

## SUPPLEMENTARY MATERIAL

### **Oxidative stress and inflammatory markers in abdominal aortic aneurysm.**

*Running title:* Novel markers for AAA

Authors: David Sánchez-Infantes<sup>1,2</sup>, Meritxell Nus<sup>3,4</sup>, Miquel Navas-Madroñal<sup>5,6</sup>, Joan Fité<sup>7</sup>, Belén Pérez<sup>8</sup>, Antonio J. Barros-Membrilla<sup>9</sup>, Begoña Soto<sup>7</sup>, José Martínez-González<sup>4,10</sup>, Mercedes Camacho<sup>4,5,6</sup>, Cristina Rodríguez<sup>4,5,6</sup>, Ziad Mallat<sup>3</sup>, María Galán<sup>4,5,6</sup>.

<sup>1</sup>Department of Basic Sciences of Health, Area of Biochemistry and Molecular Biology, University Rey Juan Carlos , Alcorcón, Spain.

<sup>2</sup>Centro de Investigación Biomédica en Red-Fisiopatología de la Obesidad y Nutrición (CIBEROBN), ISCIII, Madrid, Spain.

<sup>3</sup>Division of Cardiovascular Medicine, University of Cambridge. Cambridge, United Kingdom.

<sup>4</sup>Centro de Investigación en Red de Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain.

<sup>5</sup>Institut de Recerca del Hospital de la Santa Creu i Sant Pau, Barcelona, Spain. 08041.

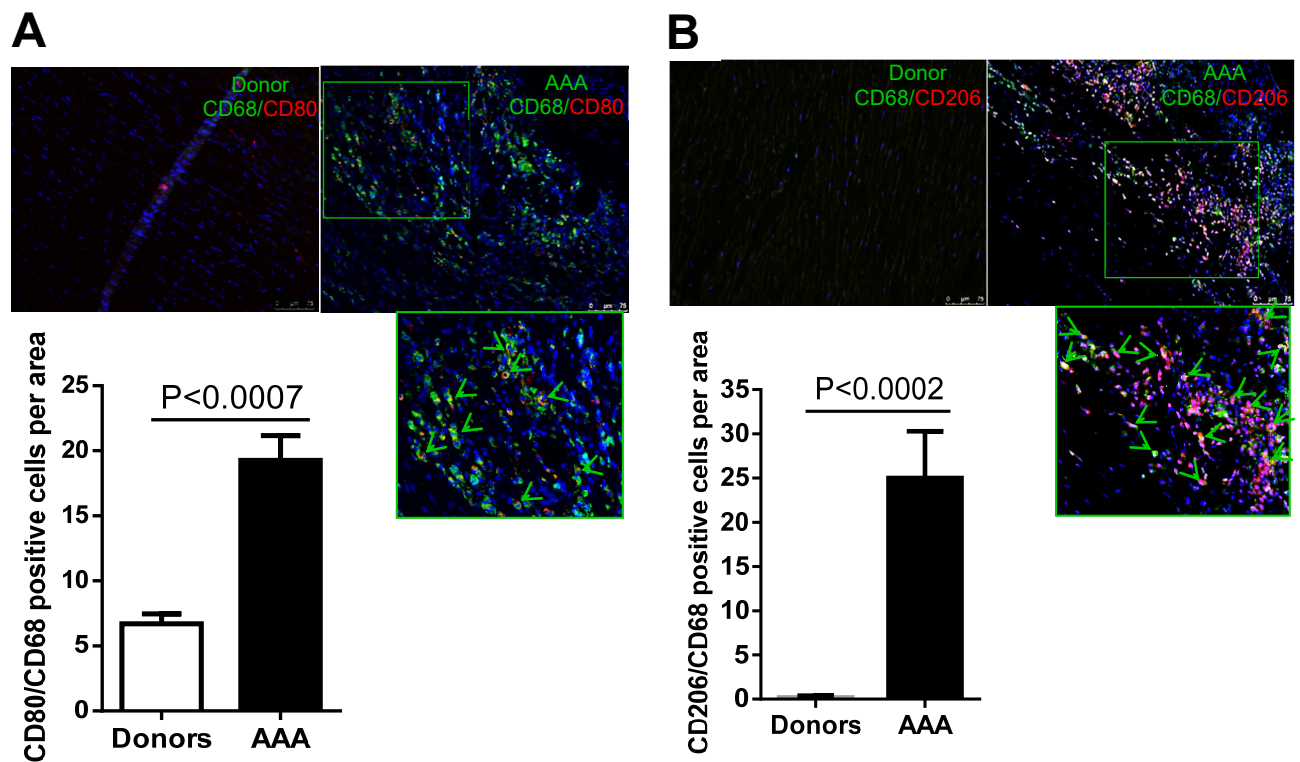
<sup>6</sup>Instituto de Investigación Biomédica Sant Pau (IB Sant Pau), Barcelona, Spain.

<sup>7</sup>Servicio de Angiología, Cirugía Vascular y Endovascular, Hospital de la Santa Creu i Sant Pau. Barcelona, Spain.

<sup>8</sup>Faculty of Medicine, Universidad Autónoma de Barcelona, Bellaterra. Barcelona, Spain.

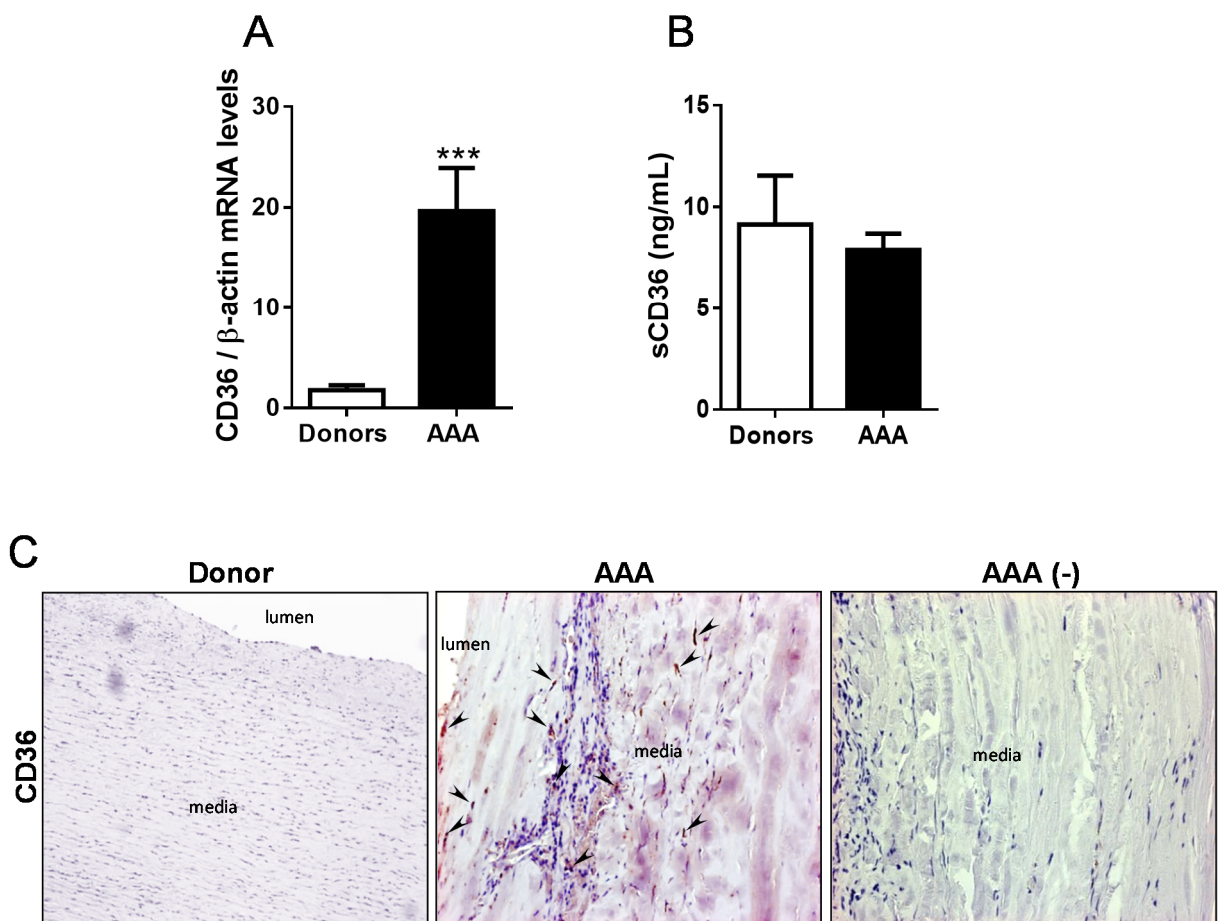
<sup>9</sup>Unidad Funcional de Patología de la Aorta (UPA), Servicio de Cardiología, Hospital de la Santa Creu i Sant Pau. Barcelona, Spain.

<sup>10</sup>Instituto de Investigaciones Biomédicas de Barcelona (IIBB-CSIC), IIB Sant Pau, Barcelona, Spain.



**Figure Supplementary 1. Presence of M1 and M2 infiltrated macrophages in AAA tissue.** A-B) Representative images of immunostaining assays performed in abdominal aorta sections from donors and AAA patients targeting M1 (CD68 CD80) and M2 (CD68 CD206) macrophages (n=10). The histograms below show

the quantification of the number of positive cells per aortic area (10x). Results are expressed as mean  $\pm$  SEM.



**Figure Supplementary 2. CD36 protein is increased in aneurysmal tissue but not in the circulation.** **A)** Human abdominal aortic mRNA levels of CD36 measured by quantitative real Time PCR analysis and normalized to  $\beta$ -actin in healthy donors (n=15) and patients (AAA) (n=80); **B)** Plasma levels of CD36 in AAA (n=32) vs healthy donors (n=21); **C)** Representative images of immunostaining assays performed in abdominal aorta sections from donors and AAA patients targeting CD36 (n=10; Scale bars: 100  $\mu$ m). Arrows indicate the positively stained cells. AAA (-) indicates negative control for immunohistochemistry. Results are expressed as mean  $\pm$  SEM. \*\*\*  $p < 0.001$  vs. AAA.

**Supplementary Table S1. Logistic regression analysis.**

	Covariate	OR	95 % C.I. for EXP (B)		P value
			Lower	Upper	
Unadjusted	<b>IgG</b>	28.007	7.529	104.187	< 0.0001*
Adjusted for age		20.863	3.719	117.028	0.00045*
Adjusted for sex		27.185	7.211	102.483	< 0.0001*
Adjusted for HTA		12.861	3.206	51.589	< 0.0001*
Adjusted for DM		28.696	7.581	108.625	< 0.0001*
Adjusted for smoking		25.944	6.443	104.467	< 0.0001*
Adjusted for all factors		8.221	0.670	100.951	0.043*
Unadjusted	<b>IgM</b>	0.096	0.020	0.454	0.003*
Adjusted for age		0.018	0.001	0.254	0.004*
Adjusted for sex		0.097	0.020	0.478	0.004*
Adjusted for HTA		0.143	0.021	0.987	0.048*
Adjusted for DM		0.1	0.021	0.987	0.004*
Adjusted for smoking		0.068	0.012	0.408	0.003*
Adjusted for all factors		0.008	0.0001	0.578	0.027*

Unadjusted	<b>ROS</b>	1.314	1.168	1.466	< 0.0001*
Adjusted for age		1.816	0.999	3.301	0.050*
Adjusted for sex		1.376	1.197	1.582	< 0.0001*
Adjusted for HTA		1.495	1.137	1.966	0.004*
Adjusted for DM		1.324	1.179	1.488	< 0.0001*
Adjusted for smoking		1.348	1.180	1.540	< 0.0001*
Adjusted for all factors		1.708	1.012	2.884	0.989
Unadjusted	<b>CD38</b>	6.447	2.201	18.885	0.001*
Adjusted for age		6.927	2.058	23.315	0.002*
Adjusted for sex		6.617	2.247	19.485	0.001*
Adjusted for HTA		6.361	1.850	21.879	0.003*
Adjusted for DM		6.619	2.205	19.869	0.001*
Adjusted for smoking		7.188	2.274	22.728	0.001*
Adjusted for all factors		10.119	1.813	56.485	0.008*
Unadjusted	<b>GDF15</b>	24.653	9.215	65.957	< 0.0001*
Adjusted for age		13.755	4.587	41.248	< 0.0001*
Adjusted for sex		20.749	7.696	55.942	< 0.0001*
Adjusted for HTA		18.903	6.239	57.275	< 0.0001*
Adjusted for DM		24.721	9.191	66.490	< 0.0001*
Adjusted for smoking		24.608	8.653	69.982	< 0.0001*
Adjusted for all factors		5.793	1.767	18.993	0.004*
Unadjusted	<b>S100A4</b>	0.093	0.025	0.344	0.0004*
Adjusted for age		0.094	0.020	0.350	0.020*
Adjusted for sex		0.091	0.024	0.350	0.0005*
Adjusted for HTA		0.114	0.025	0.521	0.005*
Adjusted for DM		0.094	0.025	0.350	0.0004*
Adjusted for smoking		0.079	0.018	0.341	0.001*
Adjusted for all factors		0.112	0.007	1.747	0.118

OR: Odd Ratio; 95% CI: 95 confidence interval. \*P<0.05 vs. Donors: statistically significant.