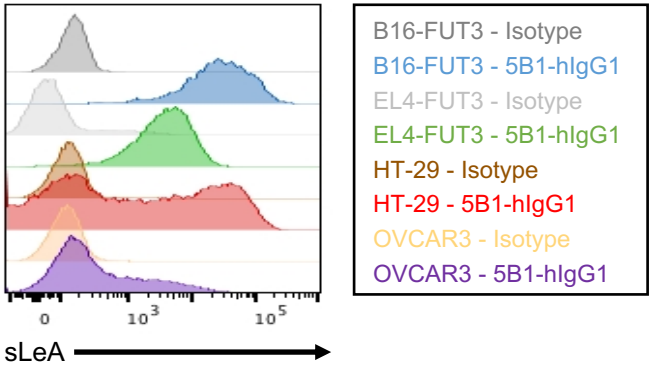
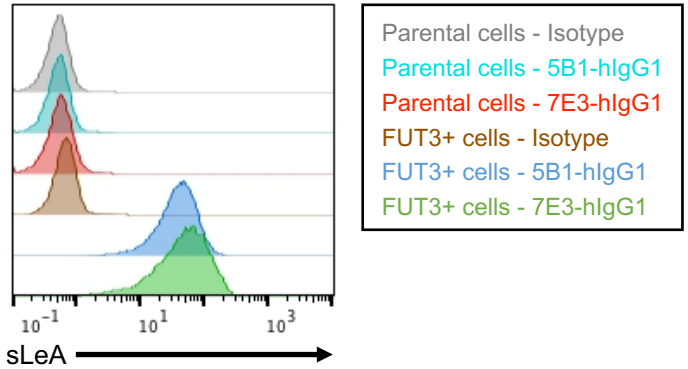
(A) sLeA expression on murine and human tumor cells



(B) Binding of different sLeA-targeting Ab clones to sLeA+ tumor cells



(C) Binding of different sLeA-targeting Ab clones to synthetic sLeA - indirect ELISA

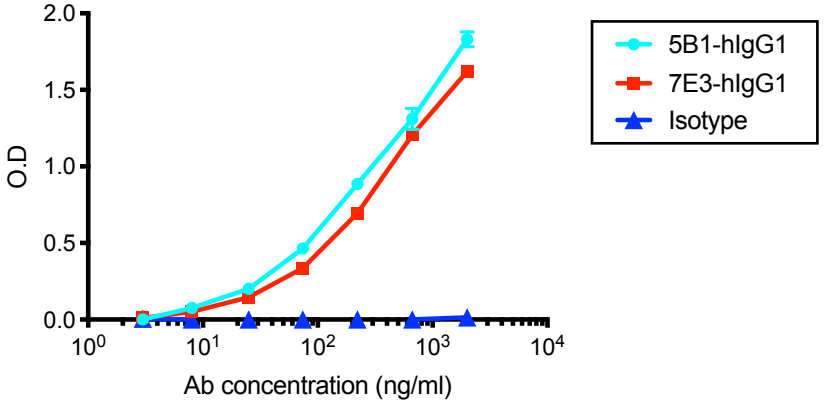


Figure S1

(A) Murine tumor cells were transduced to stably express the human enzyme Fucosyltransferase III (FUT3), which synthesizes sLeA. *FUT3*-transduced murine tumor cells and human tumor cells (HT-29 and OVCAR3) were labeled with an Anti-sLeA primary Ab (clone 5B1-hIgG1) followed by Alexa-488-conjugated-goat anti-human IgG. The panel shows a representative experiment (n>3), showing similar results. **(B)** To validate binding of 5B1 and 7E3 Ab clones to surface sLeA - cells were labeled with an Anti-sLeA primary Abs followed by Alexa-488-conjugated-goat anti-human IgG. The panel shows a representative experiment (n>3), showing similar results. **(C)** Assay plates were coated and incubated with synthetic sLeA, blocked and incubated with serial dilutions of clones 5B1-hIgG1, 7E3-hIgG1 or non-relevant hIgG1 isotype control Abs. HRP-conjugated goat anti-human IgG Abs, followed by addition of TMB substrate were used for detection.

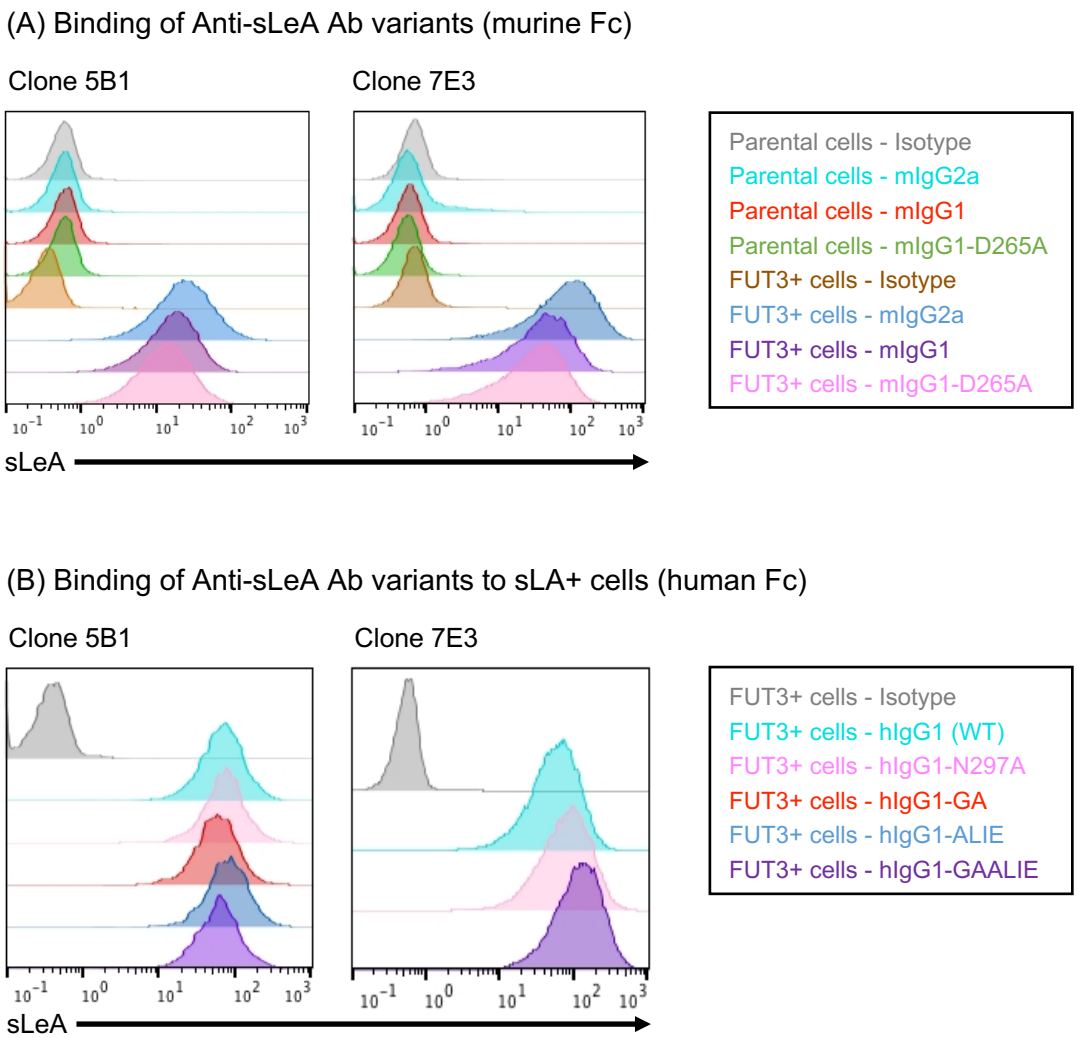
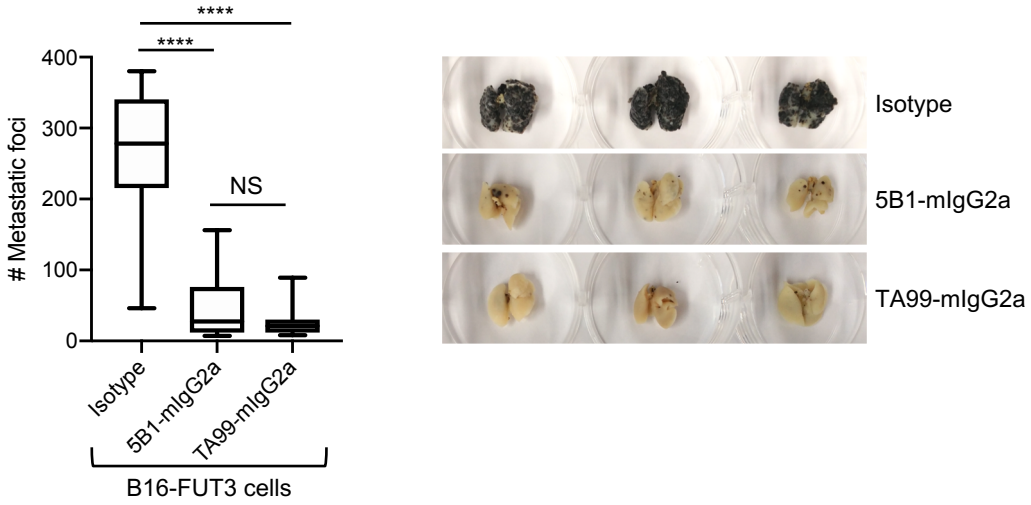


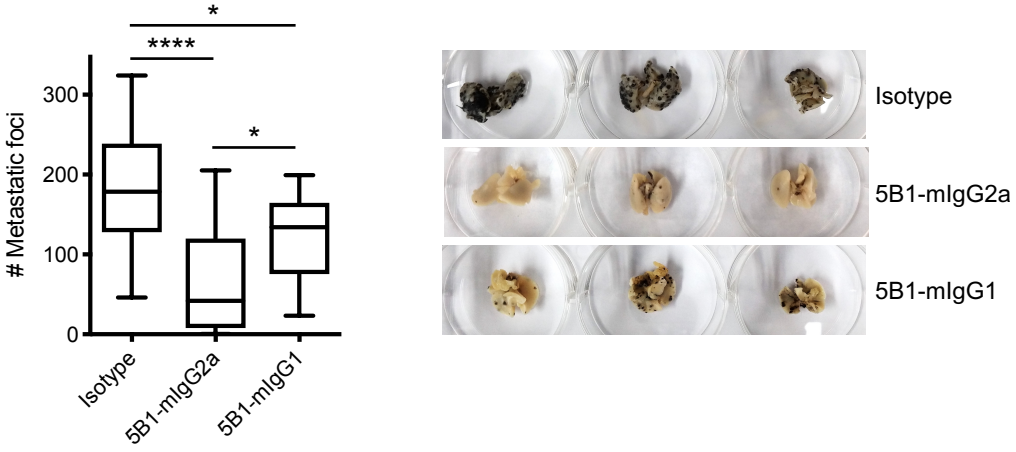
Figure S2

Murine tumor cells were transduced to stably express the human enzyme Fucosyltransferase III (FUT3), which synthesizes sLeA. To validate binding of various sLeA-targeting Abs with a murine Fc **(A)** or human Fc **(B)** - cells were labeled with an Anti-sLeA primary Abs followed by Alexa-488-conjugated-goat anti-human IgG or anti-mouse IgG and analyzed by flow cytometry. Both panels show a representative experiment (n>3), showing similar results.

(A) sLeA-targeting Abs protect mice from sLeA+ tumor challenge comparably to gp75-targeting Abs



(B) 5B1 Ab-mediated anti-tumor effect relies on activating FcγRs



(C) 5B1-hIgG1 activity relies on FcγR engagement

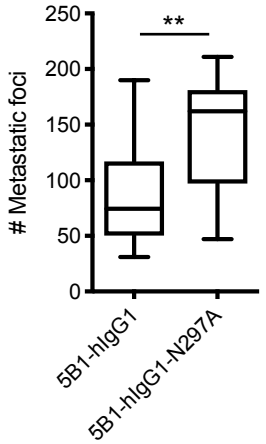


Figure S3

WT C57BL/6 mice **(A, B)** or FcγR-humanized mice **(C)** were inoculated IV with $5*10^5$ B16-FUT3 tumor cells. 100 µg of anti-sLeA Abs (5B1-mIgG2a or 5B1-mIgG1 or 5B1-hIgG1 or 5B1-hIgG1-N297A), anti-gp75 (TA99-mIgG2a) or isotype-matched control Abs were administered IP on days 1,4,7 and 11. 14 days post-inoculation, mice were euthanized, lungs were excised and fixed, and metastatic foci were counted. For all panels, data pooled from n=2-3 experiments, n≥11/group. Panels A, B show representative images of three excised lungs from each group. * p<0.05, ** p<0.01, **** p<0.0001. For all panels, the box extends from 25th to 75th percentile, the line within the box represents the median value and the whiskers correlate to 5-95 percentiles.

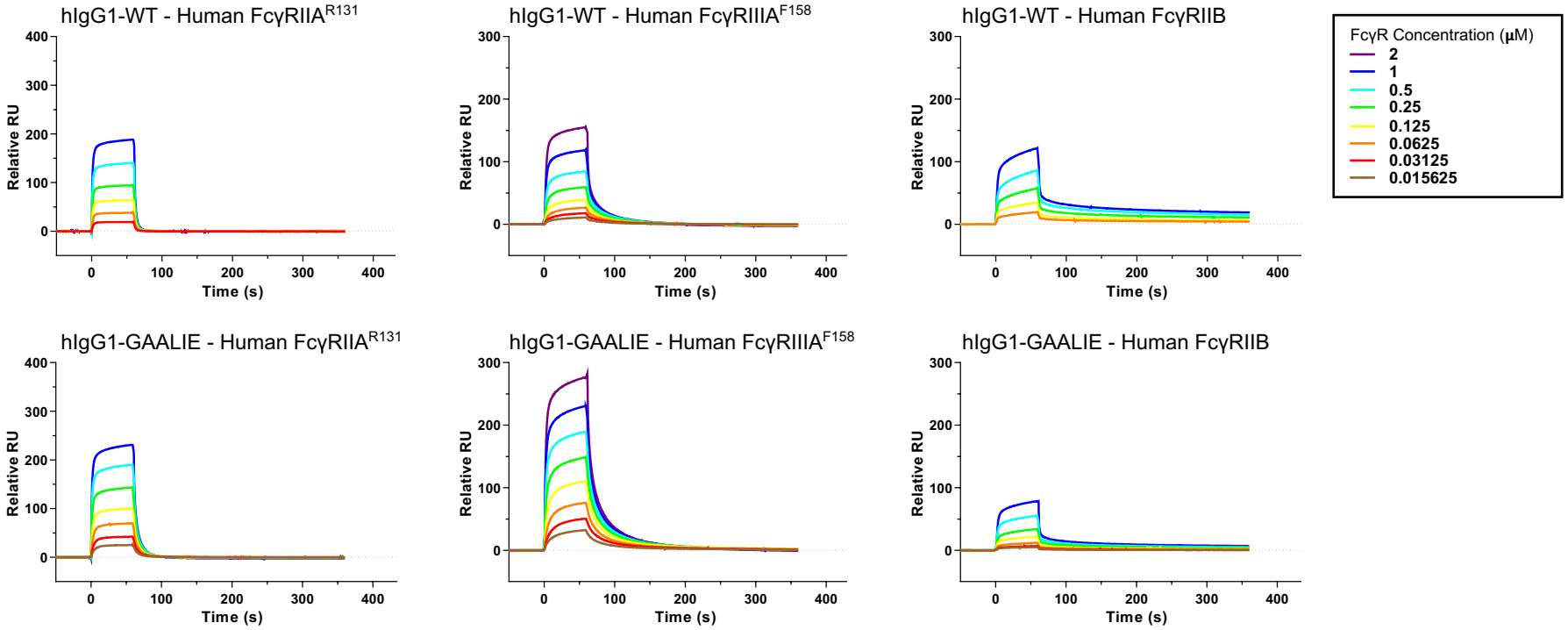


Figure S4

Binding affinity of hIgG1 Fc variants to human FcγRs was determined by SPR analysis. Representative SPR sensorgrams of hIgG1-WT and hIgG1-GAALIE variants binding to the various classes of hFcγRs (see table 1 for the affinity measurements and fold change in affinity over hIgG1-WT).

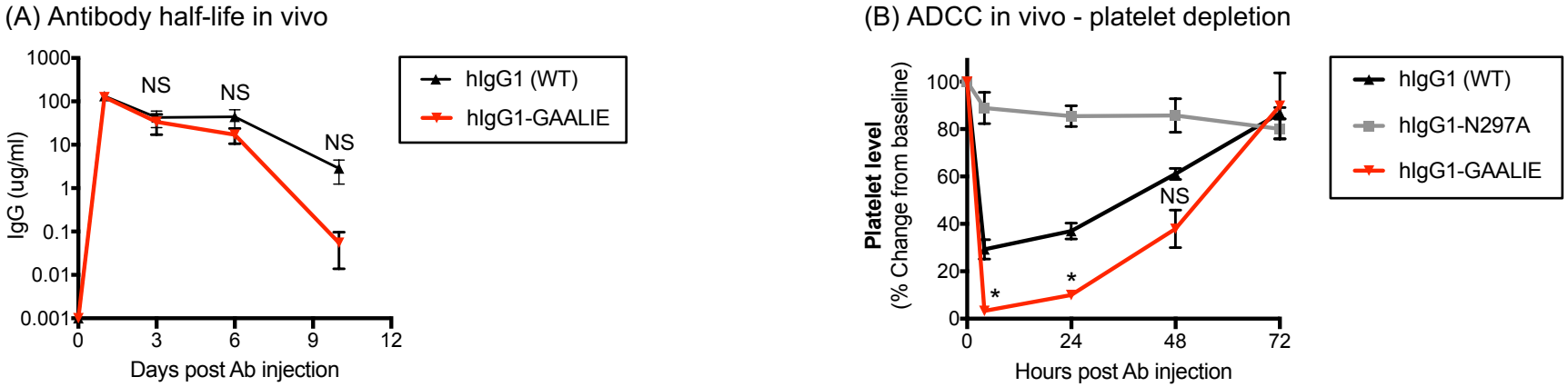
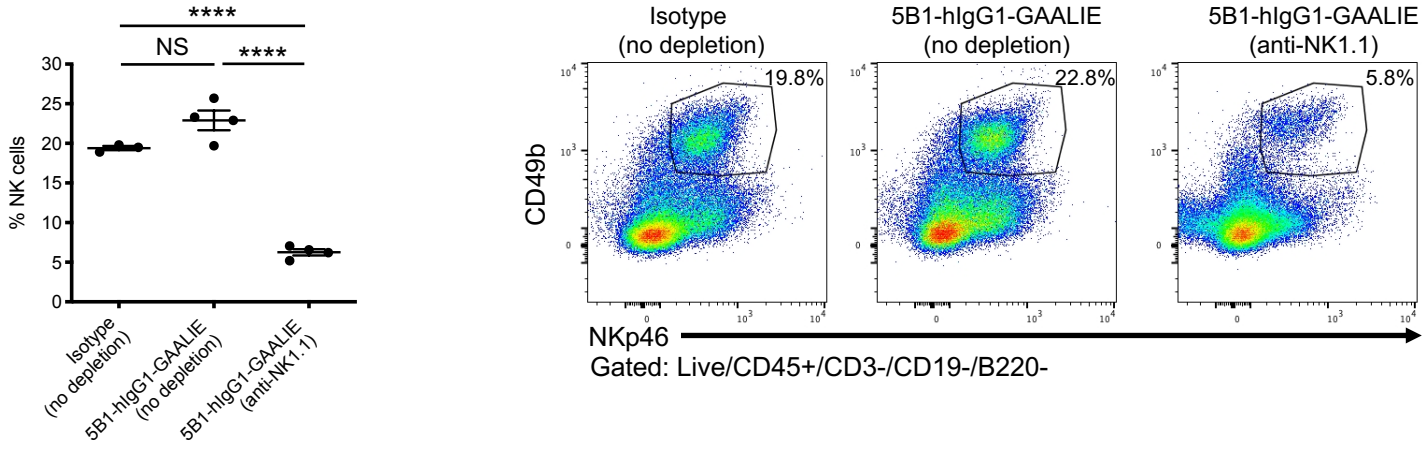


Figure S5

(A) Half-life of hIgG1 variants in FcγR-humanized mice – FcγR-humanized mice were injected IV with 100 µg of hIgG1 Fc variants, bled at indicated time points, and serum IgG levels were determined by ELISA. n=4/group. **(B) ADCC in vivo** – FcγR-humanized mice were injected intravenously with 10 µg of hIgG1 Fc variants (clone 6A6, targeting a platelet-associated antigen). hIgG1-N297A (was included as control. Mice were bled at the indicated time points and platelet numbers were analyzed using an Advia 120 hematology system. Values represent the mean (± SEM) percentage change in platelet number relative to 0h. n=3/group. For hIgG1-WT vs. hIgG1-GAALIE at 4h and 24h - * $p<0.05$.

(A) Validation of NK cell depletion in murine lungs



(B) NK cell depletion does not hamper anti-tumor effect of sLeA-targeting Abs in FcγR-humanized mice

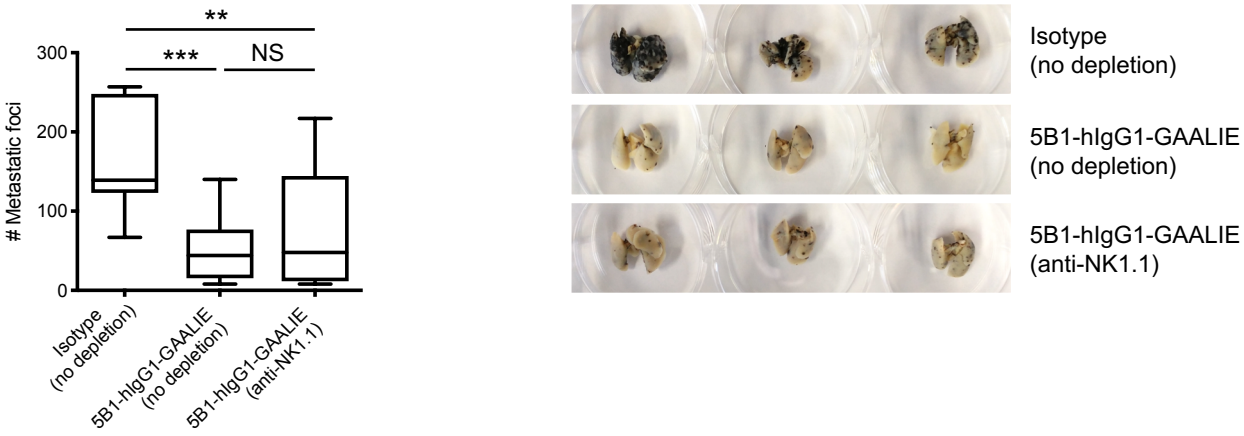


Figure S6

FcγR-humanized mice were inoculated IV with 5*10^5 B16-FUT3 tumor cells. 100 µg of anti-sLeA Abs (5B1-hIgG1-GAALIE with G236A/A330L/I332E mutations) or isotype-matched control Abs were administered IP on days 1,4,7 and 11. NK cells were depleted using Intra-nasal administrations of 300 µg of anti-NK1.1 Abs on days -3, -1, 1, 4, 7, and 11. For all panels, 14 days post-inoculation, mice were euthanized, and lungs were excised. **(A) Validation of NK cell depletion** – Lungs were perfused, dissociated and stained for flow cytometry. NK cells were gated as follows: Live/CD45+/CD3-/CD19-/B220-/NKp46+/CD49b+ cells, and are presented as Mean ± SEM. n≥4/group. **** p<0.0001 (one-way ANOVA with Bonferroni's post-test). The panel summarizes the data obtained for all mice (left), and shows representative dot plots for a single mouse from each group (right). **(B) NK cell depletion does not hamper anti-tumor effect of sLeA-targeting Abs** – Lungs were excised and fixed, and metastatic foci were counted. Data pooled from n=2 experiments, n≥11/group, and shows representative images of three excised lungs from each group. ** p<0.01, *** p<0.001 (one-way ANOVA with Bonferroni's post-test). The box extends from 25th to 75th percentile, the line within the box represents the median value and the whiskers correlate to 5-95 percentiles.

anti-sLeA and anti-gp75 Abs demonstrate differential hFcγR-engagement requirements to confer anti-tumor activity

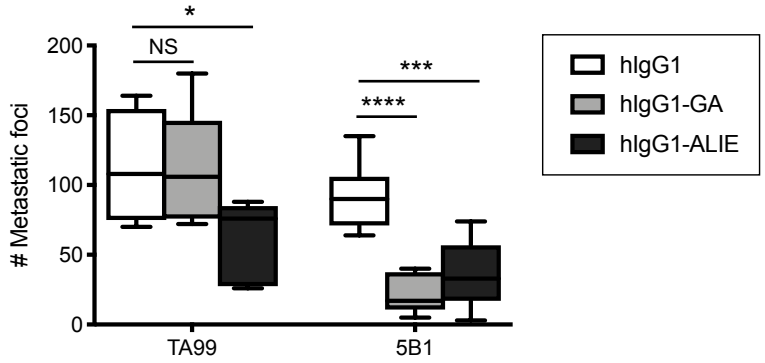


Figure S7

FcγR-humanized mice were inoculated IV with $5*10^5$ B16-FUT3 tumor cells. 100 μg of anti-sLeA Abs (5B1-hIgG1 or 5B1-hIgG1-GA or 5B1-hIgG1-ALIE) or anti-gp75 (TA99-hIgG1 or TA99-hIgG1-GA or TA99-hIgG1-ALIE) or isotype-matched control Abs were administered IP on days 1,4,7 and 11. 14 days post-inoculation, mice were euthanized, lungs were excised and fixed, and metastatic foci were counted. For 5B1 - data pooled from n=2 experiments, n=10-12/group. For TA99 - n=5/group. * $p<0.05$, *** $p<0.001$, **** $p<0.0001$ (one-way ANOVA with Bonferroni's post-test). The box extends from 25th to 75th percentile, the line within the box represents the median value and the whiskers correlate to 5-95 percentiles.