

Figure S1: Genotyping of mice used in this study. Related to Figure 1.

(A-C) Genotyping gels for  $\mathit{Mis18}\alpha^{\mathit{fl/fl}}$  mice for both  $\mathit{Cre}$  drivers.

## Generating cKO females using Gdf9-cre (equivalent crosses were done for Zp3-Cre)

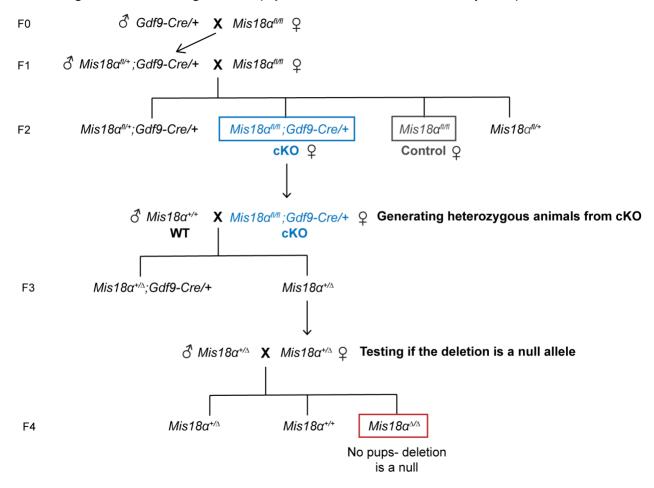
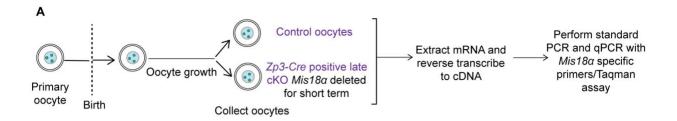


Figure S2 : Cross scheme to combine the *Cre* recombinases with the *Mis18* $\alpha$  floxed allele. Related to Figures 1- 4.

Crosses are shown for Gdf9-Cre, and equivalent crosses were done for Zp3-Cre. The blue and grey boxes designate cKO and control females used for CENP-A measurements in oocytes (Figure 4). The cKO mothers were also used to generate heterozygous animals, which were then utilized to confirm that  $Mis18\alpha$  deletion is a null allele (also see Figure 1D).



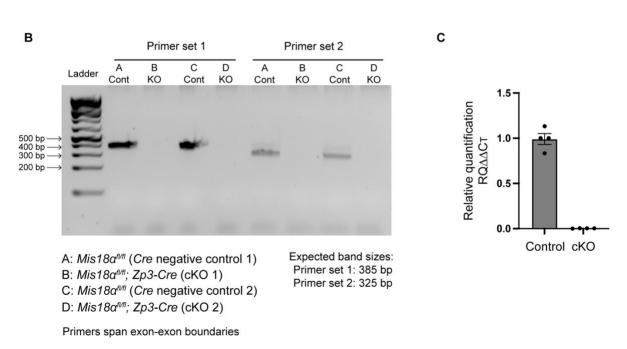


Figure S3:  $Mis18\alpha$  gene expression in oocytes. Related to Figure 2.

(A) Schematic of  $Mis18\alpha$  gene expression assay for control and late cKO oocytes. (B) Standard RT-PCR gives no amplification from late cKO oocytes compared to the control. (C) Quantification of real time PCR for  $Mis18\alpha$  gene expression using a Taqman assay against  $Mis18\alpha$ , with H2A as the endogenous control. Expression was quantified using the Livak method<sup>S1</sup>.  $Mis18\alpha$  mRNA is undetectable by both methods. Error bars: S.E.M.

## **Supplemental Reference:**

S1. Livak, K.J., and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods *25*, 402–408. 10.1006/meth.2001.1262.