Expanded View Figures

Figure EV1. The complex comprising FTCD, p47, and p97.

A Binding of p97 to p47 enhances the binding affinity of p47 to FTCD. His-tagged FTCDwt was biotinylated using EZ-Link Sulfo-NHS-LC-biotin and bound to Dynabeads M280 Streptavidin to generate FTCD-beads. The FTCD-beads (1.0 μg) were incubated in the presence of AMP-PNP (1 mM) together with the indicated proteins (p97wt, 2.0 μg; p97(V722k, E724K), 2.0 μg; p47wt, 1.0 μg; p47(E86K, E88K), 1.0 μg) in buffer (100 mM KCl, 20 mM Hepes, 1 mM $MgCl_2$, 1 mM DTT, 0.5% Triton X-100, 10% glycerol, pH 7.4). The blots were probed with HRP-conjugated avidin and antibodies to p97 and p47. The amount of p97 bound to the FTCD-beads was not changed in the presence of p47(E86K, E88K), which lacked binding affinity to FTCD (upper panel, lane 2). In contrast, the amount of p47 bound to the FTCD-beads was increased in the presence of p97(V722k, E724K), which lacked binding affinity to FTCD (middle panel, lane 4). These results indicate that the binding of p97 to p47 enhances the binding affinity of p47 to FTCD.

B Localization of FTCD. GM130, a cis-Golgi marker, and FTCD were visualized as described in Fig 3A. HA-tagged NAGT I, a medial/trans-Golgi marker, and HA-tagged SialylT, a trans-Golgi/TGN marker, were expressed in HepG2 cells and visualized with anti-HA antibodies after fixation. Arrowheads indicate differences in the staining patterns. Scale bar = 5 μm.

C Depletion of p47 by p47 siRNA duplexes. HepG2 cells were either mock transfected with water or transfected with siRNA duplexes specific to human p47. After incubation for 48 h, the cells were analyzed by Western blotting with antibodies to p47 and α-tubulin.

D Isolation of the complex comprising p97, p47, and FTCD. The p97/p47 complex was incubated with an excess amount of FTCD in the presence of 1 mM AMP-PNP, and the complex comprising p97, p47, and FTCD was isolated using 5%-40% sucrose gradient centrifugation. Proteins in the fractions were separated by SDS–PAGE and stained with Coomassie Brilliant Blue (upper panel). As a control, FTCD alone was used (lower panel). The amounts of p97, p47, and FTCD in fraction no. 18 (indicated by the asterisk) were analyzed by a densitometer and used for the stoichiometric estimation of the complex. The results demonstrated a ratio of p97 hexamer:p47 trimer:FTCD octamer = 1:1:1.8, indicating that each complex comprises a p97 hexamer, a p47 trimer and two FTCD octamers or a p97 hexamer, a p47 trimer and one FTCD octamer. This is consistent with the results from the negative staining EM (Fig 7D).

E p97/p47-mediated aggregation of FTCDwt-beads is nucleotide-dependent. FTCDwt-beads were incubated together with p97 (0.1 μg/μl) and p47 (0.1 μg/μl) in the presence of the indicated nucleotides (1 mM). Scale bar = 50 μm. The results of quantification are presented in Fig 7G.

F p47 and p97 mutants that lack binding affinity inhibit p97/p47-mediated aggregation of FTCDwt-beads. FTCDwt-beads were incubated with p97wt/mutant (0.1 μg/μl) and p47wt/mutants (0.1 μg/μl) in the presence of AMP-PNP. Scale bar = 50 μm. The results of quantification are presented in Fig 7H.

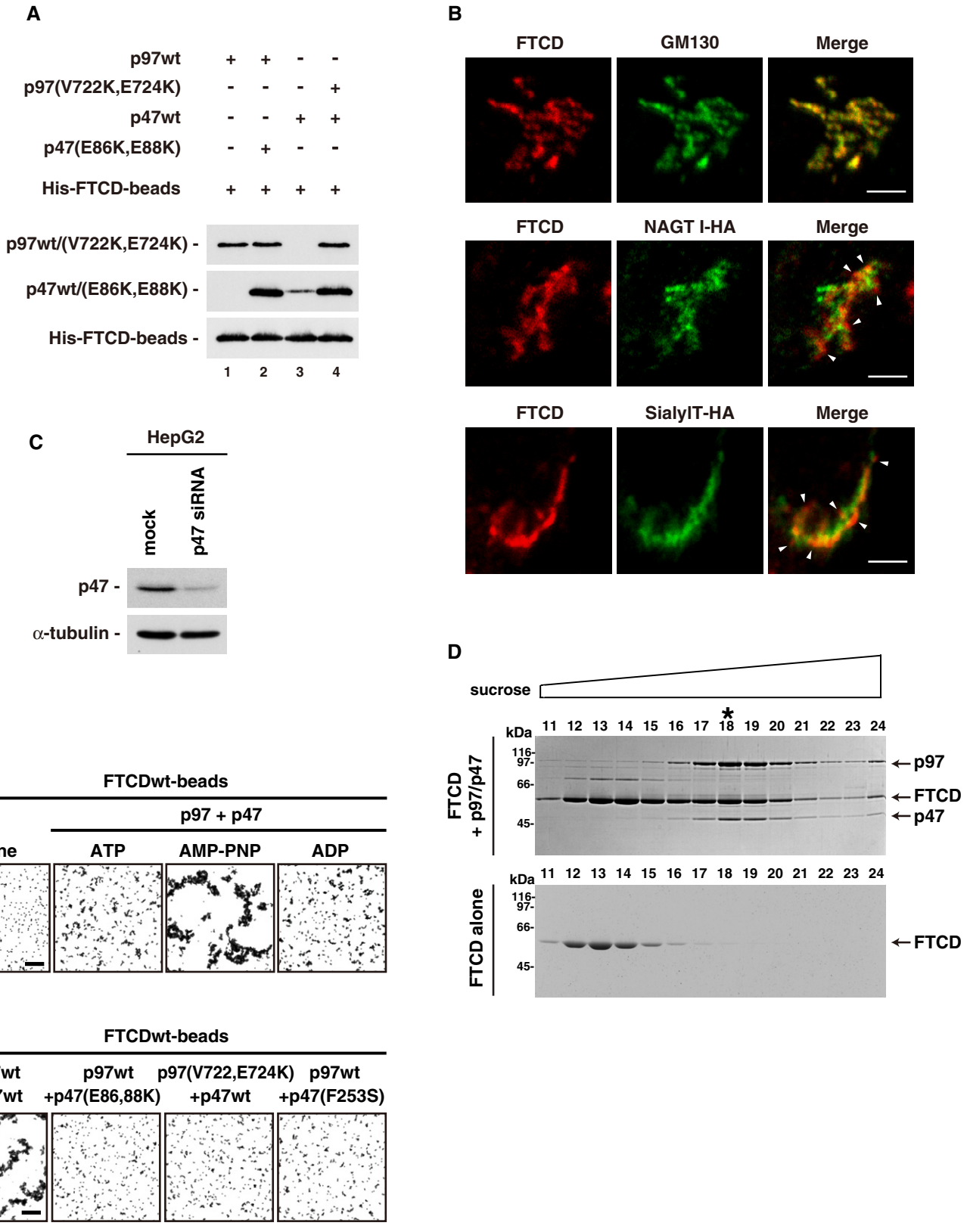

Figure EV1.

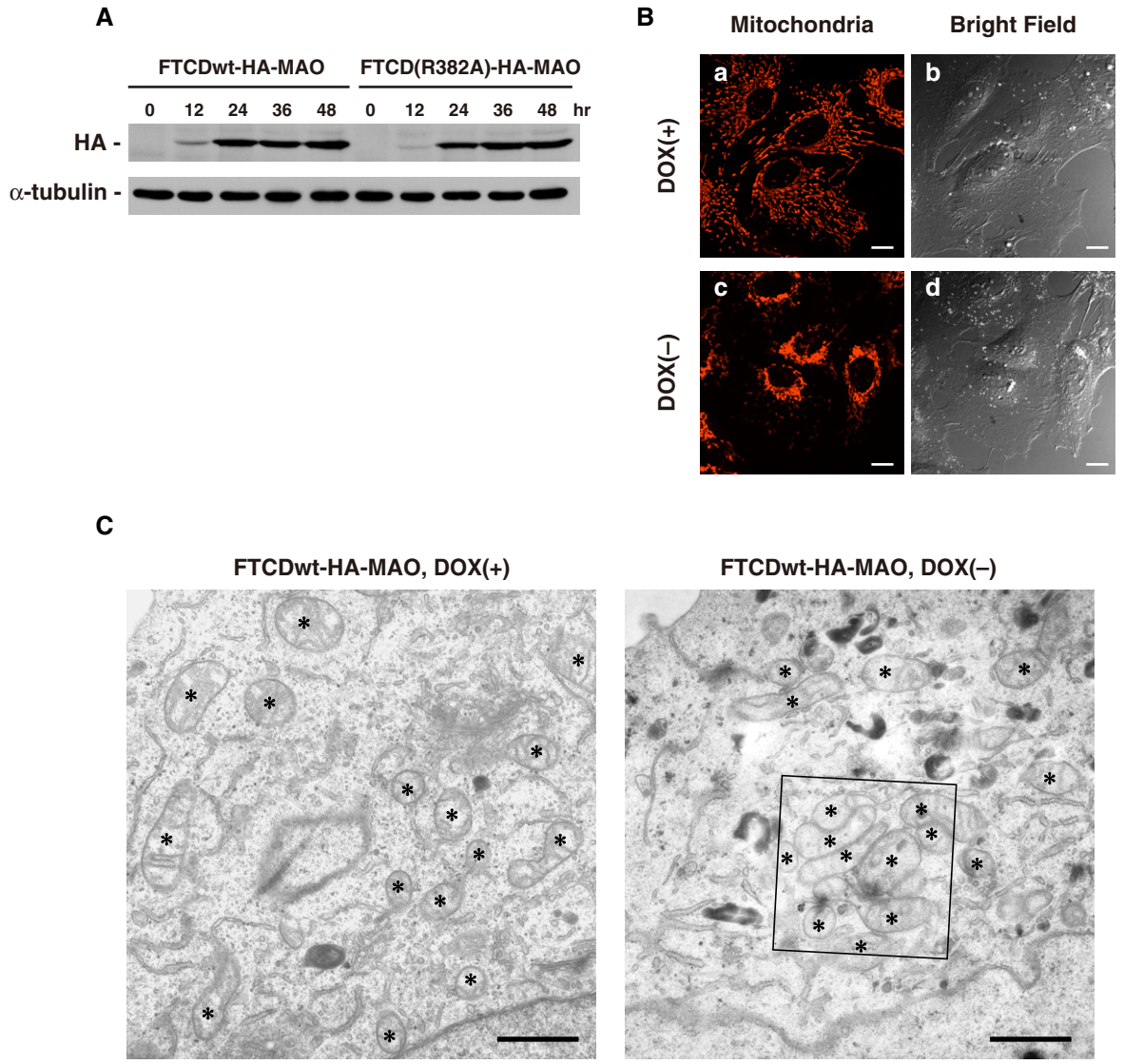

Figure EV2. HeLa Tet-off cells inducibly expressing either FTCDwt-HA-MAO or FTCD(R382A).

A Time course of the expression of either FTCDwt-HA-MAO or FTCD(R382A)-HA-MAO in Tet-off HeLa cells. HeLa Tet-off cells inducibly expressing either FTCDwt-HA-MAO or FTCD(R382A)-HA-MAO were cultured in DOX-free medium for the indicated times. The cells were trypsinized immediately before the medium change. Their expression levels were determined by Western blotting with antibodies to their HA-tags. The expression level was very low at the 12-h time point and reached a maximum at the 24-h time point.

B Images of cells expressing FTCDwt-HA-MAO. The HeLa Tet-off cells inducibly expressing FTCDwt-HA-MAO were cultured for 48 h in the presence or absence of DOX. Living cells were stained with MitoTracker for the visualization of the mitochondria and observed by confocal microscopy and differential interference contrast microscopy. The cells were incubated in a stage top CO2 incubator during the observation. Scale bar = 10 μm. The expression of FTCDwt-HA-MAO had no effect on the size of the cell, but caused mitochondria aggregation. Mitochondria were aggregated around the nucleus and were rarely observed in the peripheral region of cells.

C Representative EM images of a cell expressing FTCDwt-HA-MAO. The HeLa Tet-off cells inducibly expressing FTCDwt-HA-MAO were cultured for 48 h in the presence or absence of DOX and used for EM observation. The asterisks show mitochondria. Scale bar = 1 μm. The inset is presented in Fig 8C (panel a).

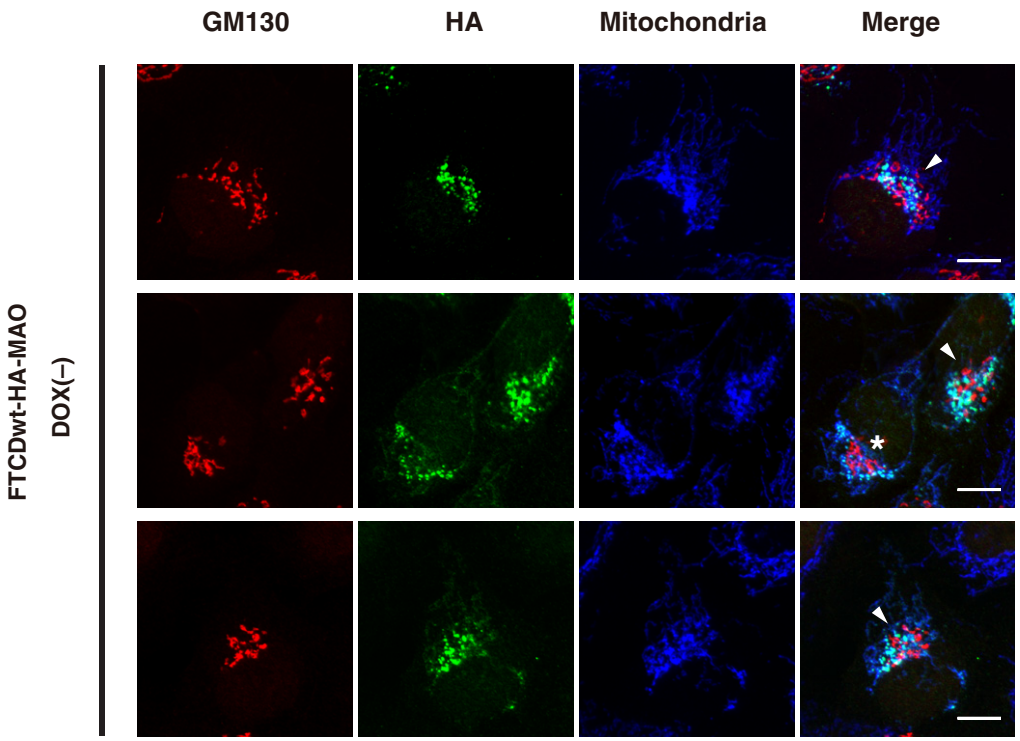

Figure EV3. Aggregated mitochondria were located close to the Golgi and were partially intermixed with the Golgi in the *in vivo* mitochondria aggregation assay.

HeLa Tet-off cells inducibly expressing FTCDwt-HA-MAO were cultured in DOX-free medium for 24 h. The cells were fixed, stained with a monoclonal antibody to mitochondria and polyclonal antibodies to GM130 and HA, and observed by confocal microscopy. Scale bar = 10 μm. The asterisk shows that aggregated mitochondria surround the Golgi. The arrowheads indicate aggregated mitochondria, which are partially intermixed with the Golgi, and consequently, the Golgi is somewhat dispersed.

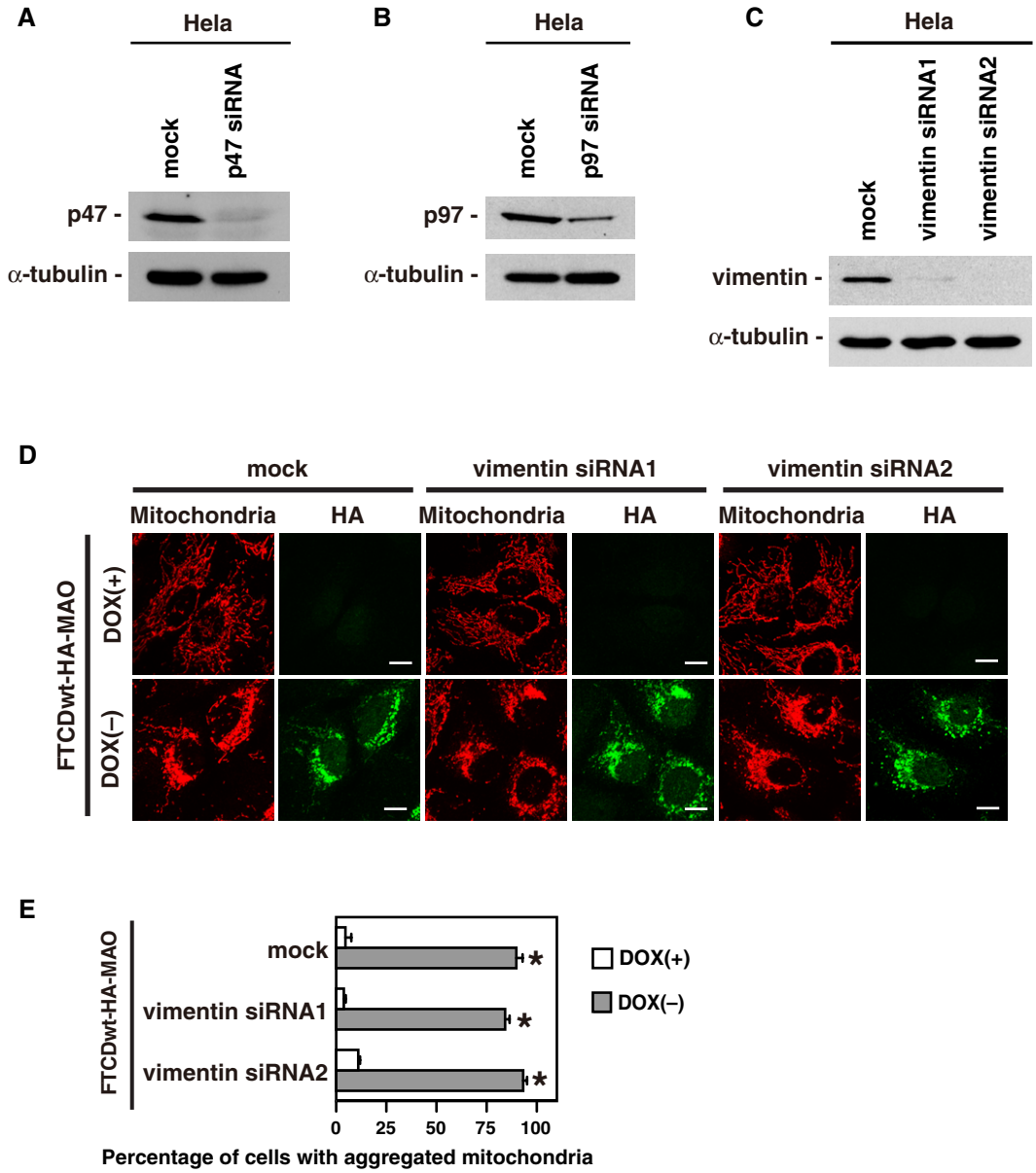

Figure EV4. The siRNA treatment specific to p47, p97, and vimentin in the mitochondria aggregation assay.

A Depletion of p47 by p47 siRNA duplexes. HeLa cells were either mock transfected with water or transfected with siRNA duplexes specific to human p47. After incubation for 48 h, the cells were analyzed by Western blotting with antibodies to p47 and α-tubulin.

B Depletion of p97 by p97 siRNA duplexes. HeLa cells were either mock transfected with water or transfected with siRNA duplexes specific to human p97. After incubation for 48 h, the cells were analyzed by Western blotting with antibodies to p97 and α-tubulin.

C Depletion of vimentin by vimentin siRNA duplexes. Human vimentin was targeted with the following independent siRNAs: 5′-CUGAGUACCGGAGACAGGUdTdT-3′ (vimentin siRNA1) and 5′- GGAUUCACUCCCUCUGGUUdTdT-3′ (vimentin siRNA2). HeLa cells were either mock transfected with water or transfected with siRNA duplexes (2.5 nM) specific to human vimentin. After incubation for 48 h, the cells were analyzed by Western blotting with antibodies to vimentin (V9, Sigma) and α-tubulin.

D Endogenous vimentin is unnecessary for mitochondria aggregation mediated by FTCDwt-HA-MAO. HeLa Tet-off cells inducibly expressing FTCDwt-HA-MAO were transfected with either mock, vimentin siRNA1, or vimentin siRNA2 duplexes and cultured for 24 h. The cells were further cultured in DOX-free medium for 48 h for the induction of FTCDwt-HA-MAO and then analyzed as in Fig 8D. Scale bar = 10 μm.

E The results of quantification of (D). Results are shown as the mean ± SD of five sets of independent experiments, with 100 cells counted in each group in each independent experiment. Asterisks indicate a significant difference at $P < 0.01$ compared with each DOX(+) group (Bonferroni method).

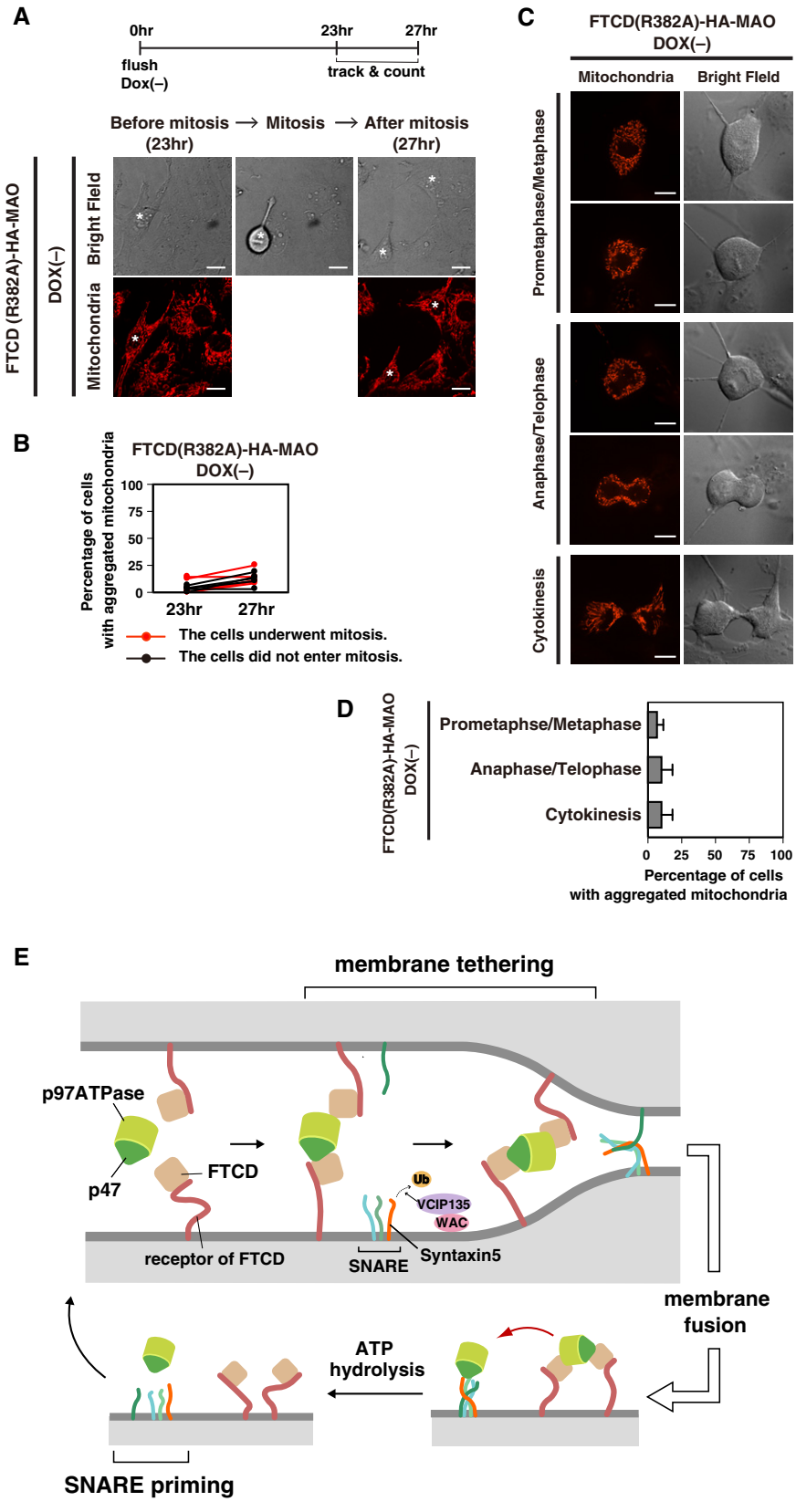

Figure EV5. FTCD(R382A)-HA-MAO expression did not cause mitochondria aggregation during mitosis.

A A single living cell expressing FTCD(R382A)-HA-MAO was tracked during mitosis. Mitotic cells were collected by flushing from HeLa Tet-off cells inducibly expressing FTCD(R382A)-HA-MAO and cultured in DOX-free medium. The cells were stained with MitoTracker at the 22-h time point, and confocal images of 30–40 cells were randomly taken at the 23-h time point. From the 23- to 27-h time point, cells were tracked in the bright field every 30 min. At the 27-h time point, the confocal images of the 23–39 cells that were successfully tracked were taken. Asterisks indicate the tracked cells. Scale bar = 10 μm.

B The results of quantification of (A). The experiment was repeated five times and each line shows the average percentage of an independent experiment.

C In the FTCD(R382A)-HA-MAO-expressed cells, mitochondria aggregation was rarely observed during mitosis. Mitotic cells were collected from HeLa Tet-off cells inducibly expressing FTCD (R382A)-HA-MAO and cultured in DOX-free medium. The cells were stained with MitoTracker at the 23-h time point and observed by confocal microscopy from the 24- to 26-h time point. Scale bar = 10 μm.

D The results of quantification of (C). Cells were classified by their cell cycle phases into the following three categories: prometaphase/metaphase, anaphase/telophase, and cytokinesis. The values are from three sets of independent experiments. The results are shown as the mean ± SD of three sets of independent experiments, with 10 cells counted for each category in each independent experiment.

E The p97/p47 complex plays the dual roles of membrane tethering and SNARE priming in the membrane fusion process. Prior to membrane fusion, the p97/p47 complex tethers two equal Golgi fragments by forming FTCD-p97/p47-FTCD complex. After membrane fusion, the tethering complex is dissociated and then the p97/p47 complex is utilized for SNARE priming.