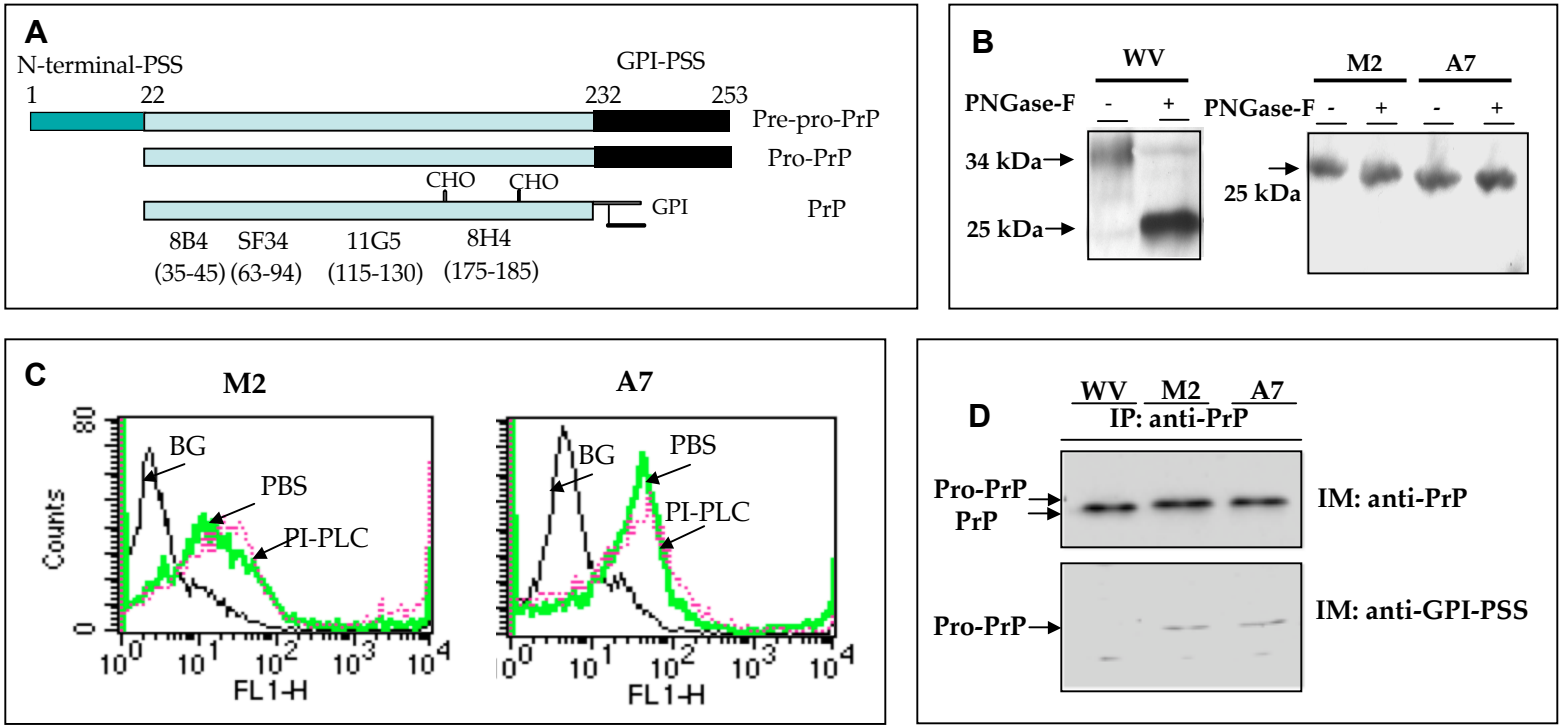

A
N-terminal-PSS
GPI-PSS
1
22
232
253
Pre-pro-PrP
Pro-PrP
CHO
CHO
GPI
PrP
8B4
(35-45)
SF34
(63-94)
11G5
(115-130)
8H4
(175-185)
B
WV
M2
A7
PNGase-F
-
+
-
+
-
+
34 kDa
25 kDa
25 kDa
C
M2
A7
BG
PBS
PI-PLC
Counts
80
0
FL1-H
D
WV
M2
A7
IP: anti-PrP
Pro-PrP
PrP
IM: anti-PrP
Pro-PrP
IM: anti-GPI-PSS

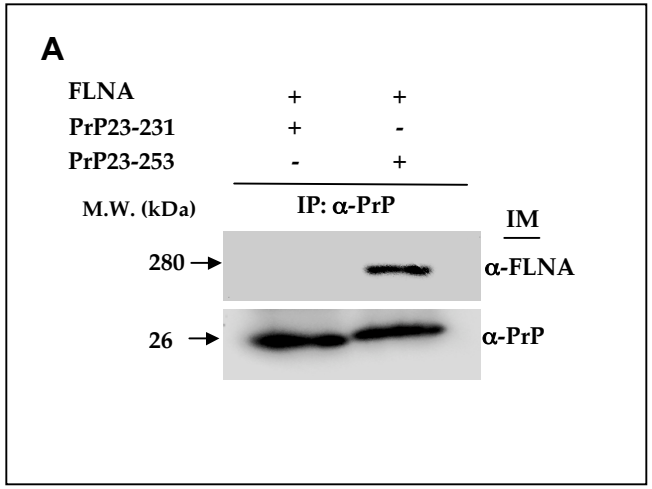
Fig. S1. PrP exists as pro-PrP in M2 and A7 cells

A). A diagrammatic drawing on the processing of PrP, and the location of the epitopes of the anti-PrP monoclonal antibodies (mAbs).

B). Immunoblots show that a glycosylated PrP from WV cells is sensitive to treatment with PNGase, but PrP from M2 and A7 cells is resistant to PNGase. Hence, the PrP in M2 and A7 cell has no N-linked glycans. To prepare cell lysate, cells were seeded overnight, and lysates were prepared in lysis buffer containing 20 mM Tris (pH7.5), 150 mM NaCl, 1mM EDTA, 1mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1mM β-glycerolphosphate, 1 mM Na_3VO_4. 1mM PMSF, and EDTA-free protease inhibitor cocktail was added just before cell lysis. PrP was subjected to PNGase-F treatment according to the protocols provided by the provider. After treatment, samples were separated on SDS-PAGE and immunoblotted with an anti-PrP mAb.

C). Histograms show that the PrP on the cell surface of M2 and A7 cells is resistant. Thus, cell surface PrP on M2 and A7 cells lacks the GPI-anchor. (BG, background, cells stained with irrelevant mAb D7C7).

D). Immunoblots show that affinity purified PrP from WV cells does not react with an anti-serum that is specific for the GPI-PSS of PrP. On the other hand, the PrP from M2 and A7 cells does react with the anti-serum. Thus, the PrP in M2 and A7 cells retains its GPI-PSS (IP, immunoprecipitation; IM, immunoblot). To detect GPI-PSS on PrP, mAb 8B4 affinity purified PrP was separated and detected with affinity purified rabbit anti PrP-GPI-PSS.


A
FLNA
PrP23-231
PrP23-253
+ +
+ -
- +
M.W. (kDa)
IP: α-PrP
IM
280
α-FLNA
26
α-PrP

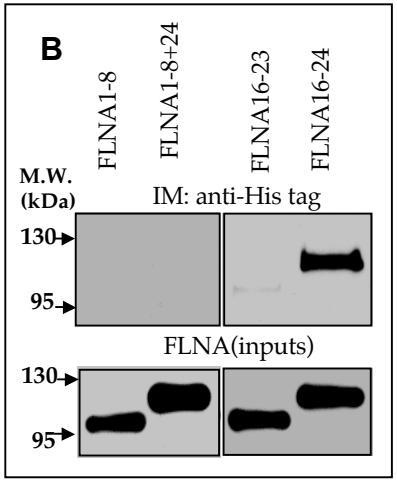

B
FLNA1-8
FLNA1-8+24
FLNA16-23
FLNA16-24
M.W. (kDa)
IM: anti-His tag
130
95
FLNA(inputs)
130
95

Fig. S2. Identification of the pro-PrP binding motif on FLNA

A). Immunoblots show that in an *in vitro* pull-down assay, FLNA binds pro-PrP(23-253) but not mature PrP(23-231).

B). Immunoblots show that in *in vitro* pull-down assays, pro-PrP does not bind to the N-terminus domains 1 to 8 of FLNA, domains 1 to 8 linked to 24, or domains 16 to 23. On the other hand, pro-PrP does bind to domains 16 to domain 24 of FLNA. Therefore, pro-PrP does not bind domain 24 alone, it binds to the C-terminus between domains 16 and 23 of FLNA.

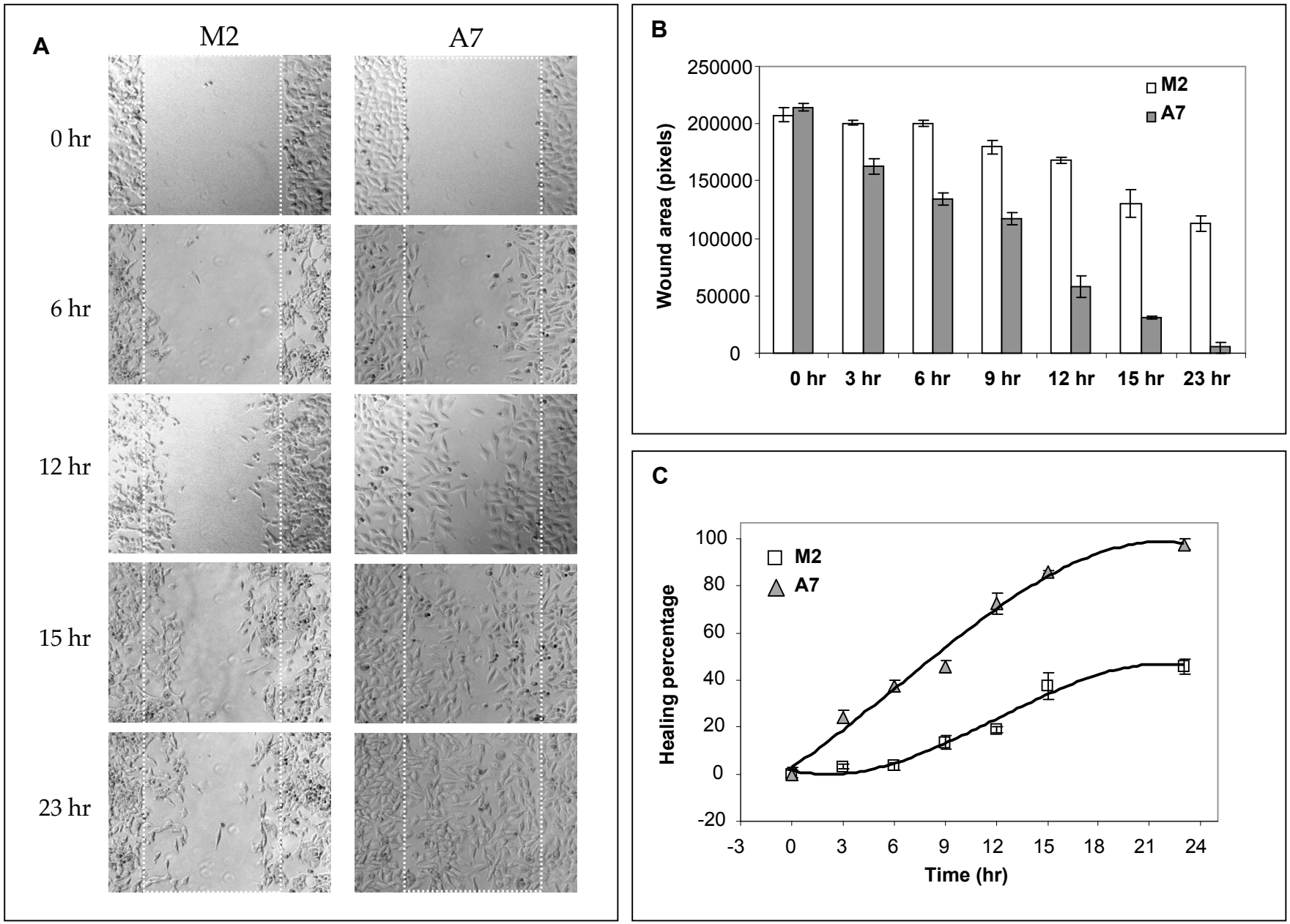

A
M2
A7
0 hr
6 hr
12 hr
15 hr
23 hr
B
Wound area (pixels)
250000
200000
150000
100000
50000
0
M2
A7
0 hr
3 hr
6 hr
9 hr
12 hr
15 hr
23 hr
C
Healing percentage
100
80
60
40
20
0
-20
M2
A7
-3
0
3
6
9
12
15
18
21
24
Time (hr)

Fig. S3. M2 cells are deficient in cell spreading and migration compared to A7 cells

A). Microscopic photographs show that M2 cells are deficient in spreading and migration comparing to A2 cells. Photograph taken at various times after wound healing from two representative wells. The two dashed white lines mark the boundary of the wound. Magnification: 10×10.

B and C). Quantification results of the would-healing assay; results presented were the average +/- S.E. of the triplicate wells.

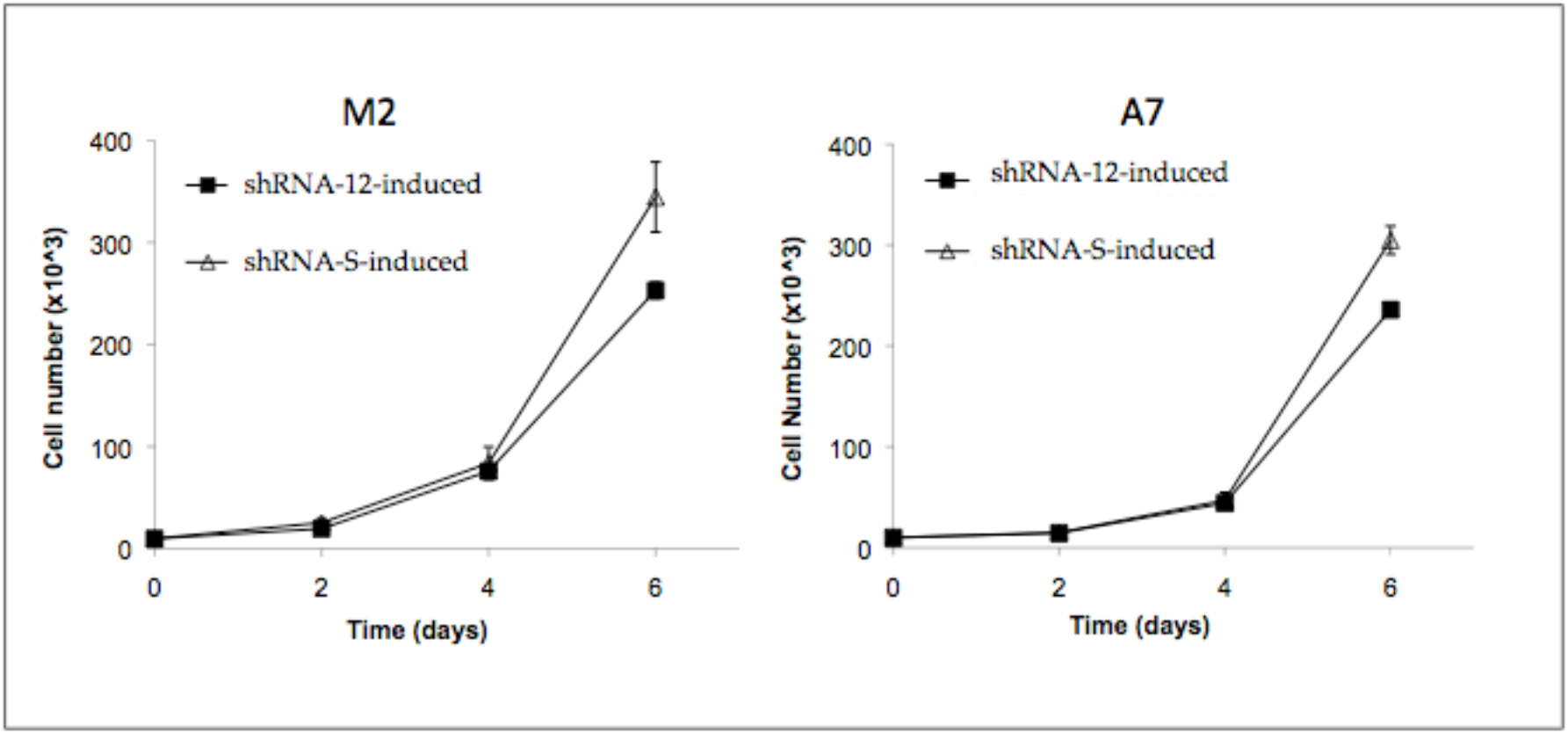
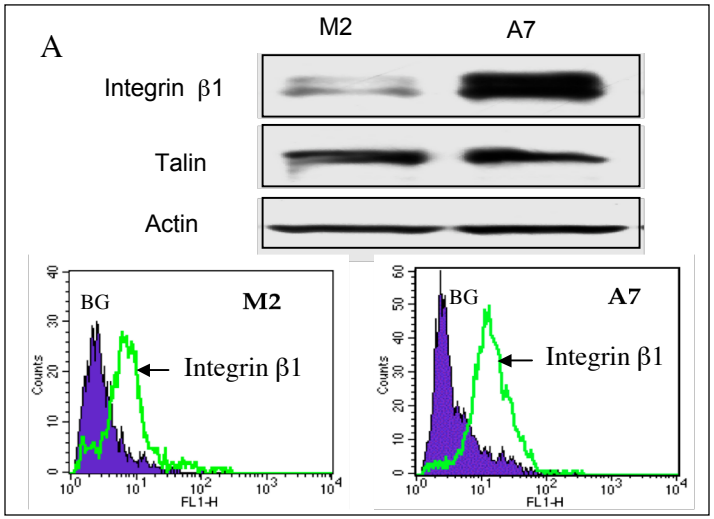

M2
400
300
200
100
0
Cell number (x10^3)
shRNA-12-induced
shRNA-S-induced
0
2
4
6
Time (days)
A7
400
300
200
100
0
Cell Number (x10^3)
shRNA-12-induced
shRNA-S-induced
0
2
4
6
Time (days)

Fig. S4. Down regulation of PrP did not alter the proliferation of M2 and A7 cells: Single cell suspension of the tumor cells were prepared and counted. Equal numbers of the tumor cells ($1X10^4$) were then platted in a 96 well tissue culture plates in triplicates. At different times after culture, the cells from each well were harvested and counted. The results presented were the mean +/- S.E. of two experiments. The proliferation of PrP down regulated and control cells are comparable at 2 and 4 days after platting. The numbers of PrP down regulated M2 and A7 cells are reduced slightly at 6 days after platting. Results presented were the average +/- S.E. of the triplicate wells.


A
M2
A7
Integrin β1
Talin
Actin
BG
M2
Integrin β1
Counts
FL1-H
BG
A7
Integrin β1
Counts
FL1-H

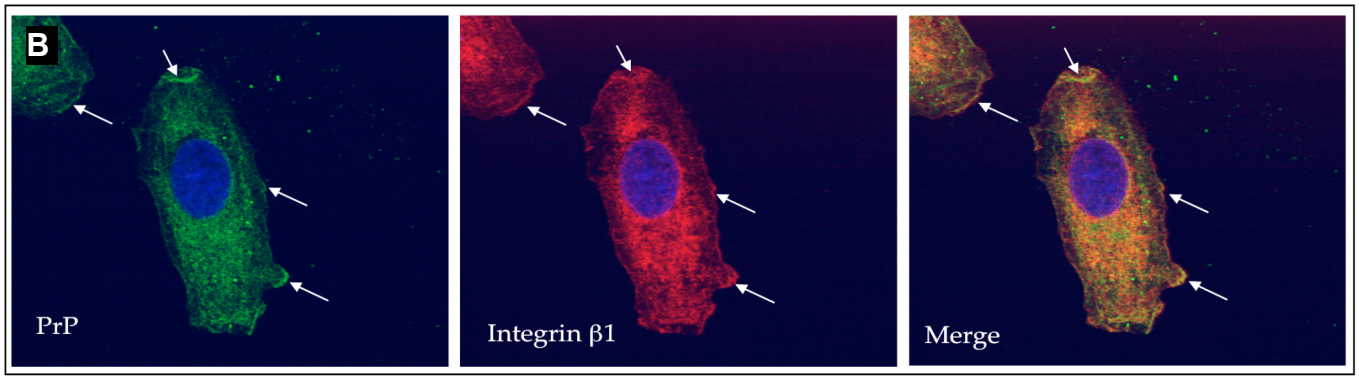

B
PrP
Integrin β1
Merge

Fig. S5. Co-purification of FLNA and integrin β1 and the effects of down-regulation of PrP on integrin β1 and FLNA association:

A. Immunoblots show that A7 cells have more integrin β1 than M2 cells, but the levels of talin in the two cell lines are comparable. Histograms show that A7 cells also have more cell surface integrin β1 than M2 cells.

B. Confocal microscopic photos show that integrin β1 and PrP are partially co-localized in A7 cells (Arrows mark the areas of co-localization). Original magnification: 10×10.

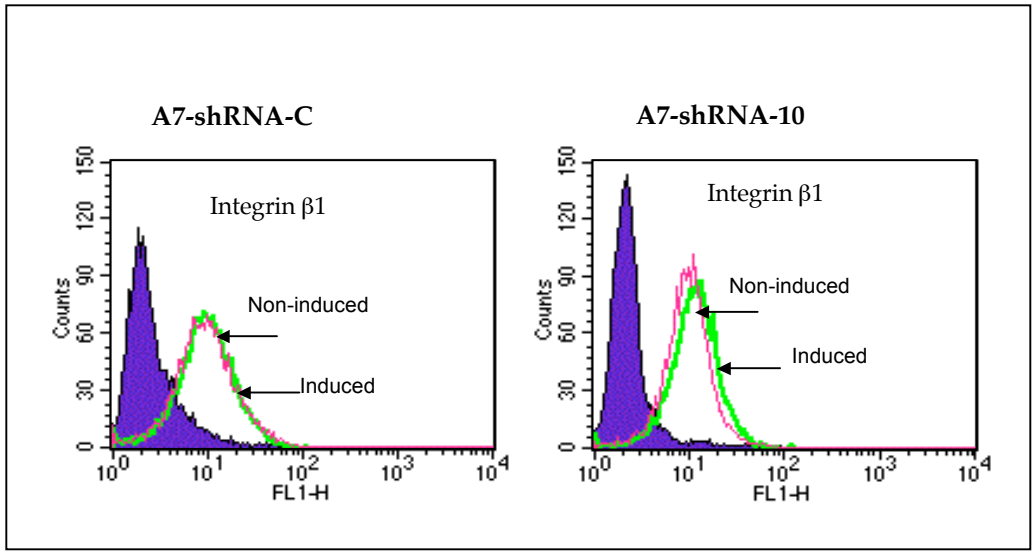
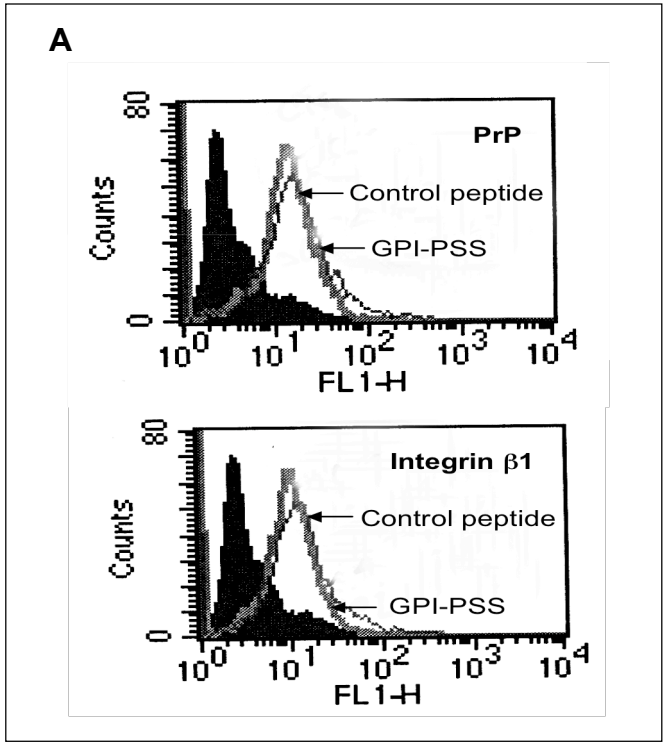

A7-shRNA-C
Integrin β1
Non-induced
Induced
Counts
FL1-H
A7-shRNA-10
Integrin β1
Non-induced
Induced
Counts
FL1-H

Fig. S6. Histograms show that down regulation of PrP does not change the expression levels of cell surface integrin β1 on A7 cells:

Single cell suspension of A7 or PrP down regulated A7 cells are stained with an anti-integrin β1 mAb followed by a FITC conjugated goat anti-mouse antibody. Stained cells are then analyzed in a flow cytometry. (BG, background staining with irrelevant, control Mab D7C7).


A
PrP
Control peptide
GPI-PSS
Counts
80
0
10^0 10^1 10^2 10^3 10^4
FL1-H
Integrin β1
Control peptide
GPI-PSS
Counts
80
0
10^0 10^1 10^2 10^3 10^4
FL1-H

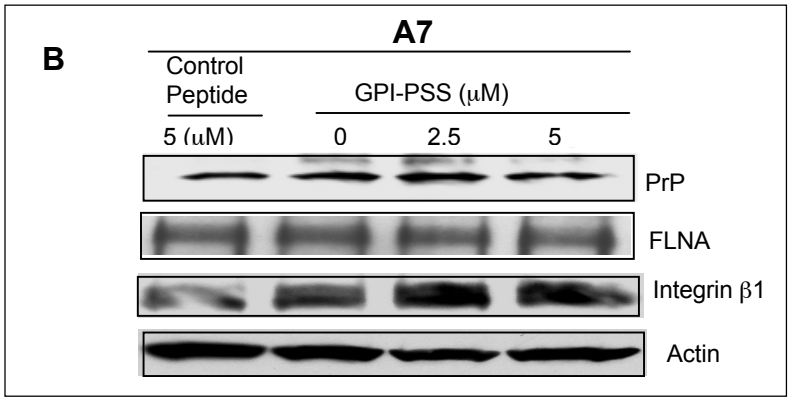

B
A7
Control Peptide
GPI-PSS (μM)
5 (μM)
0
2.5
5
PrP
FLNA
Integrin β1
Actin

Fig. S7. A PrP-GPI-PSS inhibits A7 cell spreading and migration

A. Histograms show that incubation of A7 cells with the KKRPK-PrP-GPI-PSS (5mM) for 15 hrs did not alter the cell surface expression levels of either PrP or integrin β1.

B. Immunoblots show that incubation of A7 cells with the KKRPK-PrP-GPI-PSS (5μM) for 15 hrs does not alter the expression of total PrP, FLNa and integrin β1.