

**Computed tomography imaging of macrophage phagocytic activity in abdominal aortic aneurysm**

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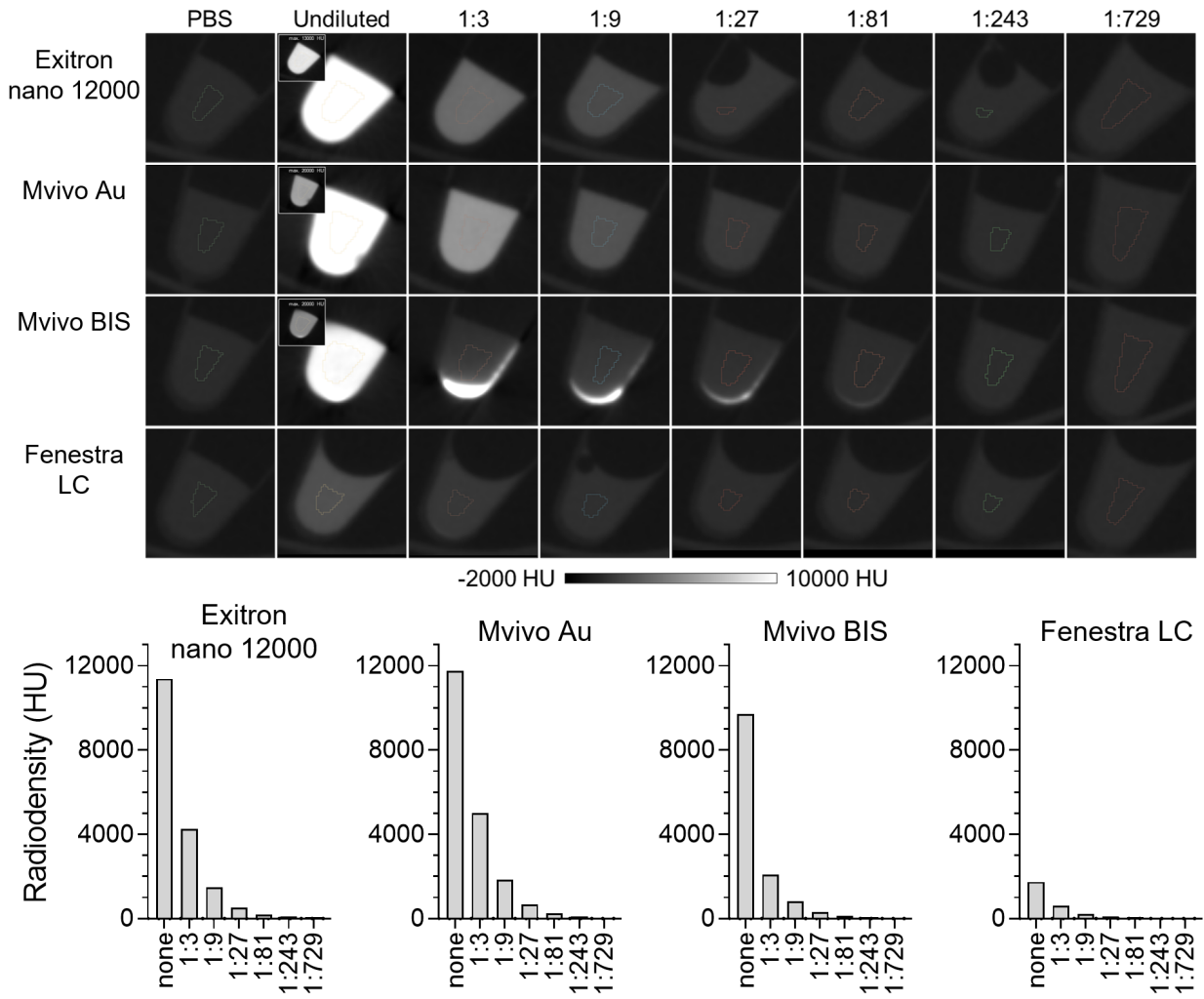
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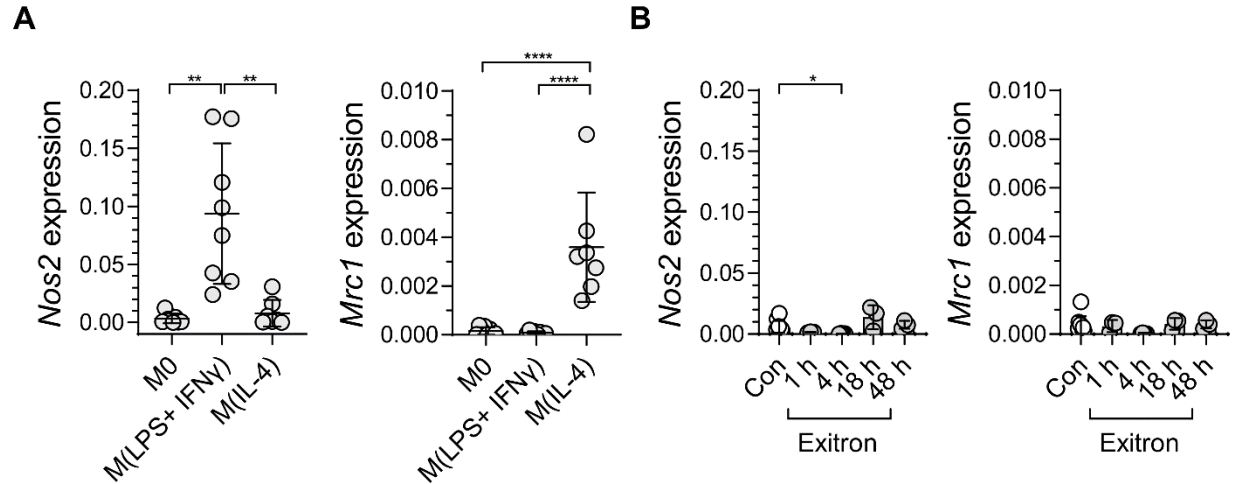
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## Supplementary Material



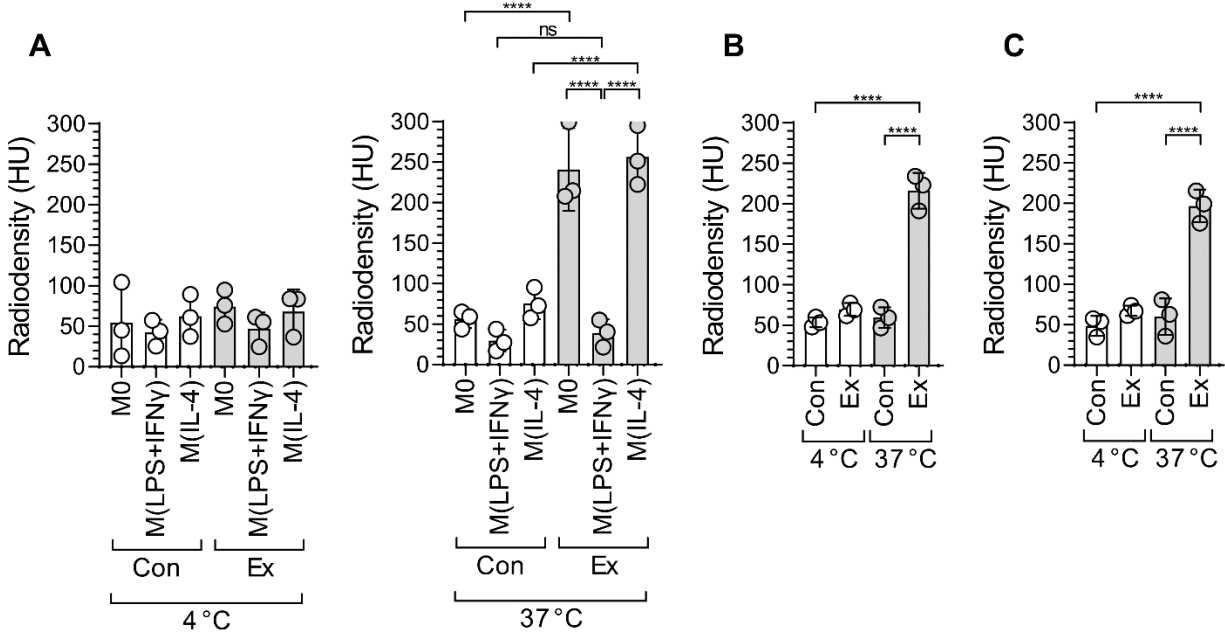
**Figure S1**

Radiodensity of CT contrast agents. CT images and radiodensity quantification of serial dilutions of selected nanoparticle contrast agents. PBS: Phosphate buffered saline, HU: Hounsfield units.



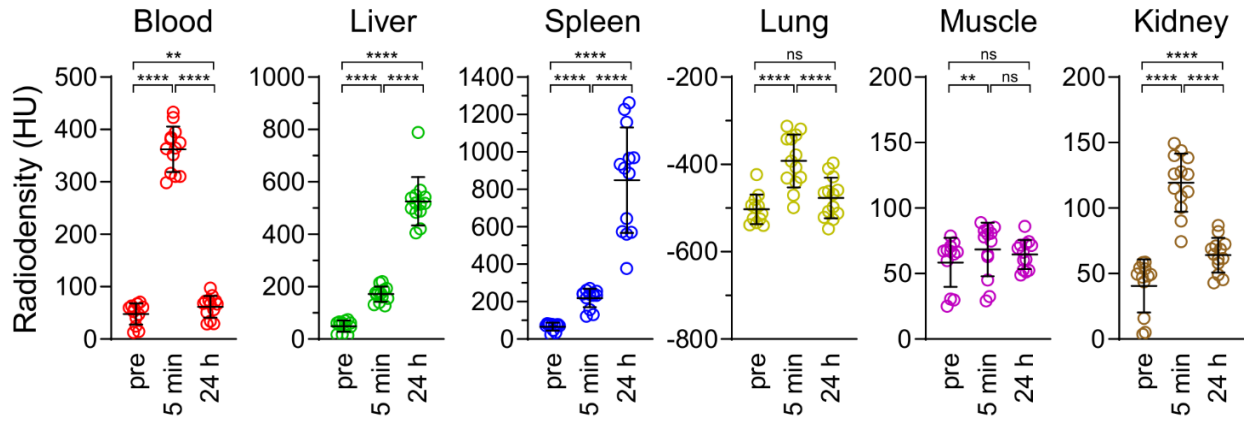
**Figure S2**

RAW 264.3 macrophage polarization and impact of Exitron nano 12000 exposure on polarization. **(A)** Gene expression analysis of RAW 264.7 cell polarization markers *Nos2* and *Mrc1* in untreated cells (M0), after LPS and IFN $\gamma$  stimulation [M(LPS+ IFN $\gamma$ )] and IL-4 stimulation [M(IL-4)] (n = 7–8). Statistical significance was assessed by Kruskal-Wallis test with Dunn's corrections and one-way ANOVA for *Nos2* and *Mrc1* gene expression, respectively. **(B)** Evaluation of polarization markers *Nos2* and *Mrc1* in RAW 264.7 cell without and after 1 h, 4 h, 18 h and 48 h of Exitron nano 12000 exposure (n = 3–4 for Exitron, n = 10 for Control). Statistical significance was assessed by Dunn's multiple comparison test.



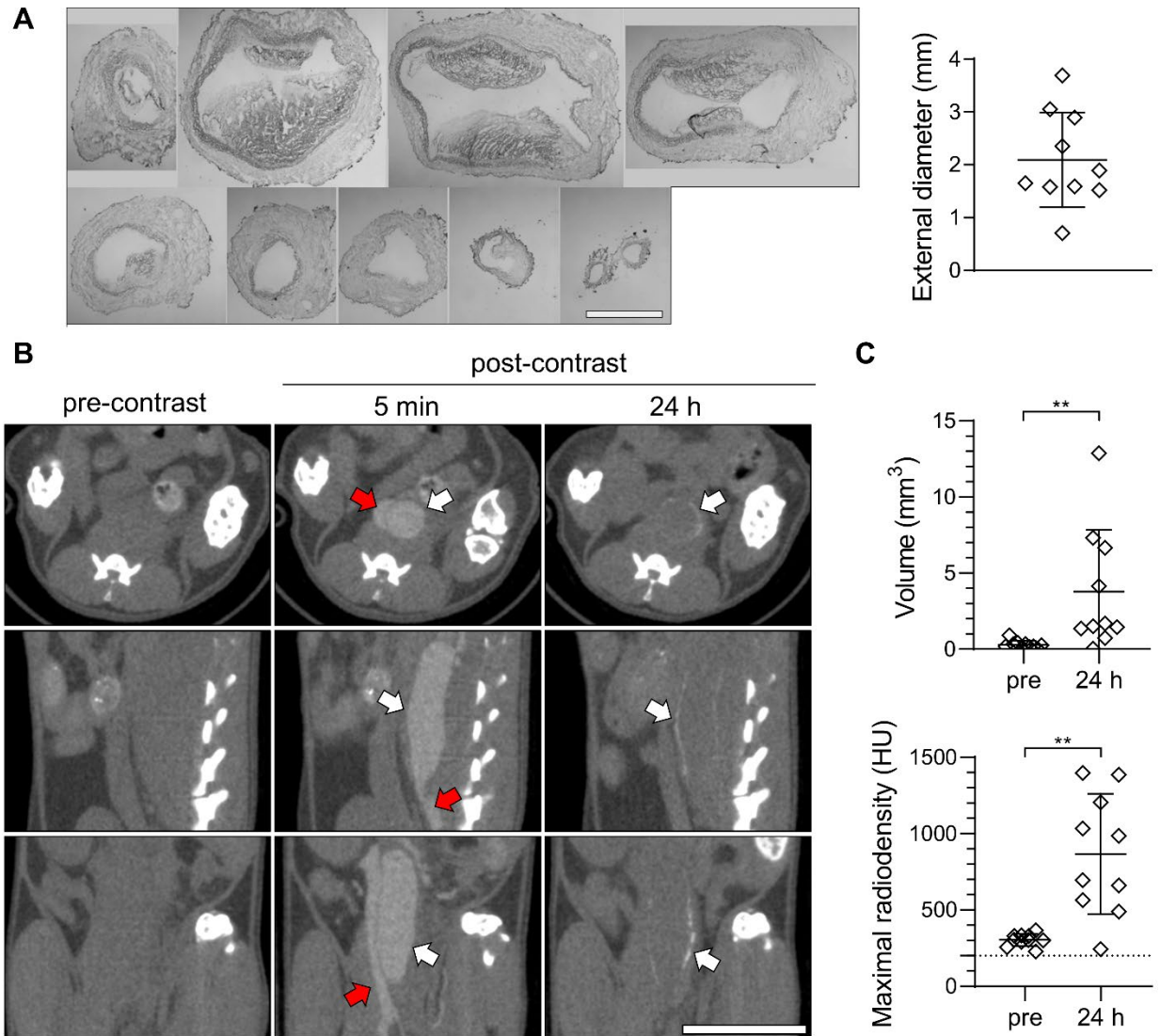
**Figure S3**

In vitro evaluation of Exitron nano 12000 uptake in unpolarized (M0) and polarized [M(LPS+IFN $\gamma$ ) and M(IL-4)] RAW 264.7 macrophages (**A**), b.END3 endothelial cells (**B**) and MOVAS aortic smooth muscle cells (**C**) at 4 and 37 °C (n = 3). Statistical significance was assessed by Dunn's multiple comparison test (left panel in A) or one-way ANOVA (all other panels). Ex: Exitron nano 12000; HU: Hounsfield units.



**Figure S4**

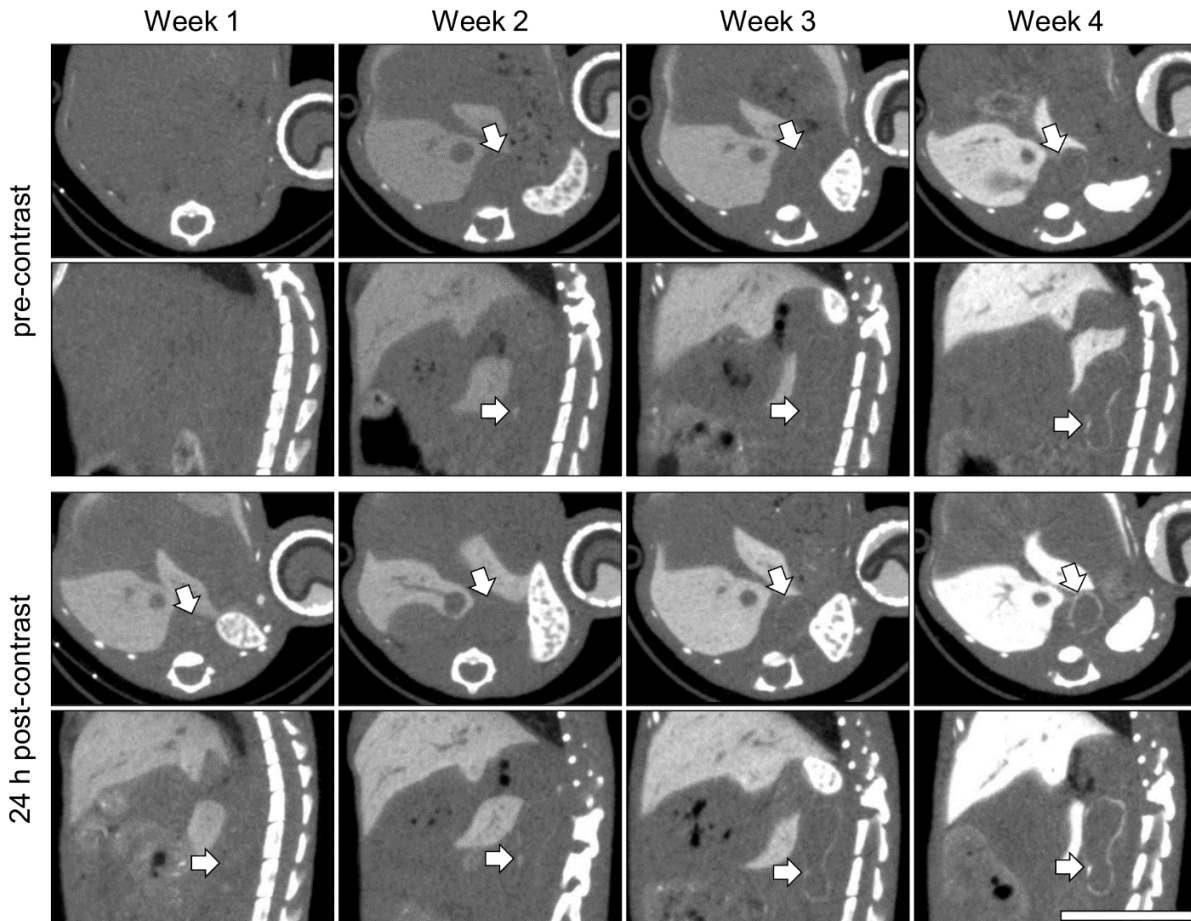
Exitron nano 12000 biodistribution in Ang II-infused *Apoe*<sup>-/-</sup> mice. Average tissue radiodensity quantified on in vivo CT images of *Apoe*<sup>-/-</sup> mice pre-, and at 5 min and 24 h post-Exitron administration. Statistical significance was assessed by one-way ANOVA with Tukey's correction (n=13). \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , \*\*\*\*  $P < 0.0001$ , ns: not significant. HU: Hounsfield Units.



**Figure S5**

Exitron nano 12000 uptake in topical elastase-induced murine AAA. **(A)** Brightfield images of serial sections of infra-renal abdominal aorta (proximal to distal from left to right) from a C57Bl/6J mouse at 6 weeks after topical elastase application (scale bar: 1 mm), and maximal external diameter of the infra-renal aorta at 4 to 6 weeks after topical elastase application in  $\beta$ -aminopropionitrile-treated C57Bl/6J mice. **(B)** CT images of a representative C57Bl/6J mouse at

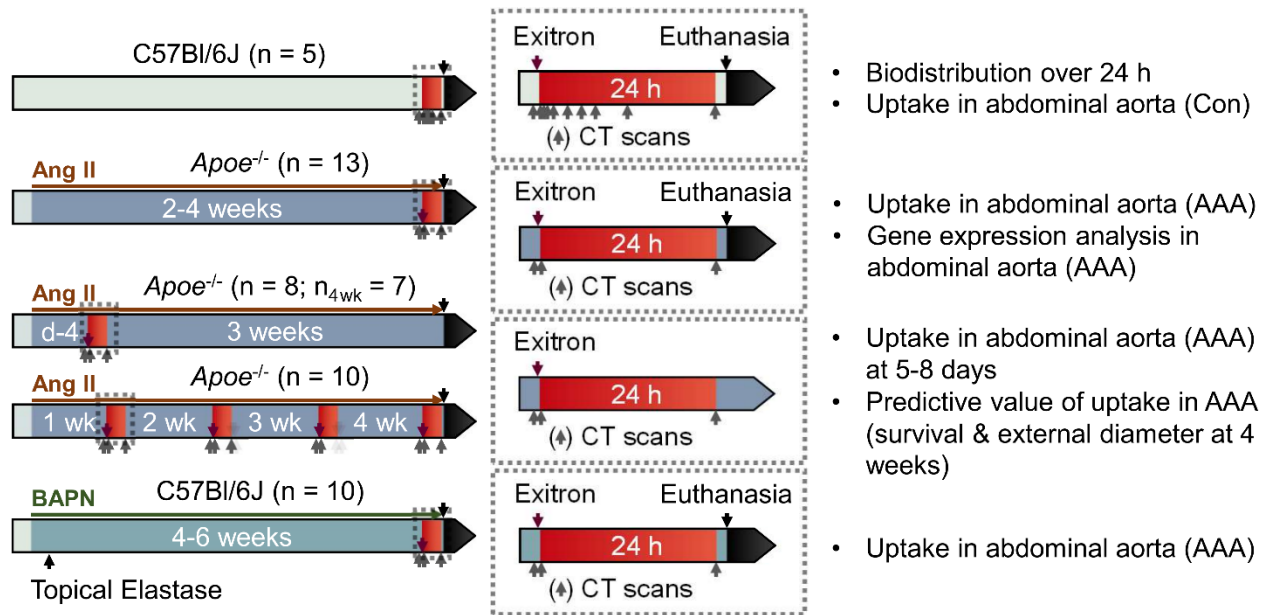
4 weeks after topical elastase application pre- (left panels), and at 5 min (middle panels) and 24 h (right panels) post-Exitron nano 12000 administration. White arrows: AAA; red arrows: inferior vena cava. Scale bar: 1 cm. CT scale: -750 to 1250 HU. (C) Quantification of the CT signal presented as enhancement volume (top panel) and maximal radiodensity (bottom panel). Statistical analysis was performed using Wilcoxon signed-rank test. \*\*  $P < 0.01$ . HU: Hounsfield Units.



**Figure S6**

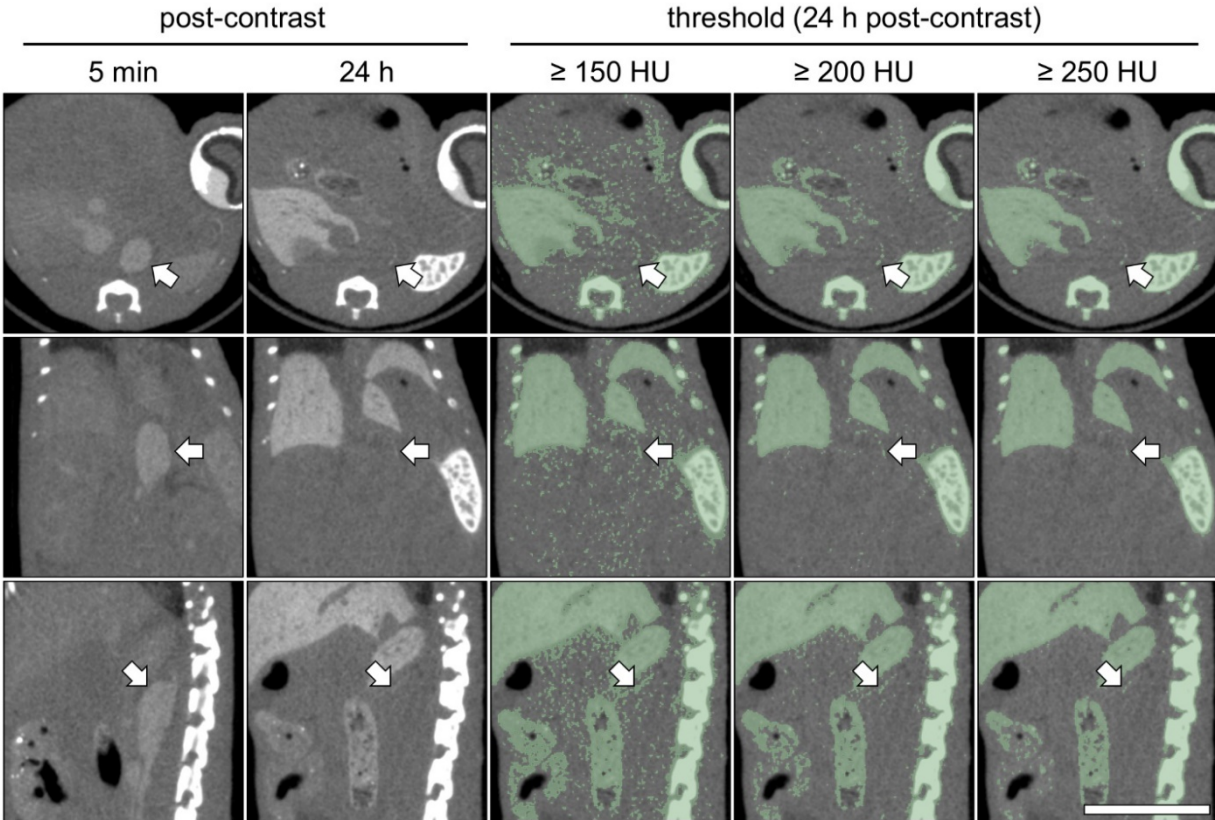
Evolution of CT signal with AAA progression. Serial CT images obtained weekly before (top panels) and 24 h after Exitron nano 12000 administration (bottom panels) from an *ApoE*<sup>-/-</sup> mouse infused with Ang II for 4 weeks. White arrows: AAA. Scale bar: 1 cm. CT scale: -750 to 1250 Hounsfield Units.





**Figure S7**

Flow chart of animal studies.



**Figure S8**

Thresholding of CT images acquired 24 h post-Exitron administration. CT images of a representative Ang II-infused *ApoE*<sup>-/-</sup> mouse acquired at 5 min and 24 h after Exitron nano 12000 administration (left panels). The pixels with radiodensities  $\geq 150$  Hounsfield Unit (HU), 200 HU and 250 HU thresholds are colored in green on delayed CT images (right panels). White arrow: AAA. CT scale: -750 to 1250 HU. Scale bar: 1 cm. HU: Hounsfield units.