

# **N-helix and cysteines inter-regulate human mitochondrial VDAC-2 function and biochemistry**

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## **SUPPLEMENTAL FIGURES**

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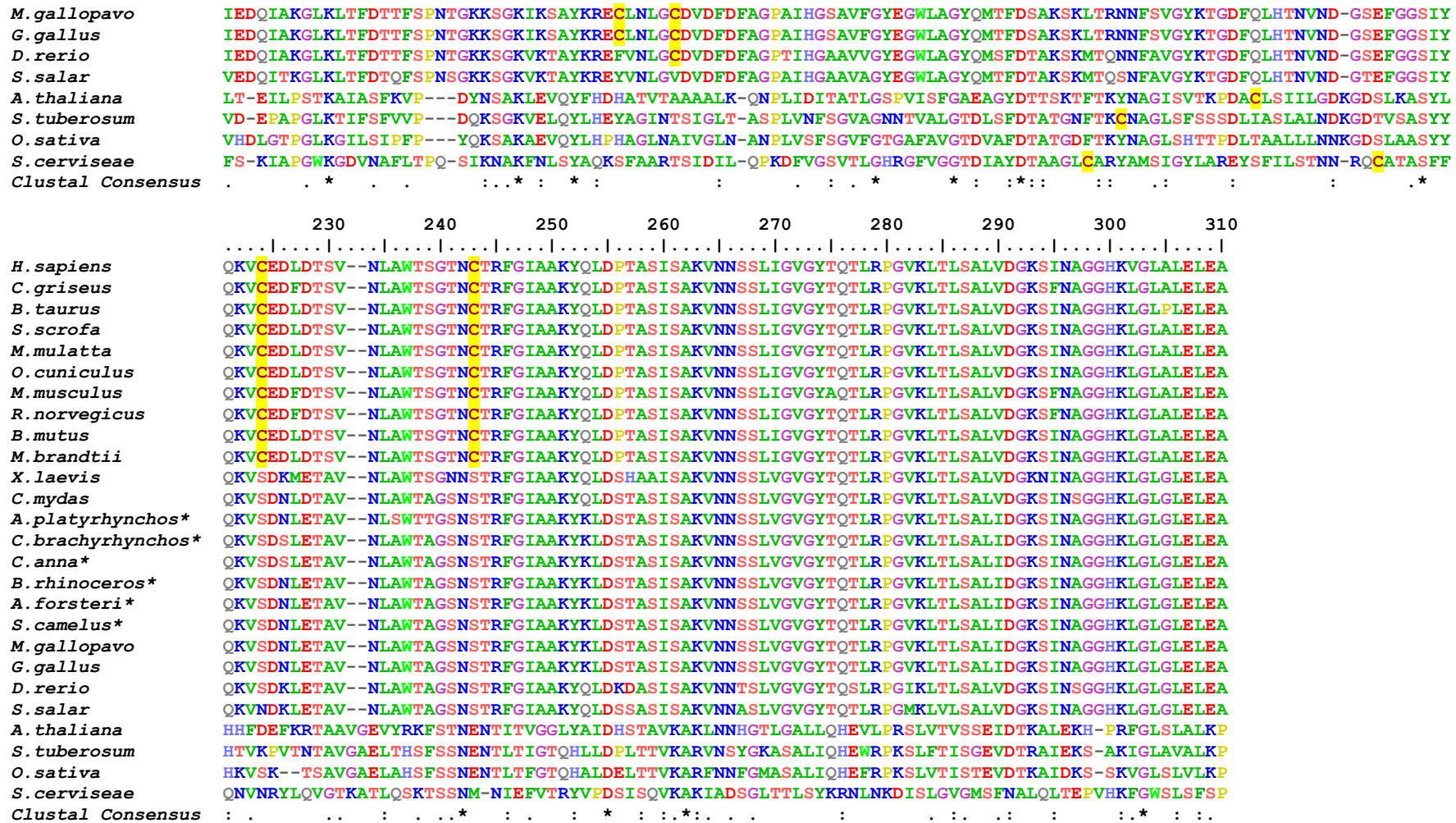
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H.sapiens       -----MATHGQTCARPMCIPPSYADLGKAARDIFNKGFGF-GLVKLDVTKSCSGVEFSTSGSSNTDTGKVVTGTTLETQYKWCYGLTTFTEKWNNDNLTGTEIA
C.griseus      -----MLTIECFFLLAMCIPPYADLGKAARDIFNKGFGF-GLVKLDVTKSCSGVEFSTSGSSNTDTGKVSGTLETQYKWCYGLTTFTEKWNNDNLTGTEIA
B.taurus       -----MATHGQNCARPMCIPPSYADLGKAARDIFNKGFGF-GLVKLDVTKSCSGVEFSTSGSSNTDTGKVVTGTTLETQYKWCYGLTTFTEKWNNDNLTGTEIA
S.scrofa       -----MATHGQTCPRPMCIPPSYADLGKAARDIFNKGFGF-GLVKLDVTKSCSGVEFSTSGSSNTDTGKVVTGTTLETQYKWCYGLTTFTEKWNNDNLTGTEIA
M.mulatta      -----MATYQTCARPMCIPPSYADLGKAARDIFNKGFGF-GLVKLDVTKSCSGVEFSTSGSSNTDTGKVVTGTTLETQYKWCYGLTTFTEKWNNDNLTGTEIA
O.cuniculus    -----MATHGQTCARPMCIPPSYADLGKAARDIFNKGFGF-GLVKLDVTKSCSGVEFSTSGSSNTDTGKVVTGTTLETQYKWCYGLTTFTEKWNNDNLTGTEIA
M.musculus     -----MAECCVPVCPRPMCIPPSYADLGKAARDIFNKGFGF-GLVKLDVTKSCSGVEFSTSGSSNTDTGKVSGTLETQYKWCYGLTTFTEKWNNDNLTGTEIA
R.norvegicus   -----MAECCVPVCQRPICIPPSYADLGKAARDIFNKGFGF-GLVKLDVTKSCSGVEFSTSGSSNTDTGKVSGTLETQYKWCYGLTTFTEKWNNDNLTGTEIA
B.mutis        MSWQGELRLLALKPHSIRGLESMPICPPSYADLGKAARDIFNKGFGF-GLVKLDVTKSCSGVEFSTSGSSNTDTGKVVTGTTLETQYKWCYGLTTFTEKWNNDNLTGTEIA
M.brandtii     -----MCIPPSYADLGKAARDIFNKGFGF-GLVKLDVTKSCSGVEFSTSGSSNTDTGKVVTGTTLETQYKWCYGLTTFTEKWNNDNLTGTEIA
X.laevis       -----MAVPPSYADLGKSARDIFNKGFGF-GLVKLDVTKSATGVEFTTSGSNTDTSKVNGLSLETQYKWCYGLTTFTEKWNNDNLTGTEIA
C.mydas        -----MAIPPSYADLGKSARDIFNKGFGF-GLVKLDVTKSASGVEFTTSGSNTDTSKVNGLSLETQYKWCYGLTTFTEKWNNDNLTGTEIA
A.platyrrhynchos* -----PMAIPPSYADLGKSARDIFNKGFGF-GLVKLDVTKSASGVEFTTSGSNTDTSKVNGLSLETQYKWCYGLTTFTEKWNNDNLTGTEIA
C.brachyrrhynchos* -----PMAIPPSYVDLGKPARDFNKGFGF-GLVKLDVTKSASGVEFTTSGSNTDTSKVNGLSLETQYKWCYGLTTFTEKWNNDNLTGTEIA
C.anna*         -----PMAIPPSYVDLGKSARDIFNKGFGF-GLVKLDVTKSATGVEFTTSGSNTDTSKVNGLSLETQYKWCYGLTTFTEKWNNDNLTGTEIA
B.rhinoceros* -----PMAIPPSYVDLGKSARDIFNKGFGF-GLVKLDVTKSASGVEFTTSGSNTDTSKVNGLSLETQYKWCYGLTTFTEKWNNDNLTGTEIA
A.forsteri*     -----PMAIPPSYVDLGKSARDIFNKGFGF-GLVKLDVTKSASGVEFTTSGSNTDTSKVNGLSLETQYKWCYGLTTFTEKWNNDNLTGTEIA
S.camelus*      -----PMAIPPSYADLGKSARDIFNKGFGF-GLVKLDVTKSASGVEFTTSGSNTDTSKVNGLSLETQYKWCYGLTTFTEKWNNDNLTGTEIA
M.gallopavo    -----AIPPSYADLGKSARDIFNKGFGF-GLVKLDVTKSASGVEFTTSGSNTDTSKVNGLSLETQYKWCYGLTTFTEKWNNDNLTGTEIA
G.gallus       -----MAIPPSYADLGKSARDIFNKGFGF-GLVKLDVTKSASGVEFTTSGSNTDTSKVNGLSLETQYKWCYGLTTFTEKWNNDNLTGTEIA
D.rerio        -----MAVPPAYADLGKSARDIFNKGFGF-GMVKLDVTKSASGVEFTTSGSNTDTSKVNGLSLETQYKWCYGLTTFTEKWNNDNLTGTEIN
S.salar        -----MAVPPSYGDLGKSARDIFNKGFGF-GLVKLDVTKSASGVEFTTSGSNTDTSKVNGLSLETQYKWCYGLTTFTEKWNNDNLTGTEIT
A.thaliana     -----MSKGGPLFTDIGKKAKDLTRDYS-D-QKFSISTYSASGVALTSTALKGGVH--AADVATQYKY--KNALFDVKIDTDSVLTETIT
S.tuberosum    -----MVGKGPLYSDIGKKARDLLYRDYVS-D-HKFTVTTYSTTGVAITASGLKKGELF--LADVSTQLKN--KNITVDKVDTNSNVYTTIT
O.sativa       -----MAAAAAPPAGPGLYSDIGKKARDLLYRDYHT-D-QKFTLLTYAANGAAITVAGTKKNESI--FSEIQSQVKN--NNVSDVVKATSDSKLITTFIT
S.cerviseae    -----MALRFFNDISRDVNLGNRDFHTNPLSLNISTTENGVNFTLKAQGVTEGPIQTSVEGRFYDRKEGVSLSQSWSNQNRNLNRIE
Clustal Consensus
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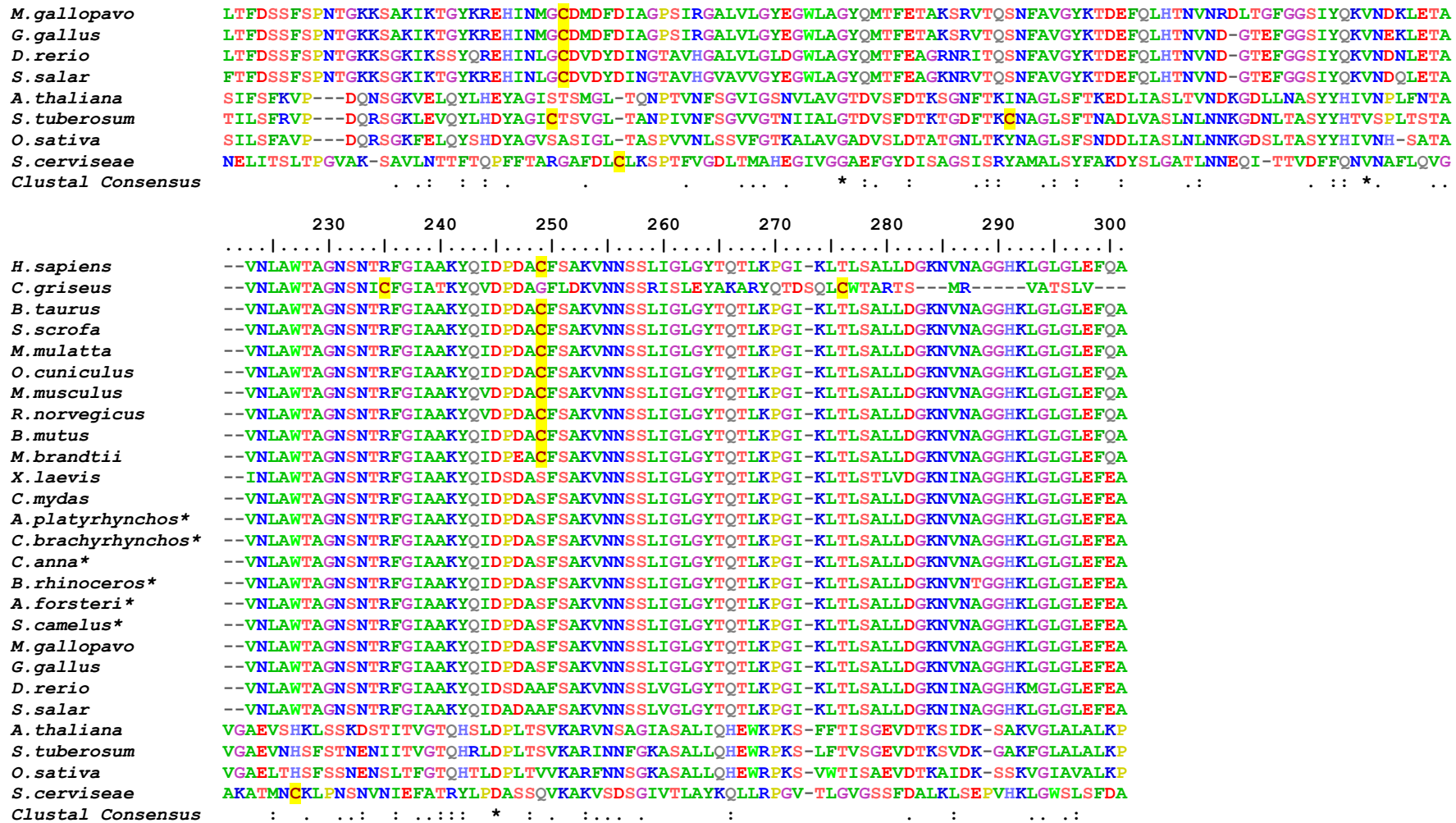
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...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|. ...|.
H.sapiens       IEDQIQGLKLTFDITTFSPNTEGKSGKIKSSYKRECNLGCDDVDFDFAGPAIHGSAVFGEYEGWLQYMTFDSAKSKLTRNNFAVGYRTGDFQLHTNVND-GTEFGGSYI
C.griseus       IEDQIQGLKLTFDITTFSENETGKSGKIKSAYKRECNLGCDDVDFDFAGPAIHGSAVFGEYEGWLQYMTFDSAKSKLTRNSFAVGYRTGDFQLHTNVNN-GTEFGGSYI
B.taurus       IEDQIQGLKLTFDITTFSPNTEGKSGKIKSSYKRECNLGCDDVDFDFAGPAIHGSAVFGEYEGWLQYMTFDSAKSKLTRNNFAVGYRTGDFQLHTNVND-GTEFGGSYI
S.scrofa       IEDQIQGLKLTFDITTFSENETGKSGKIKSSYKRECNLGCDDVDFDFAGPAIHGSAVFGEYEGWLQYMTFDSAKSKLTRNNFAVGYRTGDFQLHTNVND-GTEFGGSYI
M.mulatta      IEDQIQGLKLTFDITTFSENETGKSGKIKSSYKRECNLGCDDVDFDFAGPAIHGSAVFGEYEGWLQYMTFDSAKSKLTRNNFAVGYRTGDFQLHTNVND-GTEFGGSYI
O.cuniculus    IEDQIQGLKLTFDITTFSENETGKSGKIKSAYKRECNLGCDDVDFDFAGPAIHGSAVFGEYEGWLQYMTFDSAKSKLTRNSFAVGYRTGDFQLHTNVNN-GTEFGGSYI
M.musculus     IEDQIQGLKLTFDITTFSENETGKSGKIKSAYKRECNLGCDDVDFDFAGPAIHGSAVFGEYEGWLQYMTFDSAKSKLTRNSFAVGYRTGDFQLHTNVNN-GTEFGGSYI
R.norvegicus   IEDQIQGLKLTFDITTFSENETGKSGKIKSAYKRECNLGCDDVDFDFAGPAIHGSAVFGEYEGWLQYMTFDSAKSKLTRNSFAVGYRTGDFQLHTNVNN-GTEFGGSYI
B.mutis        IEDQIQGLKLTFDITTFSENETGKSGKIKSSYKRECNLGCDDVDFDFAGPAIHGSAVFGEYEGWLQYMTFDSAKSKLTRNNFAVGYRTGDFQLHTNVND-GTEFGGSYI
M.brandtii     IEDQIQGLKLTFDITTFSENETGKSGKIKSSYKREWNLGCDVDFDFAGPAIHGSAVFGEYEGWLQYMTFDSAKSKLTRNNFAVGYRTGDFQLHTNVND-GTEFGGSYI
X.laevis       IEDQIAKGLKLTFDITTFSPNTEGKSGKVKAAAYKQYEVNLGCDVDFDFAGPAIHGSAVVGEYEGWLQYMTFDSAKSKLTKNNFAVGYKTGDFQLHTNVND-GSEFAGSYI
C.mydas        IEDQIAKGLKLTFDITTFSENETGKSGKIKSSYKRECNLGCDDVDFDFAGPAIHGSAVVGEYEGWLQYMTFDSAKSKLTKNNFVSVGYKTGDFQLHTNVND-GSEFGGSYI
A.platyrrhynchos* IEDQIAKGLKLTFDITTFSENETGKSGKIKSAYKRECNLGCDDVDFDFAGPAIHGSAVFGEYEGWLQYMTFDSAKSKLTKNNFVSVGYKTGDFQLHTNVND-GSEFGGSYI
C.brachyrrhynchos* IEDQIAKGLKLTFDITTFSENETGKSGKIKSAYKRECNLGCDDVDFDFAGPAIHGSAVFGEYEGWLQYMTFDSAKSKLTKNNFVSVGYKTGDFQLHTNVND-GSEFGGSYI
C.anna*         IEDQIAKGLKLTFDITTFSENETGKSGKIKSAYKRECNLGCDDVDFDFAGPAIHGSAVFGEYEGWLQYMTFDSAKSKLTKNNFVSVGYKTGDFQLHTNVND-GSEFGGSYI
B.rhinoceros*   IEDQIAKGLKLTFDITTFSENETGKSGKIKSAYKRECNLGCDDVDFDFAGPAIHGSAVFGEYEGWLQYMTFDSAKSKLTKNNFVSVGYKTGDFQLHTNVND-GSEFGGSYI
A.forsteri*     IEDQIAKGLKLTFDITTFSENETGKSGKIKSAYKRECNLGCDDVDFDFAGPAIHGSAVFGEYEGWLQYMTFDSAKSKLTKNNFVSVGYKTGDFQLHTNVND-GSEFGGSYI
S.camelus*      IEDQIAKGLKLTFDITTFSENETGKSGKIKSAYKRECNLGCDDVDFDFAGPAIHGSAVFGEYEGWLQYMTFDSAKSKLTKNNFVSVGYKTGDFQLHTNVND-GSEFGGSYI

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**SUPPLEMENTAL FIGURE 1.** Evolutionary conservation of NTE and cysteines in various VDAC-2 proteins. Multiple sequence alignment (MSA) of VDAC-2 sequence from various organisms, generated using Clustal Omega (1,2). Cysteines are highlighted in yellow. Note how those VDAC-2 sequences with an abundance of cysteines also possess additional residues at the N-terminal region (this corresponds to the N-terminal extension (NTE), which we describe in this study). The length of this NTE region varies across various species. Also notable is the existence of sequence variation in the NTE, although all cysteines are highly conserved. NTE could therefore have been acquired by the Cys-containing VDAC-2 barrel, in the course of evolution. Organisms for which only partial sequence was available have been marked with \*.





**SUPPLEMENTAL FIGURE 2.** Evolutionary conservation of cysteines in various VDAC-1 proteins. MSA of VDAC-1 sequence from various organisms generated using Clustal Omega (1,2). Cysteines are highlighted in yellow. The first conserved cysteine (C127 in hVDAC-1) is retained in most species, except in a few plants and yeast. The second conserved cysteine (C232 in hVDAC-1) is restricted to higher mammals, with the notable exception of *C. griseus* (Chinese hamster), which also shows an overall lower sequence similarity to other mammalian VDAC-1 sequences. Organisms for which only partial sequence was available have been marked with \*.

10 20 30 40 50 60 70 80 90 100 110

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*H. sapiens* -----MCNTPTTYCDLGAAKDVFNKGYGFGMVKIDLRTKSCSGVEFSTSGHAYTDTGKASGNLETKYKVCNYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*C. griseus* -----MCNTPTTYCDLGAAKDVFNKGYGFGMVKIDLRTKSCSGVEFSTSGHAYTDTGKASGNLETKYKVCNYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*B. taurus* -----MCNTPTTYCDLGAAKDVFNKGYGFGMVKIDLRTKSCSGVEFSTSGHAYTDTGKASGNLETKYKVCNYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*S. scrofa* -----MCNTPTTYCDLGAAKDVFNKGYGFGMVKIDLRTKSCSGVEFSTSGHAYTDTGKASGNLETKYKVCNYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*M. mulatta* -----MCNTPTTYCDLGAAKDVFNKGYGFGMVKIDLRTKSCSGVEFSTSGHAYTDTGKASGNLETKYKVCNYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*O. cuniculus* -----MCNTPTTYCDLGAAKDVFNKGYGFGMVKIDLRTKSCSGVEFSTSGHAYTDTGKASGNLETKYKVCNYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*M. musculus* -----MCNTPTTYCDLGAAKDVFNKGYGFGMVKIDLRTKSCSGVEFSTSGHAYTDTGKASGNLETKYKVCNYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*R. norvegicus* -----MCSTPTTYCDLGAAKDVFNKGYGFGMVKIDLRTKSCSGVEFSTSGHAYTDTGKASGNLETKYKVCNYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*B. mutus* -----MCNTPTTYCDLGAAKDVFNKGYGFGMVKIDLRTKSCSGVEFSTSGHAYTDTGKASGNLETKYKVCNYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*M. brandtii* -----MLKSQMCNTPTTYCDLGAAKDVFNKGYGFGMVKIDLRTKSCSGVEFSTSGHAYTDTGKASGNLETKYKVCNYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*X. laevis* -----MAVPPITYADLGKSARDVFNKGYGFGVLKLEVKTKSSSGVEFTSSGSSNTDTGKASGNLETKYKMKETGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*C. mydas* -----MIKLELTKSSSGVEFTTSSGSSNTDTGKASGNLETKYKIKDYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*A. platyrhynchos\** FAYYWNGDKCSYPMAVPPSYSDLGKSARDVFNKGYGFGMVKLELTKSSSGVEFTTSSGSSNTDTGKASGNLETKYKIKDYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*C. brachyrhynchos\** -----GFGMVKLELTKSSSGVEFTTSSGSSNTDTGKASGNLETKYKIKDYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*C. anna\** -----PMAVPPSYSDLGKSARDVFNKGYGFGMVKLELTKSSSGVEFTTSSGSSNTDTGKASGNLETKYKIKDYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*B. rhinoceros\** -----PMAVPPSYSDLGKSARDVFNKGYGFGMVKLELTKSSSGVEFTTSSGSSNTDTGKASGNLETKYKIKDYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*A. forsteri\** -----PMAVPPSYNDLGKCSARDVFNKGYGFGMVKLELTKSSSGVEFTTSSGSSNTDTGKASGNLETKYKIKDYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*S. camelus\** -----GFGMVKLELTKSSSGVEFTTSSGSSNTDTGKASGNLETKYKIKDYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*M. gallopavo* -----MAVPPSYSDLGKAARDVFNKGYGFGMVKLELTKSSSGVEFTTSSGSSNTDTGKALGNLETKYKIKDYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*G. gallus\** -----PMAVPPSYSDLGKAARDVFNKGYGFGMVKLELTKSSSGVEFTTSSGSSNTDTGKALGNLETKYKIKDYGLTFTQKWN TDNTLGT EISWENKLA EGLKL

*D. rerio* -----MAVPPAYADLGKSAKIDFSGKYGFGVTKLDLTKSQSGVEFTTSSGSSNTDTGKAAGNLETKYKVKELGSLNQKWN TDNTLGT EISWENKLA EGLKL

*S. salar* -----MAVPPAYSDDLGAAKDIFGKYGFGIVKLDLTKAQSVEFATSSGSSNTDTGKAAGNLETKYKVKELGSLNQKWN TDNTLGT EISWENKLA EGLKL

*A. thaliana* -----MVKGPGLYTEIGKARDLRYDYQG-DQKFSVTTYSSGTVAITTTGTNKG--LFLGDVAT--QVKNNFADVKVSTDSLLTTLTFDE-PAPGLK

*O. sativa* -----MAPGLYTDIGKTRDLYRDTGT-HHKFTLTTCTPEGVTTAAGTRKNE--SVFGELOQT--QLNKKLTVDVKANSESDLLTTLTVDFEFTPGLKS

Clustal Consensus \* : \* : \*\* :: \* . \* : \* :: : : \* : : : \* \* : : : \*\*\*

120 130 140 150 160 170 180 190 200 210 220

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*H. sapiens* TLDTIFVPNTGKKSGLKASYKRD CFSVGSNV DIFSGPTIYGWAVLAFEGWLAGYQMSFD TAKSKLSQNNFALGYKAADFQLH THVND-GTEFGGS IYQVNEKIETS I

*C. griseus* TLDTIFVPNTGKKSGLKASFRRDC FSLGSNV DIFSGPTIYGWAVLAFEGWLAGYQMSFD TAKSKLSQNNFALGYKAADFQLH THVND-GTEFGGS IYQVNEKVETS V

*B. taurus* TLDTIFVPNTGKKSGLKASYKRD CFSVGSNV DIFSGPTIYGWAVLAFEGWLAGYQMSFD TAKSKLSQNNFALGYKAADFQLH THVND-GTEFGGS IYQVNEKIETS I

*S. scrofa* TLDTIFVPNTGKKSGLKASYKRE CFSIGSNV DIFAGPTIYGWAVLAFEGWLAGYQMSFD TAKSKLSQNNFALGYKAADFQLH THVND-GTEFGGS IYQVNEKIETS I

*M. mulatta* TLDTIFVPNTGKKSGLKASYKRD CFSVGSNV DIFSGPTIYGWAVLAFEGWLAGYQMSFD TAKSKLSQNNFALGYKAADFQLH THVND-GTEFGGS IYQVNEKIETS I

*O. cuniculus* TLDTIFVPNTGKKSGLKASYKRD CFSVGSNV DIFSGPTIYGWAVLAFEGWLAGYQMSFD TAKSKLSQNNFALGYKAADFQLH THVND-GTEFGGS IYQVNEKIETS I

*M. musculus* TLDTIFVPNTGKKSGLKASYRRD CFSVGSNV DIFSGPTIYGWAVLAFEGWLAGYQMSFD TAKSKLSQNNFALGYKAADFQLH THVND-GTEFGGS IYQVNERIETS I

*R. norvegicus* TVD TIFVPNTGKKSGLKASYRRD CFSVGSNV DIFSGPTIYGWAVLAFEGWLAGYQMSFD TAKSKLQNNFALGYKAADFQLH THVND-GTEFGGS IYQVNEKIETS I

*B. mutus* TLDTIFVPNTGKKSGLKASYKRD CFSVGSNV DIFSGPTIYGWAVLAFEGWLAGYQMSFD TAKSKLSQNNFALGYKAADFQLH THVND-GTEFGGS IYQVNEKIETS I

*M. brandtii* TLDTIFVPNTGKKSGLKASYKRD CFSVGSNV DIFSGPTIYGWAVLAFEGWLAGYQMSFD TAKSKLSQNNFALGYKAADFQLH THVND-GTEFGGS IYQVNEKIETS I

*X. laevis* SLDTTFVPNTGKKSGLKASYKRDYANLGDIDL DLAGPTIYGWVGLGYQWLAGYQMAFD TAKSRLAQN FALGYTAGDFQLH THVND-GTEFGGS IYQVNEKIETS I

*C. mydas* TLDTIFVPNTGKKSGLKASYKREYVNLG CNIDV DLSGPTIYGWAVLAFEGWLAGYQMAFD TAKSRLAQN FALGYTAGDFQLH THVND-GTEFGGS IYQVNEKIETS I

*A. platyrhynchos\** ALDTIFVPNTGKKSGLKASYKREYVNLG CNIDV DLSGPTIYGWAVLAFEGWLAGYQMAFD TAKSRLAQN FALGYTAGDFQLH THVND-GTEFGGS IYQVNEKIETS I

*C. brachyrhynchos\** ALDTIFVPNTGKKSGLKASYKRDYVHLG CNIDV DLSGPTIYGWAVLAFEGWLAGYQMAFD TAKSRLAQN FALGYTAGDFQLH THVND-GTEFGGS IYQVNEKIETS I

*C. anna\** ALDTIFVPNTGKKSGLKASYKRDYVNLG CNIDV DLSGPTIYGWAVLAFEGWLAGYQMAFD TAKSRLAQN FALGYTAGDFQLH THVND-GTEFGGS IYQVNEKIETS I

*B. rhinoceros\** ALDTIFVPNTGKKSGLKASYKRDYVNLG CNIDV DLSGPTIYGWAVLAFEGWLAGYQMAFD TAKSRLAQN FALGYTAGDFQLH THVND-GTEFGGS IYQVNEKIETS I

*A. forsteri\** ALDTIFVPNTGKKSGLKASYKREYVNLG CNIDV DLSGPTIYGWAVLAFEGWLAGYQMAFD TAKSRLAQN FALGYTAGDFQLH THVND-GTEFGGS IYQVNEKIETS I

*S. camelus\** ALDTIFVPNTGKKSGLKASYKREYVNLG CNIDV DLSGPTIYGWAVLAFEGWLAGYQMAFD TAKSRLAQN FALGYTAGDFQLH THVND-GTEFGGS IYQVNEKIETS I

*M. gallopavo* ALDTIFVPNTGKKSGLKASYKREYVNLG CNIDV DLSGPTIYGWAVLAFEGWLAGYQMAFD TAKSRLAQN FALGYTAGDFQLH THVND-GTEFGGS IYQVNEKIETS I

*G. gallus\** ALDTIFVPNTGKKSGLKASYRRD YVNLG CNIDV DLSGPTIYGWAVLAFEGWLAGYQMAFD TAKSRLAQN FALGYTAGDFQLH THVND-GTEFGGS IYQVNEKIETS I

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D.rerio          GLDTSFVPNTGKKSAKLKTGYKREYMNVGCDLDFDLAGPTVHAAAVLGYEGWLAGYQMAFDTAKSKLAQNNFALGYKAGDFQLHTNVND-GTEFGGSIYQKVNQLETAV
S.salar          ALDSSFVPNTGKKSAKLKTGYKRDFINLGCDLDFMAGPTVHAAAVLGYEGWLAGYQMAFDTAKSKLAQNNFALGYKAGDFQLHTNVND-GTEFGGSIYQKVNCHLETAI
A.thaliana       IVQAKL---PDHKSGAAEVQYFHDYAGISTSVGFT-ATPIVNFSGVVGTNGLSLGTDVAYNTESGNFKHFNAGFNFTKDDLTASLILNDKGEKLNASYYQIVSP--STVV
O.sativa         ILSLVV---PDQRSGKLELQYLHEYAGINASVGLN-SNPMVNLSGVFGSKELSSVGVDVAFDTATSNFTKYNAALSLTNSDLIASLHLNHGDTLIASYYHLVKHHSNTAV
Clustal Consensus  .. .          ::*.* : : : : : : : : * : .*. : * : : : : * : . : * : . * : : * : * : . * * : . * * : * :

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          230      240      250      260      270      280      290
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H.sapiens      NLA--WTAGSNNTRFGIAAAKYMLDCRTSLSAKVNNASLIGLGYTQTLRPGVKLTLSALIDGKNFSAGGHKVGLGFELEA
C.griseus      NLA--WTAGSNNTRFGIAAAKYKLDCRTSLSAKVNNASLIGLGYTQTLRPGVKLTLSALIDGKNFNAGGHKVGLGFELEA
B.taurus       NLA--WTAGSNNTRFGIAAAKYKLDCRTSLSAKVNNASLIGLGYTQTLRPGVKLTLSALIDGKNFNAGGHKVGLGFELEA
S.scrofa       NLA--WTAGSNNTRFGIAAAKYKLDCRTSLSAKVNNASLIGLGYTQTLRPGVKLTLSALIDGKNFSAGGHKVGLGFELEA
M.mulatta      NLA--WTAGSNNTRFGIAAAKYKLDCRTSLSAKVNNASLIGLGYTQTLRPGVKLTLSALIDGKNFSAGGHKVGLGFELEA
O.cuniculus    NLA--WTAGSNNTRFGIAAAKYKLDCRTSLSAKVNNASLIGLGYTQTLRPGVKLTLSALIDGKNFNAGGHKVGLGFELEA
M.musculus     NLA--WTAGSNNTRFGIAAAKYKLDCRTSLSAKVNNASLIGLGYTQTLRPGVKLTLSALIDGKNFNAGGHKVGLGFELEA
R.norvegicus   NLA--WTAGSNNTRFGIAAAKYRLDCRTSLSAKVNNASLIGLGYTQSLRPGVKLTLSALVDGKNFNAGGHKVGLGFELEA
B.mutus        NLA--WTAGSNNTRFGIAAAKYKLDCRTSLSAKVNNASLIGLGYTQTLRPGVKLTLSALIDGKNFNAGGHKVGLGFELEA
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X.laevis       SLA--WSAGSNNTRFGIGAKYQLDSNTTSIAKVNNASLIGVGYTQTLRPGVKLTLSALVNGKNFSAGGHKVGLGFELEA
C.mydas        NLA--WTAGSNNTRFGIATKYQLDENTSIAKVNNASLIGIGYTQTLRPGVKLTLSGLVDGKNFSAGGHKVGLGFELEA
A.platyrrhynchos* NLA--WTAGSNNTRFGIAAAKYKLDEKTSIVAKVNNASLIGIGYTHALRPGVKLTLSGLIDGKNFSAGGHKVGLGFELEA
C.brachyrrhynchos* NLA--WTAGSNNTRFGIAGKYQLDEKTSIVAKVNNASLIGIGYTHALRPGVKLTLSGLIDGKNFSAGGHKIGLGFELEA
C.anna*        NLA--WTAGSNNTRFGIAAAKYQMDEKTSVVAKVNNASLIGIGYTHALRPGVKLTLSGLIDGKNFSAGGHKVGLGFELEA
B.rhinoceros*  NLA--WTAGSNNTRFGLAAKYQLDEKTSIVTTKVNNASLIGIGYTHALRPGVKLTFSGLIDGKNFSAGGHKVGLGFELEA
A.forsteri*    NLA--WTAGSNNTRFGIAAAKYQLDEKTSIVAKVNNASLIGIGYTHALRPGVKLTLSGLIDGKNFSAGGHKVGLGFELEA
S.camelus*     NLA--WTAGSNNTRFGIAAAKYQLDEKTSIVAKVNNASLIGIGYTSHALRPGVKLTLSGLIDGKNFSAGGHKVGLGFELEA
M.gallopavo    NLA--WTAGSNNTRFGIAAAKYQLDEKTSIVAKVNNASLIGIGYTLALRPGVKLTLSGLIDGKNFSAGGHKVGLGFELEA
G.gallus*      NLA--WTAGSNNTRFGIAAAKYQLDEKTSIGAKVNNASLIGIGYTLALRPGVKLTLSGLIDGKNFSAGGHKVGLGFELEA
D.rerio        NLA--WTAGSNNTRFGIAAAKYQLKDSSVSASAKVNNASLIGVGYTQSLRPGVKLTLSALIDAKNFNAGGHKVGMGFELEV
S.salar        NLA--WTAGSNNTRFGIGAKYQLDKNASLSAKVNNASLIGVGYTQALRPGVKLTLSALIDAKNFNAGGHKVGMGFELEA
A.thaliana     GAELSHNFTTKENAITVGTQHALDPLTTVKARVNNAGVANALIQHEWRPKSFFTVSGEVDSKAIDK-SAKVGIALALKP
O.sativa       GAELSHSFSRNESTLIFGSQHSLDPHTTVKARFNNYGMASALVQHEWRPKSLITISGEVDTKAIEK-STKVGLSLVLKH
Clustal Consensus  .          :. : . : : * : : : : : * . : * : : * * : . : * : * : * :

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**SUPPLEMENTAL FIGURE 3.** Evolutionary conservation of cysteines in various VDAC-3 proteins. MSA of VDAC-3 sequence from various organisms generated using Clustal Omega (1,2). Cysteines are highlighted in yellow. The first ten organisms (higher mammals) show conservation of all six cysteines, while lower organisms and plants primarily have one conserved cysteine, which is replaced by a conserved serine in the first ten organisms. Organisms for which only partial sequence was available have been marked with \*.

## SUPPLEMENTAL REFERENCES

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