Title:

MicroRNA-140-5p inhibits hepatocellular carcinoma by directly targeting the unique isomerase Pin1 to block multiple cancer-driving pathways

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Key Words: miR-140-5p; Pin1; hepatocellular carcinoma; metastasis

Supplementary Tables.

Table S1. MiRNA candidates potentially targeting the 3'-untranslated region of Pin1 mRNA were predicted by at least two of the five bioinformatics databases

Predicted miRNAs / Predicted databases	miRnada	PITA	TargetScan
has-miR-140		√	√
has-miR-140-5p	√	√	√
has-miR-200b	√	√	√
has-miR-200c	√	√	√
has-miR-429	√	√	√
has-miR-488	√	√	
has-miR-450a	√	√	
has-miR-370	√	√	
has-miR-548a		√	√
has-miR-876-3p		√	√
has-miR-1244		√	√

Table S2. Clinicopathological characteristics of studied patients and expression of Pin1 and miR-140-5p in HCC

Factor	NO.	(%)
Gender		
Male	22	88
Female	3	12
Age (years)		
≤60	16	64
>60	9	36
Cirrhosis		
Presence	20	80
Absence	5	20
Tumor size (cm)		
≤5	9	36
>5	16	64
Tumor nodule number		
Solitary	18	72
Multiple (≥2)	7	28
Capsular formation		
Presence	15	60
Absence	10	40
Edmondson-Steiner grade		
I-II	6	24
III-IV	19	76
Vein invasion		
Presence	18	72
Absence	7	28
TNM stage		
Stage I	3	12
Stage II	8	32
Stage III	14	56
HBV		
Negative	2	8
Positive	23	92
Pin1 expression		
Low expression	9	36
High expression	16	64
MiR-140-5p expression		
Low expression	17	68
High expression	8	32

Table S3. Sequence of oligonucleotides for plasmid construct, siRNA, qRT-PCR and miRNAs oligonucleotide

Assay		Sequence 5' →3’
PCR		
Pin1	F	AAGATGGCGGACGAGGAG
	R	CACTCAGTGCGGAGGATGAT
GAPDH	F	ACCCACTCCTCCACCTTTG
	R	CTCTTGTGCTCTTGCTGGG
Plasmid construction		
pmirGLO-Pin1-3’UTR-WT	F	CTAGTTGTTTAAACGAGCTCGGGTGGGGAGC CCAGGCCTG
	R	ATGCCTGCAGGTCGACGCAGAGCAGTGGTTC TGGGT
pmirGLO-Pin1-3’UTR-Mut	F	CTAGTTGTTTAAACGAGCTCGGGTGGGGAGC CCAGGCCTG
	R	ATGCCTGCAGGTCGACGCAGAGCTCACCAA CTGGGT
miRNAs oligonucleotide		
miRNA-200b mimics		UAAUACUGCCUGGUAAUGAUGA AUCAUUACCAGGCAGUAUUAUU
miRNA-200c mimics		UAAUACUGCCGGGUAAUGAUGGA CAUCAUUACCCGGCAGUAUUAUU
miRNA-429 mimics		UAAUACUGUCUGGUAAAACCGU GGUUUUACCAGACAGUAUUAUU
miRNA-140-5p mimics		CAGUGGUUUUACCCUAUGGUAG ACCAUAGGGUAAAACCACUGUU
Negative control		UUCUCCGAACGUGUCACGUTT ACGUGACACGUUCGGAGAATT

Supplementary Figures.

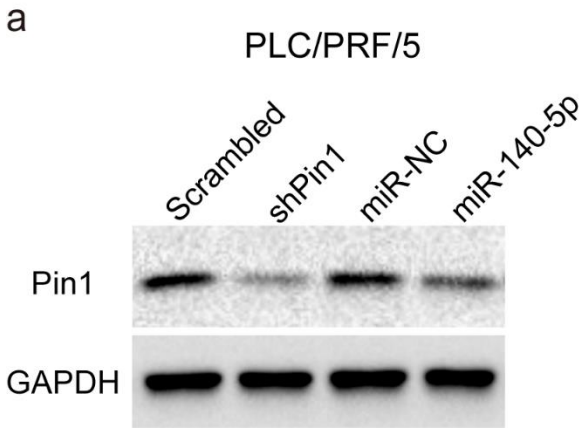

Figure S1. Effect of miR-140-5p overexpression on the protein levels of Pin1 in PLC/PRF/5 cells.

(a). Like stable Pin1 knockdown, moderate overexpression of miR-140-5p downregulates Pin1 expression in PLC/PRF/5 cells, as detected by immunoblotting analysis. GAPDH served as loading control.

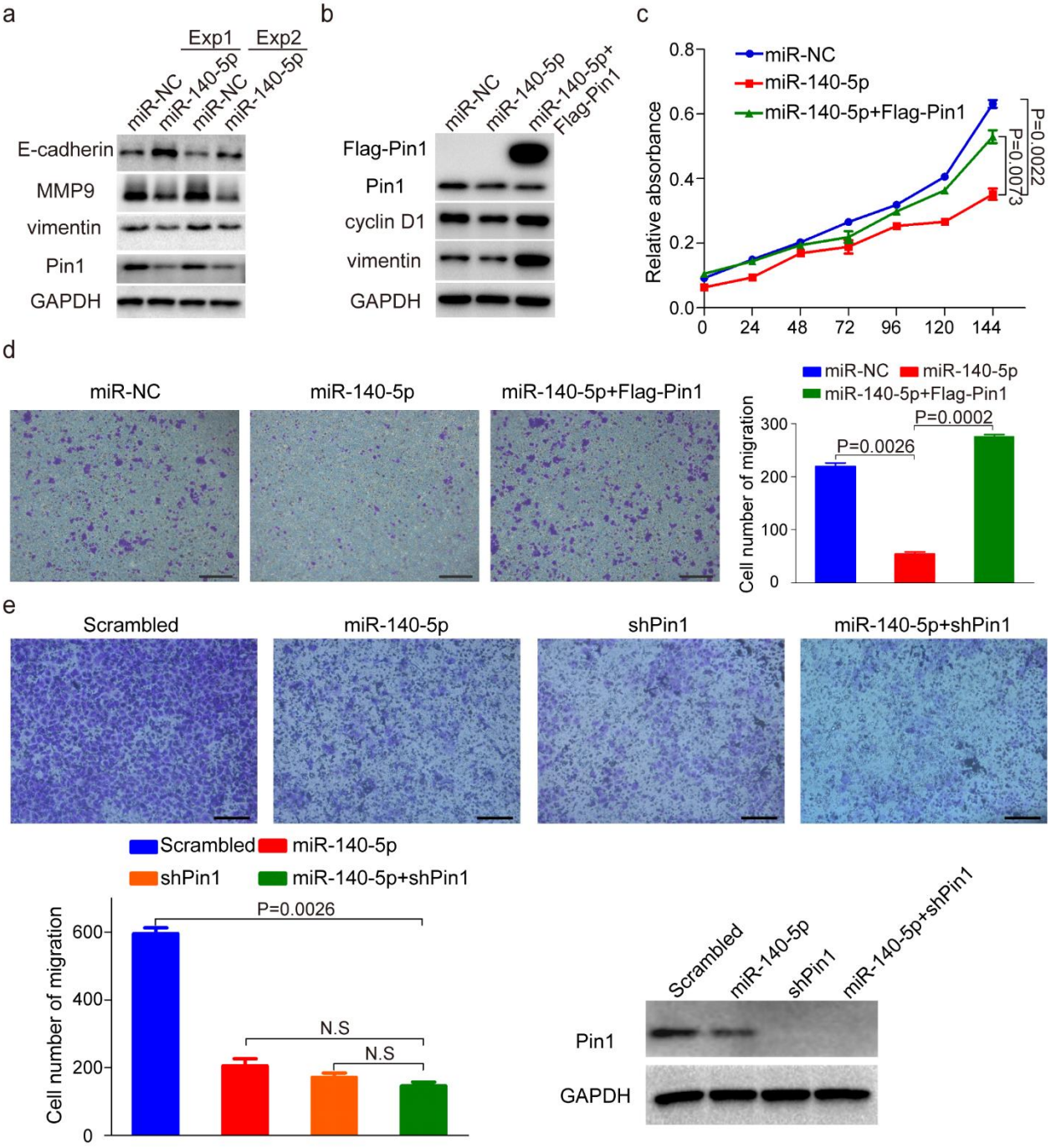

Figure S2. Effect of Pin1 overexpression or stable Pin1 knockdown on the function of miR-140-5p.

(a). The protein levels of Pin1 and EMT markers in Huh7 cells after miR-140-5p overexpression were detected by Western blot assay. GAPDH served as loading control.

(b). The protein levels of Pin1, cyclinD1 and vimentin in PLC/PRF/5 cells after co-transfected with miR-140-5p and Flag-Pin1 were detected by Western blot assay. GAPDH served as loading control.

(c). Cell proliferation of PLC/PRF/5 cells with NC, miR-140-5p or miR-140-5p combined with expression of Flag-Pin1 resistant to miR-140-5p was detected by MTT.

(d). Migration of PLC/PRF/5 cells infected with NC, miR-140-5p or miR-140-5p combined with expression of Flag-Pin1 resistant to miR-140-5p were assayed by transwell experiments. Scale bars, 100 μm.

(e). Migration of Huh7 cells infected with Scrambled, miR-140-5p, shPin1 or shPin1 combined with expression of miR-140-5p were assayed by transwell experiments. Scale bars, 100 μm. The protein levels of Pin1 in Huh7 cells were detected by Western blot assay. GAPDH served as loading control.

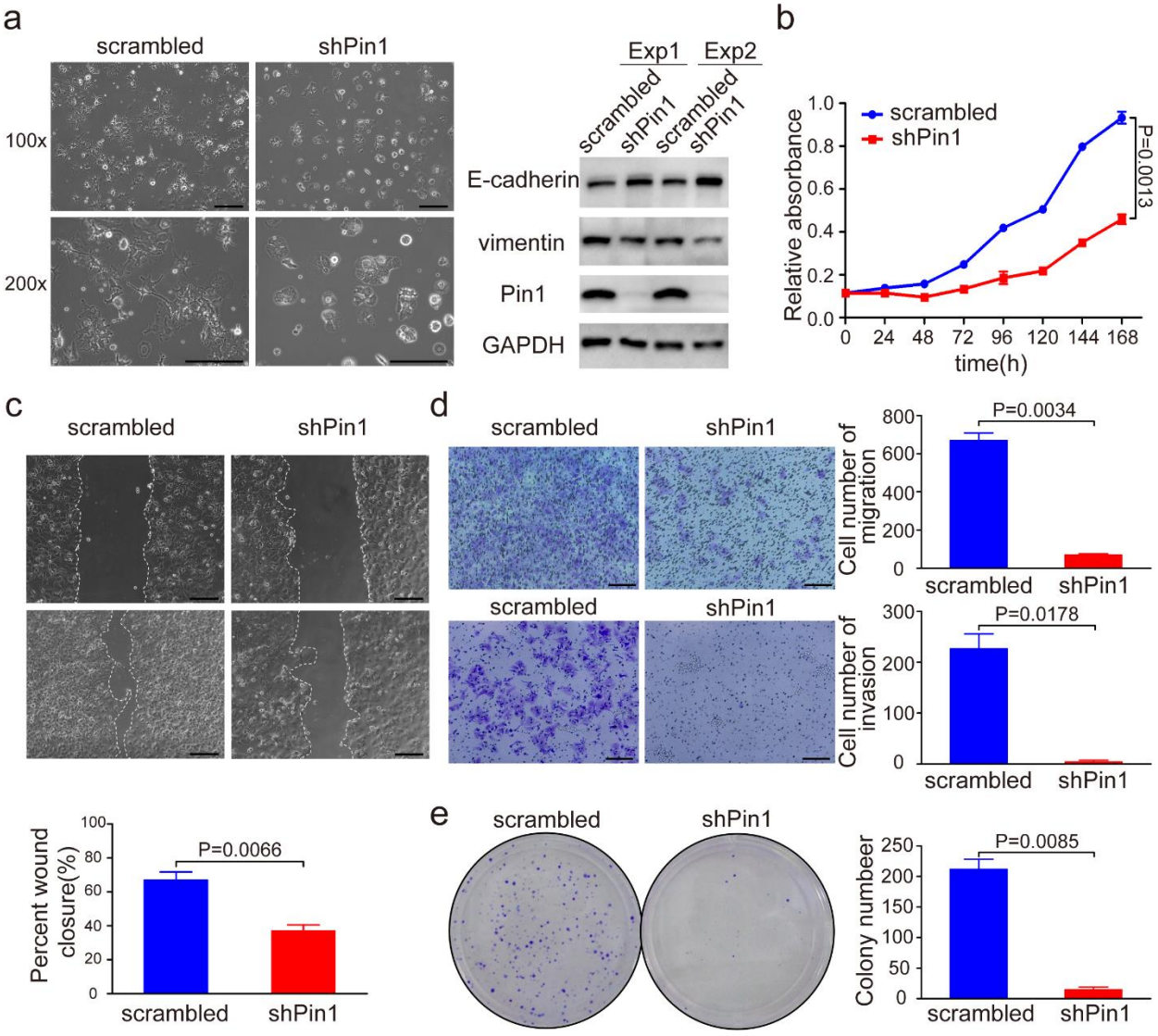

Figure S3. Stable knockdown of Pin1 inhibits migration, invasion, growth and colony formation of human HCC cells in Huh7.

(a). Phase-contrast micrographs of indicated Huh7 cells. Scale bars, 100 μm. The protein levels of EMT markers and Pin1 in two indicated Huh7 cells. GAPDH served as loading control.

(b). The growth of indicated Huh7 cells was performed by using MTT assay.

(c,d). The wound-healing assay (c) and transwell assay (d) of Huh7 cells infected with lentivirus. The invasion assay was measured with Matrigel-coated transwell experiments. Scale bars, 100 μm.

(e). The colonies of indicated Huh7 cells were counted and compared with that of scrambled.

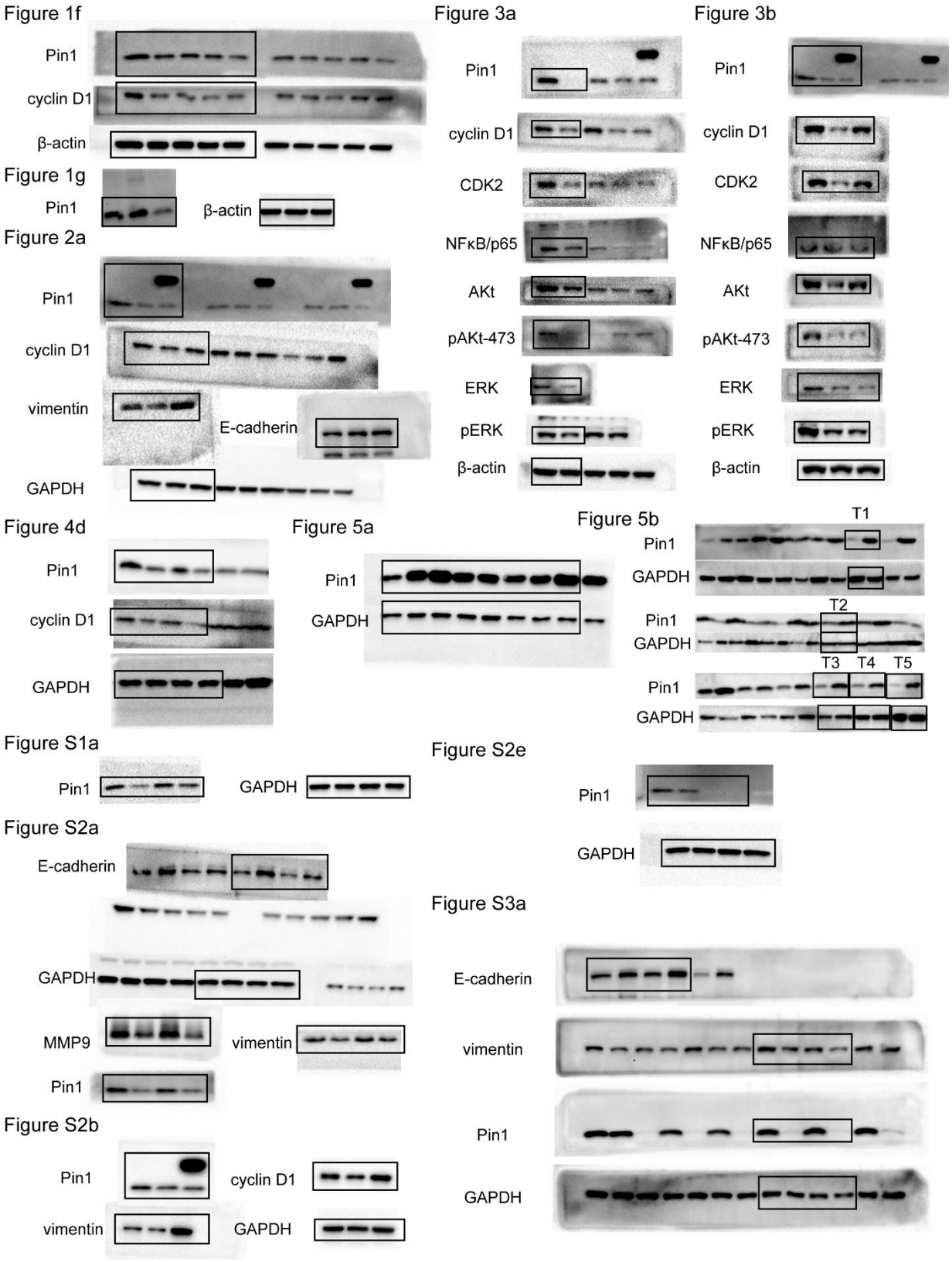

Figure S4. Uncropped images for the key experiments in the main figure.