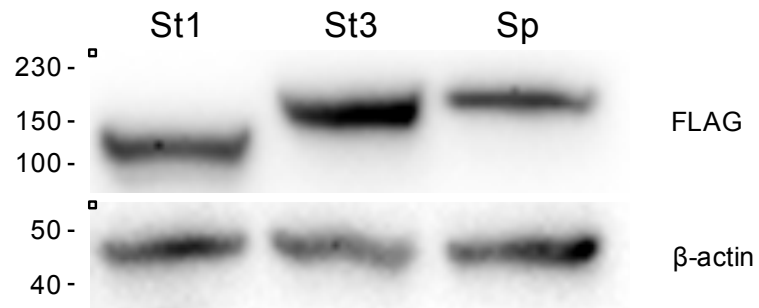


## ***Streptococcus thermophilus* CRISPR-Cas9 systems enable specific editing of the human genome**

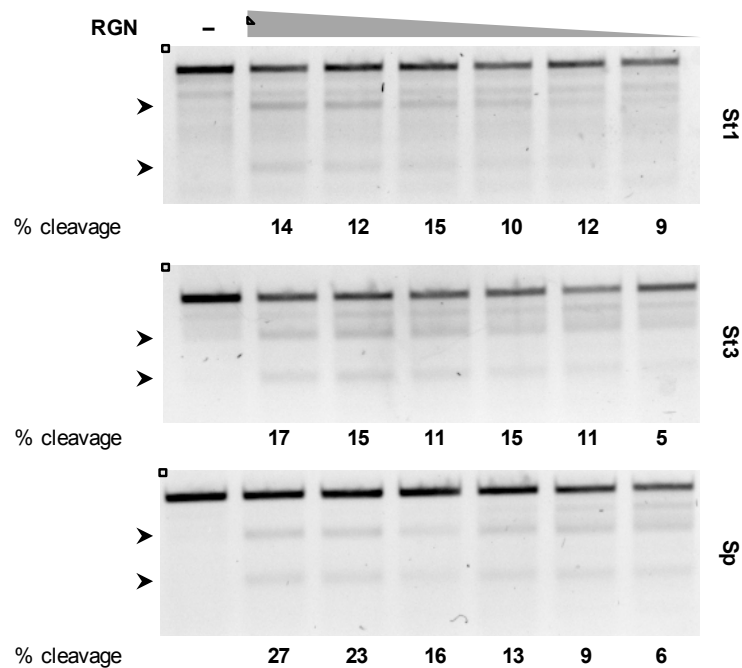
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### **SUPPLEMENTARY MATERIAL**

- Figure S1.** Expression levels of Cas9 orthologs.
- Figure S2.** Titration of *CARD11*-specific RGN activity.
- Figure S3.** Amino acid sequences of Cas9 proteins used in this study.
- Figure S4.** DNA sequences of gRNAs used in this study.
- Table S1.** Cas9 orthologs used in this study.
- Table S2.** Sequencing results for *PRKDC*-specific RGNs.
- Table S3.** Sequencing results for *CARD11*-specific RGNs.
- Table S4.** Oligonucleotides used in this study.
- Table S5.** Sequencing primers for *PRKDC*-specific RGNs.
- Table S6.** Sequencing primers for *CARD11*-specific RGNs.



**Supplementary Figure S1. Expression levels of Cas9 orthologs.** The expression levels of the three different Cas9 proteins was assessed in HEK293T cells, two days post-transfection. Cell lysates were assayed using anti-FLAG tag antibody that specifically recognizes FLAG-tagged Cas9 proteins and anti- $\beta$ -actin antibody to monitor the total protein load. Numbers on the left indicate the protein ladder (kDa).



**Supplementary Figure S2. Titration of *CARD11*-specific RGN activity.** The correlation between RGN activity and amount of expression plasmid transfected was assessed in HEK293T cells via T7 endonuclease I (T7EI) assay three days post-transfection. Different amounts of plasmid DNA expressing the Cas9 and the gRNA were transfected using a constant 1:3 ratio. Arrows indicate the positions of the expected cleavage products. Numbers at the bottom show the average percentages of modified alleles (n=3).

***Streptococcus thermophilus* CRISPR1 Cas9 (St1Cas9)**

MSDLVLGLD**I**DIGIGSVGVGILNKVTGEI**I**HKNSRIFPAAQAENNLVRRTRNRQGRRLTRRKKHRRVRLNRLFEEESGLITDFTKIS  
 INLNPYQLRVKGLTDELSNEELFIALKNMVKHRGISYLDASDDGNSSIGDYAQIVKENSQLETKTPGQIQQLERYQTYGQLR  
 GDFTEVKDGKKHRLINVFPTSAYRSEALRILQTTQEEFNQITDEFINRYLEILTGKRKYHGPNEKSRDYGRYRTSGETLD  
 NIFGILIGKCTFPYDFRAAKASYTAQEFNLLNDLNLVPTETKKSKEQKNQIINYVKNEKAMGPAKLFKYIAKLLSCDVA  
 DIKGYRIDKSGKAEIHTFEAYRKMKTLETLDIEQMDRETLDKLAYVLTNTEREGIQEAELEHEFADGSFSQKQVDELVQFRKA  
 NSSIFGKGWHNFSVKLMMELIPELYETSEEQMTILTRLGKQKTTSSSNKTKYIDEKLLTEEIYNPVAKSVRQAIIKIVNAAIK  
 EYGDNDNIVIEMARETNEDEKKAIQKIQKANKDEKDAAMLKAANQYNGKAELPHSVFHGHKQLATKIRLWHQOGERCLYTGK  
 TISIHDLINNSNQFEVD**H**ILPLSITFDDSLANKVLVYATANQEKQRTPYQALDSMDDAWSFRELKAFVRESKTLNKKKEYL  
 LTEEDI SKFDVRKKFIERNLVDTRYASRVVNLALQEHFRAHKIDTKVSVVRGQFTSQRLRRHWGIEKTRDTYHHHAVDALI IAA  
 SSQNLNLWKKQKNTLVSYSEDQLLDIETGELISDDEYKESVFKAPYQHFDVTLKSKEFEDSILFSYQVDSKFNKISDATIYAT  
 RQAKVGKDKADETYVLGKIKDIYTDQGYDAFMKIYKDKSKFLMYRHDPTFEKVIEPILENYPNKQINEKGEVPCNPFLKY  
 KEEHGYIRKYSKKGNGPEIKSLKYDYSKLGNHIDITPKDSNNKVVLSVSPWRADVFNKTTGKYEILGLKYADLQFEKGTGT  
 YKISQEKYNDIKKKEGVSDSEFKFTLYKNDLLLKVDTEKQQLFRFLSRTMPKQKHVELKPYDKQKFEFGGEALIKVLGNV  
 ANSGQCKKGLGKSNISYKVRTDVLGNQHI IKNEGDKPKLDF

***Streptococcus thermophilus* CRISPR3 Cas9 (St3Cas9)**

MTKPYSIGLD**I**DIGTNSVGAVITDNYKVPSSKMKVLGNTSKYIKKNLLGVLLFDSGITAEGRRLKRTARRRYTRRRNRILYLQ  
 EIFSTEMATLDDAFFQRLDDSFVLPDDKRDSKYP IFGNLVEEKVYHDEFPTIYHLRKYADSTKKADLRLVYALAHMIKYRG  
 HFLIEGEFNSKNNDIQKNFQDFLDYNAIFESDLSLENSKQLEEVKDKISKLEKKDRILKLFPGKNSGIFSEFLKLVGNQ  
 ADRKCFNLDEKASLHFSKESYDEDELETLGGYIGDYSDFVLKAKKLYDAIILSGFLTVDNETEAPLSSAMIKRYNEHKEDL  
 ALLKEYIRNISLKYNEVFKDDTKNGYAGYIDGKTNQEDFYVYLNLLAEFEGADYFLEKIDREDFLRKQRTFDNGSIPYQIH  
 LQEMRAILDQAKFYPPFLAKNKERIEKILTFRIPIYVVGPLARGNSDFAWSIRKRNEKITPWNFEDVIDKESSAEAFINRMTSF  
 DLYLPEEKVLPKHSLLYETFNVYNELTKVRFIAESMRDQYFLDSKQKDIVRLYFKDKRKTVDKDIIEYLHAIYGYDGIELKG  
 IEKQFNSSLSTYHDLNIINDKEFLDSSNEAIEEIIHTLTI FEDREMIKQRLSKFENIFDKSVLKKLSRRHYTGWGKLSAK  
 LINGIRDEKSGNTILDYLIIDDGISNRNFMQLIHDDALSFKKKIQKAQIIGDEDKGNIEVVKSLPGSPAIKKILQSIKIVDE  
 LVKVMGGRKPEISVEMARENQYTNQKSNQRLKRLKLEKSKELGSKILKENIPAKLSKIDNNALQNDRLYLYLQNGKDMY  
 TGDDLDIDRLSNYDIDHIIPQAFKDNSIDNKVLSVSS**N**RGKSDDFPSLEVVKKRKTFWYQLLKSCLIQRKFDNLTKAERG  
 GLLPEDKAGFIQRQLVETRQITKHVARLLDEKFNKKDENNRRAVRTVKIITLKSTLVSQFRKDFELYKREINDFHHAHDAYL  
 NAVIASALLKKYPKLEPEFVYGDYPKYNSFRERKSATEKVYFYSNIMNIFKKSISLADGRVIERPLIEVNEETGESVWNKESD  
 LATVRRVLSYPQVNVVKKVEEQNHGLDRGPKGLFNANLSSKPKPNSNENLVGAKEYLDPKKYGGYAGISNSFAVLVKGTEK  
 GAKKIKTNVLEFQGISILDRINRYRDKLNFLLKGYKDIELIIELPKYSLEFELSDGSRMLASILSTNNKRGEIHKGNQFLS  
 QKFVKLLYHAKRISNTINENHRKYVENHKKFEFEELFYILEFNENYVGAKKNGKLLNSAFQSQWQNSIDELCSSFIGPTGSE  
 R KGLFELTSRGSAADEFELGVKIPRYRDYTPSSLLKDATLIHQSVTGLYETRIDLAKLGE

***Streptococcus pyogenes* Cas9 (SpCas9)**

MDKKYSIGLD**I**DIGTNSVGAVITDEYKVPSSKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQ  
 EIFSNEMAKVDDSFHRLLEESFLVEEDKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTDKADLRLIYLALAHMIKFRG  
 HFLIEGLNPDNSVDKFLFIQLVQTYNQLFEENPINASGVDAKAIL SARLSKSRLENLIAQLPGEKKNGLFGNLIALSILGLT  
 PNFKSNFDLAEDAQLQSKDQYDQYADLFLAANKLSDAIIILSDILRVNTEITKAPLSASMIKRYDEHHDQL  
 TLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKNLREDDLRRQRTFDNGSIPYQIH  
 LGELHAILRRQEDFYPPFLKDNREKIEKILTFRIPIYVVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGSASAQSFIERMTNF  
 DKNLPNEKVLKHSLLYEFYVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTRKRVTVKQLKEDYFKKIECFDSVEIS  
 GVEDRFNASLGTYHDLKIKKDKFDLNEENEDILEDIVLTLTLFEDREMIERLKYAHLFDKVMKQLKRRRYTGWGRLSR  
 KLINGIRDKQSGKITLDFLKSDFANRNFMLIHDDSLTFKEDIQKAQVSGQDLSHEHIANLAGSPAIKKILQTVKVVDEL  
 VKVMGRHKPENIVIEMARENQTTQKQKNSRERMKRIEIEGKELGSQLKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDI  
 NRLSDYD**V**D**H**IVPQSFVKDDSDNKVLTSDKNRGSNDVPSSEVVKMKNYWRQLLNKLIQTKRFDNLTKAERGGLSELDK  
 AGFIKRLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVIITLKSCLVSDFRKDFQFYKREINNYHHAHDAYLNAVVGTA  
 LIKKYPKLESEFVYGDYKVDVRKMIKSEQEI GKATAKYFFYSNIMNFKTEITLANGEIRKRPLIETNGETGEIVWDKGRD  
 FATVRKVL SMPQVNI VVKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVAKVEKGSKLLKSVKE  
 LLGITIMERSSEFKNPIDFLEAKGYKEVKKDLI IKLPKYSLEFELNGRKRMLASAGELQKGNELALPSKYVNFYLYASHYEKL  
 KGSPEQNEQQLFVEQHKHYLDEIEQISEFSKRVILADANLDKVL SAYNKHRDKPIREQAENI IHLFTLTLNLGAPAAFKYFD  
 TTIDRKRYTSTKEVLDATLIHQSIITGLYETRIDLSQLGGD

**Supplementary Figure S3. Amino acid sequences of Cas9 proteins used in this study.** The protein sequences of the Cas9 orthologs used in this study are shown. Amino acid residues highlighted in bold and underlined have been changed to Alanine to generate catalytically 'dead' Cas9 proteins (dCas9).

**gRNAs for St1Cas9**PRKDC

**GCGTATCCAGCACTTGTGGT**GTTTTTGTACTCTCAAGATTCAATAATCTTGCAGAAGCTACAAAGATAAGGCTTCATGCC  
GAAATCAACACCCTGTCATTTTATGGCAGGGTGT

CARD11

**GATGAAGAGGACGCCTTGTG**GTTTTTGTACTCTCAAGATTCAATAATCTTGCAGAAGCTACAAAGATAAGGCTTCATGCC  
GAAATCAACACCCTGTCATTTTATGGCAGGGTGT

**gRNAs for St3Cas9**PRKDC

**GGCGTATCCAGCACTTGTGGG**GTTTTTAGAGCTGTGTTGTTTGTAAAACAACACAGCGAGTTAAAATAAGGCTTAGTCCGT  
ACTCAACTTGAAAAGGTGGCACCATTTCGGTGT

CARD11

**GGATGAAGAGGACGCCTTGTG**GTTTTTAGAGCTGTGTTGTTTGTAAAACAACACAGCGAGTTAAAATAAGGCTTAGTCCGT  
ACTCAACTTGAAAAGGTGGCACCATTTCGGTGT

**gRNAs for SpCas9**PRKDC

**GGCGTATCCAGCACTTGTGGG**GTTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGT  
GGCACCAGTTCGGTGCTTTTTTTT

CARD11

**GGATGAAGAGGACGCCTTGTG**GTTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGT  
GGCACCAGTTCGGTGCTTTTTTTT

**Supplementary Figure S4. DNA sequences of gRNAs used in this study.** The individual spacers are highlighted in bold.

**Supplementary Table S1. Cas9 orthologs used in this study.**

<b>Acronym</b>	<b>Organism</b>	<b>PAM</b>	<b>Ref</b>
Sp	<i>Streptococcus pyogenes</i>	5'-NGG	9
St1	<i>Streptococcus thermophilus</i>	5'-NNAGAAW	32
St3	<i>Streptococcus thermophilus</i>	5'-NGGNG	30

Supplementary Table S2. Sequencing results for *PRKDC*-specific RGNs

Nuclease ID	OT ID	target sequence <sup>1</sup>	mismatches	closest gene	region	hg19 coordinates	empty vector			low activity				high activity			
							indels	total	mutation frequency	indels	total	mutation frequency	p-value <sup>2</sup>	indels	total	mutation frequency	p-value <sup>2</sup>
<b><i>PRKDC</i>-specific RGNs</b>																	
SpCas9	Target	GGCGTATCCAGCAGCTTGTGGTGG	0	<i>PRKDC</i>	Exon	chr8:48710914	224	38887	0,58	<b>4687</b>	<b>38571</b>	<b>12,15</b>	<b>0,000</b>	<b>7101</b>	<b>30443</b>	<b>23,33</b>	<b>0,000</b>
	Sp_OT_01	AaaGtTCCAGCAGCTTGTGGTGG	3	<i>CASC10</i>	Intergenic	chr10:21717537	12	49453	0,02	2	31125	0,01	0,096	1	24940	0,00	0,073
	Sp_OT_02	CtatTACCAGCAGCTTGTGGAGG	4	<i>CGSER1</i>	Intron	chr4:92219980	5	31853	0,02	15	55573	0,03	0,357	4	22455	0,02	1,000
	Sp_OT_03	AagGagTCCAGCAGCTTGTGGAGG	4	<i>C17orf51</i>	Intergenic	chr17:21409948	3	17471	0,02	1	24458	0,00	0,315	3	30351	0,01	1,000
	Sp_OT_04	AagGagTCCAGCAGCTTGTGGAGG	4	<i>FLJ36000</i>	Intergenic	chr17:21958996	4	31707	0,01	4	39715	0,01	1,000	3	23366	0,01	1,000
	Sp_OT_05	CaCtTtTaCAGCAGCTTGTGGAGG	4	<i>LOC389033</i>	Intergenic	chr2:130424558	3	19903	0,02	3	18642	0,02	1,000	1	11896	0,01	1,000
	Sp_OT_06	AaaGTATCCAGCAGCTTGTGGAGG	3	<i>LINC01384</i>	Intron	chr20:22587913	1	30259	0,00	6	32989	0,02	0,127	<b>10</b>	<b>23518</b>	<b>0,04</b>	<b>0,002</b>
	Sp_OT_07	GcCGctCCAGCAGCTTGTGGAGG	4	<i>BRD1</i>	Intron	chr22:50180357	5	7611	0,07	<b>15</b>	<b>8293</b>	<b>0,18</b>	<b>0,045</b>	2	5424	0,04	0,707
	Sp_OT_08	GctGTAGCCAACACTTGTGGTGG	4	<i>AMOTL1</i>	Intergenic	chr11:94649052	12	38733	0,03	20	35226	0,06	0,111	<b>27</b>	<b>34619</b>	<b>0,08</b>	<b>0,006</b>
	Sp_OT_09	CcCaTACCAGCAGCTTGTGGTGG	3	<i>PABPC4L</i>	Intergenic	chr4:135380087	9	38250	0,02	12	44525	0,03	0,829	8	45010	0,02	0,631
	Sp_OT_10	TttGTACCAGCAGCTTGTGGTGG	4	<i>LINC01591</i>	Intergenic	chr8:136240411	5	11271	0,04	3	18437	0,02	0,165	4	18134	0,02	0,318
	Sp_OT_11	GaCcTgTCCAGCAGCTTGTGGCAG	4	<i>PSMD13</i>	Intron	chr11:250126	5	47957	0,01	34	277657	0,01	1,000	8	36716	0,02	0,263
	Sp_OT_12	CtCcTAgCCAGACTTGTGGGAG	4	<i>TMEM45B</i>	Intergenic	chr11:129673387	19	6690	0,28	7	6239	0,11	0,031	18	5276	0,34	1,000
	Sp_OT_13	GtaaT-TCCAGCAGCTTGTGGAGG	3, del 15	<i>MYH10</i>	Intron	chr17:8419116	146	34171	0,43	98	21599	0,45	1,000	120	30474	0,39	0,538
	Sp_OT_14	GtCaTc-CCAGCAGCTTGTGGAGG	3, del 14	<i>PAFAH2</i>	Intergenic	chr1:26269301	3	22097	0,01	7	27384	0,03	0,528	5	46150	0,01	1,000
	Sp_OT_15	TGC-TggCCAGCAGCTTGTGGTGG	2, del 17	<i>EPB41L3</i>	Intergenic	chr18:5718306	1	43393	0,00	2	36822	0,01	0,597	<b>6</b>	<b>28198</b>	<b>0,02</b>	<b>0,017</b>
	Sp_OT_16	CtgGTgTC-AGCAGCTTGTGGTGG	3, del 12	<i>RP2A2</i>	Intron	chr11:990375	13	27774	0,05	10	29611	0,03	0,533	9	23668	0,04	0,675
	Sp_OT_17	GGC-aATtCAGCAGCTTGTGGGGG	2, del 17	<i>RM26</i>	Intergenic	chr13:79664515	47	39999	0,12	32	28021	0,11	1,000	33	34940	0,09	0,370
	Sp_OT_18	GGC-aATtCAGCAGCTTGTGGGGG	2, del 17	<i>RM26</i>	Intergenic	chr13:79669657	45	42631	0,11	57	46663	0,12	0,489	28	25953	0,11	1,000
	Sp_OT_19	GGgGTcTC-AGCAGCTTGTGGGGG	2, del 12	<i>PDE4A</i>	Intron	chr19:10540553	23	32130	0,07	27	32353	0,08	0,672	28	25050	0,11	0,121
	Sp_OT_20	ActGT-TgCAGCAGCTTGTGGAGG	3, del 15	<i>SNAP29</i>	Exon	chr22:21243551	3	41052	0,01	<b>13</b>	<b>41038</b>	<b>0,03</b>	<b>0,013</b>	<b>13</b>	<b>32574</b>	<b>0,04</b>	<b>0,004</b>
	Sp_OT_21	GcCtTA-gCAGCAGCTTGTGGGGG	3, del 14	<i>ANAPC1</i>	Intergenic	chr2:112442281	53	39046	0,14	53	36381	0,15	0,771	29	29041	0,10	0,219
	Sp_OT_22	AatGTcTCCAGCAGCTTGTGGTGG	4	<i>CORIN</i>	Intron	chr4:47652995	35	28595	0,12	38	29274	0,13	0,816	30	26196	0,11	0,805
	Sp_OT_23	GtgGTtTCCAGCAGCTTGTGGTAG	4	<i>TBX22</i>	Intergenic	chrX:79087058		PCR Failure			PCR Failure				PCR Failure		
Sp_OT_24	GtaaT-TCCAGCAGCTTGTGGGAG	3, del 15	<i>RAH1F</i>	Intergenic	chr1:202845889	3	20052	0,01	3	24970	0,01	1,000	4	27173	0,01	1,000	
St1Cas9	Target	GGGTATCCAGCAGCTTGTGGGAGAAA	0	<i>PRKDC</i>	Exon	chr8:48710909	224	38887	0,58	<b>3362</b>	<b>35995</b>	<b>9,34</b>	<b>0,000</b>				
	St1_OT_01	CttTATttAGCAGCTTGTGGTAGAGAAA	4	<i>LINC00669</i>	Intergenic	chr18:36515102	42	28357	0,15	50	35777	0,14	1,000				
	St1_OT_02	TtcTtTCCAGCAGCTTGTGGTAGAGAAA	4	<i>SOX2-OT</i>	Intron	chr3:180924313	3	3235	0,09	10	9084	0,11	1,000				
	St1_OT_03	TgaTcaCCATcACTTGTGGTCAGGAAA	5	<i>SORCS1</i>	Intron	chr10:108706808	1	21224	0,00	7	37166	0,02	0,272				
	St1_OT_04	AcATaaCatCACTTGTGGTAGGAAA	5	<i>MGAT4C</i>	Intergenic	chr12:86347672	9	77347	0,01	4	41711	0,01	1,000				
	St1_OT_05	CtGTAgacACcACTTGTGGTTAGGAAA	4	<i>MCR2</i>	Intron	chr18:13890178	4	22065	0,02	12	34146	0,04	0,311				
	St1_OT_06	TaGcATaCAGtACTTGTGGTTAAGAAA	4	<i>BAZ1A</i>	Intergenic	chr14:35367791	11	27203	0,04	9	39675	0,02	0,255				
	St1_OT_07	TttTtTCCAttACTTGTGGTCCAGGAAA	5	<i>VWA5B1</i>	Intergenic	chr1:20682682	3	5704	0,05	6	7279	0,08	0,740				
	St1_OT_08	gAGTtCCAGCAGCTTGTGGTAGGAAA	4	<i>LINC01603</i>	Intergenic	chr8:70291893	4	5627	0,07	8	7814	0,10	0,771				
	St1_OT_09	TgGcAgCCAGCAGCTTGTGGTAGGAAA	4	<i>NUBPL</i>	Intergenic	chr14:32426712	6	26641	0,02	5	38117	0,01	0,377				
	St1_OT_10	GgaTATgCAGctCTTGTGGTCTAGAAAT	5	<i>MRPL3</i>	Intergenic	chr3:131150014	11	14953	0,07	5	31190	0,02	0,005				
	St1_OT_11	ACTaaTAc-GCACTTGTGGTTCAGGAA	3, del 12	<i>HLF</i>	Intron	chr17:53357071	4	17830	0,02	4	40608	0,01	1,000				
	St1_OT_12	TCaTtT-CAaCACTTGTGGTGTGGAAAG	3, del 14	<i>MCHR2-AS1</i>	Intron	chr6:100513422	8	30733	0,03	7	41283	0,02	0,442				
	St1_OT_13	TtaTcTC-AaCACTTGTGGTAAGGAAAG	4, del 13	<i>SERPINA13P</i>	Intergenic	chr14:95154225	2	15147	0,01	2	2124	0,09	0,077				
	St1_OT_14	GgGTAg-CAaCACTTGTGGTTAGGAAAG	3, del 14	<i>LOC101929413</i>	Intergenic	chr20:10813238	4	23752	0,02	2	40044	0,00	0,204				
	St1_OT_15	GgagATCCAGgAgTGTGGTGGGGAAG	5	<i>UBA1</i>	Intergenic	chrX:47075905	2	6650	0,03	11	14019	0,08	0,246				
	St1_OT_16	GgcTtTCCACcAGcGTGGTGTGGAAAG	5	<i>IL33</i>	Intergenic	chr9:6125137	3	5528	0,05	6	8480	0,07	1,000				
	St1_OT_17	AtGTATaCatCAcATGTGGTTCAGGAT	4	<i>CRBM</i>	Intron	chr10:35499012	1	25594	0,00	3	33056	0,01	0,637				
	St1_OT_18	CaGTATaCAGaAtTGTGGTGTAGGAG	4	<i>MDGA2</i>	Intron	chr14:47818229	0	27314	0,00	5	36154	0,01	0,075				
	St1_OT_19	TatgATCC-cCACTTGTGGTTCAGGAAA	4, del 12	<i>SUDS3</i>	Intergenic	chr12:119091119	2	20666	0,01	8	50462	0,02	0,734				
	St1_OT_20	TttgAaCCAG-ActTGTGGTGGAGGAG	4, del 10	<i>SORCS3</i>	Intron	chr10:106464716	11	18359	0,06	6	30082	0,02	0,042				
	St1_OT_21	CCtaAttCAG-ActTGTGGTAAAGAAA	3, del 10	<i>MIR1468</i>	Intergenic	chrX:63011205	14	23440	0,06	17	47124	0,04	1,000				
	St1_OT_22	TaaTtCCAG-ActTGTGGTAAAGGAA	4, del 10	<i>RORA</i>	Intron	chr15:61403537	5	28153	0,02	10	39590	0,03	0,607				
	St1_OT_23	CtGAgCCAGC-CTTGTGGTGTGGAAA	3, del 9	<i>CXCR4</i>	Intergenic	chr2:137257834	3	229996	0,00	<b>3</b>	<b>23553</b>	<b>0,01</b>	<b>0,013</b>				
St1_OT_24	TgGTACCAGtAcCTTGTGGTAGAGAAAG	4	<i>MIR7641-2</i>	Intergenic	chr4:56953086	3	23579	0,01	2	24050	0,01	0,684					





Supplementary Table S3. Sequencing results for *CARD11*-specific RGNs

Nuclease ID	OT ID	target sequence <sup>1</sup>	mismatches	closest gene	hg19 coordinates	empty vector			low activity				high activity			
						indels	total	mutation frequency	indels	total	mutation frequency	p-value <sup>2</sup>	indels	total	mutation frequency	p-value <sup>2</sup>
<b><i>CARD11</i>-specific RGNs</b>																
SpCas9	Target	GGATGAAGAGGACGCCCTGTGGG	0	<i>CARD11</i> Exon	chr7:2987359	12	31050	0,04	<b>3829</b>	<b>65385</b>	<b>5,86</b>	<b>0,00</b>	<b>4241</b>	<b>41966</b>	<b>10,11</b>	<b>0,00</b>
	Sp_OT_01	GGAcGAAGAGGtCGCCTGTGGG	2	<i>FOXA1</i> Intron	chr14:38063708	3	41570	0,01	<b>412</b>	<b>34650</b>	<b>1,19</b>	<b>0,00</b>	<b>1117</b>	<b>38987</b>	<b>2,87</b>	<b>0,00</b>
	Sp_OT_02	AcATGtgGAGcAGCCTGTGGG	4	<i>PTPRB</i> Intron	chr12:70947248	7	8726	0,08	11	67916	0,02	1,00	7	38030	0,02	1,00
	Sp_OT_03	AttgGAAGAGGatGCCTGTAGG	5	<i>DPH5</i> Intron	chr1:101481224	4	33058	0,01	<b>13</b>	<b>33113</b>	<b>0,04</b>	<b>0,00</b>	<b>13</b>	<b>21429</b>	<b>0,06</b>	<b>0,05</b>
	Sp_OT_04	TtcTGAtGAGGcCGCCTGTGcAG	4	<i>SEC16B</i> Intergenic	chr1:177866906	5	39059	0,01	2	33106	0,01	0,29	9	35652	0,03	0,46
	Sp_OT_05	TcAgGTAGAGGAaGCCTGTGTCG	4	<i>ABTB1</i> Intron	chr3:127396848	13	45497	0,03	6	19405	0,03	0,08	1	18879	0,01	1,00
	Sp_OT_06	TATGAAAaAGGAaGCCTGTGGG	3	<i>SRPK2</i> Intron	chr7:104892019	10	76934	0,01	14	45199	0,03	1,00	5	41376	0,01	0,04
	Sp_OT_07	GGAgGAAGAGGAgCCCTGTGTAG	2	<i>HIBADH</i> Intergenic	chr7:27554525		PCR Failure			PCR Failure				PCR Failure		
	Sp_OT_08	CGAgGAAGAGGAaGCCTGTGAG	2	<i>LINC00642</i> Intergenic	chr14:90952872	3	7565	0,04	8	46484	0,02	1,00	10	41792	0,02	1,00
	Sp_OT_09	TaATGgAaAGGAGcCCCTGTAGG	4	<i>NEFM</i> Intergenic	chr8:24660369	6	37796	0,02	9	27531	0,03	1,00	6	35255	0,02	0,19
	Sp_OT_10	TcATGAAGATGAaGCCTGTAGG	3	<i>DTNA</i> Intergenic	chr18:32487684	2	9080	0,02	7	53739	0,01	1,00	7	40971	0,02	1,00
	Sp_OT_11	AGGagAAGAGGACTcCCTGTAGG	3	<i>ABHD4</i> Intergenic	chr14:23124178	5	36747	0,01	<b>19</b>	<b>29092</b>	<b>0,07</b>	<b>0,00</b>	<b>26</b>	<b>32413</b>	<b>0,08</b>	<b>0,00</b>
	Sp_OT_12	AGcTGA-tAGGACGCCCTGTGTCG	2, del 14	<i>KHDRBS3</i> Intergenic	chr8:136462133	1	12750	0,01	4	19226	0,02	1,00	4	38138	0,01	0,65
	Sp_OT_13	GGAT-AGGAGGACGCCCTGTAG	1, del 16	<i>LOC101927577</i> Intergenic	chr2:66951479	8	33550	0,02	12	34669	0,03	1,00	8	39269	0,02	0,50
	Sp_OT_14	AtA-GAgTGGAGCCTGTAGG	3, del 17	<i>LGSN</i> Intergenic	chr6:63903490		PCR Failure			PCR Failure				PCR Failure		
	Sp_OT_15	TtAgGgAGAGGAaGCCTGTGAG	4	<i>ADGRF1</i> Intergenic	chr6:47082815	7	36825	0,02	14	41067	0,03	0,57	5	40248	0,01	0,27
	Sp_OT_16	GGAgGTAGAGGATGCCTGTGTAAG	3	<i>STX11</i> Intergenic	chr6:144537633	11	49603	0,02	<b>84</b>	<b>52271</b>	<b>0,16</b>	<b>0,00</b>	<b>130</b>	<b>33403</b>	<b>0,39</b>	<b>0,00</b>
	Sp_OT_17	TcAgGgAGAGGAaGCCTGTGAG	4	<i>GRID1</i> Intron	chr10:87865710	2	25315	0,01	1	42681	0,00	1,00	2	37912	0,01	0,56
	Sp_OT_18	TtATGcAGATGAaGCCTGTGAG	4	<i>LINC00971</i> Intergenic	chr3:83562980	3	5115	0,06	4	51800	0,01	1,00	4	42912	0,01	1,00
	Sp_OT_19	TcATGATAGATGAaGCCTGTGAG	4	<i>CHRD1</i> Intergenic	chrX:110081893	3	9495	0,03	0	28589	0,00	1,00	4	35906	0,01	0,02
	Sp_OT_20	GtATGAGATGAGcCCTGTGAGG	4	<i>RSU1</i> Intron	chr10:16719011	5	40320	0,01	5	30087	0,02	1,00	6	43230	0,01	0,75
	Sp_OT_21	TcATGAAAaGAaGCCTGTGTCG	4	<i>ANK2</i> Intron	chr4:114110211	7	6461	0,11	7	40294	0,02	1,00	9	39736	0,02	1,00
	Sp_OT_22	AaATGAAAGAGGAcCCTGTGAG	3	<i>LINC01335</i> Intergenic	chr5:73434648		PCR Failure			PCR Failure				PCR Failure		
	Sp_OT_23	GGA-atAGAGGACGCCCTGTGTAAG	2, del 17	<i>SCML4</i> Intron	chr6:108054086	11	41478	0,03	9	45387	0,02	0,50	9	48415	0,02	0,66
Sp_OT_24	AGAgcAAGAGGACTcCCTGTGTAAG	3	<i>MIR7641-2</i> Intergenic	chr6:4322363	10	33715	0,03	1	25682	0,00	0,47	7	36837	0,02	0,03	
St1Cas9	Target	GATGAAGAGGACGCCCTGTGGGAGAT	0	<i>CARD11</i> Exon	chr7:2987354	12	31050	0,04	<b>4785</b>	<b>42265</b>	<b>11,32</b>	<b>0,000</b>				
	St1_OT_01	AggcAAGAGGATGCCTGTGTAGAAA	4	<i>DLG1</i> Intron	chr3:196969818	9	29243	0,03	6	38090	0,02	0,205				
	St1_OT_02	AtaGAAGAtGATGCCTGTGTGGAAA	4	<i>LINC01467</i> Intergenic	chr14:83571802	5	30983	0,02	5	47490	0,01	1,000				
	St1_OT_03	AcTGAAttGGAAcCCTGTGTAGAAA	4	<i>DRG1</i> Intron	chr22:31819425	3	23361	0,01	3	34770	0,01	1,000				
	St1_OT_04	TATaAGATGAaGCCTGTGAGAGGAT	3	<i>VGLL2</i> Intergenic	chr6:117437944	4	10707	0,04	4	19115	0,02	1,000				
	St1_OT_05	AATGAAGAGatgCCCTGTGTCAGGAA	3	<i>TTL4</i> Intergenic	chr2:219621643	0	25822	0,00	<b>7</b>	<b>35292</b>	<b>0,02</b>	<b>0,024</b>				
	St1_OT_06	AtgGAgGAGGACGctTTGTGTGAGGAG	4	<i>WIPF1</i> Intergenic	chr2:175557440		PCR Failure			PCR Failure						
	St1_OT_07	AgTGAAGAGccCCTGTGCAAGAAT	4	<i>SYNGR1</i> Exon	chr22:39779459	5	24766	0,02	8	26840	0,03	0,585				
	St1_OT_08	GATGAAGAAGGAaGCCTGTGAAAGAAC	1, ins 12	<i>SRRM4</i> Intergenic	chr12:119365733	2	22014	0,01	5	25801	0,02	0,463				
	St1_OT_09	AgTGAaAAGGAaGcCTGTGTAAGAAT	4	<i>CDH9</i> Intergenic	chr5:26779606	4	34026	0,01	3	37233	0,01	0,716				
	St1_OT_10	AATGAAAaAGA-GCCTGTGGGAGGAA	2, del 9	<i>C3orf56</i> Intergenic	chr3:126876605	9	9423	0,10	14	9863	0,14	0,407				
	St1_OT_11	TgTGAATAG-AtGCCTGTGcCCAGGAT	3, del 11	<i>MIR1973</i> Intergenic	chr4:116779724	0	18683	0,00	2	21005	0,01	0,502				
	St1_OT_12	GtTgG-GAGGACcCCTGTGcCCAGGAT	3, del 15	<i>SAMM50</i> Intron	chr22:44361944	7	9483	0,07	9	10422	0,09	0,807				
	St1_OT_13	GcaGgAGAGGACGC-TTGTGAGGAAAG	3, del 6	<i>LINC01399</i> Exon	chr22:35627030	3	15718	0,02	3	16550	0,02	1,000				
	St1_OT_14	AATGgAGAGG-tGCCTGTGGGGAAAC	2, del 10	<i>TEX26</i> Intergenic	chr13:31573663	263	24124	1,09	405	42267	0,96	1,000				
	St1_OT_15	AtTGAAAAGAGaGCCTGTGTAGAAC	3, ins 11	<i>FSHP</i> Intergenic	chr2:49393909	5	30414	0,02	2	35053	0,01	0,262				
	St1_OT_16	CtTGAAGAc-ACaCCTGTGCTGGAAG	3, del 11	<i>GSAP</i> Intron	chr7:77013931	3	42627	0,01	7	42172	0,02	0,224				
	St1_OT_17	AcT-AAGAGtACGACTGTGAGAGAAC	3, del 17	<i>PRDX4</i> Intergenic	chrX:23635637	0	20008	0,00	2	32168	0,01	0,527				
	St1_OT_18	TgTGAAGAGGcCAtcCTGTGGGAGAAA	4	<i>BCAS3</i> Intron	chr17:59390228	4	34594	0,01		PCR Failure						
	St1_OT_19	GcTGAAGAGGAtGC-TTGTGTGAGAAA	2, del 6	<i>DNMT3A</i> Intron	chr2:25540417	3	19235	0,02		PCR Failure						
	St1_OT_20	AcTGAAGAGGAaGC-TTGTGAGGAAAA	2, del 6	<i>LINC01571</i> Intergenic	chr16:51695491	5	24726	0,02		PCR Failure						
	St1_OT_21	AATGAA-AGGAgCCTGTGTAGGAAAC	2, del 14	<i>RPRM</i> Intergenic	chr2:154276079	2	23332	0,01		PCR Failure						
	St1_OT_22	TtTGAAGATGAaCCTGTGGGAGGAT	3, ins 9	<i>SPATA6L</i> Intergenic	chr9:4595955	8	32891	0,02		PCR Failure						
	St1_OT_23	GcgGAAGAGGA-GcCTGTGTAGGAAAC	3, del 9	<i>ZC3H12C</i> Exon	chr11:110035898	5	6604	0,08		PCR Failure						
St1_OT_24	AtTGA-GAGGATcTCTGTGGAAGAAT	3, del 15	<i>LOC440390</i> Intergenic	chr16:87140262	3	12471	0,02		PCR Failure							

Target	GGATGAAGAGGACGCCTTGTGGGAG	0	CARD11	Exon	chr7:2987357	12	31050	0,04	2131	25153	8,47	0,000
St3_OT_01	<b>Att</b> TGgAGAGaACGCCTTGTGGATC	4	LOC101927630	Intergenic	chr7:17684983	5	33343	0,01	3	47591	0,01	0,287
St3_OT_02	G <b>at</b> GT <b>ct</b> GAGG <b>at</b> GCCTTGTGG <b>AA</b> G	4	GRIA1	Intron	chr5:153008828	2	5913	0,03	3	9270	0,03	1,000
St3_OT_03	C <b>ac</b> GAAG <b>at</b> g <b>act</b> c <b>ct</b> TGTGG <b>TT</b>	4	SOS1	Intron	chr2:39333929	9	6410	0,14	24	9164	0,26	0,114
St3_OT_04	T <b>at</b> GT <b>at</b> G <b>at</b> G <b>act</b> c <b>ct</b> TGTGG <b>AG</b> G	4	MYO3B	Intron	chr2:171251698	1	31680	0,00	6	40737	0,01	0,146
St3_OT_05	C <b>ag</b> GAAG <b>g</b> g <b>ct</b> GCCTTGTGG <b>G</b> A	4	KCNK9	Intergenic	chr8:140381132	1	8959	0,01	7	21110	0,03	0,450
St3_OT_06	AGATGA <b>ac</b> AG <b>ct</b> c <b>ct</b> TGTGG <b>AG</b> G	3	LHX5-AS1	Intergenic	chr12:114036786	7	26500	0,03	10	42902	0,02	1,000
St3_OT_07	T <b>ag</b> GAAG-GG <b>c</b> CGCCTTGTGG <b>G</b> CT	3, del 12	CACNA1I	Intron	chr22:40011069	5	28372	0,02	5	37384	0,01	1,000
St3_OT_08	T <b>ag</b> GAAG <b>tg</b> g <b>ac</b> g <b>ct</b> TGTGG <b>GG</b> C	4	SEPT8	Intergenic	chr5:132121907	91	31912	0,29	115	39092	0,29	0,834
St3_OT_09	AGAT <b>ct</b> AGAGGA-GCCTTGTGG <b>G</b> AT	2, del 8	PRELID2	Intergenic	chr5:144741267	4	31859	0,01	9	33392	0,03	0,269
St3_OT_10	A <b>ag</b> GA <b>aa</b> AGGA-GCCTTGTGG <b>G</b> CT	3, del 8	UBASH3B	5'UTR	chr11:122526384	4	23619	0,02	7	31134	0,02	0,767
St3_OT_11	C <b>t</b> AT <b>ca</b> AGAGGA <b>at</b> c <b>ct</b> TGTGG <b>TT</b>	4	RPP21	Intergenic	chr6:30331958	10	7992	0,13	10	9132	0,11	1,000
St3_OT_12	A <b>act</b> GAAG <b>t</b> GGA-GCCTTGT <b>AG</b> AAG	3, del 8	LAMA3	Exon	chr18:21495363	3	32144	0,01	6	34363	0,02	0,510
St3_OT_13	T <b>at</b> GT <b>ag</b> GAGG <b>ag</b> a <b>ct</b> TGT <b>GA</b> TG	4	DNAJC6	Intron	chr1:65865047	6	39645	0,02	8	42348	0,02	0,792
St3_OT_14	G <b>ca</b> GAAG-GG <b>aa</b> GCCTTGTGG <b>AG</b> G	3, del 12	C10orf11	Intron	chr10:77750620	3	31917	0,01	7	30309	0,02	0,216
St3_OT_15	C <b>ca</b> TG <b>aa</b> cAGG <b>ag</b> c <b>ct</b> TGTGG <b>TT</b> C	4	RORA	Intron	chr15:61454627	9	28723	0,03	11	32487	0,03	1,000
St3_OT_16	T <b>at</b> GT <b>at</b> G <b>g</b> GGA-GCCTTGTGG <b>AG</b> G	3, del 8	PTPN3	Intron	chr9:112246123	6	43260	0,01	7	35407	0,02	0,584
St3_OT_17	A <b>aat</b> GAAGAG <b>ag</b> t <b>cg</b> c <b>ct</b> TGTGG <b>AG</b> G	2, ins 10	CTTNBP2	Intron	chr7:117394240	4	33195	0,01	7	42586	0,02	0,765
St3_OT_18	TGAG <b>G</b> AA <b>aa</b> AGGAC-CCTTGT <b>AG</b> GTG	2, del 7	MIR8054	Intergenic	chr11:23242566	5	37873	0,01	18	53192	0,03	0,058
St3_OT_19	C <b>ca</b> TG <b>t</b> AGAG- <b>ag</b> GCCTTGTGG <b>AG</b> G	3, del 10	PLS1	Intron	chr3:142404412	8	24561	0,03	4	36289	0,01	0,079
St3_OT_20	GGATG <b>ag</b> G <b>ag</b> GA-GCCTTGT <b>GG</b> TG	2, del 8	NKAIN2	Intron	chr6:124316384	8	10467	0,08	16	13983	0,11	0,413
St3_OT_21	G <b>aa</b> GAAG <b>C</b> AGGA <b>a</b> c <b>ct</b> TGT <b>AG</b> GGG	3, ins 12	ADGRF5	Intron	chr6:46903615	35	36624	0,10	45	50773	0,09	1,000
St3_OT_22	T <b>at</b> GT <b>aa</b> <b>aa</b> AGGA-GCCTTGTGG <b>AG</b> C	3, del 8	C3orf56	Intergenic	chr3:126876608	3	12510	0,02	9	36707	0,02	1,000
St3_OT_23	AGATG <b>ca</b> AG <b>AG</b> G <b>aa</b> c <b>ct</b> TGT <b>GG</b> AG	2, ins 11	PABPC4L	Intergenic	chr4:134772265	2	6293	0,03	9	8865	0,10	0,137
St3_OT_24	C <b>tg</b> TGAAG <b>G</b> AGG <b>act</b> c <b>ct</b> TGTGG <b>GG</b> C	3, ins 12	PSD4	Intron	chr2:113953582	33	15703	0,21	18	5762	0,31	1,000

<sup>1</sup> PAM sequence is underlined. Differences to the target sequence are highlighted as follows: mismatches (lower-case bold), insertions (bold-italics), deletions (-).

<sup>2</sup> Statistically significant indel events are highlighted in bold.

Supplementary Table S4. Oligonucleotides used in this study

Primer ID	Sequence	Target	Application
#13	TCACGGGGATTTCCAAGTCTC	GFP reporter	T7EI assay
#77	AAGTCGTGCTGCTTCATGTGGT	GFP reporter	T7EI assay
#2397	TCAACCGCAGGATTTTCCT	CARD11	T7EI assay
#2398	CCTGCTCGGTTGATCTTGA	CARD11	T7EI assay
#2187	GGCCTCTTAAGCAACATGACCTAC	PRKDC	T7EI assay
#2188	CATGCACTGCACACACTAACGCG	PRKDC	T7EI assay
#1904	ACACCGGATGAAGAGGACGCCTTGTG	CARD11	gRNA cloning
#1905	AAAACACAAGGCGTCTCTTCATCCG	CARD11	gRNA cloning
#2059	ACACCGATGAAGAGGACGCCTTGTGG	CARD11	gRNA cloning
#2060	AAAACCACAAGGCGTCTCTTCATCG	CARD11	gRNA cloning
#2201	ACACCGATGAAGAGGACGCCTTGTG	CARD11	gRNA cloning
#2202	AAAACACAAGGCGTCTCTTCATCG	CARD11	gRNA cloning
#2203	ACACCGTGAAGAGGACGCCTTGTG	CARD11	gRNA cloning
#2204	AAAACACAAGGCGTCTCTTCACG	CARD11	gRNA cloning
#2205	ACACCGGAAGAGGACGCCTTGTG	CARD11	gRNA cloning
#2206	AAAACACAAGGCGTCTCTTCCG	CARD11	gRNA cloning
#2207	ACACCGTGAAGAGGACGCCTTGTGG	CARD11	gRNA cloning
#2208	AAAACCACAAGGCGTCTCTCACG	CARD11	gRNA cloning
#2209	ACACCGGAAGAGGACGCCTTGTGG	CARD11	gRNA cloning
#2210	AAAACCACAAGGCGTCTCTTCCG	CARD11	gRNA cloning
#2211	ACACCGAAGAGGACGCCTTGTGG	CARD11	gRNA cloning
#2212	AAAACCACAAGGCGTCTCTTCG	CARD11	gRNA cloning
#1906	ACACCGCGTATCCAGCACTTGTGGG	PRKDC	gRNA cloning
#1907	AAAACCCACAAGTGCTGGATACGCCG	PRKDC	gRNA cloning
#2061	ACACCGCGTATCCAGCACTTGTGGTG	PRKDC	gRNA cloning
#2062	AAAACACCACAAGTGCTGGATACGCCG	PRKDC	gRNA cloning
#2189	ACACCGCGTATCCAGCACTTGTGGG	PRKDC	gRNA cloning
#2190	AAAACCCACAAGTGCTGGATACGCCG	PRKDC	gRNA cloning
#2191	ACACCGTATCCAGCACTTGTGGG	PRKDC	gRNA cloning
#2192	AAAACCCACAAGTGCTGGATACG	PRKDC	gRNA cloning
#2193	ACACCGGTATCCAGCACTTGTGGG	PRKDC	gRNA cloning
#2194	AAAACCCACAAGTGCTGGATACCG	PRKDC	gRNA cloning
#2195	ACACCGTATCCAGCACTTGTGGTG	PRKDC	gRNA cloning
#2196	AAAACACCACAAGTGCTGGATACG	PRKDC	gRNA cloning
#2197	ACACCGGTATCCAGCACTTGTGGTG	PRKDC	gRNA cloning
#2198	AAAACACCACAAGTGCTGGATACCG	PRKDC	gRNA cloning
#2199	ACACCGATCCAGCACTTGTGGTG	PRKDC	gRNA cloning
#2200	AAAACACCACAAGTGCTGGATCG	PRKDC	gRNA cloning
#1898	TAACCCGGGGATGAAGAGGACGCCTTGTGGAGAATA	CARD11	GFP reporter cloning
#1899	CCGGTATTCTCCACAAAGGCGTCTCTTCATCCCCGGGTTAAT	CARD11	GFP reporter cloning
#1900	TAACCCGGGGCGTATCCAGCACTTGTGGTGAGAAAA	PRKDC	GFP reporter cloning
#1901	CCGGTTTTCTCCACCAAGTGCTGGATACGCCCGGGTTAAT	PRKDC	GFP reporter cloning

Supplementary Table S5. Sequencing primers for *PRKDC*-specific RGNs.

Nuclease ID	OT ID	Closest Gene	PCR Primer Left	PCR Primer Right
SpCas9	Target	<i>PRKDC</i>	CACCAACCTCTTTGTGCATGAGGC	CCTGGTGTTTGGTGGTAAAGTGGTAG
	Sp_OT_01	<i>CASC10</i>	TACAGGGTCTCACTCTGTGGCC	GCAAGACCCCGCCTAAAAACG
	Sp_OT_02	<i>CCSER1</i>	GTTGAGCATGGAGGCATCCTG	GGGTAGAGGTTCCAAAGTATTTGGCC
	Sp_OT_03	<i>C17orf51</i>	GGAAATTGCCTAAGCAGTGTGG	CCAGGGGACACAAACACTTAGC
	Sp_OT_04	<i>FLJ36000</i>	GGGAAATTACCCTAACAGGGTGTG	CAGGGGACACAAACCGTTAGCA
	Sp_OT_05	<i>LOC389033</i>	AGTCTTTGTGTGAACGCTCATGCC	GGTGACAGAACAGACTCCATCTC
	Sp_OT_06	<i>LINC01384</i>	TTGCAACCAGACCTGCCAGGTA	TCTGGCACTAGAGAAGGTGGC
	Sp_OT_07	<i>BRD1</i>	AGTACTGCATTTACCACAGGGGC	AACTCCTGGCCTCTGACTCC
	Sp_OT_08	<i>AMOTL1</i>	GCTGCAGTTGCTGCATTTAAGGAG	GGCTAGTTCAGTTTCTGTTTGAATTCAC
	Sp_OT_09	<i>PABPC4L</i>	CACCCAGGCTTCAACATAAATGC	ACATGCTCTTCTCACTTGGCCAC
	Sp_OT_10	<i>LINC01591</i>	GAACACATTCCAAAGCTCTGGGG	GTGCCACCACACCCAGCTAA
	Sp_OT_11	<i>PSMD13</i>	ACGCTTCTGCAAGATATGCATCATCTCTTC	GGTGACACTCAGAAAGGAGATGG
	Sp_OT_12	<i>TMEM45B</i>	CCCATTCTGCCTGACACCA	TACTGTGCCTCCCACACAGC
	Sp_OT_13	<i>MYH10</i>	CTACAGGTGTGCCACCACATG	GCACAAAGAACCAACTAGGGCAG
	Sp_OT_14	<i>PAFAH2</i>	CACTGAAGCCTTGAATCTCTGGG	AGGCGTGCAGGGAAGGTTCTCTTA
	Sp_OT_15	<i>EPB41L3</i>	TCTGTAATGTGACCCGGGTGACT	TGGGCATGGCTATCTGGAAACC
	Sp_OT_16	<i>AP2A2</i>	CCAGGACCTCAAATCTGCACAAC	AAGTTGAGGGGGACAGGGAC
	Sp_OT_17	<i>RBM26</i>	GAGTTTTCTCCAGTTTCTCCACAG	CACTTGGCACCAGAAAGTGGTATC
	Sp_OT_18	<i>RBM26</i>	TTTGCTTAAGCTACATTTCTAGTTGCCTGC	GCTGTTATATGCCATTCATAGTAGTAGGG
	Sp_OT_19	<i>PDE4A</i>	CAGAAATCCCCACACCCACA	AGACTGCTTAGCAGCTGGAGAG
	Sp_OT_20	<i>SNAP29</i>	ACAGAAAGCTCACCTPGCCTATGG	GAGCCACAAGGCCCTTGTCT
	Sp_OT_21	<i>ANAPC1</i>	TAAACTGTATGTAACAAACGGGTGTTGTGAAG	GGCCAGAAAACATAAATATCATACCAGGCAC
	Sp_OT_22	<i>CORIN</i>	CATATCGGATTTGCACAATTCATCCACAC	GCTCCCTCAGAATTTCTAACAAAGTTATTGAGT
	Sp_OT_23	<i>TBX22</i>	CTCTGGACAGTGGTTATGGCATAG	GTGCAAGGTTCCAGCATGCTTATG
Sp_OT_24	<i>RAB1F</i>	TATTTCTAAGCTCTGTGGGCTGAGG	ACTGCAGCCTCAACCTCCCA	
St1Cas9	Target	<i>PRKDC</i>	CACCAACCTCTTTGTGCATGAGGC	CCTGGTGTTTGGTGGTAAAGTGGTAG
	St1_OT_01	<i>LINC00669</i>	GAACCCAGCCTATCCTGGTTA	CATGTTGGAATCGTTTCTGTGTTGGGTAG
	St1_OT_02	<i>SOX2-OT</i>	GTCCCCATTTGAGAGGGTCTG	CAGCCAGTTCCTCATTCATGGAC
	St1_OT_03	<i>SORCS1</i>	GCAATAATAGTTTGCAGATTTCCCCAGGC	GCATGGCATACAGGTAGGCTAAG
	St1_OT_04	<i>MGAT4C</i>	GATGGAACACAGGCATACACACAC	CAAGTAGCACATTTAGGTCATGACTAATGAG
	St1_OT_05	<i>MCR2</i>	GGCCACAGTGTGCTAATCTATC	GGCTCCAAAATGATTAATCTTAGCCGCTCC
	St1_OT_06	<i>BAZ1A</i>	GACCTAGCTGGTCTCAGATTC	GGCACTGTCAAGGGTAATGCA
	St1_OT_07	<i>VMA5B1</i>	AGGCAGAGAGTCTGGGACTGT	TCTTGCTCCAGGCTCCAGG
	St1_OT_08	<i>LINC01603</i>	CCAAAGTGTGGGATTACAGGAG	CCAGGGAGCTGAAAGAGAGATC
	St1_OT_09	<i>NUBPL</i>	TGTTCCAGGCCACAGCATGTCAG	CCAGCTTCAATATCCGCCAGCCC
	St1_OT_10	<i>MRPL3</i>	GGGATTCGGGGAGGATGAT	ACACAGGGCAGAACTCAGCTGG
	St1_OT_11	<i>HLF</i>	TGGTGGGGGGAATGGAGAA	AACCGAGTGAGACCCCATCTCTA
	St1_OT_12	<i>MCHR2-AS1</i>	GATCTCGAATCCCAAGCTCAAG	GGAAAGCCTCTGGCCTTCACTTCT
	St1_OT_13	<i>SERPINA13P</i>	TGGATTACAGATGTGAGCCACTGC	GGTGACAGAGCAAGACTCCCTCAA
	St1_OT_14	<i>LOC101929413</i>	TGCACTGAAACCAGCCTCCATG	GGCTCTGTGCCCTTCTATATCC
	St1_OT_15	<i>UBA1</i>	GTAAAATGGAATGAGGTGGAGGGG	TCTAGAGTACCCTAGACACTCCAC
	St1_OT_16	<i>IL33</i>	CTCAAATACAGCATCTGCTGCC	GCAGAGAAAGCTAAATTTGCTGCC
	St1_OT_17	<i>CREM</i>	CTGCCCTTGGAGTAGATCACCC	CCATTGTTCTGGCTAGGAAGCA
	St1_OT_18	<i>MDGA2</i>	TTCGTTCACTCAAGTAGGCTCTAGG	GAGGGAGTTAGTTTGCAGTGGAG
	St1_OT_19	<i>SUDS3</i>	GTTGGGAAGAGAGAAAGAACTTACATTCTGTG	GCATTCCATAGAGCTGTTCTTCTGTAATATTG
	St1_OT_20	<i>SORCS3</i>	CCAAGAATTGGTCTCCACCATAAAAATACAAATGACT	CCAGCCATACCAACTCAATTTGCTGAG
	St1_OT_21	<i>MIR1468</i>	CGGTAGGGCTTGCAGGAAATG	CTGGGTTAGATTCTTGGTCCAC
	St1_OT_22	<i>RORA</i>	CCAGAGAAGAAATGTATAGCTTACCCTCA	GAACAGTTACCCTCAAGAGCCG
	St1_OT_23	<i>CXCR4</i>	CAGTCTTGTGCTCCCCCAT	AGAGTGAGACTCTATCTGTCTCGC
St1_OT_24	<i>MIR7641-2</i>	CCCCATCAATGCCACGTGGT	CCAGATCACACCCACACACTC	
St3Cas9	Target	<i>PRKDC</i>	CACCAACCTCTTTGTGCATGAGGC	CCTGGTGTTTGGTGGTAAAGTGGTAG
	St3_OT_01	<i>CCSER1</i>	TTTGTGAGCATGGAGGCATCCTG	TCACCAGTTACAGATGCTGCTGC
	St3_OT_02	<i>LOC101927845</i>	TCATCTCCAGGCCAGCTGT	GTGGGGATCAGATGTGTTTCCAG
	St3_OT_03	<i>PSMB7</i>	CTTGTGCTGCACCGAAGAGCAAT	GCTCTGTGATCTTTGGGGGATC
	St3_OT_04	<i>LY86</i>	CGGAGAGCAAGCAATCAGTAAGTG	GCCTCATCTCAATTTCTGTCTGG
	St3_OT_05	<i>ACO1</i>	TTATCTCTGTGGCAGAGCTCCTCC	ACAGGCTTCAATTTTGGGAAGGTGG
	St3_OT_06	<i>ZCWPW2</i>	CTGATTTGCTGCAGTCTCAGACC	GGAGTGGAGATTGCTGAGGC
	St3_OT_07	<i>TMEM108</i>	CTCTTTTGCCTTGACTCCAAGAAAGCTC	CTGTGGTAAGCTCCAGACATGC
	St3_OT_08	<i>SLC16A7</i>	TGCAGTAACTAACCTGACATCTCAATGCC	CATCAGGTGCCACTTTCATCTAATGTC
	St3_OT_09	<i>FLT1</i>	CCCAGCCAGACCTTGGCCAAT	GATCGTGCCACTGCCTCCA
	St3_OT_10	<i>ADAMTSL3</i>	ACAGTCTGCTCCGGCACAGGACTT	AAGAATGGCAGCAGGTAGGGC
	St3_OT_11	<i>KCNU1</i>	GCCTCCCATTAAGCTGTAGTTGC	GGCAGTAAGAGGAGTTGAATACG
	St3_OT_12	<i>PITCHD1</i>	GACTTGTGCATATGCTCTCTGGGG	GGGGCTGTGATTTTAGTTTCTGTGC
	St3_OT_13	<i>MLH3</i>	TGGACTATATCCGATGCTTCTGGG	GGGGAAAAGAGGGAGTCTGTG
	St3_OT_14	<i>STK24</i>	GCCTCACACTGATGAGCCCT	TGGCTTTAACCCCGAGGTC
	St3_OT_15	<i>MECOM</i>	TTACAGCCACCTAGAGTAACCAGC	TTAGGGAAAGTGGCAGGCACC
	St3_OT_16	<i>LINC00968</i>	CACAAGATTCTGTGCACTTCCAGG	GCAAGAGGTAGCAGAAGTTGTATGGATAG
	St3_OT_17	<i>RIMBP2</i>	GTAARACACAAGCCATTTAAAGAAAGAGGGTC	TTAGGCCGAGACAGTTAGTCTTGG
	St3_OT_18	<i>JARID2</i>	TGATTAGCTCCTTGCAGGGAC	GCTAGCCGAAGGGGAGACTGA
	St3_OT_19	<i>PARD3B</i>	AAGGAGTCAAGTAACTTTGTGGC	GCCTCTGCTTAAAGCCTCC
	St3_OT_20	<i>AGBL4</i>	CTAGTACCTGGGGAAGAGAG	GGGATGGATAACCAATTTCCCATG
	St3_OT_21	<i>TSNARE1</i>	ACAGCCGGTTAGCTGCAGA	CTTTCTGCACACTTTCGGCGGG
	St3_OT_22	<i>GFRA1</i>	CCCCGTAGCAACGCCCTAT	ATGCACCTTCCAACACTCCCCC
	St3_OT_23	<i>MYLK4</i>	CAGCAATTACCTCCCCTGCC	CCAGCCCTCAACAGAAATTCAC
St3_OT_24	<i>CCDC171</i>	TTTTAACTTGACTATTCTCATCCCCACCC	ACTGAGGCCTTCTCAGGGAC	

Supplementary Table S6. Sequencing primers for *CARD11*-specific RGNs.

Nuclease ID	OT ID	Closest Gene	PCR Primer Left	PCR Primer Right
SpCas9	Target	<i>CARD11</i>	TCTAGAGGTCACCTCCAGAGGAGTT	GTTACCTGCTCGGTGTGATCTTGG
	Sp_OT_01	<i>FOXA1</i>	GGAAACTCCGGCTTTGAAAACAG	AGGAGCACACTTCTCCCAAATAG
	Sp_OT_02	<i>PTPRB</i>	CTCCCATTTGTAGTGAACCCACTT	GCAGTGCCAAAACACACAGCAG
	Sp_OT_03	<i>DPH5</i>	GCCGAGGTTGGTTCATGAGG	GCAGCAGGGAGAATAGATTTAGC
	Sp_OT_04	<i>SEC16B</i>	CCATTGCTCCCAATTATGAGGAGG	ATGGCCCTGGGCAGGATCAA
	Sp_OT_05	<i>ABTB1</i>	CAGGGATCTGCAGCACCACA	GATGGCACACAACCTGTGCTG
	Sp_OT_06	<i>SRPK2</i>	TCCTGGGGTCAAGCTATGCTC	TGGGCCAGTATGCAAAAAGGAC
	Sp_OT_07	<i>HIBADH</i>	CCCTCTGAGGCCTCACACAT	CTGGGAAGTGTCCAGGAGAGC
	Sp_OT_08	<i>LINC00642</i>	CAAAGAGATCCTTGGGGAATGAGC	TATGACCTTGGGGATCTCTCTG
	Sp_OT_09	<i>NEFM</i>	GGAGATGAAGGAATTACCCATGGG	GCAGAGACTCTTCCAACAGGAC
	Sp_OT_10	<i>DTNA</i>	ATCCTCACAATACCCATTTTAGGAGGGTGGTAATA	TTTTCCACCACAAAAGAGAGCTCCC
	Sp_OT_11	<i>ABHD4</i>	GGAGTTTGAAGAAGGAGGAGAGAC	TTCAAGGTGGAGTGTCTCTFGG
	Sp_OT_12	<i>KHDRBS3</i>	CTTTTGTGGAGGATGCGATGTGC	CCCTGCCATCTTTCTGCTTCATC
	Sp_OT_13	<i>LOC101927577</i>	GCAGAAGTACTTGACGGCATGAC	CCAGACATAGGCCGTAGGAAC
	Sp_OT_14	<i>LGSN</i>	CATGGCCCTGCCTAGGGTA	GGCCACAAGAGCAAACTCCATC
	Sp_OT_15	<i>ADGRF1</i>	CAGGAGGTAAGTGAAGCATAGG	GAGAGTGTGTTGAGGGTTTTGG
	Sp_OT_16	<i>STX11</i>	AAACCTAAAGCTACCAGGAGTGGG	GTGTCTTCACTGCCGAGC
	Sp_OT_17	<i>GRID1</i>	CATGTGTCTAACTGGCCTGGG	GCAAAGGGTCAAGGAGGACA
	Sp_OT_18	<i>LINC00971</i>	CAAGAGTGTGGCCGAACACAG	GCCTGCAAGTCTTTTGTGGGG
	Sp_OT_19	<i>CHRD1</i>	GAAACCAGGCCTTCCCTTCTAG	AAAGTCAGGGGTGGGGGAG
	Sp_OT_20	<i>RSU1</i>	GAATCAGGGTGAATGCAGGAAGC	GTCTGCGCAGTTTAAAGAAGCTGTCTC
	Sp_OT_21	<i>ANK2</i>	GGTATCAATTTCAAGTATGCTGAGACACAC	GAGTACCTGGTGAATTAAGCAAACTCC
	Sp_OT_22	<i>LINC01335</i>	GGCTGCTTGGAACTTTTGTAGGTC	CTCCCTCCCAAGTATACT
	Sp_OT_23	<i>SCML4</i>	CGAAGTGGTGGACATCCT	CAGCAGGACTGAGTTTCCAATGAG
Sp_OT_24	<i>MIR7641-2</i>	AGTAGGTTAAGTTACTTGGCTCCCAGTTTG	ATGGAAAGTGACATCCAGCCACC	
St1Cas9	Target	<i>CARD11</i>	TCTAGAGGTCACCTCCAGAGGAGTT	GTTACCTGCTCGGTGTGATCTTGG
	St1_OT_01	<i>DLG1</i>	TGCCGTGTAATCTCAGCACTTTGGG	GGGAGGACACAGAAACAGGAAC
	St1_OT_02	<i>LINC01467</i>	CCACAGAGTTTGTATAACCAAAGTACACC	CACCTGTGTCATGTTTTGTGCTG
	St1_OT_03	<i>DRG1</i>	GATCAGCCACTGCACCTTAG	GCTGGATGCTGAAACTGTGAAGAG
	St1_OT_04	<i>VGLL2</i>	GTGCAGCAAACCTACCATGGCAG	GCCTGCAATGGCACTAGATTTTCC
	St1_OT_05	<i>TLL4</i>	GTGCAGGCTATTTCTGGAGTAGTG	AACTTTCGCAACCTGCACTGTAG
	St1_OT_06	<i>WIPF1</i>	TCAAGTTCACAAAGGATGGAACCC	CACCTGAGATGGGAAAAGAGGAG
	St1_OT_07	<i>SYNR1</i>	TGAAAACACCAGCACTGGGC	ACACAGGCAAGAGCCCTGA
	St1_OT_08	<i>SRRM4</i>	CTATTCAGGCTCATGCCATACC	CGGGGGATAGAGGATTCAGG
	St1_OT_09	<i>CDH9</i>	CACAGGGAATGCATATGGTGCT	GGCCCTCTGTTTCACTGAAACGTC
	St1_OT_10	<i>C3orf56</i>	CTGAAGGAGTCTTCCACCGCC	TGTGGACACGAGTGGGTTGG
	St1_OT_11	<i>MIR1973</i>	CCCATGCTGTTCTCTGTATAGTG	GACCTCTCTGGGCTCTTAACTC
	St1_OT_12	<i>SAMM50</i>	CTCAGCCTCCTGAGTAGCCA	CCAGGAATCCCACTTCCAGG
	St1_OT_13	<i>LINC01399</i>	GGAAACCAGTGGGAGGTGAT	GGCTGTTTACCTCTGTATTTGGTCC
	St1_OT_14	<i>TEX26</i>	GAGGAACAGATGGATAGAGAGGC	GTAAAATGAGGTGGTTGGACCAGG
	St1_OT_15	<i>FSHR</i>	GGCATGAGGAGGGAAGATGG	CCCAGAGAGATTTAGGACTGGAAG
	St1_OT_16	<i>GSAP</i>	CCAGCAGCACATTTCCCTAATC	CAGCTCTGCAAAAGCACTCAAGG
	St1_OT_17	<i>PRDX4</i>	GGTCTTTCTTCTCTGCAGGC	CCCTTTGCCACAACCATTGAC
	St1_OT_18	<i>BCAS3</i>	CTAACTTTGTACTGGCACTGGG	AGGAAGCCATCGGTATTCTGAG
	St1_OT_19	<i>DNMT3A</i>	TCGTGAAAGCAGACTTGTGACAGC	CTTTGTCTCAGCTGTGCCAAGTG
	St1_OT_20	<i>LINC01571</i>	CTCAGTGGCTCCCTTCTATG	GAGCATAAGTCCAGGAGGAGG
	St1_OT_21	<i>RPRM</i>	CTCCGTGATTCACCTATTTAACATTCAAAAATGGC	CACCTAGTTTCCCAAGTATAGC
	St1_OT_22	<i>SPATA6L</i>	ACTACTAAACAGGAGTCAAACTGGTGGC	CATACTTACTGGGCAATGAGACC
	St1_OT_23	<i>ZC3H12C</i>	CGACCCACAGCTAGAGGAGA	GAGATGCTGTCTATTTCTCGTGC
St1_OT_24	<i>LOC440390</i>	GACCTCAGCACTATGAGGAC	GGCAGCCTTCTGAGAGAGCTA	
St3Cas9	Target	<i>CARD11</i>	TCTAGAGGTCACCTCCAGAGGAGTT	GTTACCTGCTCGGTGTGATCTTGG
	St3_OT_01	<i>LOC101927630</i>	GGATGAAACAAATATAAGAGGTTGGAGGGG	GCAGAGCAGTAACCTTCACTCGGTAAGATC
	St3_OT_02	<i>GRIA1</i>	CCTGGATGGTTTGCATGCCCC	TTCCACCTGCCTTCTTCTCAC
	St3_OT_03	<i>SOS1</i>	CCCATGTATATATCTCCACTCCCAAC	CAGTAAGACAAAAGAAAACAATTCAGAAACACCCC
	St3_OT_04	<i>MYO3B</i>	CTTTAGTGTGGAGTCCAGGAGCATG	GACCATCACAATAGATCATCTGCC
	St3_OT_05	<i>KNKN9</i>	GAGTCCCTGTATCCACAGTGA	ACTGAGCTCAAGGAGTGTTTCCC
	St3_OT_06	<i>LHX5-AS1</i>	GATCTTTCATGAGCTTGGCTGGCT	GACAAGCAGCAAGATCTGCAGAG
	St3_OT_07	<i>CACNA1I</i>	CAGGAGGTAGTGGGAGGTT	CAGGCATAAGCACCACGTCTC
	St3_OT_08	<i>SEPT8</i>	TTGGGATTTCACTGGGCCAC	GGGAAGTGGAGAAGGTTGAACG
	St3_OT_09	<i>PRELID2</i>	TCCCAGGTAGGAGGACAGCT	GAGACAGACTGGTATGTAAGGGGC
	St3_OT_10	<i>UBASH3B</i>	GGATCCTCCGTCCAAGTAG	TGAGGCCACAGGAGCCAGAA
	St3_OT_11	<i>RPP21</i>	CAACTGGCAATGTTGGGAACCG	GATGGATCCACAATGGAGTACAG
	St3_OT_12	<i>LAMA3</i>	CAGCTTCCCAGTGCACATAAGTTTC	CAGGACTGCCAGTGTGCATG
	St3_OT_13	<i>DNAJC6</i>	CTGCTTCAGAAAGTTGTTGTGATAAGCCC	GGCAAGTCTGAGCCTCAGTTTTC
	St3_OT_14	<i>C10orf11</i>	CACCTTCACTTTATGGAGGAGGAG	AGAGGAAATGGTCCAGTTTGGTTAAGTGTG
	St3_OT_15	<i>RORA</i>	AATGTCAGGGCTAAAAGAGAGGGC	GGTAACCACATGATCTGGTCCCTC
	St3_OT_16	<i>PTPN3</i>	CTGATTCACAAGAACCCGACAGA	GAGAAGCAGCCTGTCCAC
	St3_OT_17	<i>CTTNBP2</i>	CAAGGAGATAAAGAGTGGGTGGAC	GGAGCTATGCCATTTCTTGGCTC
	St3_OT_18	<i>MIR8054</i>	CTTGACCGGTAACCTGGACTAG	CAGTTGGTGGAGTTCTTCAATTCCC
	St3_OT_19	<i>PLS1</i>	TGCTGTGAAAGTGTATGTTCTGGGG	TGGGTTGATGATCTTGGCTGG
	St3_OT_20	<i>NKAIN2</i>	AGAAACACGAGAATTGCATCCTCAGGAC	CATAACCAGGTTGGTGCCTTATATACATTATTCTAC
	St3_OT_21	<i>ADGRF5</i>	TGAGATAGGTGTGGAAACTGCTGC	CTGACCCAGGGAAGGAAAGG
	St3_OT_22	<i>C3orf56</i>	CTGAAGGAGTCTTCCACCGCC	TGTGGACACGAGTGGGTTGG
	St3_OT_23	<i>PABPC4L</i>	CAGGTTGCTTTCGTGCTCACTTTC	CCAGGCCATGGGATTTCTGAAAC
St3_OT_24	<i>PSD4</i>	CTTGAGGTGAGGCAAGCCAG	CCTTCTCAGGTCGGCTGTG	