Supplementary Table S3. Top 20 out of 2,074 predicted off-target sites of tru-sgRNA2 and SpCas9-VRQR

Coordinates (GRCm38/mm10)	*MM*[a]	*Target_sequence*[b]	*PAM*	*Position*	*Gene name*
chr6:115932059-115932078	1	GTGGT[GCGGAGCC**C**CTT]	CGA	Exonic	*Rho*
chr3:51534318-51534337	2	G**GT**GT[GCGGAGCCACTT]	GGA	Intronic	*Setd7*
chr15:5877846-5877865	1	GTGGT[G**T**GGAGCCACTT]	TGA	Intergenic	*Gm27760*
chr18:42216853-42216872	4	**ACCC**T[GCGGAGCCACTT]	TGA	Exonic	*Lars*
chr12:112667982-112668001	2	GTGG**G**[GC**T**GAGCCACTT]	GGA	Exonic	*Akt1*
chr17:90591810-90591829	3	G**A**G**C**T[G**A**GGAGCCACTT]	GGA	Intronic	*Nrxn1*
chr15:82952543-82952562	4	**AA**G**CC**[GCGGAGCCACTT]	TGA	Intergenic	*Mir3080*
chr16:35528273-35528292	4	**CA**G**TA**[GCGGAGCCACTT]	TGA	Intergenic	*Sema5b*
chr1:91917859-91917878	3	**C**T**T**GT[GC**A**GAGCCACTT]	GGA	Exonic	*Gm37600*
chr8:121126629-121126648	3	**A**TGG**A**[G**G**GGAGCCACTT]	GGA	Intronic	*AC127554.1*
chr14:14957653-14957672	2	GTGGT[**A**C**T**GAGCCACTT]	GGA	Intronic	*Nek10*
chr6:28464010-28464029	3	GT**TC**T[G**G**GGAGCCACTT]	CGA	Intergenic	*Gm43264*
chr1:48454326-48454345	3	GT**AA**T[G**A**GGAGCCACTT]	TGA	Intergenic	*Gm23240*
chr7:118779695-118779714	2	G**A**GGT[GCGG**G**GCCACTT]	CGA	Exonic	*9030624J02Rik*
chr12:85072399-85072418	3	**T**TGGT[**TT**GGAGCCACTT]	GGA	Intergenic	*Ylpm1*
chr15:41792995-41793014	4	**AGT**GT[G**A**GGAGCCACTT]	TGA	Intronic	*Oxr1*
chr16:43727298-43727317	4	**AAA**GT[G**G**GGAGCCACTT]	TGA	Intergenic	*Drd3*
chr19:38652109-38652128	3	G**A**GGT[**AA**GGAGCCACTT]	GGA	Exonic	*Plce1*
chr10:114571848-114571867	3	G**A**GGT[**TG**GGAGCCACTT]	GGA	Intronic	*Trhde*
chr4:22491277-22491296	4	**CA**G**T**T[G**G**GGAGCCACTT]	CGA	Intronic	*RP23-381K3.5*
chr4:100378125-100378144	3	GT**A**G**A**[GC**A**GAGCCACTT]	TGA	Intronic	*Gm12706*

[a] *In silico* predicted off-target sites with one to five mismatches (MM) by CCTop-CRISPR-Cas9 target online predictor using input sequence GTGGTGCGGAGCC**A**CTTCGA and NGA PAM sequence.

[b] The sequences in the brackets are the seed sequences; bold nucleotides indicate mismatched positions.