Supplementary information

Supplementary Fig S1

Structural comparison and interaction of zNanos$^{91\text{-}144}$ molecules in the asymmetric unit

A) Superimposition of the four structures in the asymmetric unit shown by tube representation.

For clarification, zinc ions located in the molecular interface are omitted. Colors correspond to those in **Fig 2A**. Residue numbers, 91 and 144 are labeled.

B) Zinc ions located at the molecular interface.

In the structure determination process, ten zinc atoms were found in the asymmetric unit. Of these, eight zinc atoms were assigned as zinc ions in ZF1 and ZF2. The remaining two, which are probably from the buffer, are bound to molecular interfaces and contribute to crystal packing. They are coordinated by H97, C151 and symmetry-related residues. H97 and C151 from different molecules interact with a zinc ion, as shown by a white sphere. Colors correspond to those in **Fig 2A**. The purple cage is the electron density (10σ) of the zinc ion generated by difference anomalous Fourier calculation using Zn-Peak data at 2.5 Å resolution. Interactions are shown by white dots. These interactions must be an artifact of the crystallization conditions, because the residues are not conserved in Nanos of other species.

Supplementary Fig S2

Formation of the Nanos dimer in solution

A) Structure of the Nanos dimer.

For clarification, only the core structures are shown and the colors correspond to those in **Fig 2A**. The N- and C-terminal ends are labeled. Water molecules located in the dimer interface are shown by red spheres.

B) Analytical ultracentrifugation.

Sedimentation velocity experiments were performed using an Optima XL-I ultracentrifuge and an An-50 Ti rotor (Beckman Coulter) at 20°C. The concentrations of the protein solutions used in the sedimentation velocity experiment were 0.2, 0.4 and 0.8 mg/mL in a reference buffer (5 mM HEPES-NaOH pH7.4 and 100 mM NaCl). Absorbance (OD_{280}) scans were collected during sedimentation at 40,000 rpm. Data analysis was performed with the program SEDIFIT (Schuck, 2000; Schuck et al, 2002) and SEDNTERP (Laue et al., 1992). At low protein concentration (0.2 mg/mL), the sedimentation coefficient distribution, C(s) showed one peak corresponding to a zNanos$^{59\text{-}159}$ monomer. By contrast, the C(s) at high protein concentration (0.8 mg/mL) showed two peaks that correspond to the zNanos$^{59\text{-}159}$ dimer and monomer. This result suggests that the dimer formation depends on the protein concentration.

Supplementary Fig S3

EMSA using ssRNA with or without the Nanos preferential sequence.

A) Interaction between zNanos$^{59\text{-}159}$ and ssRNA.

A 10-mer (lane 1-6), 6-mer (lane 7-12) or 4-mer (lane 13-18) of ssRNA either including wild type (WT) or the lacking Nanos preferential sequence (MT) (Kadyrova et al, 2007) was used in this assay **(supplementary Table S1)**. RNA (WT or MT) and zNanos$^{59\text{-}159}$ wild type were mixed in storage buffer and incubated for 1 hr on ice. The concentration of RNA in the mixed solution was 13.3 μM. The concentration of zNanos$^{59\text{-}159}$ wild type in the mixed solution was 6.7 (lane 2, 5, 8, 11, 14 and 17) or 13.3 μM (lane 3, 6, 9, 12, 15 and 18). The solutions were analyzed by a procedure similar to that used in Fig 3C. Because SYBR Gold (Invitrogen) has high specificity for uracil and low specificity for cytosine, 6-mer or 4-mer ssRNA in the absence of Nanos might not be detected (lane 10 or 16). These results clearly indicate that zNanos$^{59\text{-}159}$ binds to ssRNA with no sequence specificity. In these assays, super-shift was observed with an increasing amount of Nanos. Because the super-shift was observed even with 4-mer ssRNA, it might be cased by protein-concentration dependent dimerization.

B) Interaction between zNanos$^{59\text{-}159}$ and ssRNA in the presence of the Pumilio RNA-binding domain.

The cDNA encoding the Pumilio RNA-binding domain from zebrafish (residues 822-1206) was sub-cloned into the EcoRI-XhoI site of the pGEX6P-1 vector (GE Healthcare). The RNA-binding domain of zebrafish Pumilio (zPumilio$^{822\text{-}1206}$) was expressed in *E. coli* BL21(DE3) by IPTG induction (0.2 mM) at 20°C. zPumilio$^{822\text{-}1206}$ was purified by a procedure similar to that used for zNanos$^{59\text{-}159}$ and concentrated in the storage buffer. A 20-mer of ssRNA (WT_20 or MT_20) including the Pumilio binding site was used in this EMSA **(supplementary Table S1)**. Initially, RNA and zPumilio$^{822\text{-}1206}$ were mixed in storage buffer and incubated for 1.5 hr on ice (lane 2 and 5). Next, zNanos$^{59\text{-}159}$ was added to the mixed solution, which was additionally incubated for 1.5 hr at 277 K (lane 3 and 6). The final concentrations of RNA, zPumilio$^{822\text{-}1206}$ and zNanos$^{59\text{-}159}$ were 8.3, 16.7 and 16.7 μM, respectively. The solutions were analyzed by a procedure similar to that used in Fig 3C.

Supplementary Table S1

Sequences of the ssRNA used in EMSA.

These RNAs were purchased from Operon Biotechnology. The Nanos preferential sequence is shown in bold green (Kadyrova et al, 2007). The Pumilio-binding site is shown in bold red.

WT_20	5′ gac**uauuugua**au**uuau**auc 3′
MT_20	5′ gacccaa**ugua**au**uuau**auc 3′
WT_10	5′ gac**uauuugu** 3′
MT_10	5′ gacccaa**ugu** 3′
WT_6	5′ c**uauu**u 3′
MT_6	5′ cccaau 3′
WT_4	5′ **uauu** 3′
MT_4	5′ ccaa 5′

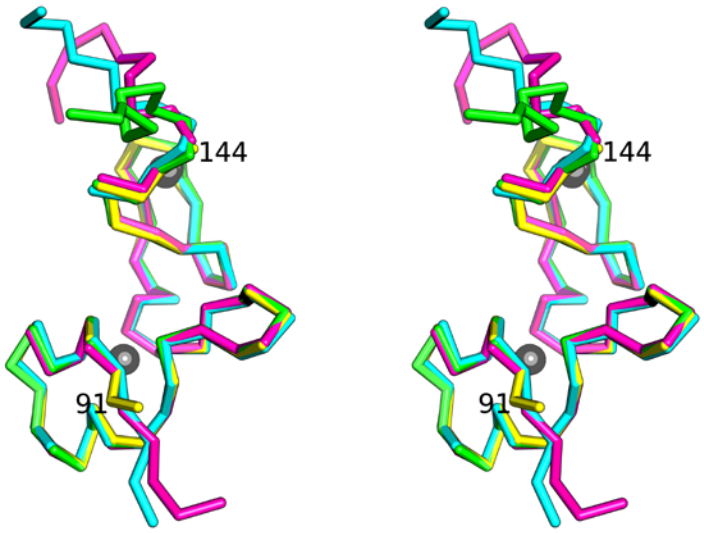
Supplementary References

Kadyrova LY, Habara Y, Lee TH, Wharton RP (2007) Translational control of maternal Cyclin B mRNA by Nanos in the Drosophila germline. *Development (Cambridge, England)* **134**(8)**:** 1519-1527

Schuck P (2000) Size-distribution analysis of macromolecules by sedimentation velocity ultracentrifugation and lamm equation modeling. *Biophys J* **78**(3)**:** 1606-1619

Schuck P, Perugini MA, Gonzales NR, Howlett GJ, Schubert D (2002) Size-distribution analysis of proteins by analytical ultracentrifugation: strategies and application to model systems. *Biophys J* **82**(2)**:** 1096-1111

A



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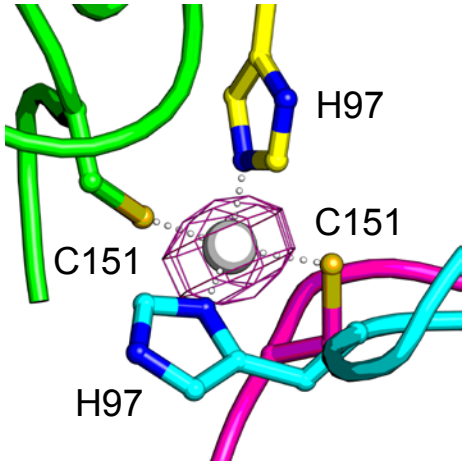
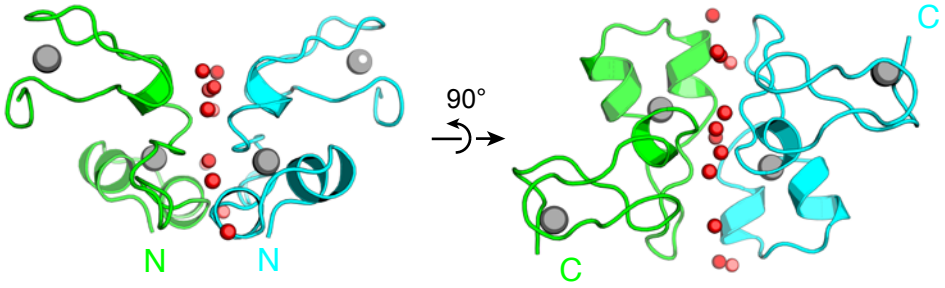


Supplementary Figure S1
Hashimoto et al.

A



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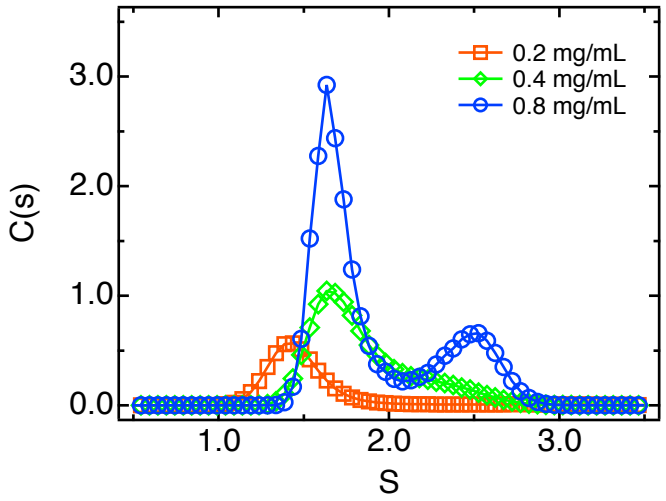

Supplementary Figure S2
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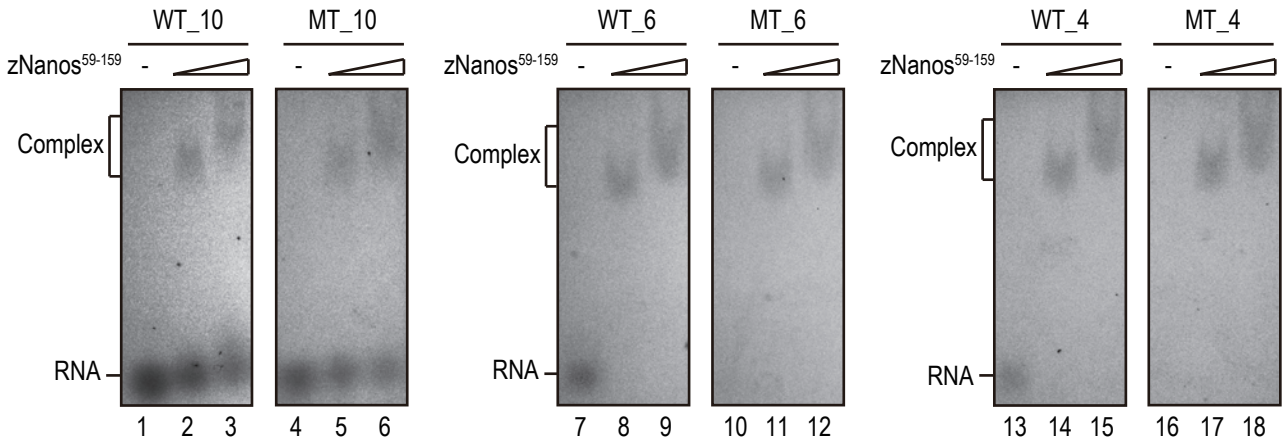
A



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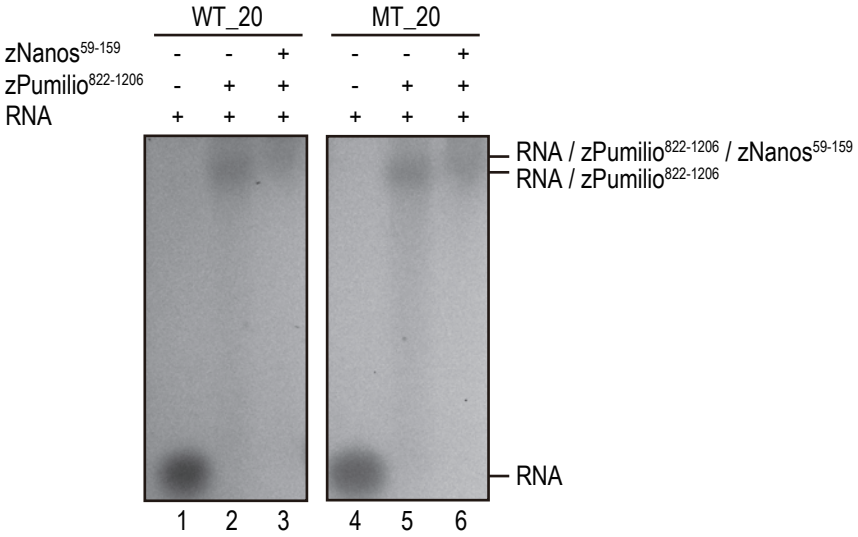

Supplementary Figure S3
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