ONLINE SUPPLEMENTARY MATERIAL (Norris et al., 2009)

Fig. S1. Concentration-response curves for cGi500 for cGMP and cAMP, measured in vitro using lysates from HEK293A cells expressing the sensor. 24 hours after calcium phosphate transfection, the cells were suspended in 5 mM Tris, 2 mM EDTA (pH = 7.4), disrupted with an Ultra-Turrax homogenizer (IKA-Werke GmbH & Co., Staufen, Germany) and centrifuged for 20 min at 80,000 rpm (see Nikolaev et al., 2004). Fluorescence emission spectra of the supernatant were measured as described in the Materials and Methods. The EC_{50} value for cGMP is 345 ± 16 nM (mean ± s.e.m., n = 3).

Fig. S2. The cyclic nucleotide sensors did not alter cAMP or cGMP concentrations in the oocyte. (**A**) The change in YFP/CFP emission ratio after injecting 1 mM cAMP into preantral follicle-enclosed oocytes was the same for oocytes containing 5 μM or 2 μM Epac2-camps300. Preantral follicles (140-180 μm diameter) were used for these experiments because they provided a technically easier system. (**B,C**) The cGMP concentration measured in antral follicle-enclosed oocytes did not depend on the amount of cGi500 protein that was expressed following RNA injection. Measured cGMP values were plotted as a function of the laser power required to get a CFP intensity of ~110-140 units. Lower laser power indicates that the oocyte image was brighter, corresponding to more cGi500 protein expression (although differential absorption of light by the follicle, which depends on the depth of the oocyte in the follicle, is another contributing factor). Data points correspond to the data sets shown in Fig. 5C, for no LH (**B**), or for 1-1.3 hours LH treatment (**C**).

Fig. S3. 5 μM of Epac2-camps300, or an approximately similar concentration of cGi500, did not change the kinetics of NEBD in response to LH. (**A**) Epac2-camps300. (**B**) cGi500.

Fig. S4. 10 μM U0126 inhibits LH-stimulated phosphorylation of MAP kinase in mouse antral follicles. Follicles were incubated under 3 different conditions: no treatment, 1 hour with LH, or 1 hour with 10 μM U0126 followed by 1 hour with 10 μM U0126 + LH. Follicle proteins were separated by SDS-PAGE and immunoblotted for phospho-MAP kinase. pMAPK band intensities were quantitated using a LICOR Odyssey system. Values obtained with LH or (U0126 + LH) treatment were divided by values obtained with no treatment, and the ratios are presented as mean ± s.e.m. These results were reported by Norris et al. (2008), but without the quantitation shown here. The immunoblotting and densitometry were done by Paul Lampe (Fred Hutchinson Cancer Research Center, Seattle, WA). Methods are described by Norris et al. (2008).

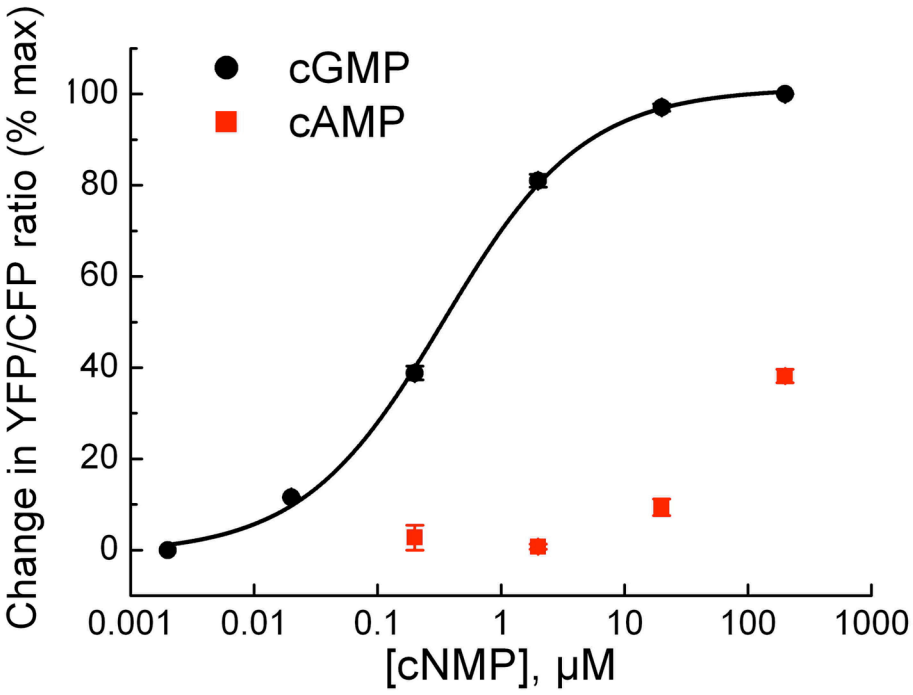

cGMP
cAMP
Change in YFP/CFP ratio (% max)
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
1000
[cNMP], μM

Fig. S1

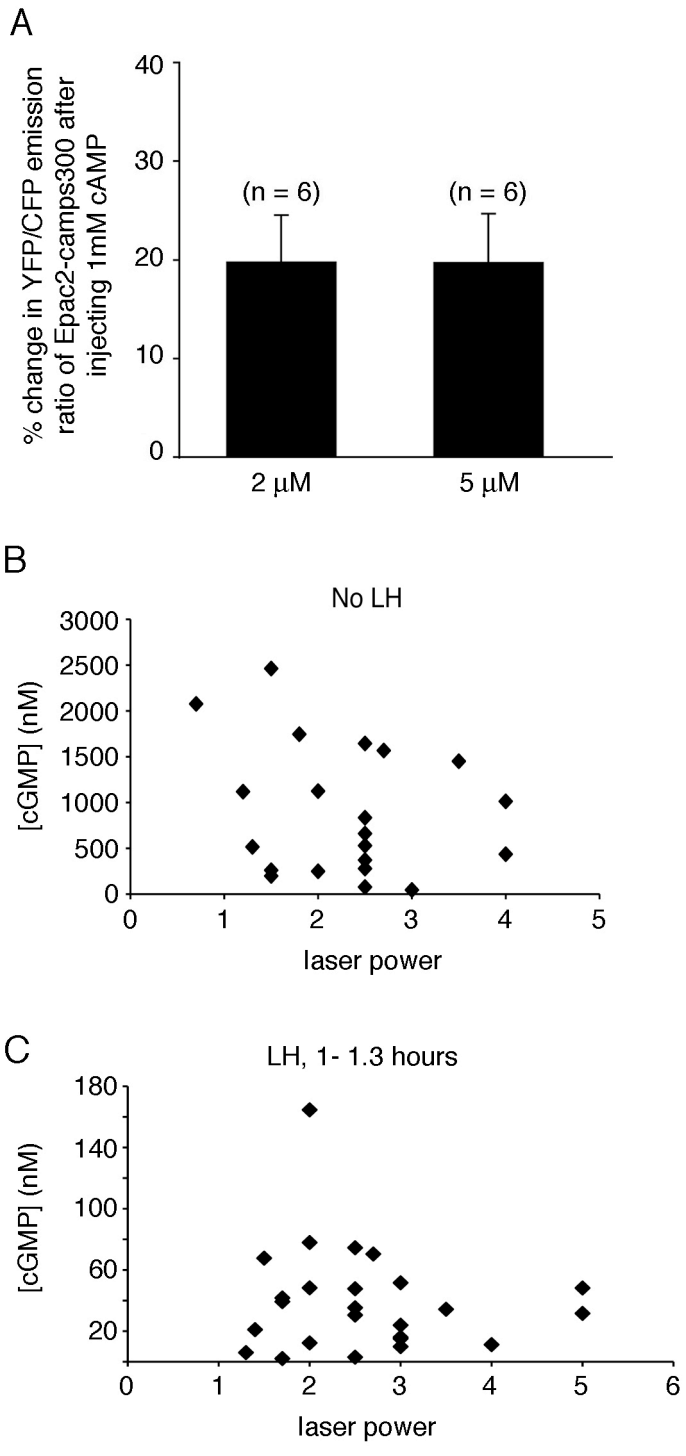
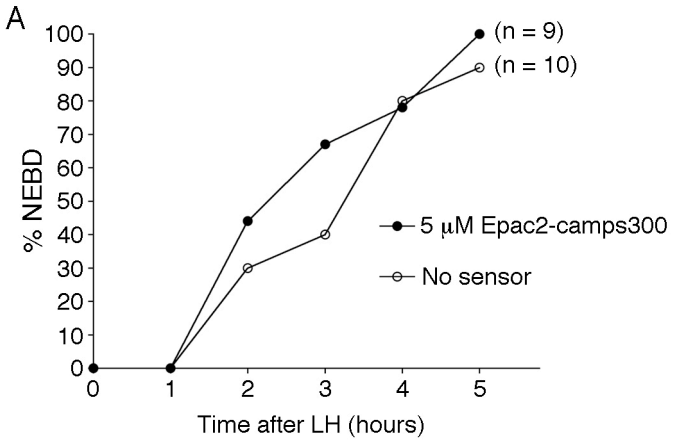

A
% change in YFP/CFP emission ratio of Epac2-camps300 after injecting 1mM cAMP
40
30
20
10
0
(n = 6)
(n = 6)
2 μM
5 μM
B
No LH
[cGMP] (nM)
3000
2500
2000
1500
1000
500
0
0
1
2
3
4
5
laser power
C
LH, 1- 1.3 hours
[cGMP] (nM)
180
140
100
60
20
0
1
2
3
4
5
6
laser power

Fig. S2


A
100
90
80
70
60
50
40
30
20
10
0
% NEBD
0
1
2
3
4
5
Time after LH (hours)
(n = 9)
(n = 10)
5 μM Epac2-camps300
No sensor

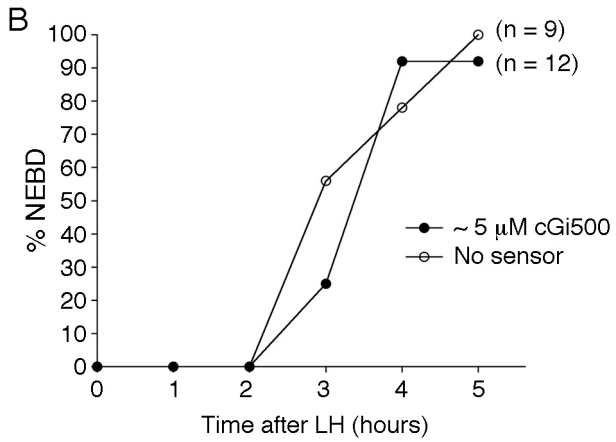

B
100
90
80
70
60
50
40
30
20
10
0
% NEBD
0
1
2
3
4
5
Time after LH (hours)
(n = 9)
(n = 12)
~ 5 μM cGi500
No sensor

Fig. S3

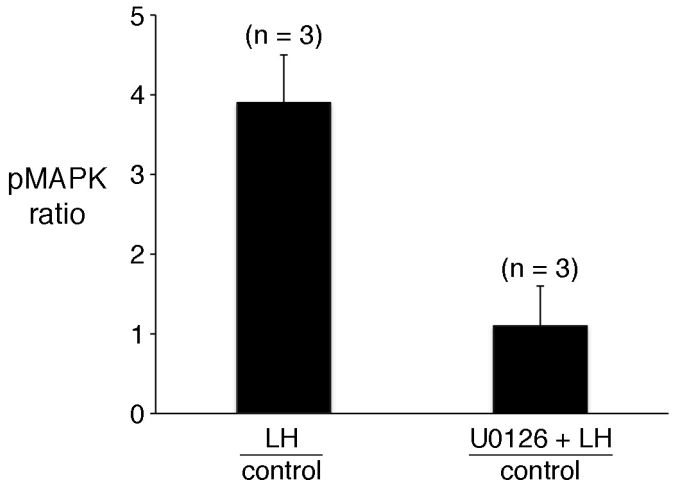


Fig. S4