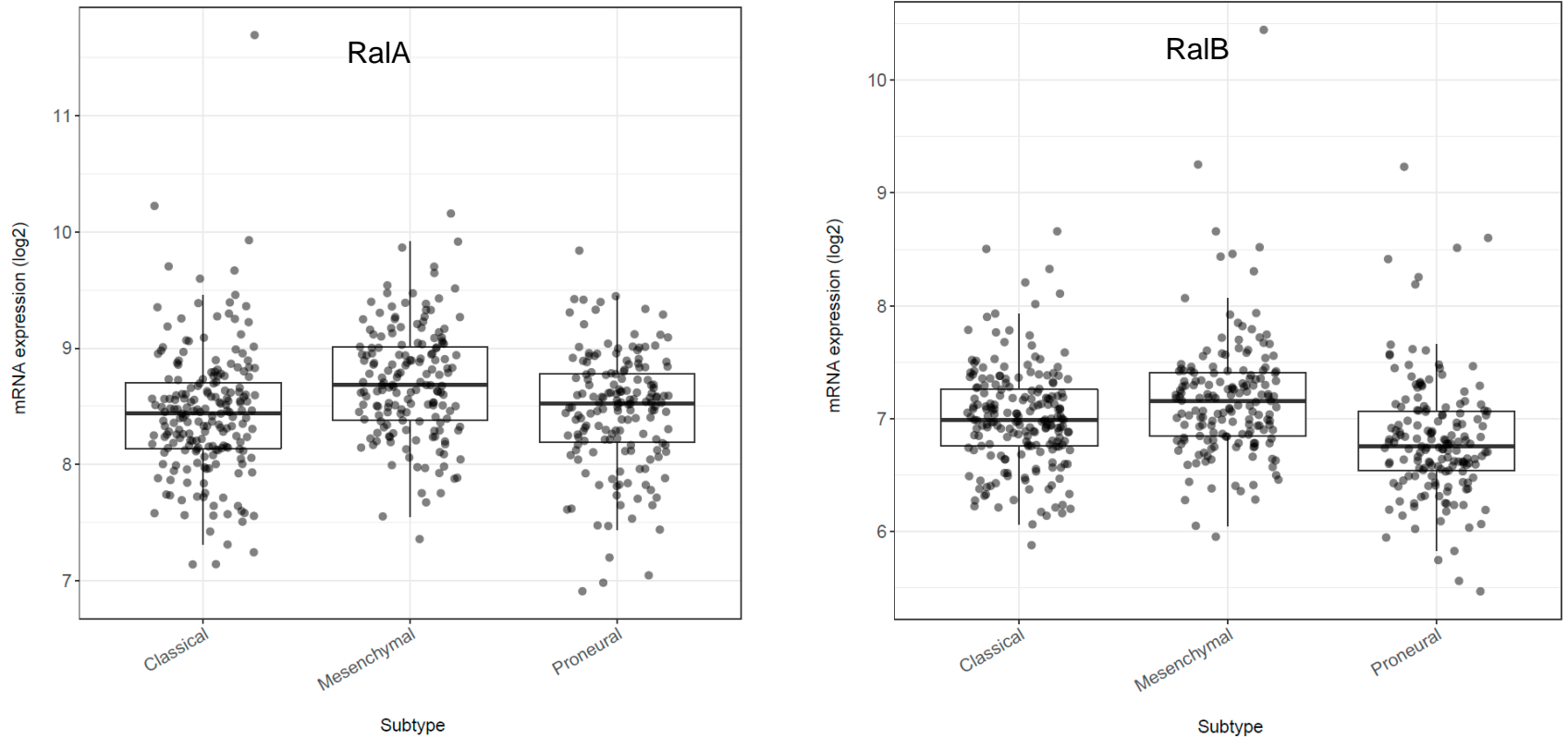


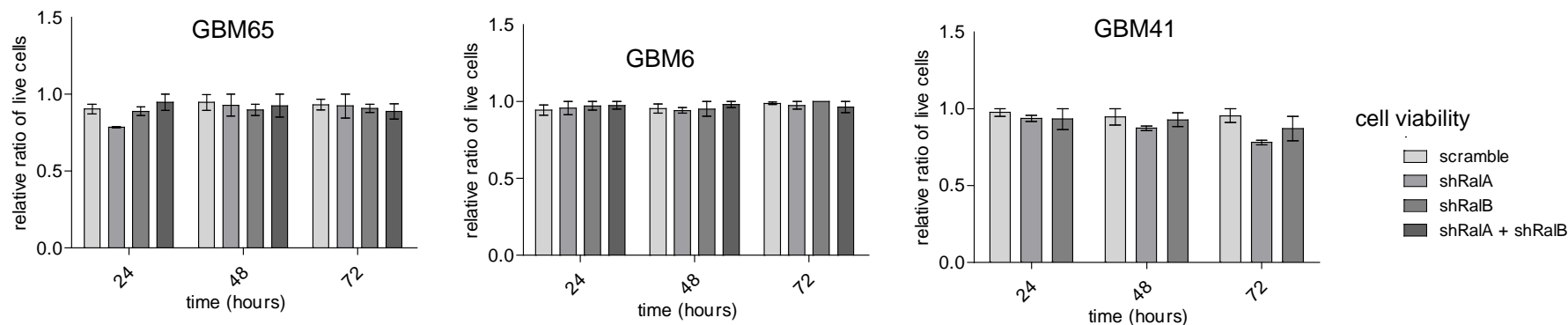
Figure S1.



**Figure S1 . *RalA* and *RalB* expression in different GBM subtypes.** Analysis of *RalA* and *RalB* mRNA expression levels from Gliovis database comparing three GBM types, Mesenchymal, Classical and Proneural (TGCA-GBM; platform HG-U133A). Classical n=199; Mesenchymal n=165; Proneural n=162. (<http://gliovis.bioinfo.cnio.es/>).

Figure S2.

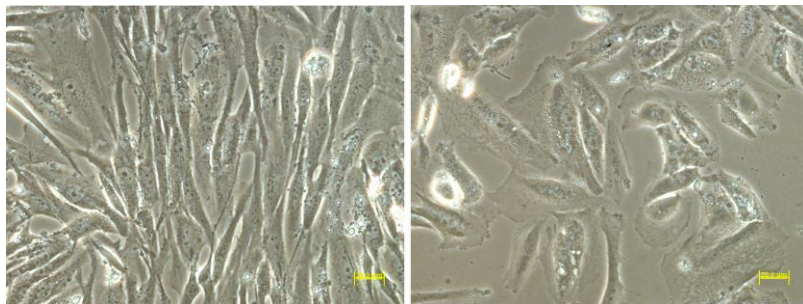
(a)



(b)

scramble

shRalB

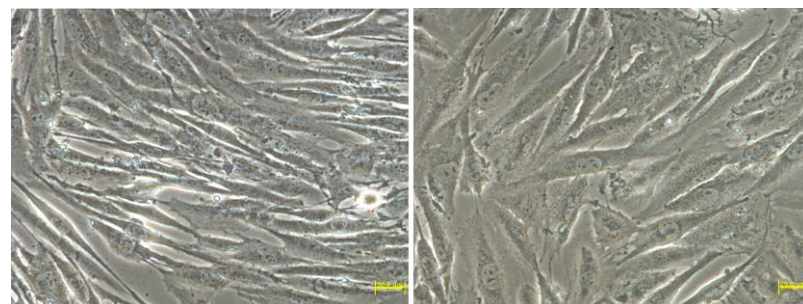


GBM6

(c)

scramble

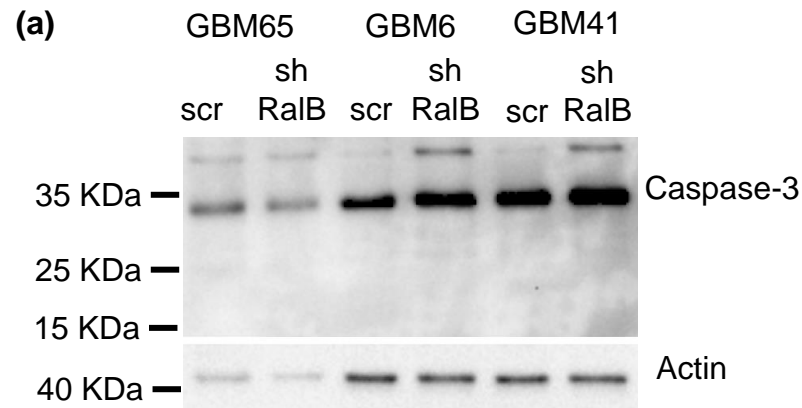
shRalB



GBM41

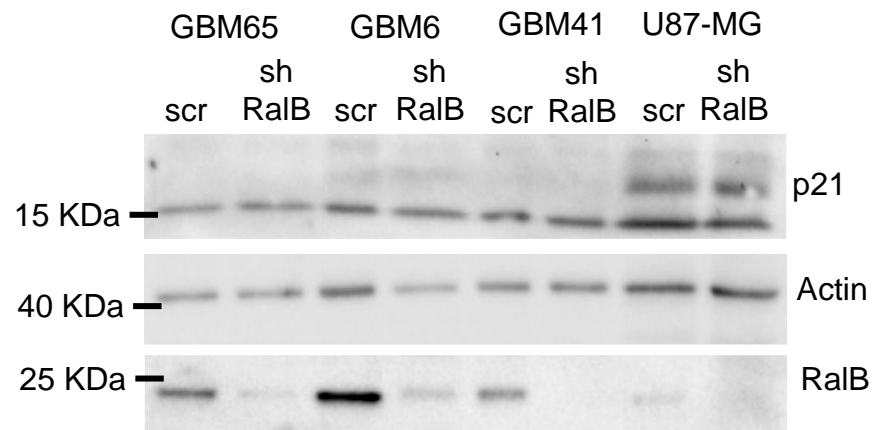
**Figure S2.** Knockdown of *Ral* GTPases promotes a reduction of cell growth without affecting cell viability. Primary GBM cells were infected with lentivirus driving interference RNA against *RalA* (shRalA) or *RalB* (shRalB) or both. Scramble shRNA was used as a control. Three days after infection, cells were seeded and counted every 24 h for 3 days. **(a)** Graphics and bar diagrams representing trypan blue-negative GBM cells. Data is represented as mean  $\pm$  SEM ( $n=3$ ; three independent experiments). There was no significant differences. **(b, c)** Representative phase-contrast images of GBM6 (b) and GBM41 (c) cells were taken after 4 days of infection (20  $\mu$ m Bar).

Figure S3



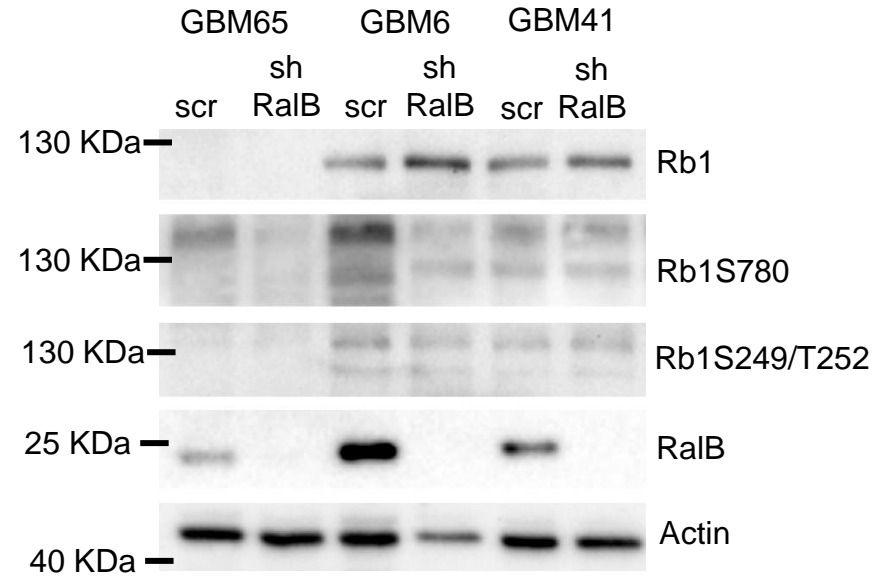
**Figure S3.** *Caspase-3 was not processed after downregulation of RalB in glioblastoma cells.* RalB was downregulated by RNA interference (shRalB). GBM cells were infected by lentiviral vectors harboring shRalB. Scramble shRNA was used as a control. Five days after infection cells were processed for immunoblot to detect total and cleaved caspase-3. Actin is used as a loading control

Figure S4



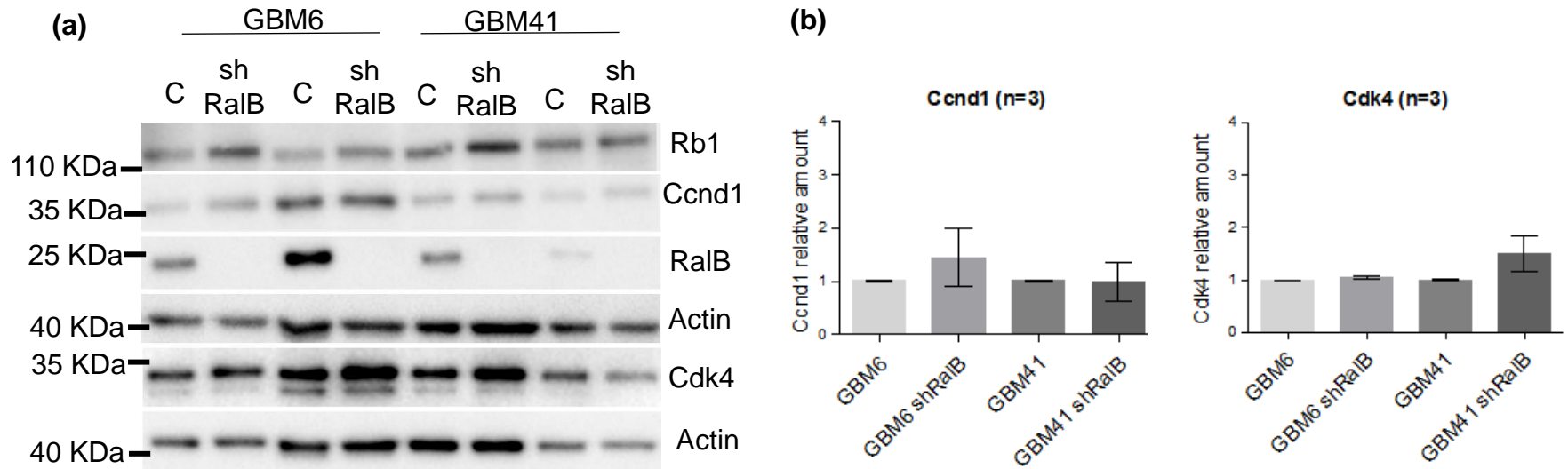
**Figure S4.** *p21* was not detected by immunoblot in the primary glioblastoma cells used in this work. Primary GBM cells were infected with lentivirus driving interference RNA against RalB (shRalB). Five days after infection, cells were processed for immunoblot. Scramble shRNA was used as a control. Immunoblots of p21, actin (loading control) and RalB. The U87-MG GBM cell line was used as a positive control of p21 detection.

Figure S5



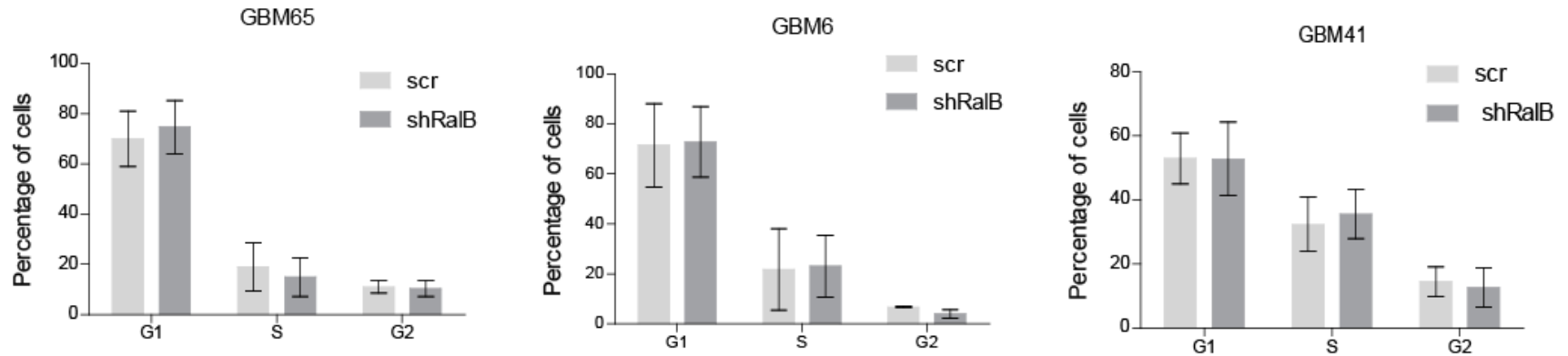
**Figure S5.** *Rb1 was hypophosphorylated in primary GBM cells after RalB-knockdown.* Primary GBM cells were infected with lentivirus driving interference RNA against RalB (shRalB). Five days after infection, cells were processed for immunoblot. Scramble shRNA was used as a control. Specific antibodies against Rb1, Rb1-phosphoS780, Rb1-phosphoS249/T252, actin (loading control) and RalB were used.

Figure S6



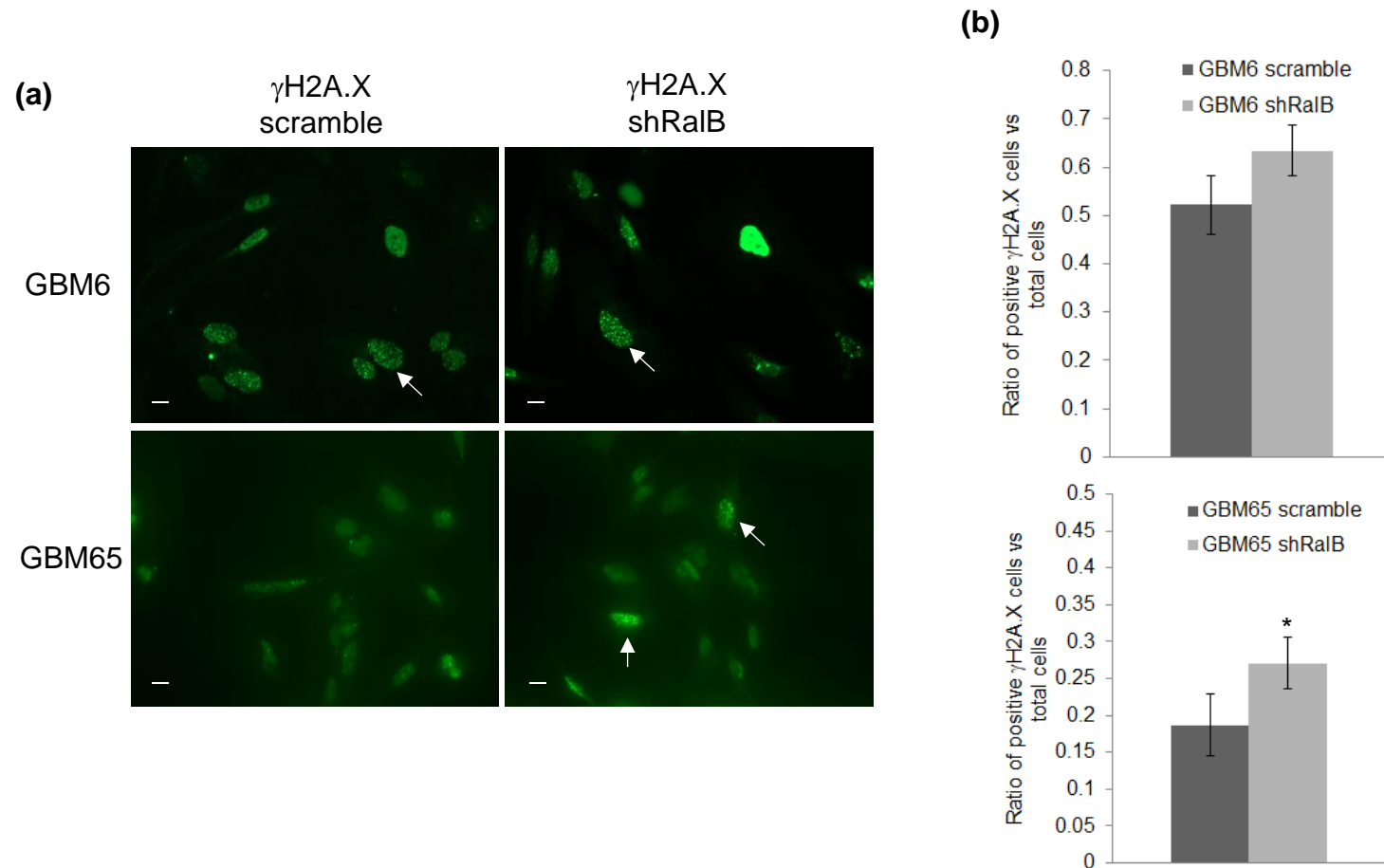
**Figure S6.** Cyclin d1 and Cdk4 levels were not altered in primary GBM cells after RalB-knockdown. Primary GBM cells were infected with lentivirus driving interference RNA against RalB (shRalB). Five days after infection, cells were processed for immunoblot. Scramble shRNA was used as a control. (a) Specific antibodies against Rb1, Ccnd1, Cdk4, actin (loading control) and RalB were used. (b) Bar diagrams representing the relative amount of Ccnd1 and Cdk4. Data is represented as mean  $\pm$  SEM (n=3; three independent experiments) There was no significant differences.

Figure S7



**Figure S7.** *RalB*-knockdown did not produced a G1 arrest in primary GBM cells. Primary GBM cells were infected with lentivirus driving interference RNA against *RalB* (sh*RalB*). Five days after infection, cells were processed for FACS analyses. Scramble shRNA was used as a control. Bar diagrams representing the cell distribution in cell cycle phases examined by staining with PI. Data is represented as mean  $\pm$  SEM (n=3; three independent experiments). There was no significant differences.

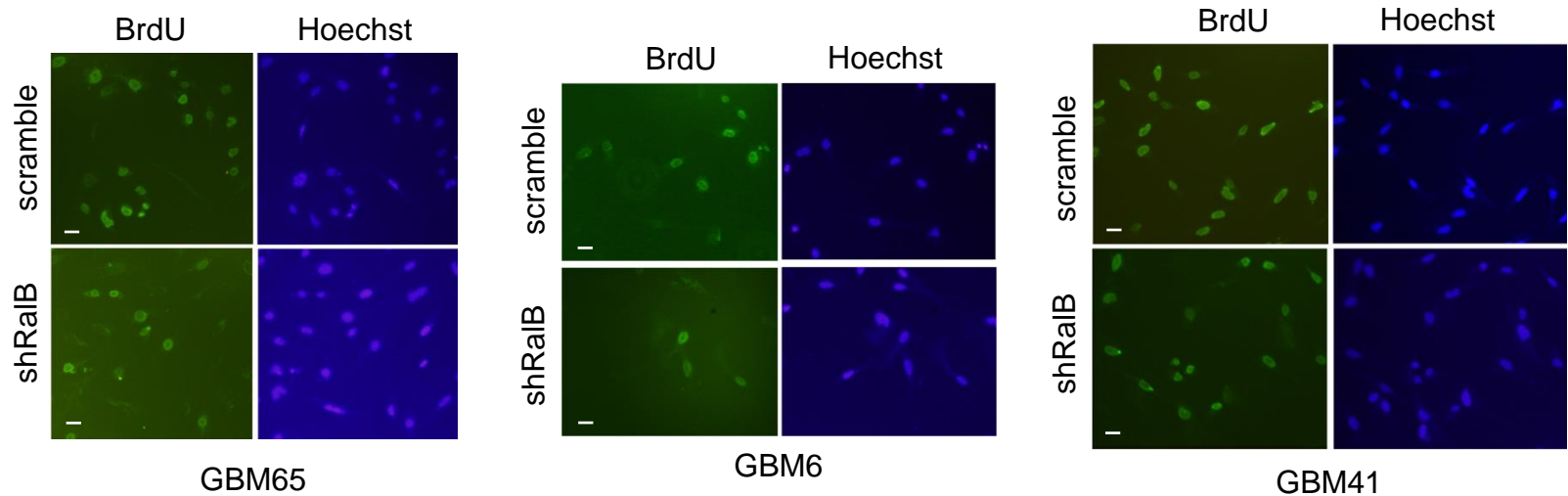
Figure S8



**Figure S8.** DNA damage in glioblastoma cells after downregulation of RaiB. Primary GBM cells growing under the same conditions as in figure S4 were analyzed. (a) Representative images of cells positive for  $\gamma$ -H2A.X staining (20  $\mu$ m Bar) (b) Cells with more than five foci were counted as positive. Graphs represent the ratio of positive cells versus total cells ( $n > 255$ ). The confidence intervals of proportions are shown ( $p \leq 0.05$ , \*).

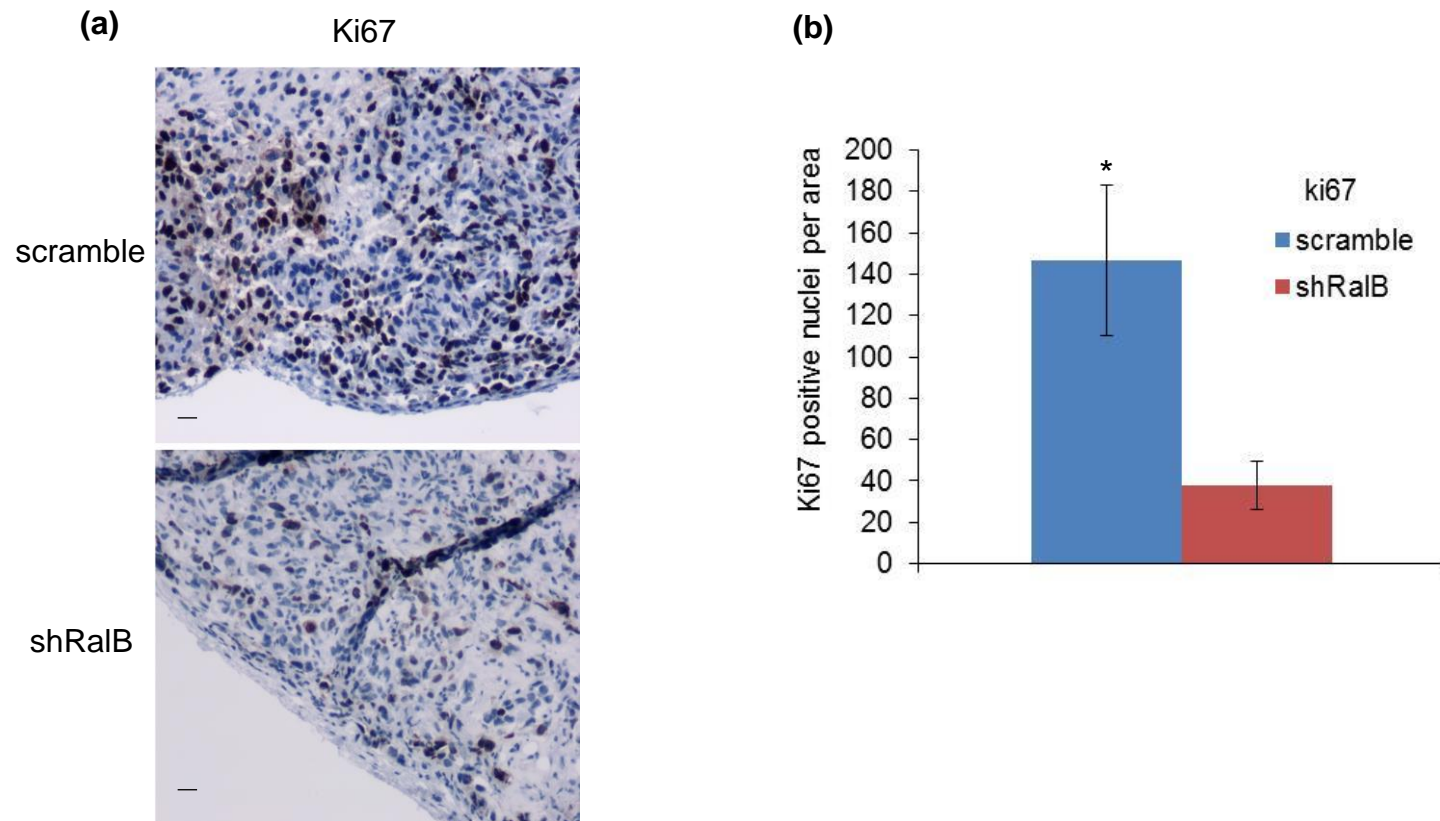


Figure S9



**Figure S9.** *Downregulation of RaIB promotes a proliferation arrest in glioblastoma cells.* RaIB was downregulated by RNA interference (shRaIB). Scramble shRNA was used as a control. GBM cells were infected by lentiviral vectors harboring the shRNAs. Five days after infection cells were processed for BrdU analysis. Representative images (20 μm Bar).

Figure S10



**Figure S10.** RalB downregulation reduces proliferation of subcutaneous-derivative GBM tumor. Human U251-MG cells were infected with lentiviruses harboring scramble or shRalB. Infected cells were inoculated subcutaneously in immunodeficient SCID male mice. Mice were euthanized seven weeks after injection and the tumors excised. Proliferation was analyzed by Ki67 staining. **(a)** Representative images of cells positive for ki67 staining (40 µm Bar) **(b)** Quantification of Ki67 positive nuclei. Data is mean  $\pm$  SEM. Significance was calculated by t test ( $p < 0.05$ ).

Figure S11. Uncropped blots of Figure 1a

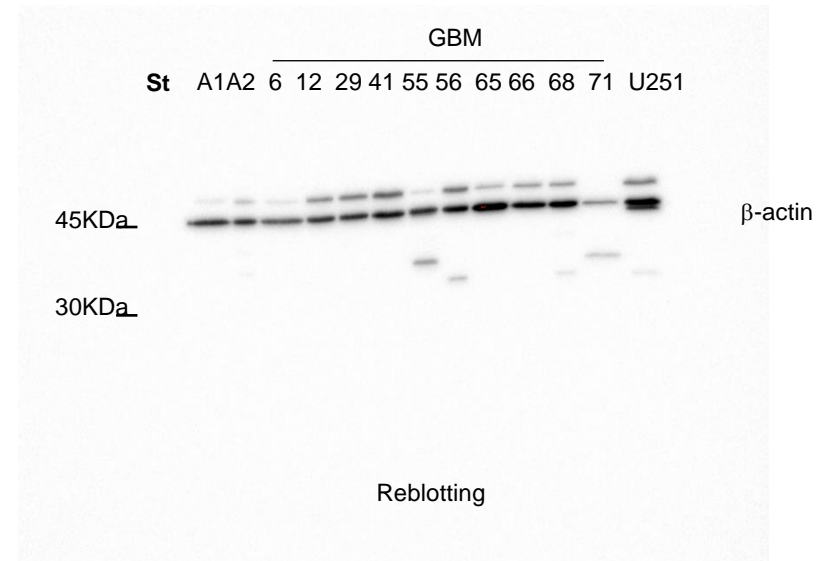
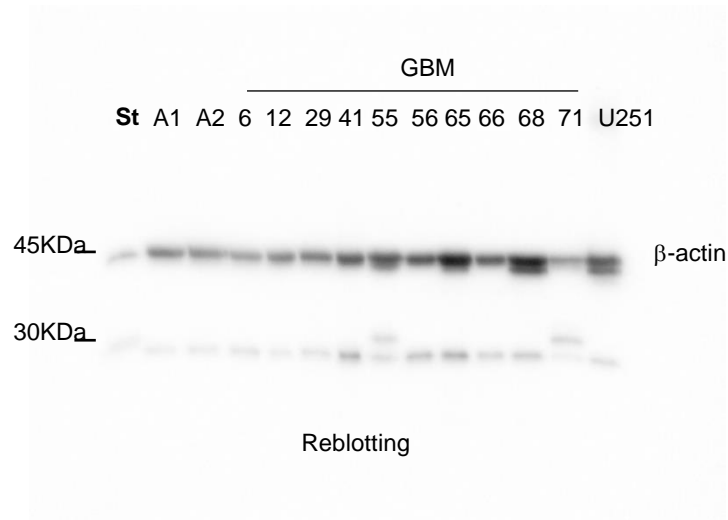
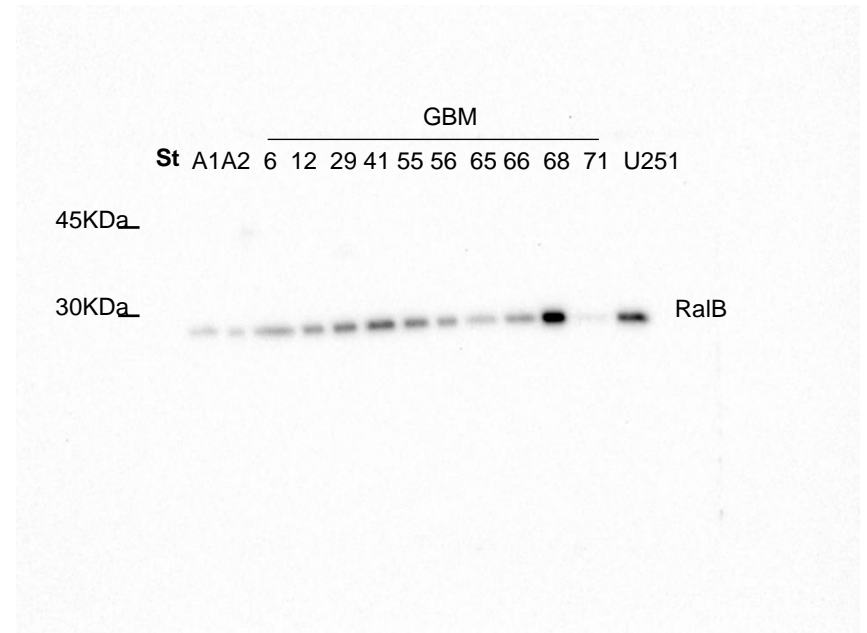
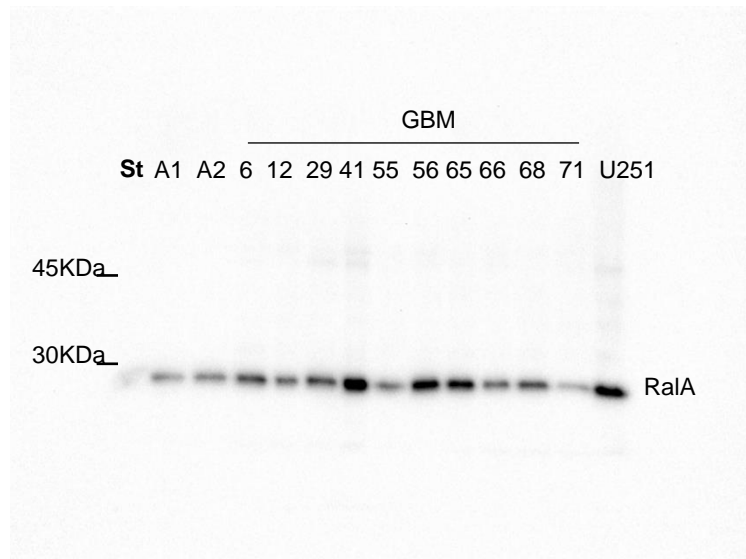


Figure S12. Uncropped blots of Figure 1b

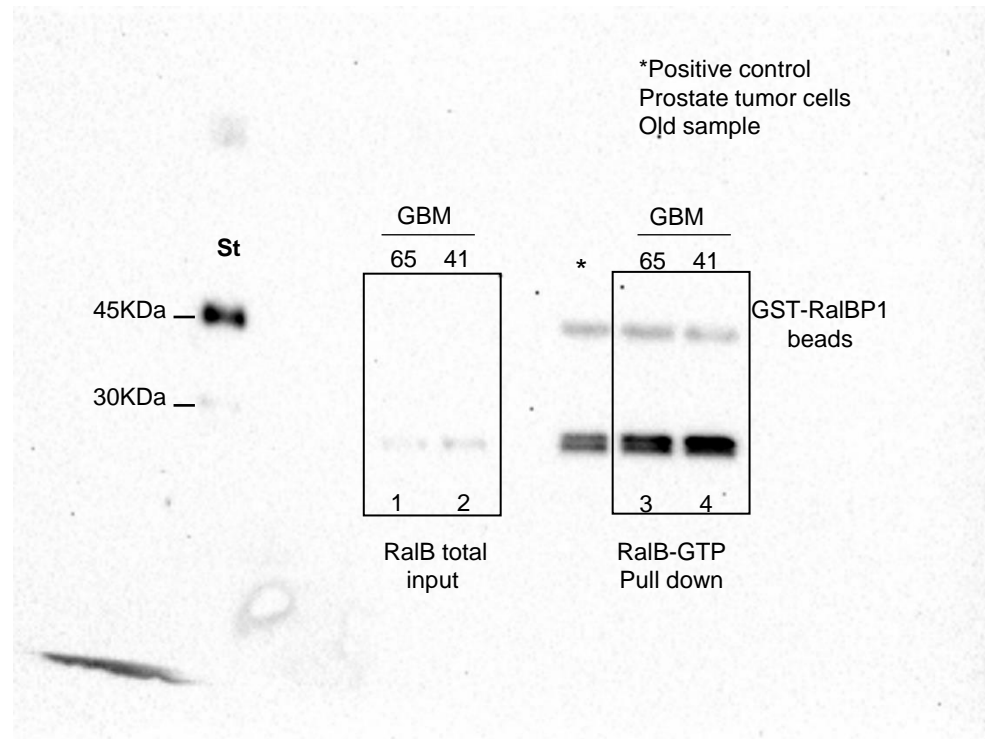


Figure S13. Uncropped blots of Figure 2b

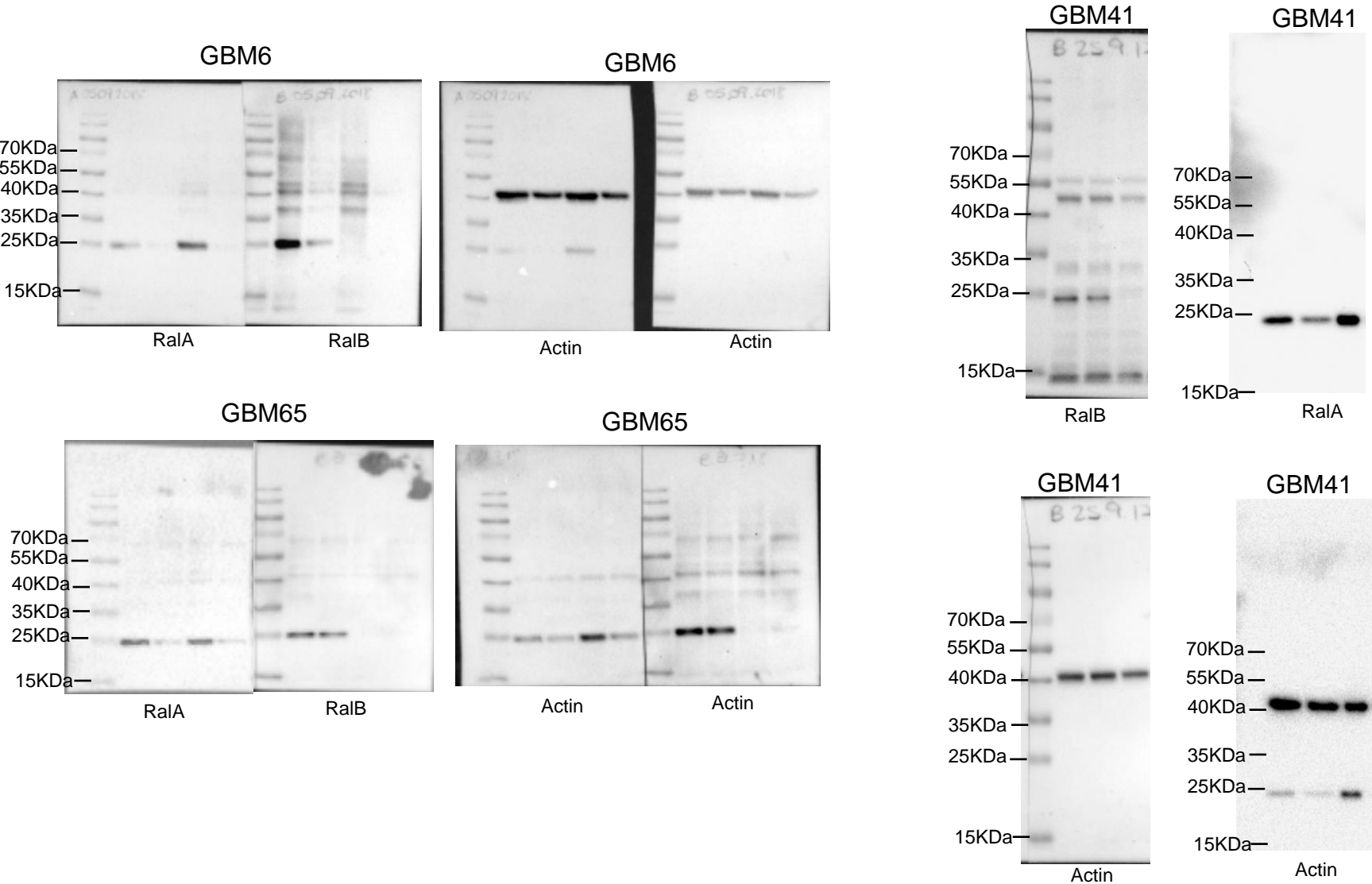


Figure S14. Uncropped blots of Figure 5a

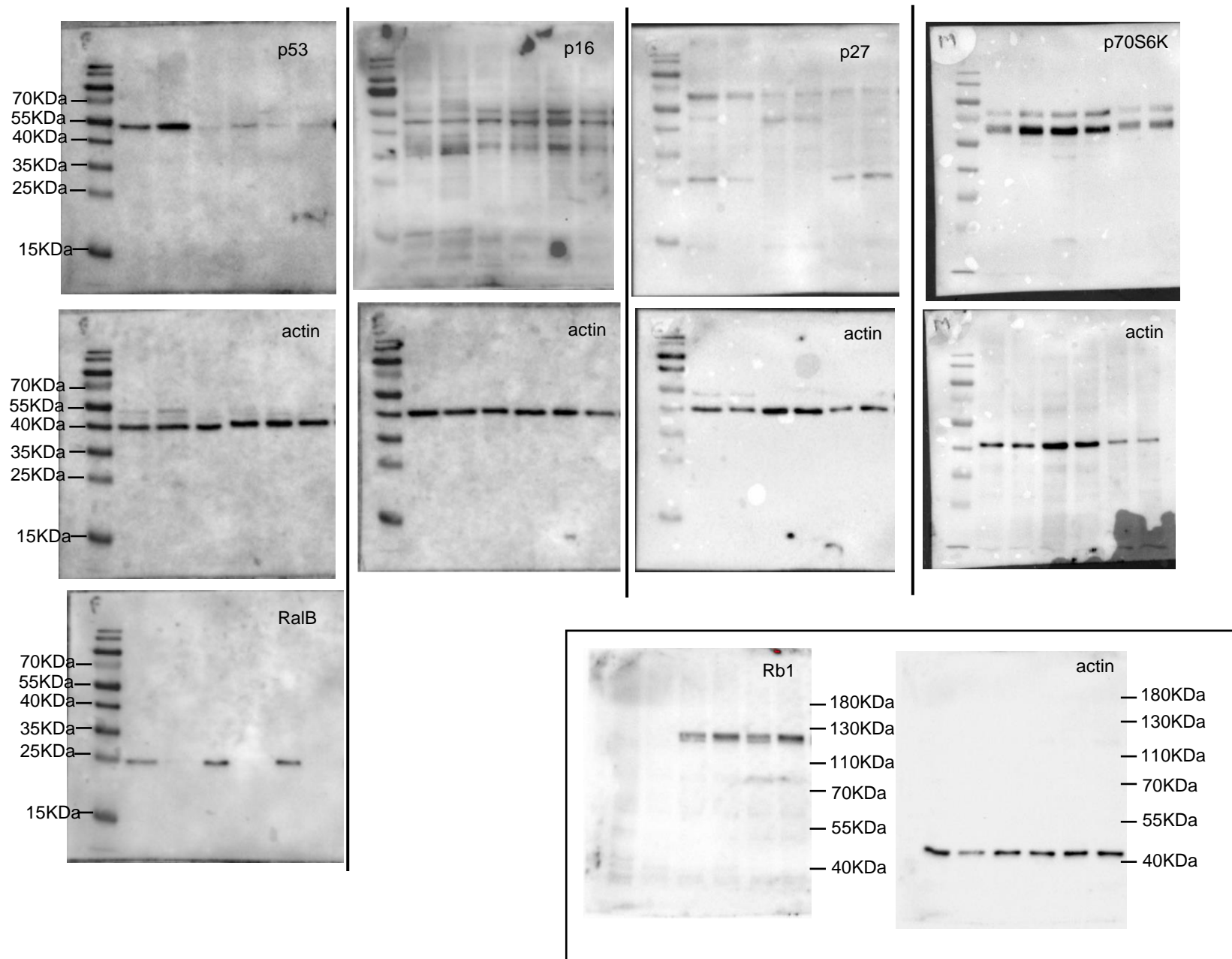


Figure S15. Uncropped blots of Figures S3 and S4

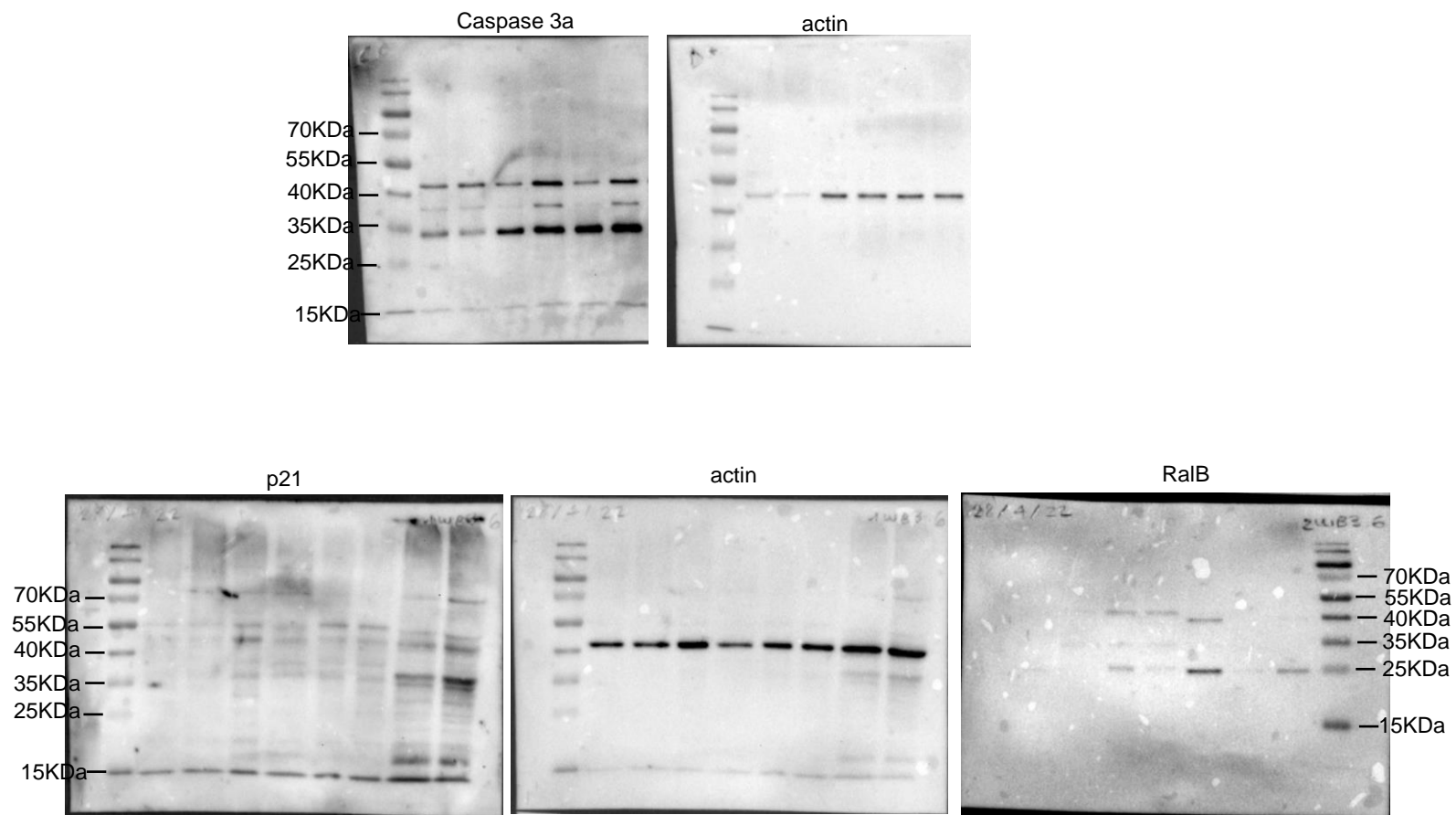




Figure S16. Uncropped blots of Figures S5 and S6

Rb1

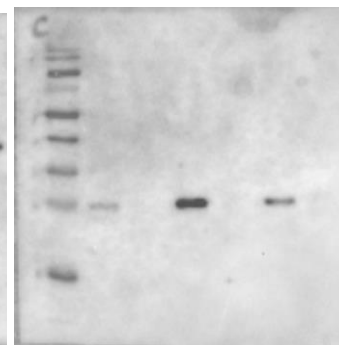
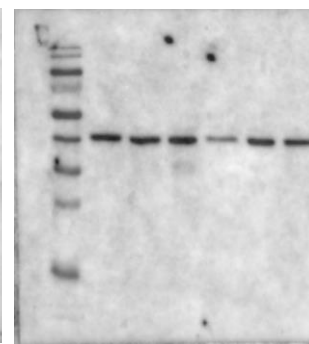
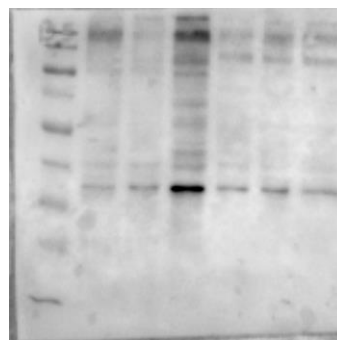
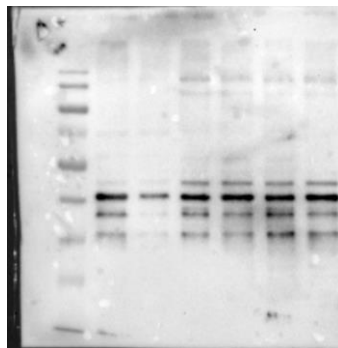
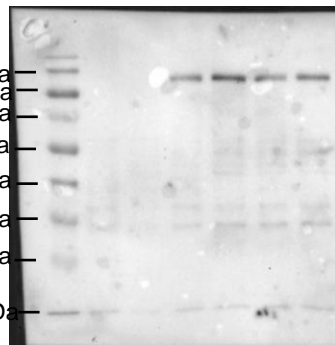
Rb1 S249/T252

Rb1 S780

actin

RalB

130KDa  
110KDa  
70KDa  
55KDa  
40KDa  
35KDa  
25KDa  
15KDa



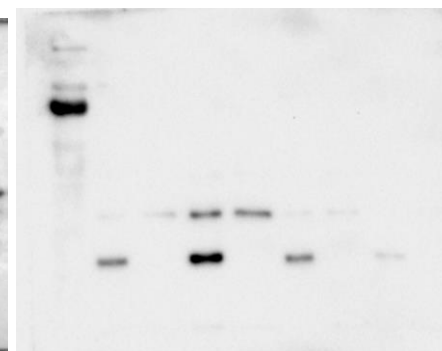
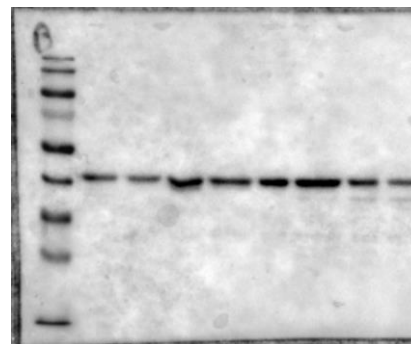
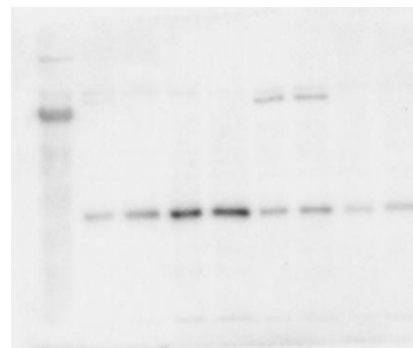
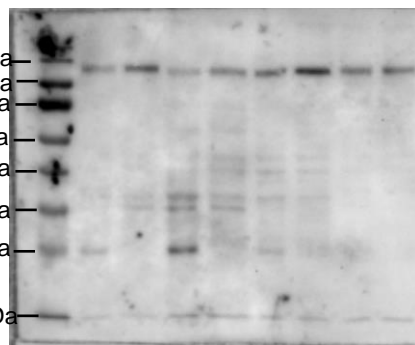
Rb1

Ccnd1

actin

RalB

130KDa  
110KDa  
70KDa  
55KDa  
40KDa  
35KDa  
25KDa  
15KDa



Cdk4

actin

130KDa  
110KDa  
70KDa  
55KDa  
40KDa  
35KDa  
25KDa  
15KDa

