

Supplementary Figure 2

a

Potential off-target site 1:

Chromosome: 12

Query 9 TCCCCTGCACCAGG 23
 |||
 Sbjct 23955230 TCCCCTGCACCAGG 23955244

Potential off-target site 3:

Query 9 TCCCCTGCACCAGG 23
 |||
 Sbjct 75049055 TCCCCTGCACCAGG 75049041
 Chromosome 5

Potential off-target site 5:

Query 7 TGTCCTGCACCAGG 23
 |||
 Sbjct 184835830 TGTCCTGCACCAGG 184835846
 Chromosome 1

Potential off-target site 7:

Query 8 GTCCCTGCACCAGG 23
 |||
 Sbjct 47164181 GTCCCTGCACCAGG 47164166
 Chromosome 15

Potential off-target site 9:

Query 9 TCCCCTGCACCAGG 23
 |||
 Sbjct 153963836 TCCCCTGCACCAGG 153963822
 Chromosome 2

Potential off-target site 11:

Query 1 TCCCCTGCACCAGG 15
 |||
 Sbjct 70556960 TCCCCTGCACCAGG 70556946
 Chromosome 13

Potential off-target site 2:

Chromosome: 5

Query 10 CCCCTGCACCAGG 23
 |||
 Sbjct 23957304 CCCCTGCACCAGG 23957291

Potential off-target site 4:

Query 7 TGTCCTGCACCAGG 23
 |||
 Sbjct 184622440 TGTCCTGCACCAGG 184622424
 Chromosome 1

Potential off-target site 6:

Query 8 GTCCCTGCACCAGG 23
 |||
 Sbjct 304696826 GTCCCTGCACCAGG 304696841
 Chromosome 1

Potential off-target site 8:

Query 8 GTCCCTGCACCAGG 23
 |||
 Sbjct 57260515 GTCCCTGCACCAGG 57260530
 Chromosome 15

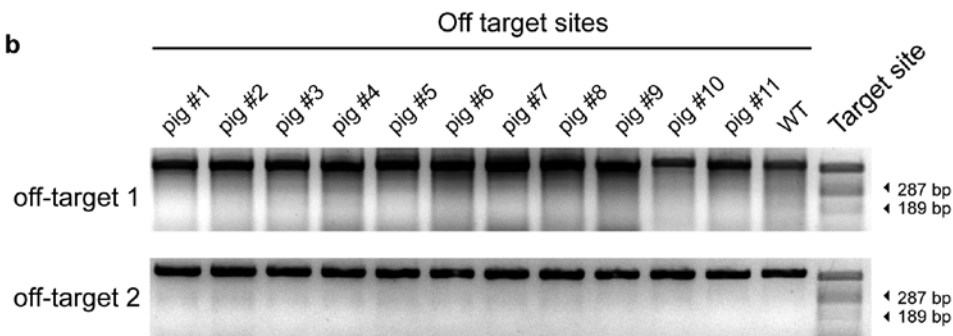
Potential off-target site 10:

Query 9 TCCCCTGCACCAGG 23
 |||
 Sbjct 28242978 TCCCCTGCACCAGG 28242964
 Chromosome 10

Potential off-target site 12:

Query 1 TCCCCTGCACCAGG 15
 |||
 Sbjct 26399419 TCCCCTGCACCAGG 26399405
 Chromosome 13

b



C

Wild-type control	GATTCAACAAATATTTTCAAAGCT <u>CCCCCTGCACC</u> AGG AATATAATGTTACAGAACA	
Pig #9	GATTCAACAAATATTTTCAAAGCTCCCCCTGCACCAGGAATATAATGTTACAGAACA	wt
Pig #10	GATTCAACAAATATTTTCAAAGCTCCCCCTGCACCAGGAATATAATGTTACAGAACA	wt
Pig #11	GATTCAACAAATATTTTCAAAGCTCCCCCTGCACCAGGAATATAATGTTACAGAACA	wt
Wild-type control	GTTAATGAGATAAGATGTCTTT <u>GTCCCCCTGCACC</u> AGG TCCCATCCAGTGCCTACAG	
Pig #9	GTTAATGAGATAAGATGTCTTTGTCCCCCTGCACCAGGTCCCATCCAGTGCCTACAG	wt
Pig #10	GTTAATGAGATAAGATGTCTTTGTCCCCCTGCACCAGGTCCCATCCAGTGCCTACAG	wt
Pig #11	GTTAATGAGATAAGATGTCTTTGTCCCCCTGCACCAGGTCCCATCCAGTGCCTACAG	wt
Wild-type control	GTTAATGAGATAAGATGTCTTT <u>GTCCCCCTGCACC</u> AGG TCCCATCCAGTGCCTACAG	
Pig #9	GTTAATGAGATAAGATGTCTTTGTCCCCCTGCACCAGGTCCCATCCAGTGCCTACAG	wt
Pig #10	GTTAATGAGATAAGATGTCTTTGTCCCCCTGCACCAGGTCCCATCCAGTGCCTACAG	wt
Pig #11	GTTAATGAGATAAGATGTCTTTGTCCCCCTGCACCAGGTCCCATCCAGTGCCTACAG	wt
Wild-type control	TGCTGCTGTCAGTTTGGGTACCGT <u>CCCCCTGCACC</u> AGG GTGGGAATATGCCCTGG	
Pig #9	TGCTGCTGTCAGTTTGGGTACCGTCCCCCTGCACCAGGGTGGGAATATGCCCTGG	wt
Pig #10	TGCTGCTGTCAGTTTGGGTACCGTCCCCCTGCACCAGGGTGGGAATATGCCCTGG	wt
Pig #11	TGCTGCTGTCAGTTTGGGTACCGTCCCCCTGCACCAGGGTGGGAATATGCCCTGG	wt
Wild-type control	GGCCAACCTGCAGCATATGGAGGT <u>CCCCCTGCACC</u> AGG GATTGAATCCAAGCTGCAG	
Pig #9	GGCCAACCTGCAGCATATGGAGGTCCCCCTGCACCAGGGATTGAATCCAAGCTGCAG	wt
Pig #10	GGCCAACCTGCAGCATATGGAGGTCCCCCTGCACCAGGGATTGAATCCAAGCTGCAG	wt
Pig #11	GGCCAACCTGCAGCATATGGAGGTCCCCCTGCACCAGGGATTGAATCCAAGCTGCAG	wt
Wild-type control	TTATTTTCATTGAGATTACCCAAGT <u>CCCCCTGCACC</u> AGG TCCCCTTTCCAGTTGCTGT	
Pig #9	TTATTTTCATTGAGATTACCCAAGTCCCCCTGCACCAGGTCCCCTTTCCAGTTGCTGT	wt
Pig #10	TTATTTTCATTGAGATTACCCAAGTCCCCCTGCACCAGGTCCCCTTTCCAGTTGCTGT	wt
Pig #11	TTATTTTCATTGAGATTACCCAAGTCCCCCTGCACCAGGTCCCCTTTCCAGTTGCTGT	wt
Wild-type control	GAGCTGTGAGCTGGCGGTGTTTCT <u>CCCCCTGCACC</u> AGG ACACCCGACCCAAGGATCT	
Pig #9	GAGCTGTGAGCTGGCGGTGTTTCTCCCCCTGCACCAGGACACCCGACCCAAGGATCT	wt
Pig #10	GAGCTGTGAGCTGGCGGTGTTTCTCCCCCTGCACCAGGACACCCGACCCAAGGATCT	wt
Pig #11	GAGCTGTGAGCTGGCGGTGTTTCTCCCCCTGCACCAGGACACCCGACCCAAGGATCT	wt
Wild-type control	GGACTTATGGGTGGCGGGGCCACT <u>CCCCCTGCACC</u> AGG CAGAGGGCCAGACTGCTC	
Pig #9	GGACTTATGGGTGGCGGGGCCACTCCCCCTGCACCAGGCAGAGGGCCAGACTGCTC	wt
Pig #10	GGACTTATGGGTGGCGGGGCCACTCCCCCTGCACCAGGCAGAGGGCCAGACTGCTC	wt
Pig #11	GGACTTATGGGTGGCGGGGCCACTCCCCCTGCACCAGGCAGAGGGCCAGACTGCTC	wt
Wild-type control	TGTTTTAACCCCATTTGGATGTTTT <u>CCCCCTGCACC</u> AGG CCATTTCTCCATCCTGTGG	
Pig #9	TGTTTTAACCCCATTTGGATGTTTTCCCCCTGCACCAGGCCATTTCTCCATCCTGTGG	wt
Pig #10	TGTTTTAACCCCATTTGGATGTTTTCCCCCTGCACCAGGCCATTTCTCCATCCTGTGG	wt
Pig #11	TGTTTTAACCCCATTTGGATGTTTTCCCCCTGCACCAGGCCATTTCTCCATCCTGTGG	wt
Wild-type control	CAGCTGCAACCTCAGATACTTTAT <u>CCCCCTGCACC</u> AGG CCAGGGACTGAACCTGCAT	
Pig #9	CAGCTGCAACCTCAGATACTTTATCCCCCTGCACCAGGCCAGGGACTGAACCTGCAT	wt
Pig #10	CAGCTGCAACCTCAGATACTTTATCCCCCTGCACCAGGCCAGGGACTGAACCTGCAT	wt
Pig #11	CAGCTGCAACCTCAGATACTTTATCCCCCTGCACCAGGCCAGGGACTGAACCTGCAT	wt

Supplementary Figure S2 Off-targets analysis in *vWF* mutant pigs. (a) Based on the prediction method, the potential off-target sites of sgRNA-*vWF* are indicated, and PAM site is shown in red

bold letter. (b) T7EI analysis of two potential off-target sites in all the 11 mutant piglets, and no off-target cleavage was detected. (c) Sanger sequencing of 10 randomly selected off-target sites in three biallelic mutant pigs.