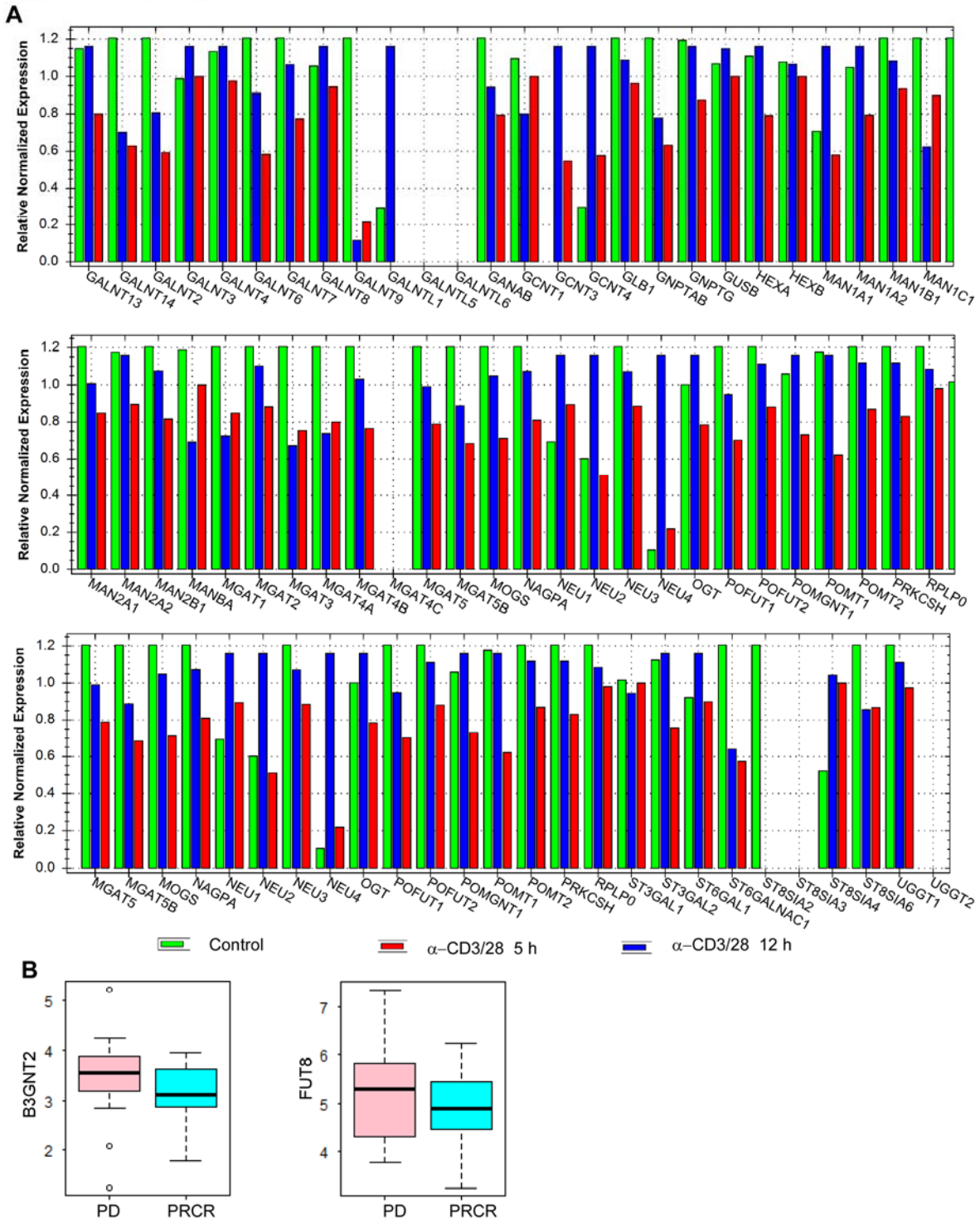
Supplementary Information

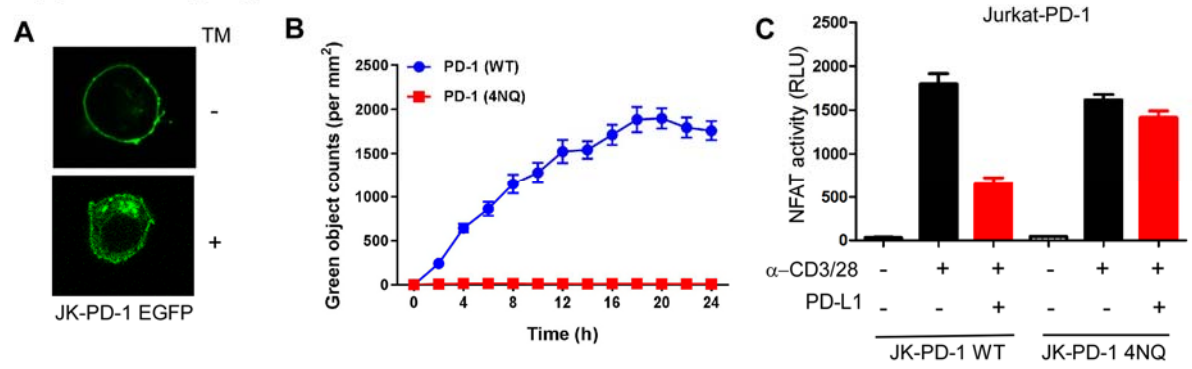
Supplementary Figure 1



Supplementary Figure.1 Evaluation of the mRNA levels of glycosyltransferases upon TCR activation and their correlations with PD-1 therapy. (A) Quantification of mRNA levels of different glycosyltransferases by real-time PCR in Jurkat T cells

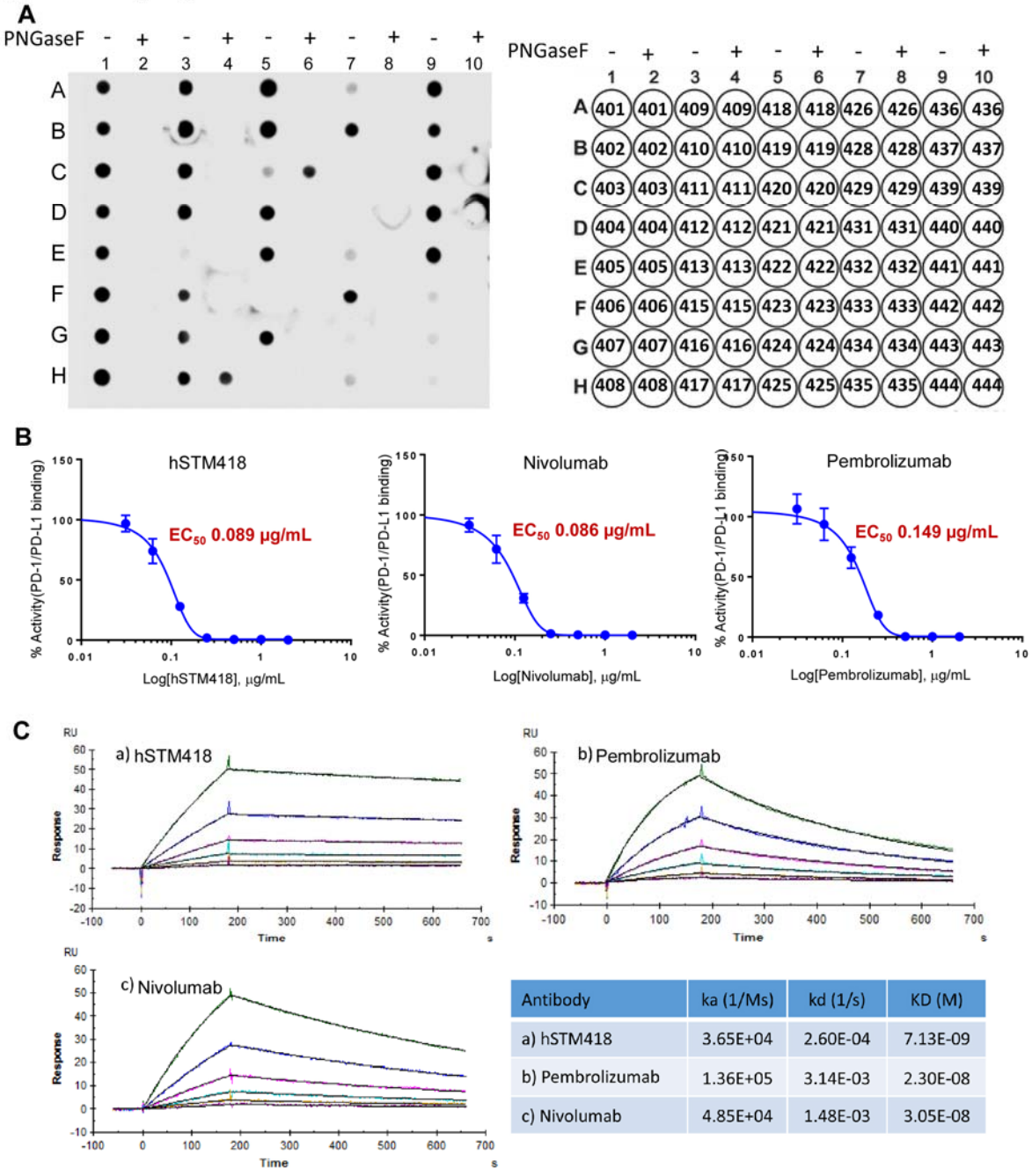
stimulated with or without anti-CD3/CD28 for the indicated time points. (B) Analysis of the correlation of B3GNT2 or Fut8 expression levels with the outcome of PD-1 therapy by using GSE78220 dataset.

Supplementary Figure 2



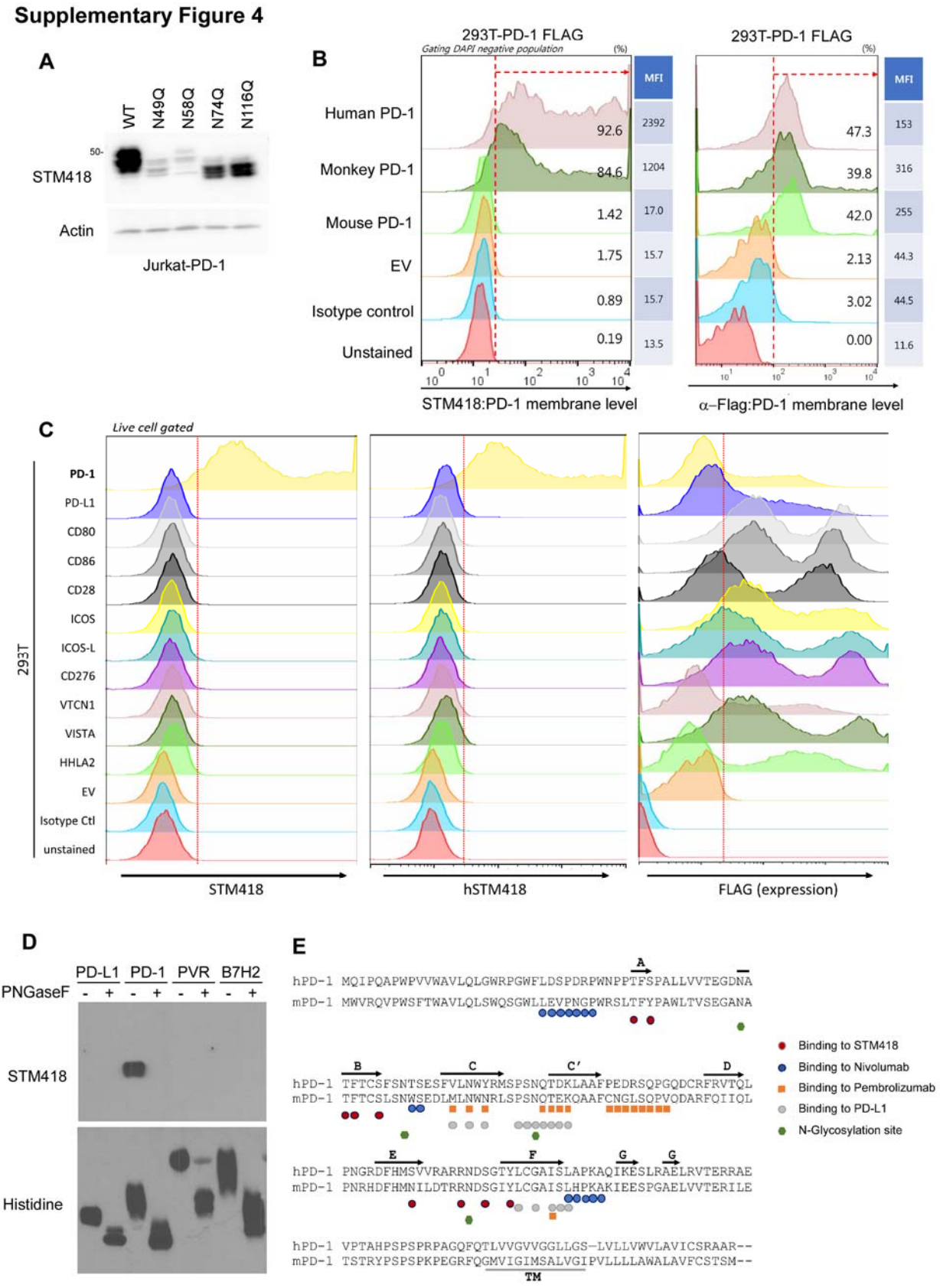
Supplementary Figure.2 Glycosylation of PD-1 is important for maintaining its membrane expression and immunosuppressive function.(A) Immuno-fluorescence analysis of EGFP-PD-1 expressed in Jurkat T cells treated with or without tunicamycin (TM) overnight.**(B)** Quantification of the dynamic interaction between green fluorescent-labeled PD-L1-Fc and PD-1 WT or 4NQ expressed in 293T cells. **(C)**Normalized luminescence of PD-1 WT- or 4NQ-expressing Jurkat T cells transiently transfected with an NFAT-Luc reporter construct and stimulated with α-CD3/CD28/IgG or α-CD3/CD28/PD-L1.

Supplementary Figure 3



Antibody	ka (1/Ms)	kd (1/s)	KD (M)
a) hSTM418	3.65E+04	2.60E-04	7.13E-09
b) Pembrolizumab	1.36E+05	3.14E-03	2.30E-08
c) Nivolumab	4.85E+04	1.48E-03	3.05E-08

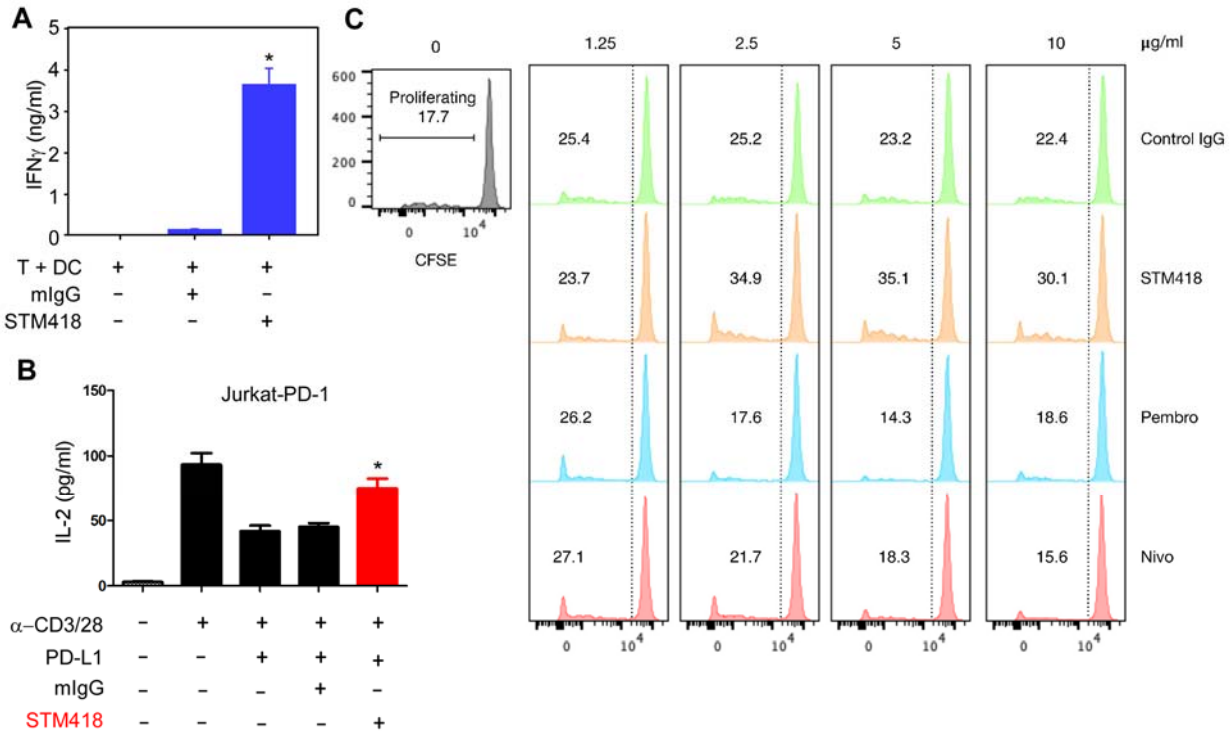
Supplementary Figure.3 Characterization of STM418 antibody. (A) Dot blot analysis of PD-1 antibodies using purified PD-1 or PNGase F-treated PD-1. **(B)** EC_{50} calculations for hSTM418, nivolumab and pembrolizumab by GraphPad Prism software. **(C)** KD determination for hSTM418, nivolumab and pembrolizumab.



Supplementary Figure 4. Evaluation of the glycan specificity of STM418. (A)

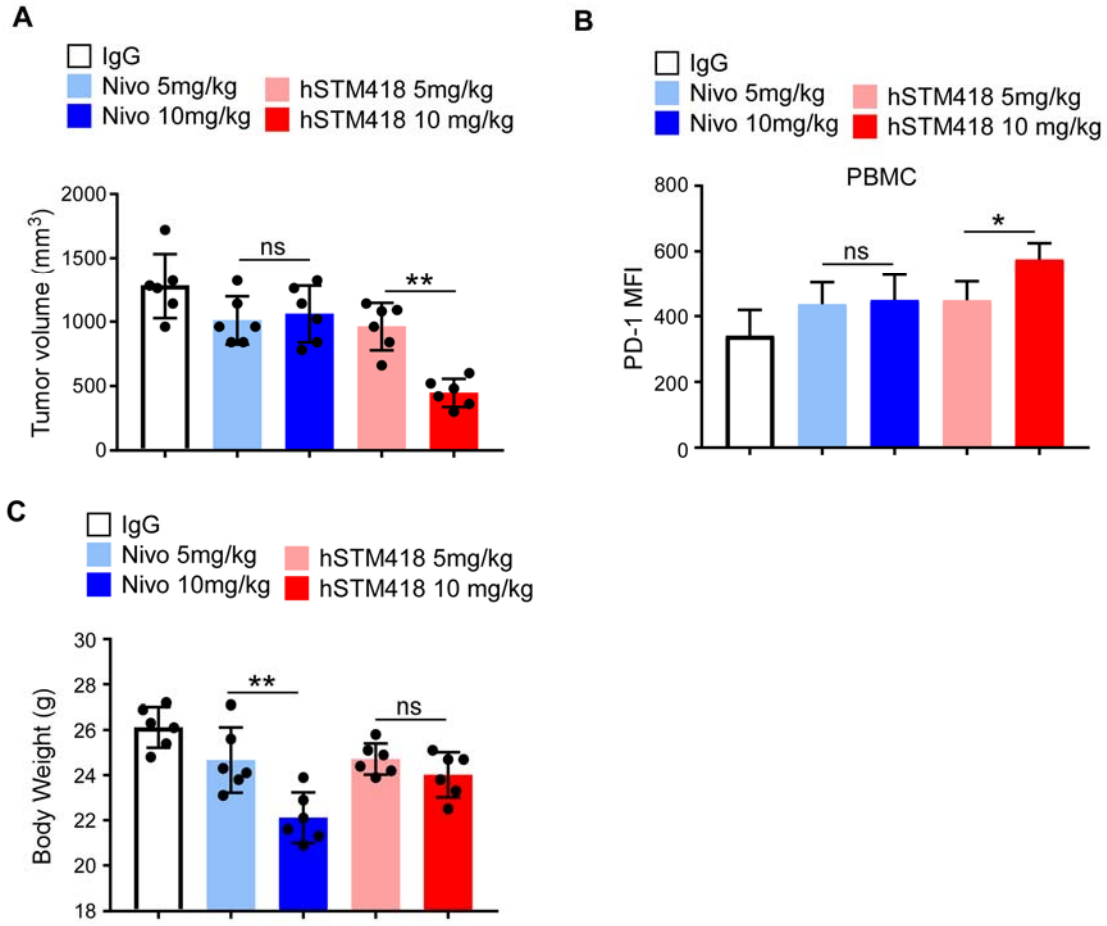
Immunoblot of PD-1 protein using STM418 in Jurkat cells overexpressing PD-1 WT or the indicated NQ mutants **(B)** Flow cytometric analysis of human, monkey or mouse PD-1 expressed in 293T cells. **(C)** Cross-reactivity evaluation of STM418 (left panel) and hSTM418 (middle panel) with cell surface CD28 or B7 family proteins transiently expressed in 293T cells by flow cytometric analysis. FLAG expression was used for detecting the protein expression (right panel). **(D)** Cross-reactivity evaluation of STM418 with PNGase F-treated glycoproteins by immuoblot. **(E)** Schematic diagram of PD-1 binding sites for STM418, nivolumab, pembrolizumab, and PD-L1.

Supplementary Figure 5



Supplementary Figure.5 STM418 promotes T cell activation and proliferation in vitro. (A) Quantification of IFNγ secretion by ELISA. T cells were co-cultured with DCs in the presence or absence of STM418 (10 μg/mL). Supernatants were collected on day 5 and subjected to ELISA. **(B)** Quantification of IL2 levels by ELISA from Jurkat-PD-1 cells stimulated with α-CD3/CD28/IgG or α-CD3/CD28/PD-L1-Fc conjugated beads in the presence or absence of STM418 (20 μg/mL). **(C)** Flow cytometric analysis of the proliferation of CFSE-labeled T cells co-cultured with dendritic cells (DCs) in the presence of indicated dose of IgG, STM418, nivolumab or pembrolizumab.

Supplementary Figure 6



Supplementary Figure 6. Therapeutic efficacy of hSTM418 in humanized mice. **(A)** The tumor volume was measured as indicated. NOD-SCID-IL2Rg-null mice were engrafted with human PBMCs to remodel and humanize the immune system seven days before inoculating human cancer cells. MDA-MB-231 cells (5×10^4) were implanted into the humanized mice, followed by the administration of IgG1, nivolumab (5mg/kg or10 mg/kg), or hSTM418 (5 mg/kg or10 mg/kg).Tumors were measured at the experimental endpoint. **(B)** PD-1 expression on PBMC was evaluated by FACS as indicated. Experiments were conducted as described in (A). **(C)** The body weight was measured as indicated at the experimental endpoint. Experiments were conducted as described in (A). All error bars represent mean ± SD. *, $P < 0.05$; **, $P < 0.01$; NS, not significant.