

Supplementary figure legends

Supplementary Figure 1. The performance of CIBERSORT and ssGSEA for characterizing immune phenotype from TCGA. (A) The percent of 22 types of adaptive and innate immune cells in each glioma sample. (B) Kaplan-Meier analysis for the prognostic value of 22 tumor-infiltrating immune cells in glioma. HR, hazard ratio; CI, confidence interval. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (C) The difference of immune infiltration among different grades of glioma. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (D) Heatmap of 29 gene sets correlated with glioma microenvironment signatures in TLX_low and TLX_high by ssGSEA analysis. (E) Heatmap of 29 gene sets correlated with glioma microenvironment signatures in PD-L1_low and PD-L1_high by ssGSEA. TumorPurity, ESTIMATEScore, ImmuneScore and StromalScore were evaluated by ESTIMATE.

Supplementary figure 2. TLX and PD-L1 expression, correlation and clinical outcome in gliomas in CGGA database. (A) TLX and PD-L1 mRNA expression levels in patients with WHO grade II ($n=291$), WHO grade III ($n=334$) and glioblastoma multiforme (WHO grade IV, $n=388$). (B) Correlation of TLX and PD-L1 in glioma. High expression of PD-L1 is correlated positively with TLX. $p < 0.0001$. (C) Overall survival according to expression levels of TLX (NR2E1) and PD-L1 (CD274) in the tumors. Median survival for NR2E1 high-expression group was 753 days versus 1669 days for the low. Median survival for CD274 high-expression group was 718 days versus 1669 days for the low. (D) TLX and PD-L1 mRNA expression levels in patients with IDH-mutant vs. IDH-wild-type in TCGA. ** $p < 0.01$, **** $p < 0.0001$

Supplementary figure 3. (A) CD8 immunohistochemistry; representative micrographs show CD8 immunostaining in normal brain tissues or tumor adjacent normal tissues and glioma tissues with different grades. Magnification, $\times 40$. Scale bar 300 μm . (B) No difference in infiltration of CD8⁺ TILs in TMA among three groups (one-way ANOVA, $p=0.3229$ versus Normal/ANT tissues). (C) Single channel of multiplex immunofluorescence staining of TLX (yellow), PD-L1 (red), PD-1 (green), CD8 (bright blue), CD163 (purple) and DAPI (blue) on GBM tissue (15X).

Supplementary figure 4. shTLX rescued *in vivo* antitumor immune response in glioma. (A) Photos of dissected tumors formed by GL261-Scramble and shTLX infectants grown subcutaneously in C57 mice. shTLX cells formed smaller tumors than Scramble control cells. (B-D) FCM analysis of TILs (CD3⁺CD4⁺ and CD3⁺CD8⁺), PD-L1⁺ population and TAMs (F4/80⁺CD163⁺ and F4/80⁺CD86⁺) population in GL261 allograft. (B) PD-L1⁺ population was significantly decreased in GL261-shTLX tumors, * $p < 0.05$ versus scramble control tumors. (C) CD3⁺ and CD8⁺ populations were significantly accumulated in GL261-shTLX tumors, * $p < 0.05$ versus scramble control. (D) M2 macrophage (F4/80⁺CD163⁺) but not M1 macrophage (F4/80⁺CD86⁺) population was remarkably decreased in GL261-shTLX tumors. * $p < 0.05$ versus scramble control tumors.

Supplementary Figure 5. TLX reverse agonist induce tumor growth arrest. (A) Schematic diagram of TLX reverse agonist ATRAL treatment. Mouse glioma CT2A cells (2×10^6) were subcutaneously

inoculated into C57 mouse. ATRAL at 20mg/kg and vehicle (4% DMSO) were intraperitoneal injected since 7th day post tumor implantation. Tumor size and weight were monitored every two days. (B) No obvious difference of mice body weight between ATRAL and vehicle group. (C) Photos of dissected tumors from ATRAL and vehicle treated mice. Tumor size was much smaller in ATRAL group. (D-E) Statistic analysis of tumor size from two groups. n=3. At least two independent experiments were performed.

Supplementary Figure 6. TLX overexpression enhances PD-L1 expression. (A) mRNA level of PD-L1 in PC3-TLX infectants by RT-qPCR analysis. Data are mean \pm SEM. * $p < 0.05$, *** < 0.001 . (B) Protein level of PD-L1 in PC3-TLX infectants by immunoblot analysis. (C) FACS-based measurement of PD-L1 expression (phycoerythrin (PE)-conjugated) by PC3-TLX cells compared with PC3-Scramble cells. (D) Frequency of PD-L1⁺ cells among PC3-Scramble cells compared PC3-TLX cells. Data are mean \pm SEM. ** $p < 0.01$.