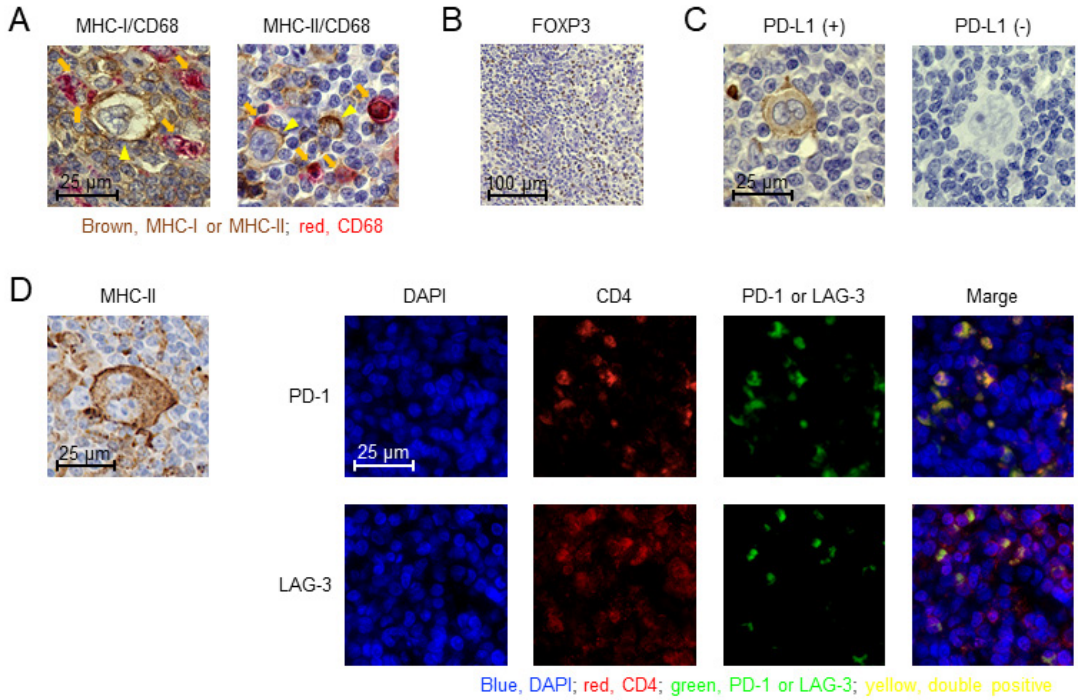
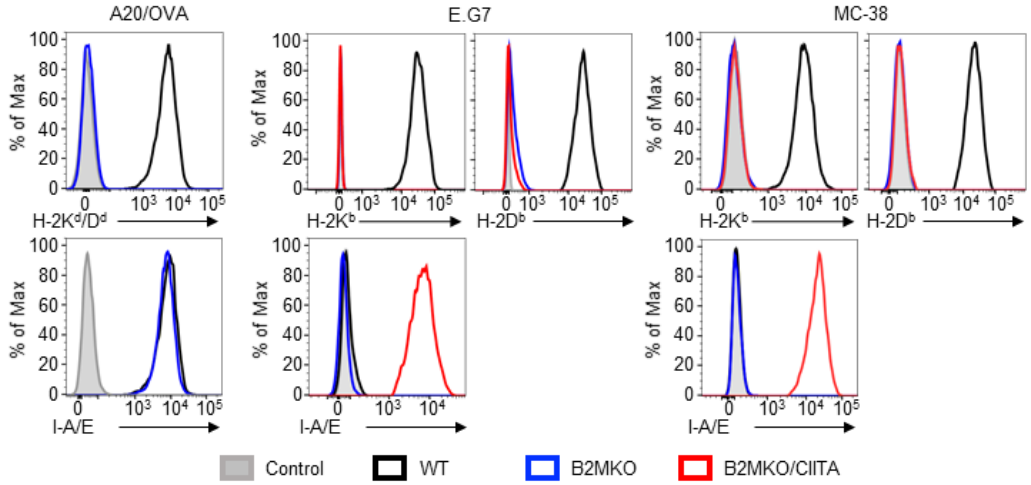
Supplemental Figures

Supplemental Figure 1. IHC in cHL.



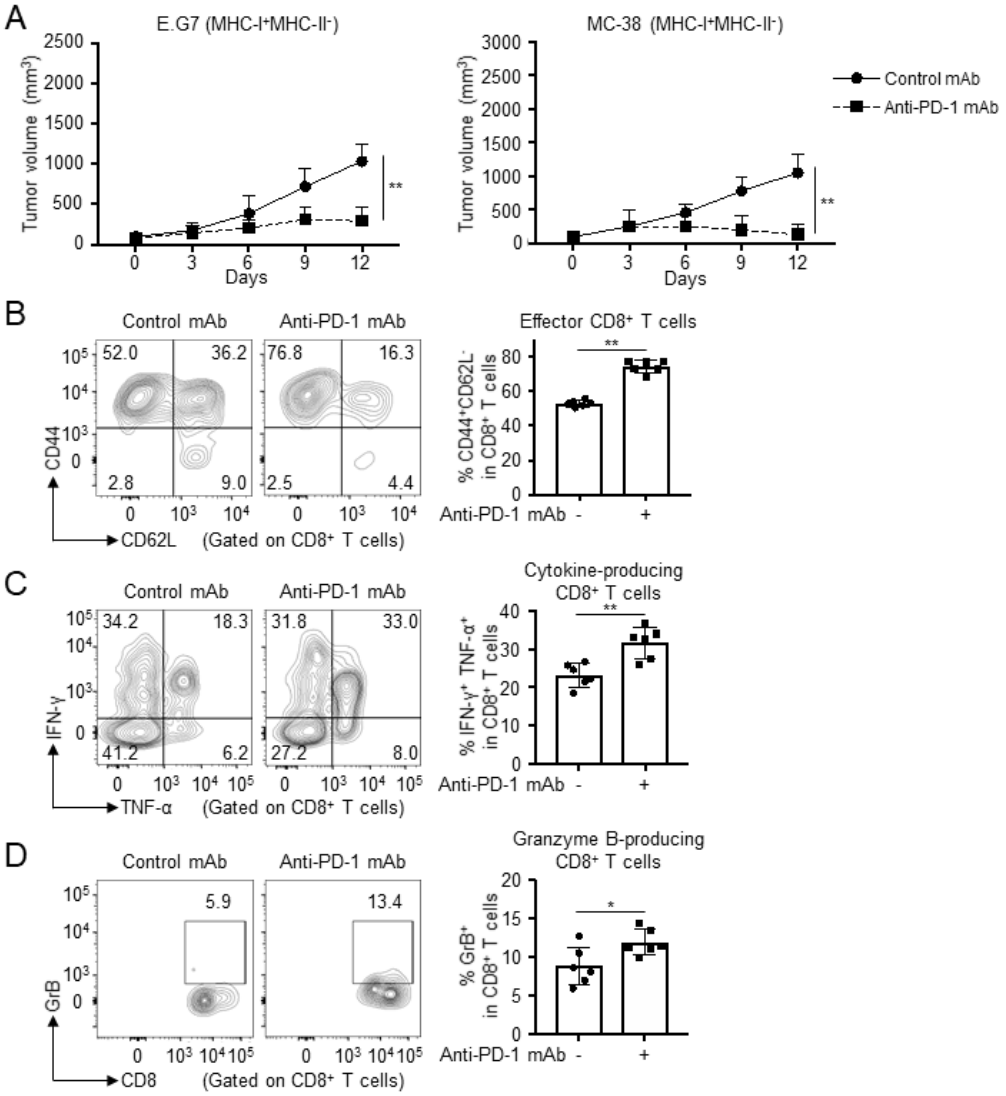
IHC was conducted using FFPE sections (3 μm). **(A)** Double staining for MHC-I or MHC-II and CD68. To discriminate HRS cells from macrophages, we co-stained MHC-I or MHC-II (brown) and CD68 (red). Representative staining pictures are shown. Yellow arrows, HRS cells; orange arrows, $MHC^{+}CD68^{+}$ cells. **(B** and **C)** FOXP3 and PD-L1 staining. Representative staining pictures for FOXP3 (**B**) and PD-L1 (**C,** left: positive, right: negative) are shown. **(D)** Multiplex fluorescent IHC for CD4 and PD-1 or LAG-3. MHC-II staining (left) and multiplex fluorescent CD4 (red) and PD-1 or LAG-3 (green) staining pictures (right) in a patient are shown.

Supplemental Figure 2. MHC expression in each tumor cell line.



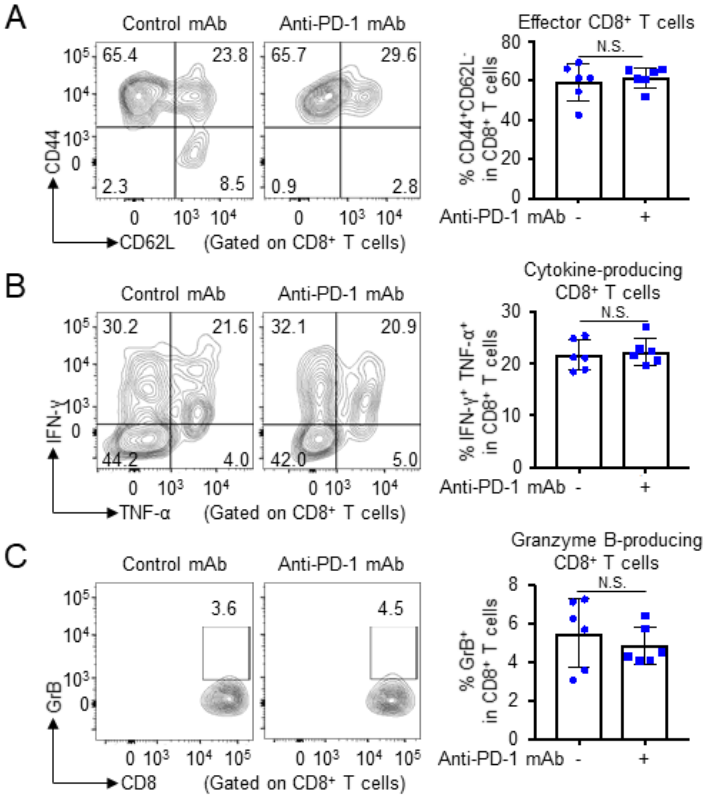
The expression of MHC-I and MHC-II was analyzed after treatment with IFN-γ (1,000 IU/mL) with flow cytometry. Representative data from three independent experiments are shown. Gray, control; black, wild-type cell lines (WT); blue, *B2M*-knockout cell lines (B2MKO); red, *B2MKO*-knockout and *CIITA*-overexpressing cell lines (B2MKO/CIITA).

Supplemental Figure 3. *In vivo* antitumor efficacy of anti-PD-1 mAb against E.G7 or MC-38 tumors.



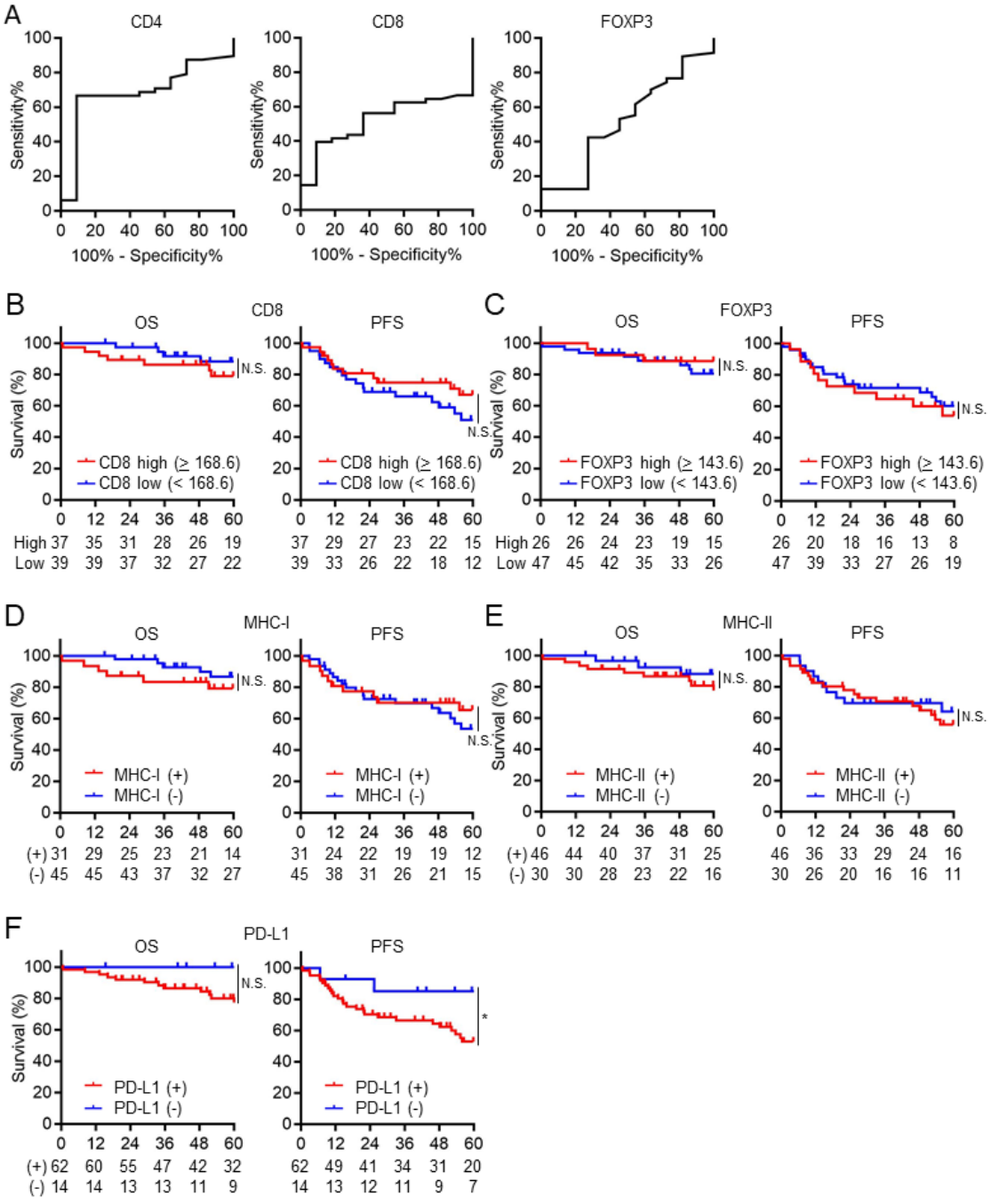
(A). *In vivo* antitumor efficacy of anti-PD-1 mAb against E.G7 or MC-38 tumors. E.G7 (5.0×10^6) or MC-38 (1.0×10^6) cells were inoculated subcutaneously. Mice were grouped when the tumors reached approximately 100 mm^3 (day 0), and anti-PD-1 mAb was administered on days 0, 3, and 6 (n = 6 per group). Tumor growth was monitored every 3 days. Circle, control Ab; square, anti-PD-1 mAb. **(B-D)** TILs were prepared from E.G7 tumors 14 days after tumor inoculation, and the frequencies of $CD44^+CD62L^-$ effector/memory $CD8^+$ T cells (**B**), $TNF\text{-}\alpha^+IFN\text{-}\gamma^+CD8^+$ T cells **(C)**, and GrB^+CD8^+ T cells **(D)** were analyzed with flow cytometry. Representative staining (left) and summaries for the frequency of each cell population (right) are shown. All *in vivo* experiments were performed twice with similar results. Circle, control Ab; square, anti-PD-1 mAb; *, $P < 0.05$; **, $P < 0.01$.

Supplemental Figure 4. The frequencies of CD44$^+$CD62L$^-$ effector/memory CD8$^+$ T cells, TNF-α$^+$IFN-γ$^+$CD8$^+$ T cells, and GrB$^+$CD8$^+$ T cells in the TIL population from E.G7/B2MKO tumors.



(A-C) E.G7/B2MKO cells (5.0×10^6) were inoculated subcutaneously. Mice were grouped when the tumors reached approximately 100 mm^3 (day 0), and anti-PD-1 mAb was administered on days 0, 3, and 6 (n = 6 per group). TILs were prepared from tumors 14 days after tumor inoculation, and the frequencies of CD44$^+$CD62L$^-$ effector/memory CD8$^+$ T cells **(A)**, TNF-α$^+$IFN-γ$^+$CD8$^+$ T cells **(B)**, and GrB$^+$CD8$^+$ T cells **(C)** in the TIL population were analyzed with flow cytometry. Representative staining (left) and summaries for the frequency of each cell population (right) are shown. All *in vivo* experiments were performed twice with similar results. Circle, control mAb; square, anti-PD-1 mAb; N.S., not significant.

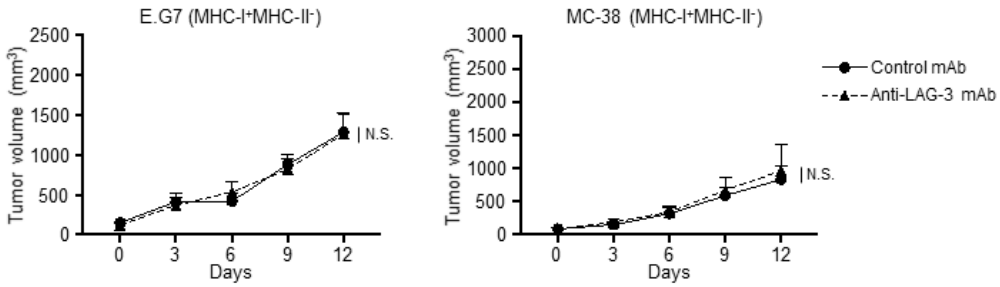
Supplemental Figure 5. Survival of cHL patients stratified according to CD8$^+$ T cell infiltration, FOXP3$^+$ regulatory T cell infiltration, MHC-I expression, MHC-II expression and PD-L1 expression.



(A) ROC curves. The survival of 76 cHL patients who received first-line standard chemotherapy (adriamycin, bleomycin, vinblastine, and dacarbazine) was examined. We employed 5-year survival for ROC curves. ROC curves for CD4 (left), CD8 (middle), or FOXP3 (right) are shown. **(B-F)** OS and PFS. The cohort was divided by each cut-off

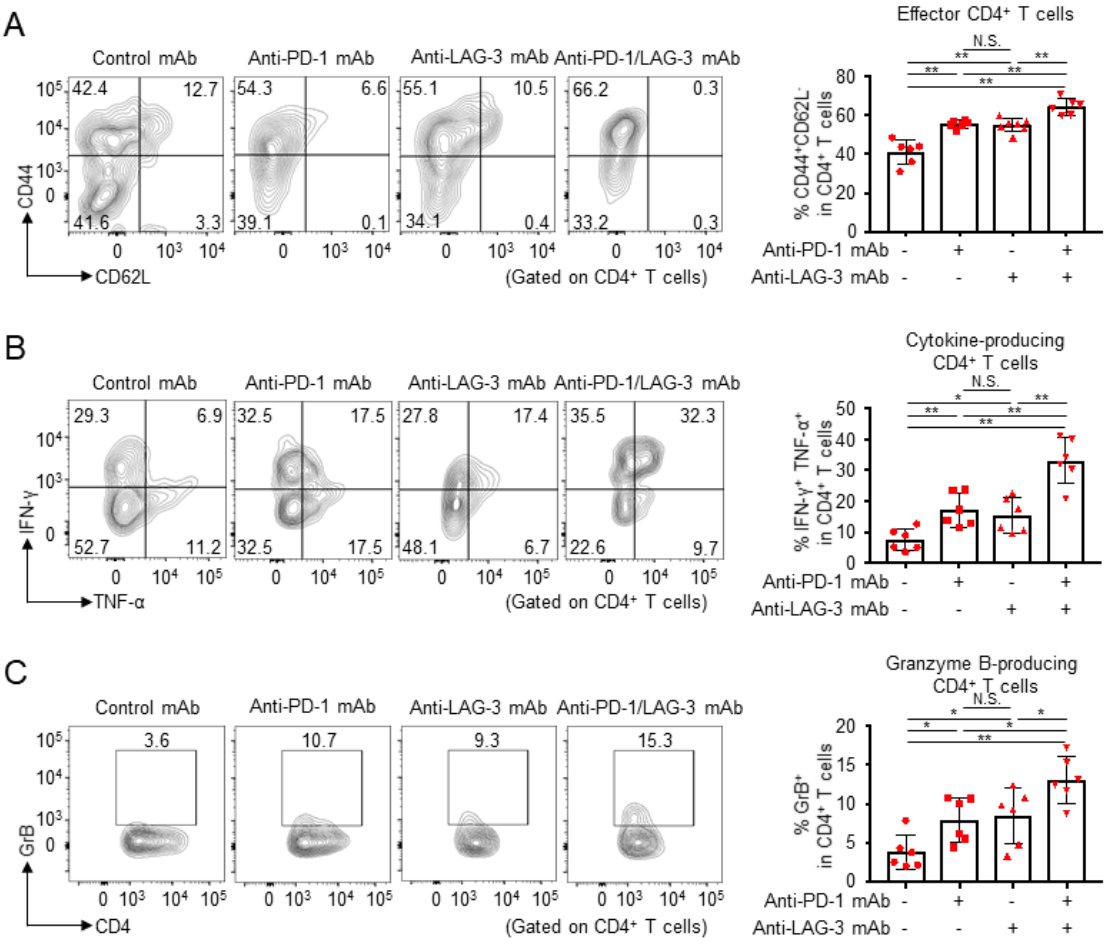
value (CD8 high, ≥ 168.6 vs. low, < 168.6 and FOXP3 high, ≥ 143.6 vs. low, < 143.6) determined by the ROC curves. OS (left) and PFS (right) according to IHC for CD8 **(B)**, FOXP3 **(C)**, MHC-I **(D)**, or MHC-II (**E**), or PD-L1 (**F**) are shown. N.S., not significant; *, $P < 0.05$.

Supplemental Figure 6. *In vivo* antitumor efficacy of anti-LAG-3 mAb against E.G7 or MC-38 tumors.



E.G7 (left; 5.0×10^6) or MC-38 (right; 1.0×10^6) cells were inoculated subcutaneously. Mice were grouped when the tumors reached approximately 100 mm^3 (day 0), and anti-LAG-3 mAb was administered on days 0, 3, and 6 (n = 6 per group). Tumor growth was monitored every 3 days. All *in vivo* experiments were performed twice with similar results. Circle, control mAb; triangle, anti-LAG-3 mAb; N.S., not significant.

Supplemental Figure 7. The frequencies of CD44$^+$CD62L$^-$ effector/memory CD4$^+$ T cells, TNF-α$^+$IFN-γ$^+$CD4$^+$ T cells, and GrB$^+$CD4$^+$ T cells in the TIL population of E.G7/B2MKO/CIITA tumors.



(A-C) Tumor cells (1.0×10^6) were inoculated subcutaneously. Mice were grouped when the tumors reached approximately 100 mm^3 (day 0), and ICIs were administered on days 0, 3, and 6 (n = 6 per group). TILs were prepared from E.G7/B2MKO/CIITA tumors 14 days after tumor inoculation, and the frequencies of CD44$^+$CD62L$^-$ effector/memory CD4$^+$ T cells **(A)**, TNF-α$^+$IFN-γ$^+$CD4$^+$ T cells **(B)**, and GrB$^+$CD4$^+$ T cells **(C)** in the TIL population were analyzed with flow cytometry. Representative staining (left) and summaries for the frequency of each cell population (right) are shown. All *in vivo* experiments were performed twice with similar results. Circle, control mAb; square, anti-PD-1 mAb; triangle, anti-LAG-3 mAb; inverted triangle, combination of anti-PD-1 mAb and anti-LAG-3 mAb; N.S., not significant; *, $P < 0.05$; **, $P < 0.01$.

Supplemental Table 1. Summary of the antibodies used in flow cytometry analyses.

Molecule	Clone	Tag	Company
CD3	17A2	AF700	Biolegend
CD8	53-6.7	V500	BD Biosciences
CD4	RM4-5	BV786	BD Biosciences
CD44	IM7	PE-Cy7	BD Biosciences
CD62L	MEL-14	PerCP-Cy5.5	Biolegend
IFN-γ	XMG1.2	APC	BD Biosciences
TNF-α	MP6-XT22	BV421	Biolegend
GrB	NGZB	PE-Cy7	Thermo Fisher Scientific
I-A/E	M5/114.15.2	AF488	Biolegend
H-2K^{b}	AF6-88.5	PE	Biolegend
H-2L^{d}/D^{b}	28-14-8	PE	Biolegend
H-2K^{d}/D^{d}	34-1-2S	APC	Biolegend

Supplemental Table 2. Clinicopathological features in relation to $CD4^+$ T cell infiltration.

Features	CD4		
	High, ≥ 425 (46)	**Low, < 425 (39)**	***P***
Age [median] (range)	44 (15-81)	52 (15-88)	0.081
Sex Male/female	35/11	23/14	0.24
Sampling 1st diagnosis/relapse	44/2	36/3	0.66
Histology NS/MC/others	16/22/8	20/17/2	0.36¶
EBV Positive/negative	16/14	14/15	0.80
Performance status 0 or 1/2 or 3	46/0	37/2	0.21
Ann Arbor stage I or II/III or IV	23/23	16/23	0.51
B symptom Yes/no	13/33	12/27	0.82
LDH [median U/L] (range)	229.5 (68-554)	205 (122-410)	0.34
MHC class I Positive/negative	18/28	18/21	0.66
MHC class II Positive/negative	37/9	17/22	< 0.01
CD8 [median] (range)	140.7 (11-400)	187.4 (6.2-437.4)	0.64
FOXP3 [median] (range)	93.8 (11-568.6)	100 (6.4-368.6)	0.22
PD-L1 Positive/negative	37/9	34/5	0.56
Best response to ABVD CR/PR, SD, or PD	36/5	24/11	0.051

NS, Nodular sclerosis; MC, Mixed cellularity; ABVD, adriamycin, bleomycin, vinblastine, and dacarbazine; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

¶, NS vs. MC

Supplemental Table 3. Univariate and multivariate analyses for OS.

Features	Univariate			Multivariate		
	HR	**95% CI**	***P***	**HR**	**95% CI**	***P***
Age (< 45/≥ 45 years)	< 0.01	-0.11	< 0.01	< 0.01	-0.17	< 0.01
Sex (female/male)	1.28	0.44-3.41	0.63			
Histology (the others/MC)	0.50	0.17-1.35	0.17			
EBV (negative/positive)	0.51	0.16-1.48	0.22			
Performance status (0 or 1/2 or 3)	0.075	0.019-0.49	0.012	0.36	0.085-2.45	0.25
Ann Arbor stage (I or II/III or IV)	0.23	0.052-0.70	< 0.01	0.30	0.069-0.97	0.044
B symptom (no/yes)	0.52	0.20-1.39	0.19			
LDH (≤ upper limit/> upper limit)	0.62	0.23-1.75	0.35			
MHC class I (positive/negative)	1.39	0.52-3.65	0.50			
MHC class II (positive/negative)	1.36	0.50-4.31	0.56			
CD4 (high/low)	0.18	0.049-0.51	< 0.01	0.26	0.072-0.79	0.016
CD8 (high/low)	1.47	0.56-4.07	0.43			
FOXP3 (high/low)	0.52	0.15-1.48	0.23			
PD-L1 (positive/negative)	3.63	0.74-65.61	0.13			

Supplemental Table 4. Clinical characteristics of 3 patients who received anti-PD-1 mAb.

Case	CCC-43	CCC-66	NCCHE-24
Age	64	56	58
Sex	Male	Male	Male
Histology	Mixed cellularity	Mixed cellularity	Mixed cellularity
EBV	Positive	Positive	Positive
Performance status	3	0	2
Treatment line	4th	4th	4th
Treatment	Nivolumab	Nivolumab	Nivolumab
Best response	PR	PR	SD
PFS	More than 2 years	6 months	4 months
MHC class I	Positive	Positive	Negative
MHC class II	Positive	Positive	Positive
CD4	750	493.8	137.6
CD8	337.4	125	193.8
FOXP3	62.6	106.2	31.2
PD-L1	Positive	Positive	Positive

PR, partial response; SD, stable disease; PFS, progression-free survival.