

Membrane and Nuclear Estrogen Receptor α actions: From Tissue Specificity to Medical Implications

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Conflict of interest : Financial support from Mithra Pharma (Be)

Pubmed search : estrogen receptor (ER) > 80 000 papers....

Unresolved/pending questions ?

1- Medical issues of estrogens / ER ?

2- Respective roles of ER alpha and beta ?

3- ER α is a nuclear receptor, but is it only a nuclear receptor ?

4- ER α is also a membrane receptor: *in vivo* evidences and physiological significance in vessels ?

5- New modulation/modulators of ER in medicine ?

1- Medical issues of hormonal estrogens (E) treatments ?


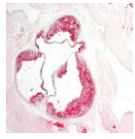
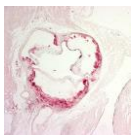
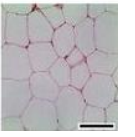
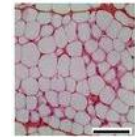
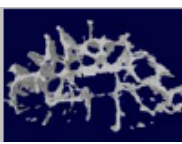
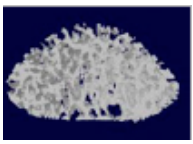
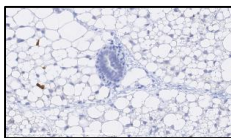
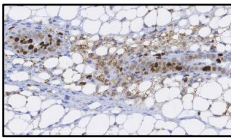
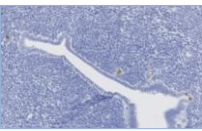
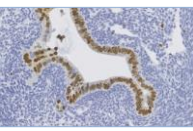
- **Menopause** : E remain the most efficient treatment for:
 - the climateric symptoms,
 - the prevention of bone demineralization and fractures;

but E increase the risk of breast cancer (with progestins).

- **Contraception** : E increase the risk of VTE
- **Breast cancer** : benefit of anti-E: tamoxifen (agonist in other tissues such as bone).
- **New treatments with an increased benefit/risk ratio ?**

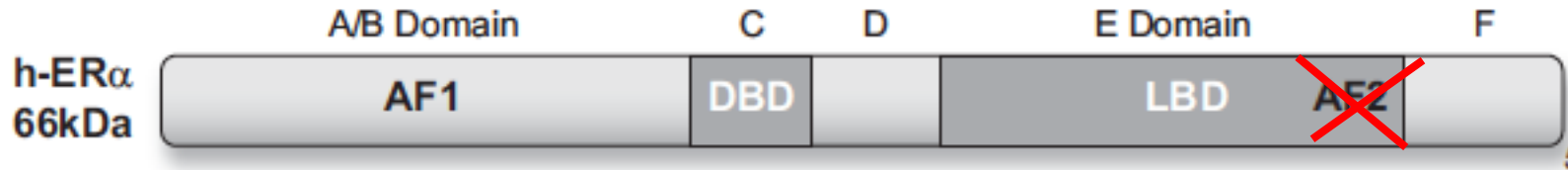
2- Roles of ER alpha and beta?

Mouse models are relevant to study estrogens effects in women

		 Mouse models	OVX (ovariectomized)	+ E2	
Benefits/Prevention	HT(E) Women				
	Coronary events (early atheroma)	Atheroma (HChol)			Aortic Sinus
	Type 2 Diabetes	Glucose intolerance and Insulino- resistance (HFD)			Obesity/Adipocyte size
	Osteoporosis	Bone demineralization			Trabecular bone
Risks	Breast Cancer (+ progestin)	Epithelial cell proliferation			Mammary gland (Ki67 staining)
	Endometrial Cancer (- progestin)	Epithelial cell proliferation			Uterus (Ki67 staining)

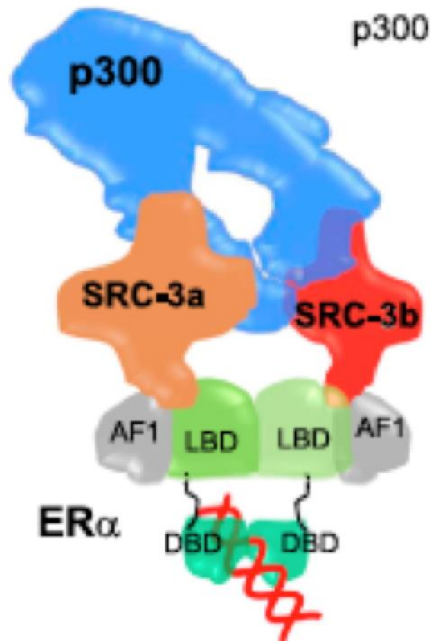
All these effects are abolished in ER α -/-, but not in ER β -/-, demonstrating the key role of ER α

3- ER α as a nuclear transcription factor : role of activation function AF2

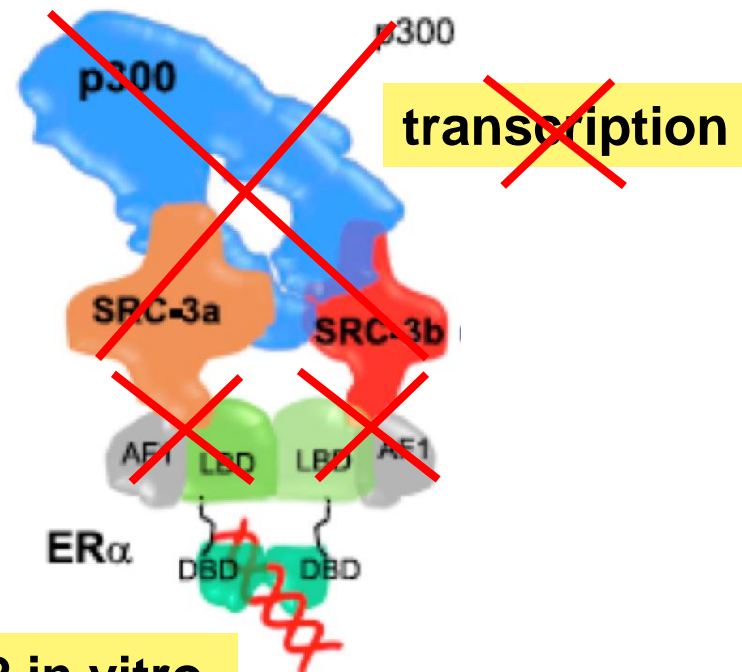


O'Malley et al. Mol Cell 2014

cryomicroscopy



Activation of
AF2 =>
Recruitment of
CoA and then
RNA-pol-II =>
transcription



Structure and key role of AF2 in vitro

Role of AF2 in vivo?



ER α -AF2⁰

**Deletion of 7 AA (543–549) in helix 12
=> AF2-deficient mice = ER α -AF2⁰**

Uterus

**Uterine samples from ER α -AF2⁰ mice or WT controls
treated with E2 (8 μ g/kg, 6 h) or vehicle (Veh)**

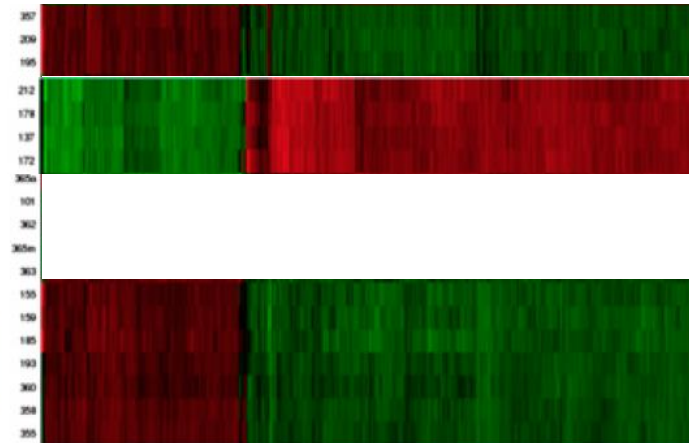
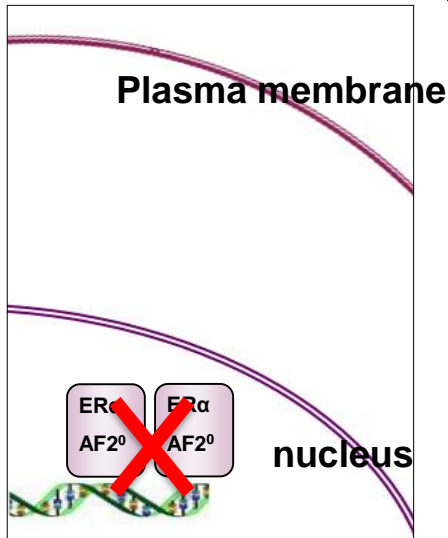


WT + Veh

Gene expression profiles (microarray analysis, Agilent)



WT + E2



WT + Veh

WT + E2

AF2⁰ + Veh

AF2⁰ + E2



AF2⁰ + E2

AF2⁰ abrogates nuclear, transcriptional and proliferative actions of E2 in the uterus

Comparison of the E2 effects in ER α -/- and in AF2 $^{\circ}$ -ER α

Effect of E2 action on:	ER α -/- mice	AF2 $^{\circ}$ ER α mice	References
Atheroma	0	0	Billon et al. Circ. 2009 Billon et al. PNAS 2011
T2 Diabetes	0	0	Handgraaf et al. Diabetes 2013
Bone demineralization	0	0	Börjesson et al. PNAS 2011
Endometrial proliferation	0	0	Abot et al. Endocrinology 2013
Breast proliferation	0	0	In preparation

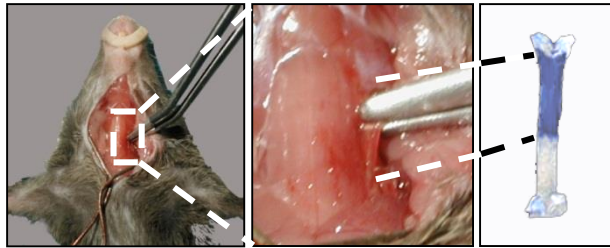
0 means abrogation of the effect of E2 compared to WT

AF2 $^{\circ}$ - ER α abrogate all the in vivo actions of E2 ... but one...

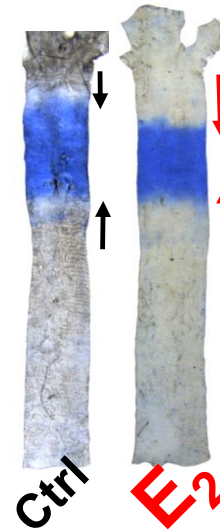
Model of Reendothelialization = endothelial healing

Electric injury model of the common carotid artery in ovariectomized C57Bl/6 mice

Brouchet et al. Circulation 2001

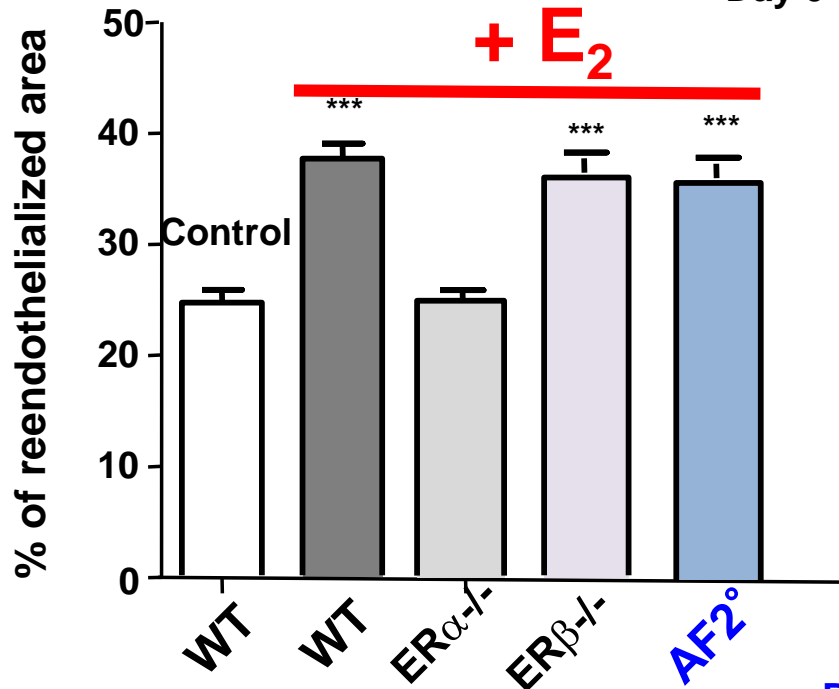


Day 0



Day 3 post-injury :

E2 accelerates of reendothelialization in WT mice



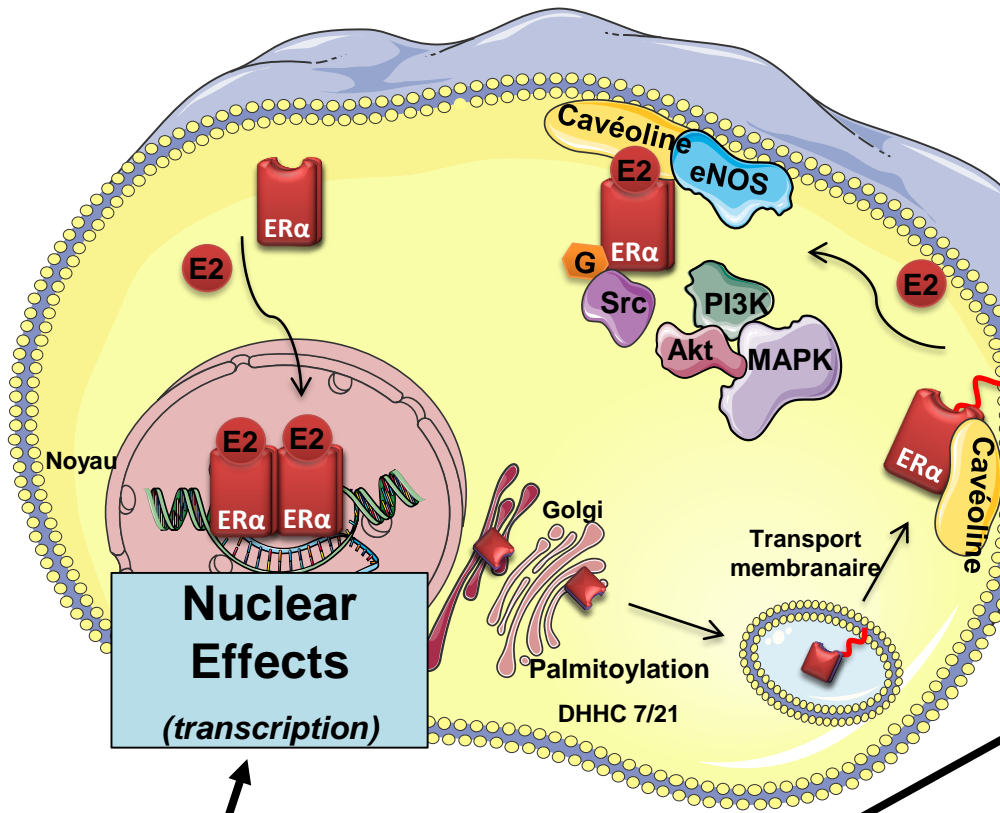
AF2 is dispensable for the accelerative effect of E2 on reendothelialization, although ER α is necessary.

Billon et al.

Brouchet et al. Circulation 2001

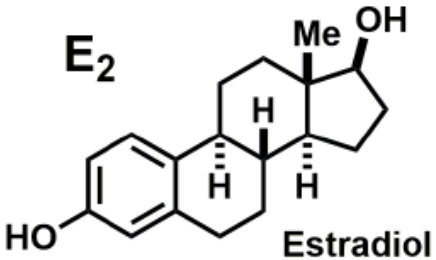
PNAS USA 2009 and 2011

4- ERα is also a membrane receptor

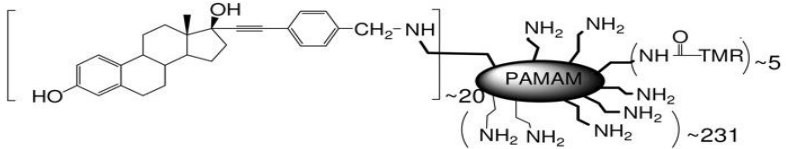


Non genomic = Rapid = membrane initiated steroid signals (MISS)
 => *Rapid activation of :*
Kinases : MAPK, PI3K,..
2nd mess: cAMP, Ca⁺⁺,..
NO synthase...

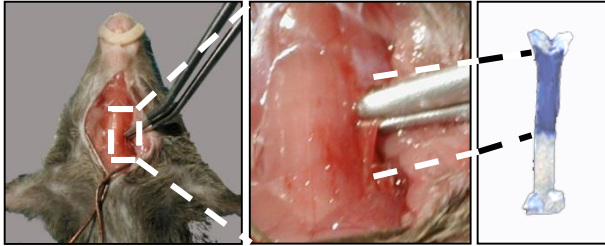
Nuclear Effects
(transcription)



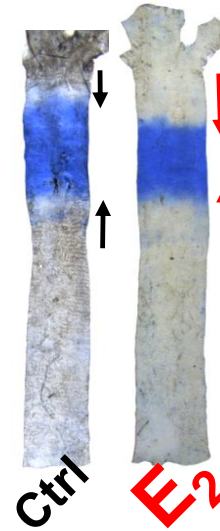
J and B Katzenellenbogen et al.
Estrogen Dendrimer Conjugate EDC



Reendothelialization

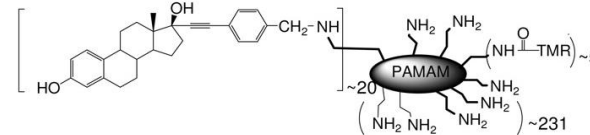
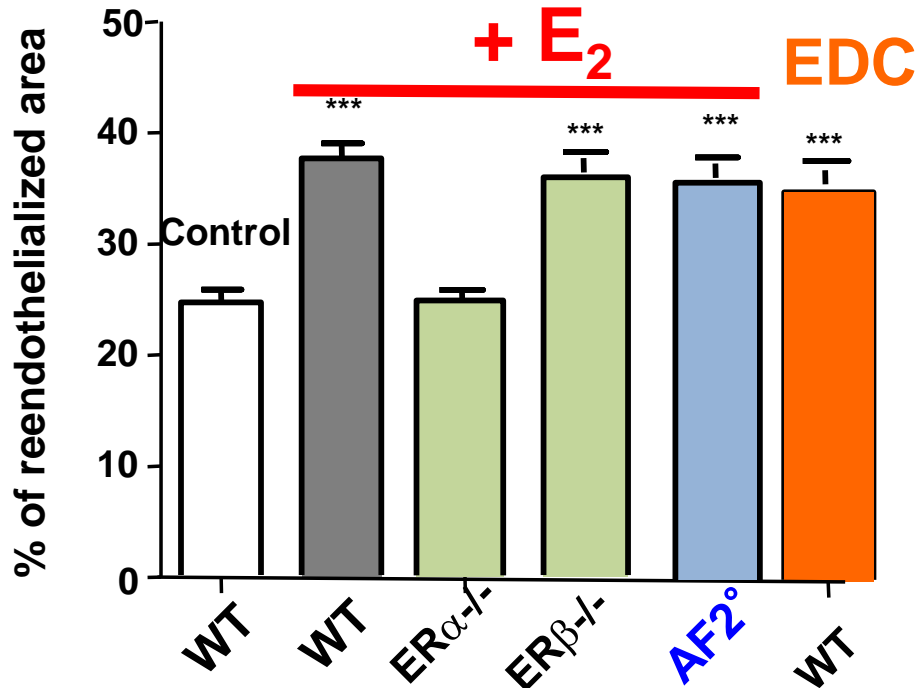


Day 0



Day 3

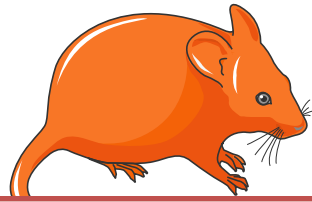
E2 accelerates of reendothelialization



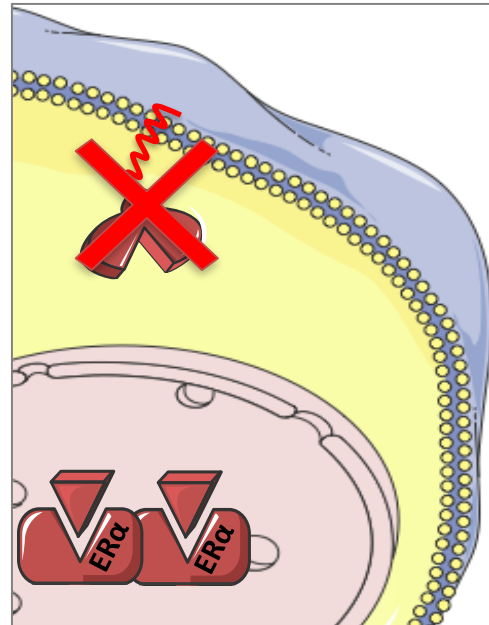
Chambliss Katzenellenbogen J, Shaul. JCI 2010

Pharmacological activation of membrane ER α is sufficient to accelerate reendothelialization.

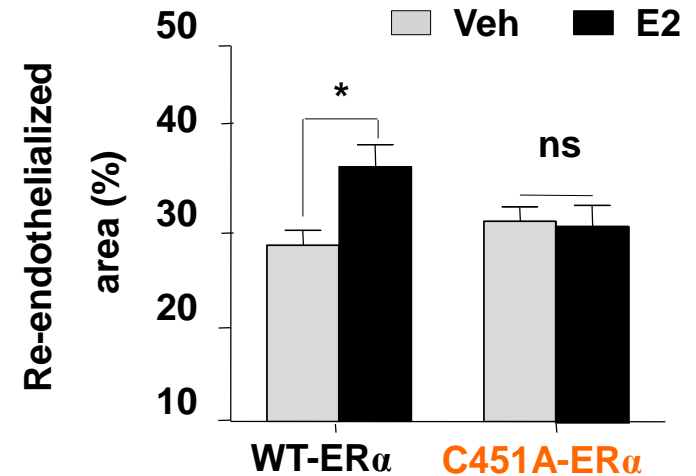
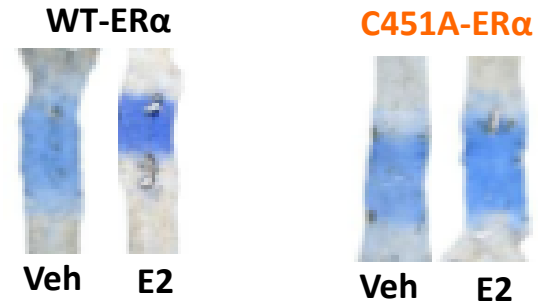
But is membrane ER α necessary for this effect ?

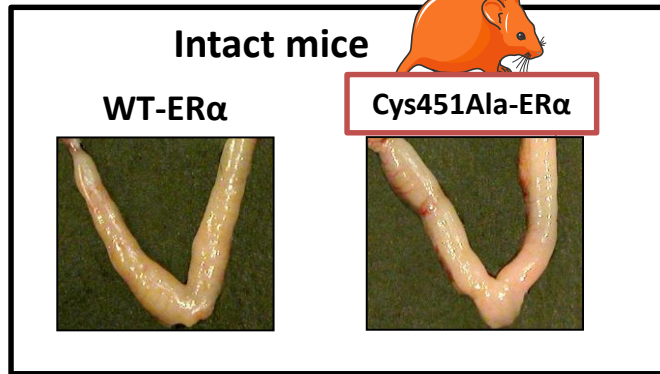


**First Model of Selective
inactivation of membrane ER α =
Membrane ER α loss-of-function
Cys451Ala-ER α = Palm^o
mice**



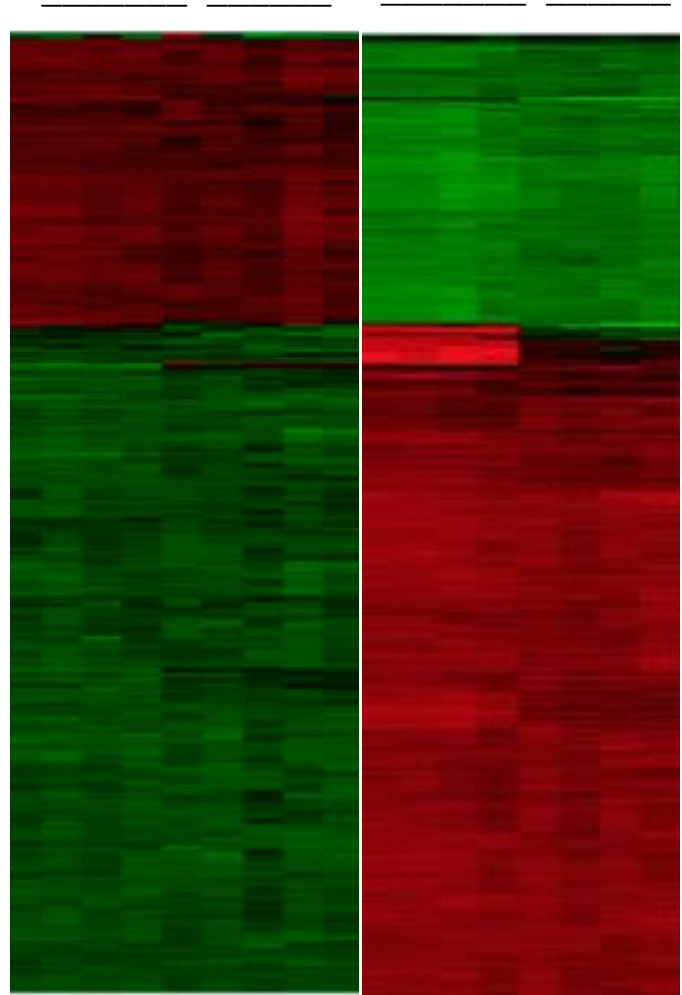
Abrogation of the E2-induced acceleration of reendothelialization



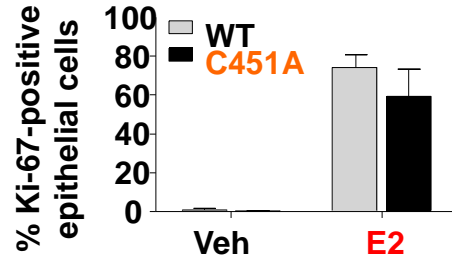
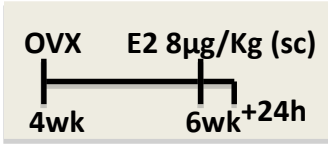


Palm^o mice : preservation of E2-induced uterine growth and gene expression

Vehicle E2 6 h
 WT C451A WT C451A



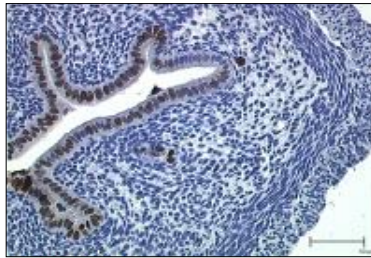
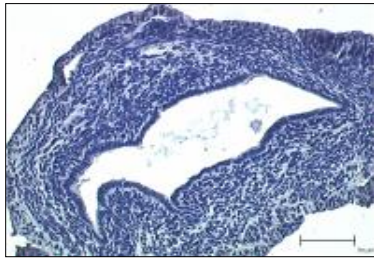
Ovariectomized mice



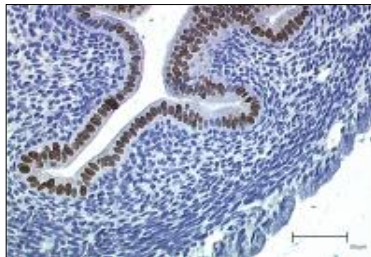
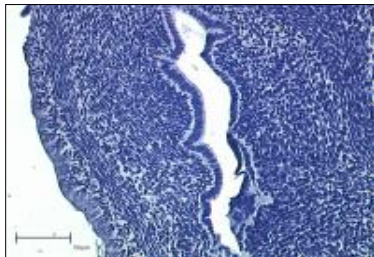
Vehicle

E2 24h

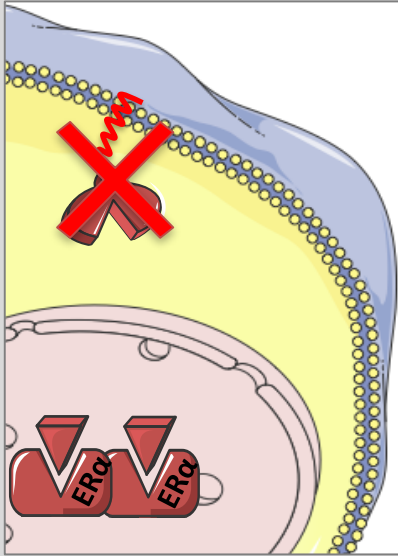
WT-C451



C451A-ER α



Summary : Specific roles of membrane and nuclear ER α in vascular and uterus effects (tissue-specificity+++)



Cys451Ala-ER α = Palm $^{\circ}$

Loss of function of membrane effects of ER α

WT-ER α

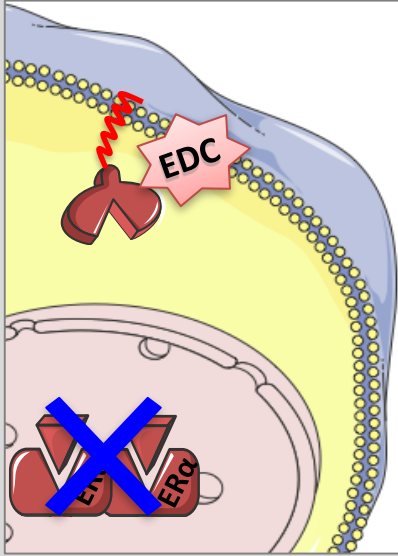
C451A-ER α

WT-ER α Veh
WT-ER α E2

C451A-ER α Veh
C451A-ER α E2

Normal uterine response

Lack of endothelial response



ER α -AF2 $^{\circ}$

Loss of function of nuclear effects of ER α

WT-AF2

ER α -AF2 $^{\circ}$

ER α -AF2 $^{\circ}$ Veh
ER α -AF2 $^{\circ}$ E2
ER α -AF2 $^{\circ}$ EDC

Normal endothelial response

Lack of uterine response

Physiological Role of E2/ER α in the endothelium ?

Physiological Remodelling of the Maternal Uterine Circulation during Pregnancy

Maurizio Mandala^{1,2} and George Osol²

¹Department of Cellular Biology, University of Calabria, Arcavacata di Rende (CS), Italy, and ²Department of Obstetrics, Gynecology and Reproductive Sciences, University of Vermont, Burlington, VT, USA

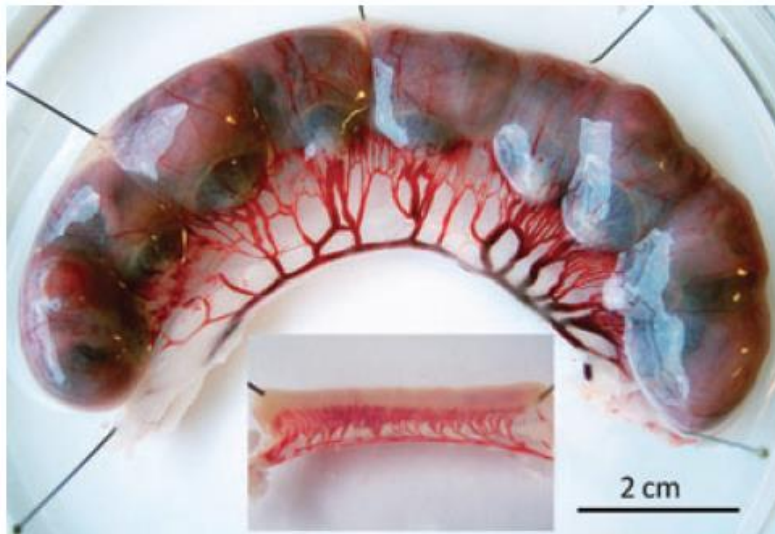


Fig. 1. Extent of uterine vascular remodelling during pregnancy in the rat. Photograph showing one uterine horn from a pregnant (top) versus age-matched nonpregnant (inset, bottom) rat showing the extent of vascular growth that occurs during gestation. The main uterine arteries and veins run parallel to the uterine wall and are connected to it by the smaller arcuate and radial vessels. In the pregnant uterus, nine placentas and foetuses are also visible. Both photographs are equally scaled.

Does E2 help
the remodelling
process ?

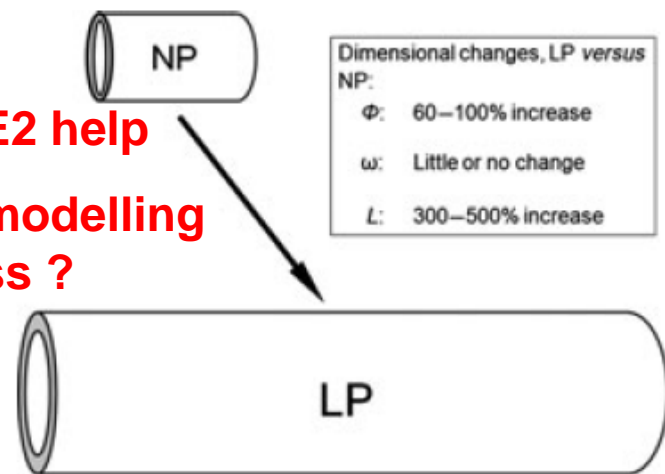
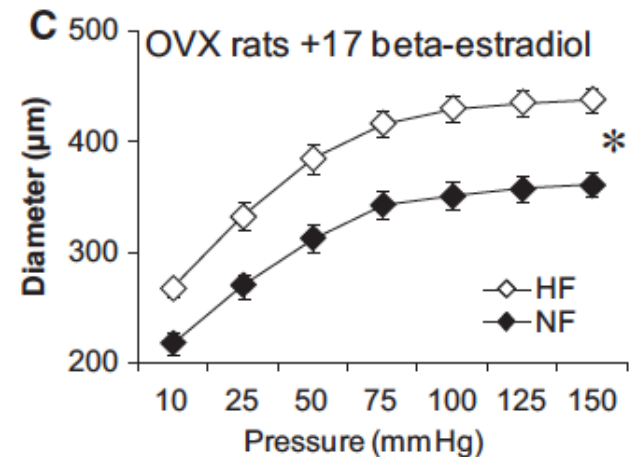
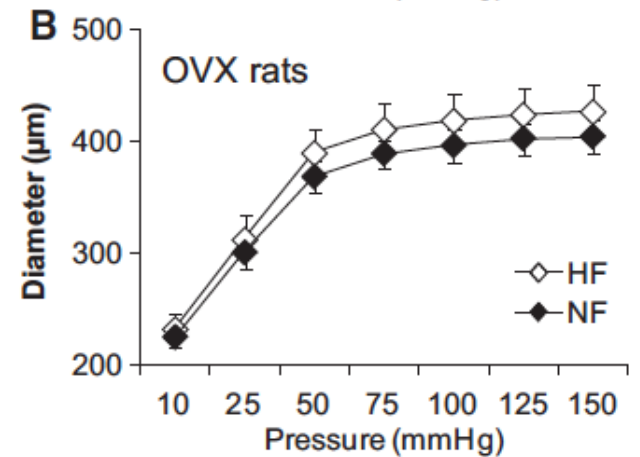
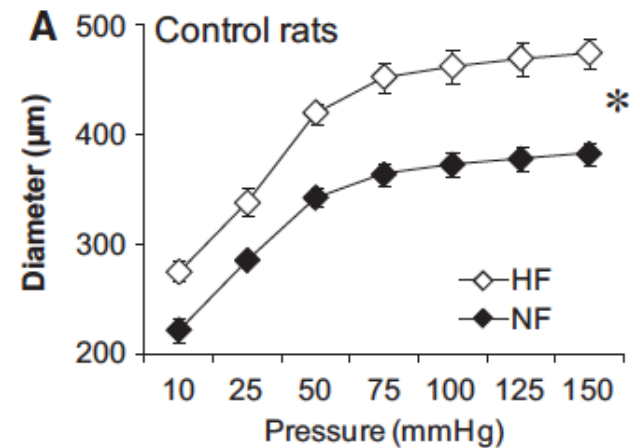
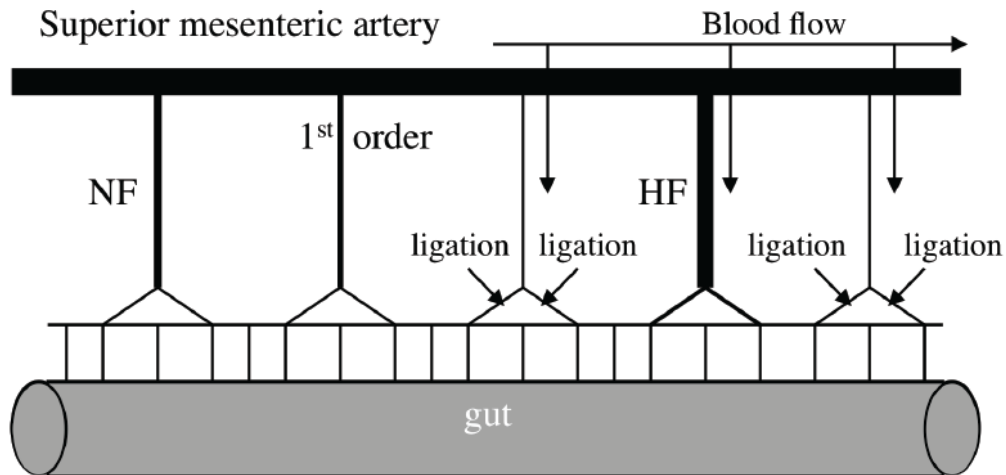


Fig. 2. Three-dimensional pattern of gestational uterine artery remodelling. Based on the published data, this drawing shows the approximate extent of uterine radial artery widening (circumferential growth), lengthening (axial elongation) but not wall thickness during gestation in the rat. Φ = diameter; ω = wall thickness; L = axial length; LP = late pregnant (day 20/22); NP = age-matched nonpregnant.

Role of estrogen in the rodent model of Blood Flow Mediated Remodelling of resistance arteries.

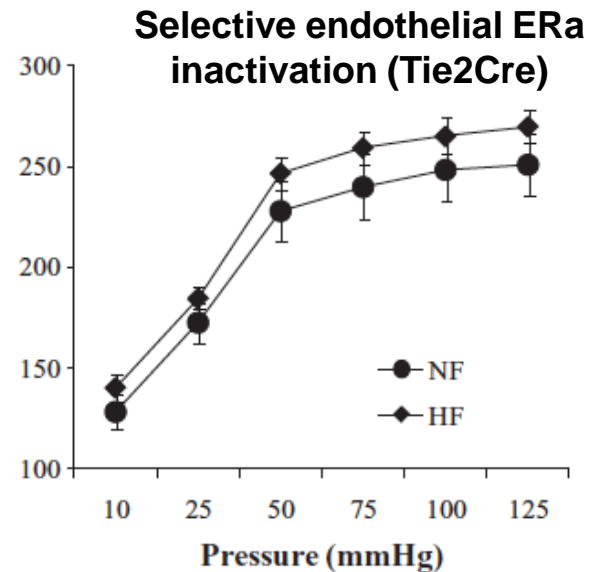
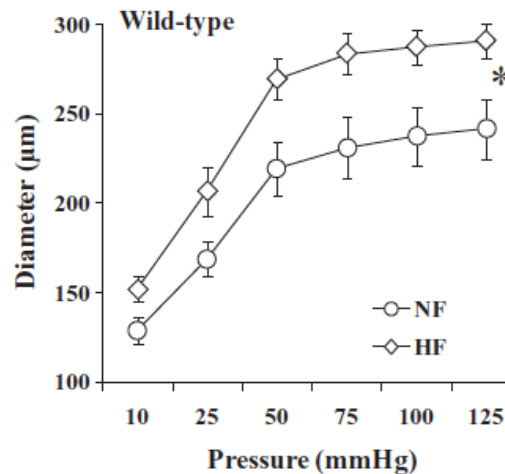
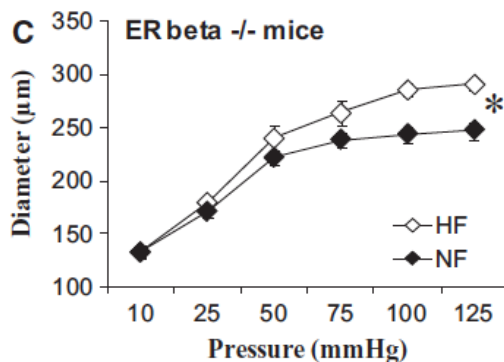
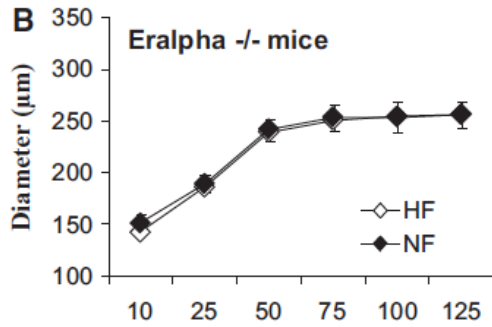
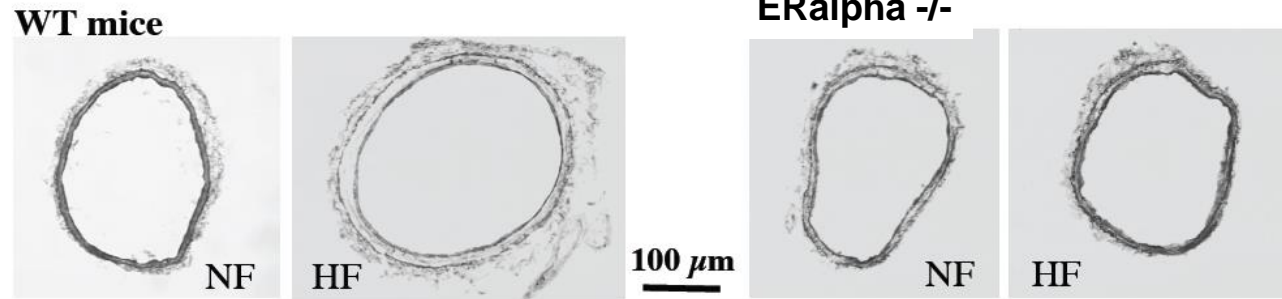
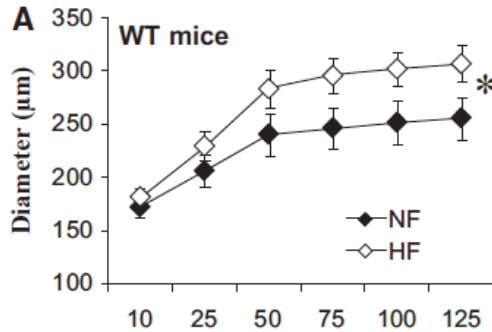
Daniel Henrion et al. , INSERM et Université d' Angers



Key Role of Estrogens and Endothelial Estrogen Receptor α in Blood Flow–Mediated Remodeling of Resistance Arteries

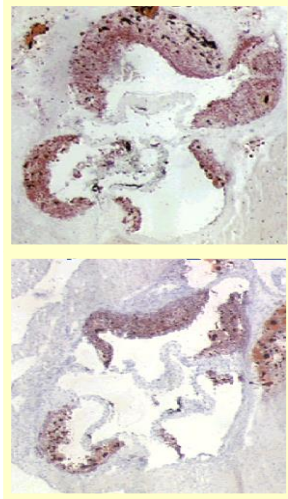
K. Tarhouni, A. L. Guihot, M. L. Freidja, B. Toutain, B. Henrion, C. Baufreton, F. Pinaud, V. Procaccio, L. Grimaud, A. Ayer, L. Loufrani, F. Lenfant, J. F. Arnal, D. Henrion

Arterioscler Thromb Vasc Biol. 2013;33:605-611

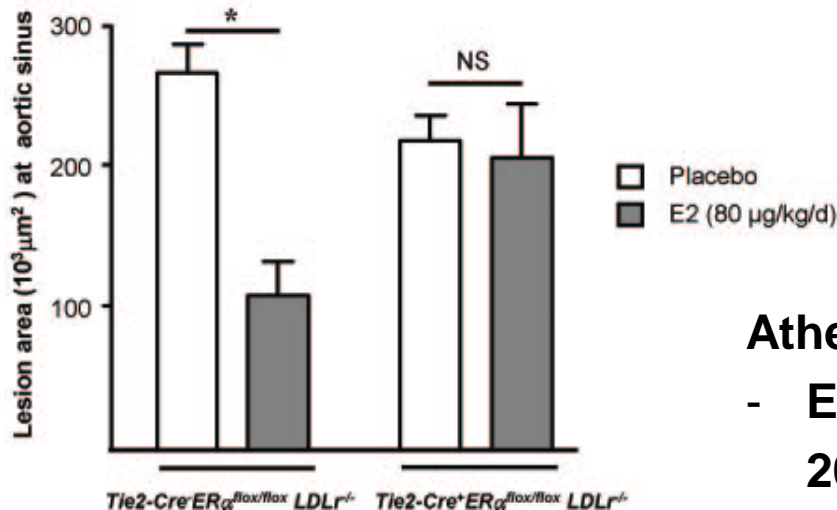
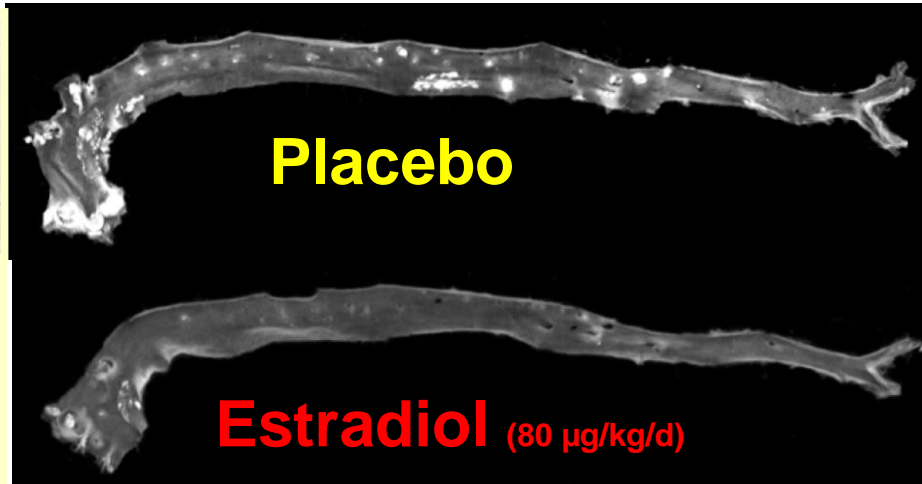


Endogenous and exogenous E2 prevents early atheroma in all models of hypercholesterolemic animals : from mouse to monkey...

Aortic root



« En face » aorta: atheroma deposit in white



Atheroprotection by E2 in mice through:

- Endothelial ERα (Tie2-Cre; Circulation 2009)
- Nuclear ERα (AF2° ; PNAS 2011)

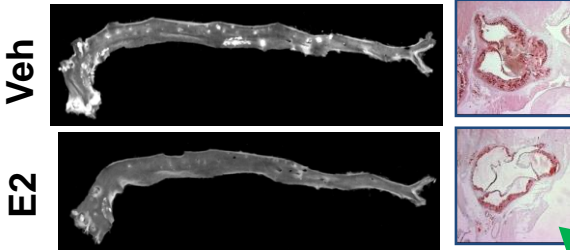


Vascular effects of E2 : Key role of endothelial ER α

Atheroprotection

Maeda, Korach et al. 2000

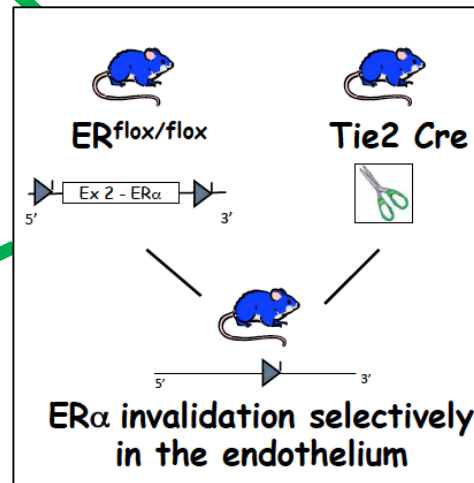
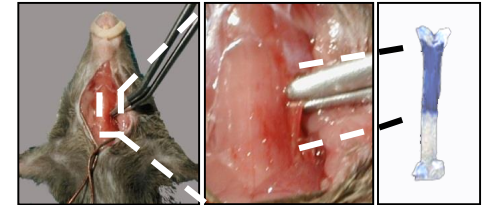
Billon Gales et al. Circulation 2009



Endothelial healing

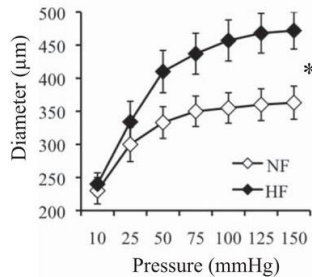
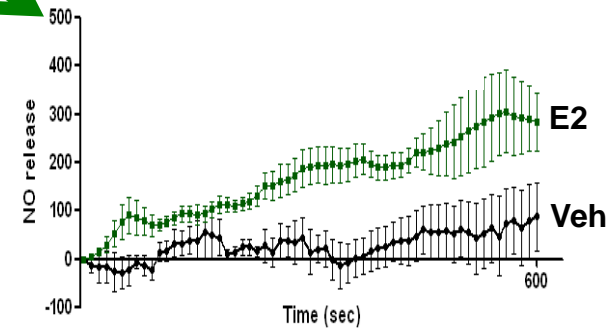
Brouchet et al. Circulation 2001

Toutain CE et al. ATVB 2009



Leads to abrogation of most of the vascular effects of E2

Endothelial NO production

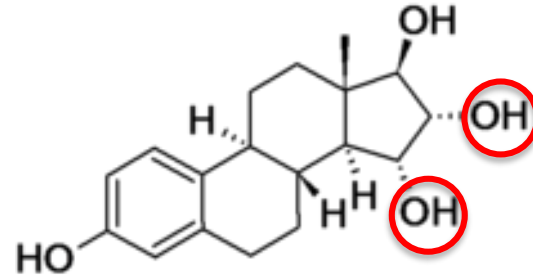


Flow mediated remodeling

Tarhouni K. et al. ATVB 2013

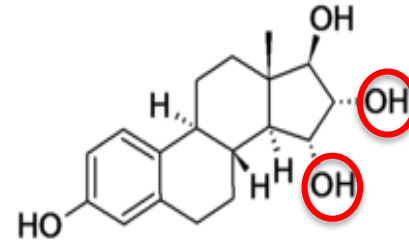
5- Medical implications: SERMs uncoupling membrane /nuclear actions ?

Estetrol (E4)



- is a natural estrogen, that is produced only by the primate fetal liver during pregnancy.
- circulates at high concentrations (up to 30 nM) in the foetal plasma.
- has a very long half-life (30 hours in women).

**Estetrol (E4) : fetal estrogen
(Mithra Pharma, Belgium)**



**In combination with a progestin (drospirenone or levonorgestrel),
E4 blocks ovulation**

**E4 has less effect on hemostatic biomarkers : « liver friendly »
compared with ethinylestradiol (EE)**

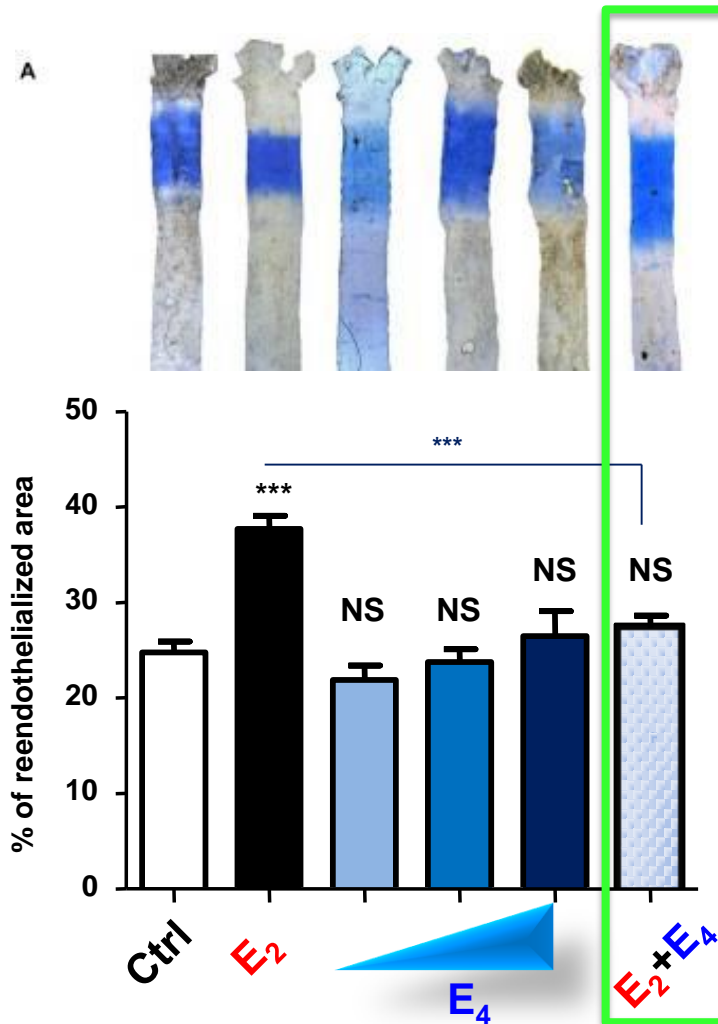
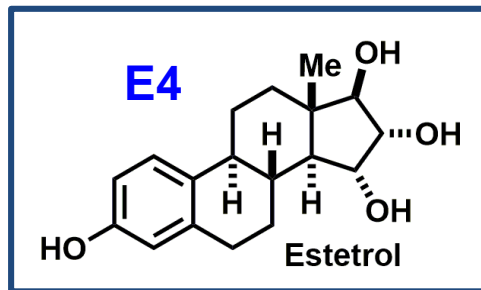
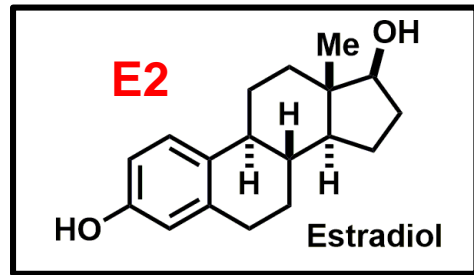
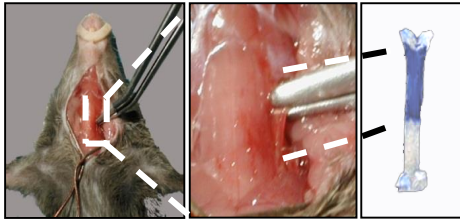
E4 could be safer than EE in terms of thromboembolic risk.

E4 (20 mg) currently developed :

- **in oral contraceptive (clinical phase III in progress)**
- **at menopause , as E4 prevents Hot Flushes (clinical phase II)**

Q : What are the mechanisms of action of E4 ?

However, E4 does not accelerate endothelial healing, thus appears devoid of membrane actions...

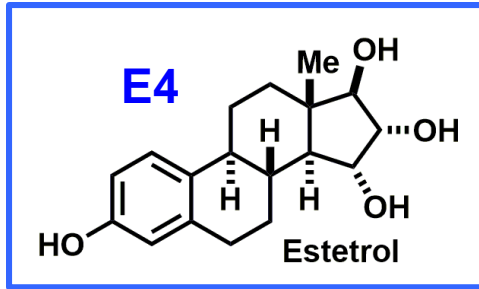


Suggests that Membrane and Nuclear ER α could have different conformations and binding affinities

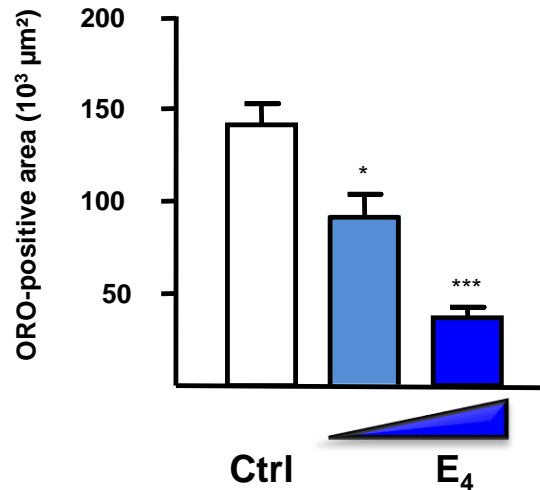
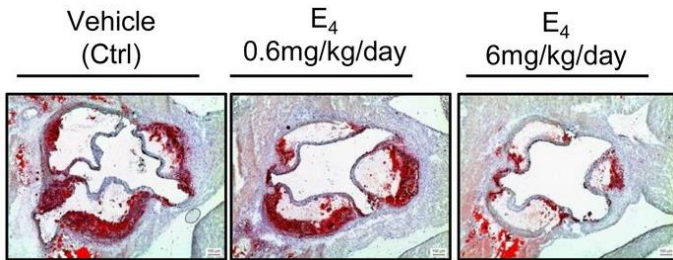
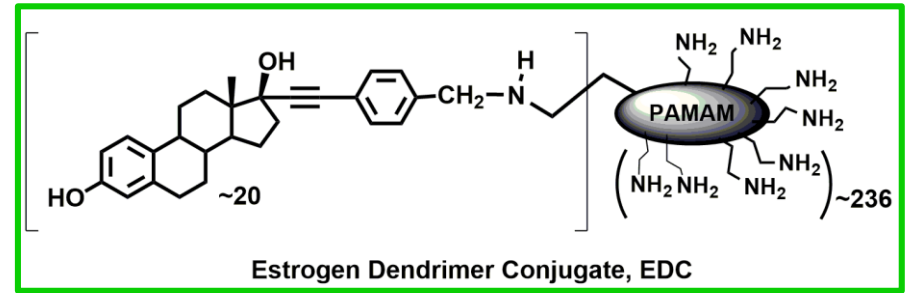
... and even antagonizes the accelerative effect of E2

Nuclear activation of ER α by E4 is sufficient to induce atheroprotection

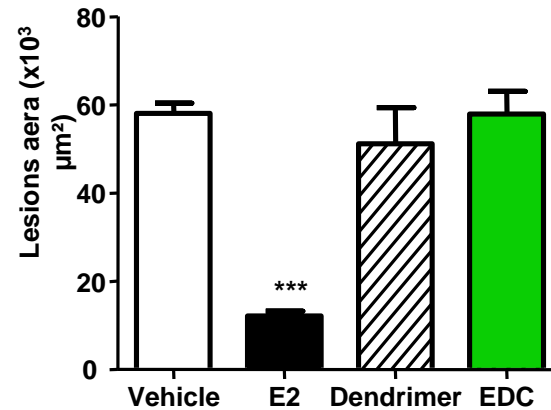
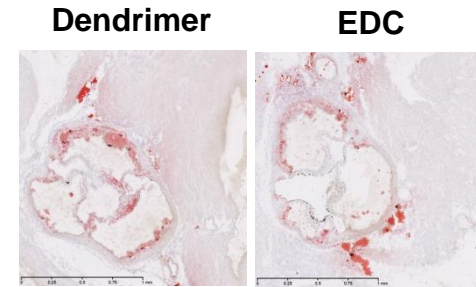
Membrane activation of ER α by EDC is not sufficient to induce atheroprotection



LDLR^{-/-}



Abot et al. EMBO Mol Med 2014



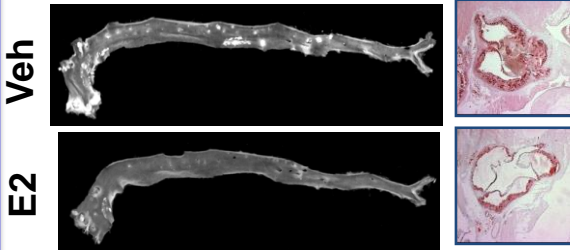
Unpublished data

Vascular effects of E2 depend on either **membrane** or **nuclear** ERa

Atheroprotection

Maeda, Korach et al. 2000

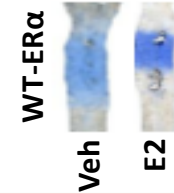
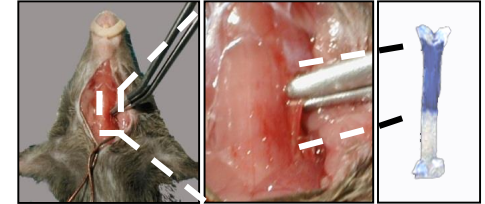
Billon Gales et al. Circulation 2009



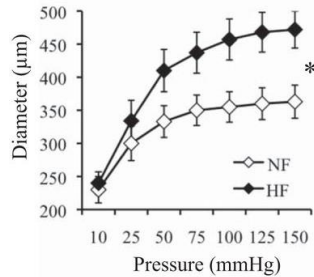
Endothelial healing

Brouchet et al. Circulation 2001

Toutain CE et al. ATVB 2009



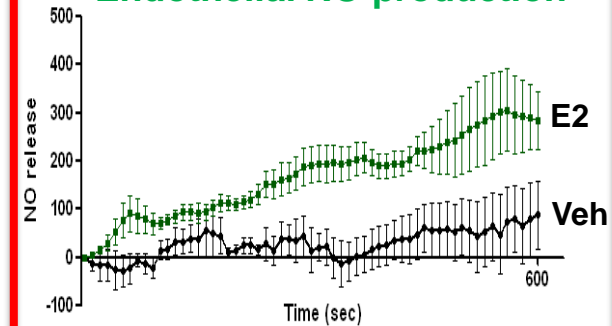
E2



Flow mediated remodeling

Tarhouni K. et al. ATVB 2013

Endothelial NO production



Nuclear ERa-dep

Membrane ERa-dep

Conclusions – Take home messages

Combination of transgenic mouse models and pharmacological tools have begun to delineate the respective roles of **Nuclear** and **Membrane** ER α in some tissues/functions:

1- Female **fertility** requires both.

2- **Uterus growth** : mainly Nuclear ER α actions.

3- Endothelial effects mediate most of the vasculoprotective actions of E2 :

- Some effects are (**NO production and reendothelialization**) are dependent on **Membrane ER α**

- But others (**atheroprotection and FMR**) rely on **Nuclear ER α**

5- **Estetrol (E4)** is a unique fetal estrogen

- ER α **nuclear agonist**/membrane antagonist : a new natural SERM !

- May be a new oral contraceptive or treatment for menopause ?



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MEMBRANE AND NUCLEAR ESTROGEN RECEPTOR ALPHA ACTIONS: FROM TISSUE SPECIFICITY TO MEDICAL IMPLICATIONS

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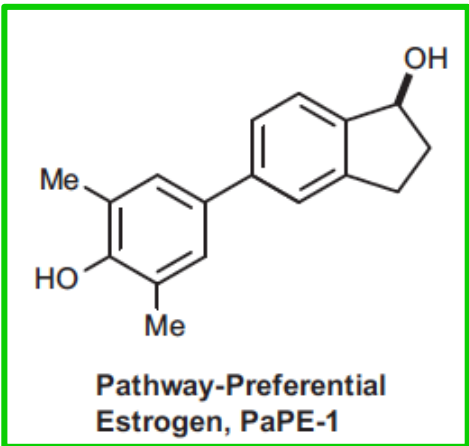
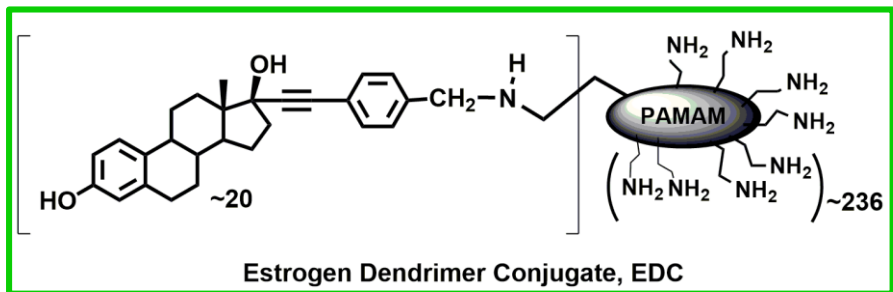
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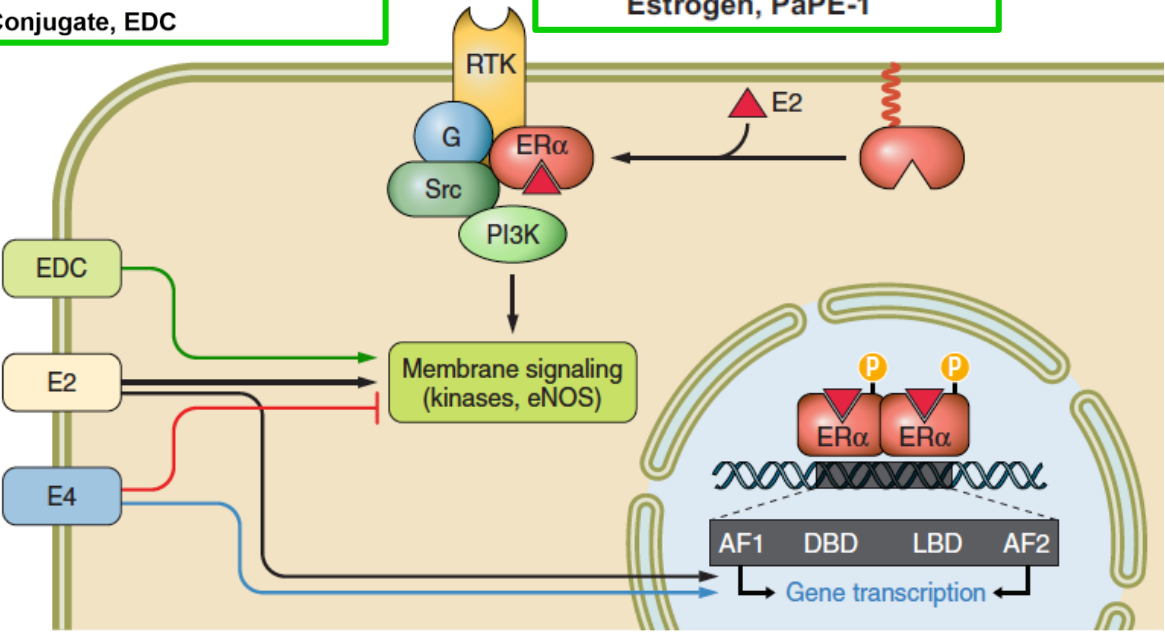
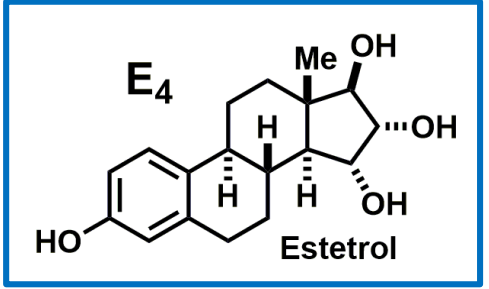
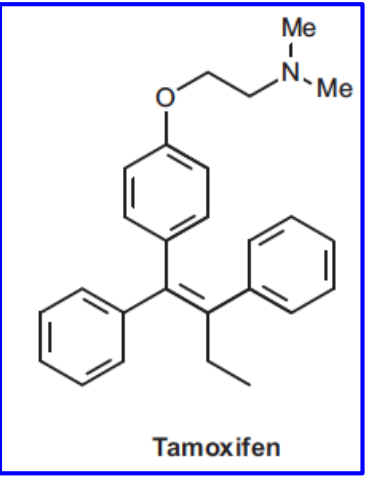
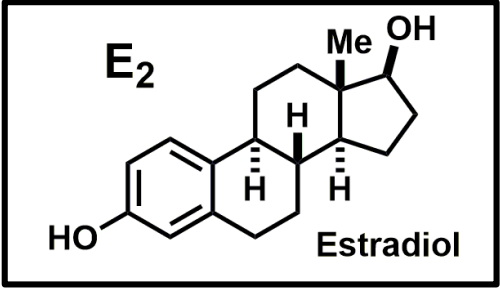
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JM Foidart (Mithra, Liège Be)
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Profile of old and new SERMs



J Katzenellenbogen
Z Madak-Erdogan
Sci Sign. 2016



Nuclear effects