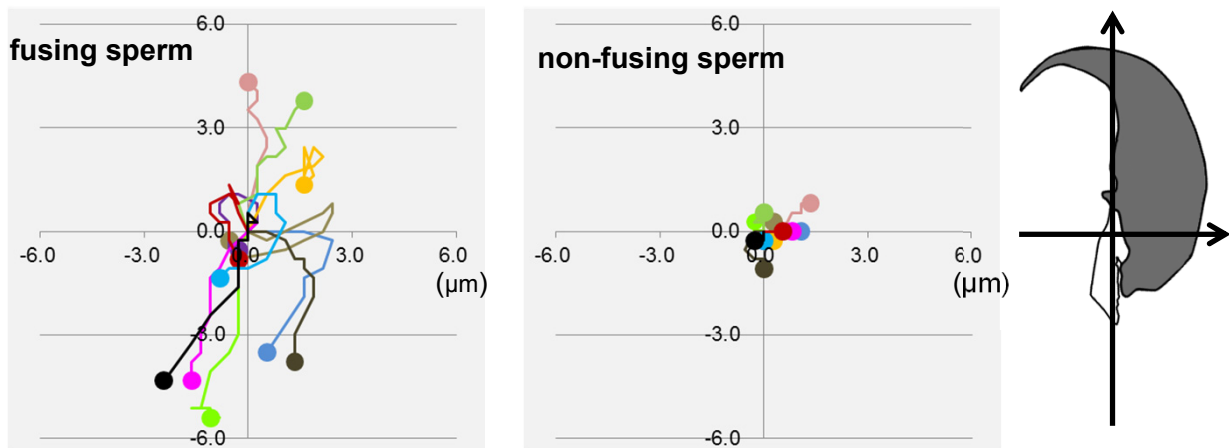
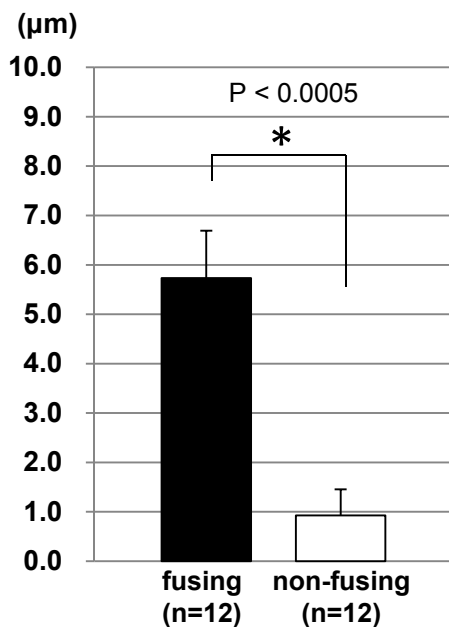


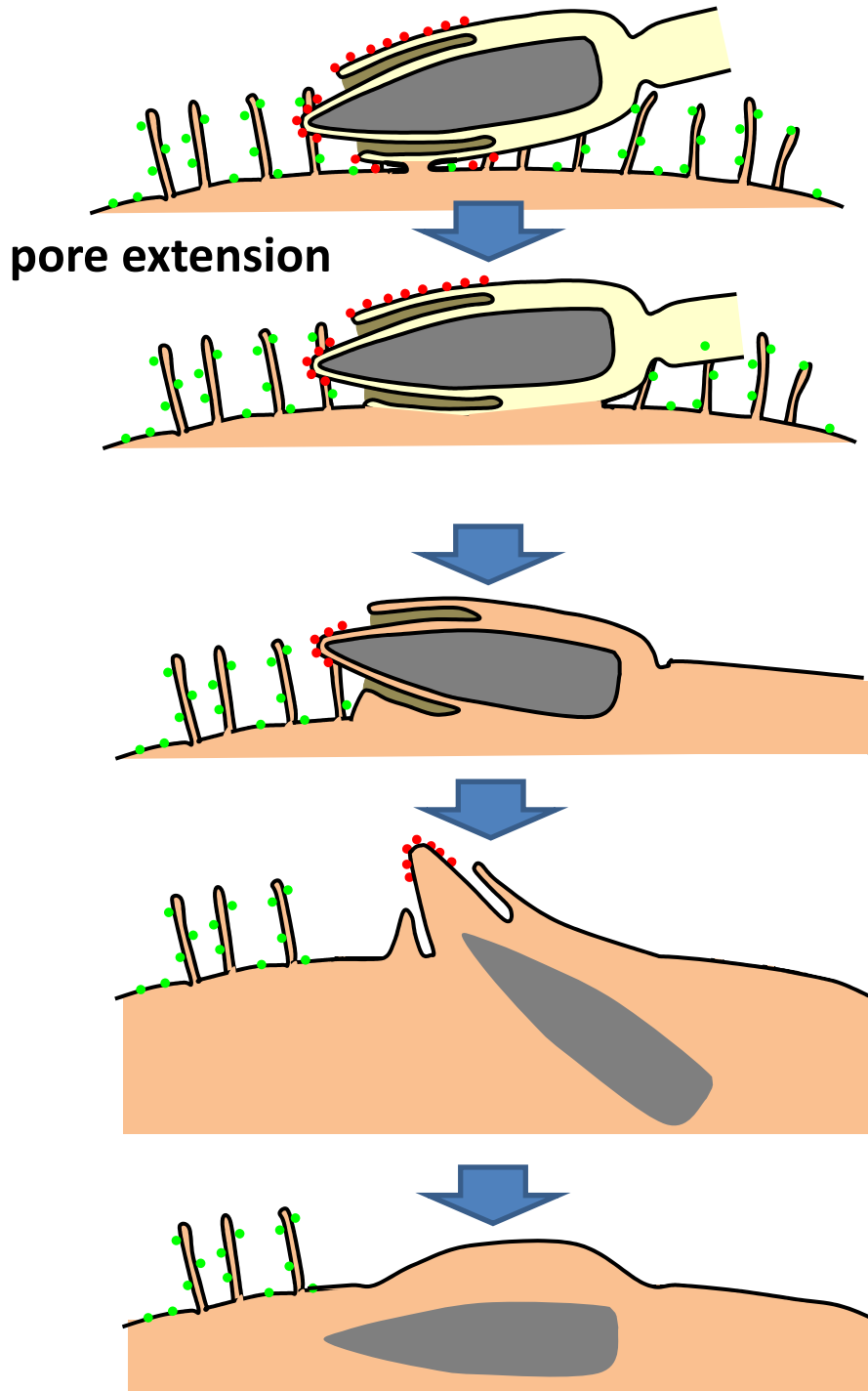
**A****B**

**Figure S1. The fusing point drifts during the sperm-egg fusion process.**

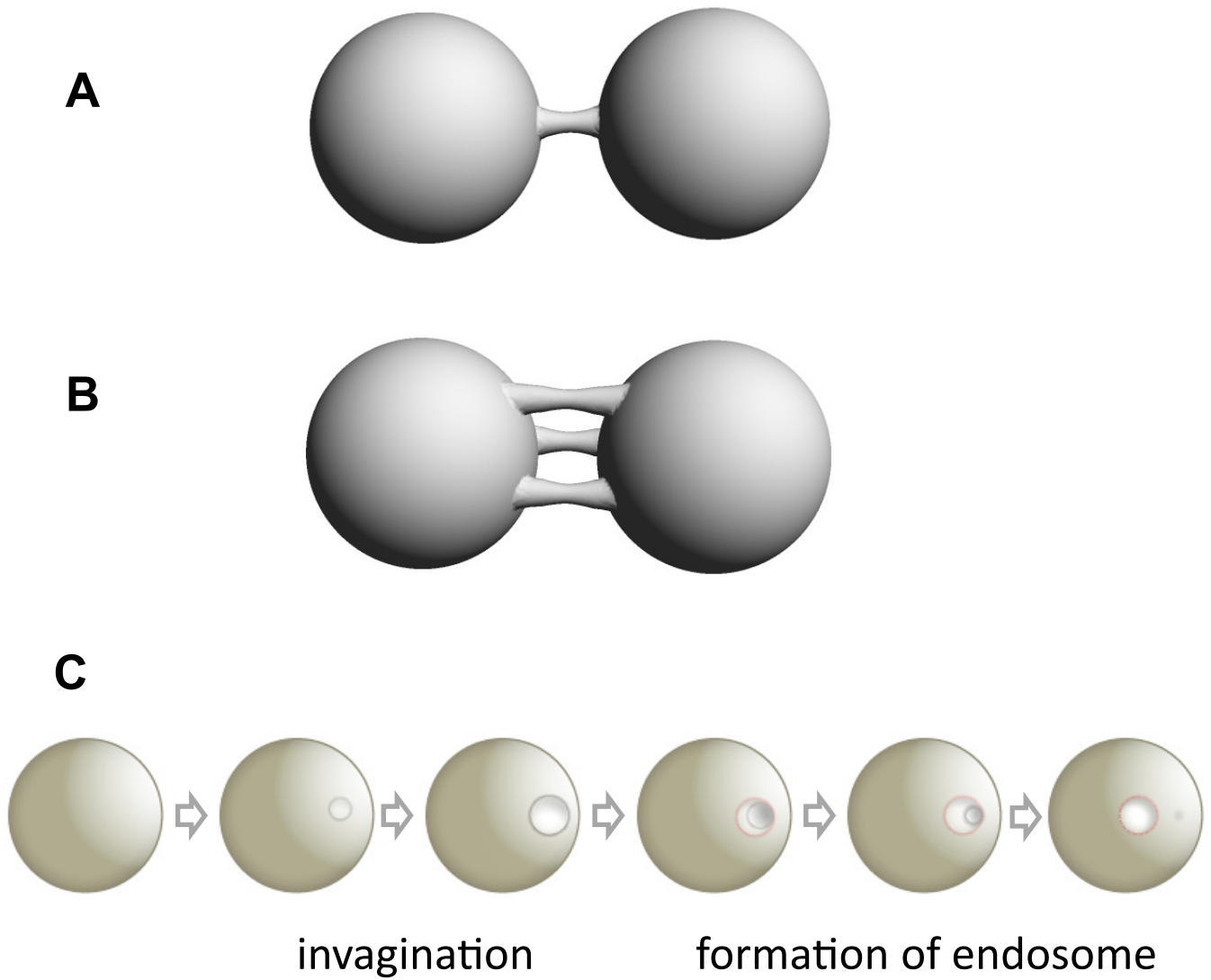
(A) The neck point of sperm head was plotted in the center with the sperm head to the 12 o'clock direction. Then the same neck point of each fusing sperm (left figure) or non-fusing sperm (right figure) was plotted every 30 seconds for a further 5 minutes. The direction of the drift showed the tendency towards the antero-posterior axes rather than the left-right axes. (B) The total distance which sperm drifted. The fusing sperm drifted a significantly greater distance ( $5.73 \mu\text{m} \pm 0.96$ ; mean  $\pm$  SD) than the sperm that bound on the egg surface ( $0.93 \mu\text{m} \pm 0.53$ ).

## sperm-egg fusion

● IZUMO1 (sperm)  
● CD9 (egg)



**Fig. S2. An estimated outcome of fertilization by a single cell-cell fusion.**  
The entire sperm and egg membrane mingle together and no internalized vesicle such as shown in figure 4C would appear.



**Fig. S3.**

(A) A cell-cell fusion joins two membranes into one. (B) The additional fusion at multiple points does not change the number of membrane planes. (C) To form an internalized vesicle, the membrane must fuse in a topographically different manner, with a single membrane divided into two separate parts.

**Table S1. The disappearance of  $AC_{dim}$  pattern in conditions where fusion was impaired using  $Cd9^{-/-}$  eggs**

| egg         | percentile of distribution pattern of Red-Iz |      |      |            | No. of spermatozoa examined |
|-------------|--|------|------|------------|-----------------------------|
|             | AI   | H    | EQ   | $AC_{dim}$ |                             |
| wild-type   | 57.9   | 22.1 | 11.5 | 8.5        | 366                         |
| $Cd9^{-/-}$ | 57.0   | 24.2 | 18.8 | 0.0        | 505                         |

The spermatozoa were mixed with zona-free wild-type and  $Cd9^{-/-}$  eggs.