

Supplemental Figures

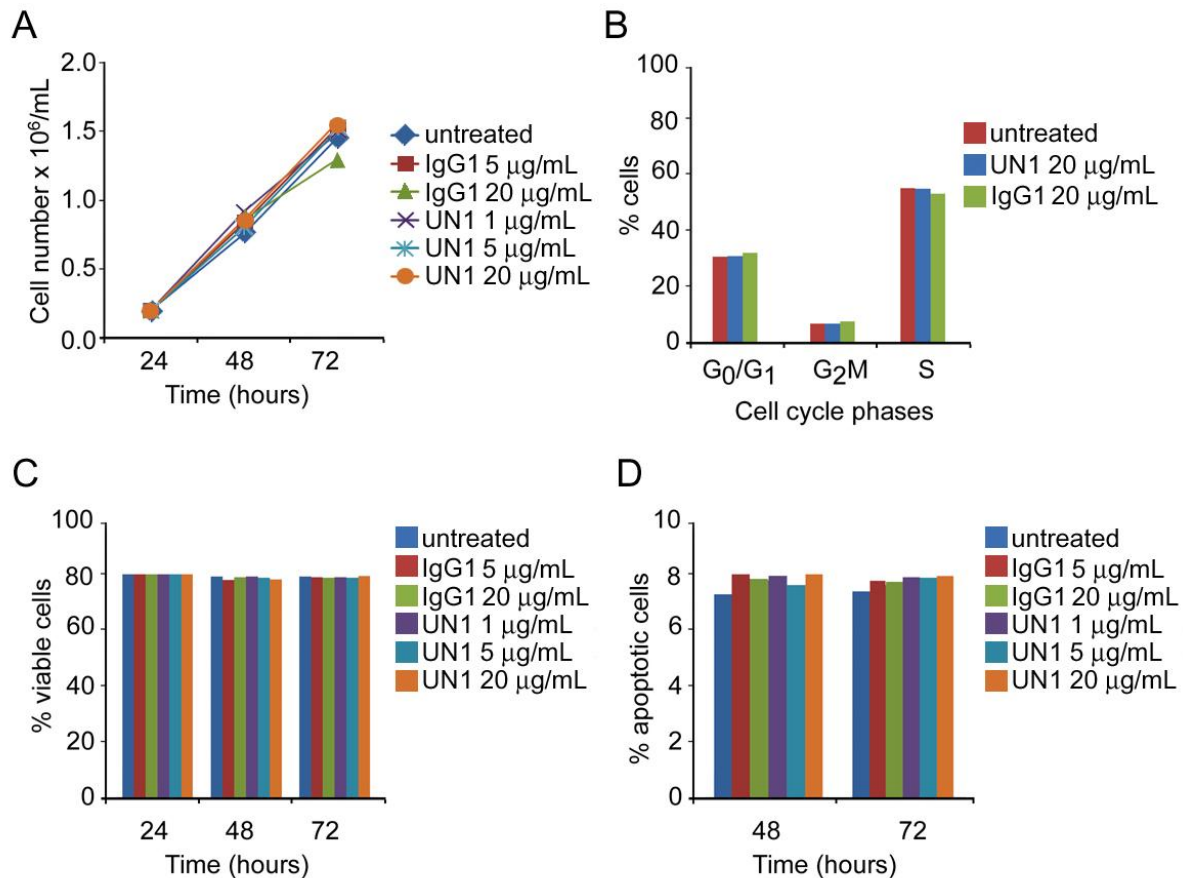


Figure S1. UN1 mAb did not affect the proliferation rate, cell cycle, viability and apoptosis of HPB-ALL cells.

HPB-ALL cells ($2 \times 10^5/\text{ml}$) were incubated with the indicated amounts of antibody and then analyzed for proliferation, cell-cycle profile, viability, and apoptosis. (A) Proliferation was measured at the indicated time by cell counting. The number of viable cells of a representative experiments out of three is shown. (B) Cell cycle phase distribution of HPB-ALL cells treated as described in A and harvested at 72 hours from treatment. Cell cycle phase distribution was obtained by performing a flow cytometric analysis of the DNA content. Representative histograms for cell-cycle profile of 1 out of 3 independent experiments with similar results are shown. (C) Viability of HPB-ALL cells treated as described in A, assessed by Trypan blue dye exclusion. (D) Percentage of sub-G1 (apoptotic) HPB-ALL cells treated as described in A and measured by propidium iodide staining and flow cytometry.

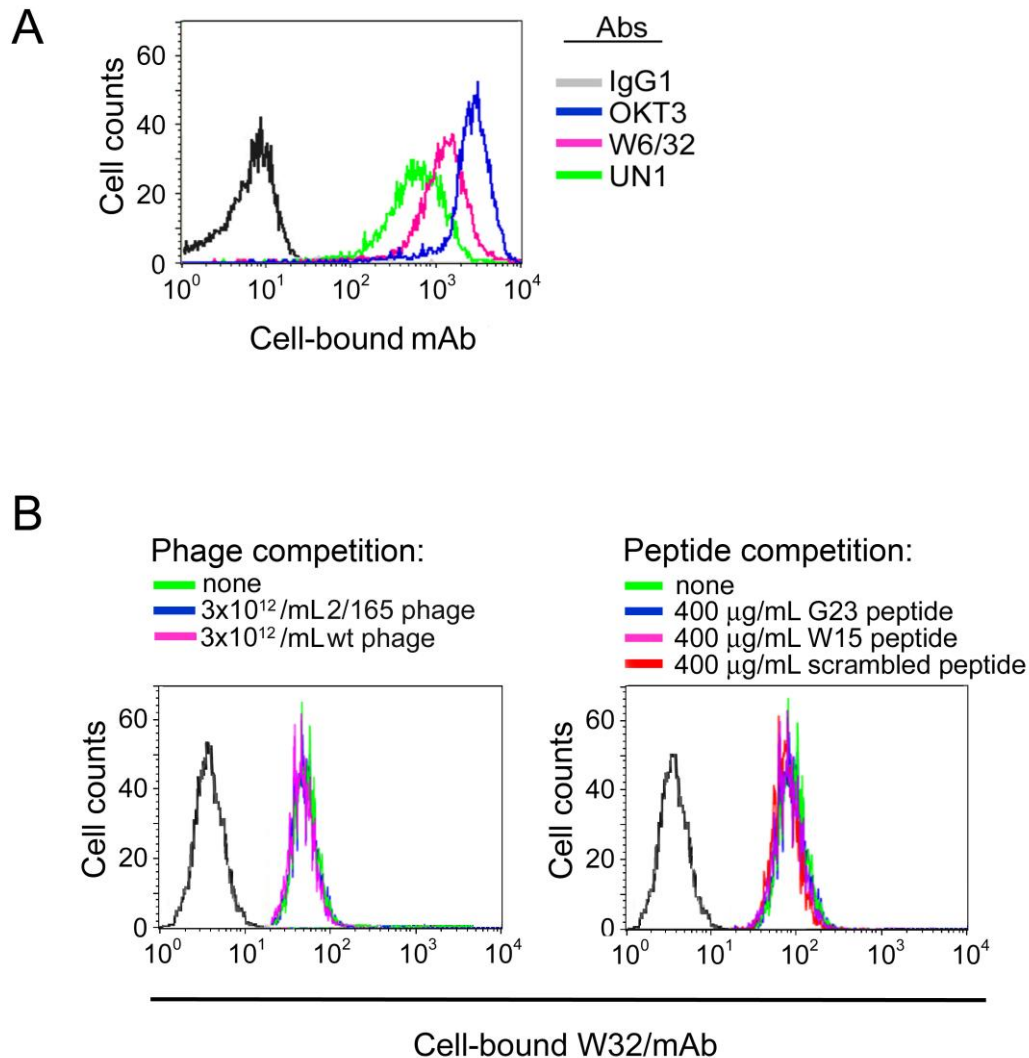
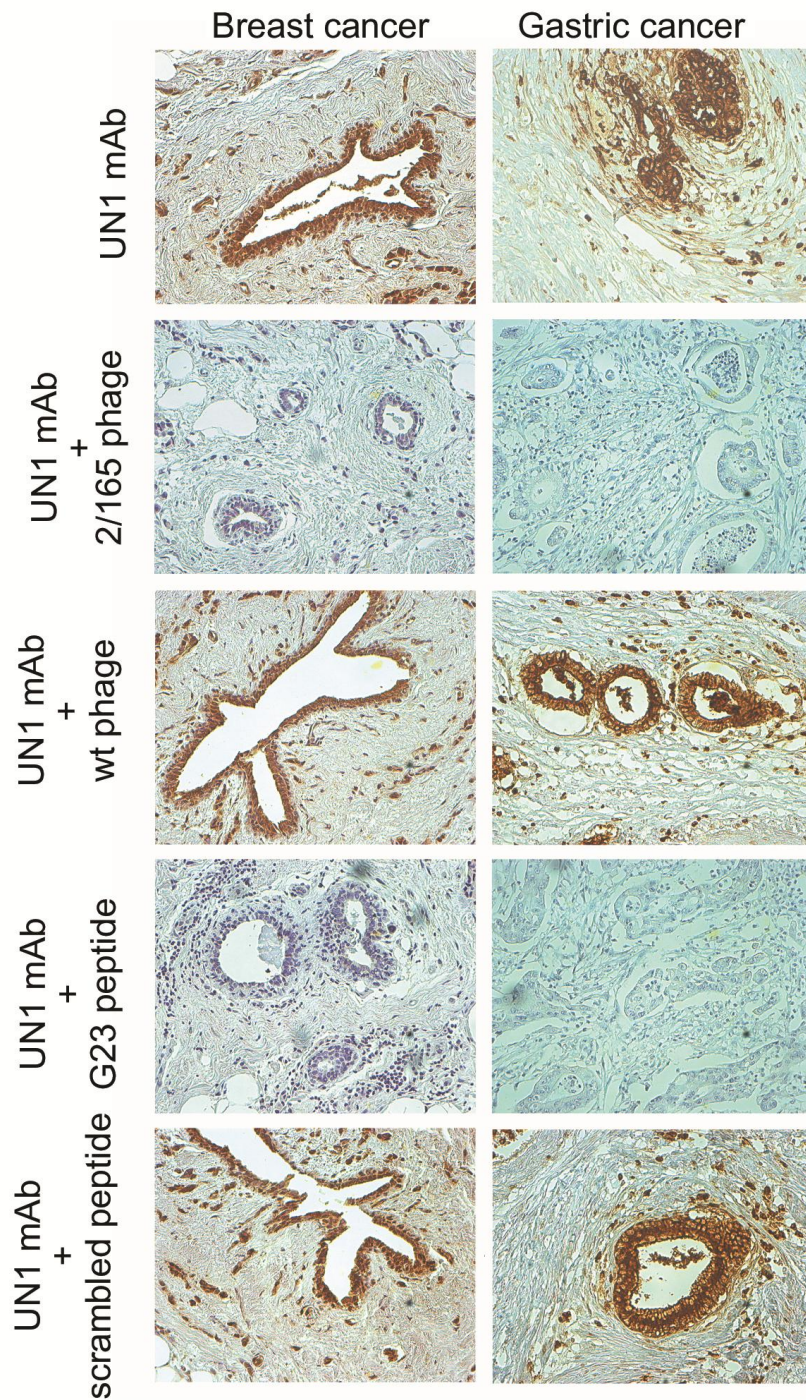


Figure S2. The 2/165 phage clone and synthetic peptides do not inhibit the binding of mAb W6/32 to HPB-ALL cells.

(A) The indicated Abs were incubated with HPB-ALL cells (5×10^5) and cell-bound mAbs were revealed by flow cytometry. Histogram overlays of the mAbs fluorescence intensities are shown. The black histogram represents fluorescence associated to an isotype control IgG.

(B) The mAb W6/32 (0.37 μ g/ml) was pre-incubated overnight with the indicated doses of phages (left panel), or synthetic peptides (right panel), and then added to HPB-ALL cells (5×10^5). Cell-bound W6/32 mAb was revealed by flow cytometry. Histogram overlays of the W6/32 mAb fluorescence intensity are shown. The black histogram represents fluorescence associated to an isotype control IgG.



Supplementary Figure S3. Competition of the UN1 mAb-binding to UN1-positive breast and gastric cancer tissues by the 2/165 phage and G23 peptide.

Serial sections of surgical specimens derived from breast and gastric cancer tissues were stained with UN1 mAb, pre-incubated overnight at 4°C with the indicated phage (2.5×10^{13} phage particles/mL) or peptide (500 µg/mL), according to peroxidase-antiperoxidase method. Original magnification x200.

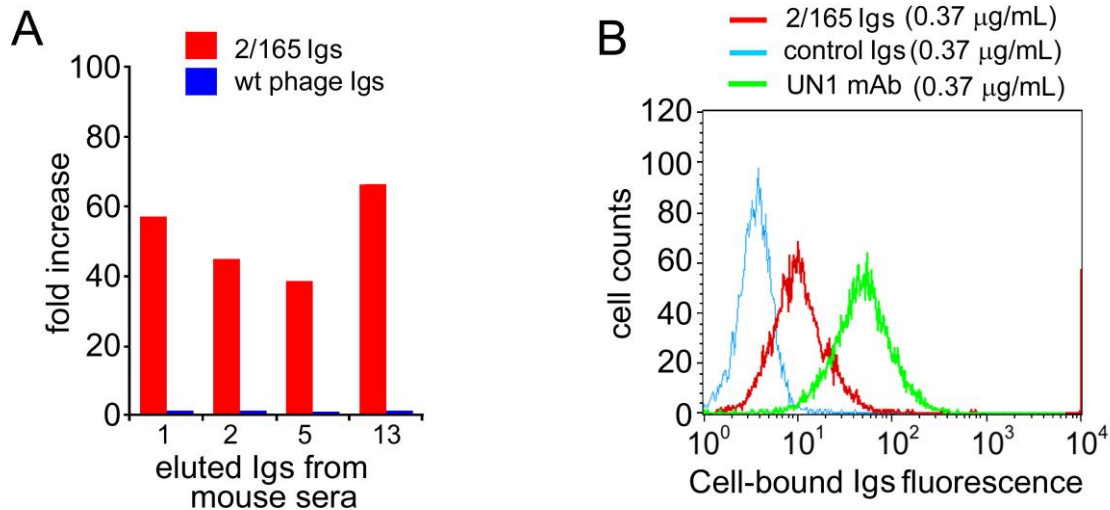


Figure S4. Characterization of affinity-purified antibodies from sera of 2/165 phagotope-immunized mice.

Antibodies from pre-immune or 2/165-immunized mice sera were affinity-purified by using 2/165 (2/165 Igs) or wild type phage (wt Igs) as ligand. (A) Reactivity of the affinity-purified Igs to G23 peptide analysed by ELISA. Each affinity purified Igs was tested in duplicate and the relative absorbance was calculated as the difference between $\text{OD}_{405\text{nm}}$ and $\text{OD}_{620\text{nm}}$. Fold increase is ratio of the mean OD value of each immunized mouse to the mean OD value of the corresponding pre-immune serum.

(B) Binding of the affinity-purified Igs to HPB-ALL cells. The affinity-purified Igs from a representative serum (mouse 2) or from a pre-immune mouse serum (control Igs) were tested for reactivity against HPB-ALL cell line by flow cytometry. As control, a comparable amount of UN1 mAb is also shown.