

Supplementary Material

Sperm gamma-aminobutyric acid type A receptor delta subunit (GABRD) and its interaction with purinergic P2X₂ receptors in progesterone-induced acrosome reaction and male fertility

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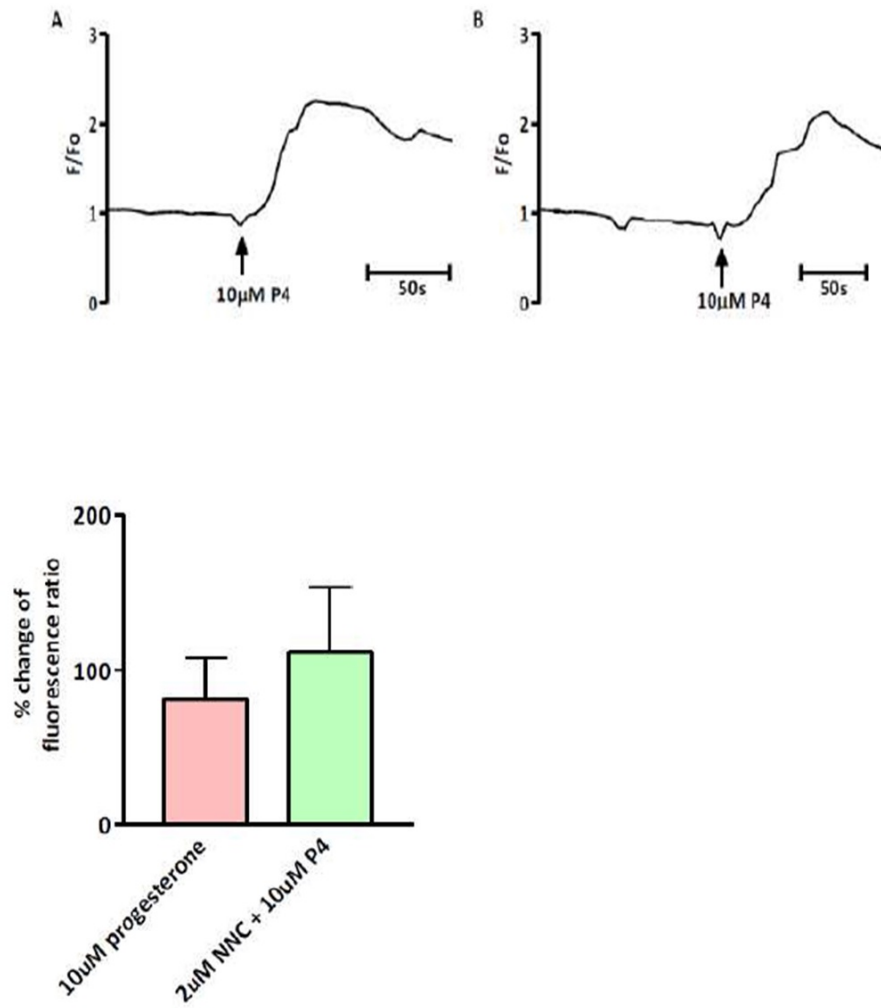


Fig. S1. Mice sperm calcium measurement result shows that NNC55-0396 cannot inhibit the progesterone (10 µM) induced calcium increase.

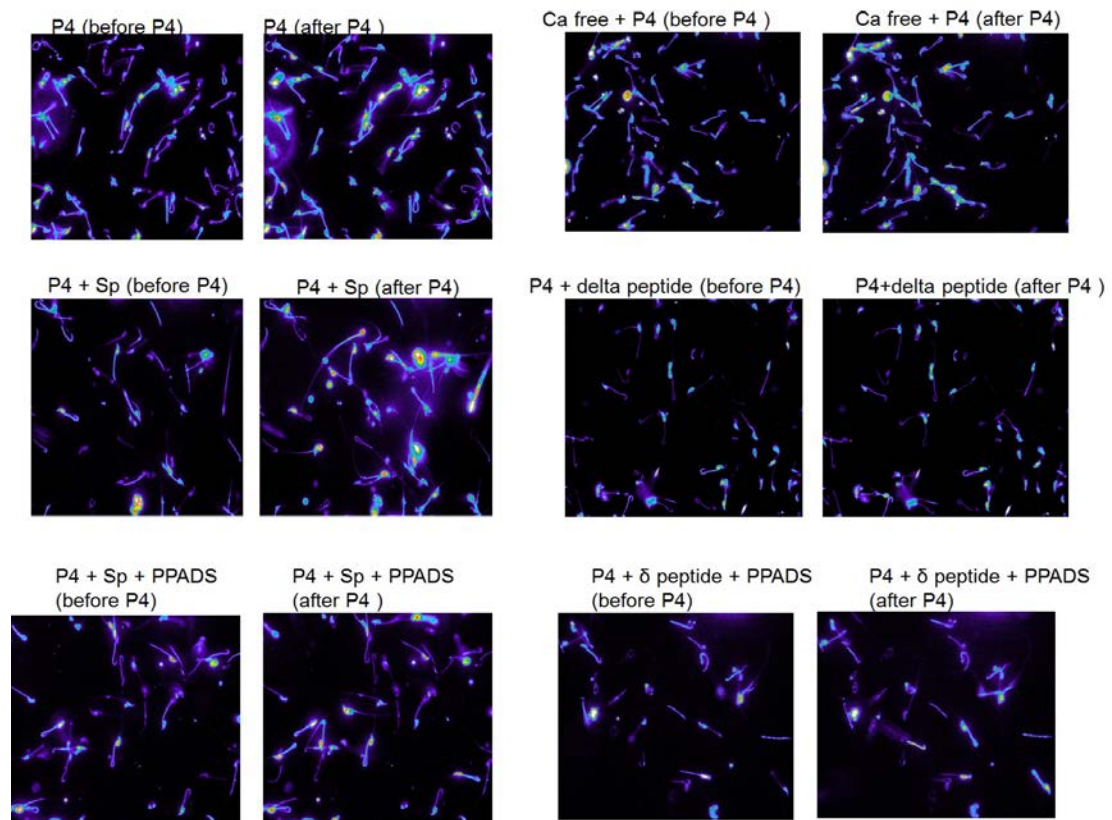


Fig. S2. Original calcium pictures for the Figure 3 in the main text.

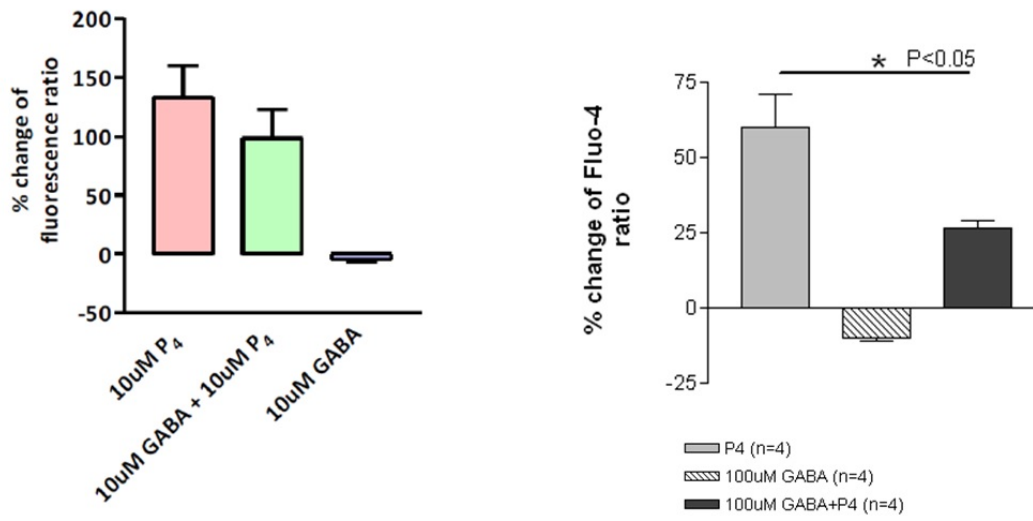


Fig. S3. Calcium measurement showed that Low level of GABA is inhibitory for the calcium increase; Adding GABA and progesterone together also have no significant different effect compared with progesterone treatment.

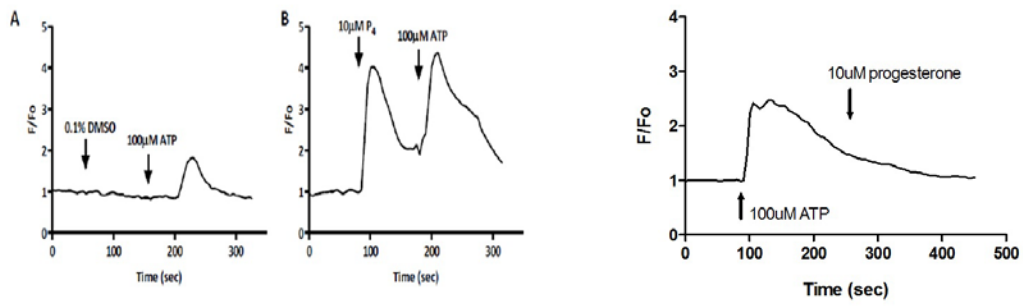


Fig. S4. (A) ATP itself can induce the calcium increase in mice sperm; while add progesterone show no additive effect on the calcium response. (B) Adding Progesterone (10 µM) and ATP (100 µM) sequentially can induce a higher level of calcium increase, indicating progesterone and ATP can modulate calcium channel P2X2 either indirectly or directly, leading to calcium increase.

Table S1**Percentage of sperm responding to progesterone and also to the inhibitors**

Fig 2B

	no. of experiment	Total sperm	Responding sperm	% of responding sperm
Sp + P4	4	84	33	39.28571429
dp + P4	4	81	38	46.91358025

Fig 3B

	no. of experiment	Total sperm	Responding sperm	% of responding sperm
Ca free + P4	3	43	30	69.76744186
P4	8	203	159	78.32512315
10-PPADS + P4	5	137	120	87.59124088
100-PPADS + P4	6	196	148	75.51020408

Fig 3C

	no. of experiment	Total sperm	Responding sperm	% of responding sperm
p4	7	94	75	79.78723404
PPADS + P4	7	78	56	71.79487179
Sp + P4	5	66	51	77.27272727
dp + P4	6	83	62	74.69879518
Sp+PPDAS+P4	5	75	60	80
dp+PPADS+P4	5	84	70	83.33333333