

Supplementary Figure Legends.

Supplementary 1. Western blots of mouse eggs using the MAPK antibody. In A, lane 1 used 50 control eggs, and lane 2 used 50 eggs that were injected with MAPK_{AR} cRNA and incubated for 4hr. In B a similar experiment is shown where lane 1 shows a blot of 50 control eggs, and then two there two more separate groups of 50 eggs that were injected with MAPK_{AR} cRNA and then either treated (+) or not treated with (-) U0126. The methods are as described in Fig.2. The relative levels of MAPK_{AR} are much less than the endogenous ERK1/2 which can be seen as doublet in both blots.

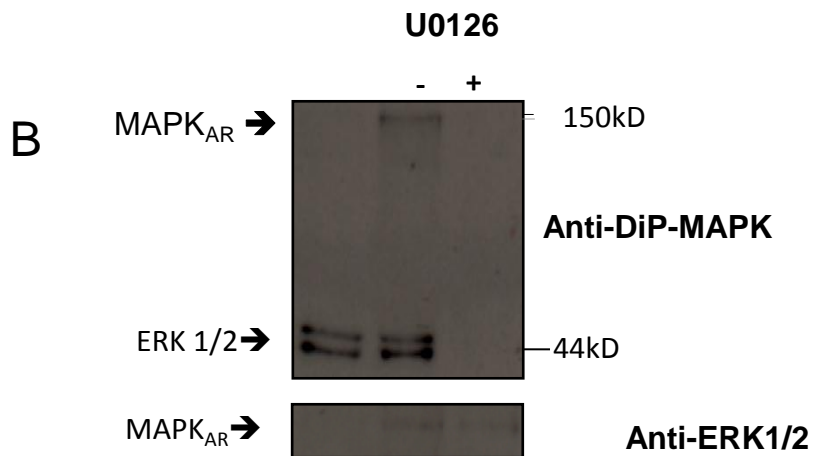
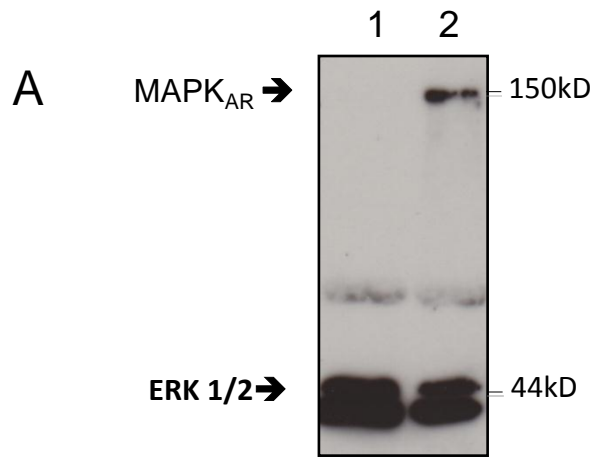
Supplementary 2. Luminescence from an egg expressing MAPK_{AR}Δ4, but that did not fertilize. Shown is one of 7 eggs that had been injected with MAPK_{AR}Δ4 RNA and OGBD. The conditions are otherwise the same as in Fig 3. There are no Ca²⁺ oscillations and the luminescence recording from MAPK_{AR}Δ4 shows only a gradual drift in signal with time.

Supplementary 3. MAPK-DM control trace and fertilization. In both cases the conditions were otherwise the same as in Fig.3 with recording of luminescence from MAPK-DM (that cannot be phosphorylated) and fluorescence from OGBD. In A is shown an example of one of 12 control eggs where no sperm were added to eggs and no Ca²⁺ oscillations occurred. In B an example is shown of one of 14 eggs where sperm were added (at the arrow) and Ca²⁺ oscillations occurred. The luminescence of MAPK-DM did not show the marked (~10 fold change) characteristic of MAPK_{AR} and MAPK_{AR}Δ4.

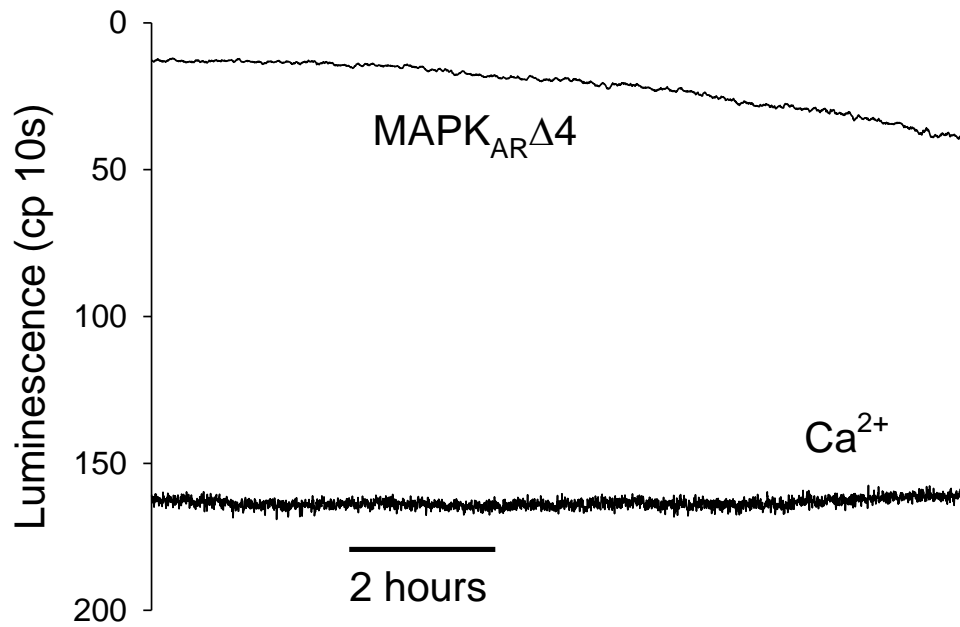
Supplementary 4. Activation of eggs by roscovitine. In A there is a trace of one of 5 eggs following the addition of roscovitine (50μM), with the luminescence of MAPK_{AR}Δ4 and the fluorescence of OBGD to measure Ca²⁺. The conditions were the same as Fig 6 except that measurements of Ca²⁺ were made and showed no substantial Ca²⁺ increase in response to roscovitine. B shows an image of some eggs activated by roscovitine that have formed single pronuclei compared to eggs that formed two pronuclei after fertilization. The presence of pronuclei is most evident by the nucleoli in the appropriate focal plane. The fertilization experiment was at the end of a ~15 hours recording.

Supplementary 5. The effect of okadaic acid on eggs undergoing a decrease in MAPK. The conditions are the same as in Fig.7 except that the MAPK_{AR} probe was used. The example shown is one of 13 eggs that underwent Ca²⁺ oscillations at fertilization, and then once the MAPK_{AR} signal has started to change, 50μM okadaic acid was added (OA at the arrow). After a delay the MAPK_{AR} luminescence returns to pre-fertilization levels and the Ca²⁺ oscillations return.

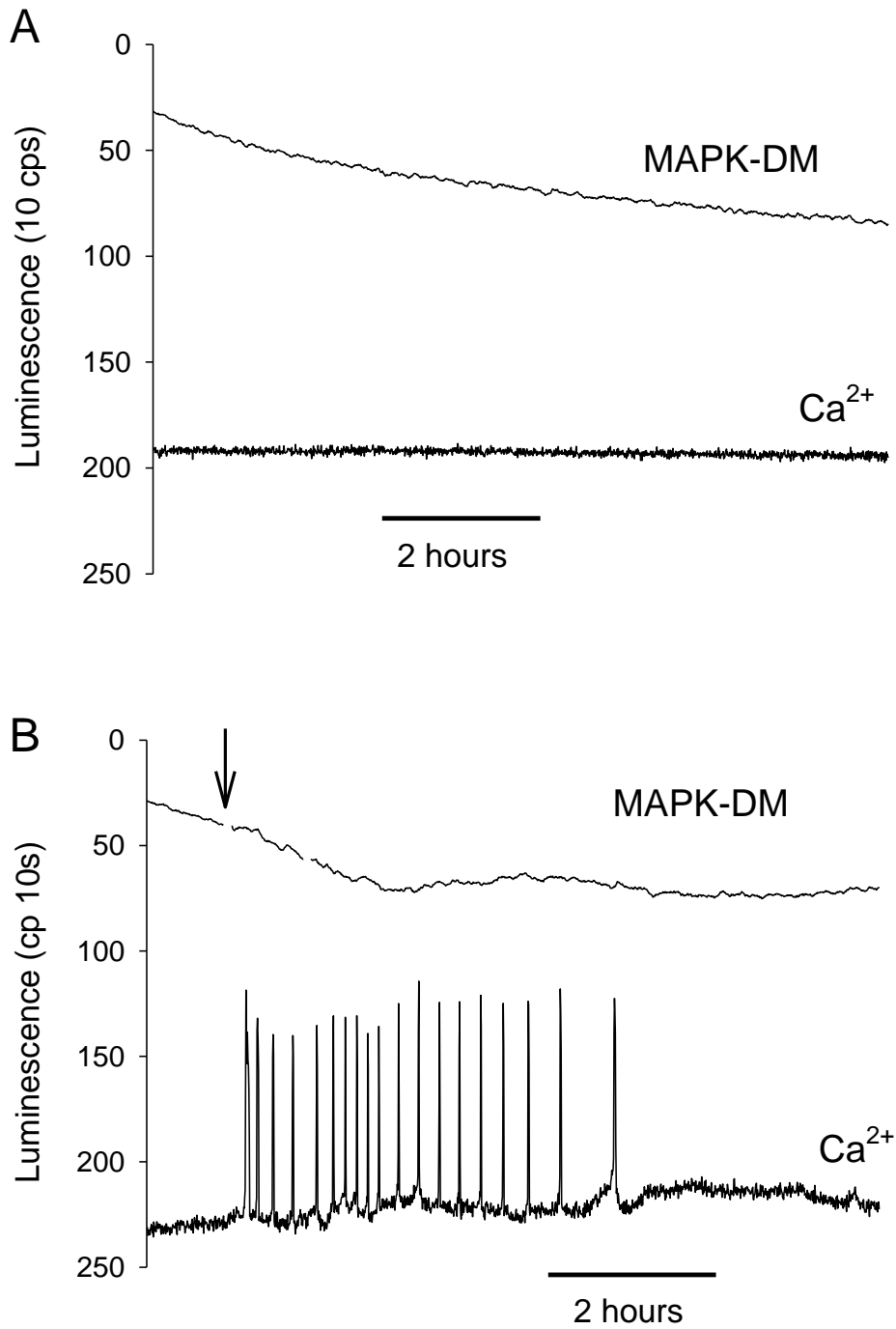
Supplementary 1. Western blots of MAPK_{AR} injected eggs.



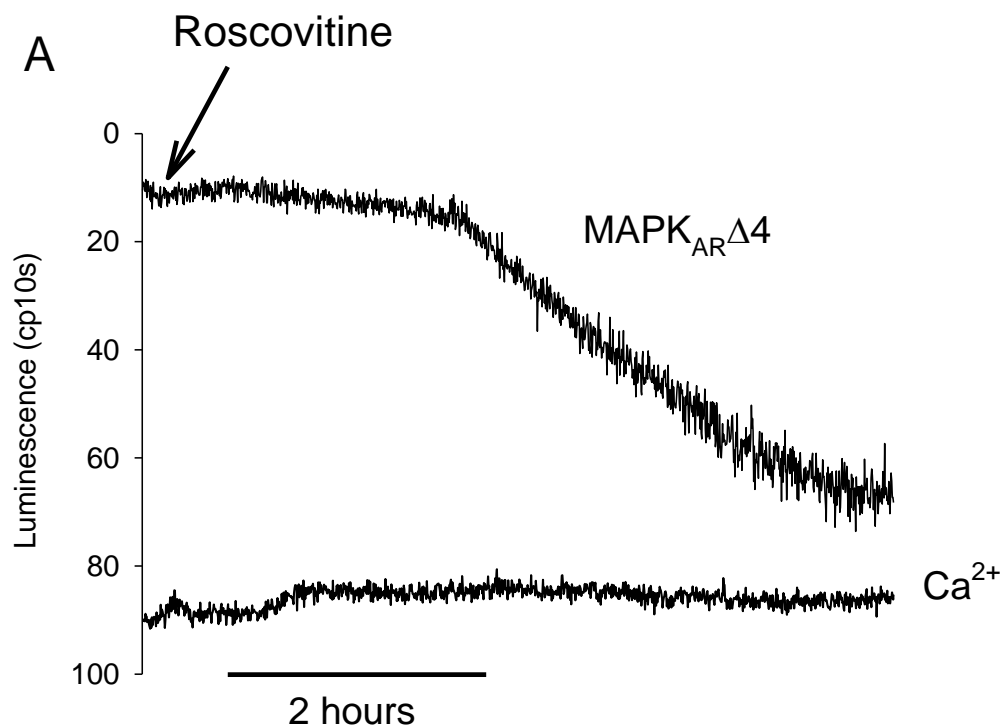
Supplementary 2. MAPK_{AR}Δ4 control traces without fertilization



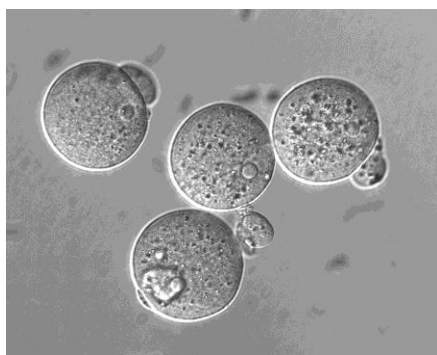
Supplementary 3. MAPK-DM control trace and fertilization.



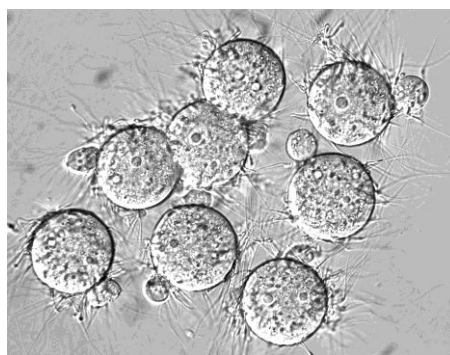
Supplementary 4. Activation of eggs by roscovitine.



B



Roscovitine



Fertilization

Supplementary 5.

The effect of okadaic acid on eggs undergoing a decrease in MAPK

