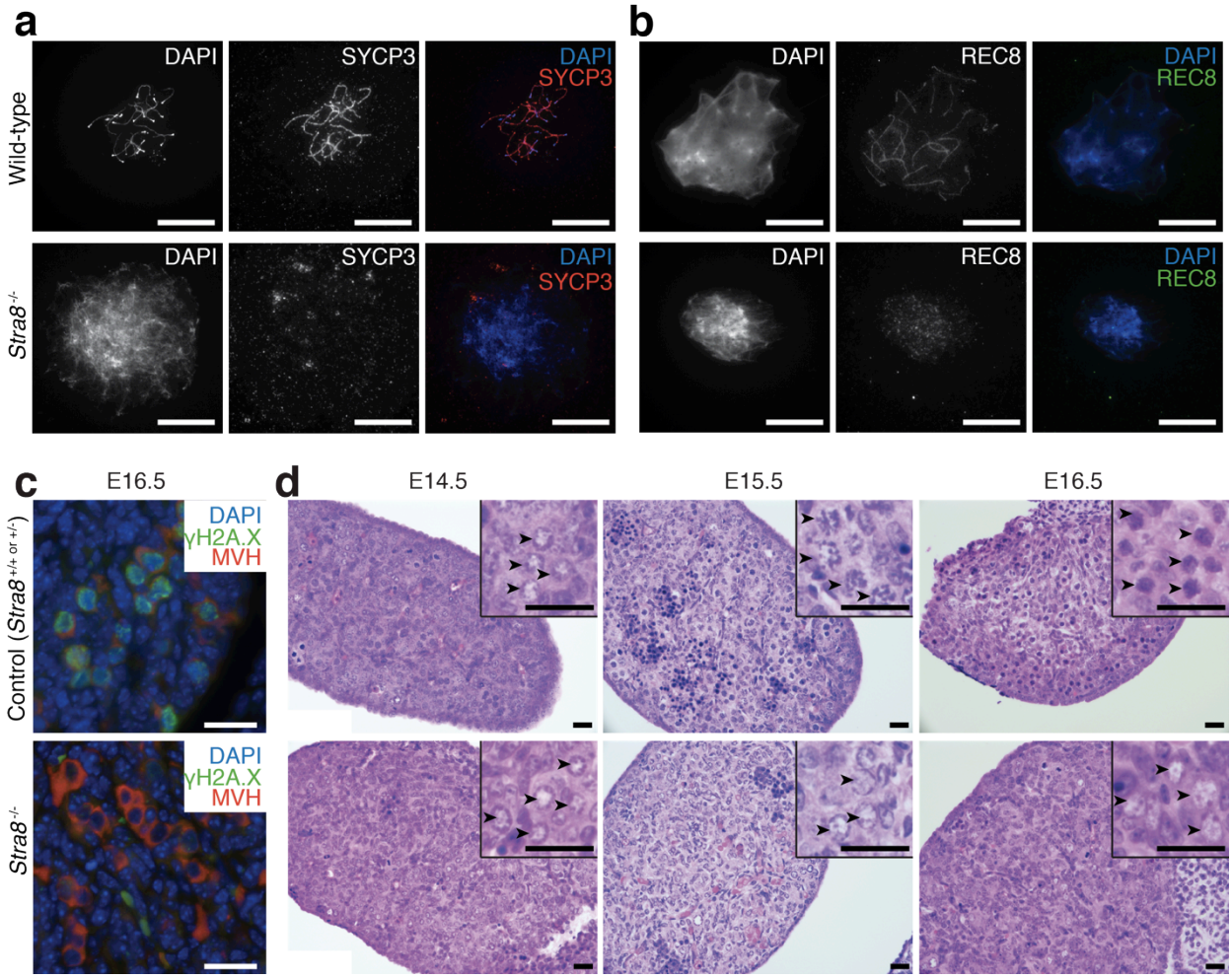
Supplementary information for:

Oocyte differentiation is genetically dissociable from meiosis in mice

Gregoriy A. Dokshin[1], Andrew E. Baltus[1], John J. Eppig[2] & David C. Page[1,*]

[1] Whitehead Institute, Howard Hughes Medical Institute, and Department of Biology, Massachusetts Institute of Technology, 9 Cambridge Center, Cambridge, Massachusetts 02142, USA.
[2] The Jackson Laboratory, Bar Harbor, ME 04609, USA.

[*]Correspondence: dcpage@wi.mit.edu



Supplementary Figure 1 *Stra8* is required for meiotic initiation in ovarian germ cells on C57BL/6 inbred background employed throughout this study.

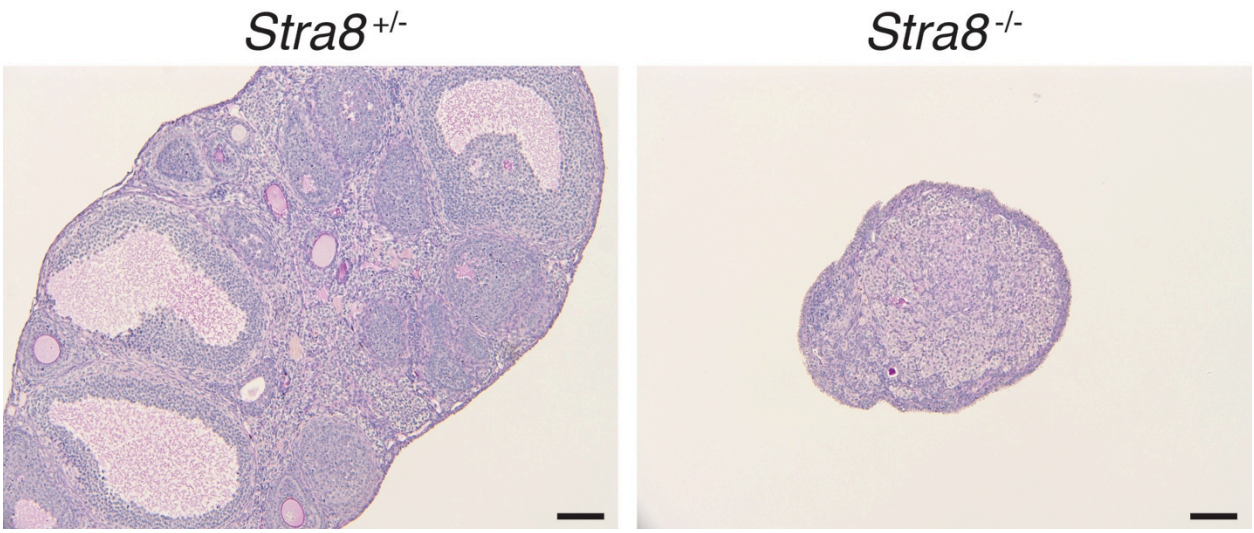
(**a**, **b**) Immunofluorescent staining of chromosome spreads of E16.5 wild-type and *Stra8*-deficient germ cells for (**a**) SYCP3 and (**b**) REC8 proteins. In merged images, DAPI is shown in blue, SYCP3 in red, and REC8 in green.

(**c**) Immunohistochemical staining for double-strand break response marker γH2A.X (nuclear), in green, and germ cell marker mouse vasa homolog (MVH; cytoplasmic), in

red, on sections of wild-type and *Stra8*-deficient E16.5 ovaries. In the wild-type ovary, γH2A.X signal is present in MVH-positive cells, while in the mutant the γH2A.X signal is absent from MVH-positive cells.

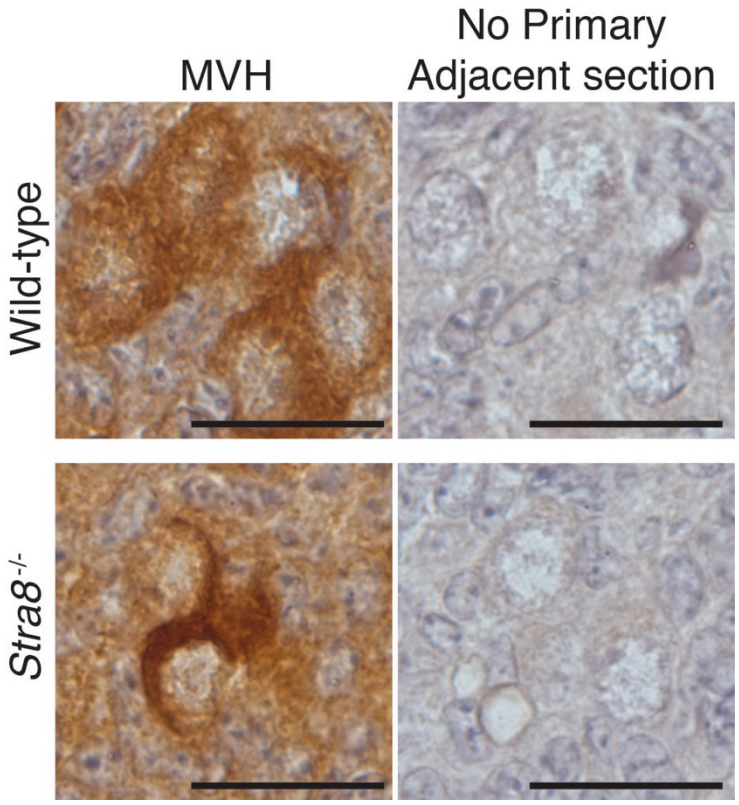
(**d**) Photomicrographs of sections from control (wild-type or *Stra8*-heterozygous) and *Stra8*-deficient ovaries at E14.5, E15.5, and E16.5 stained with hematoxylin and eosin. Insets show higher magnification and arrows indicate representative germ cells. While wild-type germ cells condense their chromosomes as they progress through meiotic prophase at E15.5 and E16.5, *Stra8*-deficient germ cells maintain pre-meiotic nuclear morphology.

Scale bars represent 20 μm.



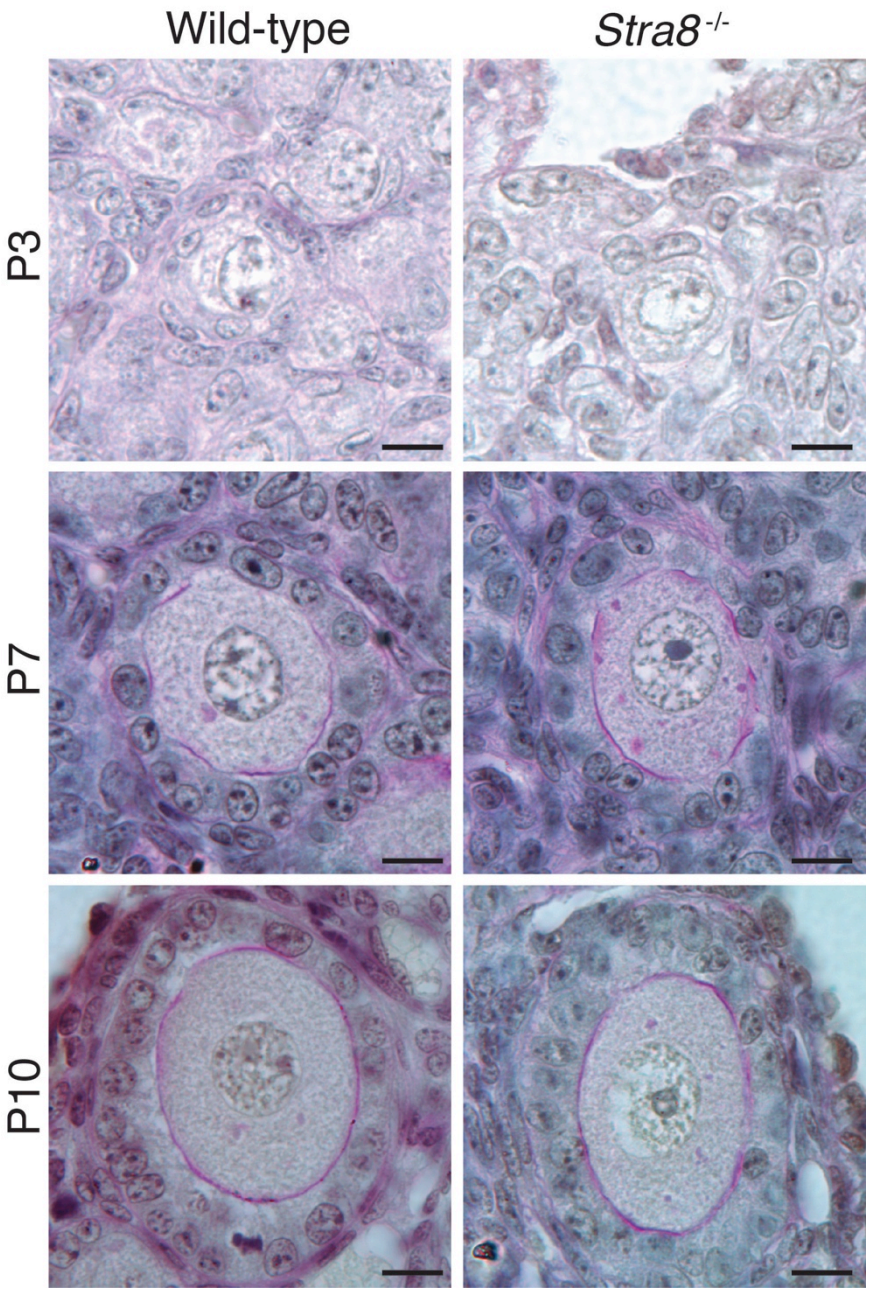
Supplementary Figure 2 *Stra8*-deficient ovaries are depleted of germ cells by six weeks of age.

Photomicrographs of sections from control (*Stra8*-heterozygous) and *Stra8*-deficient ovaries at six weeks stained with periodic acid-Schiff (PAS) and hematoxylin. *Stra8*-deficient ovaries are significantly smaller than control ovaries and contain no germ cells or follicle structures. Scale bars represent 100 µm.



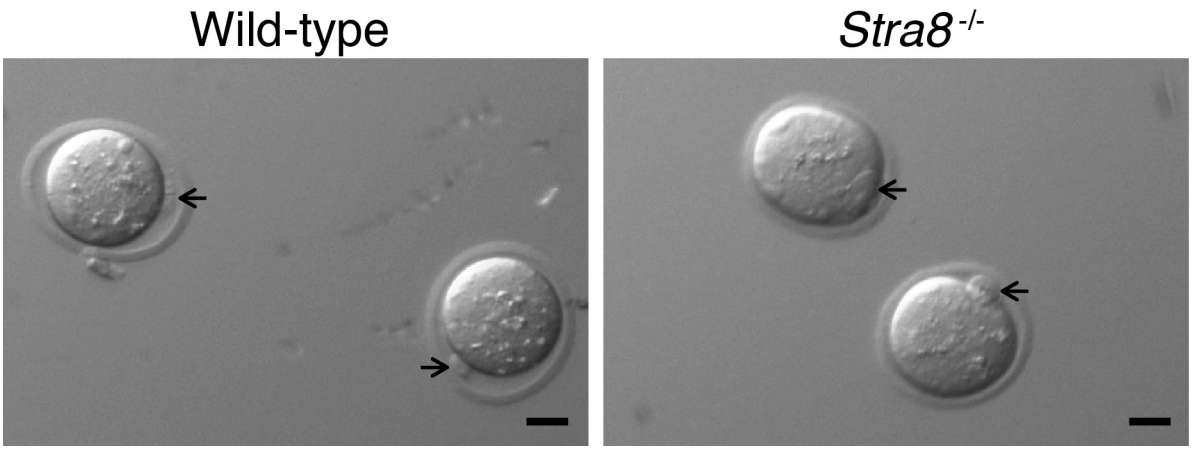
Supplementary Figure 3 *Stra8*-deficient ovarian germ cells survive postnatally.

Immunohistochemical staining of P2 wild-type and *Stra8*-deficient ovary sections for germ cell marker MVH; counter-stained with hematoxylin. As controls, sections adjacent to those stained for MVH were counter-stained with hematoxylin to reveal germ cell morphology but were not treated with primary antibody. Scale bars represent 20 μm.



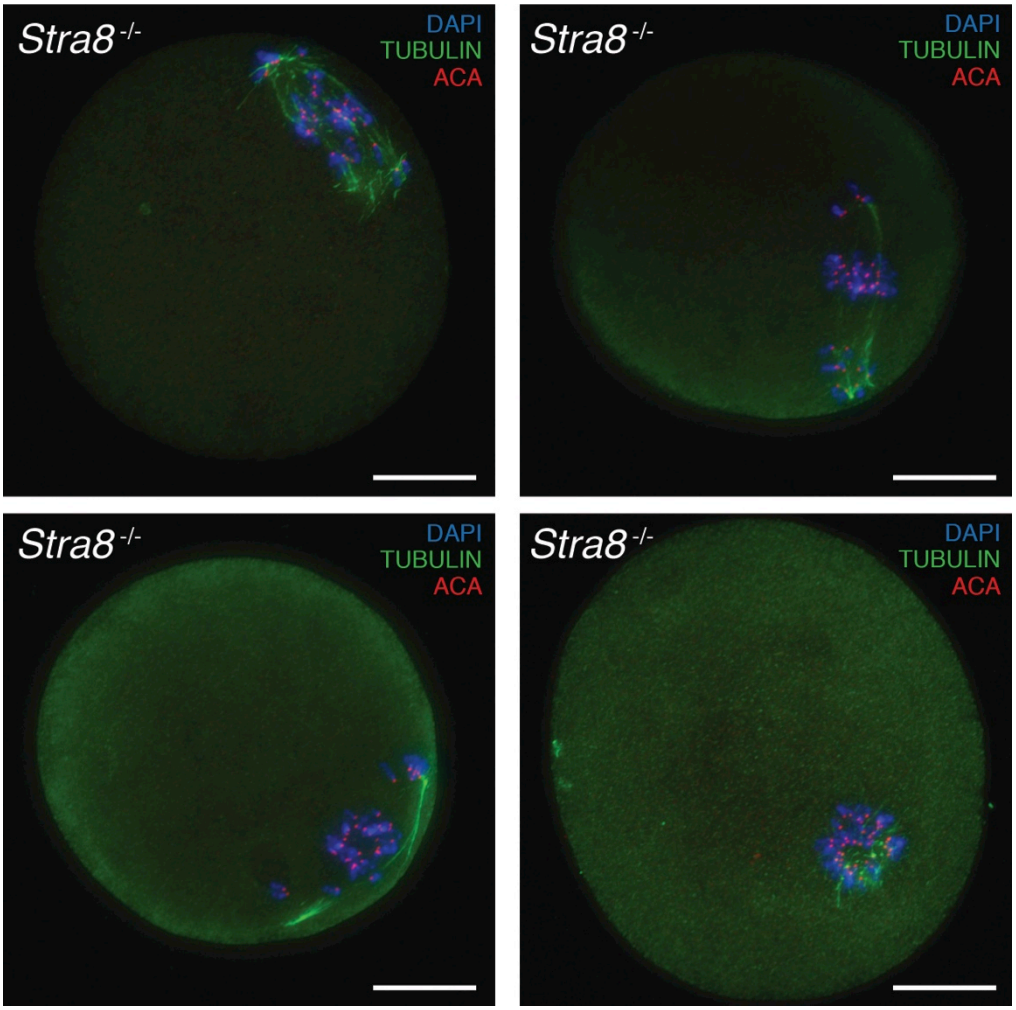
Supplementary Figure 4 In *Stra8*-deficient ovaries, germ cells grow and differentiate during first postnatal week.

Photomicrographs of sections from wild-type and *Stra8*-deficient ovaries at P3, P7, and P10 stained with PAS and hematoxylin. Scale bars represent 10 μm.



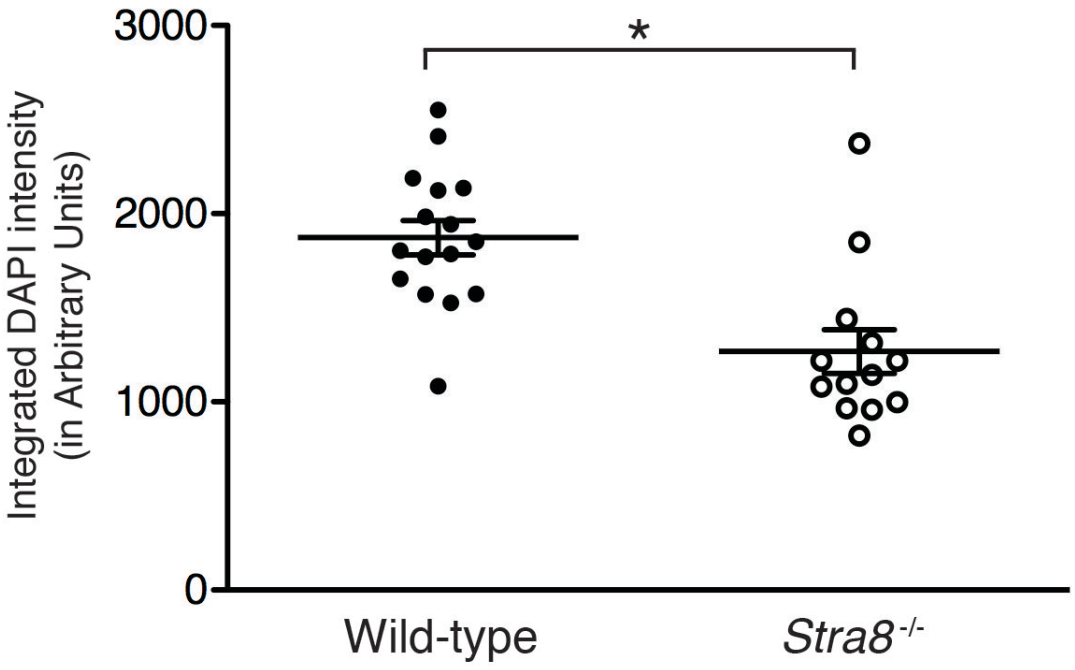
Supplementary Figure 5 *Stra8*-deficient oocyte-like cells form polar bodies upon ovulation.

Photomicrographs of ovulated wild-type oocytes and *Stra8*-deficient oocyte-like cells following cumulus cell removal by hyaluronidase treatment. Arrows indicate polar bodies. Hyaluronidase treatment is necessary to free ovulated oocytes from the sticky cumulus cell mass but has been suspected to induce artificial polar body extrusion[40]. To exclude the possibility of artificial polar body extrusion we also performed *in vitro* maturation experiments (Fig. 5). Scale bars represent 20 μm



Supplementary Figure 6 Some *Stra8*-deficient oocyte-like cells exhibit spindle assembly defects resulting from absence of meiotic prophase.

Deconvolved, projected Z-stacks of images of *in vitro*-matured *Stra8*-deficient oocyte-like cells that underwent GVB but failed to extrude a polar body. Cells were immunofluorescently labeled with anti-tubulin antibody (green) and anti-centromere antibody (ACA) (red). Chromosomes stained with DAPI (blue). In these oocytes the spindles are abnormally elongated with chromatids scattered along the spindle. Scale bars represent 20 μm.



Supplementary Figure 7 *Stra8*-deficient oocyte-like cells contain significantly less DNA than wild-type oocytes at GV stage.

Integrated DAPI intensity (in arbitrary units) of GV-stage wild-type oocytes and *Stra8*-deficient oocyte-like cells. Error bars represent standard error of the mean (SEM). *$p < 0.001$ (Wilcoxon test). Note: DAPI intensity depends in part on the degree of chromatin condensation and, therefore, may not track linearly with DNA content when cells whose chromatin condensation differs are compared.