NC 3Rs

NIHR | Leicester Biomedical Research Centre

UNIVERSITY OF LEICESTER

Institute for Precision Health

UKRI Medical Research Council

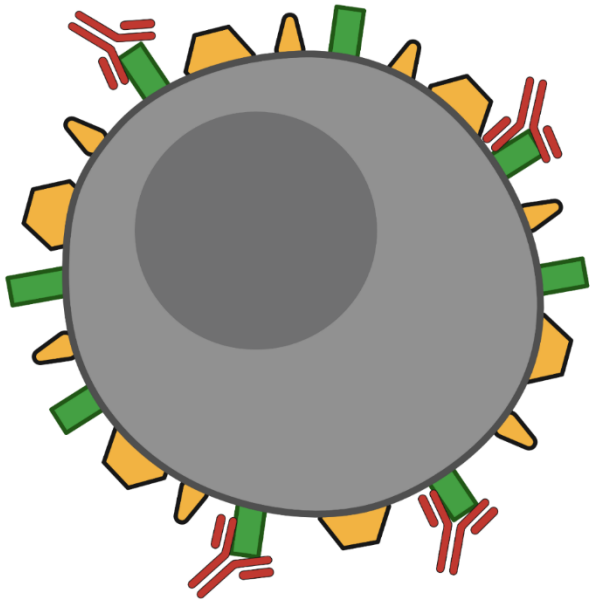
THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH

YCHAROS
ANTIBODY CHARACTERIZATION THROUGH OPEN SCIENCE

What is an antibody?

- Y shaped protein

- They are used to:
  - Understand the involvement of proteins in disease mechanisms.
  - Therapeutics
  - Diagnostics/ prognostics
    - Biomarkers

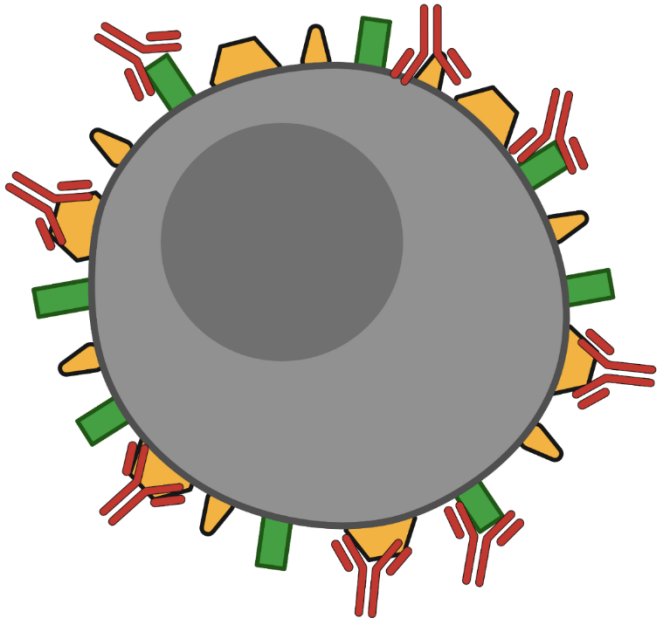


OGA
Only Good Antibodies

What is an antibody?

- Y shaped protein

- They are used to:
  - Understand the involvement of proteins in disease mechanisms.
  - Therapeutics
  - Diagnostics/ prognostics
    - Biomarkers

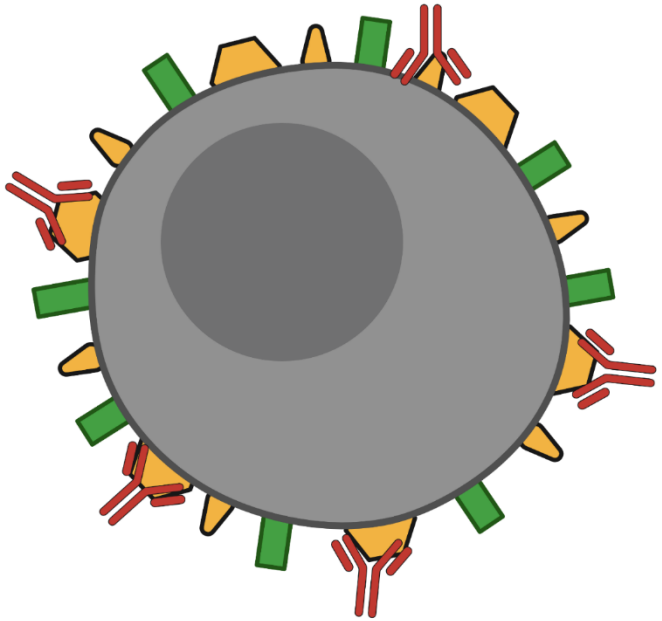


OGA
Only Good Antibodies

What is an antibody?

- Y shaped protein

- They are used to:
  - Understand the involvement of proteins in disease mechanisms.
  - Therapeutics
  - Diagnostics/ prognostics
    - Biomarkers



OGA
Only Good Antibodies

OGA

Only Good Antibodies

Mission: make best practices in antibody choice and use more feasible, easy and rewarded.

Projects: Better Antibodies, Better Science and Better Patient Outcomes.

About: Non-profit grass roots community

YCHAROS

ANTIBODY CHARACTERIZATION THROUGH OPEN SCIENCE

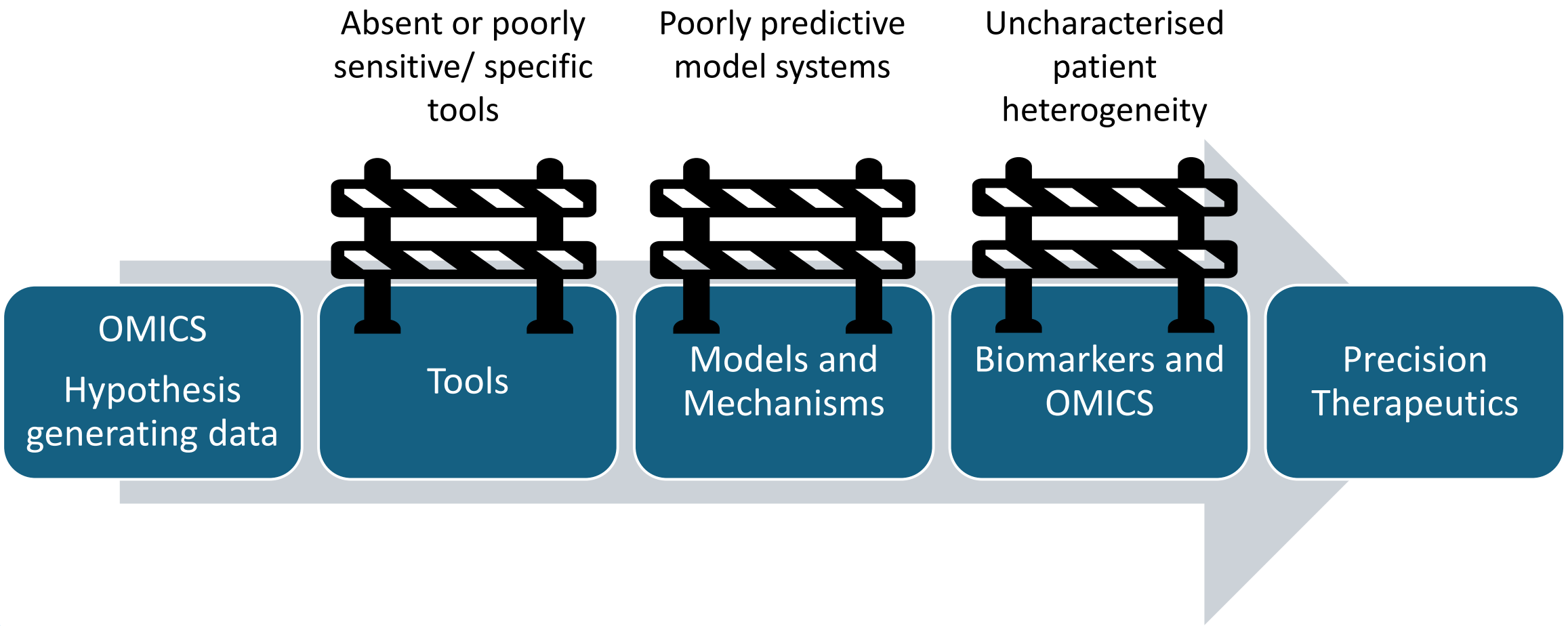
Mission: identify selective antibodies for every human protein

Projects: KO characterisation of antibodies used to study proteins of interest in…

About: An incorporated Open Science public good company that facilitates the collaboration between antibody manufacturers and academic labs.

OGA
Only Good Antibodies

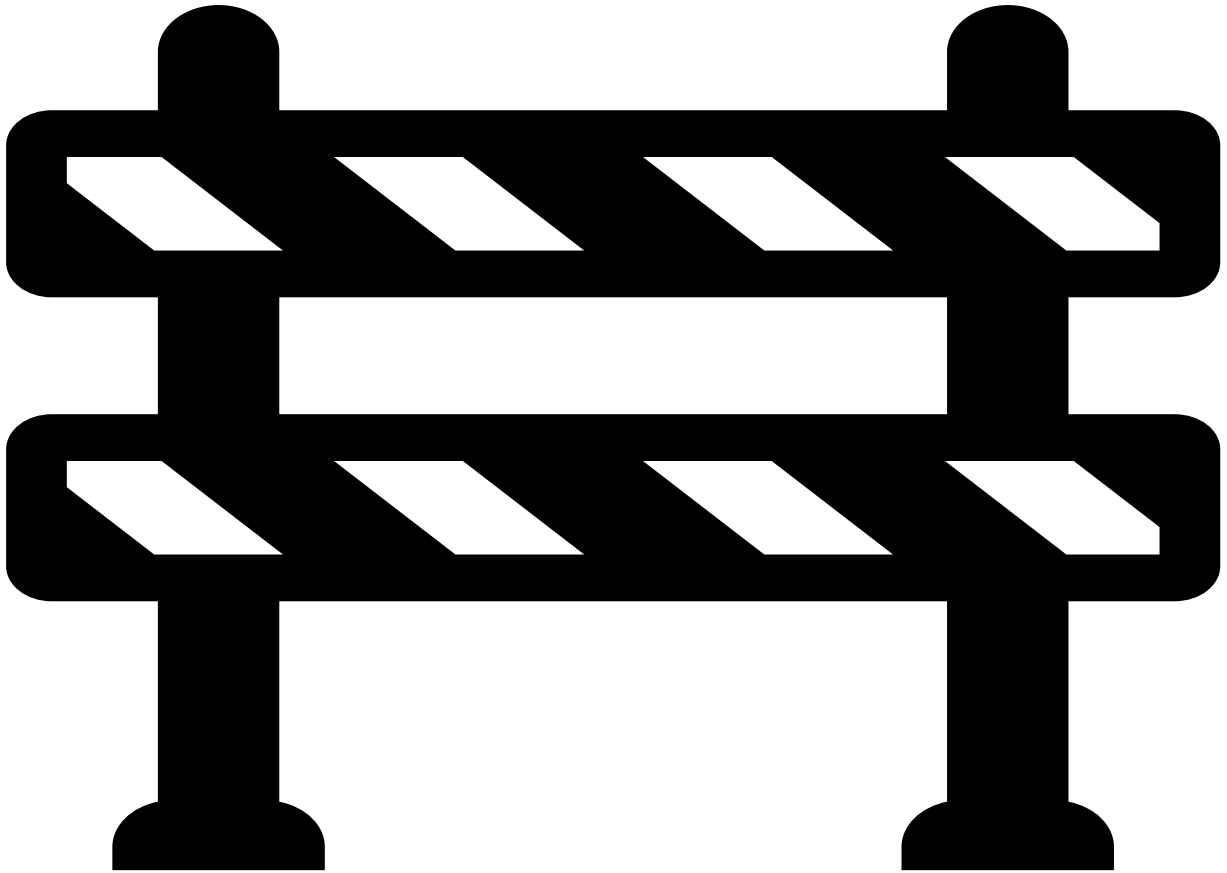
Why is drug development slow, expensive and risky?



OGA
Only Good Antibodies

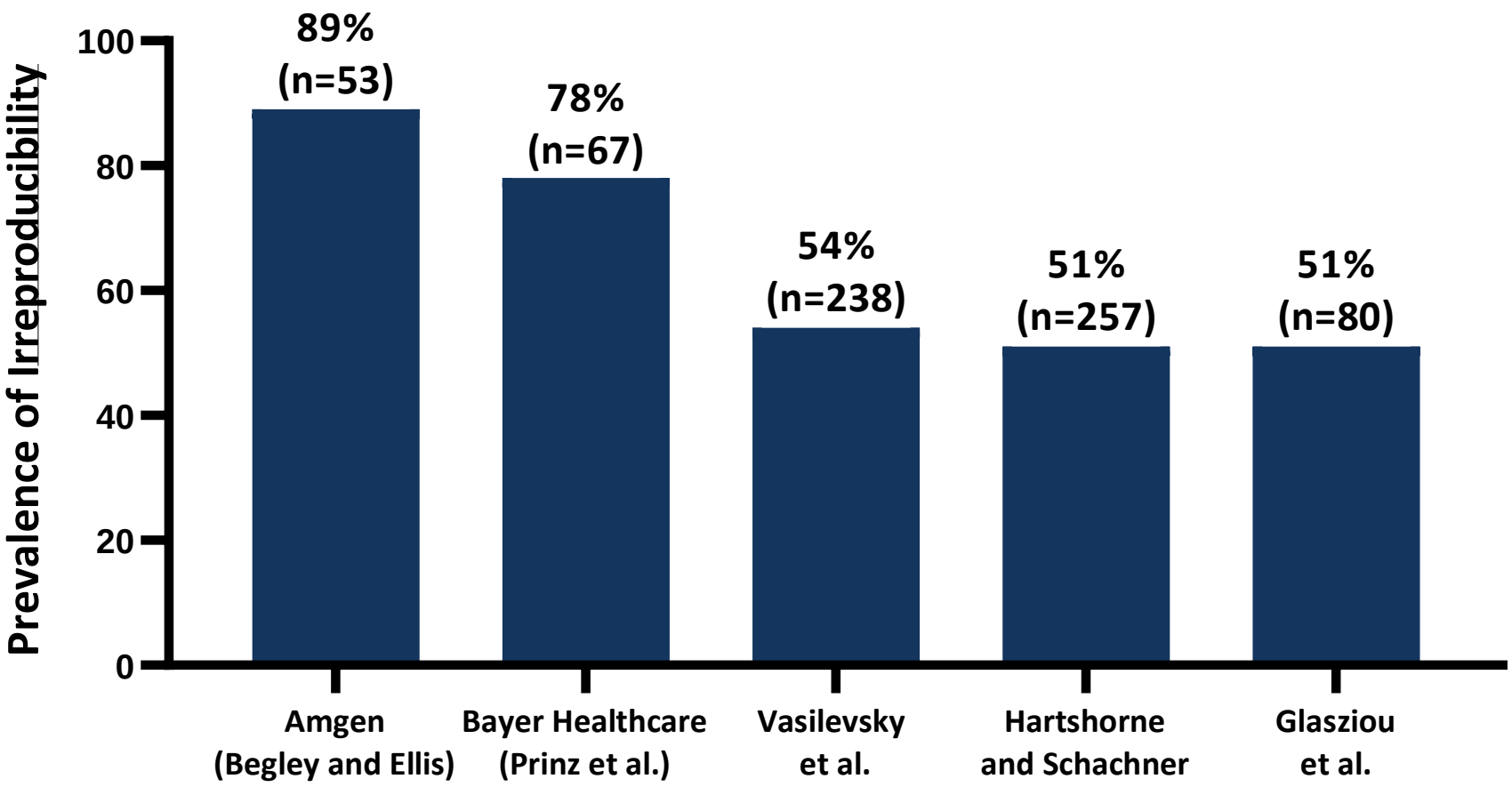
Why is drug development slow, expensive and risky?

Irreproducibility and lack of quality



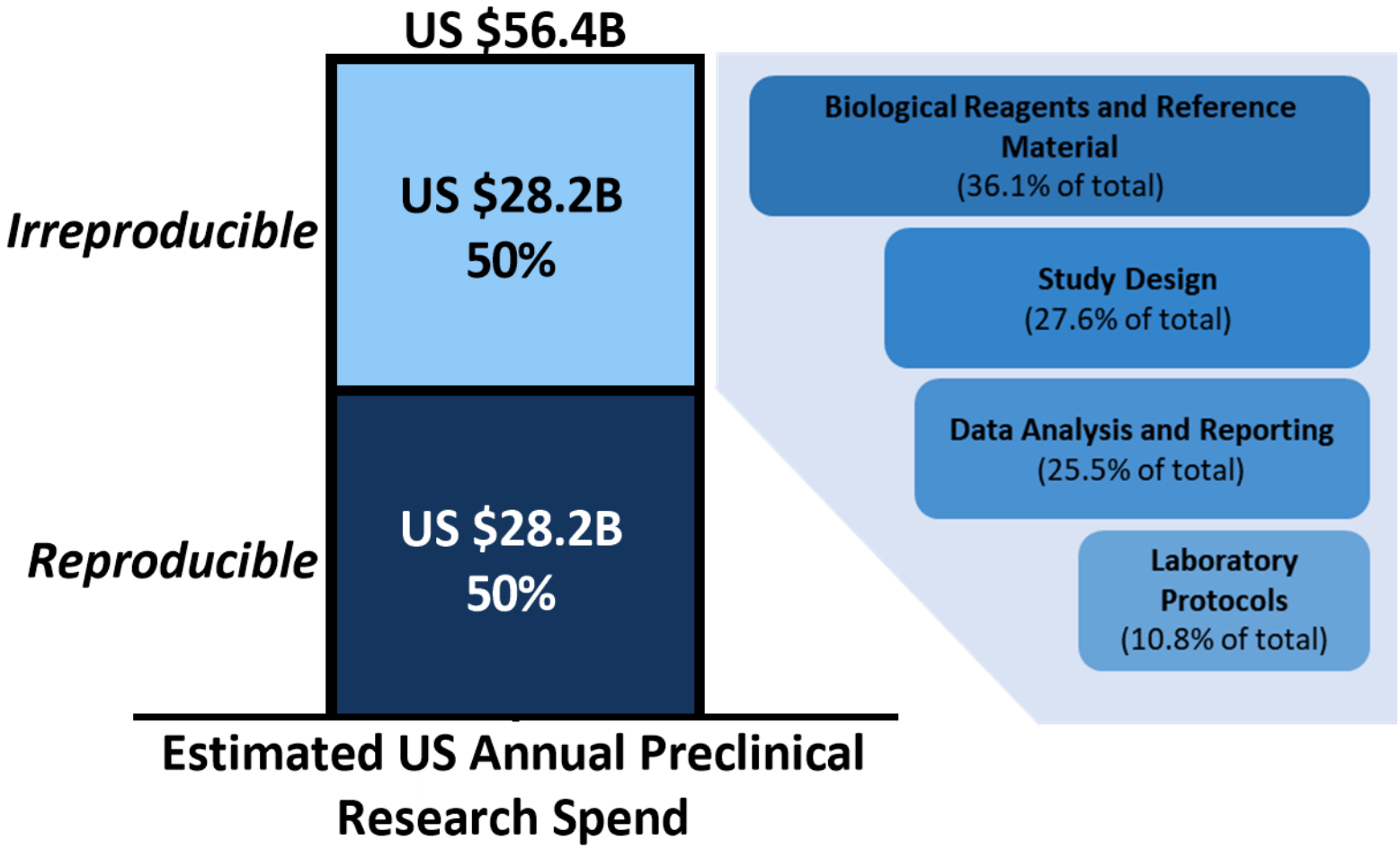
OGA
Only Good Antibodies

The problem



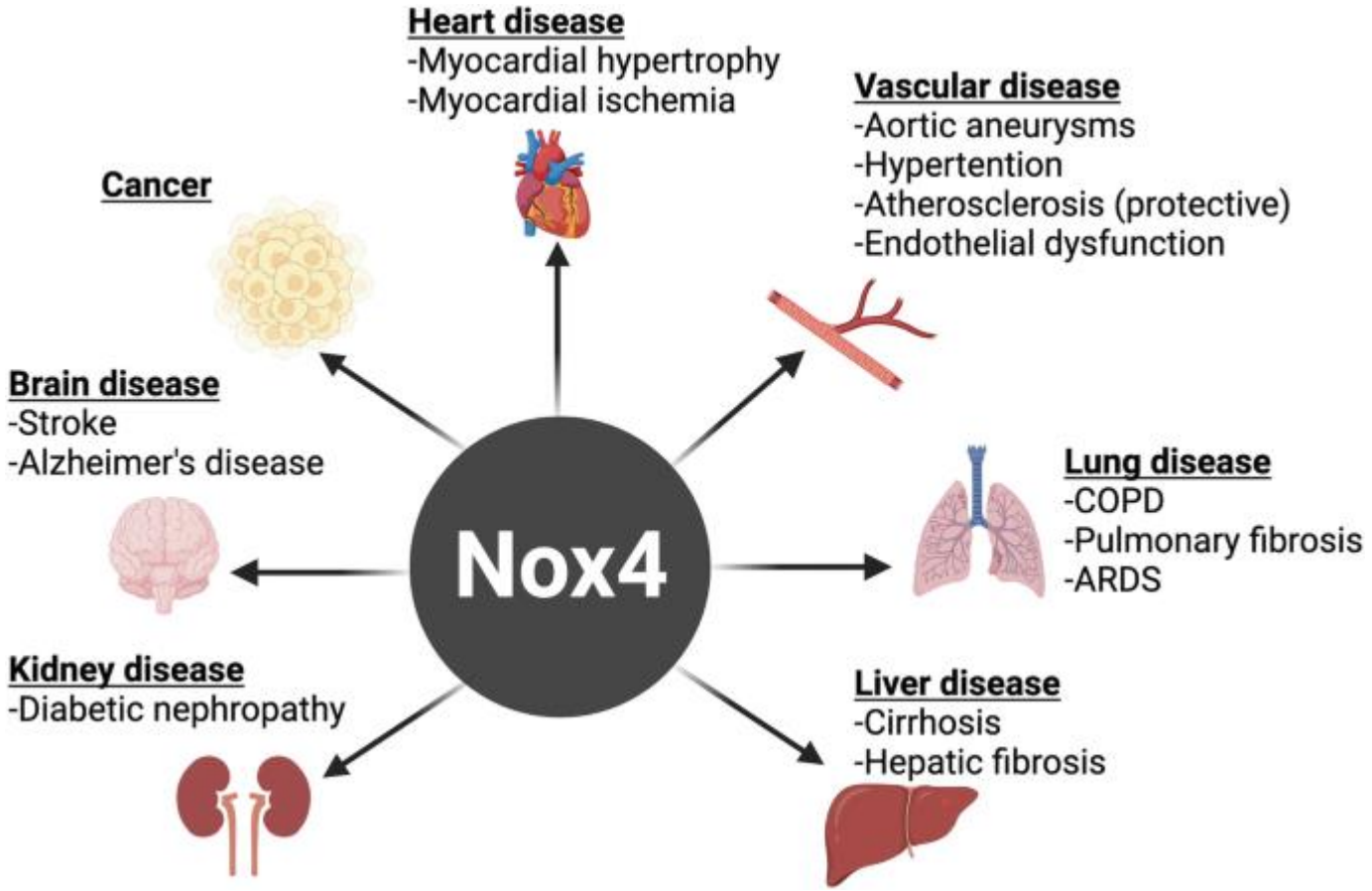
OGA
Only Good Antibodies

The problem



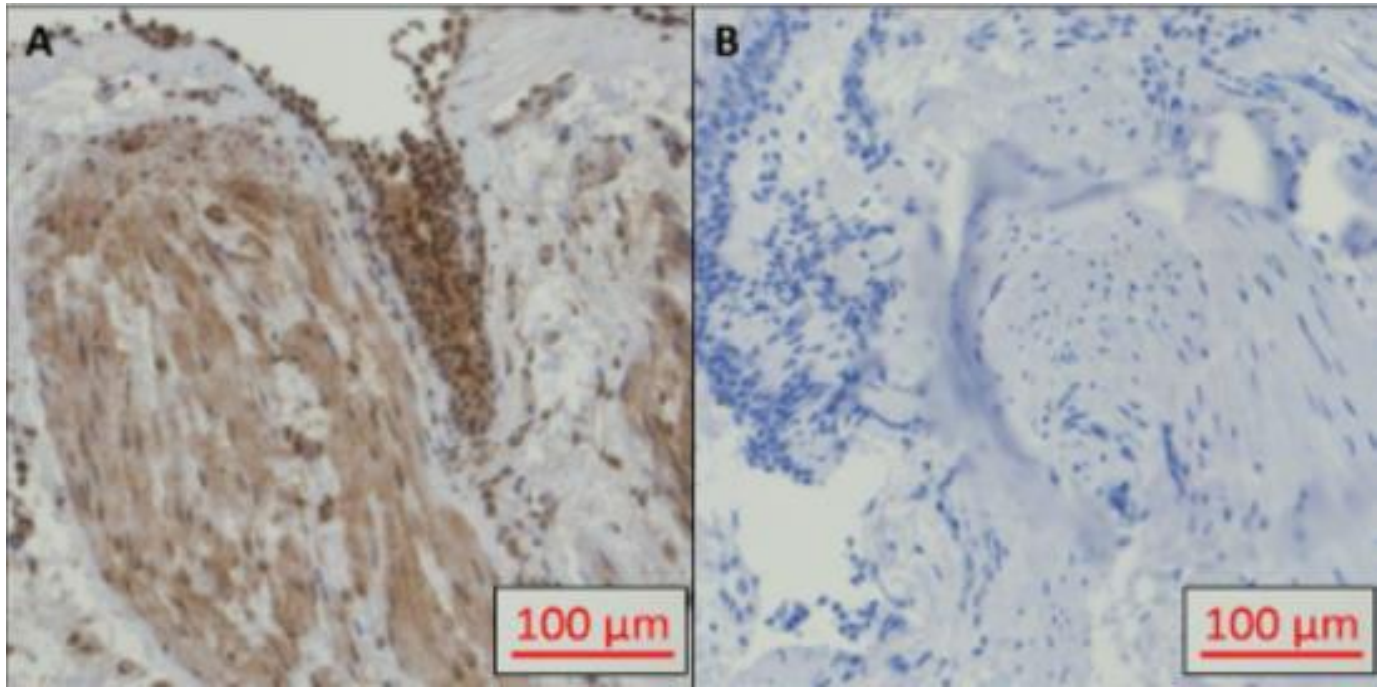
OGA
Only Good Antibodies

My personal motivation



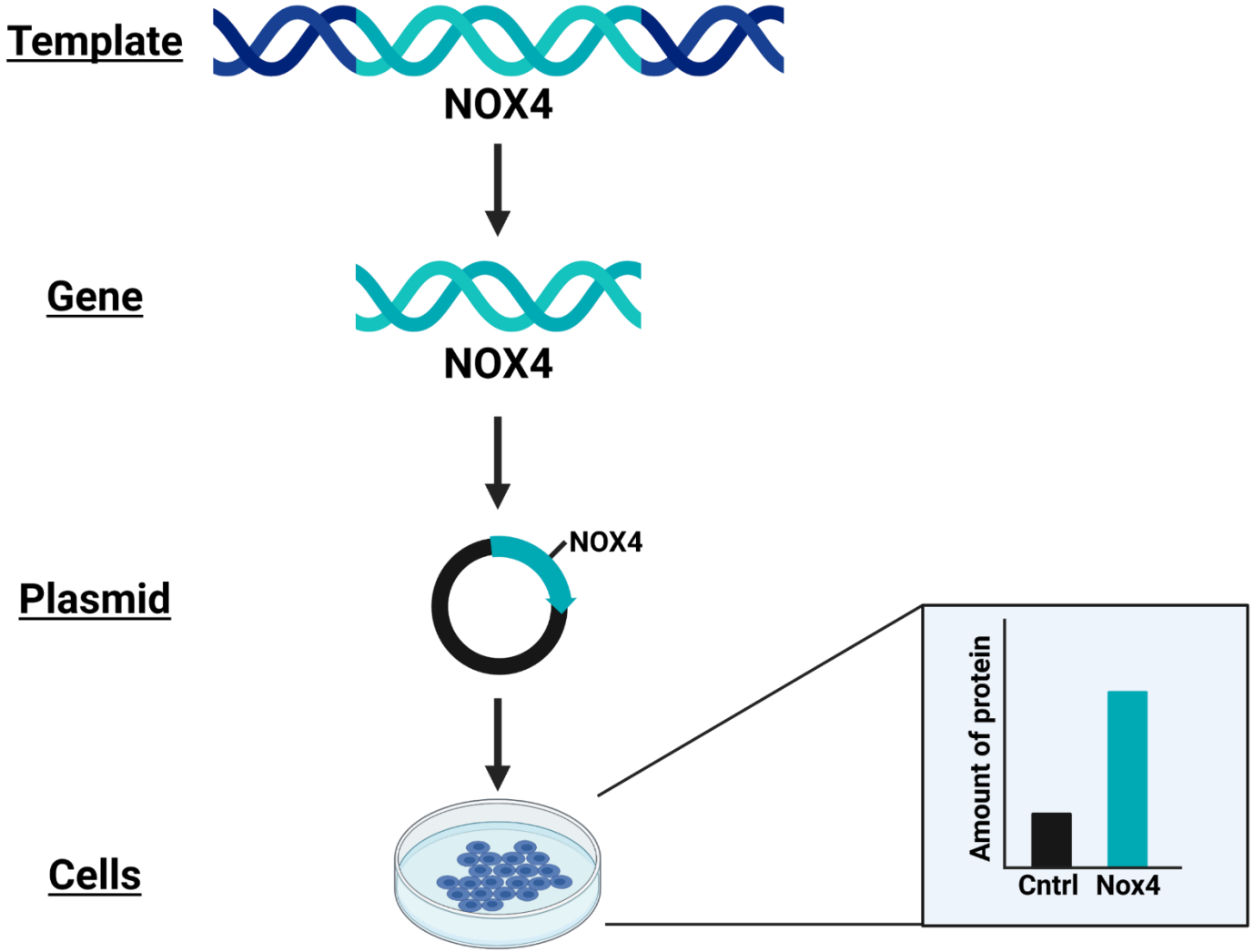
OGA
Only Good Antibodies

My personal motivation



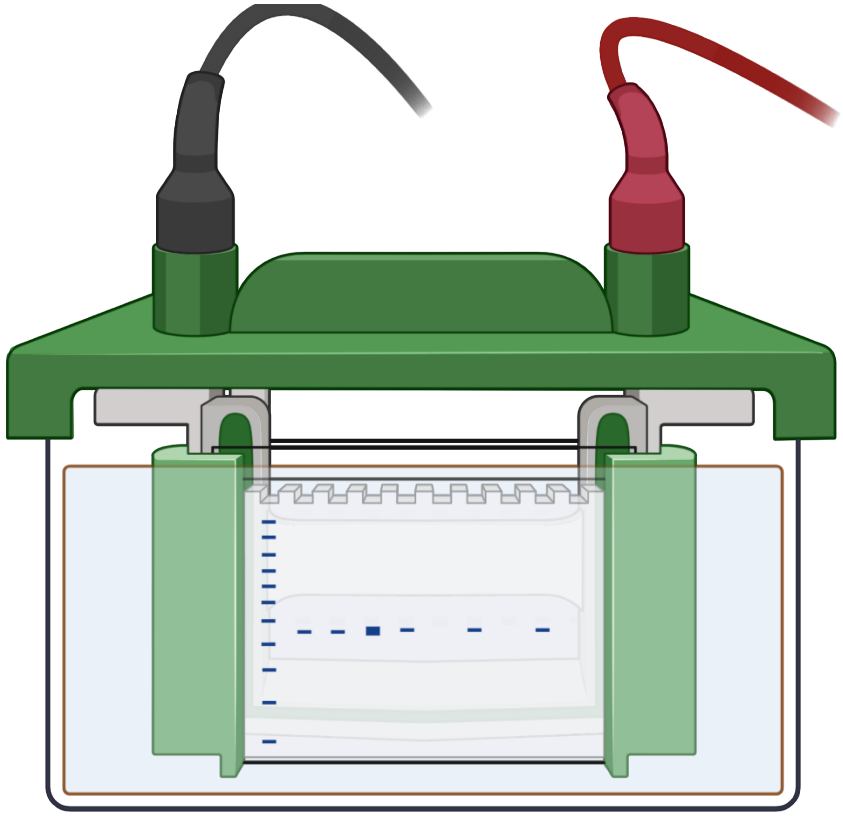
OGA
Only Good Antibodies

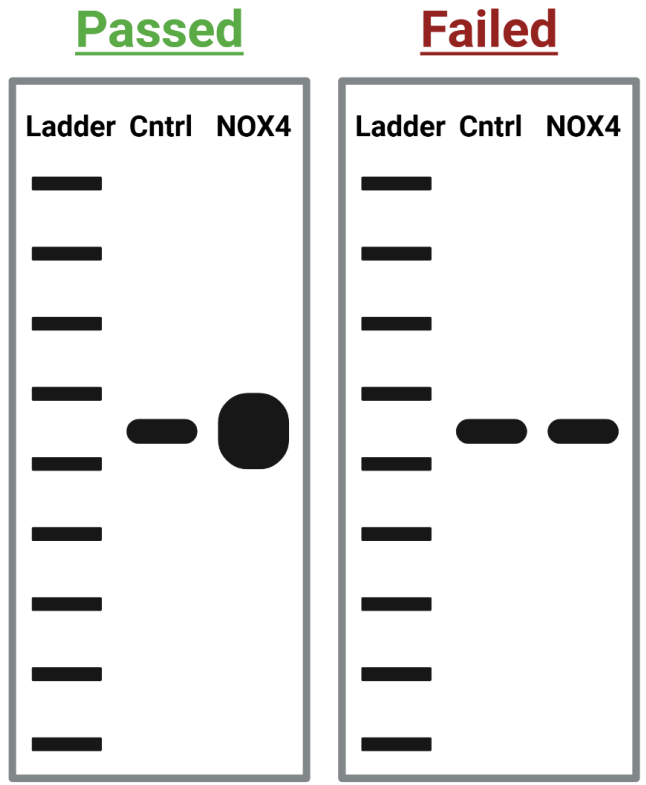
Check the antibody?



OGA
Only Good Antibodies

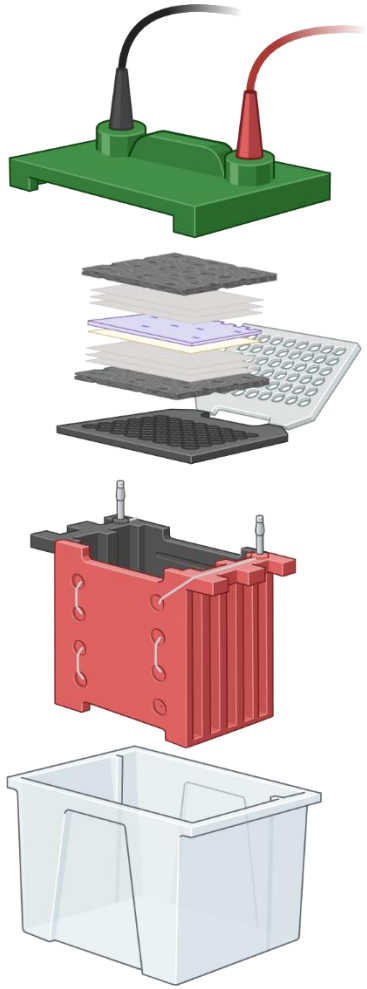
Check the antibody?

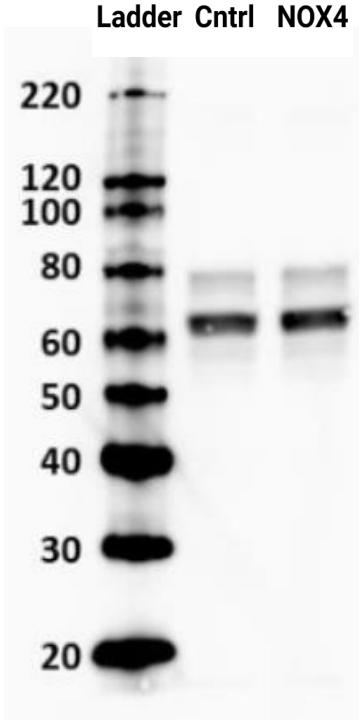




OGA
Only Good Antibodies

Check the antibody?





Failed

OGA
Only Good Antibodies

Check multiple antibodies?

CiteAb

952 results for 'nox4' antibodies

Antibodies resold by many suppliers

Which ones to pick?

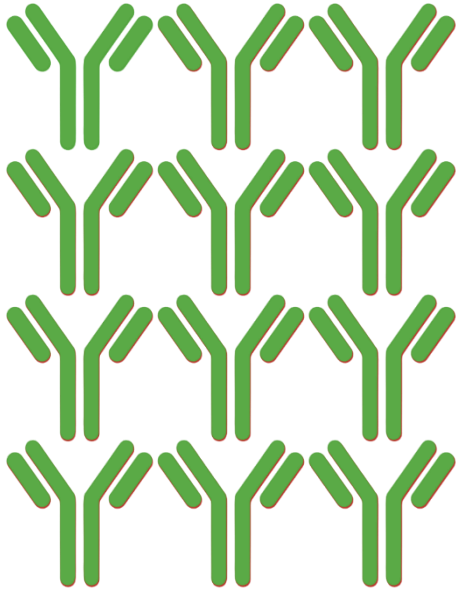
Citations?

Validation data?

Recommended technique?

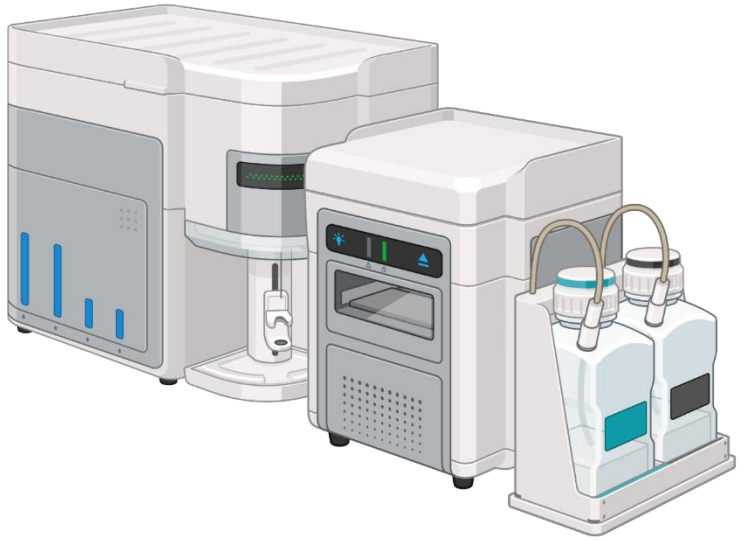
OGA
Only Good Antibodies

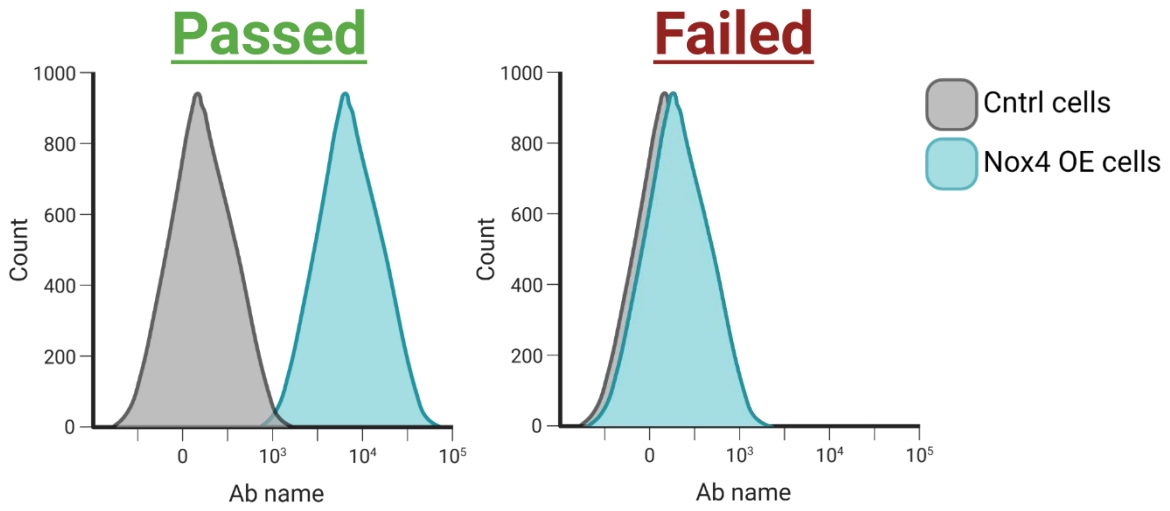
Check multiple antibodies?



12 antibodies

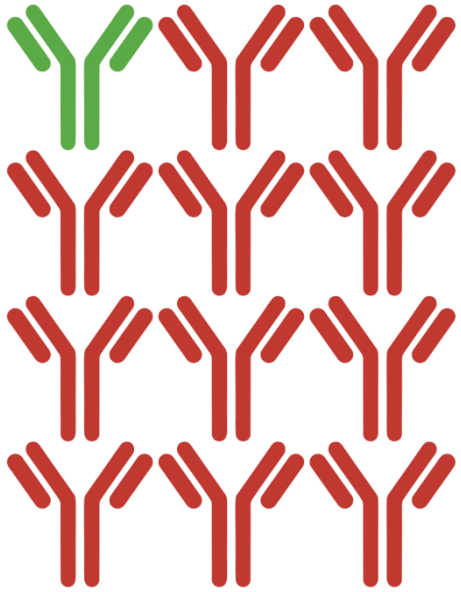
£4000





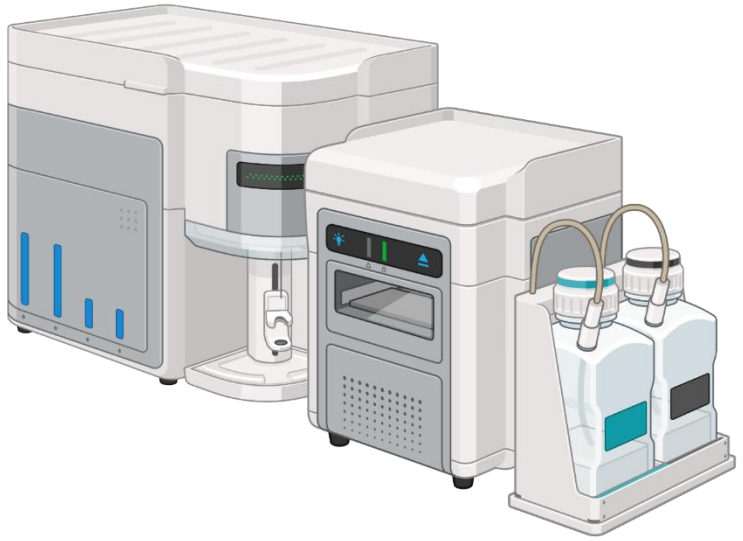
OGA
Only Good Antibodies

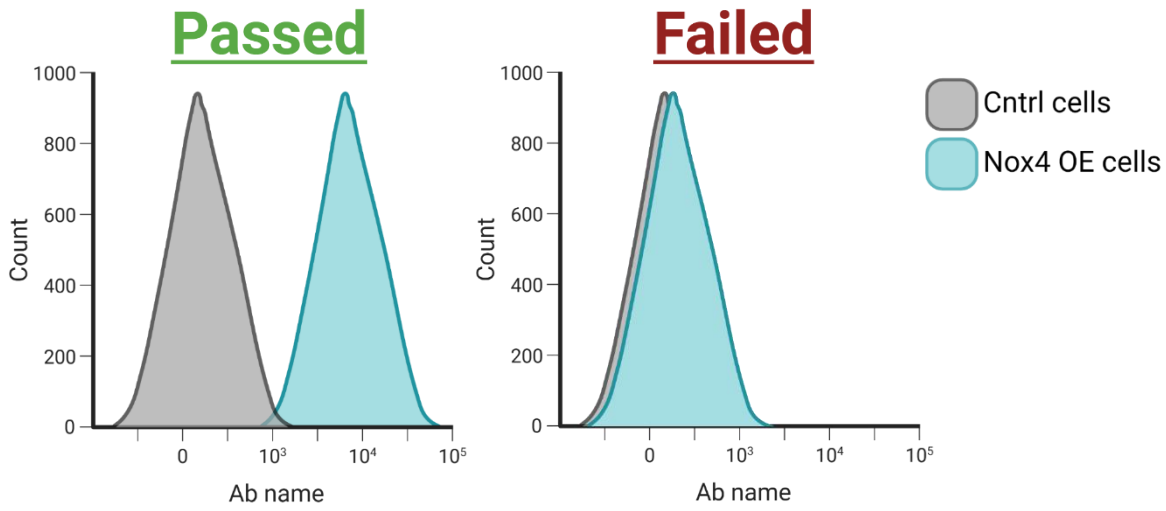
Check multiple antibodies?



12 antibodies

£4000

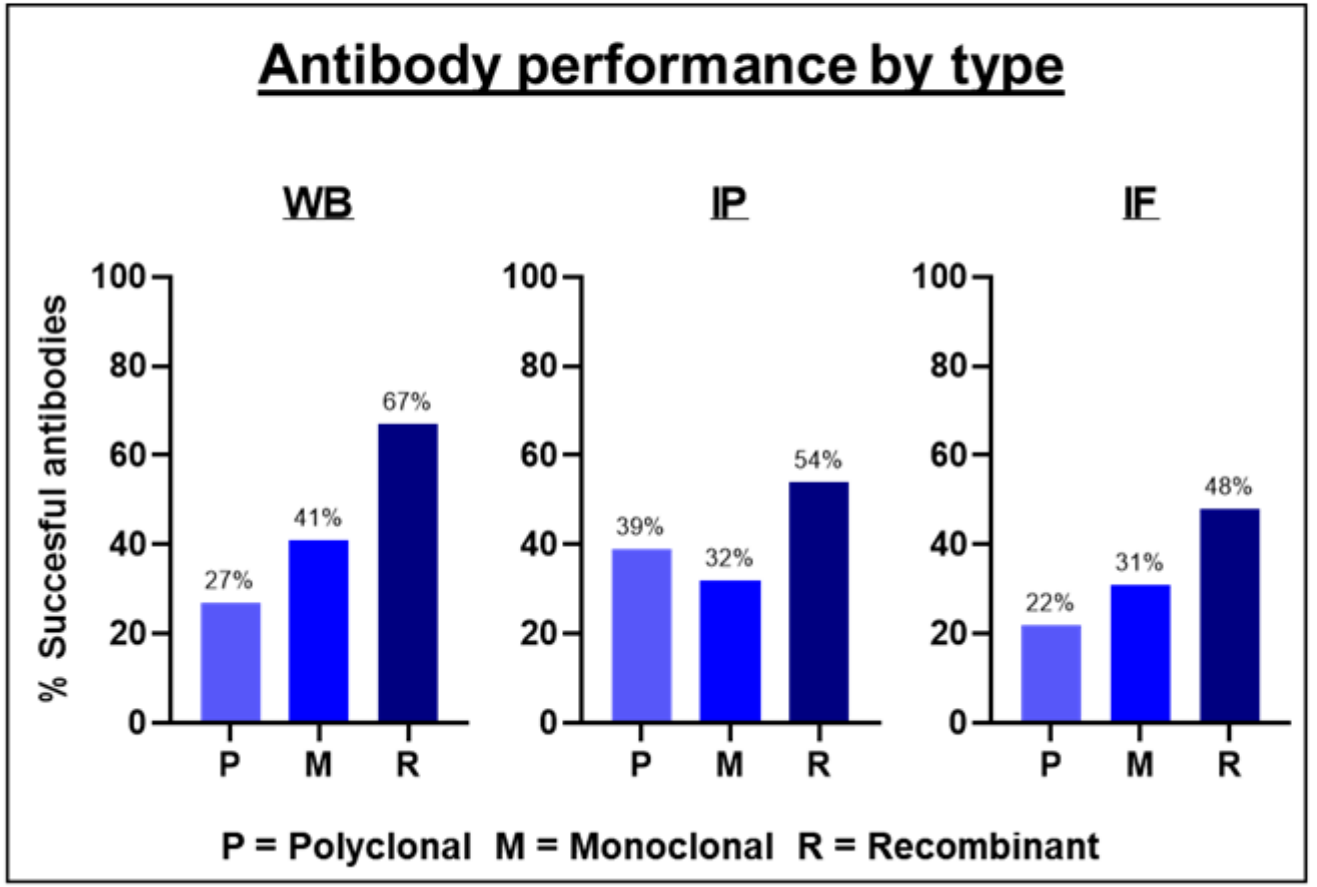


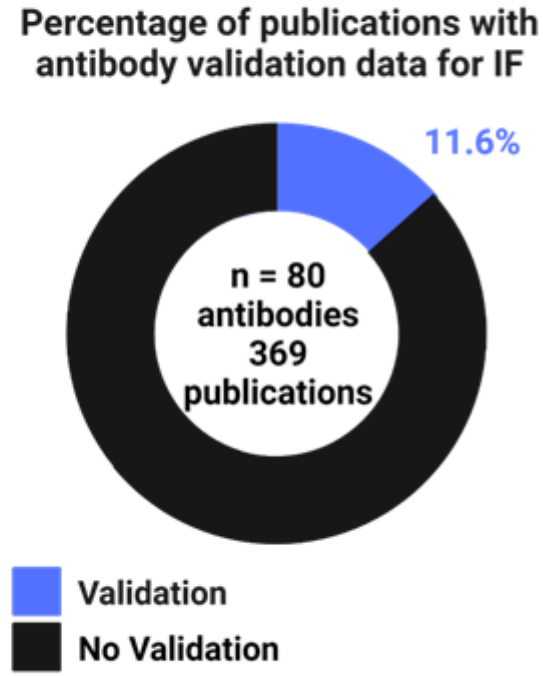


OGA
Only Good Antibodies

The problem

YCHAROS
ANTIBODY CHARACTERIZATION THROUGH OPEN SCIENCE

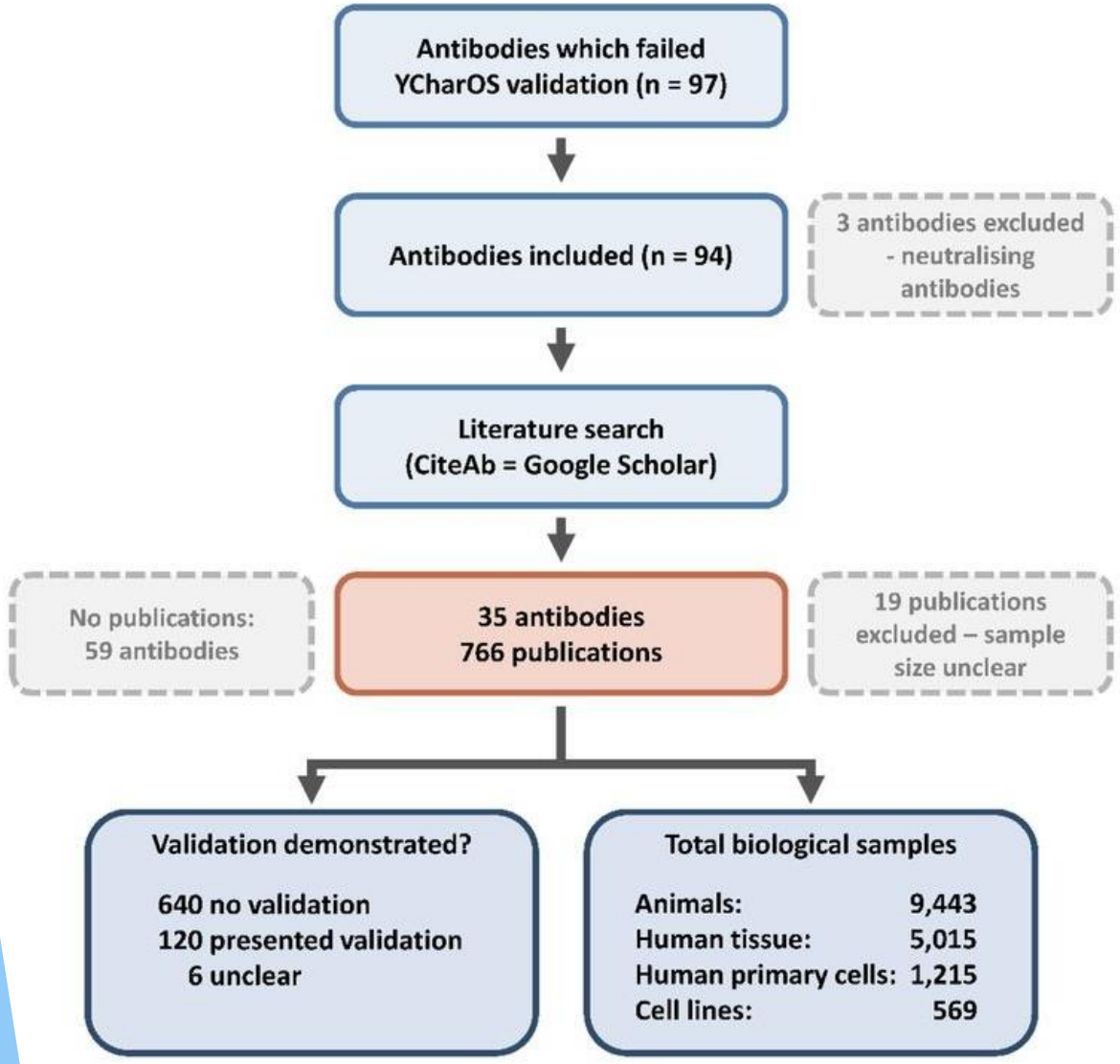


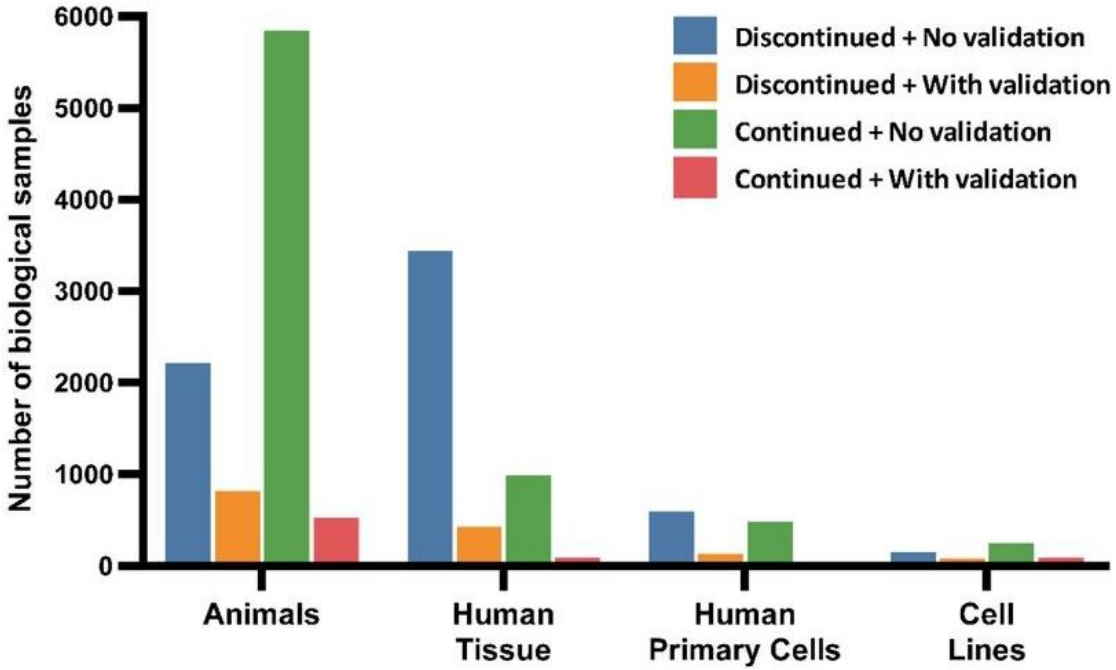


https://doi.org/10.7554/eLife.91645.2

OGA
Only Good Antibodies

The problem





OGA
Only Good Antibodies

Only Good Antibodies

Community and Forum

Our Mission

The OGA community will **work with** all stakeholders to accelerate scientific and drug discovery research by:

1. Increasing the availability and use of high performing antibodies

2. Eliminating the use of poorly performing antibodies

Only Good Antibodies

Community and Forum

About us

- We are a diverse **interdisciplinary** collaboration of basic, clinical, and behavioural scientists and technicians from academia, industry, and non-profit organisations.
- We are actively **engaging all stakeholders**
- We support **open science** and transparency, and we believe a **no blame** culture helps facilitate better collaboration and progress

Working together to improve biomedical research

Research institutions and end users

UK RN

UK Reproducibility Network

OGA Only Good Antibodies

University of Leicester

Institute for Precision Health

Experts in antibody production and validation

YCHAROS Antibody Characterization Through Open Science

AstraZeneca

GeneTex

abcam progress happens together

DSHB Antibodies for Research

Institute for Protein Innovation

addgene

Cell Signaling Technology

proteintech®

Antibody Society

Research funding agencies

NC3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

The Michael J. Fox Foundation for Parkinson's Research

EU-PFF European Pulmonary Fibrosis Federation

mnda motor neurone disease association

Reagent database providers

RRID

Chemical Probes.org

Biocompare The Buyer's Guide for Life Scientists

CiteAb

Publishers and journals

F1000

eLife

nature protocols

The Company of Biologists

Biology Open

Journal of Cell Science

Development

Disease Models & Mechanisms

WILEY

Journal of Experimental Biology

Potential drivers of the problem

Availability of robust characterisation data

- Lack of standardised approach
- Potential for conflict of interest
- Expensive to produce

End-user antibody choice and use

- Lack of awareness and education
- Resource constraints
- Lack of rewards for best practices

Variability in antibody technologies

- Slow adoption of recombinant technologies
- Lack of unique identification
- Characterisation data applicable to old batch

OGA
Only Good Antibodies

Raising awareness and education

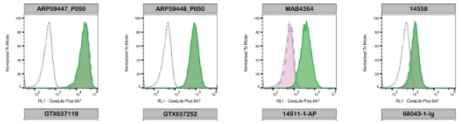
addgene Blog

Knockout cell line approach

Genetic knockout-based validation can conclusively prove the ability of an antibody to detect the antigen of interest when expressed at endogenous levels (Laflamme et al., 2019). CRISPR-Cas9 approaches are commonly used to produce KO cell lines, some of which are commercially available. Various proteomic and transcriptomic datasets can be used to select a candidate cell line (e.g. DepMap) – you'll need to ensure that the parental cell lines of your chosen KO line are likely to express the target at a robust level, similar to that in samples of interest. If you are working with cells that are amenable to genetic knockout, this represents a very robust approach to antibody validation. If your protein of interest is essential to the cell's function, it likely is not amenable to knockout approaches. In these cases, we would recommend a knockdown approach instead.

In this approach, you're looking for an antibody that detects the target in the parental cell line, but not in the iso-genic knockout control. This can involve cell surface labeling and/or intracellular labeling (or both). It is important to test for the protocol you intend to use in your experiment. An antibody that demonstrates selectivity for cell surface labeling may become less selective if used in intracellular labeling because the intracellular compartment may contain cross-reacting antigens not present on the cell surface. The presentation of antigens can also be affected by cell fixation and permeabilization techniques — and so it may be necessary to try multiple techniques.

For example, we have found that some antibodies perform well in a PFA-saponin fixation and permeabilization protocol, but very poorly in a methanol fixation-permeabilization protocol. Although the expected location of the target is an important factor in deciding which protocol is likely to work, we have found that it is often best to determine this by testing them. For intracellular targets, we usually test 3 different fix/perms (methanol, PFA-saponin, PFA-triton). The fix/perm solutions can make a significant difference in the efficacy of your antibody. When compared, 4% PFA and 0.1% saponin; 4% PFA and 0.1% Triton X-100; and methanol, we found that the optimal perm/fix solution depended on the specific antibody:target combination of interest (Figure 1). It can therefore be helpful to try all three when looking to label an intracellular target.



OnlyGoodAntibodies Community and forum

Great to have 55 PhD students from University of Leicester, University of Birmingham, University of Warwick, University of Aston and University of Nottingham at our first ever Antibodies and Research Reproducibility Masterclass. Thank you to Dr Adam Wright, Panayiota Stylianou, PhD, Michael Biddle, Harvinder Virk and Lisa Finch for organisation and delivery.
Thank you to all the students for attending.

NC3Rs 20 YEARS
Pioneering Better Science

OGA Only Good Antibodies

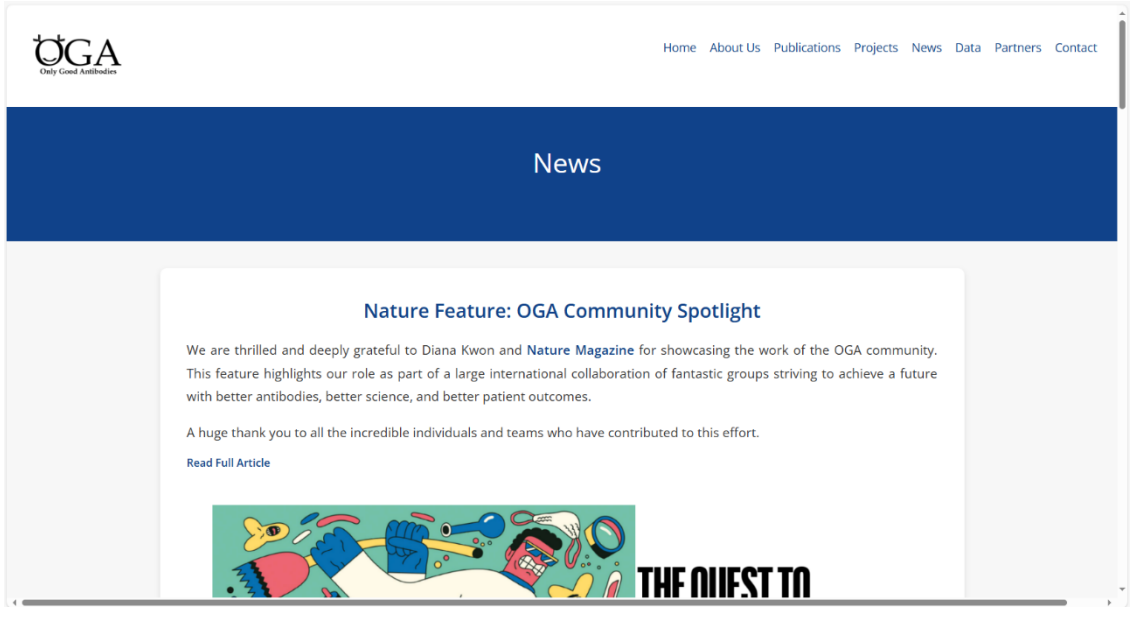
Defining the role of antibodies in improving research reproducibility

NC3Rs and Only Good Antibodies community meeting report

Authors:
Dr Harvinder Virk, University of Leicester, UK (hsv5@leicester.ac.uk)
Dr Rachel Eyre, NC3Rs, UK (rachel.eyre@nc3rs.org.uk)

With support from:
Dr Anthony Holmes, NC3Rs, UK
Dr Michael Biddle, University of Leicester, UK
Dr Eva Krockow, University of Leicester, UK

Meeting held: 27 February 2024, London, UK
Report published: 17 July 2024



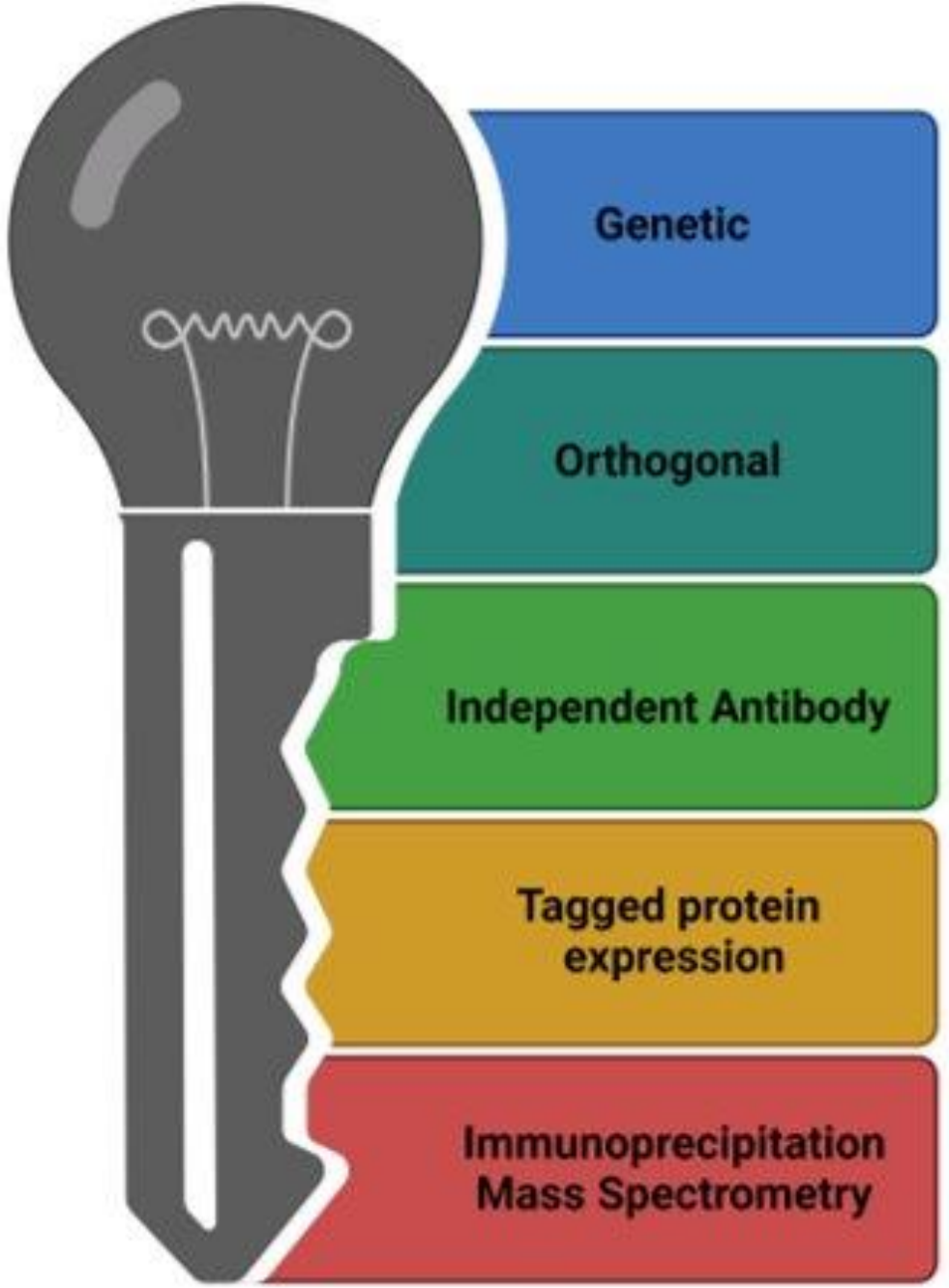
OGA Only Good Antibodies

Antibody Characterisation

International Working Group for Antibody Validation proposed "**Five conceptual pillars**".

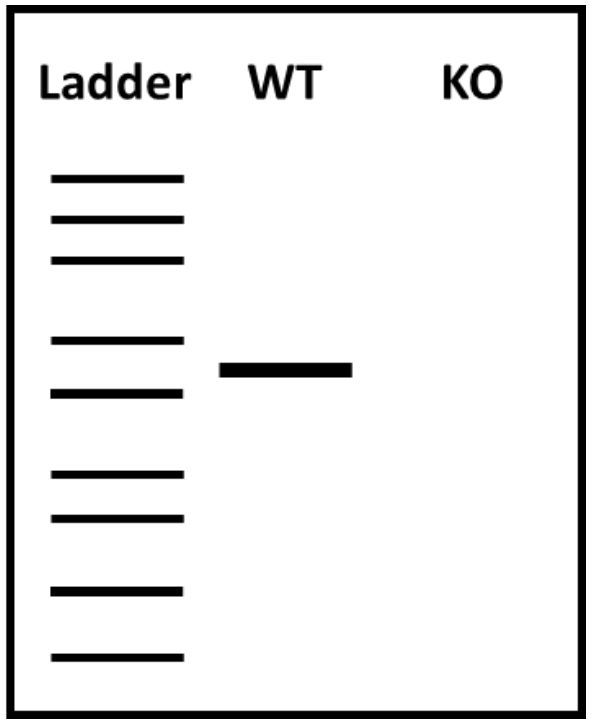
A **high selective antibody** recognises its target with **minimal cross reactivity** within a given application.

Any **proposed pillar** adopted should therefore provide **evidence** of **antibody-target binding** and allow the **evaluation of cross-reactivity**.



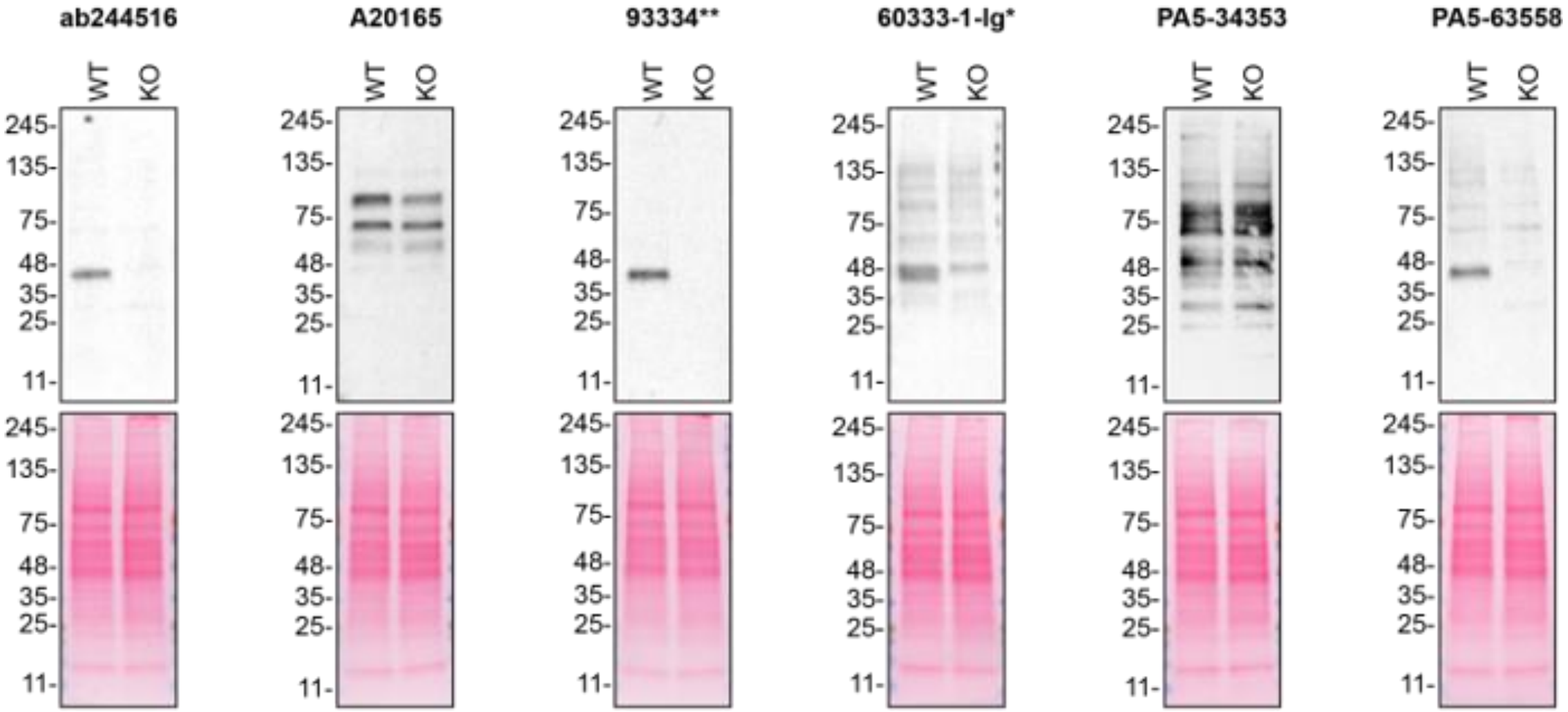
OGA
Only Good Antibodies

What is a knockout cell line?



OGA
Only Good Antibodies

The problem



OGA
Only Good Antibodies

YCHAROS

ANTIBODY CHARACTERIZATION
THROUGH OPEN SCIENCE

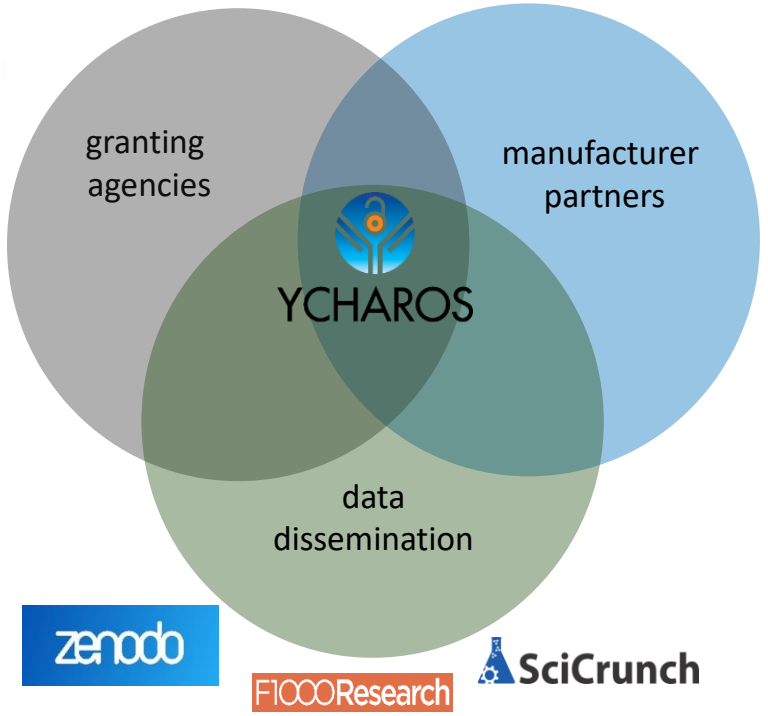
OGA
Only Good Antibodies

YCHAROS
ANTIBODY CHARACTERIZATION THROUGH OPEN SCIENCE

OGA
Only Good Antibodies

Identification of selective renewable antibodies

Creation of a trusted open science ecosystem to test antibodies

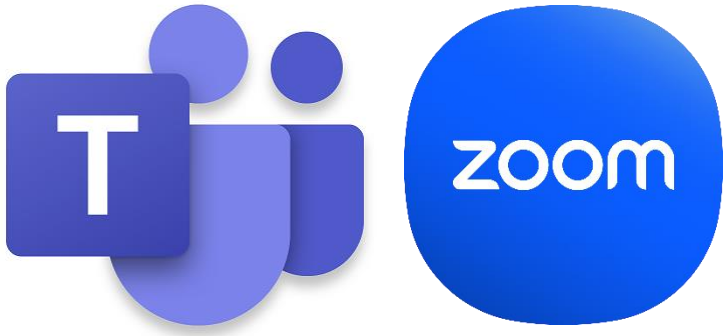


- Total # of public antibody reports: **120**
- Total # of antibodies tested: **1200**

zenodo.org/communities/ycharos
f1000research.com/ycharos
antibodyregistry.org

Only Good Antibodies

Building relationships with commercial partners



OGA
Only Good Antibodies

Building relationships with commercial partners

What do they benefit from joining

Highlight your best performing products

- Best performing products stand out from the competition
- Data is distributed to the relevant scientific communities
- Available to use in your marketing material

Reduce catalogue size and product maintenance cost

- Removal of sub-optimal antibodies increase catalogue quality and allows focus on a smaller number of quality products.

Identify new applications for your antibodies

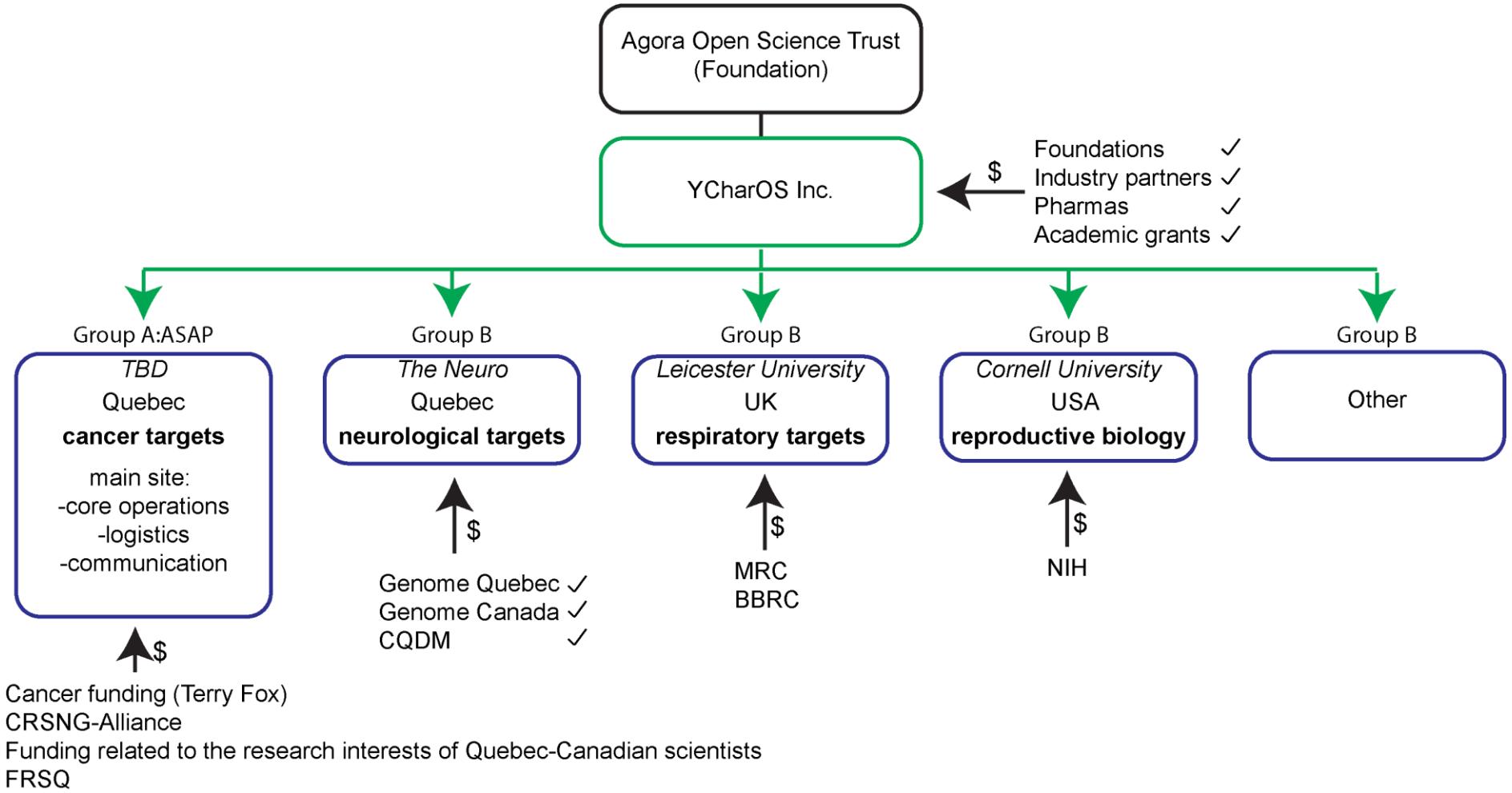
Better focused research effort

- YCharOS will identify gaps in the market where there is a lack of high-quality products

OGA
Only Good Antibodies

Building relationships with commercial partners

International collaborations allow selective communities to uptake the data



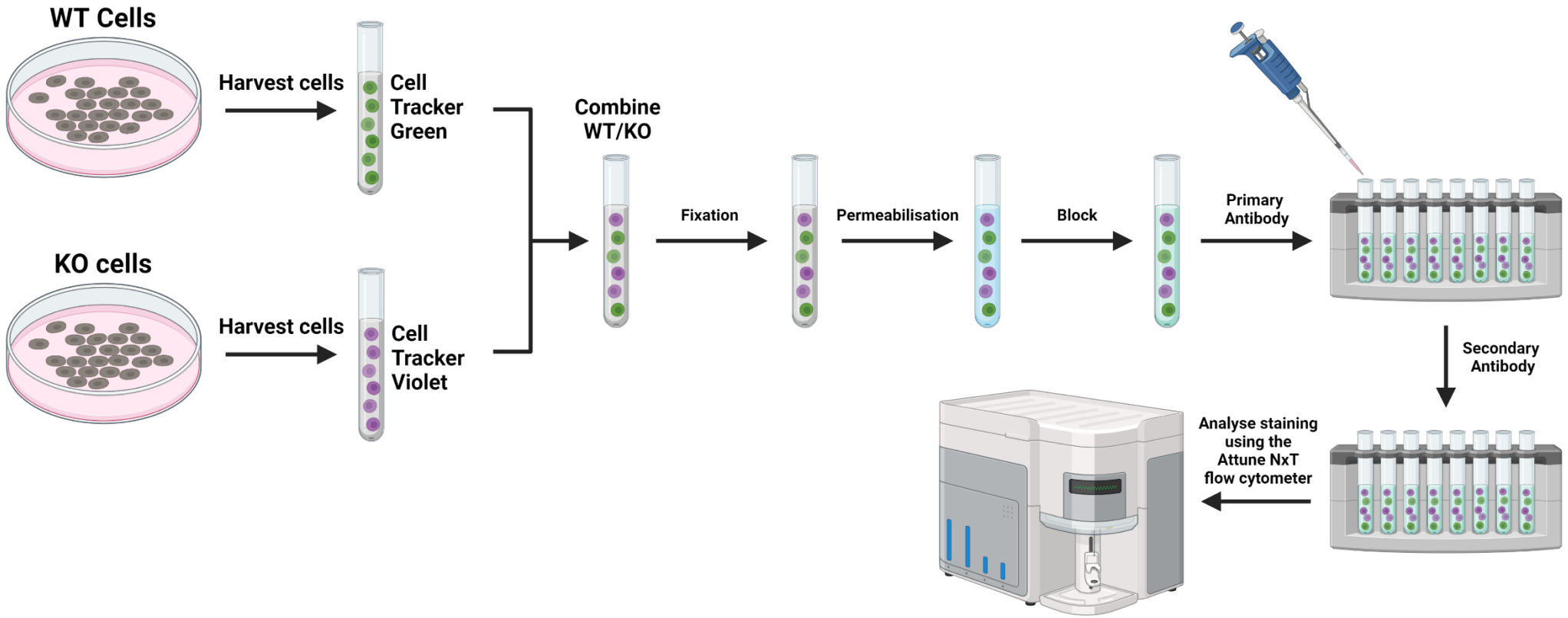
OGA
Only Good Antibodies

Transparent and Open Dissemination Process

- First, we prepare antibody characterization reports and share with all our partners.
- Second, we look carefully at all the reports during our monthly advisory meeting to answer any issues or questions.
- Third, once unanimously approved by all partners, we release the reports on Zenodo.
- Fourth, we contact the funding agencies (NIH, ALS associations, etc.) and they highlight the release of the reports to their scientific communities.

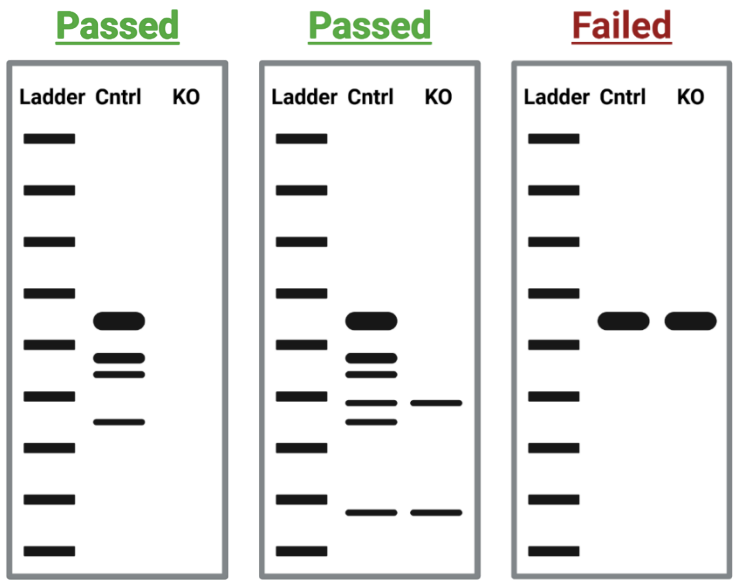
OGA
Only Good Antibodies

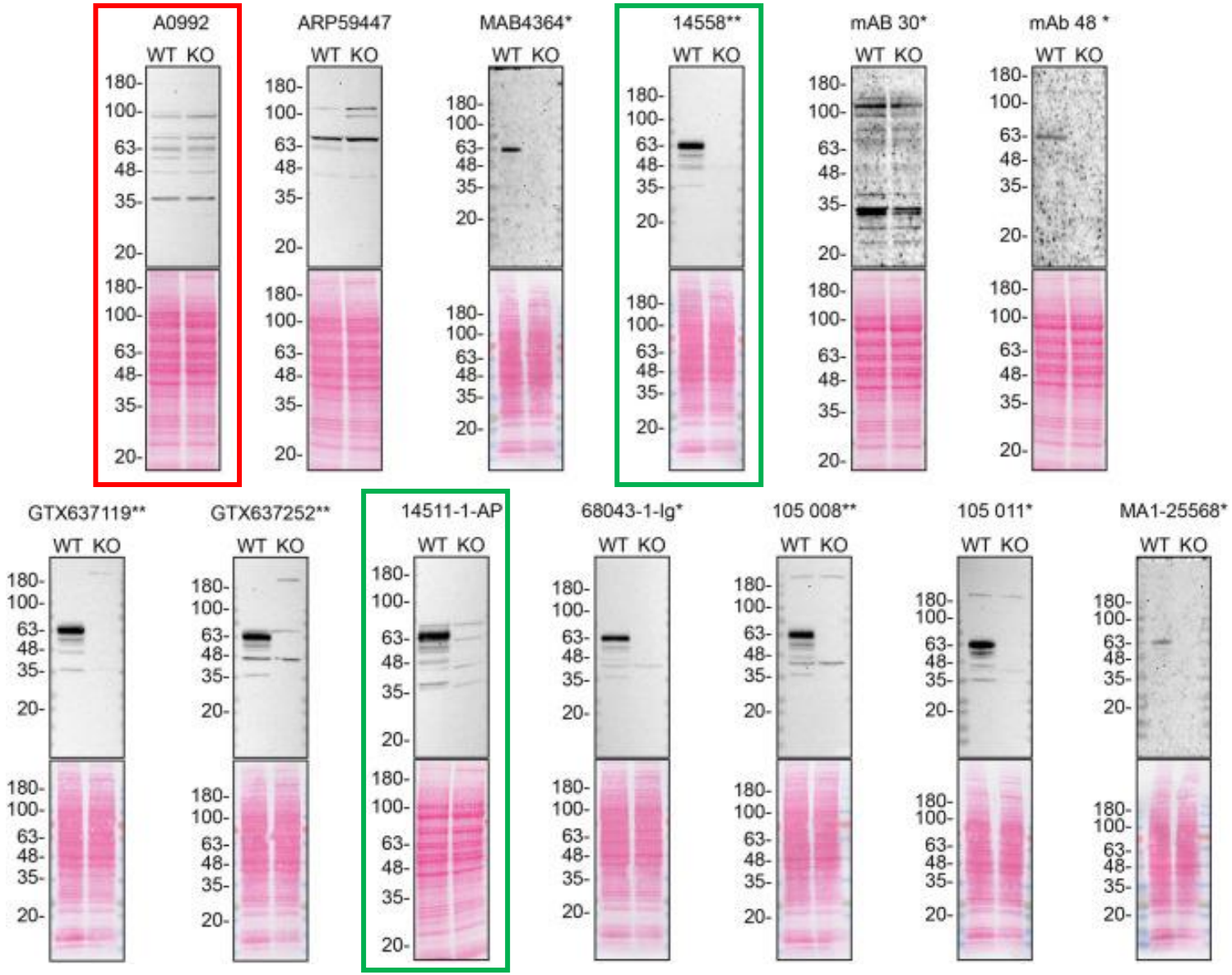
Flow cytometry – PFA/ Saponin, PFA/ Triton X-100 summary



OGA
Only Good Antibodies

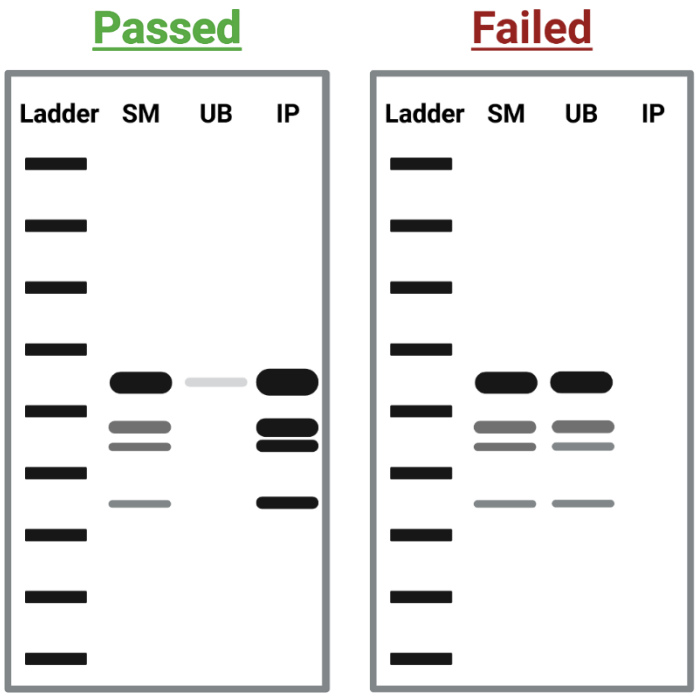
Western blot

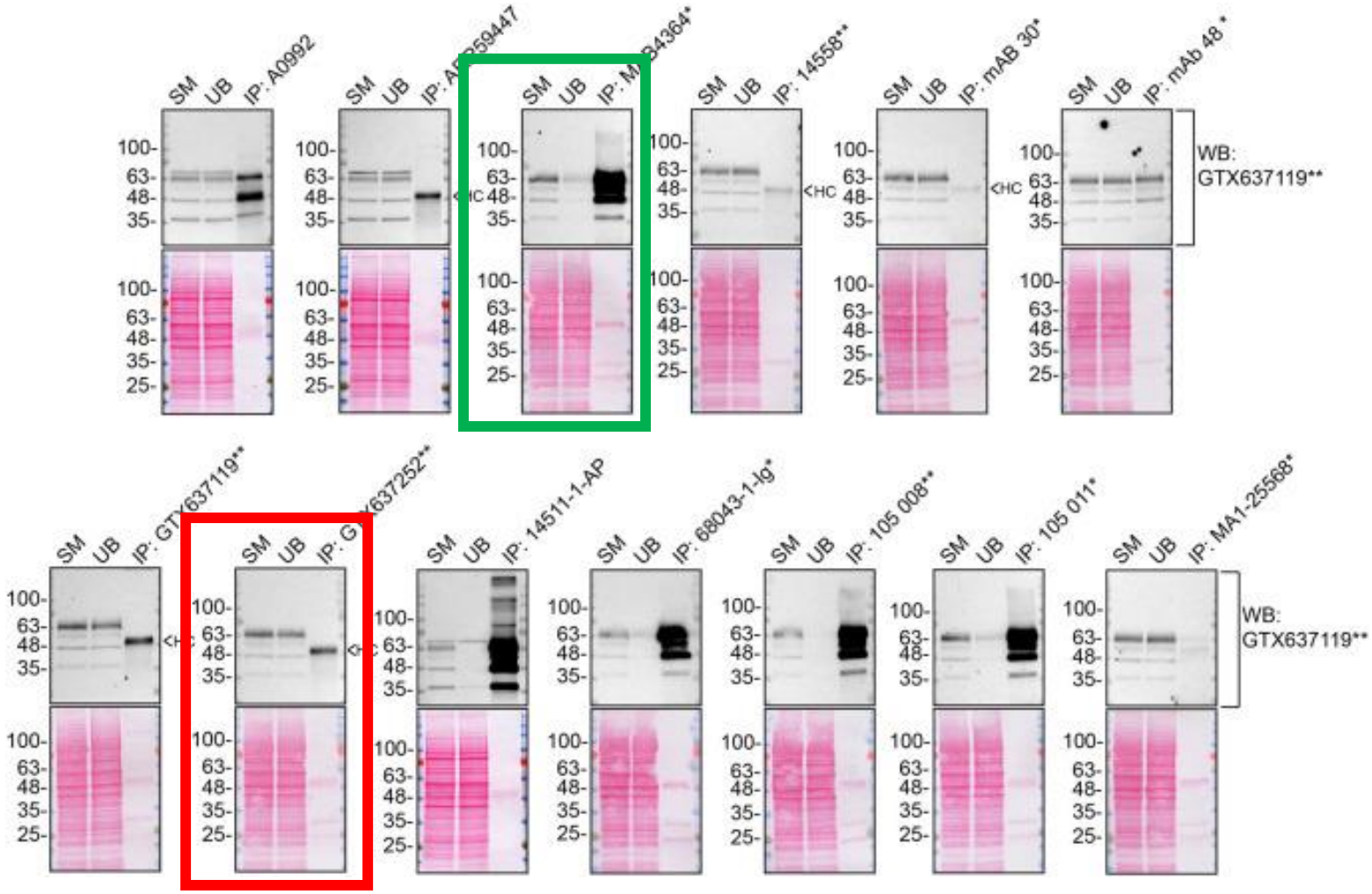




OGA
Only Good Antibodies

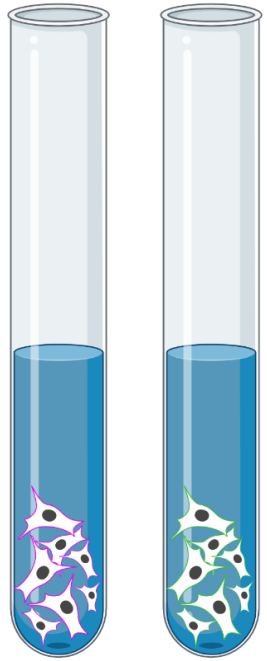
Immunoprecipitation

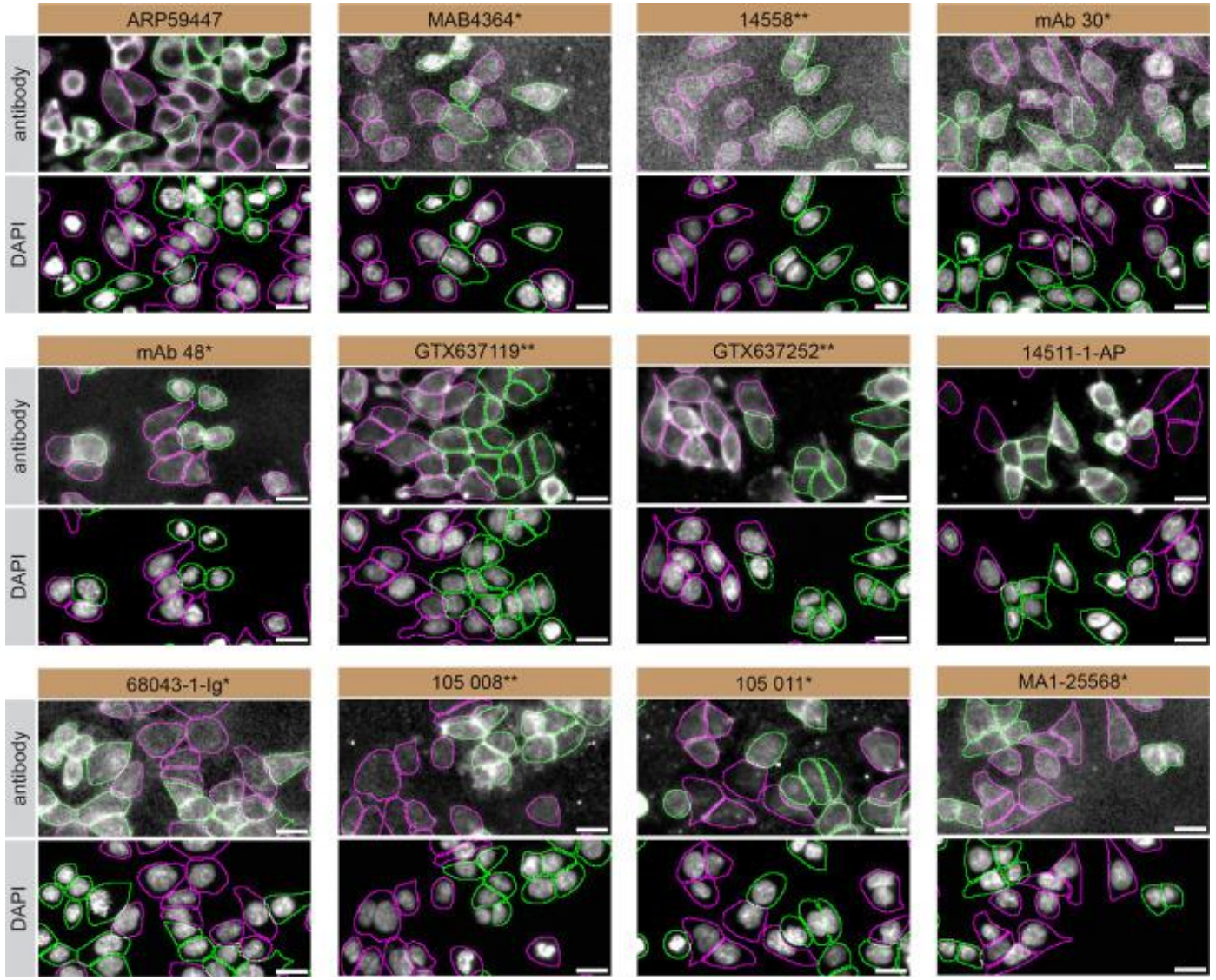




Only Good Antibodies

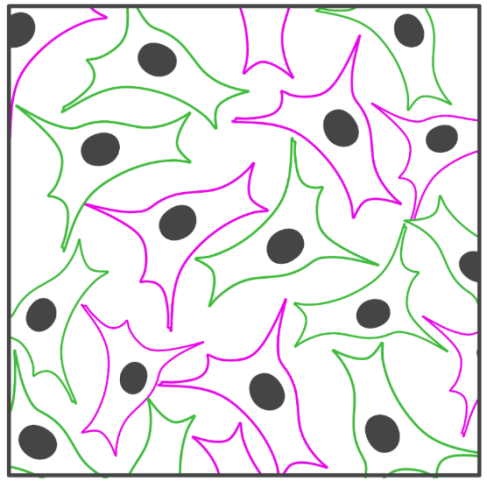
Immunofluorescence

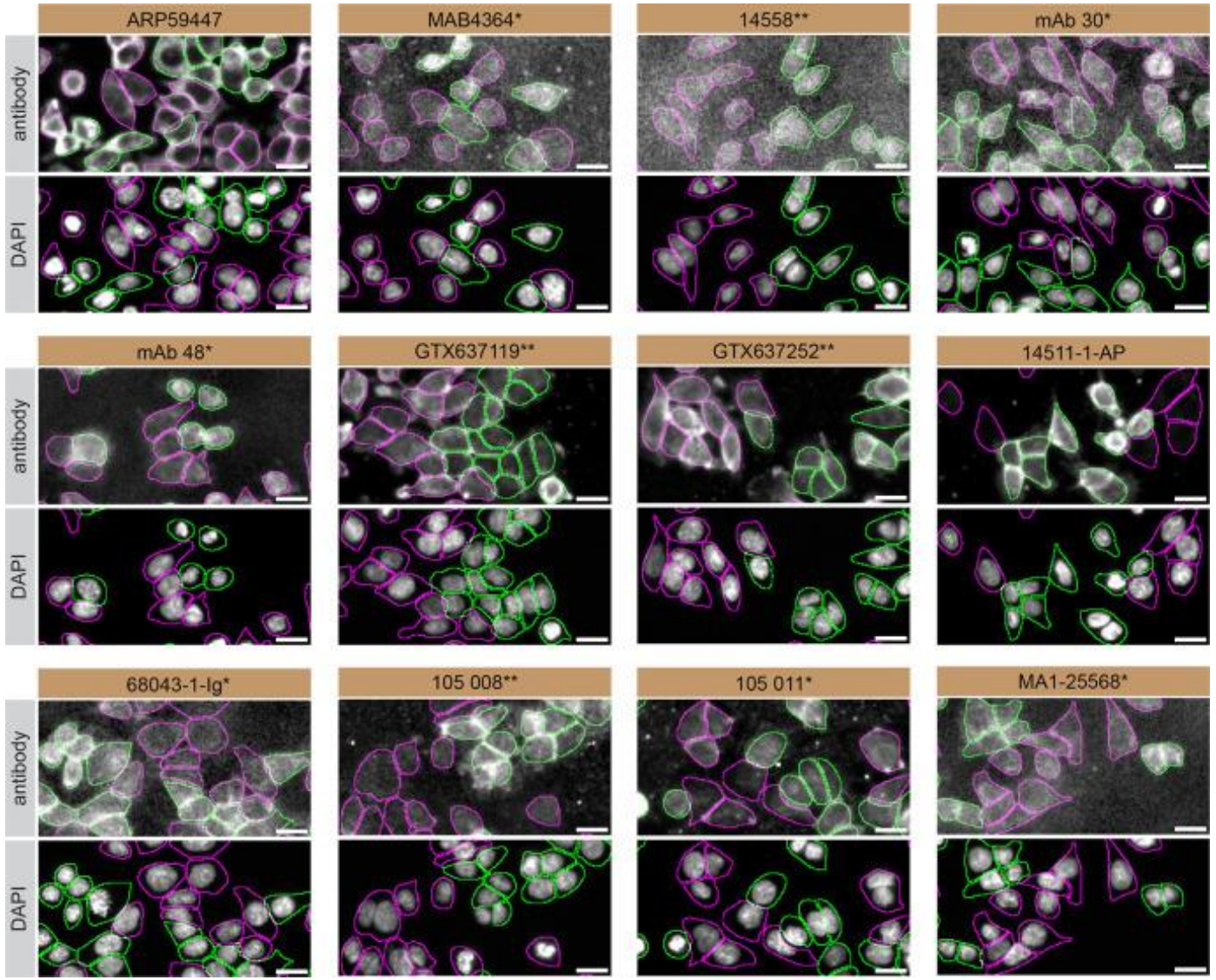




OGA
Only Good Antibodies

Immunofluorescence

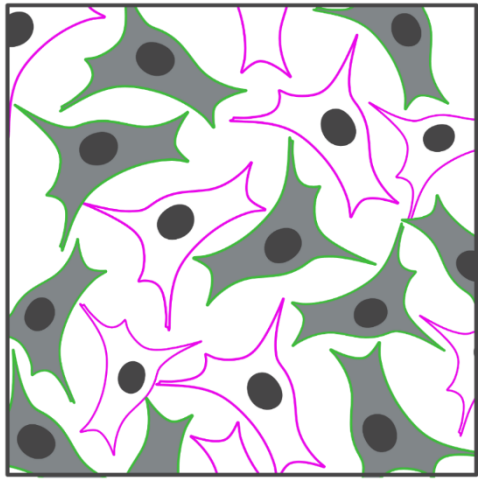


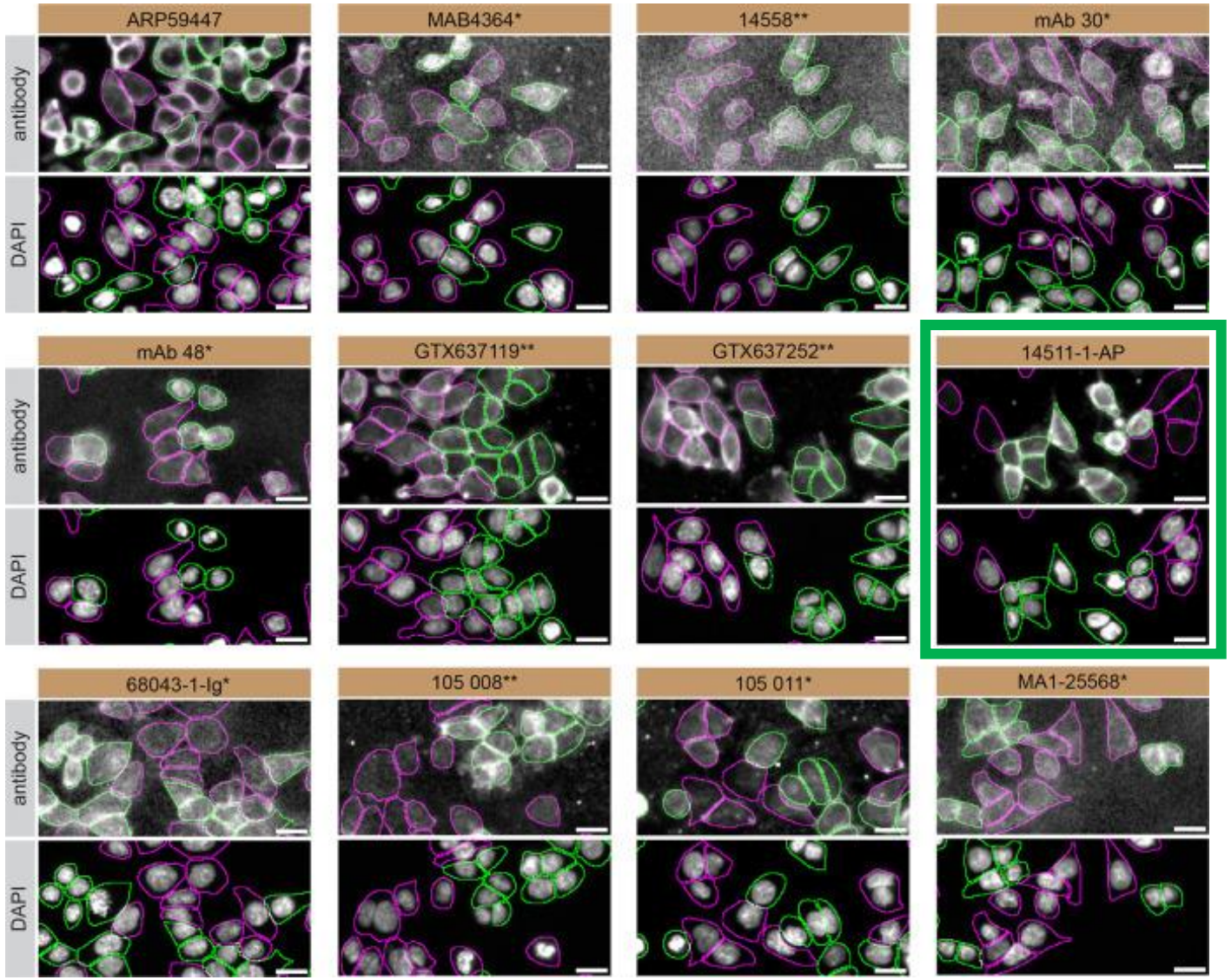


OGA
Only Good Antibodies

Immunofluorescence

Passed

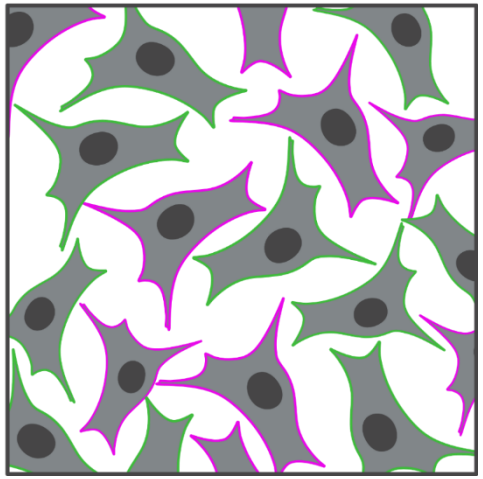


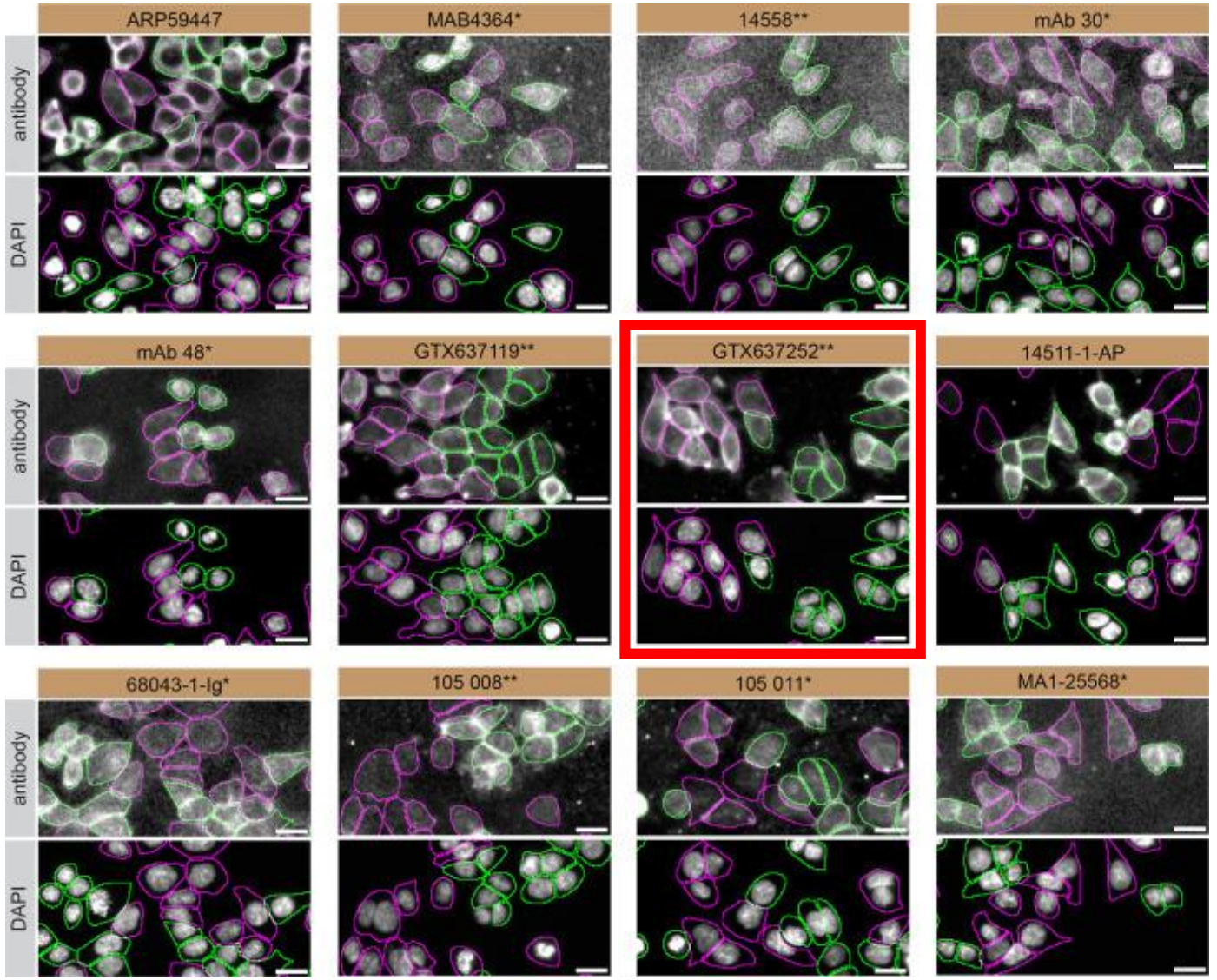


OGA
Only Good Antibodies

Immunofluorescence

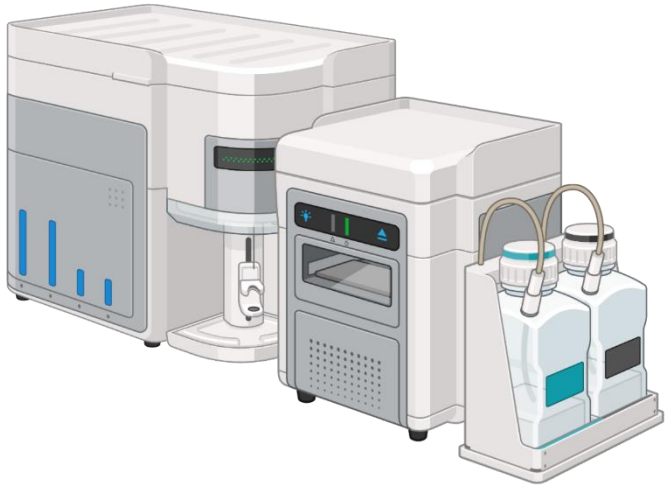
Failed

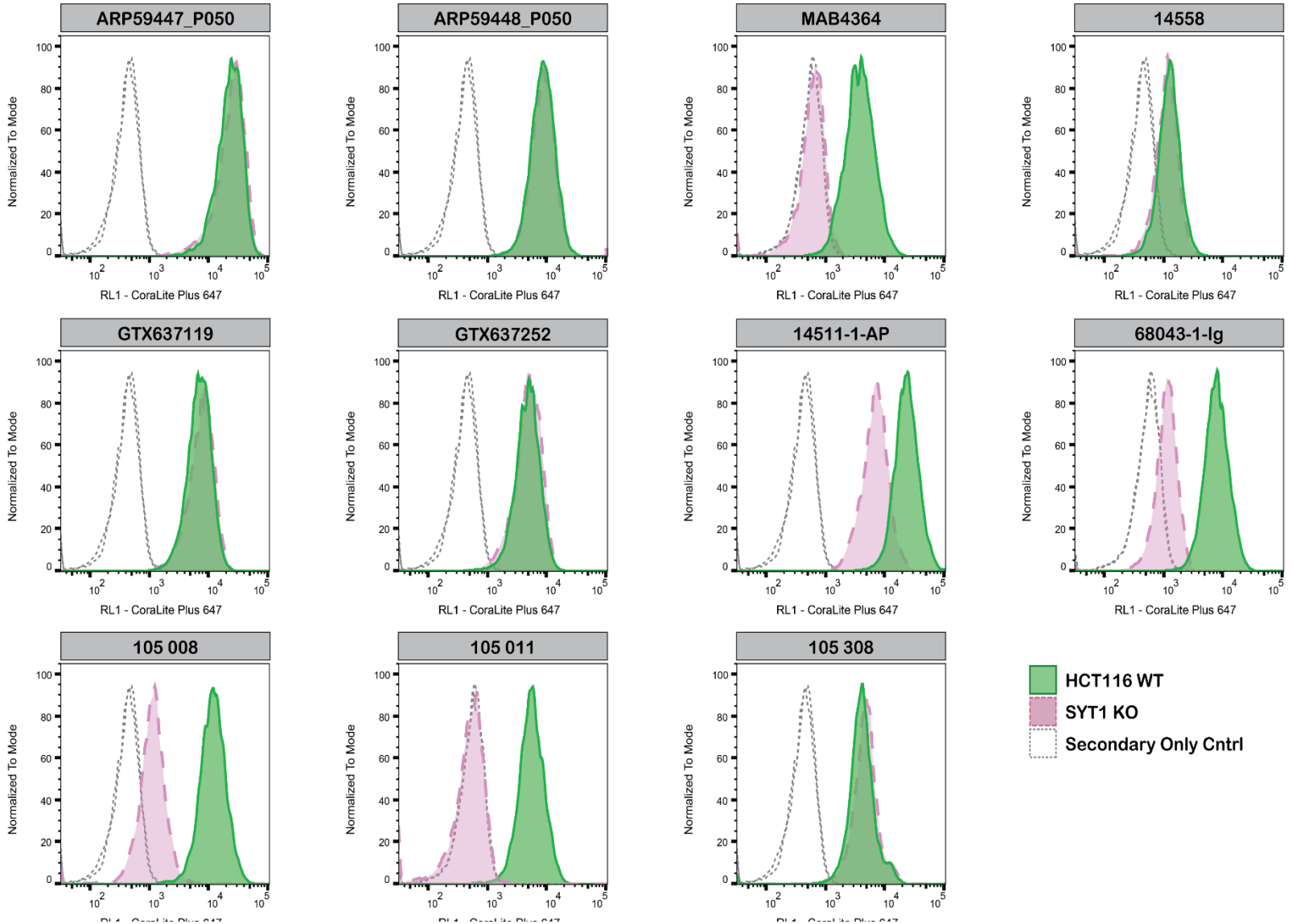




OGA
Only Good Antibodies

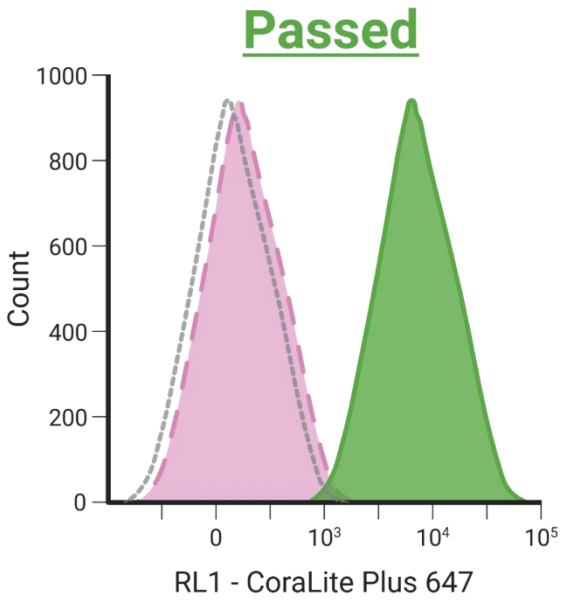
Flow cytometry

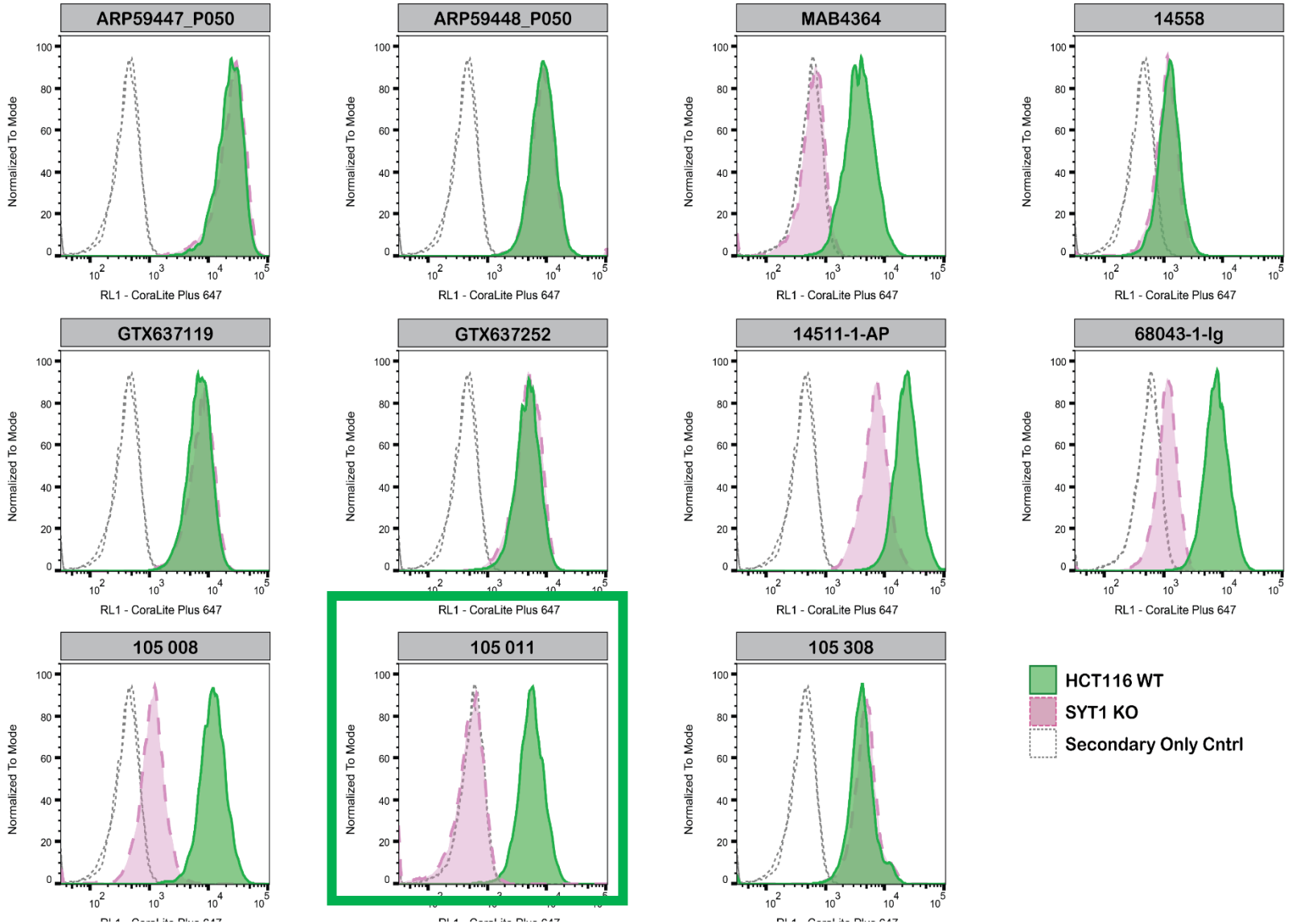




OGA
Only Good Antibodies

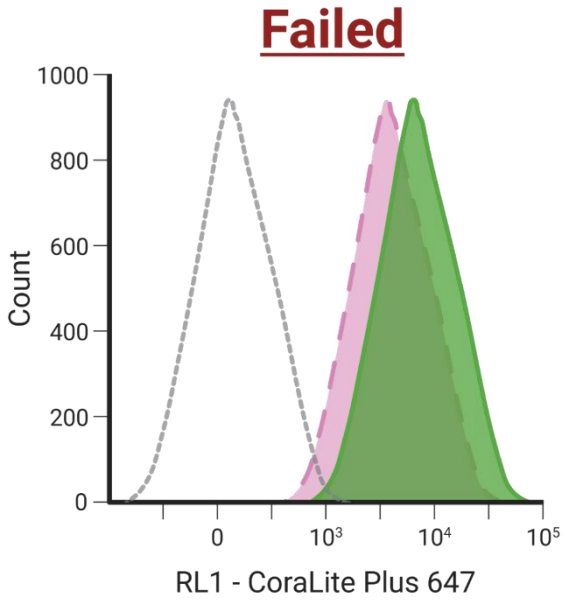
Flow cytometry

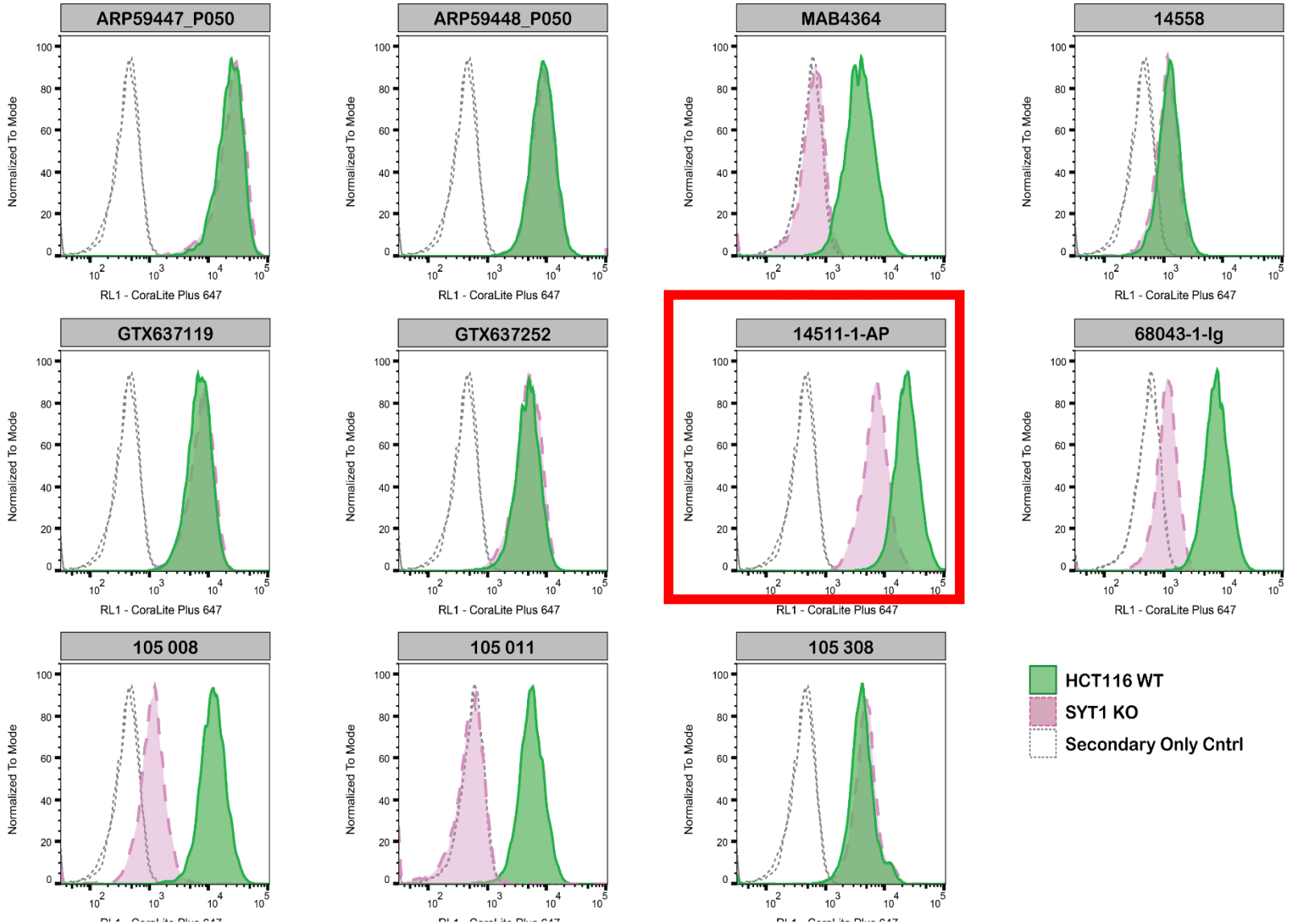




OGA
Only Good Antibodies

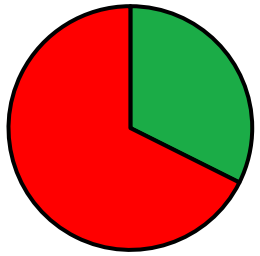
Flow cytometry



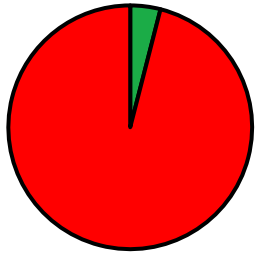


OGA
Only Good Antibodies

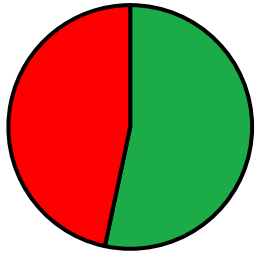
Flow data



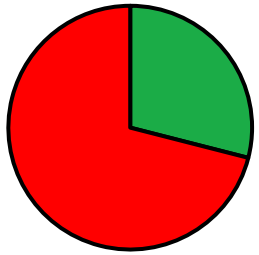
32.4% of antibodies are selective in flow cytometry (45/139).



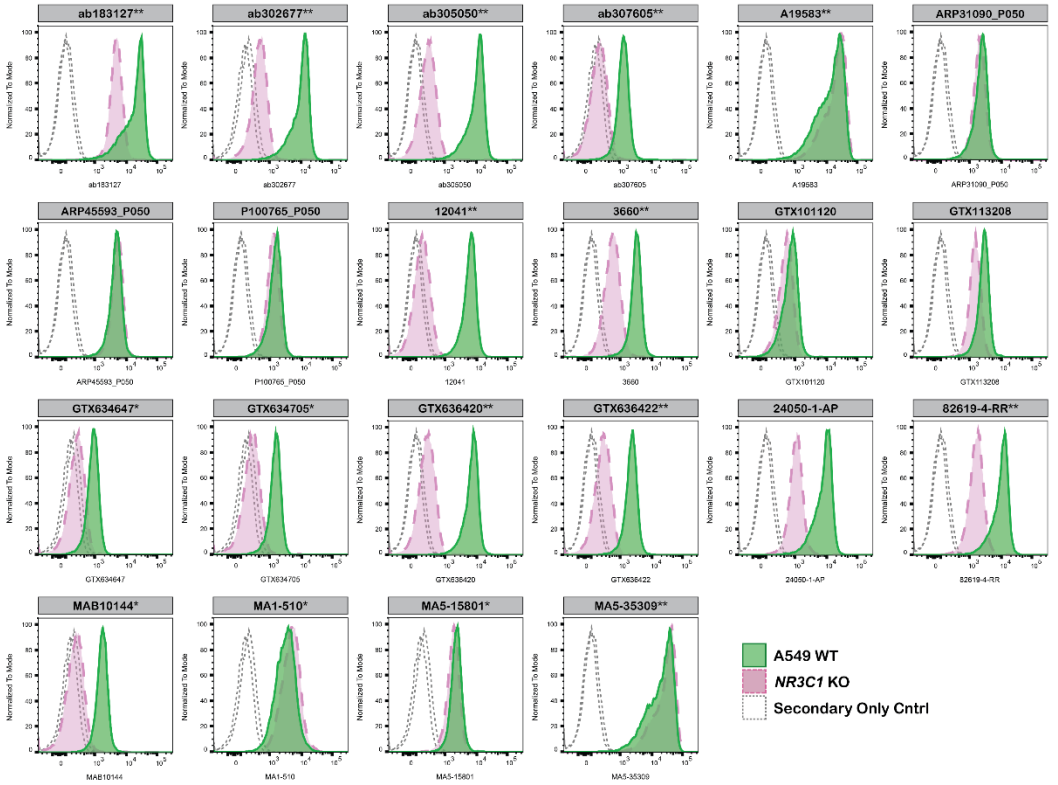
Polyclonal antibodies
4% are selective (1/25)



Monoclonal antibodies
53% are selective (24/45)

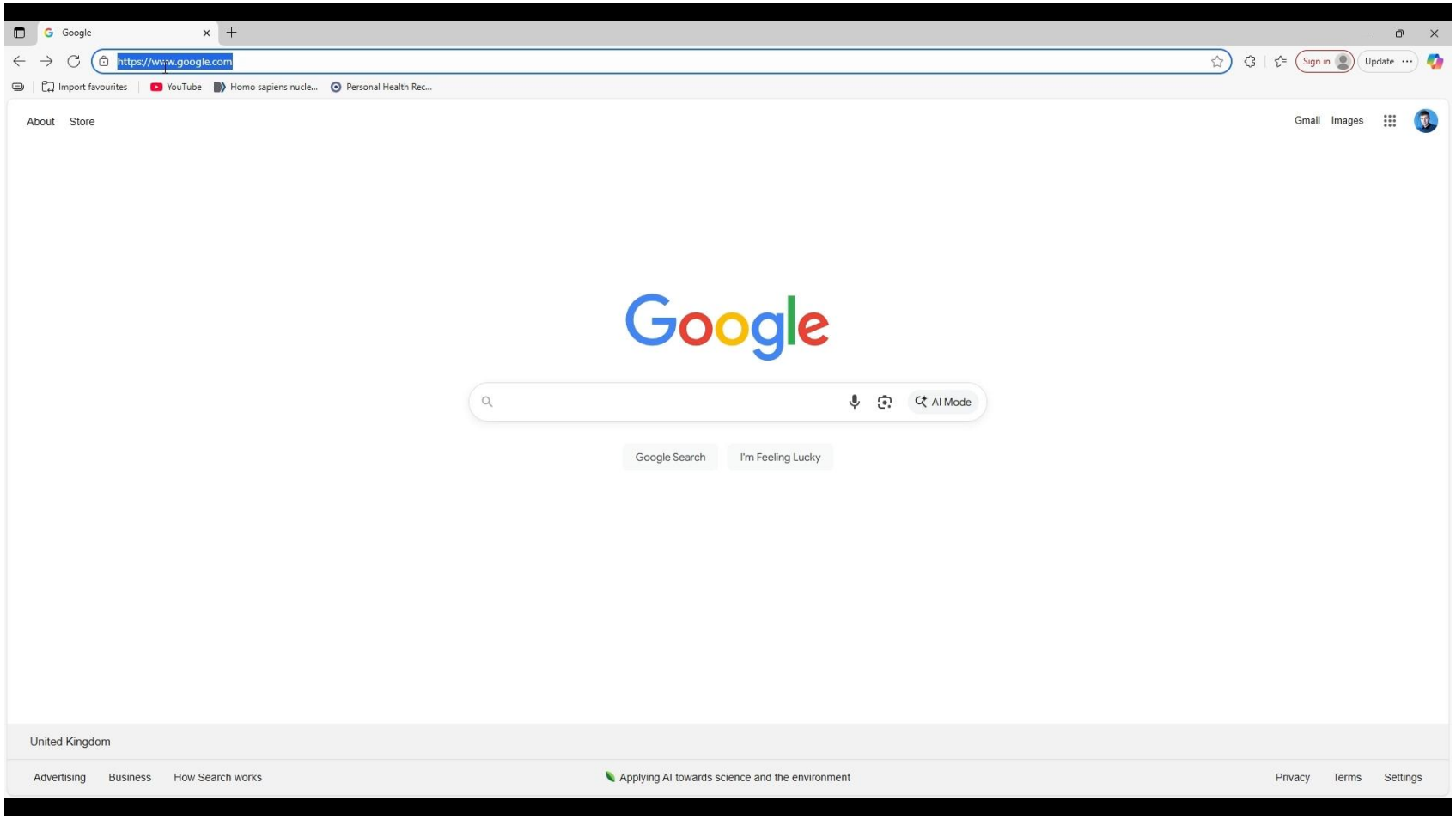


Recombinant antibodies
29% are selective (20/69)

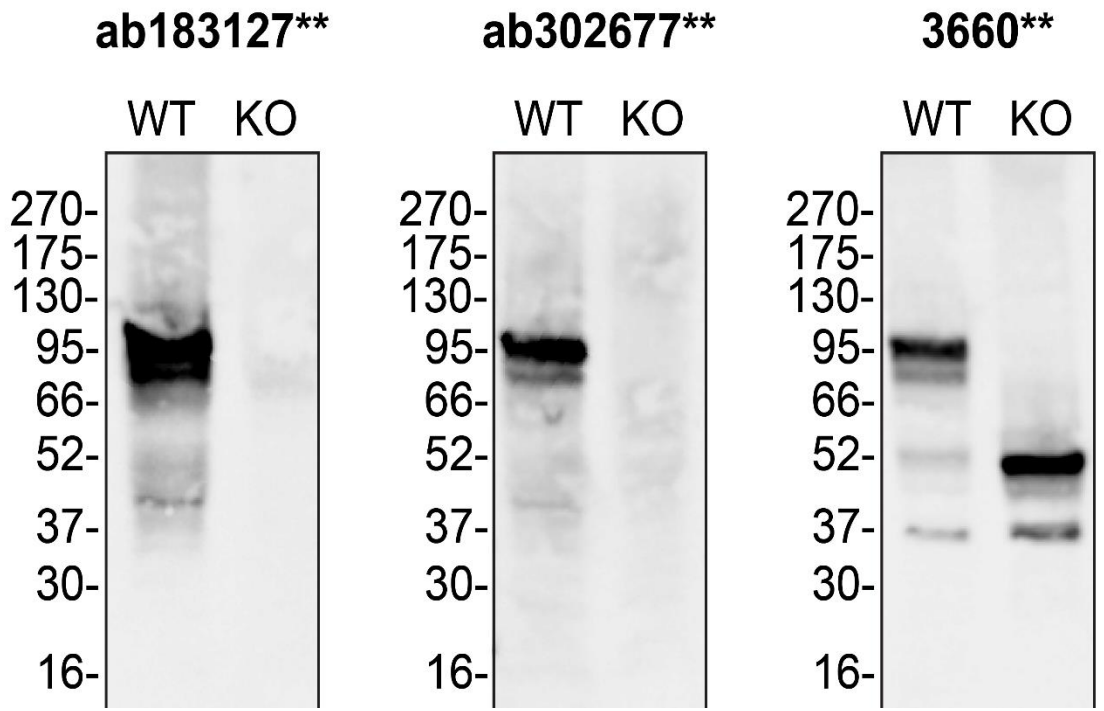


OGA
Only Good Antibodies

www.onlygoodantibodies.co.uk



OGA
Only Good Antibodies



- Glucocorticoid receptor (*NR3C1*)
  - commercially available KO
  - contains either increased expression of alternative splice variant, or truncated protein
  - only able to determine this with multiple antibodies with different epitopes
  - this is an issue we see 25 – 50% of the time depending on the target

OGA
Only Good Antibodies

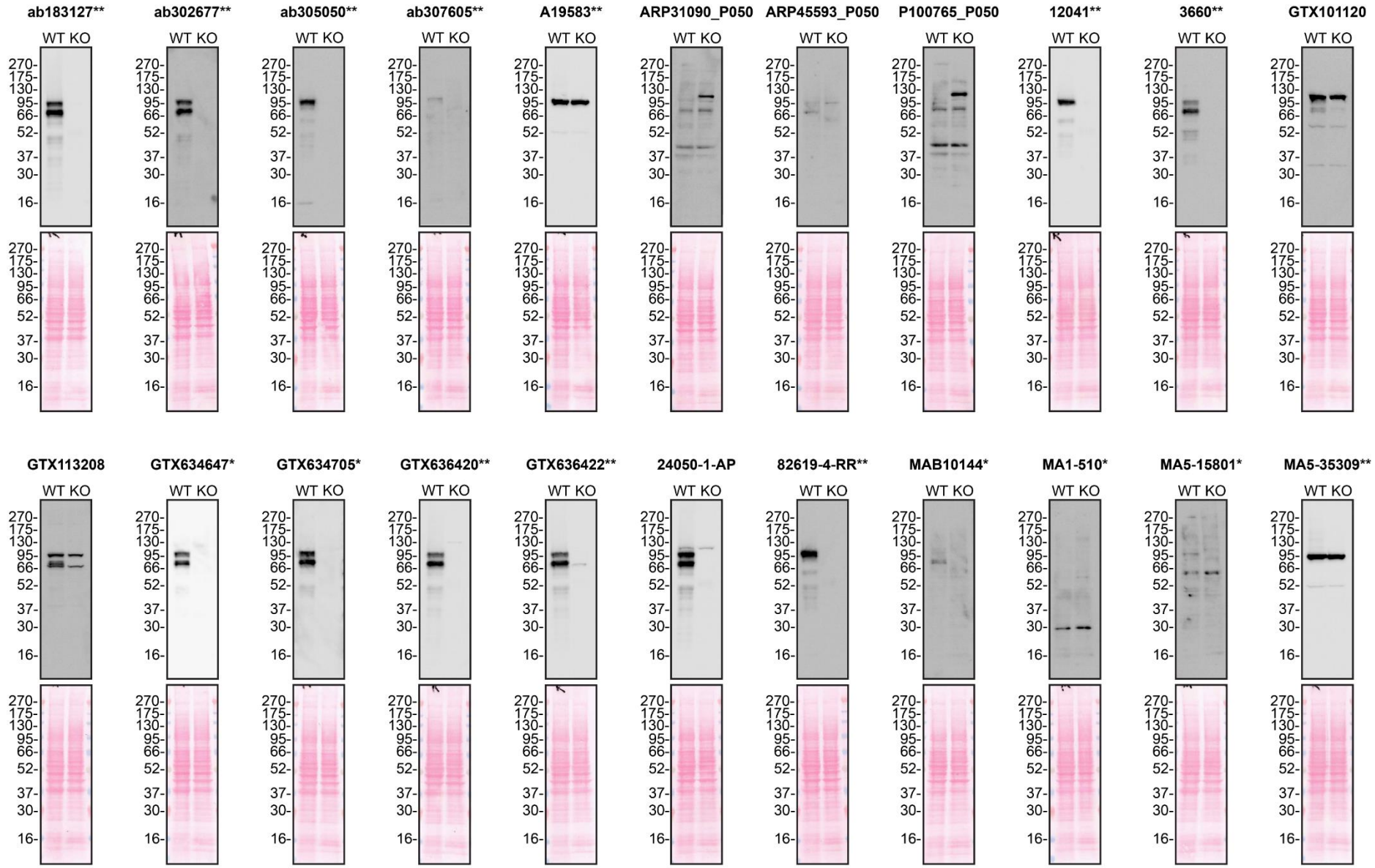


Figure 1: Glucocorticoid receptor antibody screening by immunoblot

OGA
Only Good Antibodies

Conclusion

Better antibodies
Better science
Better patient outcomes

Reach out to us for help!

If you want to know more, please get in touch.

msb65@leicester.ac.uk

OGA
Only Good Antibodies

UNIVERSITY OF LEICESTER

Institute for Precision Health