**Supporting information**



**Figure S1.** Effect of external cations on self- and nonself-fertilization. (A) Self- or nonself-fertilization rate with different concentrations of Ca2+ (*n* = 5), Mg2+ (*n* = 5), or K+ (*n* = 5) using intact eggs (with follicle cells) of *C. intestinalis*. (B) Fertilization rate with the condition of osmotic pressure-adjusted artificial seawater (ASW) using intact eggs of *C. intestinalis*. Ca2+ is replaced by Mg2+ (*n* = 6), Mg2+ is replaced by Ca2+ (*n* = 5), and K+ is replaced by Na+ (*n* = 5). (C) Fertilization rate with different concentrations of Ca2+ (*n* = 5), Mg2+ (*n* = 6), or K+ (*n* = 6) using VC-free eggs of *C. intestinalis*. Data (% fertilization) are expressed as the mean value ± standard deviation (SD).

Table S1Composition of artificial seawater

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | NaCl | CaCl2 | MgCl2 | KCl | HEPES pH 8.2 |
| ASW | 462 mM | 11 mM | 48 mM | 9.4 mM | 10 mM |
| Ca2+-free ASW | 462 mM | – | 48 mM | 9.4 mM | 10 mM |
| Mg2+-free ASW | 462 mM | 11 mM | – | 9.4 mM | 10 mM |
| K+-free ASW | 462 mM | 11 mM | 48 mM | – | 10 mM |
| Ca2+-replaced ASW | 462 mM | – | 59 mM | 9.4 mM | 10 mM |
| Mg2+-replaced ASW | 462 mM | 59 mM | – | 9.4 mM | 10 mM |
| K+-replaced ASW | 471.4 mM | 11 mM | 48 mM | – | 10 mM |