# AP-MS

Affinity purification followed by mass spectrometry (AP-MS) can be used for protein complex identification. Excellent examples can be found in studies of the SREBP pathway[1](#_ENREF_1),[2](#_ENREF_2), the mTOR complex[3-7](#_ENREF_3) and the mitochondrial pyruvate carrier[8](#_ENREF_8),[9](#_ENREF_9) and Ca2+ uniporter[10](#_ENREF_10).

## Affinity purification

* A protein of interest is fused to a tag (TAP[11](#_ENREF_11),[12](#_ENREF_12), FLAG[13](#_ENREF_13), HA[14](#_ENREF_14), 14-3-3[15-17](#_ENREF_15), etc.)
* Tagged protein complexes are purified with affinity columns.
* Proteins are digested into peptide fragments and separated by LC or 2D PAGE. Gel-free LC analysis is preferred because 2D PAGE is laborious, and protein comigration is an issue[18-22](#_ENREF_18).

## Mass spectrometry

* See [Broad Institute proteomics tutorials](https://www.broadinstitute.org/scientific-community/science/platforms/proteomics/tutorials-and-workshops) for general introduction.
* See [Mass spectrometry-based proteomics at Nature Methods](http://blogs.nature.com/methagora/2014/09/mass-spectrometry-based-proteomics-at-nature-methods.html) for general mass spec history.
* Mass spectrometry-based proteomic approaches are used to identify proteins that co-purify with the tagged proteins of interest.
* Create ions from peptides
* Separate ions by charge and mass
* Detect ions and determine m/z
* A tandem MS can be used to provide additional information.
  + - **MS3: MS1 (precursor ion detection), MS2 (peptide sequencing), MS3 (quantification)**
    - **The whole cycle takes 2 seconds in an orbitrap fusion tribrid.**
* Database analysis to identify peptides and proteins
* After analysis of MS data, interactions are usually identified and scored with an algorithm such as SAInt (Significance Analysis of Interactions)[23](#_ENREF_23),[24](#_ENREF_24).

## Quantification

* Absolute quantification can be achieved with SILAC (stable isotopic labeling of amino acids in culture) or TMT (tandem mass tagging).
  + **SILAC** cells are grown with labeled amino acids. Different isotopes can be used for different experimental conditions, allowing comparison among treatments.
  + **TMT** was developed by Steve Gygi’s lab and does not require labeled amino acids, making it superior to SILAC. TMTs are used to label primary amine groups for multiplexing. They consist of an amine-reactive NHS-ester group, a spacer arm and a mass reporter. Ten different tags are available, allowing ten samples in the same mass spectrometry run. The tags are isobaric (meaning that they elute at the same time during LC, and have the same mass during MS1 acquisition), but after MS2 peptide sequencing, they fragment into unique ion masses during MS3 reporter ion quantification. **TMT is vastly superior to SILAC. It does not require growth of cells in labeled amino acids. SILAC can only differentiate between 2-3 groups, whereas TMT can quantify 10 samples in the same run, reducing the effects of inter-assay variability.**
  + From thermo quantitative proteomics handbook: "Isobaric chemical tags are powerful tools that enable concurrent identification and quantitation of proteins in different samples using tandem mass spectrometry. These tags contain reactive groups that covalently label peptide amino termini, peptide cysteine amino acid side changes or glycopeptides, depending on the chemistry used. During the tandem mass spectrometry (MS/MS) analysis, the isobaric tag produces a unique reporter ion signature that makes quantitation possible. In the first MS analysis, the labeled peptides are indistinguishable from each other; however, in the tandem MS mode during which peptides are isolated and fragmented, the tag generates a unique reporter ion. Protein quantitation is then accomplished by comparing the intensities of the six reporter ions in the MS/MS spectra."
  + TMTs with cross-linkers: **The tandem mass tags used for quantification react with primary amine groups. Most cross-linkers also react with amine groups. Even if reversible, this could affect the ability of the peptide to bind a TMT for quantification. We could try a carboxy-reactive cross linker.**

## Post-translational modifications

* + See [“Global PTM analysis”](https://www.broadinstitute.org/partnerships/education/broade/proteomics-everything-you-always-wanted-know-were-afraid-ask) Broad lecture
  + PTMs of consistent mass (phosphorylation, acetylation[25](#_ENREF_25), methylation and ubiquitination[26](#_ENREF_26)) can be detected by observing a mass shift
  + Many PTMs occur at low cellular abundance, requiring antibody-based peptide enrichment before LC-MS/MS.
  + Glycosylation can be detected using lectin affinity chromatography[27](#_ENREF_27). Glycosylation analysis is complicated because, unlike phosphorylation, glycan chains are heterogeneous and labile. A new method called IsoTaG may offer improved mass-independent detection of glycosylation[28](#_ENREF_28).
  + [PhosphoSite Plus](http://www.phosphosite.org/) is a database of PTMs, primarily phosphorylation[29](#_ENREF_29). The majority of PTM sites have been identified in high-throughput proteome-wide studies.

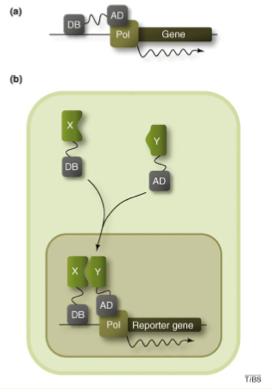
## AP-MS variations

* AP-MS is not typically great with membrane proteins. Modifications can improve results.
  + Gavin et al.[30](#_ENREF_30),[31](#_ENREF_31) used modified purification procedures for membrane proteins.
  + Babu et al.[32](#_ENREF_32) used non-denaturing detergents in conjunction with a TAP tag AP-MS approach to characterize the membrane protein complexome in yeast.
  + BioID[33](#_ENREF_33" \o "Roux, 2012 #844): Similar to the DamID DNA-protein interaction method. A biotin ligase is fused to the protein of interest. Other proteins that interact with the protein of interest will be stably biotinylated. Biotinylated proteins can then be affinity purified and identified with mass spectrometry. In comparison with conventional AP-MS analysis, this method works better for membrane proteins and identifies many interactions that would be missed[13](#_ENREF_13).
* APEX proximity biotin labeling[34-37](#_ENREF_34): developed at MIT and the Broad. Interesting approach to map protein interactions in a native cellular context using ascorbate peroxidase. I hadn’t previously proposed this because it has not been as widely used as AP-MS, and may not also be applicable to animal models. Also, we would have to work with the Broad, and based on input I received they would be slow and would not prioritize our work. You still have to purify the proteins anyway with this method.

## Notes

* Mass spectrometry can be performed in either discovery mode, which is not as reproducible but allows for unbiased identification of a large number of proteins, or targeted mode, where a pre-selected set of proteins is detected. Pre-selecting a set of proteins increases reproducibility and lowers the limit of detection.
* Both “bottom up” and “top down” approaches can be used[22](#_ENREF_22),[38](#_ENREF_38). See next page and [Broad proteomics workshops](https://www.broadinstitute.org/partnerships/education/broade/proteomics-everything-you-always-wanted-know-were-afraid-ask).
  + “Bottom-up” proteomics is the most common technique. Proteins are fragmented into peptides by enzymatic digestion, and the peptides are matched to proteins based on bioinformatic prediction of fragmentation patterns.
  + For “top-down” proteomics, intact proteins are separated by chromatography without initial digestion into peptides. Sequence coverage is inherently higher than bottom-up proteomics, because the proteins are not fragmented prior to analysis. This may allow more comprehensive analysis of post-translational modifications. It is challenging to reduce sample complexity due to the wide range of protein sizes and expression levels. Bioinformatics tools are not as well developed as for bottom-up.
* High-throughput[22](#_ENREF_22) (multiple reaction monitoring can be used in targeted proteomics to detect 200 proteins in multiplex[39](#_ENREF_39), hyperplexing can detect 3000 proteins[40](#_ENREF_40), SWATH-MS can detect 4-5000 proteins[23](#_ENREF_23),[27](#_ENREF_27),[41](#_ENREF_41),[42](#_ENREF_42)).
* Standardization of procedures can result in highly reproducible results[43](#_ENREF_43),[44](#_ENREF_44).
* Detects stable, long-term interactions. Cross-linking can also be used to keep complexes together[3-6](#_ENREF_3),[45-48](#_ENREF_45). Hybrid assays are better for transient or low-affinity interactions (see below).
* Overexpression of tagged proteins may result in false-positive interactions that do not occur at physiological expression levels[49](#_ENREF_49).

# Hybrid assays

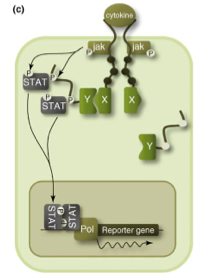


Liebens TiBS 2009

* Method[50-55](#_ENREF_50):
  + **This method tests binary (yes/no) interactions between protein pairs and uses a transcriptional reporter readout.**
  + One protein, the “bait,” is fused to the DNA binding domain of a transcription factor such as Gal4 in yeast.
  + The second protein, the “prey,” is fused to the transcriptional activation domain of the same transcription factor.
  + If the two proteins interact, the reporter gene will be transcribed.
  + Particularly good at identifying transient and low-affinity interactions[53](#_ENREF_53),[54](#_ENREF_54).
  + Limitations[51](#_ENREF_51),[53](#_ENREF_53): traditional Y2H assays are somewhat artificial systems. Proteins of interest must be expressed and post-translationally processed the same way in yeast as in the original species. Both proteins must be soluble and able to enter the nucleus, so the basic Y2H system is not suitable for membrane proteins. Variations have been developed to address this limitation (see below). Y2H systems can be prone to false positives if corrective methods are not employed[56](#_ENREF_56).
  + BioGRID definition: “Bait protein expressed as a DNA binding domain (DBD) fusion and prey expressed as a transcriptional activation domain (TAD) fusion and interaction measured by reporter gene activation.”
* Hybrid assays are being used to develop the [Human Interactome Database](http://interactome.dfci.harvard.edu/index.php?page=home)[57](#_ENREF_57).

### Hybrid assay variations

* MAPPIT (mammalian protein-protein interaction trap)[51](#_ENREF_51),[58](#_ENREF_58)
  + A hybrid mammalian cell assay using a split cytokine receptor, with signal pathway-dependent transcriptional activation as the output.



Liebens TiBS 2009

* + Description[51](#_ENREF_51): “Bait X is coupled to a signalling-deficient cytokine receptor (via substituting tyrosine residues in the receptor tail which are critical for recruiting signal transducer and activator of transcription (STAT) proteins (brown dots)). The prey Y is tethered to a portion of another receptor containing intact recruitment sites. Upon bait–prey interaction, a functional receptor is reconstituted and it can be activated by the appropriate cytokine ligand. Upon ligand binding, pre-associated Janus kinases (JAK) are activated by cross-phosphorylation (P). Activated JAKs phosphorylate (P) tyrosine residues in the receptor fragment coupled to the prey (white dots), which then act as docking sites for STATs. Recruited STATs are, in turn, phosphorylated by the JAKs, leading to their activation and subsequent dissociation and translocation to the nucleus. In the nucleus, STAT dimers induce STAT-dependent reporter gene transcription. In the MASPIT setup, dihydrofolate reductase (DHFR) is coupled to the signalling deficient receptor chain, and a chemical compound is fused to methotrexate, a molecule that binds to DHFR with very high affinity. Addition of such a methotrexate fusion compound to cells expressing the DHFR-coupled receptor results in the chemical compound being displayed as a bait.”
* Split ubiquitin[59](#_ENREF_59)
  + Bait and prey proteins are fused to halves of ubiquitin. A point mutation is introduced into the ubiquitin molecule to prevent spontaneous reassociation, and a reporter transcription factor is conjugated to the bait fragment. When a prey protein interacts with the membrane-bound bait, ubiquitin is reconstituted, targeted and cleaved by deubiquitinases, and the reporter transcription factor travels to the nucleus.
* A split ubiquitin variant called the mating-based split-ubiquitin system (mbSUS) has been used to interrogate the membrane protein interactome of *Arabidopsis*[60](#_ENREF_60).
* iMYTH (integrated split ubiquitin membrane yeast two-hybrid)[61](#_ENREF_61),[62](#_ENREF_62): An improved version of the earlier membrane Y2H method (MYTH)[63](#_ENREF_63),[64](#_ENREF_64) optimized for membrane proteins.
* **MaMTH (mammalian membrane two-hybrid)**[**65**](#_ENREF_65)
  + **MaMTH is a split ubiquitin hybrid screen for membrane protein interactions in mammalian cells. It tests binary (yes/no) interactions between protein pairs and uses a transcriptional reporter readout. Susan Lindquist’s lab at the Whitehead Institute collaborated on the MaMTH paper.**
  + **Method**
    - **HEK-293 cells are engineered to stably express a luciferase or GFP reporter**
    - **A membrane “bait” protein (Nrf1) is tagged with the c-terminal half of ubiquitin (Cub) and a chimeric transcription factor (such as ErbB)**
    - **Other “prey” proteins (either cytosolic or membrane-bound) are tagged with the N-terminal half of ubiquitin (Nub)**
    - **When a prey protein interacts with the membrane-bound bait, ubiquitin is reconstituted, targeted and cleaved by deubiquitinases, and the reporter transcription factor travels to the nucleus, generating a reporter readout.**
* Fluorescence 3 hybrid[66](#_ENREF_66): similar to a FRET approach. A GFP-binding nanobody is fused to another protein with stable subcellular localization (such as the nucleus). It can then recruit a bait protein labeled with GFP. Candidate prey proteins are labeled with RFP. An interaction is visible with fluorescence microscopy. The method can be adapted for any cell type and subcellular location.

# Other methods

* **FRET**
  + Forster/Fluorescence Resonance Energy Transfer
  + FRET is used to detect protein-protein interactions in real-time with high sensitivity.
  + Attach two different fluorescent proteins (e.g. CFP and YFP) to two different interacting protein of interest (such as a nuclear receptor and a coactivator). One, designated the “donor,” gets CFP, “acceptor” gets YFP.
  + Excite the donor with the appropriate wavelength of light (433 nm for CFP). If the two proteins are not interacting, the donor protein will emit fluorescence. However, if the two proteins are interacting, CFP and YFP will be in close proximity. Resonance energy will be transferred from the donor (CFP) to the acceptor (YFP). The acceptor then decays to its ground state by fluorescence at its own wavelength (527 nm for YFP).
  + Advantages: detects transient and dynamic interactions
  + Limitations: low-throughput, technically complex
  + BioGRID: “An interaction is inferred when close proximity of interaction partners is detected by fluorescence resonance energy transfer between pairs of fluorophore-labeled molecules, such as occurs between CFP (donor) and YFP (acceptor) fusion proteins.”
* LUMIER[51](#_ENREF_51),[53](#_ENREF_53): a form of affinity purification where one protein is FLAG tagged and the other protein conjugated to luciferase. FLAG is captured by affinity purification, and an interaction will be indicated with luminescence.
* Microfluidics: a microfluidic system was developed to address some of the limitations of AP-MS and Y2H experiments. It is basically a miniaturized immunoprecipitation assay, so it has the same limitations as low-throughput immunoprecipitation assays. The initial system could perform 640 reactions per chip and was used in the initial publication to map the protein-protein interactions of 43 *Streptococcus pneumonia* proteins[67](#_ENREF_67). The technique was later refined to perform 4000 reactions per chip and used by the same group to map the interactome of proteins with unknown function in the same species[68](#_ENREF_68). Another group used a different microfluidic system in combination with imaging ellipsometry to determine SNARE protein interactions[69](#_ENREF_69), but the advantages of this system are not clear.
* Microarrays[70](#_ENREF_70),[71](#_ENREF_71): protein microarrays were created by spotting purified proteins onto a microarray, with BSA used as a stabilizer. This system is primarily for purified proteins, and while useful for high-throughput drug screening, does not test protein-protein interactions *in vivo*. It has not been widely adopted.

# NFE2L1/Nrf1 background

**See Nrf1 background document**

# NFE2L1 interacting proteins

**HCFC1**

HCFC is a coactivator and has been previously identified as an Nrf1-interacting protein (see Tsuchiya Y *MCB* 2013 supplementary table 1, [BioGRID](http://thebiogrid.org/110851/table/homo-sapiens/nfe2l1.html" \t "_blank)).

**OGT**

Chen j FEBS letters 2015

Tsuchiya 2013 supplementary data

2/27 mass spec SEE LABGURU

* I replicated the HCFC1 and OGT interactions that we saw in our previous analysis. This, in combination with their previous detection in other published datasets, makes them relatively high-confidence interactions. **The HCFC1 and OGT interactions have been demonstrated previously in the literature, but their functional significance is not known.**
* Both HCFC1 and OGT were identified as Nrf1-interacting proteins in an Nrf1-Flag AP-MS dataset (Tsuchiya Y MCB 2013 supplementary table 1, think this is COS7 cells but it's not clear from the article).
* Bugno BBA 2015 p.1265 says that OGT glycosylates Nrf1 in the ER to regulate its ER membrane retention. They cite Zhang PLoS One 9:e93458, 2014, but this paper does not even mention OGT. Zhang cites other papers that describe OST (Oligosaccharyltransferase), such as Shibatani Biochemistry 2005.
* Chen et al. FEBS Letters 2015 also showed that O-GlcNAcylation by OGT increases Nrf1 turnover, but did not determine the specific cellular compartment in which this occurs.
* OGT and HCFC1 also interact with each other. ***FINISH***

**Casein Kinase**

TSUCHIYA REFS

2013 paper cites 2011 paper. Neither one describes mass spec methods.

2011 paper has no supplementary data

2013 paper has interacting proteins in supplementary data

**Hrd1**

Tsuchiya 2011

Note that this is not listed in BioGRID.

**FBXW11**

Tsuchiya 2013 supplementary data

The existing interactome hits are mostly transcription factors or proteasome-related proteins. The hits may represent Nrf1 in its various forms (ER bound or transcriptionally active). Membrane-bound proteins are difficult to study, and few if any of these interactions were detected using methods optimized for them. Additionally, the Vidal group had a recent paper showing how other literature-curated databases are not always reliable[72](#_ENREF_72).

* Human interactome database
  + KEAP1
  + MAFF
  + CREBZF
* IntAct
  + CD6
  + Cdk9
  + fadL
  + KEAP1
  + lysU
  + recC
  + TULP3
  + 3mgh\_bacan: Putative 3-methyladenine DNA glycosylase ([UniProt](http://www.uniprot.org/uniprot/Q81UJ9))
  + q7cjc9\_yerpe: uncharacterized protein from Yersinia pestis ([UniProt](http://www.uniprot.org/uniprot/Q7CJC9))
* [BioGRID](http://thebiogrid.org/110851/table/homo-sapiens/nfe2l1.html?sort=official)
  + Bach2[73](#_ENREF_73): repressor of Nrf transcriptional activity[74](#_ENREF_74)
  + CAPN1[75](#_ENREF_75): calpain 1. [UniProt](http://www.uniprot.org/uniprot/P07384): Calcium-regulated non-lysosomal thiol-protease which catalyze limited proteolysis of substrates involved in cytoskeletal remodeling and signal transduction.
  + CD6[76](#_ENREF_76): adhesion protein
  + CREBZF[73](#_ENREF_73): coactivator of HCFC1
  + **FBXW7**[**77**](#_ENREF_77)**,**[**78**](#_ENREF_78)**: ubiquitin ligase.**
  + GSK3B[78](#_ENREF_78): glycogen synthase kinase 3 β
  + HCFC1[79](#_ENREF_79): coactivator involved in transcriptional regulation during herpes infection. Has also been shown to interact with Nrf3.
  + JUN[80](#_ENREF_80): transcription factor and oncogene
  + KEAP1[76](#_ENREF_76),[81-83](#_ENREF_81): NFE2L1 sequestered by cytoplasmic inhibitor with same mechanism as NFE2L2/Nrf2. Wang et al[76](#_ENREF_76) identified NFE2L1 as a node in the ROS subnetwork of the human liver interactome.
  + MAF F/G[73](#_ENREF_73),[84](#_ENREF_84): the interaction with Maf proteins regulates transcription[74](#_ENREF_74),[85](#_ENREF_85)
  + NFE2L3[73](#_ENREF_73)
  + **SUMO2**[**86**](#_ENREF_86)**: small ubiquitin-like modifier. Not directly involved in proteasomal degradation, but may play a role in protein quality control**[**86**](#_ENREF_86)**.**
  + **UBC**[**14**](#_ENREF_14)**,**[**26**](#_ENREF_26)**,**[**77**](#_ENREF_77)**,**[**78**](#_ENREF_78)**,**[**83**](#_ENREF_83)**,**[**87-92**](#_ENREF_87)**: polyubiquitin C.**

## Full list from BioGRID

**Downloaded 02/05/2016**

| [**Interactor**](javascript:changeSort(%20'table',%20'interaction-body',%20'interactor'%20)) | [**Role**](javascript:changeSort(%20'table',%20'interaction-body',%20'role'%20)) | [**Organism**](javascript:changeSort(%20'table',%20'interaction-body',%20'organism'%20)) | [**Experimental Evidence Code**](javascript:changeSort(%20'table',%20'interaction-body',%20'system'%20)) | [**Dataset**](javascript:changeSort(%20'table',%20'interaction-body',%20'publication'%20)) | [**Throughput**](javascript:changeSort(%20'table',%20'interaction-body',%20'throughput'%20)) |
| --- | --- | --- | --- | --- | --- |
| [BACH2](http://thebiogrid.org/121913/table/homo-sapiens/bach2.html?sort=official) | HIT | H. sapiens | **FRET** | [Reinke AW (2013)](http://thebiogrid.org/156691/publication/networks-of-bzip-protein-protein-interactions-diversified-over-a-billion-years-of-evolution.html) | High |
| [BRD9](http://thebiogrid.org/122430/table/homo-sapiens/brd9.html?sort=official) | HIT | H. sapiens | **Affinity Capture-MS** | [Hein MY (2015)](http://thebiogrid.org/188719/publication/a-human-interactome-in-three-quantitative-dimensions-organized-by-stoichiometries-and-abundances.html) | High |
| [BTRC](http://thebiogrid.org/114457/table/homo-sapiens/btrc.html?sort=official) | BAIT | H. sapiens | **Affinity Capture-MS** | [Coyaud E (2015)](http://thebiogrid.org/185962/publication/bioid-based-identification-of-skp-cullin-f-box-scf-trcp12-e3-ligase-substrates.html) | High |
| [BTRC](http://thebiogrid.org/114457/table/homo-sapiens/btrc.html?sort=official) | BAIT | H. sapiens | **Proximity Label-MS** | [Coyaud E (2015)](http://thebiogrid.org/185962/publication/bioid-based-identification-of-skp-cullin-f-box-scf-trcp12-e3-ligase-substrates.html) | High |
| [C8ORF33](http://thebiogrid.org/122420/table/homo-sapiens/c8orf33.html?sort=official) | HIT | H. sapiens | **Affinity Capture-MS** | [Hein MY (2015)](http://thebiogrid.org/188719/publication/a-human-interactome-in-three-quantitative-dimensions-organized-by-stoichiometries-and-abundances.html) | High |
| [CAPN1](http://thebiogrid.org/107273/table/homo-sapiens/capn1.html?sort=official) | BAIT | H. sapiens | **Two-hybrid** | [Jiang LQ (2002)](http://thebiogrid.org/154117/publication/screening-the-proteins-that-interact-with-calpain-in-a-human-heart-cdna-library-using-a-yeast-two-hybrid-system.html) | Low |
| [CD6](http://thebiogrid.org/107361/table/homo-sapiens/cd6.html?sort=official) | BAIT | H. sapiens | **Two-hybrid** | [Wang J (2011)](http://thebiogrid.org/151081/publication/toward-an-understanding-of-the-protein-interaction-network-of-the-human-liver.html) | High |
| [CREBZF](http://thebiogrid.org/121817/table/homo-sapiens/crebzf.html?sort=official) | BAIT | H. sapiens | **FRET** | [Reinke AW (2013)](http://thebiogrid.org/156691/publication/networks-of-bzip-protein-protein-interactions-diversified-over-a-billion-years-of-evolution.html) | High |
| [FBXW7](http://thebiogrid.org/120581/table/homo-sapiens/fbxw7.html?sort=official) | HIT | H. sapiens | **Affinity Capture-Western** | [Biswas M (2011)](http://thebiogrid.org/126014/publication/the-fbw7-tumor-suppressor-regulates-nuclear-factor-e2-related-factor-1-transcription-factor-turnover-through-proteasome-mediated-proteolysis.html) | Low |
| [FBXW7](http://thebiogrid.org/120581/table/homo-sapiens/fbxw7.html?sort=official) | HIT | H. sapiens | **Affinity Capture-Western** | [Biswas M (2013)](http://thebiogrid.org/154931/publication/glycogen-synthase-kinase-3-regulates-expression-of-nuclear-factor-erythroid-2-related-transcription-factor-1-nrf1-and-inhibits-pro-survival-function-of-nrf1.html) | Low |
| [FBXW11](http://thebiogrid.org/116887/table/homo-sapiens/fbxw11.html?sort=official) | BAIT | H. sapiens | **Affinity Capture-MS** | [Low TY (2014)](http://thebiogrid.org/187503/publication/a-systems-wide-screen-identifies-substrates-of-the-scftrcp-ubiquitin-ligase.html) | High |
| [GSK3B](http://thebiogrid.org/109187/table/homo-sapiens/gsk3b.html?sort=official) | BAIT | H. sapiens | **Affinity Capture-Western** | [Biswas M (2013)](http://thebiogrid.org/154931/publication/glycogen-synthase-kinase-3-regulates-expression-of-nuclear-factor-erythroid-2-related-transcription-factor-1-nrf1-and-inhibits-pro-survival-function-of-nrf1.html) | Low |
| [GSK3B](http://thebiogrid.org/109187/table/homo-sapiens/gsk3b.html?sort=official) | BAIT | H. sapiens | **Biochemical Activity** | [Biswas M (2013)](http://thebiogrid.org/154931/publication/glycogen-synthase-kinase-3-regulates-expression-of-nuclear-factor-erythroid-2-related-transcription-factor-1-nrf1-and-inhibits-pro-survival-function-of-nrf1.html) | Low |
| [HCFC1](http://thebiogrid.org/109304/table/homo-sapiens/hcfc1.html?sort=official) | BAIT | H. sapiens | **Two-hybrid** | [Ravasi T (2010)](http://thebiogrid.org/105677/publication/an-atlas-of-combinatorial-transcriptional-regulation-in-mouse-and-man.html) | High |
| [JUN](http://thebiogrid.org/109928/table/homo-sapiens/jun.html?sort=official) | BAIT | H. sapiens | **Affinity Capture-Western** | [Venugopal R (1998)](http://thebiogrid.org/5373/publication/nrf2-and-nrf1-in-association-with-jun-proteins-regulate-antioxidant-response-element-mediated-expression-and-coordinated-induction-of-genes-encoding-detoxifying-enzymes.html) | Low |
| [JUN](http://thebiogrid.org/109928/table/homo-sapiens/jun.html?sort=official) | BAIT | H. sapiens | **Reconstituted Complex** | [Venugopal R (1998)](http://thebiogrid.org/5373/publication/nrf2-and-nrf1-in-association-with-jun-proteins-regulate-antioxidant-response-element-mediated-expression-and-coordinated-induction-of-genes-encoding-detoxifying-enzymes.html) | Low |
| [KEAP1](http://thebiogrid.org/115156/table/homo-sapiens/keap1.html?sort=official) | HIT | H. sapiens | **Affinity Capture-Western** | [Wang W (2006)](http://thebiogrid.org/146889/publication/nrf1-is-targeted-to-the-endoplasmic-reticulum-membrane-by-an-n-terminal-transmembrane-domain-inhibition-of-nuclear-translocation-and-transacting-function.html) | Low |
| [KEAP1](http://thebiogrid.org/115156/table/homo-sapiens/keap1.html?sort=official) | BAIT | H. sapiens | **Affinity Capture-Western** | [Wang W (2006)](http://thebiogrid.org/146889/publication/nrf1-is-targeted-to-the-endoplasmic-reticulum-membrane-by-an-n-terminal-transmembrane-domain-inhibition-of-nuclear-translocation-and-transacting-function.html) | Low |
| [KEAP1](http://thebiogrid.org/115156/table/homo-sapiens/keap1.html?sort=official) | BAIT | H. sapiens | **Affinity Capture-Western** | [Niture SK (2013)](http://thebiogrid.org/161805/publication/oncogene-pkcvarepsilon-controls-inrf2nrf2-interaction-in-normal-and-cancer-cells-through-inrf2-phosphorylation.html) | Low |
| [KEAP1](http://thebiogrid.org/115156/table/homo-sapiens/keap1.html?sort=official) | BAIT | H. sapiens | **Two-hybrid** | [Yu M (2011)](http://thebiogrid.org/115827/publication/nuclear-factor-p65-interacts-with-keap1-to-repress-the-nrf2-are-pathway.html) | High |
| [KEAP1](http://thebiogrid.org/115156/table/homo-sapiens/keap1.html?sort=official) | BAIT | H. sapiens | **Two-hybrid** | [Wang J (2011)](http://thebiogrid.org/151081/publication/toward-an-understanding-of-the-protein-interaction-network-of-the-human-liver.html) | High |
| [MAFF](http://thebiogrid.org/117264/table/homo-sapiens/maff.html?sort=official) | BAIT | H. sapiens | **Affinity Capture-MS** | [Huttlin EL (2015)](http://thebiogrid.org/186338/publication/the-bioplex-network-a-systematic-exploration-of-the-human-interactome.html) | High |
| [MAFF](http://thebiogrid.org/117264/table/homo-sapiens/maff.html?sort=official) | HIT | H. sapiens | **FRET** | [Reinke AW (2013)](http://thebiogrid.org/156691/publication/networks-of-bzip-protein-protein-interactions-diversified-over-a-billion-years-of-evolution.html) | High |
| [MAFF](http://thebiogrid.org/117264/table/homo-sapiens/maff.html?sort=official) | BAIT | H. sapiens | **FRET** | [Reinke AW (2013)](http://thebiogrid.org/156691/publication/networks-of-bzip-protein-protein-interactions-diversified-over-a-billion-years-of-evolution.html) | High |
| [MAFF](http://thebiogrid.org/117264/table/homo-sapiens/maff.html?sort=official) | BAIT | H. sapiens | **Reconstituted Complex** | [Johnsen O (1996)](http://thebiogrid.org/5375/publication/small-maf-proteins-interact-with-the-human-transcription-factor-tcf11nrf1lcr-f1.html) | Low |
| [MAFG](http://thebiogrid.org/110272/table/homo-sapiens/mafg.html?sort=official) | HIT | H. sapiens | **FRET** | [Reinke AW (2013)](http://thebiogrid.org/156691/publication/networks-of-bzip-protein-protein-interactions-diversified-over-a-billion-years-of-evolution.html) | High |
| [MAFG](http://thebiogrid.org/110272/table/homo-sapiens/mafg.html?sort=official) | BAIT | H. sapiens | **Reconstituted Complex** | [Johnsen O (1996)](http://thebiogrid.org/5375/publication/small-maf-proteins-interact-with-the-human-transcription-factor-tcf11nrf1lcr-f1.html) | Low |
| [NFE2L1](http://thebiogrid.org/110851/table/homo-sapiens/nfe2l1.html?sort=official) | HIT | H. sapiens | **FRET** | [Reinke AW (2013)](http://thebiogrid.org/156691/publication/networks-of-bzip-protein-protein-interactions-diversified-over-a-billion-years-of-evolution.html) | High |
| [NFE2L3](http://thebiogrid.org/114967/table/homo-sapiens/nfe2l3.html?sort=official) | BAIT | H. sapiens | **FRET** | [Reinke AW (2013)](http://thebiogrid.org/156691/publication/networks-of-bzip-protein-protein-interactions-diversified-over-a-billion-years-of-evolution.html) | High |
| [RUSC2](http://thebiogrid.org/115187/table/homo-sapiens/rusc2.html?sort=official) | HIT | H. sapiens | **Affinity Capture-MS** | [Hein MY (2015)](http://thebiogrid.org/188719/publication/a-human-interactome-in-three-quantitative-dimensions-organized-by-stoichiometries-and-abundances.html) | High |
| [WDFY3](http://thebiogrid.org/116647/table/homo-sapiens/wdfy3.html?sort=official) | HIT | H. sapiens | **Affinity Capture-MS** | [Hein MY (2015)](http://thebiogrid.org/188719/publication/a-human-interactome-in-three-quantitative-dimensions-organized-by-stoichiometries-and-abundances.html) | High |

# References

1. Yang T, Espenshade PJ, Wright ME, Yabe D, Gong Y, Aebersold R, Goldstein JL, Brown MS. Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. *Cell* 110:489-500 (2002).

2. Goldstein JL, DeBose-Boyd RA, Brown MS. Protein sensors for membrane sterols. *Cell* 124:35-46 (2006).

3. Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, Sabatini DM. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* 320:1496-1501 (2008).

4. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell* 141:290-303 (2010).

5. Bar-Peled L, Schweitzer LD, Zoncu R, Sabatini DM. Ragulator is a GEF for the rag GTPases that signal amino acid levels to mTORC1. *Cell* 150:1196-1208 (2012).

6. Bar-Peled L, Chantranupong L, Cherniack AD, Chen WW, Ottina KA, Grabiner BC, Spear ED, Carter SL, Meyerson M, Sabatini DM. A tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. *Science* 340:1100-1106 (2013).

7. Chantranupong L, Wolfson RL, Orozco JM, Saxton RA, Scaria SM, Bar-Peled L, Spooner E, Isasa M, Gygi SP, Sabatini DM. The Sestrins interact with GATOR2 to negatively regulate the amino-acid-sensing pathway upstream of mTORC1. *Cell Rep.* 9:1-8 (2014).

8. Bricker DK, Taylor EB, Schell JC, Orsak T, Boutron A, Chen YC, Cox JE, Cardon CM, Van Vranken JG, Dephoure N *et al.* A mitochondrial pyruvate carrier required for pyruvate uptake in yeast, Drosophila, and humans. *Science* 337:96-100 (2012).

9. Divakaruni AS, Murphy AN. Cell biology. A mitochondrial mystery, solved. *Science* 337:41-43 (2012).

10. Sancak Y, Markhard AL, Kitami T, Kovacs-Bogdan E, Kamer KJ, Udeshi ND, Carr SA, Chaudhuri D, Clapham DE, Li AA *et al.* EMRE is an essential component of the mitochondrial calcium uniporter complex. *Science* 342:1379-1382 (2013).

11. Rigaut G, Shevchenko A, Rutz B, Wilm M, Mann M, Seraphin B. A generic protein purification method for protein complex characterization and proteome exploration. *Nat. Biotechnol.* 17:1030-1032 (1999).

12. Kwon Y, Vinayagam A, Sun X, Dephoure N, Gygi SP, Hong P, Perrimon N. The Hippo signaling pathway interactome. *Science* 342:737-740 (2013).

13. Couzens AL, Knight JD, Kean MJ, Teo G, Weiss A, Dunham WH, Lin ZY, Bagshaw RD, Sicheri F, Pawson T *et al.* Protein interaction network of the mammalian Hippo pathway reveals mechanisms of kinase-phosphatase interactions. *Sci. Signal.* 6:rs15 (2013).

14. Sha Z, Goldberg AL. Proteasome-mediated processing of Nrf1 is essential for coordinate induction of all proteasome subunits and p97. *Curr. Biol.* 24:1573-1583 (2014).

15. Andersen JL, Thompson JW, Lindblom KR, Johnson ES, Yang CS, Lilley LR, Freel CD, Moseley MA, Kornbluth S. A biotin switch-based proteomics approach identifies 14-3-3zeta as a target of Sirt1 in the metabolic regulation of caspase-2. *Mol. Cell* 43:834-842 (2011).

16. Chen S, Synowsky S, Tinti M, MacKintosh C. The capture of phosphoproteins by 14-3-3 proteins mediates actions of insulin. *Trends Endocrinol. Metab.* 22:429-436 (2011).

17. Collins BC, Gillet LC, Rosenberger G, Rost HL, Vichalkovski A, Gstaiger M, Aebersold R. Quantifying protein interaction dynamics by SWATH mass spectrometry: application to the 14-3-3 system. *Nat. Methods* 10:1246-1253 (2013).

18. Wilkins MR, Pasquali C, Appel RD, Ou K, Golaz O, Sanchez J-C, Yan JX, Gooley AA, Hughes G, Humphery-Smith I *et al.* From proteins to proteomes: large scale protein identification by two-dimensional electrophoresis and amino acid analysis. *Nat. Biotechnol.* 14:61-65 (1996).

19. Link AJ, Eng J, Schieltz DM, Carmack E, Mize GJ, Morris DR, Garvik BM, Yates JR, 3rd. Direct analysis of protein complexes using mass spectrometry. *Nat. Biotechnol.* 17:676-682 (1999).

20. Rabilloud T. Two-dimensional gel electrophoresis in proteomics: old, old fashioned, but it still climbs up the mountains. *Proteomics* 2:3-10 (2002).

21. Patterson SD, Aebersold RH. Proteomics: the first decade and beyond. *Nat. Genet.* 33 Suppl:311-323 (2003).

22. Zhang Z, Wu S, Stenoien DL, Pasa-Tolic L. High-throughput proteomics. *Annu. Rev. Anal. Chem.* 7:427-454 (2014).

23. Lambert JP, Ivosev G, Couzens AL, Larsen B, Taipale M, Lin ZY, Zhong Q, Lindquist S, Vidal M, Aebersold R *et al.* Mapping differential interactomes by affinity purification coupled with data-independent mass spectrometry acquisition. *Nat. Methods* 10:1239-1245 (2013).

24. Morris JH, Knudsen GM, Verschueren E, Johnson JR, Cimermancic P, Greninger AL, Pico AR. Affinity purification-mass spectrometry and network analysis to understand protein-protein interactions. *Nat. Protoc.* 9:2539-2554 (2014).

25. Weinert BT, Wagner SA, Horn H, Henriksen P, Liu WR, Olsen JV, Jensen LJ, Choudhary C. Proteome-wide mapping of the Drosophila acetylome demonstrates a high degree of conservation of lysine acetylation. *Sci. Signal.* 4:ra48 (2011).

26. Povlsen LK, Beli P, Wagner SA, Poulsen SL, Sylvestersen KB, Poulsen JW, Nielsen ML, Bekker-Jensen S, Mailand N, Choudhary C. Systems-wide analysis of ubiquitylation dynamics reveals a key role for PAF15 ubiquitylation in DNA-damage bypass. *Nat. Cell Biol.* 14:1089-1098 (2012).

27. Liu Y, Hüttenhain R, Surinova S, Gillet LCJ, Mouritsen J, Brunner R, Navarro P, Aebersold R. Quantitative measurements of N-linked glycoproteins in human plasma by SWATH-MS. *Proteomics* 13:1247-1256 (2013).

28. Woo CM, Iavarone AT, Spiciarich DR, Palaniappan KK, Bertozzi CR. Isotope-targeted glycoproteomics (IsoTaG): a mass-independent platform for intact N- and O-glycopeptide discovery and analysis. *Nat. Methods* 12:561-567 (2015).

29. Hornbeck PV, Zhang B, Murray B, Kornhauser JM, Latham V, Skrzypek E. PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. *Nucleic Acids Res* 43:D512-520 (2015).

30. Gavin AC, Bosche M, Krause R, Grandi P, Marzioch M, Bauer A, Schultz J, Rick JM, Michon AM, Cruciat CM *et al.* Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* 415:141-147 (2002).

31. Gavin AC, Aloy P, Grandi P, Krause R, Boesche M, Marzioch M, Rau C, Jensen LJ, Bastuck S, Dumpelfeld B *et al.* Proteome survey reveals modularity of the yeast cell machinery. *Nature* 440:631-636 (2006).

32. Babu M, Vlasblom J, Pu S, Guo X, Graham C, Bean BD, Burston HE, Vizeacoumar FJ, Snider J, Phanse S *et al.* Interaction landscape of membrane-protein complexes in Saccharomyces cerevisiae. *Nature* 489:585-589 (2012).

33. Roux KJ, Kim DI, Raida M, Burke B. A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells. *J. Cell Biol.* 196:801-810 (2012).

34. Rhee HW, Zou P, Udeshi ND, Martell JD, Mootha VK, Carr SA, Ting AY. Proteomic mapping of mitochondria in living cells via spatially restricted enzymatic tagging. *Science* 339:1328-1331 (2013).

35. Hung V, Zou P, Rhee HW, Udeshi ND, Cracan V, Svinkina T, Carr SA, Mootha VK, Ting AY. Proteomic mapping of the human mitochondrial intermembrane space in live cells via ratiometric APEX tagging. *Mol. Cell* 55:332-341 (2014).

36. Lam SS, Martell JD, Kamer KJ, Deerinck TJ, Ellisman MH, Mootha VK, Ting AY. Directed evolution of APEX2 for electron microscopy and proximity labeling. *Nat. Methods* 12:51-54 (2015).

37. Jing J, He L, Sun A, Quintana A, Ding Y, Ma G, Tan P, Liang X, Zheng X, Chen L *et al.* Proteomic mapping of ER-PM junctions identifies STIMATE as a regulator of Ca influx. *Nat. Cell Biol.* (2015).

38. Ntai I, Kim K, Fellers RT, Skinner OS, Smith ADt, Early BP, Savaryn JP, LeDuc RD, Thomas PM, Kelleher NL. Applying label-free quantitation to top down proteomics. *Anal. Chem.* 86:4961-4968 (2014).

39. Wolf-Yadlin A, Hautaniemi S, Lauffenburger DA, White FM. Multiple reaction monitoring for robust quantitative proteomic analysis of cellular signaling networks. *Proc. Natl. Acad. Sci. USA* 104:5860-5865 (2007).

40. Dephoure N, Gygi SP. Hyperplexing: a method for higher-order multiplexed quantitative proteomics provides a map of the dynamic response to rapamycin in yeast. *Sci. Signal.* 5:rs2 (2012).

41. Gillet LC, Navarro P, Tate S, Röst H, Selevsek N, Reiter L, Bonner R, Aebersold R. Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: a new concept for consistent and accurate proteome analysis. *Mol. Cell. Proteomics* 11:O111.016717 (2012).

42. Toprak UH, Gillet LC, Maiolica A, Navarro P, Leitner A, Aebersold R. Conserved peptide fragmentation as a benchmarking tool for mass spectrometers and a discriminating feature for targeted proteomics. *Mol. Cell. Proteomics* 13:2056-2071 (2014).

43. Varjosalo M, Sacco R, Stukalov A, van Drogen A, Planyavsky M, Hauri S, Aebersold R, Bennett KL, Colinge J, Gstaiger M *et al.* Interlaboratory reproducibility of large-scale human protein-complex analysis by standardized AP-MS. *Nat. Methods* 10:307-314 (2013).

44. Braun P. Reproducibility restored--on toward the human interactome. *Nat. Methods* 10:301, 303 (2013).

45. Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH. RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell* 78:35-43 (1994).

46. Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* 110:163-175 (2002).

47. Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr. Biol.* 14:1296-1302 (2004).

48. Jin Y, McFie PJ, Banman SL, Brandt C, Stone SJ. Diacylglycerol acyltransferase-2 (DGAT2) and monoacylglycerol acyltransferase-2 (MGAT2) interact to promote triacylglycerol synthesis. *J. Biol. Chem.* 289:28237-28248 (2014).

49. Gibson TJ, Seiler M, Veitia RA. The transience of transient overexpression. *Nat. Methods* 10:715-721 (2013).

50. Fields S, Song O. A novel genetic system to detect protein-protein interactions. *Nature* 340:245-246 (1989).

51. Lievens S, Lemmens I, Tavernier J. Mammalian two-hybrids come of age. *Trends Biochem. Sci.* 34:579-588 (2009).

52. Sardiu ME, Washburn MP. Building protein-protein interaction networks with proteomics and informatics tools. *J. Biol. Chem.* 286:23645-23651 (2011).

53. Baker M. Proteomics: The interaction map. *Nature* 484:271-275 (2012).

54. Ryan CJ, Cimermancic P, Szpiech ZA, Sali A, Hernandez RD, Krogan NJ. High-resolution network biology: connecting sequence with function. *Nat. Rev. Genet.* 14:865-879 (2013).

55. Vidal M, Fields S. The yeast two-hybrid assay: still finding connections after 25 years. *Nat. Methods* 11:1203-1206 (2014).

56. Braun P, Tasan M, Dreze M, Barrios-Rodiles M, Lemmens I, Yu H, Sahalie JM, Murray RR, Roncari L, de Smet AS *et al.* An experimentally derived confidence score for binary protein-protein interactions. *Nat. Methods* 6:91-97 (2009).

57. Rolland T, Tasan M, Charloteaux B, Pevzner SJ, Zhong Q, Sahni N, Yi S, Lemmens I, Fontanillo C, Mosca R *et al.* A proteome-scale map of the human interactome network. *Cell* 159:1212-1226 (2014).

58. Lievens S, Peelman F, De Bosscher K, Lemmens I, Tavernier J. MAPPIT: a protein interaction toolbox built on insights in cytokine receptor signaling. *Cytokine Growth Factor Rev.* 22:321-329 (2011).

59. Johnsson N, Varshavsky A. Split ubiquitin as a sensor of protein interactions in vivo. *Proc. Natl. Acad. Sci. USA* 91:10340-10344 (1994).

60. Jones AM, Xuan Y, Xu M, Wang RS, Ho CH, Lalonde S, You CH, Sardi MI, Parsa SA, Smith-Valle E *et al.* Border control--a membrane-linked interactome of Arabidopsis. *Science* 344:711-716 (2014).

61. Paumi CM, Menendez J, Arnoldo A, Engels K, Iyer KR, Thaminy S, Georgiev O, Barral Y, Michaelis S, Stagljar I. Mapping protein-protein interactions for the yeast ABC transporter Ycf1p by integrated split-ubiquitin membrane yeast two-hybrid analysis. *Mol. Cell* 26:15-25 (2007).

62. Snider J, Kittanakom S, Damjanovic D, Curak J, Wong V, Stagljar I. Detecting interactions with membrane proteins using a membrane two-hybrid assay in yeast. *Nat. Protoc.* 5:1281-1293 (2010).

63. Stagljar I, Korostensky C, Johnsson N, te Heesen S. A genetic system based on split-ubiquitin for the analysis of interactions between membrane proteins in vivo. *Proc. Natl. Acad. Sci. USA* 95:5187-5192 (1998).

64. Iyer K, Burkle L, Auerbach D, Thaminy S, Dinkel M, Engels K, Stagljar I. Utilizing the split-ubiquitin membrane yeast two-hybrid system to identify protein-protein interactions of integral membrane proteins. *Sci. STKE* 2005:pl3 (2005).

65. Petschnigg J, Groisman B, Kotlyar M, Taipale M, Zheng Y, Kurat CF, Sayad A, Sierra JR, Mattiazzi Usaj M, Snider J *et al.* The mammalian-membrane two-hybrid assay (MaMTH) for probing membrane-protein interactions in human cells. *Nat. Methods* 11:585-592 (2014).

66. Herce HD, Deng W, Helma J, Leonhardt H, Cardoso MC. Visualization and targeted disruption of protein interactions in living cells. *Nat. Commun.* 4:2660 (2013).

67. Gerber D, Maerkl SJ, Quake SR. An in vitro microfluidic approach to generating protein-interaction networks. *Nat. Methods* 6:71-74 (2009).

68. Meier M, Sit RV, Quake SR. Proteome-wide protein interaction measurements of bacterial proteins of unknown function. *Proc. Natl. Acad. Sci. USA* 110:477-482 (2013).

69. Qi C, Zhang H, Liu L, Yang Y, Kang T, Hao W, Jin G, Jiang T. Analysis of interactions between SNARE proteins using imaging ellipsometer coupled with microfluidic array. *Sci. Rep.* 4:5341 (2014).

70. MacBeath G, Schreiber SL. Printing proteins as microarrays for high-throughput function determination. *Science* 289:1760-1763 (2000).

71. Service RF. Biochemistry. Protein arrays step out of DNA's shadow. *Science* 289:1673 (2000).

72. Cusick ME, Yu H, Smolyar A, Venkatesan K, Carvunis AR, Simonis N, Rual JF, Borick H, Braun P, Dreze M *et al.* Literature-curated protein interaction datasets. *Nat. Methods* 6:39-46 (2009).

73. Reinke AW, Baek J, Ashenberg O, Keating AE. Networks of bZIP protein-protein interactions diversified over a billion years of evolution. *Science* 340:730-734 (2013).

74. Sykiotis GP, Bohmann D. Stress-activated cap'n'collar transcription factors in aging and human disease. *Sci. Signal.* 3:re3 (2010).

75. Jiang LQ, Wen SJ, Wang HY, Chen LY. Screening the proteins that interact with calpain in a human heart cDNA library using a yeast two-hybrid system. *Hypertens. Res.* 25:647-652 (2002).

76. Wang J, Huo K, Ma L, Tang L, Li D, Huang X, Yuan Y, Li C, Wang W, Guan W *et al.* Toward an understanding of the protein interaction network of the human liver. *Mol. Syst. Biol.* 7:536 (2011).

77. Biswas M, Phan D, Watanabe M, Chan JY. The Fbw7 tumor suppressor regulates nuclear factor E2-related factor 1 transcription factor turnover through proteasome-mediated proteolysis. *J. Biol. Chem.* 286:39282-39289 (2011).

78. Biswas M, Kwong EK, Park E, Nagra P, Chan JY. Glycogen synthase kinase 3 regulates expression of nuclear factor-erythroid-2 related transcription factor-1 (Nrf1) and inhibits pro-survival function of Nrf1. *Exp. Cell Res.* 319:1922-1931 (2013).

79. Ravasi T, Suzuki H, Cannistraci CV, Katayama S, Bajic VB, Tan K, Akalin A, Schmeier S, Kanamori-Katayama M, Bertin N *et al.* An atlas of combinatorial transcriptional regulation in mouse and man. *Cell* 140:744-752 (2010).

80. Venugopal R, Jaiswal AK. Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes. *Oncogene* 17:3145-3156 (1998).

81. Wang W, Chan JY. Nrf1 is targeted to the endoplasmic reticulum membrane by an N-terminal transmembrane domain. Inhibition of nuclear translocation and transacting function. *J. Biol. Chem.* 281:19676-19687 (2006).

82. Yu M, Li H, Liu Q, Liu F, Tang L, Li C, Yuan Y, Zhan Y, Xu W, Li W *et al.* Nuclear factor p65 interacts with Keap1 to repress the Nrf2-ARE pathway. *Cell. Signal.* 23:883-892 (2011).

83. Niture SK, Gnatt A, Jaiswal AK. Oncogene PKCepsilon controls INrf2-Nrf2 interaction in normal and cancer cells through phosphorylation of INrf2. *J. Cell Sci.* 126:5657-5669 (2013).

84. Johnsen O, Skammelsrud N, Luna L, Nishizawa M, Prydz H, Kolsto AB. Small Maf proteins interact with the human transcription factor TCF11/Nrf1/LCR-F1. *Nucleic Acids Res.* 24:4289-4297 (1996).

85. Marini MG, Chan K, Casula L, Kan YW, Cao A, Moi P. hMAF, a Small Human Transcription Factor That Heterodimerizes Specifically with Nrf1 and Nrf2. *J. Biol. Chem.* 272:16490-16497 (1997).

86. Tatham MH, Matic I, Mann M, Hay RT. Comparative proteomic analysis identifies a role for SUMO in protein quality control. *Sci. Signal.* 4:rs4 (2011).

87. Danielsen JM, Sylvestersen KB, Bekker-Jensen S, Szklarczyk D, Poulsen JW, Horn H, Jensen LJ, Mailand N, Nielsen ML. Mass spectrometric analysis of lysine ubiquitylation reveals promiscuity at site level. *Mol. Cell. Proteomics* 10:M110 003590 (2011).

88. Wagner SA, Beli P, Weinert BT, Nielsen ML, Cox J, Mann M, Choudhary C. A proteome-wide, quantitative survey of in vivo ubiquitylation sites reveals widespread regulatory roles. *Mol. Cell. Proteomics* 10:M111 013284 (2011).

89. Kim W, Bennett EJ, Huttlin EL, Guo A, Li J, Possemato A, Sowa ME, Rad R, Rush J, Comb MJ *et al.* Systematic and quantitative assessment of the ubiquitin-modified proteome. *Mol. Cell* 44:325-340 (2011).

90. Emanuele MJ, Elia AE, Xu Q, Thoma CR, Izhar L, Leng Y, Guo A, Chen YN, Rush J, Hsu PW *et al.* Global identification of modular cullin-RING ligase substrates. *Cell* 147:459-474 (2011).

91. Chepelev NL, Bennitz JD, Huang T, McBride S, Willmore WG. The Nrf1 CNC-bZIP protein is regulated by the proteasome and activated by hypoxia. *PloS one* 6:e29167 (2011).

92. Steffen J, Seeger M, Koch A, Kruger E. Proteasomal degradation is transcriptionally controlled by TCF11 via an ERAD-dependent feedback loop. *Mol. Cell* 40:147-158 (2010).