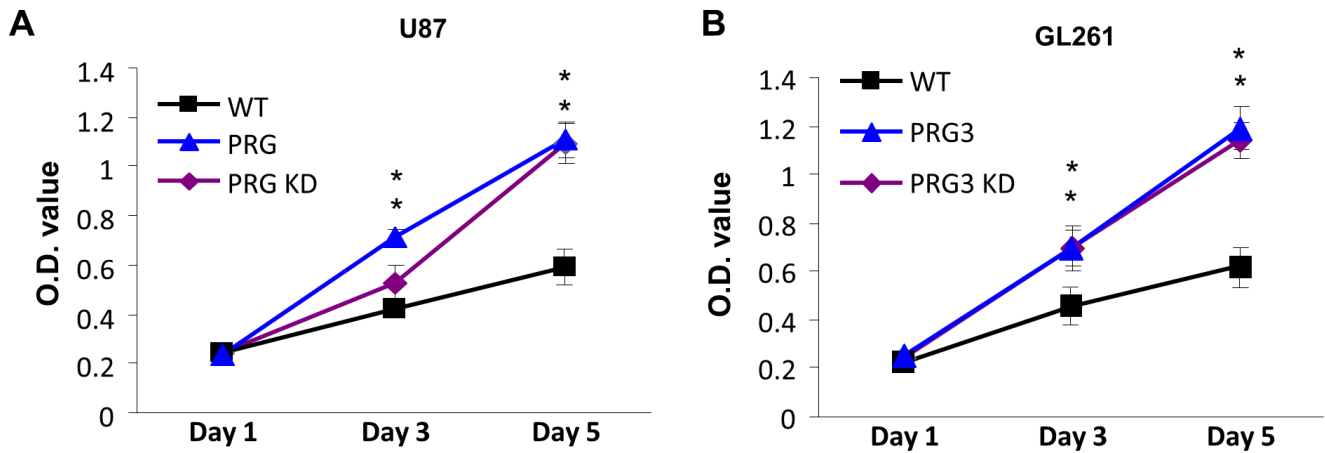


## PRG3 induces Ras-dependent oncogenic cooperation in gliomas

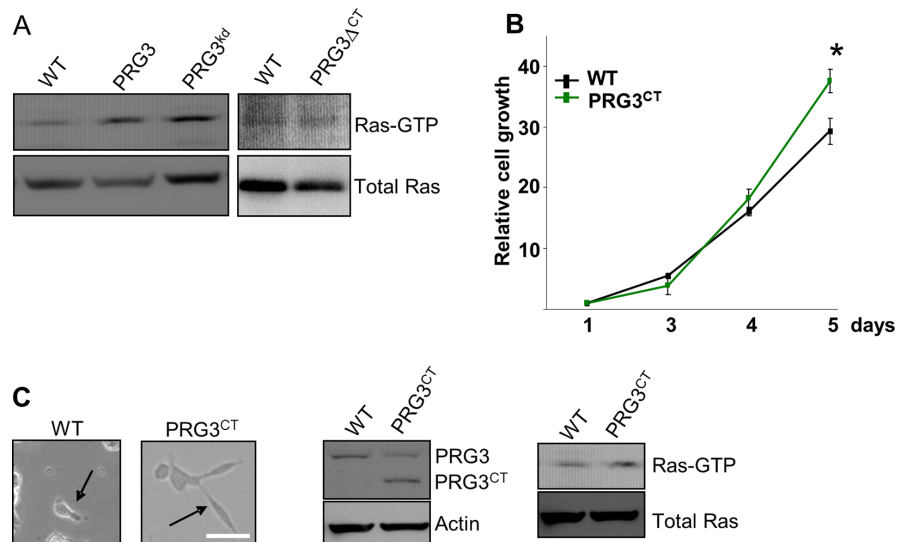
### Supplementary Materials

	10	20	30	40	50	60
hPRG-3	MAVGNNTQRS	YSIIPCFFIV	ELVIMAGTVL	LAYYFECTDT	FQVHIQGFFC	QDGDLMKPYP
mPRG-3	MAVENNTQRS	YSIIPCFFIV	ELVIMAGTVL	LAYYFECTDT	FQVHIQGFFC	QDGDLMKPYP
rPRG-3	MAVENNTQRS	YSIIPCFFIV	ELVIMAGTVL	LAYYFECTDT	FQVHIQGFFC	QDGDLMKPYP
	TM2 70	80	90	100	110	120
hPRG-3	GTEEESFITP	LVLYCVLAAT	PTAIFIGEI	SMYFIKSTRE	SLIAQEKTIIL	TGECCYLNPL
mPRG-3	GTEEESFISP	LVLYCVLAAT	PTAIFIGEI	SMYFIKSTRE	SLIAEEKMIL	TGDCCYLSPL
rPRG-3	GTEEESFISP	LVLYCVLAAT	PTAIFIGEI	SMYFIKSTRE	SLIAEEKMIL	TGDCCYLSPL
	TM3 130	140	150	160	170	180
hPRG-3	LRRIRFTGV	FAFGLFATDI	FVNAGQVVTG	HLTPYFLTVC	KPNYTSADCQ	AHQFINNGN
mPRG-3	LRRIRFIGV	FAFGLFATDI	FVNAGQVVTG	HLTPYFLTVC	QPNYTSTDCR	AHQFINNGN
rPRG-3	LRRIVRFIVG	FAFGLFATDI	FVNAGQVVTG	HLTPYFLTVC	QPNYTSTDCR	AHQFINNGN
	190	200	TM4 210	220	230	TM5 240
hPRG-3	ICTGDLEVIE	KARRSFPSKH	AALSIYSALY	ATMYITSTIK	TKSSRLAKPV	LCLGTLCTAF
mPRG-3	ICTGDLEVIE	KARRSFPSKH	AALSIYSALY	ATMYITSTIK	TKSSRLAKPV	LCLGTLCTAF
rPRG-3	ICTGDLEVIE	KARRSFPSKH	AALSIYSALY	ATMYITSTIK	TKSSRLAKPV	LCLGDLCTAF
	250	TM6 260	270	280	290	300
hPRG-3	LTGLNRVSEY	RNHCSVDVIAG	FILGTAVALF	LGMCVVHNEK	GTQGSPSKPK	PEDPRGVPLM
mPRG-3	LTGLNRVSEY	RNHCSVDVIAG	FILGTAVALF	LGMCVVHNER	GTQGSPSKPK	PEDPRGVPLM
rPRG-3	LTGLNRVSEY	RNHCSVDVIAG	FILGTAVALF	LGMCVVHNEK	GTQGSASKPK	PEDPRGVPLM
	310	320	330			
hPRG-3	AFPRIESPLE	TLSAQNHSAS	MTEVT.....			
mPRG-3	AFPRIESPLE	TLSAQNHSAS	MTEVT*.....			
rPRG-3	AFPRIESPLE	TLSAQNHSAS	MTEVT.....			

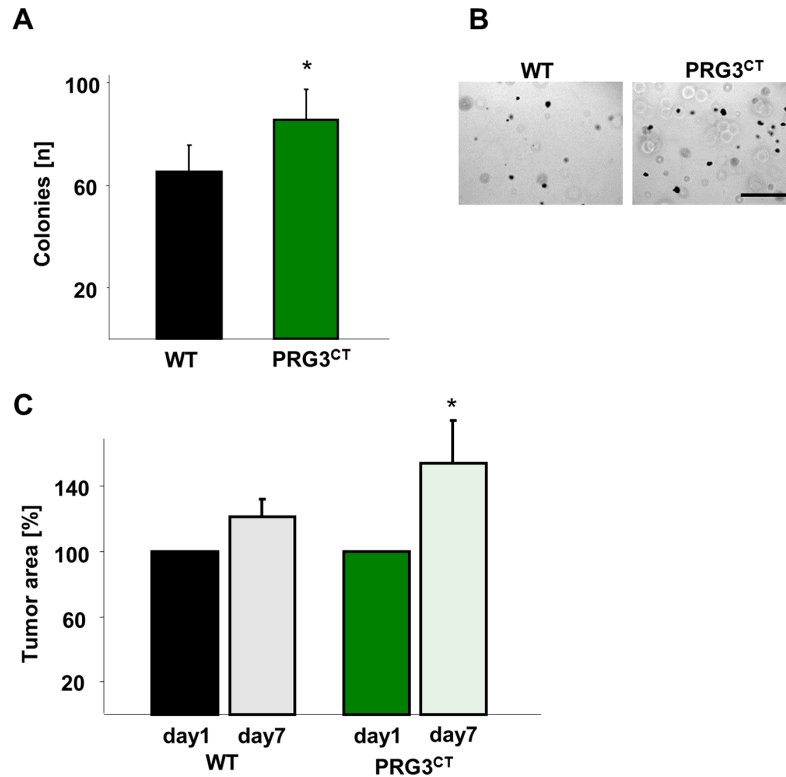
**Supplementary Figure S1: PRG3 amino acid sequence alignment of human, rat and mouse species.** Amino acid sequence of human PRG3 (GenBank Accession no. AY337718) aligned to mouse PRG3 (GenBank Accession no. AY345342) and rat PRG3 (GenBank Accession no. AY299399). Putative transmembrane domains TM1 - TM6 are boxed (black lines).



**Supplementary Figure S2: PRG3 expression enhances human tumor proliferation.** (A) PRG3 boosts growth in human glioma cells. Quantitative growth analysis of human glioma cells (U87) over time. Note that PRG3 overexpressing cells grow significantly faster than wild-type (WT) gliomas. Statistical significance was calculated with Student's *t*-test (mean  $\pm$  SD,  $n > 12$  per group;  $*P < 0.05$ ). (B) PRG3 boosts growth in murine glioma cells. Quantitative analysis of cell growth in murine glioma cells (GL-261) over time. Note that PRG3 overexpressing cells grow significantly faster than RFP control transfected cells. Statistical significance was calculated with Student's *t*-test (mean  $\pm$  SD,  $n = 12$  per group;  $*P < 0.05$ ). Abbreviation: OD, optical density.



**Supplementary Figure S3: (A) Left, Perturbed PRG3 expression leads to elevated Ras activation.** Upper blot gives levels of activated GTP-Ras in wild-type (WT), PRG3 overexpressing (PRG3) and PRG3-knockdown (PRG3<sup>kd</sup>) gliomas. Total Ras served as controls for equal loading (bottom panel). *Right*, C-terminal tail deleted PRG3 construct does not affect Ras signaling. (B) PRG3 promotes cell growth through its C-terminal domain. Quantitative analysis of cell growth over time of parental (WT) and PRG3-C-terminal domain expressing (PRG3<sup>CT</sup>) glioma cells. Statistical significance was calculated with Student's *t*-test (mean  $\pm$  SD,  $n = 4$  per group;  $*P < 0.005$ ). (C) PRG3<sup>CT</sup> expression in gliomas alters cellular morphology and activates Ras. *Left*, representative images showed PRG3-C-terminal domain expressing (PRG3<sup>CT</sup>) glioma cells exert an elongated cell shapes comparable to PRG3 full length expressing gliomas. Middle, Upper blot shows PRG3<sup>CT</sup> expression levels in wild-type (WT) and PRG3<sup>CT</sup> glioma cells. Actin served as a control for equal loading (bottom panel). *Right*, Upper panel shows activated Ras-GTP in wild-type (WT) and PRG3<sup>CT</sup> glioma cells. Total Ras served as a control for equal loading (bottom panel). Scale bar represents 50  $\mu$ m.



**Supplementary Figure S4: The C-terminal domain of PRG3 promotes oncogenesis and glioma growth.** Oncogenic amplification of glioma cells after expression of the C-terminal domain of PRG3. **(A)** Quantitative analysis of colony formation in parental wild-type glioma cells (WT) and PRG3 C-terminal domain expressing gliomas (PRG3<sup>CT</sup>). Quantification is given from three independent experiments. Values are given as mean  $\pm$  SD, \* $P < 0.001$  (Student's unpaired  $t$ -test). **(B)** Representative images of colonies formed in soft agar from wild-type glioma cells (WT) and PRG3 C-terminus expressing gliomas (PRG3<sup>CT</sup>). Scale bar, 500  $\mu$ m. **(C)** Expression of PRG3 C-terminal domain in gliomas (PRG3<sup>CT</sup>) fosters tumor expansion into brain parenchyma. GFP+ glioma cells (WT) and PRG3 C-terminal domain expressing gliomas (PRG3<sup>CT</sup>) were implanted in brain slices and tumor expansion was evaluated after various days. Quantitative analysis of tumor expansion (bright colored columns represent tumor expansion at day 1; faintly colored columns represent tumor expansion at day 7). Statistical significance was calculated with Student's unpaired  $t$ -test (mean  $\pm$  SD,  $n = 6$  per group; \* $P < 0.005$ ).