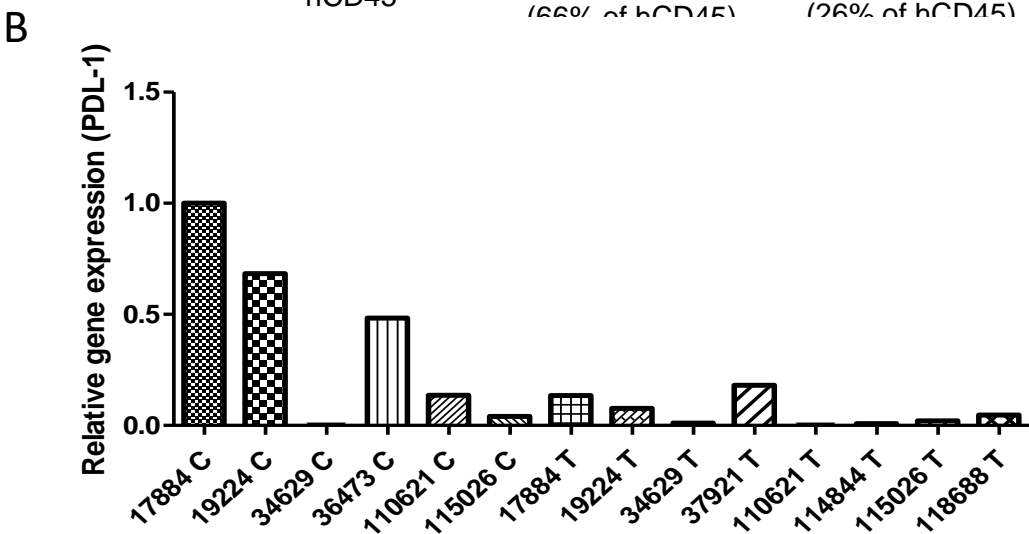
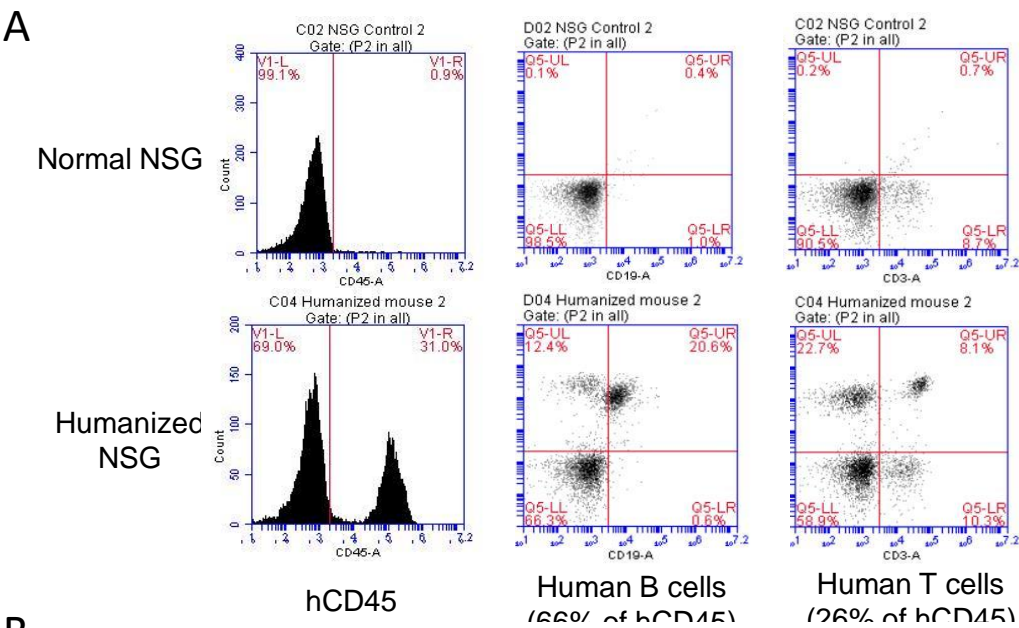


# Supplementary Material for

siRNA nanoparticle targeting PD-L1 activates tumor immunity and abrogates pancreatic cancer growth in humanized, orthotopic preclinical model

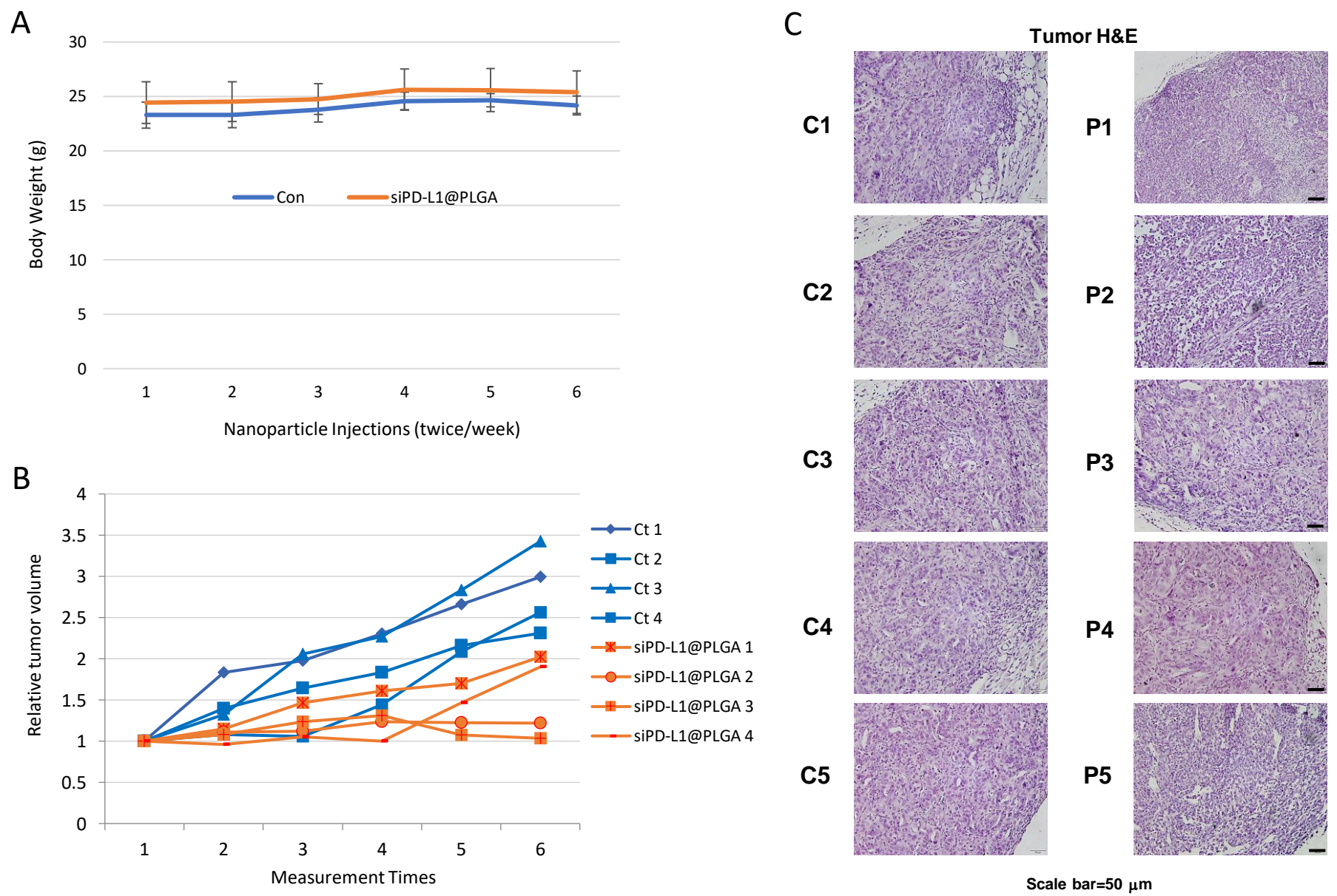
- This file contains 6 supplementary figures and 1 supplementary table.

Supplementary Figure 1

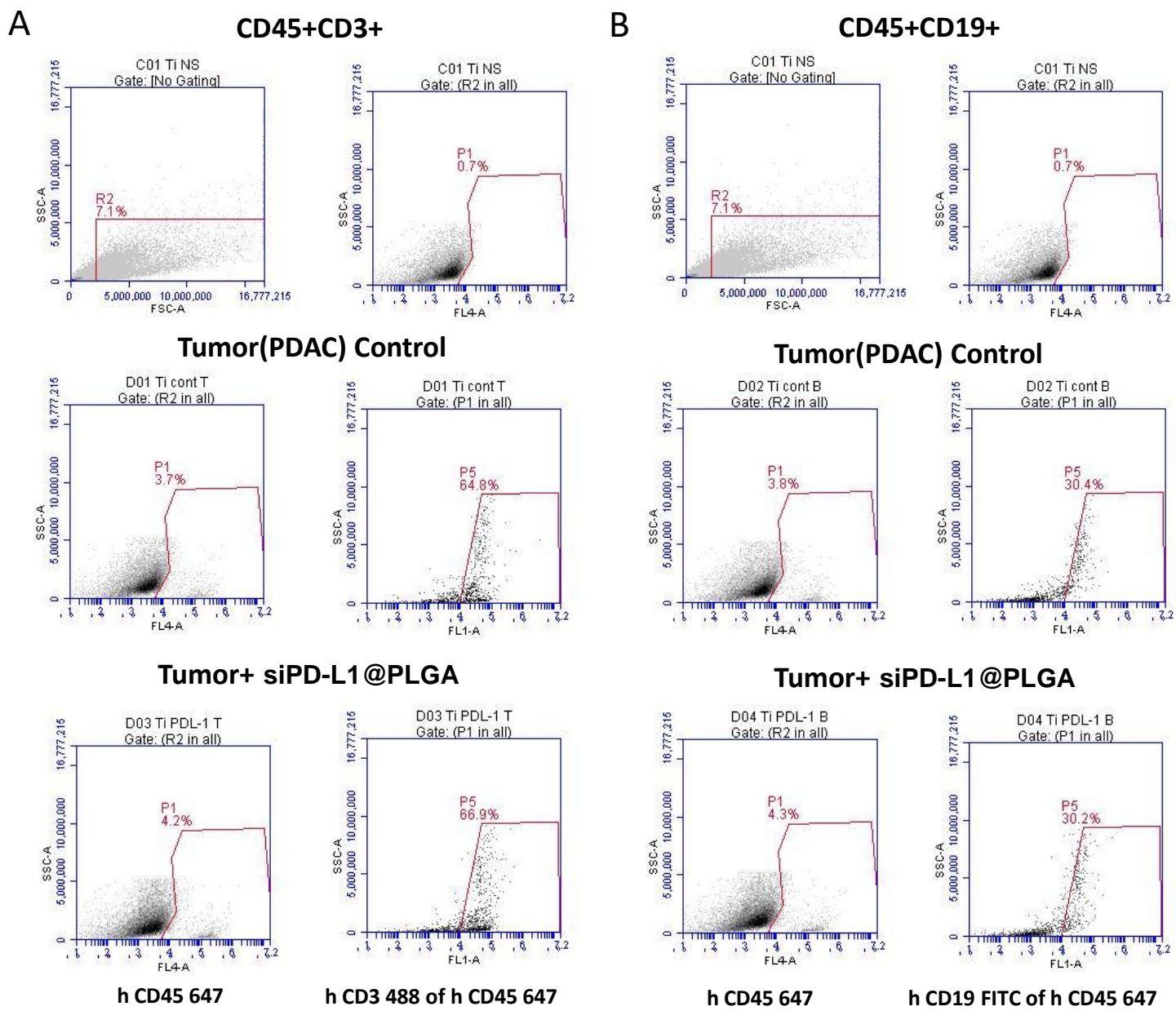


**Figure S1.** A. Representative flow cytometry plots showing human hematopoietic cells (hCD45<sup>+</sup>), human T lymphocytes (hCD45<sup>+</sup>hCD3<sup>+</sup>) and human B lymphocytes (hCD45<sup>+</sup>hCD19<sup>+</sup>) in the blood of humanized normal mice. B. Expression profile of PDAC primary cells (marked as C) and tumors (marked as T).

Supplementary Figure 2

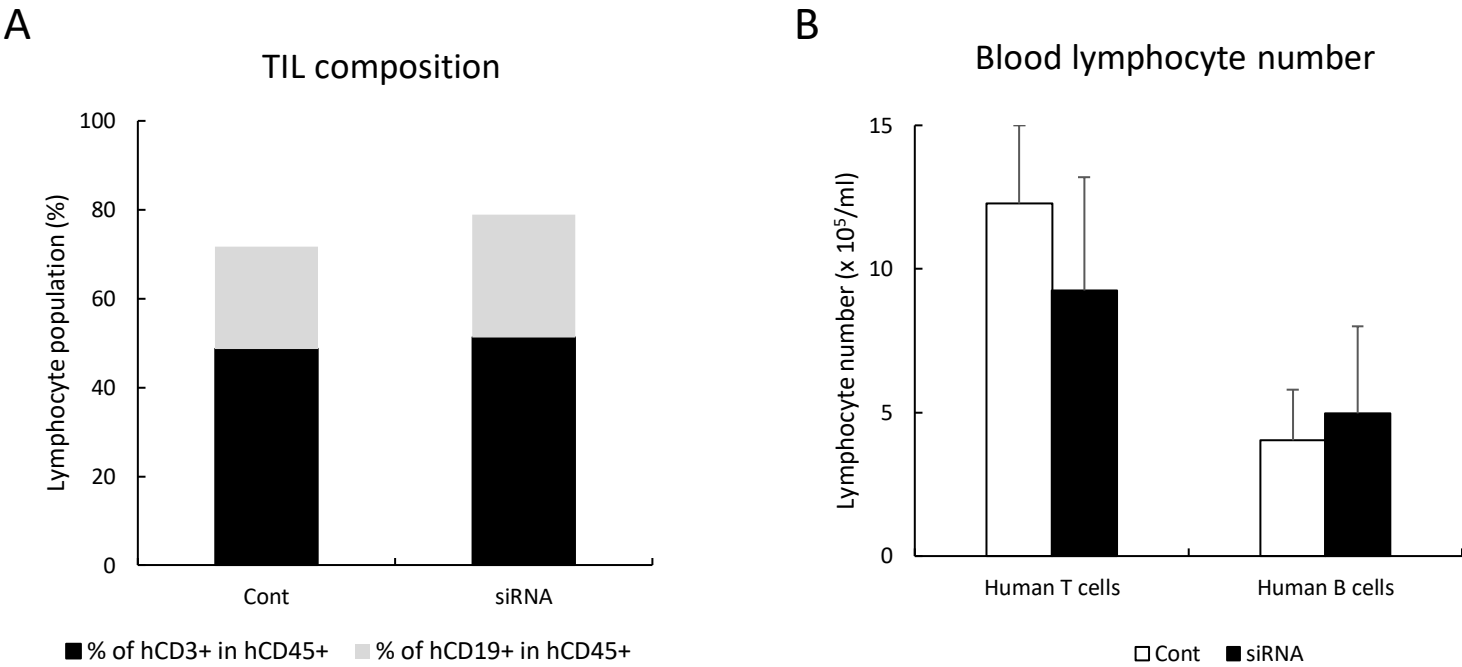


**Figure S2.** A. Graph for the body weight changes during the siPD-L1@PLGA treatment (in orange). B. Relative tumor volume of the individual mouse of control group (in blue) or siPD-L1@PLGA treated group (in orange). C. H&E staining of each tumor



**Figure S3.** Representative flow cytometry plots and gatings for the tumor infiltrated immune cell analysis. Panels in A represent the gating for Cd45+Cd3+ cells, whereas panels in B represent Cd45+Cd19+ cells.

Supplementary Figure 4

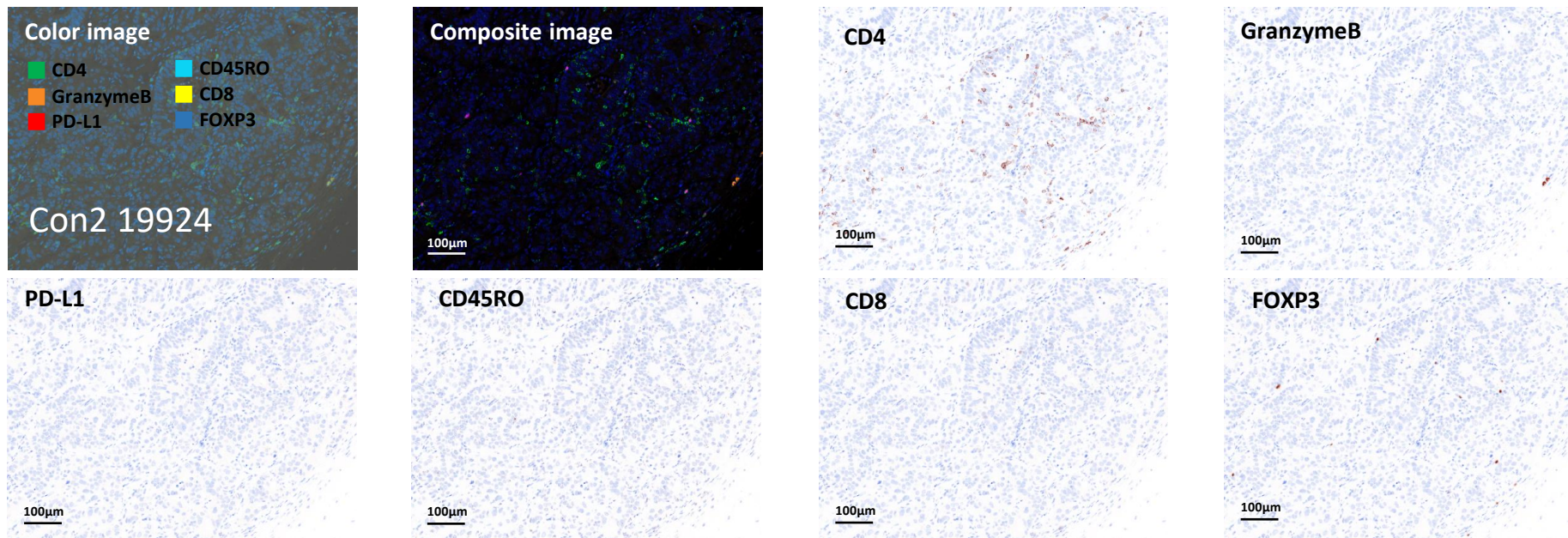


**Figure S4.** Human lymphocyte composition in tumor (A) and human lymphocyte count in the blood (B) of humanized NSG mice bearing PDAC tumors treated with vehicle or nano-PD-L1 siRNA. hCD45<sup>+</sup>hCD3<sup>+</sup> and hCD45<sup>+</sup>hCD19<sup>+</sup> represent human T lymphocytes and human B lymphocytes, respectively. Data are expressed as the mean  $\pm$  SE (n=4-5 mice/group).

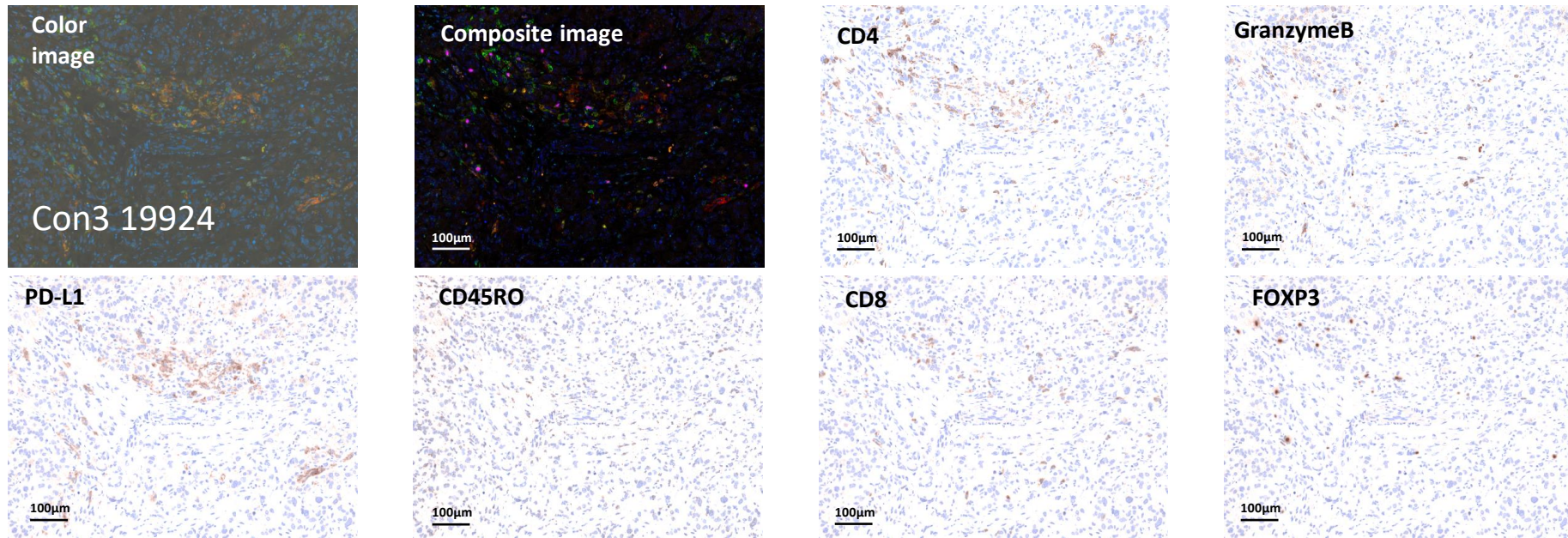


# Supplementary Figure 5

A



B



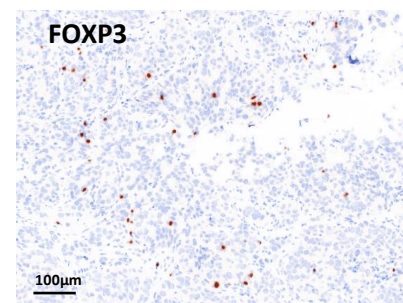
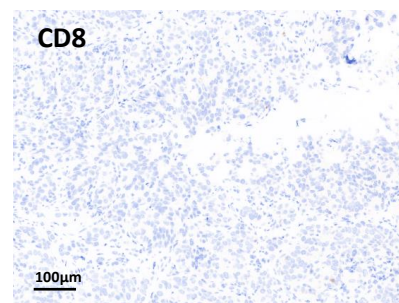
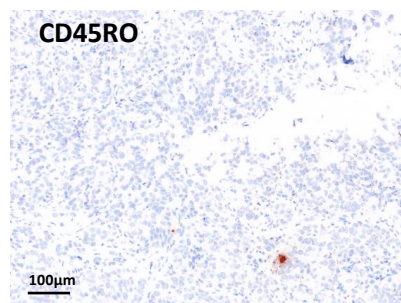
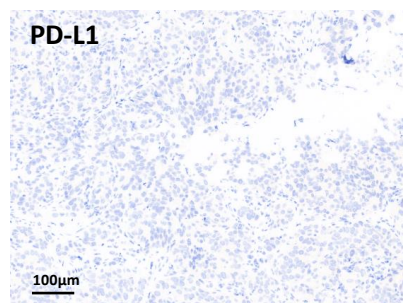
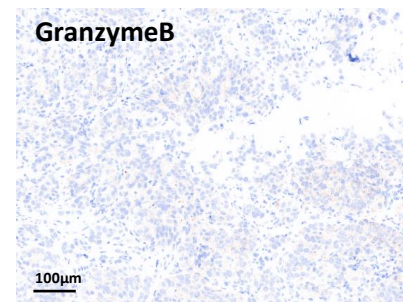
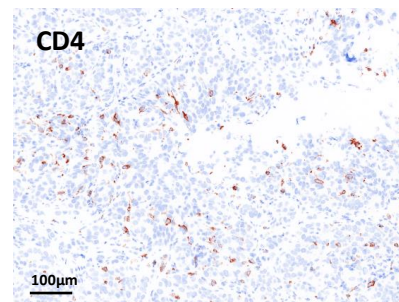
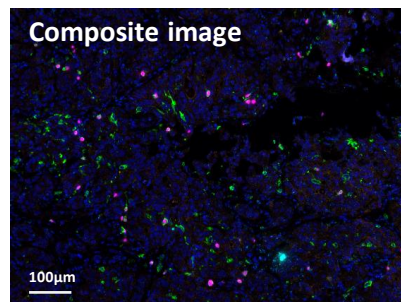
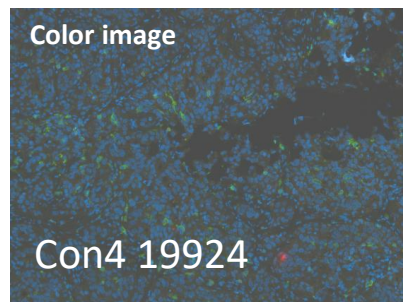
**Figure S5.** Raw data of OPAL images shown in Figure 5B and 5C. (A-D) present control tumors and (E-H) present siPD-L1 @PLGA treated tumors



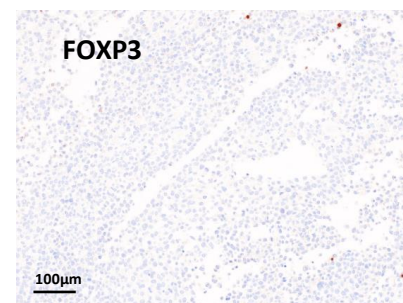
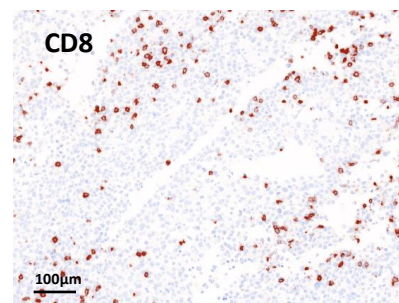
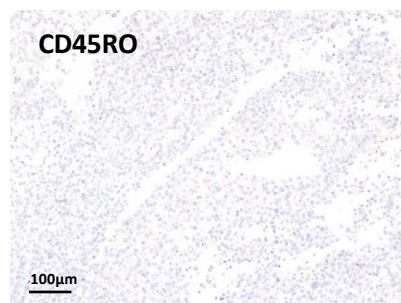
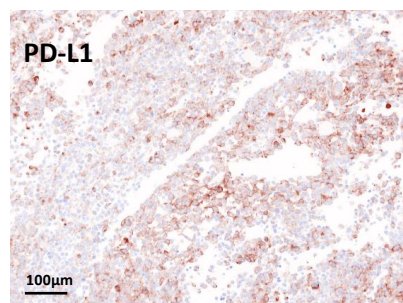
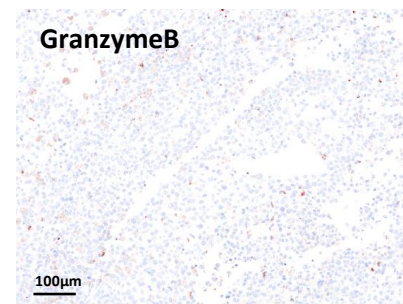
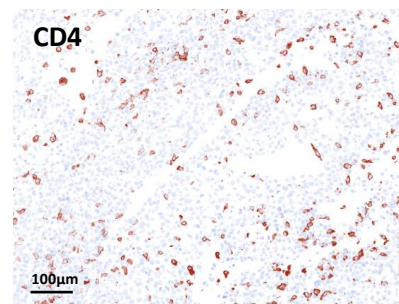
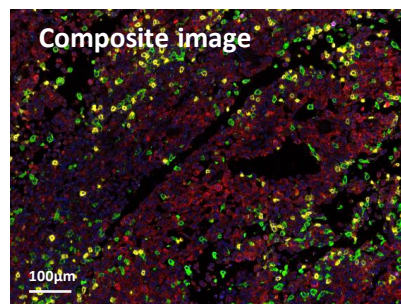
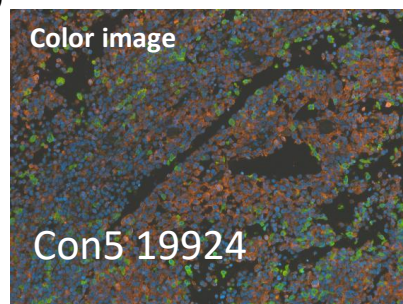
# Supplementary Figure 5

C

CD4  
GranzymeB  
PD-L1  
CD45RO  
CD8  
FOXP3



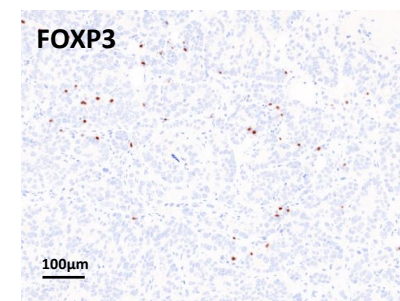
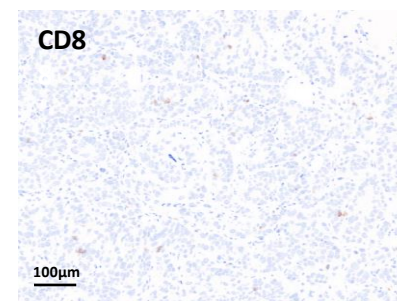
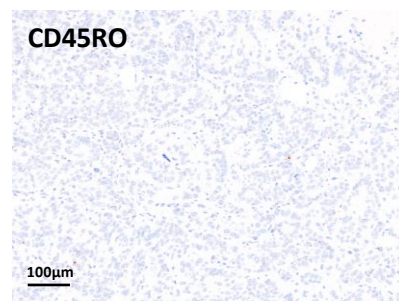
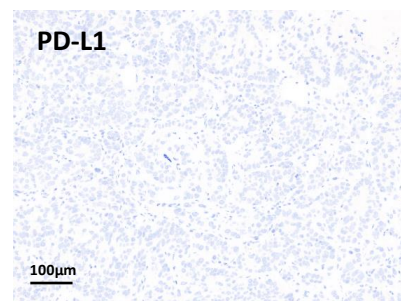
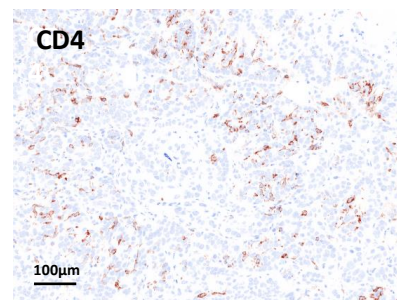
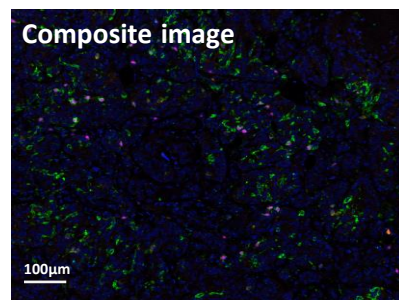
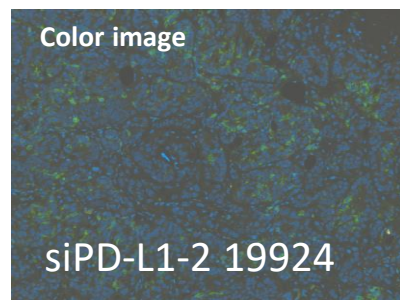
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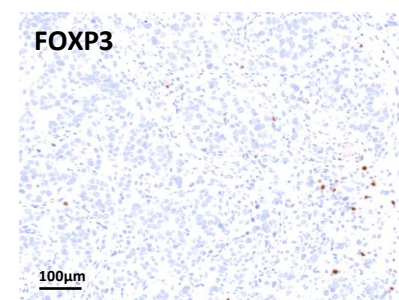
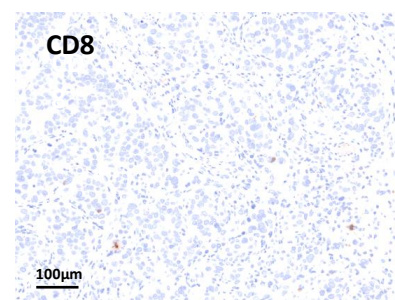
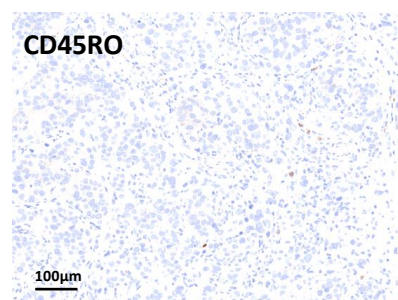
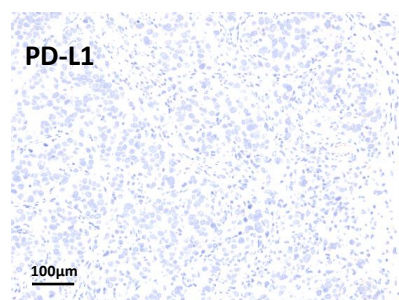
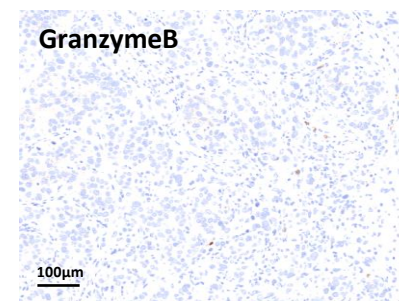
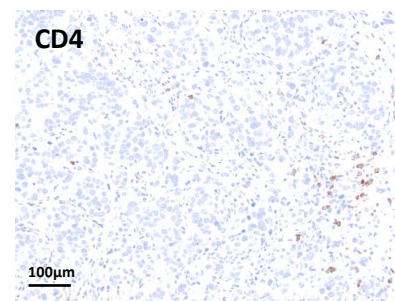
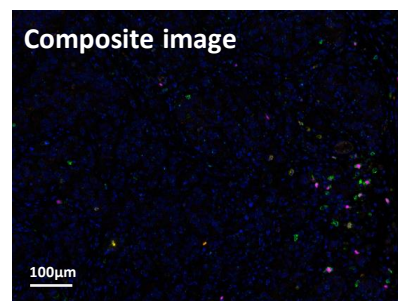


# E

# E



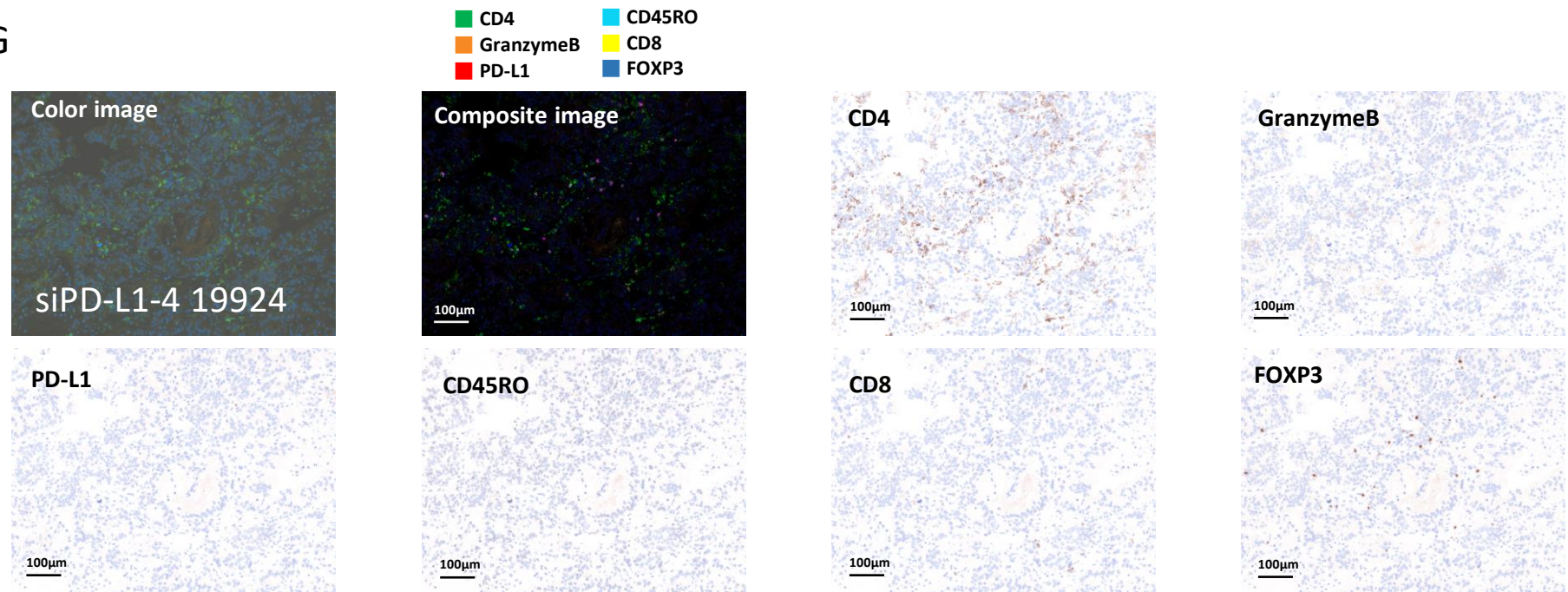
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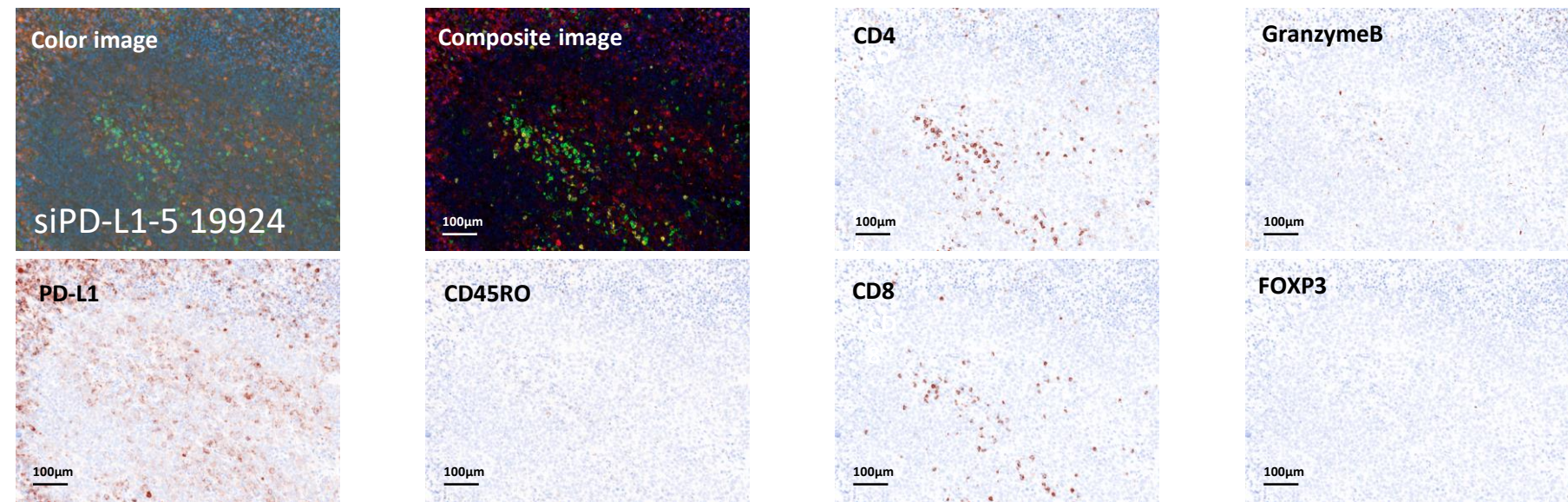


Supplementary Figure 5

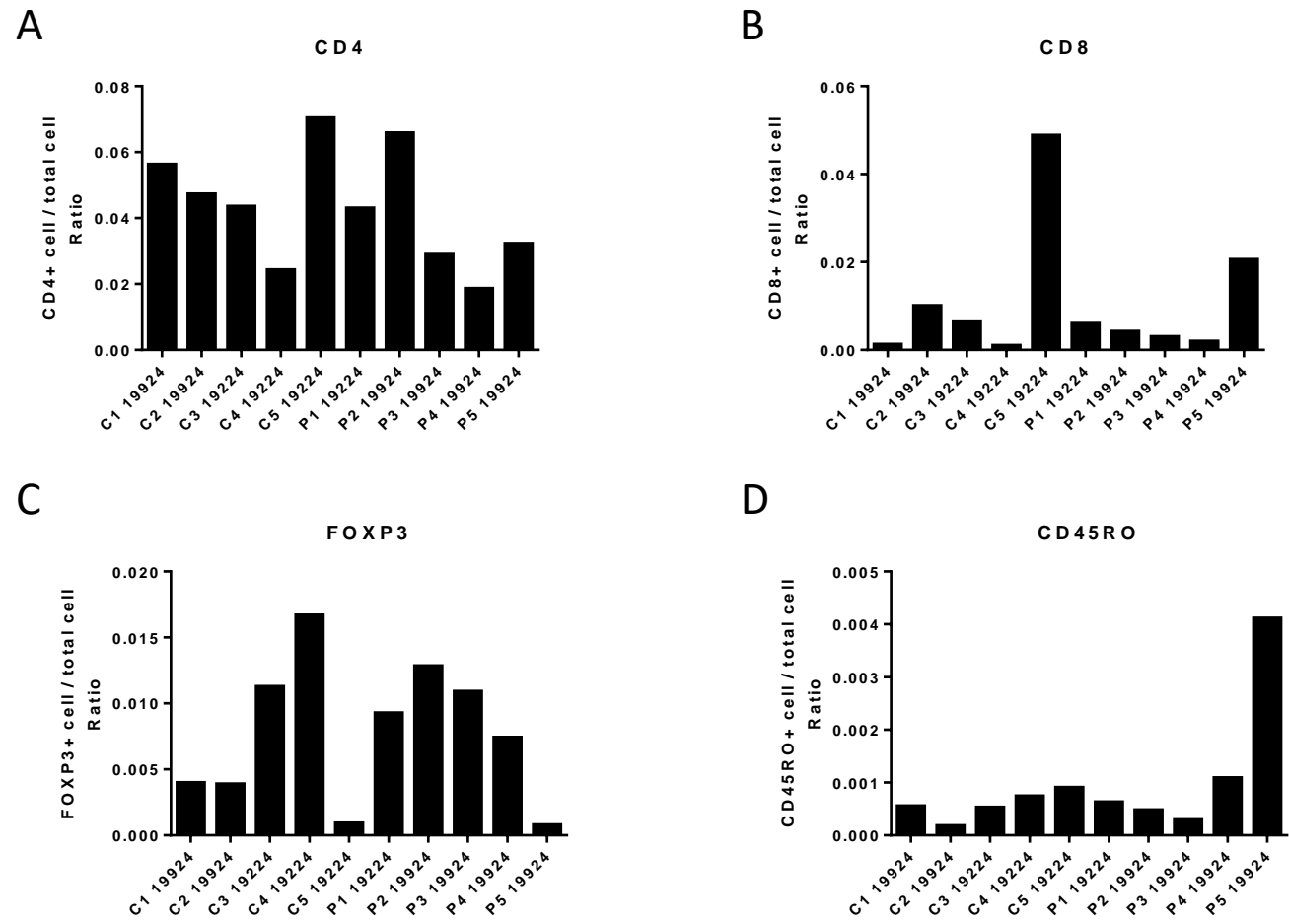
G



H



Supplementary Figure 6



**Figure S6.** The ratio of infiltrated immune cells in the individual PDAC tumors. CD4(A), CD8(B), FoxP3(C) and CD45RO(D) were analyzed. C1-C5 indicate control tumors whereas P1-P5 indicate siPD-L1@PLGA treated tumors



**Supplementary Table S1.** Density of immune cells in the blood of normal NSG and humanized NSG mice. Representative flow cytometry analysis of the percentage of human hematopoietic cells (hCD45+), human T lymphocytes (hCD45+hCD3+) and human B lymphocytes (hCD45+hCD19+) in the blood of humanized normal mice

Mouse	Total cells (/200ul)	hCD45+	hCD45+ hCD19+	hCD45+ hCD3+
NSG 1	8.6 x 10 <sup>5</sup>	1.1 %	1.0 %	1.0 %
NSG 2	8.5 x 10 <sup>5</sup>	0.9 %	0.4 %	0.7 %
Hu NSG 1	3.8 x 10 <sup>5</sup>	27.1%	21.7 % (80 %)	4.6 % (17 %)
Hu NSG 2	3.9 x 10 <sup>5</sup>	30.8 %	20.6 % (66.9 %)	8.1 % (26.3 %)