Identification of Survival Genes in Human Glioblastoma Cells Using siRNA Screening

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Molecular Pharmacology

SUPPLEMENTARY FIGURES

Supplementary Figure S1. Validation of survival gene hits. Survival gene candidates were re-assayed with the 3 individual unpooled siRNA sequences to validate the efficacy of each targeting siRNA. Percent viabilities following inhibition with targeting siRNAs were normalized to in-plate negative control wells (scrambled). Negative control well viability was normalized to untreated cells. A positive control (siRNA that causes cell death) was chosen for its significant cytotoxic effect in this cell line. *PSMA3, PSMC3, PSMD14,* and *RAN* revalidated with 3 unpooled sequences; *PSMB4* revalidated with 2 unpooled sequences; *DDX39* revalidated with only 1 unpooled sequence. Each experiment was performed at least in triplicate, and error bars represent SEM.

Supplementary Figure S2. Expression of PSMB1, PSMB2, and PSMB5 in human glioma, endothelial cells and astrocytes. Protein levels were determined from cell lysates by Western blotting and quantified in two blots using tubulin as the loading control.

Supplementary Figure S3. Biological pathways represented by survival genes. IPA analysis revealed the biological pathways represented by survival genes. Significance values refer to the negative log of the *p*-value. These pathways included protein ubiquitination (*PSMA1, PSMA2, PSMA3, PSMA4, PSMA5, PSMA6, PSMB2, PSMB4, PSMC3, PSMC5, PSMC6, PSMD14;*

significance: 11.6), purine metabolism (*DDX39, KIF20B, POLR2A, POLR2F, POLR2G, PSMC3, PSMC5, PSMC6, RRM1;* 5.9), nucleotide excision repair (*POLR2A, POLR2F, POLR2G* ; 3.4), pyrimidine metabolism (*POLR2A, POLR2F, POLR2G, RRM1;* 2.6), and NF-κB (*AKT3, ITGB3, PRKCD;* 2.5). Twelve of 55 survival genes were proteasome components.