The role of Lipoxin A_4 in the endometrium and in endometriosis

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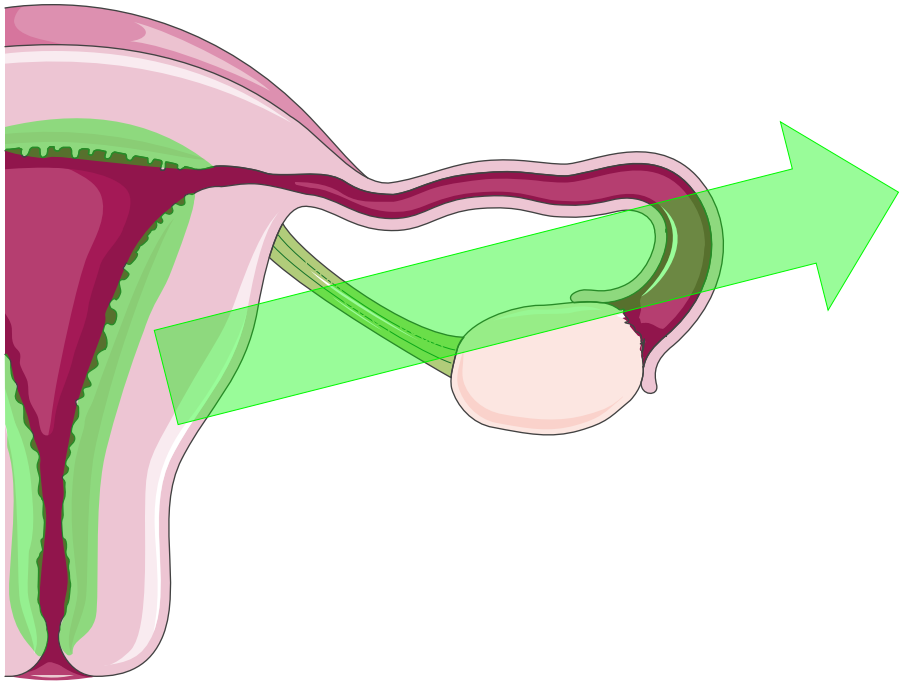
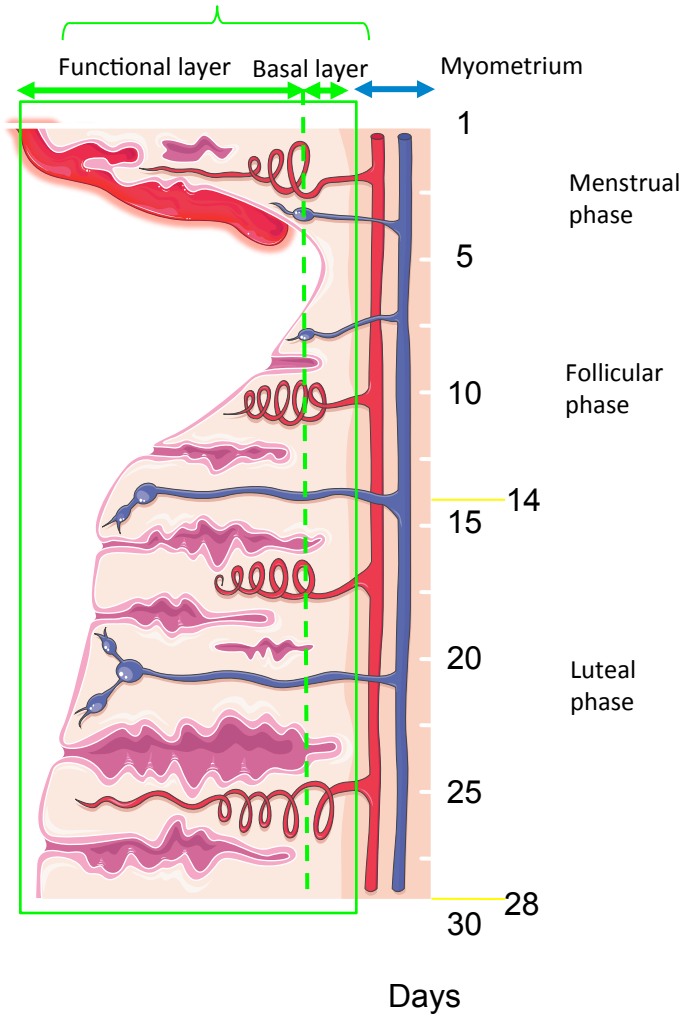
Presentation overview

- Introduction: the endometrium, endometriosis, ER signalling, inflammation and its resolution; Lipoxin A_4
- Results: *in vitro*, *in vivo*, studies using clinical samples
- Conclusion

The Endometrium – a unique tissue

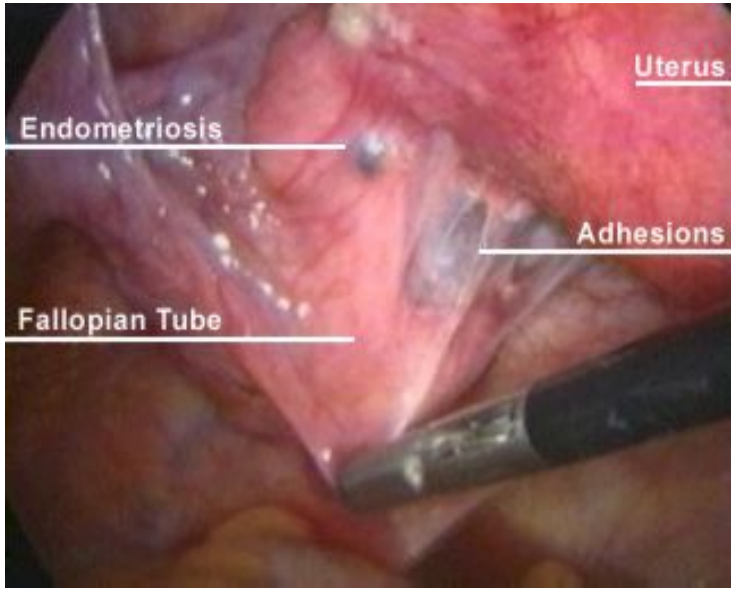
The endometrial lining undergoes cyclic regeneration under the influence of hormones and immune mediators





Endometriosis

- Presence of endometrial tissue outside the uterine cavity
- An estrogen-dependent, inflammatory disease
- Affects up to 10% of women of reproductive age (176 million worldwide)
- Delay of up to 11 years prior to diagnosis, no specific biomarkers

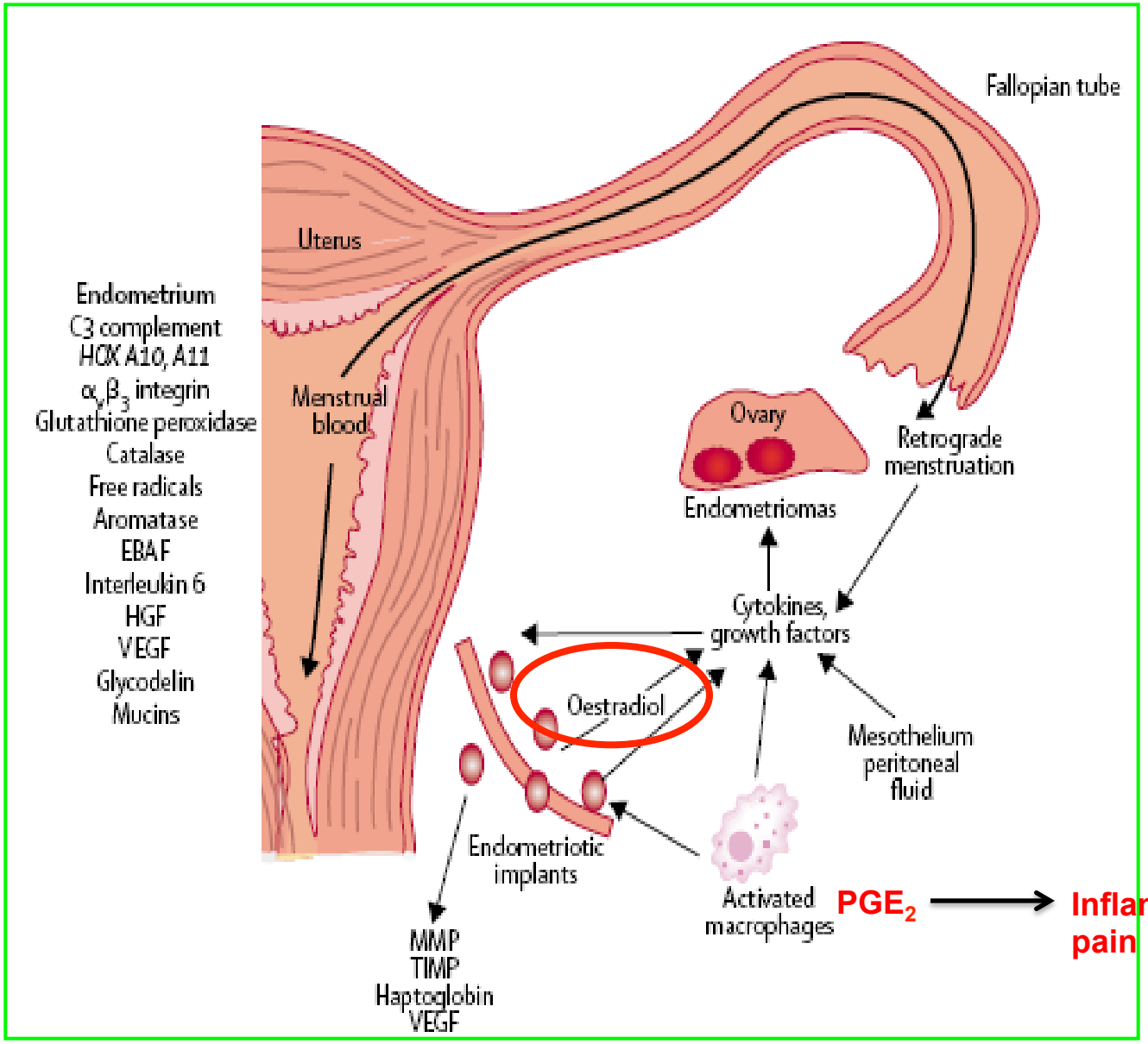


The main clinical features are:

- chronic pelvic pain
- dysmenorrhea
- abdominal pain
- dysuria
- dyschezia
- infertility

- Infertility has been reported in more than 30% of women affected with the disorder

Endometriosis: proliferation and inflammation



From Giudice L.C., and Kao. (2004).

Treatment strategies for endometriosis

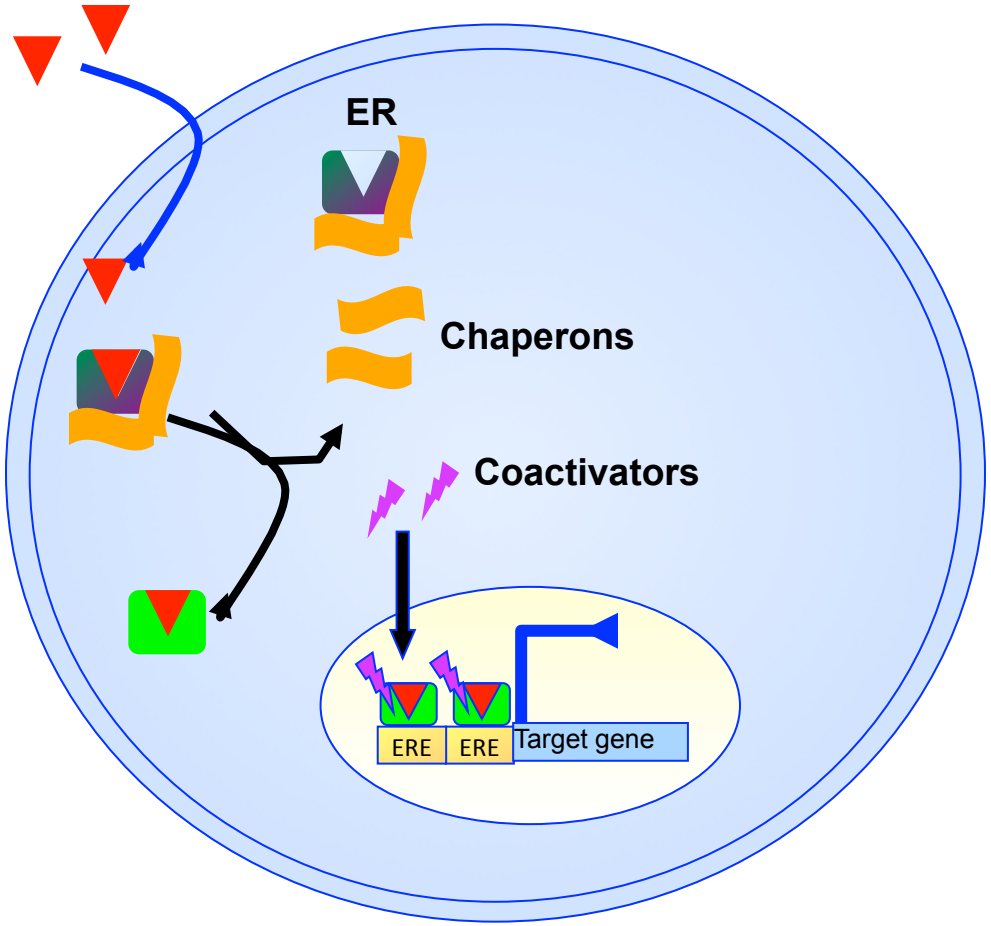
Current strategies aim to stop disease progression and reduce pain through:

- surgical intervention
- reducing estrogen levels through the use of aromatase inhibitors
- gonadotrophin-releasing hormone (GnRH) analogues
- oral contraceptive pills
- progestins e.g. Dienogest
- anti-inflammatory therapies: more studies needed

Limitations associated with these drugs include a **negative impact on fertility and undesireable side effects**.

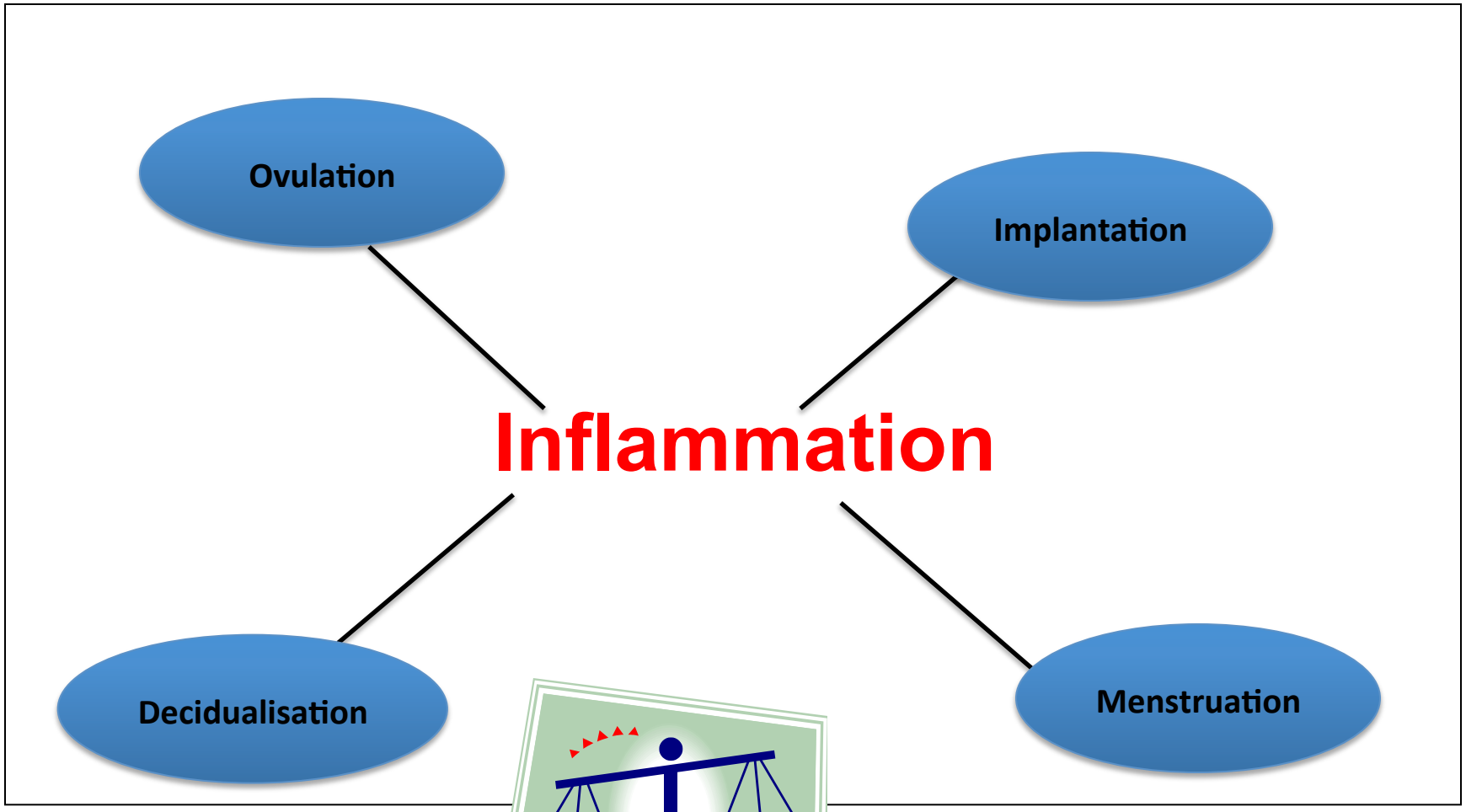
A molecule with a dual anti-estrogenic effect (or which negatively impacts E2 metabolism) and anti-inflammatory effect represents a potential therapeutic.

Estrogen receptor signalling



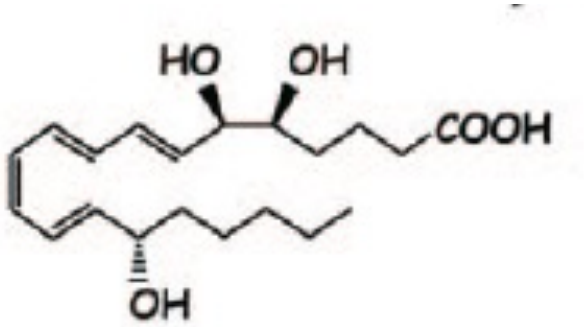
Target genes: Progesterone Receptor, GREB1, cyclin D1, pS2, cathepsin D, c-fos, c-myc

Inflammation is an integral part of endometrial physiology



Lipoxin A_4

- The term Lipoxin is an acronym for Lipoxygenase interaction products
- Arachidonic acid metabolite with potent anti-inflammatory and pro-resolution properties
- Produced in **nanomolar** levels by transcellular biosynthesis during specific cell to cell interactions at sites of inflammation

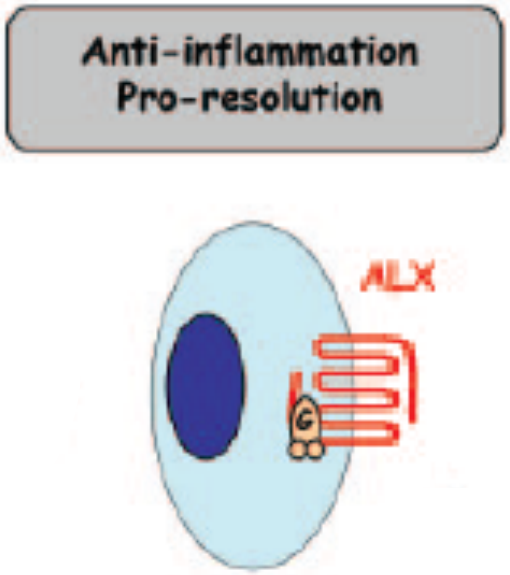


LXA_4

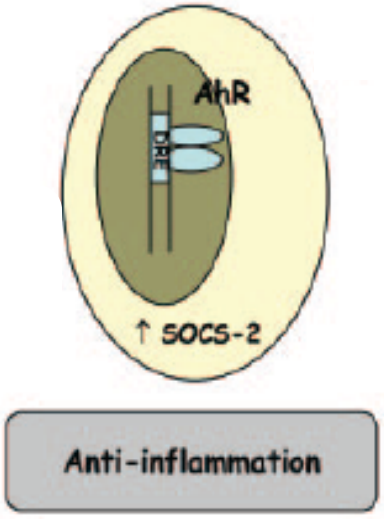
(Serhan C and Sheppard,1990; Serhan, 2007; Spite & Serhan, 2010)

LXA_4 receptors

1. Formyl peptide receptor 2 / Lipoxin A_4 receptor (FPR2/ALX)

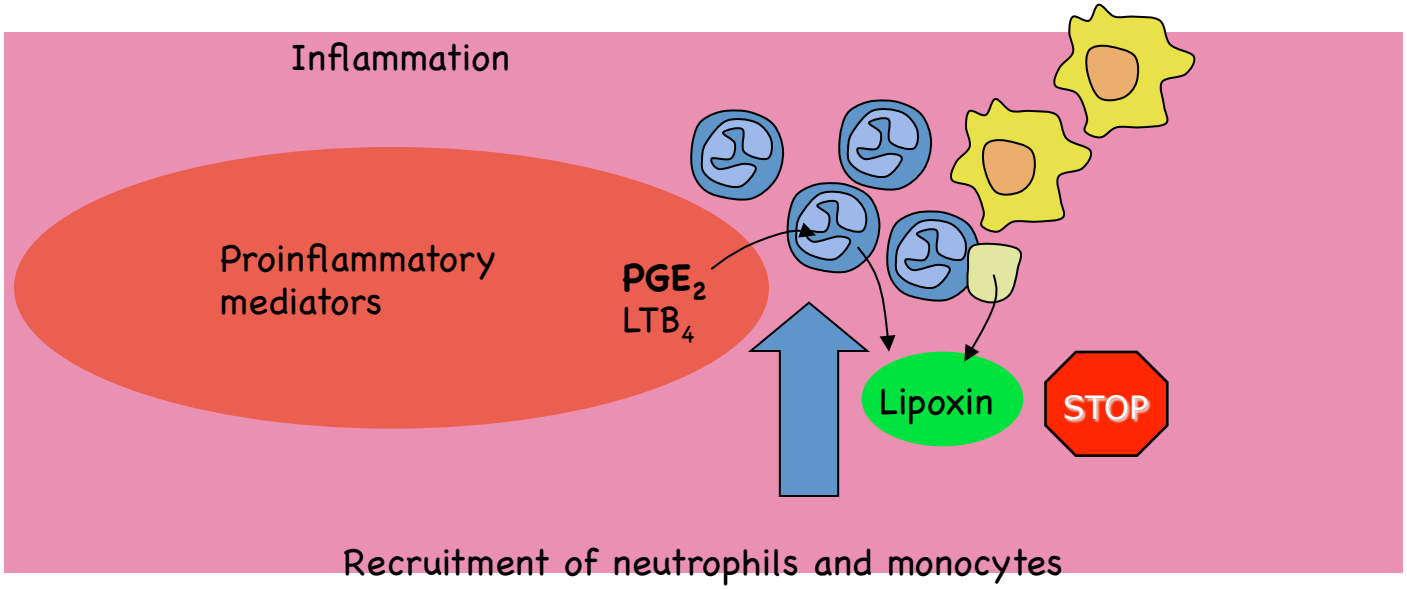


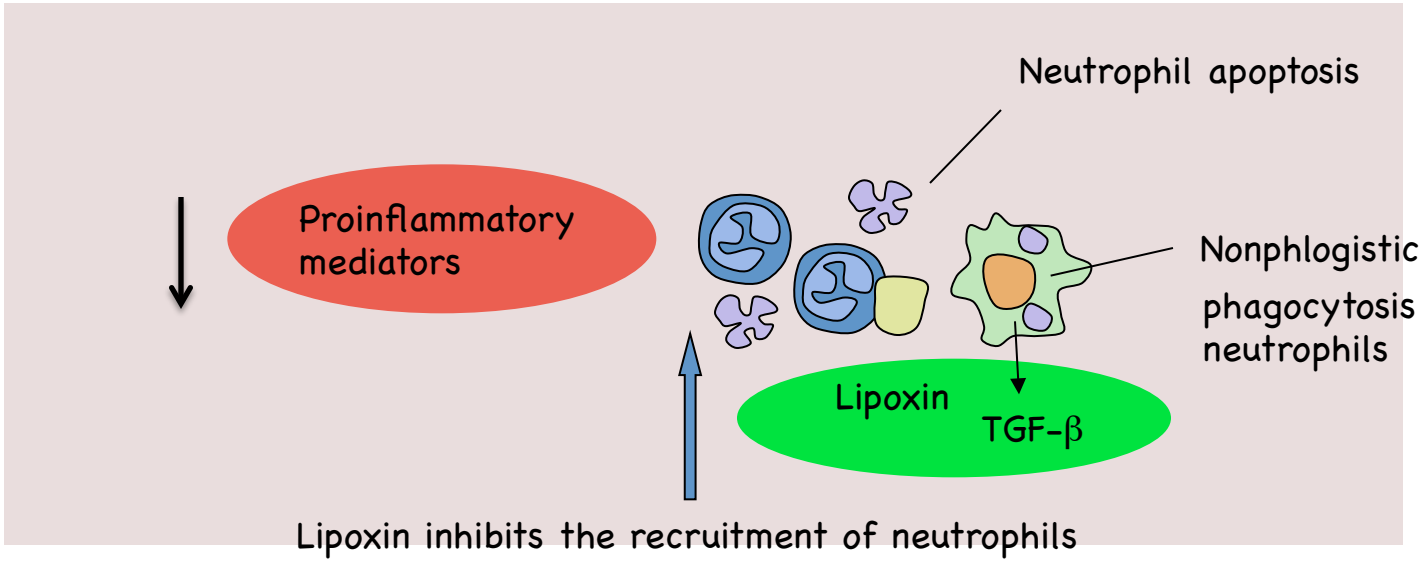
2. Aryl hydrocarbon receptor (AhR)



Fiore *et al.,* 1994; Schaldach et al., 1999

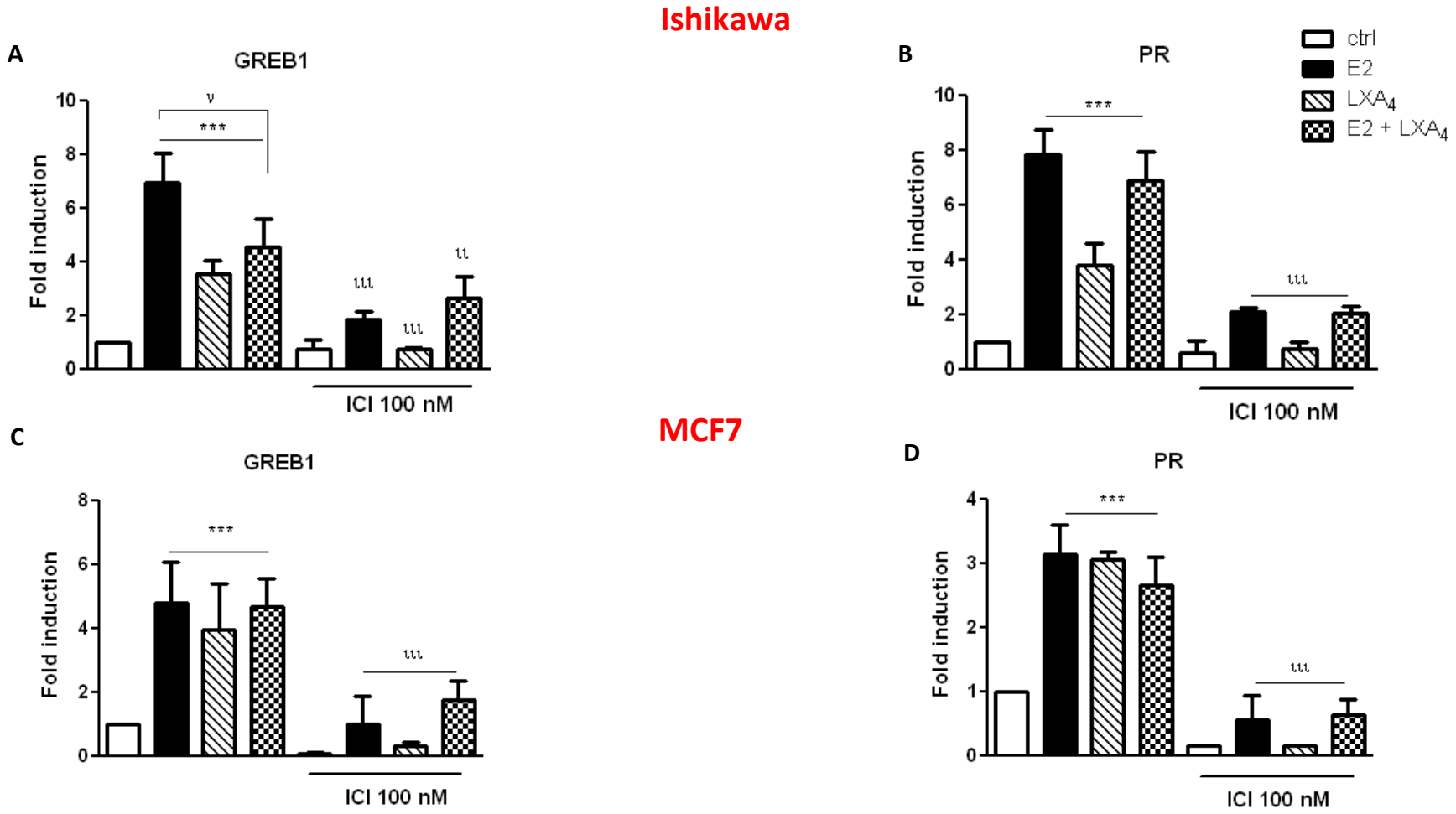
Role of Lipoxin A_4 in the resolution of inflammation





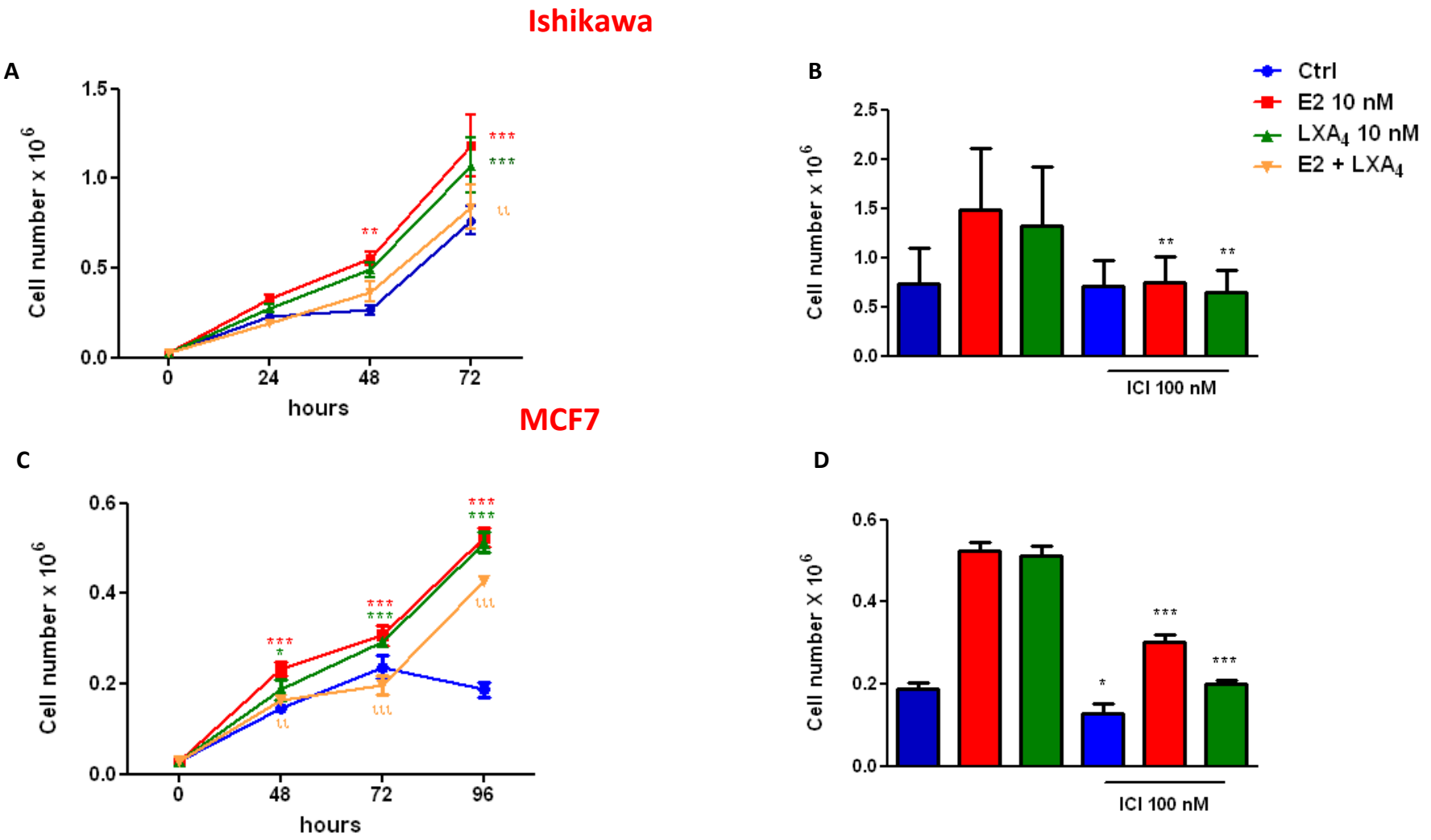
Return to tissue homeostasis

LXA_4 modulates endogenous estrogen-regulated gene expression

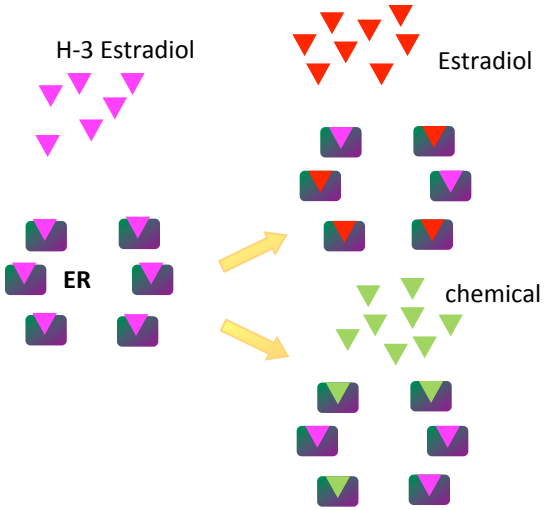


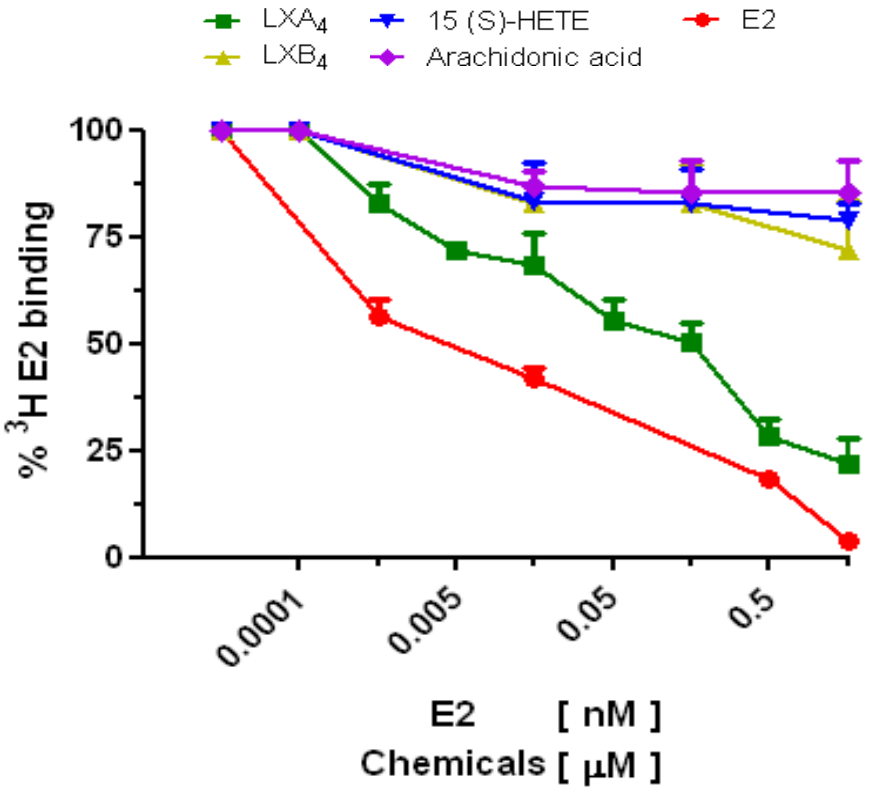
LXA_4 alters the expression of estrogen-regulated genes specifically through ER

LXA_4 alters endometrial and breast epithelial cell proliferation



LXA_4 binds to ER



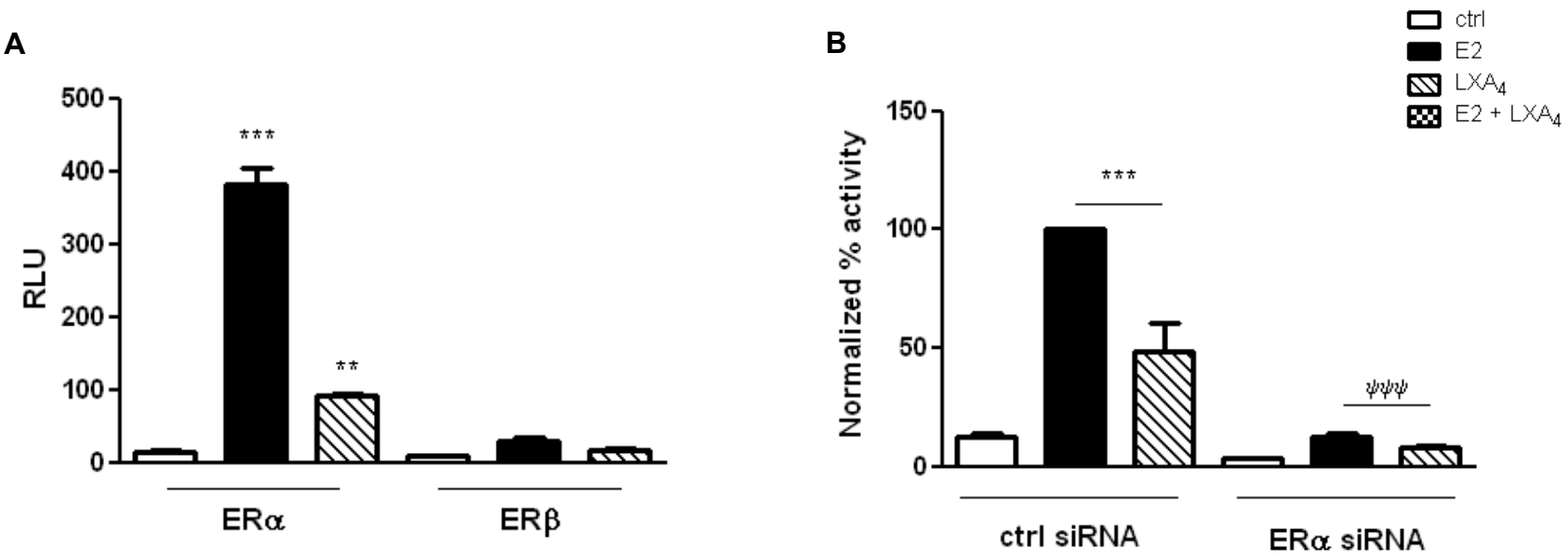


Only LXA_4 directly binds ER at physiologically relevant concentrations with an IC_{50} of 46 nM

LXA_4 signals via ERα

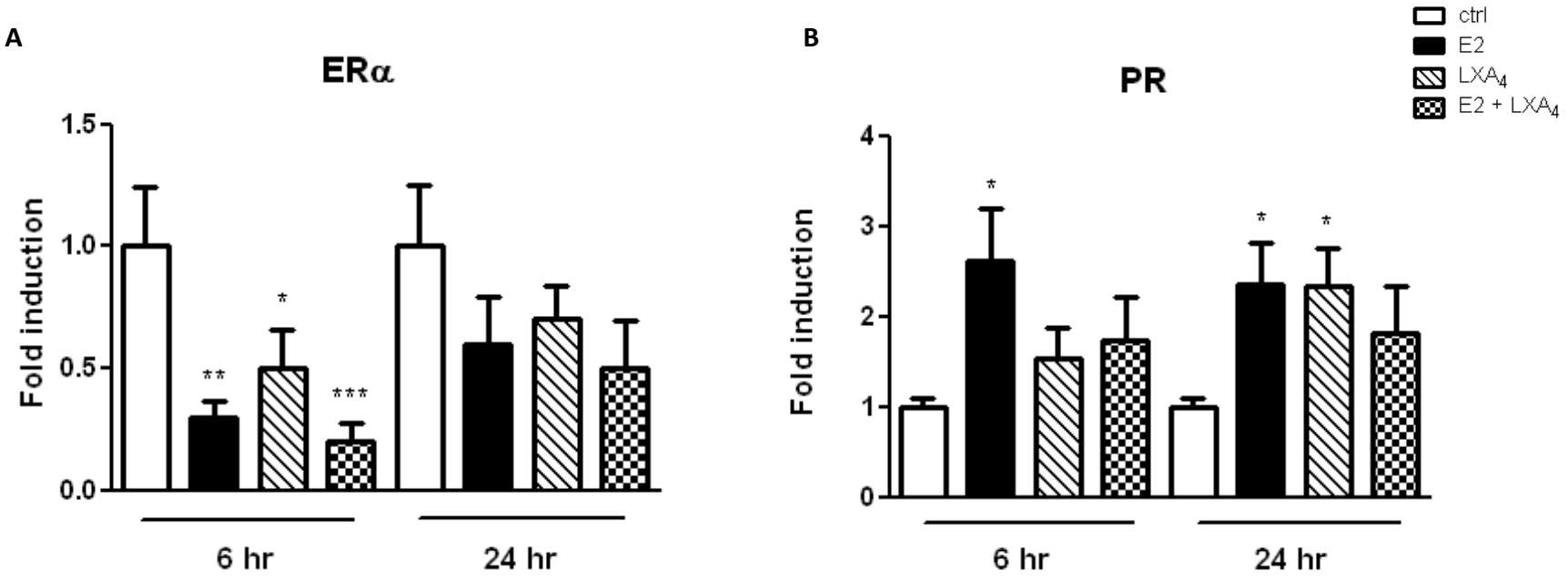
HeLa: ER negative cells

Ishikawa



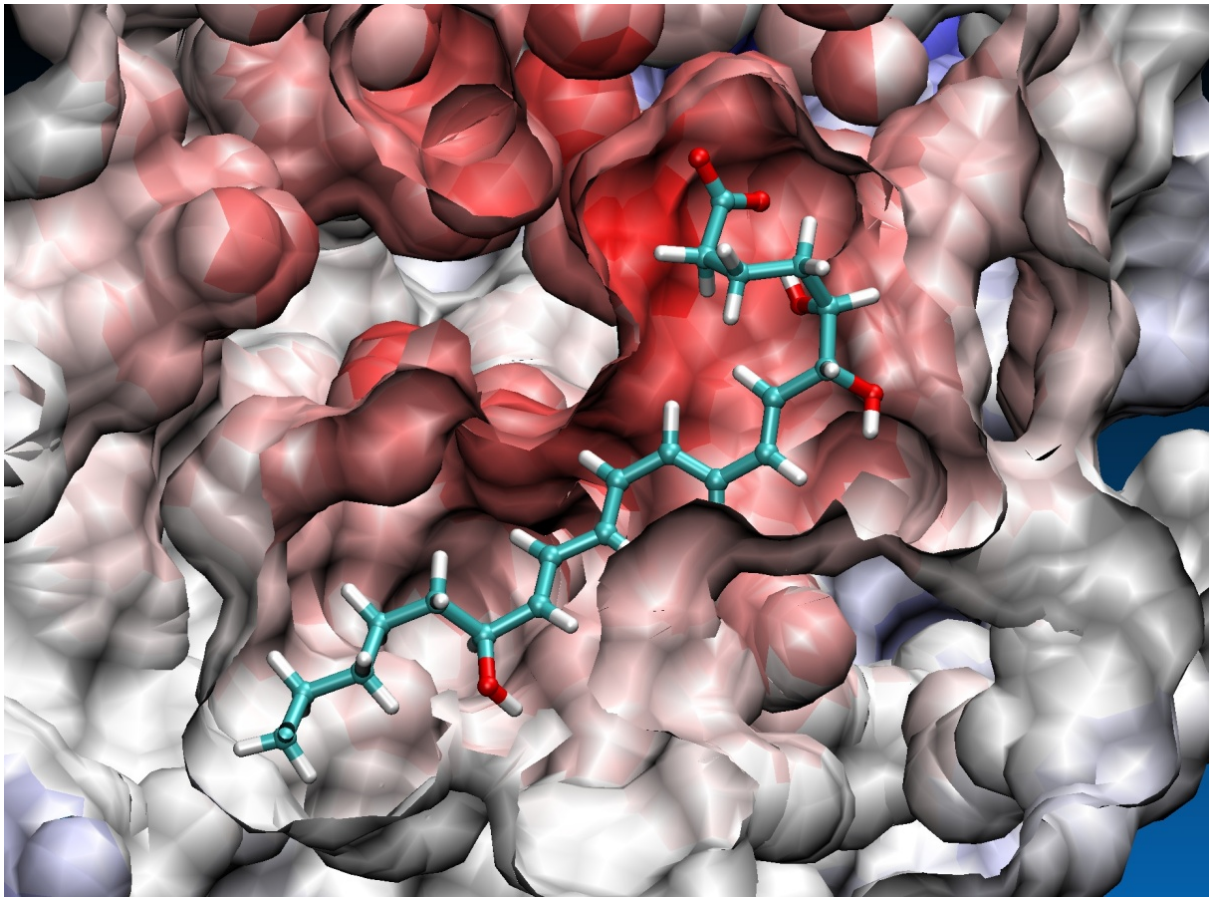
FPR2/ALX and AhR not involved.

LXA_4 exhibits estrogenic activity *in vivo*



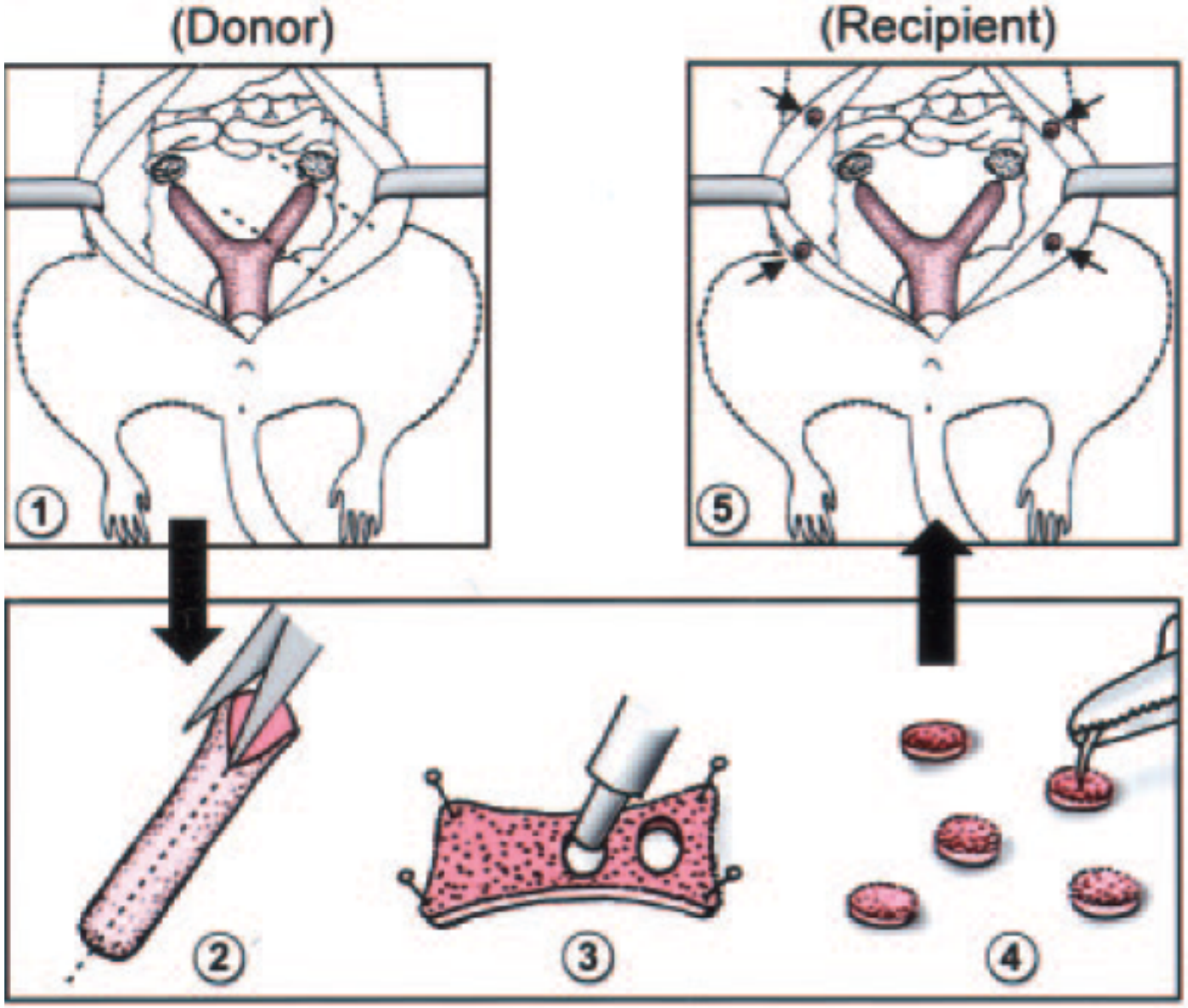
Russell, Gori et al. 2011 FASEB J

Computer generated model of Lipoxin A_4 interaction with ERα



> 85% structural similarity between LXA4 and Estriol (E3)

Mouse model of peritoneal endometriosis



Is LXA_4 protective?

C57BL/6 wild type female mice

Sham

PBS/EtOH (IP)
Once/day

Surgical induction of endometriosis

PBS/EtOH (IP)
Once/day

5 µg/Kg LXA_4 in PBS/EtOH (IP)
Once/day

-1 | 0 | +7 | +14 | +21

Start treatment

Surgery and weighing

weighing

weighing

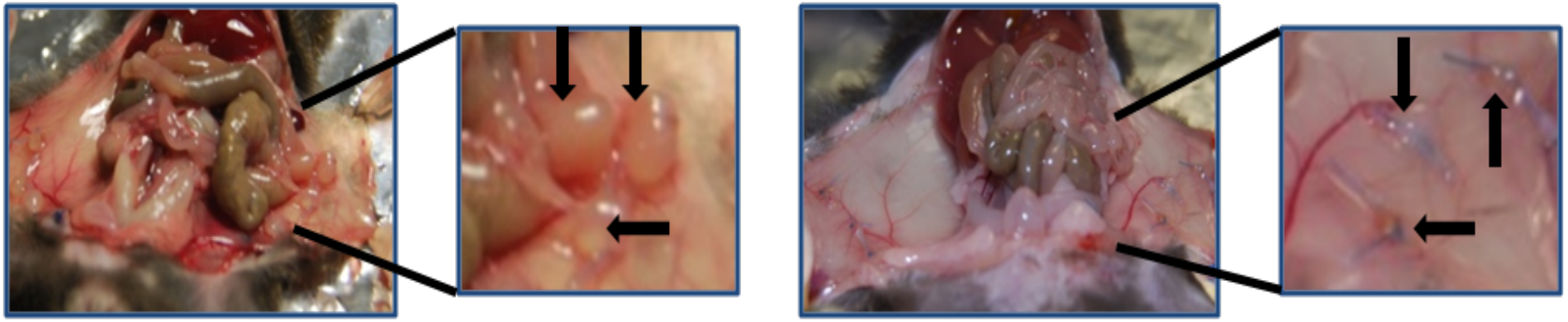
Weighing
Sacrificing
Lesion measurement

Tissue collection

Gene expression profiling, ELISA, IHC

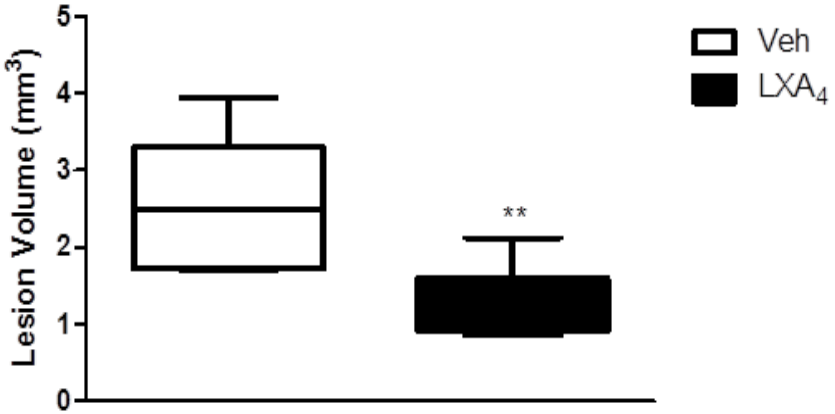
LXA_4 reduces peritoneal lesion volume

A



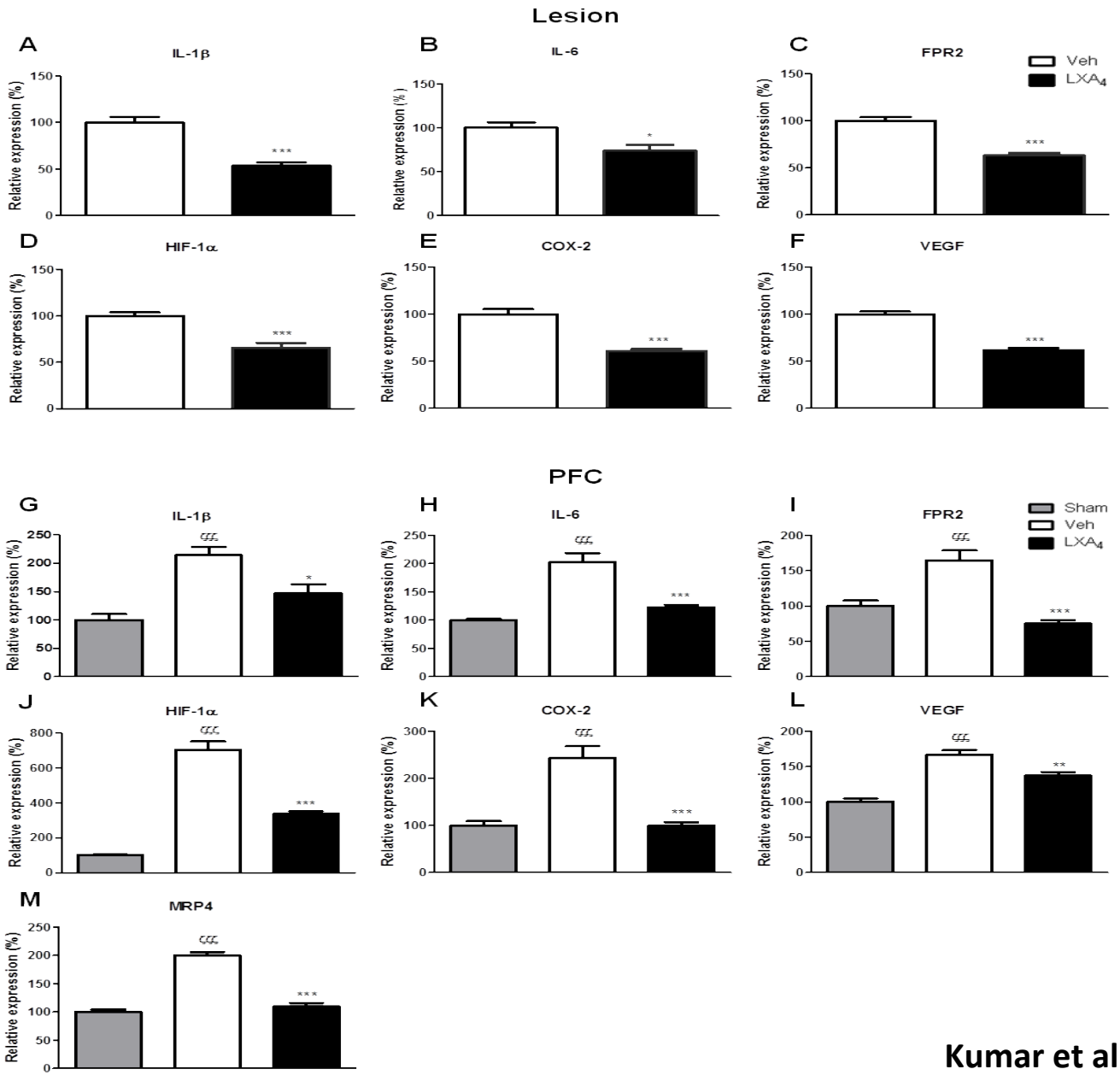
Veh LXA4

B



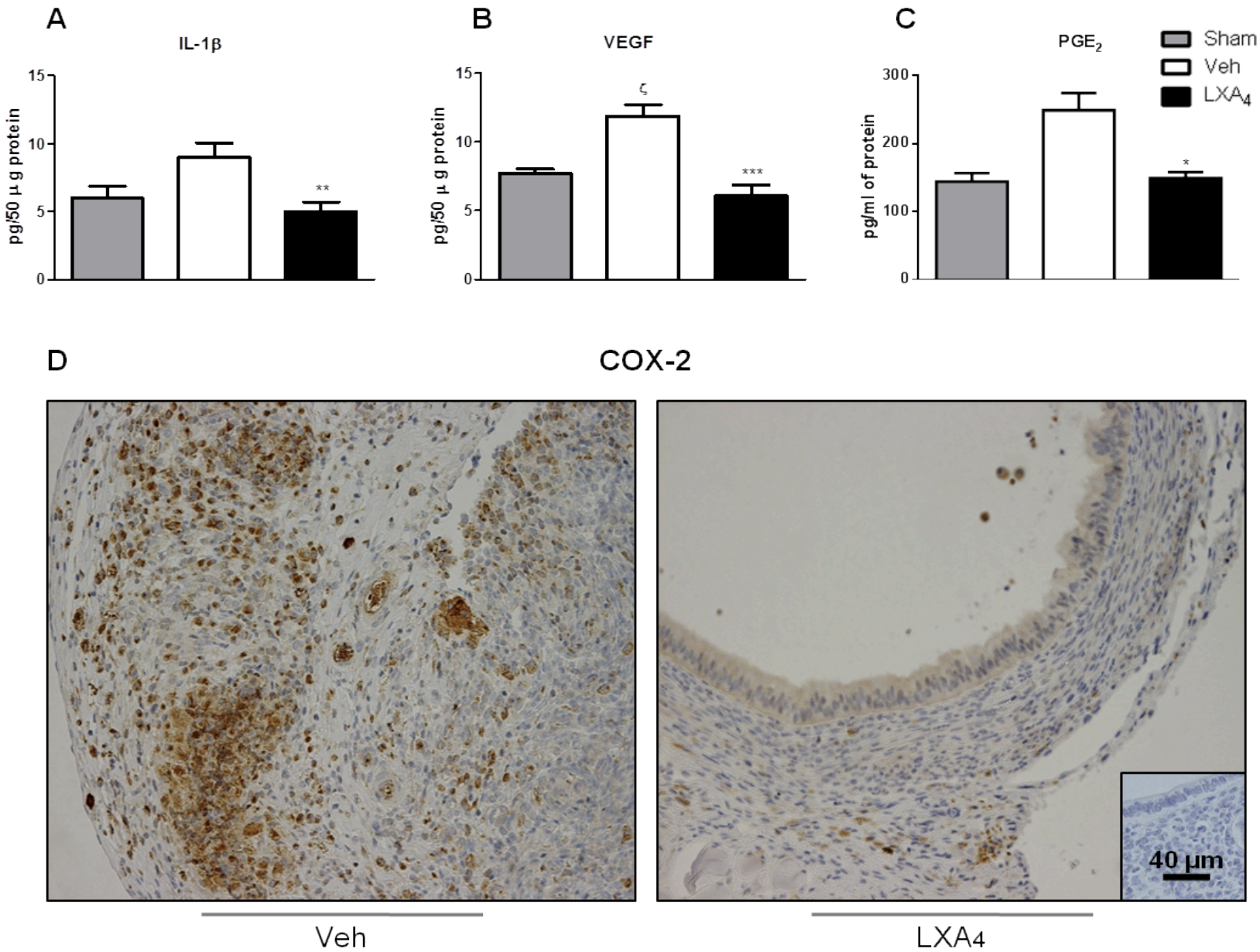
Kumar et al. 2014

LXA_4 targets anti-inflammatory pathways

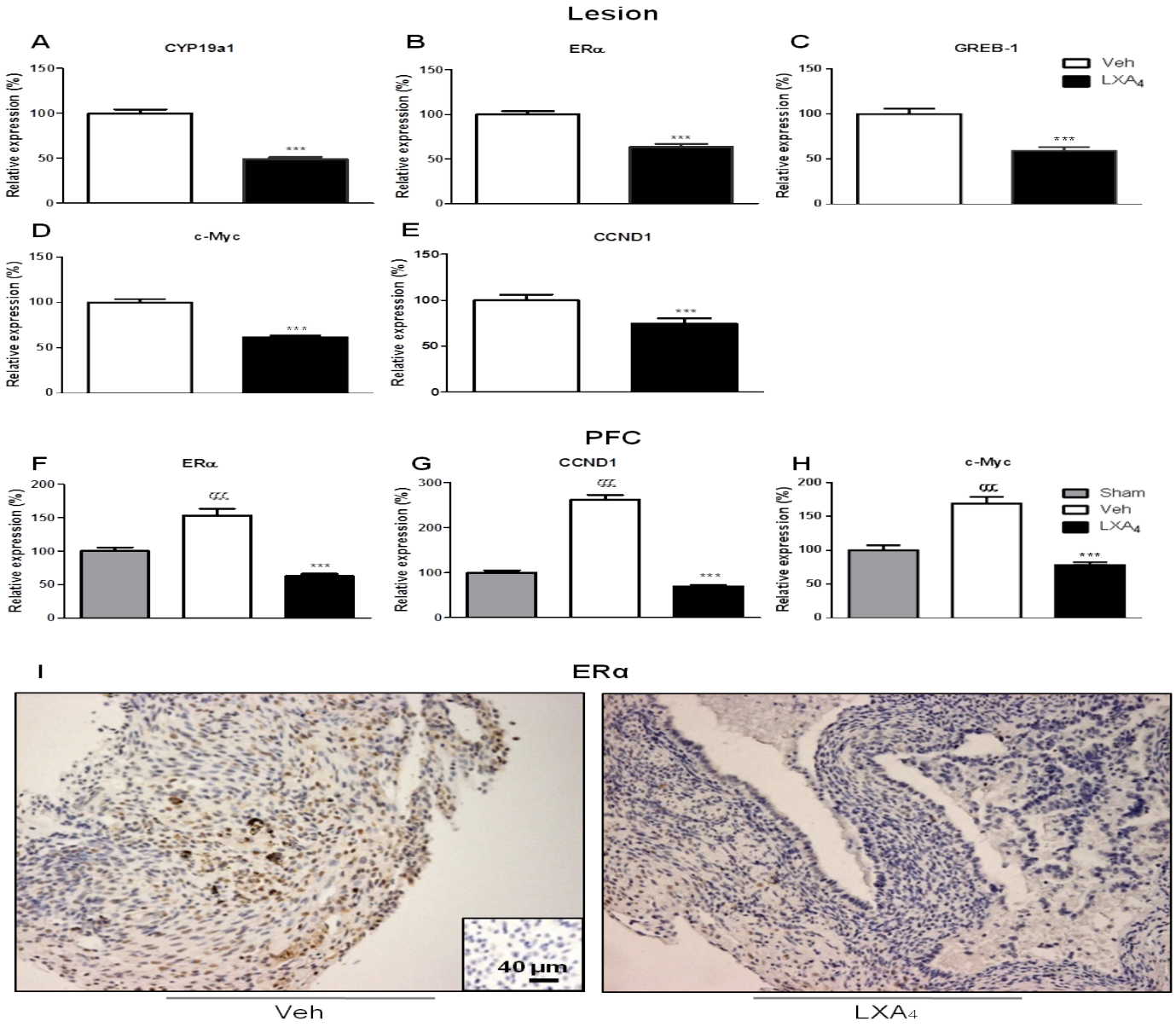


Kumar et al. 2014

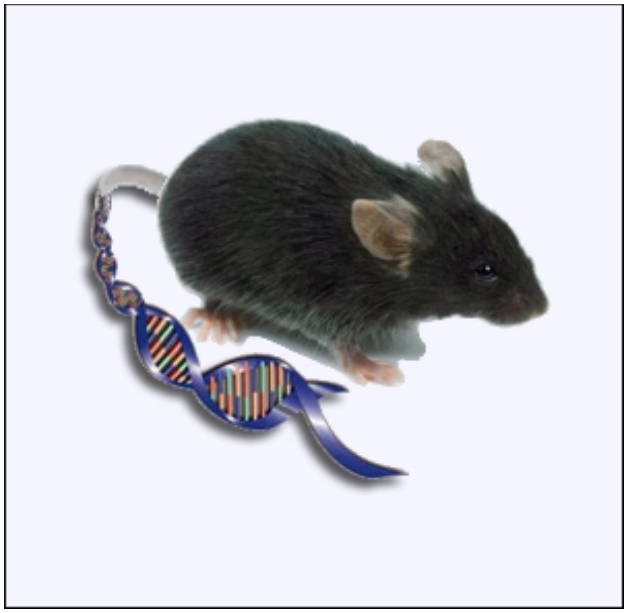
LXA_4 decreases IL-1β, VEGF and PGE_2 production



LXA_4 attenuates E2 production and ER signaling



Experimental models



In vitro

In vivo

Bench to bedside…and back…

Animal Experiments

C57BL/6 wild type female mice

Surgical induction of endometriosis

5 µg/Kg LXA_4 in PBS/EtOH (IP)
Once/day

-1 | 0 | +6 | +14 | +21

Start treatment *De novo*

Surgery and weighing

Start treatment *Established*

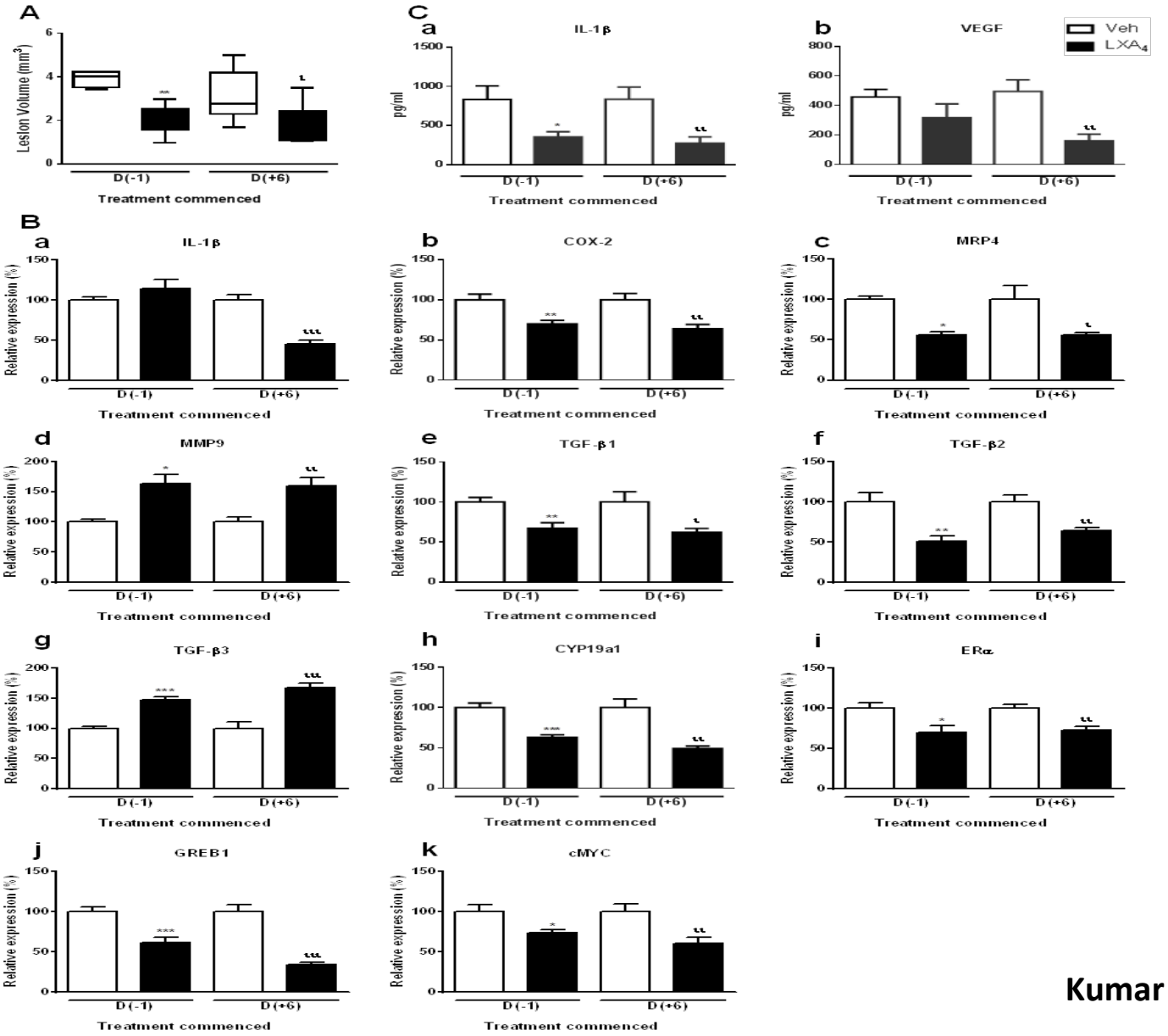
weighing

Weighing
Sacrificing
Lesion measurement

Tissue collection

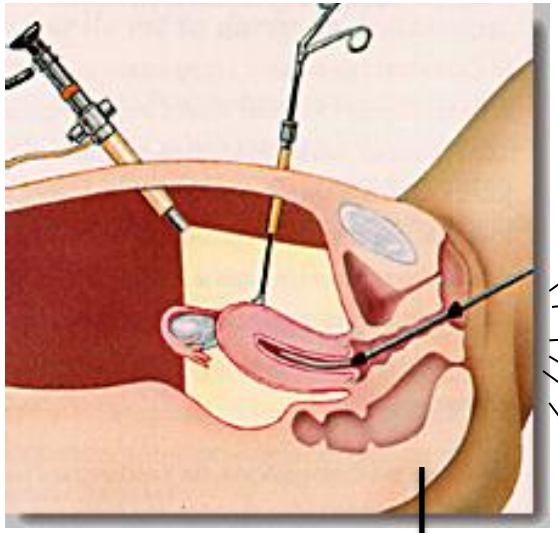
Gene expression profiling, ELISA, IHC

LXA_4 inhibits the progression of *de novo* and established endometriosis

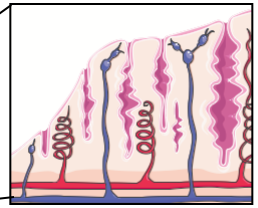


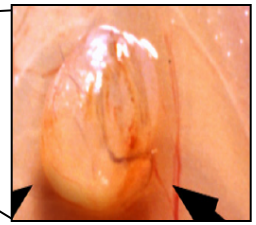
Kumar et al. 2014

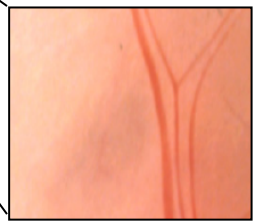
Clinical samples



Peritoneal fluid



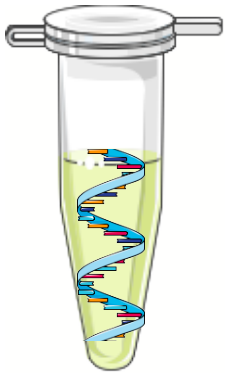




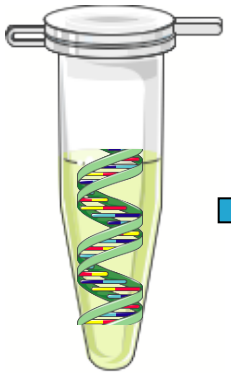
Endometrium (from age- and BMI-matched endometriosis patients and ctrl subjects)

Peritoneum (from age- and BMI-matched endometriosis patients and ctrl subjects)

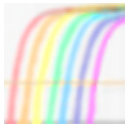
Peritoneal lesions (from endometriosis patients)



RT reaction

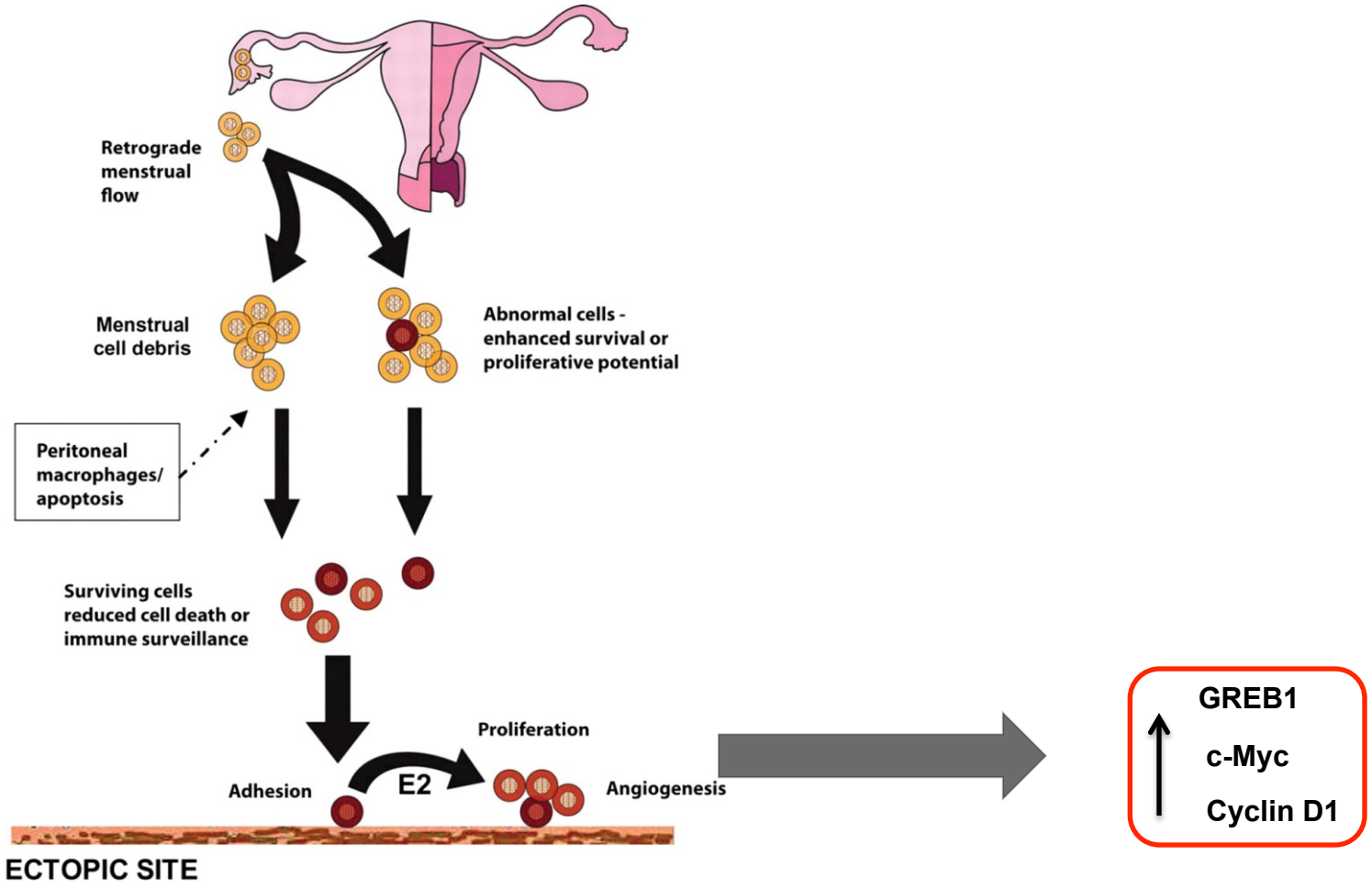


qPCR



Gene expression analysis and IHC

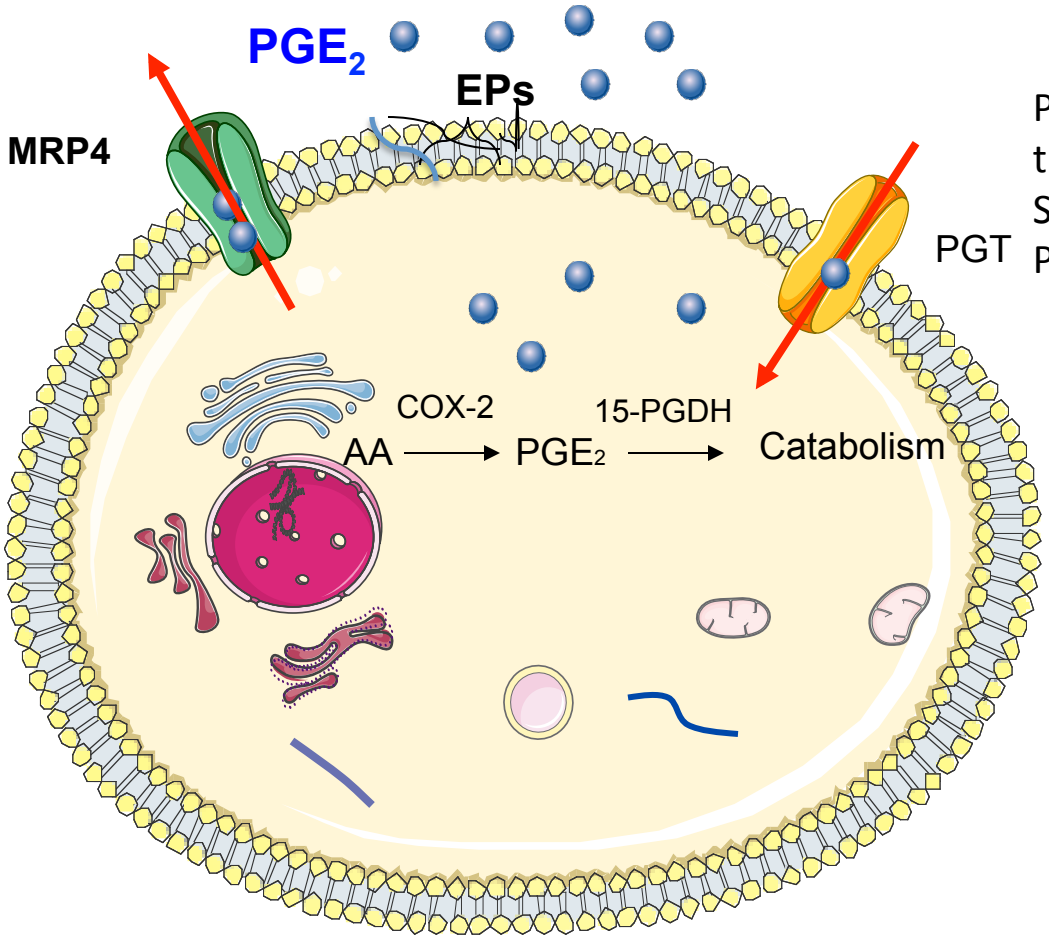
Estrogens and proliferation of ectopic tissue



Pellegrini et al. 2012 Fertility and Sterility

PG metabolism and transport

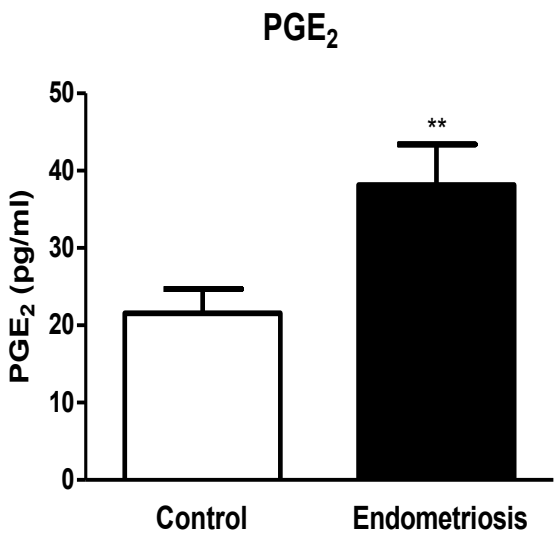
MRP4 (ABCC4): multidrug resistant protein 4, responsible for the release of PG from the cell



Prostaglandin transporter (PGT = SLCO2A1): transports PG into the cell

Augmented peritoneal fluid PGE_2 levels and increased MRP4 expression in peritoneal lesions

A



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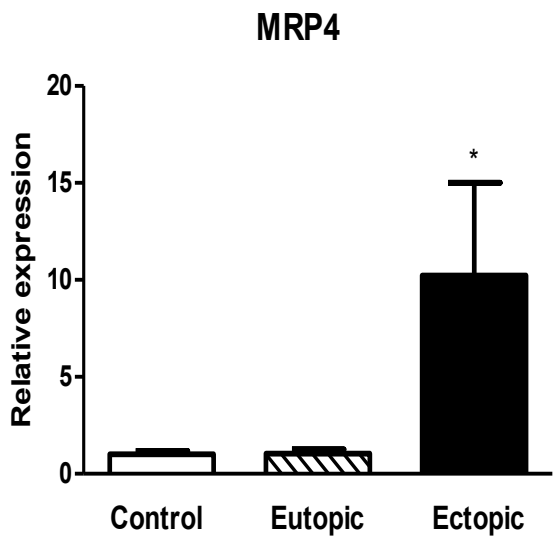
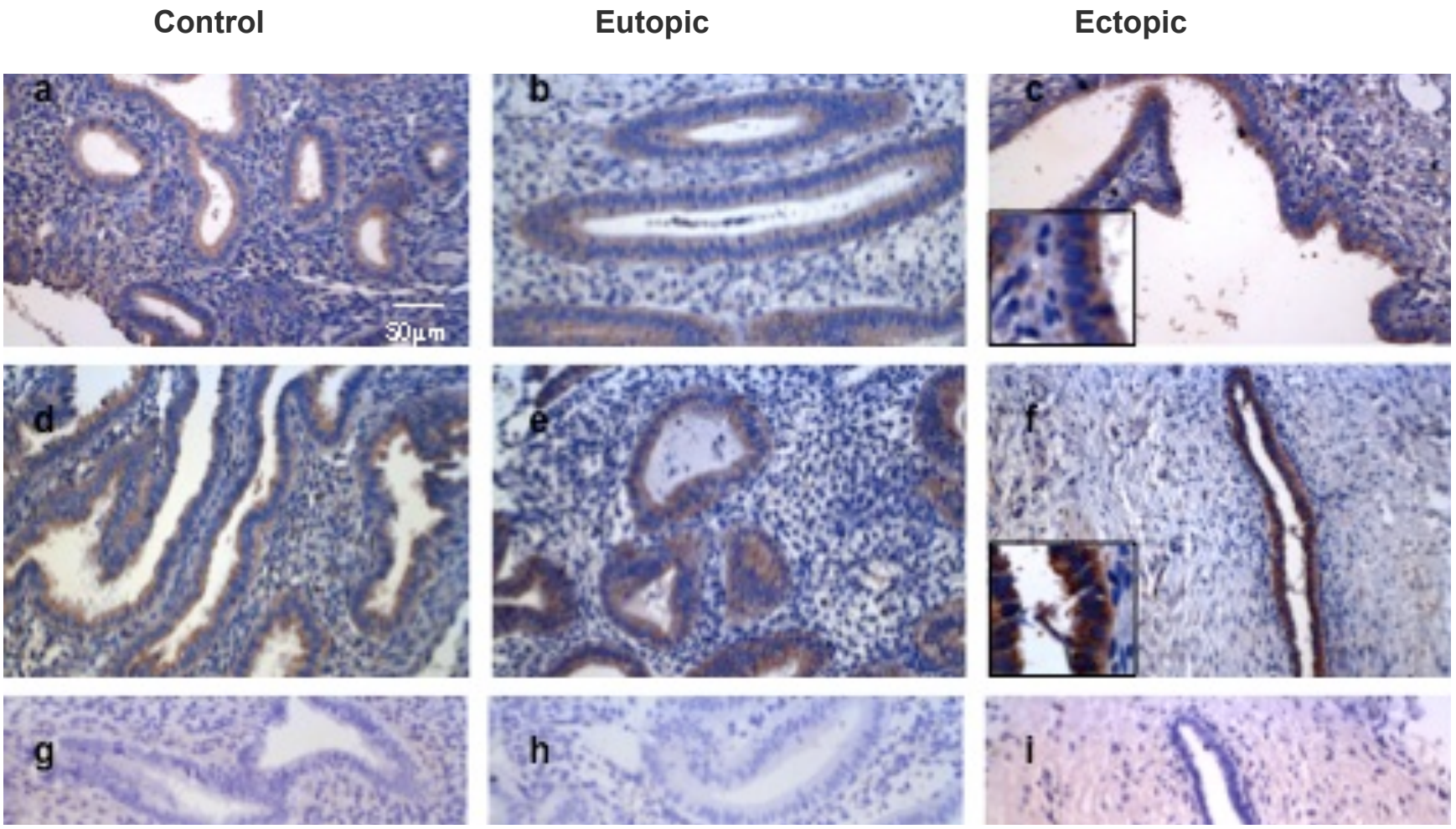


Gori et al. Fertility and Sterility 2013

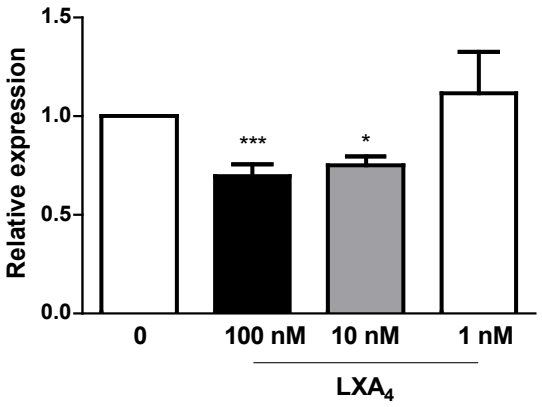
Localisation of MRP4 in eutopic and ectopic endometrial tissue



Gori et al. Fertility and Sterility 2013

LXA_4 inhibits MRP4 expression in endometriotic epithelial cells in a dose-dependant manner (via ERα) and reduces PGE_2 production

A



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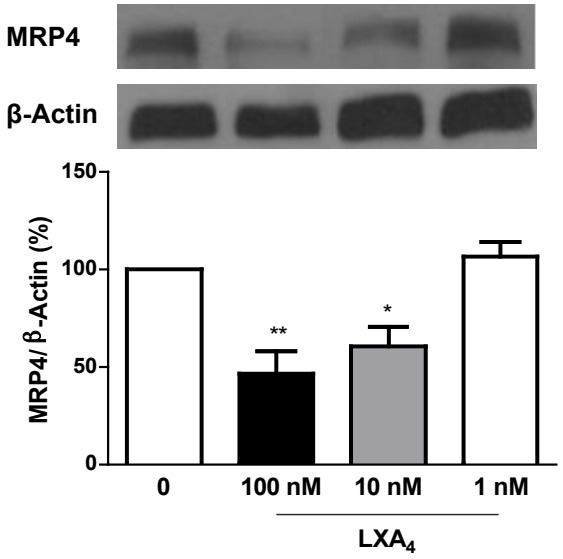

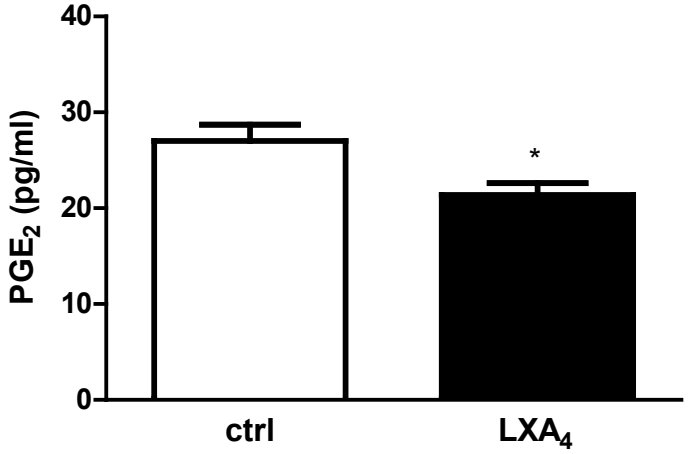

Gori et al. Fertility and Sterility 2013

Conclusions

- LXA_4 is an estrogen receptor agonist
- LXA_4 decreases the expression of mediators involved in inflammation and proliferation *in vitro* and *in vivo* *(MRP4- novel biomarker)*
- LXA_4 is protective in a mouse model of endometriosis, both for *de novo* and established disease via dual actions on inflammatory and ER signalling pathways

Future research challenges

- Well designed studies with **well characterised patient groups and sufficient patient numbers**
- Deep Infiltrating Endo: not well understood
- Screening -> novel molecules, originality
- Mechanistic studies (not just observational): linking biology with symptoms
- Transdisciplinary research: E health, online surveys, Med Tech etc. : **the patient is a partner**
- Personalised medicine

Thank you for your attention