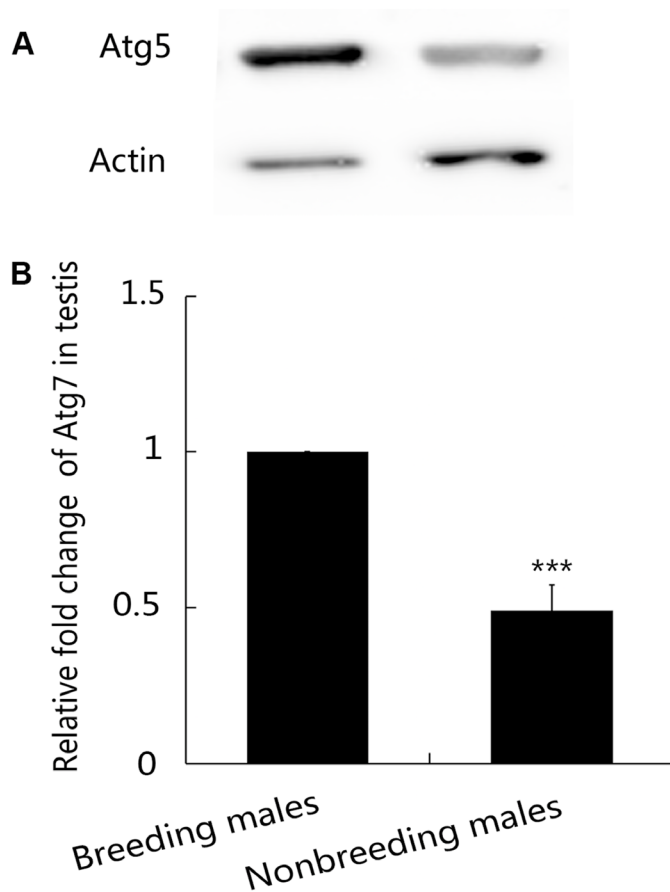
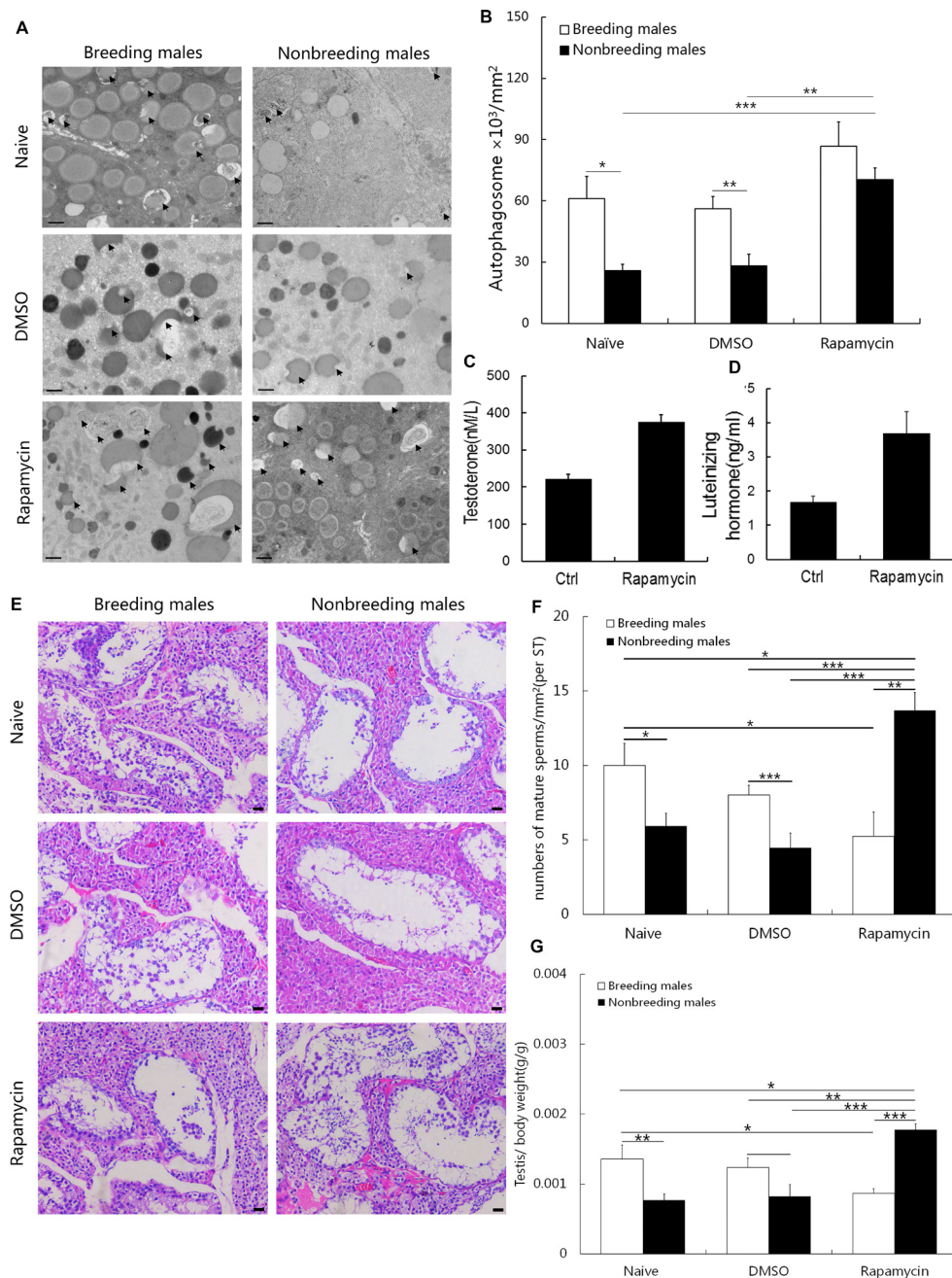


# Levels of Leydig cell autophagy regulate the fertility of male naked mole-rats

## SUPPLEMENTARY MATERIALS



**Supplementary Figure 1: Atg5 levels in breeding and nonbreeding male NMRs.** (A) Western blot analysis of Atg5 levels in breeding and nonbreeding male NMRs. (B) Quantification of Atg5 levels in breeding and nonbreeding male NMRs ( $P = 0.000879$ ) ( $n = 6$  in each group). Data are presented as means  $\pm$  SEM.



**Supplementary Figure 2: Rapamycin treatment restored the fertility of nonbreeding male NMRs.** (A) TEM was used to visualise Leydig cells from breeding and nonbreeding male NMRs. (B) Quantification of the autophagosomes (arrows) shown in (A) ( $n = 6$  in each group). (C and D) Quantification of testosterone ( $P = 0.0002910$ ) and LH ( $P = 0.006257$ ) concentrations in nonbreeding male NMRs ( $n = 6$  in each group), which were IP injected with the control or rapamycin. Data are presented as means  $\pm$  SEM. (E) HE staining was used to visualise sperm maturation status from breeding and nonbreeding male NMRs. (F) Quantification of the mature sperms shown in (E) ( $n = 6$  in each group) (breeding males vs nonbreeding males in naive group,  $P = 0.0146$ , breeding males vs nonbreeding males in DMSO group,  $P = 0.0007713$ , breeding males in naive group vs in rapamycin group,  $P = 0.0203$ , breeding males vs nonbreeding males in rapamycin group,  $P = 0.001995$ , breeding males in naive group vs nonbreeding males in rapamycin group,  $P = 0.02839$ , breeding males in DMSO group vs nonbreeding males in rapamycin group,  $P = 0.0001150$ , nonbreeding males in DMSO group vs in rapamycin group,  $P = 0.0005040$ ) (G) Quantification of testes weights from breeding and nonbreeding male NMRs under different treatment ( $n = 6$  in each group) (breeding males vs nonbreeding males in naive group,  $P = 0.009860$ , breeding males vs nonbreeding males in DMSO group,  $P = 0.02905$ , breeding males in naive group vs in rapamycin group,  $P = 0.02927$ , breeding males vs nonbreeding males in rapamycin group,  $P = 0.0001300$ , nonbreeding males in naive group vs in rapamycin group,  $P = 0.0001310$ , breeding males in DMSO group vs nonbreeding males in rapamycin group,  $P = 0.004080$ , nonbreeding males in DMSO group vs in rapamycin group,  $P = 0.009300$ ), which were IP injected with the control or rapamycin. Data are presented as means  $\pm$  SEM.