Expanded View Figures

A

FLAG IP

HA-GRWD1
HA-GRWD1-FLAG

B

<table>
<thead>
<tr>
<th>Annotation</th>
<th>Mass</th>
<th>MASCOT score (Gel slice number)</th>
<th>Peptides matched</th>
<th>MASCOT score (Gel slice number)</th>
<th>Peptides matched</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPL5</td>
<td>34569</td>
<td>499 (23)</td>
<td>14</td>
<td>139 (23)</td>
<td>3</td>
</tr>
<tr>
<td>RPL11</td>
<td>20468</td>
<td>369 (30)</td>
<td>12</td>
<td>100 (30)</td>
<td>2</td>
</tr>
<tr>
<td>RPL23</td>
<td>14970</td>
<td>1602 (33)</td>
<td>29</td>
<td>258 (33)</td>
<td>4</td>
</tr>
</tbody>
</table>

Figure EV1. Identification of RPL5, RPL11, and RPL23 as candidate GRWD1 interactors by a combination of FLAG-GRWD1 immunopurification and mass spectrometric analysis.

A 293T cells transfected with either HA-GRWD1-FLAG or HA-GRWD1 as a control were subjected to immunoprecipitation with anti-FLAG antibody. The immunoprecipitates were eluted with FLAG peptide and separated by SDS-PAGE followed by silver staining. The gel slice numbers are shown on the right.

B The gel was cut into 33 slices mainly according to the visible bands, numbered as shown in (A). Proteins in each gel slice were digested with trypsin and the obtained peptides were detected by liquid chromatography–MS/MS analysis.
Figure EV2. Effects of GRWD1 silencing or overexpression on p53 pathway.

A HCT116 cells were transfected with control (mixture of control Ds scrambledNeg, siLuci, and siGFP) or another GRWD1-targeting (siGRWD1-1) siRNAs for 36 h, treated with 5 nM actinomycin D, and harvested at the indicated times. Whole-cell extracts were analyzed by immunoblotting with the indicated antibodies. CBB staining serves as a loading control.

B HCT116 cells were transfected with control (mixture of control siLuci and siGFP) or GRWD1-targeting (siGRWD1-1) siRNAs for 24 h, treated with 8 μM bleomycin, and harvested at the indicated times. Whole-cell extracts were analyzed by immunoblotting with the indicated antibodies.

C p53-null H1299 cells were transfected with control (mixture of control siLuci and siGFP) or GRWD1-targeting (mixture of siGRWD1-3 and 4) siRNAs for 36 h, treated with 5 nM actinomycin D, and harvested at the indicated times. Whole-cell extracts were analyzed by immunoblotting with the indicated antibodies. HCT116 cells treated with 8 μM bleomycin for 24 h were also analyzed.

D Another example of data for half-life of p53. HCT116 cells were transfected with control (mixture of control Ds scrambledNeg, siLuci, and siGFP) or GRWD1-targeting (mixture of siGRWD1-3 and 4) siRNAs for 24 h, treated with 50 μg/ml cycloheximide, and harvested at the indicated times. Whole-cell extracts were analyzed by immunoblotting with the indicated antibodies.
Figure EV3. Effects of RPL5 or GRWD1 overexpression on p53 levels.

A. GRWD1 overexpression alone does not affect p53 levels. H1299 cells in 12-well plates were transiently transfected with expression vectors (p53, 6 ng; HA-GRWD1-FLAG, 200 ng) using Lipofectamine 2000. At 24 h after transfection, cells were subjected to immunoblotting.

B. RPL11 but not RPL5 increases MDM2 and p53 levels when overexpressed. H1299 cells were co-transfected with the indicated expression vectors (p53, 7.5 ng; HA-Ub, 0.5 μg; His-Xpress-MDM2, 2 μg; RPL11-FLAG, 1 μg; RPL5-FLAG, 1.5 μg; HA-GRWD1-FLAG, 1.5 or 1 μg; GFP, 0.04 μg) for 48 h and then analyzed by immunoblotting with the indicated antibodies, as in Fig 4A. GFP serves as a control protein to show equal transfection efficiencies.

Figure EV4. GRWD1 overexpression is associated with poor prognosis in cancer patients.

Association of expression levels of GRWD1 with prognosis of bladder cancer patients in an expression profile study from GEO #GSE13507 (left panel) and of lung cancer from jacob-00182-MSK (right panel) was investigated using the PrognoScan database [36].
Figure EV5. The abilities of GRWD1 to inhibit nucleolar stress response and promote tumorigenesis require interaction with RPL11 via the N-terminal domain.

A Signal intensities of the His-Xpress-MDM2 bands in Fig 6C were quantified and normalized to those of GFP. The means and SDs of the His-Xpress-MDM2 levels are shown alongside those from cells transfected with His-Xpress-MDM2 only set 1 (n = 7).

B In vivo ubiquitination assay to detect MDM2 autoubiquitination was performed with the indicated expression vectors (His-Xpress-MDM2, 2 µg; HA-Ub, 0.5 µg; RPL11-FLAG, 1 µg; HA-GRWD1-FLAG WT, 1.5 µg; HA-GRWD1-FLAG Δ1–136, 1.5 µg) as in Fig 4C. Immunoprecipitates (IPs) and inputs were immunoblotted with the indicated antibodies. SE, short exposure.

C Confirmation of specificity of our anti-RPL11 antibody. HCT116 cells were transfected with control (mixture of control siLuci and siGFP) or RPL11-targeting (siRPL11-1 or 2) siRNAs for 48 h. Whole-cell extracts were analyzed by immunoblotting with the anti-RPL11 antibody.