

**S3 Table. List of constructs**

Name	<sup>(1)</sup> Protein	Residues	Mutations	Vector	<sup>(2)</sup> Tag	Expression in	Origin
Trx-MYOT	myotilin	1-498	-	pETM-20	N-Trx-His <sub>6</sub> -TEV	<i>E. coli</i>	this study
Trx-MYOT-NEECK	myotilin	1-498	F96E, L97E, L101E	pETM-20	N-Trx-His <sub>6</sub> -TEV	<i>E. coli</i>	this study
Ig1Ig2 <sup>185-498</sup>	myotilin	185-498	-	pETM-20	N-Trx-His <sub>6</sub> -3C C-Strep	<i>E. coli</i>	this study
<sup>(3)</sup> Ig1Ig2 <sup>185-498*</sup>	myotilin	185-498	-	pDB-HisGST	N-His <sub>6</sub> -GST-TEV	<i>E. coli</i>	this study
Ig1Ig2 <sup>220-452</sup>	myotilin	220-452	-	pETM-14	N-His <sub>6</sub> -3C	<i>E. coli</i>	this study
Ig1Ig2 <sup>220-452 R405K</sup>	myotilin	220-452	R405K	pETM-14	N-His <sub>6</sub> -3C	<i>E. coli</i>	this study
<sup>(4)</sup> Ig1Ig2 <sup>250-498</sup>	myotilin	250-498	-	pETM-14	N-His <sub>6</sub> -3C	<i>E. coli</i>	this study
Ig1Ig2 <sup>250-444</sup>	myotilin	250-444	-	pETM-14	N-His <sub>6</sub> -3C	<i>E. coli</i>	[22]
Ig1Ig2 <sup>K354A</sup>	myotilin	250-444	K354A	pETM-14	N-His <sub>6</sub> -3C	<i>E. coli</i>	this study
Ig1Ig2 <sup>Q356A</sup>	myotilin	250-444	Q356A	pETM-14	N-His <sub>6</sub> -3C	<i>E. coli</i>	this study
Ig1Ig2 <sup>K358A</sup>	myotilin	250-444	K358A	pETM-14	N-His <sub>6</sub> -3C	<i>E. coli</i>	this study
Ig1Ig2 <sup>K359A</sup>	myotilin	250-444	K359A	pETM-14	N-His <sub>6</sub> -3C	<i>E. coli</i>	this study
Ig1Ig2 <sup>K367A</sup>	myotilin	250-444	K367A	pETM-14	N-His <sub>6</sub> -3C	<i>E. coli</i>	this study
Ig1Ig2 <sup>R405K</sup>	myotilin	250-444	R405K	pETM-14	N-His <sub>6</sub> -3C	<i>E. coli</i>	this study
Ig1Ig2 <sup>K411A</sup>	myotilin	250-444	K411A	pETM-14	N-His <sub>6</sub> -3C	<i>E. coli</i>	this study
Ig1Ig2 <sup>K354/359A</sup>	myotilin	250-444	K354A, K359A	pETM-14	N-His <sub>6</sub> -3C	<i>E. coli</i>	this study
Ig1Ig2 <sup>K354/358/359A</sup>	myotilin	250-444	K354A, K358A, K359A	pETM-14	N-His <sub>6</sub> -3C	<i>E. coli</i>	this study
Ig1 <sup>250-344</sup>	myotilin	250-344	-	pET-3d(+)	N-His <sub>6</sub> -TEV	<i>E. coli</i>	this study
Ig2 <sup>349-459</sup>	myotilin	349-459	-	pET-3d(+)	N-His <sub>6</sub> -TEV	<i>E. coli</i>	this study
MYOT <sup>WT</sup>	myotilin	1-498	-	pEGFP-N1	N-EGFP	C2C12	this study
MYOT <sup>K354A</sup>	myotilin	1-498	K354A	pEGFP-N1	N-EGFP	C2C12	this study
MYOT <sup>K359A</sup>	myotilin	1-498	K359A	pEGFP-N1	N-EGFP	C2C12	this study
MYOT <sup>K354/359A</sup>	myotilin	1-498	K354A, K359A	pEGFP-N1	N-EGFP	C2C12	this study
MYOT <sup>K354/358/359A</sup>	myotilin	1-498	K354A, K358A, K359A	pEGFP-N1	N-EGFP	C2C12	this study
ACTN2-WT	α-actinin-2	1-894	-	pET-3d(+)	N-His <sub>6</sub> -TEV	<i>E. coli</i>	[7]
ACTN2-NEECK	α-actinin-2	1-894	R268E, I269E, L273E	pET-3d(+)	N-His <sub>6</sub> -TEV	<i>E. coli</i>	[7]
ACTN2-EF14	α-actinin-2	746-894	-	pETM-14	N-His <sub>6</sub> -3C	<i>E. coli</i>	this study
palladin Ig3	palladin	1022-1126	-	pTBSG	N-His <sub>6</sub> -TEV	<i>E. coli</i>	[46]
Doc2b	Doc2b	125-412	-	pGEX4T1	N-GST-Thr	<i>E. coli</i>	[47]
DVD-actin	actin-5C	1-376	D287A, V288A, D289A	pFastBacHT	N-His <sub>6</sub>	<i>Sf9</i>	[30]
tropomyosin	tropomyosin					<i>E. coli</i>	[33]

(1), myotilin (human, Uniprot-ID Q9UBF9); α-actinin-2 (human, P35609), palladin (Mus musculus, Q9ET54), Doc2b (Rattus norvegicus, P70610), actin-5C (Drosophila melanogaster, P10987), tropomyosin (Tpm1.1st, Homo sapiens, NP\_001018005.1)

(2), in many cases removed during purification; N, N-terminal fusion; Trx, Thioredoxin-tag; His<sub>6</sub>, 6xHis-tag, TEV; TEV protease cleavage site; 3C, HRV-3C protease cleavage site; C, C-terminal fusion; Strep, Strep-tag II; Thr, thrombin cleavage site, GST, Glutathione s-transferase-tag; EGFP, Enhanced green fluorescent protein-tag

(3), Ig1Ig2<sup>185-498\*</sup> was used to produce Ig1Ig2<sup>185-454</sup>, and GST

(4), Ig1Ig2<sup>250-498</sup> was used to produce Ig1Ig2<sup>250-466</sup>