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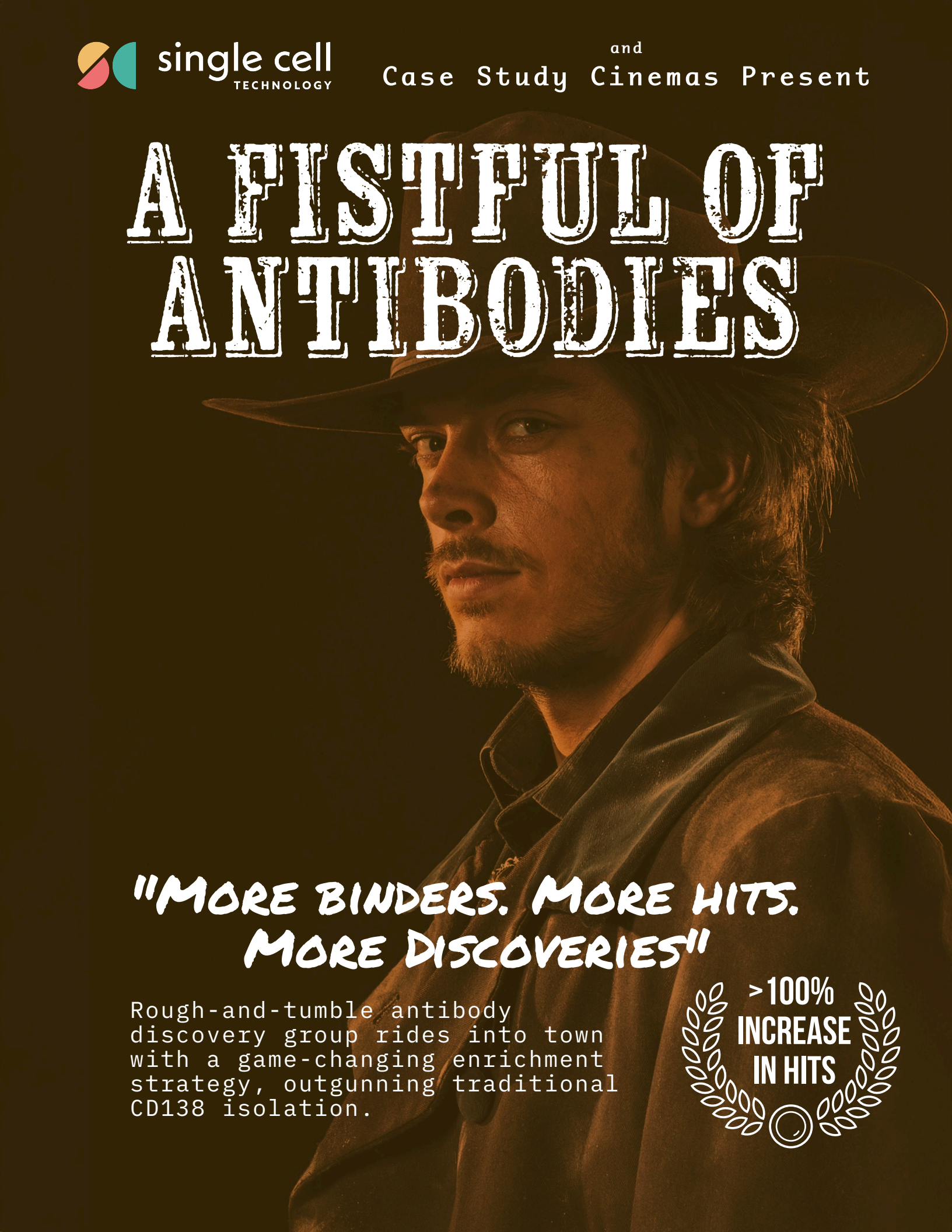
and

Case Study Cinemas Present

A FISTFUL OF ANTIBODIES



"More binders. More hits. More discoveries"

Rough-and-tumble antibody discovery group rides into town with a game-changing enrichment strategy, outgunning traditional CD138 isolation.

>100% INCREASE IN HITS

single cell TECHNOLOGY

A Fistful of Antibodies:
Innovative Cell Isolation to Drive More Hits

The Problem

Certain targets in antibody discovery present challenges because they produce few hits. This can be due to several reasons: immunogenicity of the target, immunomodulatory effects of the target during immunization, the immunization conditions used, and/or the discovery methods used. Even still, some programs demand differentiated activity for their antibody drugs, making it diffcult to discover the target profile. Exploring a large set of hits helps start a campaign with the most options.

The Solution

CD138 is a marker for plasma cells, and commercial CD138+ cell isolation kits work well to isolate antibody-secreting cells (ASCs) from mouse. The expression profile of CD138 in immunized mouse lymphocytes consistently shows a large negative cell population, a low-mid positive shoulder, and a positive population with higher SSC.

We used a microfluidic-based cell sorter (Miltenyi MACSQuant Tyto) to sort 3 populations of CD138+ cells from immunized wild-type mice (Fig. 1). All 3 populations show a majority express IgG (Fig. 1, right plot). We further enriched the CD138-hi population for CD138-hi and the antigen, Human Serum Albumin (HSA) in a 4th population.

The secretion rate of all 4 sorted populations was confirmed using AbTheneum's antibody capture & screen workflow (Fig. 2). The results confirm that CD138-hi has the highest hit rate (HSA-positive/all IgGs). We also confirmed that most CD138-hi cells also express IgG on the surface, and further enriching for antigen increases the hit rate.

We used the CD138 experiment to develop a two step cell sorting workflow to enrich antigen-specific antibody-secreting cells. The workflow, called FACS antigen+, was applied in head-to-head comparisons with commercial CD138+ cell isolation for several therapeutic targets (Table 1).

All trials using Single Cell's FACS antigen+ cell isolation delivered more hits (antibody sequences of screened antigen-positive antibodies), even in frozen or low titer samples, compared to traditional CD138 cell isolation kits. Three of the 6 trials delivered >100% hits compared to magnetic CD138+ cell isolation, highlighting the power of FACS antigen+ workflow with AbTheneum antibody discovery.

Learn more at
singlecelltechnology.com

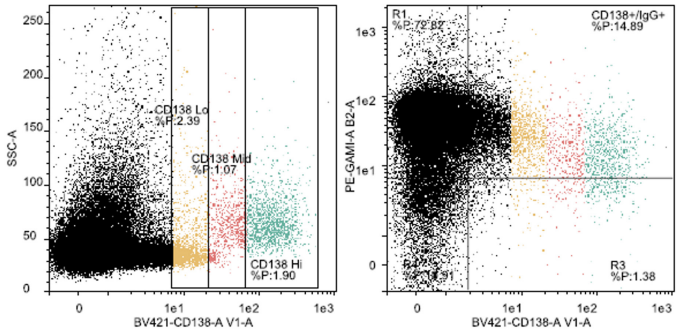


Figure 1. CD138 cell sorting experiment in mouse lymphocytes. CD138+ vs. SSC (left) and CD138+ vs. IgG (right) showing 3 sorted populations: CD138-lo (yellow), CD138-mid (pink), and CD138-hi (green).

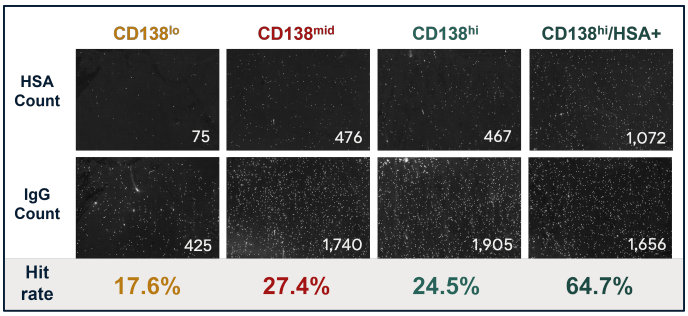


Figure 2. Sorted CD138 cells screened for IgG secretion and hit rate of antigen-specific antibodies (HSA hits).

Table 1. Six diverse IO targets were tested in head-to-head comparisons of commercial CD138+ cell isolation kits vs. FACS antigen+ workflow. Hit rate are number of screened antibody sequences delivered for each campaign.

Antibody Campaign	Cell Isolation	Immunization Duration	Fresh/ Frozen	Hits Delivered	% increase in hits
IO Target 1 Transgenic	MACS CD138+	35 Days	Fresh	351	40%
	FACS antigen+	56 Days	Fresh	491	
IO Target 2 Transgenic	MACS CD138+	35 Days	Fresh	456	109%
	FACS antigen+	35 Days	Frozen	954	
IO Target 3 Transgenic	MACS CD138+	49 Days	Fresh	201	50%
	FACS antigen+	63 Days	Frozen	302	
IO Target 4 Transgenic	MACS CD138+	63 Days	Fresh	265	102%
	FACS antigen+	91 Days	Frozen	535	
IO Target 5 WT	MACS CD138+	35 Days	Fresh	456	165%
	FACS antigen+	35 days	Frozen	1,210	
IO Target 6 Transgenic	MACS CD138+	49 Days	Fresh	364	54%
	FACS antigen+	49 days	Fresh	562	