Reciprocal transcriptional regulation of serum amyloid A versus apolipoprotein A-I and paraoxonase-1 by inflammation in murine hepatocyte

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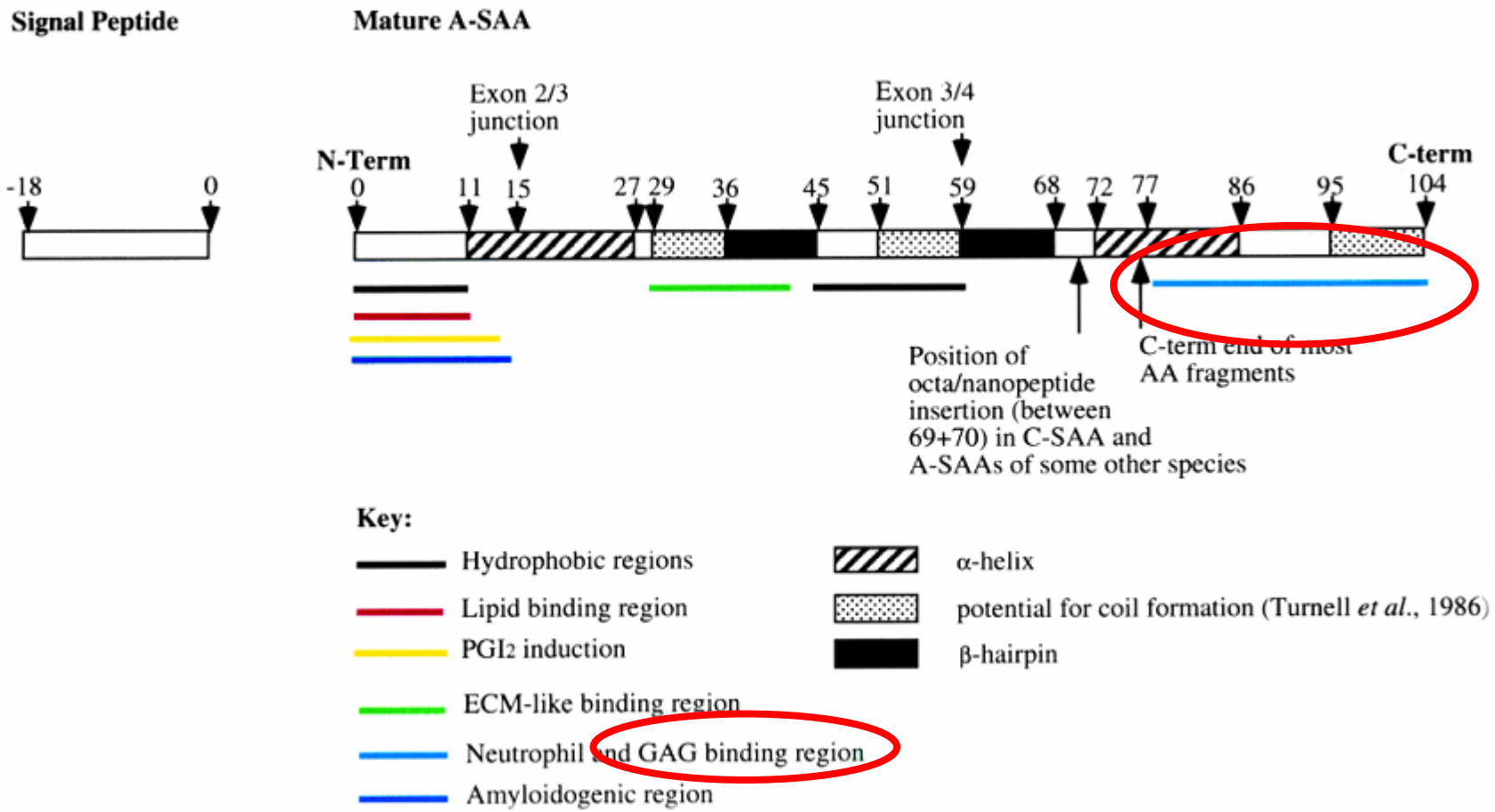
Human and Mouse SAA Genes

Current Nomenclature	Alternate Name/Features
Human	
SAA1	**A-SAA (Acute-phase SAA)**
SAA2	
SAA4	**C-SAA (Constitutive SAA)**
Mouse	
Saa1	**A-SAA**
Saa2	
Saa3	**Extrahepatic: mØ, SMC, AT**
Saa4	**C-SAA**
$Saa_{CE/J}$	**Non-amyloidogenic**

Structure of Human A-SAA Protein



(Uhlar and Whitehead, *Eur. J. Biochem* 1999;265:501-523)

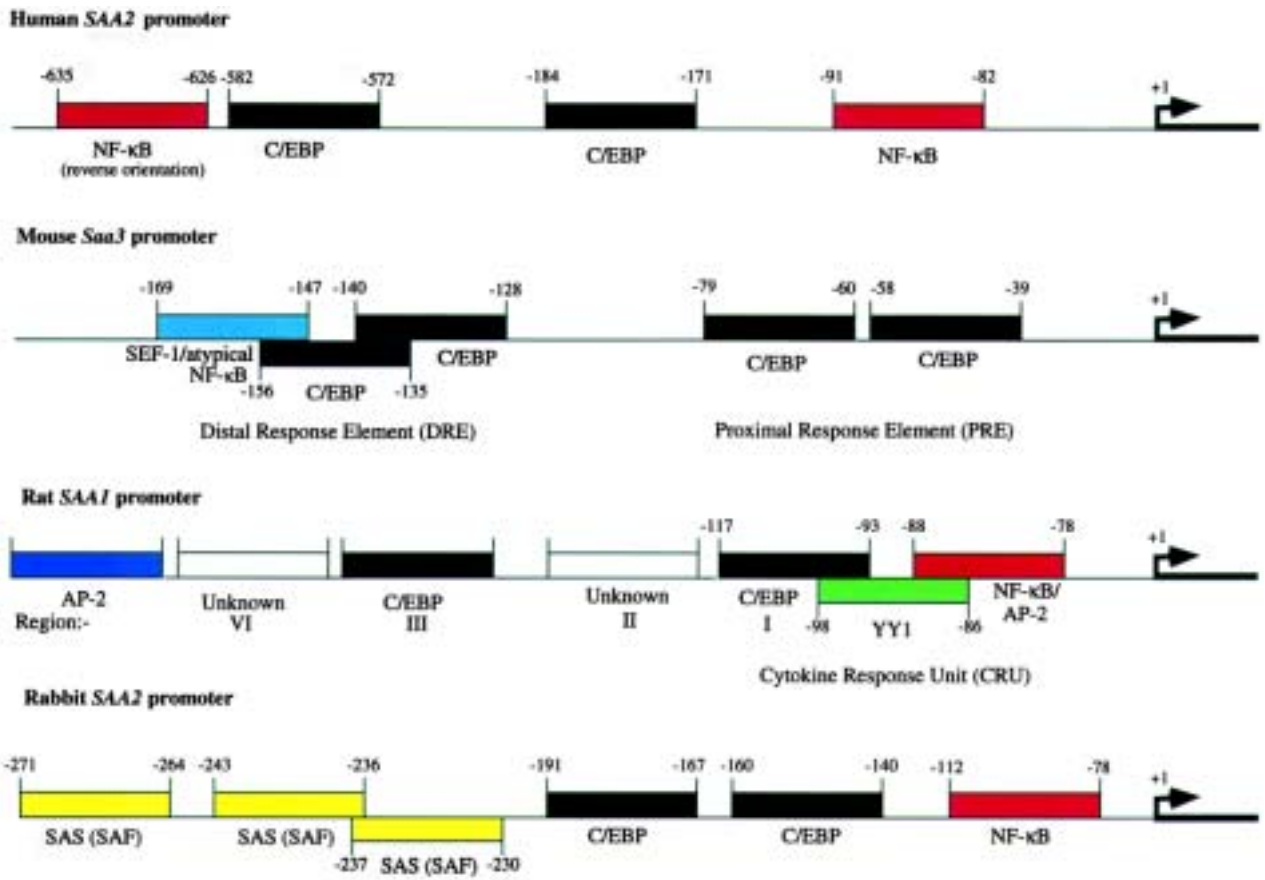
Sites of Synthesis

1. **SAA1/2 – mainly liver but also extrahepatic, incl. artery wall**

2. **Mouse SAA3 – extrahepatic, mainly macrophages and adipocytes**

3. **SAA4 – mainly liver, but also extrahepatic incl. artery wall**

Regulatory elements of mammalian A-SAA promoters



(Uhlar and Whitehead, *Eur. J. Biochem* 1999;265:501-523)

Serum amyloid A (SAA)

1. SAAs are major acute-phase reactants
2. SAAs have been shown to exhibit higher risk relationship with CVD
3. Acute-phase SAAs (SAA1,2) and constitutive SAA(SAA4)

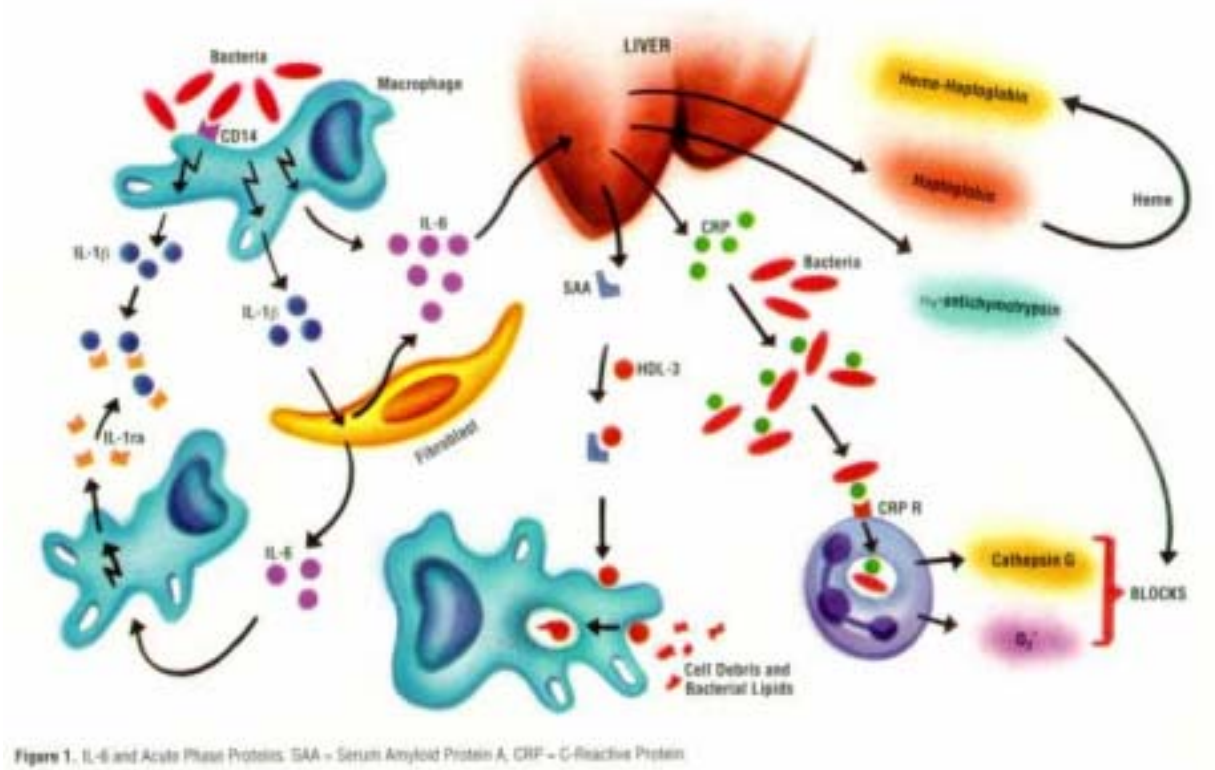


Figure 1. IL-6 and Acute Phase Proteins. SAA = Serum Amyloid Protein A, CRP = C-Reactive Protein

Differences Between Acute and Chronic Elevations of Inflammatory Molecules

1. Acute:
   - –Levels increase up to 1000 fold
   - –Plays a role in host defenses
2. Chronic
   - –Examples: rheumatoid arthritis, obesity, metabolic syndrome/insulin resistance
   - –Levels considerably lower
   - –Could have detrimental effect

Role of SAA in Atherogenesis

1. “Displacement” of apo A-I result in decreased HDL and imparied reverse cholesterol transport.

2. Displacement of antioxidant enzyme from HDL

3. Induction of infiltration of monocyte-macrophage

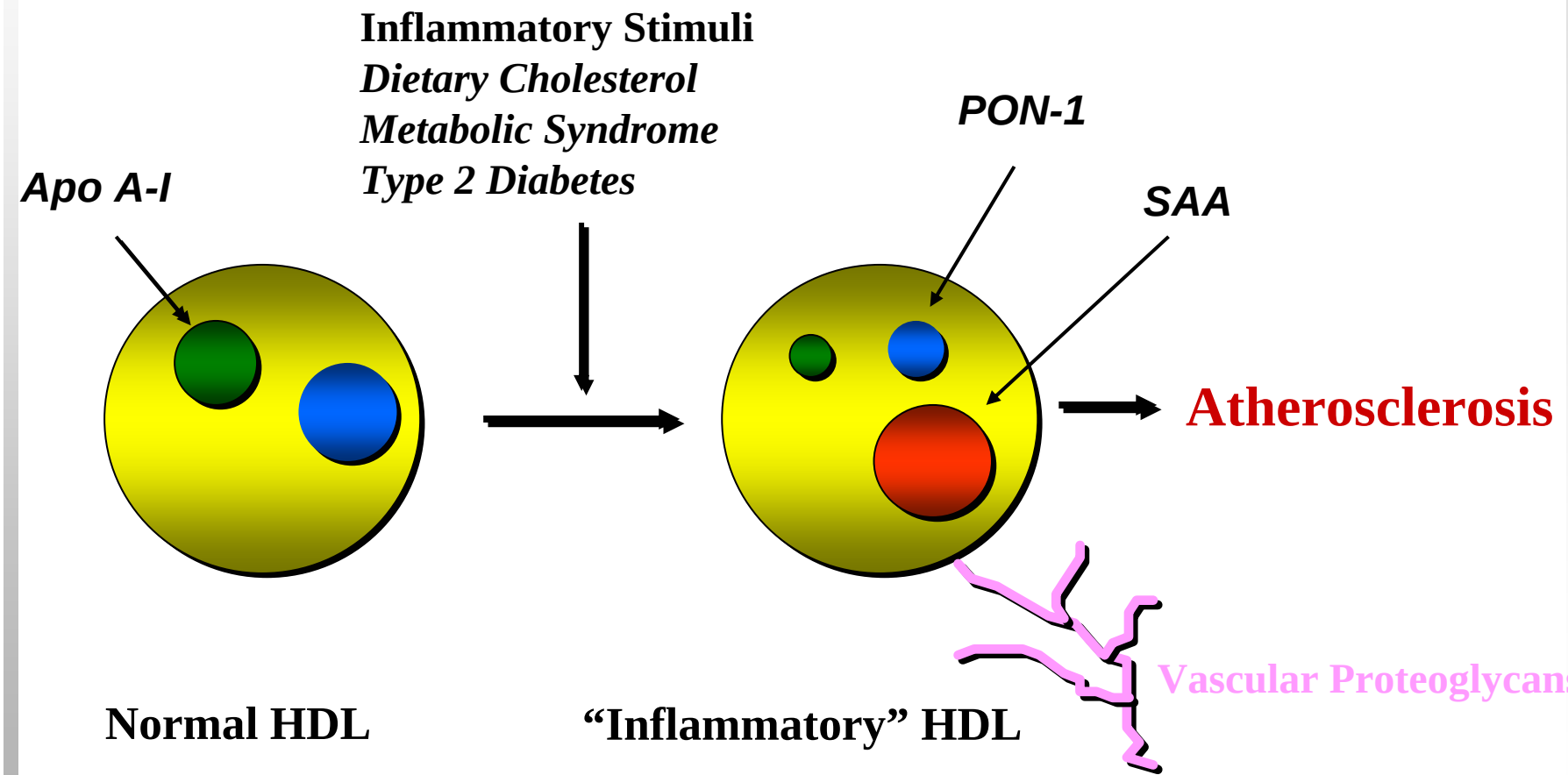
4. Increase of retention of SAA containing lipoprotein by proteoglycan

Paraoxonase-1 (PON-1)

- 1. Apolipoprotein present in HDL
- 2. Has been suggested to inhibit LDL oxidation
- 3. Transgenic mice overexpressing PON-1 are protected against atherosclerosis.
- 4. PON-1 deficient mice have increased atherosclerosis

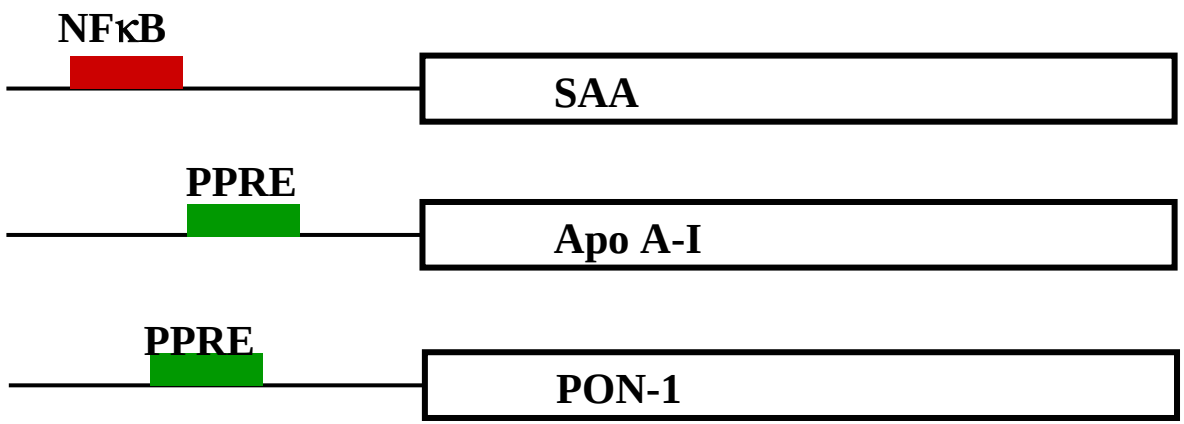
Focus on this study

SAA as a Mediator of Atherosclerosis



Aims of this study

1. To investigate the role of cytokines on the expression of SAA, apoA-I and PON-1 in hepatic cells and to evaluate the mechanism converting HDL into pro-atherogenic HDL.


NFκB
SAA
PPRE
Apo A-I
PPRE
PON-1

Cytokines up-regulate SAA while simultaneously down-regulating apo A-I and PON-1 in hepatocytes

A. Time Course

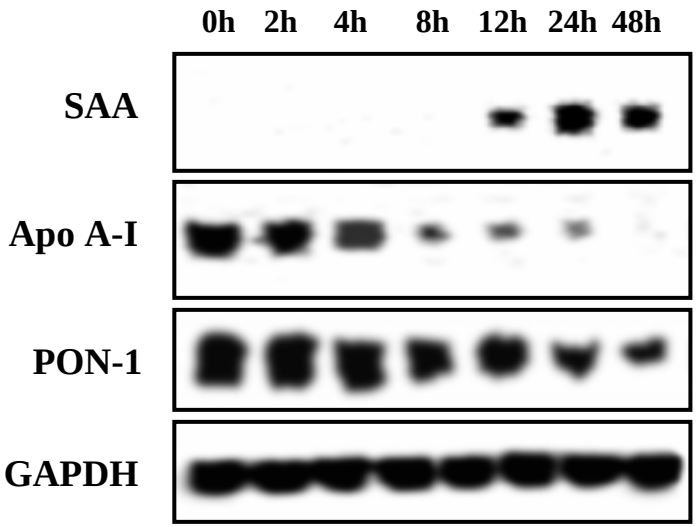


Figure 1. Total RNA from Hepa 1-6 (panel A), AML12 and NMH (panel B) hepatocytes treated with a mixture of cytokines (10ng/ml IL-1, 10ng/ml TNF-α, 10ng/ml IL-6) for the indicated time periods (panel A) or 24h (panel B) were extracted and subjected to Northern blot analysis using SAA, apo A-I and PON-1 cDNA as probes.

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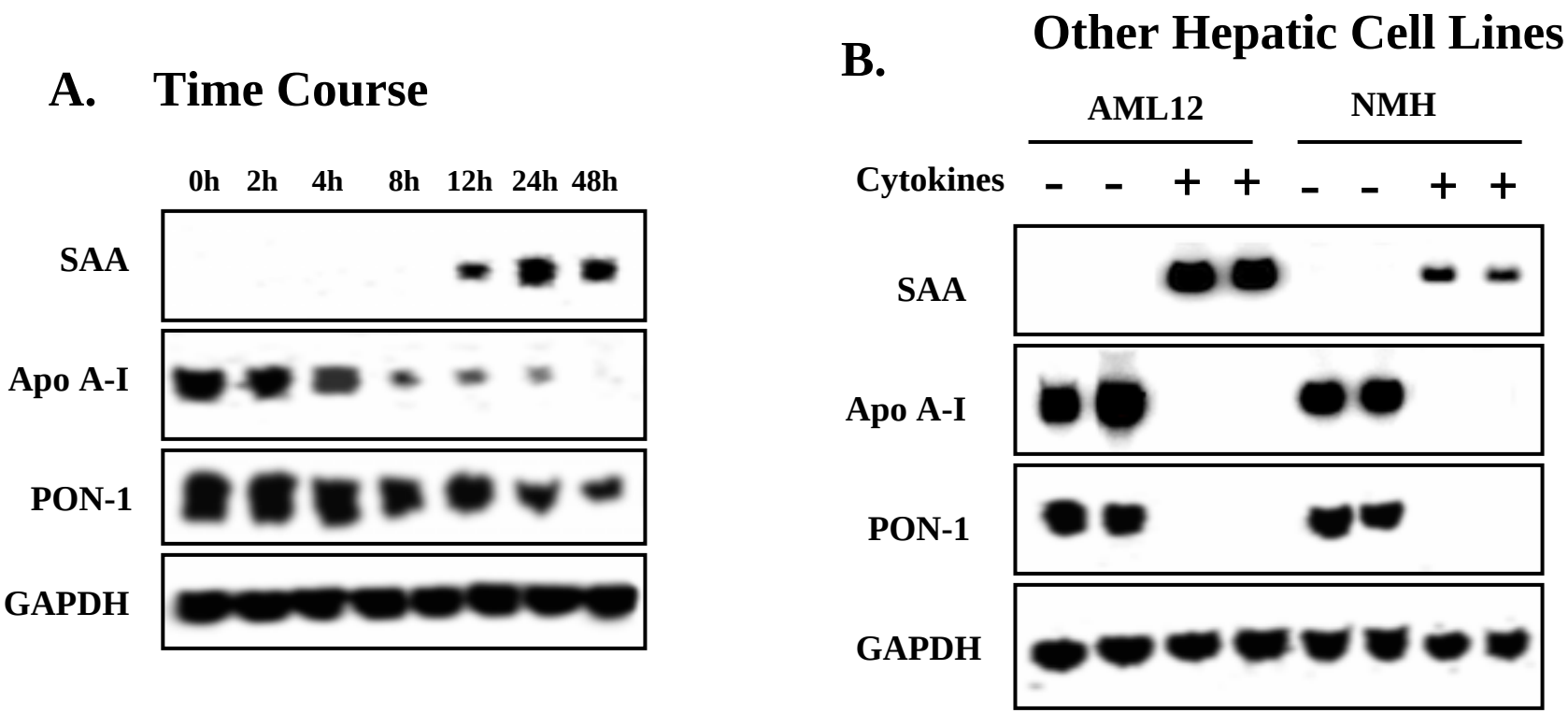

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Injection of LPS increases SAA gene expression, while simultaneously decreasing apo A-I and PON-1 gene expression in mouse liver

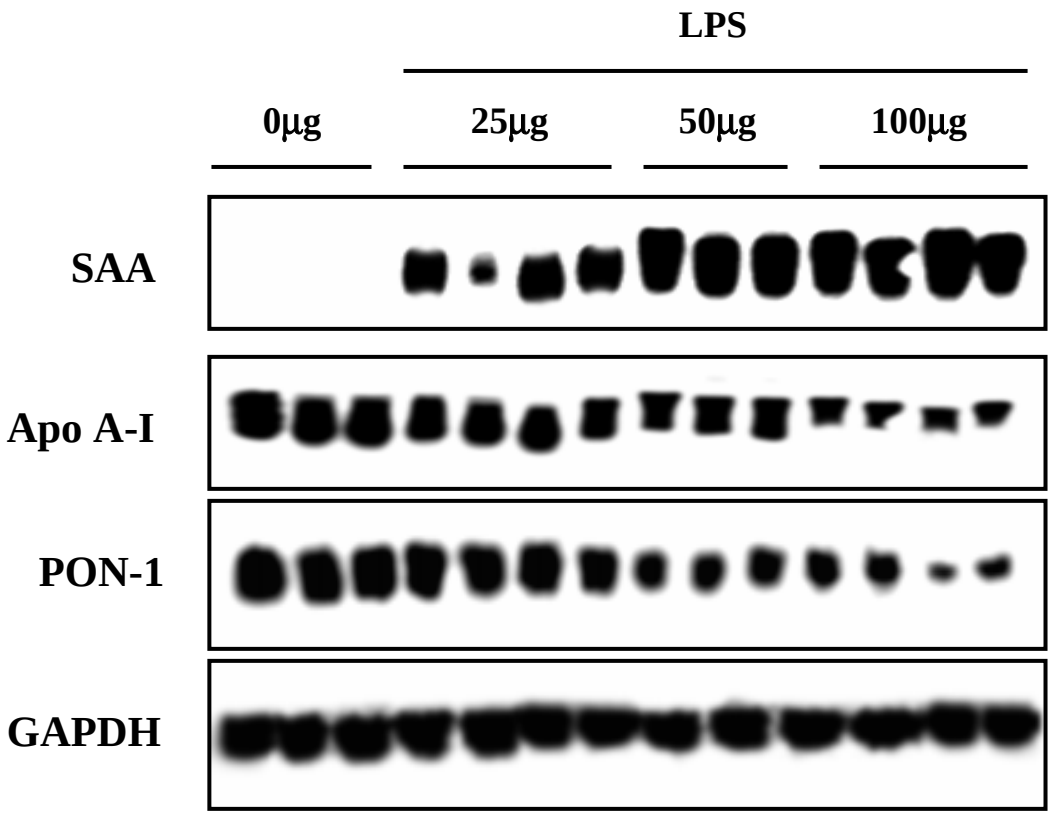


Figure 2. LPS was injected intraperitineally into C57BL/6 mice at the indicated doses. After 24h, total RNA from mouse liver was isolated and analyzed by Northern blot analysis using SAA, apo A-I and PON-1 cDNA as probes.

NFκB inhibitors antagonize the alteration of SAA, apo A-I and PON-1 induced by cytokines

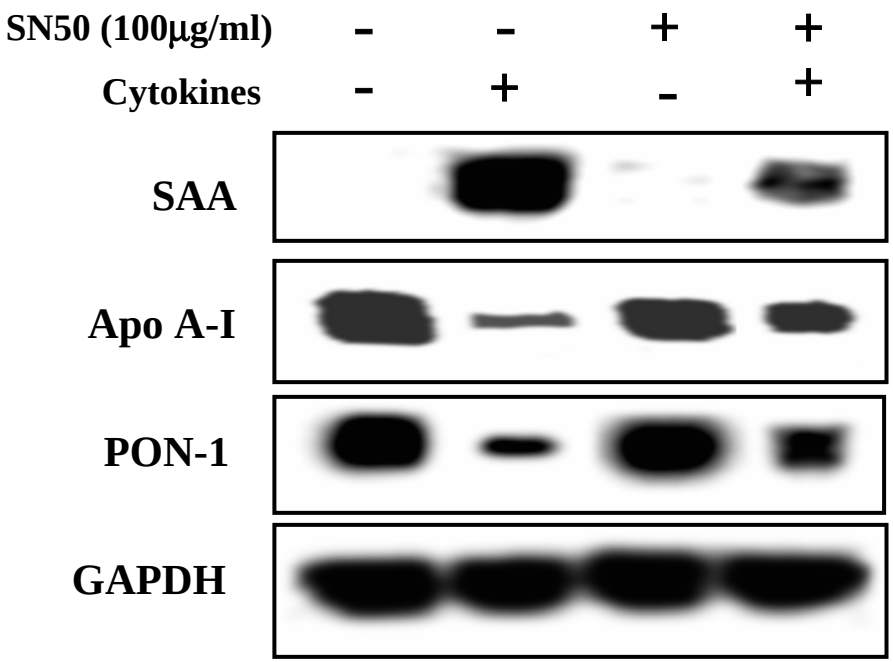
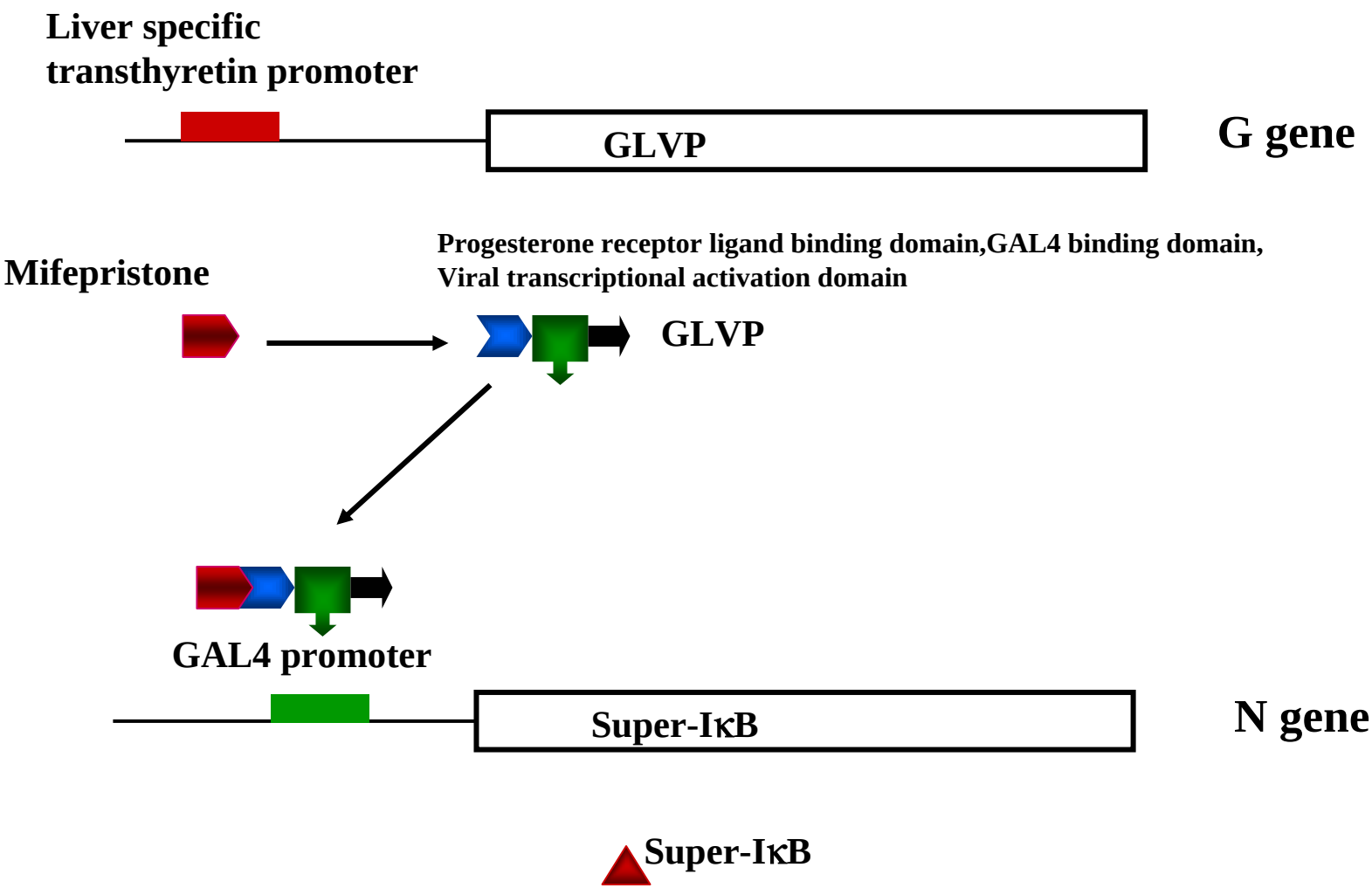


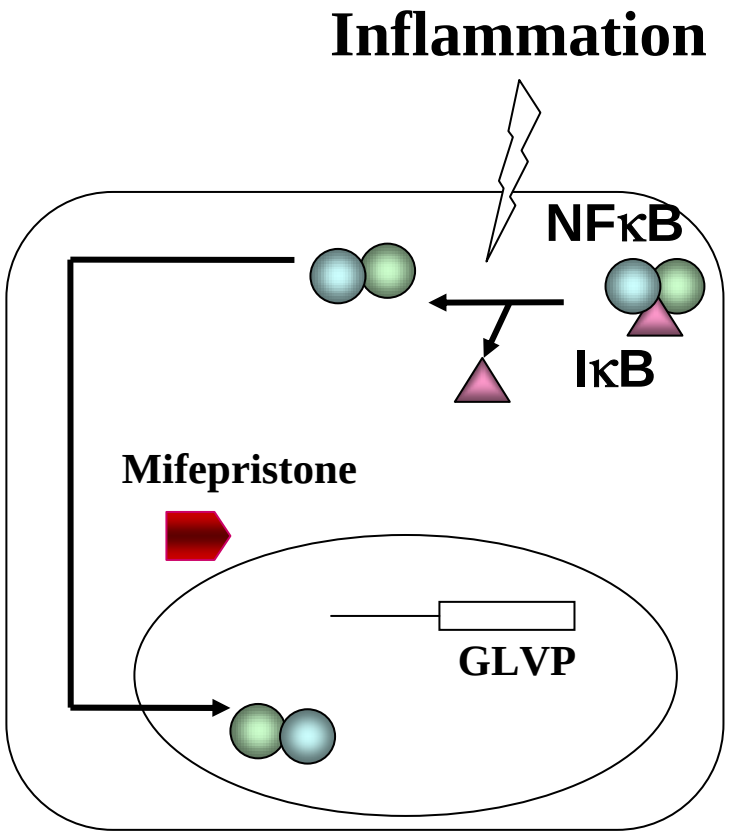
Figure 3. Total RNA was extracted from Hepa 1-6 cells treated with a mixture of cytokines and the NFκB inhibitor SN50 for 24 hr and was analyzed by Northern blot as described

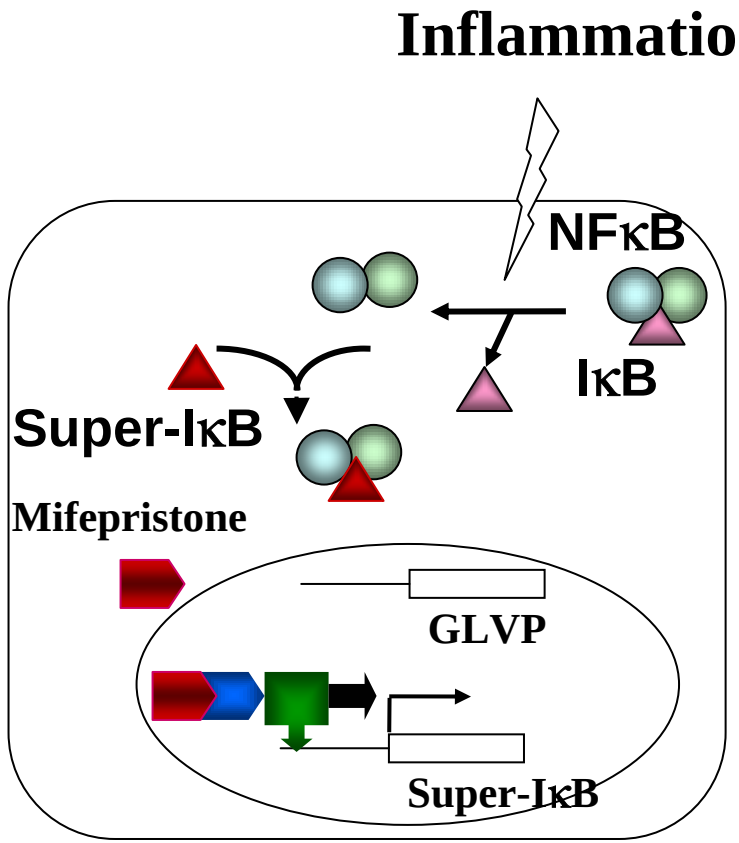
Liver specific NFκB Turn on/off system



Single transgenic mice (G mice)

Double transgenic mice (GN mice)





Normal NFκB activity

NFκB deficiency

An IκB super-repressor antagonizes the alterations of SAA, apo A-I and PON-I induced by injection of TNF-α in GLVP/*ΔN-IκB* transgenic mice.

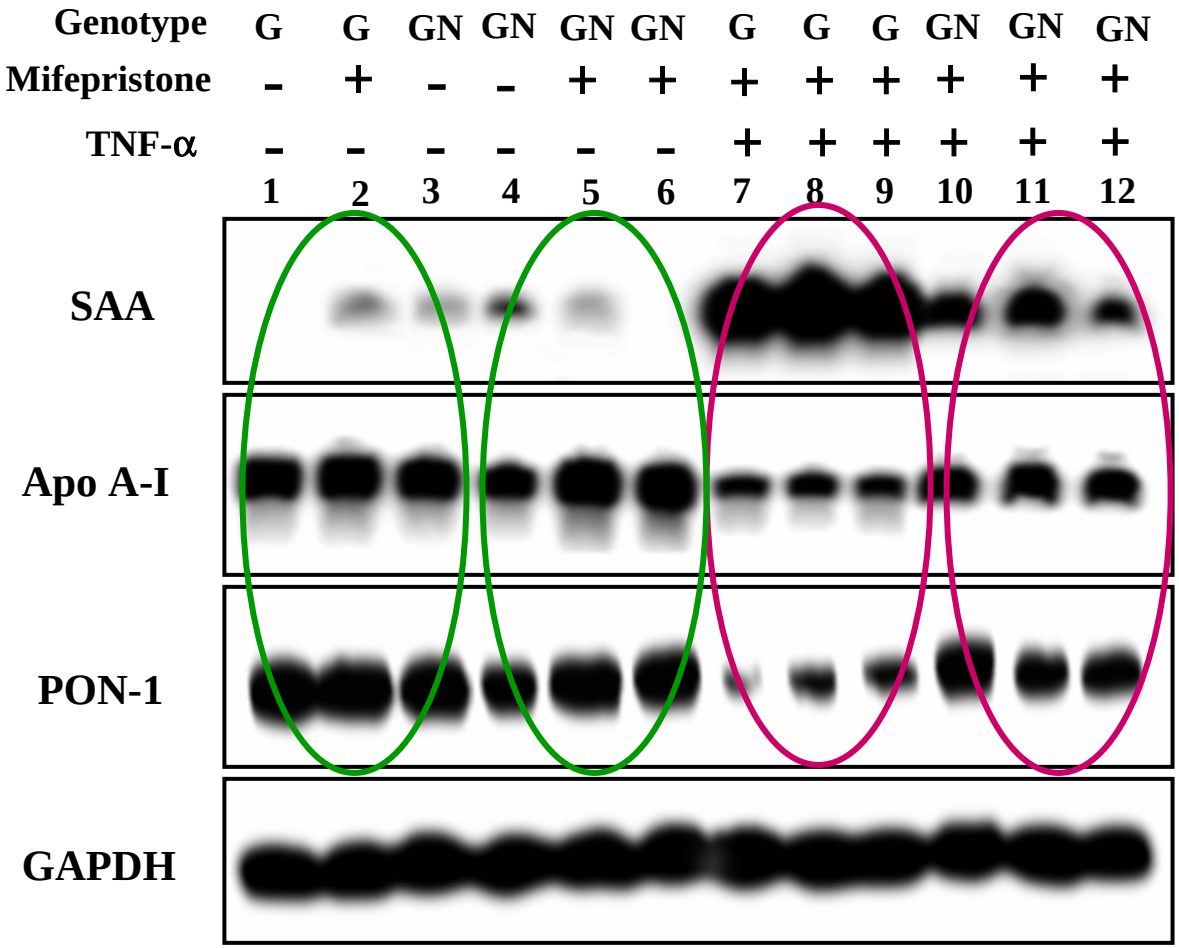


Figure 5. Single transgenic (G) and double transgenic (GN) were injected with mifepristone (5 mg/kg) or sesame seed oil for 3 hr prior to injection with TNF-α (25 μg/kg) or saline as indicated. Liver tissue was harvested before TNF-α injection (control or 0 hr) or 24 hr after TNF-α injection. Total RNA was prepared and Northern blot analysis was performed as described in Fig. 1. Each lane represents a single mouse

PPARα agonists block the alteration of SAA, apo A-I and PON-1 induced by cytokines

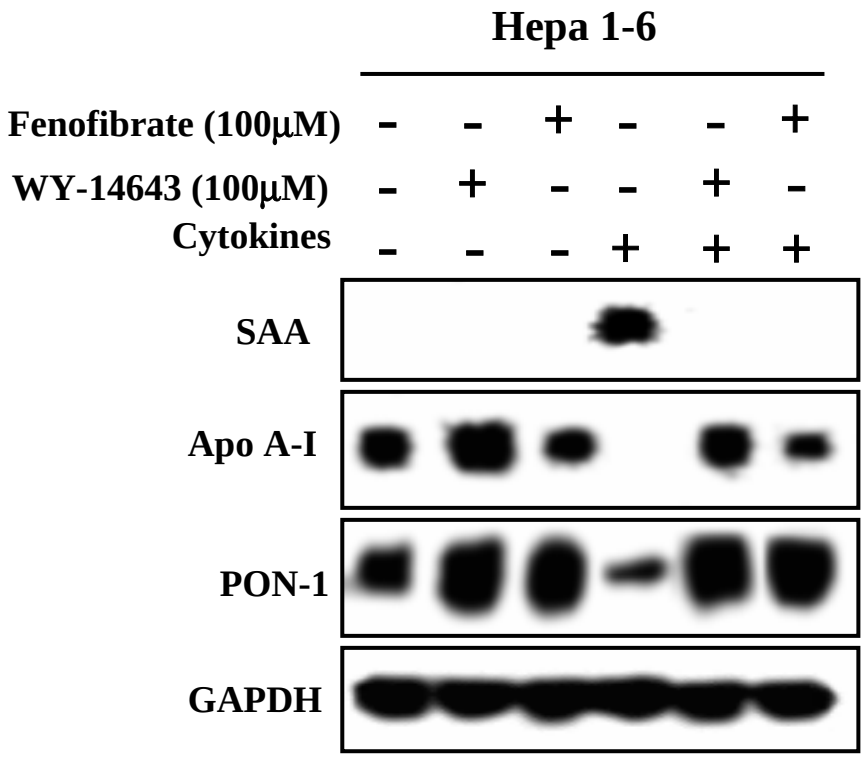


Figure 4. Hepa 1-6 and AML12 cells were treated with the PPARα ligands, WY-14643 (100 μM) and fenofibrate (100 μM), and/or a mixture of cytokines as indicated. Total RNA after 24 hr. exposure was analyzed by Northern blot as described in Fig. 1.

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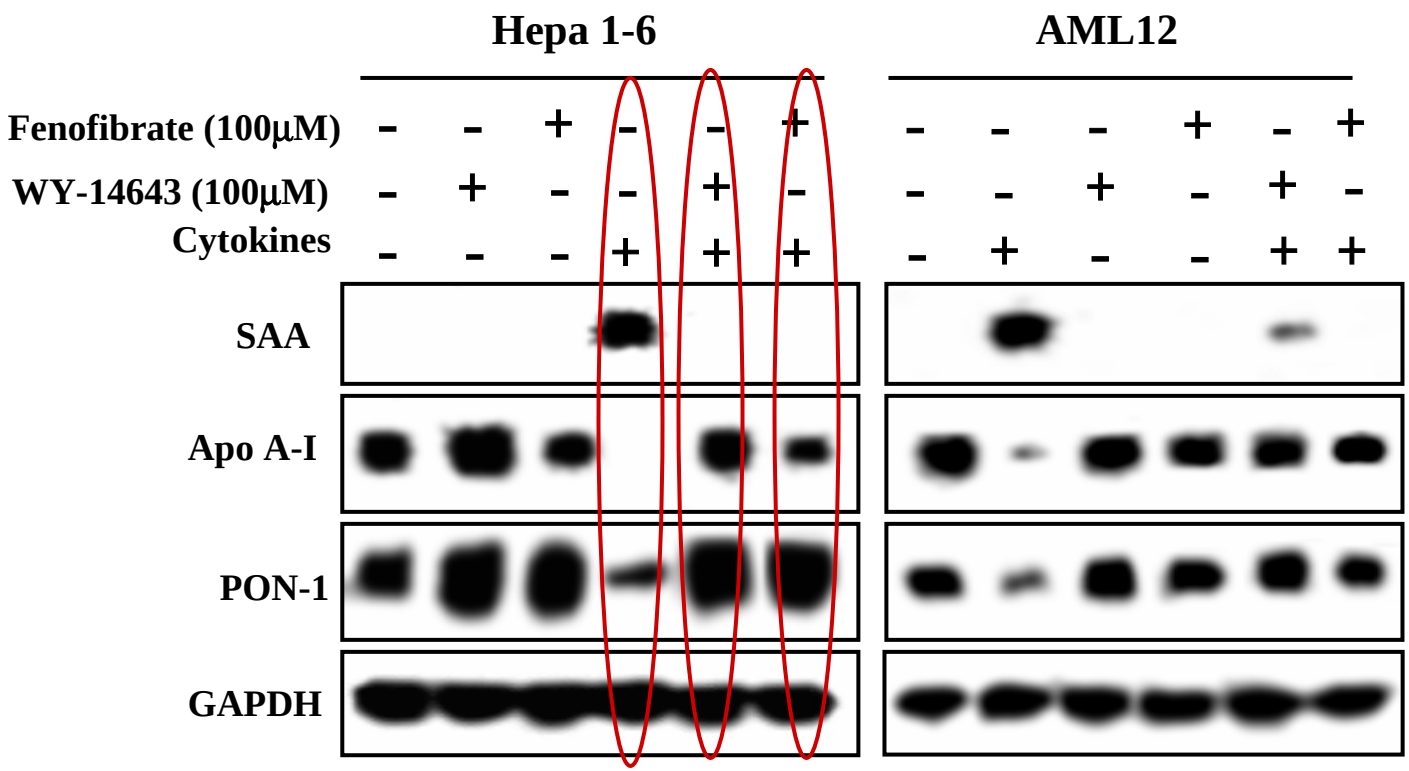
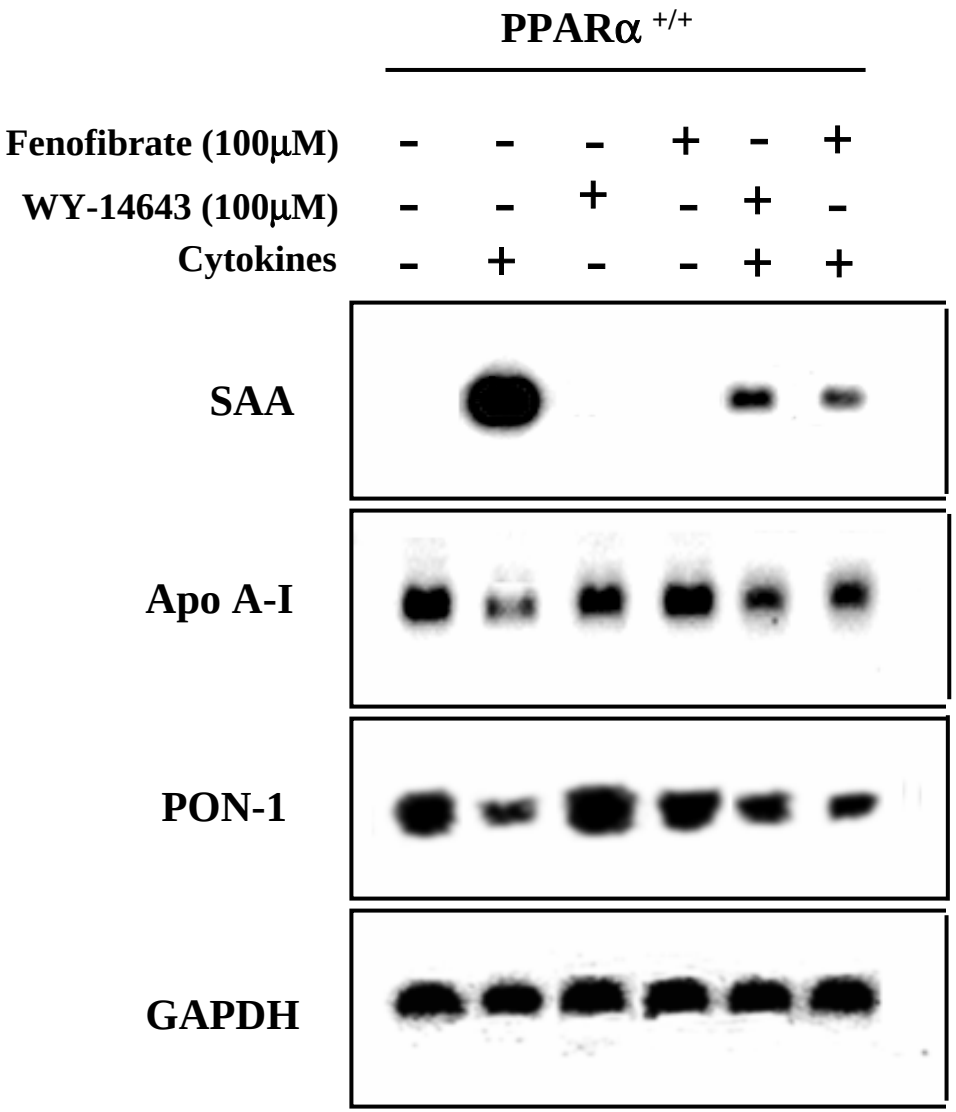
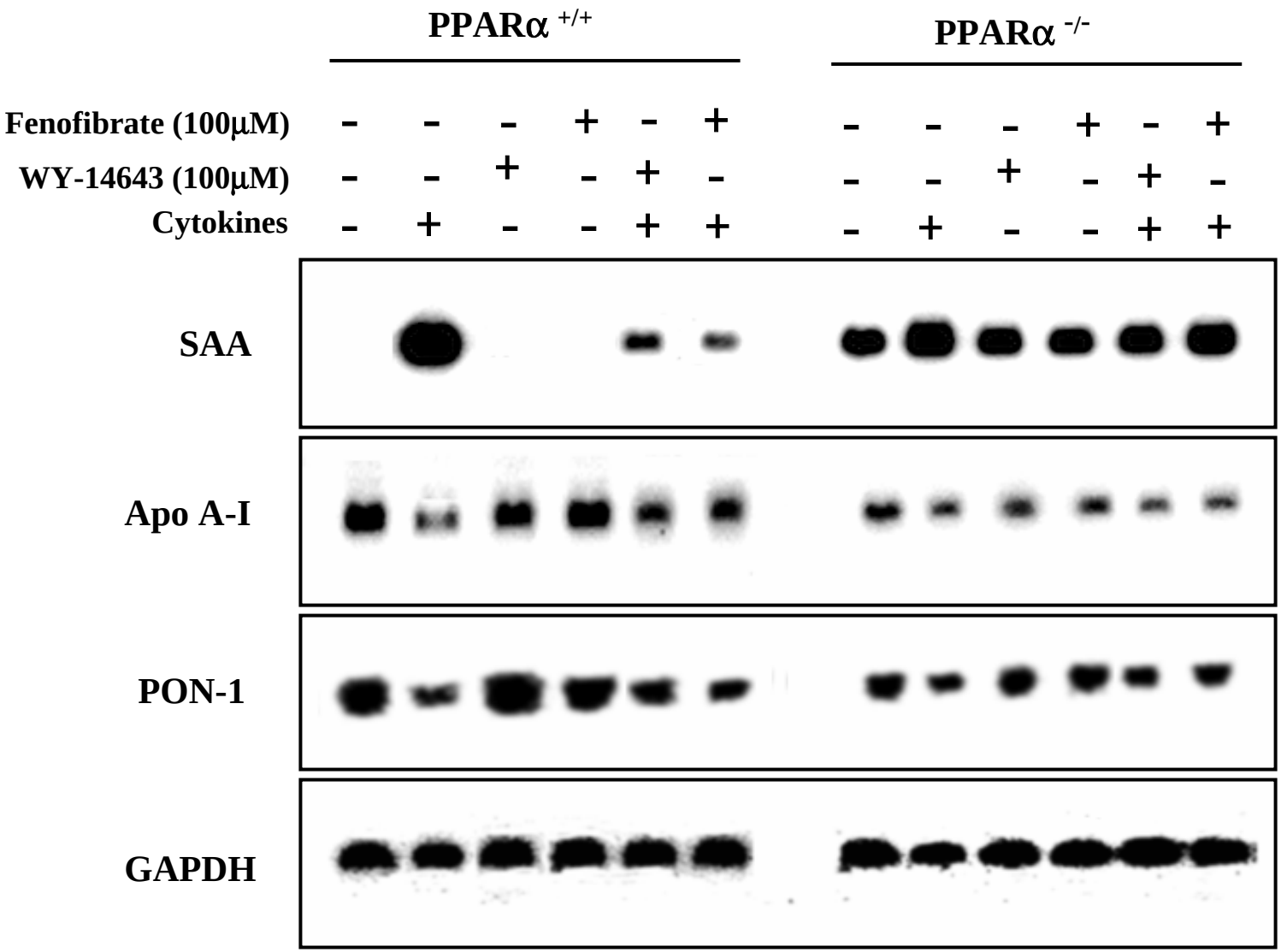
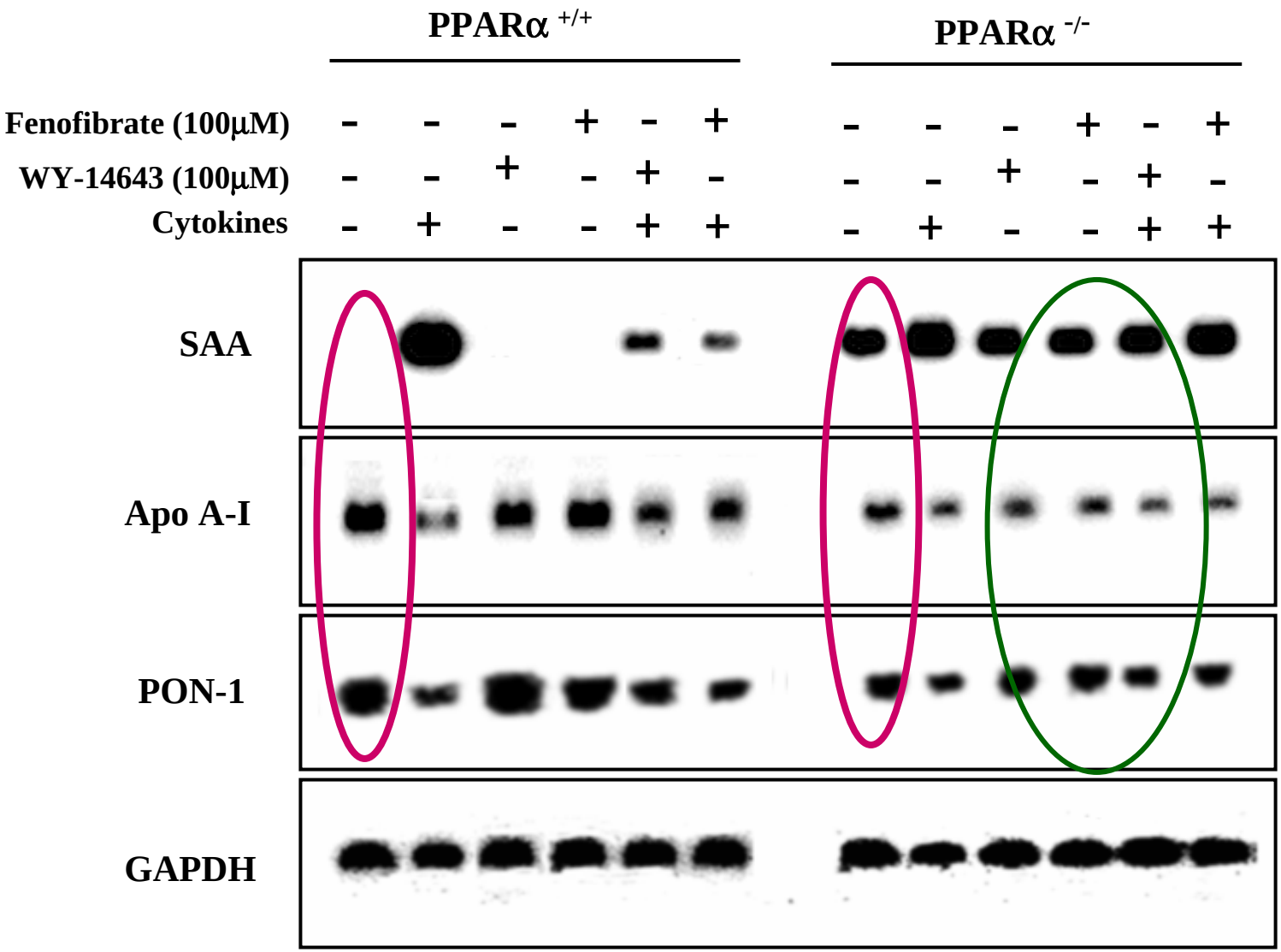


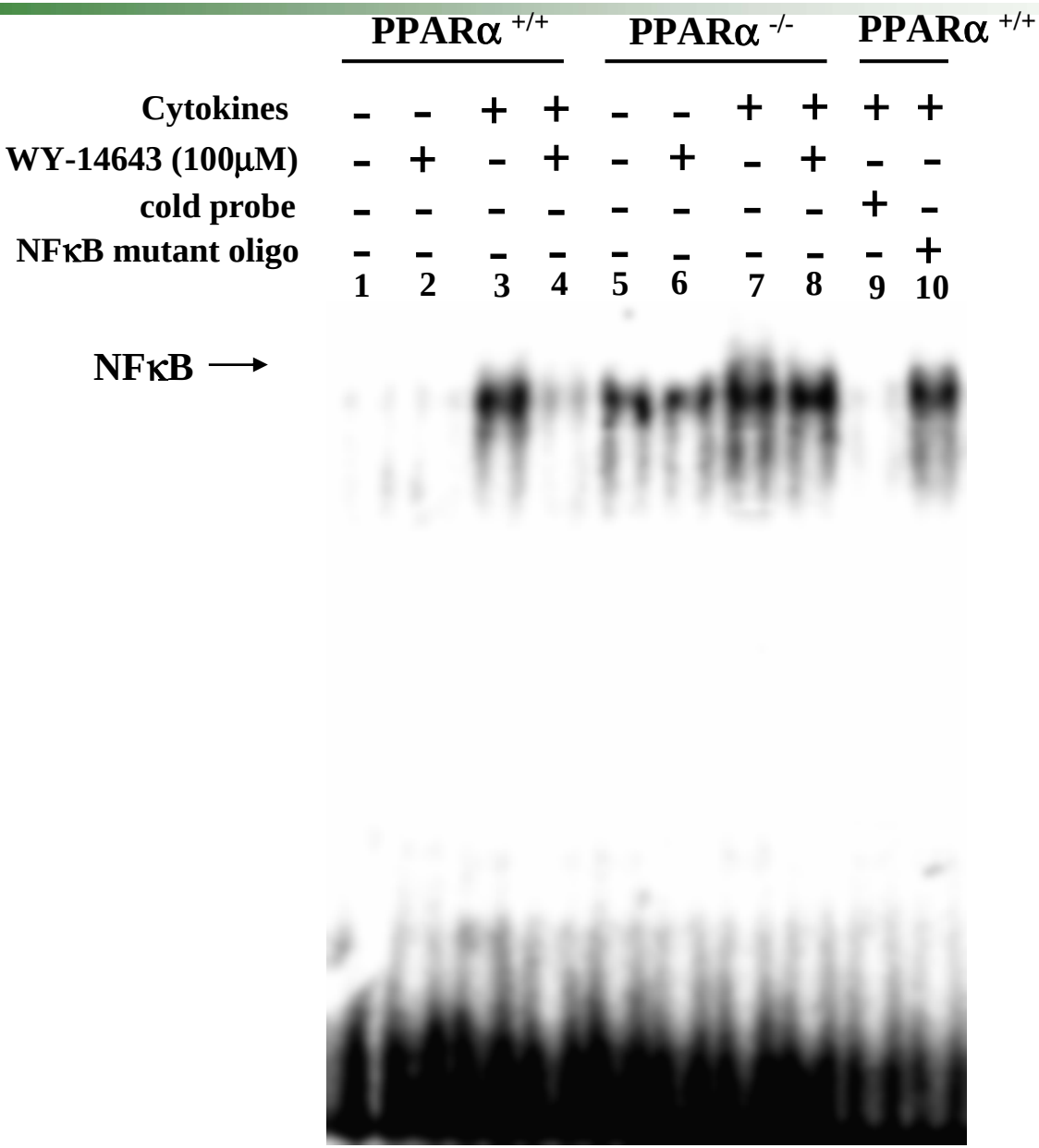
Figure 4. Hepa 1-6 and AML12 cells were treated with the PPARα ligands, WY-14643 (100 μM) and fenofibrate (100 μM), and/or a mixture of cytokines as indicated. Total RNA after 24 hr. exposure was analyzed by Northern blot as described in Fig. 1.

Cytokine regulation of SAA, apo A-I and PON-I gene expression by hepatocytes from PPARα$^{+/+}$ and PPARα$^{-/-}$ mice




PPARα +/+
PPARα -/-
Fenofibrate (100μM) - - - + - + - - - + - +
WY-14643 (100μM) - - + - + - - - + - + -
Cytokines - + - - + + - + - - + +
SAA
Apo A-I
PON-1
GAPDH

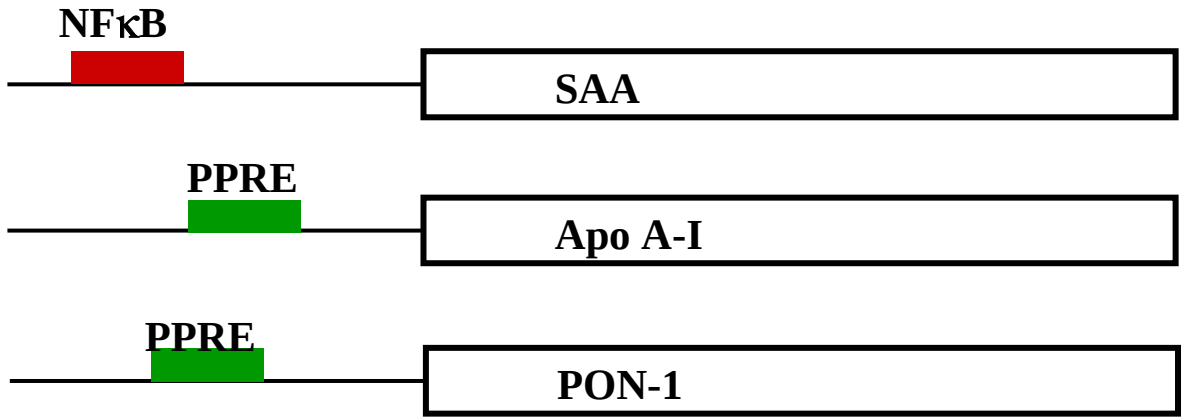

PPARα +/+
PPARα -/-
Fenofibrate (100μM) - - - + - + - - - + - +
WY-14643 (100μM) - - + - + - - - + - + -
Cytokines - + - - + + - + - - + +
SAA
Apo A-I
PON-1
GAPDH


PPARα +/+
PPARα -/-
PPARα +/+
Cytokines - - + + - - + + + +
WY-14643 (100μM) - + - + - + - + - -
cold probe - - - - - - - - + -
NFκB mutant oligo - - - - - - - - - +
1 2 3 4 5 6 7 8 9 10
NFκB

Summary

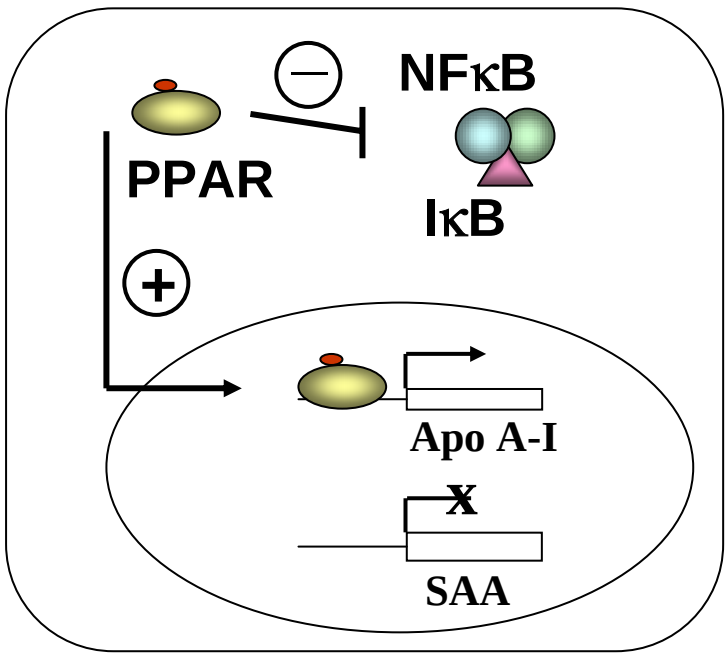
1. **Cytokines increase the expression level of SAA through NFκB transactivation while simultaneously decreasing the expression of apo A-I and PON1 through inhibition of PPARα activation.**
2. **Cytokines may play a critical role in converting atheroprotective HDL into a pro-atherogenic form by coordinately and inversely regulating the hepatic expression of SAA versus apo A-I and PON1.**
3. **The formation of SAA-enriched and apo A-I and PON1-depleted HDL during inflammation may be the result of changes in the hepatic regulation of these two apolipoproteins, rather than to displacement of apo A-I by SAA.**
4. **PPARα exerts a chronic "braking" effect on limiting inflammation in the basal state**
5. **Cross-talk between NFκB, activated by cytokines (inflammation) and PPARα, activated by ligands (fibrates), affects the expression of genes other than those directly activated by either NFκB or PPARα. Thus, NFκB activated by cytokines can influence the expression of apo A-I and PON-1, and ligand activation of PPARα can regulate the expression of SAA.**

A

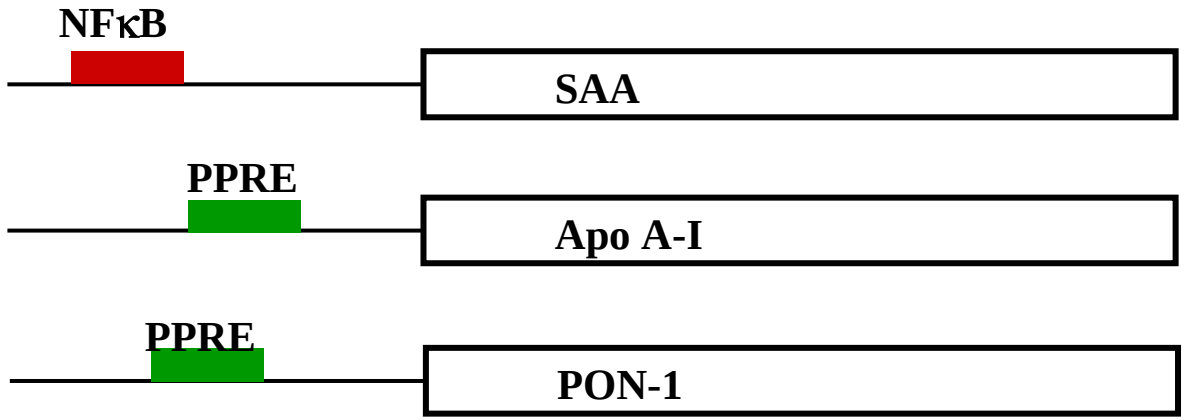


B

Basal

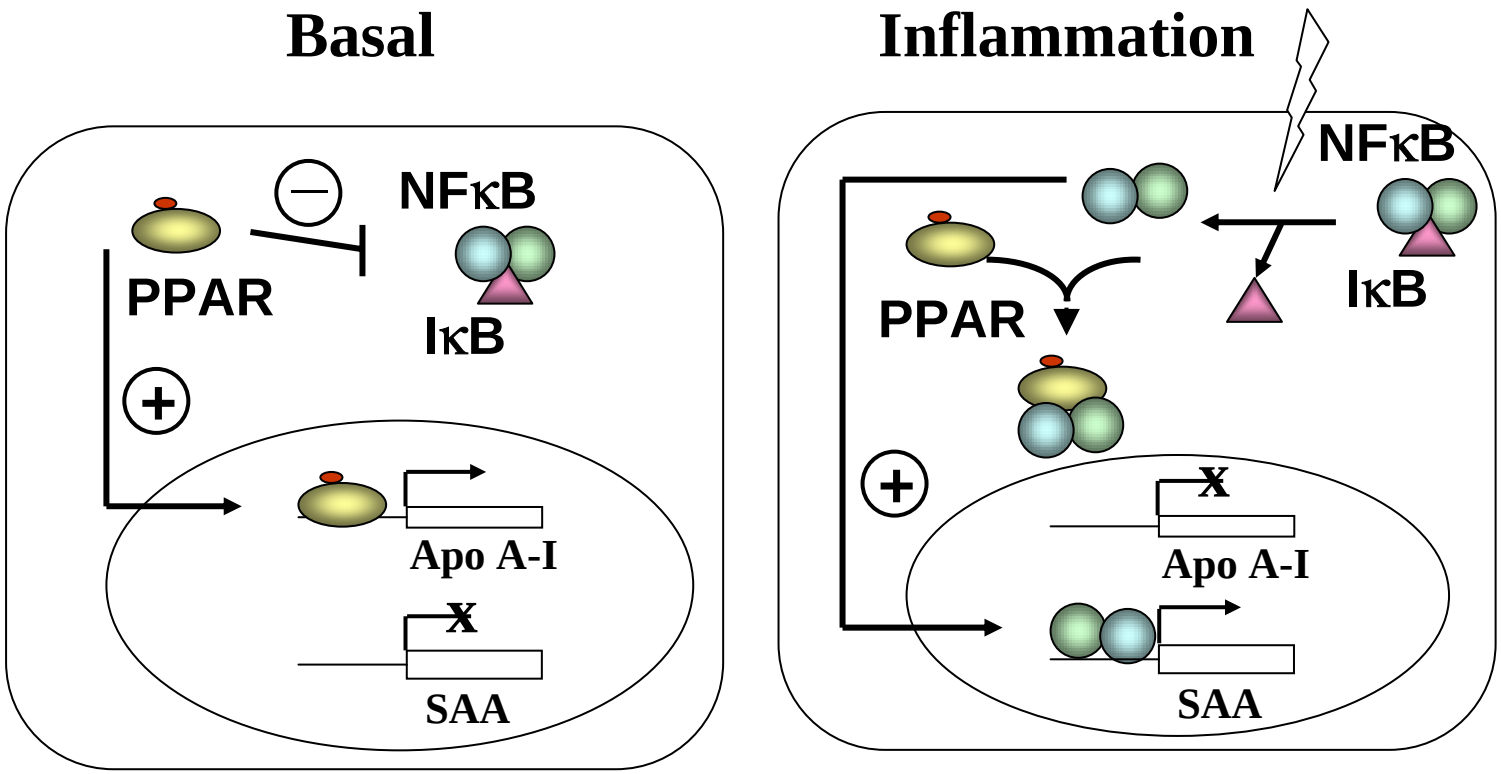


A



IL-1, IL-6,TNF-α

B



Collaborators

- **Nelson Fausto**
- **Jean Campbell**

- **Jorge Plutzky**
- **Gabriela Orasanu**