DATA SUPPLEMENT

Article

Cystathionine gamma lyase is regulated by flow and controls smooth muscle migration in human veins

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**Supplementary Table S1: Antibodies**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Target antigen** | **Vendor** | **Catalog #** | **Working**  **concentration** | **RRID** |
| **Goat anti-Rabbit IgG Secondary Antibody, Alexa Fluor 593** | Thermo Fisher Scientific | A21109 | 1/250 (ICC) | AB\_2535758 |
| **Goat anti-Rabbit IgG Secondary Antibody, Alexa Fluor 488** | Thermo Fisher Scientific |  |  |  |
| **Anti-Rabbit HRPO** | Thermo Fisher Scientific | 31460 | 1/20000 (WB) | AB\_228341 |
| **Anti-mouse HRPO** | Jackson ImmunoResearch Labs | 115-035-146 | 1/15000 (WB) | AB\_2307392 |
| **BrdU** | BD Biosciences | 555627 | 1/200 (ICC) | AB\_10015222 |
|  |  |  |  |  |
|  |  |  |  |  |
| **VWF** | DAKO (now Agilent) | A0082 | 1/20000 (IHC-P) |  |
| **eNOS** | BD Biosciences | 610297 | 1/1000 (WB) |  |
| **CSE** | Proteintech | 12217-1-AP | 1/1000 (WB)  1/250 (IHC-P) | AB\_2087497 |
| **CBS** | Santa Cruz | sc-133154 | 1/1000 (WB)  1/100 (IHC-P) | AB\_2244094 |
| **3-MST** | Sigma-Aldrich | HPA001240 | 1/500 (IHC-P) |  |
| **3-MST** | Novus Biologicals | NBP1-82617 | 1/2000 (WB) | AB\_11014969 |

**Supplementary Table S2: Reagents**

|  |  |  |  |
| --- | --- | --- | --- |
| **Kit/product** | **Vendor** | **Catalog #** | **link** |
| BrdU | Sigma-Aldrich | B5002 | https://www.sigmaaldrich.com/CH/en/product/sigma/b5002 |
| Immobilon Western Chemiluminescent HRP Substate | Millipore | WBKLS0050 | [https://www.merckmillipore.com/CH/de/product/Immobilon-Western-Chemiluminescent-HRP-Substrate,MM\_NF-WBKLS0050](https://www.merckmillipore.com/CH/de/product/Immobilon-Western-Chemiluminescent-HRP-Substrate,MM_NF-WBKLS0050?ReferrerURL=https%3A%2F%2Fwww.google.com%2F) |
| Immobilon-P transfer membrane | Millipore | IPVH00010 | <https://www.merckmillipore.com/CH/de/product/Immobilon-P-PVDF-Membrane,MM_NF-IPVH00010> |
| EnVision®  + Dual Link System-HRP (DAB+) | DAKO (now Agilent) | K4065 | <https://www.agilent.com/cs/library/packageinsert/public/PD04048EFG_01.pdf> |
| Antifade Mounting Medium with DAPI | Vectashield | H-1200 | <https://vectorlabs.com/products/mounting/vectashield-with-dapi> |
| Pierce reversible protein Stain Kit for PDGF membranes | Thermo Fisher Scientific | 24585 | <https://www.thermofisher.com/order/catalog/product/24585#/24585> |
|  |  |  |  |
| RPMI-1640 Glutamax I | Gibco | 61870-010 | <https://www.thermofisher.com/RPMI> |
| Gelatin type B | Sigma-Aldrich | G9391 | [G9391 Sigma](https://www.sigmaaldrich.com/catalog/product/sigma/g9391?lang=fr&region=CH&gclid=EAIaIQobChMI1aaDp4HZ6QIVhrHtCh1rbQ7ZEAAYASAAEgJWf_D_BwE) |
| Tripure | Roche | 11667157001 | [Roche\_tripure](https://www.sigmaaldrich.com/catalog/product/roche/tripurero?lang=fr&region=CH) |
| DAz-2 | Cayman Chemicals | 13382 | [www.caymanchem.com/product/13382](https://www.caymanchem.com/product/13382) |
| Cyanine 5.5 alkyne | Lumiprobe | C70B0 | https://www.lumiprobe.com/p/cy55-alkyne |
| 4-Chloro-7-Nitrobenzofurazan | Sigma-Aldrich | 163260 | <https://www.sigmaaldrich.com/catalog/product/aldrich/163260?lang=fr&region=CH> |
| SF7-AM fluorescent probe | Sigma-Aldrich | 748110 | [Sigmaaldrich\_748110](https://www.sigmaaldrich.com/catalog/product/aldrich/748110?lang=fr&region=CH) |
| Resorcine-Fuchsine Weigert | Waldeck | 2E-030 | <https://www.reactolab.ch/boutique/chroma/resorcin-fuchsine-weigert-solution-500-ml/> |
| Hematoxylin crist. | Merck | 1.04302.0025 | [Hematoxylin-cryst](https://www.merckmillipore.com/CH/de/product/Hematoxylin-cryst.-C.I.-75290,MDA_CHEM-104302?ReferrerURL=https%3A%2F%2Fwww.google.com%2F) |
| Acid Fuchsin | Sigma-Aldrich | F8129 | <https://www.sigmaaldrich.com/catalog/product/sigma/f8129?lang=fr&region=CH> |
| Picric acid | Sigma-Aldrich | 197378 | <https://www.sigmaaldrich.com/catalog/product/aldrich/197378?lang=fr&region=CH> |
| Neo-Clear | Merck | 1.09843.5000 | [Neo-Clear](https://www.merckmillipore.com/CH/de/product/Neo-Clear,MDA_CHEM-109843?ReferrerURL=https%3A%2F%2Fwww.google.com%2F) |
| Ethanol absolu | Merck | 1.00983.2500 | [Ethanol](https://www.merckmillipore.com/CH/de/product/Ethanol,MDA_CHEM-100983?ReferrerURL=https%3A%2F%2Fwww.google.com%2F) |
| Phosphate Buffered Saline (PBS) | Bichsel: | 100 0 324 | [https://www.bichsel.ch/](https://www.bichsel.ch/details-bichsel-news-146/items/brochure-bichsel.html) |
| L-cysteine | Sigma-Aldrich | C7352 |  |
| Sodium dodecyl sulfate (SDS) | Promega | H5114 | [https://ch.promega.com/products/biochemicalssodium-dodecyl-sulfate](https://ch.promega.com/products/biochemicals-and-labware/biochemical-buffers-and-reagents/sodium-dodecyl-sulfate_-molecular-biology-grade-_sds_/?catNum=H5113) |
| Tween-20 | Applichem | A1389 | [https://www.applichem.com/tween](https://www.applichem.com/en/shop/product-detail/as/tweenreg-20-fuer-die-molekularbiologie/) |
| Triton x-100 | Sigma-Aldrich | T8787 | <https://www.sigmaaldrich.com/catalog/product/sigma/t8787?lang=fr&region=CH> |
| Lead Acetate trihydrate | Acros Organics (Now Thermo Fisher Scientific) | 423841000 | https://www.acros.com/DesktopModules/Acros\_Search\_Results/Acros\_Search\_Results.aspx?search\_type=CatalogSearch&SearchString=lead%20acetate |
| Pyridoxal 5’-phosphate monohydrate | Sigma-Aldrich | 82870 |  |
| Bovine Serum albumin (BSA) | Applichem | A1391 | [https://www.applichem.comalbumin-fraktion-v](https://www.applichem.com/en/shop/product-detail/as/albumin-fraktion-v-ph-70/) |
| *DC*™ Protein Assay Kit I | Bio-Rad Laboratories | 5000111 | [Bio-rad dc-protein-assay](https://www.bio-rad.com/en-ch/product/dc-protein-assay?ID=22faf97a-6b8d-4763-8b97-3dc530dcab66) |
| Fast SYBR™ Green Master Mix | Applied Biosystems | 4385618 |  |
| PrimeScript RT Reagent Kit (Perfect Real Time) | Takara Bio | RR037B | https://www.takarabio.com/products/real-time-pcr/reverse-transcription-prior-to-qpcr/primescript-rt-reagent-kit |

**A close-up of a microscope slide

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**Figure S1. 3MST is mainly expressed in endothelial cells in large artery and vein segments.**

Representative VGEL staining or 3-MST immunostaining in freshly isolated human aorta (a) and saphenous vein (b). Images are representative of 4 to 5 different arteries/veins. IH: intimal hyperplasia

**A screenshot of a cell phone

Description automatically generated with low confidence****Figure S2. Static vein culture promotes CSE-derived H2S production.**

Lead acetate assay of freshly isolated human vein segments (D0) or after 7 days (D7) in static culture. 4 different veins/patients in triplicates. P value as determined by bilateral paired t-test.

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**Figure S3. Static vein culture promotes 3-MST expression in the media of saphenous veins**

**a**) 3-MST immunostaining in freshly isolated human saphenous vein (D0) or after 7 days (D7) in static culture. Representative of 5 different veins/patients. **b**) 3-MST immunostaining in freshly isolated human saphenous vein (D0) or after 7 days in culture under arterial conditions (D7-HP).

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**Figure S4. CSE modulation does not regulate P-ERK or P-S6RP levels**

**a**) VSMC were infected with an adenovirus encoding GFP (AdGFP) or CSE (AdCTH) at MOI 10 or 20, as indicated. **b**) VSMC were transfected with a control siRNA (siCtrl) or two distinct CTH siRNA (siCTH 1 and 2), as indicated. **a-b**) Reprsentative Western blot analyses and quantitative assessment of P-ERK over total ERK and P-S6RP over S6RP. Data shown as mean ± SEM of 6 to 7 independent experiments. No statistical differences as determined by repeated measures one-way ANOVA.