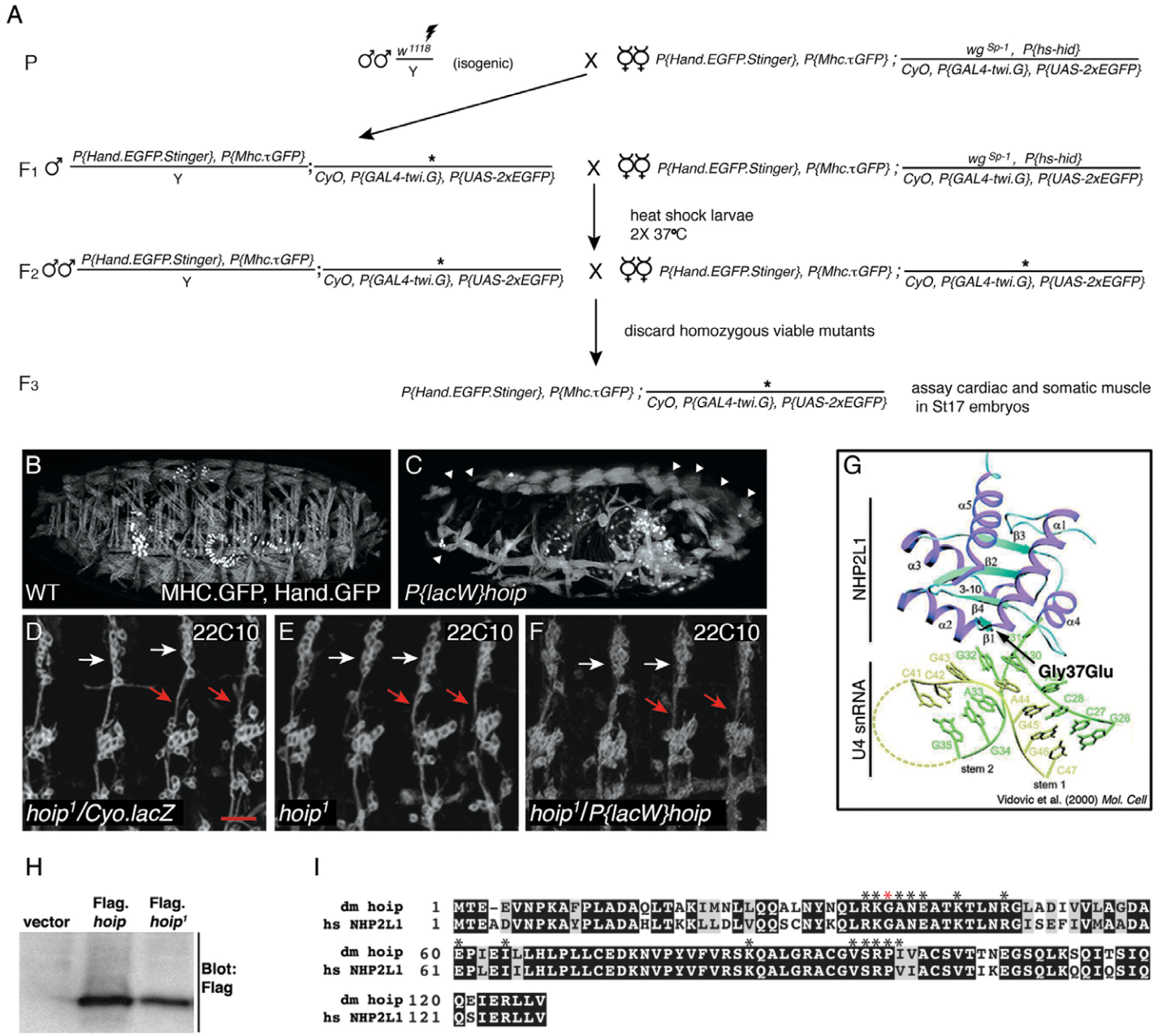

Fig. S1. A forward genetic screen identified Hoip as a novel regulator of mesoderm development. (**A**) Crossing scheme to generate EMS mutants in a double GFP reporter background. Over 10,000 mutagenized genomes were screened. (**B**,**C**) MHC.τGFP, Hand.n-GFP expression in St17 embryos. Compared with wild-type embryos (B), *P{lacW}hoip*^{k07104} homozygous embryos (C) show severe muscle defects and apparent segmentation defects (white arrowheads). (**D-F**) St16 embryos stained with the PNS marker 22C10. Micrographs show four dorsal neuron clusters. The organization of the dorsal clusters (white arrows) and the pathway of the descending nerve (red arrows) are comparable among *hoip*^{1}*/Cyo* (D), *hoip*^{1} (E) and *hoip*^{1}*/P{lacW}hoip*^{k07104} (F) embryos. (**G**) Crystal structure of hNHP2L1 bound to U4 snRNA as reported previously (Vidovic et al., 2000), except the position of the 37th residue (mutated in *hoip*^{1} embryos) is shown. (**H**) α-Flag western blot from COS cells transfected with empty pcDNA.Flag, pcDNA.Flag.Hoip or pcDNA.Flag.Hoip^{1}. (**I**) Alignment of Hoip and hNHP2L1 protein sequence. The two proteins are 79% identical and 89% similar. hNHP2L1 amino acids required for RNA binding are indicated with an asterisk (Schultz et al., 2006). The red asterisk shows the position of the *hoip*^{1} missense mutation. Scale bar: 20μm.

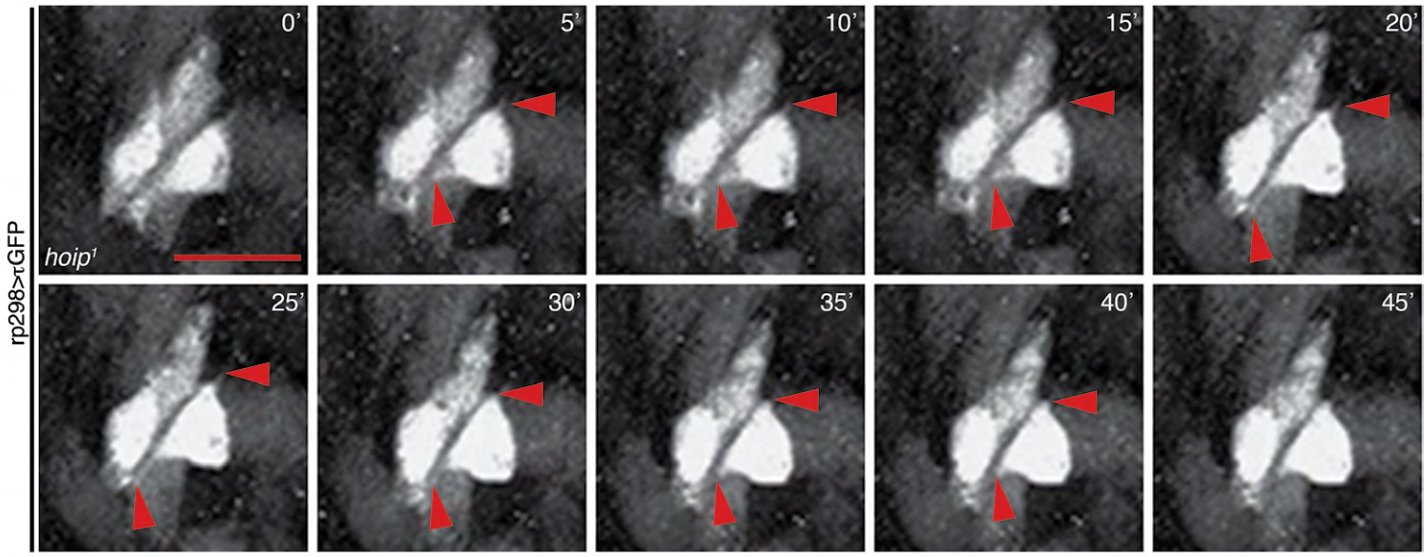

Fig. S2. Somatic muscles in *hoip* embryos extend filapodia in the direction of polarization. Time-lapse images of rp298. gal4>τGFP *hoip*^{1} somatic muscles beginning at late St12. Filapodia extend in the axis of myotube polarization (red arrowheads) and then retract. Scale bar: 10 μm.

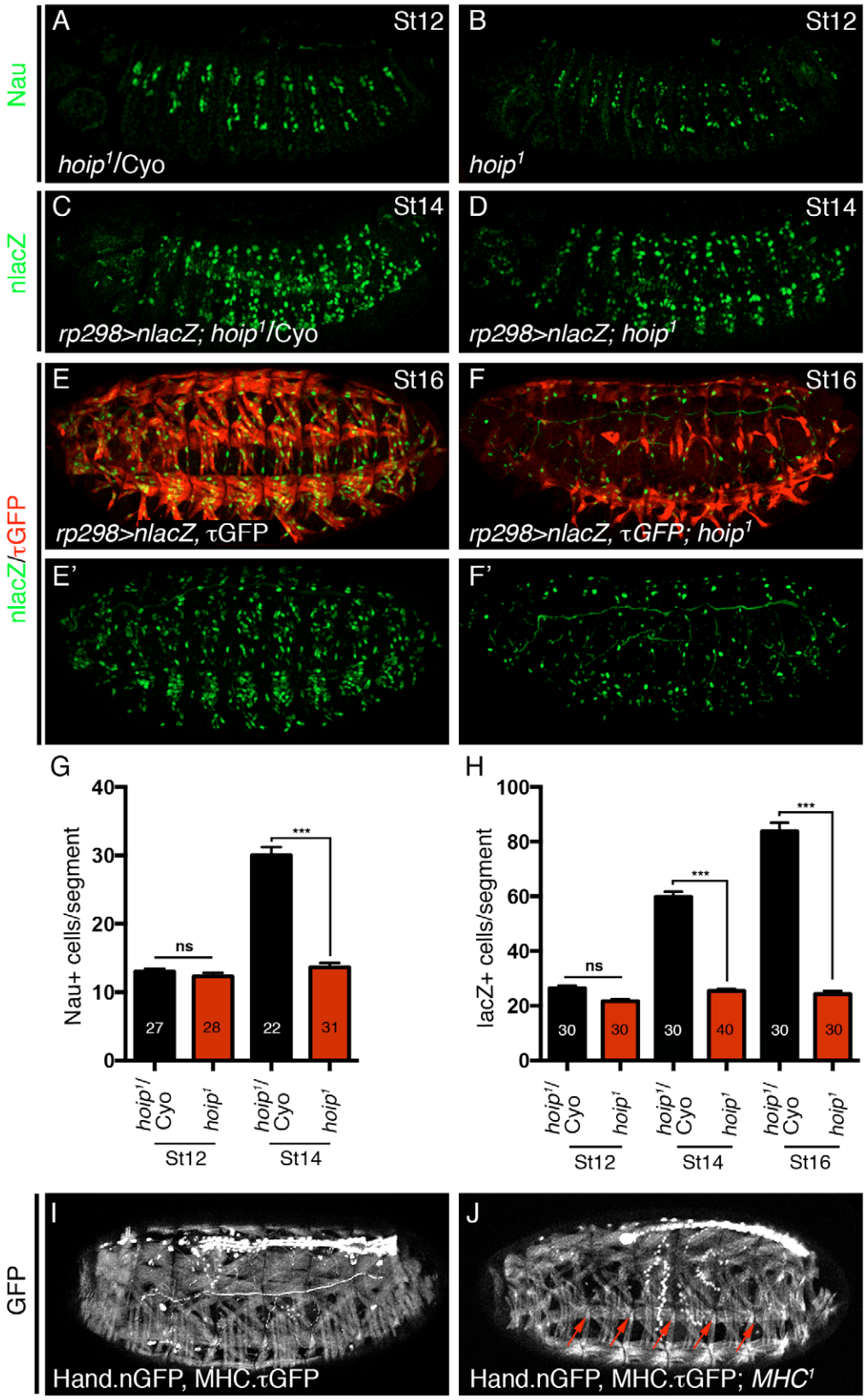

Fig. S3. Muscle morphology and identity gene expression. (**A**,**B**) Nau expression in St12 embryos. The number of Nau+ nuclei is comparable between control and *hoip*^{1} embryos. (**C**,**D**) rp298.lacZ expression in St12 embryos. The number of lacZ^{+} nuclei is comparable between control and *hoip*^{1} embryos. (**E**,**F**) St16 rp298.gal4>τ.GFP, rp298.nlacZ embryos double-labeled for GFP (red) and lacZ (green). *hoip*^{1} embryos showed a significant reduction in the number of lacZ^{+} nuclei (F) compared with wild type (E). (**G**,**H**) Quantification of Nau^{+} and rp298.lacZ^{+} positive nuclei in the dorsal mesoderm. The number of segments quantified is given for each genotype and time point. Unpaired *t*-tests were performed to establish significance. Error bars represent s.e.m. (**I**,**J**) MHC.τGFP, Hand.nGFP expression in St16 embryos. (I) Wild-type and (J) *MHC*^{1} embryos show similar somatic muscle morphology. In particular LL1/DO5 muscles have elongated and attached in all segments (red arrows). ns, not significant; ***P<0.001.

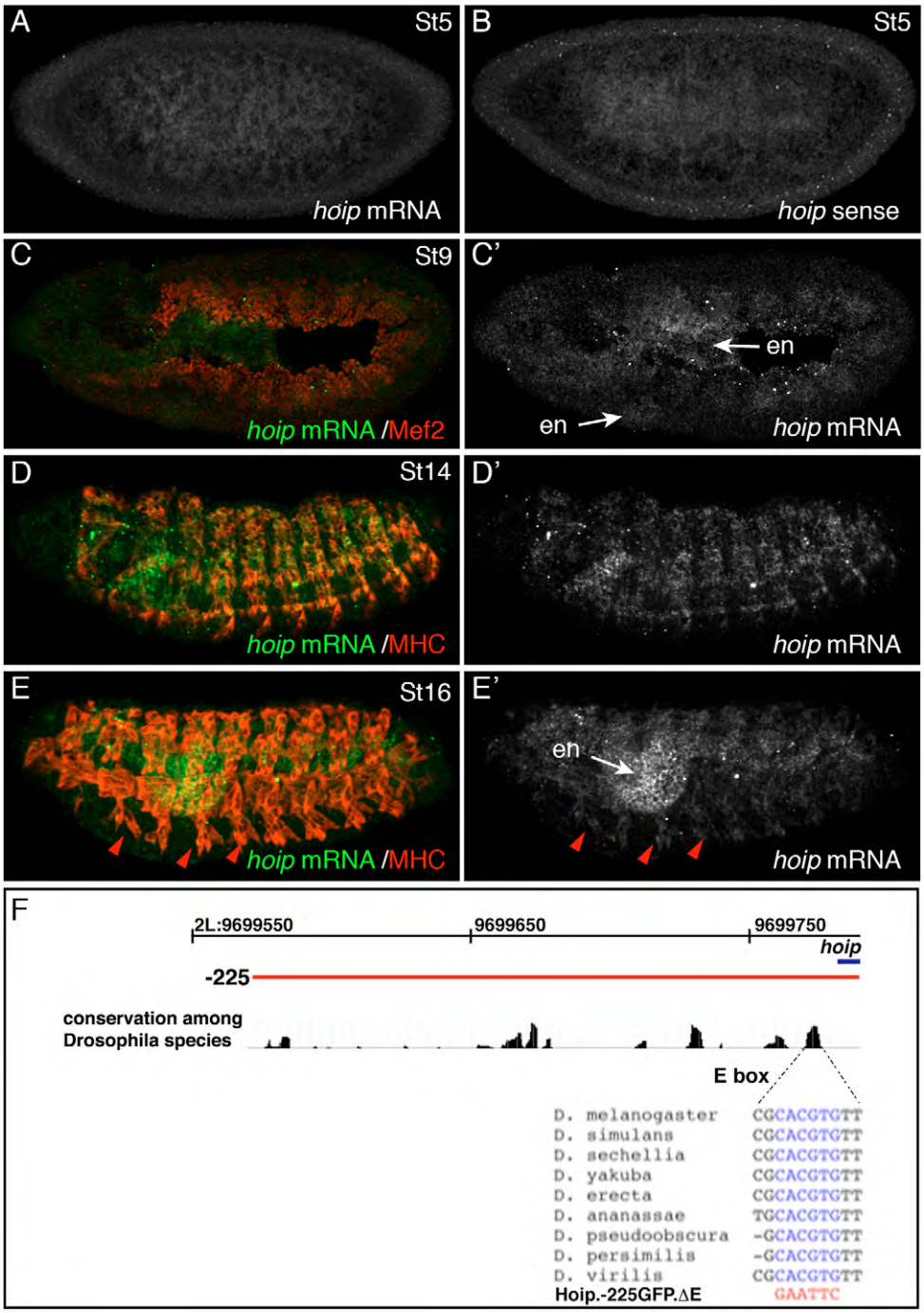

Fig. S4. *hoip* expression during embryonic development. (A-E) Wild-type embryos hybridized with RNA probes antisense (A,C-E) or sense (B) to the *hoip* mRNA. (**A**,**B**) Post-cellularization blastoderm embryos. Fluorescent intensity is comparable between the antisense and sense probes. (**C**) St9 embryo co-labeled for *hoip* (green) and Mef2 (red). Weak *hoip* expression has initiated in the endoderm and mesoderm. (**D**,**E**) St14 (D) and St16 (E) embryos co-labeled for *hoip* (green) and MHC (red). *hoip* is expressed at high levels in the endoderm (en) and at lower levels in the somatic musculature (red arrowheads). (**F**) Genomic conservation 225 bp 5′ to the *hoip* transcriptional start site. The highly conserved E-box sequence and mutations in Hoip.-225.ΔEGFP are shown. Genomic coordinates refer to base pair positions along chromosome 2L.

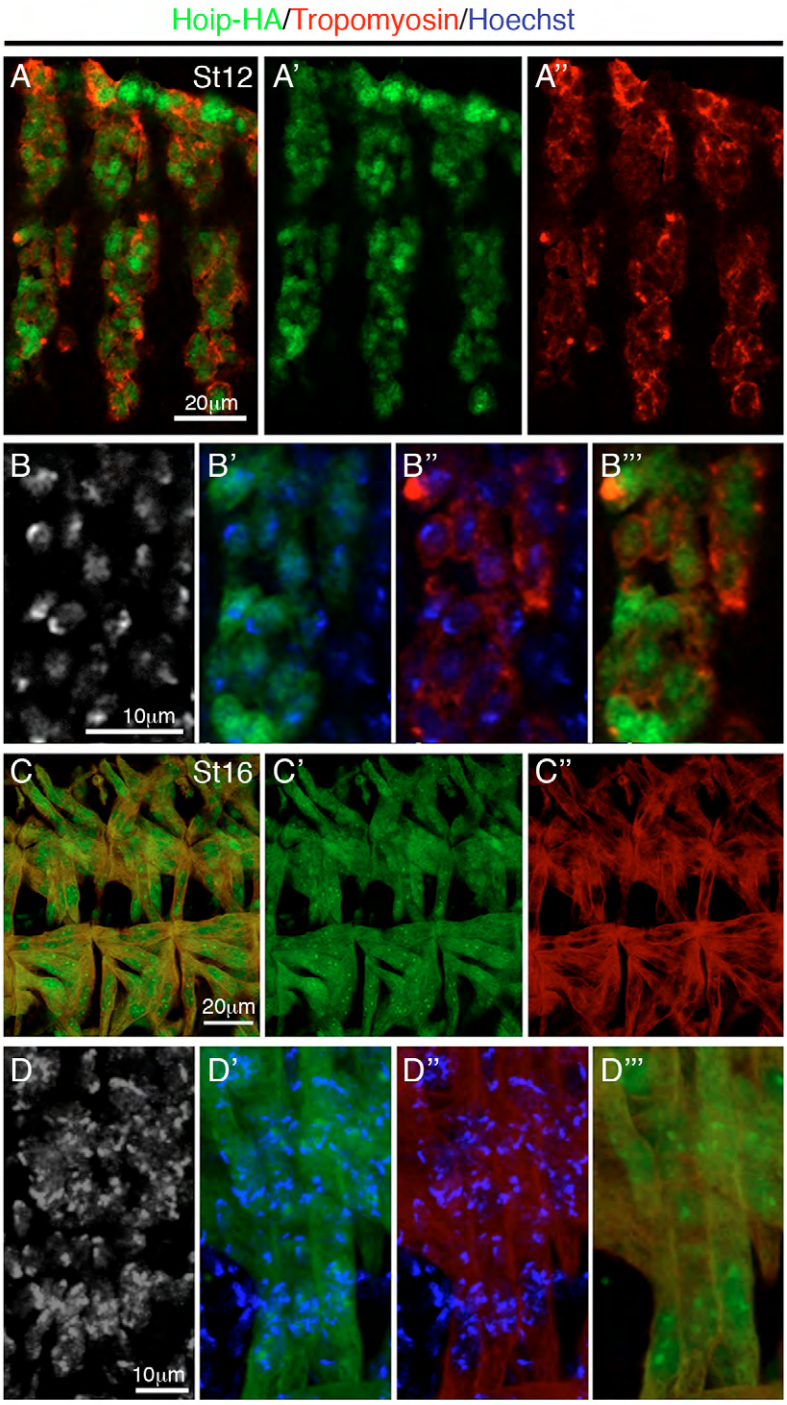

Fig. S5. Hoip localizes to both the nucleus and the cytoplasm. (A-D) Mef2>Hoip-HA embryos co-labeled for HA (green) and Tropomyosin (red). (**A**,**B**) Hoip is largely localized to the nucleus in St12 embryos, although some cytoplasmic staining is present. (**C**,**D**) Hoip is localized throughout the myofibers of St16 embryos. Enhanced localization is apparent in a subnuclear domain.

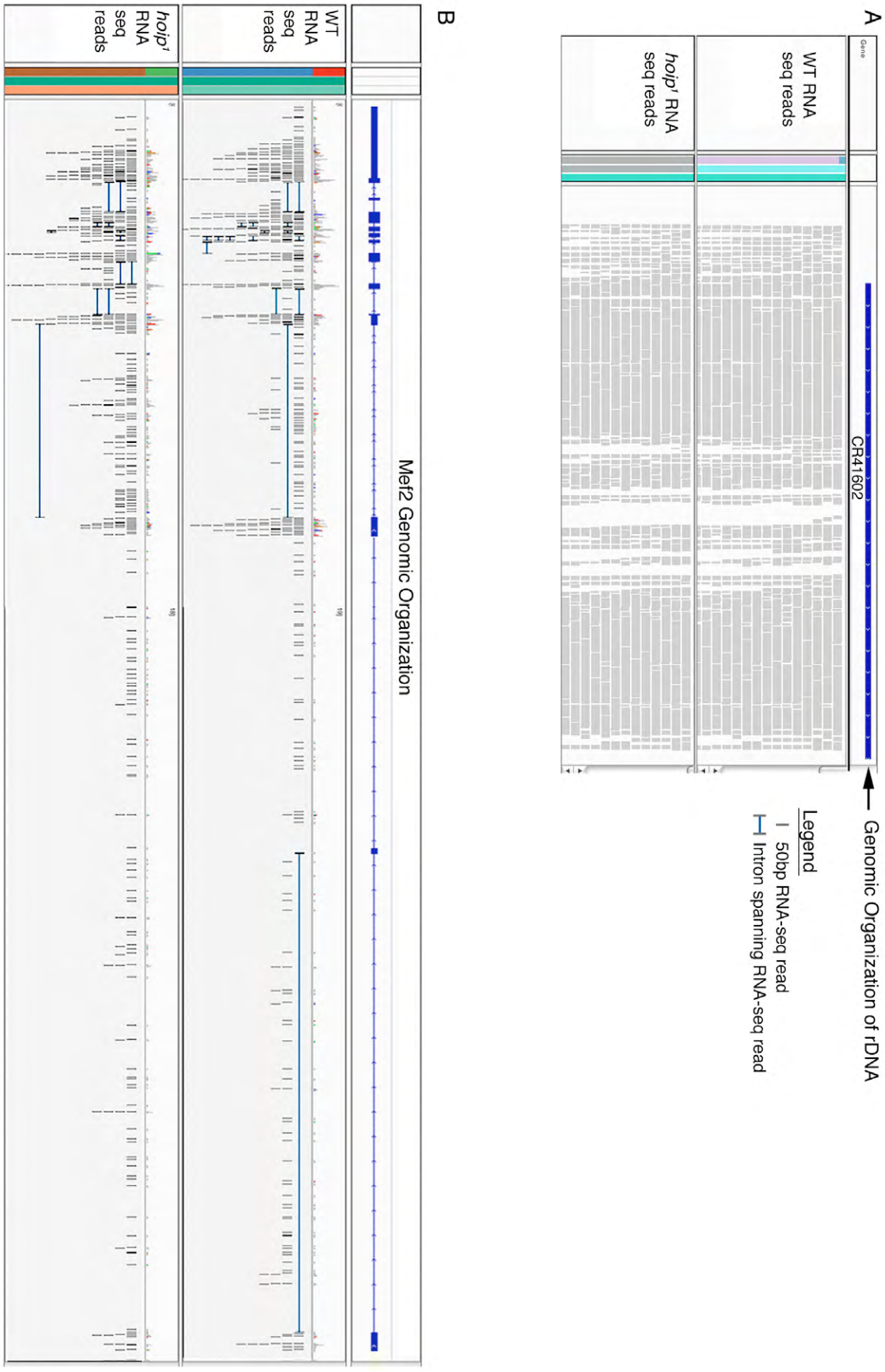

Fig. S6. rRNA processing and *Mef2* expression are not affected in *hoip*^{1} embryos. (**A**,**B**) Screen shot from Integrative Genome Viewer at the rDNA locus (A; CR41602) and the Mef2 locus (B) showing RNA-seq reads from wild-type and *hoip*^{1} embryos. Vertical grey bars represent a single sequencing read. Horizontal blue lines indicate a sequencing read across an intron. The number and positions of RNA-seq reads is comparable between genotypes at both loci.

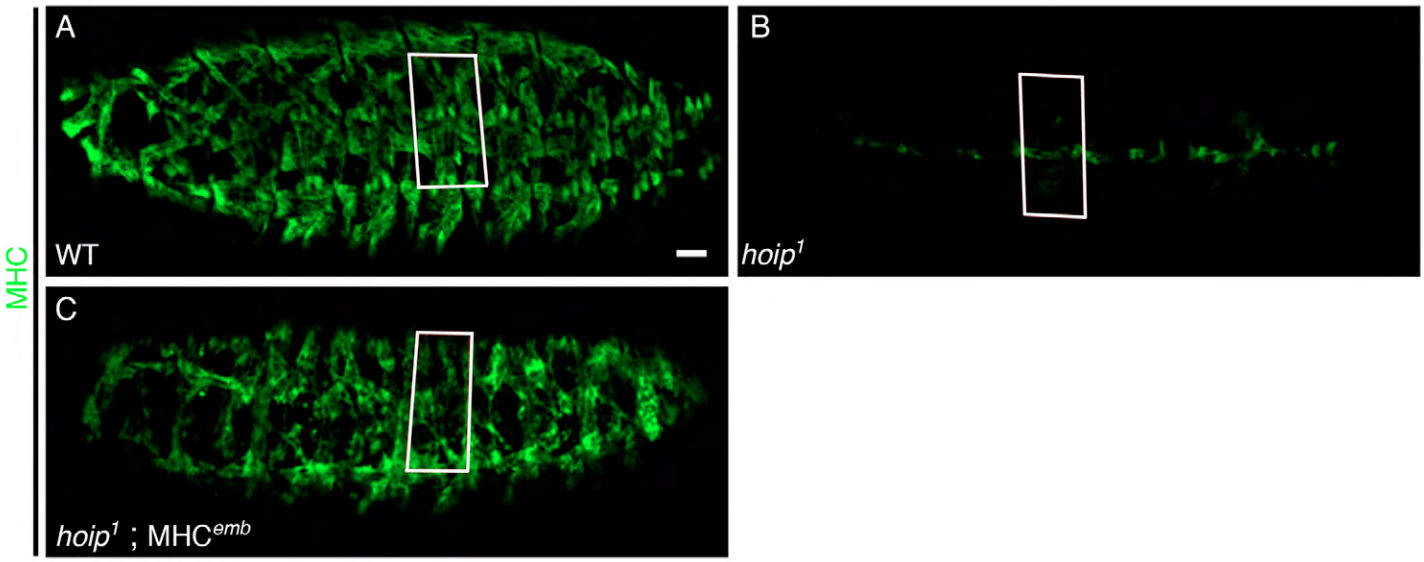

Fig. S7. Method to quantify MHC expression by mean fluorescence intensity. (**A-C**) Z-stacks including only the somatic muscles from St16 embryos stained for MHC. MHC expression was clearly restored in *hoip*^{1} embryos by *MHC*^{emb}; however, muscle morphology was not (compare A with C). To quantify MHC expression in the somatic musculature (SM), we used the Zeiss LSM Zen 2011 software to trace the medial region of a segment using SBMs to define anterior-posterior position of each segment (white boxes). The software then calculated mean fluorescent intensity (MFI) within each boxed region. We also measured fluorescent intensity in the visceral musculature (VM). To normalize MHC expression, we divided SM MFI by VM MFI to obtain relative fluorescence for each segment.

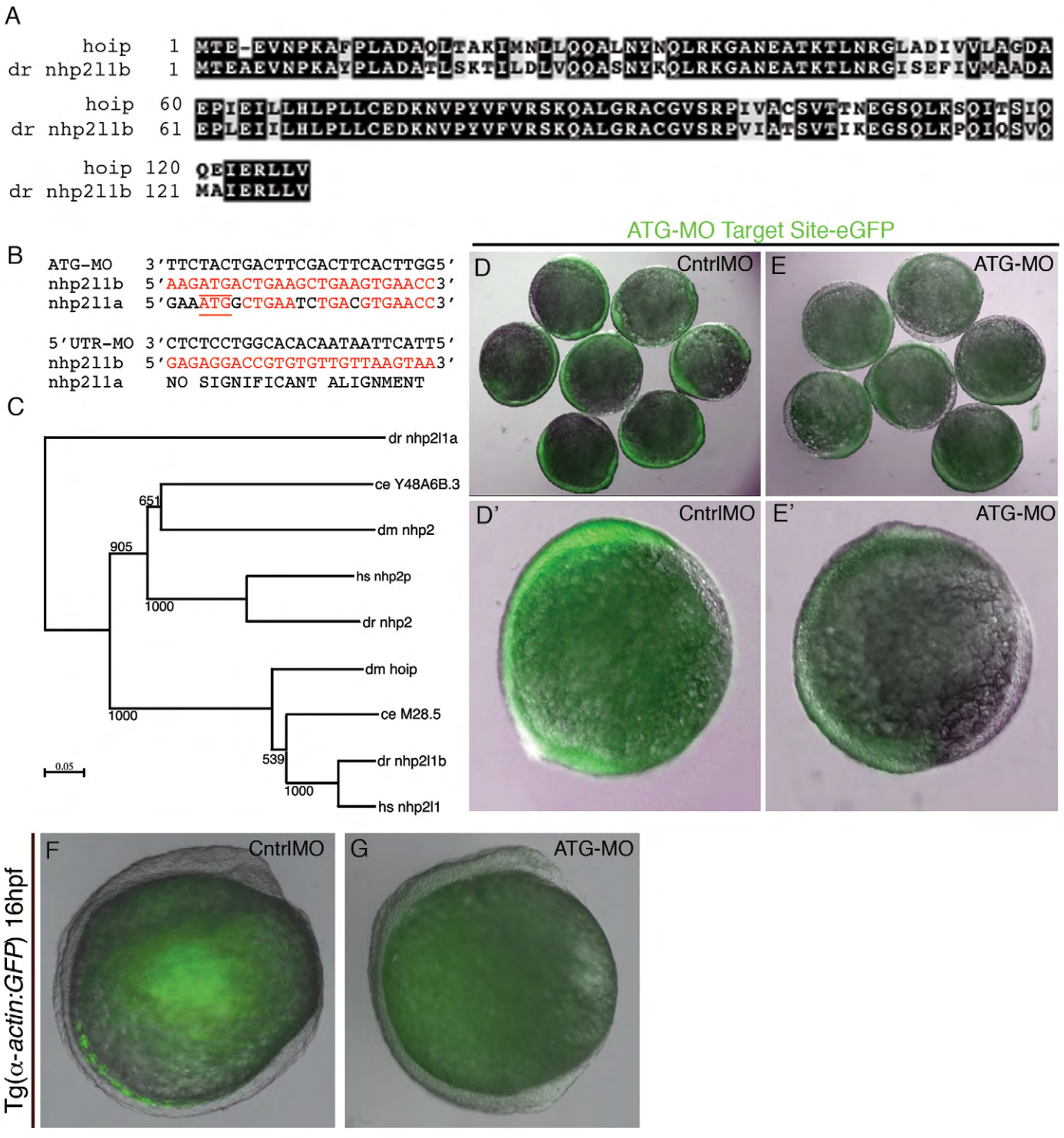

Fig. S8. *nhp2l1b* is a Hoip orthologue and the *nhp2l1b* ATG-MO efficiently blocks translation. (**A**) Alignment between Hoip and zebrafish *nhp2l1b* protein sequences. Identical amino acids are shaded black; similar amino acids are shaded gray. (**B**) Alignment of the *nhp2l1a/b* translational start sequences with the ATG-MO and 5'UTR-MO. Target nucleotides are red. The beginning of the ORF is underlined. (**C**) Phylogenetic analysis of NHP2 and NHP2L1 proteins in worms (ce), flies (dm), zebrafish (dr) and humans (hs). Bootstrap values are given. (**D**) Zebrafish embryos co-injected with Control-MO and an eGFP construct containing the *nhp2l1b* target site in the 5′UTR show robust eGFP expression at 12 hpf. (**E**) Embryos co-injected with ATG-MO and the same eGFP construct as in D show a significant reduction in eGFP expression. (**F**,**G**) Tg(α-*actin:GFP*) embryos injected with ATG-MO did not develop beyond 16 hpf.

Table S1. Muscle development in *hoip* embryos

Muscle*	*DO1*	*DO2*	*DA 3*	*DO 3*	*DO 4*	*DO 5*	*LT1-4*	*DT 1*	*LO 1*	*SBM*	*VO 1-3*	*VA 1-2*
hoip^{1}	97 (36)	94 (36)	33 (36)	13 (36)	38 (36)	19 (36)	47 (144)	91 (36)	47 (36)	94 (36)	61 (90)	64 (62)
hoip^{1}/ Df(2L)ED690	77 (31)	100 (31)	38 (31)	16 (31)	54 (31)	32 (31)	41 (124)	93 (31)	41 (31)	67 (31)	52 (93)	77 (62)
hoip^{1}/*P{lacW}hoip*^{K07104}	100 (34)	100 (34)	53 (34)	14 (34)	44 (34)	41 (34)	33 (136)	76 (34)	50 (34)	91 (34)	71 (66)	75 (48)
P{lacW}hoip^{K07104}	86 (22)	78 (22)	9 (22)	0 (22)	18 (22)	18 (22)	4 (88)	36 (22)	4 (22)	59 (22)	16 (66)	23 (44)

*Muscles extending across the segment scored positive

Data are percentage of positive muscles (number of muscles scored)

Table S2. Differential gene expression in 6-10 hour *hoip*^{1} embryos

Gene	Wild-type value	*hoip* value	Fold change	*P* value
abba	25.7008	0.3275	0.01274	0.00002
Acsl	193.6320	82.4571	0.42584	0.00143
Actn	235.0040	103.6920	0.44124	0.00014
Adh	308.0260	45.7923	0.14866	0.00000
Adk1	66.7169	5.9337	0.08894	0.00033
Ahcy13	355.8390	152.4240	0.42835	0.00401
alphaTub85E	99.3420	25.6589	0.25829	0.00004
arg	285.5680	34.4369	0.12059	0.00445
Atf6	184.9760	74.7937	0.40434	0.00113
ATPsyn-g	620.1150	296.6300	0.47835	0.00164
betaTub97EF	124.1460	13.9371	0.11226	0.00000

BM-40-SPARC	197.7130	54.6709	0.27652	0.00022
bt	248.1740	13.6823	0.05513	0.00000
Ca-alpha1D	27.9455	10.2013	0.36505	0.00417
Ca-beta	24.3753	2.5030	0.10268	0.00062
cals	521.7950	248.3170	0.47589	0.00810
CaMKI	182.9920	64.2499	0.35111	0.00020
CAP	86.9704	36.7742	0.42284	0.00011
Caps	179.0760	75.8946	0.42381	0.00000
Cda4	140.2440	65.9648	0.47036	0.00617
Cf2	66.7754	20.9347	0.31351	0.00101
CG10249	65.6698	27.0462	0.41185	0.00023
CG10591	2167.7900	628.6480	0.29000	0.00000
CG10625	96.9136	2.9092	0.03002	0.00000
CG10737	142.7700	42.1398	0.29516	0.00000
CG11155	26.3139	6.6147	0.25138	0.00258

CG11198	81.6923	31.6125	0.38697	0.00047
CG11255	199.1450	88.5562	0.44468	0.00039
CG11658	124.5830	58.0435	0.46590	0.00199
CG11883	17.8655	1.6973	0.09500	0.00033
CG12769	27.3271	8.2212	0.30084	0.00727
CG13124	34.9772	12.4803	0.35681	0.00720
CG13183	30.3052	5.5422	0.18288	0.00318
CG13397	70.7670	21.9354	0.30997	0.00209
CG13698	145.5600	53.7993	0.36960	0.00039
CG14265	191.3600	0.0000	0.00000	0.00023
CG14817	589.0110	97.1897	0.16500	0.00833
CG14869	32.6878	11.2547	0.34431	0.00039
CG15093	157.8800	12.2214	0.07741	0.00000
CG15251	34.8088	1.9145	0.05500	0.00643
CG15309	53.2676	15.7721	0.29609	0.00827

CG15618	29.7964	9.6678	0.32446	0.00003
CG15822	45.2577	17.9422	0.39644	0.00026
CG1607	68.4284	23.6532	0.34566	0.00357
CG1674	104.1200	3.5355	0.03396	0.00001
CG16884	156.7830	1.3108	0.00836	0.00000
CG16885	247.5510	2.8119	0.01136	0.00004
CG17549	104.3110	13.2131	0.12667	0.00017
CG17598	164.8950	69.0480	0.41874	0.00311
CG1764	62.8433	21.1004	0.33576	0.00455
CG17816	34.8959	12.4392	0.35647	0.00207
CG1824	81.5638	40.0639	0.49120	0.00400
CG18522	38.5264	18.1660	0.47152	0.00566
CG18661	32.8536	5.0490	0.15368	0.00362
CG18675	15.8026	1.5630	0.09891	0.00653
CG2219	143.0870	56.4341	0.39441	0.00381

CG2930	66.8025	6.7124	0.10048	0.00001
CG2950	68.0822	34.2304	0.50278	0.00443
CG2962	372.1370	100.6710	0.27052	0.00003
CG30101	96.4232	11.8547	0.12294	0.00004
CG3011	106.0890	34.2676	0.32301	0.00327
CG30458	44.2715	0.7229	0.01633	0.00007
CG30460	78.2668	31.4999	0.40247	0.00002
CG31004	88.4885	17.5975	0.19887	0.00000
CG31140	58.8638	19.0361	0.32339	0.00000
CG31323	20.3243	1.9758	0.09721	0.00058
CG3164	354.7930	173.0310	0.48769	0.00035
CG31999	54.3378	10.3593	0.19064	0.00000
CG32352	17.0136	1.9147	0.11254	0.00009
CG32373	26.2502	3.7408	0.14250	0.00380
CG32581	33.2508	2.7799	0.08360	0.00151

CG32813	97.8948	46.8468	0.47854	0.00294
CG33144	47.1899	23.2984	0.49371	0.00270
CG33205	151.1240	9.7741	0.06468	0.00000
CG33977	733.7690	54.7722	0.07465	0.00085
CG3588	54.3898	5.6839	0.10450	0.00001
CG3793	69.7514	16.3636	0.23460	0.00510
CG40263	77.1697	14.6049	0.18926	0.00005
CG42326	24.2489	0.4969	0.02049	0.00018
CG42492	84.9767	30.5867	0.35994	0.00000
CG42527	185.2060	89.3761	0.48258	0.00129
CG42533	45.7777	19.2573	0.42067	0.00501
CG42673	77.4045	32.9339	0.42548	0.00614
CG42741	20.4978	1.1266	0.05496	0.00641
CG4467	40.2310	18.5803	0.46184	0.00049
CG4562	34.0110	2.9619	0.08709	0.00001

CG4692	912.1890	414.2920	0.45417	0.00773
CG4822	63.7431	15.4715	0.24272	0.00014
CG5080	476.0120	126.7830	0.26634	0.00000
CG5322	36.5403	7.3002	0.19978	0.00082
CG5381	46.1005	12.6364	0.27411	0.00561
CG5804	267.2140	0.0000	0.00000	0.00174
CG5830	162.1450	80.2979	0.49522	0.00334
CG5853	88.4155	26.1122	0.29533	0.00096
CG5910	39.0743	3.5508	0.09087	0.00227
CG5958	1026.3700	428.3170	0.41732	0.00144
CG6043	32.5517	6.9578	0.21374	0.00032
CG6388	92.7134	28.3814	0.30612	0.00301
CG6672	151.9640	72.3595	0.47616	0.00080
CG6972	86.6755	10.0643	0.11611	0.00012
CG7203	103.2540	0.0000	0.00000	0.00001

CG7203	103.2540	0.0000	0.00000	0.00001
CG7298	226.4770	2.8344	0.01252	0.00000
CG7675	82.4901	34.9946	0.42423	0.00001
CG7715	105.4550	0.0000	0.00000	0.00035
CG7920	179.5330	61.1720	0.34073	0.00001
CG7998	352.3630	75.3197	0.21376	0.00000
CG8086	16.4205	4.7136	0.28705	0.00745
CG8193	835.7910	220.8040	0.26419	0.00007
CG8303	39.5537	12.0205	0.30390	0.00285
CG8630	41.7814	1.5594	0.03732	0.00001
CG9184	2626.3900	896.2130	0.34123	0.00000
CG9331	163.9160	31.4306	0.19175	0.00010
CG9380	101.9050	26.2261	0.25736	0.00000
CG9416	159.5170	72.8624	0.45677	0.00637
CG9527	52.0325	12.0834	0.23223	0.00155

CG9603	1277.9900	593.6690	0.46453	0.00003
CG9619	23.3602	4.1184	0.17630	0.00006
CG9743	121.2990	43.6324	0.35971	0.00018
CG9747	109.8010	38.4442	0.35013	0.00073
CG9850	16.3071	1.7034	0.10446	0.00034
CG9990	38.8872	3.9755	0.10223	0.00001
cher	268.4920	90.8359	0.33832	0.00000
chrb	524.0690	248.8120	0.47477	0.00065
Cht3	183.3590	39.5191	0.21553	0.00000
Cpr62Bc	73.4912	3.4134	0.04645	0.00006
Cpr65Ea	768.1140	33.2480	0.04329	0.00000
cpx	64.3182	14.6317	0.22749	0.00010
CRMP	86.8474	31.3974	0.36152	0.00227
CYLD	137.7420	46.6807	0.33890	0.00405
Cyp309a2	51.1353	13.0595	0.25539	0.00027

deltaCOP	280.8840	141.0960	0.50233	0.00001
dpr7	45.0698	6.2485	0.13864	0.00272
drpr	80.2505	40.6775	0.50688	0.00000
emp	91.7095	23.7796	0.25929	0.00040
Epac	44.0771	13.2918	0.30156	0.00006
eya	64.8562	23.1432	0.35684	0.00510
fau	135.1110	0.0000	0.00000	0.00000
fok	231.0960	100.3170	0.43409	0.00547
Gad1	24.8103	1.6205	0.06531	0.00002
Galpha73B	30.3547	0.9903	0.03262	0.00615
Gasp	1254.2300	379.6490	0.30270	0.00000
Gel	113.5200	36.9592	0.32557	0.00059
Gfat1	112.0150	30.1142	0.26884	0.00008
Gfat2	148.8290	67.0459	0.45049	0.00280
Glut1	64.3970	25.5972	0.39749	0.00155

Got2	92.7271	24.2257	0.26126	0.00179
Gpo-1	42.2637	4.4773	0.10594	0.00011
grn	103.0120	50.6430	0.49162	0.00188
haf	127.1860	60.3035	0.47414	0.00462
ia2	24.9502	0.9657	0.03870	0.00000
ImpL3	354.4180	65.5088	0.18484	0.00000
iotaTry	23222.1000	7344.8800	0.31629	0.00000
kdn	406.4710	191.7070	0.47164	0.00196
l(2)01289	34.9121	1.5863	0.04544	0.00000
l(2)03709	453.0830	212.6080	0.46925	0.00169
l(2)efl	139.5990	5.9545	0.04265	0.00003
LamC	181.5150	37.5050	0.20662	0.00000
mas	155.4540	60.9205	0.39189	0.00084
mbl	182.6290	86.3384	0.47275	0.00398
Megalin	194.0080	47.3070	0.24384	0.00000

Mf	160.0220	1.6157	0.01010	0.00000
Mhc	726.6050	38.8825	0.05351	0.00000
Mipp1	223.4110	112.1830	0.50214	0.00691
Mlc1	837.2200	56.5194	0.06751	0.00000
Mlc2	738.3910	68.5564	0.09285	0.00000
Mob2	63.0313	25.5342	0.40510	0.00001
Msp-300	156.9930	43.6981	0.27834	0.00000
Msr-110	183.3070	40.4618	0.22073	0.00000
mura	149.8970	53.8473	0.35923	0.00007
n-syb	38.6363	3.4608	0.08957	0.00022
Nckx30C	18.8468	2.6763	0.14200	0.00002
Nep2	121.8840	43.4931	0.35684	0.00157
Nnf1a	162.6400	45.1467	0.27759	0.00213
Nurf-38	324.2540	155.6600	0.48005	0.00255
Oatp33Ea	44.0468	12.5061	0.28393	0.00338

Obp44a	206.1100	0.0000	0.00000	0.00008
obst-E	62.6213	0.9349	0.01493	0.00005
pgant3	67.1310	26.2108	0.39044	0.00028
Pgm	730.4410	149.3760	0.20450	0.00588
Pif1A	60.5008	21.7064	0.35878	0.00001
PKD	77.6680	24.3038	0.31292	0.00075
PQBP-1	78.8846	38.2711	0.48515	0.00206
ps	360.4450	174.2810	0.48352	0.00000
Rab-RP4	495.6050	62.7785	0.12667	0.00262
Rab7	496.8930	236.2550	0.47546	0.00496
Rbp6	68.6119	9.4344	0.13750	0.00001
rec	24.9292	6.0120	0.24116	0.00236
RhoGAP100F	38.6191	15.9435	0.41284	0.00011
rut	47.1320	20.9665	0.44485	0.00042
Rya-r44F	62.7417	27.0400	0.43097	0.00065

salm	131.1010	62.0813	0.47354	0.00000
scaf	101.2600	16.3263	0.16123	0.00000
SelG	148.5730	47.7733	0.32155	0.00167
serp	422.5820	155.8250	0.36875	0.00000
sgl	271.7720	127.0570	0.46751	0.00501
slo	17.1657	4.4506	0.25927	0.00414
Spn77Ba	21.9980	1.1494	0.05225	0.00544
Strn-Mlck	25.6701	6.5128	0.25371	0.00000
sv	61.5652	26.8702	0.43645	0.00333
synaptogyrin	24.1509	2.5238	0.10450	0.00089
Syt1	26.7950	7.0848	0.26441	0.00062
Tace	57.4618	16.9370	0.29475	0.00417
TepII	85.7617	36.8163	0.42929	0.00575
Tina-1	376.2530	92.1554	0.24493	0.00006
Tm1	1179.4600	590.3450	0.50052	0.00000

Tm2	236.8290	23.4468	0.09900	0.00000
Tpi	85.7148	26.3455	0.30736	0.00735
TpnC47D	1137.7500	43.3073	0.03806	0.00000
TpnC73F	310.8360	45.3916	0.14603	0.00000
trol	92.6705	14.6083	0.15764	0.00000
Tsp42Ea	98.0492	46.6541	0.47582	0.00832
TwdlL	187.6780	0.0000	0.00000	0.00000
Uhg5	276.9450	101.8660	0.36782	0.00362
Unc-89	17.9081	1.4018	0.07828	0.00000
up	496.6070	36.6903	0.07388	0.00000
usnp	239.4150	96.9262	0.40484	0.00781
Vha13	599.8410	275.5040	0.45929	0.00003
Vha68-1	82.3499	33.4698	0.40643	0.00045
vig	385.8230	153.7520	0.39851	0.00000
vkg	156.3580	31.1849	0.19945	0.00000

wupA	387.3000	27.5334	0.07109	0.00000
Zasp66	87.0525	1.1663	0.01340	0.00003
Zn72D	139.6570	57.0876	0.40877	0.00230
zormin	47.2647	10.4859	0.22185	0.00000
Acph-1	43.4594	241.1100	5.54793	0.00000
Aef1	146.4810	297.2880	2.02954	0.00478
aPKC	121.9500	279.3850	2.29097	0.00001
Art1	242.4790	531.7740	2.19307	0.00118
Ate1	39.0081	73.1226	1.87455	0.00308
aub	56.4823	98.7710	1.74871	0.00793
ball	171.4560	333.7020	1.94628	0.00034
Bap60	421.5060	697.0230	1.65365	0.00036
bcd	63.8502	160.6040	2.51533	0.00078
BicC	27.4546	107.4950	3.91537	0.00001
BicD	111.1150	206.2440	1.85613	0.00057

borr	313.7790	629.6920	2.00680	0.00021
Bsg25D	146.2920	276.9470	1.89311	0.00038
BubR1	80.5678	180.9800	2.24630	0.00005
bur	180.8900	338.4700	1.87113	0.00121
CG10083	102.4790	201.0980	1.96234	0.00012
CG11164	76.2473	199.2020	2.61257	0.00722
CG12391	161.1550	321.4480	1.99464	0.00737
CG12734	73.7598	145.6700	1.97493	0.00006
CG14435	36.5679	99.6366	2.72470	0.00030
CG14764	163.1420	353.1810	2.16487	0.00004
CG15141	163.9630	351.3140	2.14264	0.00609
CG1518	247.8010	384.9000	1.55326	0.00167
CG16970	4.5993	23.3453	5.07585	0.00528
CG1962	198.9030	413.9530	2.08118	0.00175
CG2051	205.5650	419.1920	2.03922	0.00818

CG2924	364.3240	729.1350	2.00133	0.00000
CG2938	75.4931	176.2400	2.33451	0.00173
CG30085	125.2360	343.5470	2.74318	0.00000
CG30497	278.0410	538.4850	1.93671	0.00016
CG31755	24.9920	69.3976	2.77679	0.00188
CG31875	17.2983	103.0400	5.95669	0.00063
CG3227	144.5160	332.4690	2.30057	0.00345
CG32365	63.6367	164.4680	2.58449	0.00220
CG3238	66.1893	193.1280	2.91780	0.00036
CG33181	55.1074	139.6530	2.53420	0.00146
CG42351	16.7495	62.3572	3.72293	0.00515
CG42732	5.1984	16.5247	3.17884	0.00448
CG4300	321.5820	670.3470	2.08453	0.00000
CG4911	243.5320	419.8970	1.72420	0.00541
CG5003	117.4080	221.5270	1.88682	0.00664

CG5098	51.4592	113.8990	2.21339	0.00398
CG5568	22.7570	61.9223	2.72102	0.00439
CG6051	128.0830	383.6580	2.99538	0.00000
CG6241	55.6652	133.7950	2.40357	0.00432
CG6425	43.2409	117.9910	2.72869	0.00411
CG6461	30.9774	127.6630	4.12116	0.00000
CG7357	89.1638	221.8540	2.48816	0.00419
CG7433	115.4730	434.6690	3.76427	0.00000
CG7824	64.8566	184.4170	2.84345	0.00450
CG8116	154.2400	285.7130	1.85239	0.00471
CG8173	159.6780	380.9250	2.38560	0.00012
CG8177	123.7370	243.6640	1.96922	0.00000
CG8290	73.7468	142.1180	1.92711	0.00721
CG8944	109.7400	194.5750	1.77306	0.00573
CG9062	82.6832	185.9320	2.24873	0.00020

CG9393	135.6090	303.3170	2.23671	0.00002
chif	171.8770	342.3200	1.99166	0.00000
cnn	178.3990	374.4030	2.09868	0.00095
coro	201.5780	359.2250	1.78206	0.00018
Cpsf160	86.3761	182.3080	2.11064	0.00151
crm	101.2880	178.3820	1.76114	0.00168
CycA	552.3510	939.0740	1.70014	0.00003
CycB	1287.3700	2954.1100	2.29469	0.00023
Edc3	114.6820	241.3340	2.10437	0.00226
exu	16.0356	63.6793	3.97110	0.00044
fl(2)d	200.3200	343.5310	1.71491	0.00055
for	168.6020	330.3640	1.95943	0.00352
fs(2)ltoPP43	149.0240	291.3330	1.95493	0.00133
G-ialpha65A	322.3930	630.7580	1.95649	0.00003
Gap69C	131.7660	218.4400	1.65778	0.00320

hang	222.0300	391.2560	1.76218	0.00346
heix	161.8620	264.4980	1.63410	0.00008
HP5	146.5290	289.1320	1.97320	0.00474
l(1)G0269	106.2820	165.2180	1.55453	0.00305
Lk6	434.1760	776.2680	1.78791	0.00809
LpR2	33.5198	78.2008	2.33297	0.00120
Mapmodulin	485.4570	951.0330	1.95905	0.00023
mol	116.6800	285.0380	2.44289	0.00000
mrj	201.0180	371.7790	1.84948	0.00130
Msh6	27.7488	67.7988	2.44332	0.00697
muskelin	106.5290	340.6590	3.19780	0.00001
ncd	178.4470	379.7040	2.12783	0.00009
Nlp	1328.1400	2396.1700	1.80415	0.00820
norpA	13.8622	41.4837	2.99258	0.00265
Not1	363.3890	603.0990	1.65965	0.00000

Nuf2	99.5041	183.2350	1.84148	0.00297
Obp56a	0.0000	161.2760	und	0.00157
otu	18.2523	56.1326	3.07537	0.00350
PEK	150.9750	288.4890	1.91084	0.00427
Pink1	110.5080	203.3170	1.83983	0.00350
Pmm45A	111.6540	296.5350	2.65584	0.00034
Poc1	81.7573	196.7340	2.40632	0.00711
poe	133.7730	214.7520	1.60534	0.00003
proPO-A1	43.5178	222.5680	5.11442	0.00000
ptip	65.7316	118.5520	1.80357	0.00011
ptr	22.4477	43.1218	1.92099	0.00781
Pxt	26.5660	117.9900	4.44139	0.00003
Rcd1	197.9670	319.0070	1.61142	0.00193
RhoGAP54D	30.3514	72.4985	2.38864	0.00750
rig	58.3268	108.5820	1.86161	0.00732

Ripalpha	106.0340	392.3240	3.69996	0.00003
RPA2	104.9070	372.4870	3.55067	0.00091
RpII215	232.9340	372.1600	1.59771	0.00281
Rpt4	327.0040	623.6410	1.90714	0.00051
SMC2	96.7567	200.4190	2.07136	0.00005
smg	750.2190	2228.2500	2.97014	0.00000
ssp3	47.9181	104.9210	2.18959	0.00326
sti	185.6460	353.5520	1.90444	0.00323
Su(var)2-10	322.9430	560.4340	1.73540	0.00000
Su(var)2-HP2	112.7700	207.5120	1.84013	0.00519
Taf12	179.4620	399.9310	2.22851	0.00021
thr	115.3340	328.6780	2.84980	0.00001
tlk	246.1990	403.7060	1.63976	0.00001
tor	38.6324	94.7882	2.45360	0.00091
Trf4-1	160.3040	315.3580	1.96725	0.00279

tum	258.8920	528.6690	2.04205	0.00212
twe	138.3430	396.1770	2.86374	0.00000
Uba2	244.0670	419.2780	1.71788	0.00008
UbcD2	318.5530	673.5540	2.11442	0.00027
Uch-L3	169.4910	324.7130	1.91581	0.00775
Uev1A	574.7470	923.3250	1.60649	0.00280
Usp7	202.0050	337.6360	1.67142	0.00000
vlc	283.0970	505.1150	1.78425	0.00001
wapl	115.2550	253.6930	2.20115	0.00041
wdn	127.8800	229.4830	1.79452	0.00280
yuri	19.1355	71.9366	3.75932	0.00024

Downregulated transcripts are shaded red; upregulated transcripts are shaded green.

Table S3. Differential isoform expression in 6-10hr *hoip*^{1} embryos

Gene	Wild-type value	*hoip* value	Fold change	*P* value	Transcript defect
aub	56.48230	98.77100	1.74871	0.00793	Enhanced splicing
ptip	65.73160	118.55200	1.80357	0.00011	Enhanced splicing
sti	185.64600	353.55200	1.90444	0.00323	Enhanced splicing
CG8177	123.73700	243.66400	1.96922	0.00000	Enhanced splicing
CG42748	175.85800	90.52090	0.51474	0.00432	Exon inclusion
kcc	89.84850	46.96120	0.52267	0.00085	Exon inclusion
Ser	84.99020	44.45880	0.52311	0.00064	Exon inclusion
Ppn	555.02200	306.51800	0.55226	0.00000	Exon inclusion
Ca-P60A	498.64100	275.82100	0.55315	0.00723	Exon inclusion
CG32000	480.65000	268.36300	0.55834	0.00024	Exon inclusion
hth	835.08400	505.59300	0.60544	0.00059	Exon inclusion
Mbs	167.98500	107.22400	0.63830	0.00395	Exon inclusion

tlk	246.19900	403.70600	1.63976	0.00001	Exon inclusion
Su(var)2-HP2	112.77000	207.51200	1.84013	0.00519	Exon inclusion
rig	58.32680	108.58200	1.86161	0.00732	Exon inclusion
ptr	22.44770	43.12180	1.92099	0.00781	Exon inclusion
CG8290	73.74680	142.11800	1.92711	0.00721	Exon inclusion
chif	171.87700	342.32000	1.99166	0.00000	Exon inclusion
bip2	311.99200	203.80600	0.65324	0.00047	Exon skipping
Atpalpha	619.91700	429.29900	0.69251	0.00385	Exon skipping
dikar	85.61790	120.58700	1.40843	0.00286	Exon skipping
CG1518	247.80100	384.90000	1.55326	0.00167	Exon skipping
Su(var)2-10	322.94300	560.43400	1.73540	0.00000	Exon skipping
vlc	283.09700	505.11500	1.78425	0.00001	Exon skipping
Ate1	39.00810	73.12260	1.87455	0.00308	Exon skipping
for	168.60200	330.36400	1.95943	0.00352	Exon skipping

shot	177.77600	112.71300	0.63401	0.00722	Exon skipping/exon inclusion
mrj	201.01800	371.77900	1.84948	0.00130	Exon skipping/exon inclusion
sano	95.19750	52.42650	0.55071	0.00662	Exon skipping/inclusion
CG34417	31.43320	16.63610	0.52925	0.00058	No splicing
mrt	79.34430	42.20550	0.53193	0.00438	No splicing
sm	266.06100	135.89400	0.51076	0.00830	Reduced splicing
crol	319.13200	169.49200	0.53110	0.00000	Reduced splicing
fz2	300.78100	160.47500	0.53353	0.00062	Reduced splicing
if	86.65180	48.43270	0.55893	0.00119	Reduced splicing
VhaSFD	325.35600	182.20800	0.56003	0.00003	Reduced splicing
CG14425, Sxl	368.62100	209.39300	0.56804	0.00010	Reduced splicing
CG14767	311.92900	178.67400	0.57280	0.00070	Reduced splicing
PNUTS	187.48500	107.55800	0.57369	0.00145	Reduced splicing
Pdp1	154.50300	89.82400	0.58137	0.00835	Reduced splicing

A2bp1	203.49100	118.55600	0.58261	0.00818	Reduced splicing
ct	94.26300	56.17210	0.59591	0.00489	Reduced splicing
rictor	83.02180	49.61840	0.59766	0.00072	Reduced splicing
nkd	215.54700	130.30700	0.60454	0.00083	Reduced splicing
Cirl	438.24000	271.95700	0.62057	0.00068	Reduced splicing
par-1	190.83200	124.93800	0.65470	0.00019	Reduced splicing
Aats-glupro	212.19200	140.44800	0.66189	0.00034	Reduced splicing
grh	178.67900	123.00500	0.68841	0.00292	Reduced splicing
Rcd1	197.96700	319.00700	1.61142	0.00193	Reduced splicing
hang	222.03000	391.25600	1.76218	0.00346	Reduced splicing
Nlp	1328.14000	2396.17000	1.80415	0.00820	Reduced splicing
fs(2)ltoPP43	149.02400	291.33300	1.95493	0.00133	Reduced splicing
G-ialpha65A	322.39300	630.75800	1.95649	0.00003	Reduced splicing

cpo	180.11500	105.18600	0.58399	0.00013	Reduced splicing/exon inclusion
Pvr	126.49100	75.74530	0.59882	0.00122	Reduced splicing/exon inclusion
Eip75B	341.18800	207.52200	0.60823	0.00418	Reduced splicing/exon skipping
l(3)82Fd	159.89400	100.41200	0.62799	0.00255	Reduced splicing/exon skipping
Imp	551.30400	374.13400	0.67863	0.00303	Reduced splicing/exon skipping
Dscam	147.12600	112.67700	0.76585	0.00043	Reduced splicing/exon skipping

Table S4. Muscle elongation, target recognition and attachment mRNA expression in *hoip*^{1} embryos

Gene	Muscle function	Fold change in *hoip*^{1} embryos*	Stage of muscle phenotype	Reference
Dynein heavy chain 64C	Elongation	NC	Stage 15	(Folker et al., 2012)
pavarotti	Elongation	NC	Stage 16	(Guerin and Kramer, 2009)
tumbleweed	Elongation	2.04	Stage 14/15	(Guerin and Kramer, 2009)
derailed	Recognition	NC	Stage 14	(Callahan et al., 1996)
echinoid	Recognition	NC	Stage 16	(Swan et al., 2006)
kon-tiki	Recognition	NC	Stage 13	(Schnorrer et al., 2007)
MSP-300	Recognition	0.27	Stage 16	(Rosenberg-Hasson et al., 1996)
perdido	Recognition	NC	Stage 13	(Estrada et al., 2007)
roundabout	Recognition	NC	Stage 14	(Steigemann et al., 2004)
Slit	Recognition	0.66	Stage 14	(Steigemann et al., 2004)
Stripe	Recognition/	NC	Stage 14	(Frommer et al., 1996)

	attachment			
Glutamate Receptor Binding Protein	Attachment	NC	Stage 13	(Estrada et al., 2007)
inflated	Attachment	0.56	Stage 17	(Bloor and Brown, 1998)
Leucine-rich tendon-specific protein	Attachment	NC	Stage 16	(Wayburn and Volk, 2009)
mind bomb 2	Attachment	NC	Stage 15	(Carrasco-Rando and Ruiz-Gomez, 2008)
multiple edematous wings	Attachment	NC	Stage 16	(Chanana et al., 2007)
myospheroid	Attachment	NC	Stage 16	(Brown, 1994)
rhea	Attachment	NC	Stage 16	(Brown et al., 2002)
slowdown	Attachment	NC	Stage 16	(Gilsohn and Volk, 2010a)
Thrombospondin	Attachment	NC	Stage 16	(Subramanian et al., 2007)
Tiggrin	Attachment	NC	Stage 16/17	(Bunch et al., 1998)
vein	Attachment	NC	Stage 16	(Yarnitzky et al., 1998)

*Fold change compared with wild-type embryos

Table S5. Possible muscle elongation regulators misexpressed in *hoip*^{1} embryos

Gene	Molecular function	Fold change in *hoip*^{1} embryos
Gelsolin	Actin binding	0.32
Rab7	GTPase	0.13
Rab18	GTPase	0.48
RhoGAP54D	Rho GAP	2.30
RhoGAP100F	Rho GAP	0.41
Tetraspanin 42Ea	Transmembrane protein	0.48
Unc-89	Rho GEF	0.07

Table S6. Primer sequences

Primer	Forward primer	Reverse primer
Site-directed mutagenesis		
hoip[1]	ATCAACTGCGCAAGGAAGCCAACGAGGCCAC	GTGGCCTCGTTGGCTTCCTTGCGCAGTTGAT
Hoip.-225.GFP*	TCTAGAACTCGCGGATGCGC	GGTACCAAGTGCTTTAAAATGCAACCTCTC
Hoip.-225ΔE.GFP	TTTCTAACGCTAACGGAATTCTTTGAAAGCGATTTC	GAAATCGCTTTCAAAGAATTCCGTTAGCGTTAGAAA
qPCR		
if	CGATCCTTCCCACGAGATTA	GCAGTGGATTCTACGGCAAT
Mf	TCCAAAGCAGGTTGTCATTG	GCCACTCGGTATCTTTGAGC
MHC	CTGTTCAAGTGGCTGGTGAA	AATGGGTGTTGGTCAGCTTC
Mlc2	CAGCAACTGGGTGAAGTTGA	CAGCCAGAGATTCAGTGTGC
Tm2	CAGCTGACCAACCAGTTGAA	AAATCGTCGCAGATTGCTTT
TpnC 47D	TTTATTCAAGCCTCGGGTGT	CAGTCGTTAGCGGTGATCG
TpnC 73F	CAACAGCTTCGATCACCAGA	AAGTGGGTGGTGTTTTCCAG
smyhc1	GAGGAACCAGCAGAGAGTGG	CAGATTGTTGCGTCTCTCCA
smyhc3	TGGAGAGACGCAACAATCTG	CTGCCTCCTCAACCTCAGTC

tpma	CCCGTAAGCTGGTGATTGTT	ACGAGTCTCAGCCTCCTTCA
tpm2	TGCAGATGCTCAAACTGGAC	TTGAGCGCGATCTAACTCCT
Mef2a	GGCTCTCCAGGGCTCTCTAT	CATCGTAGGAGGAGCAGGAG
Mef2d	GAGAATGCTCAGCGGTTAGG	TGTTGCCAAGTGGTCATGTT
notch1	CGAGCCCTTGTCATTATGGT	TCGCAGACACACTCATAGCC
GAPDH	GATACACGGAGCACCAGGTT	AACAGCAAAGGGGTCACATC
Rpl32	TTACTCGTTCTCTTGAGAACGC	CTTCAAGATGACCATCCGC

*The PCR product was digested with Kpn/*Eco*RI to generate the Hoip.-225 fragment