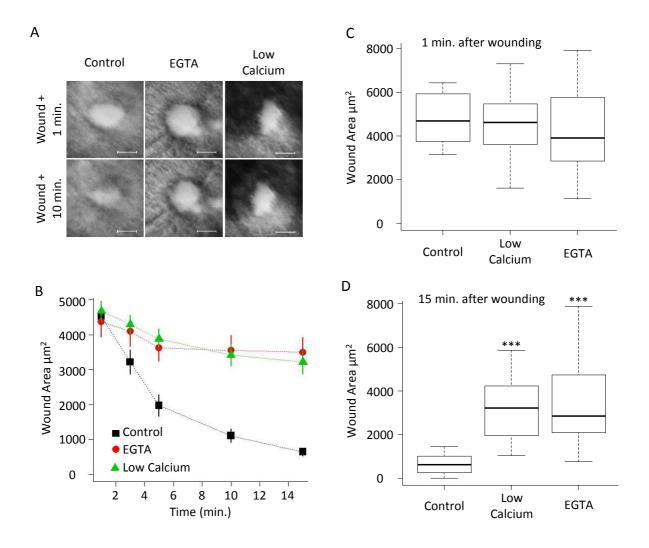
Electronic Supplementary Material (ESI) for Integrative Biology. This journal is © The Royal Society of Chemistry 2014

Supplementary Figures



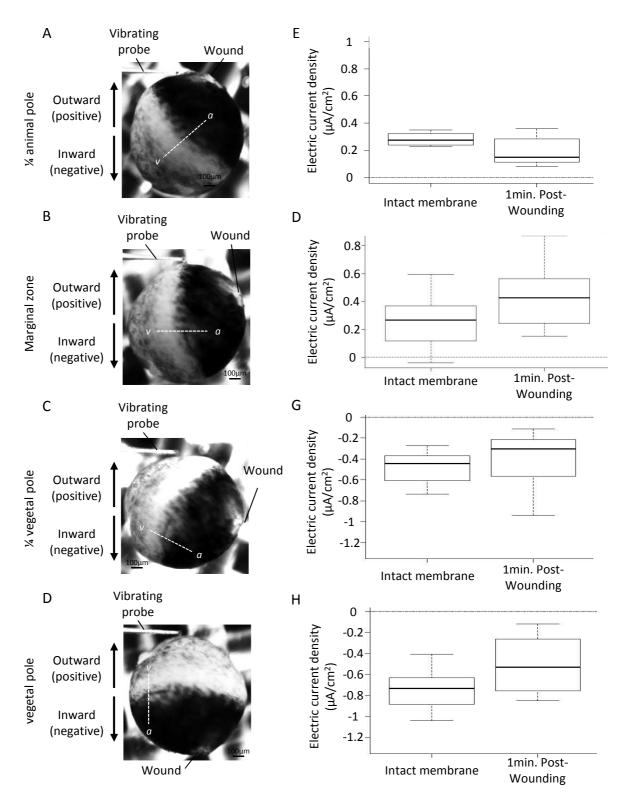
## Supplementary fig. 1 Extracellular Calcium is required for *Xenopus laevis* oocyte cell membrane re-sealing

A. Representative photographs of *Xenopus laevis* oocyte cell membrane wounds at one and ten minutes after wounding, scale bar is 50µm length.

B. Diagram showing means and standard error of wound area at different times in control condition (black squares, n=20), in presence of 5mM EGTA (red dots, n=21) or in presence of low extracellular calcium concentration (green triangles, n=17).

C. Box Plot showing wound area distributions one minute after wounding in the same conditions as (B).

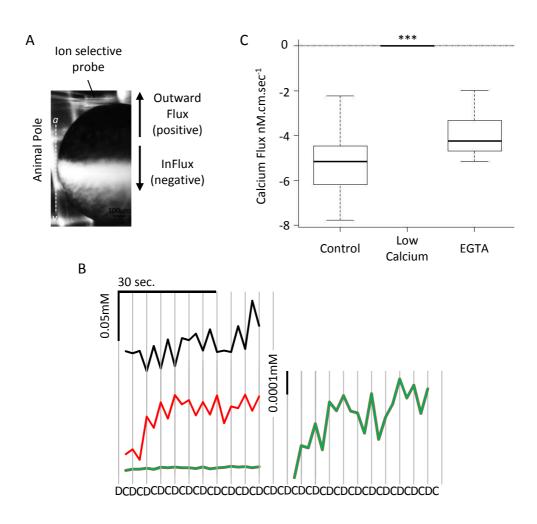
D. Box Plot showing wound area distributions fifteen minutes after wounding in the same conditions as (B).



## Supplementary Fig. 2 *Xenopus laevis* oocyte cell membrane wounding induces local change of electric current density around the wound.

A-D. Photographs illustrating electric current density measurements at specific position along the animal vegetal axis after wounding of the cell membrane. Arrows indicates the direction of the electric current represented by positive value when outward and negative value when inward in (E-H). Positions were approximately 45° (A,E), 90° (B,F), 135° (C,G), or 180° (D,H) from the wound.

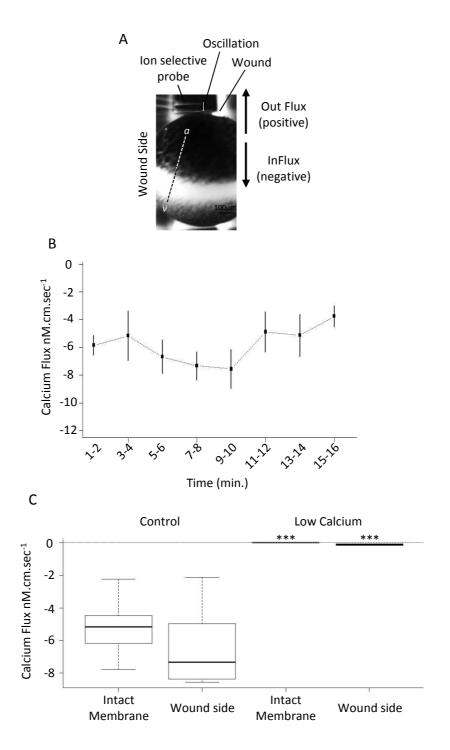
E-H. Box Plots showing the electric current density distributions at intact membranes and at one minute after wounding in control condition (n=15 for intact membrane measurements and 8-10 for wounded membrane measurements).



## Supplementary Fig. 3: Extracellular calcium concentration affects calcium flux at the intact cell membrane at the animal pole.

A. Photograph illustrating the calcium fluxes measurements at the animal pole of *Xenopus laevis* oocyte cell membrane. Arrows indicate the direction of the calcium flux representing outflow (positive value) or influx (negative value) in (C).
B. Example of ion selective electrode measurement traces of calcium concentration obtained at the animal pole of *Xenopus laevis* oocyte cell membrane in control condition (black), in presence of 5mM EGTA (red) or in presence of low extracellular calcium concentration (green).

C. Box Plot showing calcium flux at the animal pole of *Xenopus laevis* oocyte cell membrane wounds in control condition (n=21), in presence of 2mM EGTA (n=8) or in presence of low extracellular calcium concentration (n=12).

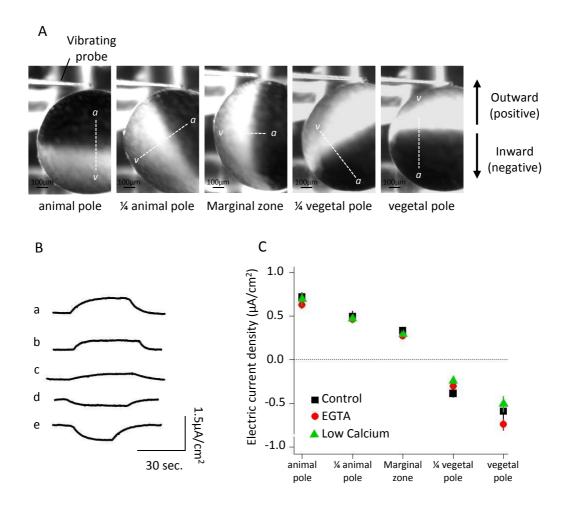


## Supplementary Fig. 4 *Xenopus laevis* oocyte wounding does not affect calcium flux at wound side.

A. Photograph illustrating the calcium flux measurements at cell membrane wound side (ion selective electrode is placed  $100\mu$ m from the wound edge using a micromanipulator). Arrows indicate the direction of the calcium flux representing outflow (positive value) or influx (negative value) in (B), (C) and (D).

B. Diagram showing means and standard error of calcium flux at the wound side at different times in control condition (n=8).

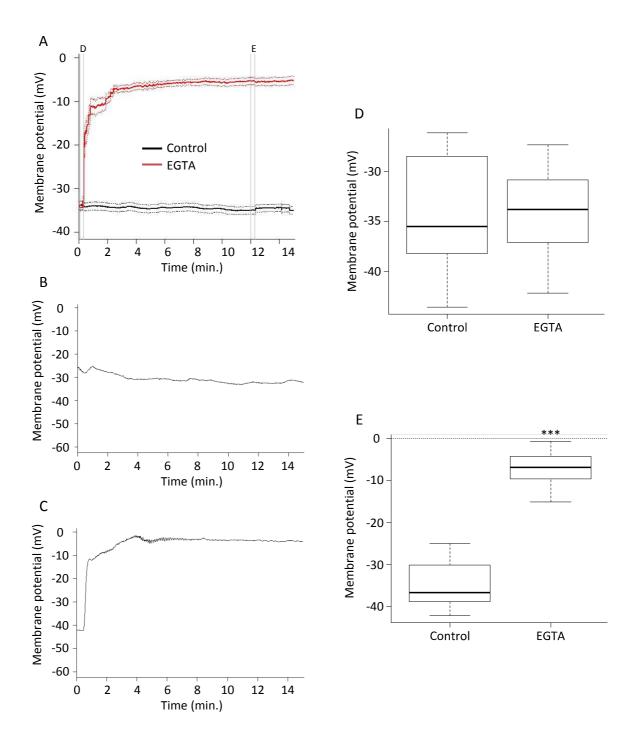
C. Box Plot showing the calcium flux at the intact membrane compare to wound side in control condition (n=8 and 21) or in presence of low extracellular calcium concentration (n=8 and 12). Asterisks represent the statistical differences between control intact membranes versus low calcium intact membrane or control wound side versus low calcium wound side.



# Supplementary fig.5 The heterogeneous electric current density distribution at cell membrane along the animal-vegetal axis is not affected by low extracellular calcium concentration.

A. Photograph illustrating the electric current density measurements at *Xenopus laevis* oocyte cell membrane at different cell membrane domains along the animal-vegetal axis. Arrows indicate the direction of the electric current represented by positive value when outward and negative value when inward in (C).

B. Example of electric current measurement traces obtained at different cell membrane domains along the animal-vegetal axis in control condition.
C. Diagram showing means and standard error of electric current density at different cell membrane domains along the animal-vegetal axis in control condition (black squares, n=15), in presence of 5mM EGTA (red dots, n=15) or in presence of low extracellular calcium concentration (green triangles, n=15).



## Supplementary Figure 6: Extracellular calcium concentration affects cell membrane potential

A. Diagram showing means (solid lines) and standard error (dashed lines) of *Xenopus laevis* oocyte cell membrane potential measurements versus time in control condition (black, n=19) or after addition of 5mM EGTA at 30 seconds (red, n= 22). Letters show positions used to measure data shown in graphs (D) and (E).

B and C. Examples of *Xenopus laevis* oocyte cell membrane potential measurements over the time in control condition (B), and in the presence of 5mM EGTA (C).

D and E. Box Plot showing the distribution of *Xenopus laevis* oocyte cell membrane potential measurements over a 20 seconds period prior to addition of EGTA (D) or eleven minutes after (E).

Supplementary Tables

	Control	Low Ca	EGTA
Wound center at 1min.	-39.4 ± 2.8	15 ± 3 (***)	-0.8 ± 0.8 (***)
Wound center at 3min.	-43.2 ± 2.4	11.8 ± 1.7 (***)	0.3 ± 0.8 (***)
Wound center at 15min.	0.1 ± 0.3	5.5 ± 1.2 (***)	0.06 ± 1 (NS)
Wound side at 1min.	2.7 ± 0.4	-1.7 ± 0.3 (***)	-0.4 ± 0.1 (***)
Wound side at 3min.	3.2 ± 0.4	-1.4 ± 0.3 (***)	-1 ± 0.4 (***)
Wound side at 9min.	0.7 ± 0.08	-0.5 ± 0.1 (***)	0.3 ± 0.1 (*)

### Supplementary table 1: Extracellular calcium is required for electric current circuitry

Values represent means and standard error of electric current density (in  $\mu$ A/cm<sup>2</sup>) at different times in control condition (Center n=21; side n=18), in presence of 5mM EGTA (Center n=20; side n=19), or in presence of low extracellular calcium concentration (Center n=20; side n=19). Asterisks show statistical significant differences between control versus cow calcium and control versus EGTA; NS indicates no significant difference.

	prior wounding	1min. after wounding	4min. after wounding	15min. after wounding
Control	-35+/- 1.2	-13.3+/- 0.7	-22.6+/-1.8	-35+/-0.5
EGTA	-34.1+/-0.7 (NS)	-13.8+/-1.4 (NS)	-6.5+/-1.4 (***)	-2.7+/-1.8 (***)

#### Supplementary Table 2: Extracellular calcium is required for cell membrane re-

**polarization.** Values represent means and standard error of *Xenopus laevis* oocyte cell membrane potential measurements (in mV) before and after wounding at specific times in control condition (n=19) or in presence of 5mM EGTA (n= 22). Wound was made thirty seconds after the measurement starts and EGTA added thirty seconds after wounding. Asterisks show significant differences between control versus EGTA; NS indicates no significant difference.

	Control	
Intact animal	-5.2 ± 0.4	
	512 2 011	
Wound center 1-2min	-176.7 ± 24.6 (***)	
Wound Side 1-2min	-5.8 ± 1.8 (NS)	
Wound center 3-4min	-97.7 ± 11.1 (***)	
Wound center 15-16min	-21.6 ± 4.7 (***)	

### Supplementary table 3: Dynamic of calcium flux around the

**wounds.** Values represent means and standard error of calcium flux (in nM/cm<sup>2</sup>/sec) at different times in control condition (n=15). Asterisks show significant differences between intact membranes versus the different conditions; NS indicates no significant difference.

	Control	Low Ca	EGTA
Intact animal	-5.2 ± 0.4	-0.003 ± 0.008 (***)	-4 ± 0.4 (NS)
Wound center 1-2min	-176.7 + 24.6	-0.1 ± 0.03 (***)	-124.4 ±15.3 (*)
	1,00, 22,00	, <i>, , , , , , , , , , , , , , , , , , </i>	12 10.00 ( )
Wound Side 1-2min.	-6.4 ± 1	-0.1 ± 0.02 (***)	ND

**Supplementary table 4: Extracellular calcium is required for calcium influx through the wounds.** Values represent means and standard error of calcium flux (in nM/cm<sup>2</sup>/sec) at different times in control condition (n=15), in presence of 5mM EGTA (n=8) or in presence of low extracellular calcium concentration (n=16). Asterisks show significant differences between control versus low calcium and control versus EGTA; NS indicates no significant difference; ND indicates not determined.

	prior to +/- EGTA	20sec after +/- EGTA	14 min after+/- EGTA
Control	-34+/-1.5	-34.2+/-1.4	-35.4+/-1.4
EGTA	34.2+/-1.1 (NS)	-16.3+/-3.2 (***)	-5.2+/-1.5 (***)

**Supplementary Table 5: Extracellular calcium concentration affects cell membrane potential**. Values represent means and standard error of *Xenopus laevis* oocyte cell membrane potential measurements (in mV) at specific times in control condition (n=19) or after addition of 5mM EGTA (n= 22). Asterisks show significant differences between control versus EGTA; NS indicates no significant difference.

	Control	low calium	EGTA
Intact cell membrane electric current	0	0	0
Intact cell membrane potential	0	ND	-
Intact cell membrane calcium flux	0		0
Wound healing	0		
Wound center electric current	++	Х	
Wound side electric current	+	Х	
Wounded cell membrane potential	-/0	ND	- /
Wound center calcium flux	++		-
Wound side calcium flux	0		0

**Supplementary Table 6: Summary of the effects of low extracellular calcium concentration on electric current, cell membrane potential, calcium fluxes and wound healing.** 0 (no change); - (decrease); + (increase), x (reversal), / (distinguish two time points); ND indicates not determined.