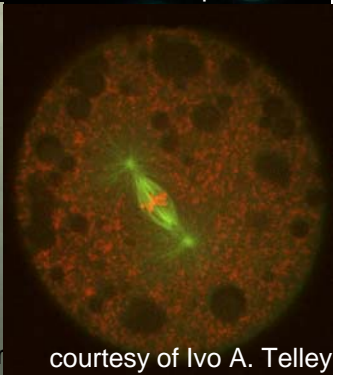
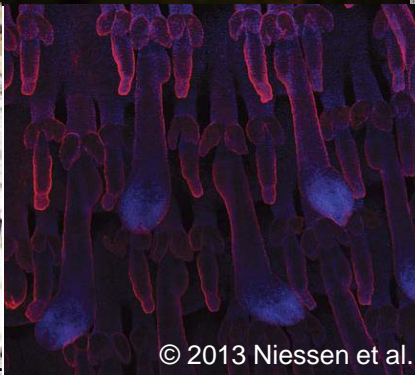
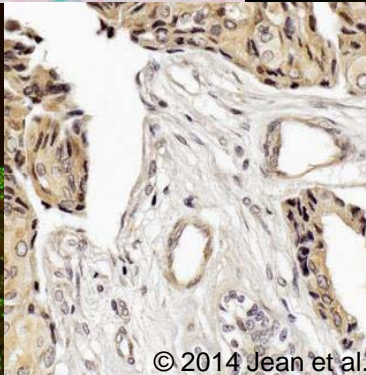
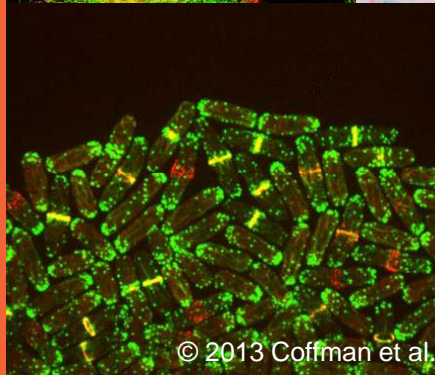
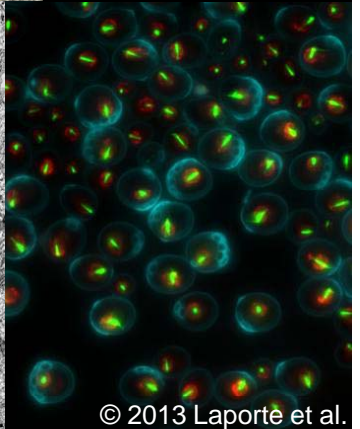
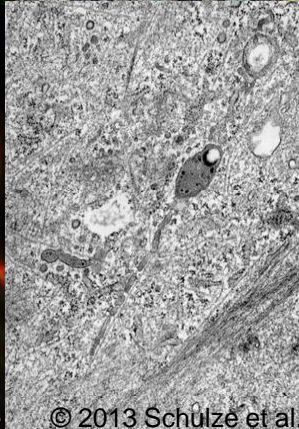
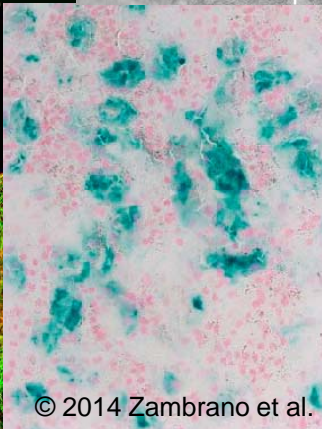
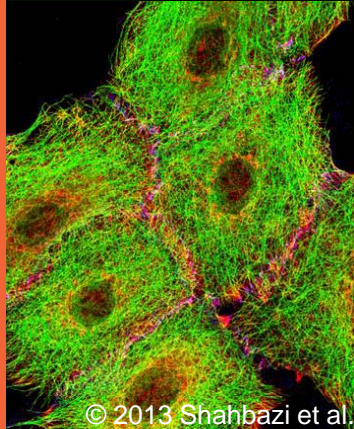
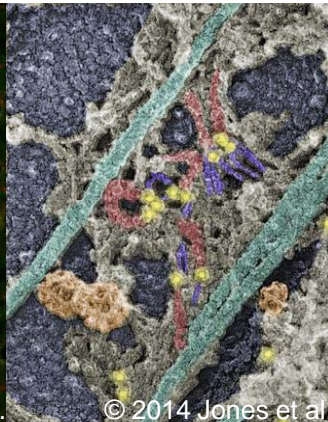
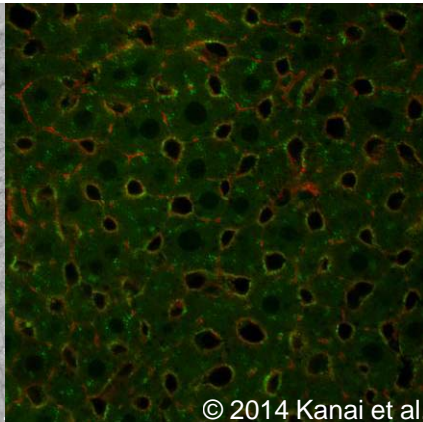
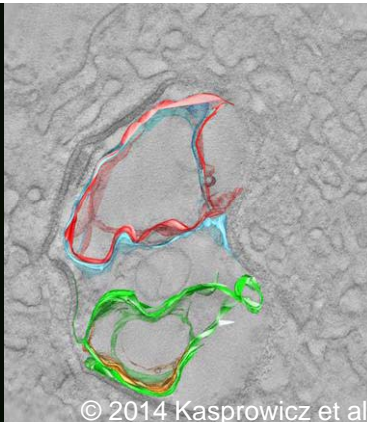
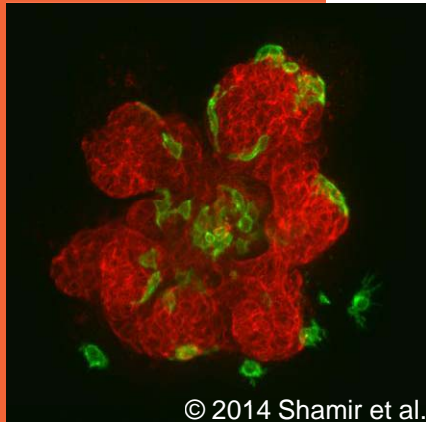


# JCB Data Viewer

Bringing New Dimensions  
to Published Image Data

Liz Williams, PhD  
Executive Editor, *The Journal of Cell Biology*  
lwilliams@rockefeller.edu

**Why would a publisher be interested in  
OME/OMERO?**



# Image data in *JCB*

## The challenges:

- 1. How can we make complex image data accessible in a meaningful and interactive way to users of *JCB* content?**
2. How can we ensure the longevity and accessibility of those data as part of the published record?
3. Can we leverage those data to ensure the integrity of the scientific record?

Published October 15, 2007

JCB: REPORT

## Bod1, a novel kinetochore protein required for chromosome biorientation

Iain M. Porter,<sup>1</sup> Sarah E. McClelland,<sup>2</sup> Guennadi A. Khoudoli,<sup>1</sup> Christopher J. Hunter,<sup>2</sup> Jens S. Andersen,<sup>4</sup> Andrew D. McAinsh,<sup>3</sup> J. Julian Blow,<sup>1</sup> and Jason R. Swedlow<sup>1</sup>

<sup>1</sup>Division of Gene Regulation and Expression and <sup>2</sup>Medical Research Council Protein Phosphorylation Unit, College of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, UK; <sup>3</sup>Chromosome Segregation Laboratory, Marie Curie Research Institute, Oxford, Surrey RH8 0TL, England, UK; <sup>4</sup>Center for Experimental Biomedicine, University of Southern Denmark, DS-5230 Odense, Denmark

We have combined the proteomic analysis of *Xenopus laevis* in vitro-assembled chromosomes with RNA interference and live cell imaging in HeLa cells to identify novel factors required for proper chromosome segregation. The first of these is Bod1, a protein conserved throughout metazoans that associates with a large macromolecular complex and localizes with kinetochores and spindle poles during mitosis. Small interfering RNA depletion of Bod1 in HeLa cells produces elongated mitotic spindles with severe biorientation defects. Bod1-depleted cells form syntelic attachments that can oscillate and generate enough force to separate sister

kinetochores, suggesting that microtubule-kinetochore interactions were intact. Releasing Bod1-depleted cells from a monastrol block increases the frequency of syntelic attachments and the number of cells displaying biorientation defects. Bod1 depletion does not affect the activity or localization of Aurora B but does cause mislocalization of the microtubule depolymerase mitotic centromere-associated kinesin and prevents its efficient phosphorylation by Aurora B. Therefore, Bod1 is a novel kinetochore protein that is required for the detection or resolution of syntelic attachments in mitotic spindles.

### Introduction

Mitotic chromosome segregation requires the coordination of both regulatory and mechanical molecular machines and culminates in the delivery of two complete sets of chromosomes to two daughter cells. Chromosomes contain long, continuous strands of DNA that are folded and assembled into higher order structures, which, in human cells, results in a 10–20,000-fold linear compaction of DNA (Swedlow and Hirano, 2003). Besides the core histones, many nonhistone chromosomal proteins have been identified (Uchiyama et al., 2005), but a full identification and functional characterization of chromosomal proteins has so far been unavailable.

Chromosomes assemble specific structures called kinetochores that serve as the molecular machines to mediate attachment, checkpoint signaling, and force generation at the ends of spindle microtubules (Cleveland et al., 2003; Tanaka et al., 2005). Kinetochores are built either at the primary constriction of

centric chromosomes or along the whole length of holocentric chromosomes. The molecular components of kinetochores are best characterized in *Saccharomyces cerevisiae*, and many of the components of yeast kinetochores are highly conserved (De Wulf et al., 2003; Westermann et al., 2003; Cheeseman et al., 2004; Meraldi et al., 2006). Nonetheless, a full inventory of the components of the animal cell kinetochore is still lacking.

Cell-free cytoplasmic extracts from *Xenopus laevis* eggs have previously been used for functional studies of chromosomes and kinetochores (Hirano and Mitchison, 1994; Desai et al., 1997; Funabiki and Murray, 2000; Emanuele et al., 2005). This system targets many chromosome and kinetochore proteins to chromatin in a cell cycle-dependent fashion and has the advantage of providing a method of preparing chromatin and chromosomes that are largely free of cytoplasmic contaminants. We have previously developed methods for preparing a soluble fraction of chromatin and chromosome-associated proteins (Murnion et al., 2001) and have used two-dimensional gel electrophoresis of these preparations to reveal >350 distinct polypeptides associated with in vitro-assembled mitotic chromosomes, although the exact number depended on the resolution of the gel system (Khoudoli et al., 2004).

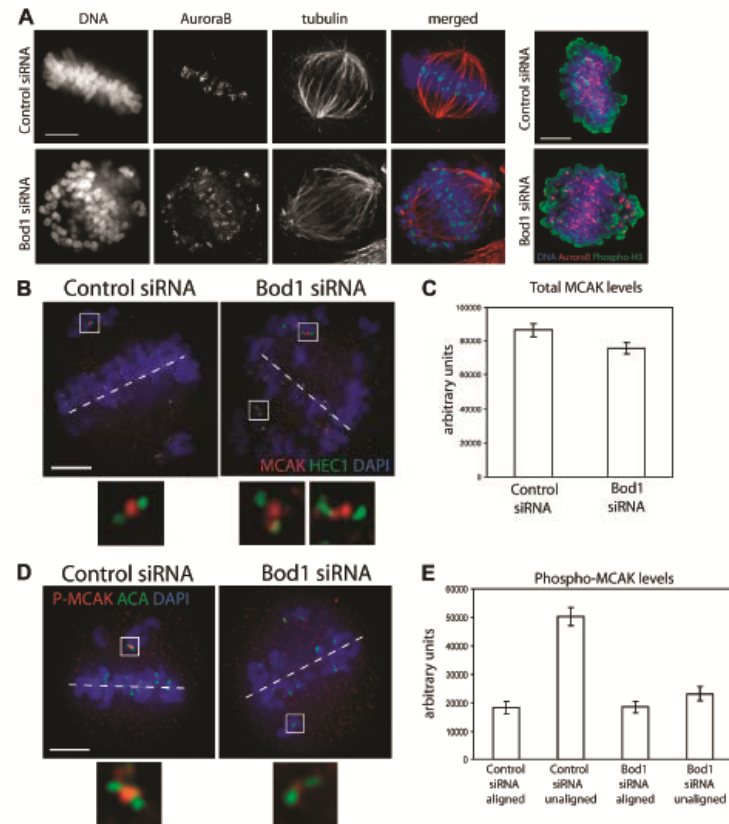
Correspondence to: Jason R. Swedlow: jason@fresci.dundee.ac.uk  
Abbreviations used in this paper: ACA, anticentromere antibody; CENP, centromere protein; MCAK, mitotic centromere-associated kinesin; siRNA, short hairpin RNA.  
The online version of this article contains supplemental material.

© The Rockefeller University Press \$30.00  
The Journal of Cell Biology, Vol. 179, No. 2, October 22, 2007 187–197  
http://www.jcb.org/cgi/doi/10.1083/jcb.200704098

Supplemental Material can be found at:  
http://www.jcb.org/cgi/content/full/179/2/187/DC1.html  
Original image data can be found at:  
http://dx.doi.org/10.1083/jcb.200704098

JCB 187

Published October 15, 2007



**Figure 5. MCAK is not efficiently phosphorylated in Bod1<sup>siRNA</sup> cells.** (A) Aurora B is not delocalized in Bod1-depleted cells. Phospho-Ser10-histone H3 staining in control and Bod1 siRNA cells indicating Aurora B activity. (B–E) Cells were transfected with control or Bod1 siRNA. After 72 h, cells were treated with monastrol for 3 h and released into media containing MG132 for 1 h before fixing. (B and C) Cells were stained for total MCAK population, and levels at kinetochores were quantified. Boxed areas are magnified below the main images. (D and E) Cells were stained for phospho-Ser92-MCAK, and levels at aligned and unaligned kinetochores were quantified. Dashed lines indicate orientation of the metaphase plate. Error bars represent SD. Bars, 5  $\mu$ m.

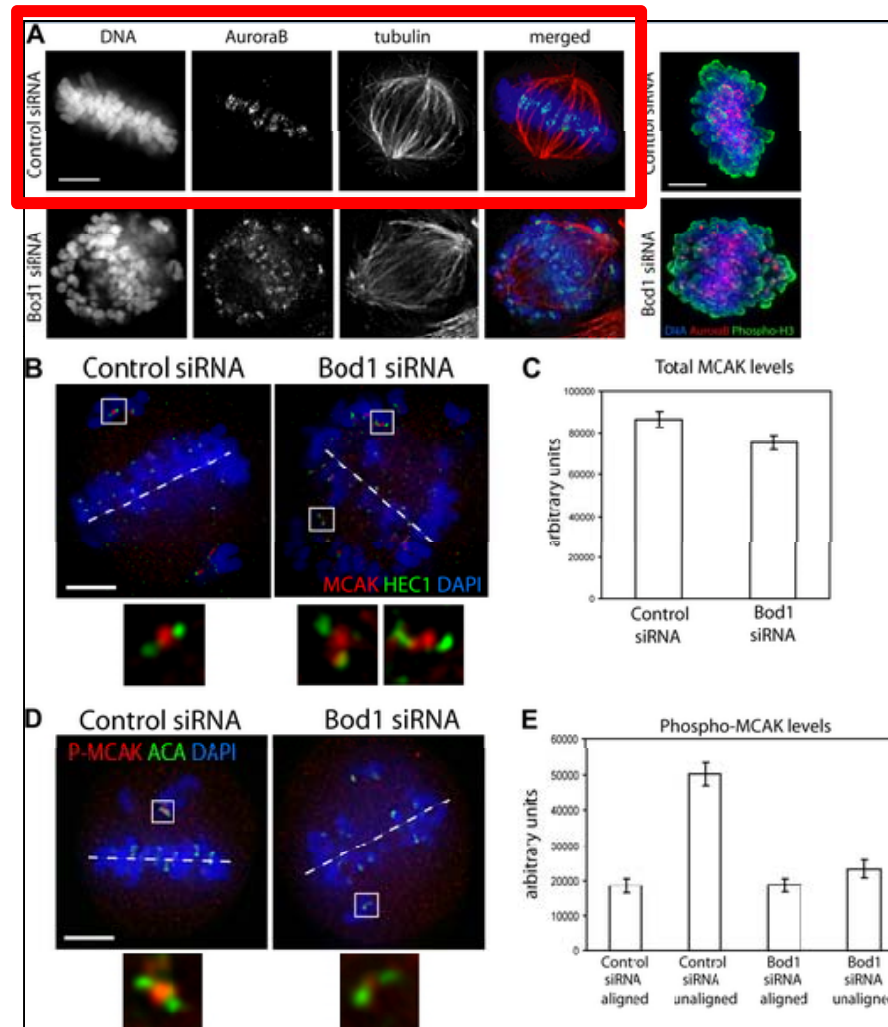
Mouse anti- $\alpha$ -tubulin DM1A (Sigma-Aldrich), rabbit anti-HEC1 antibody (Abcam), mouse anti-Aurora B antibody AIM-1 (BD Biosciences), and mouse anti-Bub1 (Chemicon) were used at 1:500. Rabbit anti-Aurora A (Abcam), mouse anti-Eg5 (BD Biosciences), and human CREST autoantiserum (ACA; a gift from W.C. Earnshaw, University of Edinburgh, Edinburgh, Scotland, UK) were used at 1:1,000. Sheep anti-MCAK and anti-phospho-MCAK antibodies [Andrews et al., 2004] were used at 1  $\mu$ g/ml. Rabbit

anti-phospho-H3 [Ser10; Upstate Biotechnology] was used at 1:200. Fluorescently labeled secondary antibodies were all obtained from Jackson ImmunoResearch Laboratories.

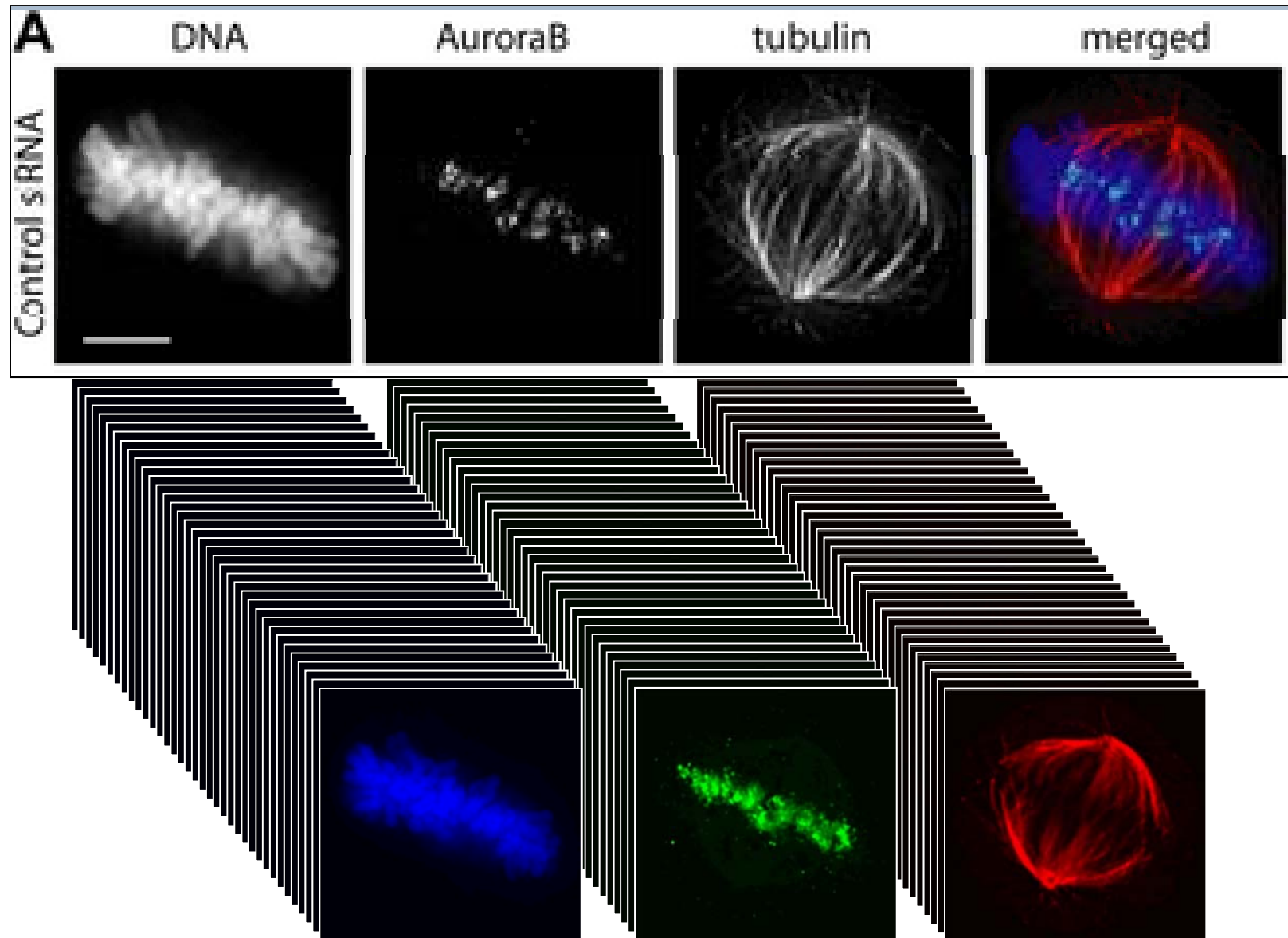
#### Online supplemental material

Fig. S1 shows the use of shRNA and live cell imaging to identify candidate proteins. Fig. S2 shows the relative expression levels of Bod1-GFP. Fig. S3

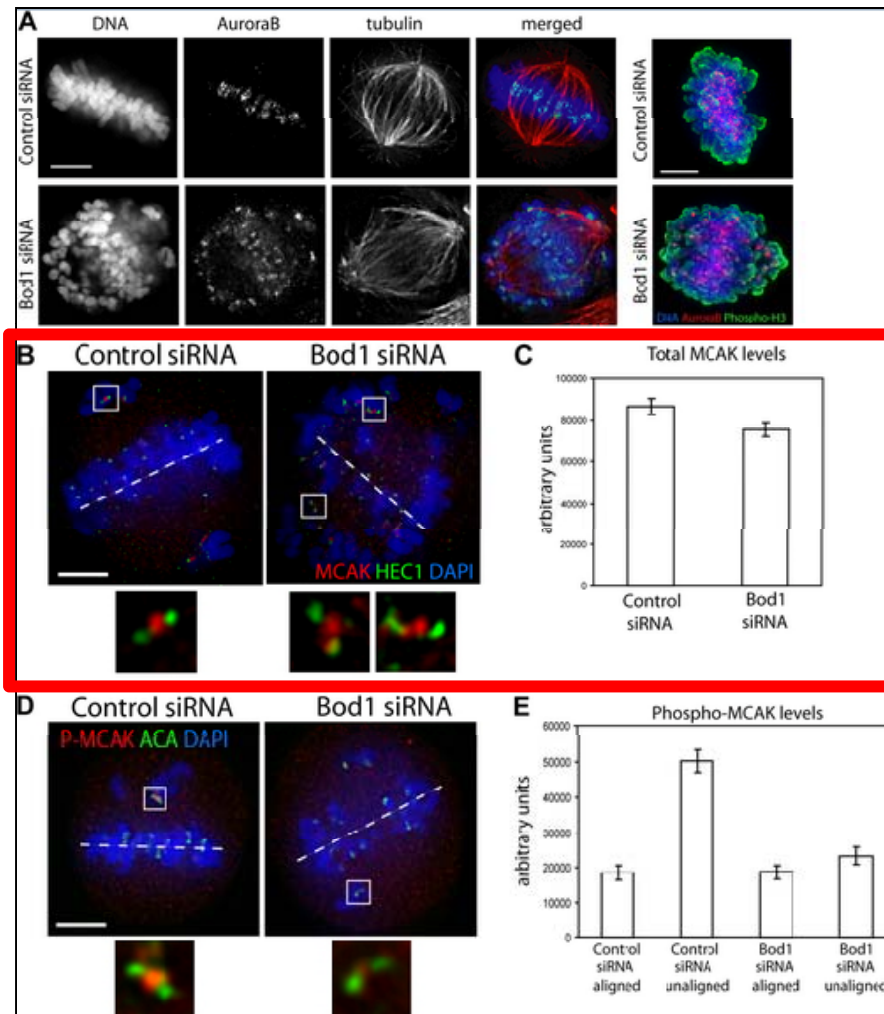
# Image data in *JCB*



# Image data in *JCB*



# Image data in *JCB*





# Image data in *JCB*

## The challenges:

1. How can we make complex image data accessible in a meaningful and interactive way to users of *JCB* content?
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3. Can we leverage those data to ensure the integrity of the scientific record?

# Image data in *JCB*

## What repositories exist:

### Domain-Specific:

- Structural data (Protein Data Bank)
- Sequence data (Genbank)
- Gene expression data (GEO)
- Proteomics data (Pride)

### General

- Figshare
- Dryad

### Institutional (general and domain-specific)

## Image data in *JCB*

### What these repositories lack:

- Dynamic connectivity to the published paper
- Tools to make diverse data types accessible to all users

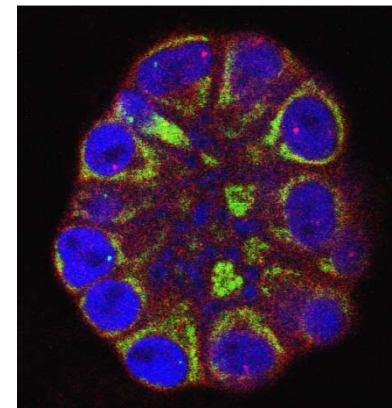
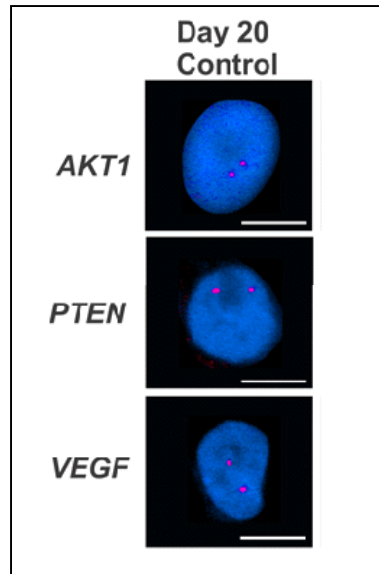
# Image data in *JCB*

## The challenges:

1. How can we make complex image data accessible in a meaningful and interactive way to users of *JCB* content?
2. How can we ensure the longevity and accessibility of those data as part of the published record?
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# Image data in *JCB*

## Data access and validation



# Image data in *JCB*

## The challenges:

1. How can we make complex image data accessible in a meaningful and interactive way to users of *JCB* content?
2. How can we ensure the longevity and accessibility of those data as part of the published record?
3. Can we leverage those data to ensure the integrity of the scientific record?

- An OMERO-based, browser-based application for archiving, viewing, and sharing raw image files associated with *JCB* articles.
- Enables presentation of over 125 proprietary file types from various light microscope and gel-documentation systems.
- Allows users (editors, reviewers, readers) to perform simple analyses of the data within the browser and to download the data in OME-TIFF format for more detailed analysis with their software of choice.
- Enables multidimensional, interactive publishing – far beyond what is possible with standard PDFs and html.
- Fulfills the criteria laid out in the 2013 U.S. government public-access mandate for data resulting from federally-funded research.



<http://jcb-dataviewer.rupress.org>

- Accessibility
  - Within a standard browser
  - Without the need for proprietary software
- Interactivity
  - Within a standard browser
  - Via data downloading
- Multidimensionality
- Seamless linking between the published paper and the raw data



<http://jcb-dataviewer.rupress.org>



The screenshot shows the JCB Data Viewer homepage in a Mozilla Firefox browser. The address bar displays [jcb-dataviewer.rupress.org](http://jcb-dataviewer.rupress.org). The page features a search bar and navigation links for Home, About, Contact, and JCB. A welcome message states: "Welcome to the JCB DataViewer! The JCB DataViewer facilitates viewing, analysis, and sharing of multi-dimensional image data associated with articles published in *The Journal of Cell Biology*." Below this, a "View" section offers "List" and "Gallery" options. The main content area displays a grid of six article thumbnails, each with a representative microscopy image and a brief title and author list.

Thumbnail 1	Thumbnail 2	Thumbnail 3
<p><b>Conserved and divergent features of kinetochores and spindle microtubule ends from five species</b> J. Richard McIntosh, (...) Ekaterina L. Grishchuk jcb. 2013. 200:169-174 DOI: 10.1093/jcb.201209164</p>	<p><b>Obscurin is required for ankyrinB-dependent dystrophin localization and sarcolemma integrity</b> Davide Rancazzo, (...) Vincenzo Sorrentino jcb. 2013. 200:523-536 DOI: 10.1093/jcb.201205116</p>	<p><b>Condensin II initiates sister chromatid resolution during S phase</b> Takao Ono, Daikoku Yamashita, Tetsuya Hirano jcb. 2013. 230:420-441 DOI: 10.1093/jcb.201200000</p>
<p><b>Robust polarity establishment occurs via an endocytosis-based cortical corralling mechanism</b> Miri Jossé, (...) Derek McCusker jcb. 2013. 200:169-174 DOI: 10.1093/jcb.201209164</p>	<p><b>APC binds intermediate filaments and is required for their reorganization during cell migration</b> Yasunika Sekemoto, Balázs Dobó, Sandrine Etienne-Manneville jcb. 2013. 200:169-174 DOI: 10.1093/jcb.201209164</p>	<p><b>Myosin VI small insert isoform maintains exocytosis by tethering secretory granules to the cortical actin</b> Vanessa M. Tomatis, (...) Frédéric A. Heunicher jcb. 2013. 200:169-174 DOI: 10.1093/jcb.201209164</p>

<http://jcb-dataviewer.rupress.org>

## **1. single image analysis**

<http://jcb-dataviewer.rupress.org>

## The Mini Viewer

The screenshot displays the JCB Data Viewer web interface in a Mozilla Firefox browser. The page title is "JCB Data Viewer - Manuscript". The URL in the address bar is "jcb-dataviewer.rupress.org/jcb/browse/5902/138C6/". The page features a search bar, navigation links (Home, About, Contact, JCB, log in), and a list of publications. The selected publication is "Lkb1 regulates organogenesis and early oncogenesis along AMPK-dependent and -independent pathways" by Eryan LC, Geraldine Strasser, Meredith Sagolla, Cary D. Austin, Melissa Junttila, and Ira Mellman, published in JCB vol. 199 (no. 7) 117-117 on Dec 24, 2012. The article DOI is 10.1083/jcb.201208080 and the DataViewer DOI is 10.1083/jcb.201208080.dv. A "Full Viewer" button is visible next to the article title. Below the article information, the "Figure 6 :: 4 images" section is highlighted, with a "Download Figure" button. The main image area shows a large, colorful fluorescence microscopy image of a z-stack of a PanIN-like lesion in an Lkb1 MG/MG pancreatic explant. The image is labeled with "z coordinates" on the vertical axis and "timepoints" on the horizontal axis. To the right of the image is an "Image Details" panel with a "Description" and a "Legend". The description states: "z-stack of a PanIN-like lesion in an Lkb1 MG/MG pancreatic explant". The legend states: "Shown is a z-stack of a mouse E16.5 Lkb1 MG/MG pancreatic explant cultured on transwell filters for 8 days in vitro with 1µM 1NMPP1 and labeled for EpCAM (green), actin (red), and DNA (blue)". Below the main image are two smaller thumbnail images and a "Download Part" button. On the right side of the page, the "Original Data" section is visible, with a "Download All Data (12)" button and a list of figures: "Figure 3 [6]", "Figure 6 [4]", and "Figure 7 [2]". Below the list is a grid of small image thumbnails.

<http://jcb-dataviewer.rupress.org>

Lo et al (2012)

## The Mini Viewer

Attribution & Maneuverability

### Lkb1 regulates organogenesis and early oncogenesis along AMPK-dependent and -independent pathways

Bryan Lo, Geraldine Strasser, Meredith Sagolla, Cary D. Austin, Melissa Junttila, Ira Mellman

JCB vol. 199 no. 7 1117-1118 Article DOI: [10.1083/jcb.201238080](https://doi.org/10.1083/jcb.201238080) DataViewer DO: [10.1083/jcb.201208080.dv](https://doi.org/10.1083/jcb.201208080.dv)

Figure 6 :: 4 images

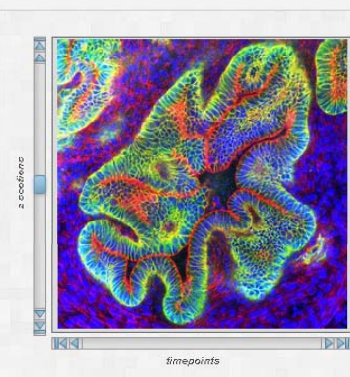
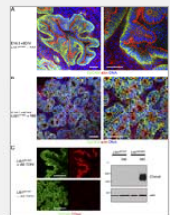


Image Details

Description  
z-stack of a PanIN-like lesion in an Lkb1 MG/MG pancreatic explant

Legend  
Shown is a z-stack of a mouse E16.5 Lkb1 MG/MG pancreatic explant cultured on transwell filters for 8 days in vitro with 1 μM 1NMPP1 and labeled for EpCAM (green), actin (red), and DNA (blue).

Figure 7 [2]



## The Mini Viewer

**JCB Data Viewer - Manuscript - Mozilla Firefox**

File Edit View History Bookmarks Tools Help

JCB Data Viewer - Manuscript

Search the JCB Data Viewer

Home About Contact JCB | log in

**Lkb1 regulates organogenesis and early oncogenesis along AMPK-dependent and -independent pathways**

Published 24 Dec 2012

Full Viewer

Eryan LC, Geraldine Strasser, Meredith Sagolla, Cary D. Austin, Melissa Junttila, Ira Mellman

JCB vol. 199 no. 7 1177-1177 Article DOI: 10.1083/jcb.201208080 DataViewer DOI: 10.1083/jcb.201208080.dv

**Figure 6 :: 4 images**

Download Figure

**Image Details**

**Description**

z-stack of a PanIN-like lesion in an Lkb1 MG/MG pancreatic explant

**Legend**

Shown is a z-stack of a mouse E16.5 Lkb1 MG/MG pancreatic explant cultured on transwell filters for 8 days in vitro with 1µM 1NMPP1 and labeled for EpCAM (green), actin (red), and DNA (blue).

z coordinate

timepoints

Full Viewer Download Image

**Original Data**

Download All Data (12)

Figure 3 [6]

Figure 6 [4]

Figure 7 [2]

Download Part

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## The Mini Viewer

The screenshot shows the JCB Data Viewer interface in a Mozilla Firefox browser. The main content area displays a manuscript titled "Lkb1 regulates organogenesis and early oncogenesis along AMPK-dependent and -independent pathways" by Eryan LC, Geraldine Strasser, Meredith Sagolla, Cary D. Austin, Melissa Junttila, and Ira Mellman. The article is published in JCB, volume 199, issue 7, pages 1107-1117, with a DOI of 10.1083/jcb.201208080. The interface includes a search bar, navigation links (Home, About, Contact), and a "log in" option. A large microscopy image of a z-stack of a PanIN-like lesion is the central focus. To the right, there is a section for "Original Data" with a "Download All Data (12)" button and a list of figures: Figure 3 [6], Figure 6 [4], and Figure 7 [2]. Below the main image, there is a "Figure 6 :: 4 images" section with a "Download Figure" button. A "Full Viewer" button is also present. The interface is annotated with a box labeled "Annotation" pointing to the "Image Details" section.

**Annotation**

**Image Details**

**Description**  
a z-stack of a PanIN-like lesion in an Lkb1 MG/MG pancreatic explant

**Legend**  
Shown is a z-stack of a mouse E16.5 Lkb1 MG/MG pancreatic explant cultured on transwell filters for 8 days in vitro with 1µM 1NMPP1 and labeled for EpCAM (green), actin (red), and DNA (blue).

<http://jcb-dataviewer.rupress.org>

## The Mini Viewer

The screenshot displays the JCB Data Viewer web interface. At the top, there is a search bar and navigation links (Home, About, Contact, JCB, log in). The main content area shows a manuscript titled "Lkb1 regulates organogenesis and early oncogenesis along AMPK-dependent and -independent pathways" by Eryan LC, Geraldine Strasser, Meredith Sagolla, Cary D. Austin, Melissa Junttila, and Ira Mellman. Below the title, there is a "Full Viewer" button. The figure section shows "Figure 6 :: 4 images" with a "Download Figure" button. A large image viewer displays a z-stack of a PanIN-like lesion in an Lkb1 MG/MG pancreatic explant. The image details include a description and legend. The legend states: "Shown is a z-stack of a mouse E16.5 Lkb1 MG/MG pancreatic explant cultured on transwell filters for 8 days in vitro with 1µM 1NMPP1 and labeled for EpCAM (green), actin (red), and DNA (blue)." There are "Full Viewer" and "Download Image" buttons for the main image. Below the main image, there are two smaller thumbnail images and a "Download Part" button. On the right side, there is an "Original Data" section with "Download All Data [12]" and a list of figures: "Figure 3 [6]", "Figure 6 [4]", and "Figure 7 [2]". A callout box on the right highlights "Sharing & Re-analysis" with buttons for "Download Image", "Download Figure", and "Download All Data [957]".

<http://jcb-dataviewer.rupress.org>

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## The Mini Viewer

The screenshot displays the JCB Data Viewer web interface in a Mozilla Firefox browser. The page title is "JCB Data Viewer - Manuscript". The URL in the address bar is "jcb-dataviewer.rupress.org/jcb/browse/5902/138C6/". The page features a search bar with the text "Search the JCB DataViewer" and a "Search" button. Navigation links for "Home", "About", "Contact", and "JCB" are visible, along with a "log in" link.

The main content area displays a manuscript entry for the article "Lkb1 regulates organogenesis and early oncogenesis along AMPK-dependent and -independent pathways" by Eryan LC, Geraldine Strasser, Meredith Sagolla, Cary D. Austin, Melissa Junttila, and Ira Mellman. The article is published in JCB, vol. 199, no. 7, 117-117, with an Article DOI of 10.1083/jcb.201208080 and a DataViewer DOI of 10.1083/jcb.201208080.dv. A "Full Viewer" button is present next to a small thumbnail image of the organ explant.

Below the article information, the interface shows "Figure 6 :: 4 images" with a "Download Figure" button. A large, detailed image of the organ explant is displayed, with a "Full Viewer" and "Download Image" button above it. To the right of the image is an "Image Details" section containing a "Description" and a "Legend".

**Description**  
z-stack of a PanIN-like lesion in an Lkb1 MG/MG pancreatic explant

**Legend**  
Shown is a z-stack of a mouse E16.5 Lkb1 MG/MG pancreatic explant cultured on transwell filters for 8 days in vitro with 1µM 1NMPP1 and labeled for EpCAM (green), actin (red), and DNA (blue).

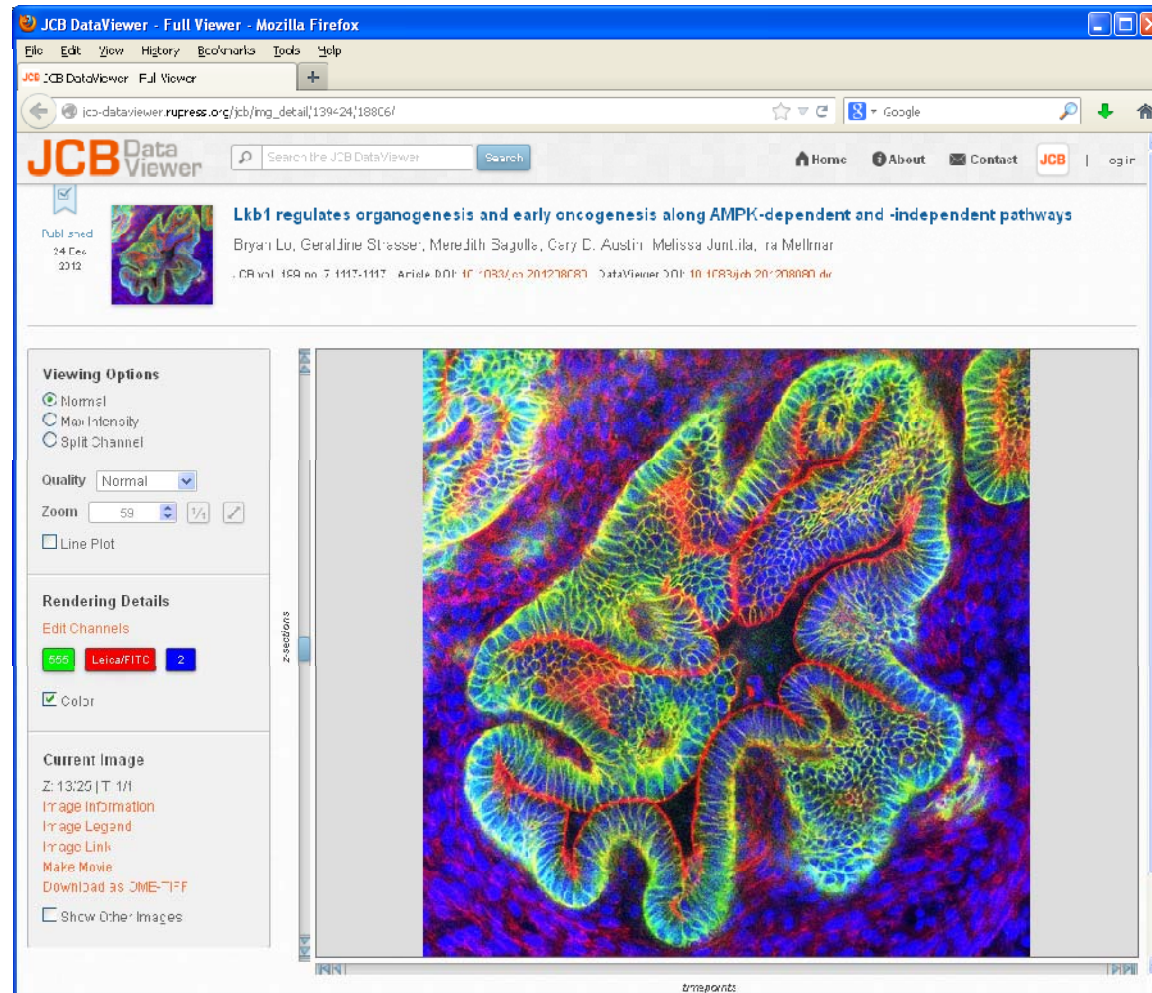
Below the main image, there are two smaller thumbnail images labeled "A". A "Download Part" button is located below the thumbnails.

On the right side of the page, there is an "Original Data" section with a "Download All Data (12)" button. Below this, there are links for "Figure 3 [6]", "Figure 6 [4]", and "Figure 7 [2]". A grid of small image thumbnails is shown below these links.

At the bottom of the page, there is a search bar with the text "Search the JCB DataViewer" and a "Search" button. A blue box with the word "Mining" is overlaid on the bottom right of the page.



## The Full Viewer



<http://jcb-dataviewer.rupress.org>

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The Full Viewer

Annotation

**Image Information**

**Basic Information**

**Image name:** a z-stack of a PanIN-like lesion in an Lkb1 MG/MG pancreatic explant

**Author:** Bryan Lo, Geraldine Strasser, Meredith Sagolla, Cary D. Austin, Melissa Junttila, Ira Mellman

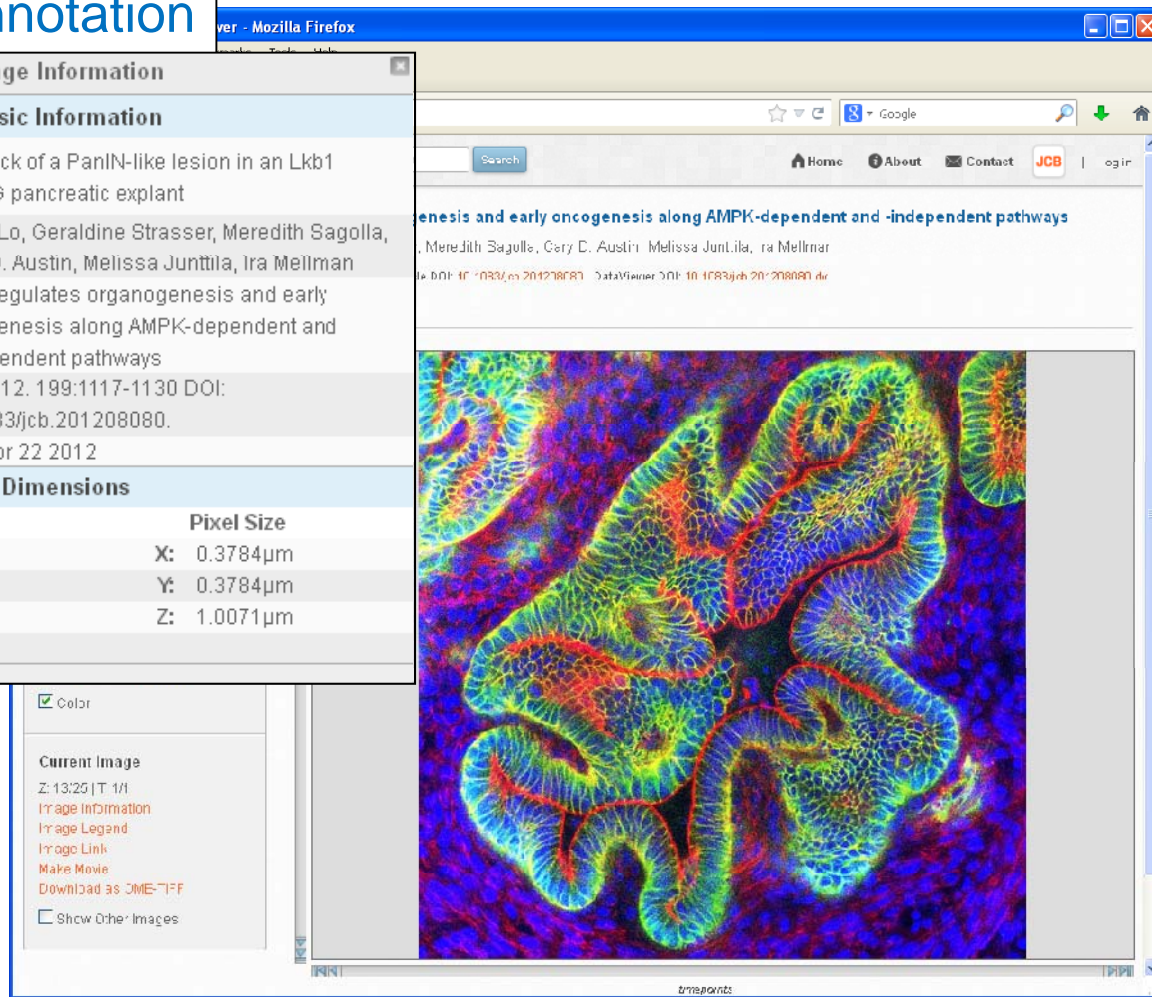
**Publication:** Lkb1 regulates organogenesis and early oncogenesis along AMPK-dependent and -independent pathways

**Publication ID:** jcb. 2012. 199:1117-1130 DOI: 10.1083/jcb.201208080.

**Created on:** Sun Apr 22 2012

**Dimensions**

Image Size	Pixel Size
<b>X:</b> 1024px	<b>X:</b> 0.3784µm
<b>Y:</b> 1024px	<b>Y:</b> 0.3784µm
<b>Z:</b> 25	<b>Z:</b> 1.0071µm
<b>T:</b> 1	



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Lo et al (2012)

## The Full Viewer

The screenshot displays the JCB Data Viewer interface in a Mozilla Firefox browser window. The address bar shows the URL: [http://jcb-dataviewer.rupress.org/jcb/img\\_detail/139424/18806/](http://jcb-dataviewer.rupress.org/jcb/img_detail/139424/18806/). The page title is "JCB Data Viewer - Full Viewer". The main content area features a search bar, navigation links (Home, About, Contact, JCB, login), and a featured article titled "Lkb1 regulates organogenesis and early oncogenesis along AMPK-dependent and -independent pathways" by Bryan Lu, Geraldine St'essier, Meredith Bagulle, Cory D. Austin, Melissa Junttila, and Ira Mellman. Below the article is a thumbnail image of the featured microscopy image. The central part of the interface is a large microscopy image of intestinal crypts, rendered in a 3D-like perspective with various channels highlighted in green, red, and blue. To the left of the image is a "Viewing Options" panel with radio buttons for "Normal" (selected), "Max Intensity", and "Split Channel". Below this is a "Quality" dropdown set to "Normal", a "Zoom" slider set to 59, and a "Line Plot" checkbox. The "Rendering Details" section includes "Edit Channels" with three colored buttons (green, red, blue) and a "Color" checkbox. A "Link to This Page" dialog box is open in the foreground, showing the URL: [http://jcb-dataviewer.rupress.org/jcb/img\\_detail/139424/18806/?c=110:255%00f](http://jcb-dataviewer.rupress.org/jcb/img_detail/139424/18806/?c=110:255%00f).

Attribution & Maneuverability

<http://jcb-dataviewer.rupress.org>

Lo et al (2012)

## The Full Viewer

### Data Presentation

The screenshot displays the JCB Data Viewer interface in a Mozilla Firefox browser. The main window shows a 3D visualization of a cell structure, likely a Drosophila wing, with different channels highlighted in green, red, and blue. The interface includes a search bar, navigation buttons (Home, About), and a 'Make Movie' dialog box. The dialog box is titled 'Make Movie' and contains the following options:

- Axis: Z (dropdown menu)
- Format: Quiddtime .mov (Mac) (dropdown menu)
- Frames per second: 3 (spinners)
- Make Movie (button)

The main window also features a 'Viewing Options' panel on the left with the following settings:

- Normal (selected radio button)
- Max Intensity (radio button)
- Split Channel (radio button)
- Quality: Normal (dropdown menu)
- Zoom: 59 (spinners)
- Line Plot (checkbox, unchecked)
- Rendering Details: Edit Channels (button), 556 (green), Leica/FITC (red), 2 (blue), Color (checkbox, checked)
- Current Image: Z: 13/25 | T: 1/1, Image Information, Image Legend, Image Link, Make Movie, Download as COME-TIFF, Show Other Images (checkbox, unchecked)

<http://jcb-dataviewer.rupress.org>

Lo et al (2012)

## The Full Viewer

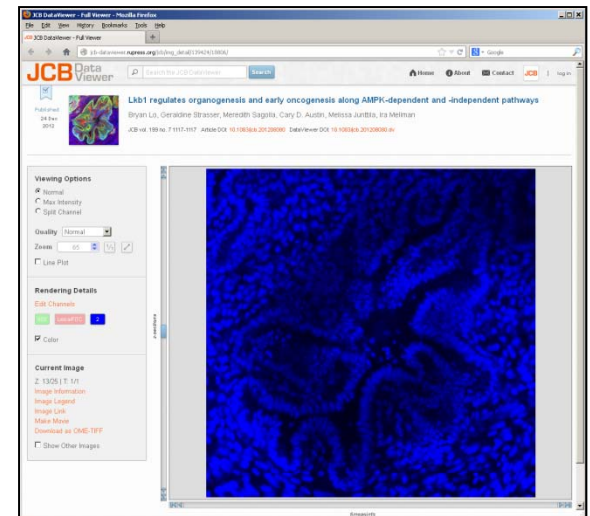
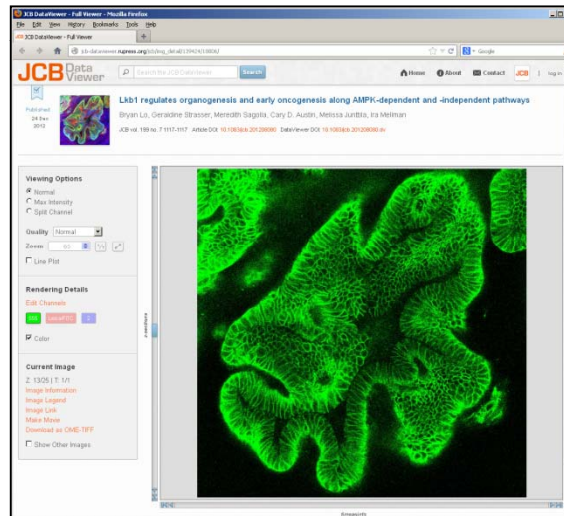
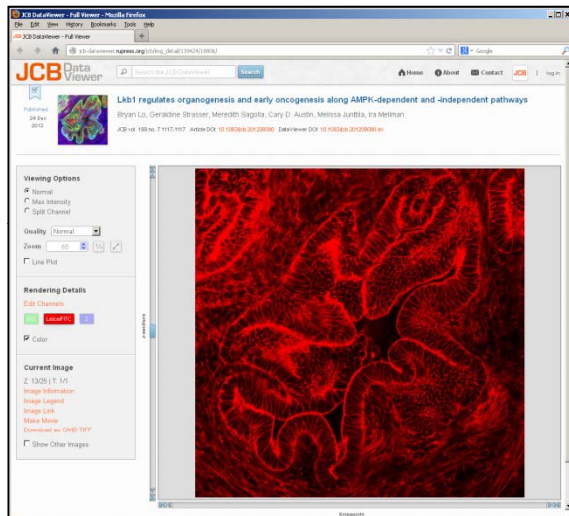
The screenshot displays the JCB Data Viewer interface in a Mozilla Firefox browser window. The browser address bar shows the URL [http://jcb-dataviewer.rupress.org/jcb/img\\_det.all.139424.18806/](http://jcb-dataviewer.rupress.org/jcb/img_det.all.139424.18806/). The page title is "JCB Data Viewer - Full Viewer". The main content area features a search bar, navigation links (Home, About, Contact, JCB), and a login option. Below the navigation is a featured article titled "Lkb1 regulates organogenesis and early oncogenesis along AMPK-dependent and -independent pathways" by Bryan Lu, Geraldine St'essier, Meredith Bagulle, Cory D. Austin, Melissa Junttila, and Ira Mellman. The article is published in JCB, Vol. 159, no. 5, 1117-1117, Article DOI: 10.1083/jcb.201208081, DataViewer DOI: 10.1083/jcb.201208081.dv. The central image is a microscopy image showing a complex, branching structure with multiple color channels (red, green, blue, yellow). To the left of the image is a "Viewing Options" panel with radio buttons for "Normal" (selected), "Max Intensity", and "Split Channel". It also includes a "Quality" dropdown set to "Normal", a "Zoom" slider at 59, and a "Line Plot" checkbox. Below this is a "Rendering Details" section with "Edit Channels" (556, Leica/FITC, 2) and a "Color" checkbox. The "Current Image" section shows "Z: 13/25 | T: 1/1" and links for "Image Information", "Image Legend", "Image Link", "Make Movie", and "Download as OME-TIFF". A "Show Other Images" checkbox is at the bottom. To the right of the image is another "Viewing Options" panel with radio buttons for "Normal" (selected), "Max Intensity", and "Split Channel". It includes a "Quality" dropdown set to "Normal", a "Zoom" slider at 59, a "Line Plot" checkbox (checked), and an "Axis" dropdown set to "Horizontal". A "Y = 983" input field is at the bottom.

Data Analysis

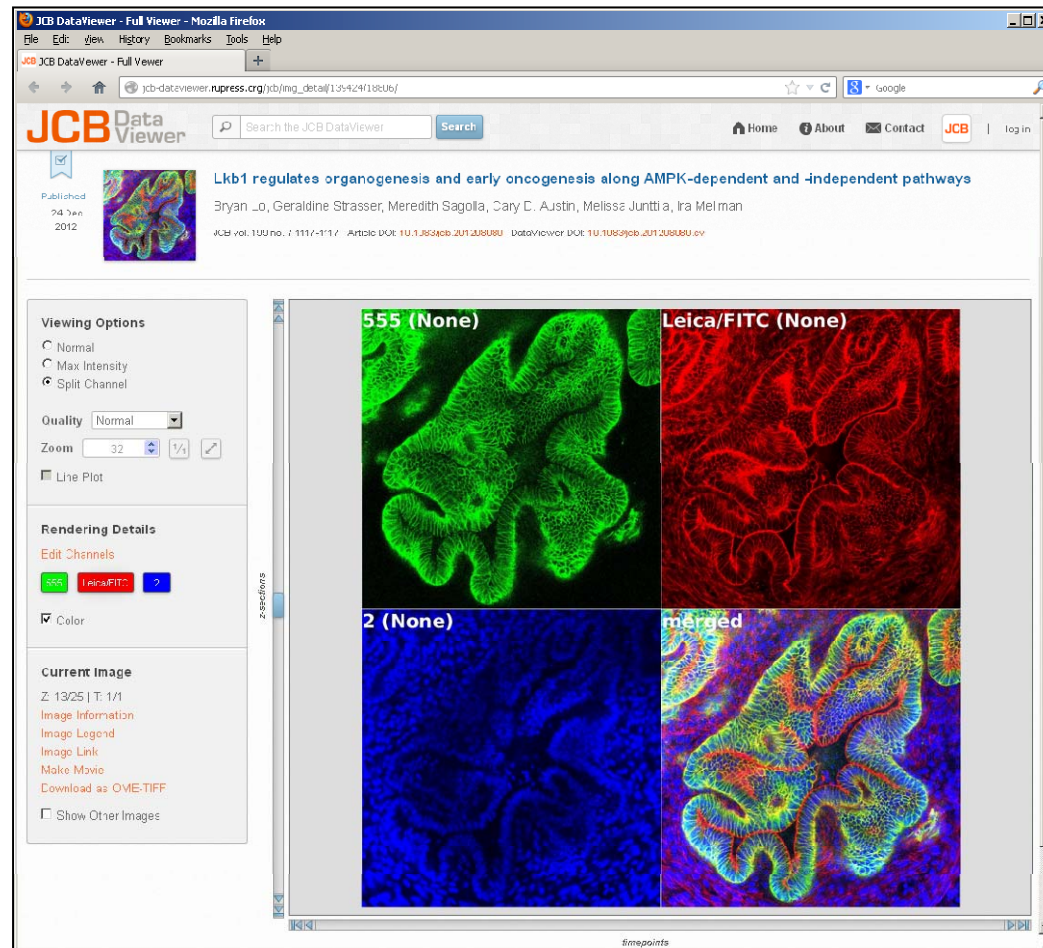
<http://jcb-dataviewer.rupress.org>

Lo et al (2012)

## Split-Channel View



## Split-Channel View



<http://jcb-dataviewer.rupress.org>

Lo et al (2012)

## **2. ultra-large image presentation**

<http://jcb-dataviewer.rupress.org>

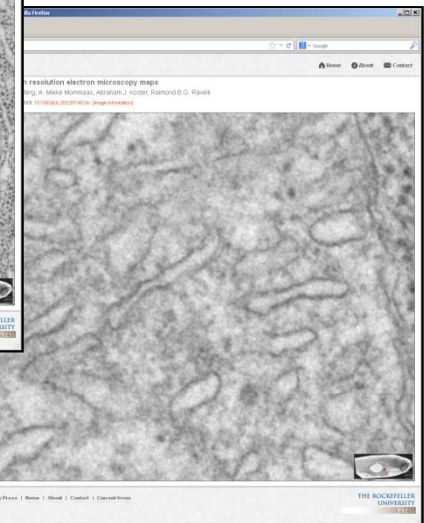
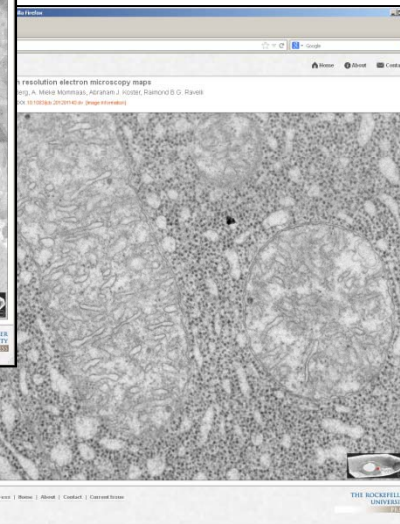
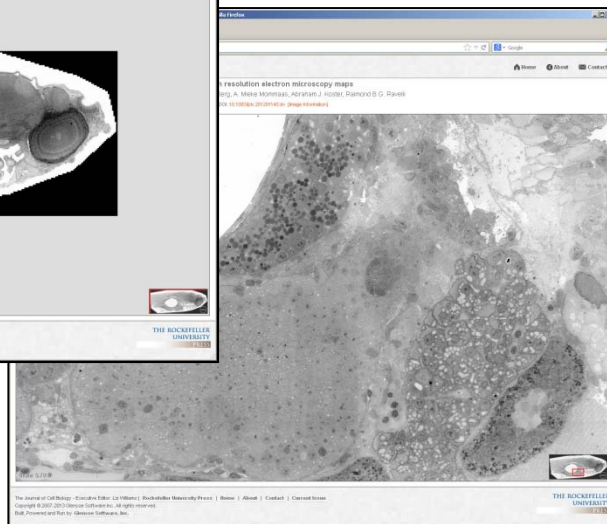
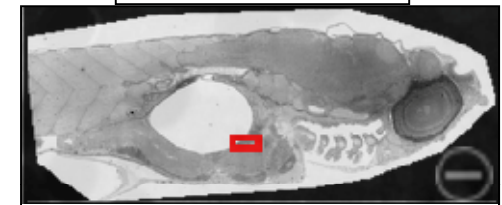
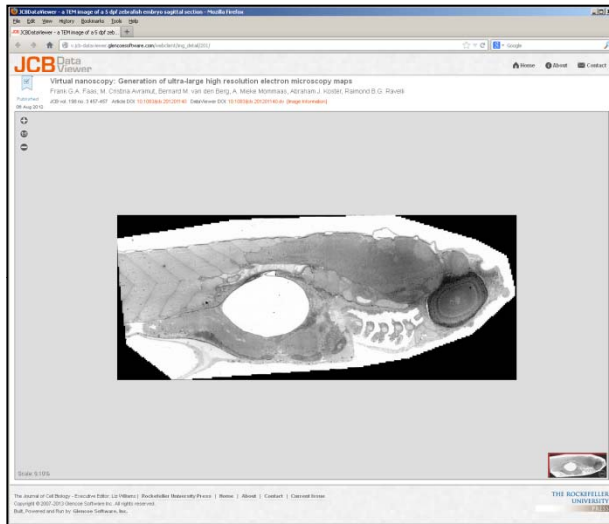


## What do we mean by an “ultra-large” image?

- 26,000 tiled EMs
- 921,600 pixels x 380,928 pixels
- 281 gigapixels
- 1.6 nm resolution (16 million dpi)

## Ultra-large, high-resolution, tiled images

Navigation



<http://jcb-dataviewer.rupress.org>

Faas et al (2012)

## Ultra-large Image Presentation



## Ultra-large Image Presentation



- 90,000 unique visitors in one week
- a peak of 38,000 unique visitors in one day
- 32 million image tiles served in one week

### **3. high-content screen analysis**

<http://jcb-dataviewer.rupress.org>

## **Eight high-content screens hosted to date:**

from “small”:

Srikumar et al (2013)

- 12 384-well plates (only 25% full)
- 3 fields per well
- 2 channels per field
- 2 quantitative datapoints per gene scored as a ‘hit’ (290 total)

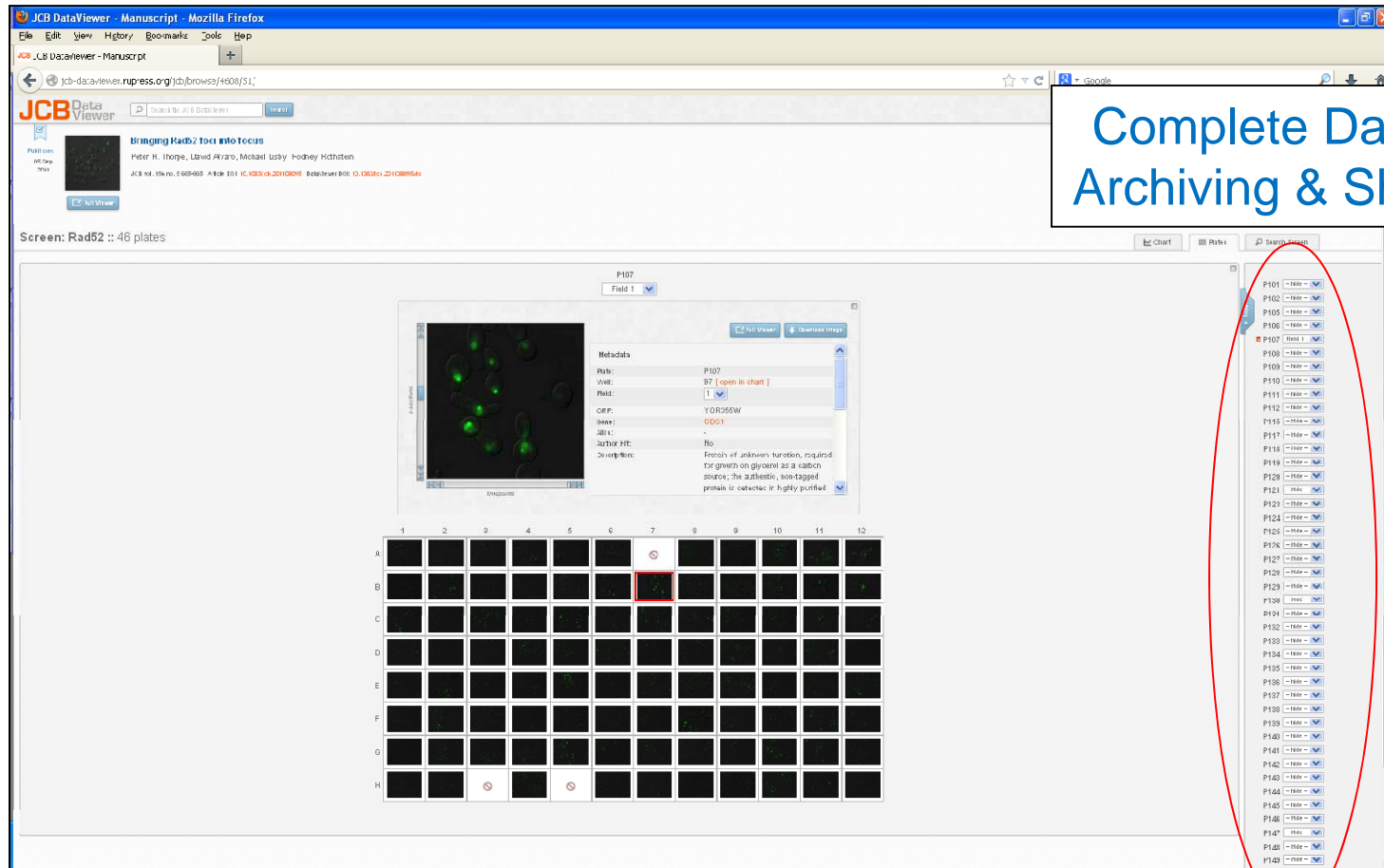
to “large and complicated”:

Breker et al (2013)

- 85 384-well plates
- 3 fields per well
- 3 channels per field (brightfield and 2 fluorescence channels)
- 97,920 total images
- cross-plate datasets (2 untreated and 3 treated datasets per strain)
- 1 qualitative & 1 quantitative datapoint per strain per treatment (26,650 total)

<http://jcb-dataviewer.rupress.org>

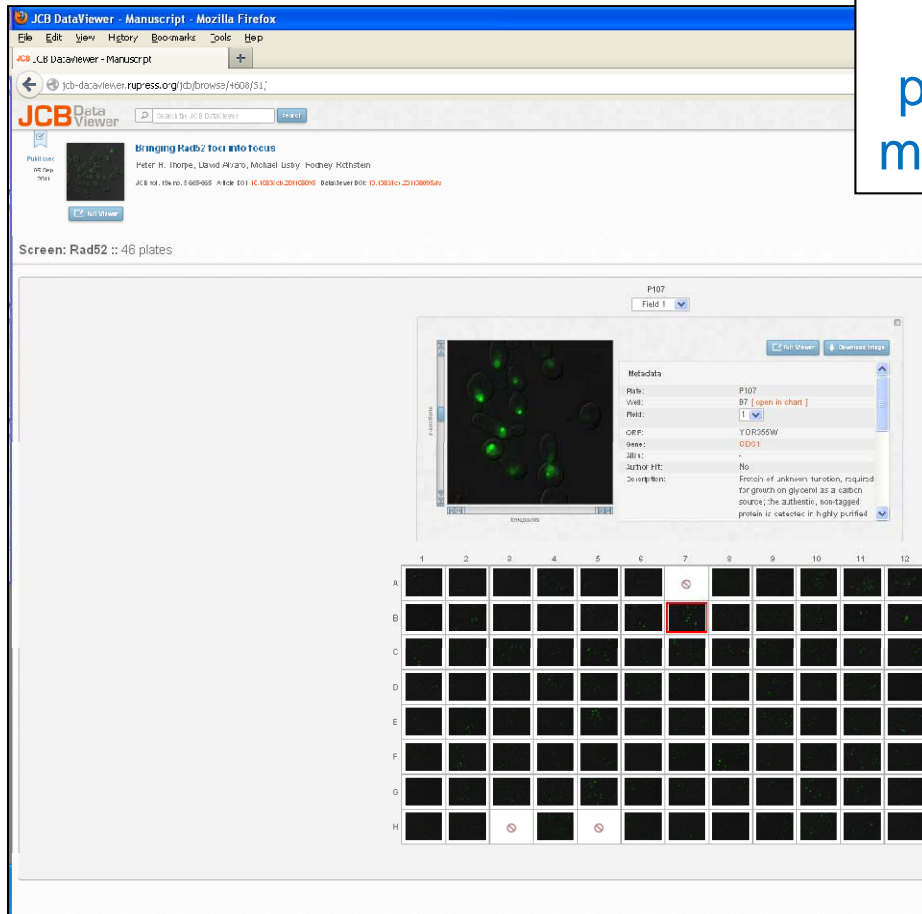
## HCS Data – Plate View



Complete Dataset  
Archiving & Sharing

HCS Data – Plate View

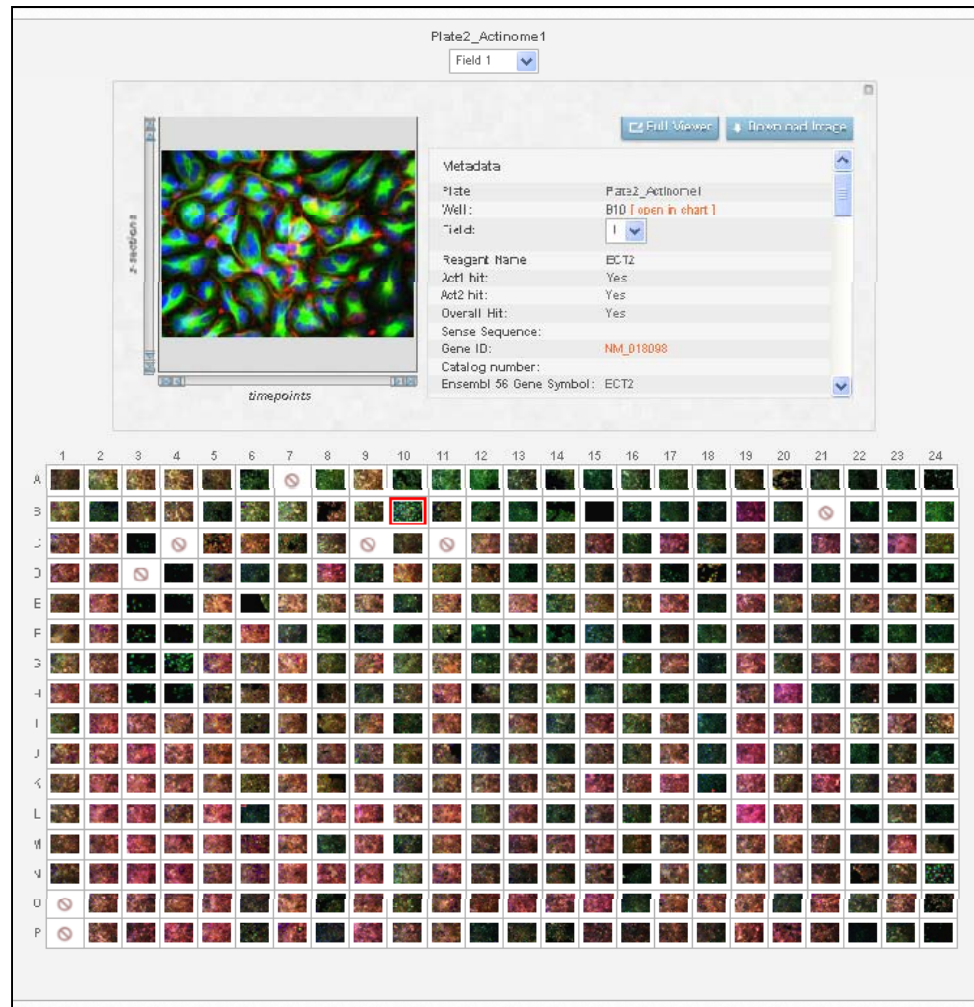
Maneuverability between plate array data, image data, metadata, and phenotypic data



Metadata	
Plate:	P107
Well:	B7 [ open in chart ]
Field:	1
ORF:	YOR355W
Gene:	GDS1
Alias:	-
Author Hit:	Nu
Description:	Protein of unknown function, required for growth on glycerol as a carbon source; the authentic, non tagged protein is detected in highly purified mitochondria in high-throughput studies
GO Biological Process:	generation of precursor metabolites and energy, cellular respiration
GO Molecular Function:	not available
GO Cellular Component:	nucleus, mitochondrion, cytoplasm
GO Term:	cellular respiration, cytoplasm, generation of precursor metabolites and energy, mitochondrion, nucleus
Record no.:	
Strain:	
Batch:	
Cells:	198
Foci:	24
% of Foci:	12



## HCS Data – Plate View



Maneuverability between plate array, image data, metadata, and phenotypic data

HCS Data – Plate View

Plate2\_Actinome1  
Field 1

Full Viewer | Download

Metadata

Plate:	Plate2_Actinome1
Well:	B10 <a href="#">[open in chart]</a>
Field:	2
Reagent Name:	ECT2
Act1 hit:	Yes
Act2 hit:	Yes
Overall Hit:	Yes
Sense Sequence:	
Gene ID:	NM_018098
Catalog number:	
Ensembl Gene Symbol:	ECT2
More actin:	No
More peripheral actin:	No
More cytoplasmic actin:	Yes
More actin over nucleus:	Yes
More filopodia:	No
Increased width of lamellae:	Yes
Less actin:	No
Fewer filopodia:	No
Decreased width of lamellae:	No
Nuclear actin ring:	No
More actin puncta:	Yes
More actin stress fibers:	No
More transverse actin stress fibers:	No
More cortical actin stress fibers:	No
More zigzag actin stress fibers:	Yes
Disorganised peripheral actin:	Yes
Increased cell size:	Yes
Decreased cell size:	No
Variable cell size:	No
Cell shape round:	No
Cell shape spiky:	No
Cell shape bipolar or elongate:	No
Cell shape geometric:	No
Cell shape variable:	No
Decreased cell number:	Yes
Increased cell number:	No
More multinucleate cells:	Yes
Increased DNA area:	Yes
Decreased DNA area:	No
Misshapen DNA:	Yes
Apoptotic DNA:	No
Increased mitotic index:	No
Microtubules disorganised:	Yes
Microtubule processes:	No
Microtubule clumps:	No
Microtubule nuclear ring:	No
Microtubule nuclear bracket:	No
More microtubules:	No
Loss of cell monolayer:	Yes
Motile lamellae:	No

timepoints

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

A B C D E F G H I J K L M N O P

Maneuverability between plate array, image data, metadata, and phenotypic data

HCS Data – Plate View

[ open in chart ]

Plate2\_Actinom1  
Field 1

Full Viewer Download

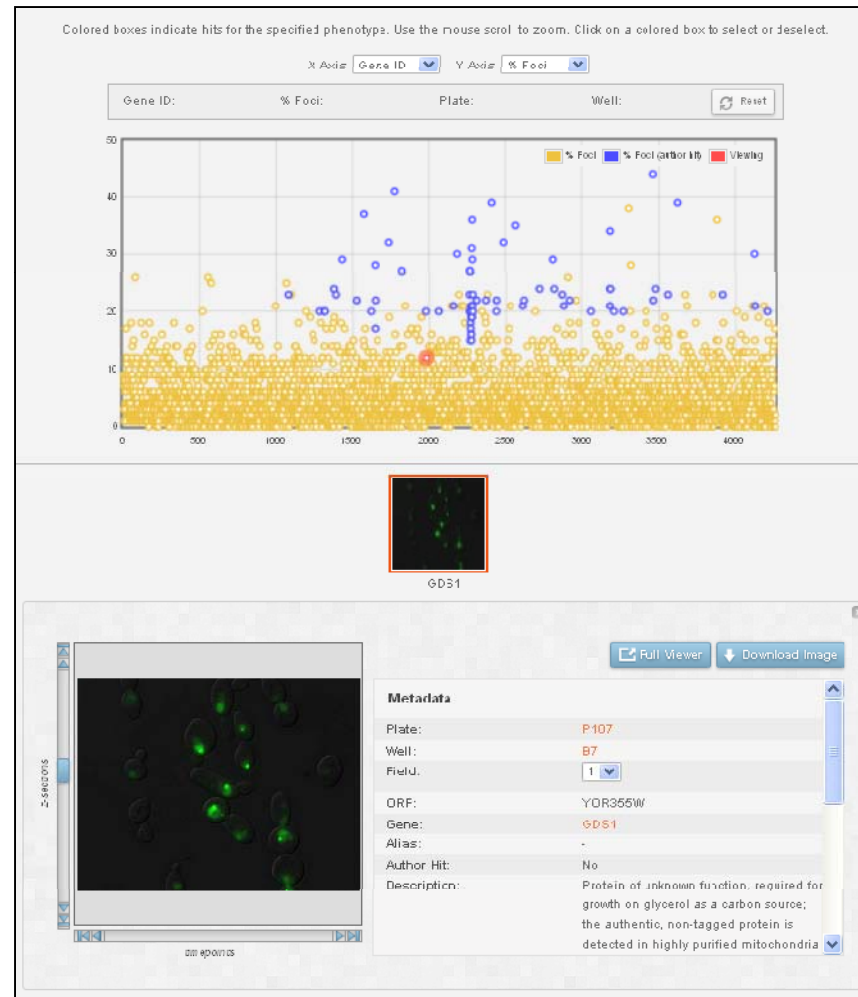
Metadata

Plate: Par22\_Actinom1  
Well: B10 [open in chart 1](#)  
Field: 1

Reagent Name: ECT2  
Act1 hit: Yes  
Act2 hit: Yes  
Overall Hit: Yes  
Sense Sequence:  
Gene ID: NM\_018098  
Catalog number:  
Ensembl 56 Gene Symbol: ECT2

Reagent Name:	ECT2
Act1 hit:	Yes
Act2 hit:	Yes
Overall Hit:	Yes
Sense Sequence:	
Gene ID:	NM_018098
Catalog number:	
Ensembl 56 Gene Symbol:	ECT2
More actin:	No
More peripheral actin:	No
More cytoplasmic actin:	Yes
More actin over nucleus:	Yes
More filopodia:	No
Increased width of lamellae:	Yes
Less actin:	No
Fewer filopodia:	No
Decreased width of lamellae:	No
Nuclear actin ring:	No
More actin puncta:	Yes
More actin stress fibers:	No
More transverse actin stress fibers:	No
More cortical actin stress fibers:	No
More zigzag actin stress fibers:	Yes
Disorganised peripheral actin:	Yes
Increased cell size:	Yes
Decreased cell size:	No
Variable cell size:	No
Cell shape round:	No
Cell shape spiky:	No
Cell shape bipolar or elongate:	No
Cell shape geometric:	No
Cell shape variable:	No
Decreased cell number:	Yes
Increased cell number:	No
More multinucleate cells:	Yes
Increased DNA area:	Yes
Decreased DNA area:	No
Misshapen DNA:	Yes
Apoptotic DNA:	No
Increased mitotic index:	No
Microtubules disorganised:	Yes
Microtubule processes:	No
Microtubule clumps:	No
Microtubule nuclear ring:	No
Microtubule nuclear bracket:	No
More microtubules:	No
Loss of cell monolayer:	Yes
Motile lamellae:	No

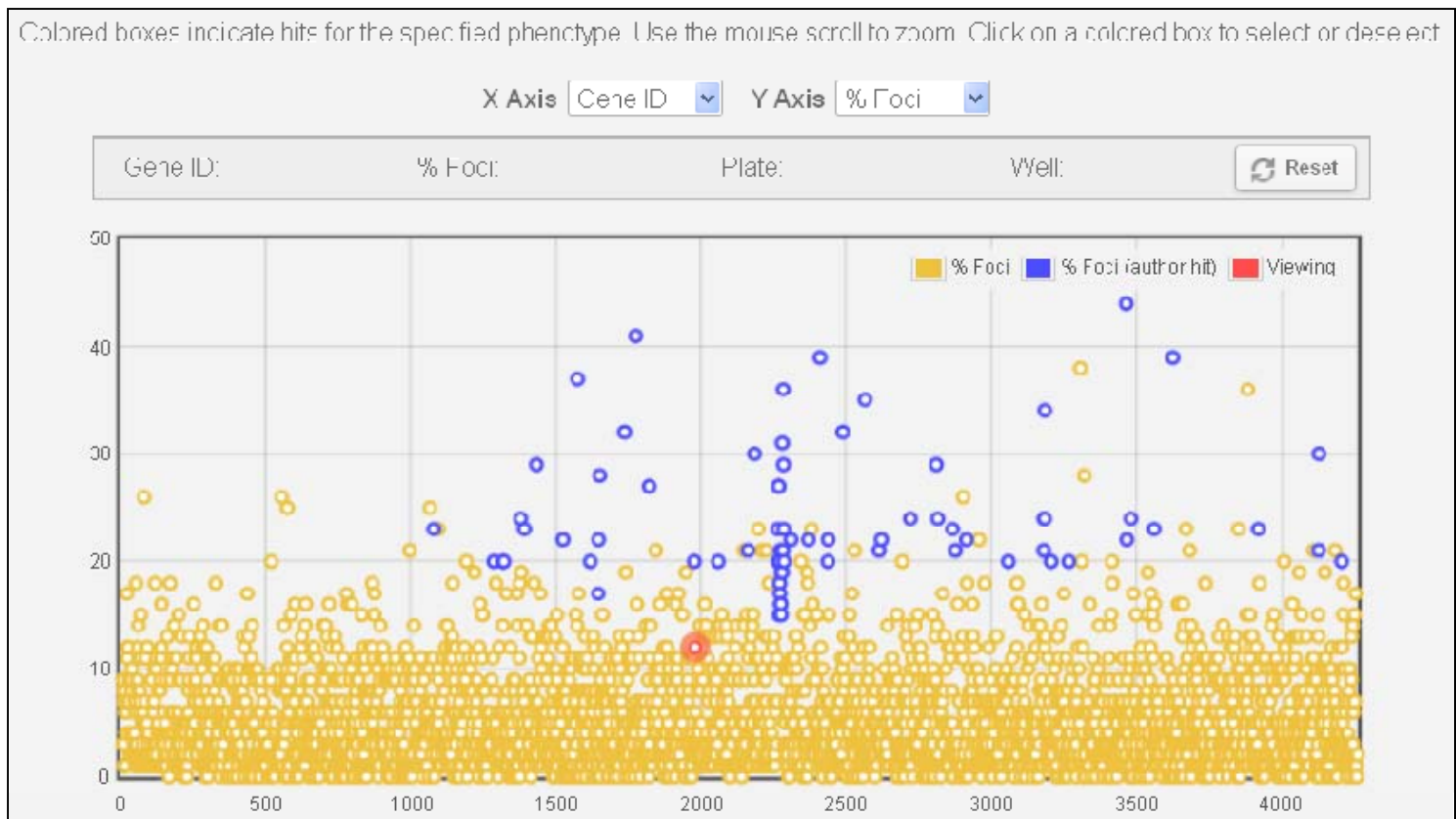
## HCS Data – Chart View (Quantitative)



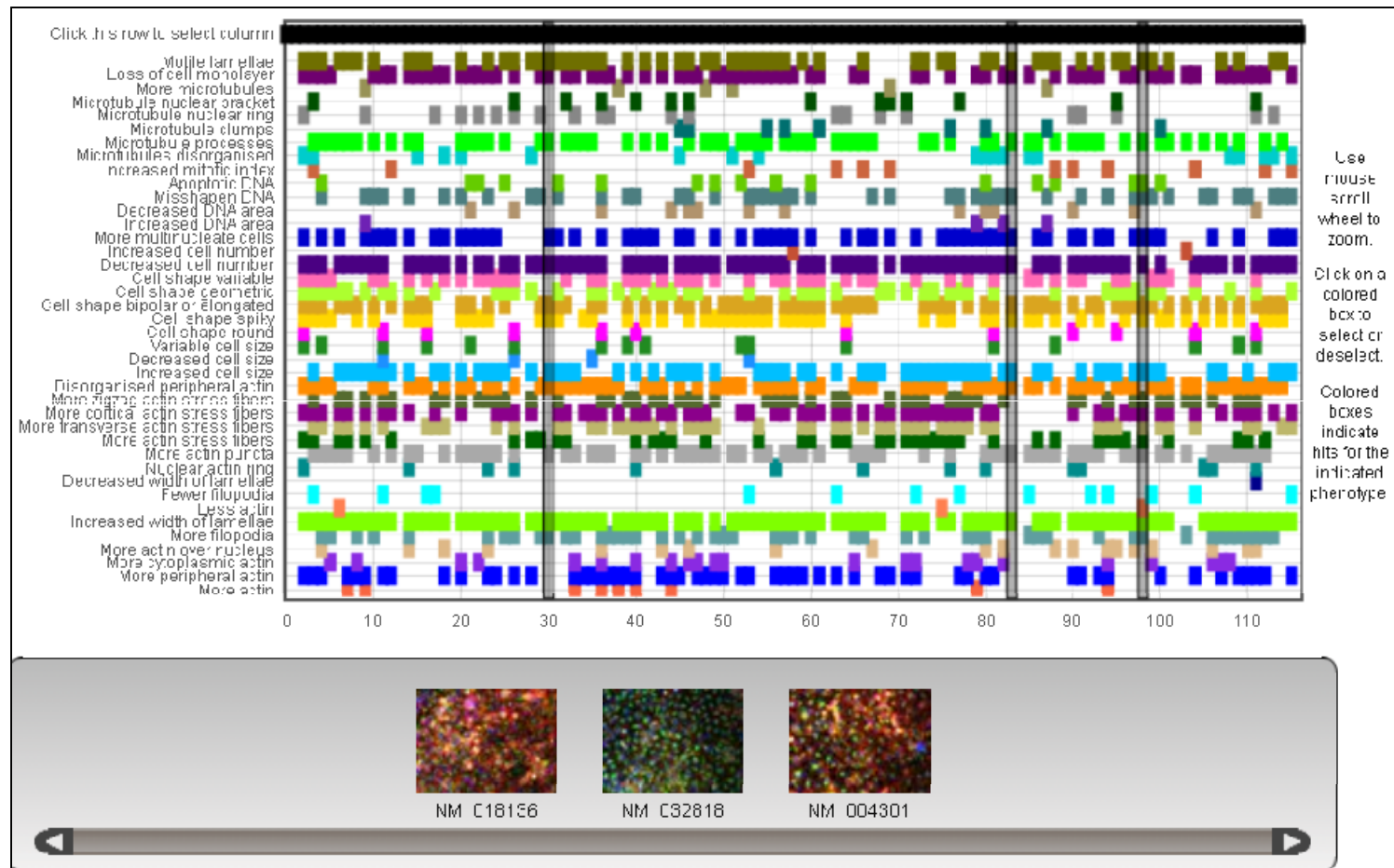
<http://jcb-dataviewer.rupress.org>

Thorpe et al (2011)

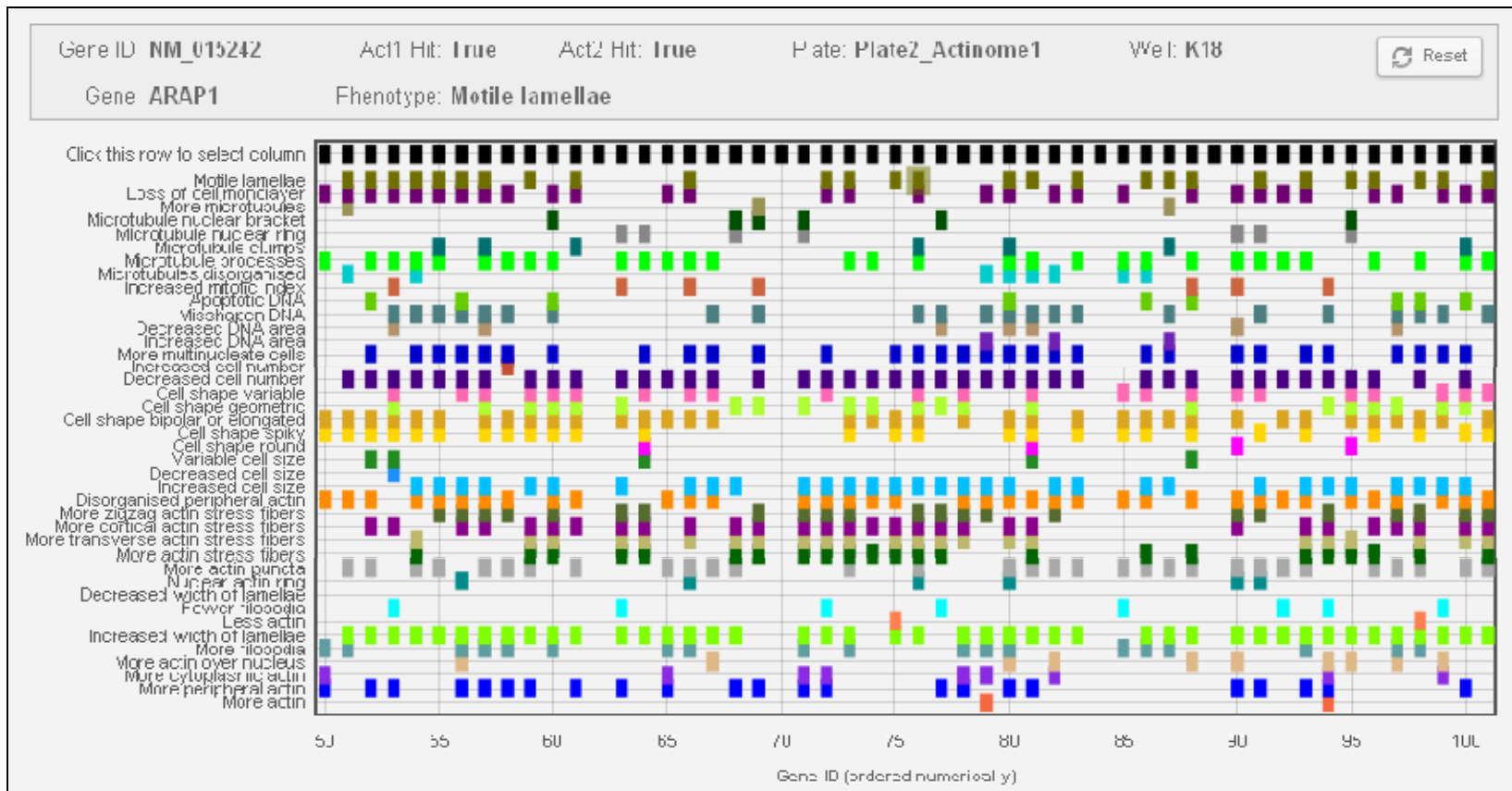
## HCS Data – Chart View (Quantitative)



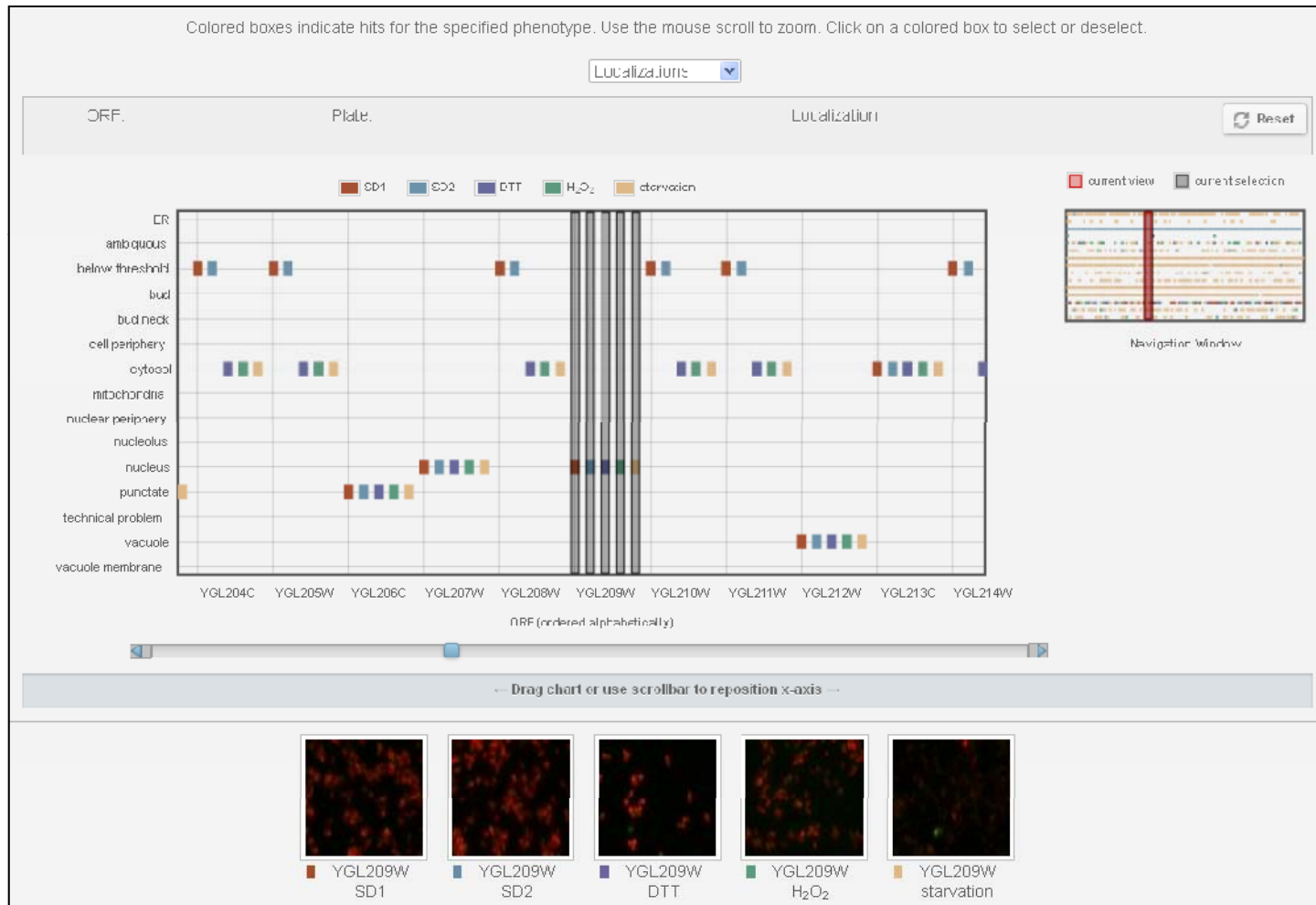
## HCS Data – Chart View (Qualitative)



## HCS Data – Chart View (Qualitative)



## HCS Data – Chart View (Qualitative)

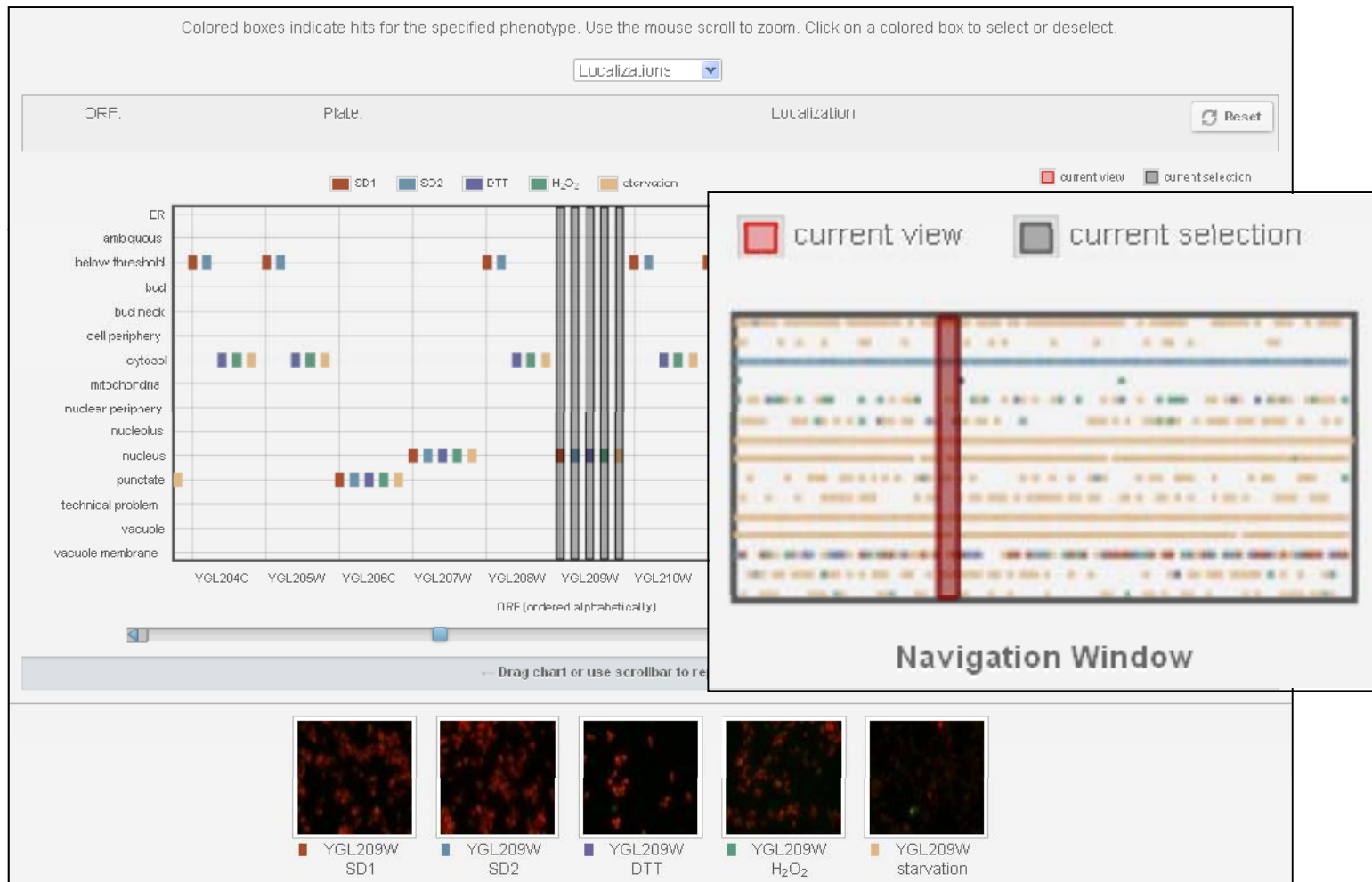




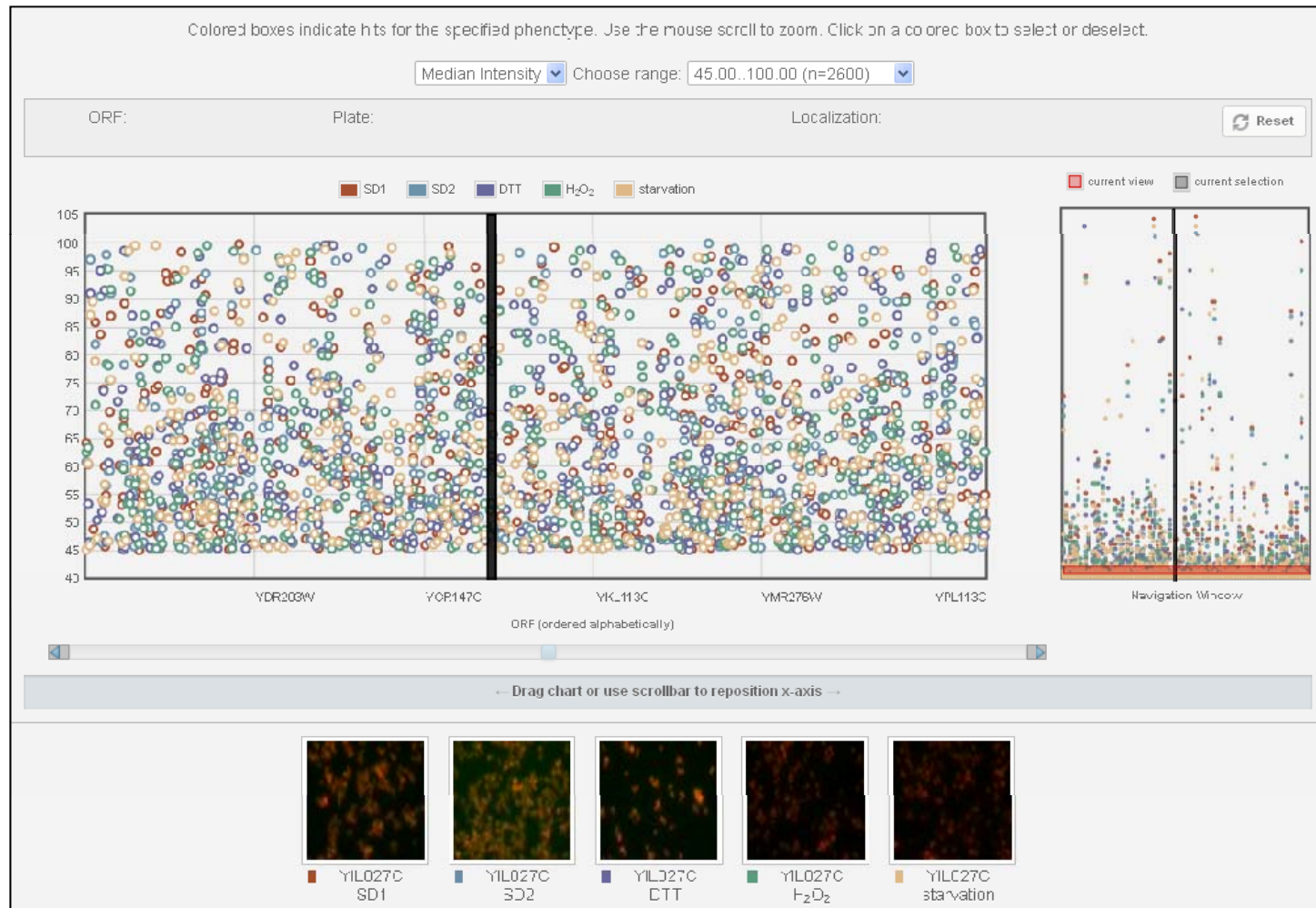
## HCS Data – Chart View (Qualitative)



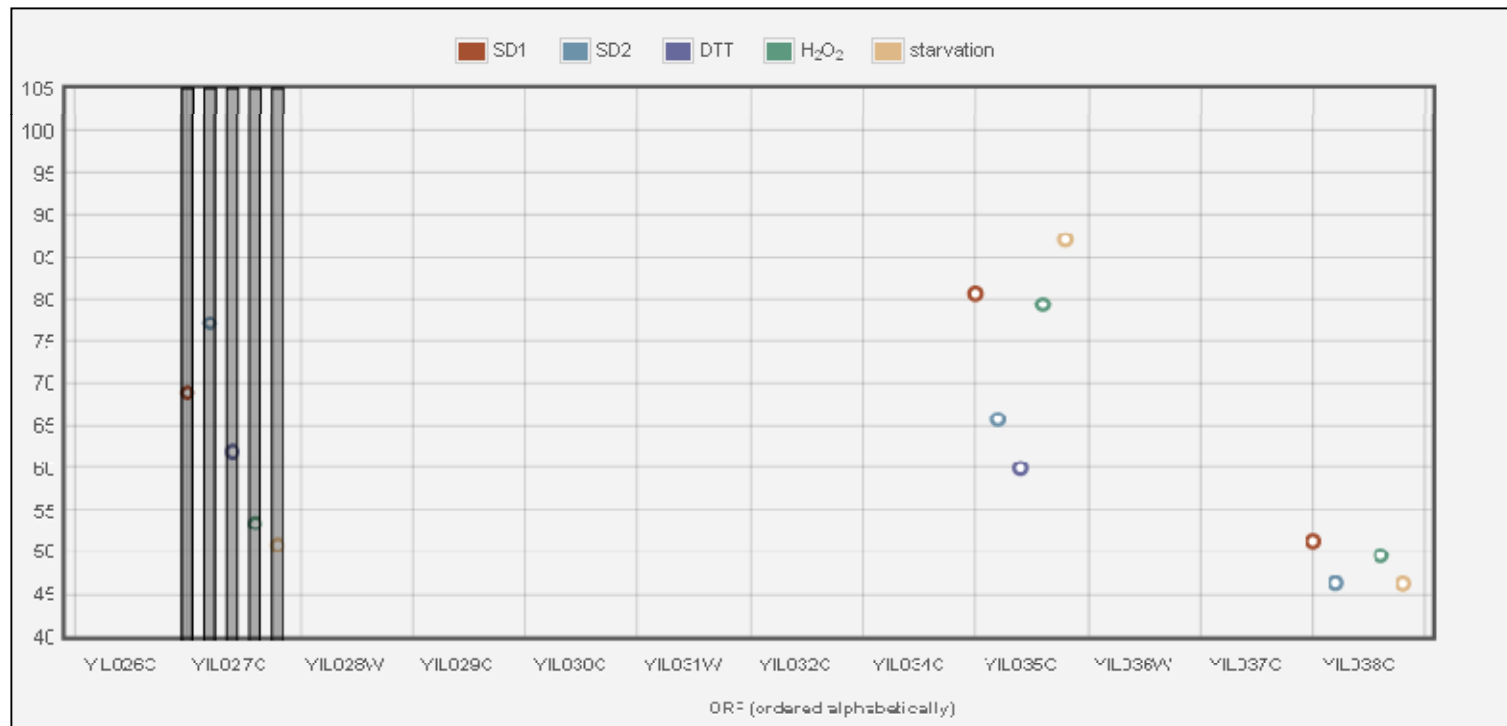
## HCS Data – Chart View (Qualitative)



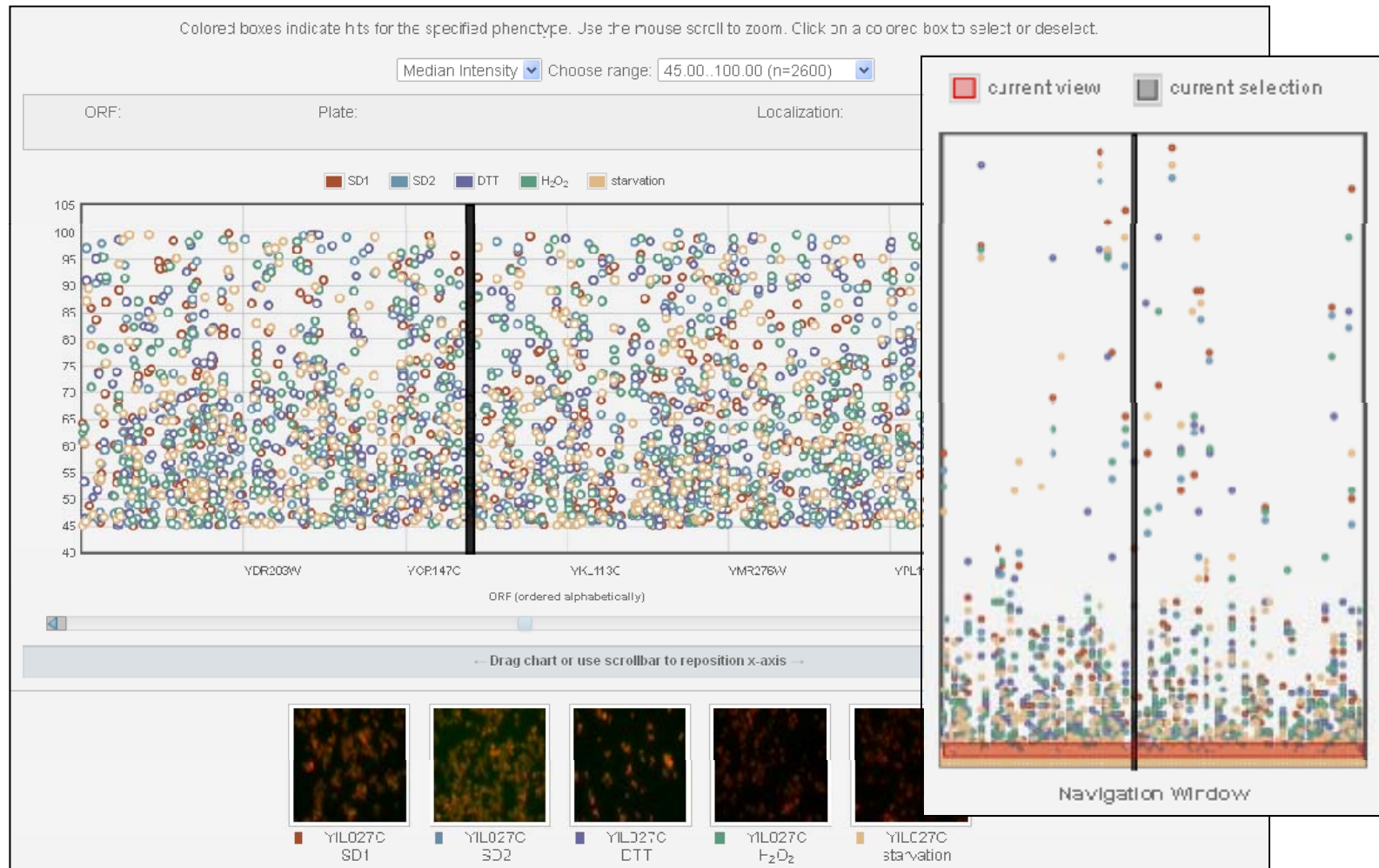
## HCS Data – Chart View (Quantitative)



## HCS Data – Chart View (Quantitative)



## HCS Data – Chart View (Quantitative)



## Summary of Features

- Browser-based viewing of original, raw image files in >125 PFFs (from single images to massively tiled images to plate-arrayed HCS datasets)
- Dynamic linking of image data to metadata to the published paper; seamless integration with publication workflows (e.g., citation information, data DOIs, easy paper-data linking)
- Built-in tools for single-image, ultra-large image, and complex HCS dataset presentation and analysis
- Raw image downloading in OME-TIFF format
- Database-wide search functionality
- Fulfillment of the criteria laid out in the 2013 U.S. government public-access mandate for data resulting from federally-funded research.

## Multidimensional, Interactive Publishing

difference in the amount of Aurora B in unaligned and apparently aligned chromosomes (Fig. 5A). We detected no change in chromosome staining with anti-phosphohistone H3 (Fig. 5A) or anti-phospho-CENP-A (not depicted) after Bod1 depletion. Because both are markers of Aurora B activity (Zeitlin et al., 2001), these results suggest that Aurora B kinase activity was not dramatically impaired by the loss of Bod1. To further assay the function of Aurora B, we determined the localization of MCAK, which localizes to the inner centromere in its phosphorylated form but concentrates at kinetochores in its dephosphorylated state (Andrews et al., 2004). At unaligned sister kinetochores or in kinetochore pairs not yet fully under tension, MCAK is predominantly located at the inner centromere (Fig. 5B; Andrews et al., 2004). In Bod1<sup>siRNA</sup> cells, we observed that although total MCAK present at unaligned centromeres was similar to control cells (Fig. 5C), its precise localization was abnormal, forming multiple foci stretching out to one or both sister kinetochores.

**Figure 5. MCAK is not efficiently phosphorylated in Bod1<sup>siRNA</sup> cells.** (A) Aurora B is not delocalized in Bod1-depleted cells. Phospho-Ser10-histone H3 staining in control and Bod1 siRNA cells indicating Aurora B activity (B–E). Cells were transfected with control or Bod1 siRNA. After 72 h, cells were treated with monastrol for 3 h and released into media containing MG132 for 1 h before fixing. (B and C) Cells were stained for total MCAK population, and levels at kinetochores were quantified. Boxed areas are magnified below the main images. (D and E) Cells were stained for phospho-Ser92-MCAK, and levels at aligned and unaligned kinetochores were quantified. Dashed lines indicate orientation of the metaphase plate. Error bars represent SD. Bars, 5  $\mu$ m.

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Because MCAK localization to centromeres and kinetochores depends on the state of MCAK phosphorylation, we examined the levels of phosphorylated MCAK using an anti-phospho-Ser92 MCAK antibody (Andrews et al., 2004). Phosphorylation of MCAK was substantially reduced at the inner centromere of unaligned chromosomes in Bod1<sup>siRNA</sup> cells compared with the control cells (Fig. 5, D and E). These results suggest that Bod1 depletion impairs the formation of bioriented attachments across sister kinetochores, possibly by impairing the correct localization of MCAK at centromeres and, thereby, preventing its phosphorylation and timely correction of syntelic attachments. We have not detected any effect of Bod1 on the *in vitro* phosphorylation of MCAK by Aurora B (unpublished data), so Bod1 may modulate MCAK phosphorylation by interacting with other proteins. Aurora B activity and kinetochore oscillations are necessary for syntelic correction (Lampson et al., 2004), and our data further suggest that syntelic correction may require MCAK phosphorylation. Whether there is any subtle perturbation in kinetochore oscillations in Bod1-depleted cells is not yet known and will require much higher resolution live cell imaging.

In summary, by using a cell cycle-dependent analysis of the *Xenopus* chromatin proteome, we have identified a novel protein required for proper chromosome biorientation called Bod1. Bod1 is a member of the FAM44 protein family and is highly conserved throughout metazoans. Depletion of Bod1 in human cells causes severe biorientation defects, although kinetochores appear to

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difference in the amount of Aurora B in unaligned and apparently aligned chromosomes (Fig. 5A). We detected no change in chromosome staining with anti-phosphohistone H3 (Fig. 5A) or anti-phospho-CENP-A (not depicted) after Bod1 depletion. Because both are markers of Aurora B activity (Zeitlin et al., 2001), these results suggest that Aurora B kinase activity was not dramatically impaired by the loss of Bod1. To further assay the function of Aurora B, we determined the localization of MCAK, which localizes to the inner centromere in its phosphorylated form but concentrates predominantly located in control cells (Fig. 5C).

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**Figure 5. MCAK is not efficiently phosphorylated in Bod1<sup>siRNA</sup> cells.** (A) Aurora B is not delocalized in Bod1-depleted cells. Phospho-Ser10-histone H3 staining in control and Bod1 siRNA cells indicating Aurora B activity (B–F). Cells were transfected with control or Bod1 siRNA. After 72 h, cells were treated with monastrol for 3 h and released into media containing MG132 for 1 h before fixing. (B and C) Cells were stained for total MCAK population, and levels at kinetochores were quantified. Boxed areas are magnified below the main images. (D and E) Cells were stained for phospho-Ser92-MCAK, and levels at aligned and unaligned kinetochores were quantified. Dashed lines indicate orientation of the metaphase plate. Error bars represent SD. Bars, 5  $\mu$ m.



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Because MCAK localizes to the inner centromere in its phosphorylated form, we compared the correct localization of MCAK in control cells with the correct localization of MCAK in cells treated with the Aurora B inhibitor, AZD1775. In vitro phosphorylation of MCAK is necessary for its localization to the inner centromere, and oscillations are necessary for chromosome segregation. In summary, by using a combination of genetic and biochemical approaches, we have shown that MCAK is a member of the FAZ complex.

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**Bod1, a Novel Kinetochores Protein Required for Chromosome Biorientation**  
I.M. Porter, S.E. McClelland, G.A. Khoudoli, C.J. Hunter, J.S. Andersen, A.D. McAlinh, J.J. Blow, J.R. Swedlow  
JCB vol. no. - Article DOI: 10.1083/jcb.200704089 DataViewer DOI: 10.1083/jcb.200704089 dv

Original Data  
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Figure 4 (5)  
Figure 5 (8)

Figure 5 :: 8 images

A

B

D

Figure 5. MCAK is not in control and Bod1 siRNA and released into me. Boxed areas are magnified. Dashed line

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difference in the amount of Aurora B in unaligned and apparently aligned chromosomes (Fig. 5A). We detected no change in chromosome staining with anti-phosphohistone H3 (Fig. 5A) or anti-phospho-CENP-A (not depicted) after Bod1 depletion. Because both are markers of Aurora B activity (Zeitlin et al., 2001), these results suggest that Aurora B kinase activity was not dramatically impaired by the loss of Bod1. To further assay the function of Aurora B, we determined the localization of MCAK, which localizes to the inner centromere in its phosphorylated form but concentrates predominantly located control cells (Fig. 5C).

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Published 22 Oct 2007  
JCB vol. no. - Article DOI: 10.1083/jcb.200704098 DataViewer DOI: 10.1083/jcb.200704098

**Figure 5 :: 8 images**

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**Figure 5 :: 8 images**

**Image Details**  
**Description**  
Control siRNA Aurora B

**Legend**  
Figure 5. MCAK is not efficiently phosphorylated in Bod1 siRNA cells. (A) Aurora B is not delocalized in Bod1-depleted cells. Phospho-Ser10-histone H3 staining in control and Bod1 siRNA cells indicating Aurora B activity. (B-E) Cells were transfected with control or Bod1 siRNA. After 72 h, cells were treated with microtubule for 5 h, and reassembled.

**Original Data**  
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[Figure 4 \[5\]](#)  
[Figure 5 \[8\]](#)  
[Download Figure](#)

**Figure 5. MCAK is not efficiently phosphorylated in Bod1 siRNA cells.** (A) Aurora B is not delocalized in Bod1-depleted cells. Phospho-Ser10-histone H3 staining in control and Bod1 siRNA cells indicating Aurora B activity. (B-E) Cells were transfected with control or Bod1 siRNA. After 72 h, cells were treated with microtubule for 5 h, and reassembled.

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**Figure 5. MCAK is not efficiently phosphorylated in Bod1 siRNA cells.** (A) Aurora B is not delocalized in Bod1-depleted cells. Phospho-Ser10-histone H3 staining in control and Bod1 siRNA cells indicating Aurora B activity. (B-E) Cells were transfected with control or Bod1 siRNA. After 72 h, cells were treated with microtubule for 5 h, and reassembled.

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difference in the amount of Aurora B in unaligned and apparently aligned chromosomes (Fig. 5A). We detected no change in chromosome staining with anti-phosphohistone H3 (Fig. 5A) or anti-phospho-CENP-A (not depicted) after Bod1 depletion. Because both are markers of Aurora B activity (Zeitlin et al., 2001), these results suggest that Aurora B kinase activity was not dramatically impaired by the loss of Bod1. To further assay the function of Aurora B, we determined the localization of MCAK, which localizes to the inner centromere in its phosphorylated form but concentrates predominantly located control cells (Fig. 5C).

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Because MCAK localizes to the inner centromere in its phosphorylated form but concentrates predominantly located control cells (Fig. 5C). In summary, by using a member of the FA

Figure 5. MCAK is not in control and Bod1 siRNA and released into me Boxed areas are magnified. Dashed line

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**Bod1, a Novel Kinetochores Protein Required for Chromosome Biorientation**

Published 22 Oct 2007

I.M. Porter, S.E. McClelland, G.A. Khoudji, C.J. Hunter, J.S. Anderson, A.D. McAinsh, J.J. Blow, J.R. Swedow

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