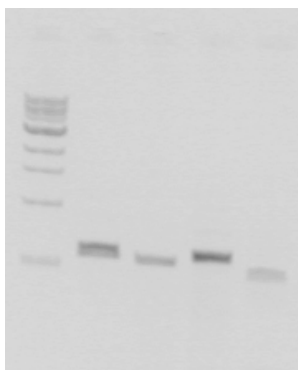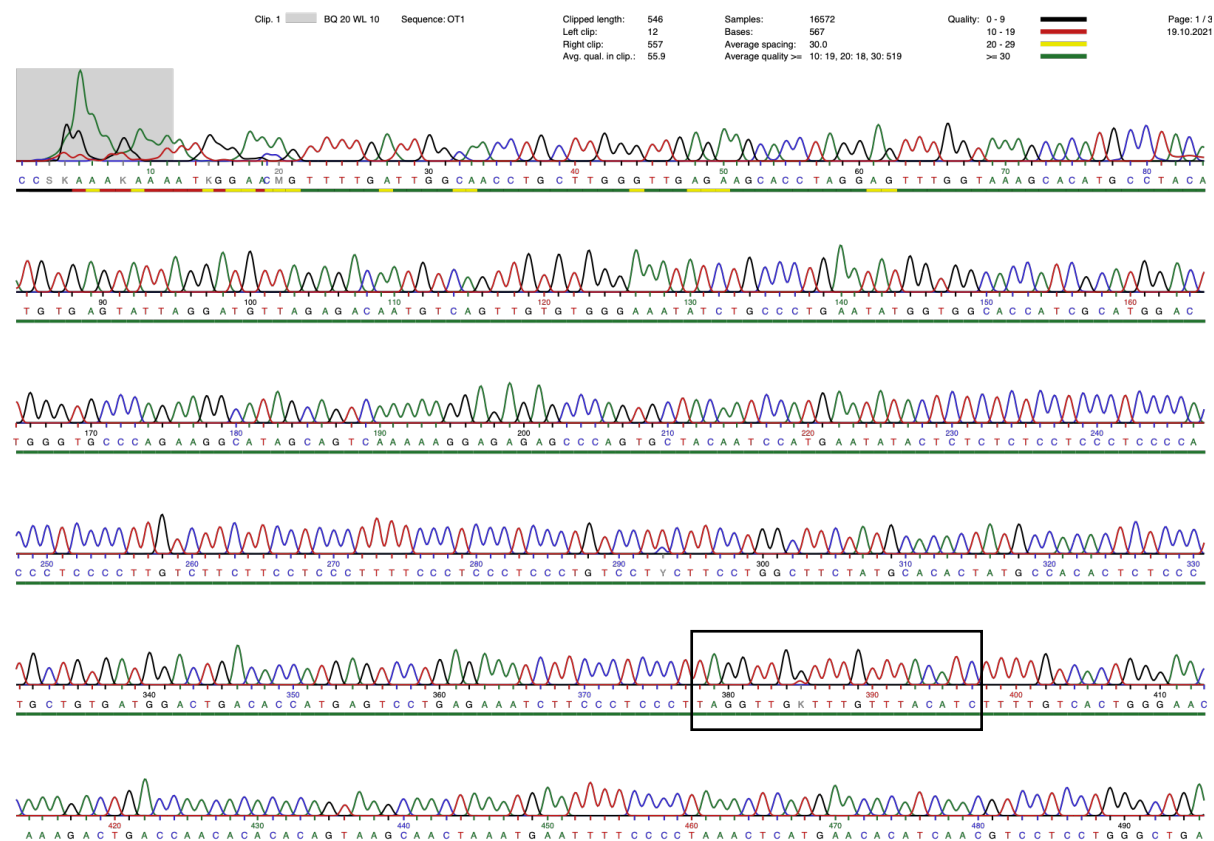# Supplementary Material

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.
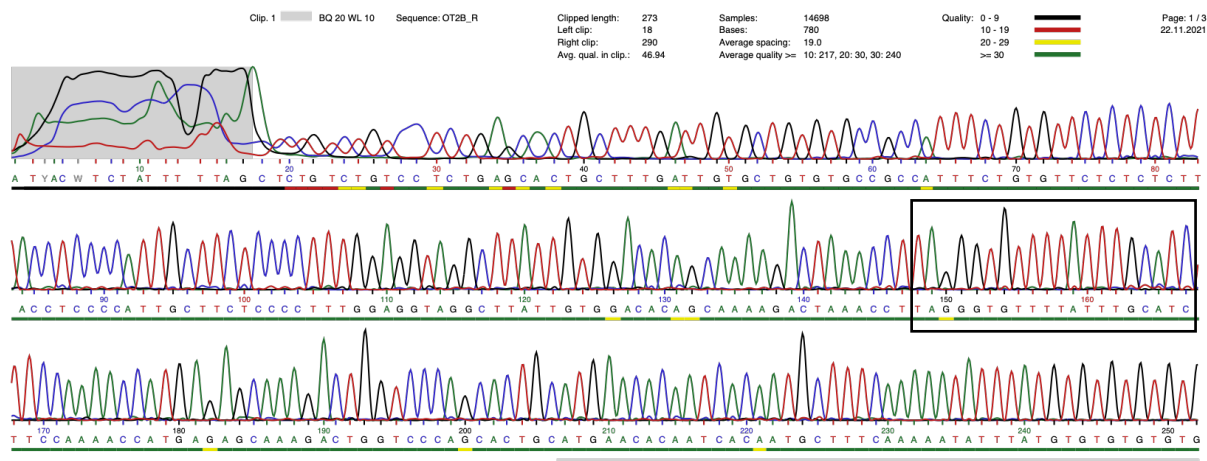
Off-Target 1

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

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.
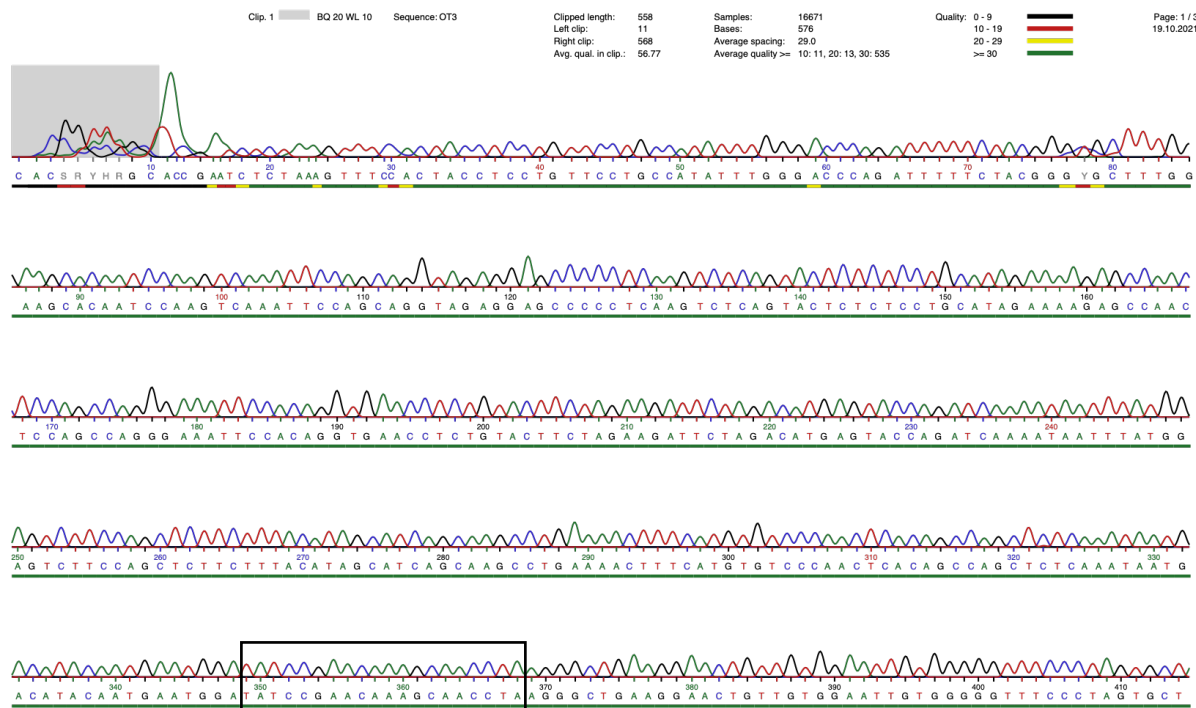
Off-Target 2

A T YAC W T CT AT TT T TA G C TC TG TC T G T CC TC T G AG C AC T G C T T T G AT T G T G C T G T G T G C C G C C A T T T C T G T G T T C T C T C T C T T

A C C T C C C C A T T G C T T C T C C C C T T T G G A G G T A G G C T T A T T G T G G A C A C A G C A A A A G A C T A A A C C T A G G G T G T T T T A T T T G C A T C

T T C C A A A A C C A T G A G A G C A A A G A C T G G T C C C A G C A C T G C A T G A A C A C A A T C A C A A T G C T T T C A A A A A T A T T T A T G T G T G T G T G

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        GACTAAACCTTAGGGTGTTTTATTTGCATCTTCCAAAACCATGAGAGCAAAGACTGGTCC  179
                  ||||||||| |||||||||||||||||||| |||||||||||||||||||||||||||||
Sbjct  91342378   GACTAAACCTTAGGGTGTTTTATTTGCATCTTCCAAAACCATGAGAGCAAAGACTGGTCC  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.

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.
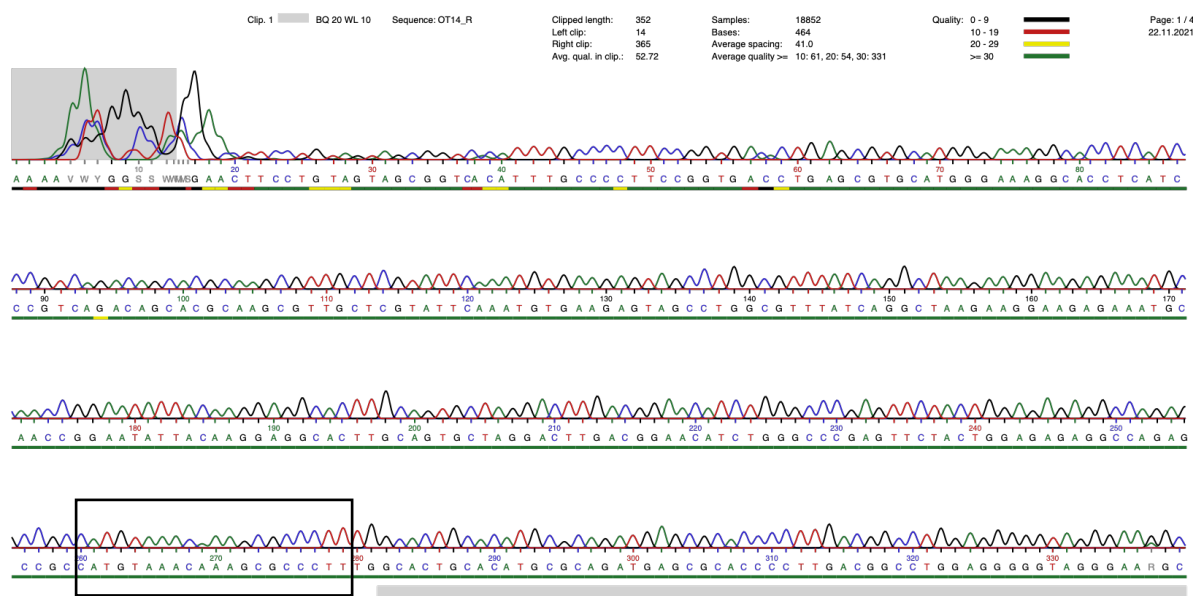
Off-Target 13

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

```
Query  3         ACTTCCTGTAGTAGCGGTCACATTTGCCCCTTCCGGTGAC-CTGAGCGTGCATGGGAAAG  61
                 |||||||||||||||||||||||||||||||||||||||| |||||||||||||||||||
Sbjct  26941743  ACTTCCTGTAGTAGCGGTCACATTTGCCCCTTCCGGTGACACTGAGCGTGCATGGGAAAG  26941684

Query  62        GCACCTCATCCCGTCAGACAGCACGCAAGCGTTGCTCGTATTCAAATGTGAAGAGTAGCC  121
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  26941683  GCACCTCATCCCGTCAGACAGCACGCAAGCGTTGCTCGTATTCAAATGTGAAGAGTAGCC  26941624

Query  122       TGGCGTTTATCAGGCTAAGAAGGAAGAGAAATGCAACCGGAATATTACAAGGAGGCACTT  181
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  26941623  TGGCGTTTATCAGGCTAAGAAGGAAGAGAAATGCAACCGGAATATTACAAGGAGGCACTT  26941564

Query  182       GCAGTGCTAGGACTTGACGGAACATCTGGGCCCGAGTTCTACTGGAGAGAGGCCAGAGCC  241
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  26941563  GCAGTGCTAGGACTTGACGGAACATCTGGGCCCGAGTTCTACTGGAGAGAGGCCAGAGCC  26941504

Query  242       GCCATGTAAACAAAGCGCCCTTTGGCACTGCACATGCGCAGATGAGCGCACCCCTTGACG  301
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  26941503  GCCATGTAAACAAAGCGCCCTTTGGCACTGCACATGCGCAGATGAGCGCACCCCTTGACG  26941444

Query  302       GCCTGGAGGGGGTAGGGAARGCGAgggggggggg       335
                 |||||||||||||||||||||||||
Sbjct  26941443  GCCTGGAGGGGGTAGGGAAAGCGAGGGGGGGGGG       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	CCTCGGTGGCATACCTTCAA	AGGTGTAAAGGGTGGGTCT
Off-Target 13	CGTAACGCACCGAAGTGCTA	TCTTACACGGCAGGGAACCT

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

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