
Figures and figure supplements

Scaling of an antibody validation procedure enables quantification of antibody performance in major research applications

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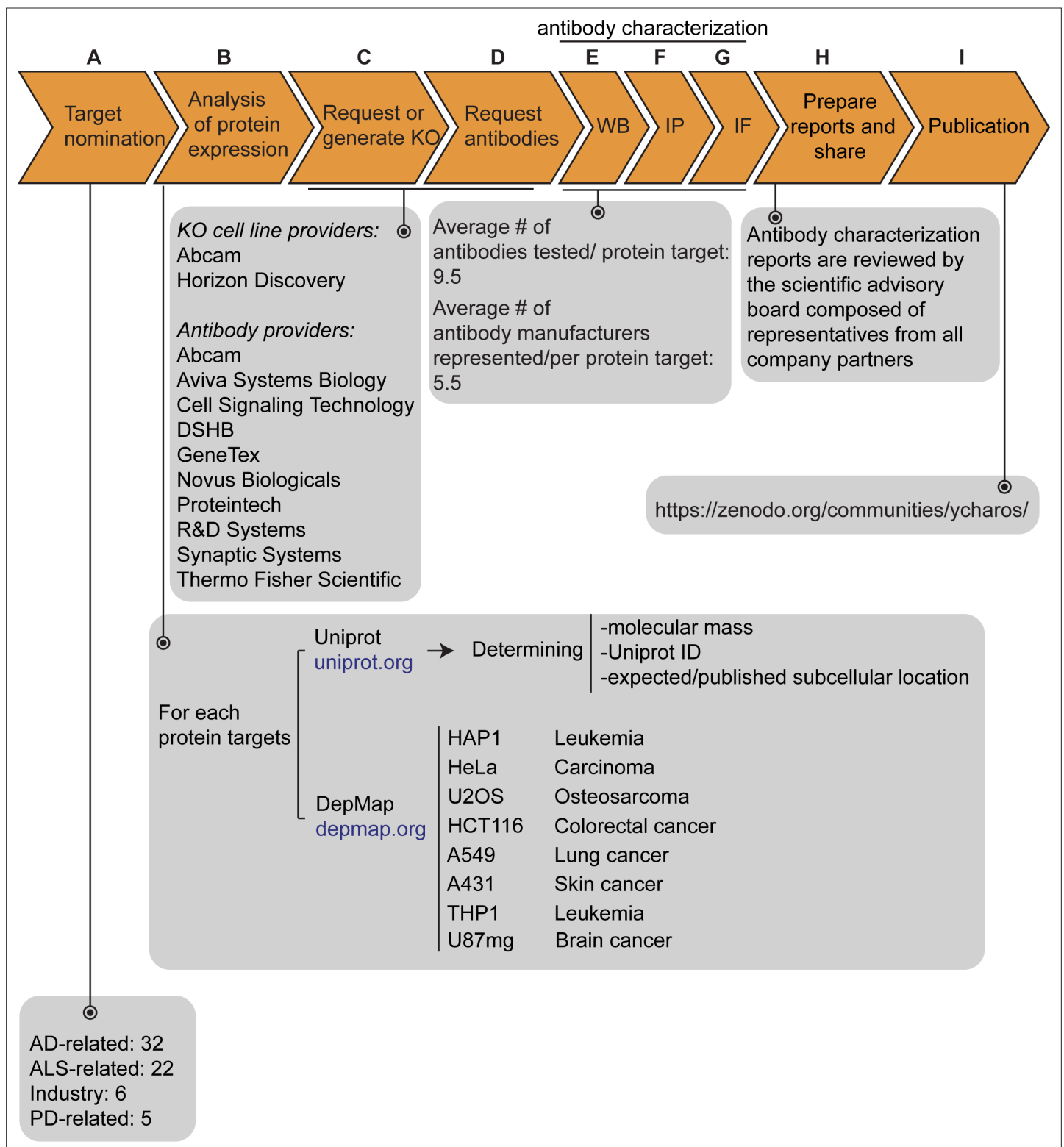


Figure 1. Antibody characterization platform. **(A)** The funders of the targets analyzed in this study and the number of targets proposed by each are indicated. **(B)** Bioinformatic analyses of nominated proteins using Uniprot to determine their molecular mass, unique Uniprot ID and published/expected subcellular distribution. In parallel, analyses of the Cancer Dependency Map ("DepMap") portal provided RNA sequencing data for the designated target, which guided our selection of cell lines with adequate expression for the generation of custom KO cell lines. A subset of cell lines amenable for genome engineering were prioritized. **(C)** Receive relevant KO cell lines or generate custom KO lines and **(D)** receive antibodies from manufacturing partners. All contributed antibodies were tested in parallel by **(E)** WB using WT and KO cell lysates ran side-by-side, **(F)** IP followed by WB using a KO-

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validated antibody identified in (E) and by (G) IF using a mosaic strategy to avoid imaging and analysis biases. (H) Antibody characterization data for all tested antibodies were presented in a form of a protein target report. All reports were shared with participating companies for their review. (I) Reviewed reports were published on ZENODO, an open access repository. ALS-RAP=amyotrophic lateral sclerosis-reproducible antibody platform, AD = Alzheimer's disease, MJFF = Michael J. Fox Foundation. KO = knockout cell line.

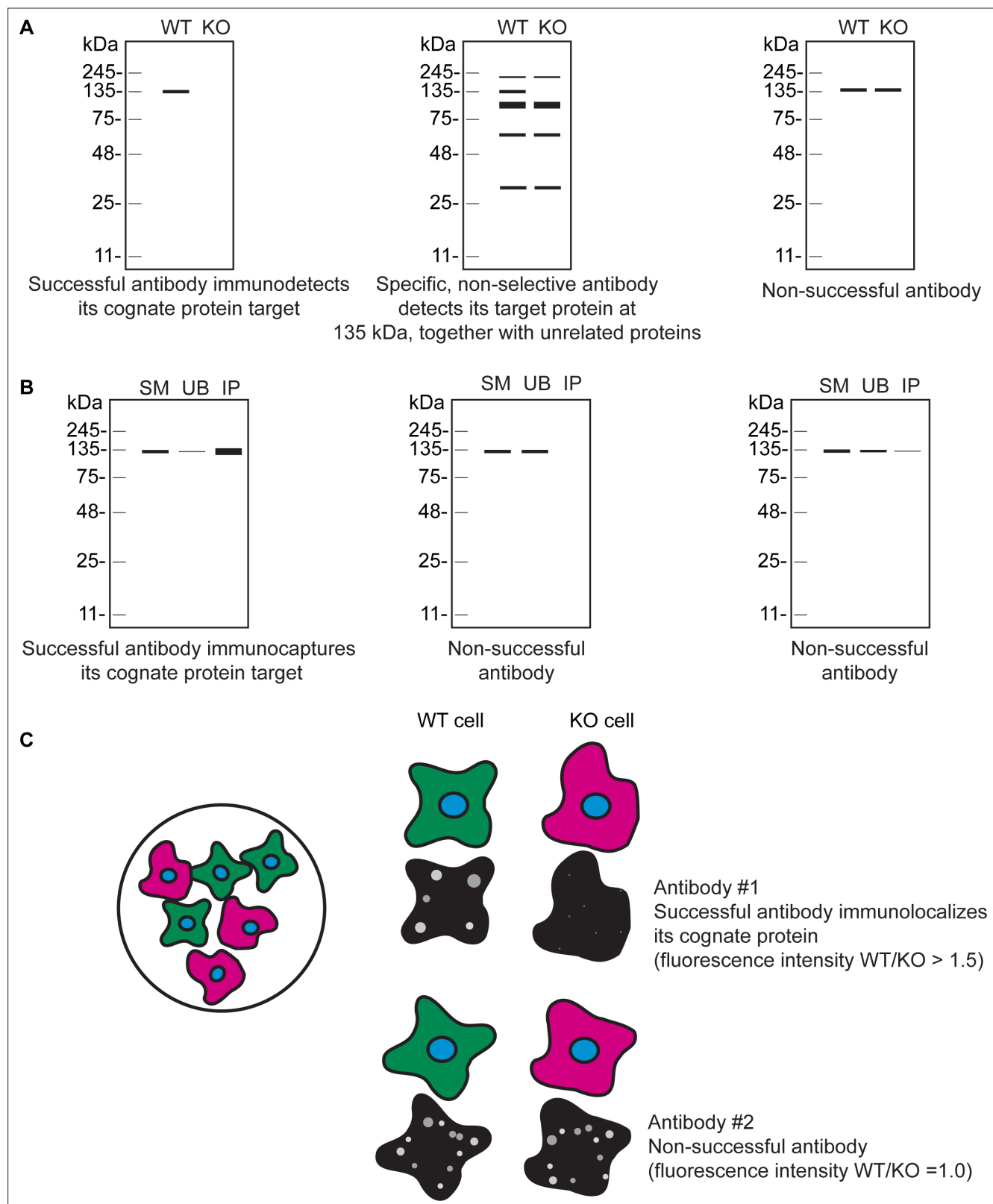


Figure 1—figure supplement 1. Schematic representations of antibody performance. (A) Schematic representations of a successful antibody (left schematic), specific, non-selective antibody (middle schematic), and a non-successful antibody (right schematic) for WB. (B) Schematic representations of a successful antibody (left schematic) and non-successful antibodies (middle and right schematics) for IP. (C) Schematic representation of the mosaic strategy used (left schematic). WT cells are labelled with a fluorescent cell dye (green), and KO cells are labelled with a different fluorescent cell dye

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(magenta) plated together as a mosaic. Schematic representations of a successful antibody (antibody #1) and a non-successful antibody (antibody #2) for IF are shown.

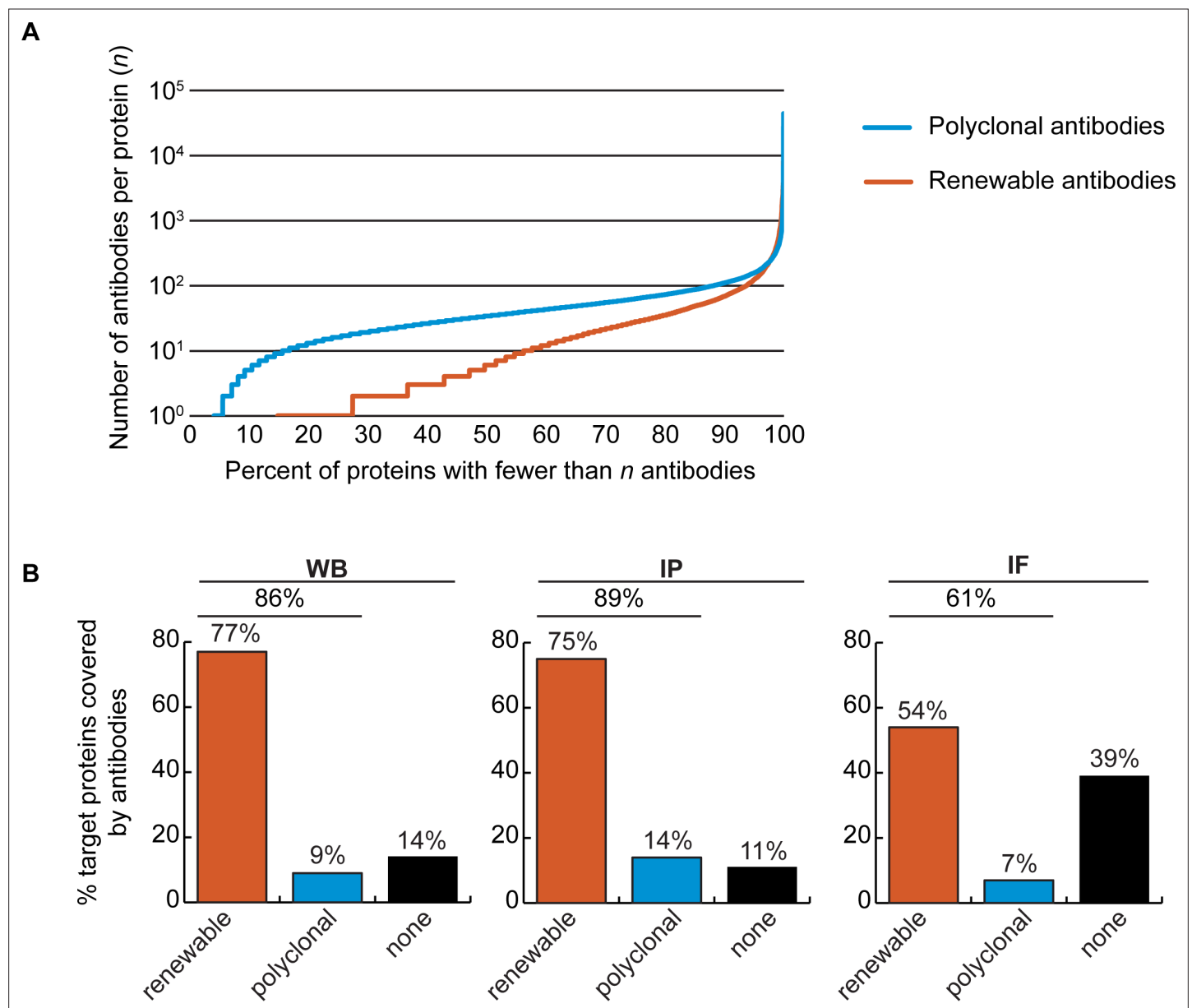


Figure 2. Analysis of human protein coverage by antibodies. **(A)** Cumulative plot showing the percentage of the human proteome that is covered by polyclonal antibodies (blue line) and renewable antibodies (monoclonal + recombinant; orange line). The number of antibodies per protein was extracted from the Antibody Registry database. **(B)** Percentage of target proteins covered by minimally one renewable successful antibody (orange column) or covered by only successful polyclonal antibodies (blue column) is shown for each indicated applications using a bar graph. Lack of successful antibody ('none') is also shown (black column).

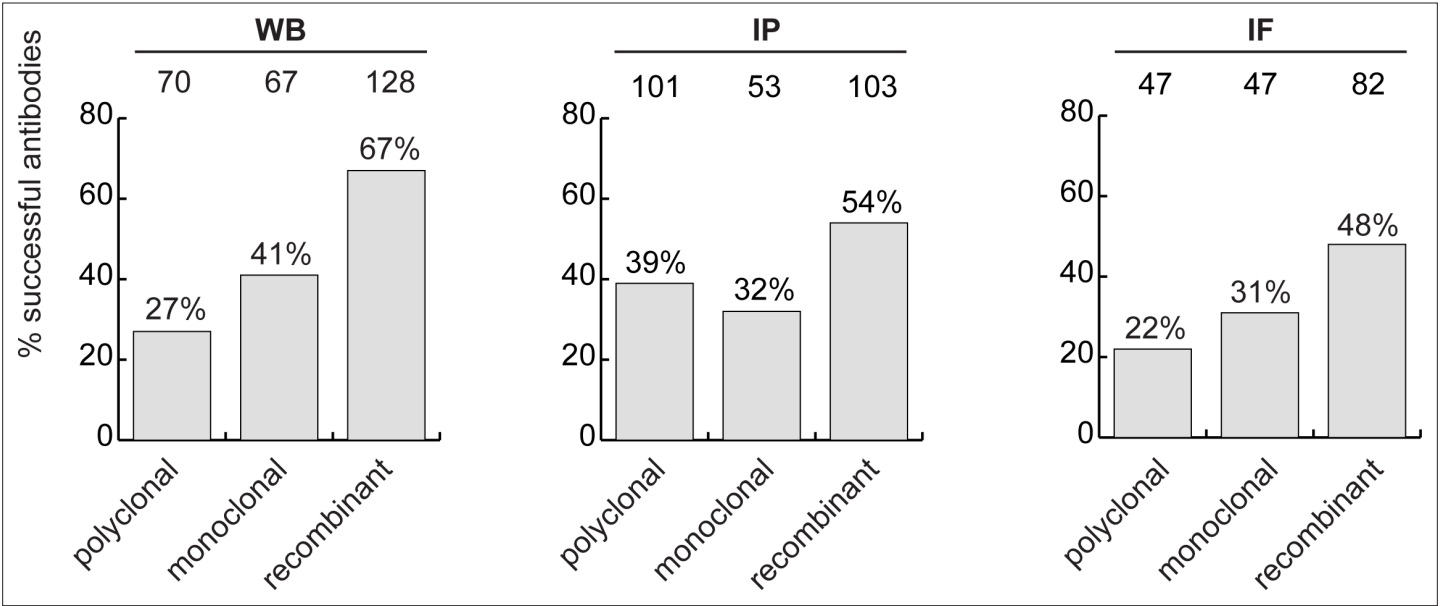


Figure 3. Analysis of antibody performance by antibody types. The percentage of successful antibodies based on their clonality is shown using a bar graph, for each indicated application. The number of antibodies represented in each category is indicated above the corresponding bar.

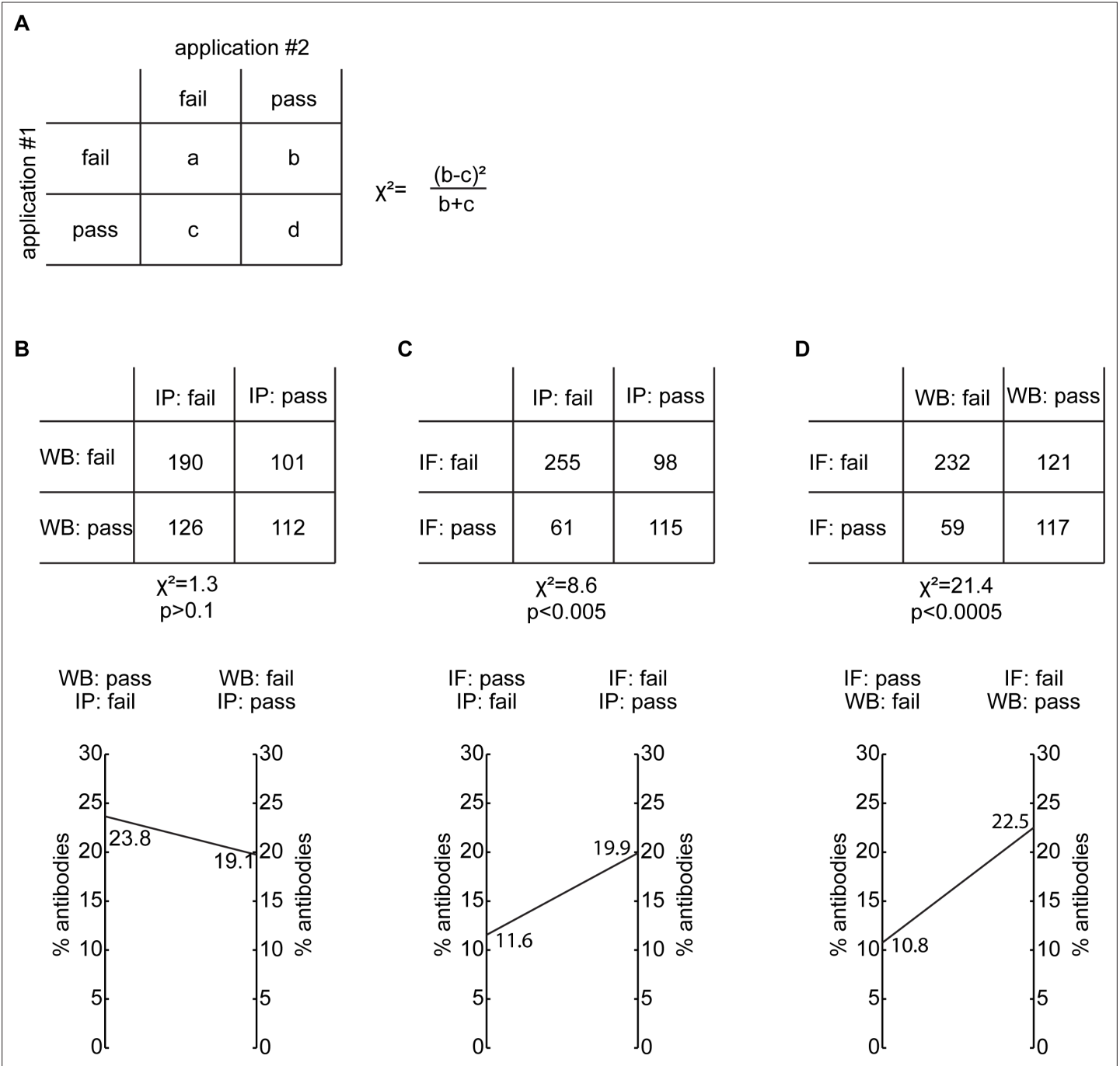


Figure 3—figure supplement 1. Correlation of antibody performance between applications. **(A)** Representation of a 2x2 contingency table used to apply the McNemar Test as well as the equation of the chi-square (χ^2) statistic used. Analysis of antibody performance correlation represented as a contingency table and as a double y-axis graph between **(B)** WB and IP, **(C)** IF and IP and **(D)** IF and WB. n/s=non-significant.

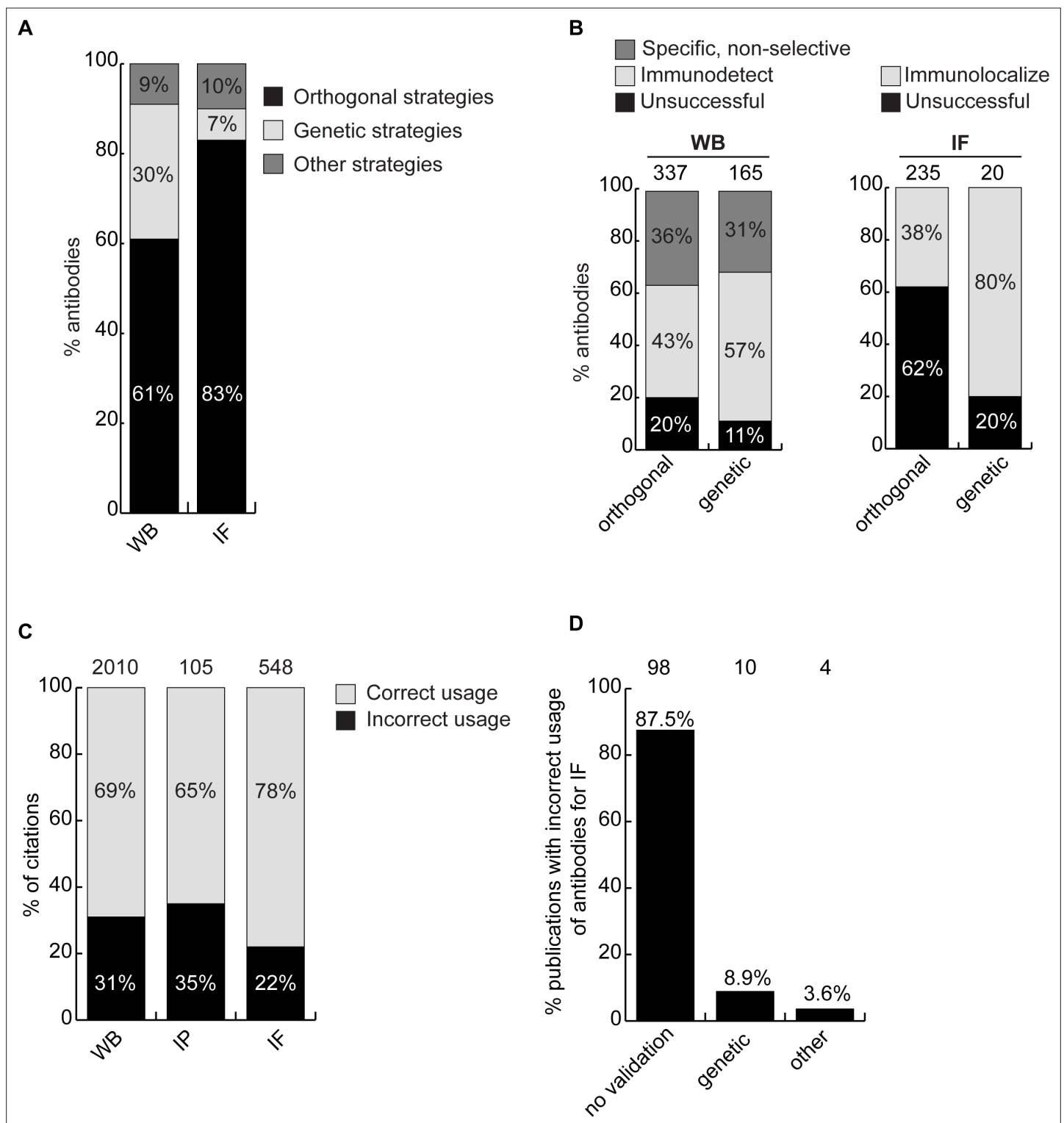


Figure 4. Scientific value of antibody characterization methods and research usage. (A) Percentage of antibodies validated by suppliers using one of the indicated methods for WB or IF showed using a bar graph with stacked columns. The percentage corresponding to each section of the bar graph is shown directly in the bar graph. Orthogonal = orthogonal strategies, genetic = genetic strategies. (B) Percentage of successful (light gray), specific, non-selective (dark gray-only for WB) and unsuccessful (black) antibodies according to the validation method used by the manufacturer for WB and IF as compared to the KO strategy used in this study. Data are shown using a bar graph with stacked columns. The percentage corresponding to each section of the bar graph is shown directly in the bar graph. The number of antibodies analyzed corresponding to each condition is shown above each bar. (C) Percentage of publications that used antibodies that successfully passed validation (correct usage) or to antibodies that were unsuccessful

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in validation (incorrect usage) showed using a bar graph with stacked columns. The number of publications was found by searching CiteAb. The percentage corresponding to each section of the bar graph is shown in the bar graph and the number of publications represented in each category is shown above the corresponding bar. **(D)** Percentage of publications that used an unsuccessful antibody for IF from **(C)** that provided validation data for the corresponding antibodies. Data is shown as a bar graph. The number of publications represented in each category is shown above the corresponding bar.

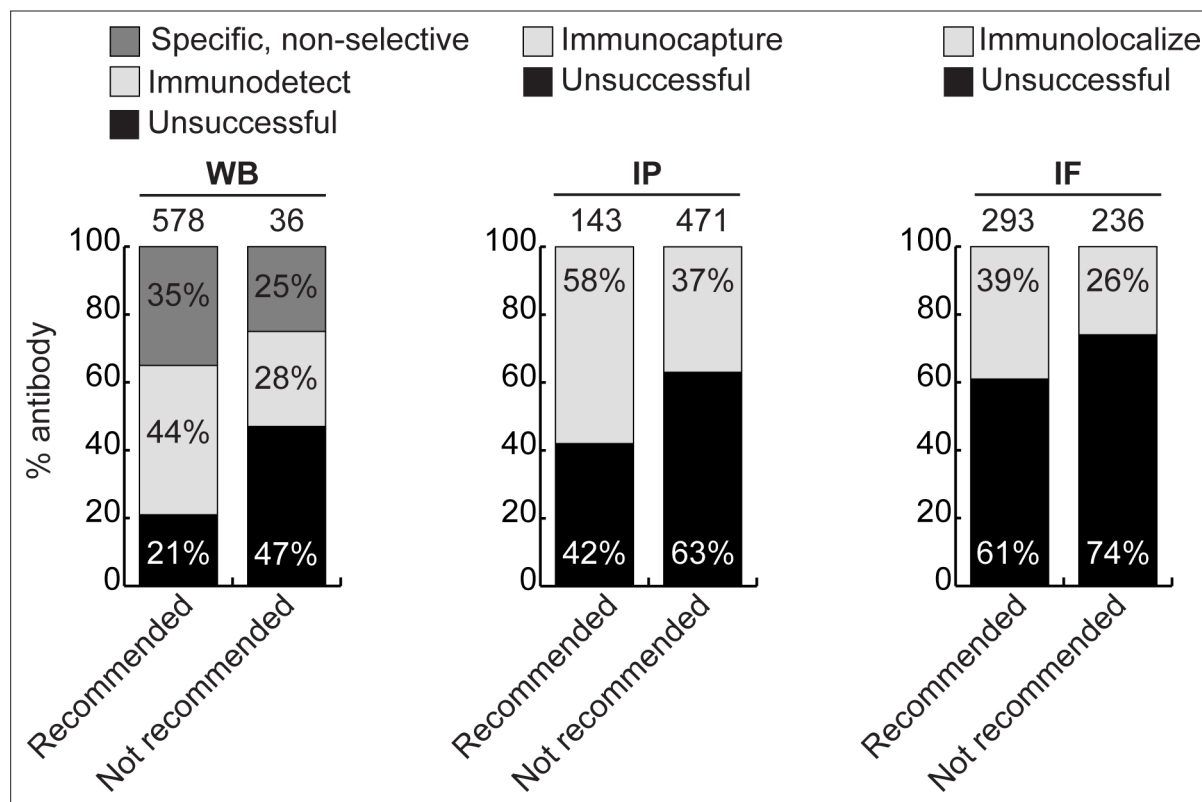


Figure 4—figure supplement 1. Analysis of antibody performance by manufacturer's catalogue recommendation. Percentage of successful or unsuccessful antibodies for the indicated applications are shown using a bar graph with stacked columns. Antibodies were divided according to whether they were recommended or not recommended by the manufacturers for the indicated applications. The percentage corresponding to each section of the bar graph is shown in the graph, and the total number of antibodies represented in each category is indicated above the corresponding bar.

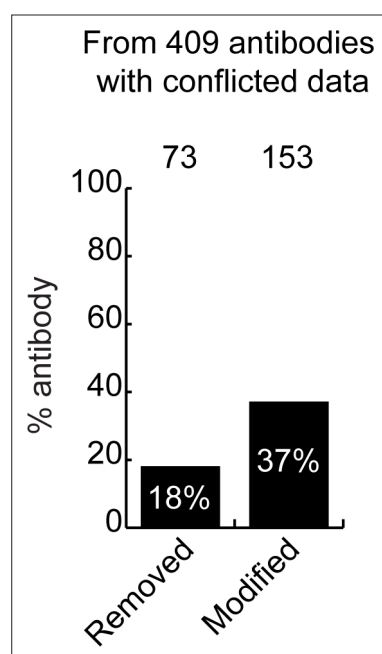


Figure 4—figure supplement 2. Actions taken from participating companies. The percentage of antibodies removed from the market, or for which catalogue recommendations were modified following assessment of our data by our antibody manufacturing partners. The number of antibodies represented in each category is indicated above the corresponding bar.

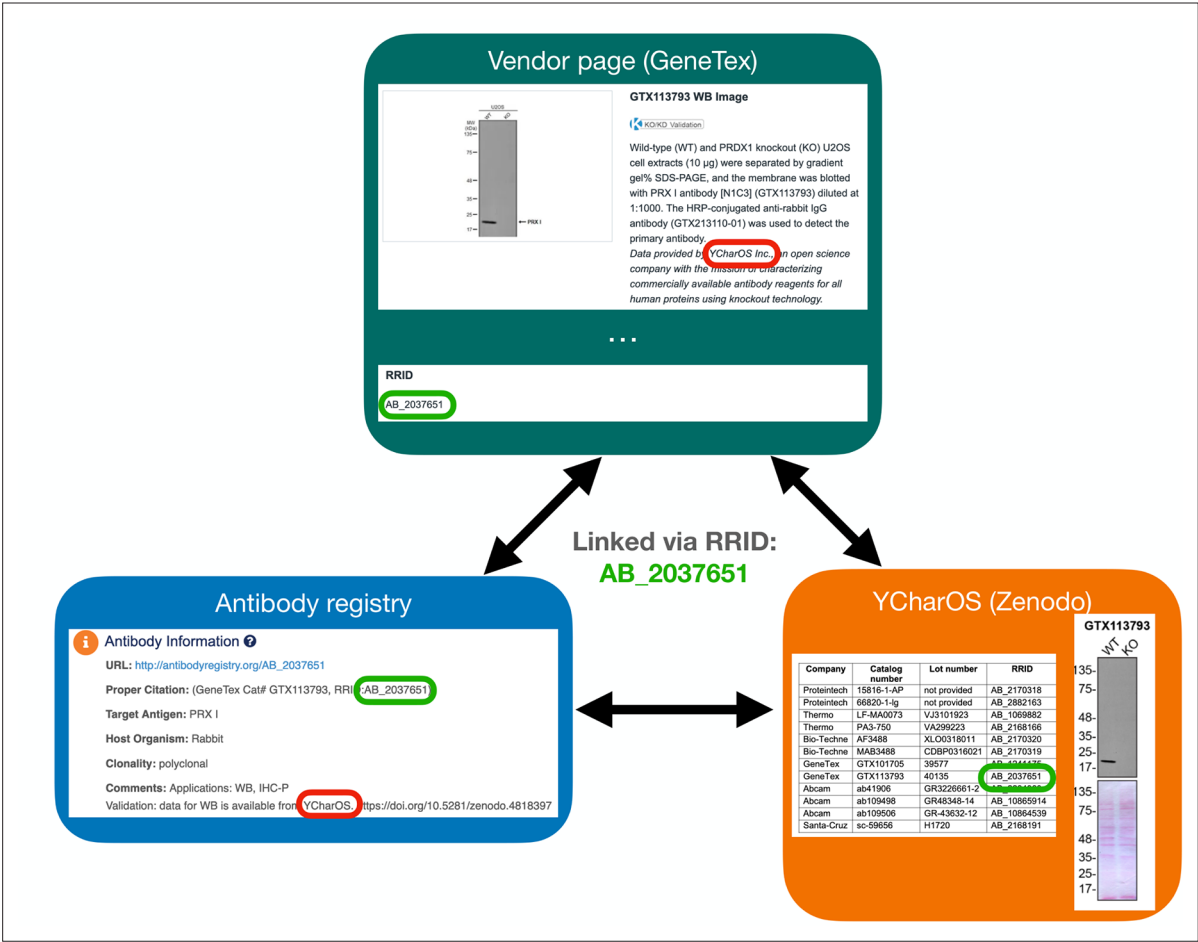


Figure 5. Accessing antibody characterization data using RRIDs. An antibody RRID can be used to search characterization studies across various databases, such as the vendor page, the Antibody Registry and on the YCharOS community page on ZENODO. AB_2037651 is given as an example.