

- ✓ BiCell Scientific has designed an algorithm to predict the antigenic peptide antigen.
- ✓ BiCell Scientific antibodies are raised against short fragments of peptide (13-19 amino acid long). Short antigen sequences allow antibodies to be used for mutation or modification specific recognition.
- ✓ BiCell Scientific has developed a “smart” selection approach to identify antibody-secreting hybridoma cells based upon antigen specific binding to make monoclonal antibodies.

THE LARGER ISSUE:

Site specific changes in protein vital for diseases are hard to detect with existing methods.

- 1 Point mutations in proteins, esp. cancer proteins or Alzheimer’s proteins underlies disease progression
- 2 Point modifications in proteins, such as phosphorylation, acetylation, etc were overlooked in disease diagnostics due to lack of reliable antibodies

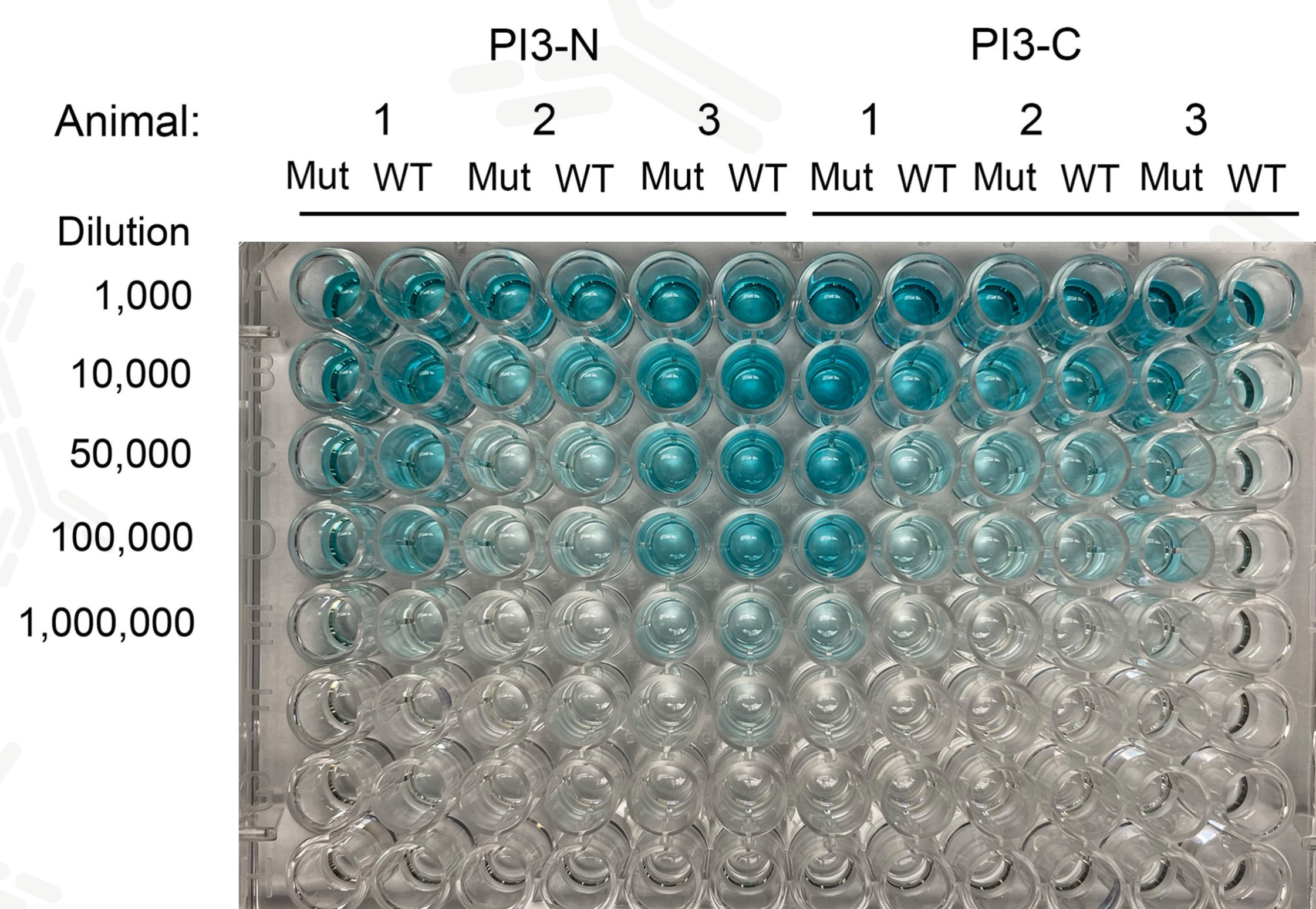
OUR SOLUTION:

“Develop point specific recognition antibodies with novel short-peptide antigen technique and hybridoma sorting technique.”

Our Approach & Its Demonstration

Objective: Developing PIK3CA-H1047R cancer mutation specific antibody

Step 1: Polyclonal antibody development based upon depletion chromatography



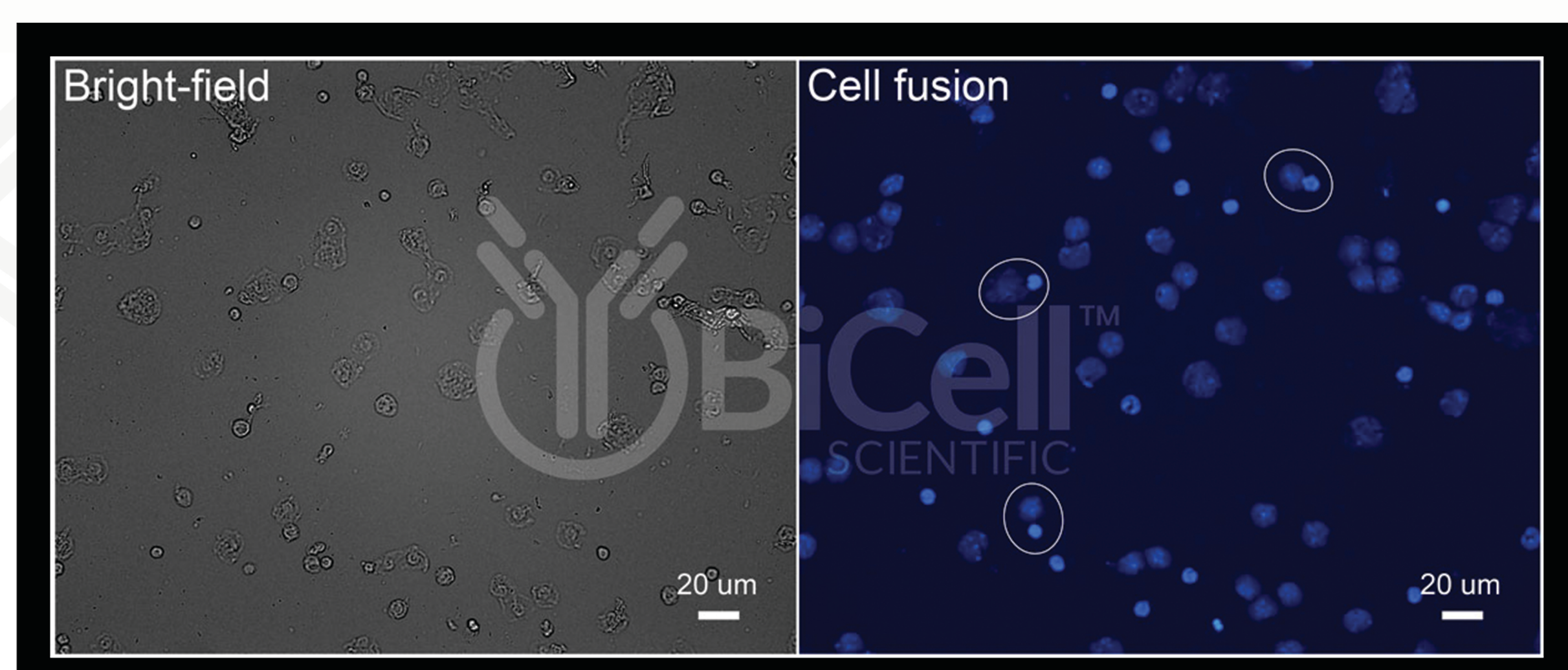
APPROACH:

- 1 Two mutant peptides (N and C, both harboring the H1047R mutation) were injected and ELISA was performed with antiserum against mutant or wildtype (homologous sequence with no mutation) peptide side by side.
- 2 Both peptides generate ELISA titer >100,000 in at least one animal within each experimental group.
- 3 PI3-N peptide fails to show difference between Mutant peptide and wildtype peptide.
- 4 PI3-C peptide showed pronounced difference between Mutant peptide and WT peptide (Animal #1 and #3).

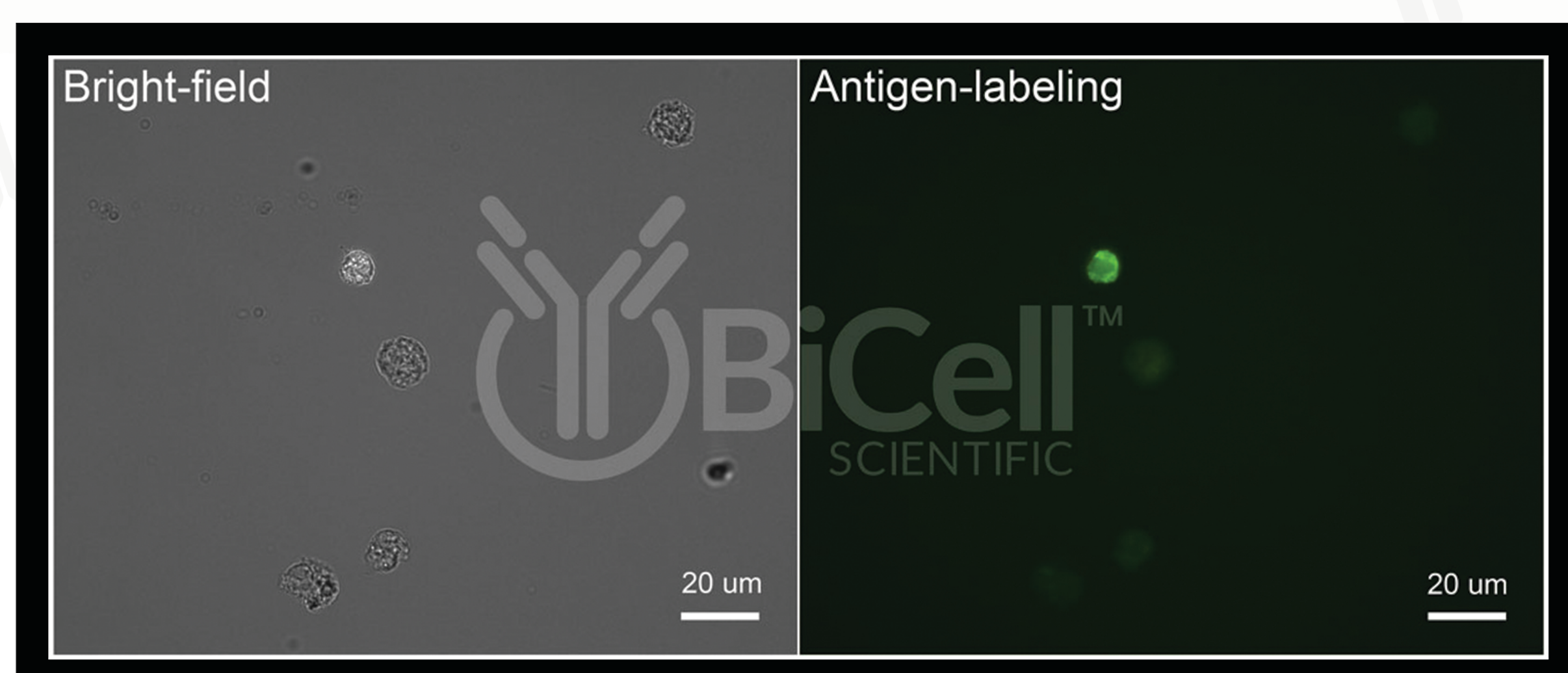
Step 2: Monoclonal antibody development based upon hybridoma cell sorting

APPROACH:

- 1 PI3C-Animal #1 splenocytes were fused with myeloma cells.
- 2 The resultant hybridoma cells were labeled with wildtype peptide and depleted with magnetic bead sorting.
- 3 The depleted hybridoma cells were labeled with mutant peptide and selected with magnetic bead sorting.



Electrofusion efficiently fuses myeloma cells with splenocytes. Circle indicates fusion event. Myeloma cells: large nuclei; splenocytes: small nuclei.



Antigen labeling of hybridoma cells. Hybridoma cells are transiently crosslinked to immobilize antibodies on the cell surface. FITC-labeled antigen binds to and decorates the hybridoma cell that secretes the correct antibody.