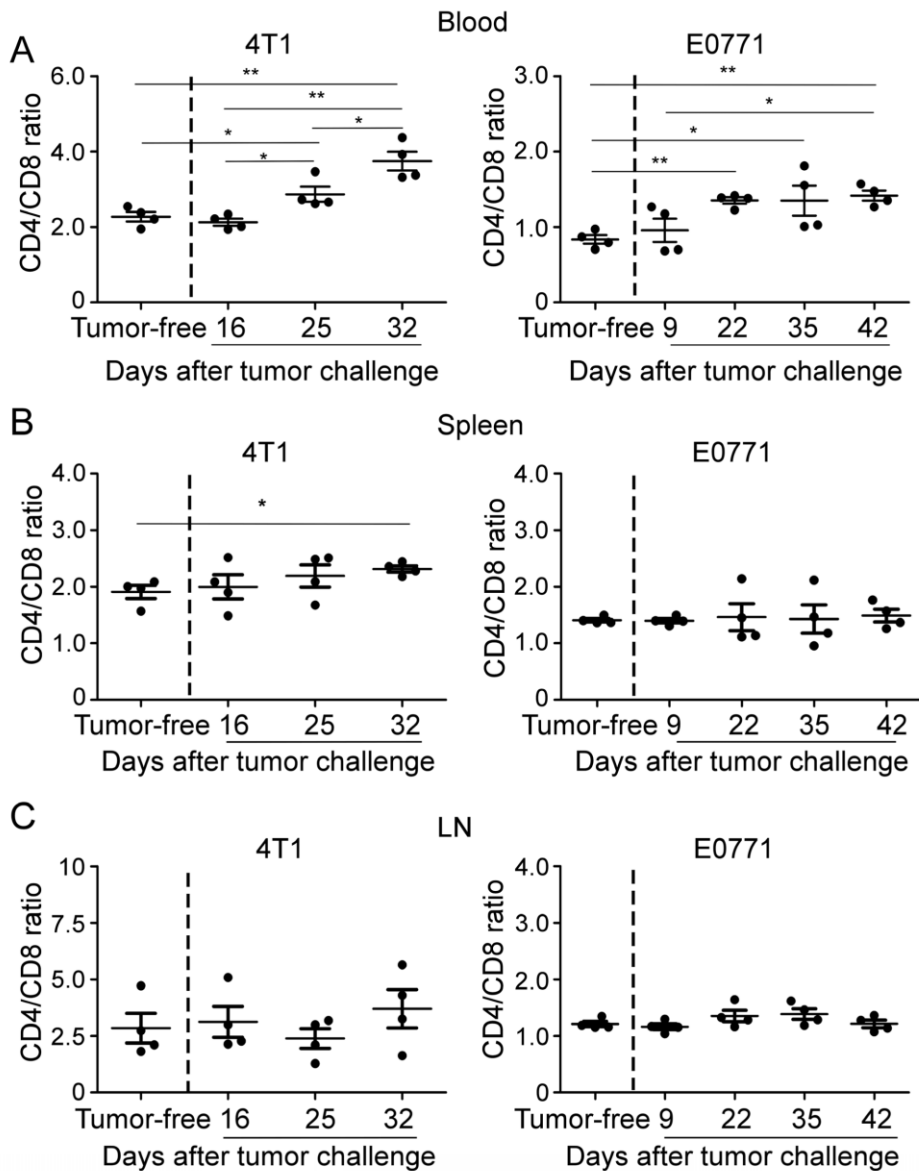


# CD4<sup>+</sup> and CD8<sup>+</sup> T cells have opposing roles in breast cancer progression and outcome

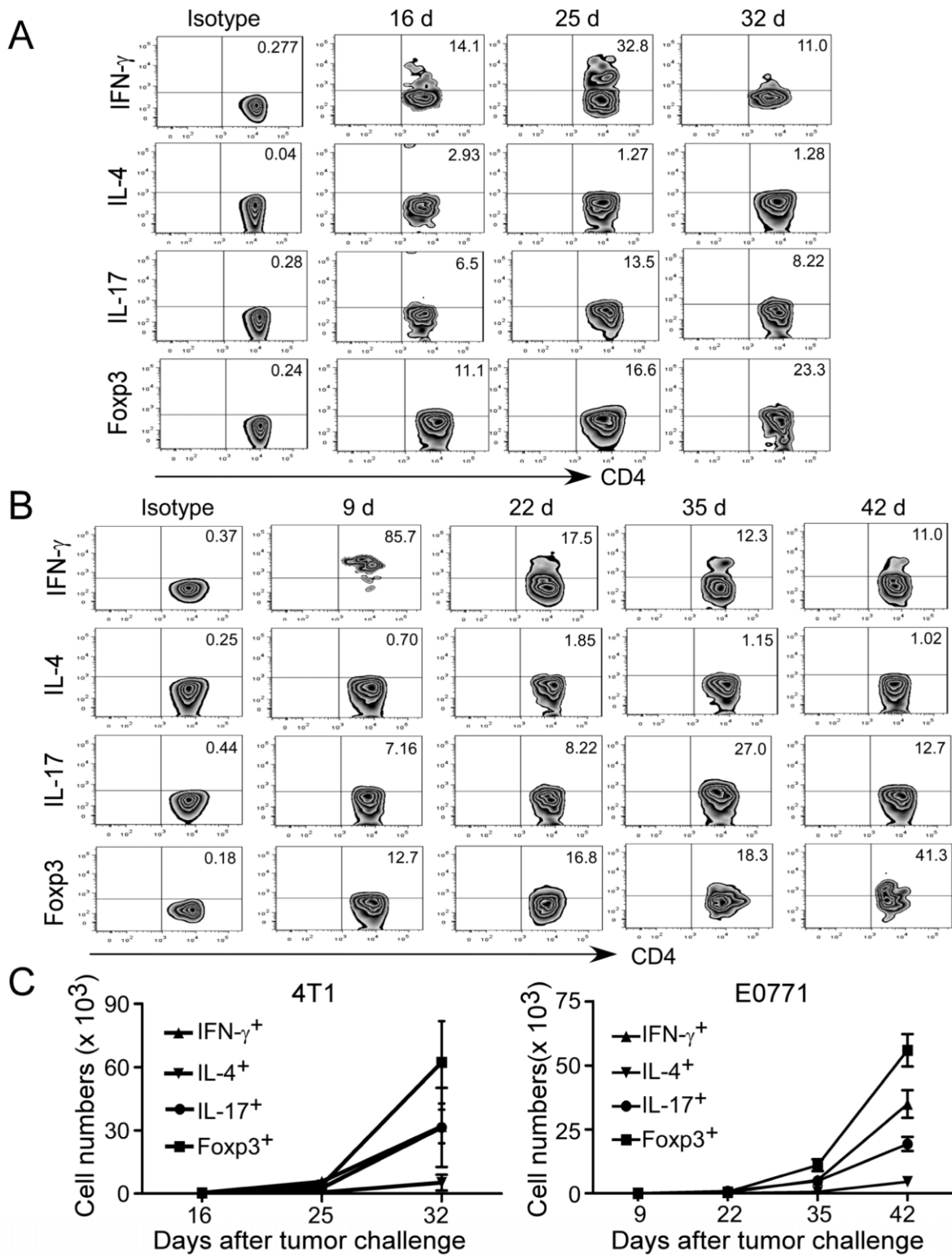
## Supplementary Material



## Supplemental Figure 1

(A) to (C) Dynamic changes of CD4<sup>+</sup> to CD8<sup>+</sup> T cell ratios in peripheral blood (A), spleen (B) and draining lymph nodes (C) at the different stages of tumor development in two models. Tumor-free BALB/c (4T1) and C57BL/6 (E0771) mice were included as controls. The treatment procedure was identical as that described in Figure 1. T cells were isolated from the organs and proportions of CD4<sup>+</sup> and

CD8<sup>+</sup> T cells detected at the indicated time points using flow cytometry analyses by gating CD3<sup>+</sup> population. Results shown in (A) to (C) are mean  $\pm$  SE from four individual mice in each time point. \*  $p < 0.05$  and \*\*  $p < 0.01$  between the indicated two groups determined by paired student's T-test.

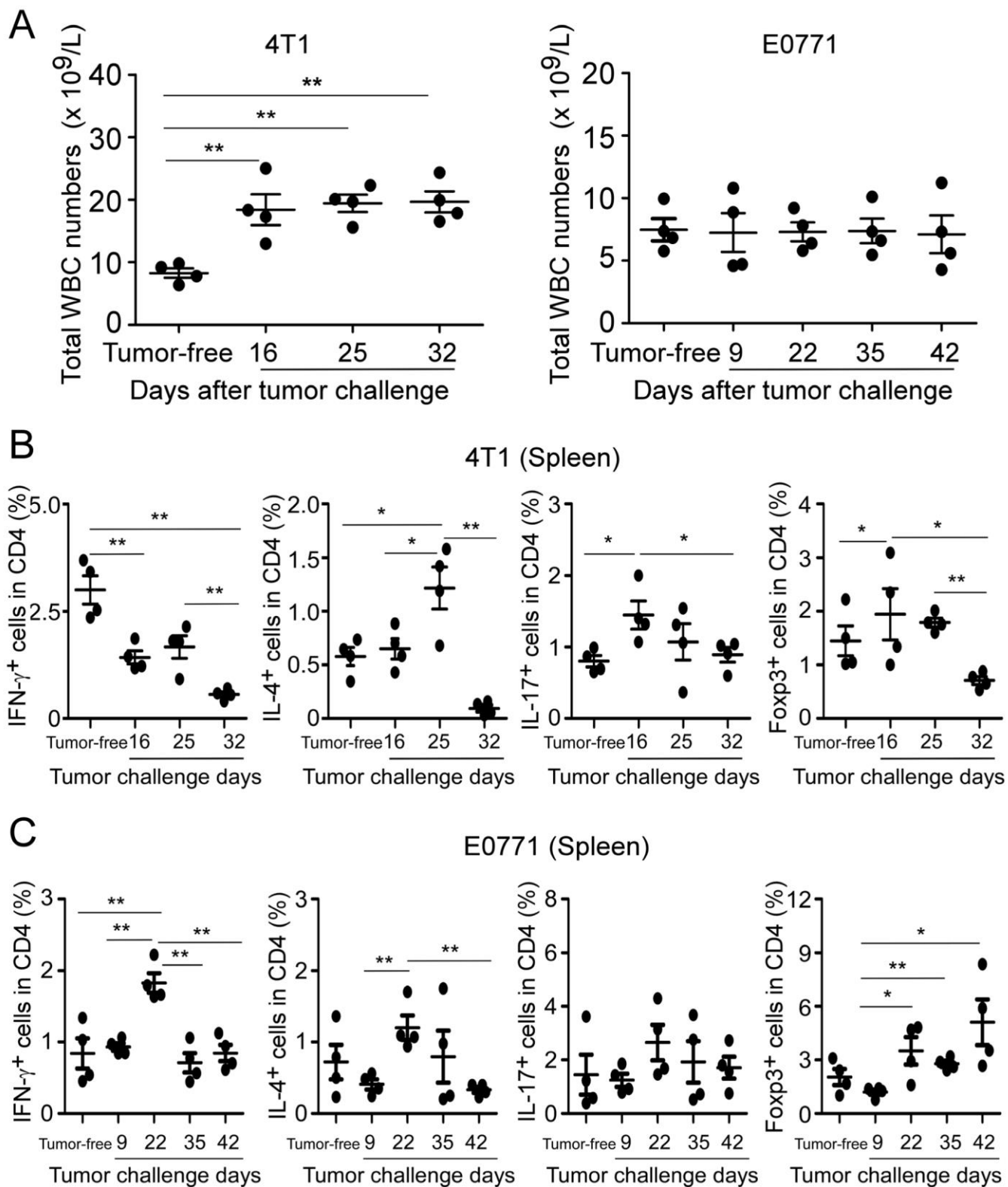


**Supplemental Figure 2**

**Dynamic analyses of tumor-infiltrating CD4 $^+$  T cell subsets in mouse breast cancer models.**

(A) and (B) Proportions of CD4 $^+$  T cell subsets in TILs were analyzed at the indicated time points using flow cytometry analyses by gating CD4 $^+$  population. Representative FACS graphs from 4T1-bearing mice (A) and E0771-bearing mice (B) are shown. The treatment procedure was identical as that described in

Figure 1. TILs were isolated and intracellular staining performed after stimulation with PMA and ionomycin for 5 hours. **(C)** Total cell numbers of CD4<sup>+</sup> T cell subsets were concluded. Data shown are mean  $\pm$  SE from four individual mice in each group.



**Supplemental Figure 3**

**Distributions of CD4 $^+$  T cell subsets in blood and spleens in breast tumor-bearing mice.**

**(A)** Peripheral blood from two mouse breast tumor models were collected and total white blood cell numbers counted. The mouse treatment procedure was identical as that described in Figure 1. **(B)** and **(C)** The dynamic distributions of CD4<sup>+</sup> T cell subsets in spleens from breast tumor-bearing mice in two models were analyzed at the indicated time points using flow cytometry analyses by gating CD4<sup>+</sup> population. The treatment procedure was identical as that described in Figure 1. T cells were isolated from the organs and intracellular staining performed after stimulation with PMA and ionomycin for 6 hours. Results shown in (A) to (C) are mean  $\pm$  SE from four individual mice in each time point. Tumor-free BALB/c (4T1) and C57BL/6 (E0771) mice were included as controls. \*  $p < 0.05$  and \*\*  $p < 0.01$  between the indicated two groups determined by paired student's T test.

Supplemental Table 1.

**A. Univariate analyses of factors associated with Relapse-free Survival and Overall Survival in breast cancer patients (n=81).**

Variables	Relapse-free Survival			Overall survival		
	HR	95% CI	p	HR	95% CI	p
<b>CD4 (&gt;16)</b>	3.92	1.48 to 10.36	<b>0.005</b>	2.61	1.17 to 5.82	<b>0.02</b>
<b>CD8 (&gt;13)</b>	0.06	0.01 to 0.47	<b>6.71E-05</b>	0.32	0.13 to 0.79	<b>0.007</b>
<b>CD4/CD8 (&gt;1.2)</b>	10.87	2.46 to 47.23	<b>5.01E-05</b>	3.73	1.48 to 9.41	<b>0.002</b>
<b>Stage ( III vs I + II)</b>	11.94	4.31 to 33.07	<b>1.53E-06</b>	6.72	2.88 to 15.71	<b>1.65E-05</b>
<b>Nodal (Positive)</b>	16.71	3.76 to 74.26	<b>1.35E-06</b>	6.97	2.58 to 18.79	<b>1.15E-05</b>

Note: Results obtained using the Cox proportional hazard regression model. Boldface indicates the significance of the *p* value.

**B. Multivariate analyses of Hazard Ratios with Relapse-free Survival and Overall Survival in breast cancer patients (n=81).**

Variables	Relapse-free Survival			Overall survival		
	HR	95% CI	p	HR	95% CI	p
<b>CD4 (&gt;16)</b>	1.37	0.35 to 5.25	0.65	1.37	0.49 to 3.81	0.55
<b>CD8 (&gt;13)</b>	0.05	0.01 to 0.51	<b>0.01</b>	0.28	0.09 to 0.93	<b>0.04</b>
<b>CD4/CD8 (&gt;1.2)</b>	0.65	0.08 to 5.48	0.68	0.69	0.17 to 2.78	0.60
<b>Stage ( III vs I + II)</b>	4.54	1.04 to 22.02	<b>0.04</b>	2.76	0.99 to 7.71	<b>0.05</b>
<b>Nodal (Positive)</b>	9.46	1.19 to 74.74	<b>0.03</b>	4.61	1.29 to 16.44	<b>0.02</b>

Note: Results obtained using the Cox proportional hazard regression model. Boldface indicates the significance of the *p* value.