SUPPLEMENTARY INFORMATION

Characterization of SMG-9, an essential component of the nonsense-mediated mRNA decay SMG1C complex

Israel S. Fernández, Akio Yamashita, Ernesto Arias-Palomo, Yumi Bamba, Ruben A. Bartolomé, M. Angeles Canales, Joaquín Teixidó, Shigeo Ohno and Oscar Llorca

SUPPLEMENTARY FIGURES

Supplementary Figure S1. Sequences alignment of SMG-9 of four vertebrates (human, mouse, cow and Xenopus laevis).

Supplementary Figure S2. Analysis of hydropathy versus mean net charge for each segment of the protein as provided by PONDR (http://www.pondr.com). The graphic superimposes the known values for several proteins with defined characteristics of disordered (red dots) and folded (blue squares) conformations. The NT-SMG-9 region (B, green diamond) occupied a position in the graph that unambiguously placed it in the region of disordered proteins. The C-terminal domain of SMG-9 (D, green diamond) fitted into the region of folded domains.

Supplementary Figure 3. **Hydrodynamic analysis of NT-SMG-9¹²⁻¹⁸⁰**. (A) Analytical gel filtration chromatography in a Superdex-75 16/20 column. NT-SMG-9¹²⁻¹⁸⁰ eluted from the column as a single sharp peak with a retention volume of 1.7 mL compatible with a molecular mass of around 20kDa after calibration of the column, the molecular mass calculated for monomeric NT-SMG-9¹²⁻¹⁸⁰. (B and C) Sedimentation velocity

experiment of NT-SMG-9¹²⁻¹⁸⁰ performed using a low ionic strength buffer (50mM NaCl). (B) A representative array of scans of the run. (C) The sedimentation coefficient distributions after data analysis. The estimated Svedberg coefficient (S) was 1.1 compatible with a globular protein of aprox. 20KDa. (D) Sedimentation equilibrium experiments were performed at two different speeds, 17000 rpm (not shown) and 19000 rpm. Continuous lines represent the best-fit single-species model that accounts for the experimental data as described in the text. The lower panel shows the best-fit residuals distribution. From these data, a molecular mass of 19.5 +/- 0.4 KDa was estimated for NT-SMG-9¹²⁻¹⁸⁰.

Supplementary Figure S4. **Cells expressing N-terminal or C-terminal fragments of SMG-9 have increased susceptibility to apoptosis**. HEK-293 cells were transfected with N-terminal, C-terminal or full-length SMG-9 vectors, or with empty vector alone (mock), and transfectants analyzed by RT-PCR (A), or incubated with the indicated concentrations of cisplatin, and subjected to cell apoptosis analyses by flow cytometry (B). (C) Transfectants were analyzed by immunoblotting using antibodies against the indicated proteins. The antibody anti-PARP-1 recognizes both the intact and the cleaved form of the protein. Protein loading was assessed with anti-RhoA antibodies.

SMG-1 has been implicated in a broad range of biological functions, including NMD, but also genome stability, cell proliferation and apoptosis, sometimes in cooperation with other PIKKs (1). SMG-1 seems to cooperate with DNA damage responders (Brumbaugh *et al.*, 2004) and, given that the functions of SMG-9 take place as part of SMG-1-containing complexes, we tested if expression of the distinct fragments of SMG-9 could interfere with the normal response of the cell to stress.

We transfected HEK-293 cells with vectors coding for SMG-9 fragments corresponding to the N-terminal domain, the C-terminal domain or to the complete SMG-9 protein (Supplementary Figure S4A), and tested them for susceptibility to cisplatin, a DNA alkylating agent known to cause cell apoptosis. Flow cytometry experiments indicated that cisplatin treatment led to a dose-dependent increase in cell apoptosis that was of higher extent in cells overexpressing the N-terminal or C-terminal domains of SMG-9 than in cells expressing the full length protein or than in mock cells. This effect was associated with reduction in procaspase-3 levels and with increased processing of PARP-1 to yield the cleaved fragment in cells expressing the SMG-9 protein (Supplementary Figure S4C). In contrast, cell proliferation was similar in cells transfected with the N- or C-terminal fragments of SMG-9 or with the full protein, as assessed by MTT assays (data not shown).

METHODS for Supplementary Figure S3

Analytical gel-filtration and analytical ultracentrifugation

A Superdex-75 16/20 2.4mL column was used for analytical gel filtration. The column was equilibrated with 5 column volumes of 20 mM sodium phosphate (pH 7.2) and 300mM NaCl. The sample was injected in the same buffer at a concentration of 25uM. Absorbance was recorded at 280nm.

Sedimentation velocity was performed at 19 000 rpm and 20 °C in an XL-A analytical ultracentrifuge (Beckman Coulter, Inc.) equipped with a UV–VIS optics detection system, using an An60Ti rotor and 12-mm double-sector centerpieces. Sedimentation profiles were recorded every 2 min at the selected wavelength (255 nm). The

sedimentation coefficient distributions were calculated by least squares boundary modeling of sedimentation velocity data using the c(s) method as implemented in the SEDFIT program.

METHODS for Supplementary Figure S4

Cell apoptosis assays

SMG-9 constructs corresponding to full-length SMG-9, SMG-9¹⁻¹⁸¹ and SMG-9¹⁷⁷⁻⁵²⁰ were cloned in pcDNA3.1 plasmid (Invitrogen). Cells were lysed in Tri-Reagent (Sigma-Aldrich Co., St. Louis, MO), and RNA was extracted and reverse-transcribed. PCR was performed using TaqDNA polymerase (Invitrogen Corp., Carlsbad, CA) and CGGGAGAGGGGACTACATTGC-3' following primers: 5'-5'the and CCCGGTTCTGGATCTGGTAC-3' for N-terminal domain of Smg9; 5'-CATCCTGAGCCCTTCTATCC-3' and 5'-CTCTTGGTGGGTTTTCACTC-3' for Cterminal domains of Smg9; and 5'-CCCATCATCCTCTCAAAACC-3' and 5'-ACCAACCACCAACAACATCAG-3' for full-length SMG-9. PCR profile was 35 cycles of 30-second denaturation at 94°C, 30-second annealing at 56°C (60°C for Nterminal domain) and 1-minute polymerization at 72°C. As cDNA loading control, aliquots of each sample were amplified with human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers.

Cells were transfected using jetPRIME reagent (Polyplus, Illkirch, France), according to manufacturer's instructions. Transfectants were exposed for 24 h to cisplatin, and subsequent to cell detachment, transfectants were incubated with annexin-V-FITC (Miltenyi Biotec, Bergisch Gladbach, Germany) and propidium iodide, and analyzed in a FACScan cytofluorometer (Becton Dickinson, Mountain View, CA).

Following cell lysis, extracts were resolved by SDS-PAGE and proteins blotted onto membranes that were sequentially incubated with primary antibodies and horseradish peroxidase-conjugates secondary antibodies. Proteins were visualized using Immobilon Western chemiluminescent substrate (Millipore Corp., Billerica, MA).

For reagents (antibodies)

Anti-PARP-1 (Santa Cruz Biotechnology, Santa Cruz, CA) anti-pro-caspase-3 (Cell Signaling Technologies, Danvers, MA), and anti-RhoA antibodies (Santa Cruz Biotechnology) were obtained commercially.

 Oliveira V, Romanow WJ, Geisen C, Otterness DM, Mercurio F, Wang HG, Dalton WS, Abraham RT. (2008) A protective role for the human SMG-1 kinase against tumor necrosis factor-alpha-induced apoptosis. J Biol Chem. 2008 283(19):13174-84.

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|----------------------------------------|----------------------------------------------------|----------------------------------|----------------------------------------|----------------------------------|-------------------------------------|----------------------------------------------|-------------------------------------|----------------------------------|----------------------------------------------|-------------------------------------------------------------------------|-------------------------------------|----------------------------------------|----------------------------------------------|----------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|-------------------------------------------------------------------------------------------|
| SMG9_HUMAN SMG9_MOUSE SMG9_BOVIN | MSESGHSQP MSESGHSQP MS <mark>E</mark> SGHSQP | GLYGIERI GLYGIERI GLYGIERI | RRRWKEPGSG RRRWKEPGSS RRRWKEPGPG | GPQNLSGP GPQNLSGP GPQNLSGP | GGRERDYIA GGRERDYIA GGRERDYIA | ♥ WERERRDASE ♥ WERERRDGSE ♥ WERERRDGSE | ETSTSVMQK DPSTNVMQK ETSTAVMQK | PIILSKPP PIILSKPP PIILSKPP | AERSKQPPPPT# AERSKQPPPST# AERSKQPPPPA# | A P A A P P A P A P I A P A A P P A P A P I A P A A P P A P A P I | EKPIVLMKP EKPIVLMKP EKPIVLMKP | EEGKGPVAVI EEGKGPVAGI EEGKGPAATI | GASTPEGTAPPI GASTPEGTAPPI STSTPEGTAPPI | РА А Р А РР К РТ А Р А РР К РА А Р У РР К | G <mark>EKEGQ</mark> RPTÇ GEKEGQRPTÇ GEKEGQRPTÇ | Q P V Y Q I Q N R GMG TAA P Q P V Y Q I Q N R GMG TAA P Q P V Y Q I Q N R GMG TAA P |
| SMG9_XENLA | MSDSGHSQP | N VF IP GRNRI | RRRWMDQGP A | GNLNLSEP | Gr ek dsv lr i | RDQERWEGND | EGCGY TIQK | PIILAKPA | SDRAK | AATPAAPAPI | EKPIVLMKAR | EEGK | P TA PA BGAA VPI | . AAAVAK | VEKEGQRPTO | QPVYQIQNRGMG <mark>A</mark> AAS |
| | | | | | | | | | | | | | | | | |
| 1 | 6 Q | 17 <u>0</u> | 180 | 190 | 200 | 210 | 2 2 o | 230 | 240 | 250 | 260 | 27 <u>0</u> | 280 | 2 9 0 | зоо | 31 Q |
| SMG9_HUMAN | A.AMDPVV | GQAKLLPPE | RMKHSIKLVI | DOMNWCDS | AIEYLLDQTI | VLVVGVLGLC | GTGKSMVMSI | LSANTPEE | DQRTYVFRAQS | EMKERGGNQT | SGIDFFITQE | RIVFLDTQPI | LSPSILDHLIN | IDRKLPPEY | NLPHTYVEM | SLQIAAFLFTVCHVV |
| SMG9_MOUSE SMG9_BOVIN | A. AMDPVV | GQAKLLPPEI | RMKHSIKLVI | DOMNWCDS | AIEYLLDQTI | VLVVGVLGLČ | Q T G K S M V M S I | LSANTPEE | DQRAYVFRAQS | EMKERGGNQI | SGIDFFITQE | RIVFLDTQPI | LSPSILDHLIN | IDRKLPPEI | NLPHTYVEMQ | OSLQIAAFLFTVCHVV OSLQIAAFLFTVCHVV |
| SMG9_XENLA | AGGSVDPVV | GQAKLLPPOI | MKHSIKLVI | EYMNWCDS | AIEFLLDQTI | VLVVGVLGLÇ | QGTGKST I MSI | LSANSPDD | DQRSYVFRMQS | QEVRERAGNQI | SGIDFFISQE | RI I FLDTQPI | LSPAILDHLIN | IDRKLPPEY | ΝLΡΗΤΥνεμά | QSLQIAAFLFTVCHVV |
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| | 320 | 330 | 340 | 350 | 360 | 370 | 380 | 390 | 400 | 410 | 420 | 430 | 440 | 450 | 460 | 470 |
| SMG9_HUMAN | IVVQDWFTD | LSLYRFLQT | AEM <mark>V</mark> KPSTPS | PSHESSS | SGSDEGTEYY | PHLVFLQNKA | RREDFCPRKI | ROMHLMID | QLM <mark>A</mark> HSHLRYKO | TLSMLOCNVE | PGLPPDFLDS | EVNLFLVPFM | DSEAE S E NPP R | GPGS <mark>SPLF</mark> | SLLPGYRGHE | SFQSLVS <mark>K</mark> LRSQ <mark>V</mark> MS |
| SMG9_MOUSE SMG9 BOVIN | IVVQDWFTD IVVQDWFTD | LSLYRFLQT2 LSLYRFLQT2 | AEMVKPSTPS AEMVKPSTPS | PSHESSSS | AGSDEGTEYY SGSEEGAEYY | PHLVFLQNKA PHLVFLQNKA | RREDFCPRKI | ROMHLMID ROMHLMID | QLMAHSHLRYKG OLMAHSHLRYKG | TLSMLOCNVE TLSMLOCNVE | PGLPPDFLD | EVNLFLVPFM EVNLFLMPFM | DSEAENENPPR DSETESETPPR | AGPGSSPLF AGPGS SPLF | SLLP GYRGHI SLLP GYRGHI | SFOSLVSKLRSQVMS SFOSLVSKLRSQVMS |
| SMG9_XENLA | IVVQDWFTD | FNLYRFLQT | AEM <mark>L</mark> KPSTPS | PSHESSGS | SGSDEGIEYY | PHIVFVQNKS | KREDFCPRTI | KOMHTVMD | KLMLHSHLRYKO | TLSMLDCNIE | PGLPYDFVES | EVNLFLIPIM | ESDAD.ETISR | AGTSG iplf | SLLPAYKGHE | SFHSLVS <mark>R</mark> LRSQ I MS |
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| SMG9_HUMAN | MARPQLS | HTILTEKNW | FHYAARIWD | GVRKSSALAE | YSRLLA |
| SMG9_MOUSE | MARPQLS | HTILTEKNW | FHYAARIWD | GVKKSSALAE | YSRLLA |
| SMG9_BOVIN | MARPQLS | HTI LTEKNW | FHYAARIWD | GVKKSSALAE | YSRLLA |
| SMG9_XENLA | MSRPQLS | HTILTEKNW | FHYAARIWD | GVKKSSALSE | YSRLLG |

Fernández et al. Suppl-Figure S1



Fernández et al. Suppl-Figure S2



Fernández et al. Supplementary Figure S3



Suppl-Figure S4