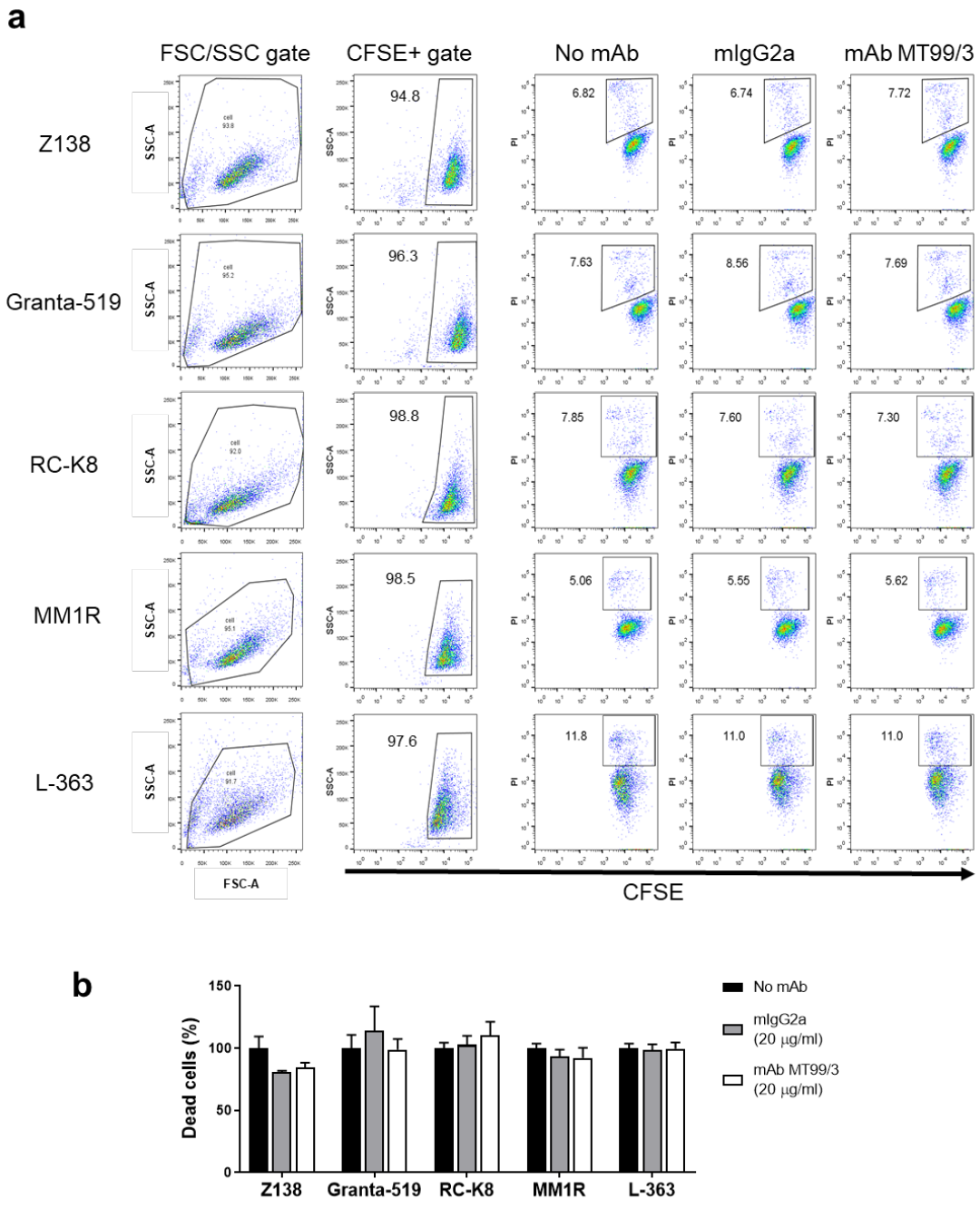
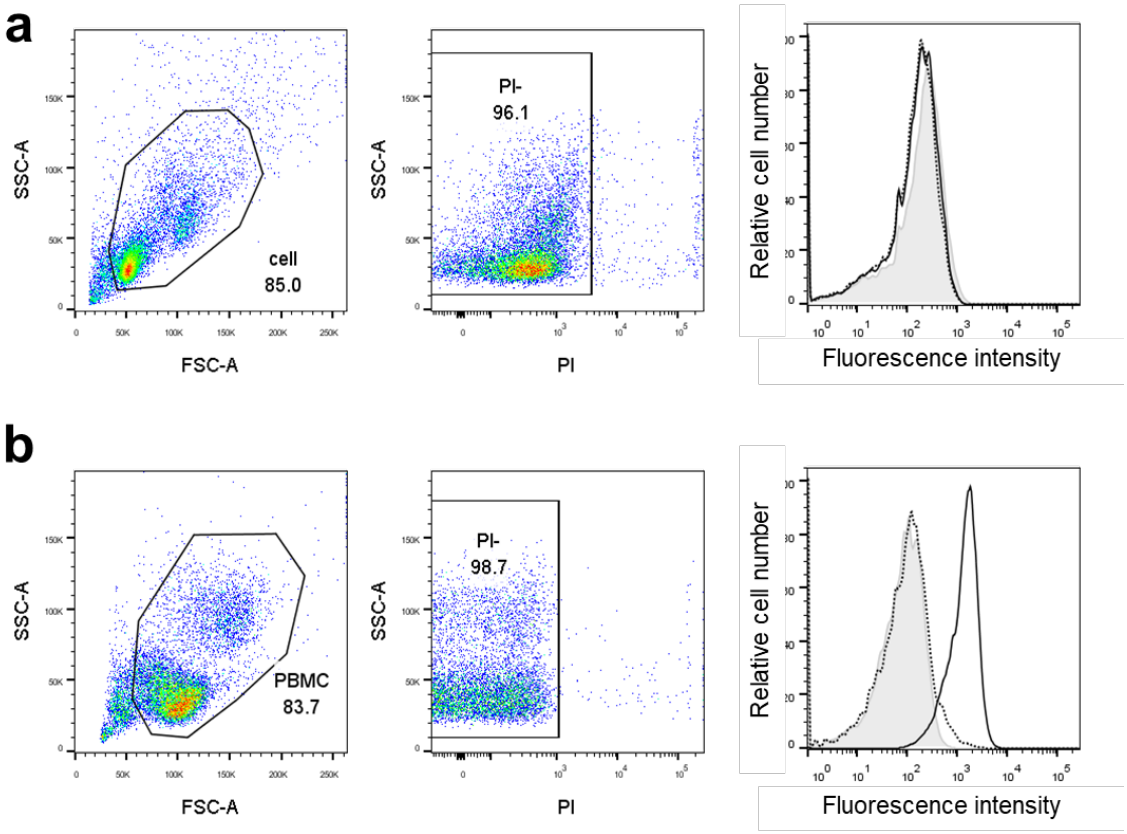
Supplementary Figure 1



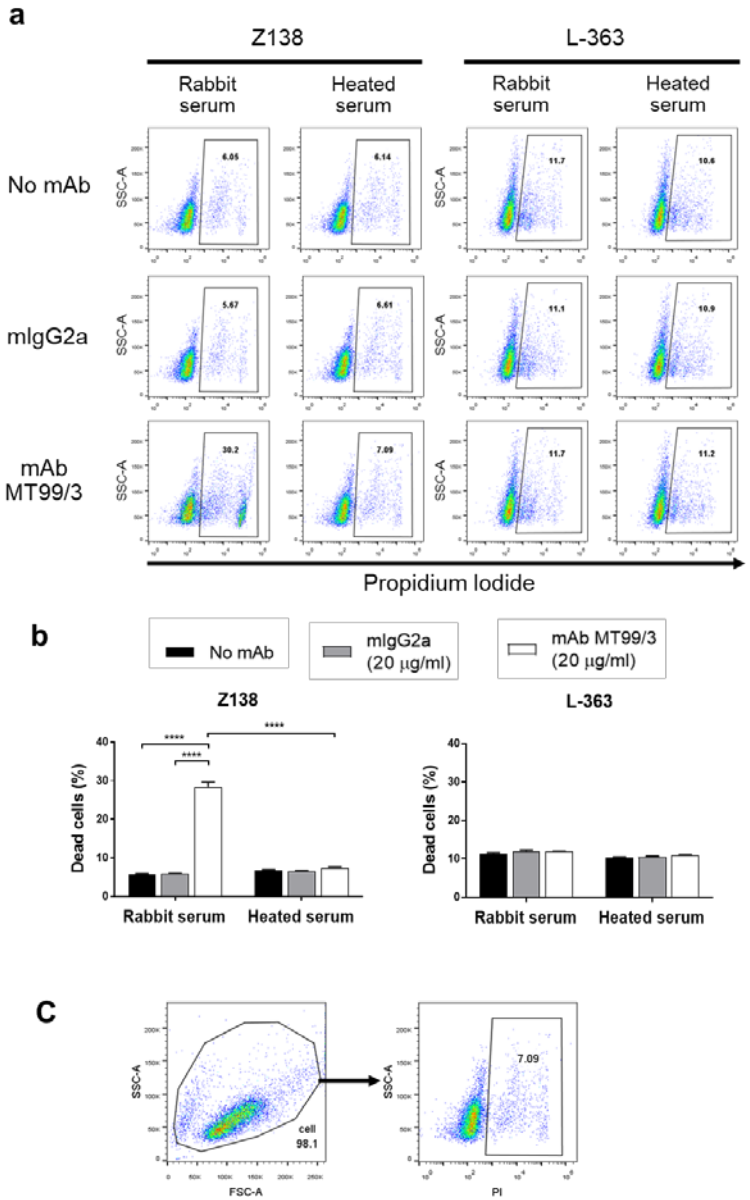
Supplementary Fig. 1 Direct cytotoxic effect of mAb MT99/3 on malignant B cell lines. CFSE labeled cell lines were treated with 20 μg/ml of anti-CD99 mAb (mAb MT99/3) or isotype-matched control mAb (mIgG2a) or kept in culture medium (no mAb) for 4 h at 37°C. Cell death was determined by PI staining. CFSE positive cells (CFSE+ gate) were gated from FSC/SSC gate. The dead cells (CFSE+PI+) were measured by flow cytometry. **a** The dead cells (%) in the indicated conditions are shown as the representative result from one of the triplicate. **b** The dead cells (%) of each condition were normalized using no mAb condition as 100%. The values are shown as mean ± SEM.

Supplementary Figure 2



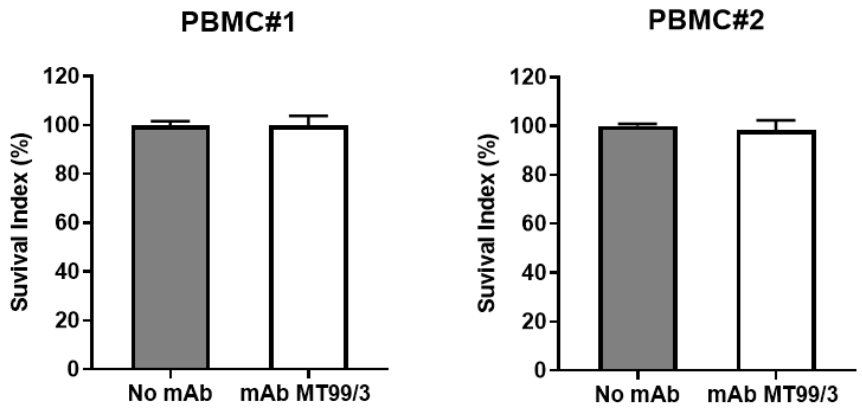
Supplementary Fig. 2 Determining cross-reactivity of anti-human CD99 mAb (MT99/3) to mouse CD99. **a** Mouse splenocytes from nude mice or **b** human PBMCs were stained with mAb MT99/3 (white peak with solid line), isotype-matched control mAb (white peak with dotted line) or no mAb (gray peak) followed by Alexa Flour-488-conjugated anti-mouse IgG (H+L) Abs. Stained cells were suspended in propidium iodide (PI) solution (0.5 μg/ml) and analyzed by a FACSCelesta flow cytometer. Live cells (negative staining for PI) were gated to determine CD99 expression using FlowJo software.

Supplementary Figure 3



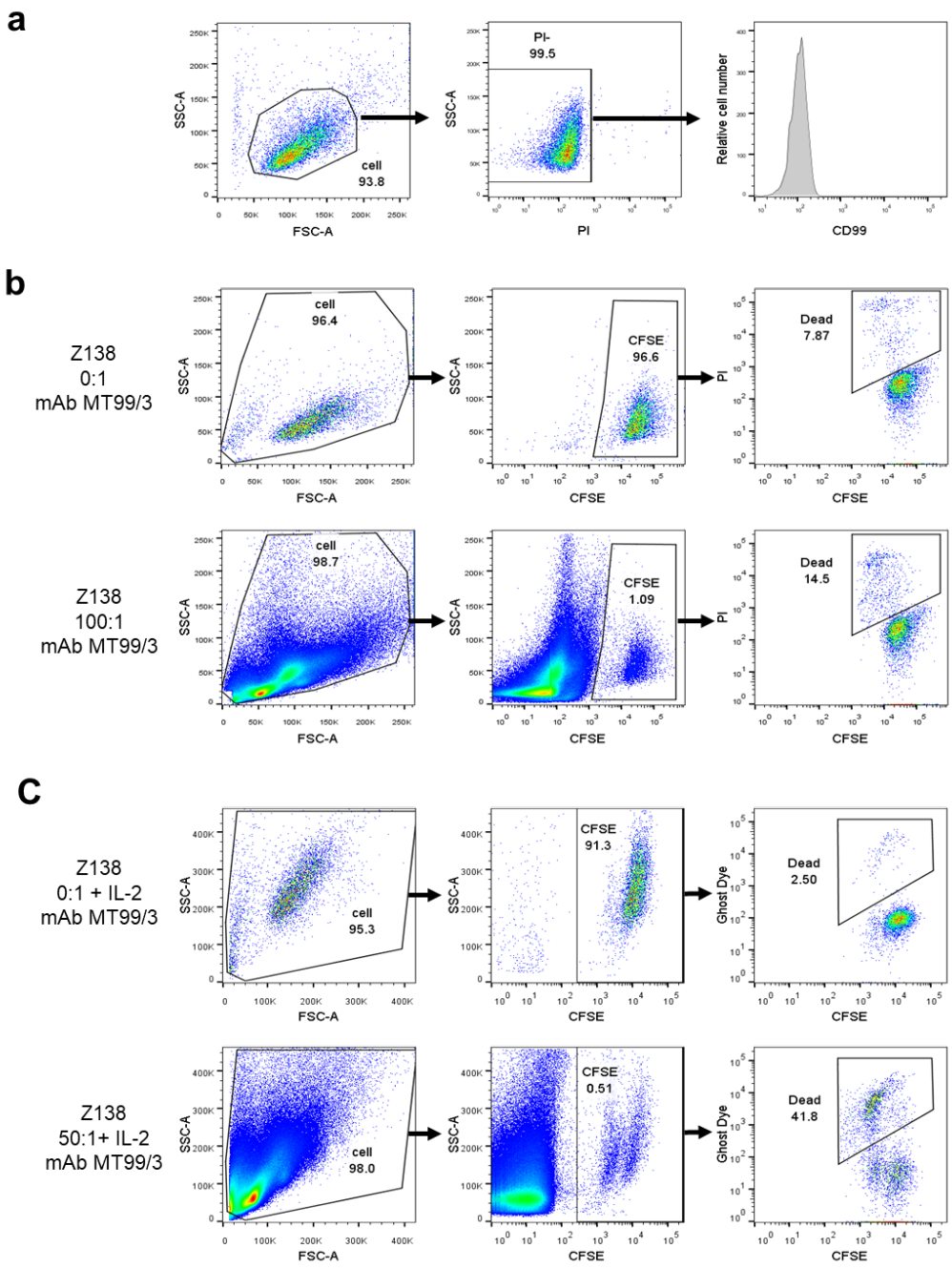
Supplementary Fig. 3 CDC activities of anti-CD99 mAb MT99/3 in Z138 and L-363 cell lines. CD99 positive Z138 and CD99 negative L-363 cell lines were plated into RPMI-1640 medium supplemented with 5% rabbit serum or heat-inactivated rabbit serum (Heated serum) in 96-well plate. Anti-CD99 mAb (mAb MT99/3) or isotype-matched control (mIgG2a) at 20 μg/ml were added or kept in culture medium (no mAb) and incubated for 2 h at 37°C. After cultivation, dead cells were determined by staining with PI solution and analyzed by flow cytometry. **a** The dead cells (%) in the indicated conditions are shown as the representative result from one of the triplicate. **b** The bar graphs of % dead cells are shown as the mean ± SEM. Two-way ANOVA followed by Tukey's multiple comparisons test was used for comparison, ****P<0.0001. **c** The gating strategy is exhibited.

Supplementary Figure 4



Supplementary Fig. 4 CDC activities of anti-CD99 mAb MT99/3 in CD99 expressing primary cells. Human peripheral blood mononuclear cells (PBMC; n=2) were plated into RPMI-1640 medium supplemented with 5% rabbit serum in 96-well plate in the presence or absence of mAb MT99/3. Cells were incubated for 2 h at 37°C. Survival cells were determined by MTT assay. The survival index (%) were calculated by the absorbance of each condition normalized to mean absorbance of no mAb condition as 100%. The bar graphs of % survival index are shown as the mean ± SD of triplicated wells.

Supplementary Figure 5



Supplementary Fig. 5 Gating strategies and flow cytrometric analysis. **a** Gating strategy of immunofluorescence staining of CD99 expression on the representative cell line is shown. Live cells (negative staining for PI) were gated from FCS/SSC gate then CD99 expression levels were determined as histogram graphs. **b** The representative results of ADCC assay by freshly isolated mouse splenocytes are shown. CFSE positive target cells were gated from FCS/SSC gate then dead target cells were determined by $CFSE^{+}PI^{+}$. **c** The representative results of ADCC assay by IL-2 activated mouse splenocytes are shown. CFSE positive target cells were gated from FCS/SSC gate then dead target cells were determined by $CFSE^{+}$ Ghost Dye^{+}.