Supporting Information

Liu et al. 10.1073/pnas.1013676108

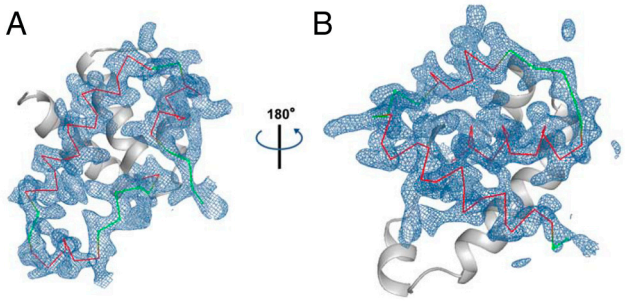

Fig. S1. Electron density maps showing the two sides of Orc6 molecule A in an asymmetric unit. (*A*) Electron density map showing the region covering helices 1, 2, and 3; (*B*) electron density map showing the region covering helices 4, 5, and 6. Electron density is shown as blue net lines and the protein is shown in red (for helices) and green (for loops) lines.

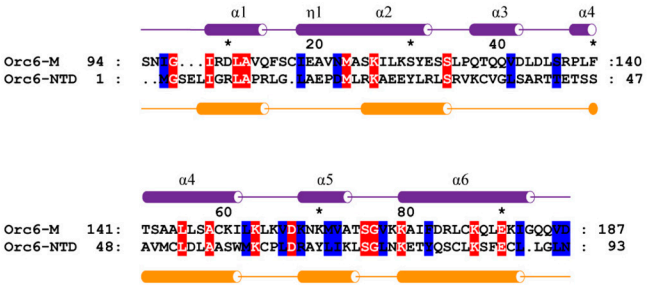

Fig. S2. Sequence alignment between two fragments [Orc6 middle region (Orc6-M, residues 94–187) and Orc6 amino-terminal domain (Orc6-NTD, residues 1–93)] of human Orc6. The secondary structure of Orc6-M is shown in cylinders colored in violet, whereas the predicted secondary structure of Orc6-NTD by PSIPRED server (http://bioinf4.cs.ucl.ac.uk:3000/psipred/) is shown in cylinders colored orange.

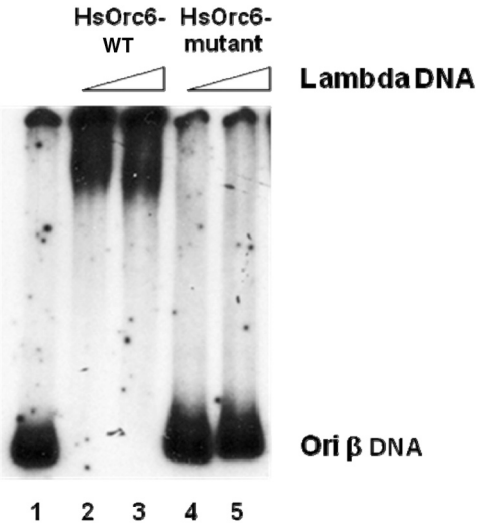

Fig. S3. DNA binding of human Orc6 to radiolabeled DNA fragment containing *Drosophila* ori-β origin was monitored by electrophoretic mobility shift assays. Human Orc6 wild-type protein (Orc6-WT) (lanes 2 and 3) and Orc6 mutant protein containing mutations R137A, Q129A, and K168A (lanes 4 and 5) (50 ng each) were incubated with DNA fragment in the presence of increasing amounts of competitor lambda DNA. The amount of competitor was 50 and 100 ng. No protein was added to DNA fragment in lane 1.

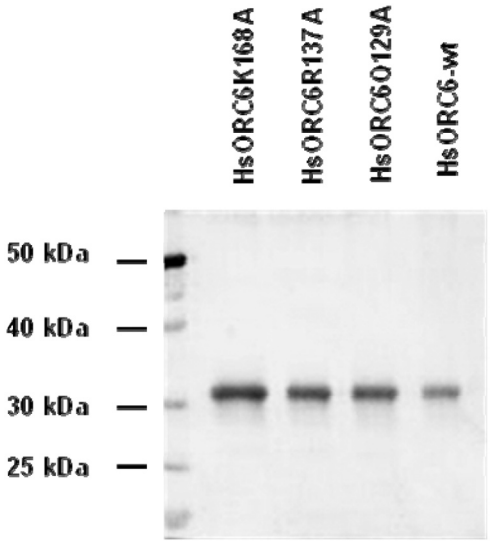

Fig. S4. Silver stained SDS-PAGE gel of purified recombinant wild-type and mutant human Orc6 proteins used for electrophoretic mobility shift assays in Fig. 3*A*.

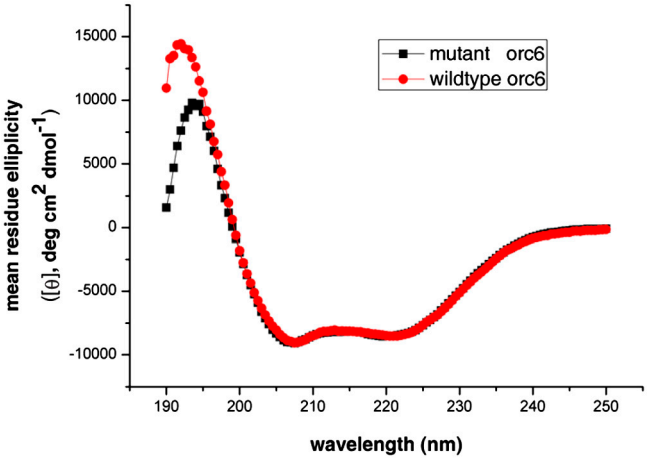

Fig. S5. CD analysis of wild-type (red circle line) and a mutant Orc6 protein with Q129A/R137A/K168A triple mutations (black square line) shows that the two proteins have similar overall structures. CD spectra data were collected on a BIO-Logic MOS450 CD spectrometer at room temperature using a 1-mm path-length cell. Proteins were dissolved in 10 mM dipotassium hydrogen phosphate (pH 7.3) with 100 mM $(NH_4)_2SO_4$. Protein concentrations were estimated from the calculated molar extinction coefficient at 280 nm (1). The corresponding concentrations were 0.489 mg/mL and 0.326 mg/mL, respectively. Both samples were scanned three times and the averaged data were finally used.

1 Pace CN, Vajdos F, Fee L, Grimsley G, Gray T (2001) How to measure and predict the molar absorption coefficient of a protein. *Protein Sci* 4:2411–2423.

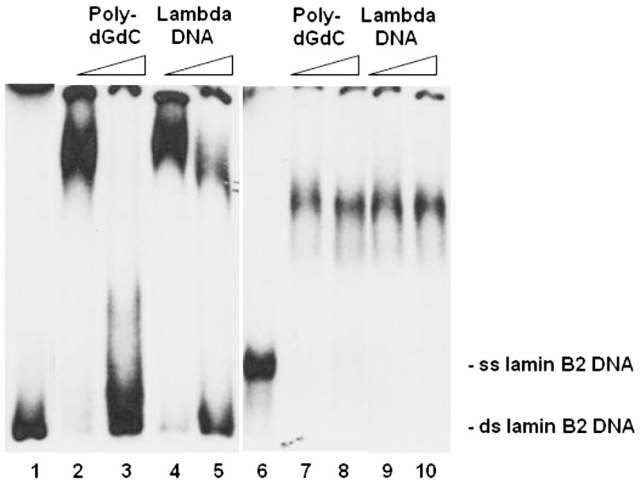

Fig. S6. DNA binding of human Orc6 to radiolabeled dsDNA (lanes 1–5) and ssDNA (lanes 6–10) fragments containing lamin B2 origin was monitored by electrophoretic mobility shift assays. Wild-type Orc6 (50 ng) was incubated with DNA in the presence of increasing amounts of competitor poly(dG-dC) or lambda DNA (100 and 200 ng). No protein was added to DNA fragment in lanes 1 and 6.

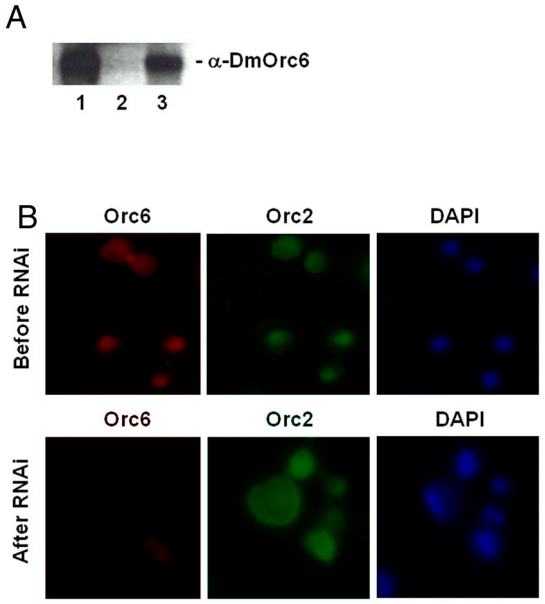

Fig. S7. (*A*) Western immunoblotting analysis of extracts from *Drosophila* cells treated with dsGFP RNA (lane 1) or dsRNA complementary to the 3′UTR of Orc6 (lanes 2 and 3). In lane 3, extract was prepared from cells expressing Orc6 triple amino acid mutant [Orc6 middle region (Orc6-M)] under control of inducible MT promoter. (*B*) Ablation of Orc6 from *Drosophila* cells results in mislocalization of Orc2. Cells were stained with antibodies against *Drosophila* Orc6 and Orc2 proteins before (*Upper*) and after (*Lower*) Orc6 RNAi.

Table S1. BrdU incorporation in *Drosophila* L2 cells expressing GFP-fused Orc6 wild-type and mutants proteins

	pMT-GFP	Orc6-WT	Orc6-E128A	Orc6-H136A	Orc6-R167A	Orc6-3M
GFP	312	518	368	412	318	284
BrdU	21	186	63	87	48	25
BrdU/GFP, %	6.7	36	17.1	21.1	15	8.8

Combined data from three independent experiments are shown. See text for more details. Orc6-3M, Orc6 triple amino acid mutant.