Supplementary Material: Reproduction, Fertility and Development, 2019, 31(9), 1444–1456.

## **Supplementary Material**

## Mitogen-activated protein kinase kinase kinase 8 (MAP3K8) mediates the LH-induced stimulation of progesterone synthesis in the porcine corpus luteum

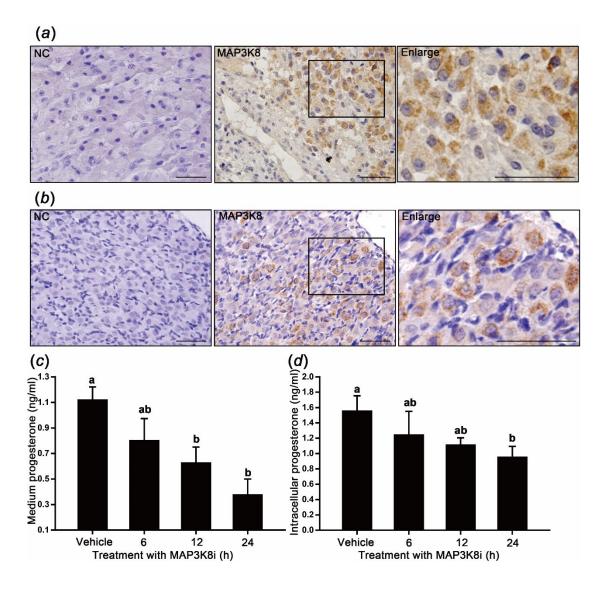
Di Zhang<sup>A</sup>, Ying Liu<sup>A</sup>, Yan Cui<sup>B,C</sup> and Sheng Cui<sup>A,C</sup>

<sup>A</sup>State Key Laboratory of Agrobiotechnology, College of Biological Sciences, China Agricultural University, Beijing, 100094, PR China.

<sup>B</sup>The 306th Hospital of People's Liberation Army, Beijing, 100101, PR China.

<sup>C</sup>Corresponding authors. Emails: cuisheng@cau.edu.cn; dryancui@163.com

MAP3K8 is expressed in primate CL and mediates  $P_4$  syntheses in human SVOG cell line. We investigated the expression of MAP3K8 in primate CL, including women and monkeys. IHC showed the localization of MAP3K8 protein in the corpus luteal (Figure S1.a and b), suggesting that MAP3K8 may also participate in  $P_4$  synthesis in primate CL. A human granulosa-lutein SVOG cell line was used to support our hypothesis. Cultured SVOG cells were incubated with 10 μM MAP3K8i for 0 hour, 6 hours, 12 hours and 24 hours, and  $P_4$  concentrations were measured by RIA at the end of incubation. Results demonstrated that MAP3K8i inhibited  $P_4$  synthesis at a certain degree in the SVOG cell line (P < 0.05; Figure S1.c and d).



**Fig. S1.** MAP3K8 is expressed in primate CL and mediates  $P_4$  synthesis in human SVOG cell line. (*a*) and (*b*) IHC staining of MAP3K8 in woman and monkey CL respectively. Bars in all pictures represent 50 μm. (*c*) and (*d*) RIA analysis of  $P_4$  levels in cultured medium and intracells of cultured human SVOG cell line treated with MAP3K8i for 0 h, 6 h, 12 h and 24 h. Data represent the means  $\pm$  s.d.,  $n \ge 3$ . Data are analysed by one-way ANOVA. Different superscript letters indicate significant difference (P < 0.05), bars with the same letter are not significantly different.