SUPPLEMENTAL DATA

THE *sld* GENETIC DEFECT: INTRONIC INSERTION OF TWO CA-REPEATS THAT PROMOTE INSERTION OF THE SUBSEQUENT INTRON AND mRNA DECAY

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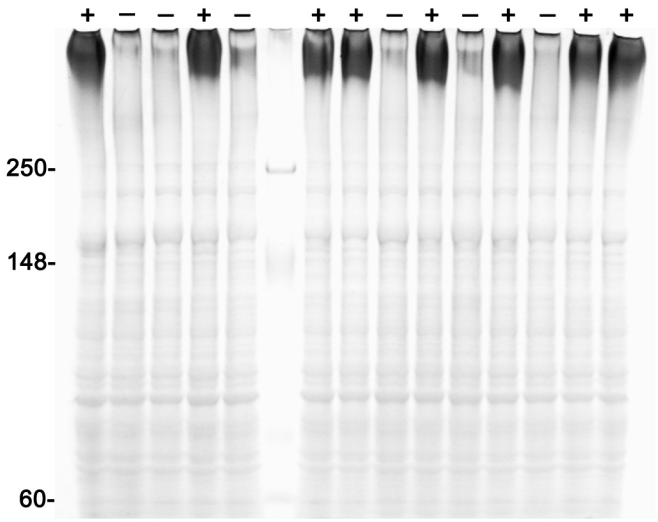

Figure 1S. Mapping the *sld* mutation: Phenotyping mice. To identify the *sld* mutant phenotype, we prepared homogenates from frozen excised sublingual glands of F2 mice killed at 3 weeks of age. Homogenates were assayed for the presence or near absence (mutant phenotype) of high molecular weight mucins by SDS-PAGE on gradient gels (3% - 8%). Gels were stained using Alcian blue with subsequent silver enhancement of dye staining. Lane 6 contains molecular weight markers with size (kd) indicated at far left. *sld* phenotype, -; wild type phenotype, +.

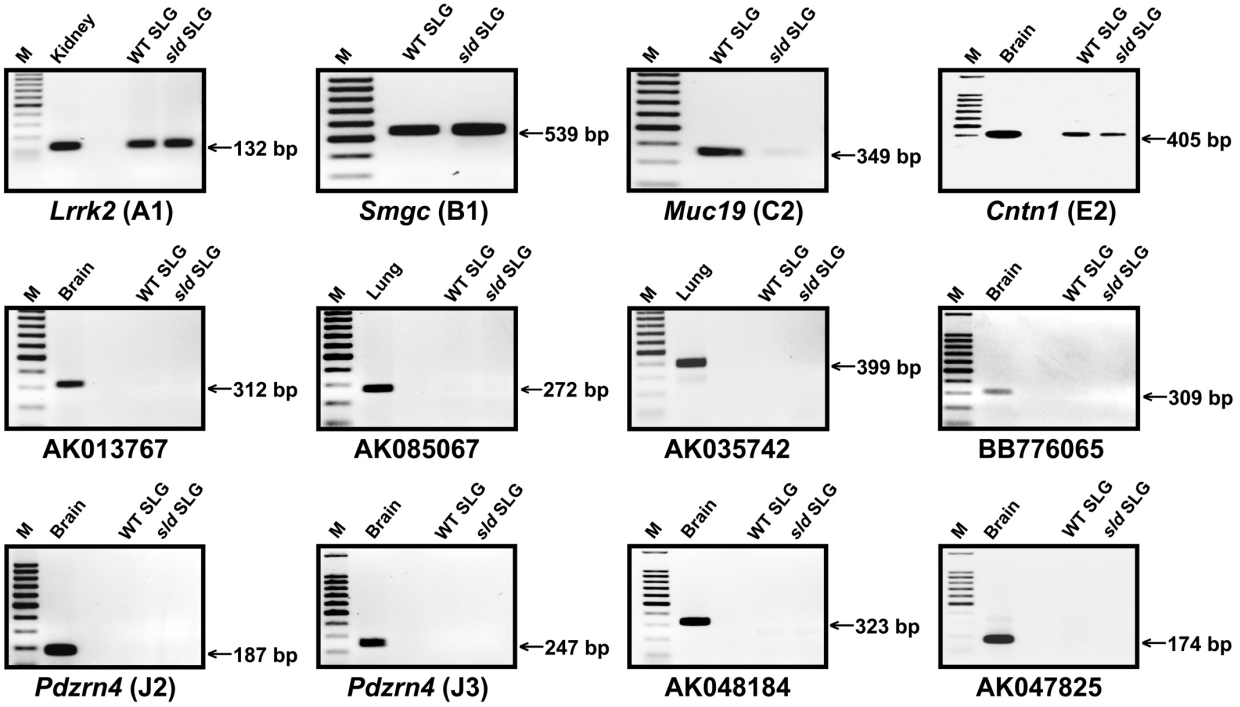

Figure 2S. Expression of transcripts within the critical interval in sublingual glands of wild type (WT) and *sld* mutant mice as assessed by RT-PCR. Top row are representative gels from known genes within the critical interval. Bottom two rows are results for EST-based transcripts and *Pdzrn4*, all of which consistently displayed no expression in sublingual glands. In parentheses is the primer set identification for transcripts with more than one amplicon (see Supplemental Table S4). Control tissues other than sublingual glands are identified. SLG, sublingual gland.

Table 1S. Sequence Tagged Sites (STS) used in chromosomal localization of the *sld* mutation. Sequences of primers for each STS marker are available from the UniSTS database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unists). The total length of each chromosome (Chr) and the positions of STS markers are given in centiMorgans (cM, MGI genetic map position) and in mega-base pairs (Mbp) based on the NCBI genomic sequence, Build 36.1. CAST, CAST/EiJ mouse strain; *sld*, NFS/N-*sld* mouse strain; bp, base pair. *, D6Mit42 has multiple alignments in a highly repetitive region of 100 kb.

Chr (cM) [Mbp]	STS (cM, Mbp)	Product (bp)		Chr (cM) [Mbp]	STS (cM, Mbp)	Product (bp)	
		CAST	*sld*			CAST	*sld*
1 (127.5)	D1Mit171 (20.2, 36.7)	184	150	**10** (77.5)	D10Mit4 (19.0, 25.7)	147	134
[197]	D1Mit7 (41.0, 74.9)	152	105	[130]	D10Mit226 (43.0, 79.7)	172	95
	D1Mit140 (70.0, 136.1)	158	150		D10Mit121 (62.0, 114.4)	217	190
	D1Mit116 (100.5, 180.2)	176	146				
				11 (80.0)	D11Mit268 (19.0, 40.7)	112	142
2 (115.0)	D2Mit33 (17.0, 30.4)	384	182	[122]	D11Mit35 (47.6, 83.5)	234	216
[182]	D2Mit247 (44.0, 74.9)	142	121		D11Mit253 (71.0, 114.1)	115	85
	D2Mit484 (65.5, 118.3)	146	124				
	D2Mit170 (96.0, 162.3)	129	141	**12** (67.0)	D12Mit2 (19.0, 36.0)	178	149
				[120]	D12Mit116 (35.0, 80.9)	88	115
3 (120.0)	D3Mit333 (22.0, 44.4)	144	126		D12Mit100 (50.0, 104.9)	111	91
[160]	D3Mit104 (52.5, 107.9)	118	90				
	D3Mit219 (84.9, 154.9)	203	151	**13** (80.0)	D13Mit38 (19.0, 37.7)	156	145
				[121]	D13Mit27 (42.0, 88.2)	186	205
4 (84.0)	D4Mit214 (17.9, 45.7)	154	129		D13Mit76 (61.0, 111.7)	118	90
[155]	D4Mit297 (38.0, 80.7)	136	115				
	D4Mit68 (66.0, 136.4)	130	102	**14** (69.5)	D14Mit26 (19.5, 54.2)	142	126
				[124]	D14Mit32 (32.5, 69.3)	270	235
5 (93.0)	D5Mit4 (20.0, 35.9)	238	195		D14Mit9 (54.5, 111.8)	244	237
[152]	D5Mit89 (53.0, 100.5)	180	148				
	D5Mit32 (78.0, 136.2)	148	124	**15** (81.5)	D15Mit165 (21.1, 38.0)	166	144
				[103]	D15Mit2 (46.9, 80.1)	107	84
6 (75.0)	D6Mit42 (20.4, 47.6*)	168	178		D15Mit42 (59.2, 98.9)	170	184
[150]	D6Mit36 (46.0, 104.5)	178	195				
	D6Mit111 (63.7, 133.8)	180	150	**16** (72.0)	D16Mit103 (22.2, 31.9)	123	103
	D6Mit15 (74.0, 146.4)	220	150	[98]	D16Mit64 (38.0, 57.5)	245	220
					D16Mit6 (63.2, 89.2)	171	185
7 (75.4)	D7Mit69 (24.5, 48.9)	274	234				
[145]	D7Mit281 (52.4, 112.0)	207	139	**17** (82.0)	D17Mit21 (18.6, 38.9)	105	122
	D7Mit177 (72.0, 143.8)	161	136	[95]	D17Mit203 (34.3, 59.4)	158	122
					D17Mit190 (54.6, 85.6)	121	110
8 (82.0)	D8Mit63 (15.0, 34.3)	244	222				
[132]	D8Mit252 (43.0, 93.8)	138	166	**18** (60.0)	D18Mit104 (20.0, 42.0)	159	135
	D8Mit13 (67.0, 126.7)	114	98	[91]	D18Mit152 (37.0, 62.1)	124	145
					D18Mit25 (57.0, 89.7)	146	128
9 (79.5)	D9Mit2 (17.0, 37.1)	159	182				
[124]	D9Mit8 (42.0, 76.2)	180	194	**19** (56.0)	D19Mit73 (22.0, 24.1)	166	141
	D9Mit16 (61.0, 114.8)	194	178	[61]	D19Mit11 (41.0, 44.4)	118	150

Table 2S. Sequence Tagged Sites (STS) derived from genomic sequence between D15Mit34 and D15Mit223. The position in mega-base pairs (Mbp) of each STS on chromosome 15 is given in parentheses and is based on NCBI genomic sequence, Build 36.1, released February 2006. PCR reactions (20 μl) contained 50 ng genomic DNA (DNeasy Kit, Qiagen), 50 ng primers and 10 μl Taq Polymerase Master Mix kit (Qiagen) with a cycling profile of 95°C for 5 min, 35 cycles (95°C, 30 sec; 55°C, 120 sec; 72°C, 120 sec) and 7 min at 72°C. Products were analyzed on 4% gels (NuSieve 3:1 agarose; Cambrex Bio Science). bp, base pair.

STS (Mbp)	GenBank Accession Number	Forward/Reverse Primers	PCR Product (bp)	
			CAST/EiJ	NFS/N-*sld*
D15Roc1 (91.159)	GI:154937296	5'-CCTGATGCTGGAGATGACAAATTC-3' 5'-GGCAGAAATGAGAGGGAGATTGAG-3'	231	201
D15Roc2 (91.408)	GI:154937297	5'-GGAGCCAGAAGATTAAAACAGATGC-3' 5'-GCTCAAACTCCTCTCCACACTAACTG-3'	266	195
D15Roc3 (91.609)	GI:154937298	5'-TTTTAGTCCCAATGTTTCCCATCAC-3' 5'-CCAAGCACAAGAATACACAGACGC-3'	325	283
D15Roc4 (91.631)	GI:154937299	5'-TCCTGACTTCTGCTGCTTGCTATTG-3' 5'-TGGTGCTATATTGATGTCTGCATTCAAG-3'	328	300
D15Roc5 (91.700)	GI:154937300	5'-GGCTGCTTTGCTTTCAGTTTTGTAG-3' 5'-CCCAGTTACTAAGTGGTTTTTTTGCTTG-3'	290	200
D15Roc6 (91.763)	GI:154937301	5'-GCACAAAGGTGGATGGATTG-3' 5'-TCAAGGTAGAAGAAAGAGGCAAG-3'	275	224
D15Roc7 (91.890)	GI:154937302	5'-CAGATCCTCACAAGGCTTTTGACAG-3' 5'-CATAATTTCTCCCTTTCCTCCTTCC-3'	320	370
D15Roc8 (92.215)	GI:154937303	5'-GGGTTCTCAACCATTTGCTTTTCAG-3' 5'-AACAATCCAAGGAGAGAGAGAGGGAG-3'	89	171
D15Roc9 (92.392)	GI:154937304	5'-GGTCCTATTCCTACATTCTCAGGTTTTG-3' 5'-CCCACCACACAGGTTTTTCTGAAAG-3'	192	175
D15Roc10 (92.456)	GI:154937307	5'-ATGGTGTTCAAGAGTCTCAGTAAGCAAC-3' 5'-TGAGGCAGGAGGATTGTGGTTGAG-3'	260	237
D15Roc11 (92.533)	GI:154937306	5'-TGTTCGGTTGCCTGTTACAGAAGTG-3' 5'-GTGCATTACACACAAGGAAACTCGTC-3'	287	334
D15Roc12 (92.638)	GI:154937305	5'-ATCACCAGGGTCCTAGACTTTCTGTATC-3' 5'-TGACAACGGGTCATCAATATGTTAATTG-3'	220	176

Table 3S. RT-PCR primers used to detect transcripts within the critical interval. Known transcripts and computer models are identified by their official symbol from NCBI Entrez Gene (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db) whereas ESTs are identified by their locus designation from NCBI Entrez Nucleotide (http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db). The positions of primers is based the GenBank Accession Number (given in parentheses or the EST designation). Amp ID, amplicon identification; Amp (bp), amplicon length in base pairs; Binding Site, location in which primer binds; PCR, three different PCR cycling profiles (A - C) were incorporated to amplify cDNA (10 ng): A, 95°C for 15 min, 25 cycles (94°C, 60 sec; 55°C, 60 sec; 72°C, 60 sec) and 10 min extension at 72°C; B and C, same as A but for 30 and 35 cycles, respectively; *Lrrk2*, leucine-rich repeat kinase 2; *Muc19*, mucin 19; *Smgc*, submandibular gland protein C; *Cntn1*, contactin 1 *Pdzrn4*, PDZ domain containing RING finger 4.

Transcript	Primer Sequence	Binding Site	Amp ID	Amp (bp)	PCR
Lrrk2 (NM_025730)	5'-GCCACATCTTACTCCTGGATCTTTC-3'	Exon 49	A1	132	B
	5'-TGTACCCCAAAACCAGCATGAC-3'	Exon 50			
	5'-GGAGGAGGTTCTGAGAAGTTGATAG-3'	Exon 1	A2	361	B
	5'-GACAGGTTTACATTGGCGTGATG-3'	Exon 4			
Smgc (NM_198927)	5'-TAATGCACAATCAAGCAACAAC-3'	Exon 14	B1	539	A
	5'-AATTATGGCTTCCTGAGTCACTC-3'	Exons 17/18			
	5'-GCTCCTCAGCAGTAGACTCCACAG-3'	Exon 17	B2	241	A
	5'-GGGTCAGCAGGTTCGTTTTTG-3'	Exon 18			
Muc19 (NM_207243)	5'-CATCTCAGGAATGTTCAGACACAGTAAC-3'	Exon 25	C1	288	A
	5'-CAGGGCAGAGGTTTGGCTTTC-3'	Exons 26/27			
	5'-GATTATGCGATTGGTTCATCCT-3'	Exons 39/40	C2	349	A
	5'-GTGCAATGTCCCTGAACTCATA-3'	Exon 43			
LOC667753 (XM_992195)	5'-TGCGTCTGCGAGGAAAGTTG-3'	Exon 1	D1	143	B
	5'-GCGACACCTATGACACCAATCAG-3'	Exon 1			
Cntn1 (NM_007727)	5'-GCCAGCCCATTTCCAGTTTAC-3'	Exon 4	E1	378	B
	5'-GCCGTTGGTCTGAGACACAAATC-3'	Exon 7			
	5'-CCAATCAATGCTGACATCACTGTTG-3'	Exon 14	E2	405	B
	5'-CGTCTTGGTCTGGATCGTGTATTTAG-3'	Exon 16			
AK035742	5'-GATGTAGGCAGGGGACTGATAGTTC-3'	Exon 1	F1	399	C
	5'-CCCTAACGGTGTTGATAATGCTCC-3'	Exon 3			
AK013767	5'-GCCTCCTTTGTCTTGTTTGCTGAC-3'	Exon 1	G1	312	B
	5'-GGAGATTGATGCTTTCTTTTGGC-3'	Exon 1			
AK085067	5'-GCAAGAGCGAAAATGGCAAAAC-3'	Exon 1	H1	272	B
	5'-TCCCAGGCACTAAACCACCAAC-3'	Exon 1			
BB776065	5'-CACCTAGAAGGGTGTGAACACGG-3'	Exon 1	I1	309	B
	5'-GGAGGCTGTGGGCTTCATATTG-3'	Exon 1			

Table 3 S continued

Pdzrn4 (XM_992218)	5'-TCTGGTTGTCGCCTGCTGTC-3'	Exon 1	J1	212	B
	5'-TCCTATTATGTTGAATCCCAAAGTGTC-3'	Exon 3			
	5'-CCGTTCACTGTTGTGTTGGAAAG-3'	Exon 3	J2	187	B
	5'-GCCTTACCGCAATGATTTTGTC-3'	Exon 4			
	5'-GAGCCCATAGTGGTACAGGTGTTAAG-3'	Exon 5	J3	247	B
	5'-AGACGTACTCATGGTCTTCATAAAACC-3'	Exon 6			
	5'-AAGAAGACACGGGCATTTATG-3'	Exon 7	J4	256	C
	5'-CATTCATTCACGAACTTGGTG-3'	Exon 9			
	5'-GCAAGGAGGAGAGAAAGCAACAC-3'	Exon 11	J5	277	B
	5'-GTCACCGACAGAAAGGCATTATG-3'	Exon 11			
	5'-TGAAGCAGCACCCCTTGAAC-3	Exon 11	J6	220	B
	5'-CCGTGGGCAAAGAAAATAACAGTC-3'	Exon 11			
AK048184	5'-CATTGGTTGCCAGAAACTACACTG-3'	Exon 3	K1	323	B
	5'-TCAGGTGACCATCCAACAGCAC-3'	Exon 3			
AK048652	5'-GAGTCTCTGTGTTGGCAAATGAATC-3'	Exon 1	L1	216	B
	5'-TTGTGGGAGGCACTGGGTAGATGAAC-3'	Exon 1			
AK137852	5'-GGCTAGACGGTGACAAGGCAATC-3'	Exon 1	M1	233	B
	5'-ATGGAACATGGAACTCCCTGAAG-3'	Exon 1			
AK081689	5'-GACGCTCTCTCCTGGATTCTGTAG-3'	Exon 1	N1	233	B
	5'-AAGTCGGCTTCTGGCAACACAC-3'	Exon 1			
AK047825	5'- TGAAGGCTGGCTGGAAGATG -3'	Exon 1	O1	174	B
	5'- TGGTTGTTGGAAGAGGAGGTGG -3'	Exon 1			
AK016367	5'-GCAGAAGGAATGAGAATGGACATC-3'	Exon 1	P1	122	B
	5'-AGTTGGCTTTTGCTGTGCTGAC-3'	Exon 1			

Table 4S. *Muc19* cDNA clones. Overlapping clones cover sequence from exon 1 to the 5'-end of exon 50. Clones from the 3'-end of exon 50 to each of two polyadenylation sites were produced by 3'-RLM RACE. PCR cycling profiles for 5'-end clones were: 95°C for 15 min followed by 40 cycles (94°C, 1 min; 55°C, 1 min; 72°C, 1 min) and a final 10 min extension at 72°C. PCR cycling profiles for 3'-end clones were: 94°C for 3 min followed by 35 cycles (94°C, 1 min; 66°C, 1 min; 72°C, 1 min) and a final 2 min extension at 72°C.

Mouse Strain	**Forward/Reverse Primers**	**Sequence Length (bp)**	**Included Exons**	**GenBank Accession Number**
sld	5'-GCCTGAACAGTCTCTACACTTAGG-3' 5'-TCTGCTTGCATGTACGAAGAG-3'	1,526	1, 19-31	HM132022
sld	5'-TGAACACTGGCGAGATGACTC-3' 5'-CTTGGGTCCAGGATAACTGATAC-3'	2,094	25-41	HM132023
NCR & *sld*	5'-CGAGACCGAAGCTGTGATATAG-3' 5'-GGTGATGTGTGCTTGTGCTTTAC-3'	1,797	36-50	HM132024
NCR & *sld*	5'-CCTCATCTTTCATTTCCCCATCTG-3' 5'-GCGAGCACAGAATTAATACGACT-3'	974	50-60	HM132025
NCR & *sld*	5'-CCTCATCTTTCATTTCCCCATCTG-3' 5'-GCGAGCACAGAATTAATACGACT-3'	1,079	50-60	HM132026

Table 5S. Genomic clones within the *Muc19/Smgc* locus. Clones were prepared from both NFS/NCr and NFS/N-*sld* DNA. Sequences of clones from both mouse strains were identical using primer sets 1-10, whereas clones from primers sets 11-14 contain variations between the two strains. Typically 25 ng of genomic DNA was amplified with 1 Unit of Accuprime™ *Taq* high fidelity DNA polymerase using 0.25 µM primers and a cycling profile of 95°C for 5 min followed by 35 cycles (95°C, 60 sec; 55°C 60 sec; 68°C, 2-10 min) and 7 min extension at 68°C.

Primer Set	GenBank Accession Number	Mouse Strain	Forward/Reverse Primers	Binding Site	Size (kb)
1	HM132005	NCr & *sld*	5'-GGTGCATCTCTCTTGCAGCTTTC-3' 5'-GTGACGATCTGGGACCTAAGTGTAG-3'	5' Flank Exon 1	7,762
2	HM132006	NCr & *sld*	5'-ATTGCCTTTGCCTTGCTGCG-3' 5'-TGGTCCAGGTCCCTTGTCTC-3'	5' Flank Intron 5	6,670
3	HM132007	NCr & *sld*	5'-GCTAGGGAATACACACTGTAG-3' 5'-TGGTCCATGATCCTCTGTC-3'	Intron 4 Exon 7	3,080
4	HM132008	NCr & *sld*	5'-GGATCATGGACCAACAAG-3' 5'-GCTTTAAGATTCTCGACAGC-3'	Exon 7 Exon 10	3,481
5	HM132009	NCr & *sld*	5'-GGACAGAACTTGTGTCTTC-3' 5'-GTGACAGACTGTTGAATGTG-3'	Intron 9 Exon 13	4,469
6	HM132010	NCr & *sld*	5'-AAGAGAGGCAAAGAATCAGG-3' 5'-CTCACAAAGACTGATTCGGTAG-3'	Intron 12 Intron 13	2,070
7	HM132011	NCr & *sld*	5'-AAGGATGGATCTAGGTTAGGTG-3' 5'-GGAGAGAGTATGCTCAGAACTTGATG-3'	Intron 13 Intron 14	1,110
8	HM132012	NCr & *sld*	5'-GAATGGCAGAAGGCATTTG-3' 5'-CCCAAAGATGAAGACCAAC-3'	Intron 14 Exon 18	4,200
9	HM132013	NCr & *sld*	5'-TAGTGGCAGAGTTACCTGTCCTACTG-3' 5'-TGGCGGGCTTAACAATAGACTAGC-3'	Exon 18 Intron 18	3,594
10	HM132014	NCr & *sld*	5'-CAGTGGTTGAATAGCCAGGAACAG-3' 5'-AGAGGGATACCTTTGGAAGCACAG-3'	Intron 18 Intron 18	3,675
11	HM132015	NCr	5'-GCTTCCAAAGGTATCCCTCTTAGTGAAGAC-3' 5'-GCCACAGACAAGCATTGAAACTGGACTATC-3'	Intron 18 Intron 21	5,559
11	HM132016	*sld*	5'-GCTTCCAAAGGTATCCCTCTTAGTGAAGAC-3' 5'-GCCACAGACAAGCATTGAAACTGGACTATC-3'	Intron 18 Intron 21	5,563
12	HM132017	NCr & *sld*	5'-GGGCATTGATCCTGGAATCATCTA-3' 5'-CCTGGAGTGACTGCAGATACCAAAT-3'	Intron 51 Intron 53	1,763
13	HM132018	NCr	5'-TTAAAGCATCTGGGAGGGATGGAAC-3' 5'-TGCAAAGATCAGACTGAAAATCGAG-3'	Intron 52 Intron 56	3,273
13	HM132019	*sld*	5'-TTAAAGCATCTGGGAGGGATGGAAC-3' 5'-TGCAAAGATCAGACTGAAAATCGAG-3'	Intron 52 Intron 56	3,277
14	HM132020	NCr	5'-GCCTCACATAAAAAGCAACACGAC -3' 5'-GACCCATAACCTCTAAAAGTGGTGC-3'	Intron 56 3' Flank	6,486
14	HM132021	*sld*	5'-GCCTCACATAAAAAGCAACACGAC -3' 5'-GACCCATAACCTCTAAAAGTGGTGC-3'	Intron 56 3' Flank	6,488

Table 6S. Primer pairs used to PCR amplify genomic DNA from NFS/NCr and NFS/N-*sld* mice for direct sequence comparisons. Amplicons of the appropriate molecular weight were isolated and sequenced directly. Sequences of amplicons from both mouse strains were identical for each primer set. PCR reactions incorporated 25 ng genomic DNA, 1 Unit of Accuprime™ *Taq* high fidelity DNA polymerase and 0.25 μM primers with a cycling profile of 95°C for 5 min, 35 cycles (95°C, 60 sec; 55°C, 60 sec; 68°C, 2-6 min) and 5 min extension at 68°C.

Primer Set	Forward/Reverse Primers	Binding Site	Size (kb)
1	5'-CGAAGTCCCACTACGACTAGAACAC-3' 5'-GAGAGTTTGTCCTTGCTCCACTTTAG-3'	Exon 19 Exon 22	2,779
2	5'-GCAAGGCAACCACTATTC-3' 5'-AAGGGCTGGGGCAGTCATTG-3'	Exon 22 Exon 24	1,460
3	5'-TGAGCATATTGCCAGTAGTAAG-3' 5'-CAAACCACAGCAGAGTCATC-3'	Exon 24 Exon 25	3,211
4	5'-GCAGACTACGTGTGATACCTAC-3' 5'-GGACTTTTCCTGGCTTGTAC-3'	Exon 25 Exon 28	1,408
5	5'-ACATTGGAGAGAAAGGAAAG-3' 5'-AGCCAAGAAGTGGCAGTC-3'	Exon 27 Exon 27	1,746
6	5'-GGTCTTGCACTGAAGCTCGATG-3' 5'-CTCCATCCTTTGTGACTGTC-3'	Exon 28 Exon 30	3,336
7	5'-AGGAGTTGGTTCTGTGAATGGAAG-3' 5'-GTGTACTTCAGACAGTTCTGCCTCC-3'	Intron 29 Intron 32	3,290
8	5'-AACACAGCCAATCCCCTTGC-3' 5'-AACTTTTCTCTTGCCCCACTTAGG-3'	Intron 32 Intron 35	3,760
9	5'-ACAGCAGATTGCACAGACGTTTC-3' 5'-CCATGACAGAAGTCAGCAGAGTTG-3'	Exon 35 Intron 39	4,827
10	5'-AATCAACTTGCCATGTCTACGGAG-3' 5'-TGACACCAGGATAACTCACACGTTC-3'	Exon 39 Intron 41	4,444
11	5'-ATCACAGCAATGGTAACCCTGG-3' 5'-CTTAGTTCAACCCGATGGAAACC-3'	Intron 41 Intron 48	5,938
12	5'-GAGTCCTGCATCCAATCTGTTAGTC-3' 5'-AGCTGAGGTCTCATTAGAGGCTGC-3'	Intron 48 Exon 50	4,089
13	5'-CCTCGTCCAGTGTGTCATCTAC-3' 5'-GCAGGAATCATGTATGCAGTG-3'	Exon 50 Intron 50	4,980
14	5'-AGACTGAGCCCCCGATTC-3' 5'-TCTAAAAGCCAGAAGGGTC-3'	Intron 50 Intron 51	6,117
15	5'-CCTCTTTGTTTTCCGACTG-3' 5'-TTGTGACTCCAGGTGCTAC-3'	Intron 51 Exon 52	4,981

Table 7S. Primers and PCR conditions used in the production and genotyping of Muc19 KO mice. The produce homology arms and to screen ES cells and F1 mice (outside 3'-arm primer set) we used Accuprime™ High fidelity Taq DNA polymerase with PCR cycling profiles of 94ºC for 2 min followed by 30-35 cycles (94°C, 1 min; 55-58°C, 1 min; 68°C, 5-13 min) and 10-20 min at 68°C.

To produce Southern probe templates, PCR reactions were run in Taq PCR Master Mix (Qiagen) for 3 min at 98°C followed by 35 cycles (94°C, 30 s; 60°C, 30 s; 74°C, 30 s) and 3 min at 72°C. PCR reactions to genotype KO mice were run in the FailSafe PCR buffer system (Epicentre). Conditions were: buffer B; 3 min at 94 °C followed by 30 cycles (94°C, 30 s; 55°C, 30 s; 72°C, 90 s) and 5 min at 72°C. The his3-dedl Region is within the STOP cassette from pBS302.

Primers to Produce Homology Arms		
Location	**Forward/Reverse Primers**	**Product Size (bp)**
5'-Arm	5'-GGACCCATTTGATTGCCTCG-3' 5'-GCTTCTGAGTATGTGGGAGTCGC-3'	5,693
3'-Arm	5'-GCCCTTTGAGCAGGAGCAATG-3' 5'-CGAGTGTAAGTGTGCCCTGAAGTTG-3'	4,390
Primers to Screen ES Cells and F1 Mice		
934 bp 5' of 5'-Arm Within PGK Promoter	5'-TGAGGCATCTCAAGGGTAGC-3' 5'-TTCCTGACTAGGGGAGGAGTAG-3'	5,977
Within neo 84 bp 3' of 3'-Arm	5'-TCGGCTATGACTGGGCACAACAGAC-3' 5'-TGGAGCACGTTGGAGGATTTGAAG-3'	5,028
Primers to Produce Southern Probes		
Pvu II Probe	5'-TGCCATCTCTAATAGTTTCAGTGC-3' 5'-CTCTTGAAGGGGACACCCTAAACC-3'	308
Sph I Probe	5'-TTCGCGATTCACAAGTGTGCATG-3' 5'-GCACATTAATTAGCTTGGAGCAC-3'	332
Primer Set to Genotype KO Mice		
Intron 24-Deleted Region In his3-dedl Region In 3'-arm	For: 5'-CAGACCCCTGAGGACATTCTTG-3' For: 5'-TGAGGAGGAACATAACCATTCTCG-3' Rev: 5'-GGCTTGGAGTGACAGTTTGGATAC-3'	509 (Wild Type) 1,263 (Muc19 KO)

Table 8S. TaqMan gene expression assays. Standard assays used for evaluating transcripts in the critical interval are given, as well as a custom assay to detect *Muc19* heteronuclear RNA. In all cases, assays were performed in triplicate. Site, assay location along transcript (GenBank Accession Number in parentheses); context sequence, target sequence where TaqMan probe hybridizes (see https://products.appliedbiosystems.com/ for more information); Exons, exons in which the primers bind; Amp, amplicon length in base pairs; *Lrrk2*, leucine-rich repeat kinase 2; *Muc19*, mucin 19; *Smgc*, submandibular gland protein C; *Cntn1*, contactin 1; *Actb*, beta actin; *Hprt1*, hypoxanthine guanine phosphoribosyl transferase 1; *Rn18s*, 18S ribosomal RNA.

Standard TaqMan Assays					
Transcript	**Assay ID**	**Exons**	**Site**	**Context Sequence**	**Amp**
Muc19 (NM_207243)	Mm01306462_m1	51-52	21,866	5'-ACCAGTATGCCAGCTTCCACCTCGG-3'	82
Smgc (NM_198927)	Mm01303795_g1	17-18	2,091	5'-GTGACTCAGGAAGCCATAATTTGTC-3'	93
Lrrk2 (NM_025730)	Mm00481934_m1	47-48	7,027	5'-AAAACCAACCAGCTGTTTTCTTACG-3'	88
Cntn1 (NM_007727)	Mm00514374_m1	23-24	3,233	5'-AAGTCAAAATTTCAGGCGTGTCCAC-3'	64
Actb (NM_007393)	Mm00607939_s1	6-6	1,230	5'-TACTGAGCTGCGTTTTACACCCTTT-3'	115
Hprt1 (NM_013556)	Mm00446968_m1	6-7	630	5'-GTTAAGGTTGCAAGCTTGCTGGTGA-3'	65
B2m (NM_009735)	Mm00437762_m1	1-2	117	5'-GTATGCTATCCAGAAAACCCCTCAA-3'	77
Rn18s (NR_003278)	Hs99999901_s1	1-1	609	5'-TGGAGGGCAAGTCTGGTGCCAGCAG-3'	187

Custom TaqMan Assay to *Muc19* Heteronuclear RNA			
Assay ID	**Location**	**Context Sequence**	**Amp**
MUC19HNRNA-MU19	Intron 19 - Exon 20	5'-TCAACACAAGAGAGAAATAA-3'	93
Forward Primer	5'-CCTTGGGAATACAATTTGAATCAATGCA-3'		
Reverse Primer	5'-GCTTCTGGAACATGGGATGCTT-3'		

Primers to Prepare Standard Templates for each TaqMan Assay	
Transcript	**Forward/Reverse Primers**
Muc19	5'-TTCATTTCCCCATCTGGACATC-3' 5'-GTCTGGACACTCTGGCTGAGATATG-3'
Smgc	5'-TAATGCACAATCAAGCAACAAC-3' 5'-AATTATGGCTTCCTGAGTCACTC-3'
Lrrk2	5'-AGGGTGTGGCACAAAGGTCTTC-3' 5'-TGGCAATATACAGGGCTGTGTCTAC-3'
Cntn1	5'-CGAGTAGACAAGAAAGCCAAGGGAG-3' 5'-TTCCGAGTAGACAAGAAAGCCAAG-3'
Actb	5'-GGCTGCTTTGCTTTCAGTTTTGTAG-3' 5'-CCCAGTTACTAAGTGGTTTTTTTGCTTG-3'
Hprt1	5'-TGGAAAGAATGTCTTGATTG-3' 5'-CTTGTCTGGAATTTCAAATC-3'
Rn18s	5'-ACGGCTACCACATCCAAGGAAG-3' 5'-GGACACTCAGCTAAGAGCATCGAG-3'
Muc19 hnRNA	5'-GGAACACGACATTTAGCGTCTCAC-3' 5'-CTGGAACATGGGATGCTTTTTC-3'

Table 9S. Primer pairs to create minigenes and for detecting spliced and unspliced products from minigenes and in sublingual glands. PCR reactions to create minigenes incorporated 25 ng genomic DNA, 1 Unit of Accuprime *Taq* high fidelity DNA polymerase and 0.25 μM primers with a cycling profile of 95°C for 5 min, 35 cycles (95°C, 60 sec; 55°C, 60 sec; 68°C, 2 min) and 2 min final extension at 68°C.

PCR reactions to detect splicing products used random-primed cDNA (5 ng or more) as template and 1 Unit of Accuprime *Taq* high fidelity DNA polymerase and 0.25 μM primers. Cycling profiles were 94C for 5 min followed by 25-30 cycles (94°C, 1 min; 60-62°C, 3 min; 68°C, 1 min) with 5 min final extension at 68°C.

To detect neo and β-actin cDNA we used a cycling profile of 94°C for 3 min followed by 30 cycles (94°C, 30 sec; 56°C 30 sec; 68°C, 2 min) with no final extension.

Cycling conditions for SYBR Green qPCR assays (20 μl reactions) for correctly and aberrantly spliced *Muc19* transcripts using primers F4/R4 and F5/R5, respectively, and *Actb* primers (endogenous control) were 95°C for 10 min followed by 40 cycles (95°C, 15 sec; 60°C, 1 min; 68°C, 1 min). These same primers were used to prepare standard templates for quantification of copy numbers per reaction.

Primer pairs to create minigenes	
Genomic Location	**Forward/Reverse Primers**
Intron 52 Intron 55	5'-ACCTTGAGAGGCTAACCTGTGTT-3' 5'-CGTATTTAGTTCATCTGGTGTGTTACA-3'
Intron 56 Intron 58	5'-CCCCACTTAACTTGCAGATT-3' 5'-TATTTTTCTATATACAGAAGTCAACTTACA-3'
Top Strand of 99 bp Inserted into Multiple Cloning Site of pEGFP-N3	
5'-TTAGCTAGCTACCGGACTCAGACAACCTTCTCGAGAAAGAATCTTAGTC CTGCTACTGGTGGCTCTGCAACACAACAGTCTAATTTAGATGGATCCTCC-3'	
Primers for PCR of cDNA from expressed minigenes and in sublingual glands	
Primer ID	**Genomic Location**	**Forward/Reverse Primers**
F1	Exon 2	5'-GAGTTCTGGTGGGAGCAAAGATTAC-3'
R1	EGFP	5'-ACGTTGTGGCTGTTGTAGTTGTACTC-3'
F2	Exon 57	5'-GGTTCATCCTTTGATGACCCAAG-3'
R2	Exon 58	5'-TTATTTGAATCGTAGATTCTCTCTTC-3'
F3	Exon 53	5'-GTGTCCAGACTCACTCCCACCAAC-3'
R3	Exon 55	5'-TTTTTCGGTACAGGTACACTGATG-3'
F4	Exons 51/52	5'-GAACCACCAGTATGCCAGCTT-3'
R4	Exons 55/54	5'-TACGTCCCCAGGAGATTTCTCT-3'
F5	Exons 50/51	5'-CCTTCAGTCATTAAAACAGGAGGGACGACT-3'
R5	Intron 54/Exon 54	5'-GAGAGACCAGAACTGCATGCTTACAGATTTCT-3'
F6	Exon 1	5'-GATCGTCACCATGAAGCTGATA-3'
R6	Exons 22/21	5'- GTGGCTATAAAGAATGAGTGAGAGT-3'
Neo-F	*neo*	5'-GATCTGGACGAAGAGCATCAG-3'
Neo-R	*neo*	5'-CCATGATATTCGGCAAGCAG-3'
β-actin-F	*Actb* Exon 1	5'-GAGCACAGCTTCTTTGCAGCTC-3'
β-actin-R	*Actb* Exon 3	5'-CACCCACATAGGAGTCCTTCTGAC-3'