

Supplementary Figure 1

A

M, 6H5, 6E11, 4E11, 4E6, 4D1, BGG

170 kDa

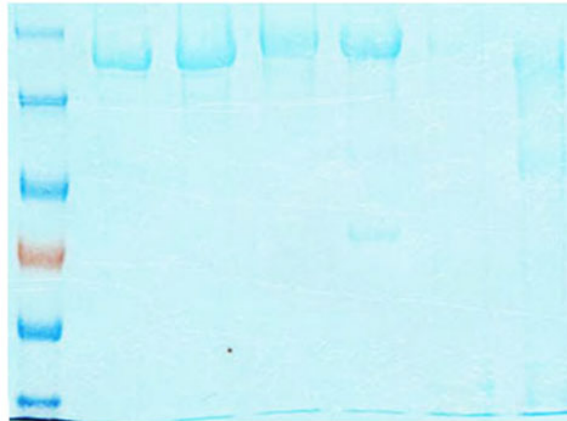
130 kDa

95 kDa

72 kDa

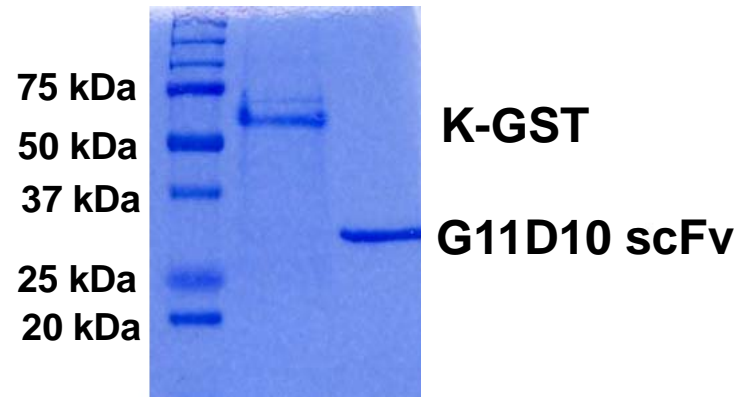
56 kDa

43 kDa

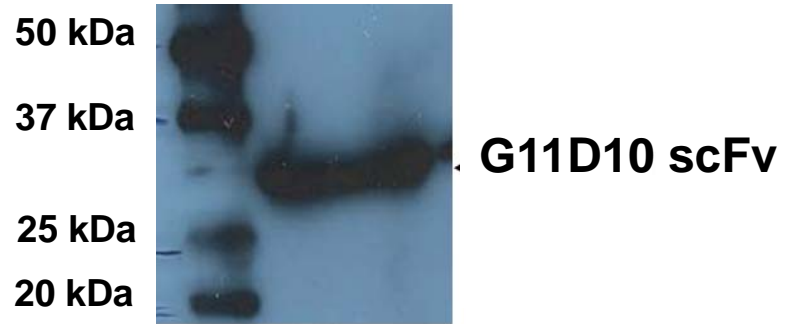


mAb

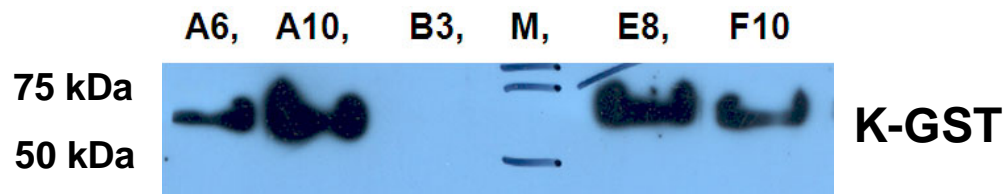
B



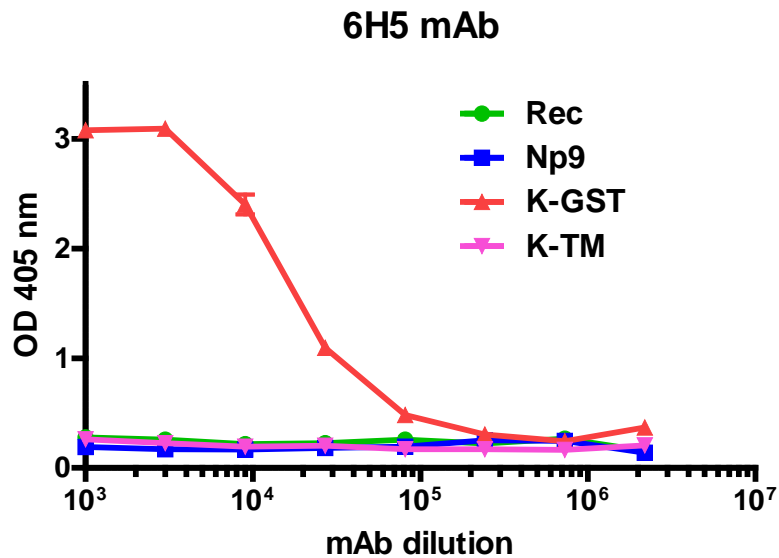
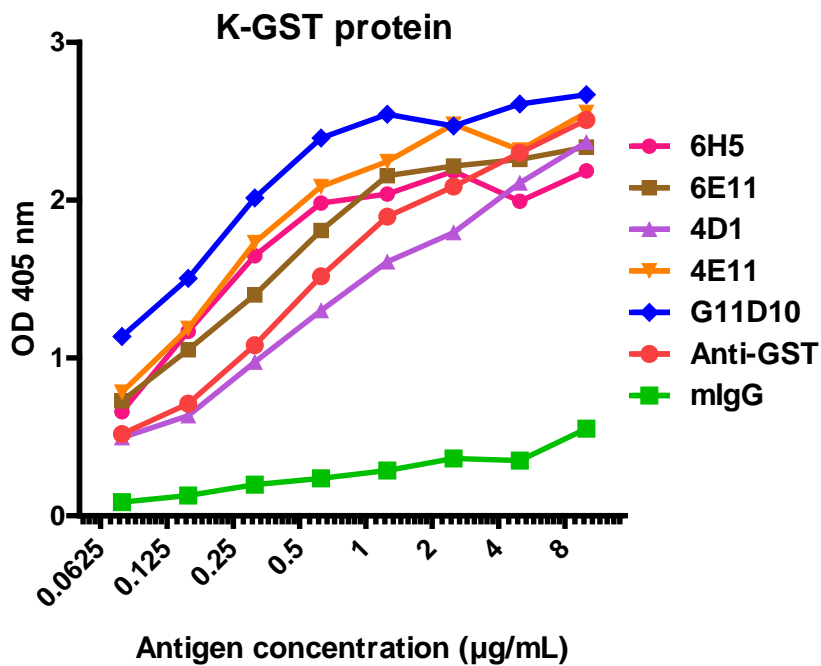
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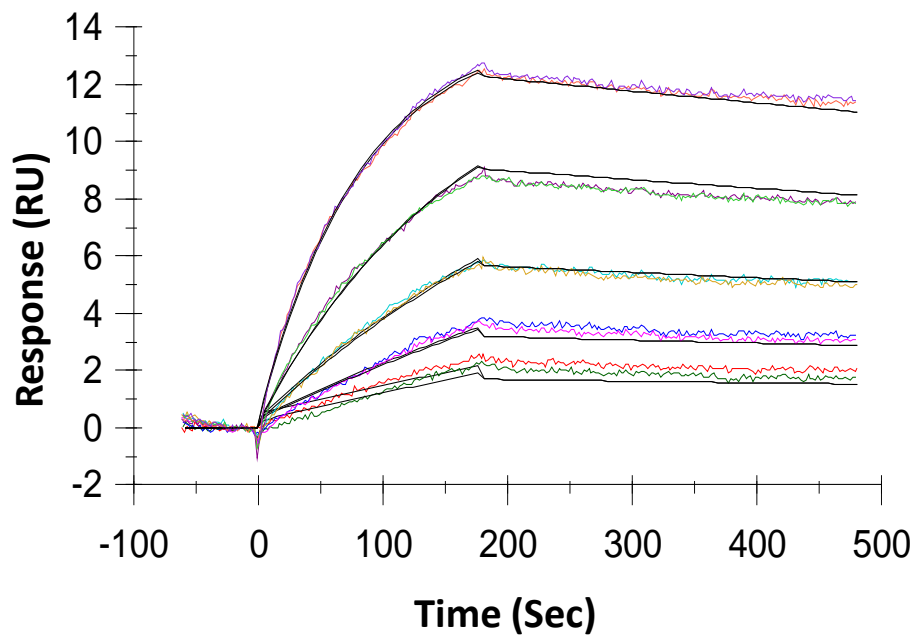
D



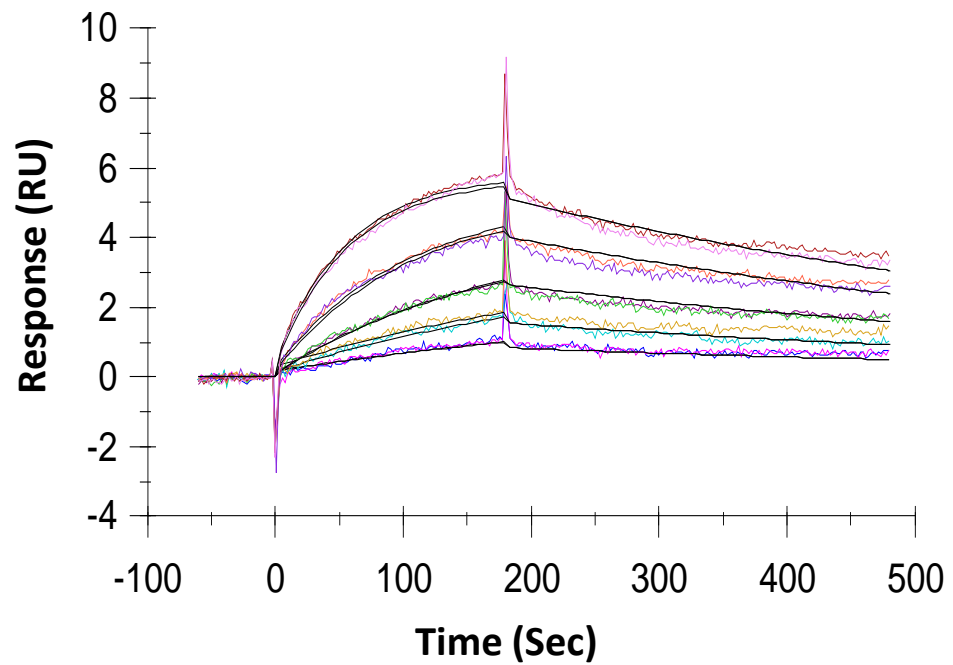
E



F **6H5 mAb**



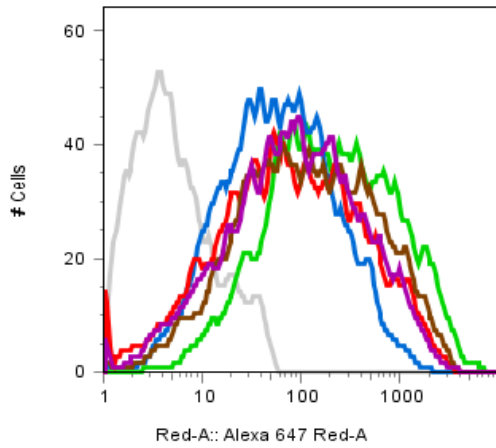
G11D10 scFv



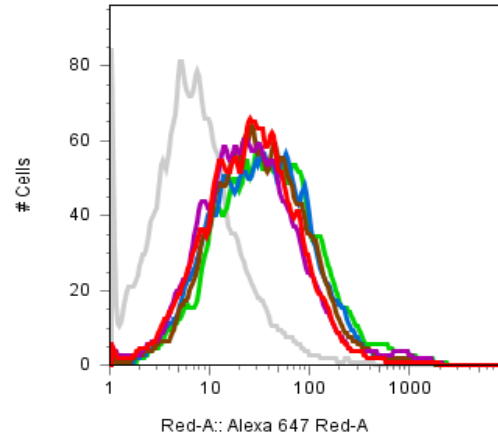
G

**6H5
mAb**

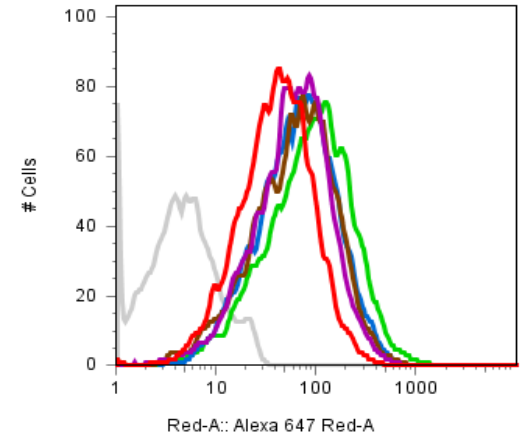
MCF-7



MDA-MB-231

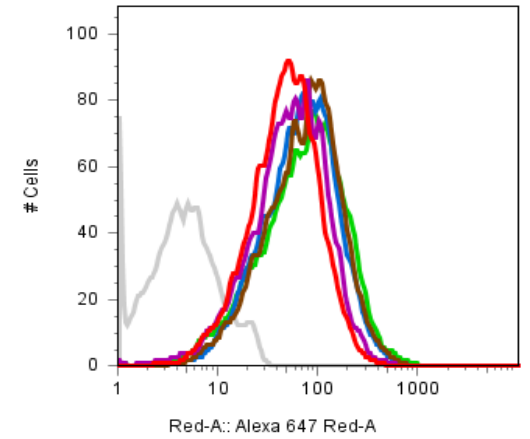
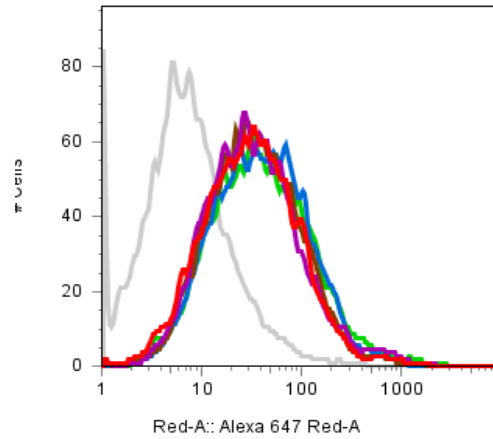
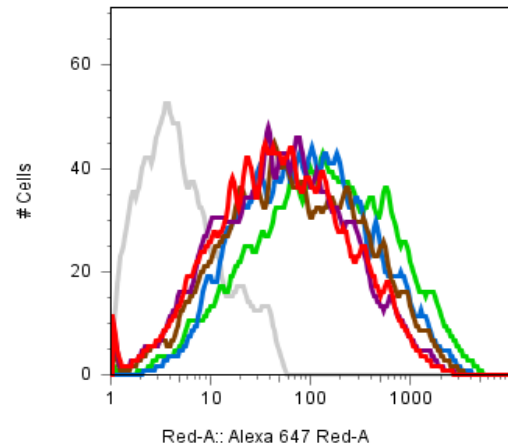


T47D



**4D1
mAb**

Mean Fluorescence Intensity



HERV-K env protein

Supplementary Figure 1. Characterization of anti-HERV-K antibody. HERV-K fusion proteins were expressed in *E. coli* cells and affinity purified, and cell lysates were loaded onto polyacrylamide gels. **A)** Antibody purification. Polyacrylamide gel stained with Coomassie blue showing purified anti-HERV-K monoclonal antibodies 6H5, 6E11, 4E11, 4E6, and 4D1 (10 μ g per lane). Bovine gamma globulin (BGG) (10 μ g) was used as a control for equal protein loading. **B)** Purity of HERV-K and single chain antibody. HERV-K env SU fusion protein (K-GST) (center lane) and a single-chain antibody generated from 6H5 mAb (G11D10 scFv) (right lane), were loaded on a polyacrylamide gel and detected by Coomassie blue staining. Left lane is a molecular weight marker (Bio-Rad). One representative blot of two independent experiments is shown. **C)** Detection of single chain G11D10 scFv antibody. The G11D10 scFv protein, purified using the Recombinant Phage Antibody System (GE Healthcare), was detected by immunoblot using anti-E-tag antibody. One representative blot of two independent experiments is shown. **D)** Immunoblot detection of K-GST. Several single chain G11D10 scFv antibody subclones (A6, A10, E8, and F10) were used to detect K-GST. These subclones were obtained after *E. coli* cells were infected with recombinant phage containing the antibody scFv gene insert, and plated onto growth medium. One representative blot of two independent experiments is shown. **E)** Determination of antibody sensitivity and specificity. Enzyme-linked immunosorbent assay was used to determine the sensitivity (left panel) and specificity (right panel) of anti-HERV-K antibody for HERV-K protein. Left panel: The sensitivity of anti-HERV-K antibodies was tested by enzyme-linked immunosorbent assay using a serial dilution of K-GST (10 μ g/mL to 0.078125 μ g/mL) and a constant concentration of 10 μ g/mL of 6H5 mAb, 6E11 mAb, 4D1 mAb, 4E11 mAb, and G11D10 scFv as primary antibodies. Anti-GST mAb and mIgG were used as positive and negative controls, respectively. Right panel: HERV-K102 Np9 protein (Np9), HERV-K

provirus Rec protein (Rec), HERV-K transmembrane env protein (K-TM), and HERV-K surface subunit (SU) (KSU) proteins were used as antigens (10 $\mu\text{g}/\text{mL}$, 100 μl per well). Antibody (1 μg) was diluted 1:1000 to 1:2187000. Mouse IgG (mIgG) and anti-GST antibody were used as negative and positive controls, respectively. One representative ELISA result of two independent experiments is shown. **F**) Biacore assay. Association rate constant (K_a), dissociation rate constant (K_d) and affinity constant (K_D) analysis of 6H5 mAb (left panel) and G11D10 scFv (right panel). **G**) Cycling of HERV-K env protein between the cell surface and intracellular stores in breast cancer cells. Time-dependent (red = 45 minutes, purple = 15 minutes, brown = 5 minutes, blue = 1 minutes, green = 0 minutes) internalization of HERV-K env protein was evaluated in MCF-7, MDA-MB-231, and T47D cells. Internalization after binding to 6H5 mAb (top panel) and to conformation-dependent 4D1 antibody (bottom panel) is shown. The internal fluorescence was then calculated as total mean fluorescence intensity (MFI) at 0 minutes minus surface MFI at each time point. Each panel is a representative result of duplicate independent experiments.

Supplementary Figure 2

A

6H5 (Non-perm)

6H5(Perm)

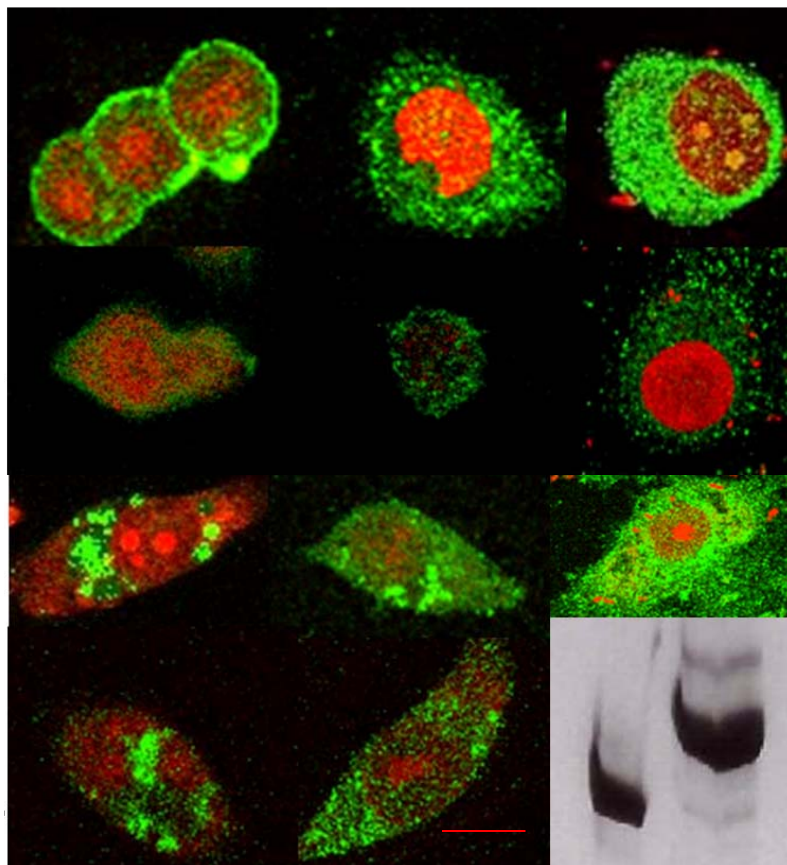
anti-rGel

MCF-7

MCF-10A

MDA-MB-231

MDA-MB-453



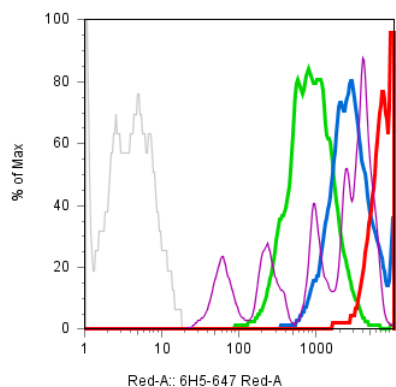
← **6H5-rGel**

← **6H5 mAb**

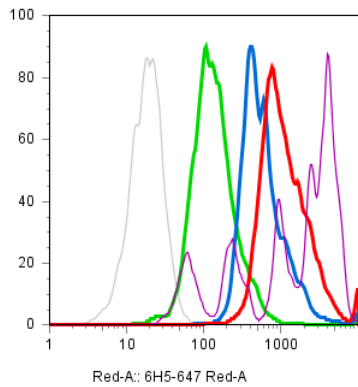
⊞

Mean Fluorescence Intensity

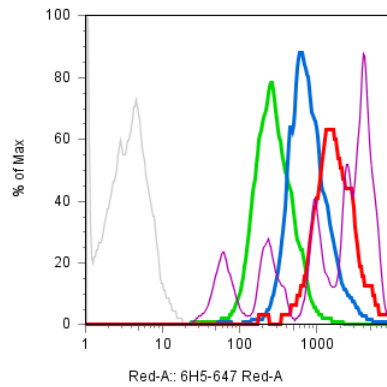
MCF-7



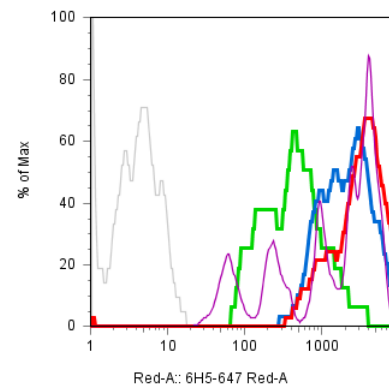
SKBR3



MDA-MB-231



MDA-MB-435 EB1



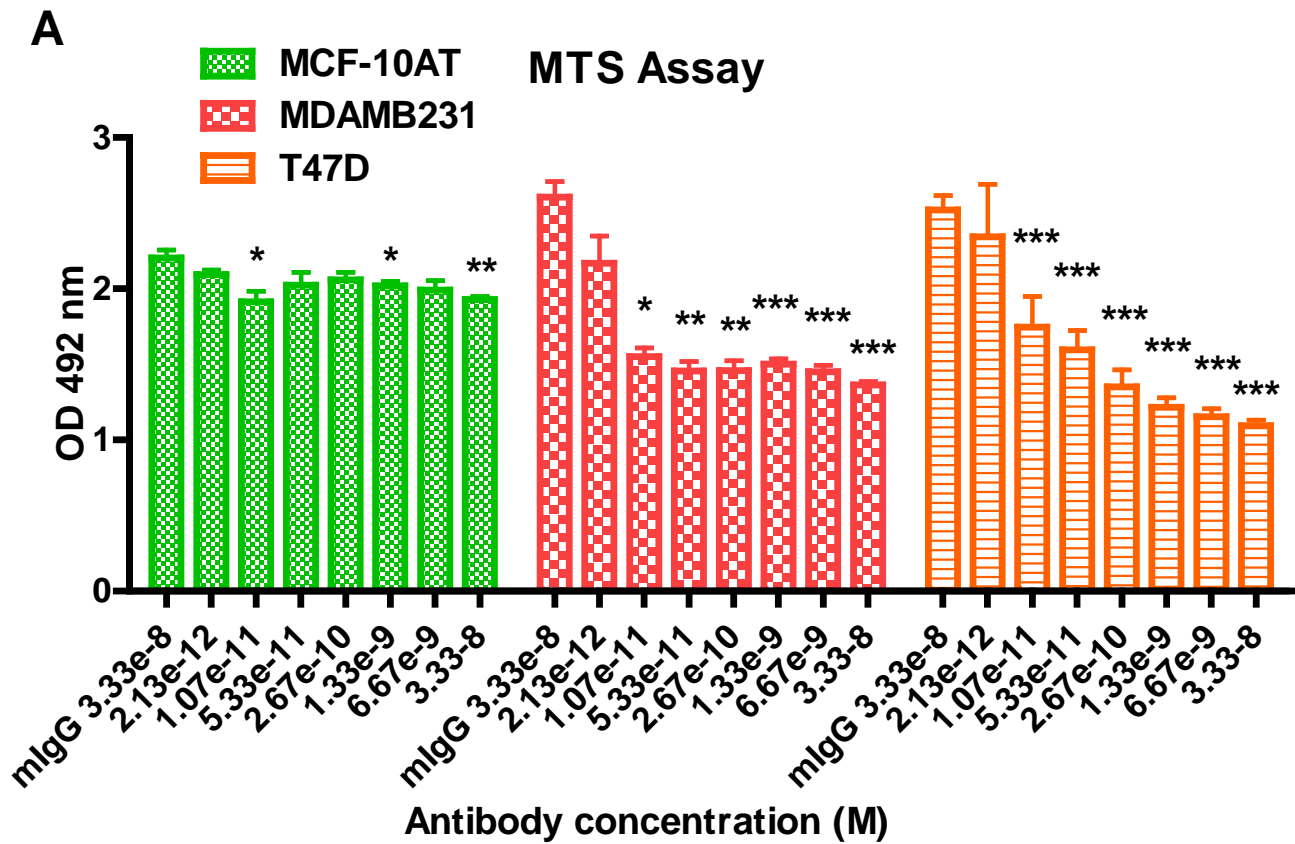
HERV-K env protein

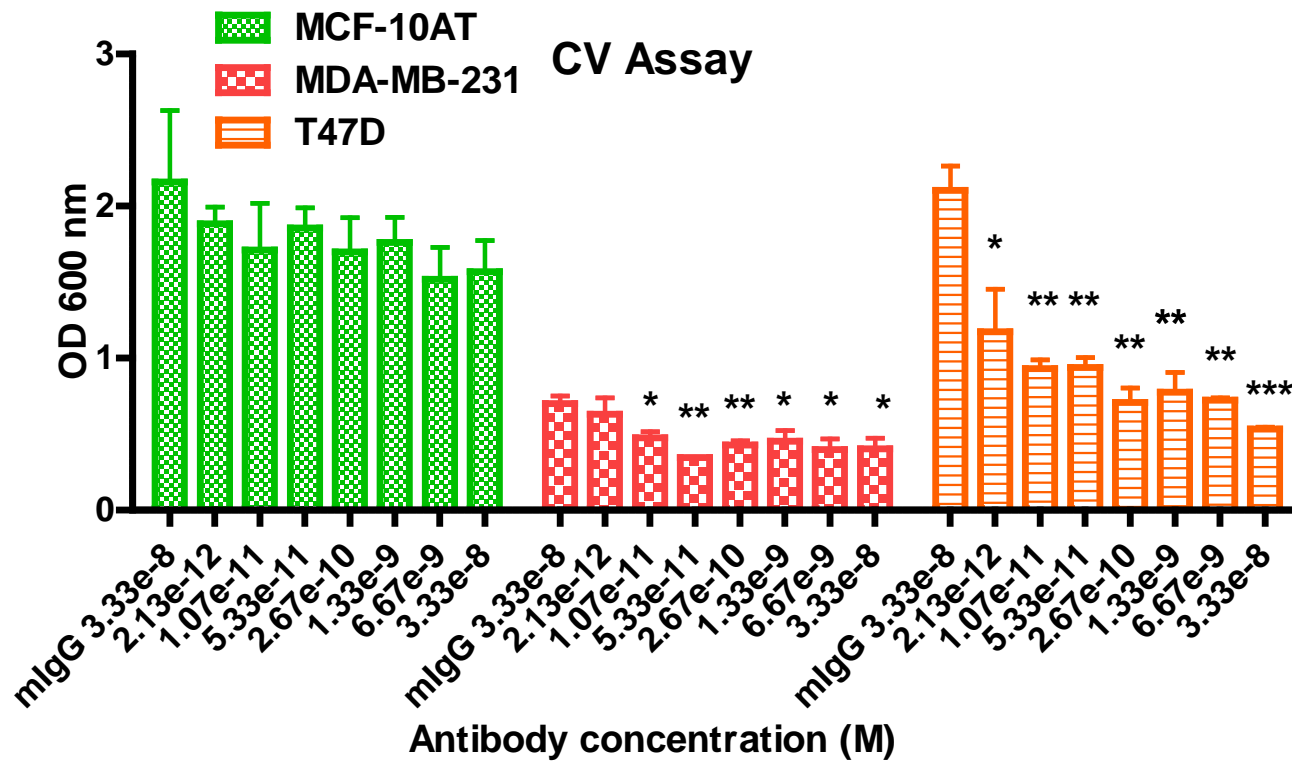
Supplementary Figure 2. Specific delivery, binding and internalization of mAb in breast cancer cells. **A)** Expression of r-Gel in MCF-7, MDA-MB-231 breast cancer and MCF-10A

immortalized breast cells was evaluated using anti-r-Gel antibody (anti-r-Gel), followed by anti-rabbit IgG FITC after 4 hours internalization. Scale bar = 20 μm . A non-reducing gel showing purified 6H5 mAb (150 kDa) and a 6H5-r-Gel conjugate (178 kDa) is (bottom right panel). One representative blot of two independent experiments is shown.

B) Flow cytometry assay of surface molecules of HERV-K env protein on breast cell lines MCF-7, SKBR3, MDA-MB-231, and MDA-MB-435EB1, as a function of dosage with 6H5 mAb (1 μg , green scan; 10 μg , blue scan; and 20 μg , red scan). The mean fluorescence intensity (MFI) of HERV-K for each cell line was calculated according to the calibration equation using QIFI beads (purple scan). Each of the five purple peaks from left to right represents a standard numbers of beads—1700, 11,000, 54,000, 194,000, and 561,000 beads, respectively. Cells treated with isotype control (IgG2a), followed by anti-mIgG-AF64 treatment were used as control (gray). These experiments were performed in duplicate with similar results.

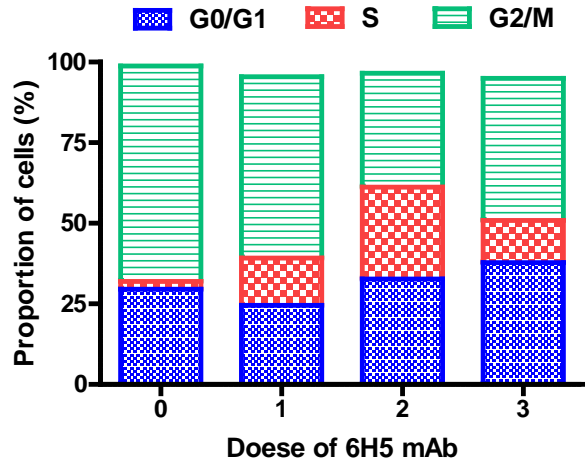
Supplementary Figure 3



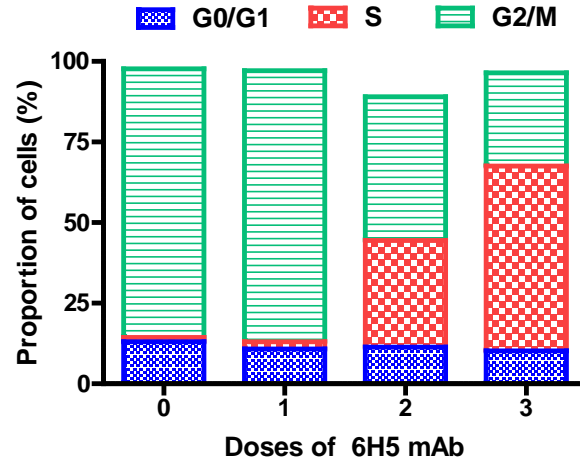
B

C

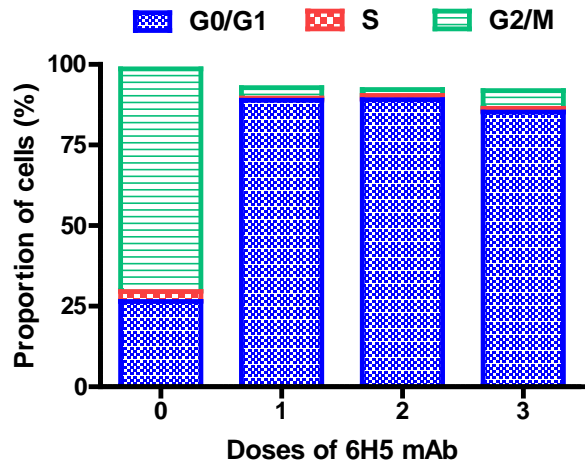
MCF-7



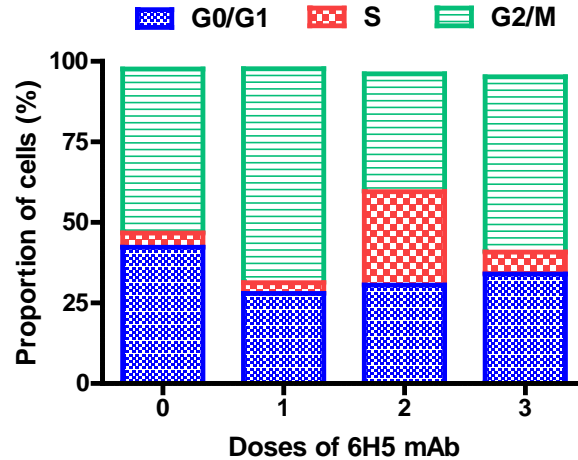
MDA-MB-231



T47D



ZR-75-1



Supplementary Figure 3. Effect of 6H5 mAb treatment on cell proliferation, cytotoxicity, and cell cycle. **A)** MCF-10AT, MDA-MB-231 and T47D breast cells were treated with 6H5 mAb or mIgG (10 µg/mL) on day 0, and cell proliferation was measured by MTS assay (OD 492 nm) after 72 hours. Cells treated with mIgG (10 µg/mL) were used as control. Means and 95% confidence intervals are presented. These experiments were performed in duplicate with similar results. **B)** Cytotoxicity of 6H5 mAb. A crystal violet assay (OD 600 nm) was also used to evaluate the cytotoxicity of mAb after 72 hours of 6H5 mAb treatment. Means and 95% confidence intervals are presented. These experiments were performed in duplicate with similar results. **C)** Cell cycle phase of 6H5 mAb-treated cells. Cells in G0/1, S or G2/M cell-cycle phases were defined by their total cellular DNA levels (7-AAD staining intensities). 7-AAD was used for total cellular DNA staining. This two-color flow cytometric analysis quantitates cells that are actively synthesizing DNA (BrdU incorporation) in terms of their cell cycle position, which is defined by 7-AAD staining intensities. Cells were untreated or treated once per day with 6H5 mAb (10 µg/mL). Doses indicate number of times cells were dosed with antibody. The data represent the proportion of cells (%) in each phase for each cell line treated with 6H5 mAb. These experiments were performed in at least duplicate with similar results. OD = optical density; MTS = 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium); CV = crystal violet.

Supplementary Figure 4

A

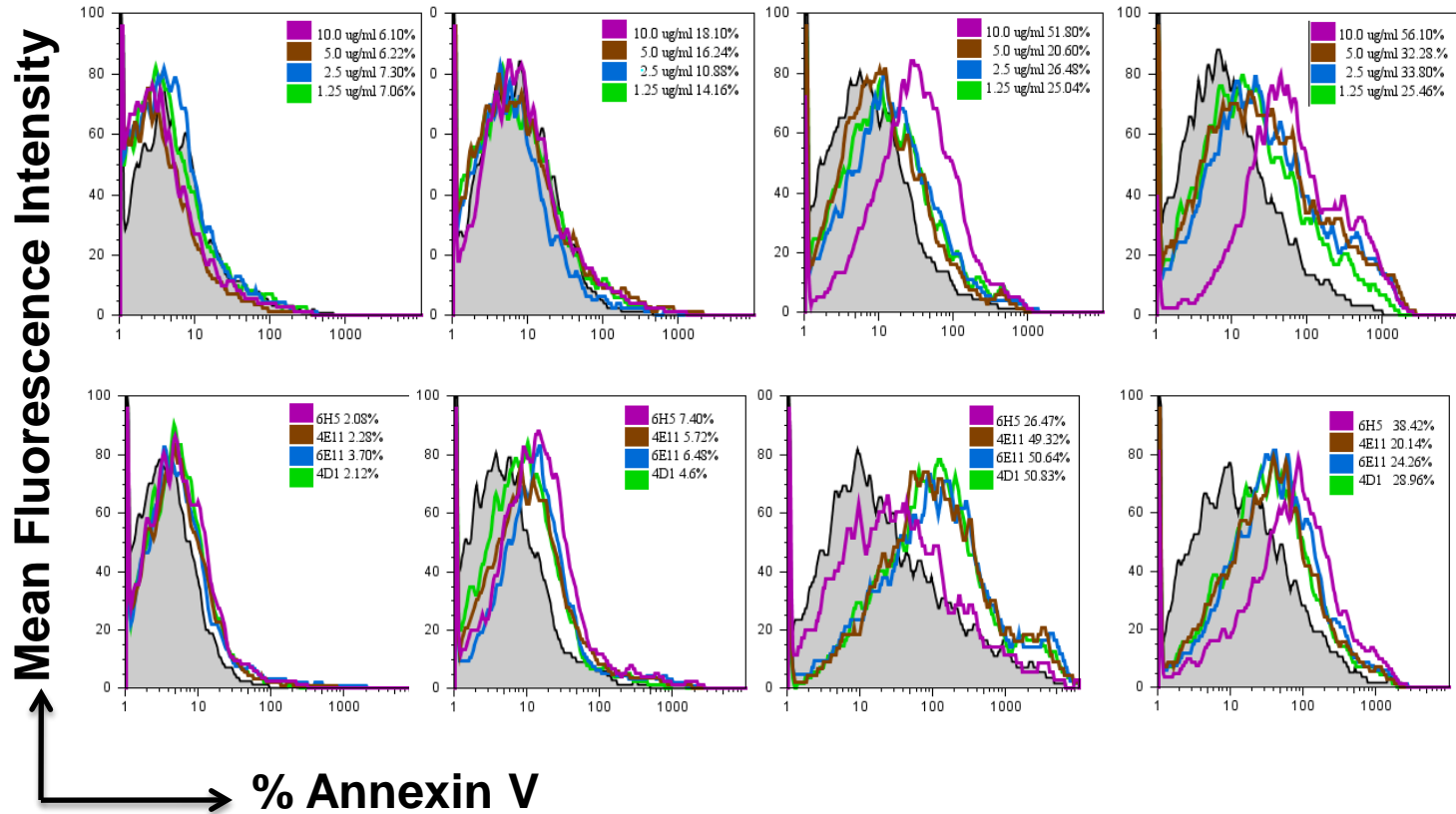
**6H5
mAb**

MCF-10A

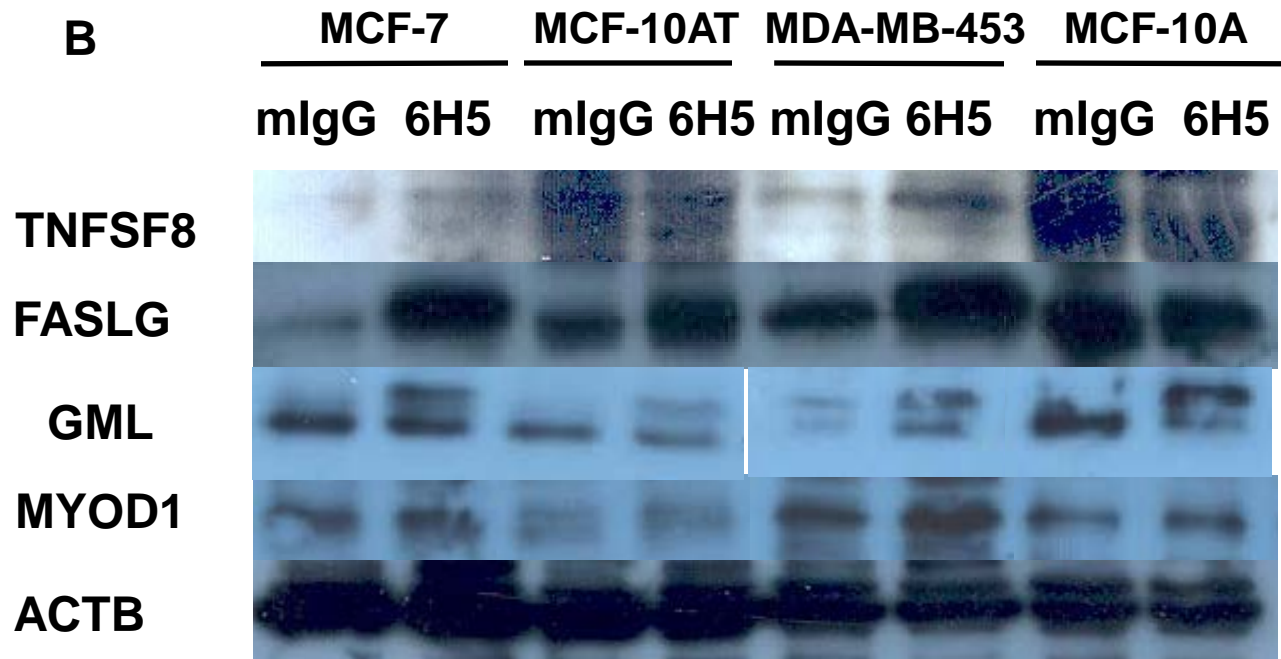
MCF-10AT

T47D

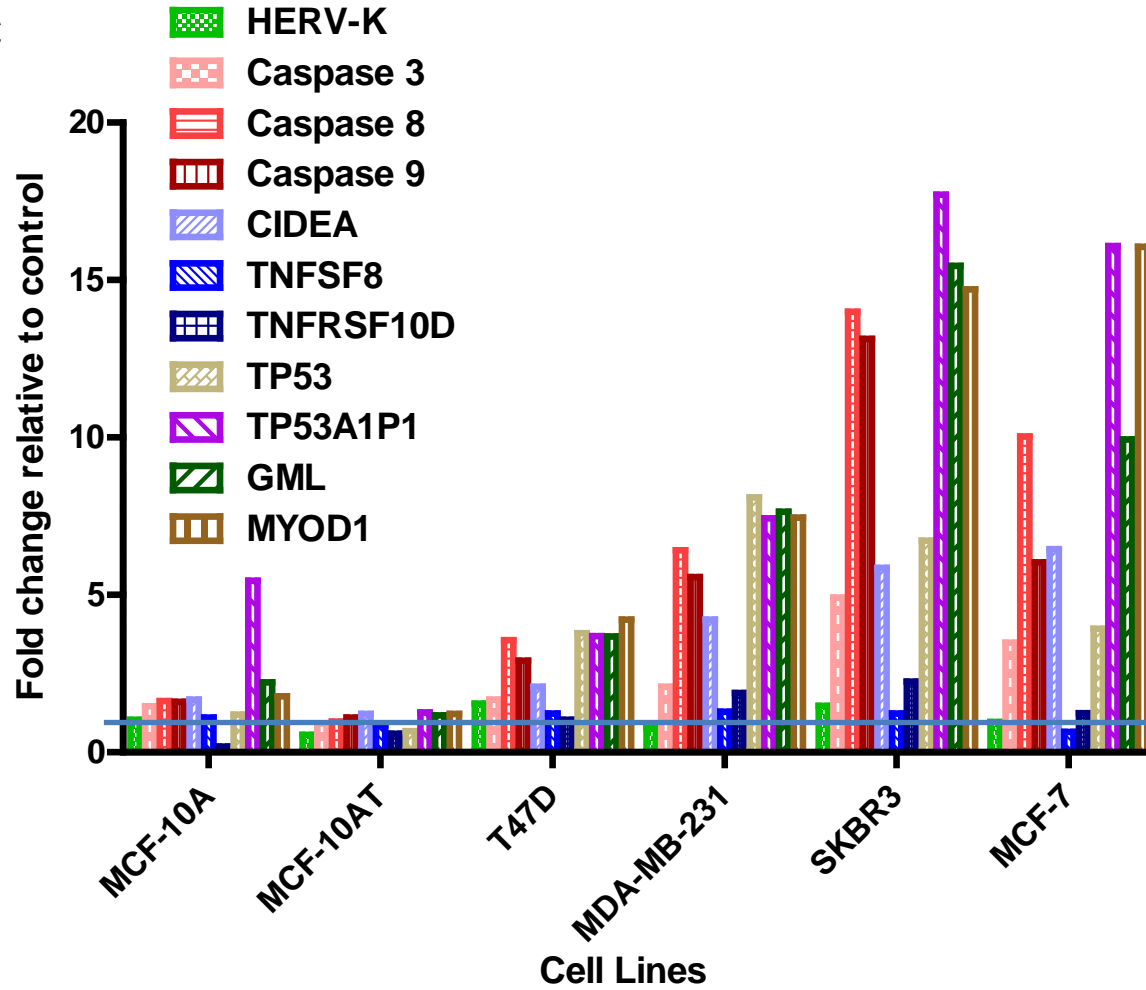
MCF-7



**6H5
4E11
6E11
4D1**



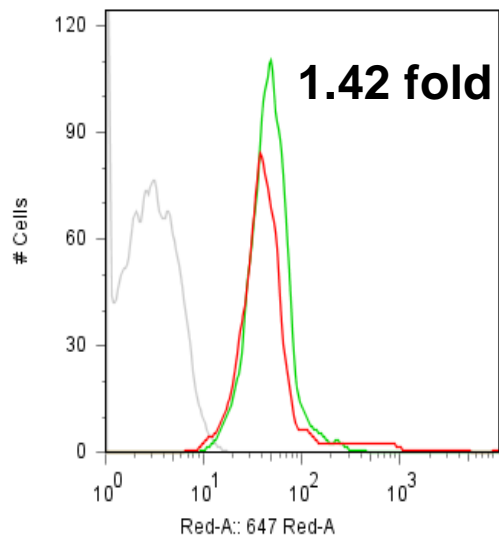
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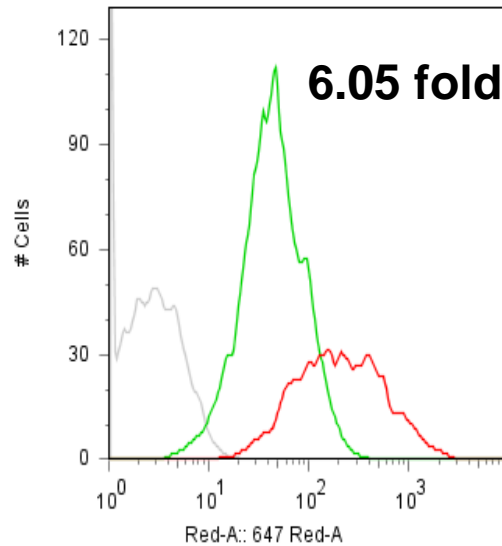
D

Caspase 3

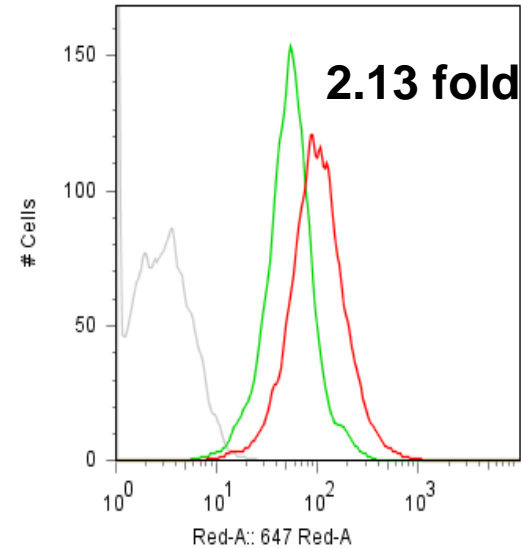
MCF-10A



SKBR3

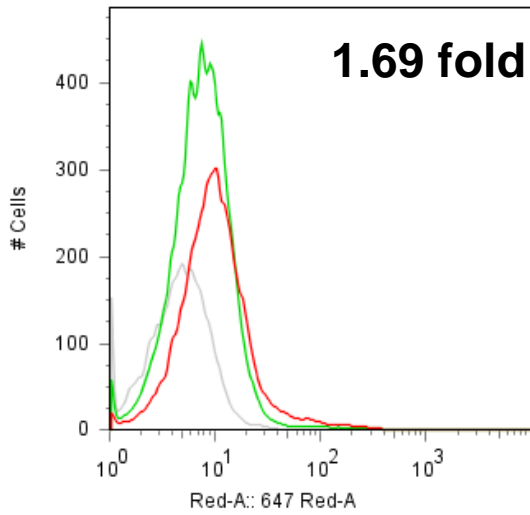


MDA-MB-231

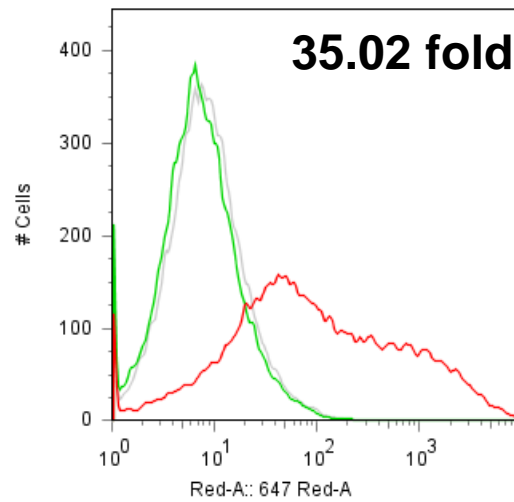


Caspase 7

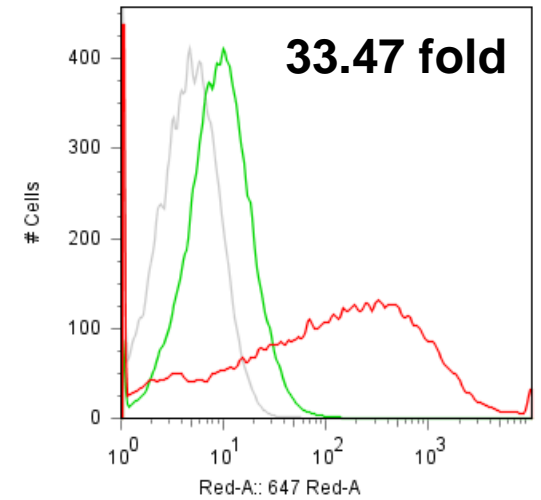
MCF-10A



SKBR3

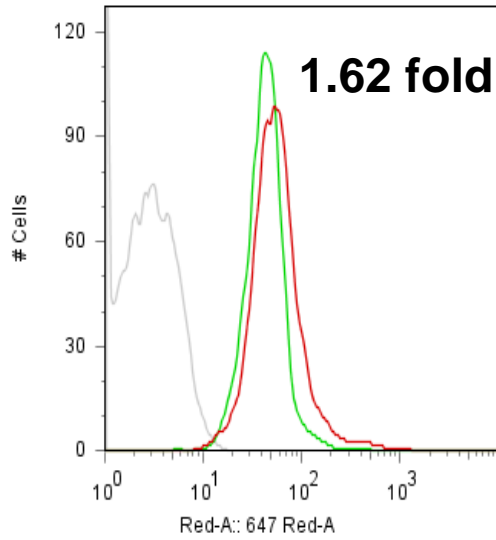


MDA-MB-231

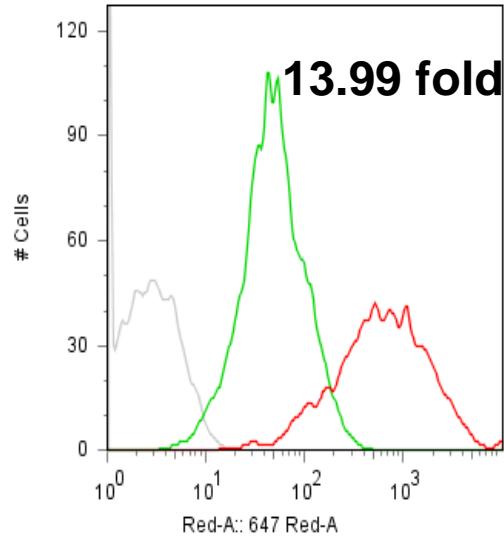


Caspase 8

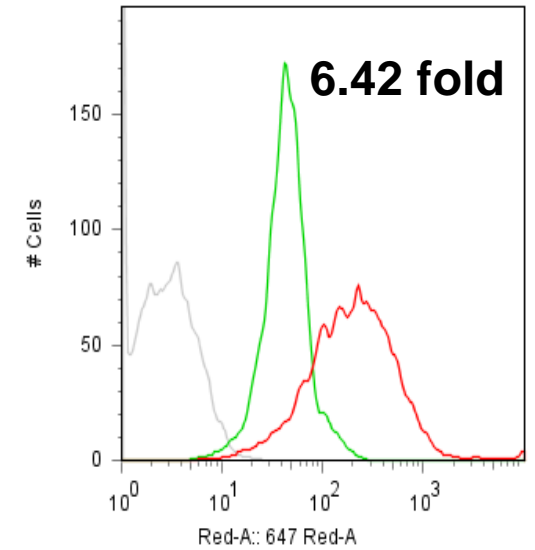
MCF-10A



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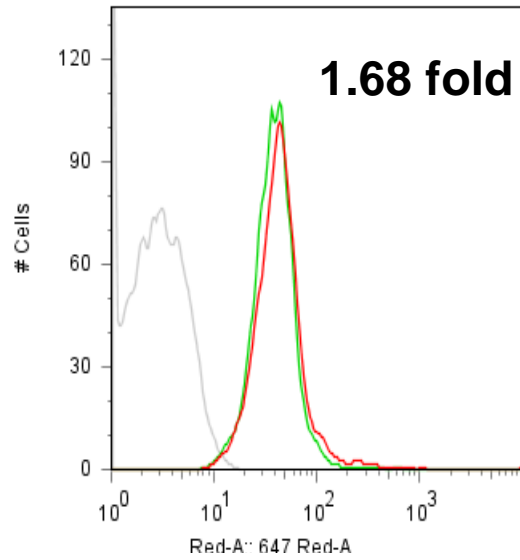


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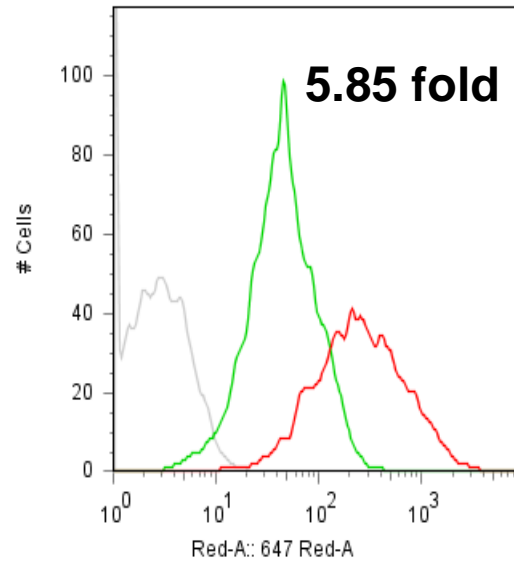


CIDEA

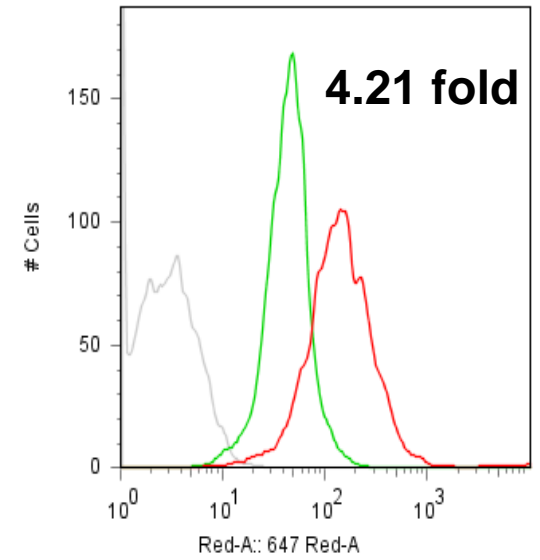
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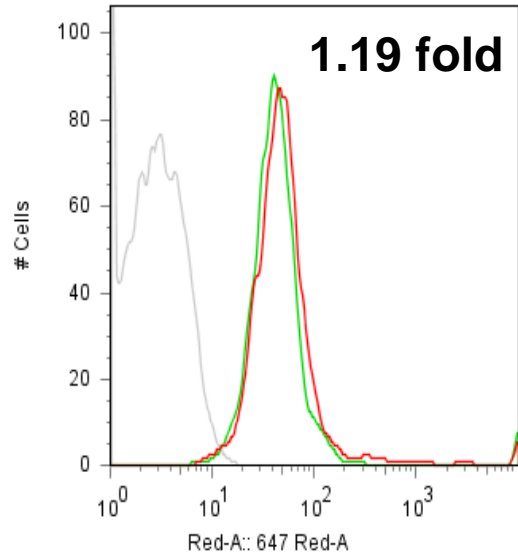


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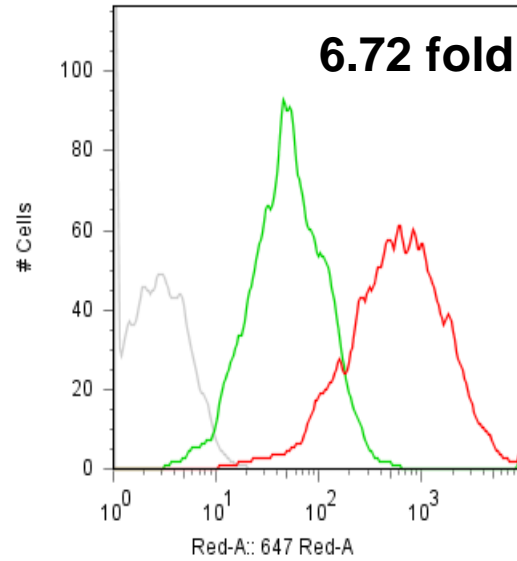


TP53

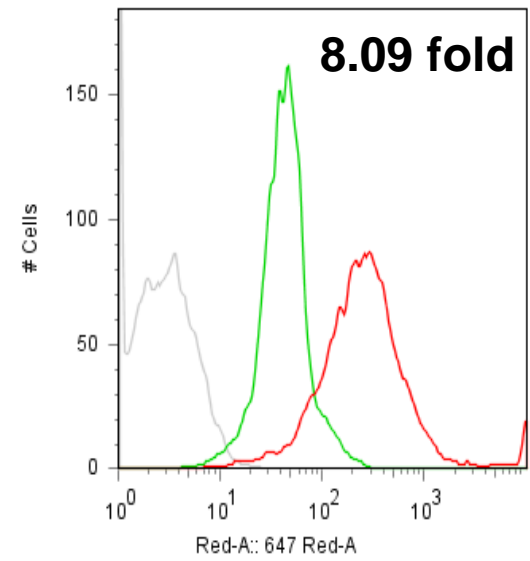
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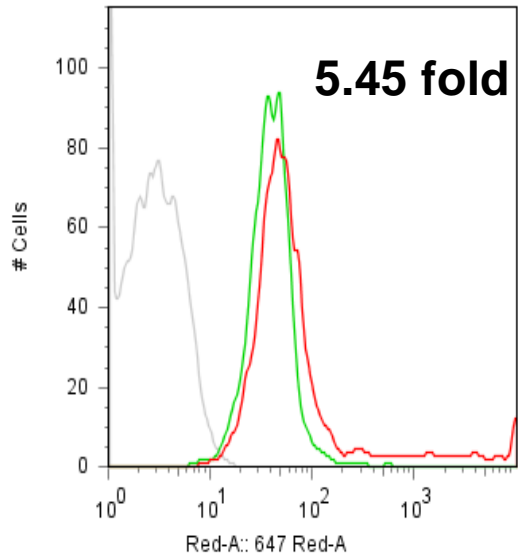


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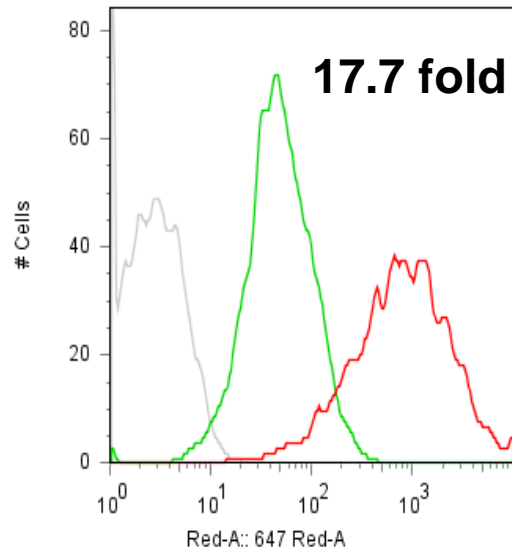


TP53 AIP1

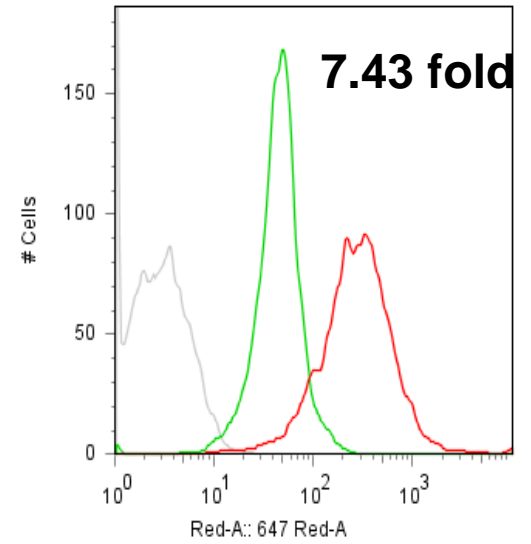
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SKBR3

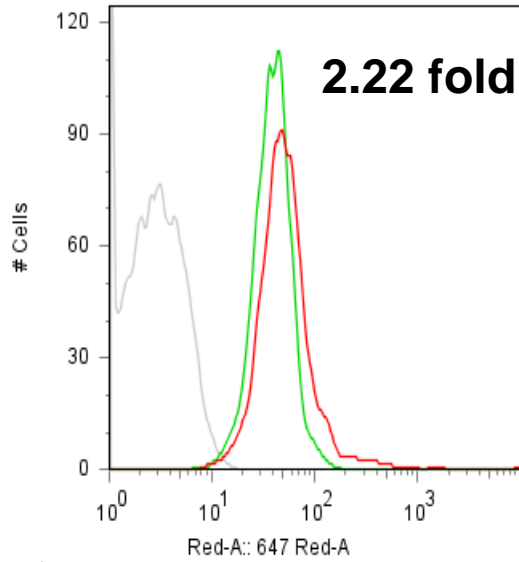


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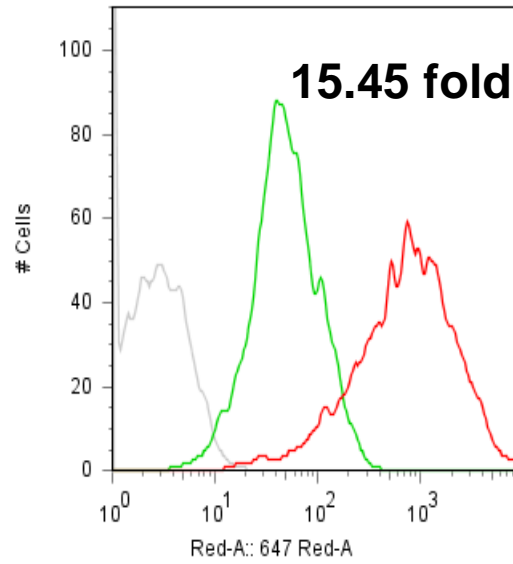


GML

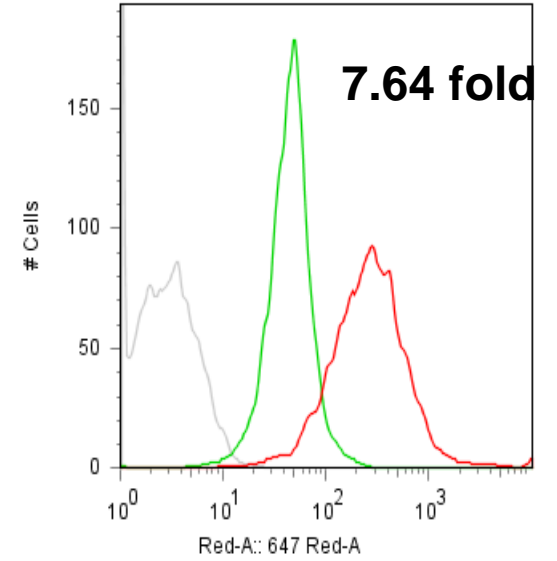
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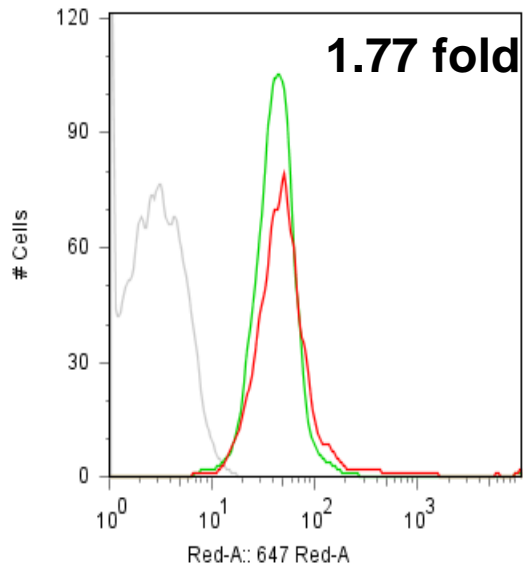


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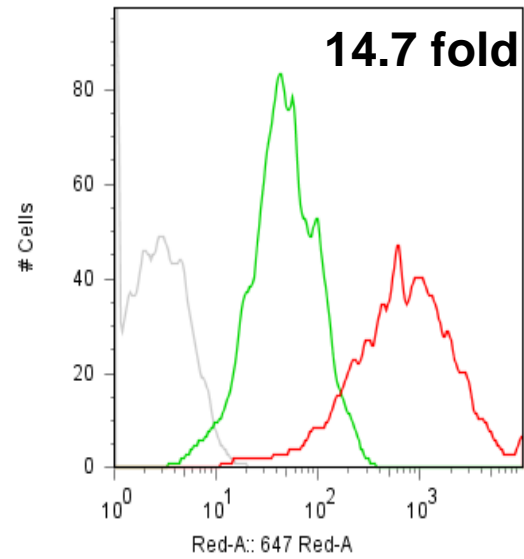


MYOD1

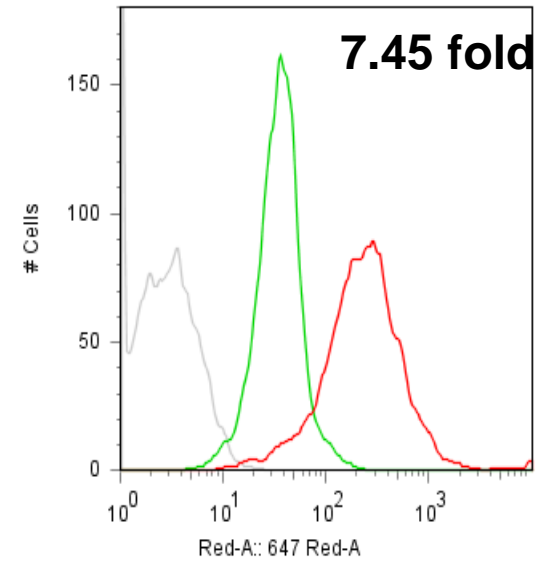
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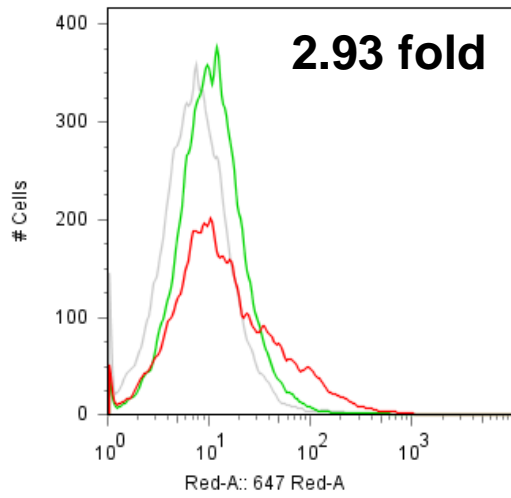


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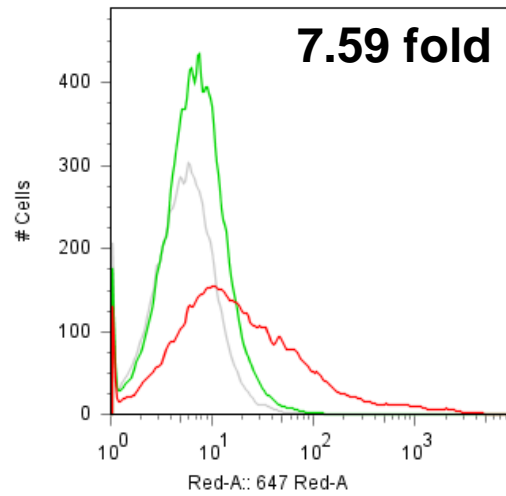


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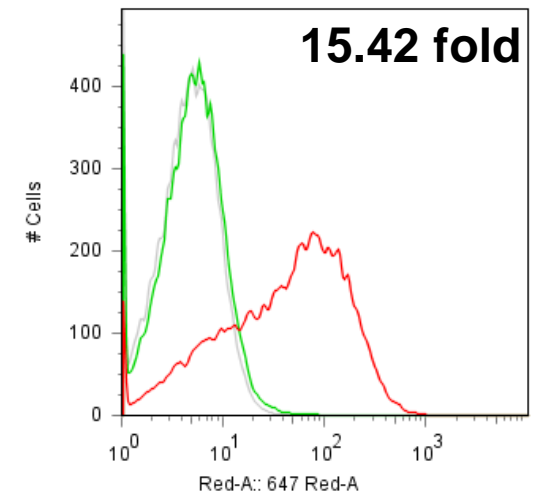
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SKBR3

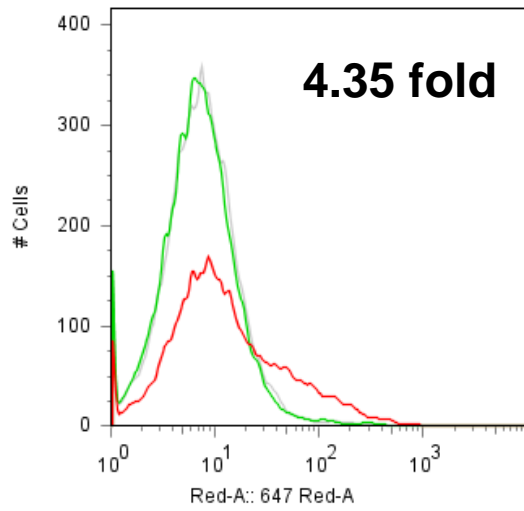


MDA-MB-231

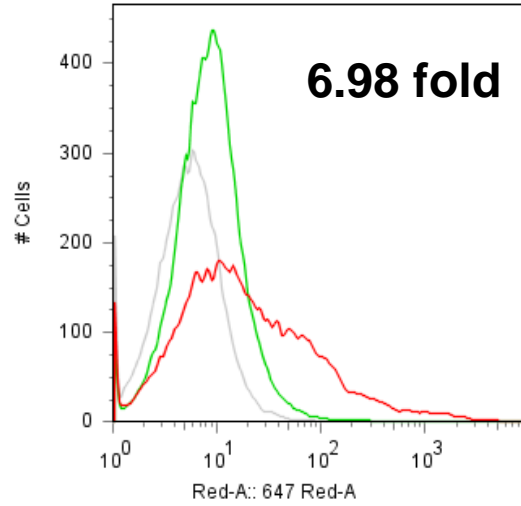


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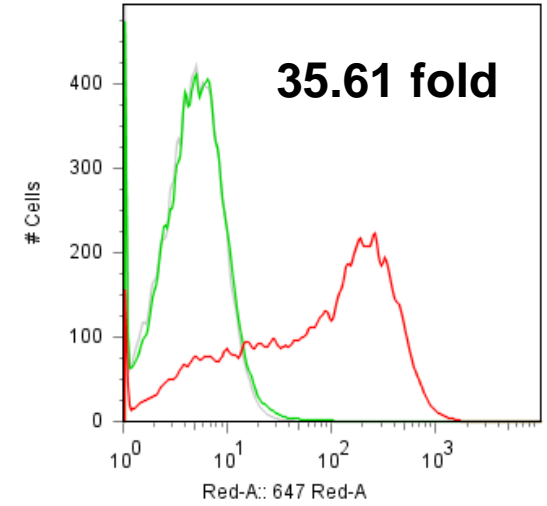
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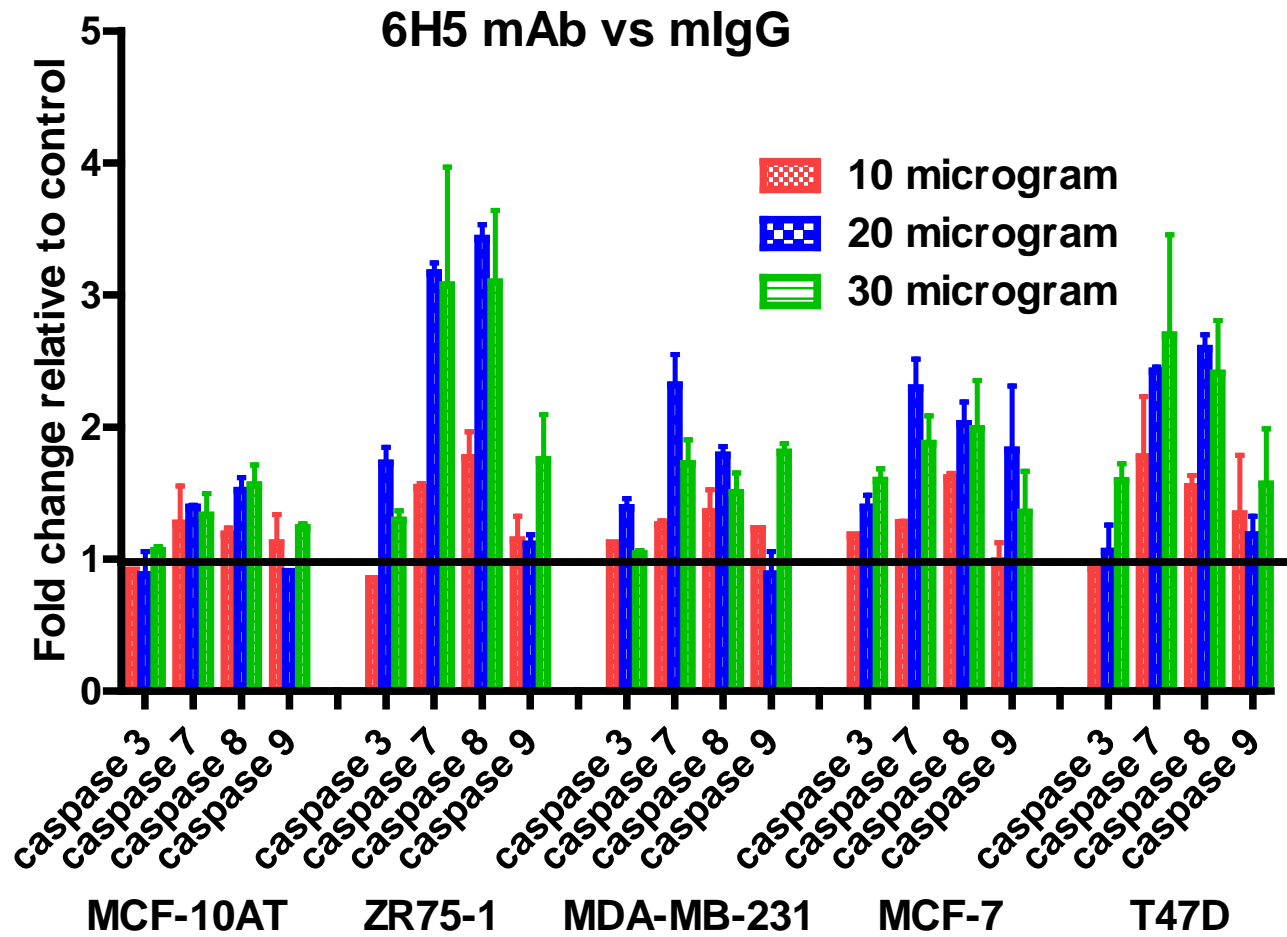


SKBR3



MDA-MB-231



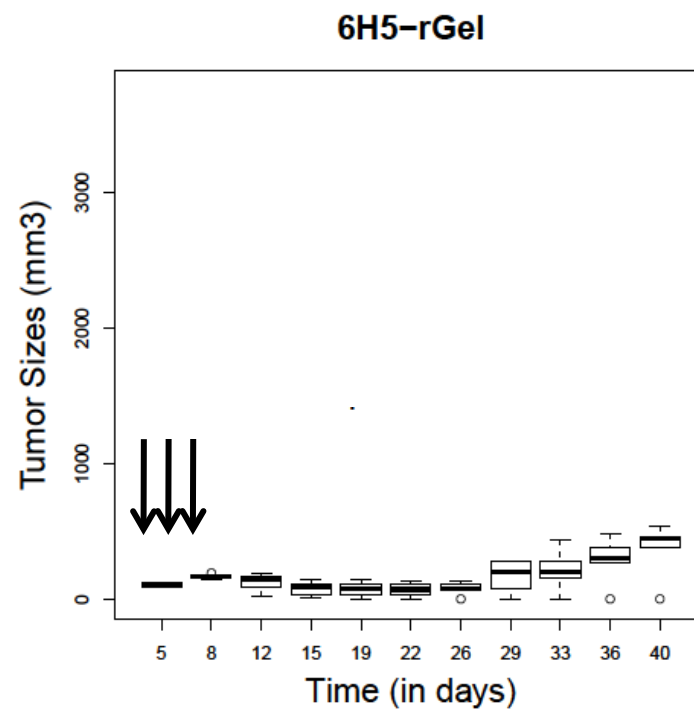
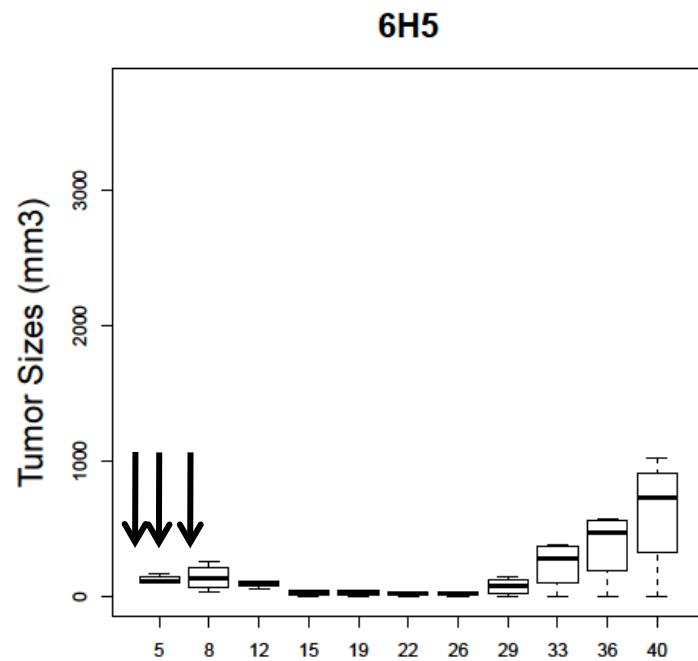
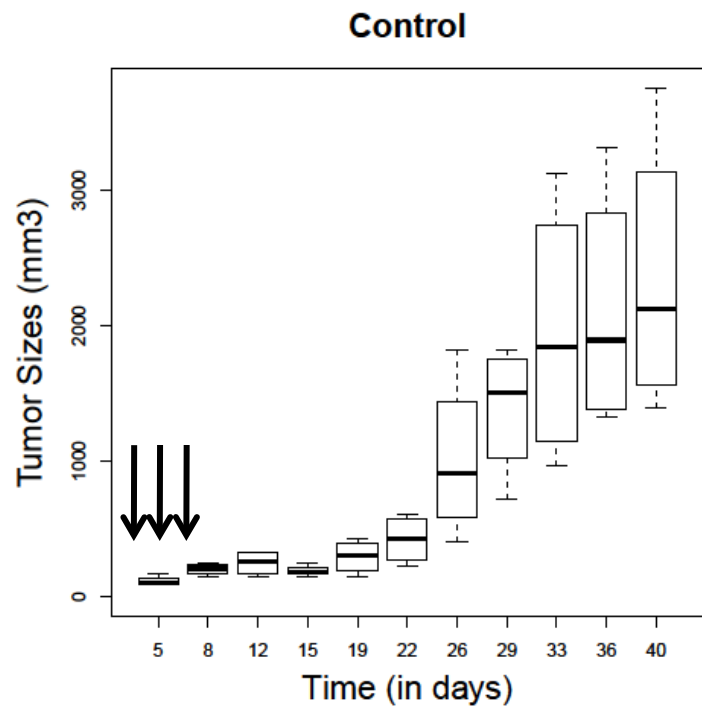
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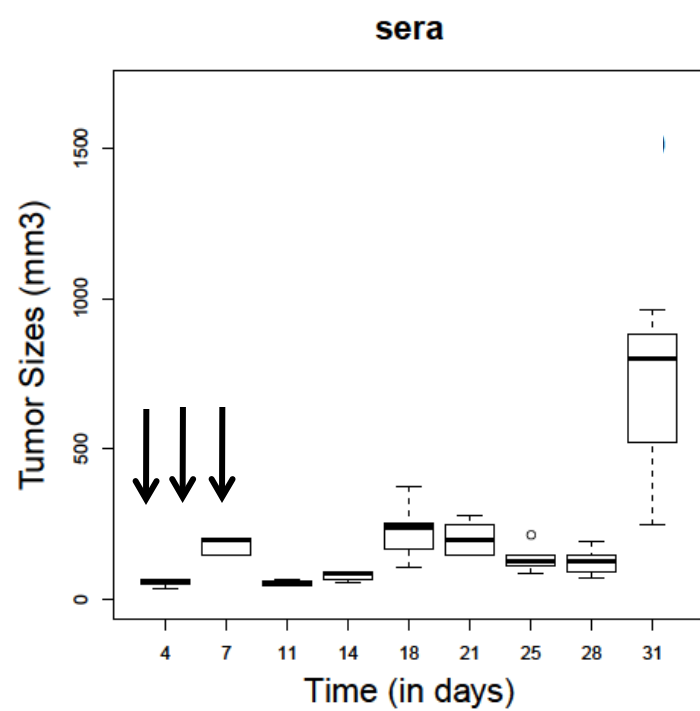
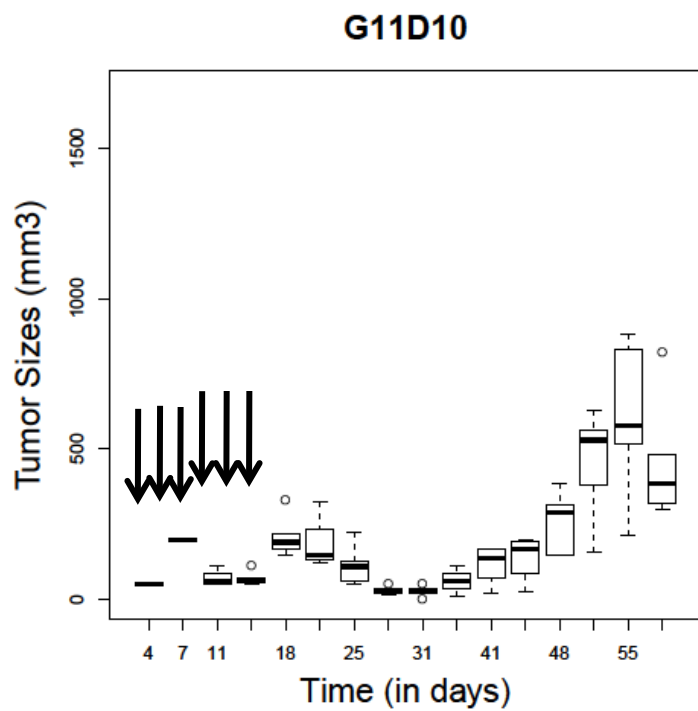
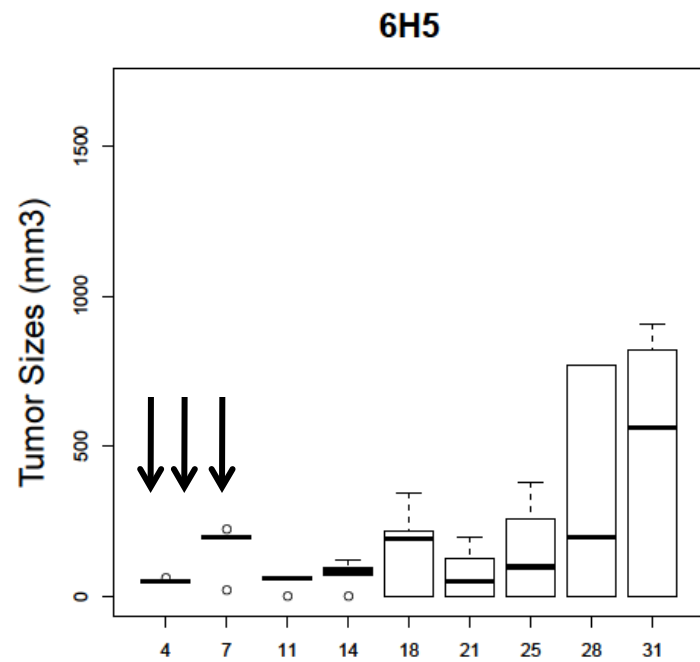
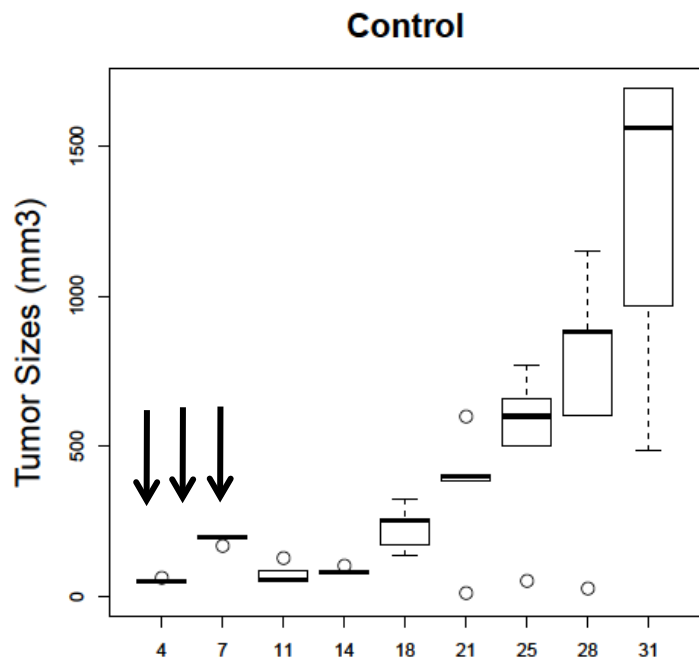
Supplementary Figure 4. Induction of apoptosis in breast cancer cells treated with mAbs. **A)** Summary of antibody dose response (top panel) and effect of various HERV-K mAbs (bottom panel) on induction of breast cell apoptosis, assessed by annexin V flow cytometric assays. Effect of dose of 6H5 mAb (1.25, 2.5, 5.0 and 10.0 $\mu\text{g}/\text{mL}$) on induction of apoptosis in MCF-10A, MCF-10AT, T47D and MCF-7 breast cells, in comparison to the same cells not treated with 6H5 mAb. Breast cells were treated with three additional HERV-K mAbs (4D1 mAb, green scan; 6E11 mAb, blue scan; and 4E11, brown scan) and the effect on apoptosis was compared to the same cells treated with 6H5 mAb (purple scan; 10 $\mu\text{g}/\text{mL}$) or with no mAb (gray scan; anti-mIgG only). Each flow cytometry assay was carried out in two independent experiments with similar results. **B)** Immunoblot analysis of protein expression in 6H5 mAb-treated cells. Expression of TNFSF8, FASLG, GML, and MYOD1 proteins was evaluated in MDA-MB-453 and MCF-7 breast cancer cells or MCF10A and MCF-10AT nonmalignant breast cells treated with 6H5 mAb compared with mIgG by immunoblot. Each immunoblot is representative of duplicate independent experiments. **C)** Flow cytometric analysis of protein expression in 6H5 mAb-treated cells. One representative result is depicted of data from at least three independent experiments obtained from flow cytometric assays of multiple breast cell lines treated with 6H5 mAb (10 $\mu\text{g}/\text{mL}$) or mIgG (10 $\mu\text{g}/\text{mL}$). Antibodies targeting the genes caspase 3, 8, and 9, CIDEA, TNFRSF8, TNFRSF10D, TP53, TP53PIA1, GML, and MYOD1 were employed. **D)** Flow cytometric analysis of expression of individual proteins in 6H5 mAb-treated cells. The fold change in expression of caspase 3, caspase 7, caspase 8, CIDEA, TP53, TP53AIP1, GML, MYOD1, CDK5, and CDKN1A protein was compared among MCF-10AT, SKBR3, and MDA-MB-231 cells treated with either 6H5 mAb (red color) or mIgG (10 $\mu\text{g}/\text{mL}$, green color) for 24 hours. IgG2a (gray color) was used as an isotype control. Individual flow

results are shown for each gene. **E)** Expression of caspase proteins after 6H5 mAb treatment.

Flow cytometry was used to determine the change in expression of caspases 3, 7, 8, and 9 in breast cancer cells (ZR-75-1, MDA-MB-231, MCF-7, and T47D) and MCF-10AT cells treated with increasing doses of 6H5 mAb (10, 20, and 30 $\mu\text{g}/\text{mL}$), in comparison to cells treated with mIgG. The fold changes (obtained from three independent experiments) for each caspase in each cell line are shown here. Each protein assay was carried out in at least three independent experiments with similar results.

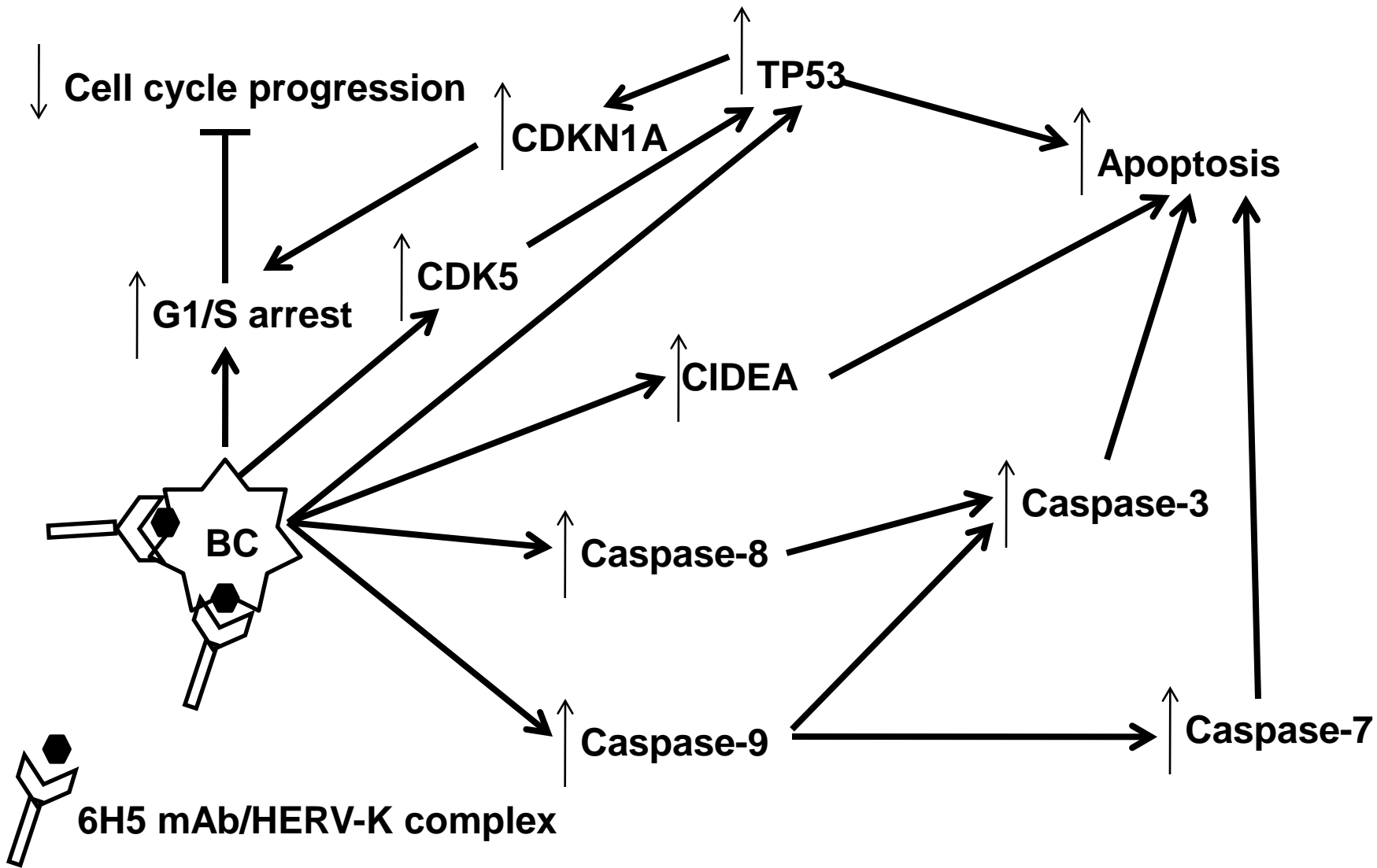
Supplementary Figure 5

A

B

Supplementary Figure 5. Tumor growth in mouse xenografts treated with 6H5. **A)** Tumor sizes were compared in MDA-MB-231 xenografts treated with 6H5 mAb or 6H5-r-Gel to sizes in xenograft mice treated with mIgG (3 solid arrows, left panel) using a linear mixed-effects model. The tumor sizes from mice ($n = 5$) on a particular day are shown, using a box-and-whiskers plot format. The horizontal line in the “box” indicates the median tumor size, the box represents interquartile range (25th and 75th percentiles) and the ends of the vertical lines or "whiskers" indicate the minimum and maximum data values. Open circles are outliers or suspected outliers. Error bars represent 95% confidence intervals from experiments performed two independent times with similar results. **B)** Tumor sizes were also compared in MCF-7 xenografts treated with 6H5, G11D10 scFv or anti-HERV-K sera (200 μ L per dose) obtained from mice immunized with HERV-K fusion proteins to sizes in xenograft mice treated with mIgG (top left panel). Error bars represent 95% confidence intervals from experiments performed two independent times with similar results.

Supplementary Figure 6



Supplementary Figure 6. Summary of the influence of underexpression of HERV-K env protein induced by 6H5 mAb treatment on breast cancer cell signaling pathways. An upward arrow indicates overexpression and a downward arrow indicates underexpression.