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Supplementary Material

Platelet-activating factor acetylhydrolase 1B3 (PAFAH1B3) is required for the formation of the meiotic spindle during *in vitro* oocyte maturation

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Table S1. Primers used in this study: product size, annealing temperature

List of primers: NCBI Genebank accession number, GeneID, sequence of the forward and reverse primer, size of the amplicon and optimal primer annealing temperature: PAFAH1B3, platelet-activating factor acetylhydrolase 1B subunit 3

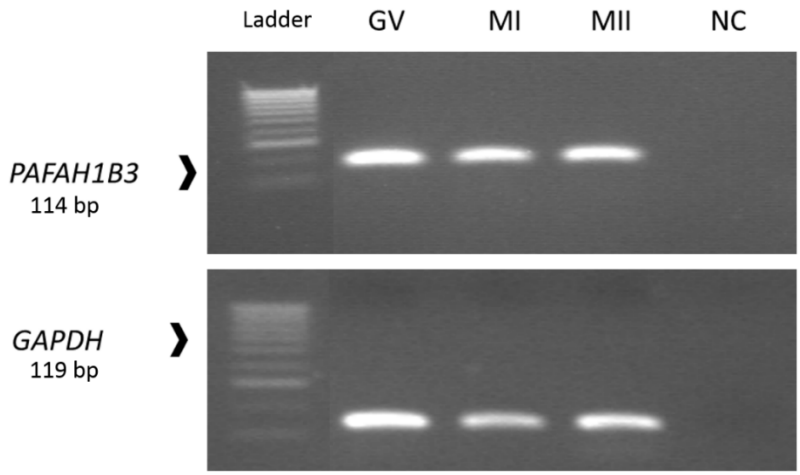
Gene name (GeneID)	**Genebank accession**	**Sequence** (5'→ 3')	**Product size** (bp)	**Ta** (°C)
PAFAH1B3 ***(282515)***	NM_174665.2	*F:AGCAAAGATAAGGAACCCGAAGTCGT* *R:GCCAAAGTTAAGTGCGTGCAGAGG*	114	65

Table S2. Percentages of aberrant spindle configuration in bovine metaphase II oocytes treated with P11

Bovine oocytes with a clear polar body after maturation for 24 h, were treated with 10 µM P11 during 6 h. Afterwards, the oocytes were processed for immunofluorescence confocal microscopy to assess spindle morphology (alpha-tubulin staining). No significant difference between the treatment group and the control group (0.001% DMSO) could be observed. Data of separate components were compared with the control groups and are presented as ± s.e.m.

Treatment			**No. oocytes**	**Metaphase II (%) No. aberrant spindles**
	DMSO	**P11**		
MII + 6 h	0.001%	10 µM	65	1 (1.53 ± 0.18)
Control	0.001%	0 µM	42	1 (2.4 ± 0.20)

(*a*)



(*b*)

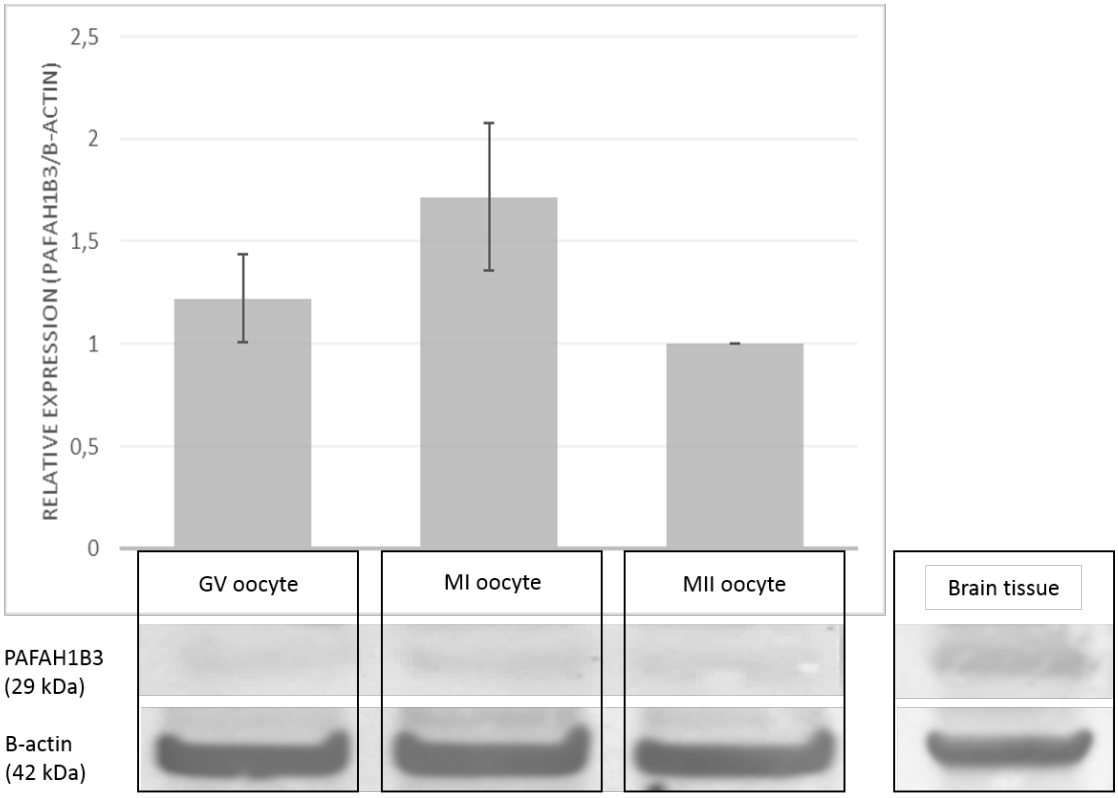


Fig. S1. (*a*) Expression of PAF acetylhydrolase 1B3 in bovine oocytes, as detected by RT–PCR. Oocytes were collected at GV (0 h), MI (12 h) and MII (24 h) stage. Each sample contains five oocytes (total: $n = 15$) and was repeated two times. First lane: 1 kb + DNA Ladder (Promega, Madison, WI). GAPDH was used as an internal control and a negative control (NC) with no template was included. (*b*) Relative expression of PAF acetylhydrolase 1B3 in bovine oocytes, as detected by western blot analysis. Ninety oocytes per stage (GV, MI and MII) were collected. Bovine brain tissue was used as positive control; β-actin as loading control. The relative expression of PAFAH1B3 is shown. The data are expressed as the mean ± s.e.m., student *t*-test was used to analyse the data ($P < 0.05$ was considered significant).

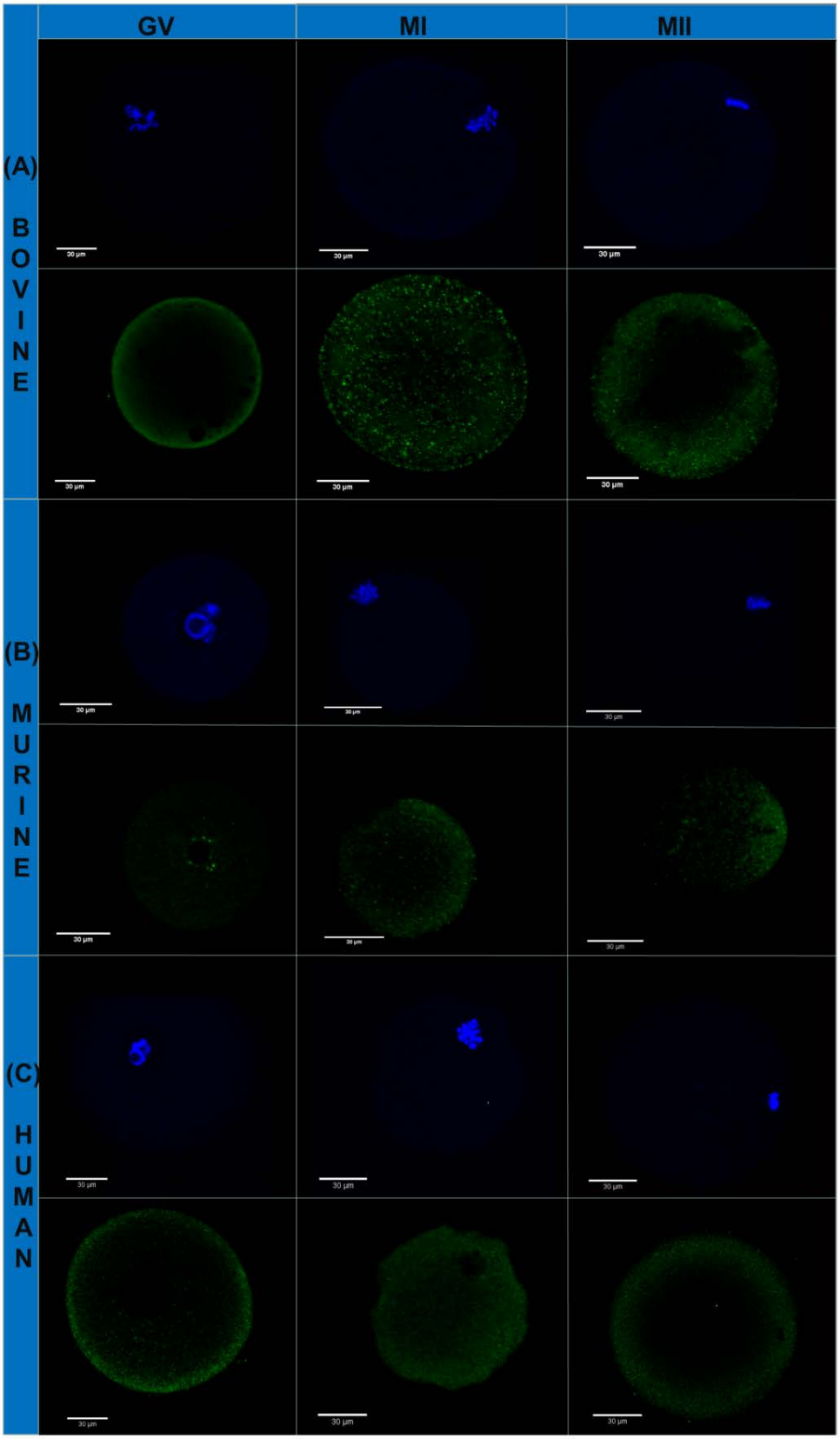


Fig. S2. Negative controls of immunofluorescence experiments in bovine, murine and human oocytes. Representative images of oocytes incubated with whole molecule IgG antibody as replacement for the specific primary antibody in the corresponding dilutions, in order to assess non-specific binding. Z-projected sections of confocal images. Chromatin is labeled with Hoechst 33342. The non-immune IgG is labeled in green. Scale bars = 30 µM. (A) Bovine oocytes (germinal vesicle (GV), metaphase I (MI) and metaphase II (MII)) processed for

immunofluorescent confocal microscopy and labeled with non-immune IgG. (B) Murine oocytes (germinal vesicle (GV), metaphase I (MI) and metaphase II (MII)) processed for immunofluorescent confocal microscopy and labeled with non-immune IgG. (C) Human oocytes (germinal vesicle (GV), metaphase I (MI) and metaphase II (MII)) processed for immunofluorescent confocal microscopy and labeled with non-immune IgG.

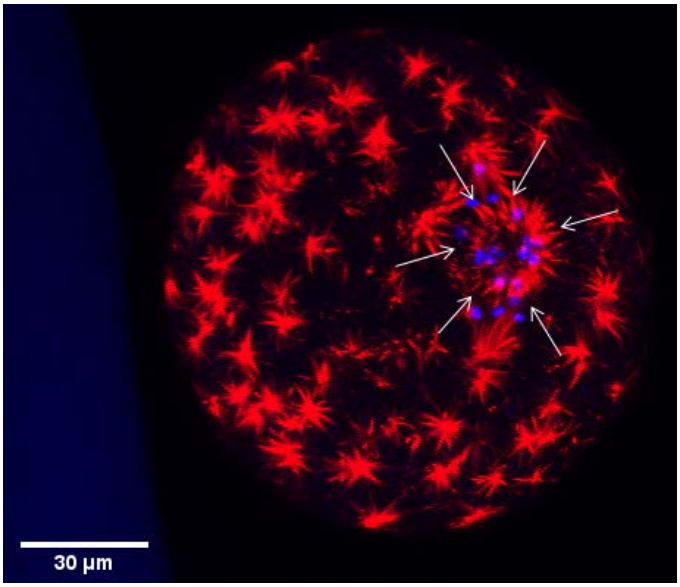


Fig. S3. Taxol treatment of bovine oocytes. Mature (MII) bovine oocytes were treated with the microtubule-stabilising agent taxol (10 µM) in TCM199 medium for 45 min. After zona digestion, the oocytes were subjected to treatment, and subsequently fixed in 2% PFA for 20 min. After fixation, the oocytes were processed for immunofluorescent staining and the spindle was visualised with an alpha-tubulin antibody (Molecular Probes, A11126; 1 : 200). The treatment was successful if multiple asters in the cytoplasm and an enlarged and irregular meiotic spindle (encircled by arrows) was present. Alpha-tubulin, red; DNA, blue. Bar = 30 µM.

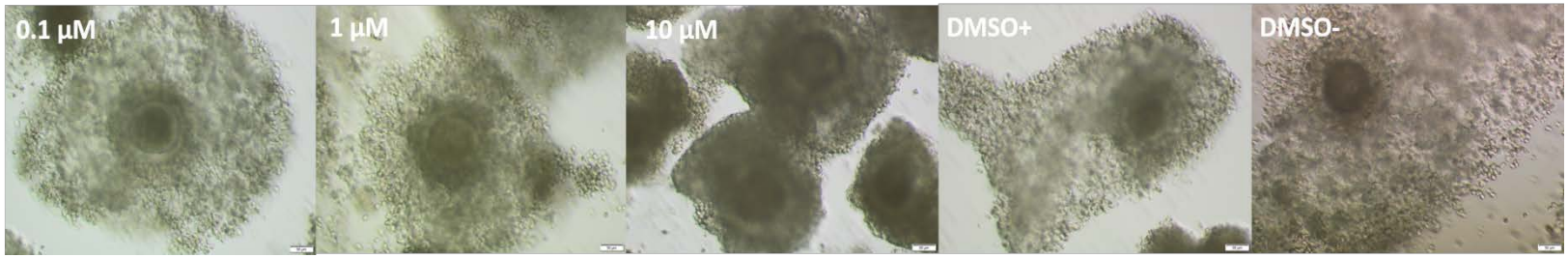


Fig. S4. Representative pictures of bovine cumulus-oocyte complexes treated with PAFAH1B3 inhibitor P11 and controls. Bovine cumulus-enclosed GV oocytes were treated with different concentrations of P11 (0.1 – 1 – 10 µM). Treatment groups and their respective controls: with 0.001% DMSO (DMSO+) or without DMSO (DMSO-) were evaluated based on cumulus expansion (light microscopy). Scale bar = 50 µM.