Luteinizing hormone causes MAP kinase-dependent phosphorylation and closure of connexin 43 gap junctions in mouse ovarian follicles: one of two paths to meiotic resumption

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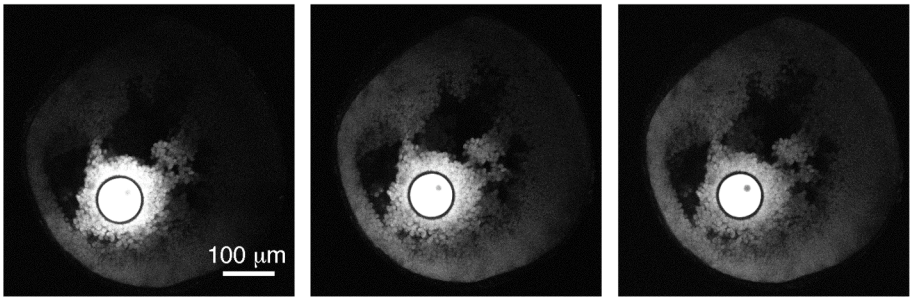

Fig. S1. Alexa-350 diffuses from the cumulus cells into the mural cells through the region of contact between the cumulus mass and mural epithelium. Images were taken at 10, 15, and 20 minutes after injecting 2.5 mM Alexa-350 into the oocyte of an antral follicle.

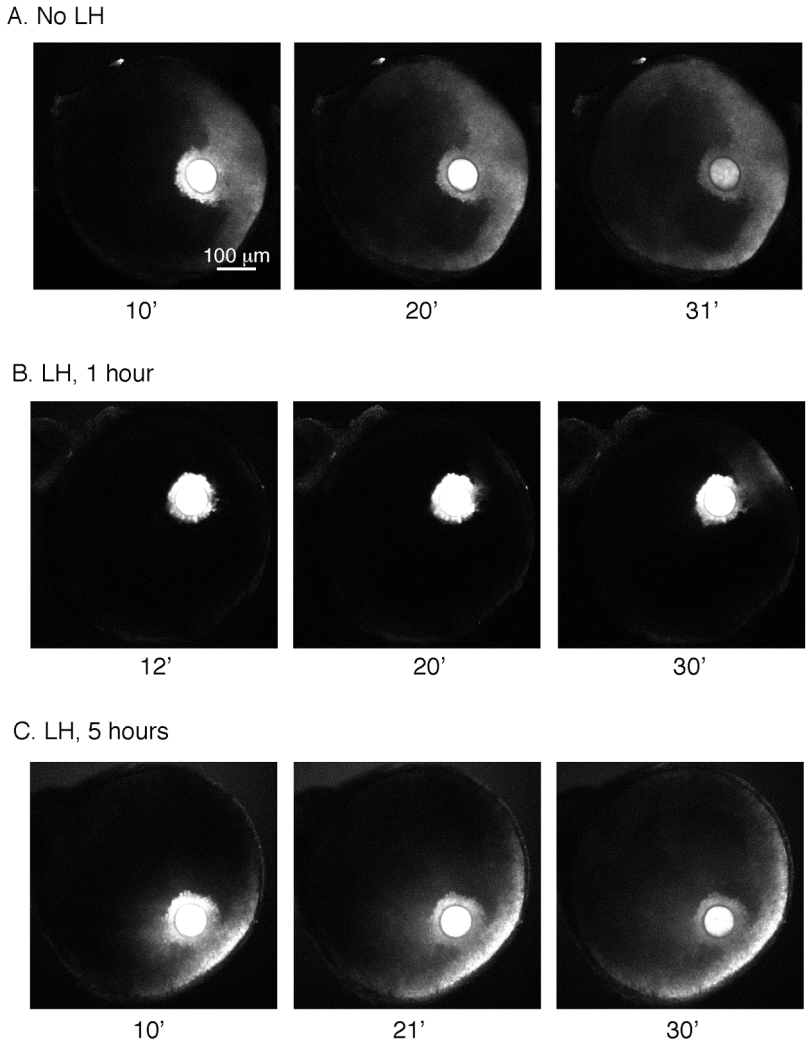

Fig. S2. In follicles that were isolated from PMSG-injected mice, LH causes a transient reduction in gap junction permeability, similar to that shown in Fig. 1 for follicles that were exposed to FSH in vitro. **(A)** No LH, images at 10, 20, and 31 minutes after injecting 5 mM Alexa-350 into the oocyte. The tracer diffuses from the oocyte into the cumulus mass, and then into the mural epithelium. Images are representative of 3 similar injections into oocytes in follicles without LH exposure. **(B)** 1 hour LH exposure, images at 12, 20, and 30 minutes after Alexa-350 injection. The tracer diffuses from the oocyte into the cumulus cells, but its diffusion into the mural cells is greatly reduced. Images are representative of 4/5 similar injections into oocytes in follicles that had been exposed to LH for 65-72 minutes. **(C)** 5 hours LH exposure, images at 10, 21, and 30 minutes after Alexa-350 injection. Images are representative of 3 similar injections into oocytes in follicles that had been exposed to LH for 5 hours.

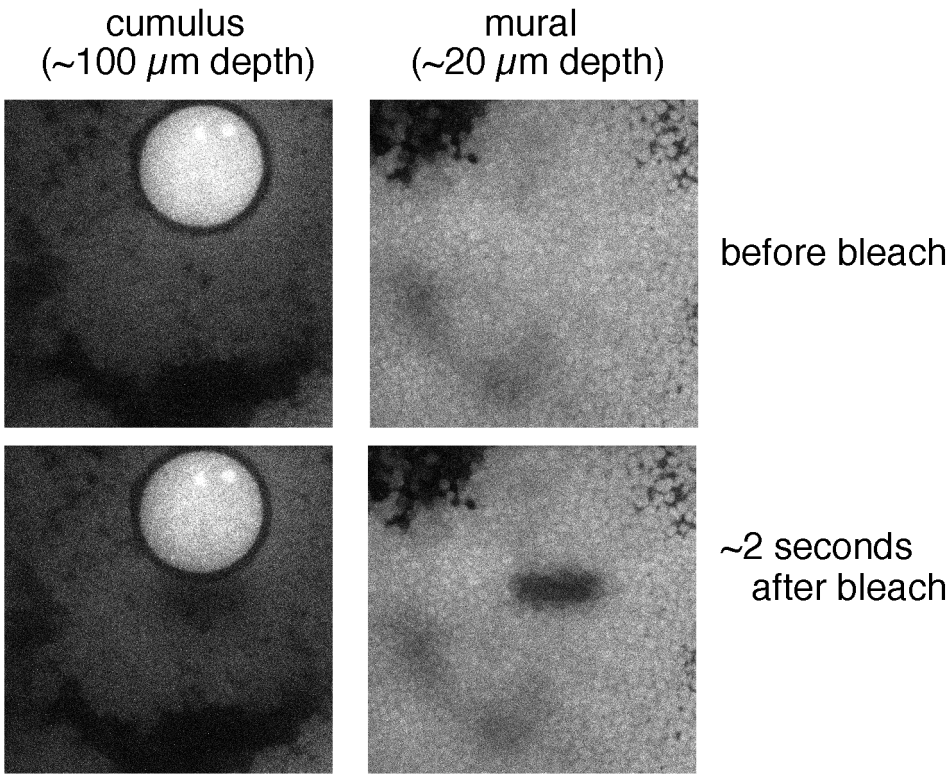

Fig. S3. Ineffective photobleaching in the cumulus cell layer. The images show a follicle in which the oocyte was injected 3 hours previously with Alexa-488 and which was then exposed to the full intensity of the 488 and 514 lines of an Argon laser for 4.5 seconds. When the beam was focused at the level of the cumulus cells (~100 μm deep, bottom left), the image was much dimmer, and the bleach was much weaker than when it was focused at the level of the mural cells (~20 μm deep, bottom right). The difference is due to light scattering in the specimen. We also attempted to photobleach using 2-photon excitation, since this method is better for imaging deep within tissues (Helmchen and Denk, 2005) and has the important additional advantage of restricting the excitation to a thinner optical section; however, we found that at the intensity level required for photobleaching, 2-photon excitation resulted in cell damage.

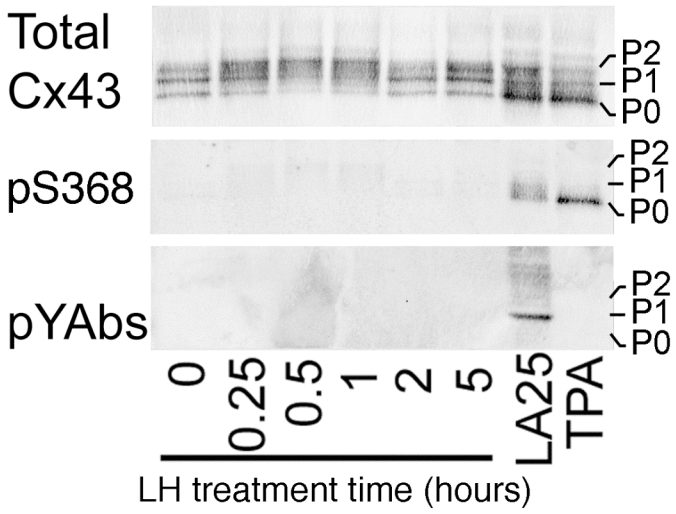

Fig. S4. LH does not lead to an increase in Cx43 phosphorylation at S368, Y247 or Y265. Immunoblots of follicles, ± LH for various times, were probed for total Cx43 and for phosphorylation on S368 or on Y247 and/or Y265 (the latter two antibodies were applied together). Positive controls include normal rat kidney (NRK) cells treated with 12-*O*-tetradeconylphorbol-13-acetate (TPA) or LA-25 cells that contain temperature sensitive v-src grown at the permissive temperature (Solan and Lampe, 2008). The migration positions of the different phosphorylation forms P0, P1, and P2 are marked. Similar results were obtained in 3 independent experiments.