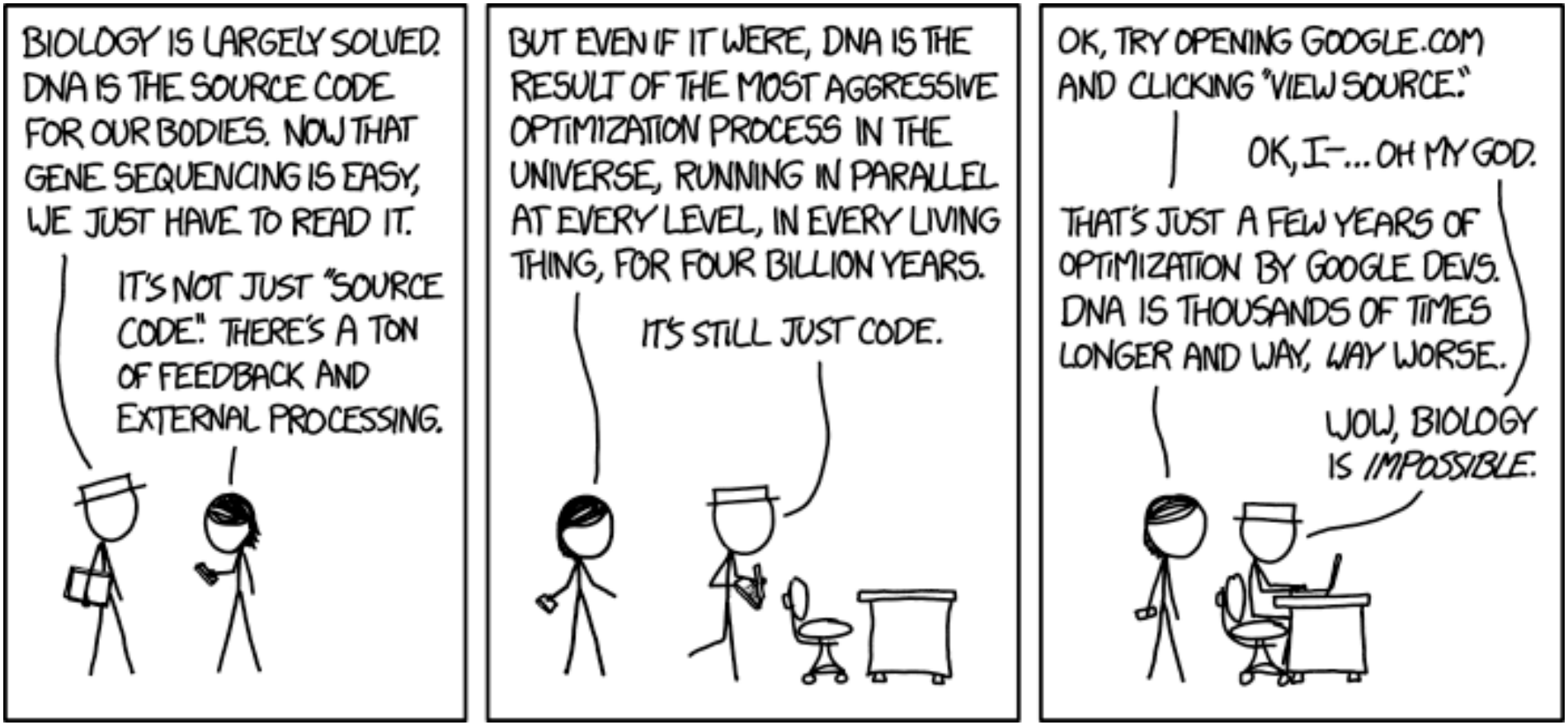


https://xkcd.com/1605/

The evolution of gene expression in the term placenta of viviparous mammals

Don L Armstrong, Michael R. McGowen, Amy Weckle, Jason Caravas, Dalen Agnew, Kurt Benirschke, Sue Savage-Rumbaugh, Eviatar Nevo, Chong J Kim, Günter P. Wagner, Roberto Romero, and Derek E. Wildman

Institute for Genomic Biology, Computing Genomes for Reproductive Health, University of Illinois, Urbana-Champaign

February 22, 2016

Function and Evolution of Placenta

- Placenta functions in the transfer of nutrients, oxygen, and waste between mother and offspring
- Placenta is highly variable structure and form in mammals, even though major function is conserved
- Placenta likely arose before the origin of therian mammals
- Form relatively well characterized, but diversity of molecular environment of the placenta is only recently being understood
- Possible role of evolutionary interplay between maternal and fetal strategies

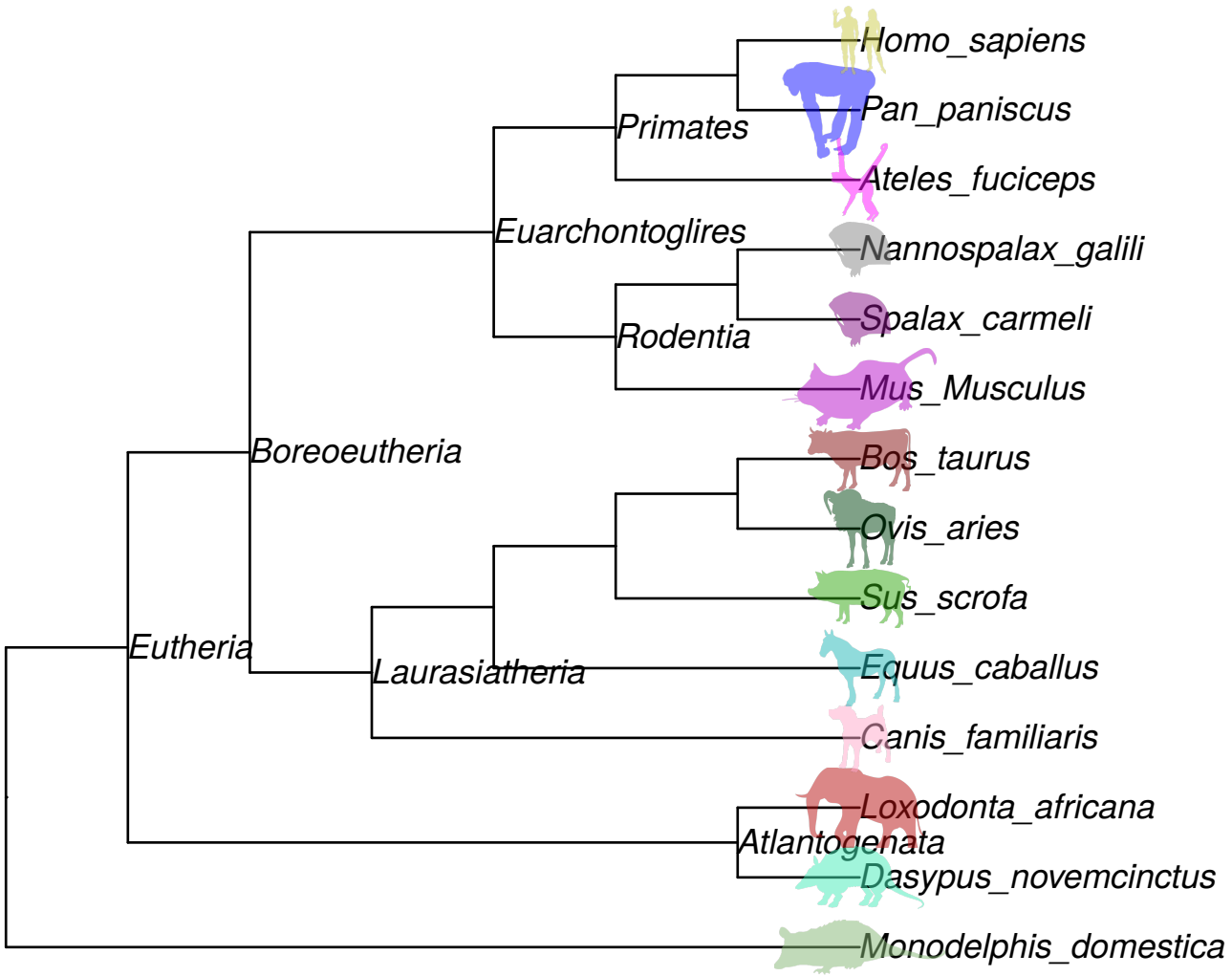
Impacts on Human Health?

- Humans possess a deeply invasive trophoblast layer that can penetrate the myometrium of the uterus[1].
- Defects in placental growth can lead to pre-eclampsia, which can cause hypertension, proteinuria, and mortality of mother and/or infant[2].
- Pre-eclampsia has been seen in *Pan troglodytes*[3], and *Gorilla gorilla*[4]
- Far higher incidence in *Homo sapiens*, with as many as 4% of pregnancies United States affected[2].

Overall Hypotheses

- Gene expression patterns correlate with placenta morphology
- Altered expression of genes correlates with invasiveness, and therefore pre-eclampsia
- Maternal/Fetal evolutionary strategies will be reflected in gene expression patterns

Species Studied



Variation in Gestational Periods and Litter Size

Species	Gestation (days)	Litter Size	Source
Ateles fusciceps	224	1	Ernest [5]
Bos taurus	278	1	Kiltie [6]
Canis familiaris	61	4	Kiltie [6]
Dasypus novemcinctus	150	4	Smith & Doughty [7]
Equus caballus	336	1	Kiltie [6]
Loxodonta africana	644	1	Ernest [5]
Homo sapiens	270	1.1	Kiltie [6]
Monodelphis domestica	15	7.1	Harder *et al.* [8]
Mus musculus	20	6.1	Kiltie [6]
Nannospalax galili	28-42	4	Nevo [9]
Ovis aries	151	1.35	Kiltie [6]
Pan paniscus	223	1	Ernest [5]
Spalax carmeli	28-42	4	Nevo [9]
Sus scrofa	114	5.8	Kiltie [6]

Variations in Placenta Morphology

Species	Interhemal Membrane	Pattern
A. fusciceps	hemochorial	trabecular
B. taurus	epitheliochorial	villous
C. familiaris	endotheliochorial	labyrinthine
D. novemcinctus	hemochorial	trabecular
E. caballus	epitheliochorial	villous
L. africana	endotheliochorial	labyrinthine
H. sapiens	hemochorial	villous
M. domestica	epitheliochorial	choriovitelline
M. musculus	hemotrichorial	labyrinthine
N. galili	hemotrichorial	labyrinthine
O. aries	epitheliochorial	villous
P. paniscus	hemochorial	villous
S. carmeli	hemotrichorial	labyrinthine
S. scrofa	epitheliochorial	diffuse

Collection of Placental Tissue

- Collected from 8 mammalian species
- Only fetal portion was sampled
- Sequenced using GAII with an insert size of 230 bp
- Four additional species and additional *M. musculus* and *H. sapiens* samples were obtained from SRA

Assembly, Alignment, Annotation and Quantification

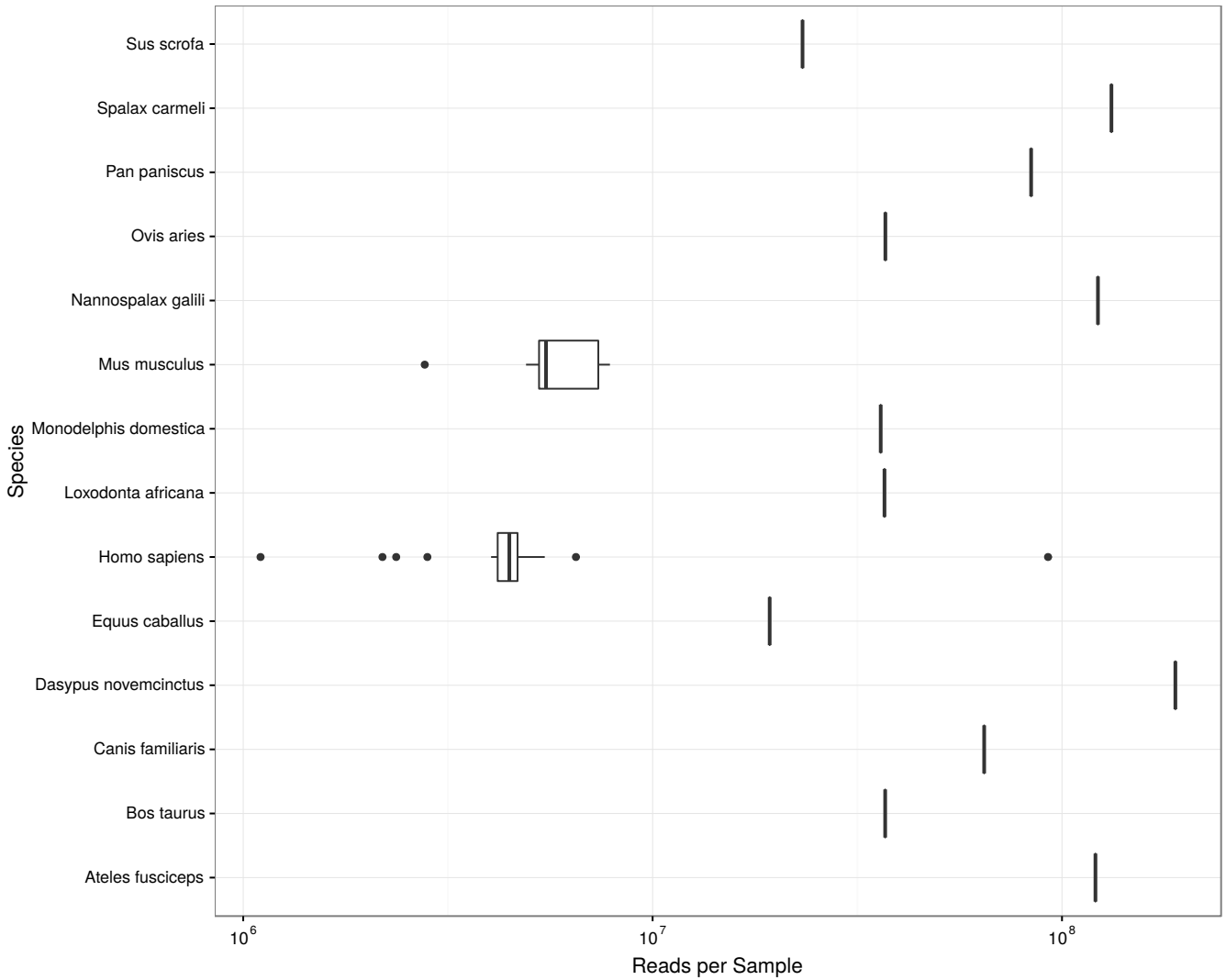
- QC Using FASTQC v0.11.2
- Transcriptomes without suitable reference (*A. fusciceps*, *N. galili*, *S. carmeli*)
  - assembled using Trinity
  - annotated using Diamond against *H. sapiens* or *M. musculus*
- Aligned to the reference (or assembled genome) using STAR
- Expression values were quantified using Cufflinks

Additional Methods

All of the code used to produce every analysis presented in this poster is available at `https://github.com/uiuc-cgm/placenta-viviparous.git`.
All of the sequences used are or will be deposited in SRA.

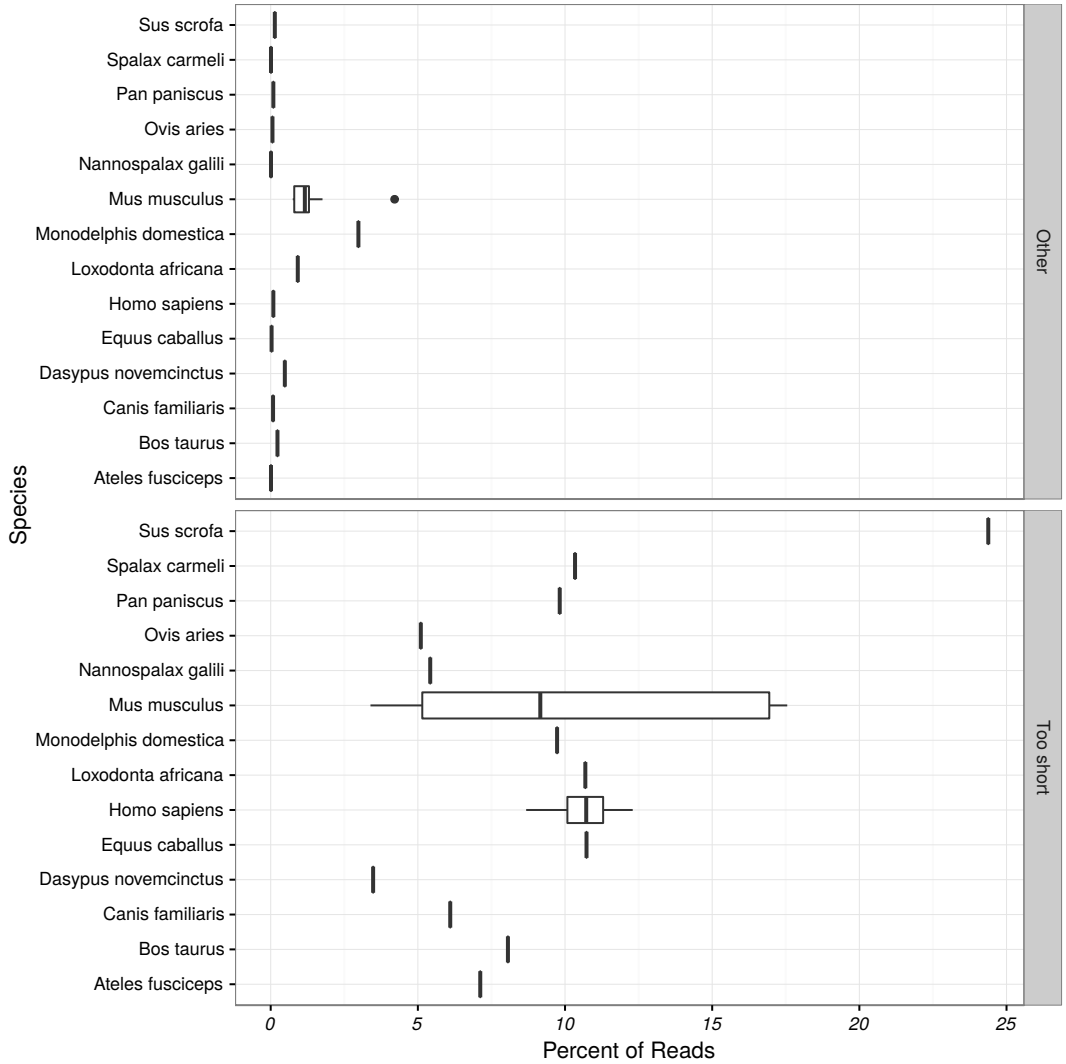
CARL R. WOESE
INSTITUTE FOR
GENOMIC BIOLOGY
Where Science Meets Society

Transcriptome Mapping Statistics: Reads



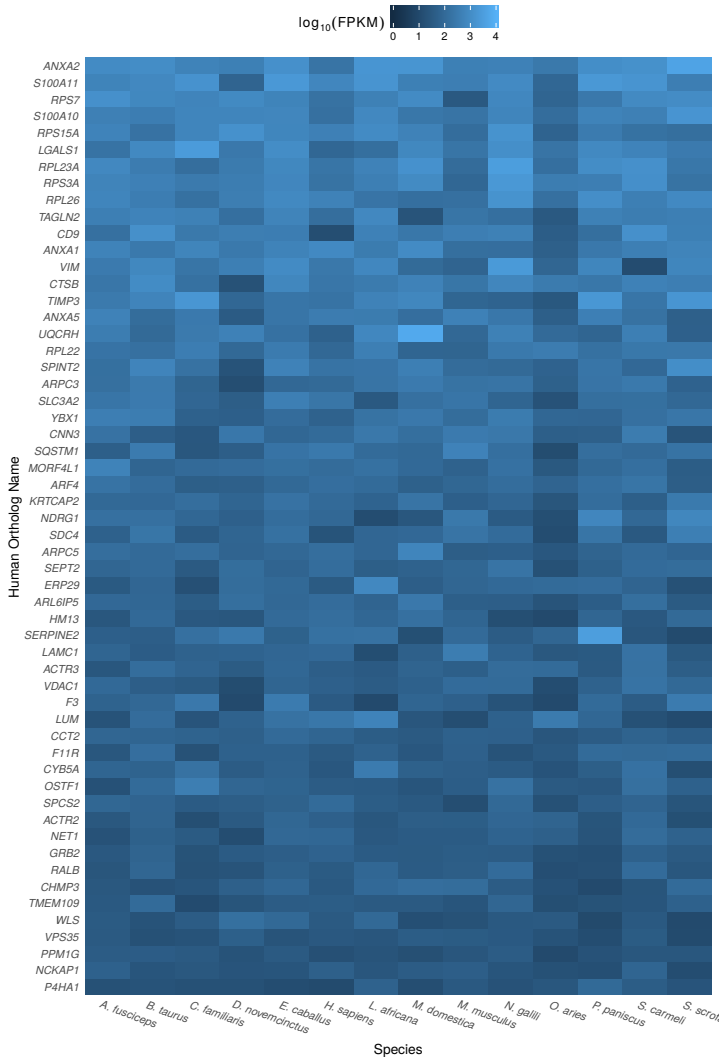
CARL R. WOESE
INSTITUTE FOR
GENOMIC BIOLOGY
Where Science Meets Society

Transcriptome Mapping Statistics: Unmapped



CARL R. WOESE
INSTITUTE FOR
GENOMIC BIOLOGY
Where Science Meets Society

Core Placenta Transcriptome



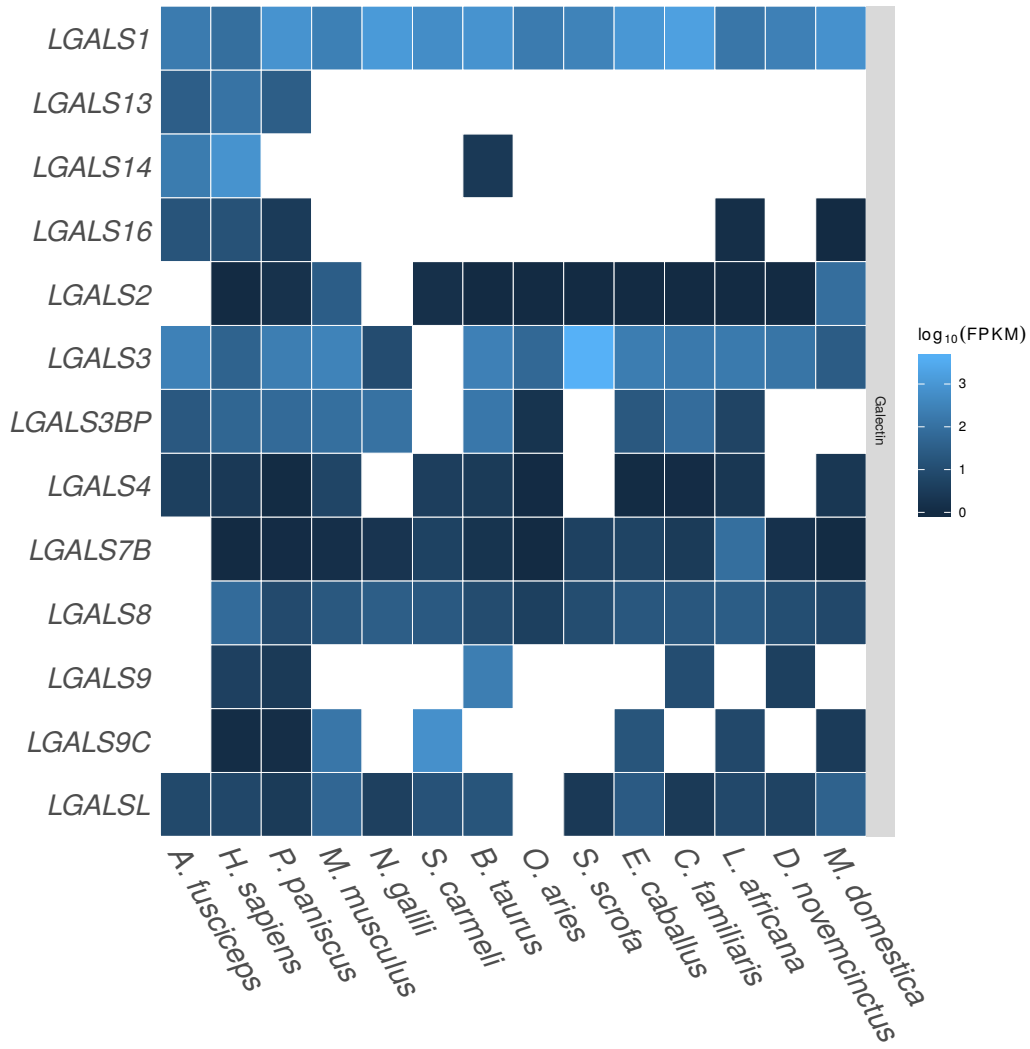
CARL R. WOESE
INSTITUTE FOR
GENOMIC BIOLOGY
Where Science Meets Society

Core placenta transcriptome

- Gene is core to the placenta transcriptome if a 1:1 ortholog is expressed with $\mathrm{FPKM} \geq 10$ in all species studied and was not a human housekeeping gene according to Eisenberg & Levanon [10].
- Multiple components of annexin complexes are included in the core transcriptome, including *ANXA2*, *ANXA1*, *S100A11*, *S100A10*.
- ANXA1 is involved in resolution of inflammation by inhibiting phospholipase A2 activity and signaling via formyl peptide receptor family
- hypothesized to be important for the maintenance of the anti-inflammatory during pregnancy[11].
- ANXA2 interacts with S100A10, S100A6, S100A11, and S100A4[12], and there is evidence for its interaction with S100P as well
- ANXA2 involved in cell-cell interaction as well as vesicle trafficking and von Willibrand Factor secretion.
- *TIMP3* has been implicated in pre-eclampsia[13,14].

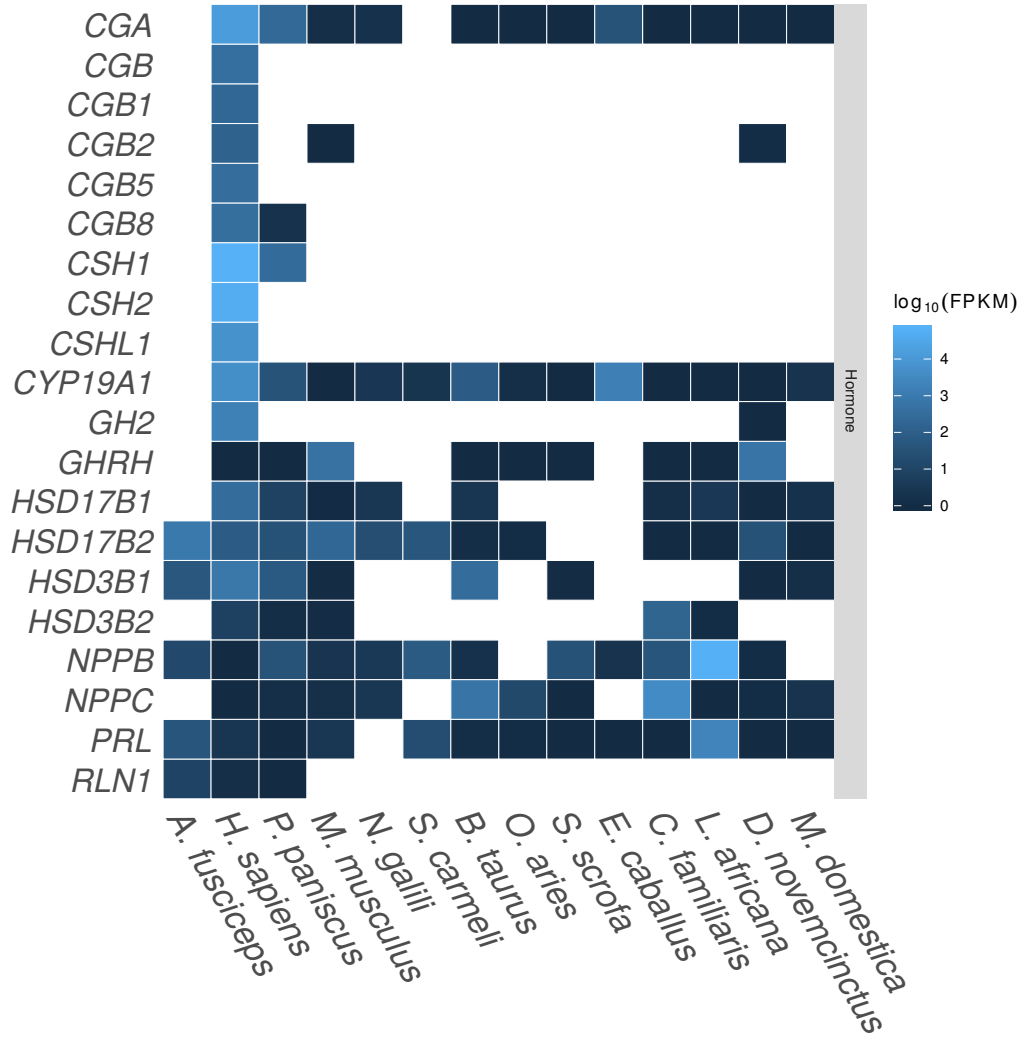
Galectin Expression

- Galectins are major regulators of pregnancy in fetal and maternal tissues[15]
- *LGALS1* expressed everywhere
- Primate expansion of *LGALS13* and *LGALS14*
- Likely present in ancestral placenta of therian mammals as it is expressed even in *M. domestica*



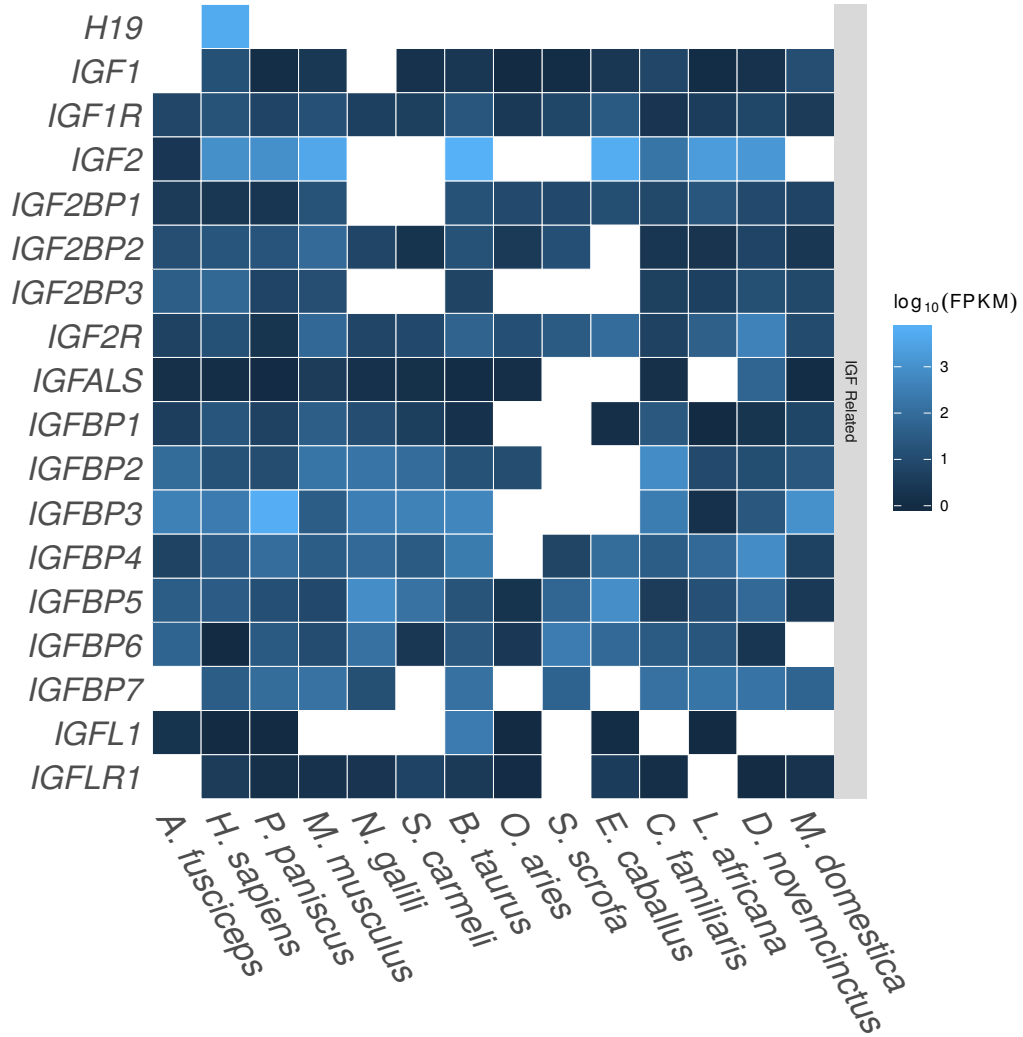
Hormone Expression

- *H. sapiens* specific high expression of *CGA*
- *H. sapiens* expansion of *CGB*
- Vasodilation-involved proteins (*NPPB* & *NPPC*) highly-expressed in *L. africana* and *C. familiaris*, but not expressed elsewhere
- *PRL* highly expressed in *L. africana*



IGF Related

- *IGF2* is highly expressed in most clades
- *IGF2* promotes the growth and division of cells; highly active during fetal development
- *IGF2* binding partners are also highly expressed (*IGFBP3*, *IGFBP5*)



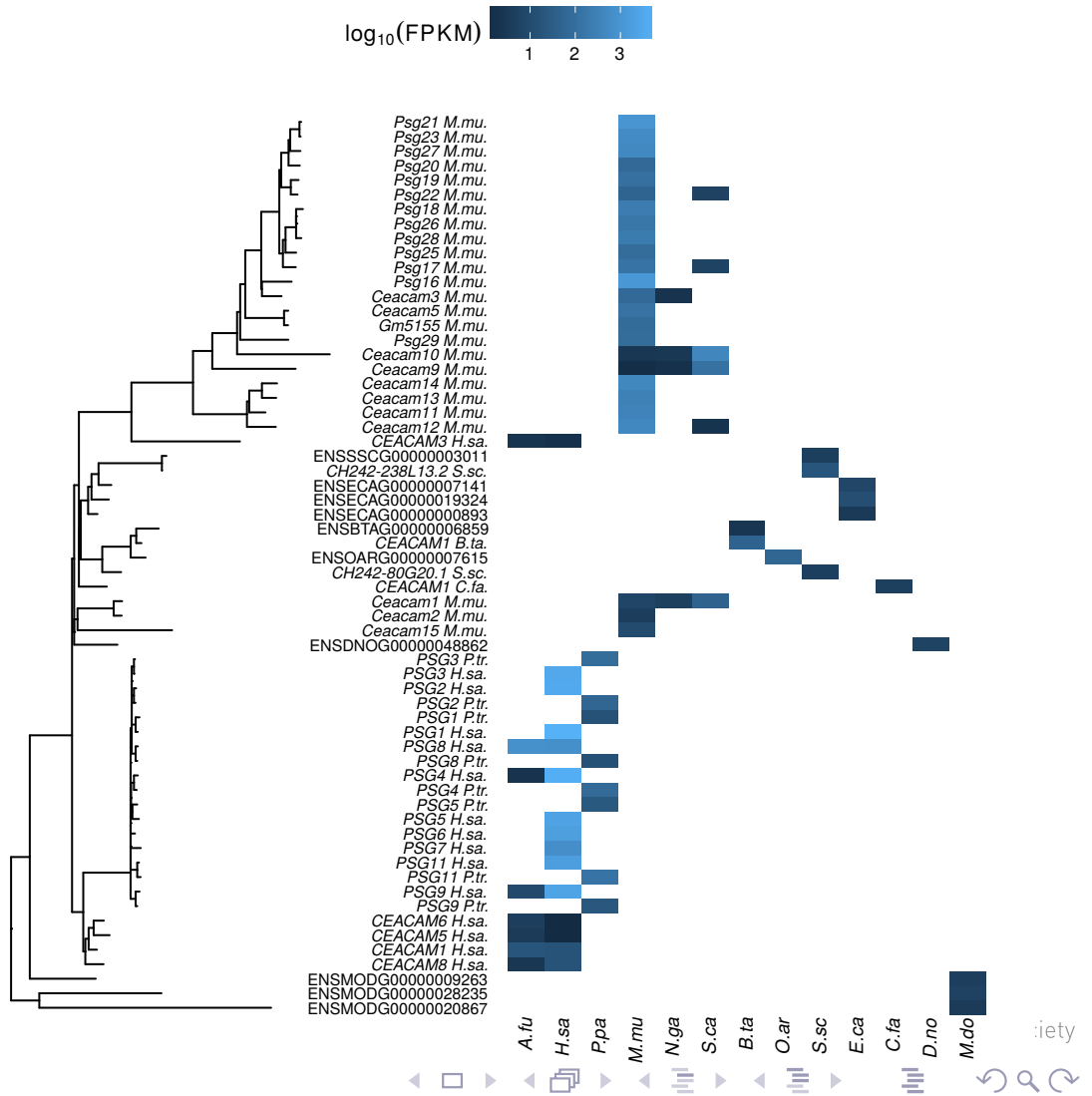
Primate Placenta Transcriptome

Human Symbol	Human Name	p (t-test)	FDR	Fold Change
FSTL1	follistatin like 1	1.06×10^{-6}	0.0011	3.45
GNB2L1	guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1	1.84×10^{-3}	0.65	2.04
CORO1B	coronin, actin binding protein, 1B	3.17×10^{-3}	0.65	-3.94
ATP1B1	ATPase, Na+/K+ transporting, beta 1 polypeptide	3.39×10^{-3}	0.65	-4.77
ACTR3	ARP3 actin-related protein 3 homolog (yeast)	6.05×10^{-3}	0.65	-2.36

CARL R. WOESE
INSTITUTE FOR
GENOMIC BIOLOGY
Where Science Meets Society

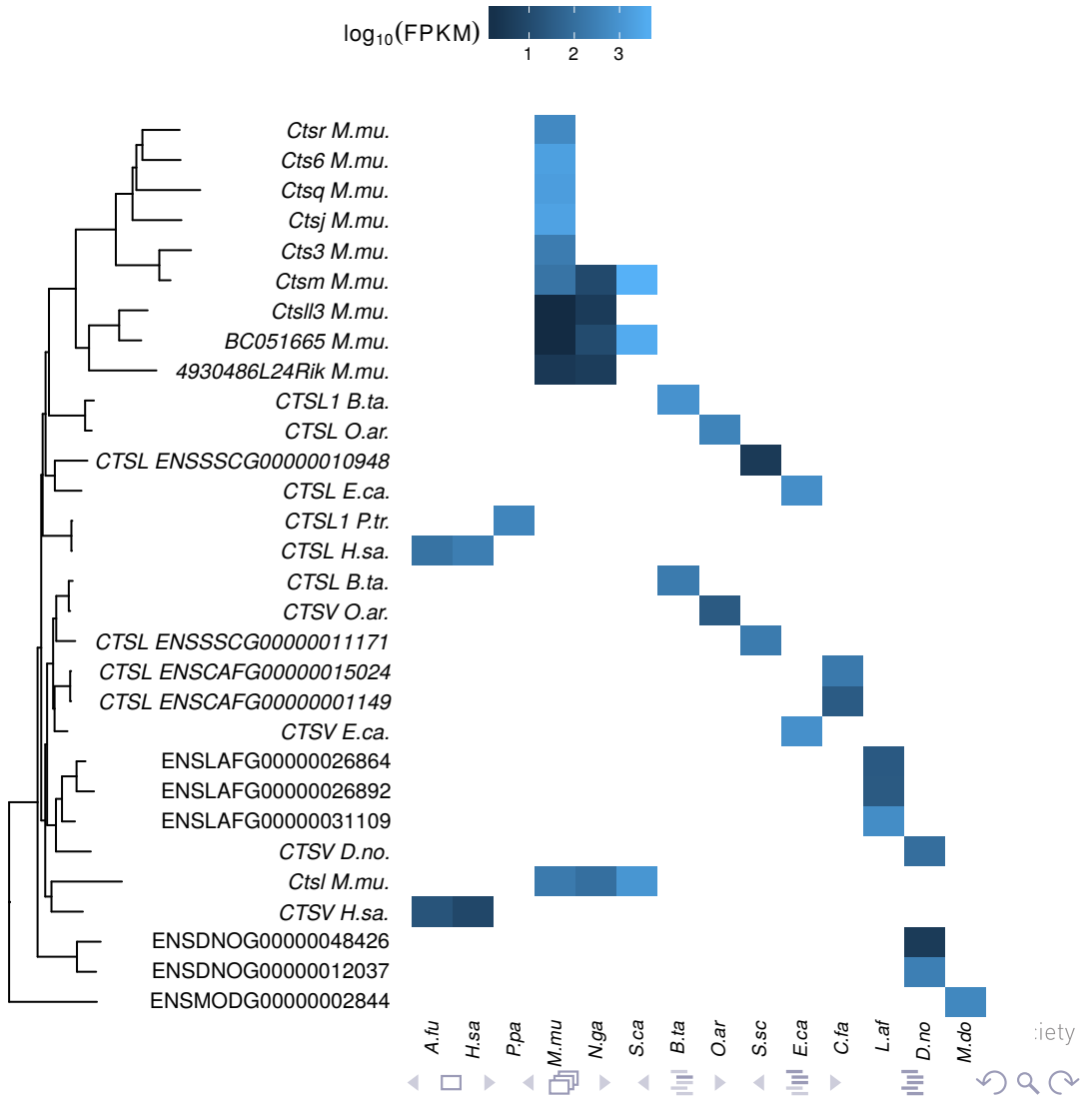
Pregnancy-Specific Glycoprotein Expansion

- 11 highly expressed *PSG* genes in *H. sapiens* and some expression in *A. fusciceps* and *P. paniscus*
- *M. musculus* specific expansion and high expression of *PSG* as well
- No other species have high expression of *PSG* or *CEACAM*



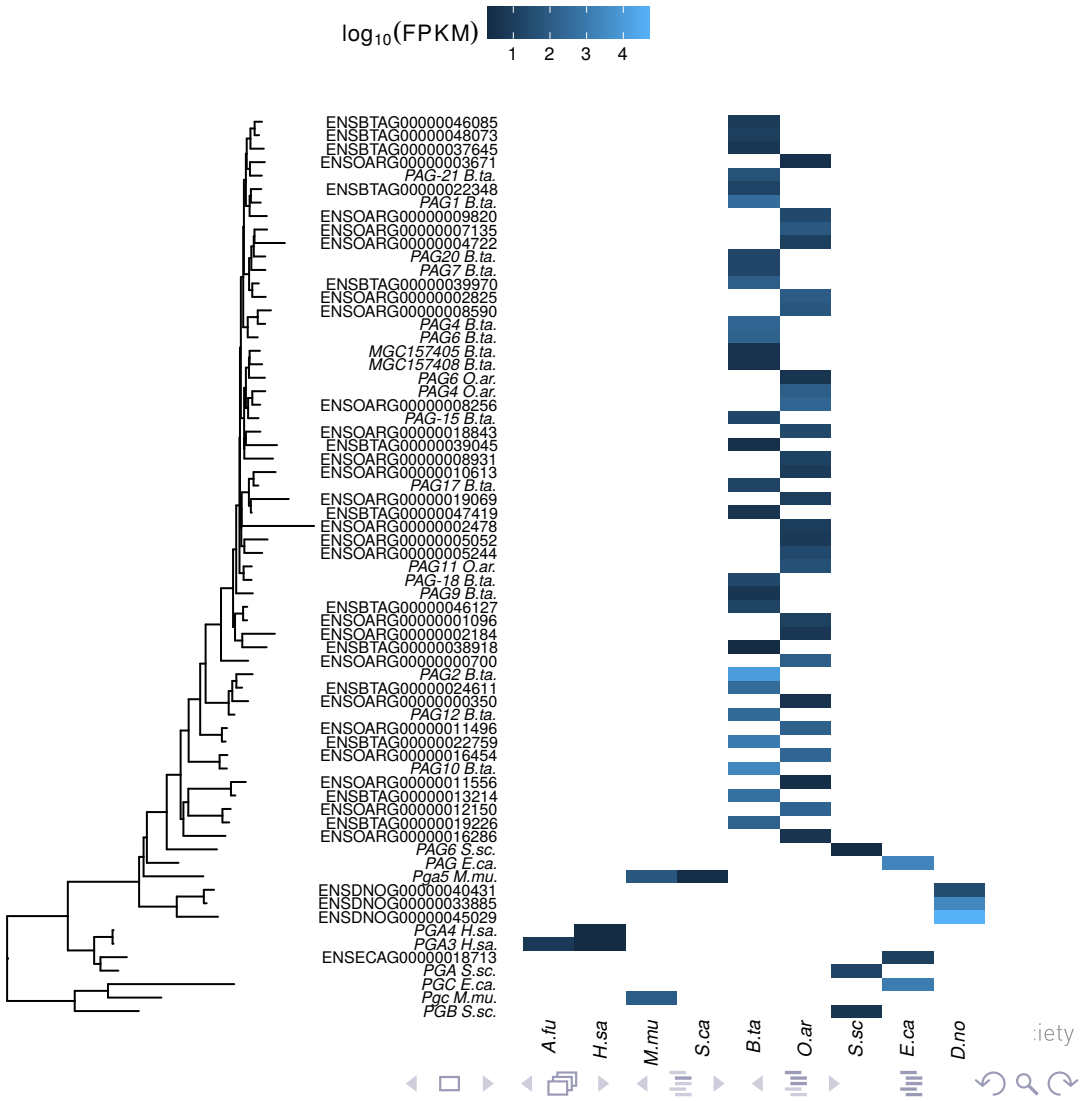
Cathepsin-L expansion

- Cathepsins are highly expressed in many placentas
- Expanded in *M. musculus* and *N. galili* and possibly also *S. carmeli* to a lesser degree
- Intracellular protease which degrades collagen, elastin, and in IGF1R



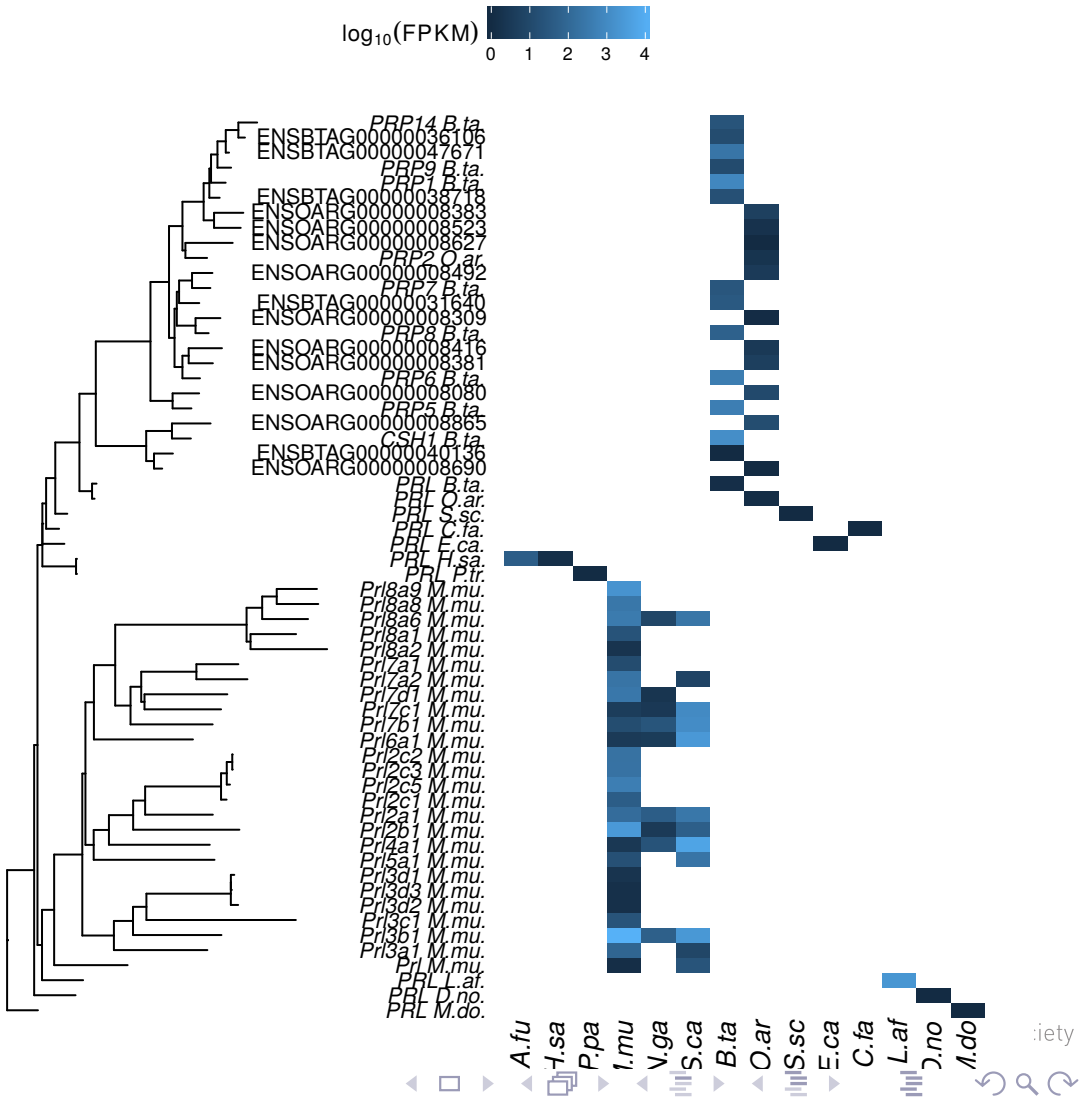
Pregnancy associated glycoprotein

- Massive expansion in ruminants (*B. taurus* and *O. aries*)
- 28 members of this family have $\mathrm{FPKM} \geq 1$ in *B. taurus*
- *PAG2* $= 9730$ FPKM
- *PAG10* $= 2146$ FPKM



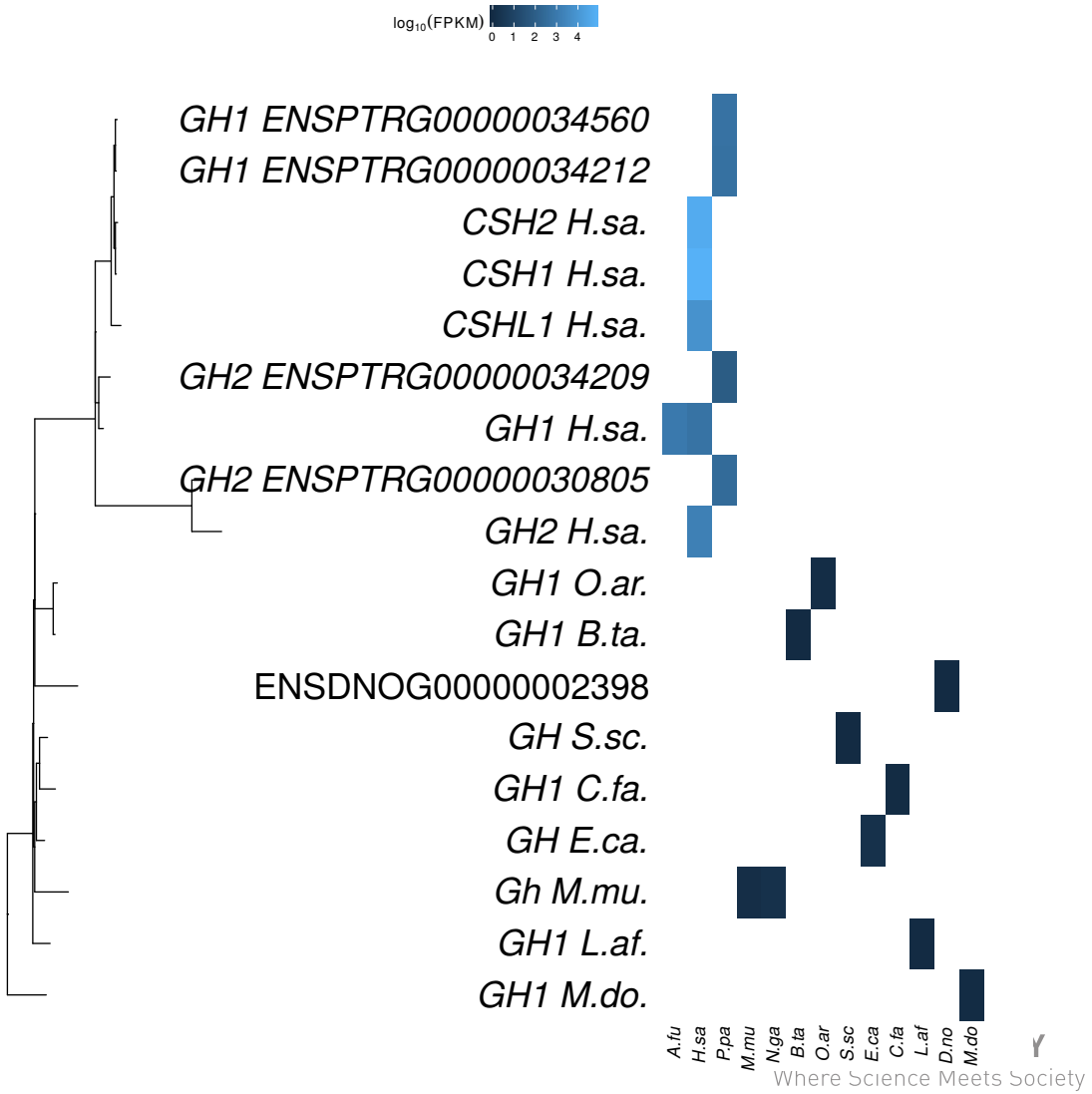
Prolactin

- *L. africana* is only analyzed mammal with high prolactin expression (*PRL*) in the placenta
- However, *M. musculus*, *S. carmeli*, and *B. taurus* have duplications of *PRL* with expression



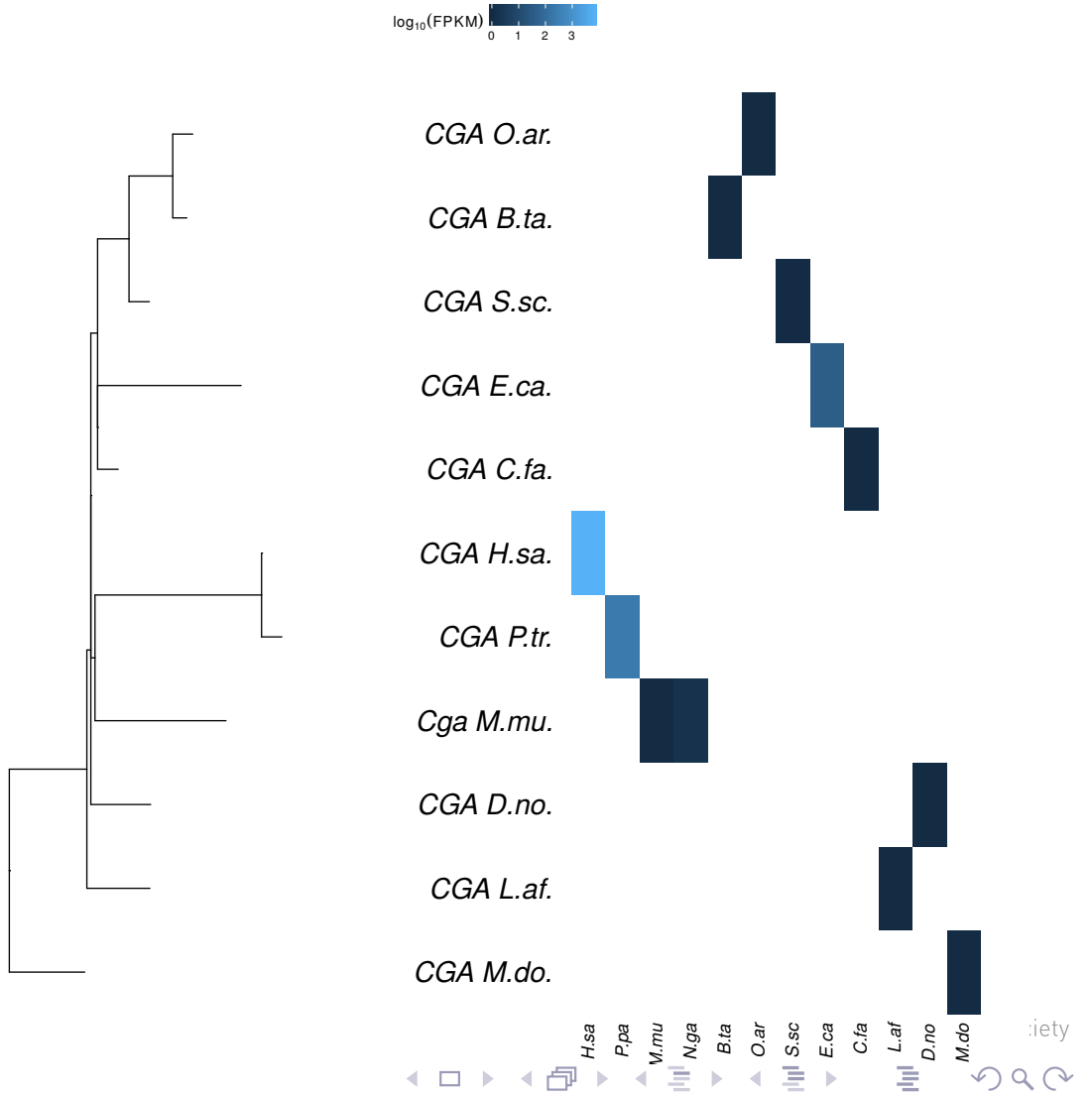
Growth Hormone (GH) family

The GH family has expanded in anthropoid primates, resulting in approximately 5 genes in *H. sapiens* (*GH1*, *GH2*, *CSH1*, *CSH2*, *CSHL1*) and at least three in *P. paniscus* (two *GH2* and *GH1*; owing to the similarity between the two *GH1* in *Pan troglodytes* we cannot reliably distinguish transcripts between the two).



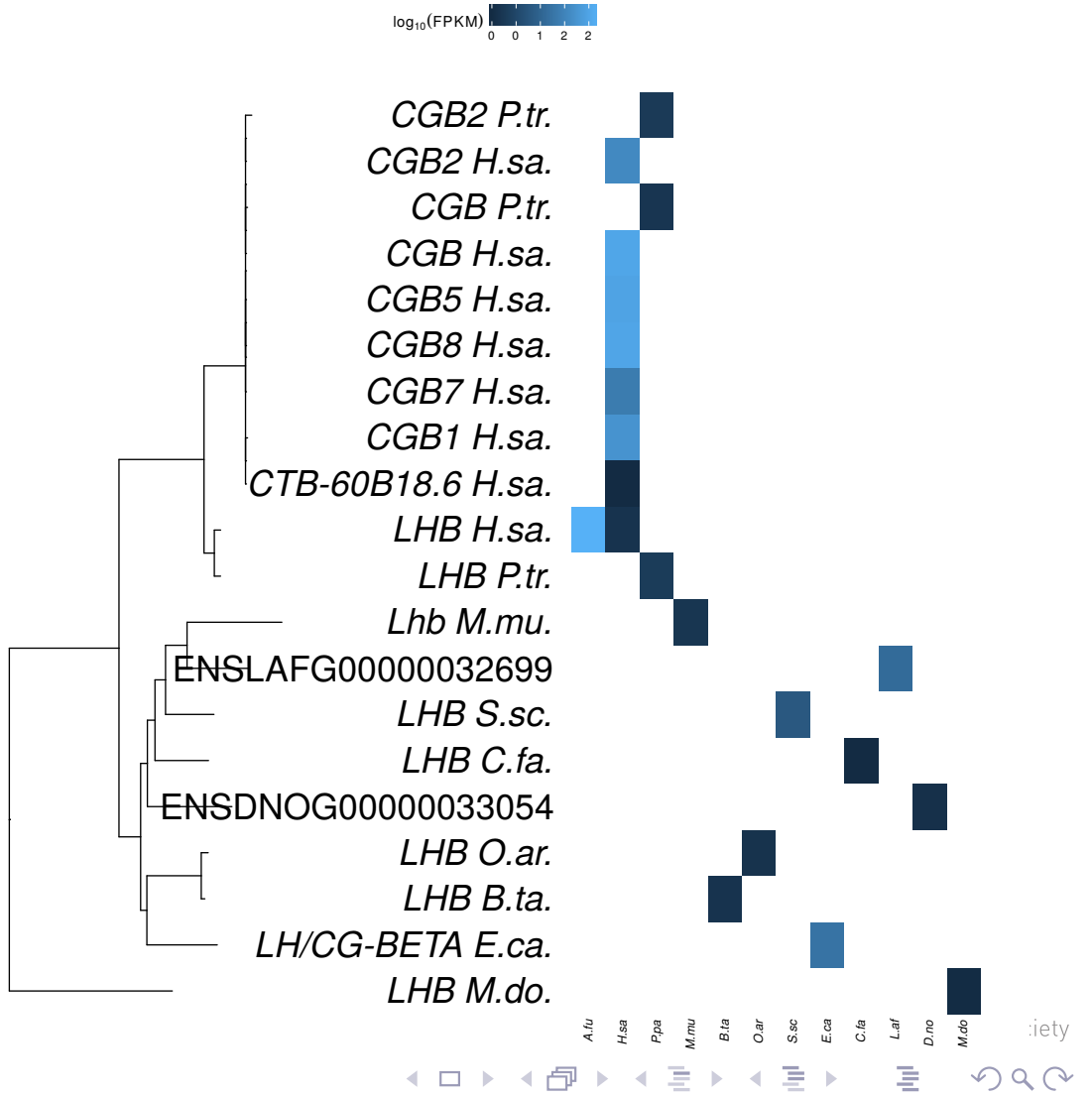
CGA

- *CGA* codes for the alpha subunit of multiple hormones as well as numerous chorionic gonadotropins with variable beta subunits[16].
- Chorionic gonadotropins prevent the regression of the corpus luteum[17].
- *CGA* only expressed in the placentas of *H. sapiens* and *P. paniscus*



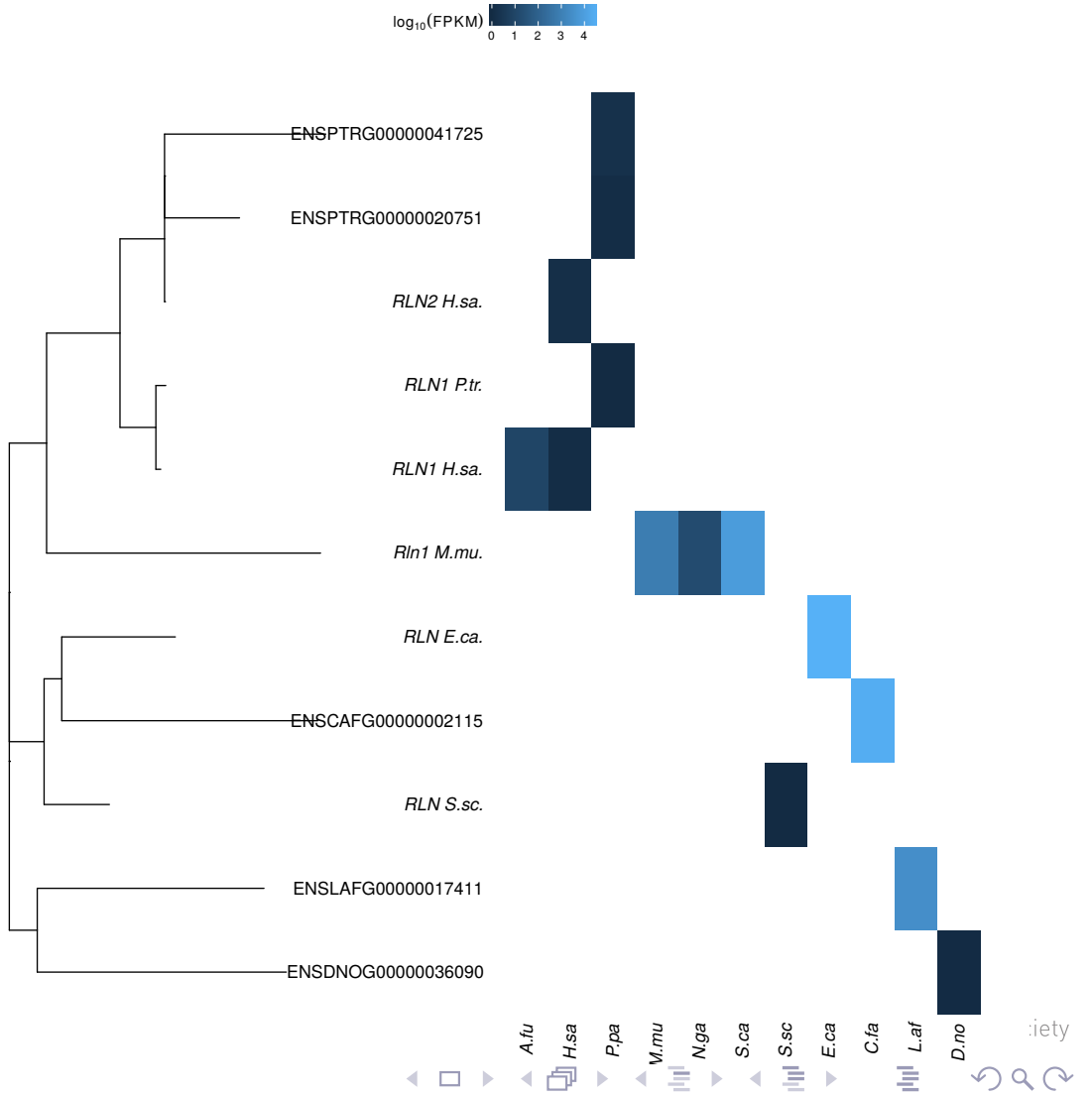
CGB

- *CGB* is highly expressed in *H. sapiens*
- Some expansion of *CGB* in *P. paniscus*, but not highly expressed
- High expression of *LHB* in *A. fusciceps*
  - May be indicative of expansion
  - Would have to examine assembled transcripts to tell



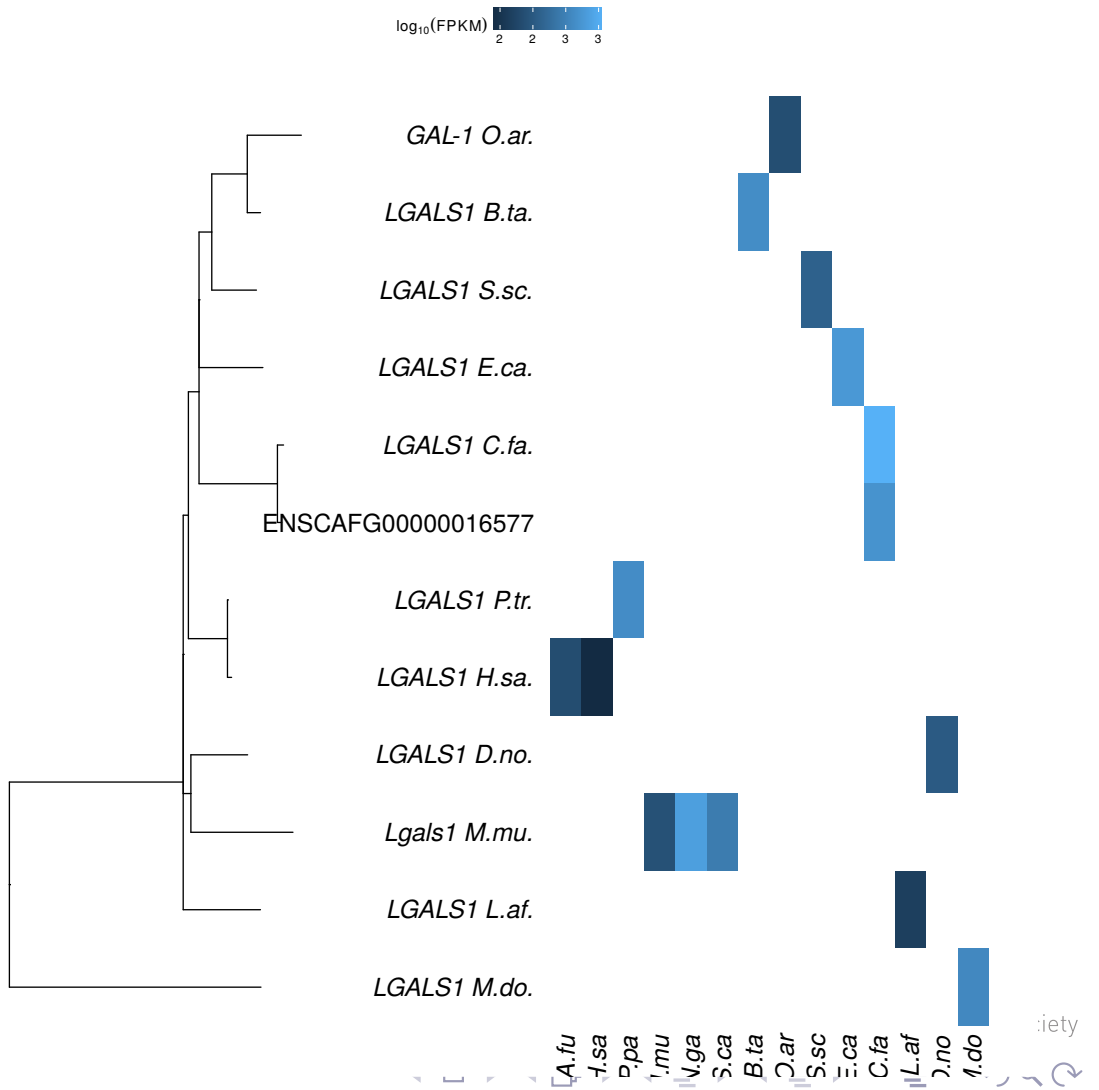
Relaxin

- *RLN* increasing maternal blood flow, widening the pubic bone and relaxing uterine musculature in preparation for labor
- We show that the placenta is a source of *RLN* in *C. familiaris*, *L. africana*, *M. musculus*, *S. carmeli*, and *E. caballus*.

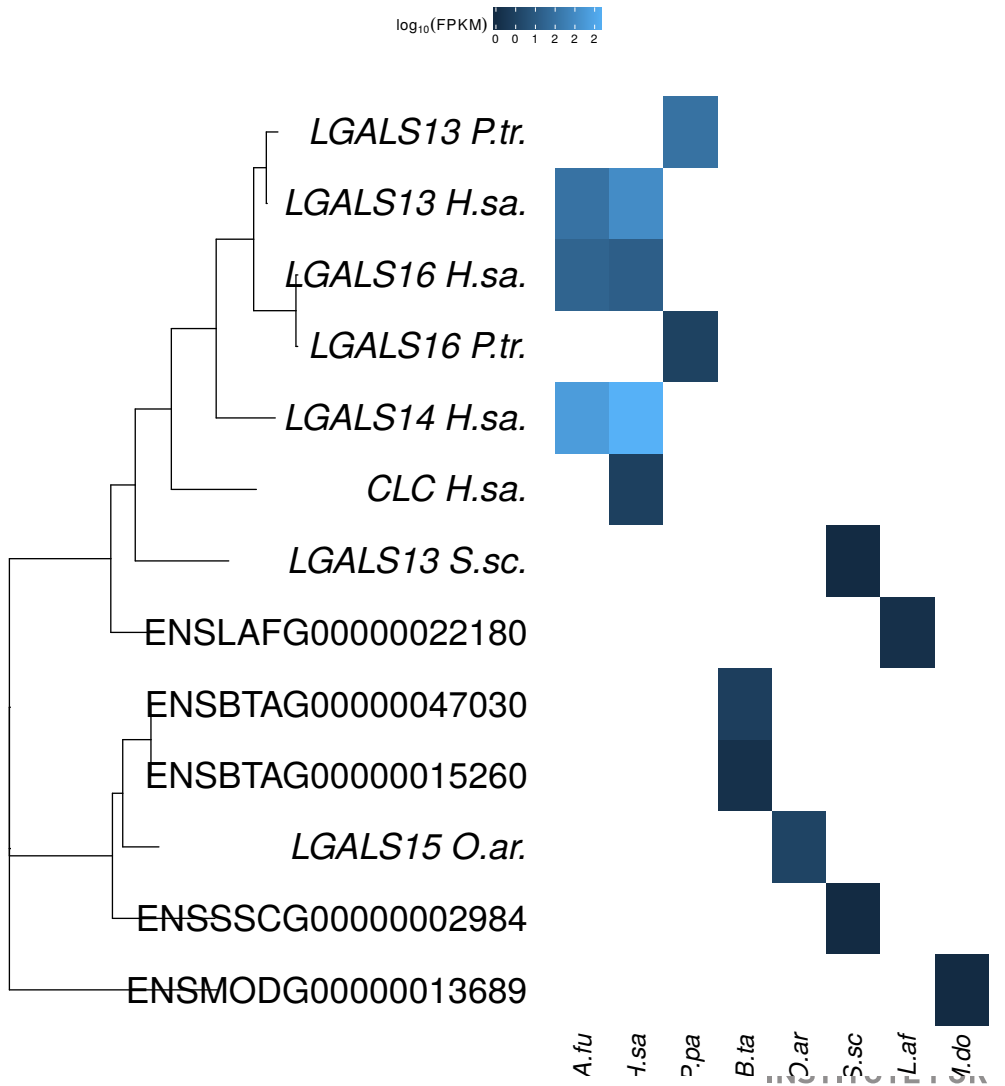


Galectins

- Galectins are major regulators of pregnancy in fetal and maternal tissues
- *LGALS1* mediates maternal-fetal immune tolerance
- substantial expression of galectins in the *M. domestica*
- consistent with ancestral placenta may have maternal-fetal immune tolerance mediated by galectins



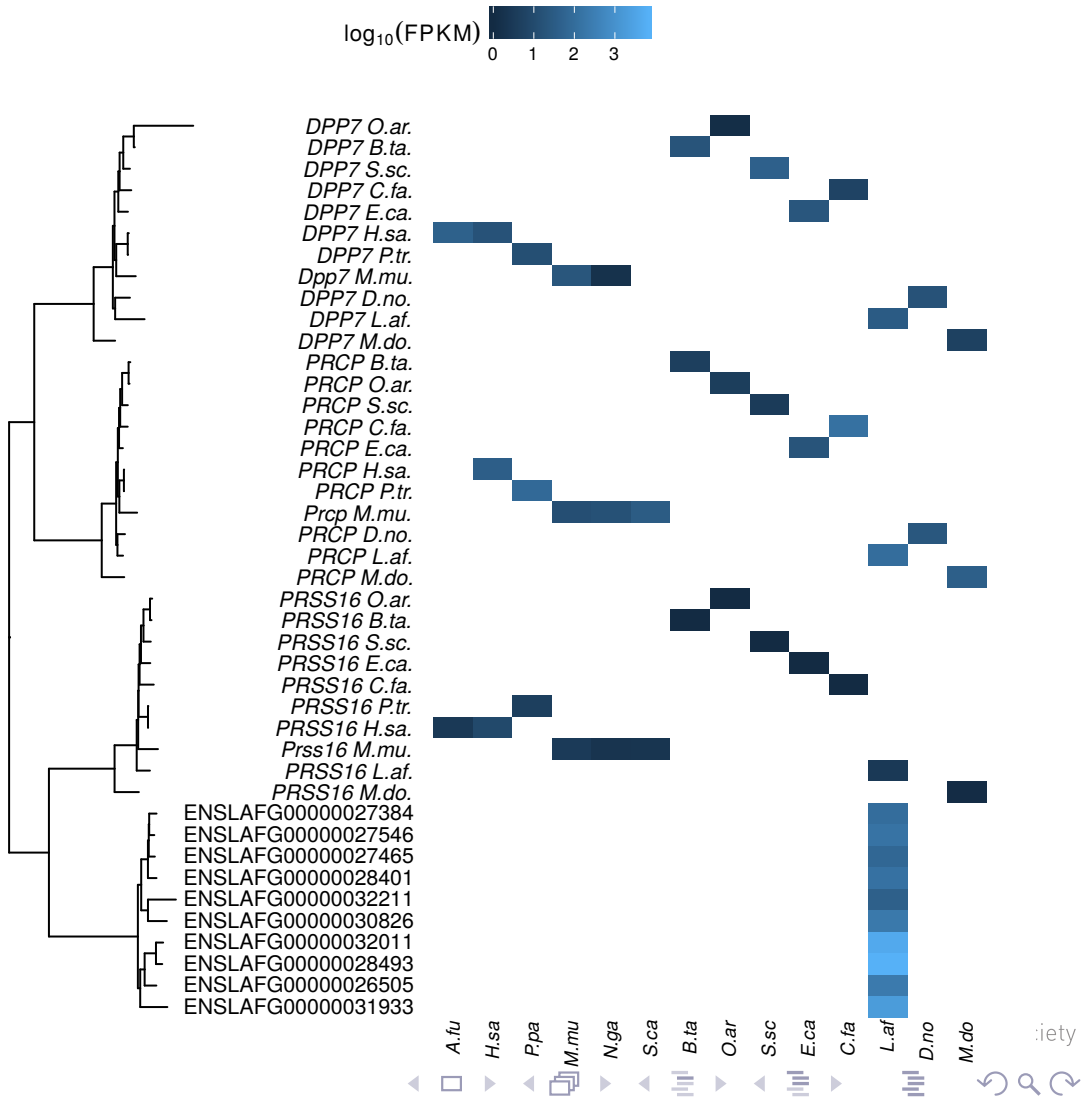
- Evolution of new lineage-specific galectins has occurred in both primates and ruminants, with the genes *LGALS13*, *LGALS14*, and *LGALS16* recognized as being placenta-specific in *H. sapiens* and regulating immune responses by inducing apoptosis of maternal T-cells[18]



- The *M. domestica* placenta shows expression of genes that either do not exist in eutherians or are not found in the term eutherian placenta
- The existence of embryonic forms of hemoglobin in fetal tissues at term, as evidenced by the expression of *HBE* and *HBZ* is a feature of marsupials that is generally due to their very short gestation periods.
- Embryonic hemoglobins in marsupials have a lower affinity for oxygen and allow for a lower rate of oxygen uptake from maternal tissues[19].
- One novel uncharacterized gene that shows high expression (*LOC10001195*) is not found in eutherians; Ensembl gene trees reveal that homologs of this gene exist in other amniotes such as birds and turtles, but are not present in eutherian mammals.
- *WFDC2* is a member of the whey acidic protein (WAP) family and involved in innate immunity.

PRSS16

- *PRSS16*, a thymus-specific serine protease that regulates the presentation of self-peptides in CD4+ T-lymphocytes[20].
- Possibly involved in the mediation of maternal-fetal immune tolerance in *L. africana*



Conclusion

- Sequenced placentas of 14 species
- lineage-specific expansions of gene families and differential gene expression
- Identified common expression patterns of the mammalian expression
- Still much more to learn about the changes in placenta expression during placentation
- Many clades are only represented by a single species; need to have more information to make stronger inferences about evolution of placenta expression
- Lots to learn about what is actually driving the changes in expression

References I

Carter, A. M. & Pijnenborg, R. Evolution of invasive placentation with special reference to non-human primates. *Best Pract Res Clin Obstet Gynaecol* **25,** 249–257 (June 2011).

Chaiworapongsa, T., Chaemsaithong, P., Yeo, L. & Romero, R. Pre-eclampsia part 1: current understanding of its pathophysiology. *Nat Rev Nephrol* **10,** 466–480 (Aug. 2014).

Stout, C. & Lemmon, W. B. Glomerular capillary endothelial swelling in a pregnant chimpanzee. *Am. J. Obstet. Gynecol.* **105,** 212–215 (Sept. 1969).

Thornton, J. G. & Onwude, J. L. Convulsions in pregnancy in related gorillas. *Am. J. Obstet. Gynecol.* **167,** 240–241 (July 1992).

Ernest, S. K. M. Life history characteristics of placental non-volant mammals. *Ecology* **84,** 3402 (2003).

Kiltie, R. A. Intraspecific Variation in the Mammalian Gestation Period. *J. Mamm.* **63,** 646–652 (Nov. 1982).

Smith, L. L. & Doughty, R. W. *The Amazing Armadillo: Geography of a Folk Critter* ISBN: 0-292-70375-9 (University of Texas Press, Austin, 1984).

Harder, J. D., Stonerook, M. J. & Pondy, J. Gestation and placentation in two New World opossums: Didelphis virginiana and Monodelphis domestica. *J. Exp. Zoolog. Part A Comp. Exp. Biol.* **266,** 463–479 (Aug. 1993).

Nevo, E. *Mosaic evolution of subterranean mammals: Regression, progression, and global convergence* ISBN: 0-19-857526-6 (Oxford University Press, 1999).

References II


Eisenberg, E. & Levanon, E. Y. Human housekeeping genes, revisited. *Trends Genet.* **29,** 569–574 (Oct. 2013).


Hutchinson, J. L., Rajagopal, S. P., Sales, K. J. & Jabbour, H. N. Molecular regulators of resolution of inflammation: potential therapeutic targets in the reproductive system. *Reproduction* **142,** 15–28 (July 2011).


Liu, Y., Myrvang, H. K. & Dekker, L. V. Annexin A2 complexes with S100 proteins: structure, function and pharmacological manipulation. *Br. J. Pharmacol.* **172,** 1664–1676 (Apr. 2015).


Ma, R., Gu, B., Gu, Y., Groome, L. J. & Wang, Y. Down-regulation of TIMP3 leads to increase in TACE expression and TNFα production by placental trophoblast cells. *Am. J. Reprod. Immunol.* **71,** 427–433 (May 2014).


Xiang, Y. *et al.* Promoter hypomethylation of TIMP3 is associated with pre-eclampsia in a Chinese population. *Mol. Hum. Reprod.* **19,** 153–159 (Mar. 2013).


Than, N. G. *et al.* Galectins: guardians of eutherian pregnancy at the maternal-fetal interface. *Trends Endocrinol. Metab.* **23,** 23–31 (Jan. 2012).


Maston, G. A. & Ruvolo, M. Chorionic gonadotropin has a recent origin within primates and an evolutionary history of selection. *Mol. Biol. Evol.* **19,** 320–335 (Mar. 2002).


Hanson, F. W., Powell, J. E. & Stevens, V. C. Effects of HCG and human pituitary LH on steroid secretion and functional life of the human corpus luteum. *J. Clin. Endocrinol. Metab.* **32,** 211–215 (Feb. 1971).

References III

Than, N. G. *et al.* A primate subfamily of galectins expressed at the maternal-fetal interface that promote immune cell death. *Proc. Natl. Acad. Sci. U.S.A.* **106,** 9731–9736 (June 2009).

Holland, R. A. & Gooley, A. A. Characterization of the embryonic globin chains of the marsupial Tammar wallaby, Macropus eugenii. *Eur. J. Biochem.* **248,** 864–871 (Sept. 1997).

Fornari, T. A., Marques, M. M., Nguyen, C., Carrier, A. & Passos, G. A. Transcription profiling of Prss16 (Tssp) can be used to find additional peptidase genes that are candidates for self-peptide generation in the thymus. *Mol. Biol. Rep.* **39,** 4051–4058 (Apr. 2012).