

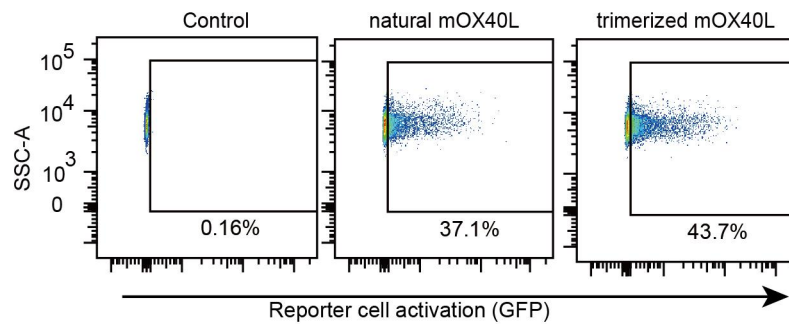
## **Supplementary Materials**

Supplementary Figure and Figure Legends

Supplementary Table

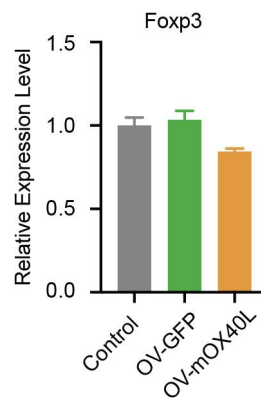
## Supplementary Figures and Figure Legends

### Figure S1



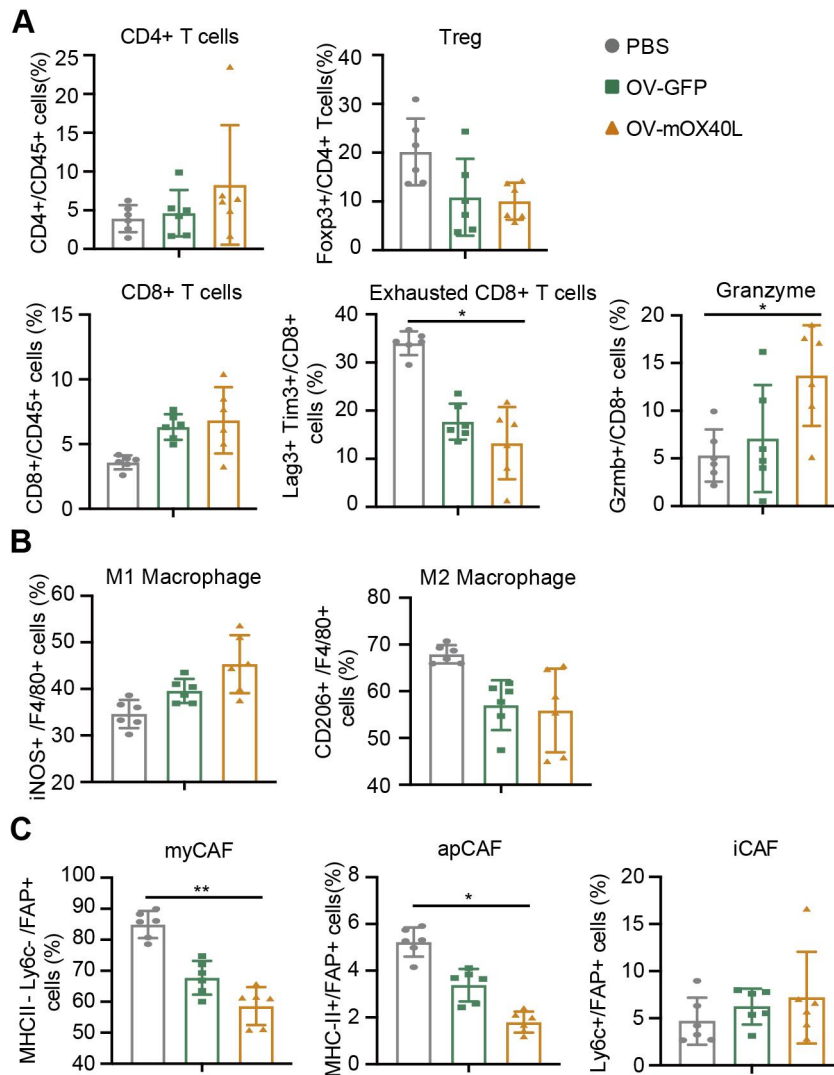
**Figure S1. Agonistic activity of the natural mOX40L and trimerized mOX40L.** HEK293FT cells were transfected with the whole natural mOX40L or trimerized membrane bound mOX40L and cocultured with Jurkat-NF- $\kappa$ B-GFP-OX40 reporter cells, which express GFP upon activation of the OX40-OX40L signaling pathway. The GFP expression of the reporter cells was analyzed by flow cytometry.

### Figure S2



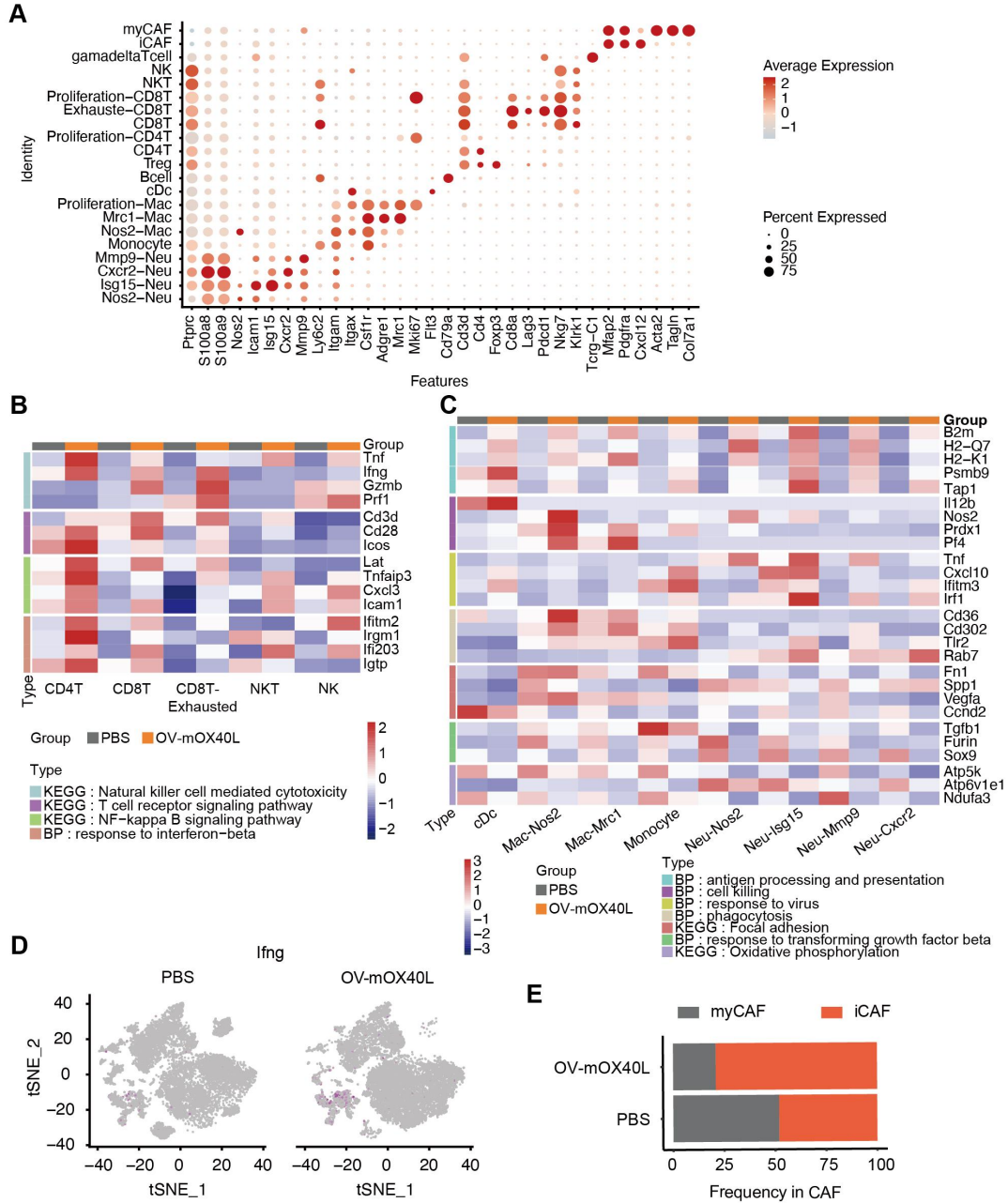
**Figure S2. Effect of OV-mOX40L on Foxp3 expression of Tregs.** Tregs were cocultured with OV-infected KPC cells in 48-well plate for 24 hours. Foxp3 expression was detected by quantitative real-time PCR.

**Figure S3**



**Figure S3. Flow cytometry analysis of tumor-infiltrating immune cells and fibroblasts after the treatment with oncolytic viruses.** Mice were subcutaneously implanted with KPC cells and treated with oncolytic viruses or PBS. Tumor tissues were collected 3 days after the last treatment. A-C) The single-cell suspension was prepared from tumor, stained with the fluorescent antibodies and subjected to flow cytometry analysis for the profiling of T cells (A), macrophages (B) and fibroblasts (C). Statistical significance was determined by ANOVA tests and multiple comparisons. Figure S3 was a repeated experiment for Figure 3.

**Figure S4**



**Figure S4. Analysis of intratumoral immune cells by scRNA-seq. A)** Bubble plots showed the expression of cell markers in each cluster. **B)** Heatmap displayed the expression of the selected genes in each pathway of lymphocyte cluster. **C)** Heatmap displayed the expression of the selected genes in each pathway of neutrophil, macrophage and cDC cluster. **D)** The expression of IFN- $\gamma$  from PBS and OV-mOX40L group was shown by the t-SNE plot. **E)** The proportions of CAF clusters.

## Supplementary Table

**Table S1. List of anti-mouse antibodies for flow cytometry analysis**

Cell marker	Catalog number	Vendor	Fluorescence
CD45	103107	biolegend	FITC
CD4	100428	biolegend	Pacific Blue™
CD8a	100752	biolegend	Brilliant Violet 510™
CD366 (Tim-3)	134006	biolegend	Alexa Fluor® 647
CD223 (LAG-3)	125226	biolegend	PE/Cyanine7
CD279 (PD-1)	135228	biolegend	PE/Dazzle™ 594
Ki-67	151210	biolegend	PE
IFN-γ	505846	biolegend	PE/Dazzle™ 594
FOXP3	320014	biolegend	Alexa Fluor® 647
Granzyme B	372208	biolegend	PE
CD11b	101224	biolegend	Pacific Blue™
I-A/I-E (MHCII)	107614	biolegend	APC
F4/80	123149	biolegend	Brilliant Violet 650™
CD206 (MMR)	141732	biolegend	PE/Dazzle™ 594
Nos2 (iNOS)	696806	biolegend	PE
Ly-6C	128033	biolegend	Brilliant Violet 510™
FAP	bs-5758R-AF647	Bioss	Alexa Fluor® 647