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Supplemental information

Testis electroporation coupled with autophagy inhibitor to treat non-obstructive azoospermia

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Supplemental Information

Supplemental Figures and Figure legends

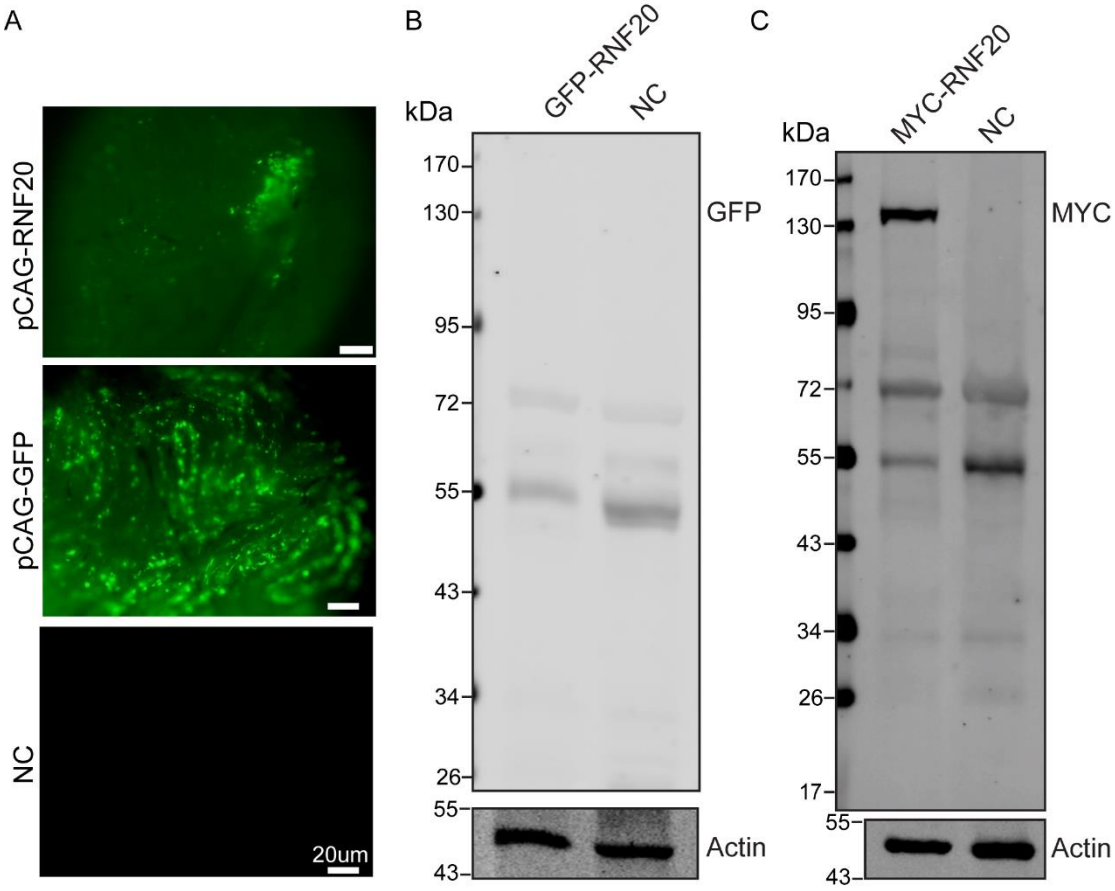

Figure S1. Expression of exogenous RNF20 in testes after electroporation.

(A) Representative images of the expression of GFP-RNF20 in testes after electroporation. Scale bar, 20um.

(B-C) Western blot analysis of testis extracts after electroporation. Actin served as a loading control.

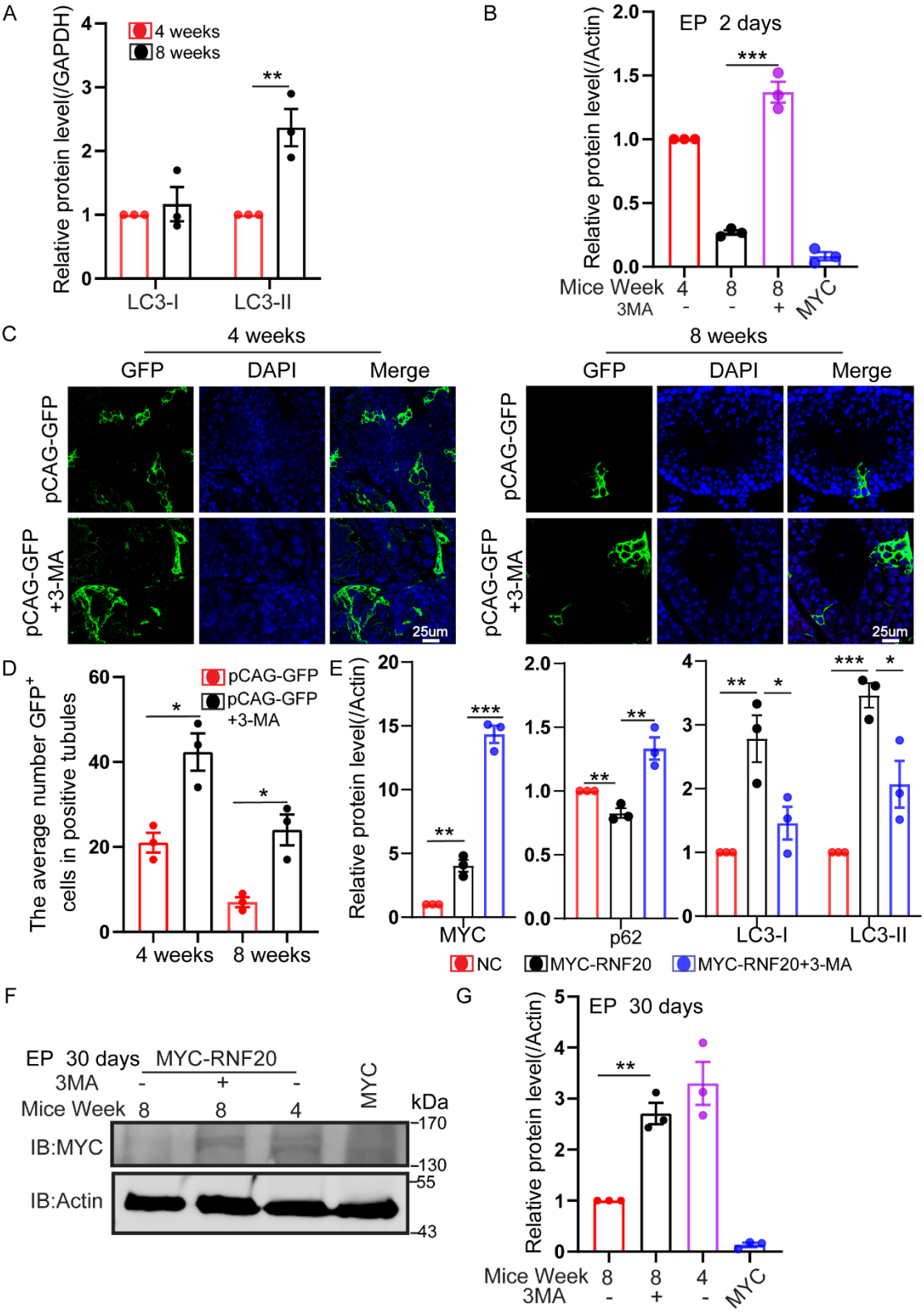

Figure S2. The efficiency of electroporation is increased by adding autophagy inhibitor 3-MA in 4-week-old and 8-week-old mouse testes after electroporation.

(A) Relative protein levels of LC3 in 4-week-old and 8-week-old mouse testes. n=3

biological replicates. Data are presented as mean ± SEM. **p < 0.01. Statistical analysis was performed with two-tailed unpaired student's t test.

(B) Quantification of the MYC-RNF20 levels in testes after 2 days of electroporation. n=3 biological replicates. Data are presented as mean ± SEM. ***p < 0.001. Statistical analysis was performed with two-tailed unpaired student's t test.

(C)The efficiency of electroporation is increased by adding autophagy inhibitor 3-MA in 4-week-old and 8-week-old mouse testes. Scale bar, 25um.

(D) Quantification of the GFP positive signals in (C). n=3 biological replicates. Data are presented as mean ± SEM. *p < 0.05. Statistical analysis was performed with two-tailed unpaired student's t test.

(E) Quantification of the MYC-RNF20, LC3 and p62 levels in 8-week-old mouse testes after 2 days of electroporation. n=3 biological replicates. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01 and ***p < 0.001. Statistical analysis was performed with two-tailed unpaired student's t test.

(F) Western blotting analysis of testis extracts after electroporation. Actin served as a loading control.

(G) Quantification of the MYC-RNF20 levels in (F). n=3 biological replicates. Data are presented as mean ± SEM. **p < 0.01. Statistical analysis was performed with two-tailed unpaired student's t test.

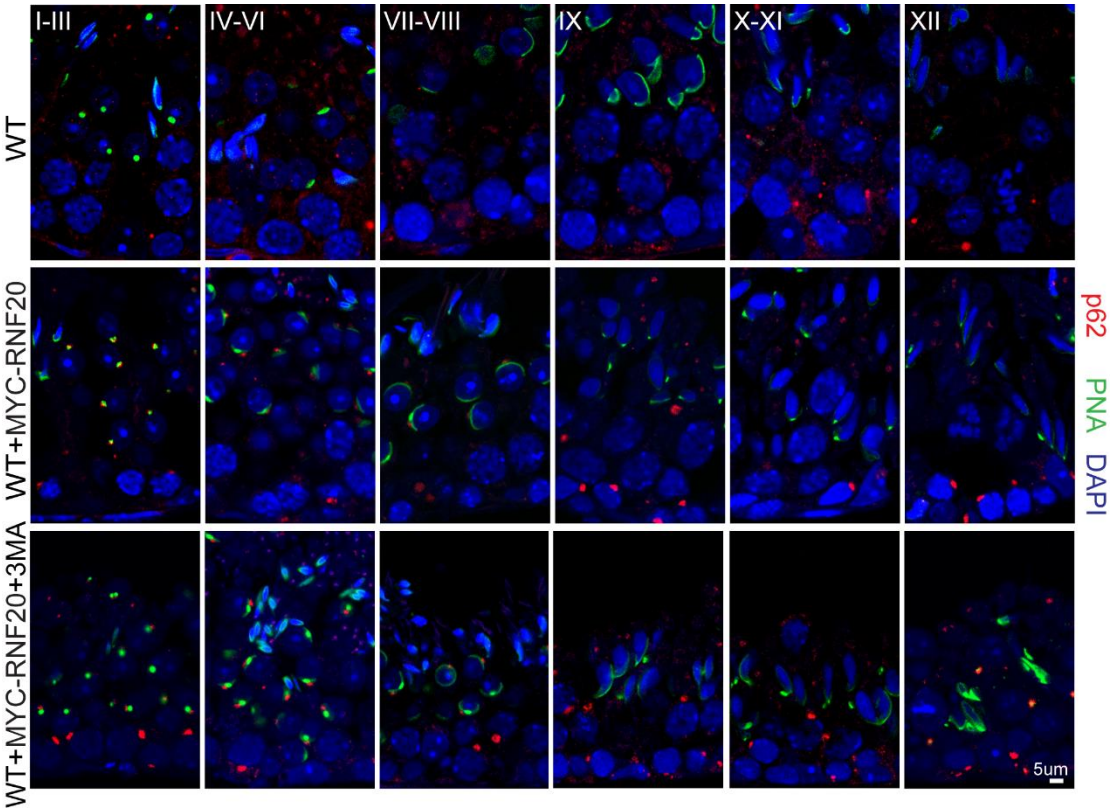

Figure S3. Expression levels of p62 in mouse testes.

Expression levels of p62 are increased in mouse testes treated with autophagy inhibitor 3-MA after electroporation. Scale bar, 5um.

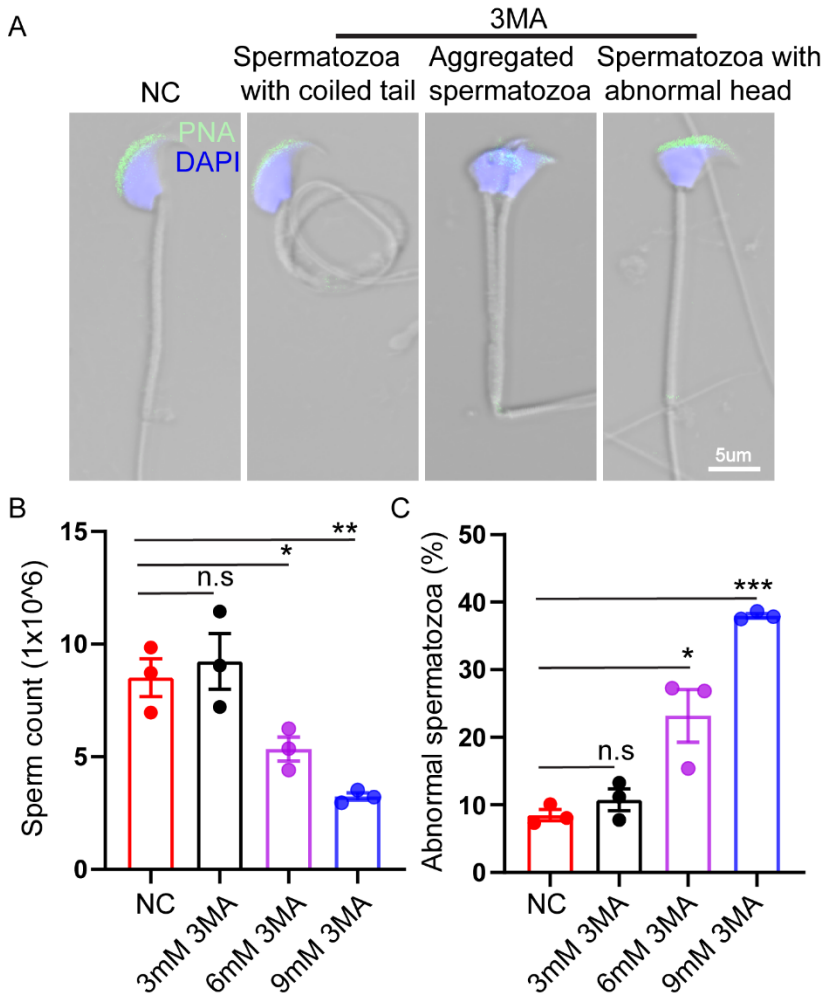

Figure S4. High concentration of 3-MA results in abnormal spermatozoa.

(A) PNA staining of spermatozoa from the epididymis of 0.9% NaCl (NC group) or 3-MA injected mice for 3 weeks. Scale bar, 5um.

(B) Sperm counts in the caudal epididymidis were detected in 3mM, 6mM and 9mM 3-MA injected mice. n=3 biological replicates. Data are presented as mean ± SEM. n.s, non-significant, *$p < 0.05$ and **$p < 0.01$. Statistical analysis was performed with two-tailed unpaired student's t test.

(C) Quantification of abnormal spermatozoa in (B). n=3 biological replicates. Data are presented as mean ± SEM. n.s, non-significant, *$p < 0.05$ and ***$p < 0.001$. Statistical analysis was performed with two-tailed unpaired student's t test.