



## Supplemental Table and Figures for:

## Acetylcholine Receptor Activation as a Modulator of Glioblastoma Invasion

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		Mutation	Amino	Chromosome
Sample	Gene	Turno	Acid	
		Type	change	
TCGA.76.6660	CHRM1	Missense	G450D	11
TCGA.02.2485	CHRM2	Missense	K376E	7
TCGA.28.5207	CHRM2	Missense	G238R	7
TCGA.76.6285	CHRM2	Missense	R363H	7
TCGA.06.0155	CHRM3	Missense	A495V	1
TCGA.06.5858	CHRM4	Missense	R473Q	11
TCGA.32.4210	CHRM4	Missense	A263T	11
TCGA.06.0649	CHRNA10	Missense	R387Q	11
TCGA.19.2629	CHRNA10	Missense	M303V	11
TCGA.06.0744	CHRNA4	Missense	G625D	20
TCGA.76.4931	CHRNB2	Missense	Q59*	1
TCGA.06.0645	CHRNB4	Missense	D259N	15
TCGA.19.5953	CHRNB4	Missense	V448I	15
TCGA.06.2570	CHRND	Missense	T84K	2
TCGA.19.2631	CHRND	Missense	Y239H	2
TCGA.16.1048	CHRND	Missense	R244H	2
TCGA.76.6657	CHRNG	Missense	A44T	2

Table S1. Annotated mutations of AChRs in TCGA GBM dataset.

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**Figure S1.** Expression of AChRs in GBM cell and xenograft lines. qPCR analysis of AChR expression (*CHRM1-5; CHRNA3-7; CHRNB2-4*) in adherent GBM cell lines (D54 and U251) and patient-derived xenograft (PDX) lines (PDX14 and PDX22). Gene expression is expressed relative to internal reference gene *IPO8* and then relative to control tissue from human brain cortex (Human Ctx). Expression was not found for *CHRNA3* in the PDX14 and PDX22 lines, *CHRNA4* in the U251, PDX14, and PDX22 lines, *CHRNA7* in the PDX22 line, *CHRNB3* in the U251 line, *CHRNB4* in all cell and PDX lines.



**Figure S2.** Calcium responses to AChR stimulation in D54 and PDX22 lines. Representative graphs of  $[Ca^{2+}]_i$  changes with 1mM ACh application in the D54 and PDX22 lines (**a**, **b**).



Figure S3. Calcium response to 1µM ACh. [Ca<sup>2+</sup>]i changes in U251 line with 1µM ACh application.



**Figure S4.** Calcium response to AChR stimulation in D54-GCaMP cell line. [Ca<sup>2+</sup>]<sub>i</sub> changes with application of 1mM ACh in the D54 cells utilizing genetically encoded calcium sensor, GCaMP3.



**Figure S5.** MMP-9 activity is not affected by AChR inhibition. AChR inhibitors (AM, 1 $\mu$ M atropine and 10 $\mu$ M mecamylamine) were applied to U251 cells and the conditioned media was collected at 24hr for gelatin zymography analysis. MMP-9 activity was not significantly difference between the control and AM condition. A student's test was used for statistical analysis, p=0.8045.



**Figure S6.** Increased CHRM3 expression correlates to decreased survival times. Kaplan-Meier survival curve of TCGA GBM patients grouped by *CHRM3* mRNA expression (HG-U133A), high vs. low expression was defined by calculating the optimal cutoff value of 5.76 with maximally selected rank statistics. The log-rank test was used to compare survival differences between GBM patients with high vs. low *CHRM3* expression, p<0.001\*\*\*.