTCCCTTCCCTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTC
8461 CTTCGTCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCCTCCCGCGGCCTGCTGCC
8521 GGCTCTGCGCCCTTCCGCGTCTTCGCTTCGCTTCGCTCAGACGAGTCCGATCTCCCTTG
8581 GGCCGCTCCCGCTCATGATACCGTCCGACCTCGATCGAGACCTAGAAAAACATGGAGCAA
8641 TCACAAGTAGCAATACAGCAGCTACCAATGCTGATTGTGCCTGGCTAGAAGCACAAGAGG
8701 AGGAGGAGGTGGGTTTTCCAGTACACCTCAGGTACCTTTAAGACCAATGACTTACAAGG
8761 CAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGGGACTGGAAGGGCTAATTCCT
8821 CCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTACTTCCCTG
8881 ATTTGGCAGAACTACACACCAGGGCCAGGGATCAGATATCCACTGACCTTTGGATGGTGT
8941 ACAAGCTAGTACCAGTTGAGCAAGAGAAGGTAGAAGAAGCCAATGAAGGAGAGAACACCC
9001 GCTTGTACACCTGTGAGCCTGCATGGGATGGATGACCCGGAGAGAGAAGTATTAGAGT
9061 GGAGGTTTGACAGCCGCCTAGCATTTTCATCACATGGCCCCGAGAGCTGCATCCGGACTGTA
9121 CTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACC
9181 CACTGCTTAAGCCTCAATAAAGCTTGCCCTGAGTGTCTCAAGTAGTGTGTGCCCGTCTGT
9241 TGTGTGACTCTGGTAACTAGAGATCCCTCAGACCTTTTAGTCAGTGTGGAAAATCTCTA
9301 GCAGCATCTGAGCAAAAAGGCCAGCAAAAAGGCCAGGAACCGTAAAAAGGCCCGCTGTG
9361 CGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGA
9421 GGTGGCGAAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCCCTGGAAGCTCCCTCG
9481 TGCGCTCTCCTGTTCCGACCCCTGCCGCTTACCAGGATACCTGTCCGCTTTTCTCCCTTCGG
9541 GAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTT
9601 GCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCCGACCGCTGCGCCTTATCCG
9661 GTAACATATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCA
9721 CTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGT
9781 GGCCTAACACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAG
9841 TTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCG
9901 GTGGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATC
9961 CTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTT
10021 TGGTCATGAGATTATCAAAAAGGATCTTCCACTAGATCCTTTTAAATTAAAAATGAAGTT
10081 TTAAATCAATCTAAAGTATATATAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCA
10141 GTGAGGCACCTATCTCAGCGATCTGTCTATTTGTTTCATCCATAGTTGCCTGACTCCCCG
10201 TCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATAC
10261 CGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGG
10321 CCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCC
10381 GGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTA
10441 CAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCATTTCAGCTCCGTTCCCAAC
10501 GATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTC
10561 CTCCGATCGTTGTGCAAGTAAGTTGGCCGCACTGTTATCACTCATGGTTATGGCAGCAC
10621 TGCATAATTCCTTACTGTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGTGACTACT
10681 CAACCAAGTCATCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGGCCGGCGCTCAA
10741 TACGGGATAAATACCGGCCACATAGCAGAACTTTAAAAGTGTCTCATCTTGGAAAACGTT
10801 CTTCCGGGCGAAAACTCAAGGATCTTACCCTGTTGAGATCCAGTTTCGATGTAACCCA
10861 CTCGTGCACCCAACTGATCTTACGATCTTTTACTTTTACCAGCGTTTTTGGGTGAGCAA
10921 AAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAATGTTGAATAC
10981 TCATACTCTTCTTTTCAATATTTATGAAGCATTTATCAGGGTTATTGTCTCATGAGCG
11041 GATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCC
11101 GAAAAGTGCCACCTGAC

2622-3276 bp: CMV promoter
3348-4064 bp: TALE N-terminus
4065-6433 bp: ccdB cassette
6434-6967 bp: TALE C-terminus
6968-7009 bp: NLS
7010-7174 bp: VP64
7181-7243 bp: 2A peptide
7244-7951 bp: mCherry

SUPPLEMENTARY METHODS

Artificial system for RVD screening

The artificial screening system was composed of four reporters and a TALE-VP64 expression library in which the RVDs of three consecutive monomers in the middle of an artificial TALE array were encoded by the same 6 randomly synthesized nucleotides (TALE-(XX')₃). TALE-(XX')₃ contained 14.5 repeats fused with the VP64 trans-activation domain and 2A peptide-linked mCherry. The variable diresidues (XX') for testing were placed in the 7th - 9th repeat modules, and the variable RVD-carrying TALE modules were purposely designed as triplets to augment the DNA binding capability. X and X' represents the 12th and 13th amino acids in the 7th - 9th repeat modules, respectively. Four reporters consist of TALE-(XX')₃ binding sites CTGGCCNNNTACGTA, in which N represents A, T, C or G, was located immediately upstream of a minimal CMV promoter (P_{minCMV}) and its downstream EGFP gene. TALE-Ctrl was constructed to have the identical backbone as TALE-(XX')₃ except that its TALE repeats (16.5-mers) are different, not matching with any reporters. The mCherry-normalized EGFP level of TALE-Ctrl co-transfected with reporter served as the corresponding basal level. For each sample, EGFP fluorescence intensity was normalized to mCherry intensity. Fold induction is calculated as the result of normalized sample EGFP intensity divided by normalized basal level shown as follows.

$$\text{Fold Induction} = \frac{(\prod_{i=1}^n \text{Exp}_{\text{EGFP}_i})^{1/n}}{(\prod_{i=1}^n \text{Ctrl}_{\text{EGFP}_i})^{1/n}} \bigg/ \frac{(\prod_{i=1}^n \text{Exp}_{\text{mCherry}_i})^{1/n}}{(\prod_{i=1}^n \text{Ctrl}_{\text{mCherry}_i})^{1/n}}$$

Notes:

$(\prod_{i=1}^n \text{Exp}_{\text{EGFP}_i})^{1/n}$: Geometric mean of Exp_{EGFP} of cells from FACS (n = number of cells)

Exp_{EGFP} : EGFP intensity of HEK293T cells co-transfected with TALE-(XX')_m plus reporter (m = 3, 6 and 12, corresponding to TALE-(XX')₃, TALE-(XX')₆ and TALE-(XX')₁₂, respectively)

$\text{Exp}_{\text{mCherry}}$: mCherry intensity of HEK293T cells co-transfected with TALE-(XX')_m plus reporter

$\text{Ctrl}_{\text{EGFP}}$: EGFP intensity of HEK293T cells co-transfected with TALE-Ctrl plus reporter

$\text{Ctrl}_{\text{mCherry}}$: mCherry intensity of HEK293T cells co-transfected with TALE-Ctrl plus reporter

Construction of the TALE-(XX')₃ library

A 102-nt monomer encoding a standard TALE repeat unit was synthesized with the following sequences: 5'-GCTATGGCTACAACCTGTTTCGGGGGTCAACCCATGAGCCT GACACAGTACTGGGAGCAGGCGCTGCACGGTTTCCAGTGCCTGCTTACCTCCNNNN NNAGAA-3'. The six random nucleotides (underlined Ns) corresponding to the RVD-encoding region were purposely placed near the 3' end to ensure unbiased oligonucleotide synthesis. This single-stranded DNA was cyclized using linker primer 1 (5'-GAACAGGTT GTAGCCATAGCTTCT-3') and T4 DNA ligase (NEB). Using the agarose gel-purified single stranded circular DNA as template, rolling-circle amplification was conducted with phi29 DNA polymerase (NEB) and primer 1 (30°C, 90 min) followed by primer extension using primer 2 (5'-AGGTTGTAGCCATAGCT-3') and phi29 (30°C, 90 min) to acquire long dsDNA. After ultrasonic shearing (270 W, work 10 s, pause 10 s, 10 cycles) and T4 DNA polymerase (NEB) treatment to blunt the ends of the DNA fragments (12°C, 15 min, with 400 μM dNTP mix), 250-400 bp DNA fragments were harvested by gel purification. These DNA fragments were then cloned into a pre-made entry vector using the ligase-independent cloning (LIC) method to create an entry library. BsmBI digestion of entry

library clones produced ~300 bp DNA fragments that were subsequently cloned, via the Golden Gate approach¹, into a pre-made RVD library vector, which was constructed using the ULtiMATE protocol previously developed by our group². Each plasmid in the final RVD library was verified through sequencing analysis. About 350 kinds of TALE-(XX')₃ constructs were obtained from this approach.

Individual construction of TALE-(XX')₃

A complementary primer (5'-aaCGTCTCaGTTTCGGGGGTCAACCCATGAGCCTGACACAGTACTGGGAGCAGGGCGCTGCACGGTTTCCAGTGCCTGCTT-3') and a specific primer (5'-tCGTCTCaGAACAGGTTGTAGCCATAGCTTCTNNNNNNGGAGGTAAGCAGGCACTGGAA-3'; NNNNNN indicates the RVD codons) were annealed and PCR extended to generate a 102 bp monomer with BsmBI sites at each end. The monomer was ligated via a 6 cycles of the Golden Gate method¹ to generate repeats. The repeat product was PCR amplified using the primers G-lib-F (5'-TAGCTATACGTCTCATTGACCCCCGAACAGGTTGTAGCC-3') and G-lib-R (5'-TAGCTATACGTCTCACCCATGAGCCTGACACAGTACTGGGAGCA-3') and Taq Hifi (Transgen, Inc.). The 3-repeat fragment was gel purified and subsequently cloned into a pre-made RVD library vector via the Golden Gate method¹. Trans1-T1 competent cells (Transgen, Inc.) were used for bacterial transformation. About 50 kinds of TALE-(XX')₃ constructs were obtained from this approach.

Design and construction of TALE-(XX')₆ and TALE-(XX')₁₂

For TALE-(XX')₆, the customized TALEs (17.5-mer) are the same as TALE-(XX')₃ except that there are six identical repeats containing the variable RVDs. Accordingly, four reporters were constructed, consisting of TALE-(XX')₆ binding sites with six consecutive nucleotides (A, T, C or G) substituted at positions 7 - 12 in front of a minimal CMV promoter and its downstream EGFP gene. The 7th - 9th repeats in TALE-(XX')₃ were PCR

amplified using the primers G-lib-seq-F (5'-TCTAGGTACCAAGCCCACGGATTGA-3') and G-lib-seq-R (5'-ATCGATCGTCCGGAGTGAGCCCA-3'). The 300 bp PCR product was purified and further PCR amplified using the primers G-lib-F and G-lib-R. The product of the 6-repeat fragment was gel purified and cloned into a pre-made RVD library vector via the Golden Gate method¹ to construct the final TALE-(XX')₆ plasmid.

For TALE-(XX')₁₂, the customized TALEs (15.5-mer) contained four ACTC-targeting TALE repeat modules followed by 11.5 repeat units with variable RVDs to be tested. Accordingly, four reporters were constructed, consisting of TALE-(XX')₁₂ binding sites with 12 consecutive nucleotides (A, T, C or G) substituted at positions 5 - 16 in front of a minimal CMV promoter and its downstream EGFP gene. TALE-(XX')₁₂ plasmids were constructed with a similar method using TALE-(XX')₆ as a PCR template and applying the ULtiMATE protocol².

Backbone plasmid	10 ng
6-repeat fragment	5 ng
10X Tango buffer (Thermo)	1 µl
10 mM ATP	1 µl
BsmBI (Thermo)	0.75 µl
T4 ligase (NEB)	0.25 µl
ddH ₂ O	up to 10 µl

37°C	5 min	\
		10 cycles
16°C	5 min	/
37°C	5 min	

Cell culture, transfection and flow cytometric analysis

HEK293T cells (from Stanley Cohen lab at Stanford University) were cultured in DMEM medium with 10% FBS and 1% penicillin-streptomycin at 37°C and 5% CO₂. Cells were

seeded 24 h prior to transfection in 24-well plates at a density of 10^5 cells per well. The cells in each well were co-transfected with 0.2 μg TALE-(XX')₃ plasmid and 0.4 μg reporter plasmid using polyethylenimine (PEI)³. At 48 h post-transfection, the cells were collected and analyzed on a BD LSR II flow cytometer (BD Biosciences). Lasers with wavelengths of 488 nm and 561 nm were used to quantify EGFP and mCherry protein expression, respectively. At least 10,000 events were collected from every sample to obtain sufficient data for analysis. Majority of cells showed mCherry fluorescence intensity of 5×10^4 – 5×10^5 , thus were gated for further analysis. The binding efficiencies and specificities of the variable RVDs (XX') in each customized TALE were assayed by comparing the fold induction of EGFP reporters with the basal level of EGFP in HEK293T cells transfected with the reporter plasmid and a customized TALE plasmid containing unmatched TALE repeats (Supplementary Sequences). The EGFP fluorescence intensity assayed from FACS analysis was normalized to the corresponding mCherry fluorescence intensity.

Generation of a heat map illustrating the base-preference of RVDs

The heat map was generated from library screening of TALE-(XX')₃ using four reporters (3A, 3T, 3C, and 3G), thereby reflecting the base preference of 400 RVDs. EGFP activities from different reporters are coded by different colors representing the reporter identities (3A, green; 3T, red; 3C, blue; and 3G, yellow). The brightness of the colors indicates the fold induction of the reporters by TALE-(XX')₃ compared to their basal levels.

Criteria for selection of novel RVDs from TALE-(XX')₃ for intensive study

The standards are as follows: (1) the highest fold induction among four reporters is at least equal to NK for G recognition; and (2) the second highest fold induction is lower than half of the highest one. In addition to these simple rules, we have also included groups of

RVDs that displayed some unique patterns, i.e. seven RVDs ended with Ala (KA, CA, FA, YA, RA, PA, and AA) that showed preference for T-recognition, and RVDs recognizing both A and G (NN and HN).

Statistical analysis

For comparison between the induction level of TALE-(XX')₁₂ and the basal level (Fig. 1f), two-sample, one-tailed t-test was performed assuming unequal variance. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.005$.

Construction of TALENs

TALENs with natural RVDs (i.e. NI, NG, HD and NN) were constructed using ULtiMATE system as previously described². For TALEN repeats using novel RVDs KN (in place of NN), NH (in place of NN) or RG (in place of NG), TALE monomers containing new RVDs were individually synthesized. The final assembly of these TALENs constructs was conducted using the same ULtiMATE protocol as above.

Assessment of TALENs-mediated indels

HEK293T cells were seeded in 6-well plates at a density of 3×10^5 cells per well and incubated at 37°C with 5% CO₂. For each well, a pair of TALEN plasmids and pmaxGFP (Lonza Group Ltd.) were co-transfected at a ratio of 9:9:2 (0.9 µg : 0.9 µg : 0.2 µg) using PEI method. The transfected cells were cultured at 37°C for one day followed by 3 days of incubation at 30°C (cold shock) before flow cytometric sorting for GFP positive cells. TALENs-targeting regions were PCR-amplified from the genome DNA of the isolated GFP positive cells. The TALENs-mediated indels were analyzed by mismatch-sensitive T7 endonuclease I (T7E1; New England Biolabs) as described previously⁴.