Supplementary Information

Vesicle amine transport-1 regulates hepatocellular carcinoma progression by EGF-induced STAT3 signalling

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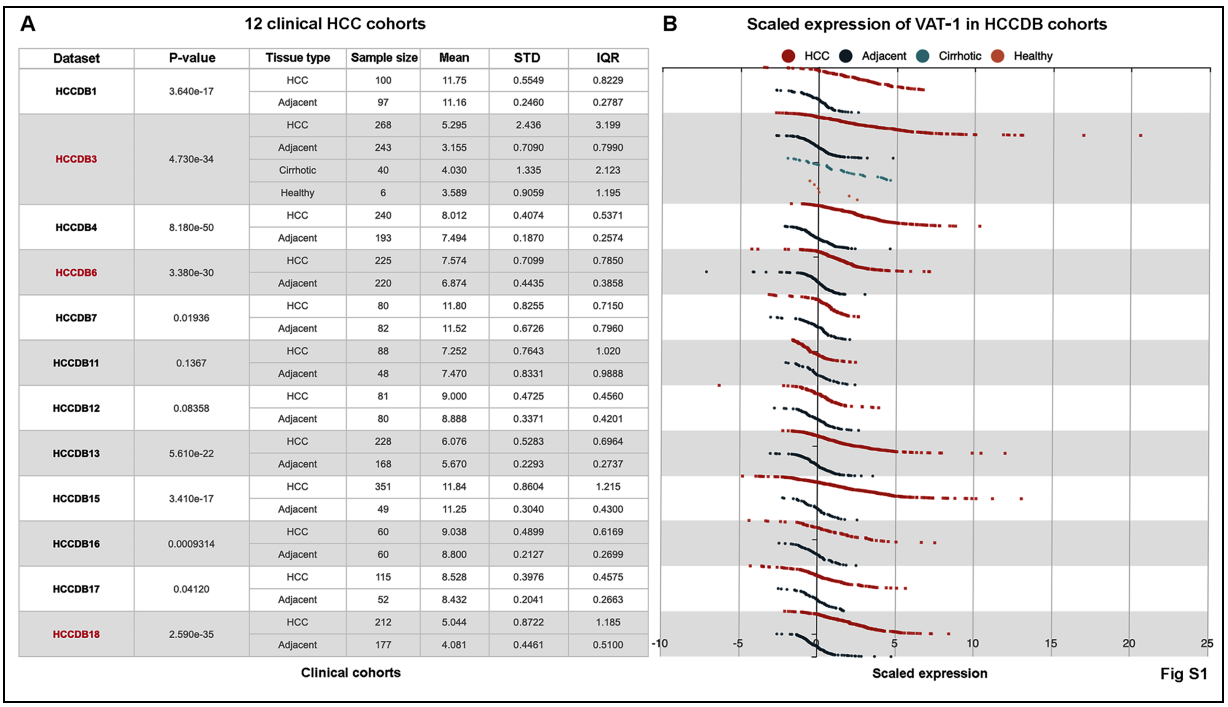
Supplementary figure S1-S6

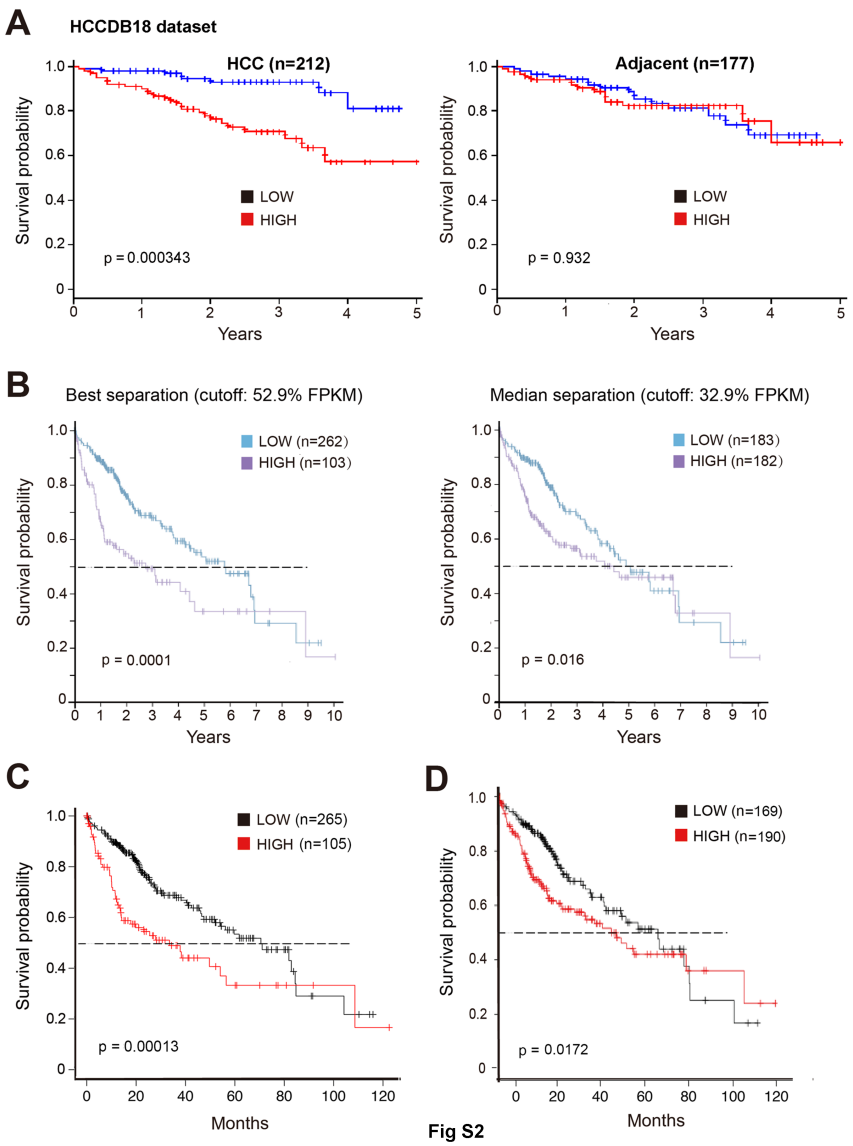


Dataset	P-value	Tissue type	Sample size	Mean	STD	IQR
HCCDB1	3.640e-17	HCC	100	11.75	0.5549	0.8229
		Adjacent	97	11.16	0.2460	0.2787
HCCDB3	4.730e-34	HCC	268	5.295	2.436	3.199
		Adjacent	243	3.155	0.7090	0.7990
		Cirrhotic	40	4.030	1.335	2.123
		Healthy	6	3.589	0.9059	1.195
HCCDB4	8.180e-50	HCC	240	8.012	0.4074	0.5371
		Adjacent	193	7.494	0.1870	0.2574
HCCDB6	3.380e-30	HCC	225	7.574	0.7099	0.7850
		Adjacent	220	6.874	0.4435	0.3858
HCCDB7	0.01936	HCC	80	11.80	0.8255	0.7150
		Adjacent	82	11.52	0.6726	0.7960
HCCDB11	0.1367	HCC	88	7.252	0.7643	1.020
		Adjacent	48	7.470	0.8331	0.9888
HCCDB12	0.08358	HCC	81	9.000	0.4725	0.4560
		Adjacent	80	8.888	0.3371	0.4201
HCCDB13	5.610e-22	HCC	228	6.076	0.5283	0.6964
		Adjacent	168	5.670	0.2293	0.2737
HCCDB15	3.410e-17	HCC	351	11.84	0.8604	1.215
		Adjacent	49	11.25	0.3040	0.4300
HCCDB16	0.0009314	HCC	60	9.038	0.4899	0.6169
		Adjacent	60	8.800	0.2127	0.2699
HCCDB17	0.04120	HCC	115	8.528	0.3976	0.4575
		Adjacent	52	8.432	0.2041	0.2663
HCCDB18	2.590e-35	HCC	212	5.044	0.8722	1.185
		Adjacent	177	4.081	0.4461	0.5100

Supplementary Figure S1. VAT-1 transcripts are elevated in HCC tissues versus adjacent non-tumour tissues in HCCDB (Integrative Molecular Database of Hepatocellular Carcinoma)

A, Analysis of HCCDB database (including 12 clinical HCC cohorts) demonstrated that VAT-1 expression in 10 out 12 clinical cohorts is significantly higher in HCC tissues than in adjacent non-tumour tissues with *P*-value from 1.936e-2 (HCCDB7) to 8.180e-50 (HCCDB4).

B, Scaled expression of VAT-1 in all 12 clinical HCC cohorts of HCCDB (http://lifeome.net/database/hccdb/search.html).



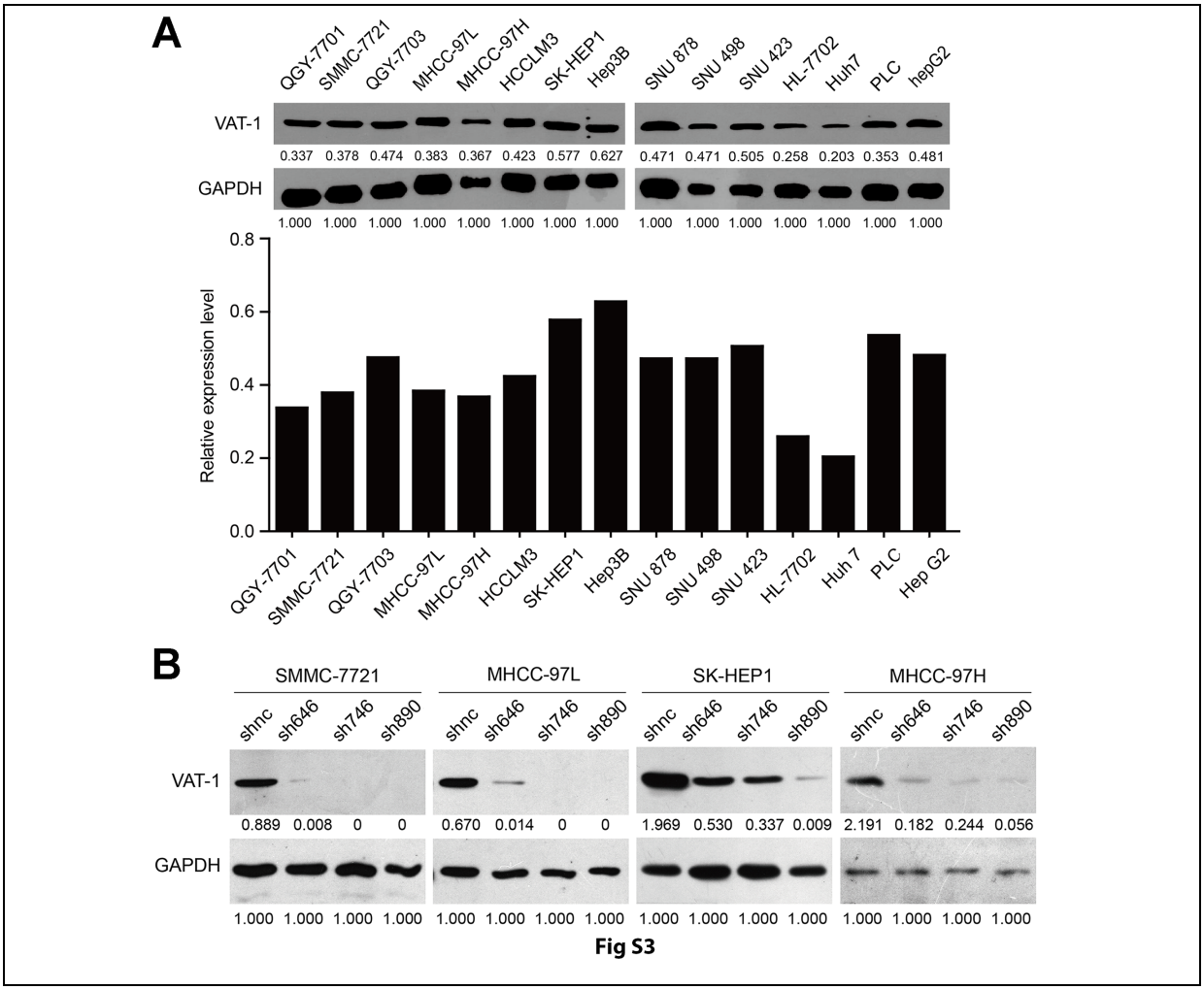
Supplementary Figure S2. Upregulation of VAT-1 transcripts is significantly associated with overall survival of HCC patients.

A, High expression of VAT-1 in tumour tissues (left) but not in adjacent non-tumour tissues (right) of HCCDB18 clinical cohort is reversely associated with poor overall survival (http://lifeome.net/database/hccdb/search.html).

B, High expression of VAT-1 is reversely associated with poor overall survival in Human Protein Altas (https://www.proteinatlas.org/ENSG00000108828-VAT-1/pathology/liver).

C, High expression of VAT-1 is reversely associated with poor overall survival in Kaplan Meier plotter database (http://kmplot.com/analysis/index.php?p=service&start=1).

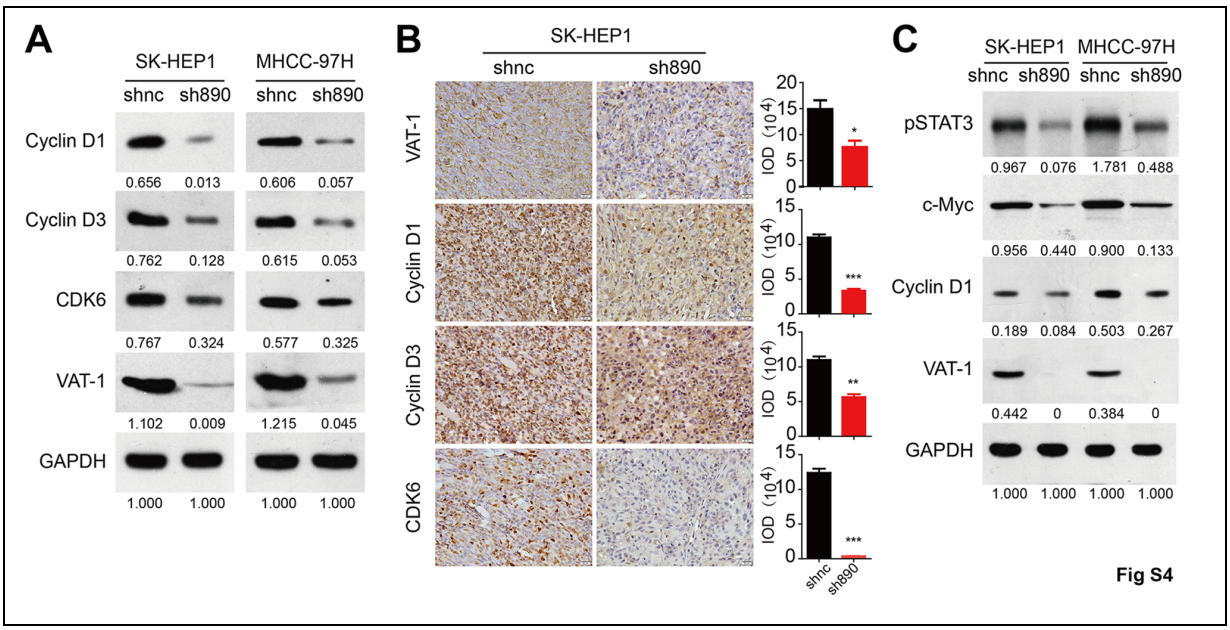
D, High expression of VAT-1 is reversely associated with poor overall survival in OncoLnc database(http://www.oncolnc.org/kaplan/?lower=47&upper=53&cancer=LIHC&gene_id=10493&raw=VAT1&species=mRNA).



Supplementary Figure S3. Expression of VAT-1 protein in various HCC cell lines.

A, Expression of endogenous VAT-1 protein in human normal hepatocyte (HL-7702) and 14 HCC cell lines as revealed by Western blotting. GAPDH was used as a protein loading control. Numbers indicate relative protein ratio measured by Image Pro Plus 6.0 software and normalized to GAPDH.

B, Downregulation of VAT-1 expression in 4 HCC cell lines (SMMC-7721, MHCC-97L, SK-HEP1 and MHCC-97H) by lentivirus transductions containing lentivirus vectors encoding shRNA against VAT-1 (sh646, sh745 or sh890) or control shRNA (shnc) respectively as revealed by Western blotting.

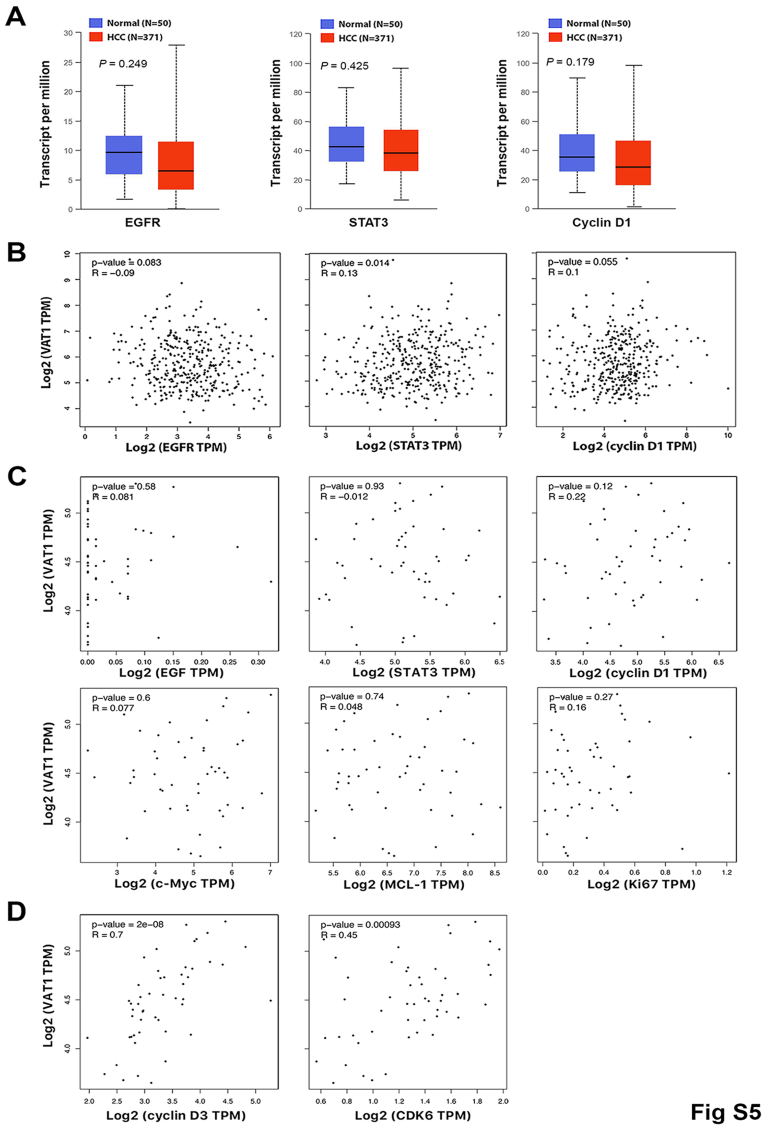


Supplementary Figure S4. Downregulation of VAT-1 decreases STAT3 signalling and expressions of cyclin D1, cyclin D3, CDK6, c-Myc and pSTAT3-Y705 in HCC cells.

A, Western blotting showing that downregulation of VAT-1 reduces the expressions of cyclin D1, cyclin D3 and CDK6 in both SK-HEP1 and MHCC-97H cell lines. GAPDH was used as a protein loading control. Numbers indicate relative protein ratio measured by Image Pro Plus 6.0 software and normalized to GAPDH.

B, IHC staining showing that downregulation of VAT-1 decreases the expressions of cyclin D1, cyclin D3 and CDK6 in SK-HEP1 xenograft sections (400× magnifications, scale bar 20μm). Quantification of IHC staining for the expressions of VAT-1, cyclin D1, cyclin D3 and CDK6 was performed by Image Pro Plus 6.0 software. Mean ± SEM, N=5, paired t-test; ***P*<0.01, ****P*<0.001

C, Western blotting showing that downregulation of VAT-1 reduces the expressions of pSTAT3-Y705, c-Myc and cyclin D1 in both SK-HEP1 and MHCC-97H cell lines. GAPDH was used as a protein loading control. Numbers indicate relative protein ratio measured by Image Pro Plus 6.0 software and normalized to GAPDH.



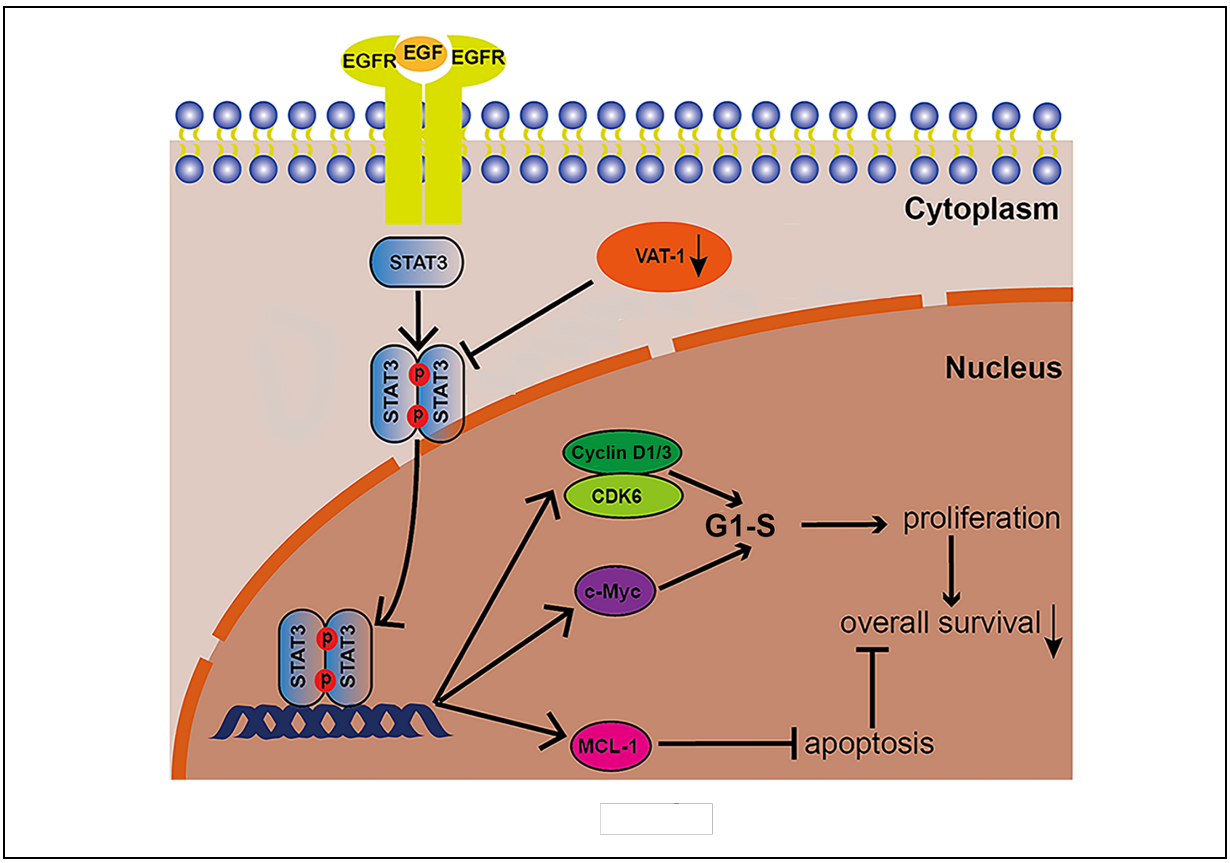
Supplementary Figure S5. Expression and correlation of VAT-1 with EGF, STAT3, c-Myc, MCL-1, cyclin D3, CDK6 and Ki67 in TCGA tumour and normal liver specimens of HCC.

A, Expressions of EGF, STAT3 and cyclin D1 in 371 TCGA tumour specimens versus 50 normal liver samples of HCC by analyzing the Ualcan dataset.

B, Spearman correlation analyses via the GEPIA server show that VAT-1 expression is significantly correlated with STAT3 (*P*=0.014) but not with EGFR (*P*=0.083) or cyclin D1 (*P*=0.055) in TCGA tumour samples of HCC.

C, Spearman correlation analyses demonstrate that VAT-1 expression is not significantly correlated with EGF, STAT3, cyclin D1, c-Myc, MCL-1 and Ki67 in TCGA normal liver samples (LIHC normal).

D, Spearman correlation analyses reveal that VAT-1 expression is significantly correlated with cyclin D3 and CDK6 in TCGA normal liver samples (LIHC normal).



Supplementary Figure S6. Summary of key results in the working model for VAT-1.

VAT-1 may interact with and modulate the activity of enzyme(s) that regulate phosphorylation or dephosphorylation of STAT3 at Y705, pSTAT3-Y705 dimerization and nuclear translocation of pSTAT3-Y705 in the EGF-induced STAT3 pathway, regulate the expression of MCL-1, c-Myc, cyclin D1, cyclin D3 and CDK6, and control the cell cycle progression from G1-S phase. Therefore, VAT-1 regulates tumour cell proliferation and apoptosis, and thereby affects the disease progression and overall survival of HCC patients. In clinical HCC specimens, VAT-1 regulates cell cycle, ErbB signalling pathway, EGFR TKI resistance and JAK-STAT signalling pathway, and highly correlates with the expression of EGF, STAT3, c-Myc, MCL-1, cyclin D3, CDK6 and Ki67, highlighting the role of VAT-1 in regulation of EGF-EGFR-STAT3-cyclin D/CDK6 signalling, carcinogenesis and disease progression of HCC.