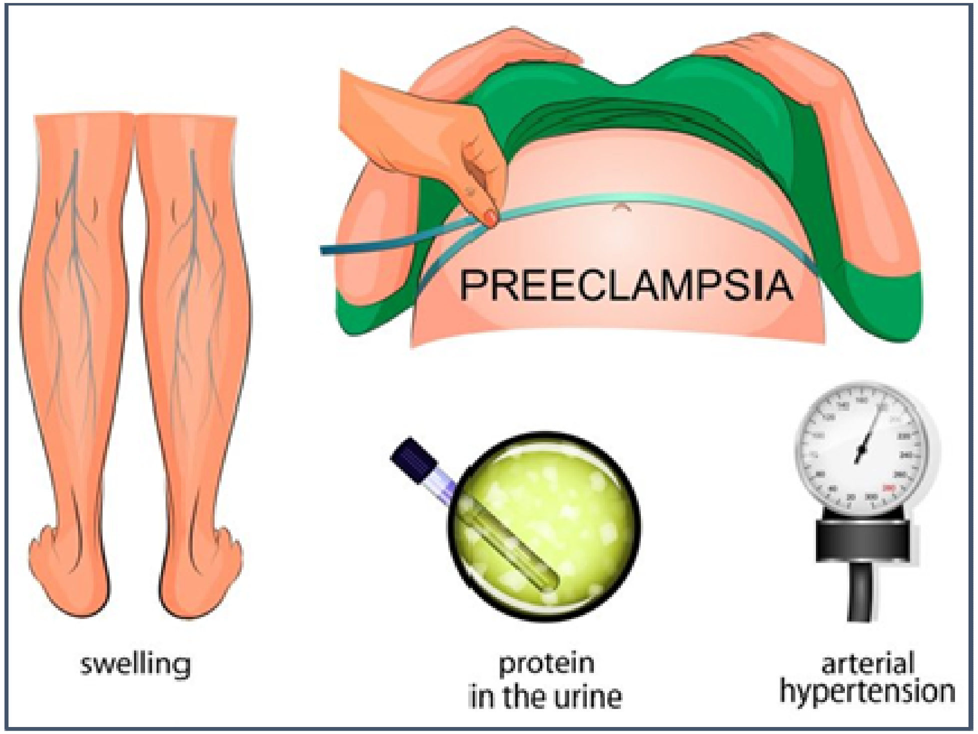
Modèles murins de la pré-éclampsie :
des visions différentes et complémentaires de la pathologie humaine
Murine models of preeclampsia:
bringing a different and complementary vision to the human disease

Daniel VAIMAN, Institut Cochin, U1016 INSERM, UMR8104 CNRS, Paris

Physiopathology of Preeclampsia



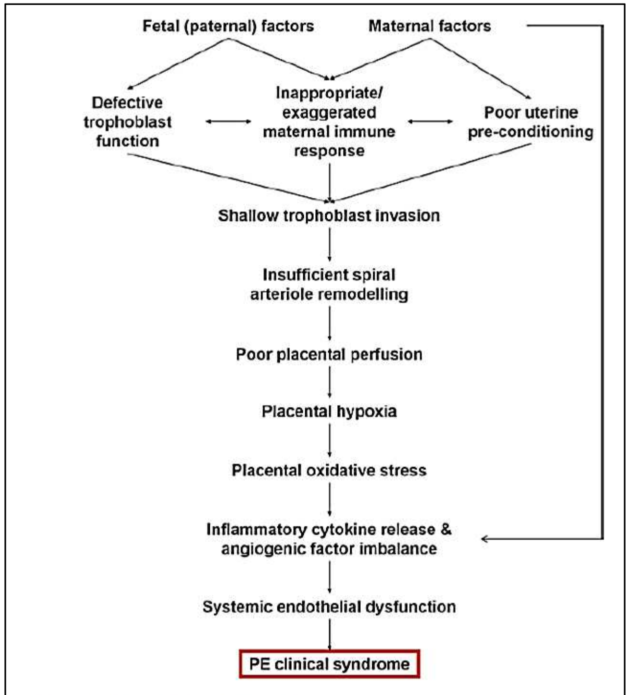
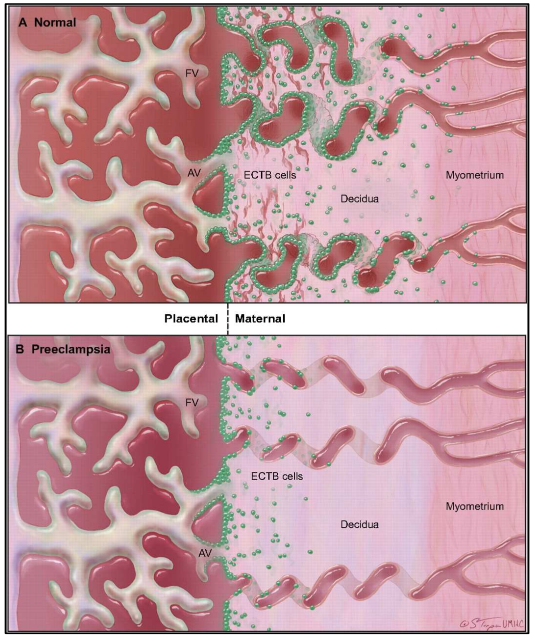
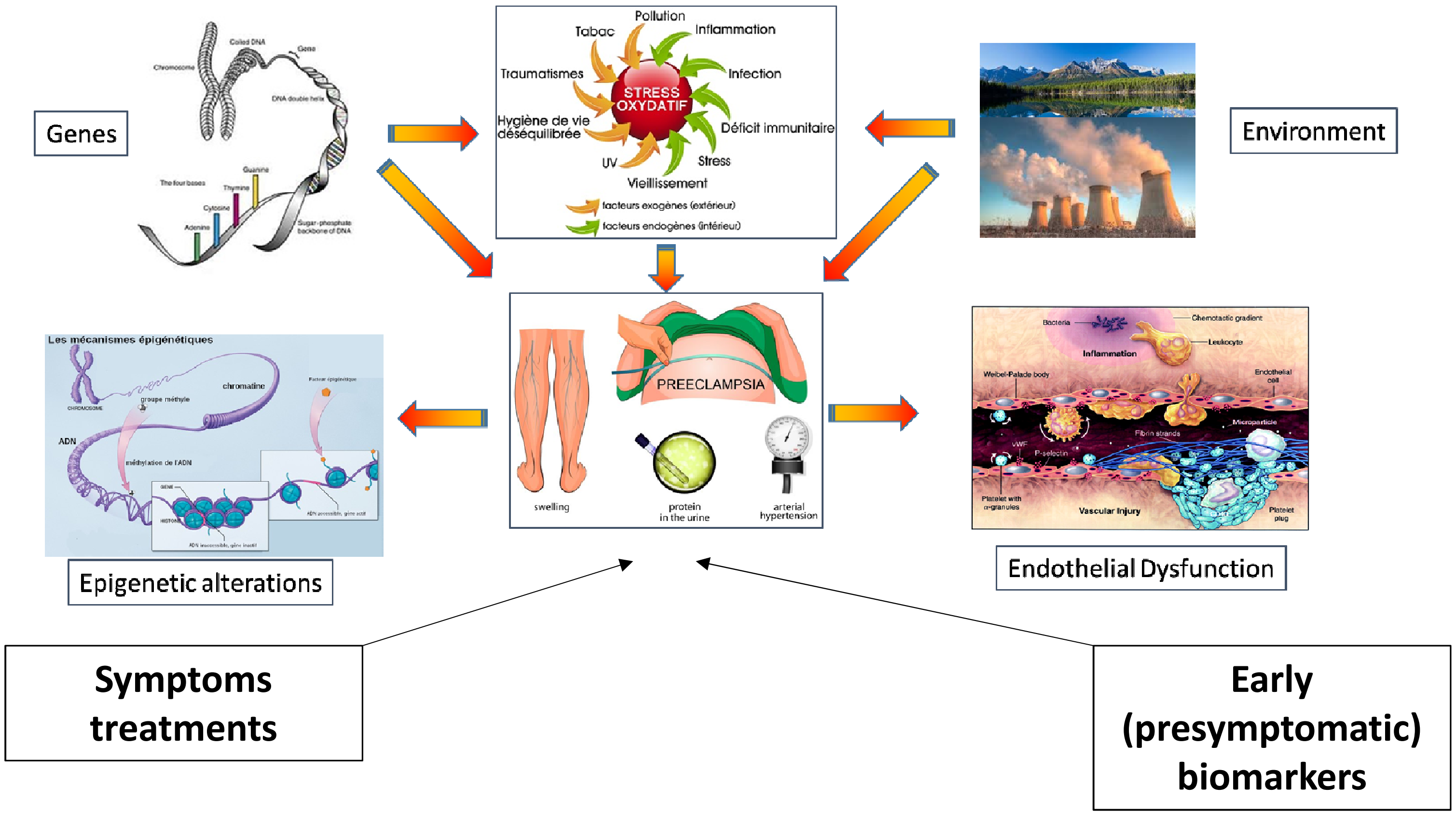


Fig. 1 Pathophysiology of preeclampsia



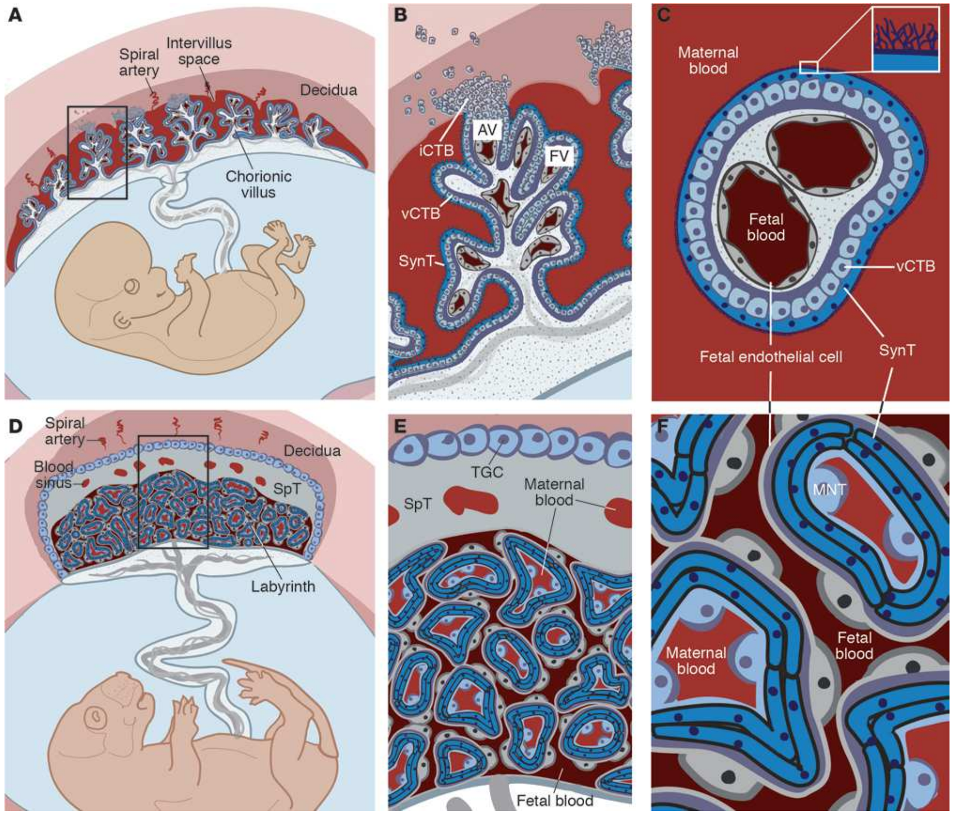
Padma Murthi and Cathy Vaillancourt (eds.), *Preeclampsia: Methods and Protocols*, Methods in Molecular Biology, vol. 1710, https://doi.org/10.1007/978-1-4939-7498-6_5, © Springer Science+Business Media LLC 2018
Pennington, K.A. *et al*, Dis. Mod. Mech., 5. 9-18, 2012

Reseach questions in preeclampsia



Mouse versus human placentation

- A discoïd shape, hemochorial. Three cell layers separting maternal and fetal circulations.

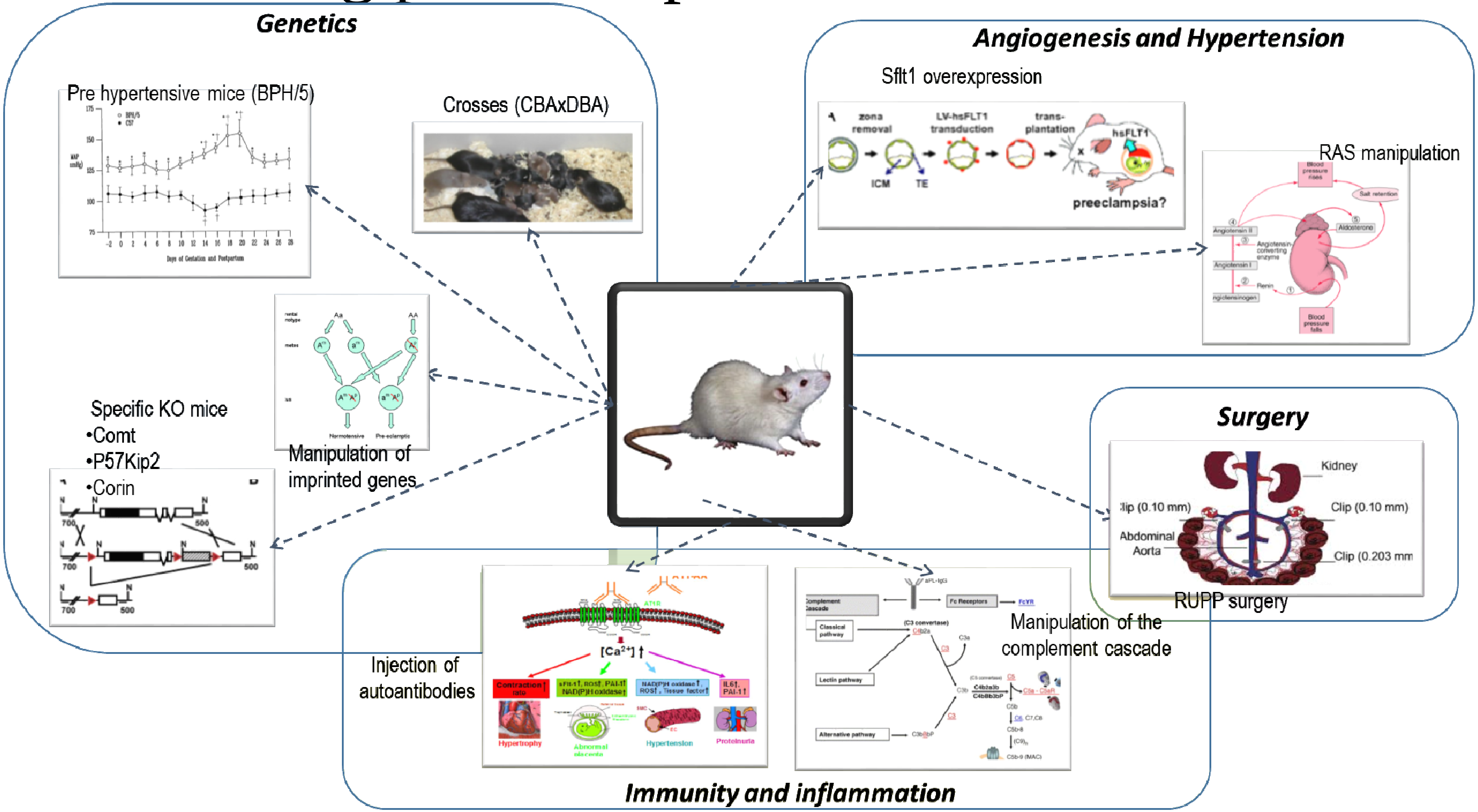


Maltepe, E. *et al*, J. Clin. Invest., 120. 1016-1025, 2010

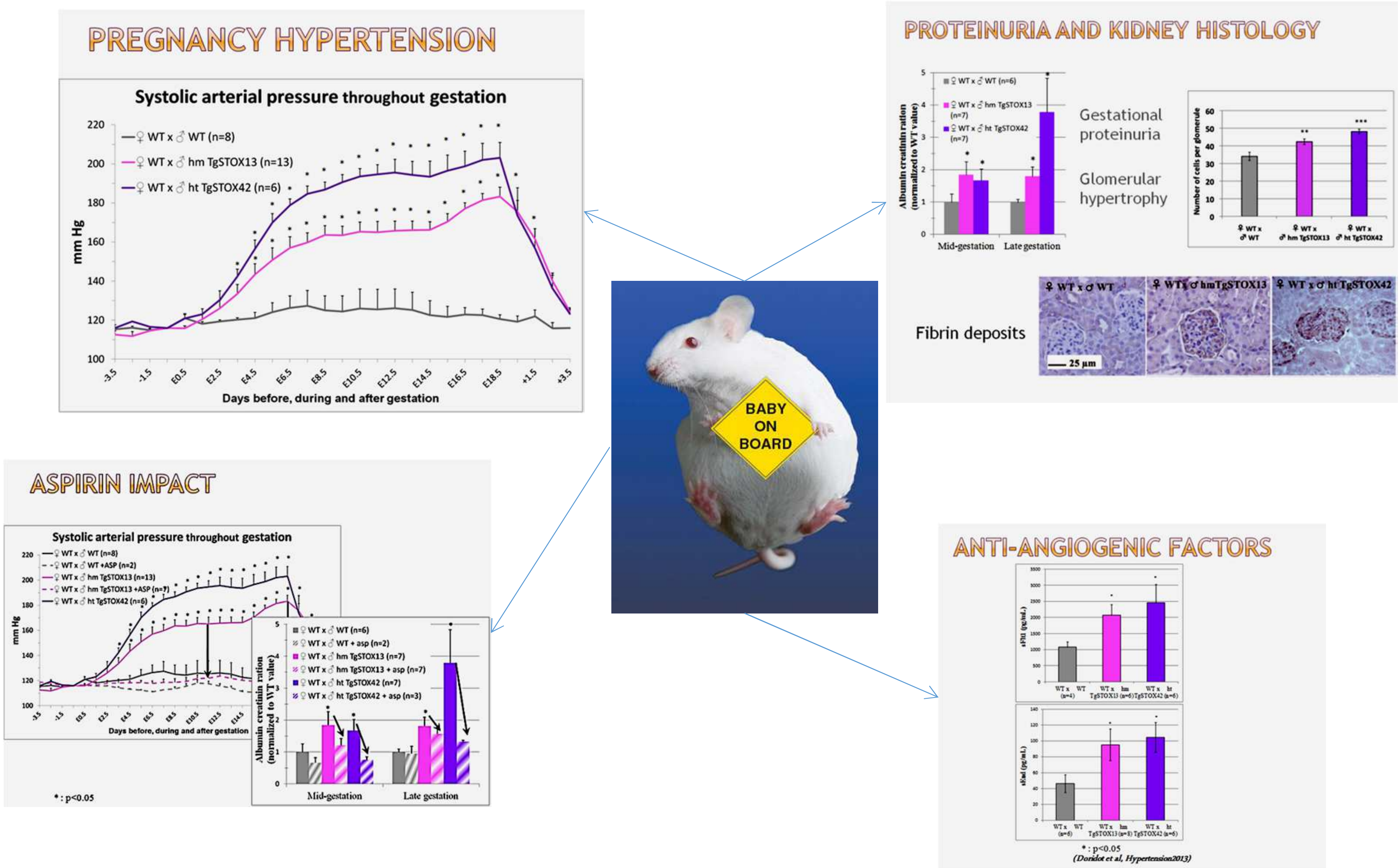
- The human placenta: monochorial, existence of placental villi in the intervillous space

- The murine placenta: Hemo-Trichorial, but the maternal blood accesses to the syncytiotrophoblast bilayer. Specific structure: junctional zone, labyrinth

Emulating preeclampsia in mice

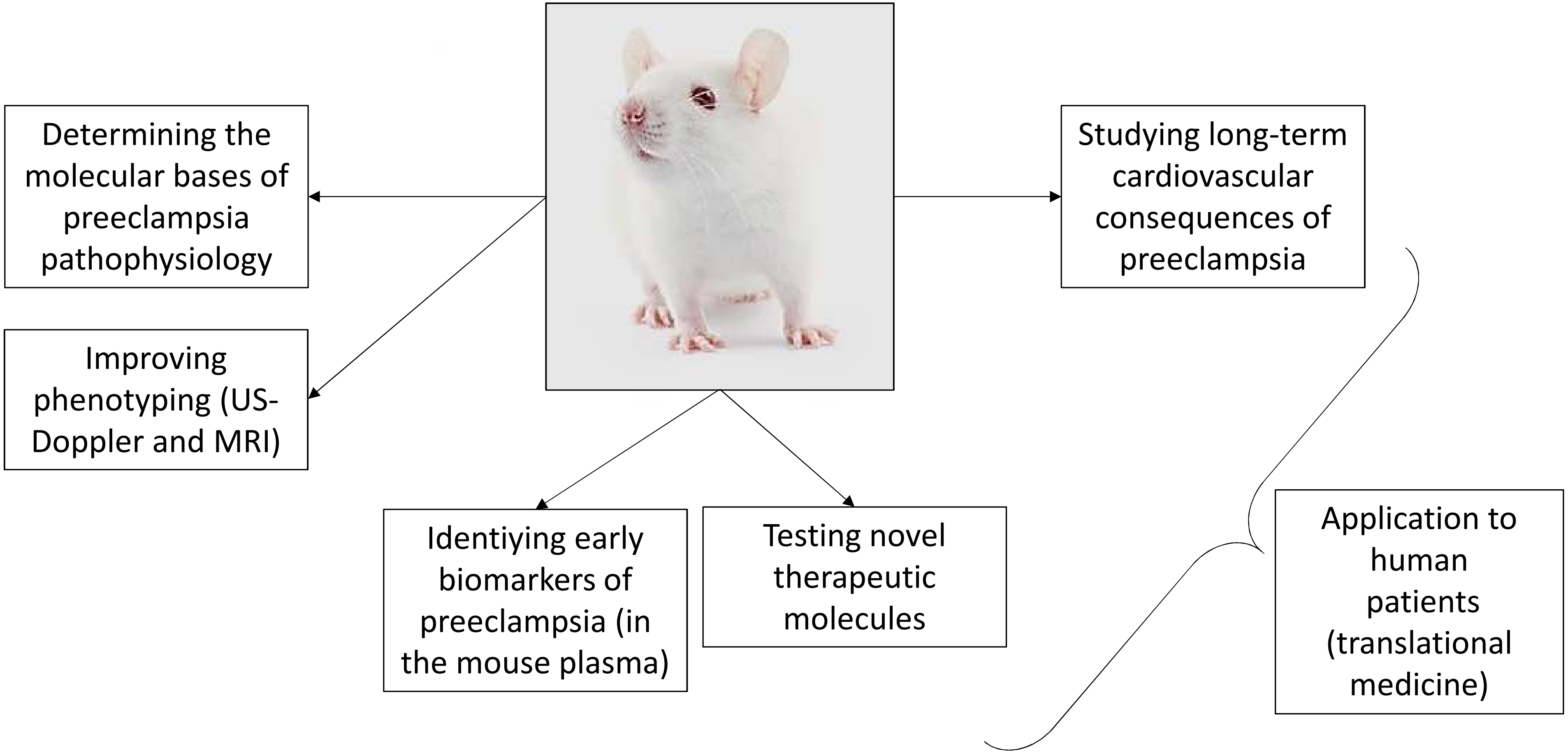


Foeto-placental expression of *STOX1* induces preeclampsia in mice



(Doridot et al, Hypertension, 2013)

Novel ongoing projects using preeclamptic STOX1 mice



Massive alterations of the placental tanscriptome affects especially mitochondrial genes

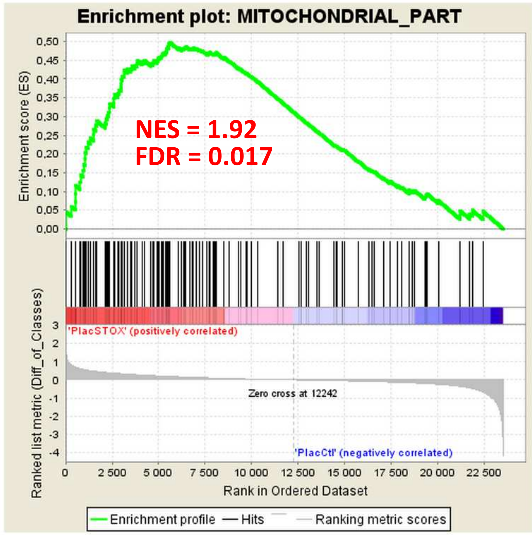
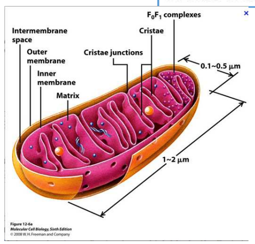
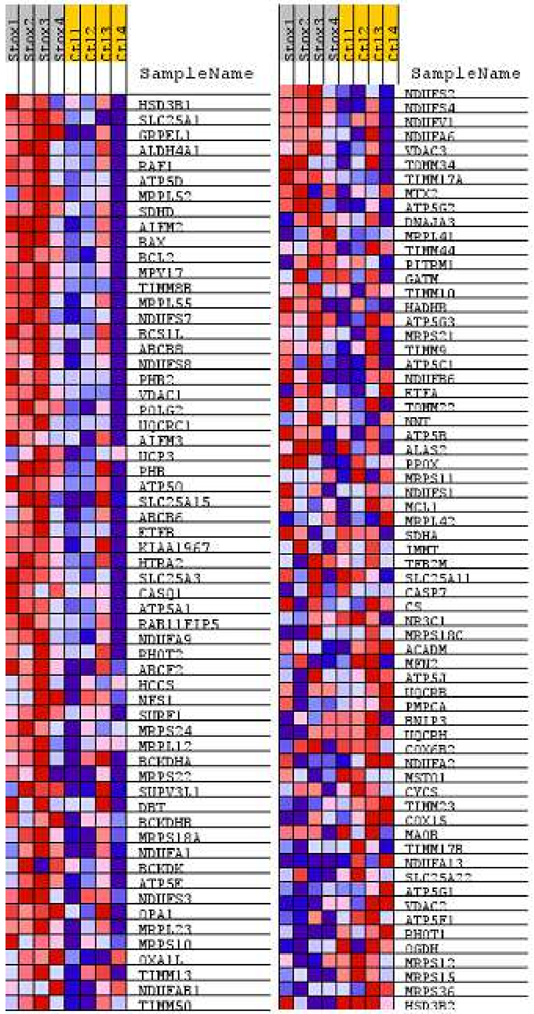


Fig 1: Enrichment plot: MITOCHONDRIAL_PART
Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List

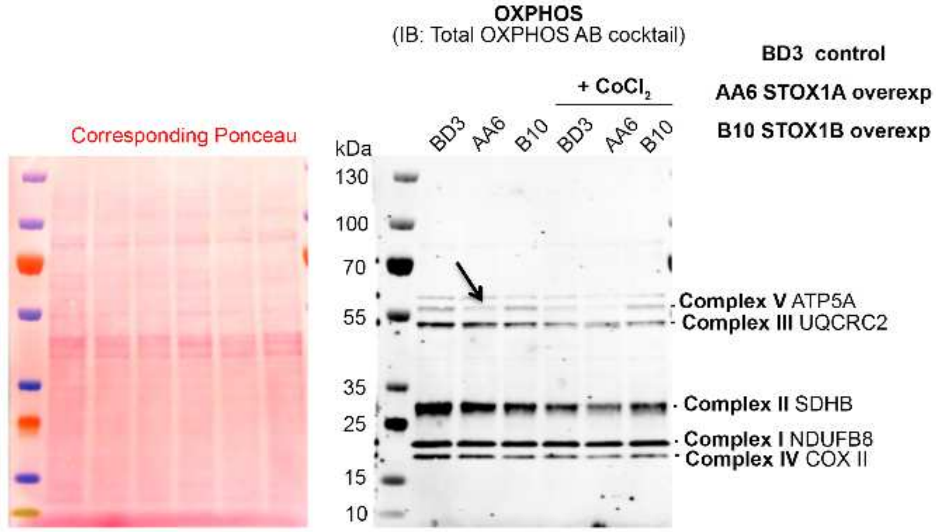




(Doridot et al, Antioxidant and Redox Signaling, 2014)

Specific alterations of mitochondrial complexes in *STOX1* overexpressing cells

STOX1A and STOX1B direct/indirect effect on OXPHOS complexes



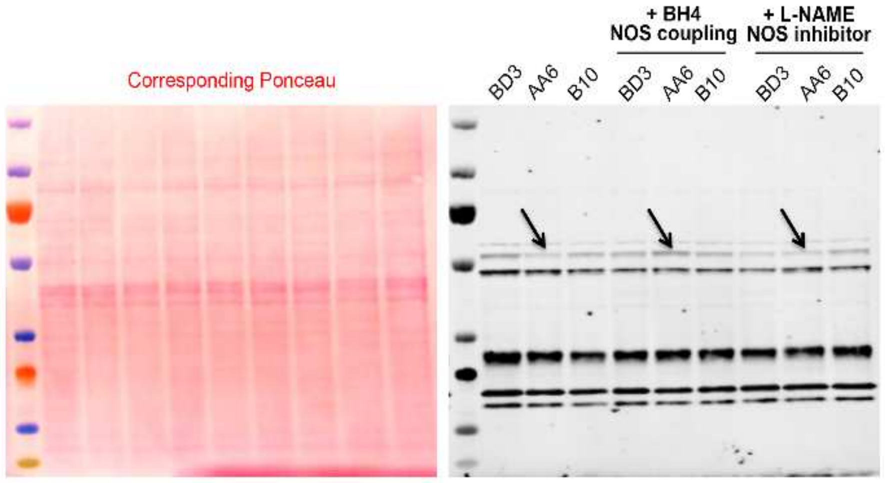
Complex I: no effect of STOX1A/STOX1B

Complex II: decrease due to STOX1A and STOX1B

Complex III:decrease due to STOX1A and STOX1B

Complex IV: decrease due to STOX1A and STOX1B

Complex V: decrease only due to STOX1A

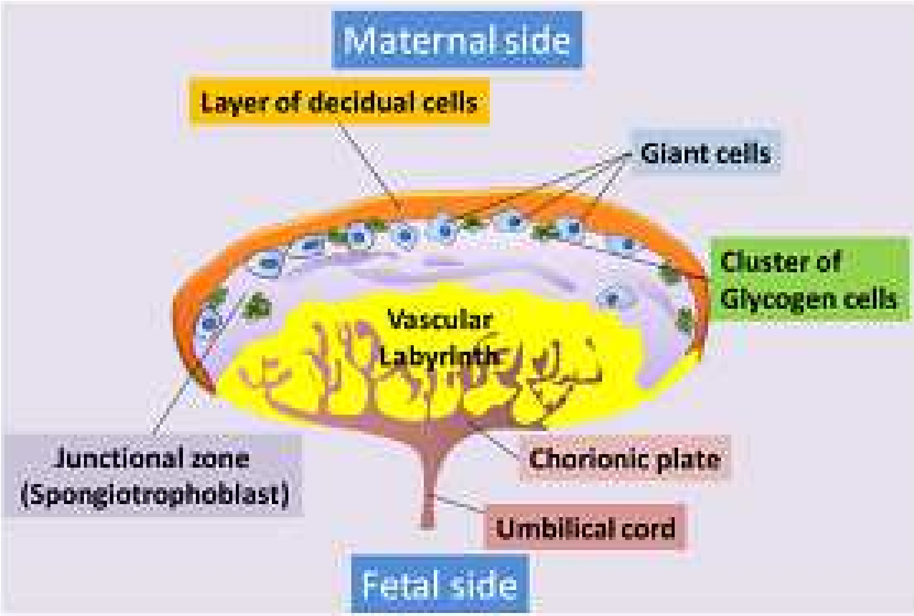


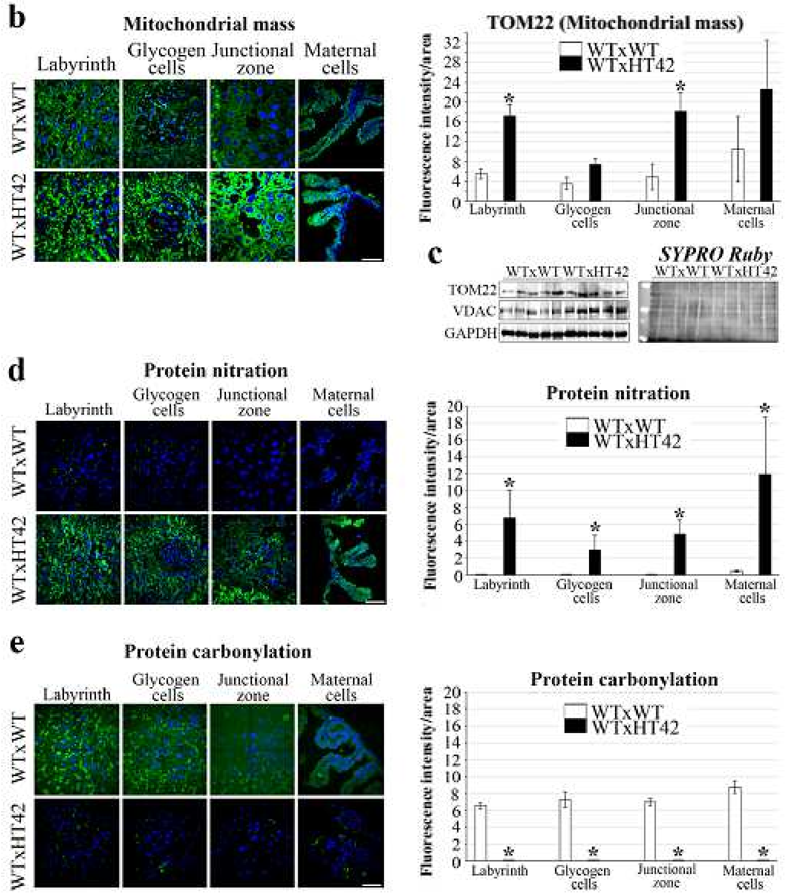
BH4 treatment induces Complex V increase in AA6 (STOX1A overexp)

L-NAME treatment modifies the OXPHOS complexes in all cells types

(Chatre, Ducat et al in preparation)

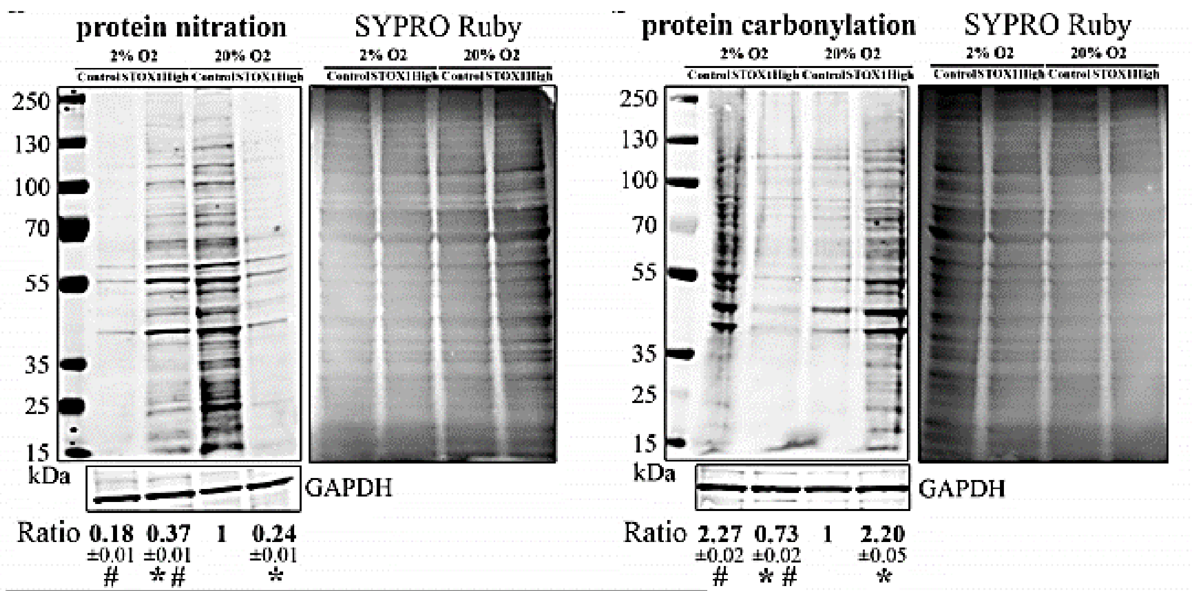
At the placental level the oxidative/nitrosative stress balance is perturbed



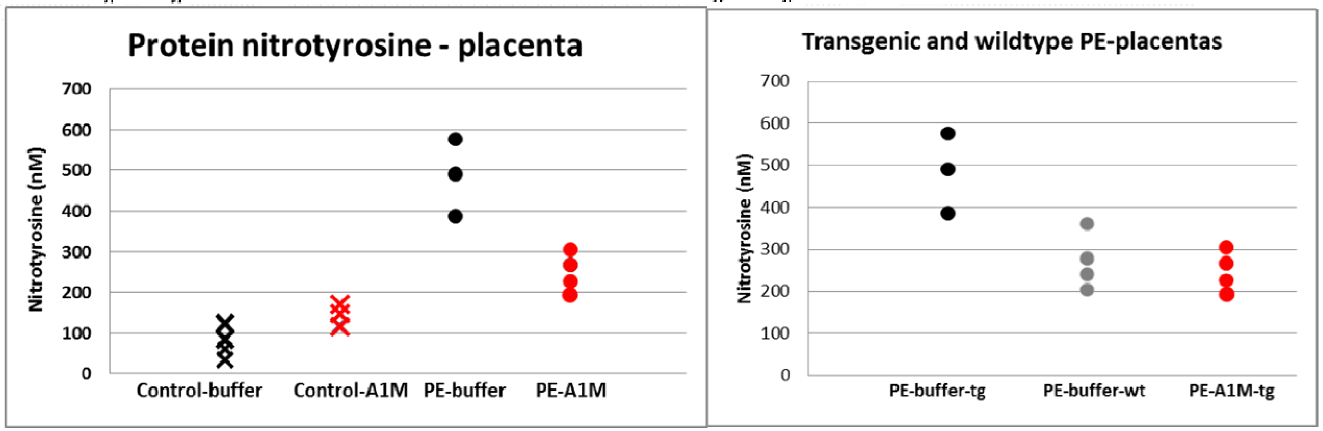


(Doridot et al, Antioxidant and Redox Signaling, 2014)

Oxidative / Nitrosative stress : a mirror image in cells

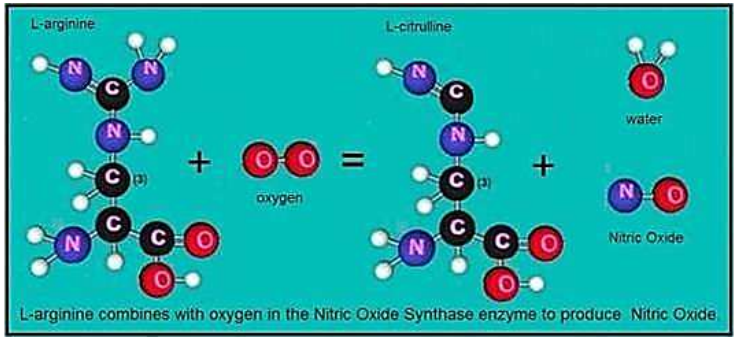


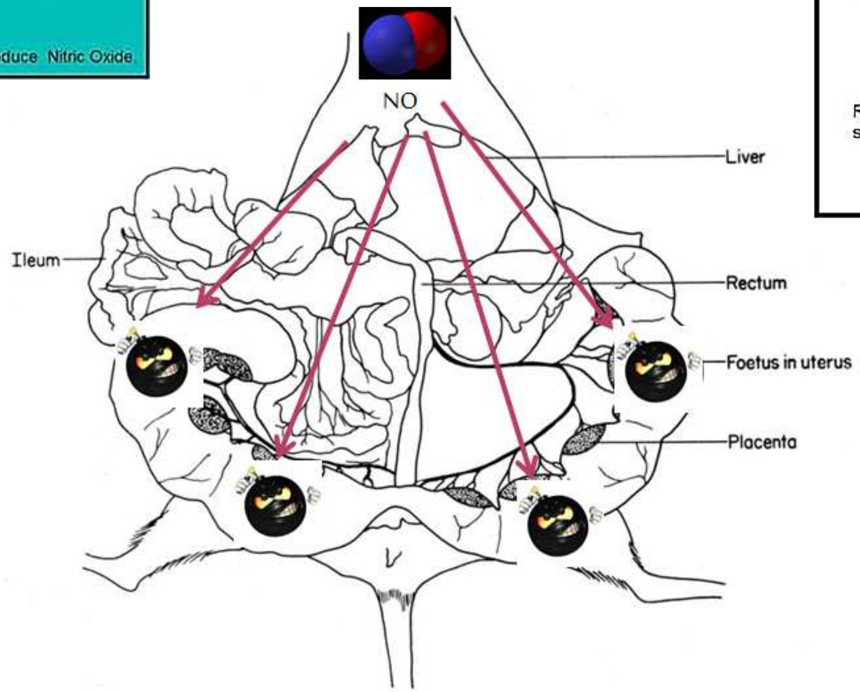
(Doridot et al, Antioxidant and Redox Signaling, 2014)

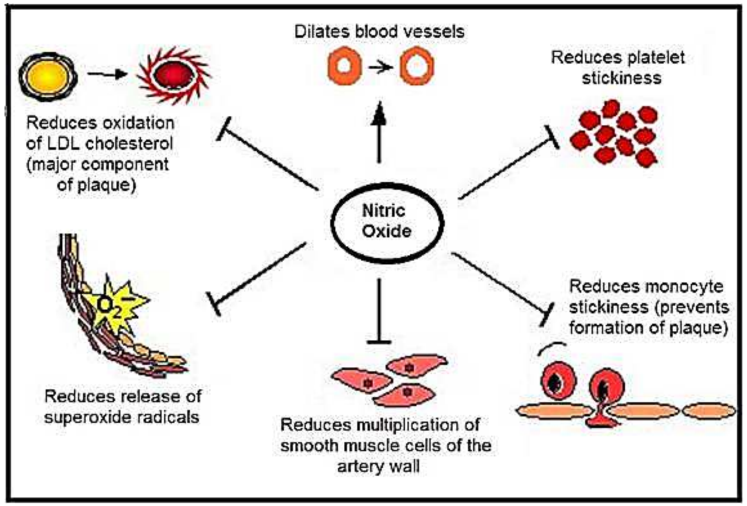


(Erlandsson et al, unpublished results)

Pumping out NO in *STOX1* overexpressing placentas

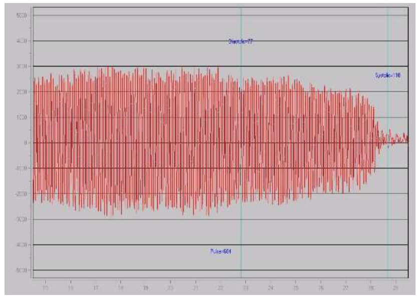


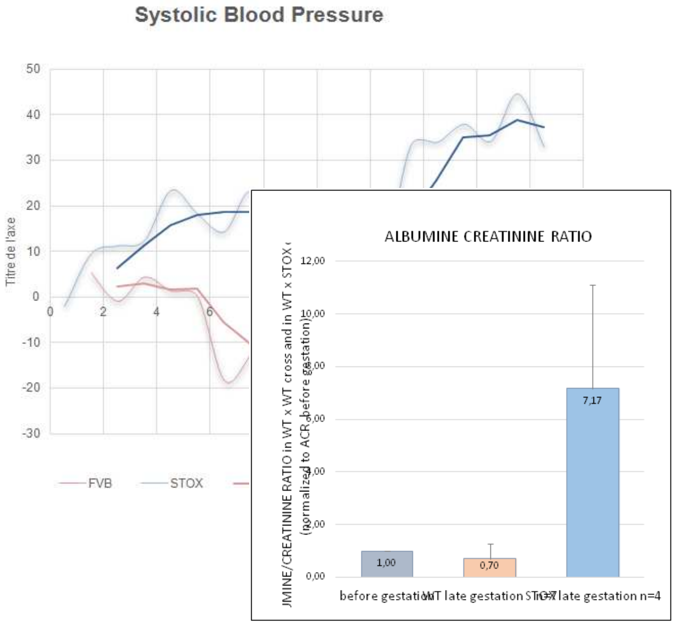


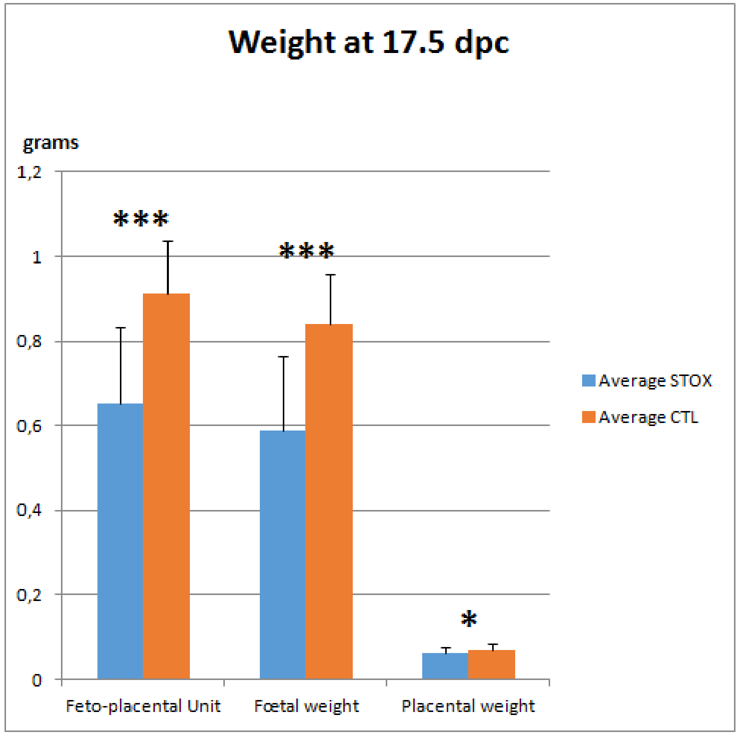


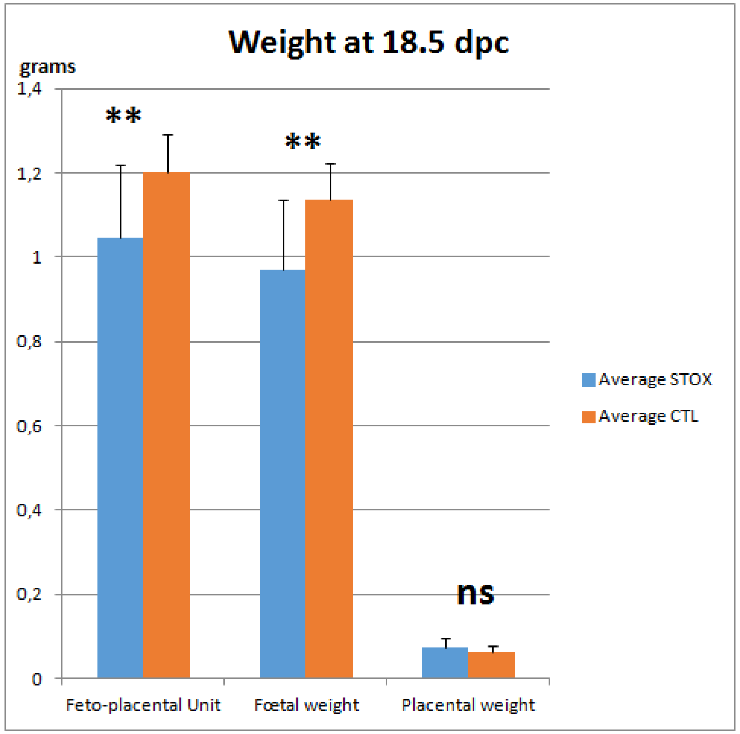
Improved phenotyping: IUGR in the STOX1 mouse model

(Collinot et al, submitted)



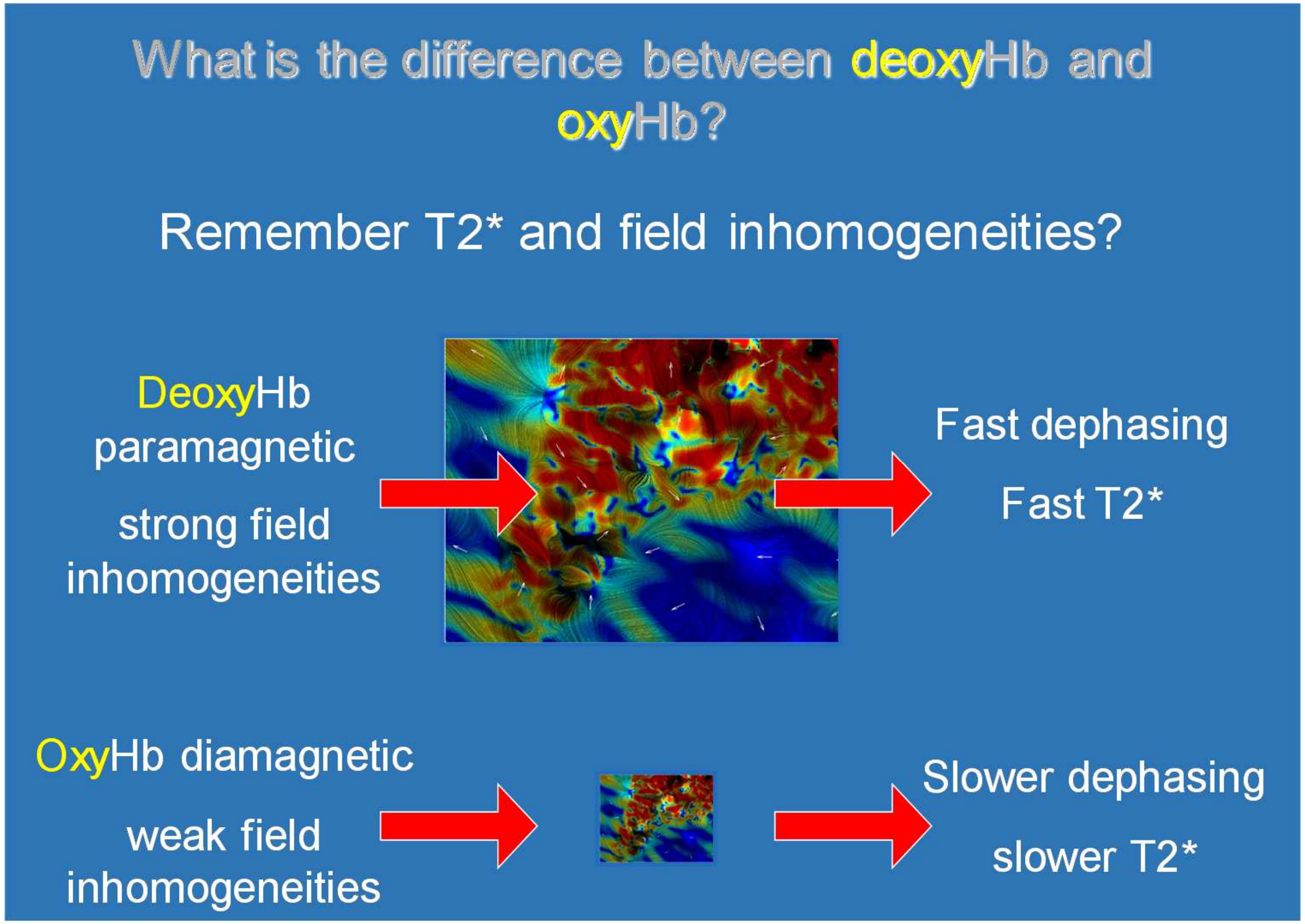






Mesure de la pression par photopléthysmographie

BOLD(Blood-Oxygen-Level-Dependent) MRI analysis



Specific values of the T2* and ΔT2* parameter

Organ	N	$T2^*_{\text{ambient air period}}$ in msec (mean ± SD)	$T2^*_{\text{hyperoxygenation}}$ in msec (mean ± SD)	P value
Maternal liver	27	11.9 ± 4.1	14.5 ± 5.2	<0.001
WT x TgSTOX1 mice				
Placental inner layer	83	14.9 ± 4.9	23.05 ± 8.2	<0.001
Placental outer layer	65	8.4 ± 3.3	14.5 ± 6.9	<0.001
Fetal brain	67	38.1 ± 8.0	43.4 ± 10.4	<0.001
Fetal liver	93	8.9 ± 1.8	11.3 ± 4.2	<0.001
WT x WT mice				
Placental inner layer	57	14.8 ± 4.1	20.7 ± 6.3	<0.001
Placental outer layer	52	8.2 ± 2.0	12.1 ± 3.6	<0.001
Fetal brain	39	41.4 ± 6.1	50.6 ± 9.9	<0.001
Fetal liver	53	9.5 ± 1.8	12.04 ± 3.0	<0.001

Mice	Placenta Inner Layer $T2^*_{\text{ambient air period}}$ in msec Median (Q1-Q3)	Placenta Outer Layer $T2^*_{\text{ambient air period}}$ in msec Median (Q1-Q3)	P value
WT x TgSTOX1 mice	15.2 (11.7-19.7)	7.9 (6.4-10.3)	<0.001
WT x WT mice	14.3 (11.9-16.9)	7.4 (6.1-9.8)	<0.001

Placental zone	WT x TgSTOX1 mice $T2^*_{\text{ambient air period}}$ in msec Median (Q1-Q3)	WT x WT mice $T2^*_{\text{ambient air period}}$ in msec Median (Q1-Q3)	P value
Placental inner layer (n=140)	15.2 (11.7-19.7) (n=83)	14.3 (11.9-16.9) (n=57)	0.924
Placental outer layer (n=117)	7.9 (6.4-10.3) (n=65)	7.4 (6.1-9.8) (n=52)	0.316

Placenta	WT x TgSTOX1 mice ΔT2*(ms) (mean ± SD	WT x WT mice ΔT2*(ms) (mean ± SD)	P value
Inner layer (n = 140)	8.2 ± 5.9 (n=83)	5.8 ± 5.9 (n=57)	0.025
Outer layer (n=117)	6.1 ± 5.1 (n=65)	3.9 ± 3.1 (n=52)	0.005

Organ	WT x TgSTOX1 mice ΔT2*(ms) (mean ± SD)	WT x WT mice ΔT2*(ms) (mean ± SD)	P value
Fetal brain (n=106)	5.3 ± 6.1 (n=67)	9.1 ± 8.0 (n=39)	0.004
Fetal liver (n=140)	2.4 ± 3.7 (n=93)	2.5 ± 2.6 (n=53)	0.25

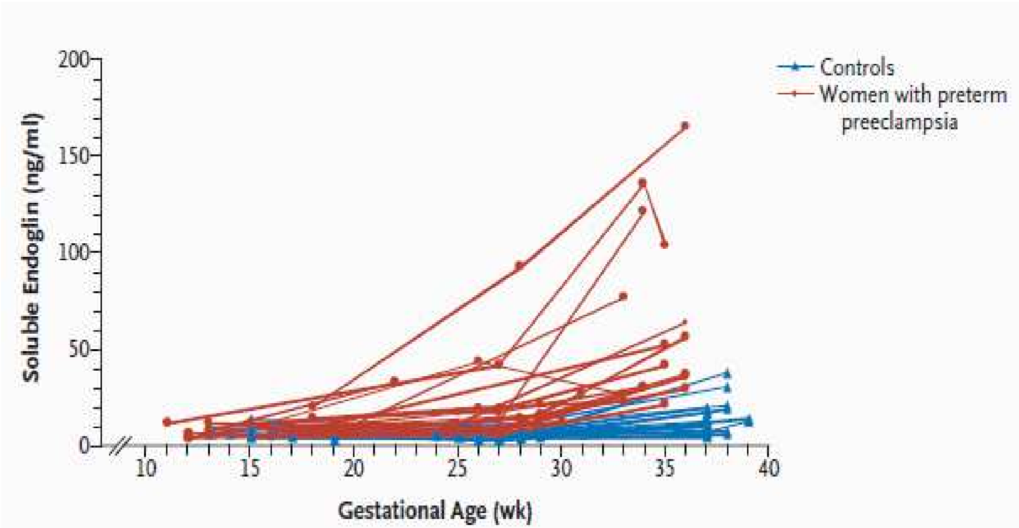
Diagnosis and Prognosis of preeclampsia using markers

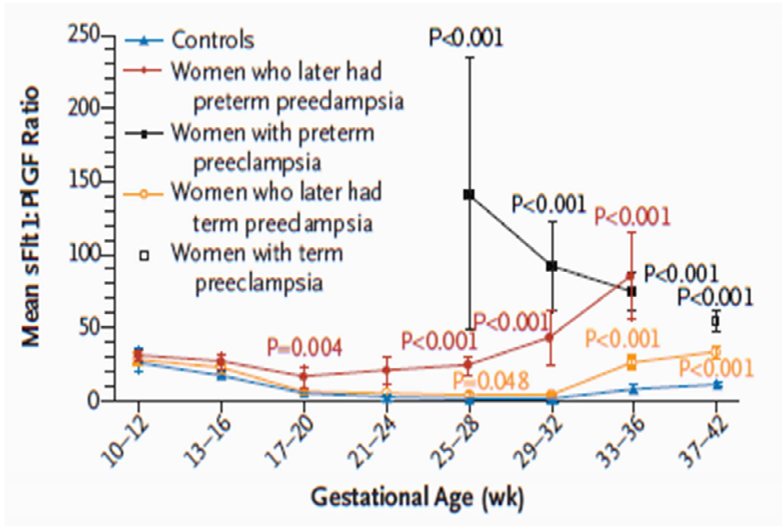
Context

• Low-dose aspirin before the 16th week is efficient to protect from preeclampsia.

• Plasmatic presymptomatic markers of preeclampsia have been identified (sFLT1, sENG, PLGF, PAPPA); at best the difference is detected after the 20th week of pregnancy.

• Novel earlier marker are warranted.

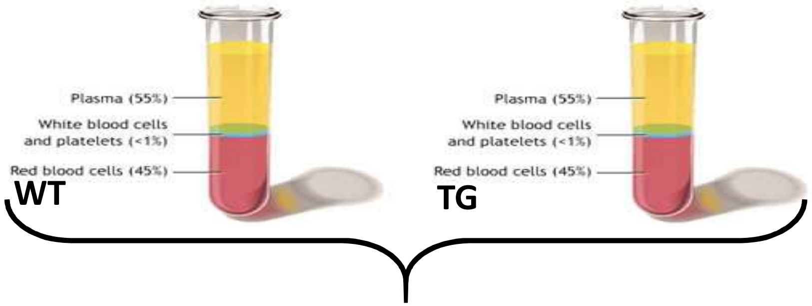




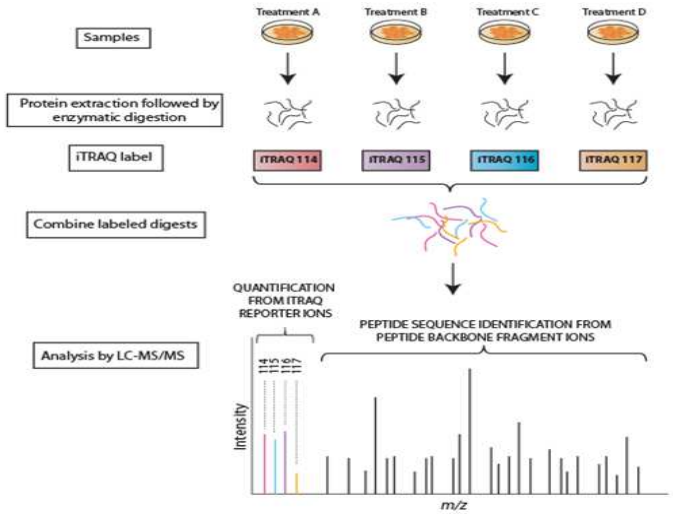
Innovation à l'Institut Cochin; 16 juin 2017

Levine et al, NEJM 2006

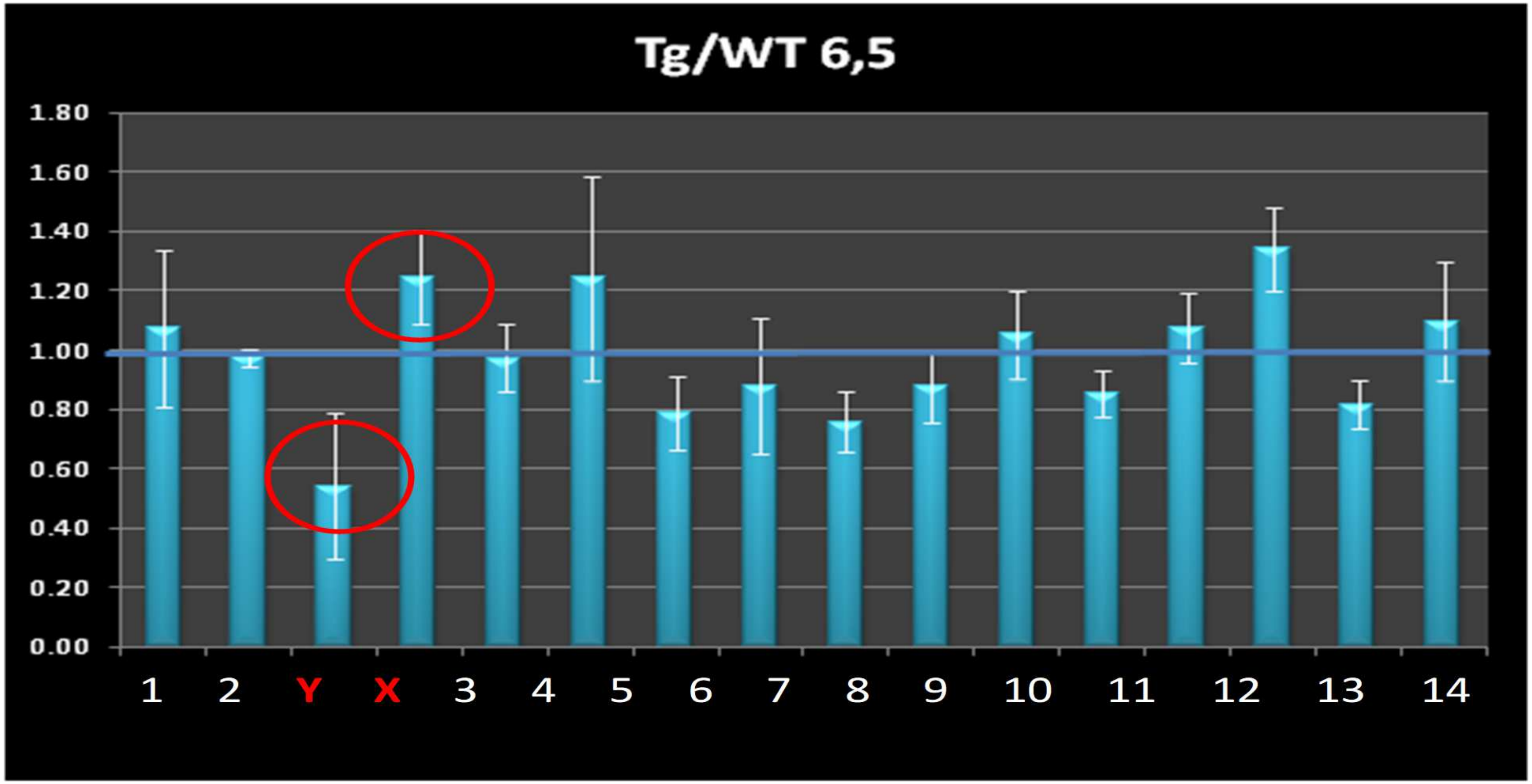
Using the STOX1 mouse model to identify novel markers



iTRAQ analysis 6.5 days post-coïtus



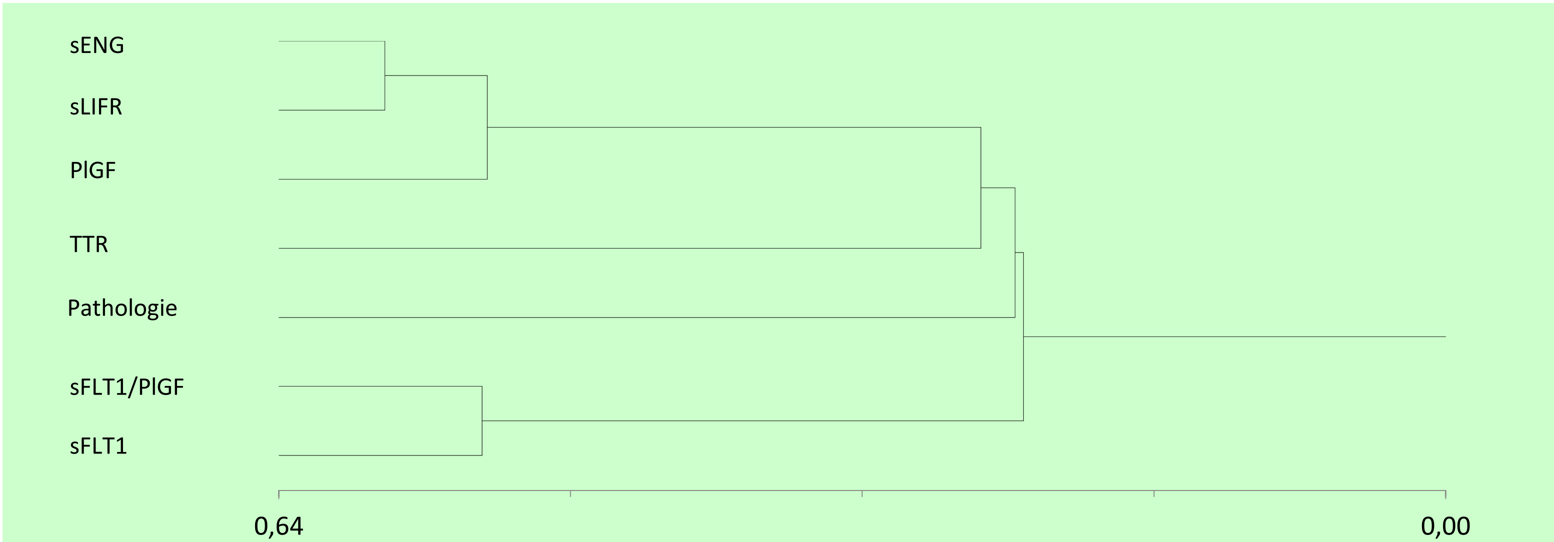
iTRAQ results

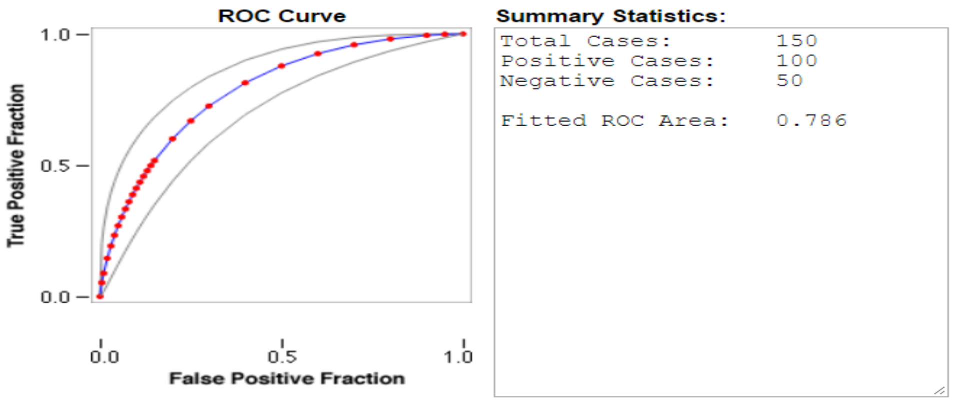


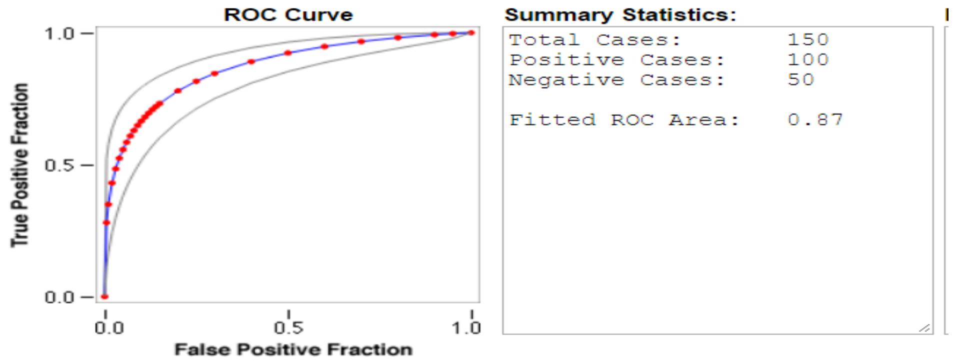
Five markers were analyzed in 150 human plasma sample (Lund University)

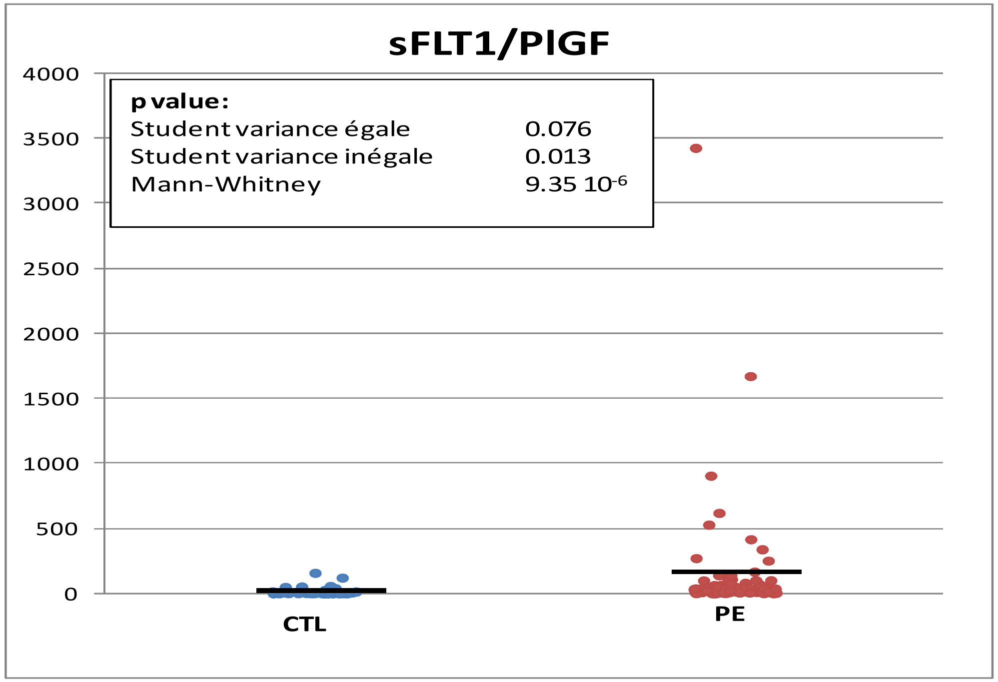
Pearson Correlations and p-values

Pathologie	-	0.232	-0.220	-0.153	0.236	0.078	0.146
sFLT1	0.004	-	0.047	0.003	0.105	0.147	0.528
TTR	0.007	0.568	-	0.255	0.017	0.221	0.065
PLGF	0.061	0.967	0.002	-	0.526	0.511	-0.097
sLIFR	0.004	0.200	0.839	4.99E-12	-	0.582	-0.062
sENG	0.340	0.073	0.007	2.42E-11	5.81E-15	-	0.128
sFLT1/PLGF	0.074	3.64E-12	0.426	0.238	0.454	0.119	-

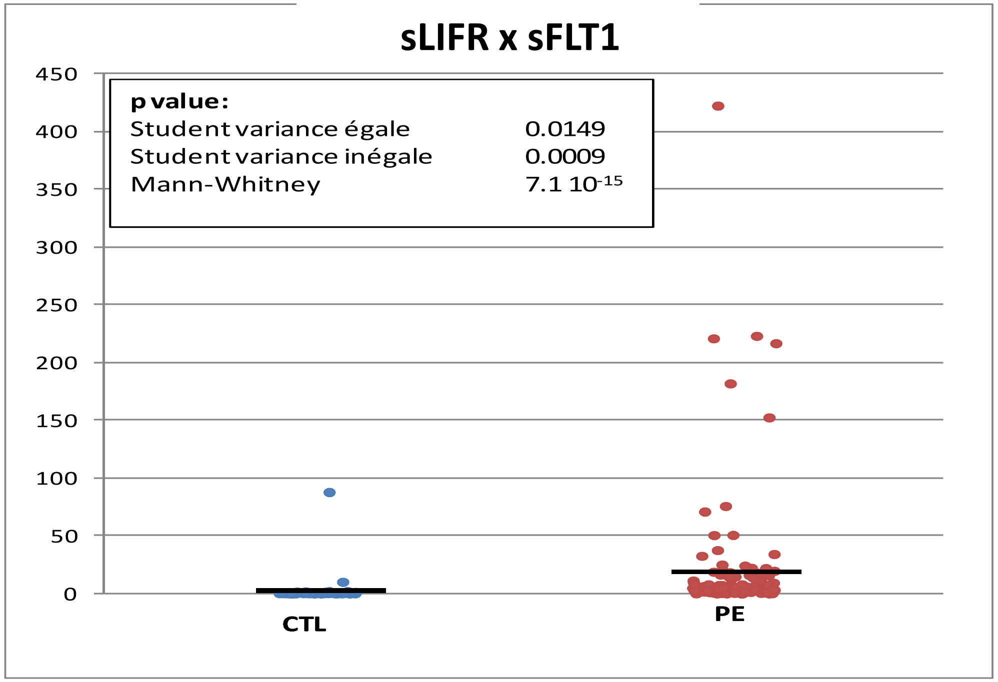








Mean: 14 vs 114



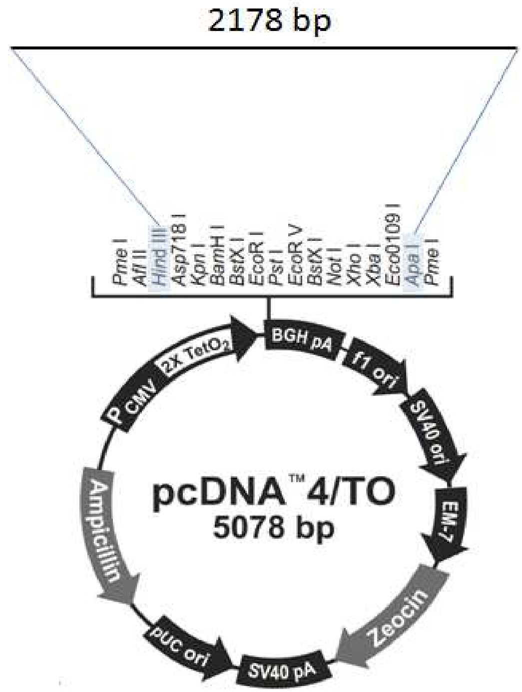
Mean: 2.4 vs 23.4

Follow-up

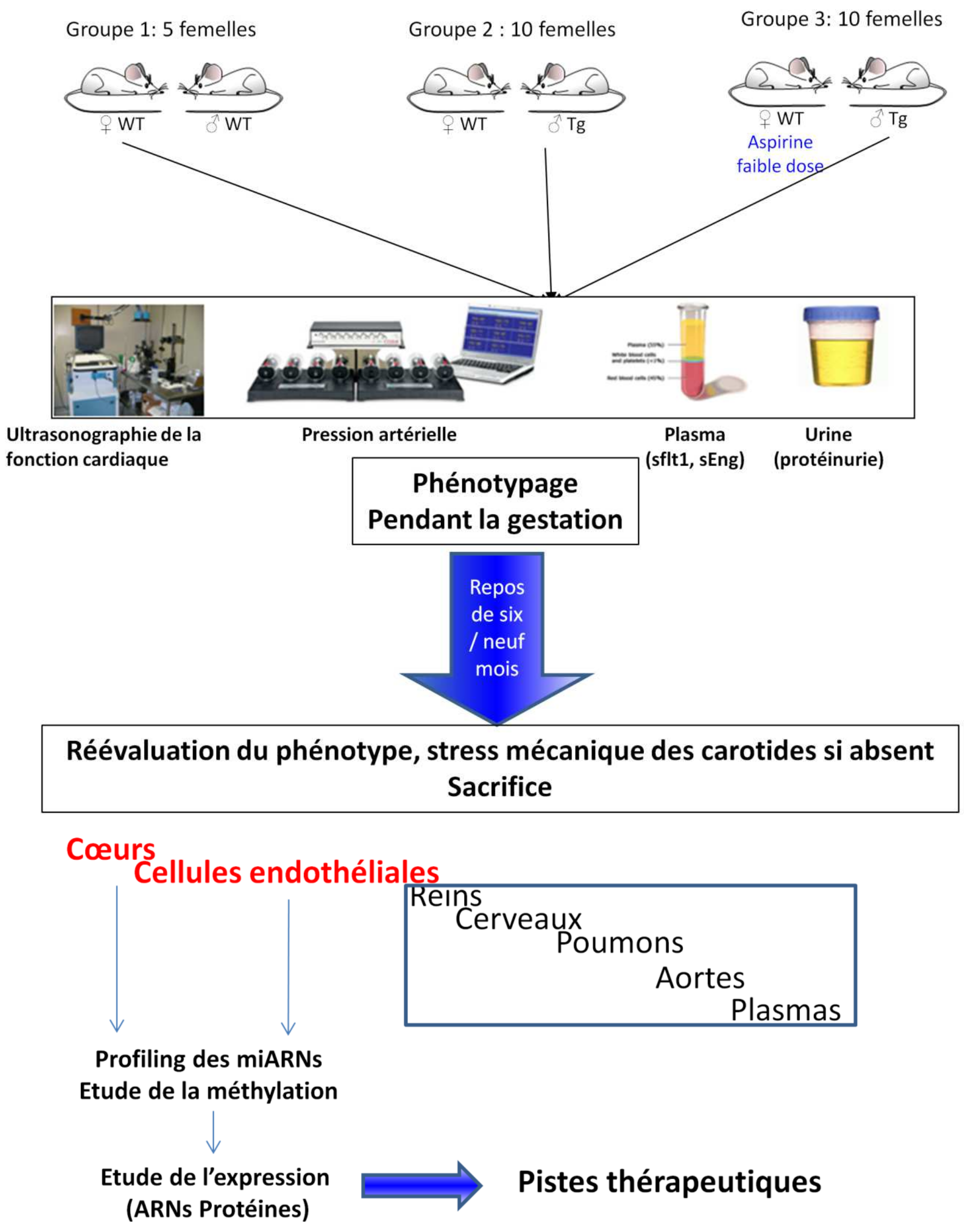
1. Analysis of other additional human cohorts.

2. Generation of a Doxycyclin-inducible mouse model of overexpression of sLIFR ()

3. Analysis of the phenotype (arterial hypertension/embryonic resorption, proteinuria) in function of the induction moment of the transgene



Long-term effects of preeclampsia



Increased mouse heart weight 8 months after a preeclamptic pregnancy

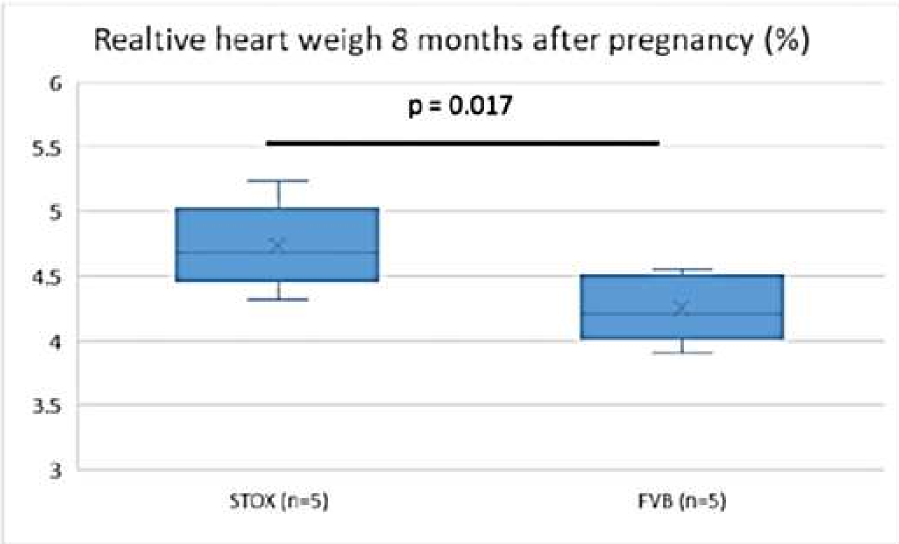
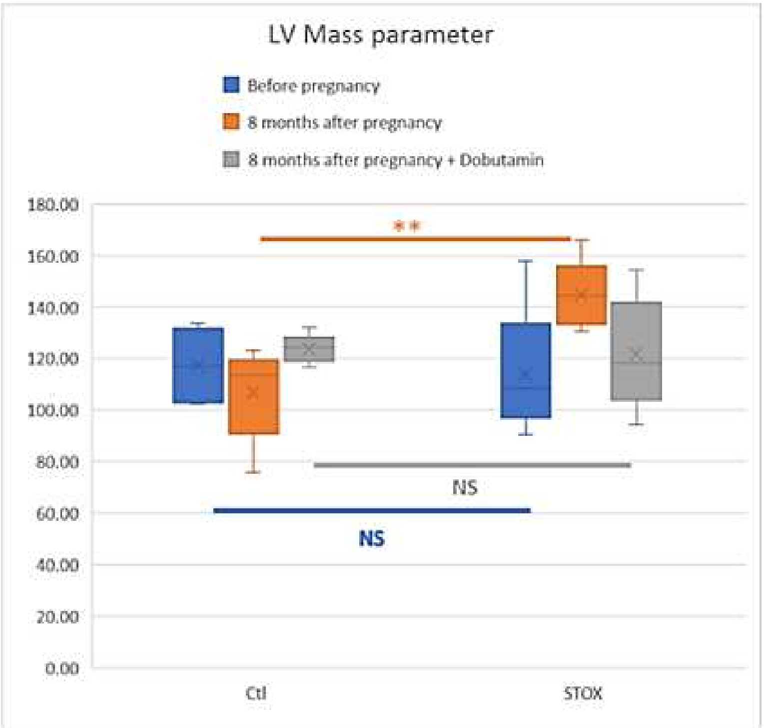
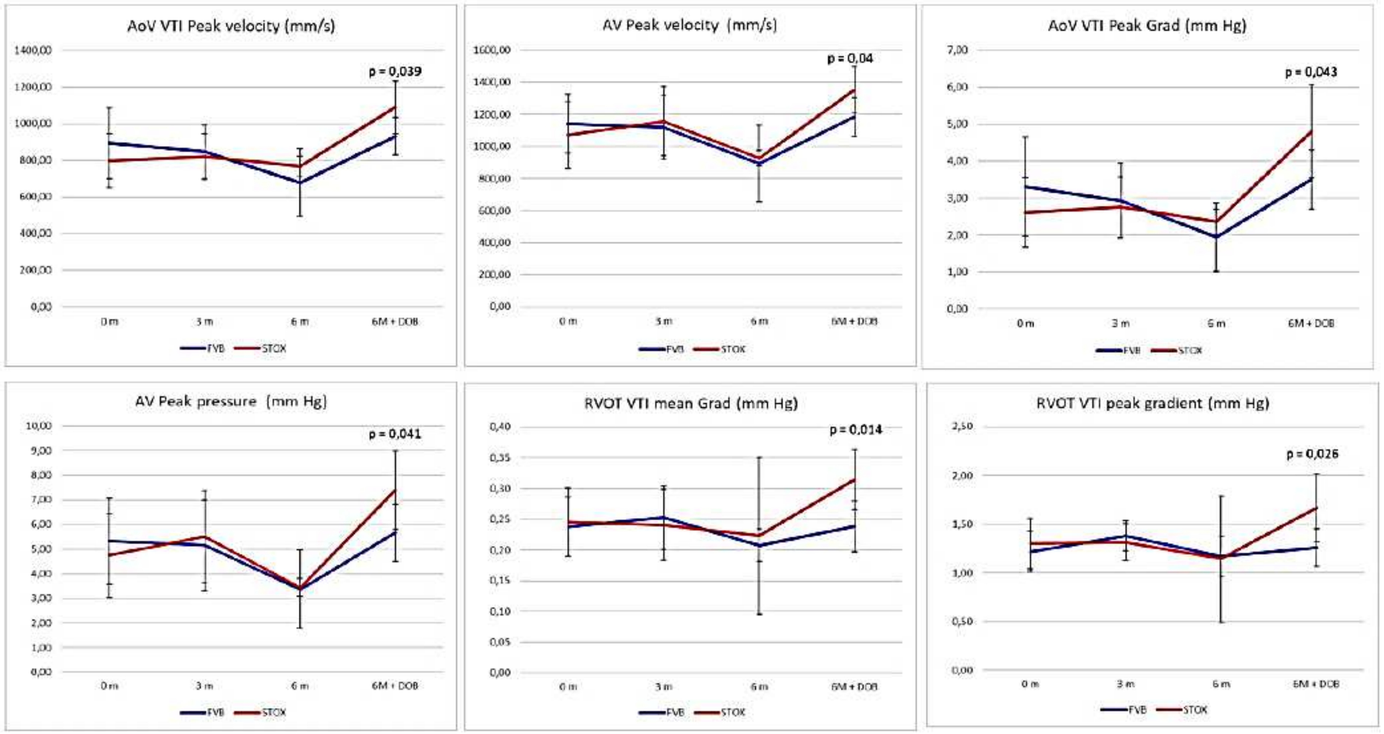


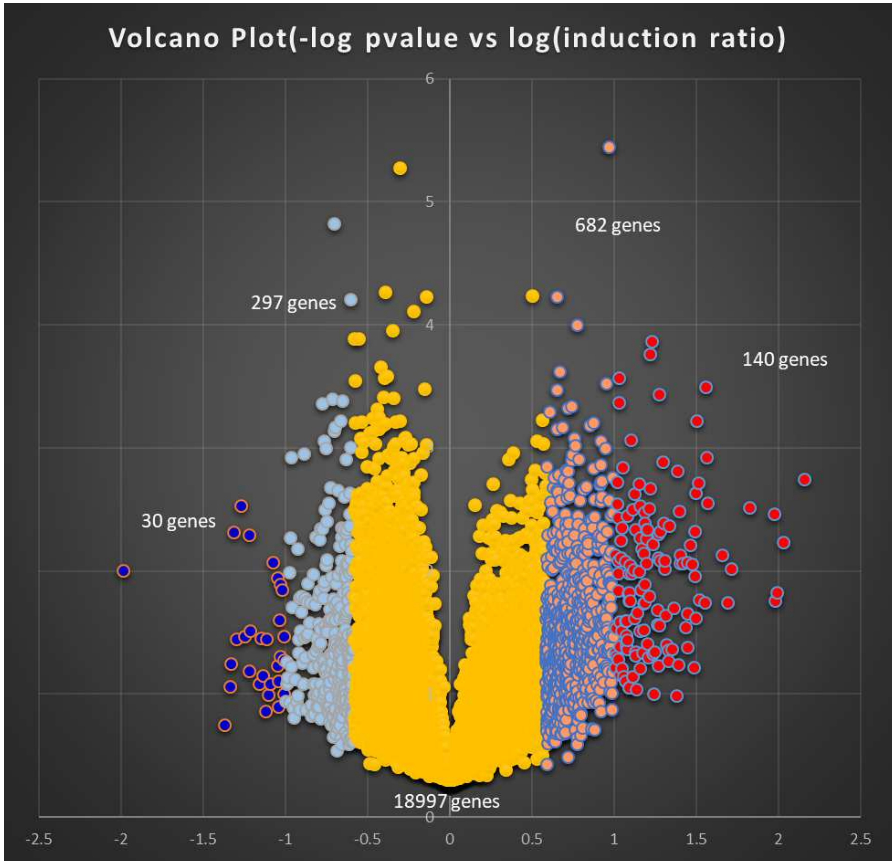
Figure 1 : Mouse heart weight is durably increased following a preeclamptic pregnancy. The difference is about 11% after correction for the mouse total weight. This difference was similar to the one observed at the end of pregnancy (Ducat et al, 2016) suggesting that the alterations were not reversible.

An altered response to stress stimuli at the heart level (Doppler US analysis)





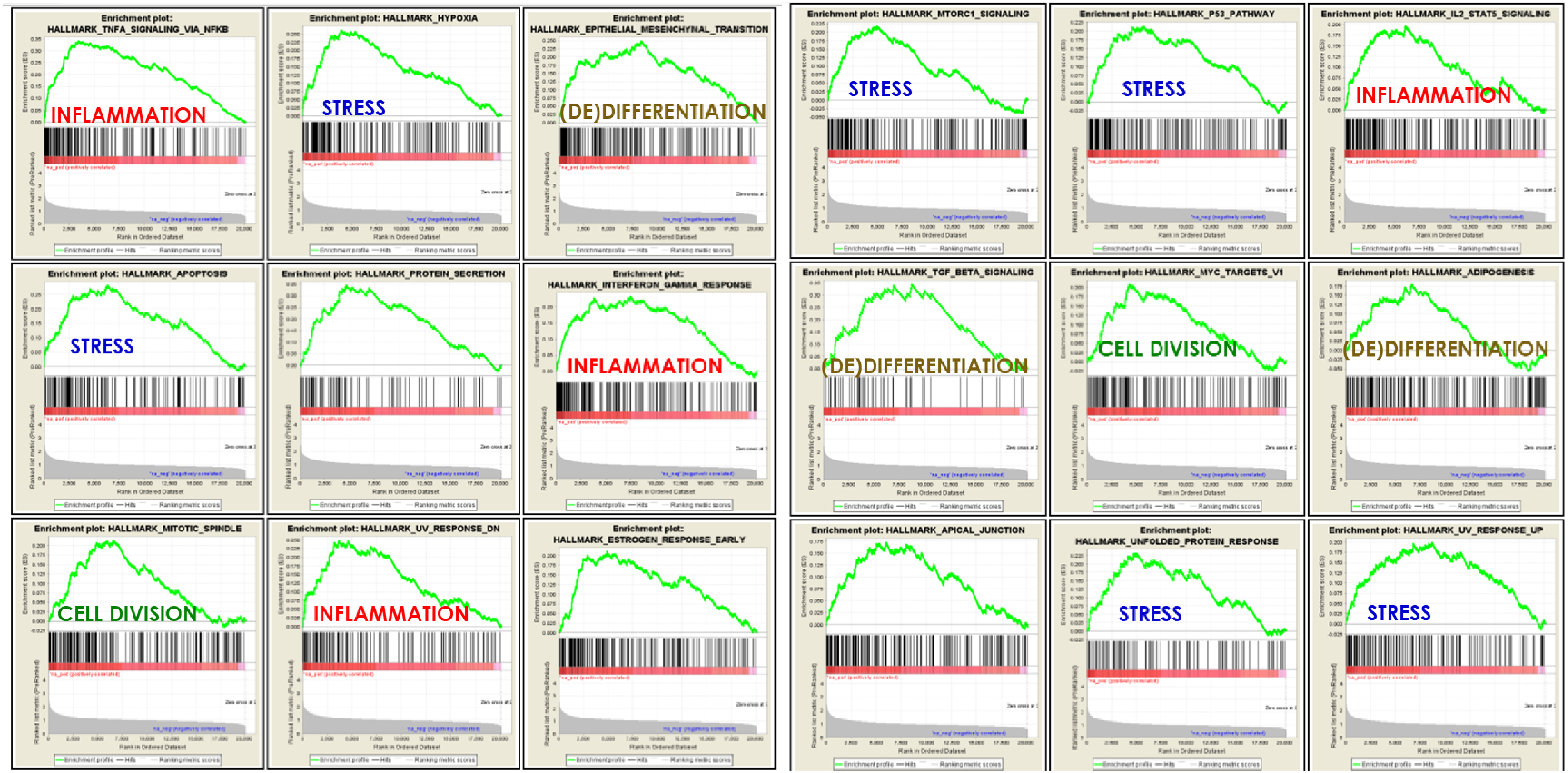
Massive alteration of the endothelial cell transcriptome



- RIKEN cDNA 1190007I07 gene
- proteoglycan 2, bone marrow
- CD27 antigen
- cDNA sequence AB124611
- ribosomal protein S27-like
- deltex 3, E3 ubiquitin ligase
- RIKEN cDNA 2900060B14 gene
- developmentally regulated GTP binding protein 2
- CD59a antigen
- xeroderma pigmentosum, complementation group A
- oxysterol binding protein-like 1A
- lymphocyte cytosolic protein 1
- RIKEN cDNA I830077J02 gene
- DEAH (Asp-Glu-Ala-His) box polypeptide 8
- intraflagellar transport 22
- killer cell lectin-like receptor, subfamily D, member 1
- thymocyte expressed, positive selection associated 1
- protein tyrosine phosphatase, receptor type, C
- RIKEN cDNA 1110059G10 gene
- sarcospan
- coiled-coil domain containing 6
- TSSK6 activating co-chaperone
- S100 calcium binding protein A8 (calgranulin A)
- histone cluster 1, H2bc
- tectonic family member 3
- DEAH (Asp-Glu-Ala-His) box polypeptide 32
- G protein-coupled receptor kinase-interactor 1
- potassium voltage-gated channel, subfamily G, member 1
- transmembrane protein 159
- tryptophan hydroxylase 1

- MKL/myocardin-like 2
- muscle glycogen phosphorylase
- actinin, alpha 1
- glutathione S-transferase, mu 1
- plexin domain containing 2
- colony stimulating factor 2 receptor, beta 2, low-affinity (granulocyte-macrophage)
- dynamin 3
- peripheral myelin protein 22
- src homology 2 domain-containing transforming protein C1
- apolipoprotein D
- sprouty homolog 4 (Drosophila)
- developmentally regulated GTP binding protein 1
- activating transcription factor 1
- A kinase (PRKA) anchor protein (gravin) 12
- insulin receptor
- mitogen-activated protein kinase kinase kinase 3
- decorin
- TCDD-inducible poly(ADP-ribose) polymerase
- thrombospondin 1
- GTP binding protein (gene overexpressed in skeletal muscle)
- thrombospondin, type I, domain containing 7A
- voltage-dependent anion channel 2
- matrix-remodelling associated 8
- a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 4
- protein phosphatase 3, regulatory subunit B, alpha isoform (calcineurin B, type I)
- retinol saturase (all trans retinol 13,14 reductase)
- RAS p21 protein activator 4
- interleukin 6
- ribosomal protein L7-like 1
- RAB8B, member RAS oncogene family

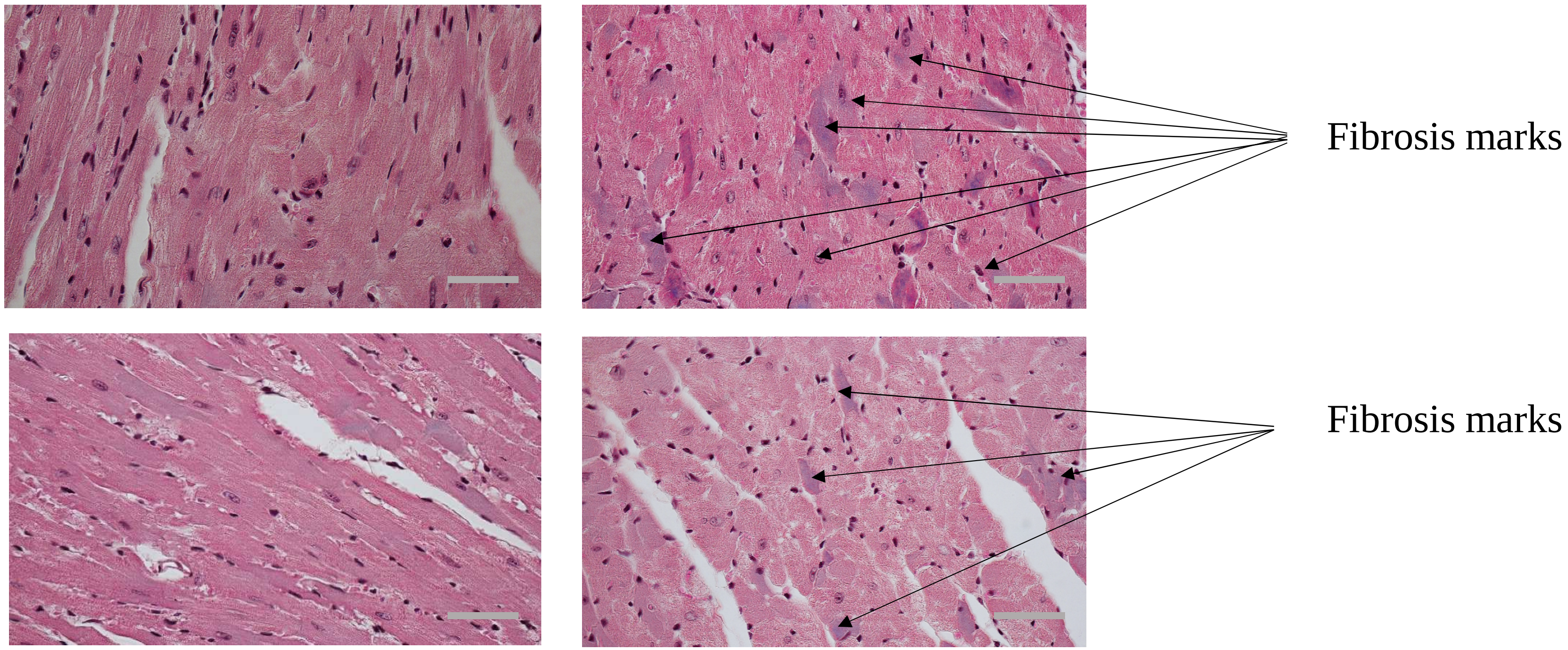
Transcriptome functional clustering



NES : 5.49 to 2.75

Preliminary Results on Cardiac histology

CTL pregnancy | STOX pregnancy



Trichrome Masson staining (x40, bar = 50µm)

Thank you for your attention

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  - Ludivine Doridot, PhD
  - Aurélien Ducat, PhD
  - Rosa Calicchio, PhD
  - Betty Couderc, Master Student
  - Irène Gaillard, Master Student
  - Sophia Palfray
- **Today**
  - Louise Biquard
  - Rajaa Aouache
  - Francisco Miralles
  - Céline Méhats
- **Analysis of the oxidative stress**
  - Christiane Chéreau
  - Fréderic Batteux

Institut Pasteur (Mitochondrie et stress oxydatif)

- Laurent Châtre
- Miria Ricchietti

INRA (Jouy en Josas)

- Bruno Passet
- Johan Castille
- Marthe Vilotte
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Lund University

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- Grigorios Karampas
- Eva Hansson

Glasgow University

- Dylis Freeman
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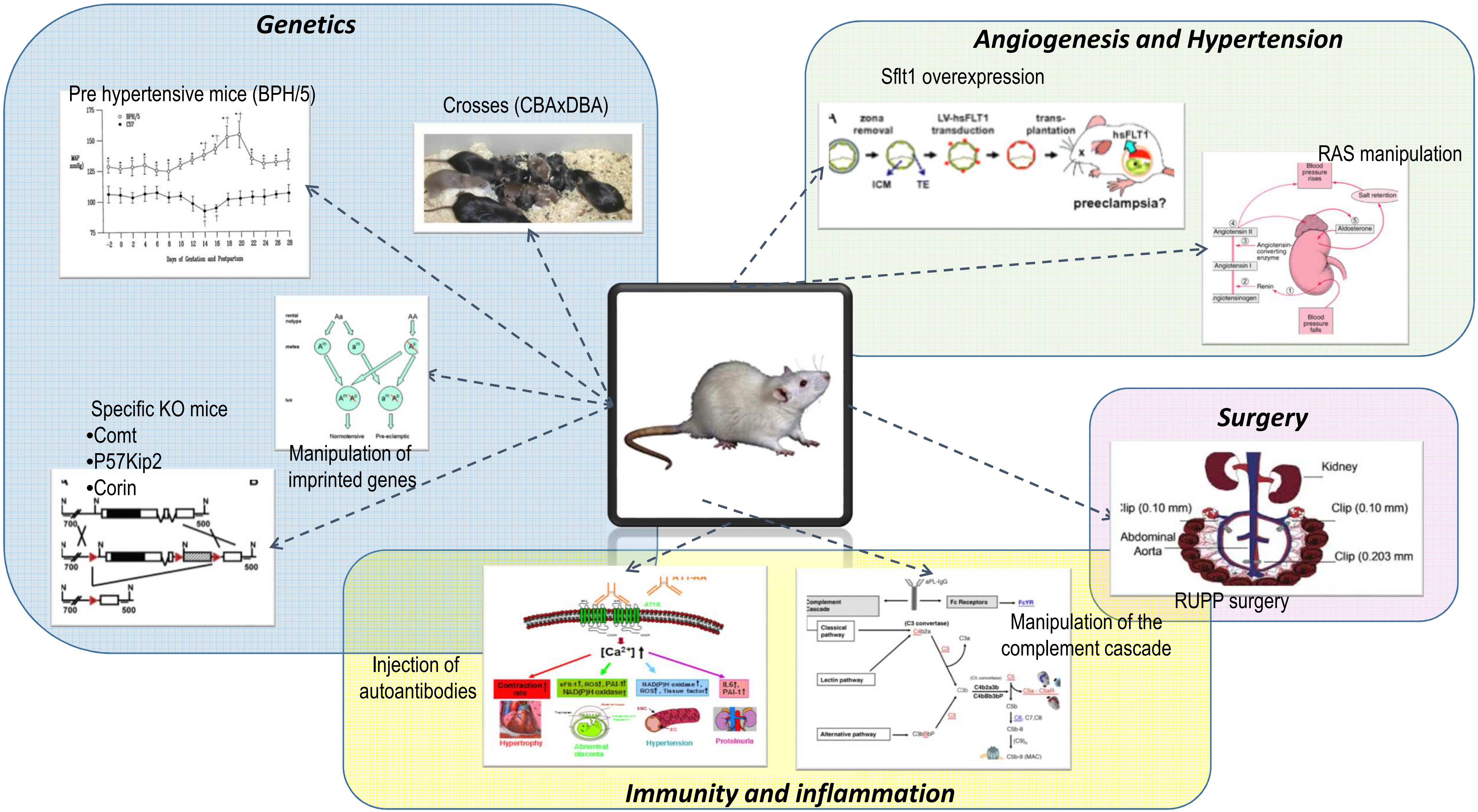
Genetics
Pre hypertensive mice (BPH/5)
Crosses (CBAxDBA)
Manipulation of imprinted genes
Specific KO mice
•Comt
•P57Kip2
•Corin
Angiogenesis and Hypertension
Sflt1 overexpression
zona removal
LV-hsFLT1 transduction
trans-plantation
hsFLT1
ICM
TE
preeclampsia?
RAS manipulation
Surgery
Kidney
Clip (0.10 mm)
Abdominal Aorta
Clip (0.203 mm
RUPP surgery
Injection of autoantibodies
Manipulation of the complement cascade
Immunity and inflammation