

Supplementary Tables

Supplementary Table 1. Summary of off-targeting comparison for *RAG2*. Target gene (*RAG2*) guide sequence compared to potential off-target sequence. Location on chromosome, intron or exon region and presence of PAM site is indicated.

Gene	Sequence	Location on chromosome	Intron/Exon?	PAM
<i>RAG2</i>	GTATAGTCGAGGGAAAAGTA	NC_010444.4 (24547605-24553944)	Exon	Yes
<i>SLC04C1</i>	GTATAGTTGAGGGAAAATAA	NC_010444.3 (111923785-111924901)	Intron	No
<i>PLCXD3</i>	GTATAGTCAAGGAAAAGTA	NC_010458.3 (27465590-27466609)	Intron	No
<i>FAM174B</i>	GTATAGTCGAGGGAAACCTC	NC_010449.4 (92221611-92222625)	Intron	No
<i>DPYD</i>	ATATAGTAGAGGGAAAAGTC	NC_010446.4 (132532671-132533688)	Exon	No
<i>DPY19L2</i>	GTATAGTAGAGTGAAAAGTA	NC_010460.3 (42568459-42569440)	Intron	No
<i>UNC13C</i>	ATATAGTGGAGGGAAAAGCC	NC_010443.4 (130045664-130046680)	Intron	No
<i>ITSN2</i>	GTATAGTTGAGGGAAATGTA	NC_010445.3 (121743732-121744717)	Intron	Yes
<i>3' to GNPDA2</i>	ATATAGTCGTGGGAAAAGTT	NC_010450 (35839672-35840689)	Intron	No
<i>3' to LPGAT1</i>	GTATAGGCAAGGGAAAAGTA	NC_010451.4 (131421672-131422691)	Intron	No

Supplementary Table 2. Summary of off-targeting comparison for *IL2RG*. Target gene (*IL2RG*) guide sequence compared to potential off-target sequence. Location on chromosome, intron or exon region and presence of PAM site is indicated.

Gene	Sequence	Location on chromosome	Intron/Exon?	Pam
<i>IL2RG</i>	GGGACTCTCAACGTTTCCAC	NC_010461.5 (57143568-57151242)	Exon	Yes
<i>CCNB3</i>	TGA A CTCT G AACGTTTCCAC	NC_010461.4 (48865012-48865996)	Intron	No
<i>ZNF189</i>	TGA A CTCTCA A CTTTTCCAC	NC_010443.4 (271662850- 271663866)	Intron	No
<i>MYOM1</i>	GGGACTCTCAA A GTTT T AGC	NC_010448.3 (96567814-96568829)	Intron	Yes
<i>P2RX7</i>	GGGACTCTCA A TGTTT C TCC	NC_010456.4 (33314885-33315869)	Intron	Yes
<i>NTNG1</i>	GGGACTCT G AACGTT C CCAC	NC_010446.4 (123215716- 123216697)	Intron	No
<i>TRPV1</i>	A GGACTCTCAA G GTTT C CCA	NC_010454.3 (51553951-51554967)	Intron	Yes
<i>5' to LOC102157658</i>	GGGACTCT C TACGTT C TG	NC_010461.5 (117490221-117491238)	Intron	No
<i>5' to DCBLD2</i>	GGGACTCTCA A TGTTTCCAC	NC_010455.5 (160064437-160156205)	Intron	No

Supplementary Table 3. Summary of off-targeting comparison for *Ig heavy chain*. Target gene (*Ig heavy chain*) guide sequence compared to potential off-target sequence. Location on chromosome, intron or exon region and presence of PAM site is indicated.

Gene	Sequence	Location on Chromosome	Intron/Exon?	Pam
<i>Ig Heavy Chain</i>	ACACCCCCCAGGTTTTTGTG	NW_018084979.1 (3676148-3841291)	Exon	Yes
<i>5' to LRRFIP1</i>	CAACCCCCCAGCTTTTTGTG	NC_010457 (137255672- 137256655)	Intron	Yes
<i>EDIL3</i>	TAACCCCCCTAGGTTTTTGTG	NC_010444 (92541026 – 92542043)	Intron	No
<i>AR</i>	TCTCCCCCCAGGTTTTTGTG	NC_010461.5 (53713243- 53714259)	Intron	Yes
<i>MYLK3</i>	ACACCCACCAGGTTTTTGTG	NC_010448.4 (37860344- 37861325)	Intron	No
<i>RBFOX1</i>	ACACCTCCCAGGCTTTTTGTG	NC_010445 (34998525- 34999544)	Intron	Yes
<i>CCDC60</i>	GCACCCCCCTAGGTTTTTGTG	NC_010456 (33512327- 33513309)	Intron	No

Supplementary Table 4. Summary of off-targeting comparison for *SCD5*. Target gene (*SCD5*) guide sequence compared to potential off-target sequence. Location on chromosome, intron or exon region and presence of PAM site is indicated.

<i>Gene</i>	Sequence	Location on Chromosome	Intron/Exon?	PAM
<i>SCD5</i>	ACCGCTCTGGGTGTGACAGC	NC_010450.4 (135507346-135634031)	Exon	Yes
<i>SDK1</i>	CCTGCTCTGGGTGTGACAGT	NC_010445.3 (3521346- 3522331)	Intron	No
<i>KLHL29</i>	AGAGCTCTGGGTGTGGCAGC	NC_010445.3 (122252390- 122253406)	Intron	No
<i>ACLY</i>	CCAGCTCTGGGAGTGACAGC	NC_010454.3 (21092639- 21093655)	Intron	Yes
<i>5' to RAB3A</i>	CCCGCTCTGCGTGTGACAGT	NC_010444.4 (59602517- 59603534)	Intron	Yes

Supplementary Table 5. Summary of off-targeting results for *RAG2*. Five pigs were tested for each potential off-target gene. No off-targeting events were found.

<i>RAG2</i>		
Gene	Number pigs	Number off-target events
<i>SLCO4C1</i>	5	0
<i>PLCXD3</i>	5	0
<i>FAM174B</i>	5	0
<i>DPYD</i>	5	0
<i>DPY19L2</i>	5	0
<i>UNC13C</i>	5	0
<i>ITSN2</i>	5	0
<i>3' to GNPDA2</i>	5	0
<i>3' to LPGAT1</i>	5	0

Supplementary Table 6. Summary of off-targeting results for *IL2RG*. Five pigs were tested for each potential off-target gene. No off-targeting events were found.

<i>IL2RG</i>		
Gene	Number pigs	Number off-target events
<i>CCNB3</i>	5	0
<i>ZNF189</i>	5	0
<i>MYOM1</i>	5	0
<i>P2RX7</i>	5	0
<i>NTNG1</i>	5	0
<i>TRPV1</i>	5	0
<i>5' to LOC102157658</i>	5	0
<i>5' to DCBLD2</i>	5	0

Supplementary Table 7. Summary of off-targeting results for *SCD5*. Six pigs were tested for each potential off-target gene. No off-targeting events were found.

SCD5		
Gene	Number pigs	Number off-target events
<i>SDK1</i>	6	0
<i>KLHL29</i>	6	0
<i>ACLY</i>	6	0
<i>5' to RAB3A</i>	6	0

Supplementary Table 8. Embryo transfer to generate SCD5 knockout fetuses. Day 5-6 embryos were transferred into surrogates. A total of 6 fetus were obtained from one surrogate.

Surrogate ID	Number of embryos generated	Number of embryos transferred into a recipient	pregnancy	Number of fetuses
27-5	448	65 (day-6)	Yes	6
27-2		50 (day-5)	No	-

Supplementary Table 9. Genotype of SCD5 knockout fetuses. No wild-type allele was identified from the fetuses.

Fetuses	Genotype
1	9 base pair deletion/ 14 base pair deletion
2	2 base pair change, 1 base pair deletion/ 9 base pair deletion
3	14 base pair deletion/ 3 base pair deletion, 1 base pair change
4	4 base pair deletion/ 16 base pair deletion, 3base pair change
5	2 base pair insertion
6	9 base pair deletion, 1 base pair change/ 9 base pair deletion

Supplementary Table 10. Summary of primers used to amplify genes tested for potential off-targeting sites as well as original *RAG2* target gene.

Gene	Forward	Reverse	Expected Size
<i>RAG2</i>	AAGGATTCCTGCTACCTTCCTCCT	AGATAGCCCATCTTGAAGTTCTGG	426
<i>SLCO4C1</i>	GTAAACTGATAGGCATTCTAATCCAGAACTAACC	CTGGCCCATCTCCTCCATTC	506
<i>PLCXD3</i>	GAGGGCCGAATCAGTACTTGC	CTCCACAAGACAGGGATGACATC	478
<i>FAM174B</i>	CAGAGGTTGCAGGACAATGGC	GCAATGAACATGCGGGTGC	369
<i>DPYD</i>	CCCTGTGCTTAAACTCCCACATC	CTAAAGTGACACCGGTTAAGCCC	502
<i>DPY19L2</i>	GGA GTATCTGAAC TCCACTTTT GGT	AGA AGT GCA ACC AAG GCC AG	496
<i>UNC13C</i>	GTGGGTTTTGTGGGAGTGTAGTG	CCACTGTACTCAAAGAATCCAGATTGAC	410
<i>ITSN2</i>	CAATGAAAGACAGGAAAGACCAGAGGCTG	GCTACAGCATGATGTTTCCTTGAGAG	767
<i>3' to GNPDA2</i>	CCTGCTCTGTTTCGCCTCAG	CTTTGTACAGAGGTTGGCTCTTCT	575
<i>3' to LPGAT1</i>	GGGGTCTCTTGGTTTGCTCC	GCTTGTAGGCCGGCTTCTTCAG	463

Supplementary Table 11. Summary of primers used to amplify genes tested for potential off-targeting sites as well as original *IL2RG* target gene.

Gene	Forward	Reverse	Expected Size
<i>IL2RG</i>	CTGGACTATTAGAAGGATGTGGGC	ATATAGTGGGAAGCCTGGGATGCT	417
<i>CCNB3</i>	GCCAGATCCACAGGACACTTTG	GAGGTGACAGCTAAAGGTACAGGG	469
<i>ZNF189</i>	GGCTTTTCTTTCTTCTAGTCTCCACTGG	CCTGGCTGTGAAGTGAACAAATAGTAGG	439
<i>MYOM1</i>	GGTTCCATTCATCTGTCTTTGCTCTC	GGCTGTGGTATAGGCTGGC	452
<i>P2RX7</i>	CTTTGTCACTCTTGCCATCGGG	CTACCCAACAAACTATCCTGAACCTGG	441
<i>NTNG1</i>	GCTGTAATTGGTAAGGCAATGGCTT	GGCCTCTCCCTGCTTCTC	447
<i>TRPV1</i>	GCAGCCTCTGGGTGCTAAG	CCAGGGTGCTTCGCTTGTC	467
<i>5' to LOC102157658</i>	GTGTGCAGATTTCTTCTATAACGGC	GTTTCAACATTAACATCATGGGGTCCC	400
<i>5' to DCBLD2</i>	CCAGGGTCCCCCTTCCTG	CATGGGTACTGGAACACAAAAGAGGG	466

Supplementary Table 12. Summary of primers used to amplify genes tested for potential off-targeting sites as well as original *Ig heavy chain* target gene.

Gene	Forward	Reverse	Expected size
<i>Ig heavy chain</i>	GACACTTTGGAGGTCAGGAACGGGAG	CTTCTCTCTCCGACATGGCTCTTTCAGAC	848
<i>5' to LRRF1P1</i>	GAAGTCACACTCCGCCGGG	GTGAAGAATCTGGAAGGCCCAAGG	390
<i>EDIL3</i>	CATCTTCCTATTCTCCAGCAGGGC	CACATATCACACCTAGAATCTGTGGGAG	462
<i>AR</i>	CTAGAAGATGGTGAATCCTAGTTCCCATTCTC	GAGTGAAGAGGACACTGATATGCC	650
<i>MYLK3</i>	CACACTCTTCTGTGGTTACTAATGAGAGCC	CCCTGAGACGCCAGGTGG	392
<i>RBFOX1</i>	GCTCTCTCCTCCCTGAGAGG	CAAAGCCATGCTGCCATCAGGAG	401
<i>CCDC60</i>	GCTGTCCTTCTCTCAGTCCCAC	CCTGTGACCTTGGGCAGG	400

Supplementary Table 13. Summary of primers used to amplify genes tested for potential off-targeting sites as well as original *SCD5* gene.

Gene	Forward	Reverse	Expected Size
<i>SCD5</i>	GCAGGAAACAGTCGCAAAGA	GGGGTACTGTCAGGGTCAA	756
<i>SDK1</i>	GCACGTTTTGTAATGGGGCCG	CCCCCAGTATTTAGCACAGAGCC	352
<i>KLHL29</i>	GGCGCAGCTGGTGTCAATC	GATGCTCACTCTCGAGGGCG	342
<i>ACLY</i>	GGCCAGGGAAAGCAGAGC	CCAGAATTCTAGACGGGGTGGGAG	384
<i>5' to RAB3A</i>	CGAGAGGAGGGCCGTGACAATC	GGCACTCGGGAGCTCACC	467