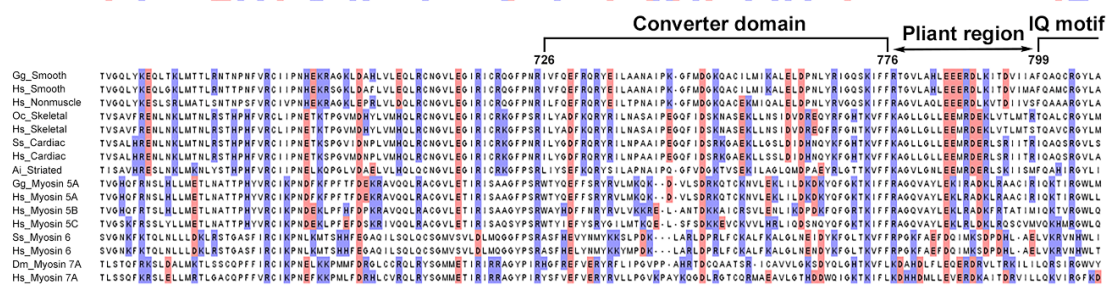
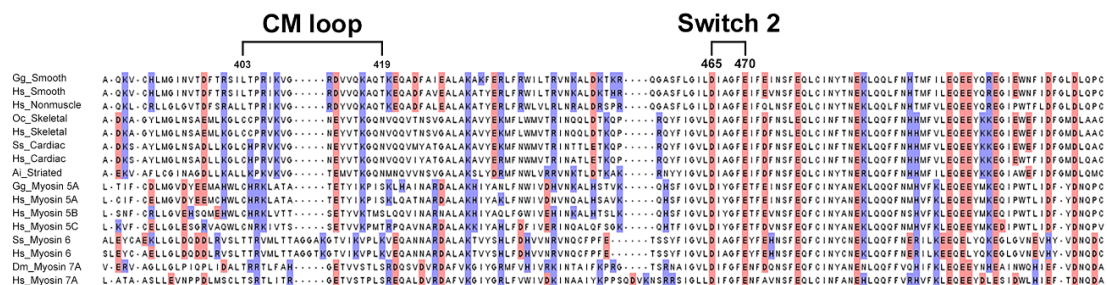
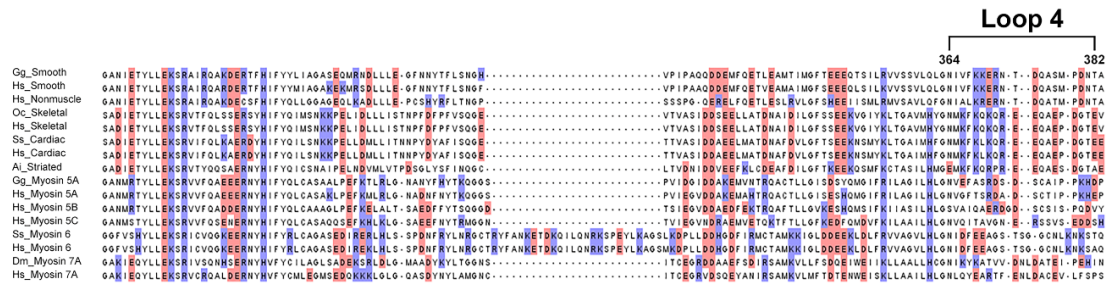
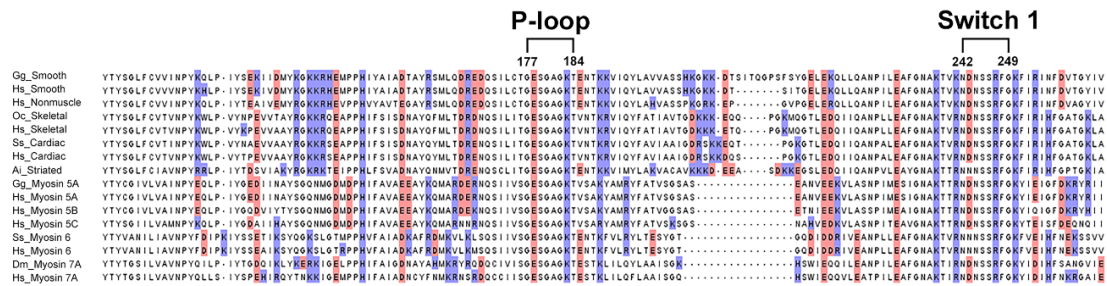


Supplementary Material

Gg_Smooth	MSQR	PL-S5	DEKFLF		VDNHVF	N	PLAOD	WSAK	HLWNP	SEKHOF	ASAIKE	EKDEVT	VELQ	ENKKYV	T	LSK		DDIO	LMMPFF	SVDEMAEL	TLCLAE	SVLHML	REVF	SGLI				
Gg_Smooth	MSQR	GL-S5	DEKFLF		VDNHVF	IR	S	PVAD	AWAK	HLWNP	SEKHOF	ASAIKE	EKDEVT	VELQ	ENKKYV	T	VOK		DDIO	LMMPFF	SVDEMAEL	TLCLAE	SVLHML	REVF	SGLI			
Gg_Nonmuscle	MAAV	TM	SVPRKAP	RRP	PPVPE	AAQPL	FLTP	PPSA	AGOG	PSOS	POVEV	ATSRK	VLNP	SELF	HLF	AAALR	S	EGEAE	VELAE	ESRRR	L	LP	LR					
Oc_Skeletal	MSDAEM	AI	FGEA	APYL	RKPEKE		R	LEA	GNP	FFDK	ACAFV	DKEUWY	KGMIQ	REND	VTVT	LDLRT	T	LNS		DDVF	MMMPFF	KDIED	AMITL	HLREPAV	LHKERTA	AWMI		
Ss_Skeletal	MSDAEM	AI	FGEA	APYL	RKPEKE		R	LEA	GNP	FFDK	ACAFV	DKEUWY	KGMIQ	REND	VTVT	LDLRT	T	LNS		DDVF	MMMPFF	KDIED	AMITL	HLREPAV	LHKERTA	AWMI		
Ss_Skeletal	MYDA	EM	AIFGEA	APYL	RKPEKE		R	LEA	GNP	FFDK	ACAFV	DKEUWY	KALIS	REND	VTVT	LDLRT	T	LVN		DDVF	QDMPFF	KDIED	AMITL	HLREPAV	LHKERTA	AWMI		
Gg_Smooth	MSQR	PL-S5	DEKFLF		VDNHVF	IR	S	PVAD	AWAK	HLWNP	SEKHOF	ASAIKE	EKDEVT	VELQ	ENKKYV	T	LSK		DDIO	LMMPFF	SVDEMAEL	TLCLAE	SVLHML	REVF	SGLI			
Gg_Smooth	MSQR	GL-S5	DEKFLF		VDNHVF	IR	S	PVAD	AWAK	HLWNP	SEKHOF	ASAIKE	EKDEVT	VELQ	ENKKYV	T	LSK		DDIO	LMMPFF	SVDEMAEL	TLCLAE	SVLHML	REVF	SGLI			
Al_Striated	ML	DFSS	DF		QYLVA	RRK	L	ME	ATAF	AK	GNWNP	DEKQFA	SAIOS	S	DOE	VTVL	IA	ADST	T	VKM		DDIO	LMMPFF	SVDEMAEL	TLCLAE	SVLHML	REVF	SGLI
Gg_Myosin 5A	MAAS	EL						YTK	YR	WVP	DPDEWV	S	AE	L	NDY	K	PK	VL	LR	E	E	Q	L					
Hs_Myosin 5A	MAAS	EL						YTK	YR	WVP	DPDEWV	S	AE	L	NDY	K	PK	VL	LR	E	E	Q	L					
Hs_Myosin 5B	MSVQ	EL						YTK	YR	WVP	DPDEWV	S	AE	L	NDY	K	PK	VL	LR	E	E	Q	L					
Hs_Myosin 5C	MAVA	EL						YTK	YR	WVP	DPDEWV	S	AE	L	NDY	K	PK	VL	LR	E	E	Q	L					
Ss_Myosin 6	MBP							YTK	YR	WVP	DPDEWV	S	AE	L	NDY	K	PK	VL	LR	E	E	Q	L					
Hs_Myosin 6	MBP							YTK	YR	WVP	DPDEWV	S	AE	L	NDY	K	PK	VL	LR	E	E	Q	L					
Dm_Myosin 7A	MYIV	T						YTK	YR	WVP	DPDEWV	S	AE	L	NDY	K	PK	VL	LR	E	E	Q	L					
Hs_Myosin 7A	MVIL	Q						YTK	YR	WVP	DPDEWV	S	AE	L	NDY	K	PK	VL	LR	E	E	Q	L					



Negatively charge
Positively charge

Figure S1 Amino acid sequence alignment of heavy chains from myosin 7A and other myosins. Nucleotide binding sites of myosin 7A: Phosphate loop (G156 – T163), Switch-1 (N205 – G212), and Switch-2 (D431 – E436). Actin binding sites of myosin 7A: Loop 4 (N328 – N349), Cardiomyopathy loop (T369 – S385), Helix-loop-helix motif (I500 – S528), Loop 3 (H529 – S542), Loop 2 (I586 - P602). Converter domain of myosin 7A (H670 -L720). The pliant region is indicated at the junction between the converter domain and lever arm. The residue number in this figure corresponds to the original residues from skeletal myosin 2. Positively and negatively charged residues are shown in red and blue, respectively. Species codes: *Gg* *Gallus gallus*; *Hs* *Homo sapiens*; *Oc* *Oryctolagus cuniculus*; *Ss* *Sus scrofa*; *Ai* *Argopecten irradians*; *Dm* *Drosophila melanogaster*.

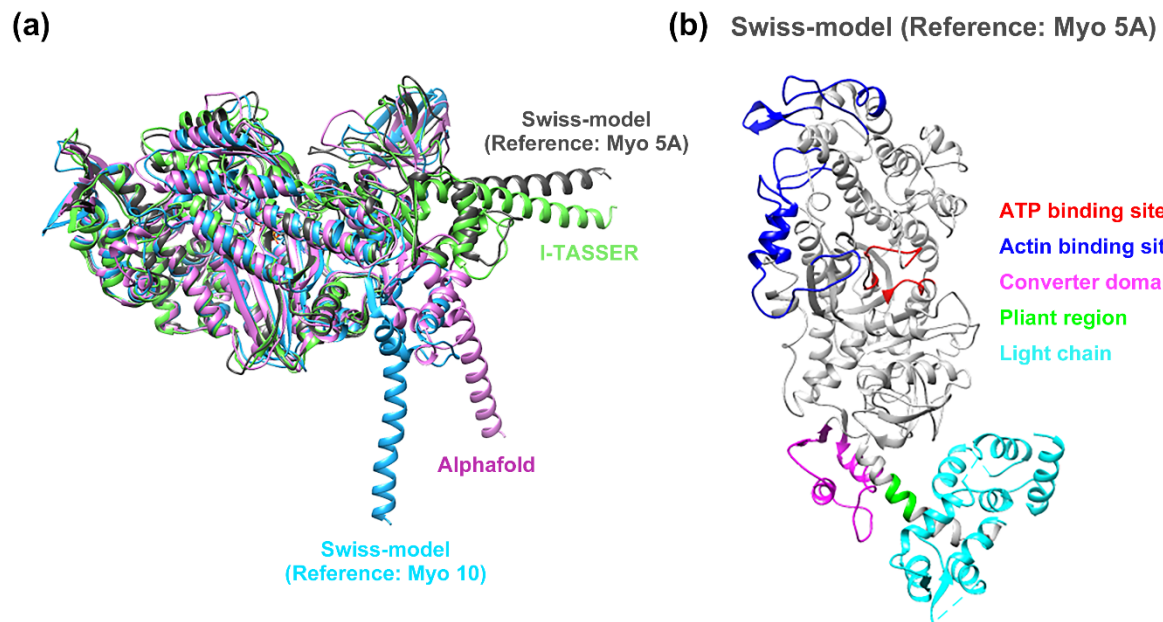


Figure S2 Homology modeling and comparative analysis of myosin 7A using AlphaFold (AF-Q9V3Z6-F1), I-TASSER, and SWISS-MODEL. (a) The modeling results, leveraging differences in software and reference selection, indicate a high structural similarity in the motor domain, with the exception of the flexible regions around the neck area. (b) Utilized a homology model based on the reference of the representative unconventional myosin, myosin 5A. The key active sites shown as different colors respectively.

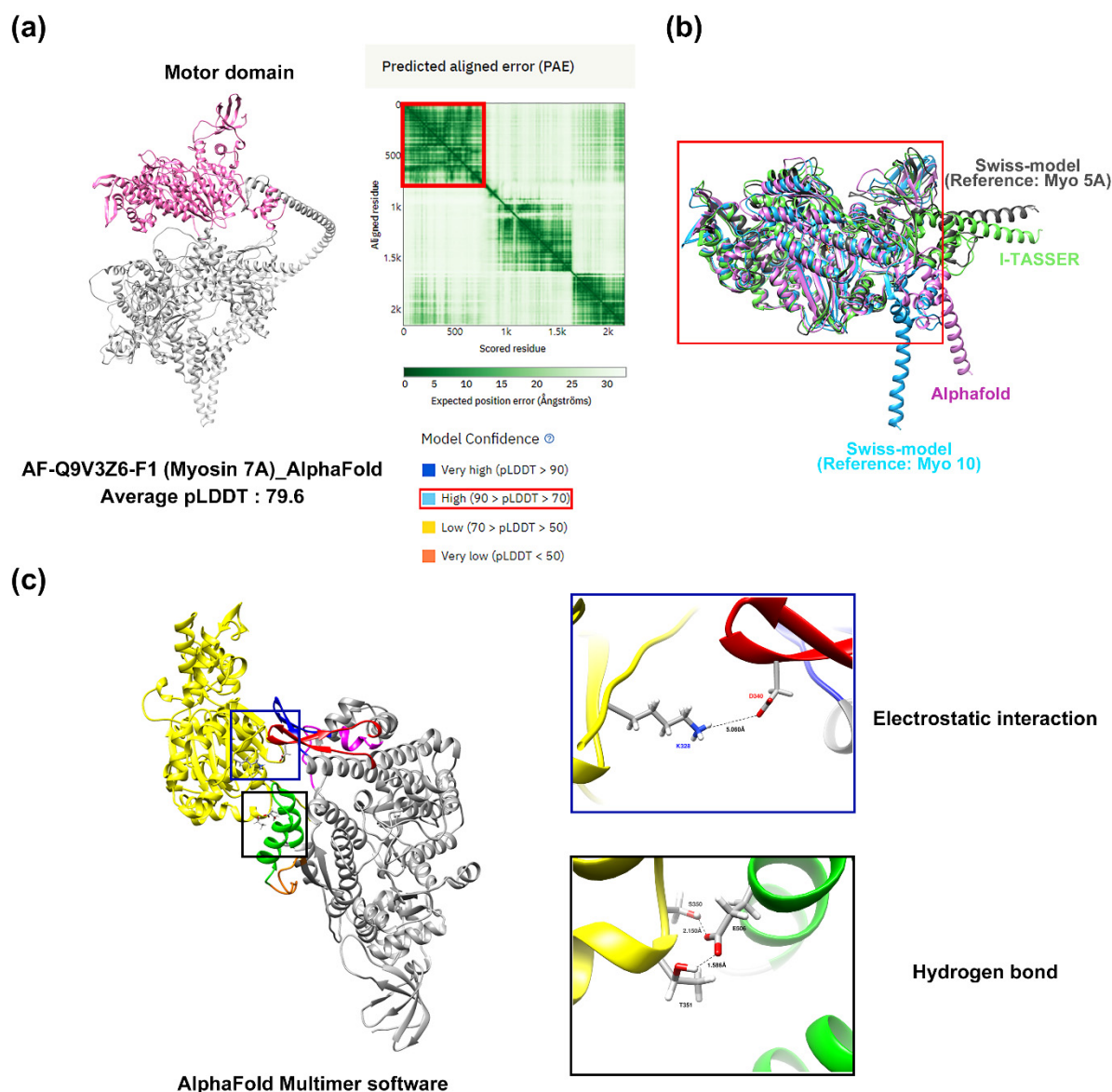


Figure S3 Assessment of Prediction Models: Evaluating Structural Integrity. (a) The Myosin 7A AlphaFold structure (Uniprot: Q9V3Z6) has very low predicted aligned error (PAE) and per-residue local distance difference test (pLDDT) values in its motor domain, indicating high confidence in the model's reliability. (b) Comparing myosin 7A homology models generated by other software (SWISS-MODEL and I-TASSER) with the AlphaFold model confirms their high structural similarity in the motor domain, ensuring the reliability of all predicted homology models. (c) The AlphaFold Multimer software predicted the actomyosin-7A structure, revealing the same core interaction between actin and myosin 7A as previously proposed by HDock software. The structural predictions for *Drosophila* myosin 7A and alpha skeletal actin were acquired from the AlphaFold protein structure database. These predictions were generated by submitting the respective protein sequences to AlphaFold, utilizing default settings without template assistance. Subsequently, the final structural complex was visualized using UCSF Chimera for comprehensive analysis.