Supplementary Material for:

Crystal structure of the EndoG/EndoGI complex: Mechanism of EndoG inhibition

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Table S1. Model completeness of the individual polypeptide chains (Ala311 is encoded from the vector backbone.)

dEndoG				
chain A	Thr67	Ala311		
chain B	Ser65	Ala311		
dEndoGI				
chain C / Dom1	Ala21	Arg174		
chain C / Dom2	Leu219	Val230	Ile234	Glu353

Table S2. Interaction between the dimeric dEndoG (chain A and chain B): van der Waals (vdW) interactions, hydrogen bonds (HB), and salt bridges (SB). Distances are given in Å with a distance cut-off of 2.4 Å to 3.3 Å for hydrogen bonds and salt bridges.

chain A (dEndoG)	chain B (dEndoG)	distance	type
Pro82O	Arg99Nη2	2.8	HB
Arg99Nη2	Pro82O	2.9	HB
Arg100Nη2	Asp98Oδ2	2.8	SB
Arg102Nη1	Arg287O	2.9	HB
Arg102Nη2	Arg287O	2.7	HB
Glu221Oε2	Lys229Nζ	2.8	SB
Ser226O	Val232N	3.0	HB
Val228O	Tyr230N	2.8	HB
Lys229Nζ	Glu221Oε1	2.7	SB
Lys229Nζ	Glu221Oε2	3.2	SB
Tyr227	Lys229, Glu231		vdW
Try230	Val228		vdW
Tyr230O	Val228N	2.8	HB
Val232N	Ser226O	3.1	HB
Arg287Nη1	Phe138O	2.7	HB
Arg287Nη1	Asn236Oδ1	3.0	HB
Arg287Nη2	Asn236Oδ1	2.7	HB
Arg287NH2	Ala235O	2.9	HB
Gly290O	Arg99Nε	3.3	HB
Leu292	Phe139		vdW

Table S4. Interaction between dEndoGI and dEndoG. Hydrogen bonds (HB), salt bridges (SB), and van der Waals (vdW) interactions between the two dEndoGI domains and the respective dEndoG molecule are listed. Distances are given in Å with a distance cut-off of 2.4 Å to 3.3 Å for hydrogen bonds and salt bridges.

dEndoGI Dom1 chain C	**dEndoG chain A**	**distance**	**type**	**dEndoGI Dom2 chain C**	**dEndoG chain B**	**distance**	**type**
-	-	-	-	Asp336Oδ1	Arg124Nη1	3.0	SB
Leu146O	Arg124Nη2	3.2	HB	Tyr331O	Arg124Nη2	2.8	HB
Asp151Oδ2	Arg124Nη2	2.7	SB	Asp336Oδ2	Arg124Nη2	3.0	SB
Lys56Nζ	Asp128Oδ2	2.7	SB	-	-	-	-
Asp52Oδ2	Gln131N	3.2	HB	-	-	-	-
Asp52Oδ2	Gln131Nε2	3.1	HB	-	-	-	-
Val50O	Gln131Nε2	2.9	HB	-	-	-	-
Asp98Oδ1	Arg147Nε	3.3	HB	-	-	-	-
Gly97O	Arg147Nη2	3.3	HB	-	-	-	-
Asp98Oδ1	Arg147Nη2	2.6	SB	-	-	-	-
Glu94Oε2	Arg148Nη2	3.0	SB	-	-	-	-
Phe129	Leu146		vdW	Phe129	Tyr331		vdW
Asp145Oδ2	Arg153N	2.8	HB	Asp330Oδ1	Arg153N	2.9	HB
Asp145Oδ2	Arg153Nε	3.1	HB	Asp330Oδ1	Arg153Nε	3.1	HB

Arg147, Arg153	Phe101		vdW	Arg147, Arg153	Phe101		vdW
Phe101O	Arg153Nη2	2.9	HB	Phe286O	Arg153Nη2	2.8	HB
Asp145Oδ1	Arg153Nη2	3.0	SB	Asp330Oδ2	Arg153Nη2	2.9	SB
Arg142O	Gln184Nε2	3.2	HB	-	-	-	-
Ser141O	Arg188Nη2	2.9	HB	-	-	-	-
Arg142O	Arg188Nη2	3.1	HB	Ala326O	Arg188Nη2	3.1	HB
Ser149N	Arg188O	2.9	HB	Asn334N	Arg188O	2.9	HB
-	-	-	-	Asn334Nδ2	Asp189Oδ1	3.2	HB
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
Asp150Oδ2	Asn192Nδ2	3.1	HB	Asp333Oδ2	Asn192Nδ2	3.3	HB
Ser149Oγ	Asn192Nδ2	2.9	HB	-	-	-	-
-	-	-	-	Glu335Oε1	Arg199Nη2	2.7	SB

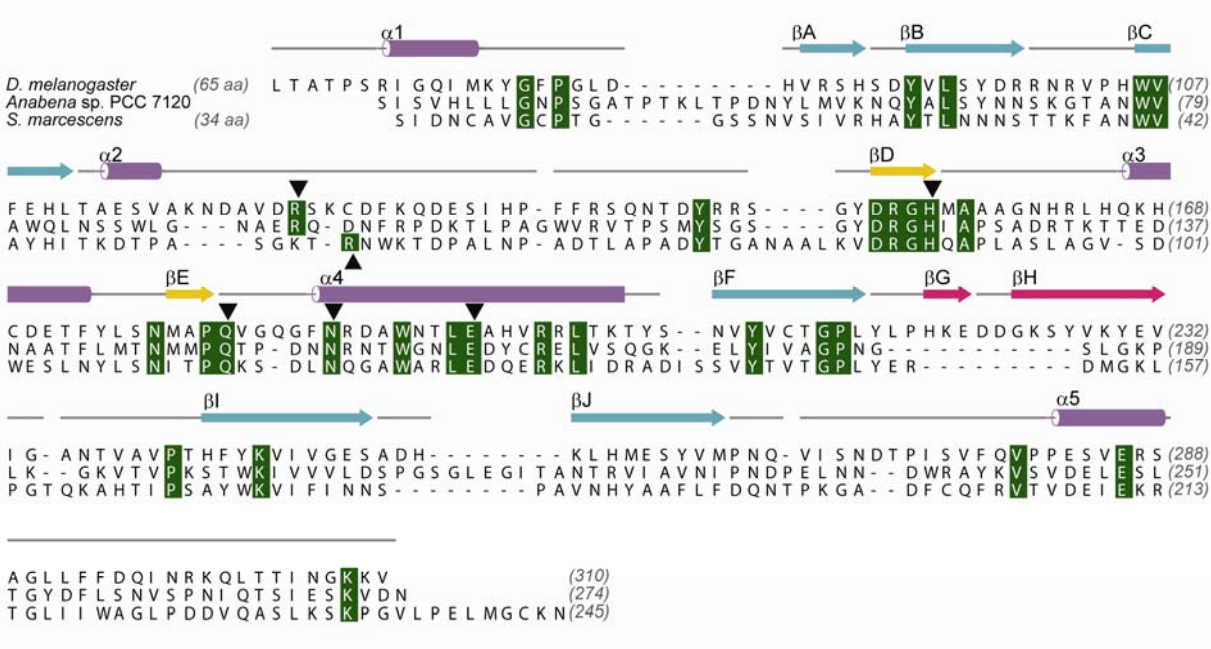

Fig. S1. Structure based sequence alignment of EndoG from *D. melongaster* (NP_609781), NucA from *Anabena* sp. PCC 7120 (YP_227663) and endonuclease from *Serratia marcescens* (P_13717). Identical residues are colored dark green. Secondary structure elements are colored according to Secondary structure elements are shown above the amino acid sequences with α-helices as brown cylinders. The small two-stranded β-sheet (β-strands D and E) involved in metal ion binding is highlighted in yellow. The two wings forming an intermolecular β-sheet are shown in red (β-strands G and H). Residues of dEndoG directly involved in catalysis are marked with black triangles above the amino acid sequence. The guanidinium function of Arg57 of the *Serratia* nuclease (black triangle below the sequence) and Arg124 in dEndoG occupy the same position even though the trace of the polypeptide main chain differs within both structures.

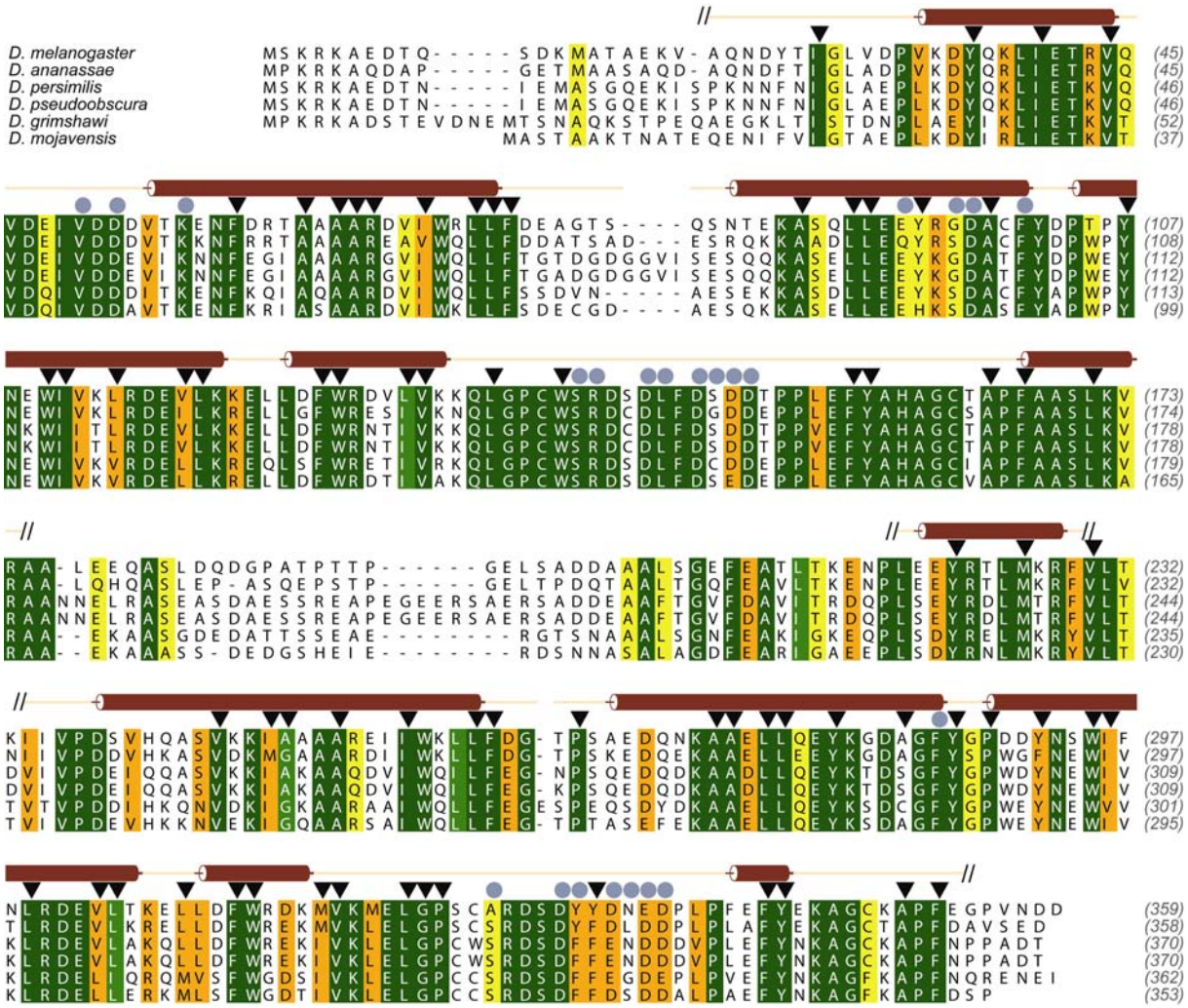

Fig. S2. Sequence alignment of dEndoGI with other *Drosophila* species for *D. melongaster* (NP_609781), *D. ananassae* (XP_1961417), *D. persimilis* (NP_2018773), *D. pseudoobscura* (XP_1357384), *D. grimshawi* (XP_1992859), and *D. mojavensis* (XP_2002941). Identical residues are colored dark green and according to the decreasing similarity from light green through orange to yellow. Secondary structures are shown above the primary sequences with α-helices as brown cylinders. Residues which are involved in hydrogen bonds, salt bridges or hydrophobic interaction in the dEndoG/dEndoGI interface are labeled with grey circles. The black triangles indicate residues which establish the hydrophobic core of Dom1 and Dom2 of dEndoGI.

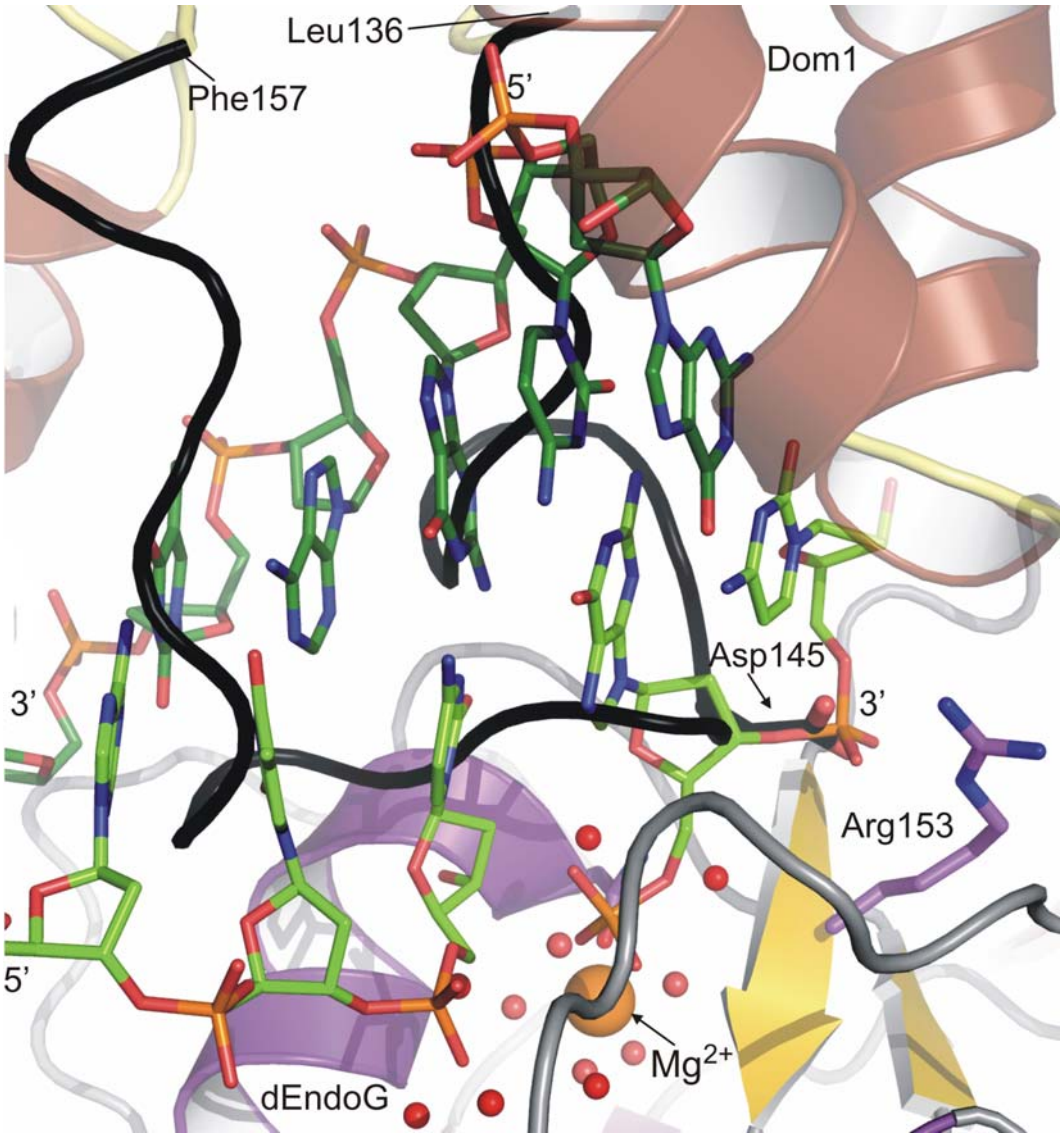

Fig. S3. Superposition of dEndoG/dEndoGI with Vvn enduculease bound to DNA. A superposition of dEndoG (residues 151-162 and 178-188) with the structure of the Vvn endonuclease (residues 76-87 and 119-129) in complex with a cleaved DNA oligonucleotide (PDB code 1oup); The DNA strand is drawn in light green (sense) and dark green (antisense). Helices of dEndoG are illustrated as purple ribbons and strands of the central β-sheet in cyan. The small two-stranded β-sheet (β-strands D and E) involved in metal ion binding is highlighted in yellow, and the bound metal ion is shown as an orange sphere. The black colored loop region (residues 136-157) illustrates the mimicry of the DNA oligonucleotide phosphate backbone by dEndoGI/Dom1. The negative charged carboxyl group of Asp145 of Dom1 (black sticks) occupies similar position as a phosphate moiety of the DNA and forms in the dEndoG/dEndoGI complex a salt bridge to Arg153 (side chains are shown as stick model).