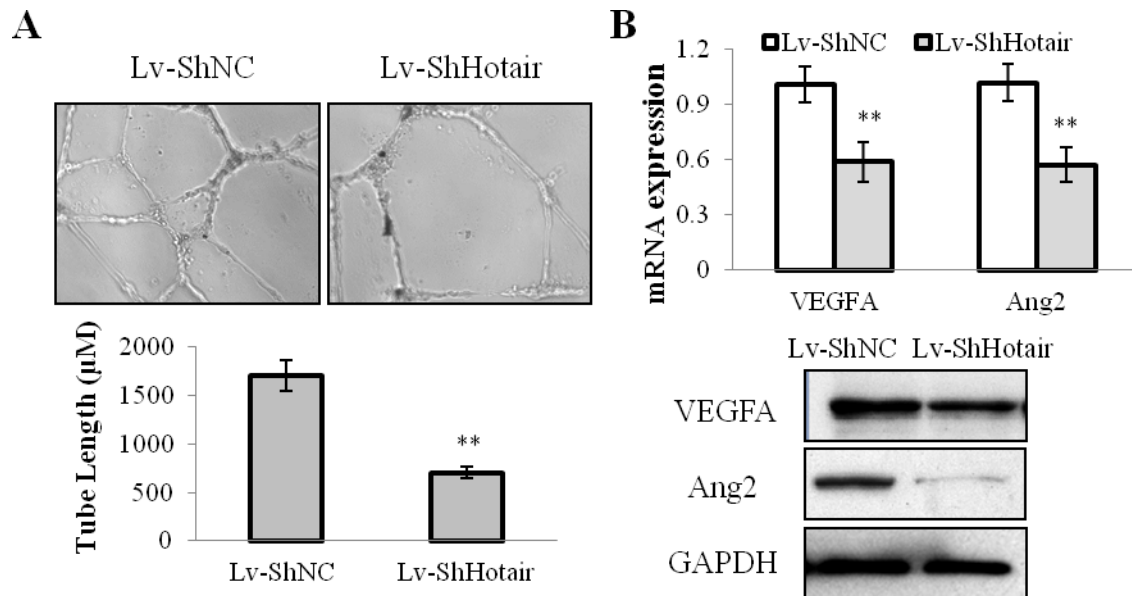
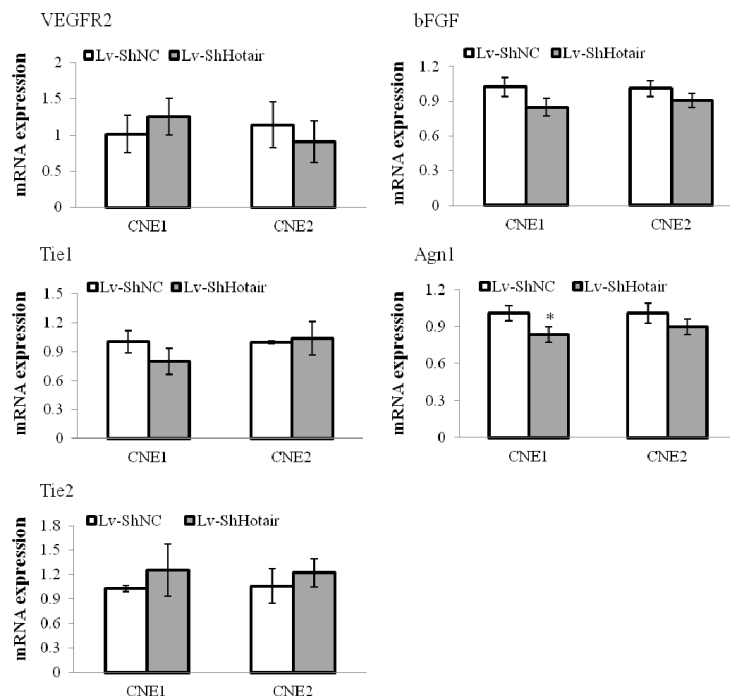


Long noncoding RNA hotair mediated angiogenesis in nasopharyngeal carcinoma by direct and indirect signaling pathways

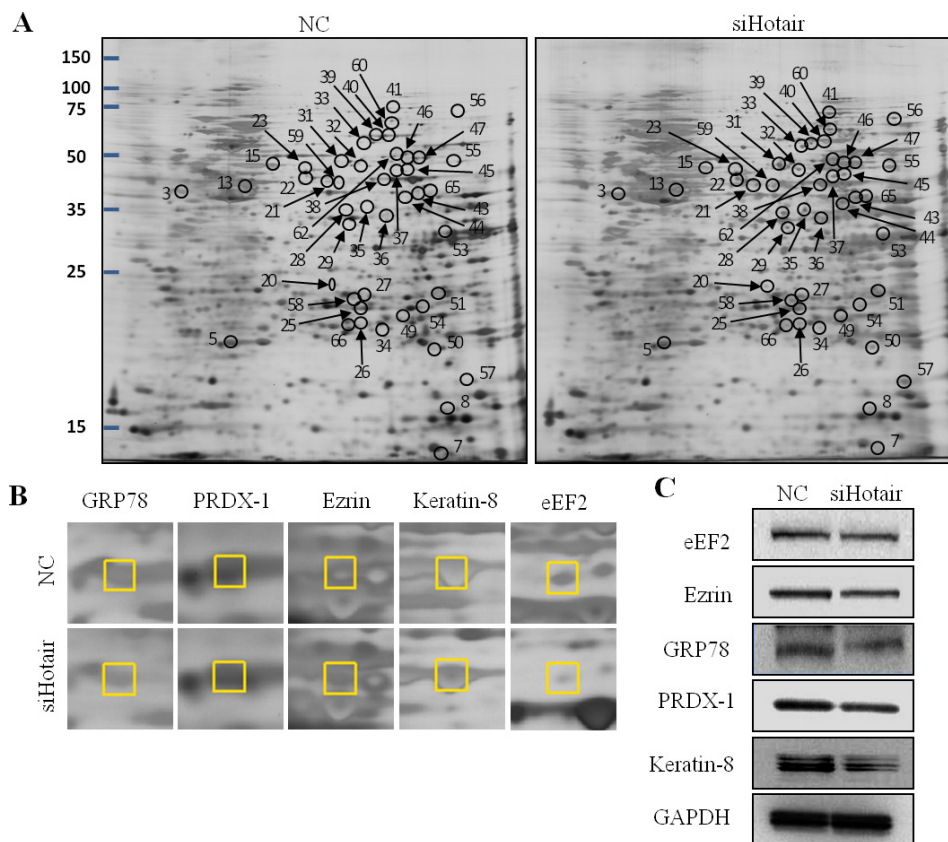
Supplementary Materials



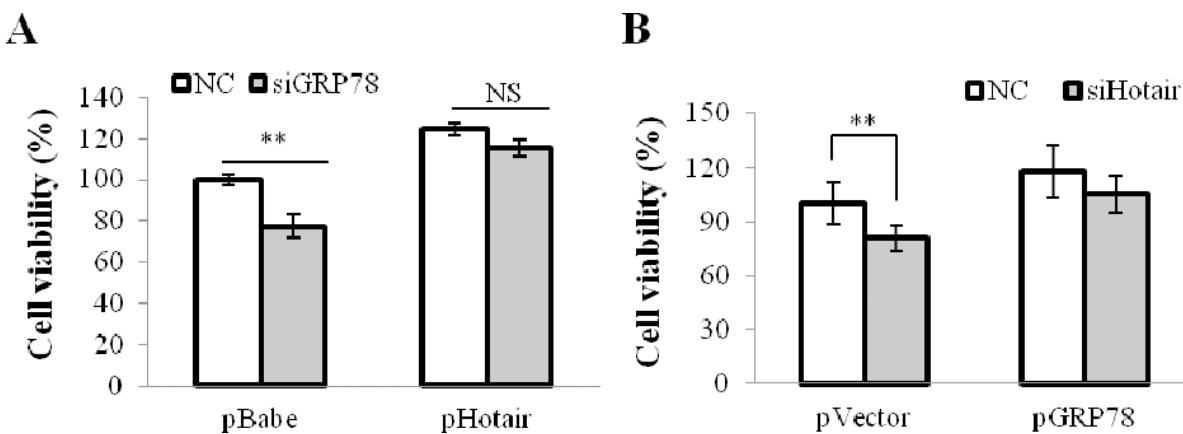
Supplementary Figure S1: Hotair mediated angiogenesis in CNE2 cells. (A) the ability of endothelial cell tube formation was suppressed in the Lv-ShHotair-infected condition medium, as compared to the Lv-ShNC infected condition medium. (B) the angiogenic growth factors VEGFA and Ang2 were down-regulated in the Lv-ShHotair infected CNE2 cells at mRNA and protein levels. * $P < 0.05$; ** $P < 0.01$.



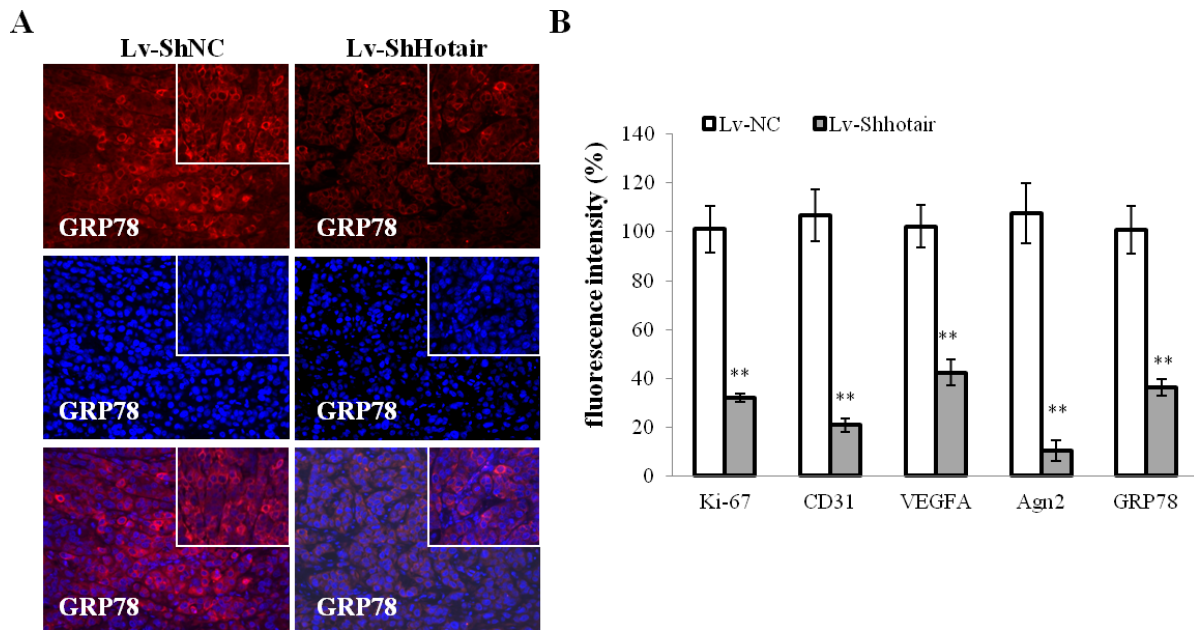
Supplementary Figure S2: The expression of other angiogenic factors was not significantly affected at mRNA level by Hotair knockdown.



Supplementary Figure S3: GRP78 was a direct target of Hotair in NPC cells. (A) the representative silver-stained 2-DE images of CNE1 cells treated with siHotair or NC by using proteomic profiling ($n = 3$). The protein of spot 3 was identified to be human GRP78 by protein database search. (B) enlarged images of differentially expressed protein in the two groups. (C) the five proteins were confirmed by using Western blotting. $*P < 0.05$; $**P < 0.01$.



Supplementary Figure S4: GRP78 mediated cell growth in CNE2 cells. (A) Hotair overexpression reversed the suppression of cell viability by siGRP78. (B) GRP78 overexpression rescued the siHotair-induced growth inhibition. $*P < 0.05$; $**P < 0.01$.



Supplementary Figure S5: The immunofluorescence double staining analyses. (A) GRP78 expression was suppressed in Lv-ShHotair treated group. (B) the quantitative analyses for the immunofluorescence staining of Ki-67, CD31, VEGFA, Agn2 and GRP78. * $P < 0.05$; ** $P < 0.01$.

Supplementary Table S1: Differentially expressed proteins identified by 2-DE and MS analysis between siHotair-treated and NC-treated CNE1 cells

Spots No.	Accession No.	Protein name	MW	PI	Score	change fold
Cytoskeleton						
49	Q12955	Ankyrin-3 (ANK3)	482394	6.07	61	(+)
36	P11532	Dystrophin	428479	5.65	58	3.4x ↓
13	P15311	Ezrin	69484	5.94	66	27.2x ↓
7	P07737	Profilin-1	15216	8.44	122	(+)
15	P05787	Keratin, type II cytoskeletal 8	53671	5.52	165	30x ↓
62	P13647	Keratin, type II cytoskeletal 5	62568	7.59	78	(-)
39	P02545	Lamin-A/C	74380	6.57	244	3.4x ↓
RNA processing and modification						
58	P31942	Heterogeneous nuclear ribonucleoprotein H3	36960	6.37	151	(-)
65	Q14103	Heterogeneous nuclear ribonucleoprotein D0	38581	7.62	127	(-)
53	P22626	Heterogeneous nuclear ribonucleoproteins A2/B1	37464	8.97	70	4.2x ↑
Regulation of transcription and translation						
45	P13639	Elongation factor 2	96246	6.41	70	4.3x ↓
41	Q92945	Far upstream element-binding protein 2	73443	6.84	123	3.9x ↓
60	Q96AE4	Far upstream element-binding protein 1	67690	7.18	103	(-)
35	Q15365	Poly(rC)-binding protein 1	37987	6.66	62	3.2x ↓
32	Q9Y265	RuvB-like 1	50538	6.02	80	4.5x ↓
Post-translational modification						
29	A2A3K4	Protein tyrosine phosphatase domain-containing protein 1	85543	7.35	109	3.3x ↓
21	O75439	Mitochondrial-processing peptidase subunit beta	55073	6.38	88	3.5x ↓

Chaperones and stress response						
3	P11021	78 kDa glucose-regulated protein	72402	5.07	109	18.6x ↓
51	P11142	Heat shock cognate 71 kDa protein	71082	5.37	358	3.0x ↑
5	Q06830	Peroxiredoxin-1	22324	8.27	61	7.6x ↓
25	P30041	Peroxiredoxin-6	25133	6	67	3.4x ↑
66	P61758	Prefoldin subunit 3	22815	6.63	60	(+)
33	P40227	T-complex protein 1 subunit zeta	58444	6.23	105	5.7x ↓
31	P78371	T-complex protein 1 subunit beta	57794	6.01	157	3.8x ↓
Chaperone regulators						
26	O95816	BAG family molecular chaperone regulator 2	23928	6.25	68	3.2x ↑
Metabolic enzymes and regulators						
38	P06733	Alpha-enolase (ENO1)	47481	7.01	78	6.0x ↓
55	P25705	ATP synthase subunit alpha, mitochondrial	59828	9.16	358	3.4x ↑
43	P22695	Cytochrome b-c1 complex subunit 2, mitochondrial	48584	8.74	56	3.9x ↓
52	P04406	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	36201	8.57	122	6.0x ↑
57	P51970	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8	20548	7.57	34	3.9x ↓
8	P22392	Nucleoside diphosphate kinase B	17401	8.52	83	(+)
44	P00558	Phosphoglycerate kinase 1	44985	8.3	74	4.2x ↓
27	P18669	Phosphoglycerate mutase 1	28900	6.67	106	4.7x ↓
22	P14618	Pyruvate kinase isozymes M1/M2	58470	7.96	220	21.5x ↓
46	Q9NR45	Sialic acid synthase	40738	6.29	55	5.2x ↑
54	P60174	Triosephosphate isomerase	26938	6.45	82	(+)
41	P53007	Tricarboxylate transport protein, mitochondrial	34333	9.91	63	3.9x ↓

Growth regulation						
56	O60566	Mitotic checkpoint serine/threonine-protein kinase BUB1 beta	120781	5.2	69	4.2x ↓
59	Q9UQ80	Proliferation-associated protein 2G4	44101	6.13	50	(-)
40	Q9BXU1	Serine/threonine-protein kinase 31	116647	5.04	56	47x ↓
Miscellaneous functional proteins						
52	P07355	Annexin A2	38808	7.57	72	6.0x ↑
50	P30086	Phosphatidylethanolamine-binding protein 1	21158	7.01	178	3.6x ↑
23	Q9H0H3	Ectoderm-neural cortex protein 2	66964	6.1	60	3.7x ↓

With a 2-fold difference cutoff in intensity, 52 spots were selected for further MALDI-TOF-MS/MS-MS analysis, and 43 proteins were finally identified. CI, confidence interval

Supplementary Table S2: Primers for the qPCR analysis

Gene	Primer sequence (5'-3')
GAPDH	<i>Forward:</i> TATTCAGGAAGGTGTTACTTAA
	<i>Reverse:</i> TAAGGCACCCTTCTGAGTAGA
Hotair	<i>Forward:</i> CAGTGGGGAAGTCTGACTCG
	<i>Reverse:</i> GTGCCTGGTGCTCTCTTACC
VEGFA	<i>Forward:</i> CTACCTCCACCATGCCAAGT
	<i>Reverse:</i> GCGCTGCGCTGATAGAAGTA
Ang2	<i>Forward:</i> CTGGGAAGGGAATGAGGCTT
	<i>Reverse:</i> CATGCATCAAACCACCAGCC
VEGFR2	<i>Forward:</i> TGATGTGGTCTGAGTCCGT
	<i>Reverse:</i> AGACTGGGTTTTAGGTCTCGG
Tie-1	<i>Forward:</i> TGGCTGCTCTTGTGGATCTG
	<i>Reverse:</i> TCTCACAGTGCACTCCATGC
Tie-2	<i>Forward:</i> TTCACCAGGCTGATAGTCCG
	<i>Reverse:</i> ACACGTCCTTCCCATAAACCC