PNAS

Supporting Information for
Redox states in the endoplasmic reticulum directly regulate the activity of calcium channel, inositol 1,4,5-trisphosphate receptors

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This PDF file includes:

Figures S1 to S3

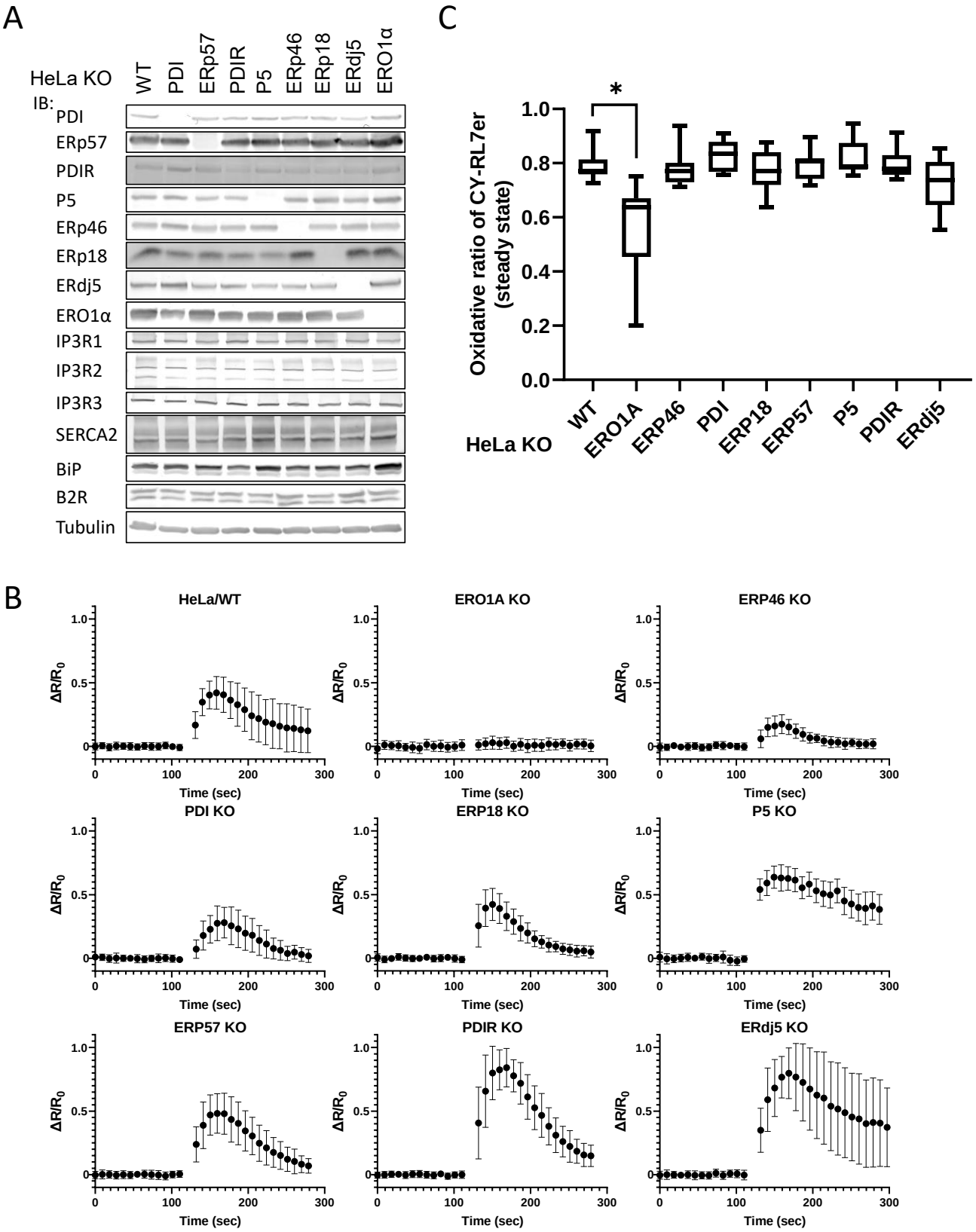

Fig. S1. The establishment of PDI gene-deficient HeLa cells.

(A) To confirm the expression of the indicated proteins in each cell line, proteins in cell lysates were separated by SDS-PAGE, and immunoblotting was performed with each antibody.

(B) Effect of PDIs KO on BK-induced Ca^{2+} release. Each tracing represents the mean ± SD.

(C) Measurement of the relative oxidative ratio in the ER using CY-RL7er, a Forster resonance energy transfer (FRET)-based redox imaging sensor. Steady-state redox conditions in the indicated knockout (KO) cell lines were determined by the titration of sequential treatments with diamide, an oxidizing agent, and dithiothreitol (DTT), a reducing agent. The horizontal line within the box represents the median value, the upper and lower edges of the box represent 75 and 25% values, and the whiskers represent the total range. *, $P < 0.05$.

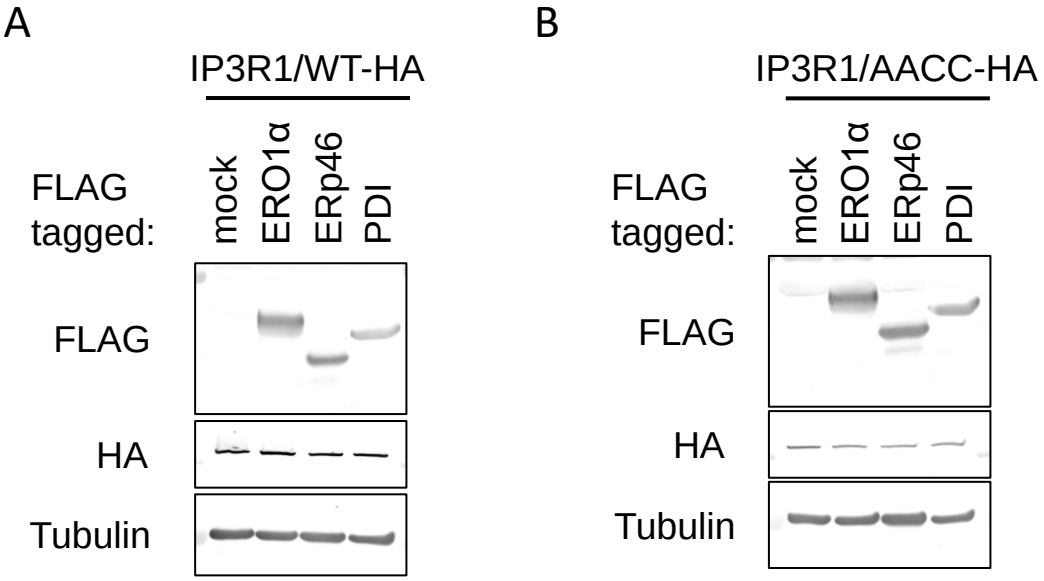

Fig. S2. The expression of IP3R1 was unaffected by the overexpression of the indicated oxidases. (A, B) Forty-eight hours after transfection of FLAG-tagged oxidases with Yellow Cameleon 3.6 (YC3.6) into hemagglutinin (HA)-tagged stably expressing inositol 1,4,5-trisphosphate receptor 1 (IP3R1)/WT (A) or AACC mutant (B) cells, the proteins in cell lysates were separated by SDS-PAGE and immunoblotted with each antibody.

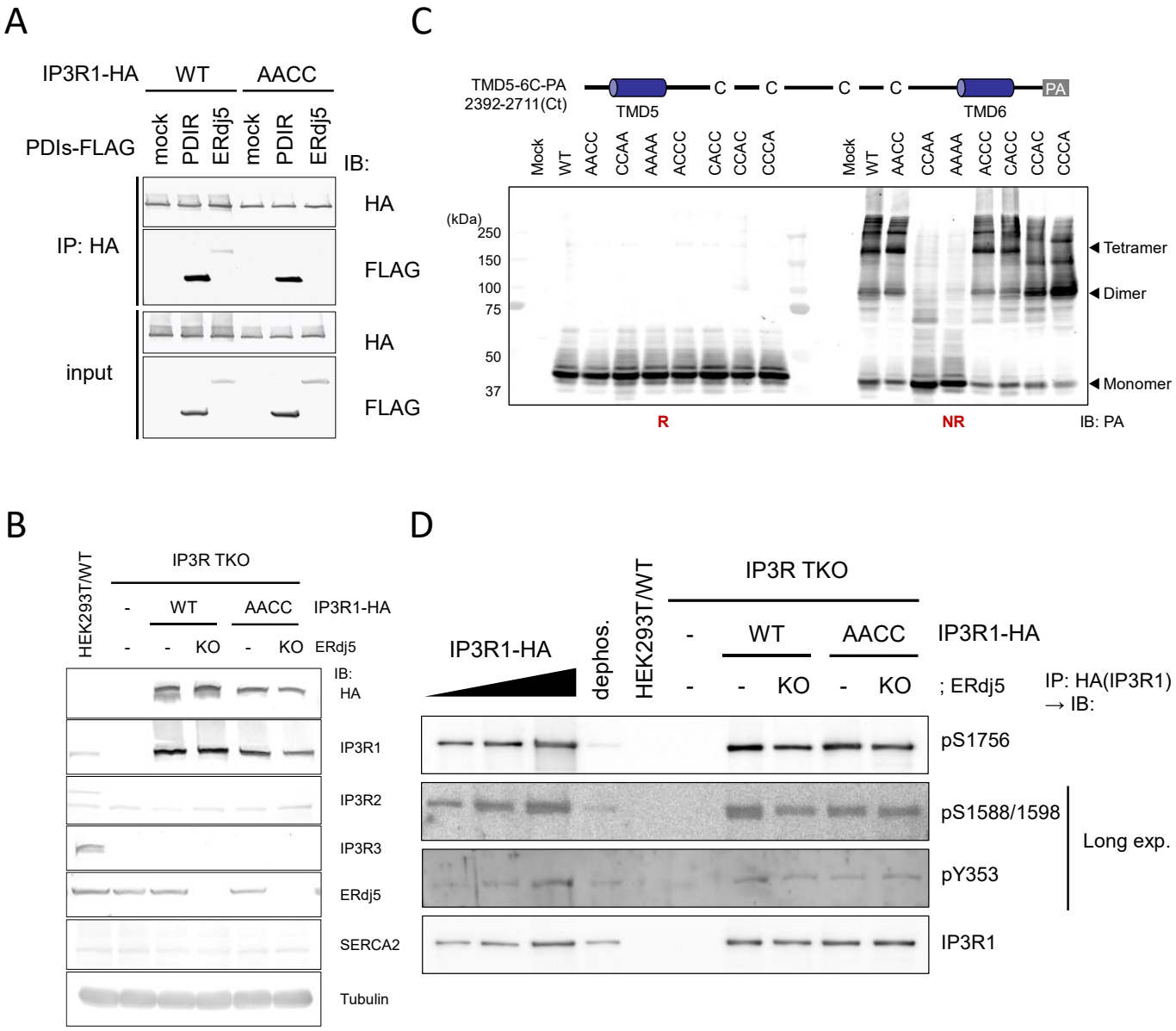

Fig. S3. The establishment of ERdj5 deficient IP3R1-expressing cells and an expression system for IP3R1 truncation mutants.

(A) After the transfection of indicated FLAG-tagged PDIs, immunoprecipitates were prepared from the lysates of (HA)-tagged stably expressing inositol 1,4,5-trisphosphate receptor 1 (IP3R1) cells and detected by immunoblotting using the antibodies against the indicated peptide tags.

(B) To confirm the expression of the indicated proteins in each cell line, the proteins in the cell lysates were separated by SDS-PAGE and immunoblotted with each antibody.

(C) Truncated mutants of IP3R1 were expressed in HEK-293T cells. Proteins in the cell lysates were separated by reducing (R) or nonreducing (NR) SDS-PAGE and immunoblotted.

(D) To detect the phosphorylation of IP3R1 in each cell line, immunoprecipitates were separated by SDS-PAGE and immunoblotted with indicated antibodies. dephos.; Lambda PP treated IP3R1-HA