Supporting Information

Ying et al. 10.1073/pnas.0914930107

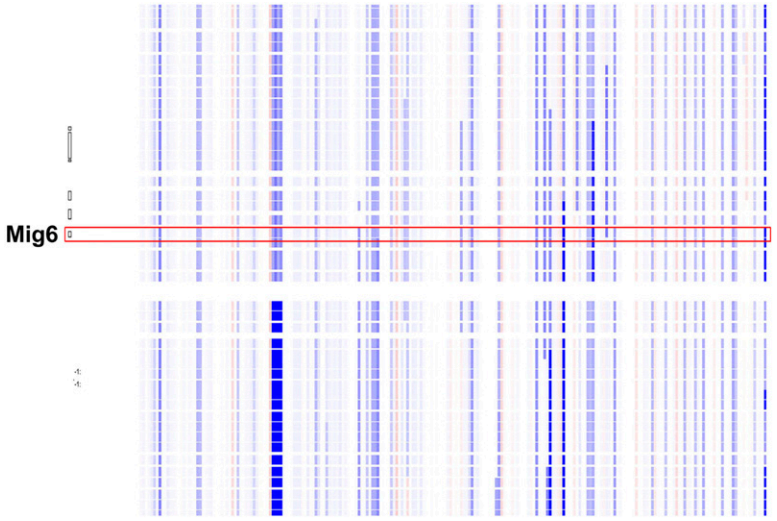
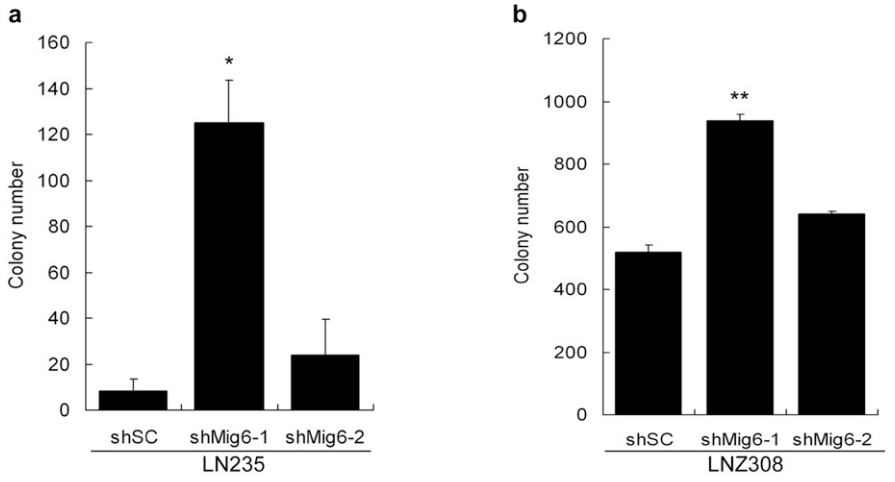


Fig. S1. Deletion of Mig-6 in TCGA dataset. Array-CGH heat map details Mig-6 deletion at chromosome 1p36 in TCGA dataset. Regions of genomic amplification and deletion are denoted in red and blue, respectively.



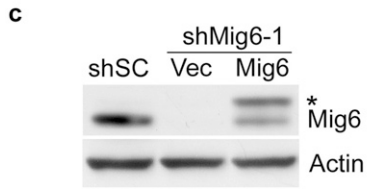


Fig. S2. Knocking down of Mig-6 promotes anchorage-independent growth. Knocking down of Mig-6 expression in (*A*) LN235 and (*B*) LNZ308 cells promotes anchorage-independent growth in soft agar. Error bars indicate ±SD (*, $P = 0.013$; **, $P = 0.003$; $n = 3$). (*C*) Mig-6 expression was reconstituted in U87 cells expressing shRNA against Mig-6. *, HA-tagged Mig-6.

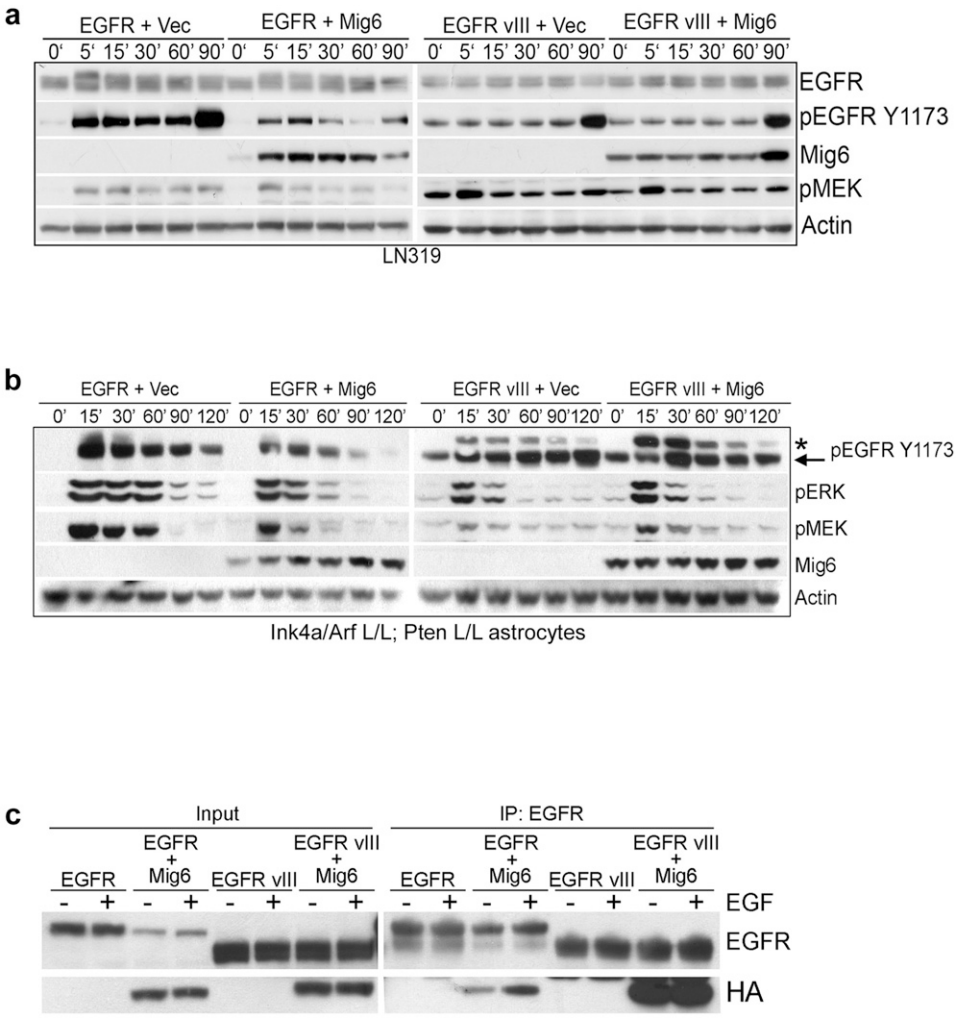


Fig. S3. Mig-6 fails to regulate EGFRvIII activation and downstream signaling. Ectopic expression of HA-tagged Mig-6 in (*A*) LN319 cells and (*B*) murine *INK*$^{-/-}$; *Pten*$^{-/-}$ astrocytes stably expressing EGFRvIII shows no effect on EGFR phosphorylation or activation of downstream signaling pathways, whereas expression of Mig-6 in cells with wild-type EGFR inhibits EGFR activation and downstream signaling induced by EGF treatment. *, endogenous EGFR; arrow, EGFRvIII. (*C*) Mig-6 binds with EGFRvIII and the interaction is not regulated by EGF stimulation. Murine *INK*$^{-/-}$; *Pten*$^{-/-}$ astrocytes expressing HA-tagged Mig-6 together with wild-type or vIII forms of EGFR were treated with EGF (20 ng/mL) for 30 min. Cell lysates were subjected to IP with human-specific anti-EGFR antibody and immunoblotted with the indicated antibodies.

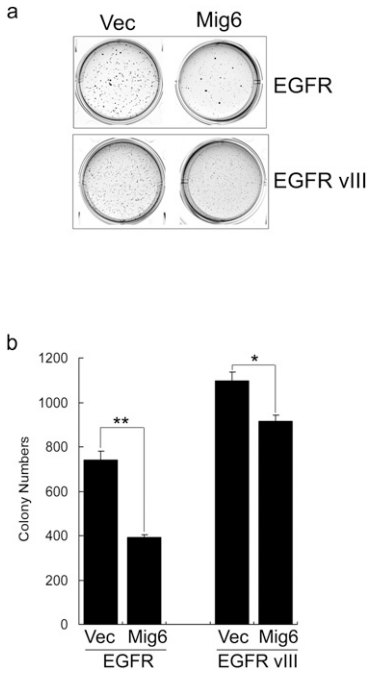


Fig. S4. GBM cells with EGFRvIII are more resistant to Mig-6 expression. (*A*) Reconstitution of Mig-6 expression in LN319 cells expressing wild-type or the vIII mutant form of EGFR attenuates anchorage-independent growth in soft agar. (*B*) Histogram quantification. Error bars indicate ±SD (**, $P = 0.007$; *, $P = 0.03$; $n = 3$).

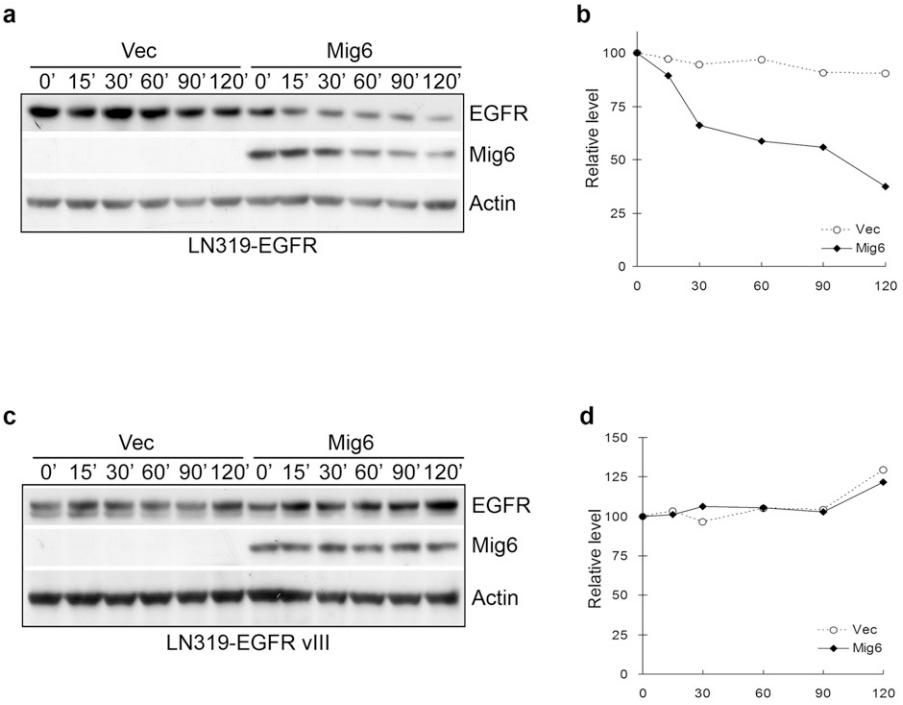


Fig. S5. Mig-6 fails to regulate EGFRvIII degradation. (*A*) Reconstitution of Mig-6 expression in LN319 cells expressing wild-type EGFR promotes EGFR degradation induced by EGF stimulation. Cells were pretreated with cycloheximide (CHX) (10 μg/mL) for 1 h before being treated with EGF (20 ng/mL) in the presence of CHX for the indicated times and cell lysates were subjected to immunoblotting with the indicated antibodies. (*B*) Histogram quantification of EGFR level (normalized with actin level) in *A*. (*C*) Reconstitution of Mig-6 expression in LN319 cells expressing EGFRvIII fails to promote EGFR degradation in response to EGF stimulation. Cells were treated as described in *A* and lysates were subjected to immunoblotting with the indicated antibodies. (*D*) Histogram quantification of EGFR level (normalized with actin level) in *C*.

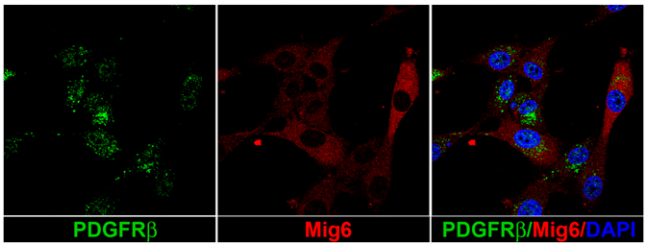


Fig. S6. Mig-6 does not colocalize with PDGFRβ in vesicle structures. U87 cells were treated with PDGF-BB (10 ng/mL) for 30 min and subjected to immunofluorescence staining with anti-PDGFRβ (green), anti-Mig-6 (red), and DAPI (blue).

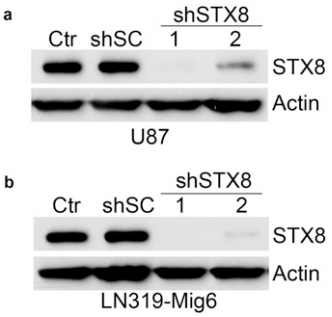
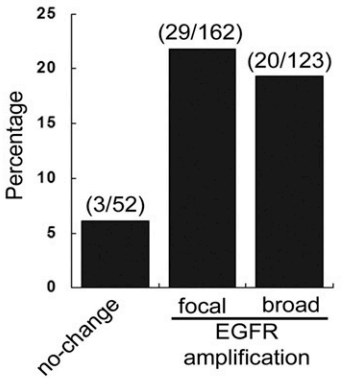


Fig. S7. Depletion of STX8 expression by shRNA. STX8 expression in (*A*) U87 and (*B*) LN319-Mig-6 cells was knocked down by two independent shRNAs (shSTX8-1 and shSTX8-2).

a



b

	EGFR vIII	EGFR others	
Mig6 deletion/loss	9	24	
No Mig6 change	10	112	
Percentage	47%	18%	p=0.006

Fig. S8. Genetic correlation between Mig-6 and EGFR in human GBM samples. (*A*) Incidence of Mig-6 deletion/loss in three groups of samples with different EGFR status. Numbers are presented as number of samples with Mig-6 deletion/loss over total sample numbers in each group. (*B*) Incidence of Mig-6 deletion/loss and EGFRvIII mutation in samples with EGFR focal amplification.

Table S1. Summary of yeast two-hybrid interactors

Gene	Description	Gene ID
ACBD3	Acyl-CoA binding domain containing 3	64746
AOX1	Aldehyde oxidase 1	316
ARRDC5	Arrestin domain containing 5	645432
C13orf18	Chromosome 13 ORF 18	80183
CRHBP	Corticotropin releasing hormone binding protein	1393
DCTN6	Dynactin 6	10671
EFEMP1	EGF-containing fibulin-like extracellular matrix protein 1	2202
FAM96B	Family with sequence similarity 96, member B	51647
GAS1	Growth arrest-specific 1	2619
GNPDA2	Glucosamine-6-phosphate deaminase 2	132789
GRB2	Growth factor receptor-bound protein 2	2885
GTF2H2	General transcription factor IIH, polypeptide 2	2966
HNRPA3	Heterogeneous nuclear ribonucleoprotein A3	220988
HYDIN	Hydrocephalus inducing homolog	54768
IL2RG	Interleukin 2 receptor, gamma	3561
JAB1	*Homo sapiens* mRNA for COP9 signalosome subunit 5 variant protein	10987
LOC401397	Hypothetical LOC401397	401397
MFSD5	Major facilitator superfamily domain containing 5	84975
MMP26	MMP26 matrix metallopeptidase 26	56547
NEU1	Sialidase 1 (lysosomal sialidase)	4758
NPC2	Niemann–Pick disease, type C2	10577
OSTF1	Osteoclast stimulating factor 1	26578
PAPSS1	3′-Phosphoadenosine 5′-phosphosulfate synthase 1	9061
PCCA	Propionyl CoA carboxylase, alpha polypeptide	5095
PCNA	Proliferating cell nuclear antigen	5111
PDCD2	Programmed cell death 2 isoform 1	5134
PHYHIPL	Phytanoyl-CoA 2-hydroxylase interacting protein-like	84457
PLEKHA3	Pleckstrin homology domain containing, family A	65977
PPIA	Peptidylprolyl isomerase A (cyclophilin A)	5478
PSMA7	Proteasome (prosome, macropain) subunit, alpha type, 7	5688
PSMB6	Proteasome (prosome, macropain) subunit, beta type, 6	5694
RAB18	RAB18, member RAS oncogene family	22931
SH3YL1	SH3 domain containing, Ysc84-like 1	26751
SLC7A13	Solute carrier family 7	157724
STX8	Syntaxin 8	9482
TC2N	TC2N tandem C2 domains, nuclear	123036
TIMP4	Tissue inhibitor of metalloproteinase 4	7079
TM2D3	TM2 domain containing 3	80213
TMDCII	ADAM metallopeptidase domain 5 pseudogene	255926
TMEM165	Transmembrane protein 165	55858
TMEM59	Transmembrane protein 59	9528
WDR61	WD repeat domain 61	80349
YWHAB	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide	7529
ZPBP	Zona pellucida binding protein	11055

Interactors identified two times or more are shown in bold.