Supplemental Information

A Non-genetic Mechanism Involving the Integrin β4/Paxillin Axis Contributes to Chemoresistance in Lung Cancer

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Supplemental Table and Figure Legends

Table S1. Genes upregulated in cisplatin-resistant lung adenocarcinoma cell line from Molecular Signatures Database (MSigDB), Broad Institute. Related to Figure 1.

Table S2. Cox proportional hazards model: Survival ~ Sex + Stage + Age + PXN * ITGB4. Related to Figure 1F.

Table S3. DAVID analysis of 96 unique genes downregulated in double knockdown of PXN and ITGB4. Related to Figure 5D-F.

Table S4. DAVID analysis of 206 common genes downregulated in single and double knockdown of PXN and ITGB4. Related to Figure 5D-F.

Table S5. MiRNAs differentially regulated by single and double knockdown of PXN and ITGB4. Related to Figure 8B-D.

Table S6. SiRNA sequences used for knockdown experiments. Related to Figures 2-7.

Figure S1. Expression of ITGA6 in NSCLC cell lines. Related to Figure 1B. Protein expression of ITGA6 and PXN in the 5 KRAS mutant and 5 KRAS WT NSCLC cell lines. SW480 cells were used as positive control for ITGA6 expression.

Figure S2. Variability of expression of PXN and ITGB4 in tumor tissue samples. Related to Figure 1G.
A) Immunohistochemistry staining of lung adenocarcinoma tumor tissue showed that Case 1 had high PXN and intermediate ITGB4 expression. In Case 2, PXN expression is low and ITGB4 expression is high. In Case 3, both PXN and ITGB4 expression are low. B) and C) Multiplex staining of ITGB4 and PXN staining in 5 City of Hope patients. The images were deconvoluted using QuPath software, and percentage of area was calculated for either ITGB4 or PXN or both. Data are represented as mean +/- SD.

Figure S3. ITGB4 knockdown attenuates cell proliferation in commonly used NSCLC cell lines. Related to Figure 2.
A) Stable cell lines were generated to express nuclear mKate2 red fluorescent protein (RFP) using the NucLight Red Lentivirus Reagent (Essen BioScience). Upon selection with puromycin, cells were analyzed with the IncuCyte software to create a mask around each individual nucleus and obtain accurate real-time cell counts for proliferation assays. B) Effect of 3 different ITGB4 siRNA constructs (A, B, C) on cell proliferation over the course of 72 h in H358 cells. C) Effect of 3 different ITGB4 siRNA constructs (A, B, C) on cell proliferation over the course of 72 h in A549 cells. D) Immunoblot confirming ITGB4 knockdown upon Si ITGB4 transfection in H358 and A549 cells. Data are represented as mean +/- SD. (****p<0.0001)
**Figure S4.** ITGB4 knockdown has a specific effect in NSCLC compared to other integrin forms. Related to Figure 2.  
**A)** Doubling times for H2009 and H1993 cells transfected with scramble siRNA (siControl) were measured and compared to that of cells with ITGB4 knockdown (siITGB4). **B)** H2009 cells transfected with 10 nM of siRNA constructs A, B, and C, targeting ITGA7 had minimal effect on proliferation. **C)** Knocking down ITGB4 increased mRNA expression of other integrin beta forms such as ITGB1, ITGB2, and ITGB3 but had no significant effect on the expression of ITGA7. **D)** In order to nullify the effect of ITGB3 rescue, H2009 cells transfected with siRNA ITGB4 were treated with ITGB3 inhibitors. There was no significant effect in the fold change in proliferation compared to the ITGB4 knockdown cells. **E)** Scratch wound healing assays were performed by creating an initial scratch wound with the WoundMaker tool. Wound closure was quantitated by monitoring cells that migrated to fill the initial wound. Cells transfected with scramble siRNA (Si Scramble) were able to completely fill the scratch wound by day 3 whereas ITGB4 knockdown cells (Si ITGB4) were not. **F)** H2009 and H1993 cells treated with 10 µM cisplatin over the course of 72 h. Data are represented as mean +/- SD.

**Figure S5.** ITGB4 knockdown induces cell death in NSCLC. Related to Figure 2.  
**A)** Proliferation and apoptosis assays were executed with stable cell lines expressing nuclear RFP and the IncuCyte Caspase-3/7 Green Reagent (Essen BioScience), which emits green fluorescence when cleaved by activated caspase-3/7. Apoptosis is induced with knockdown of ITGB4 (Si ITGB4) by day 4 and enhanced with added cisplatin. **B)** In H1993 cells with ITGB4 knockdown (red), apoptosis increased significantly compared to control cells (black). **C)** ITGB4 knockdown and double knockdown of PXN and ITGB4 induced apoptosis in H1993 and rendered cells more prone to toxic effects of cisplatin. (**p=0.03, **p=0.002, ****p<0.0001 Two-way ANOVA) **D)** H2009 cells did not show significant increase in apoptosis with ITGB4 knockdown. **E)** Double knockdown of PXN and ITGB4 in H2009 cells induced strong apoptosis and in combination with cisplatin, caspase activity increased at an earlier time point of 24 h. (**p=0.02, ***p=0.0002, ****p<0.0001 Two-way ANOVA) **F)** Nuclear RFP-expressing cell line H1650 was transfected with ITGB4 siRNA (Si ITGB4) and monitored in real-time with the IncuCyte. Over the course of 10 h, ITGB4 knockdown induced cells with an intact membrane were observed to undergo anoikis-like bursting. **G)** ITGB4 knockdown (red) in H1650 cells attenuated cell proliferation compared to control (black). Data are represented as mean +/- SD.

**Figure S6.** ITGB4 knockdown enhances cisplatin sensitivity and double knockdown with PXN impedes spheroid viability. Related to Figure 3.  
**A)** Effect of ITGB4 knockdown (3 different siITGB4 constructs) and cisplatin 2.5 µM on cell proliferation in H358 cells. **B)** Effect of ITGB4 knockdown (3 different siITGB4 constructs) and cisplatin 2.5 µM on cell proliferation in A549 cells. **C)** Expression of C-MET at the mRNA and protein level upon ITGB4 knockdown (red) compared to control (black). Data are represented as mean +/- SD.

**Figure S7.** PXN and ITGB4 knockdown induces caspase activity in spheroid culture. Related to Figure 3G-J.
A) H2009 cells expressing nuclear RFP were seeded in an ultra-low attachment 96-well plate to facilitate spheroid formation. After 4 h, double knockdown of PXN and ITGB4 impeded cells from forming a compact spheroid as observed in control and single knockdown conditions. B) and C) Spheroids with ITGB4 single knockdown and PXN/ITGB4 double knockdown were sensitized to cisplatin (10 μM) treatment indicated by a decrease in red fluorescence area and mean intensity. (****p<0.0001 Two-way ANOVA) D) In H2009 cells, single knockdown of ITGB4 (Si ITGB4) significantly induced apoptosis and double knockdown of PXN and ITGB4 (Si ITGB4+Si PXN) had an enhanced effect. (*p=0.014, ***p=0.0002) E) Double knockdown spheroids treated with cisplatin had the greatest cytotoxic effect indicated by the green fluorescence in the confocal images acquired by a Zeiss LSM 880 microscope. (**p<0.002, ***p<0.0009 Two-way ANOVA). Data are represented as mean +/- SD.

**Figure S8. Control experiments for proximity ligation assay (PLA).** Related to Figure 4F and G. Each antibody (FAK, ITGB4, and PXN) used in the PLA experiment was tested separately as a negative control.

**Figure S9. Supporting data for Figures 6-8.** Related to Figures 6-8. A) The selected constructs were tested for induction of caspase-3/7 activity by tracking green fluorescence using live cell imaging and analysis for 72 h. (****p<0.0001) B) H2009 and H1993 cells treated with ML323, a USP1 inhibitor, did not undergo significant changes in proliferation compared to untreated cells (black). C) Spare respiratory capacity, which is the ratio of maximal respiration vs. basal respiration, did not show any significant change between control and knockdown cells. D) RACIPE ensemble results (n=100,000) show ITGB4 and miR-1-3p exhibit bimodality: two distinct subpopulations of cells, as shown via z-score distributions of ITGB4 and miR-1-3p. E) FACS analysis using an anti-ITGB4 antibody showed enrichment of high ITGB4 population upon cisplatin treatment in H2009 cells. Data are represented as mean +/- SD. (****p<0.0001)

**Figure S10. Knocking down ITGB4 with shRNA.** Related to Figure 2. A) Effect of knocking down ITGB4 with shRNA on cell proliferation over the course of 3 days. B) Images of H2009 cells stably transfected with plasmid pGFP-V-RS only or expressing ITGB4 shRNA at 20X magnification.
| Original Member | Entrez Gene Id | Gene Symbol | Gene Description |
|-----------------|----------------|-------------|------------------|
| AF010316        | 9536           | PTGES       | Prostaglandin E Synthase |
| AF019770        | 9518           | GDF15       | Growth Differentiation Factor 15 |
| J04164          | 8519           | IFITM1      | Interferon Induced Transmembrane Protein 1 |
| M29366          | 2065           | ERBB3       | V-erb-b2 ErythroblasticLeukemia Viral Oncogenesis |
| M29870          | 5879           | RAC1        | Ras-related C3BotulinumToxin Substrate 1 |
| M33882          | 4599           | MX1         | Myxovirus(Influenza Virus) Resistance1 |
| S80437          | 2194           | FASN        | Fatty Acid Synthase |
| U09579          | 1026           | CDKN1A      | Cyclin-dependent Kinase Inhibitor 1A |
| U14588          | 5829           | PXN         | Paxillin |
| X53587          | 3691           | ITGB4       | Integrin, Beta 4 |
| X74295          | 3679           | ITGA7       | Integrin, Alpha 7 |

Table S2.

|                | Estimate | Standard Error | Relative Risk | P-Value  |
|----------------|----------|----------------|---------------|----------|
| gender[1]      | -0.04918 | 0.154775       | 0.952008      | 0.750665 |
| stage[S2]      | 0.738091 | 0.194204       | 2.09194       | 0.000144 |
| stage[S3]      | 1.17346  | 0.192492       | 3.23316       | 1.09E-09 |
| stage[S4]      | 1.31524  | 0.284479       | 3.72563       | 3.78E-06 |
| age            | 2.36E-05 | 2.14E-05       | 1.00002       | 0.271914 |
| ENSG00000089159*| 0.000156 | 3.03E-05       | 1.00016       | 2.47E-07 |

Table S3.

| Category        | Term                                                                 | Fold Enrichment | Count | PValue  |
|-----------------|----------------------------------------------------------------------|-----------------|-------|---------|
| G0006355        | GO:006355-regulation of transcription,DNA-templated                | 2.232978723     | 17    | 0.0029  |
| G0003158        | GO:0003158-endothelium development                                   | 65.85098039     | 2     | 0.0296  |
| G0000086        | GO:0000086--G2/M transition of mitotic cell cycle                   | 5.767969085     | 4     | 0.0314  |
| G0006351        | GO:0006351--transcription,DNA-templated                             | 1.717851662     | 17    | 0.0316  |
| G0015677        | GO:0015677--copper ion import                                        | 56.44369748     | 2     | 0.0345  |
| G0002544        | GO:0002544--chronic inflammatory response                            | 43.90065359     | 2     | 0.0441  |
| G0007156        | GO:0007156--homophilic cell adhesion via plasma membrane adhesion molecules | 5.001340283     | 4     | 0.0448  |
| hsa04350        | hsa04350:TGF-beta signaling pathway                                 | 8.471674877     | 3     | 0.0454  |
| hsa00450        | hsa00450:Selenocompound metabolism                                  | 27.90669371     | 2     | 0.0671  |
| hsa001701       | hsa001701--in utero embryonic development                            | 4.225731362     | 4     | 0.0673  |
| G0008284        | G0008284--positive regulation of cell proliferation                | 2.543600101      | 6     | 0.0841  |
| G0010971        | G0010971--positive regulation of G2/M transition of mitotic cell cycle | 21.9503268       | 2     | 0.0864  |
| Category            | Term                                                                 | Fold Enrichment | Count | PValue   |
|---------------------|-----------------------------------------------------------------------|-----------------|-------|----------|
| GOTERM_BP_DIRECT    | GO:0006334~nucleosome assembly                                        | 5.759560967     | 8     | 4.79E-04 |
| GOTERM_BP_DIRECT    | GO:0007265~Ras protein signal transduction                            | 6.119535328     | 5     | 0.00891  |
| KEGG_PATHWAY        | hsa04810:Regulation of actin cytoskeleton                            | 3.04717608      | 8     | 0.01475  |
| GOTERM_BP_DIRECT    | GO:001525~angiogenesis                                                | 3.073487691     | 8     | 0.01553  |
| KEGG_PATHWAY        | hsa05034:Alcoholism                                                   | 3.163381947     | 7     | 0.02182  |
| GOTERM_BP_DIRECT    | GO:0017085~response to insecticide                                    | 85.67346939     | 2     | 0.02309  |
| KEGG_PATHWAY        | hsa05322:Systemic lupus erythematosus                                 | 3.58156889      | 6     | 0.02467  |
| GOTERM_BP_DIRECT    | GO:0032093~dGTP catabolic process                                     | 57.11564626     | 2     | 0.03444  |
| KEGG_PATHWAY        | hsa00470:Phosphatidylinositol signaling system                        | 4.061039393     | 5     | 0.03263  |
| GOTERM_BP_DIRECT    | GO:0009611~response to wounding                                       | 5.439585358     | 4     | 0.03684  |
| KEGG_PATHWAY        | hsa045815~positive regulation of gene expression, epigenetic         | 5.527320606     | 4     | 0.04327  |
| GOTERM_BP_DIRECT    | GO:0009611~response to wounding                                       | 5.439585358     | 4     | 0.03684  |
| GOTERM_BP_DIRECT    | GO:0043928~exonucleolytic nuclear-transcribed mRNA catabolic process  | 8.862772695     | 3     | 0.04437  |
| GOTERM_BP_DIRECT    | GO:0035404~histone-serine phosphorylation                             | 42.83673469     | 2     | 0.04565  |
| GOTERM_BP_DIRECT    | GO:0007052~mitotic spindle organization                               | 8.567346939     | 3     | 0.04718  |
| GOTERM_BP_DIRECT    | GO:0030855~epithelial cell differentiation                             | 4.895626822     | 4     | 0.04791  |
| GOTERM_BP_DIRECT    | GO:0006335~DNA replication-dependent nucleosome assembly              | 8.03188755      | 3     | 0.053    |
| GOTERM_BP_DIRECT    | GO:0061551~trigeminal ganglion development                            | 34.26938776     | 2     | 0.05674  |
| KEGG_PATHWAY        | hsa04152:AMPK signaling pathway                                       | 3.251559841     | 5     | 0.06526  |
| GOTERM_BP_DIRECT    | GO:0042060~wound healing                                             | 4.283673469     | 4     | 0.06621  |
| GOTERM_BP_DIRECT    | GO:0032958~inositol phosphate biosynthetic process                    | 28.55782313     | 2     | 0.06769  |
| GOTERM_BP_DIRECT    | GO:0071455~cellular phosphate to hyperoxia                            | 24.47813411     | 2     | 0.07852  |
| GOTERM_BP_DIRECT    | GO:0051290~protein heterotetramerization                             | 6.119535328     | 3     | 0.08541  |
| GOTERM_BP_DIRECT    | GO:0007160~cell-matrix adhesion                                      | 3.807709751     | 4     | 0.08722  |
| GOTERM_BP_DIRECT    | GO:0051597~response to methylmercury                                 | 21.41836735     | 2     | 0.0923   |
| GOTERM_BP_DIRECT    | GO:0030514~negative regulation of BMP signaling pathway              | 5.711564626     | 3     | 0.09605  |
| GOTERM_BP_DIRECT    | GO:0060627~regulation of vesicle-mediated transport                   | 19.03854875     | 2     | 0.09981  |
| GOTERM_BP_DIRECT    | GO:0030903~notochord development                                      | 19.03854875     | 2     | 0.09981  |

Table S4.
### Table S5.

| SI/RNA Sequence | Target Gene | Locus ID | RefSeq | Construct | Sequence (5'-3') |
|-----------------|-------------|----------|--------|-----------|------------------|
| PXN KD          |             |          |        | A         | AGCUACAGUGAUAACUAGAGT |
|                 |             |          |        | B         | GGAGCAAAAGCCCAACUAAGACAG |
|                 |             |          |        | C         | CAGCAAGUUAAGAAUAGCAAGCAG |

* Denotes optimal siRNA construct used for downstream experiments

### Table S6.

| Target Gene | Locus ID | RefSeq | Predicted Activation | Activation Score | P-value |
|-------------|----------|--------|----------------------|------------------|---------|
| ITGB4       | 5829     | NM_001080855.2, NM_001243756.1, NM_002859.3, NM_025157.4 | Activated | 2.236 | 0.0985 |
| PXN KD      |          |        |                      | Activated | 0.00772 |
| PXN/ITGB4 KD|          |        |                      | Activated | 0.00352 |
| PXN KD      |          |        |                      | Activated | 0.0000133 |
| PXN KD      |          |        |                      | Activated | 0.0015 |

* Denotes optimal siRNA construct used for downstream experiments
Fig. S1

| kDa  | SW480 | H23  | H358 | SW1573 | H441 | H2009 | H522 | H1650 | H596 | H1437 | H1993 |
|------|-------|------|------|--------|------|-------|------|-------|------|-------|-------|
| 150  |       |      |      |        |      |       |      |       |      |       |       |
| 50   |       |      |      |        |      |       |      |       |      |       |       |

ITGA6

β-Actin
Fig. S2

A

Case3

1

2

Case4

1

2

Case5

1

2

B

Dual Positive

ITGB4 Positive

PXN Positive

C

Percentage of area

Case1  Case2  Case3  Case4  Case5

Case1  Case2  Case3  Case4  Case5

Case1  Case2  Case3  Case4  Case5
**Fig. S3**

**A**

- NucLight Red Lentivirus Transduction Reagent
- Nuclear restricted mKate2 expression (Stable cell line selection with puromycin)

**B**

- **H358+ Si ITGB4**

  - siScramble
  - siITGB4 (A)
  - siITGB4 (B)
  - siITGB4 (C)

  - Fold change in cell count vs. elapsed time in hours

**C**

- **A549+ Si ITGB4**

  - siScramble
  - siITGB4 (A)
  - siITGB4 (B)
  - siITGB4 (C)

  - Fold change in cell count vs. elapsed time in hours

**D**

- **H358+ Si ITGB4**

  - Scr
  - A
  - B
  - C

  - ITGB4
  - PXN
  - γH2AX
  - β-Actin

- **A549+ Si ITGB4**

  - Scr
  - A
  - B
  - C

  - kDa
    - 250
    - 75
    - 20
    - 50

**Legend:**

- siScramble
- siITGB4 (A)
- siITGB4 (B)
- siITGB4 (C)
**Fig. S5**

A) Untreated vs Cisplatin

B) Fold change in caspase activity for H1993 + Si ITGB4

C) Caspase 3/7 activity for H1993

D) Fold change in caspase activity for H2009 + Si ITGB4

E) Caspase 3/7 activity for H2009

F) H1650 + Si ITGB4

G) Fold change in cell count for H1650 + Si ITGB4
Fig. S6

(A) H358 + SiITGB4 + Cisplatin 2.5 µM

(B) A549 + SiITGB4 + Cisplatin 2.5 µM

(C) H1993

- mRNA expression

- C-Met

H2009

- mRNA expression

- C-Met

- ITGB4

- Actin

- Western Blot

- H1993

- H2009
Fig. S7

A

Si Scramble | Si PXN | Si ITGB4 | Si PXN + Si ITGB4

0h

4h

B

C

D

E

Spheroid Area

Red Fluorescence Intensity

Fold Change in Caspase Activity

Nuclear mKate2 | Caspase-3/7 | Merge

Spheroid Caspase Activity

Caspase-3/7 activity

0 | 24 | 48 | 72 | 96 | 120

Si Scramble | Si PXN | Si ITGB4 | Si ITGB4 + Si PXN

0 | 24 | 48 | 72 | 96 | 120
**Fig. S9**

**A**

H2009 + SiRNA

- siScramble
- SIVDAC1-A
- SiG3BP1-C
- SiUSP1-B

**B**

H2009 + ML323

- H1993 + ML323

**C**

Spare respiratory capacity

- OCR (pmol/min/1000cells)

**D**

- Caspase3/7 Activity

**E**

- Fold change in Cell Count

- DMSO Cisplatin

- miR-1-3p (z-score)

- ITGB4 (z-score)

- Population Percentage

- ITGB4 LOW

- ITGB4 HIGH

- 48.8 49.7

- 34.9 63.6
Fig. S10

A

H2009+ ShITGB4

Fold change in cell count

Control
shRNA 50ng/24 well
ShRNA100ng/24 well

Day0 Day3 Day0 Day3 Day0 Day3

0 1 2 3 4 5

B

pGFP-V-RS Stable Cells (4 weeks selection)

Brightfield
GFP
Magnification 20X

pGFP-V-RS-ShRNA ITGB4 Stable Cells (4 weeks selection)

Brightfield
GFP
Magnification 20X
**Transparent Methods**

**Cell lines and reagents**

Lung cancer cell lines (A549, H23, H358, SW1573, H441, H2009, H522, H1650, H596, H1437, and H1993) were obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA). All cell lines were cultured in RPMI 1640 medium (Corning) supplemented with fetal bovine serum (FBS) (10%), L-glutamine (2 mM), penicillin/streptomycin (50 U/ml), sodium pyruvate (1 mM), and sodium bicarbonate (0.075%) at 37°C, 5% CO₂. Cisplatin was provided by City of Hope National Medical Center clinics.

**Antibodies**

Antibodies against ITGB4 (cat #: 4707), FAK (cat #: 3285), phospho-FAK (Y397) (cat #: 8556), γH2AX (cat #: 2557), p27 (cat #: 3686), phospho-Rb (S807/811) (cat #: 8516), USP1 (cat #: 8033), and ITGA6 (cat #: 3750) were purchased from Cell Signaling Technology (Danvers, MA, USA). Antibodies against ITGA7 (cat #: sc-515716), ITGA6 (cat #: sc-53356), PXN (cat #: sc-5574), MET (cat #: sc-10), G3BP1 (cat #: sc-365338), and VDAC1 (cat #: sc-39096), and agarose-conjugated antibodies (ITGB4, FAK, PXN, IgG) were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Cyclin D1 antibody was purchased from Invitrogen (cat #: MA5-14512) (Waltham, MA, USA). Phospho-PXN (Y31) (cat #: ab4832) antibody was purchased from Abcam (Cambridge, UK). β-actin antibody was purchased from Sigma-Aldrich (cat #: A5441) (St. Louis, MO, USA).

**Western blotting**

Cell lysates were prepared with 1X RIPA buffer (MilliporeSigma) and denatured in 1X reducing sample buffer at 95°C for 5 min. Protein samples (15 μg) were run on 4-15% TGX gels (Bio-Rad, Hercules, CA, USA) and transferred onto nitrocellulose membranes (Bio-Rad). Blots were blocked with 5% non-fat milk in TBS-T for 1 hour at room temperature and probed with primary antibody diluted in 2.5% BSA in TBS-T overnight at 4°C. After three washes with TBS-T, blots were incubated with HRP-conjugated secondary antibodies for 2 hours at room temperature. After three more washes, bands of interest were visualized via chemiluminescence using WesternBright ECL HRP substrate (Advansta, Menlo Park, CA, USA) and imaged with the ChemiDoc MP imager (Bio-Rad).

**Quantitative real-time PCR and RNAseq**

Quantitative real-time PCR (qPCR) reactions were performed using TaqMan Universal PCR Master Mix (Thermo Fisher Scientific, Waltham, MA) and analyzed by the QuantStudio7 Real-time PCR system (Life Technologies, Grand Island, NY). Total RNA isolation and on-column DNase digestion from cells were performed basing on the manufacturer’s protocol RNeasy Plus Mini Kit (Qiagen Cat #: 74134). 1 ug of RNA was used to synthesize the cDNA according to the one step cDNA synthesis kit from QuantaBio (Cat#: 101414-106). TaqMan probes for HS99999905 –GAPDH, HS00236216-ITGB4, HS01104424-PXN, HS00174397-ITGB1, HS00164957-ITGB2,
HS01001469-ITGB3, HS01565584-MET, HS04978484-VDAC1, HS00428478-G3BP1 and HS00163427-USP1 were purchased from ThermoFisher (Waltham, MA). The mRNA expression was analyzed using multiplex PCR for the gene of interest and GAPDH as reference using two independent detection dyes FAM probes and VIC probes respectively. Relative mRNA expression was normalized to GAPDH signals and calculated using the delta delta Ct method.

RNA was extracted from both single and double knockdown H2009 cells 48 h post siRNA transfection, and total RNAseq was performed by the Integrative Genomics Core at City of Hope. RSeQC showed no substantial bias in the coverage of RNAseq reads. A total of 30 million reads were analyzed for each condition.

**siRNA and shRNA Transfection**

Knockdown of ITGB4 (Cat #: SR302473C), FAK (Cat #: SR303877C), USP1 (Cat #: SR305052B), and VDAC1 (Cat #: SR305067C) at the mRNA level was executed using siRNAs purchased from OriGene Technologies (Rockville, MD, USA). Knockdown of PXN was achieved by siRNA purchased from Life Technologies Corporation (Cat #: 4392421). JetPRIME transfection reagent (Polyplus Transfection, Illkirch, France) was used to transfect the siRNAs according to the manufacturer’s protocol. Cells were seeded in 6-well plates (200,000 cells/well) and allowed to adhere overnight. Next day, 10 nM siRNA was transfected with 4 μl jetPRIME reagent in complete growth medium for each well. Cell growth medium was changed the next day and expression was detected 72 h post-transfection by immunoblot. Similarly, the plasmid pGFP-V-RS expressing shRNA against ITGB4 was used to transfect H2009 cells, and puromycin (ThermoFisher) was used to generate a stable cell line. The sequences of all the siRNAs used are described in Table S6.

**Cell viability assay**

Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies (Rockville, MD, USA). Cells were seeded on a 96-well plate and allowed to adhere in complete medium for 24 hours. Test compounds were added to 100 μl of medium at the indicated concentrations for 72 hours. Ten μl of the CCK-8 reagent were added to each well and absorbance at 450 nm was measured using a Tecan Spark 10M multimode microplate reader.

**Immunohistochemistry (IHC)**

Dual IHC stain for ITGB4 and PXN was performed on Ventana Discovery Ultra (Ventana Medical Systems, Roche Diagnostics, Indianapolis, USA) automated IHC stainer. Briefly, tissue samples were sectioned at a thickness of 5 μm and mounted on positively charged glass slides. The slides were loaded on the machine and deparaffinization, rehydration, endogenous peroxidase activity inhibition and antigen retrieval were performed. Then, the two antigens were sequentially detected, and heat inactivation was used to prevent antibody cross-reactivity between the same species. Following each primary antibody incubation, DISCOVERY anti-mouse HQ or DISCOVERY anti-Rabbit NP and DISCOVERY anti-HQ-HRP or anti-NP-AP were incubated. The stains were then visualized with DISCOVERY Purple and DISCOVERY Yellow Kit, respectively,
counterstained with hematoxylin (Ventana) and cover slipped. The slides were scanned using the Motic Easy Scanner and 40X images were analyzed using the QuPath software.

**Scratch wound healing assay**

Cells were seeded on a 96-well ImageLock (Essen BioScience, Ann Arbor, MI, USA) plate to reach 90% confluence by the next day. After cell adherence, 96 uniform wounds were created simultaneously using the WoundMaker (Essen BioScience) tool. Cells were washed once with serum-free medium and replenished with complete medium. To monitor wound healing, the plate was placed in the IncuCyte S3 Live Cell Imaging System (Essen BioScience) and images were acquired every hour. Data analysis was generated by the IncuCyte software using a set confluence mask to measure relative wound density over time.

**Cell proliferation and apoptosis assay**

Cell proliferation assays were performed using cell lines stably transfected with NucLight Red Lentivirus (Essen BioScience) to accurately visualize and count the nucleus of a single cell. Cells were seeded on a 96-well plate and allowed to adhere for 24 h. Test compounds were added at indicated concentrations. Caspase-3/7 Green Apoptosis Reagent (Essen BioScience) was also added as a green fluorescent indicator of caspase-3/7-mediated apoptotic activity. To monitor cell proliferation and apoptosis over time, the plate was placed in the IncuCyte S3 Live Cell Imaging System (Essen BioScience) and images were acquired every 2 hours. Data analysis was generated by the IncuCyte software using a red fluorescence mask to accurately count each cell nucleus and a green fluorescence mask to measure apoptosis over time.

**3D spheroid assay**

3D spheroid experiments were performed using cell lines stably transfected with NucLight Red Lentivirus (Essen BioScience) to visualize red fluorescence as an indicator of cell viability. Cells were seeded on a 96-well ultra-low attachment plate and allowed to form spheroids overnight. Drug treatment was added as indicated along with Cytotox Green Reagent (Essen BioScience), used as a green fluorescence indicator of cell death due to loss of cell membrane integrity. To monitor cell proliferation and apoptosis over time, the plate was placed in the IncuCyte S3 Live Cell Imaging System (Essen BioScience) and images were acquired every 2 hours. Data analysis was generated by the IncuCyte software using a red fluorescence mask to accurately measure intensity and area of red fluorescence, indicating spheroid viability and a green fluorescence mask, indicating cell death.

**Cell cycle analysis**

H2009 cells were harvested and pelleted after 72 h following siRNA transfection. Ice cold 70% ethanol was added to the pellet with mild vortexing to fix the cells. The fixed cells were kept at 4°C for PI staining. FxCycle™ PI/RNase Staining solution from Invitrogen was used for staining the DNA according to the manufacturer's protocol prior
the FACS analysis. Univariate model of Watson (Pragmatic) was used for cell cycle analysis.

Confocal microscopy

3D spheroids were seeded and imaged in 96-well clear ultra-low attachment microplates (Corning) using Zeiss LSM 880 confocal microscope with Airyscan at the Light Microscopy/Digital Imaging Core Facility at City of Hope. Images were processed using ZEN software and analyzed using ImageJ (Schneider et al, 2012).

Co-immunoprecipitation (Co-IP)

For all co-IP experiments, cells were lysed in the Pierce™ IP Lysis Buffer (Thermo Fisher Scientific) and 1 mg of protein was allowed to bind overnight in 4°C to agarose-conjugated antibodies (Santa Cruz Biotechnology): ITGB4 (Cat #: sc-13543 AC), FAK (Cat #: sc-271195 AC), PXN (Cat #: sc-365379 AC). IP beads were washed 5 times with 1X RIPA buffer and denatured in 2X reducing sample buffer at 95°C for 5 min. Western blots according to aforementioned protocol were performed to determine IP results. Multiple gels were run using the same lysates to detect all the specific antibodies. Exogeneous expression of ITGB4 and PXN was achieved in HEK293 cells by transfecting 7 ug of both pRK5 beta 4 and pBMN-PXN-HA-IRE5-Hygro with Lipofectamine 3000 (Invitrogen) in a 10 cm dish. pRK5 beta4 was a gift from Filippo Giancotti (Dans et al, 2001) (Addgene plasmid # 16037; http://n2t.net/addgene:16037; RRID: Addgene16037). Cells were lysed 72h post transfection and co-IP was performed according to above protocol.

Proximity ligation assay (PLA)

To perform a complete Duolink® PLA in situ experiment, we used two primary antibodies (PLA, Immunofluorescence validated) that recognize PXN, ITGB4 or FAK epitopes. The starter kit from SIGMA supplies all other necessary reagents for Duolink® PLA reactions, which include a pair of PLA probes (Anti-Rabbit PLUS and Anti-Mouse MINUS), red detection reagents, wash buffers, and mounting medium. The primary antibodies used came from the same species as the Duolink® PLA probes for ITGB4 (Cell Signaling Technology, cat #: 4707)/PXN or FAK (Cell Signaling Technology, cat #: 3285) /PXN (clone 5H11, Invitrogen, cat #: MA5-13356) PLA. Analysis was carried out using standard immunofluorescence assay technique. We used a confocal microscope (LSM880) to capture images.

Seahorse XF Cell Mito Stress Test metabolic assay

Cells were seeded in complete growth medium on a Seahorse XF Cell Culture Microplate (Agilent Technologies, Santa Clara, CA, USA) to reach 90% monolayer confluence by the next day. One day prior to assay, 5 μM cisplatin was added for 24 h. On the day of the assay, mitochondrial inhibitor compounds were added to injection ports of the XFe96 FluxPak sensor cartridge at a final concentration of: oligomycin 1 μM, FCCP 1 μM, rotenone/antimycin A 1 μM each. Culture medium was changed to assay medium:
Seahorse XF RPMI medium supplemented with 1 mM sodium pyruvate, 2 mM L-glutamine, and 10 mM glucose. After completion of assay, cells were immediately stained with Hoechst dye and imaged using BioTek. Images were analyzed with QuPath (Bankhead et al, 2017) to obtain number of cells in each well and normalize data according to cell number.

**ROS production assay**

Cells were seeded in a 96-well plate and placed in an incubator at 37°C for 72 h. 50 μl of medium from each well was transferred to another 96-well plate to measure ROS production with ROS-Glo™ H₂O₂ Assay (Promega, Madison, WI, USA). Remaining cells on the first plate were used to perform CellTiter-Glo® Luminescent Cell Viability Assay (Promega) to normalize ROS data to number of viable cells. Luminescence was measured using a Tecan Spark 10M multimode microplate reader.

**γH2AX foci staining and analysis**

Cells were seeded (50,000 cells/well) on glass cover slips coated with 0.1% gelatin (Millipore) in a 12-well plate. Next day, 5 μM cisplatin was added for 24 h. Cells were fixed in 4% formaldehyde for 30 min at room temperature and blocked. Primary antibody against γH2AX (Cell Signaling Technology) was incubated in 4°C overnight. Then secondary antibody was incubated for 2 hours at room temperature. Cover slips were mounted on glass slides and imaged with Zeiss LSM 880 confocal microscope at the Light Microscopy/Digital Imaging Core Facility at City of Hope. Using QuPath (Bankhead et al, 2017), green fluorescent subcellular particles were counted in each nucleus to obtain γH2AX foci count per cell.

**Chromatin immunoprecipitation (ChIP)**

Briefly, five million formaldehyde-fixed cells were lysed in 200 μl of SDS lysis buffer and diluted to 2 ml in ChIP dilution buffer in the presence of protease inhibitors. Lysates were sonicated using Bioruptor PICO for 3 cycles and each cycle has 10 repeats of 30 sec pulse and 30 sec break. Lysates were precleared in salmon sperm DNA and protein A agarose by centrifugation. Prior to addition of antibody, 10% of the lysate was used for input and the remaining lysate was divided into two equal parts, one for IgG control and other for H3K27 acetylated antibody from Diagenode. Downstream processing of the chromatin bound antibody was done as per the manufacturer’s protocol for EZ-magna ChIP A/G (Millipore, Temecula, CA). The extracted DNA was used for SYBR green based qPCR assay using the primers sequences Upstream USP1R-5’-AGGTTTCACAGCATTTCTCAATCC-3’, Upstream USP1F-CAGTGCCCTGTGAAAACCTTTGGA, Promoter USP1F-CTCAGCTCTACAGCACCTCGC and Promoter USP1R-GGCCATCCAATGAGACAAGG. The data was analyzed based on the percentage of input.

**Fluorescence-activated cell sorting (FACS) and analysis**
Cells were trypsinized and resuspended (5 million) in PBS with 2% FBS. Cells were stained with ITGB4 antibody conjugated to Alexa Fluor® 488 (5 μl/1 million cells) (R&D Systems, Minneapolis, MN, USA) and Propidium Iodide Ready Flow™ Reagent (1 drop/1 million cells) (Invitrogen) for 30 min at 4°C. The Analytical Cytometry Core Facility at City of Hope carried out and assisted all FACS sorting and analysis experiments. Gates were set to sort cell populations having low 10% and high 10% expression of ITGB4 using the FACSAria™ Fusion (BD Biosciences, San Jose, CA, USA). Sorted cells were immediately cultured in 12-well plates and treated with cisplatin (1 μM) for 48 h. Then, equal number of untreated and treated cells were collected and stained with same reagents as above. FACS analysis was performed to determine shifts in cell population using the Attune Nxt Flow Cytometer (Invitrogen).

Mathematical modeling

Bifurcation diagram was obtained using MATCONT (Dhooge et al, 2003). Next, Random circuit perturbation (RACIPE) algorithm was run on the two-node network – ITGB4/ miR-1-3p. The continuous gene expression levels were obtained as output with randomly chosen parameters for the regulatory links. The algorithm was used to generate 100,000 mathematical models, each with a different set of parameters for the following ODEs:

\[ u = G_u H^s(I, I^0_u, n_{iu}, \lambda^-_{iu}) - k_u u \]
\[ I = G_i H^s(u, u^0_i, n_{ai}, \lambda^-_{ai}) - k_i I \]

where, \( u \) denotes miR-1-3p and \( I \) denotes ITGB4. \( G_u \) and \( G_i \) are the maximum production rates of miR-1-3p and ITGB4 respectively. And, \( k_u \) and \( k_i \) are their innate degradation rates respectively.

Equations:

\[ \mu^* = g_\mu \mu^* H^s(I, \lambda_{i,\mu^*}) - m_\mu Y_u(\mu_{3p}) - k_\mu \mu_{3p} \]
\[ m_i^* = g_m H^s(C, \lambda_{i,m}) - m_i Y_m(\mu_{3p}) - k_m m_i \]
\[ I^* = g_i m_i L(\mu_{3p}) - k_i I \]

where \( H^s \) is the shifted Hill function, defined as \( H^s(B, \lambda) = H^-(B) + \lambda H^+(B), \)
\( H^-(B) = 1/(1 + (B / B_0)^n) \), \( H^+(B) = 1 - H^-(B) \) and \( \lambda \) is the fold change from the basal synthesis rate due to protein B. \( \lambda >1 \) for activators, while \( \lambda <1 \) for inhibitors.

The total translation rate:

\[ m_i L(\mu_{3p}) = m \sum_{(i=0)}^n l_i C_i M_i^+(\mu) \]
The total mRNA active degradation rate:

\[ m_i Y_m (\mu_{3p}) = m \sum_{i=0}^{n} \gamma_{m_i} C_{m_i}^i M_{m_i}^i (\mu) \]

The total miR active degradation rate is

\[ m_i Y_\mu (\mu_{3p}) = m \sum_{i=0}^{n} \gamma_{m_i} C_{m_i}^i M_{m_i}^i (\mu) \]

Parameters used in panel A:

| n (\# of miR binding sites) | 0 | 1 | 2 | 3 |
|-----------------------------|---|---|---|---|
| L only | \( l_i \) | 1.0 | 0.5 | 0.2 | 0.02 |
| Y only | \( \gamma_{mi} \) (Hour\(^{-1}\)) | 0.3 | 1.5 | 7.5 |
| Both, L stronger | \( l_i \) | 1.0 | 0.6 | 0.3 | 0.1 |
| | \( \gamma_{mi} \) (Hour\(^{-1}\)) | 0.04 | 0.2 | 1.0 |
| ---- | \( \gamma_{mi} \) (Hour\(^{-1}\)) | 0.005 | 0.05 | 0.5 |

| Parameter | Value |
|-----------|-------|
| \( k_{\mu_{3p}} \) \( \text{(hour}^{-1} \text{)} \) | 0.05 |
| \( k_{mi} \) \( \text{(hour}^{-1} \text{)} \) | 0.5 |
| \( k_l \) \( \text{(hour}^{-1} \text{)} \) | 0.1 |
| \( g_{\mu_{3p}} \) \( \text{(molecules/hour)} \) | 2900 |
| \( g_{mi} \) \( \text{(molecules/hour)} \) | 30 |
| \( g_l \) \( \text{(molecules/hour)} \) | 100 |
| \( g_{\mu_{3p}} \) \( \text{(hour}^{-1} \text{)} \) | 100 |
| Parameter         | Value  |
|-------------------|--------|
| I₀,µ3p            | 6000   |
| (molecules)       |        |
| µ3p₀              | 10000  |
| (molecules)       |        |
| C₀,ml             | 250000 |
| (molecules)       |        |
| n₁,µ3p            | 3      |
| nµ3p              | 6      |
| nC,ml             | 3      |
| λ₁,µ3p            | 0.3    |
| λC,ml             | 10     |

The feedback loop was constructed based on data reported in the manuscript (ITGB4 inhibits miR-1-3p) and publicly available data (miR-1-3p inhibits ITGB4) - [https://www.genecards.org/cgi-bin/carddisp.pl?gene=ITGB4](https://www.genecards.org/cgi-bin/carddisp.pl?gene=ITGB4).

The parameters for microRNA-mediated dynamics were estimated from our previous models for microRNA-mediated regulation of EMT (Lu et al. 2013).
Supplemental References

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