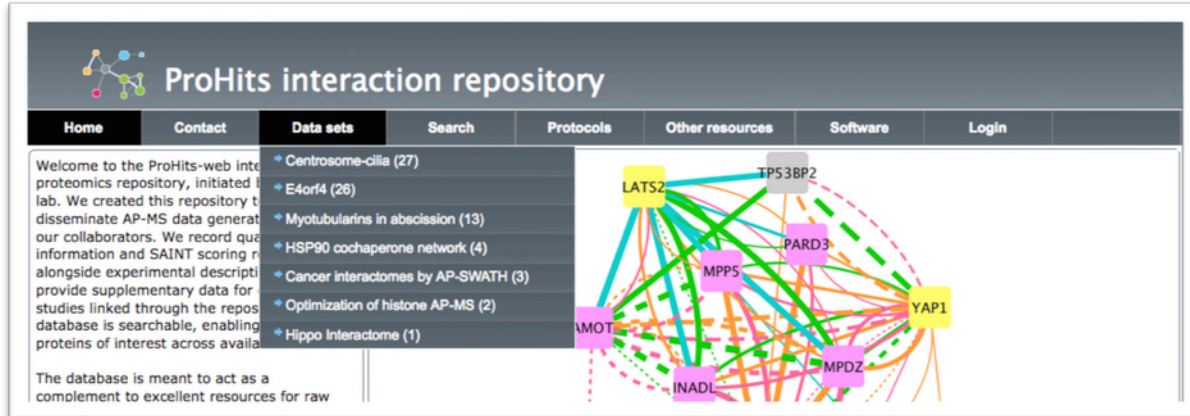


Quick guide to the “ProHits-web” interaction proteomics repository at prohits-web.lunenfeld.ca

Website designed and maintained by JP Zhang and G Liu; tutorial prepared by AC Gingras, June 29th, 2013; with revisions on August 31st, 2016, August 1st, 2017 and February 1st, 2018. Please contact Anne-Claude Gingras (Gingras@lunenfeld.ca) for any questions.

This repository was created to store and disseminate interaction proteomics data generated by the Gingras laboratory and collaborators. The repository contains separate “Data sets” pages for each of the specific projects performed. These consist of published¹⁻⁹, submitted and ongoing projects, with unpublished data being password-protected. The data is recorded primarily as spectral counts following assessment of the interaction significance using SAINT tools^{10,11}; data are automatically transferred from instances of ProHits-LIMS¹² after selection of the high-confidence thresholds, and in more recent relevant projects, bait information is provided through OpenFreezer¹³. Within each project, the data are structured by “bait” (most often expressed at the level of genes), and additional information about the baits is provided through links to different resources (see below). Search functions enable one to look for all instances of a gene symbol within a specific project or across all accessible projects. Within a project, multiple baits can be selected for visualization of the results using ProHits-viz¹⁴, and the hits associated with individual baits can be downloaded as text files, or further explored directly within the system. Links to interactions annotated by BioGRID¹⁵ or IntAct (now MIntAct¹⁶) are provided for each bait-prey pair (links point to the NCBI PubMed¹⁷ pages for each publication). Each project/dataset is also associated with specific Supplementary data and/or other functionality that are described on the home page of the project. Information regarding the location of the primary mass spectrometry data in public repositories such as MassIVE¹⁸ and the publication (when available) is also provided on the home page of each project.

1) Access the website at prohits-web.lunenfeld.ca. From the top menu bar, select “Data sets” and scroll to the desired dataset. All published datasets are available from this dropdown menu without login; to access submitted or unpublished projects, please first login with your username and password (this will give you access to the projects you have been authorized to view and/or edit). Recent datasets are also listed on the home page in the bottom left box. A unique identifier for each project (project number) is shown in parentheses at the end of each line.



2) Navigate through the selected project home page using the left menu bar. Each project is associated with a short abstract describing the project/dataset, as well as a number of notes and links. On the left menu, several options are available.

The Supplementary Data section of the project provides manuscript information as detailed in the corresponding publication(s), organized into folders. Additional Results files available only on the web are also provided for several of the projects/datasets. These may consist of downloadable files (for example complete SAINT results output files^{10,11}, Cytoscape files¹⁹, files for our visualization tool, ProHits-viz²⁰) and additional documents that could not be included in the publication. A link to the mass spectrometry data repository MassIVE¹⁸ is provided for each of the datasets included in the publication, each accompanied by clear descriptions of the samples included and the experimental procedure used for sample generation and analysis. Links to the data deposition in public interaction databases^{15,16} as well as links to the publications are also provided.

ProHits interaction repository

Home Contact **Data sets** Search Protocols Other resources Software Admin Log out

DataSets / Project home (Hippo Interactome)

Project home
Explore baits
Expert zone
Search
Supplementary data
Project participants
Funding
Publication

Protein Interaction Network of the Mammalian Hippo Pathway Reveals Mechanisms of Kinase-Phosphatase Interactions

First Author: Couzens AL, Knight JDR, Kean MJ, Teo G, Weiss A, Dunham WH, Lin ZY, Bagshaw RD, Sicheri F, Pawson T, Wrana JL, Choi H, Gingras AC

Abstract:

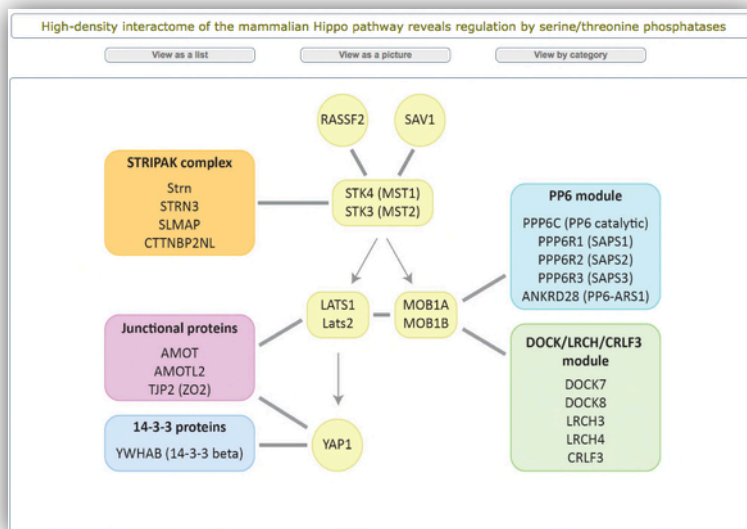
The Hippo pathway regulates organ size and tissue homeostasis in response to multiple stimuli, including cell density and mechanotransduction. Pharmacological inhibition of phosphatases can also stimulate Hippo signaling in cell culture. Here, we defined the Hippo protein-protein interaction network with and without inhibition of serine and threonine phosphatases by okadaic acid. We identified 749 protein interactions, including 599 previously unrecognized interactions, and demonstrated that several interactions with serine and threonine phosphatases were phosphorylation-dependent. Mutation of the T-loop of MST2 (mammalian STE20-like protein kinase 2) that prevented autophosphorylation disrupted its association with STRIPAK (Striatin-Interacting Phosphatase and Kinase complex). Deletion of the N-terminal forkhead associated domain of SLMAP (sarcolemmal membrane-associated protein), a component of the STRIPAK complex, prevented its association with MST1 and MST2. Phosphatase inhibition produced temporally distinct changes in proteins that interacted with MOB1A and MOB1B (Mps one binder kinase activator-like 1A and 1B) and promoted interactions with upstream Hippo pathway proteins, such as MST1 and 2, and with the trimeric protein phosphatase 6 complex (PP6). Mutation of three basic amino acids that are part of a phospho-serine and threonine-binding domain in human MOB1B prevented its interaction with MST1 and PP6 in cells treated with okadaic acid. Collectively, our results indicated that changes in phosphorylation orchestrate interactions between kinases and phosphatases in Hippo signaling, providing a putative mechanism for pathway regulation.

Manuscript available at [Science Signaling](#). Also see accompanying [Perspective article](#).

Interaction data was deposited in [IntAct](#) and [BioGRID](#)

The raw mass spectrometry data was contributed to [ProteomeXchange](#) through the [MassIVE repository dataset 1](#), [dataset 2](#), [dataset 3](#).

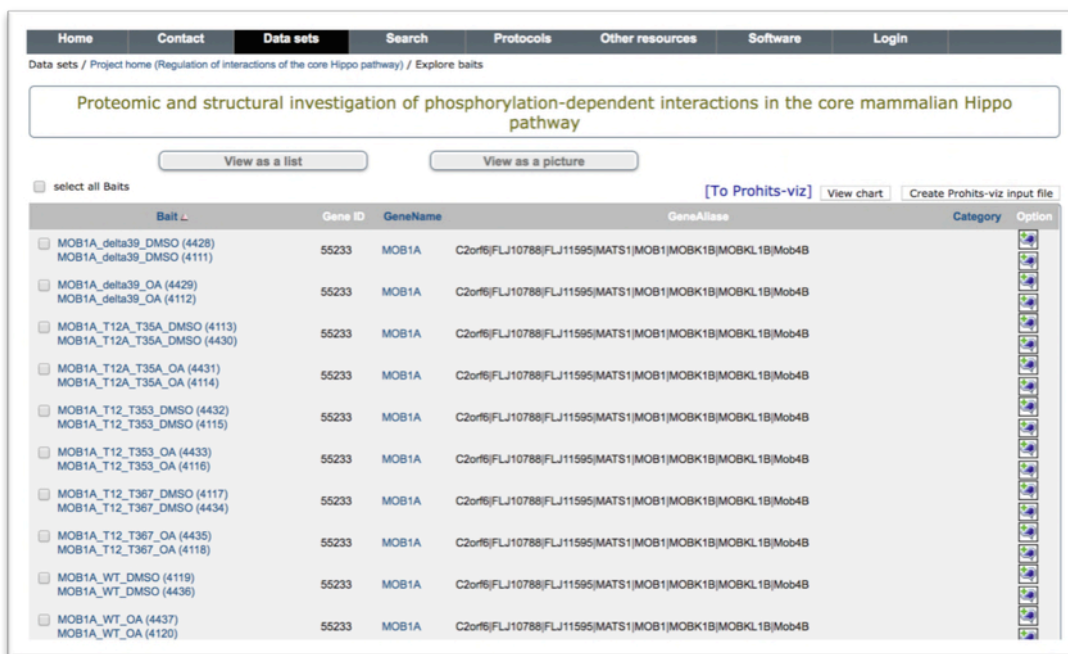
3) Navigate through the bait-prey interaction data by selecting “Explore Baits”. This will take you by default to a pictorial view of the baits analyzed in the projects if available, or to an alphabetically sorted bait list. (Alternatively, bait may be organized by categories defined by the experimentalist, e.g. protein families or baits with specific subcellular localizations). Clicking on any of the bait names will take you (by default) to the list of high confidence interactors for the given bait (here in the in the Hippo interactome dataset¹).



For example, the top of the interaction list for STK4 (MST1) is shown below. The upper left corner contains a zoomable version of the images presented the manuscript by Couzens et al.¹; different types of images are presented to support different projects (these could be microscopy data, results from a functional screen or, as here, a physical interactome). The upper right corner, which is common to all projects, is a dynamically generated network of the bait-prey interactions.

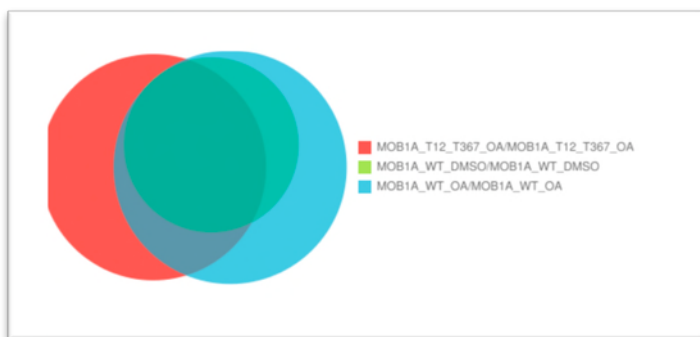
5) Data comparison tools

While we offer a bait-by-bait exploration of the dataset and simple search functions, we also enable comparisons of data between baits. Those are selected from the Explore baits → view as a list page; baits to be compared are selected through the checkboxes to the left.



| Bait | Gene ID | GeneName | GeneAlias | Category | Option |
|---|---------|----------|---|----------|--------|
| <input type="checkbox"/> MOB1A_delta39_DMSO (4428) MOB1A_delta39_DMSO (4111) | 55233 | MOB1A | C2orf6 FLJ10788 FLJ11595 MATS1 MOB1 MOBK1B MOBK1B Mob4B | | |
| <input type="checkbox"/> MOB1A_delta39_OA (4429) MOB1A_delta39_OA (4112) | 55233 | MOB1A | C2orf6 FLJ10788 FLJ11595 MATS1 MOB1 MOBK1B MOBK1B Mob4B | | |
| <input type="checkbox"/> MOB1A_T12A_T35A_DMSO (4113) MOB1A_T12A_T35A_DMSO (4430) | 55233 | MOB1A | C2orf6 FLJ10788 FLJ11595 MATS1 MOB1 MOBK1B MOBK1B Mob4B | | |
| <input type="checkbox"/> MOB1A_T12A_T35A_OA (4431) MOB1A_T12A_T35A_OA (4114) | 55233 | MOB1A | C2orf6 FLJ10788 FLJ11595 MATS1 MOB1 MOBK1B MOBK1B Mob4B | | |
| <input type="checkbox"/> MOB1A_T12_T353_DMSO (4432) MOB1A_T12_T353_DMSO (4115) | 55233 | MOB1A | C2orf6 FLJ10788 FLJ11595 MATS1 MOB1 MOBK1B MOBK1B Mob4B | | |
| <input type="checkbox"/> MOB1A_T12_T353_OA (4433) MOB1A_T12_T353_OA (4116) | 55233 | MOB1A | C2orf6 FLJ10788 FLJ11595 MATS1 MOB1 MOBK1B MOBK1B Mob4B | | |
| <input type="checkbox"/> MOB1A_T12_T367_DMSO (4117) MOB1A_T12_T367_DMSO (4434) | 55233 | MOB1A | C2orf6 FLJ10788 FLJ11595 MATS1 MOB1 MOBK1B MOBK1B Mob4B | | |
| <input type="checkbox"/> MOB1A_T12_T367_OA (4435) MOB1A_T12_T367_OA (4118) | 55233 | MOB1A | C2orf6 FLJ10788 FLJ11595 MATS1 MOB1 MOBK1B MOBK1B Mob4B | | |
| <input type="checkbox"/> MOB1A_WT_DMSO (4119) MOB1A_WT_DMSO (4436) | 55233 | MOB1A | C2orf6 FLJ10788 FLJ11595 MATS1 MOB1 MOBK1B MOBK1B Mob4B | | |
| <input type="checkbox"/> MOB1A_WT_OA (4437) MOB1A_WT_OA (4120) | 55233 | MOB1A | C2orf6 FLJ10788 FLJ11595 MATS1 MOB1 MOBK1B MOBK1B Mob4B | | |

At the simplest level, this exploits the Google chart Venn diagram function (“View chart” above the table on the right). This enables to quickly have a look at the overlaps between the baits that are compared to one another.



Selecting instead the “Create ProHits-viz input file” function downloads a text file that is already formatted for input in our ProHits-viz.lunenfeld.ca visualization toolkit¹⁴. In ProHits-viz, there are a number of tools available, including the dotplot tool shown below. Selecting baits from ProHits-web will enable download of all preys (whether they are detected with high confidence or not) associated with all these baits. ProHits-viz has default cut-offs (typically 1% calculated FDR) for selecting preys to be displayed. Once a prey passes the FDR cutoff with one bait, all its quantitative information across all prey is retrieved for display. Score thresholds are showed as outside circle color intensity, while center color intensity maps to raw abundance values (spectral counts in ProHits-web) and circle size to the relative abundance of a given prey across all bait purifications. ProHits-viz also performs data clustering, and enables dynamic viewing and reorganization of the images as well as analysis, e.g. of GO terms enriched with select baits or preys. Detailed instructions are available at ProHits-viz.lunenfeld.ca, as well as in the manuscript by Knight et al., *Nature Methods*, 2017 (please contact JKnight@lunenfeld.ca for questions concerning ProHits-viz¹⁴).

Resources automatically linked to ProHits-web

| Resource name | Home page | Function | Ref |
|----------------|---|---|---------------|
| NCBI Gene | http://www.ncbi.nlm.nih.gov/gene/ | Detailed information on bait/prey gene (and associated mRNAs and proteins). | ²¹ |
| BioGRID | http://thebiogrid.org/ | Curated interaction repository | ¹⁵ |
| IntAct/MIntAct | http://www.ebi.ac.uk/intact/ | Curated interaction repository | ¹⁶ |
| Uniprot | http://www.uniprot.org/ | Comprehensive resource for protein sequence and annotation data | ²² |
| ProteinAtlas | http://www.proteinatlas.org/ | Tissue atlas (expression) and subcellular atlas (localization) | ²³ |
| ProteomicsDB | https://www.proteomicsdb.org/ | Mass spectrometry-based human proteome resource | ²⁴ |
| GeneCards | http://www.genecards.org/ | Integrative database for human genes | ²⁵ |
| DEPOD | http://www.koehn.embl.de/depod/ | Specialized database for human phosphatases | ²⁶ |

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