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OME Users' Meeting
June 24, 2013

JCB Data Viewer

Bringing New Dimensions to Published Image Data

Liz Williams, PhD
Executive Editor, *The Journal of Cell Biology*
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THE ROCKEFELLER UNIVERSITY PRESS
QUALITY AND INTEGRITY

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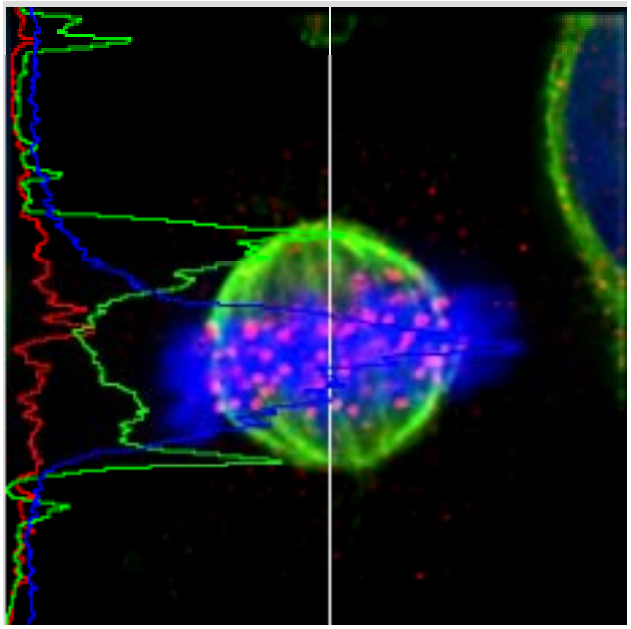
Why would a publisher be interested in OME/OMERO?

Our goals as a publisher:

- To ensure that our authors have an effective medium for publishing their image data.
- To ensure that our readers have an effective medium for accessing and assessing published image data.
- To ensure the integrity of the scientific record.



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JCB Data Viewer

- An OMERO-based, browser-based application for viewing, sharing, and archiving original, unprocessed image data associated with *JCB* papers.
- Enables presentation and sharing of image data acquired using a wide variety of light microscope and gel documentation systems.
- Allows users (editors, reviewers, readers) to perform simple analyses of the data within the application and to download the data in the open OME-TIFF format for more in-depth analysis with their software of choice.
- Enables multidimensional publishing – far beyond what is possible with standard PDFs and html.

OME

GLENCOE SOFTWARE

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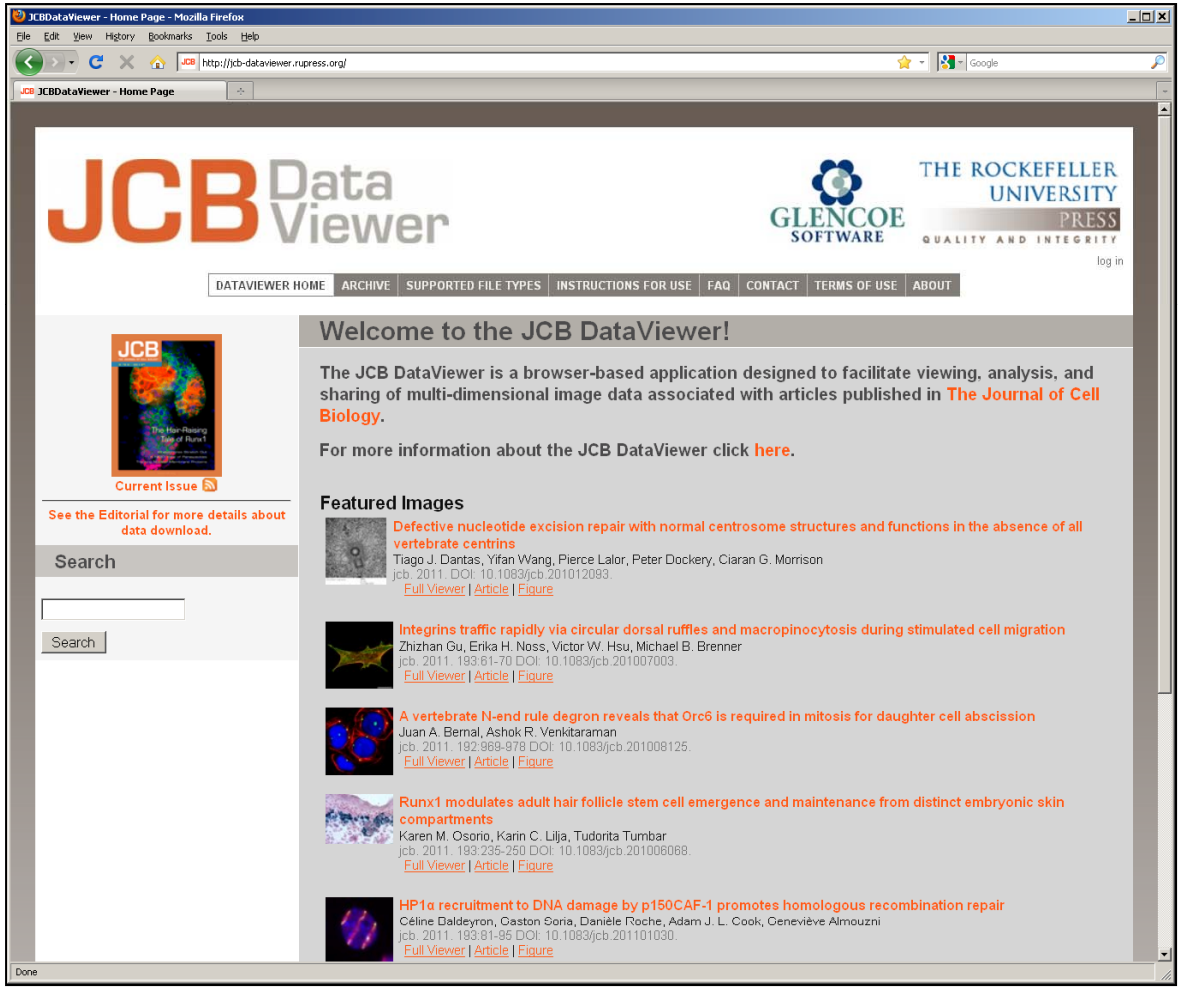
circa 2012



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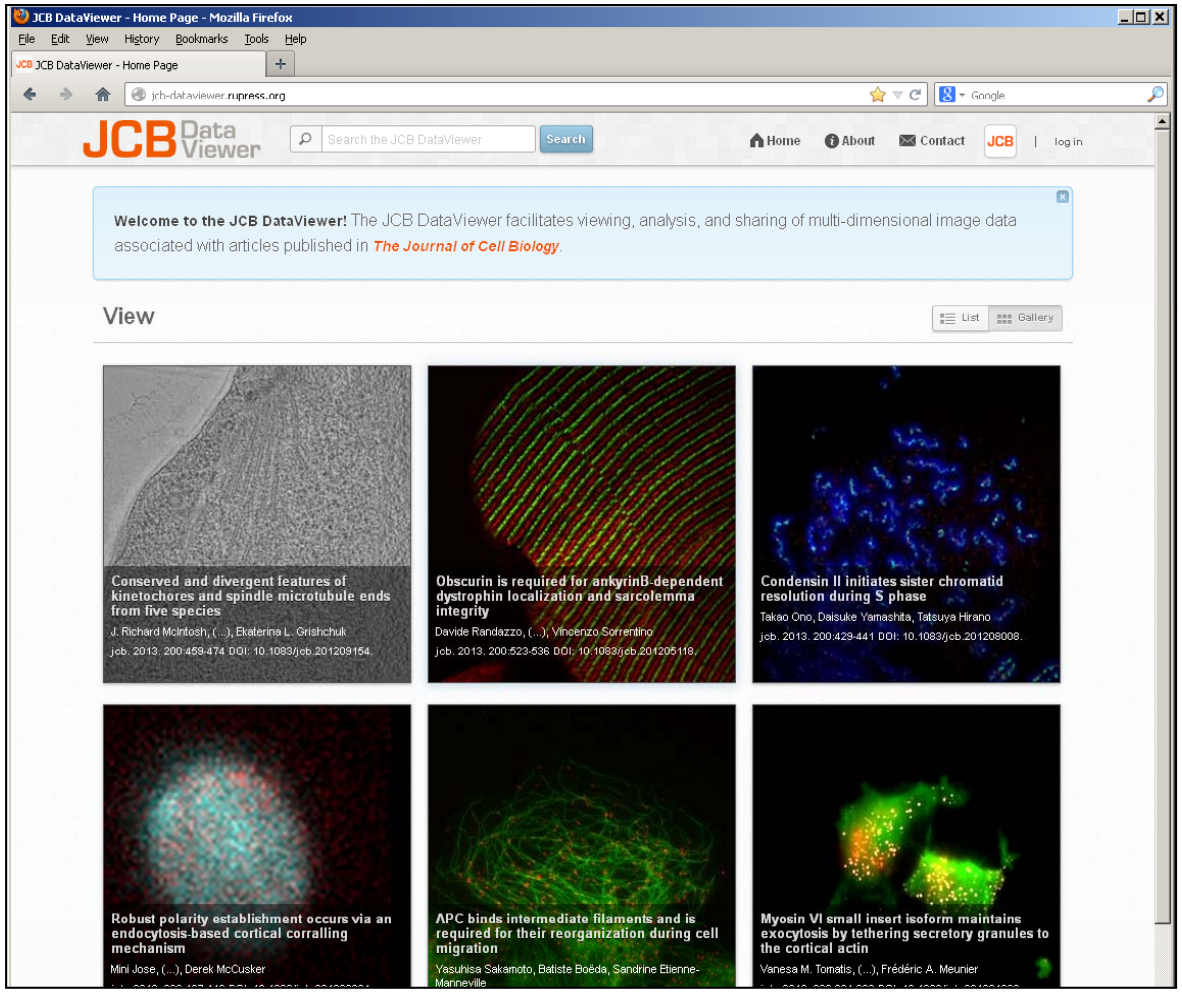
circa 2013



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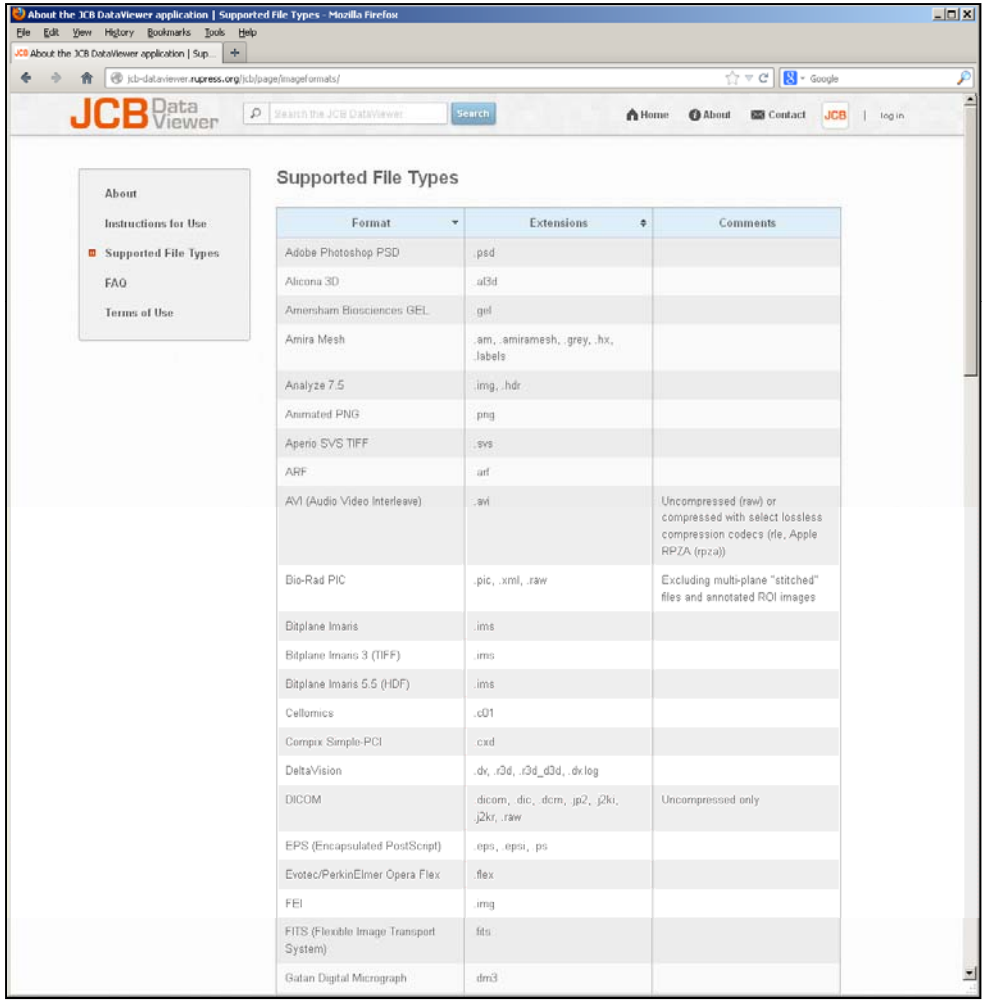
The JCB DataViewer: Supported File Types



Format	Extensions	Comments
Adobe Photoshop PSD	.psd	
Alicona 3D	.al3d	
Amersham Biosciences GEL	.gel	
Amira Mesh	.am, .amiramesh, .grey, .hx, .labels	
Analyze 7.5	.img, .hdr	
Animated PNG	.png	
Aperio SVS TIFF	.svs	
ARF	.arf	
AVI (Audio Video Interleave)	.avi	Uncompressed (raw) or compressed with select lossless compression codecs (rle, Apple RPZA (rpza))
Bio-Rad PIC	.pic, .xml, .raw	Excluding multi-plane "stitched" files and annotated ROI images
Bitplane Imaris	.ims	
Bitplane Imaris 3 (TIFF)	.ims	
Bitplane Imaris 5.5 (HDF)	.ims	
Cellomics	.c01	
Compix Simple-PCI	.cxd	
DeltaVision	.dv, .r3d, .r3d_d3d, .dv.log	
DICOM	.dicom, .dic, .dcm, .jp2, .j2ki, .j2kr, .raw	Uncompressed only
EPS (Encapsulated PostScript)	.eps, .epsi, .ps	
Evotec/PerkinElmer Opera Flex	.flex	
FEI	.img	
FITS (Flexible Image Transport System)	.fits	
Gatan Digital Micrograph	.dm3	

Using Bioformats, more than 75 PFFs are supported.

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1. single image analysis

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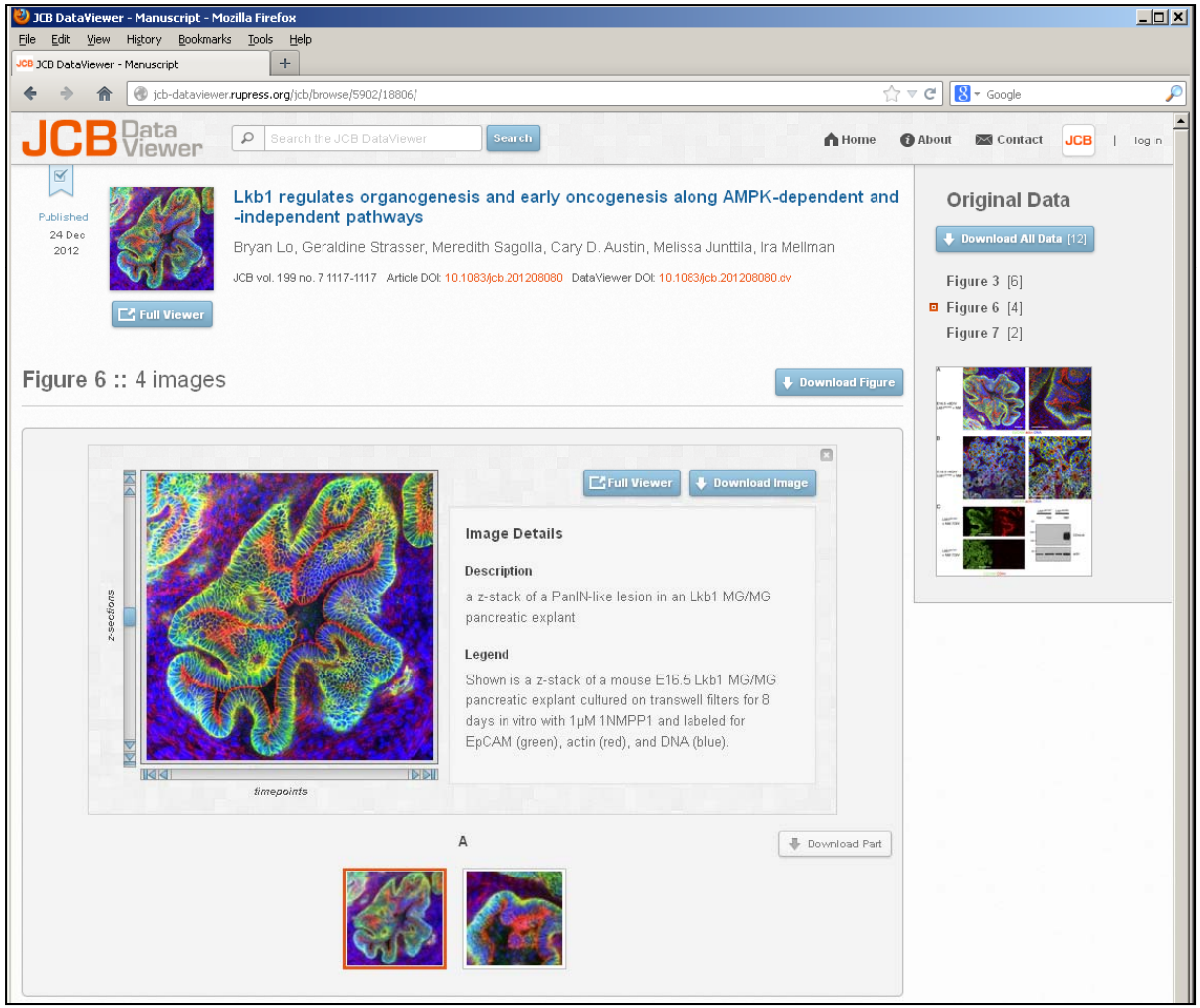
The Mini Viewer



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Lo et al (2012)

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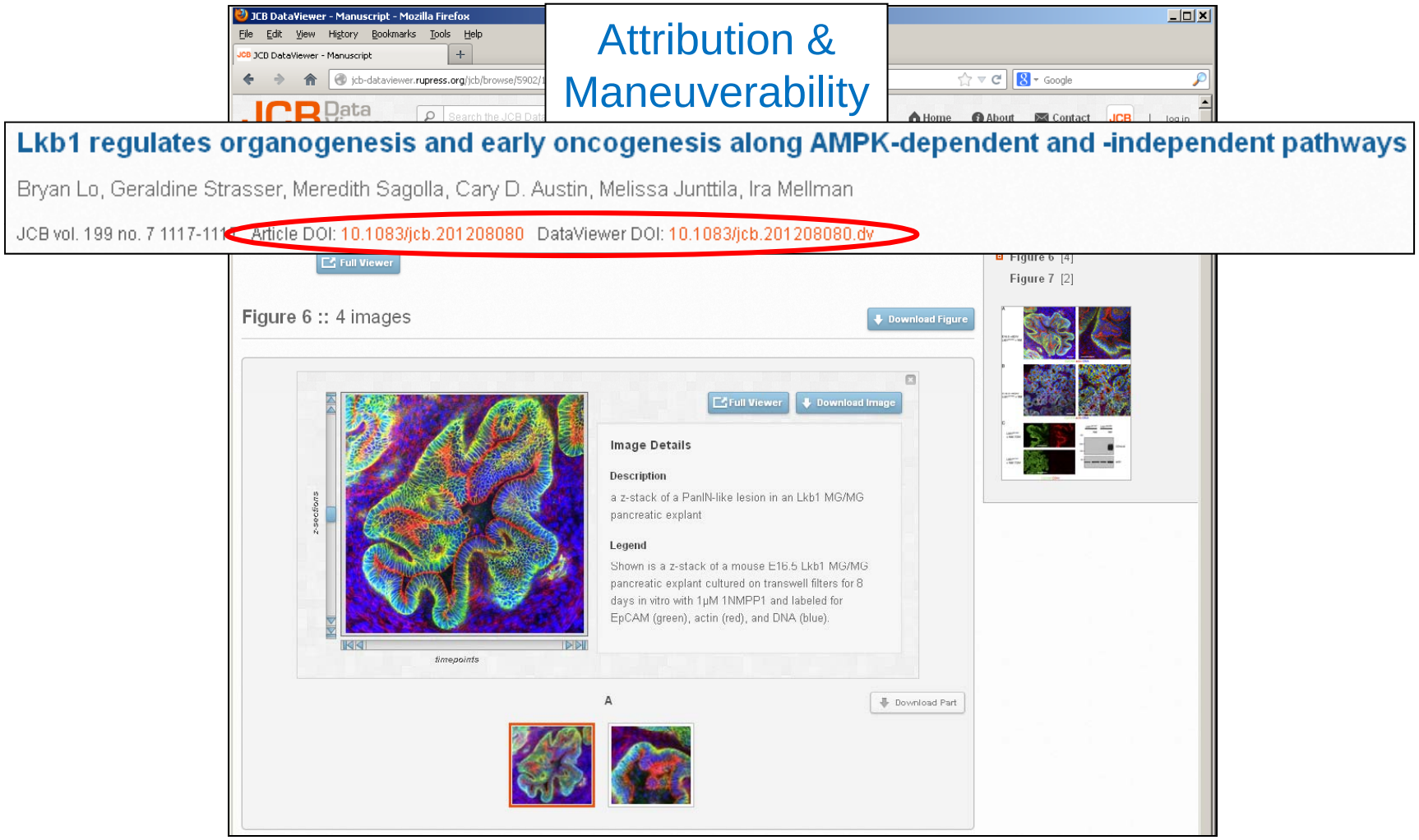

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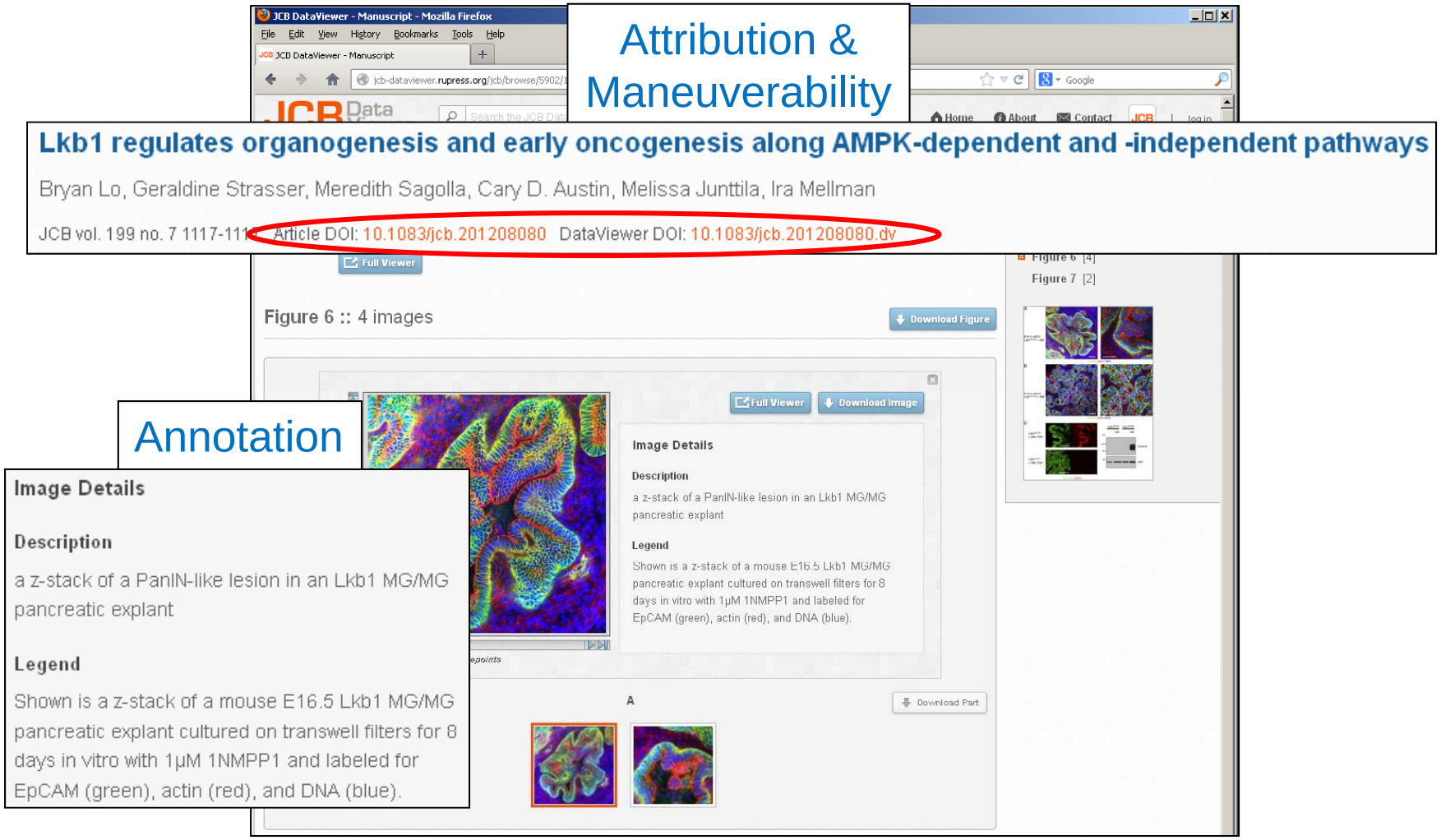

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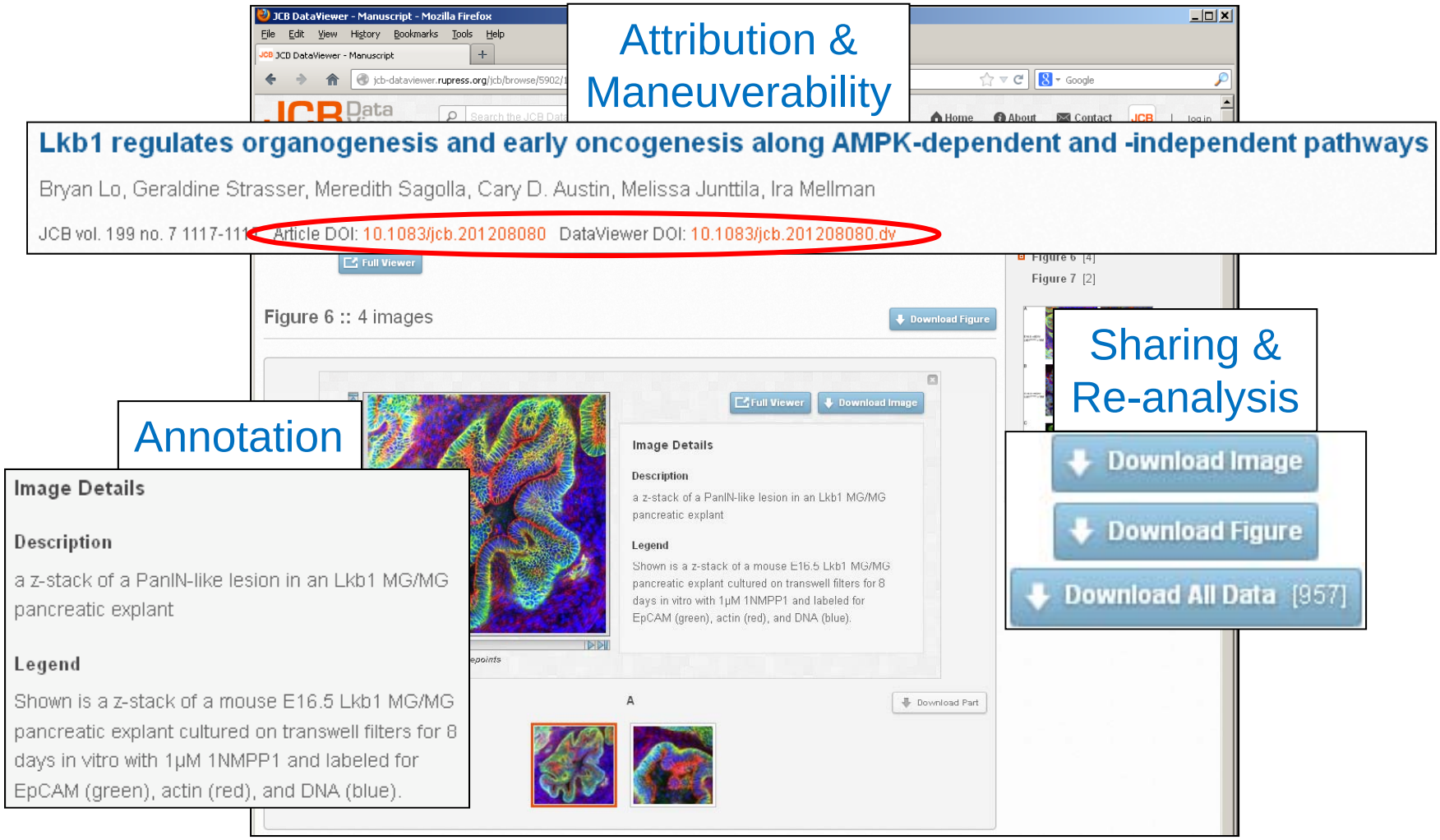

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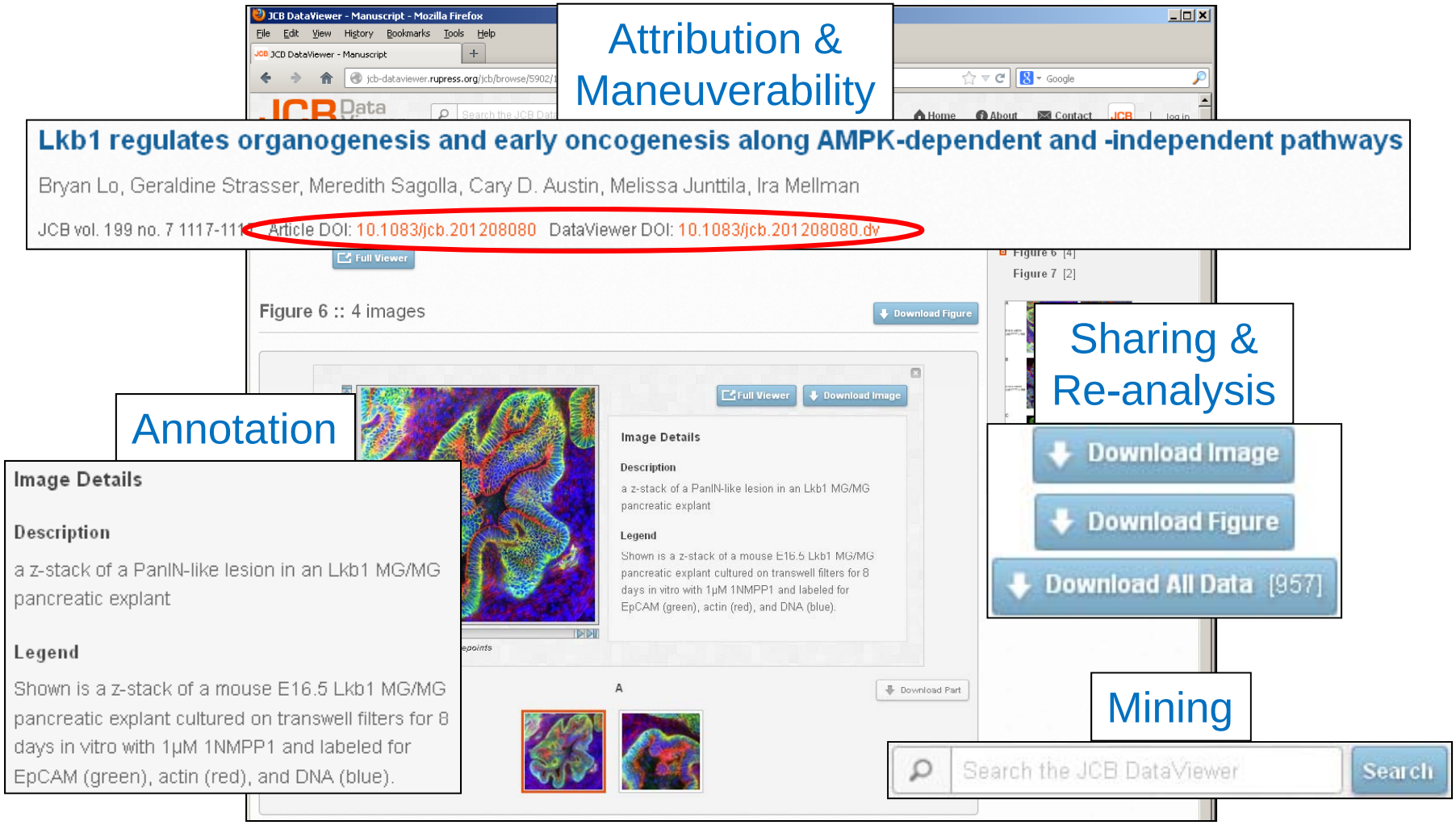
The Mini Viewer



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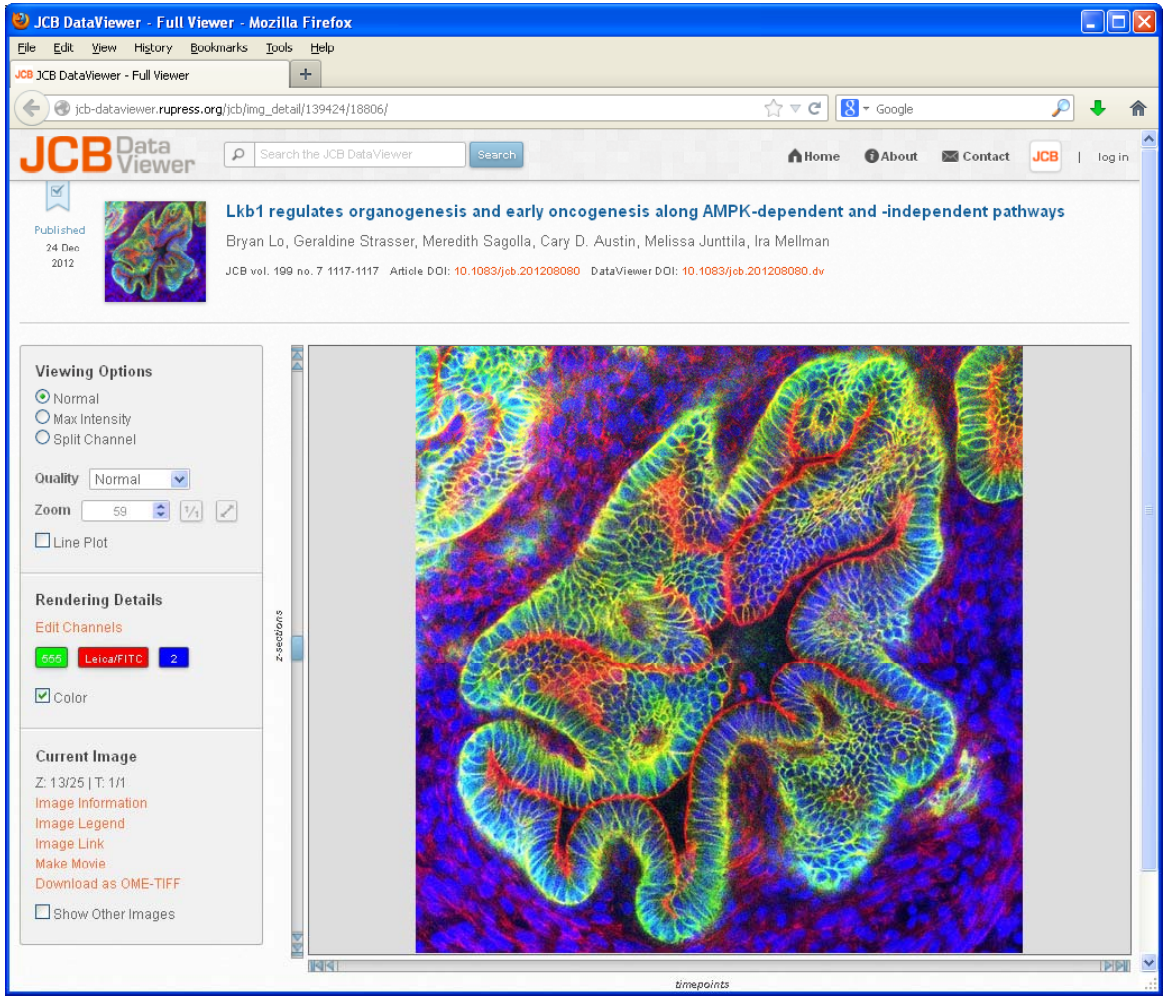
The Full Viewer



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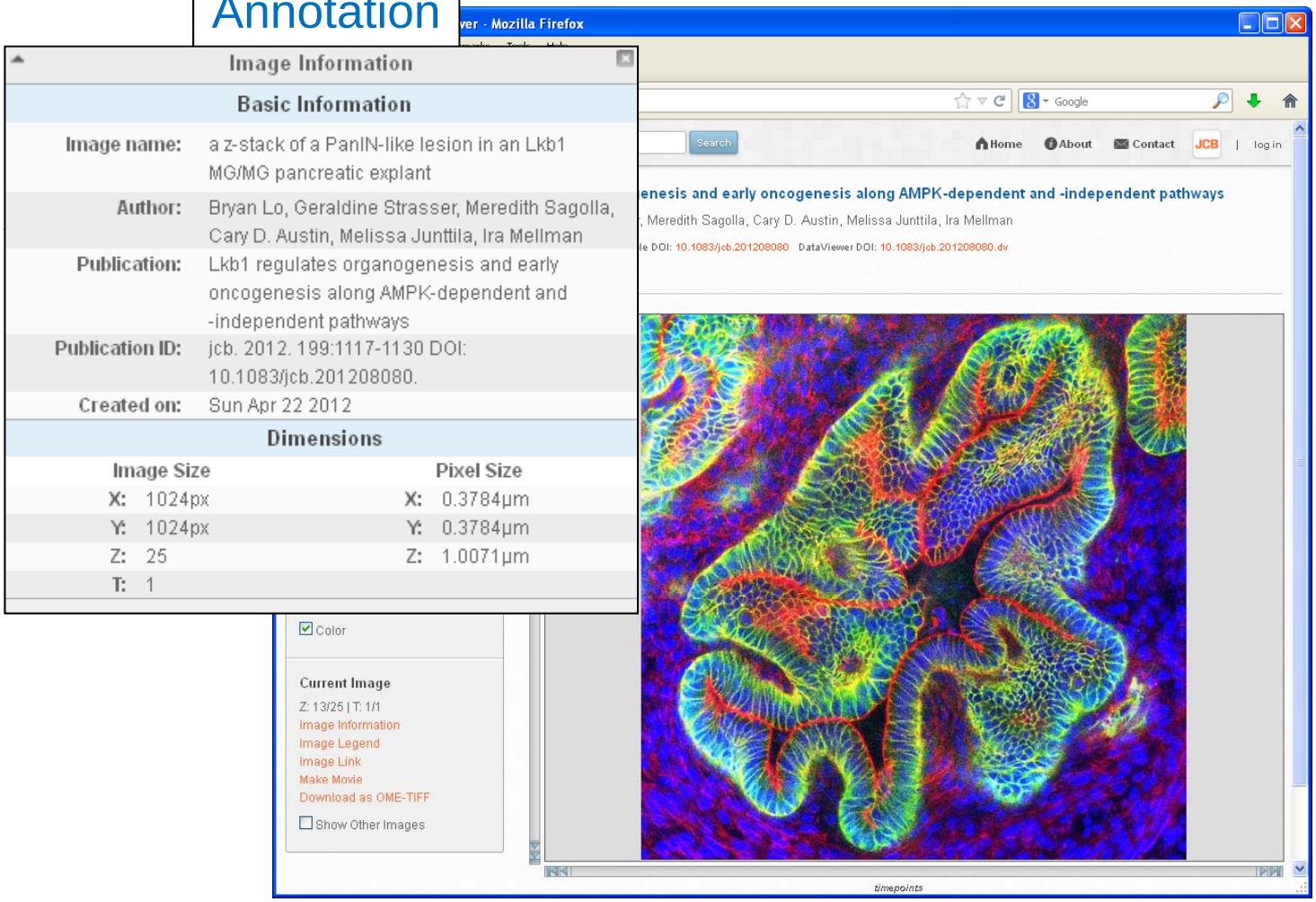
The Full Viewer

Annotation



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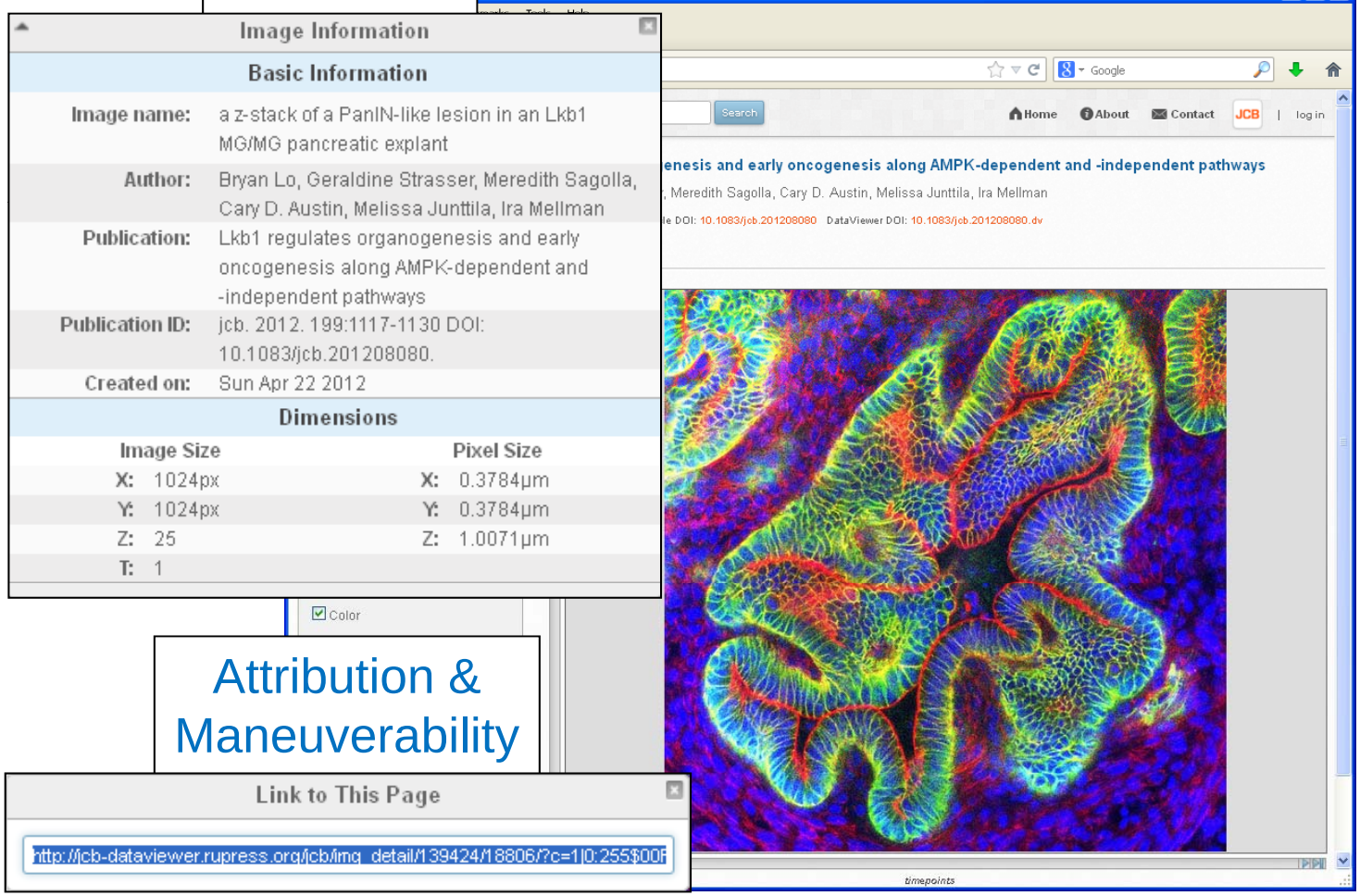
The Full Viewer

Annotation



Attribution & Maneuverability

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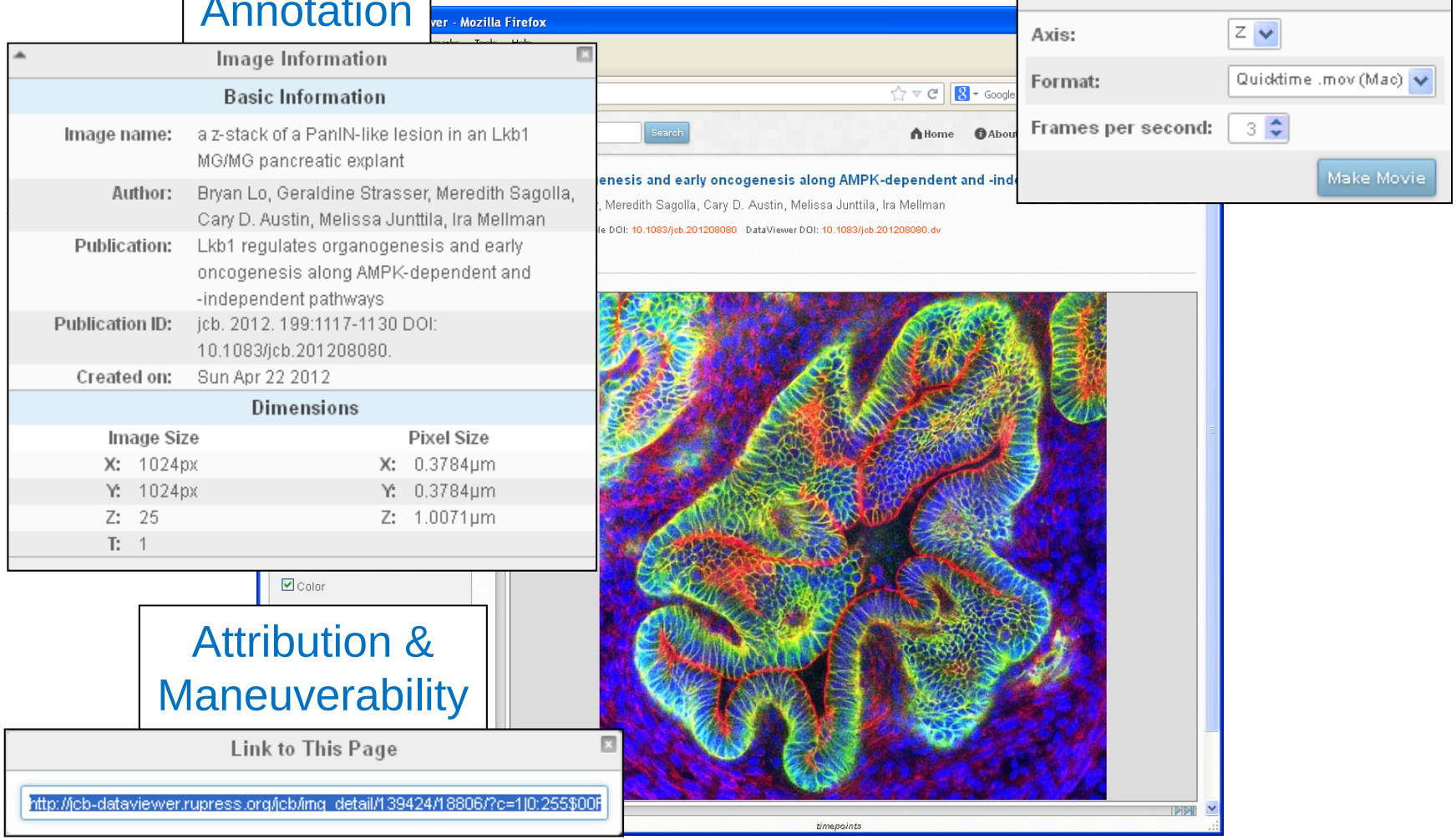
The Full Viewer

Data Presentation

Annotation



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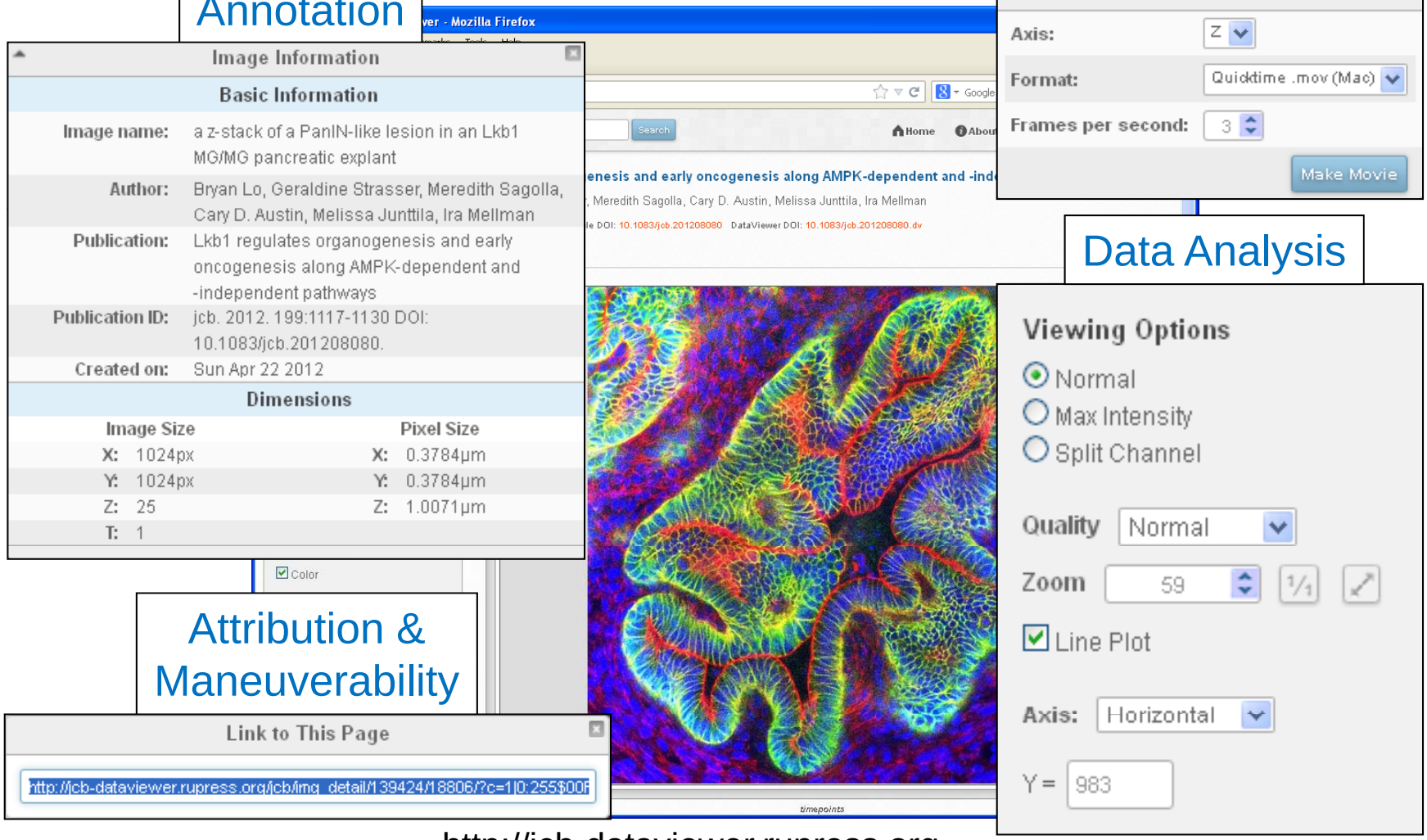
The Full Viewer

Data Presentation

Annotation



Data Analysis

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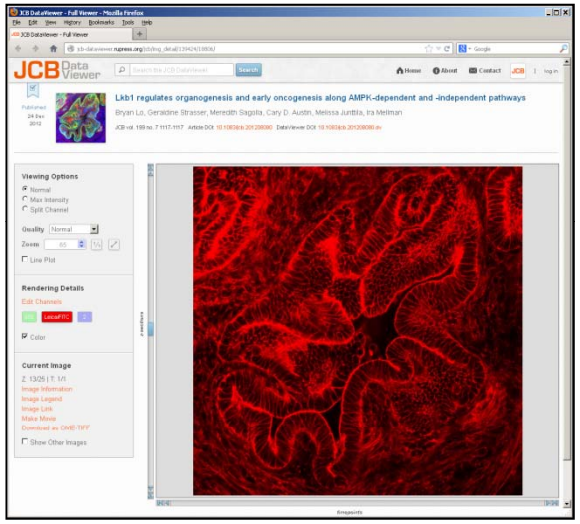
Split-Channel View



Published 24 Dec 2012

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

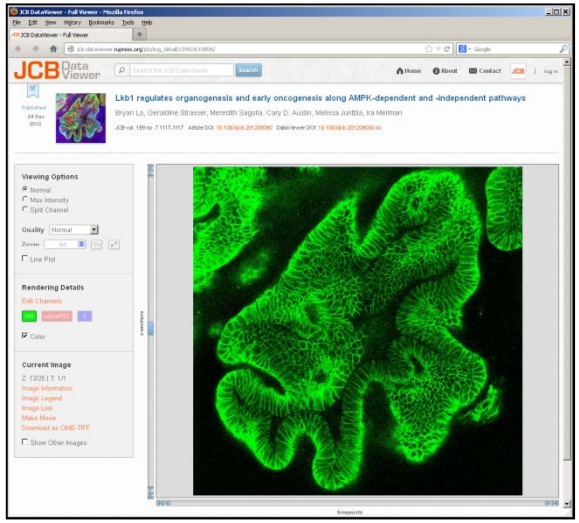
JCB vol. 199 no. 7 1117-1117 Article DOI 10.1083/jcb.201208080 DataViewer DOI 10.1083/jcb.201208080.dv

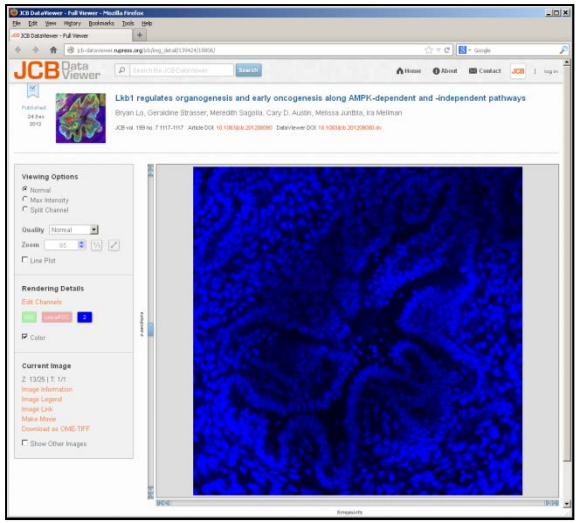
Viewing Options
- ● Normal
- ○ Max Intensity
- ○ Split Channel

Quality: Normal
Zoom: 65
☐ Line Plot

Rendering Details
Edit Channels
LeicaFITC
☑ Color

Current Image
Z: 13/25 | T: 1/1
Image Information
Image Legend
Image Link
Make Movie
Download as OME-TIFF
☐ Show Other Images


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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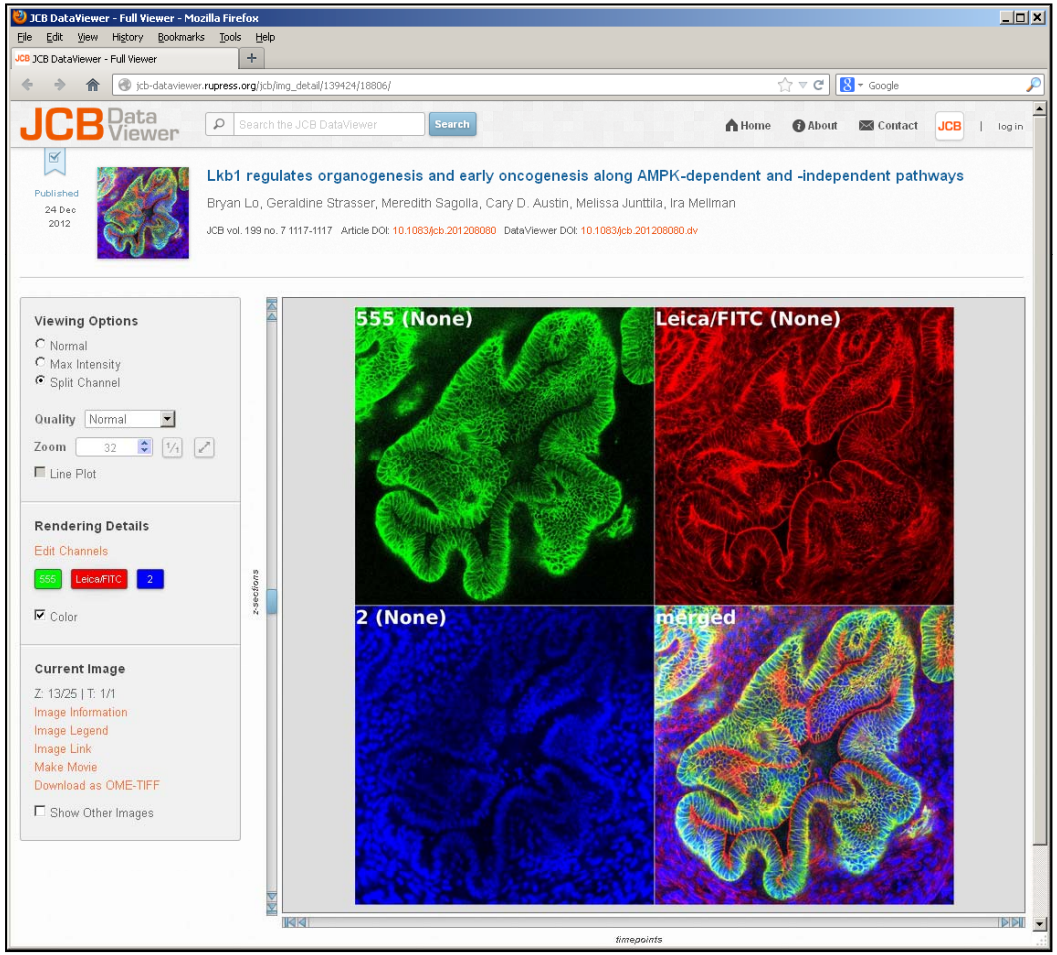
Split-Channel View



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2. ultra-large, tiled image hosting

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

- 26000 tiled EMs
- 921600 pixels x 380928 pixels
- 281 gigapixels
- 1.6 nm resolution (16 million dpi)

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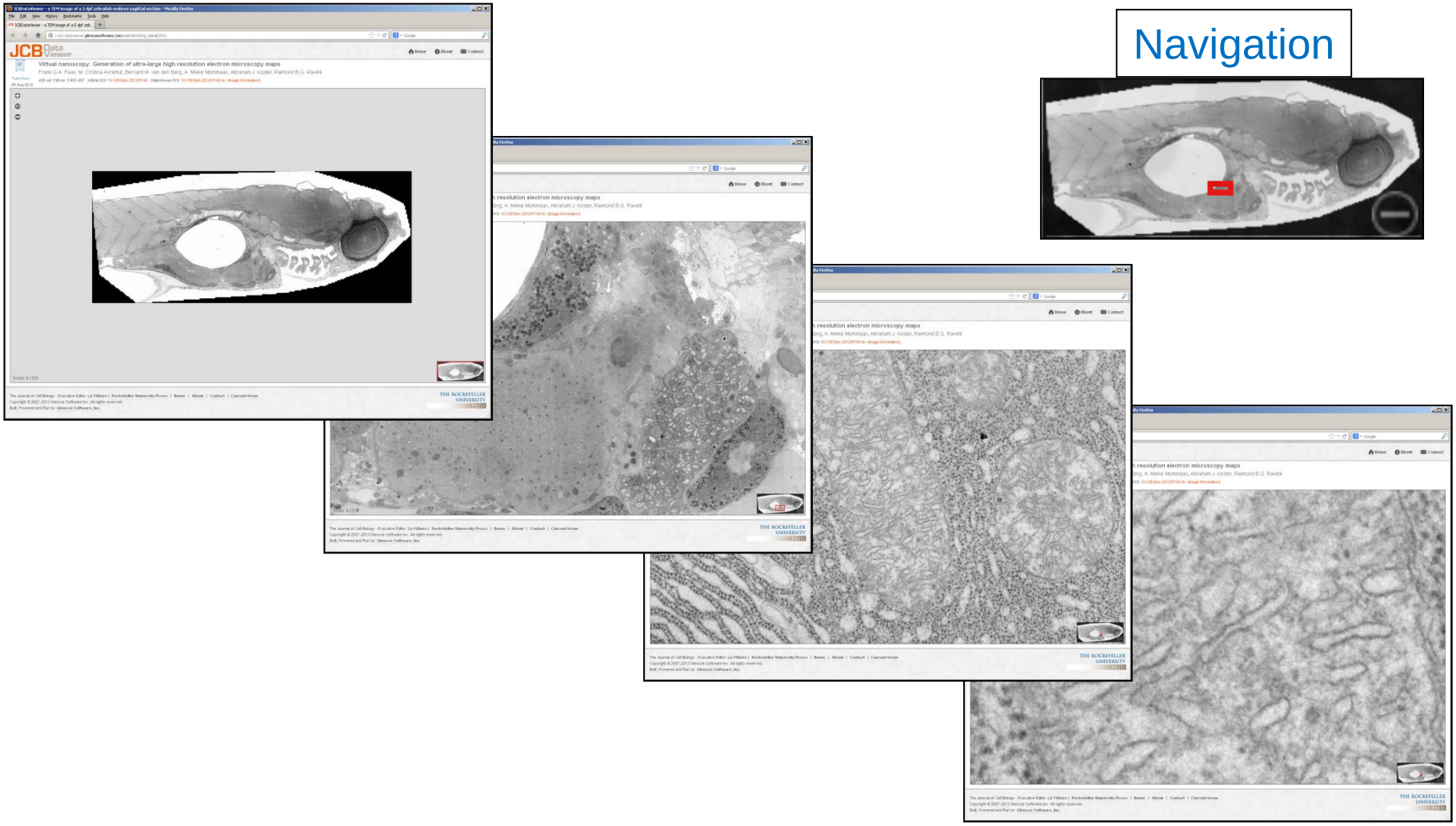
Ultra-large, high-resolution, tiled images



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Faas et al (2012)

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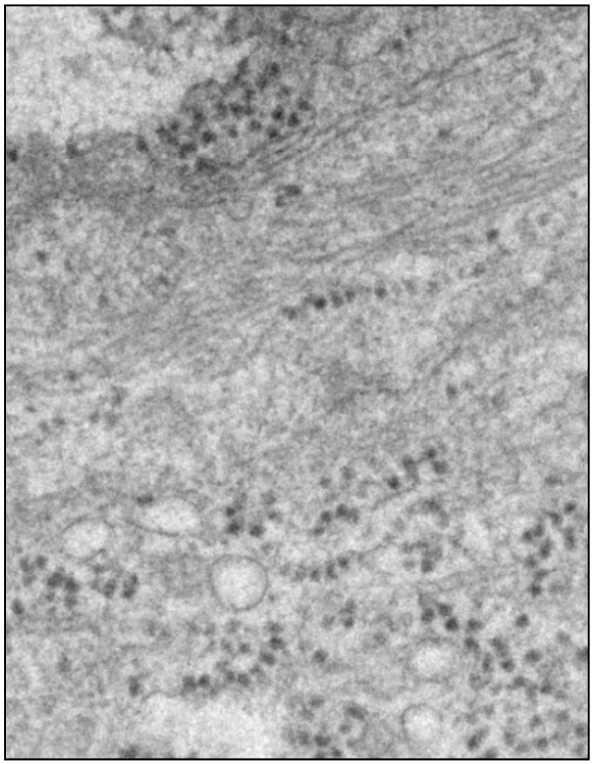
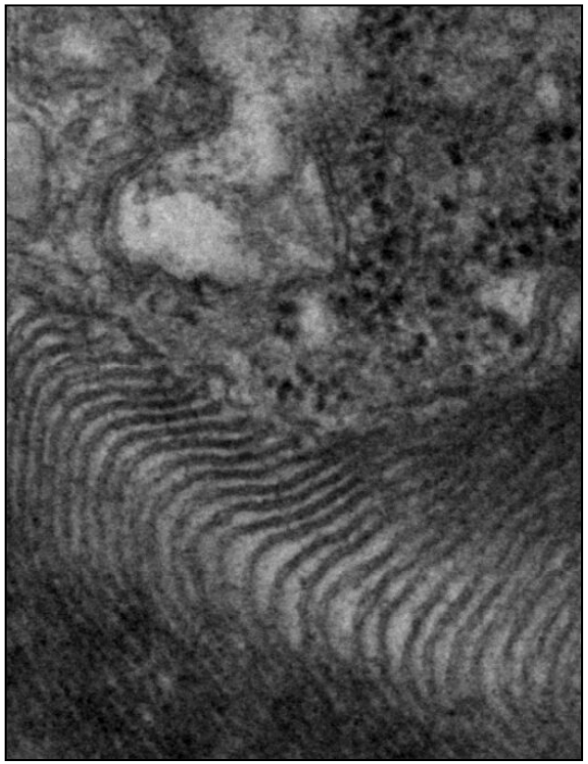
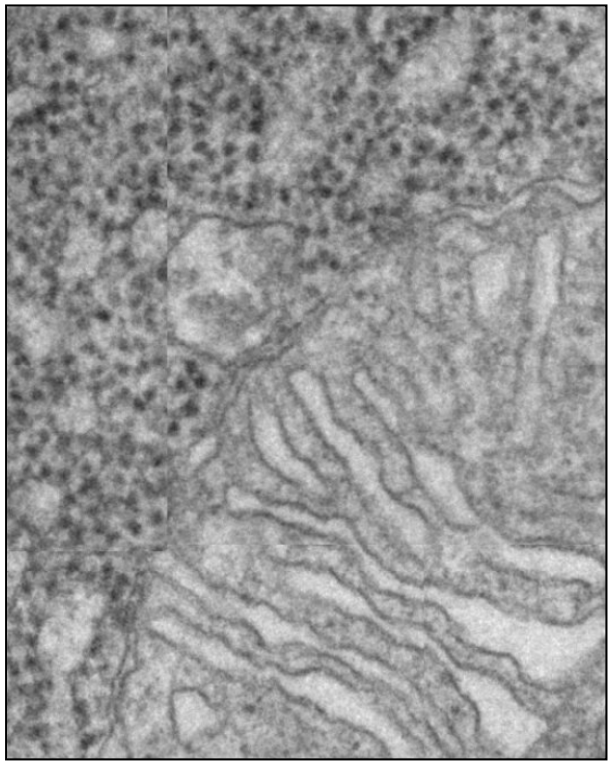
Ultra-large, high-resolution, tiled images







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Faas et al (2012)

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3. high-content screen analysis

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Six 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)
- 97920 total images
- 1 qualitative & 1 quantitative datapoint per strain per treatment (26650 total)
- cross-plate datasets (2 untreated and 3 treated datasets per strain)

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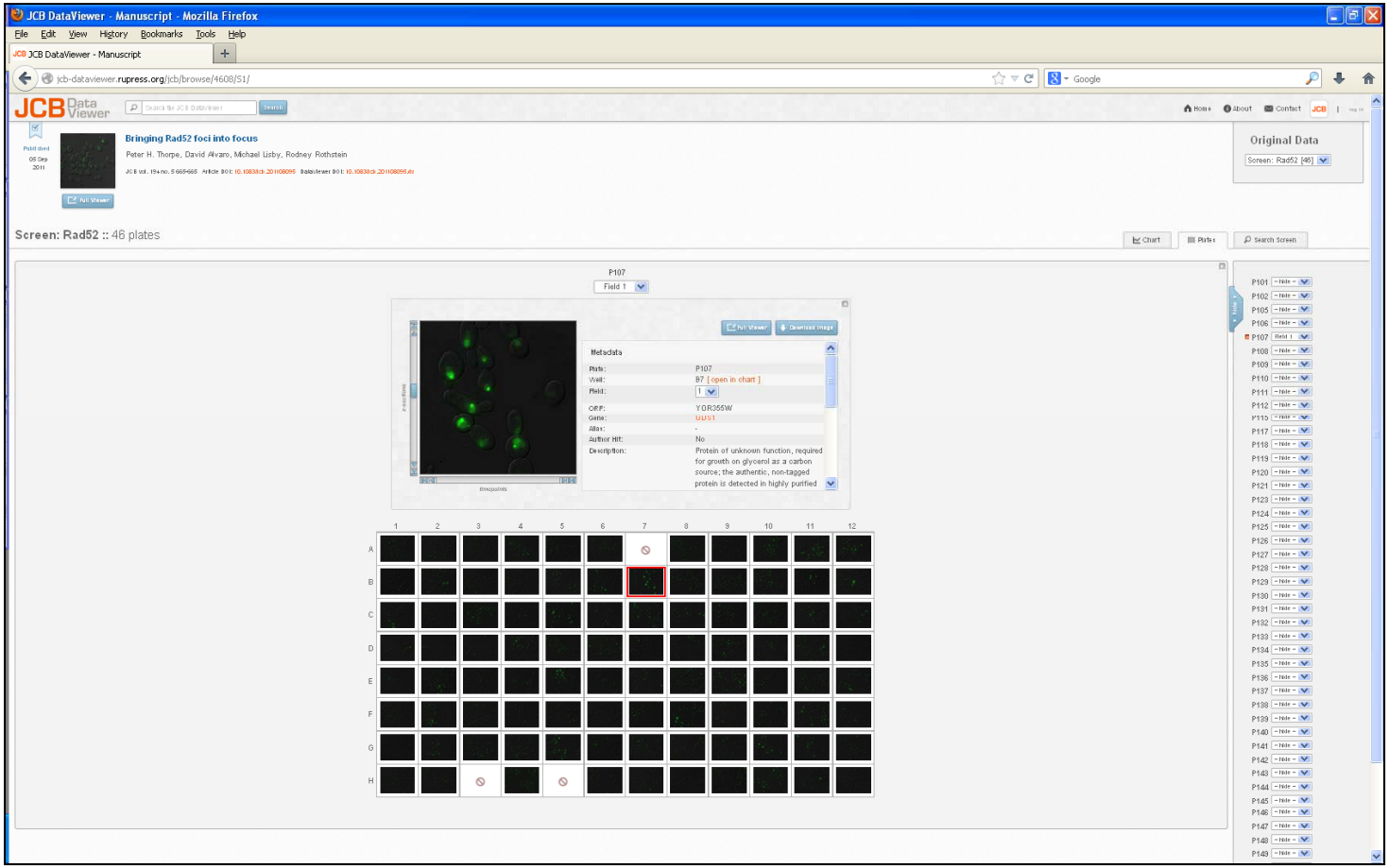
HCS Data – Plate View



http://jcb-dataviewer.rupress.org

Thorpe et al (2011)

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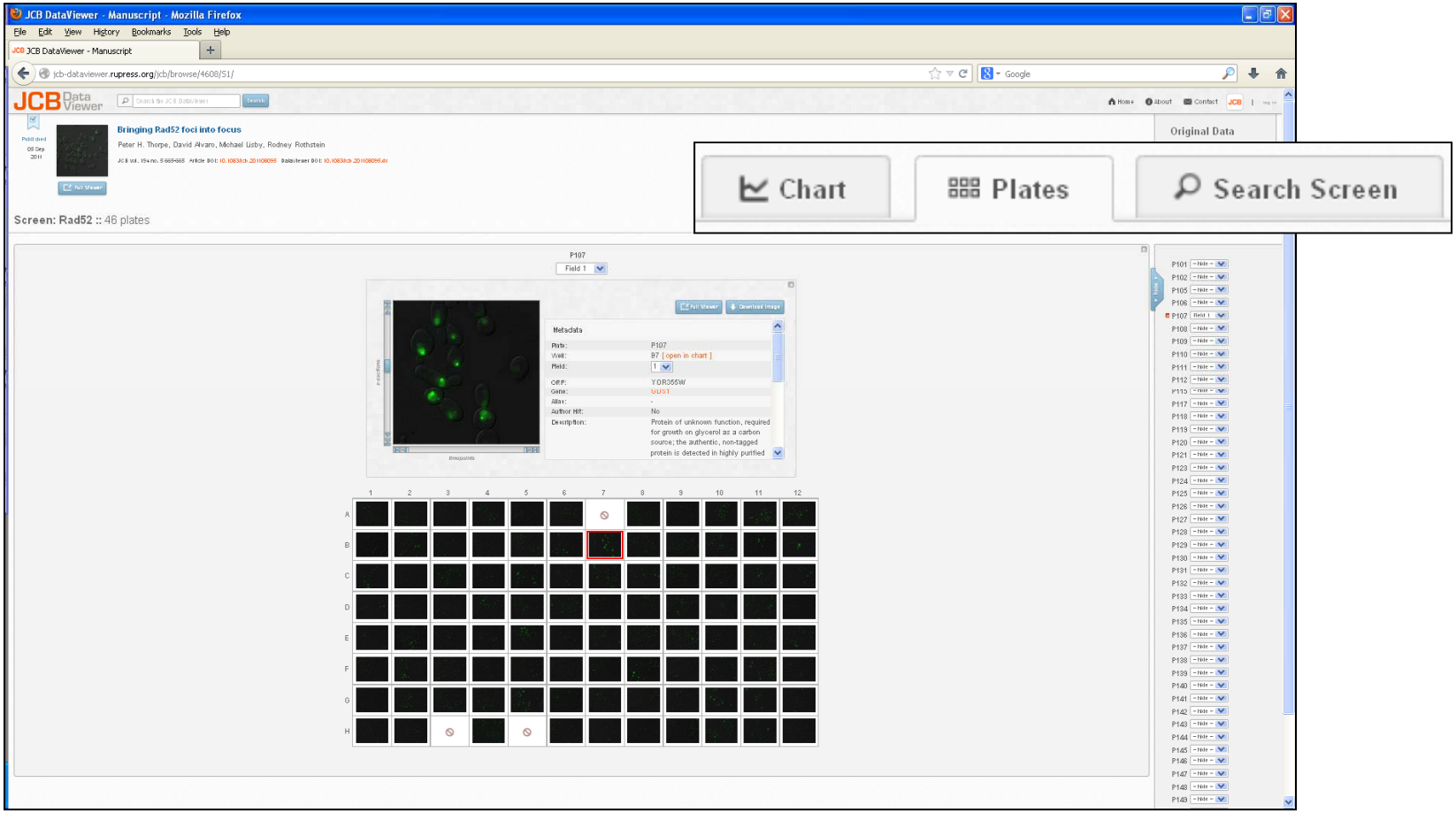
HCS Data – Plate View



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Thorpe et al (2011)

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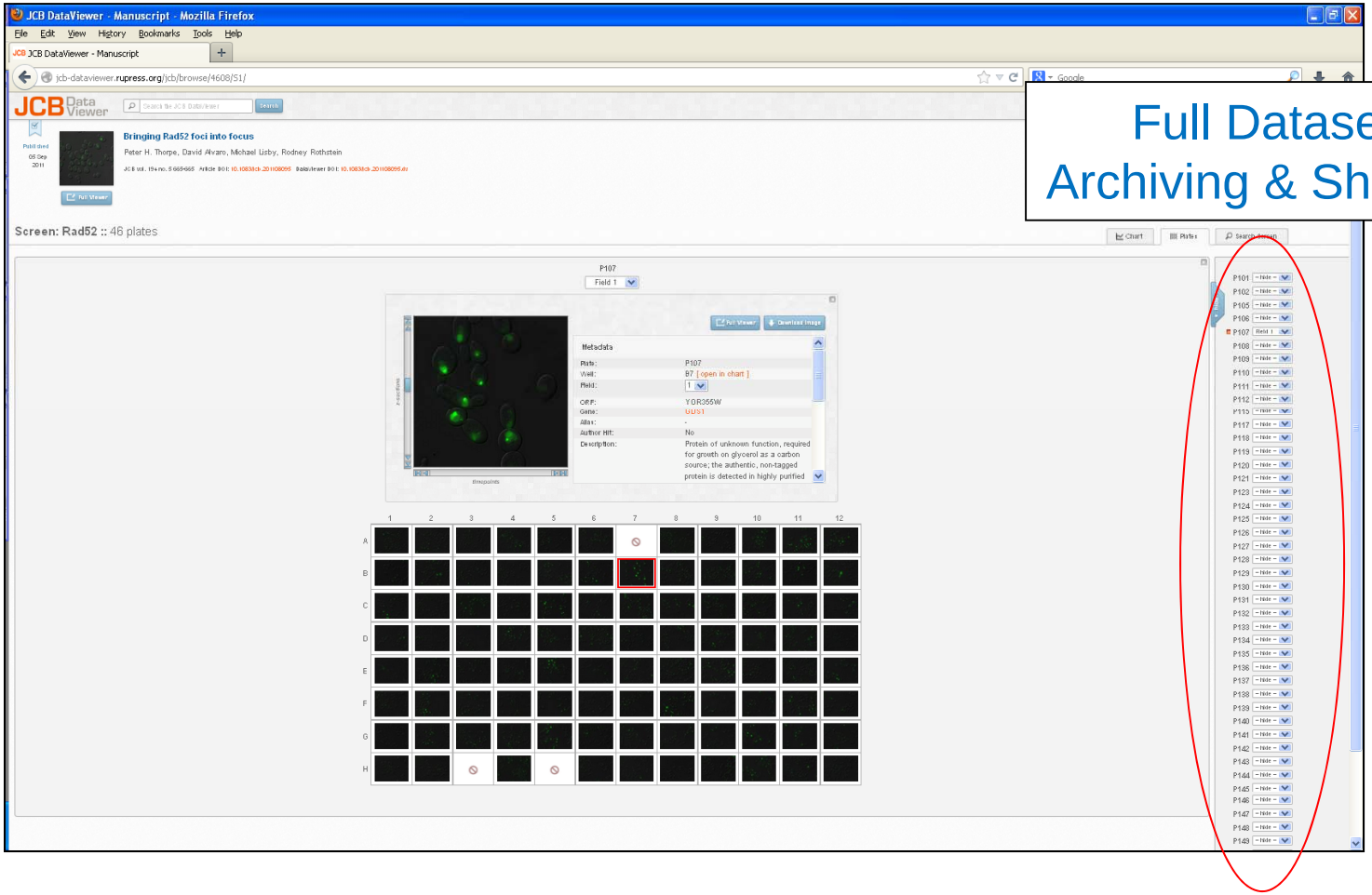
HCS Data – Plate View



Full Dataset Archiving & Sharing

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Thorpe et al (2011)

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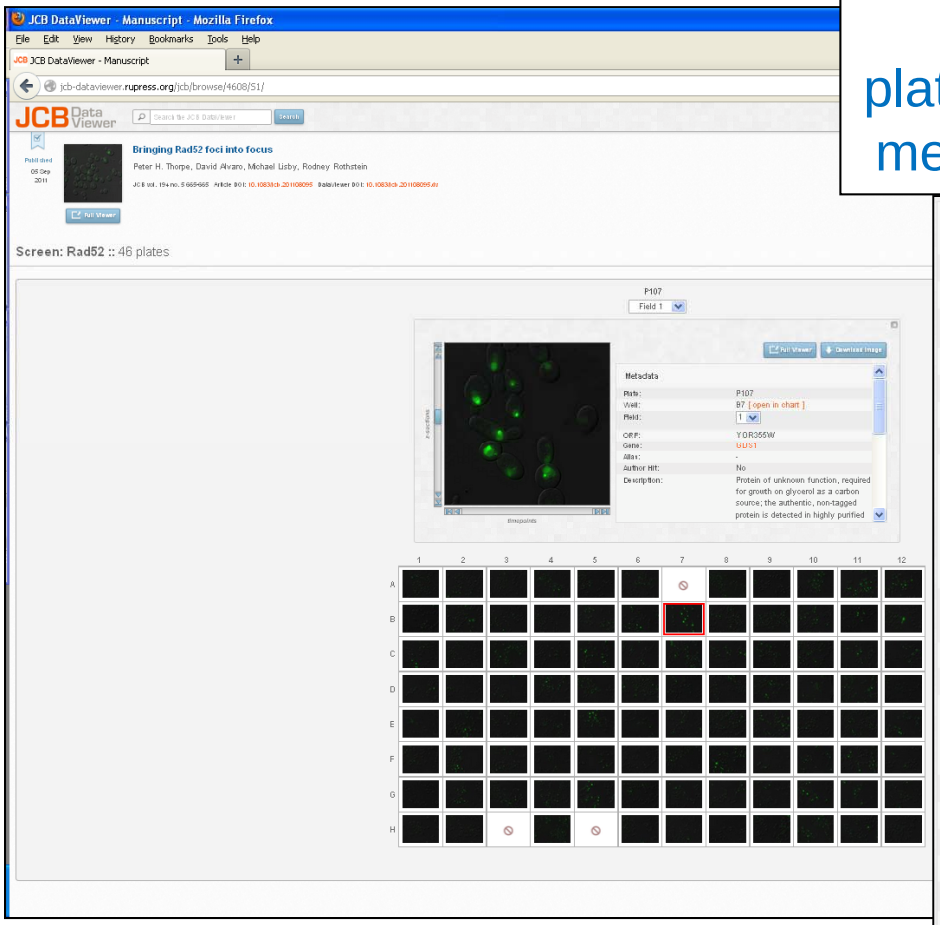
HCS Data – Plate View



Maneuverability between plate arrays, single 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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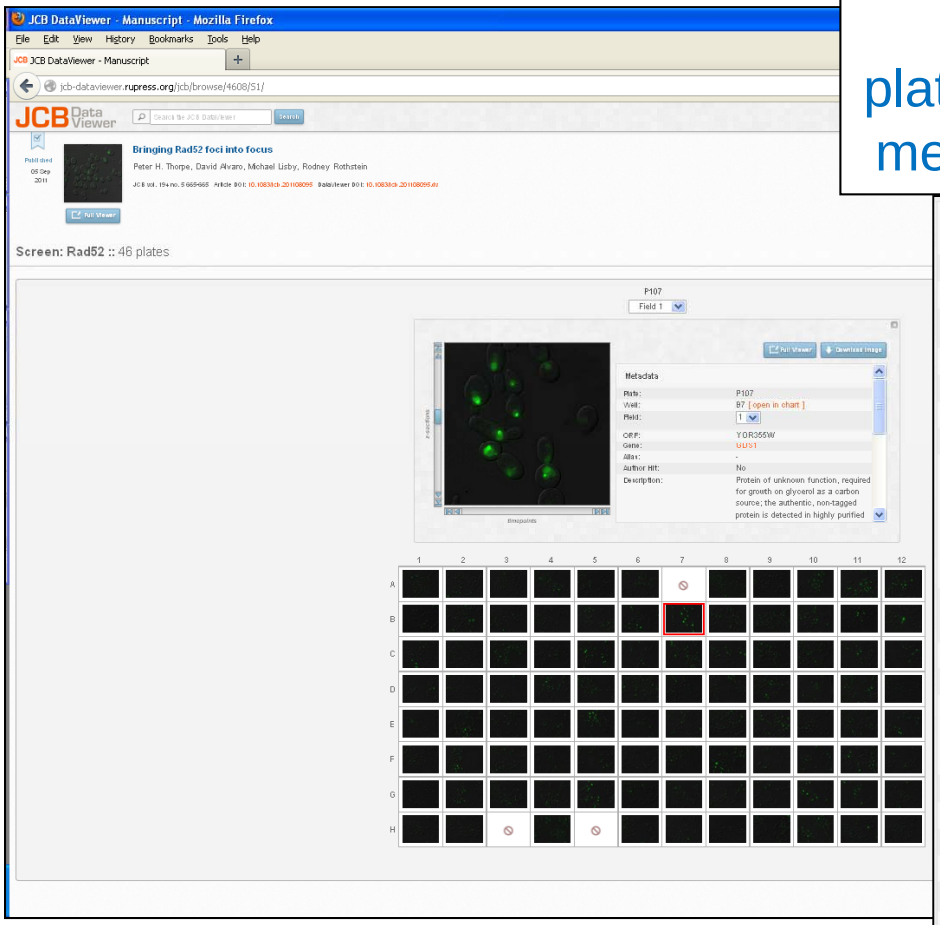
HCS Data – Plate View

Maneuverability between plate arrays, single image data, metadata, and phenotypic data



Metadata

Plate:	
Well:	[open in chart]
Field:	
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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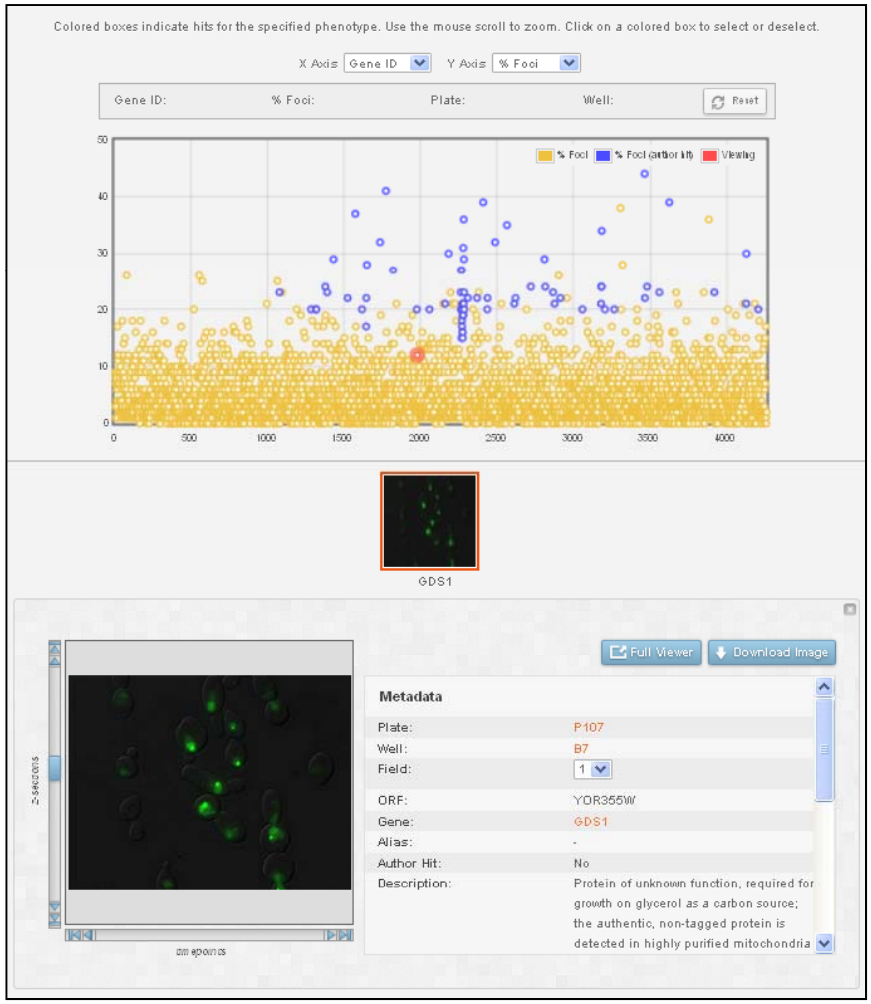
HCS Data – Chart View (quantitative)



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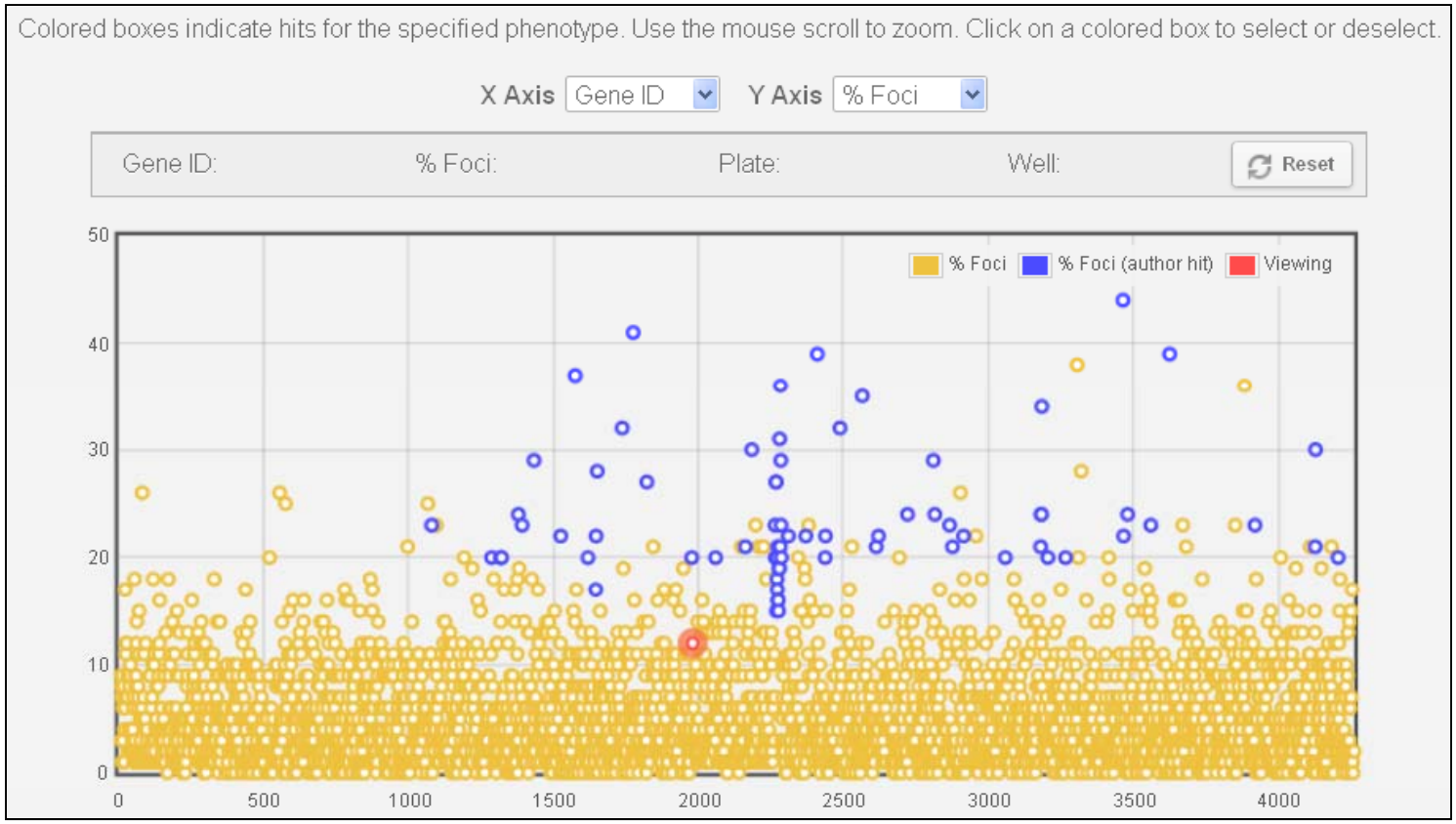
HCS Data – Chart View (quantitative)



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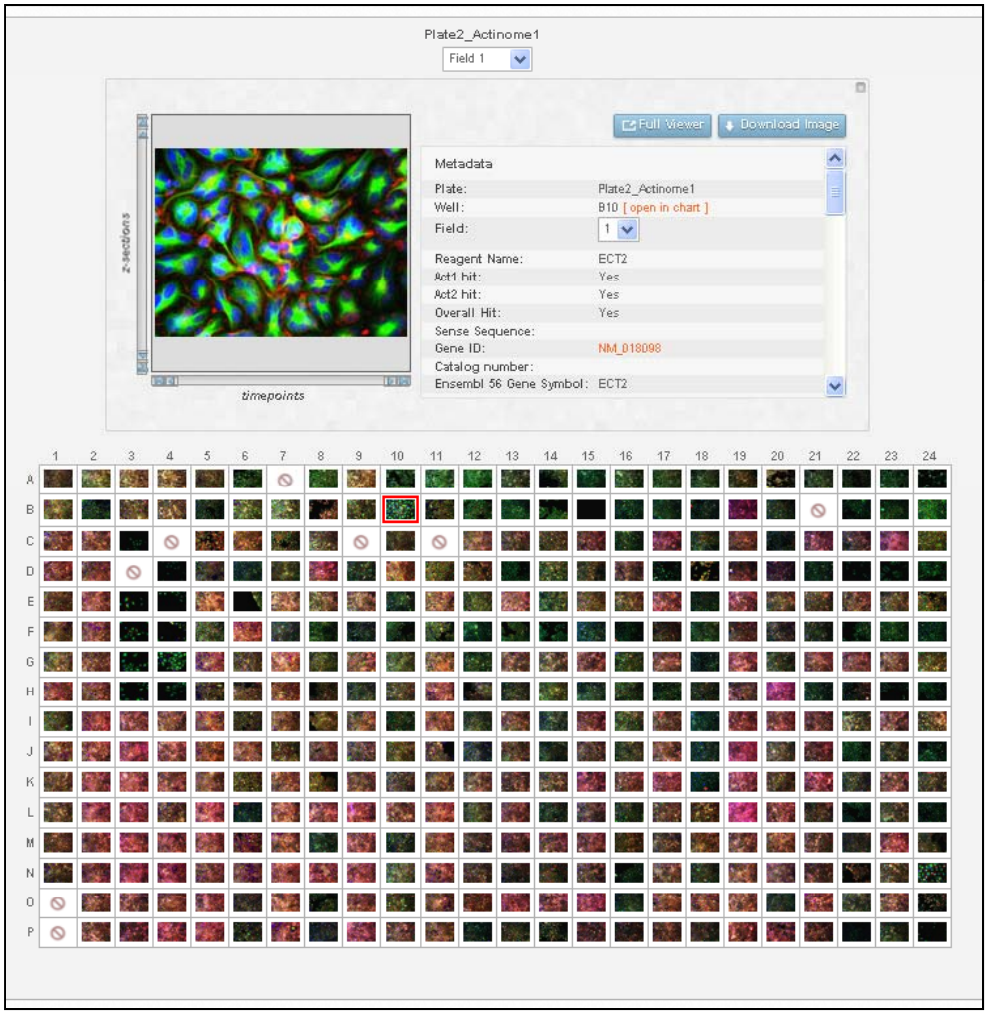
HCS Data – Plate View



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Rohn et al (2011)

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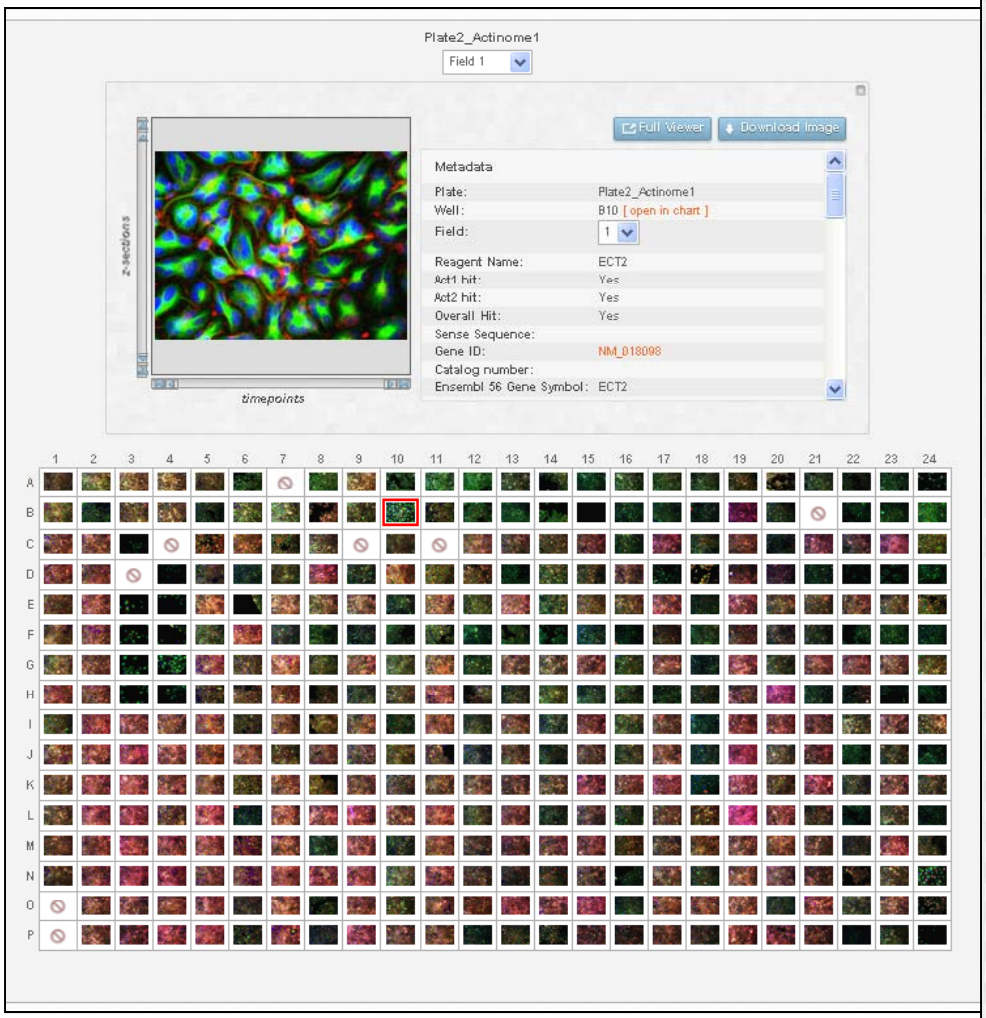

Metadata

Plate:	Plate2_Actinome1
Well:	B10 [open in chart]
Field:	2
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 elongated:	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

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Rohn et al (2011)

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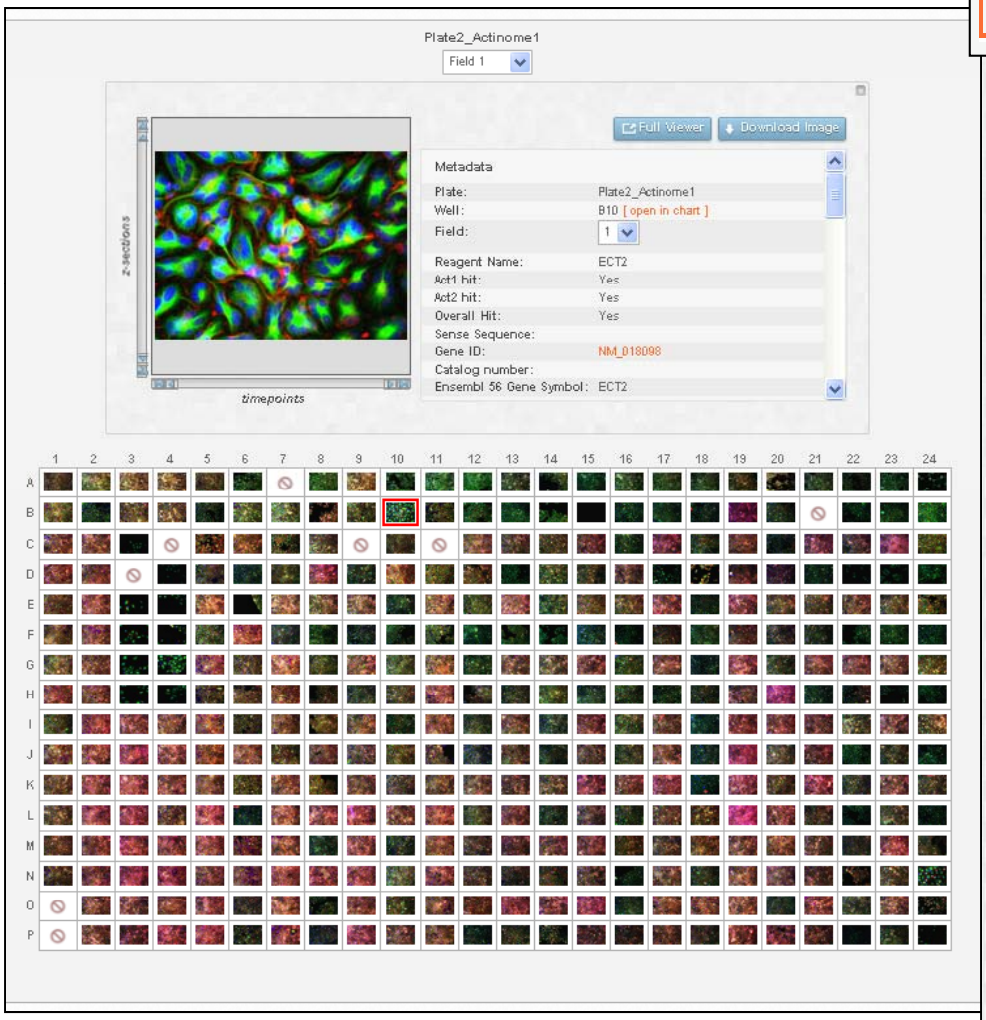
HCS Data – Plate View



[open in chart]

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 elongated:	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

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Rohn et al (2011)

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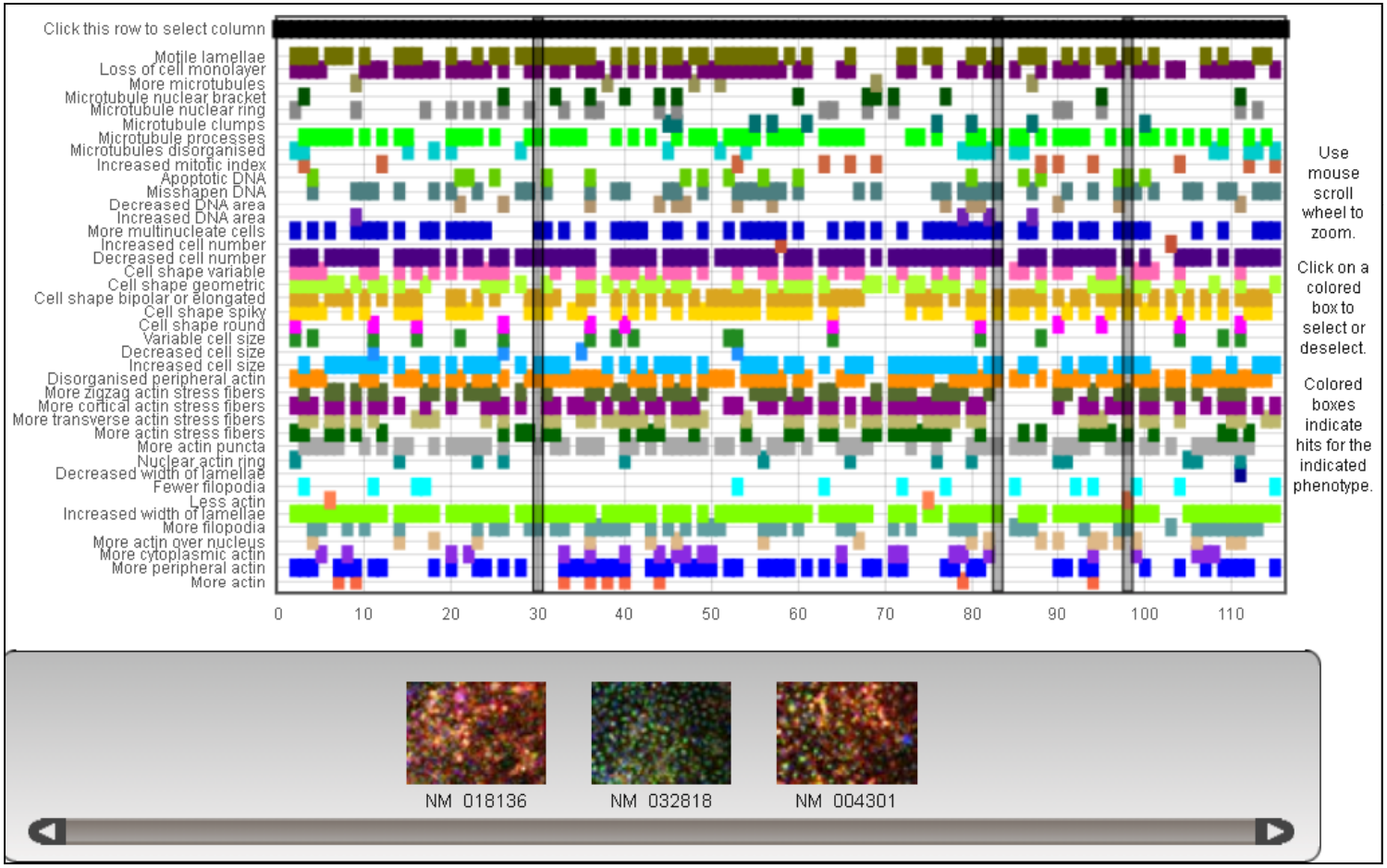

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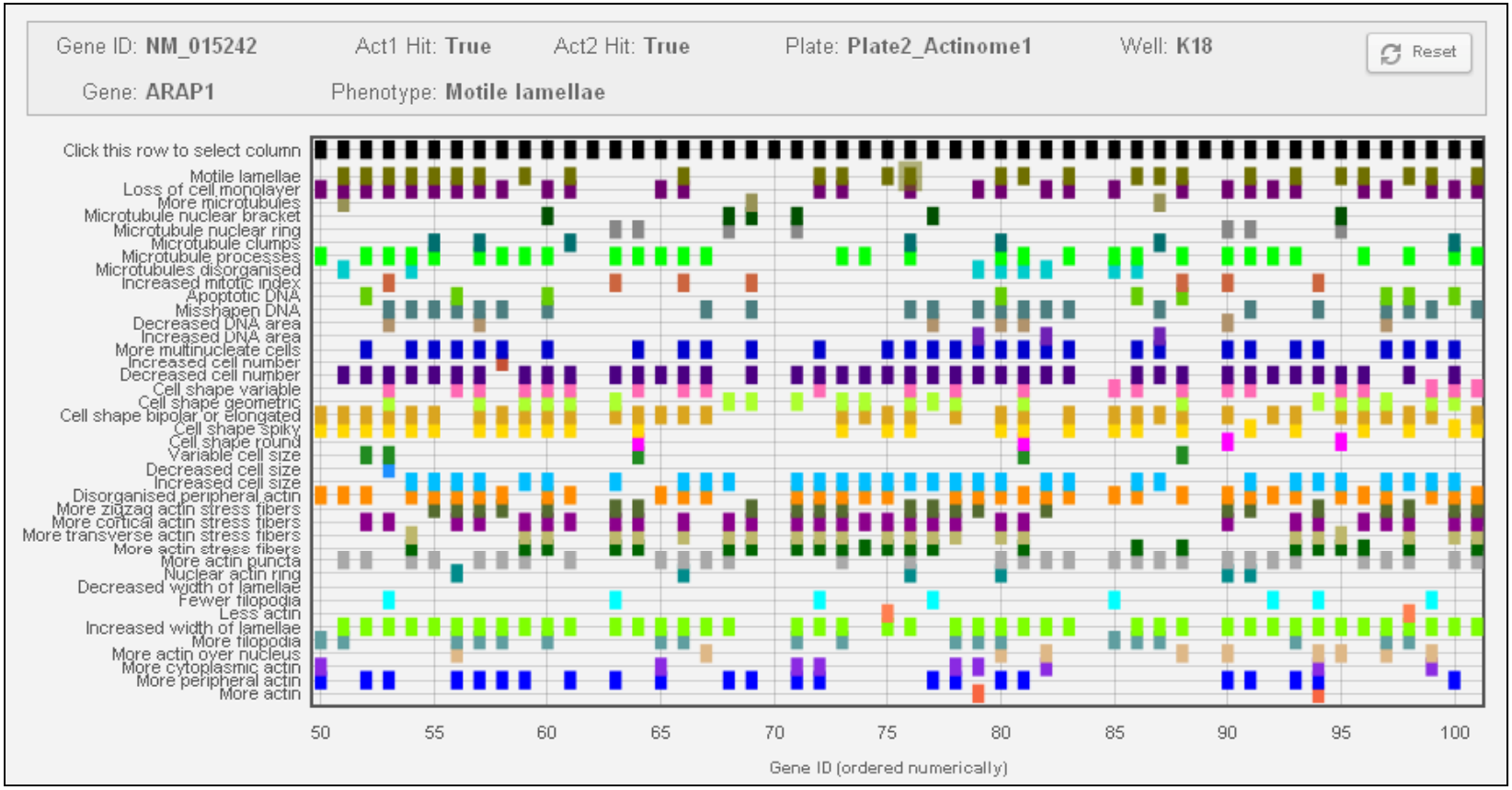

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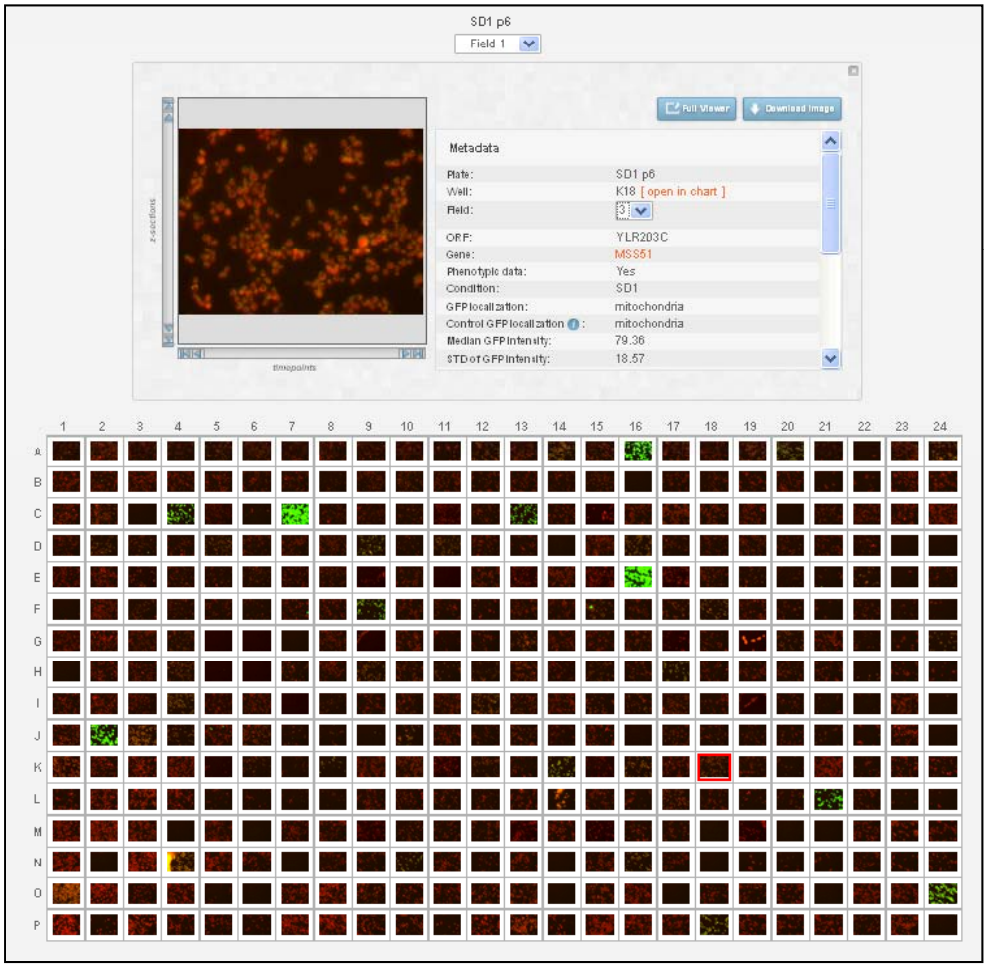

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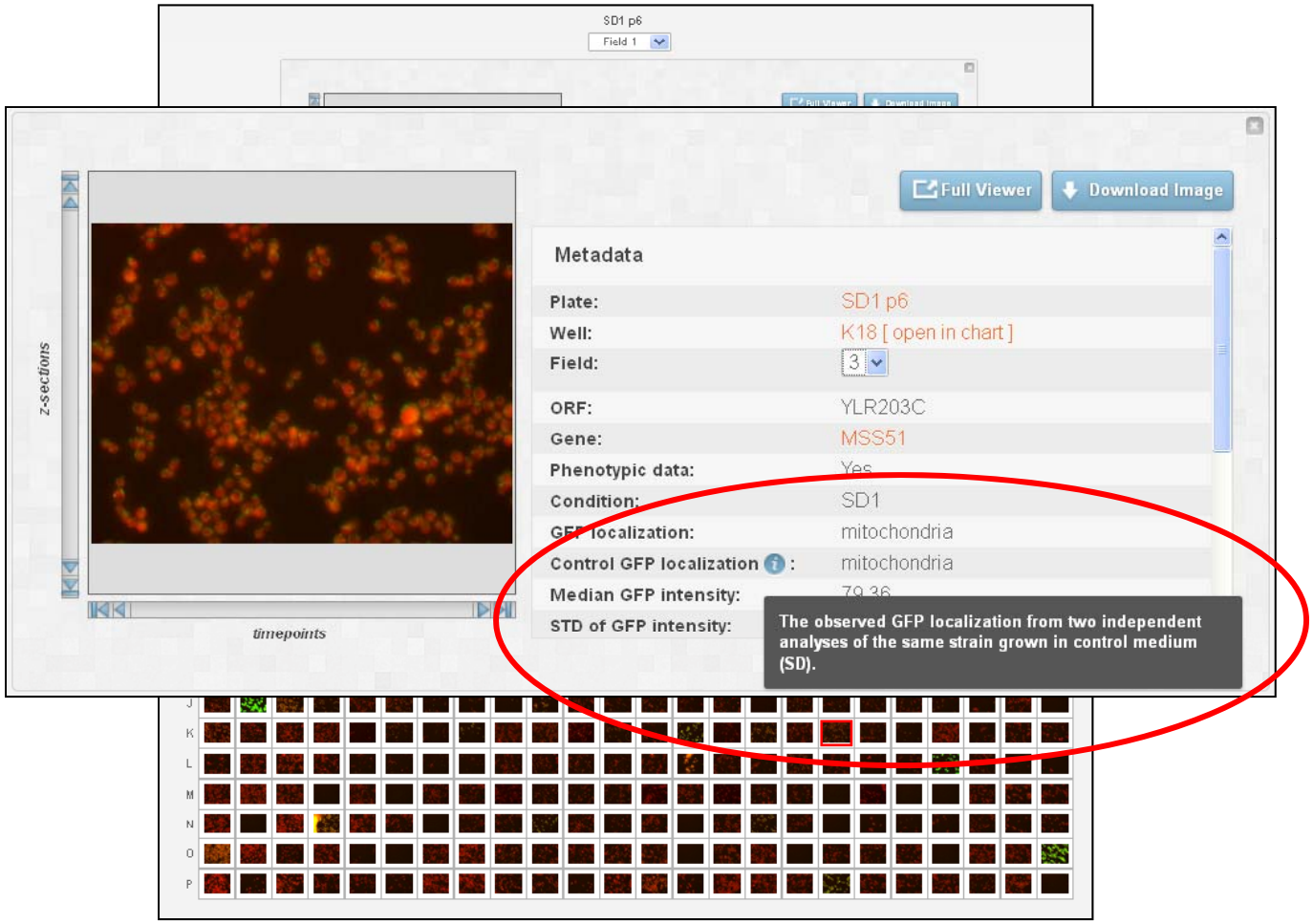

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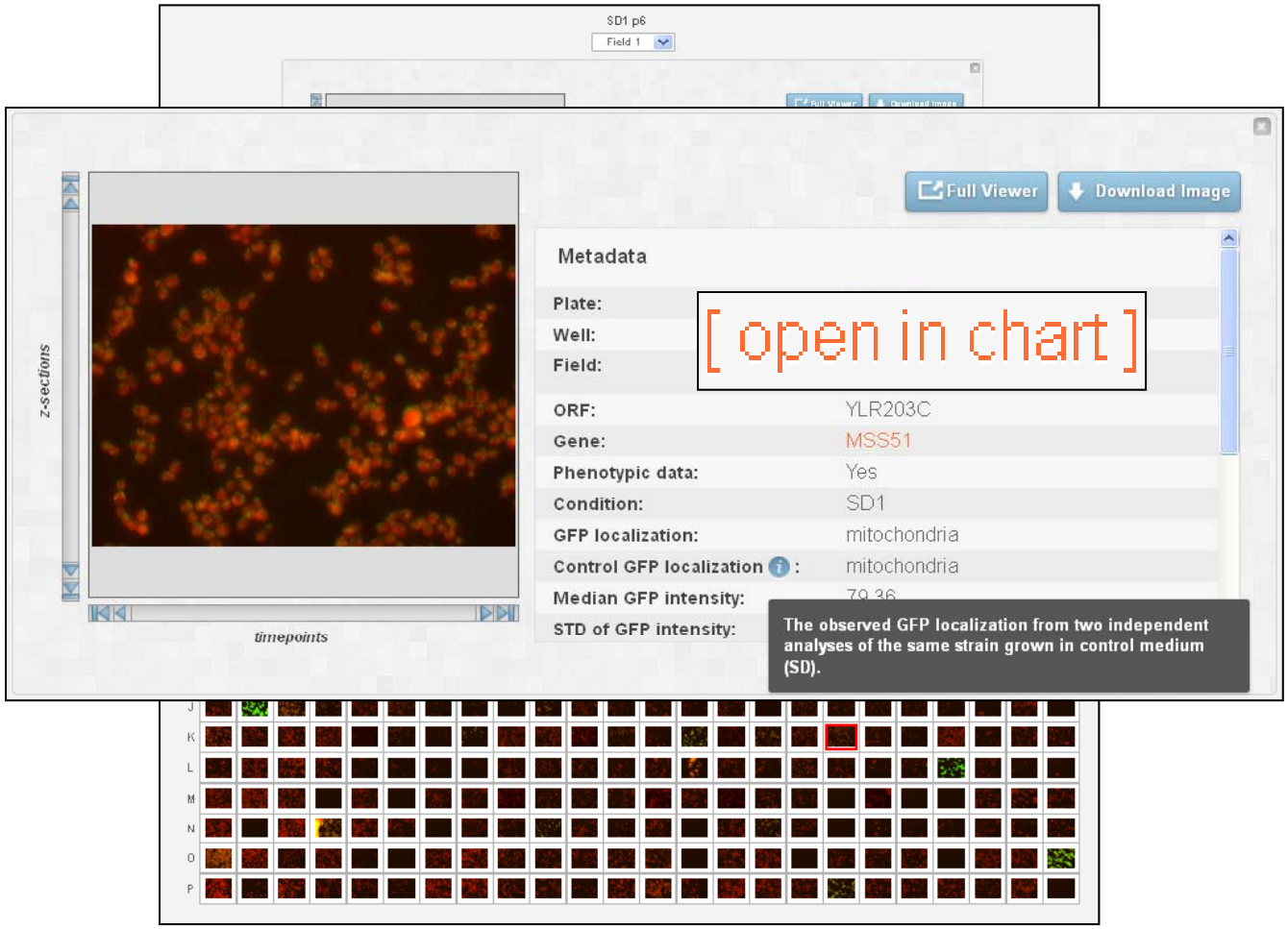

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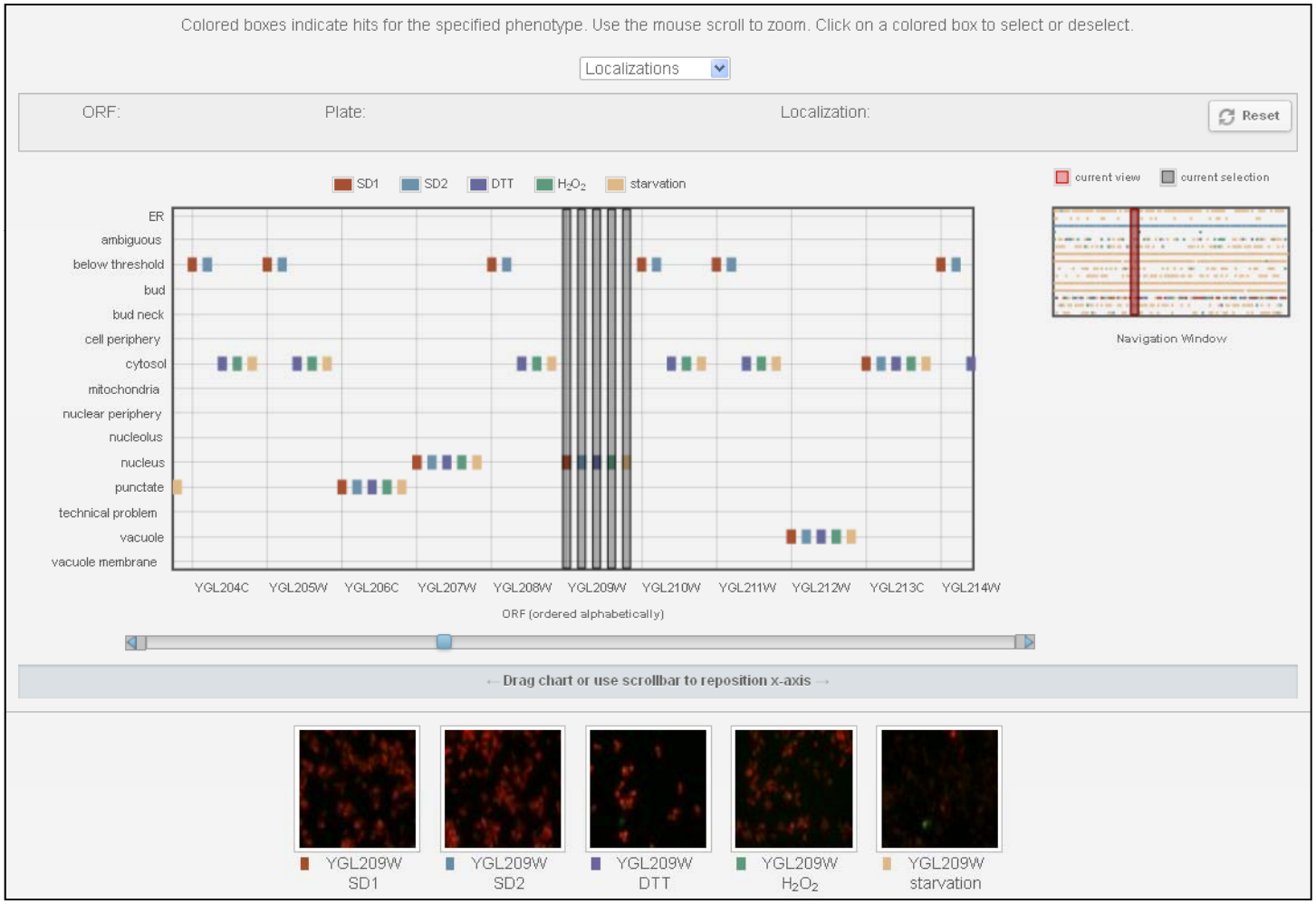

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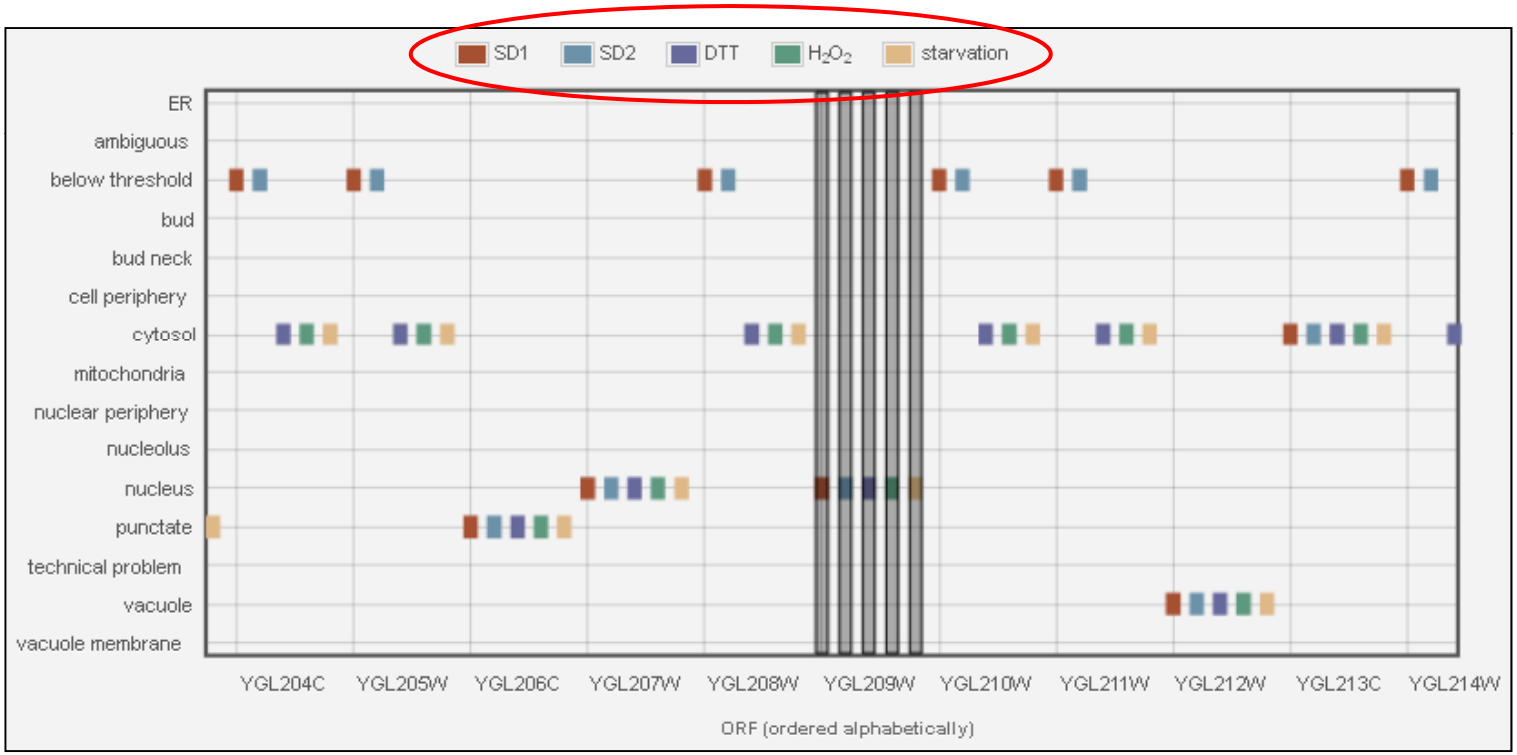

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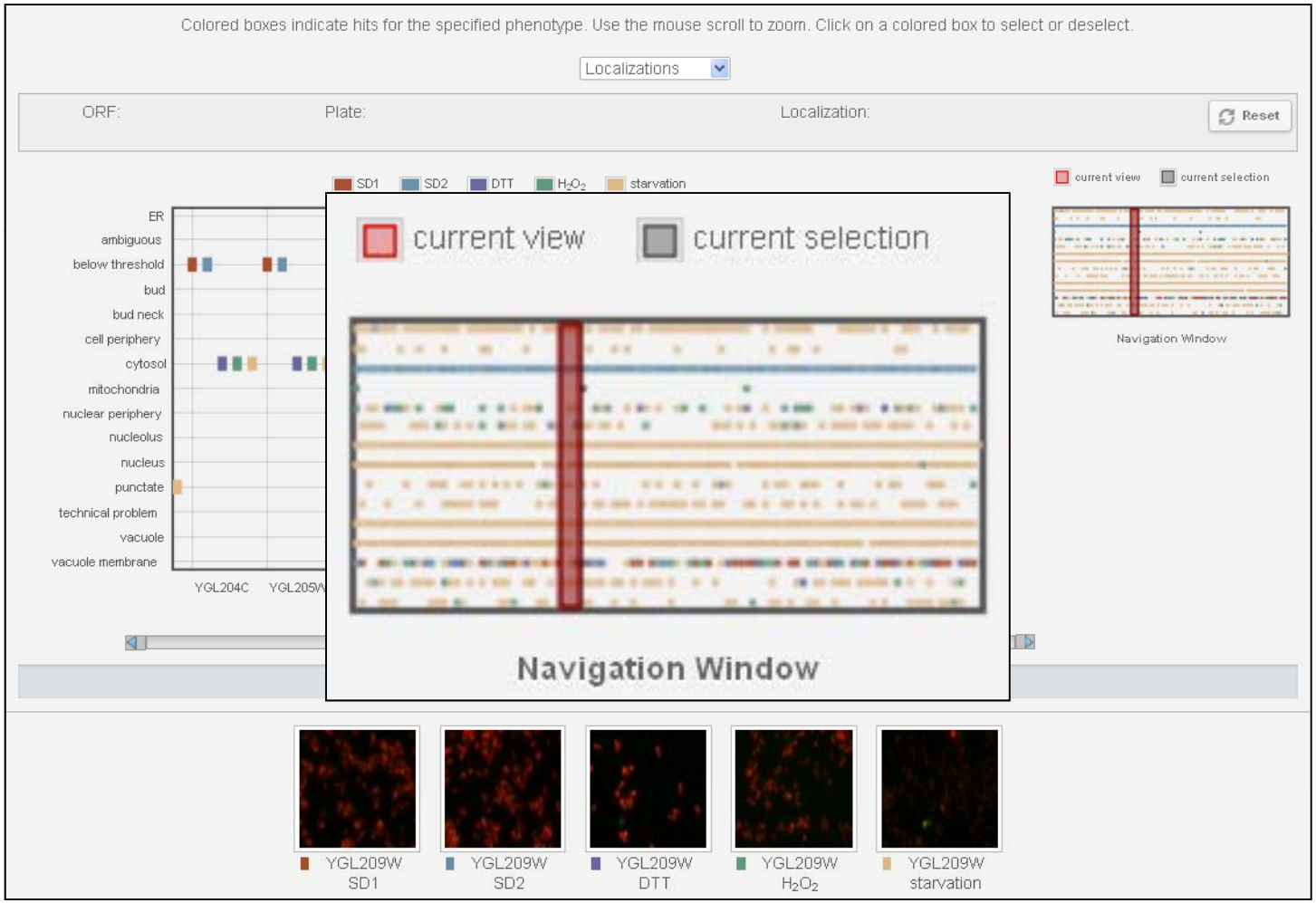

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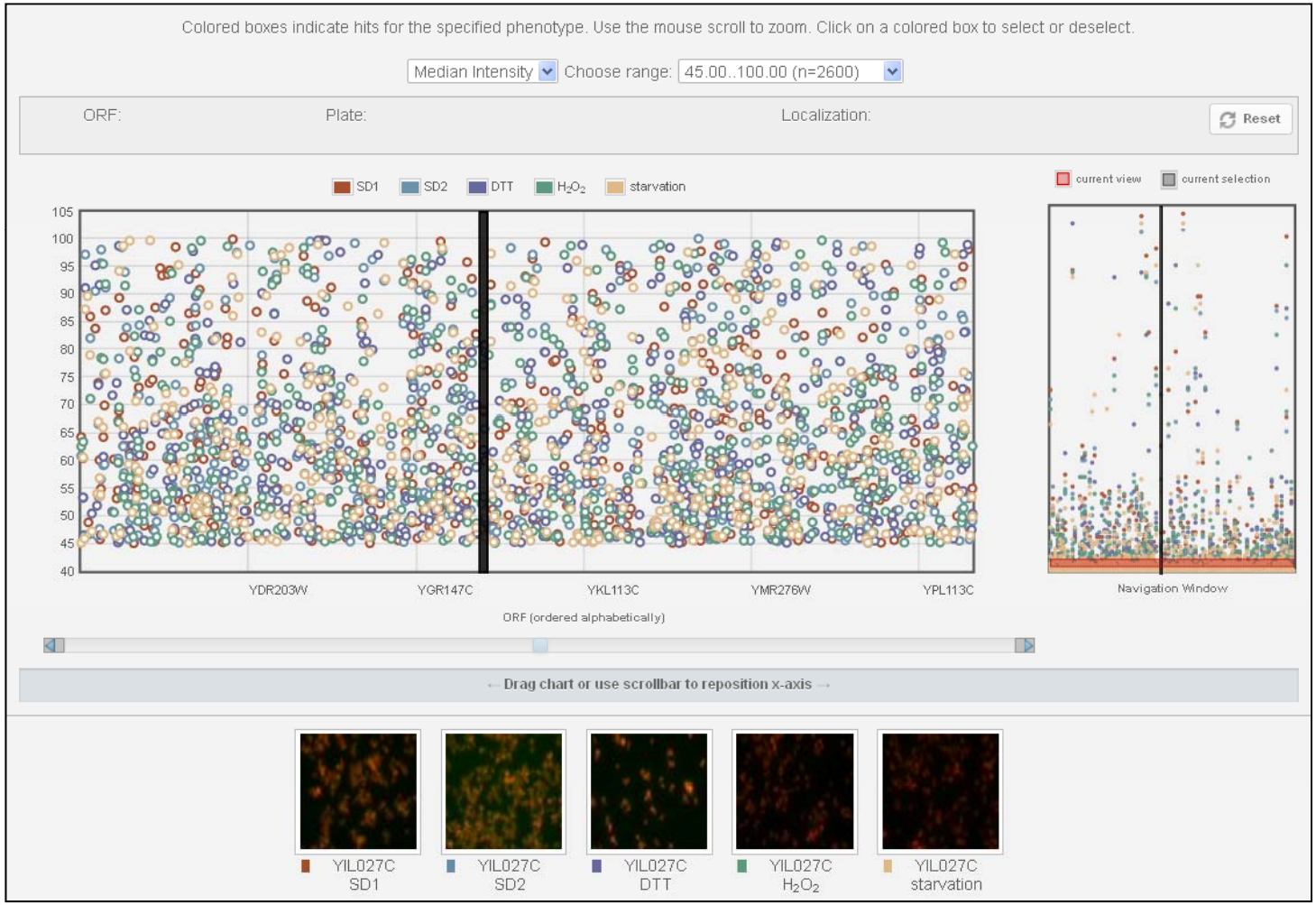

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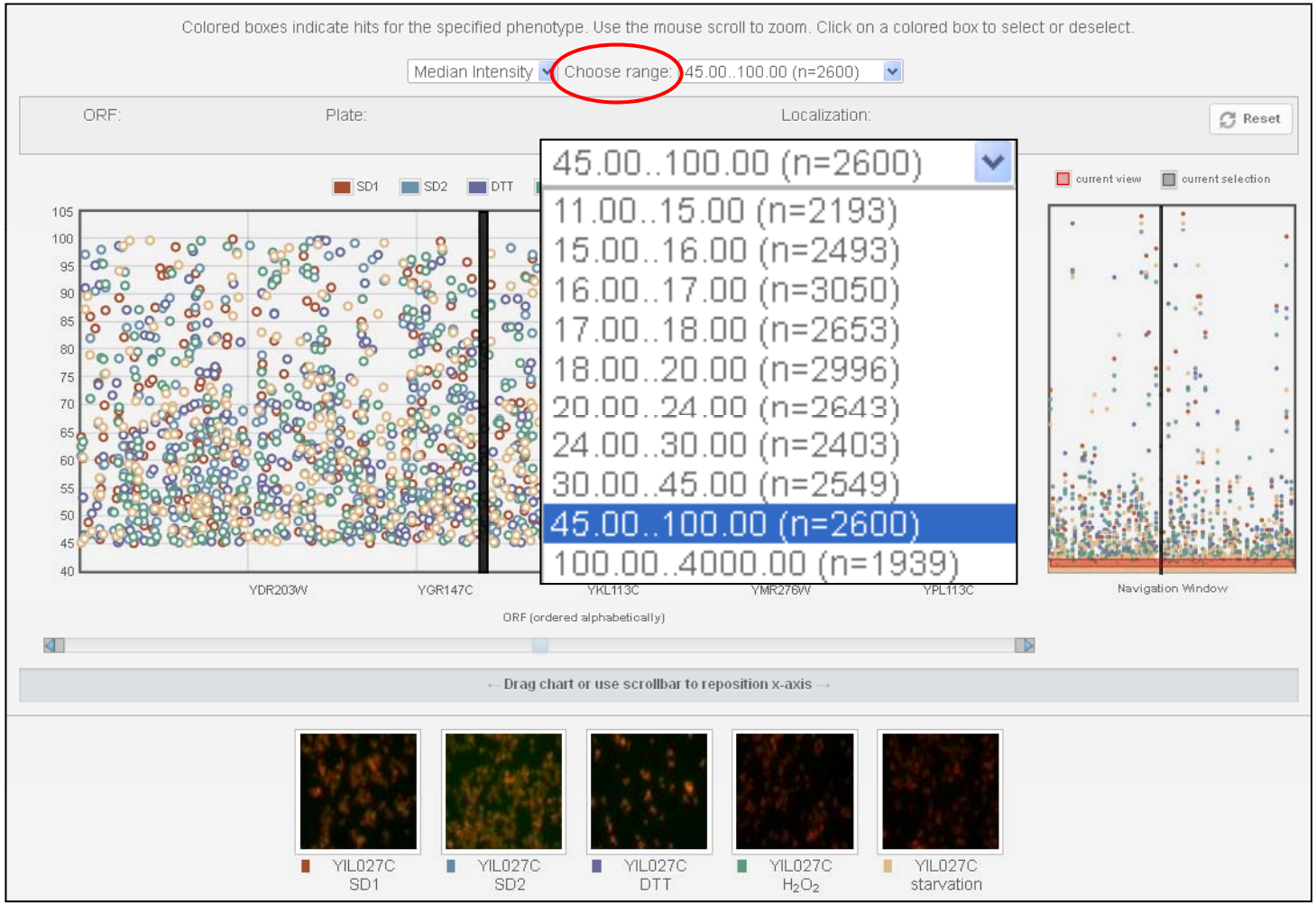

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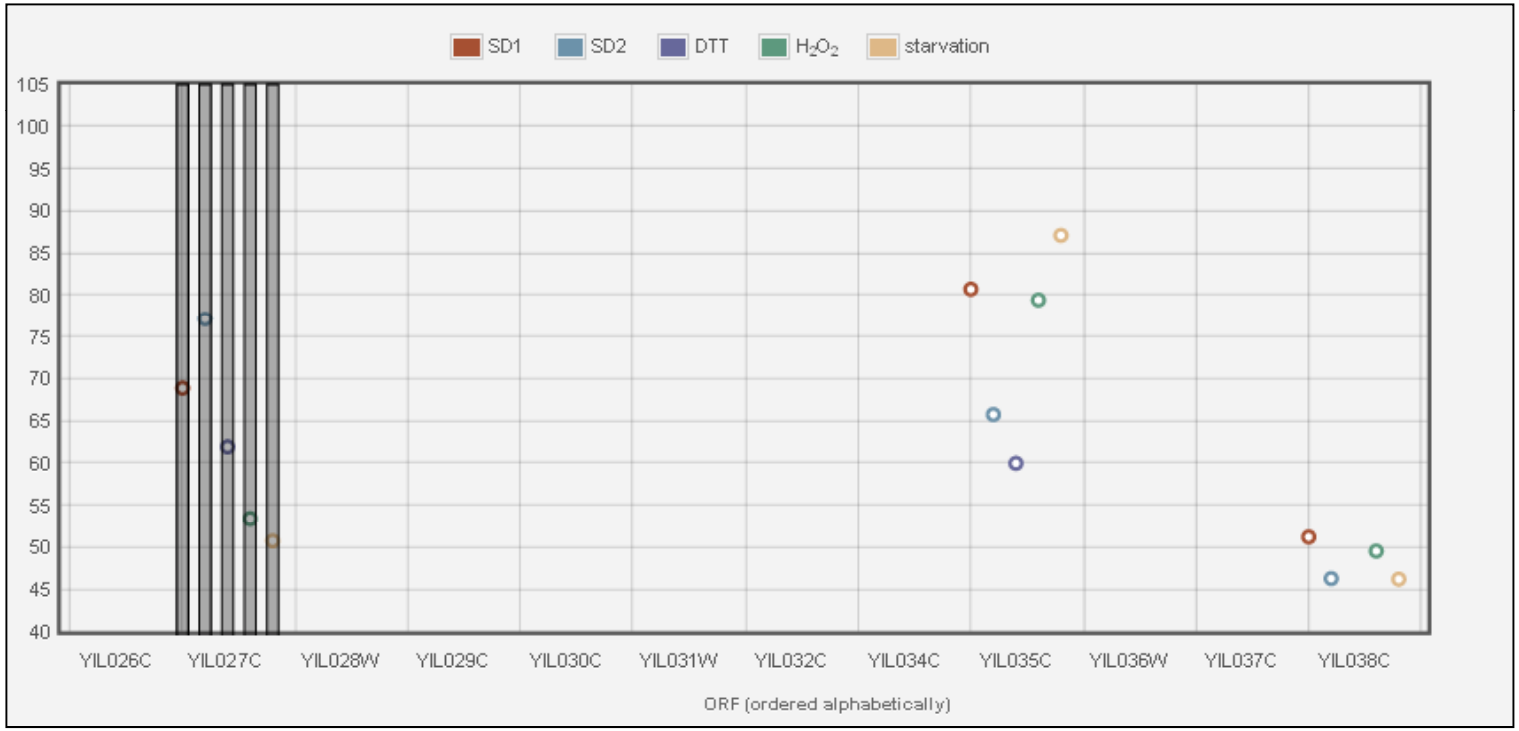

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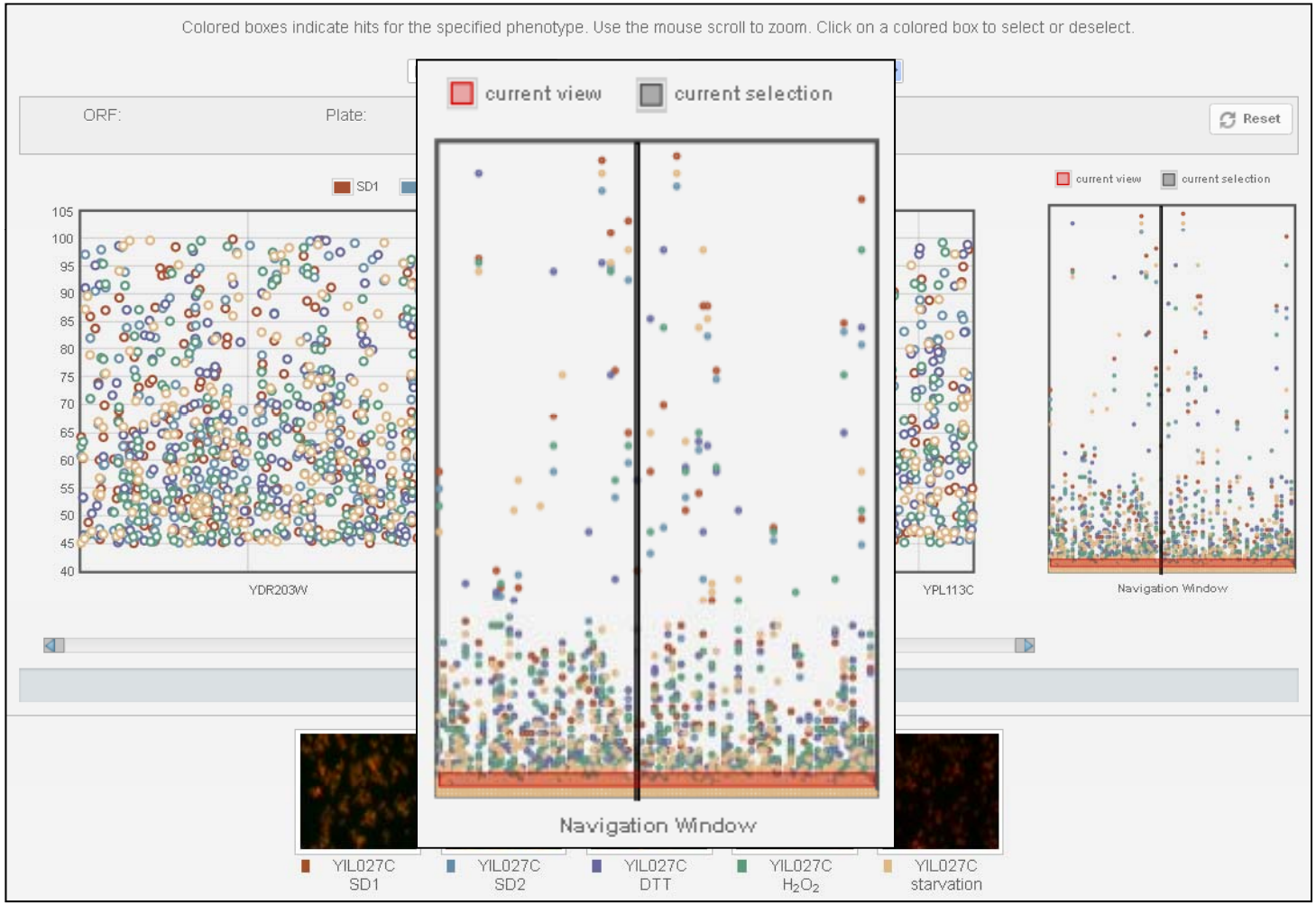

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Summary of Features

- Browser-based viewing of original, raw image files in >75 PFFs
  (from single images to massively tiled images to plate-arrayed HCS datasets)
- Image data-metadata linking
- Tools for single image analysis
- Tools for complex HCS dataset visualization and analysis
- Tools to enhance image data-published data maneuverability
  (e.g., direct image links, citation information and links, data DOIs)
- Raw image downloading in OME-TIFF format
- Database-wide search functionality.

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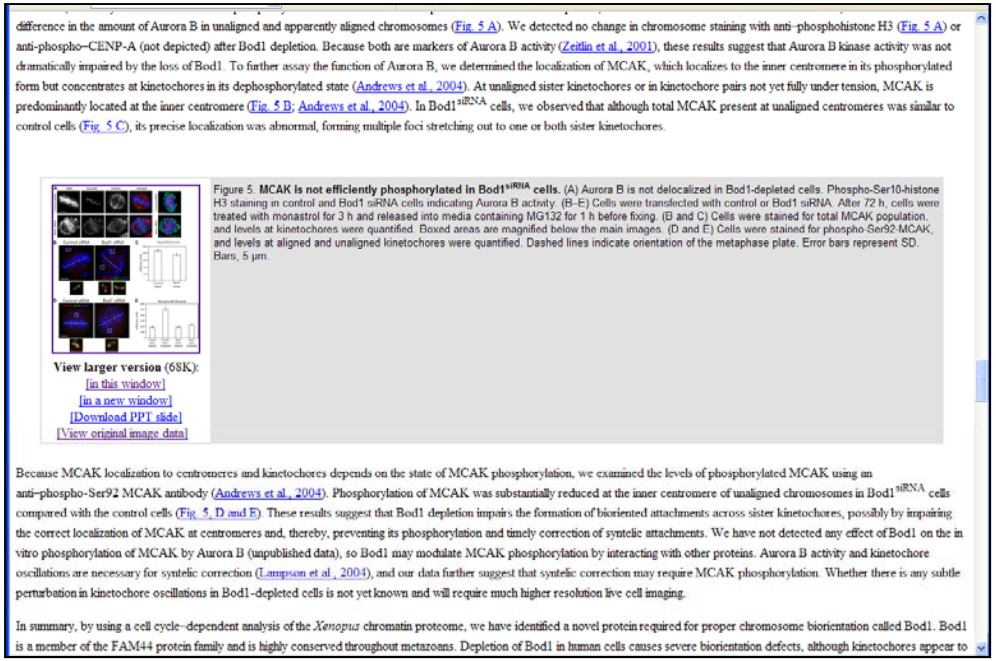

difference in the amount of Aurora B in unaligned and apparently aligned chromosomes (Fig. 5 A). We detected no change in chromosome staining with anti–phosphohistone H3 (Fig. 5 A) or anti–phospho–CENP-A (not depicted) after Bod1 depletion. Because both are markers of Aurora B activity (Zeitlin et al., 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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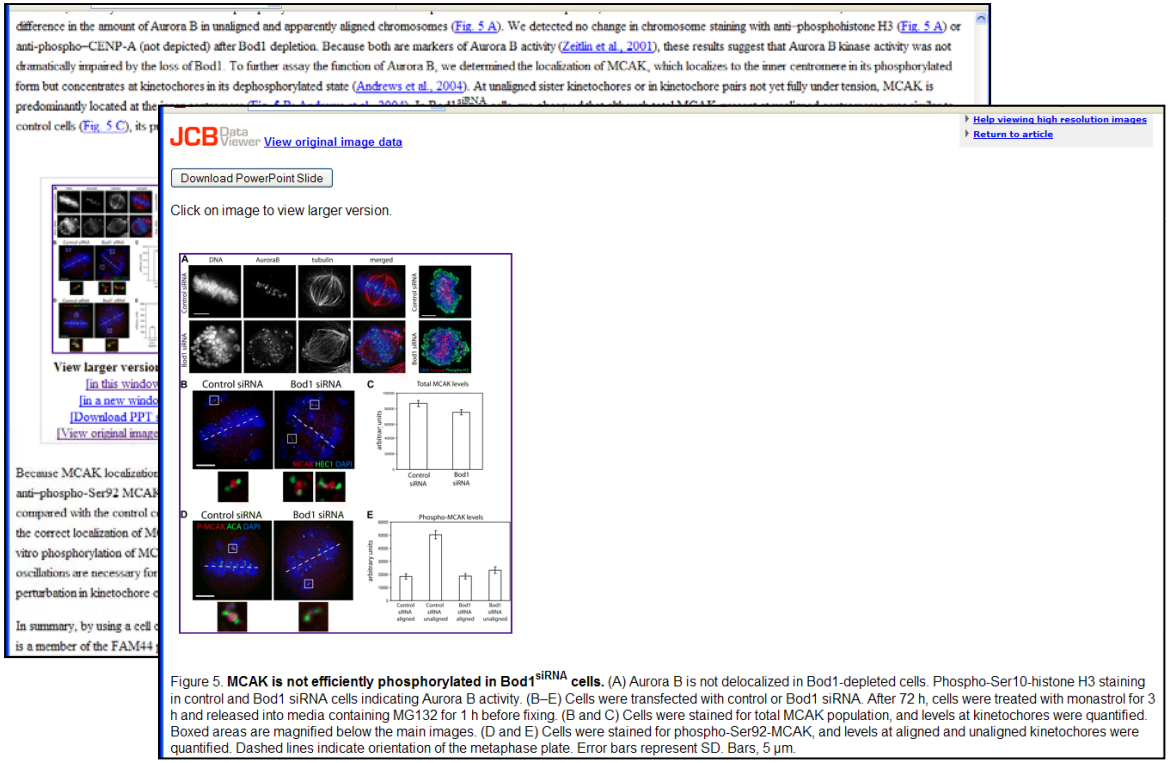

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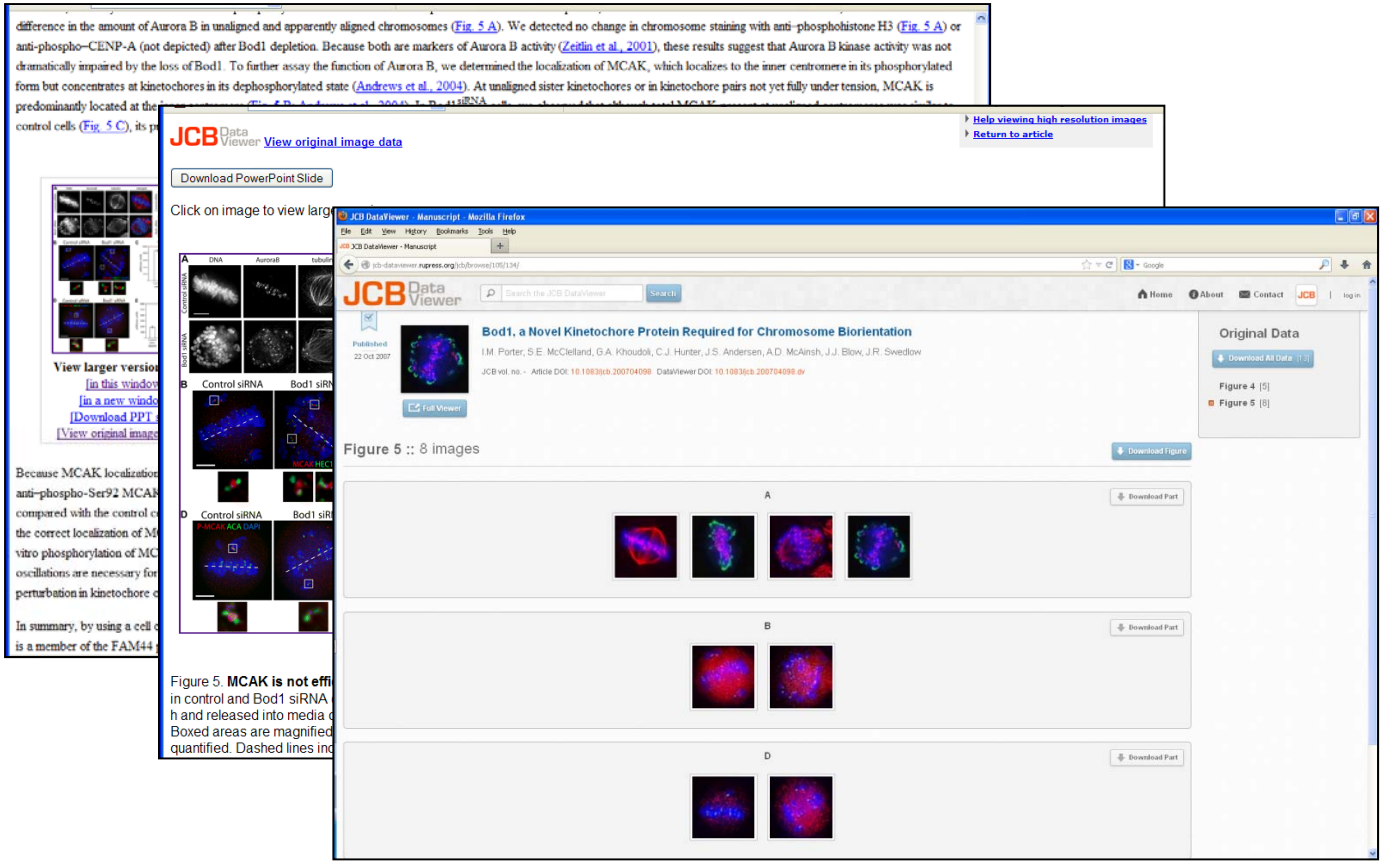

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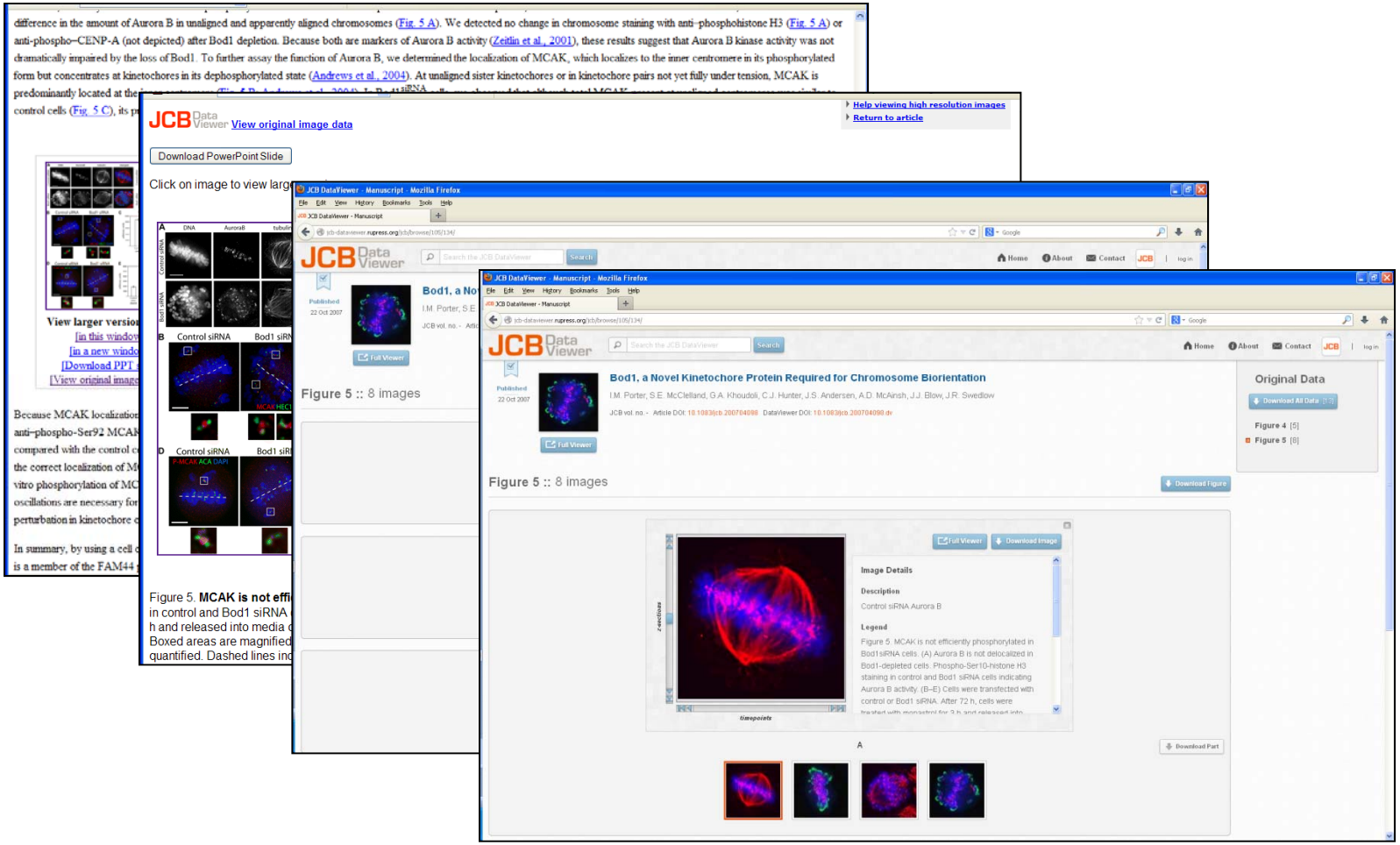

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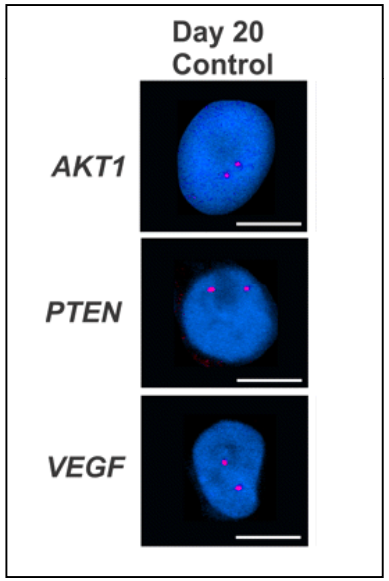

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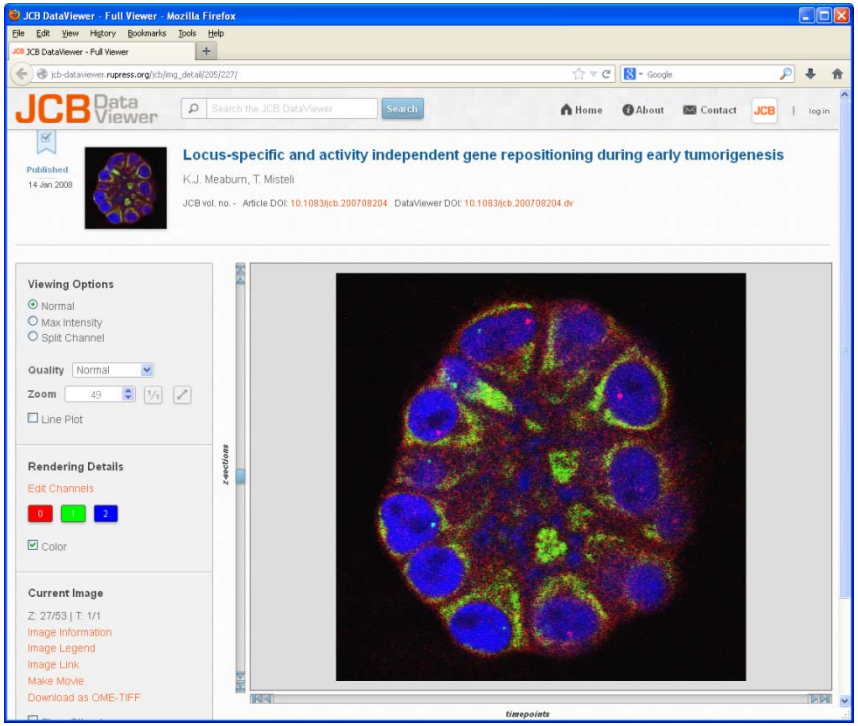

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2.03 TB of image data (published and unpublished)

→313 published manuscripts

→ 1164 published figures

→ 204,935 published images

→ 1,309,621 individual image frames

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Where do we go from here?

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

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

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