

966 **SUPPLEMENTAL FIGURE LEGENDS**

967 **Supplemental Figure 1** Siglec-7 and -9 ligand expression on tumor cell lines and
968 primary tumors assessed by flow cytometry **(A,B)** or confocal microscopy **(C,D)**. **(A)**
969 Histograms depict differential staining for ligands of Siglec-3, -7 and -9 on melanoma
970 line A375, head and neck squamous cell carcinoma (HNSCC) cell line LAU2106, and
971 colon adenocarcinoma line HCT116. Grey-shaded histograms represent control
972 staining with secondary antibody. **(B)** Geometric mean fluorescence intensity (GMFI)
973 ratios of Siglec-7 (open symbols) and Siglec-9 (filled symbols) ligand expression on
974 CLL (n=3) and AML (n=3) leukemia cells. **(C,D)** Paraffin-embedded tissue biopsy
975 sections of malignant melanoma lesions in dermal skin layers **(C)** or healthy dermal
976 skin **(D)**, co-stained for the melanoma marker Melan-A and Siglec-7 or Siglec-9
977 ligands, respectively. Scale bar, 50 μm **(C,D)**. Data are representative of at least
978 three **(A)** independent experiments, and of six individual subjects **(C,D)**.

979

980 **Supplemental Figure 2.** Neuraminidase treatment has no effect on 721.221 cells.
981 Cytotoxicity of isolated peripheral blood NK cells from healthy donors (n=5) against
982 721.221 as assessed in a ^{51}Cr -release assay, without (open circles) or with
983 neuraminidase-treatment (filled circles) of target cells. Cytotoxicity was evaluated at
984 the indicated effector to target (E/T) ratios.

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986 **Supplemental Figure 3.** Flow cytometric intracellular cytokine measurement in NK
987 cells exposed to neuraminidase treated or untreated K562 cells (10/1 E/T ratio, n=7).
988 *P<0.05 and **P<0.005 (Student's *t*-test).

989 **Supplemental Figure 4.** Circulating NK cells of (hu)-NSG mice express high levels
990 of Siglec-7. Flow cytometry of NK cells from peripheral blood of hu-NSG mice (n=13)
991 for expression of human Siglec-7 and Siglec-9. Frequency (left of each pair) and
992 representative histograms (right of each pair) are shown. Specific staining (black
993 line), and isotype-matched control (shaded).

994

995 **Supplemental Figure 5.** Siglec-7 and Siglec-9 ligands on K562 cells are not
996 reexpressed within 24 hours following neuraminidase treatment. Expression of
997 Siglec-7 and Siglec-9 ligands on K562 cells before (t=0) or after neuraminidase-
998 treatment, as assessed by flow cytometry over a period of 24 hours. Values are
999 expressed as GMFI ratio compared to control.

1000

1001 **Supplemental Figure 6.** The combination of Siglec-7 and -9 Fab fragments has no
1002 enhanced effect on NK cell cytotoxicity against K562 target cells, as assessed by a
1003 ⁵¹Cr assay at an E/T ratio of 10/1 (n=7). **P<0.005 (Student's *t*-test).

1004

1005 **Supplemental Figure 7.** Effects of targeting Siglec-7 and -9 on NK cell cytotoxicity,
1006 survival and proliferation. (A,B) Cytotoxicity of isolated peripheral blood NK cells from
1007 healthy donors against the K562 cell line assessed in a ⁵¹Cr-release assay in the
1008 presence of indicated mAbs (n=2 for Clone 191240, n=10 for Clone E10-286). E/T
1009 ratio 20/1. (B) Cytotoxicity of sorted human CD56^{dim} CD16⁺ Siglec-9⁻ NK cells against
1010 the K562 cell line assessed in a ⁵¹Cr-release assay in the presence of E10-286 mAb
1011 or an isotype control (n=3). (C,D) Flow cytometric analysis of NK cell survival (C) and

1012 proliferation (D). (C) AnnexinV-GFP/PI staining of 24-hour cultures in the presence of
1013 indicated mAbs at 10 $\mu\text{g/ml}$. Similar results were achieved at lower mAb
1014 concentrations. (D) CFSE dilution upon stimulation with IL-2 and IL-15 for 9 days and
1015 culture in the presence of anti-Siglec-7 mAb at 4 $\mu\text{g/ml}$, anti-Siglec-9 mAb at 3 $\mu\text{g/ml}$,
1016 or isotype-matched control mAbs. Data are representative of four (C) or three (D)
1017 independent experiments.

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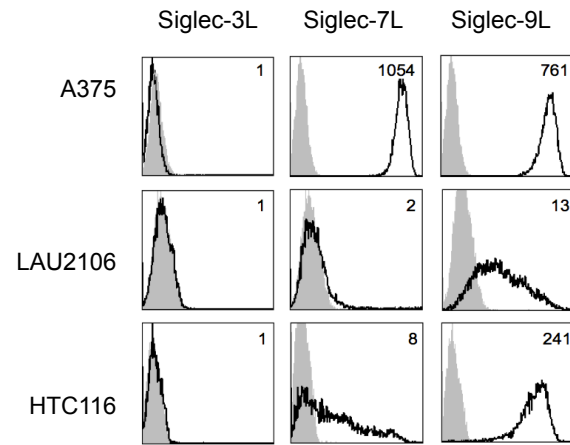
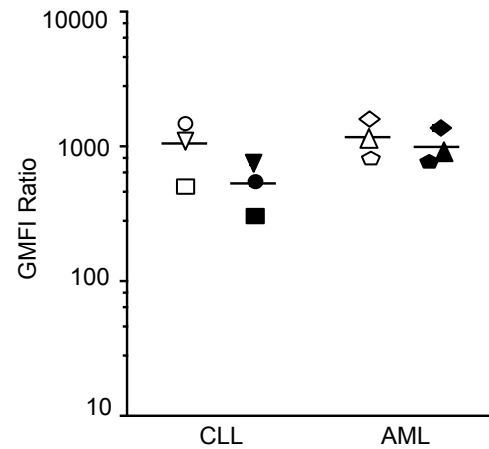
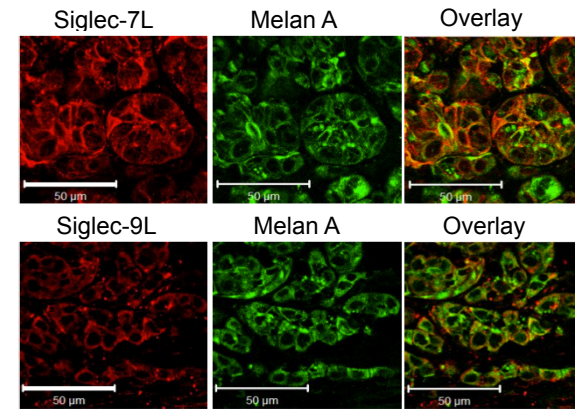
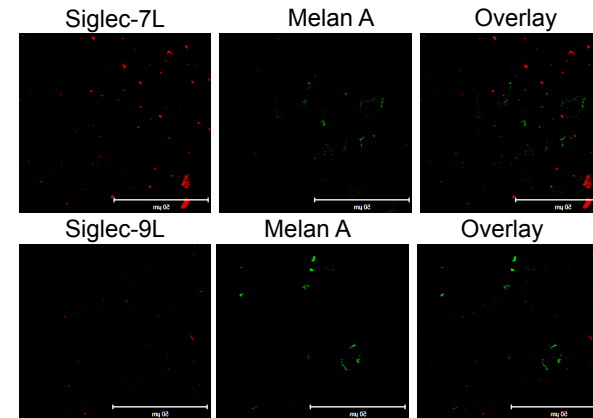
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1020 cytometry of surface siglec receptor expression on peripheral blood NK cells of adult
1021 healthy donors with and without neuraminidase treatment. Specific staining (black
1022 line), neuraminidase treated cells (dashed line) and isotype-matched control
1023 (shaded) (n=3). (B) Representative examples of Siglec-7 and Siglec-9 expression on
1024 $\text{CD56}^{\text{bright}} \text{CD16}^{\text{dim/-}}$ and $\text{CD56}^{\text{dim}} \text{CD16}^{\text{+}}$ NK cell subsets with or without unmasking
1025 by neuraminidase treatment (removal of sialic acid residues). **(C,D) Siglec-9**
1026 **expression on NK cells in presence of NK cell-relevant cytokines as assessed by flow**
1027 **cytometry. C) Geometric mean fluorescence intensity (GMFI) ratios of gated Siglec-**
1028 **9⁺ NK cells from total NK cell cultures. D) Histograms are representative of freshly**
1029 **fluorescence-activated cell sorted NK cell Siglec-9⁺ or Siglec-9⁻ CD56^{dim} NK cell**
1030 **subsets (dotted line), isotype control (shaded), 24 (dashed line), 48 (solid line) or 120**
1031 **(long dashed line) hour-cultures. Data are representative of at least three (A,D), six**
1032 **(C), or 14 (B), experiments.**

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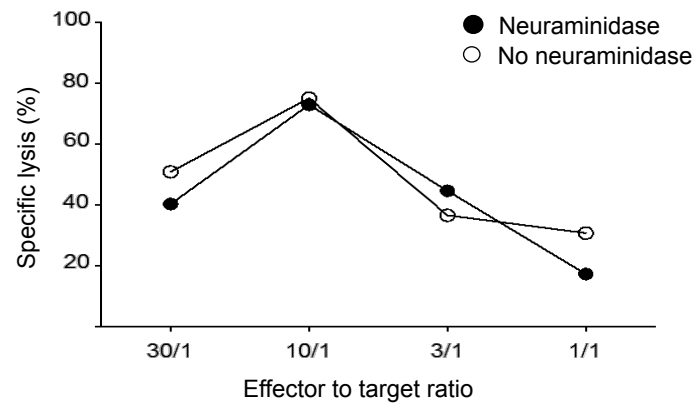
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1036 surface expression of NKG2D and NCRs.

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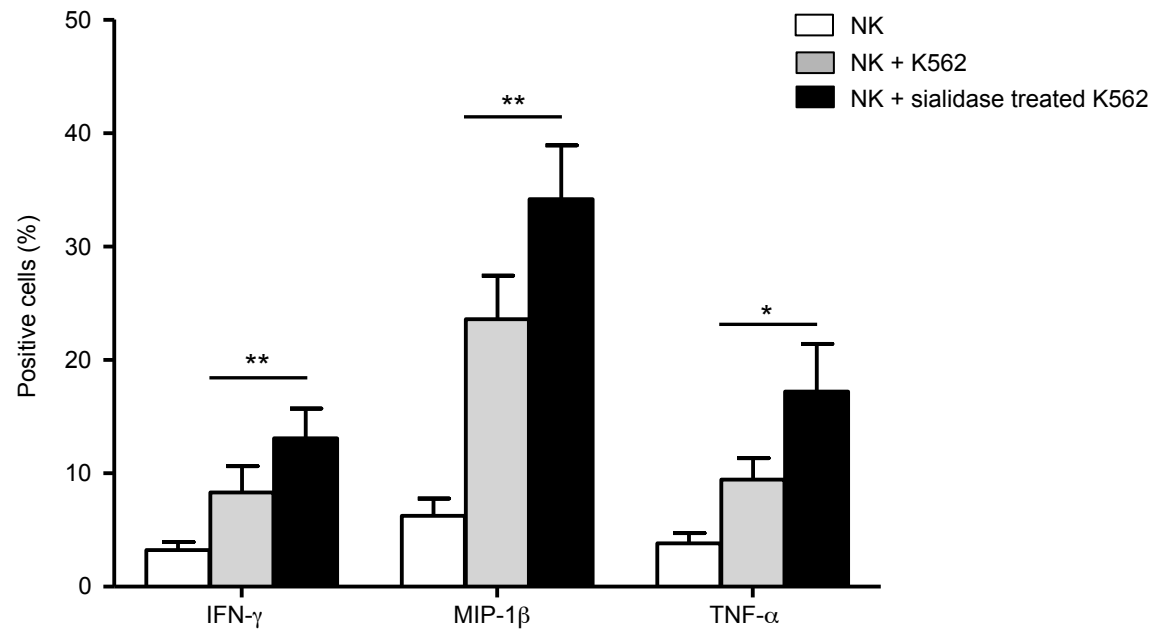
1038 **Supplemental Figure 10.** Frequency and Siglec-7 expression on peripheral blood
1039 NK cells in cancer. **(A)** NK cell numbers in the peripheral blood of melanoma and
1040 colon adenocarcinoma (CoACa) patients. **(B)** Cytotoxicity of NK cells from healthy
1041 donors (HD) and melanoma patients, as assessed in a ⁵¹Cr release assay of K562
1042 target cells at an E/T ratio of 20/1 (n=7). **(C)** Expression of Siglec-7 on peripheral
1043 blood NK cells of healthy donors (HDs) and cancer patients. Shown are frequency
1044 and GMFI ratio of specific staining to isotype-matched control, without (white bars) or
1045 with (grey bars) neuraminidase treatment. *P<0.05, and **P<0.005 (Student's t-test).

A**B****C****D**

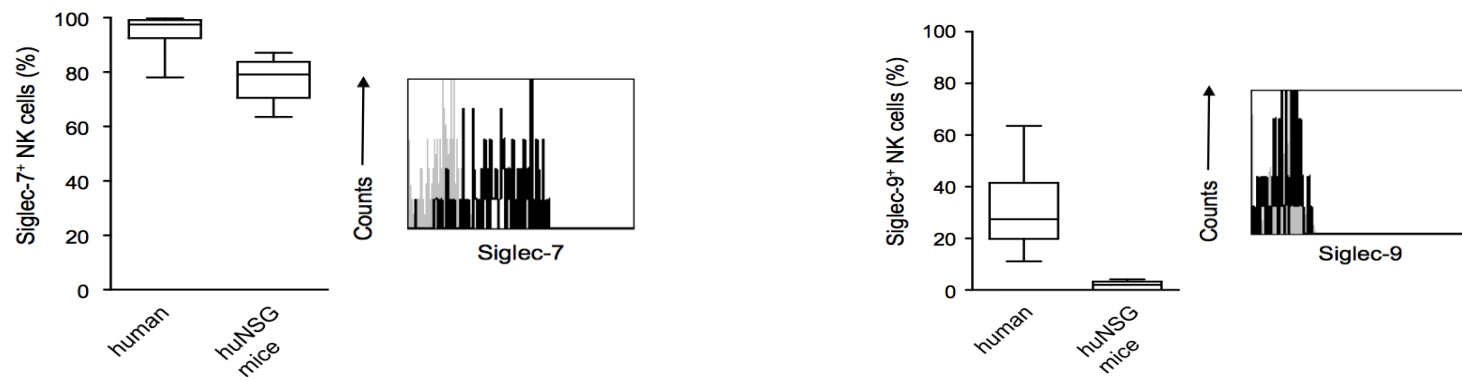
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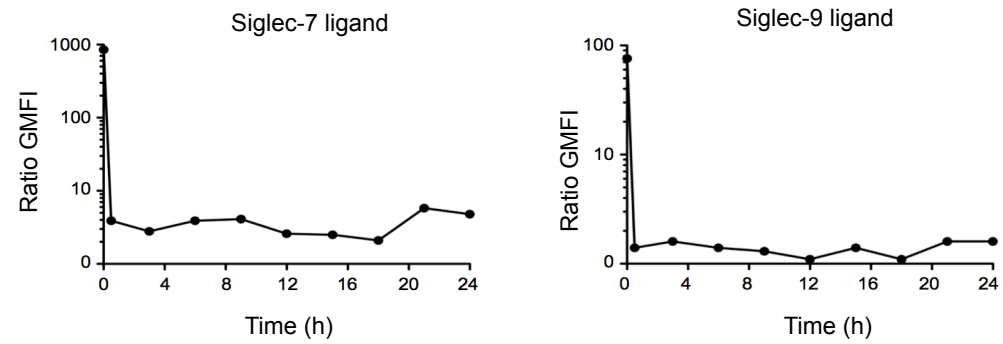
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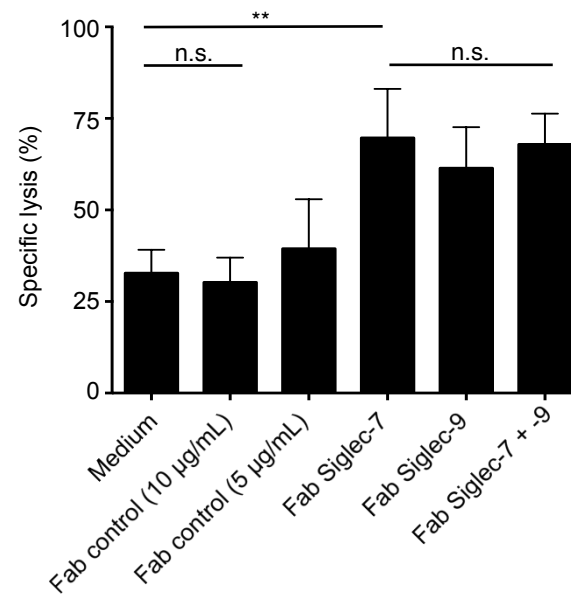
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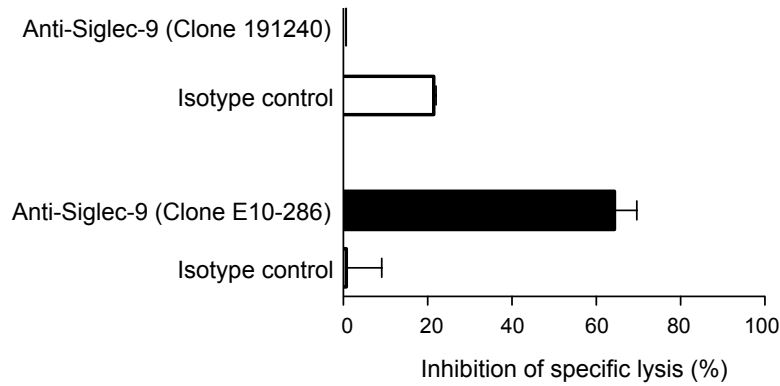
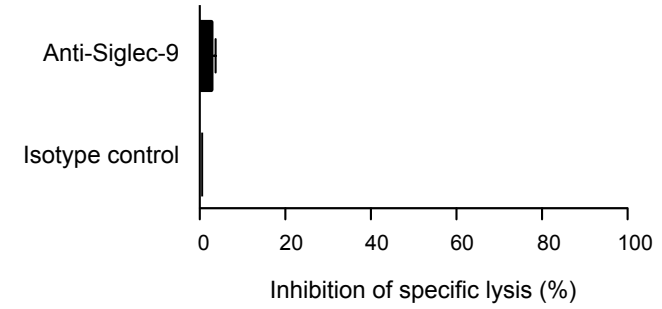
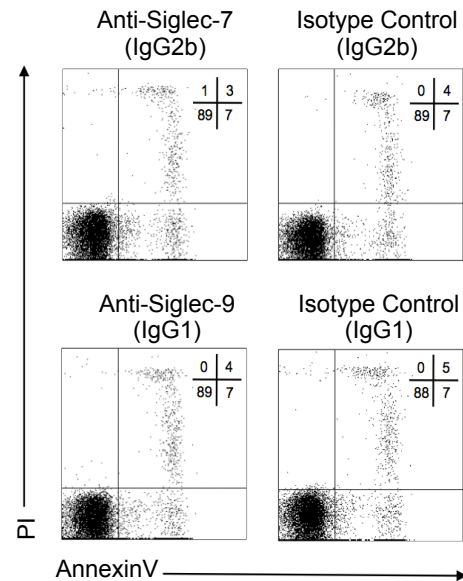
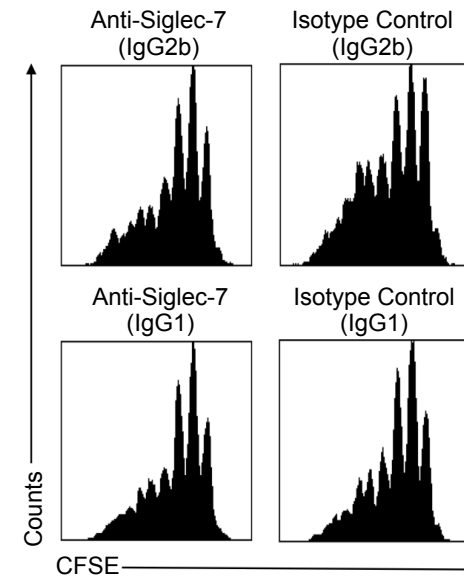
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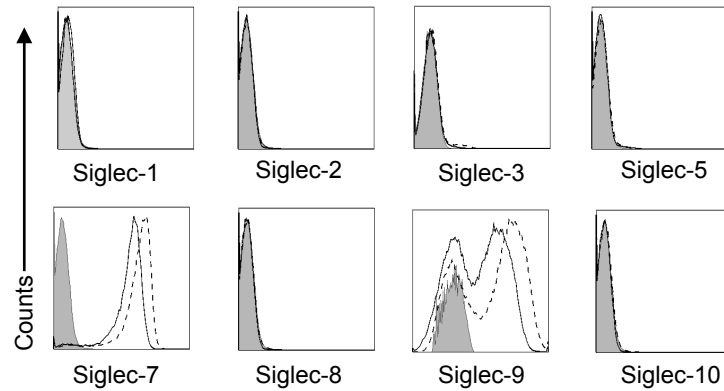
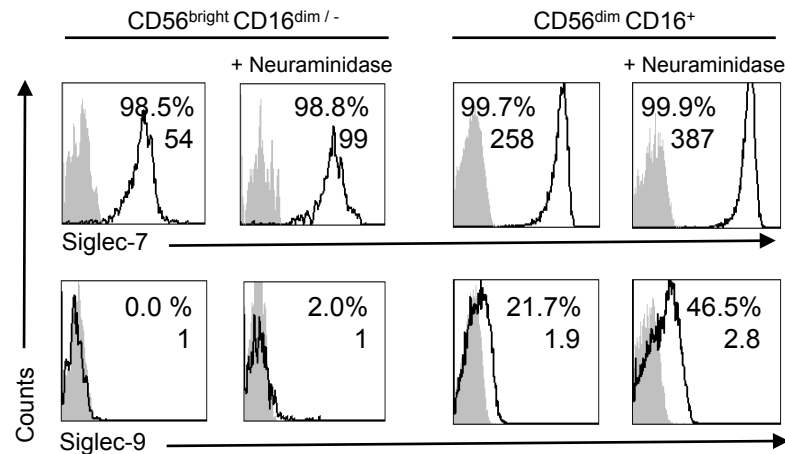
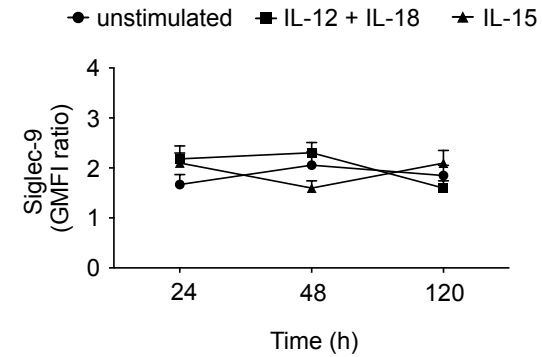
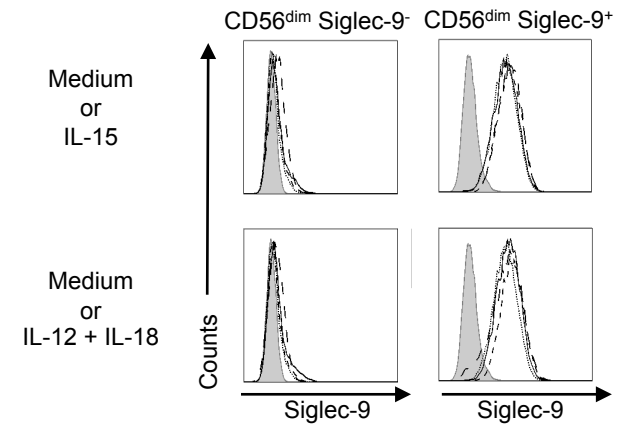
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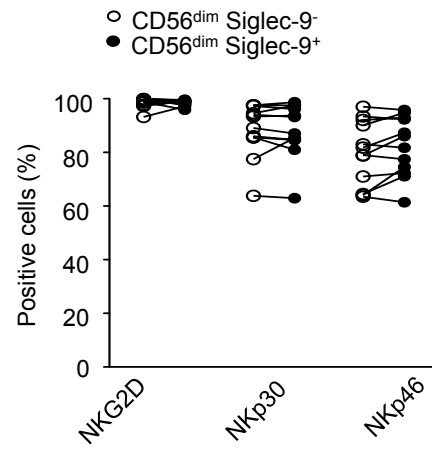
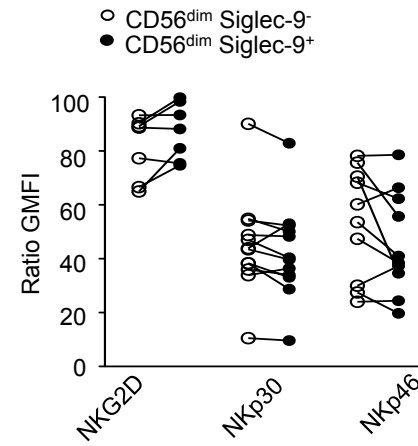
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A**B****C****D**

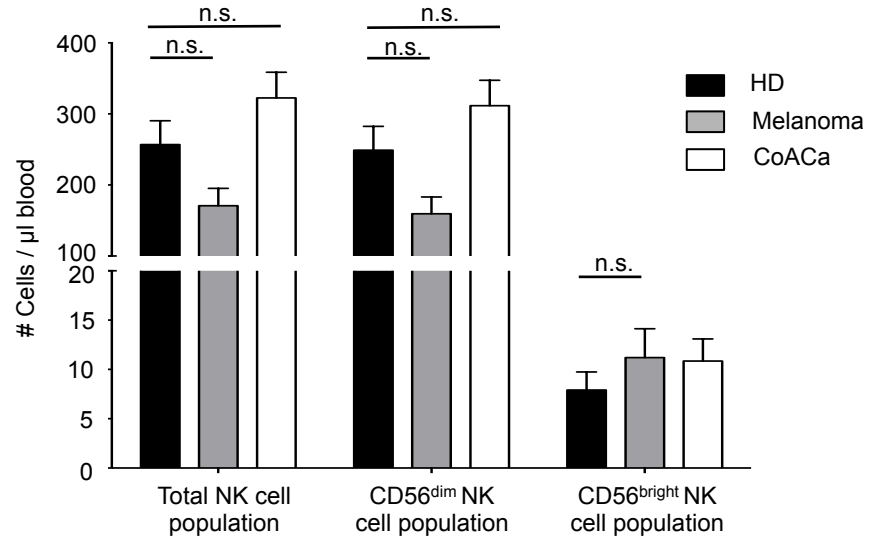
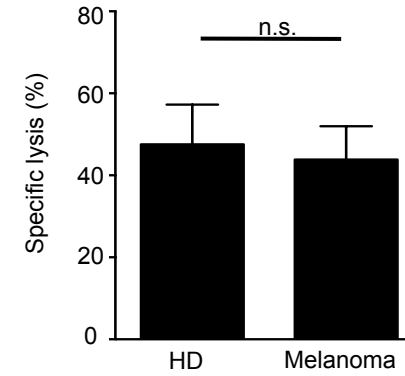
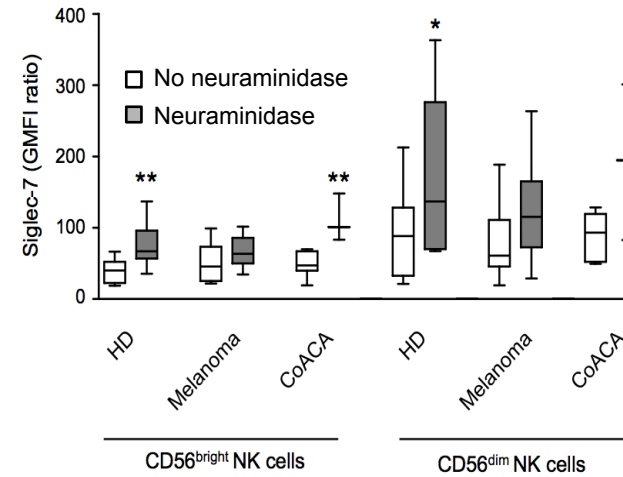
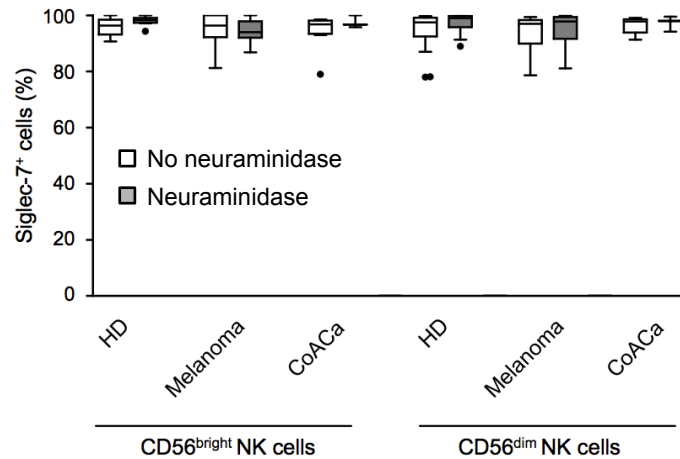
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A**B****C****D**

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A**B**

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A**B****C**

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