WT	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y21	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y21	CGTCAGGGAGGGGCGGGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y21	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y22	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y22	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y22	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y23	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y23	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y23	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y24	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y24	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y24	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y24	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y25	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y25	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y25	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y25	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y26	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y26	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y26	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA
Y26	CGTCAGGGCGGGGGGTGCTGCTAACGTGCTCCTGGTCCTGCAGGTGGGCTGCTGGCATTCGCTATGGAGGAGGACTGGGACACGGAGCTGGA

Table S1. Sequence of the non-targeted allele in Z^{GFP}Z PGC lines

The six independently targeted male PGCs lines show in Fig 1D were PCR amplified and sequenced at the non-targeted DDX4 allele to detect potential indel mutations. Red; TALEN binding sites.

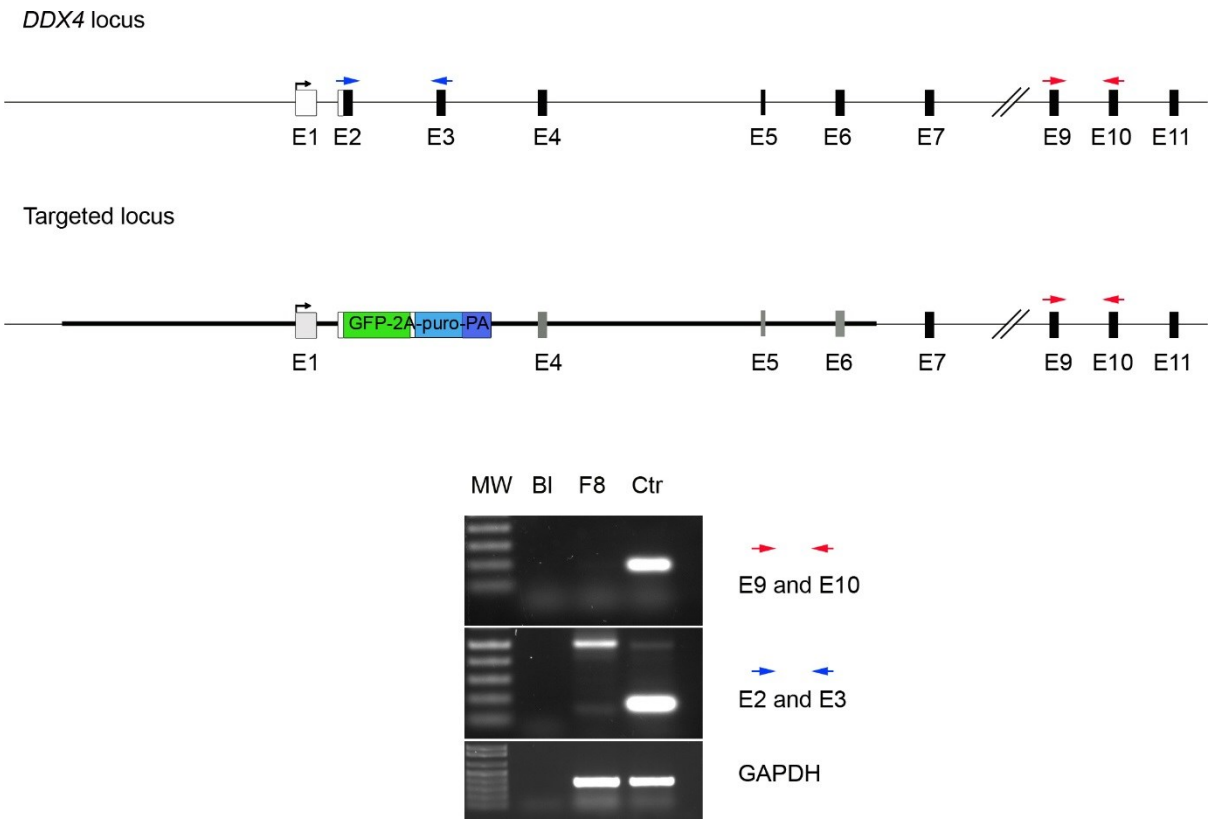

Figure S1. DDX4 expression in targeted female PGCs

RT-PCR was carried out on cDNA from cultured chicken cells lines. Bl, Blank, F8, *DDX4* targeted female cell line 8; Ctr, untargeted female cell line 8.

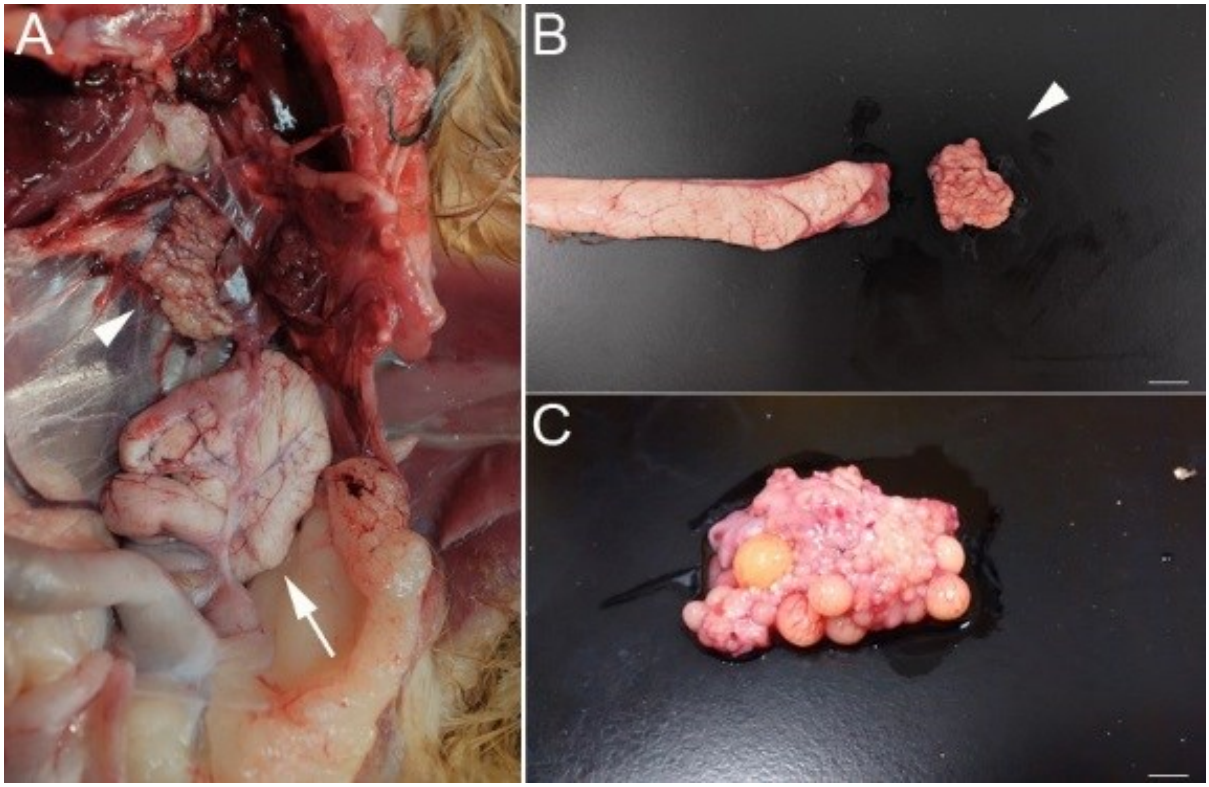

Figure S2. Follicles are absent in $Z^{GFP}W$ hens.

(a) Oviduct (arrow) and immature ovary (arrowhead) in a 29 week old $Z^{GFP}W$ hen.

(b) Ovary (arrowhead) of a 29 week old $Z^{GFP}W$ hen. Bar, 1 cm.

(c) Ovary containing developed follicles of a ZW control hen. Bar, 1 cm.

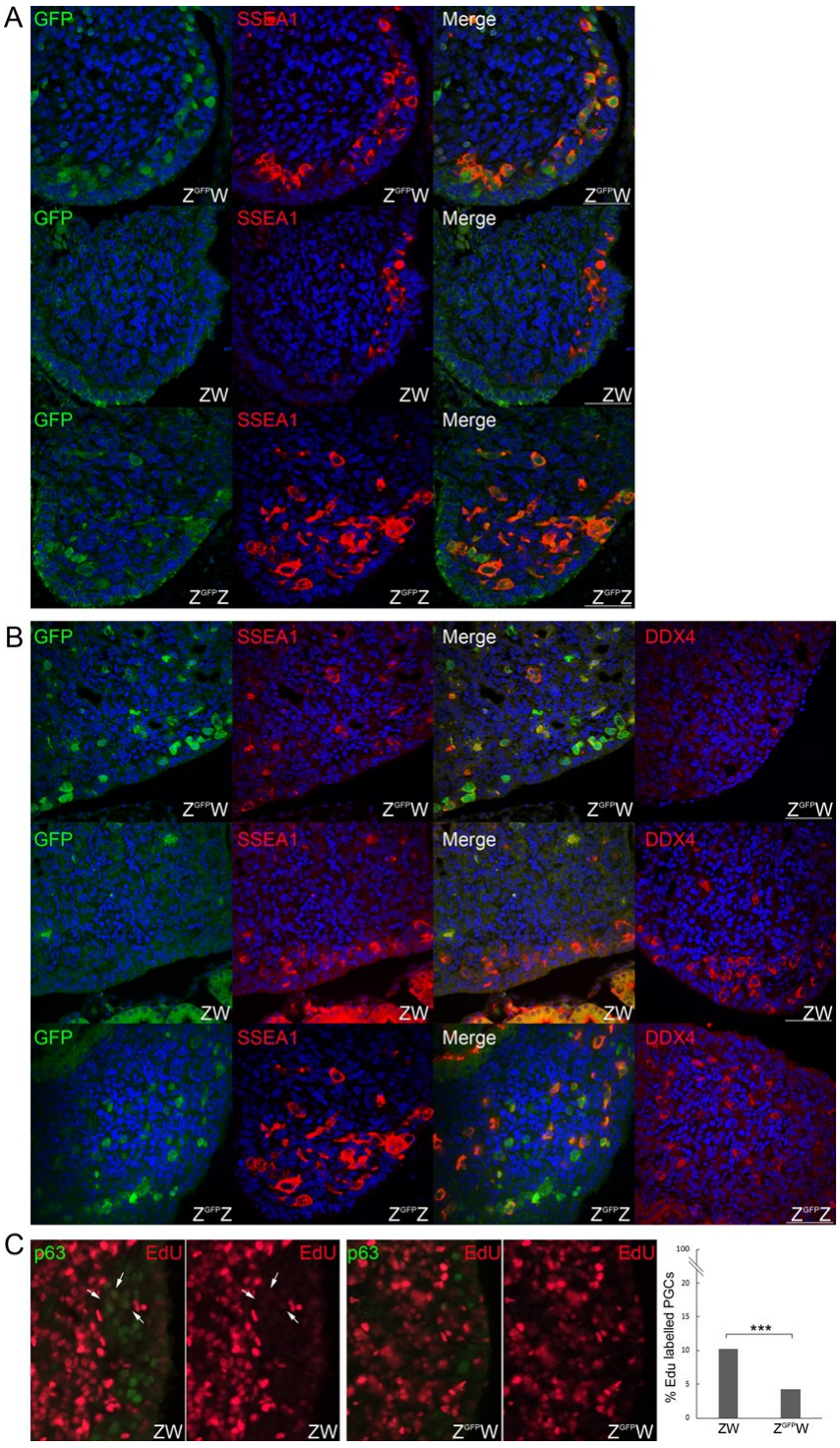

Figure S3. PGCs are present in $Z^{GFP}W$ embryos

Analysis of GFP fluorescence and immunostaining for SSEA1and DDX4 at A) day 6 and B) day 9 of incubation. Blue, nuclear stain. Scale bar, 100 μm.

C) Proliferation of PGCs in day 10.5 gonads using EdU labelling. Representative sections from ZW and $Z^{GFP}W$ embryos. PGCs were identified by immunostaining for p63 and were scored as EdU^+ or EdU^-. ***, $p < 0.001$ using a Fisher's exact test two-tailed P value.

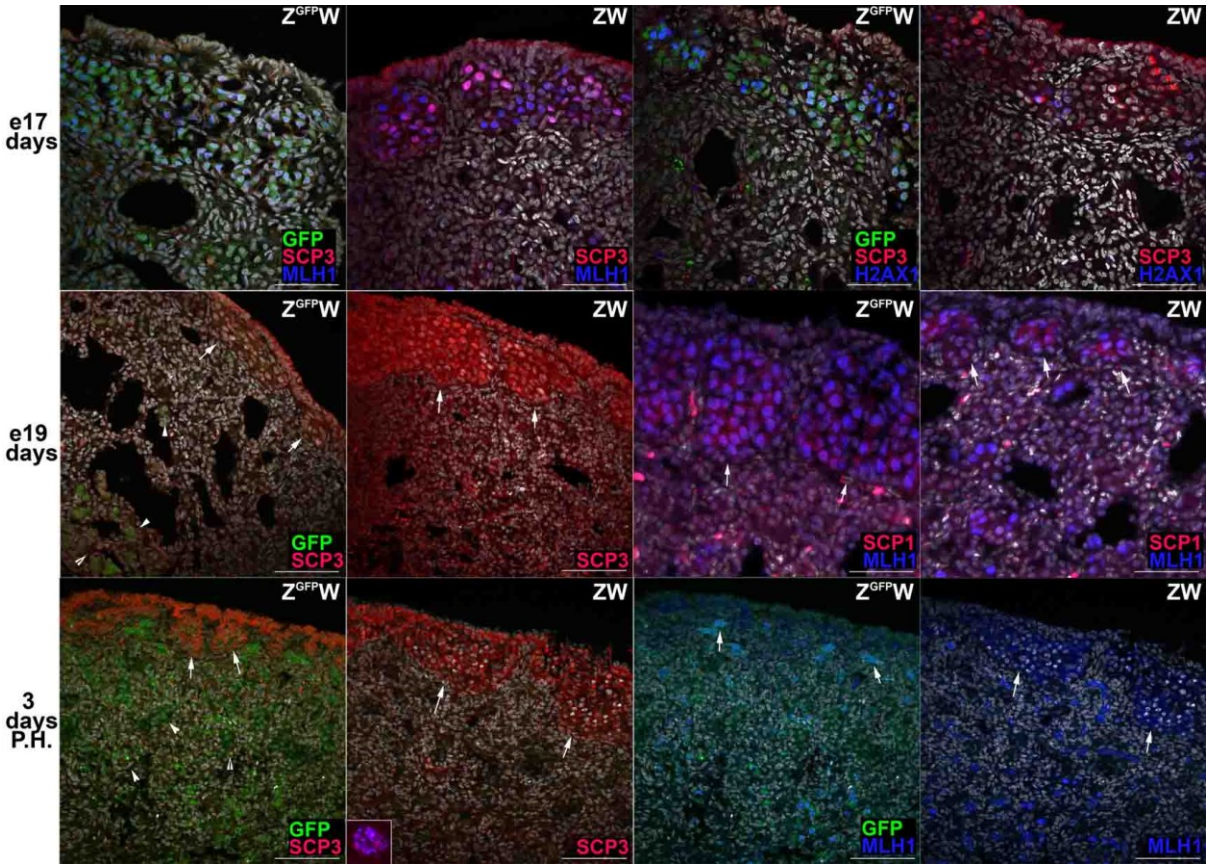

Figure S4. $Z^{GFP}W$ germ cells fail to progress through meiosis and are lost postnatally.

Immunostaining of SCP3, SCP1, γH2AX, and MLH1 and GFP fluorescence in embryonic day 17 and 19 (e17 and e19) embryos and 3 days PH chicks. Arrowheads indicate germ cells located in the medulla of the ovary, arrows indicate germ cells located in the cortex of the ovary. Insert in 3 days PH ZW image is the enlargement of a single cell showing punctate staining pattern of SCP3. Nuclear stain, white. Bar, 50 um.

Supplementary materials and methods:

Immunohistochemistry

Primary antibodies were: rabbit anti-DDX4 antibody (kind gift from Craig Smith (Monash University, Melbourne), SCP1 (Novus, NB300-229, rabbit, 4 µg/ml), SCP3 (Abcam, ab15093, rabbit, 3 µg/ml), MLH1 (BD, 550838, mouse, 5 µg/ml), γH2AX (Millipore, 05-636, mouse, 3 µg/ml), p63 (Abcam, ab124762, rabbit, 5 µg/ml). Sections were washed with PBT for 30 min, and incubated with secondary antibodies conjugated with Alexa-Fluor 488 555, or 647 (Thermo Fisher Scientific, 4 µg/ml) for 1 h at room temperature. Samples were washed PBT for 30 min, and stained with Hoechst (Sigma) to visualize nuclei. Cells were mounted under coverslips in PBS and cryosections were mounted in Diamond Gold (Thermo Fisher Scientific) and visualized using a Zeiss LSM 710 inverted confocal microscope. Images were captured using Zen Black software (Zeiss). For EdU staining 0.5 ml of 0.4 mM EdU solution was injected near the amniotic sac of day 10.5 embryos. The eggs were sealed, incubated for 6 hours and the embryos were fixed and processed for immunohistochemistry. The Click-iT EdU Alexa Fluor 555 kit (Thermo Fisher Scientific, C10337) was used to stain and PGCs were then co-stained using the p63 antibody. Three embryos for each genotype were analyzed. Images were taken on a Leica DMLB microscope.

RT PCR analysis

Total RNA was isolated from control and targeted female PGCs using the RNeasy Mini Kit (Qiagen). Total RNA (500 ng) was reverse-transcribed. PCR conditions were 94 °C for 5 min, 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s for 30 cycles. PCR conditions for exon 2 and 3 reactions were 94 °C for 5 min, 94°C for 30 s, 50 °C for 30 s, and 72 °C for 1 min for 30 cycles Reaction products were resolved on 1% agarose gels and visualised using a transilluminator. Primer sequences were:

GAPDH: fwd, CCTCTCTGGCAAAGTCCAAG rev, CATCTGCCCATTTGATGTTG

Exon2&3: fwd, ATGGAGGAGGACTGGGACA rev, AGCCAAAGAAGGGGCTGT

Exon9&10: fwd, TCCATCTTTGCATGTTATCAGTCAGG rev, AATCCCGCCCTGCTTGTATAACAG