SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Tandem mass spectrometry coupled to liquid chromatography (LC-MS/MS) - Excised protein bands following SDS-PAGE were digested in trypsin (10 µg/mL) at 37°C overnight. LC-MS/MS analyses of in-gel trypsin digested(1) protein bands were carried out using a quadrupole ion trap ThermoFinnigan LCQ DECA XP PLUS (San Jose, CA) equipped with a Michrom Paradigm MS4 HPLC, and a nanoelectrospray source. Peptides were eluted from a 15 cm pulled tip capillary column (100 um I.D. x 360 um O.D; 3-5 um tip opening) packed with 7 cm Vydac C18 (Hesperia, CA) material (5 µ, 300Å pore size), using a gradient of 0-65% solvent B (98% methanol/ 2% water/ 0.5% formic acid/ 0.01% triflouroacetic acid) over a 60-min period at a flow rate of 350 nl/min. The LCQ DECA XP PLUS electrospray positive mode spray voltage was set at 1.6 kV, and the capillary temperature at 200°C. Dependent data scanning was performed by the Xcalibur v 2.0 SR2 software(2) with a default charge of 2, an isolation width of 1.5 amu, an activation amplitude of 35%, activation time of 30 msec, and a minimal signal of 10,000 ion counts. Global dependent data settings were as follows, reject mass width of 1.5 amu, dynamic exclusion enabled, exclusion mass width of 1.5 amu, repeat count of 1, repeat duration of 1 min, and exclusion duration of 5 min. Scan event series included one full scan with mass range 350 – 2000 Da, followed by 3 dependent MS/MS scan of the most intense ion. Dynamic exclusion was turned on for a period of 60 sec. Tandem MS spectra of peptides were analyzed with Turbo SEQUEST™ v 3.1, a program that allows the correlation of experimental tandem MS data with theoretical spectra generated from known protein sequences(3). The peak lists (dta files) for the search were generated by Xcalibur 2.0 SR2. Parent peptide mass error tolerance was set at 1.5 amu and fragment ion mass tolerance was set at 0.5 amu during the search. The criteria that were used for a preliminary positive peptide identification are the same as previously described, namely peptide precursor ions with a +1 charge having a Xcorr >1.8, +2 Xcorr > 2.5 and +3 Xcorr > 3.5. A dCn score > 0.08 and a fragment ion ratio of experimental/theorical >50% were also used as filtering criteria for reliable matched peptide identification (4). All matched peptides were confirmed by visual examination of the spectra. All spectra were searched against the latest version of the non-redundant protein database downloaded July 14, 2005, from NCBI. At the time of the search the non-redundant protein database from NCBI contained 2,662,317 entries.

Tandem mass spectrometry coupled to tandem liquid chromatography (LC-LC-MS/MS) - For LC-LC-MS/MS analyses, a microbore HPLC system (Surveyor, Thermo Fisher Scientific, San Jose, CA) was used with two separate strong cation exchange (SCX) and reversed phase (RP) columns: a 100 µm I.D. capillary packed with 3.5 cm of 5 µm PolySulfoethyl-Asp strong cation exchanger (SCX, PolyLC Inc., Columbia, MD) and a separate 100 µm I.D. capillary packed with 8cm of 5 µm Zorbax Eclipse XDB-C18 material (Agilent, Santa Clara, CA). The samples were acidified using TFA and manually injected by pressure packing onto the SCX column, the effluent from the column being fed through RP column. Peptides were eluted in a gradient using Buffer A (0.1% formic acid), Buffer B (acetonitrile/0.1% formic acid), Buffer C (250 mM ammonium acetate), and Buffer D (1.5 M ammonium acetate) at a flow rate of 400 nL/min. Twelve steps were then performed as follows: (step 1) 0% C with a gradient of 5-50% B over 90 minutes followed by a column clean-up of 5 min. 50-98% B and an equilibration of 20 min. 5% B. (steps 2-11) X% C (where X = 10-100% C increased in increments of 10) loaded over 4 minutes and then washed with 5% B for 7 minutes followed by a gradient of 5-50% B over 60 minutes. Each gradient was followed by a column clean-up of 5 min. 50-98% B and an equilibration of 20 min. 5% B. (step 12) 50% D loaded over 4 minutes and then washed with 5% B for 7 minutes followed by a gradient of 5-50% B over 60 minutes. The flow rate was 1.0 uL/min for the 7-minute wash following each salt bump and for each final 5% B equilibration. Peptides were directly eluted into a custom-built nanoelectrospray ionization source of a ThermoFinnigan LTQ linear ion trap mass spectrometer (Thermo Fisher Scientific, San

Jose, CA). Electrospray voltage of 2.0 kV was applied using a gold electrode via a liquid junction up-stream of the column. Spectra were scanned over the range 400-1500 amu. Automated peak recognition, dynamic exclusion, and daughter ion scanning of the top seven most intense ions were performed using the Xcalibur v 1.4 SR1 data system (Thermo Fisher Scientific, San Jose, Ca).

Database searching - Tandem mass spectra were extracted, charge state deconvoluted and deisotoped by Mascot Distiller version 2.0. All MS/MS samples were analyzed using Sequest (Thermo Fisher Scientific, San Jose, CA, USA; version 27, rev. 12) and X! Tandem (The GPM, thegpm.org; version 2007.01.01.1). X! Tandem was set up to search a subset of the ipi.RAT.v3.68 database also assuming trypsin. Sequest was set up to search the ipi.RAT.v3.68 database (unknown version, 39687 entries) assuming the digestion enzyme trypsin. Sequest and X! Tandem were searched with a fragment ion mass tolerance of 1.00 Da and a parent ion tolerance of 2.0 Da. Oxidation of methionine and iodoacetamide derivative of cysteine were specified in Sequest and X! Tandem as variable modifications.

Criteria for protein indentification - Scaffold (version Scaffold_2_06_01, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 20.0% probability as specified by the Peptide Prophet algorithm (5). Peptide identifications were also required to exceed specific database search engine thresholds. Sequest identifications required at least deltaCn scores of greater than 0.08 and XCorr scores of greater than 1.8, 2.5, 3.5 for singly, doubly, triply charged peptides. X! Tandem identifications required at least -Log(Expect Scores) scores of greater than 2.0. Protein identifications were accepted if they could be established at greater than 80.0% probability and contained at least 5 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm (6). Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.

SUPPLEMENTAL REFERECES

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Supplemental Figure Legends

Suppl. Figure 1. *TNF mediated inhibition of Phex gene expression may not requires de novo synthesis of a transrepressor and does not correlate with significant cytotoxicity***.** (**A**) Real-time RT-PCR analysis of *Phex* expression in TNF-treated UMR-106 cells in the absence or presence of cycloheximide (CHX); statistical significance ($p<0.05$) depicted as * (Ctrl *vs.* TNF), # (Ctrl *vs.* CHX) and ○ (Ctrl *vs.* CHX+TNF). The effect of TNF treatment on (**B**) apoptosis (Caspase-Glo 3/7 Assay Systems; Promega), and (**C**) adenylate kinase release (ToxiLight BioAssay kit; Lonza) as a measure of cytotoxicity. Values are means ±SE from three independent experiments. (D) Real-time RT-PCR analysis of IκBα (NFKBAI; nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha) expression in TNF-treated UMR-106 cells in the absence or presence of cycloheximide (CHX); statistical significance ($p<0.05$) depicted as * (Ctrl *vs.* respective treatement).

Suppl. Figure 2. *Peptide coverage maps for the scoring protein matches: (PARP-1 (113 kDa, **A&B**) and S120 (88 kDa, **C&D**).* (NTT-number of expected enzymatic termini; DAMU – actual minus calculated peptide mass (AMU); DPPM – actual minus calculated peptide mass (PPM); Start- peptide start index; Stop – peptide stop index).