

B

| | | | | | |
|--|---------|--|--|--|----------|
| <i>Cnot6</i> (+/-) x <i>Cnot6</i> (+/-) | | <i>Cnot6</i> (+/+) | <i>Cnot6</i> (+/-) | <i>Cnot6</i> (-/-) | 13 pairs |
| | 4 weeks | 26 | 41 | 22 | |
| <i>Cnot6</i> l(+/-)/ <i>Cnot6</i> (-/-) x <i>Cnot6</i> l(+/-)/ <i>Cnot6</i> (-/-) | | <i>Cnot6</i> l(+/-)/ <i>Cnot6</i> (-/-) | <i>Cnot6</i> l(+/-)/ <i>Cnot6</i> (-/-) | <i>Cnot6</i> l(-/-)/ <i>Cnot6</i> (-/-) | 17 pairs |
| | 4 weeks | 22 | 53 | 21 | |
| <i>Cnot8</i> (+/-) x <i>Cnot8</i> (+/-) | | <i>Cnot8</i> (+/+) | <i>Cnot8</i> (+/-) | <i>Cnot8</i> (-/-) | 5 pairs |
| | P0 | 11 | 16 | 0 | |
| | 4 weeks | 20 | 18 | 0 | 6 pairs |

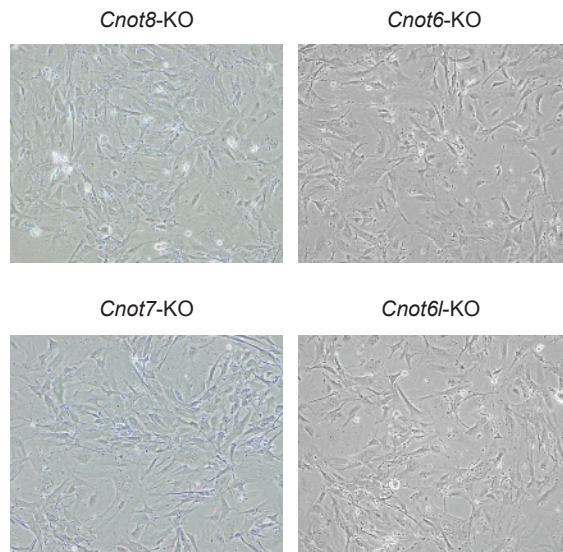
Supplementary Figure 1. Generation of *Cnot6* or *Cnot8* conditional and knockout (KO) mice and viability of their embryos

(A) Schematic representation of wild-type, the targeted, the flox and the KO allele.

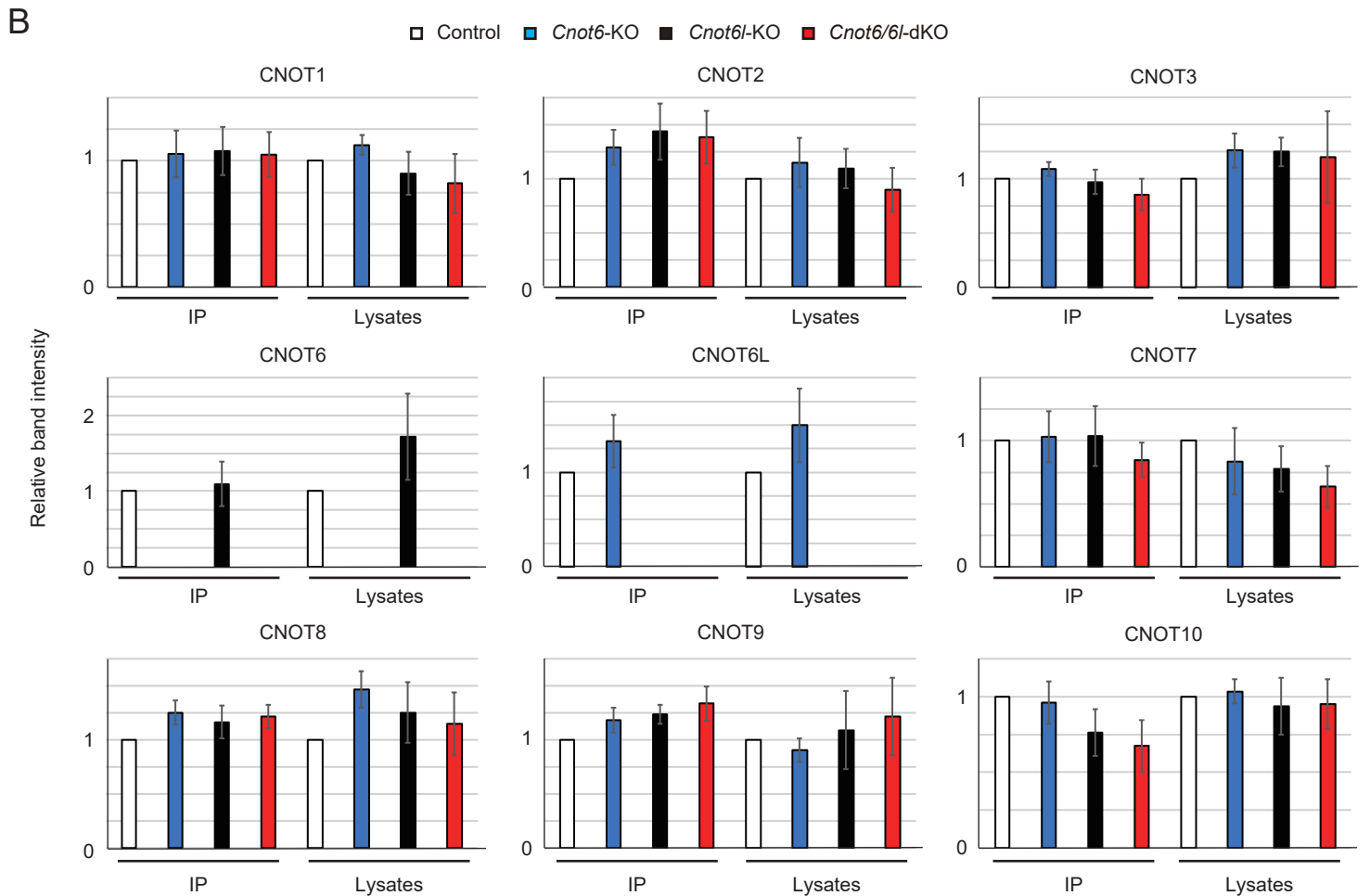
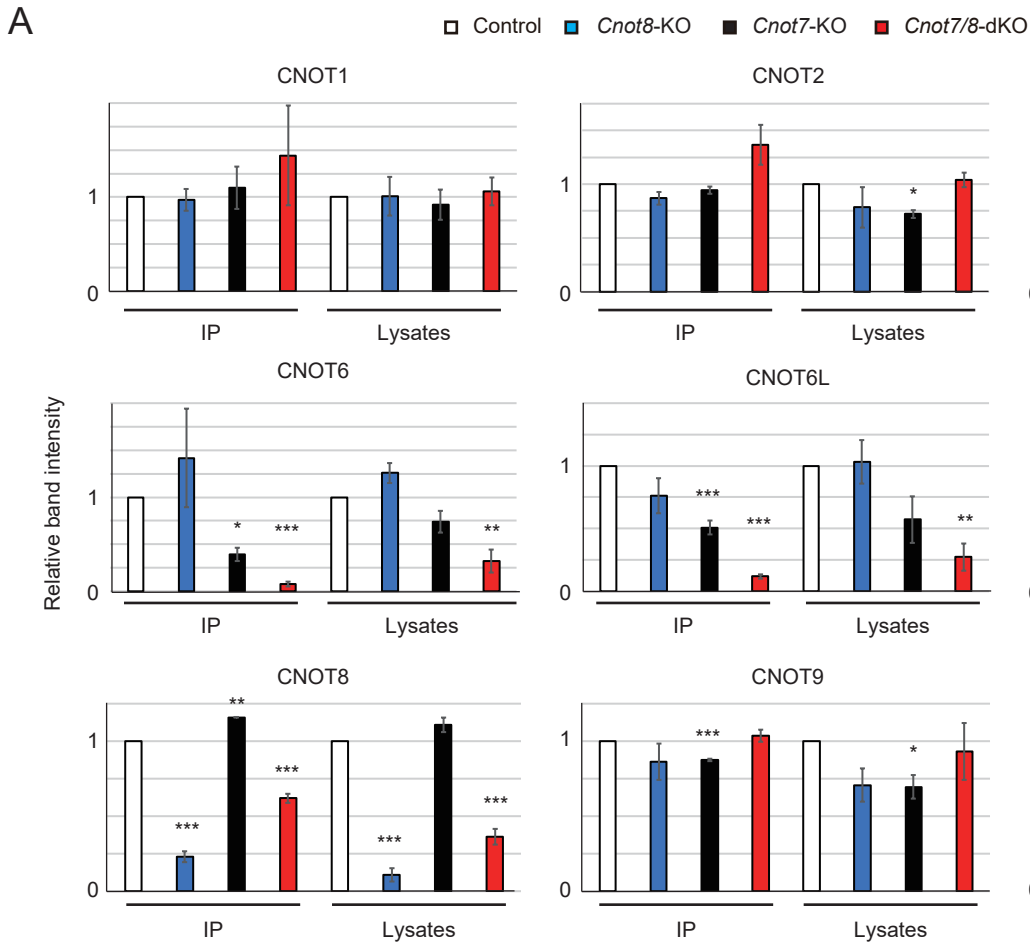
Exons (white boxes), loxp sequences (black triangles), frt sequences (gray triangles), and the neomycin-resistance gene cassette are shown.

(B) *Cnot8*-KO, but not *Cnot6*-KO and *Cnot6/6l*-dKO mice die at early embryonic days.

The tables show the number of embryos and pups obtained from mating of the indicated pairs



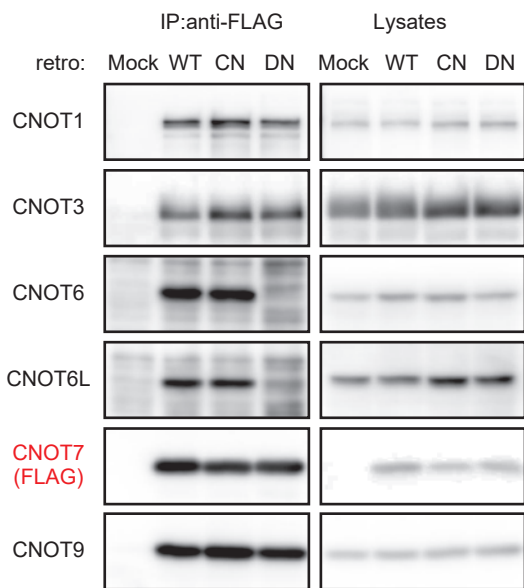
Supplementary Figure 2. Morphology of MEFs lacking *Cnot6*, *6l*, *7* or *8* genes. *Cnot8*-KO and *Cnot7*-KO MEFs represent *Cnot8*-floxed MEFs infected with retrovirus expressing Cre and *Cnot7*-KO/*Cnot8*-floxed MEFs with mock infection, respectively.



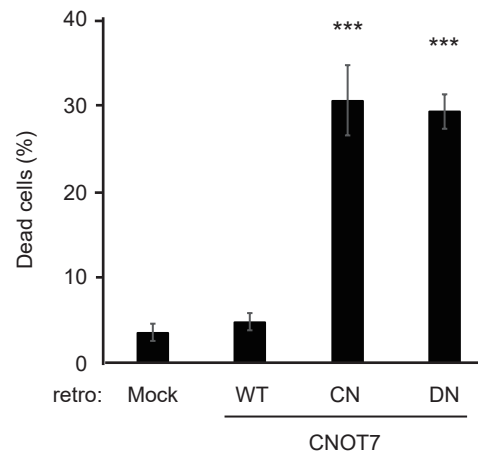
Supplementary Figure 3

(A) Quantification of the data shown in Fig. 2C. Relative band intensities normalized to those of IP or lysates in control MEFs were shown. (B) Quantification of the data shown in Fig. 2D. Relative band intensities normalized to those of IP or lysates in control MEFs were shown. n=3 for each genotype. Values represent mean \pm S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

A



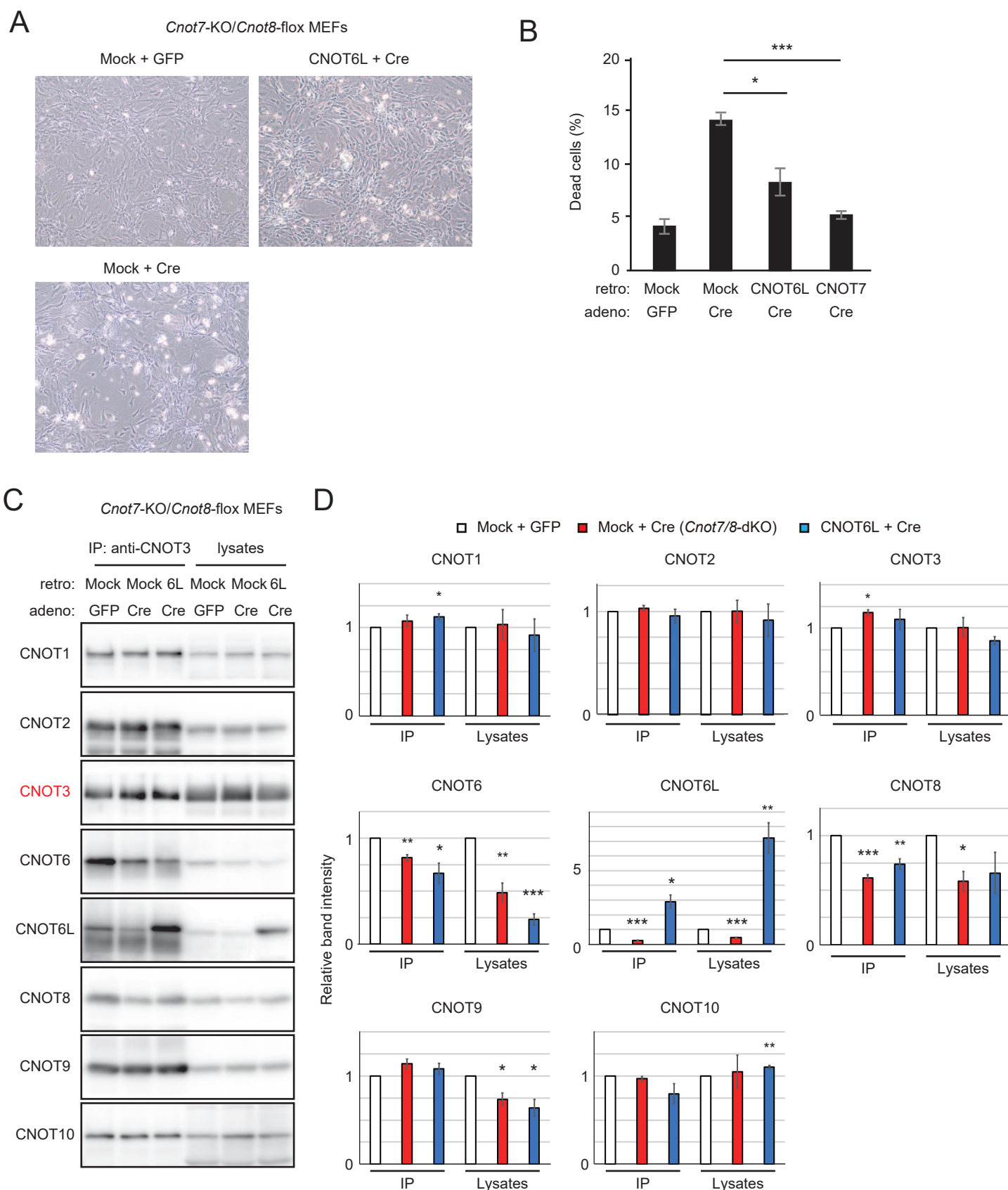
B



Supplementary Figure 4 Characterization of CNOT7 mutants

(A) Lysates were prepared from WT MEFs, which were infected with the indicated retrovirus and subjected to immunoprecipitation with anti-FLAG antibody. Immunoprecipitates (IP) and lysates were analyzed by immunoblot with the indicated antibodies.

(B) Cell death was assessed by propidium iodide uptake using flow cytometry (n=3). Values in the graphs represent mean \pm S.E.M. *** $P < 0.001$.



Supplementary Figure 5. Exogenous CNOT6L expression partly recovers viability of *Cnot7/8*-dKO MEFs

(A) Morphology of *Cnot7*-KO/*Cnot8*-floxed MEFs infected with the indicated (retro + adeno) viruses.

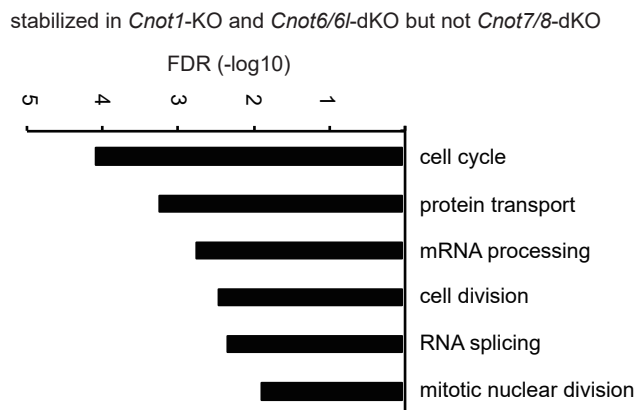
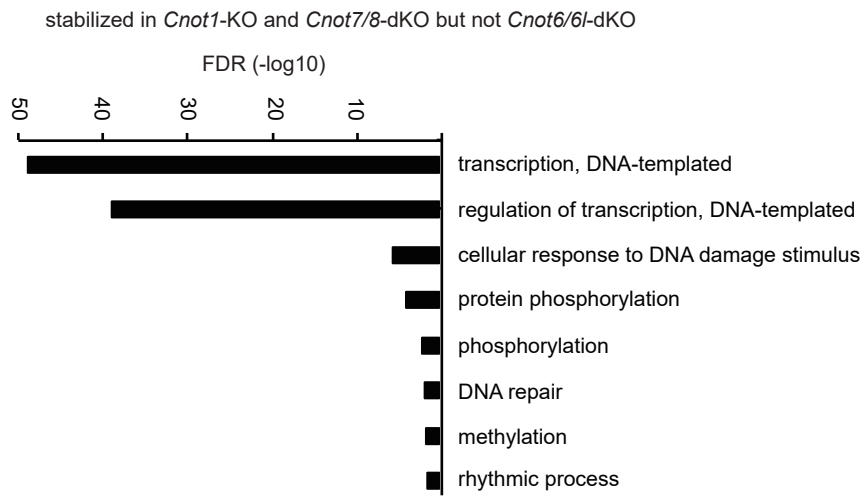
(B) Cell death was assessed as in Fig. 1C (n=3). Values represent means \pm S.E.M.

Reintroduction of CNOT7 was also performed to compare roles in cell viability between CNOT6L and CNOT7.

(C) Lysates were prepared from *Cnot7*-KO/*Cnot8*-floxed MEFs infected with the indicated viruses and subjected to immunoprecipitation with anti-CNOT3 antibody. Lysates and IP were analyzed by immunoblot with the indicated antibodies.

(D) Quantification of the immunoblot data in Supplementary Fig. 5C. Relative band intensities normalized to those of IP or lysates in control MEFs were shown (n=3). Values represent means \pm S.E.M.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.



Supplementary Figure 6

GO analysis of mRNAs stabilized in *Cnot1*-KO and *Cnot7/8*-dKO but not *Cnot6/6l*-dKO (upper), or *Cnot1*-KO and *Cnot6/6l*-dKO but not *Cnot7/8*-dKO MEFs (lower).

Bar charts of the GO terms (Biological Process) ranked by FDR (< 0.05) are shown.

Gene lists included in each GO term are summarized in Supplementary table 1.