SUPPLEMENTARY METHODS

Antibody printing on microarray substrates.

The bioactivity of covalently anchored antibodies was studied by secondary antibody staining on the arrays post antibody printing. The microarray substrates were soaked for 2 hrs in sterile PBS prior to contact printing. The primary monoclonal antibodies were diluted to 200 μg/mL, 100 μg/mL, 50 μg/mL, and 25 μg/mL in sterile PBS. Nanoliter volumes of antibodies were printed with a Calligrapher BioOdyssey microarrayer (as stated in methods). After printing, the microarrays were stored at 4°C for 48 hrs. They were then soaked in sterile PBS for 1 hr and given a final rise in MilliQ water to remove any excess (non-covalently bound) antibodies. The arrays were then incubated with the secondary antibody (FITC – goat anti-mouse IgG/IgM, BD Biosciences) diluted to10 μg/mL in PBS for 1 hr and rinsed in MilliQ thrice. The arrays were imaged with a Nikon Eclipse 50i fluorescence microscope using 4x, 10x and 20x objectives. At the higher concentrations of printed antibodies, we observed a greater fluorescence intensity after secondary antibody incubation. This is due to the higher surface immobilization achieved with higher printing concentrations (Supplementary Fig. 1).

Tuning cell attachment and binding density.

Cell attachment was tuned by adjusting cell seeding density, varying the concentration of the arrayed capture antibodies, varying the incubation time, and the area of arrayed antibodies can be modified by using different printing pins on the microarrayer. Four different concentrations of antibody were used to study the effects of factor concentrations on cell attachment of lymphocytes: 200 μg/mL, 100 μg/mL, 50 μg/mL and 25 μg/mL (all diluted in PBS). For cell attachment studies, B lymphoblastoid cell line HRIK in RPMI media (10% FBS and 1% penicillin and streptavidin) was used to determine optimum attachment density

for adaptation with the CBMN assay. The cell seeding density was adjusted to 1×10^6 cells/mL. A wax barrier pen (Dako) was used to encase the arrayed antibody area and to help minimize cell volumes required. 1mL of cell suspension was pipetted onto the surface and incubated on the arrays for 30 min, 1 hr, and 2 hrs. The arrays were rinsed in PBS and fixed with 3.7% formaldehyde in PBS for 10 mins followed by staining with Hoechst 33342. Finally, digital images were obtained using a fluorescence microscope (Supplementary Fig. 2).

7 day incubation of cell microarrays.

The utility of long-term cell culturing with the microarrays substrates was determined. The microarrays slides were soaked in PBS containing pen-strep [Sigma, 120/200 μg/mL] and antifungal agent Amphotericin B [Sigma, 5μg/mL] for 2 hrs immediate prior to lymphocyte seeding. The arrays were seeded with lymphocytes (HRIK B cells) at a concentration of 2×10^6 cells/mL and incubated for 2 hrs to allow the cells to attach the corresponding antibody. The remaining cells in suspension that had not attached to the antibody were removed by gently rinsing the microarray in PBS (3x's). The incubation chamber was flooded with fresh, complete media and returned to the incubator for the next 7 days. The media was replaced with fresh, complete media every 2 days. On day 7, the arrays were fixed in 3.7% formaldehyde (Sigma) in PBS and stained with Hoechst 33342. The arrays were imaged with a Nikon fluorescent microscope with 10x and 20x objectives (Supplementary Fig. 3)

In situ cell sorting on cell microarrays.

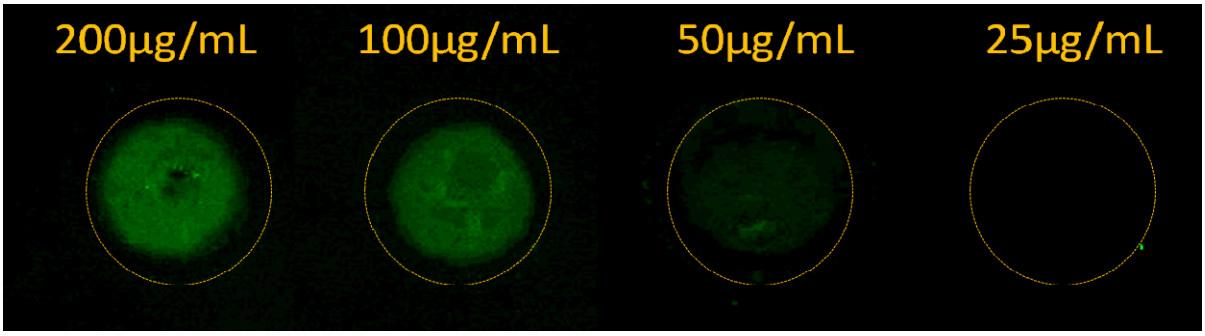
The effectiveness of the lymphocyte sorting on the arrays was determined by separate lymphocyte B and T cell microarrays. B (HRIK) and T (Jurkat) cell lines were cultured in RPMI media (10% FBS and 1% pen-strep). The two cell lines were stained with CellTracker

orange and CellTracker blue (Invitrogen) fluorescent stains separately. The two cell lines were then mixed together and incubated on microarrays (2 hrs) containing arrayed anti-CD2 and anti-CD20. A Nikon Eclipse 50i fluorescent microscope was used to manually count the number of cells per arrayed antibody and determine the percent selectivity of the corresponding antibodies. The results demonstrate ~95% separation efficiency of both the B cells residing on the anti-CD20 spots and the T cells on the arrayed anti-CD2 spots.

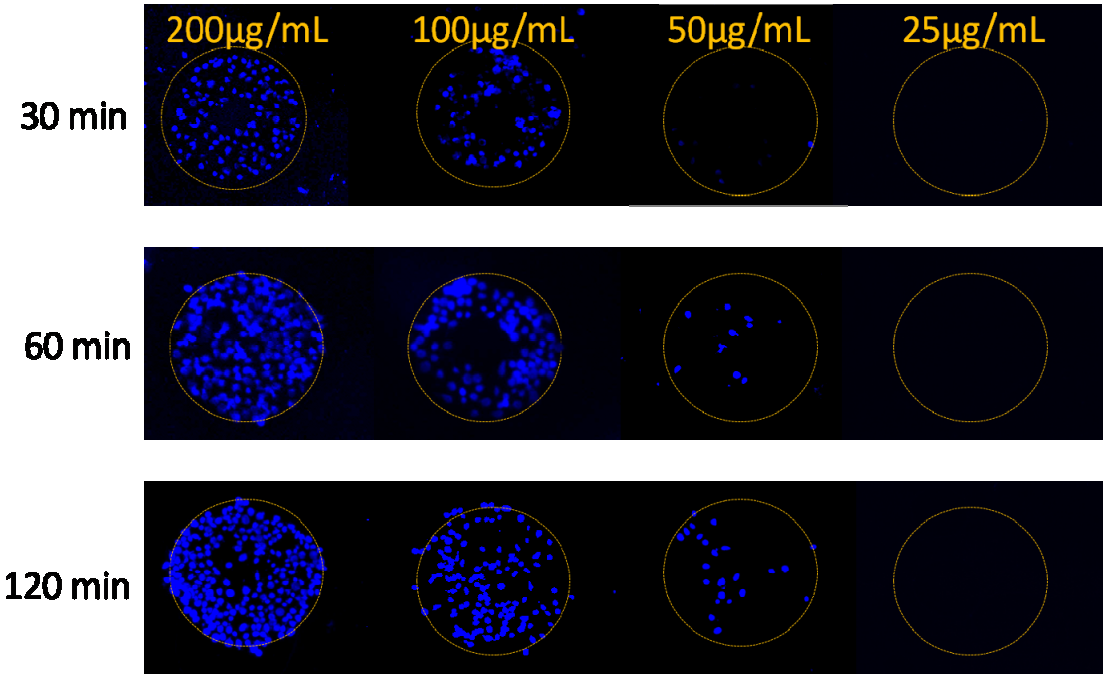
Flow cytometric phenotyping of freshly isolated peripheral blood lymphocytes and γ-irradiated lymphocytes.

A FACSAria (BD, San Jose, CA) flow cytometer equipped with a 488nm laser was used for determination of percent subsets. Peripheral blood mononuclear cells were isolated as stated in the methods. The cells were labelled with pre-determined concentrations of purified CD19, CD8, CD4, CD2, CD45 and CD20 (Rituximab) monoclonal antibodies. A secondary goat anti-mouse IgG/IgM FITC or rabbit anti-human IgG FITC was used to detect the primary antibody. Incubation of cells and antibody were performed at room temperature for 30 min. After washing, cells were analyzed by gating on FSC and SSC. The percentages of positive cells staining for each antibody in this population were recorded. Purified monoclonal antibodies and goat anti-mouse IgG/IgM FITC were purchased from BD Biosciences, San Diego, CA. Rabbit anti-human IgG FITC was purchased from Dako, Glostrup, Denmark and Rituximab was sourced from Roche, Basel, Switzerland.

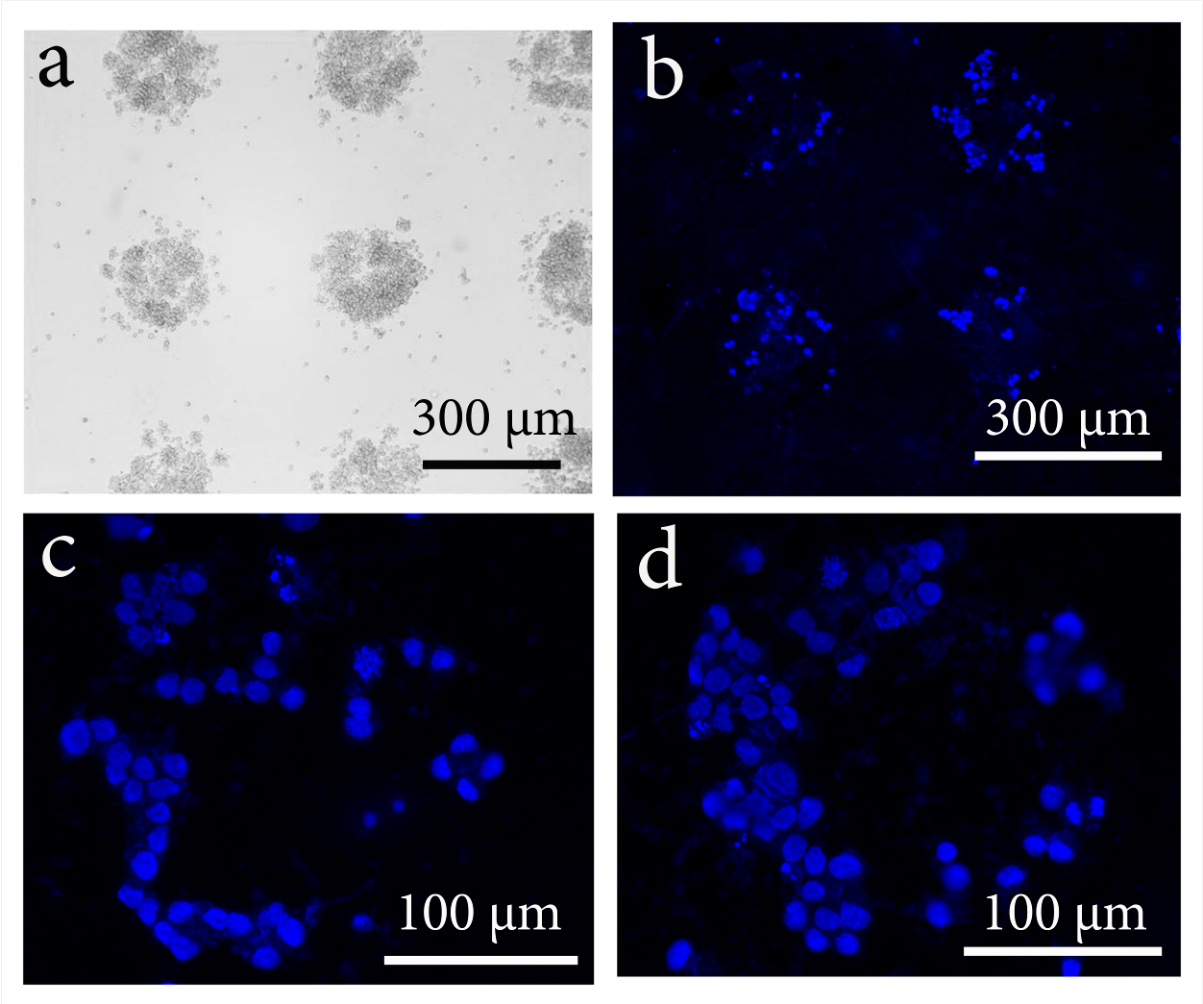
Supplementary Figures



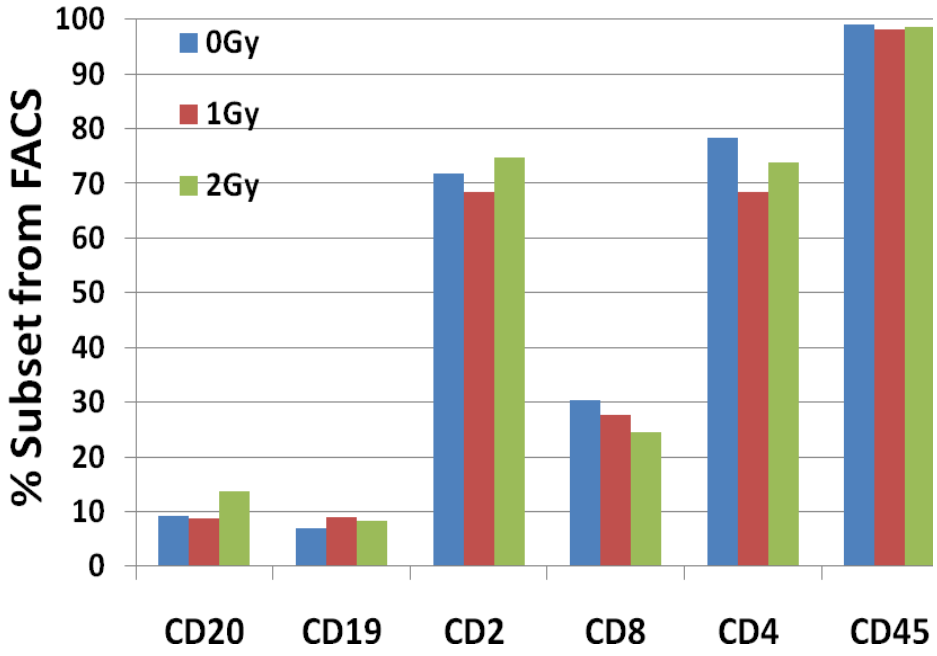
Supplementary Figure 1. Monoclonal antibody CD2 arrayed at various concentrations on microarray substrates. The monoclonal antibody was printed with a contact printer at 200, 100, 50, and 25 µg/mL concentrations. A fluorescently labeled secondary antibody was then incubated with the array for 1 hr. The increased antibody binding density is apparent at higher concentrations.



Supplementary Figure 2. Tuning cell attachment density on the microarrays. The cell density can be tuned by adjusting the incubation time and the printing concentrations of the arrayed antibodies. Four different concentrations of printed antibody (anti-CD20 in this image) have been incubated with B cells (HRIK) at various incubation times (30 min, 60 min and 120 min). Cell attachment increases with antibody concentration and incubation time. The highest cell attachment is observed for an antibody concentration of 200 µg/mL after incubation with B cells for 2 hrs.



Supplementary Figure 3. Microscopic images of lymphocyte microarrays after 3 and 7 days incubation. The arrays were treated with pen-strep and amphotericin B antifungal agent prior to seeding of lymphocytes in order to discourage contaminant growth. The microarrays were incubated for up to 7 days. (**a)** represents a typical living lymphocyte microarray that has been in culture for 3 days obtained in bright-field from an inverted microscope. (**b - d)** are images from the lymphocyte microarrays after 7 days in culture. The arrays were fixed and stained with Hoechst and imaged with 10x (b) and 20x objectives (c-d).



Supplementary Figure 4. Flow cytometric determination of lymphocyte subsets after various doses of ionizing radiation. A non-labeled monoclonal antibody (CD20, CD19, CD2 CD8, CD4, or CD45) was incubated with a heterogeneous mixture of freshly isolated lymphocytes and subsequently labeled with a secondary FITC labeled antibody for FACS analysis. The results indicate little effect caused by radiation exposure of up to 2 Gy on flow cytometry phenotyping results. Each measurement was generated with 20,000 cells.