Supplementary Methods

Primers used in the RNAi knockdown experiments. The following pairs of primers were used to amplify dsRNAs of Orc1, Orc4, and Orc6:

Orc1: 5'-CGACTCACTATAGGGAGAATTGTTCAGCAGACTCCCGA-3' and 5'-CGACTCACTATAGGGAGAAGTTCGGATTTGCTGGAATC-3';

Orc4: 5'-CGACTCACTATAGGGAGAGTTACCTGTCGCCTCGACGT-3' and 5'-CGACTCACTATAGGGAGATCTGCACCTTACCTACGCCC-3';

Orc6: 5'-CGACTCACTATAGGGAGAACCGAACTAGTACGCCTCCT-3' and 5'-CGACTCACTATAGGGAGAGCTGGCTATCCATATGCGAT-3'.

Supplementary Figures

Fig. S1. (A) The specificity of antibodies used in the immunostaining experiments was tested by the Western blot analysis of the nuclear extract from *Drosophila* embryos. Lanes treated with the preimmune serum (PI), immune antiserum (Im), and an affinity purified antibody (Ab) against the specified antigen (above the line) are shown in each blot. (B) Embryonic nuclear extract was subjected to several conventional chromatographic steps and then fractionated on a Superose 6 gel filtration column. The fractions were analyzed for the presence of TREX-2 and ORC components by Western blotting with the corresponding antibodies (indicated on the left). Fraction numbers and positions of the molecular weight markers (arrowheads) are indicated on the top. Void volume was eluted in fraction 14. (C) Outputs from the immunoprecipitation experiments are shown in Fig. 2A. The antibodies used in each immunoprecipitation are indicated.

Fig. S2. (A) ORC subunits colocalize with the NPC. Immunostaining of the *Drosophila* S2 cells with the antibodies against Orc3, Orc4, Xmas-2 (red), NPC (green), and the merged images of single cells and fields of cells are shown. (B) Colocalization of Xmas-2 with Orc3, or Orc4 subunits in the S2 cells. Here, and in (C), the recombinant proteins expressed in the cells were stained with the antibodies against FLAG or HA tag. Antibodies used to stain the endogenous proteins are indicated. Single-cell and two-cell images are shown. (C) Colocalization of Nxf1 with Orc3, or Orc4 subunits in S2 cells. The recombinant proteins expressed in the S2 cells were stained with the antibodies against FLAG or HA tag. Antibodies used to stain the endogenous proteins are indicated. The one cell and two cell images are shown. Asterisk indicates the cell that contains no recombinant protein. Scale bar, 10 μm.

Fig. S3. (A) The levels of proteins precipitated in the RNA immunoprecipitations experiments (IP) identified by the Western-blotting. The ratio of the material loaded, to the Input and the IP lanes is 1:10. (B) The level of the studied mRNAs (of ras2, actin, tubulin, and Hpr1) in the original S2 cells, versus the cells subjected to the X-mas2, Orc3, and Orc5 RNAi knock-down. The level of the corresponding RNAs is normalized to that in the original S2 cells. (C) The effect of the RNAi-mediated Hpr1 knockdown on the association of Xmas-2, Thoc5, and ORC subunits with the *ras2* mRNA. The RNA immunoprecipitation was performed on the nuclear fraction of the S2 cells treated with the dsRNA of GFP (control) or dsRNA of the corresponding protein. The results (gray bars) are shown relative to the control RNAi level (black bars). The horizontal gray line shows the level of the Ras2 mRNA coprecipitated with the IgG. The levels of the tested proteins in the control and the RNAi-depleted nuclear extracts are shown below the figure. (D) The effect of the RNAi-mediated Orc3 and Orc5 knockdown on the *ras2* mRNA export. RNA FISH was carried out using an Alexa 488-labeled anti-sense probe to identify *ras2* RNA. The nuclei were stained with DAPI. Representative examples of the stained cells are shown. Bar, 10μm.

Fig. S4. (A) The time course of the newly synthesized mRNA export from the nuclei in the control cells and the cells with the RNAi knockdown of Xmas-2 or Orc5. The S2 cells were incubated with 5-ethynyluridine (EU) for 3 hrs, washed (this point is taken as 0 hrs) cultivated in the medium without EU, and analyzed by the immunostaining at different time points after that. The newly synthesized mRNA was labeled with EU (red staining) as described (77), and the nuclei were stained with DAPI (false-colored green); merged images (EU + DAPI) are also shown. Scale bar, 10 μm. In the control cells, the newly synthesized RNA was initially concentrated in the nuclei but then migrated to the cytoplasm, and almost no cells with a strong nuclear signal were detected at 2 hrs after the labeling. On the other hand, a considerable proportion of cells with the Xmas-2 or Orc5 knocked down still showed a strong nuclear signal at this time point, indicating a block of the mRNA export. Each RNAi knockdown experiment was performed in four replicates, 300 cells were examined in blind count for every replicate, and the mean value was calculated (see Fig. 5D). (B) Percentages of the S2 cells in which mRNA have remained in the nucleus after the RNAi knockdowns of Orc1, Orc4, or Orc6, with the RNAi knockdowns of GFP or Trf2 used as the negative controls. Each RNAi knockdown was performed in four replicates. 300 cells were examined in blind count for every replicate, and the mean value was calculated. **(C)** Percentages of the S2 cells in which mRNA have remained in the nucleus at 2 hrs after the EU labeling, after RNAi knockdowns of Orc1, Orc4, or Orc6, with the RNAi knockdowns of GFP or Trf2 used as the negative controls. Each RNAi knockdown experiment was performed in four replicates, 300 cells were examined in blind count for every replicate, and the mean value was calculated.

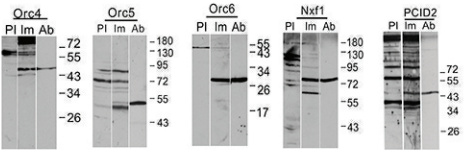
Supplementary Table

Table S1 The list of the most relevant proteins identified by the MALDI-TOF MS analysis. Following a chromatographic purification, the protein complex was precipitated with the antibodies against ENY2 immobilized on the protein A-Sepharose. Next, the proteins eluted from the anti-ENY2--protein A-Sepharose were resolved by the SDS-PAGE and stained with Coomassie. The protein bands were cut out from the gel and subjected to an in-gel trypsin digestion. MALDI-TOF MS was performed using a Bruker Ultraflex II mass spectrometer (Bruker Daltonics, United States). The complete information list for every band is provided in the Supplementary Tables 1-8.

Supplementary Files

Files S1-8 MALDI-TOF MS analysis. The complete information list for every protein band eluted from the SDS-PAGE.

A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55
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Orc5
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43
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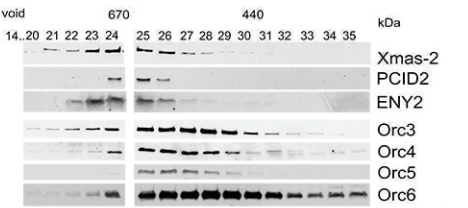
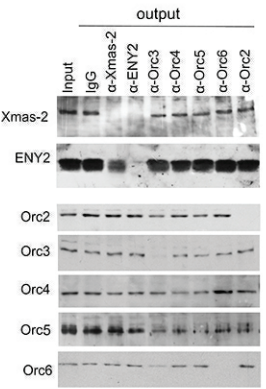
PCID2
Pl Im Ab
72
55
43
34
26

B


void
670
440
kDa
14..20 21 22 23 24
25 26 27 28 29 30 31 32 33 34 35
Xmas-2
PCID2
ENY2
Orc3
Orc4
Orc5
Orc6

C


output
Input
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α-ENY2
α-Orc3
α-Orc4
α-Orc5
α-Orc6
α-Orc2
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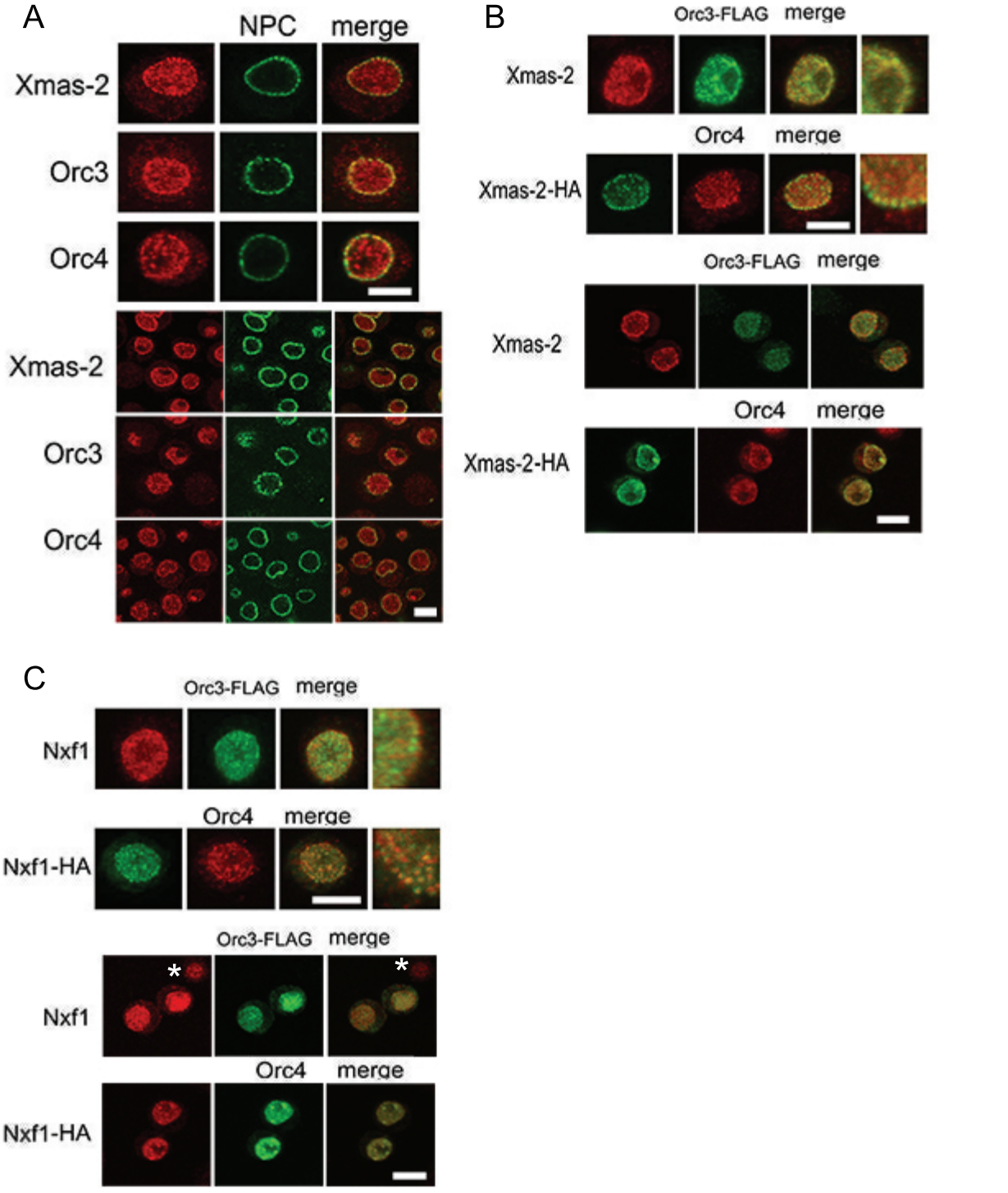
ENY2
Orc2
Orc3
Orc4
Orc5
Orc6

Figure S2


A
NPC
merge
Xmas-2
Orc3
Orc4
Xmas-2
Orc3
Orc4
B
Orc3-FLAG
merge
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Orc4
merge
Xmas-2-HA
Orc3-FLAG
merge
Xmas-2
Orc4
merge
Xmas-2-HA
C
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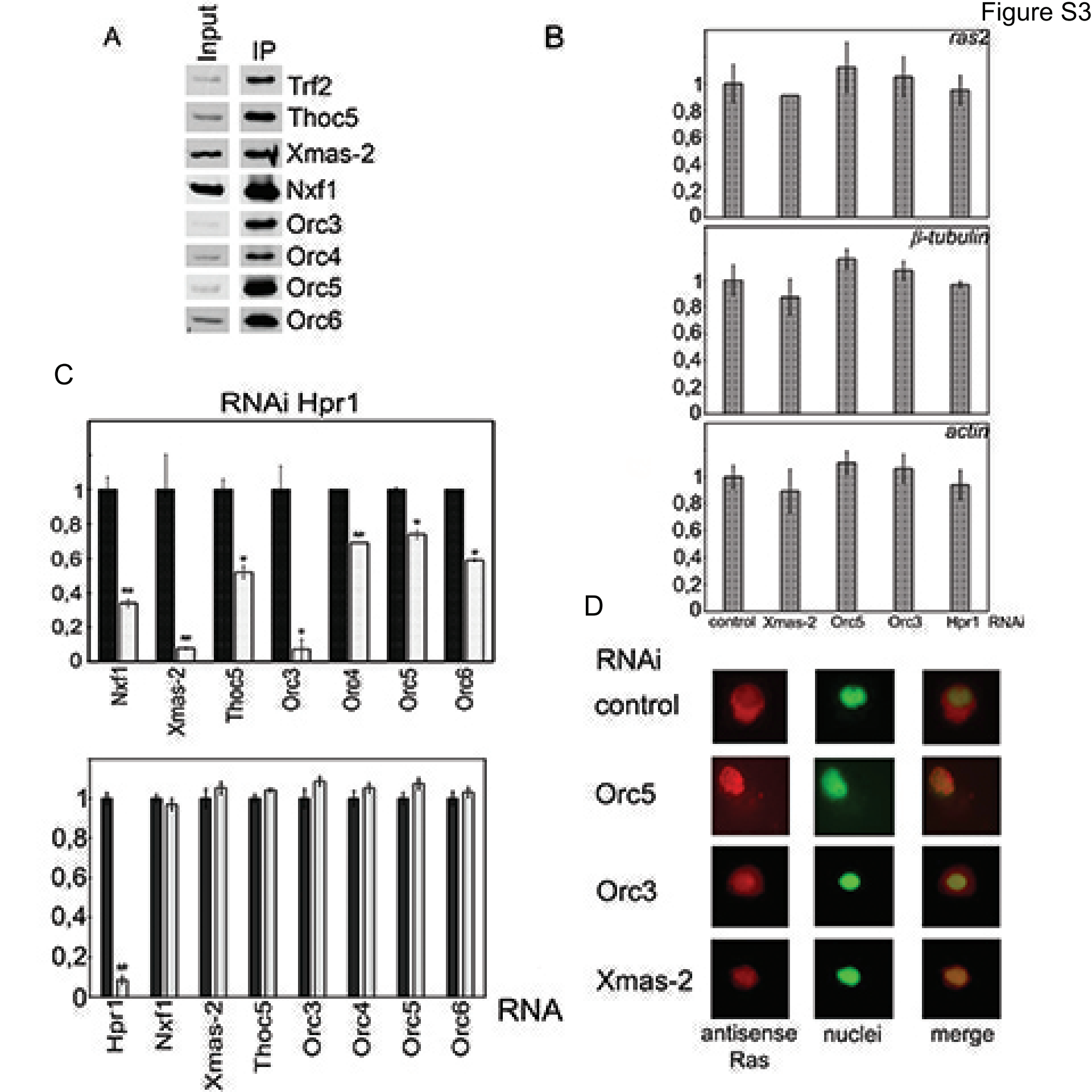
merge
Nxf1-HA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Input
IP
Trf2
Thoc5
Xmas-2
Nxf1
Orc3
Orc4
Orc5
Orc6
B
ras2
β-tubulin
actin
control
Xmas-2
Orc5
Orc3
Hpr1
RNAi
C
RNAi Hpr1
Nxf1
Xmas-2
Thoc5
Orc3
Orc4
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antisense Ras
nuclei
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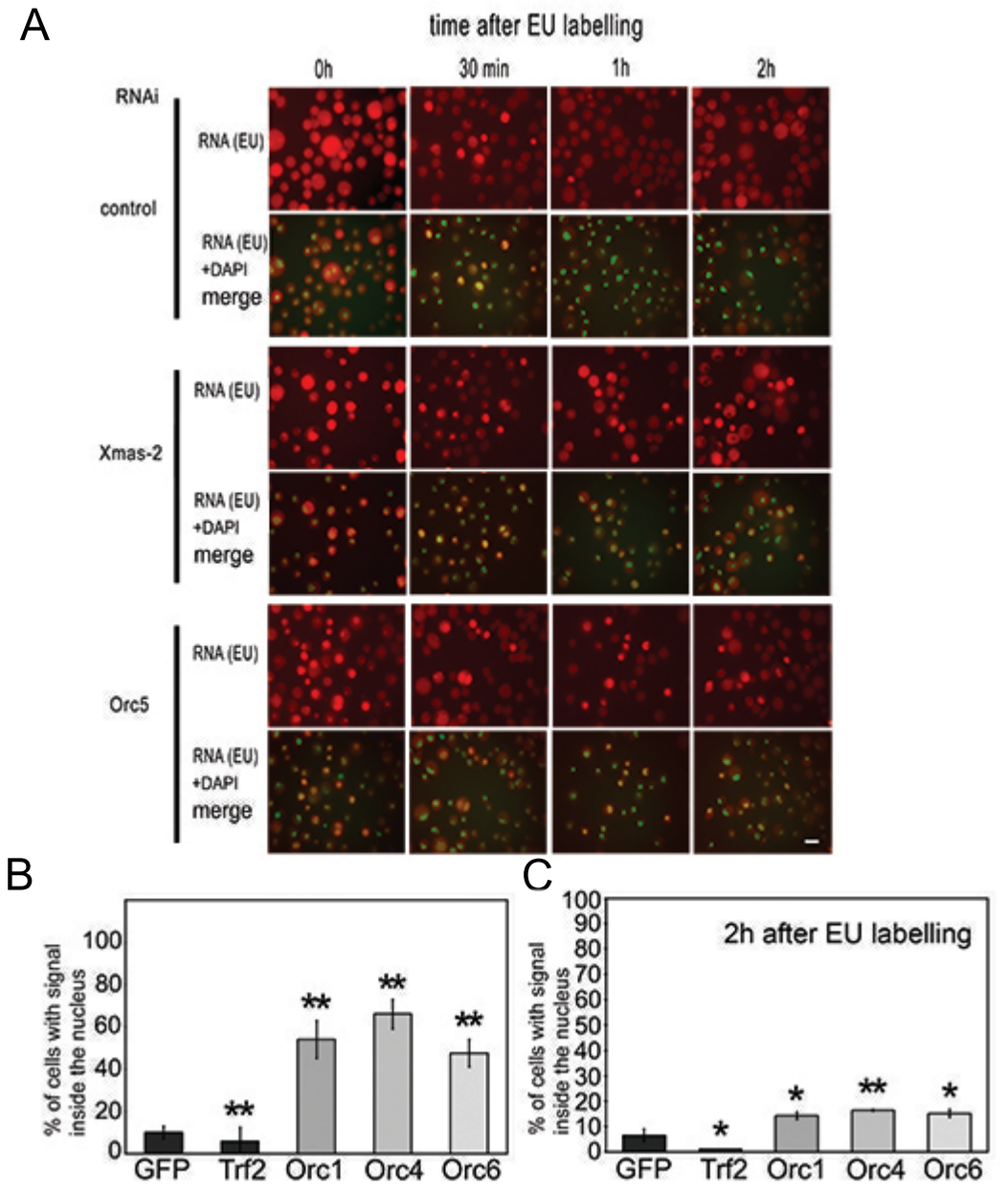
A
time after EU labelling
0h
30 min
1h
2h
RNAi
control
Xmas-2
Orc5
RNA (EU)
RNA (EU)
+DAPI
merge
B
% of cells with signal
inside the nucleus
100
80
60
40
20
0
**
GFP
Trf2
Orc1
Orc4
Orc6
C
2h after EU labelling
% of cells with signal
inside the nucleus
100
90
80
70
60
50
40
30
20
10
0
*
**
GFP
Trf2
Orc1
Orc4
Orc6

CG number	Protein	mass	score	Number of peptides
CG1584-PA	Orc6	29220	128	15
CG7351-PA	PCID2	45150	123	22
CG2917-PA	Orc4	51903	101	27
CG2917-PA	Orc4	51733	91	13
CG2979-PA	Yolk protein 2	51622	67	15
CG7833-PA	Orc5	52082	140	15
CG4088-PA	Orc3	82120	100	13
CG4088-PA	Orc3	82151	100	13
CG10667-PA	Orc1	103217	158	29
CG10667-PA	Orc1	103233	158	29
CG32562-PA	Xmas-2	158081	61	18
CG32562-PA	Xmas-2	158081	147	23
CG32562-PA	Xmas-2	137181	102	18