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Supplemental Information

Regulation of Mother-to-Offspring Transmission of mtDNA Heteroplasmy

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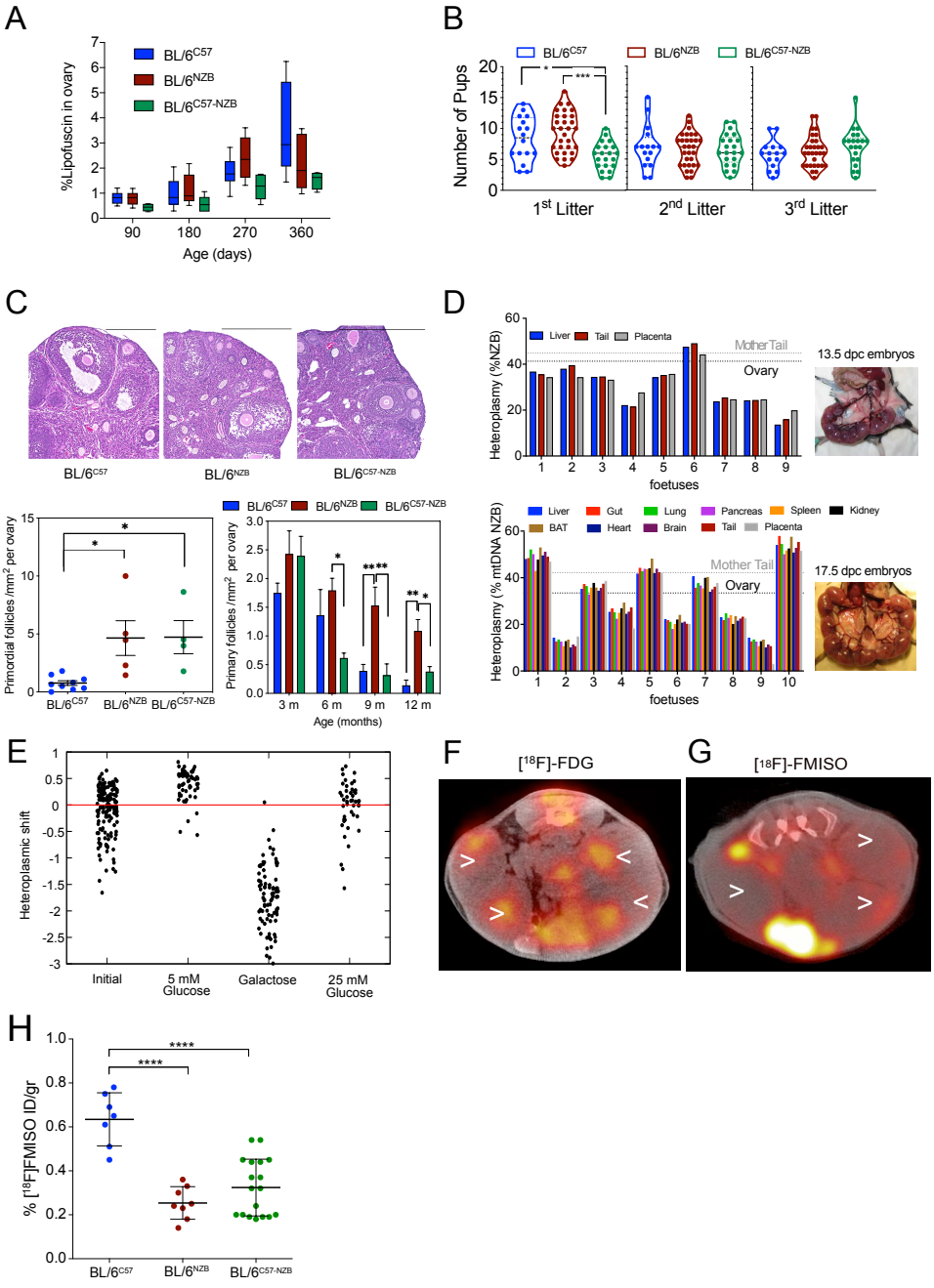

Figure S1.- Related to figures 1 and 2. (A) Lipofuscin accumulation throughout the reproductive life of homoplasmic and heteroplasmic females (n =5-11). Data of homoplasmic females from (Latorre-Pellicer et al., 2016). **(B)** Litter size in homoplasmic and heteroplasmic mice. Each dot represents a different litter, only females with three litters are considered (mean ± sd, one-way ANOVA test, **p-value* < 0.05, ****p-value* < 0.001). **(C)** H&E histological sections of ovaries from homoplasmic (BL/6^{C57}, BL/6^{NZB}) and heteroplasmic (BL/6$^{C57\text{-}NZB}$) strains (top). Analysis of the oocyte maturation (primordial and primary follicles) in ovaries of the indicated ages and mouse strains (bottom). (Mean ± sem, one-way ANOVA test, **p-value* < 0.05, ***p-value* < 0.01). **(D)** Variability in heteroplasmy between tissues of individual embryos of the same litter at 13.5 (n=9, top) or 17.5 (n=10, bottom) dpc and between different tissues. **(E)** Heteroplasmy shifts in MEF single cell-clones from the mean of the initial MEF

population set (n=149 single cell-clones) after 60 days of treatment (n= 46 clones with 25mM Glucose, p = 0.30 against identity to initial state, 89 clones with 5mM Glucose, p = 2.4×10^{-17} against identity to initial state, and 86 clones with 5mM Glucose, p = 2.2×10^{-24} against identity to initial state). Significant non-zero segregation for 5 mM glucose and galactose treatments is observed. (**F-G**) PET-CT representative *in vivo* images of (F) [^{18}F]-FDG uptake or (G) [^{18}F]-FMISO in 12.5 dpc heteroplasmic pregnant females. (**H**) Ex vivo determination of [^{18}F]-FMISO uptake measured by gamma counter in 12.5 dpc embryos of homoplasmic (either C57 or NZB mtDNA) and heteroplasmic animals. Each dot represents an individual embryo. (mean ± sd, one-way ANOVA test, ****p-value < 0.0001).

Table S1. Related to Fig 2. List of antibodies used

Antibody	Features		Source	Reference no.	mtETC
NDUFA9	Monoclonal	Mouse IgG1	Abcam	Ab14713	**CI**
SDHA (Fp70)	Monoclonal	Mouse IgG1	ThermoFisher	459200	**CII**
UQCRC2 (Core2)	Polyclonal	Rabbit IgG2a	ProteinTech	14742-I-AP	**CIII**
COI	Monoclonal	Mouse IgG2a	Invitrogen	459600	**CIV**
ATP-β	Monoclonal	Mouse IgG1	Abcam	Ab14730	**CV**
GAPDH	Monoclonal	Mouse IgG1	Abcam	Ab8245	

Table S2. Related to Fig 2. Padlock probes and padlock probe primers

Probe	Sequence
ppC57	5'-P-ATTACTCTCTTCTGGTTCCTTTTACGA**CCTCAATGCTGCTGCTGTAC TAC**CTTCACAGTGTACAGGTTA -3'
ppNZB	5'-P- ATTACTCTCTTCTGG**CCTTTCCTACGACCTCAATGCACATGTTTG GC**TCCTCTT ACAGTGTACAGGTTC -3'
Lin33 (ppC57)	5'-Cy3CCTCAATGCTGCTGCTGTACTAC-3'
Lin16 (ppNZB)	**5'-FITC-CCTCAATGCACATGTTTGGCTCC-3'** **5'-Cy5-CCTCAATGCACATGTTTGGCTCC-3'**