Cancer Cell, Volume *35*

Supplemental Information

Targeting Tumors with IL-10 Prevents Dendritic Cell-Mediated $CD8^+$ T Cell Apoptosis

Jian Qiao, Zhida Liu, Chunbo Dong, Yan Luan, Anli Zhang, Casey Moore, Kai Fu, Jianjian Peng, Yang Wang, Zhenhua Ren, Chuanhui Han, Ting Xu, and Yang-Xin Fu

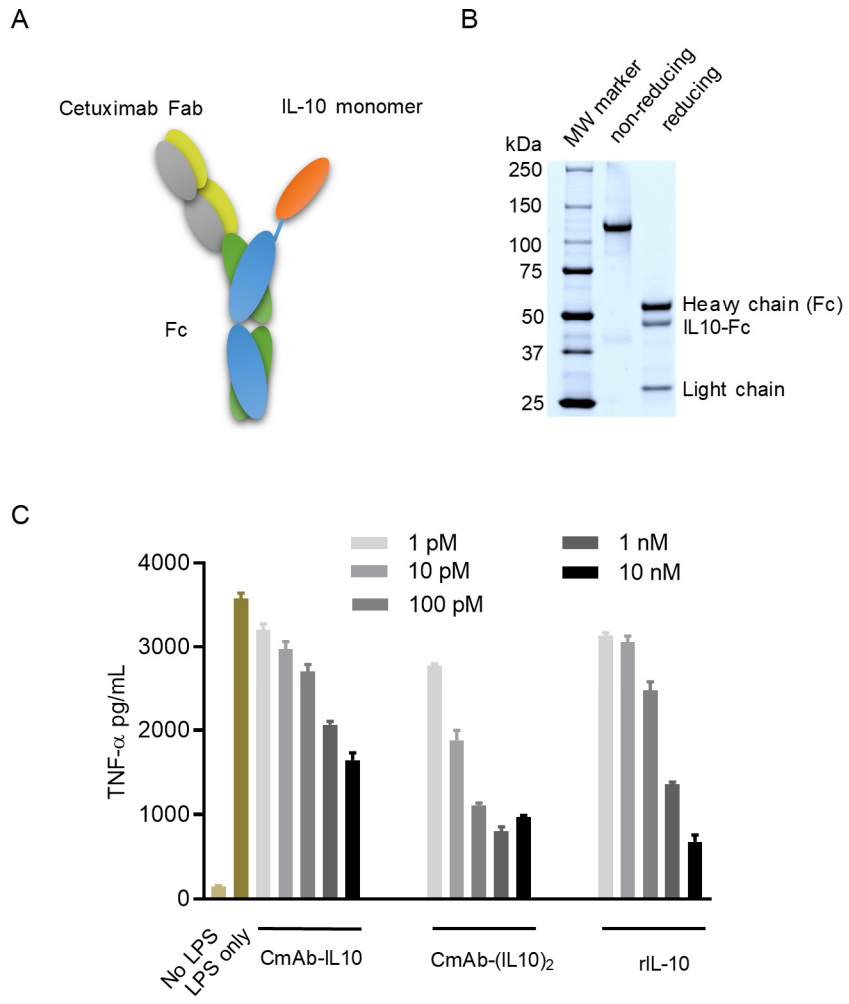

Figure S1. CmAb-(IL10)$_2$ Has Better Activities than CmAb-IL10 by Measuring Inhibition of TNF-α Production. Related to Figure 1.

(A) Schematic structure of CmAb-IL10. The Fab fragment of Cetuximab or the IL-10 monomer was fused to Fc region, respectively. These two fusion proteins form a bispecific CmAb-IL10 protein.

(B) SDS-PAGE analysis of CmAb-IL10.

(C) TNF-α production of bone marrow-derived DCs (BMDCs) (1x10^5) from C57BL/6J mice incubated with LPS (1 μg/mL) in the presence or absence of CmAb-IL10, CmAb-(IL10)$_2$ or rIL-10 at indicated concentrations, detected by CBA assay. Data are shown as mean ±SEM.

Table S1. Pharmacokinetic Parameters of CmAb-(IL10)$_2$. Related to Figure 1.

Animal ID	Rsq_ adjusted	$T_{1/2}$	T_{max}	C_{max}	AUClast	AUCall	V	Cl	MRT
		hr	hr	μg/mL	hr*mg/mL	hr*mg/mL	mL/kg	mL/hr/kg	hr
1	0.98	40.90	0.25	36.65	0.89	0.89	50.67	0.86	38.18
2	0.99	41.37	0.25	23.17	0.61	0.61	72.27	1.21	41.58
3	0.64	42.48	0.25	32.76	0.65	0.65	74.67	1.22	36.21
4	0.92	74.52	0.25	27.38	0.65	0.65	96.24	0.90	40.58

Note: Rsq_adjusted: R-squared adjusted. $T_{1/2}$: Half-life. T_{max}: Time of Maximum concentration. C_{max}: Maximum concentration. AUClast: Area under Curve of last time point. AUCall: Area under Curve of all. V: Volume of distribution. Cl: Clearance rate. MRT: Mean Residence Time.

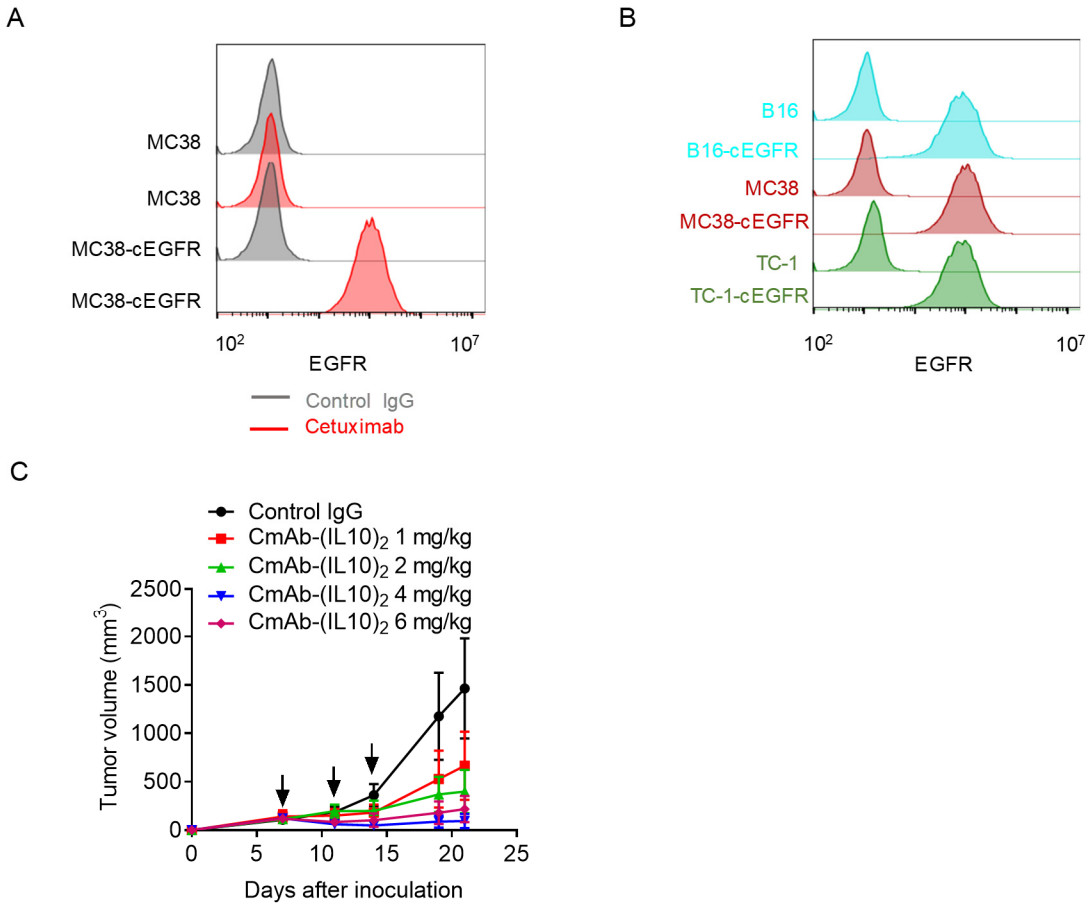

Figure S2. Establishment of Murine Tumors Expressing a Chimeric EGFR and Dose-dependent Antitumor Effects of CmAb-(IL10)$_2$. Related to Figure 2.

(A) Assessment of cEGFR binding to Cetuximab, detected in MC38 and MC38-cEGFR cells by flow cytometry.

(B) Assessment of cEGFR binding to CmAb-(IL10)$_2$, detected in several tumor cell lines and their derivatives expressing c-EGFR by flow cytometry.

(C) Tumor growth in C57BL/6J mice (n=6-8) bearing B16-hEGFR-SIY tumors treated with control IgG or different dose of CmAb-(IL10)$_2$ (i.p., indicated by arrows). Data are shown as mean ±SEM.

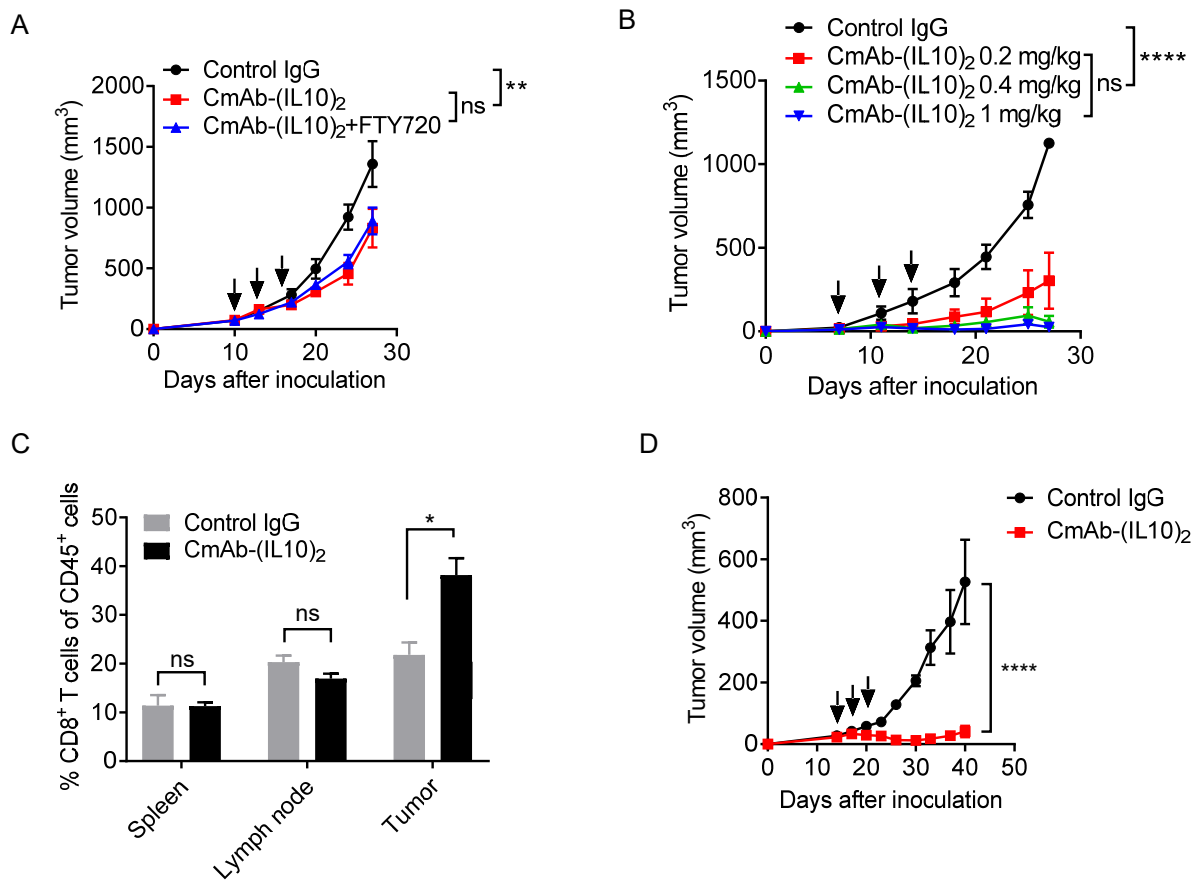

Figure S3. CmAb-(IL10)$_2$ Inhibits Tumor Growth through Its Effects on Intratumoral Immune Cells. Related to Figure 3.

(A) Tumor growth in C57BL/6J mice (n=5) bearing B16-hEGFR-SIY tumors treated with FTY720, control IgG or CmAb-(IL10)$_2$ (i.p., indicated by arrows).

(B) Tumor growth in C57BL/6J mice (n=5) bearing B16-hEGFR-SIY tumors treated with control IgG or different dose of CmAb-(IL10)$_2$ (i.t., indicated by arrows).

(C) Quantification of CD8$^+$ T cells in tumor tissues collected from B16-cEGFR tumor bearing C57BL/6J mice (n=3) treated twice by i.t. injection with control IgG or CmAb-(IL10)$_2$. Tumor tissues were collected 7 days after first treatment and analyzed by flow cytometry.

(D) Tumor growth in C57BL/6J mice (n=4-5) bearing MC38-cEGFR tumors treated with control IgG or CmAb-(IL10)$_2$ (i.t., indicated by arrows).

(A-D) Data are shown as mean ±SEM. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, ns, not significant.

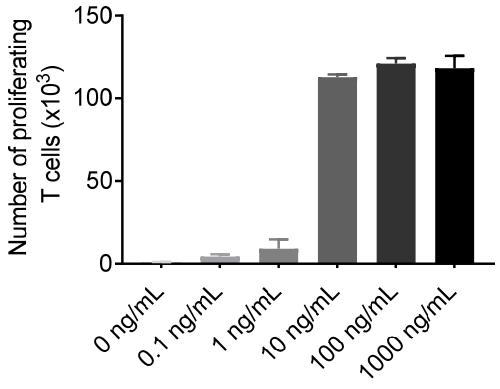

Figure S4. CmAb-(IL10)$_2$ Promotes CD8$^+$T Cell Proliferation in a Dose-dependent Manner. Related to Figure 4.

Cell number of proliferating CD8$^+$ OT1 T cells co-cultured with BMDCs from C57BL/6J mice in the presence of OVA treated with the indicated dose of CmAb-(IL10)$_2$, assessed by flow cytometry at 72 hr after co-culture. Data are shown as mean ±SEM.

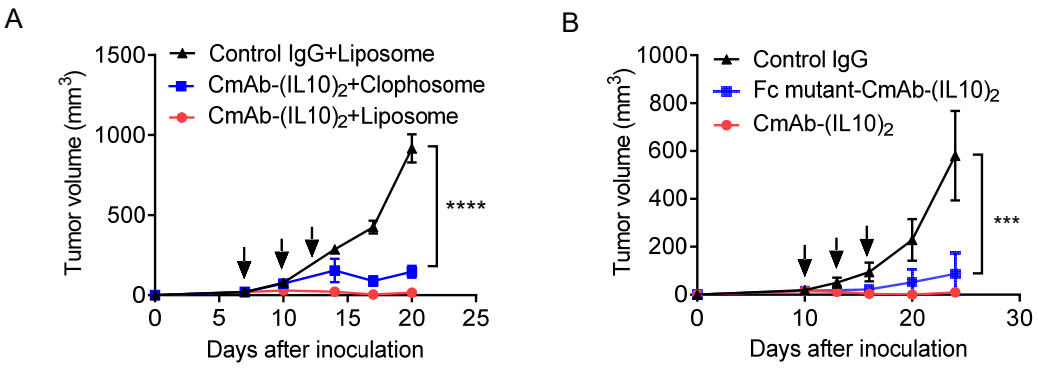

Figure S5. Macrophage and Fc Receptor Binding are not Required for the Anti-tumor Effects of CmAb-(IL10)$_2$. Related to Figure 4.

(A) Tumor growth in C57BL/6J mice (n=5) bearing B16-cEGFR tumors treated with Clophosome or control liposome (i.p.), control IgG or CmAb-(IL10)$_2$ (i.t., indicated by arrows).

(B) Tumor growth in C57BL/6J mice (n=5) bearing B16-cEGFR tumors treated with control IgG, CmAb-(IL10)$_2$ or Fc mutant CmAb-(IL10)$_2$ (Fc region was mutated to ablate its binding capacity to the receptor) (i.t., indicated by arrows).

(A-B) Data are shown as mean ±SEM. ***$p < 0.001$, ****$p < 0.0001$.

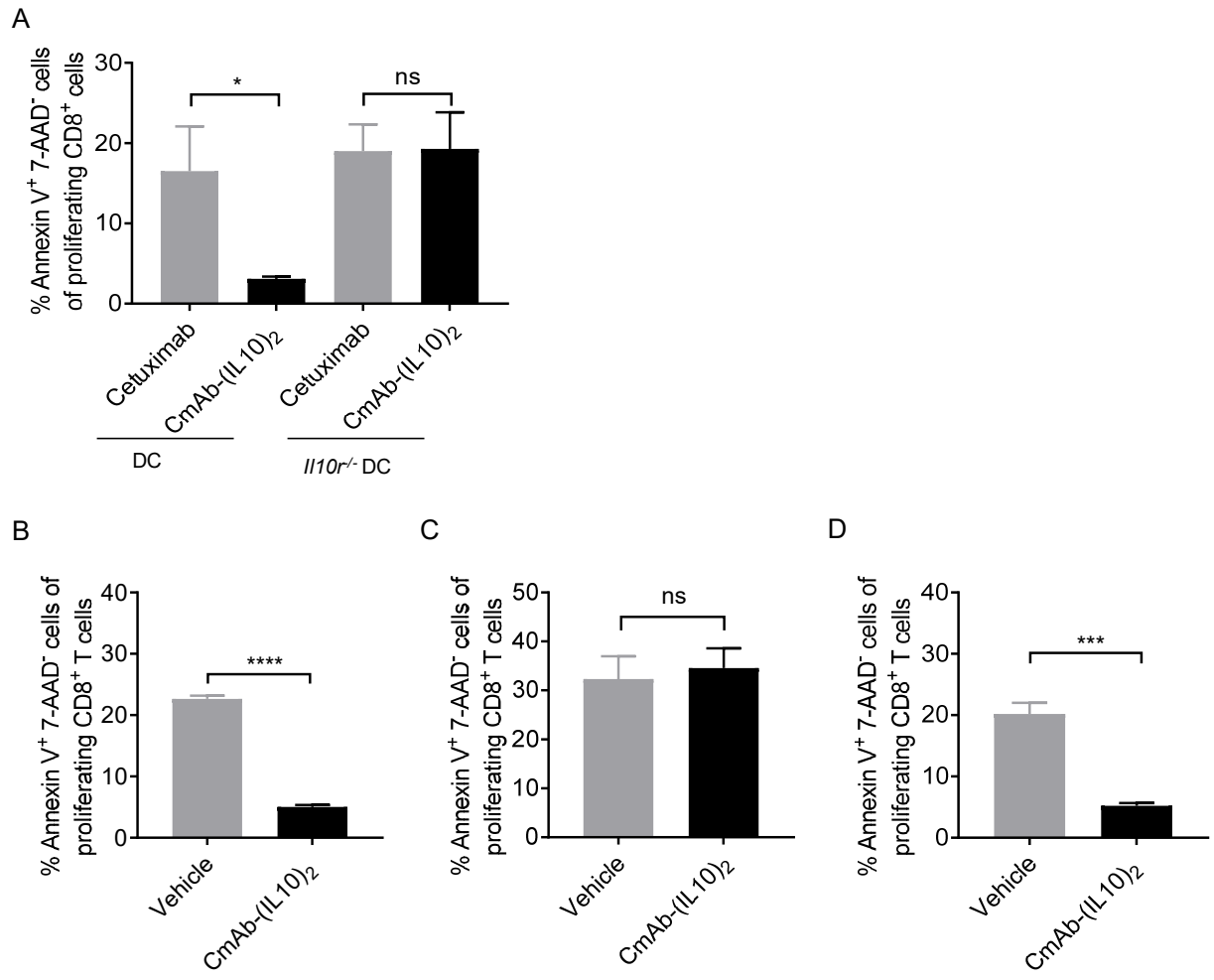

Figure S6. IL-10R Signaling on DCs, rather than on T cells is Required to Prevent Antigen-Specific CD8$^+$ T cell Apoptosis. Related to Figure 5.

(A) Apoptosis of proliferating CD8$^+$ T cells from B6.Cg-*Thy1*^{a}/Cy Tg(TcraTcrb)8Rest/J mice (Pmel-1 TCR transgenic mice) co-cultured with BMDCs from WT or *Il10r*$^{-/-}$ mice in the presence of 2.5 μg/mL gp100 peptide and 10 ng/mL LPS and treated with CmAb-(IL10)$_2$ or Cetuximab, assessed by flow cytometry at 72 hr after co-culture.

(B-D) Apoptosis of proliferating CD8$^+$ T cells from OT1 (B and C) or *Il10r*$^{-/-}$ OT1 (D) transgenic mice co-cultured with BMDCs from WT (B and D) or *Il10r*$^{-/-}$ (C) mice, in the presence of OVA treated with CmAb-(IL10)$_2$ or vehicle, assessed by flow cytometry at 72 hr after co-culture.

(A-D) Data are shown as mean ±SEM. *p < 0. 05, ***p < 0. 001, ****p < 0.0001, ns, not significant.

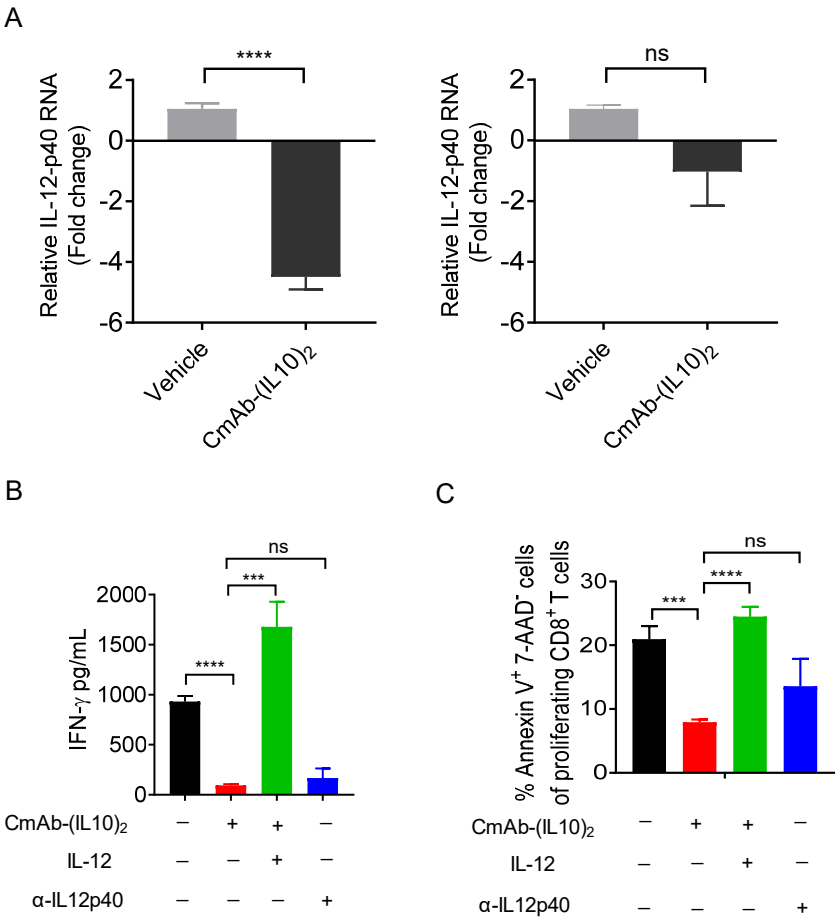

Figure S7. CmAb-(IL10)$_2$ Regulates IL-12 Production by DC, Which Results in Reduced IFN-γ Production and Antigen-specific T cell Apoptosis. Related to Figure 6.

(A) IL-12p40 expression by BMDCs incubated with supernatants from co-culture of CD8$^+$ OT1 T cells with BMDCs from WT (left) or *Il10r*$^{-/-}$ (right) mice in the presence of OVA treated with CmAb-(IL10)$_2$ or vehicle, assessed by RT-PCR at 24 hr after incubation.

(B-C) IFN-γ concentration in supernatants (B) and apoptosis (C) of proliferating CD8$^+$T cells detected from co-culture of CD8$^+$ OT1 T cells with BMDCs from WT mice in the presence of OVA treated with CmAb-(IL10)$_2$, IL-12 (2.5 ng/mL) or anti-IL12p40 (10 μg/mL), quantified by CBA assay (B) and flow cytometry (C) at 72 hr after co-culture.

(A-C) Data are shown as mean ±SEM. ***p < 0.001, ****p < 0.0001, ns, not significant.