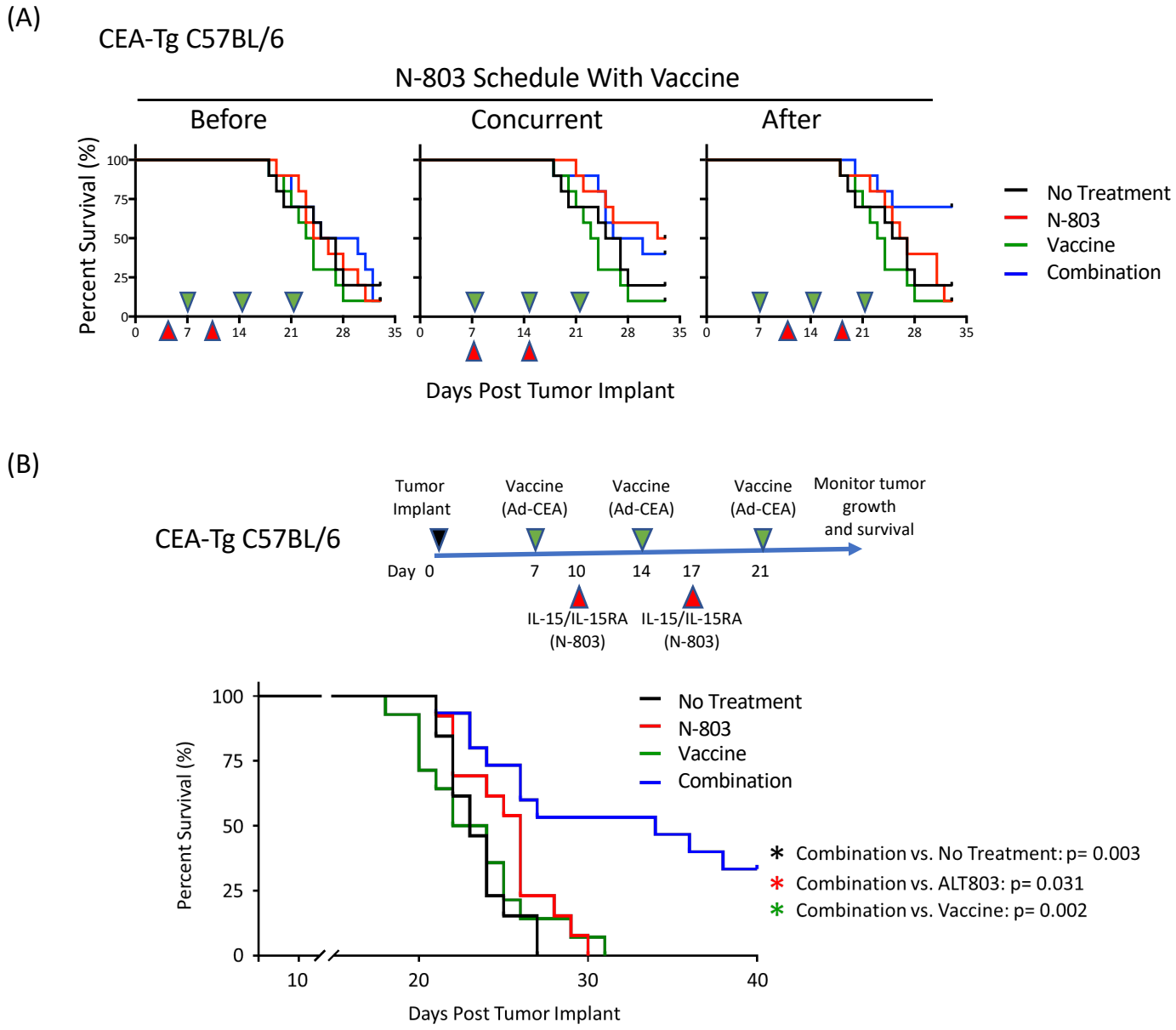
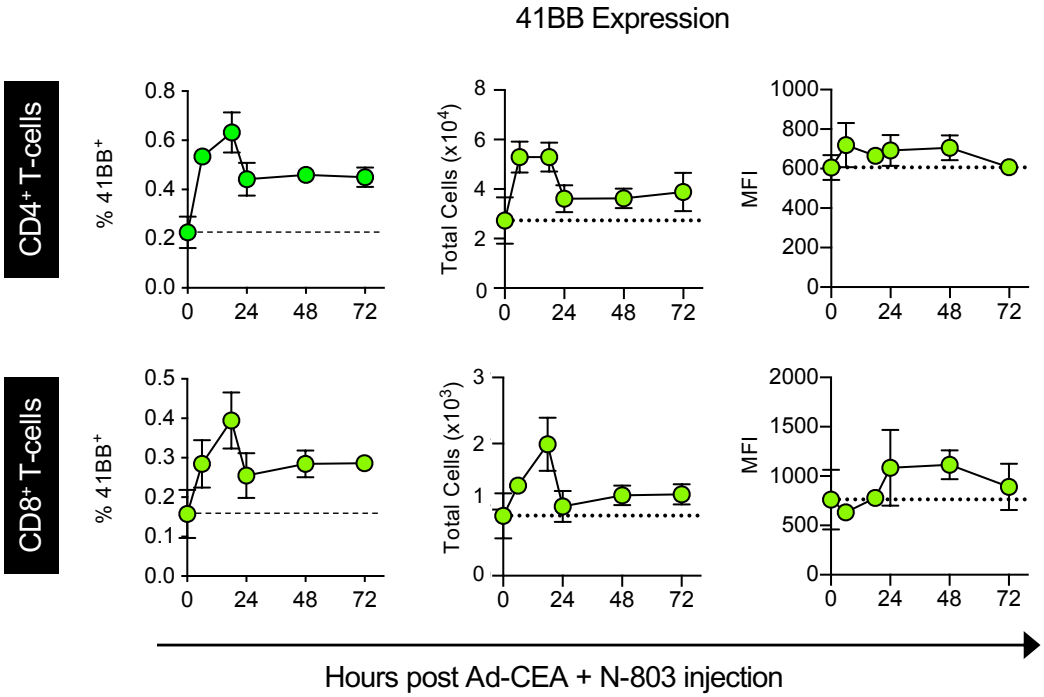
Supplementary Figure S1



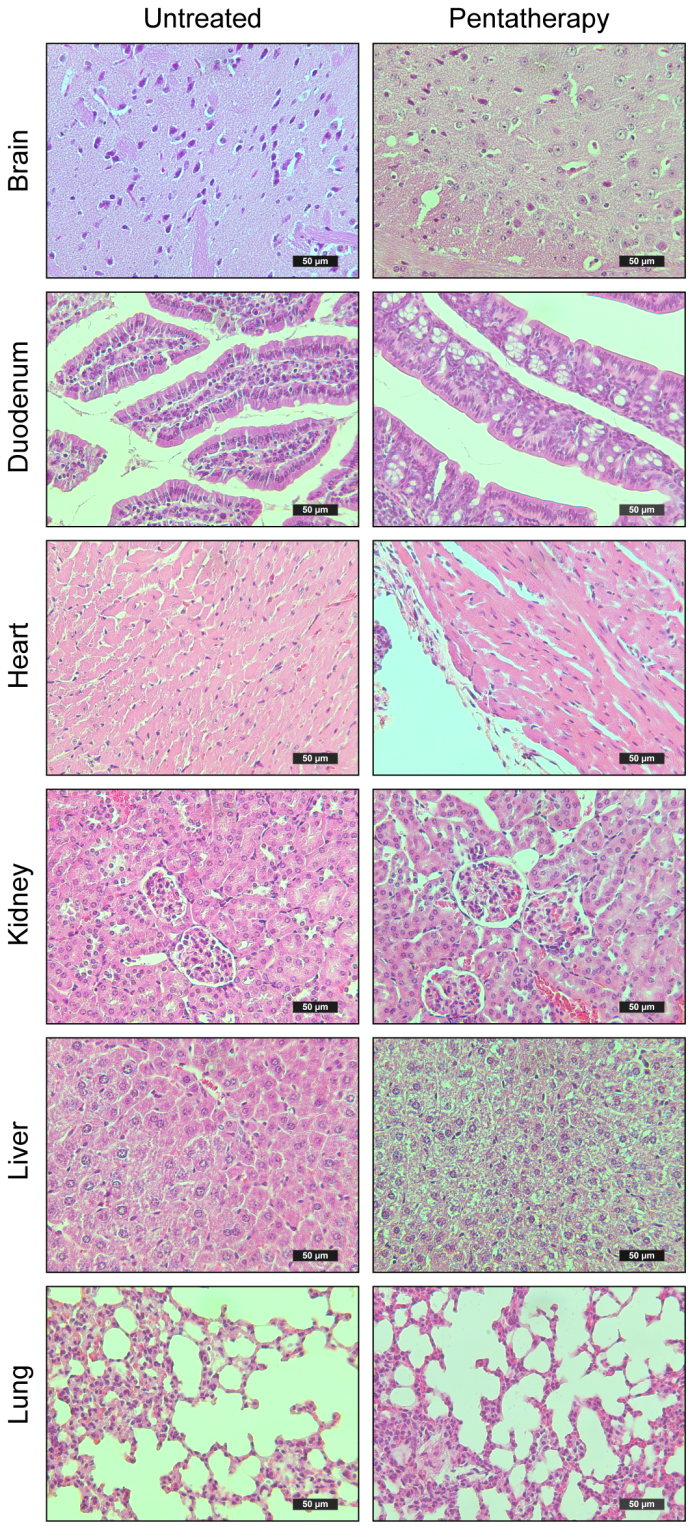
Supplemental Figure 1.
Timing of N-803 in relation to vaccine is crucial to antitumor activity and overall survival in a self-antigen CEA-Tg tumor model. (A) CEA-Tg C57BL/6 mice (n=10/group) were implanted on day 0 with MC38-CEA tumor cells. Mice were administered Ad-CEA vaccine (red triangles) on days 7, 14 and 21. Subgroups were administered N-803(green triangles) either before vaccine (on days 4, and 11), concurrent with vaccine (days 7 and 14), or after vaccine (days 10 and 17). Depicted are Kaplan-Meier survival curves. **(B)** N-803 administered after vaccine significantly improves overall survival. CEA-Tg C57BL/6 mice (n=10/group) were implanted on day 0 with MC38-CEA tumor cells. Mice were administered Ad-CEA vaccine on days 7, 14 and 21. N-803 was administered after vaccine (days 10 and 17). Depicted are Kaplan-Meier survival curves.

Supplementary Figure S2



Supplemental Figure 2. The combination of Ad-CEA and N-803 temporally upregulates 4-1BB expression in CD4⁺ and CD8⁺ splenocytes. Naive female C57BL/6-CEA-Tg mice were injected concurrently with Ad-CEA (s.c.) and N-803 (s.c.). Spleens were collected 0, 8, 18, 24, 48, and 72 hours after the injection. Frequency, total cell number, and MFI of 4-1BB on CD4⁺ and CD8⁺ splenocytes were analyzed via flow cytometry. s.c., subcutaneous.

Supplementary Figure S3



Supplementary Figure S3. Histopathology of organ tissues show minimal toxicity in the MC38-CEA-bearing mice treated with the Ad-CEA, N-803, OX40, GITR, an IDOi combination (pentatherapy). Brain, duodenum, heart, kidney, liver, and lungs were collected from MC38-CEA-bearing mice in the untreated and pentatherapy-treated cohorts. H&E slides were prepared and examined for abnormalities and signs of combination-related toxicities. H&E, hematoxylin and eosin.

Supplementary Table S1

Test	Normal ranges	None[a] (n = 5)	Pentatherapy[b] (n = 5)
Weight (g)[c]	18-26	25.3± 2.1	24.5 ± 2.2
Blood assays[d]			
WBC (K/ul)	5.3-14.8	6.2	7.2
LY (K/ul)	3.5-11.7	5.1	4.5
MO (K/ul)	0.3-1.4	0.3	0.3
EO (K/ul)	0.01-0.3	0.10	0.12
BA (K/ul)	0.0-0.2	0.02	0.01
LY (%)	53.0-84.2	55.4	55.1
MO (%)	3.7-14.6	5.2	4.5
EO (%)	0.1-4.0	3.3	2.0
BA (%)	0.0-1.6	0.7	0.3
RBC (M/ul)	8.0-11.8	10.3	7.6
Hb (g/dl)	12.6-19.2	15.3	12
HCT (%)	43.5-67.3	50.3	61.4
MCV (fl)	50.8-64.2	58.9	56.9
PLT (K/ul)	390-1633	834	780
Serum assays[e]			
BUN (mg/dl)	6-32	17	14
CRE (mg/dl)	0.2-0.7	0.1	0.1
AST (u/l)	67-393	269	366
ALT (u/l)	35-172	85	133
ALK (u/l)	65-399	74	130
TPR (g/dl)	4.9-7.8	5.5	6.4

Twelve week-old CEA-Tg mice were implanted with MC38-CEA tumors on day 0. Mice received [a]no treatment or were treated with [b]anti-GITR on day 5, vaccine (ad-CEA), N803, and anti-OX40 on days 7, 14, and 21, and daily epacadostat (in chow) beginning on day 7 (pentatherapy). [c]Weights were measured on day 28. [d]Blood was drawn on day 28 and equal-volume pooled. [e]Serum was drawn on day 28 and equal-volume pooled.

Supplementary Table S1.
Blood analysis show minimal toxicity in the MC38-CEA-bearing mice treated with the Ad-CEA, N-803, OX40, GITR, and IDOi combination (pentatherapy).
Blood and serum were collected and pooled from pentatherapy-treated animals (n=3) and analyzed for blood cells and liver enzymes, respectively.