36, 49, and 62, respectively. The mouse monoclonal antibody or antigen binding fragment that specifically binds to human TIGIT can also comprise light chain variable regions, CDR1, CDR2, and CDR3 sequences comprising amino acid sequences of SEQ ID NOs: 75, 88, and 101, respectively. In one embodiment, the anti-human TIGIT mouse monoclonal antibody of the present invention comprises the heavy chain and light chain variable domains of 83G6H11C12 comprising amino acid sequences of SEQ ID NOs: 10 and 23. It covers the sequences sharing at least 80%, 85%, 90% and 95% sequence identity with these disclosed sequence. In another embodiment, the mouse monoclonal antibody, 83G6H11C12, or an antigen binding fragment comprise the following functional characteristics: (a) binds to human TIGIT with a K_D of 20 nM or less as determined by surface plasmon resonance (BIAcore); (b) has cross-reactivity to cynomolgous TIGIT; (c) block the interaction between human TIGIT and its ligand, CD155; (d) activate T cell in a reporter assay; (e) stimulate IL-2 production in Jurkat cells.

[23] The invention provides a mouse monoclonal antibody (92E9D4B4) or an antigen binding fragment that specifically binds to human TIGIT and comprise heavy chain variable regions, CDR1, CDR2, and CDR3 sequences comprising amino acid sequences of SEQ ID NOs: 37, 50, and 63, respectively. The mouse monoclonal antibody or antigen binding fragment that specifically binds to human TIGIT can also comprise light chain variable regions, CDR1, CDR2, and CDR3 sequences comprising amino acid sequences of SEQ ID NOs: 76, 89, and 102, respectively. In one embodiment, the anti-human TIGIT mouse monoclonal antibody of the present invention comprises the heavy chain and light chain variable domains of 92E9D4B4 comprising amino acid sequences of SEQ ID NOs: 11 and 24. It covers the sequences sharing at least 80%, 85%, 90% and 95% sequence identity with these disclosed sequence. In another embodiment, the mouse monoclonal antibody, 92E9D4B4, or an antigen binding fragment comprise the following functional characteristics: (a) binds to human TIGIT with a K_D of 20 nM or less as determined by surface plasmon resonance (BIAcore); (b) has cross-reactivity to cynomolgous TIGIT; (c) block the interaction between human TIGIT and its ligand, CD155; (d) activate T cell in a reporter assay; (e) stimulate IL-2 production in Jurkat cells.

[24] The invention provides a mouse monoclonal antibody (100C4E7D11, also referred to herein as "100C4") or an antigen binding fragment that specifically binds to human TIGIT and comprise heavy chain variable regions, CDR1, CDR2, and CDR3 sequences comprising amino acid sequences of SEQ ID NOs: 38, 51, and 64, respectively. The mouse monoclonal antibody or antigen binding fragment that specifically binds to human TIGIT can also

comprise light chain variable regions, CDR1, CDR2, and CDR3 sequences comprising amino acid sequences of SEQ ID NOs: 77, 90, and 103, respectively. In one embodiment, the anti-human TIGIT mouse monoclonal antibody of the present invention comprises the heavy chain and light chain variable domains of 100C4E7D11 comprising amino acid sequences of SEQ ID NOs: 12 and 25. The humanized VH sequences of SEQ ID NOs: 105-108 have the CDRs of SEQ ID Nos: 38, 51, and 64 and the humanized VL sequences of SEQ ID NOs: 113-117 have the CDRs of SEQ ID NOs: 77, 90, and 103. It covers the sequences sharing at least 80%, 85%, 90% and 95% sequence identity with these disclosed sequence. In another embodiment, the mouse monoclonal antibody 100C4E7D11, the antibodies made by the combinations of one of the VH sequences of SEQ ID NOs:12, and 105-108 and one of the VL sequences of SEQ ID NOs:25, and 113-117, or an antigen binding fragment comprise the following functional characteristics: (a) binds to human TIGIT with a K_D of 20 nM or less as determined by surface plasmon resonance (BIAcore); (b) has cross-reactivity to cynomolgous TIGIT; (c) block the interaction between human TIGIT and its ligand, CD155; (d) activate T cell in a reporter assay; (e) stimulate IL-2 production in Jurkat cells.

[25] The invention provides a mouse monoclonal antibody (64G1E9B4, also referred to herein as "64G1") or an antigen binding fragment that specifically binds to human TIGIT and comprise heavy chain variable regions, CDR1, CDR2, and CDR3 sequences comprising amino acid sequences of SEQ ID NOs: 39, 52, and 65, respectively. They also comprise light chain variable regions, CDR1, CDR2, and CDR3 sequences comprising amino acid sequences of SEQ ID NOs: 78, 91, and 104, respectively. In one embodiment, the anti-human TIGIT mouse monoclonal antibody of the present invention comprises the heavy chain and light chain variable domains of 64G1E9B4 comprising amino acid sequences of SEQ ID NOs: 13 and 26. In one embodiment, the antibody comprises one amino acid substitution in the heavy chain CDR of SEQ ID NO: 52, wherein the substitution is made at position 7D, and wherein residue 7D is substituted to G. In one embodiment, the antibody comprises one amino acid substitution in the heavy chain CDR of SEQ ID NO: 65, wherein the substitution is made at position 8M, and wherein residue 8M is substituted to F or L. In one embodiment, the antibody comprises one amino acid substitution in the light chain CDR of SEQ ID NO: 78, wherein the substitution is made at position 1K, and wherein residue 1K is substituted to R. The humanized VH sequences of SEQ ID NOs: 109 -112 have the CDRs of SEQ ID NOs: 39, 52, and 65 and the humanized VL sequence of SEQ ID NO:

Example 5: The competitive binding on human TIGIT over-expressed in CHO-K1 cells between the mouse anti-human TIGIT monoclonal antibodies and PVR recombinant protein

[286] The competitive binding on human TIGIT between the mouse anti-human TIGIT monoclonal antibodies and PVR recombinant protein was assessed using a FACS assay. To assess the competitive binding on human TIGIT between the mouse anti-human TIGIT monoclonal antibodies and PVR, the mouse anti-human TIGIT monoclonal antibody samples were prepared (starting at 500 nM, 3-fold serial dilution with 10 concentrations). CHO cells expressing human TIGIT were dissociated from adherent culture flasks and mixed with varying concentrations of each mouse anti-human TIGIT monoclonal antibody and 5 μg/ml human TIGIT-Fc fusion protein having a biotin label. The mixture was equilibrated for 30 minutes at room temperature, and washed three times with FACS buffer (PBS containing 1% BSA). PE/Cy5 Streptavidin secondary antibody was then added to the mixtures and incubated for 15 minutes at room temperature. Subsequently, the cells were washed with FACS buffer and analyzed by flow cytometry. Data was analyzed with PRISM™ (GraphPad Software, San Diego, CA) using non-linear regression, and IC_{50} values were calculated (FIGS. 4A-4B and Table 6). The competition FACS study demonstrated the ability of the mouse anti-human TIGIT monoclonal antibodies to block the binding between human TIGIT and human PVR with IC_{50} values range from ~~0.75~~0.6 nM to 73.5 nM, except for 16F10H12C11.

Table 6: Anti-human TIGIT monoclonal antibodies' blocking on binding of TIGIT/PVR

Antibody	**Best-fit values**		
	Bottom	**Top**	**IC_{50} (nM)**
70A11A8E6	19.6	113.4	0.7
11D8E12A4	21.2	107.9	73.5
16F10H12C11	N.A		
8F2D8E7	11.2	130.6	0.9
48B5G4E12	64.6	131.7	1.6
139E2C2D2	10.4	73.8	0.7
128E3G7F5	8.8	69.7	1.1
121C2F10B5	11.8	68.8	0.6
104G12E12G2	8.9	56.9	2.4
83G6H11C12	12.9	70.2	0.7
92E9D4B4	10.3	65.5	2.0
100C4E7D11	12.9	66.5	1.2

64G1E9B4	9.5	67.1	1.0

Example 6: Epitope binding of the mouse anti-human TIGIT monoclonal antibodies against human TIGIT

[287] An epitope binding test on 128E3G7F5, 121C2F10B5, 104G12E12G2, 83G6H11C12, 92E9D4B4, 100C4E7D11, and 64G1E9B4 was performed on an Octet RED96 instrument (ForteBio; Menlo Park, CA). All measurements were performed at 30°C. One antibody of interest was immobilized onto the biosensors using amine coupling method. The antigen protein TIGIT was diluted in PBST buffer (1x PBS, pH 7.4, and 0.05% Tween-20) as used as analyte 1. The mixture of antigen protein TIGIT (concentration same as in analyte 1) and the second antibody was used as analyte 2. The coated biosensors were first dipped into analyte 1 and then dipped into analyte 2 after regeneration and equilibration. The sensorgrams of analyte 1 and analyte 2 were compared. If the binding level of analyte 1 is significantly higher than that of analyte 2, the second antibody was considered to be able to compete with the immobilized antibody for binding the target protein TIGIT. If the binding level of analyte 1 is significantly lower than that of analyte 2, the second antibody was considered not able to compete with the immobilized antibody for binding the target protein TIGIT. The experiment was repeated until all the antibodies were analyzed. 128E3G7F5, 121C2F10B5, 104G12E12G2, 83G6H11C12, 92E9D4B4, 100C4E7D11, and 64G1E9B4 were mapped into three groups (Table 7). The mouse anti-human TIGIT monoclonal antibodies in each group have the closely related or same epitope on human TIGIT.

Table 7: Epitope binning of the mouse anti-human TIGIT antibodies against human TIGIT

Antibody ID	**Group**
64G1E9B4	I
100C4E7D11	II
83G6H11C12	II
92E9D4B4	II
104G12E12G2	I
121C2F10B5	III
128E3G7F5~~128E3F10F3F2~~	III

Example 7: The neutralization effects of the mouse anti-human TIGIT antibodies on T cell activations inhibited by binding of PVR on TIGIT overexpressed in T cells

[288] CHO-K1 cells were engineered to be the artificial dendritic cells (APC) by stably transfecting a T cell receptor (TCR) activator. CHO-K1 APC was then overexpressed with PVR. The Jurkat cell line was stably transfected with an NFAT-inducible Lucia reporter construct to generate a Jurkat/NFAT reporter cell line. The Jurkat/NFAT reporter cell line was then overexpressed with human TIGIT. The CHO-K1 APC/PVR cells were co-cultured with the Jurkat/NFAT reporter/TIGIT cells to evaluate the neutralization activities of the mouse anti-human TIGIT monoclonal antibodies. The Jurkat/NFAT reporter/TIGIT cells were pre-incubated with serial dilution of each mouse anti-human TIGIT monoclonal antibodies for 30 minutes before adding the CHO-K1 APC/PVR cells. After a couple hours of interaction, 20 μl of supernatant were collected from each well for IL-2 measurement and then ONE-STEP™ Luciferase reagent was added to the system for measuring the NFAT activity. The activation of Jurkat/NFAT reporter/TIGIT cells was evaluated by the intensity of luciferase signal or the secretion of IL-2. The binding of PVR on TIGIT between two cells inhibited Jurkat/NFAT reporter/TIGIT cells activation. The mouse anti-human TIGIT monoclonal antibodies blocked the interaction between PVR and TIGIT, and then neutralized the inhibition of PVR on TIGIT. The more neutralization antibody was added, the more Jurkat/NFAT reporter/TIGIT cells were activated, the more luciferase signal or the more IL-2 was secreted (FIGS 5A-5B, Table 8, and Table 9). Data was analyzed with PRISM™ (GraphPad Software, San Diego, CA) using non-linear regression, and EC_{50} values were calculated on both normalized luciferase signal and IL-2 secretion.

Table 8: The mouse anti-human TIGIT antibodies on luciferase signal in T cell activations

Antibody	Best-fit values		
	Bottom	Top	EC_{50} (nM)
64G1E9B4	5.7	124.2	1.64
100C4E7D11	11.6	117.0	3.46
83G6H11C12	9.6	145.0	4.40
92E9D4B4	3.4	140.7	3.90
104G12E12G2	6.0	147.0	6.42
121C2F10B5	5.4	152.7	4.38
128E3G7F5~~128E3F10F3F2~~	4.5	99.0	8.54

Table 9: The mouse anti-human TIGIT antibodies on IL-2 secretion in T cell activations

Antibody	Best-fit values		
	Bottom	Top	EC_{50} (nM)
64G1E9B4	111.4	282.6	0.82
100C4E7D11	107.1	191.4	0.98
83G6H11C12	104.3	170.7	0.83
92E9D4B4	106.1	274.1	3.36
104G12E12G2	108.2	189.2	3.31
121C2F10B5	96.18	197.2	1.02

Example 8: Binding of the chimeric anti-human TIGIT monoclonal antibodies or humanized anti-human TIGIT monoclonal antibodies to human TIGIT recombinant protein

[289] The chimeric anti-human TIGIT monoclonal antibodies (100C4E7D11 chimeric or 64G1E9B4 chimeric) were made by fusing variable domains of the heavy and light chains of 100C4E7D11 or 64G1E9B4 with the constant region of human IgG1.

[290] The humanized anti-human TIGIT monoclonal antibody (100C4E7D11VH1_VL1) of 100C4E7D11 was made by fusing the humanized variable domains of the heavy chain (SEQ ID NO: 105) and light chain (SEQ ID NO: 113) of 100C4E7D11 with the constant region of human IgG1.

[291] The humanized anti-human TIGIT monoclonal antibody (64G1E9B4VH1_VL1.M1) of 64G1E9B4 was made by fusing the humanized variable domains of the heavy chain (SEQ ID NO: 109) and light chain (SEQ ID NO: 118) of 64G1E9B4 with the constant region of human IgG1. The humanized 64G1E9B4VH1_VL1.M1 monoclonal antibody comprises one amino acid substitution in the light chain CDR of SEQ ID NO: 78, wherein the substitution is made at position 1K, and wherein residue 1K is substituted to R.

[292] The humanized anti-human TIGIT monoclonal antibody (64G1E9B4VH1.M1_VL1.M1) of 64G1E9B4 was made by fusing the humanized variable domains of the heavy chain (SEQ ID NO: 109) and light chain (SEQ ID NO:118) of 64G1E9B4 with the constant region of human IgG1. The humanized 64G1E9B4VH1.M1_VL1.M1 monoclonal antibody comprises one amino acid substitution in the heavy chain CDR of SEQ ID NO:52, wherein the substitution is made at position 7D, and wherein residue 7D is substituted to G. It also comprises one amino acid substitution in the light chain CDR of SEQ ID NO: 78, wherein the substitution is made at position 1K, and wherein residue 1K is substituted to R.

[293] Binding kinetics of the chimeric anti-human TIGIT monoclonal antibodies and the humanized anti-human TIGIT monoclonal antibodies to human TIGIT were determined using a Surface Plasmon Resonance (SPR) biosensor, BIACORE® T200 (GE Healthcare). Different concentrations of the chimeric anti-human TIGIT monoclonal antibodies or humanized anti-human TIGIT monoclonal antibodies were prepared starting at 50 nM with a 3-fold serial dilution. Each chimeric anti-human TIGIT monoclonal antibody or each humanized anti-human TIGIT monoclonal antibody was immobilized on the sensor chip through the Fc capture method. Human TIGIT protein with a HIS tag was used as the analyte. The dissociation (k_d) and association (k_a) rate constants were obtained using the BIACORE® T200 evaluation software. The apparent equilibrium dissociation constants (K_D) were calculated from the ratio of k_d over k_a. As shown in Table 10, the chimeric anti-human TIGIT monoclonal antibodies and the humanized anti-human TIGIT monoclonal antibodies had comparable binding kinetics to human TIGIT protein (Table 10).

Table 10: Binding of chimeric or humanized anti-human TIGIT antibodies on human TIGIT

~~Ligand~~Antibody	k_a (1/Ms)	k_d (1/s)	K_D (M)	Rmax (RU)	Chi² (RU²)
64G1 Chimeric	9.10E+05	6.40E-05	7.00E-11	31.36	0.037
64G1VH1_VL1.M1	5.90E+05	8.80E-05	1.50E-10	45.42	0.038
64G1VH1.M1_VL1.M1	6.30E+05	1.00E-04	1.70E-10	40.16	0.041
100C4 Chimeric	9.10E+05	1.60E-04	1.70E-10	39.53	0.098
100C4VH1_VL1	6.10E+05	3.50E-04	5.70E-10	34.68	0.092

Example 9: Binding of the chimeric anti-human TIGIT monoclonal antibodies and the humanized anti-human TIGIT monoclonal antibodies to human or cynomolgus TIGIT expressed in CHO-K1 cells

[294] Binding affinities of the chimeric anti-human TIGIT monoclonal antibodies and the humanized anti-human TIGIT monoclonal antibodies to human or cynomolgus TIGIT over-expressed on CHO-K1 cells were determined using a fluorescence-activated cell sorting (FACS)-based assay. The chimeric anti-human TIGIT monoclonal antibodies and the humanized anti-human TIGIT monoclonal antibodies were prepared (starting at 100 μM for human TIGIT and 55.6 μM for cynomolgus TIGIT, 3-fold serial dilution with 10 concentrations) as primary

100C4E7D11 chimeric	4881	23687	1.03

Example 10: The competitive binding on human TIGIT over-expressed in CHO-K1 cells between the chimeric anti-human TIGIT monoclonal antibodies or the humanized anti-human TIGIT monoclonal antibodies and PVR recombinant protein

[3] The competitive binding on human TIGIT between the chimeric anti-human TIGIT monoclonal antibodies or the humanized anti-human TIGIT monoclonal antibodies and PVR recombinant protein was assessed using a FACS assay. To assess the competitive binding on human TIGIT between the chimeric anti-human TIGIT monoclonal antibodies or the humanized anti-human TIGIT monoclonal antibodies and PVR, the chimeric anti-human TIGIT monoclonal antibody or the humanized anti-human TIGIT monoclonal antibody samples were prepared (starting at 300 nM, 3-fold serial dilution with 10 concentrations). CHO cells expressing human TIGIT were dissociated from adherent culture flasks and mixed with varying concentrations of each chimeric anti-human TIGIT monoclonal antibody or the humanized anti-human TIGIT monoclonal antibody and 5 μg/ml human TIGIT-Fc fusion protein having a biotin label. The mixture was equilibrated for 30 minutes at room temperature and washed three times with FACS buffer (PBS containing 1% BSA). PE/Cy5 Streptavidin secondary antibody was then added to the mixtures and incubated for 15 minutes at room temperature. Subsequently, the cells were washed with FACS buffer and analyzed by flow cytometry. Data was analyzed with PRISM™ (GraphPad Software, San Diego, CA) using non-linear regression, and IC_{50} values were calculated (FIG. 7 and Table 13). The competition FACS study demonstrated the ability of the mouse anti-human TIGIT monoclonal antibodies' capabilities on blocking to the binding between human TIGIT and human PVR with IC_{50} values ranging from 0.4 nM to 1.3 nM.

Table 13 Anti-human TIGIT monoclonal antibodies' blocking on binding of TIGIT/PVR

Antibody	**Best-fit values**		
	Bottom	**Top**	**EC_{50} (nM)**
64G1E9B4 VH1_VL1.M1	138.6	819.6	0.79
64G1E9B4 VH1.M1_VL1.M1	200.6	508.3	0.35
100C4E7D11 VH1_VL1	150.1	755.4	1.29
64G1E9B4 chimeric	197.1	771.5	0.51
100C4E7D11 chimeric	170.1	677.7	0.49