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Supplemental Information

COPS2 Antagonizes OCT4 to Accelerate the G2/M Transition of Mouse Embryonic Stem Cells

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Running title: *COPS2 promotes the G2/M transition*

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Inventory of Supplemental Information

Supplemental Figures and Legends

Figure S1. COPS2 attenuates the interaction between OCT4 and CDK1 in mouse ESCs. (related to Figure 4A)

Supplemental Tables

Table S1. COPS2 and COPS5 interacting proteins identified by co-IP and mass spectrometric analysis. (Table S1.xlsx, related to Figure 2A and 2B)

Table S2. Proteins interacting with COPS2, but not COPS5. (Table S2.xlsx, related to Figure 2B)

Supplemental Experimental Procedures

Supplemental Figures and Legends

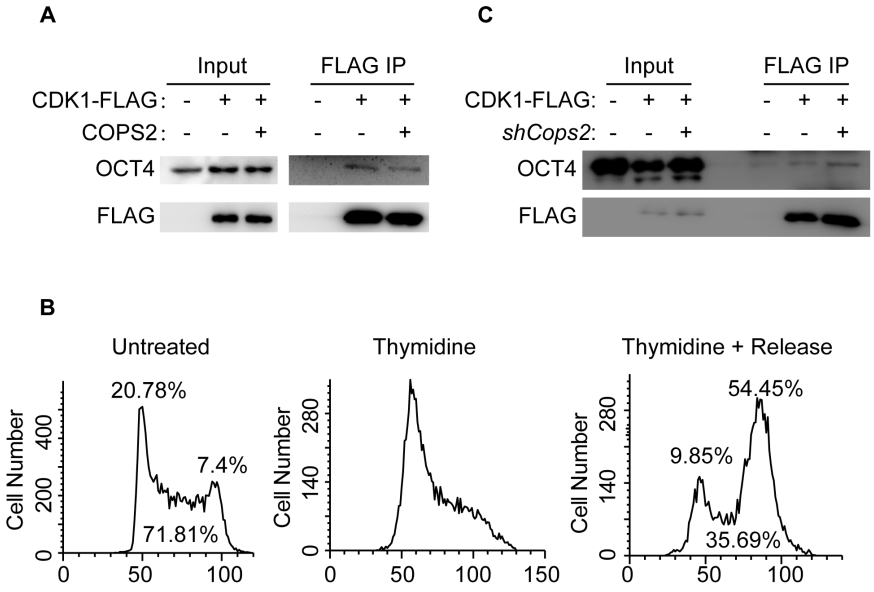

Figure S1. COPS2 attenuates the interaction between OCT4 and CDK1 in mouse ESCs.

(A) Co-IP experiment to detect the interaction between OCT4 and CDK1 in mouse ESCs, with or without COPS2 overexpression. Mouse ESCs expressing CDK1-FLAG were transfected with COPS2 expressing plasmid or empty vector. Forty-eight hours after transfection, cells were harvested for co-IP experiments. (B) To enrich for G2/M cells, mouse ESCs were treated with 2.5 mM thymidine for 14 hours, and then the cells were washed with PBS and cultured in mouse ESC medium for 7 hours. The resulting cells were subjected to cell cycle analysis by flow cytometer. (C) Co-IP experiment to detect the interaction between Oct4 and Cdk1 in G2/M enriched mouse ESCs, with or without COPS2 knockdown. Mouse ESCs expressing CDK1-FLAG were transfected with shRNA plasmids targeting *GFP* or *Cops2*. Fifty-one hours after transfection, cells were treated with 2.5 mM thymidine for 14 hours and released for 7 hours. The resulting G2/M enriched ESCs were collected for co-IP experiments.

Supplemental Experimental Procedures

Cell Culture and Cell Cycle Synchronization

V6.5 ESCs were cultured in medium consisting of 85% Dulbecco's modified Eagle's medium (high glucose DMEM, GIBCO), 15% fetal bovine serum (FBS, Hyclone), 2 mM L-glutamine, 5000 U/ml penicillin and streptomycin, 0.1 mM nonessential amino acids (Invitrogen), 0.1 mM 2-mercaptoethanol (Sigma), and 1000 units/ml LIF (Chemicon). HEK293T and HeLa cells were cultured in DMEM (GIBCO) supplemented with 10% FBS (Hyclone), penicillin and streptomycin (5000 U/ml).

For G2/M phase enrichment, mouse ESCs were treated with 2.5 mM thymidine (Sigma) for 14 hours, washed three times with PBS, and released into fresh medium for 7 hours. According to cell cycle analysis, more than 50% of ESCs were arrested at G2/M phase (Figure S1B).

shRNA knockdown

The shRNA plasmids were constructed using the pSuper-puro system (Oligoengine). The targeting sequence of *Cops2* shRNA was 5'-GTGCATACTGGATAACACTAT-3'.

Transfection

ESCs, HEK293T, and HeLa cells were transfected with plasmids using Lipofectamine 3000 Transfection Reagent (Invitrogen), according to the manufacturer's protocol. At 24 hours after transfection, transfected cells were selected with 1.25 μM puromycin, until cells were harvested.

Western blot

Cells were lysed, and total protein concentration was measured using BCA Protein Assay Kit (Beyotime) to ensure equal loading. Samples were resolved by SDS-PAGE followed by transferring onto a PVDF membrane (Millipore). Membranes were probed with primary antibodies, including anti-FLAG (Sigma, F1804-1 mg), anti-β-TUBULIN (Huada, AbM59005-37B-PU), anti-COPS2 (Bethyl, IHC-00179), anti-OCT4 (Santa Cruz, Santa Cruz, sc-5279), anti-CDK1 (Santa Cruz, 610037), anti-HIS (THETM, A00186), anti-CYCLIN A2 (Abcam, ab181591), and anti-CYCLIN B1 (Abcam, ab181593). Bound primary antibodies were recognized by HRP-linked secondary antibodies (GE Health-care, HA934-1 ML). HRP activity was detected by ECL Plus (Beyotime). Digital images were taken by the automatic chemiluminescence imaging analysis system (Tanon).

Co-immunoprecipitation

Cells were lysed in lysis buffer (20 mM Tris-HCl pH 8.0, 137 mM NaCl, 10% glycerol, 1% NP-40, and 2 mM EDTA) with protease inhibitor (Roche) on ice for 30 minutes. After centrifugation at 17,000 g for 30 minutes, the supernatant was collected and incubated with anti-FLAG M2 magnetic beads (Sigma, M8823-5 mL) at 4°C overnight. The beads were washed three times with lysis buffer, and the bound proteins were released from the beads by boiling in 2×SDS loading buffer for 5 minutes. Western blot was performed to detect the proteins in IP samples.

Recombinant Protein Preparation

Open reading frames of *Cops2* and *Oct4* were inserted to pET28a vector, and transformed into *Escherichia coli* BL21 plus strain. 0.2 mM IPTG was added to induce protein expression for 8 hours at 16°C. Recombinant proteins were purified by Ni-NTA

immobilized metal affinity chromatography (GE-Healthcare) following manufacturer's instructions. Recombinant proteins were concentrated using Vivaspin spin columns (GE-Healthcare), and dissolved in binding buffer (20 mM NaH_2PO_4 [pH=7.4], 500 mM NaCl). Protein concentration was measured by NanoDrop 2000. The proteins in 20% glycerol were stored at -80°C until use.

CDK1 kinase Assay

In vitro CDK1 kinase assays were performed with HeLa cell extract (1 μl, 5 mg/ml), with or without CYCLIN B (300 nM, GeneTex), OCT4 (1800 nM), and/or COPS2 (1800 nM) purified recombinant proteins. Cdk1 kinase activities were measured with the MESACUP® CDC2/CDK1 Kinase Assay Kit (MBL International).