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Supplementary Material

Cumulus cell-conditioned medium supports embryonic stem cell differentiation to germ cell-like cells

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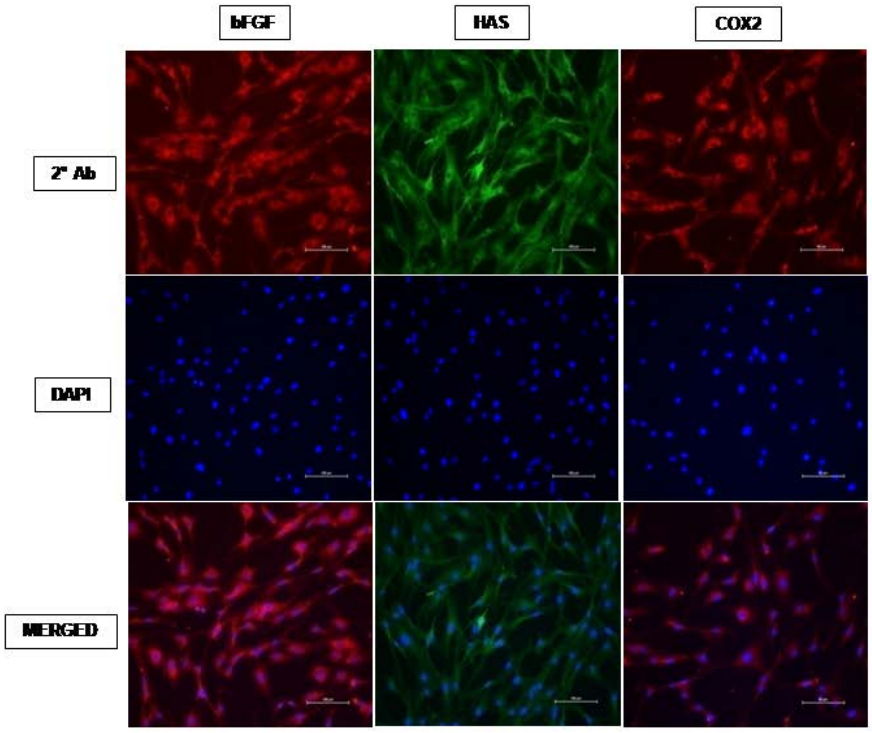

Fig. S1. Immunocytochemical characterization of cumulus cell cultures for cumulus cell markers (bFGF, HAS and COX2). DAPI, nuclear stain; 2˚Antibody, PE-conjugated secondary antibody against 1°-antibody; M, merged images of Hoechst and 2° antibody (Magnification = 200×, Scale bar = 100 µm).

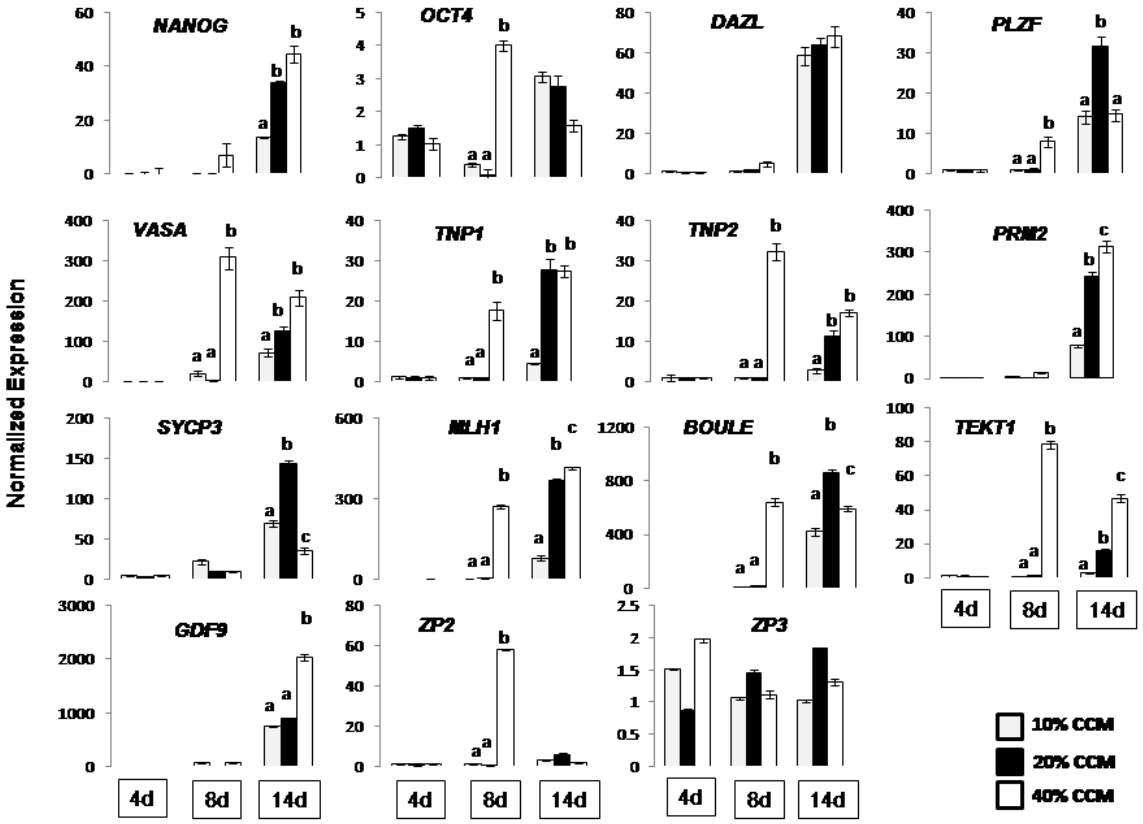

Fig. S2. qPCR analysis of normalised expression of key genes involved in ES cell differentiation to germ cell lineage upon cumulus cell conditioned medium (CCM) induction over 14 days of monolayer culture. Bars represent $2^{-\Delta\Delta cT}$ values ± s.e. of mean, and are calibrated against the corresponding normalised values of undifferentiated ES cell colonies. Bars with different superscripts differ significantly ($P < 0.05$).

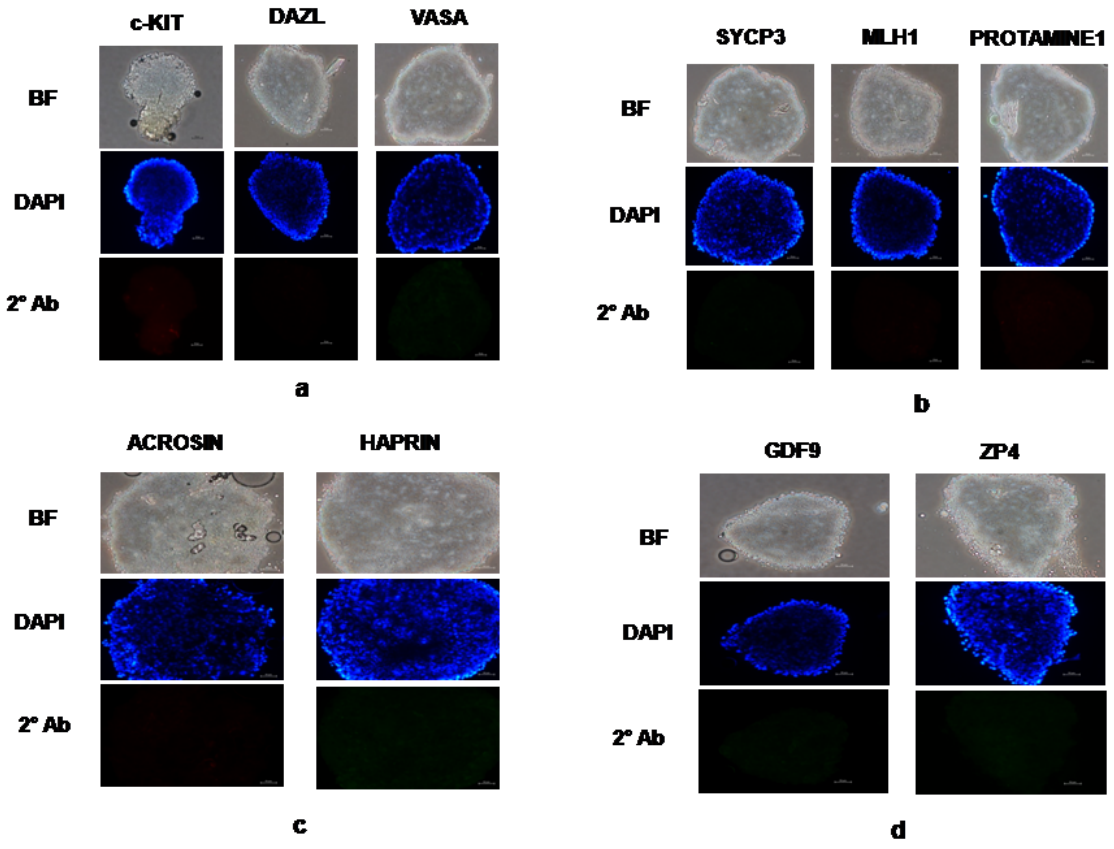

Fig. S3. Immunocytochemical characterisation of undifferentiated ES cell colonies for: (a) Primordial germ cell (PGCs) markers (c-KIT, DAZL and VASA); (b) meiotic markers (SYCP3, PROTAMINE1 and MLH1); (c) spermatocyte markers (ACROSIN and HAPRIN); (d) oocyte markers (GDF9 and ZP4). BF, Bright field images; DAPI, nuclear stain; 2° Antibody, PE-conjugated secondary antibody against 1° antibody; (Magnification = 200×, Scale bar = 100 µm).

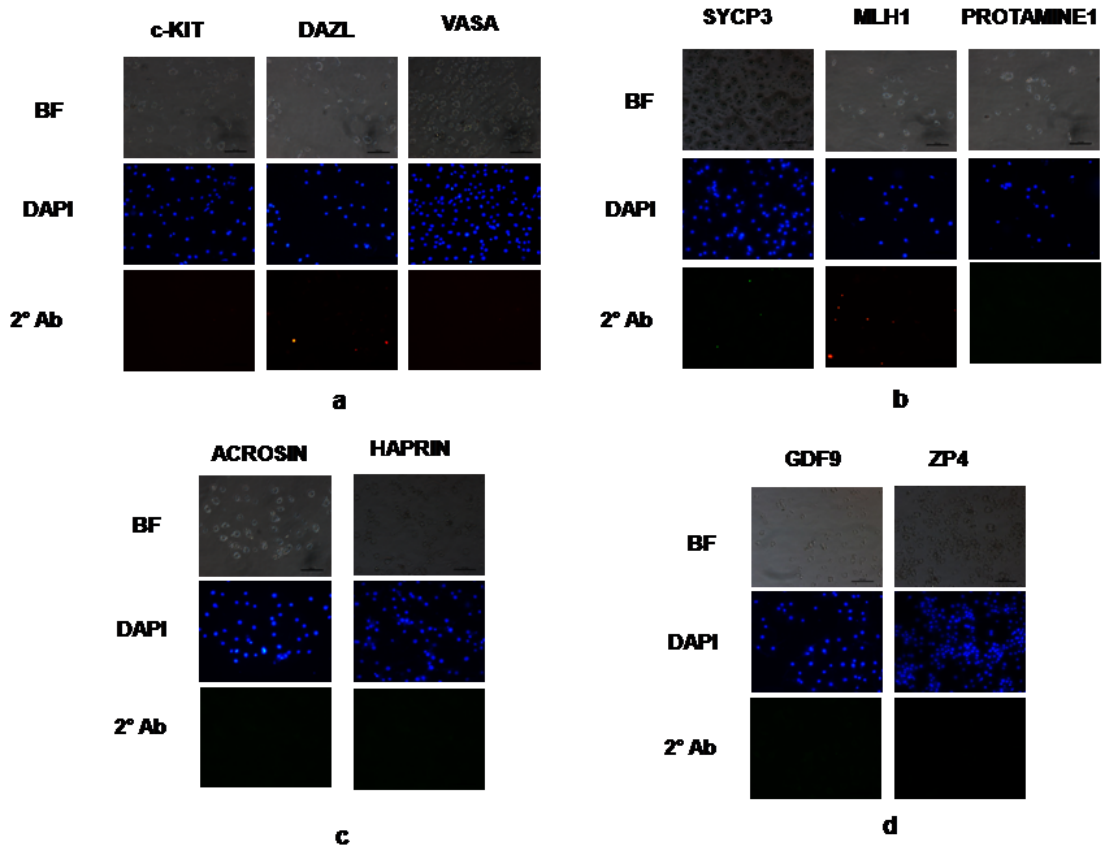

Fig. S4. Immunocytochemical characterisation of undifferentiated ES cell monolayers for: (a) Primordial germ cell (PGCs) markers (c-KIT, DAZL and VASA); (b) meiotic markers (SYCP3, PROTAMINE1 and MLH1); (c) spermatocyte markers (ACROSIN and HAPRIN); (d) oocyte markers (GDF9 and ZP4). BF, bright field images; DAPI, nuclear stain; 2° Antibody, PE-conjugated secondary antibody against 1° antibody; (Magnification = 200×, Scale bar = 100 µm).