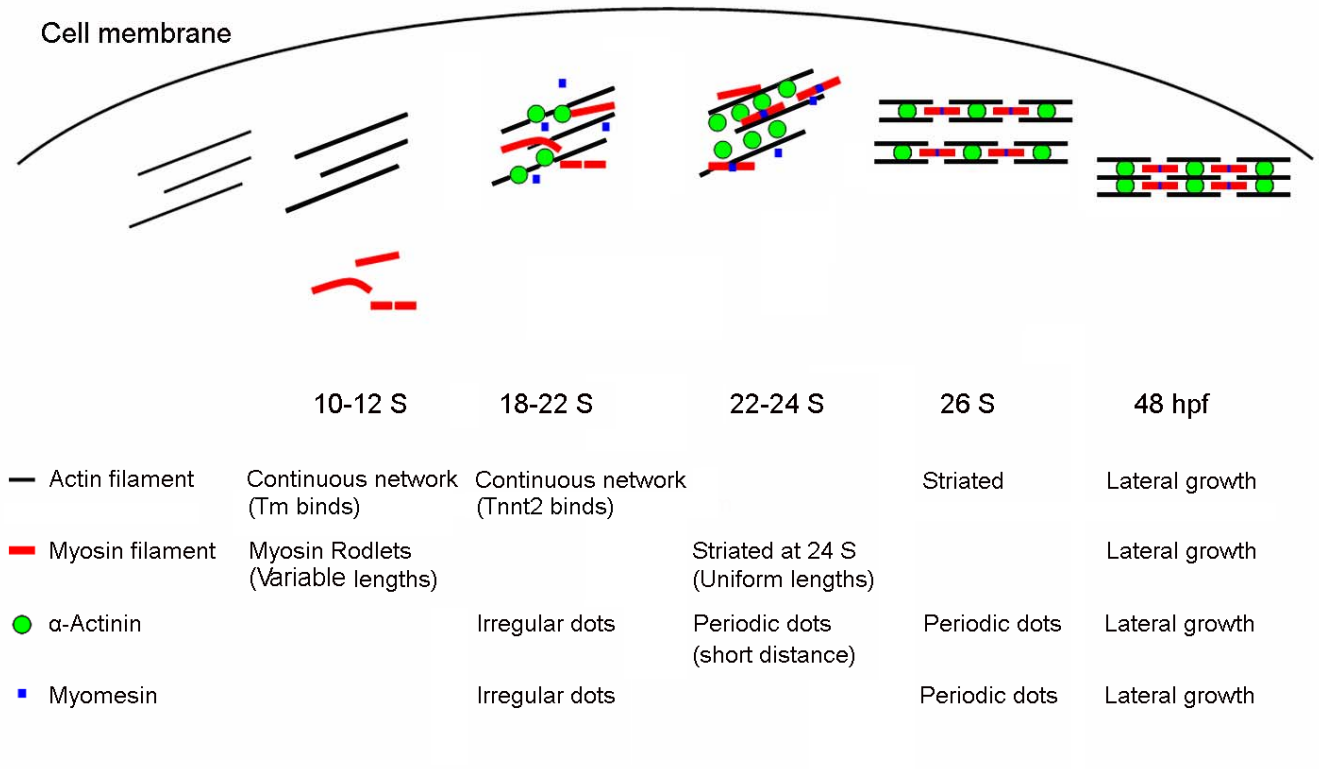
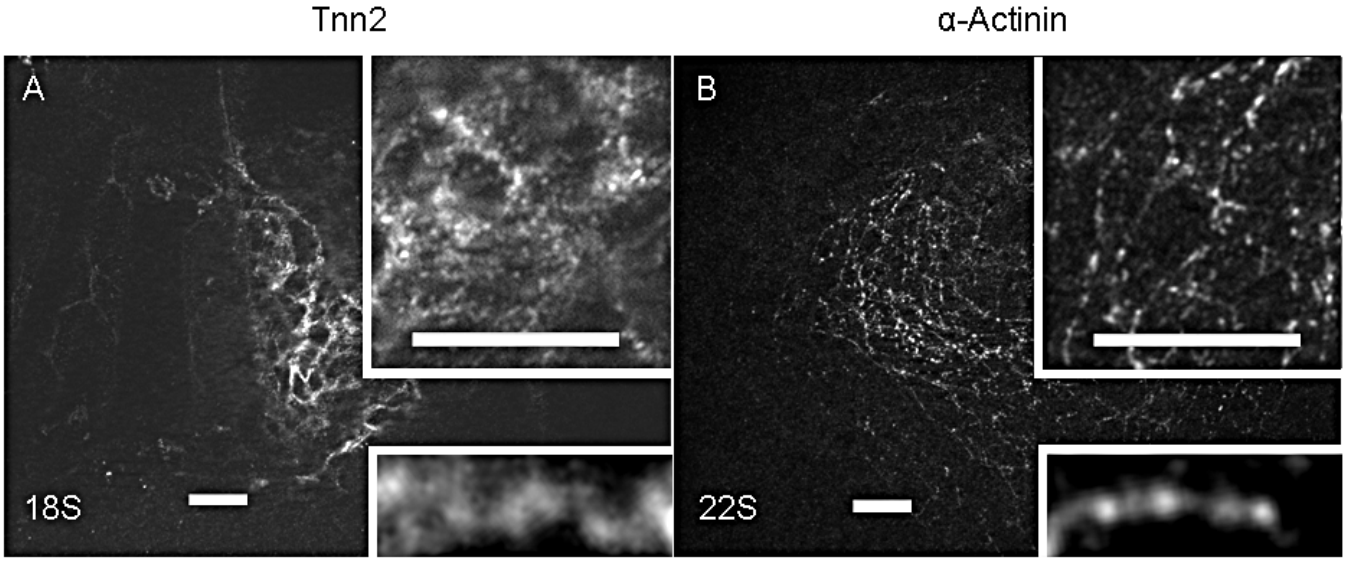


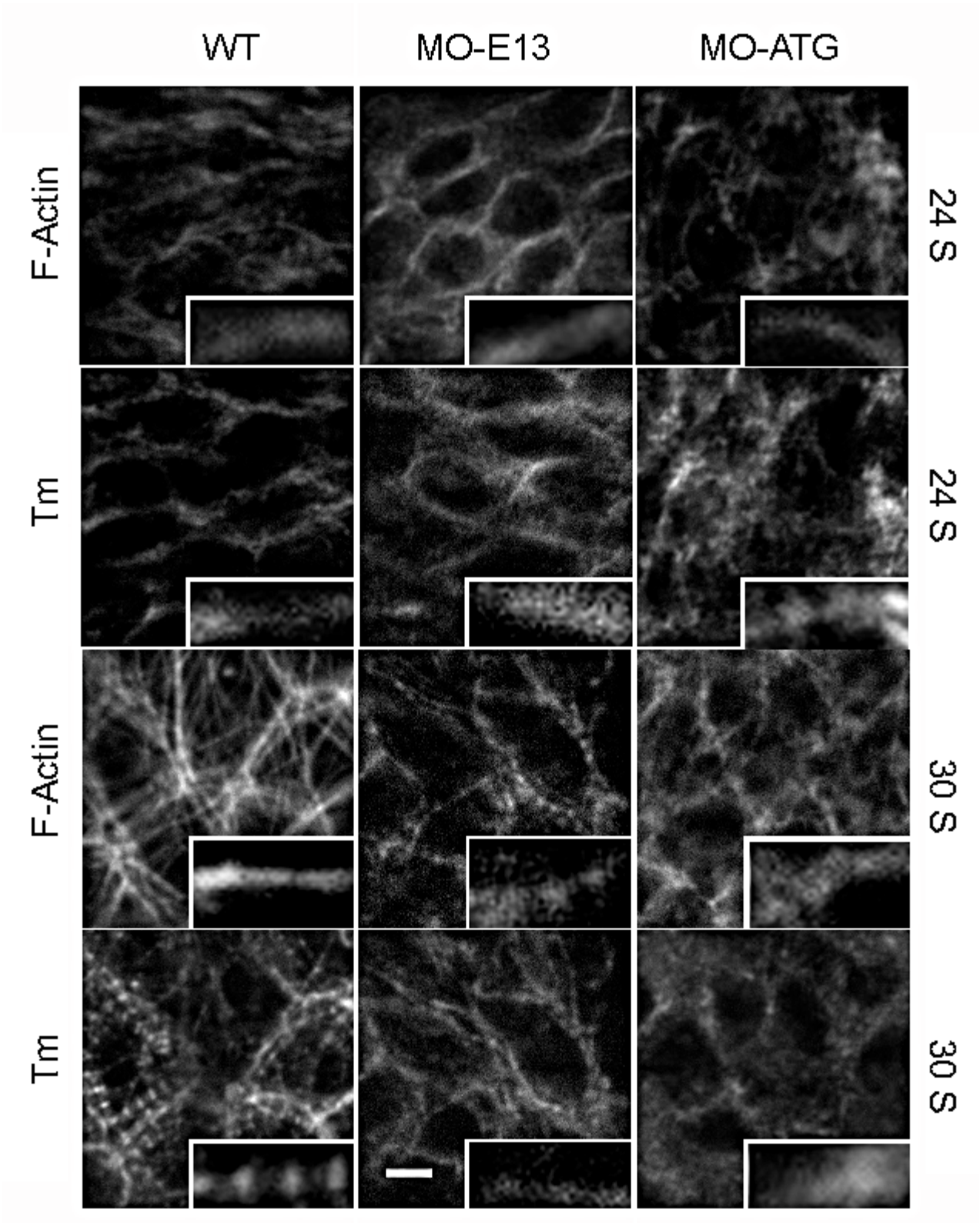
Supplemental Fig. S1. Summary of myofibrillogenesis in the zebrafish heart. At least five different stages can be resolved during heart development: (1) independent assembly of thin and thick filaments; (2) appearance of irregular a-actinin and myomesin dots; (3) striation of a-actinin separated by a shorter distance and integration of thick filaments into the thin filament network; (4) expansion of the Z-disc, M-line, and thin filaments; and (5) lateral growth to form a mature sarcomere.

Supplemental Fig. 1



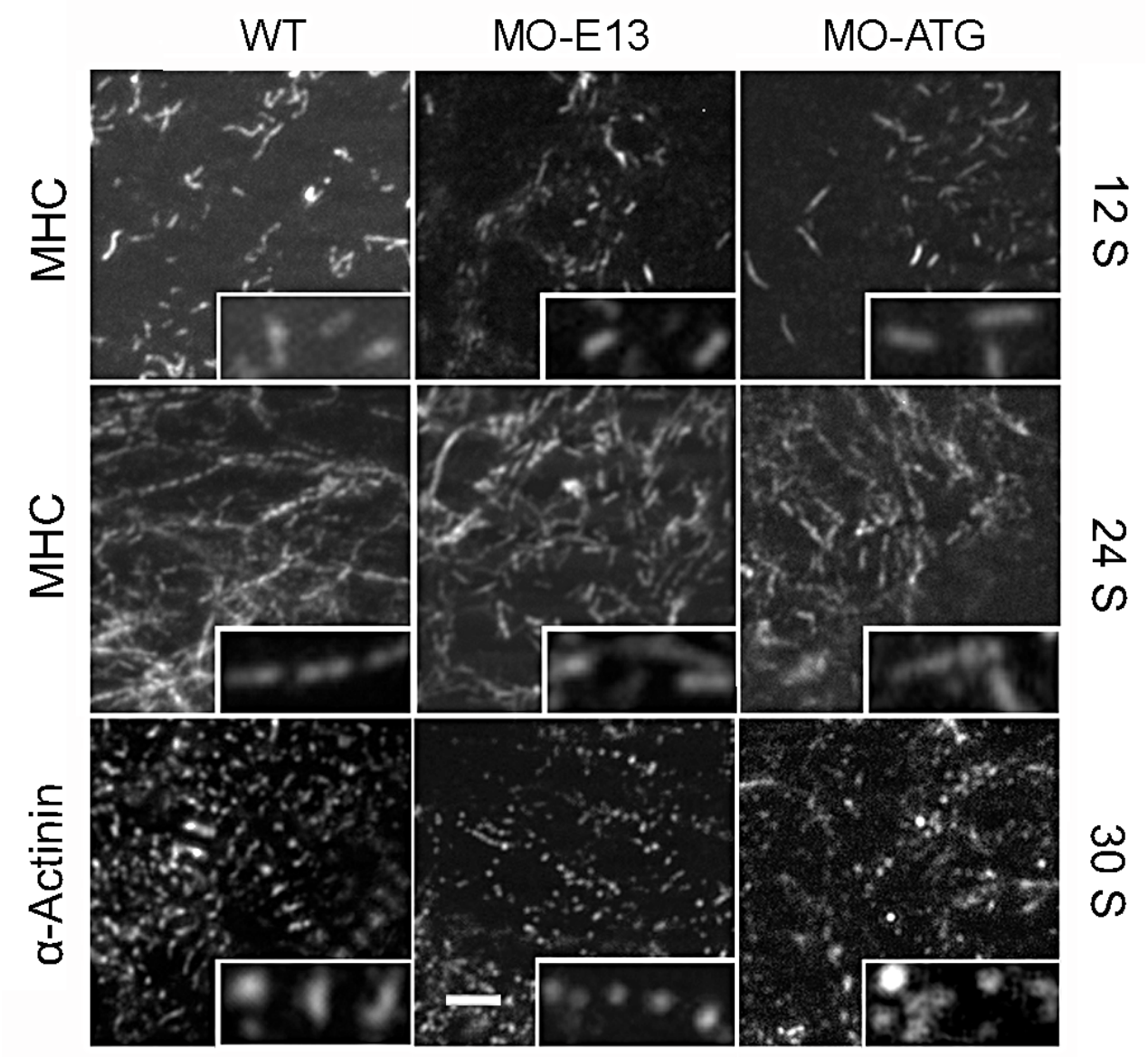
Supplemental Fig. S2. Immunostaining for tnnt2 (A) and α-actinin (B) in the WT heart at early stages. At 18 S, tnnt2 appears as dotted pattern that associates with the thin filament network in perimembrane region. At 22 S, α-actinin dots start to be periodic, but show a shorter distance between two neighboring α-actinin dots than at 26 S. Insets are of the same image of higher magnification to reveal networks (at top), and a myofibril (at bottom). Scale bar = 20 μm.

Supplemental Fig. 2



Supplemental Fig. 3

Supplemental Fig. S3. Thin filament assembly in morphants at an early stage revealed by immunostaining for F-actin and Tm. Assembly of continuous thin filaments was not affected in either MO-ATG or MO-E13 morphants at 24 S. Thin filaments remained as continuous filaments at 30 S in MO-ATG morphants while both F-actin and Tm exhibit a striated pattern in the WT control and MO-E13 morphants. Scale bar = 5 μm.



Supplemental Fig. S4. Thick filament and α-actinin assembly in morphants at early stages revealed by immunostaining for MHC and α-actinin, respectively. Myosin rodlets with variable lengths are assembled normally in both morphants at 12 S. However, they fail to be striated and do not integrate into the thin filament network at 24 S, as does the WT control. Irregular α-actinin dots were detected in MO-ATG morphants at 30 S, while periodic dots can be detected in both the WT control and MO-E13 morphants. Scale bar = 5 μm.

Zebrafish *	Exon Length (bp)	Ensembl Database Number	Human #	Exon Length (bp)	Comments
			Exon 1	55	
Exon 1	32	ENSDARE00000738634			
Exon 2	11	ENSDARE00000738453			
Exon 3	7	ENSDARE00000738464			Translation start codon
Exon 4	54	ENSDARE00000738613	Exon 2	55	
			Exon 3	11	
			Exon 4'	15	
			Exon 5'	30	
Exon 5	60	ENSDARE00000738452	Exon 6	66	
Exon 6	30	ENSDARE00000738595			
Exon 7	25	ENSDARE00000595106	Exon 7	36	
			Exon 8	34	
Exon 8	64	ENSDARE00000401007	Exon 9	61	
Exon 9	117	ENSDARE00000008500	Exon 10	117	
Exon 10	78	ENSDARE00000082232	Exon 11	78	
Exon 11	108	ENSDARE00000401005	Exon 12	111	
			Exon 13a'	9	
			Exon 13b'	6	
Exon 12	110	ENSDARE00000034878	Exon 14	110	
Exon 13	90	ENSDARE00000738495	Exon 15	91	
Exon 14	41	ENSDARE00000738479	Exon 16	41	
Exon 15	20	ENSDARE00000738442			
Exon 16	23	ENSDARE00000738440			
Exon 17	31	ENSDARE00000738450			
Exon 18	183	ENSDARE00000738459			Translation stop codon
			Exon 17	242	

* Zebrafish exons were confirmed experimentally by our data, except exon 3, 16, and 17, which were predicted in the Ensemble database.
# Human exons were named according to Genebank sequences: NM_000364, NM_001001430, NM_001001431, NM_001001432
' Alternatively spliced in human

Supplemental Table 1