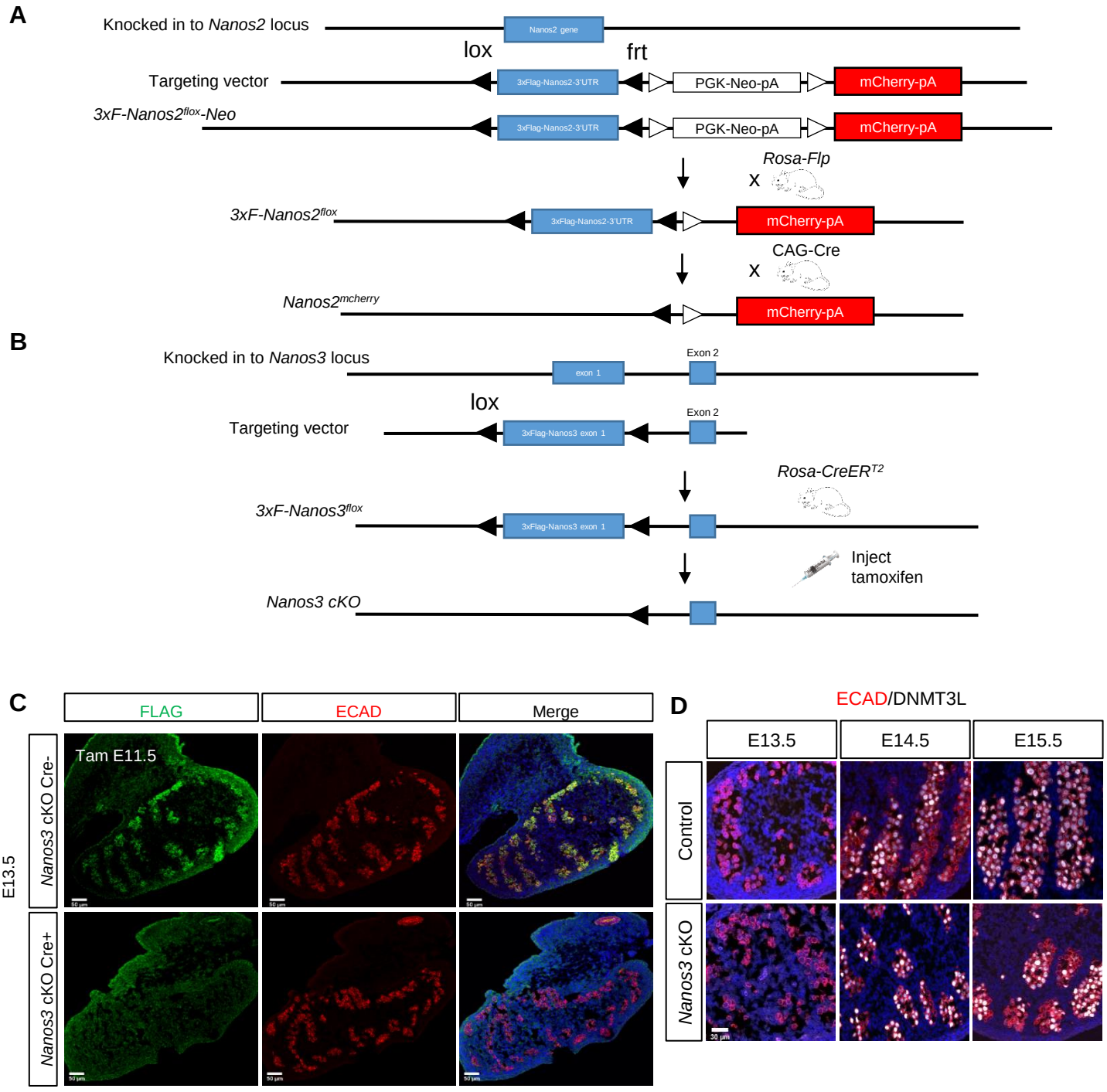

Fig. S1. A) Schema of the creation of *Nanos2*^{mcherrry} mice. The neomycin cassette was removed by crossing with *Rosa-Flp* mice[15]. To create *Nanos2*^{mcherry}, the mice were crossed with CAG-Cre mice to remove *3xFlag-Nanos2*. B) Schema of the creation of *Nanos3* cKO mice. Tamoxifen was injected to remove *3xF-Nanos3*. C) Testis sections from E13.5 *Nanos3* cKO mice administered tamoxifen at E11.5 were stained with anti-FLAG and anti-E-cadherin to assess the Cre recombination efficiency. No germ cells in Cre+ mice expressed 3xF-NANOS3 at E13.5. D) Sections of E13.5, E14.5 and E15.5 control and *Nanos3* cKO testes were stained with antibodies against E-cadherin and DNMT3L to mark male-type differentiation. Scale bar 30 µm. DNA was stained with DAPI.

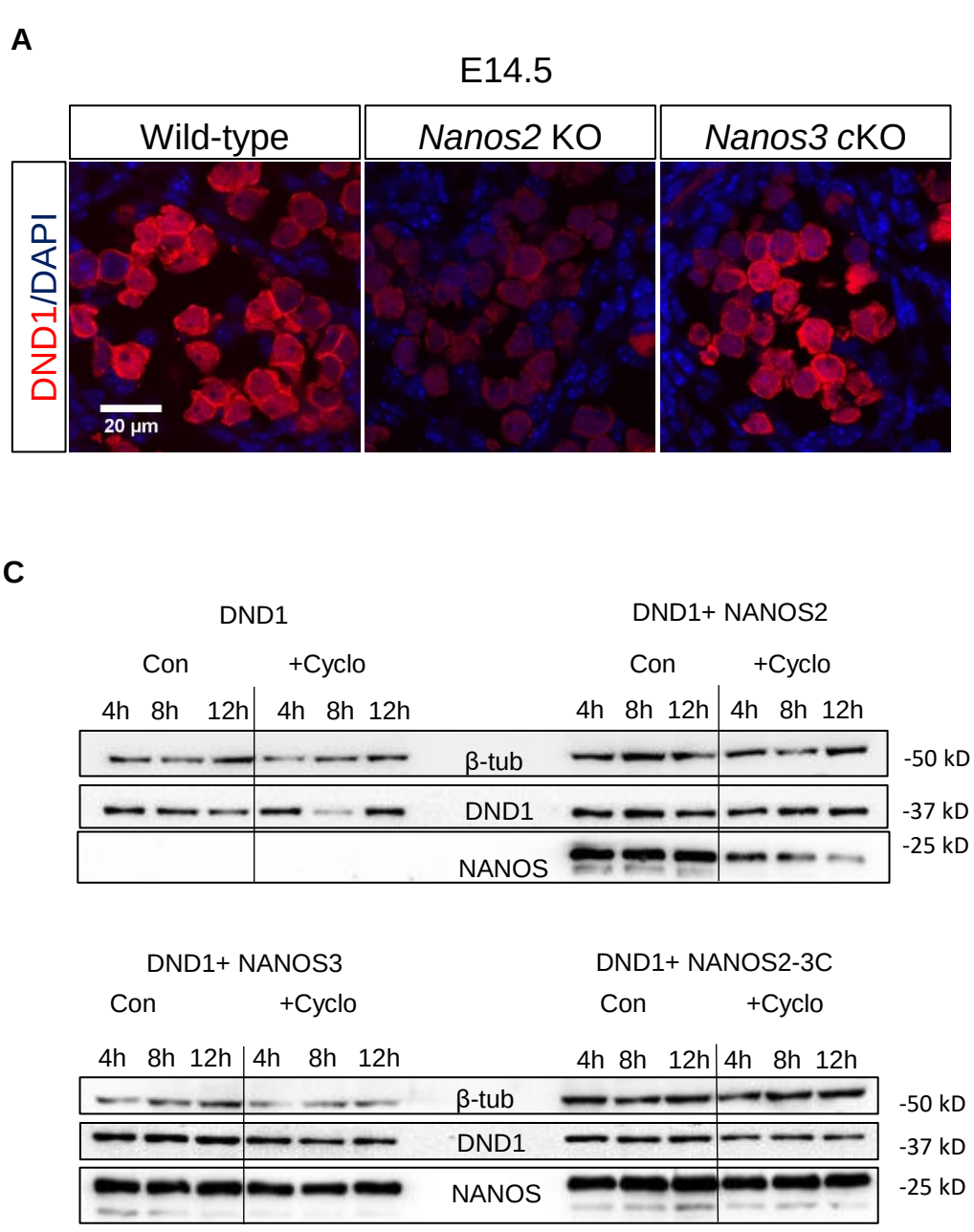

Fig. S2. A) DND1 expression in wild-type, *Nanos2* KO and *Nanos3* cKO germ cells. Sections of testesfrom E14.5 wild-type, *Nanos2* KO or *Nanos3* cKO embryos were stained with anti-DND1 (red). DNA was stained with DAPI (blue). Scale bar 20 µm. B) Single-cell RNA sequencing results for *Actb* and *Dnd1* using E13.5 and E14.5 control and *Nanos2* KO germ cells (data were obtained from Shimada et al. 2020 preprint). Violin plots were generated using the Seurat package for R (Butler et al., 2018). Black dots indicate the median. C) Western blotting of HEK293T cells expressing HA-DND1 alone or together with 3xF-NANOS2, NANOS3 or NANOS2-3C cultured with or without cycloheximide for the indicated times. β-tubulin was used as a control.

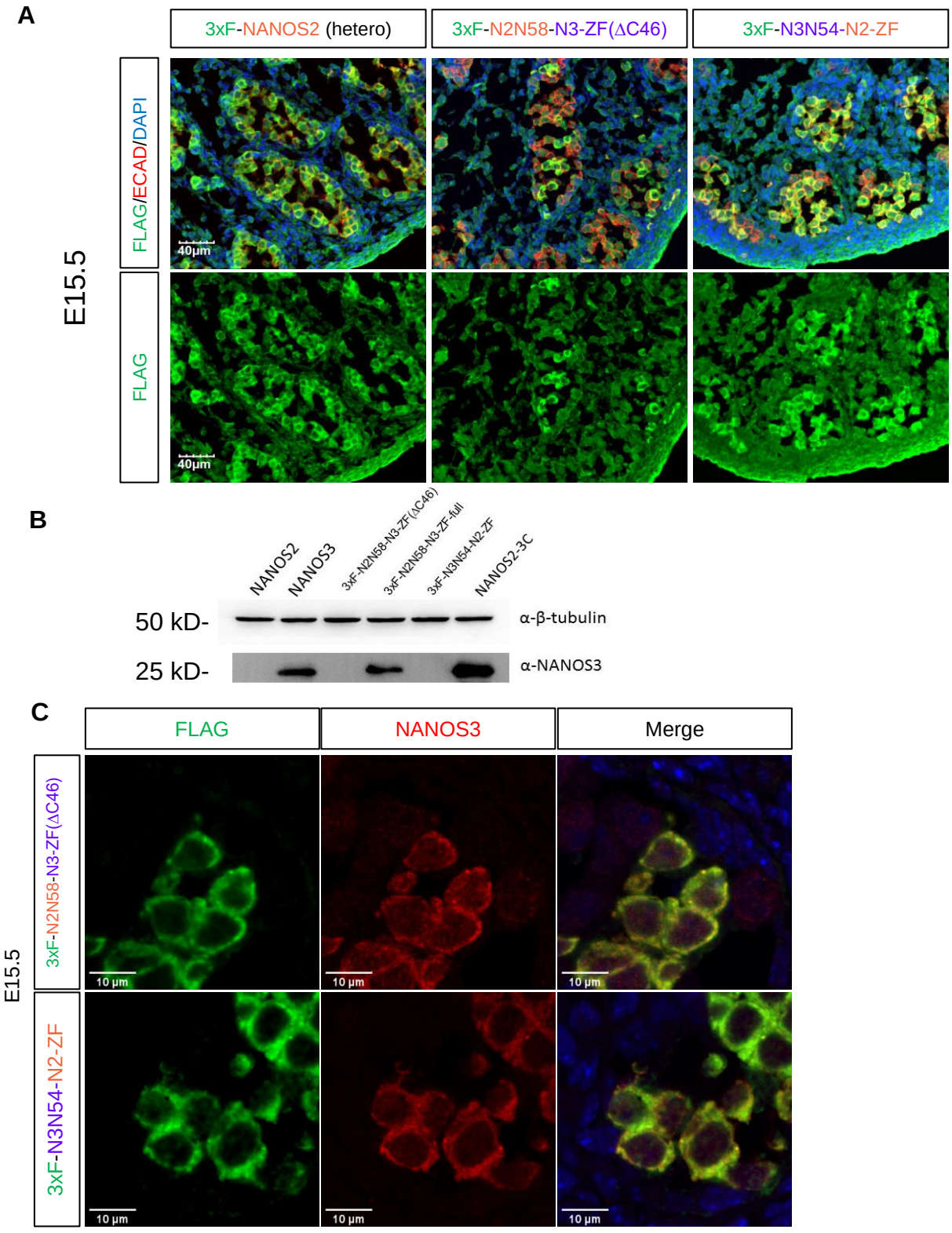

Fig. S3. A) Comparison of protein expression among 3xF NANOS2, 3xF-NANOS2N58-NANOS3-ZF(ΔC46) and 3xF-NANOS3N54-NANOS2-ZF. E15.5 testis sections were stained with anti-FLAG and anti-E-cadherin. Scale bar 40 μm. B) Western blotting of HEK293T cells transfected with the indicated proteins using anti-NANOS3 antibody. This antibody detects the C-terminal of NANOS3, and therefore can be used to assess endogenous NANOS3 expression in chimera. C) Endogenous NANOS3 is still upregulated in chimeric germ cells. Sections of testes from E15.5 3xF-Nanos2N58-Nanos3-ZF(ΔC46) (top) or 3xF-Nanos3N54-Nanos2-ZF (bottom) were stained with antibodies against FLAG (green) and NANOS3 (red). DNA was labeled with DAPI (blue). Scale bar 10 μm.

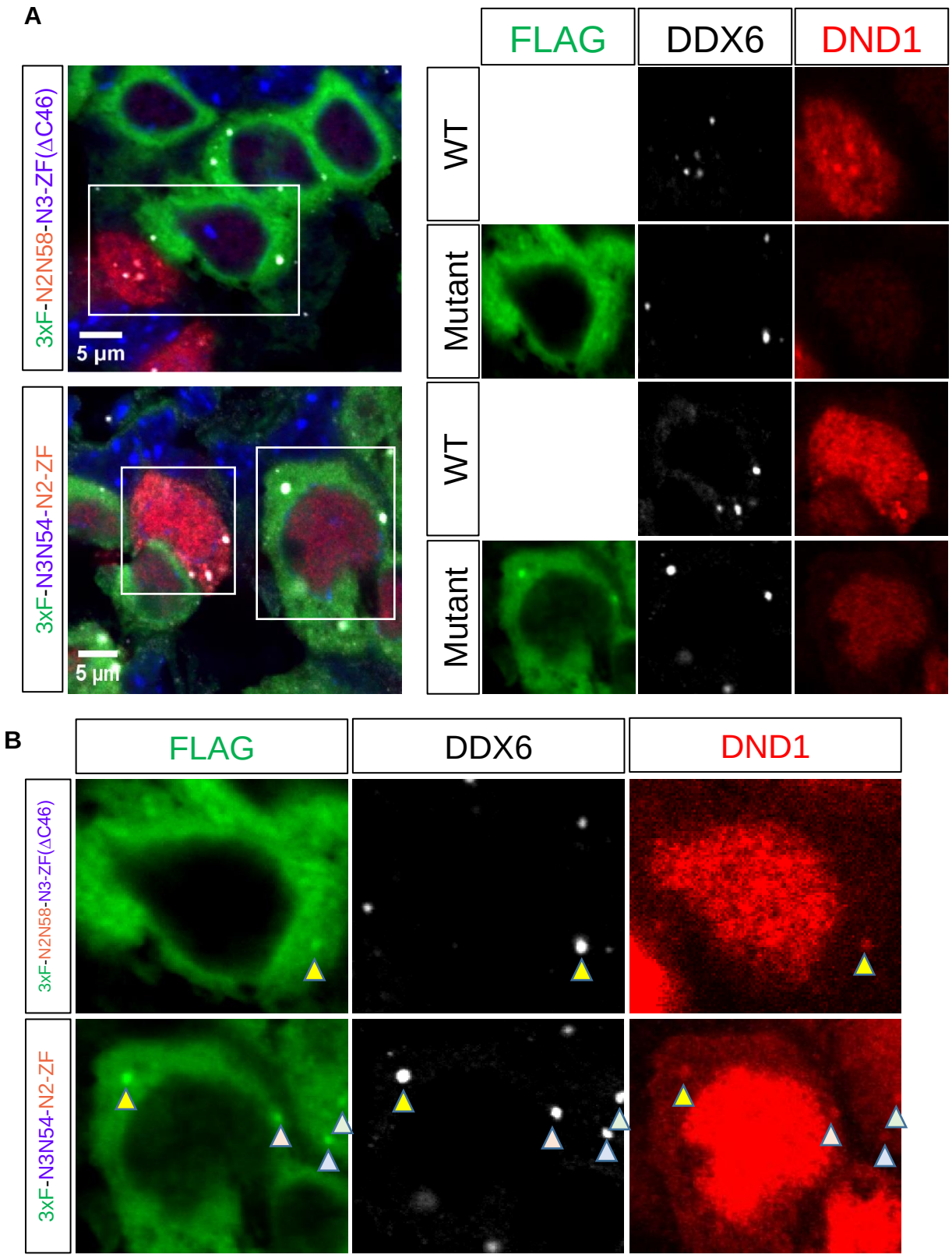

Fig. S4. A) Sections of testes from E15.5 3xF-Nanos2N58-Nanos3-ZF(ΔC46) (top) or 3xF-Nanos3N54-Nanos2-ZF (bottom) chimeras were stained with antibodies against FLAG (green), DDX6 (white) and DND1 (red). DNA was labeled with DAPI (blue). Scale bar 5 μm. B) The intensity of DND1 immunofluorescence images shown in A was intentionally increased to visualize granules. FLAG and DDX6 signals are unchanged from those in A. P-bodies merged with FLAG and DND1 are indicted by arrowheads.

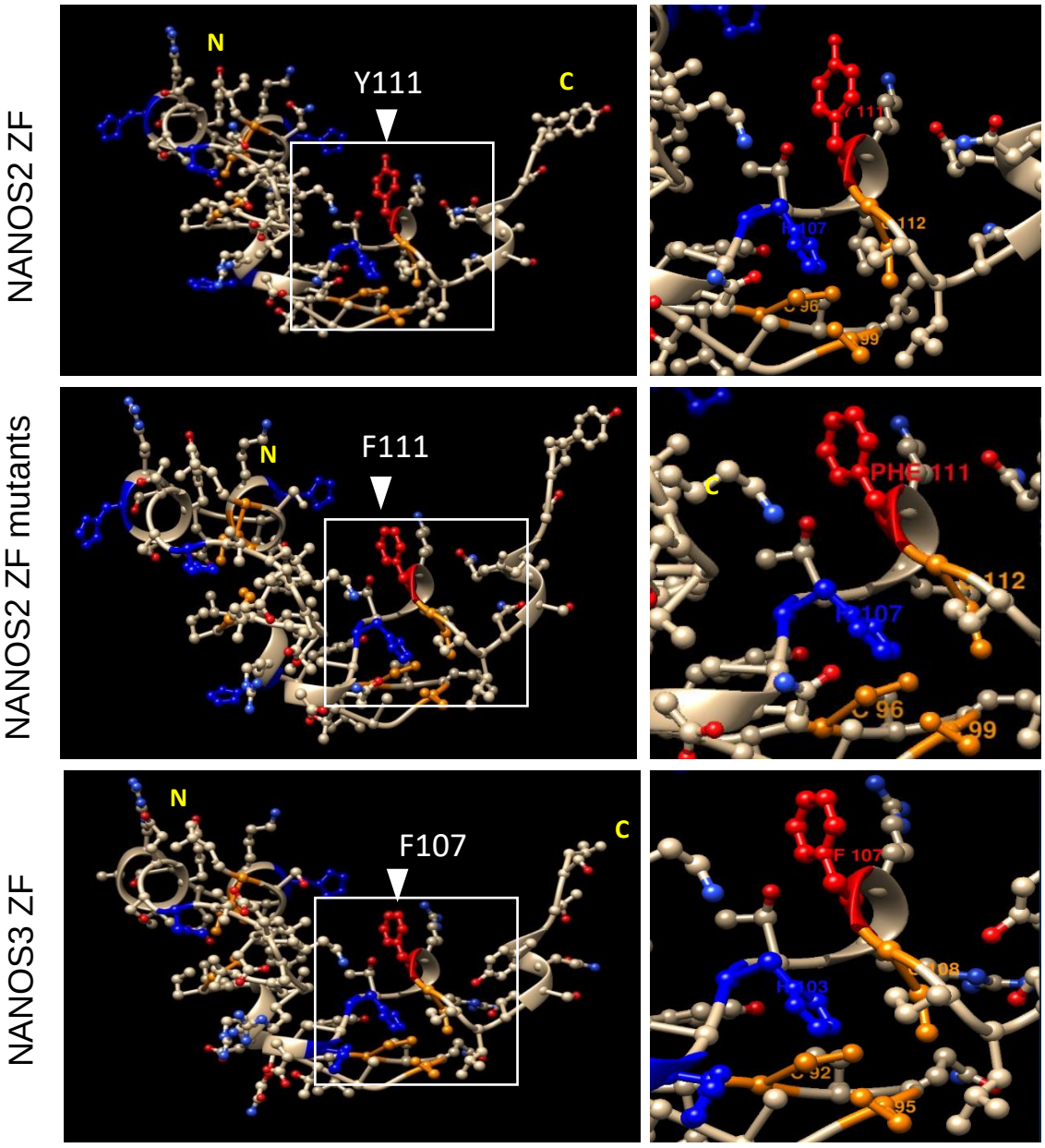

Fig. S5. Comparison of mouse NANOS2, NANOS2 Y111F and NANOS3 zinc finger domains. Three-dimensional models were created using amino acid sequences of the respective proteins in the Phyre2 engine. Orange indicates cysteine and blue indicates histidine comprising the CCHC motif. The red amino acid is the position equivalent to Y111 in NANOS2, i.e., F107 in mouse NANOS3. When NANOS2 Y111 is mutated to F111, the side chain orientation becomes similar to that of NANOS3 F107.

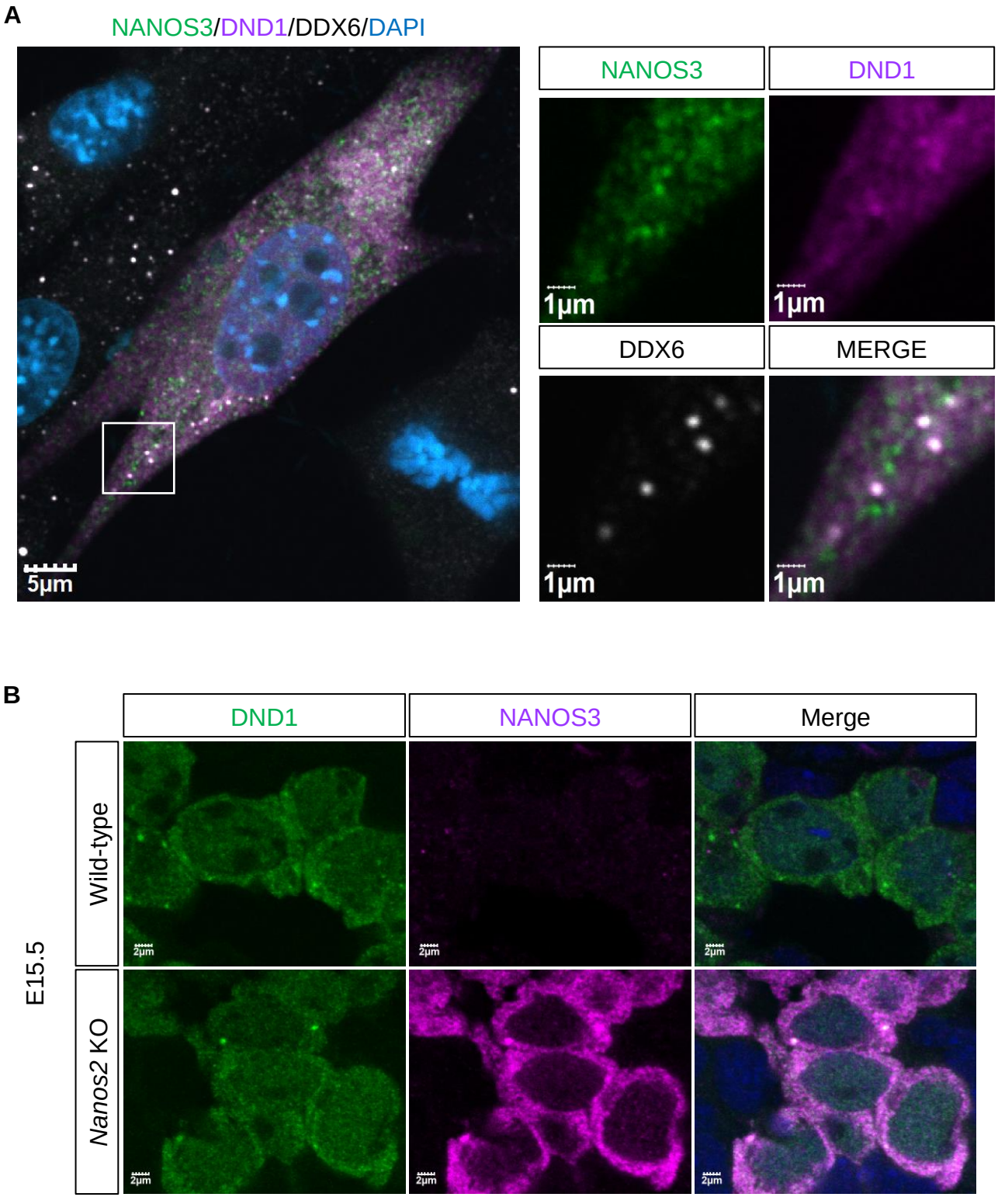

Fig. S6. A) Immunostaining of NIH3T3 cells expressing 3xF-NANOS3 (green) and HA-DND1 (magenta). P-bodies were stained with anti-Rck/p54 (white). DNA was stained with DAPI (blue). Enlarged images of the boxed area are shown on the right. Magnification 100x. Scale bars 5 µm, 1 µm. B) E15.5 WT and *Nanos2* KO testes were stained with antibodies against NANOS3 (magenta) and DND1 (green). DNA was stained with DAPI (blue). Magnification 200x. Scale bar 2 µm.