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Supplemental Data

Mutations in *INPP5K* Cause a Form of Congenital Muscular Dystrophy Overlapping Marinesco-Sjögren Syndrome and Dystroglycanopathy

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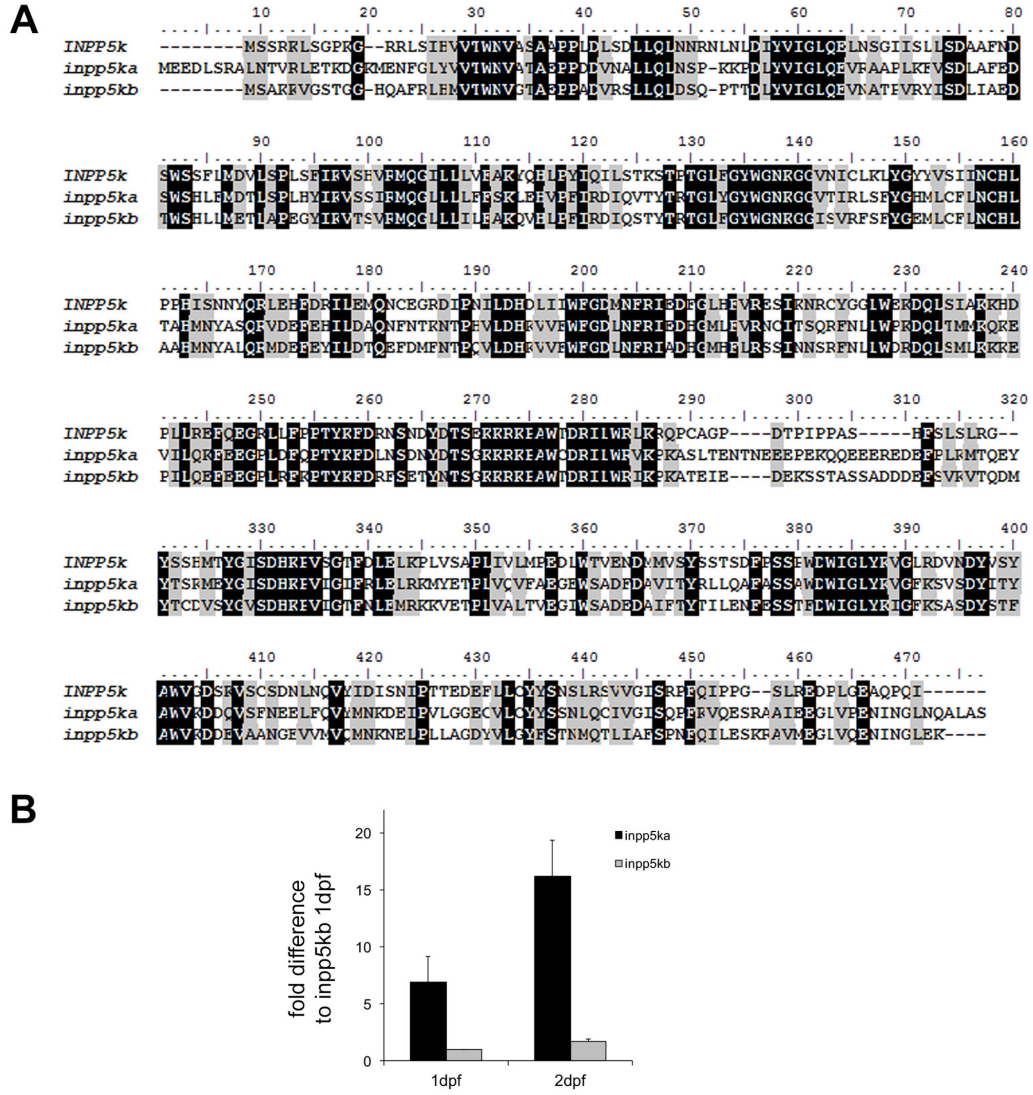
Supplemental Material

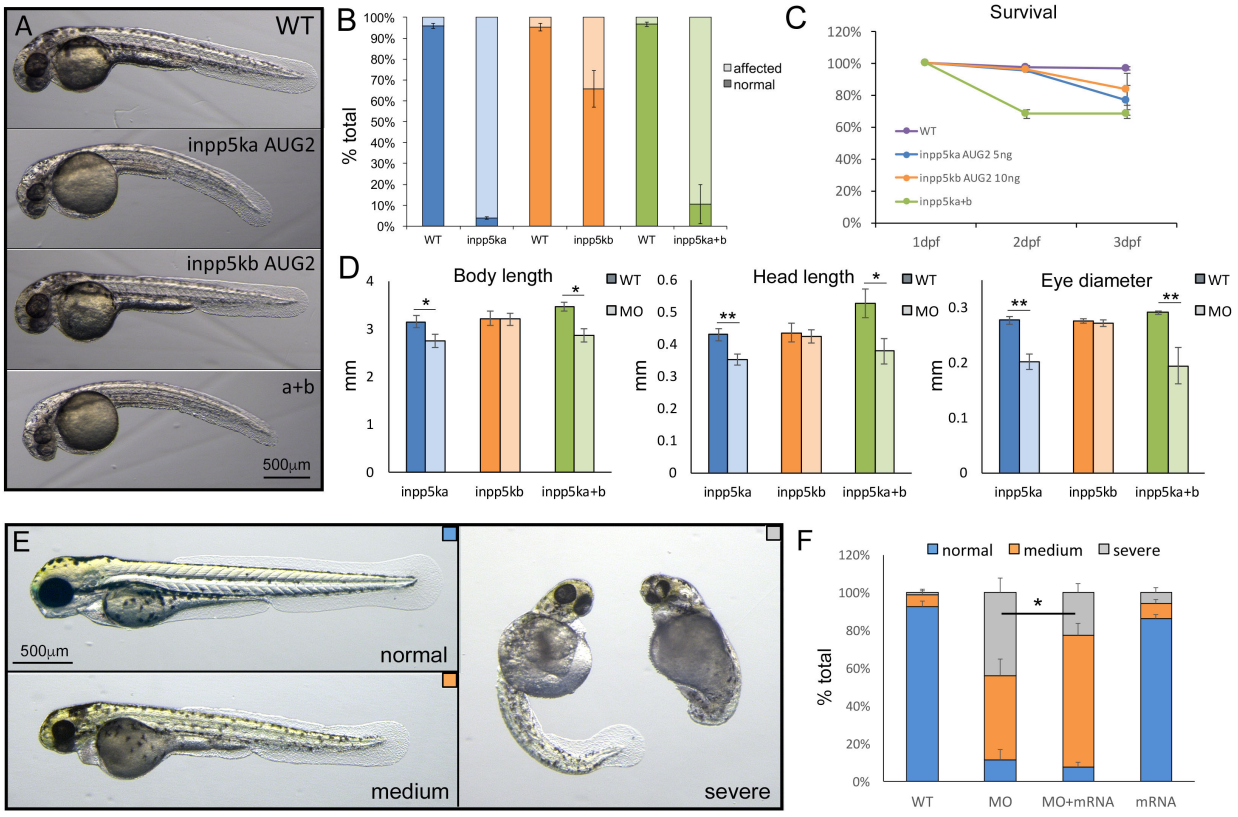


Supplemental Figure 1. A. Protein alignment of INPP5K with inpp5ka and inpp5kb showing that protein conservation is higher for inpp5ka. **B.** Quantitative PCR (qPCR) measurement of *inpp5ka* and *inpp5kb* expression on RNA extracted from zebrafish embryos at 1 and 2 dpf shows that *inpp5ka* expression is higher than *inpp5kb*. RNA was extracted from frozen embryos using ReliaPrep RNA Miniprep System (Promega). cDNA generated iScript Reverse Transcription Supermix (Bio-Rad) and qPCR was performed via a SYBR Green-based approach using Sso Fast EvaGreen Supermix (Bio-Rad) on a CFX 384 Real Time PCR Detection system (Bio-Rad).

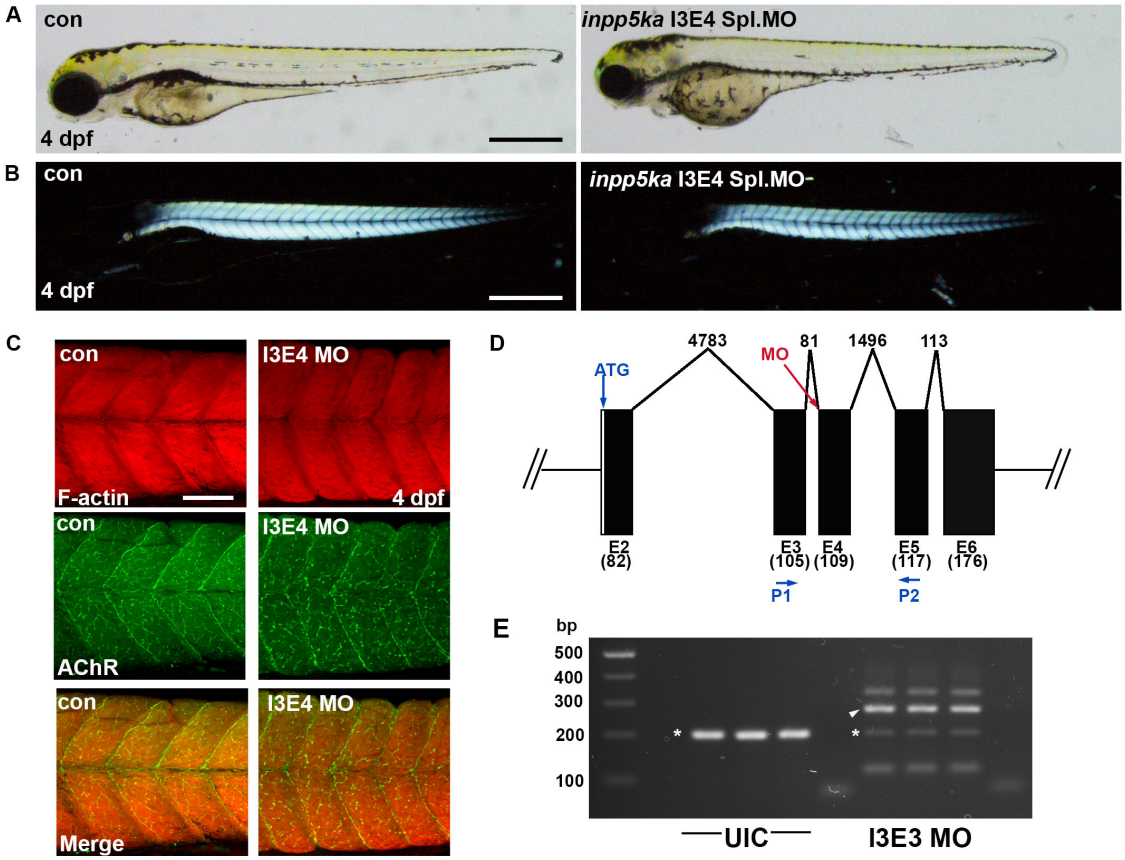
PCR primers:

inpp5ka For GGAGTCACCTCTTCATGGAC; Rev TCCAGCTTGGAAAAGAAAAG

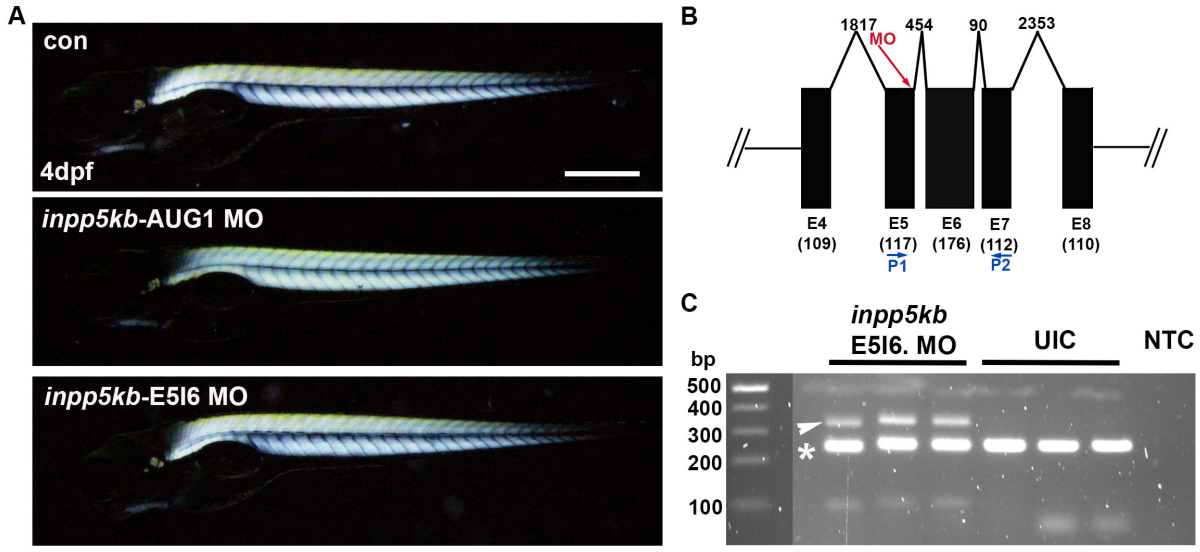
inpp5kb For TGGGACTGGATTGGGTTAT; Rev GCTCCTCATTGAAAGACACC



Supplemental Figure 2. *inpp5ka* morphants shows a more severe phenotype than *inpp5kb* morphants A. Average presentation of 2 dpf embryos injected with 5ng *inpp5ka* AUG2 MO, 10ng *inpp5kb* AUG2 MO or a combination of the two MOs (5ng for *inpp5ka* and 10ng *inpp5kb*). Scale bar: 500µm **B.** Quantification across multiple independent injections of 50-150 embryos/condition showed what most *inpp5ka* AUG2 morphants are affected by 2 dpf, but *inpp5kb* AUG2 morphants are less severely affected. N= 6 for *inpp5ka*, 5 for *inpp5kb* and 3 for a+b. Data is shown as average±s.e.m. **C.** Survival for the three MO injections**. D.** Quantification of total body length, head length from the most rostral point to the otic vesicle, and anterior to posterior eye diameter show that *inpp5ka* morphants and a+b morphants are equally affected, while *inpp5kb* morphants do not differ from uninjected clutch-mates. Darker shaded bar represents wild-type (WT) and lighter bar morphants (MO). Data is shown as average±s.e.m. **E.** 3dpf phenotypes scored for rescue analysis showing examples of medium and severe morphants presentation. Scale bar: 500µm **F.** Quantification of 4 independent experiments shows that injecting 200pg of human *INPP5K* mRNA improves the *inpp5ka* morphants phenotype, while mRNA alone has no effect. One-way ANOVA followed by Tukey's post-hoc test indicates that phenotypic improvement between MO and rescue (MO+mRNA) is significant p=0.034. Data is shown as average±s.e.m. *INPP5K* cDNA (clone BC004362, Transomic) was cloned into pCS2P+ (Addgene) and capped mRNA was generated using the mMessage mMachine SP6 Transcription kit (Ambion). For each experiment MO, mRNA and MO+mRNA injections were performed on oocytes from the same clutch. In double injections MO and mRNA were injected sequentially using the same needles and conditions employed for the single injections.



Supplemental Figure 3. Knockdown of *inpp5ka* using a splice morpholino further confirms myogenic and ocular defects. (A) Injections of a MO targeting the Intron3-Exon4 splice border causes defects in embryonic length and eye size. Scale bar: 500 µm. **(B)** Birefringence analysis of skeletal muscle organization reveals reduced but not absent signal, myotomes are small with reduced chevron shaped appearance. Scale bar: 500 µm. **(C)** F-actin (Red) staining of muscle fibers reveals reduction of myotome width and fiber disorganization. NMJs (Green) are present but notably more punctate in morphants compared to control embryos. Scale bar: 100 µm. **(D)** Schematic representation of morpholino target site. **(E)** RT-PCR validation of gene specific morpholino knockdown shown for three groups of MO or control MO injected embryos at 48 hpf. Expected size of endogenous *inpp5ka* product: 204 bp (asterisk), predicted product size if Exon 4 excluded: 95 bp, predicted product size if intron 3 included: 285 bp (arrow head).



Supplemental Figure 4. Morpholino knockdown of *inpp5kb* has no discernable effect on skeletal muscle integrity. (A) Birefringence assay in control non-injected or standard MO treated embryos, compared to a translational blocking or splice inhibiting MOs at 4 dpf. Scale bar: 500 μm. **(B)** Schematic representation of splice MO target site, at the exon5-intron6 splice border. Primers for RT-PCR knockdown were localized to exon 5 and exon7, forward and reverse, respectively. **(C)** RT-PCR showing the predicted endogenous PCR product of 274 bp (asterisks) and the presence of a second band in morphant embryos at 364 bp, (arrow head), consistent with an intron 6 inclusion, and premature stop codon. Experiments were completed on 3 independent injections. NTC: no template control.

PCR primers:

inpp5kb For: GTACATACACACGCACAGGC

inpp5kb Rev: CGGAGAAAGTGCATCCCATG

Supplemental Table 1. Information on exome sequencing

ID	Sequencing center	Instrument	Library	Exome capture	Reads	Mapped reads	% Mapped	Mean coverage	Coverage 30X
S1.1	Otogenetics Corporation (Norcross, GA, USA	Illumina HiSeq 2500	SureSelect XT Target Enrichment	SureSelect AllExon v5	37,899,311	37,728,210	99.55%	59.5	84.90%
S2	Broad Institute (Cambridge, MA, USA)	Illumina HiSeq 2000	SureSelect XT Target Enrichment	SureSelect AllExon v2	107,223,634	64,252,668	86.13%	86.89	80.13%
S3	Broad Institute (Cambridge, MA, USA)	Illumina HiSeq 2000	SureSelect XT Target Enrichment	SureSelect AllExon v2	131,302,974	98,035,909	92.30%	115.62	87.47%
S4	GeneDX (Gaithersburg, MD, USA)	Illumina HiSeq 4000	proprietary capture chemistry using Agilent Clinical Research Exome probes	Agilent Clinical Research Exome	61,481,105	61,451,744	99.95%	117.34	93.18%

Supplemental information on variant annotation methods

S1.1 - Reads were aligned to genome assembly hg19 with Burrows-Wheeler Aligner (BWA,V.0.5.87.5). High quality indel and single nucleotide variant calling and annotation were performed using GATK Lite 2.3.10 using standard filtering criteria (read depth >=10%, genotype quality score >=30). Candidate genes were prioritized by searching for homozygous variants with a minor allele frequency < 1% in 300 in-house ethnically-matched Iranian control exomes, dbSNP, 1000 Genomes and ExAC.
S2-3 - Reads were aligned to genome assembly hg19 with BWA and annotation was performed using GTAK as listed above. Data was loaded in a SQL database and was filtered for rare (<1%in 1000Genomes, <1% in Exome Variant Server), recessive (homozygous or compound heterozygous) and protein-altering (non synonymous, nonsense, frameshift, splicing) variants. Filtered variants were visually confirmed on IGV.

S4 - DNA sequence was mapped to the published human genome build UCSC hg19/GRCh37 reference sequence using Burroughs Wheeler Aligner (BWA) with the latest internally validated version at the time of sequencing, progressing from BWA v0.5.8 through BWA-Mem v0.7.8. The variant interpretation protocol has been previously described (Tanaka et al., Am J Hum Genet, 2015. 97(3): p. 457-64)

Supplemental Table 2. List of potential candidate genes identified by exome sequencing See Excel file

Supplemental Table 3. Estimation of pathogenic relevance for INPP5K mutations identified by exome sequencing
Human INPP5K sequence IDs: GenBank transcript NM_016532.3; GenBank Protein NP_057616.2; UniProt Q9BT40; Ensembl transcript ENST00000421807; Ensembl protein ENSP00000413937.2

	S1.1, S1.2	S2, S3	S2	S4	S4
DNA change	c.277A>G	c.67G>A	c.805G>A	c.418G>A	c.1251-1252delCA
Protein change	p.Met93Val	p.Val23Met	p.Asp269Asn	p.Gly140Ser	p.Asn417Lysfs*26
ExAc het/total	0	1/119,622	1/124,461	1/120,892	0
gnomAD het/total	0	2/251,228	2/250,742	3/252,326	0
SIFT prediction	damaging	damaging	damaging	damaging	
score	0.01	0	0	0	
Polyphen2 prediction	benign	damaging	damaging	damaging	
score	0.19	1	1	1	
MutationTaster prediction	disease causing	disease causing	disease causing	disease causing	
score	0.999993771	1	1	1	
CADD score	22.7	16.7	34	28.6	

Supplemental Table 4. Morpholino oligonucleotide (MO) sequences

MO name	MO sequence (5'>3')	Target
inpp5ka (ENSDART00000169037.1)		
inpp5ka-AUG1	CACGAGACAAATCTTCCTCCATCCT	Start
inpp5ka-I3E4	ACTGAAGAGGAGCAGCATTCAAACA	Intron 3 - Exon 4
inpp5ka-AUG2	GCACGAGACAAATCTTCCTCCATCC	Start
inpp5ka-I1E2	TCGCCTGTCAATCATAACATCGTGT	Intron 1 – Exon 2
inpp5kb (ENSDART00000148480.1)		
inpp5kb-AUG1	CGCTCATACTCAAACCCTTCAGCCT	Start
inpp5kb-E5I6	ATAATAATGAACTGACTTGTGGTCT	Exon 5 - Intron 6
inpp5kb-AUG2	CTCAAACCCTTCAGCCTCGTCTAGC	Start
inpp5kb-I1E2	AAACCTGAGCACACAAACACCATGT	Intron 1 – Exon 2