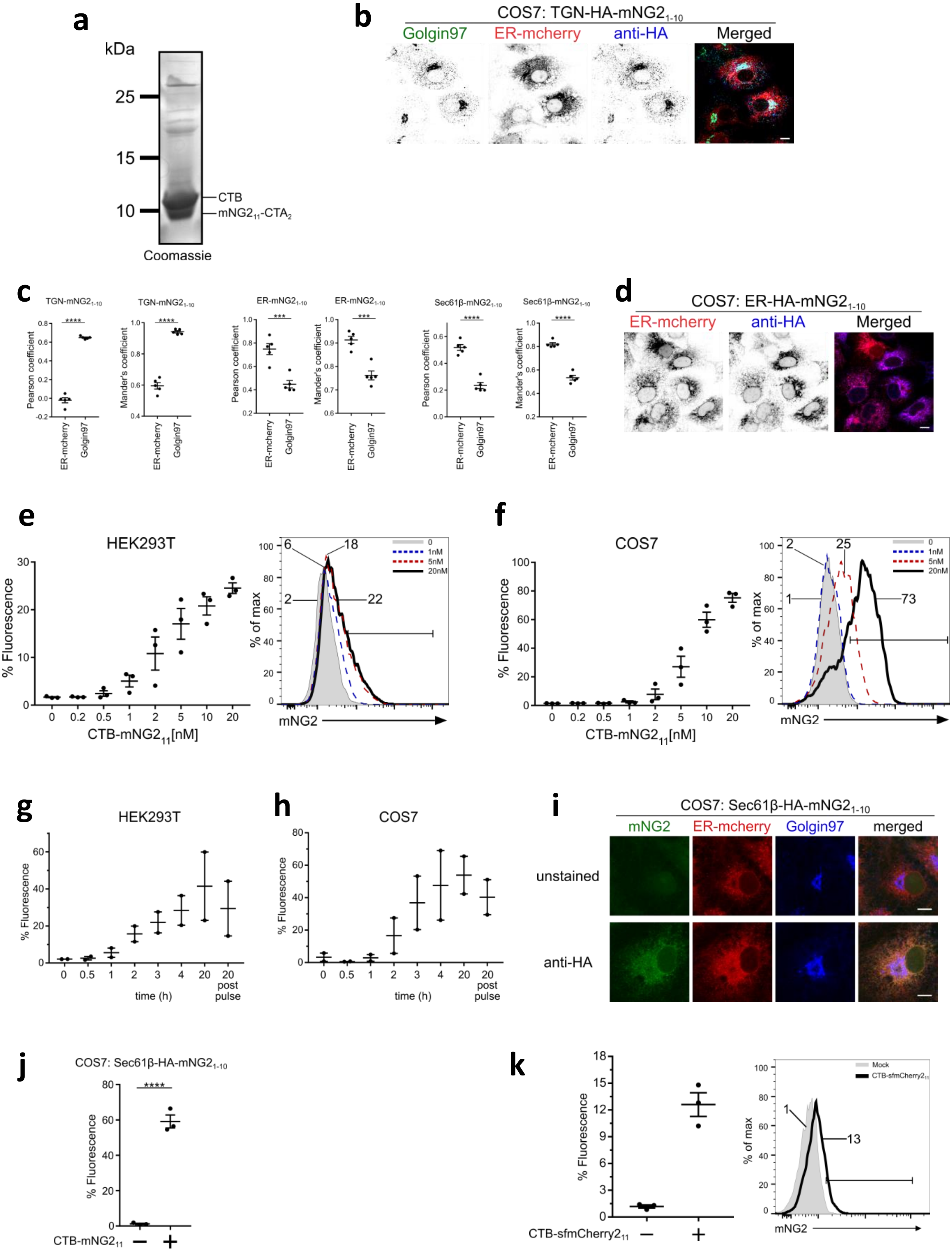
Supplemental Materials

Molecular Biology of the Cell

Luong et al.

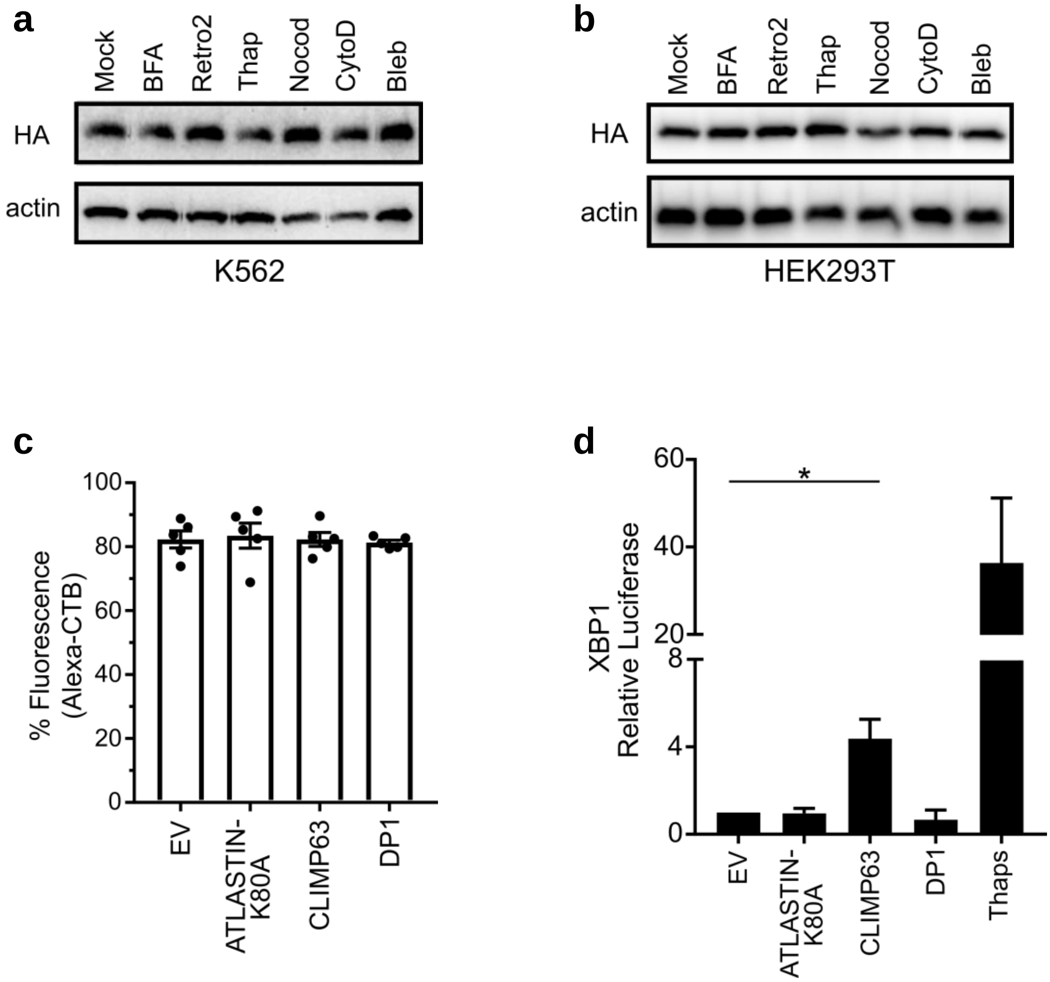
Supplementary Figure 1



Supplementary Figure 1.

Retrograde trafficking of CTB-mNG2_{11} from the plasma membrane to the ER. **a** Coomassie stained gel of recombinant purified CTB-mNG2_{11}. **b** Confocal microscopy of HA-tagged split neon green 2 (HA-mNG$2_{1\text{-}10}$) to trans-Golgi (TGN-HA-mNG$2_{1\text{-}10}$) in COS7. TGN is marked by immunocytochemistry of Golgin97 and ER is marked by expression of luminal ER-mCherry. Anti-HA detects the split HA-mNG$2_{1\text{-}10}$ constructs. **c** Pearson's and Mander's coefficient calculations for TGN-HA-mNG$2_{1\text{-}10}$, ER-HA-mNG$2_{1\text{-}10}$ and Sec61β-HA-mNG$2_{1\text{-}10}$ with ER-mcherry and Golgin97. n=5 individual images. **d** Confocal performed as in **b** but with ER-HA-mNG$2_{1\text{-}10}$. **e, f** Retrograde trafficking in HEK293T and COS7 cells expressing ER-HA-mNG$2_{1\text{-}10}$. Cells were treated with indicated concentrations of CTB-mNG2_{11} for 4 hours at 37°C. Representative flow cytometry graph is displayed highlighting CTB-mNG2_{11} concentrations at 0, 1nM, 5nM and 20nM. n=3 independent experiments each performed with 3 biologic replicates and mean marked as one point for each dose. **g, h** Flow cytometry time course of retrograde trafficking into the ER in HEK293T and COS7 cells with CTB-mNG2_{11}, respectively. Cells were treated with 10nM CTB-mNG2_{11} continuously at 0, 0.5, 1, 2, 4, 20-hour and a condition where cells were treated with a 2-hour toxin pulse and chase for a total of 20 hours. n=2 independent experiments with 3 biological replicates per experiment. CT-1XmNG2_{11} CT-2X mNG2_{11} CT-5XmNG2_{11}. **i** Confocal images as in **b** but with Sec61β-HA-mNG$2_{1\text{-}10}$. **j** PM-ER retrograde trafficking in COS7 cells stably expressing Sec61β-HA-mNG$2_{1\text{-}10}$ incubated for 5 hours with 20nM CTB-mNG2_{11}. n=3 independent experiments. **k** Retrograde trafficking of CTB-sfmCherry2_{11} in K562 cells stably expressing trans-Golgi localized sfmCherry$2_{1\text{-}10}$. Cells were incubated with 20nM CTB-sfmCherry2_{11} for 5 hours at 37°C. n=3 independent experiments. Error bars indicate ± SEM. Scale bars are 10μm.

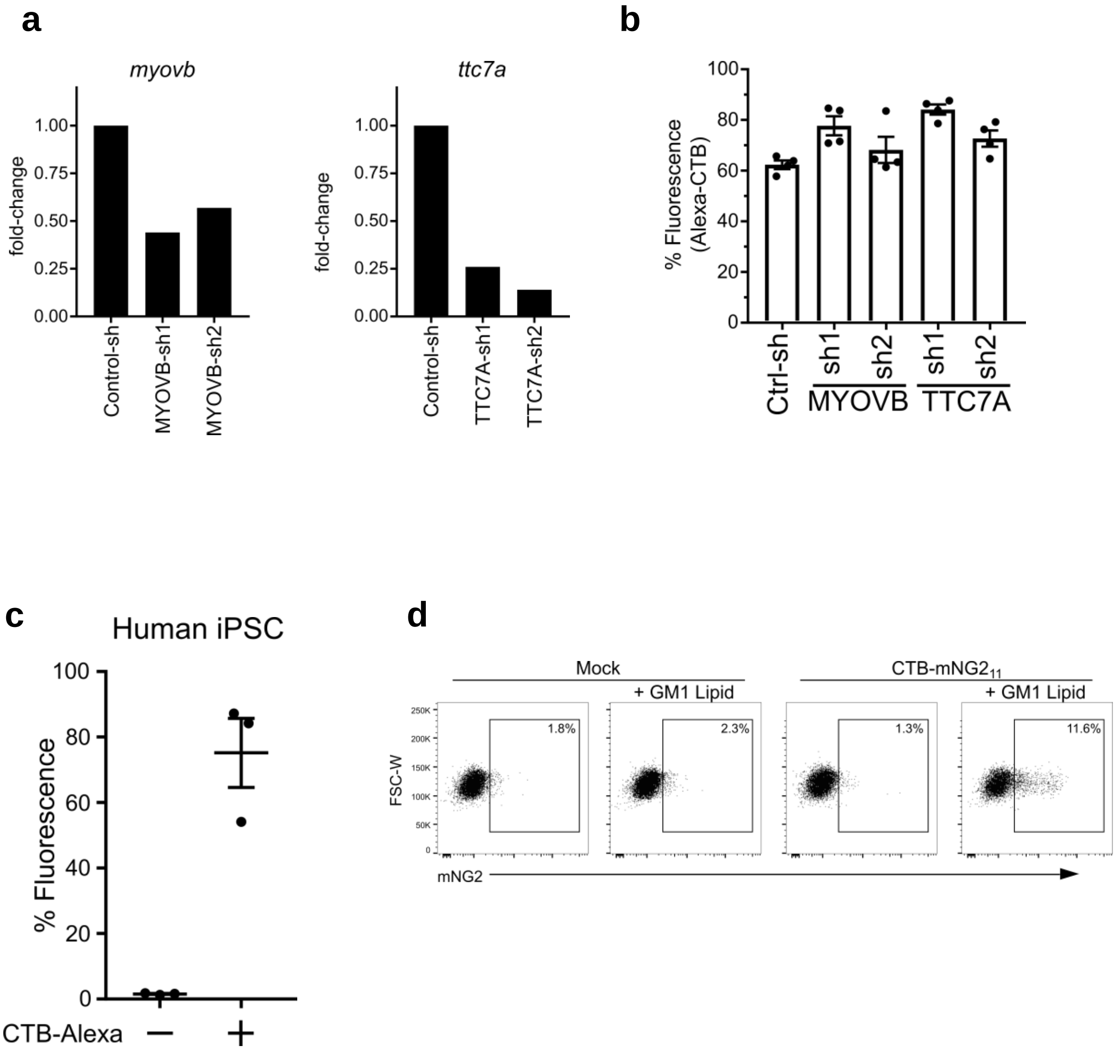
Supplementary Figure 2



Supplementary Figure 2.

Toxin binding and XBP1 stress response to ER shape gene expression. **a, b** Immunoblots for ER-HA-mNG$2_{1\text{-}10}$ in K562 (a) and HEK293T (b) cells treated or not with the indicated compounds used as above. Immunoblots for β-actin provide loading controls. **c** Binding control experiment displays equal loading of CTB-Alexa Fluor 488 in HEK293T cells expressing empty vector (EV), and ER shape genes ATLASTIN1-K80A, CLIMP63 and DP1. Cells were incubated with 2nM CTB-Alexa Fluor 488 for 5 hours 37°C, and then harvested and assayed by flow cytometry. n=5 independent experiments. **d** XBP1 luciferase reporter assay in cells expressing vector (EV), and ER shape genes ATLASTIN1-K80A, CLIMP63 and DP1. Cells were treated with thapsigargin (Thaps) at 300nM for 4 hours as positive control to induce ER stress. Graph displays average of n=3 independent experiments. Error bars indicate ± SEM, $*p<0.05$.

Supplementary Figure 3



Supplementary Figure 3.

mRNA expression and toxin binding CTB-Alexa Fluor 488 in cells with knockdown of monogenic disease genes. **a** Relative mRNA expression to control by qPCR of MYOVB and TTC7A in HEK293T cells expressing shRNA against indicated gene. Actin is used as expression control. **b** Binding control experiment displays equal loading of CTB-Alexa Fluor 488 in HEK293T expressing control shRNA or shRNA targeting MYOVB or TTC7A. Cells were incubated with 2nM CTB-Alexa Fluor 488 for 5 hours 37°C, and then harvested and assayed by flow cytometry. **c** CTB-Alexa Fluor 488 binding controls for human iPSCs loaded with GM1 C12:0 lipid, Incubation conditions same as above. n=3 independent experiments. **d** Representative flow cytometry graph from Fig 3e. Error bars indicate ± SEM.

Supplementary Table1 :	**Sequence of split-FP constructs**
CTA2-sfmcherry211	TACACCATCGTGGAGCAGTACGAGAGAGCCGAGGCCAGACACAGCACCGGAGGTGGAGGTTCCTCGATGAGTAATACTTGCGATGAAAAAACCCAAAGTCTAGGTGTAAAATTCCTTGACGAATACCAATCTAAAGTTAAAAGACAAATATTTTCAGGCTATCAATCTGATATTGATACACATAATAGAATTAAGGATGAATTA
CTA2-mNG211	ACCGAGCTCAACTTCAAGGAGTGGCAAAAGGCCTTTACCGATATGATGGGAGGTGGAGGTTCCTCGATGAGTAATACTTGCGATGAAAAAACCCAAAGTCTAGGTGTAAAATTCCTTGACGAATACCAATCTAAAGTTAAAAGACAAATATTTTCAGGCTATCAATCTGATATTGATACACATAATAGAATTAAGGATGAATTA
ER-HA-mNG21-10	ATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGCGCGCACTCCGGAGAACAGAAGCTGATCTCAGAGGAGGACCTGGGAGGTATGGTGAGCAAGGGTGAGGAGGATAACATGGCCTCTCTCCCAGCGACTCATGAGTTACACATCTTTGGCTCCATCAACGGTGTGGACTTTGACATGGTGGGTCAGGGTACCGGCAATCCAAATGATGGTTATGAGGAGTTAAACCTGAAGTCCACCAAGGGTGACCTCCAGTTCTCCCCCTGGATTCTGGTCCCTCATATCGGGTATGGCTTCCATCAGTACCTGCCCTACCCTGACGGGATGTCGCCTTTCCAGGCCGCCATGGTAGATGGCTCCGGATACCAAGTCCATCGCACAATGCAGTTTGAAGATGGTGCCTCCCTTACTGTTAACTACCGCTACACCTACGAGGGAAGCCACATCAAAGGAGAGGCCCAGGTGATGGGGACTGGTTTCCCTGCTGACGGTCCTGTGATGACCAACACGCTGACCGCTGCGGACTGGTGCATGTCGAAGAAGACTTACCCCAACGACAAAACCATCATCAGTACCTTTAAGTGGAGTTACACCACTGTAAATGGCAAACGCTACCGGAGCACTGCGCGGACCACCTACACCTTTGCCAAGCCAATGGCGGCTAACTATCTGAAGAACCAGCCGATGTACGTGTTCCGTAAGACGGAGCTCAAGCACTCCATGGGAGAGAAGGATGAACTG
TGN-HA-mNG21-10	ATGAGGCTTCGGGAGCCGCTCCTGAGCGGCAGCGCCGCGATGCCAGGCGCGTCCCTACAGCGGGCCTGCCGCCTGCTCGTGGCCGTCTGCGCTCTGCACCTTGGCGTCACCCTCGTTTACTACCTGGCTGGCCGCGACCTGAGCCGCCTGCCCCAACTGGTCGGAGTCTCCACACCGCTGCAGGGCGGCTCGAACAGTGCCGCCGCCATCGGGCAGTCCTCCGGGGAGCTCCGGACCGGAGGGGCCAAGGATCCACCGGTCGCCACCTACCCATACGATGTTCCAGATTACGCTGGAGGTATGGTGAGCAAGGGTGAGGAGGATAACATGGCCTCTCTCCCAGCGACTCATGAGTTACACATCTTTGGCTCCATCAACGGTGTGGACTTTGACATGGTGGGTCAGGGTACCGGCAATCCAAATGATGGTTATGAGGAGTTAAACCTGAAGTCCACCAAGGGTGACCTCCAGTTCTCCCCCTGGATTCTGGTCCCTCATATCGGGTATGGCTTCCATCAGTACCTGCCCTACCCTGACGGGATGTCGCCTTTCCAGGCCGCCATGGTAGATGGCTCCGGATACCAAGTCCATCGCACAATGCAGTTTGAAGATGGTGCCTCCCTTACTGTTAACTACCGCTACACCTACGAGGGAAGCCACATCAAAGGAGAGGCCCAGGTGATGGGGACTGGTTTCCCTGCTGACGGTCCTGTGATGACCAACACGCTGACCGCTGCGGACTGGTGCATGTCGAAGAAGACTTACCCCAACGACAAAACCATCATCAGTACCTTTAAGTGGAGTTACACCACTGTAAATGGCAAACGCTACCGGAGCACTGCGCGGACCACCTACACCTTTGCCAAGCCAATGGCGGCTAACTATCTGAAGAACCAGCCGATGTACGTGTTCCGTAAGACGGAGCTCAAGCACTCCATG
Sec61β-HA-mNG21-10	ATGCCTGGTCCGACCCCCAGTGGCACTAACGTGGGATCCTCAGGGCGCTCTCCCAGCAAAGCAGTGGCCGCCCGGGCGGCGGGATCCACTGTCCGGCAGAGGAAAAATGCCAGCTGTGGGACAAGGAGTGCAGGCCGCACAACCTCGGCAGGCACCGGGGGGATGTGGCGATTCTACACAGAAGATTCACCTGGGCTCAAAGTTGGCCCTGTTC

	CAGTATTGGTTATGAGTCTTCTGTTCATCGCTTCTGTATTTATGTTGCACATTTGGGGCAAGTACACTCGTTCGGGAGGAGGATACCCATACGATGTTCCAGATTACGCTATGGTGAGCAAGGGTGAGGAGGATAACATGGCCTCTCTCCCAGCGACTCATGAGTTACACATCTTTGGCTCCATCAACGGTGTGGACTTTGACATGGTGGGTCAGGGTACCGGCAATCCAAATGATGGTTATGAGGAGTTAAACCTGAAGTCCACCAAGGGTGACCTCCAGTTCTCCCCCTGGATTCTGGTCCCTCATATCGGGTATGGCTTCCATCAGTACCTGCCCTACCCTGACGGGATGTCGCCTTTCCAGGCCGCCATGGTAGATGGCTCCGGATACCAAGTCCATCGCACAATGCAGTTTGAAGATGGTGCCTCCCTTACTGTTAACTACCGCTACACCTACGAGGGAAGCCACATCAAAGGAGAGGCCCAGGTGATGGGGACTGGTTTCCCTGCTGACGGTCCTGTGATGACCAACACGCTGACCGCTGCGGACTGGTGCATGTCGAAGAAGACTTACCCCAACGACAAAACCATCATCAGTACCTTTAAGTGGAGTTACACCACTGTAAATGGCAAACGCTACCGGAGCACTGCGCGGACCACCTACACCTTTGCCAAGCCAATGGCGGCTAACTATCTGAAGAACCAGCCGATGTACGTGTTCCGTAAGACGGAGCTCAAGCACTCCATG
TGN-FLAG-sfmcherry21-10	ATGCGCCTTAGAGAGCCATTGCTTTCAGGCTCAGCTGCCATGCCGGGTGCATCCTTGCAACGGGCTTGTCGCTTGTTGGTCGCAGTGTGCGCACTGCATCTTGGTGTCACGCTCGTATATTACTTGGCCGGGCGCGACTTGAGTCGACTGCCACAGTTGGTAGGGGTAAGCACCCCATTGCAGGGCGGGTCTAATTCAGCGGCTGCGATTGGCCAGTCTTCAGGGGAACTGAGAACGGGAGGTGCTGATTATAAAGATGACGATGACAAAATGGAAGAAGACAATATGGCCATAATCAAGGAGTTCATGAGGTTCAAGGTTCATATGGAAGGTAGTGTGAACGGGCATGAGTTTGAAATCGAGGGAGAGGGCGAGGGGCACCCTTATGAGGGTACCCAGACGGCCAAATTGAAGGTAACAAAAGGGGGGCCACTTCCTTTCGCATGGGATATACTCAGCCCCCAGTTTATGTATGGTTCTAAGGCTTATGTAAAACATCCGGCTGACATACCCGATTATCTCAAGCTCTCATTCCCCGAGGGCTTTACTTGGGAGCGGGTGATGAATTTCGAAGATGGAGGAGTGGTTACGGTTACGCAGGACAGTTCTCTTCAAGACGGGCAATTCATTTACAAAGTTAAACTCCTCGGGATAAACTTCCCTAGTGATGGCCCTGTCATGCAGAAGAAGACTATGGGGTGGGAAGCCAGCACGGAGAGGATGTATCCGGAGGATGGTGCCTTGAAAGGTGAGATCAACCAACGCCTCAAGCTCAAAGATGGTGGACACTATGATGCCGAGGTTAAGACCACCTATAAAGCCAAGAAACCGGTCCAGCTCCCCGGTGCATACAACGTTGATATAAAGCTCGATATAACATCTCACAATGAGGAC
CTA1-(EE/DD)-1XmNG2	AATGATGATAAGTTATATCGGGCAGATTCTAGACCTCCTGATGAAATAAAGCAGTCAGGTGGTCTTATGCCAAGAGGACAGAGTGAGTACTTTGACCGAGGTACTCAAATGAATATCAACCTTTATGATCATGCAAGAGGAACTCAGACGGGATTTGTTAGGCACGATGATGGATATGTTTCCACCTCAATTAGTTTGAGAAGTGCCCACTTAGTGGGTCAAACTATATTGTCTGGTCATTCTACTTATTATATATATGTTATAGCCACTGCACCCAACATGTTTAACGTTAATGATGTATTAGGGGCATACAGTCCTCATCCAGATGACCAAGACGTTTCTGCTTTAGGTGGGATTCCATACTCCCAAATATATGGATGGTATCGAGTTCATTTTGGGGTGCTTGATGAACAATTACATCGTAATAGGGGCTACAGAGATAGATATTACAGTAACTTAGATATTGCTCCAGCTGCAGATGGTTATGGATTGGCAGGTTTCCCTCCGGAGCATAGAGCTTGGAGGGAAGAGCCGTGGATTCATCATGCACCGCCGGGTTGTGGGAATGCTGGTGGAGGAGGTGGAACCGAGCTCAACTTCAAGGAGTGGCAAAAGGCCTTTACCGATATGATG
CTA1-(EE/DD)-2XmNG2	AATGATGATAAGTTATATCGGGCAGATTCTAGACCTCCTGATGAAATAAAGCAGTCAGGTGGTCTTATGCCAAGAGGACAGAGTGAGTACTTTGACCGAGGTACTCAAATGAATATCAACCTTTATGATCATGCAAGAGGAACTCAGACGGGATTTGTTAGGCACGATGATGGATATGTTTCCACCTCAATTAGTTTGAGAAGTGCCCACTTAGTGGGTCAAACTATATTGTCTGGTCATTCTACTTATTATATATATGTTATAGCCACTGCACCCAACATGTTTAACGTTAATGATGTATTAGGGGCATACAGTCCTCATCCAGATGACCAAGACGTTTCTGCTTTAGGTGGGATTCCATACTCCCAAATATATGGATGGTATCGAG

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CTA1-(EE/DD)-5XmNG2	AATGATGATAAGTTATATCGGGCAGATTCTAGACCTCCTGATGAAATAAAGCAGTCAGGTGGTCTTATGCCAAGAGGACAGAGTGAGTACTTTGACCGAGGTACTCAAATGAATATCAACCTTTATGATCATGCAAGAGGAACTCAGACGGGATTTGTTAGGCACGATGATGGATATGTTTCCACCTCAATTAGTTTGAGAAGTGCCCACTTAGTGGGTCAAACTATATTGTCTGGTCATTCTACTTATTATATATATGTTATAGCCACTGCACCCAACATGTTTAACGTTAATGATGTATTAGGGGCATACAGTCCTCATCCAGATGACCAAGACGTTTCTGCTTTAGGTGGGATTCCATACTCCCAAATATATGGATGGTATCGAGTTCATTTTGGGGTGCTTGATGAACAATTACATCGTAATAGGGGCTACAGAGATAGATATTACAGTAACTTAGATATTGCTCCAGCTGCAGATGGTTATGGATTGGCAGGTTTCCCTCCGGAGCATAGAGCTTGGAGGGAAGAGCCGTGGATTCATCATGCACCGCCGGGTTGTGGGAATGCTGGTGGTtctGGAGGAACCGAGCTCAACTTCAAGGAGTGGCAAAAGGCCTTTACCGATATGATGGGCtctggaAGCGGGACTGAATTAAATTTTAAAGAATGGCAGAAAGCATTCACTGACATGATGGGagcaggtggatcaACaGAGCTaAACTTtAAGGAaTGGCAAAAGGCtTTTACaGATATGATGggtgggtctgcaggaACgGAGCTgAAtTTCAAaGAGTGGCAgAAGGCCTTTACgGAcATGATG

Supplementary Table2:	**shRNA and qPCR oligo sequences**
MYOVB shRNA-1 Forward	TTTgcgtgttacagccgatgatTTCAAGAGAatcatcggctgtaacacgcTTTTTA
MYOVB shRNA-1 Reverse	AGCTTAAAAAgcgtgttacagccgatgatTCTCTTGAAatcatcggctgtaacacg
MYOVB shRNA-2 Forward	TTTgatggtgattcctgtagtataTTCAAGAGAtatactacaggaatcaccatcTTTTTA
MYOVB shRNA-2 Reverse	AGCTTAAAAAgatggtgattcctgtagtataTCTCTTGAAtatactacaggaatcaccat
TTC7A shRNA-1 Forward	TTTgcaagatgaattgcaccggAATTCAAGAGATTccggtgcaattcatcttgcTTTTTA
TTC7A shRNA-1 Reverse	AGCTTAAAAAgcaagatgaattgcaccggAATCTCTTGAATTccggtgcaattcatcttg
TTC7A shRNA-2 Forward	TTTgcgtttggagaatttcaccttTTCAAGAGAaaggtgaaattctccaaacgcTTTTTA
TTC7A shRNA-2 Reverse	AGCTTAAAAAgcgtttggagaatttcaccttTCTCTTGAAaaggtgaaattctccaaacg
TTC7A qPCR Primer Forward	CATCTATGACCTCCTGAGCATC
TTC7A qPCR	CTGACTTCCCACAAGCCA

Primer Reverse	
MYOVB qPCR Primer Forward	TGCTGAGTGACCTTTCCATTC
MYOVB qPCR Primer Reverse	CCAGATAGACCCTGAATGCTC