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OME Users' Meeting
June 5, 2014

# JCB Data Viewer

# Bringing New Dimensions to Published Image Data

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

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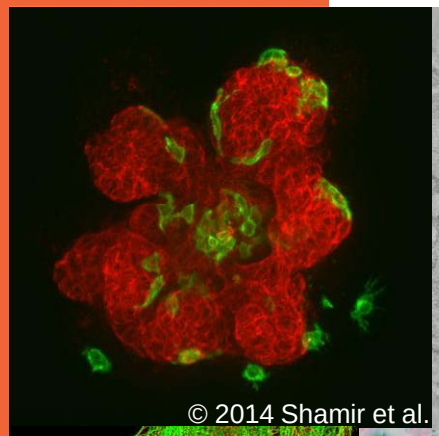
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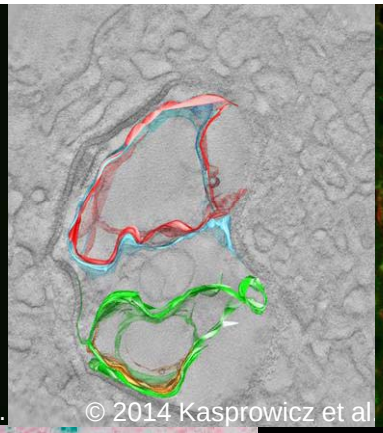
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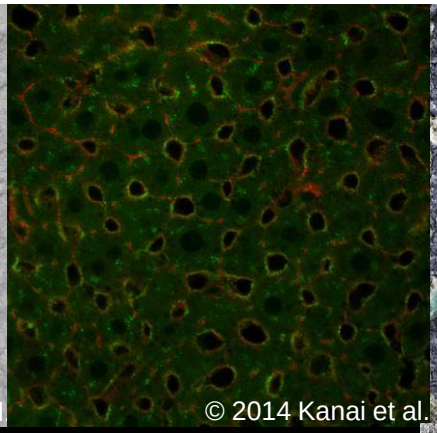
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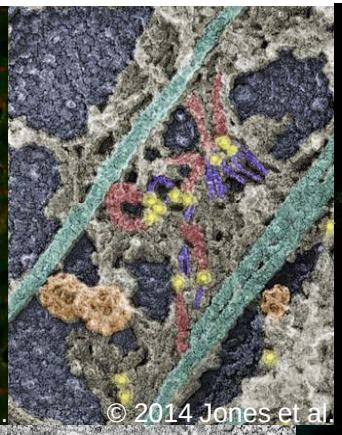
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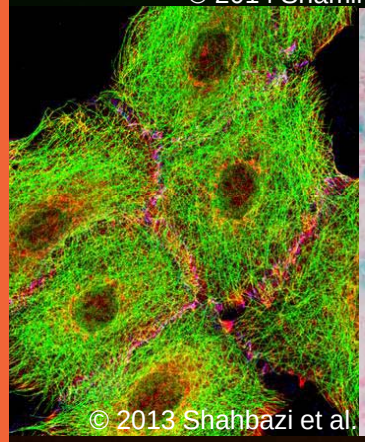
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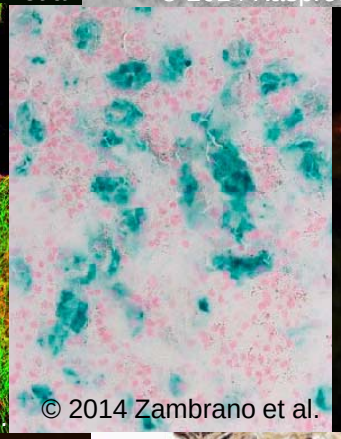
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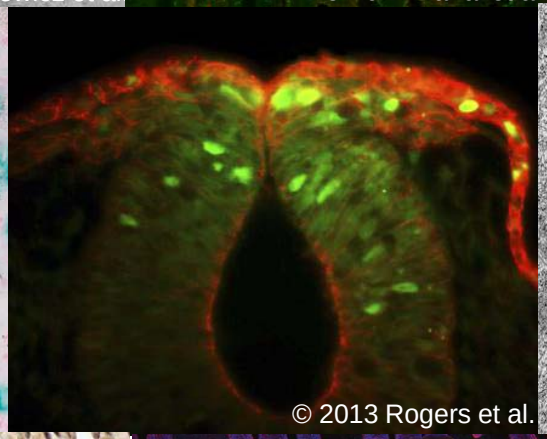
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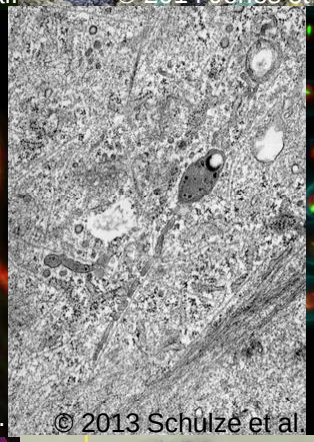
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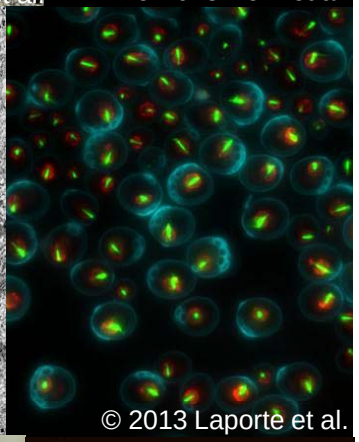
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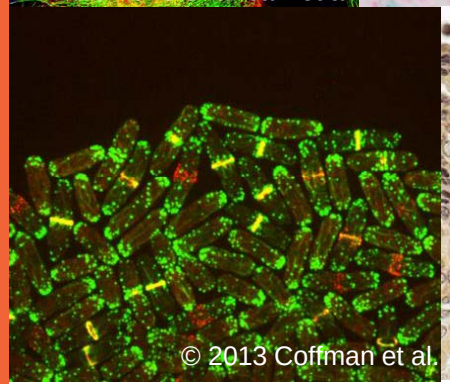
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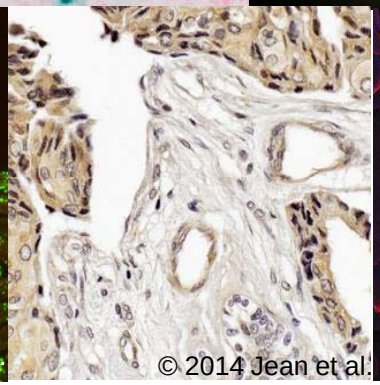
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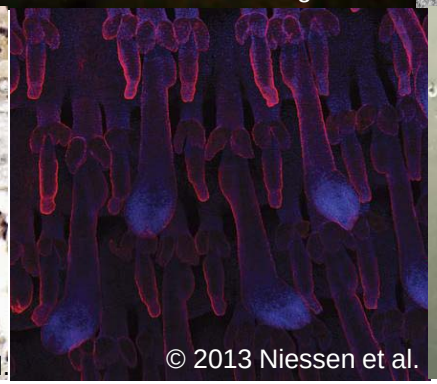
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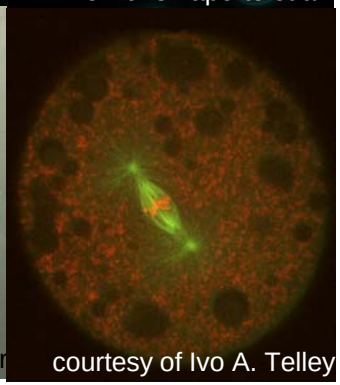
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## The challenges:

**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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Published October 15, 2007



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

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

[1]Division of Gene Regulation and Expression and [2]Medical Research Council Protein Phosphorylation Unit, College of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, UK
[3]Chromosome Segregation Laboratory, Marie Curie Research Institute, Oxted, Surrey RH8 0TL, England, UK
[4]Center for Experimental Bioinformatics, University of Southern Denmark, DK-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@lifesci.dundee.ac.uk

Abbreviations used in this paper: ACA, anticentromere antibody; CENP, centromere protein; MCAK, mitotic centromere-associated kinesin; shRNA, short hairpin RNA.

The online version of this article contains supplemental material.


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http://www.jcb.org/cgi/doi/10.1083/jcb.200704098

Supplemental Material can be found at:
http://jcb.rupress.org/content/suppl/2007/10/15/jcb.200704098.DC1.html
Original image data can be found at:
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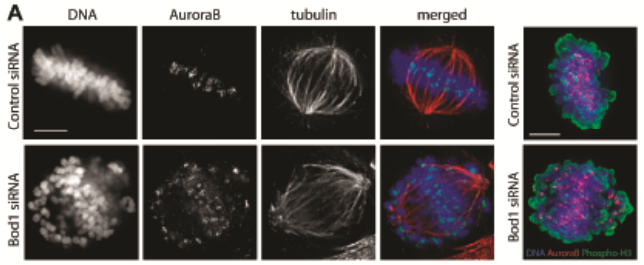


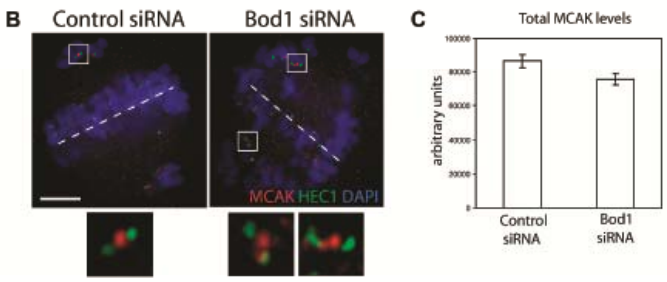


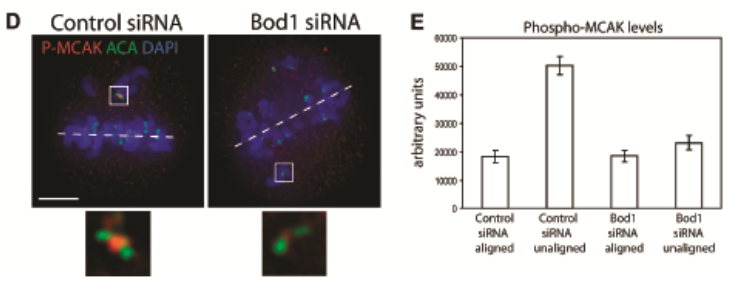


Figure 5. **MCAK is not efficiently phosphorylated in Bod1$^{siRNA}$ cells.** (A) Aurora B is not delocalized in Bod1-depleted cells. Phospho-Ser10-histone H3 staining in control and Bod1 siRNA cells indicating Aurora B activity. (B–E) Cells were transfected with control or Bod1 siRNA. After 72 h, cells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

Mouse anti–α-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 autoantisera (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 μg/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 shRNAi and live cell imaging to identify candidate proteins. Fig. S2 shows the relative expression levels of Bod1-GFP. Fig. S3



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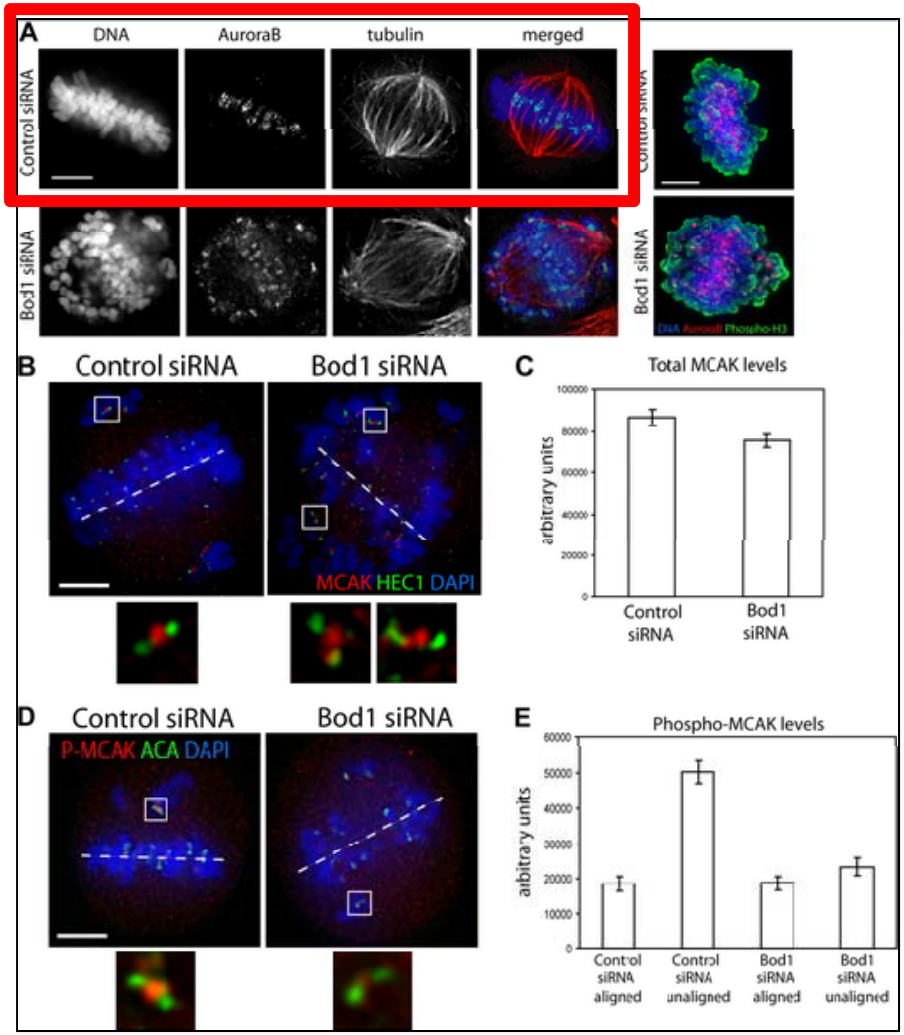


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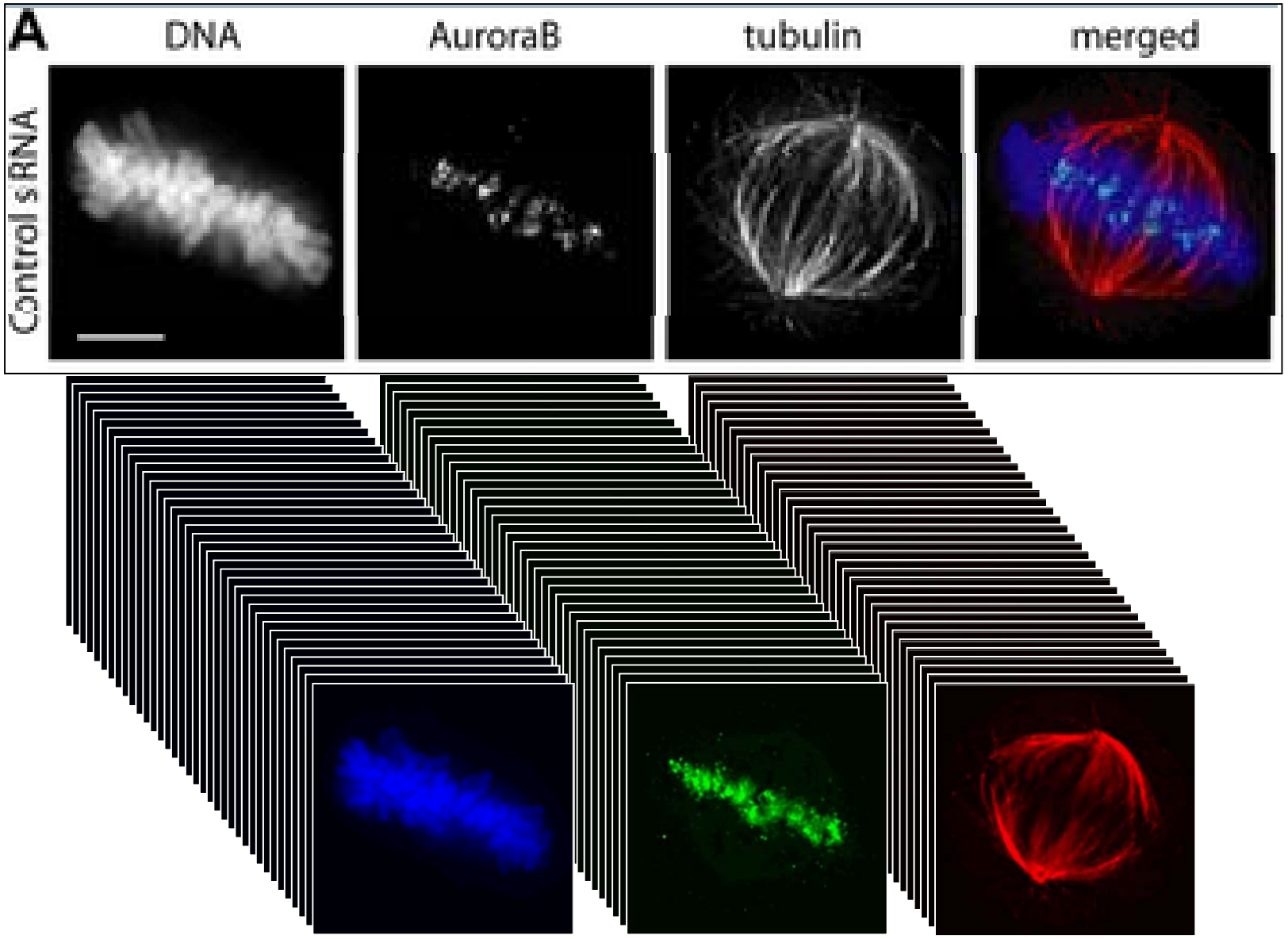


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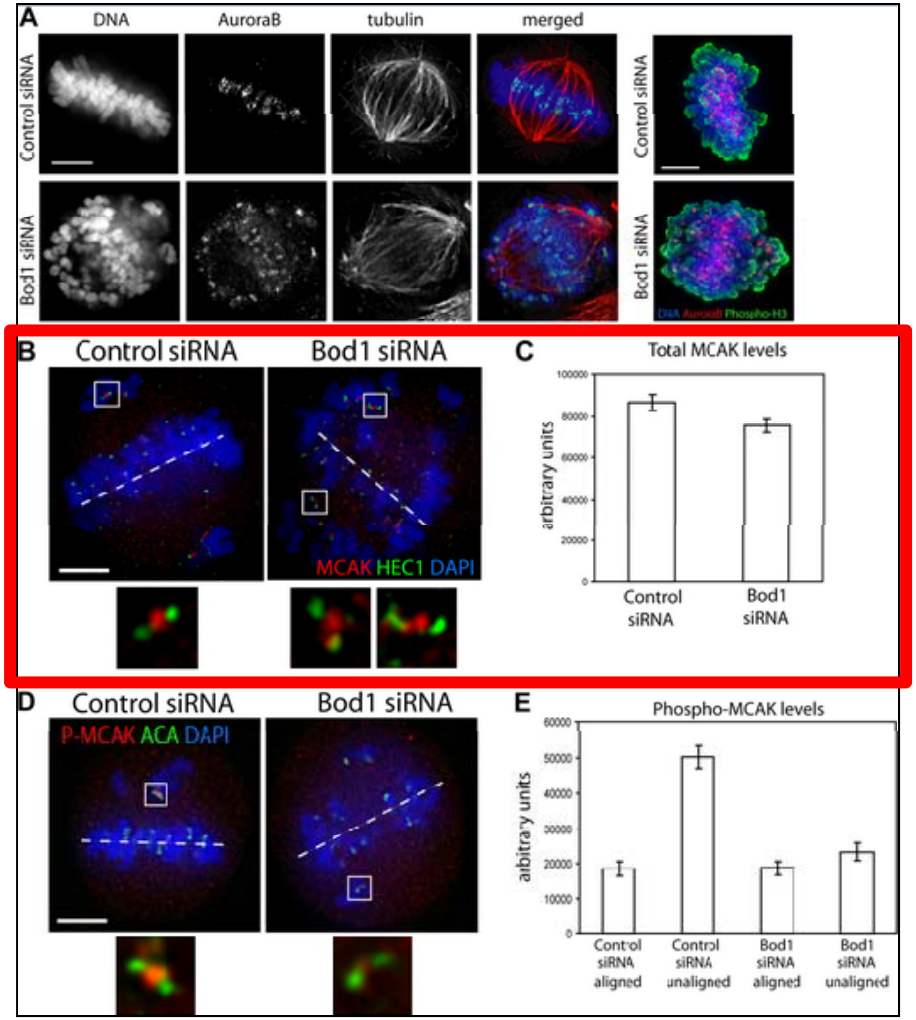


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## What repositories exist:

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)

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## What these repositories lack:

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

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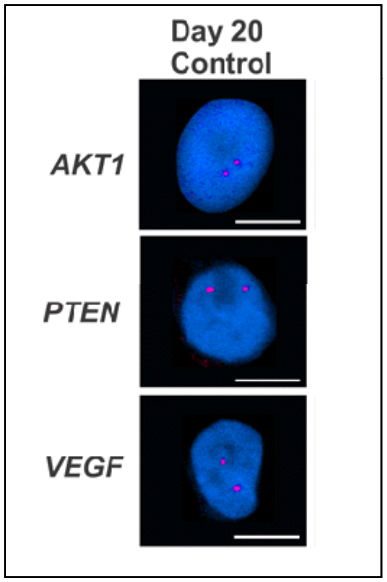
## Data access and validation

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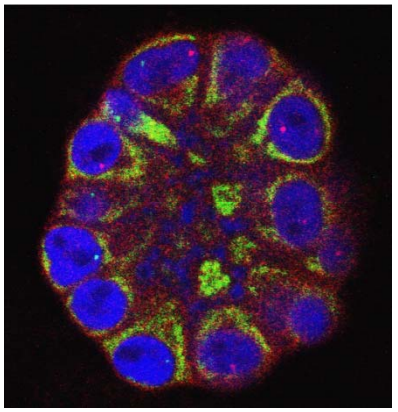

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- An OMERO-based, browser-based application for archiving, viewing, and sharing raw image files associated with *JCB* articles.
- Enables presentation of over 125 proprietary file types from various light microscope and gel-documentation systems.
- 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.



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- Accessibilty
  - 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

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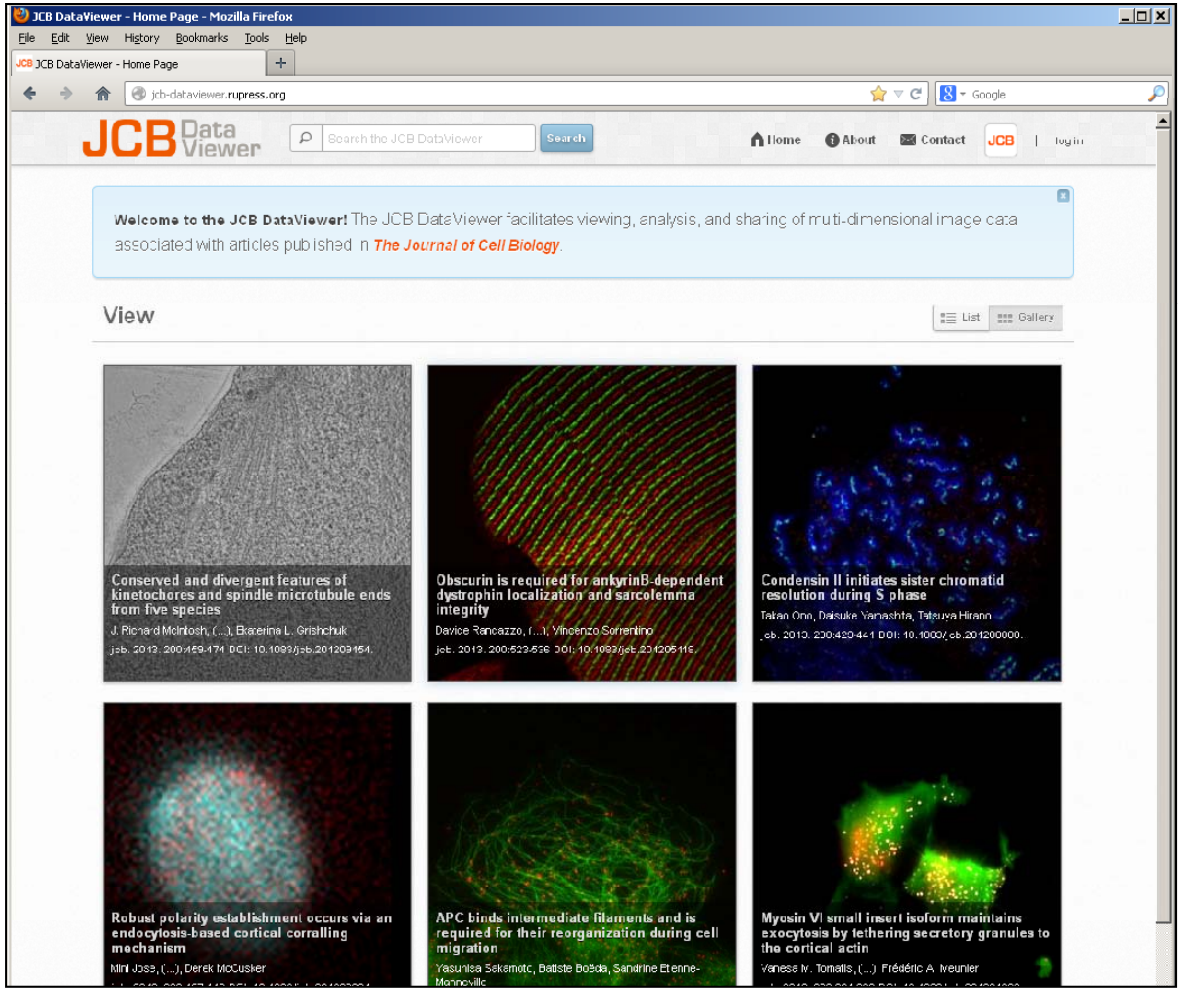


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# 1. single image analysis

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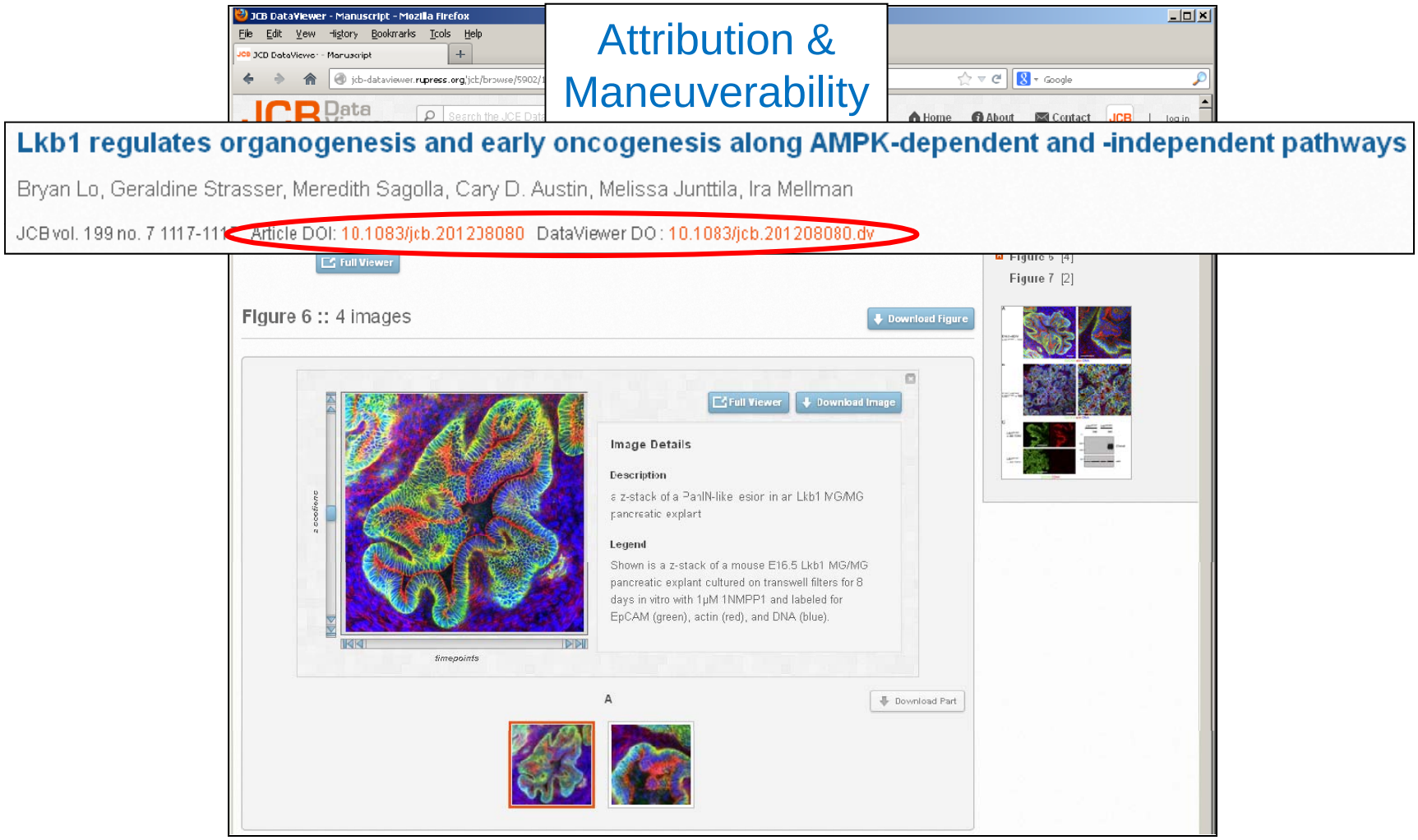


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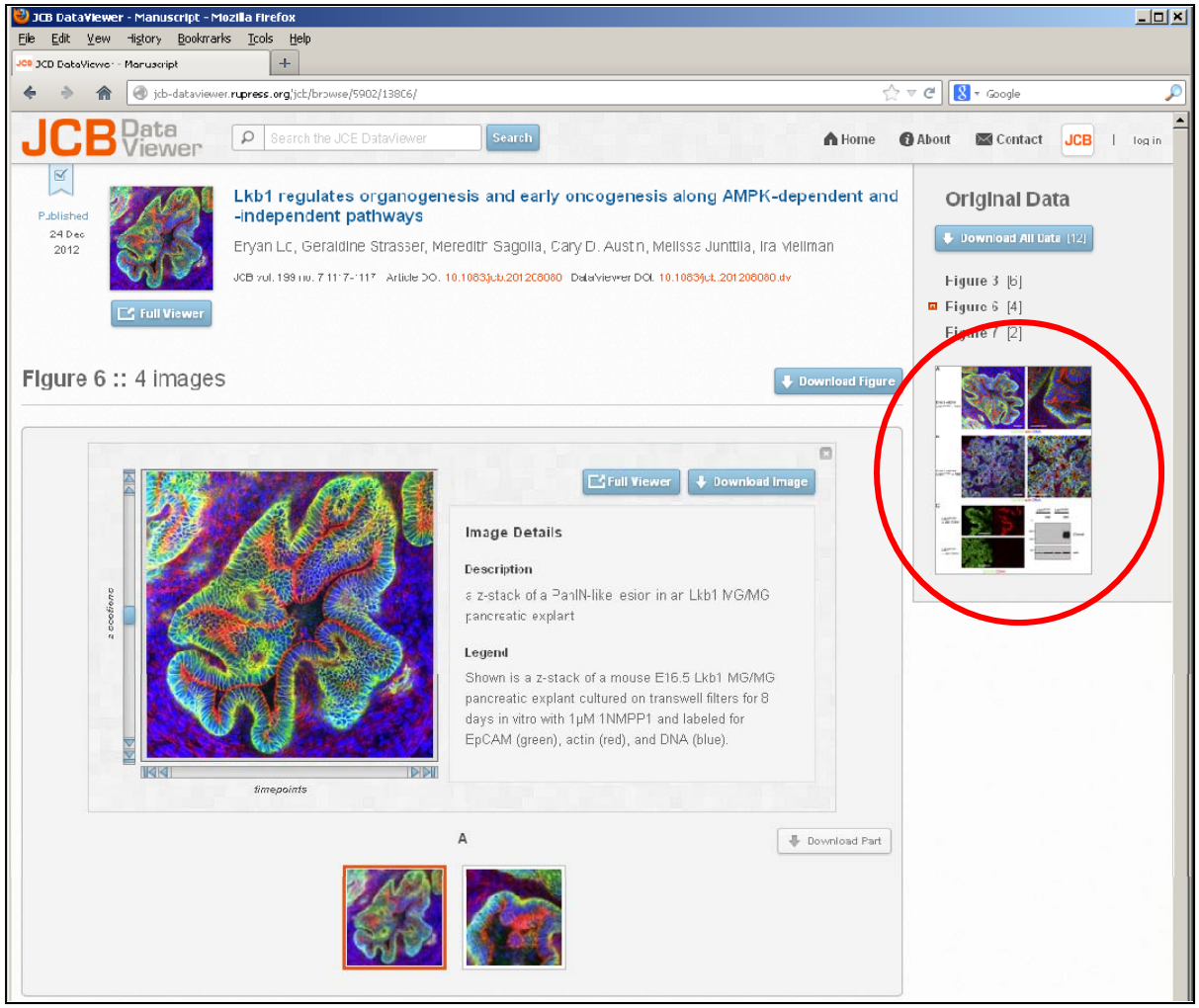


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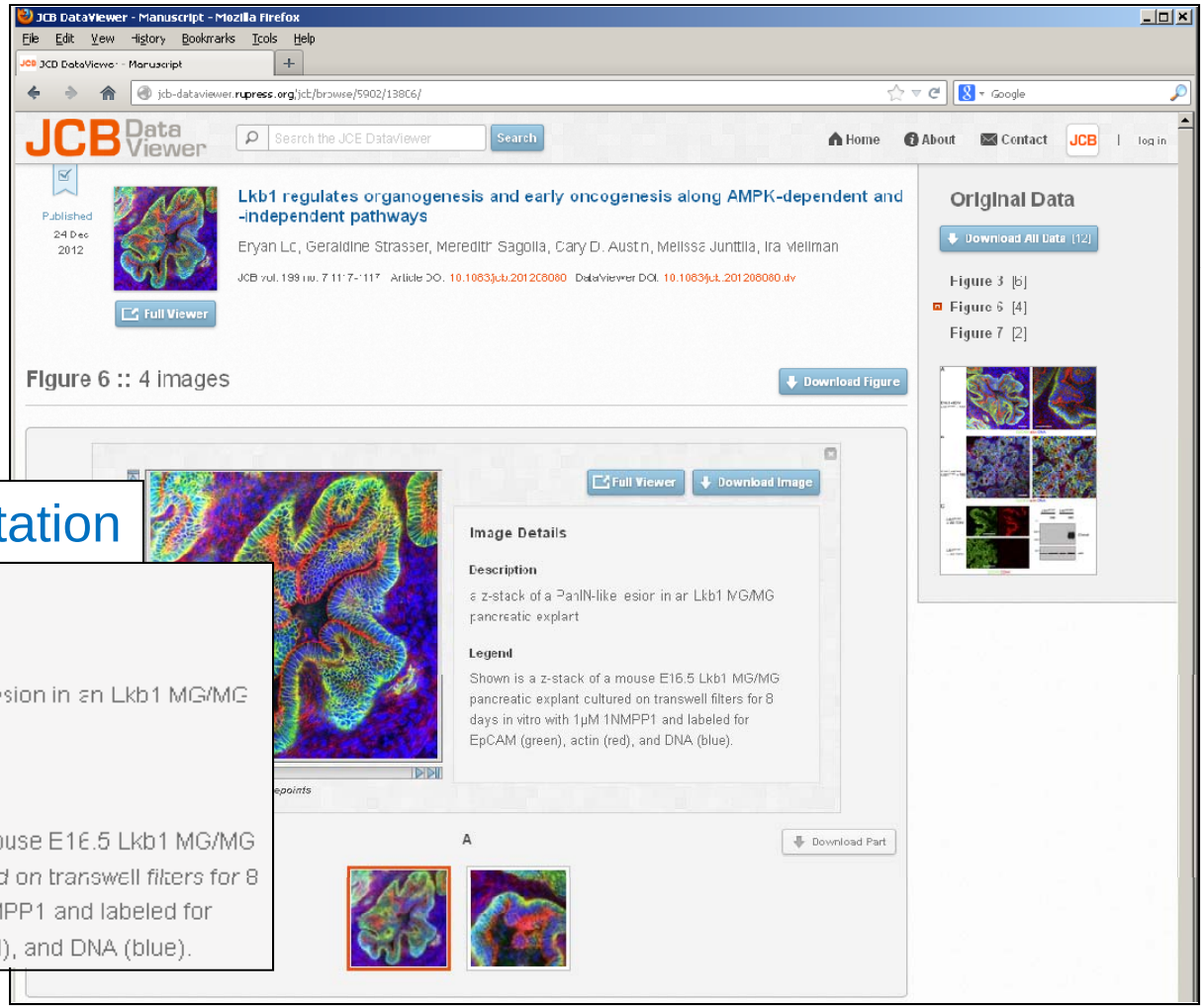


Annotation

**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).

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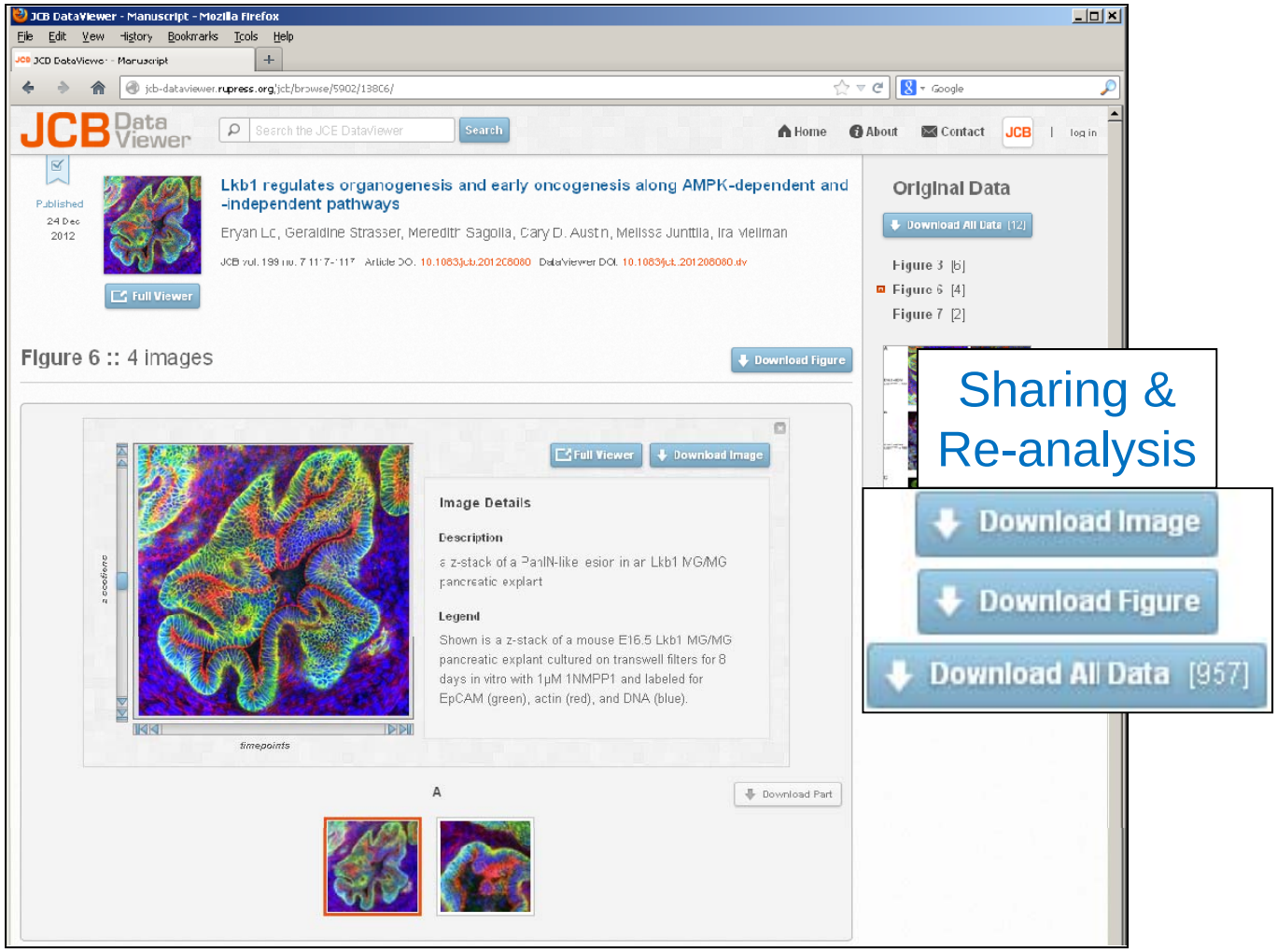


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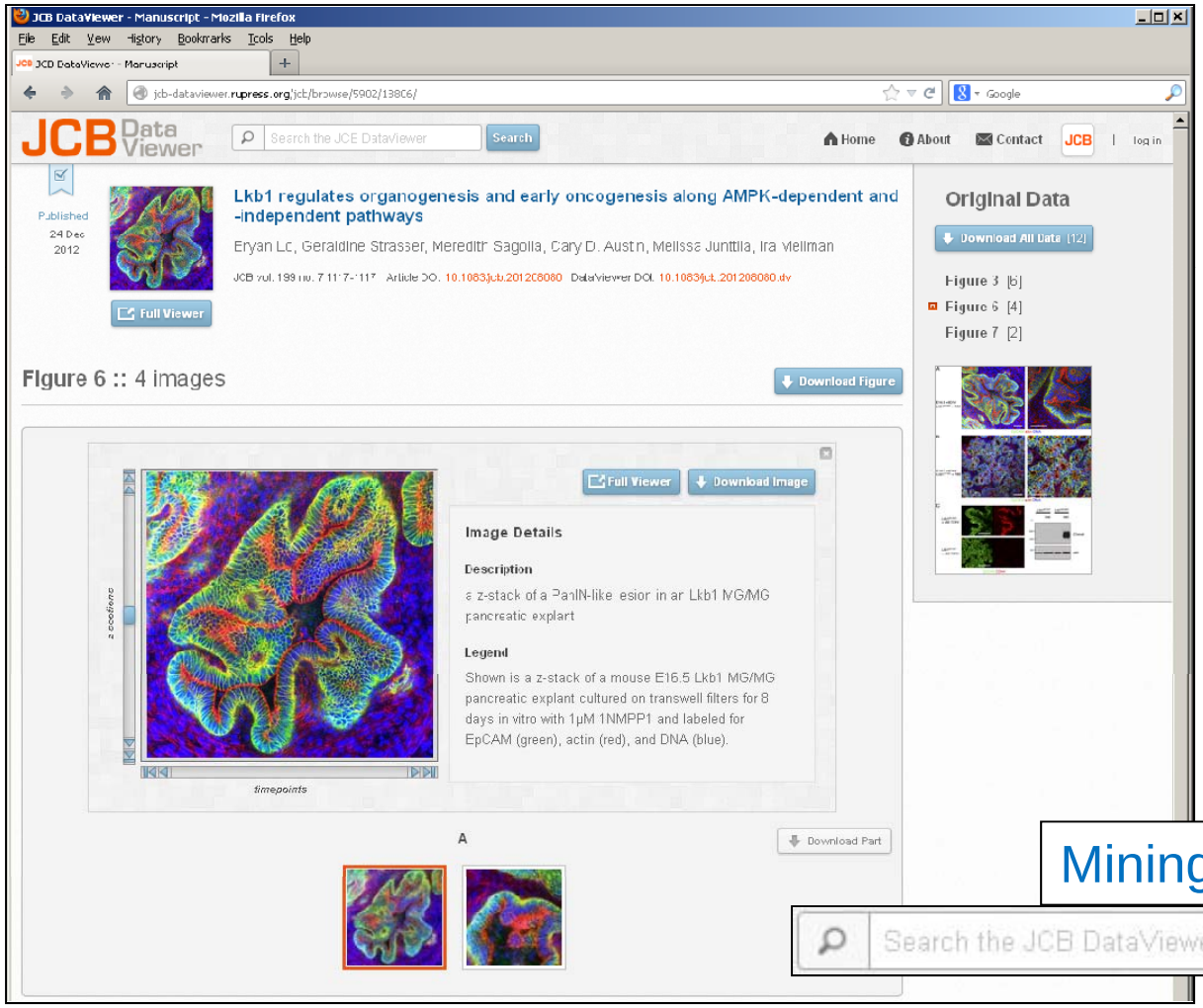


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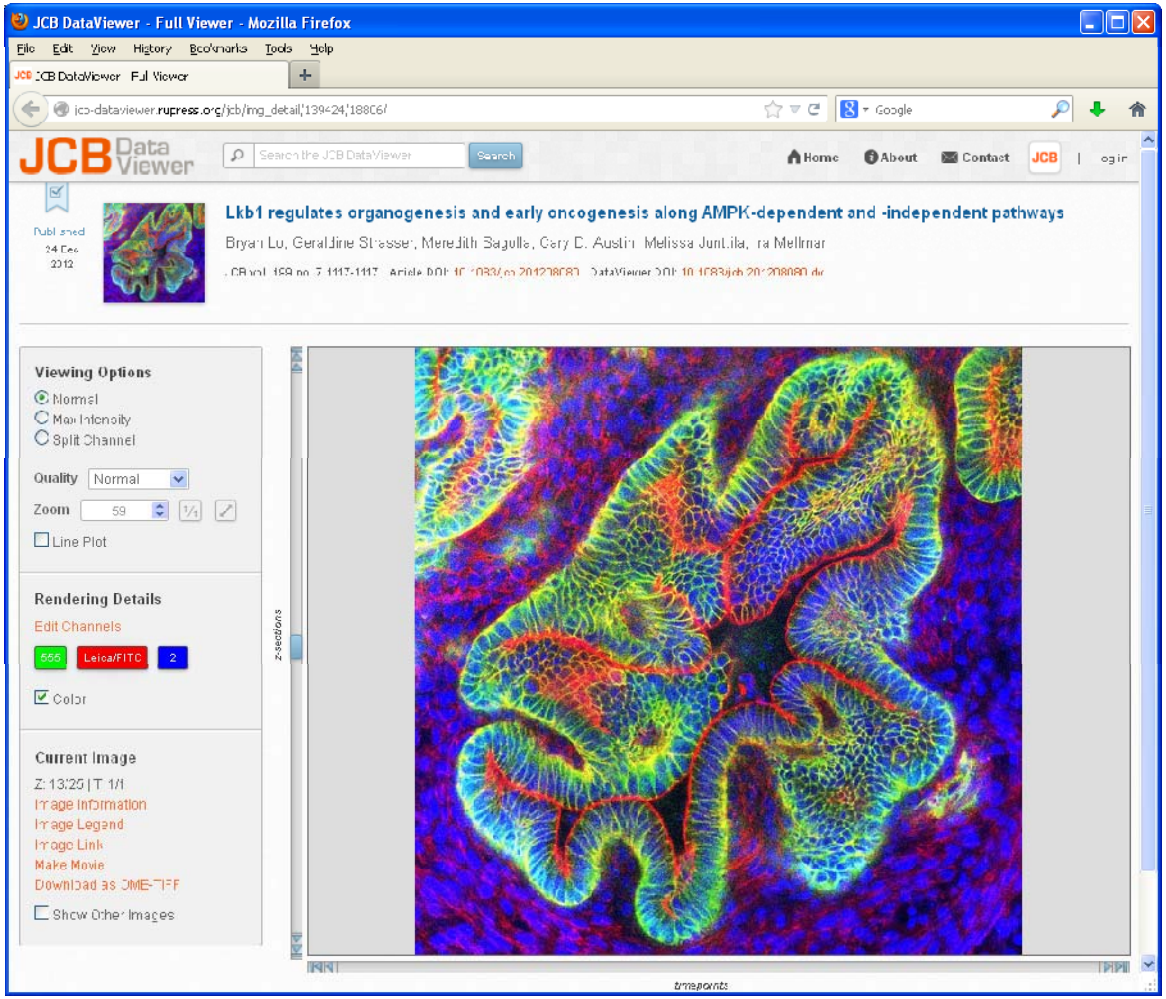


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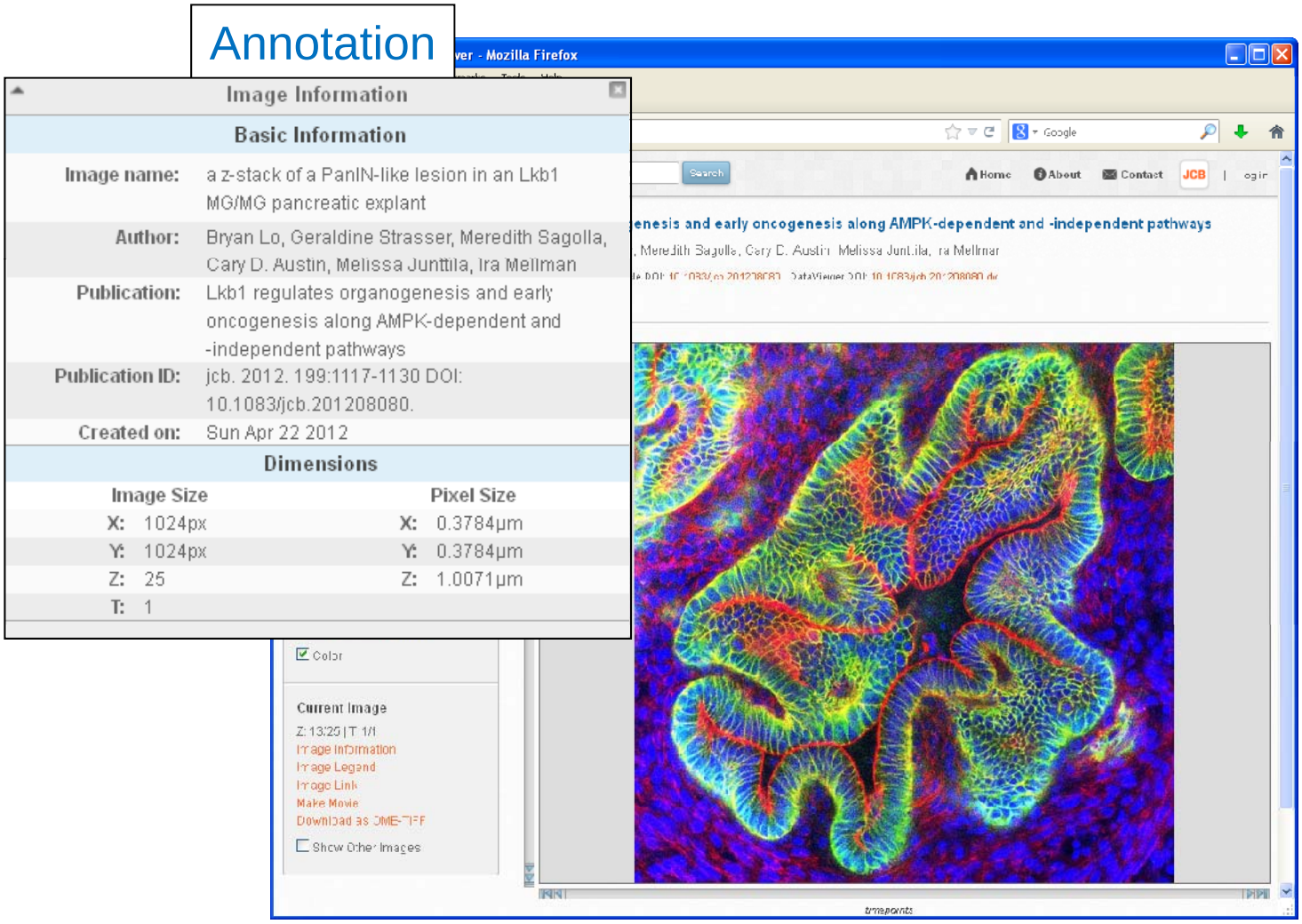


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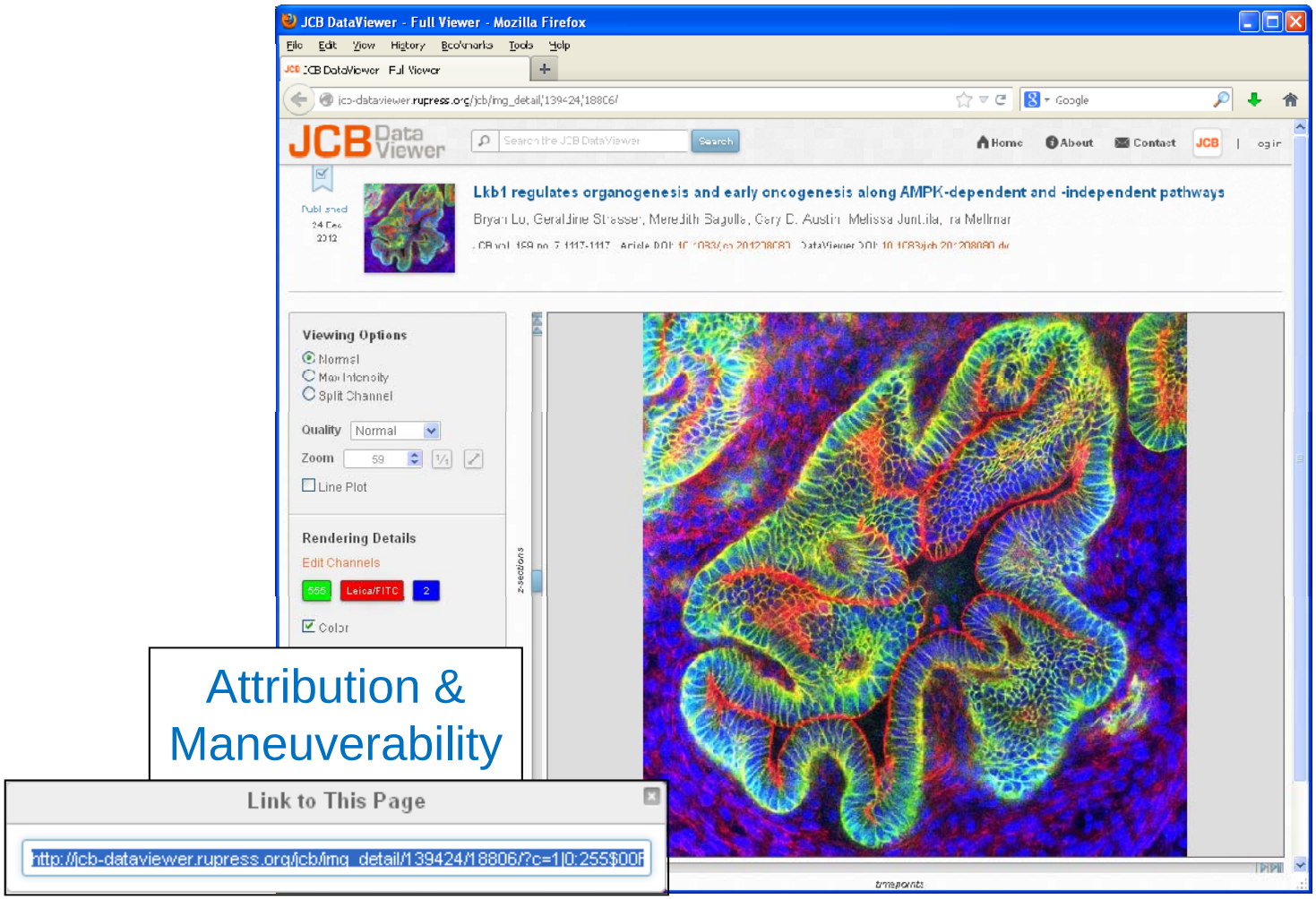


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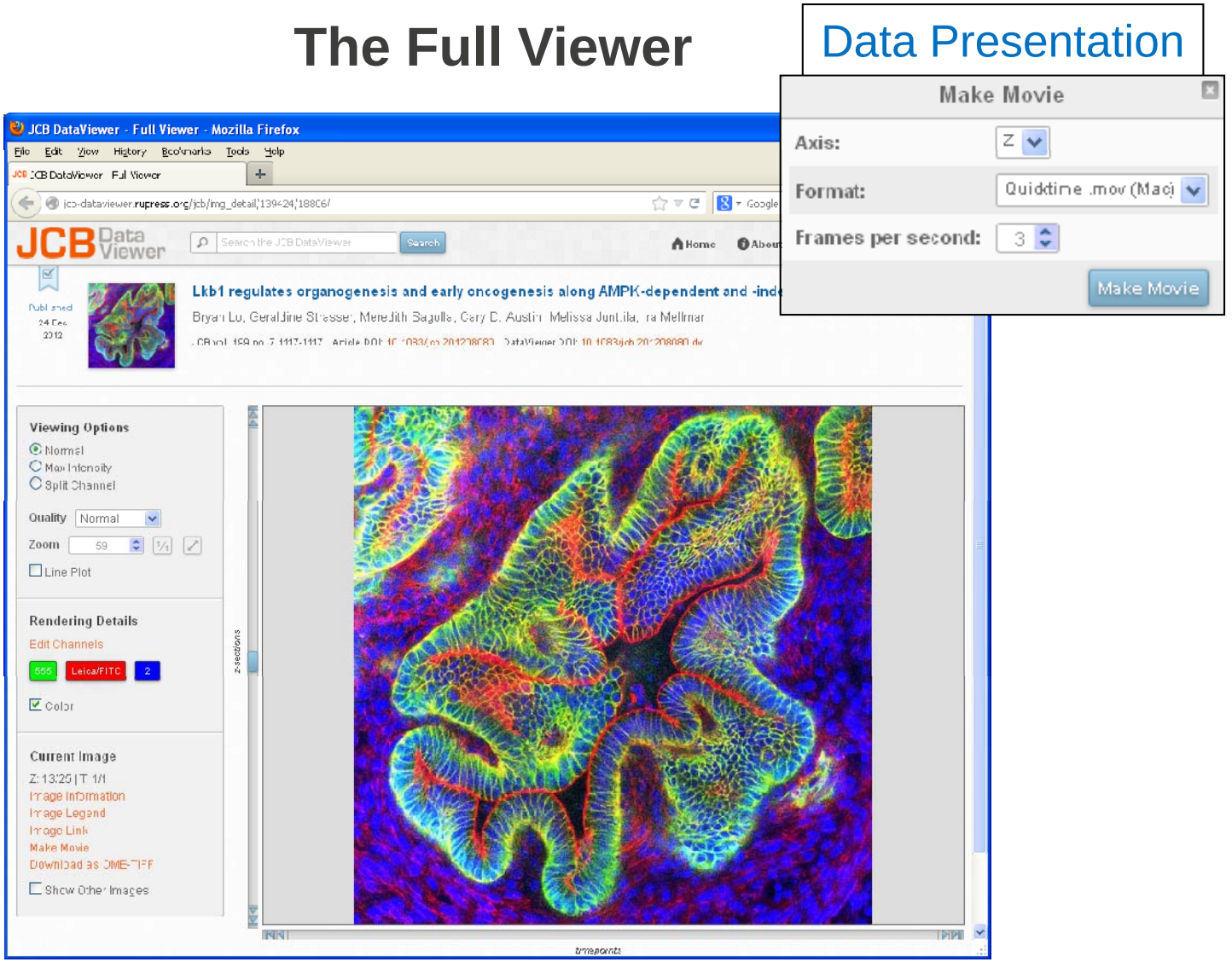
Data Presentation

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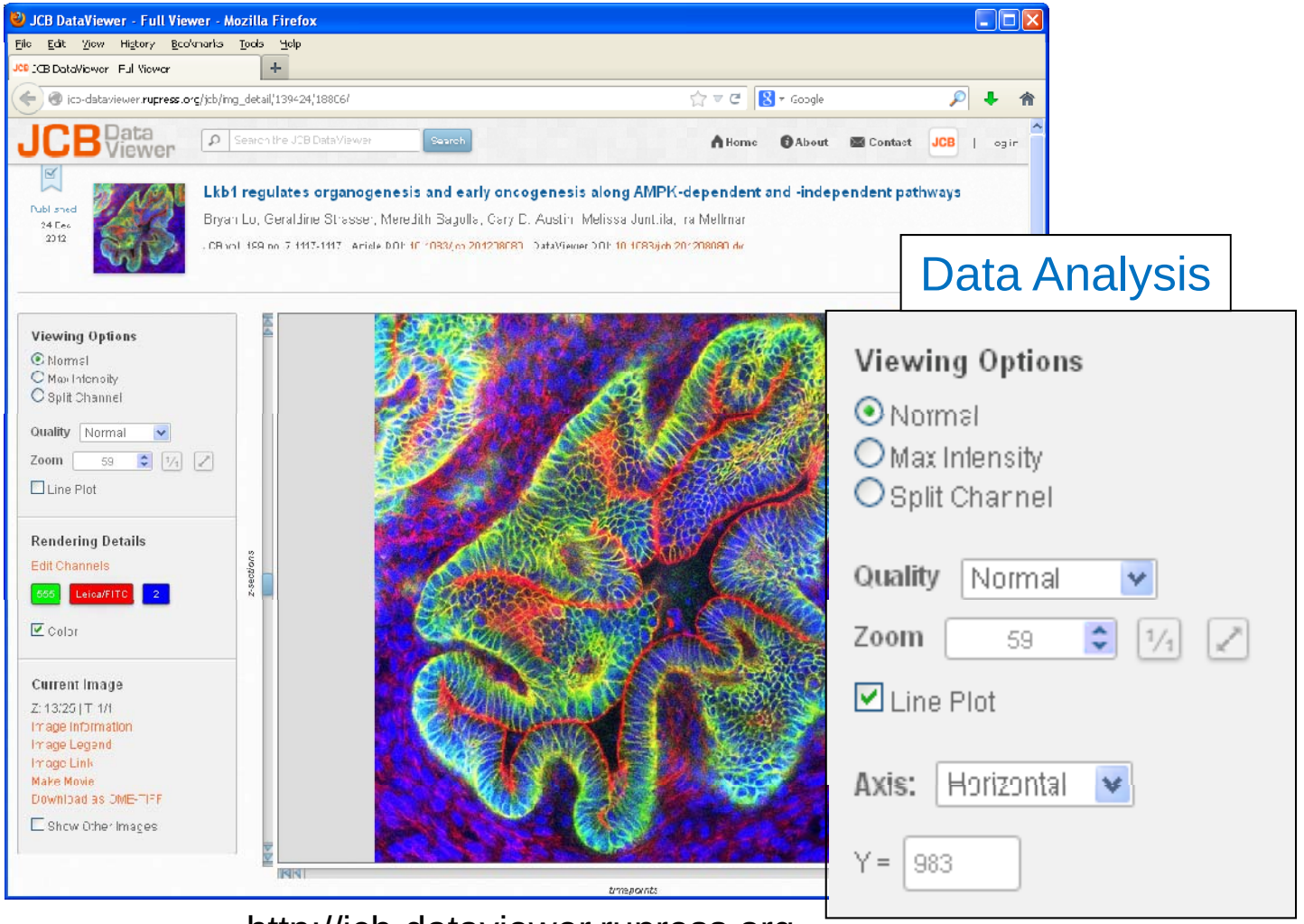


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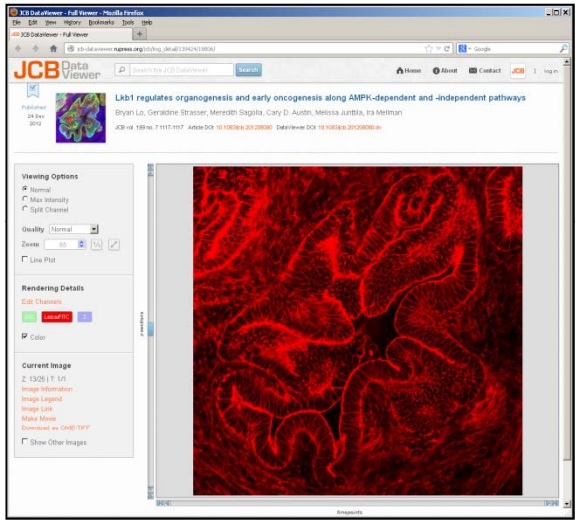
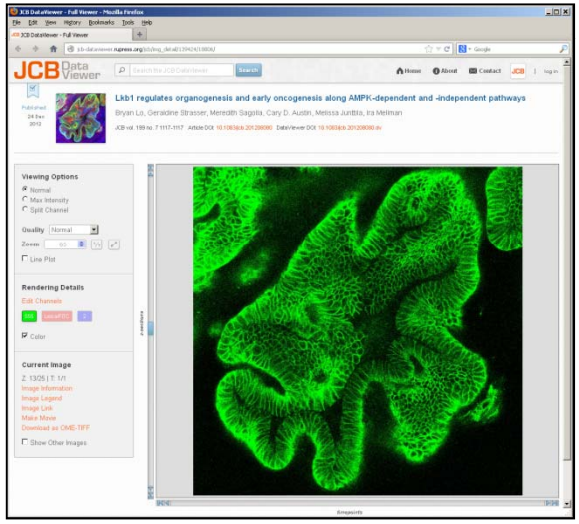
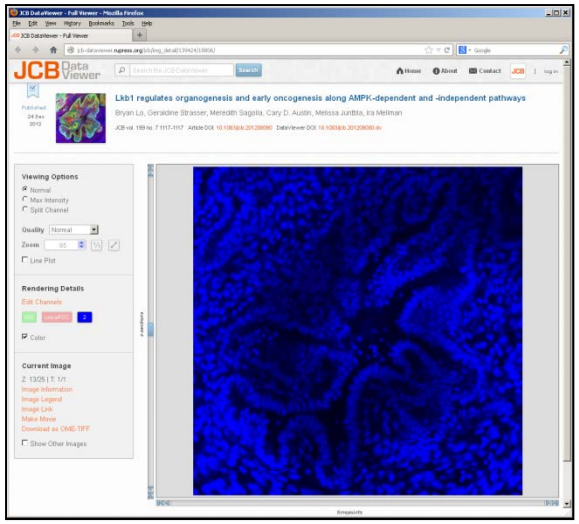
# Split-Channel View

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Lo et al (2012)

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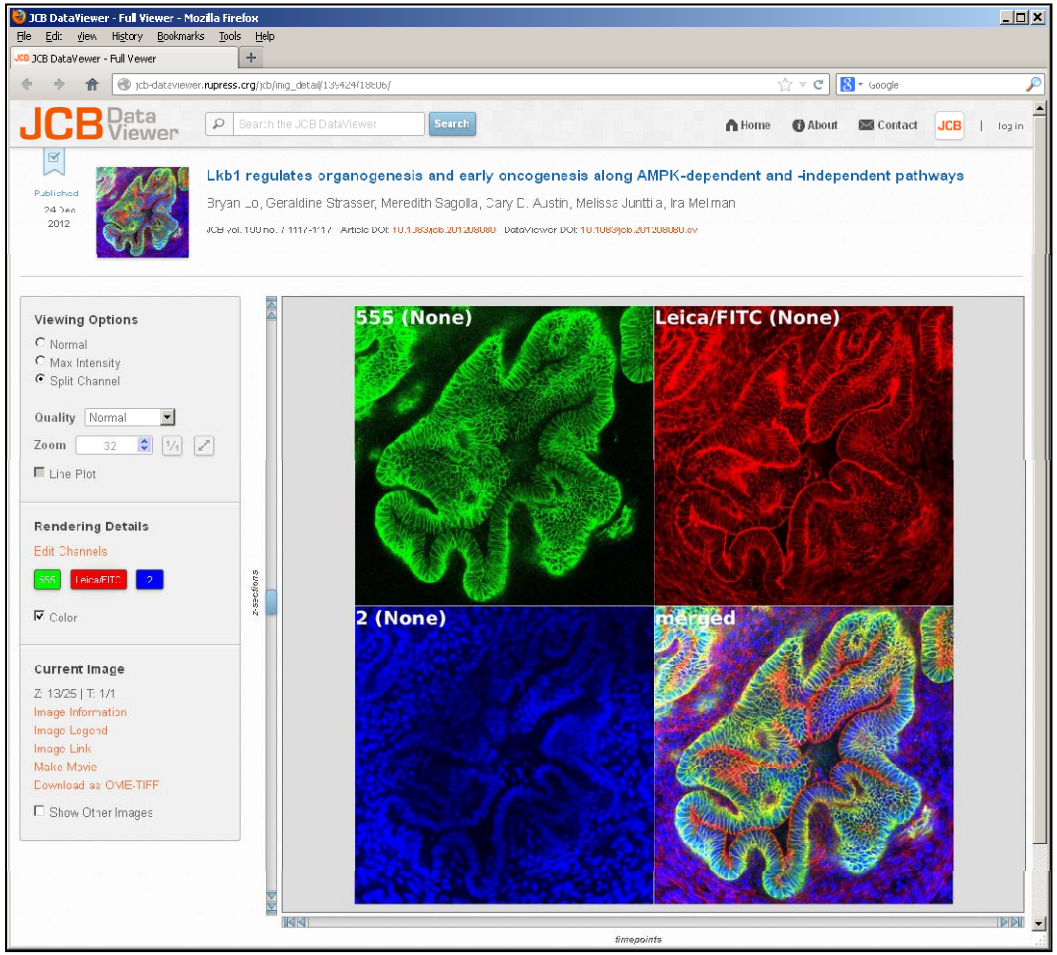
# Split-Channel View

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# 2. ultra-large image presentation

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**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)

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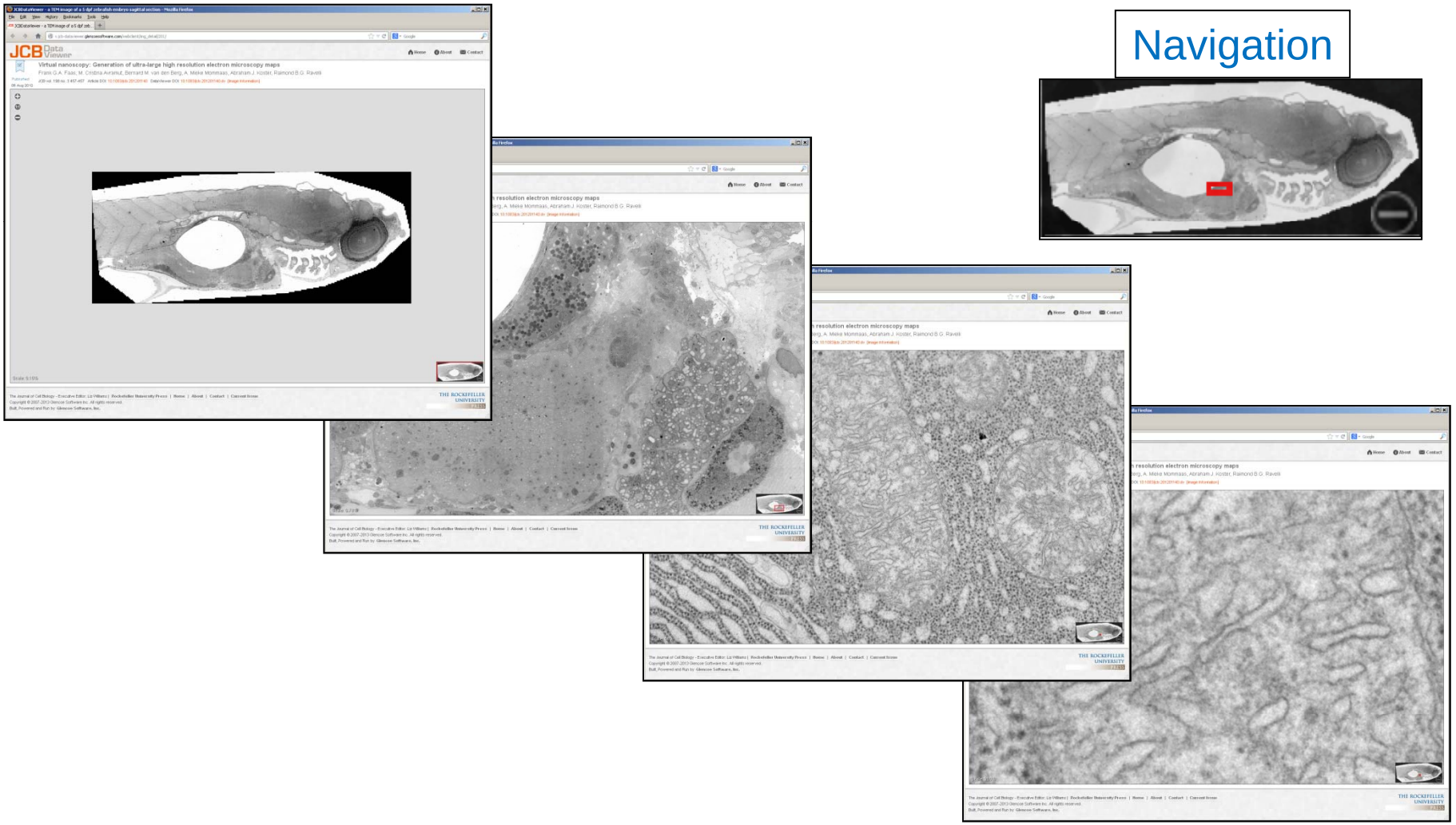
## Ultra-large, high-resolution, tiled images

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Faas et al (2012)

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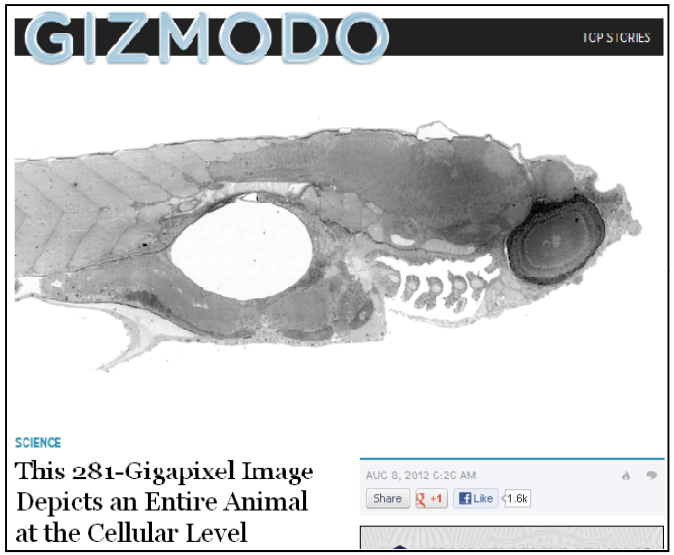
## Ultra-large Image Presentation




NewScientist

NBC NEWS


reddit

WIRED

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# 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

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# 3. high-content screen analysis

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## 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)

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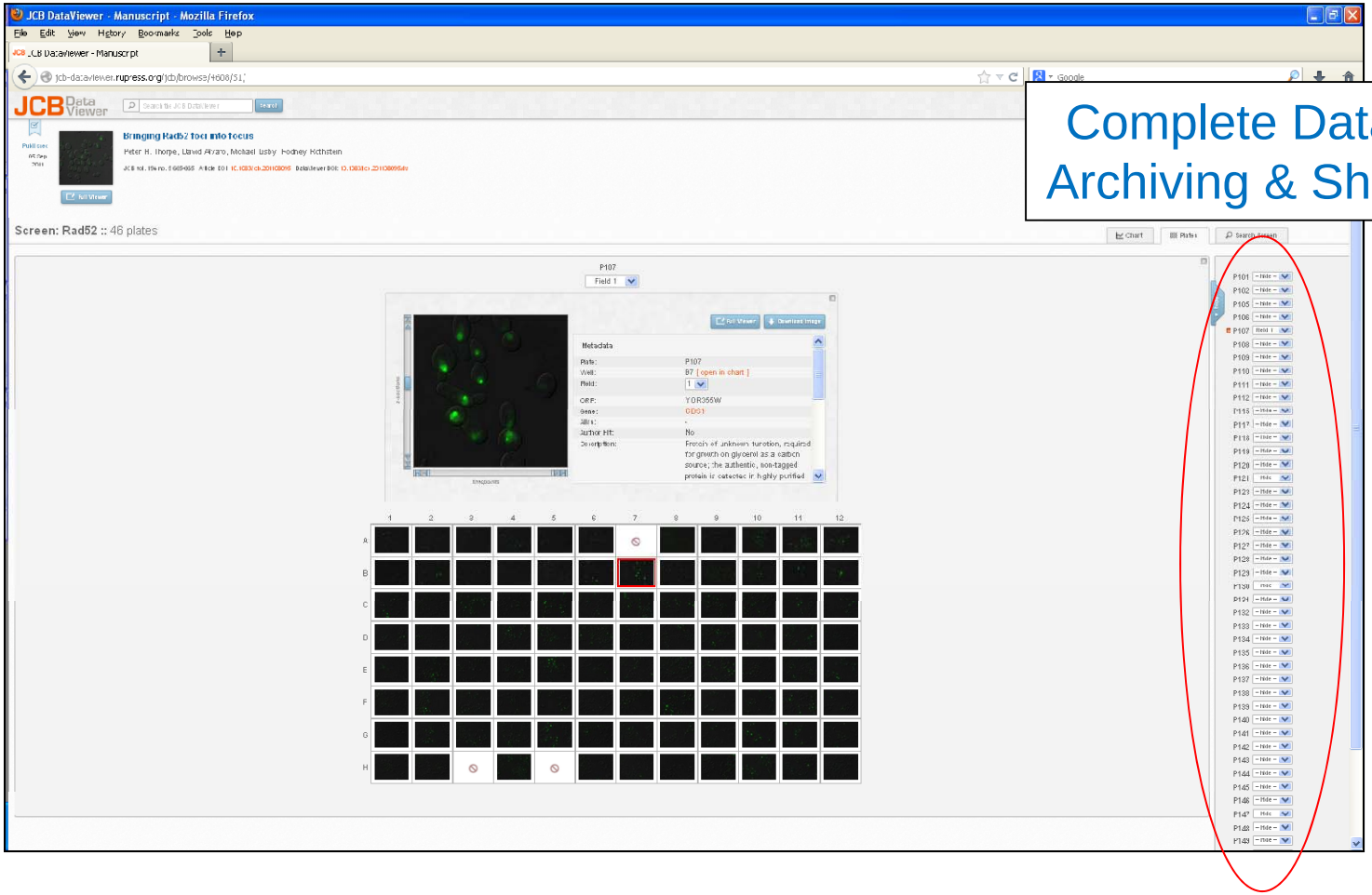
# HCS Data – Plate View

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Thorpe et al (2011)

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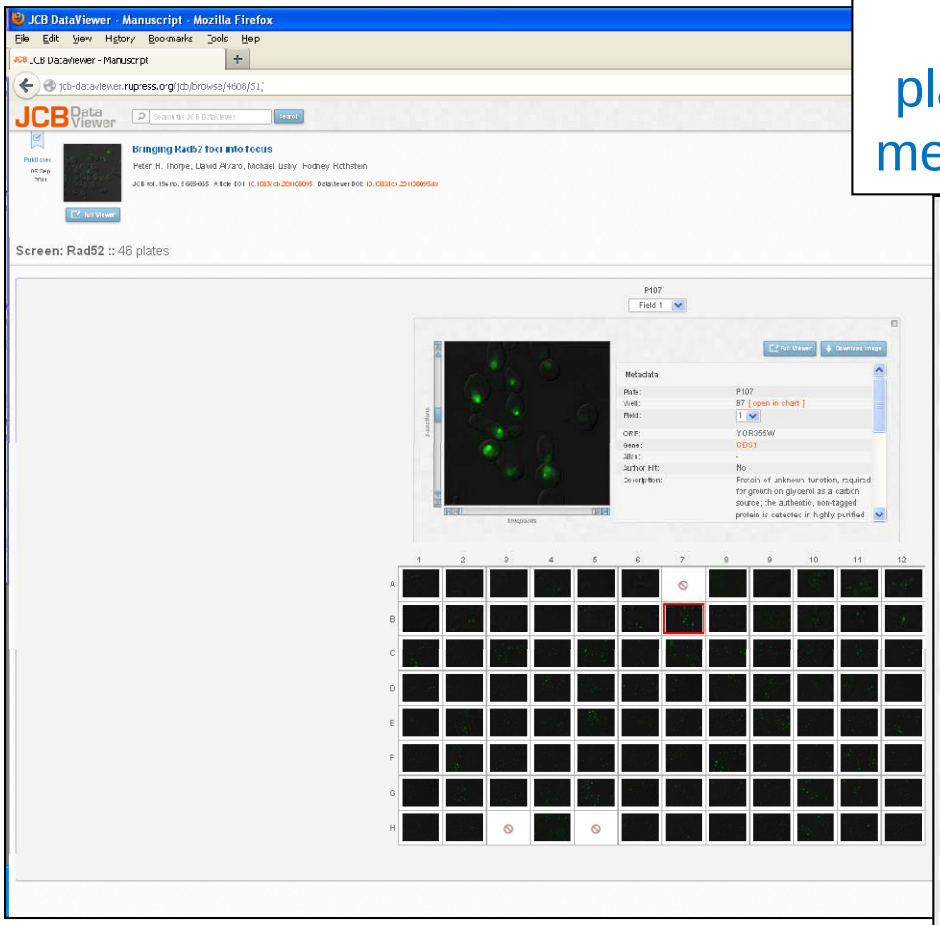
# HCS Data – Plate View




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: | No |
| 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 |

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Thorpe et al (2011)

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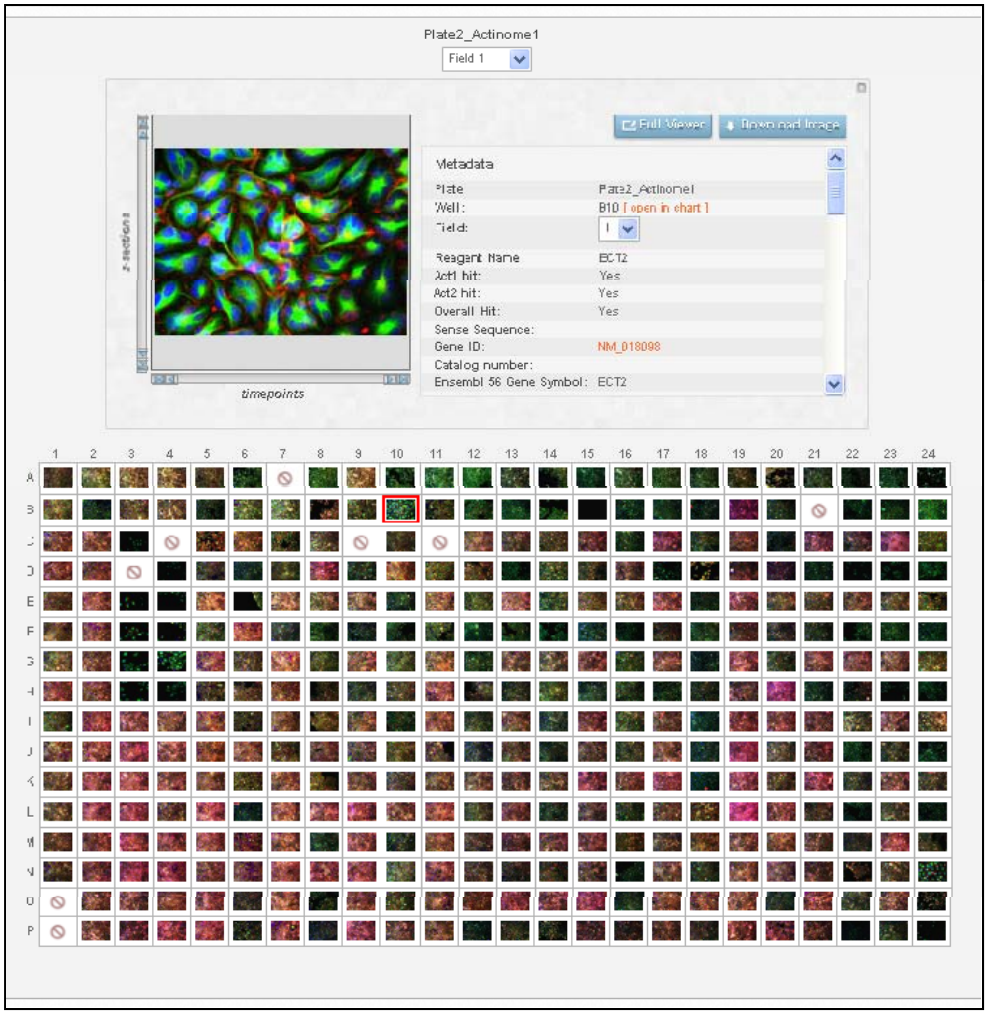
# HCS Data – Plate View

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Rohn et al (2011)

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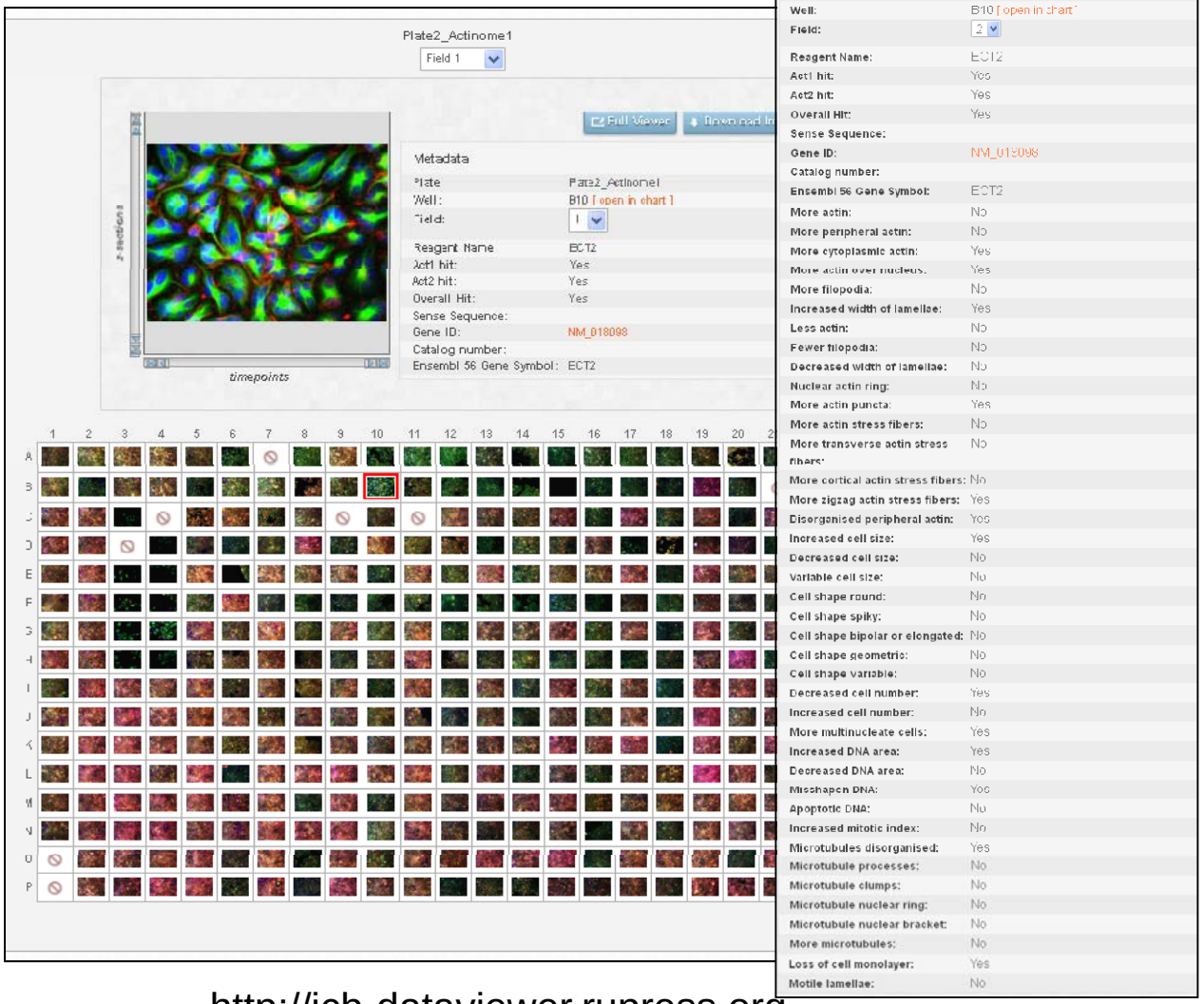
Maneuverability between plate array, image data, metadata, and phenotypic data

# HCS Data – Plate View

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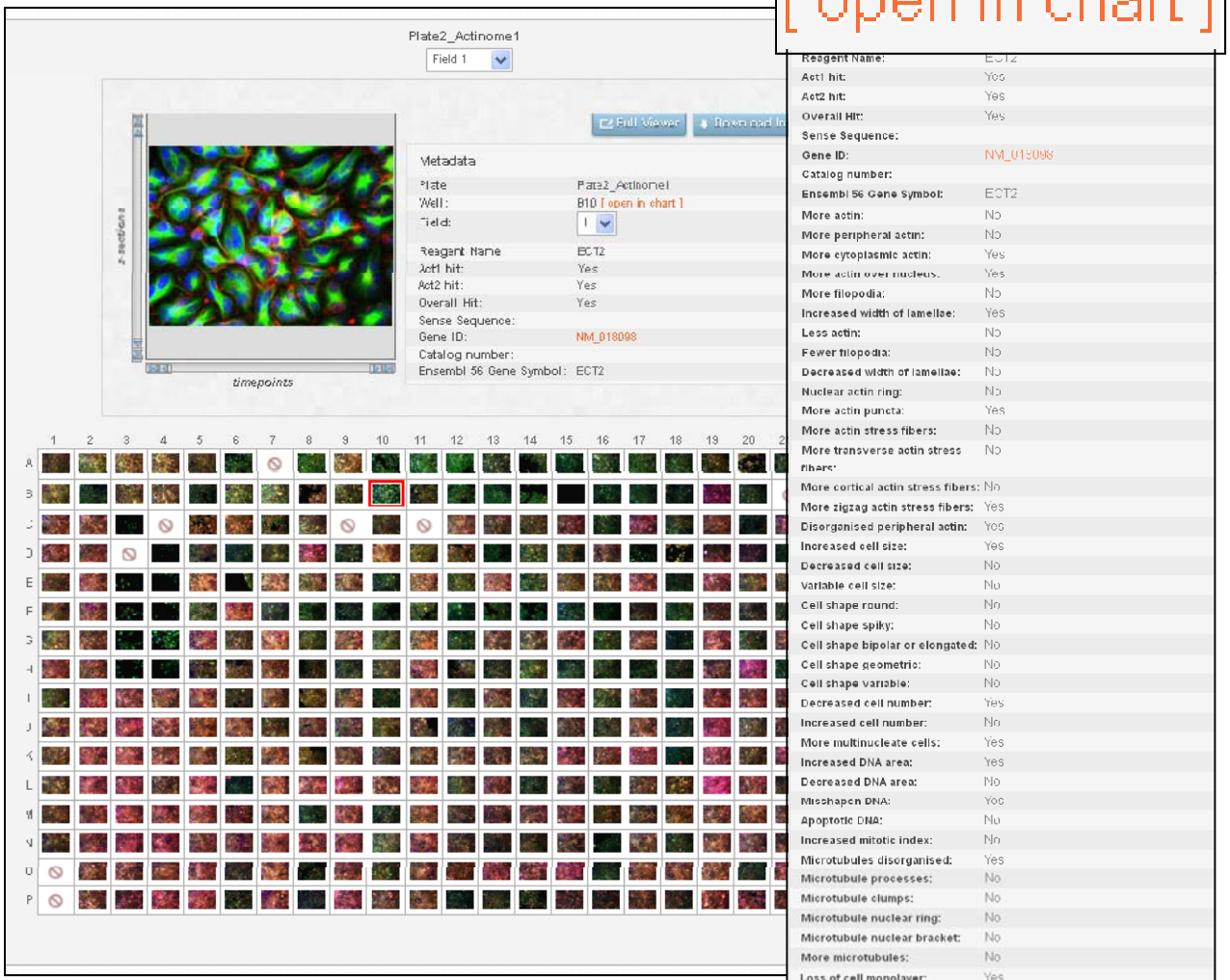
Maneuverability between plate array, image data, metadata, and phenotypic data

# HCS Data – Plate Vie

[ open in chart ]

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Rohn et al (2011)

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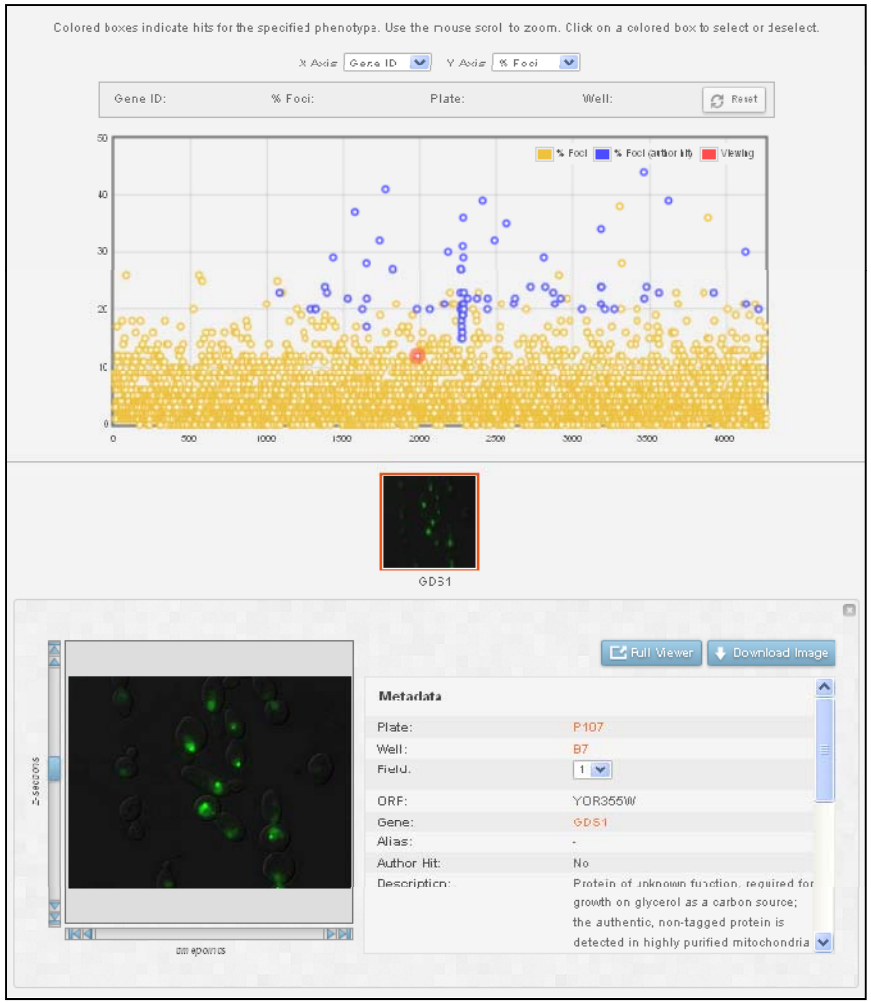
# HCS Data – Chart View (Quantitative)

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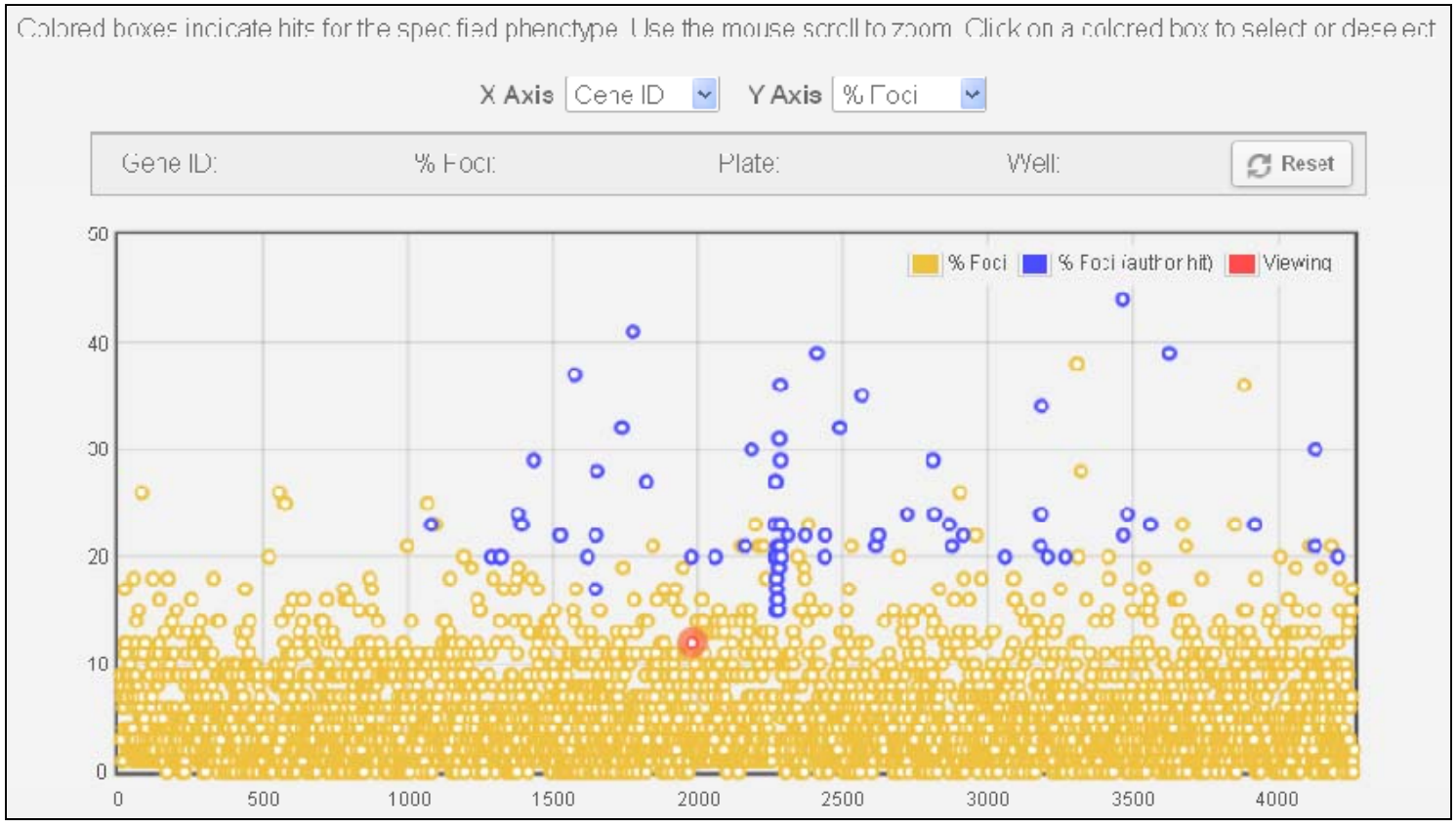


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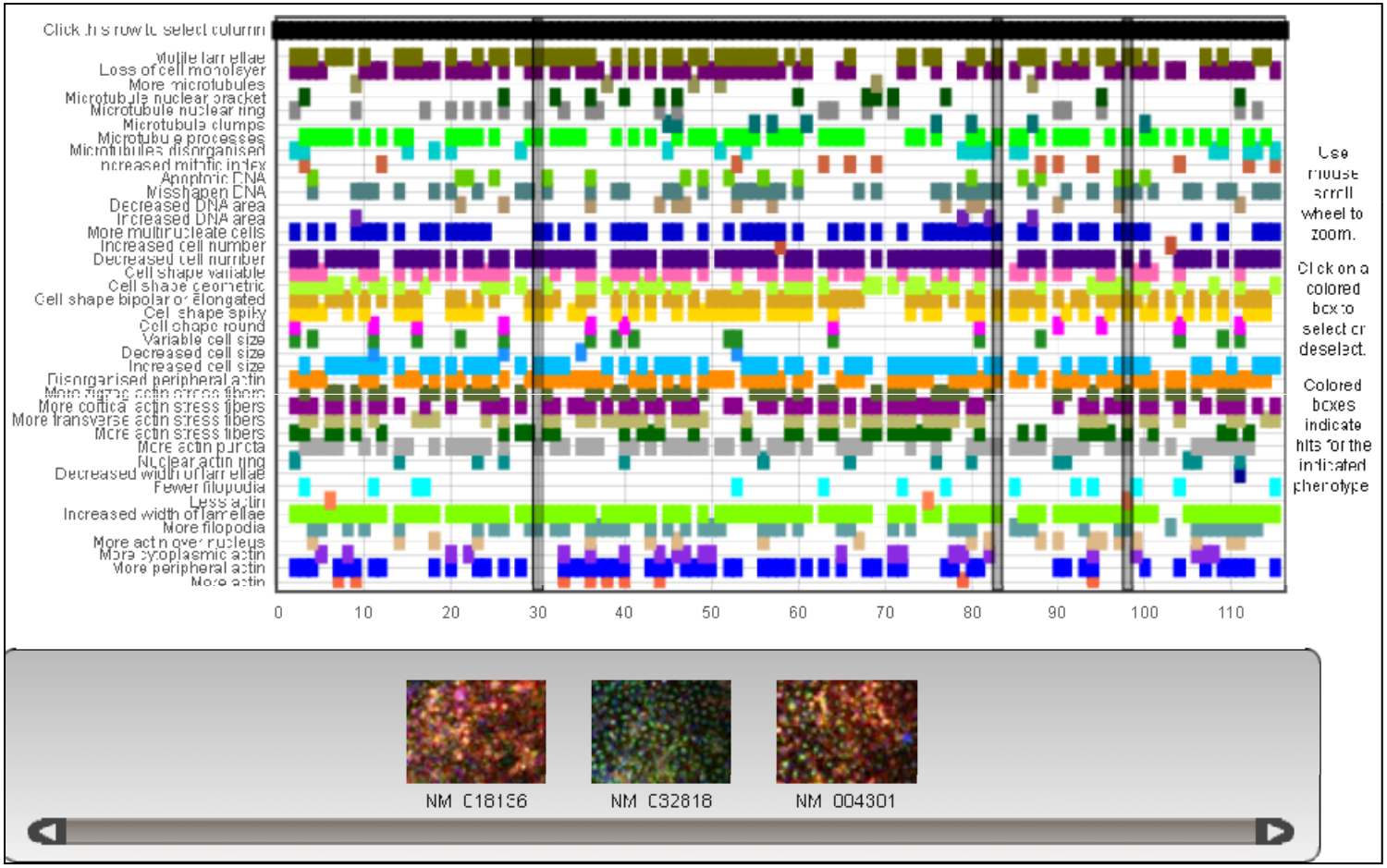
# HCS Data – Chart View (Qualitative)

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Rohn et al (2011)

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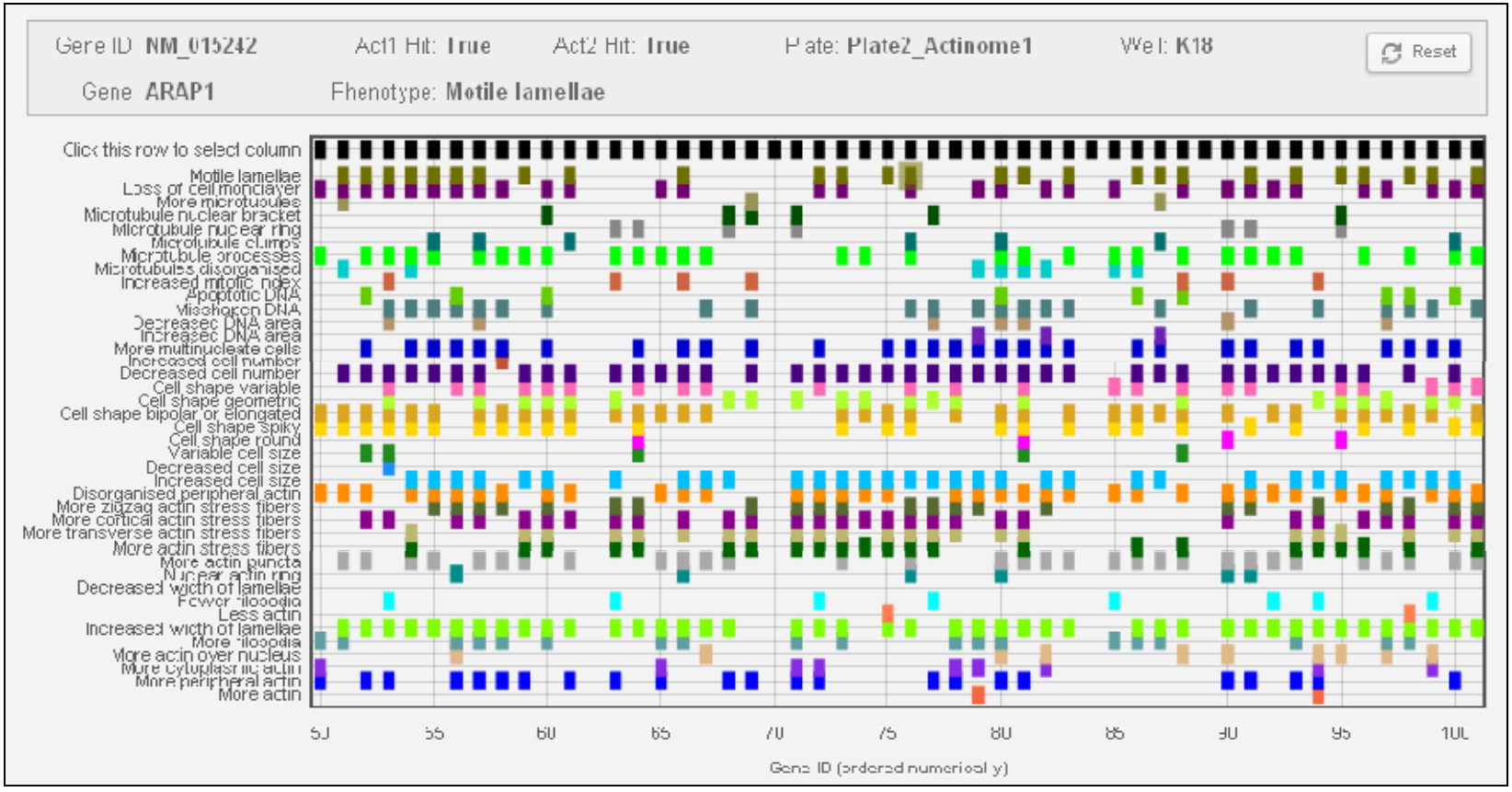
# HCS Data – Chart View (Qualitative)

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Rohn et al (2011)

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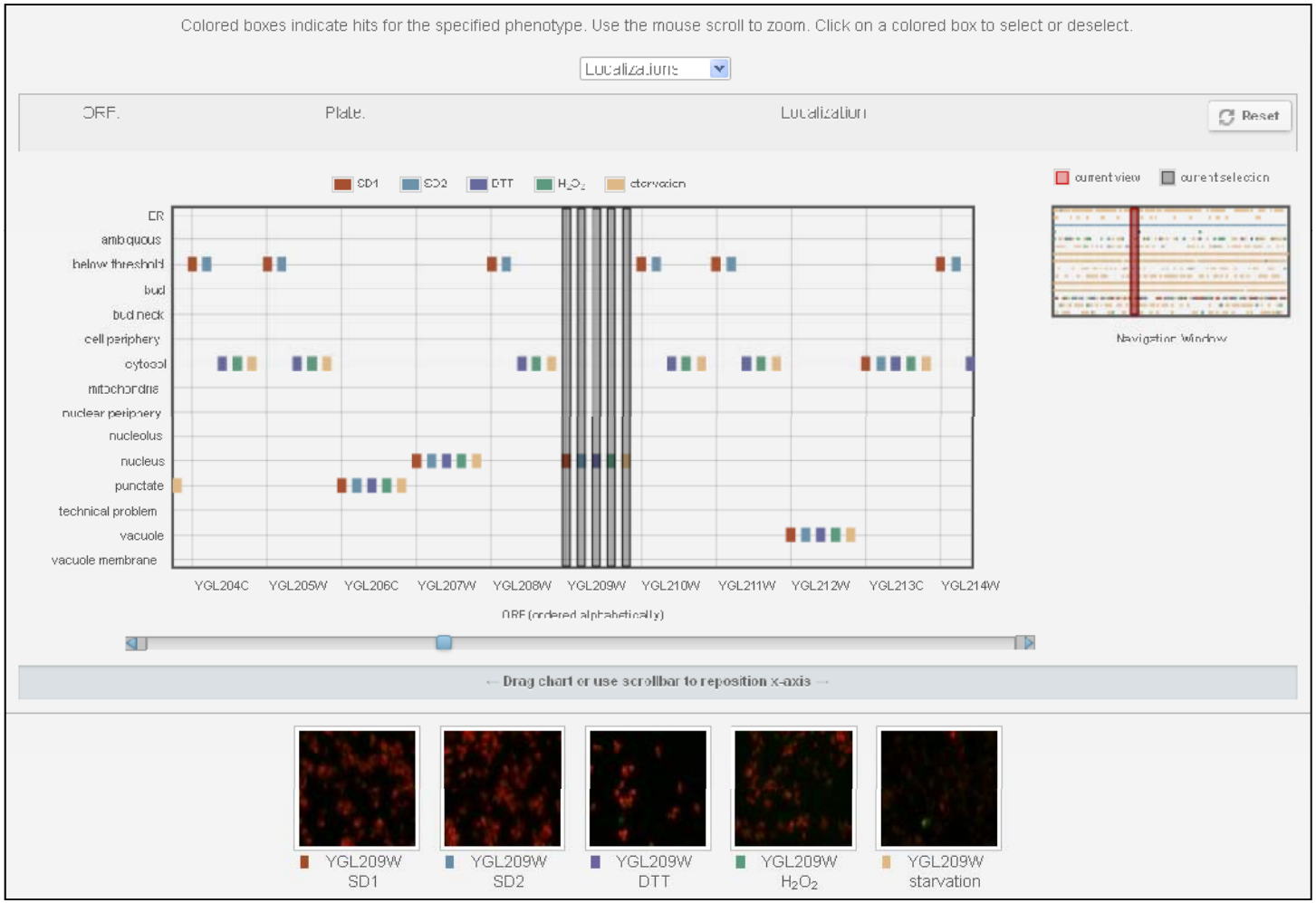


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Breker et al (2013)

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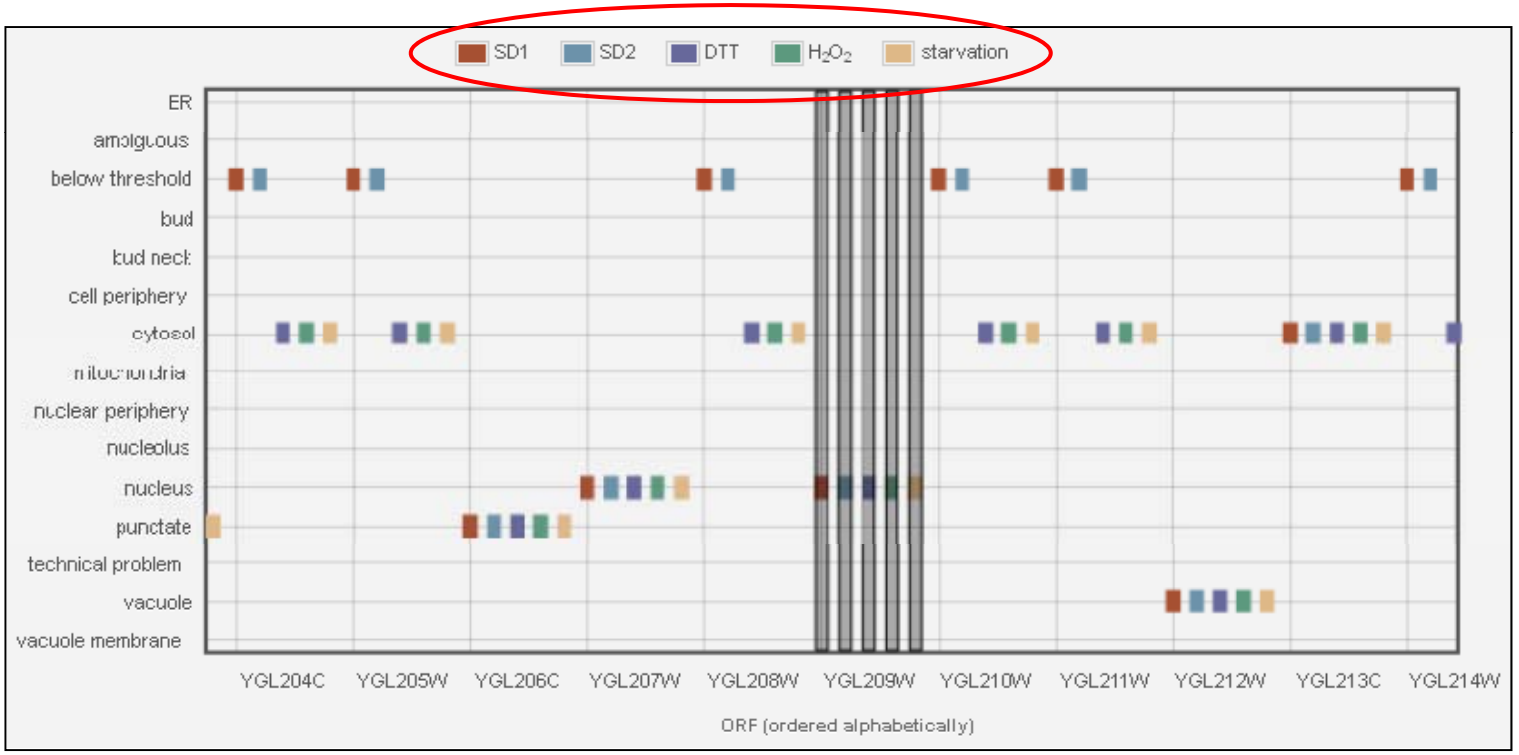


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Breker et al (2013)

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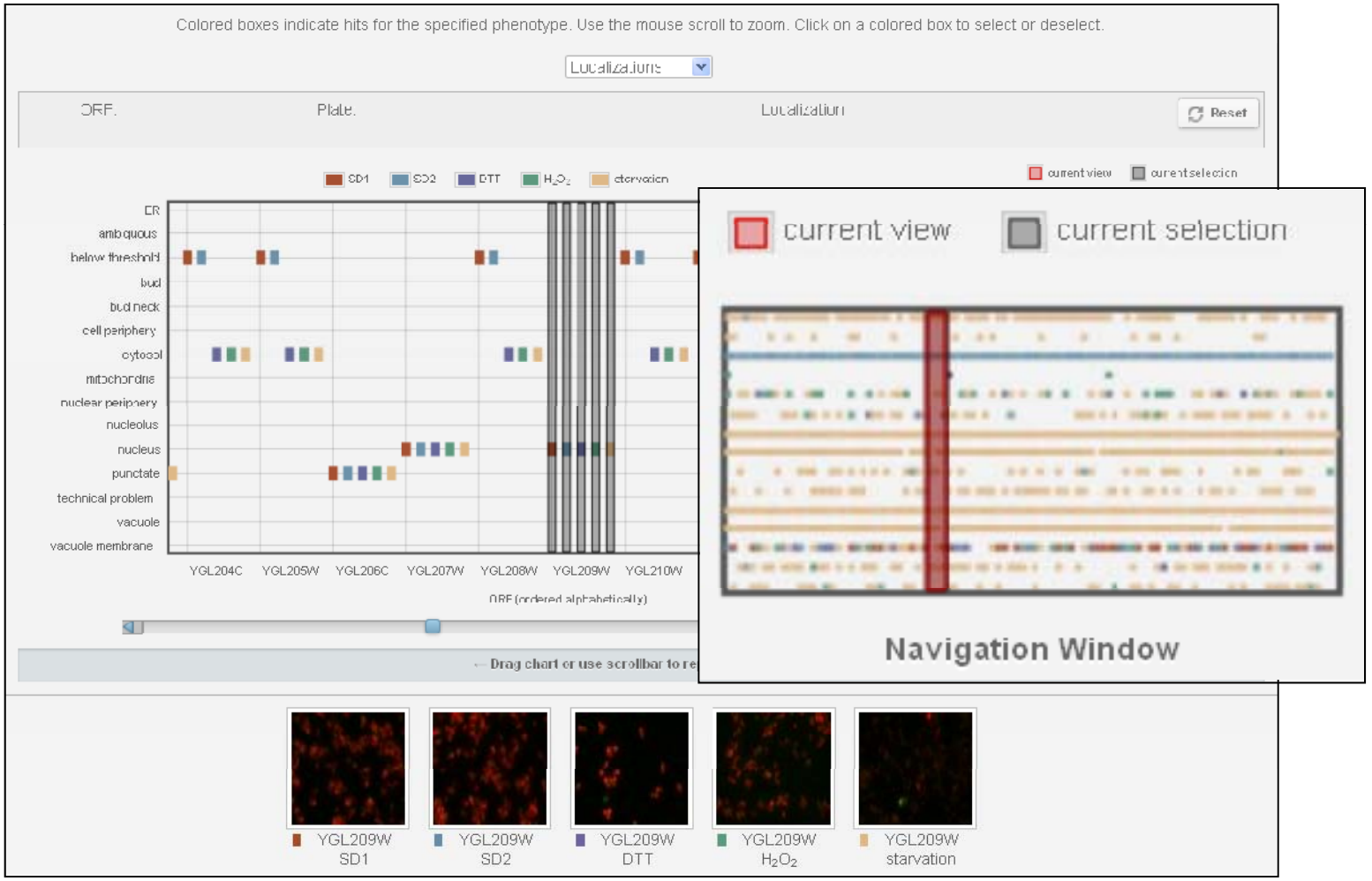


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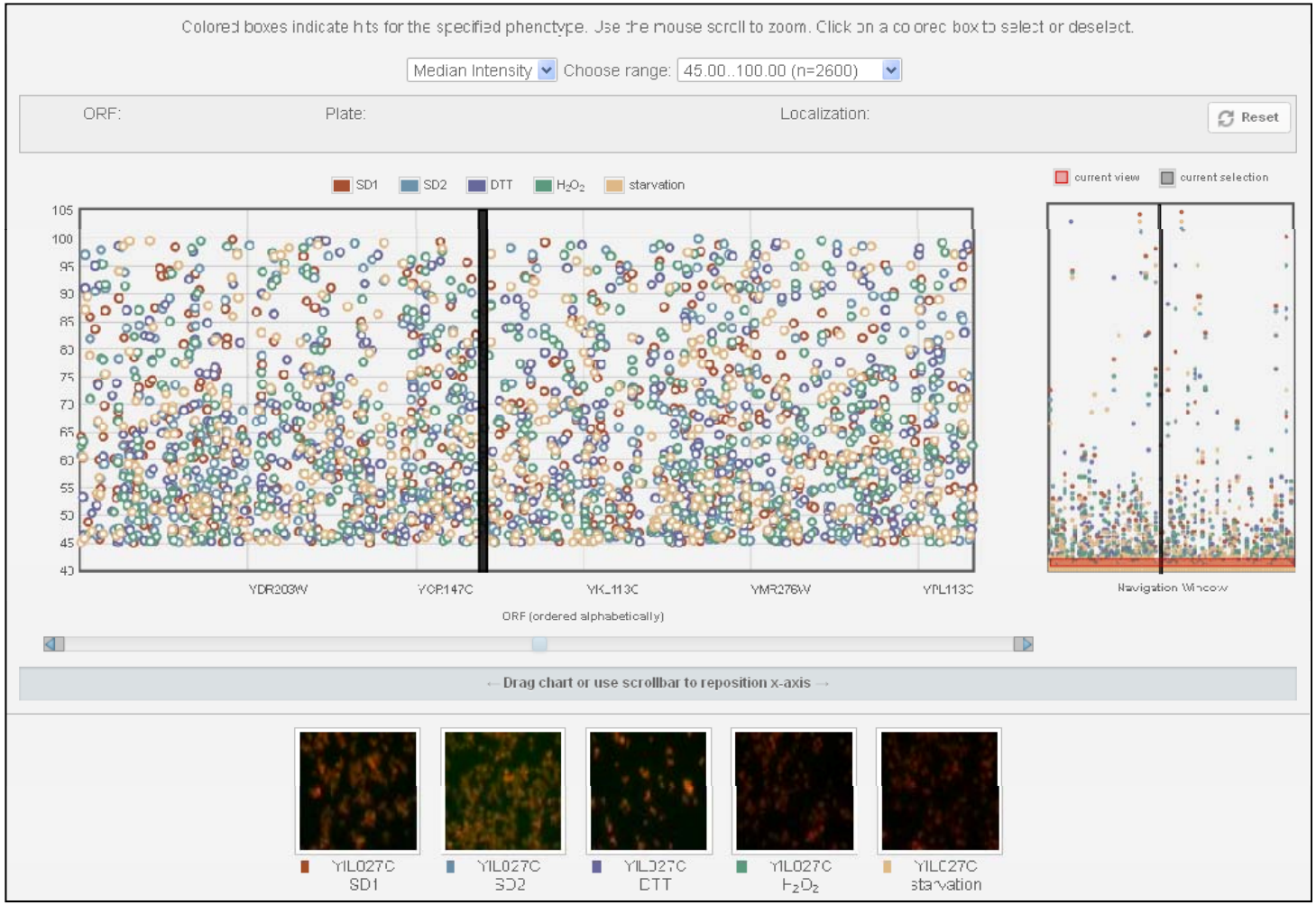


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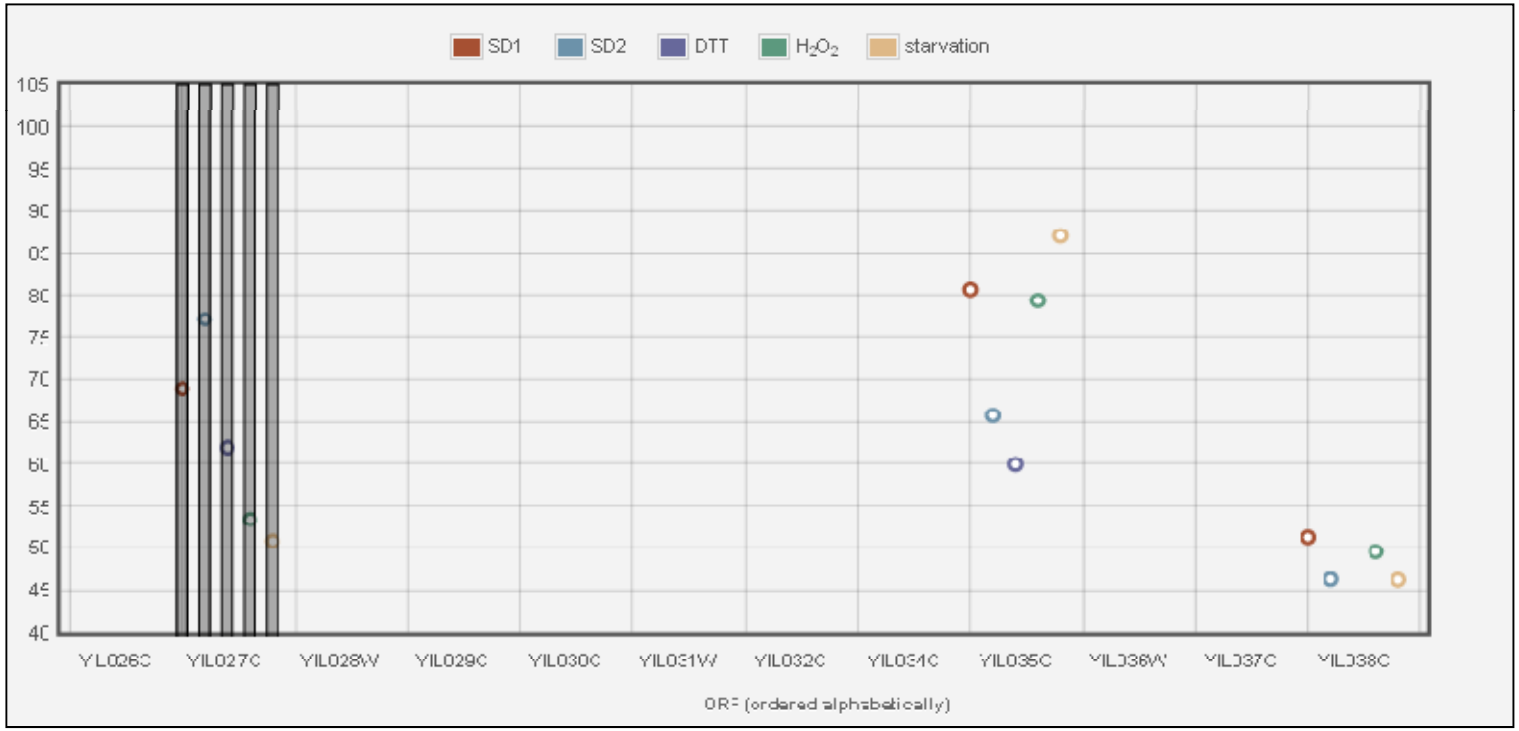


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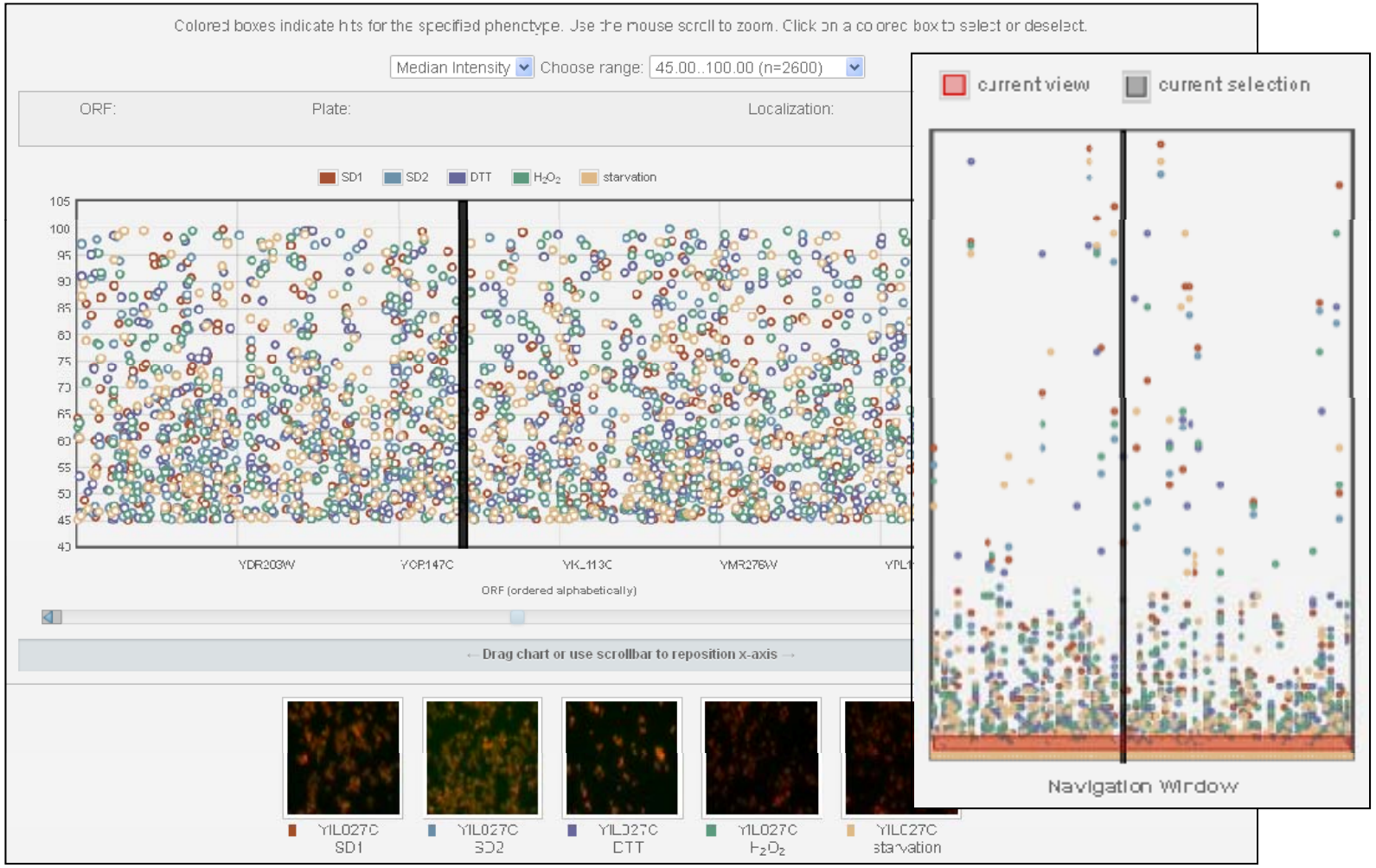


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## Summary of Features

- 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.

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# Multidimensional, Interactive Publishing

difference in the amount of Aurora B in unaligned and apparently aligned chromosomes (Fig. 5 A). We detected no change in chromosome staining with anti–phosphohistone H3 (Fig. 5 A) 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. 5 B; Andrews et al., 2004). In Bod1$^{siRNA}$ cells, we observed that although total MCAK present at unaligned centromeres was similar to control cells (Fig. 5 C), its precise localization was abnormal, forming multiple foci stretching out to one or both sister kinetochores.

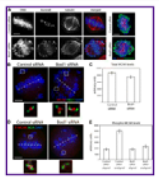

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Figure 5. **MCAK is not efficiently phosphorylated in Bod1$^{siRNA}$ cells.** (A) Aurora B is not delocalized in Bod1-depleted cells. Phospho-Ser10-histone H3 staining in control and Bod1 siRNA cells indicating Aurora B activity. (B–E) Cells were transfected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

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$^{siRNA}$ 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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Porter et al. (2007)

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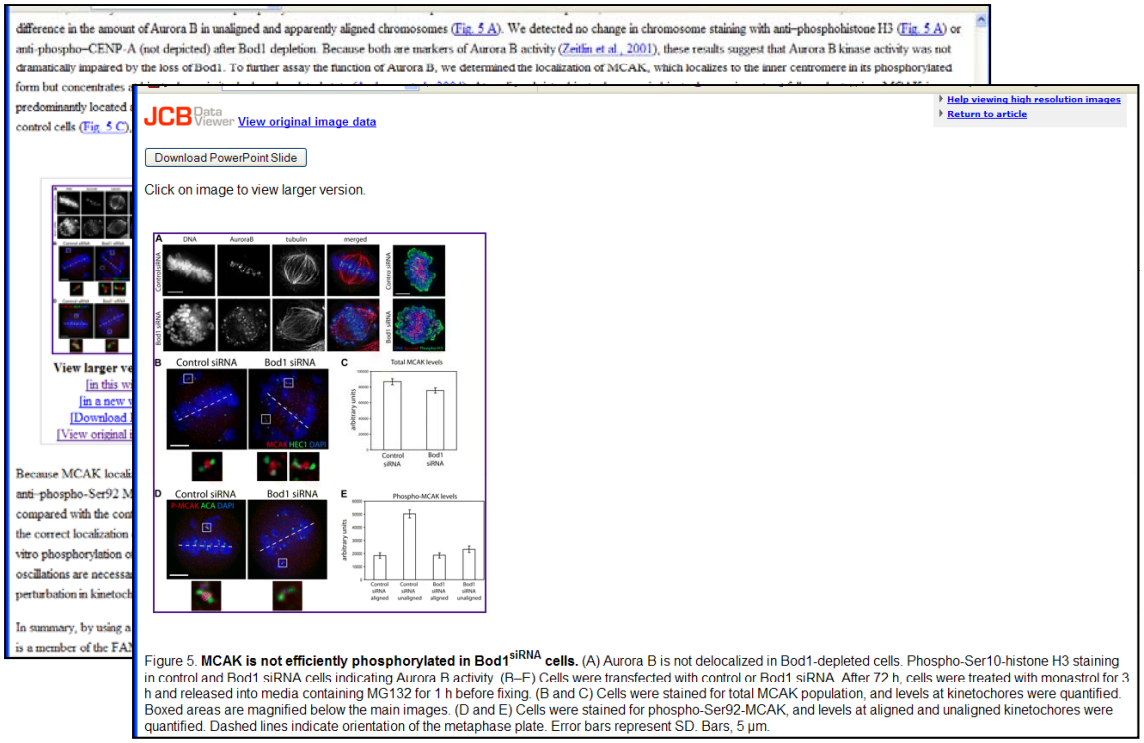


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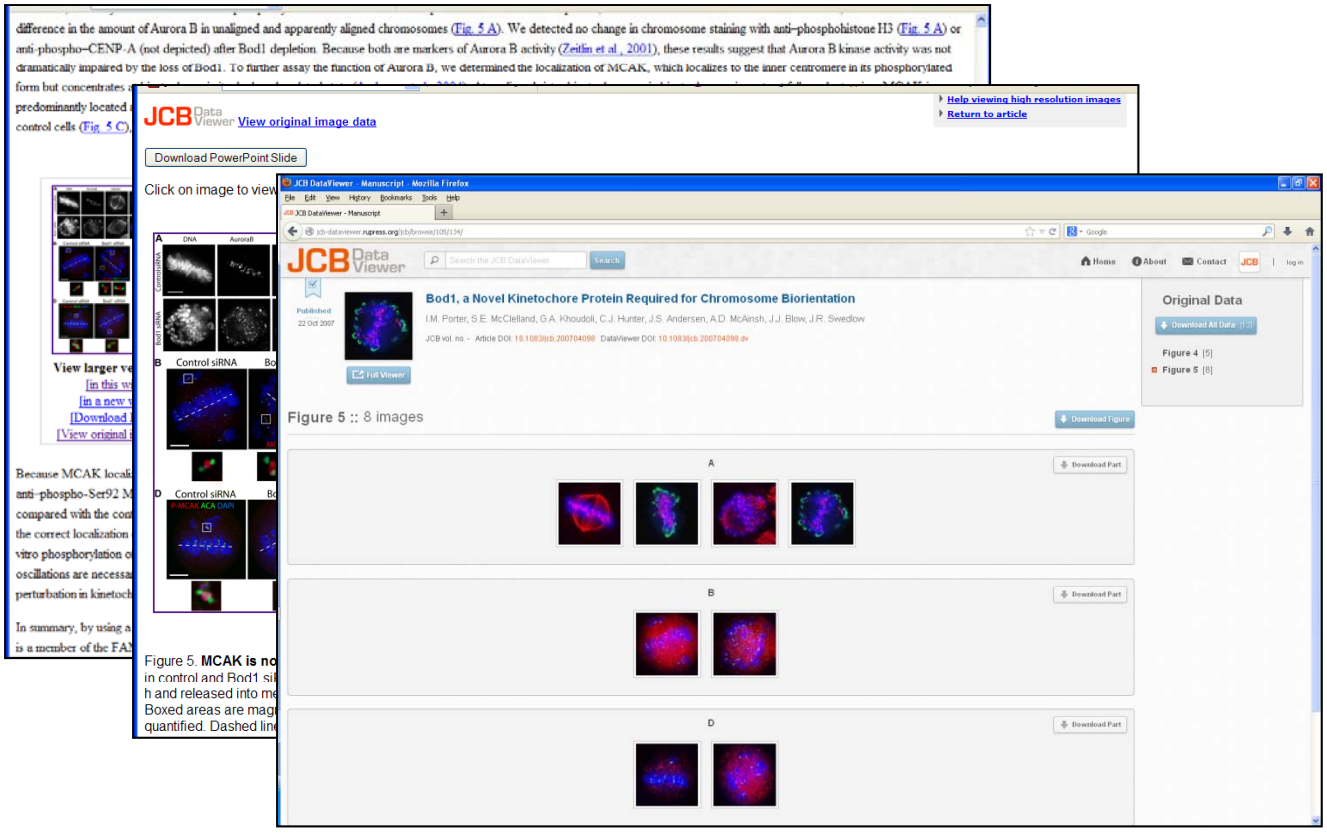


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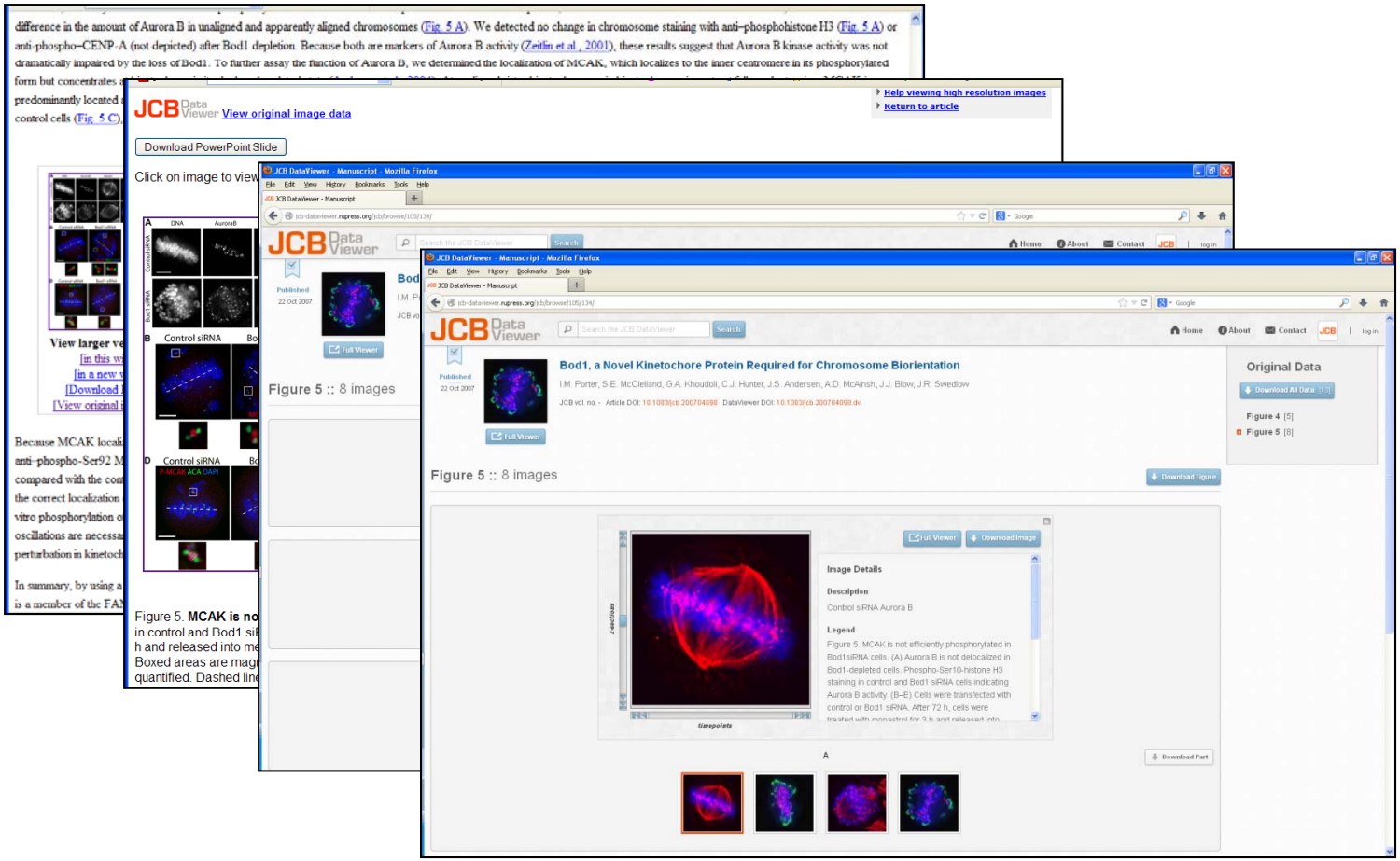


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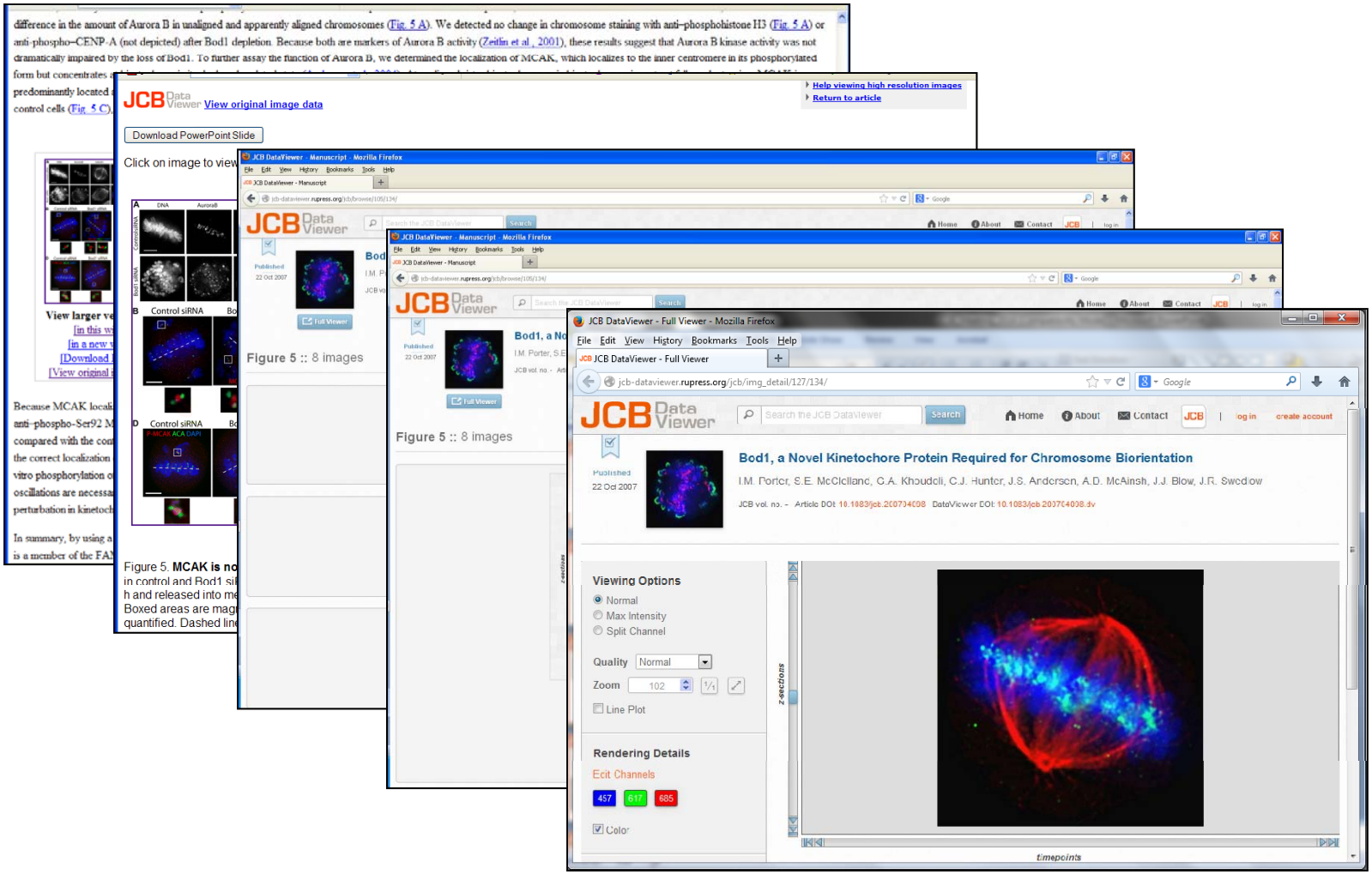


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# Voluntary participation rate

As of April, 2014:

3.54 TB of image data (published and unpublished)

→388 published manuscripts (28%)

→ 1451 published figures

→ 494,039 published images

→ 2,975,065 individual image frames

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# Usage Statistics

- Accessed by ~14,000 unique visitors per month

- ~80% of users access the JCB DataViewer via our online journal site

- Reported reasons for using the JCB DataViewer:
  - evaluating the conclusions of a *JCB* paper
  - data mining / generating new hypotheses for own research
  - obtaining images for educational purposes

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## Where do we go from here?

- Continue to expand the range of data we can host and the tools available within the JCB DataViewer to analyze and access those data.
- Continue to promote a new standard for sharing and archiving of published image data – for scientific integrity and for discovery.
- *…an international repository for all published image data?*

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*with special thanks to Mike Rossner, Emma Hill, and:*



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