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Inflammation in the formation and rupture of intracranial aneurysms

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ABC Win meeting 2016

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Aneurysm formation ≠ Aneurysm rupture

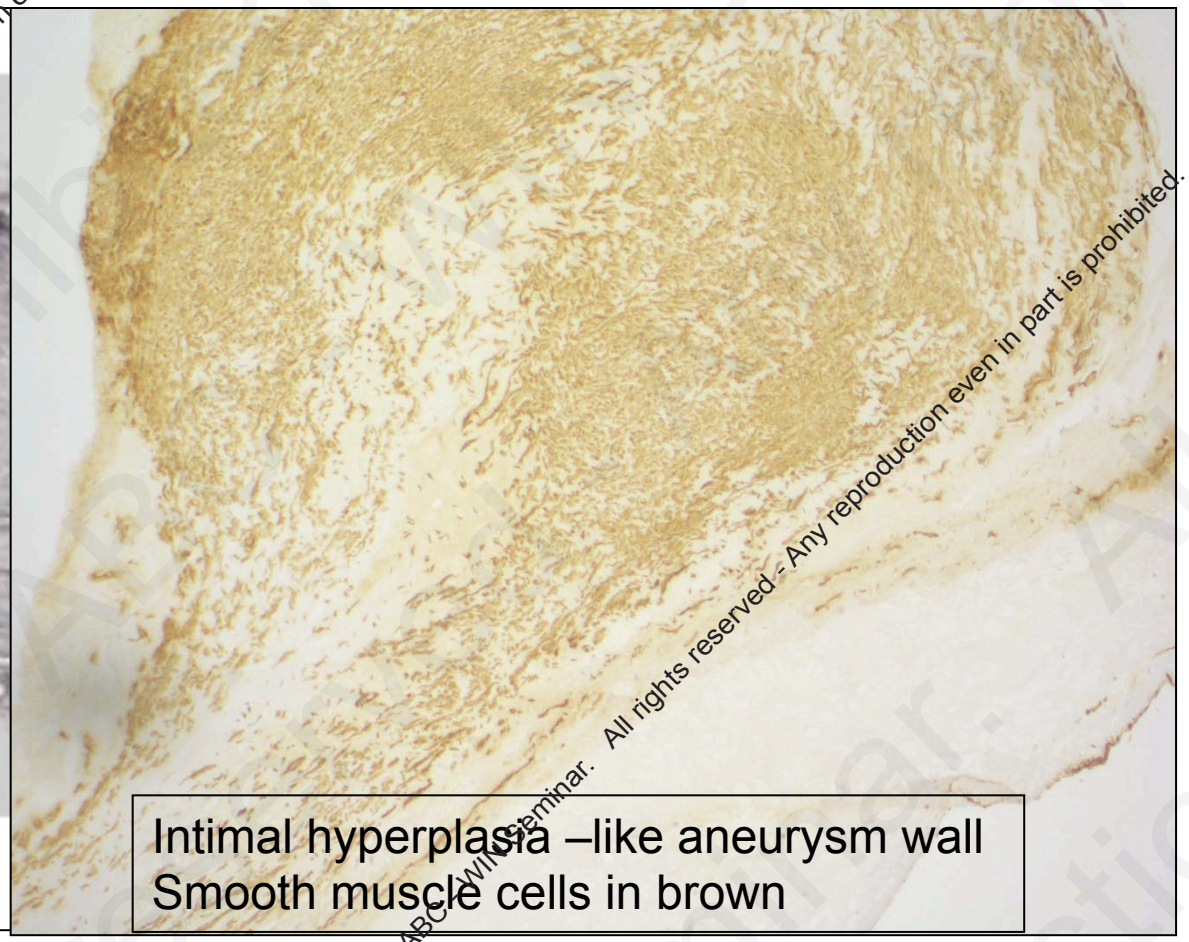
- Not all aneurysms rupture -

(autopsy studies, prospective follow-up studies, clinical experience…)

- In a life-long follow-up study of unruptured aneurysms, only 1/3 ruptured (Korja et al. Stroke 2014)

- Rupture is a rare event in animal models of induced aneurysms *(in the classical Hashimoto model 3% in 3months)*

Aneurysm wall is not ”inert rubber” – it can grow, remodel, and thicken



Intimal hyperplasia –like aneurysm wall
Smooth muscle cells in brown

Data for the role of inflammation during aneurysm formation – animal models

- Hashimoto N. group's model (Kyoto): **Rupture rare**
  - ***Induced hypertension*** (salt + renal artery ligation)
  - Inhibition of collagen crosslinking
  - *Unilateral ligation of carotid artery*

- Hashimoto T. group's model (UCSF): **Rupture common**
  - ***Induced hypertension*** (angiotensin II)
  - *Elastase injection into cisterna magna*

Cerebral artery wall is infiltrated by macrophages during formation of intracranial saccular aneurysms (unilateral carotid artery ligation model)

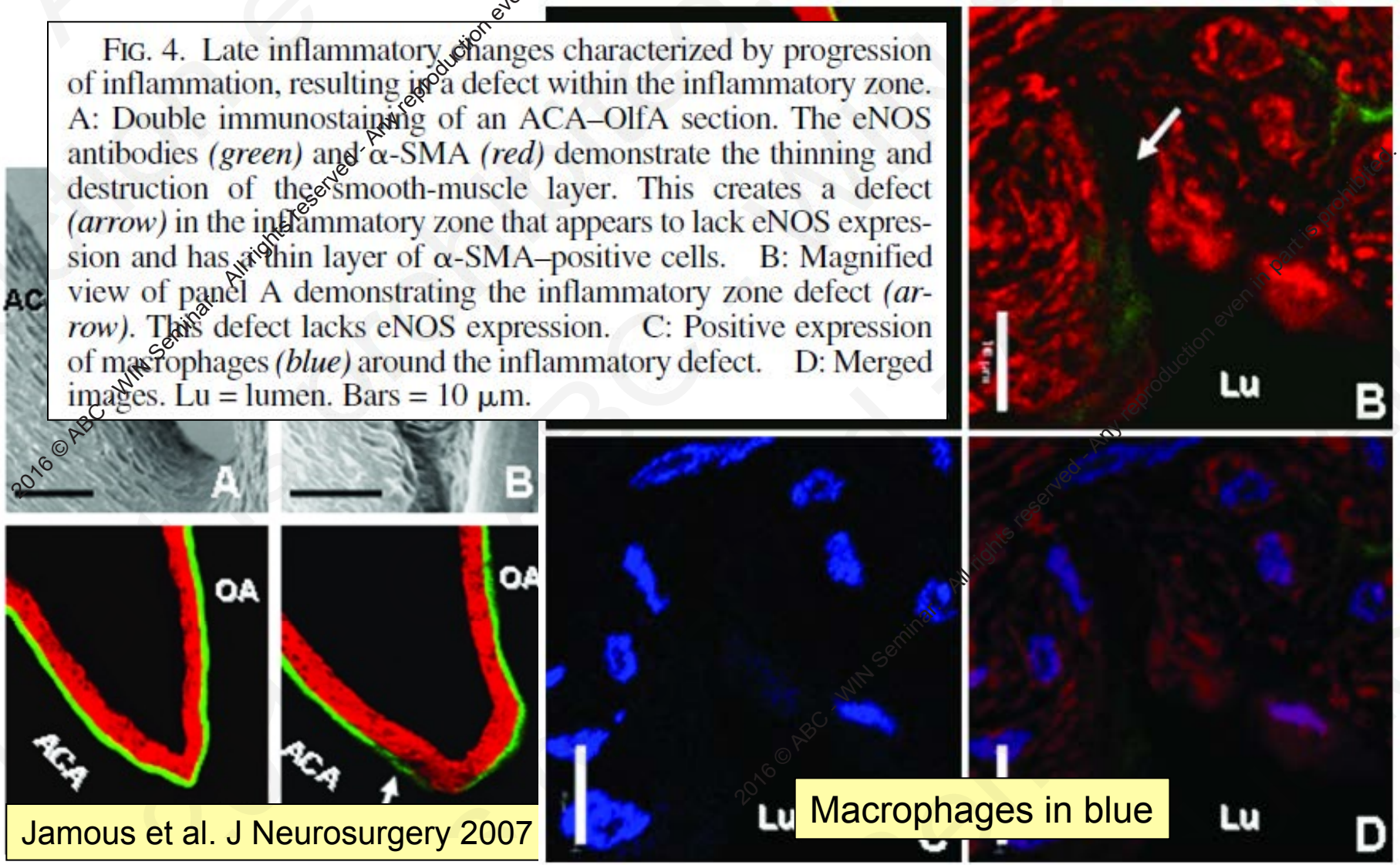
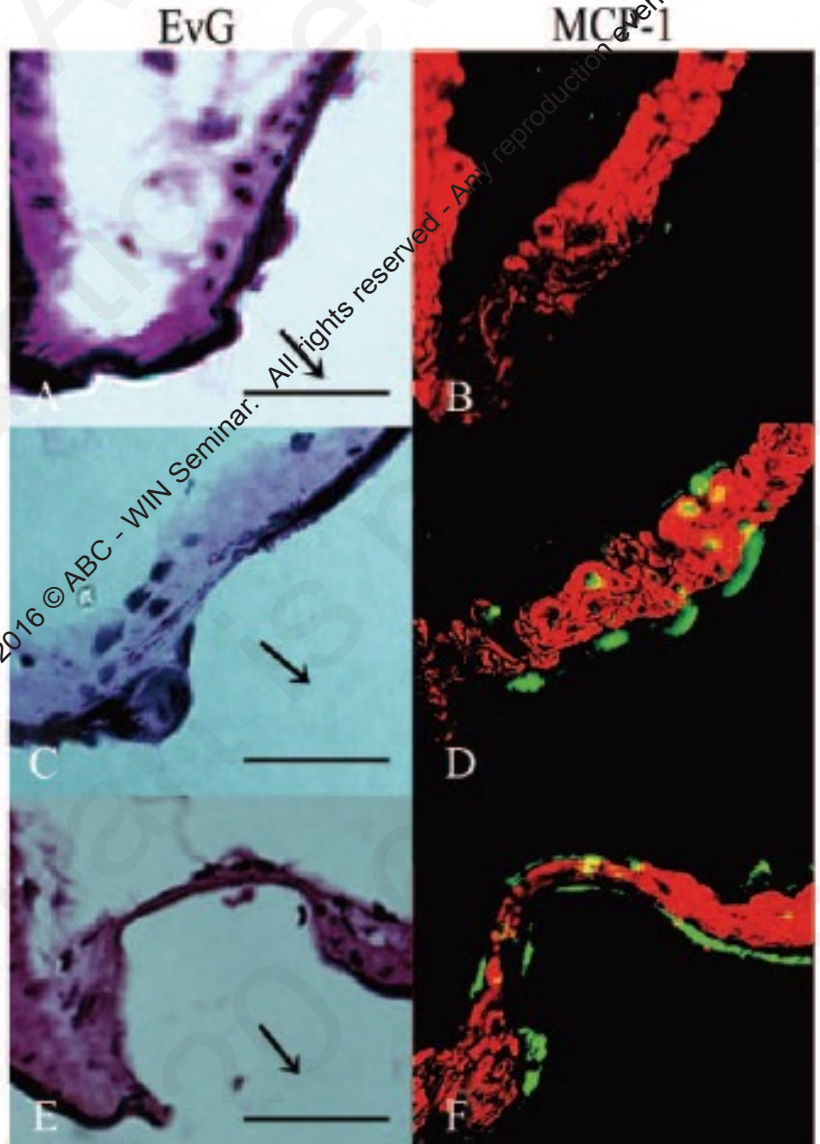


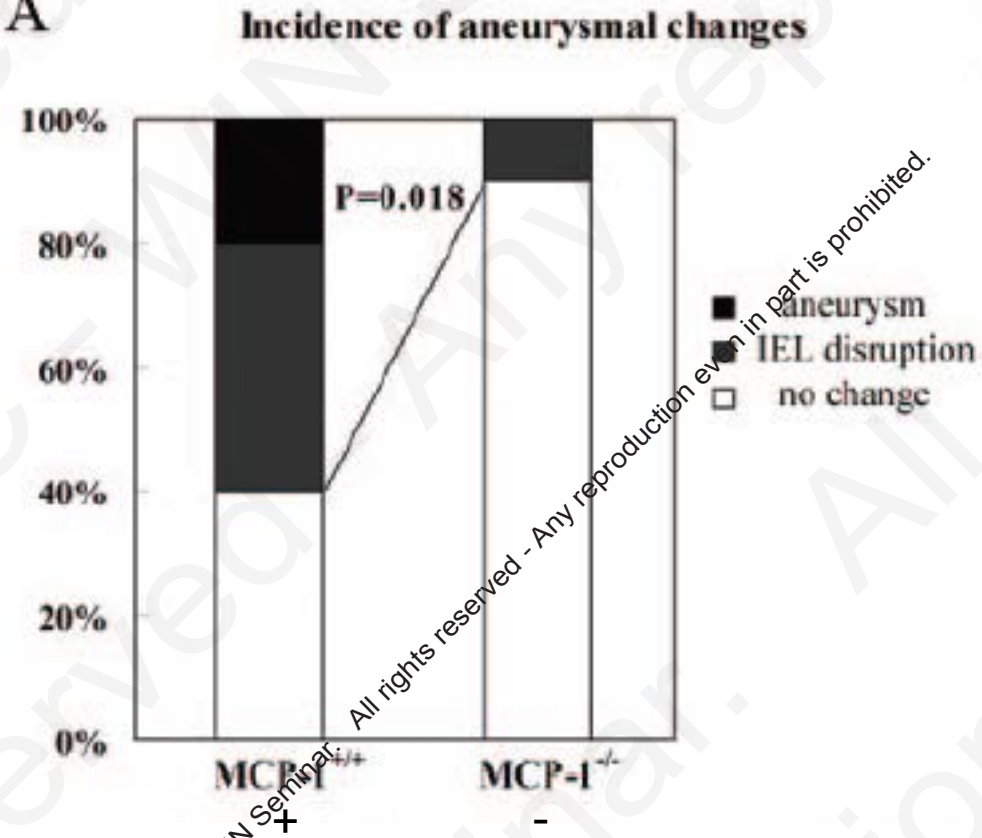
FIG. 4. Late inflammatory changes characterized by progression of inflammation, resulting in a defect within the inflammatory zone. A: Double immunostaining of an ACA–OlfA section. The eNOS antibodies *(green)* and α-SMA *(red)* demonstrate the thinning and destruction of the smooth-muscle layer. This creates a defect *(arrow)* in the inflammatory zone that appears to lack eNOS expression and has a thin layer of α-SMA–positive cells. B: Magnified view of panel A demonstrating the inflammatory zone defect *(arrow)*. This defect lacks eNOS expression. C: Positive expression of macrophages *(blue)* around the inflammatory defect. D: Merged images. Lu = lumen. Bars = 10 μm.

Macrophages in blue

Jamous et al. J Neurosurgery 2007

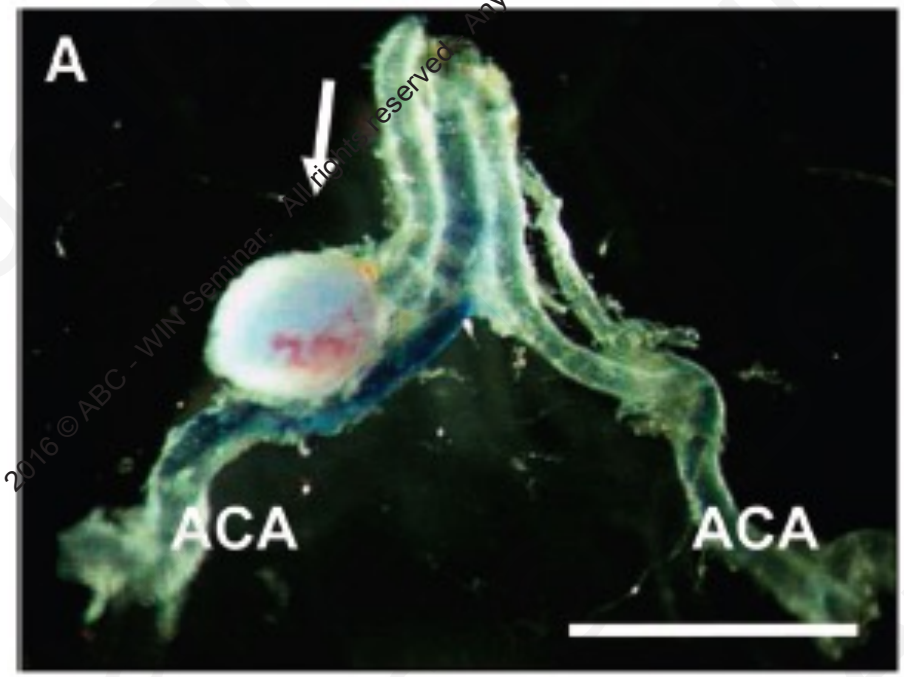
Inhibition of macrophage recruitment via MCP-1 inhibits aneurysm formation (carotid ligation model)

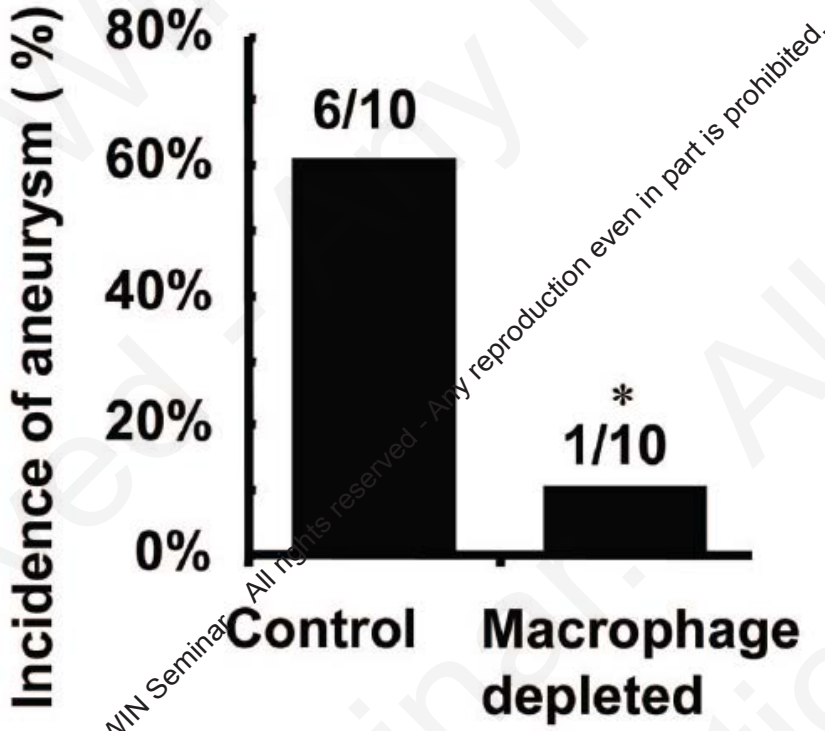




Aoki T et al. Stroke 2009

Depletion of macrophages inhibits aneurysm formation (elastase model)



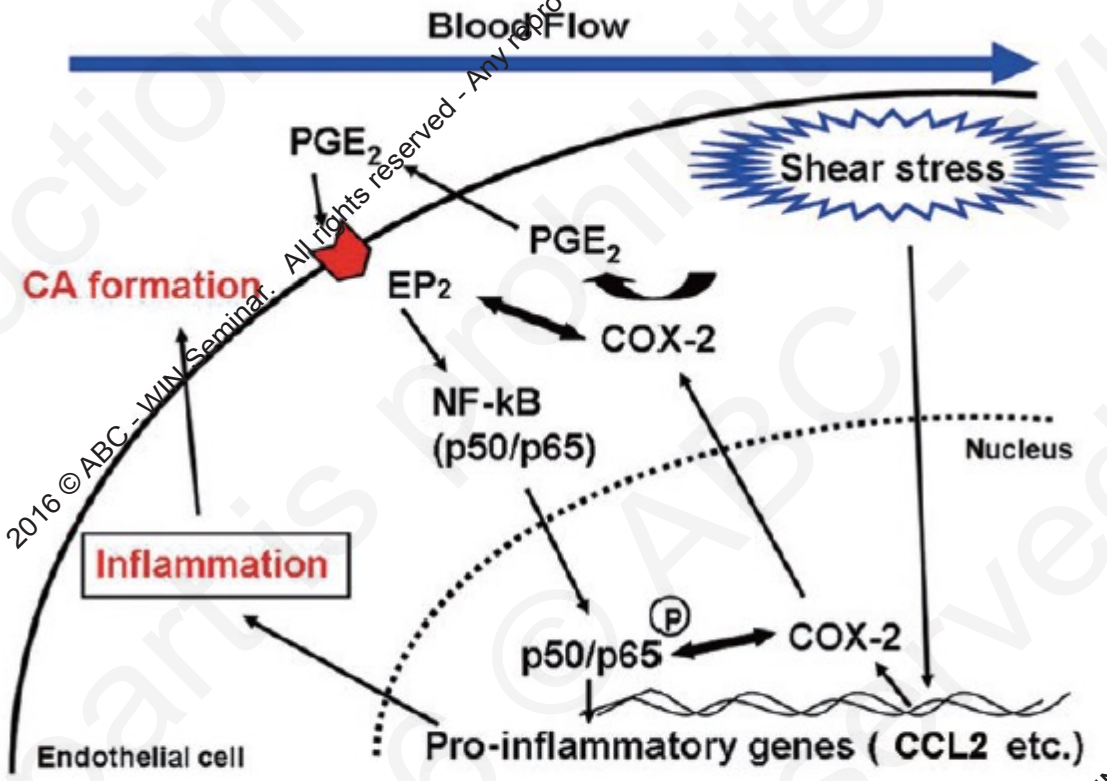


Kanematsu et al. Stroke 2011

How macrophages cause aneurysm formation?

- Secretion of matrix degrading proteases (e.g. MMP-2 and MMP-9) *(Aoki et al. Stroke 2007)*
- Induction of oxidative stress – iNOS and reactive oxygen species (Fukuda et al. Circulation 2000 and Aoki et al. Lab Invest 2009)
- *Secretion of inflammatory cytokines (! COX2 and TNFa signaling) – phenotype change in smooth muscle and endothelial cells*
- *Potentially induction of cell death in the aneurysm wall (Fas-signaling and TNFa signaling)*

Induction of macrophage activation via COX-2 and EP2 positive feed back loop during formation of intracranial aneurysms (carotid ligation model)



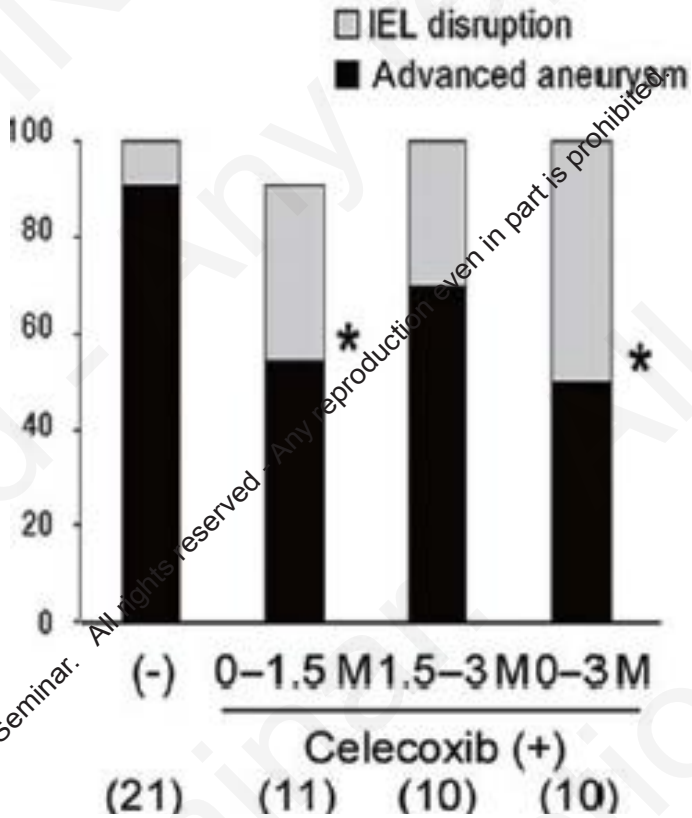
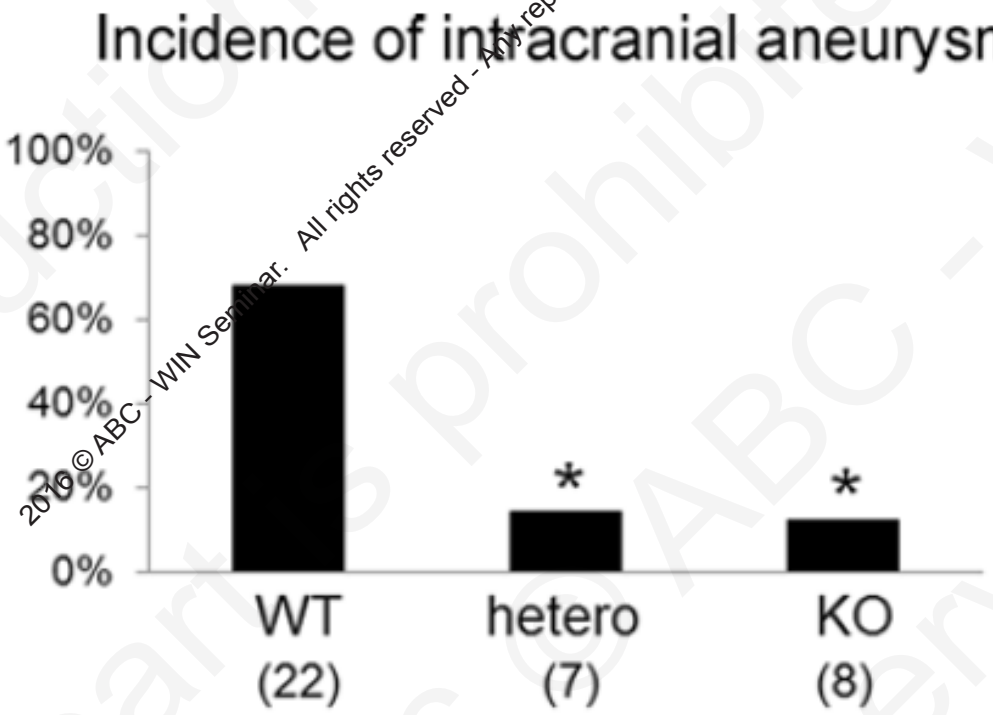


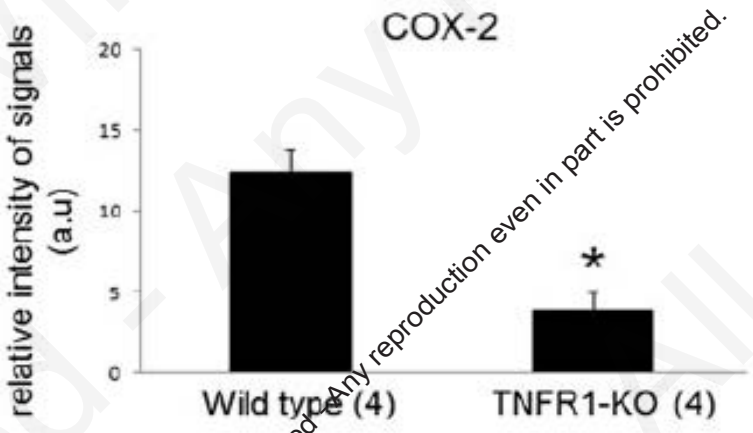
Figure 5

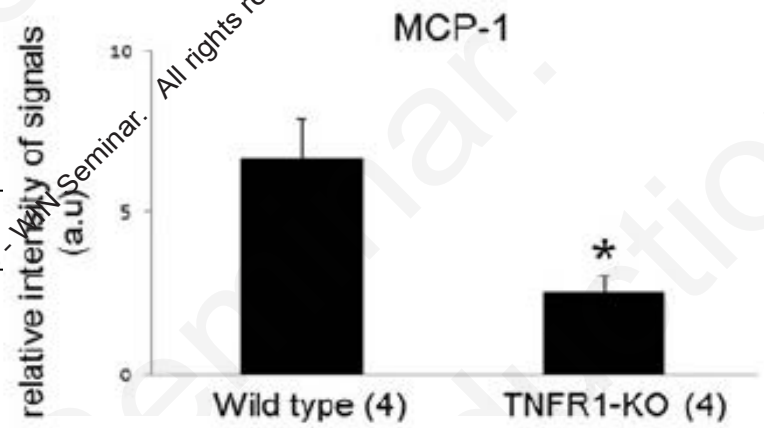
A proposed model for the formation of cerebral aneurysm induced by shear stress.

Aoki T et al. Br J Pharmacology 2011

Inhibition of TNFa signaling (TNFaR1 knock out)
1) reduces aneurysm formation and
2) MCP-1 and COX-2 expression in the aneurysm wall







TNFaR1 knock out mice (KO) vs. wild type (WT)

Aoki et al. Acta Neuropathologica Comm 2014

Conclusion: Formation of intracranial aneurysms is mediated by macrophages

1. Macrophage infiltration associates with aneurysm formation

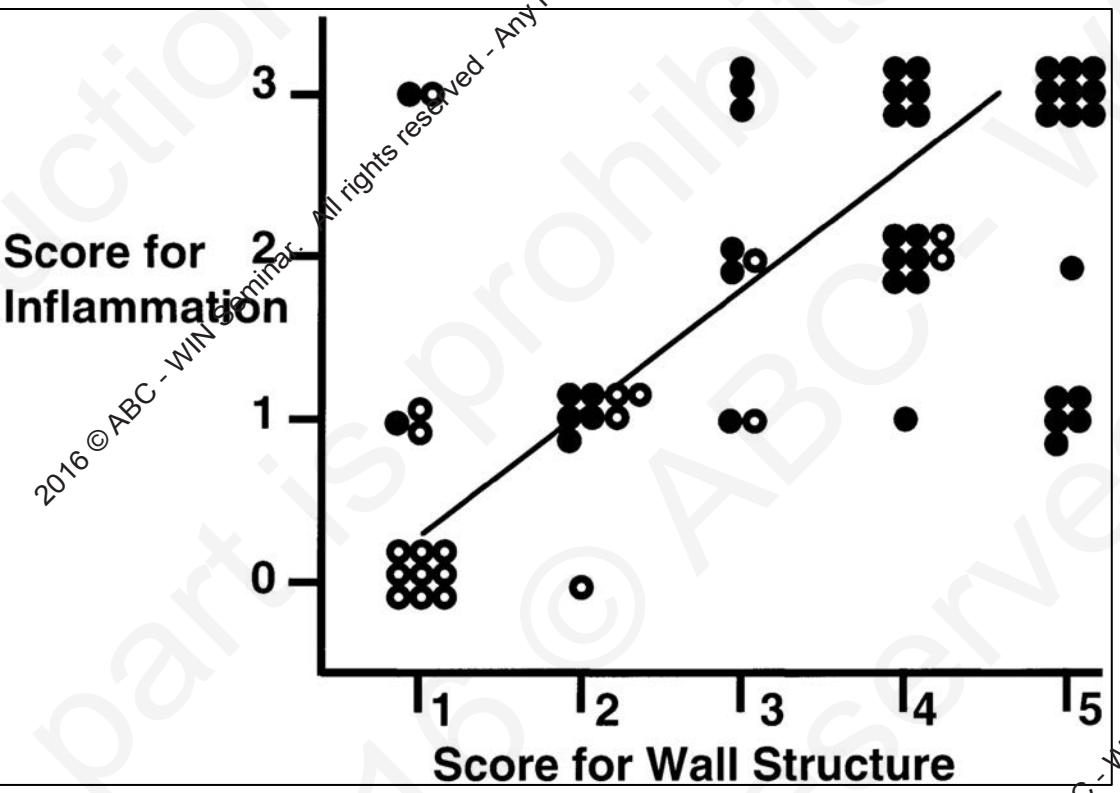
2. Blocking macrophage infiltration prevents aneurysm formation

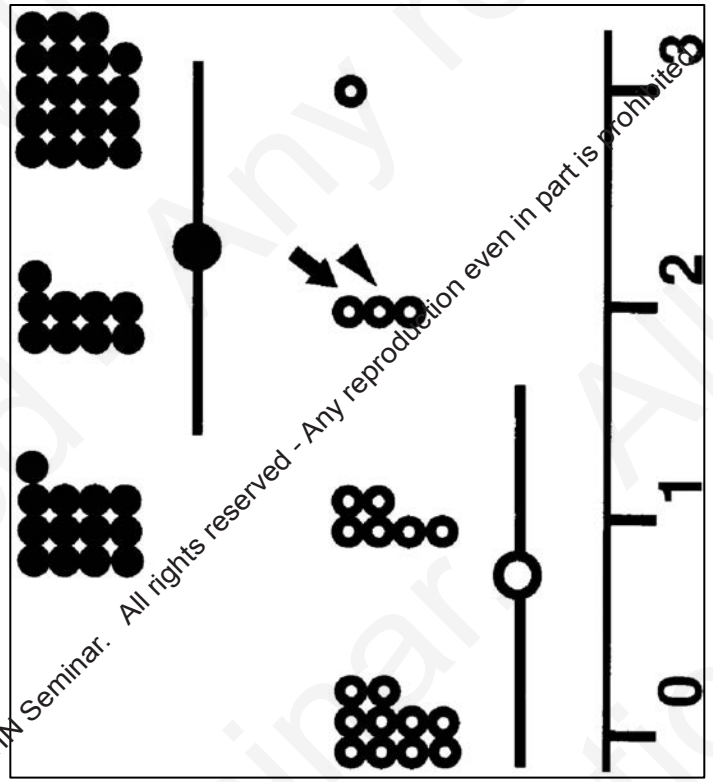
3. Inhibition of macrophage signaling (TNFa and COX-2) prevents aneurysm formation

What about aneurysm rupture?

- Data from human tissue samples available (ruptured vs. unruptured aneurysms)
- Various animal models – none that for certain replicates the human pathobiology of aneurysm rupture
  - Induced hypertension + elastase injetion model
  - Decellularized aortic graft model

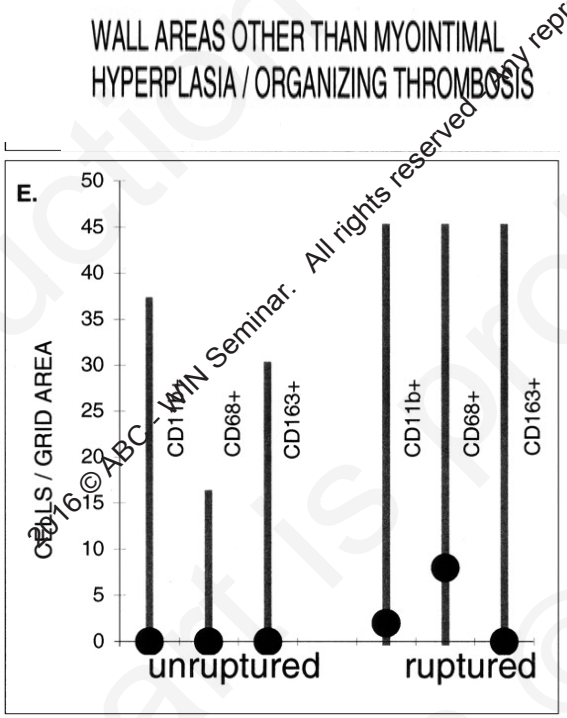
Macrophage infiltration associates with wall degeneration and rupture in human intracranial aneurysms

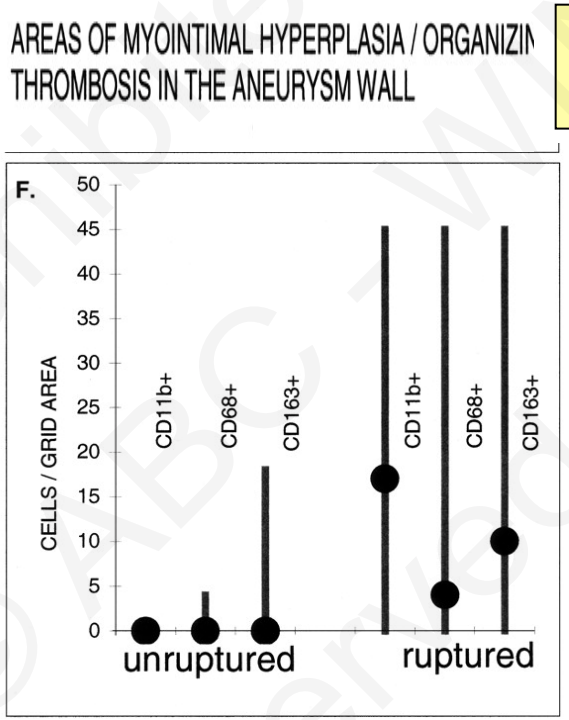




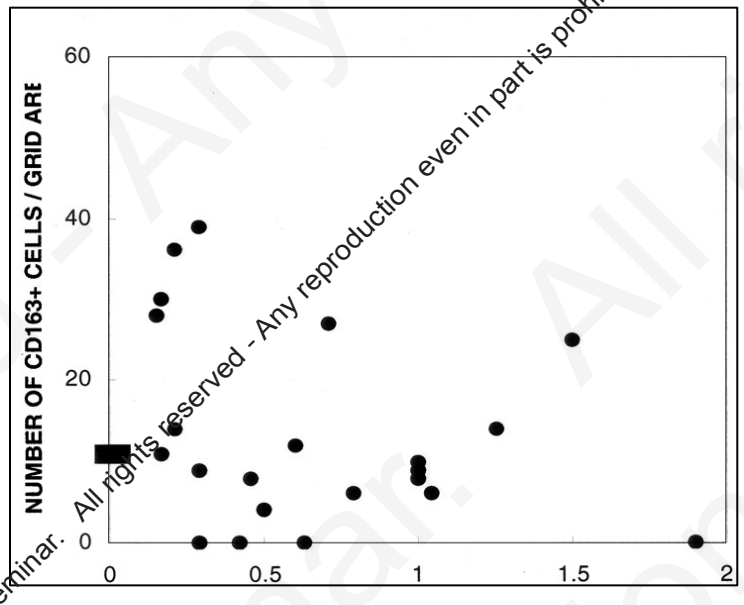
Kataoka et al. Stroke 1999

Macrophage infiltration increases with rupture and is not just a reaction to rupture





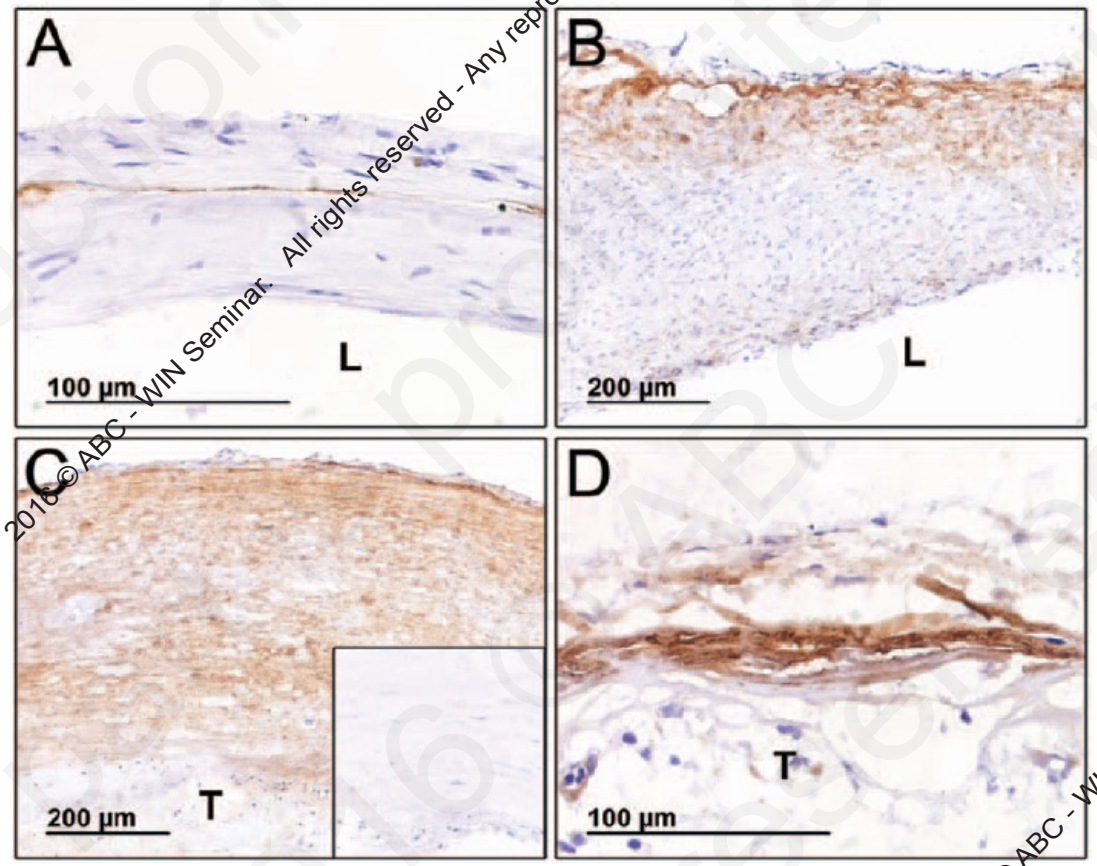
No correlation with time from rupture and macrophage infiltration

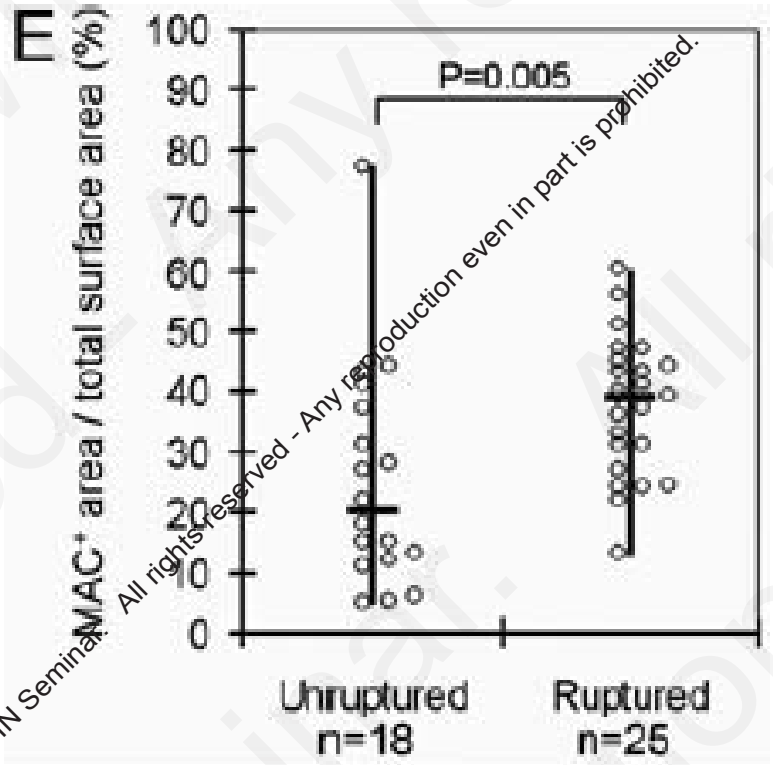


Days from rupture to sample resection

Frösen et al. Stroke 2004

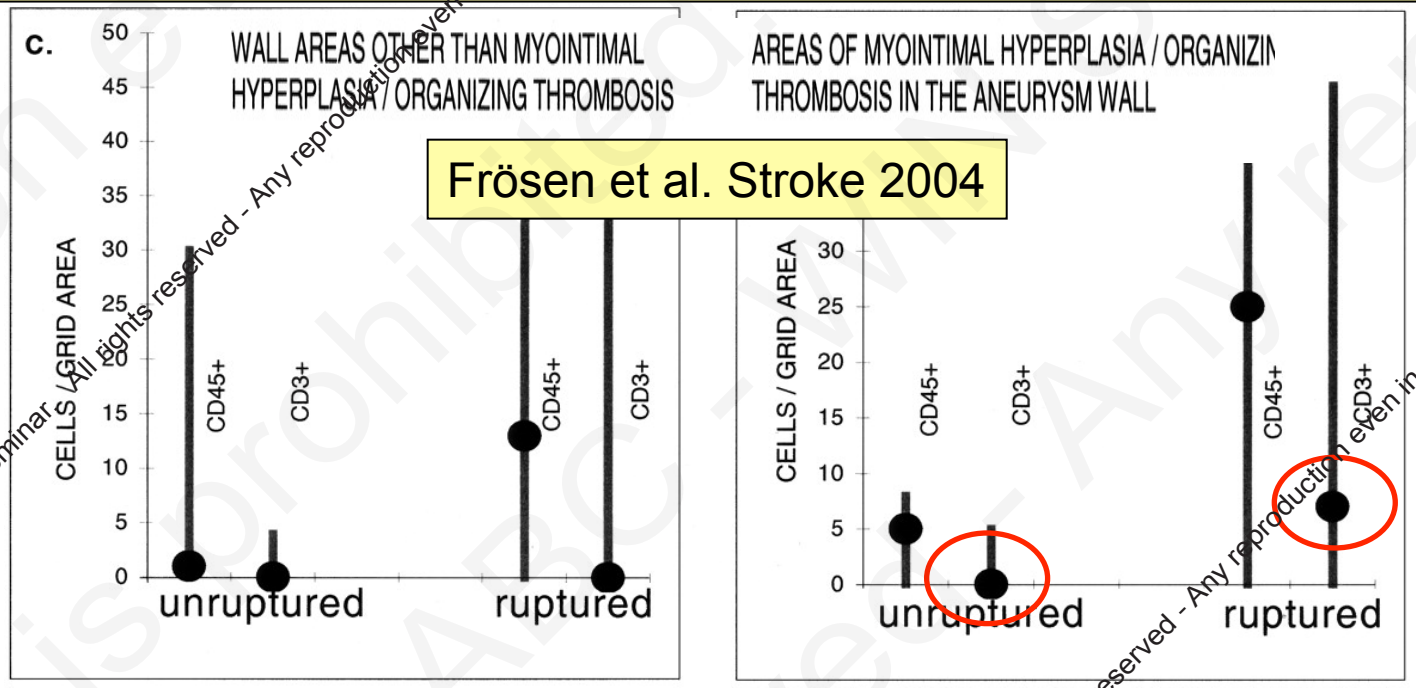
In addition to macrophage infiltration – Aneurysm wall degeneration and rupture associates with activation of the complement (humoral immune system)



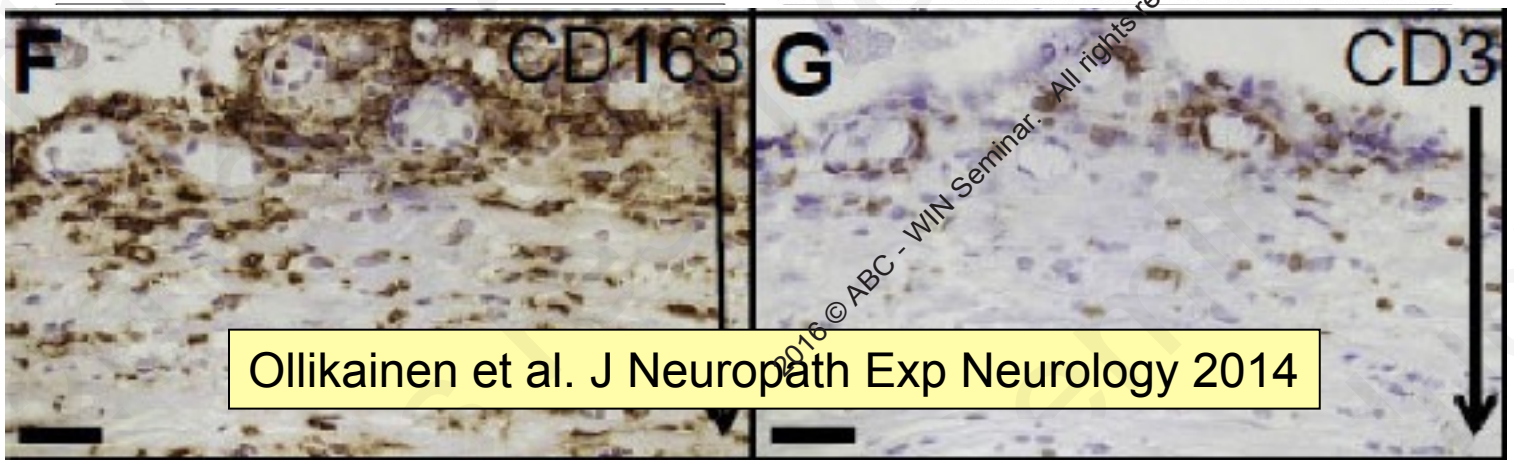


Tulamo et al. Neurosurgery 2006

Also T-cells (CD3) are present in the aneurysm wall – and associate with rupture



Frösen et al. Stroke 2004



Ollikainen et al. J Neuropath Exp Neurology 2014

…and also, *mast cells* are present in the aneurysm wall and associate with accumulation of iron and lipids as well as with macrophage and T-cell infiltration

TABLE 4. Association of Histologic Characteristics of sIAs With MCs

Variable	MCs		p
	Absent (n = 27)	Present (n = 9)	
Histology of sIA wall			
Endothelium not intact, n (%)*	17/27 (63)	9/9 (100)	0.009†
Intraluminal fresh thrombosis present, n (%)*	10/26 (38)	0/7 (0)	0.073
Intraluminal organizing thrombosis present, n (%)*	9/26 (35)	2/7 (29)	1.000
Lipid present in the EC M, n (%)*	21/27 (78)	7/9 (78)	1.000
Ratio of lipid-rich area, median (range), %‡	9 (0–37)	37 (0–69)	0.022†
Neovessels present, n (%)*	6/27 (22)	9/9 (100)	<0.001†
Distribution density of neovessels, median (range), neovessels/mm^2‡	0 (0–15)	2 (0–50)	<0.001†
Iron deposition present, n (%)*	3/27 (11)	6/9 (67)	0.003†
Strong iron deposition present, n (%)*	1/27 (4)	5/9 (56)	0.002†
Total number of inflammatory cells			
CD68-positive macrophages, median (range)‡§	15 (0–527)	224 (42–297)	0.012†
CD163-positive macrophages, median (range)‡§	72 (5–417)	158 (35–443)	0.110
CD3-positive T lymphocytes, median (range)‡§	11 (0–357)	52 (25–137)	0.010†
Further analysis of MC-positive sIAs			
Total number of MCs, median (range)¶		23 (3–170)	
Total number of degranulated MCs, median (range)¶		15 (2–110)	
Ratio of degranulated MCs to all MCs, median (range), %¶		67 (43–96)	
Distribution density of MCs, median (range), MCs/mm^2		4 (1–69)	
Total number of MCs in standard-sized areas, median (range)§		7 (2–32)	

Ollikainen et al. J Neuropath Exp Neurology 2014

Genome wide comparison of gene expression in ruptured and unruptured human aneurysms – inflammation strongly associates with rupture

TABLE 2. Biological Processes in Ruptured Saccular Intracranial Aneurysm Wall Samples[a]

		Upregulated Genes				
Gene Ontology (GO) Biological Processes	GO ID[b]	P[c]	FDR[d]	OR	Count[e]	Size[f]
Chemotaxis	GO:0006935	2.60E − 14	5.66E − 11	7	30	125
Immune response	GO:0006955	5.70E − 14	6.26E − 11	4	49	333
Response to external stimulus	GO:0009605	1.00E − 13	7.41E − 11	3	74	651
Inflammatory response	GO:0006954	1.40E − 13	7.52E − 11	4.2	46	296
Locomotory behavior	GO:0007626	1.50E − 11	6.46E − 09	4.5	35	208
Response to stress	GO:0006950	3.60E − 09	1.31E − 06	2.1	99	1224
Response to other organism	GO:0051707	4.70E − 08	1.49E − 05	5.1	18	87
Positive regulation of tumor necrosis factor production	GO:0032760	5.80E − 08	1.60E − 05	11.7	6	7
Locomotion	GO:0040011	1.30E − 07	3.18E − 05	4.7	20	114
Cytokine production	GO:0001816	2.50E − 05	5.49E − 03	3.8	16	108
Phosphate metabolic process	GO:0006796	5.30E − 05	1.06E − 02	1.8	66	893
Positive regulation of interleukin-6 production	GO:0032755	5.90E − 05	1.08E − 02	42	4	6
Regulation of cell proliferation	GO:0042127	1.80E − 04	3.10E − 02	1.9	44	550
Intracellular lipid transport	GO:0032365	2.60E − 04	3.55E − 02	21	4	8
Neutrophil chemotaxis	GO:0030593	2.70E − 04	3.55E − 02	12	5	14
Regulated secretory pathway	GO:0045055	2.70E − 04	3.55E − 02	12	5	14
Protein amino acid phosphorylation	GO:0006468	2.70E − 04	3.55E − 02	1.8	47	613
Regulation of cytokine biosynthetic process	GO:0042035	3.90E − 04	4.55E − 02	4.2	10	61
Purine ribonucleoside monophosphate biosynthetic process	GO:0009168	3.90E − 04	4.55E − 02	11	5	15

Kurki et al. Neurosurgery 2011

So
inflammation associates with rupture in human aneurysms

– but is it the cause of rupture?

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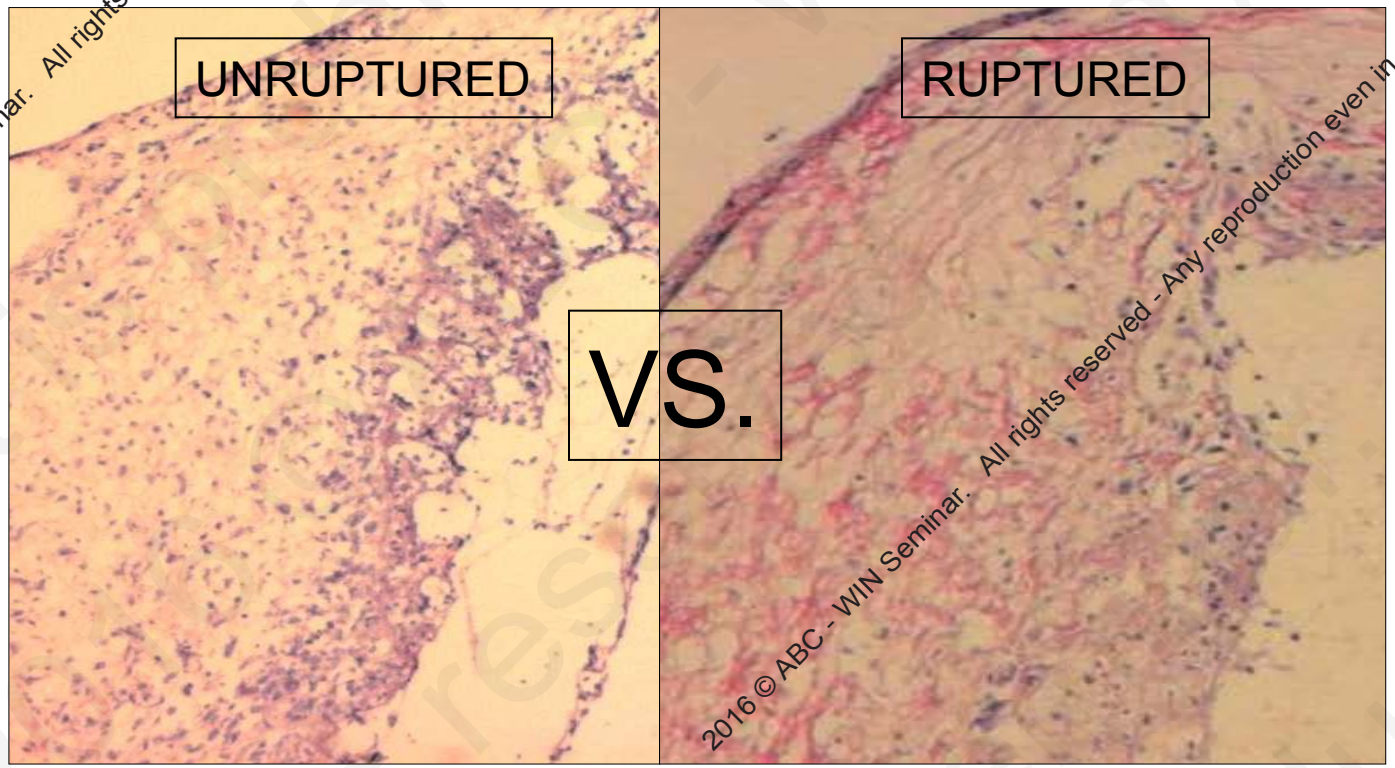
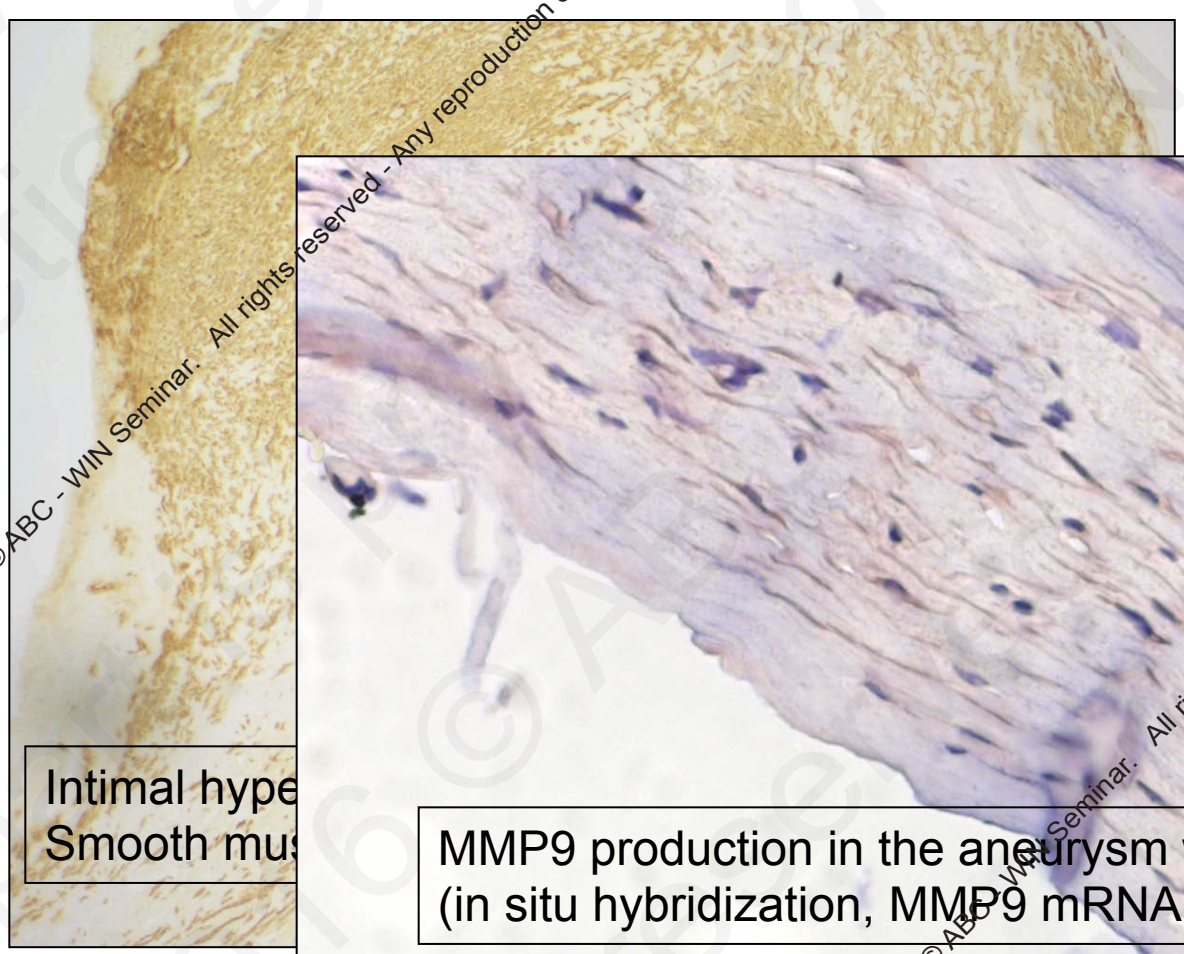
Degenerative remodeling of aneurysm wall – What characterizes a *ruptured* aneurysm wall

- Loss of smooth muscle cells

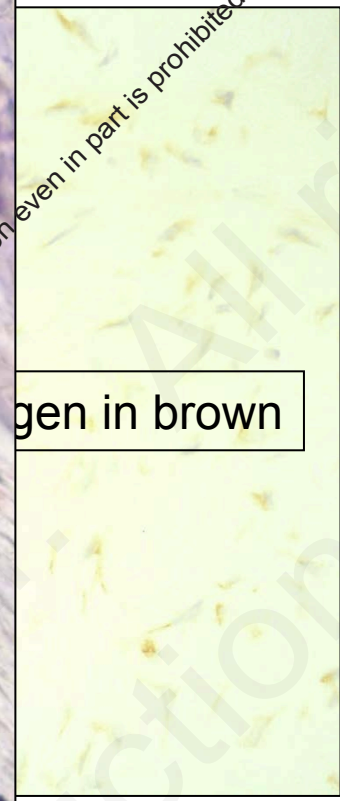


Why smooth muscle cells are important?
Capacity for matrix synthesis & adaptive remodeling



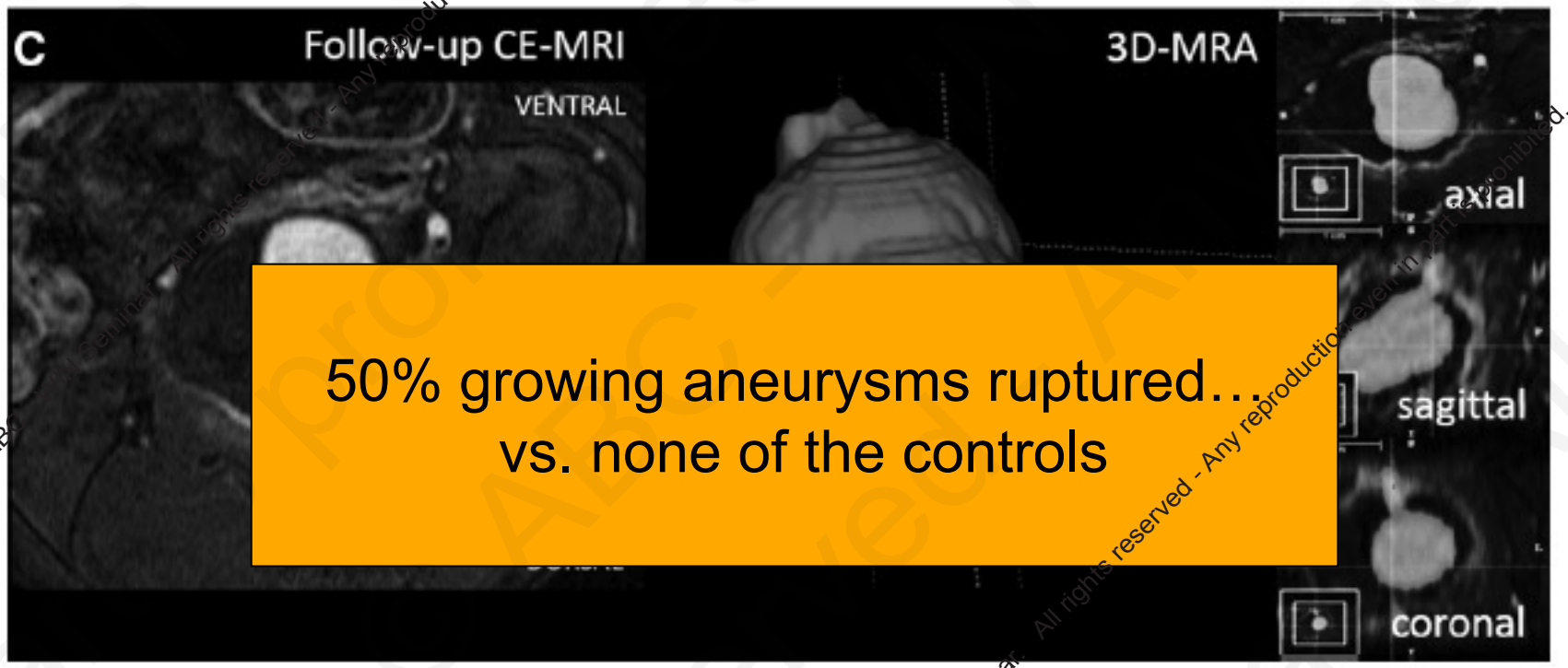
Intimal hype
Smooth mus

MMP9 production in the aneurysm wall
(in situ hybridization, MMP9 mRNA in brown

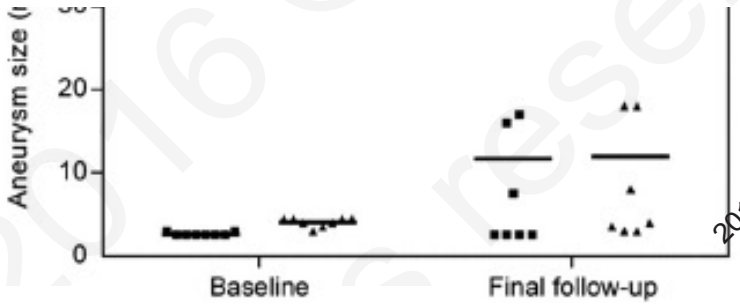


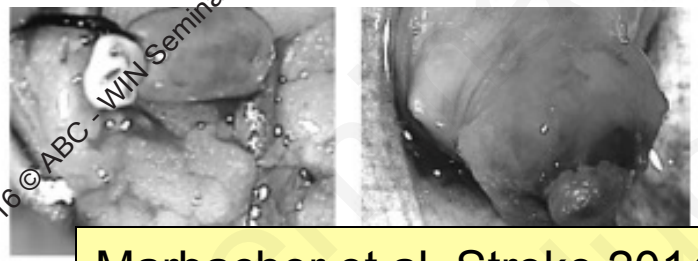
gen in brown

Loss of mural cells is what causes aneurysm growth and rupture…



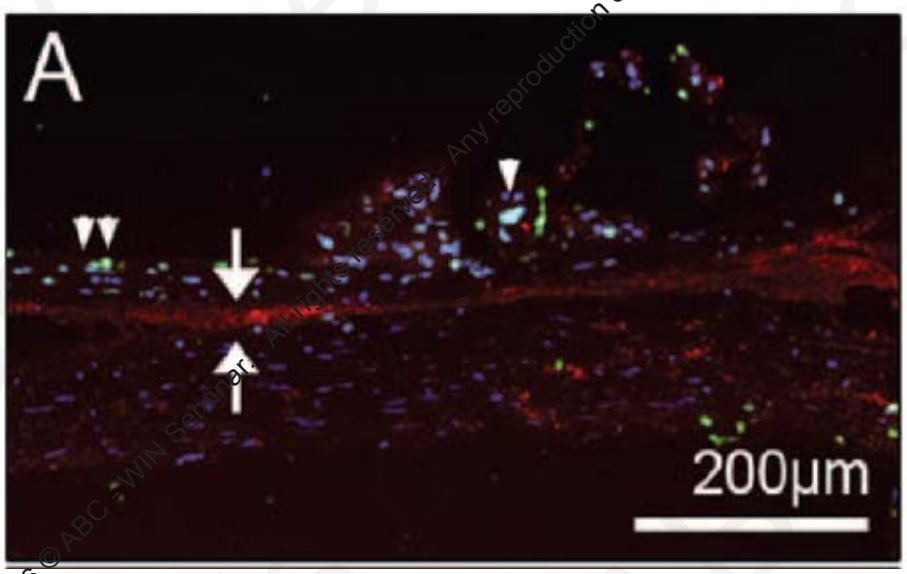
50% growing aneurysms ruptured…
vs. none of the controls

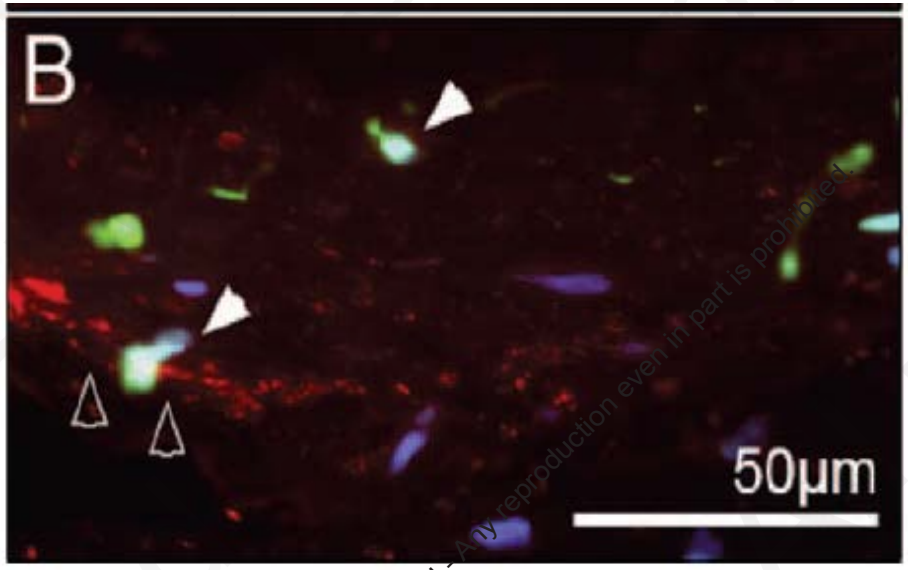




Marbacher et al. Stroke 2014

No evidence that complement acts directly on smooth muscle cells

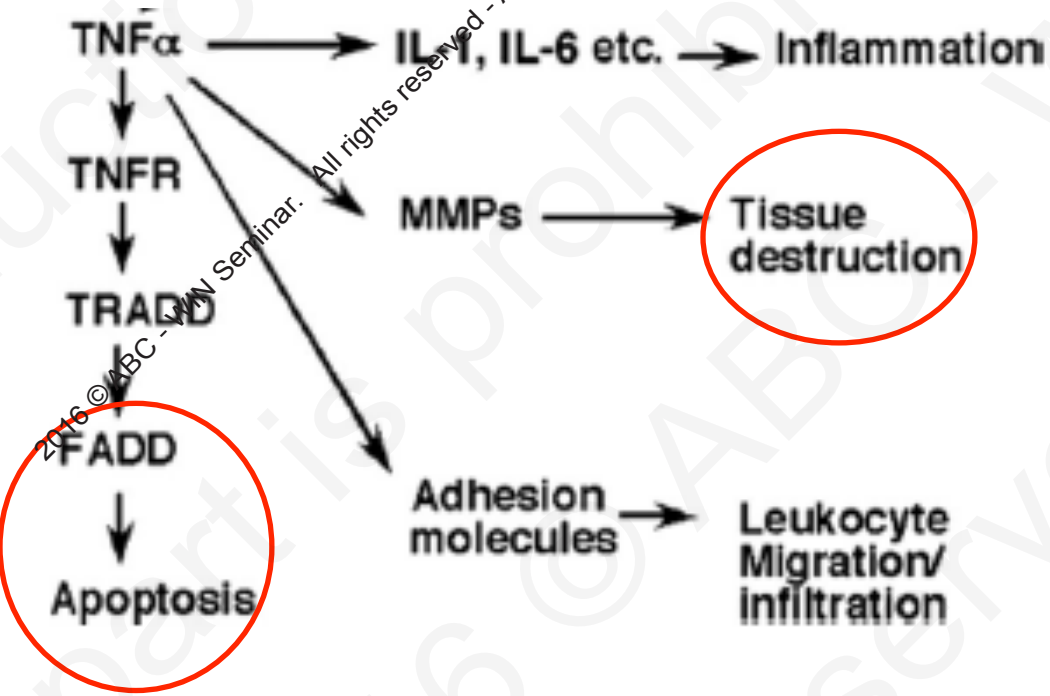




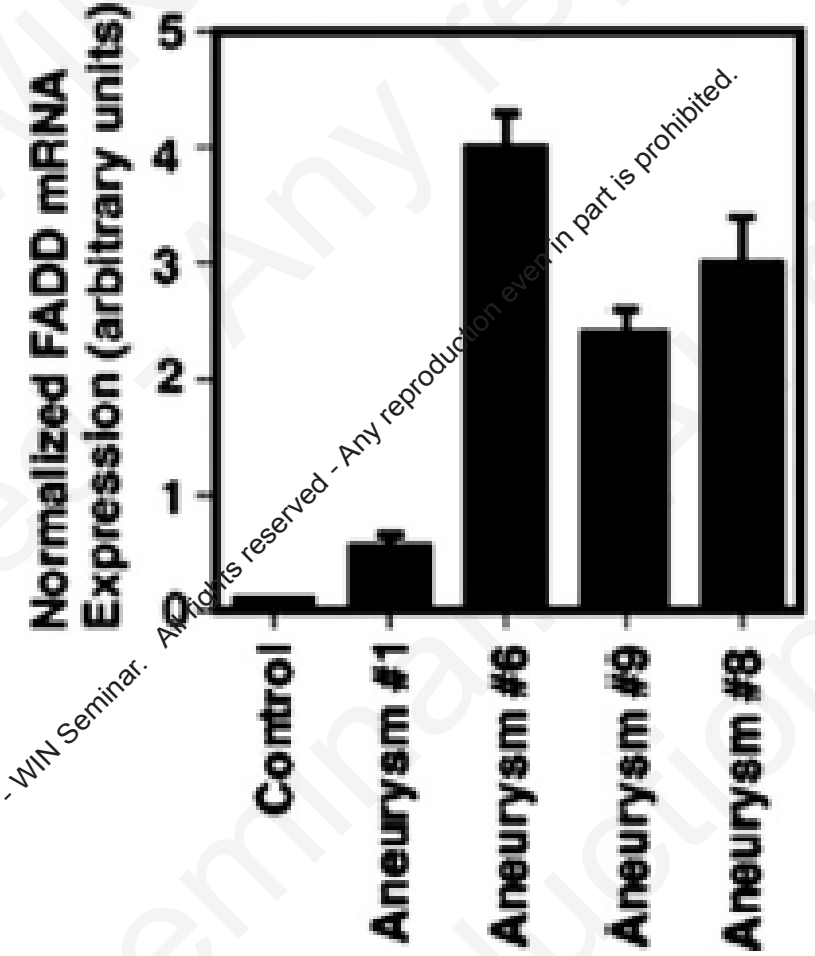
Complement activation (in red) and cell death (green/turqoise) DO NOT colocalize in the aneurysm wall

Tulamo et al. Neurosurgery 2006

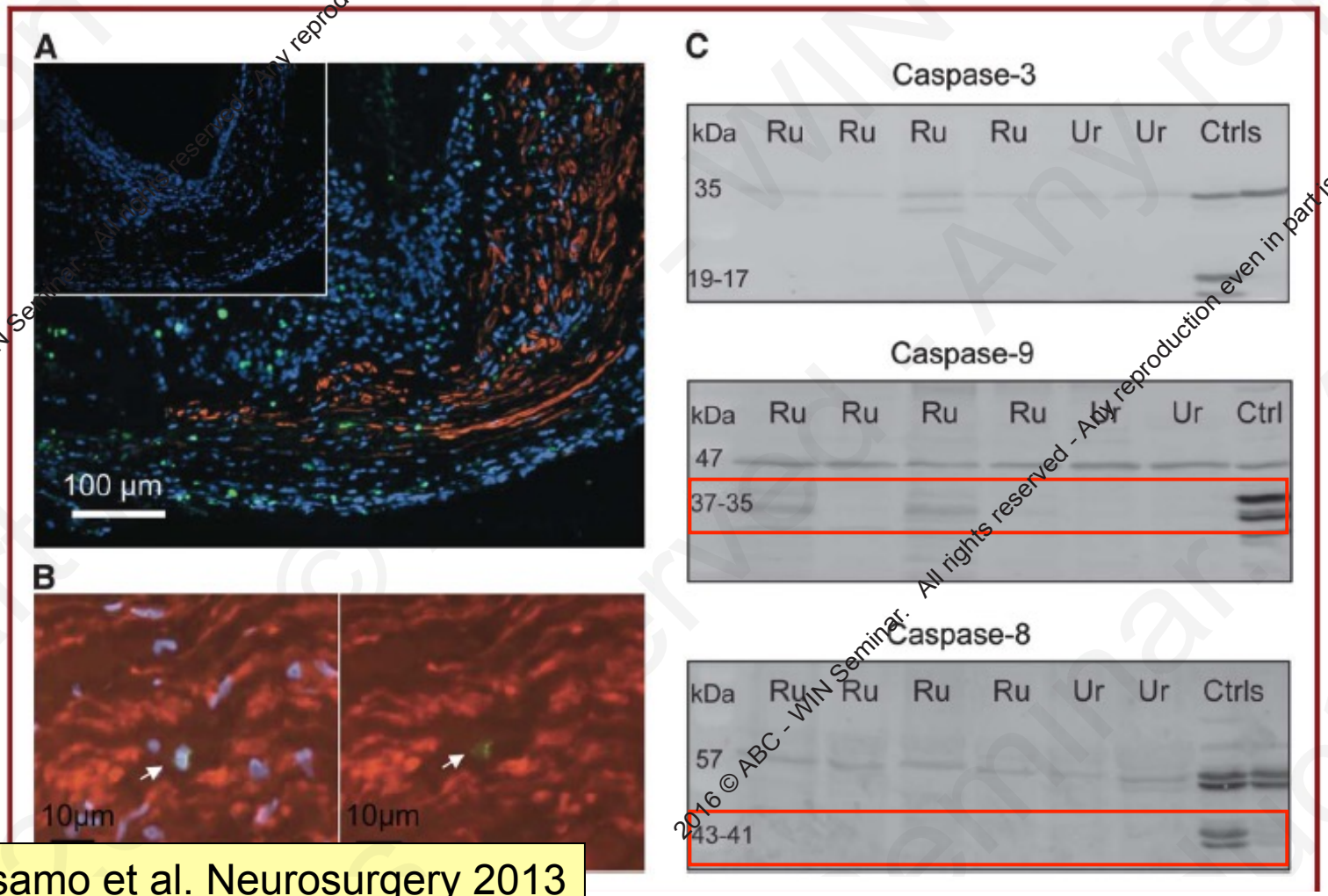
Macrophage derived cytokines – TNFa implicated in aneurysm formation in mice … what about rupture in human aneurysms?



Jayaraman et al. Neurosurgery 2006

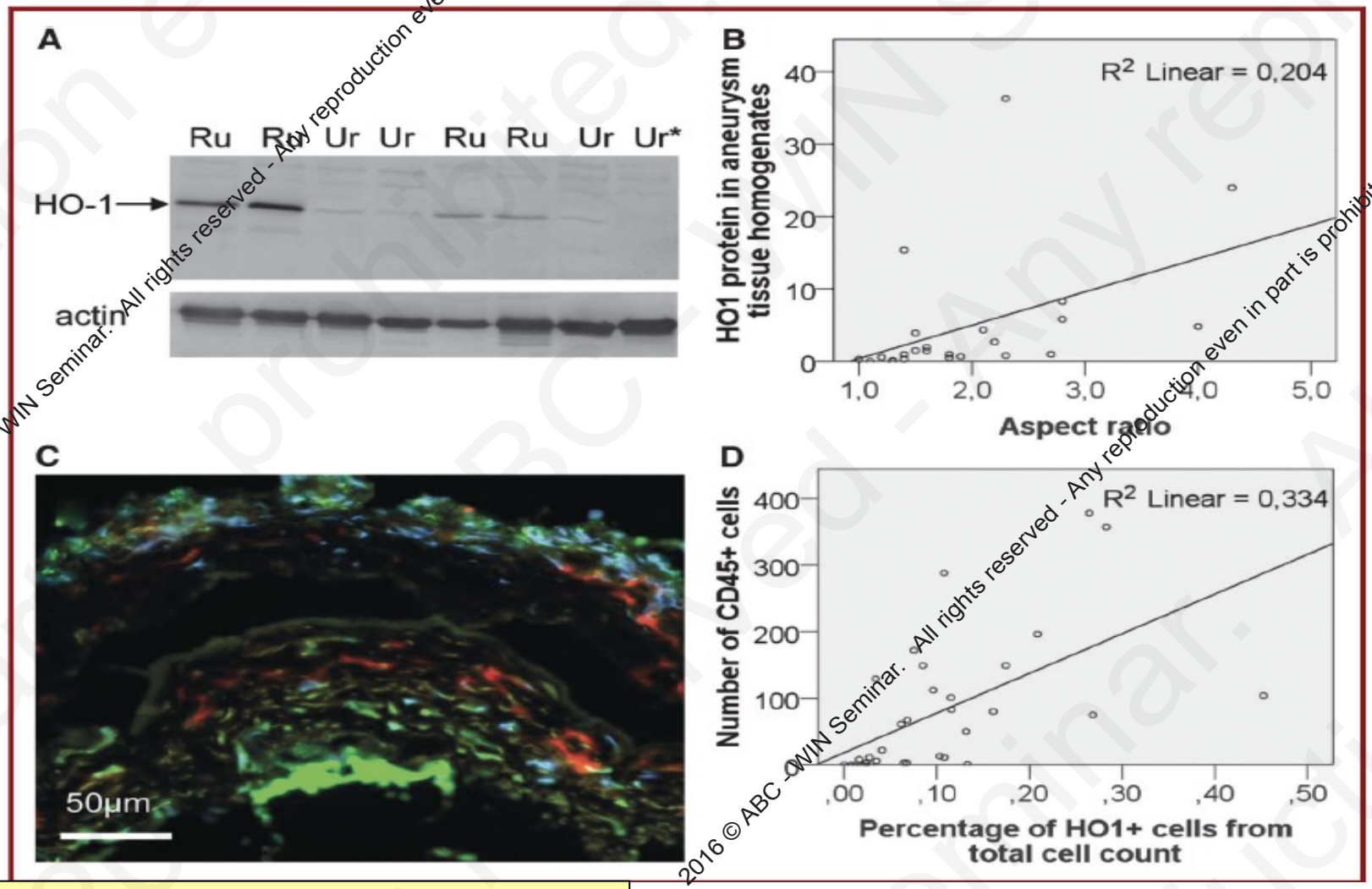


No evidence for apoptosis induced by inflammatory cells (Caspase-8 activation) in human aneurysm wall – other factors implicated (Caspase-9 activation)



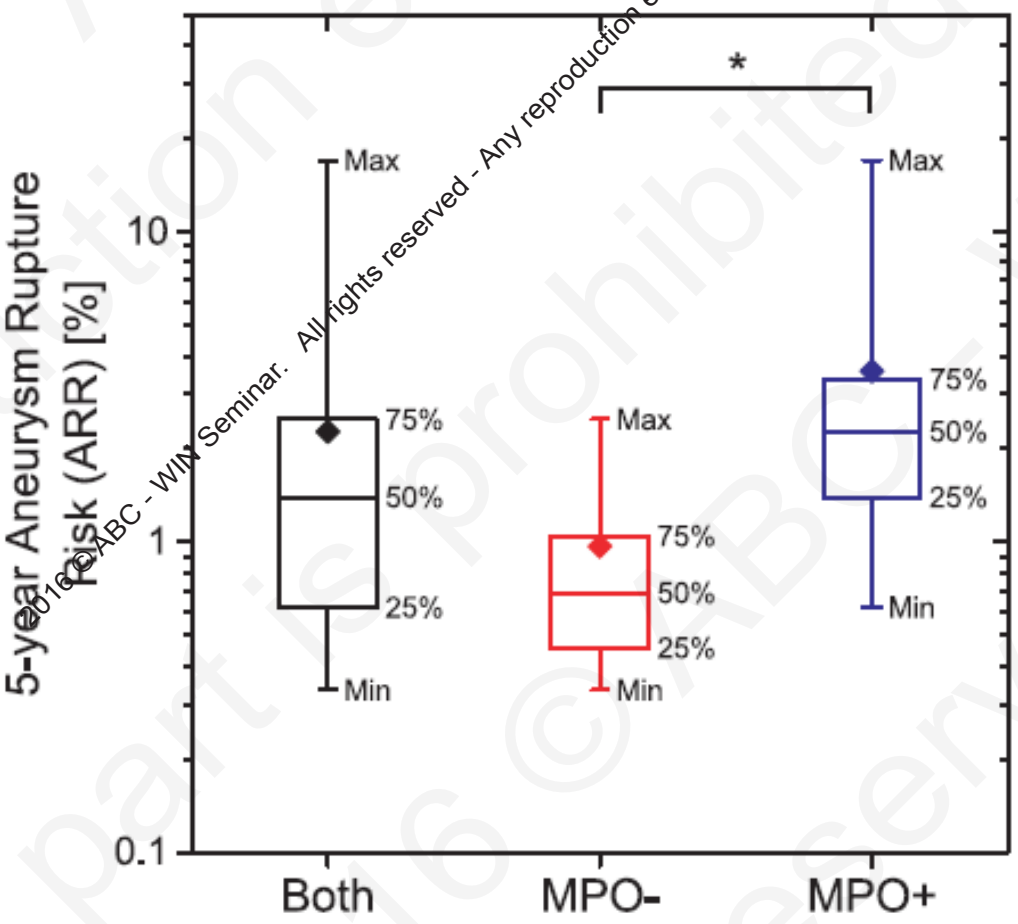
Laaksamo et al. Neurosurgery 2013

… instead apoptosis & inflammatory cell infiltration in the aneurysm wall associates with response to oxidative stress - and some inflammatory cells express hemeoxygenase-1



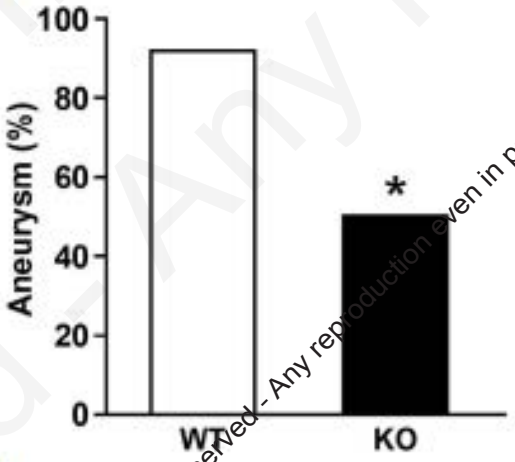
Laaksamo et al. Neurosurgery 2013

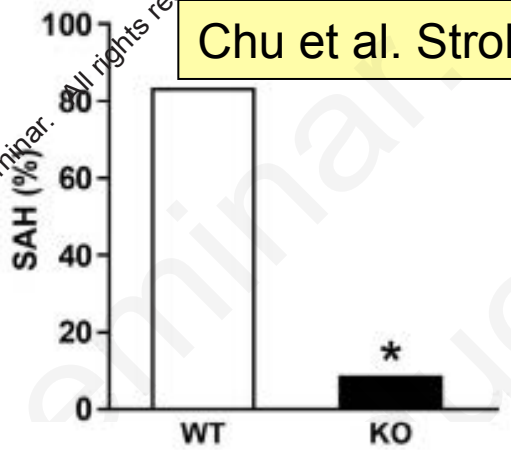
Myeloperoxidase (derived from neutrophils & macrophages) causes oxidative stress – and associates with rupture risk in aneurysms



Gounis et al. Stroke 2014

Aneurysm formation & SAH in myeloperoxidase knock out mice





Chu et al. Stroke 2015

1) So other causes – perhaps oxidative stress- rather than macrophage induced cell death are the primary cause of smooth muscle cell loss

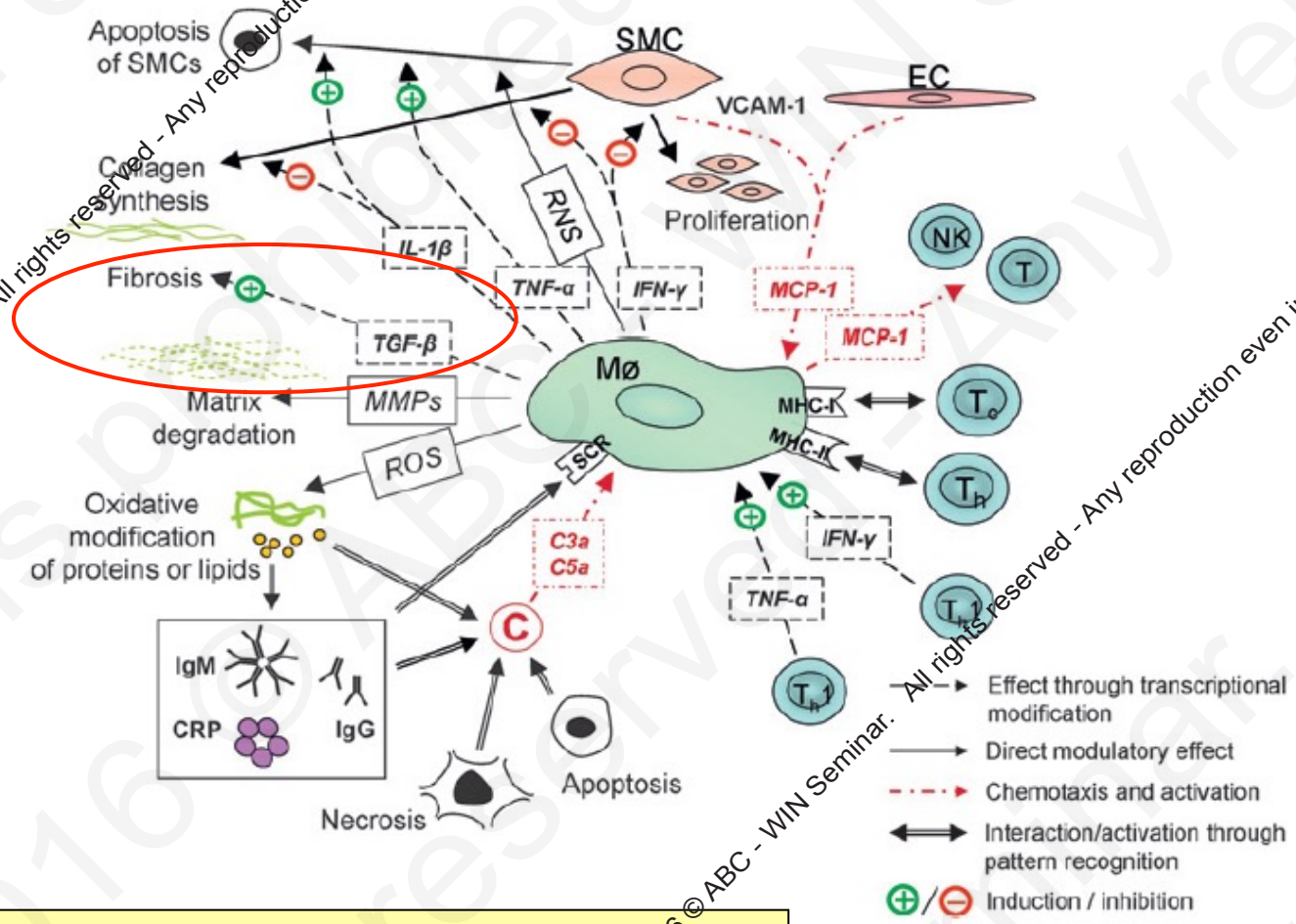
and

2) Some of the inflammatory cells perhaps cause oxidative stress

– where as others are a response to it?

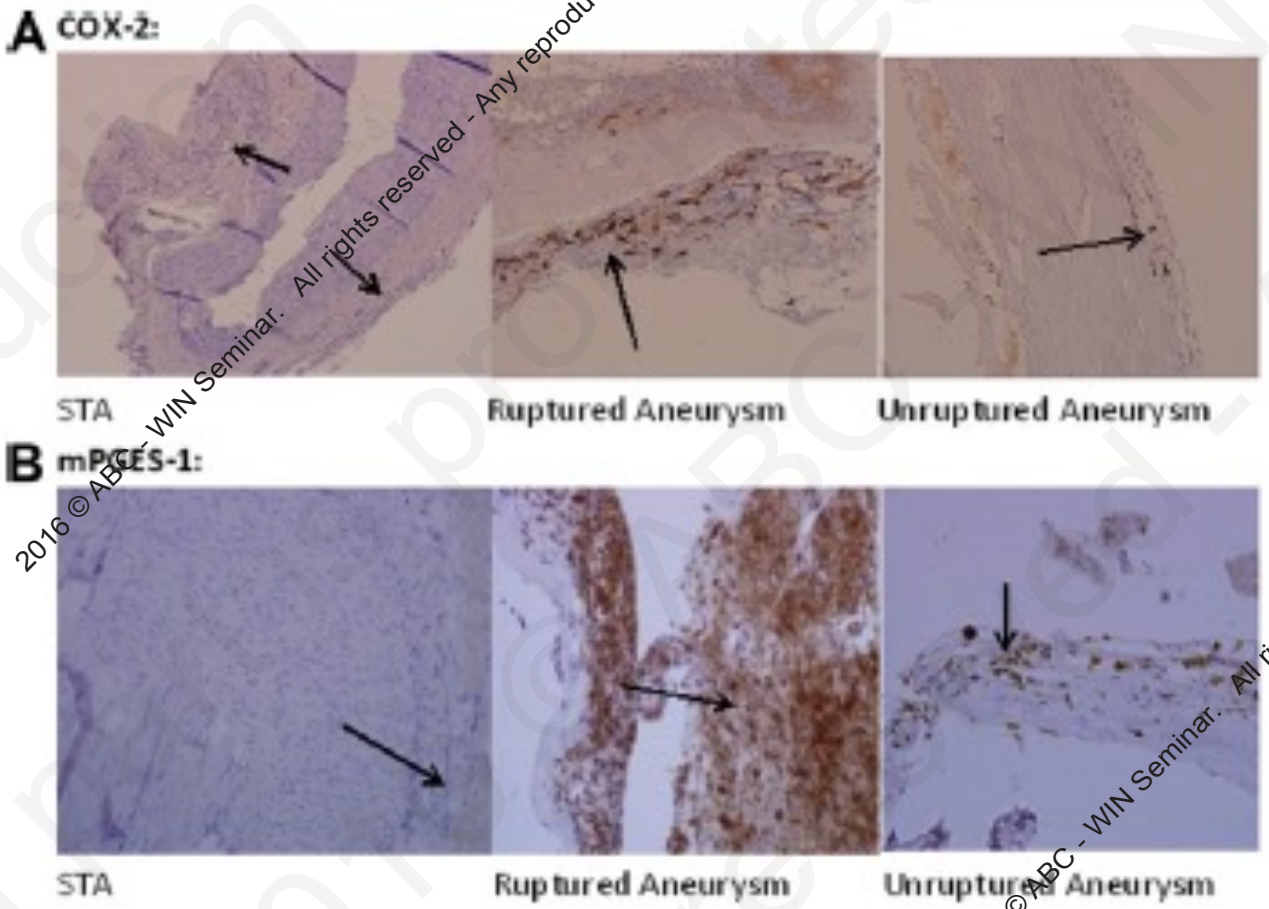
… not all inflammatory cells or macrophages in the aneurysm wall necessarily have the same function?

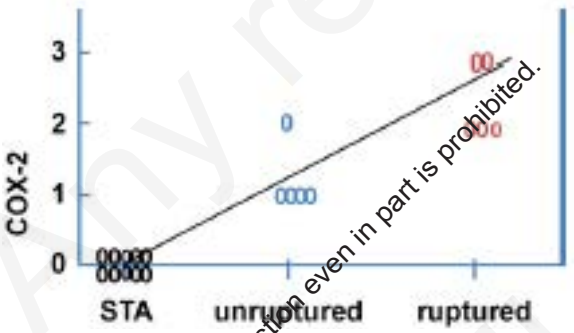
Macrophages affect smooth muscle cell function in multiple ways – and can even promote fibrosis

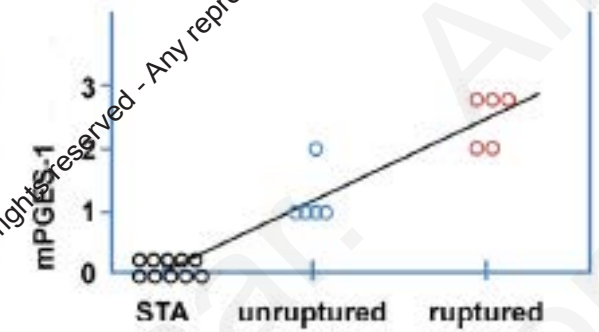


Tulamo et al. J Neurointerventional Surg 2010

Macrophage derived cytokines – COX2 signaling implicated in aneurysm formation … what about rupture in human aneurysms?







Hasan et al. Stroke 2012

There is evidence that COX inhibition reduces
1) inflammation and
2) risk of aneurysm rupture in humans

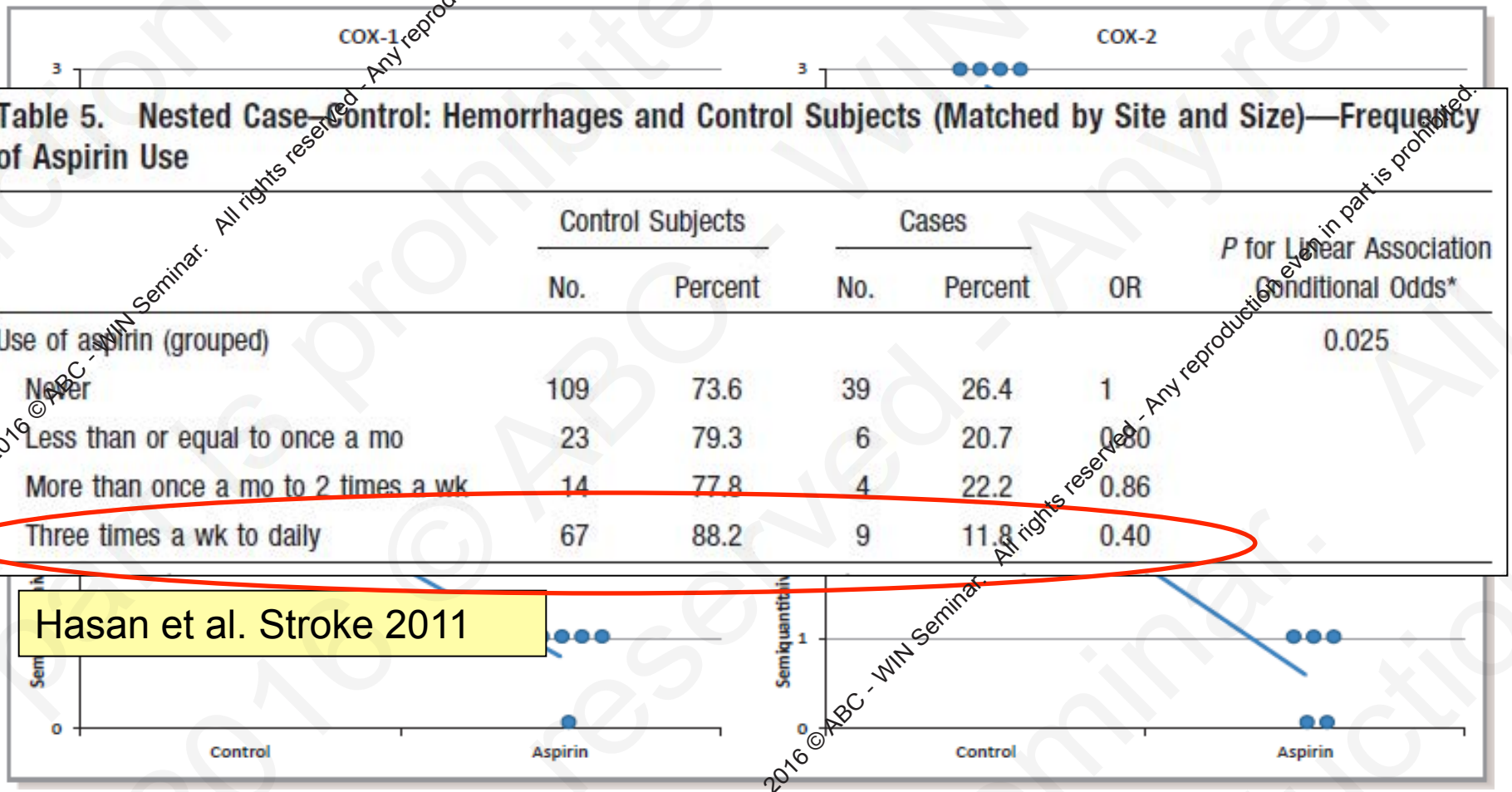


Table 5. Nested Case–Control: Hemorrhages and Control Subjects (Matched by Site and Size)—Frequency of Aspirin Use

	Control Subjects		Cases			
	No.	Percent	No.	Percent	OR	*P* for Linear Association Conditional Odds*
Use of aspirin (grouped)						0.025
Never	109	73.6	39	26.4	1	
Less than or equal to once a mo	23	79.3	6	20.7	0.80	
More than once a mo to 2 times a wk	14	77.8	4	22.2	0.86	
Three times a wk to daily	67	88.2	9	11.8	0.40	

Hasan et al. Stroke 2011

Hasan et al. JAHA 2013

Conclusion from studies on patients samples:

Inflammation does not seem to directly kill aneurysm wall cells but affects rupture risk

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What about animal models – does inflammation predispose to rupture?

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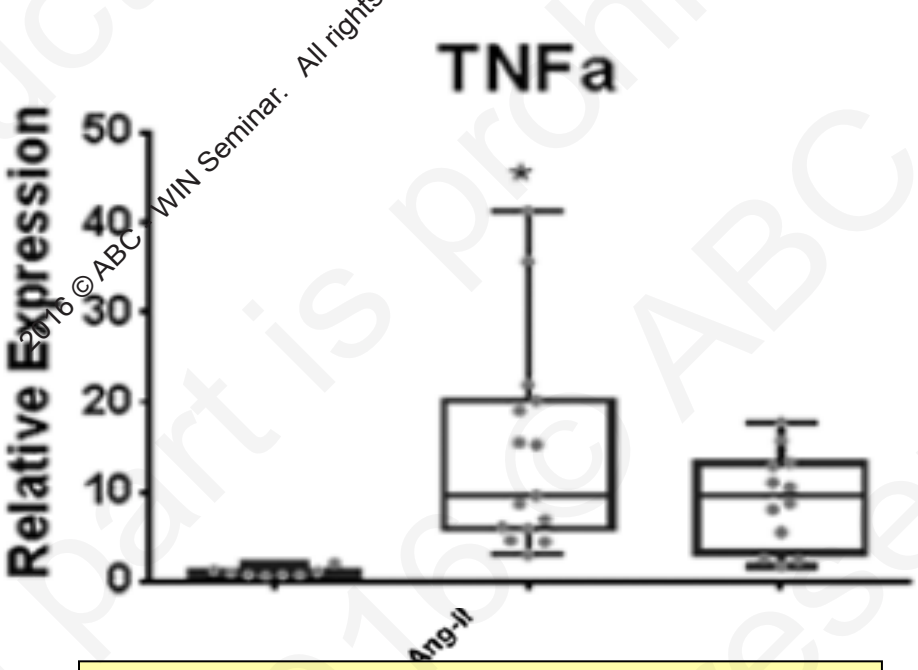
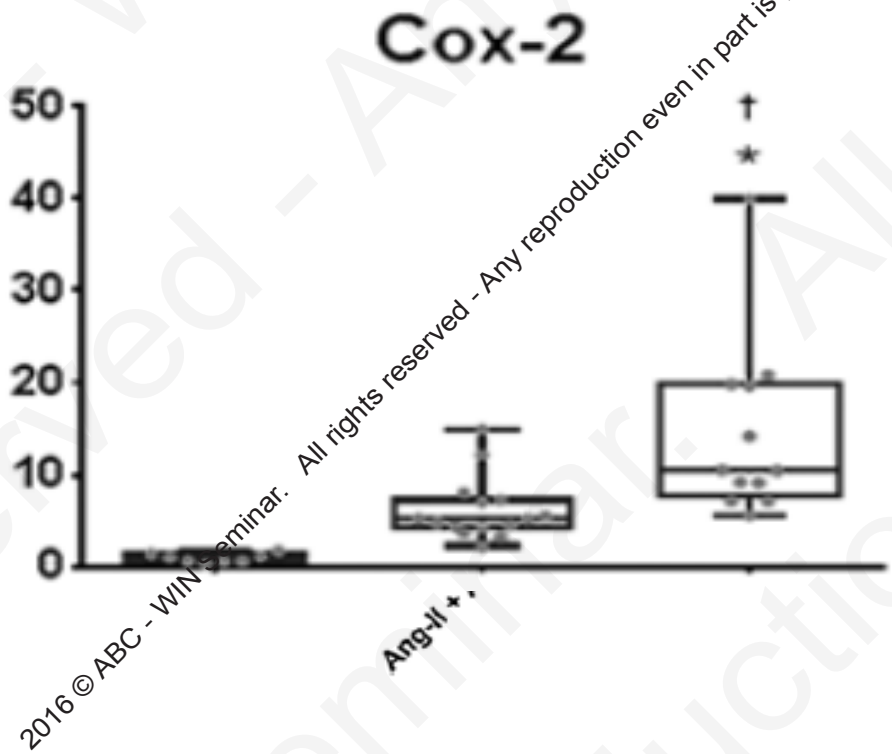
Blocking angiotensin II mediated signaling with Ang 1-7:

1) Reduced aneurysm formation
2) Reduced aneurysm rupture
3) Reduced TNFa mediated inflammation
…BUT
4) Increased COX-2 expression -?

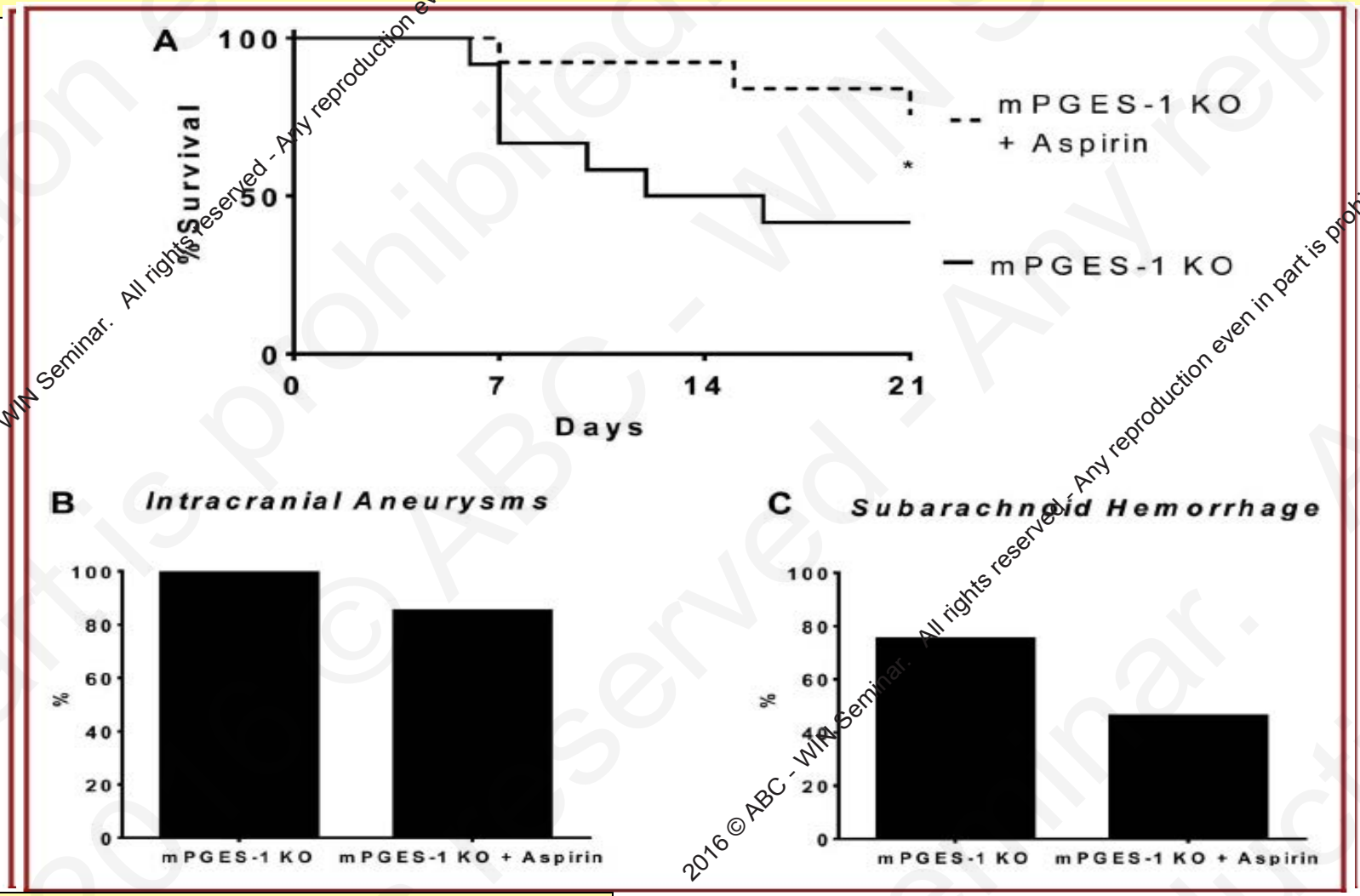




Pena Silva et al. Hypertension 2014

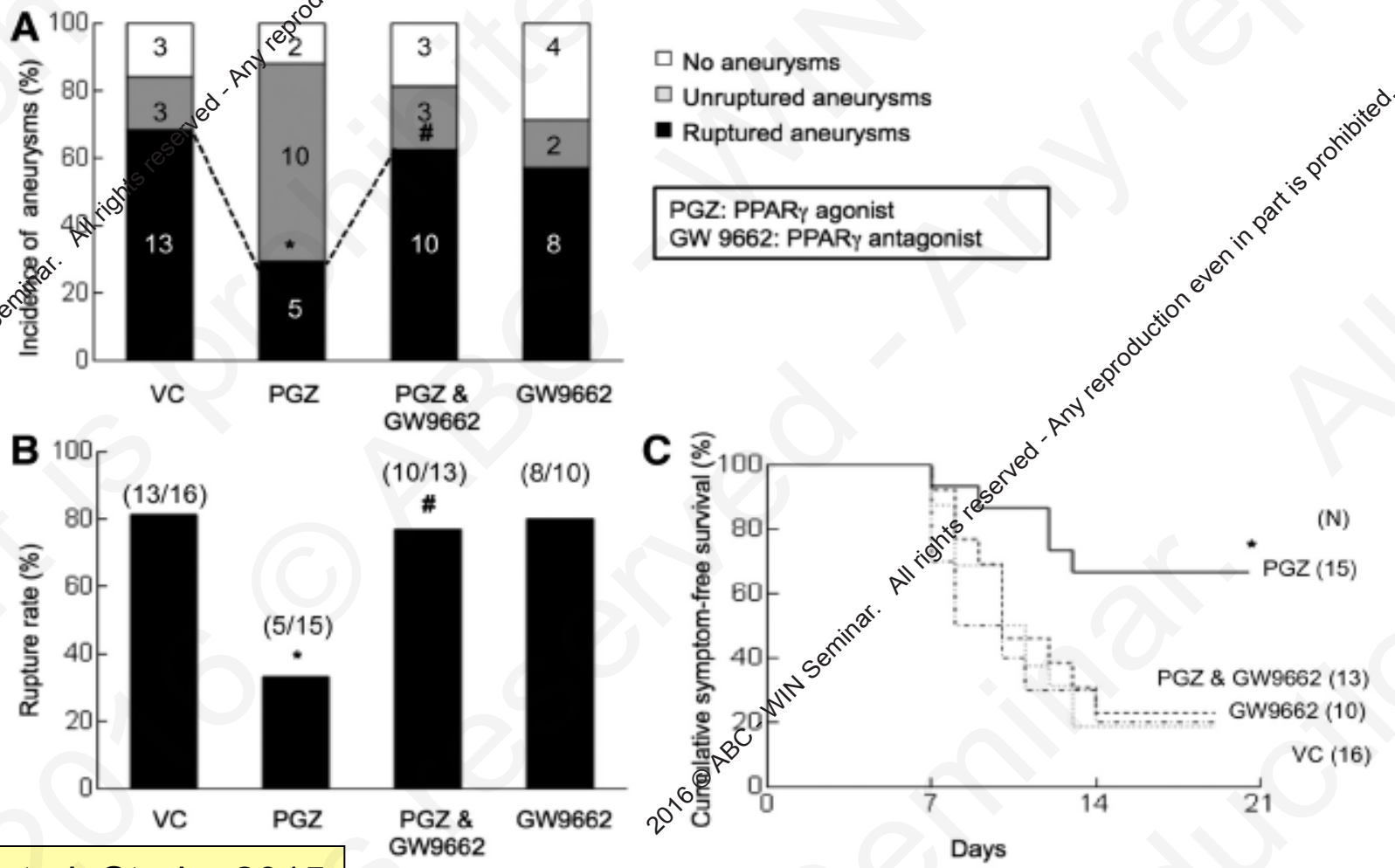
Blocking COX2 related mPGES-1 increased both aneurysm formation and rupture

- and effect reduced by aspirin



Pena Silva et al. Neurosurgery 2015

Activation of the immunomodulatory PPARg receptor in macrophages with pioglitatzone (PGZ) reduced aneurysm formation and **rupture**



Shimada et al. Stroke 2015

Studies on the aneurysms induced with hypertension + elastase demonstrate:

Modulation of the macrophage activity DOES influence the risk of aneurysm rupture

… but can also increase, not just decrease rupture risk…

Role of the inflammatory response in aneurysm healing?

Do the animal models reproduce the inflammation + pathobiology of human disease?

.. role of inflammation is multiplex and depends on the context

e.g. inflammation promotes fibrosis and wound healing

Inflammation promotes vessel wall thickening in vascular injury

Inflammation may promote plaque healing as well as plaque rupture in atherosclerosis..

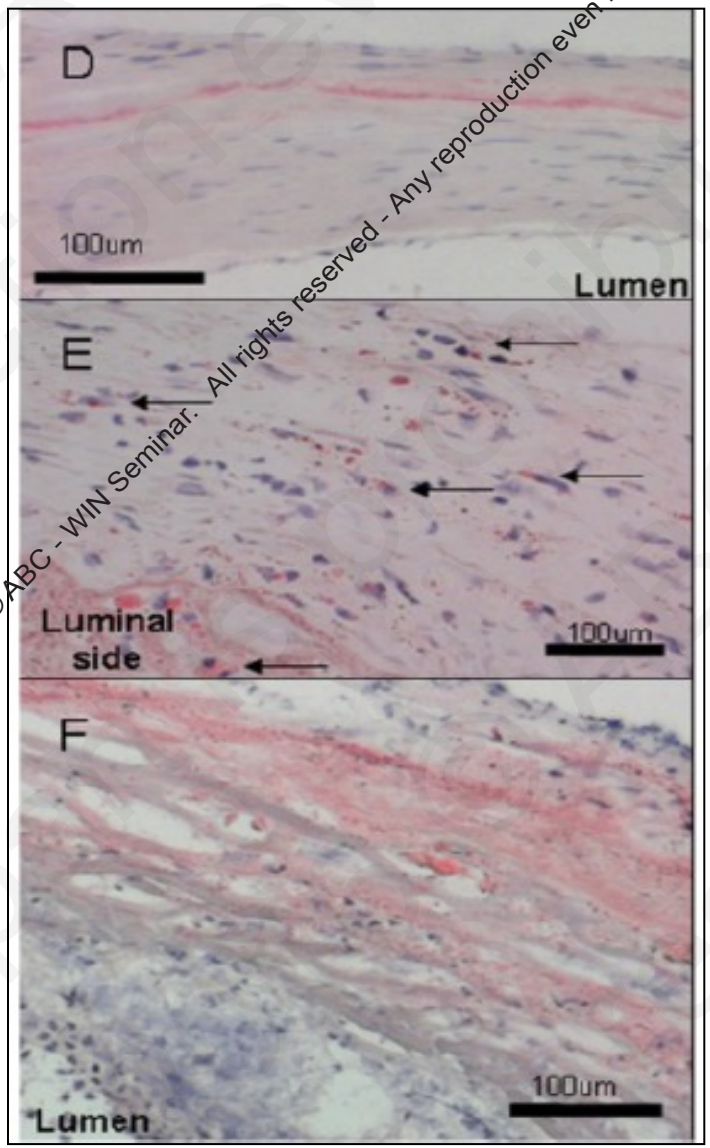
Genome wide comparison of gene expression in ruptured and unruptured human aneurysms –**also lipid accumulation** strongly associates with rupture

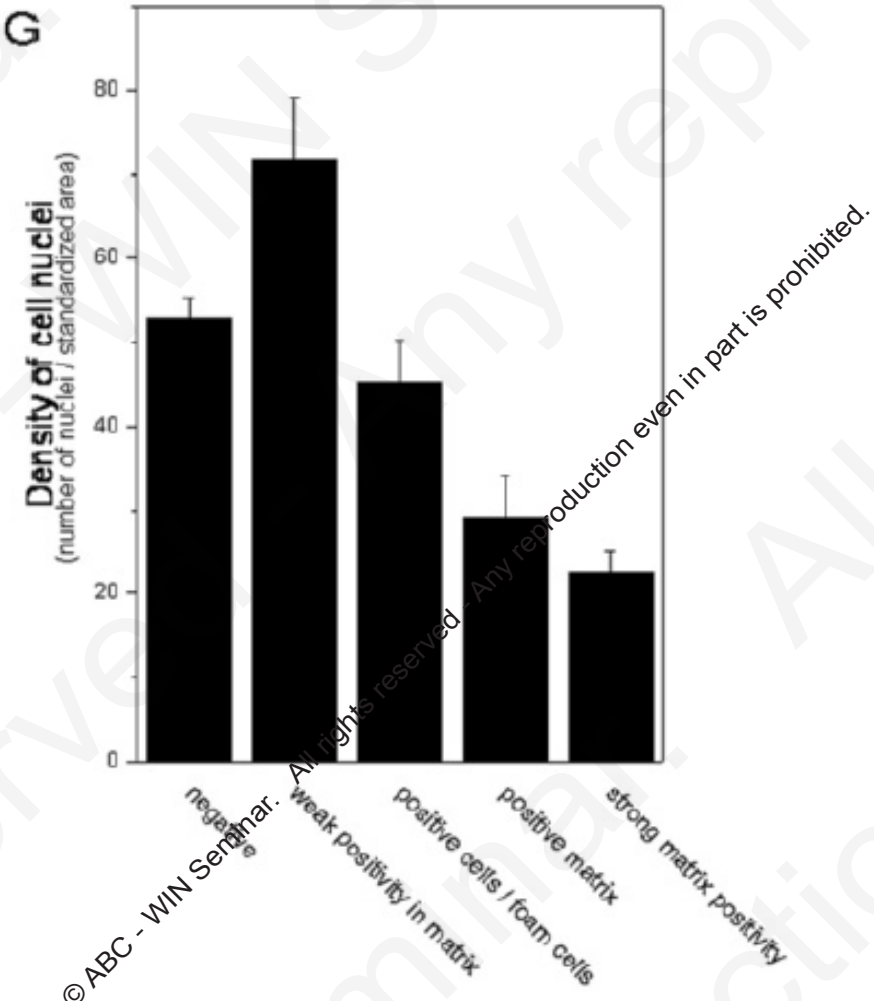
TABLE 2. Biological Processes in Ruptured Saccular Intracranial Aneurysm Wall Samples[a]

Gene Ontology (GO) Biological Processes	Upregulated Genes					
	GO ID[b]	P[c]	FDR[d]	OR	Count[e]	Size[f]
Chemotaxis	GO:0006935	2.60E − 14	5.66E − 11	7	30	125
Immune response	GO:0006955	5.70E − 14	6.26E − 11	4	49	333
Response to external stimulus	GO:0009605	1.00E − 13	7.41E − 11	3	74	651
Inflammatory response	GO:0006954	1.40E − 13	7.52E − 11	4.2	46	296
Locomotory behavior	GO:0007626	1.50E − 11	6.46E − 09	4.5	35	208
Response to stress	GO:0006950	3.60E − 09	1.31E − 06	2.1	99	1224
Response to other organism	GO:0051707	4.70E − 08	1.49E − 05	5.1	18	87
Positive regulation of tumor necrosis factor production	GO:0032760	5.80E − 08	1.60E − 05	17	6	7
Locomotion	GO:0040011	1.30E − 07	3.18E − 05	4.7	20	114
Cytokine production	GO:0001816	2.50E − 05	5.49E − 03	3.8	16	108
Phosphate metabolic process	GO:0006796	5.30E − 05	1.06E − 02	1.8	66	893
Positive regulation of interleukin-6 production	GO:0032755	5.90E − 05	1.08E − 02	42	4	6
Regulation of cell proliferation	GO:0042127	1.80E − 04	3.10E − 02	1.9	44	550
Intracellular lipid transport	GO:0032365	2.60E − 04	3.95E − 02	21	4	8
Neutrophil chemotaxis	GO:0030593	2.70E − 04	3.55E − 02	12	5	14
Regulated secretory pathway	GO:0045055	2.70E − 04	3.55E − 02	12	5	14
Protein amino acid phosphorylation	GO:0006468	2.70E − 04	3.55E − 02	1.8	47	613
Regulation of cytokine biosynthetic process	GO:0042035	3.90E − 04	4.55E − 02	4.2	10	61
Purine ribonucleoside monophosphate biosynthetic process	GO:0009168	3.90E − 04	4.55E − 02	11	5	15

Kurki et al. Neurosurgery 2011

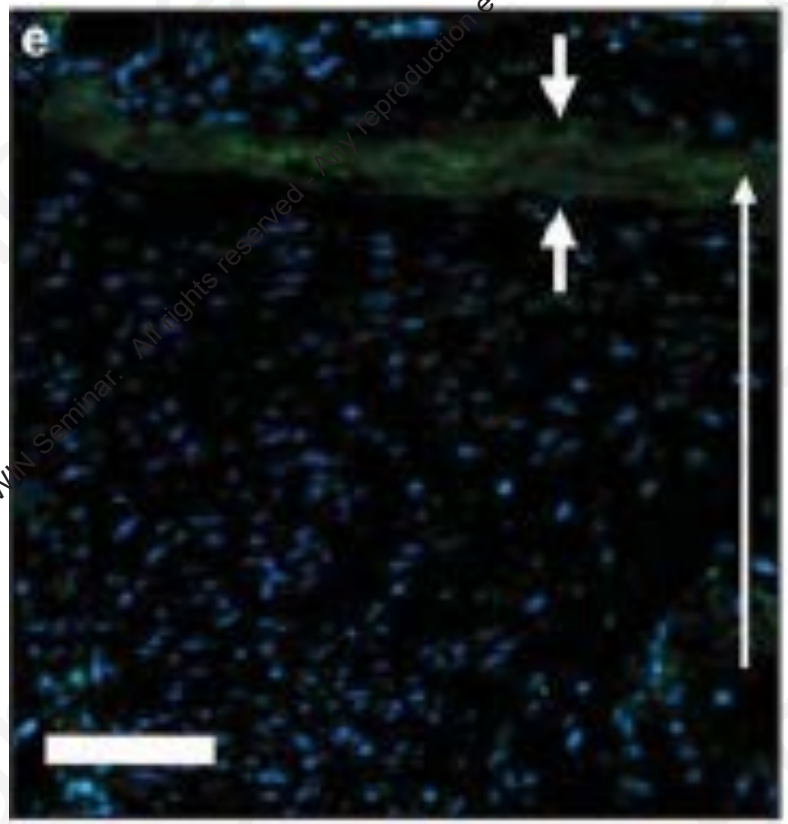
Accumulation of lipids in the aneurysm wall associates with loss of mural cells

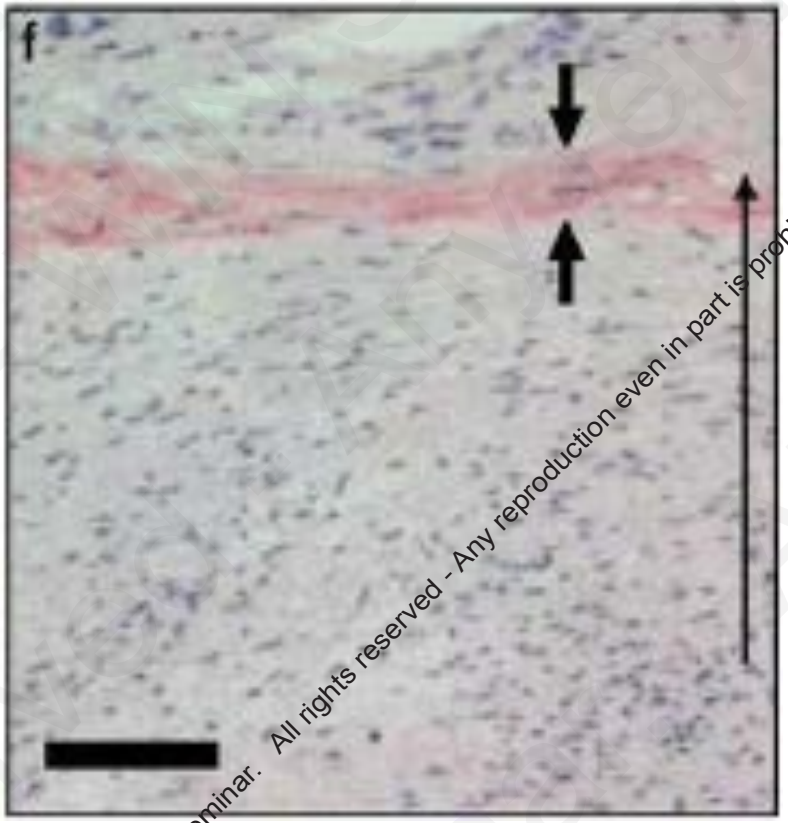




Frösen et al. Acta Neuropathologica Comm 2013

Complement activation in aneurysm wall regions with lipid accumulation





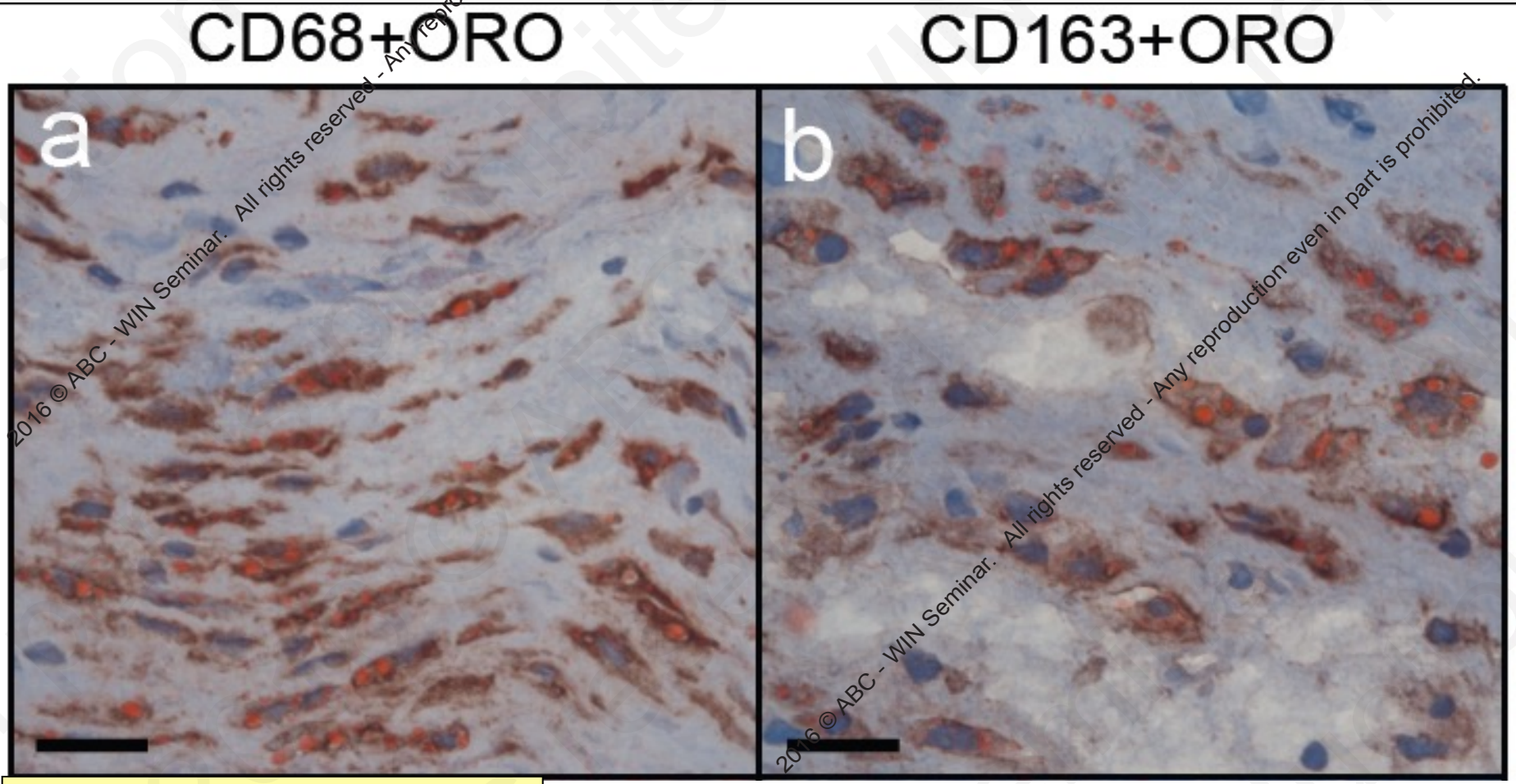
Complement in green

Lipids in red

Tulamo et al. Lab Investi 2010

Macrophage infiltration in the aneurysm wall associates with accumulation of lipids

– that the macrophages phagocytose



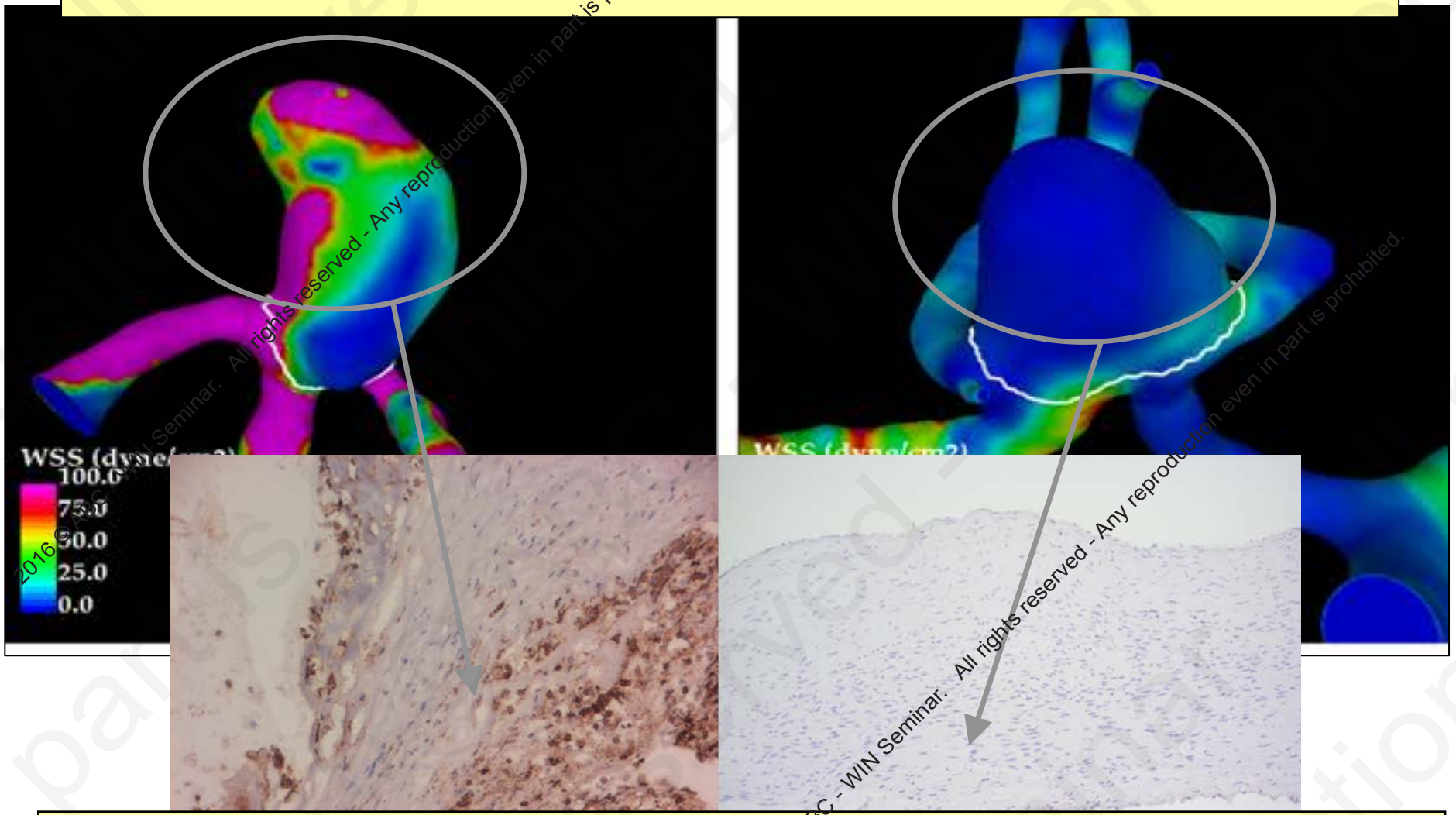
Ollikainen et al. SUBMITTED

So in human intracranial aneurysms macrophages seem to remove /clear lipids that otherwise would promote death of smooth muscle cells…

-Does lipid accumulation occur in animal models of induced aneurysms?

- How does that change the effects of anti-inflammatory therapy?

Flow dynamics in human intracranial aneurysms associate with inflammation in the wall – different flow dynamics in mouse models?



Inflammatory cells (in brown) are found especially in the walls of aneurysms with HIGH WSS - (Cebral et al. submitted)

Moreover…

Odontogenic bacteria in human aneurysm wall

– what is their role?

- how will anti-inflammatory therapy affect?

Table 2 The prevalence of bacterial DNA in the samples

	Negative	Positive
Surgical patients	11 (38%)	18 (62%)
Autopsy group	4 (57%)	3 (43%)
Total	15 (42%)	21 (58%)

Pyysalo et al. JNNP 2013

In summary -

- Inflammation mediates the formation of intracranial aneurysms

– drug therapy to reduce the formation of aneurysms?

- Inflammation of the aneurysm wall associates with degenerated wall and rupture

– marker of rupture risk?

- Inflammation is likely **not the only cause** of the degenerative remodeling but seems to modulate it

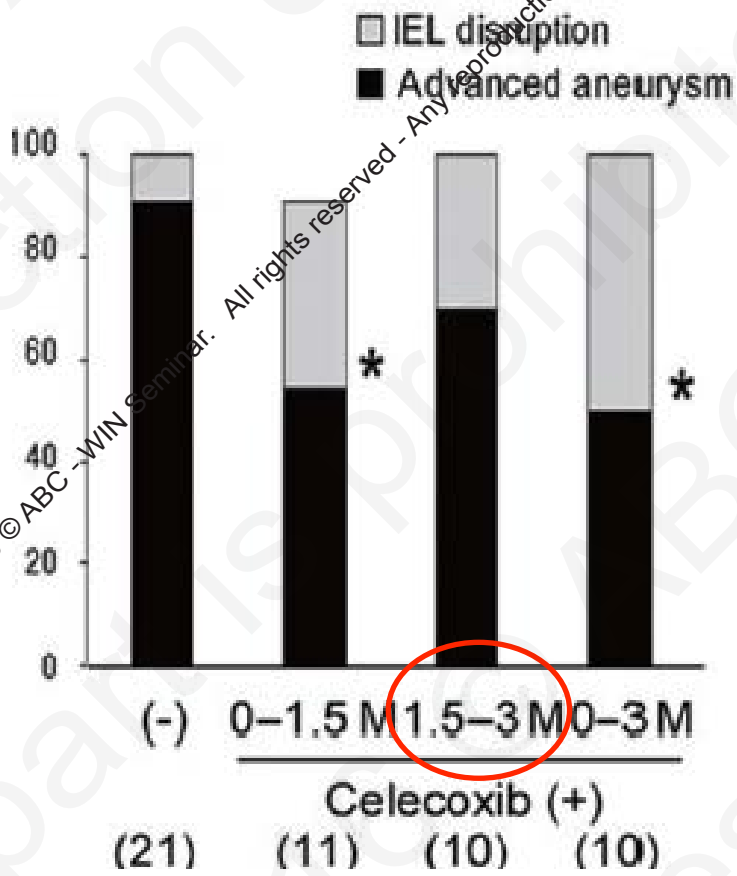
– possible target for drug therapy?

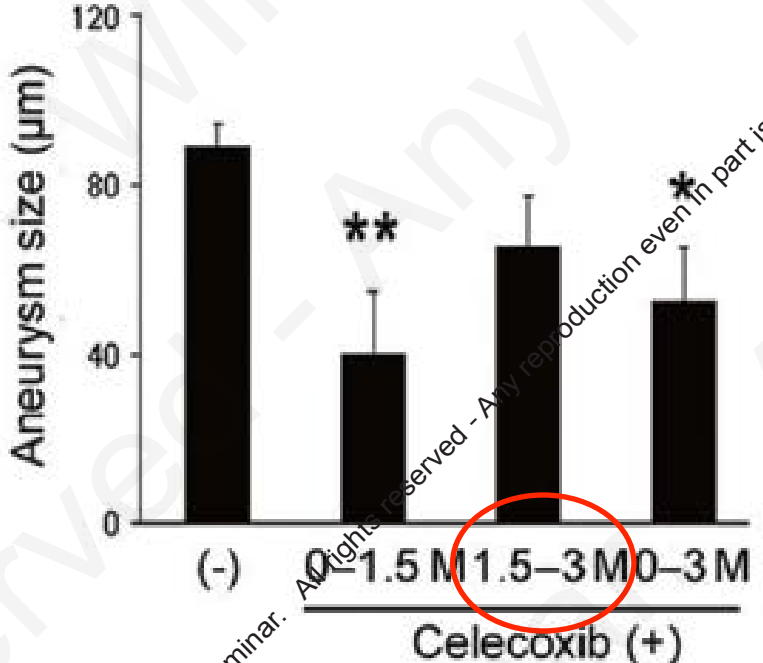
Thank you.

- Acknowledgements:
- **The Neurosurgery Research Group, Biomedicum Helsinki**
- **Professor Juha Hernesniemi**
- **Professor Mika Niemelä**
- **Dr. Riikka Tulamo**
- **Dr. Eliisa Ollikainen**

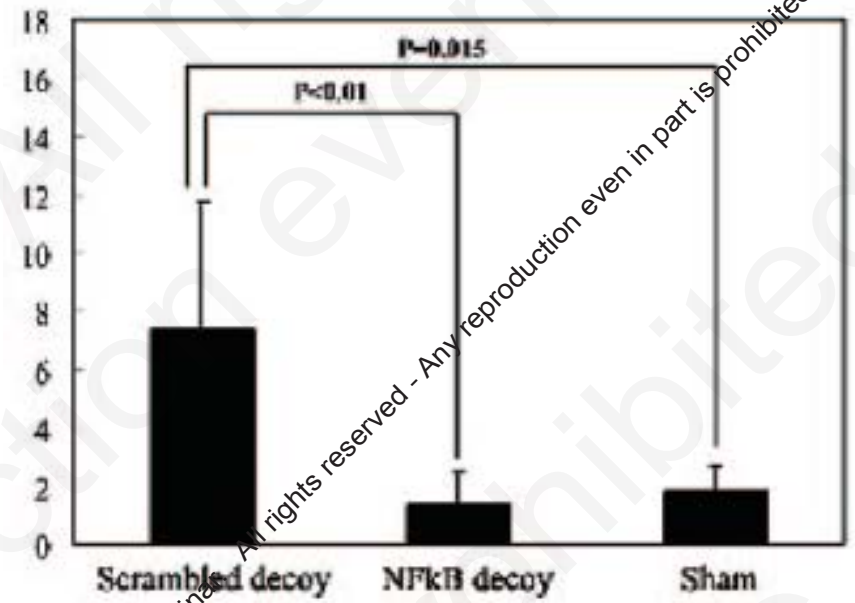
Biomedicum Helsinki

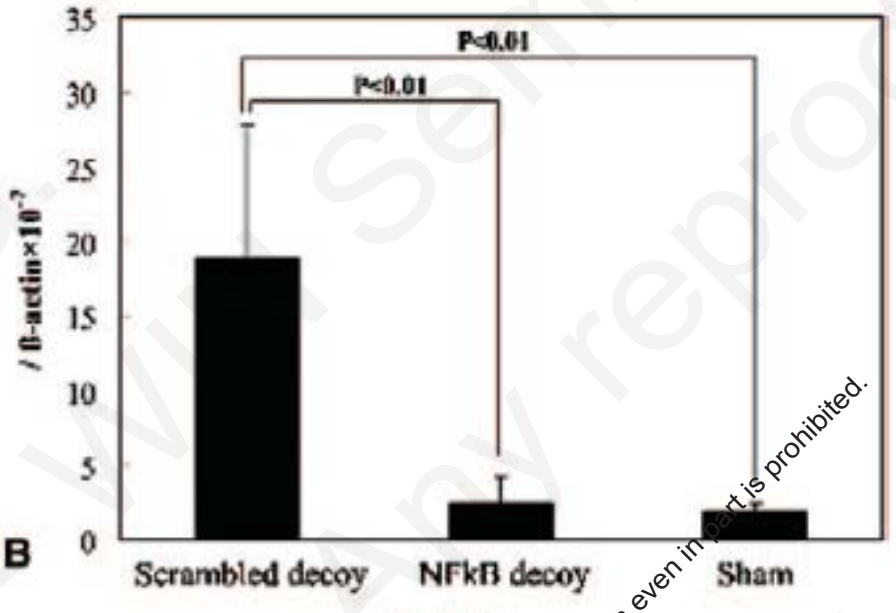
Blocking the COX2 –EP2 feedback loop in already formed aneurysms perhaps not enough?



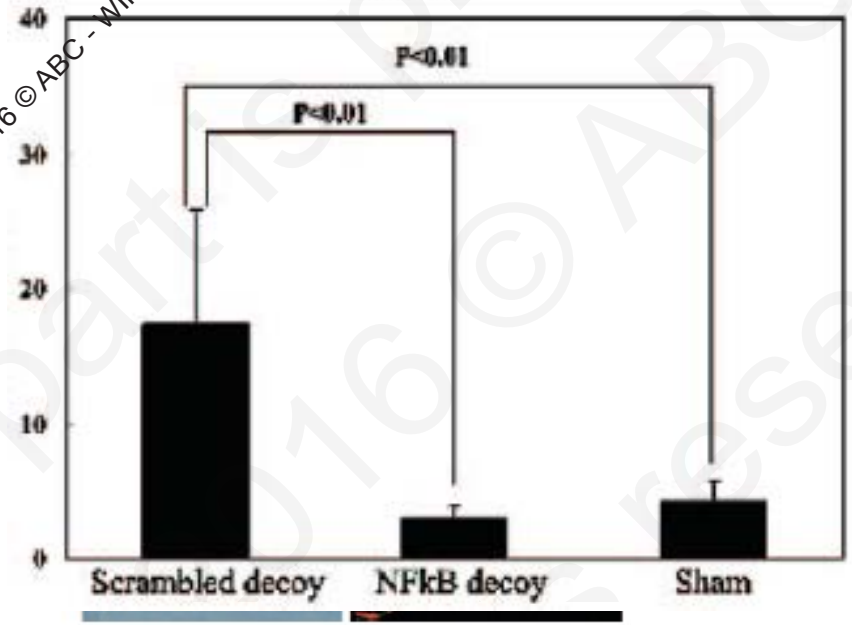


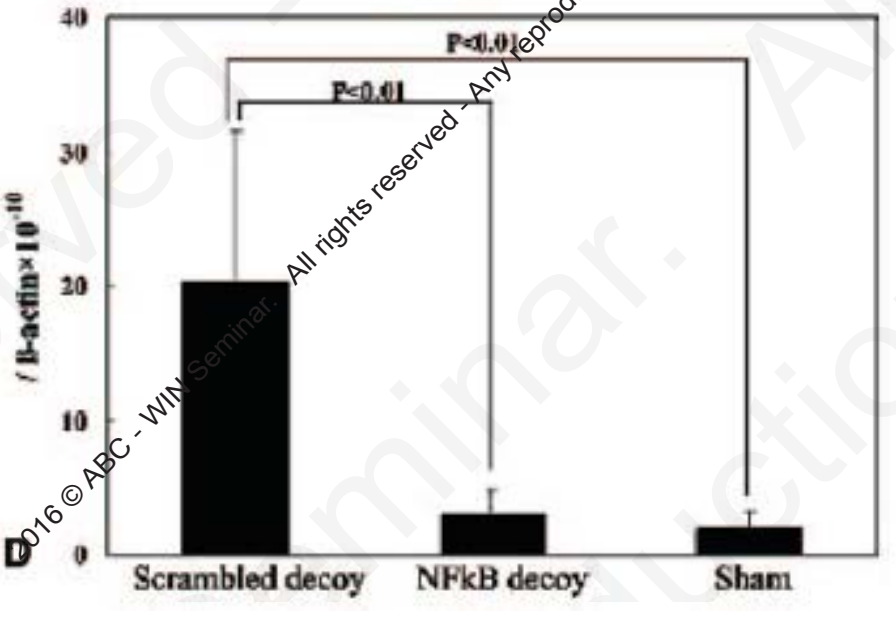
Aoki T et al. Br J Pharmacology 2011





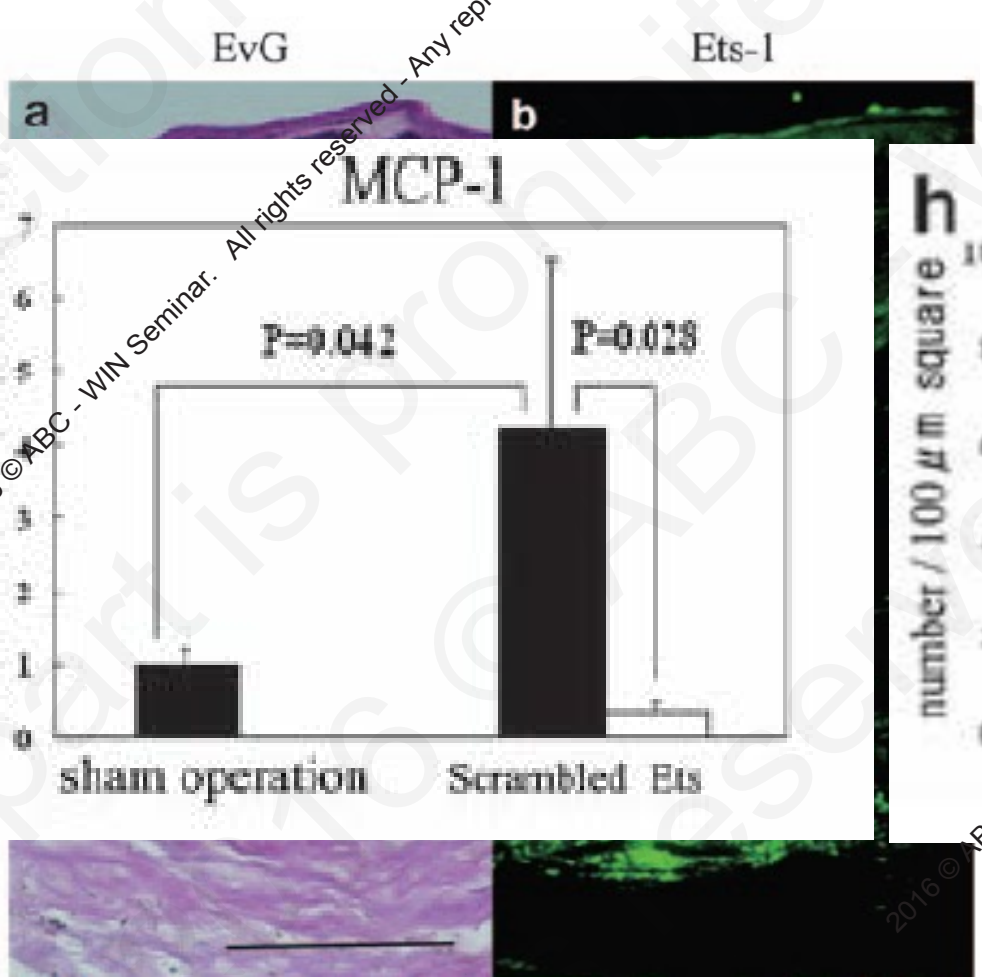
B

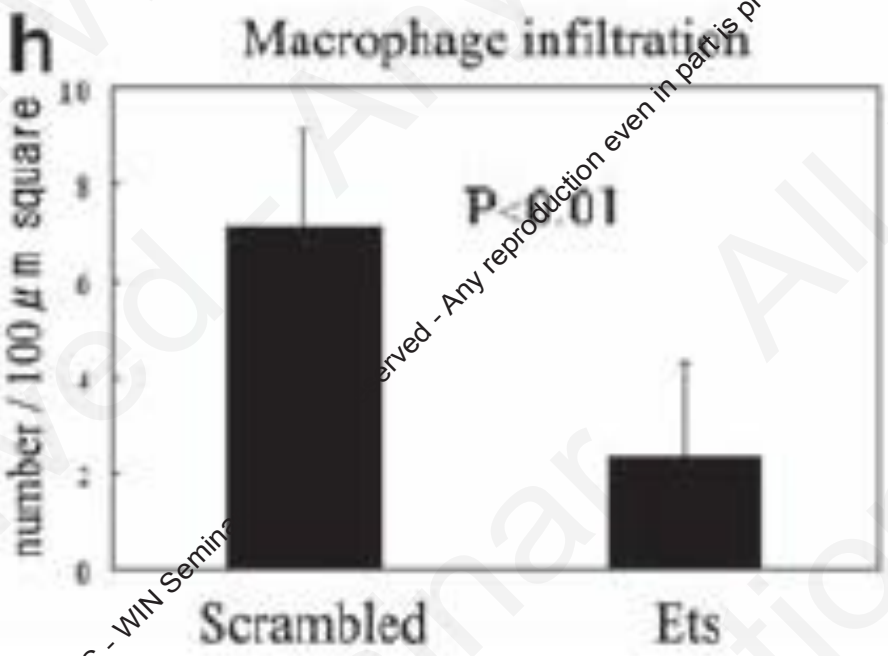




D

Also activation of Ets-1 transcription factor in SMOOTH MUSCLE CELLS mediates in the carotid ligation + hypertension model the inflammation causing aneurysm formation





Aoki et al. Gene Therapy 2010

Genome wide gene expression study:

– Inflammation mediated by activation of NFkB and ETS transcription factors in the human aneurysm wall similar to induced aneurysms in animal models

TABLE 4. In Silico Analysis of Transcription Factor Binding Sites in the −5-kb Promoter Regions of the 686 Upregulated Genes in the Ruptured Saccular Intracranial Aneurysm Walls [a]

TFs With Increased Binding Sites		Number of Binding Sites		P		
TRANSFAC[b]	HGNC[c]	686 Upregulated Genes	Human Genome[d]	Uncorrected	Bonferroni corrected	TF Family
ELF1	ELF1	411	11 549	1.66E − 08	3.32E − 06	ETS
PEA3	ETV4	714	21 887	1.66E − 07	3.32E − 05	ETS
ETS2	ETS2	413	11 968	3.64E − 07	7.28E − 05	ETS
ETS1	ETS1	728	22 501	3.87E − 07	7.74E − 05	ETS
HIF1	HIF1A	304	8663	3.13E − 06	6.26E − 04	. . .
NFKAPPAB65	RELA	96	2232	7.99E − 06	1.60E − 03	Rel/ankyrin

Kurki et al. Neurosurgery 2006