A burst of regulatory and protein innovation at the origin of placental mammals drove the emergence of placenta and underpins divergent early pregnancy strategies in modern mammals.

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Summary paragraph: The origin of live birth in mammals ~148 million years ago was a dramatic shift in reproductive strategy, yet the molecular changes that established mammal viviparity are largely unknown. Although progesterone receptor signalling predates the origin of mammals andis highly conserved in, and critical for, successful mammal pregnancy, it alone cannot explain the origin and subsequent diversity of implantation strategies throughout the placental mammal radiation. MiRNAs are known to be flexible and dynamic regulators with a well established role in the pathophysiology of mammal placenta. We propose that a dynamic core microRNA (miRNA) network originated early in placental mammal evolution, responds to conserved mammal pregnancy cues (*e.g.* progesterone), and facilitates species-specific responses. Here we identify 13 miRNAs that arose at the origin of placental mammals and were subsequently retained in all descendent lineages. The expression of these 13 miRNAs in response to early pregnancy molecules is regulated in a species-specific manner in endometrial epithelia of species with extremes of implantation strategies. Furthermore, these 13 miRNAs preferentially target 84 proteins under positive selective pressure on ancestral eutherian. Discovery of this core "live-birth" toolkit and specifically adapted proteins helps explain the origin and evolution of the placenta in mammals.

Successful pregnancy in eutheria is contingent on a developmentally competent embryo, appropriate endometrial function, and on formation of the placenta and on the molecular cross-talk across these components [1,2]. Yet placental organ morphology, and more generally, the underlying regulation of successful pregnancy, varies across mammals. Protein coding alterations along with innovations in regulatory networks drive the origin and evolution of novel traits [3]. Moreover, bursts of evolution of new microRNAs (miRNAs) are known to be associated with morphological innovation [4–6], and miRNAs are known to regulate placental function in both normal and pathophysiological conditions [7,8]. We have uncovered a core placental toolkit in mammals that evolved through a synergy of molecular events involving the emergence of new miRNAs combined with adaptive amino acid changes in key proteins. We propose that this conserved core toolkit contributes to the diversity of implantation strategies and pregnancy morphologies observed in modern eutheria (Figure 1).

53

54 **A core miRNA toolkit of 13 miRNAs arose at the origin of therian and eutherian mammals and**
55 **were never subsequently lost.**

56 Because significant miRNA family expansions have been found to correlate with major
57 transitions in animal evolution[4–6], we interrogated MirGeneDB[9] and identified 112 miRNA
58 families that originated on either the therian (6 miRNA families) or eutherian stem lineage (106
59 miRNA families). Six of these miRNA families emerged on the older therian stem lineage (mir-
60 340, mir-483, mir-671, mir-675, mir-1251, and mir-3613), of which only mir-340 is
61 phylogenetically conserved in both Theria and Eutheria (Figure 2a). Given that mir-671 is
62 present in tasmanian devil and in all Eutheria sampled, we classified mir-671 as a 'therian stem
63 lineage miRNA'. A total of 106 miRNAs emerged at the origin of eutheria, and 11 of these
64 remained conserved in all extant eutheria sampled (Figure 2a). In total, this yielded 13 miRNAs
65 that originated on stem mammal lineages and were subsequently retained in all descendent
66 lineages, henceforth referred to as "the core miRNA toolkit". Expression and function of some
67 of the core miRNA toolkit has been demonstrated for normal placenta (*e.g.* mir-433[10], mir-28[11],
68 mir-378[12]), and endometrium (*e.g*. mir-505[13] and mir-542[14]). Others have been implicated in
69 pathophysiological pregnancies, *e.g.* mir-185, mir-188, mir-423 and mir-542[15–17] have been
70 implicated in preeclampsia, mir-127[10] has been associated with placentomegaly, mir-324 is
71 associated with LGA pregnancies[18], mir-331 is associated with placenta from intra-amniotic
72 infection[19], and mir-505 can be associated with preterm birth[20]. Collectively all 13 members of
73 the core miRNA toolkit have been implicated in critical roles in placental mammal pregnancies.

74 Targets were identified in the human genome for the core miRNA toolkit using
75 TargetScan[21]. The predicted functions of the targets of the core miRNA toolkit include
76 reproductive functions (55 target transcripts), metabolic process (1476 target transcripts), and
77 Biological regulation (1269 target transcripts) (Supplementary Figure S1b). More specifically,
78 some of the targets were implicated in pathways associated with INPP5E regulation,
79 Neurophilin interactions, and VEGF interactions with its receptor (VEGR), each involved in the
80 process of angiogenesis. TGF-β signalling and p53 regulation are amongst the predicted targets
81 and are implicated in cell proliferation (Supplementary Figure S3). Both angiogenesis and

82 proliferation are required for successful pregnancy. The syncytins are a family of endogenous
83 retrovirus-derived protein coding regions that were domesticated in mammals and are
84 essential for promoting placental formation[22] and 4/13 of the core miRNA toolkit (miR-185-3p,
85 miR-188-3p, miR-423-5p and miR-433-3p) are predicted to target the syncytins with at least 7-
86 mer binding. In the case of miR-423-5p, there are two predicted target sites for *syncytin-1*, one
87 of which has a site overlapping with that of the miR-185-3p binding site, indicating
88 dynamic/competitive binding between these miRNAs and their *syncytin-1* target. The predicted
89 targets of the core miRNA toolkit also include 130 gene families that have been proposed to
90 have emerged on the eutherian stem lineage[23]. In addition, the targets included genes that
91 evolved endometrial expression on the stem eutherian lineage, and that are hypothesized to
92 have assisted in the remodelling of the uterine landscape during the evolution of the mammal
93 placenta[1].
94
95 **Evidence of positive selection on the stem eutherian lineage.**
96 Protein coding alterations (such as the birth of new genes and gene families, gene loss,
97 co-option, and selective pressure variation) along with innovations in regulatory networks drive
98 the development of novel traits[3]. A number of cases of positively selected amino acids
99 (indicative of protein functional shift) are known to have had a direct role in endometrial
100 function, *e.g.* the galectin family of proteins involved in immune modification at the maternal-
101 fetal interface[24]. We looked for evidence of adaptive evolution in single gene orthologous
102 families (SGOs) on the stem eutherian lineage. We focussed on SGOs to optimise our ability to
103 assign function to orthologs and to accurately trace evolutionary histories. We chose a total of
104 ten Eutheria that demonstrate the greatest range of diversity in placental morphology, plus
105 four outgroup species, one from each of the *Monotremata, Marsupialia, Aves* and *Teleostei*
106 (Supplementary Table S1). Annotated gene families were taken from Ensembl 90[25]. Following
107 our filtering regime we extracted a total of 1,437 SGOs. Applying codon models of evolution to
108 these SGOs, we identified signatures of positive selection on amino acid residues on the stem
109 eutherian lineage in 237 SGOs. The functions of these genes are predominantly cellular
110 processes, metabolic processes, and biological regulation (Supplementary Figure S2). Out of

111 these 237 positively selected SGOs, 115 contained positively selected amino acid residues that
112 were subsequently unaltered in all descendent lineages. The 115 SGOs are functionally
113 enriched for chromosomal maintenance, telomere activity, p53 signalling, cell cycle, and the
114 inflammatory immune response - activities that have been associated with the formation of the
115 placental tissue in pregnancy[26–28]. We then studied whether there is a significant association
116 between the core miRNA toolkit and stem-lineage positively selected proteins.
117
118 **The core miRNA toolkit preferentially targets genes under positive selection in the stem**
119 **eutherian lineage.**
120 Synergy between regulatory and protein coding innovations often drives substantial
121 phenotypic novelty[2,3,29]. Therefore, to test the hypothesis that innovation both at the level of
122 regulation and of protein coding change underpinned the origin of placentation in mammals,
123 we performed a simulation study on the targets of the 13 stem lineage miRNAs. Out of 115
124 SGOs with evidence of positive selection on the ancestral eutherian lineage, 84 were found to
125 be significantly enriched for binding sites for the 13 members of the core miRNA toolkit
126 (p=1.35618e-11), with a mean of 6.66 binding sites per transcript (median=4.0) (Figure 2b). We
127 determined if the number of binding sites in this subset of 84 positively selected SGOs was
128 significantly different than one would expect by random chance. We estimated the number of
129 binding sites per transcript for the core miRNA toolkit in 100 randomly sampled gene sets and
130 found it is significantly lower, with a mean=1.64 (median=1.0). This suggests that the 13
131 miRNAs in the core miRNA toolkit preferentially target the positively selected SGOs (6.66
132 binding sites compared to 1.64) (Figure 2b). Combined, this indicates that a co-evolutionary
133 process arose in a short time in early mammal evolution that resulted in altered protein
134 function, as well as a new miRNA-mediated regulatory network.
135 The functions of the 84 positively selected SGOs targeted by the core miRNA toolkit
136 broadly fall within the categories of cell cycle, DNA damage & DNA metabolic processes, and
137 regulation of hair cycle & hair cycle – a uniquely mammal characteristic (Supplementary Figure
138 S3). 21/84 SGOs are significantly more likely to interact with one another ($p<0.05$) in
139 comparison to any other gene in the genome (Supplementary Figure S2b). Of course there is

140 significant variation found in modern mammals in other facets such as telomere biology, cancer
141 incidence, body mass and maximum lifespan[30–33], therefore innovation at this node was not
142 expected to be entirely skewed to pregnancy (Supplementary Table S2)
143
144 **Species-specific regulation of the core miRNA toolkit by key early pregnancy molecules in**
145 **species with different implantation strategies.**
146 Implantation in eutherian mammals displays wide variation in both embryological
147 morphology and bi-lateral signalling between the embryo and maternal endometrium, and
148 degree of invasiveness (invasive in human, superficial in bovine). This variation is governed, in
149 part, by conserved signalling pathways, *e.g.* the sustained actions of the hormone progesterone
150 (P4)[34], but also diverse molecular cues such as the maternal recognition of pregnancy signal,
151 *e.g*. chorionic gonadotrophin (hCG) in human and interferon (IFNT) in bovine[35,36]. We asked the
152 question what molecules, that are involved in bi-lateral communication between the embryo
153 and endometrium, regulate the core mammal miRNA toolkit and if they are regulated in a
154 species-specific manner. We cultured endometrial epithelial cells from human and bovine[37] and
155 exposed them for 24 hr to recombinant forms of conserved (P4, CAPG, and PDI) and diverse
156 (IFNT and hCG) molecular cues important for early pregnancy success in placental mammals.
157 CAPG and PDI have recently been identified as produced by the bovine conceptus during
158 pregnancy recognition and are highly conserved (in terms of sequence identity and
159 phylogenetic distribution) across placental mammals[37,38]. We then examined the expression of
160 the 13 stem lineage miRNAs in these cells using an LNA-based approach. Treatment of bovine
161 endometrial epithelial cells with 10µg/mL of P4 during the early luteal phase resulted in
162 increased expression of miR-505-5p (Supplementary Figure S4). In summary, treatment with
163 the evolutionarily conserved early pregnancy proteins (P4, CAPG and PDI) in the endometrial
164 epithelial cells of bovine and/or human resulted in a change in expression of 11/13 of the stem
165 lineage miRNAs (Figure 1).
166 Intriguingly, addition of recombinant bovine forms of bCAPG and bPDI proteins to
167 bovine or human endometrial epithelial cells altered expression of selected miRNAs in a
168 species-specific manner (Figure 3&4 respectively). Treatment of bovine cells with recombinant

169 bCAPG resulted in increased expression of miR-331-3p, miR-324-5p and miR-505-5p in
170 endometrial epithelial cells ($p<0.05$). Whereas in human endometrial epithelial cells treated
171 with 1000ng/µl bCAPG, the expression of miR-127-3p, miR-151a-3p (a paralog of mir-28
172 originating on the Eutherian stem lineage), and miR-188-5p showed a significant decrease in
173 expression compared to vehicle control ($p<0.05$: Figure 3). Treatment with recombinant bPDI
174 decreased expression of miR-185-5p in bovine epithelial cells ($P<0.05$). In human Ishikawa
175 immortalised endometrial epithelial cells treated with 1000ng/µl bPDI the expression of miR-
176 151a-5p, miR-185-5p, miR-378a-3p and miR-532-5p (a paralogue of mir-188 originating on the
177 Eutherian stem lineage) were significantly decreased compared to vehicle control (Figure 4).
178 In contrast, addition of the species specific pregnancy recognition signals (IFNT in
179 bovine: hCG in human) to receptive endometrial epithelial cells altered expression of one
180 member of the core miRNA toolkit (Supplementary Figures S5 and S6 for IFNT and hCG data
181 respectively). These data demonstrate that the expression of the core miRNA toolkit is not
182 altered by the species-specific pregnancy recognition signals (IFNT and hCG).
183 Human and bovine represent two distinct implantation strategies for mammals and
184 these lineages last shared a common ancestor some ~92 million years ago, representing ~184
185 million years of independent evolution [39,40]. None of the 13 stem lineage miRNAs are regulated
186 by the species-specific pregnancy recognition signals (IFNT and hCG), but the expression of the
187 core miRNA toolkit is modified by proteins that are highly conserved amongst placental
188 mammals (CAPG and PDI). Combined, our results show that the preferential targeting of the
189 core mammal miRNA toolkit, and protein functional shift were essential to the establishment of
190 mammalian implantation, and that subsequent diversification of this network facilitated the
191 range of implantation strategies observed today.
192 In summary, this work identifies a core regulatory network that drove a major transition
193 - the origin of live birth in mammals. With the core now defined, future work can focus on the
194 accessory elements that drove the subsequent diversification of mammal placenta.
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197
198 **ACKNOWLEDGEMENTS**

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CONTRIBUTIONS

NF and MJOC conceived of the study and designed the experiments. AST, BC, VO and MJOC carried out all evolutionary analyses and DW carried out statistical tests. NF, AST, HT, WS, ALP, and RAS undertook all molecular analyses. NF and MJOC drafted the manuscript, all authors contributed to revising, reading, and critiquing the manuscript.

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306 **FIGURES**

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308 **Figure 1:**

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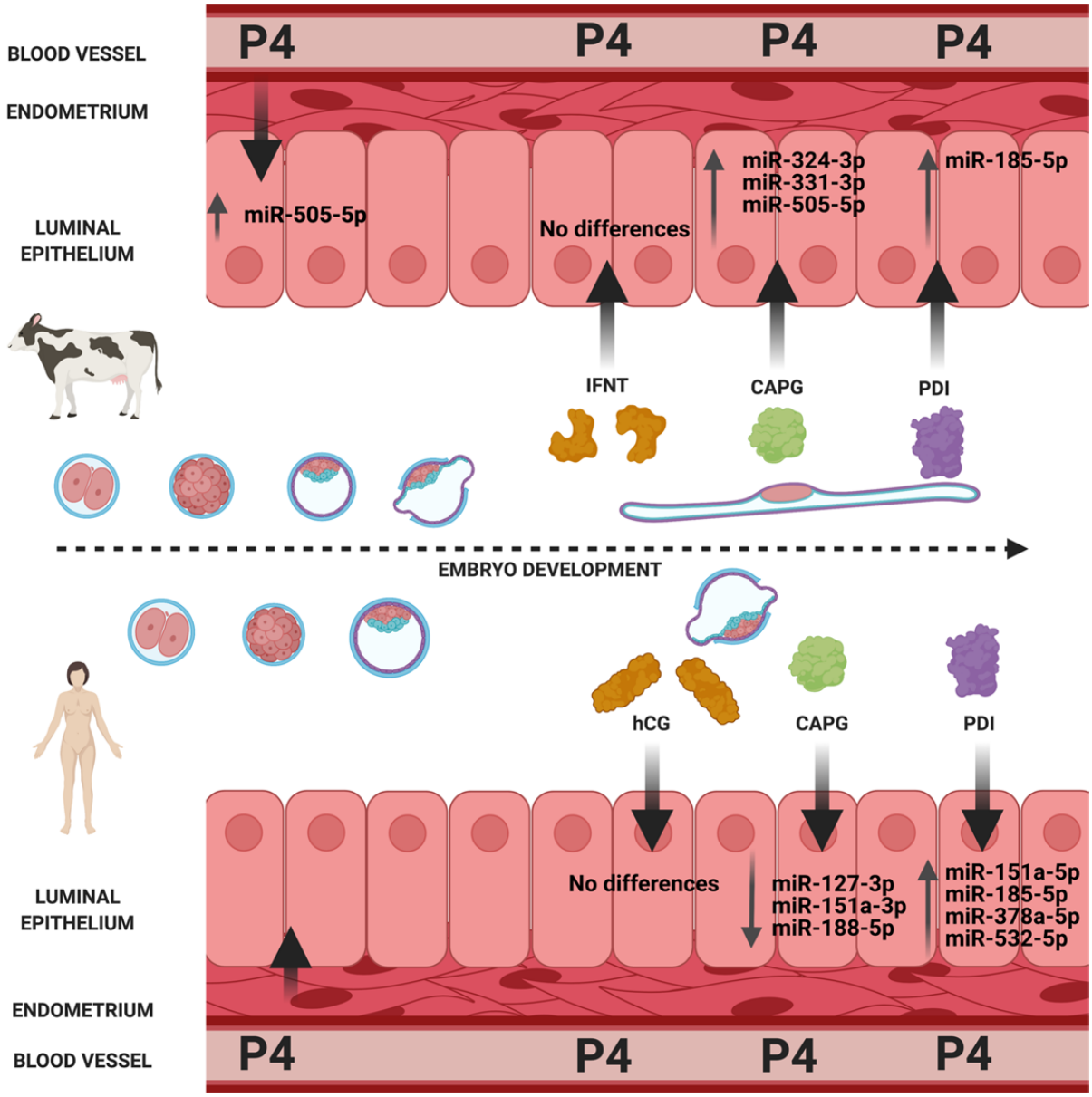

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313 ***Figure 1. Regulation of core miRNA toolkit by early pregnancy markers.*** *The endometrial*

314 *epithelium of bovine (superficial implantation strategy) (top panel) or human (invasive*

315 *implantation strategy) (bottom panel) are regulated by molecules important for endometrial*

316 *function in early pregnancy in eutheria (P4: progesterone; hCG: human chorionic*

317 *gonadotrophin; Interferon Tau: IFNT; Macrophage capping protein: CAPG, and protein disulfide*
318 *isomerase: PDI).*
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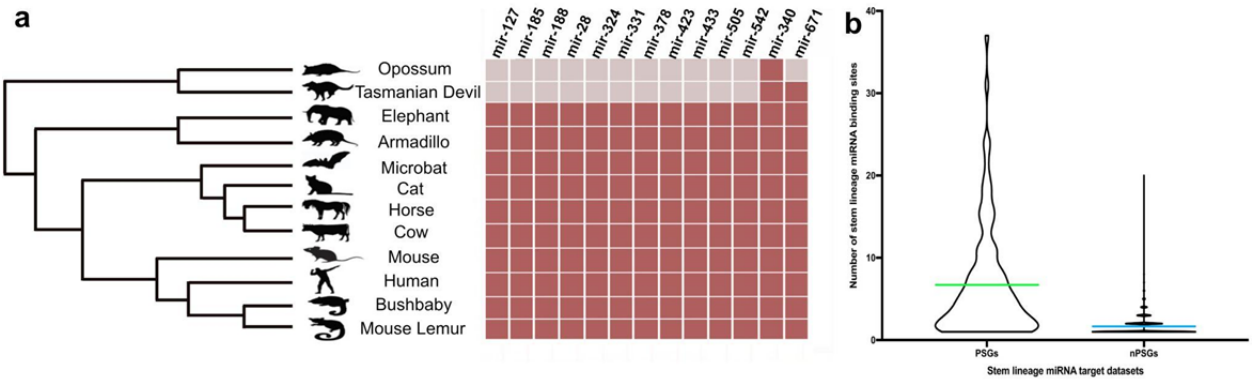
321 **Figure 2:**



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323 ***Figure 2: Phylogenetic distribution of the miRNA families and their levels of targeting in***
324 ***positively selected genes. (a)*** *Phylogenetic distribution of miRNA families specific to therian and*
325 *eutherian mammals. The mammal phylogeny displaying the species sampled in our analysis.*
326 *The corresponding matrix shows the presence (dark red) or absence (pale grey) of miRNA*
327 *families across the species sampled.* ***(b)*** *Violin plot comparing the number of target miRNA*
328 *binding sites from the core miRNA toolkit per transcript in the 84 genes that underwent positive*
329 *selection on the stem Eutherian lineage (PSGs) (left) compared to sets of randomly sampled*
330 *targets of the miRNAs that do not have signatures of positive selection on the stem Eutherian*
331 *lineage (nPSG) (right). For each of the 84 PSGs, the mean number of predicted miRNA binding*
332 *sites was determined for each target transcript in human (depicted in green). This was*
333 *compared to the mean number of binding sites for each of the nPSGs (depicted in blue). The*
334 *mean number of binding sites was determined to be significantly different between the two*
335 *datasets when $p \leq 0.05$, two-sample t-test with unequal variance.*

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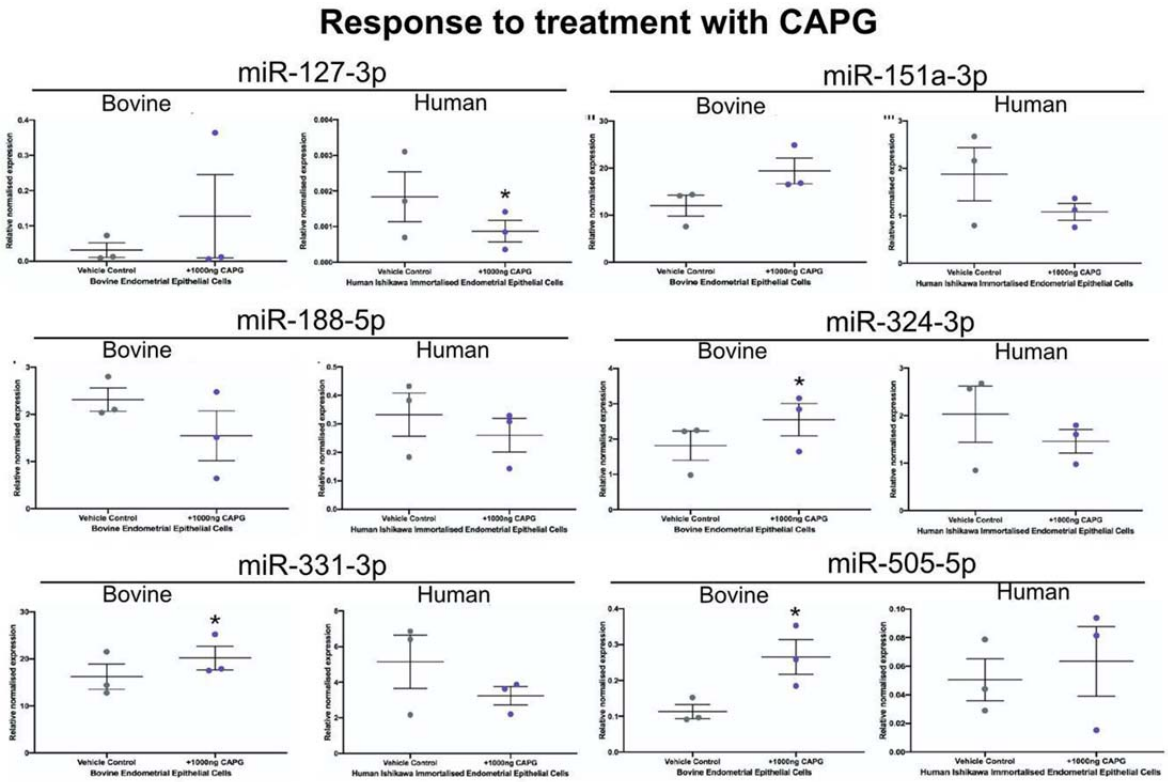
345 **Figure 3:**



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347 **Figure 3.** ***Regulation of stem lineage miRNAs in bovine and human endometrial epithelial cells***
348 ***treated with bCAPG.*** *Expression of stem lineage miRNAs miR-127-3p, miR-151a-3p, miR-188-*
349 *5p, miR-324-3p, miR-331-3p, and miR-505-5p in either bovine (left hand side of each pair) or*
350 *human (right hand side of each pair) endometrial epithelial cells. Primary bovine endometrial*
351 *epithelial cells were treated with vehicle control (grey circle), or 1000ng/µl bCAPG (purple circle)*
352 *for 24 hours. Human Ishikawa immortalized endometrial epithelial cells were treated with*
353 *vehicle control (grey circle), or 1000ng/µl bCAPG (purple circle) for 24 hours. Significant*
354 *differences in miRNA expression values determined when p≤0.05 are depicted by an asterisk (*).*

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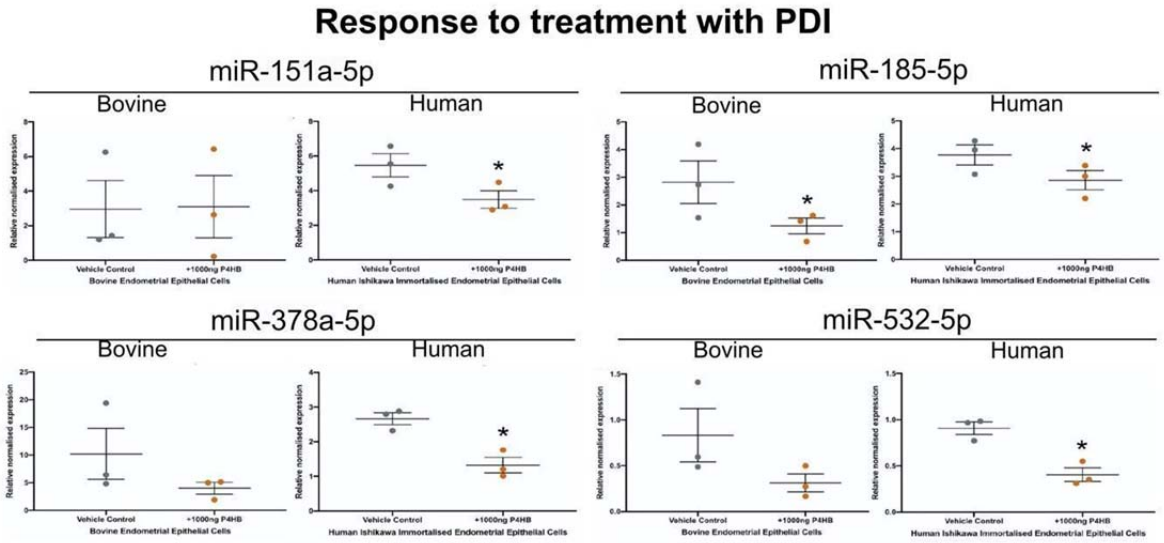
363 **Figure 4:**



364
365 **Figure 4:** ***Regulation of stem lineage miRNAs in bovine and human endometrial epithelial cells***
366 ***treated with bPDI.*** *Expression of stem lineage miRNAs miR-127-3p, miR-151a-3p, miR-188-5p,*
367 *miR-324-3p, miR-331-3p, and miR-505-5p in either bovine (left hand side of each pair) or human*
368 *(right hand side of each pair) endometrial epithelial cells. Primary bovine endometrial epithelial*
369 *cells were treated with vehicle control (grey circle), or 1000ng/µl bPDI (orange circle) for 24*
370 *hours. Human Ishikawa immortalized endometrial epithelial cells were treated with vehicle*
371 *control (grey circle), or 1000ng/µl bPDI (orange circle) for 24 hours. Significant differences in*
372 *miRNA expression values determined when p≤0.05 are depicted by an asterisk (*).*
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387 **Supplementary Figures and Tables**
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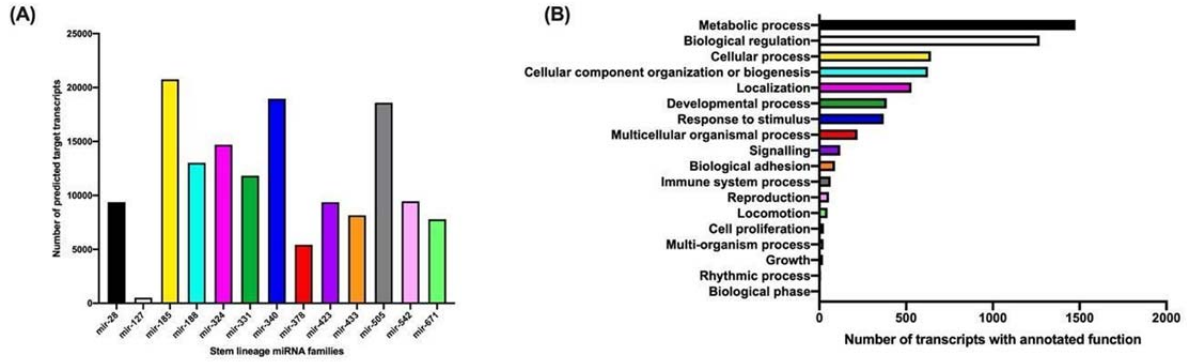
390 **Supplementary Figure S1:**



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393 ***Figure S1. Number of predicted targets for each of the 13 stem lineage miRNA family and the***
394 ***functional annotation of the target genes using Panther DB. (A)*** *Targets were predicted for*
395 *each of the 13 stem lineage miRNAs using TargetScan70 (Agarwal et al., 2015). TargetScan*
396 *output was filtered for targets with8mer-A1, 7mer-m8 and 7mer-A1 complementary binding to*
397 *the seed region.* ***(B)*** *Functional annotation of predicted targets of the 13 stem lineage miRNAs.*
398 *Filtered stem lineage miRNA target transcripts were analysed for functional enrichment using*
399 *PANTHERv.14 (Muruganujan et al., 2018), where PANTHERv.14 found functional annotations to*
400 *be significantly enriched when p≤0.05.*
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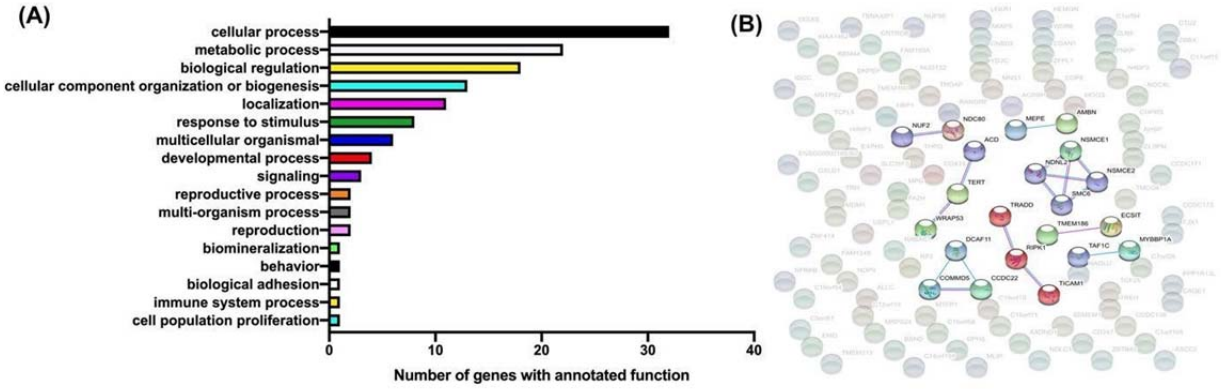
404 **Supplementary Figure S2:**



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407 ***Figure S2. Broad functions of the genes with signatures of positive selection and sample of***
408 ***their interactions. (A)*** *Functional annotation using Gene Ontology Biological Process terms of*
409 *115 SGOs that underwent positive selection on the stem eutherian lineage and where the amino*
410 *acid substitution was fixed on all extant Eutheria tested. The absolute number (out of 115) of*
411 *positively selected genes in a given category are shown in the X axis and the functional*
412 *annotations on the Y-axis.* ***(B)*** *String interaction network of the same set of 115 genes. Network*
413 *has 106 total nodes and 8 edges (expected edges =4). Background nodes, with no high*
414 *confidence interactions from experimental and database sources are faded. Nodes with high*
415 *confidence interactions from experimentally determined (pink lines) or curated database (blue*
416 *lines) sources are depicted in colour. The network was found to be significantly enriched for*
417 *gene-gene interactions (p=0.0468). Average node degree is 0.151, with an average local*
418 *clustering coefficient of 0.104. Minimum interaction score is 0.700.*
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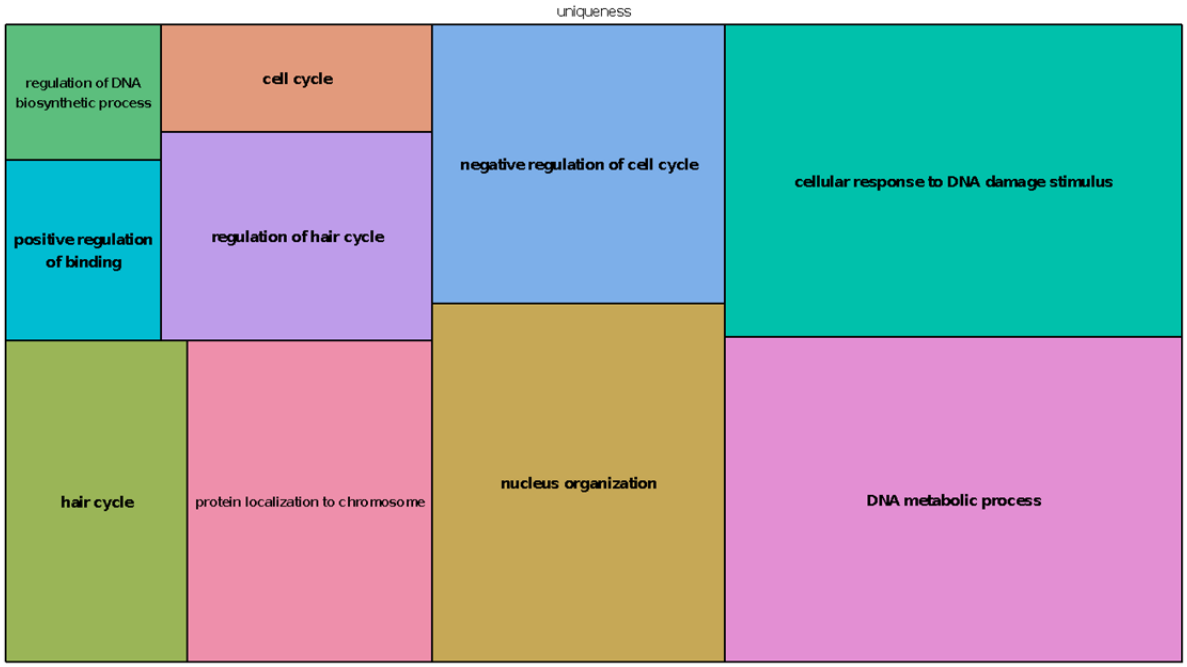
422 **Supplementary Figure S3:**



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424 ***Figure S3: A standard TreeMap from REVIGO displaying the GO biological process terms***
425 ***present in the 84 PSGs that were predicted targets of the 13 stem lineage miRNAs****. Rectangle*
426 *size represents semantic uniqueness of GO term, defined by REVIGO as the negative of average*
427 *similarity to all other terms present in human.*

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Supplementary Figure S4:

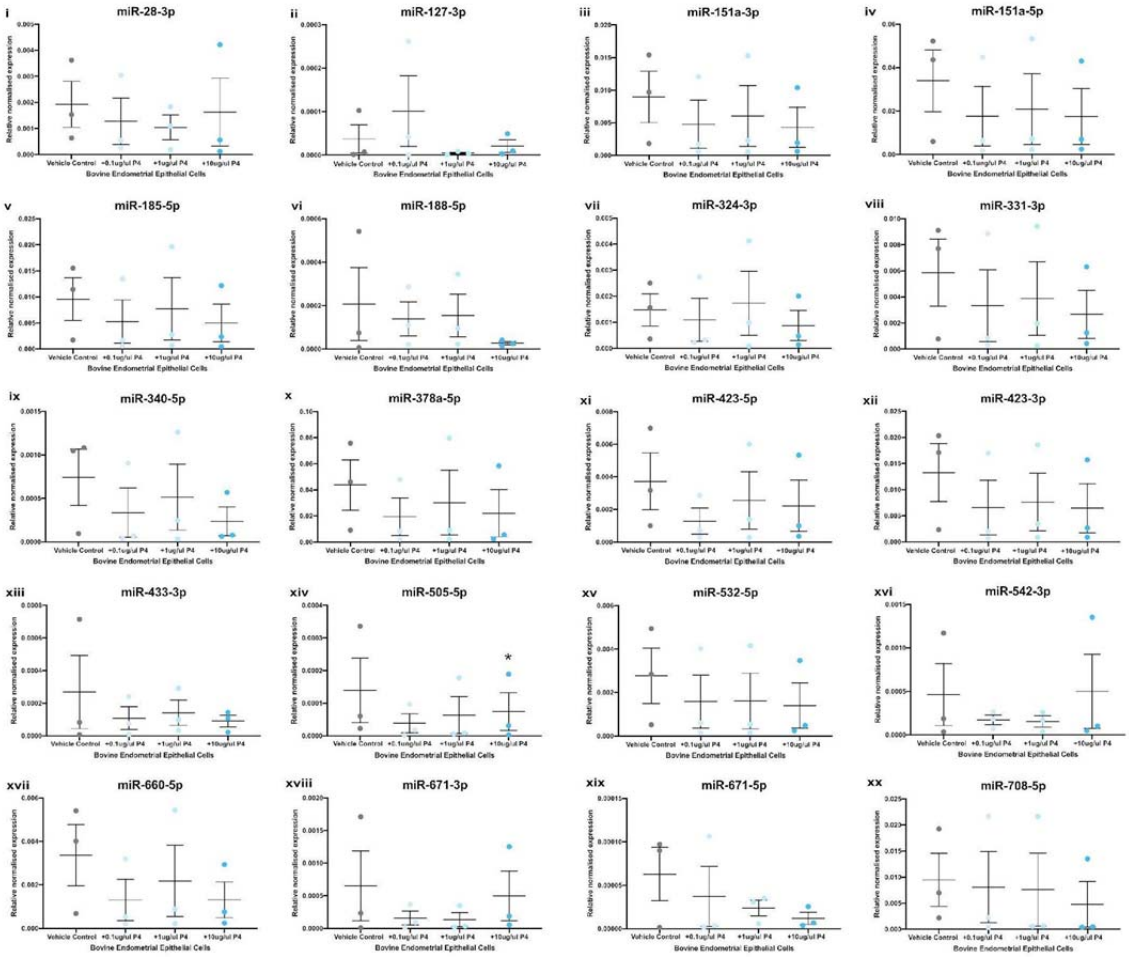

Figure S4. Expression of stem lineage miRNAs in bovine endometrial epithelial cells treated with P4. *Expression of stem lineage miRNA (i) miR-28-3p, (ii) miR-127-3p, (iii) miR-151a-3p, (iv) miR-151a-5p, (v) miR-185-5p, (vi) miR-188-5p, (vii) miR-324-5p, (viii) miR-331-3p, (ix) miR-340-5p, (x) miR-378a-5p, (xi) miR-423-3p, (xii) miR-423-5p, (xiii) miR-433-3p, (xiv) miR-505-5p, (xv) miR-532-5p, (xvi) miR-542-3p, (xvii) miR-660-5p, (xviii) miR-671-3p, (xix) miR-671-5p and (xx) miR-708-5p in bovine endometrial epithelial cells treated with vehicle control (grey circle), 0.1µg/mL (light blue), 1.0µg/mL (medium blue) or 10µg/mL P4 (dark blue circle) for 24 hours. Significant differences in miRNA expression values determined when p≤0.05 are depicted by an asterisk (*).*

454 **Supplementary Figure S5:**

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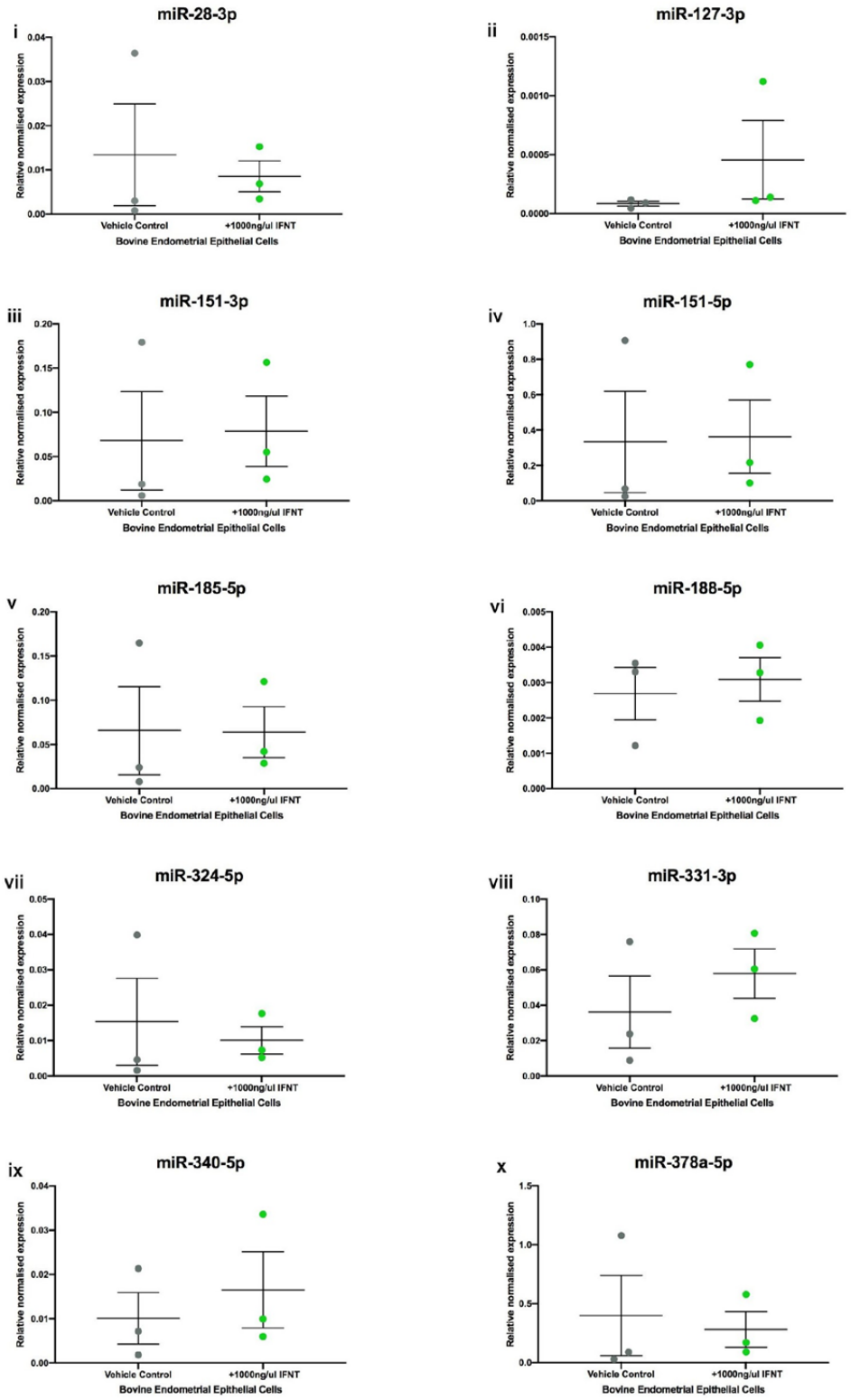

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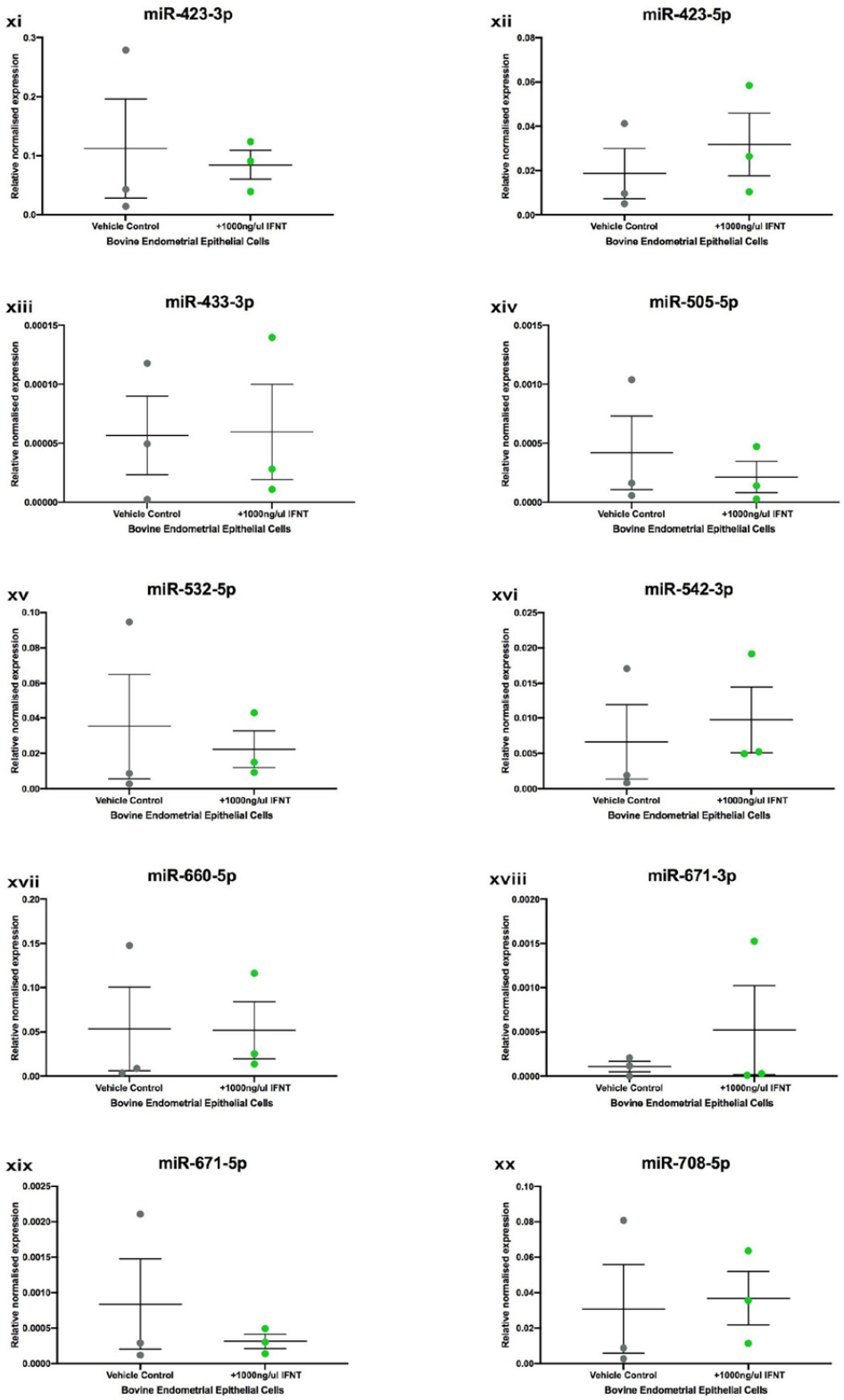

Figure S5. Expression of stem lineage miRNAs in bovine endometrial explants treated with recombinant oIFNT. Expression of stem lineage miRNA (i) miR-28-3p, (ii) miR-127-3p, (iii) miR-

151a-3p, (iv) miR-151a-5p, (v) miR-185-5p, (vi) miR-188-5p, (vii) miR-324-5p, (viii) miR-331-3p, (ix) miR- 340-5p, (x) miR-378a-5p, (xi) miR-423-3p, (xii) miR-423-5p, (xiii) miR-433-3p, (xiv) miR-505-5p, (xv) miR-532-5p, (xvi) miR-542-3p, (xvii) miR- 660-5p, (xviii) miR-671-3p, (xix) miR-671-5p and (xx) miR-708-5p in bovine endometrial explants treated with vehicle control (grey circle), or 1000ng/µl oIFNT (green circle) for 24 hours. Significant differences in miRNA expression values determined when $p<0.05$ are depicted by an asterisk (*).

471 **Supplementary Figure S6:**

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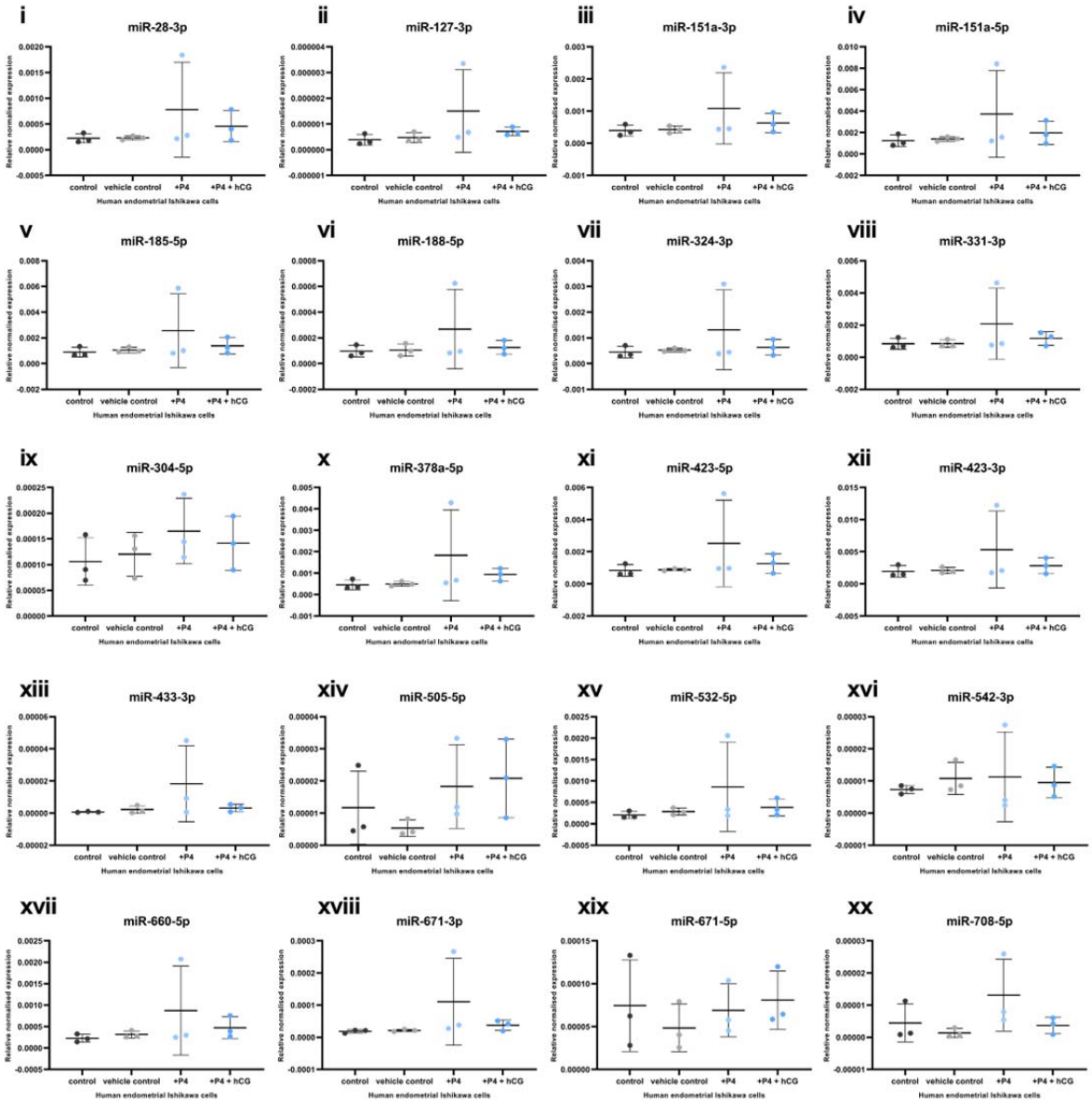

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475 **Figure S6. Expression of stem lineage miRNAs in human endometrial epithelial cells treated**
476 **with hCG.** Expression of stem lineage miRNA (i) miR-28-3p, (ii) miR-127-3p, (iii) miR-151a-3p,
477 (iv) miR-151a-5p, (v) miR-185-5p, (vi) miR-188-5p, (vii) miR-324-5p, (viii) miR-331-3p, (ix) miR-
478 340-5p, (x) miR-378a-5p, (xi) miR-423-3p, (xii) miR-423-5p, (xiii) miR-433-3p, (xiv) miR-505-5p,
479 (xv) miR-532-5p, (xvi) miR-542-3p, (xvii) miR- 660-5p, (xviii) miR-671-3p, (xix) miR-671-5p and

480 (xx) miR-708-5p in human Ishikawa immortalized endometrial epithelial cells were treated with
481 control (dark grey circle), vehicle control (light grey circle), or P4 (light blue circle), or P4+hCG
482 (darker blue circles) for 24 hours. No differences in miRNA expression were determined
483 ($p>0.05$).
484

485 **Supplementary Table S1: Vertebrate Species sampled, genome version, coverage and**
486 **completion level.**

Clade	Species	Version	Genome Quality
Fish	Zebrafish	GRCz11	Full Genome, Chromosome Level
	Chicken	Gallus_gallus-5.0	70X Coverage, Chromosome Level
Monotremes	Platypus	OANA5	6X Coverage, Chromosome Level
Metatheria	Opossum	monDom5	7.33X Coverage, Chromosome Level
Eutheria	Elephant	Loxafr3.0	7X Coverage, Scaffold Level
	Armadillo	Dasnov3.0	6X Coverage, Scaffold Level
	Mouse	GRCm38.p6	High Quality Reference Assembly
	Human	GRCh38.p12	High Quality Reference Assembly
	Mouse Lemur	Mmur_3.0	221.6X Coverage, Chromosome Level
	Bushbaby	OtoGar3	137X Coverage, Scaffold Level
	Cat	Felis_catus_9.0	72X Coverage, Chromosome Level
	Microbat	Myoluc2.0	7X Coverage, Scaffold Level
	Horse	Equ Cab 2	6.79X Coverage, Chromosome Level
	Cow	UMD3.1	9X Coverage, Chromosome Level

487 ***Table S1:*** *Set of 14 species sampled for the selective pressure analysis. Using Ensembl 92* (Yates *et al.*,
488 2016)*, a dataset of genomes representative of (i) vertebrate outgroup clades, or (ii) variations in*
489 *placental morphology in metatherian and eutherian mammals. For each clade, genomes were chosen*
490 *based on genome coverage for downstream homology searching. 'Clade' refers to the taxonomic group*
491 *of each included species. 'Species' denotes the included species, by their common name. 'Version'*
492 *denotes the genome assembly version included in this analysis. 'Genome Quality' refers to the coverage*
493 *and assembly level of each species included in this analysis.*
494

495

496 **Supplementary Table S2: Example of three functionally related proteins under positive**
497 **selection on stem eutherian lineage**

Family	Gene Name	lnL	*p*2	ω2	Positions in the alignment predicted to be positively selected.
PTHR13211	WRAP53	-10149.383	0.04821	37.80743	72, 88, 114, 225, 253, 260, 261, 331, 423, 480, 493, 506, 510, 513, 522, 528
PTHR12066	TERT	-36003.667	0.02302	954.86770	121, 122, 140, 166, 197, 508, 878, 1020, 1110
PTHR14487	ACD	-15296.106	0.10315	30.62227	138, 143, 148, 178, 185, 200, 202, 206, 208, 209, 214, 269, 278, 296, 298, 314, 347, 363, 368, 379, 390, 394, 395, 412, 414, 450, 470, 474, 524, 530, 532, 533, 545, 548, 561, 571, 575, 586, 685, 698

498 ***Table S2:*** *The panther family ID and common gene names are provided for a set of 3 proteins*
499 *extracted to illustrate the cases of positive selection identified. The LnL value associated with*
500 *the fit of the codeml model (PAML) to the data are provided as are the associated proportion of*
501 *sites (p2) that have the corresponding ω2 (or Dn/Ds ratio). The sites estimated to be positively*
502 *selected are given in the final column, these are numbered as per aligned codon position.*
503