Targeted Nanoparticle Delivery of Placenta-specific Gene Therapy for In Utero Treatment of Fetal Growth Restriction

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Fetal Growth Restriction

Occurs in 5-25% of all live births (geographically dependent)

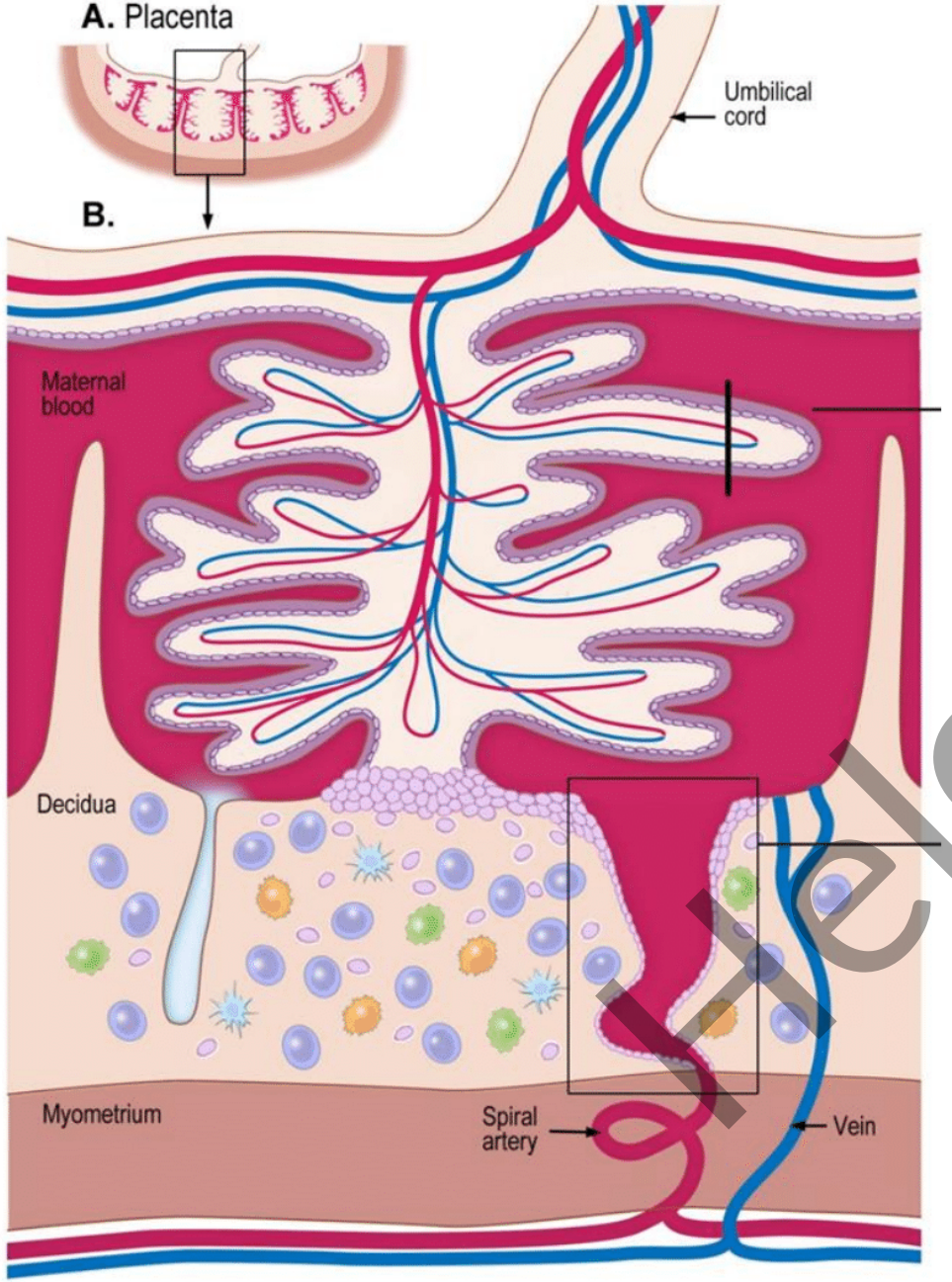
Can occur in isolation or in combination with:
Preeclampsia
Genetic syndromes
Congenital Heart Defects (birth weight major predictor of survival @5yrs)

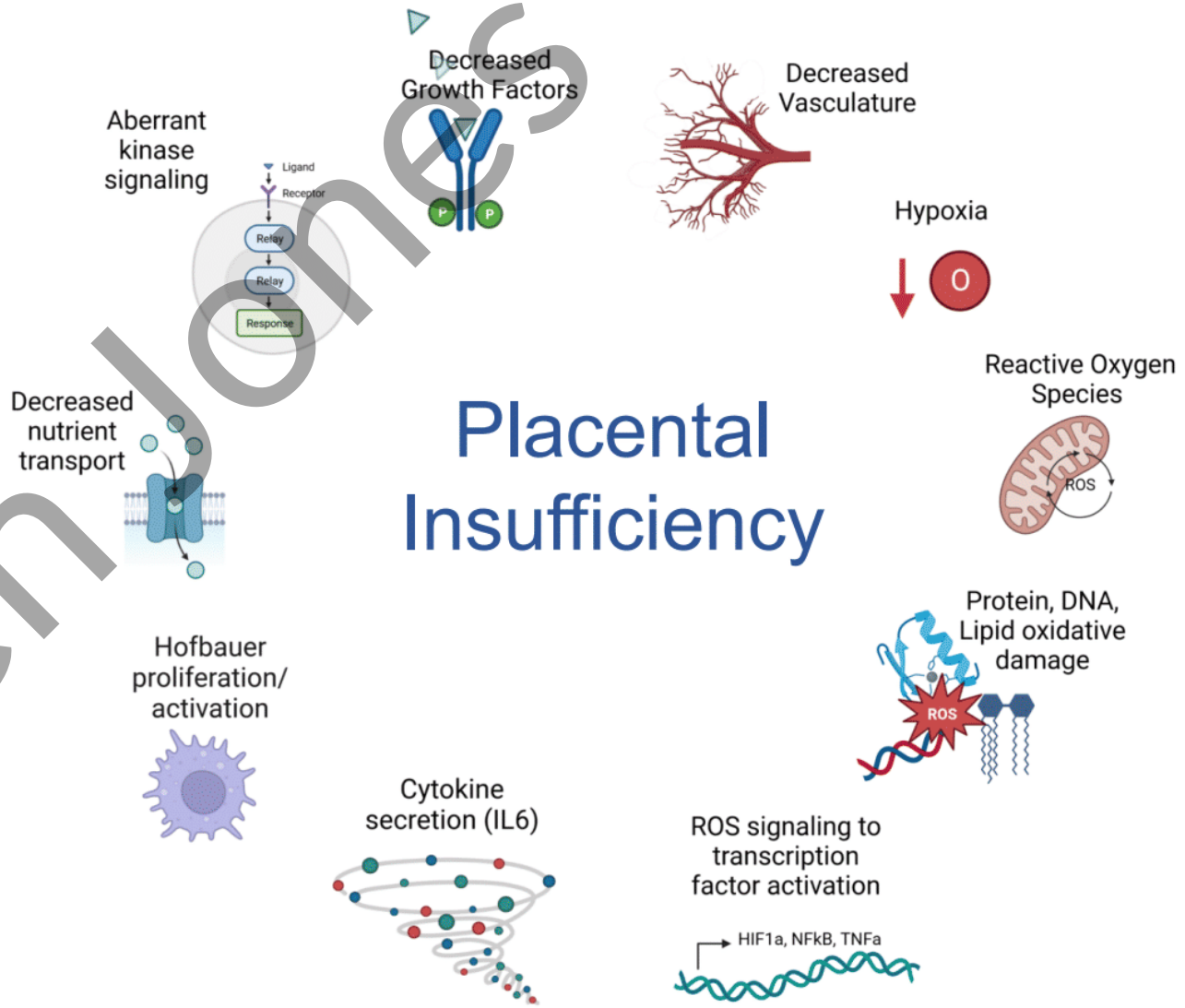
Results in Iatrogenic premature delivery and NICU/ sequelae

Developmental Origins of Health and Disease – lifelong sequelae

Majority of cases have underlying placental insufficiency

Placental Insufficiency





Therapeutic Intervention

Currently: Delivery & NICU admission

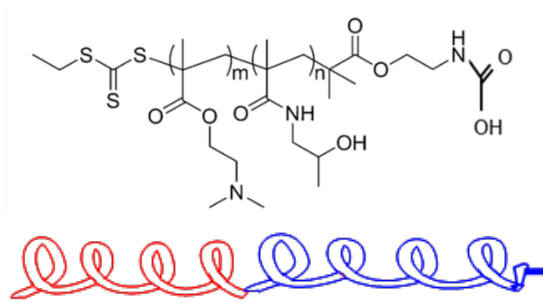
Future: Improve placental development/function– improve fetal growth?

The placenta is an ideal target for therapy:

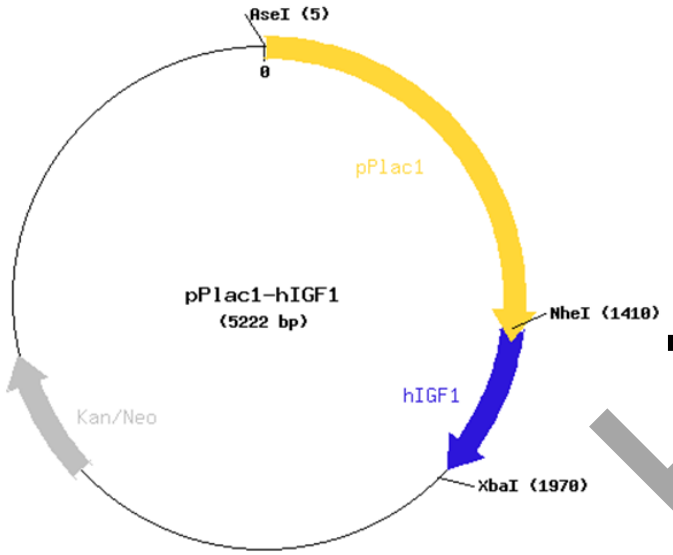
Transient
Accessible via maternal circulation
Discarded after birth

Needs to be targeted, specific, effective and safe

Nanoparticle: Trophoblast-specific promoter- hIGF-1

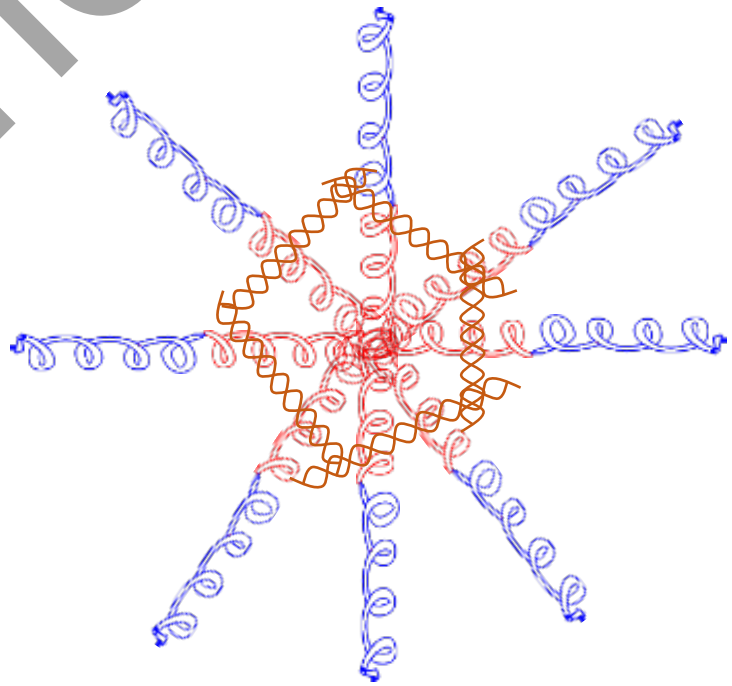


Co-polymer
(non-viral)



Transgene Plasmid
(PLAC1 or Cyp19a promotor + hIGF1 gene)

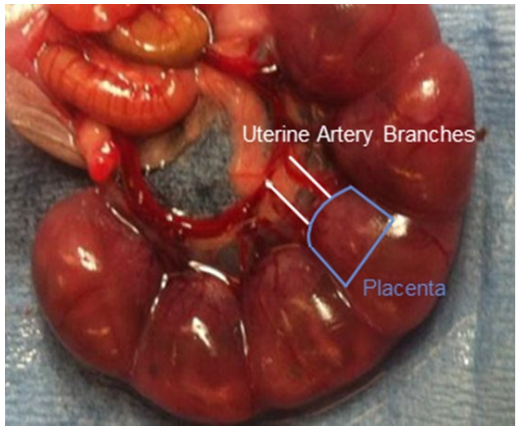
Self-assembly
1 hr @ RT

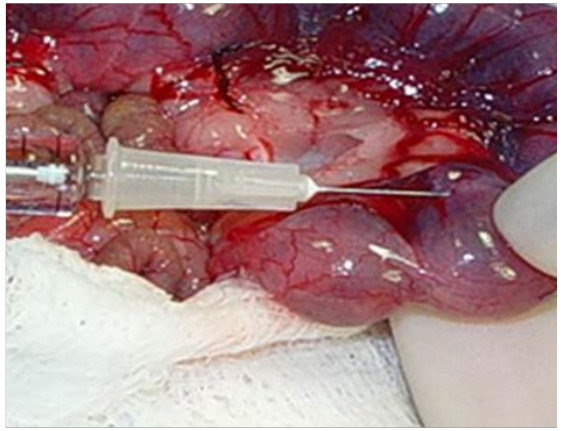


Nanoparticle (NP)

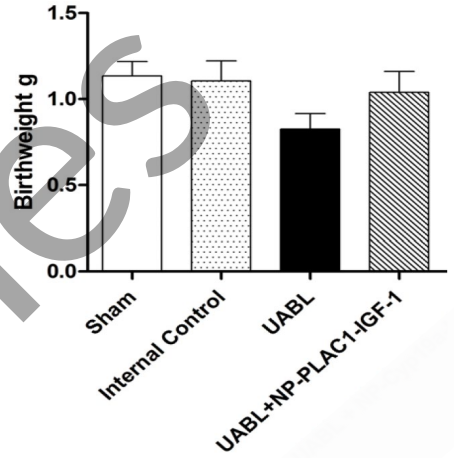
Abd Ellah NH et al., Journal of Pharmaceutical Technology & Drug Research, 2014; 3(2)

Delivery & impact in mouse model & in vitro human syncytium





96h



PLOS ONE

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

Development of Non-Viral, Trophoblast-Specific Gene Delivery for Placental Therapy

Noura Abd Ellah, Leeanne Taylor, Weston Troja, Kathryn Owens, Neil Ayres, Giovanni Pauletti, Helen Jones

Published: October 16, 2015 • https://doi.org/10.1371/journal.pone.0140879

ELSEVIER

Placenta

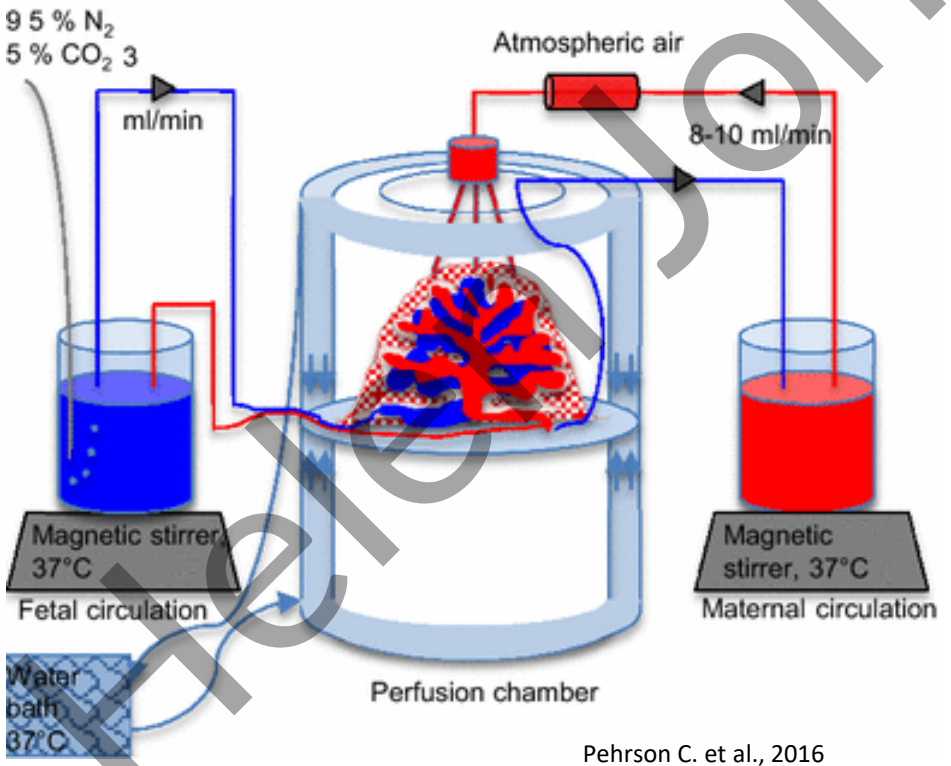
Volume 93, April 2020, Pages 1-7

Nanoparticle mediated increased *insulin-like growth factor 1* expression enhances human placenta syncytium function

Rebecca L. Wilson [a], Kathryn Owens [a], Emily K. Sumser [a], Matthew V. Fry [a], Kendal K. Stephens [a], Marcel Chuecos [b], Maira Carrillo [b], Natalia Schlabritz-Loutsevitch [b], Helen N. Jones [a]

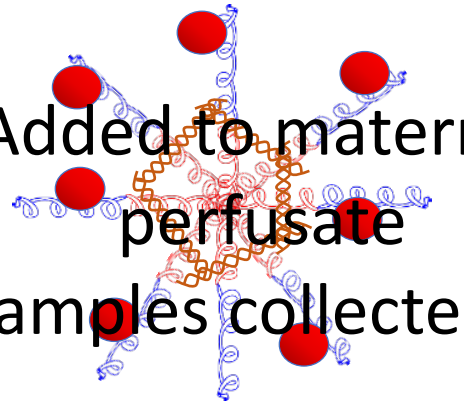
Ex vivo Human Placenta Model

- Perfused for ~2-2.5 h (samples collected)



Pehrson C. et al., 2016

n = 6 placentas



- Added to maternal perfusate (samples collected)

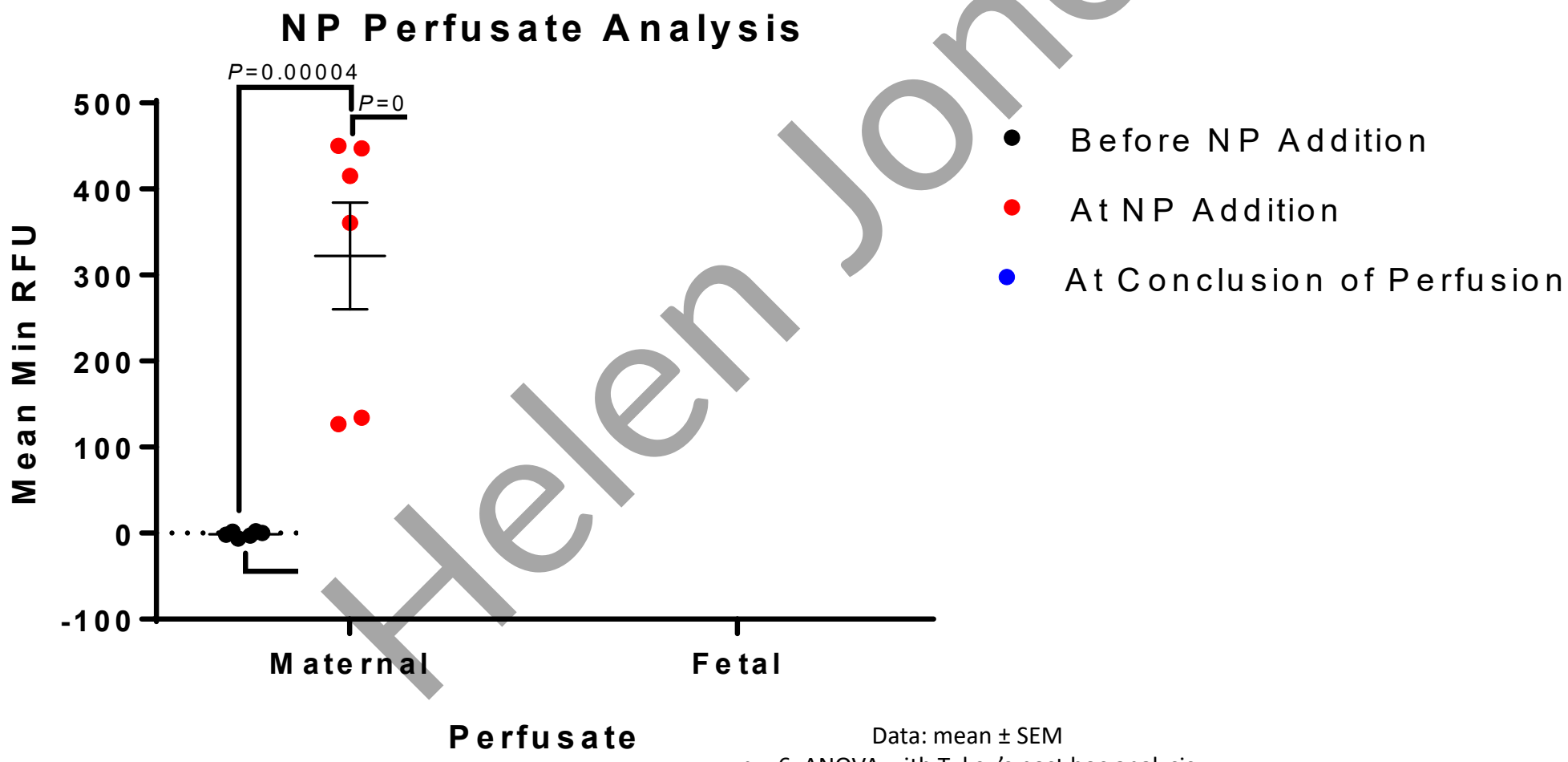
Texas Red conjugated NP

- Perfused for further ~1-1.5 h (samples collected)

Rebecca L. Wilson et al, 2020. Placenta 93:1-7.

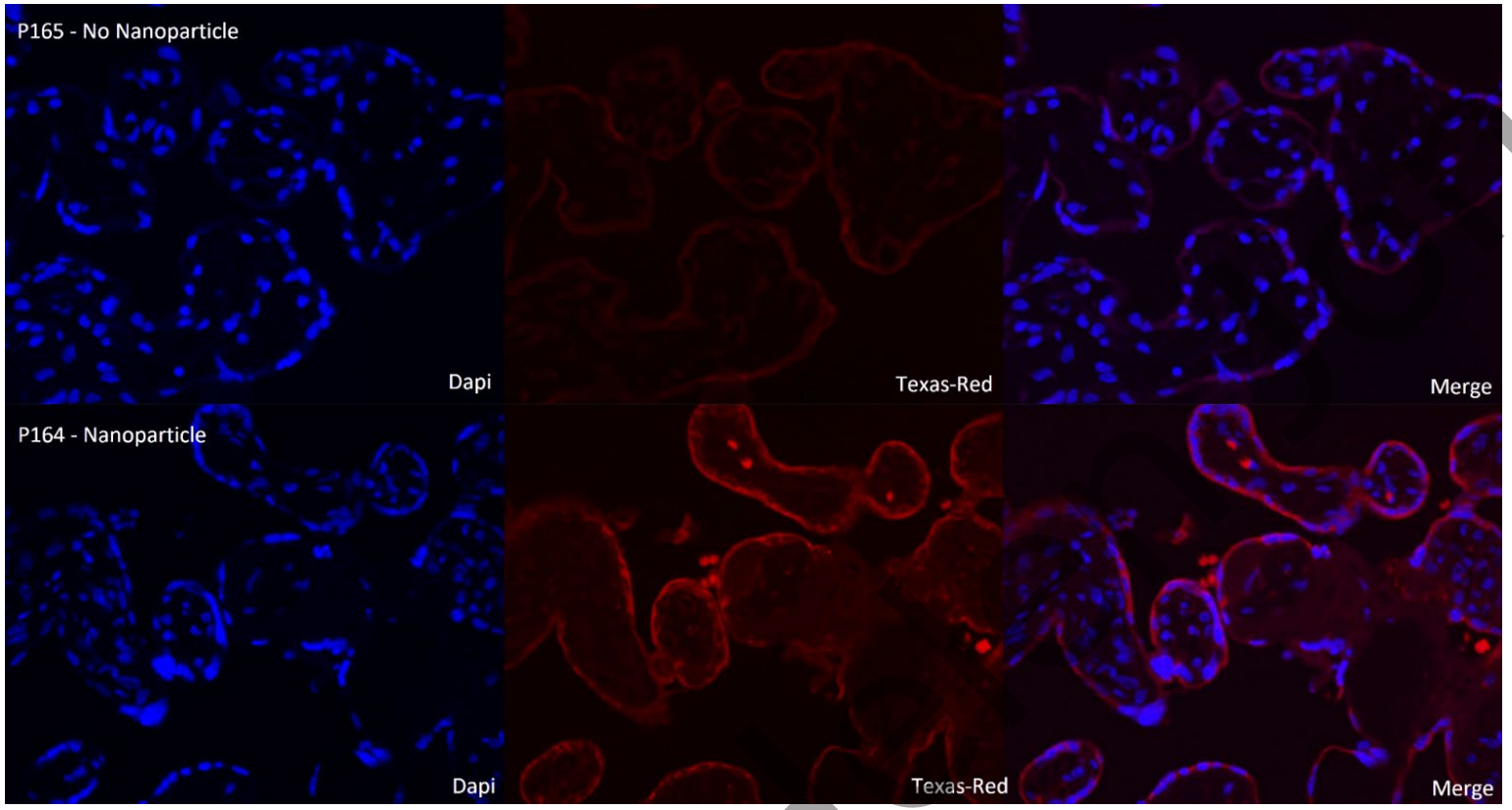
Nanoparticle is taken up by syncytium

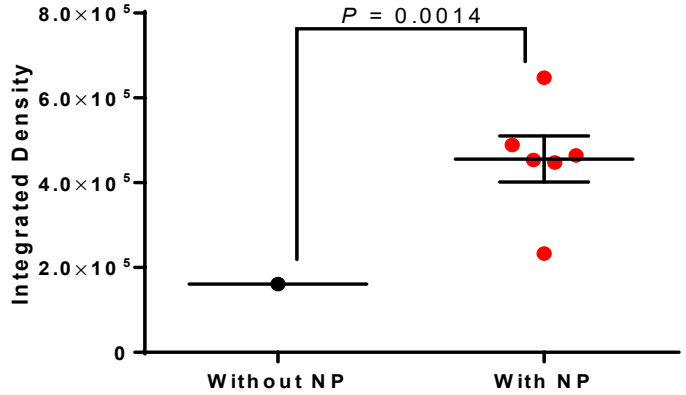
- Texas Red fluorescence measured in perfusate



Data: mean ± SEM
n = 6, ANOVA with Tukey's post hoc analysis

Syncytial uptake





Data: mean ± SEM
n = 6, Mann Whitney U Test

Next step up the ladder:

Guinea Pig FGR model, short term treatment

Non-Pregnant Female

Mate

Sacrifice

Control

Unrestricted/ Ad Libitum Food Intake

FGR

70% Food Intake

70% Food Intake

90% Food Intake

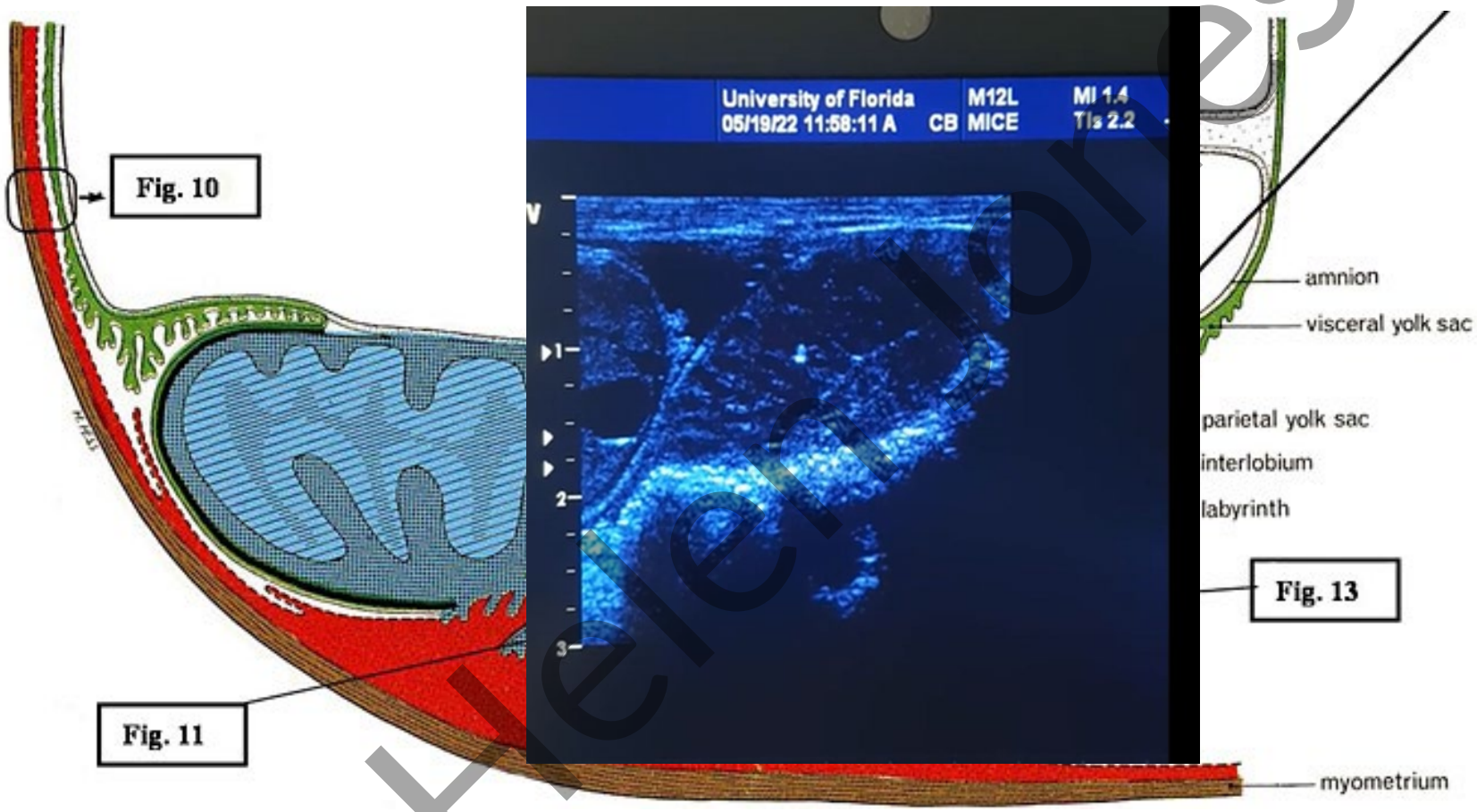
GP gestation: 30 Days Day 0 Day 30 Day 35

Human gestation Equivalency: 0 weeks 18 weeks 21 weeks

★ Pregnancy check GD20

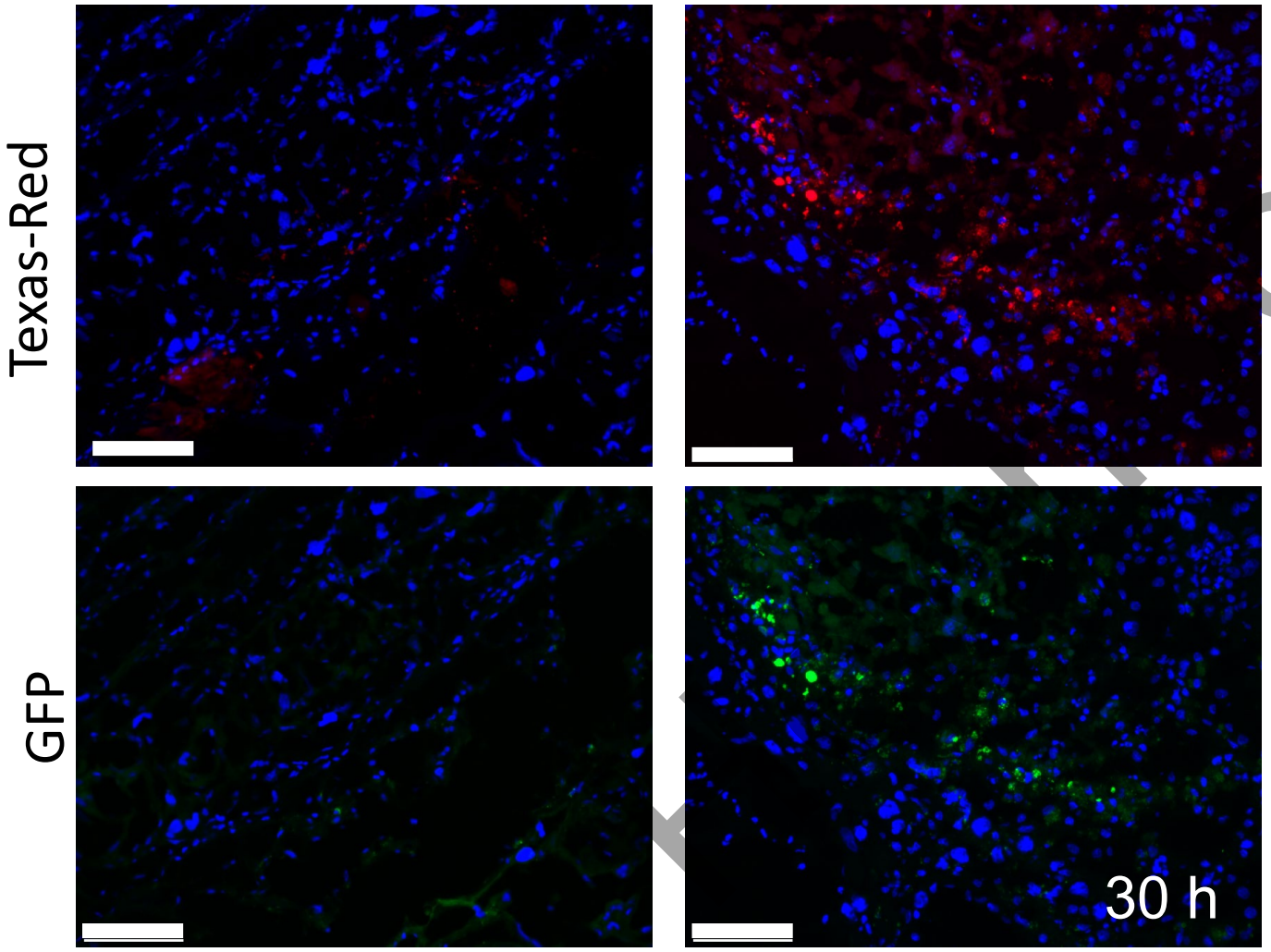
Roberts *et al.* 2001a & 2001b; Kind *et al.* 2003 & 2005; Elias *et al.* 2016 & 2017

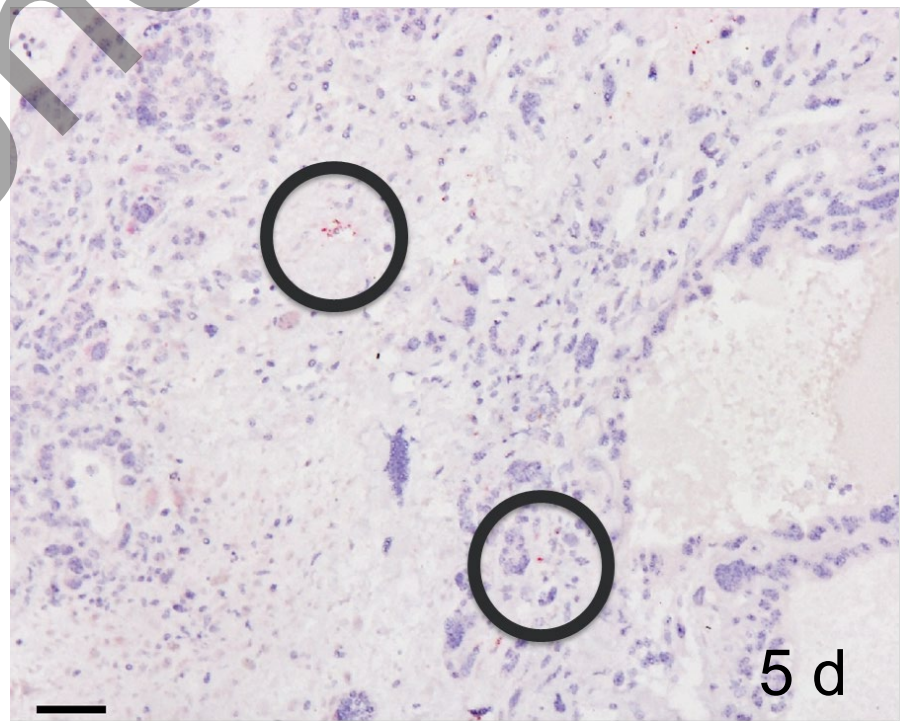
Guinea Pig placenta



Guinea Pig Cavia porcellus by Peter Kaufmann. placentation.ucsd.edu/guineabg/guinea02.htm

GP placentas successfully endocytose the nanoparticle and recognize the Cyp19a1 promotor

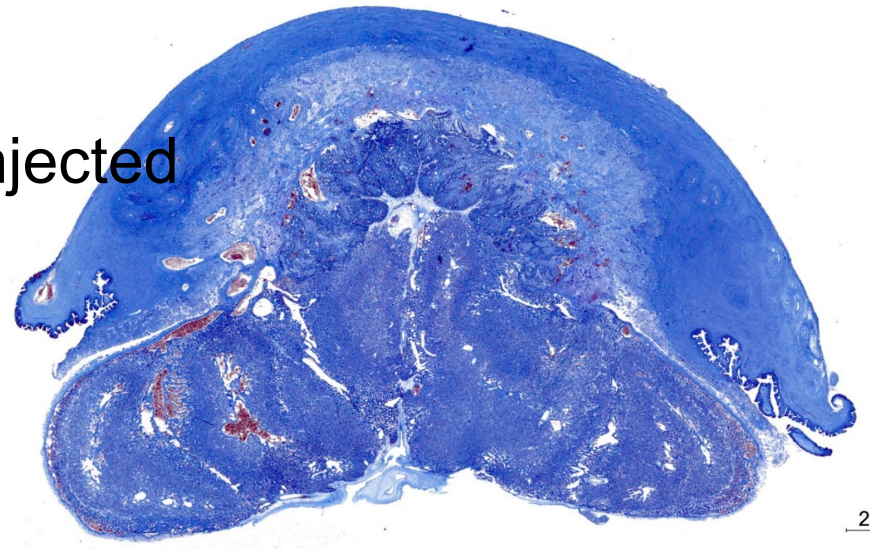




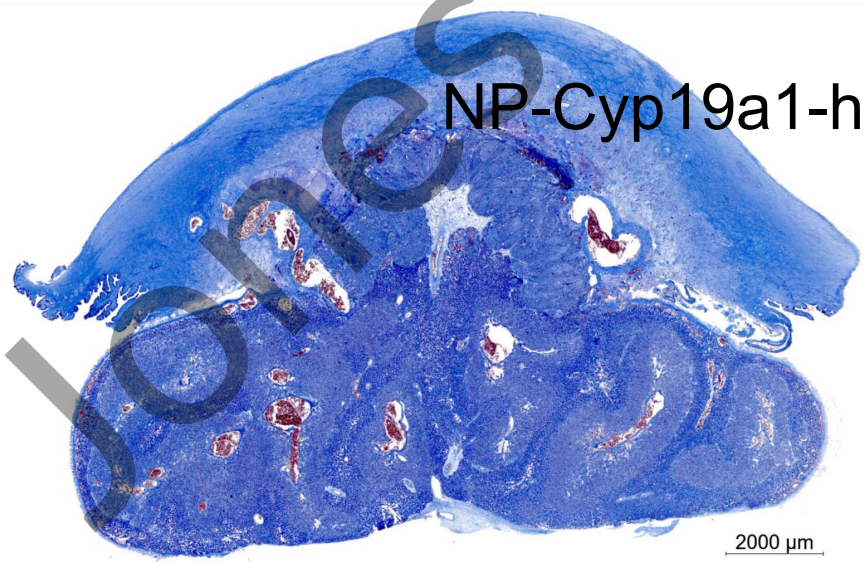
Plasmid-Specific mRNA – In situ Hybridization

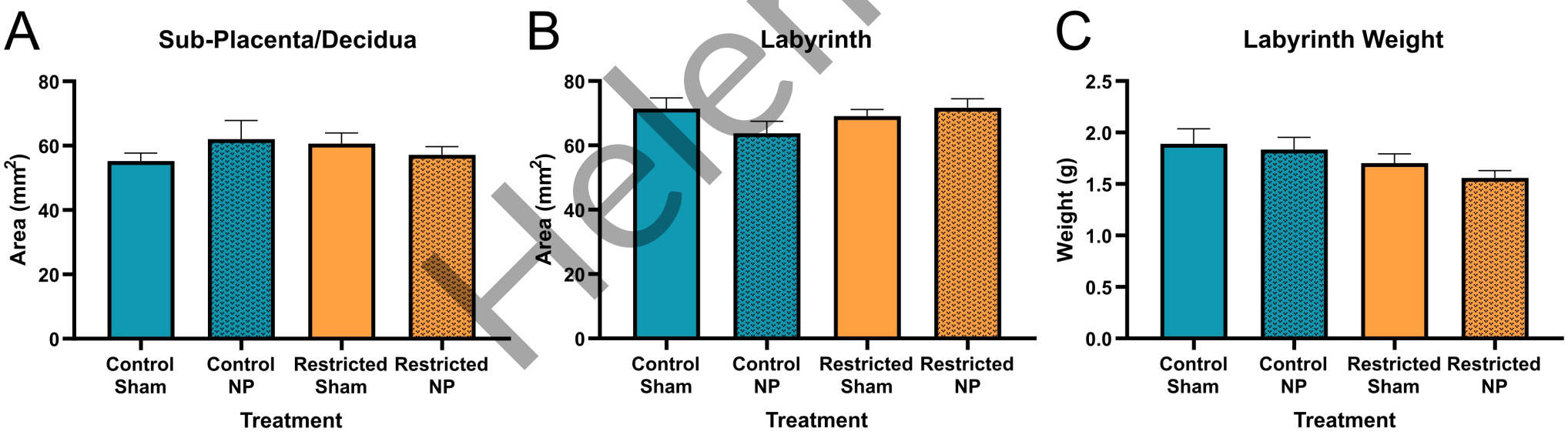
NP delivery has no adverse impact on placental or fetal development in mid-pregnancy

Sham injected



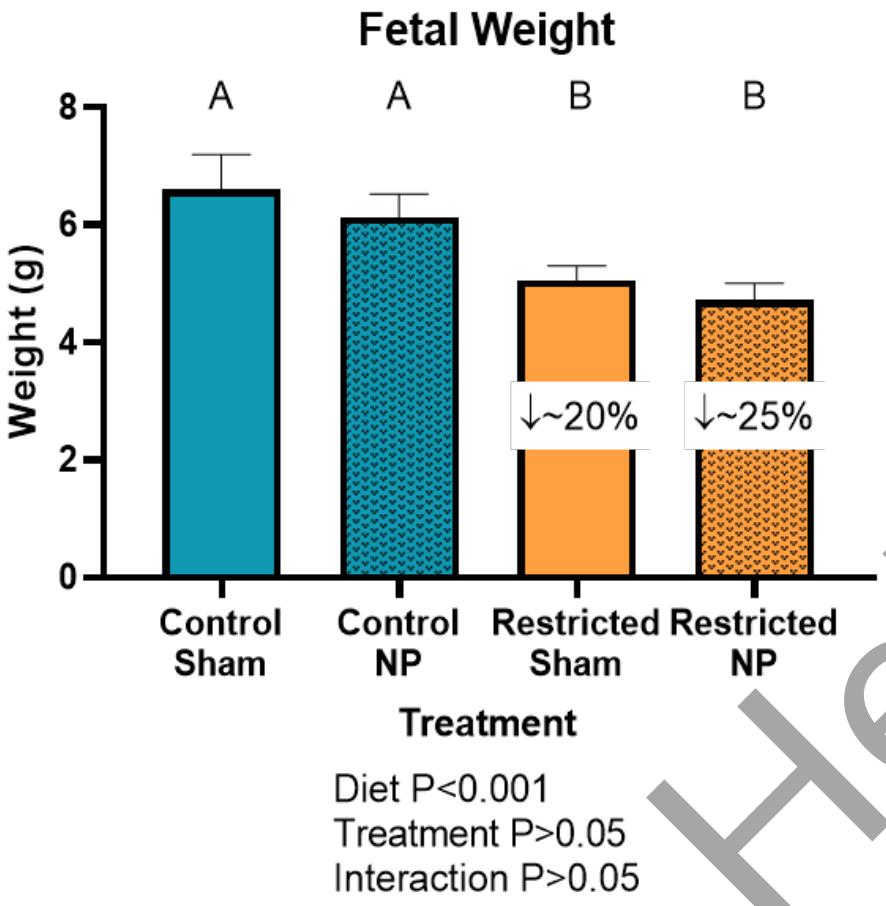
NP-Cyp19a1-hIGF-1 injected

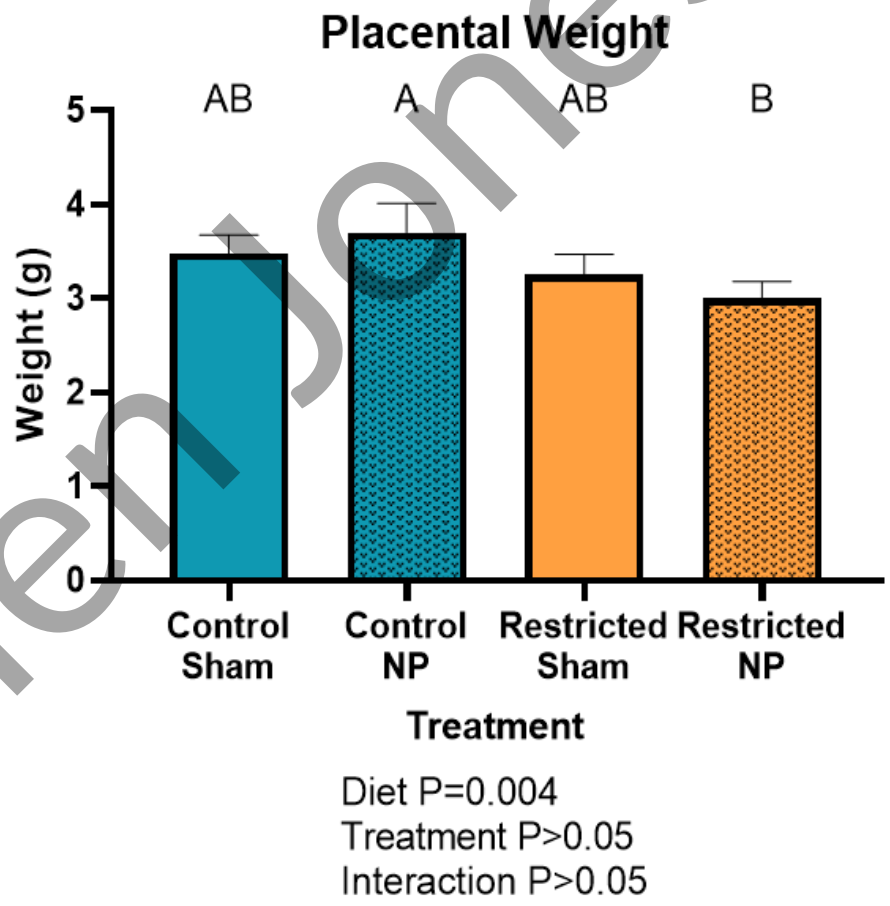




Data Estimated Means + SE Adjusted for GD & #Pups; Maternal ID treated as a random effect n = 4-7 dams per group

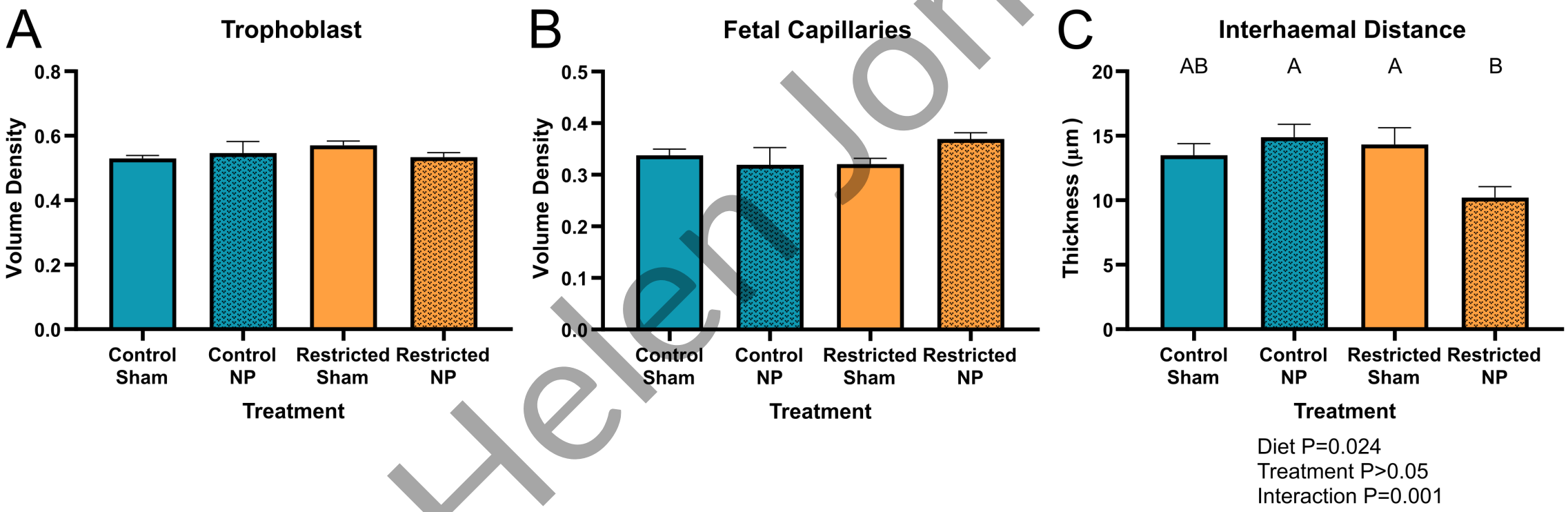
Restricted diet reduces fetal and placental weight at mid-pregnancy (5 day treatment)



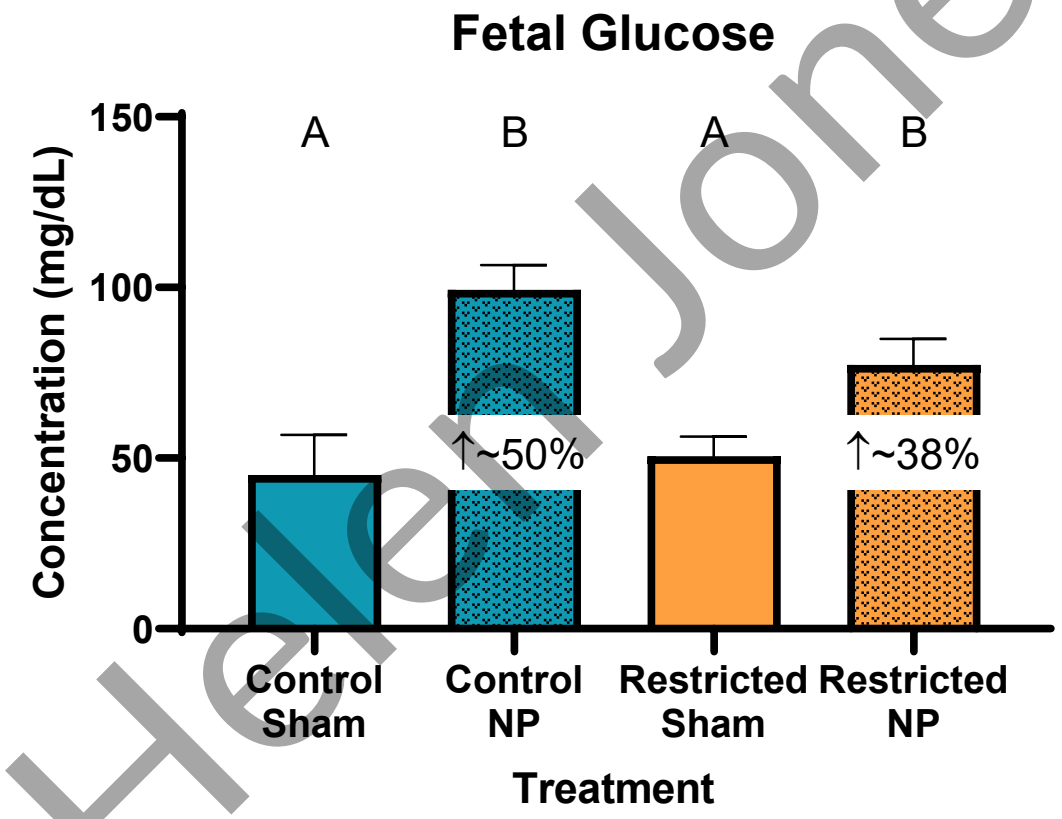


Data Estimated Means + SE Adjusted for GD & #Pups; Maternal ID treated as a random effect n = 4-7 dams per group

Interhemal distance reduced in MNR-NP placentas



Nanoparticle treatment increases fetal glucose concentrations & transporter expression in MVM of syncytiotrophoblast

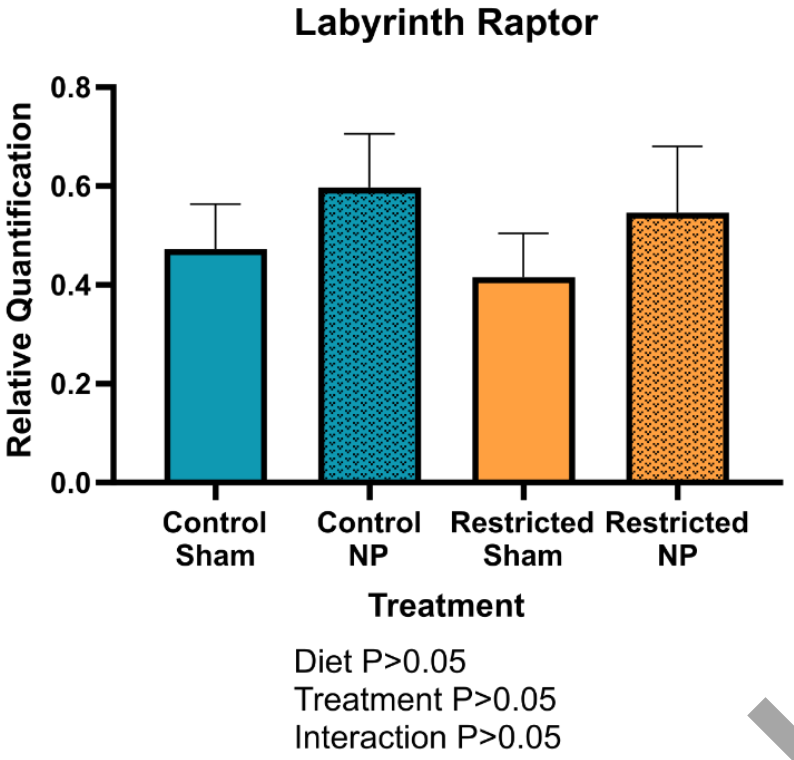


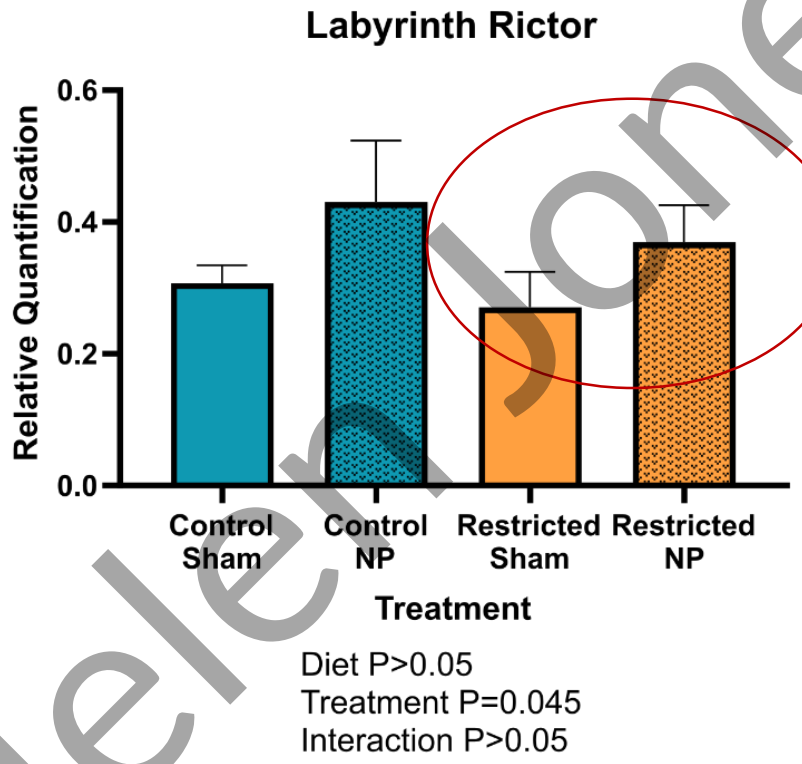
Diet P>0.05
Treatment P<0.001
Interaction P>0.05

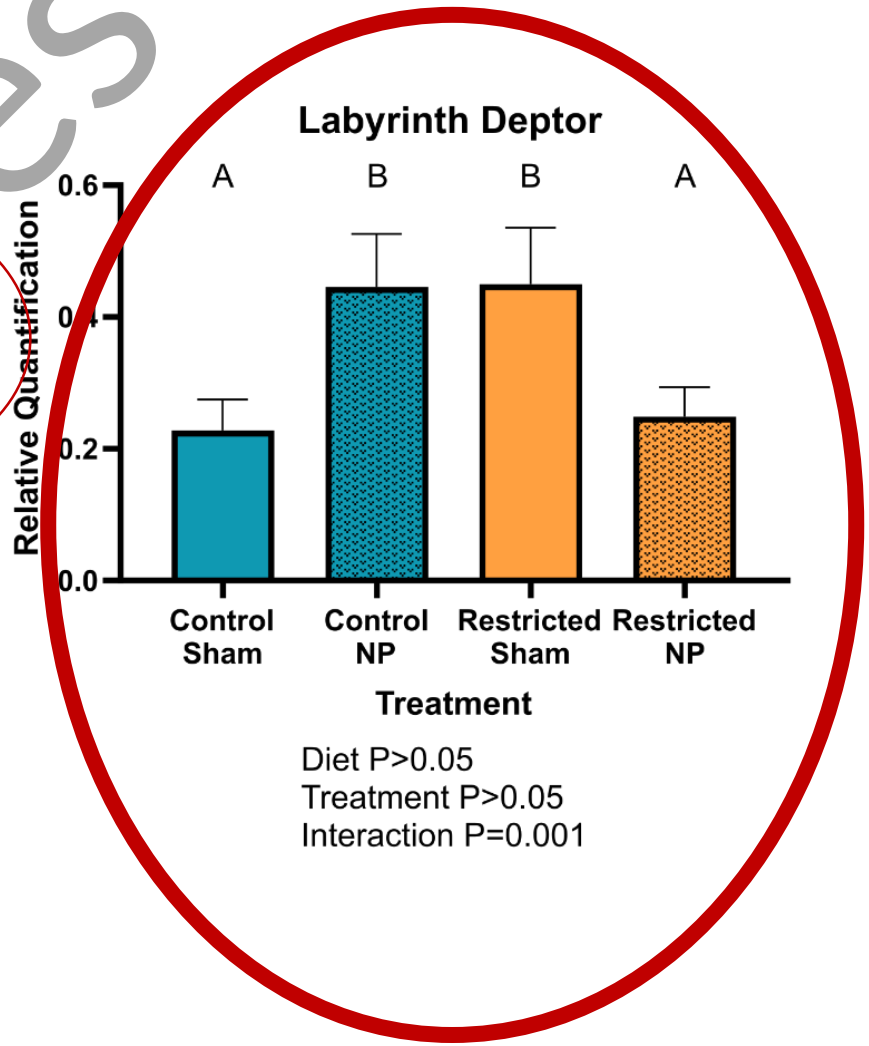
Impact of FGR model on Placenta Signaling

Function	Reactome Pathways through Panther	over-representation	p-value
Stress Response	Cellular responses to stress (R-RNO-2262752)	2.04	4.68E-02
	Cellular response to hypoxia (R-RNO-1234174)	6.16	4.83E-03

MTOR pathway response dependent on maternal diet/fetal phenotype







Treating Fetal Growth Restriction (FGR) in Animal Models

IGF-1 Nanoparticle Gene Therapy

- Does NOT cross the placenta
- < placenta interhaemal distance, ↑ oxygen diffusion to fetus
- Short term ↑ placenta nutrient transporter expression
- Short term ↑ fetal glucose concentrations in FGR fetuses

- **Human in vitro:** Wilson, R. L., et al. (2020). Placenta 93: 1-7.
- **Mouse surgical-ligation model:** Abd Ellah, N., et al. (2015). PLoS One 10(10): e0140879.
- **Mouse genetic k/o FGR model:** Wilson, R. L., et al. (2021). Am J Physiol Regul Integr Comp Physiol 320(5): R653-R662.
- **Guinea pig FGR model**:
  - Wilson, R. L., et al. (2021). Pediatr Res. 89(7): 1673-1680
  - Wilson, R. L., et al (2022). Mol Repro Develop.
  - Davenport, B.N., et al (2023). Front Physiol.

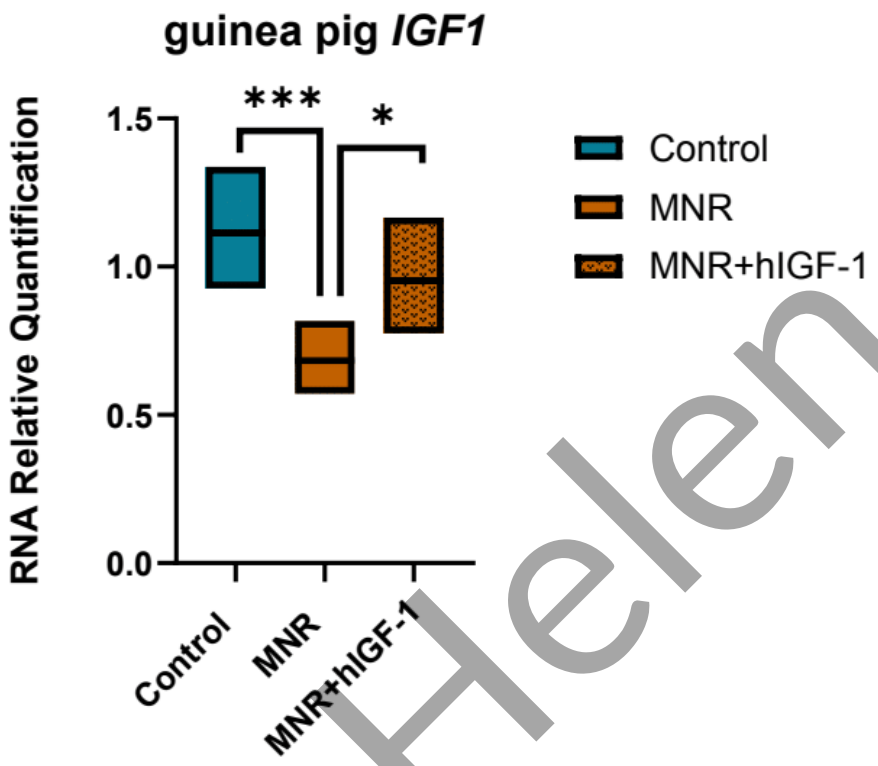
Short term treatment improves placental signaling, regulates fetal supply of oxygen, glucose, aa's,

Improvement in the *in utero* environment, impact on fetal growth?

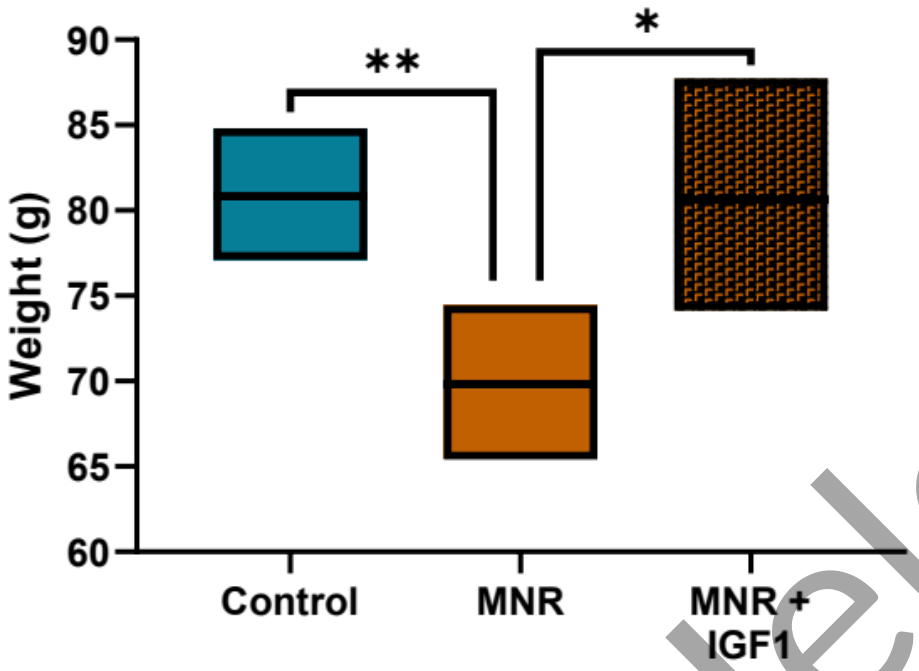
Repeated treatment protocol

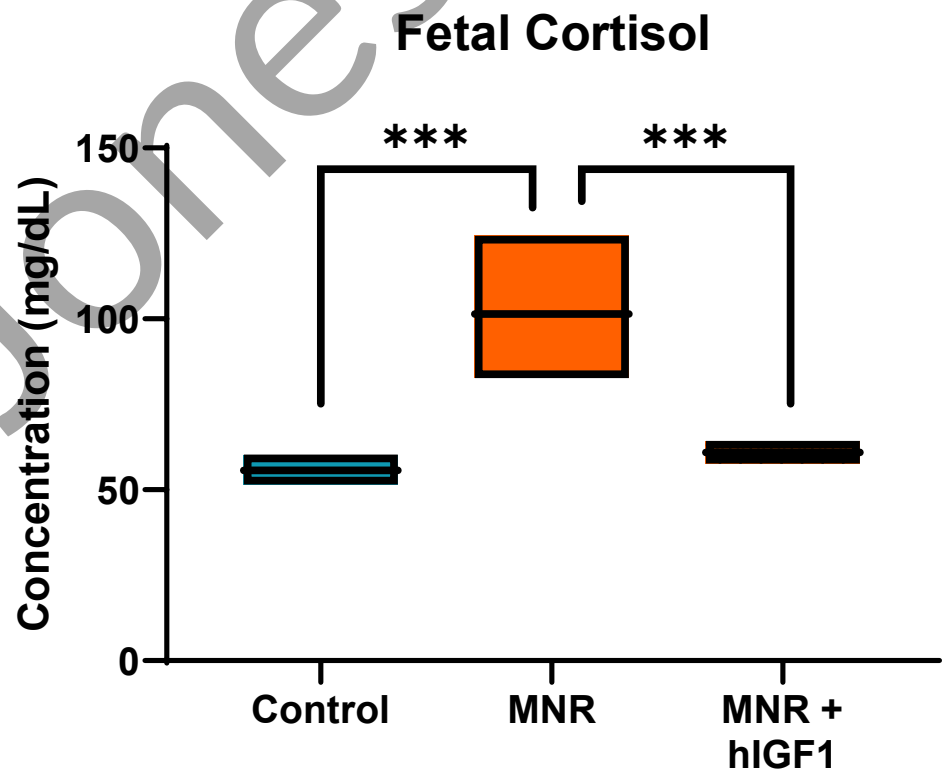
hIGF1-Nanoparticle Injections every 8 days after mid-pregnancy
C-section @ day 60

Endogenous placental IGF-1 levels are rescued after repeated treatments



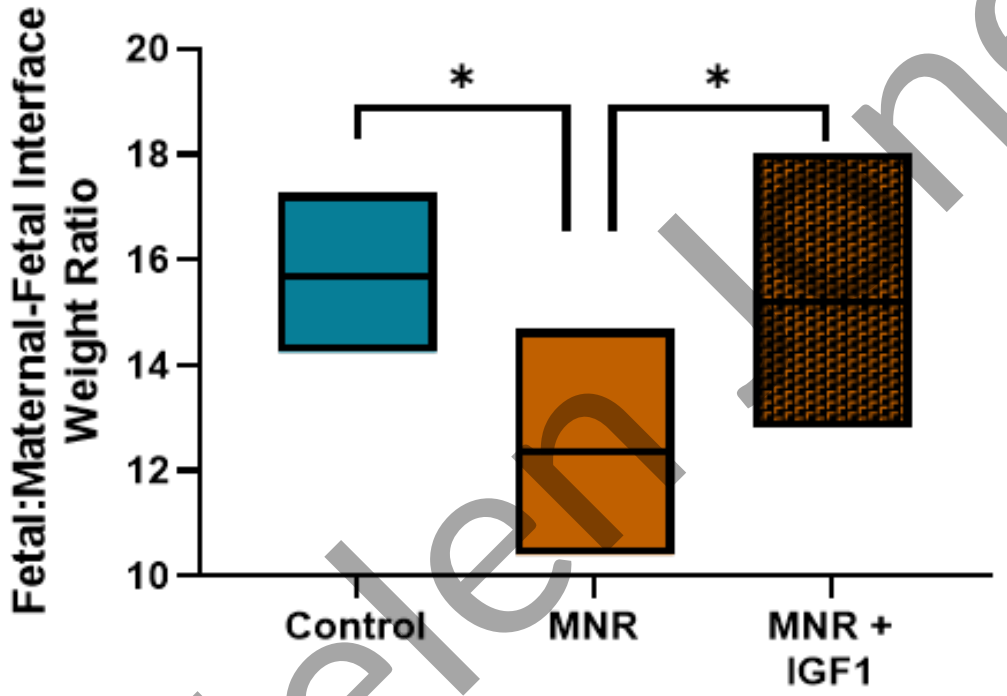
Improved birthweight, reduced stress





n = 6 Control-sham dams; 6 MNR-sham dams; 5 MNR-hIGF1 NP dams
Data are estimated marginal mean +/- 95% CI
P values calculated using generalized estimating equations. *P<0.05; **P<0.01; ***P<0.001

Placental Efficiency is normalized



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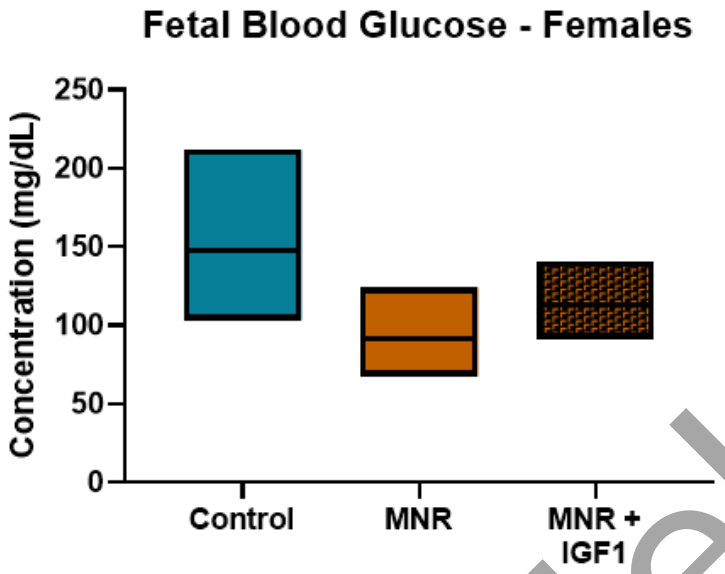
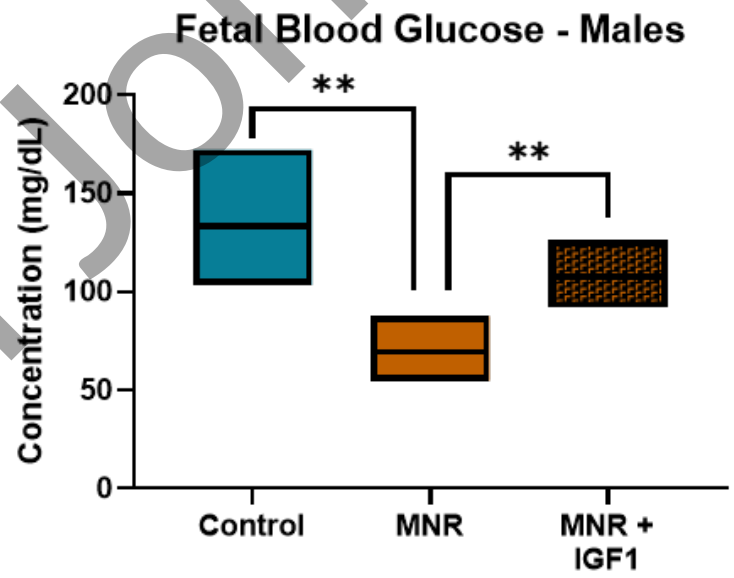
Mechanisms indicate sexual dimorphism





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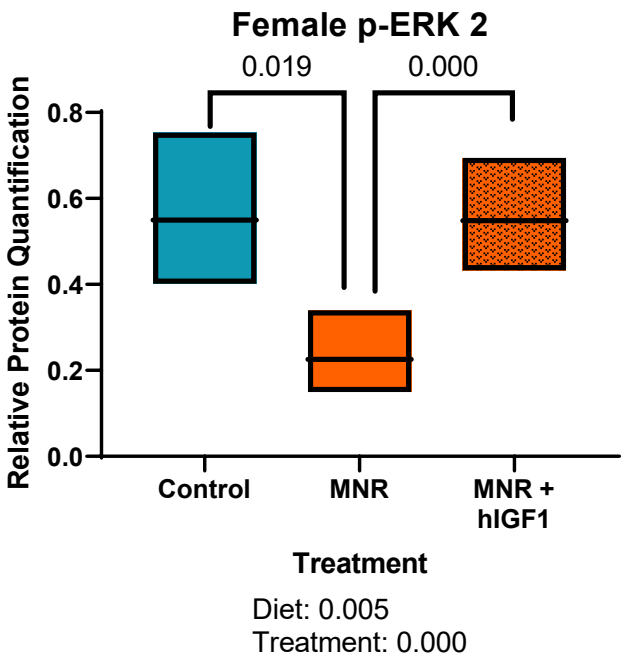
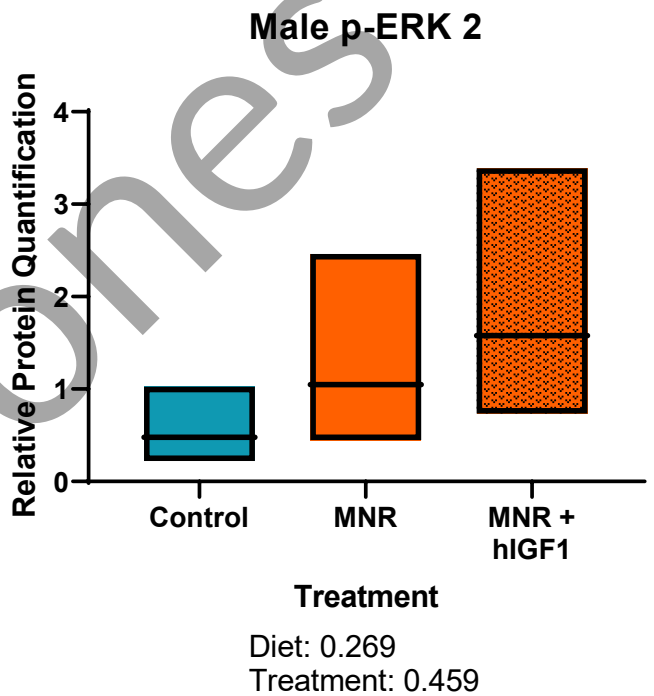
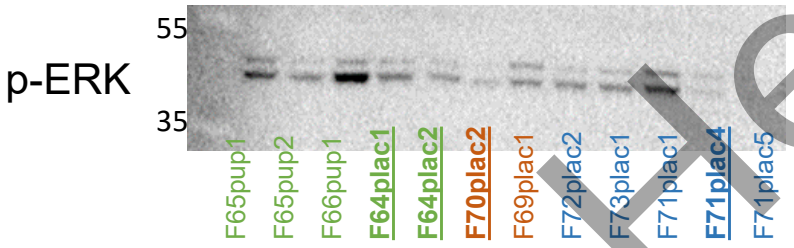
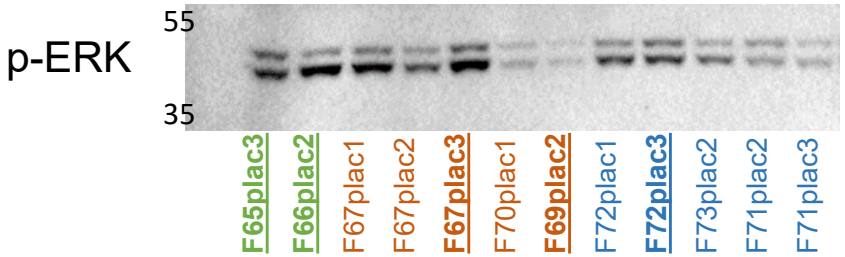
Data are estimated marginal mean +/- 95% CI
P values calculated using generalized estimating equations. *P<0.05; **P<0.01; ***P<0.001

Regulation of Intracellular signaling





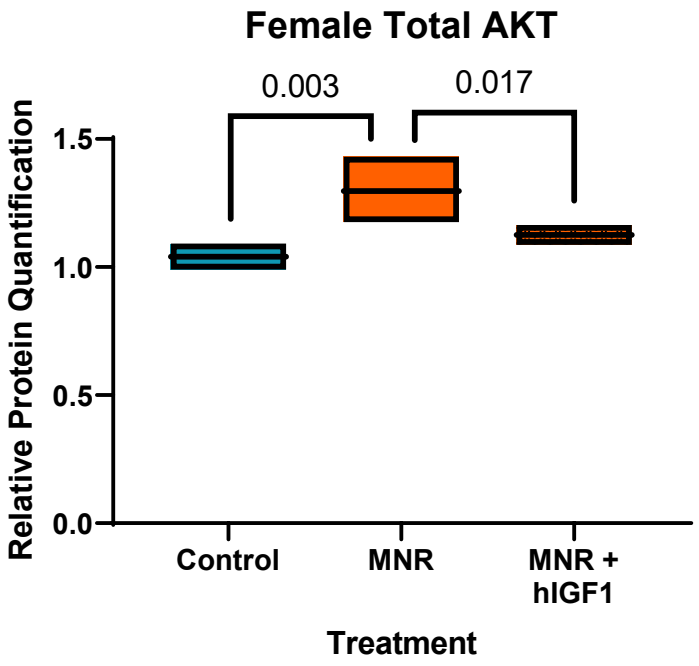




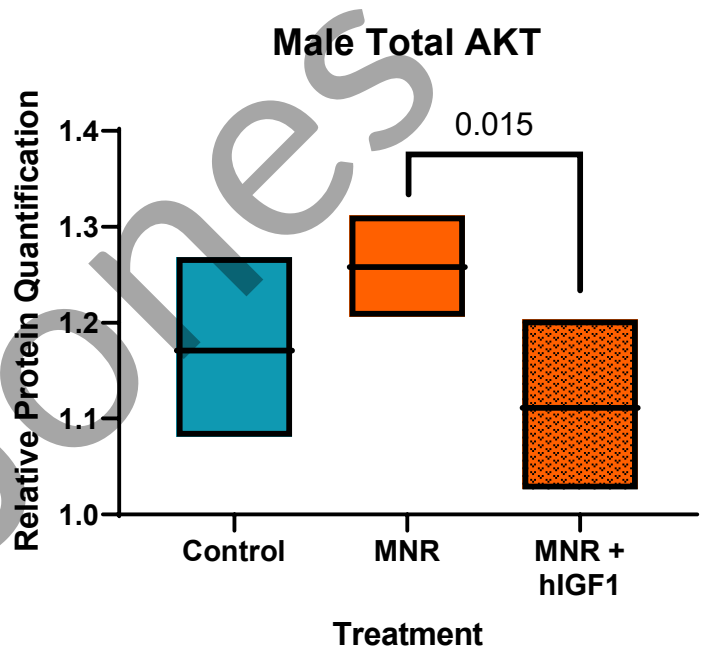
MNR + hIGF1

MNR

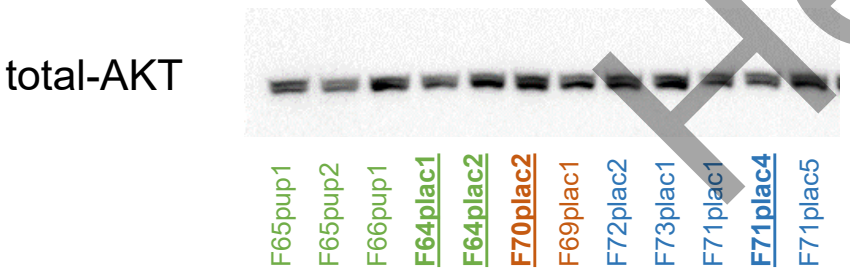
Control

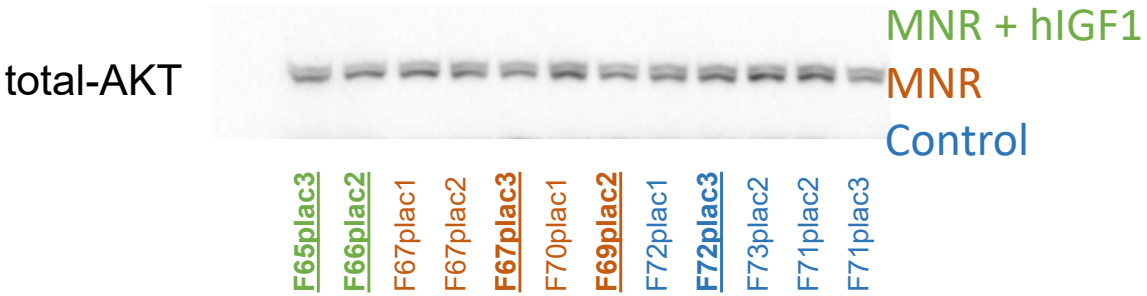


Diet: 0
Treatment: 0.003



Diet: 0.15
Treatment: 0.007



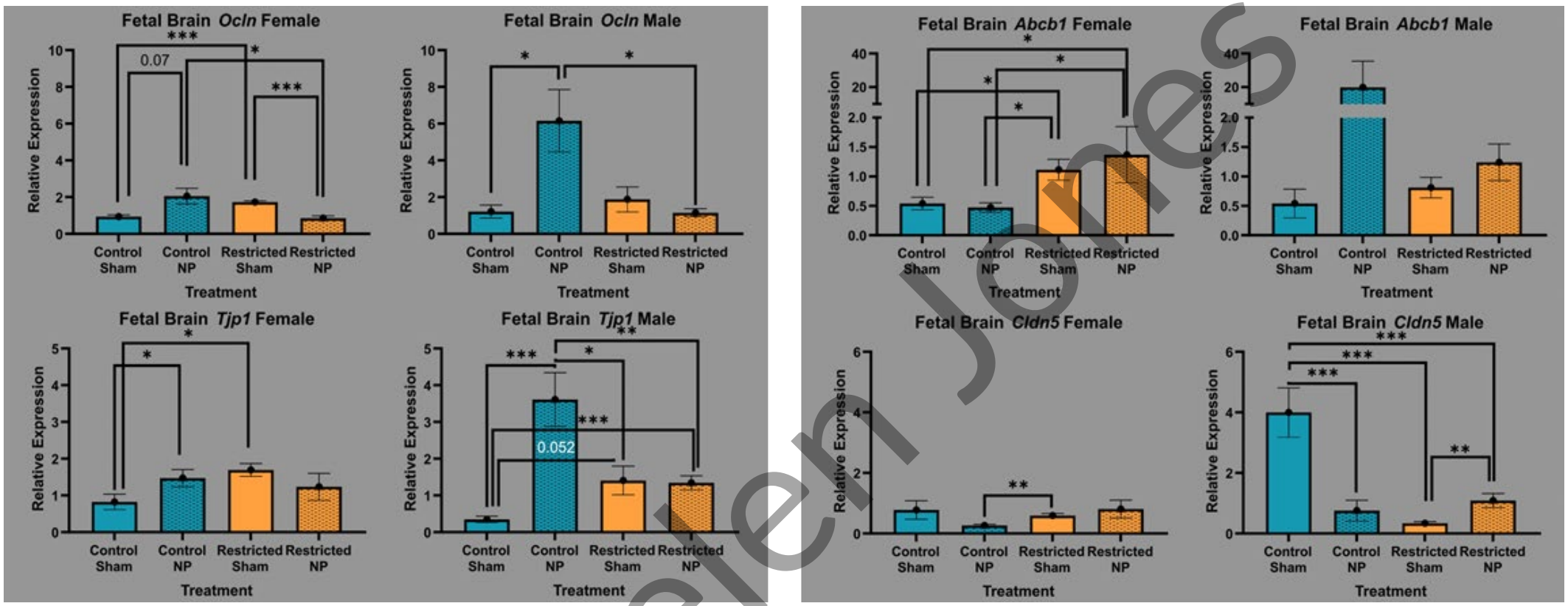


Improved placental function ameliorates mechanisms of fetal programming

- We now have evidence of signaling/structure improvement following placental treatment in guinea pigs:

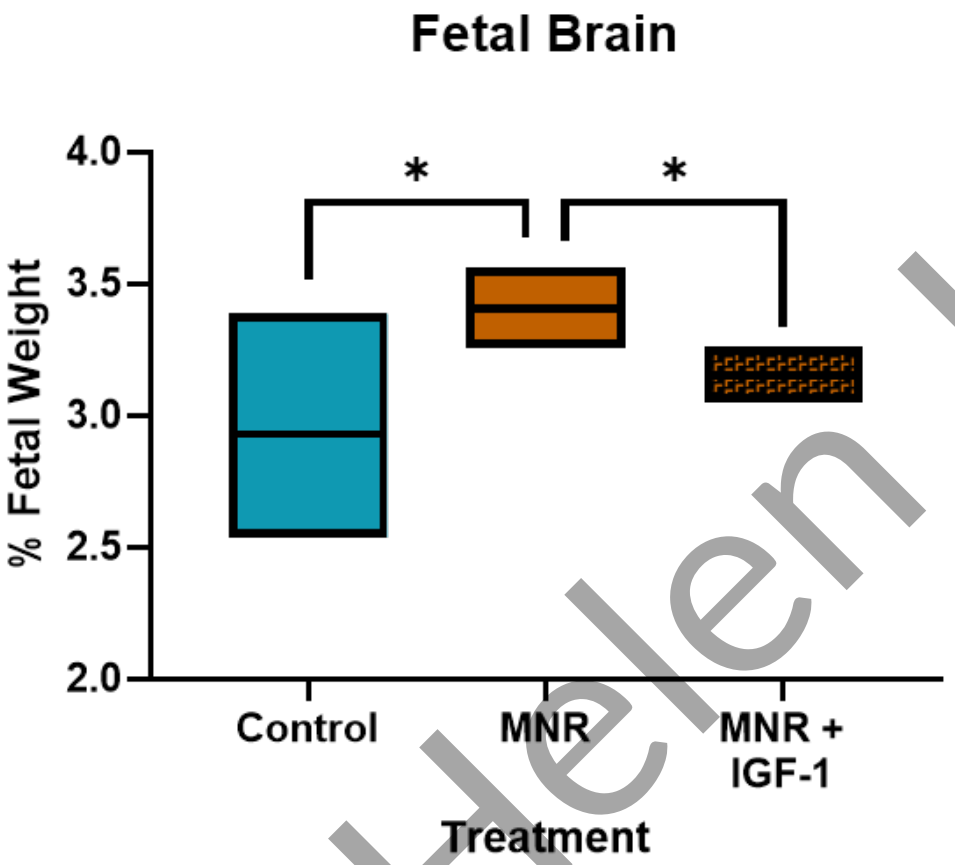
- Fetal brain
- Fetal blood-brain barrier
- Fetal liver
- Fetal kidney
- Fetal heart

Blood-brain barrier – mid-pregnancy



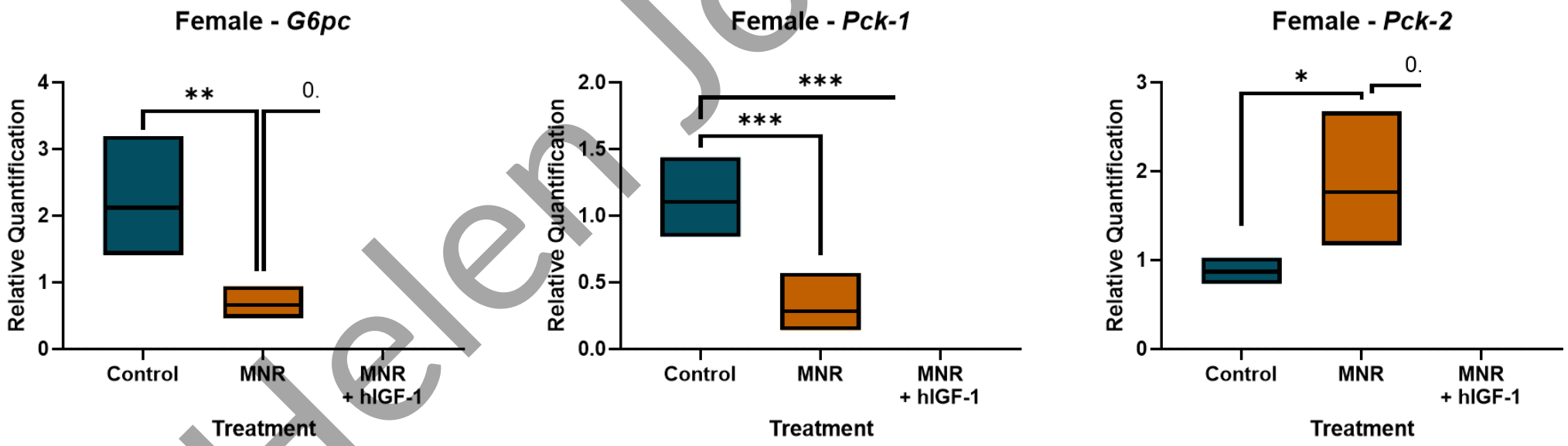
- Placenta nanoparticle treatment normalized mRNA expression of Ocln and Tjp1 in brain tissue of growth restricted females but not males
- In FGR, Tgf-β signaling may function in a sex-dependent feedback manner to maintain BBB integrity.

Multiple treatment ameliorates brain sparing



MNR changes mRNA expression of gluconeogenesis enzymes in female fetal livers, which is potentially prevented with multiple placenta nanoparticle treatments

- Near-term, multi treated, **female** fetal liver

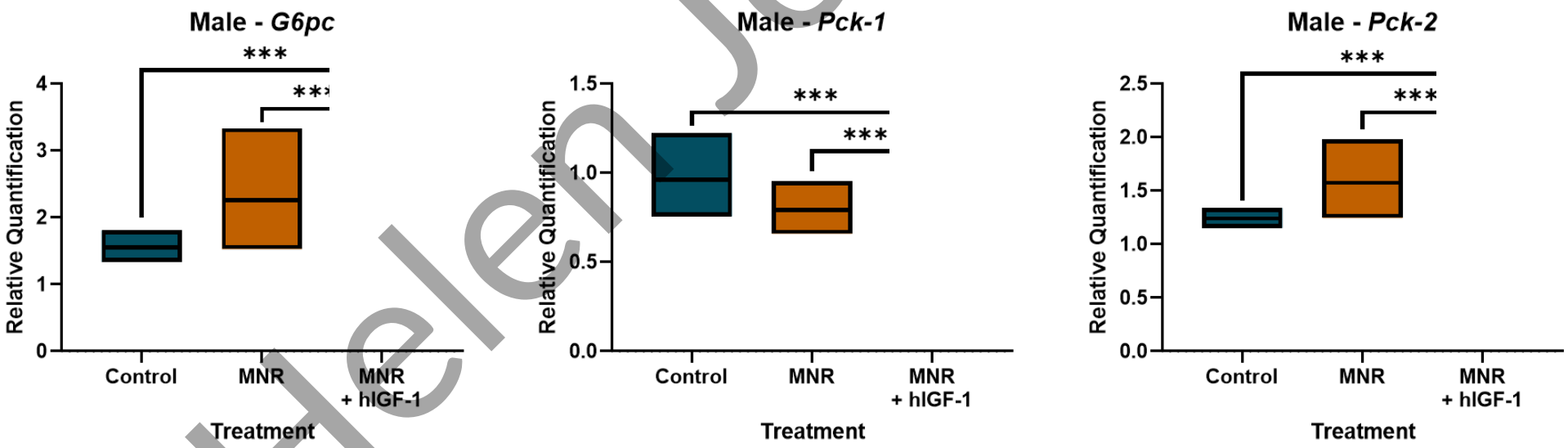


Preliminary Study **N = 3 dams per group**

Data are mean ± 95% confidence interval. P value determined using Generalized Estimating Equations

Multiple placenta nanoparticle treatments reduces expression of gluconeogenesis enzymes in male fetal livers at late pregnancy

- Near-term, multi treated, **male** fetal liver



Preliminary Study **N = 3 dams per group**

Data are mean ± 95% confidence interval. P value determined using Generalized Estimating Equations

Another step up the species ladder

NHP Macaque; Wisconsin National Primate Center

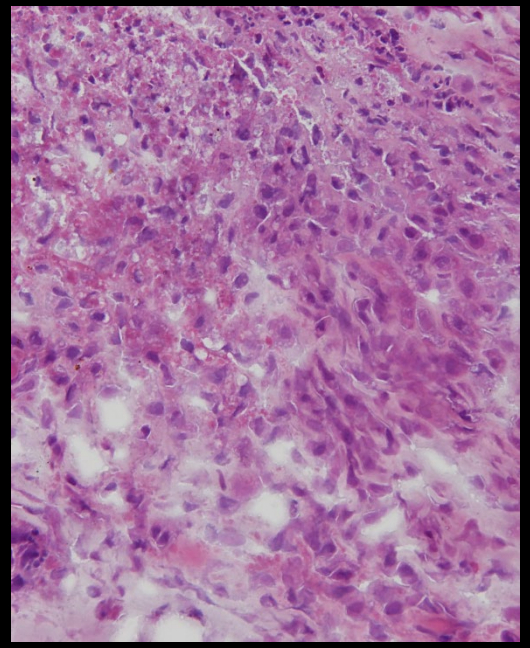
PLAC1-transgene plasmid complexed with a HPMA-DMEAMA co-polymer and delivered at approximately gestational day 100.

Fetalectomy performed

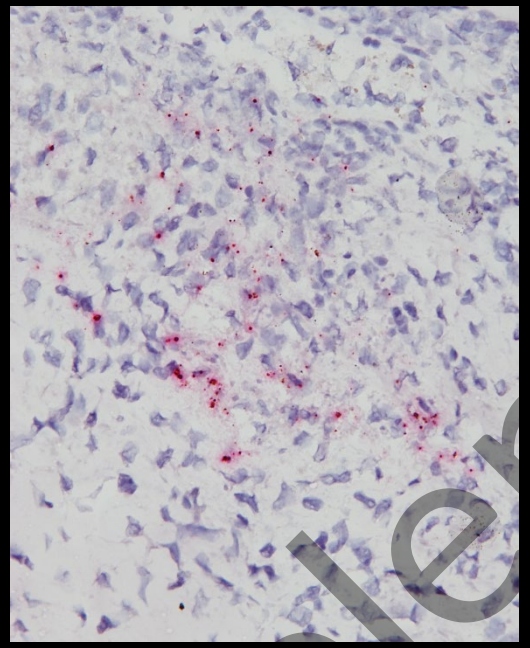
48 hours (n = 3)

10 days (n = 3)

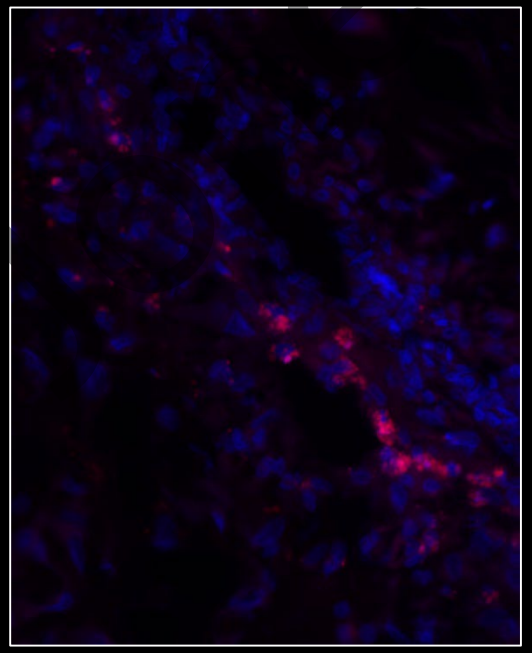
Non-Human Primate Placenta
Successful NP delivery and transgene expression @48 hours & 10 days.



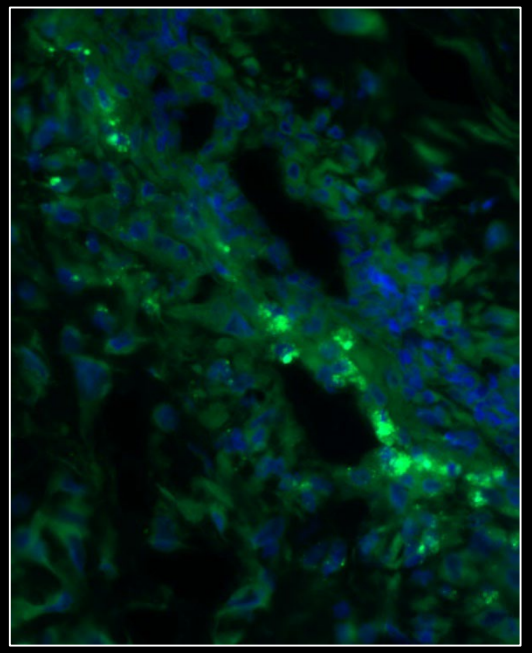
H&E



RNAscope

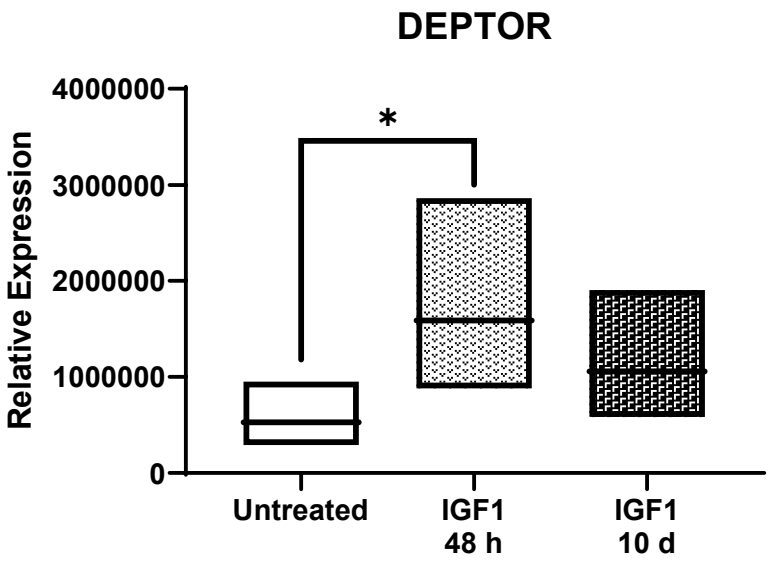


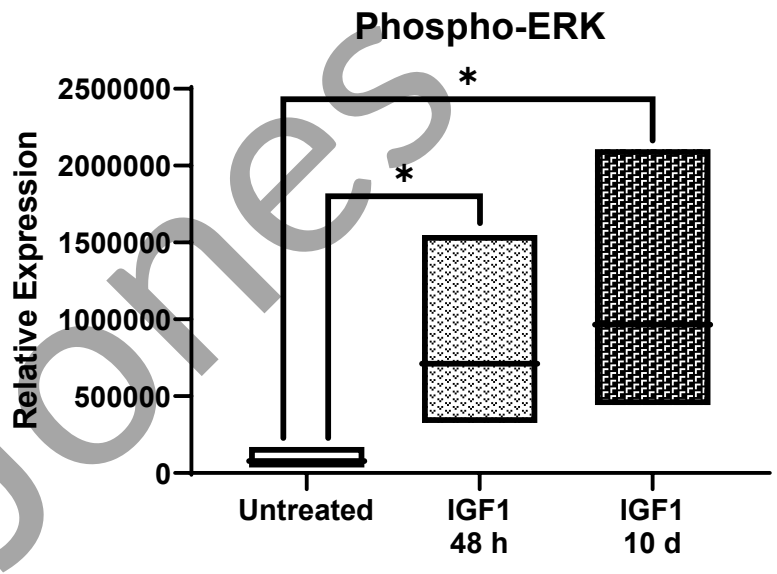
Texas Red

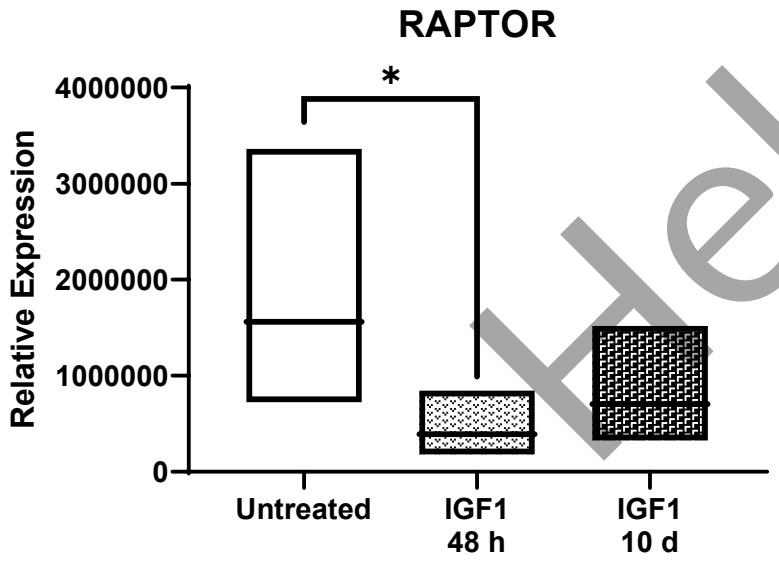


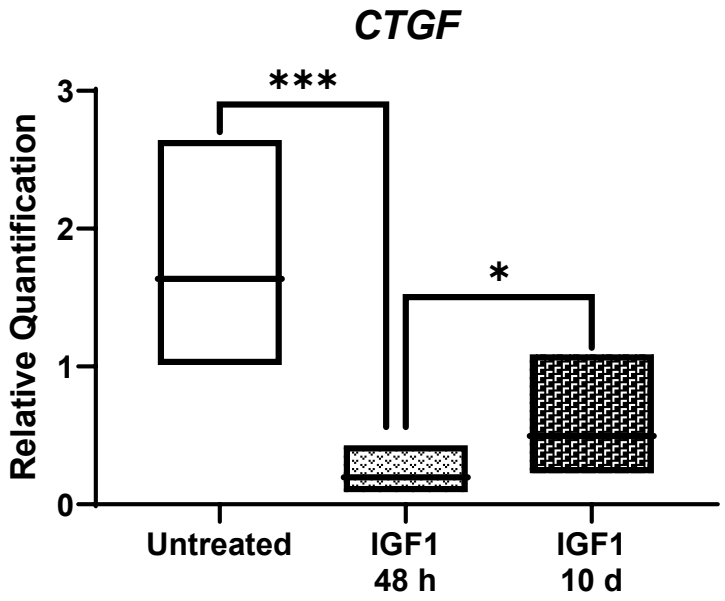
GFP

NP-IGF-1 is functional in NHP placenta









N = 3 placentas per group (mixed fetal sex). Data are estimated mean +/- 95% CI

Placental & maternal assessment

Histopathological assessment showed no difference in the occurrence of histological legions at 48 h or 10 days (independent pathology blind review)

No evidence of immune infiltration by histology and CD45 IHC

At 48 h, expression of the glucose transporter Slc2A1 increased in the MVM of syncytium, and this was sustained at 10 days.

Treatment had no impact on maternal CBC, P2 or E2 levels at either 48 h or 10 days.

Summary

- Intervention via placental therapy after establishment of fetal growth restriction is feasible
- Nanoparticle delivery is safe, effective, no off-target effects and does not cross the placenta
- Improving placental development, structure, signaling and function improves the in utero environment
- Fetal sex differences exist in intracellular signaling mechanisms of FGR and following intervention but therapy effective in both sexes
- Response to placental therapy in healthy non-human primates reflects that of the control Guinea Pigs demonstrating specificity of delivery and placental homeostasis to prevent fetal overgrowth (in cases of misdiagnosis of FGR)
- The potential for targeted placental therapy is not limited to FGR……..

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