Title

A multicentric consortium study demonstrates that dimethylarginine dimethylaminohydrolase 2 is not a dimethylarginine dimethylaminohydrolase

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Supplementary Figures

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# 2: NP_001289936.1
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
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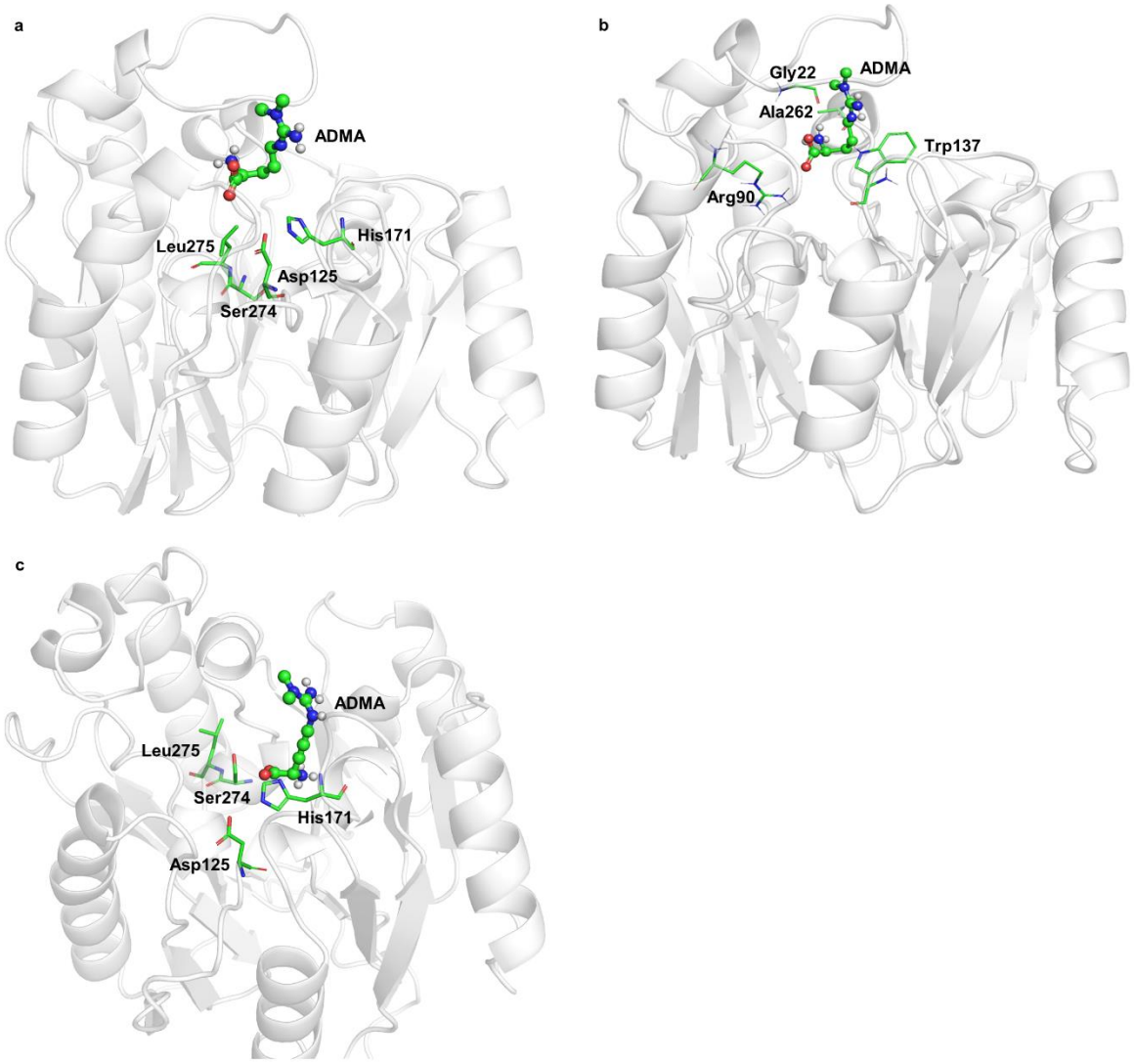
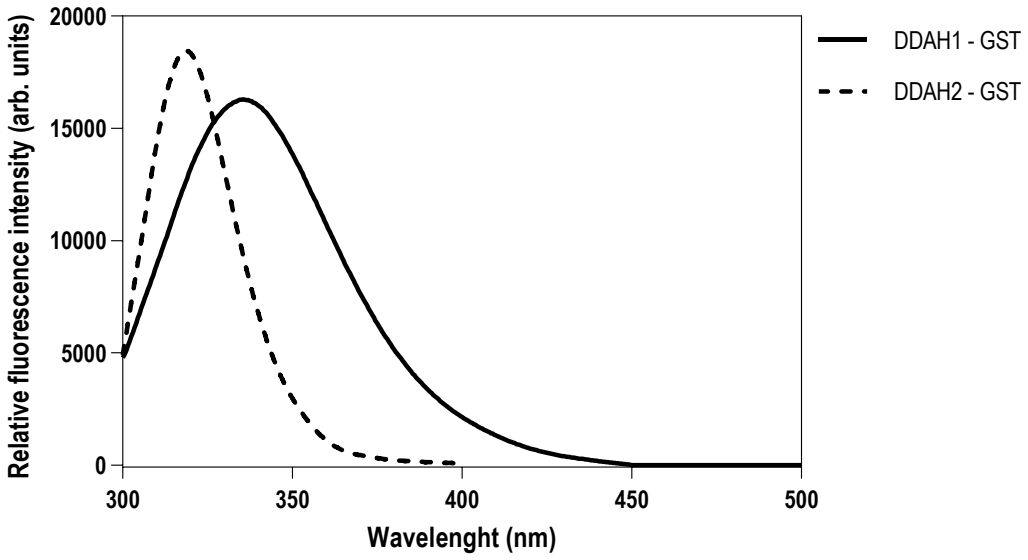
Supplementary Figure 1. Amino acid alignment of human DDAH1 and DDAH2 sequences. The amino acid sequences of DDAH1 and DDAH2 from human were aligned using the Needle Pairwise Sequence Alignment tool by EMBOSS Programs. A space identifies mismatch or gaps, a period identifies a small positive score, a colon identifies a similarity with a score of more than 1.0 while a perpendicular line identifies sequence identity. NP_036269.1 = Homo sapiens DDAH1; NP_001289936.1 = Homo sapiens DDAH2.

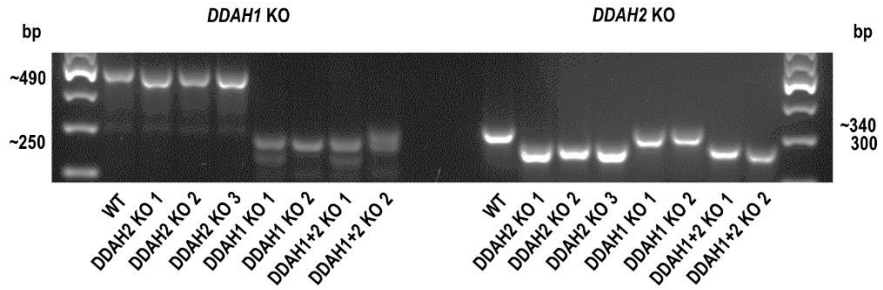


Supplementary Figure 2. Binding mode of ADMA in DDAH2 structures using molecular docking. Molecular docking of ADMA in DDAH2 Model A (SWISS-MODEL) using SYBYL (X-2.1), with **a** residues of the putative binding site and **b** interacting residues outside the

putative binding site. **c** Blind docking of ADMA in DDAH2 Model B (AlphaFold) using Flare (V6.1), with residues of the putative binding site highlighted. ADMA is shown in ball and stick, key residues are shown in sticks, and DDAH2 structures are shown as cartoon (white). C, O, N are shown in green, red, and blue, respectively. Source data are provided as a Source Data file.

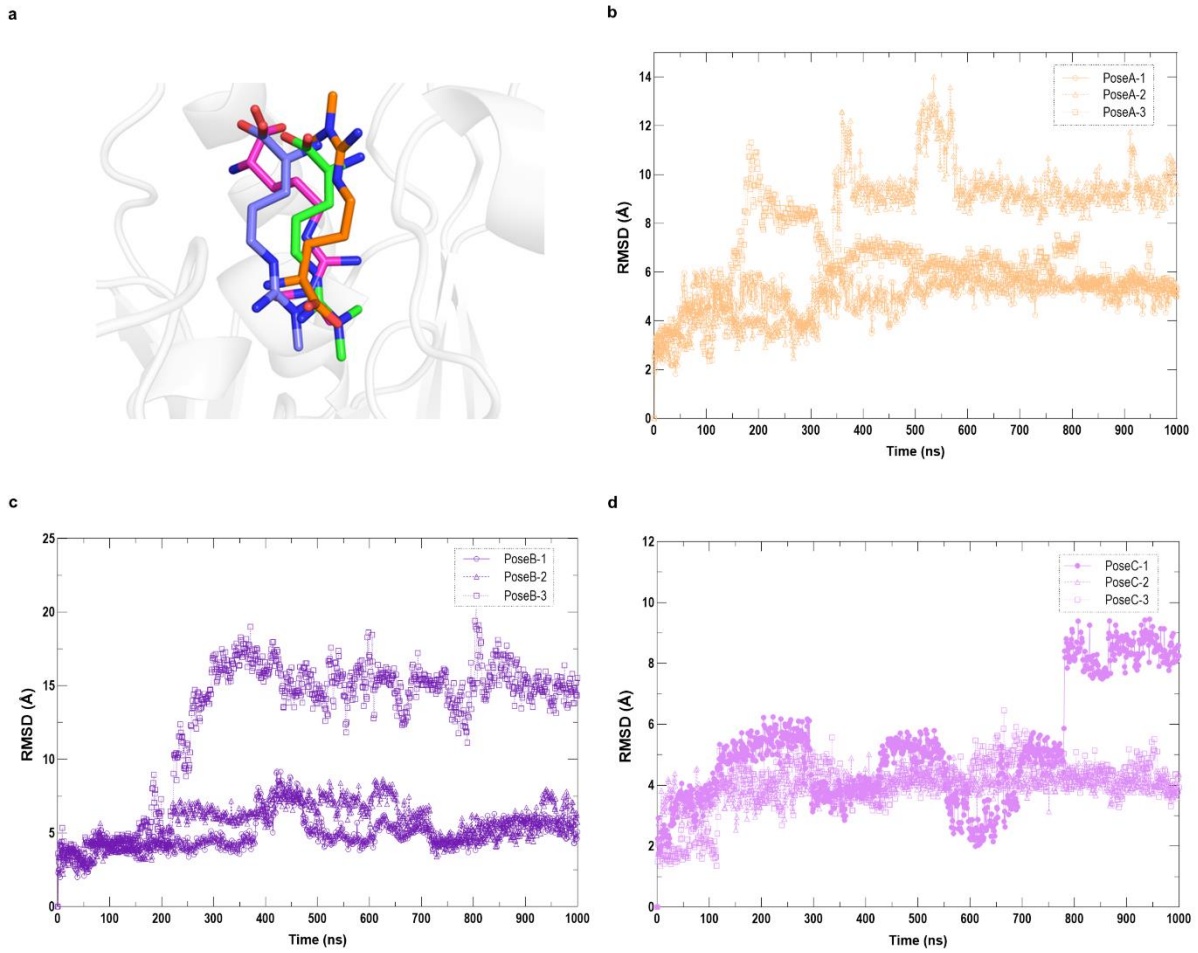


Supplementary Figure 3. Intrinsic fluorescence spectra of recombinant DDAH proteins. DDAH1-GST (straight line) and DDAH2-GST (dashed line) fluorescence spectra in 20 mM Tris-HCl, 150 mM NaCl, pH 8.5 at 1 mg/ml protein concentration were acquired. Proteins were excited at 280nm. arb. units = arbitrary units. Source data are provided as a Source Data file.



Supplementary Figure 4. Genotyping PCR results of CRISPR Cas9-mediated *DDAH* knockout MDA-MB-231 clones. Cell lines were genotyped using DDAH1 KO and DDAH2

KO primer pairs to confirm the CRISPR Cas9-mediated deletion at the respective target sites. bp = base pair. Source data are provided as a Source Data file.



Supplementary Figure 5. Molecular dynamics simulations of ADMA (different binding poses) in the DDAH2 (Model A) binding site. a Binding modes of ADMA, reference conformation ADMA (C-atoms in green, see Fig. 2) based on the citrulline conformation (2JAI), pose A (C-atoms orange), pose B (C-atoms purple), and pose C (C-atoms magenta). **b** Root mean square deviation (RMSD) of ADMA in pose A bound to the DDAH2 structure (mean RMSD ± SD of individual simulation in the triplicate run = 5.02 ± 0.78 Å, 7.72 ± 2.84 Å, 6.26 ± 1.44 Å). **c** RMSD of ADMA in pose B bound to the DDAH2 structure (mean RMSD ± SD of individual simulation in the triplicate run = 4.92 ± 1.10 Å, 5.73 ± 1.43 Å, 12.74 ± 4.62 Å). **d** RMSD of ADMA in pose C bound to the DDAH2 structure (mean RMSD ± SD of

individual simulation in the triplicate run = 5.21 ± 1.93 Å, 3.95 ± 0.40 Å, 4.07 ± 0.92 Å). Source data are provided as a Source Data file.

Supplementary Tables

Supplementary Table 1. Mean of root mean square deviation (RMSD) (Å) of ADMA bound to DDAH structures from independent MD (Molecular Dynamics) simulation runs.

Simulations	DDAH1-ADMA MD Mean RMSD ± SD (Å)	DDAH2 (Model A)-ADMA MD Mean RMSD ± SD (Å)	DDAH2 (Model B)-ADMA MD Mean RMSD ± SD (Å)
Sim. 1	1.70 ± 0.13	7.69 ± 2.21	3.44 ± 0.56
Sim. 2	1.48 ± 0.14	4.67 ± 1.14	3.79 ± 1.06
Sim. 3	1.42 ± 0.33	5.86 ± 1.13	3.71 ± 0.59
Sim. 4	1.54 ± 0.27	3.84 ± 1.34	3.86 ± 1.03
Sim. 5	1.52 ± 0.15	4.11 ± 0.70	4.24 ± 0.86
Mean Sim. 1-5	1.53 ± 0.24	5.23 ± 1.99	3.81 ± 0.88
*p-value	-	****p<0.0001 (0.000077)	**p<0.01 (0.002179)

*Statistical analysis was performed using one-way ANOVA, with multiple comparisons to DDAH1-ADMA MD simulation runs (n = 5). The mean of each simulation was calculated from snapshot frames 1-1000, with frame 0 (0-100 ps) excluded to account for the equilibration phase of the simulations. Data are presented as mean ± SD. Sim. = simulation.

Supplementary Table 2: Primer list for quantitative polymerase chain reaction (qPCR) analysis.

Genes – Models	Primers
Human *ACTB* – HEK293T cell lines	F 5'-CTTCGCGGGCGACGAT-3'
	R 5'-CCACATAGGAATCCTTCTGACC-3'
Human *ACTB* – HUVEC cell lines	F 5'-CCAACCGCGAGAAGATGA-3'
	R 5'-CCAGAGGCGTACAGGATAG-3'
Human *HPRT* – MDA-MB-231 cell lines	F 5'-TTGCGACCTTGACCATCTTTG-3'
	R 5'-CTTTGCTGACCTGCTGGATTAC-3'
Human *DDAH1* - HEK293T and MDA-MB-231 cell lines	F 5'-ATGCAACTTTAGATGGCGGAG-3'
	R 5'-CAGCCAAGATTTCAGCACCTC-3'
Human *DDAH2* - HEK293T and MDA-MB-231 cell lines	F 5'-CCACCTGAGGAGTCATTGCCGC-3'
	R 5'-CGTGATTAGGGCCGTGTCCCCTT-3'
Human *DDAH1* - HUVEC cell lines	F 5'-GTGCCCACTCCTGTTGTTTT-3'
	R 5'-GGGGTGTTGAATGAAGCAAT-3'
Human *DDAH2* - HUVEC cell lines	F 5'-GGACTCCCTTCTCCACCAA-3'
	R 5'-TTCTTGTTTCTTCACCTGTCTCC-3'
Mouse *Actb*	F 5'-ACTGTCGAGTCGCGTCCA-3'
	R 5-ATCCATGGCGAACTGGTGG-3'
Mouse *Ddah1* - *Ddah1*$^{-/-}$ and *Ddah2*$^{-/-}$ colonies	F 5'-CTACGCAGTCTCTACAGT-3'
	R 5'-TCATAACGATGGTCACTCA-3'
Mouse *Ddah2* - *Ddah1*$^{-/-}$ colony	F 5'-AAAGCAGTCAGGGCAATG-3'
	R 5'-CCAGGACGCAGAAAGAGA-3'
Mouse *Ddah2* - *Ddah2*$^{-/-}$ colony	F 5'- TCACTGCCGCTGGGACCACT-3
	R 5'-CTCGCTGCTTCCAGCCACCA-3