Title page

The Binding of PD-L1 and Akt Facilitates Glioma Cell Invasion upon Starvation via Akt/autophagy/F-actin Signaling

Ruo Qiao Chen^{1,†}, Xiao Hong Xu^{2,†}, Feng Liu², Chun Yang Li², Yuan Jun Li², Xiang

Rui Li¹, Guo Yong Jiang¹, Feng Hu³, Di Liu⁴, Feng Pan^{4*}, Xin Yao Qiu^{2*}, Xiao Qian

Chen^{2*}

¹School of Basic Medicine, Tongji Medical College, Huazhong University of Science

and Technology, Wuhan, Hubei, China

²Department of Pathophysiology, School of Basic Medicine, Tongji Medical College,

Huazhong University of Science and Technology, Wuhan, Hubei, China

³Department of Neurosurgery, Tongji hospital, Tongji medical college, Huazhong

University of Science and Technology, Wuhan, Hubei, China

⁴Department of Urology, Union Hospital, Tongji Medical College, Huazhong

University of Science and Technology, Wuhan, Hubei, China

*Correspondence: Feng Pan, panfeng@hust.edu.cn; Xin Yao Qiu,

qiu.xinyao1991@outlook.com; Xiao Qian Chen, chenxq@mails.tjmu.edu.cn

[†]These authors have contributed equally to this work

Conflicts of Interest Statement: The authors declare no conflicts of interest.

Running title: PD-L1/Akt interaction promotes GBM invasion

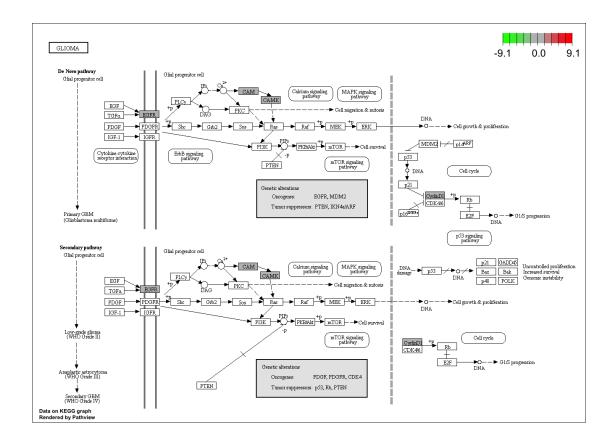


Figure S1. KEGG pathway analysis of PD-L1-altered genes in human glioma.

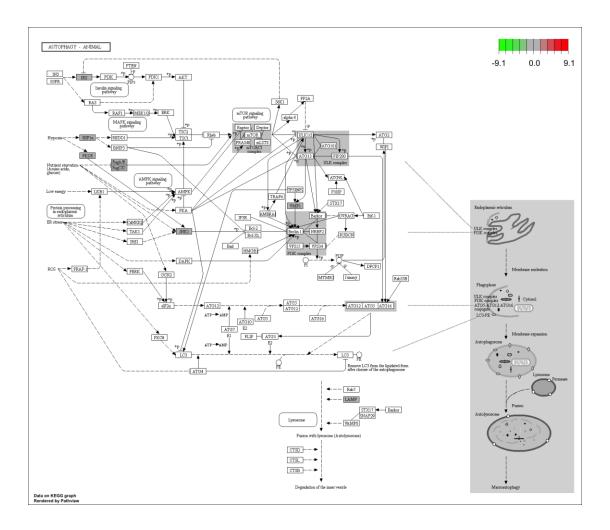


Figure S2. KEGG pathway analysis of PD-L1-altered genes in autophagy process.

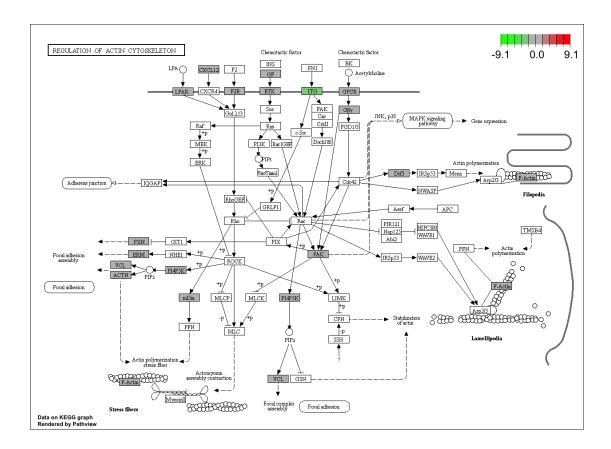


Figure S3. KEGG pathway analysis of PD-L1-altered genes in regulation of actin cytoskeleton.

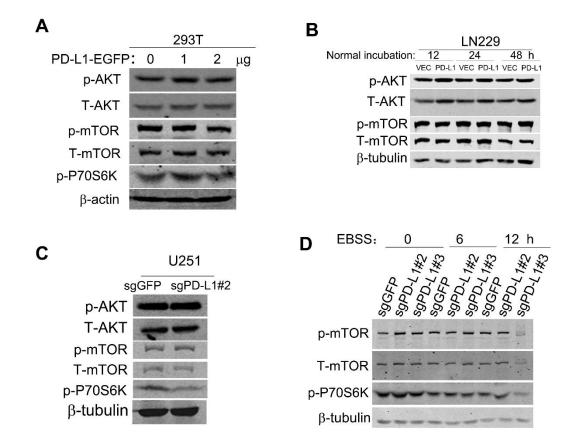


Figure S4. Effects of PD-L1 on Akt/mTOR signaling. (A) Effects of p-PD-L1-EGFP plasmid transfection of different plasmid amounts on p-Akt, Akt, p-mTOR, mTOR, p-P70S6K expression in 29T cells. (B) Effects of PD-L1 overexpression on p-Akt, Akt, p-mTOR, mTOR expression in LN229 cells at various time points after initial cell seeding. (C) Effects of PD-L1 knockout (sgCD274#2) on p-Akt, Akt, p-mTOR, mTOR, p-P70S6K expression in U251 cells. (D) Effects of PD-L1 knockout (sgCD274#2, #3) on p-mTOR, mTOR, p-P70S6K expression in U251 cells upon EBSS incubation.

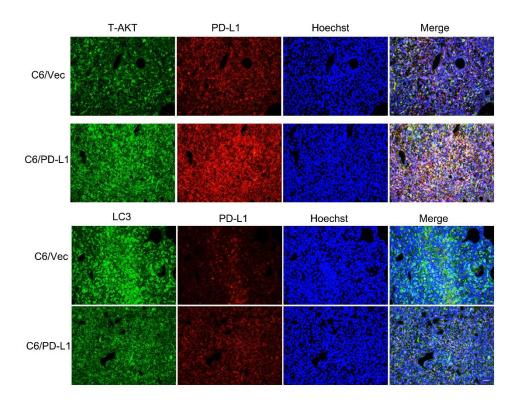


Figure S5. Double fluorescent-immunostaining shows the effects of PD-L1 overexpression on Akt and LC3 expression in orthotopic rat/C6 glioma tissues. C6 cells were lentiviral-infected for PD-L1 overexpression (C6/PD-L1). Equal amounts of C6/PD-L1 or C6/Vec were microinjected into right striatum of rats and the glioma tissues were subjected to analysis at 20 d after initial C6 cells injection. Bar, 20 μm.

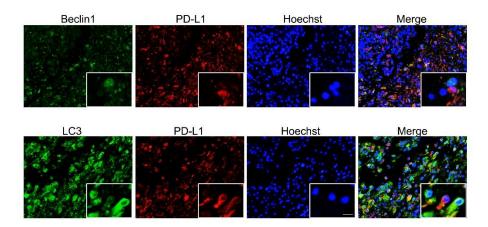


Figure S6. Double fluorescent immunostaining shows that higher PD-L1 expression in individual GBM cells is associated with lower Beclin-1 and LC3 expression in GBM patient tissue. Bar, $20~\mu m$.

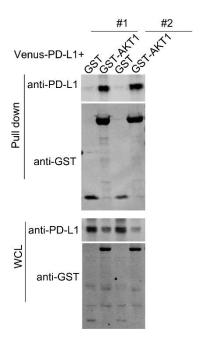


Figure S7. GST-pull down shows that PD-L1 binds to Akt. WCl, whole cell lysate.