

Figure S1. Individual oocyte and embryo lysis and qPCR workflow. Individual oocytes, cleavage stage embryos (2-cell, 4-cell, 8-cell, morula) and blastocysts were individually collected in 1 μ L sterile PBS-PVP, snap frozen and stored at -80°C until use. To lyse cells and release DNA, 9 μ L of lysis solution was added, bringing the total sample volume to 10 μ L. Due to the relative abundance of mtDNA in oocytes and embryos, 1 μ L of lysis product was removed and further diluted in 9 μ L of sterile water to be used for mtDNA analysis (bringing the mtDNA dilution factor to 100X). The remaining 9 μ L of sample was used to complete 2 technical replicates each using Rn18S primers. After lysis, ICM samples were further diluted in 10 μ L of sterile water for analysis.

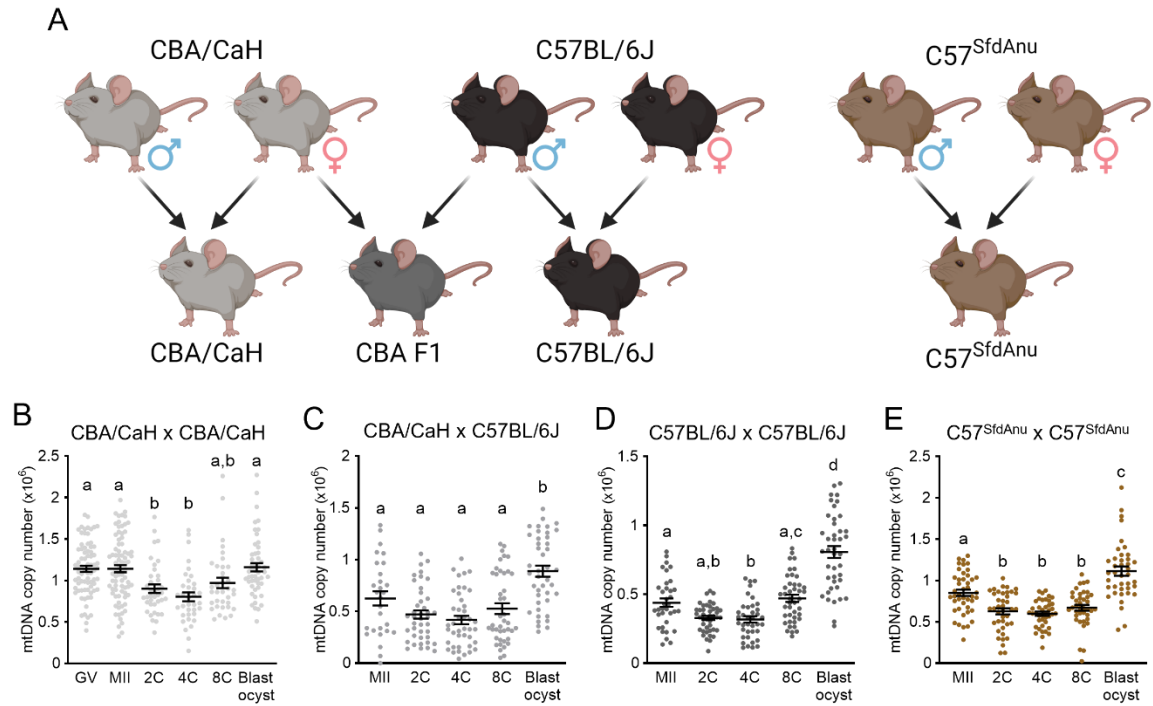


Figure S2. mtDNA copy number regulation across preimplantation embryogenesis is relatively conserved between mouse strains. **(A)** CBA/CaH female mice are crossed to C57BL/6J male mice to produce CBA F1 mice while C57^{SfdAnu} are an outbred strain with a C57BL/6J background. Total mtDNA copy number throughout a pre-implantation embryo development time course in **(B)** CBA/CaH x CBA/CaH (inbred) embryos; **(C)** CBA/CaH x C57BL/6J (hybrid) embryos; **(D)** C57BL/6J x C57BL/6J (inbred) embryos; and **(E)** C57^{SfdAnu} x C57^{SfdAnu} (outbred) embryos. $N \geq 31$ oocytes or embryos analyzed per strain per timepoint. Data analyzed using a linear mixed-effects model where different lowercase letters indicate statistical significance between groups of at least $p < 0.05$.

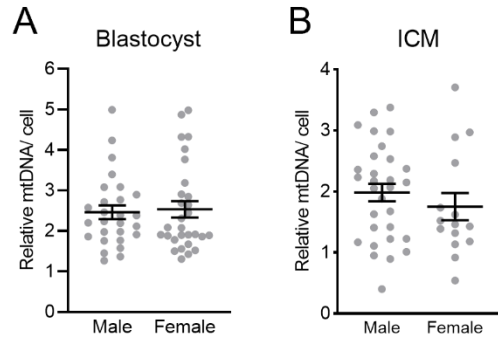


Figure S3. Embryo sex does not influence blastocyst or ICM relative mtDNA content. **(A)** Relative mtDNA content in male (n=27) and female (n=29) blastocysts. **(B)** Relative mtDNA content in the ICM of male (n=30) and female (n=15) blastocysts. Data analyzed using unpaired t-test.

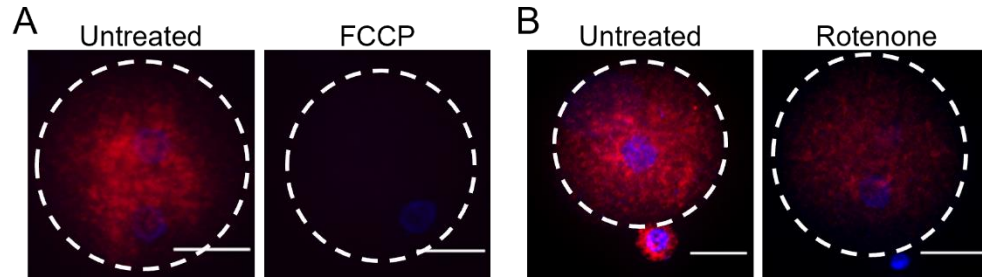


Figure S4. TMRM signal is responsive to mitochondrial uncoupling and toxicants. **(A)** To demonstrate the specificity of TMRM (red) as an indicator of MMP, after TMRM incubation (with Hoechst-3342 DNA stain (blue)), zygotes were exposed to 1 μ M FCCP for 10 minutes prior to imaging. FCCP-mediated uncoupling abrogated the TMRM/MMP signal. **(B)** Exposure of mice to a low dose of rotenone to disrupt Complex I function disrupted zygote TMRM signal. Images representative of 15-20 zygotes per group.

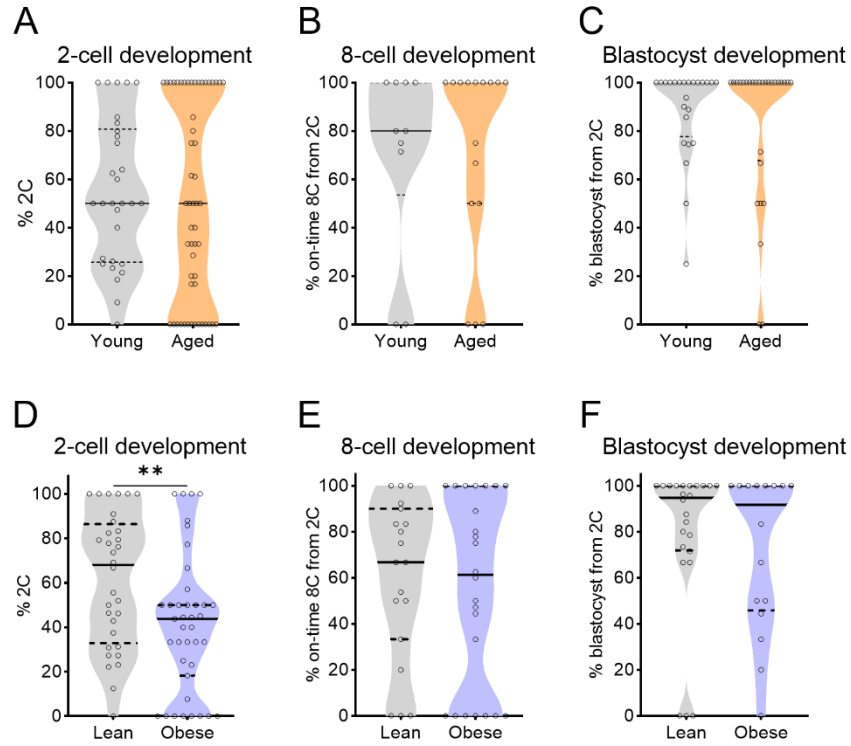


Figure S5. On-time embryo development in aged and obese female mice. On-time development to 2-cells (**A**), 8-cells (**B**) and blastocyst (**C**) following fertilization of oocytes from young or reproductively aged females. N=30 young and n=54 aged females. On-time embryos development to 2-cells (**D**), 8-cells (**E**) and blastocyst (**F**) following fertilization of oocytes from lean or obese females. N=33 lean and n=39 obese females.