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Galectin-7 dysregulates renin-angiotensin-aldosterone and NADPH oxide synthase pathways in preeclampsia.

Running title: Galectin-7 dysregulates placental RAS/ROS

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Abbreviations

ACE Angiotensin converting enzyme; AGT Angiotensinogen; AT1R Angiotensin II type 1 receptor; CYBA Cytochrome b-245 alpha chain (p22-phox); CYBB Cytochrome b-245 beta chain (p91-phox); E Embryonic Day; ICAM1 Intercellular adhesion molecule 1; NADPH Nicotinamide adenine dinucleotide phosphate ; RAAS Renin-angiotensin-aldosterone system; RAS Renin-angiotensin system; ROS Reactive oxygen species; VCAM1 Vascular cell adhesion molecule 1.

Previous presentations of work presented in this manuscript:

Figure 1A the Biovision serum concentration data was previously published (Menkhorst et al 2020, Hypertension 76:1185-1194). Permission to re-use this data has been granted by Wolters Kluwer Health, License Number 5257301002554.

Figure 1C presented at the Developmental Origins of Health and Disease conference in 2019, Melbourne Australia in the poster session.

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Abstract

Objectives: Preeclampsia is a life-threatening disorder of pregnancy unique to humans. Poor placentation in the first trimester of pregnancy is widely accepted to be an underlying cause of preeclampsia. Galectin-7 is abnormally elevated in chorionic villous samples and serum from women that subsequently develop pre-term preeclampsia. Administration of exogenous galectin-7 to pregnant mice causes preeclampsia-like features (hypertension, proteinuria), associated with dysregulation of the renin-angiotensin system (RAS). In this study investigated the mechanism by which galectin-7 induces alterations to tissue RAS homeostasis and ROS production. We hypothesized that galectin-7 induces alterations in the production of either placental RAS or NADPH oxidases (or both) to drive the dysregulated RAS and ROS production seen in preeclampsia.

Study Design: Mated female mice (n=5-6/group) received single (embryonic day [E]12/13) or multiple (E8-12) subcutaneous injections of 400ug/kg/day galectin-7 or vehicle control and killed on E13 or E18. Human first trimester placental villous and decidual tissue (n=11) was cultured under 8% oxygen 1 µg/mL galectin-7 or vehicle control for 16h.

Results: Galectin-7 administration to pregnant mice impaired placental labyrinth formation, suppressed circulating aldosterone and altered placental RAS (*Agt*, *Renin*) and NADPH oxidase (*Cyba*, *Cybb* and *Icam1*) mRNA expression. In vitro, galectin-7 regulated human placental villous RAS (*AGT*) and NADPH oxidase (*CYBA, ICAM1* and *VCAM1*) mRNA expression.

Conclusions: Overall, galectin-7 likely drives hypertension in preeclampsia via its direct regulation of multiple pathways associated with preeclampsia in the placenta. Galectin-7 may therefore be a therapeutic target to improve placental function and prevent preeclampsia.

Key words: Galectin-7, preeclampsia, renin-angiotensin system, reactive oxygen species, placenta

Introduction

Preeclampsia is a life-threatening disorder of pregnancy unique to humans. Worldwide, more than 4 million women develop preeclampsia each year [1], resulting in significant maternal and neonatal morbidity and mortality [1-3]. Preeclampsia is a complex multi-system disease, diagnosed by sudden onset hypertension (>20 weeks gestation) and at least one other associated complication including proteinuria, other maternal organ dysfunction or fetal growth restriction [4].

Poor implantation and placentation in the first trimester of pregnancy are widely accepted to be the sentinel causes of pregnancy diseases including preeclampsia. Inadequate extravillous trophoblast invasion and impaired spiral artery remodeling results in reduced uterine blood flow leading to placental ischemia [1]. The damaged placenta releases toxins into maternal blood causing systemic inflammation and widespread maternal endothelial dysfunction, resulting in the maternal syndrome of preeclampsia [1, 5].

Galectins are animal (soluble) lectins abundantly expressed at the maternal-fetal interface [6]. Dysregulated expression of galectins-1,2,3,7,9, 13 and 14 is associated with preeclampsia [6-11]. Galectin-7, expressed by first-trimester syncytiotrophoblast and extravillous trophoblast [12, 13], is abnormally elevated in chorionic villous samples [14] and first-trimester serum [12] from women who subsequently develop pre-term preeclampsia. *In vivo* administration of galectin-7 causes preeclampsia-like features including hypertension, albuminuria and impaired placentation in pregnant mice [14]. Non-pregnant mice treated with gal-7 do not develop hypertension or albuminuria, demonstrating that galectin-7 acts via the placenta to cause preeclampsia-like features in this model [14].

We previously showed that in mice, exogenous galectin-7 induces alterations to tissue renin-angiotensin-(aldosterone)-system (RAS) homeostasis and drives a pro-inflammatory placental state [14]. In a healthy pregnancy, activation of the maternal renal RAS and circulating renin-angiotensin-aldosterone system (RAAS) expands the maternal cardiovascular system, maintaining blood pressure and increasing renal

blood flow [15]. In preeclampsia, alterations to the circulating RAAS and placental RAS are clear [15, 16]: prorenin, angiotensinogen (AGT), ACE (angiotensin converting enzyme), and the AT1R (angiotensin II type 1 receptor) are all upregulated in the placenta [17-19]. Placental oxidative stress is another key driver of preeclampsia. Reactive oxygen species (ROS) have critical roles in the development of vascular disease via their involvement in endothelial dysfunction [20]. Although during uncomplicated pregnancies there is an increase in ROS production, in preeclampsia a further elevation in ROS is found in the placenta and maternal circulation causing cytokine and anti-angiogenic factor release into the maternal circulation which leads to endothelial dysfunction, a key feature in the pathophysiology of [21]. One tissue source of ROS, are the nicotinamide adenine dinucleotide phosphate (NADPH) oxidases which play a central role as 'kindling radicals' that affect other enzymes [20]. Angiotensin II (AngII) drives ROS production via NADPH oxidases in many tissues [22]. Galectin-7 treatment causes ROS accumulation in bladder cancer cell lines [23], but the mechanism leading to this accumulation remains unknown.

In this study we aimed to investigate the mechanism by which galectin-7 induces alterations to the tissue RAS homeostasis and ROS production using both human and mouse models. We hypothesized that galectin-7 induces alterations in the production of either placental RAS or NADPH oxidases (or both) to drive the dysregulated RAS and ROS production seen in preeclampsia.

Methods

Galectin-7 recombinant protein

Human galectin-7 recombinant protein from two sources was used in this project as follows - *in vivo* mouse experiments treated with one dose of galectin-7 (killed on Embryonic Day [E]13) or treated from E8-E12 and killed on E18 and *ex vivo* primary 1st trimester human placenta and decidua explant cultures: in-house prepared (WEHI) as described in [24], except the purified protein was not dialyzed against potassium phosphate (vehicle: 150 mM alpha-lactose, 10 mM Tris-HCl, 100 mM NaCl, pH 8.0 containing

0.02% sodium azide); *in vivo* mouse experiments treated daily from E8-E12 and killed on E13 (Biovision; #4647-1000; vehicle: PBS). Each batch of galectin-7 was tested for its activity as previously described [14].

In vivo mouse experiments

C57BL6/J female (virgin 8-12 weeks) and male (8-52 weeks) mice (WEHI, Kew, VIC, Australia) housed under conventional conditions, had food and water ad libitium and were maintained in a 12h: light-dark cycle. All procedures were approved by Melbourne University Animal Ethics Committee (#1814697) following the NHMRC Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Serum clearance Non-pregnant female mice received a single subcutaneous injection of 400µg/kg galectin-7 or vehicle control and were killed after 6, 16, 24 and 48 hours. Galectin-7 levels in serum peaked 24h after injection and were still detectable 48h after injection (Fig 1A).

Placental development and pregnancy outcome Mated female mice received either a single subcutaneous injection of 400ug/kg galectin-7 or vehicle control on E12/13 (E0 is day of plug detection) and killed after 8 and 16h at E13.5, or once daily sub-cutaneous injections of 400ug/kg/day galectin-7 or vehicle control from E8 to E12. Pregnant mice were killed on E13 or E18 (n=5-6/group).

Serum and tissue collection Mice were killed on E13 and E18 by tail vein injection of ketamine (235mg/kg) and xylazine (23.5mg/kg) followed by cardiac puncture to collect peripheral blood. Ketamine/xylazine overdose was chosen to kill the mice to ensure the E18 fetuses were killed at the same time as the dam. Serum was separated by centrifugation at 1500x*g* after 2h incubation at room temperature and snap-frozen until use. Implantation sites (at least 5/mouse) were dissected to obtain decidua, placenta and fetus. The decidua and placenta were weighed as a single unit and fixed in 10% neutral buffered formalin or separated and snap frozen on dry ice. The fetus was also weighed.

Blood pressure measurements Systolic Blood Pressure (sBP) was measured in conscious pregnant mice every 2-3 days from E8-17 by tail cuff plethysmography, following a procedure adapted from the manufacturer's manual (Kent Scientific). Briefly, following 15 min of stabilization on the preheated mat (Kent Scientific) before 15 (5 acclimatization, 10 regular) consecutive automated inflation−deflation cycles were performed and sBP was calculated by the software (Kent Scientific). Training prior to mating was not performed as pilot studies showed no benefit from this training, most likely as this occurred up to 3 weeks prior to the mouse becoming pregnant. Instead, mice were trained from E8-E12 in 2-3 sessions, before experimental readings were taken from E13 onwards.

Placental Morphometry Placenta/decidua (one per mouse) stained with Masson's trichrome were used to determine the area of the labyrinth, junctional and decidual zones [25]. The area of each zone was quantified using Image J software [26].

Human placenta/decidua

Human placental and decidual tissue was collected under appropriate Human Research and Ethics Committee approvals at the Royal Women's Hospital (#09317B). Written informed consent was obtained from each patient before surgery. First trimester placental villous and decidual tissue (n=11) was donated by healthy women undergoing pregnancy termination for psychosocial reasons (amenorrhea 6-13 weeks). Small pieces of first-trimester placental villous or decidua tissue were cultured under 8% oxygen in DMEM/F-12 with 1 µg/mL galectin-7 or vehicle control. Explants were collected after 16h and snap frozen.

Gene expression

RNA was extracted using the RNeasy kit (QIAGEN) according to the manufacturer's instructions. Genomic DNA was digested using the on-column kit (RNase-free DNase set, #79256, QIAGEN) according to the

manufacturer's instructions. RNA samples concentration, yield and purity were analysed by spectrophotometry (Nanodrop Thermo Scientific, Scoresby, Victoria, Australia) at an absorbance ratio of A260/280nm.

Total RNA (250ng) was reverse transcribed using Superscript III as per the manufacturer's instructions (Invitrogen) except 0.5µL Superscript III was added per reaction [27]. Real time PCR was performed using Power SYBR Green master mix (Applied Biosystems) on the Veriti 7 fast block real-time qPCR system (Applied Biosystems) in triplicate (final reaction volume, 10µl) in 384-well Micro Optical plates (Applied Biosystems). A template-free negative control in the presence of primers and RNase-free water only negative controls were added for each run. Primer sequences are shown in Tables S1 and S2 (Sigma-Aldrich). The qPCR protocol was as follows: 95 °C for 10 min and 40 cycles of 95 °C for 15s followed by 60°C for 1 min. Relative expression levels were calculated using comparative cycle threshold method (ΔΔCt) as outlined in the manufacturer's user manual, with 18S ribosomal RNA serving as the endogenous control for normalization.

ELISA

Mouse serum retention of recombinant galectin-7 was assayed by ELISA against human galectin-7 (diluted 4-fold, ELH-Galectin7-1, RayBio Technology) and mouse serum concentration of aldosterone (diluted 2-fold, #AB136933-1X, Abcam) or total renin (diluted 10-fold, #EMREN1, Thermo-Scientific) was quantified by ELISA as per the manufacturer's instructions.

Western Blot

Total protein was extracted from frozen tissue by mechanical homogenization (QIAGEN Tissue Lyser) in Universal Lysis Buffer (50 mM Tris·HCl (pH 7.5), 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 25 mM NaF, 25 mM β-glycerolphosphate, protease inhibitor mixture [Calbiochem]), centrifuged at 10,000xg to pellet cell membrane and quantified using the BCA assay (Pierce).

Denatured protein (40µg) was run on 4-15% SDS-PAGE gel (BioRad) for 45min at 150v before protein was transferred to PVDF (TransBlot Turbo Transfer System, BioRad). Membranes were blocked (5% skim milk in TBS-0.05% Tween) for 1h before being incubated overnight at 4°C with anti-Renin (1:500; Invitrogen, PA5-102432) or anti-Gapdh (1:1000 CST #2110S). Membranes were then washed 3 times (5 mins) with TBS-0.1% Tween before secondary antibody incubation (1:1000; CST 7074P2) at room temperature for 1h. After 3 further washes, membranes were incubated in ECL (Clarity™ Western ECL Substrate) for 5 mins then chemiluminescent bands visualized (iBright, BioRad). Renin band density was quantified using Image J [26] and normalized to Gapdh band density as a loading control.

Statistics

Statistical analyses were performed by GraphPad Prism version 9.2.0. $P<0.05$ was considered significant. Data were tested for normality and statistical tests (indicated in figure legends) chosen according to experimental design. A two-sided P value was calculated for all experiments.

Results

As our previous paper [14] used a commercial galectin-7 (Biovision) for the *in vivo* mouse studies and here we used an in-house purified protein (WEHI), we initially verified its in vivo activity. After 6 hours the serum concentration of the WEHI galectin-7 was significantly higher than the commercial galectin-7 (Figure 1A) but the serum retention was no different at any subsequent time-point (Figure 1A). Administration of Biovision galectin-7 to pregnant mice from E8-12 induces hypertension [14] and likewise the WEHI galectin-7 induced hypertension at E15-16 (Figure 1B). Galectin-7 treatment did not affect fetal (Figure 1C) or placental weight (Figure 1D) at E18, however the fetal:placental ratio was significantly increased (Figure 1E). Galectin-7 treated placentas showed a reduction in total area of the labyrinth zone associated with increased junctional zone area (Figure 1F&G), and a significant decrease in the labyrinth:junctional ratio (Figure 1H).

We previously showed sustained (E8-12) galectin-7 treatment dysregulated tissue RAS mRNA expression [14], however whether galectin-7 directly regulates RAS gene expression is unknown. We found a single dose of galectin-7 significantly decreased placental *Agt* expression after 8h and increased placental *Renin* expression after 16h (Figure 2A). In the decidua, galectin-7 treatment significantly increased *Agt* expression after 16h (Figure 2B). Kidney *Renin* expression was significantly increased at 8h (Figure 2C). At the protein level, active renin (37kDa) was found only in the placenta (Figure 2D-F). Daily treatment of galectin-7 from E8-12 had no effect on placental active renin expression (Figure 2D) but significantly reduced placental expression of a ~55-60kDa band, likely representing an inactive form of renin [28, 29]. Galectin-7 treatment had no effect on the large molecular weight renin in the decidua or kidney (Figure 2E,F).

In human placenta, we have previously shown that galectin-7 treatment significantly reduces placental AGT production [14]. Galectin-7 treatment for 16h significantly reduced placental *AGT* mRNA in placentas collected from pregnancies 7-11 weeks gestation but significantly increased placental *AGT* mRNA in placentas from 12+ weeks gestation (Figure 3A). There was no significant effect on decidual RAS mRNA production (Figure 3B).

To determine whether galectin-7 affects the circulating RAAS we measured circulating aldosterone and total renin in pregnant mice. Galectin-7 treatment significantly reduced circulating aldosterone at E13, but levels were restored to control at E17 (Figure 4A). There was no effect of galectin-7 on total renin (Figure 4B), but the aldosterone:renin (total) ratio was significantly reduced at E13 (Figure 4C).

To determine the mechanism by which galectin-7 may cause ROS accumulation the effect of galectin-7 on NADPH oxidase complex factors (Figure 5A/D), nitric oxidase synthases (Figure 5B) and markers of oxidative stress (Figure 5C/E) was investigated in human and murine placenta. We found galectin-7

218 treatment for 16h significantly increased human placental mRNA levels of *CYBA* (Figure 5A), *ICAM1* and
219 *VCAM1* (Figure 5C) and mouse placental mRNA levels of *Cyba*, *Cybb* (Figure 5D) and *Icam1* (Figure 5E).

220 **Discussion**

221 Here we show elevated galectin-7 directly regulated placental angiotensinogen and NADPH oxidase gene
222 expression in human and murine placenta. In mice, galectin-7 directly regulated placental, decidual and
223 kidney angiotensinogen and renin production and suppressed circulating aldosterone levels. In humans,
224 galectin-7 altered placental production of angiotensinogen in a gestation week dependent manner. In
225 both humans and mice, galectin-7 also increased placental production of the key NADPH oxidase complex
226 factor *CYBA* and the marker of oxidative stress *ICAM1*. Altogether this study demonstrates that the
227 elevated placental galectin-7 seen in women with preeclampsia [12, 14] likely drives preeclampsia by
228 dysregulating homeostasis in multiple pathways, including tissue RASs, the circulating RAAS and NADPH
229 oxidases.
230 The specific role of the RAS in preeclampsia has been difficult to study due to a lack of models that
231 demonstrate alterations to RAS homeostasis without interventions to silence/overexpress specific RAS
232 factors or surgically reducing uteroplacental perfusion. To our knowledge this model of elevated galectin-
233 7 is the only mouse model of preeclampsia to show direct regulation of RAS specific factors. In
234 preeclampsia, circulating active renin, Ang II and aldosterone are reduced [15]. In this mouse model of
235 preeclampsia we previously showed alterations to tissue RAS mRNA, including reduced placental
236 Angiotensinogen and reduced decidual renin following 5 days of galectin-7 treatment [14]. Here we
237 further show reduced placental renin protein and circulating aldosterone following 5 days of galectin-7
238 treatment. Although we saw a change in only the high molecular weight placental renin it is probable that
239 placental-released prorenin would be cleaved whilst in circulation [28]. In an uncomplicated pregnancy,
240 the RAAS supports the increasing blood volume by increasing aldosterone which increases distal sodium

241 reabsorption [30]. In preeclampsia however, circulating aldosterone is reduced [15, 31-33]. AngII
242 promotes aldosterone production [34], thus the reduced placental angiotensinogen production observed
243 in response to galectin-7 treatment both here and in [14] likely impacts aldosterone production, driving
244 the reduced circulating aldosterone seen in this mouse model of preeclampsia at E13.
245 Placental oxidative stress is a key driver of cytokine and anti-angiogenic factor release into maternal
246 circulation which leads to endothelial dysfunction. Galectin-7 causes ROS accumulation in bladder cancer
247 cell lines following treatment with cis-diamminedichloroplatinum [23] and galectin-7 drives VCAM1
248 production in human endometrial epithelial cells [35]: ICAM1/VCAM1 are induced by NADPH oxidase
249 activation [20]. We were interested therefore to determine whether galectin-7 directly disrupted
250 placental ROS production. Here we found increased galectin-7 increased the NADPH oxidase component
251 *CYBA*, also known as p22phox, in human and murine placentas. CYBA is increased in the placenta from
252 preeclamptic pregnancies [36, 37]. CYBA is a membrane protein that combines with CYBB
253 (gp91phox/NOX2) to create cytochrome b558, the catalytical core of the NADPH oxidase enzyme.
254 Following stimulation, cytochrome b558 translocates to the membrane causing the release of large
255 amounts of superoxide. Overexpression of CYBA in mice increases intracellular superoxide and H2O2
256 production [38-40], suggesting that elevated placental CYBA may cause ROS accumulation. Certainly, we
257 found galectin-7 increased placental production of *ICAM1* and *VCAM1*, providing evidence of NADPH
258 oxidase activation. Unlike AGT, galectin-7 regulation of *CYBA*, *ICAM* and *VCAM1* was not gestation week
259 dependent, emphasizing that galectin-7 dysregulation of ROS homeostasis in the placenta is independent
260 of its alterations to the RAS. Changes to both pathways are likely required to induce preeclampsia.
261 Here we found that galectin-7 dysregulated homeostasis in both the RAS and NADPH oxidase pathways,
262 suggesting that elevated galectin-7 would disrupt placental RAS and ROS. It should be noted that the
263 numbers of mice used in this study were small and future studies should include larger groups of mice.
264 Overall, data presented here and in previous studies [14] suggests galectin-7 drives hypertension in

preeclampsia via its dysregulation of multiple pathways in the placenta, resulting in altered RAAS, placental ROS accumulation, placental inflammation and placental release of anti-angiogenic factors, including sFlt-1 [14]. Galectin-7 may therefore be a therapeutic target to improve placental function and prevent preeclampsia.

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References

[1] G.J. Burton, C.W. Redman, J.M. Roberts, A. Moffett, Pre-eclampsia: pathophysiology and clinical implications, BMJ 366 (2019) I2381.

[2] **L. Poon**, **A. Shennan**, J.A. Hyett, A. Kapur, E. Hadar, H. Divakar, F. McAuliffe, F. da Silva Costa, P. von Dadelszen, H.D. McIntyre, A.B. Kihara, *G.C. Di Renzo*, *R. Romero*, *M. D'Alton*, *V. Berghella*, *K.H. Nicolaides*, *M. Hod*, **The International Federation of Gynecology and Obstetrics (FIGO) initiative on pre-eclampsia**, International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics **145 S1** (**2019**) **1**.

[3] C. Ostyon, J. Stanley, P. Barker, Potential targets for the treatment of preeclampsia, Expert Opin Ther Targets 19 (2015) 15117-1530.

[4] A. Tranquilli, G. Dekker, L. Magee, J. Roberts, B.M. Sibai, W. Steyn, G.G. Zeeman, M.A. Brown, The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP, Pregnancy Hypertension 4(2) (2014) 97-104.
[5] C.W.G. Redman, A.C. Staff, J.M. Roberts, Syncytiotrophoblast stress in preeclampsia: the convergence point for multiple pathways, Am J Obstet Gynecol doi.org/10.1016/j.ajog.2020.09.047 (2020).
[6] E. Menkhorst, N.G. Than, U. Jeschke, G. Barrientos, L. Szereday, G. Dveksler, S.M. Blois, Medawar's PostEra: Galectins Emerged as Key Players During Fetal-Maternal Glycoimmune Adaptation, Front Immunol 12(5152) (2021).
[7] H. Hao, M. He, J. Li, Y. Zhoy, J. Dang, F. Li, M. Yang, D. Deng, Upregulation of the Tim-3/Gal-9 pathway and correlation with the development of preeclampsia, European Journal of Obstetrics, Gynecology and Reproductive Biology 194 (2015) 85-91.
[8] N. Freitag, I. Tirado-Gonzalez, G. Barrientos, F. Herse, V.L.J.L. Thijssen, S.M. Weedon-Fekjaer, H. Schulz, G. Wallukat, B.F. Klapp, T. Nevers, S. Sharma, A.C. Staff, R. Dechend, S.M. Blois, Interfering with Gal-1-mediated angiogenesis contributes to the pathogenesis of preeclampsia, Proceedings of the National Academy of Sciences 110 (2013) 11451-11456.
[9] Z.H. Li, L.L. Wang, H. Liu, K.P. Muyayalo, X.B. Huang, G. Mor, A.H. Lao, Galectin-9 alleviates LPS-induced preeclampsia-like impairment in rats via switching decidual macrophage polarization to M2 subtype, Frontiers in Immunology 9 (2019) 3142.
[10] N.G. Than, O. Erez, D.E. Wildman, A.L. Tarca, S.S. Edwin, A. Abbas, J. Hotra, J.P. Kusanovic, F. Gotsch, S.S. Hassan, J. Espinoza, Z. Papp, R. Romero, Severe preeclampsia is characterized by increased placental expression of galectin-1, Journal of maternal-fetal and neonatal medicine 21(7) (2008) 429-442.
[11] N.G. Than, R. Romero, A. Balogh, E. Karpati, S.A. Mastrolia, O. Staretz-Chacham, S. Hahn, O. Erez, Z. Papp, C.J. Kim, Galectins: double-edged swords in the cross-roads of pregnancy complications and female reproductive tract inflammation and neoplasia, Journal of Pathology and Translational Medicine 49 (2015) 181-208.
[12] E. Menkhorst, K. Koga, M. Van Sinderen, E. Dimitriadis, Galectin-7 serum levels are altered prior to the onset of pre-eclampsia, Placenta 35 (2014) 281-285.
[13] L. Unverdorben, U. Jesche, L. Santoso, S. Hofmann, C. Kuhn, P. Arck, S. Hutter, Comparative analyses on expression of galectins1-4, 7-10 and 12 in first trimester placenta, decidua and isolated trophoblast cells *in vitro*, Histol Histopathol 31 (2016) 1095-1111.
[14] E. Menkhorst, W. Zhou, L. Santos, S. Delforce, T. So, K. Rainczuk, H. Loke, A. Syngelaki, S. Varshney, N. Williamson, K. Pringle, M.J. Young, K. Nicolaides, Y. St-Pierre, E. Dimitriadis, Galectin-7 impairs placentation and causes preeclampsia features in mice, Hypertension 76(4) (2020) 1185-1194.
[15] E.R. Lumbers, S.J. Delforce, A.L. Arthurs, K.G. Pringle, Causes and Consequences of the Dysregulated Maternal Renin-Angiotensin System in Preeclampsia, Frontiers in Endocrinology 10(563) (2019).
[16] F. Herse, R. Dechend, N. Harsem, G. Wallukat, Dysregulation of the circulating and tissue-based renin-angiotensin system in preeclampsia, Hypertension 49 (2007) 604-611.
[17] L. Anton, K. Brosnihan, Systemic and uteroplacental renin-angiotensin system in normal and pre-eclamptic pregnancies, Ther Adv Cardiovasc Dis 2 (2008) 349-362.
[18] S. Ito, A. Itakura, Y. Ohno, M. Nomura, T. Senga, T. Nagasaka, S. Mizutani, Possible activation of the renin-angiotensin system in the feto-placental unit in preeclampsia, J Clin Endocrinol Metab 87 (2002) 1871-1878.
[19] L. Anton, D. Merrill, L. Neves, D. Diz, J. Corthorn, G. Valdes, K. Stovall, P. Gallagher, C. Moorefield, C. Gruver, K. Brosnihan, The uterine placental bed Renin-Angiotensin system in normal and preeclamptic pregnancy, Endocrinology 150 (2009) 4316-4325.
[20] A. Konior, A. Schramm, M. Czesnikiewicz-Guzik, T.J. Guzik, NADPH oxidases in vascular pathology, Antioxidants & redox signaling 20(17) (2014) 2794-2814.

[21] P. Guerby, O. Tasta, A. Swiader, F. Pont, E. Bujold, O. Parant, C. Vayssiere, R. Salvayre, A. Negre-Salvayre, Role of oxidative stress in the dysfunction of the placental endothelial nitric oxide synthase in preeclampsia, Redox Biol 40 (2021) 101861.
[22] G. Gallo, M. Volpe, C. Savoia, Endothelial Dysfunction in Hypertension: Current Concepts and Clinical Implications, Front Med (Lausanne) 8 (2021) 798958.
[23] Y. Matsui, S. Ueda, J. Watanabe, I. Kuwabara, O. Ogawa, H. Nishiyama, Sensitizing Effect of Galectin-7 in Urothelial Cancer to Cisplatin through the Accumulation of Intracellular Reactive Oxygen Species, Cancer Research 67(3) (2007) 1212.
[24] M. Labrie, M.C. Vladoiu, B.G. Leclerc, A.A. Grosset, L. Gaboury, J. Stagg, Y. St-Pierre, A mutation in the carbohydrate recognition domain drives a phenotypic switch in the role of galectin-7 in prostate cancer, PLoS ONE 10(7) (2015) e0131307.
[25] A.K. Edwards, J. Janzen-Pang, A. Peng, C. Tayade, A. Carniato, A. Yamada, P. Lima, Tse, Microscopic anatomy of the pregnant mouse uterus during gestation, in: B.A. Croy, A.T. Yamada, F.J. DeMayo, S.L. Adamson (Eds.), The guide to the investigation of mouse pregnancy, Academic Press2014, pp. 43-67.
[26] M.A. Garcia-Gonzalez, P. Outeda, Q. Zhou, F. Zhou, L.F. Menezes, F. Qian, D.L. Huso, G.G. Germino, K.B. Piontek, T. Watnick, Pkd1 and Pkd2 are required for normal placental development, PLoS ONE 5 (2010) e12821.
[27] **A. Winship**, **K. Koga**, **E. Menkhorst**, M. Van Sinderen, K. Rainczuk, M. Nagai, C. Cuman, J. Yap, J.-G. Zhang, D. Simmons, M.J. Young, E. Dimitriadis, **Interleukin-11 alters placentation and causes preeclampsia features in mice**, **PNAS 112**(52) (**2015**) **15928**.
[28] G.M. Acker, F.X. Galen, C. Devaux, S. Foote, E. Papernik, A. Pesty, J. Menard, P. Corvol, Human chorionic cells in primary culture: a model for renin biosynthesis, J Clin Endocrinol Metab 55(5) (1982) 902-9.
[29] B.J. Leckie, A. McConnell, A renin inhibitor from rabbit kidney: conversion of a large inactive renin to a smaller active enzyme, Circ Res 36(4) (1975) 513-9.
[30] E.R. Lumbers, S.J. Delforce, A.L. Arthurs, K.G. Pringle, Causes and Consequences of the Dysregulated Maternal Renin-Angiotensin System in Preeclampsia, Front Endocrinol (Lausanne) 10 (2019) 563-563.
[31] G. Escher, M. Cristiano, M. Causevic, M. Baumann, F.J. Frey, D. Surbek, M.G. Mohaupt, High aldosterone-to-renin variants of CYP11B2 and pregnancy outcome, Nephrology Dialysis Transplantation 24(6) (2009) 1870-1875.
[32] M.N. Uddin, D. Horvat, R.O. Jones, M.R. Beeram, D.C. Zawieja, L. Perger, D.C.C. Sprague, T.J. Kuehl, Suppression of aldosterone and progesterone in preeclampsia, The Journal of Maternal-Fetal & Neonatal Medicine 28(11) (2015) 1296-1301.
[33] K. Shojaati, M. Causevic, B. Kadereit, B. Dick, J. Imobersteg, H. Schneider, E. Beinder, M. Kashiwagi, B.M. Frey, F.J. Frey, M.G. Mohaupt, Evidence for compromised aldosterone synthase enzyme activity in preeclampsia, Kidney International 66(6) (2004) 2322-2328.
[34] P. Gathiram, J. Moodley, The Role of the Renin-Angiotensin-Aldosterone System in Preeclampsia: a Review, Current Hypertension Reports 22(11) (2020) 89.
[35] E. Menkhorst, M. Griffith, M. Van Sinderen, K. Niven, E. Dimitriadis, Galectin-7 is elevated in endometroid (type 1) endometrial cancer and promotes cell migration, Oncology Letters 16 (2018) 4721-4728.
[36] E.A. Trifonova, T.V. Gabidulina, N.I. Ershov, V.N. Serebrova, A.Y. Vorozhishcheva, V.A. Stepanov, Analysis of the placental tissue transcriptome of normal and preeclampsia complicated pregnancies, Acta Naturae 6(2) (2014) 71-83.
[37] R. Dechend, C. Viedt, D.N. Müller, B. Ugele, R.P. Brandes, G. Wallukat, J.K. Park, J. Janke, P. Barta, J. Theuer, A. Fiebeler, V. Homuth, R. Dietz, H. Haller, J. Kreuzer, F.C. Luft, AT1 receptor agonistic antibodies from preeclamptic patients stimulate NADPH oxidase, Circulation 107(12) (2003) 1632-9.

[38] M.R. Manogue, J.R. Bennett, D.S. Holland, C.S. Choi, D.A. Drake, M.S. Taylor, D.S. Weber, Smooth muscle specific overexpression of p22phox potentiates carotid artery wall thickening in response to injury, Oxid Med Cell Longev 2015 (2015) 305686.
[39] D.S. Weber, P. Rocic, A.M. Mellis, K. Laude, A.N. Lyle, D.G. Harrison, K.K. Griendling, Angiotensin II-induced hypertrophy is potentiated in mice overexpressing p22phox in vascular smooth muscle, Am J Physiol Heart Circ Physiol 288(1) (2005) H37-42.
[40] C. Nagaraj, H.M. Haitchi, A. Heinemann, P.H. Howarth, A. Olschewski, L.M. Marsh, Increased Expression of p22phox Mediates Airway Hyperresponsiveness in an Experimental Model of Asthma, Antioxid Redox Signal 27(18) (2017) 1460-1472.

Figure legends

Figure 1. In-house produced galectin-7 (WEHI) induces hypertension and placental damage comparable to the commercial (Biovision) galectin-7 in mice. A. Serum concentration of galectin-7 produced by Biovision (○) or WEHI (●) following one subcutaneous injection of 400µg/kg to non-pregnant female mice (n=3/group); B-H. Blood pressure, placental morphology and fetal weight in mice following daily administration of 400µg/kg galectin-7 (○) or vehicle control (●) from embryonic day (E)8-12. B. Systolic blood pressure (sBP), C. Fetal weight at E18, D. Placenta & Decidua weight at E18, E. Fetal:placenta and decidua ratio at E18, F. Masson's trichrome stained placenta showing labyrinth (pink area in insert) and junctional zone (blue area in insert) area at E18, G. Area of labyrinth, junctional and decidual zones at E18, H. Labyrinth: junctional ratio at E18. Data shows mean±SEM from n=3-5 mice/ treatment group; B, E, G, H, student's t-test. Permission to re-use data (Biovision serum concentrations) in A from [14] granted by Wolters Kluwer Health, License Number 5257301002554.

Figure 2. A-C: Angiotensinogen and Renin mRNA and protein expression in mice. A-C. On E13 after 8 and 16h following a single dose of galectin-7 (○, 400µg/kg) or vehicle control (●). A. Placenta mRNA; B. Decidua mRNA; C. Kidney mRNA; D-F. At E13 after daily galectin-7 (○, 400µg/kg) or vehicle control (●) treatment from E8-E12. Each panel shows a representative Western Blot and graphed densitometry data. D. Placenta renin protein; E. Decidua renin protein; F. Kidney renin protein. h, hour; data shows mean±SEM from n=3-6 mice/treatment group; A-D, student's t-test.

Figure 3. *Angiotensinogen* and *Renin* mRNA expression in human placental villous explants (A) and decidua (B) following treatment for 16h with 1µg/ml galectin-7 (○) or vehicle control (●; Con). Data shows mean±SEM; n=4-7 primary villous explant culture experiments; A, student's t-test.

Figure 4. Circulating (serum) Aldosterone (A), Renin (B) and Renin:aldosterone ratio (C) in mice following daily administration of 400µg/kg galectin-7 (○) or vehicle control (●) from embryonic day (E)8-12; Data shows mean±SEM from n=5-6 mice/treatment group; A, C, student's t-test.

Figure 5. NADPH oxidase complex, NADPH oxidase synthase and oxidative stress gene expression in human (A-C) and mouse (D-E) placenta following galectin-7 treatment. A-C. Gene expression in human placental villous explants following treatment for 16h with 1µg/ml galectin-7 (○) or vehicle control (●; Con). D-E. Gene expression in mouse placenta at 8 and 16h after a single dose of galectin-7 (○, 400µg/kg) or vehicle control (●). h, hour; Data shows mean±SEM from n=5 primary villous explant culture experiments (A-C) or n=3-5 mice (D-E); student's t-test.

Supplementary Figure 1. Renin-angiotensin system component expression in mice at E13 at 8 and 16h after a single dose of galectin-7 (○, 400µg/kg) or vehicle control (●). A. Placenta mRNA; B. Decidua mRNA; C. Kidney mRNA. h, hour; Data shows mean±SEM from n=3-6 mice/treatment group; A-C, student's t-test.

Supplementary Figure 2. Renin-angiotensin system component expression in human placental villous explants (A) and decidua (B) following treatment for 16h with 1µg/ml galectin-7 (○) or vehicle control (●; Con). Data shows mean±SEM; n=4-7 primary villous explant culture experiments; A, student's t-test.

Figure 1

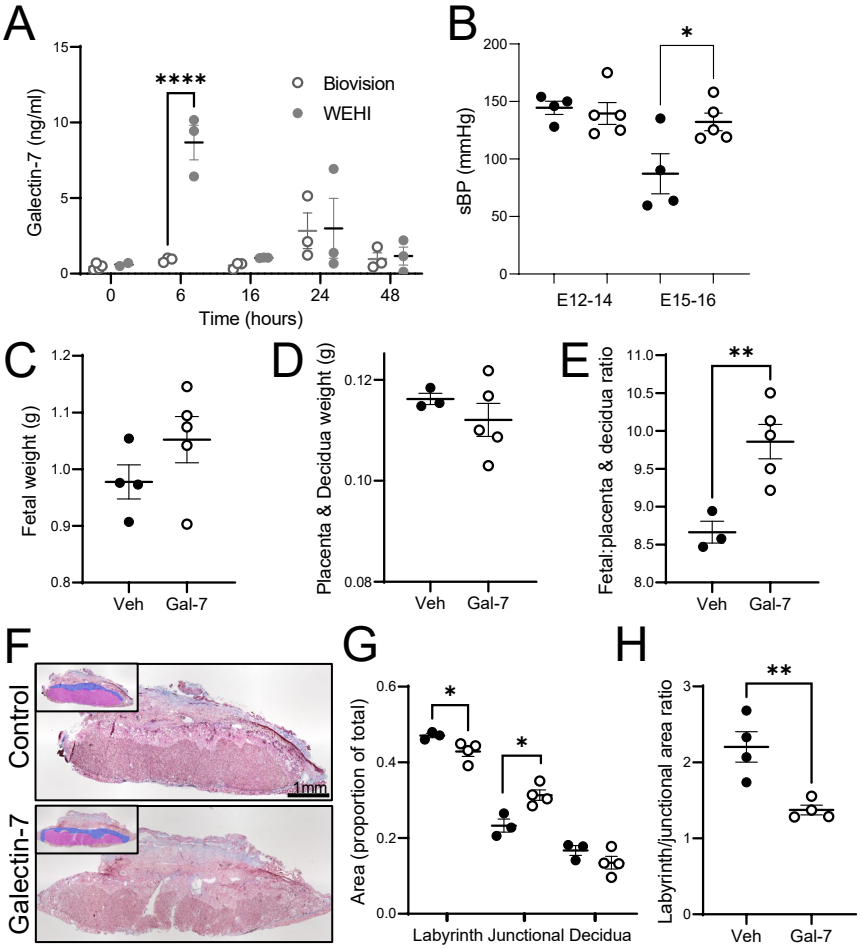

A
Galectin-7 (ng/ml)
****
Biovision
WEHI
Time (hours)
B
sBP (mmHg)
*
E12-14
E15-16
C
Fetal weight (g)
Veh
Gal-7
D
Placenta & Decidua weight (g)
Veh
Gal-7
E
Fetal:placenta & decidua ratio
**
Veh
Gal-7
F
Control
Galectin-7
1mm
G
Area (proportion of total)
*
*
Labyrinth
Junctional Decidua
H
Labyrinth/junctional area ratio
**
Veh
Gal-7

Figure 2.

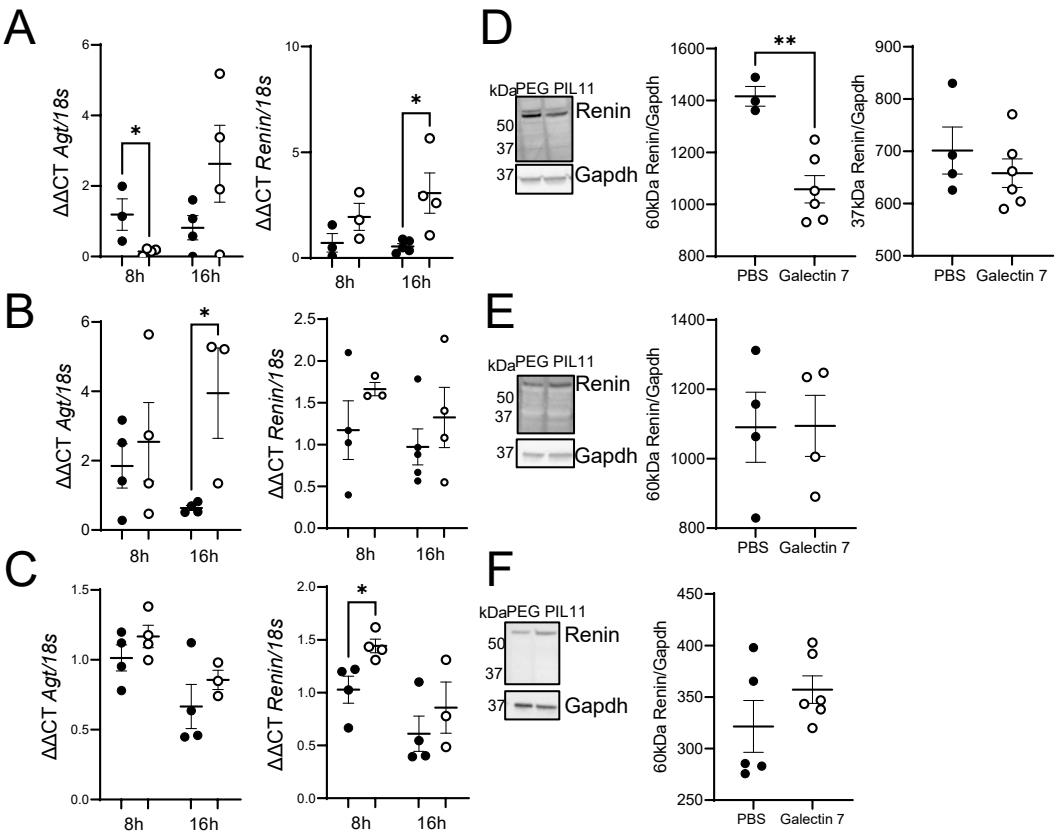

A
ΔΔCT Agt/18s
ΔΔCT Renin/18s
8h
16h
B
C
D
kDa
PEG PIL11
Renin
Gapdh
50
37
60kDa Renin/Gapdh
37kDa Renin/Gapdh
PBS
Galectin 7
E
F

Figure 3.

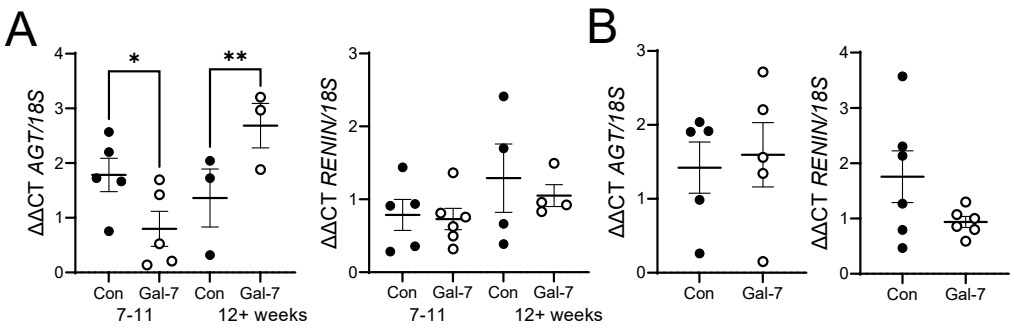


Figure 4.

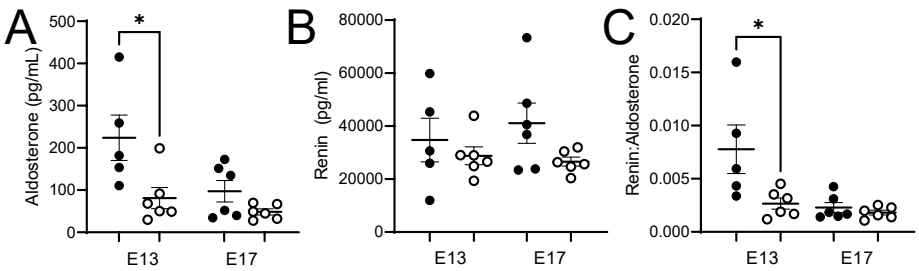
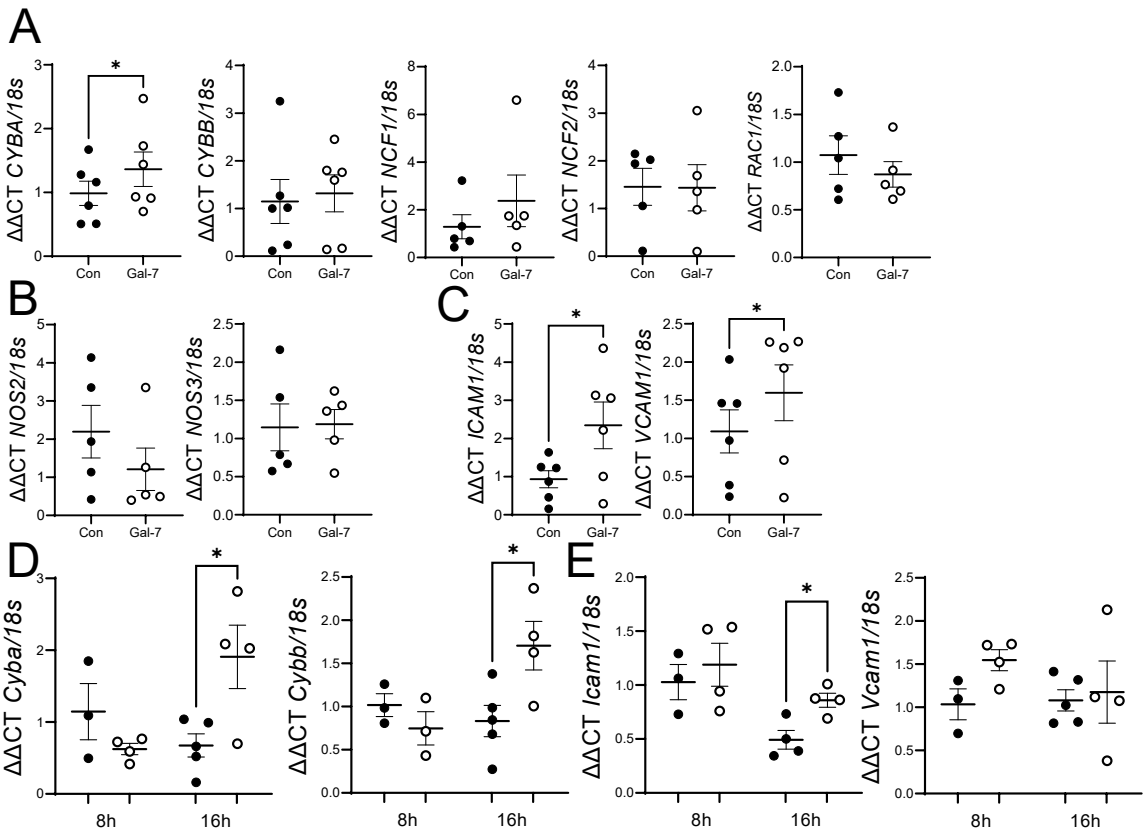
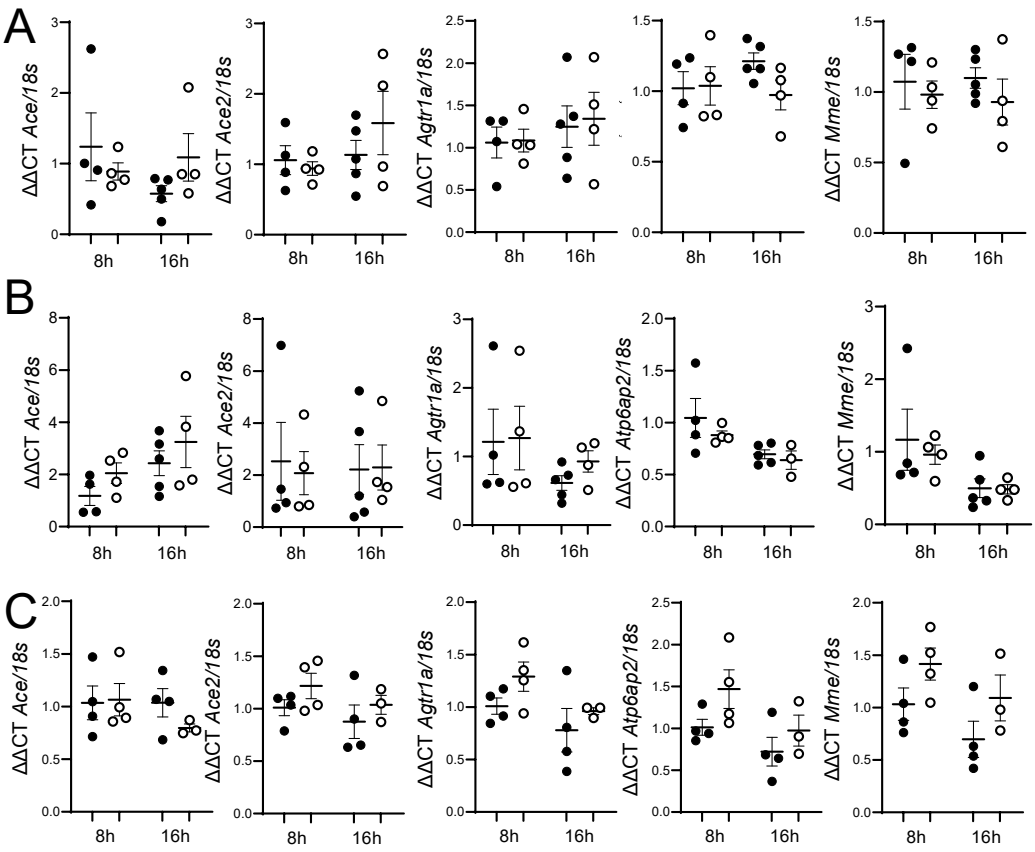

A
Aldosterone (pg/mL)
500
400
300
200
100
0
*
E13
E17
B
Renin (pg/ml)
80000
60000
40000
20000
0
E13
E17
C
Renin:Aldosterone
0.020
0.015
0.010
0.005
0.000
*
E13
E17

Figure 5.


A
ΔΔCT CYBA/18s
ΔΔCT CYBB/18s
ΔΔCT NCF1/18s
ΔΔCT NCF2/18s
ΔΔCT RAC1/18S
Con
Gal-7
B
ΔΔCT NOS2/18s
ΔΔCT NOS3/18s
C
ΔΔCT ICAM1/18s
ΔΔCT VCAM1/18s
D
ΔΔCT Cyba/18s
ΔΔCT Cybb/18s
8h
16h
E
ΔΔCT Icam1/18s
ΔΔCT Vcam1/18s

Supplementary Figure 1



Supplementary Figure 2.