

1 **Supporting Information**

2 **Bioresponsive Protein Complex of aPD1 and aCD47 Antibodies**
3 **for Enhanced Immunotherapy**

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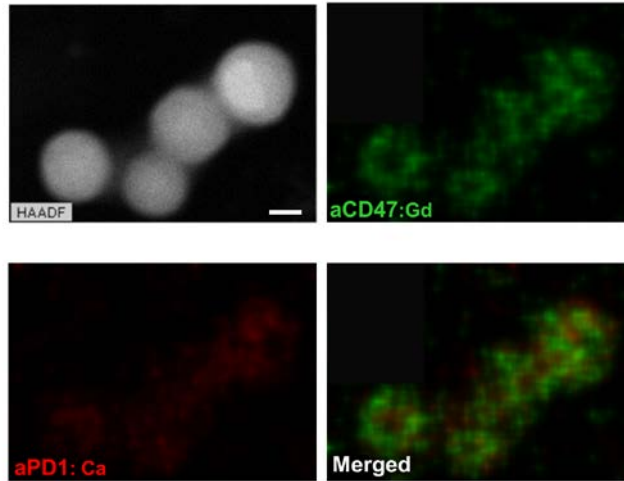
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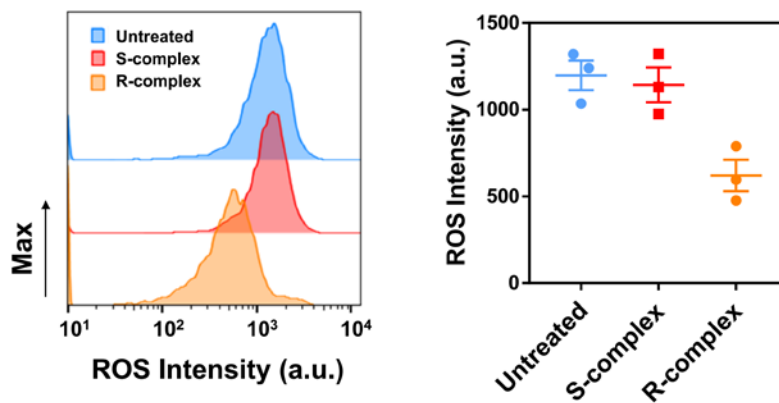
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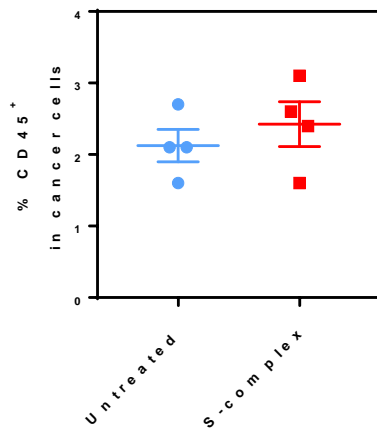
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Figure S1. Scanning TEM (STEM) images of aPD1@aCD47 complex showing the gadolinium labeled aCD47 (green) and calcium labeled aPD1 (Red) (scale bar: 100 nm). Experiments were repeated three times.

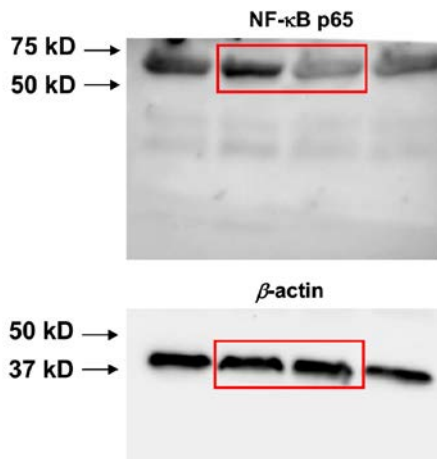


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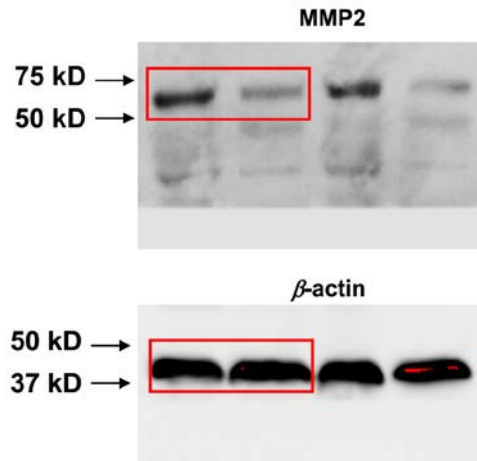
Figure S2. ROS levels in the tumor collected from mice after different treatments were measured using the CellROX® deep red reagent by flow cytometric analyses. Data presented as mean \pm s.e.m. ($n=3$). a.u., arbitrary unit. S-complex, ROS-stable complex; R-complex, ROS-responsive complex.



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 2 **Figure S3.** Percentage of CD45⁺ cells in tumors with or without ROS-stable complex (S-
 3 complex) treatment analyzed by flow cytometry. Data presented as mean ± s.e.m. (n=4).
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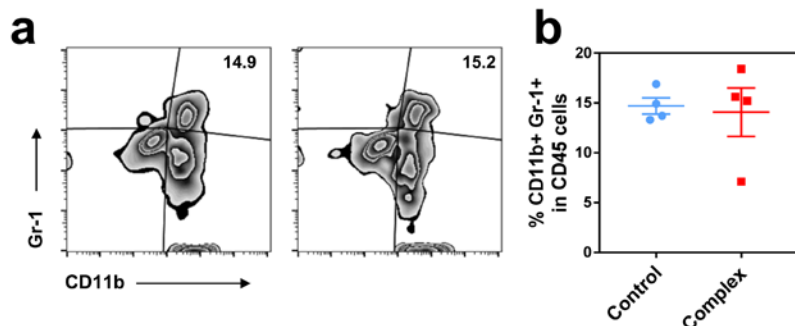


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 6 **Figure S4.** Uncropped western blots for NF-κB p65. Lanes used for Figure 2c are indicated by
 7 red rectangles. Experiments were repeated three times.
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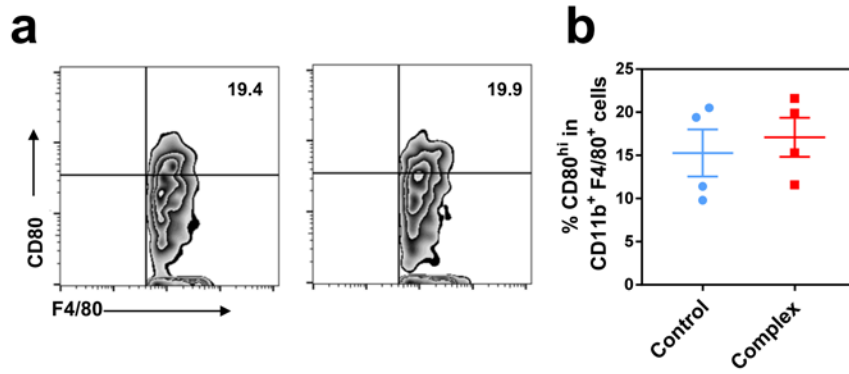
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Figure S5. Uncropped western blots for MMP2. Lanes used for Figure 2c are indicated by red rectangles. Experiments were repeated three times.



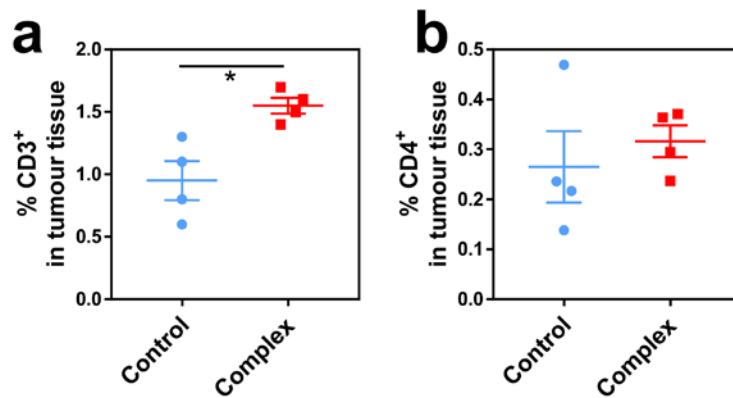
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Figure S6. Representative flow cytometric analysis images (a) and the relative quantification (b) of MDSCs (CD11b⁺Gr-1⁺) gating on CD45⁺ cells. Data are presented as mean ± s.e.m. (n=4).



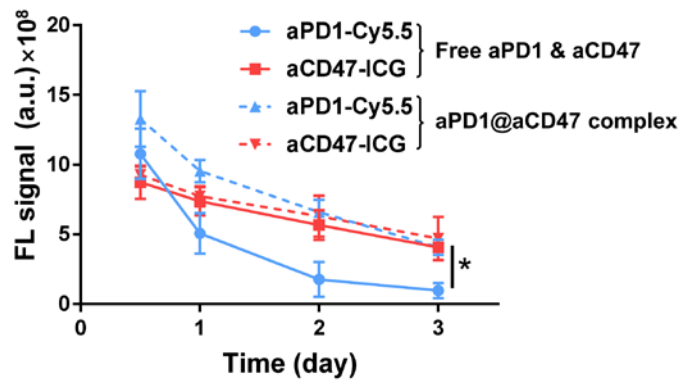
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Figure S7. Representative flow cytometric analysis images (a) and the relative quantification of M1-like macrophages (CD80^{hi}) (b) gating on F4/80⁺CD11b⁺CD45⁺ cells. Data are presented as mean \pm s.e.m. ($n=4$).

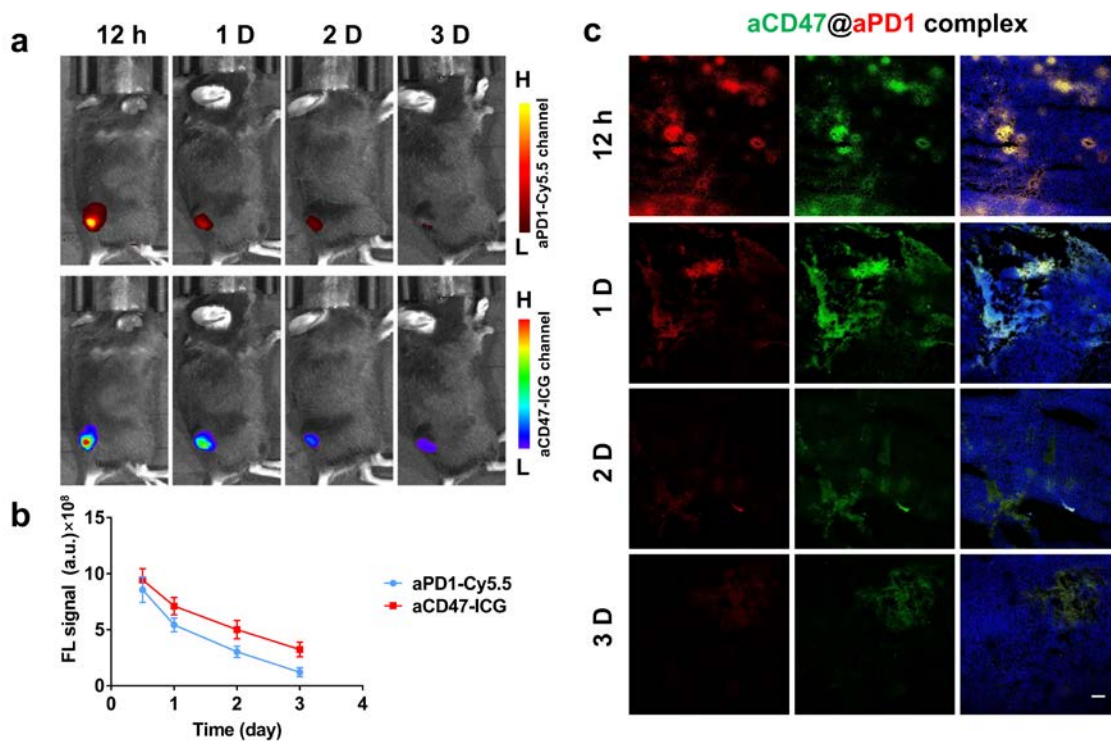


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Figure S8. Absolute percentage of CD3⁺ (a) and CD4⁺ T cells (d) within tumors after the ROS-responsive complex treatment. Data are presented as mean \pm s.e.m. ($n=4$). Statistical significance was calculated *via* two-tailed Student's *t*-test. *P* value: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

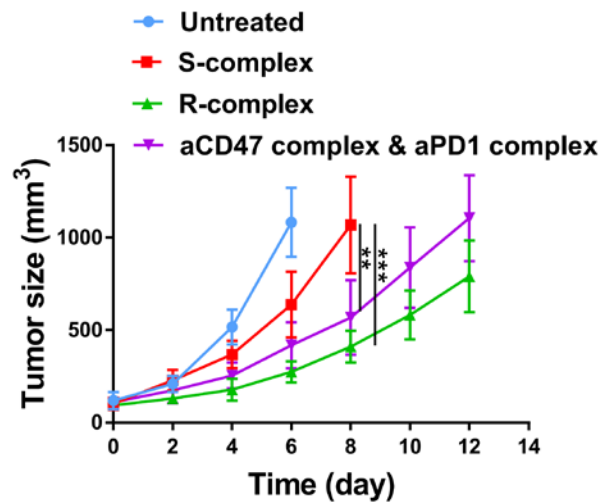


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 2 **Figure S9.** Quantification of the *in vivo* retention profile of aPD1–Cy5.5 and aCD47–ICG. The
 3 solid lines represent free aPD1 or aCD47, while the dotted lines show the behavior of
 4 aPD1@aCD47 complex. Data presented as mean ± s.e.m. ($n=3$). Statistical significance was
 5 calculated *via* one-way ANOVA with a Tukey post-hoc test. P value: $*P < 0.05$.
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 8 **Figure S10. The retention behavior of intratumoral injection of aCD47@aPD1 complex.**
 9 (a) *In vivo* fluorescence imaging to show the retention of aCD47 and aPD1 in the tumor at
 10 different time points after injection of aCD47@aPD1 complex. (b) Quantification of the *in vivo*
 11 retention profile of aPD1–Cy5.5 and aCD47–ICG. (c) Confocal immunofluorescence images
 12 of tumors collected from mice treated with aCD47@aPD1 complex at different time points
 13 (Scale bar, 200 μm). Red and green signals indicate aPD1 and aCD47, respectively.
 14 Experiments were repeated three times.
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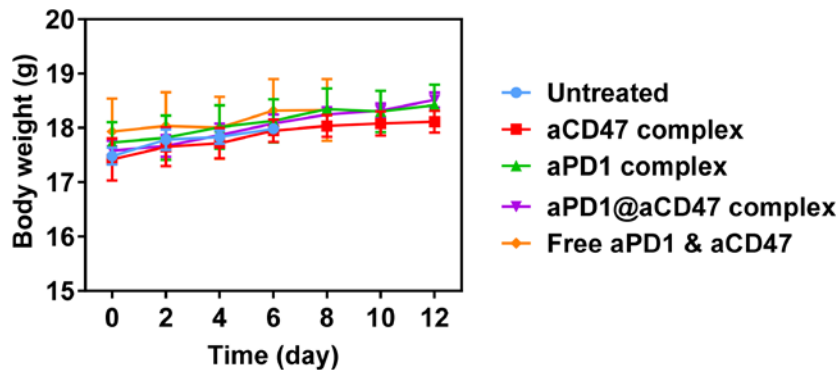
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3 **Figure S11.** Average tumor growth curves after S-aPD1@aCD47 (aPD1@aCD47 induced by
4 ROS-stable linker), or R-aPD1@aCD47 (aPD1@aCD47 induced by ROS-responsive linker),
5 or aCD47 complex & aPD1 complex treatment (aCD47:50 µg per mouse, aPD1:50 µg per
6 mouse). Data presented as mean ± s.e.m. ($n=6$). Statistical significance was calculated *via* one-
7 way ANOVA with a Tukey post-hoc test. P value: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

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12 **Figure S12.** Body weights of mice in different groups over time. Data are presented as mean ±
13 s.e.m. ($n=6$).

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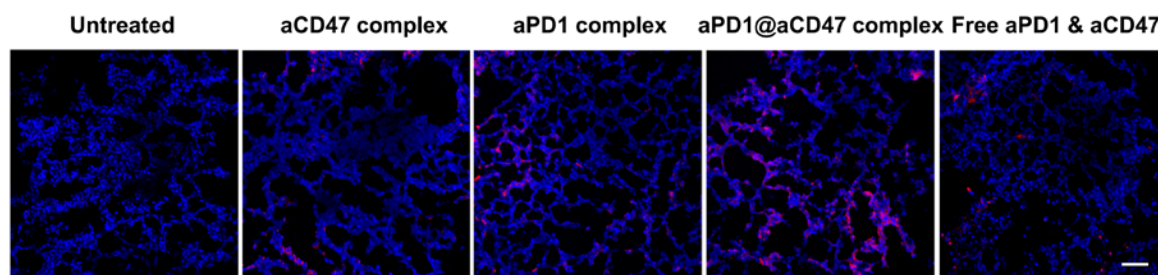


Figure S13. Representative immunofluorescence images of tumors showing CD8⁺ T cell infiltration after different treatments. Scale bar, 50 μ m. Experiments were repeated three times.

	WBC ($10^9/L$)	RBC ($10^{12}/L$)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)
Reference range	3.90~13.94	7.37~11.50	10.9~18.1	37.2~58.0	42.60~55.6	13.0~16.8
Healthy control	9.98 \pm 2.01	8.05 \pm 0.49	11.6 \pm 1.0	37.4 \pm 3.0	50.4 \pm 4.2	15.5 \pm 1.4
1D after treatment	10.07 \pm 1.89	7.88 \pm 0.56	11.1 \pm 0.8	40.5 \pm 3.4	47.3 \pm 0.9	14.1 \pm 0.3
7D after treatment	11.07 \pm 2.32	8.12 \pm 0.60	11.6 \pm 0.9	39.8 \pm 1.6	46.2 \pm 0.6	14.2 \pm 0.2

	MCHC (g/dL)	PLT ($10^9/L$)	BUN (mmol/L)	ALT (U/L)	ALP (U/L)	AST (U/L)
Reference range	26.0~35.9	565~1849	5~26	27~195	105~370	43~397
Healthy control	29.7 \pm 4.3	837 \pm 250	23.6 \pm 0.3	126 \pm 21	135 \pm 46	267 \pm 106
1D after treatment	29.8 \pm 3.2	790 \pm 218	22.5 \pm 2.8	61 \pm 10	129 \pm 28	249 \pm 51
7D after treatment	30.8 \pm 0.6	621 \pm 145	23.6 \pm 2.6	79 \pm 39	140 \pm 20	241 \pm 93

Table S1. Female C57BL/6 mice were sacrificed at 1 and 7 days after aPD1@aCD47 treatment. Untreated healthy mice were used as control. Complete blood counts: Blood levels of White blood cells (WBC), Red blood cells (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC). Serum biochemistry data including blood urea nitrogen (BUN) levels and liver function markers such as Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), and Aspartate aminotransferase (AST) were also measured. Data represented as mean \pm s.d. ($n = 3$). Reference ranges of hematology data of healthy female C57BL/6 mice were obtained from Charles River Laboratories: (<http://www.criver.com/>).