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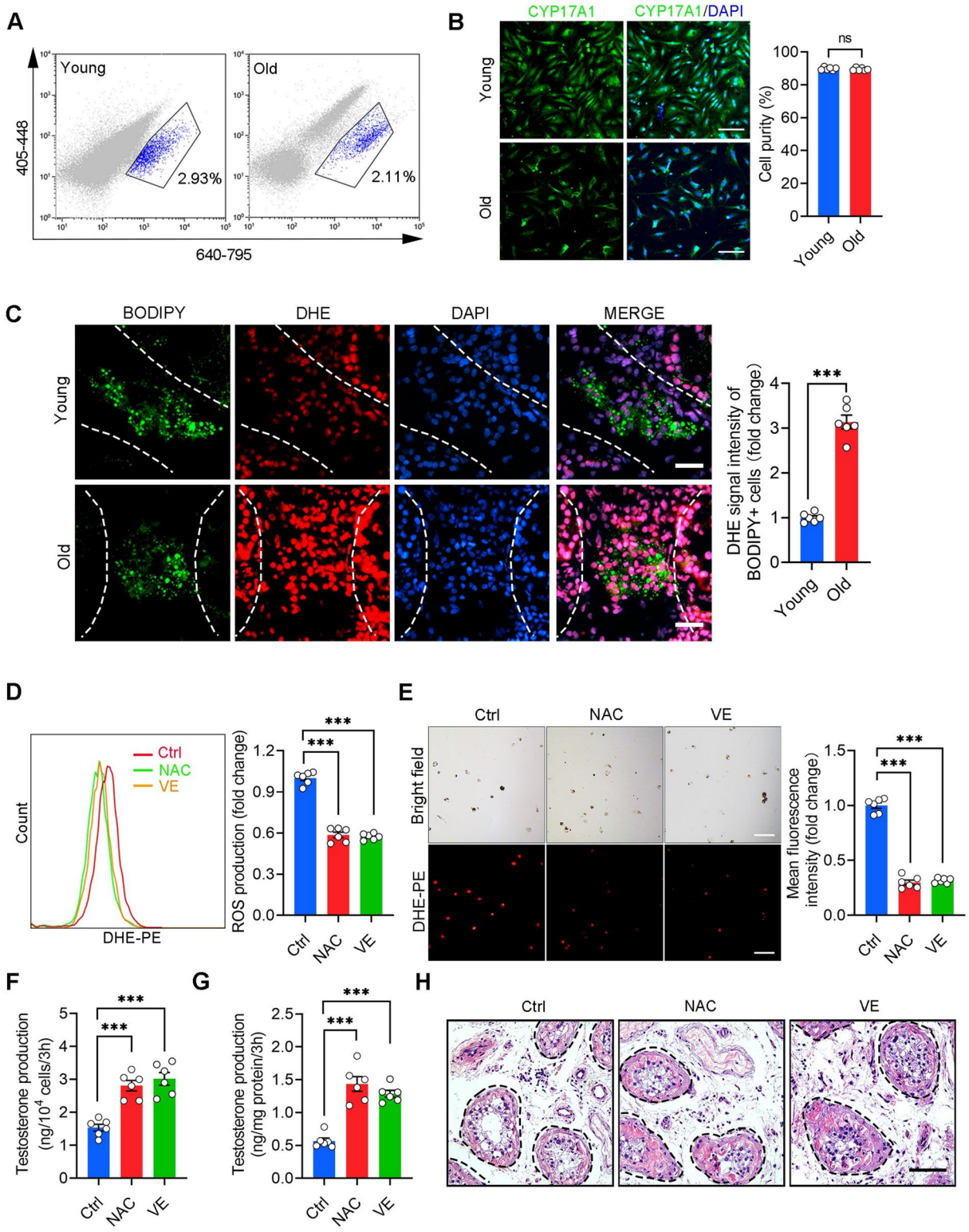
Supplementary Data



Supplementary Figure S6. Change of ROS production in LCs during testicular ageing; related to Fig. 5. (**A**) Representative gating strategy of FACS for isolating young and old human LCs. Autofluorescent cells were excited in all fluorescence channels and isolated with the 405/448 merged 640/795 channel. (**B**) Cell purity of young and old human LCs was measured by immunostaining of CYP17A1. Right, quantification of the proportion of CYP17A1+ cells. Scale bars, 100 μm. Young, n = 6 samples; Old, n = 6 samples. Data are expressed as mean±SEM. Significance was determined by

(continued)

Supplementary Figure S6. Continued
Student's *t*-test. ns = not significant. (**C**) Immunostaining and quantification of ROS production in young and old human testis sections. Left, representative fluorescent (DHE and BODIPY) images of young and old testis sections. Right, quantification of the DHE signal intensity of BODIPY+ cells in young and old human testis sections. Scale bars, 35 μm. Young, n = 6 samples; Old, n = 6 samples. Data are expressed as mean±SEM. Significance was determined by Student's *t*-test. ***$P < 0.001$. (**D**) Quantification of ROS production in primary LCs of Ctrl and antioxidant-treated (NAC or VE) groups, as measured by flow cytometry. Ctrl, n = 6 samples; NAC (10 mM), n = 6 samples; VE (50 μM), n = 6 samples. Data are expressed as mean±SEM. Significance was determined by one-way ANOVA. ***$P < 0.001$. (**E**) Representative images and quantification of ROS production by primary human LCs isolated from old testes, comparing Ctrl with antioxidant-treated (NAC or VE) groups. Left, representative bright field and DHE immunofluorescent images of primary LCs of different groups. Right, quantification of ROS production by primary LCs of Ctrl and antioxidant-treated (NAC or VE) groups. Scale bars, 100 μm. Ctrl, n = 6 samples; NAC (10 mM), n = 6 samples; VE (50 μM), n = 6 samples. Data are expressed as mean±SEM. Significance was determined by one-way ANOVA. ***$P < 0.001$. (**F**) Quantification of testosterone production by primary human LCs isolated from old testes in Ctrl and antioxidants treatment (NAC and VE) groups. Ctrl, n = 6 samples; NAC (10 mM), n = 6 samples; VE (50 μM), n = 6 samples. Data are expressed as mean± SEM. Significance was determined by one-way ANOVA. ***$P < 0.001$. (**G**) Quantification of testosterone production by old human testicular tissue in Ctrl and antioxidant-treated (NAC and VE) groups. Ctrl, n = 6 sample; NAC (10 mM), n = 6 sample; VE (50 μM), n = 6 sample. Data are expressed as mean± SEM. Significance was determined by one-way ANOVA. ***$P < 0.001$. (**H**) Changes of human testicular tissue after renal subcapsular transplantation. Representative H&E staining images of human testicular tissue in Ctrl and antioxidant-treated (NAC or VE) groups after transplantation. Scale bars, 75 μm. DHE, dihydroethidium; BODIPY, Boron-dipyrromethene; Ctrl, control group; NAC, N-acetylcysteine group; VE, vitamin E group; ROS, reactive oxygen species.