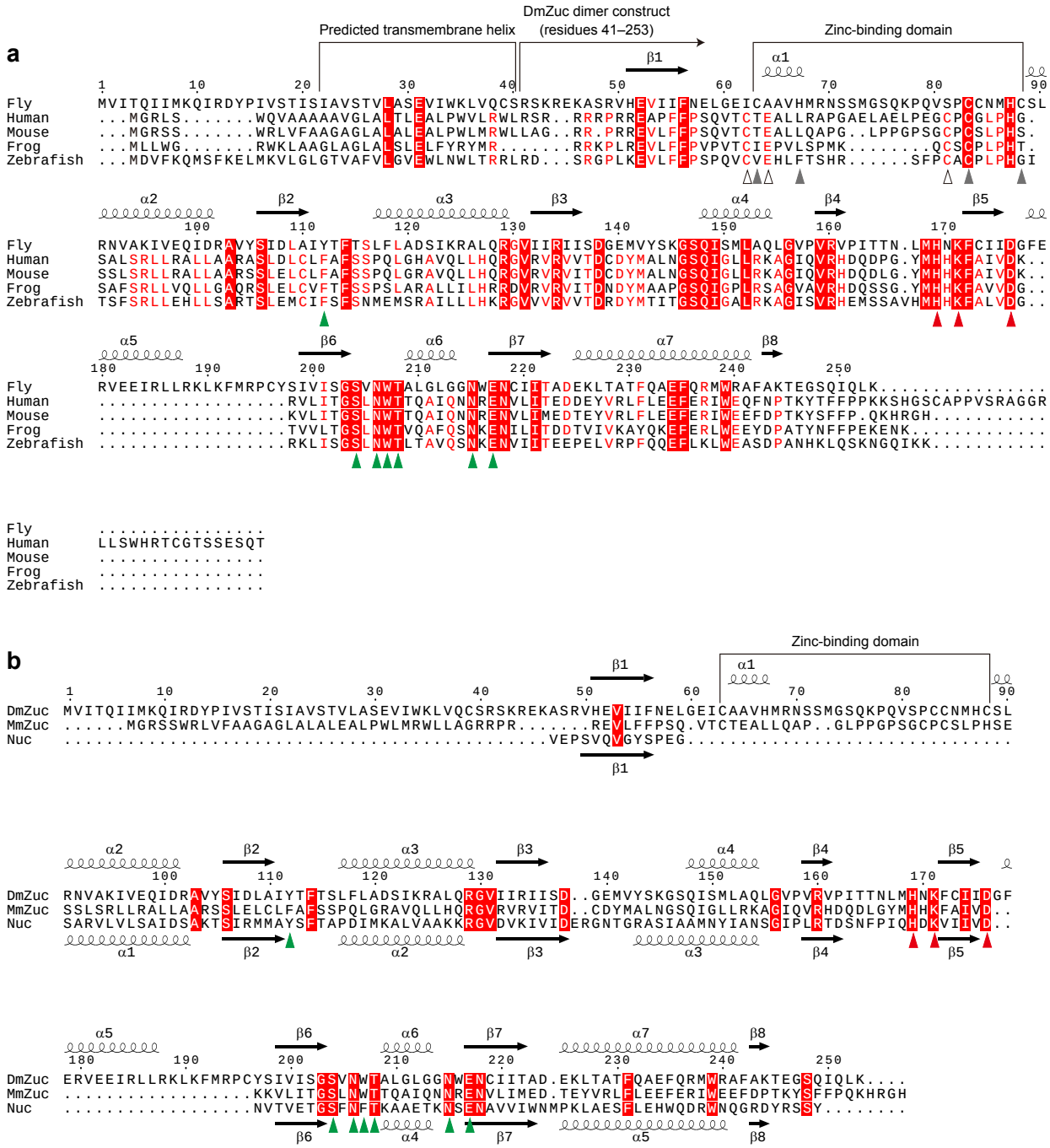
doi:10.1038/nature11509

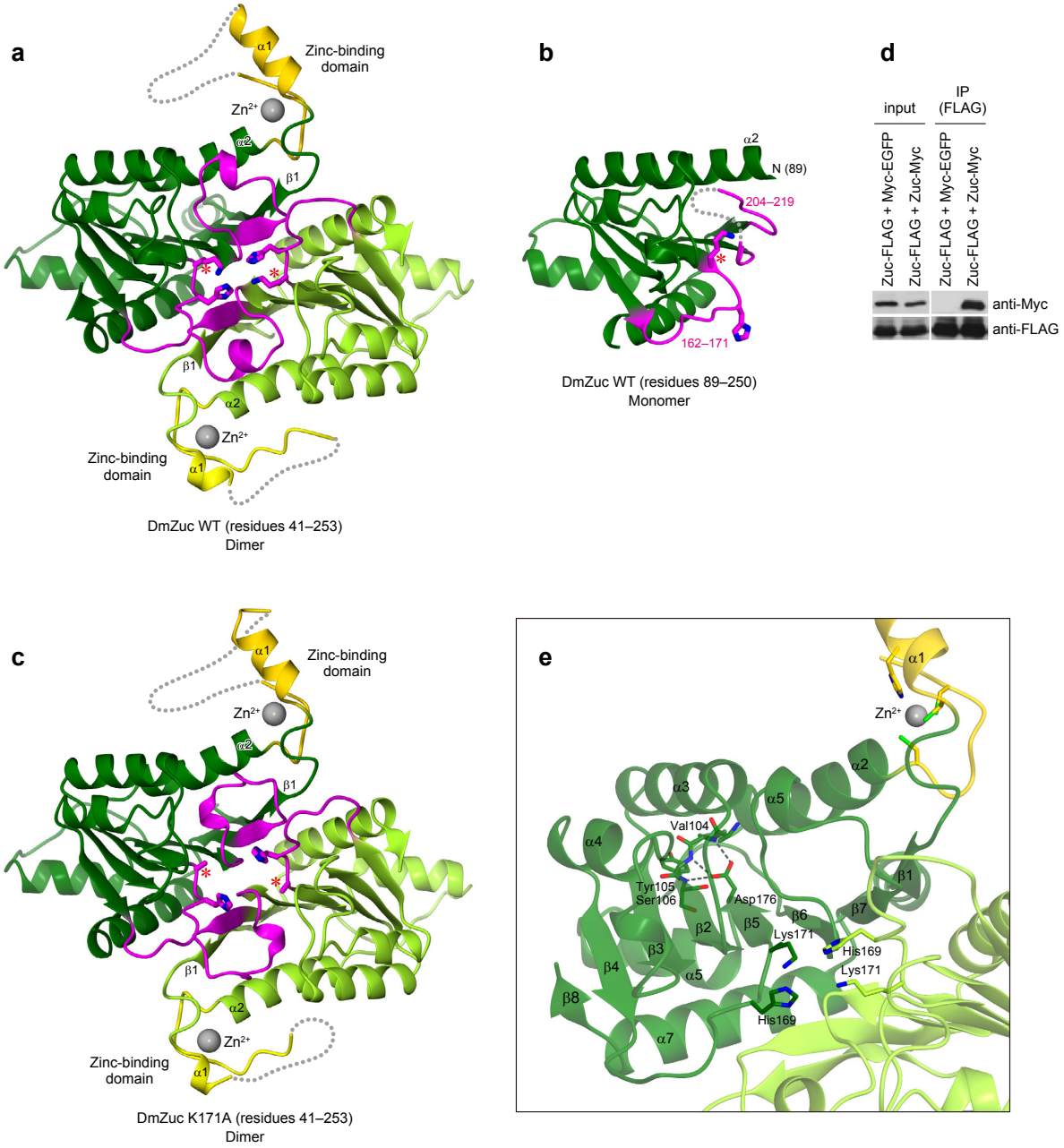
Supplementary Table 1 Data collection and refinement statistics

	DmZuc WT (residues 41–253)	DmZuc K171A (residues 41–253)	SeMet-DmZuc WT (residues 89–250)	
Data collection				
Space group	$P2_1$	$P2_1$	$P2_1$	
Cell dimensions				
a, *b*, *c* (Å)	55.0, 71.2, 56.3	55.9, 70.2, 56.9	35.3, 52.4, 38.8	
α, β, γ (°)	90, 107.9, 90	90, 108.8, 90	90, 104.9, 90	
			Peak	*Inflection*
Wavelength (Å)	1.000	1.000	0.9793	0.9800
Resolution (Å)	50.00–1.75 (1.78–1.75)	50.00–2.2 (2.24–2.20)	50.00–2.2 (2.24–2.20)	50.00–2.5 (2.54–2.50)
R_{sym}	0.064 (0.34)	0.083 (0.27)	0.14 (0.93)	0.13 (0.90)
$I/\sigma I$	27.5 (2.2)	16.6 (2.6)	22.9 (2.7)	24.5 (3.1)
Completeness (%)	97.2 (91.2)	95.0 (87.7)	100 (100)	100 (100)
Redundancy	5.8 (3.7)	2.8 (2.2)	8.4 (8.2)	8.3 (8.3)
Refinement				
Resolution (Å)	42.8–1.75	45.8–2.2	37.5–2.2	
No. reflections	39,874	19,684	6,836	
R_{work} / R_{free}	0.187 / 0.230	0.190 / 0.246	0.223 / 0.270	
No. atoms				
Protein	3,080	2,986	1,173	
Ligand/ion	31	10	1	
Water	180	96	16	
B-factors				
Protein	28.4	40.4	39.0	
Ligand/ion	33.6	35.3	24.0	
Water	34.3	38.0	30.4	
R.m.s deviations				
Bond lengths (Å)	0.008	0.005	0.002	
Bond angles (°)	1.09	0.95	0.57	

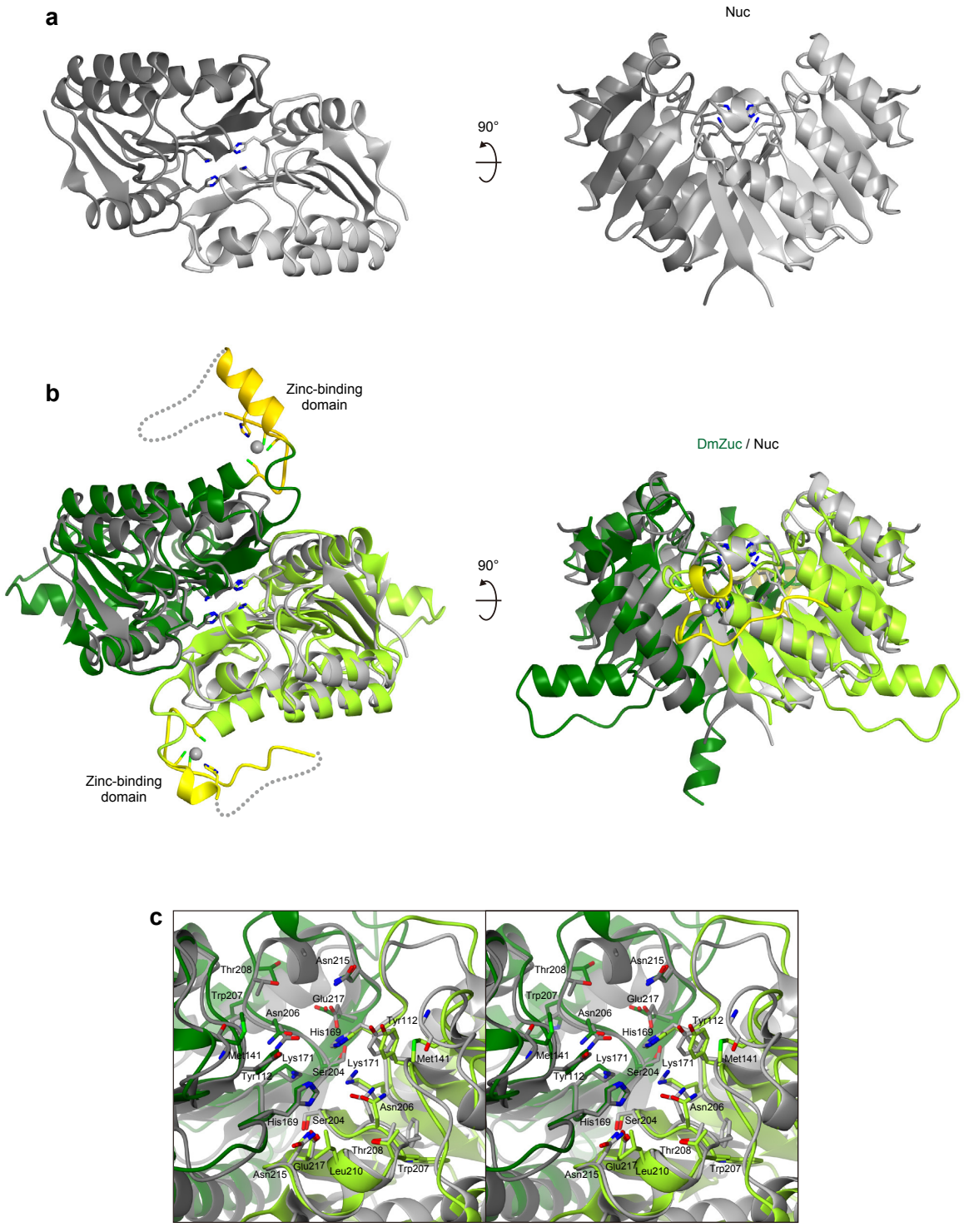
*Highest resolution shell is shown in parentheses. WT, wild-type.



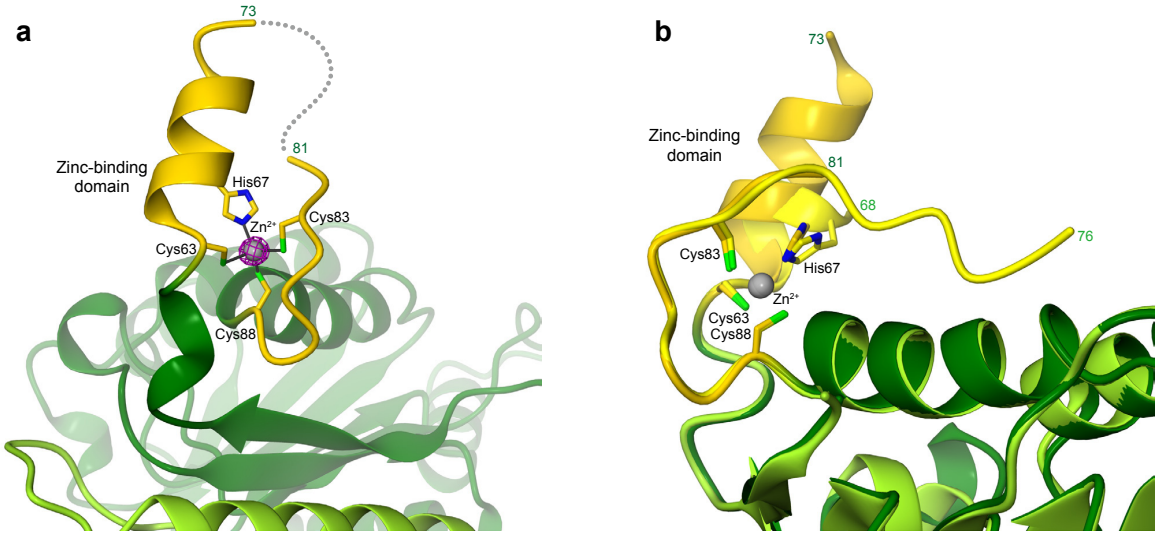
Supplementary Figure 1. Multiple amino acid sequence alignment.
a, Multiple sequence alignment of Zuc proteins from different animal species. The secondary structure of DmZuc is indicated above the sequences. **b**, Multiple sequence alignment of DmZuc, MmZuc, and *Salmonella typhimurium* Nuc. The secondary structures of DmZuc and Nuc are indicated above and below the sequences, respectively. Strictly conserved residues are highlighted in red boxes (coloured green in Fig. 2a), and highly conserved residues are indicated by red letters (coloured pale green in Fig. 2a). The HKD motif is indicated by red triangles. The zinc-binding residues in DmZuc are indicated by grey triangles, and the CEC motif is indicated by white triangles. The active-site residues are indicated by green triangles.



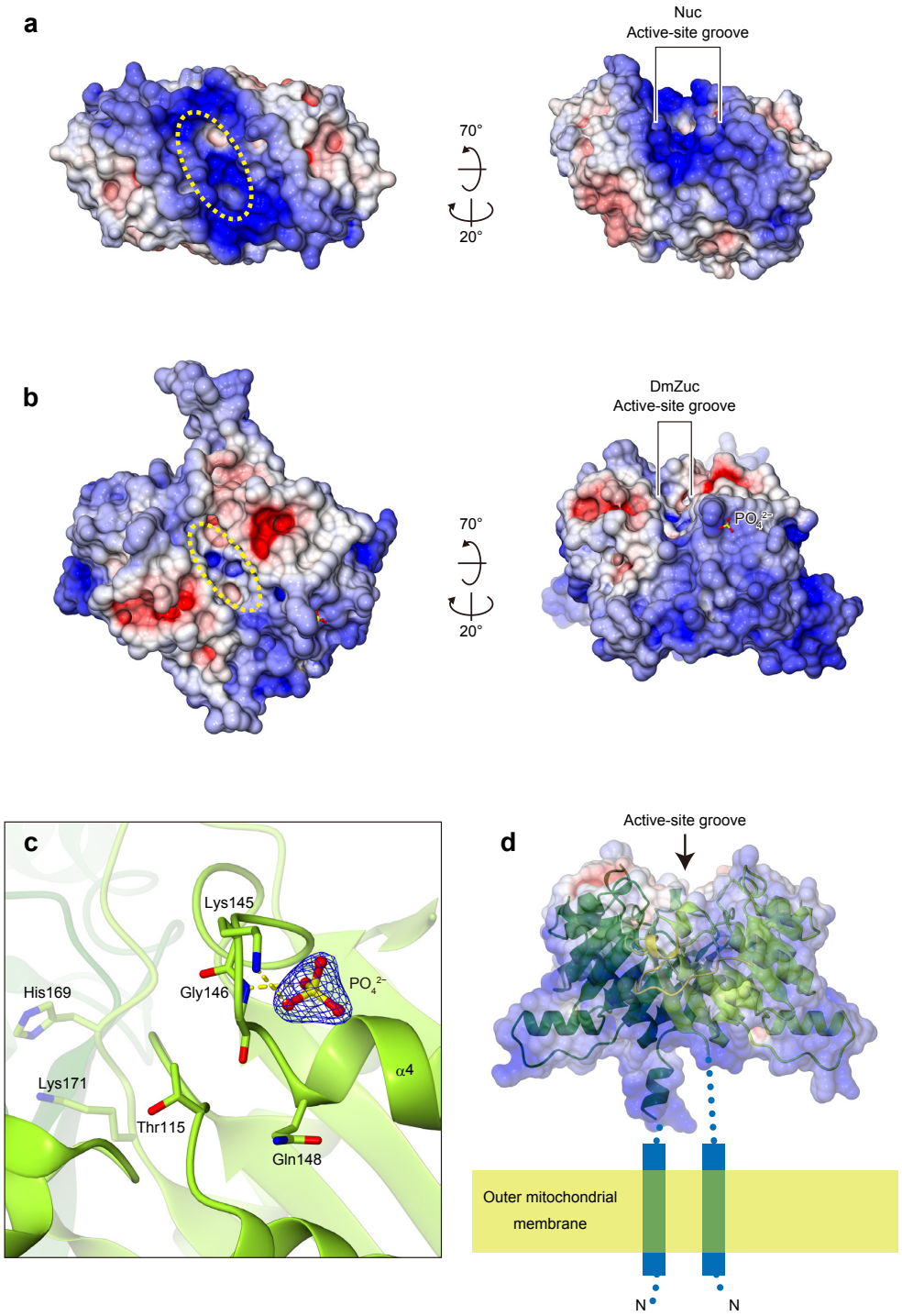
Supplementary Figure 2. Crystal structures of DmZuc.
a–**c**, Overall structures of the wild-type (WT) dimer (residues 41–253) (**a**), the WT monomer (residues 89–250) (**b**), and the K171A mutant dimer (residues 41–253) (**c**). Lys171 in the WT and Ala171 in the K171A mutant are indicated by red asterisks. Disordered regions in the zinc-binding and catalytic domains are indicated by dashed lines. In the WT dimer, residues 74–80 and 246–253 in one protomer, and residues 41–48, 69–75 and 246–253 in the other are disordered. In the K171A dimer, residues 41–43, 75–81 and 245–253 in one protomer, and residues 41–48, 68–77 and 246–253 in the other are disordered. In the WT monomer, residues 209–213 and 242–250 are disordered. The active-site loops of the WT monomer and the K171A dimer have conformations different from that of the WT dimer. **d**, Self-interaction of DmZuc in OSCs. OSCs were co-transfected with an expression vector encoding C-terminal FLAG-tagged DmZuc and either an expression vector encoding N-terminal Myc-tagged enhanced green fluorescent protein (EGFP) or an expression vector encoding C-terminal Myc-tagged DmZuc. Immunoprecipitation (IP) using an anti-FLAG M2 antibody, followed by western blotting using anti-Myc and anti-FLAG antibodies, revealed that DmZuc-FLAG interacts with DmZuc-Myc but not Myc-EGFP. **e**, Asp176 in the HKD motif helps to maintain structural integrity. Dashed lines indicate hydrogen bonds between the side chain of Asp176 and the main-chain amide groups of Val104, Tyr105 and Ser106.



Supplementary Figure 3. Structural comparison of DmZuc and Nuc.
a, Overall structure of *Salmonella typhimurium* Nuc (PDB: 1BYR). His94 and Lys96 are shown as sticks. **b**, Superposition of the overall structures of DmZuc (coloured) and Nuc (PDB: 1BYR) (grey). **c**, Superposition of the active sites of DmZuc (coloured) and Nuc (PDB: 1BYR) (grey) (stereoview). Conserved residues are shown as sticks.

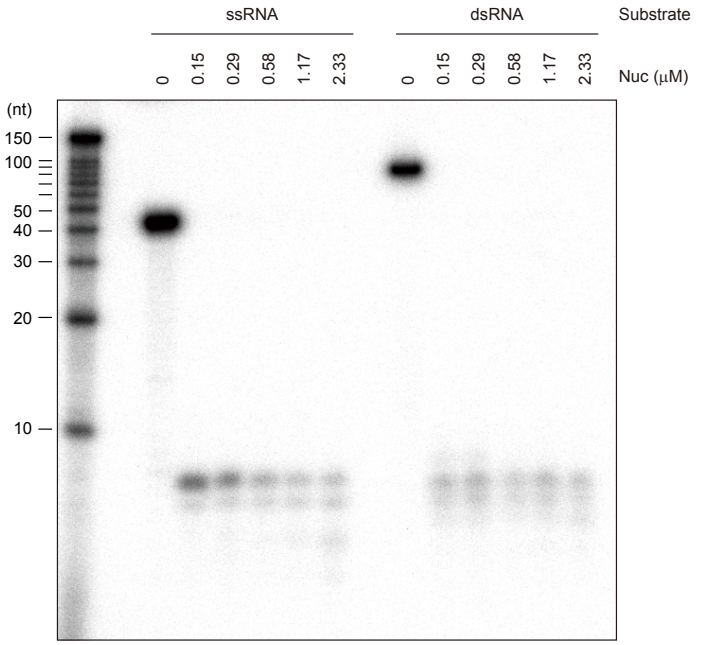


Supplementary Figure 4. Zinc-binding domain.
a, A zinc ion is coordinated by Cys63, His67, Cys83, and Cys88 in the zinc-binding domain. The bound zinc ion is shown as a grey sphere, and an anomalous difference Fourier map at 2.3-Å resolution is shown as a magenta mesh (contoured at 10σ). **b**, Superposition of the zinc-binding domains in the two protomers. The two zinc-binding domains are oriented similarly, although the disordered regions differ somewhat (residues 74–80 in one protomer and residues 69–75 in the other), because of the different crystal packing environments.

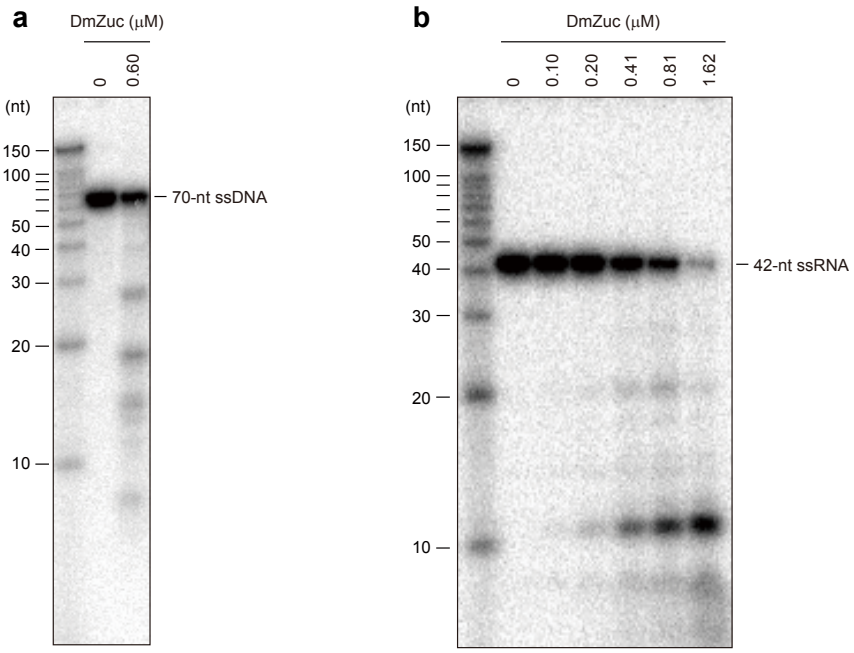


Supplementary Figure 5. Active-site groove.

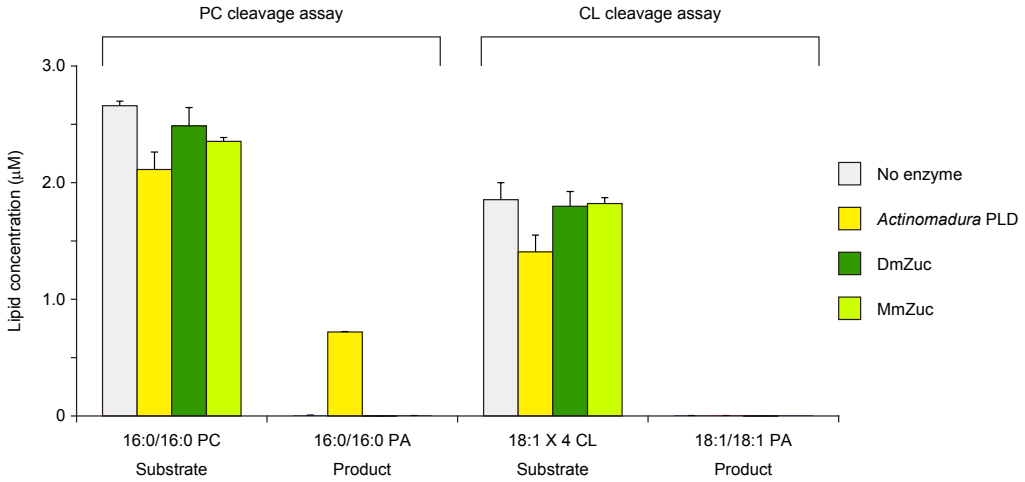
a, Electrostatic surface potential of Nuc (PDB: 1BYR). **b**, Electrostatic surface potential of DmZuc. The active sites are indicated by yellow dashed lines in **a** and **b**. **c**, Phosphate-binding site. Hydrogen bonds are indicated by yellow dashed lines, and simulated annealing F_O–F_C omit electron density map is shown as a blue mesh (contoured at 4σ). The phosphate ion interacts with the side chain of Lys145 and the main-chain amide group of Gly146, and is also surrounded by Thr115 and Gln148. Thr115, Gly146, and Gln148 are conserved in Zuc proteins (Thr115 in *Drosophila* is replaced by a serine in other animals). **d**, Model of DmZuc localization on the outer mitochondrial membrane. Electrostatic surface potential suggests that DmZuc is anchored on the mitochondrial surface through its N-terminal transmembrane helices, with the positively charged surface facing the outer mitochondrial membrane.



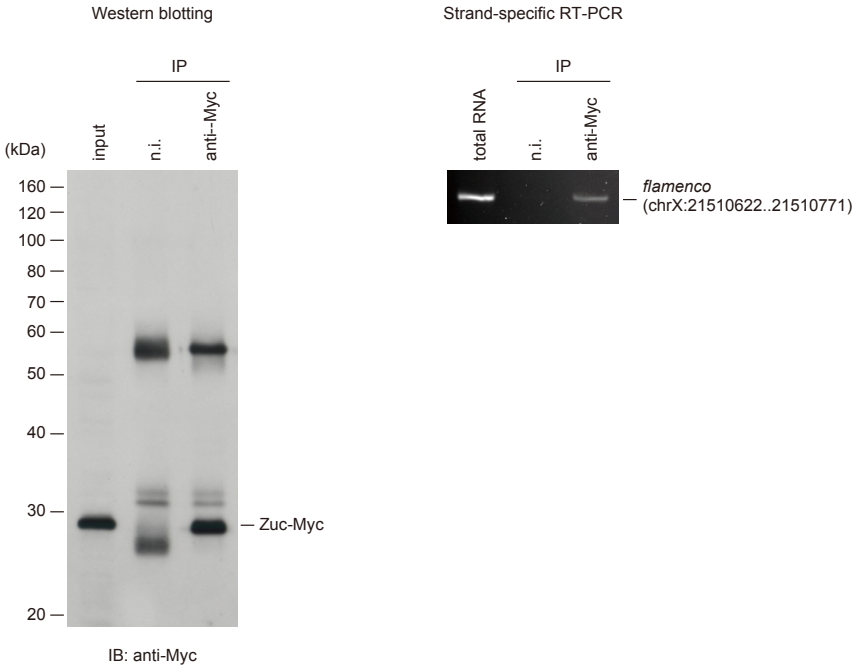
Supplementary Figure 6. Nuc cleaves both ssRNA and dsRNA.
The 42-nt ssRNA and 40-nt dsRNA were incubated with Nuc (0.15–2.33 μM) in buffer (25 mM HEPES-KOH, pH 7.4, 2.5 mM EDTA, and 5 mM DTT) at 26ºC for 1 h, and were then resolved on a 20% denaturing polyacrylamide gel.



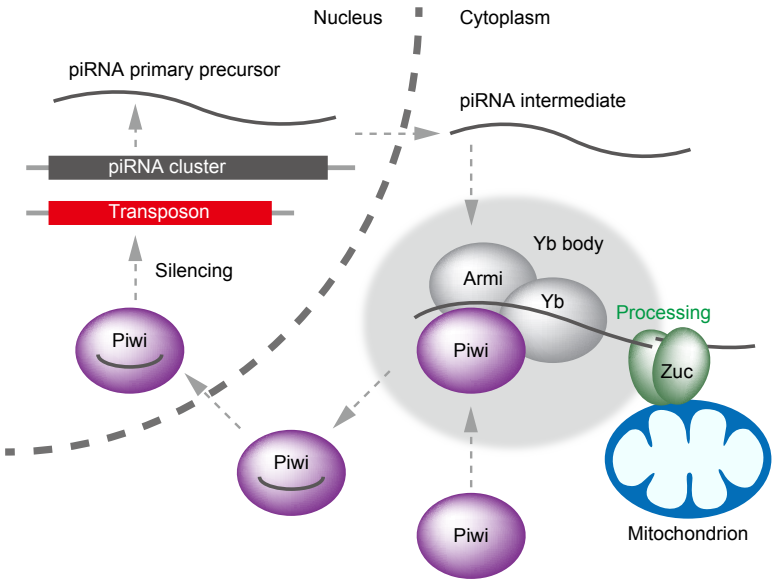
Supplementary Figure 7. Characterization of DmZuc nuclease activity *in vitro*.
a, DmZuc cleaves ssDNA. The 70-nt ssDNA labelled with ^{32}P at the 5' end was incubated with WT DmZuc (0.60 μM) for 1 h at 26ºC, and was then resolved on a 20% denaturing polyacrylamide gel. **b**, DmZuc cleaves ssRNA in a dose-dependent manner. The 42-nt ssRNA labelled with ^{32}P at the 5' end was incubated with WT DmZuc (0.10–1.62 μM) for 1 h at 26ºC, and was then resolved on a 20% denaturing polyacrylamide gel.



Supplementary Figure 8. DmZuc and MmZuc displayed no PLD activity.
Purified DmZuc, MmZuc, or *Actinomadura* PLD (positive control) was incubated with dipalmitoyl (16:0/16:0) PC or tetradioleoyl (18:1 X 4) CL, and then phospholipid products were analyzed by LC/MS/MS. *Actinomadura* PLD failed to hydrolyse tetradioleoyl CL, but hydrolysed dipalmitoyl PC to produce dipalmitoyl PA. In contrast, DmZuc and MmZuc hydrolysed neither dipalmitoyl PC nor tetradioleoyl CL.



Supplementary Figure 9. DmZuc associates with piRNA precursors in OSCs.
C-terminal Myc-tagged, full-length DmZuc was expressed in OSCs by transfection. RT-PCR was performed on RNAs isolated from the material immunoprecipitated from the DmZuc-Myc expressing cells, using an anti-Myc antibody . Western blotting confirmed that DmZuc was efficiently immunoprecipitated from the cells (left panel). RT-PCR indicated that DmZuc-Myc associates with a fragment of the *flamenco* transcript (right panel). A control experiment using non-immune IgG (n.i.) indicated that the interaction between DmZuc and the *flamenco* piRNA precursor is specific.



Supplementary Figure 10. Model of primary piRNA biogenesis in *Drosophila* OSCs.
Long, single-stranded piRNA precursors are transcribed from piRNA clusters in the genome, and are then partly processed into piRNA intermediates through an unknown mechanism. The RNA helicase Armitage (Armi) and Tudor domain-containing RNA helicase Yb localize in Yb bodies, cytoplasmic perinuclear non-membranous organelles, which are often located near mitochondria[15,33]. Armi and Yb form a complex that contains piRNA intermediates with 5'-hydroxyl and 3'-cyclicphosphate ends[15]. Nascent, piRNA-free Piwi transiently localizes at Yb bodies, and interacts with the Armi–Yb complex[15]. Zuc is anchored on the mitochondria surface, with the catalytic site facing the cytosol[12–15], and processes piRNA intermediates into piRNA fragments, which are loaded onto Piwi. In this topological environment, Zuc gains access to piRNA intermediates, which are held in the Armi–Yb complex. In this model, Zuc endoribonuclease plays a critical role in the formation of the 5' end of mature piRNAs. An enzyme participating in the formation of the 3' end remains to be identified.

Supplementary reference

33. Szakmary, A., Reedy, M., Qi, H. & Lin, H. The Yb protein defines a novel organelle and regulates male germline stem cell self-renewal in *Drosophila melanogaster*. *J. Cell Biol.* **185**, 613–627 (2009).