

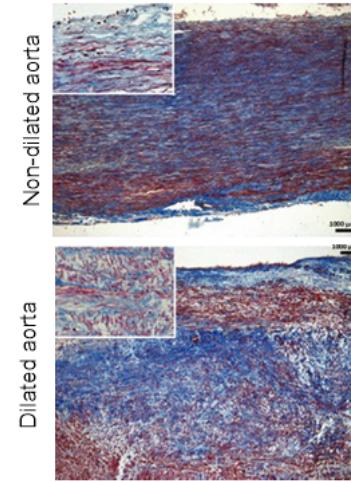
Possible role of SPP1 in Degenerative/TAV-associated Ascending Aortic Aneurysm formation

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Introduction

- Degenerative or TAV-associated ascending aortic aneurysms (AscAA) is characterized by:
 - Elastic fiber fragmentation
 - Increased collagen and proteoglycan production
 - Focal loss of SMC nuclei
- Signatures of degenerative ascending aortic aneurysms are *inflammation and fibrosis*



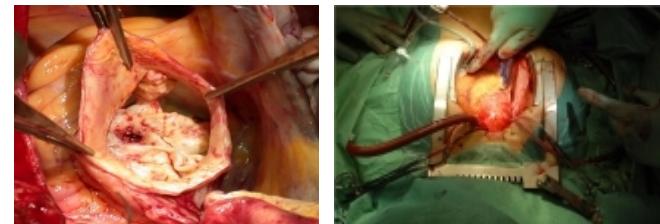
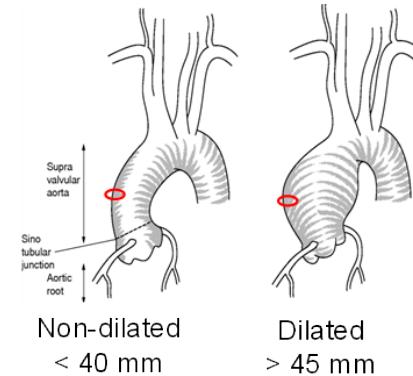
Masson trichrome staining, non-dilated and dilated ascending aorta. Wågsäter et al. JAHA 2013.

Aim

To characterize the cellular and molecular signature of degenerative AscAA from a fibrotic and inflammatory (EndMT) perspective, and identify potential regulatory elements

ASAP/DAVAACA studies of non-syndromic /degenerative TAAs

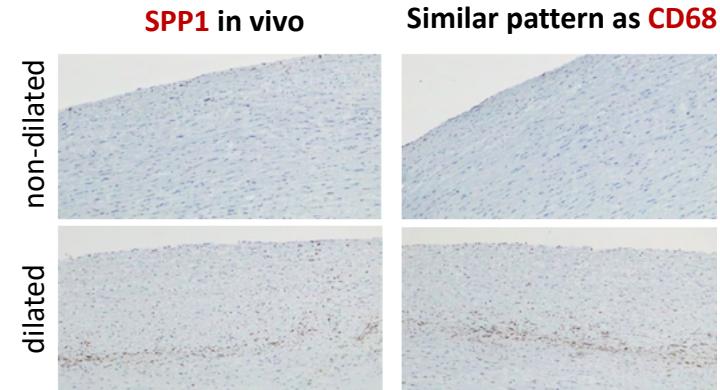
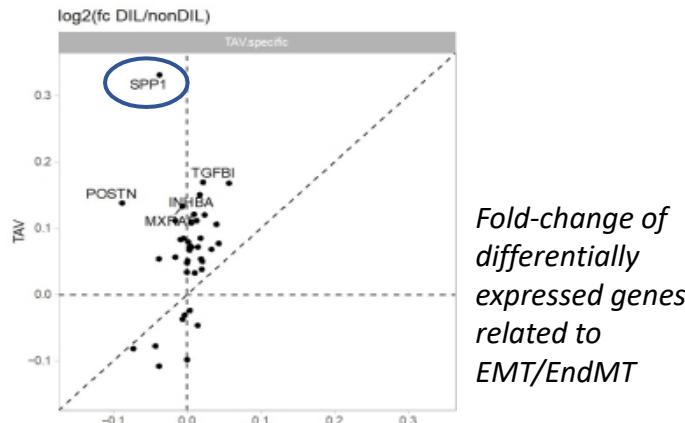
- >2100 patients being accepted for aortic valve and/or ascending aortic surgery. Syndromic forms excluded
- Tissue biopsies, **non-dilated** and **dilated ascending aortas**
- Plasma, serum and DNA from blood and anthropometric data



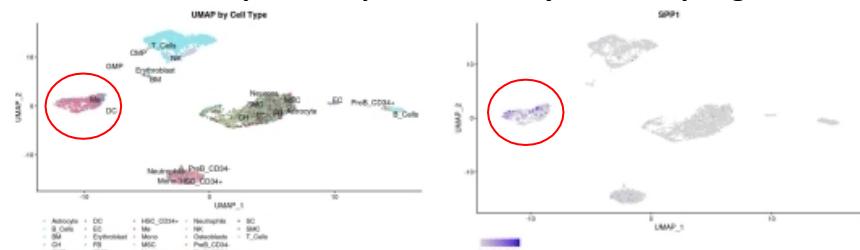
TAV specific genes associated with dilatation SPP1 possibly upregulated in intima-media macrophages

- Genes related to inflammation and fibrosis (EMT hallmark gene set, MSigDB) were selected
- Differential expression analysis (non-dilated vs. dilated) of aortic intima-media Affymetrix Human Exon 1.0 ST arrays (n=119)

SPP1 displayed the greatest fold-change of the set

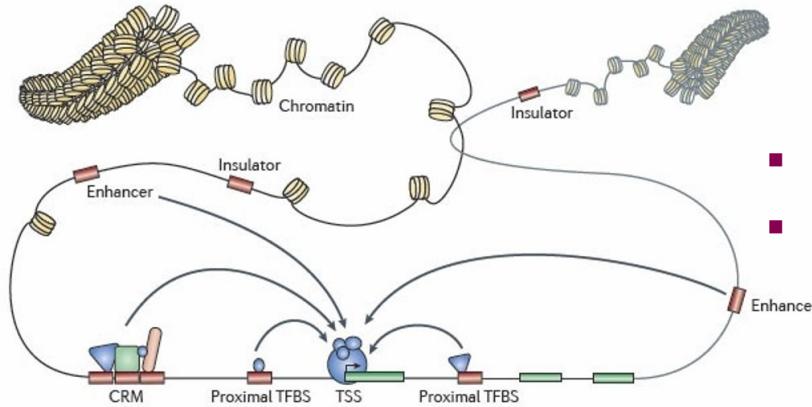


SPP1 expression predominantly in macrophages



<http://plaqview.uvadcos.io/> using sc data from Nature
Med2019;25:1280-89

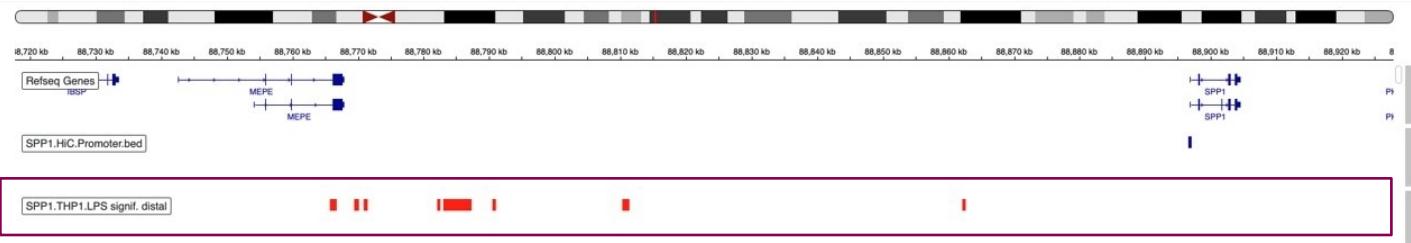
Identification of SPP1 distal regulatory elements by HiCap



- THP1-cells, differentiated by PMA
- +/- LPS-stimulation

Modified from: Lenhard et al. Nature Rev Genet 2012

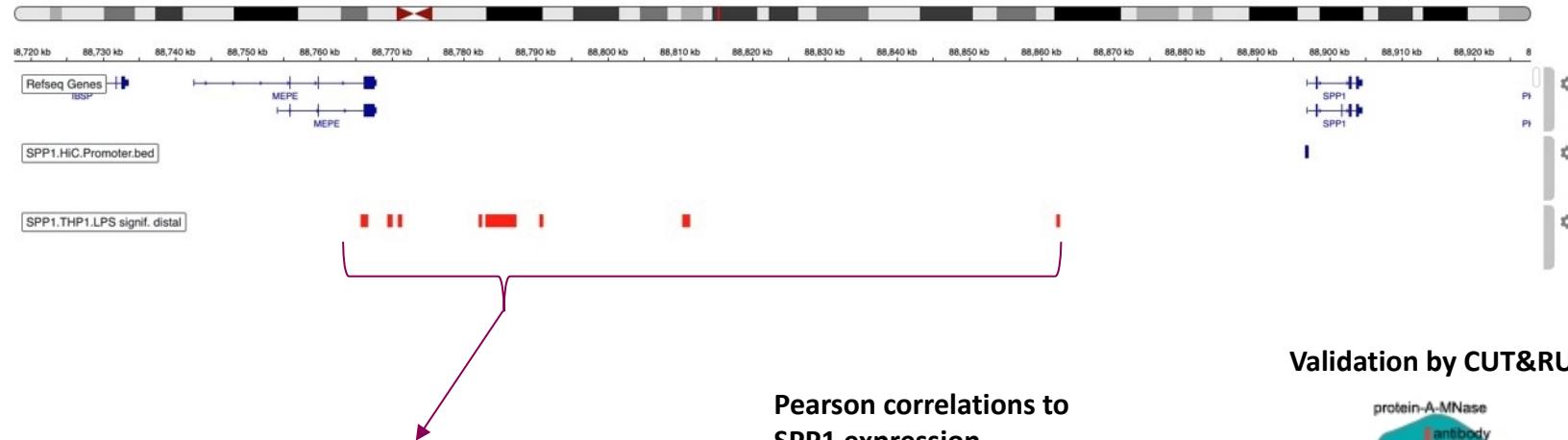
Distal regions binding to the SPP1 promoter under LPS-stimulation



Identification of TFBS and TFs regulating SPP1



Karolinska
Institutet



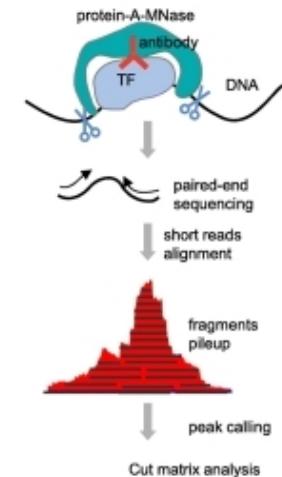
Selection criteria for further analysis:

- Identification of TFBS in distal regions (TransFac)
- DE analysis of putative TFs in ascending aortic intima-media
- Correlation with SPP1 mRNA expression

Pearson correlations to SPP1 expression

	CPEB1	<0.001	<0.001
ETS1	<0.001		<0.001
SPP1		<0.001	<0.001
	SPP1	ETS1	CPEB1

Validation by CUT&RUN

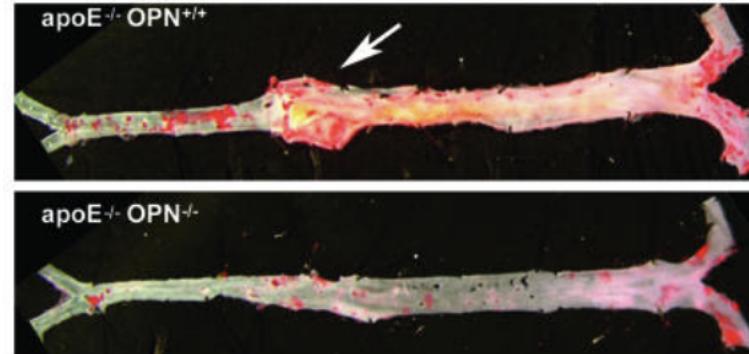


Conclusion

Our study identifies SPP1 as a potential player in degenerative AscAA development

Discussion

- SPP1 (OPN) modulates migration, proliferation and cellular survival/death (Yushi, 2016)
- OPN(-/-) decreases leukocyte infiltration (Myers, 2003) and aneurysm development in mice models (Bruemmer, 2003).



Modified from Bruemmer et al. J Clin Invest 2003

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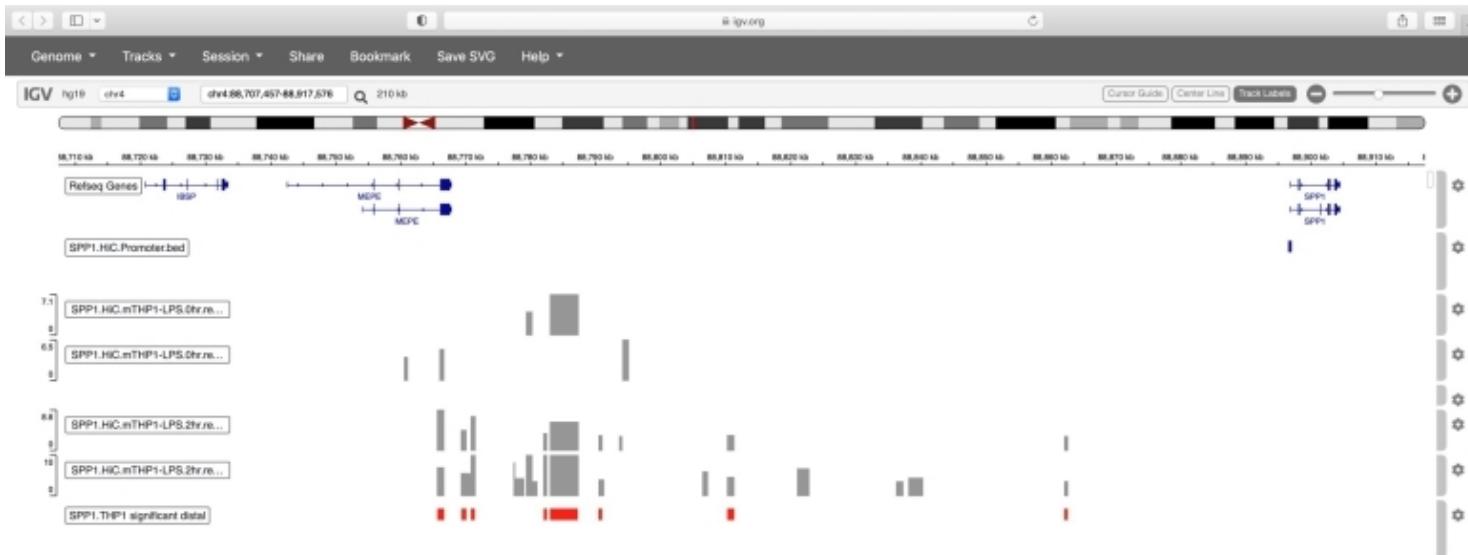
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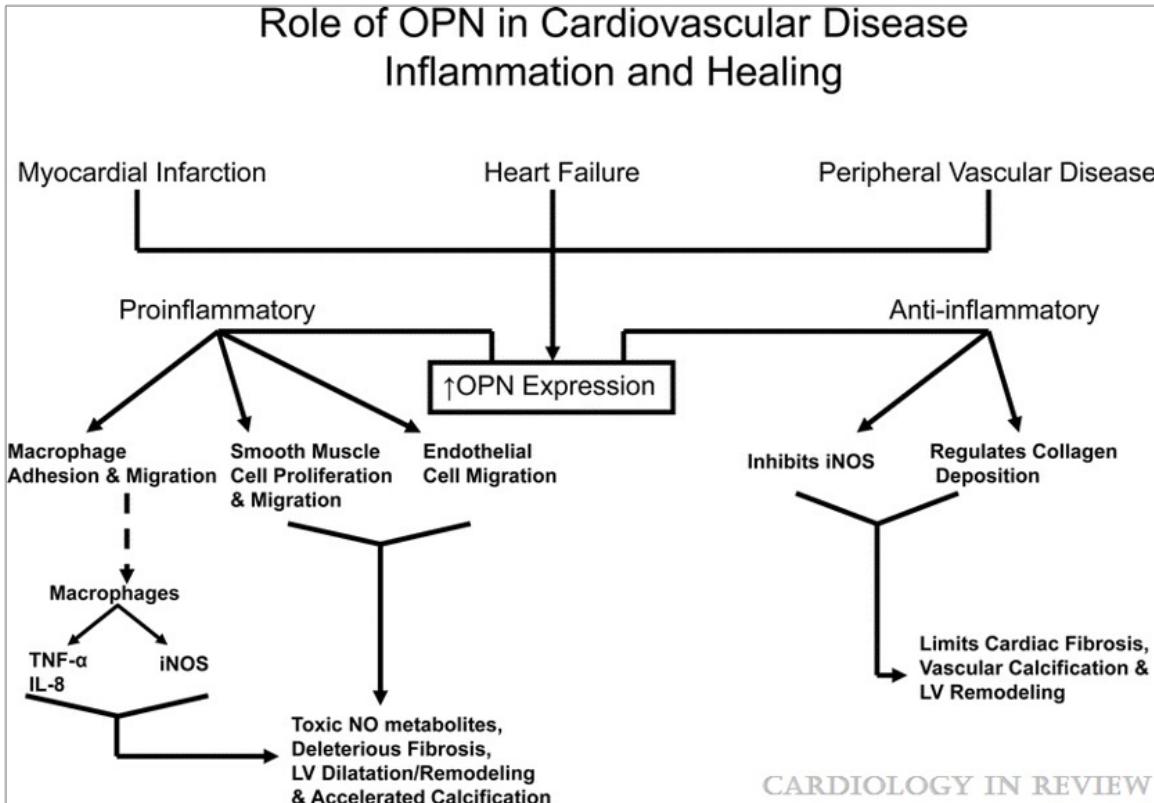
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Correlation of
inflammatory markers to
the expression of SPP1

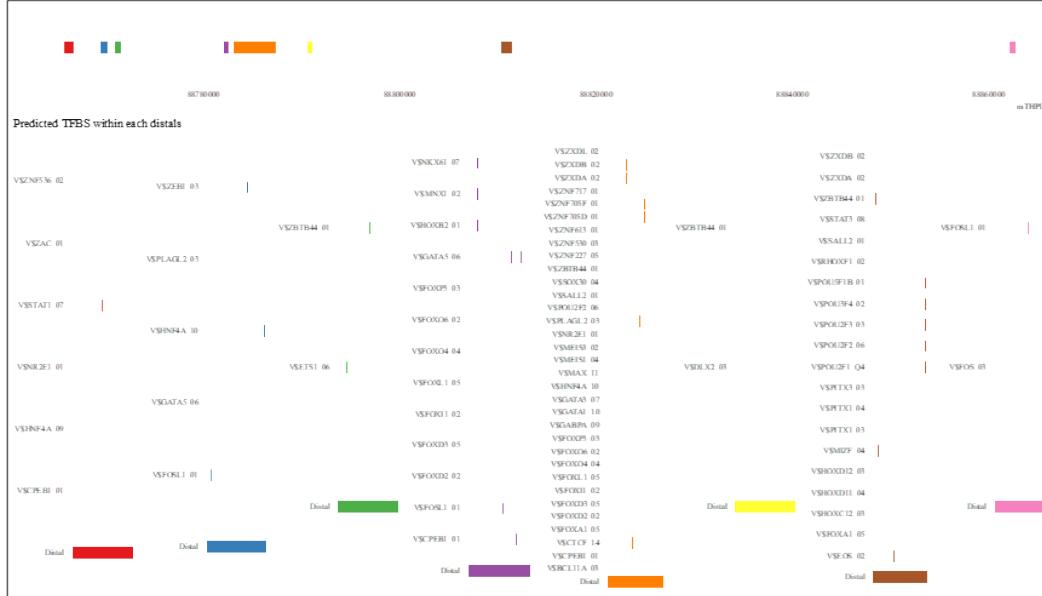
TAV

	SPP1	CD68	CCL2	NLRP3	CASP4
CASP4+	0.4221	0.6482	0.7541	0.5389	
NLRP3+	0.6728	0.8006	0.7683		0.5389
CCL2+	0.7236	0.8198		0.7683	0.7541
CD68+	0.7985		0.8198	0.8006	0.6482
SPP1+		0.7985	0.7236	0.6728	0.4221





Transfac



	factor	factor.alias	gene	p.dil.v.nondil	fc.dil.v.nondil
2	CPEB1	CPEB1	CPEB1	0.001428201	0.9506970
3	ETS1	ETS1	ETS1	0.002469294	1.0552089
4	ZNF536	ZNF536	ZNF536	0.006742784	0.9531523
5	MNX1	MNX1	MNX1	0.008515378	0.9681361
6	FOXD2	FOXD2	FOXD2	0.016480486	0.9650109
7	STAT3	STAT3	STAT3	0.016480486	1.0248709
8	PITX3	PITX3	PITX3	0.020276657	0.9655302
9	MAX	MAX	MAX	0.024793676	1.0229611
10	FOXO4	FOXO4	FOXO4	0.026477040	0.9839889
11	FOXD3	FOXD3	FOXD3	0.034206222	0.9743978
12	ZXDA	ZXDA	ZXDA	0.038727792	0.9741092
13	FOXA1	FOXA1	FOXA1	0.041168311	0.9722932
14	NKX61	NKX6-1	NKX6-1	0.043734759	0.9677991
15	NR2E1	NR2E1	NR2E1	0.043734759	0.9691834
16	SOX30	SOX30	SOX30	0.052236740	0.9731429
17	DLX2	DLX2	DLX2	0.055354258	0.9771163
18	ZNF530	ZNF530	ZNF530	0.073289390	0.9630418
19	HOXB2	HOXB2	HOXB2	0.081652236	0.9709849
20	FOXI1	FOXI1	FOXI1	0.090754672	0.9762548
21	HOXC12	HOXC12	HOXC12	0.100636656	0.9730182
22	PITX1	PITX1	PITX1	0.100636656	0.9803392
23	STAT1	STAT1	STAT1	0.117007251	1.0350888
24	ZNE613	ZNE613	ZNE613	0.117007251	1.0463595

