Supplemental Figure 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 (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.

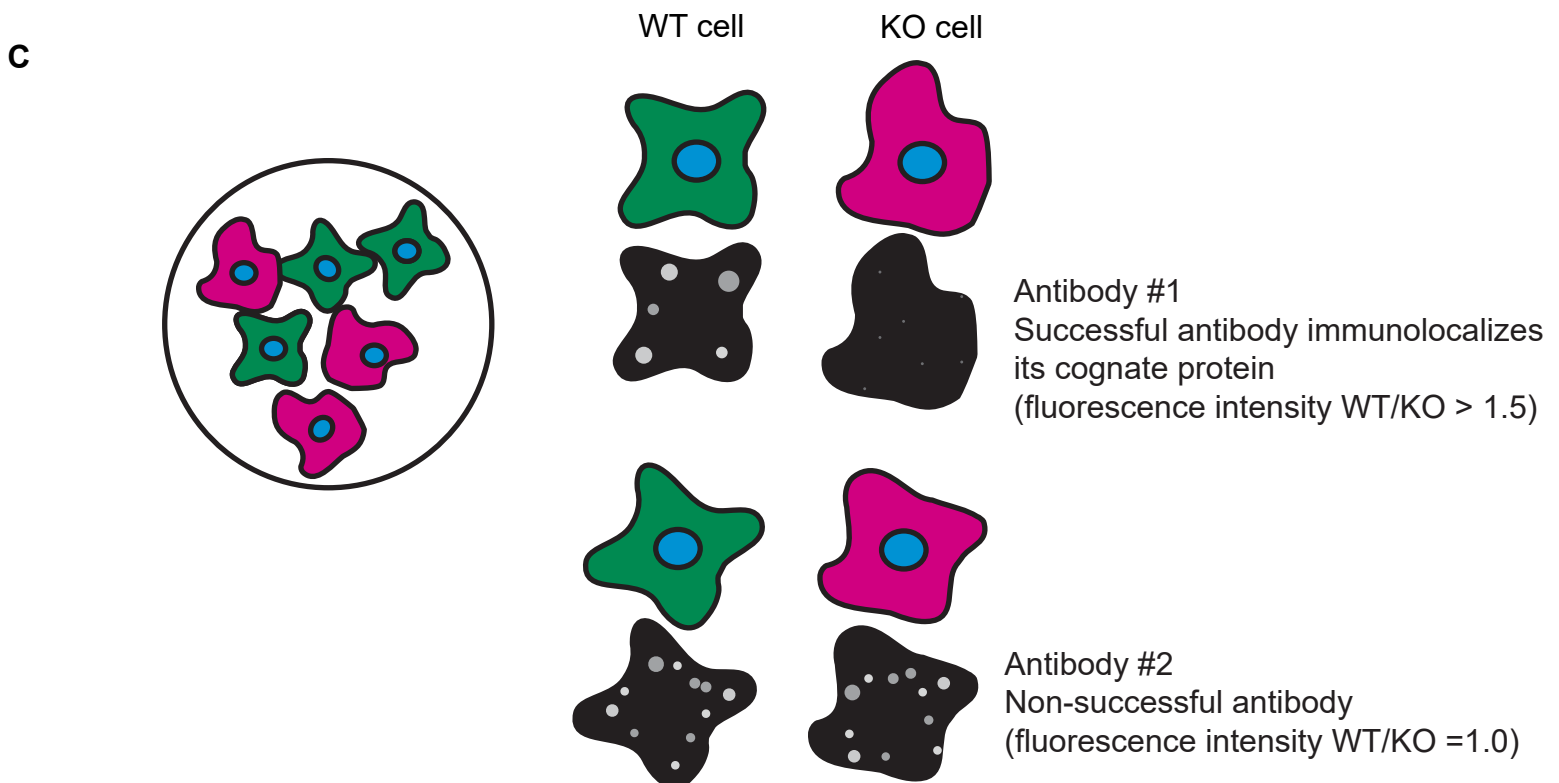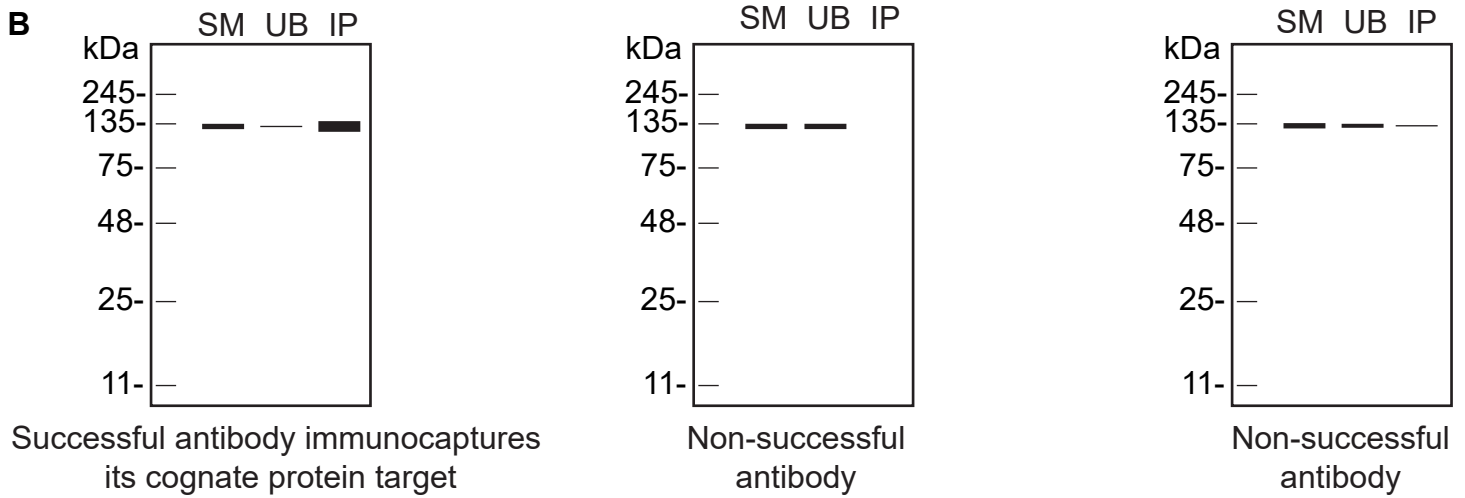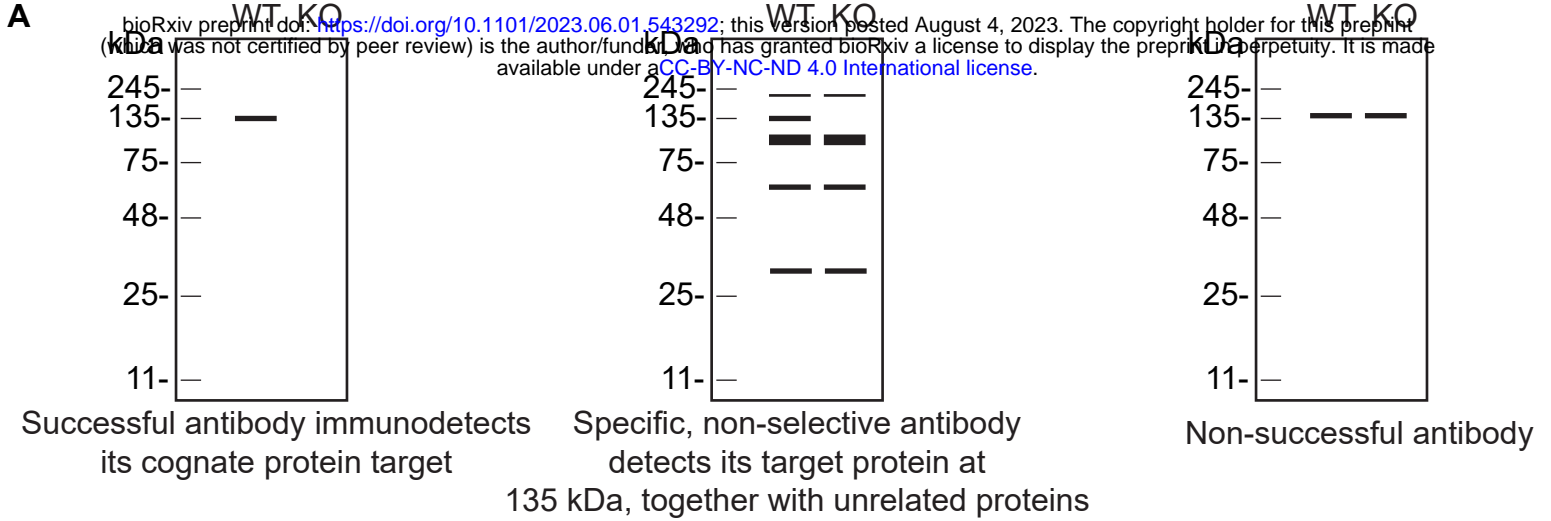**Supplemental Figure 2: Analysis of antibody performance by manufacturer's catalogue recommendation**

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.
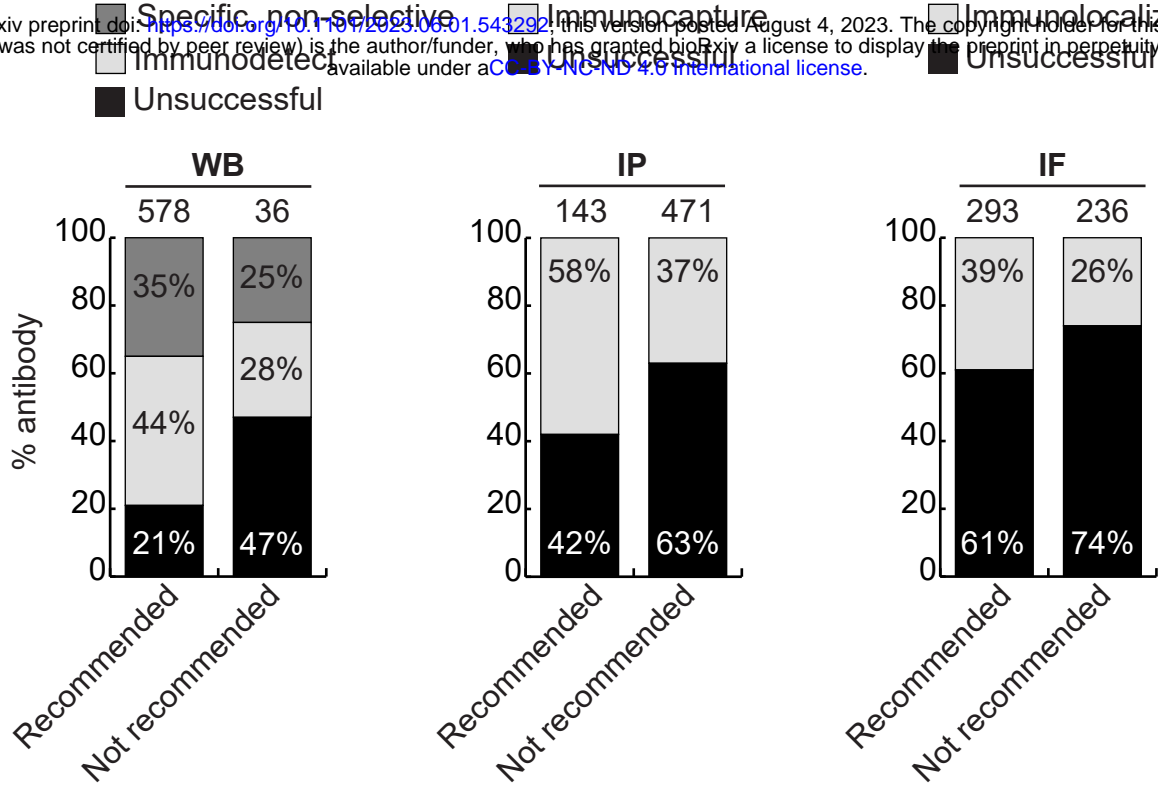
Supplemental Figure 3: Actions taken from participating companies

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.

Supplemental Figure 4: Correlation of antibody performance between applications

(**A**) Representation of a 2 x 2 contingency table used to apply the McNemar Test as well as the equation of the chi-square (X^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

A

WT KO

kDa
245-
135-
75-
48-
25-
11-

Successful antibody immunodetects
its cognate protein target

WT KO

kDa
245-
135-
75-
48-
25-
11-

Specific, non-selective antibody
detects its target protein at
135 kDa, together with unrelated proteins

WT KO

kDa
245-
135-
75-
48-
25-
11-

Non-successful antibody

B

SM UB IP

kDa
245-
135-
75-
48-
25-
11-

Successful antibody immunocaptures
its cognate protein target

SM UB IP

kDa
245-
135-
75-
48-
25-
11-

Non-successful
antibody

SM UB IP

kDa
245-
135-
75-
48-
25-
11-

Non-successful
antibody

C

WT cell   KO cell

Antibody #1
Successful antibody immunolocalizes
its cognate protein
(fluorescence intensity WT/KO > 1.5)

Antibody #2
Non-successful antibody
(fluorescence intensity WT/KO =1.0)

Ayoubi et al., Supplemental Figure 1

■ Specific, non-selective   ☐ Immunocapture   ☐ Immunolocalize
☐ Immunodetect   ■ Unsuccessful   ■ Unsuccessful
■ Unsuccessful

Ayoubi et al., Supplemental Figure 2

Ayoubi et al., Supplemental Figure 3

A

application #1

	application #2	
	fail	pass
fail	a	b
pass	c	d

$$\chi^2 = \frac{(b-c)^2}{b+c}$$

B

	IP: fail	IP: pass
WB: fail	190	101
WB: pass	126	112

$\chi^2 = 1.3$
$p > 0.1$

C

	IP: fail	IP: pass
IF: fail	255	98
IF: pass	61	115

$\chi^2 = 8.6$
$p < 0.005$

D

	WB: fail	WB: pass
IF: fail	232	121
IF: pass	59	117

$\chi^2 = 21.4$
$p < 0.0005$

Ayoubi et al., Supplemental Figure 4