THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

SUPPLEMENTARY MATERIAL

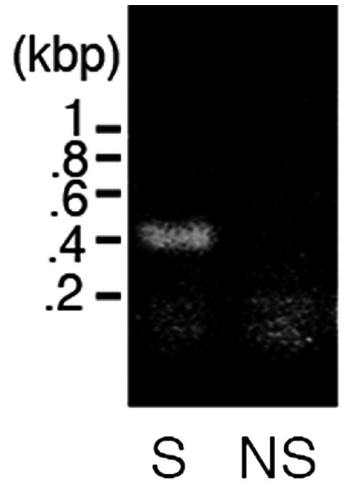
corresponding to:

Acrosome reaction-inducing substance triggers two different pathways of sperm intracellular signaling in newt fertilization

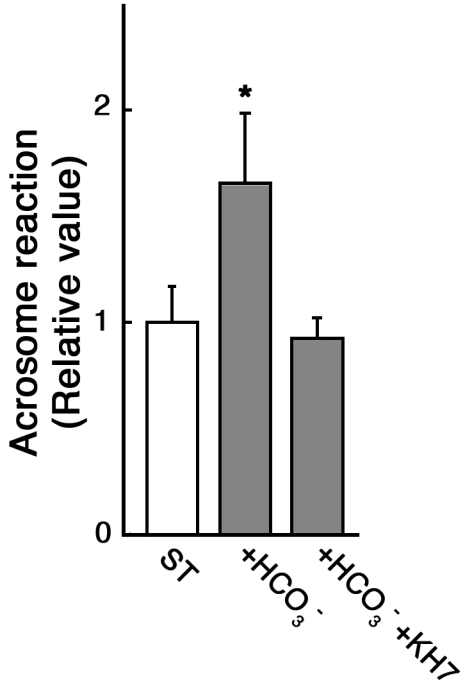
SHINNOSUKE KON, AKIO TAKAKU, FUBITO TOYAMA, ERIKO TAKAYAMA-WATANABE* and AKIHIKO WATANABE*

***Address correspondence to:** Eriko Takayama-Watanabe. Institute of Arts and Science, Yamagata University, 1-4-12 Kojirakawa, Yamagata, 9908560 Japan. Tel: +81-628-4802. E-mail: ewatanabe@kdw.kj.yamagata-u.ac.jp - https://orcid.org/0000-0001-5950-6207 or Akihiko Watanabe. Faculty of Science, Biological division, Yamagata University, 1-4-12 Kojirakawa, Yamagata, 9908560 Japan. Tel: +81-628-2619. E-mail: watan@sci.kj.yamagata-u.ac.jp - https://orcid.org/0000-0002-0382-5433

Full text for this paper is available at: https://doi.org/10.1387/ijdb.190092aw



Supplementary Fig. S1. Expression of the *CNGB3* mRNA in the testes. *Total RNA (1 μg) from spermatogenic* **(S)** *or nonspermatogenic* **(NS)** *testes was reverse-transcribed and polymerase chain reaction was performed using specific primers for the* CNGB3 *mRNA of* C. pyrrhogaster.



Supplementary Fig. S2. Inhibition of HCO_3^--induced spontaneous acrosome reaction (AR) by KH7. *Sperm were incubated in ST containing an activator and an inhibitor of soluble adenylyl cyclase,* **HCO_3^-** *and* **KH7***, respectively. The AR was evaluated by the absence of acrosome in the tip of the sperm head using a dark field microscope. Percentages of the acrosome-reacted sperm were expressed by relative values against a mean percentage of them in ST. Asterisk indicates a significant difference against ST ($P<0.05$).*