10.1071/RD16486_AC

Supplementary Material: *Reproduction, Fertility and Development*

Supplementary Material

Insulinotropic nucleobindin-2/nesfatin-1 is dynamically expressed in the haemochorial mouse and human placenta

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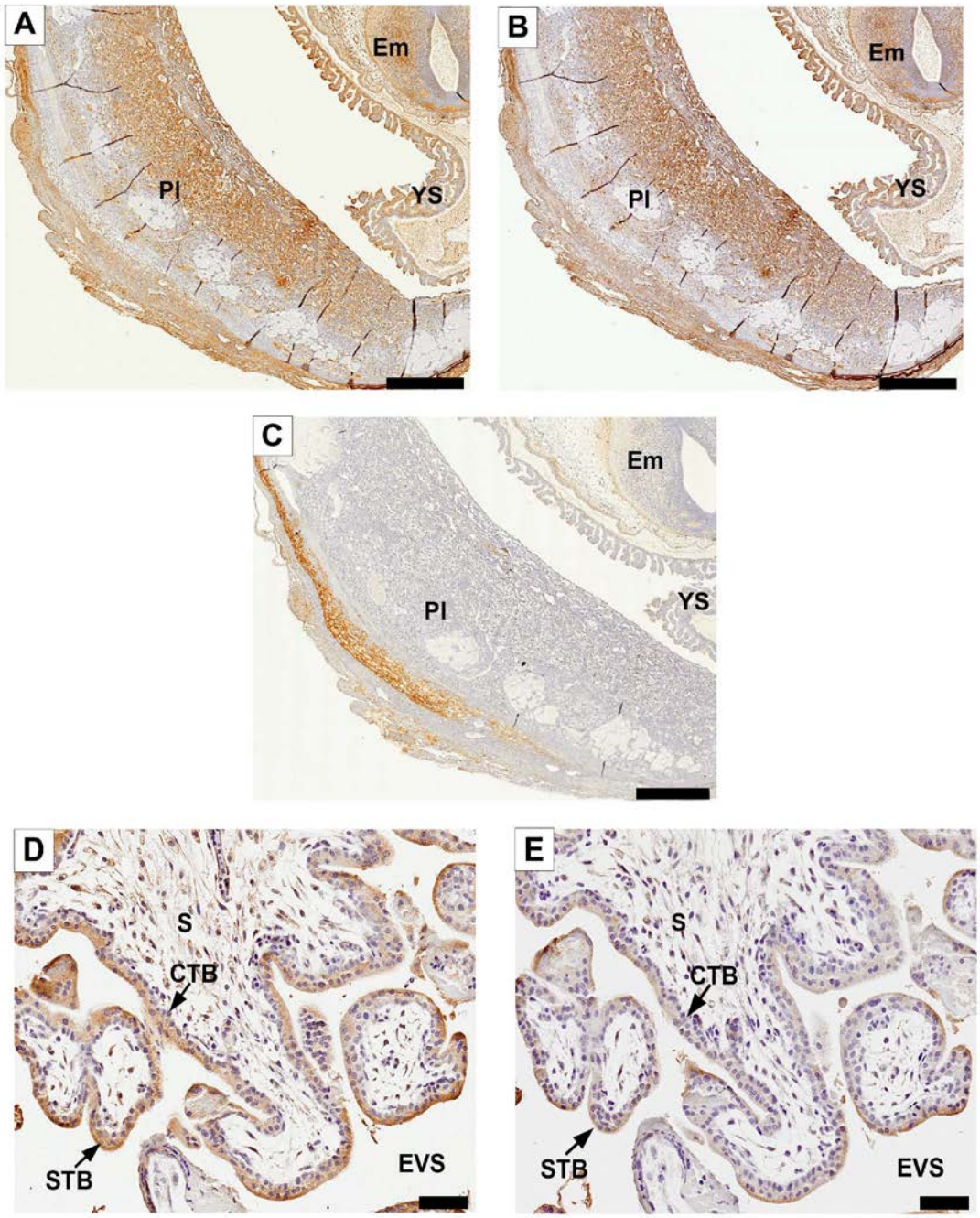

Fig. S1. Verification of anti-NUCB2/nesfatin-1 antibody specificity for immunohistochemistry experiments. (A–C) Comparison of non-purified and IgG purified NUCB2/nesfatin-1 antisera specificity in immunohistochemistry experiments. Representative experiments with E12.5 mouse placenta are shown. IgG purified

NUCB2/nesfatin-1 antisera (1:500) and non-purified NUCB2/nesfatin-1 (1:500) antisera were utilized for immunohistochemistry experiments (A and B, respectively) and demonstrated consistently similar NUCB2/nesfatin-1 expression patterns in the mouse placenta. (C) A non-specific, matched IgG control used at the same concentration as the primary antisera displayed no significant background staining within the placenta, but some minor background staining in the uterine muscle. Scale bars = 500 μm. (D, E) Verification of anti-NUCB2/nesfatin-1 specificity by peptide pre-absorption. (D) NUCB2/nesfatin-1 expression in first trimester (wk 8) chorionic villi using a NUCB2/nesfatin-1 specific antibody. (E) For pre-absorption controls, primary antisera was pre-incubated with 20 μg NUCB2/nesfatin-1 peptide prior to application to tissue sections during immunohistochemistry experiments. Peptide pre-absorption of the antibody resulted in markedly reduced detection of NUCB2/nesfatin-1 in chorionic villi. Scale bars = 20μm. Em = embryo. Pl = placenta. YS = yolk sac, CTB = cytotrophoblast, STB = syncytiotrophoblast, EVS = extravillous space, S = villous stroma.