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Supplemental information

**The molecular basis for sarcomere organization
in vertebrate skeletal muscle**

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	Ion beam voltage	Ion beam current	Thickness of lamella
Step 1	30 kV	500 pA	3 μ m
Step 2	30 kV	300 pA	1 μ m
Step 3	30 kV	100 pA	500 nm
Step 4 (polishing)	30 kV	50 pA	30-150 nm

Table S1. Milling strategy during lamellae production. Related to [Figure S1](#) and [STAR Methods](#)

Parameter	Actomyosin complex (EMDB-12289)	re-centered myosin double-head (EMDB-12291)	I-band thin filament (without troponin) (EMDB-12292)	I-band thin filament (with troponin) (EMDB-12293)
Data collection and processing				
Magnification	81000	81000	81000	81000
Voltage (kV)	300	300	300	300
Exposure dose (e ⁻ /Å ²)	130-155	130-155	130-155	130-155
Defocus range (μ m)	2.4-3.4	2.4-3.4	3.3-4	3.3-4
Pixel size (Å)	1.76	1.76	1.76	1.76
No. of tomograms	8	8	4	4
Initial no. of particles	32,421	32,421	15,153	704
Final no. of particles	18,090	4,519	15,153	704
Symmetry imposed	C1	C1	Helical (-166.6° twist, 27.9 Å rise)	C1
FSC threshold	0.143	0.143	0.143	0.143
Map resolution (Å)	10.2	15.1	10.6	19.8

Table S2. Cryo-ET data collection and sub-tomogram averaging statistics. Related to [STAR Methods](#)

Score	R	G	B	E
R	10	-1	-1	-1
G	-1	0	-1	-1
B	-1	-1	0	-1
E	-1	-1	-1	0

Table S3. Weight matrix used for multiple sequence alignment with MUSCLE algorithm. Related to [Figure S5](#) and [STAR Methods](#)