Supporting Information

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SI Materials and Methods

Expression and Purification of *Macro* Modules. The following *macro* modules were expressed and purified as described in refs. 1 and 2: recombinant, N terminus, His-tagged, full-length Af1521 wild-type and its G42E mutant; GST-tagged Af1521 wild-type; and the GST-tagged hTaf250 double bromodomain.

Cell Culture and Fractionation. The growth conditions for CHO and HL60 cells and the purification of plasma membranes were as described in ref. 3. Total membrane and the postnuclear protein fractions were prepared from cells (10^8 and 3×10^8 cells per preparation, respectively) that were pelleted at $900 \times g$ for 10 min and washed three times with PBS. The cell pellets were resuspended either in 5 ml of HES buffer (20 mM Hepes, pH 7.4, 1 mM EDTA, 250 mM sucrose and protease inhibitors) for the total membrane preparation, or in 1.5 ml of lysis buffer (20 mM Tris·HCl, pH 7.4, 0.83 mM $MgCl_2$, 1 mM EDTA, 20 mM NaCl, 1 mM DTT and protease inhibitors) for the postnuclear preparation; the cells were then lysed on ice by passage through a 25-gauge syringe. Unbroken cells and nuclei were removed by low-speed centrifugation ($400 \times g$ for 5 min) to obtain the postnuclear pellet. To obtain the total membrane preparation, this postnuclear fraction was further centrifuged for 15 min at $16{,}000 \times g$. The pellet here was resuspended in 20 mM Hepes, pH 7.4, containing 1 mM EDTA and protease inhibitors. Protein concentrations were determined using the Bio-Rad Protein Assay kit (Bio-Rad Laboratories), according to the manufacturer instructions.

ADP-Ribosylation Assay and Immunoblot Analysis. The ADP-ribosyltransferase activity was measured by following the incorporation of radioactive ADP-ribose into membrane proteins, as described in ref. 3, with some modifications. Samples (10 μg of plasma membranes) were incubated with 50 μl of ADP-ribosylation buffer [50 mM potassium phosphate buffer (PFB), pH 7.4, 5 mM $MgCl_2$, 4 mM DTT (DTT), 10 μM GTPγS, 700 μM β-NAD^+ and 4.5 μCi [^{32}P]-NAD^+ (specific activity: 1,000 Ci/mmol)] at 37 °C for 60 min, unless otherwise specified. Under these conditions, 10% of the total β subunit was mono-ADP-ribosylated; to obtain maximal modification (80–100% of total mono-ADP-ribosylated β), the incubation was prolonged to 12 h at 30 °C. The *in vitro* PT-catalyzed ADP-ribosylation was performed as described in ref. 4, using total membranes. The PT-catalyzed ADP-ribosylation was performed on intact cells by addition of 5 nM PT for 15 h under controlled atmosphere conditions (5% CO_2/95% air) at 37 °C. The immunoblot analyses were performed as described in ref. 4, using an antiserum raised against the C-terminal peptide of the β subunit (Santa Cruz Biotechnology).

Pull-Down Assay. Plasma membranes (10 μg for each sample) were ADP-ribosylated as described above, washed with 50 mM PFB, pH 7.4, to remove unbound NAD^+, and then centrifuged at $16{,}000 \times g$ for 15 min at 4 °C. The pellet was solubilized in 400 μl of RIPA buffer (100 mM Tris·HCl, pH 7.5, 1% Igepal, 0.5% deoxycholate, 0.1% SDS, and protease inhibitors) with constant rotation for 30 min at 4 °C. The mixture was briefly sonicated on ice and clarified by centrifugation at $16{,}000 \times g$ for 45 min at 4 °C. The supernatant was incubated with the indicated concentrations of the specified *macro* modules for 2 h at room temperature on a rotating wheel, and then pulled down in an overnight incubation at 4 °C, with 50 μl of 50% slurry of Ni-NTA resin that had been equilibrated with RIPA buffer. Then the resin was washed three times with RIPA buffer plus 10 mM imidazole, and finally both the washed resin and the unbound fraction (one-eighth of the total volume) were diluted in 50 μl of Laemmli buffer, boiled and analyzed by 10% SDS/PAGE. For the competition assays, the solubilized, ADP-ribosylated plasma membranes were simultaneously incubated with 30 pmol of His_6-*m*Af1521 and increasing concentrations of unlabeled free ADP-ribose (1 nM to 10 mM) for 2 h at room temperature on a rotating wheel. For the displacement assay, plasma membranes were pulled down with His_6-*m*Af1521 as described above, and then incubated with increasing concentrations of unlabeled free ADP-ribose (1 nM to 10 mM) for 4 h at room temperature on a rotating wheel. When the pull-down assay was performed using total membranes or postnuclear material, 2 mg of protein for each sample were solubilized with 1.5 ml of RIPA buffer as described above, and then adsorbed with 100 μl of a 50% slurry of either Ni-NTA beads or glutathione-Sepharose resin for 2 h at 4 °C, for the preclearing step. The supernatant underwent two sequential pull-downs, the first with 2 nmol of *m*Af1521/G42E and the second with 1 nmol of His_6-*m*Af1521. GST-tagged Af1521 wild-type and its G42E mutant were covalently cross-linked to the resin using dimethyl pimelimidate-HCl (DMP) as described in ref. 5. When resin-bound *macro* domains were used for affinity purification of ADP-ribosylated proteins (see Fig. 4), after five sequential washing, both sample and control columns were eluted with 20 mM ADP-ribose, to specifically recover the ADP-ribosylated proteins. The eluted proteins analyzed by gels underwent colloidal Coomassie blue (blue code, Pierce) or silver staining, and the bands of interest were analyzed by MALDI-TOF-MS and by LC-MS/MS.

MALDI-TOF-MS. The protein bands of interest from SDS/PAGE gels that were colloidal Coomassie blue or silver stained were excised and placed in 0.5-ml microcentrifuge tubes (Eppendorf). After washing, the disulfide bridges were reduced with DTT and alkylated with iodoacetamide (6). The samples (gels) were digested *in situ* by incubation with sequencing grade modified trypsin (Promega) in 40 mM ammonium bicarbonate, overnight at 37 °C under slight shaking on a thermomixer. The reaction was stopped with 0.1% (vol/vol) TFA/H_2O (trifluoroacetic acid) for 15 min at 30 °C. The tryptic peptides were extracted with ZipTip C18 (Millipore) reverse-phase material, and directly eluted and crystallized in a 50% (vol/vol) acetonitrile/H_2O saturated solution of β-cyano-4-hydroxycinnamic acid. The samples were analyzed in a time-of-flight mass spectrometer (Reflex IV, Bruker Daltonics) equipped with a nitrogen laser with an emission wavelength of 337 nm. Mass spectra were acquiring in positive ion Reflectron-mode with delayed extraction and an accelerating voltage of 25 kV. External calibration was performed for each measurement using a mixture of seven standard peptides (average mass accuracy >20 ppm). All of the mass spectra were acquired using a minimum number of 250 laser shots. The spectra were internally calibrated with trypsin autolysis products. A database search with the monoisotopic peptide masses was performed against the National Center for Biotechnology Information nonredundant database using the peptide search algorithm MASCOT (Matrix Science). Mass tolerance of 100 ppm, single miss cleavage site per peptide fragments, carboamidomethyl modification of cysteine residues, and the optional presence of methionine oxidation were used in the database search.

LC-MS/MS Analyses. Protein bands stained with Coomassie colloidal blue were excised from the gel and de-stained by repetitive washings with 50 mM NH_4HCO_3 pH 8.0 and acetonitrile. Samples were reduced and carboxyamidomethylated with 10 mM DTT and 55 mM iodoacetamide in 50 mM NH_4HCO_3 buffer, pH 8. Tryptic digestion of the alkylated proteins was performed at 37 °C overnight using 100 ng of trypsin for each sample.

LC-MS/MS analyses were performed on a CHIP MS system (Agilent Technologies) consisting of a LC/MSD Trap XCT Ultra equipped with a 1100 HPLC system and a Chip Cube. After loading, the peptide mixture (8 μl in 0.2% formic acid) was first concentrated and washed onto a 40 nl enrichment column (Agilent Technologies CHIP) at 4 μl/min using 0.2% formic acid in 2% acetonitrile as eluent. The sample was then fractionated on a C_{18} reverse-phase capillary column (75 μm x 43 mm in the Agilent Technologies CHIP) at a flow rate of 300 nl/min, with a linear gradient from 7% to 60% of eluent B (0.2% formic acid in 95% acetonitrile) in A (0.2% formic acid in 2% acetonitrile) in 50 min. Peptide analysis was performed using data-dependent acquisition of one MS scan (mass range from 400 to 2,000 m/z) followed by MS/MS scans of the three most abundant ions in each MS scan. Dynamic exclusion was used to acquire a more complete survey of the peptides by automatic recognition and temporary exclusion (2 min) of ions from which definitive mass spectral data had been acquired. Raw data from μLC-MS/MS analyses were used to search the National Center for Biotechnology Information non-redundant database using the Mascot software (Matrix Science).

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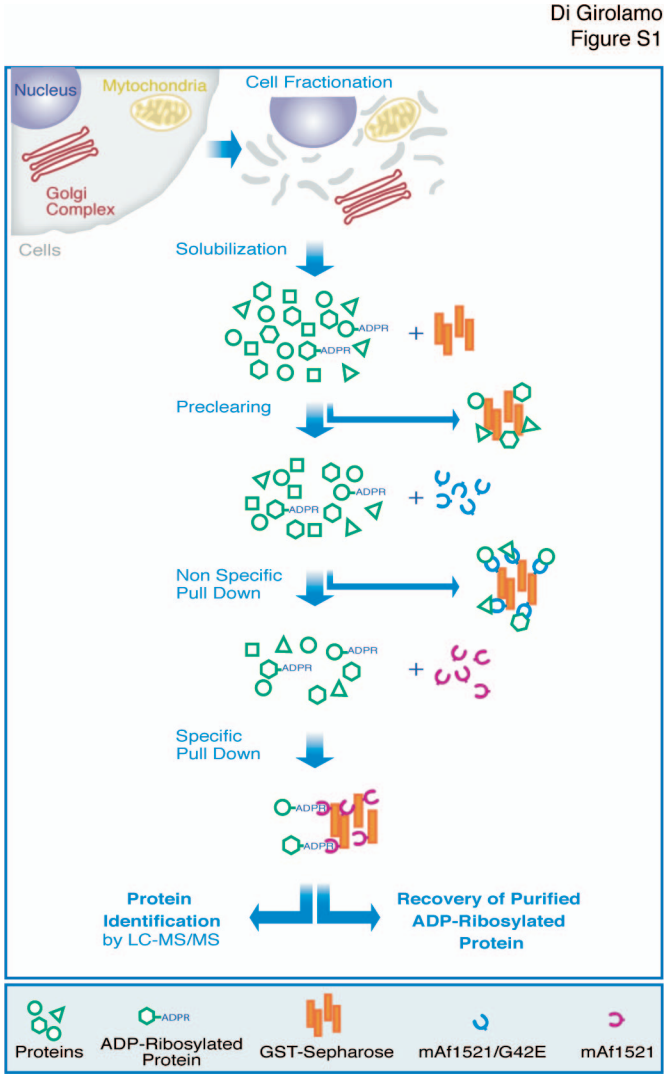

Fig. S1. Schematic representation of the pull-down protocol using *m*Af1521. After cell lysis and fractionation, the solubilized proteins are first *precleared* using GST-Sepharose resin alone. Nonspecific protein binding to the resin/*m*Af1521 is then removed by a *nonspecific pull-down* step using the resin-bound GST-*m*Af1521/G42E mutant. Finally, the *specific pull-down* of the ADP-ribosylated proteins with resin-bound GST-*m*Af1521 is followed by either *separation* by PAGE or recovery from the resin with free ADP-ribose. Samples are then *identified* by mass spectrometry or further used in biological assays.