
Figures and figure supplements

Stable transplantation of human mitochondrial DNA by high-throughput, pressurized isolated mitochondrial delivery

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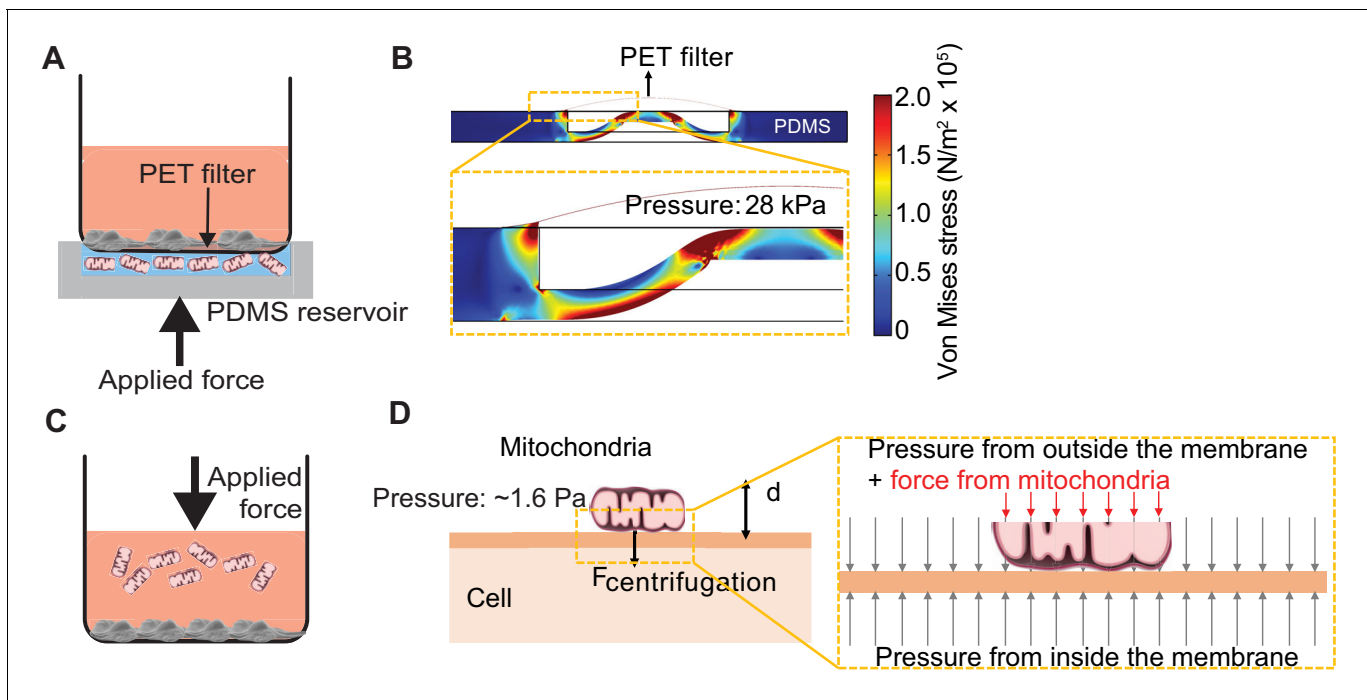


Figure 1. Pressure simulations of mitochondrial transfer tools. (A) Schematic of MitoPunch apparatus. Recipient cells (1×10^5) are seeded on a porous polyester (PET) membrane ~ 24 hr before delivery. A freshly isolated suspension of mitochondria in $1 \times$ Dulbecco's Phosphate Buffered Saline (DPBS) with calcium and magnesium, pH 7.4, is loaded into the polydimethylsiloxane (PDMS) chamber and the filter insert is sealed over the PDMS before activation of the mechanical plunger to pressurize the apparatus and deliver the mitochondrial suspension into recipient cells. (B) Numerical simulation showing the pressure inside the PDMS chamber reaching 28 kPa with piston activation. COMSOL file used to model MitoPunch pressure is available in **Figure 1—source data 1**. (C) Schematic of MitoCeption technique. Recipient cells (1×10^5) are seeded on wells of a 6-well dish ~ 24 hr before delivery. A freshly isolated suspension of mitochondria in $1 \times$ DPBS with calcium and magnesium, pH 7.4, is pipetted into the cell medium before the plate is centrifuged at $1500 \times g$ for 15 min at 4°C . The plate is incubated in a 37°C incubator for 2 hr before being centrifuged again at $1500 \times g$ for 15 min at 4°C . (D) MitoCeption pressure model and calculated pressure exerted by isolated mitochondria on recipient cells during delivery.

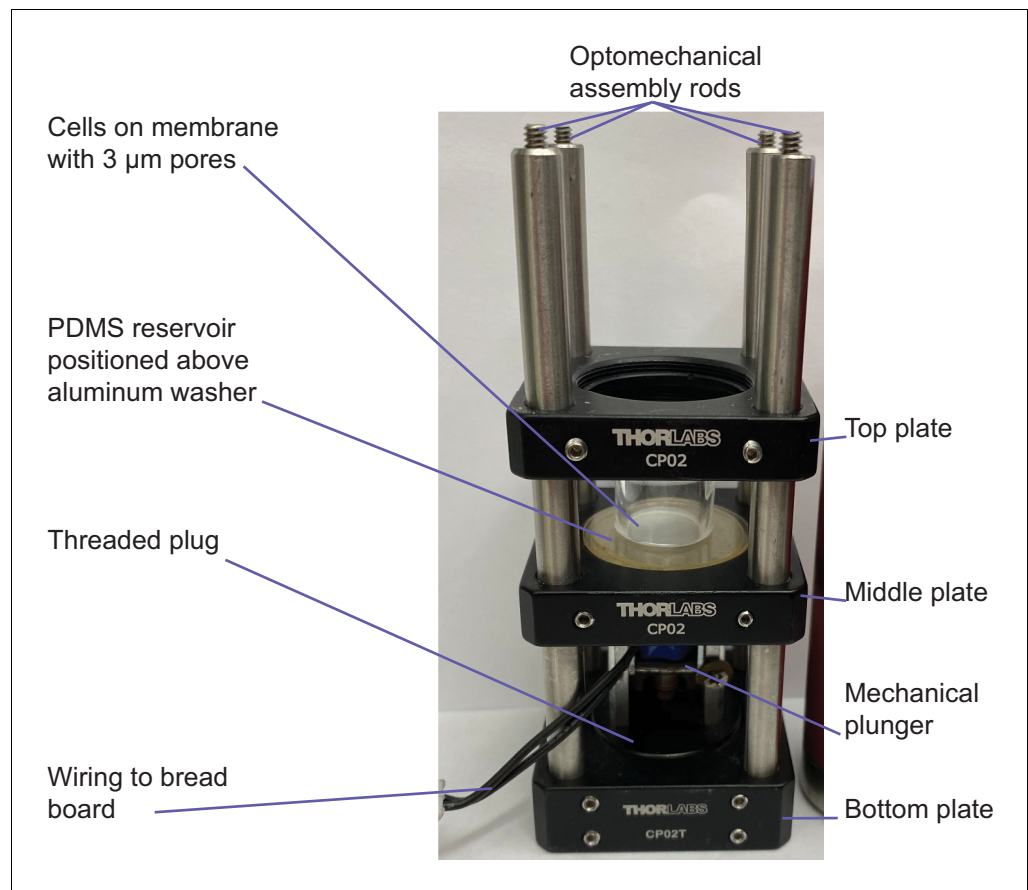


Figure 1—figure supplement 1. Annotated MitoPunch apparatus. Annotated image of the MitoPunch apparatus. Labeled parts are described in the Materials and methods to assist with construction of the apparatus.

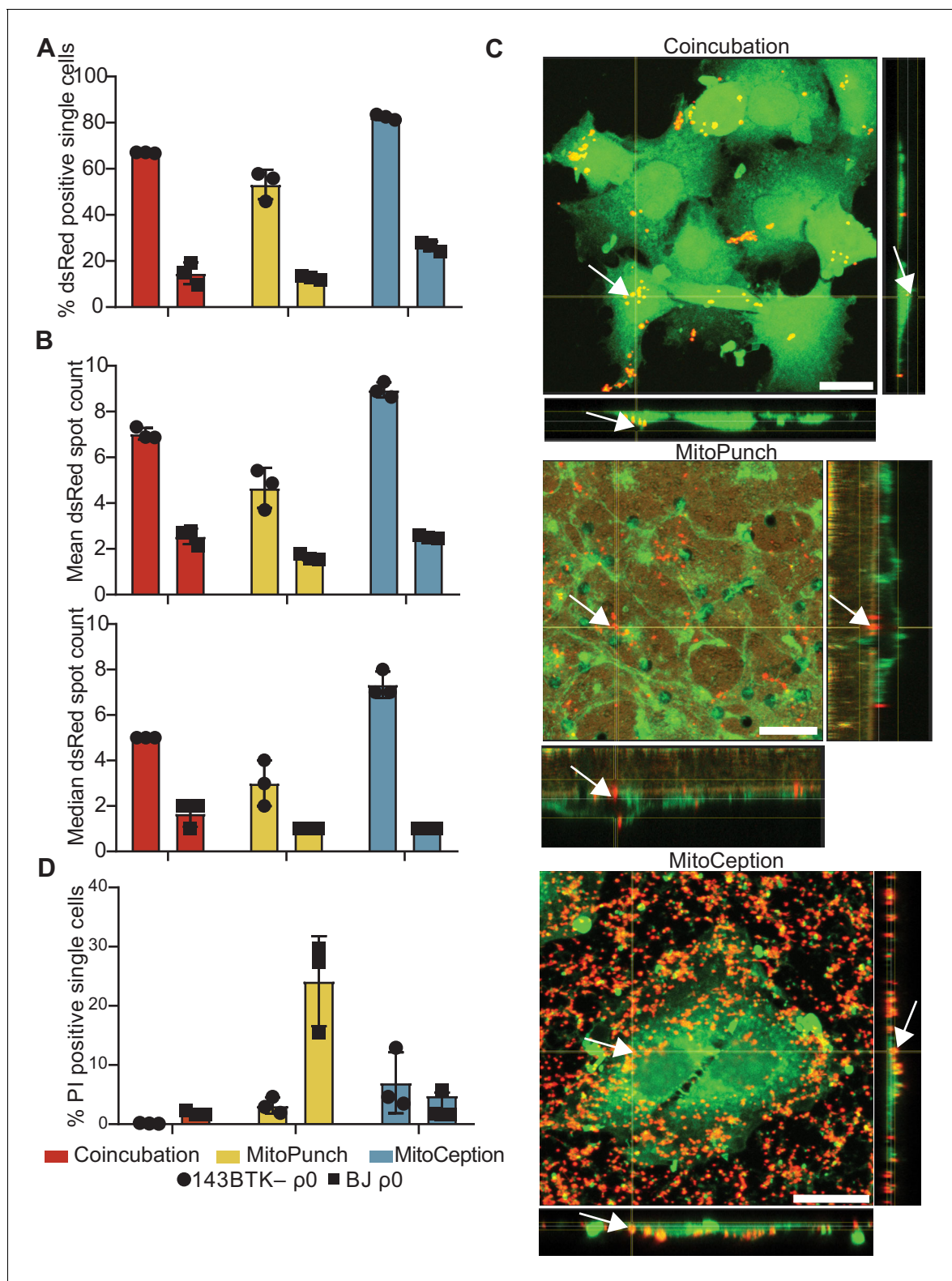


Figure 2. MitoPunch delivers isolated mitochondria to recipient cells. (A) Quantification of flow cytometry results measuring the association of dsRed mitochondria with 143BTK-p0 and BJ p0 single recipient cells following mitochondrial transfer. (B) Mean and median dsRed spot count quantification of ImageStream data. (C) Sequential Z-stacks of confocal microscopy of 143BTK-p0 cells delivered isolated HEK293T-derived dsRed mitochondria by coincubation, MitoPunch, and MitoCeption and fixed 15 min following transfer. Arrows indicate representative mitochondria interacting with recipient cells. Figure 2 continued on next page

Figure 2 continued

cells. Transferred dsRed mitochondria are labeled in red. Plasma membranes are labeled in green, stained with CellMask Green plasma membrane stain in coincubation and MitoCeption and with wheat germ agglutinin plasma membrane stain in MitoPunch. Scale bars indicate 15 μm . (D) Quantification of flow cytometry measurements of fluorescence in 143BTK– p0 and BJ p0 single cells following propidium iodide transfer by coincubation, MitoPunch, and MitoCeption. Error bars represent SD of three technical replicates in all figures.

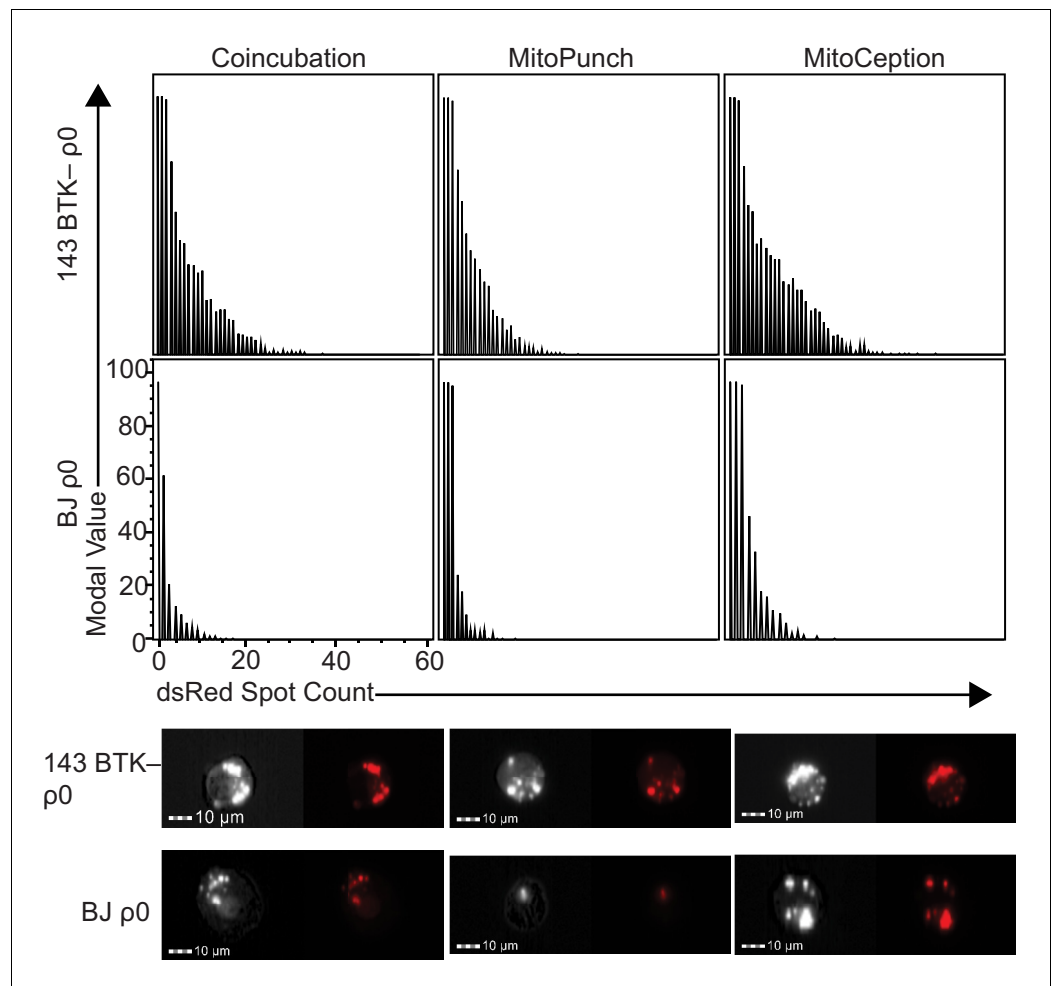


Figure 2—figure supplement 1. Mitochondrial spot quantification. Representative spot count distributions, bright-field images, and PE channel fluorescent images from ImageStream imaging flow cytometry representing the number of dsRed spots associated with 143BTK-ρ0 and BJ ρ0 cells 2 hr after mitochondrial transfer by coincubation, MitoPunch, and MitoCeption. Imaging flow cytometry data is represented as histograms normalized to the mode of each data set. Scale bars indicate 10 μm.

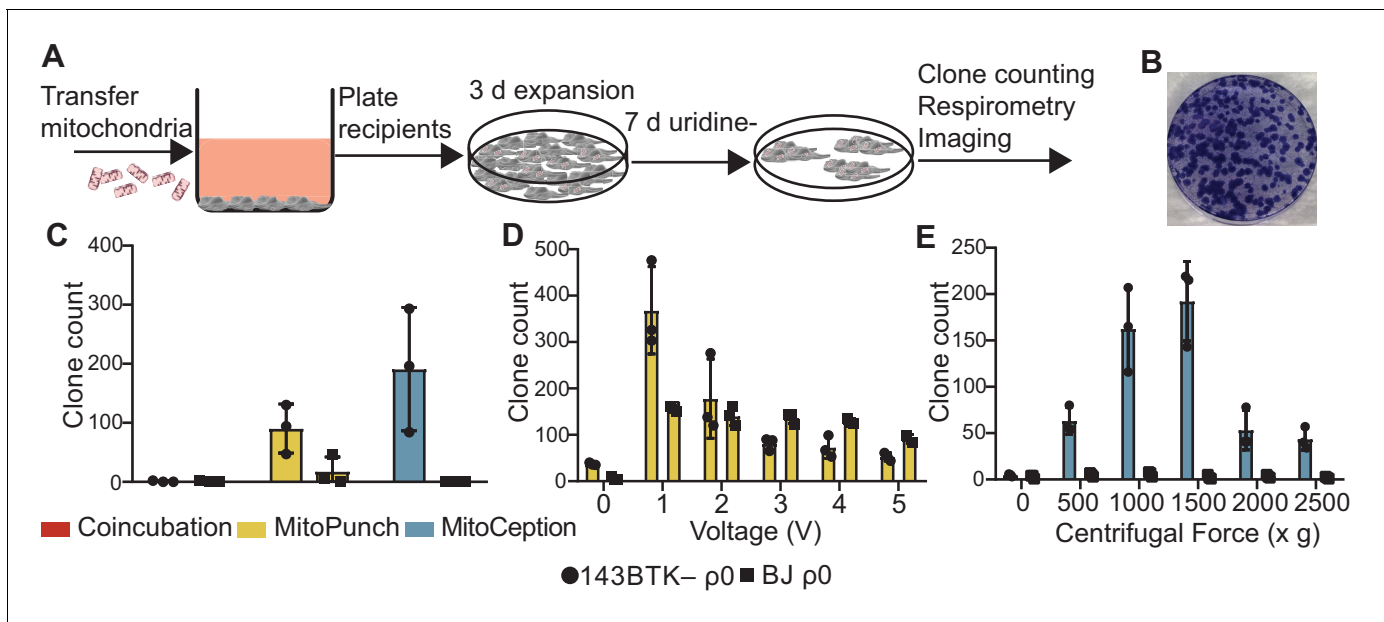


Figure 3. Stable retention of transplanted mitochondrial DNA (mtDNA) into transformed and replication-limited cells. **(A)** Workflow for stable isolated mitochondrial recipient (SIMR) cell generation by mitochondrial transfer into p0 cells. **(B)** Representative fixed and crystal violet stained 10 cm plate image following MitoPunch and SIMR cell selection used for SIMR clone generation quantification. **(C)** Quantification of crystal violet stained 143BTK-p0 and BJ p0 SIMR clones. Error bars represent SD of three technical replicates. **(D)** Quantification of crystal violet stained 143BTK-p0 and BJ p0 SIMR clones formed by MitoPunch actuated with indicated voltages after uridine-free selection. Error bars represent SD of three technical replicates with the exception of BJ p0 5 V transfer, which shows two replicates. **(E)** Quantification of crystal violet stained 143BTK-p0 and BJ p0 SIMR clones formed by MitoCeption with indicated centripetal forces after uridine-free selection. Error bars represent SD of three technical replicates.

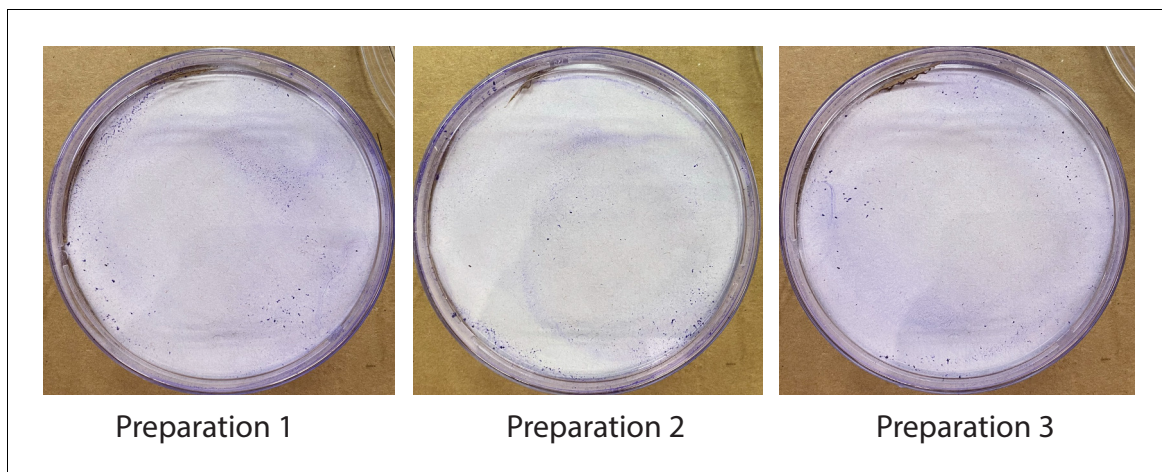


Figure 3—figure supplement 1. Verification of surviving mitochondrial donor cells following mitochondrial isolation. Images of three crystal violet stained 10 cm plates seeded with isolated mitochondria from $\sim 1.5 \times 10^7$ HEK293T dsRed donor cells taken from three independent mitochondrial isolations following dialyzed medium selection. Pictures were taken on a circular white disk matted within a cardboard frame for clarity.

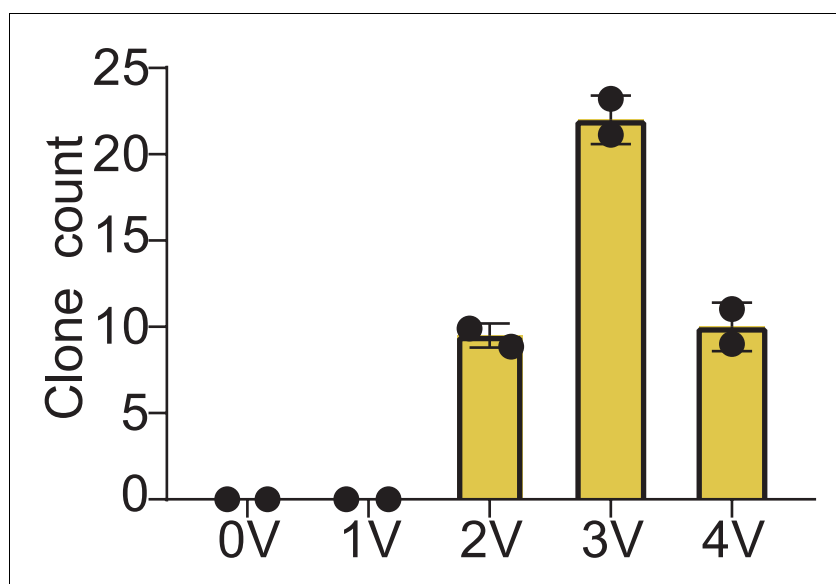


Figure 3—figure supplement 2. MitoPunch generates stable isolated mitochondrial recipient (SIMR) clones in immortalized mouse cells. Quantification of crystal violet stained B16 p0 SIMR clones formed by MitoPunch transfer of isolated L929 mitochondria actuated with indicated voltages after SIMR cell selection. Error bars indicate the range between the technical duplicates.

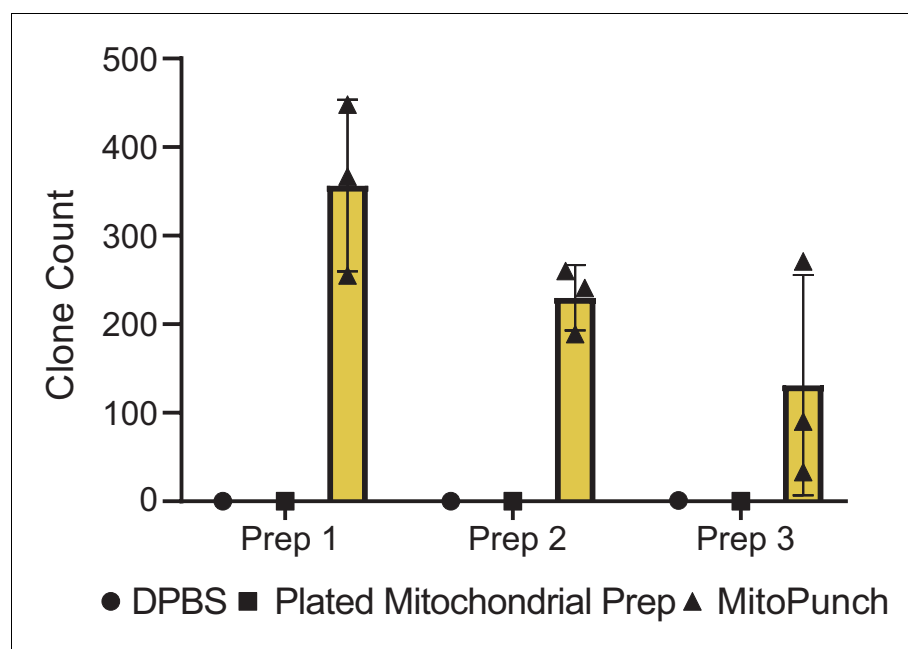


Figure 3—figure supplement 3. Quantification of MitoPunch reproducibility. Quantification of crystal violet stained 143BTK–p0 stable isolated mitochondrial recipient (SIMR) clones generated in technical triplicate from three independent HEK293T dsRed mitochondrial donor cell preparations plotted alongside technical singlet DPBS delivery and plated mitochondrial preparation controls (**Figure 3—figure supplement 1**).

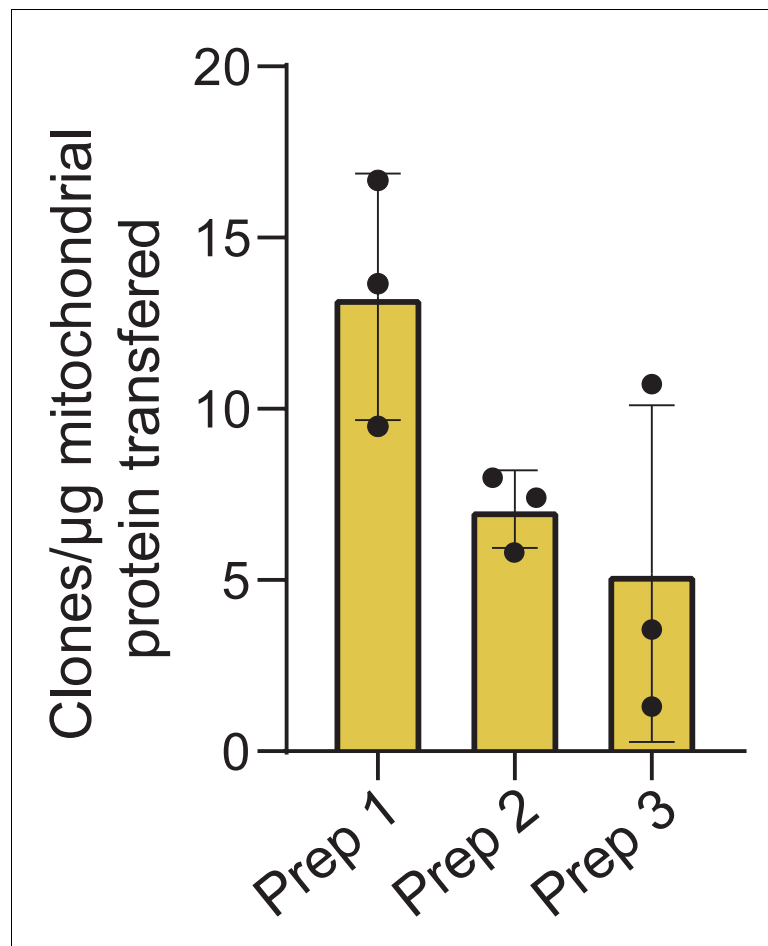


Figure 3—figure supplement 4. Quantification of MitoPunch reproducibility relative to mitochondrial mass transferred. Quantification of crystal violet stained 143BTK- p0 stable isolated mitochondrial recipient (SIMR) clones generated in technical triplicate from three independent HEK293T dsRed mitochondrial donor cell preparations plotted as number of SIMR clones generated per μg isolated mitochondrial protein loaded into the polydimethylsiloxane (PDMS) reservoir.

The mass of isolated mitochondria per 120 μL of isolated mitochondrial suspension for the three preparations are as follows: Prep 1–27 μg , Prep 2–33 μg , Prep 3–25 μg .

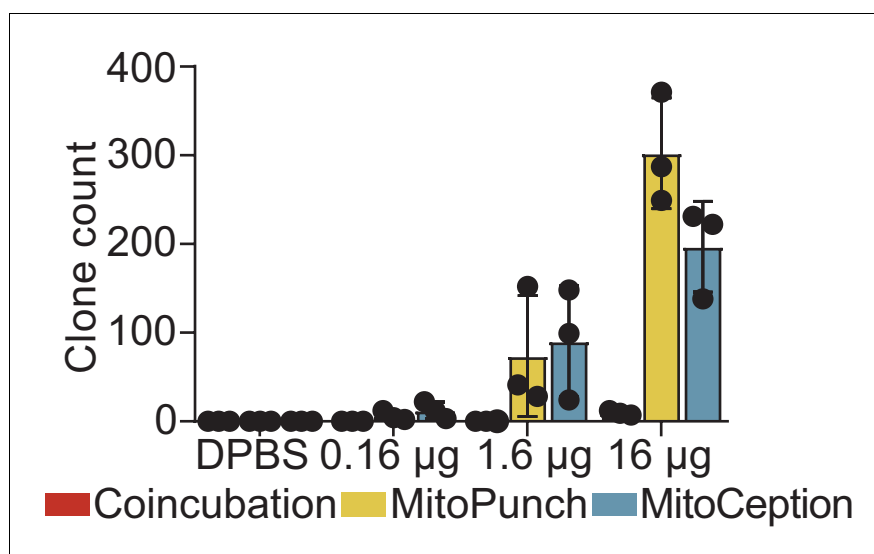


Figure 3—figure supplement 5. Quantification of stable isolated mitochondrial recipient (SIMR) generation efficiency by delivering different masses of isolated mitochondria. Quantification of crystal violet stained 143BTK-p0 SIMR clones using indicated concentrations of mitochondrial suspension following 7 days of culture in SIMR selection medium. Error bars represent SD of three technical replicates.

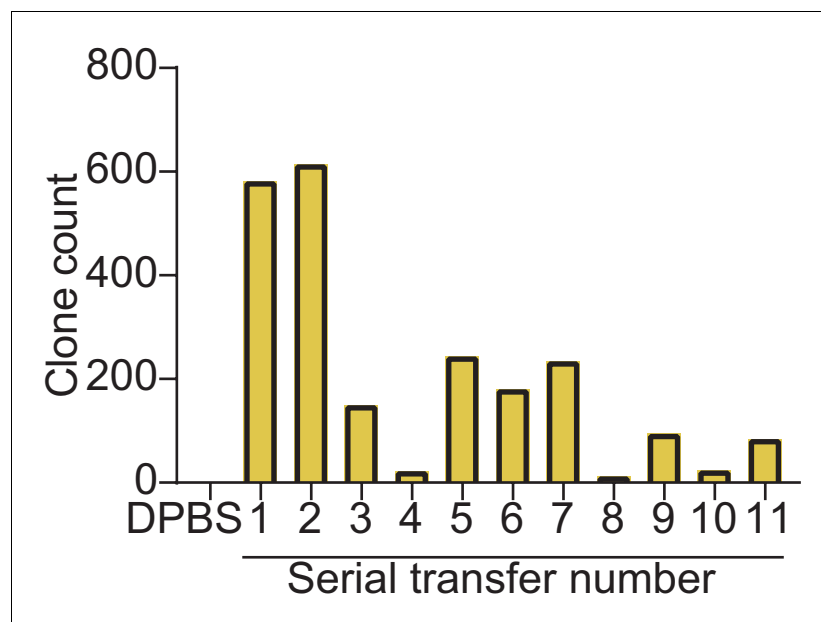


Figure 3—figure supplement 6. Quantification of MitoPunch stable isolated mitochondrial recipient (SIMR) generation by serial deliveries using one isolated mitochondrial aliquot. Quantification of crystal violet stained SIMR clones formed by serial MitoPunch deliveries of HEK293T dsRed mitochondria into 143BTK- p0 recipient cells using the same used mitochondrial sample remaining in the polydimethylsiloxane (PDMS) reservoir after the preceding delivery.

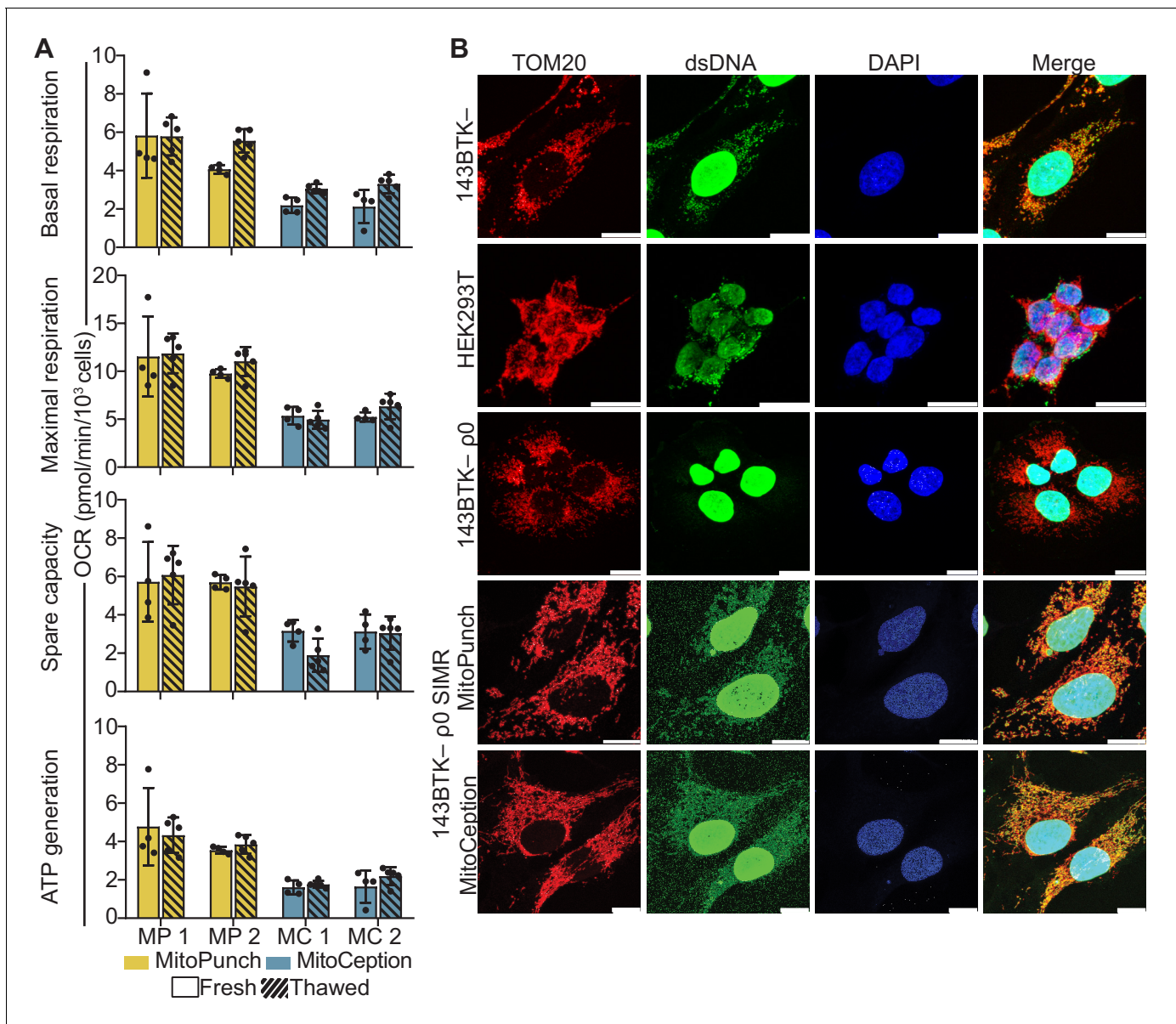


Figure 4. Mitochondrial DNA (mtDNA) transplantation rescues p0 mitochondrial phenotypes. **(A)** Oxygen consumption rate (OCR) quantification of basal and maximal respiration, spare respiratory capacity, and ATP generation from two independent 143BTK- p0 + HEK293T stable isolated mitochondrial recipient (SIMR) clones generated by MitoPunch and MitoCeption. Cross-hatched data indicate clones that were frozen and thawed twice each. Error bars represent SD of four technical replicates for fresh SIMR cell measurements and five for thawed SIMR cell measurements. **(B)** Confocal microscopy of representative 143BTK- p0 + HEK293T SIMR clones compared to 143BTK- parental, HEK293T dsRed mitochondrial donor, and 143BTK- p0 controls. Mitochondria were stained with anti-TOM20 antibody and labeled red, double-stranded DNA was stained with anti-dsDNA antibody and labeled green, and cell nuclei were stained with NucBlue (Hoechst 33342) and labeled blue. Scale bars indicate 15 μ m.

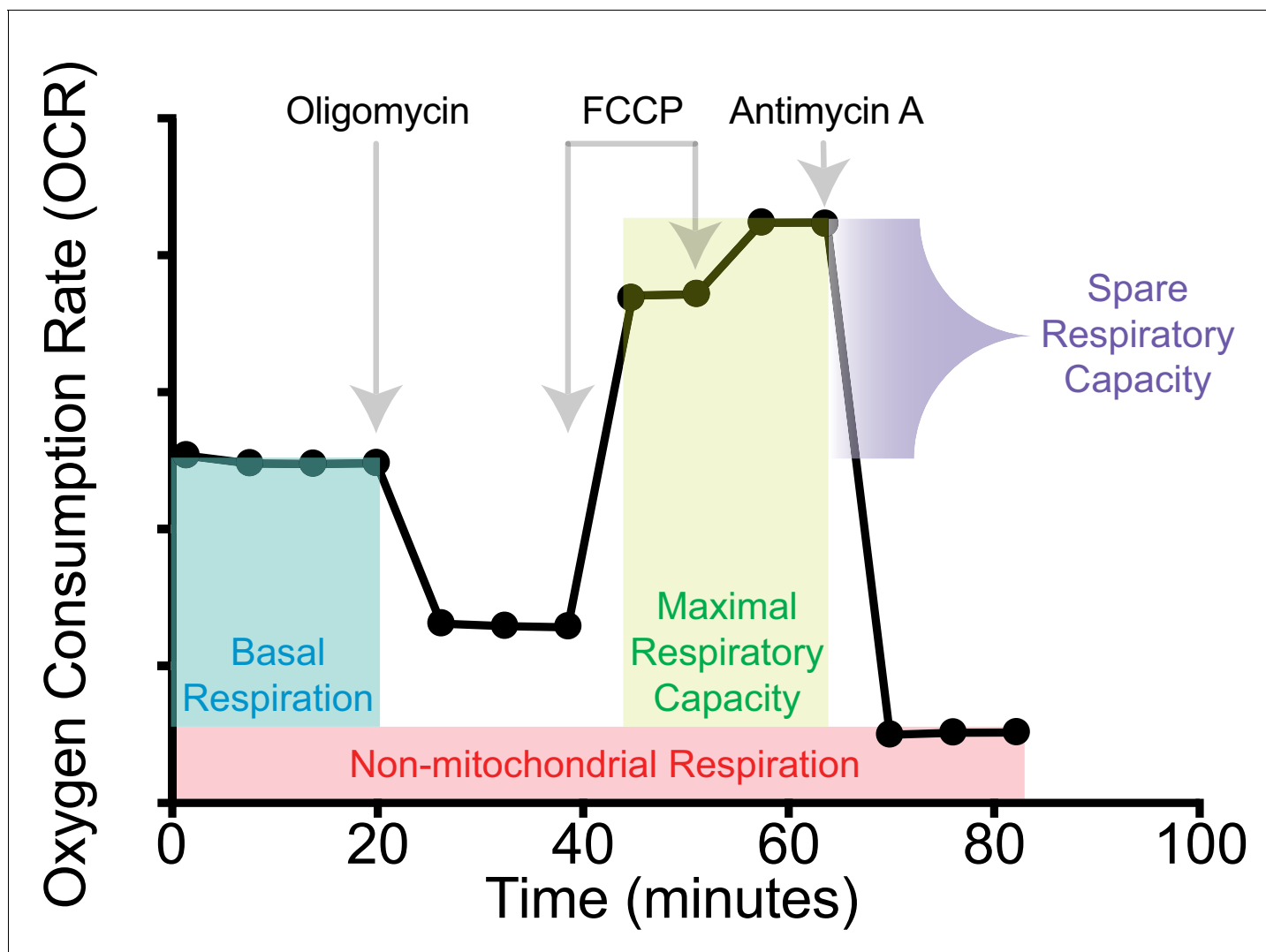


Figure 4—figure supplement 1. Schematic of the Seahorse Mito Stress Test. Annotated plot of oxygen consumption as a function of time, including the identity and timing of drugs injected over the course of a Seahorse Mito Stress Test measurement. Annotations indicate how OXPHOS parameters presented in **Figure 4A** are quantified.

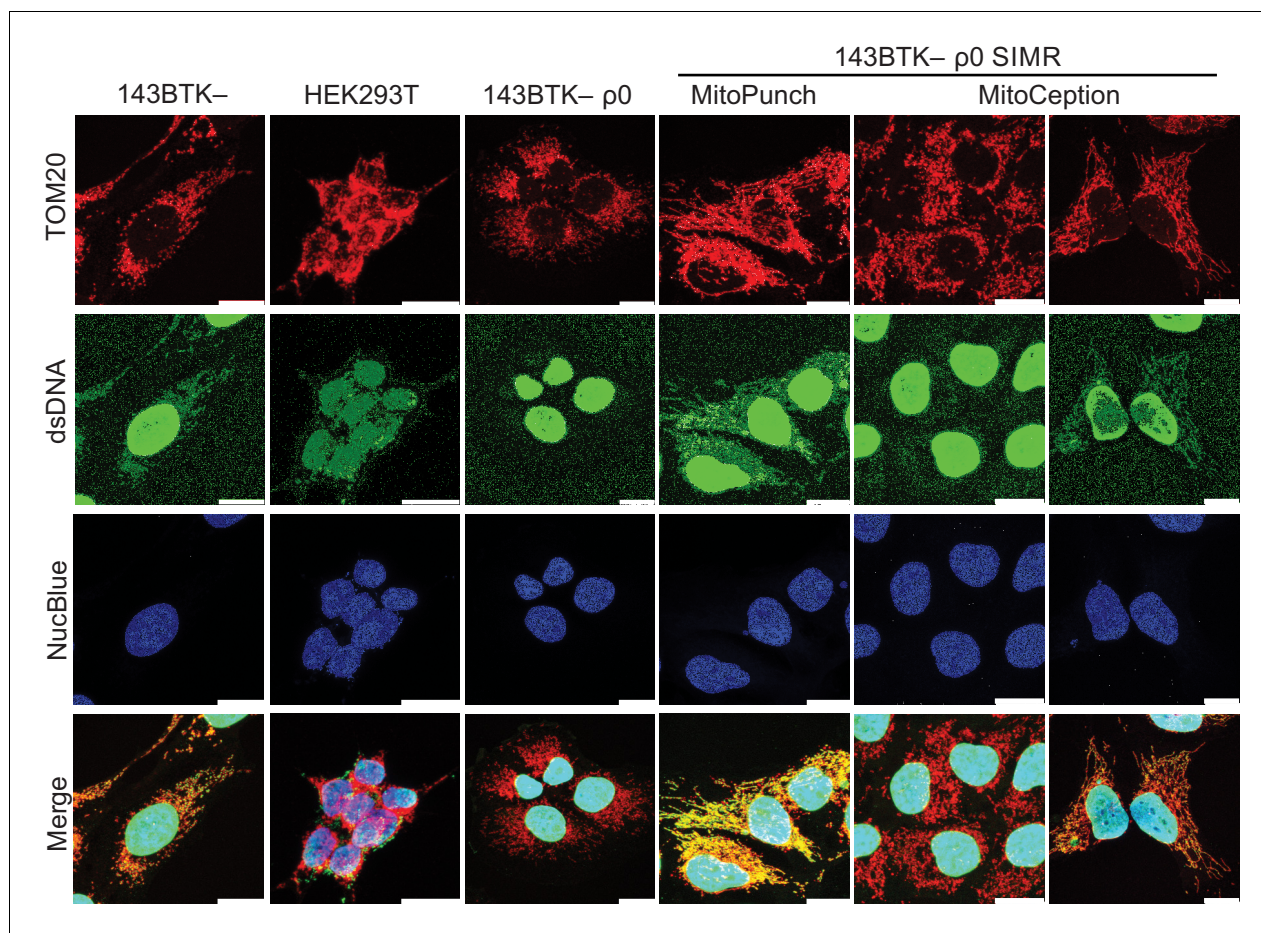


Figure 4—figure supplement 2. Confocal microscopy of stable isolated mitochondrial recipient (SIMR) lines. Confocal microscopy of SIMR clones formed in 143BTK- p0 cells with 143BTK- parental, HEK293T dsRed mitochondrial donor, and 143BTK- p0 controls. The MitoCeption SIMR clone data on the left represents the SIMR line that lost respiration following freeze-thaw. Mitochondria were stained with anti-TOM20 antibody and labeled red, double-stranded DNA was stained with anti-dsDNA antibody and labeled green, and cell nuclei were stained with NucBlue (Hoechst 33342) and labeled blue. The 143BTK-, 143BTK- p0, and HEK293T dsRed control images are the same images used in **Figure 4B**. Scale bars indicate 15 μ m.