Supplemental Materials

Molecular Biology of the Cell

Asuthkar et al.

Supplemental Figure 1S.

The side population of MIA PaCa-2 cells is rich in CD44 and CD24. MIA PaCa-2 and PANC-1 cells (2×10^6) re-suspended in 0.5 mL PBS-BSA were analyzed and the side-population (SP) cells were sorted by density-based flow cytometry (10,000 cells sorted per treatment condition with 3 replicates). Acquisition was performed on a FACS caliber flow cytometer (Becton Dickinson, San Jose, CA) and viable cells were analyzed with CellQuest software. In a separate experiment, SP cells were labeled with anti-CD44 and anti-CD24 antibodies using a similar staining protocol. The control for each sample was prepared identically except that an isotype-specific unrelated antibody was used. FACS analysis of the side population (R1) of cells stained for CD44 and CD24 indicated that these cells were enriched in CD44 and CD24 relative (27.6%) to the cells present in the R2 region (6.3%).

Supplemental Figure 2S

uPA overexpression enhances the sphere-forming ability of the side-population of MIA PaCa-2 and PANC-1 cells. Side-population (SP) and side population depleted (ΔSP) cells collected using a flow cytometer as described in the legend to Figure 1 were collected. The cells were transfected with uPA overexpressing plasmid to generate uPA over expressing cells (uPAOE). uPAOE and un-transfected ΔSP cells were cultured in serum free media for 15 days. Sphere-forming capacity was determined by plating cells on an ultralow attachment plate for 7 days at 37°C with 95% air and 5% CO_2. uPAOE but not control ΔSP cells established spheres, indicative of an anchorage independent growth pattern **(A)**. Spheres where counted and graphically represented **(B)**.

ΔSP	ΔSP+uPAOE	SP	SP+uPAOE	ΔSP	ΔSP+uPAOE	SP	SP+uPAOE
-	R^2=0.8550	R^2=0.8315	R^2=0.9467	-	R^2=0.8445	R^2=0.8366	R^2=0.8943

Supplemental Figure 3S

uPA positively regulates the proportion of side population cells in pancreatic carcinoma cell lines. MIA PaCa-2 and PANC-1 cells were transfected with uPA-encoding plasmid (pUPA OE). The uPA- overexpressing cells were further transfected with puPA (plasmid expressing uPA targeting siRNA). 10,000 cells were sorted by FACS analysis. The uPA overexpressing cells contained a higher proportion of SP cells, whereas uPA silencing lowered the proportion of SP cells in the population (MIA PaCa-2 $p<0.82$, PANC-1 $p<0.76$).

Supplemental Figure 4S.

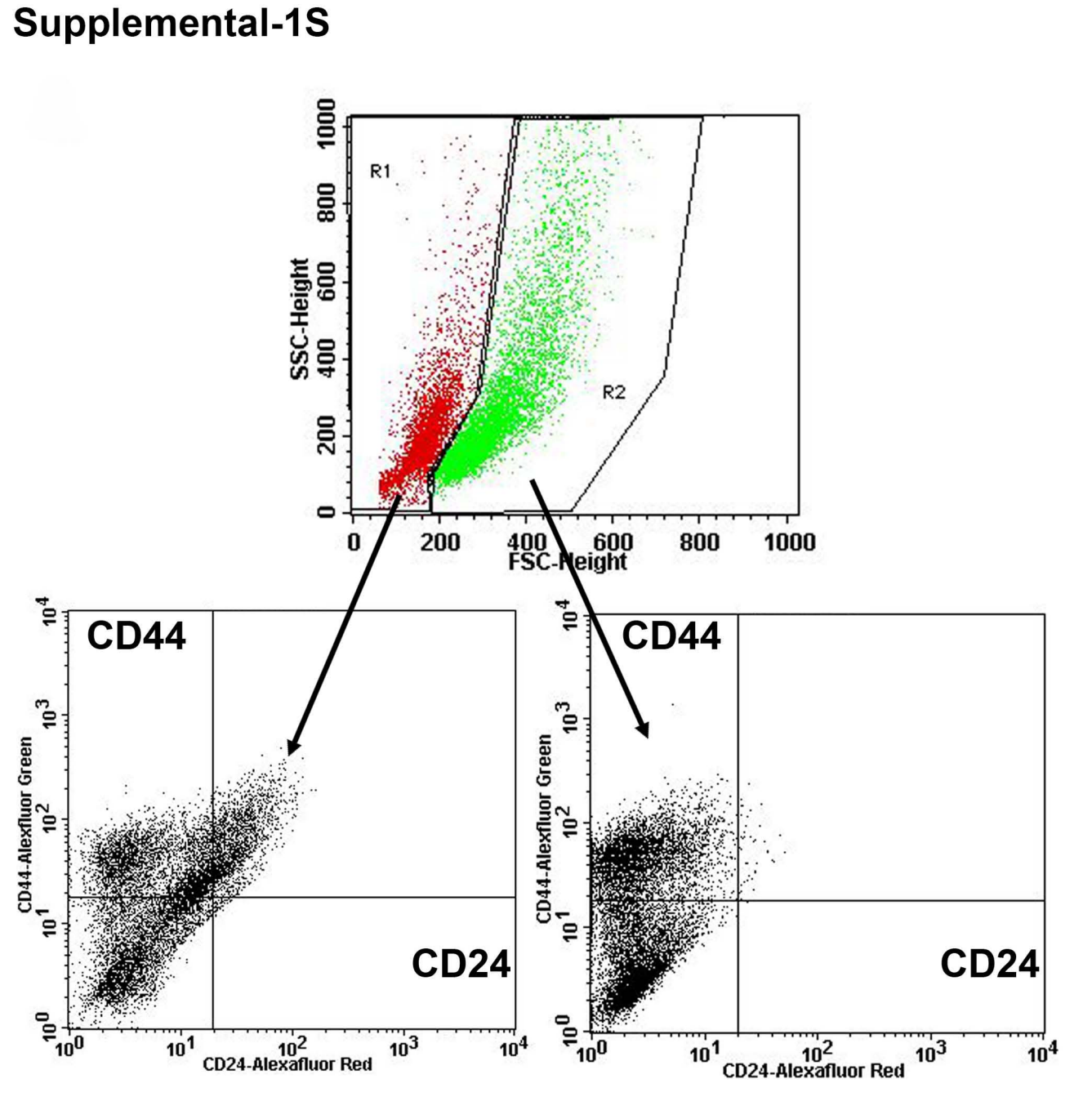
uPAR in not essential for nuclear translocation of uPA. (A) WT-uPA, ΔGFD-uPA and ΔK-uPA were isotopically labeled with $Na^{125}I$ (Stepanova et al, 2008). ^{125} I -WT-uPA, ^{125} I -

ΔGFD-uPA and ^{125}I -ΔK-uPA (10 nM each) were incubated with 10^6 PANC-1 cells for 1 hrs. Cells were washed free of unbound radioactive proteins, separated into membrane, cytoplasmic and nuclear fractions as described (Stepanova et al, 2008) the amount of radioactivity in each fraction was measured. Results are presented in fmol protein. All experiments were performed in triplicate. P<0.05. **(B)** PANC-1 cells growing exponentially on 8-well chamber slides were incubated with WT-uPA or ΔGFD-uPA for 1 h, washed, fixed in MeOH, incubated sequentially with anti-uPA rabbit polyclonal Abs and Alexa 488-conjugated secondary anti-rabbit Abs and analyzed for immunofluorescence (Stepanova et al, 2008). Nuclei were counterstained with propidium iodide (red). Green denotes positive staining for uPA. **(C)** PANC-1 cells were transduced with uPA-targeting shRNA in pLKO vector, selected with puromycin, and incubated with WT-uPA, ΔGFD-uPA and ΔK-uPA for 24 h. Cells were lysed, the lysates were subjected to SDS-PAGE and WB, and analyzed for Lhx-2 and GAPDH (loading control) as described in Methods.

Supplemental Fig. 5S.

uPA binds HOXA5 and sensitizes Capan-2 cells to gemcitabine treatment through down-regulation of p53 expression. (A) TranSignal TF Protein Array. Array Membranes (Panomics, Version II) spotted in duplicate with proteins expressed from full-length TF cDNAs were incubated with scuPA (10 nM) for 2 h. Bound scuPA was detected with HRP-conjugated rabbit α-uPA pAbs and chemoluminescent substrate. **(B)** Capan-2 cells were transduced with either uPA-targeting shRNA or scrambled shRNA in pLKO vector, selected with puromycin and gemcitabine (100 μM) was added for indicated times. Total RNA was isolated and p53 mRNA was quantified by qRT-PCR, and normalized for mRNA levels of housekeeping genes β-actin and GAPDH. **(C)** Capan-2 cells were transduced with uPA-targeting shRNA in pLKO vector and selected with puromycin. Cells (10^4 per well) were seeded onto 96-well plates in 100 μL media. The next day, gemcitabine was added in 100 μL media to the desired final concentration (0 to 100 μM) for 48 h in the presence or absence of WT-uPA, ΔGFD-uPA and ΔK-uPA. After 48 hours, Premixed WST-1 Cell proliferation Reagent (Clontech) was added at a 1:10 dilution, the plates were incubated at 37°C for 30 min, and the absorbance read at 420 nm and 600 nm. The percentage of viable cells was defined as ($A_{490}-A_{600}$) of gemcitabine divided by the ($A_{490}-A_{600}$) of cells receiving no treatment, multiplied by 100%.

Supplemental-1S



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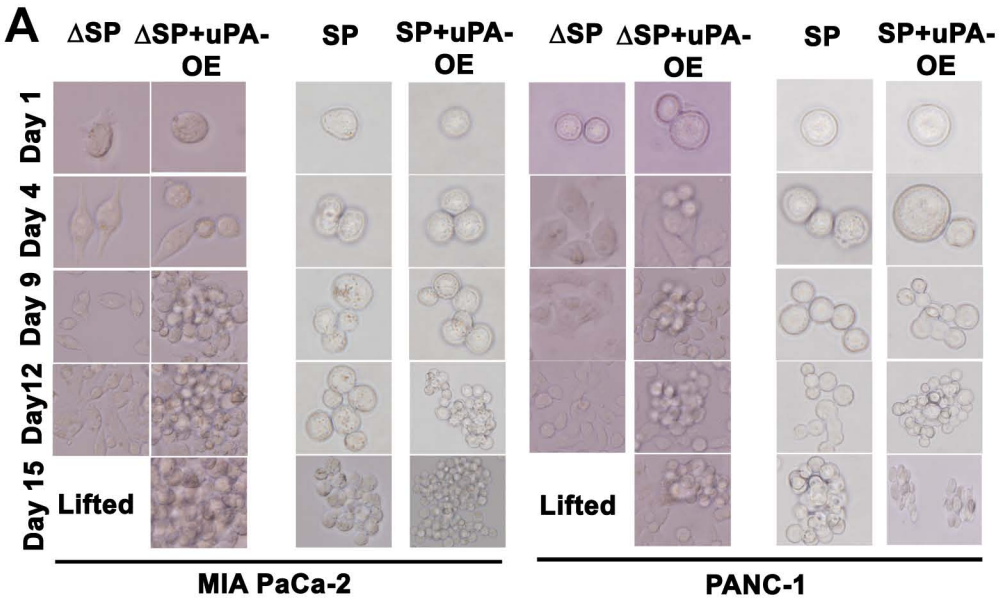

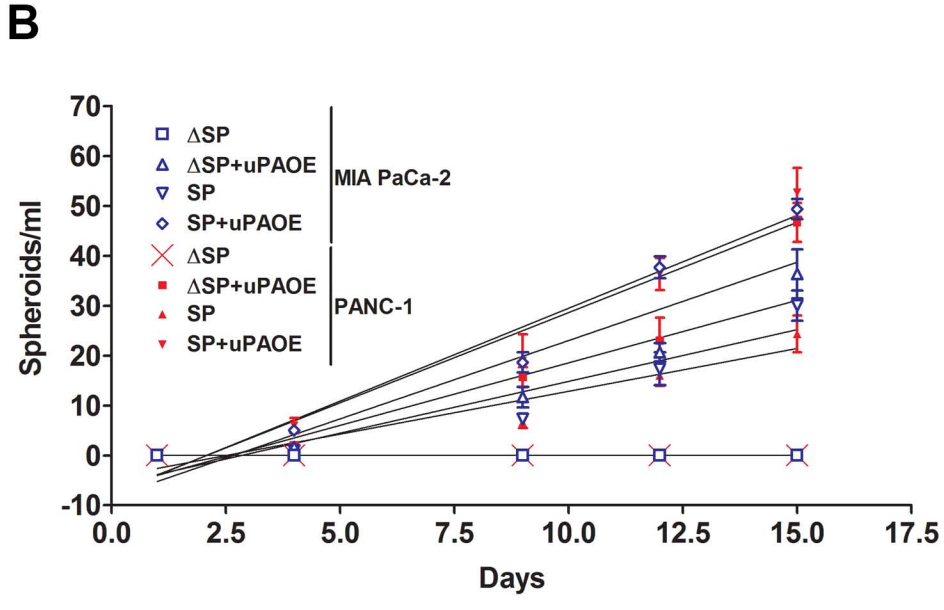

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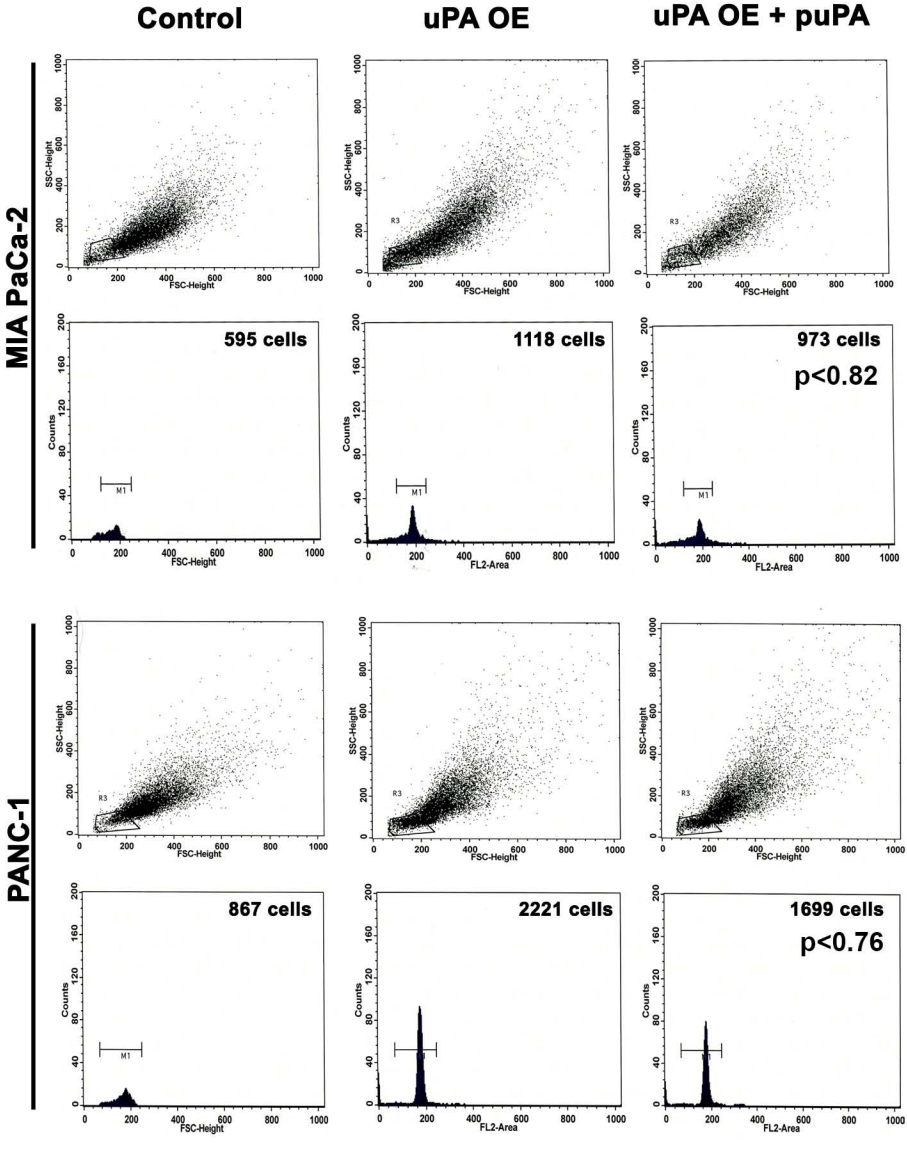

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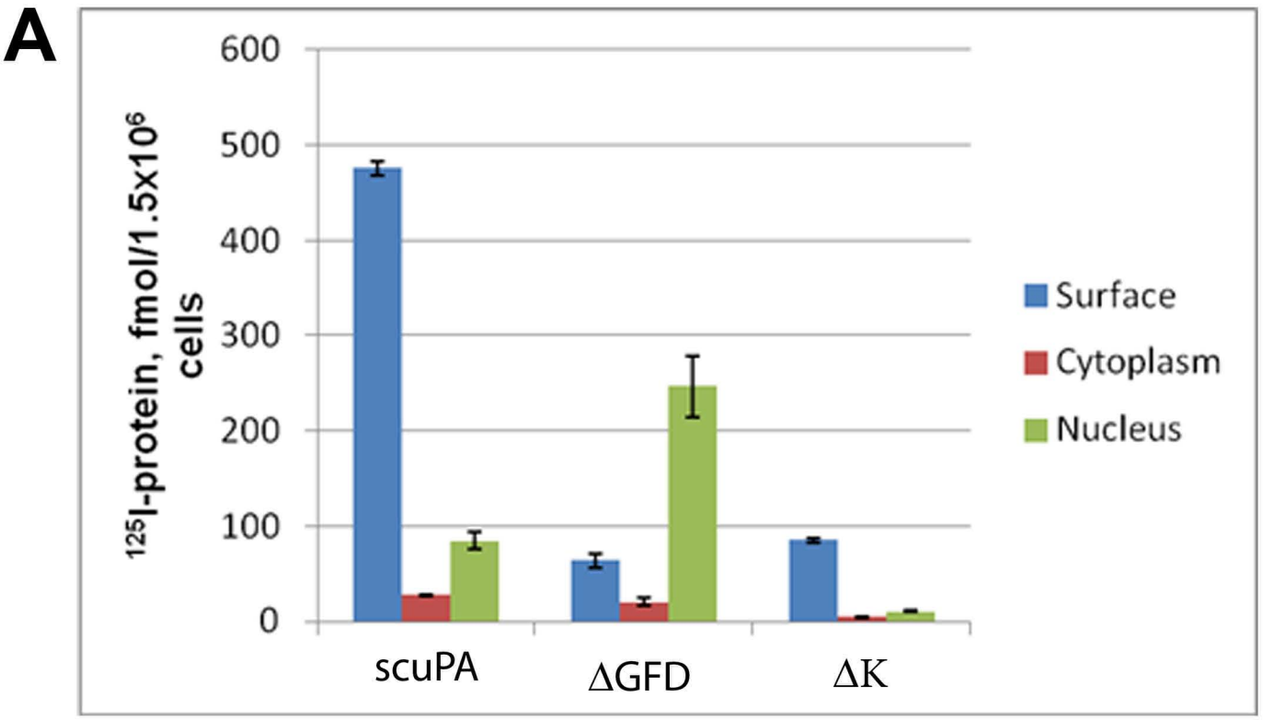
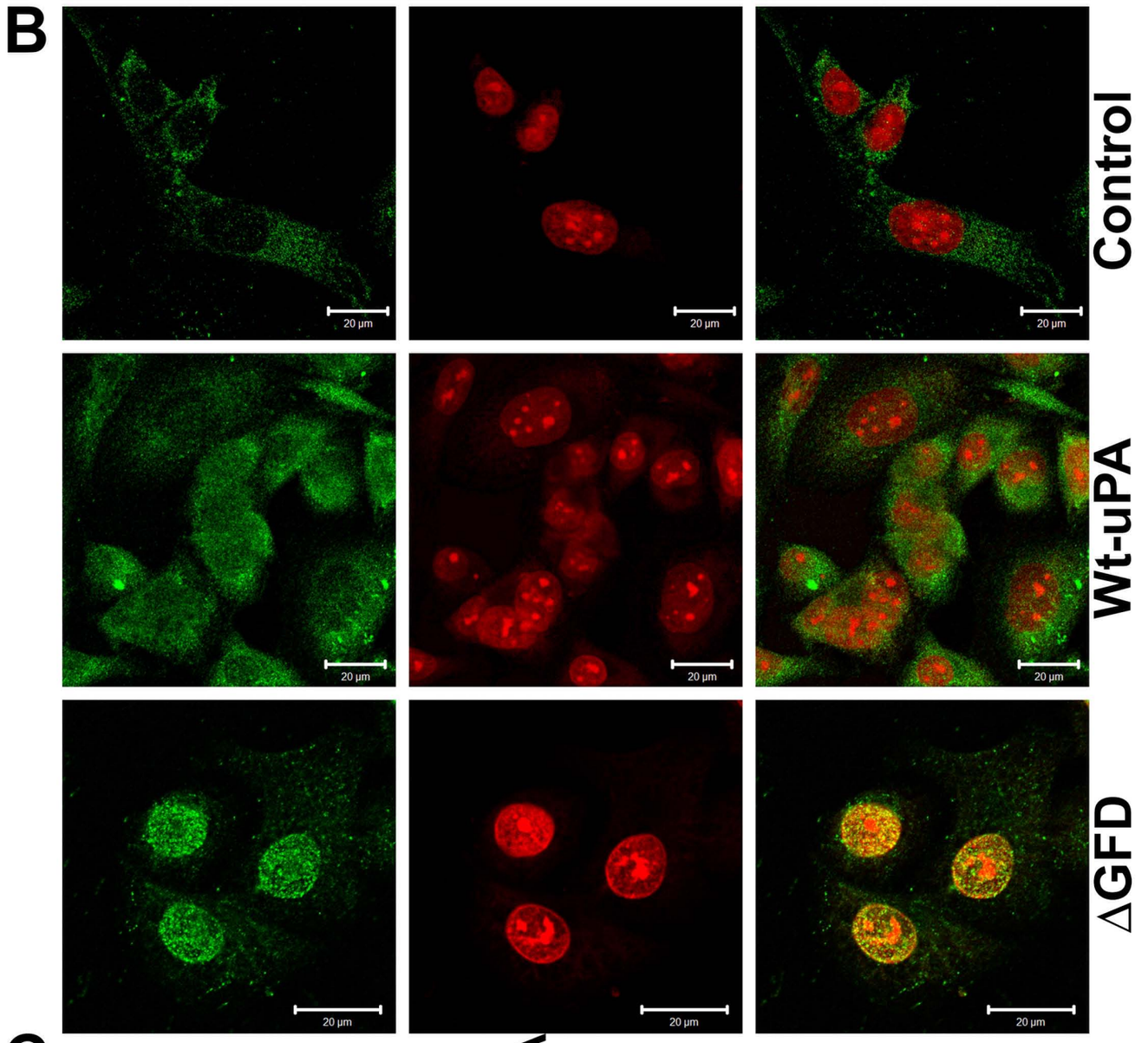
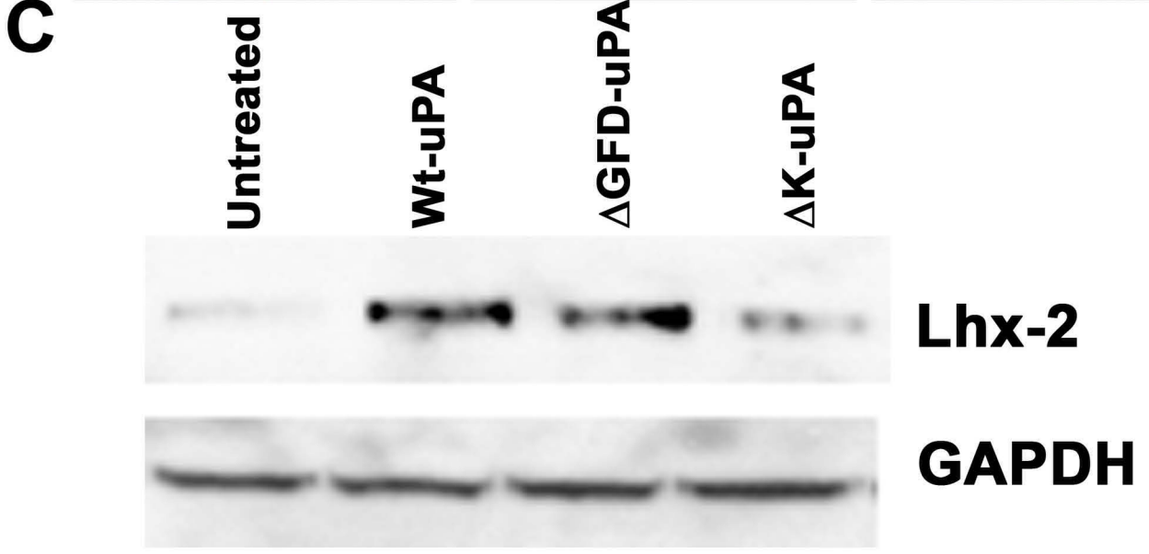
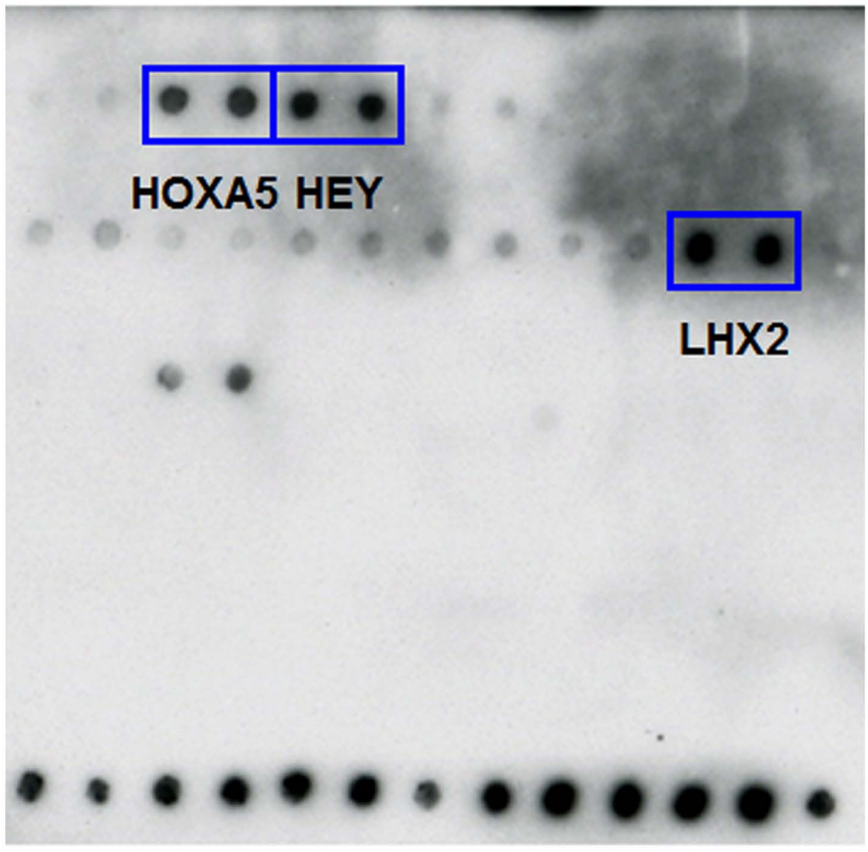






Supplemental-5S

A



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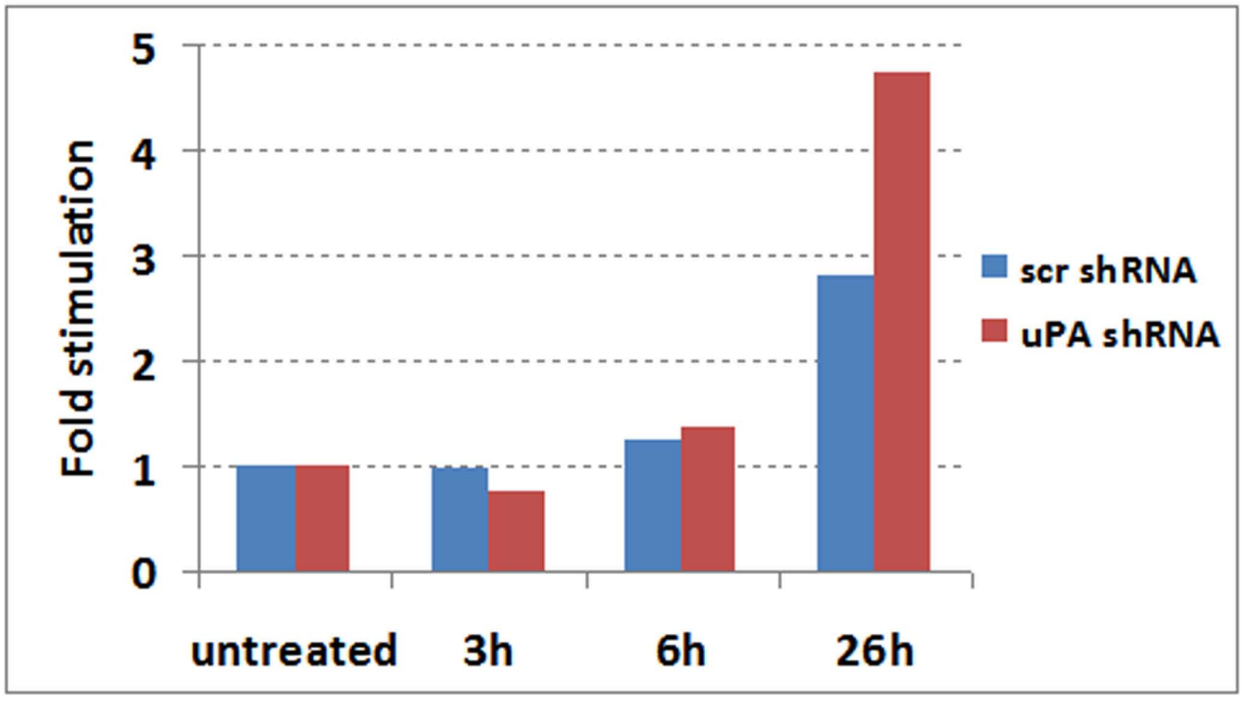

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