Supplementary Information for:
TIGIT-Fc Promotes Antitumour Immunity

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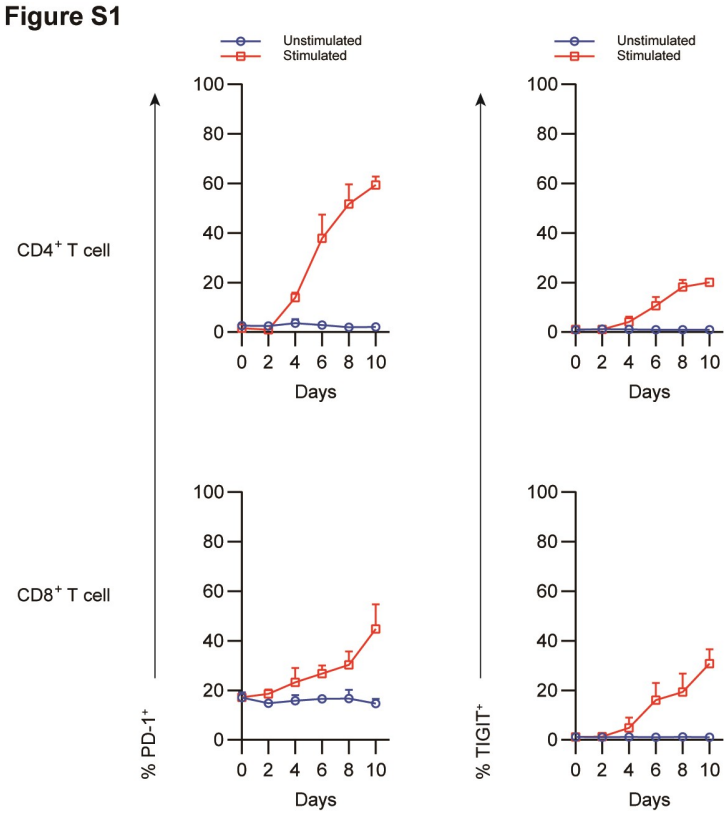

Figure S1. Expression of coinhibitory molecules on CD4 and CD8 T cells. T cells were left unstimulated or cocultured with cancer cells for 10 days. At the indicated time points, CD4 and CD8 T cells were analyzed for expression of the coinhibitory molecules PD-1 amd TIGIT.

Figure S2

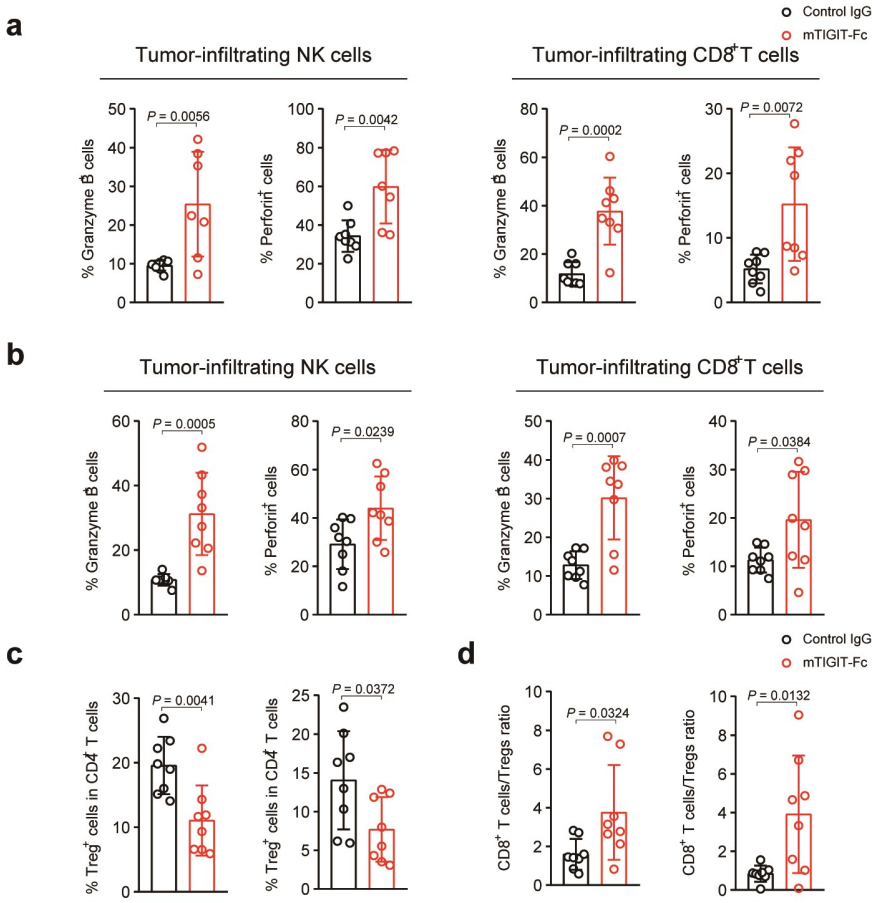

Figure S2. Characterization of Tumour-infiltrating NK cells and CD8$^+$ T cells. Frequency of cells expressing Granzyme B or Perforinamong tumour-infiltrating NK cells or T cells in MC38 mice model (**a**) and CT26 model (**b**). Percentages of intratumoral Treg cells (**c**) and CD8+ T cell:Treg ratio (**d**) in MC38 mice model and CT26 model. In MC38 model, two samples seem to be outliers. Adjust P = 0.0494 when these samples were excluded (d, left panel).

Figure S3

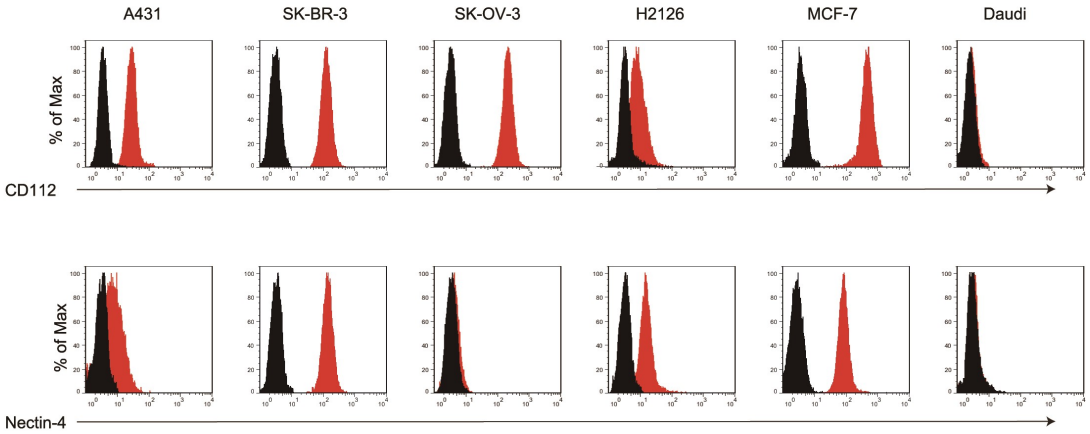

Figure S3. Characterization of TIGIT ligand expression in cancer cell lines. The expression of CD112 or Nectin-4 in different tumour cell lines was detected by staining with an antibody, followed by flow cytometry analysis. The histograms shown in black correspond to the isotype controls, whereas the red histograms indicate the positive fluorescence.

Figure S4

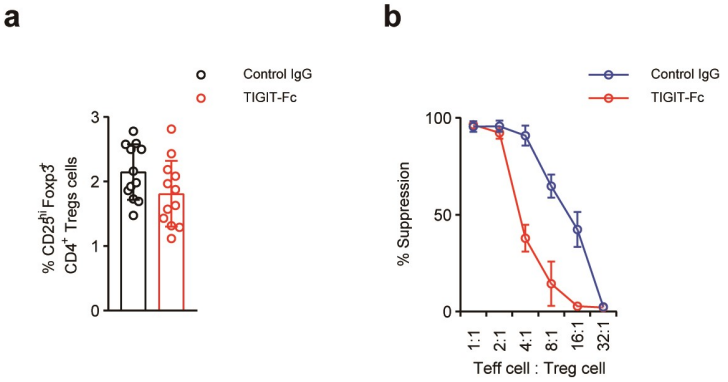

Figure S4. In vitro Treg cell characterization. a. Percentages of Treg cells in CD4+ T cell with different treatment. **b**. $CD4^{+}CD25^{+}$ Treg cells were sorted and titrated onto $CD25^{-}$effector T cells stimulated with anti-CD3/CD28 plus TIGIT-Fc or control IgG. Proliferation was measured after 72 hr by ^{3}H-thymidine incorporation. Mean ± SD;

Figure S5

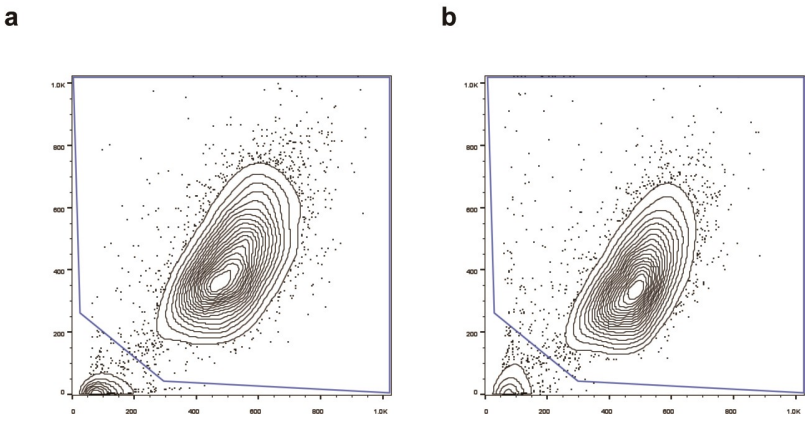

Figure S5. Gating strategies used for Flow Cytometry analysis. a. Gating strategy for cancer cells presented on Fig. 1a. **b**. Gating strategy for cancer cells presented on Fig. 3d, S3.

Supplementary Table 1. Sequences of fusion proteins

Name	Amino acid sequence
TIGIT-Fc	MMTGTIETTGNISAEKGGSIILQCHLSSTTAQVTQVNWEQQDQLLAICN ADLGWHISPSFKDRVAPGPGLGLTLQSLTVNDTGEYFCIYHTYPDGTYT GRIFLEVLESSVAEHGARFQIPEPKSCDKTHTCPPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
mTIGIT-Fc	TIDTKRNISAEEGGSVILQCHFSSDTAEVTQVDWKQQDQLLAIYSVDLG WHVASVFSDRVVPGPSLGLTFQSLTMNDTGEYFCTYHTYPGGIYKGRIF LKVQESSDDRNGLAQFQTAPLGEPRGPTIKPCPPCKCPAPNLLGGPSVFI FPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQT HREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPK GSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKT ELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHN HHTTKSFSRTPGK