# Supporting Information for

## Stretchable electrode enabled electrochemical mass spectrometry for *in situ* and complementary analysis of cellular mechanotransduction

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# 1. Experimental Sections

## 1.1 Materials and Instruments

Human umbilical vein endothelial cells (HUVEC cells) were supplied by Haixing Biosciences (Suzhou, Jiangsu, China). Endothelial cell culture medium, fetal bovine serum (FBS), penicillin and streptomycin for cell culture were obtained from ScienCell (USA). Endothelial cell growth supplement and 0.25% Trypsin-0.04% EDTA were purchased from SARSTEDT (Germany). Calcein-AM, Propidium iodide (PI) and Phosphate buffer salts (PBS) were obtained from Solaibao Biotechnology (Beijing, China). Poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS) (Clevios PH1000) was purchased from Kingtech Chemicals Co., Ltd., (Wuhan, China). Polydimethylsiloxane prepolymer, $N^G$-Nitro-L-arginine Methyl Ester Hydrochloride (L-NAME), and crosslinker was purchased from McLean Reagents. Trihydroxymethylomethane hydrochloride (Tris-HCl), dopamine hydrochloride were purchased from Sigma Aldrich (American). Potassium ferricyanide, sodium nitrite, sulphuric acid, 30% hydrogen peroxide, and potassium hydroxide were analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (China). Chromatographic grade methyl alcohol was purchased from Merck Chemical Technology Limited (Germany). All of the reagents were used as received unless stated otherwise.

Electrochemical measurements were conducted by a CHI 660e electrochemical workstation (CHI Instruments) with Ag/AgCl reference electrode and Pt counter electrode. HUVECs were cultured in a $CO_2$ constant-temperature incubator (Heracell 150i, Thermo Scientific, America). The bright field images and fluorescence images were obtained on a inverted fluorescence microscope (AxioObserver Z1, Zeiss company, Germany). Mass spectrometry experiments were conducted on Linear ion-trap mass spectrometer (LTQ XL, Thermo Fisher Scientific).

## 1.2 Fabrication of PPL/PDMS conducting film

The PPL/PDMS conducting film was prepared following the procedure outlined in a previous report.[1] Initially, a mixture of polydimethylsiloxane (PDMS) and

crosslinker (w/w =10:1) was spin-coated onto the Si substrate at 1000 rpm for 10 s. The coated substrate was then heat-cured at 80℃ for 3 h to form the PDMS membrane. Subsequently, the PDMS membrane was immersed in a dopamine hydrochloride solution (1 mg/mL, Tris-HCl buffer solution,10 mM, pH~8.5) for 24 h to enhance its hydrophilicity. To synthesize the PPL conductive film, LiTFSI (2 wt%) was added to a PEDOT:PSS aqueous solution (1 wt%), which was then vigorously stirred at 1000 rpm for 6 hours. Subsequently, the uniformly mixed solution was rotated at 1500 rpm and coated onto a PDMS film treated with polydopamine solution for 60 s. The PPL/PDMS conductive film was then formed by annealing at 130℃ for 15 minutes.

**1.3 Fabrication of stretchable electrode and ionization**

As illustrated in Fig. 1, a cubical PDMS frame (18 × 10 × 2 $mm^3$) with a triangular prism hollow (0.5 × 8 × 14 × 2 $mm^3$) and an rectangle piece of PPL/PDMS conducting film (20 × 10 $mm^2$) were initially prepared. Subsequently, the PDMS frame and PPL/PDMS conducting film were adhered together using PDMS adhesive. A Pt wire was then connected to the PPL/PDMS using conducting ink and sealed with PDMS.[2]

**1.4 Characterization of stretchable electrode and ionization**

**Characterization of the biocompatibility.** The PPL/PDMS electrode was sterilized using 75% alcohol and UV radiation for 12 h.[3] HUVECs were seeded onto the PPL/PDMS film and cultured in Endothelial cell culture medium with 10% fetal bovine serum, penicillin, and streptomycin (100 U) at 37℃ in a humidified incubator (95% air with 5% $CO_2$). After 36 h, the HUVECs were stained with Calcein-AM and PI.[4]

**Characterization of electrochemical performance**. NO solution was prepared in accordance with previous literature.[5] In brief, a 50 mL $NaNO_2$ solution (4 M) was deoxygenated by purging nitrogen for 30 minutes. Simultaneously, 25 mL deoxygenate $H_2SO_4$ solution (2 M) was added dropwise at a rate of 0.5 mL/min at room temperature. Subsequently, the generated NO underwent purification to

eliminate excess impurities using a NaOH solution (4 M). Finally, the purified NO was introduced into a 1×PBS solution and allowed to bubble and saturate for half an hour to create an NO stock solution (approximately 1.8 mM). This stock solution was stored at 5℃ under an oxygen-free atmosphere. The cyclic voltammetry of NO and $K_3[Fe(CN)_6]$ was conducted using the PPL/PDMS as working electrode, Pt wire as counter electrode, and Ag/AgCl as reference electrode. Calibration for NO was conducted by gradually adding a series of NO standard solution aliquots into the PBS solution stored in the PDMS chamber placed on the PPL/PDMS electrode (active area 0.2 × 0.2 $cm^2$). The potential on the electrode for amperometric oxidation of NO was held at +0.75 V vs. Ag/AgCl.

**Characterization of mass spectrometry performance**. 1 μL of L-Arginine at different concentrations (1 ppm to 100 ppm) was added to the stretchable PPL/PDMS electrode and allowed to dry. Subsequently, 10 μL of $CH_3OH/H_2O$ (v/v=9/1) was dripped onto the PPL/PDMS, followed by the application of +3 KV voltage to generate ions for MS detection.

**1.5 Analysis of endothelial mechanotransduction by chemical mass spectrometry**

HUVECs were seeded on the sterile PPL/PDMS electrode/ionization and cultured for 12 h. After that, the PPL/PDMS was put on a stretching tester and placed in the front of MS inlet with a distance of 1 cm between the ionization and MS inlet. Then, the ECM culture were replaced by PBS. First, the electrochemical monitoring was conducted for the mechanical stimulation. The PPL/PDMS was used as working electrode, Ag/AgCl was used as reference electrode, and Pt wire was used as counter electrode. A potential of +0.75 V was applied on the working electrode. After the baseline was stable, mechanical stimulation was carried out by stretching the PPL/PDMS.[6] After a obvious current response was observed which indicated that the endothelial mechanotransduction was triggered, removing the PBS solution and disconnecting the electrochemical station. And then, $CH_3OH/H_2O$ (v/v=9/1) was dropped on the HUVECs accompanied by applied +3 KV voltage on the PPL/PDMS to extract and ionize the metabolites for MS analysis.

**1.6 Statistical analysis**

The original data of Xcalibur was converted into Microsoft Excel spreadsheet format with *m/z* value as the independent variable and signal intensity as the dependent variable. The exported MS data were preprocessed in Matlab software and finally imported into SIMCA (version 14.0, Umetrics, Umeå, Sweden) for OPLS-DA analysis.

## 2. Supporting Figures and Table

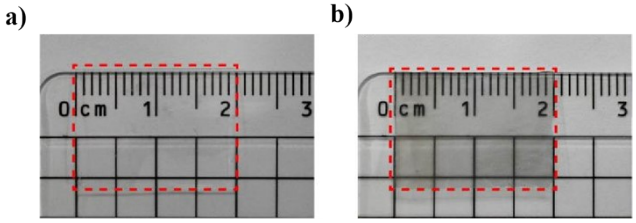


**Fig. S1** Photograph of (a) PDMS and (b) PPL/PDMS marked by red dashed box

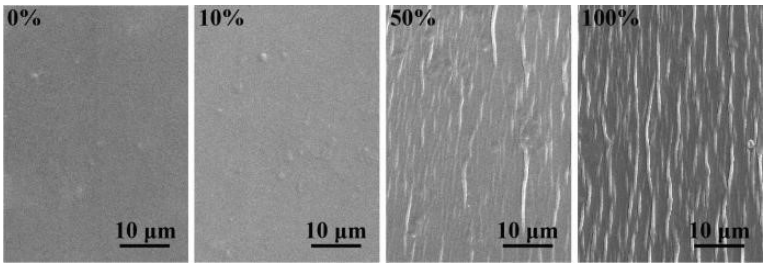


**Fig. S2** SEM images of stretchable electrodes before and after different strains

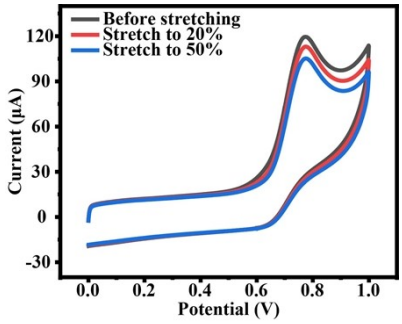


**Fig. S3** CV curves of 180 μM NO on PPL/PDMS before and after stretching

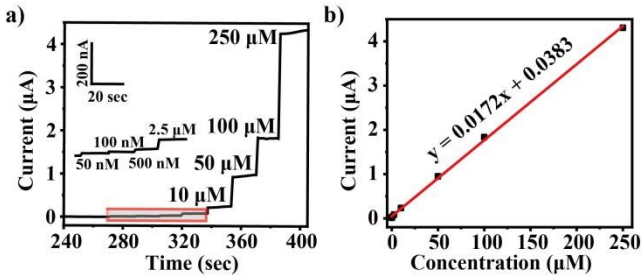


**Fig. S4** Characterization of the electrochemical performance of the stretchable electrode PPL/PDMS. (a) Amperometric response of the electrode (0.2×0.2 $cm^2$) to increasing concentration of NO at a potential of +0.75 V (vs. Ag/AgCl). (b) Corresponding calibration curve (n=3).

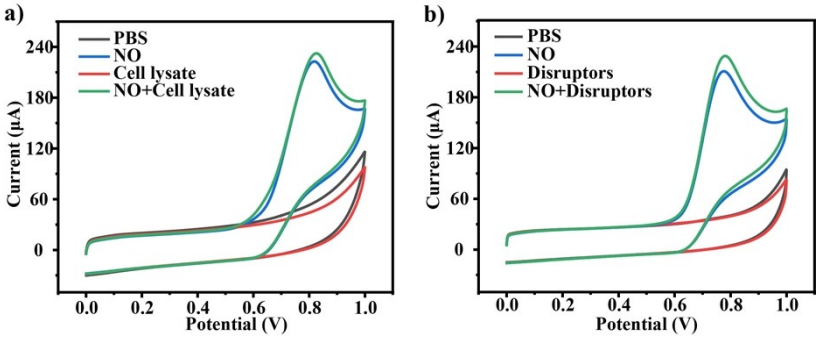


**Fig. S5** The resistance of PPL/PDMS electrode to matrix interference. (a) CV curves of PBS, 200 μM NO, cell lysate ($10^6$ cells/ml), and 200 μM NO + cell lysate ($10^6$ cells/ml). (b) CV curves of PBS, 200 μM NO, disruptors (200 μM L-arginine, L-citrulline, L-aspartic acid, and ATP), 200 μM NO + disruptors.

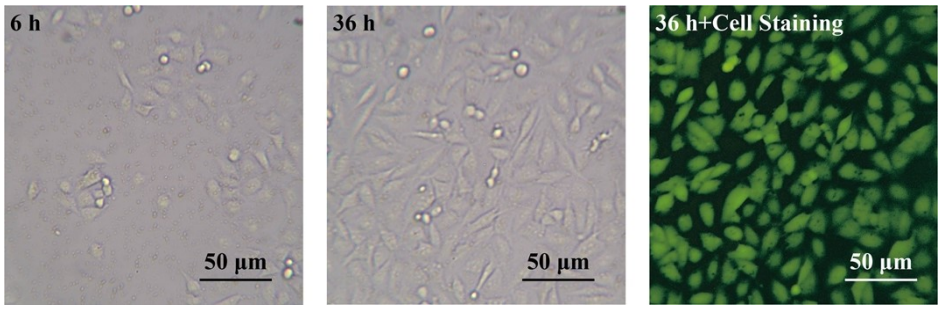


**Fig. S6** Images of HUVECs cultured on the PPL/PDMS electrode. (a) Bright field image of HUVECs after 6 h culture. (b) Bright field image and (c) fluorescence images of HUVECs after 36 h culture.

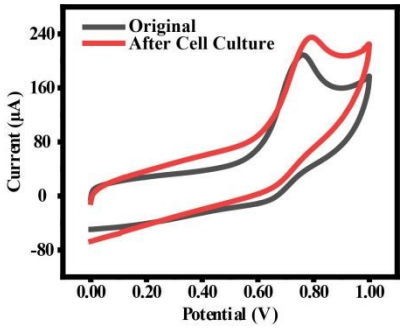


**Fig. S7** CV of 180 μM NO (PBS) before and after cell culture

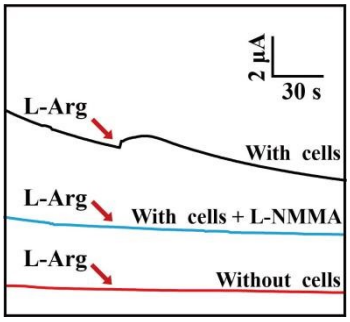


**Fig. S8** Current responses recorded from HUVECs on the PPL/PDMS electrode using L-arginine as stimulant

The remarkable capability of PPL/PDMS in detecting NO released from HUVECs was further demonstrated. L-Arginine (L-Arg), serving as the substrate for NO synthesis, can undergo catalysis by NO synthase (NOS) to generate NO. Consequently, L-Arg was employed to elicit the release of NO from HUVECs. An obvious amperometric signal was obtained after L-Arg stimulation was applied to the HUVECs. To confirm that the amperometric signal was generated by NO release, HUVECs were pre-incubated with a specific NOS inhibitor L-NAME prior to stimulation with L-Arg. Remarkably, almost no amperometric signal was observed under this condition. Furthermore, in the absence of HUVECs, even upon injection of L-Arg into the PPL/PDMS membrane, no signal was generated, thereby eliminating any potential interference caused by L-Arg. These results indicated that the PPL/PDMS electrode had the ability of real-time monitoring of NO release from HUVECs.

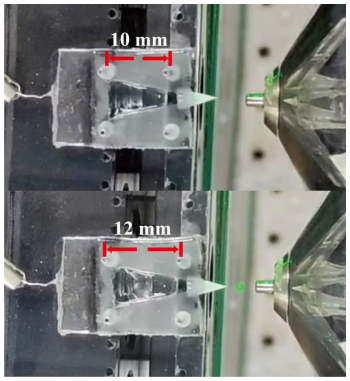


**Fig. S9** Photograph of the deformation of a stretchable ionization source

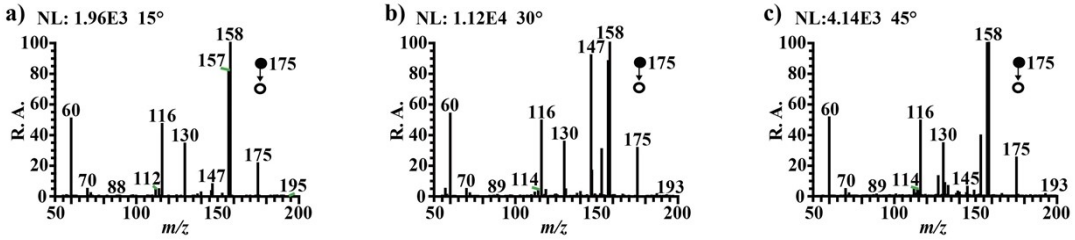


**Fig. 10** MS/MS of *m/z* 175 obtained by stretchable ionization sources with different angles. (a) 15°. (b) 30°. (c) 45°.

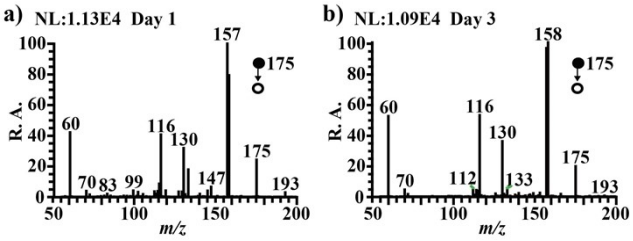


**Fig. 11** MS/MS of *m/z* 175 obtained by a same stretchable ionization source at different time. (a) Day 1. (b) Day 3.

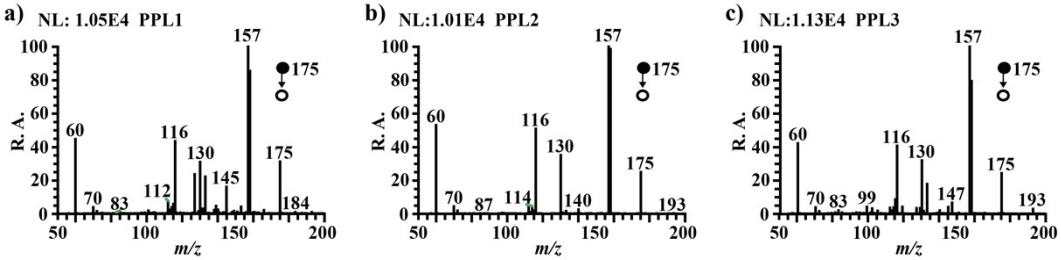


**Fig. 12** MS/MS of *m/z* 175 obtained by three stretchable ionization sources

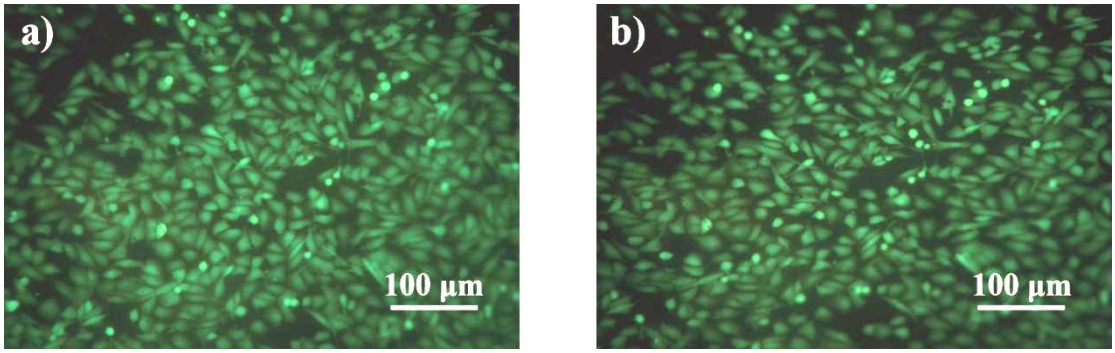


**Fig. S13** Fluorescence microscopic images of HUVECs cultured on the stretchable electrode before (a) and after (b) five cycles of 20% electrode stretching

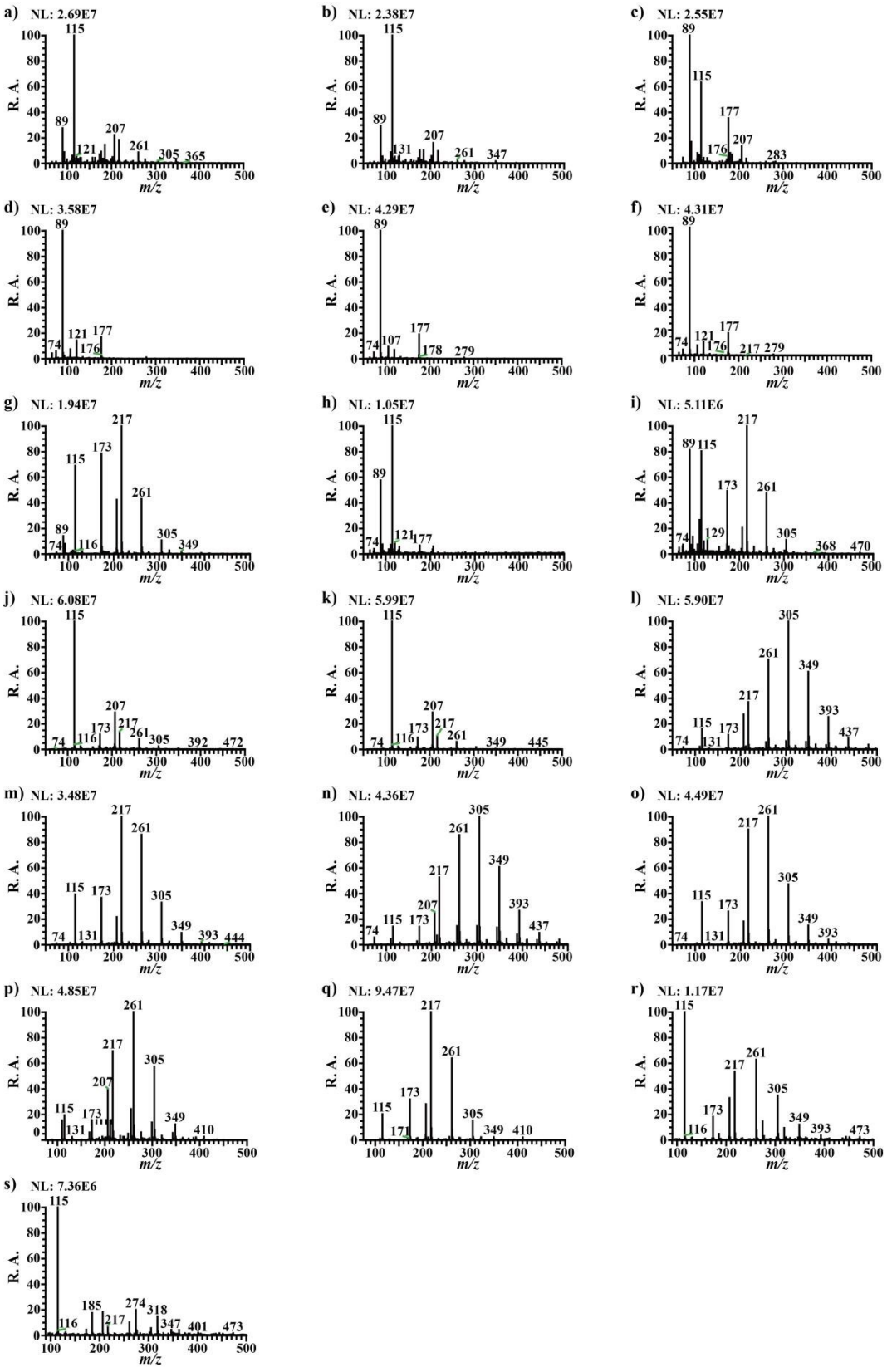


**Fig. S14** Mass spectrometry of HUVECs without mechanical stimulation

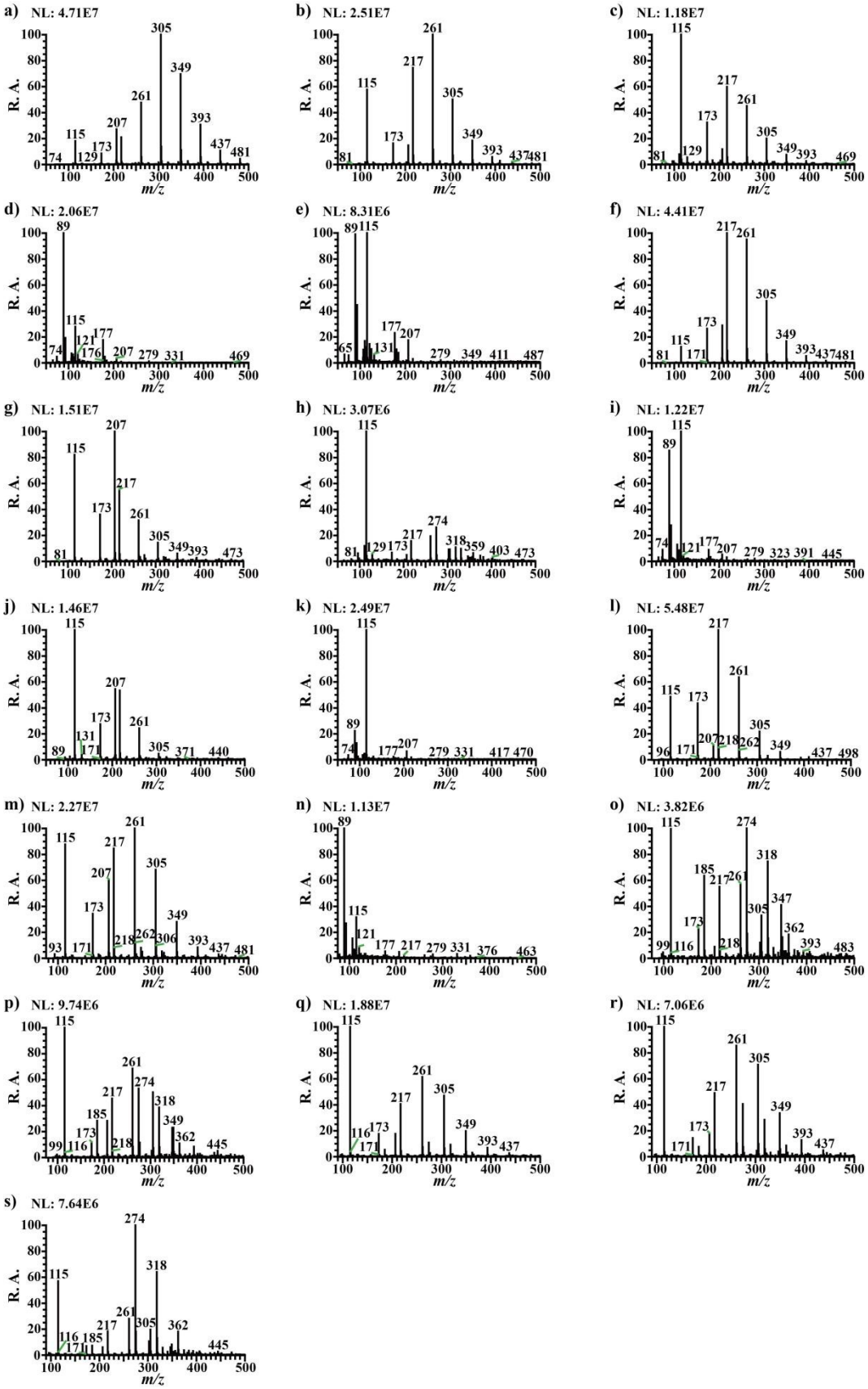


**Fig. S15** Mass spectrometry of HUVECs after transient mechanical stimulation

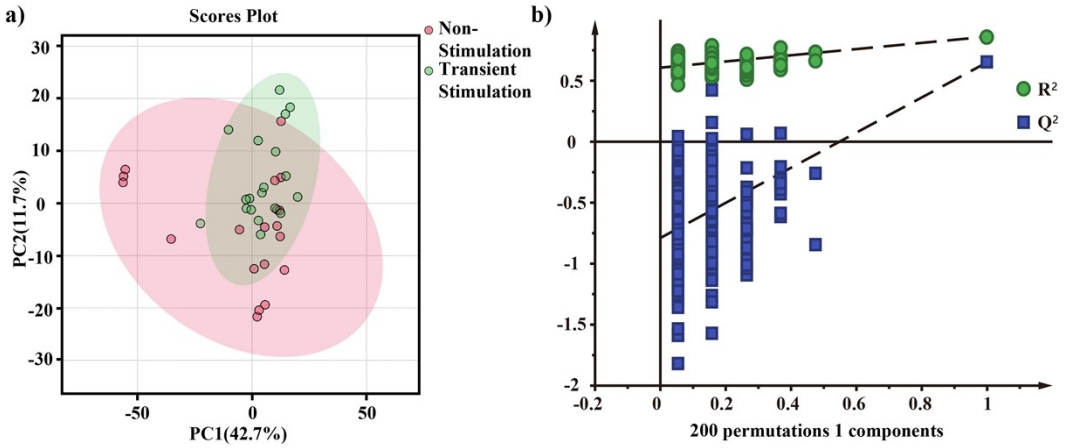


**Fig. S16** (a) PCA score scatter plot. (b) Plot of $R^2$ and $Q^2$ from 200 permutation tests in OPLS-DA model

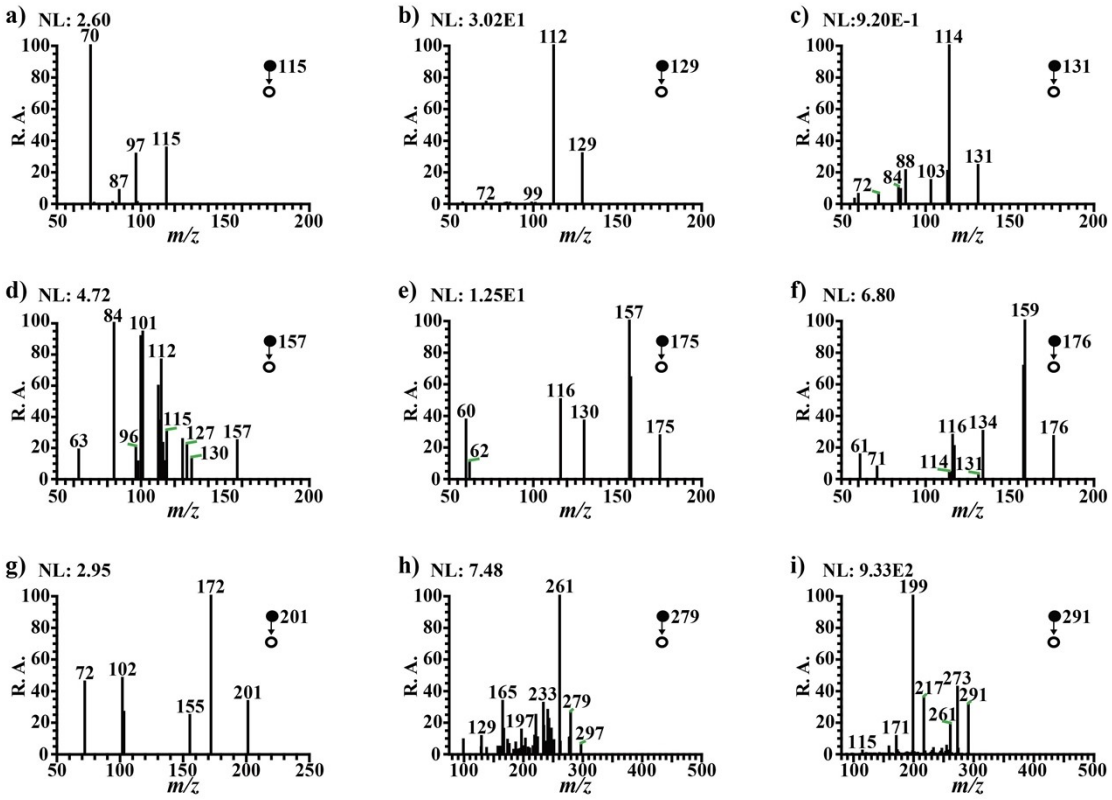


**Fig. S17** MS/MS of significantly altered metabolites in HUVECs after transient mechanical stimulation

**Table S1.** Identification of significantly altered metabolites in HUVECs

| No | Compounds | Chemical Formula | MW | Sample source | MS mode | VIP value | P value | NCE | Fragments |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Dihydrouracil | $C_4H_6N_2O_2$ | 114.1026 | Cell | Positive | 13.3572 | 0.01875 | 23 | 70<br>87<br>97 |
| 2 | (R)-Sulcatol | $C_8H_{16}O$ | 128.212 | Cell | Positive | 2.37077 | 0.01734 | 17 | 112 |
| 3 | N-Acetylputrescine | $C_6H_{14}N_2O$ | 130.1882 | Cell | Positive | 2.42321 | 4.8248E-4 | 20 | 114<br>72 |
| 4 | Decanal | $C_{10}H_{20}O$ | 156.2652 | Cell | Positive | 2.04808 | 0.01006 | 25 | 84<br>112<br>97 |
| 5 | L-Arginine | $C_6H_{14}N_4O_2$ | 174.201 | Cell | Positive | 1.45379 | 0.00095 | 30 | 157<br>116<br>60 |
| 6 | L-Citrulline | $C_6H_{13}N_3O_3$ | 175.1857 | Cell | Positive | 1.68205 | 0.02838 | 20 | 159<br>117 |
| 7 | Dodecanoic acid | $C_{12}H_{24}O_2$ | 200.3178 | Cell | Positive | 1.54936 | 0.00984 | 25 | 172<br>102<br>72 |
| 8 | γ-Linolenic acid | $C_{18}H_{30}O_2$ | 278.4296 | Cell | Positive | 1.6276 | 0.0047 | 30 | 261<br>165<br>253 |
| 9 | L-Argininosuccinate | $C_{10}H_{18}N_4O_6$ | 290.273 | Cell | Positive | 1.77447 | 0.01675 | 22 | 88<br>115<br>273 |

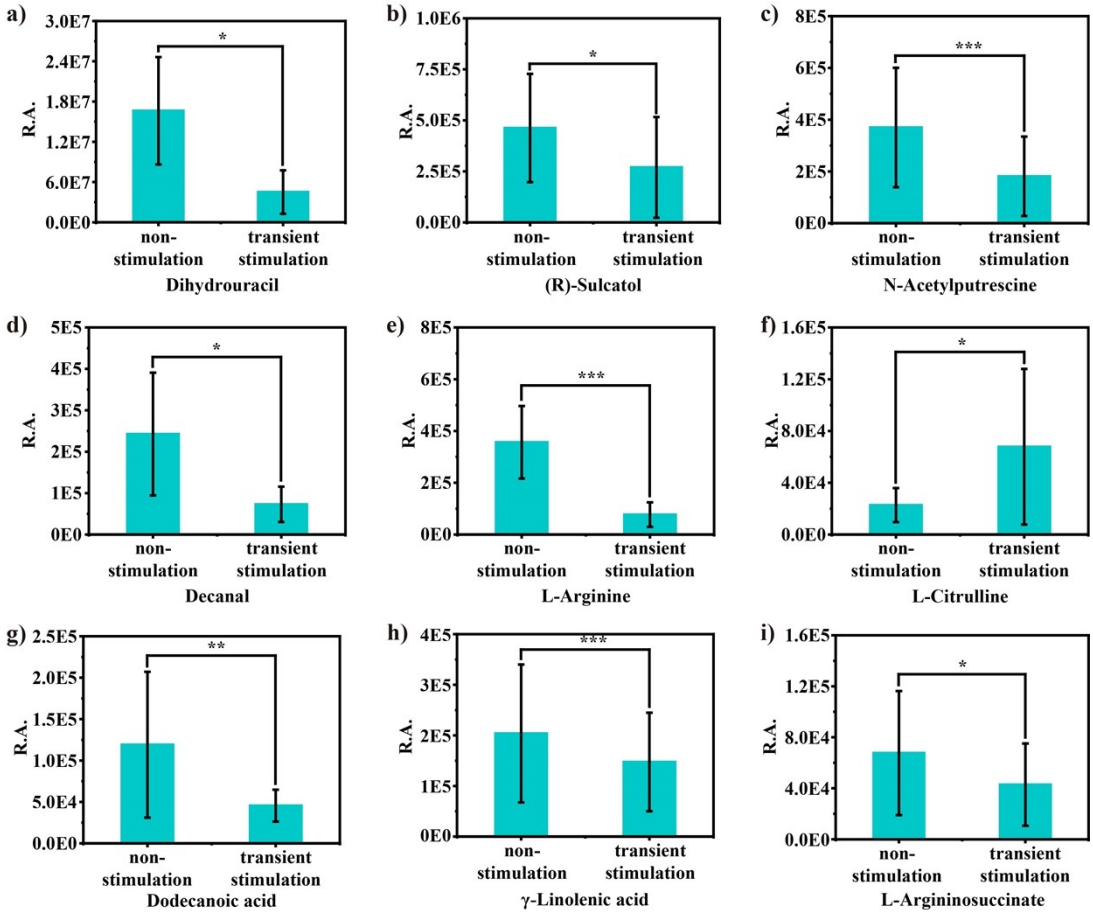


**Fig. S18** Variation tendency of the significantly altered metabolites in HUVECs after transient mechanical stimulation. (a) Dihydrouracil; (b) (R)-Sulcatol; (c) N-Acetylputrescine; (d) Decanal; (e) L-Arginine; (f) L-Citrulline; (g) Dodecanoic acid; (h) γ-Linolenic acid; (i) L-Argininosuccinate.

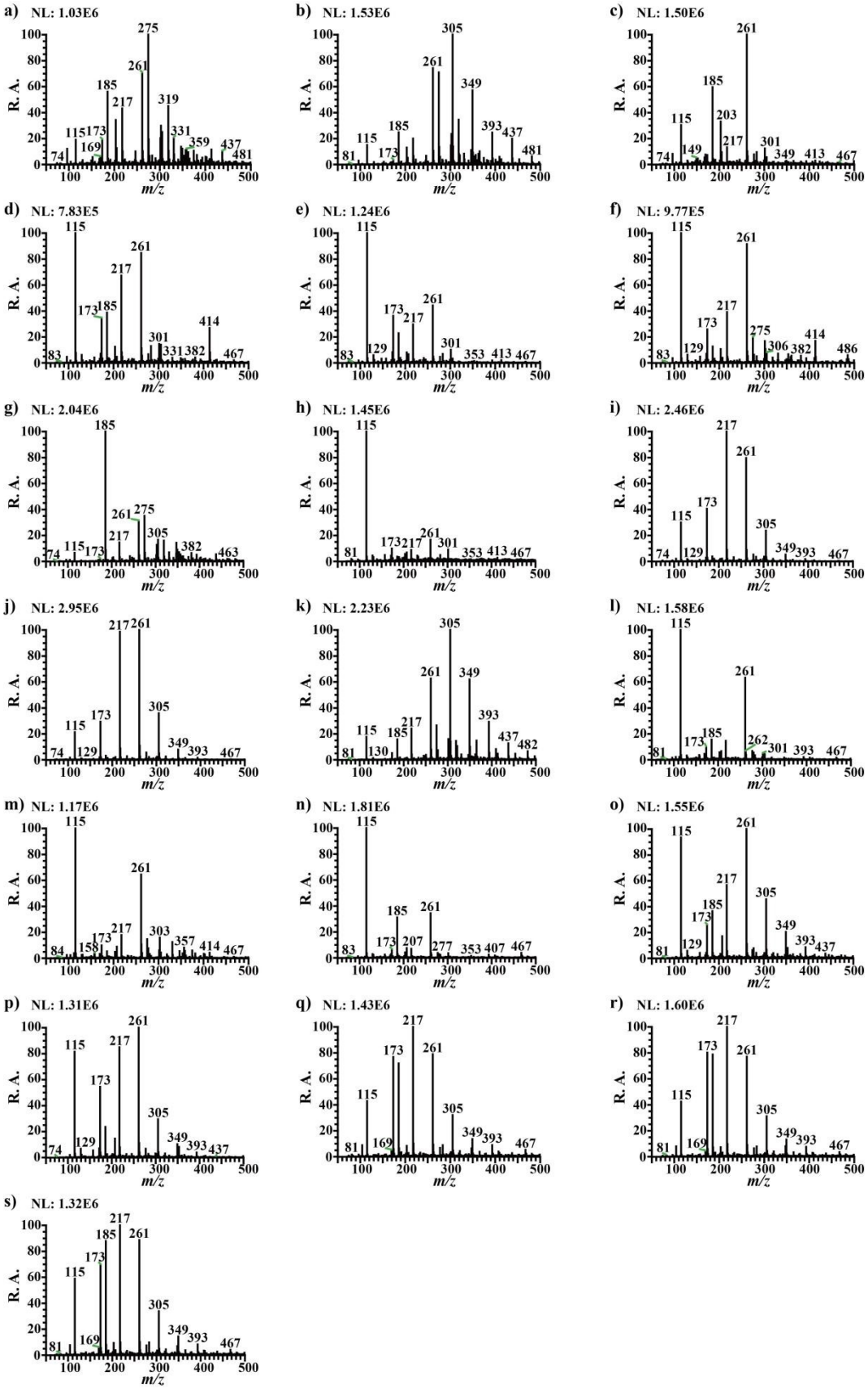


**Fig. S19** Mass spectrometry of HUVECs after prolonged mechanical stimulation

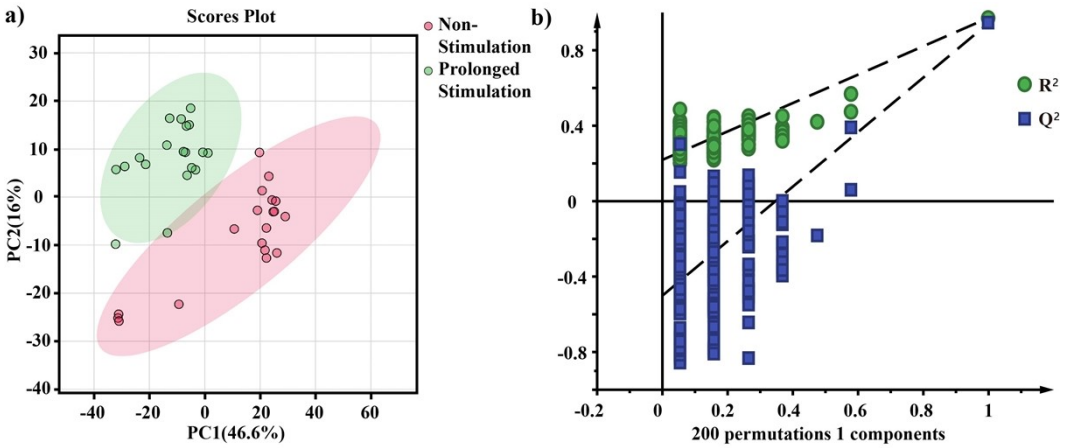


**Fig. S20** (a) PCA score scatter plot. (b) Plot of $R^2$ and $Q^2$ from 200 permutation tests in OPLS-DA model.

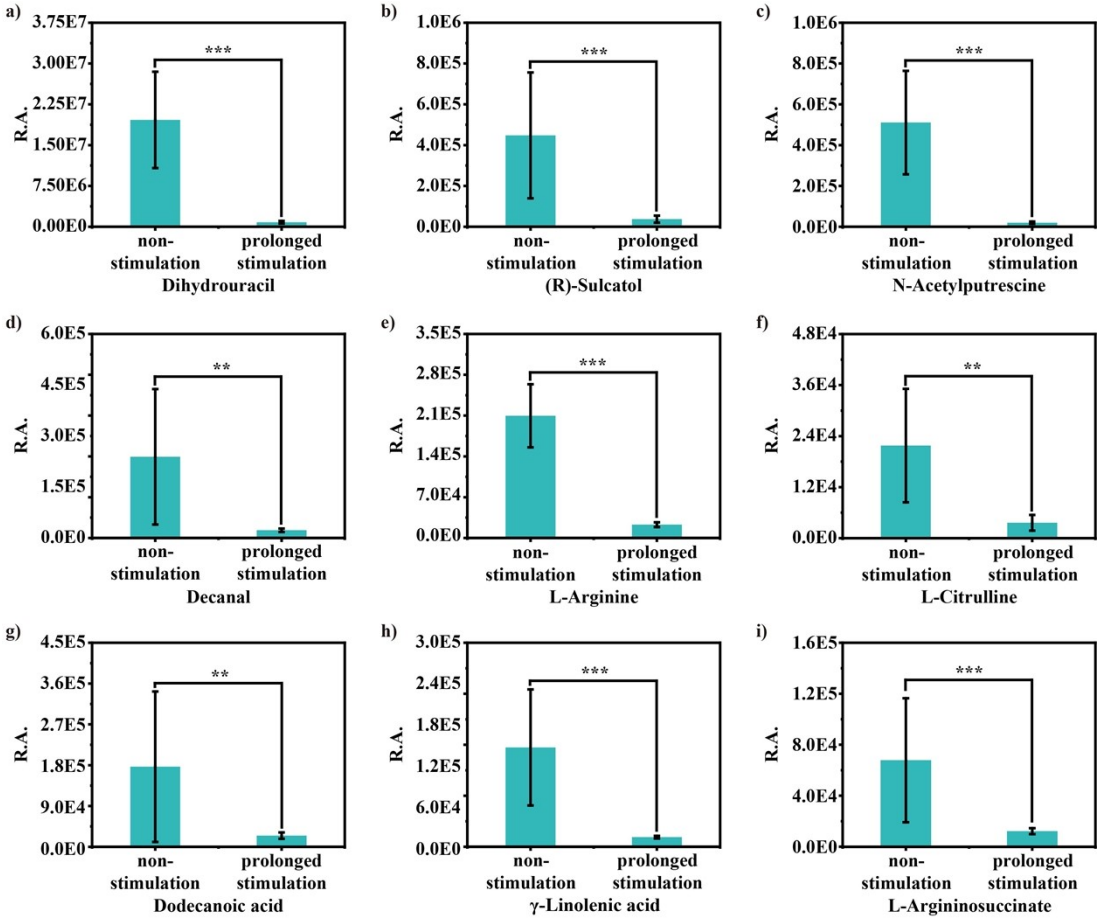


**Fig. S21** Variation tendency of the significantly altered metabolites in HUVECs after prolonged mechanical stimulation. (a) Dihydrouracil; (b) (R)-Sulcatol; (c) N-Acetylputrescine; (d) Decanal; (e) L-Arginine; (f) L-Citrulline; (g) Dodecanoic acid; (h) γ-Linolenic acid; (i) L-Argininosuccinate.

## 3. References


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