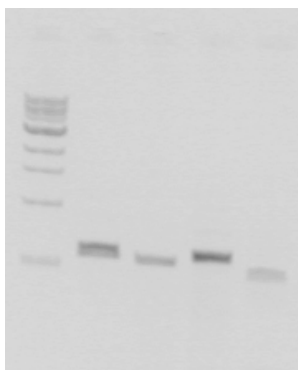


Supplementary Material

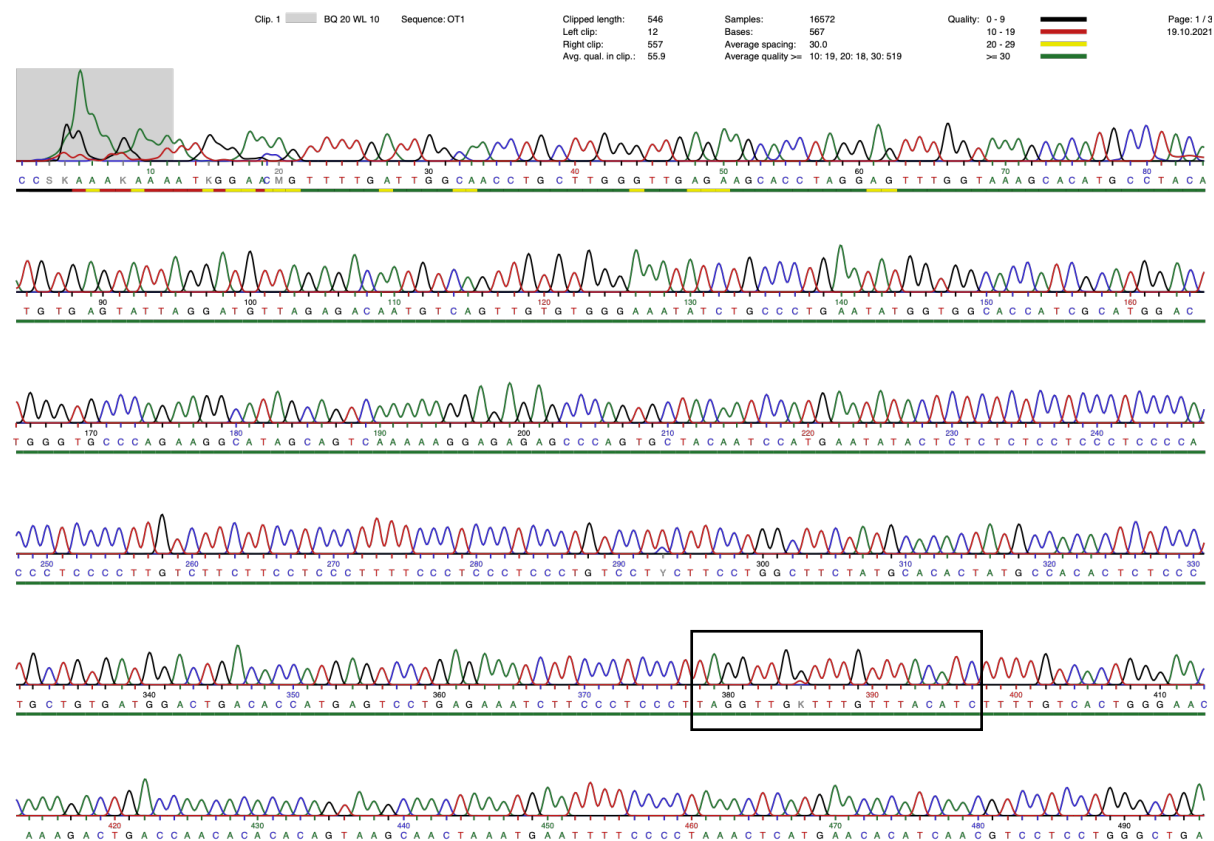
Figure S1. CRISPR Off-Target analysis.



Gel image showing genomic PCR to isolate off-target sequences. Primer sequences are listed in Supplementary Table S2.

Lane 1: 1Kb DNA ladder, Lane 2: Off-Target 1, Lane 2: Off-Target 2, Lane 4: Off-Target 3, Lane 5: Off-Target 13.

Off-Target 1

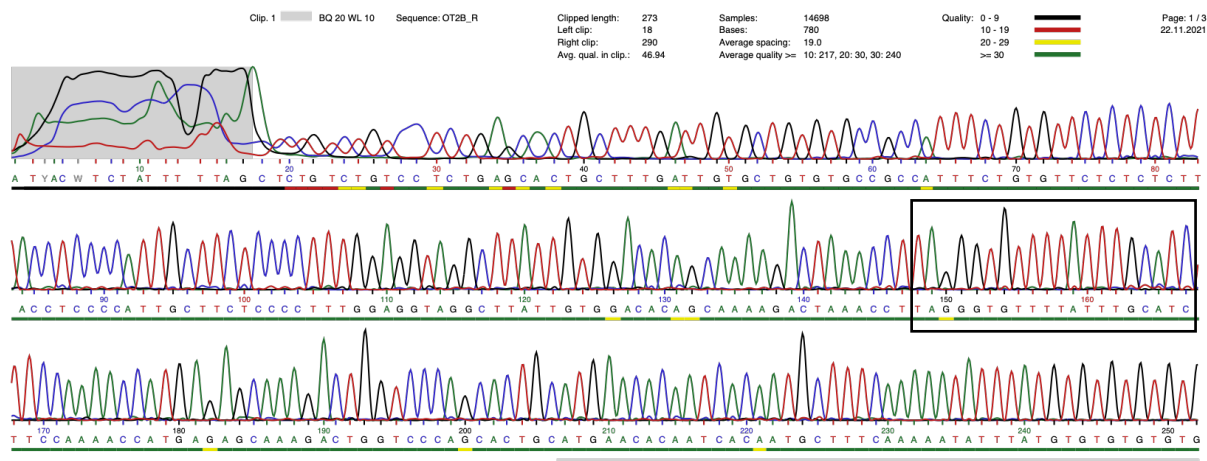


Sequencing results for Off-Target 1 on Chr14. Framed is the sequence that can be recognized by Cas9.

Query	224	CCCACcctccccctgtctcttcttctcccttttccctccctccctgtcctycttccctGGCT	283
Sbjct	77196196	CCCACCCTCCCCCTGTCTTCTTCTCCTCTTTCCCTCCCTCCCTGTCCTCCTTCTCTGGCT	77196255
Query	284	TCTATGCACACTATGCCCACTCTCCCTGCTGTGATGGACTGACACCATGAGTCCTGAGA	343
Sbjct	77196256	TCTATGCACACTATGCCCACTCTCCCTGCTGTGATGGACTGACACCATGAGTCCTGAGA	77196315
Query	344	AATCTTCCCTCCCCTAGGTTGKTTTGTGTTACATCTTTTGTCACTGGGAACAAAGACTGAC	403
Sbjct	77196316	AATCTTCCCTCCCCTAGGTTGTTTGTGTTACATCTTTTGTCACTGGGAACAAAGACTGAC	77196375
Query	404	CAACACACACAGTAAGCAACTAAATGAATTTCCCTAAACTCATGAACACATCAACGTC	463
Sbjct	77196376	CAACACACACAGTAAGCAACTAAATGAATTTCCCTAAACTCATGAACACATCAACGTC	77196435
Query	464	CTCCTGGGCTGAGTACCAGCGAAACTCAAAGGGAAGCATGCGCTCTTGAATCATAAGTG	523
Sbjct	77196436	CTCCTGGGCTGAGTACCAGCGAAACTCAAAGGGAAGCATGCGCTCTTGAATCATAAGTG	77196495
Query	524	TGGAAAT	530
Sbjct	77196496	TGGAAAT	77196502

Alignment of Off-Target 1 sequencing results with genomic DNA. Framed is the sequence that can be recognized by Cas9. The two sequences are identical. K is a known polymorphism.

Off-Target 2

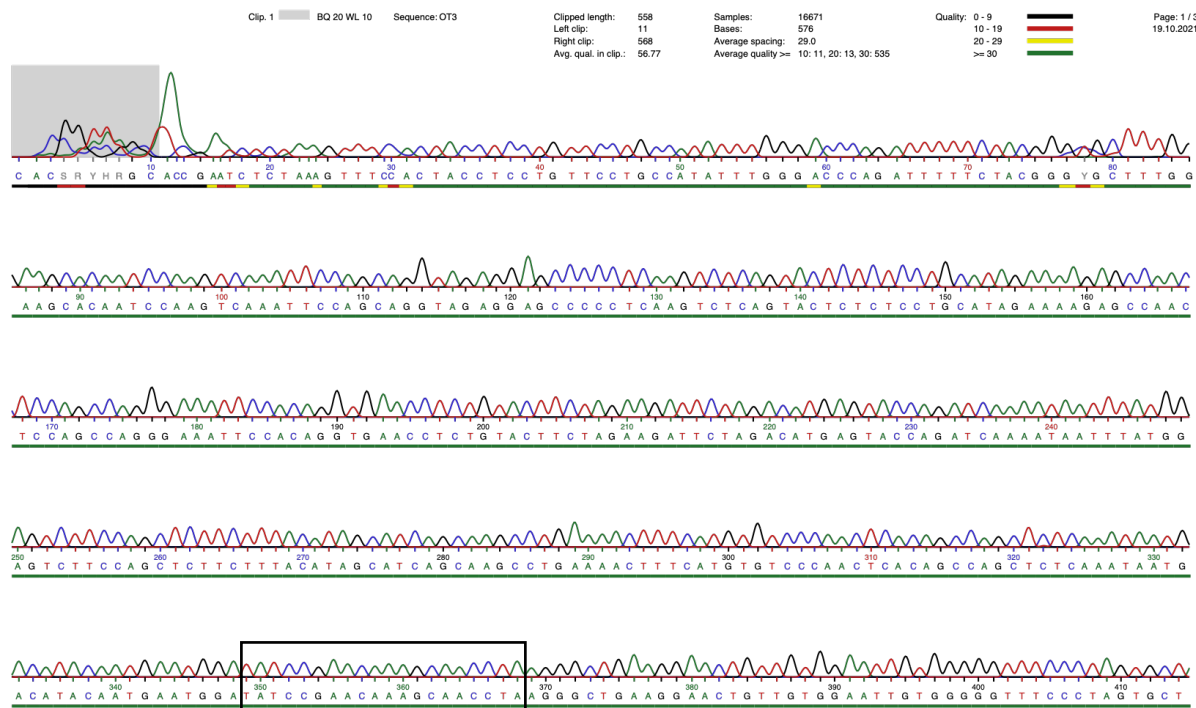


Sequencing results for Off-Target 2 on Chr16. Framed is the sequence that can be recognized by Cas9.

Query	2	CTGTCTGT-CCTCTGAGC-ACTGCTTTGATTGTGCTGTGTGCCGCCATTTCTGTGTTCTC	59
Sbjct	91342498	CTGTCTGTCCCTCTGAGCAACTGCTTTGATTGTGCTGTGTGCCGCCATTTCTGTGTTCTC	91342439
Query	60	TCTCTTACCTCCCCATTGCTTCTCCCCTTTGGAGGTAGGCTTATTGTGGACACAGCAAAA	119
Sbjct	91342438	TCTCTTACCTCCCCATTGCTTCTCCCCTTTGGAGGTAGGCTTATTGTGGACACAGCAAAA	91342379
Query	120	GACTAAACC TAGGGTGTTTTATTTGCATCTTC AAAACCATGAGAGCAAAGACTGGTCC	179
Sbjct	91342378	GACTAAACC TAGGGTGTTTTATTTGCATCTTC AAAACCATGAGAGCAAAGACTGGTCC	91342319
Query	180	CAGCACTGCATGAACACAATCACAATGCTTTCAAAAATATTTa	222
Sbjct	91342318	CAGCACTGCATGAACACAATCACAATGCTTTCAAAAATATTTA	91342276

Alignment of Off-Target 2 sequencing results with genomic DNA. Framed is the sequence that can be recognized by Cas9. The two sequences are identical.

Off-Target 3

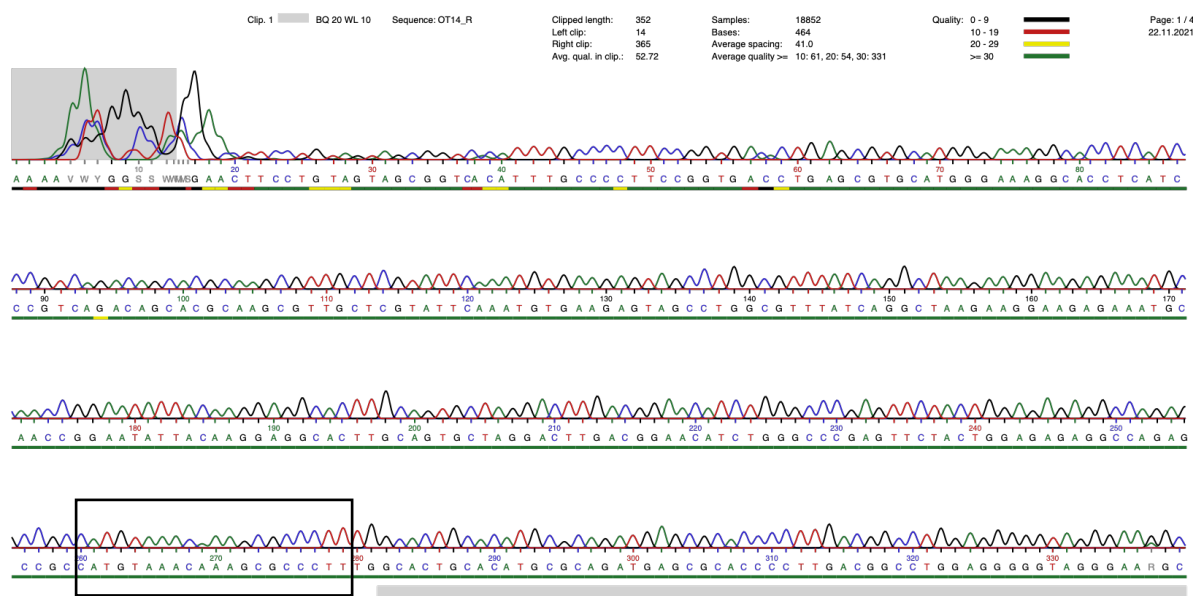


Sequencing results for Off-Target 3 on Chr1. Framed is the sequence that can be recognized by Cas9.

Query	181	GTACTTCTAGAAGATTCTAGACATGAGTACCAGATCAAAATAATTTATGGAGTCTTCCAG	240
Sbjct	42684230	GTACTTCTAGAAGATTCTAGACATGAGTACCAGATCAAAATAATTTATGGAGTCTTCCAG	42684289
Query	241	CTCTTCTTTACATAGCATCAGCAAGCCTGAAAACCTTTCATGTGTCCCAACTCACAGCCAG	300
Sbjct	42684290	CTCTTCTTTACATAGCATCAGCAAGCCTGAAAACCTTTCATGTGTCCCAACTCACAGCCAG	42684349
Query	301	CTCTCAAATAATGACATACAATGAATGGATATCCGAACAAAGCAACCTAAGGGCTGAAGG	360
Sbjct	42684350	CTTTCAAATAATGACATACAATGAATGGATATCCGAACAAAGCAACCTAAGGGCTGAAGG	42684409
Query	361	AACTGTTGTGGAATTGTGGGGGTTCCCTAGTGTAGTAATCAACAAAGRAACTCACAC	420
Sbjct	42684410	AACTGTTGTGGAATTGTGGGGGTTCCCTAGTGTAGTAATCAACAAAGRAACTCACAC	42684469
Query	421	TTGCTAGACAAGGTGTTCTACTGCTGAGCAGCCAGCTAAGTACAGGACCTTTCTAAAAG	480
Sbjct	42684470	TTGCTAGACAAGGTGTTCTACTGCTGAGCAGCCAGCTAAGTACAGGACCTTTCTAAAAG	42684529
Query	481	ACACATGATGTAAACAAACAGCATATTAGAAAGAACGGCAACATAAAGTCTAA	533
Sbjct	42684530	ACACATGATGTAAACAAACAGCATATTAGAAAGAACGGCAACATAAAGTCTAA	42684582

Alignment of Off-Target 3 sequencing results with genomic DNA. Framed is the sequence that can be recognized by Cas9. The two sequences are identical.

Off-Target 13



Sequencing results for Off-Target 13 in *Ncapd3*. Framed is the sequence that can be recognized by Cas9.

Query	3	ACTTCCTGTAGTAGCGGTCACATTTGCCCTTCCGGTGAC-CTGAGCGTGCATGGGAAAG	61
Sbjct	26941743	ACTTCCTGTAGTAGCGGTCACATTTGCCCTTCCGGTGACACTGAGCGTGCATGGGAAAG	26941684
Query	62	GCACCTCATCCCGTCAGACAGCAGCAAGCGTTGCTCGTATTCAAATGTGAAGAGTAGCC	121
Sbjct	26941683	GCACCTCATCCCGTCAGACAGCAGCAAGCGTTGCTCGTATTCAAATGTGAAGAGTAGCC	26941624
Query	122	TGGCGTTTATCAGGCTAAGAAGGAAGAGAAATGCAACCGGAATATTACAAGGAGGCACTT	181
Sbjct	26941623	TGGCGTTTATCAGGCTAAGAAGGAAGAGAAATGCAACCGGAATATTACAAGGAGGCACTT	26941564
Query	182	GCAGTGCTAGGACTTGACGGAACATCTGGGCCCGAGTTCTACTGGAGAGAGGCCAGAGCC	241
Sbjct	26941563	GCAGTGCTAGGACTTGACGGAACATCTGGGCCCGAGTTCTACTGGAGAGAGGCCAGAGCC	26941504
Query	242	GCATGTAAACAAAGCGCCCTTTGGCACTGCACATGCGCAGATGAGCGCACCCTTGACG	301
Sbjct	26941503	GCATGTAAACAAAGCGCCCTTTGGCACTGCACATGCGCAGATGAGCGCACCCTTGACG	26941444
Query	302	GCCTGGAGGGGGTAGGGAARGCGA	335
Sbjct	26941443	GCCTGGAGGGGGTAGGGAARGCGAGGGGGGGGG	26941410

Alignment of Off-Target 13 sequencing results with genomic DNA. Framed is the sequence that can be recognized by Cas9. The two sequences are identical.

Table S1. Primers used to check insertion of transgenes.

ext1-F	TGGATCTGGGGCCATAAATGCT
int1-R	ACCGGACACTTCCCTATGTG
int2-R	TGTGCTTGGTCACTGCAGAG

Table S2. Primers used in the Off-Target analysis.

	Forward (5'-3')	Reverse (5'-3')
Off-Target 1	GAAAGATGTCTCCGAGAGAGGG	CGCAGCTCGAATTTCCACACTTA
Off-Target 2	ACCGGACACTTCCCTATGTG	TGGCAGCGGAAGATAACAGG
Off-Target 3	CCTCGGTGGCATACTTCAA	AGGTGTAAAGGGTGGGTCT
Off-Target 13	CGTAACGCACCGAAGTGCTA	TCTTACACGGCAGGGAACCT

Table S3. Primers used in Real-Time RT-PCR analysis.

	Forward (5'-3')	Reverse (5'-3')
GADPH	AGGTCGGTGTGAACGGATTG	TGTAGACCATGTAGTTGAGGTCA
Nkx2.5	CTATGCCCTGTCCCTCAGAT	CTCCCGGTCCTAGTGTGGAA
GFP	GAAGCAGCACGACTTCTTCAA	AAGTCGATGCCCTTCAGCTC