

Expanded View Figures

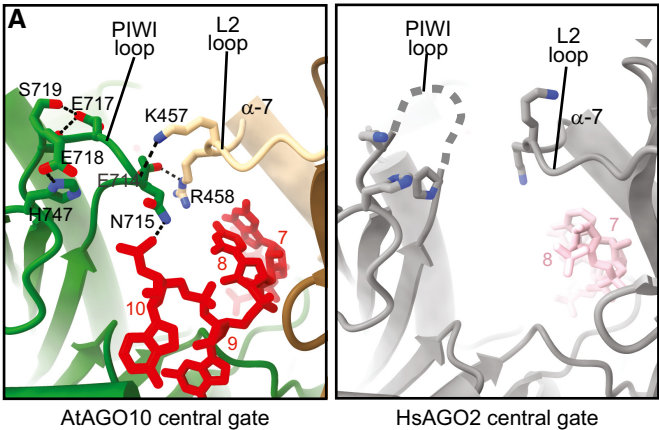


Figure EV1. Comparison of PIWI loops in diverse AGOs.

A Close-up views of PIWI-loop and L2-loop connections in AtAGO10 (PDB 7SVA) and HsAGO2 (PDB 4OLA) structures.
B Alignment of PIWI-loop sequences from diverse eukaryotes.

B

	PIWI-loop	
Arabid. AGOs	AtAgo10 GADVTHPENGEESSPSIAAVV	clade 1
	AtAgo1 GADVTHPHPGEDSSPSIAAVV	
	AtAgo5 GADVTHPQPGEDSSPSIAAVV	
	AtAgo7 GADVTHPHPFDDCSPSIAAVV	clade 2
	AtAgo2 GADVNHFAARDKMSPSIAAVV	
	AtAgo3 GADVNHFAAHDNMSPSIAAVV	
	AtAgo4 GMDVSHGSPGQSDVPSIAAVV	clade 3
	AtAgo6 GMDVSHGPPGRADVPSIAAVV	
	AtAgo9 GMDVSHGSPGQSDIPSI AAVV	
Plant clade 1 AGOs	AtAgo10 GADVTHPENGEESSPSIAAVV	thale cress (Eudicot)
	OsPNH1 GADVTHPETGEDSSPSIAAVV	rice (Monocot)
	PpAgo1 GADVTHPHPGEDSSPSIAAVV	moss (Bryophyta)
	PtAgo5 GADVTHPHPGEDSSPSIAAVV	pine (Gymnosperm)
	MpAgo1 GADVTHPHPGEDTSPSIAAVV	liverwort (Marchantiophyta)
Animal miRNA-class AGOs	HsAGO2 GADVTHPPAGDGKKPSIAAVV	human (mammal)
	DmAGO1 GADVTHPPAGDNKKPSIAAVV	fruit fly (insect)
	CeALG1 GCDITHPPAGDSRKPSIAAVV	worm (nematode)
	AcAGO1 GADVTHPPAGDTLKP SIAAVV	sea hare (gastropod)
	CgAGO2 GADVTHPPAGDTSKPSIAAVV	oyster (bivalve)
	NvAGO1 GADVTHPAAGDDKRPSIAAVV	sea anemone (Cnidarian)
Arthropod siRNA-class AGOs	EfAGO GADVTHPPAGDDKKPSIAALV	sponge (Poriferan)
	DmAgo2 GADVTHPSPDQREIPSVVGVA	Insects
	SgAgo2 GADVTHPSPDQRNIPSVAAVC	
	TcAgo2b GADVTHPSPDARDIPSVAAVT	
	BmAgo2 GADVTHPSPDQSNIP SIAAVT	Arachnids
	IsAgo2 GADVTHPAFGDKLRPSIAACV	
	PteAgo2 GADVSHAGVTDKSGISVAAVA	
Arthropod siRNA-class AGOs	LpoAgo2 GADVTHPAFGDKPETSVAAAV	Horseshoe crab

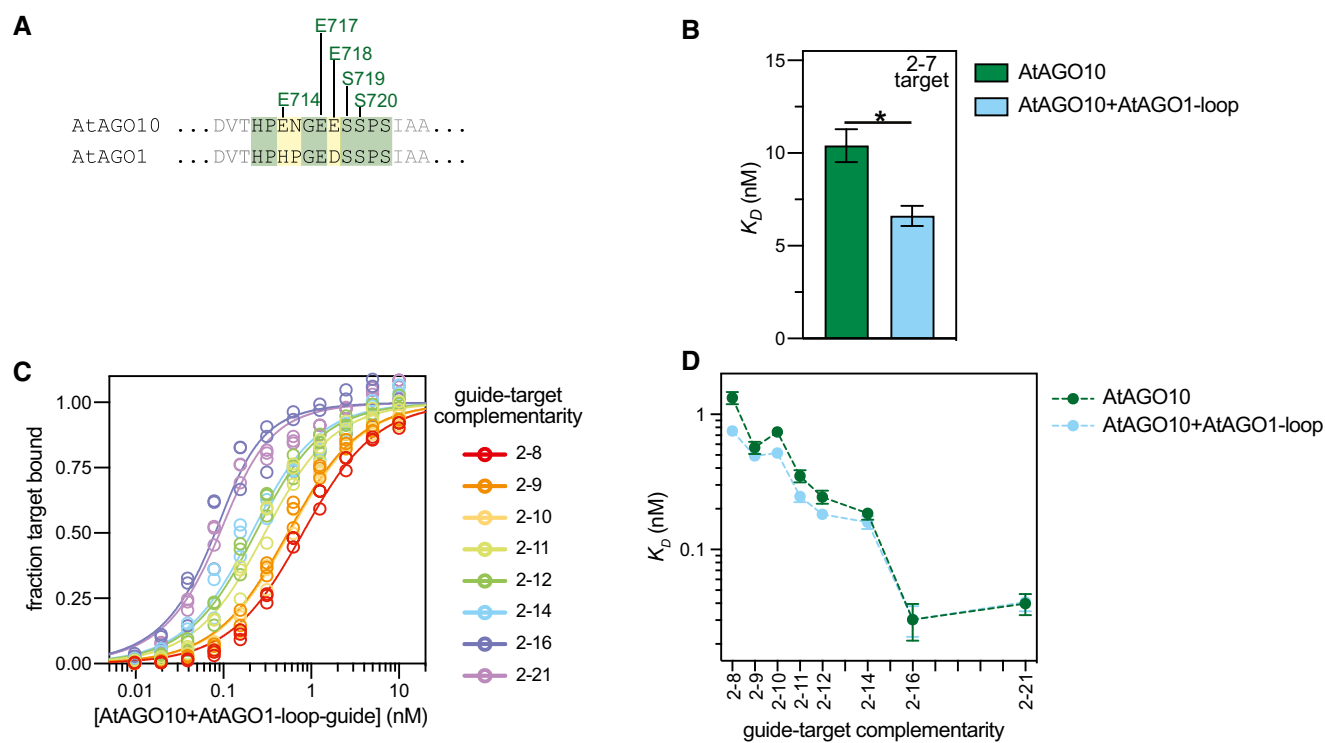


Figure EV2. PIWI loops of AtAGO1 and AtAGO10 are functionally similar.

A Detailed comparison of PIWI-loop sequences in AtAGO10 and AtAGO1.

B Dissociation constants for wild-type AtAGO10 and a mutant AtAGO10 bearing the PIWI-loop sequence from AtAGO1 (AtAGO10 + AtAGO1-loop) binding to a target RNA with complementarity to the miRNA seed region (2–7).

C Fraction target RNAs bound versus [AtAGO1 + AtAGO1-loop]. Guide and target RNAs are shown in Fig 2A.

D K_D values of wild-type AtAGO10 and AtAGO10 + AtAGO1-loop mutant for various target RNAs.

Data information: Data points in panel (C) are from $n = 3$ independent technical trials. Data in panels (B and D) represent best-fit K_D values from $n = 3$ technical trials. Error bars indicate s.e.m. Unpaired t -test P -value: *0.0219. No data points were excluded from the analyses.

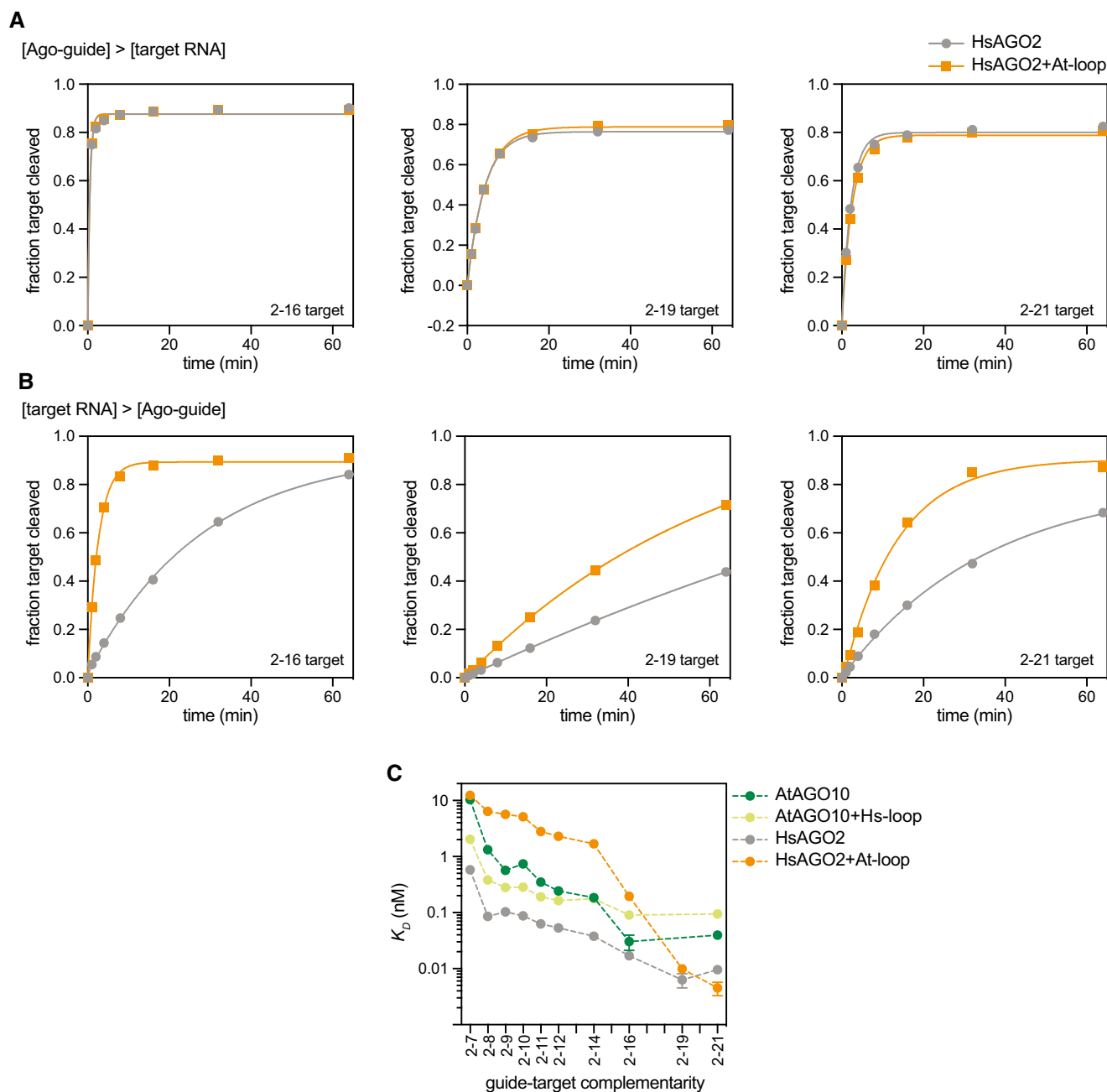


Figure EV3. Detailed comparison of HsAGO2 and HsAGO2 + At-loop biochemical properties.

A Fraction of target RNAs cleaved versus time when cleavage reaction is limited by target RNA ([Ago-guide] = 10 nM, [target RNA] = 2 nM).

B Fraction of target RNAs cleaved versus time when cleavage reaction is limited by Ago-guide ([Ago-guide] = 1 nM, [target RNA] = 5 nM).

C Comparison of K_D values of HsAGO2, HsAGO2 + At-loop, AtAGO10, and AtAGO10 + Hs-loop for various target RNAs.

Data information: Data points in panels (A and B) are values from $n = 1$ experimental trials. Data points in panel (C) are the best-fit K_D values from $n = 3$ independent technical trials, each with ≥ 11 data points. Error bars indicate s.e.m. No data points were excluded from the analyses.