

SUPPLEMENTARY DATA

CD52 Signaling via Macrophage Siglec-G Represents a Therapeutic Target for Cancer Immunotherapy

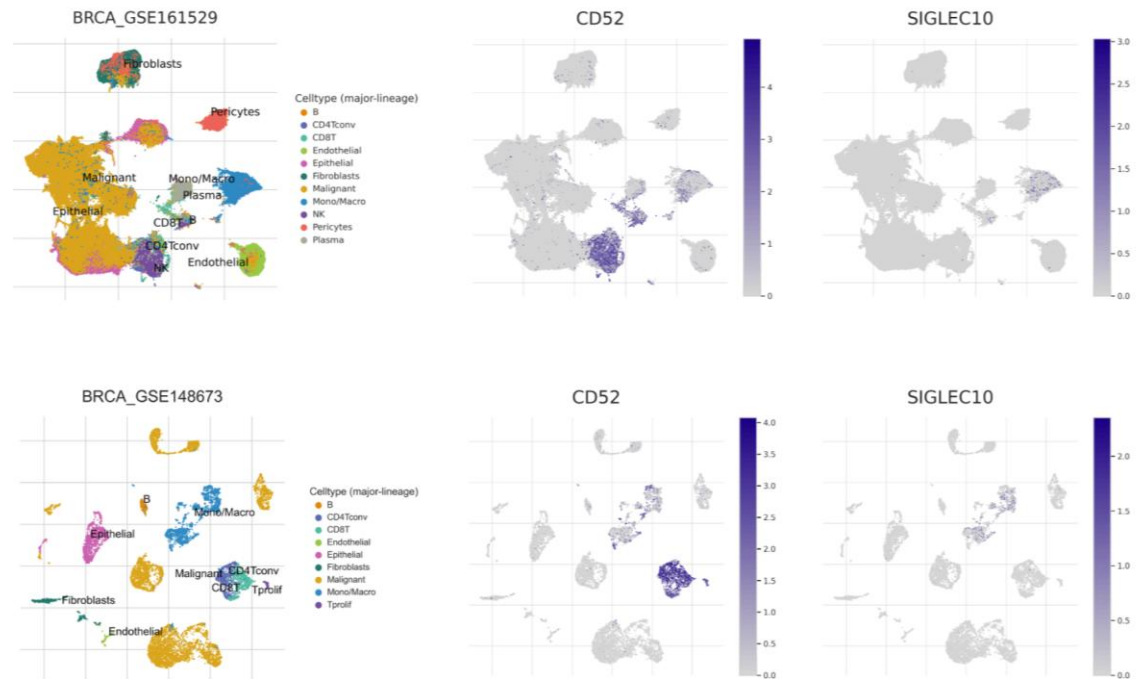
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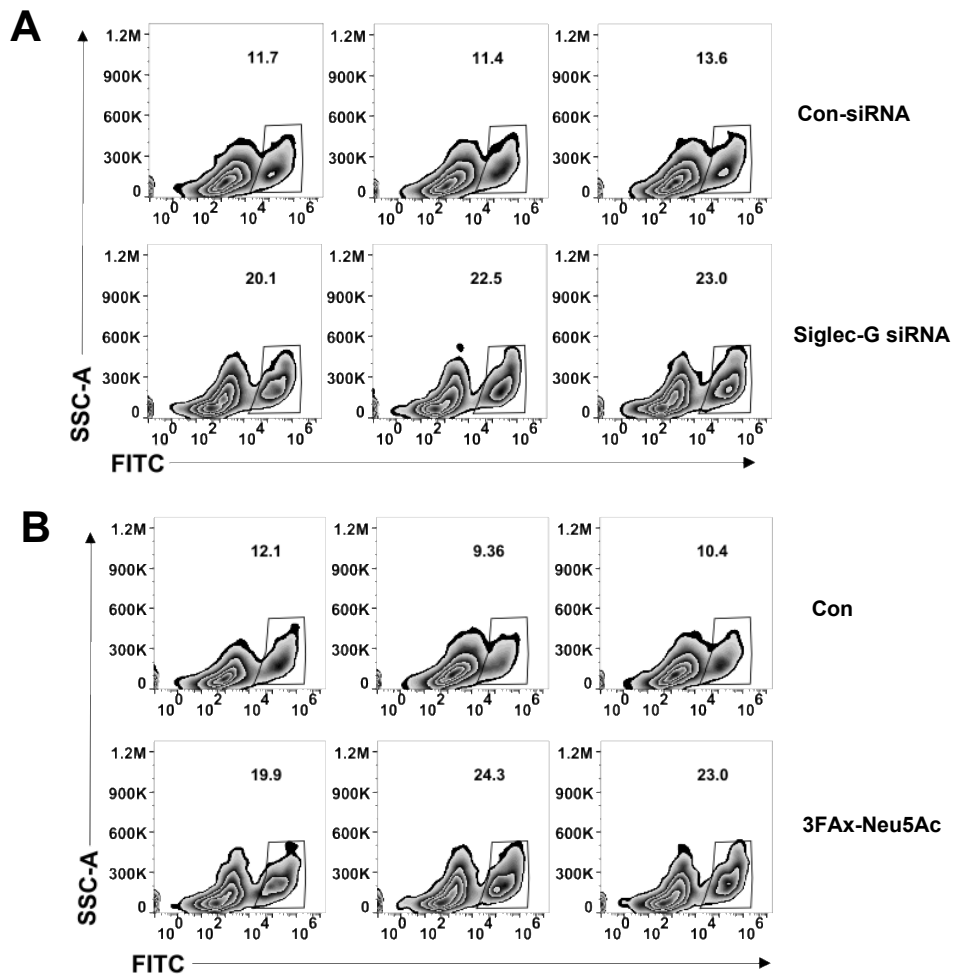
Supplementary Figures

Fig. S1 Expression patterns of CD52 and Siglec10 in breast cancer microenvironment.



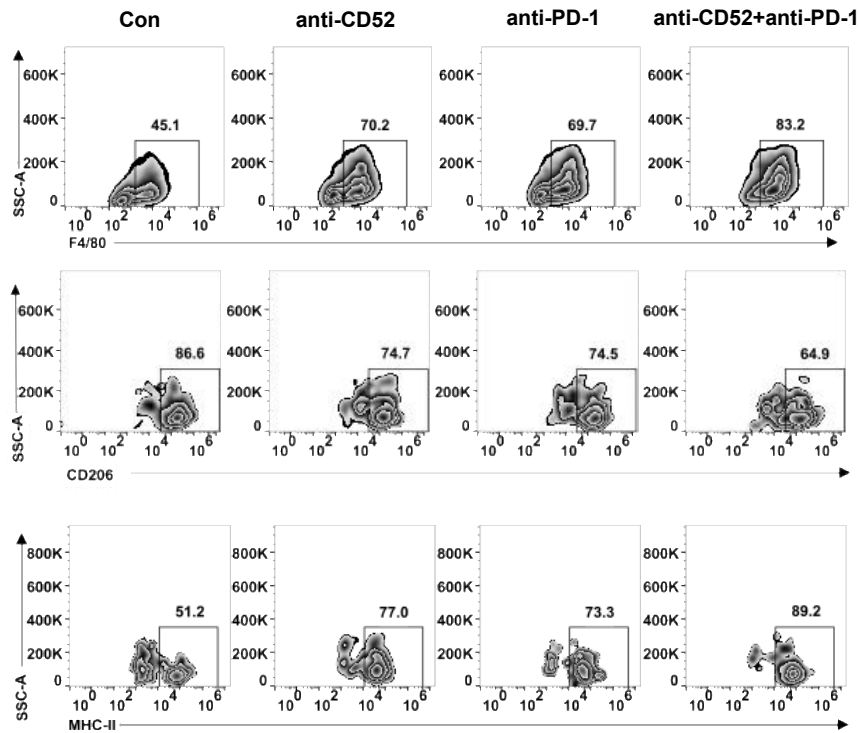
T-SNE visualization of scRNA-seq data from dataset BRCA_GSE161529 and BRCA_GSE148673, delineating immune, stromal, and malignant cell populations (Left). Feature plots displaying the expression levels of CD52 and Siglec10 (Middle and Right).

Fig. S2 Schematic diagram of flow cytometry for macrophage phagocytosis of tumor cells.



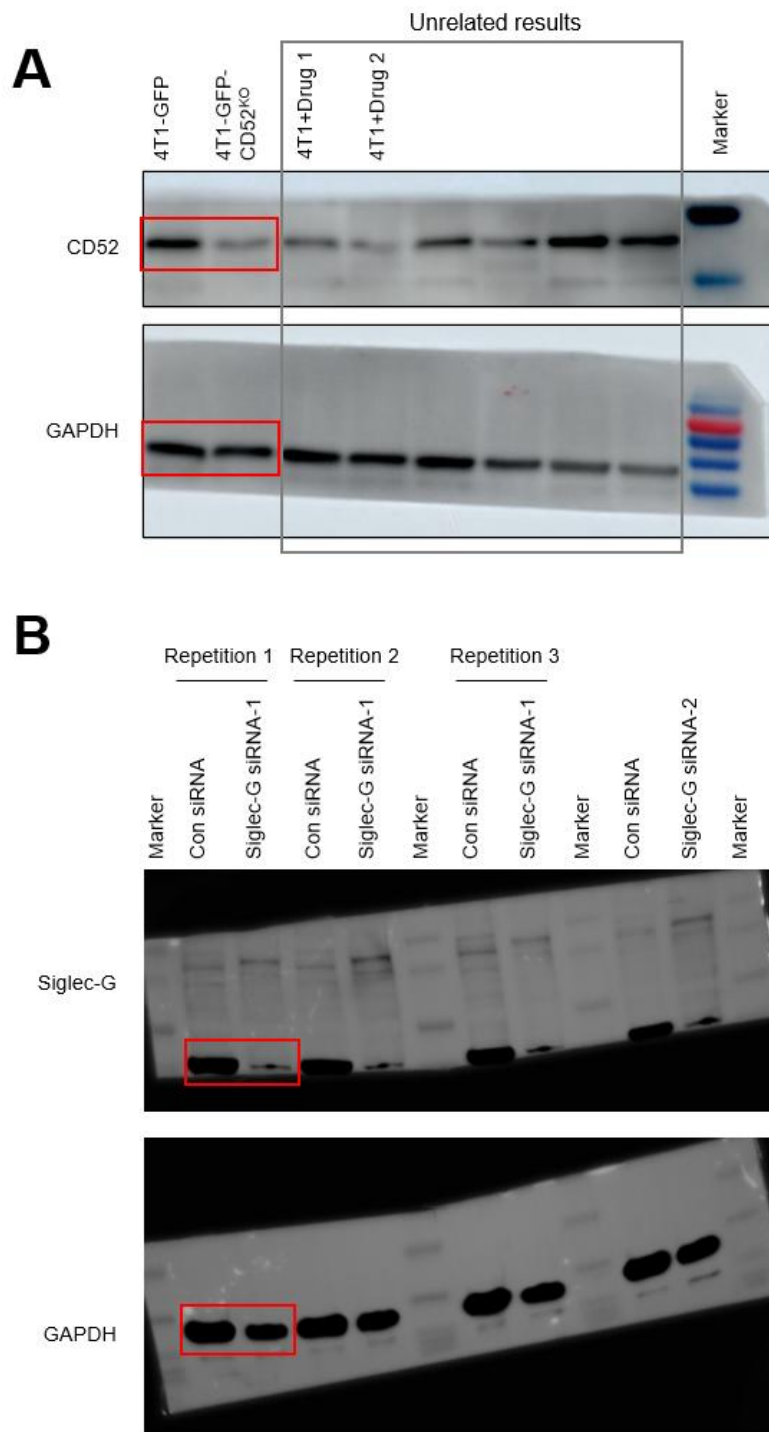
(A) Representative flow cytometry plots showing the phagocytosis of 4T1 cells by Siglec-g knockdown or control macrophages. **(B)** Representative flow cytometry plots showing the phagocytosis by macrophages of 4T1 cells pre-treated with PBS (control) or the sialic acid inhibitor 3Fax-Neu5Ac.

Fig. S3 Schematic diagram of flow cytometry for macrophages and CD8 T cells in the tumor microenvironment.



Flow cytometry analysis of MMTV-PyMT tumors from mice treated with control, anti-PD-1, anti-CD52, or a combination of anti-PD-1 and anti-CD52. Graphs show the percentage of macrophages (F4/80⁺) within the CD45⁺ immune cell population, and the expression of MHC-II and CD206 on these macrophages.

Fig. S4 The original scans of WB figures



(A) Uncropped full-length Western blots for Figure 2A showing the validation of CD52 KO in 4T1-GFP cells. (B) Uncropped full-length Western blots for Figure 3B showing the validation of Siglec-G knockdown by siRNA in bone marrow derived macrophages. The red boxes indicate the cropped regions presented in the main figures, and the grey box indicate other results unrelated to this study.

Supplementary Tables

Table. S1 Baseline characteristics of the tissue microarray cohort.

Array site	Histologic type	Lesion site	Stage	Stain H-Score
A1	Invasive Ductal Carcinoma	Primary tumor	3	1.06
A2	Invasive Ductal Carcinoma	Primary tumor	1	24.03
A3	Invasive Ductal Carcinoma	Primary tumor	1	2.69
A4	Invasive Ductal Carcinoma	Primary tumor	2	32.25
A5	Invasive Ductal Carcinoma	Primary tumor	1	70.27
A6	Invasive Ductal Carcinoma	Primary tumor	1	88.92
A7	Invasive Ductal Carcinoma	Primary tumor	2	56.75
A8	Invasive Ductal Carcinoma	Primary tumor	1	55.06
A9	Invasive Ductal Carcinoma	Primary tumor	2	27.46
A10	Invasive Ductal Carcinoma	Primary tumor	1	42.01
A11	Invasive Ductal Carcinoma	Primary tumor	2	19.85
A12	Invasive Ductal Carcinoma	Primary tumor	1	2.28
A13	Invasive Ductal Carcinoma	Primary tumor	1	89.84
A14	Invasive Ductal Carcinoma	Primary tumor	2	35.88
A15	Invasive Ductal Carcinoma	Primary tumor	1	17.82
A16	Invasive Ductal Carcinoma	Primary tumor	2	37.81
B1	Invasive Ductal Carcinoma	Primary tumor	1	59.63
B2	Invasive Ductal Carcinoma	Primary tumor	1	16.70
B3	Invasive Ductal Carcinoma	Primary tumor	2	23.35
B4	Invasive Ductal Carcinoma	Primary tumor	3	37.06
B5	Invasive Ductal Carcinoma	Primary tumor	2	14.16
B6	Invasive Ductal Carcinoma	Primary tumor	1	1.61
B7	Invasive Ductal Carcinoma	Primary tumor	1	92.69
B8	Invasive Ductal Carcinoma	Primary tumor	2	32.67

B9	Invasive Ductal Carcinoma	Primary tumor	1	20.16
B10	Invasive Ductal Carcinoma	Primary tumor	1	84.90
B11	Invasive Ductal Carcinoma	Primary tumor	2	63.18
B12	Invasive Ductal Carcinoma	Primary tumor	2	45.05
B13	Invasive Ductal Carcinoma	Primary tumor	1	64.40
B14	Invasive Ductal Carcinoma	Primary tumor	1	30.26
B15	Invasive Ductal Carcinoma	Primary tumor	1	83.62
B16	Invasive Ductal Carcinoma	Primary tumor	2	46.06
C1	Invasive Ductal Carcinoma	Primary tumor	2	1.80
C2	Invasive Ductal Carcinoma	Primary tumor	2	73.25
C3	Invasive Ductal Carcinoma	Primary tumor	2	53.81
C4	Invasive Ductal Carcinoma	Primary tumor	4	49.07
C5	Invasive Ductal Carcinoma	Primary tumor	2	59.51
C6	Invasive Ductal Carcinoma	Primary tumor	1	74.34
C7	Invasive Ductal Carcinoma	Primary tumor	2	43.03
C8	Invasive Ductal Carcinoma	Primary tumor	2	53.68
C9	Invasive Ductal Carcinoma	Primary tumor	2	69.53
C10	Invasive Ductal Carcinoma	Primary tumor	1	134.54
C11	Invasive Ductal Carcinoma	Primary tumor	2	14.19
C12	Invasive Ductal Carcinoma	Primary tumor	2	25.08
C13	Invasive Ductal Carcinoma	Primary tumor	2	80.78
C14	Invasive Ductal Carcinoma	Primary tumor	1	137.70
C15	Invasive Ductal Carcinoma	Primary tumor	1	82.46
C16	Invasive Ductal Carcinoma	Primary tumor	2	33.36
D1	Invasive Ductal Carcinoma	Primary tumor	2	55.00
D2	Invasive Ductal Carcinoma	Primary tumor	2	20.93

D3	Invasive Ductal Carcinoma	Primary tumor	2	96.44
D4	Invasive Ductal Carcinoma	Primary tumor	2	70.17
D6	Invasive Ductal Carcinoma	Primary tumor	1	73.57
D7	Invasive Ductal Carcinoma	Primary tumor	2	6.06
D8	Invasive Ductal Carcinoma	Primary tumor	1	60.21
D9	Invasive Ductal Carcinoma	Primary tumor	1	21.65
D10	Invasive Ductal Carcinoma	Primary tumor	1	25.01
D11	Invasive Ductal Carcinoma	Primary tumor	2	16.47
D12	Invasive Ductal Carcinoma	Primary tumor	3	18.85
D13	Invasive Ductal Carcinoma	Primary tumor	1	12.19
D14	Invasive Ductal Carcinoma	Primary tumor	1	187.25
D15	Invasive Ductal Carcinoma	Primary tumor	1	0.63
D16	Invasive Ductal Carcinoma	Primary tumor	2	6.41
E1	Invasive Ductal Carcinoma	Primary tumor	2	71.17
E2	Invasive Ductal Carcinoma	Primary tumor	1	9.46
E3	Invasive Ductal Carcinoma	Primary tumor	1	37.25
E4	Invasive Ductal Carcinoma	Primary tumor	2	65.26
E5	Invasive Ductal Carcinoma	Primary tumor	1	106.54
E6	Invasive Ductal Carcinoma	Primary tumor	2	70.51
E7	Invasive Ductal Carcinoma	Primary tumor	3	32.39
E8	Invasive Ductal Carcinoma	Primary tumor	1	42.96
E9	Invasive Ductal Carcinoma	Primary tumor	2	9.21
E10	Invasive Ductal Carcinoma	Primary tumor	4	0.88
E11	Invasive Ductal Carcinoma	Primary tumor	2	23.64
E12	Invasive Ductal Carcinoma	Primary tumor	3	174.43
E13	Invasive Ductal Carcinoma	Primary tumor	3	76.06

E14	Invasive Ductal Carcinoma	Primary tumor	2	88.26
E15	Invasive Ductal Carcinoma	Primary tumor	1	38.22
E16	Invasive Ductal Carcinoma	Primary tumor	1	2.99
F2	Invasive Ductal Carcinoma	Primary tumor	2	0.72
F3	Invasive Ductal Carcinoma	Primary tumor	1	11.37
F4	Invasive Ductal Carcinoma	Primary tumor	2	67.03
F5	Invasive Ductal Carcinoma	Primary tumor	1	50.33
F6	Invasive Ductal Carcinoma	Primary tumor	2	69.06
F7	Invasive Ductal Carcinoma	Primary tumor	1	0.53
F8	Invasive Ductal Carcinoma	Primary tumor	1	6.08
F9	Invasive Ductal Carcinoma	Primary tumor	3	0.51
F10	Invasive Ductal Carcinoma	Primary tumor	1	41.85
F11	Invasive Ductal Carcinoma	Primary tumor	2	8.22
F12	Invasive Ductal Carcinoma	Primary tumor	1	0.93
F13	Invasive Ductal Carcinoma	Primary tumor	2	34.19
F14	Invasive Ductal Carcinoma	Primary tumor	2	17.99
F15	Invasive Ductal Carcinoma	Primary tumor	2	20.37
F16	Invasive Ductal Carcinoma	Primary tumor	2	27.78
G2	Invasive Ductal Carcinoma	Primary tumor	3	7.21
G3	Invasive Ductal Carcinoma	Primary tumor	2	36.68
G4	Invasive Ductal Carcinoma	Primary tumor	1	133.38
G5	Invasive Ductal Carcinoma	Primary tumor	1	96.79
G6	Invasive Ductal Carcinoma	Primary tumor	1	68.92
G7	Invasive Ductal Carcinoma	Primary tumor	2	90.61
G8	Invasive Ductal Carcinoma	Primary tumor	3	0.94
G9	Invasive Ductal Carcinoma	Primary tumor	1	23.69

G10	Invasive Ductal Carcinoma	Primary tumor	1	15.10
G11	Invasive Ductal Carcinoma	Primary tumor	1	68.94
G12	Invasive Ductal Carcinoma	Primary tumor	1	4.51
G13	Invasive Ductal Carcinoma	Primary tumor	3	24.49
G14	Invasive Ductal Carcinoma	Primary tumor	2	51.88
G15	Invasive Ductal Carcinoma	Primary tumor	3	6.41
G16	Invasive Ductal Carcinoma	Primary tumor	3	77.99
H1	Invasive Ductal Carcinoma	Primary tumor	3	46.77
H2	Invasive Ductal Carcinoma	Primary tumor	2	11.05
H3	Invasive Ductal Carcinoma	Primary tumor	3	144.89
H4	Invasive Ductal Carcinoma	Primary tumor	2	44.38
H5	Invasive Ductal Carcinoma	Primary tumor	2	8.94
H6	Invasive Ductal Carcinoma	Primary tumor	1	78.53
H7	Invasive Ductal Carcinoma	Primary tumor	2	44.49
H8	Invasive Ductal Carcinoma	Primary tumor	2	51.53