10.1071/RD09265_AC

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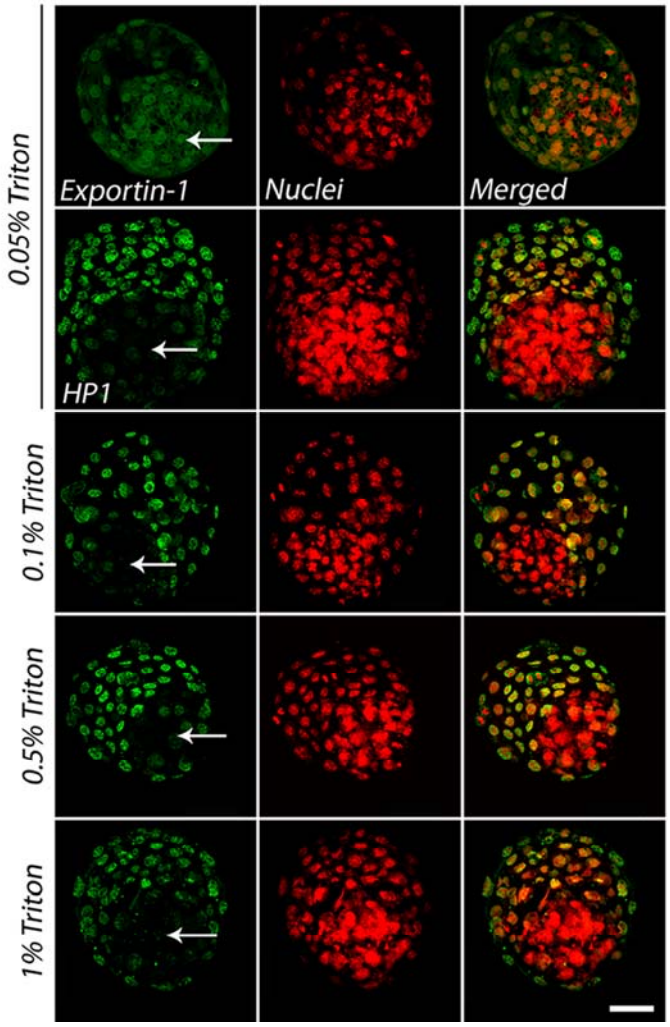

Fig. S1. Permeabilisation in 0.05% detergent is sufficient for dissemination of antibody through bovine blastocysts. Blastocysts were permeabilised in concentrations of detergent up to 1% for 10 min and stained for HP1γ or an unrelated antibody, Exportin-1. 0.05% detergent is sufficient for labelling of inner cell mass cells with an alternative antibody (arrow denotes embryonic cells, top panels). HP1γ protein was lacking in inner cell mass cells regardless of detergent concentration (arrows denote ICM cells, rows 2–5). Scale bar = 50 μm.

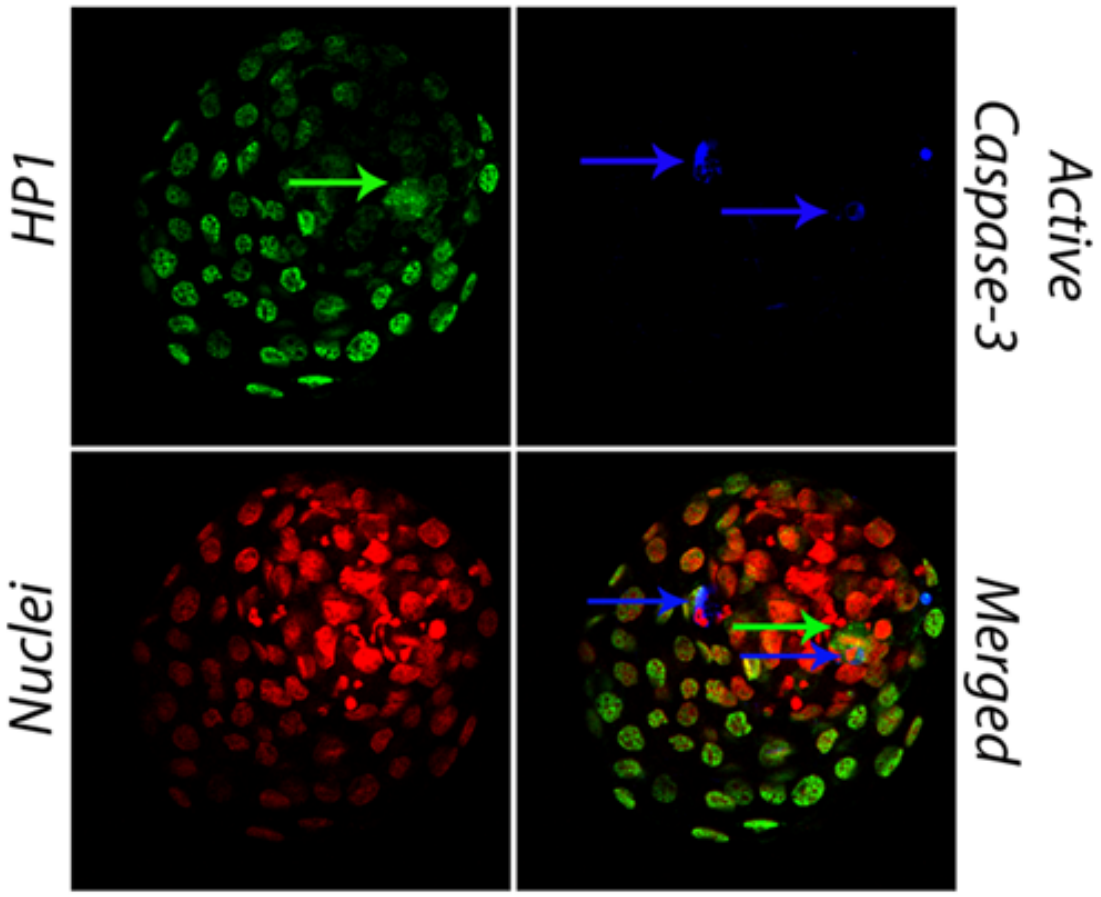

Fig. S2. Many cells with condensed chromatin staining do not express Active Caspase-3. Blastocysts were labelled with antibodies raised against HP1γ and Active Caspase-3 (*green* and *blue* images, respectively) and nuclear compartment stained with propidium iodide (*red* image). Merged image shown in bottom panel. Scale bar = 50 μm.

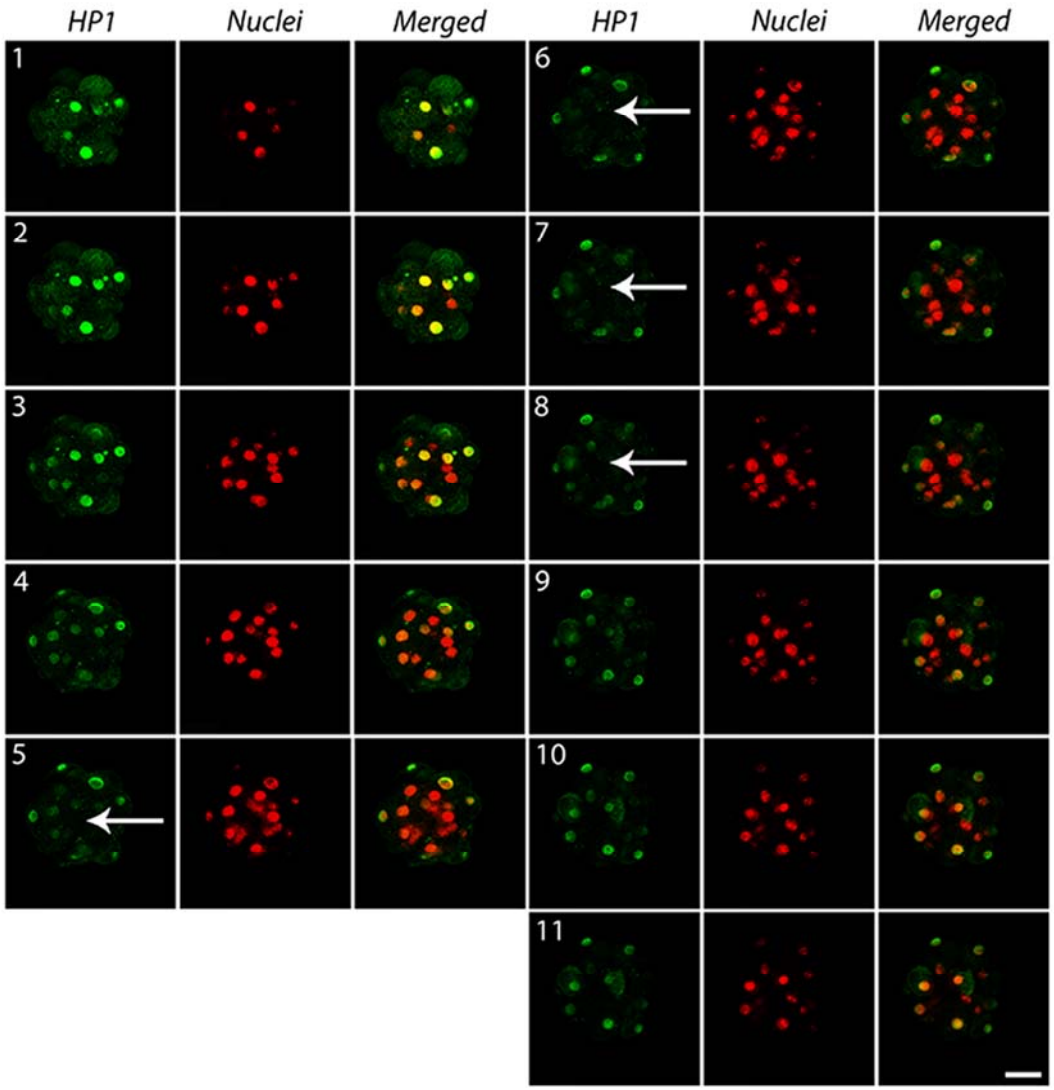

Fig. S3. Nuclear HP1γ is low or absent in cells committed to the embryonic lineage in the morula. Mounted embryos were stained for HP1γ and Z-series taken by confocal microscopy. Groups of HP1γ, nuclei and merged images are shown (numbered 1–11 in order of section), taken at 0.7-µm intervals. Arrows point to internally positioned cells that lack nuclear HP1γ protein. Scale bar = 50 µm.

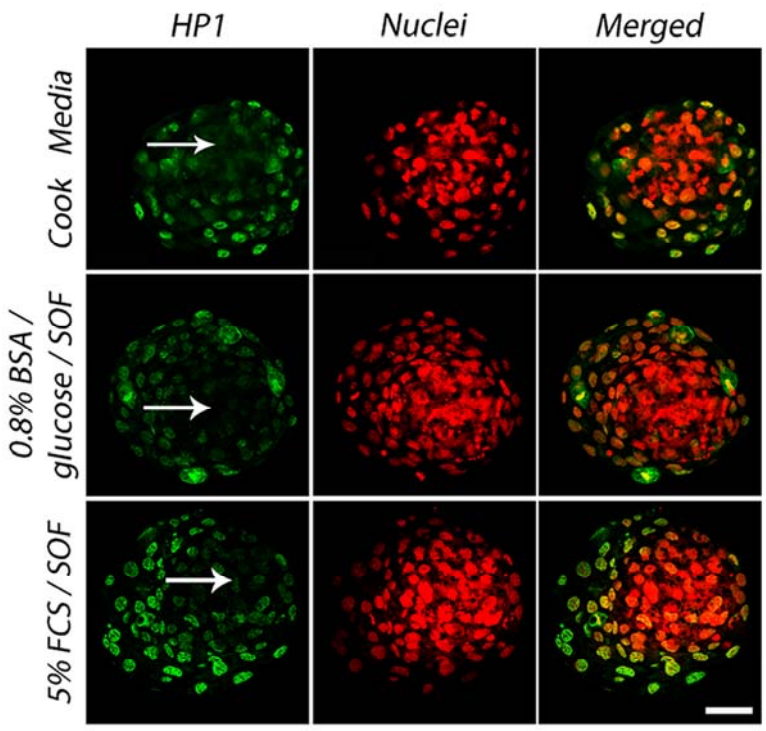

Fig. S4. Culture media had little effect on HP1γ localisation in blastocysts. Blastocysts were cultured in (i) 'Cook' culture media, or synthetic oviduct fluid (SOF) supplemented with (ii) 5% fetal calf serum or (iii) 0.8% bovine serum albumin (BSA) and 1.5 mM glucose before HP1γ antibody staining. HP1γ protein was lacking in inner cell mass cells regardless of detergent concentration (arrows denote inner cell mass cells). Scale bar = 50 µm.

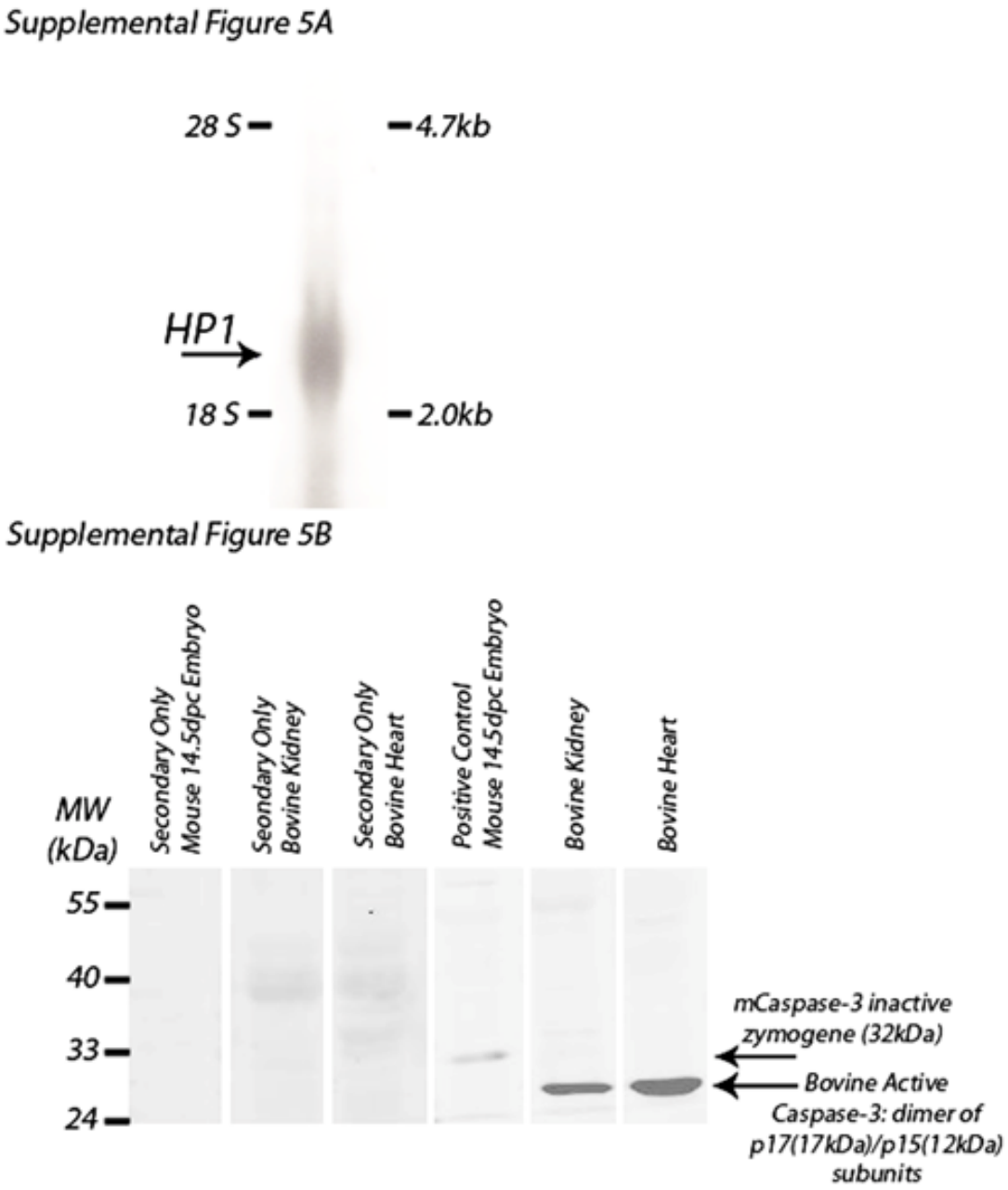

Fig. S5. Confirmation of specificity of RNA and protein probes to bovine epitopes. (A) We confirmed our HP1γ RNA probe recognises bovine RNA in bovine testis lysate by northern blot. (B) We have previously demonstrated reactivity of the HP1γ primary antibody to bovine epitopes (Ruddock-D'Cruz *et al*. 2008). We confirmed that the anti-human Active Caspase-3 antibody reacts with bovine epitope. Antibody reacts with bovine Active Caspase-3 (dimer of pro-enzyme cleavage fragments) in

bovine heart and kidney lysate. Antibody recognized pro-enzyme in mouse positive control lysate (mouse 14.5dpc embryo).

Reference

Ruddock-D'Cruz, N. T., Prashadkumar, S., Wilson, K. J., Heffernan, C., Cooney, M. A., French, A. J., Jans, D. A., Verma, P. J., and Holland, M. K. (2008). Dynamic changes in localization of chromobox (CBX) family members during the maternal to embryonic transition. *Mol. Reprod. Dev.* **75**, 477–488. doi:10.1002/mrd.20752