

Genomic Coordinates	Locus Type	Nearest Gene Name	# of Mismatches	Target Sequence at Locus (mismatches in red)	Result of Off-Target Analysis
chrX:74283651-74283673	Exon	<i>Taz</i>	0	TCCTAAAACTCCGCCACATC	Two deviant alleles detected in TAZ-KO; 81 bp deletion and single-base deletion near end of <i>Taz</i> exon 3
chr5:116193392-116193414	Intron	<i>Ccdc60</i>	4	TGCTTCAGCTCCGCCACATC	No differences detected between WT and TAZ-KO
chrX:99228578-99228600	Intergenic	<i>Efnb1</i>	4	TCCTTTTCCTCCGCCACATC	No differences detected between WT and TAZ-KO
chr7:17982183-17982205	Intergenic	<i>Ceacam11</i>	4	ACCCAAACCTCAGCCACATC	No differences detected between WT and TAZ-KO
chr4:141881515-141881537	Intergenic	<i>Fhad1os2</i>	4	TTCTCAACCTCTGCCACATC	No differences detected between WT and TAZ-KO
chr13:40369162-40369184	Intergenic	<i>Ofcc1</i>	4	GGCTATAACTCCGGCACATC	No differences detected between WT and TAZ-KO
chr15:79038802-79038824	Intron	<i>Gcat</i>	4	TCCTTATACCCTGCCACATC	No differences detected between WT and TAZ-KO
chr16:57507735-57507757	Intron	<i>Filip1l</i>	4	TTCTAAATGTCCACCACATC	No differences detected between WT and TAZ-KO
chr5:24422964-24422986	Intron	<i>Cdk5</i>	4	TCACAAAACACTCCACATC	No differences detected between WT and TAZ-KO
chr2:31561027-31561049	Intergenic	<i>Gm13426</i>	4	TCCCAAAGATCCTCCACATC	No differences detected between WT and TAZ-KO
chr6:89519351-89519373	Intron	<i>RP24-69A18.1</i>	4	TCCTGTAACCTGCACCACATC	No differences detected between WT and TAZ-KO

Supplementary Table 1. Off-target CRISPR/Cas9 effects were not detected in TAZ-KO cells. The top ten predicted off-target sites were identified using the CCTop-CRISPR/Cas9 target predictor tool based on the *Mus musculus* GRCm38 reference genome and the gRNA sequence utilized to create the TAZ-KO cell line (46,65). None of the predicted sites were located in an exonic region of the genome, and all off-target sites contained at least four mismatched bases relative to the gRNA sequence. To determine whether any of these sites were altered by Cas9 in the TAZ-KO line, whole-genome sequencing was performed on wild type (WT) and TAZ-KO cells, and differential variant analysis was run to identify deviations between the WT and TAZ-KO genomes. This analysis indicated that none of the predicted off-target sites differed between the two genomes, and also confirmed the presence of two deviant alleles at the primary gRNA target of tafazzin exon 3 (46).