

**Figure S1** | The strategy of constructing pEGFP-C1-MYO5B-wt/mut using vector pEGFP-C1 in minigene assay. The whole genomic sequence from Exon 17, intron 17 to Exon 18 was inserted into vector pEGFP-C1. The variant c.2090+3A>T shows in red. The location of the designed primers shows in purple.



**Figure S2** | Gel electrophoresis results of bacterial colony PCR and used reaction conditions. (A) Results of bacterial colony PCR for pEGFP-C1-wt/mut using primers pEGFP-C1-5' and

MYO5B-test-R. Lane 1–5: pEGFP-C1–MYO5B–wt bacterial colony, Lane 6–10: pEGFP-C1–MYO5B–mut bacterial colony. Lane M: 2000bp ladder marker. **(B)** Results of bacterial colony PCR for pcDNA3.1-wt/mut using primers MYO5B-test-F and pcDNA3.1-R. Lane 1–5: pcDNA3.1-MYO5B-wt bacterial colony, Lane 6–10: pcDNA3.1-MYO5B- mut bacterial colony, Lane M: 2000bp ladder marker. Reaction conditions for enzymatice digestion: 3  $\mu$ l 10×NEB buffer, 0.6  $\mu$ l Enzyme 1, 0.6  $\mu$ l Enzyme 2, 500 ng / 25  $\mu$ l Vector/DNA fragment, and 30  $\mu$ l ddH2O, digested two hours in 37 °C. Reaction conditions for ligation: 1  $\mu$ l 10×ligase buffer, 7  $\mu$ l digested DNA fragment (wt/mut), 1  $\mu$ l digested vector, and 1  $\mu$ l ligase reacted overnight in 4°C and then transformed to DH5a competent condition.



## С

MYSc_Myo5 - Sequence/Alignment Viewer p.Arg697 converter subdomain			converter subdomain
View Edit	Mouse Mode Unaligned Justification Imports		•
IOE9_A	FKfPFTFDEKRAVQQLRACGVLETIRISAAGFPSRWTYQ	FFSRYRVLMKQkdvlsdrkqt~~~~~~~	~~~~cknvlekli <mark>LDKDKYQFGKTKIFFR</mark> agqvayleki
IW8J_A	FKfPFTFDEKRAVQQLRACGVLETIRISAAGFPSRWTYQ	FFSRYRVLMKQkdvlsdrkqt~~~~~~~	~~~~cknvlekli <mark>LDKDKYQFGKTKIFFR</mark> agqvayleki
2DFS_A	FKfPFTFDEKRAVQQLRACGVLETIRISAAGFPSRWTYQ	FFSRYRVLMKQkdvlsdrkqt~~~~~~~	~~~~cknvlekli <mark>LDKDKYQFGKTKIFFR</mark> agqvayleki
gi 127736	DK e AWQFDNLMVL SQLRACGVLET IR I SCAGFPSRWTFE	FVLRYY1L1PHeqwdlifkkkettee~~di	i i s v v km i l d a t v <mark>KDK SKYQ I GNTK I F FK</mark> a gm l a y l e k l
gi 1929038	DKVAFKWETAKIIQQLRACGVLETVRISAAGFPSRWLYP	FYMRYQLLVYR skldkndmrl~~~~~~	-~~s c r n i vmkwi <mark>QDEDKYR FGNTQ I F FR</mark> agq vaf l eq v
gi 7498293	SK i SFDFEPKRAIQQLRACGVLETVRISAAGFPSRYPYE	FARRYRVIYTK eaalwr dkpk~~~~~~~	~~~qfaelacqqc <mark>LEEGKYAVGKTKIFLR</mark> tgqvavlerv
gi 8745416	YK1PFTFEPKRAVEQLRACGVLETIRISAAGYPSRWSYS	FFTRYRVLMVR keinkkdvrg~~~~~~~	~~~tsektlkrli <mark>VDPDKYQFGKTKlFFR</mark> agqvaylekl
gi 25573172	AKkAWELDSIQVLAQLRACGVLETIRISCAGYPSRWEFN	FAQRYL IMLHS q ewr p dmd v k q ~~~~~~~	~~~lcsailtkvlDDENQYQLGLTKIFFRpgvlallesl
gi 28950352	AK e PWKFEGPMVL SQLRACGVLETVR I SCAGYPTRWTYE	FALRYYMLVPS t qwt s e i r ema n~~~~~~	-ailtkafgastg <mark>KGLDKYQLGLTKIFFR</mark> agmlaflenl
gi 31076787	EK e AWKFDNQMVVSQLRACGVLETIKISCAGFPSRWTFD	FVSRYYMLVPSavrtteslt~~~~~~~	~~~~fskailekh <mark>ADPTKYQIGKTKIFFR</mark> sgvtpllesa
gi 32879539	AK kAWE VE PQNVLGQLRACGVLET I R I SCAGYP SRWTFA	FAERYYMLVPS <mark>drwnmsdmdkvk~~~~~</mark>	~~alathilttti <mark>TEKDKYQIGLTKIFFR</mark> agmlaqfeqr
gi 46396142	EK1PWTFSPPMVLSQLRACGVFETIRISSLGFPARFSYE	F A H R F R 1 L L S S k e w e e d n k k 1 ~~~~~~~	~~~tlnivnsvip <mark>HDNLNFQVGRSKIFFR</mark> snvignfeea
gi 94733004	LKkPFLFDPKRAVQQLRACGVLETIQISAAGYPSRWTYE	FFARYRVLLQG f v s q d d v r h s ~~~~~~~	~~~~cqstlpdli <mark>PDPEQYCFGKTKVFFR</mark> agqvavlekl
gi 156220958	TK a PFEFHPKR S I QQLRACGVLET I R I SAAGYP SRWTYR	FFARYIMLLPS <mark>kkinrkkpre~~~~~~</mark>	~~~tikliletfi <mark>KDEDMFQMGKTKIFFR</mark> agqvaylekl
gi 158598014	EK1PFTFTPKRAIQQLRACGVLETVRISAAGYPSRWMYE	FSRRYRVLYPE kklwleepri~~~~~~~	~~~~faekacnky <mark>LENKMYALGKTKVFFR</mark> tgqvalleri
gi 163777608	AKqAFAFDTPRVVQQLRACGVLETIRISAAGYPSRWSYP	FCSRYALLQSGppvstepreq~~~~~~	-~~~cksilepli <mark>EDTDKYQFGKTKLFFR</mark> agqvaylekl
gi 190340235	YKhAFTFDPKRAVQQLRACGVLETIRISAAGFPSRWTYQ	FFSRYRVLMKQkdvltdkkmt~~~~~~~	~~~~cknvleklvQDPDKYQFGKTKIFFRagqvaylekl
gi 190585207	RKaPFTFEPTLSVQQLRACGIIETIKISAAGFPSRWTYM	FYTRYRVLAKL s s d i d r n d l k g~~~~~~	~~~t c s n i v k s y i <mark>SDADK I QLGKTK I F F R</mark> p g q v a y l e k l
gi 198416146	YK1SFTFEAKRAVQQLRACGVLETVRISAAGYPSRWSYT	<b>F I AR YR VLMST</b> k d i v k k d p r k ~~~~~~~~	~~~tcekvlktli <mark>PEEDKYQPGKNKIFFR</mark> agqvaylekl
gi 212506289	FKkAFDYNPQRAVQQLRACGVLETVRISAAGFPSRWLYN	FFARYRVLCKFkdinrsdmka~~~~~~~	-~~tcskillnyi <mark>TEPDKYQFGKTKIFFR</mark> agqvaflekl
gi 270010527	SK k P F E YN P KRAVQQLRACGVLET I R I SAAGF P SRWTYV	FFYRYRVLCKFkdikrndmql~~~~~~~	~~~ t c q n i l n q y i <mark>KNKDMFQFGKTK I FFR</mark> a g q v a y l e k l
gi 291277983	AKaSFTFEPRRAVQQLRACGVLETVR I SAAGYPSRWSYH	FFVRYRLLTRSalidrtnyrr~~~~~~	~~~t c en i l knl i <mark>SDPDKYQFGNTKIFFR</mark> agqvaylekl
gi 294862453	EK1PFEFDSKR1VQQLRACGVLETIR1SAQSYPSRWTY1	FYSRYGILMTK q e l s f s d k k e~~~~~~~	-~~vekvvlhrli <mark>QDSNQYQFGKTKIFFR</mark> agqvaylekl
gi 303387470	DKmAFTFDPTRAIQQLRACGVLETVRISAAGYPSRWTYP	FFCRYRVLCHSkdivrndmrm~~~~~~~	-~~t cekiianmi <mark>NDEDKFKFGRTKIFFR</mark> agqvaymekl
gi 313219417	EKaVFTFEPSRSVEQLRACGVLETVRLSAAGFPGRWSYK	FRTRYRVLLRGkepkmeprka~~~~~~	~~~~ c e a m l t r l i <mark>PDEDKYAFGKTKIFFR</mark> a g q v a l m e k w
gi 320170369	AK e PFVFDR I HVLEQLRACGVLET I R I SAAGYP SKMTYA	FRERYRPFLTRqqavnnkanleva~~~~l	kireacslilnst <mark>FDAEQFQLGKTKIFLR</mark> agklavlerr
gi 321461417	SKeSFVFEPRRAVQQLRACGVLETVRISAAGFPSRLTYE	FIVRYRVLFHS rqcqrklrdlsvq~~~~~	-rescetvlatli <mark>TEDDKFKFGASKIFFR</mark> agqvaylekr
gi 328767577	AKvAFEFEAPMVLSQLRACGVLETIRISCAGYPNRQTFQ	F SQR F Y F L VR S v dwv a d p k q l ~~~~~~~	~~~~tetivkgli <mark>SDEDKYQIGLSKIFFR</mark> agqiayiekl
gi 339251558	EK1PFTFNNARTIQQITACSLLETLKISAAGHPTRWKYE	FFERYFLLLTMkerneqsttls~~~~~~	-~~ekcrqicerf <mark>LNNENFEFGSTKIFFR</mark> tgqialleae
gi 357629384	TKqPFQFDAARRQQFSRACGVLETIRISSAGFPSRWLYQ	FFQRYRLLCLY keidrsnika~~~~~~~	~~~tcskilekhl <mark>KDPDKFQFGATKIFFR</mark> agqvayleki
gi 363743687	SK1PFVFDSRRAVEQLRACGVLETIRISAAGYPSRWTYQ	FFERYRALLSR e e l v g d d a k q ~~~~~~~~	~~~scslalerllQDPSMYRCGKSKVFFRagqvafleel
gi 388580478	QKkAWDIDSQQVLSQLRACGVLETIKISSAGYPTRWSFA	FTDRYYPLVGS e hwl gdmk e l ~~~~~~~	~~~~clqilqvni <mark>SDEDKYQIGLSKIFFR</mark> agmlaylekl

