Izzo et al., https://doi.org/10.1083/jcb.201611012

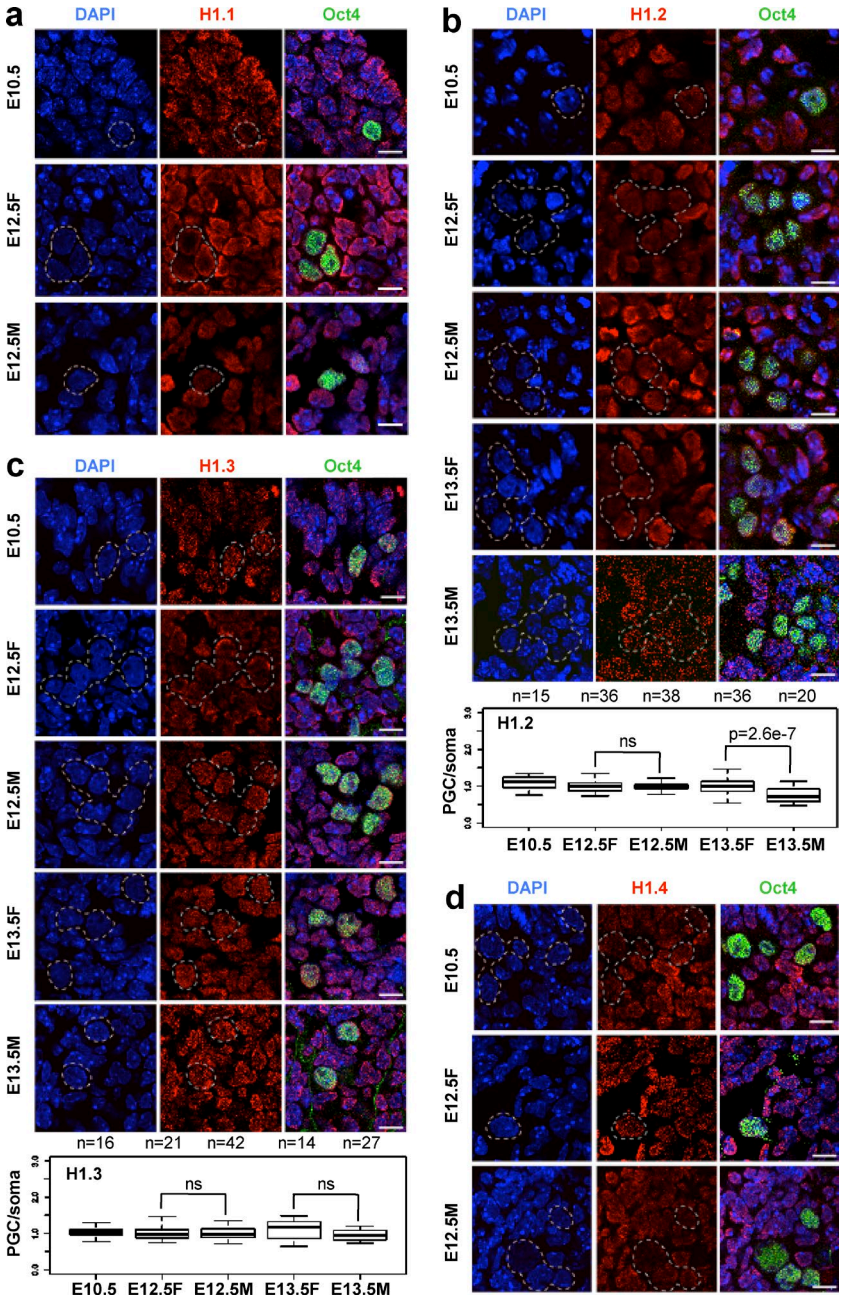

Figure S1. **H1 subtype (H1.1–H1.4) expression during PGC development.** (a) Immunofluorescence with anti-Flag antibodies (red) of cryosections of genital ridges from Flag-HA-H1.1 knockin embryos at E10.5 and E12.5; female (F) and male (M). (b and c) Immunofluorescence with anti-Flag antibodies (red) of cryosections of genital ridges from Flag-HA-H1.2 (b) and Flag-HA-H1.3 (c) at E10.5, E12.5, and E13.5. The box plots show the quantification of the signal levels of the indicated H1 subtypes in OCT4-positive cells relative to OCT4-negative cells (PGC/somatic cells [soma]) during development. Error bars correspond to the minimum and maximum values. (d) Immunofluorescence with anti-Flag antibodies (red) of cryosections of genital ridges from Flag-HA-H1.4 at E10.5 and E12.5. In all cases for the E12.5 and E13.5 time points, male and female genital ridges were stained separately. PGCs were identified using anti-OCT4 (green) antibody. DNA was counterstained with DAPI. Representative images from at least three genital ridges across three independent experiments for each stage analyzed are shown. Bars, 10 µm.

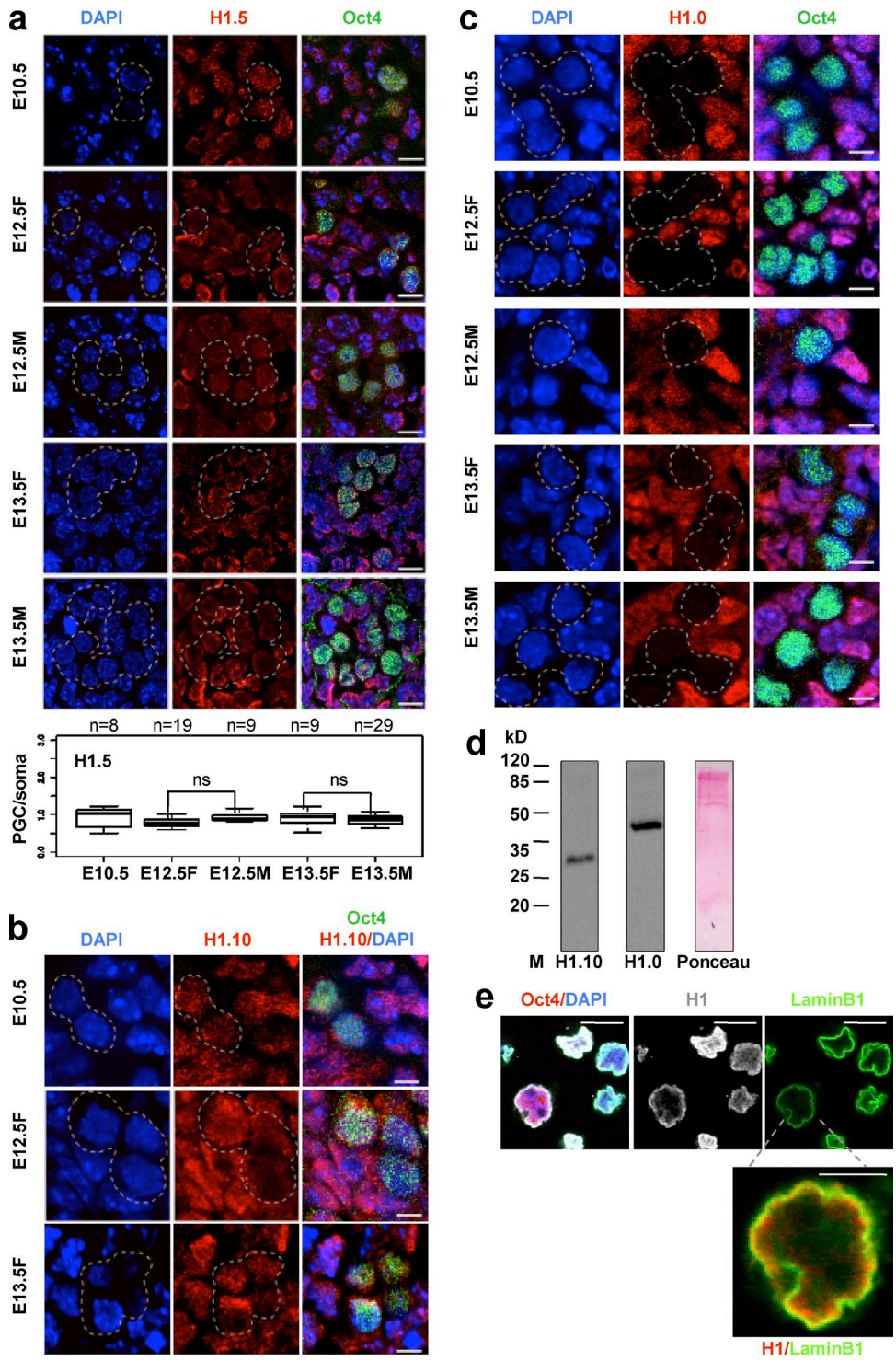

Figure S2. **H1 subtype (H1.5, H1.10, and H1.0) expression during PGC development.** (a) Immunofluorescence with anti-Flag antibodies (red) of cryosections of genital ridges from Flag-HA-H1.5 knockin male and female embryos at E10.5, E12.5, and E13.5. (b) Expression of H1.10 during PGC differentiation. Representative images of immunofluorescence using an anti-H1.10 specific antibody (red; see also Fig. S2 d) of cryosections of genital ridges from embryos at E10.5 and E12.5; female (F) and male (M). (c) Expression of H1.0 during PGC differentiation. The same as in Fig. S2 b, but an anti-H1.0 specific antibody was used. Genital ridges were embedded in paraffin. (d) Immunoblot analysis of whole-cell extract from MEFs with anti-H1.0 and anti-H1x antibodies. Note that these antibodies recognize a single band of the expected size. (e) Immunofluorescence staining of PGCs isolated from E11.25 embryos with anti-H1 and anti–lamin-B1 antibodies. Bars, 10 µm. In the higher magnification on the right, the H1 signal is displayed in the red channel showing colocalization with the laminB1 signal. Colocalization was observed in all the cells analyzed (n = 11). A representative image is shown. Bars: (A–C) 10 µm; (E) 5 µm.

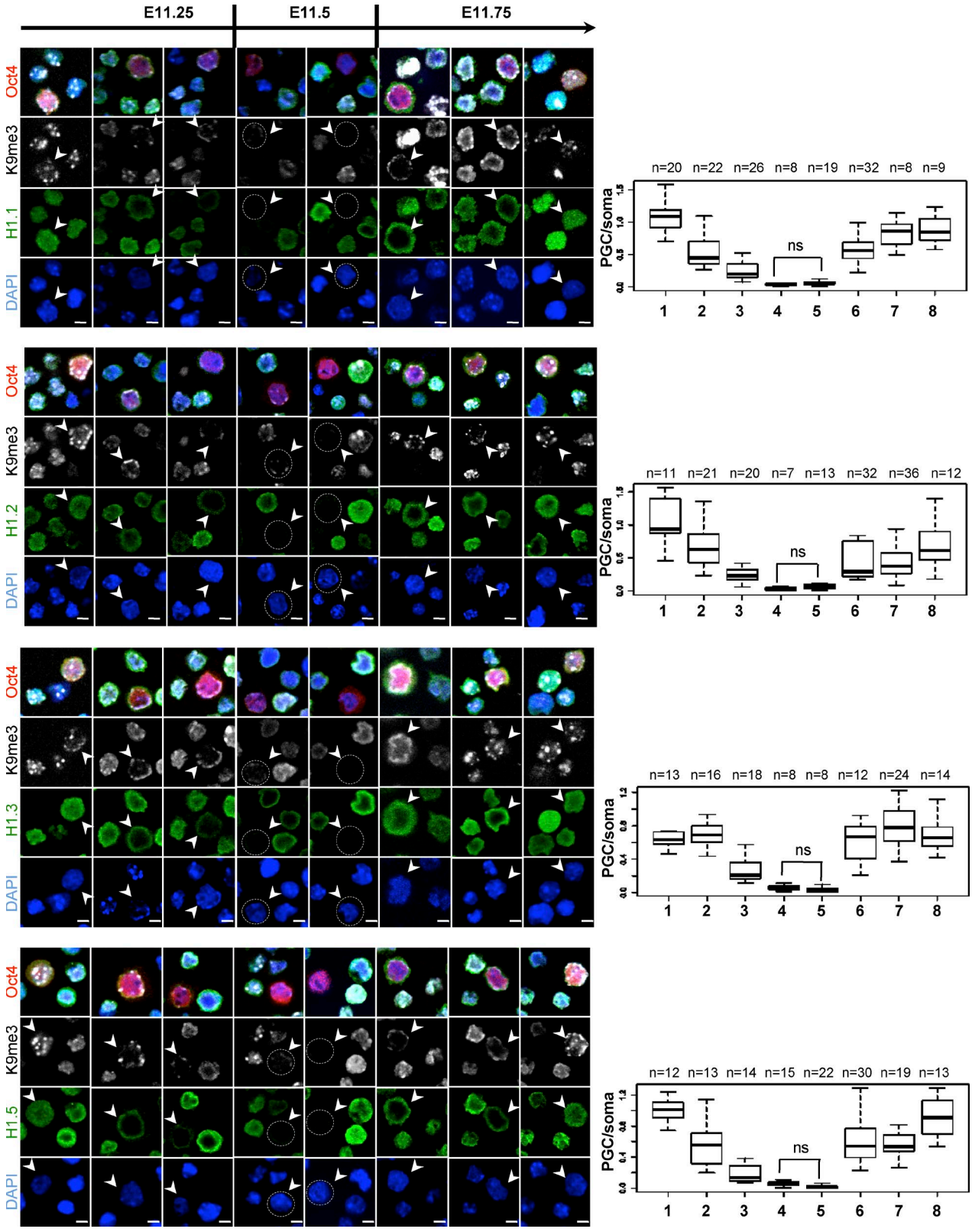

Figure S3. **Transient loss of somatic H1 subtypes at E11.5.** Kinetics of H1.1, H1.2, H1.3, and H1.5 disappearance from PGCs (arrowheads) isolated from genital ridges of embryos of the indicated Flag-HA-H1 knockin mice between early and late E11.5. OCT4 staining (red) was used as a germ cell–specific marker. H3K9me3 staining (gray) was used as internal control. The dotted line indicates the PGCs that have lost H1 signal. Bars, 10 µm. The box plots (right) show the expression level of the indicated H1 subtypes during the different stages (1–8) of epigenetic reprogramming in OCT4-positive cells relative to OCT4-negative cells (PGC/somatic cells). The p-values were calculated using a pairwise *t* test with Bonferroni correction. Only the p-values for stages 4 and 5 are shown; ns, not significant. p-values <0.05 were considered statistically significant. The number (*n*) of cells analyzed is indicated. Samples from several embryos from at least three independent litters were analyzed.