

1 **SUPPLEMENTARY FIGURE LEGEND**

2 **Supplementary Figure1 BMP4 and RA differentially induced ESC differentiation into PGC.**

3 A. qRT-PCR results showed that the *Stra8* gene was induced by RA, but not by BMP4; B, C.
4 Simultaneous addition of BMP4 and RA in the medium was not conducive to the formation of
5 embryoid bodies, Scale bar: 50µm; D. qRT-PCR results showed BMP4 and RA have obvious
6 antagonistic effects in the process of inducing germ cell differentiation.

7 **Supplementary Figure 2 Dynamic changes of key molecules in BMP4 and RA signals during**

8 **germ cell formation.** A. Dynamic expression of BMP4 in germ cell formation; B, C. Expression
9 of downstream signaling molecules and related target genes in BMP4 signaling pathway during
10 germ cell formation; D. The results of Western Blot indicated that BMP4 signaling was activated
11 during ESC differentiation into PGC, but inhibited during PGC differentiation into SSC; E. Genes
12 involved in RA synthesis: Dynamic expression of *ADH5* and *ALDH1A1* during germ cell
13 formation.

14 **Supplementary Figure 3 Acetylation and DNA methylation covalent modification of *DAZL***

15 **involved in PGC differentiation.** A. The pluripotency gene was inhibited during PGC formation
16 *in vivo*; B. *P300* gene was significantly up-regulated during PGC formation *in vivo*; C. *In vitro*
17 acetylation assay showed that the level of intracellular acetylation was significantly up-regulated
18 during PGC formation; D. qRT-PCR results showed that *DAZL* gene was activated in BMP4
19 induction model; E. Correlation analysis showed that *DAZL* expression was regulated by BMP4
20 signal; F, G. Dual luciferase assay showed that the activity of *DAZL* promoter was regulated by
21 DNA methylation and histone acetylation.

22 **Supplementary Figure 4 Morphological changes of cells.** Morphological observations showed

23 that the number of SSC-like cells induced by combination of RA and *Stra8* more than RA alone.

24 **Supplementary Figure 5 Expression of key signaling molecules of BMP4 and RA signaling**

25 **pathways during germ cell formation.** A. The expression of *BMP4* related signal molecules in

26 PGC was higher than that of SSC, except *BMP5*; B. The results of qRT-PCR showed that genes

27 involved in RA metabolism were up-regulated in the PGC phase and down-regulated in the SSC

28 phase during germ cell formation.

29 **Supplementary Figure 6 Protein interaction analysis between BMP4 and RA signals.** The

30 results showed that RA and BMP4 signals can interact with each other through BMP4,

31 ALDH1A1, ALDH1A2 and STUB1 complexes.

32 **Supplementary Figure 7 High-throughput sequencing analysis of cells at different stages in**

33 **the RA induction model.** The results of the analysis indicated that BMP4 signaling was inhibited

34 in the RA induction model.

35 **SUPPLEMENTARY TABLE LEGEND**

36 **Supplementary Table1 Induction of PGC and SSC by different inducers and their**

37 **combinations**

38 **Supplementary Table2 Primer sequence for qRT-PCR**

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