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Supplemental Material to:

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Urokinase-type plasminogen activator receptor signaling is critical in nasopharyngeal carcinoma cell growth and metastasis

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Supplementary materials

Table S1. The top 20 upregulated and downregulated genes from 2,992 genes differentially expressed between NPC vs. noncancerous nasopharyngeal tissues as sorted by *P*-values. PLAU (uPA) ranks first on the list.

Up-regulated Genes	Fold-change	*P*-value	Down-regulated Genes	Fold-change	*P*-value
PLAU	6.48	1.21E-12	SHISA3	-21.70	4.35E-15
IGSF9	8.43	7.18E-12	LTF	-76.08	5.20E-14
MMP1	41.07	8.26E-12	ZNF667	-2.94	8.38E-14
UNQ6494	12.44	1.48E-11	C16orf89	-11.42	1.20E-13
HOXC8	22.34	8.45E-11	KIAA1377	-4.07	8.28E-13
HOXA10	13.63	3.07E-10	DGKD	-2.74	1.08E-12
ZNF618	2.82	4.24E-10	C7	-40.60	1.41E-12
ZIC5	7.46	5.69E-10	EDNRB	-3.76	2.95E-12
AGRN	3.03	8.54E-10	GDF10	-8.78	3.70E-12
C22orf41	3.69	9.29E-10	FOXP1	-2.31	5.65E-12
KLRG2	7.17	1.77E-09	EDNRB	-3.49	6.25E-12
IDH1	2.16	1.84E-09	FOXP1	-2.09	6.76E-12
ABCC1	2.17	2.06E-09	PCDH9	-8.46	8.83E-12
KREMEN2	15.14	2.33E-09	LRRC34	-5.68	1.05E-11
TUBA1C	2.65	2.33E-09	CDO1	-8.59	2.52E-11
PUS7	2.04	2.89E-09	GDF10	-8.36	2.60E-11
NHS	2.35	3.02E-09	TTC21A	-3.07	2.63E-11
CLEC5A	7.87	4.38E-09	SLC26A7	-18.48	3.02E-11
BTG3	2.97	4.57E-09	KCNIP2	-2.72	3.30E-11
FGF1	4.81	1.70E-08	ADH1A	-23.69	3.50E-11

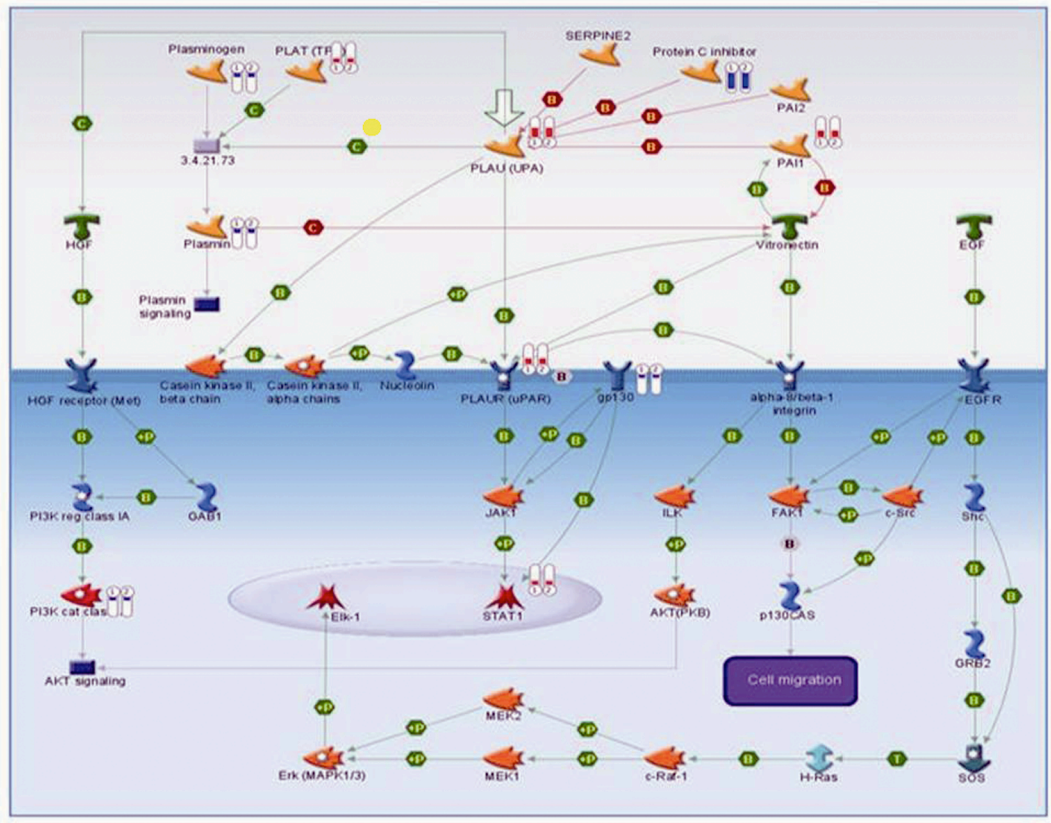

Figure S1. The PLAU signaling pathway network. The PLAU signaling pathway network generated from the GeneGo Metacore connectivity analysis suggests that Stat1 is likely upregulated in NPC. The red thermometer represents detected upregulated genes, whereas the blue thermometer represents downregulated genes.

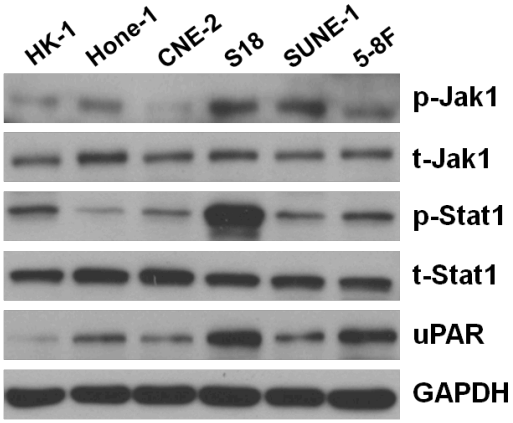

Figure S2. Phosphorylated and total Jak1 and Stat1 protein levels in NPC cells were determined by immunoblotting. The highest p-Jak1 and p-Stat1 protein levels were observed in S18 with the highest expression of uPAR, while its parental cell line CNE-2 with much lower uPAR level displayed corresponding lower expression of p-Jak1 and p-Stat1. The Jak1 and Stat1 expression levels were not exactly corresponding to the expression of uPAR in other NPC cells, indicating more complicated mechanisms are probably involved in the regulation of this pathway in different NPC cells.

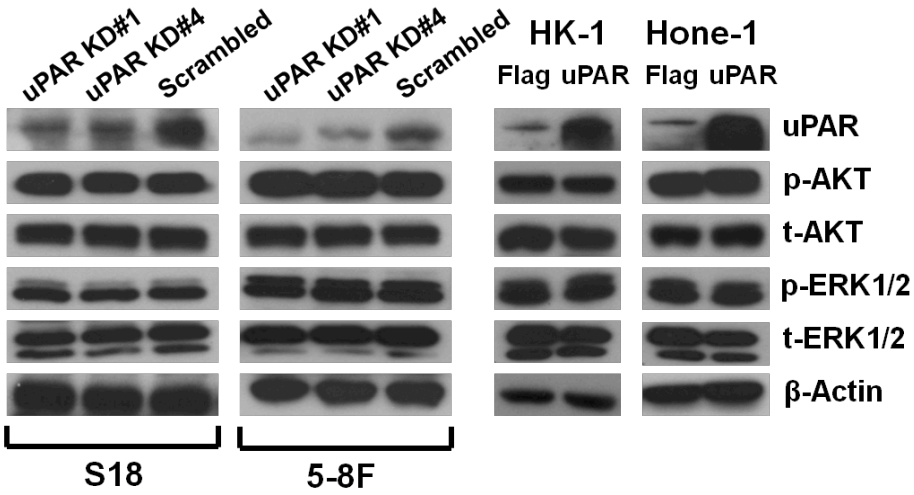

Figure S3. uPAR expression does not alter AKT or ERK expression in NPC cells. Immunoblotting of the whole cell lysates from uPAR knockdown NPC cells (left panel) or uPAR overexpressing cells (right panel) using p-AKT, t-AKT, p-ERK1/2, and t-ERK1/2 antibodies.

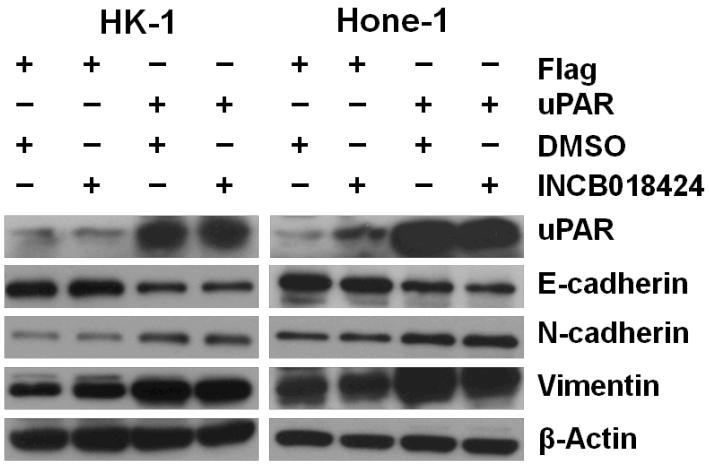

Figure S4. uPAR-induced EMT is not affected by p-Jak1 inhibition in NPC cells. Immunoblotting of EMT markers in NPC cells expressing uPAR or Flag sequences after treatment with 3 μM INCB018424 (Jak1/Jak2 inhibitor) for 48 h.

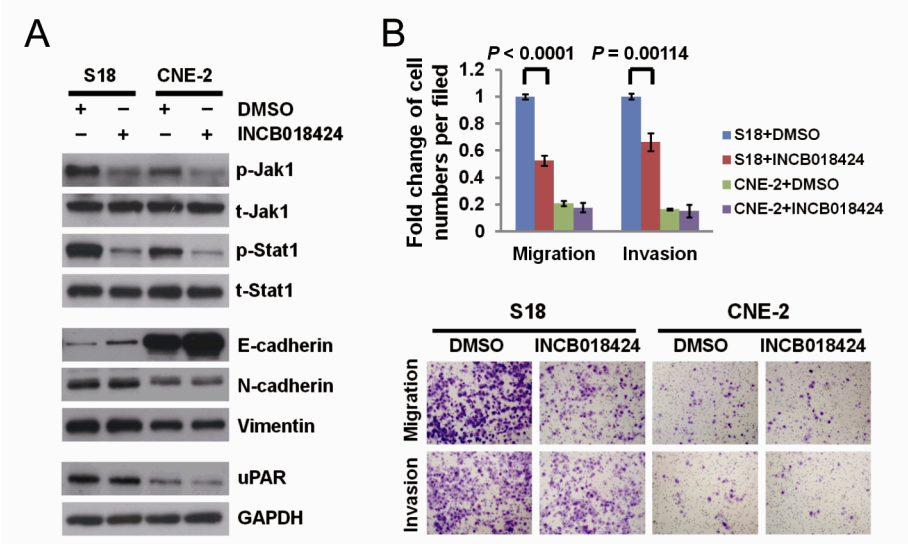

Figure S5. Inhibition of JAK-STAT pathway exerts more pronounced effect on motility of S18 compared with CNE-2, whose uPAR expression is lower than the former. **A.** Immunoblotting performed with antibodies related to JAK-STAT pathway and EMT markers in whole cell lysates from S18 and CNE-2 after treatment with 3 μM INCB018424 for 48h. **B.** The impact of INCB018424 treatment on the migration and invasion of S18 and CNE-2 as evaluated by the

Transwell assays. Photomicrographs are 100× (below). The data are presented as the mean ±SD of triplicate replicates. *P* values were calculated using the Student's *t*-test.

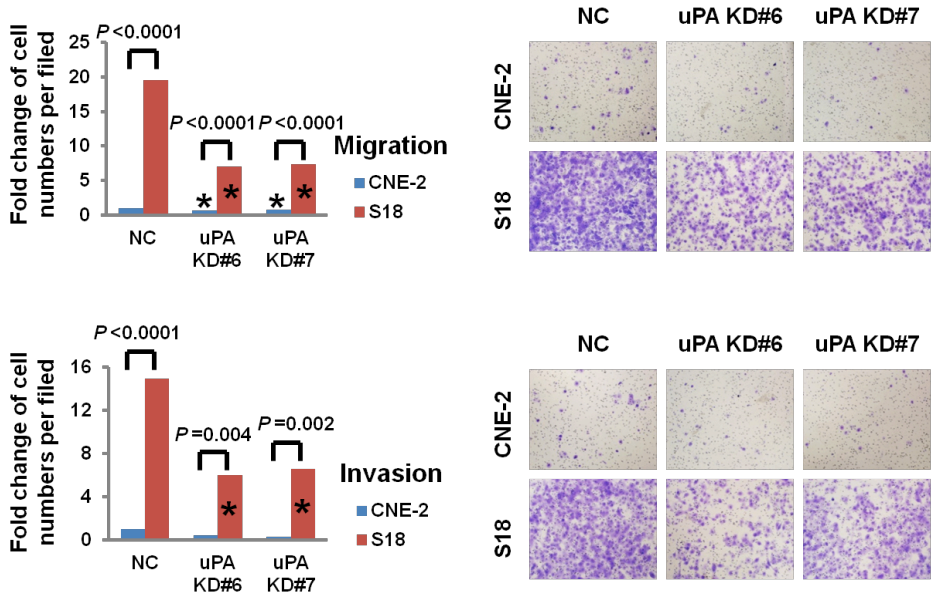

Figure S6. The impact of uPA knockdown on the migration and invasion of CNE-2 and S18 as determined by the Transwell assays. **P* < 0.005 relative to parental uPA knockdown NC controls. Photomicrographs are 100× (right panel). The data are presented as the mean±SD of triplicate replicates.

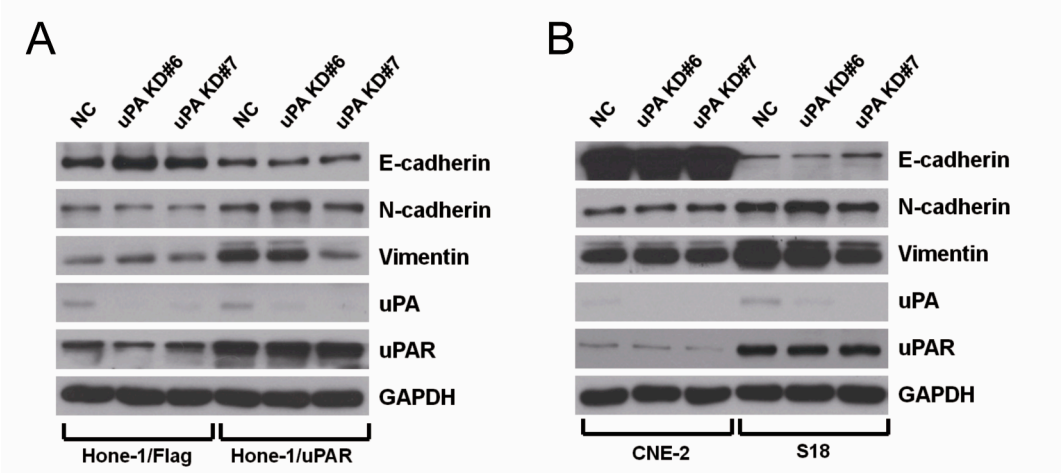

Figure S7. The mild effect of uPA on EMT in NPC cells was evaluated by knocking down uPA in Hone-1, CNE-2, and S18 cells. **A.** Immunoblotting of EMT markers in Hone-1 cells expressing uPAR or Flag sequences after uPA knockdown, uPA and uPAR expression levels were also examined. We observed that suppression of uPA in Hone-1/Flag cells resulted in slight upregulation of E-cadherin. And a mild downregulation of vimentin was observed upon uPA knockdown in Hone-1/uPAR cells. The rest of the examined markers remained unchanged. **B.** Immunoblotting of EMT markers, uPA and uPAR in CNE-2 and S18 after uPA knockdown. A mild alteration on E-cadherin and vimentin was observed after knocking down uPA in S18 cells, which was not found in CNE-2 cells.