Wu et al., http://www.jcb.org/cgi/content/full/jcb.200907047/DC1

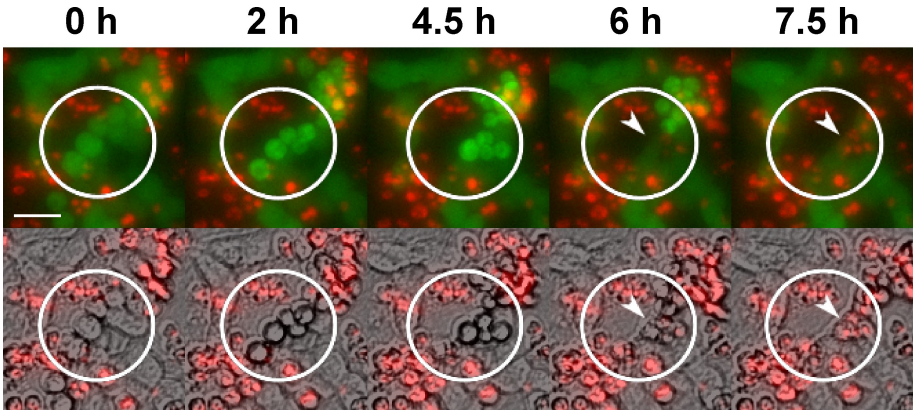


Figure S1. **A mortal population persists in long-term rat germ cell cultures.** Frames selected from a multichannel time-lapse sequence of normal GCS-EGFP germ cells that had been maintained in culture for >5 mo. (top) A field containing live germ cells expressing EGFP (green) and dead germ cells that have taken up the fluorescent cell death marker ethidium dimer (red). (bottom) Transmitted light images of the same field at each time point are overlaid with the ethidium dimer fluorescence channel. The circles highlight a group of four germ cells in the middle that died during the course of the time-lapse sequence. The first signs of cell death appear at 2 h, when the cells rounded up and their EGFP fluorescence became brighter. By 6 h, these cells had lost their EGFP fluorescence even though their cell bodies were still visible in brightfield images (arrowheads). Faint ethidium dimer fluorescence can be discerned in these cells at this time point. By 7.5 h, uptake of ethidium dimer had become evident. Bar, 20 µm.

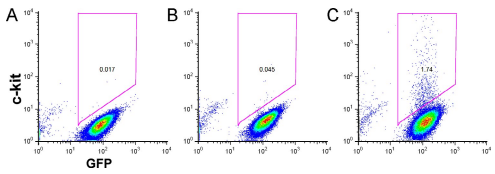
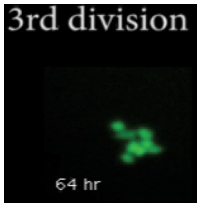
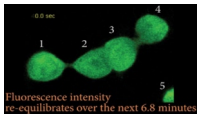


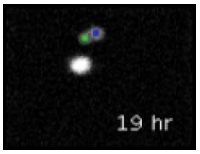
Figure S2. **Flow cytometric analysis of cultured GCS-GFP rat germ cells for c-kit expression.** Aliquots of 100 µl of ~1 × 10^6 cell suspension were treated with 0.5–1 µg of aphycoerythrin-conjugated rat monoclonal anti-c-kit antibody (Abcam, Inc.) or 0.5–1 µg of aphycoerythrin-conjugated rat IgG2b isotype control (Abcam, Inc.). (A) Unstained. (B) Control immunoglobulin. (C) Antibody specific to c-kit. Numbers in the gates specify the event percentage of total live germ cells analyzed.



Video 1. **Synchronized cell division and death in a clone of germ cells in culture.** Cultured GCS-EGFP rat germ cells (green) were freshly plated right before imaging, and were maintained at 37°C with humidity control and 5% CO_2 perfusion during time-lapse imaging with a DeltaVision RT microscope (Applied Precision). Frames were captured every 30 min. Approximately 72 h are shown in this video. Still images and detailed analysis of the video are shown in Fig. 3 B.



Video 2. **EGFP exchange between germ cells in culture through intercellular bridges.** GCS-EGFP rat germ cells (green) were cultured for 5 d before analysis. Visible intercellular bridges are labeled with arrowheads. Cell No. 2 was photobleached at time zero for 46.5 s by a confocal microscope (LSM510; Carl Zeiss, Inc.). Then, images were taken every 10 second for another 6 min. The quantitative analysis of the fluorescence intensity in each cell over time is shown in Fig. 3 D. The time compression ratio is 200:1.



Video 3. **Daughters of a single SSC dynamically sample the same microenvironment following their birth and before they divide again to form two clones of germ cells with distinct fates.** Cultured GCS-EGFP rat germ cells (green) were freshly plated right before imaging, and maintained at 37°C with humidity control and 5% CO_2 perfusion during time-lapse imaging by a DeltaVision RT microscope (Applied Precision) for 18 d. Frames shown were taken every 30 min. Approximately 98 h are shown in this video. The mother SSC is labeled by a blue dot. After the mother SSC's division at the eighth hour, one of its daughter cells is labeled with a blue dot, and the other with a green dot. A fate map of these two daughter cells' progeny is shown in Fig. 4 B, and the quantitative analysis of their relative positions over time is shown in Fig. 4 (C and D) The time compression ratio is 43,160:1.

Table S1. **Mortal germ cells in culture die synchronously as sibling groups**

Death	Sibling cells	Groups	Total cells
Synchronized	2–4	28	93
	5–8	18	134
	9–16	5	85
Isolated	NA	NA	2

A freshly plated culture was imaged by long-term time-lapse microscopy to record germ cell lineage and cell death as described in Results. From day 4–7, among germ cells that have divided at least once, cell deaths were scored and classified according to their synchrony among sibling cells. 99.4% of the cell deaths observed was synchronized; 0.6% were isolated.