Fig. 5 A 'proteome chip' composed of 6,566 protein samples representing 5,800 unique proteins, which are spotted in duplicate on a single nickel-coated glass microscope slide[39]. The immobilized GST fusion proteins were detected using a labeled antibody against GST.

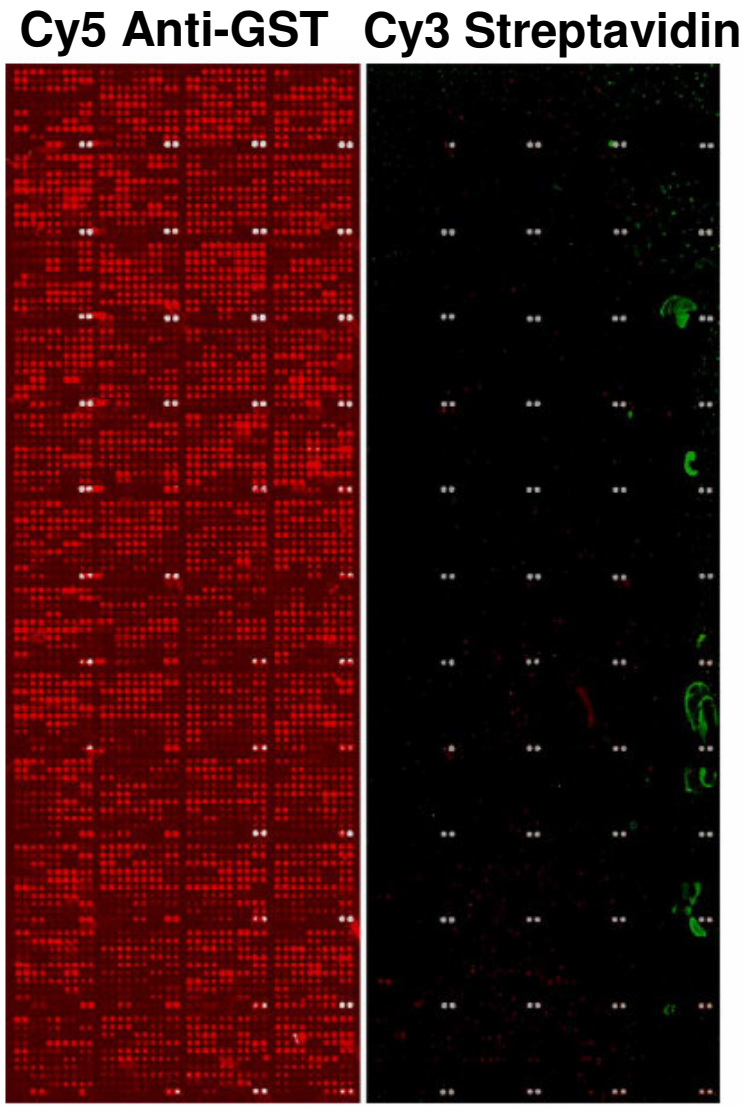

Fig. 1. Yeast protein microarray. On the left, a yeast protein microarray probed with anti-GST antibody followed by probing with Cy5-conjugated anti-rabbit antibody. The relative level of the printed GST-fused proteins can be determined. On the right, the same microarray probed Cy3-conjugated streptavidin, which recognizes the biotinylated control proteins printed on the slide.

Analyzing antibody specificity with whole proteome microarrays.

Michaud GA, Salcius M, Zhou F, Bangham R, Bonin J, Guo H, Snyder M, Predki PF, Schweitzer BI.

Protometrix, 688 East Main Street, Branford, Connecticut 06405, USA.

a


Antigen-antibody

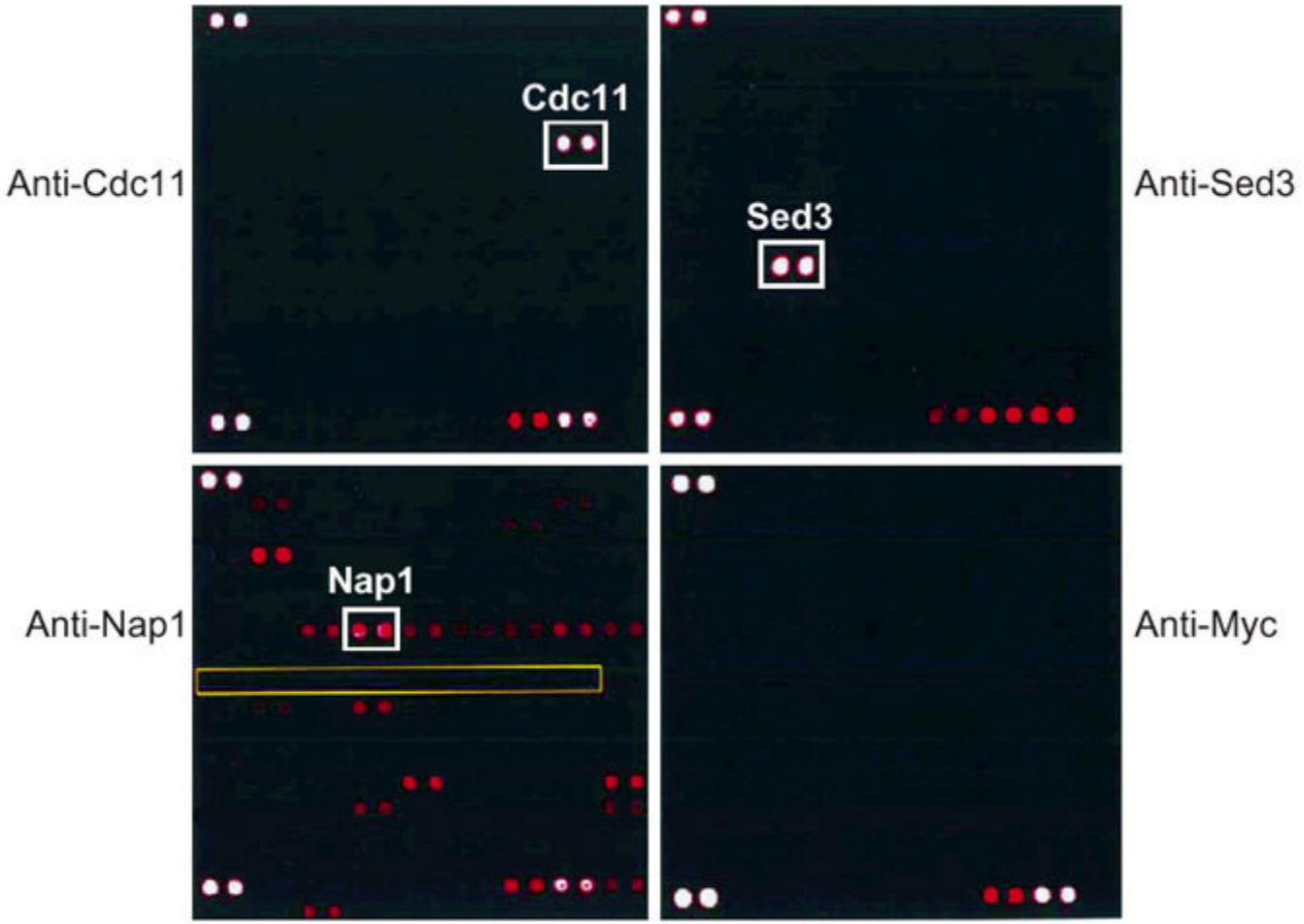
Anti-Cdc11
Cdc11
Sed3
Anti-Sed3
Nap1
Anti-Nap1
Anti-Myc

Table 1 Antibodies tested on yeast protein microarray

Antibody	Approx. amount of protein on array (pg)	Source of epitope	Immunogen	Antibody type	Antibody concentration	Number of proteins with signal/background >2.0
Ynl021W-Hda1	0.3	Yeast	Peptide$^{20\ a.a.}$	Polyclonal[a,b]	0.8	8
Ylr113W-Hog1	0.4	Yeast	Peptide$^{20\ a.a.}$	Polyclonal[a,b]	0.8	2
Yjl164C-Tpk1	1.2	Yeast	Peptide$^{20\ a.a.}$	Polyclonal[a,b]	0.8	9
Yjl030W-Mad2	2.3	Yeast	Peptide$^{20\ a.a.}$	Polyclonal[a,b]	0.8	1
Ylr229C-Cdc42	5.2	Yeast	Peptide$^{20\ a.a.}$	Polyclonal[a]	0.1	1
Ypr120C-Clb5	ND	Yeast	Peptide$^{20\ a.a.}$	Polyclonal[a,b]	0.1	1
Yjr076C-Cdc11	2.2	Yeast	Protein$^{1–415\ a.a.}$	Polyclonal[c]	0.04	7
Ykr048C-Nap1	7.2	Yeast	Protein$^{1–417\ a.a.}$	Polyclonal[c]	0.02	1,770
Ypr183W-Sed3	13.3	Yeast	Protein$^{cytosolic\ domain}$	Monoclonal[d]	2.0	1
Ygl187C-Cox4	0.9	Yeast	Protein$^{\cdot}$	Monoclonal[d]	2.0	1
Yor036W-Pep12	4.4	Yeast	Protein$^{C\ terminus}$	Monoclonal[d]	2.0	4
HA	–	Influenza	Peptide$^{12\ a.a.}$	Monoclonal[d]	1.0	0
MYC	–	Human	Protein	Monoclonal[d]	1.0	0
FLAG	–	Synthetic	Peptide	Monoclonal[d]	2.4	0

The amount of each protein on the array and the number of proteins recognized by each antibody was determined as described in Methods.

[a]Goat IgG isotype. [b]Affinity purified. [c]Rabbit IgG isotype. [d]Mouse IgG isotype. a.a., amino acids; ND, not detected.

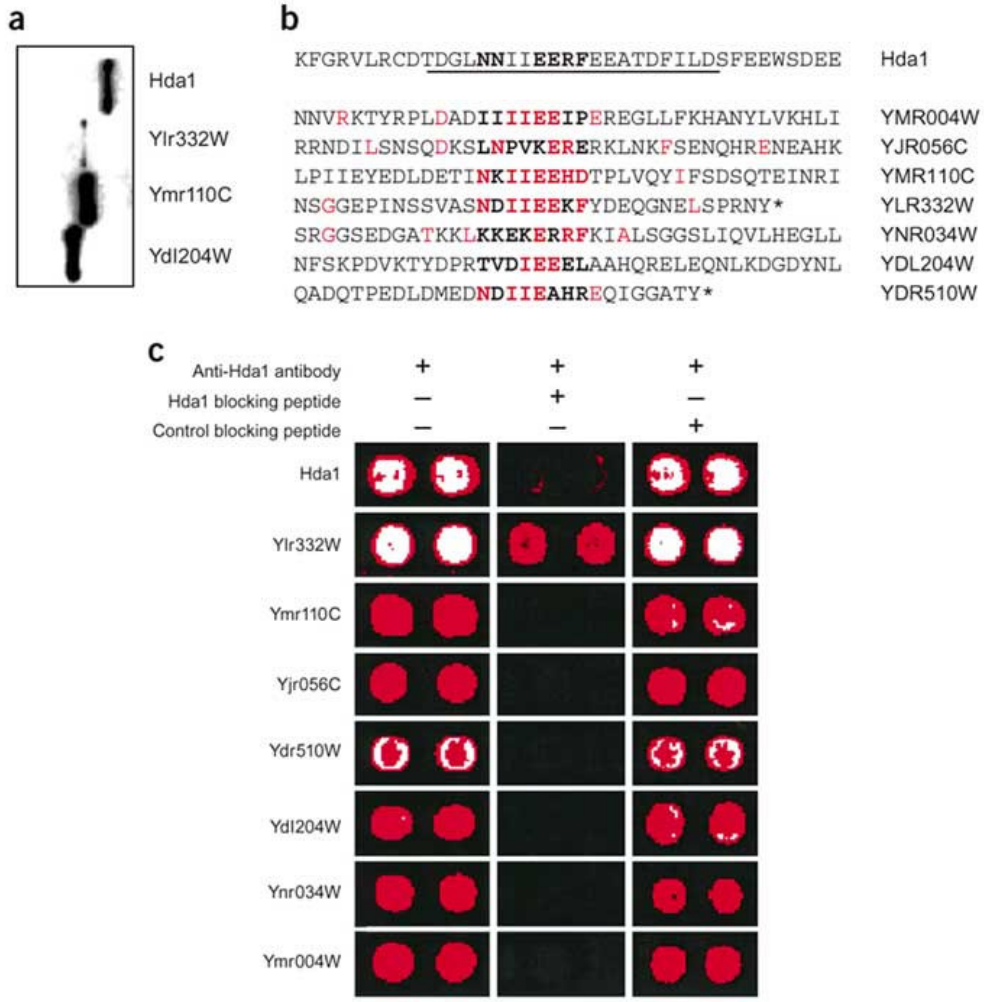

Figure 2. Analysis of anti-Hda1 binding to the yeast proteome microarray.
(**a**) Western blot analysis of proteins detected with the anti-Hda1 antibody on the yeast proteome microarray. (**b**) Sequence alignment of the Hda1 immunogenic peptide with best matches from each of the cross-reacting proteins. The immunogenic peptide is underlined in the Hda1 sequence. The eight-amino-acid window with highest maximal sequence identity between all cross-reacting proteins is shown in bold. *, C terminus. (**c**) Peptide inhibition of anti-Hda1 binding on the yeast proteome microarray. Images on the left are from an array that was probed with the anti-Hda1 antibody alone. Images in the middle are from an array that was probed in the presence of the Hda1 immunizing peptide. Images on the right are from an array that was probed in the presence of a peptide with an unrelated sequence.

Finding new components of the target of rapamycin (TOR) signaling network through chemical genetics and proteome chips

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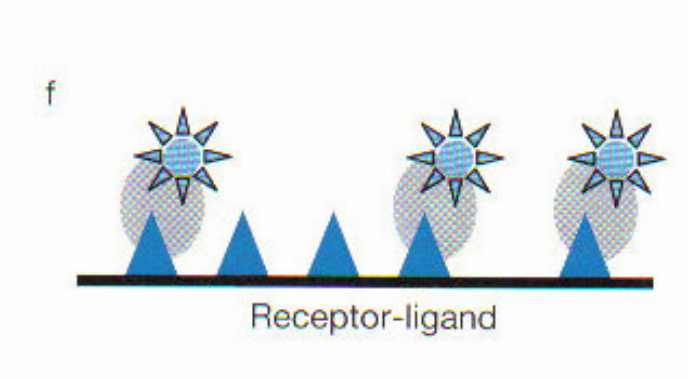
Contributed by Stuart L. Schreiber, October 11, 2004



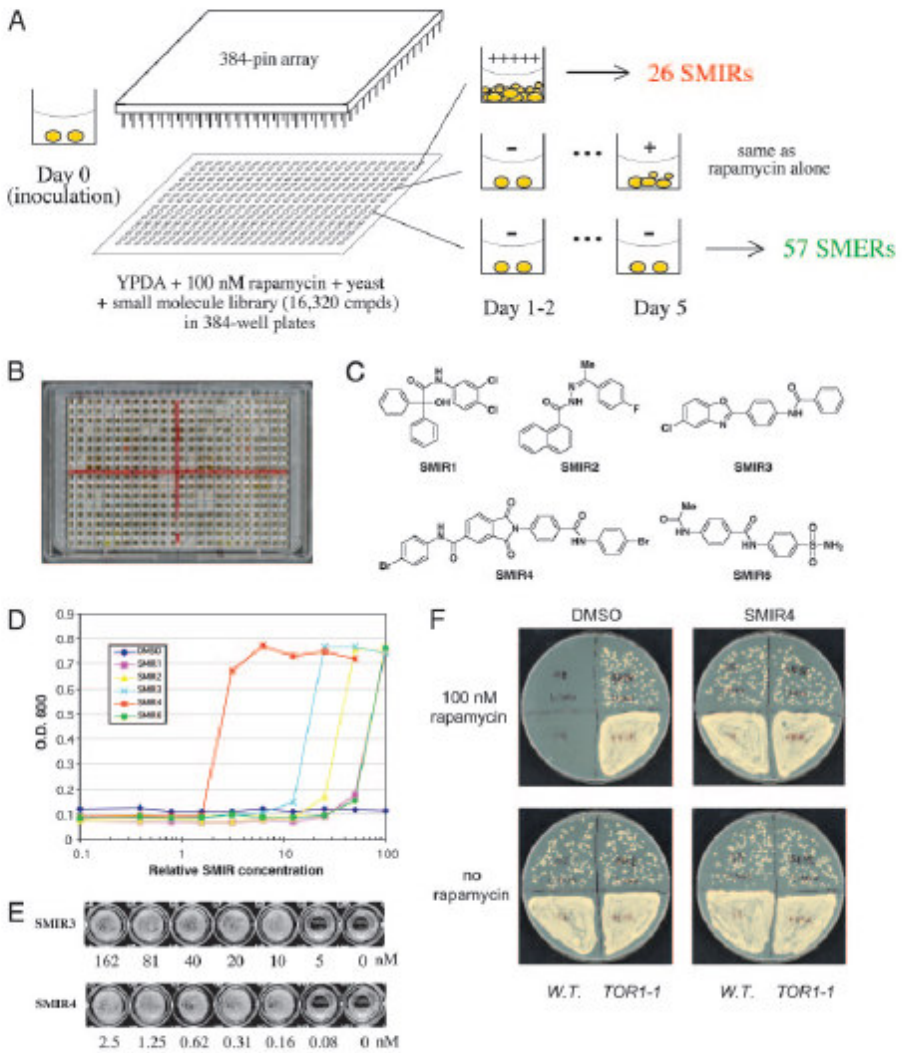


Fig. 1. A chemical genetic screen for small molecules that modulate rapamycin's antiproliferative effect in yeast. (*A*) Schematics of the screen. Compounds were transferred from library plates to assay plates (containing growth medium, rapamycin, and yeast cells) by using 384-pin arrays. SMER, small-molecule enhancers of rapamycin. (*B*) Retest of SMIRs in a 384-well plate. White wells indicate compound-induced yeast growth in the presence of rapamycin; black (transparent) wells indicate no growth. (*C*) Chemical structures of the fast-acting SMIRs (yeast growth identifiable on day 1, same as the "no rapamycin" control). (*D*) Dose–response curves for SMIRs in wild-type (rapamycin-sensitive EGY48) cells inoculated in YPDA containing 100 nM rapamycin. (*E*) Minimal concentrations of SMIR3 and SMIR4 required for yeast growth in YPDA containing 20 nM rapamycin. (*F*) SMIR4 treatment and the *TOR1-1* (S1972R) mutation (26) both confer rapamycin resistance. Cells were plated at two different densities on the upper versus lower halves of the plates (1:1,000).

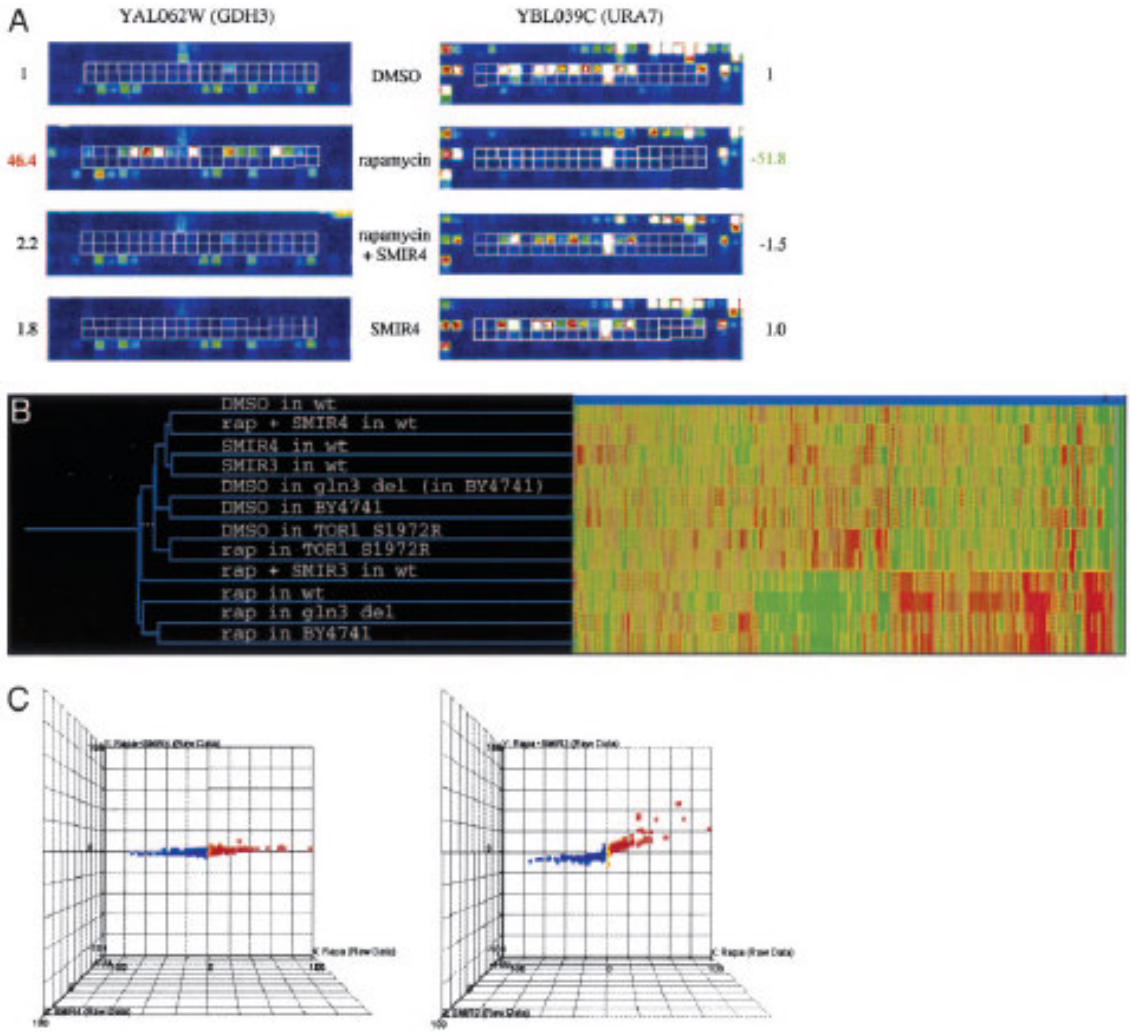
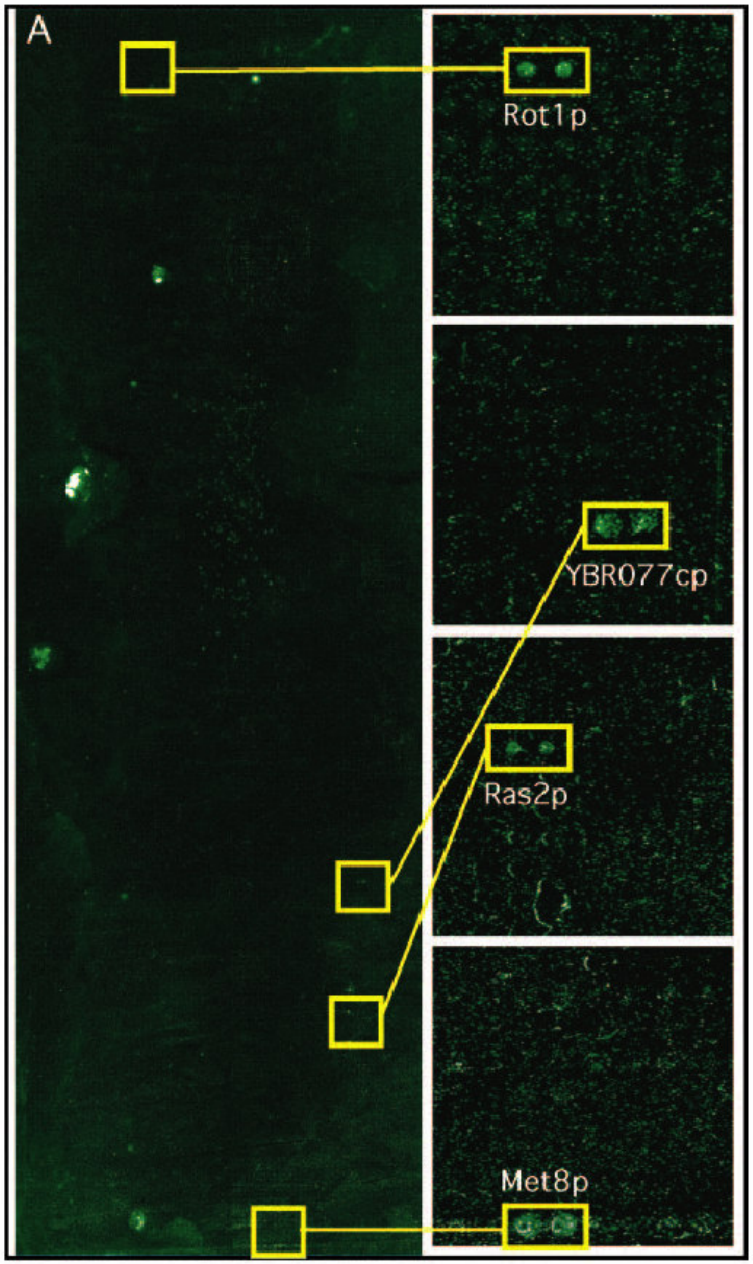
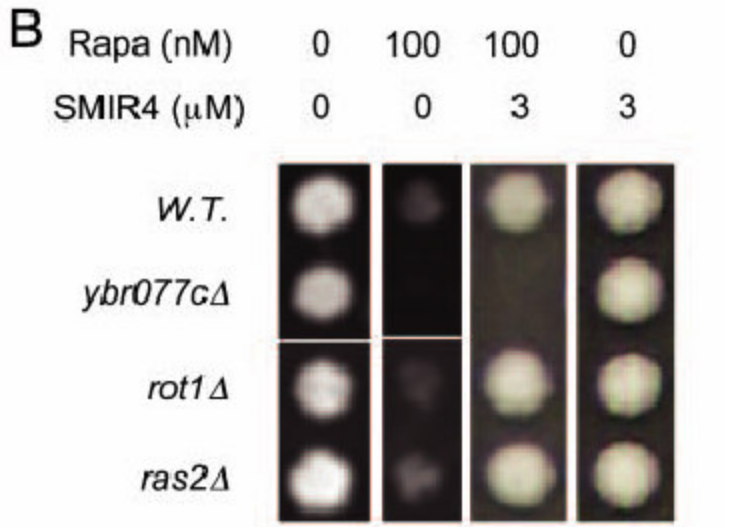


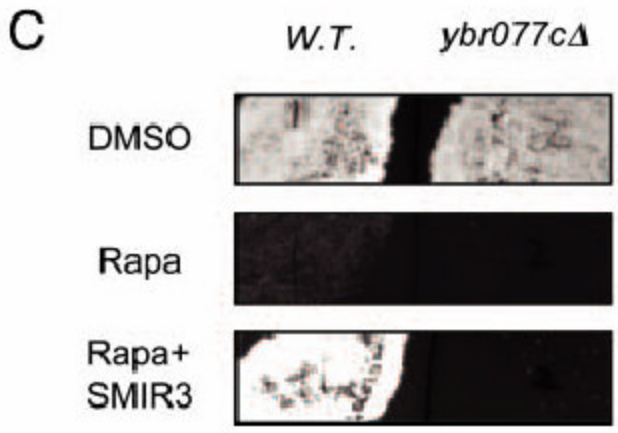
Fig. 2. SMIRs suppress rapamycin's effect in yeast on the whole-genome scale as revealed by mRNA transcript abundance analysis. (*A*) Example views of GeneChip features containing probes (outlined in white) specific for the GDH3 and URA7 transcripts (up- and down-regulated by rapamycin treatment, respectively) on the Affymetrix Ye6100 oligonucleotide arrays. (*B*) For comparing the effects of SMIRs and genetic mutations, a 2D clustergram with experiment tree and gene tree was generated in GENESPRING (Silicon Genetics, Redwood City, CA) by using the hierarchical clustering program (minimal distance, 0.001; separation ratio, 5; new tree with standard correlation). (*C*) Profiling data visualized by 3D scatter plots in GENESPRING. Fold changes over DMSO mock treatment were plotted for 6,430 genes on the Affymetrix chip. Color scaling is based on the rapamycin profile: red, increase; blue, decrease.

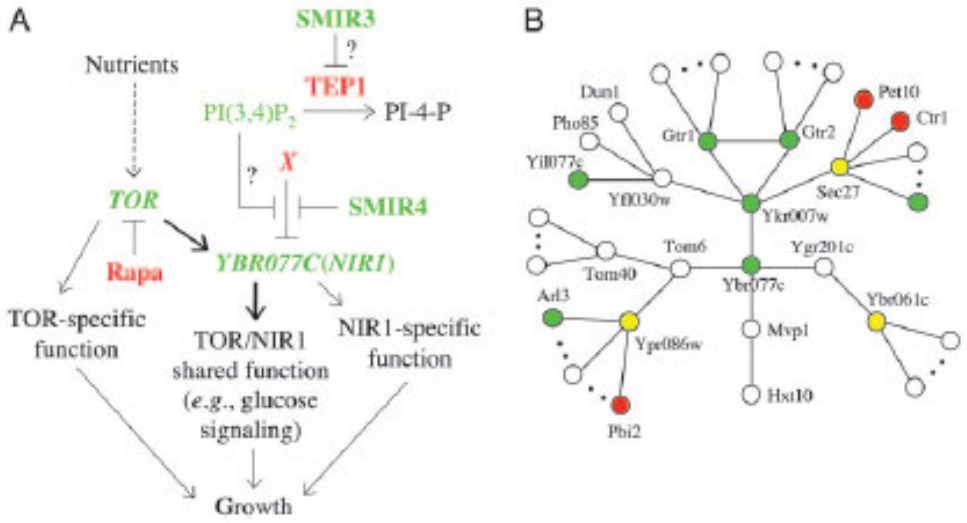
Yeast Array Probed with Biotin Tagged Drug



SMIR = Small Molecule Inhibitor of rapamyacin







C

Gene Name	Function	Fold Change over W.T.		Clusters
		W.T.+rapa	nir1Δ	
GAT1	Carbon responsive GATA factor	7.7	8.7	
GAP1	General amino acid permease	34.8	11.8	
GDH3	NADP-linked glutamate dehydrogenase	46.4	5.5	TOR/NIR1
DAL2	Allantoicase	2.2	6.8	Shared
DAL3	Ureidoglycolate hydrolase	56.8	−175.5	
DUR1,2	Urea amidolyase	27.2	11.8	
DAL5	Allantoate permease	80.2	NC	
MEP2	Ammonia transport protein	56.1	NC	
PUT1	Proline oxidase	24.8	NC	
URA7	CTP synthase	-51.8	NC	TOR
PHO3	Acid phosphatase	-16	NC	Specific
RPA190	RNA polymerase I subunit	-54.7	NC	
GCD10	Translation initiation factor eIF-3 subunit	-18.9	NC	
RAS1	Ras proto-oncogene homolog	-5.8	NC	
SPL2	Similarity to Cdk inhibitors	NC	29.6	
RPS26A	Ribosomal protein S26A	NC/D	8.3	NIR1
PAS2	Ubiquitin-conjugating protein family	NC	−119.8	Specific
YAR069C	Unknown	NC	−411.6	

Fig. 4. Ybr077cp (Nir1p) is a new component of the TOR signaling network. (*A*) Model of SMIR3 and SMIR4 suppression of the antiproliferative effect of rapamycin possibly by modulating PIs and thereby regulating Ybr077cp activity. (*B*) Graphic representation of a Ybr077cp protein interaction network and the rapamycin response phenotypes of individual deletion strains. Red, rapamycin-resistant when deleted; green, rapamycin-hypersensitive when deleted; yellow, essential genes whose heterozygous deletion did not produce detectable change in rapamycin sensitivity. (*C*) Gene-expression evidence for NIR1 function in the Tor network. NC, no change; D, decrease.

Enzyme activity profiles of the secreted and membrane proteome that depict cancer cell invasiveness

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Edited by James A. Wells, Sunesis Pharmaceuticals, Inc., South San Francisco, CA, and approved June 17, 2002 (received for review March 30, 2002)

Serine Hydrolase Activity of Secreted Proteins

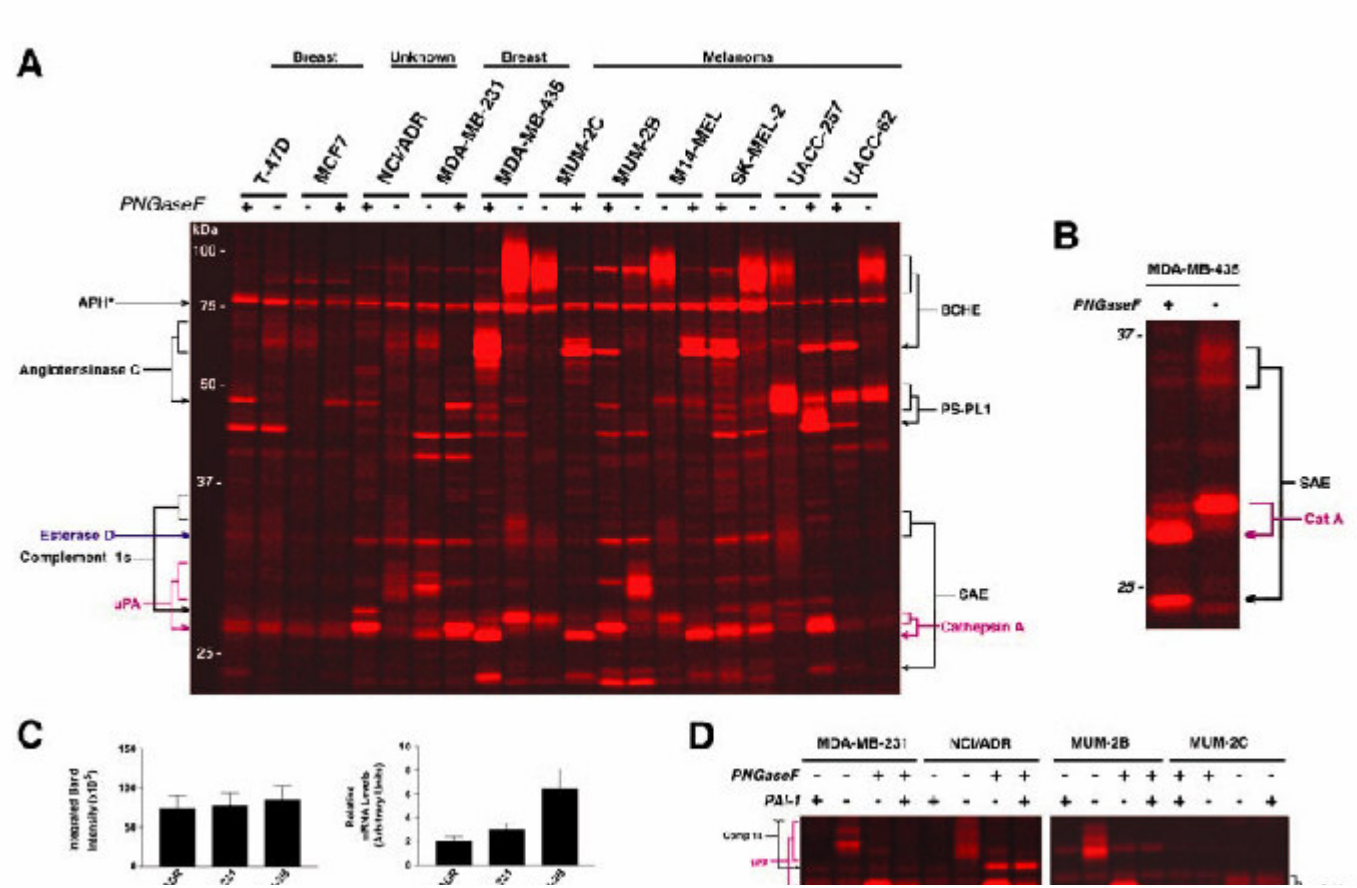


Fig. 1. Serine hydrolase activity profiles of the secreted proteomes of human cancer cell lines. (*A*) Representative in-gel fluorescence analysis of secreted serine hydrolase activity profiles obtained from reactions between cancer cell line conditioned media and a rhodamine-tagged FP. Enzyme activities are identified on either side of the gel (arrowheads point to the deglycosylated form of each enzyme; see Fig. 3*A* for complete names of proteins). Deglycosylation was accomplished by treatment of a portion of the FP-labeled proteomes with PNGaseF before analysis. APH* refers to acyl peptide hydrolase, a cytosolic protein detected in the conditioned media. (*B*) Expanded view of FP-labeled secreted MDA-MB-435 proteome highlights the increased resolution that is achieved for highly glycosylated enzyme activities (e.g., SAE) after treatment with PNGaseF. (*C*) Levels of active urokinase secreted by cancer cell lines as measured by ABPP (left panel) and urokinase mRNA levels as measured by Northern analysis ($n = 3$ or 4). mRNA levels are expressed in arbitrary units relative to an internal control. (*D*) Inhibition of urokinase (uPA) activity by PA-I. Pretreatment of each proteome with PA-I (20 μg/ml) blocked the labeling of uPA by FP-rhodamine, but did not affect the labeling of other serine proteases (e.g., Comp 1s and cat. A).

Serine Hydrolase Activity of Membrane Proteins

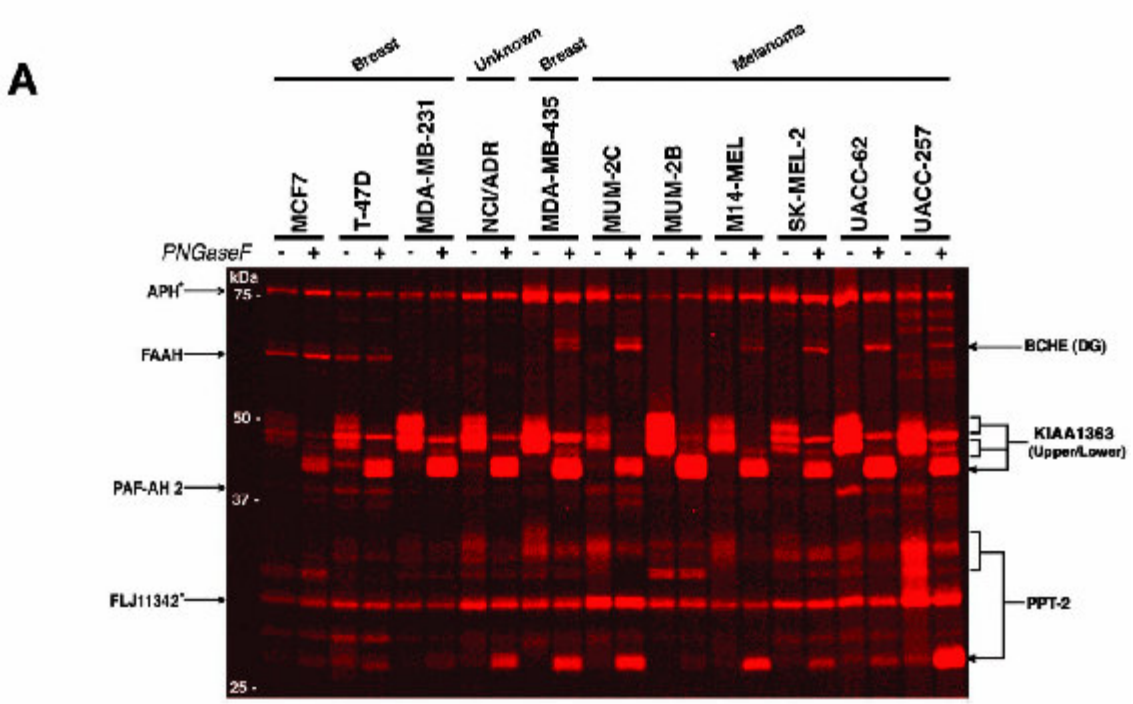


Fig. 2. Serine hydrolase activity profiles of the membrane proteomes of human cancer cell lines. (*A*) Representative in-gel fluorescence analyses of the serine hydrolase activity profiles of cancer cell membrane proteomes. Enzyme activities are identified on either side of the gels (arrowheads point to the deglycosylated form of each enzyme; see Fig. 3*A* for full names of proteins). Proteins marked with an asterisk represent soluble hydrolases also detected in the membrane proteome. DG, deglycosylated. (*B*) The activity of FAAH in breast cancer membranes as measured by ABPP (*Left*) and FAAH substrate (*Right*) assays. (*C*) Relative activity levels for upper and lower glycosylated forms of the membrane hydrolase KIAA1363 in MDA-MB-231 and MDA-MB-435 lines. Shown are a representative in-gel fluorescence analysis (*Left*) and the ratio of upper to lower glycosylated forms, expressed as ratio-1 (*Right*).

Cluster Analysis of Serine Hydrolase Activity Profiles

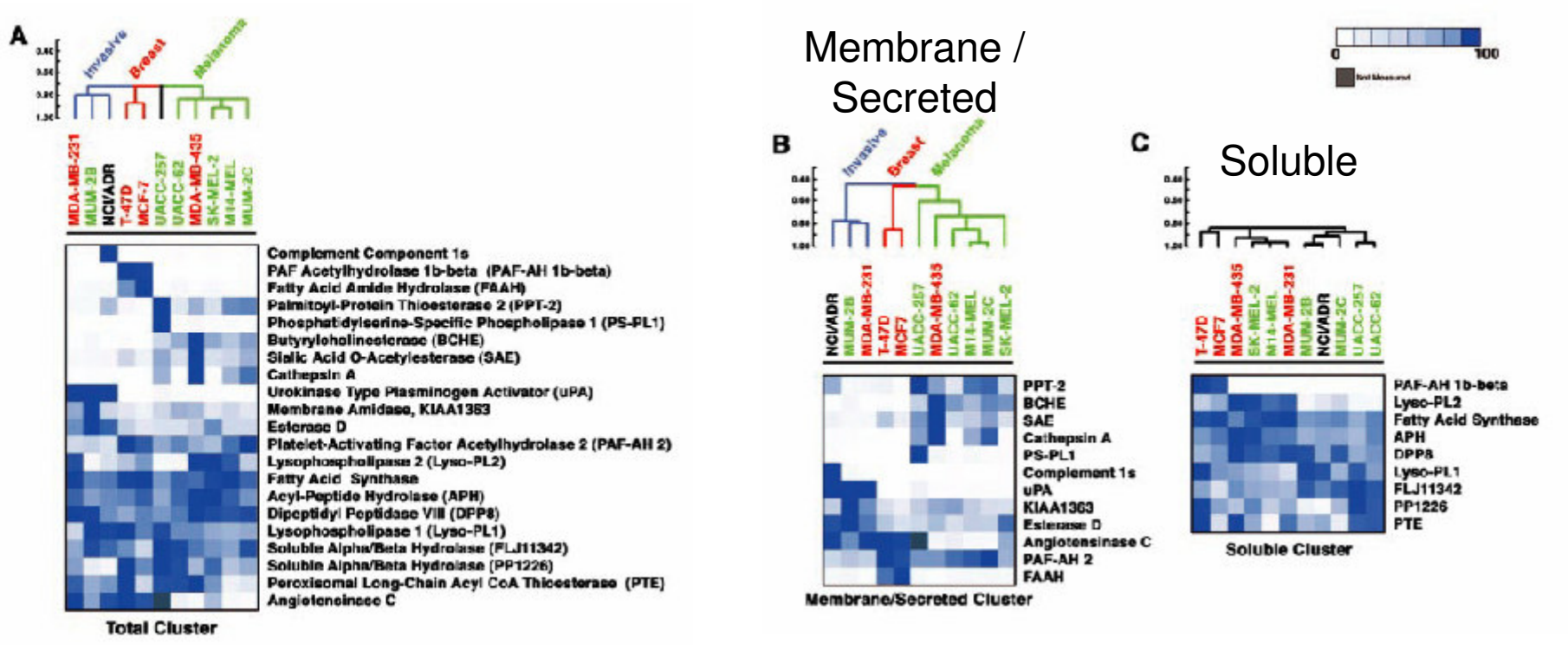
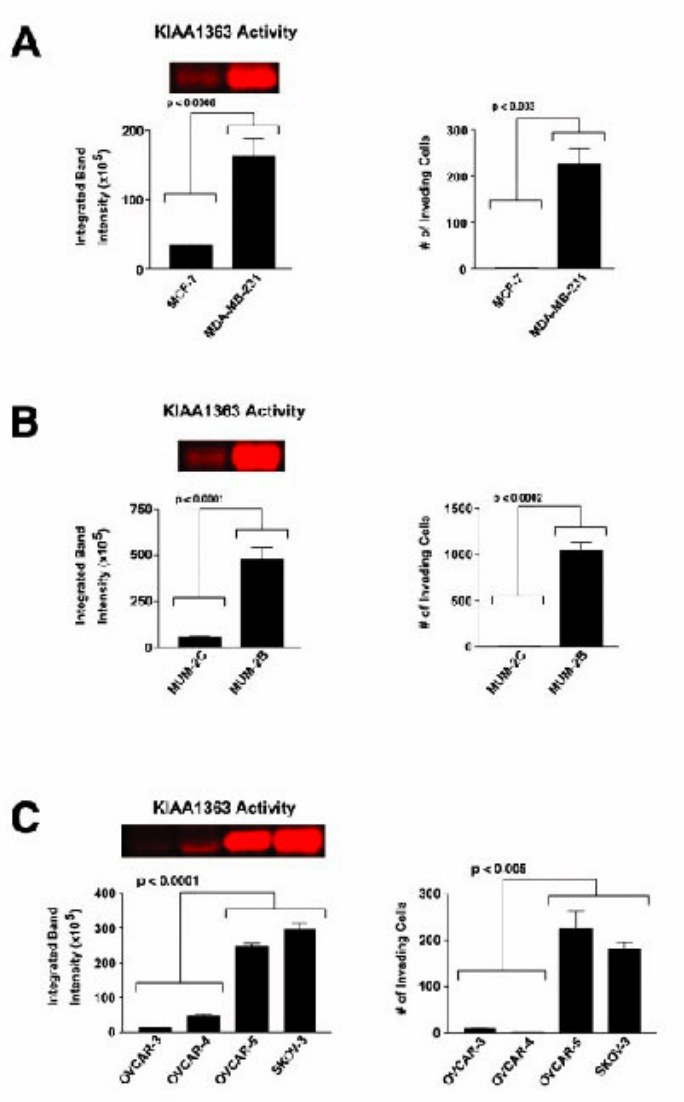


Fig. 3. Cluster analysis of the serine hydrolase activity profiles of human cancer cell lines. (*A–C*) A hierarchical clustering algorithm was applied to the cell lines by average linkage clustering using the Pearson correlation coefficient as the measure of similarity (CLUSTER computer package). Bars to the left of the dendrograms represent similarity scores. Shown are the results of cluster analyses conducted on total (*A*), membrane/secreted (*B*), and soluble (*C*) serine hydrolase activity profiles. The intensity of blue color scales directly with the relative activity of each hydrolase among the cell lines (0–100%, where for each enzyme, 100% represents the cell line with the highest activity, and the rest of the cell lines are expressed as a percentage of this highest activity to normalize the data sets). Gray, not measured. Red, breast cancer lines. Green, melanoma cancer lines. Black, NCI/ADR is of unknown origin.

Correlation Between Activity of a Protein with K1AA1363 and Invasiveness



Breast Carcinoma Lines

Melanoma Lines

Ovarian Carcinoma

- **What should be covered in your Journal Club presentation:**

  - Provide background. You need to introduce and state the problem being addressed
    - You should provide both biology background as well as experimental background as required for the audience to understand the topic
    - You may discuss older or other articles for the background section

  - Discuss the methods & experimental design used
    - Tell how they did what they did (and don't forget why) -- this section may blend into both background and results

  - Discuss the presented results
    - Don't necessarily talk about everything in the article; focus on what you think is important

  - Discuss the paper's "discussion". That is define in your mind what is important both from a mass spec point of view and from a science point of view.
    - Include:
      - The authors thoughts about what is important about the work reported
      - Your thoughts about what is important
  - Critique: during your discussion of both results and discussion, point out limitations and problems that you have identified with the paper and why

- **Use figures & tables from the article in your presentation:**

  - The figures you would like to present can be brought as either
    - Transparencies to use with an overhead projector.
    - Or a PDF or PowerPoint file. Please send me this file the day before your presentation so that I can have it on my computer
  - *Present as your first figure the title page of the article so that title, authors & affiliations, journal etc. are clear*