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Supplementary Information:

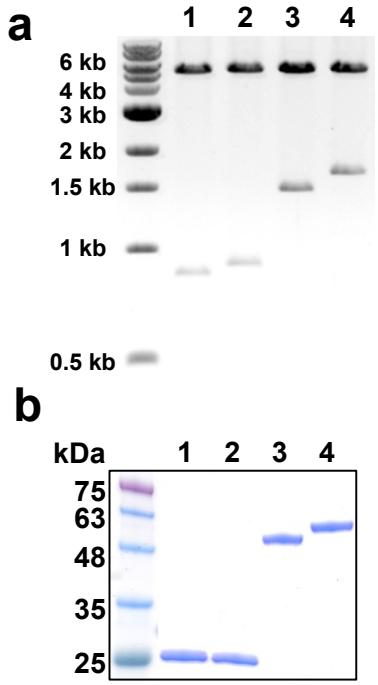


Figure S1: Analysis of the coding genes and the purified samples of the proteins used in the study.

(**a**) A photo of an agarose gel that contains the coding genes of αPD-1 (lane 1), ABD-PE (lane 2), αPD-1-PE (lane 3), and αPD-1-ABD-PE (lane 4). The lower bands of each lane were the coding genes after they were cleaved from the pET25b(+) vector (upper bands in each lane) by XbaI and BamHI. (**b**) An SDS-PAGE gel photo of purified αPD-1, ABD-PE, αPD-1-PE, and αPD-1-ABD-PE (5 μg of each sample). Lane 1: αPD-1, lane 2: ABD-PE, lane 3: αPD-1-PE, lane 4: αPD-1-ABD-PE. Studies described in this figure were repeated at least three times with similar results. Data of one repeat is shown here.

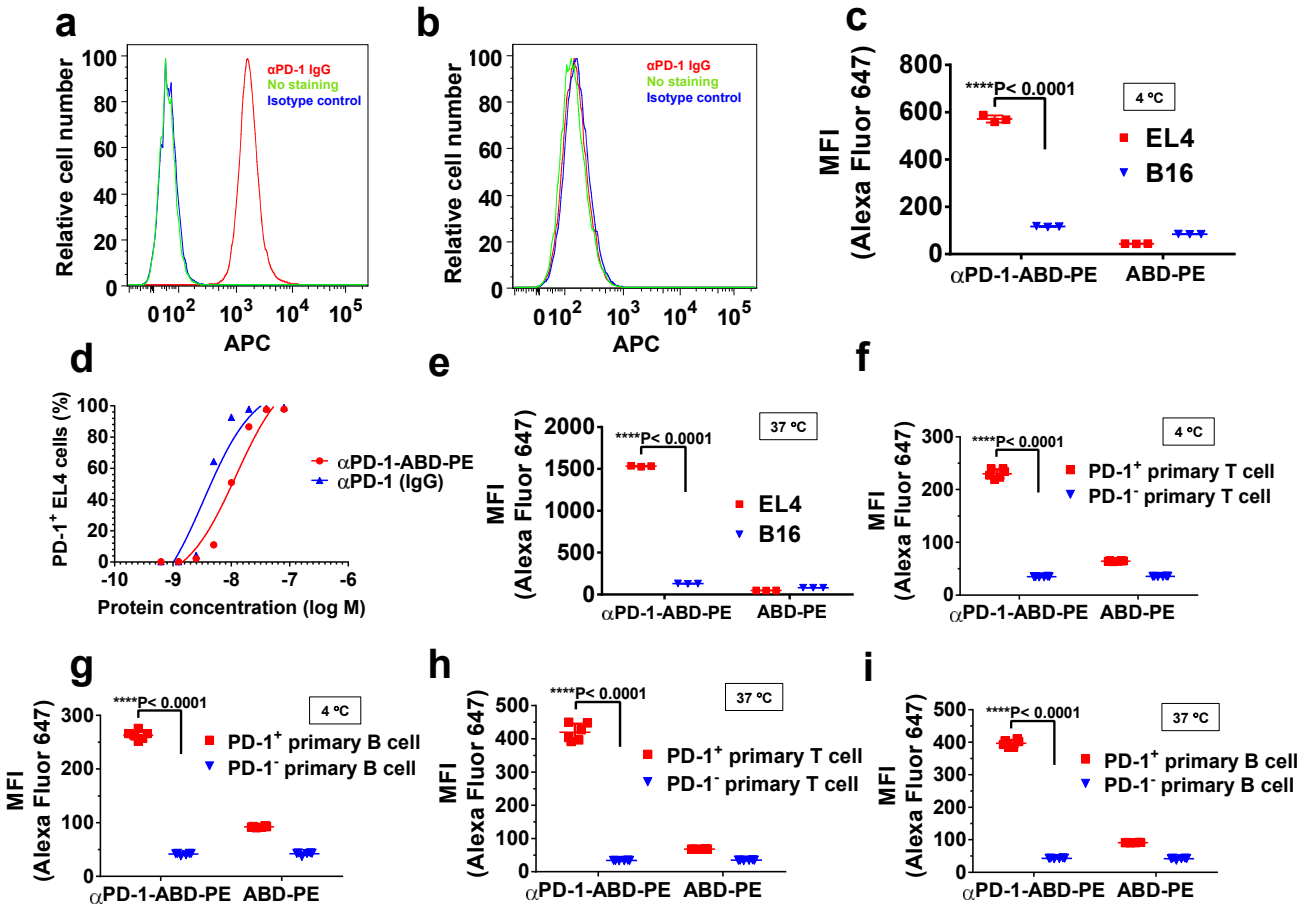


Figure S2. The selective binding and internalization of αPD-1-ABD-PE by PD-1$^+$ cells.

(a and b) Two representative histograms resulting from flow cytometry analyses of EL4 (a) and B16 cells (b). These cells were stained with either APC-labeled αPD-1 (full IgG, 100 nM, Red), APC-RatIgG2a isotype control (100 nM, Blue), or nothing (Green). Results reflected from these histograms suggest that the vast majority of EL4 cells express PD-1 on their surface, while almost no B16 cell express PD-1 on their surface. Studies described in the panels a-b were repeated at least three times with similar results. Data of one repeat is shown here. (**c**) The MFI of EL4 cells (PD-1$^+$) and B16 cells (PD-1$^-$) after the cells were incubated with Alexa Fluor 647-labeled αPD-1-

ABD-PE or Alexa Fluor 647-labeled ABD-PE at 4°C for 30 minutes. The cells were collected from NOD mice. The MFI means and their SDs are indicated (N=3 biologically independent samples; unpaired two-sided t-test). (**d**) A dose-response binding study of αPD-1-ABD-PE and αPD-1 (full IgG) with EL4 cells. 1 million EL4 cells was used for the assay at 4°C for 30 minutes. The binding affnity (Kd) were drived from sigmoidal dose-response analysis of the curves (N=6). (**e)** The MFI of EL4 and B16 cells after the cells were incubated with the labeled αPD-1-ABD-PE or the labeled ABD-PE at 37°C for 30 minutes. The MFI means and their SDs are indicated (N=3 biologically independent samples; unpaired two-sided t-test). (**f**) The MFI of PD-1^{+} and PD-1^{-} primary T cells after the cells were incubated with the labeled αPD-1-ABD-PE or the labeled ABD-PE at 4°C for 30 minutes. The cells were collected from NOD mice. The MFI means and their SDs are indicated (N=6 biologically independent samples; unpaired two-sided t-test). (**g**) The MFI of PD-1^{+} and PD-1^{-} primary B cells after the cells were incubated with the labeled αPD-1-ABD-PE or the labeled ABD-PE at 4°C for 30 minutes. The cells were collected from NOD mice. The MFI means and their SDs are indicated (N=6 biologically independent samples; unpaired two-sided t-test). **(h)** The MFI of PD-1^{+} and PD-1^{-} primary T cells after the cells were incubated with the labeled αPD-1-ABD-PE or the labeled ABD-PE at 37°C for 30 minutes. The cells were collected from NOD mice. The MFI means and their SDs are indicated (N=6). **(i)** The MFI of PD-1^{+} and PD-1^{-} primary B cells after the cells were incubated with the labeled αPD-1-ABD-PE or the labeled ABD-PE at 37°C for 30 minutes. The cells were collected from NOD mice. The MFI means and their SDs are indicated (N=6 biologically independent samples; unpaired two-sided t-test).

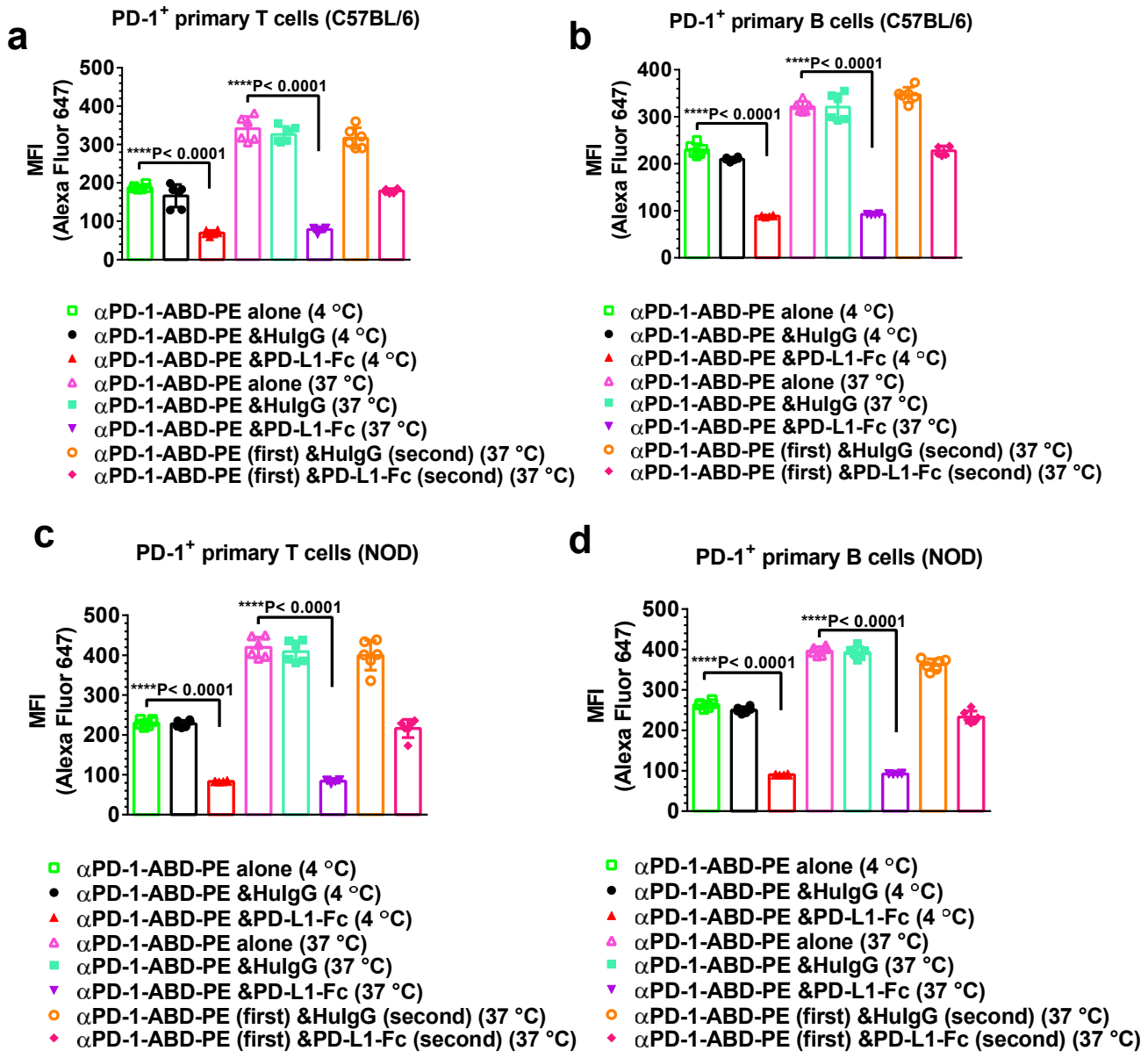


Figure S3. The selective binding and internalization of αPD-1-ABD-PE to PD-1$^+$ cells are mediated by PD-1.

(a-d) The MFI of PD-1$^+$ primary T cells (**a, c**) and PD-1$^+$ primary B cells (**b, d**) after the cells were incubated with Alexa Fluor 647-labeled αPD-1-ABD-PE under the conditions noted in the figure. The MFI means and their SDs are indicated (N=6 biologically independent samples; unpaired two-sided t-

test). Cells of “a” and “b” were collected from C57BL/6 mice; cells of “c” and “d” were collected from NOD mice.

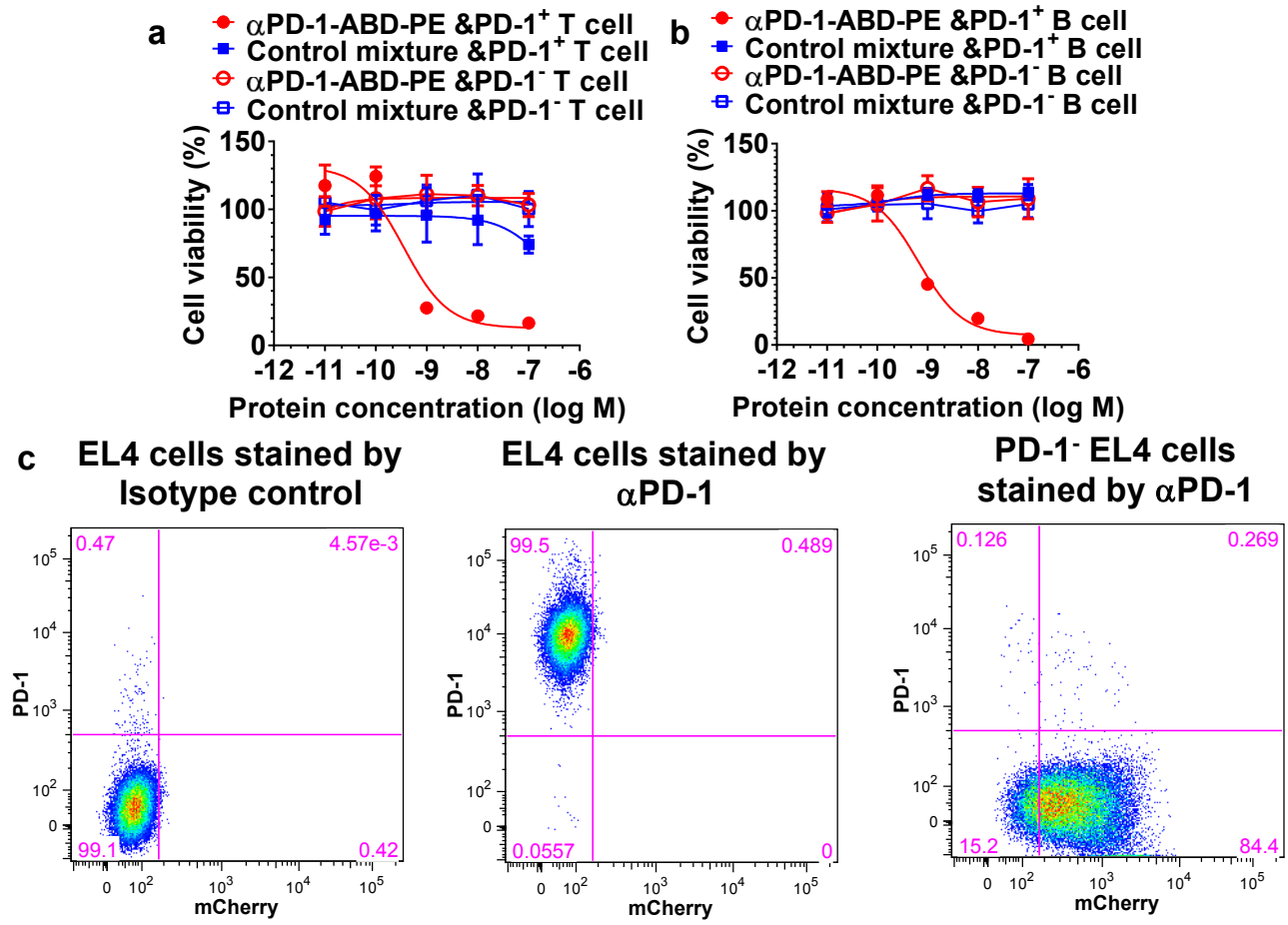
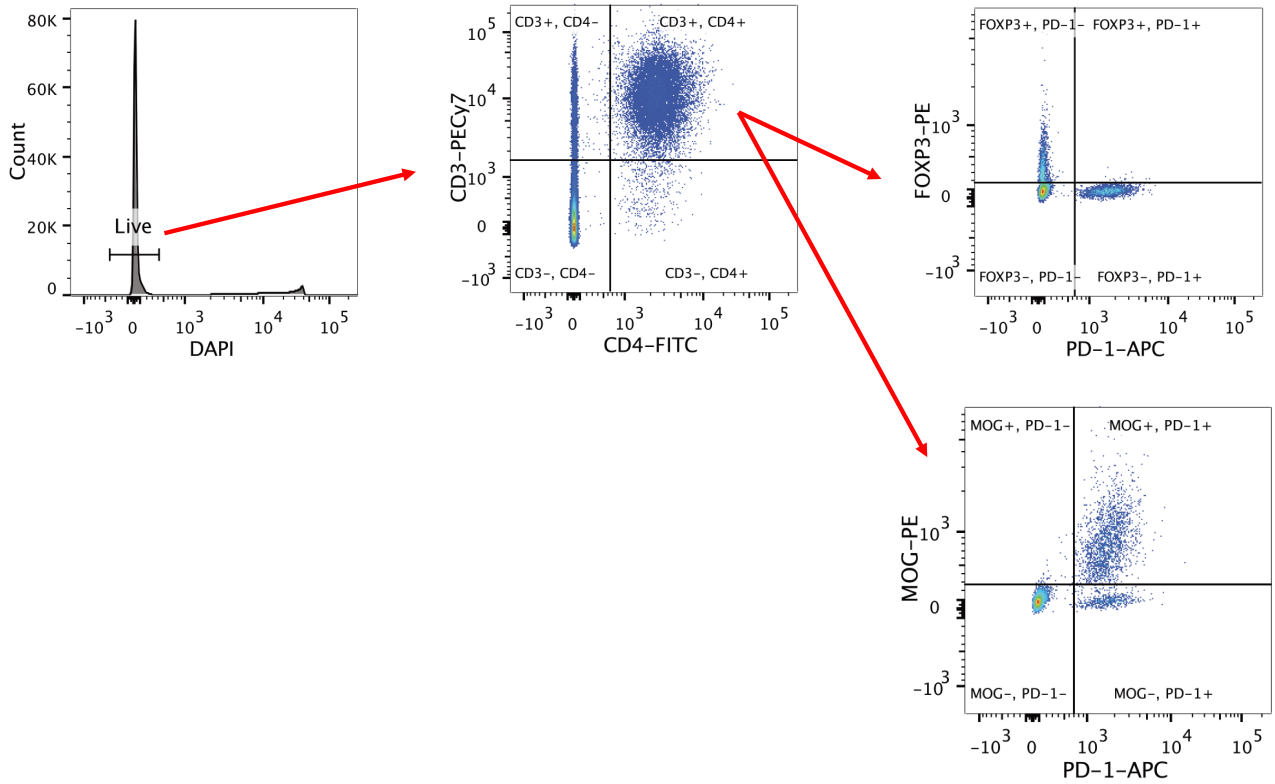


Figure S4. αPD-1-ABD-PE possesses selective toxicity to primary PD-1$^+$ lymphocytes *in vitro.*

(a-b) The relative viability of PD-1$^+$ and PD-1$^-$ primary T cells (**a**) and B cells (**b**) after they were incubated with αPD-1-ABD-PE or a control mixture of αPD-1 and ABD-PE for 72 hours. The mean viabilities and their SDs at different concentrations of αPD-1-ABD-PE and the control mixture were shown. The viability data of PD-1$^+$ primary cells after the αPD-1-ABD-PE treatment were fitted to a sigmoidal dose-response model (N=6 biologically independent samples; unpaired two-sided t-test) and the IC_{50} was obtained through the fitting. The cells were collected from NOD mice. (**c**) Representative scatterplots showing the PD-1 expression on EL4 and PD-1$^-$ EL4 cells. mCherry is the transfection marker. Left: EL4 cells stained with isotype control; middle: EL4 cells stained with αPD-1 (Clone:

RMP1-30); right: PD-1- EL4 cell stained with αPD-1. Studies described in panel were repeated at least three times with similar results.

a


Count
80K
60K
40K
20K
0
Live
DAPI
CD3+, CD4−
CD3+, CD4+
CD3−, CD4−
CD3−, CD4+
CD3-PECy7
CD4-FITC
FOXP3+, PD-1−
FOXP3+, PD-1+
FOXP3−, PD-1−
FOXP3−, PD-1+
FOXP3-PE
PD-1-APC
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MOG+, PD-1+
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MOG-PE
PD-1-APC

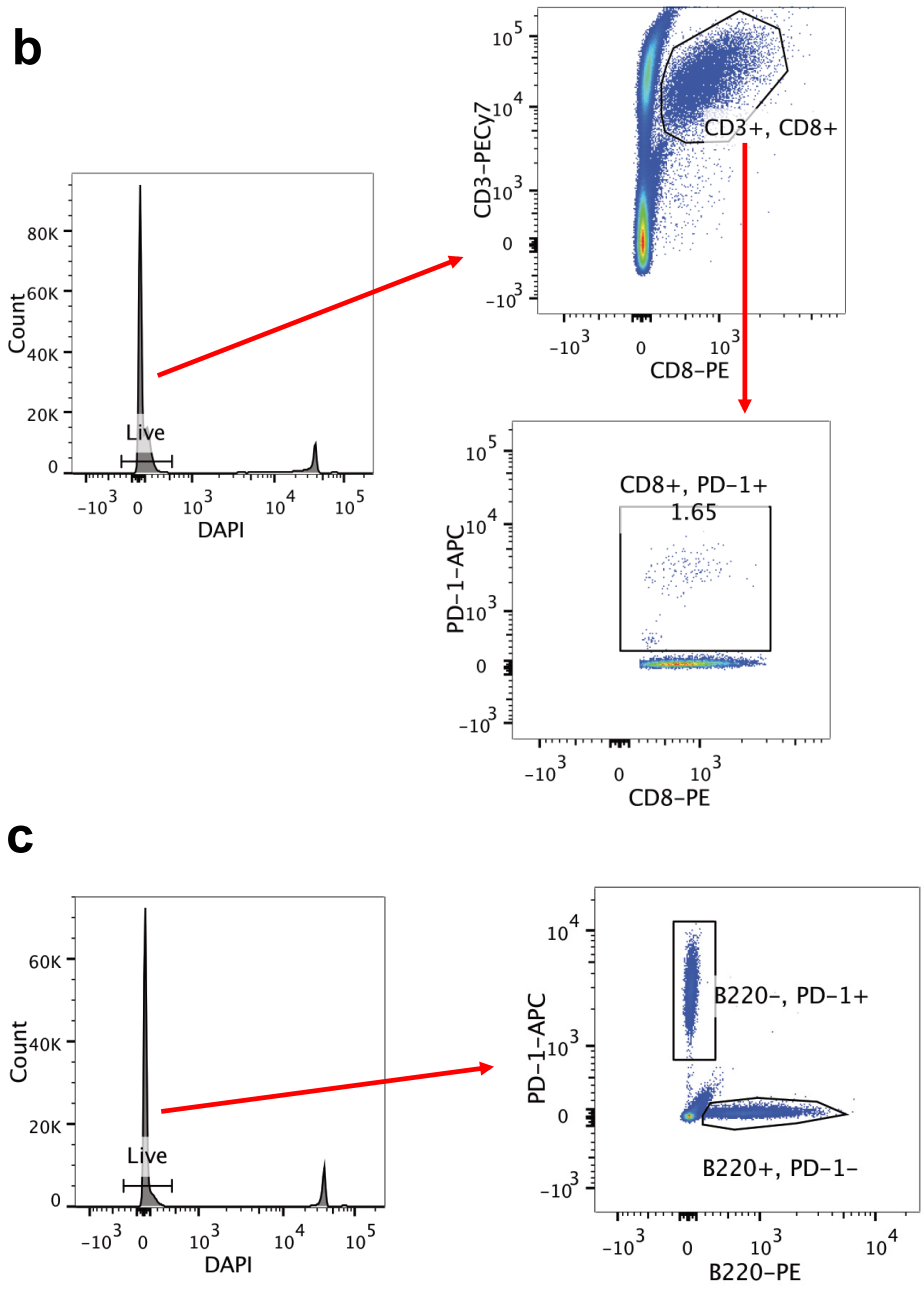

Figure S5. Gating strategies used to quantify PD-1+ CD4 T cells, Tregs, MOG_{38-49}-specific CD4 T cells.

(**a**), PD-1+ CD8 T cells (**b**), and PD-1+ B220 cells (**c**) by flowcytometry. Dead cells were first stained by 3 μM DAPI for 10 min, and then washed 3 times by a centrifugation (300 g for 5 minutes) to remove free DAPI. Then, cells were stained for markers indicated in the figures by antibodies.

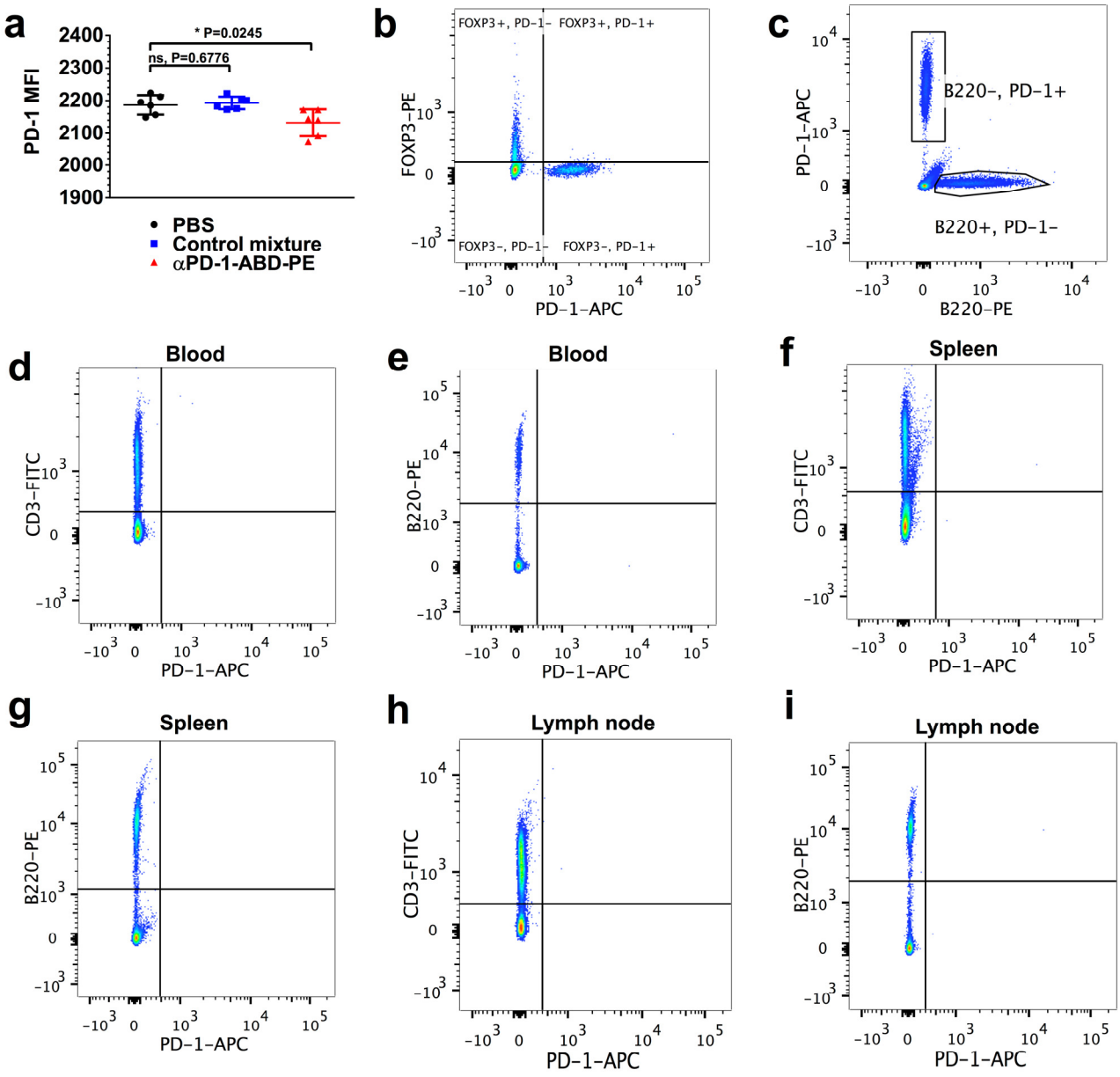

Figure S6. Analysis of immune cells in NOD mice after the αPD-1-ABD-PE treatment.

(a) The MFI (PD-1 expression) of PD-1$^+$ cells in pancreases of 18-week old NOD mice after these mice were treated with one dose of αPD-1-ABD-PE, a control mixture of αPD-1 and ABD-PE, or PBS. Each dot represents the MFI of the cells from a single mouse. The MFI means and their SDs are indicated. (N=6 mice; unpaired two-sided t-test). **(b-c)** Representative scatter plots for Tregs (**b**) and B cells (**c**) in

a pancreas that was collected from the NOD mice treated with PBS. The cells were stained with αPD-1 to show PD-1$^+$ populations. There was no PD-1$^+$ Treg or PD-1$^+$ B cell population in the pancreases. The plots represent 6 mice that were treated with PBS. Results for the mice treated with αPD-1-ABD-PE and the control mixture were the same. (**d-i).**Representative scatter plots for T cells (**d, f, h**) and B cells (**e, g, i**) in blood (**d, e**), spleens (**f, g**), and lymph nodes (**h, i**) that was collected from NOD mice treated with PBS. The cells were stained with αPD-1 to reveal PD-1$^+$ populations. There was no PD-1$^+$ T or PD-1$^+$ B cell population in these samples. The plots represent 6 mice that were treated with PBS. Results for the mice treated with αPD-1-ABD-PE and the control mixture were the same.

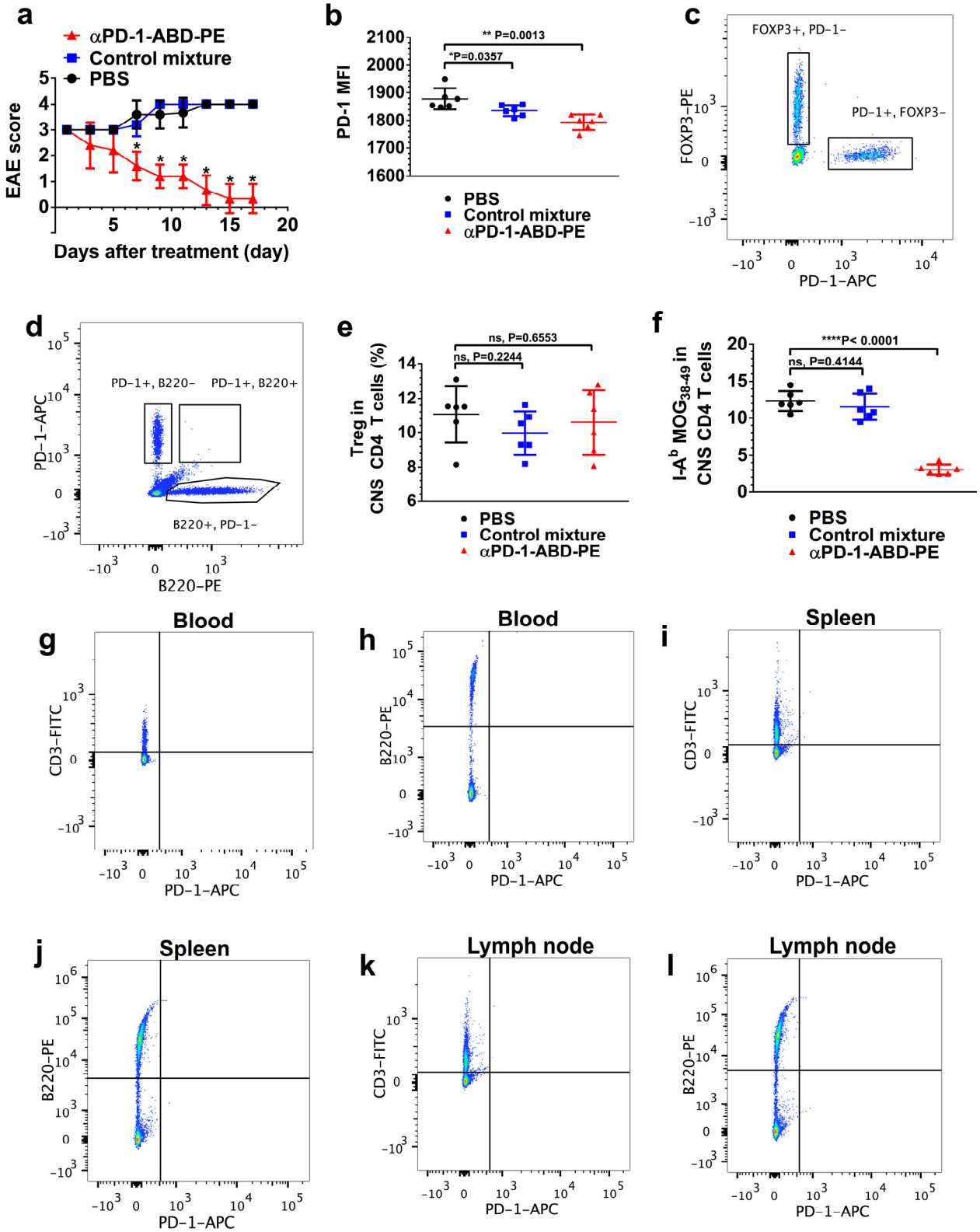
a
αPD-1-ABD-PE
Control mixture
PBS
EAE score
Days after treatment (day)
b
** P=0.0013
*P=0.0357
PD-1 MFI
PBS
Control mixture
αPD-1-ABD-PE
c
FOXP3+, PD-1−
PD-1+, FOXP3−
FOXP3-PE
PD-1-APC
d
PD-1+, B220−
PD-1+, B220+
B220+, PD-1−
PD-1−APC
B220-PE
e
ns, P=0.6553
ns, P=0.2244
Treg in CNS CD4 T cells (%)
PBS
Control mixture
αPD-1-ABD-PE
f
****P< 0.0001
ns, P=0.4144
I-Ab MOG38-49 in CNS CD4 T cells
PBS
Control mixture
αPD-1-ABD-PE
g
Blood
CD3-FITC
PD-1-APC
h
Blood
B220-PE
PD-1-APC
i
Spleen
CD3-FITC
PD-1-APC
j
Spleen
B220-PE
PD-1-APC
k
Lymph node
CD3-FITC
PD-1-APC
l
Lymph node
B220-PE
PD-1-APC

Figure S7. Analysis of immune cells in mice with EAE after the αPD-1-ABD-PE treatment.

(a) The EAE score changes of mice that were treated with one dose of αPD-1-ABD-PE, a control mixture of αPD-1 and ABD-PE, or PBS. The shown data are mean EAE clinical scores and their standard deviations at each observation time point after the induction of EAE. (N=5 mice; *P ≤ 0.01; unpaired two-sided t-test). The X-axis represents the number of days after treatment started for an individual mouse. **(b)** The MFI (PD-1 expression) of PD-1^+ cells in the CNS of the mice that were treated with one dose of αPD-1-ABD-PE, a control mixture of αPD-1 and ABD-PE, or PBS. Each dot represents the MFI of the cells from a single mouse. The MFI means and their SDs are indicated. (N=6 mice; unpaired two-sided t-test). **(c-d).** Representative scatter plots for Tregs (**c**) and B cells (**d**) in the CNS of mice with EAE and treated with PBS. The cells were stained with αPD-1 to show PD-1^+ populations. There was no PD-1^+ Treg or PD-1^+ B cell population in the CNS. The plots represent 6 mice that were treated with PBS. Results for the mice treated with αPD-1-ABD-PE and the control mixture were the same. **(e-f)** The fraction of Tregs cells (d) and MOG_{38-49}-specific CD4 T cells (e) in the collected mononuclear cells from the CNS of the mice that were treated with one dose of αPD-1-ABD-PE, a control mixture of αPD-1 and ABD-PE, or PBS. Each dot represents the fraction result of a single mouse. The fraction means and their SDs are indicated. (N=6 mice; unpaired two-sided t-test). (**g-l**) Representative scatter plots for T cells (**g, i, k**) and B cells (**h, j, l**) in blood (**g,h**), spleens (**i, j**), and lymph nodes (**k,l**) that was collected from the mice with EAE and treated with PBS. The cells were stained with αPD-1 to reveal PD-1^+ populations. There was no PD-1^+ T or PD-1^+ B cell population in these samples. The plots represent 6 mice that were treated with PBS. Results for the mice treated with αPD-1-ABD-PE and the control mixture were the same.

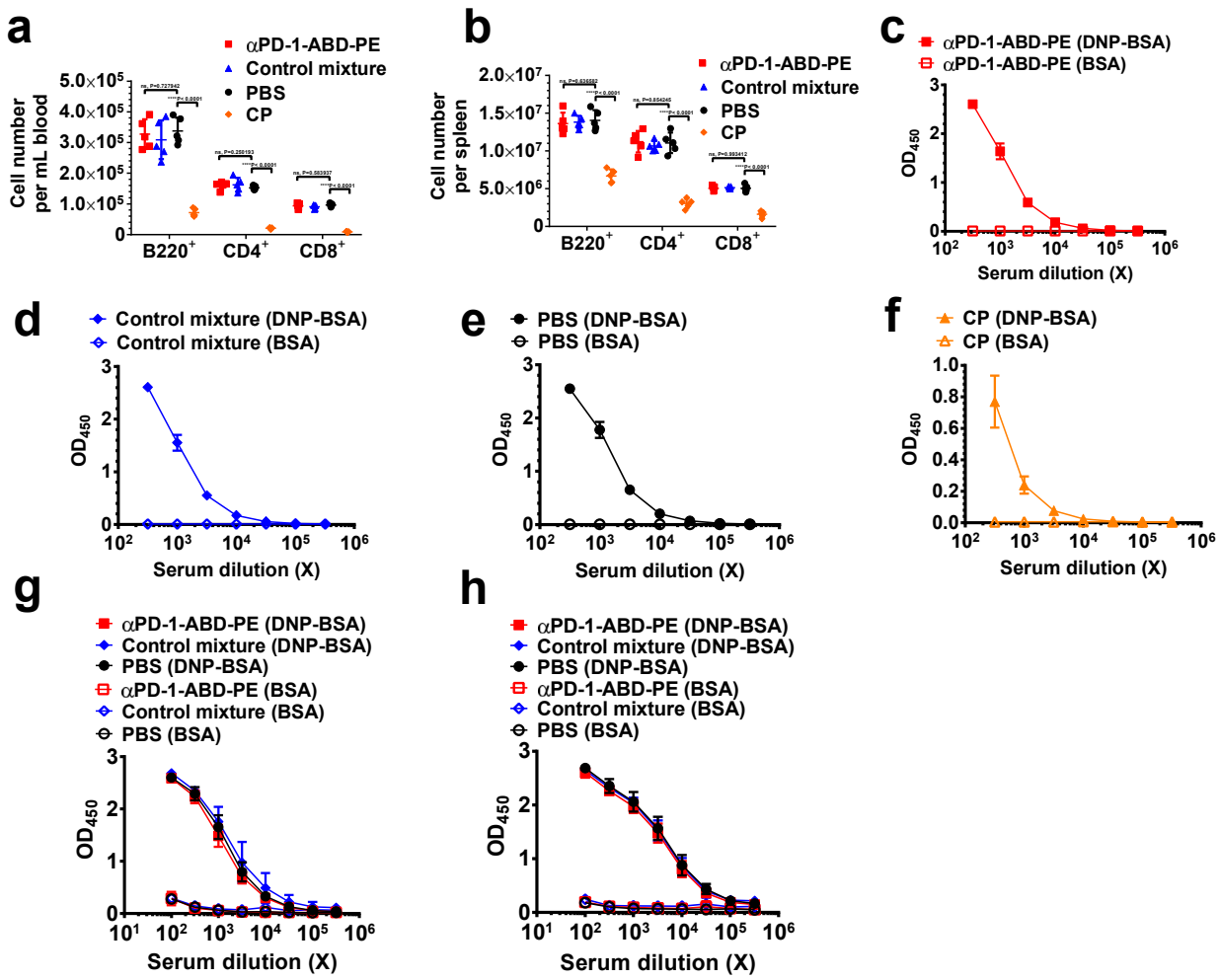

Figure S8. Analysis of normal adaptive immunity in mice after the αPD-1-ABD-PE treatment.

(a-b) B220+, CD4+, CD8+ cell numbers in blood (a) and spleens (b) in the NOD mice that were treated with one dose of αPD-1-ABD-PE, a control mixture of αPD-1 and ABD-PE, PBS, or CP. The number means and their SDs are indicated (N=6 mice; unpaired two-sided t-test). **(c-f)** The data curves in Figure 6c are separated into four subfigures based on the treatments. The subfigures are prepared to show curve details that are hidden due the overlap of these curves (N=6 mice). **(g-h)** ELISA results of the anti-DNP humoral responses in the C57BL/6 mice (**g**) and the NOD mice (**h**) that that were pre-treated with five doses of αPD-1-ABD-PE, a control mixture of αPD-1 and ABD-PE, or PBS. The results were measured by OD_{450} after a background OD_{570} subtraction. The mean±SD of OD_{450} for the serum

samples at indicated dilutions were shown. The same samples were loaded into both DNP-BSA-coated and BSA-coated (control) ELISA plates, separately. The materials used to coat the plates are written in the parentheses (N=5 mice; unpaired two-sided t-test).