

Supplemental Figures:

Supplemental figure 1. Purified GST-RhoS for the GTPase activity assay

GST-RhoS was expressed and purified as described in the methods. Purified GST-RhoS was resolved by SDS-PAGE and detected by Coomassie blue with a defined amount of GST as a homogeneity control.

Supplemental figure 2. C190S mutation leads to an abnormal subcellular location of RhoS

HEK293T cells transfected with the indicated plasmids were lysed in buffer A containing 0.5% Triton X-100. The supernatant (S) was obtained by centrifugation, and the pellet fraction (P) containing nuclei and membrane parts were harvested and resuspended in 1×SDS sample buffer. Equal amounts of supernatant (S) and pellet (P) fractions were resolved by SDS-PAGE followed by immunoblotting. The levels of GAPDH and LaminA+C were also included not only as the loading controls but also as the makers for different subcellular fractions.

Supplemental figure 3. Sequence comparison of RhoS with RhoA homologs from different species

The alignment was performed by DNAMAN. Homology levels are highlighted in different colors. Black: 100%; Pink: 75%; Blue: 50%. The residues conserved between RhoS and 4930544G11Rik (mRhoS) but significantly distinct from RhoA homologs are indicated by arrow heads. h, *Homo sapiens*; m, *Mus musculus*; r, *Rattus norvegicus*; Pa, *Pan troglodytes*; Bos, *Bos taurus*; Ga, *Gallus gallus*; Da, *Danio rerio*;

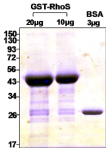
Ce, *Caenorhabditis elegans*; Dr, *Drosophila melanogaster*; Xe, *Xenopus laevis*.

Supplemental figure 4. Evaluating the association between PSMB5 precursor and three Rho isoforms (RhoA, RhoB and RhoC)

Co-immunoprecipitation of PSMB5 precursor with RhoA, RhoB or RhoC was observed and compared with RhoS.

Supplemental figure 5. Evaluating the effect of RhoA, RhoB and RhoC on the protein stability of PSMB5 precursor

HEK239T cells co-expressing the indicated proteins were treated with CHX (100µg/ml) for inhibiting the protein synthesis, and harvested at the corresponding time points. The observation performed on the group transfected with empty vector or RhoS was taken as negative and positive controls for the analysis.



Supernatant

Pellet

C190S

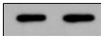
WT

C190S

WT



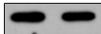
IB α -Flag



IB α -GAPDH



IB α -LaminA+C



IB α -GFP

hRhoA MAIRKKKIVVGDGACGRTCLLIVFSKDQFEVYVPTVFENYADIEVDGKQVELALNDTAGQEDYDLRRLPLSYPTDVLDMCFSIDSPDSLENIPERWT
mRhoA MAIRKKKIVVGDGACGRTCLLIVFSKDQFEVYVPTVFENYADIEVDGKQVELALNDTAGQEDYDLRRLPLSYPTDVLDMCFSIDSPDSLENIPERWT
rRhoA MAIRKKKIVVGDGACGRTCLLIVFSKDQFEVYVPTVFENYADIEVDGKQVELALNDTAGQEDYDLRRLPLSYPTDVLDMCFSIDSPDSLENIPERWT
RhoS MAIRKKKIVVGDGACGRTCLLIVFSKDQFEVYVPTVFENYADIEVDGKQVELALNDTAGQEDYDLRRLPLSYPTDVLDMCFSIGNPDSFGNIPERWT
mRhoS MAIRKKKIVVGDGACGRTCLLIVFSKDQFEVYVPTVFENYADIEVDGKQVELALNDTAGQEDYDLRRLPLSYPTDVLDMCFSIGNPDSFGNIPERWT
Pa_RhoA MAIRKKKIVVGDGACGRTCLLIVFSKDQFEVYVPTVFENYADIEVDGKQVELALNDTAGQEDYDLRRLPLSYPTDVLDMCFSIDSPDSLENIPERWT
Ga_RhoA MAIRKKKIVVGDGACGRTCLLIVFSKDQFEVYVPTVFENYADIEVDGKQVELALNDTAGQEDYDLRRLPLSYPTDVLDMCFSIDSPDSLENIPERWT
Da_rhoaa MAIRKKKIVVGDGACGRTCLLIVFSKDQFEVYVPTVFENYADIEVDGKQVELALNDTAGQEDYDLRRLPLSYPTDVLDMCFSIDSPDSLENIPERWT
Da_rhoab MAIRKKKIVVGDGACGRTCLLIVFSKDQFEVYVPTVFENYADIEVDGKQVELALNDTAGQEDYDLRRLPLSYPTDVLDMCFSIDSPDSLENIPERWT
Da_rhoad MAIRKKKIVVGDGACGRTCLLIVFSKDQFEVYVPTVFENYADIEVDGKQVELALNDTAGQEDYDLRRLPLSYPTDVLDMCFSVDSFDSLENIPERWT
Ce_rho1 MAIRKKKIVVGDGACGRTCLLIVFSKDQFEVYVPTVFENYADIEVDGKQVELALNDTAGQEDYDLRRLPLSYPTDVLDMCFSIDSPDSLENIPERWT
Dr_rho1 MGRIRKKKIVVGDGACGRTCLLIVFSKDQFEVYVPTVFENYADIEVDGKQVELALNDTAGQEDYDLRRLPLSYPTDVLDMCFSVDSFDSLENIPERWT
Xe_rhoC MAIRKKKIVVGDGACGRTCLLIVFSKDQFEVYVPTVFENYADIEVDGKQVELALNDTAGQEDYDLRRLPLSYPTDVLDMCFSIDSPDSLENIPERWT
Censusus m irkklivvgdgaagrtcllivfskdqfp vyvptvfeny adiewd kqvelalndtaggedydlrrlplsypdtvl d l cfs pds nip kw

▲▲▲▲▲

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hRhoA FEVDFHCENVP III VGNKDLRNDERTRELDANMKQEFVKPEECRIMANRIGASCMBCSAKTRGCVREVFEMATFAALCARRGKWKSGCLIVL
mRhoA FEVDFHCENVP III VGNKDLRNDERTRELDANMKQEFVKPEECRIMANRIGASCMBCSAKTRGCVREVFEMATFAALCARRGKWKSGCLIL
rRhoA FEVDFHCENVP III VGNKDLRNDERTRELDANMKQEFVKPEECRIMANRIGASCMBCSAKTRGCVREVFEMATFAALCARRGKWKSGCLIL
RhoS FEVDFHCENVP III VGNKDLRNDERTRELDANMKQEFVKPEEGDRLANSIGAREYVBCSAKTRGCVREVFEMATFAALCENRVRKWKSGCIVF
mRhoS FEVDFHCENVP III VGNKDLRNDERTRELDANMKQEFVKPEEGDRLANSIGAREYVBCSAKTRGCVREVFEMATFAALCENRVRKWKSGCIVF
Pa_RhoA FEVDFHCENVP III VGNKDLRNDERTRELDANMKQEFVKPEECRIMANRIGASCMBCSAKTRGCVREVFEMATFAALCARRGKWKSGCLIVL
Ga_RhoA FEVDFHCENVP III VGNKDLRNDERTRELDANMKQEFVKPEECRIMANRIGASCMBCSAKTRGCVREVFEMATFAALCARRGKWKSGCLIL
Da_rhoaa FEVDFHCENVP III VGNKDLRNDERTRELDANMKQEFVKPEECRIMANRIGASCMBCSAKTRGCVREVFEMATFAALCARRGKWKSGCLIL
Da_rhoab FEVDFHCENVP III VGNKDLRNDERTRELDANMKQEFVNAEECRIMANRIGASCMBCSAKTRGCVREVFEMATFAALCARRGKWKSGCLIL
Da_rhoad FEVDFHCENVP III VGNKDLRNDERTRELDANMKQEFVKPEECRIMANRIGASCMBCSAKTRGCVREVFEMATFAALCARRGKWKSGCLIL
Ce_rho1 FEVDFHCENVP III VGNKDLRNDERTRELDANMKQEFVKPEEGDRLANSIGAREYVBCSAKTRGCVREVFEMATFAALCARRGKWKSGCLIL
Dr_rho1 FEVDFHCENVP III VGNKDLRNDERTRELDANMKQEFVKPEEGDRLANSIGAREYVBCSAKTRGCVREVFEMATFAALCARRGKWKSGCLIL
Xe_rhoC FEVDFHCENVP III VGNKDLRNDERTRELDANMKQEFVKPEECRIMANRIGASCMBCSAKTRGCVREVFEMATFAALCARRGKWKSGCLIL
Censusus pev hfcenvp iiii vgn kdlr ndert rre l d anmk qef vkp ee crim an r i g a s c m b c s a k t r g c v r e v f e m a t f a a l c a r r g k w k s g c l i v l

▲▲▲▲▲

+ PSMB5-Myc

RhoA *RhoB* *RhoC* *RhoS* -

IP α -Flag



P IB α -Myc



IB α -Flag

Input (1%)



P IB α -Myc



IB α -Flag

