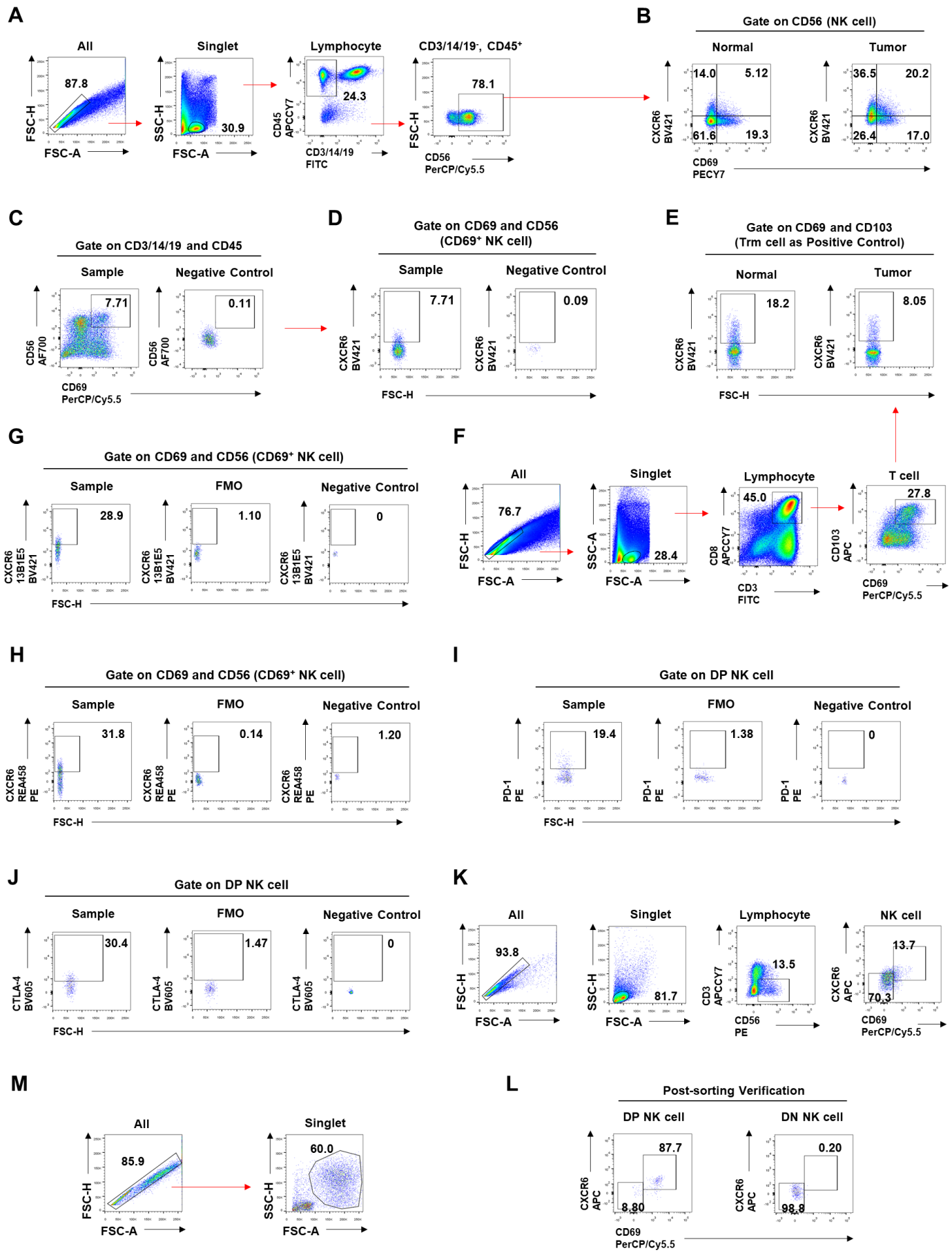


Supplementary Figure 1 | The verifications of flow cytometry analysis

A. The gating strategy for the flow cytometric analysis of NK cells.

B. The representative flow cytometric analysis of DP, SP, and DN NK cells.

C−D. The comparisons between the same sample and the negative control under different gates.

E−F. The representative flow cytometric analysis of $CXCR6^+$ Trm cells from the normal and tumor tissues as positive control. The gating strategy for the flow cytometric analysis of Trm cells is shown as (F).

G−H. The comparisons of the same sample stained with two fluorescence labelling antibodies of CXCR6 (clone 13B1E5 and clone REA458) among the normal stain, fluorescence minus one (FMO) and negative control.

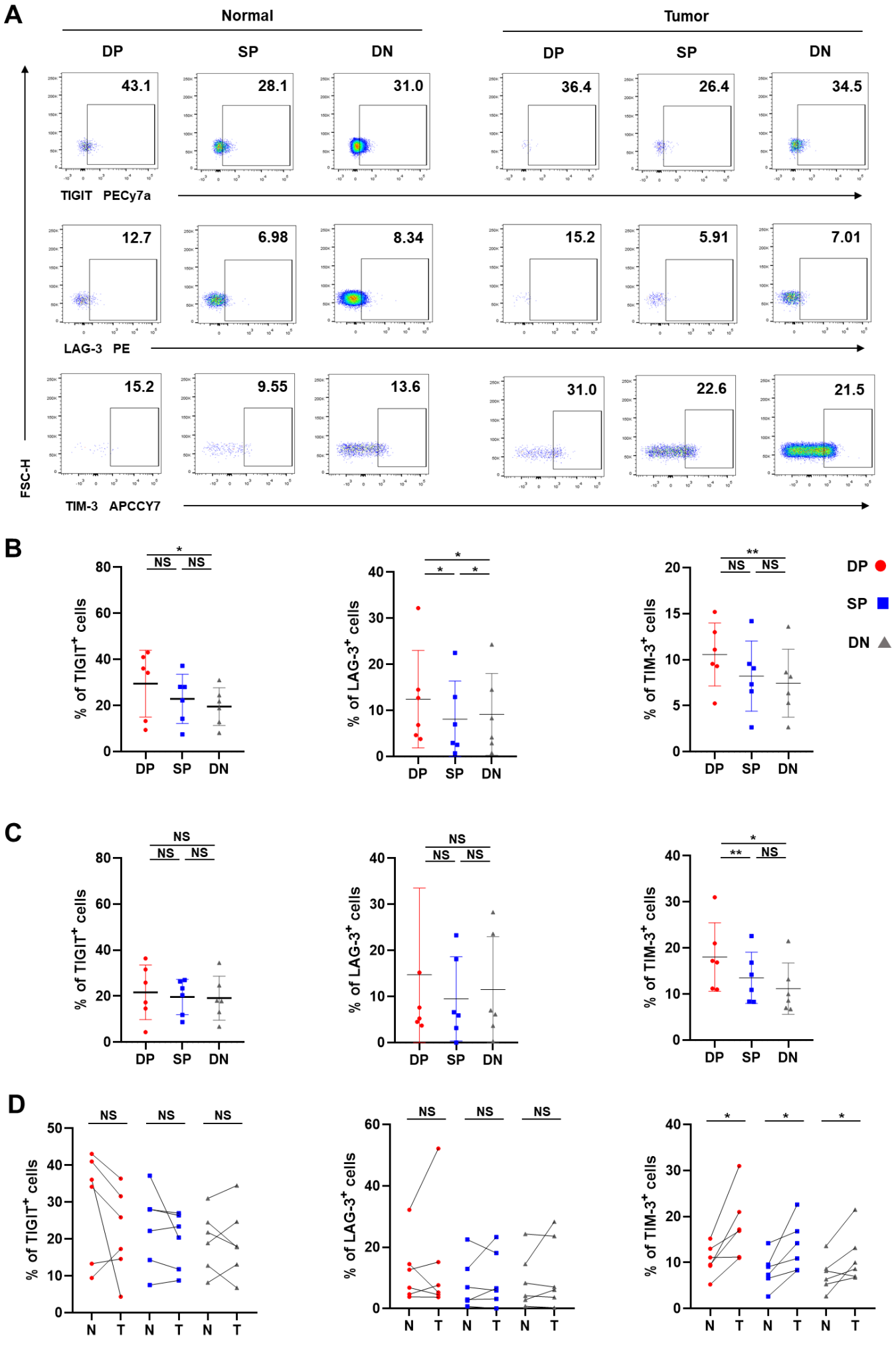
I−J. The comparisons of the samples stained with PD-1 (I) and CTLA-4 (J) among the normal stain, FMO and negative control.

K. The representative gating strategy for the flow sorting cytometric analysis of DP and DN NK cell.

L. Post-sorting verifications of DP and DN NK cell.

M. The representative gating strategy for the flow cytometric analysis of A549 lung cancer cells.

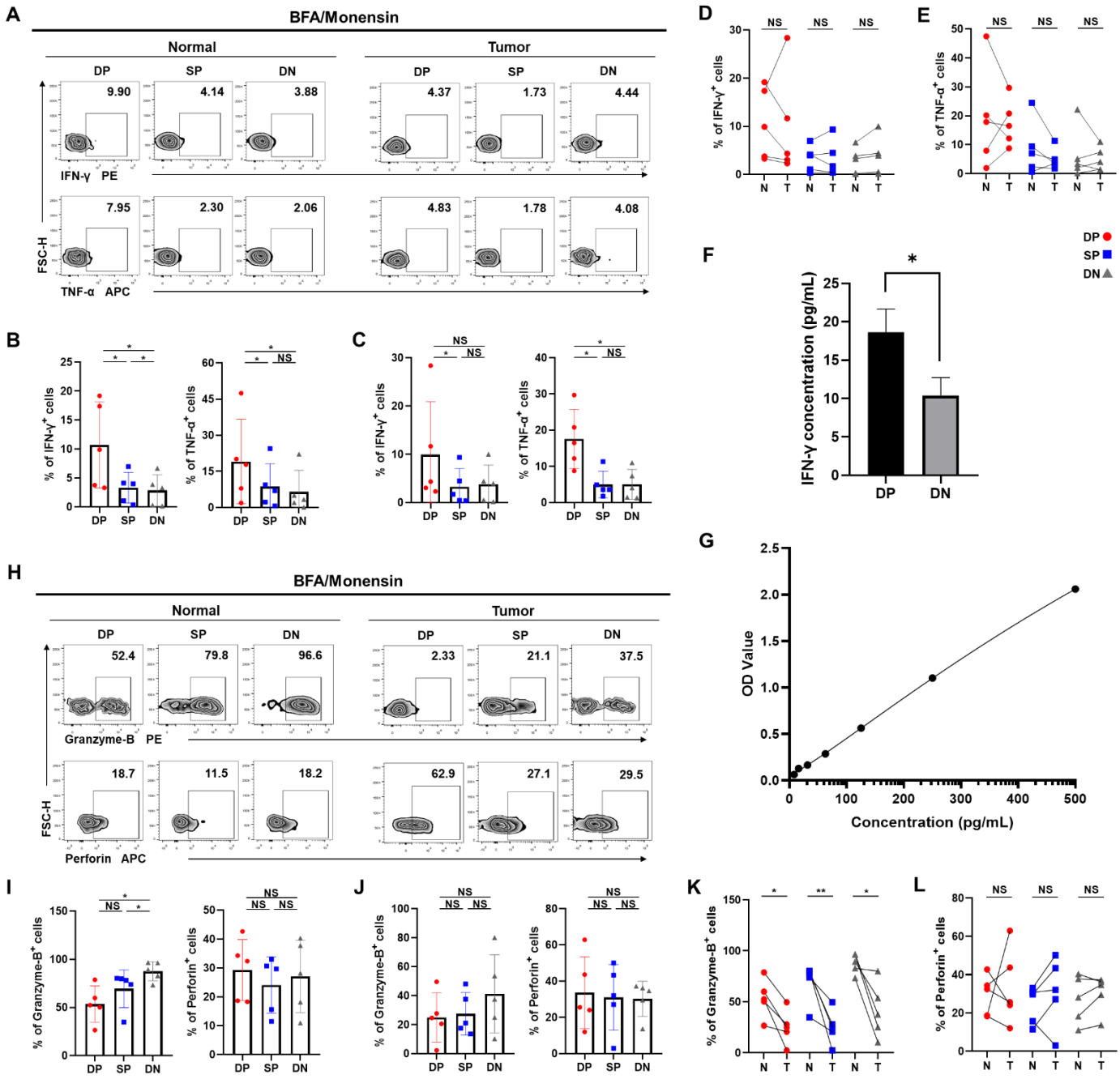
The negative control refers to as the unstained cell suspension directly uploaded to the flow cytometry. The FMO control refers to as the cell suspension stained with all the fluorescence minus one fluorescence labelling antibody to be verified. CXCR6 refers to as the clone K041E5 unless specifically marked.


A
Normal
Tumor
DP
SP
DN
43.1
28.1
31.0
36.4
26.4
34.5
TIGIT PECy7a
12.7
6.98
8.34
15.2
5.91
7.01
LAG-3 PE
15.2
9.55
13.6
31.0
22.6
21.5
FSC-H
TIM-3 APCCY7
B
% of TIGIT+ cells
% of LAG-3+ cells
% of TIM-3+ cells
NS
*
**
DP
SP
DN
C
D
N
T

Supplementary Figure 2 | The exhaustion phenotype of the trNK cells in NSCLC

The percentages of $TIGIT^+$, $LAG\text{-}3^+$, $TIM\text{-}3^+$ cells in DP, SP, and DN NK cells from the normal (B) and tumor tissues (C). The comparisons of the percentages of $TIGIT^+$, $LAG\text{-}3^+$, $TIM\text{-}3^+$ cells in DP, SP, and DN NK cells between the normal and tumor tissues (D). Data are shown as the representative flow analysis (A), mean ± SEM (B−C), and line chart (D).

Data was obtained from the normal and paired tumor tissues resected from six of 52 operative NSCLC patients, pooled from three independent experiment. *$p < 0.05$, **$p < 0.01$, ***$p<0.001$ (two-tailed t test and Wilcoxon signed rank test in B−C; two-tailed paired t test and Wilcoxon matched-pairs signed rank test in D).



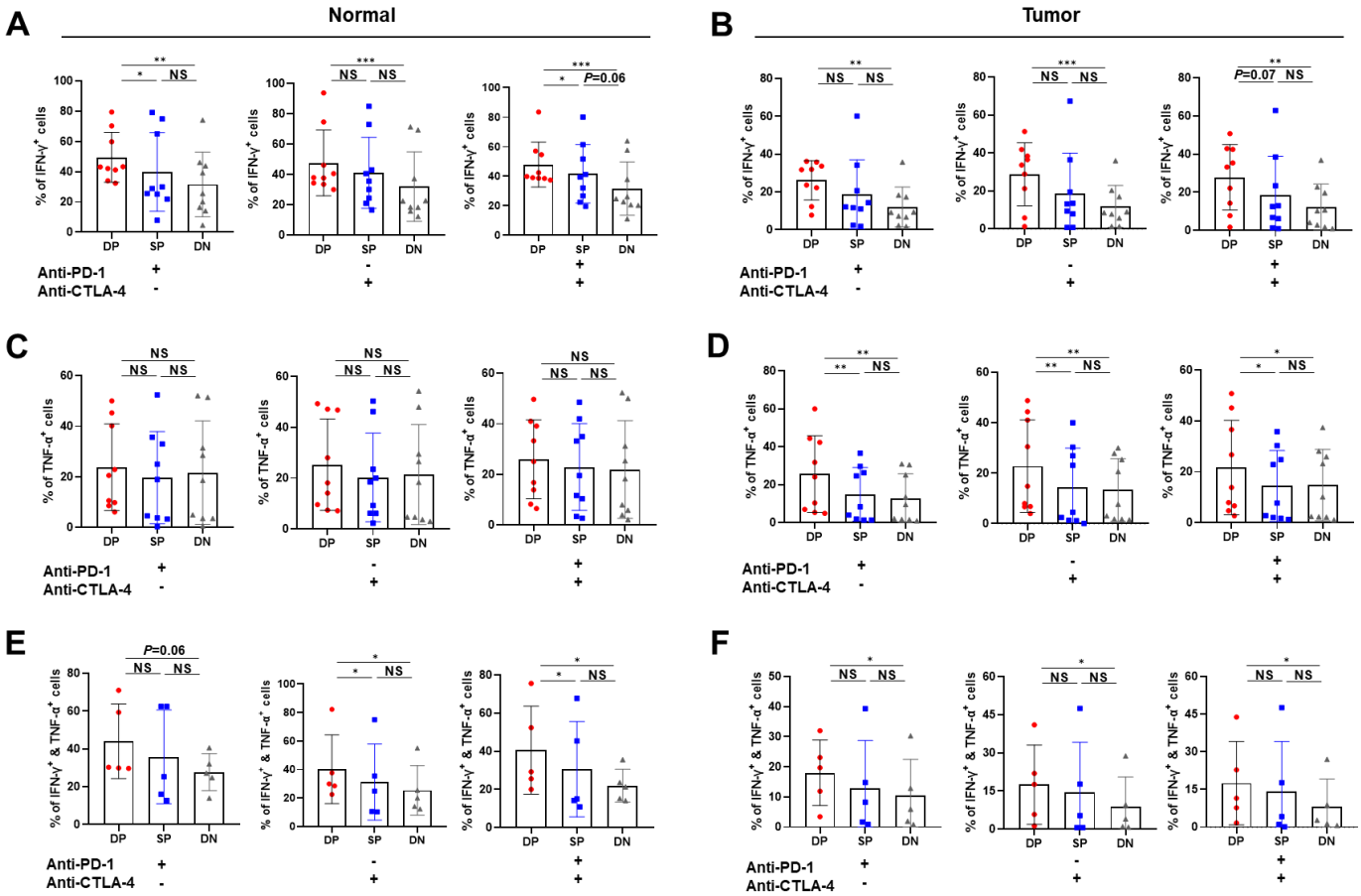
Supplementary Figure 3 | The verifications of the trNK cells' functions in NSCLC

A−E. The percentages of IFN-γ^+ and TNF-α^+ cells in DP, SP, and DN NK cells from the normal (B) and tumor tissues (C) supplemented with BFA & monensin. The comparisons of the percentages of IFN-γ^+ (D) and TNF-α^+ cells (E) in DP, SP, and DN NK cells between the normal (N) and tumor (T) tissues. Data are shown as the representative flow analysis (A), mean ± SEM (B−C), and line chart (D−E).

F−G. IFN-γ concentration in the culture supernatant of sorted DP and DN NK cell (F) and the standard curve (G). Data are shown as mean ± SEM and four parameter logistic curve fit.

H−L. The percentages of granzyme-B$^+$ cells and perforin$^+$ cells in DP, SP, and DN NK cells from the normal (I) and tumor tissues (J) supplemented with BFA & monensin. The comparisons of the percentages of granzyme-B$^+$ (K) and perforin$^+$ cells (L) in DP, SP, and DN NK cells between the normal (N) and tumor (T)

tissues. Data are shown as the representative flow analysis (H), mean ± SEM (I−J), and line chart (K−L). Data was obtained from the normal and paired tumor tissues resected from five of 52 operative NSCLC patients (A−E, H−L), pooled from three independent experiments. Data was obtained from the normal tissues resected from three operative NSCLC patients (F−G), pooled from three independent experiments. Symbols represent one individual sample. *$p < 0.05$, **$p < 0.01$, ***$p<0.001$ (two-tailed t test and Wilcoxon signed rank test in B−C, F, I−J; two-tailed paired t test and Wilcoxon matched-pairs signed rank test in D−E, K−L).



Supplementary Figure 4 | Cytokine secretion by NK cells after the blockade(s) of PD-1 and CTLA-4 in NSCLC

A–B. The percentages of IFN-γ^+ cells in DP, SP, and DN NK cells from the normal (A) and tumor tissues (B) after the blockade(s) of PD-1 and CTLA-4. Data are shown as mean ± SEM.

C–D. The percentages of TNF-α^+ cells in DP, SP, and DN NK cells from the normal (C) and tumor tissues (D) after the blockade(s) of PD-1 and CTLA-4. Data are shown as mean ± SEM.

E–F. The percentages of IFN-γ^+ and TNF-α^+ double positive cells in DP, SP, and DN NK cells from the normal (E) and tumor tissues (F) after the blockade(s) of PD-1 and CTLA-4. Data are shown as mean ± SEM.

Data was obtained from the normal and paired tumor tissues resected from nine of 52 operative NSCLC patients, pooled from three independent experiments. Symbols represent one individual sample. *$p < 0.05$, **$p < 0.01$, ***$p<0.001$ (two-tailed t test and Wilcoxon signed rank test).

Characteristics		**n = 52**
Age, mean (range)		64 (51-83)
Male, n (%)		26 (50.0)
Smoking, n (%)		18 (34.6)
TNM stage, n (%)	I	40 (76.9)
	II	7 (13.5)
	III	5 (9.62)
	IV	0
Histological type, n (%)	LUAD	40 (76.9)
	LUSC	7 (13.5)
	Others	5 (9.62)

Supplementary Table 1 | Characteristics of NSCLC patients

TNM stage is according to IASLC cancer staging manual (8th version). The information on eight normal lung tissue donators is not listed in this table.

Abbreviations: NSCLC: non-small-cell lung cancer; LUAD: lung adenocarcinoma; LUSC: squamous cell lung carcinoma.

Marker	Fluorochrome	Clone	Company
CD3	FITC	UCHT1	Biolegend
CD3	Brilliant Violet 421	UCHT1	Biolegend
CD3	APC/Cyanine7	UCHT1	Biolegend
CD8	APC/Cyanine7	HIT8a	Biolegend
CD14	FITC	HCD14	Biolegend
CD14	Brilliant Violet 421	HCD14	Biolegend
CD19	FITC	HIB19	Biolegend
CD19	Brilliant Violet 421	HIB19	Biolegend
CD45	APC/Cyanine7	HI30	Biolegend
CD45	PerCP/Cyanine5.5	HI30	Biolegend
CD45	PE-Texas Red	HI30	Thermo Fisher
CD56	PE	5.1H11	Biolegend
CD56	AlexaFluor700	5.1H11	Biolegend
CD56	PerCP/Cyanine5.5	5.1H11	Biolegend
CD69	Brilliant Violet 510	FN50	Biolegend
CD69	PE/Cyanine7	FN50	Biolegend
CD69	PerCP/Cyanine5.5	FN50	Biolegend
CXCR6	APC	K041E5	Biolegend
CXCR6	Brilliant Violet 421	K041E5	Biolegend
CXCR6	Brilliant Violet 421	13B1E5	BD Horizon
CXCR6	PE	REA458	Miltenyi Biotec
IFN-γ	PE	B27	Biolegend
IFN-γ	FITC	B27	Biolegend
IFN-γ	PE/Cyanine7	B27	Biolegend
TNF-α	PE	MAb11	Biolegend
TNF-α	APC	MAb11	Biolegend
TNF-α	PE/Cyanine7	MAb11	Biolegend
CD107a	PE	H4A3	Biolegend
Granzyme-B	PE	QA16A02	Biolegend
Granzyme-B	PerCP/Cyanine5.5	QA16A02	Biolegend
Perforin	APC	B-D48	Biolegend
CD103	PE	Ber-ACT8	Biolegend
CD103	APC	Ber-ACT8	Biolegend
CD103	PerCP/Cyanine5.5	Ber-ACT8	Biolegend
CD16	APC/Cyanine7	3G8	Biolegend
CD16	Brilliant Violet 510	3G8	Biolegend
CD16	FITC	3G8	Biolegend

Marker	Fluorochrome	Clone	Company
NKG2A	APC	REA110	Miltenyi Biotec
CD57	PE	HCD57	Biolegend
CTLA-4	Brilliant Violet 605	BNI3	Biolegend
PD-1	PE	EH12.2H7	Biolegend
PD-1	APC	EH12.2H7	Biolegend
PD-1	Brilliant Violet 421	EH12.2H7	Biolegend
TIGIT	PE/Cyanine7	A15153G	Biolegend
LAG-3	PE	7H2C65	Biolegend
TIM-3	APC/Cyanine7	F38-2E2	Biolegend

Supplementary Table 2 | Information on the fluorescence labelling antibodies used in flow cytometric analysis