Screening and Identification of Novel Anti-Siglec-15 Human Antibody 3F1 and Relevant Antitumor Activity Research

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Figure supplement Legends:

Figure S1: The results of SEC-HPLC of 5 selected antibodies under natural conditions. Except for 1B4, which has the ability to form polymers, most of the other antibodies are in the form of monomers.

Figure S2: Fitting graph of association/dissociation of the three candidate antibodies. The KD values of each antibody were listed as show in the graph.

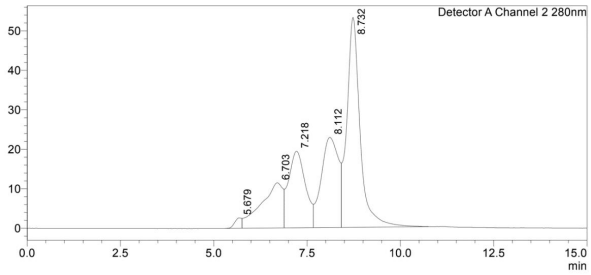
Figure S3: Analysis of blocking activity between candidate antibodies and different ligands of Siglec-15. (A) Flow cytometry was used to determine the

anti-sialyl-Tn antibody did not bind to the 293T cell line that highly expresses LRRC4C. (B) Biolayer interferometry (BLI) technology was used to determine the anti sialyl-Tn antibody did not bind to CD44 and MAG. The picture on the left is CD44 and the right is MAG. (C) The expression of sialyl-Tn on the surface of NCL-H157 was detected by flow cytometry. (D) Flow cytometry was used to determine the blocking effects of the candidate antibodies and the ligand sialyl-Tn, at a specific concentration, all three candidate antibodies could effectively compete with the interaction between Siglec-15 and sialyl-Tn antigen to the background value. (E) Flow cytometry was used to determine the blocking effect of the candidate antibody and the ligand LRRC4C, at a specific concentration, the three candidate antibodies could effectively compete with Siglec-15 and LRRC4C.

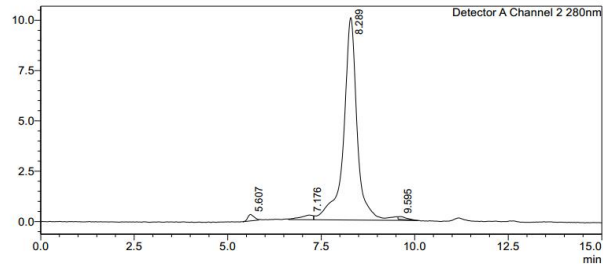
Figure S4: Evaluation of the biological activity of candidate antibodies *in vitro*. Flow cytometry method was used to evaluate the inhibitory effects of the candidate antibodies on reversing the proliferation of human PBMC mediated by Siglec-15. The candidate antibody 3F1 could effectively reverse the antigen-specific T cell response.

Figure S5: The expression level of EGFR on the cell surface of H157(S15^{+}). The commercial anti-EGFR antibody ERBITUX was used to label NCI-H157(S15^{+}), and positive rate of EGFR was 97.7%.

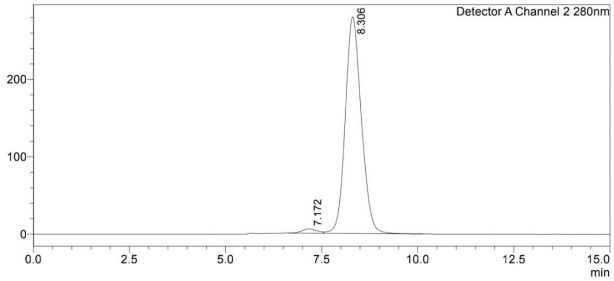
1B4



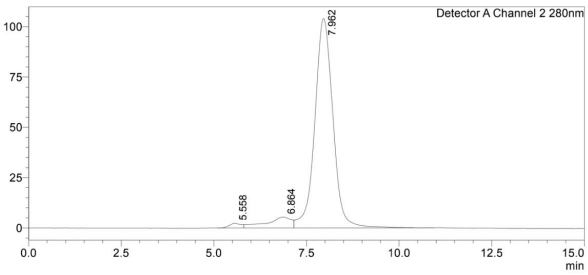
1D1



1D4



2A12



3F1

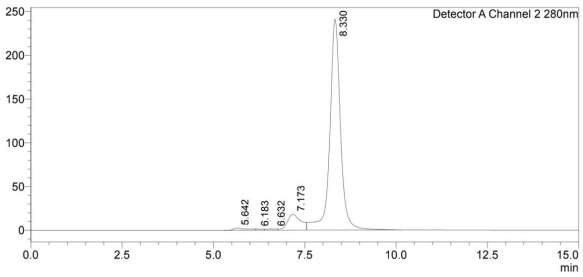


Figure S1

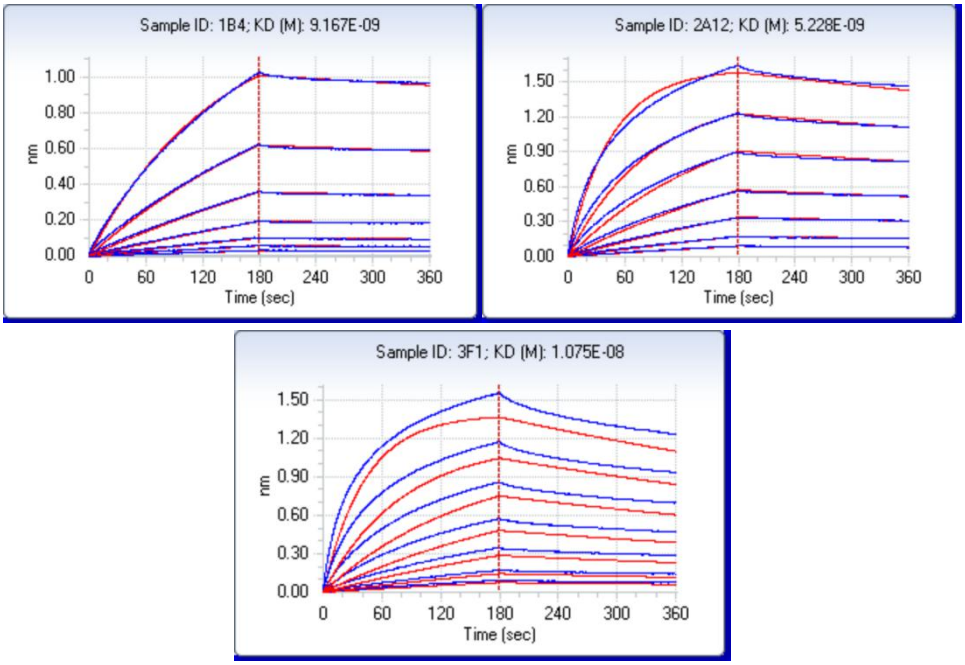


Figure S2

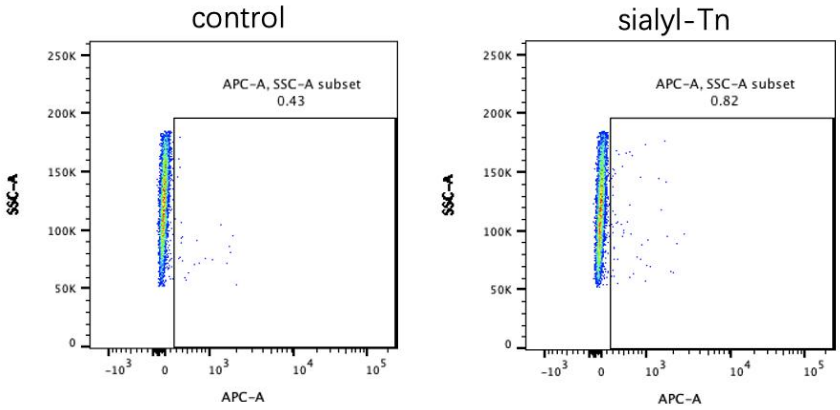


Figure S3A

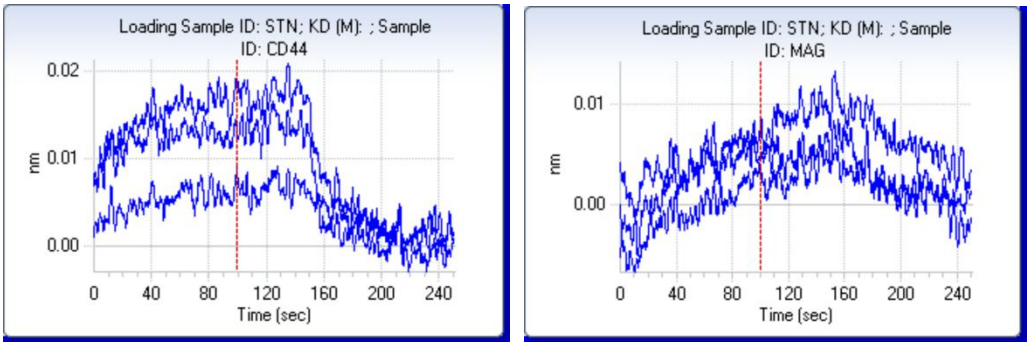
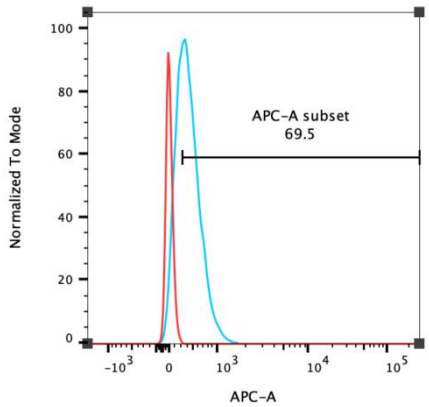
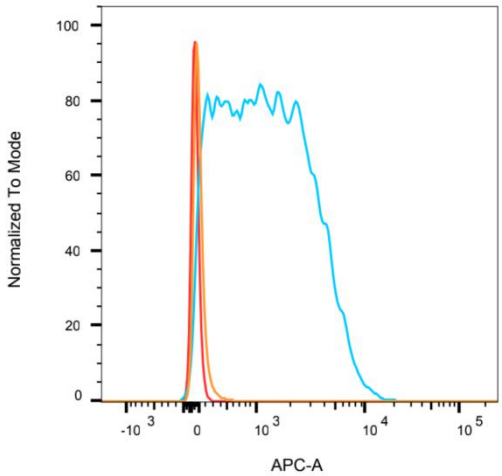


Figure S3B

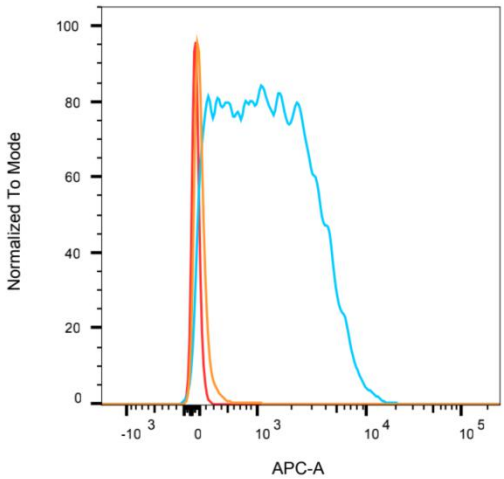


SampleID	Mean : APC-A
control	116
H157 S-Tn	511

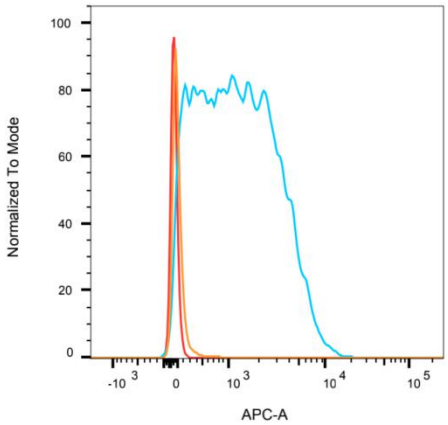
Figure S3C



SampleID	Mean : APC-A
1B4	139
Positive	1771
Control	75.8

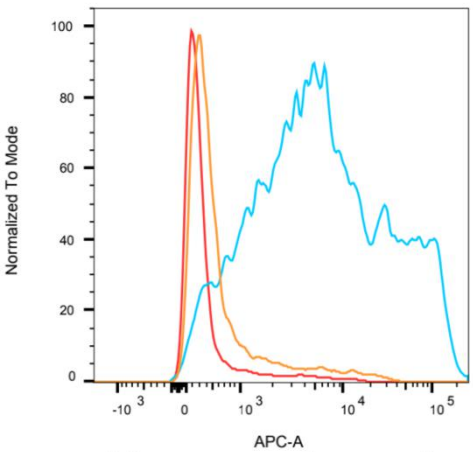


SampleID	Mean : APC-A
2A12	160
Positive	1771
Control	75.8

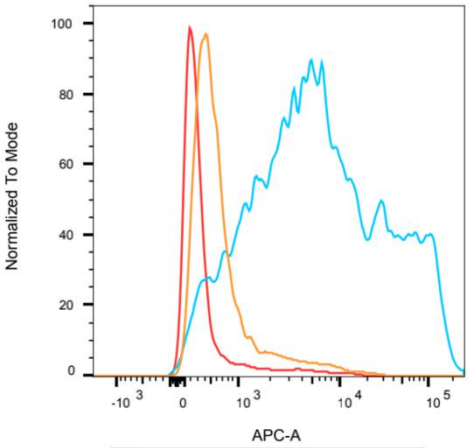


SampleID	Mean : APC-A
3F1	195
Positive	1771
Control	75.8

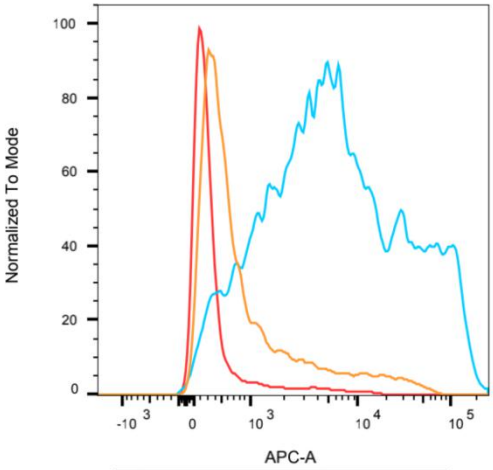
Figure S3D



SampleID	Mean : APC-A
1B4	1523
Positive	19835
Control	721

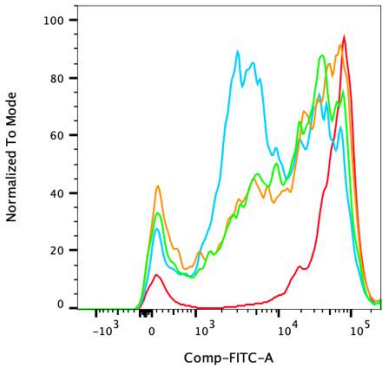


SampleID	Mean : APC-A
2A12	1182
Positive	19835
Control	721

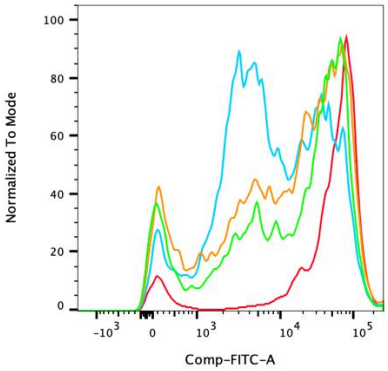


SampleID	Mean : APC-A
3F1	2889
Positive	19835
Control	721

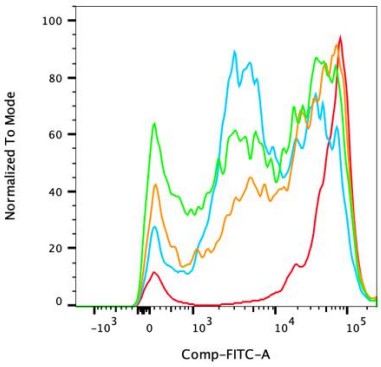
Figure S3E



SampleID	Mean : Comp-FITC-A
1B4	27598
anti-CD3+S15	30506
anti-CD3	20711
control	61523



SampleID	Mean : Comp-FITC-A
2A12	31938
anti-CD3+S15	30506
anti-CD3	20711
control	61523



	SampleID	Mean : Comp-FITC-A
	3F1	22818
	anti-CD3+S15	30506
	anti-CD3	20711
	control	61523

Figure S4

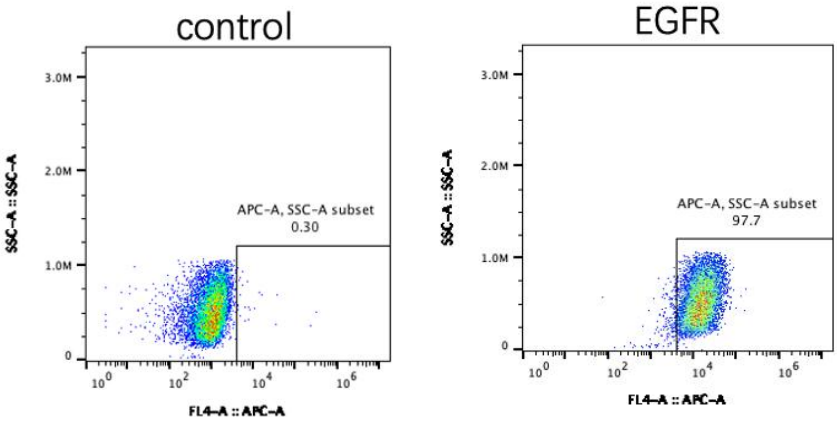


Figure S5

Table S1

Name	Amino acid sequence
SIG15_HUMAN	MEKSIWLLACLAWVLPTGSFVRTKIDTTENLLNTEVHSSPAQRWSMQVPPEVSAEAGDAAVLPCTFTHPHRHYDGPLTAIWRAGEPYAGPQVFRCAAARGSELCQTALSLHGRFRLLGNPRRNDLSLRVERLALADDRRYFCRVEFAGDVHDRYESRHGVRLHVTAAPRIVNISVLPSPAHAFRALCTAEGEPPPALAWSGPALGNSLAAVRSPREGHGHLVTAELPALTHDGRYTCTAANSLGRSEASVYLFRFHGASGASTVALLLGALGFKALLLLGVLAARAARRRPEHLDTPDTPPRSQAQESNYENLSQMNPRSPPATMCSP
SIG5_V-LIKE	MEKSIWLLACLAWVLPTGSFVRTKIDTTENLLNTEVHSSPAQRWSMQVPPEVSAEAGDAAVLPCTFTHPHRHYDGPLTAIWRAGEPYAGPQVFRCAAARGSELCQTALSLHGRFRLLGNPRRNDLSLRVERLALADDRRYFCRVEFAGDVHDRYESRHGVRLHVTAA
PDGFR	AVGQDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPR