Supplementary Information for

Role of smooth muscle activation in the static and dynamic mechanical characterization of human aortas

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Supplementary Note 1
Information on donors

Table S1. Information on donors with the following abbreviations: M = male; F = female; TBI = traumatic brain injury; HAI = hypoxic-anoxic injury; CVA = cerebrovascular accident.

| Donor | I  | II | III | IV | V  | VI | VII | VIII | IX | X  | XI | XII | XIII |
|-------|----|----|-----|----|----|----|------|------|----|----|----|-----|------|
| Age   | 32 | 48 | 55  | 40 | 25 | 30 | 68   | 42   | 68 | 58 | 47 | 59  | 60   |
| Gender| F  | M  | F   | F  | F  | M  | F    | M    | F  | M  | M  | F   | M    |
| Weight (kg) | 105 | 81 | 59  | 65 | 75 | 72 | 80.6 | 80   | 56 | 70 | 115| 76.4| 72.6 |
| Height (cm)  | 175 | 175| 175 | 173| 159| 165| 158  | 165  | 175| 163| 168| 187 | 165  |
| Cause of death | HAI | CVA| TBI | TBI| TBI| HAI| CVA  | HAI  | CVA| CVA| HAI| CVA | CVA  |
Supplementary Note 2
Isometric experiments on activation
Figure S1. Isometric activation of circumferential and longitudinal strips versus time from 13 descending thoracic aortas (identified by the roman number). Pre-stress around 50 kPa. First vasoactive agent, KCl; second vasoactive agent, noradrenaline (NA). Circ. = circumferential strip; Long. = longitudinal strip.
Supplementary Note 3
Additional results for microstructural characterization

a

b
Figure S2. Microstructural analysis of an in-plane section from the tunica media of donor XII. The three images show the same area of the tissue measuring 1600 × 1600 μm. a VSM cell orientation using DRAQ5 staining; distribution of the orientation and fitted von Mises distribution; dispersion coefficient $\kappa_{IP}^{VSM} = 0.399$. b Collagen distribution by second harmonic generation microscopy; the presence of one peak confirms the results in [1]. c Elastin distribution by two-photon microscopy. Angle 0° indicates the circumferential direction.

Supplementary Note 4
Active and passive quasi-static extension tests
Figure S3. Engineering stress versus engineering strain: quasi-static curves of circumferential and longitudinal strips of 13 descending thoracic aortas (identified by the Roman number). Maximum activation after KCl and noradrenaline. Activation of the VSM = red curve; passive behavior = blue curve; Circ. = circumferential strip; Long. = longitudinal strip.

Supplementary Note 5
Details on the material model of the passive and active aortic tissue

The strain-energy function $W$ of the aortic wall is represented as the sum of passive and active components

$$W = W^p + W^A,$$  \hspace{1cm} (6.1)

where the passive component $W^p$ is given by

$$W^p = W^p_{ISO} + W^p_{ANISO}.$$  \hspace{1cm} (6.2)

$W^p_{ISO}$ accounts for the passive hyperelastic response of the ground matrix and the elastin network and is assumed in the form of an isotropic neo-Hookean strain-energy function; $W^p_{ANISO}$ accounts for the anisotropic passive response due to collagen fibers; $W^A$ represents the contribution of the activated smooth muscles, which is mainly present in the tunica media. In particular,
\[ W_{ISO} = \frac{\mu}{2} (I_1 - 3), \]  

(6.3)

where \( \mu \) is a material parameter, \( I_1 \) is the first invariant of the right Cauchy-Green tensor, here given in matrix form as

\[
C = \begin{pmatrix}
2\varepsilon_{xx} + 1 & \gamma_{x\theta} & \gamma_{xz} \\
\gamma_{x\theta} & 2\varepsilon_{\theta\theta} + 1 & \gamma_{\theta z} \\
\gamma_{xz} & \gamma_{\theta z} & 2\varepsilon_{zz} + 1
\end{pmatrix},
\]

where \( \varepsilon \) and \( \gamma \) indicate the normal and shear Green-Lagrange strain components; \( x, \theta, z \) are the three orthogonal coordinates in the longitudinal, circumferential and radial directions, respectively. The first invariant of the tensor \( C \) is [2]

\[
I_1 = \text{tr}(C) = 2(\varepsilon_{xx} + \varepsilon_{\theta\theta} + \varepsilon_{zz}) + 3.
\]

(6.4)

Two families of fibers are used in \( W_{ANISO} \). One represents the effect of collagen fibers, which are mainly oriented in the circumferential or longitudinal direction, and an orthogonal family represents the cross-link and lateral interaction of collagen

\[
W_{ANISO} = \sum_{i=1}^{2} C^{(i)} W^{(i)}(\alpha^{(i)}),
\]

(6.5)

where the coefficient \( C^{(i)} \) has a value between 0 and 1 and describe the portion of participating fibers (i.e. the uncomressed), as introduced in [3]; it should be noted that the coefficient \( C^{(i)} \) is a function of the strain and thus varies with the load, as shown in Figure S4. On the other hand, \( C^{(2)} = 1 \). In eq. (6.5) the strain-energy function of a fiber family is represented by

\[
W^{(i)} = \frac{\mu^{(i)}_1}{\mu^{(i)}_2} \left\{ \exp\left[\frac{\mu^{(i)}_2}{\mu^{(i)}_1} (E^{(i)})^2\right] - 1 \right\},
\]

(6.6)

\[
E^{(i)} = 2\kappa^{(i)}_{op} \kappa^{(i)}_{ip} I_1 + 2\kappa^{(i)}_{op} (1 - 2\kappa^{(i)}_{ip}) I_4^{(i)} + \left(1 - 2\kappa^{(i)}_{op} (\kappa^{(i)}_{ip} + 1)\right) I_n - 1,
\]

(6.7)

where \( \mu^{(i)}_1, \mu^{(i)}_2 \) are parameters describing the fiber material, \( \kappa^{(i)}_{ip} \) is the in-plane dispersion parameter, which varies from 0 (perfectly aligned fibers) to 1/2 (in-plane isotropy), \( \kappa^{(i)}_{op} \) is the out-of-plane dispersion parameter, which can take on values between 1/3 (out-of-plane isotropy) and 1/2 (perfectly in-plane fibers). It is assumed that the mean direction of the fiber family forms the angle \( \alpha^{(i)} = 0 \) (circumferential) of \( \pi/2 \) (longitudinal) with the circumferential direction of the aorta in the plane tangential to the middle surface (in-plane). The unit vector of the mean direction is \( \{M^{(i)}\}^T = \{\sin \alpha^{(i)}, \cos \alpha^{(i)}, 0\} \) in the coordinates \( \{x, \theta, z\} \). Using the introduced notation, it is convenient to represent the pseudo-invariants \( I_4^{(i)} \) and \( I_n \) as [1, 2]

\[
I_4^{(i)} = C : M^{(i)} \otimes M^{(i)} = 1 + 2(\varepsilon_{xx} \sin^2 \alpha^{(i)} + \varepsilon_{\theta\theta} \cos^2 \alpha^{(i)} + \gamma_{x\theta} \sin \alpha^{(i)} \cos \alpha^{(i)}),
\]

(6.8)

\[
I_n = C : e_{z} \otimes e_{z} = 2\varepsilon_{zz} + 1,
\]

(6.9)

where \( \{e_{z}\}^T = \{0, 0, 1\} \) is the unit vector in the radial direction, the operation : denotes the double contraction of tensors, and \( \otimes \) is the outer product of vectors. The pseudo-invariants \( I_4^{(i)} \) and \( I_n \) measure the squares of the fiber stretches in the directions \( M^{(i)} \) and \( e_{z} \), respectively.

The change in volume during the deformation is related to the third invariant of \( C \), i.e.
\[ I_3 = \det(C) = (2\varepsilon_{xx} + 1)(2\varepsilon_{\theta\theta} + 1)(2\varepsilon_{zz} + 1) - (2\varepsilon_{xx} + 1)\gamma_{\theta\theta}^2 - (2\varepsilon_{\theta\theta} + 1)\gamma_{zz}^2 - (2\varepsilon_{xx} + 1)\gamma_{xx}\gamma_{\theta\theta}. \] (6.10)

Aortic tissue is considered incompressible (see, e.g., [4]), which gives
\[ I_3 = 1. \] (6.11)

Equations (6.10) and (6.11) allow to obtain \( \varepsilon_{zz} \) as a function of the other strains
\[ \varepsilon_{zz} = \frac{\gamma_{\theta\theta}^2 + \gamma_{zz}^2 + 2\gamma_{xx}\varepsilon_{xx} + 4\varepsilon_{xx}\varepsilon_{\theta\theta} - 2\gamma_{xx}\varepsilon_{\theta\theta}^2 + 2\varepsilon_{xx}\gamma_{\theta\theta}^2 - 2\gamma_{\theta\theta}\gamma_{xx}\gamma_{zz}}{2\left((2\varepsilon_{xx} + 1)(2\varepsilon_{\theta\theta} + 1) - \gamma_{xx}^2\right)}. \] (6.12)

Expression (6.12) is inserted into \( W \), and the resulting equation, which does not depend on \( \varepsilon_{zz} \), is denoted as
\[ \hat{W} = \hat{W}^P + \hat{W}^A. \]

Initially the parameters of the passive hyperelastic model are determined by simultaneously fitting the stress-strain curves of the longitudinal and circumferential aortic strips. The minimization functional for this case has the form
\[ f^P = \frac{1}{2} \left\{ \frac{1}{N_{\text{axial}}^P} \sum_{i=1}^{N_{\text{axial}}^P} \left( \bar{S}_{xx}^P \left( \varepsilon_{xx}^{P(i)}, \varepsilon_{\theta\theta}^{P(i)} \right) - S_{xx}^P \left( \varepsilon_{xx}, \varepsilon_{\theta\theta} \right) \right)^2 + \frac{1}{N_{\text{circ}}^P} \sum_{i=1}^{N_{\text{circ}}^P} \left( \bar{S}_{\theta\theta}^P \left( \varepsilon_{xx}^{P(i)}, \varepsilon_{\theta\theta}^{P(i)} \right) - S_{\theta\theta}^P \left( \varepsilon_{xx}, \varepsilon_{\theta\theta} \right) \right)^2 \right\}, \] (6.13)

where \( \left( \varepsilon_{xx}^{P(i)}, S_{xx}^{P(i)} \right) \) and \( \left( \varepsilon_{\theta\theta}^{P(i)}, S_{\theta\theta}^{P(i)} \right) \) are the experimentally obtained points on the stress-strain curves (second Piola-Kirchhoff stress and Green-Lagrange strain) of the non-activated longitudinal and circumferential strips, respectively, and \( N_{\text{axial}}^P, N_{\text{circ}}^P \) are the numbers of experimental points in the curves for the axial and circumferential strips, respectively. The passive second Piola-Kirchhoff stresses are determined as
\[ \bar{S}_{xx}^P \left( \varepsilon_{xx} \right) = S_{xx}^P \left( \varepsilon_{xx}, \varepsilon_{\theta\theta} \left( \varepsilon_{xx} \right) \right), \]
\[ \bar{S}_{\theta\theta}^P \left( \varepsilon_{xx} \right) = S_{\theta\theta}^P \left( \varepsilon_{xx}, \varepsilon_{\theta\theta} \left( \varepsilon_{xx} \right), \varepsilon_{xx} \right), \]
where
\[ S_{xx}^P = \frac{\partial \hat{W}^P}{\partial \varepsilon_{xx}}, \quad S_{\theta\theta}^P = \frac{\partial \hat{W}^P}{\partial \varepsilon_{\theta\theta}}. \]

The functions \( \varepsilon_{xx}^P \left( \varepsilon_{\theta\theta} \right), \varepsilon_{\theta\theta}^P \left( \varepsilon_{xx} \right) \) are determined from the conditions of absence of transverse normal stresses
\[ \varepsilon_{xx}^P \left( \varepsilon_{\theta\theta} \right): S_{xx}^P \left( \varepsilon_{xx}, \varepsilon_{\theta\theta} \right) = 0, \quad \varepsilon_{\theta\theta}^P \left( \varepsilon_{xx} \right): S_{\theta\theta}^P \left( \varepsilon_{xx}, \varepsilon_{\theta\theta} \right) = 0. \] (6.14)

The functional (6.14) is minimized by using a genetic algorithm described in [5].
Figure S4. Exclusion coefficients $C^{(1)}$ of collagen fibers for passive and active strips from donor IV during uniaxial tensile tests. a passive circumferential strip; b passive longitudinal strip; c active circumferential strip; d active longitudinal strip.

Mechanical response of the activated tissue
Experimental observations show that the difference between passive and active responses with increasing strain initially increases up to a certain strain value and then it decreases. In earlier studies this observation led to the assumption of a parabolic term in stress to describe the difference between active and passive responses. Thus, the corresponding active term in the strain-energy function was of cubic order in strain. The experiments presented in this study show that the absolute value of the tangent of the difference between passive and active responses is often much lower in the ascending part than in the descending part. Typical curves are shown in Figure S5.
This non-symmetric character of the curves can be better described by a function of the power higher than two under stress. In addition, in contrast to previous studies, it is assumed that the smooth muscle fibers are not perfectly aligned in the circumferential direction, but rather dispersed around the circumferential direction of the aorta in the plane tangential to the middle surface (in-plane) of the strip. In addition to the main family of dispersed VSMs with the dispersion parameter $\kappa_{IP}^{VSM}$, a second orthogonal family with the same dispersion is considered to describe the lateral interaction between VSM fibers; this is justified by the mutual winding of the VSM fibers shown in Figure 3(b). This second family introduces a minor active stress that is the main one. The proposed active strain-energy function is given by

$$W^A = \hat{K} \sum_{i=1}^{2} K_{i} \left( E_{i}^{VSM} + \frac{a_{i}}{2} \left( E_{i}^{VSM} \right)^{2} - \frac{b_{i}}{m_{i} + 1} \left( E_{i}^{VSM} \right)^{m_{i}+1} \right), \quad a_{i}, b_{i} > 0,$$

(6.15)

where $m_{i}$ is an integer larger than one and

$$E_{1}^{VSM} = 2 \left[ \kappa_{IP}^{VSM} \left( e_{xx} + e_{\theta\theta} \right) + (1-2\kappa_{IP}^{VSM}) e_{\theta\theta} \right],$$

(6.16a)

$$E_{2}^{VSM} = 2 \left[ \kappa_{IP}^{VSM} \left( e_{xx} + e_{\theta\theta} \right) + (1-2\kappa_{IP}^{VSM}) e_{xx} \right].$$

(6.16b)

$\hat{K}$ is the activation level coefficient with a value between zero (no activation) and 1 (maximum activation). Equations (6.16a,b) are inspired by an expression proposed in [6] for the bi-dimensional fiber dispersions. In (6.15) it is assumed that all smooth muscle fibers lie perfectly in-plane, as the experimental observations suggest. Expression (6.15) has nine parameters $K_{i}, a_{i}, b_{i}, m_{i}, \kappa_{IP}^{VSM}$. The convexity of the strain-energy function (6.1) has been verified numerically for the studied cases. The contour plots of $W$ are presented in Figure 4c for the case of donor VIII and they are convex. The first summand in (6.15) take into account the stress value at zero principal strain. In particular, $K_{i}$ is a stress-like parameter that represents the initial active stress values. These values in the axial and circumferential directions can be different, and the model captures this feature through the VSM dispersion parameter $\kappa_{IP}^{VSM}$. The second summand in (6.15) reflects the relatively slow initial increase of the stress difference, which is controlled by the parameter $a_{i}$. The third summand in (6.15), because of its higher power, represents the rapid
decrease in the difference after a certain strain value. The slope of this decrease is controlled by
the two parameters $b_i$ and $m_i$. The dispersion parameter $\kappa_{ip}^{VSM}$ is obtained by fitting a von Mises
probability distribution [1, 6], i.e.

$$\rho_{ip}(\theta) = \frac{\exp[c\cos(2\theta)]}{I_0(c)},$$

(6.17)
to the experimental histogram of the VSM distribution in Figure 3(d) to obtain the concentration
parameter $c$; $I_0$ is the modified Bessel function of the first kind of order zero. The following
normalization condition is also introduced, i.e.

$$\frac{1}{2\pi} \int_0^{2\pi} \rho_{ip}(\theta) d\theta = 1.$$

The dispersion parameters $\kappa_{ip}^{VSM}$ is given by [1, 6]

$$\kappa_{ip}^{VSM} = \frac{1}{2} - \frac{I_1(c)}{2I_0(c)},$$

(6.18)

where $I_1$ is the modified Bessel function of the first kind of order one, whereas $\kappa_{ip}^{VSM}$ takes on
values between 0 for perfectly aligned VSM fibers in the circumferential direction and 0.5 for isotropic response of the VSM.

**Identification of the material parameters for the activated tissue**

Fitting the results of uniaxial extension tests on the activated tissue to the active material model
takes place after the material parameters of the passive tissue have been identified. All material
parameters determined by minimizing the functional (6.13) for the passive behavior remain the
same. The parameters identified at this stage are $K_i, a_i, b_i, m_i$. Although active and passive
components in (6.1) are independent and the total strain-energy function is their sum, the resulting
stresses do not add up. The stresses are coupled by the equation for the orthogonal stress. Here the
case of the axial strip in the uniaxial extension test is considered. The stress in the circumferential
direction is zero during the test. In case of a passive response, the corresponding equation reads

$$S_{\theta\theta}^p(e_{xx}, e_{\theta\theta}) = 0,$$

which allows to obtain $e_{\theta\theta}^p(e_{xx})$. In the case of VSM activation, we have

$$S_{\theta\theta}^p(e_{xx}, e_{\theta\theta}) + S_{\theta\theta}^A(e_{xx}, e_{\theta\theta}) = 0,$$

(6.19)

where

$$S_{\theta\theta}^A = \frac{\partial W^A}{\partial e_{\theta\theta}}.$$

Equation (6.19) allows to obtain $e_{\theta\theta}(e_{xx})$. In general, $e_{\theta\theta}(e_{xx}) \neq e_{\theta\theta}^p(e_{xx})$. This means that

$$S_{xx}^p(e_{xx}, e_{\theta\theta}^p(e_{xx})) \neq S_{xx}^p(e_{xx}, e_{\theta\theta}(e_{xx})).$$

Therefore, the total mechanical response must be fitted by varying the parameters $K_i, a_i, b_i, m_i$ of
the active components and leaving the previously identified passive hyperelastic coefficients unchanged.

The minimization functional $f^A$ in this case has a form similar to (6.13)
\[ f^A = \frac{1}{2} \left\{ \frac{1}{N_{\text{axial}}^A} \sum_{i=1}^{N_{\text{axial}}} (\tilde{S}_{xx}(\varepsilon_{xx}^{A(i)}) - S_{xx}^{A(i)})^2 + \frac{1}{N_{\text{circ}}^A} \sum_{i=1}^{N_{\text{circ}}} (\tilde{S}_{\theta\theta}(\varepsilon_{\theta\theta}^{A(i)}) - S_{\theta\theta}^{A(i)})^2 \right\}, \]  

(6.20)

where \((\varepsilon_{xx}^{A(i)}, S_{xx}^{A(i)})\) and \((\varepsilon_{\theta\theta}^{A(i)}, S_{\theta\theta}^{A(i)})\) are the experimental points of the stress-strain curves of the activated longitudinal and circumferential strips, respectively, and \(N_{\text{axial}}^A, N_{\text{circ}}^A\) are the numbers of experimental points for the axial and circumferential strips, respectively. The second Piola-Kirchhoff stresses of the activated tissue are obtained in a similar way to the passive case, but with the total strain-energy function (6.1) instead of just the passive component

\[ \tilde{S}_{xx}(\varepsilon_{xx}) = S_{xx}(\varepsilon_{xx}, \varepsilon_{\theta\theta}(\varepsilon_{xx})), \]
\[ \tilde{S}_{\theta\theta}(\varepsilon_{xx}) = S_{\theta\theta}(\varepsilon_{xx}, \varepsilon_{\theta\theta}), \]

where

\[ S_{xx} = \frac{\partial \hat{W}}{\partial \varepsilon_{xx}}, \quad S_{\theta\theta} = \frac{\partial \hat{W}}{\partial \varepsilon_{\theta\theta}}. \]

The functions \(\varepsilon_{xx}(\varepsilon_{\theta\theta})\) and \(\varepsilon_{\theta\theta}(\varepsilon_{xx})\) are determined from the conditions of absence of the transverse normal stress (6.19) and its counterpart for the case of the circumferential strip, i.e.

\[ S_{xx}^P(\varepsilon_{xx}, \varepsilon_{\theta\theta}) + S_{\theta\theta}^P(\varepsilon_{xx}, \varepsilon_{\theta\theta}) = 0. \]

Table S2. Parameters of the passive aortic material model identified from the experiments. \(R^2\) indicates the accuracy of the model with respect to the experimental data.

| Donor | \(\mu\) (kPa) | \(\alpha^{(1)}\) | \(\mu_1^{(1)}\) (kPa) | \(\mu_2^{(1)}\) (-) | \(\kappa_{\text{OP}}\) (-) | \(\kappa_{\text{IP}}\) (-) | \(\mu_1^{(2)}\) (kPa) | \(\mu_2^{(2)}\) (-) | \(R^2\) (-) |
|------|----------------|----------------|----------------------|------------------|-----------------|-----------------|------------------|------------------|--------|
| I    | 50.21          | 0              | 16.41                | 2.327            | 0.3473          | 0.0490          | 4.400            | 1.366            | 0.997  |
| II   | 23.90          | 0              | 14.93                | 2.140            | 0.4534          | 0.3568          | 4.957            | 2.050            | 0.987  |
| III  | 13.54          | 0              | 22.88                | 1.714            | 0.4688          | 0.2668          | 6.726            | 1.675            | 0.982  |
| IV   | 32.57 \(\pi/2\) | 5.214          | 1.411                | 0.4951           | 0.1158          | 1.130           | 0.503            | 0.997            |        |
| V    | 53.25 \(\pi/2\) | 9.040          | 1.475                | 0.3463           | 0.0295          | 3.835           | 0.720            | 0.989            |        |
| VI   | 41.53 \(\pi/2\) | 56.86          | 13.39                | 0.3490           | 0.4194          | 2.032           | 0.498            | 0.975            |        |
| VII  | 19.31          | 0              | 26.46                | 4.133            | 0.4155          | 0.2064          | 3.638            | 4.106            | 0.975  |
| VIII | 32.53 \(\pi/2\) | 29.91          | 27.07                | 0.3442           | 0.4123          | 3.143           | 0.363            | 0.995            |        |
| IX   | 39.05 \(\pi/2\) | 14.79          | 98.60                | 0.3927           | 0.1438          | 7.050           | 3.501            | 0.982            |        |
| X    | 18.19          | 0              | 857.4                | 37.26            | 0.3792          | 0.4516          | 14.55            | 21.00            | 0.979  |
| XI   | 22.67 \(\pi/2\) | 70.88          | 22.00                | 0.3405           | 0.2877          | 4.323           | 1.273            | 0.966            |        |
| XII  | 28.21 \(\pi/2\) | 36.72          | 5.100                | 0.3967           | 0.2761          | 5.473           | 1.009            | 0.995            |        |
| XIII | 26.89          | 0              | 75.47                | 16.32            | 0.3973          | 0.4205          | 9.379            | 7.188            | 0.979  |
Table S3. Parameters of the active aortic material model identified from the experiments. $R^2$ indicates the accuracy of the model with respect to the experimental data.

| Donor | $K_1$ (kPa) | $a_1$ (-) | $b_1$ (-) | $m_1$ (-) | $k_{IP}^{VSM}$ (-) | $K_2$ (kPa) | $a_2$ (-) | $b_2$ (-) | $m_2$ (-) | $R^2$ (-) |
|-------|--------------|-----------|-----------|-----------|----------------|--------------|-----------|-----------|-----------|-----------|
| I     | 7.486        | 20.20     | 34.59     | 2         | 0.2960         | 4.674        | 30.44     | 63.36     | 2         | 0.962     |
| II    | 0.943        | 31.16     | 30.12     | 2         | 0.3007         | 1.589        | 34.11     | 93.88     | 2         | 0.996     |
| III   | 4.151        | 18.08     | 47.48     | 3         | 0.2807         | 1.540        | 24.50     | 57.54     | 2         | 0.941     |
| IV    | 1.249        | 31.66     | 71.27     | 2         | 0.4988         | 10.05       | $1 \times 10^{-5}$ | 4.328     | 4         | 0.994     |
| V     | 2.901        | 39.83     | 54.09     | 2         | 0.2410         | 1.052       | 39.68     | 47.60     | 2         | 0.957     |
| VI    | 5.577        | 34.05     | 22.74     | 2         | 0.4567         | 5.853       | $1 \times 10^{-5}$ | 48.16     | 2         | 0.914     |
| VII   | 5.186        | 17.76     | 49.23     | 3         | 0.2286         | 1.117       | 21.73     | 56.94     | 2         | 0.974     |
| VIII  | 8.251        | 5.955     | 8.363     | 3         | 0.3433         | 0.942       | 40.15     | 73.28     | 2         | 0.998     |
| IX    | 5.470        | 16.05     | 57.32     | 3         | 0.1576         | 0.649       | 66.17     | 48.14     | 2         | 0.969     |
| X     | 22.00        | 6.075     | 52.51     | 2         | 0.4325         | 19.40       | 2.737     | 52.64     | 3         | 0.958     |
| XI    | 6.637        | 5.622     | 3.121     | 2         | 0.3814         | 1.450       | 27.78     | 118.8     | 2         | 0.945     |
| XII   | 4.033        | 19.50     | 59.45     | 3         | 0.3989         | 2.876       | 20.09     | 36.28     | 2         | 0.958     |
| XIII  | 3.294        | 27.97     | 45.61     | 2         | 0.3188         | 3.665       | 18.73     | 49.46     | 2         | 0.992     |

Supplementary Note 6

Dynamic characterization: dynamic stiffness ratio and loss factor

A hysteresis loop of the cyclic strain is shown in Figure S6; the quasi-static stress-strain curve is also displayed in the figure. The middle curve of the loop is calculated as the mean of the upper and lower curves that make up the loop. The center of the loop is placed on this middle curve. The point on the quasi-static stress-strain curve corresponding to the center of the loop is shown in Figure S6 as the intersection of the static curve and the minimal distance line. The center of the loop should be placed on the static curve, but there may be small differences due to relaxation, creep and inelasticity that occur in the time between the quasi-static test and the dynamic test.

The tangent to the middle curve of the loop (in the middle of the loop) has the slope $a_L$; then $\tan(\alpha_L)$ is proportional to the storage modulus. The slope of the tangent to the static curve at the point corresponding to the center of the loop is $a_S$; then $\tan(\alpha_S)$ is proportional to the static modulus. The dynamic stiffness ratio $\delta$ is defined [7] as the ratio between the storage modulus and the corresponding static modulus. $\delta$ indicates the stiffness increase of the aortic strip with dynamic loading compared to a quasi-static loading and it depends on the amount of pre-stress (or pre-stretch), on the amplitude of the harmonic cyclic strain and on the frequency of the cyclic loading.

The loss factor $\eta$ can be determined from the hysteresis loop in Figure S6. For a loading cycle, the energy $\Delta W_d$ dissipated per unit volume by the cyclically loaded aortic strip is [2]

\[
\Delta W_d = \int_{cycle} \sigma \, d \varepsilon ,
\]

where $\sigma$ is the dynamic stress and $\varepsilon$ is the dynamic strain. The integral in eq. (7.1) returns the area contained within the hysteresis loop. The hysteresis loop shown in Figure S6 can be divided into
two parts: the upper half corresponds to the loading and the lower half to the unloading. They connect at the two extreme strain values of the loop, \( \varepsilon_{\text{min}} \) and \( \varepsilon_{\text{max}} \). The elastic relationship, which describes the dynamic stress-strain curve of the viscoelastic aorta under dynamic strain, is taken as the average of the upper and lower halves of the loop. This is the backbone of the loop drawn in red in Figure S6. The storage energy \( W_s \) per unit volume for a quarter of cycle [2] results as

\[
W_s = \frac{A_1 + A_2}{2},
\]

(7.2)

where \( A_1 \) and \( A_2 \) are the areas of the two curvilinear triangles under the middle curve of the loop in Figure S6. Since the dynamic elastic relationship is nonlinear, the areas \( A_1 \) and \( A_2 \) are different and an average of the two is introduced in eq. (7.2). The loss factor \( \eta \) is given by [2, 8]

\[
\eta = \frac{\Delta W_d}{2\pi W_s} = \frac{\Delta W_d}{\pi (A_1 + A_2)},
\]

(7.3)

which is a ratio of two areas in Figure S6; therefore, it is dimensionless and does not depend on the scale of the axes.

**Figure S6.** Hysteresis loop (——) and quasi-static curve (——) of a strip under harmonic load in the stress-strain diagram. The energy loss in one cycle is proportional to the area inside the loop. The mean of the areas \( A_1 \) and \( A_2 \), determined from the middle curve of the loop (——) and the horizontal line passing through the middle of the loop, is proportional to the storage energy. The slope of the tangent to the middle line of the loop (——) at the loop center is \( \alpha_t \); \( \tan(\alpha_t) \) is proportional to the storage modulus; the slope of the tangent to the static curve (——) at the point corresponding to the loop center is \( \alpha_s \) and \( \tan(\alpha_s) \) is proportional to the corresponding static module.
Supplementary Note 7
Dynamic characterization: additional data

Among the two pre-stress levels, 50 and 90 kPa, the 50 kPa (first level) is a good choice to represent the physiological condition. An approximate formula for a thin cylindrical shell under pressure gives a stress \( S = p R / h \); for a mean blood pressure \( p = 93.3 \) mmHg (obtained with a pulsatile pressure between 80 and 120 mmHg as 80+40/3 mmHg), mean aortic radius \( R = 10 \) mm and wall thickness \( h = 2 \) mm, results in 62 kPa, which is not far from 50 kPa.

Table S4. Dynamic stiffness ratio and loss factor of active and passive circumferential and longitudinal strips. 1st level pre-stress, 50 kPa; frequencies 1 and 3 Hz. N.A., not available.

|     | Circ. | Long. | Circ. | Long. | Circ. | Long. | Circ. | Long. | Circ. | Long. | Circ. | Long. |
|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|     | 1 Hz  | 3 Hz  | 1 Hz  | 3 Hz  | 1 Hz  | 3 Hz  | 1 Hz  | 3 Hz  | 1 Hz  | 3 Hz  | 1 Hz  | 3 Hz  |
| II  | 1.52  | 1.52  | 1.90  | 1.89  | 0.0694| 0.0754| 0.0781| 0.086 |       |       |       |       |
|     | 1.22  | 1.23  | 2.06  | 2.10  | 0.0731| 0.0817| 0.0787| 0.0865|       |       |       |       |
| III | 1.49  | 1.51  | 1.81  | 1.86  | 0.0731| 0.0787| 0.0885| 0.083 |       |       |       |       |
|     | 1.55  | 1.54  | 2.22  | 2.23  | 0.0673| 0.0764| 0.0828| 0.0899|       |       |       |       |
| IV  | 1.44  | 1.45  | 1.92  | 1.90  | 0.0595| 0.0689| 0.0671| 0.0778|       |       |       |       |
|     | 1.58  | 1.58  | 1.83  | 1.79  | 0.0548| 0.064  | 0.0628| 0.0719|       |       |       |       |
| V   | 1.58  | 1.49  | 2.14  | 2.25  | 0.0563| 0.0651| 0.0936| 0.0987|       |       |       |       |
|     | N.A.  | N.A.  | N.A.  | N.A.  | N.A.  | N.A.  | N.A.  | N.A.  |       |       |       |       |
| VI  | 1.32  | 1.37  | 1.81  | 1.87  | 0.0501| 0.0534| 0.0803| 0.0881|       |       |       |       |
|     | 1.55  | 1.62  | 2.33  | 2.46  | 0.0725| 0.0816| 0.0856| 0.0894|       |       |       |       |
| VIII| 1.46  | 1.54  | 2.24  | 2.27  | 0.0602| 0.0660| 0.1000| 0.1022|       |       |       |       |
| IX  | 1.60  | 1.68  | 2.22  | 2.36  | 0.0649| 0.0723| 0.0854| 0.0916|       |       |       |       |
|     | 2.06  | 2.04  | 2.32  | 2.46  | 0.0745| 0.0801| 0.0952| 0.0976|       |       |       |       |
| X   | 1.55  | 1.55  | 1.61  | 1.67  | 0.0729| 0.0802| 0.0806| 0.0877|       |       |       |       |
|     | 1.88  | 1.95  | 2.64  | 2.75  | 0.0688| 0.0763| 0.0889| 0.0931|       |       |       |       |
| XI  | 1.64  | 1.69  | 2.39  | 2.46  | 0.0649| 0.0722| 0.0823| 0.0878|       |       |       |       |
|     | 1.56  | 1.59  | 2.07  | 2.16  | 0.0667| 0.0757| 0.0896| 0.0949|       |       |       |       |
| XII | 1.66  | 1.68  | 2.00  | 2.08  | 0.0726| 0.08  | 0.0921| 0.092  |       |       |       |       |
|     | 1.52  | 1.56  | 1.88  | 1.96  | 0.0658| 0.0717| 0.0811| 0.0850|       |       |       |       |
| XIII| 2.02  | 2.11  | 2.83  | 2.94  | 0.0845| 0.0906| 0.1000| 0.0988|       |       |       |       |
|     | 1.51  | 1.55  | 2.07  | 2.16  | 0.0609| 0.0683| 0.0816| 0.0859|       |       |       |       |
|     | 1.48  | 1.51  | 1.59  | 1.65  | 0.0733| 0.0773| 0.0797| 0.0852|       |       |       |       |
Table S5. Medians of dynamic stiffness ratio and loss factor of active and passive circumferential and longitudinal strips. 1st level pre-stress, 50 kPa; frequencies 1 and 3 Hz.

|            | Dynamic stiffness ratio | Loss factor |
|------------|-------------------------|-------------|
|            | All 25-48 y. 55-68 y.   | All 25-48 y. 55-68 y. |
| Passive    | Circ. 1.52 1.51 1.56    | 0.0686 0.0656 0.0724 |
|            | Long. 1.58 1.59 1.55   | 0.0730 0.0725 0.0748 |
| Active     | Circ. 2.07 1.99 2.11    | 0.0883 0.0832 0.0872 |
|            | Long. 2.16 2.09 2.23    | 0.0861 0.0861 0.0865 |

Figure S7. Effect of frequency on the experimentally measured hysteresis loops; 1 Hz (black), 2 Hz (blue) and 3 Hz (red) for the circumferential and longitudinal strips from donor IV. Cycles at different initial pre-stretches, corresponding to about 50 and 90 kPa for the midpoint of the loop, were measured. The amplitude of each loop was about 0.07 in engineering strain. Just for representation purposes, the passive loops are centered at 0 strain, while the active loops are centered at 0.1. a circumferential strip; b longitudinal strip.

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