

**Fig. S1.** Intercellular transfer of mitochondria-related vesicles between A11 and P29 cells. mtGFPlabeled P29 cells (P29-mtGFP) and MTDR-labeled A11 cells (A11-MTDR) were cocultured for 24 h. Scale bars: 20  $\mu$ m.

## A11-MTDR



**Fig. S2.** Intercellular transfer of mitochondria-related vesicles between A11 cells and MEF cells. MTDR-labeled A11 cells (A11-MTDR) and mtGFP-labeled MEF cells (MEF-mtGFP) were cocultured for 24 h. Scale bars: 20  $\mu$ m.

## A11-MTDR + P29-mtGFP A11-MTDR + WA-mFib-mtGFP A11-MTDR + RAW264.7-mtGFP

**Fig. S3.** Red- and green-colored mitochondria-related vesicles in the coculture of A11-MTDR and P29-mtGFP, WA-mFib-mtGFP or RAW264.7-mtGFP. Note that red- and green-colored mitochondria-related vesicles exist in the areas where no cells are present. Scale bars:  $30 \mu m$ .



**Fig. S4.** PCR analysis of the presence of mtDNA in S-EVs isolated from various human cancer cell lines. S-EVs were isolated from the conditioned medium of HeLa cells, A549 cells, DLD1 cells and MIAPaCa-2 cells and subjected to PCR amplification of various mtDNA genes.



**Fig. S5.** Incorporation of A11 S-EVs into P29 cells. S-EVs isolated from the conditioned media of A11-MTDR cells were stained with PKH67, resulting in red and green two-colored PKH-67-positive S-EVs. They were then added to P29 cells and incubated for 24 h. Note that two-colored S-EVs are within P29 cells. Scale bars: 20  $\mu$ m.



**Fig. S6.** S-EV-mediated mtDNA transfer to  $\rho^0$ HeLa cells. S-EVs isolated from the conditioned media of HeLa cells were incubated with  $\rho^0$ HeLa cells in the presence or absence of EV-Entry reagent for 2 days. The cells were detached from the dishes by trypsinization and washed extensively with PBS. DNA was isolated and then subjected to PCR amplification of *ND1* and *ACTB* ( $\beta$ -actin). The primers for *ND1* were the same as those described in Fig. S4. The primers for *ACTB* were used as a loading control. As an input, one-twentieth of the amount of S-EVs added to  $\rho^0$ HeLa cells was also subjected to PCR analysis.



Fig. S7. Full-size images of Western blots.



Fig. S8. Full-size images of Western blots. The yellow dotted line indicates the cropped region.