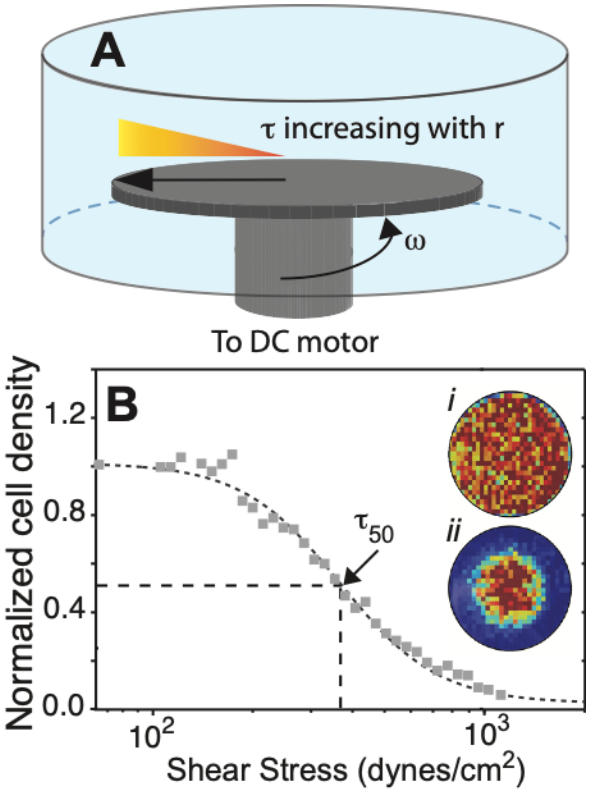

Figure S1: Spinning Disc Creates a Radially-dependent Shear Profile. (A) An illustration of the spinning disc device is shown indicating the radially-dependent shear profile (red to yellow gradient) created by the angular velocity, which causes lineage velocity at the center to effectively be zero while those at the edge move around at a high linear velocity. (B) Plot of cell density, normalized to the center of the coverslip, versus the applied shear. Data is plotted for the indicated velocities. τ_{50}, i.e. the shear to detach 50% of cells, is indicated in the plot. Inset images show heat maps of cell density. Warm (red) and cool (blue) colors indicate high and low densities, respectively.

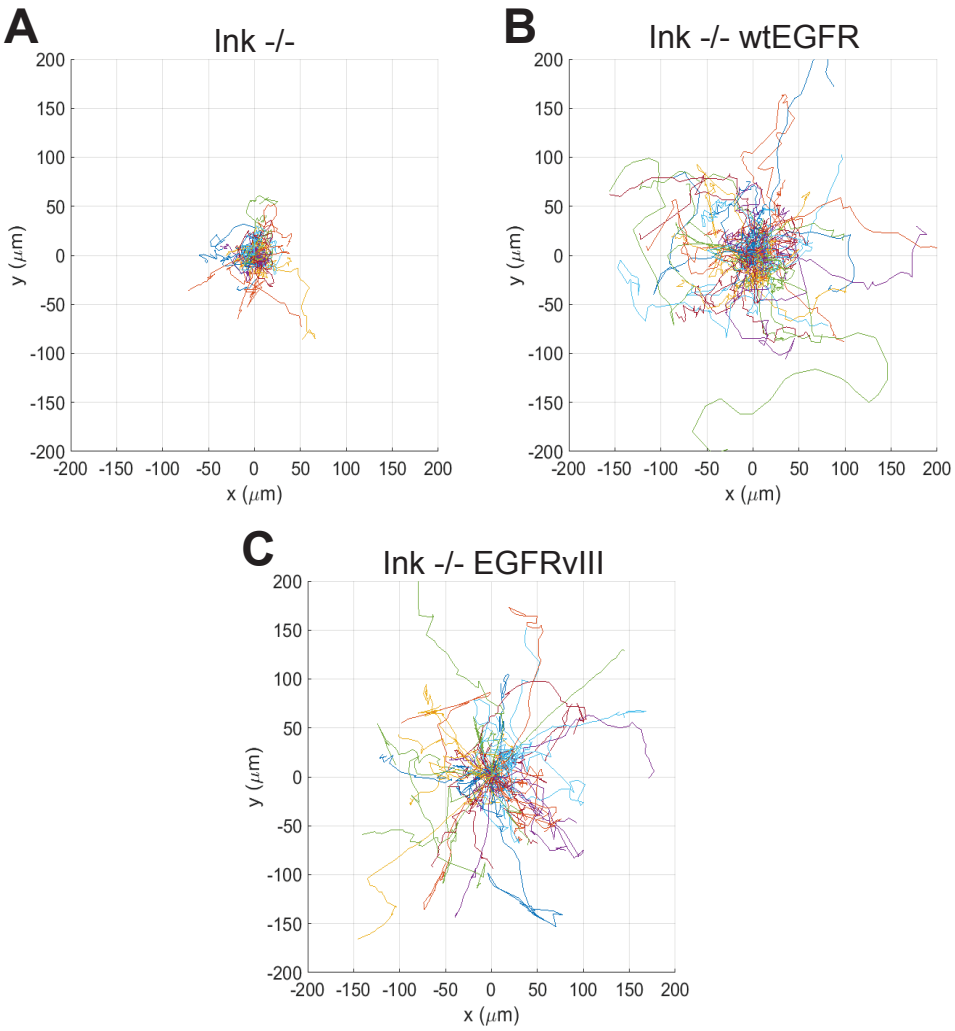

Figure S2: Migration is Driven by Receptor Truncation. Rose plots are shown for individual cells of a common genotype (each colored differently). Path length, distance traveled, and velocity were all measured from these traces. For ease of view, a limited number of traces are shown.

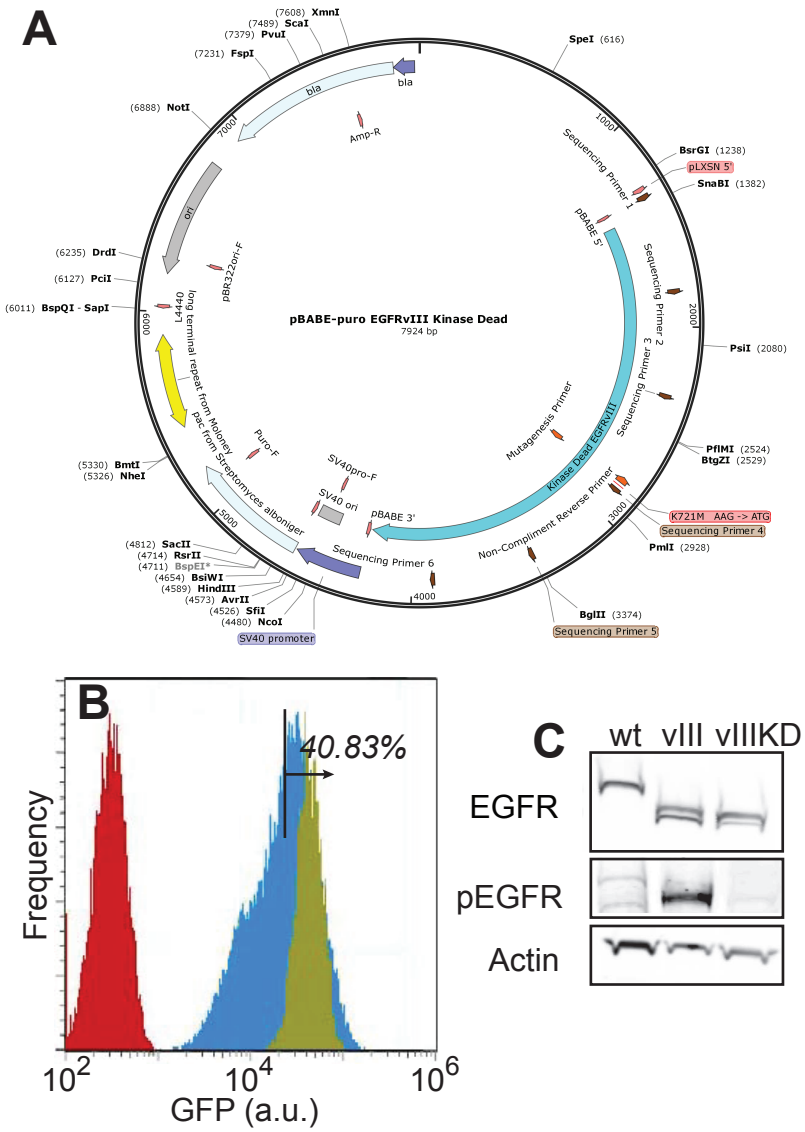

Figure S3: Construction of isogenic EGFRvIIIKD cell line. (A) Plasmid map of the puromycin-selectable lysine to methionine substitution (K721M) (Huang et al., 1997) construct for EGFR. (B) EGFR fluorescently-labeled cells were analyzed by flow cytometry for IgG only EGFRvIII (red), dual-labeled EGFRvIII (gold) and dual-labeled EGFRvIIIKD (blue). The gate indicates those cells that were serially sorted and puromycin-selected to establish a stable population. (C) Western blots of wtEGFR, EGFRvIII, and EGFRvIIIKD cells for EGFR, phosphorylated EGFR, and actin.

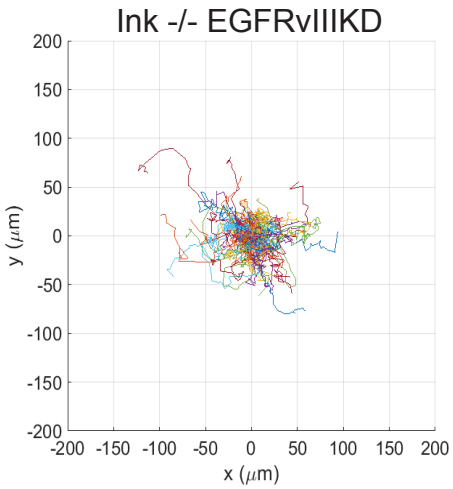

Figure S4: Migration of isogenic EGFRvIIIKD. Rose plot is shown for individual cells of EGFRvIIIKD. Path length, distance traveled, and velocity were all measured from these traces. For ease of view, a limited number of traces are shown.

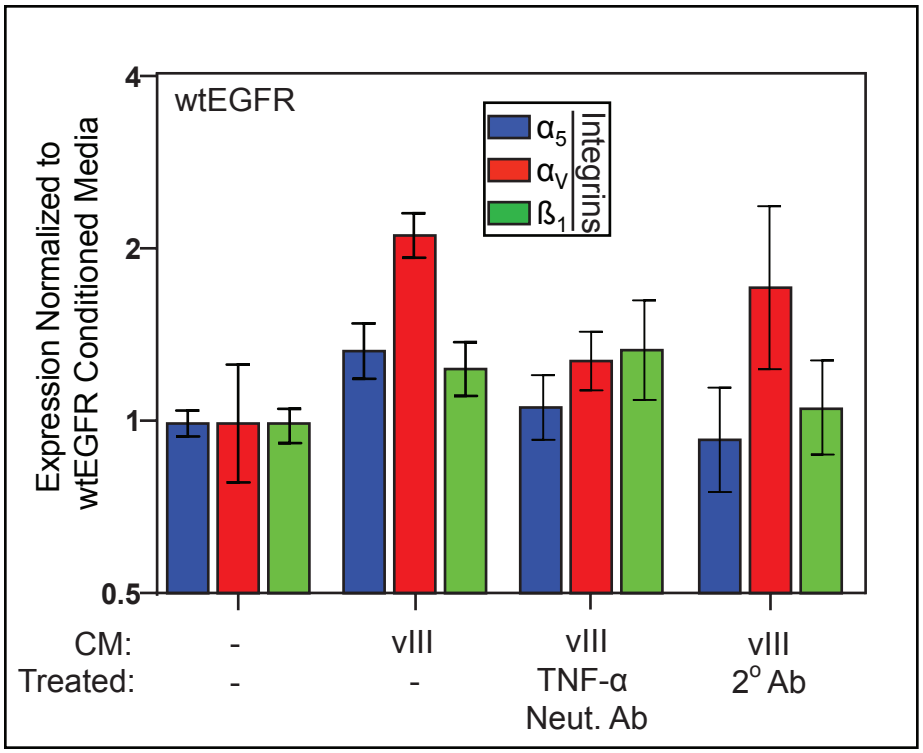

Figure S5: Integrin expression in wtEGFR cells treated with vIII conditioned media. Plot of transcript expression of the indicated integrin genes showing the fold increase in wtEGFR cells treated with the indicated conditioned media and antibodies. Data are normalized to untreated wtEGFR cells (n=3 biological replicates).

Supplemental Tables

Table S1: Mouse Astrocyte Lines. This table shows the genotypes of each line.

Ink4a/Arf	Pten	EGFR	Tumor Characteristics
-/-	+/+	N/A	Non-Tumorigenic
-/-	-/-	N/A	Tumorigenic
-/-	+/+	wt	Tumorigenic
-/-	+/+	vIII	Tumorigenic Invasive
-/-	-/-	vIII	Tumorigenic Invasive
-/-	+/+	Kinase Dead vIII	Tumorigenic

Table S2: PCR primers. This table shows all of the qPCR primers and in which figure each primer was used.

Primer Name	5'-Primer Sequence-3'
Kinase Dead EGFRvIII Forward Mutagenesis Primer	GCT ATC ATG GAA TTA AGA GAA GCA
Kinase Dead EGFRvIII Forward Mutagenesis Primer	TTT AAC TTT CTC ACC TTC TGG GAT CCA GAG T
Kinase Dead EGFRvIII Sequencing Primer 1	TTT ATC CAG CCC TCA CTC CTT CTC TAG
Kinase Dead EGFRvIII Sequencing Primer 2	GGG TTT TTG CTG ATT CAG GCT TGG
Kinase Dead EGFRvIII Sequencing Primer 3	CAG ACA ACT GTA TCC AGT GTG CCC
Kinase Dead EGFRvIII Sequencing Primer 4	AAC ATC TCC GAA AGC CAA CAA GG
Kinase Dead EGFRvIII Sequencing Primer 5	TCC ATC CTG GAG AAA GGA GAA CGC
Kinase Dead EGFRvIII Sequencing Primer 6	AAA CCA GTC CGT TCC CAA AAG G
Mouse Integrin AlphaV Forward Primer	CCG TGG ACT TCT TCG AGC C
Mouse Integrin AlphaV Reverse Primer	CTG TTG AAT CAA ACT CAA TGG GC
Mouse Integrin Alpha5 Forward Primer	CTT CTC CGT GGA GTT TTA CCG
Mouse Integrin Alpha5 Reverse Primer	GCT GTC AAA TTG AAT GGT GGT G
Mouse Integrin Beta1 Forward Primer	ATG CCA AAT CTT GCG GAG AAT
Mouse Integrin Beta1 Reverse Primer	TTT GCT GCG ATT GGT GAC ATT
Mouse Integrin Beta Actin Forward Primer	GGC TGT ATT CCC CTC CAT CG
Mouse Integrin Beta Actin Reverse Primer	CCA GTT GGT AAC AAT GCC ATG T