Supporting Information

Prasanth et al. 10.1073/pnas.1009945107

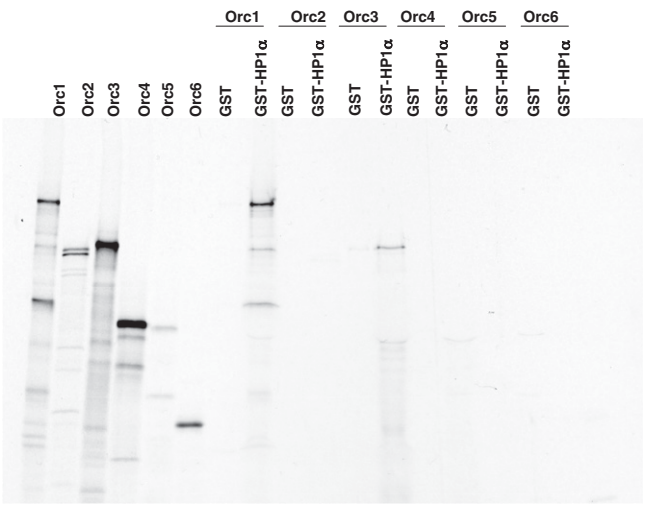

Fig. S1. Origin recognition complex 1 (Orc1) and Orc3 bind to heterochromatin protein 1α (HP1α) directly. GST pull-down assays using GST or GST-HP1α beads and incubating with in vitro transcribed and translated individual ORC subunits show direct binding of human Orc1 and Orc3 to HP1α. Pull down was conducted at 100 mM salt conditions.

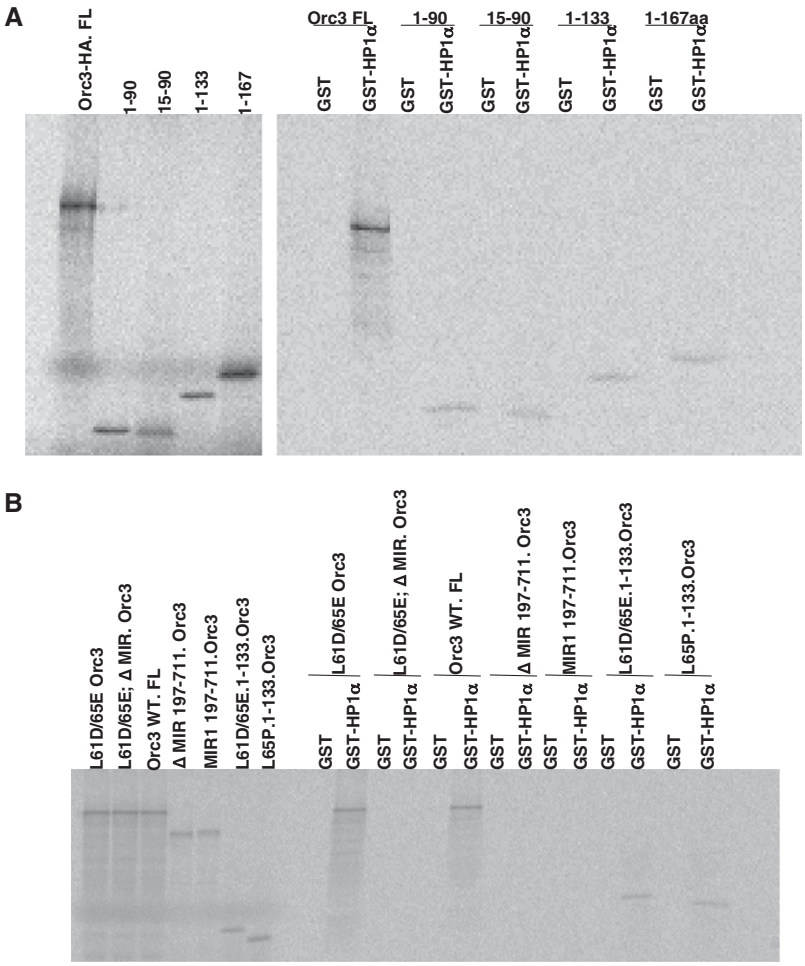

Fig. S2. Mapping the Orc3- and HP1α-interaction domain. Various deletion and mutant Orc3 constructs were in vitro transcribed and translated and used in GST pull-down assays. Note that Orc3 has two HP1α-interacting regions, one between 15 and 90 aa (containing coiled-coil region at the N terminus) and a second one containing the mod-interacting region (MIR) domain.

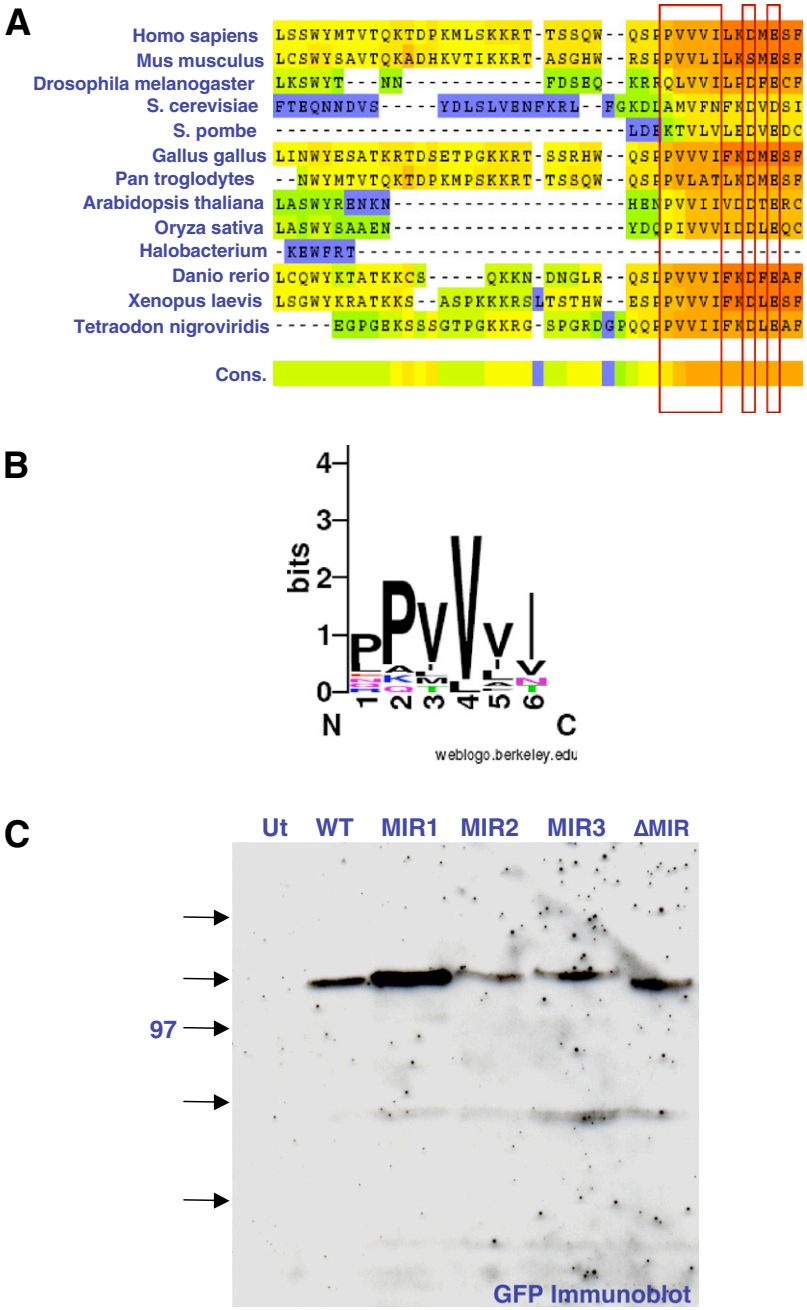

Fig. S3. Orc3 contains a MIR domain. (*A*) Sequence alignment of Orc3 in various species. The blocked region represents the PXVHH consensus of the MIR. Note also the highly conserved residues D and E in proximity to the MIR domain. (*B*) Extent of the conservation of the MIR motif of Orc3. (*C*) Immunoblot of YFP-tagged versions of various MIR mutants using GFP antibody that was expressed in MCF7 cells. Ut, untransfected cells.

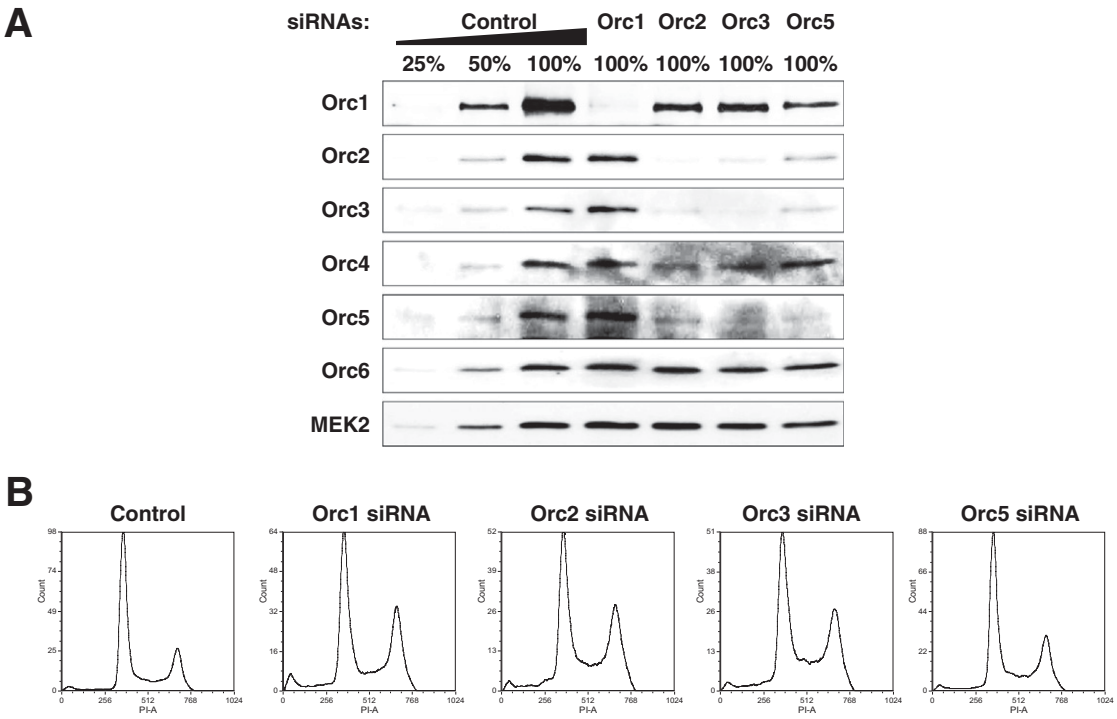

Fig. S4. Effect of depletion of individual ORC subunits on cell-cycle progression. (*A*) Efficiency of knockdown of Orc1, Orc2, Orc3, and Orc5 by immunoblot analysis. Control (luciferase), Orc1 siRNA, Orc2 siRNA, Orc3 siRNA, or Orc5 siRNA were transfected two times into cells, and cell extracts were harvested at 72 h; immunoblots for all six ORC subunits and control microtubule associated protein kinase/Erk kinase were performed. Three different amounts of control siRNA samples were loaded to determine the relative detection efficiency of each antigen in the immunoblot. Orc1 depletion does not alter levels of the other ORC subunits, suggesting that it is behaving differently from the other ORC subunits. Orc2 and Orc3 require each other for their stability. Orc4 and Orc5 levels are reduced on prolonged depletion of Orc2 or Orc3. Similarly, depletion of Orc5 only marginally reduced Orc1 levels in the cells and did not alter Orc6 levels; however, Orc2 and Orc3 levels were reduced but not to the same extent as the use of siRNAs directed against Orc2 and Orc3. (*B*) Flow-cytometric analysis after siRNA treatment for 48 h with siRNA oligonucleotides against luciferase (control), Orc1, Orc2, Orc3, and Orc5 in human HeLa cells. Note the prominent increase in G2/M peak after Orc1 (G2), Orc2 (mitotic increase), and Orc3 depletion.

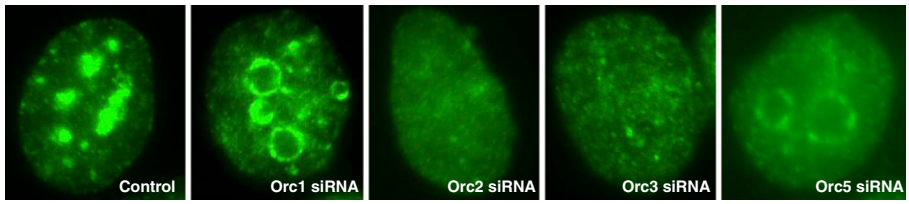

Fig. S5. Depletion of ORC results in abnormal distribution of HP1α protein. Depletion of individual ORC subunits results in aberrant organization of HP1α proteins. Depletion of Orc2 and Orc3 from human HeLa cells results in loss of HP1 from heterochromatic foci and redistribution as homogenous pool. Depletion of Orc1 and Orc5 results in the redistribution of HP1 to the pericentric heterochromatin, mostly around the nucleolar periphery. Some of these represent individual cells from Fig. 4*A* at higher magnification.

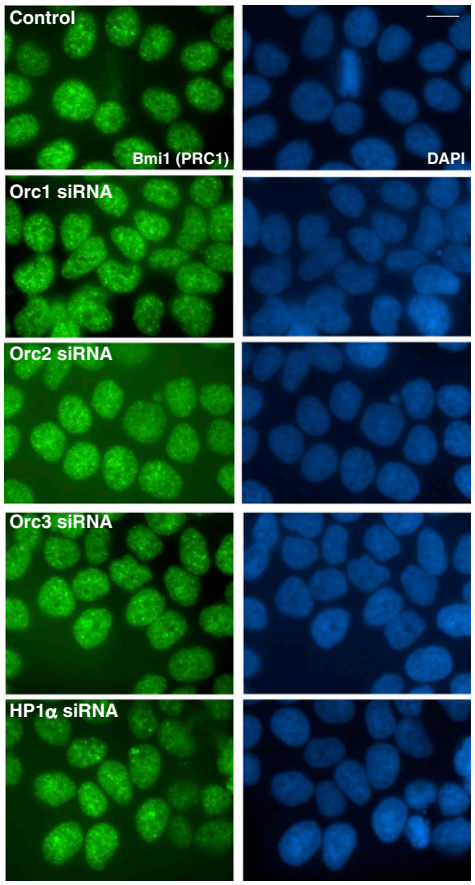

Fig. S6. Distribution of Polycomb-repressive protein Bmi1 in ORC-depleted cells. Depletion of Orc1, Orc2, Orc3, and HP1α from human cells had no change in the distribution of Polycomb-associated Bmi1 (PRC2).

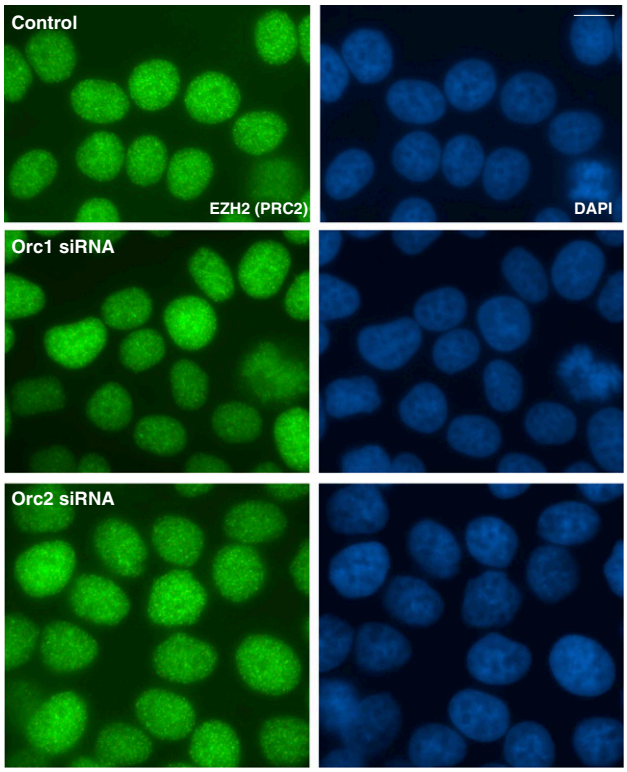

Fig. S7. Distribution of Polycomb-repressive protein EZH2 in ORC-depleted cells. Depletion of Orc1 and Orc2 from human cells had no change in the distribution of Polycomb-associated EZH2 (PRC21).