Supplementary Information Inventory

Endothelial pannexin-1 channels modulate macrophage and smooth muscle cell activation in abdominal aortic aneurysm formation

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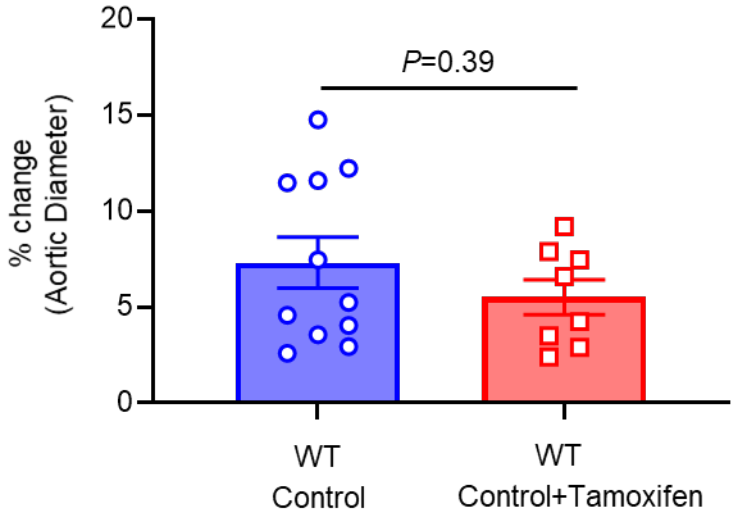
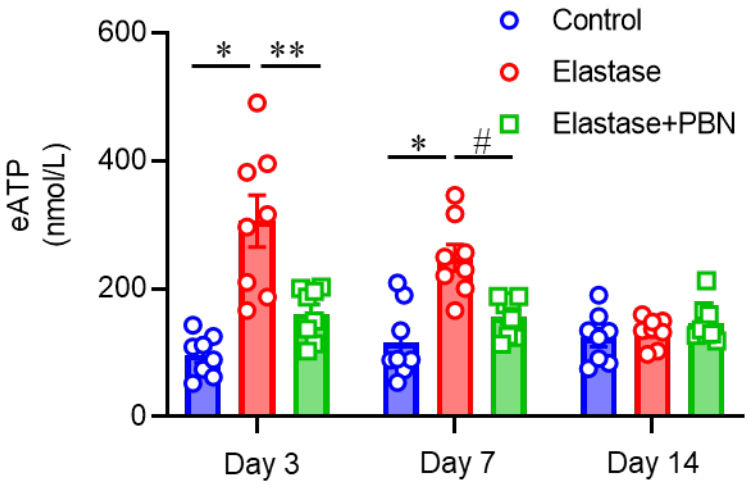
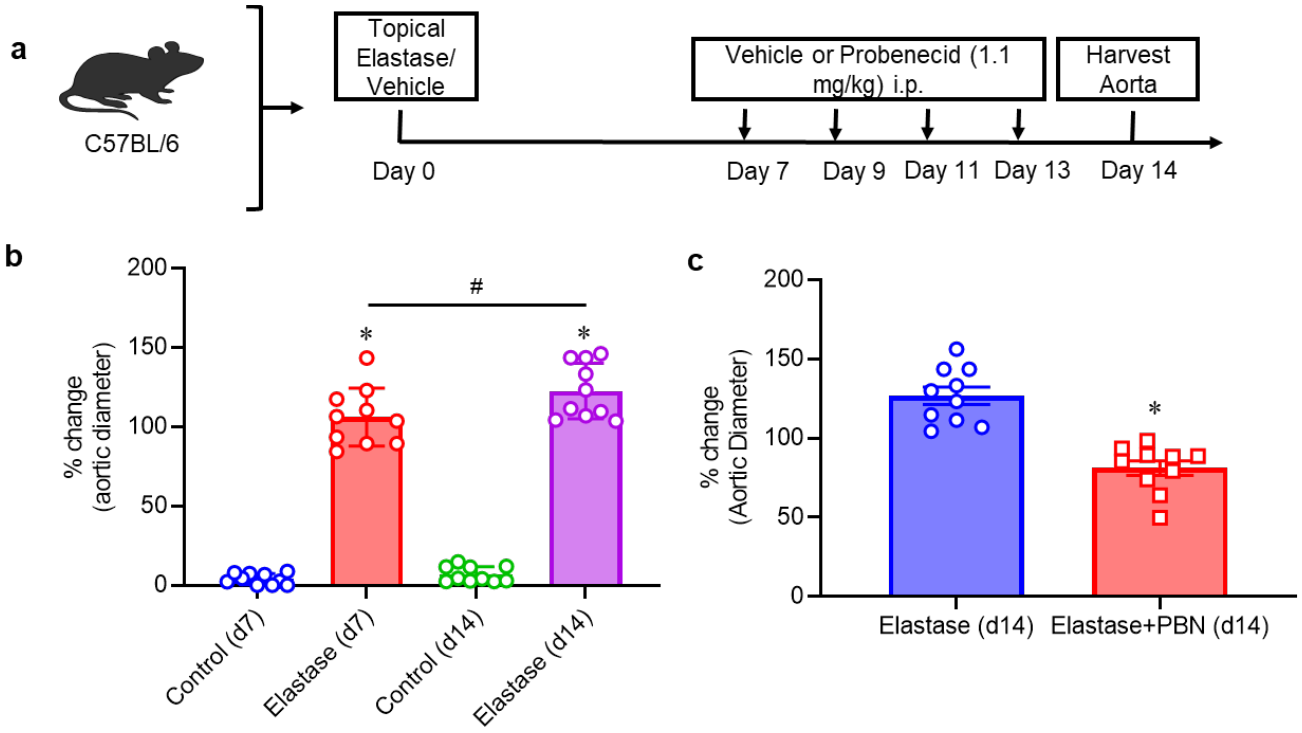


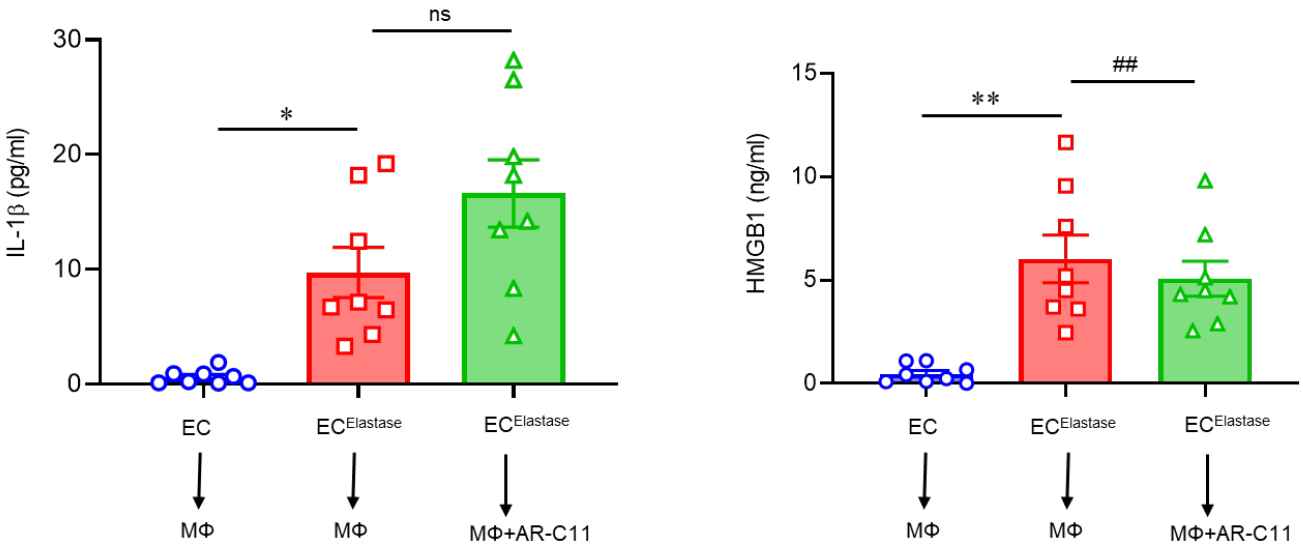
Supplementary Fig. 1 Tamoxifen treatment does not alter the aortic diameter compared to deactivated elastase-treated (control) mice on day 14. n=8-11/group. Data is represented as mean values ± SEM and comparative statistical analyses was done by two-tailed t-test. Source data are provided as a Source Data file.



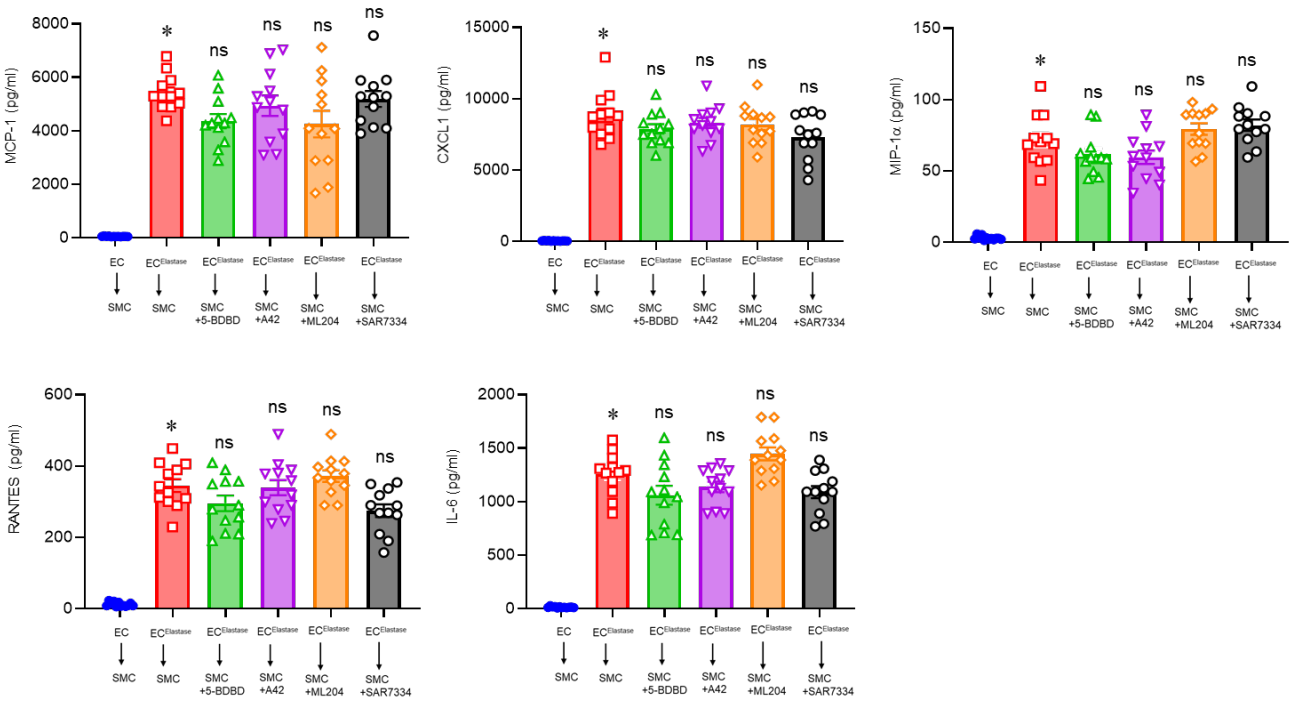
Supplementary Fig. 2 A significant increase in extracellular (e)-ATP content was observed in plasma of elastase-treated mice compared to controls. PBN-treated mice displayed a significant attenuation of eATP compared to elastase-treated mice.*P<0.0001; **P=0.001; $^{\#}P$=0.004; n=8 mice/group. Data is represented as mean values ± SEM and comparative statistical analyses was done by one-way ANOVA followed by multiple comparisons. Source data are provided as a Source Data file.



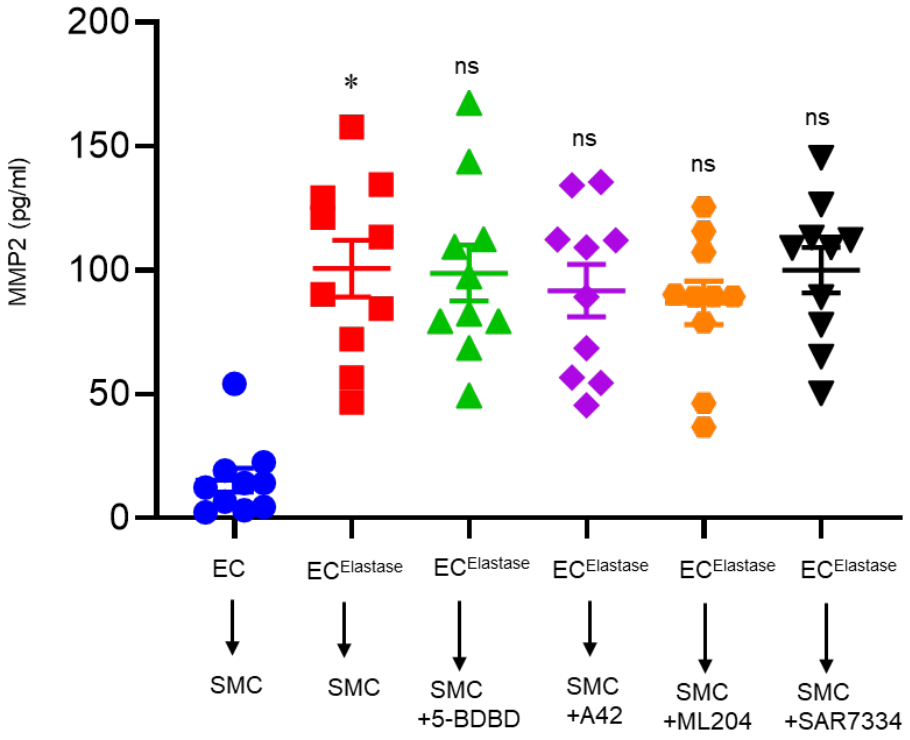
Supplementary Fig. 3 a Using the elastase AAA murine model, the protective role of Probenecid (PBN) administered **after** the formation of AAAs on day 7 was investigated. **b** AAA formation in elastase-treated WT mice was significantly increased compared to respective controls on days 7 and 14, respectively. **P*<0.0001 vs. respective controls; #*P*=0.03; n=8-10/group. Data is represented as mean values ± SEM and comparative statistical analyses was done by one-way ANOVA. **c** Treatment with PBN (after day 7) significantly attenuated aortic diameter in elastase-treated mice on day 14. **P*=0.0001; n=10/group. Data is represented as mean values ± SEM and comparative statistical analyses was done by two-tailed t-test. Source data are provided as a Source Data file.



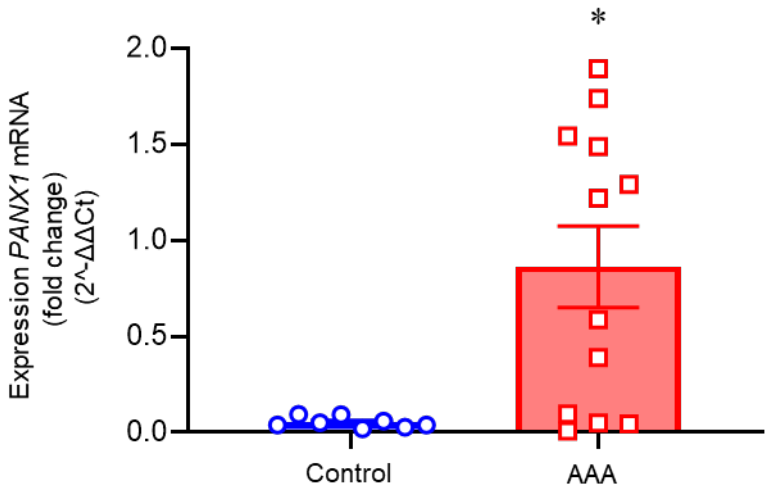
Supplementary Fig. 4 P2Y2 inhibition of macrophages fails to mitigate cytokine secretion induced by EC-dependent Panx1/ATP signaling. CMT from elastase-treated ECs to macrophages induces a significant upregulation of IL-1β and HMGB1 secretion, which was not blocked by pretreatment of macrophages with P2Y2 inhibitor (AR-C11; 10μM). *P=0.01; **P=0.0003; ns, not significant, P=0.07; $^{\#\#}P$=0.69; n=8/group. Data is represented as mean values ± SEM and comparative statistical analyses was done by one-way ANOVA. Source data are provided as a Source Data file.



Supplementary Fig. 5 P2X4, TRPV1, TRPC4 or TRPC6 inhibition of SMCs does not attenuate cytokine secretion. Activation of SMCs by CMT from elastase-treated ECs results in a multifold increase in proinflammatory cytokine production which was not inhibited by pretreatment with either P2X4 (5-BDBD; 50μM), TRPV1 (A42), TRPC4 (ML204) or TRPC6 (SAR73) inhibitors (1μM each). *P<0.001 vs. control (EC→SMC); ns, not significant vs. EC(elastase)→SMC; n=12/group. Data is represented as mean values ± SEM and comparative statistical analyses was done by one-way ANOVA. Source data are provided as a Source Data file.

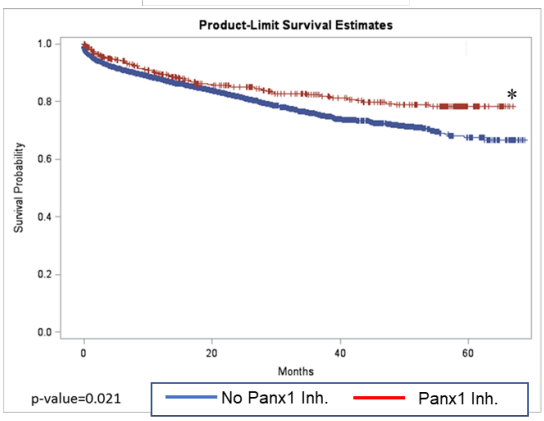


Supplementary Fig. 6 P2X4, TRPV1, TRPC4 or TRPC6 inhibition of SMCs does not attenuate MMP2 activity. Activation of SMCs by CMT from elastase-treated ECs results in a multifold increase in MMP2 activity which was not inhibited by pretreatment with either P2X4 (5-BDBD; 50μM), TRPV1 (A42), TRPC4 (ML204) or TRPC6 (SAR73) inhibitors (1μM each). **P*=0.0001; ns, not significant, *P*=0.9 vs. EC(elastase)→SMC; n=8/group. Data is represented as mean values ± SEM and comparative statistical analyses was done by one-way ANOVA. Source data are provided as a Source Data file.



Supplementary Fig. 7 *PANX1* mRNA expression is increased in AAA patients. A multi-fold increase in *PANX1* mRNA expression was observed in aortic tissue from AAA patients compared to controls. **P*=0.01; n=8-12/group. Data is represented as mean values ± SEM and comparative statistical analyses was done by two-tailed t-test. Source data are provided as a Source Data file.

Kaplan-Meier Survival Curve



Cox Proportional Hazard Model

Parameter	Hazards Ratio	p-value
Age (years)	1.05	<0.0001
Sex (Female)	0.93	0.357
Race (White)	0.94	0.605
Tobacco Use	1.57	0.014
Diabetes Mellitus	1.05	0.579
Hypertension	1.23	0.456
Chronic Obstructive Pulmonary Disease	1.76	<0.0001
Peripheral Vascular Disease	1.15	0.133
Congestive Heart Failure	1.78	<0.0001
Cancer	1.48	<0.0001
Stroke	2.89	<0.0001
Operative Repair	0.68	<0.0001
Panx1 Inhibitors	0.65	0.001

Univariate differences between groups

Variable	Panx1 Inhibitors (n=440)	No Panx1 Inhibitors (n=4109)	p value
Age (years)	66 ± 16	67 ± 16	0.198
Sex (Male)	299 (68.0%)	2773 (67.5%)	0.842
Race (White)	377 (85.7%)	3575 (87.0%)	0.435
Tobacco Use	74 (16.8%)	620 (15.1%)	0.176
Diabetes Mellitus	116 (26.4%)	868 (21.1%)	0.011
Hypertension	378 (85.9%)	3558 (86.6%)	0.737
Chronic Obstructive Pulmonary Disease	103 (23.4%)	892 (21.7%)	0.412
Peripheral Vascular Disease	82 (18.6%)	720 (17.5%)	0.560
Congestive Heart Failure	134 (30.5%)	605 (14.7%)	<0.001
Cancer	85 (19.3%)	771 (18.8%)	0.777
Stroke	27 (6.1%)	266 (6.5%)	0.784
Operative Repair	19 (4.3%)	227 (5.5%)	0.288
Probenecid	141 (3.1%)	-	
Spironolactone	313 (6.9%)	-	

Supplementary Fig. 8 Panx1 inhibitors are associated with decreased mortality in aortic aneurysm patients. Retrospective analysis of human aortic aneurysm patient data demonstrates a significantly lower risk of mortality after 5-years in patients who were receiving Probenecid or Spironolactone (Panx1 inhibitors) compared to aortic aneurysm patients without these medications. Kaplan-Meier survival analysis and Cox Proportional Hazards model was utilized to compare unadjusted and risk-adjusted long-term survival respectively. *P=0.02.