Supporting materials for

# Synergistic and Low Adverse Effect Cancer Immunotherapy by Immunogenic Chemotherapy and Locally Expressed PD-L1 Trap

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# Synergistic and Low Adverse Effect Cancer Immunotherapy by Immunogenic Chemotherapy and Locally Expressed PD-L1 Trap

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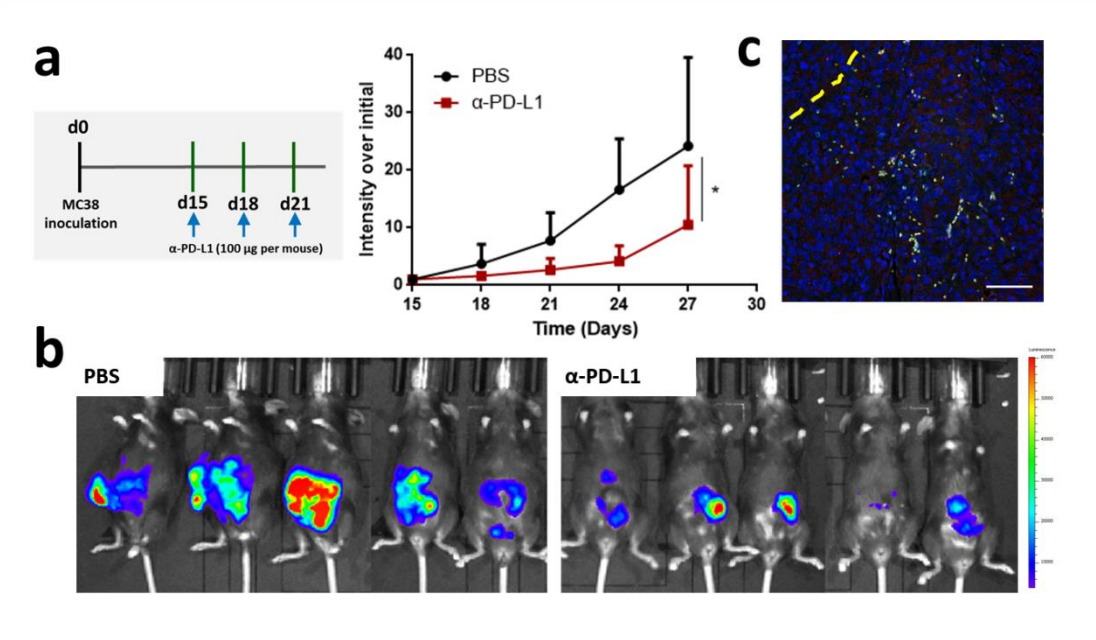
**Supplementary file includes:**

Supplementary Figures 1 to 13.

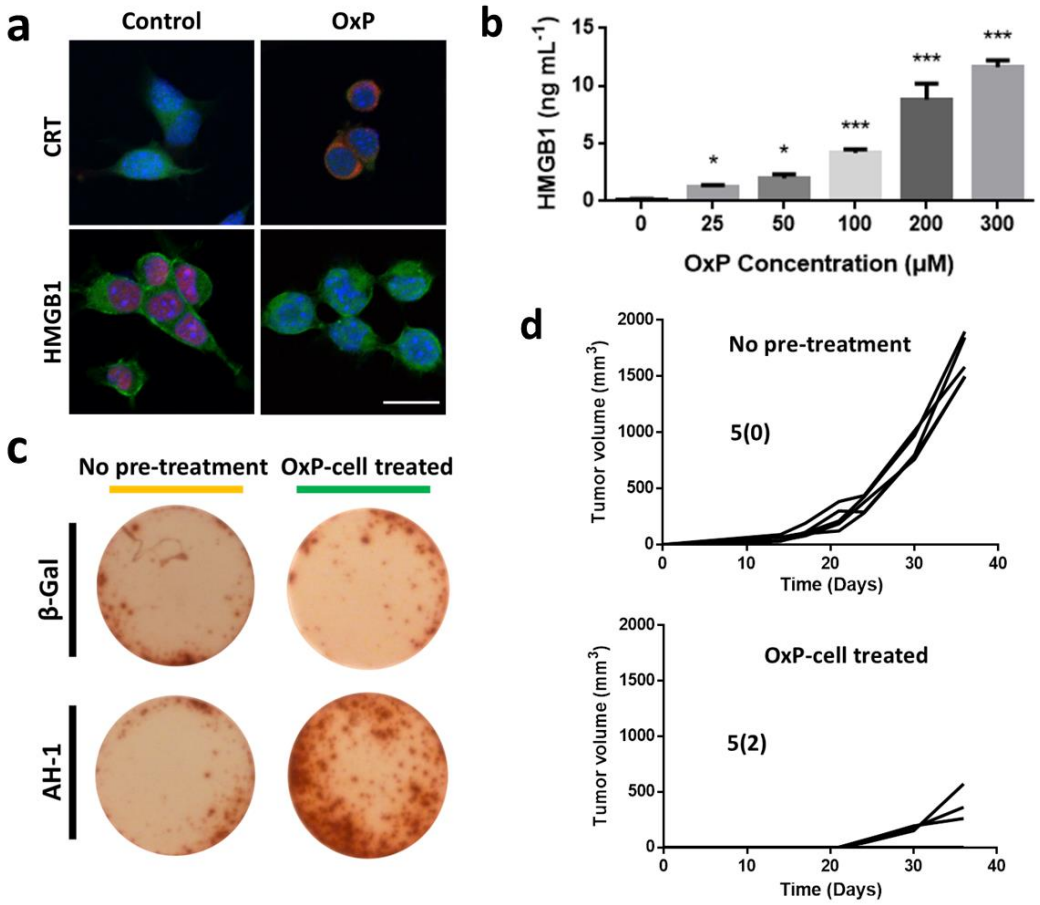
Supplementary Tables 1 to 2.

Supplementary Note 1.

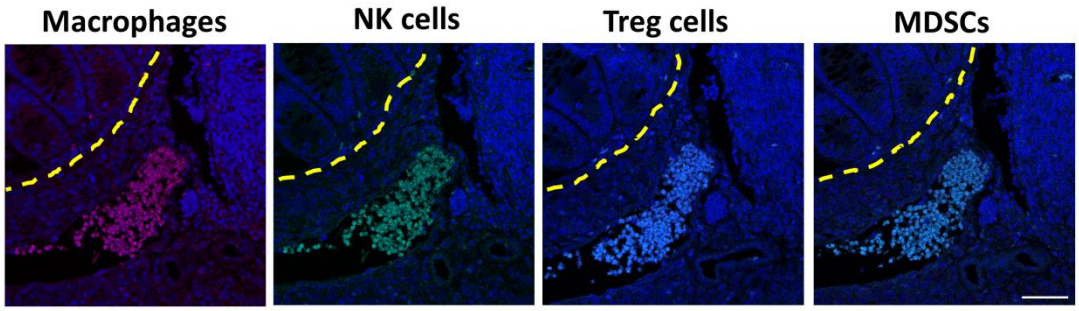
## Supplementary Figures



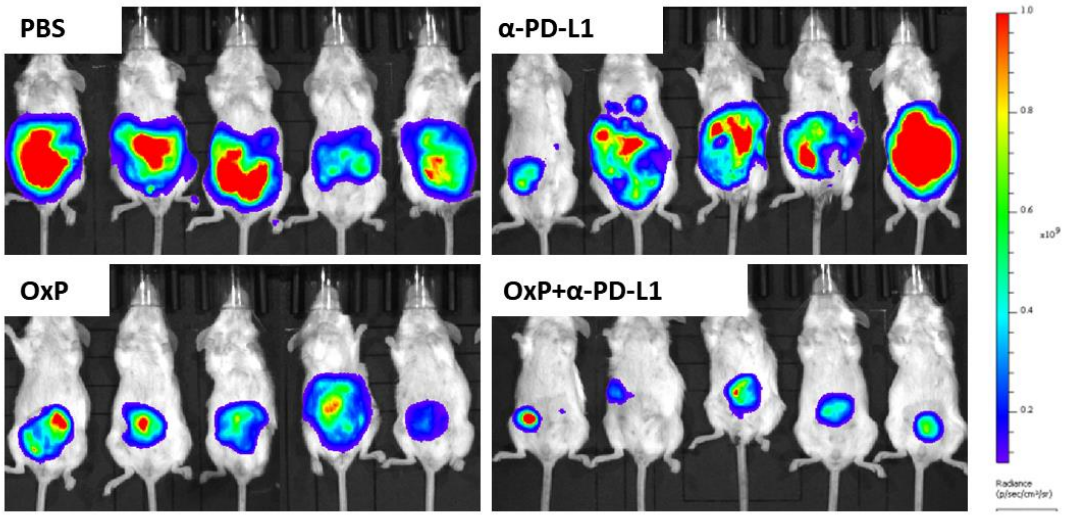

**Supplementary Figure 1.** Anti-PD-L1 mAb therapy in orthotopic MC38 model. **a** Treatment scheme and tumor growth curves of orthotopic MC38 tumors in PBS and α-PD-L1 treated groups (n = 5 mice per group). **b** IVIS images of the orthotopic MC38 tumors on day 27. **c** Immunofluorescence staining of orthotopic MC38 tumors in PBS group using DAPI (blue) and anti-CD3 antibody (red). Yellow dotted line indicates the border between intestinal mucosa and the orthotopic tumor. Scale bar represents 50 μm. Significant differences were assessed in **a** using two-way ANOVA. Results are presented as mean (SD). * $p < 0.05$.



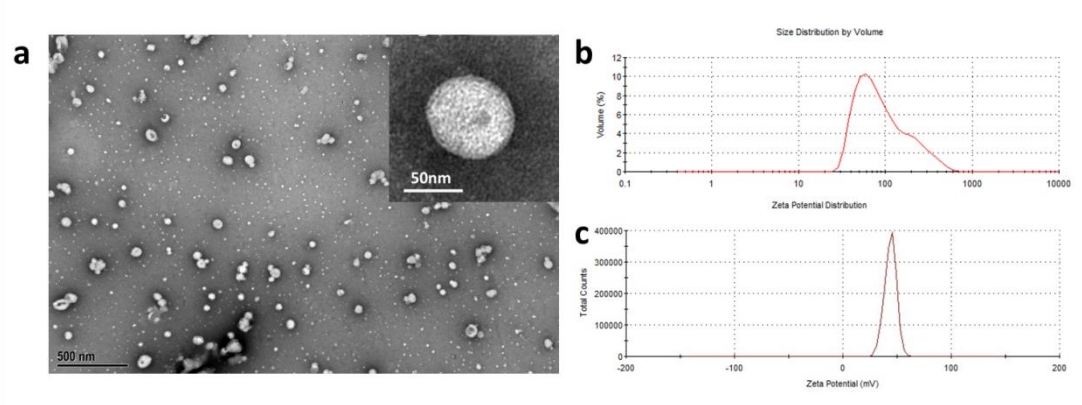

**Supplementary Figure 2.** OxP induced ICD in CT26-FL3 cells. (**a)** CRT exposure and HMGB1 release test of CT26-FL3 cells with or without incubation with OxP. Scale bar represents 20 μm. (**b)** ELISA detection of HMGB1 release in the supernatants of CT26-FL3 cells treated with different concentrations of OxP for 24 h (n = 3). (**c)** ELISpot test of the splenocytes of mice injected with or without OxP incubated CT26-FL3 cells. (**d)** OxP incubated CT26-FL3 cells efficiently vaccinated mice against re-challenging of CT26-FL3 cells. In the mice without pre-treatment, 0 out of 5 mice were tumor free. In the OxP incubated cells vaccinated group, 2 out of 5 mice were tumor free, and the tumor growth rate is slower. Significant differences were assessed in **b** using t test. Results are presented as mean (SD). *$p < 0.05$, ***$p < 0.001$.



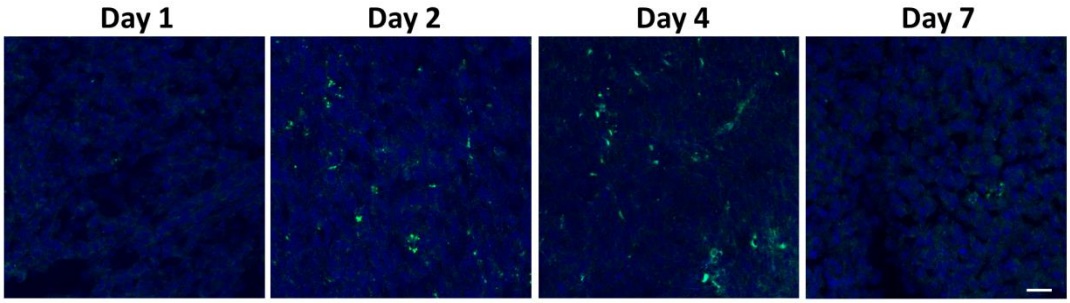

**Supplementary Figure 3.** Immunofluorescence staining of the orthotopic CT26-FL3 tumors after OxP treatment using DAPI (blue), anti-F4/80 (red), anti-NK1.1 (green), anti-CD4 (red) and anti-FoxP3 (green), anti-CD11b (green) and anti-Gr-1 (red). Yellow dotted line indicates the border between intestinal mucosa and the orthotopic tumor. Scale bar represents 50 μm.



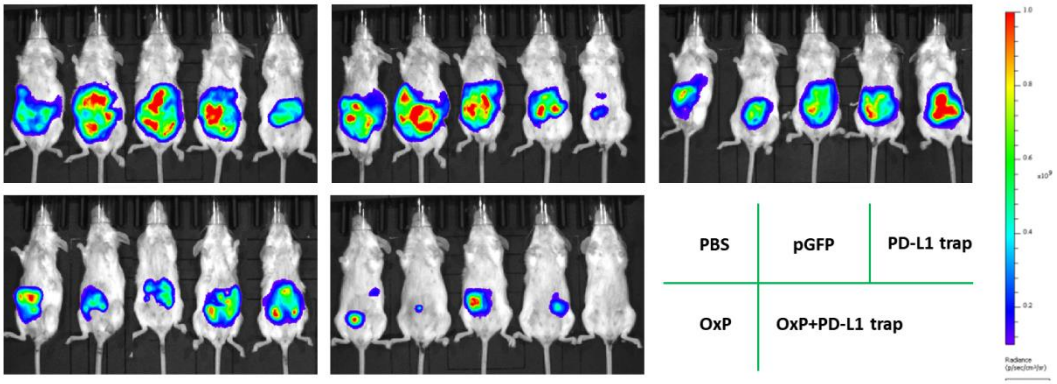

**Supplementary Figure 4.** IVIS images of the orthotopic CT26-FL3 tumor-bearing mice after various treatments on day 33.



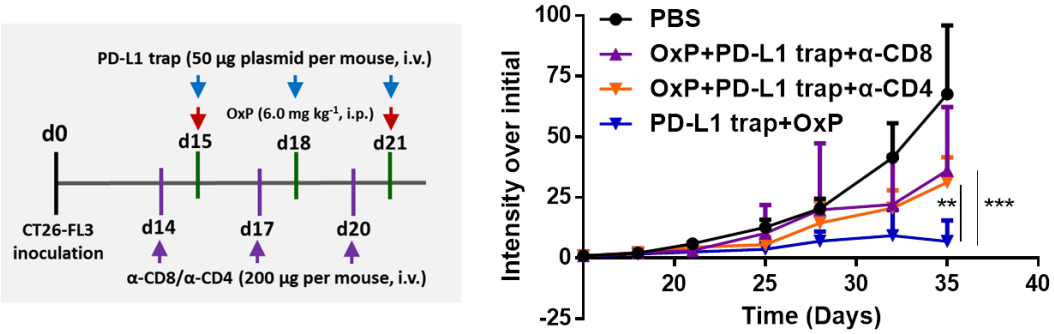

**Supplementary Figure 5.** Characterization of the prepared LPD-PD-L1 trap plasmid nanoparticles. **a** TEM images. **b** Volume average size detected by DLS. **c** Zeta potential.



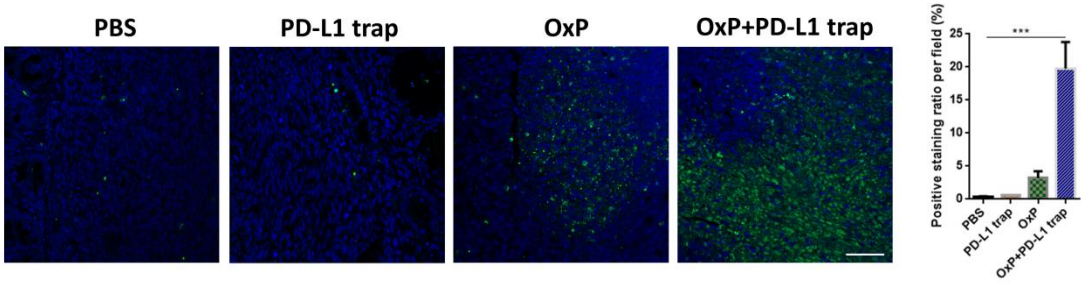

**Supplementary Figure 6.** GFP expression in tumor tissues on day 1, 2, 4 and 7. GFP plasmid loaded LPD nanoparticles were given on day 0 at a dose of 50 μg plasmid per mouse. Scale bar represents 20 μm.



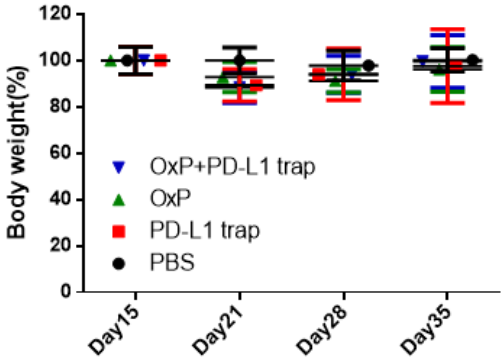

**Supplementary Figure 7.** IVIS images of the orthotopic CT26-FL3 tumor-bearing mice after various treatments on day 33.



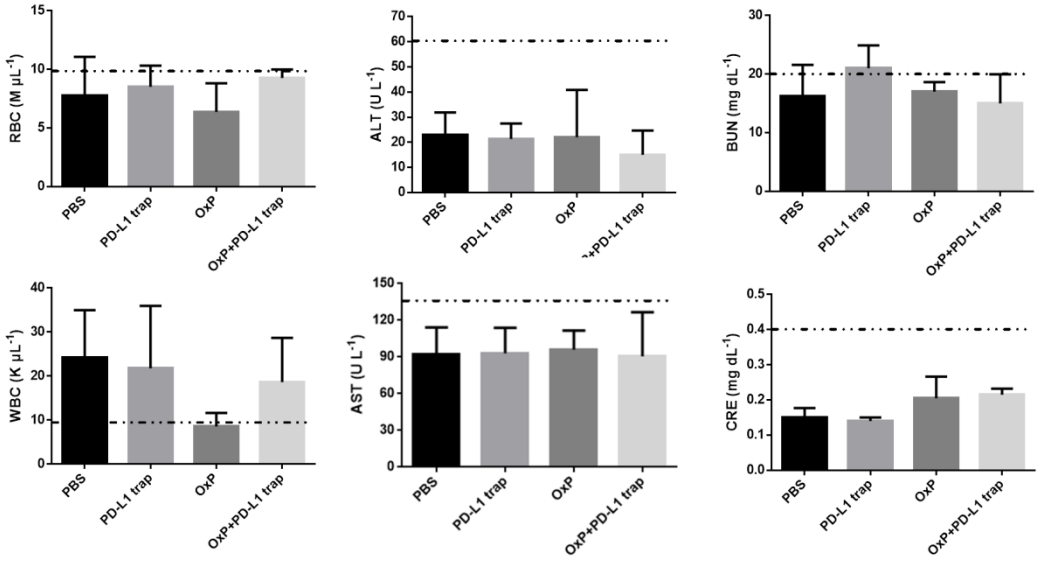

**Supplementary Figure 8.** Orthotopic CT26-FL3 tumor growth curves of the PBS, OxP+PD-L1 or combined with α-CD8/α-CD4 groups (n = 5 mice per group). Significant differences were assessed using two-way ANOVA. Results are presented as mean (SD). ** $P < 0.01$, *** $P < 0.001$.



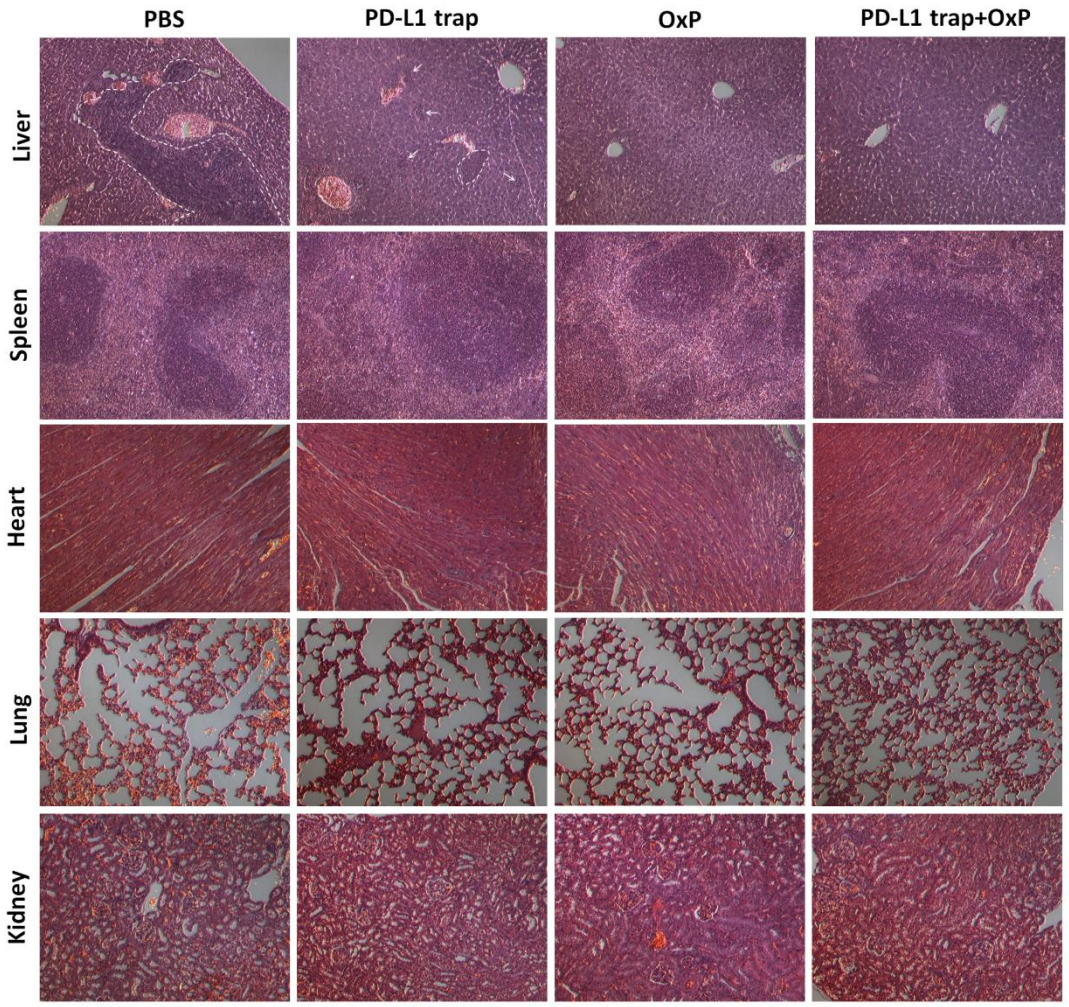

**Supplementary Figure 9.** TUNEL staining of the PBS, PD-L1 trap, OxP and OxP+PD-L1 trap treated CT26-FL3 tumor tissues on day 28. Positive ratios were quantified in 3 randomly selected fields per mouse (n = 4 mice per group). Scale bar represents 50 μm. Significant differences were assessed using t test. Results are presented as mean (SD). *** P < 0.001.



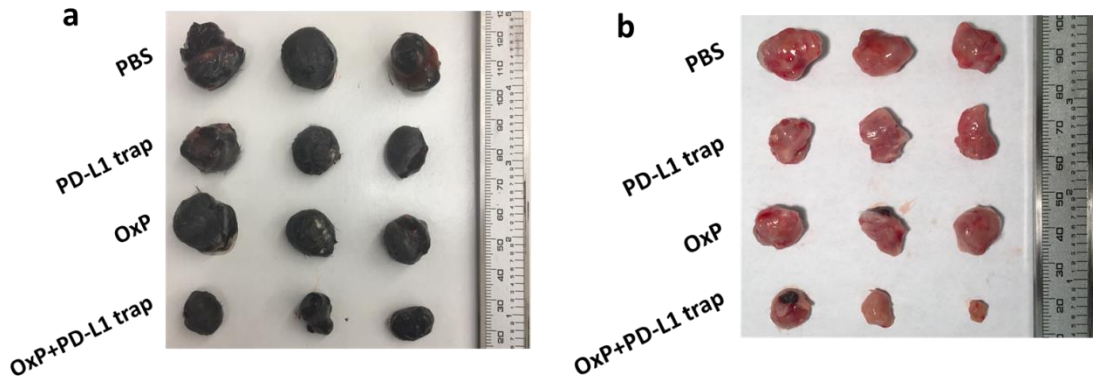

**Supplementary Figure 10.** Body weight over initial of the orthotopic CT26-FL3 tumor-bearing mice in PBS, PD-L1 trap, OxP and OxP+PD-L1 trap treated groups (n = 5).




**Supplementary Figure 11.** Complete blood count and blood chemistry analysis of the orthotopic CT26-FL3 tumor bearing mice after various treatments. Measurements were carried out on day 28 (n = 3).




**Supplementary Figure 12.** Pathological analyses of liver, spleen, heart, lung and kidney of the CT26-FL3 tumor-bearing mice in the PBS, PD-L1 trap, OxP and OxP+PD-L1 trap treated groups on day 28. The regions in the white lines or sites pointed by white arrows in liver are metastasis sites. The photos were taken at 20× magnification.




**Supplementary Figure 13.** Representative images of the B16F10 (**a**) and 4T1 (**b**) tumors at the end of observation.

# Supplementary Tables

**Supplementary Table 1.** Antibodies used in the study.

| Antibodies | Company | Catalog No. | Application | Dilution fold |
|---|---|---|---|---|
| Alexa Fluor® 488 Anti-CD45 | BioLegend | 103122 | IF | 200 |
| Alexa Fluor® 647 Anti-CD3 | BioLegend | 100209 | IF | 200 |
| Alexa Fluor® 594 Anti-CD11c | BioLegend | 117346 | IF | 200 |
| Alexa Fluor® 594 Anti-CD4 | BioLegend | 100446 | IF | 200 |
| Alexa Fluor® 488 Anti-FoxP3 | BioLegend | 126406 | IF | 200 |
| Alexa Fluor® 594 Anti-F4/80 | BioLegend | 123140 | IF | 200 |
| FITC Anti-NK1.1 | BioLegend | 108706 | IF | 200 |
| Alexa Fluor® 594 Anti-Gr1 | BioLegend | 108448 | IF | 200 |
| Alexa Fluor® 488 Anti-CD11b | BioLegend | 101217 | IF | 200 |
| APC Anti-IL-10 | BioLegend | 505010 | IF | 200 |
| Anti-HMGB1 | Abcam | ab18256 | IF | 500 |
| Anti-CRT | Abcam | ab2907 | IF | 500 |
| Goat Anti-Rabbit IgG, Alexa Fluor® 594 | Abcam | Ab150080 | IF | 500 |
| Alexa Fluor® 594 Anti-CD8a | BioLegend | 100758 | Flow | 500 |
| PE Anti-CD206 | BioLegend | 141706 | Flow | 500 |
| APC Anti-DC Marker | BioLegend | 124913 | Flow | 500 |
| APC-Cy7 Anti-CD3e | BioLegend | 557596 | Flow | 500 |
| BV605 Anti-CD274 | BioLegend | 329724 | IF, Flow | 200, 500 |
| APC Anti-mouse IL-17A | BioLegend | 506916 | Flow | 500 |
| PerCP Anti-CD45 | BD Pharmingen | 550994 | Flow | 500 |
| FITC Anti-CD4 | Santa Cruz | D3010 | Flow | 500 |
| Purified anti-human CD3 | Biolegend | 300301 | IF, IHC | 200 |
| Donkey Anti-mouse IgG, Alexa Fluor® 594 | Abcam | Ab150108 | IF | 200 |

IF: immunofluorescence. IHC: immunohistochemistry. Flow: Flow cytometry.

**Supplementary Table 2.** Primers used in this study

| **Primer** | **Applied Biosystems** |
|---|---|
| Mouse GAPDH | Mm99999915_g1 |
| Mouse IFN-γ | Mm01168134_m1 |
| Mouse TNF-α | Mm00443260_g1 |
| Mouse IL4 | Mm00445259_m1 |
| Mouse IL10 | Mm01288386_m1 |
| Mouse CXCL10 | Mm00445235_m1 |
| Mouse CXCL9 | Mm00434946_m1 |
| Mouse CCL2 | Mm00441242_m1 |
| Mouse CXCL12 | Mm00445553_m1 |
| Mouse CXCL13 | Mm04214185_s1 |

**Supplementary Note 1.** Nucleic acid sequences coding the open reading frame of the mouse PD-L1 trap (*).

ATGAAATGGGTCACCTTTATCAGCCTGCTGTTCCTGTTCAGCAGCGCCTACTCTGGATC
CTGGCTGCTGGAAGTGCCTAACGGCCCTTGGAGAAGCCTGACATTCTACCCTGCCTGG
CTGACCGTGTCTGAGGGCGCTAACGCCACCTTCACATGCAGCCTGAGCAATTGGAGCG
AGGACCTGATGCTGAACTGGAATAGACTGAGCCCCAGCAACCAGACCGAGAAGCAGG
CCGCTTTCTGCAACGGACTGTCTCAGCCTGTGCAGGACGCCAGATTCCAGATCATCCA
GCTGCCTAACAGACACGACTTCCACATGAACATCCTGGACACCAGAAGAAACGACAG
CGGCATCTACCTGTGCGGCGCCATCTCTCTGCACCCCAAGGCTAAGATCGAGGAATCTC
CTGGCGCTGAGCTGGTGGTCACCGAGAGAATTCTTGAAGGCCCGCAACCGCAACCGA
AACCGCAGCCGAAACCGGAACCGGAACCGCAACCGCAAGGCGGTTCTGAGGAAGAC
CCCTGTGCCTGTGAGTCCATACTGAAATTTGAGGCCAAGGTGGAGGGTCTGCTGCAGG
CCCTGACCAGGAAGCTGGAAGCTGTGAGCGGGCGGCTGGCTGTCCTGGAGAACAGAA
TCATCGCGGCCGCTGGCGCCCCTGTGCCTTATCCTGATCCCCTGGAACCTAGAGGCGGC
AGCCACCACCACCATCACCACTGA

*: From PCT/US2016/051966