ADAR3 Binding to Glutamate Receptor Subunit B Pre-mRNA Inhibits RNA Editing in Glioblastoma
Eimile Oakes, Ashley Anderson, Aaron Cohen-Gadol, Heather A. Hundley

Supplemental Data Included:
Figure S1
Figure S2
Table S1

Figure S1

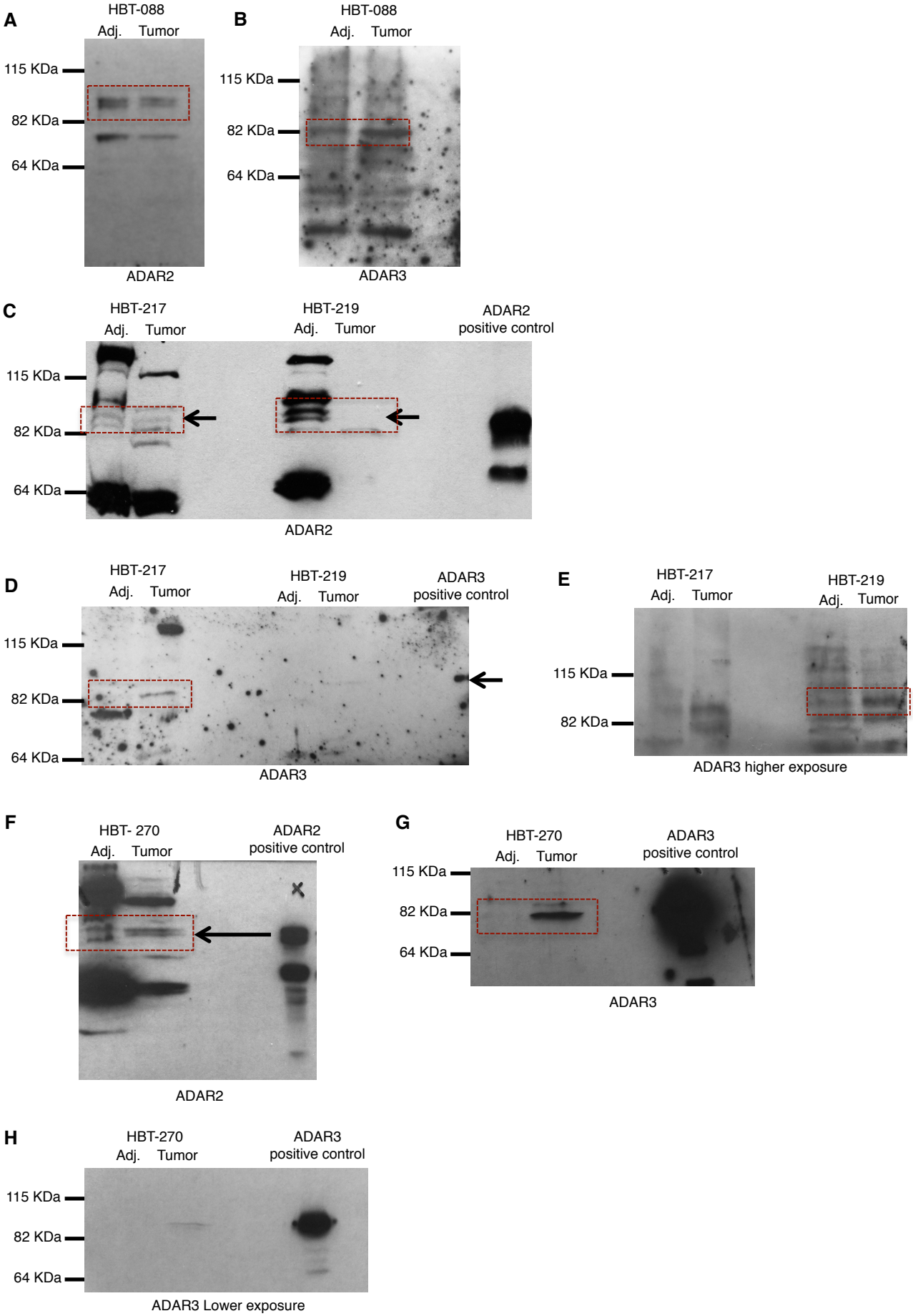
A
HBT-088
Adj. Tumor
115 KDa
82 KDa
64 KDa
ADAR2
B
HBT-088
Adj. Tumor
115 KDa
82 KDa
64 KDa
ADAR3
C
HBT-217
Adj. Tumor
HBT-219
Adj. Tumor
ADAR2
positive control
115 KDa
82 KDa
64 KDa
ADAR2
D
HBT-217
Adj. Tumor
HBT-219
Adj. Tumor
ADAR3
positive control
115 KDa
82 KDa
64 KDa
ADAR3
E
HBT-217
Adj. Tumor
HBT-219
Adj. Tumor
115 KDa
82 KDa
ADAR3 higher exposure
F
HBT- 270
Adj. Tumor
ADAR2
positive control
ADAR2
G
HBT-270
Adj. Tumor
ADAR3
positive control
115 KDa
82 KDa
64 KDa
ADAR3
H
HBT-270
Adj. Tumor
ADAR3
positive control
115 KDa
82 KDa
64 KDa
ADAR3 Lower exposure

Figure S1

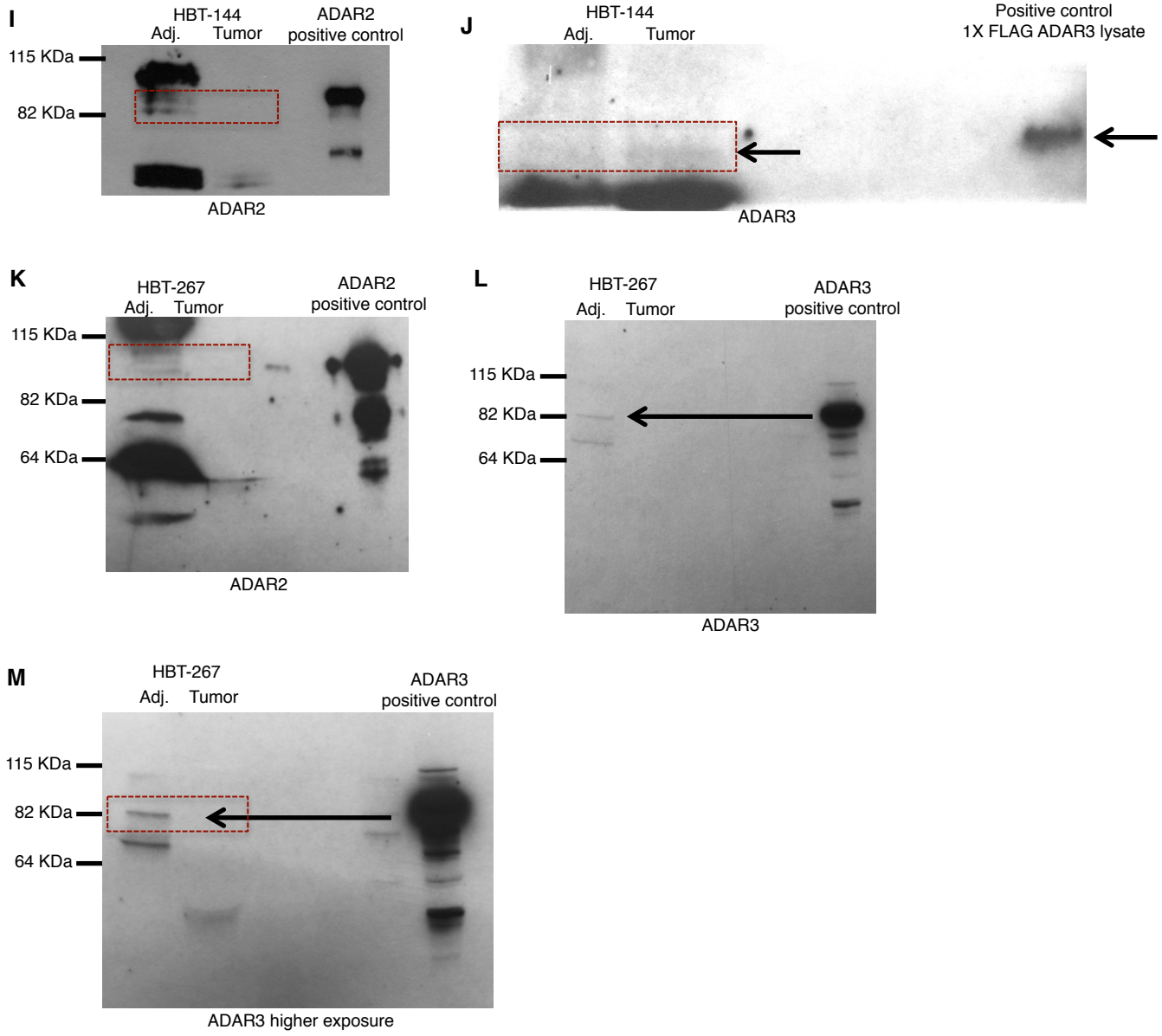

FIGURE S1. **ADAR2 and ADAR3 protein expression in glioblastoma tissues and matched adjacent tissue.** (A-M) Lysates from glioblastoma tissue (Tumor) and matched adjacent tissue (Adj.) were subjected to SDS-PAGE and immunoblotting for ADAR2 or ADAR3. The positive control for ADAR2 is 10X His-ADAR2 and the positive control for ADAR3 is lysate from a cell line expressing FLAG-ADAR3. The red-dotted line boxes indicate the portion of the final immunoblot that was cropped for Figure 5 of the manuscript.

Figure S2

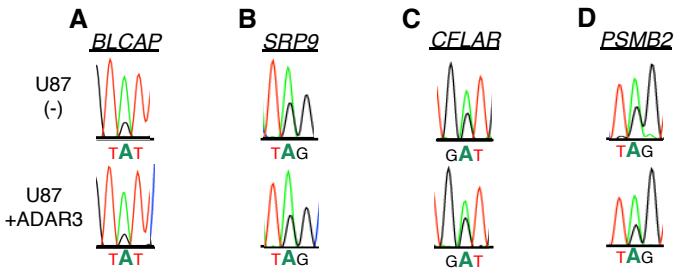

FIGURE S2. **ADAR3 expression does not effect editing at all adenosines** (A-D) Chromatograms of several edited transcripts in U87 cells transduced with retrovirus containing a neomycin resistant vector with no protein (-) or human ADAR3 expressed from the CMV promoter. Black indicates transcripts with guanosine (edited) and green indicates transcripts with adenosine (unedited). Bold A indicates the genomically encoded adenosine that is edited.

Table S1

		p-value	
Gene name	**Function**	**Replicate #1**	**Replicate #2**
FBRL	rRNA 2'-O-methyltransferase fibrillarin	0.0048	0.043
ILF3	Interleukin enhancer-binding factor 3	0.0016	0.015
NOP2	Probable 28S rRNA (cytosine(4447)-C(5))-methyltransferase	0.00056	0.015
NOP58	Nucleolar protein 58	0.0016	0.073
ADAR3	Double-stranded RNA-specific editase B2	< 0.00010	< 0.00010

TABLE S1. **ADAR3 interacting proteins identified by mass spectrometry.** Immunoprecipitates (IPs) from NHA cells transduced with retrovirus containing a neomycin resistant vector with no protein (control) or human ADAR3 with an N-terminal 3X FLAG epitope expressed from the CMV promoter were subjected to proteolytic digestion and MS/MS using an LTQ-Velos Pro Orbitrap (IUSM Proteomics Core). In both biological replicates, multiple peptides for each protein listed in the table were detected in the IPs from 3X FLAG ADAR3 cells, while no peptides were present in the IPs from the control cell line using requirements of 2 minimum peptides for each protein and filters for a 1% False Discovery Rate for each peptide and a 99% probability for each protein identified. The *p-values* were obtained using a Fisher's exact test on the total number of spectra present in the 3X FLAG ADAR3 samples compared to the control samples using Scaffold.