

**Decreased expression of CHIP leads to increased angiogenesis via VEGF-VEGFR2 pathway and poor prognosis in human renal cell carcinoma**

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Supplementary Table S1: Univariate Cox proportional regression analysis on 5-year overall and disease-specific survival of 304 renal cancer patients.

Variable *	Overall survival			Disease-specific survival		
	Hazard ratio	95% CI <sup>†</sup>	<i>P</i> *	Hazard ratio	95% CI <sup>†</sup>	<i>P</i> *
CHIP						
Negative	1.000		0.032	1.000		0.033
positive	0.738	0.559-0.974		0.722	0.536-0.974	
Age						
≤56 years	1.000		0.486	1.000		0.619
>56 years	1.103	0.836-1.455		1.079	0.800-1.453	
Tumor size						
≤7 cm	1.000		0.011	1.000		0.010
>7 cm	1.422	1.014-1.994		2.040	1.793-2.764	
pT status						
pT <sub>1</sub>	1.000		0.010	1.000		0.009
pT <sub>2</sub> - pT <sub>4</sub>	1.462	1.095-1.952		2.299	1.943-2.790	
pN status						
pN <sub>0</sub>	1.000		0.006	1.000		0.004
pN <sub>1</sub> - pN <sub>3</sub>	2.413	1.544-3.080		2.445	2.020-3.036	
TNM stage						
I	1.000		0.009	1.000		0.008
II-IV	1.497	1.106-2.026		1.933	1.369-2.628	

\* *P* values are from Log-rank test.

<sup>†</sup> CI: confidence interval.

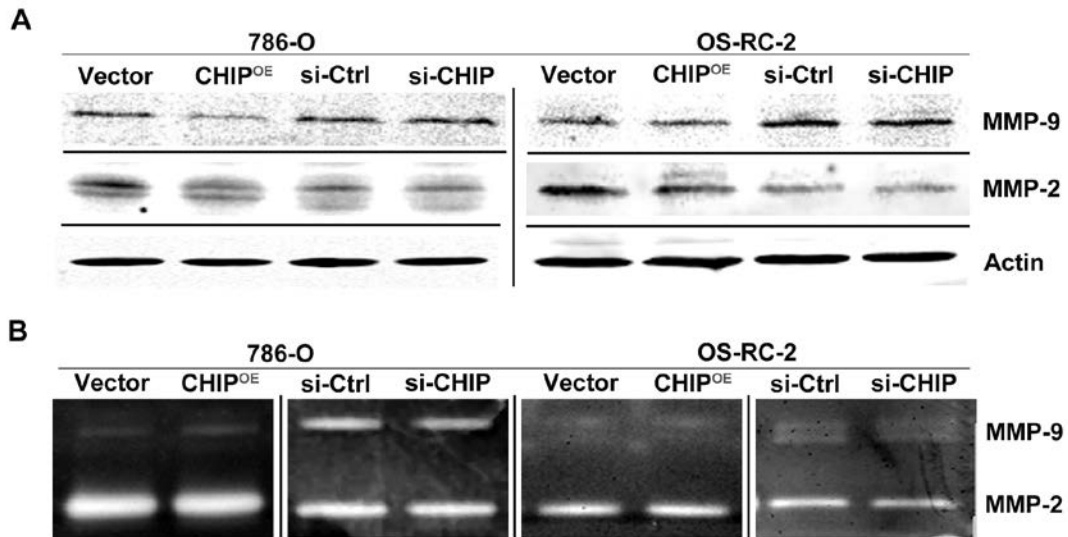
Supplementary Table S2: Multivariate Cox regression analysis on 5-year overall and disease-specific survival of 304 renal cancer patients.

Variable*	Overall survival			Disease-specific survival		
	Hazard ratio	95% CI <sup>†</sup>	<i>P</i>	Hazard ratio	95% CI	<i>P</i>
CHIP	0.739	0.595 to 1.074	0.021	0.706	0.560 to 0.947	0.024
Age	1.021	0.762 to 1.369	0.890	1.005	0.736 to 1.372	0.975
Tumor size	1.809	1.369 to 2.725	0.012	1.815	0.911 to 2.807	0.014
TNM stage	1.659	0.996 to 2.138	0.019	1.698	1.344 to 1.934	0.023

\*Coding of variables: CHIP was coded as 1 (negative), and 2 (positive). Age was coded as 1 ( $\leq 56$  years), and 2 ( $> 56$  years). Tumor size was coded as 1 ( $\leq 7$  cm), and 2 ( $> 7$  cm). TNM stage was coded as 1 (I), and 2 (II - IV).

<sup>†</sup> CI: confidence interval.

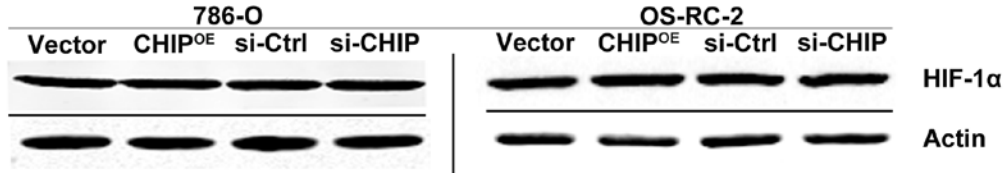
Revised Supplementary Fig. S3: CHIP levels did not affect the expression of MMP-2 and MMP-9. A Western blot analysis of the relative protein levels of MMP-2, MMP-9 and Actin after CHIP overexpression or knockdown for both 786-O and OS-RC-2 cell lines. B Gelatin zymography analysis of the enzyme activity of MMP-2 and MMP-9 after CHIP overexpression or knockdown for both 786-O and OS-RC-2 cell lines.



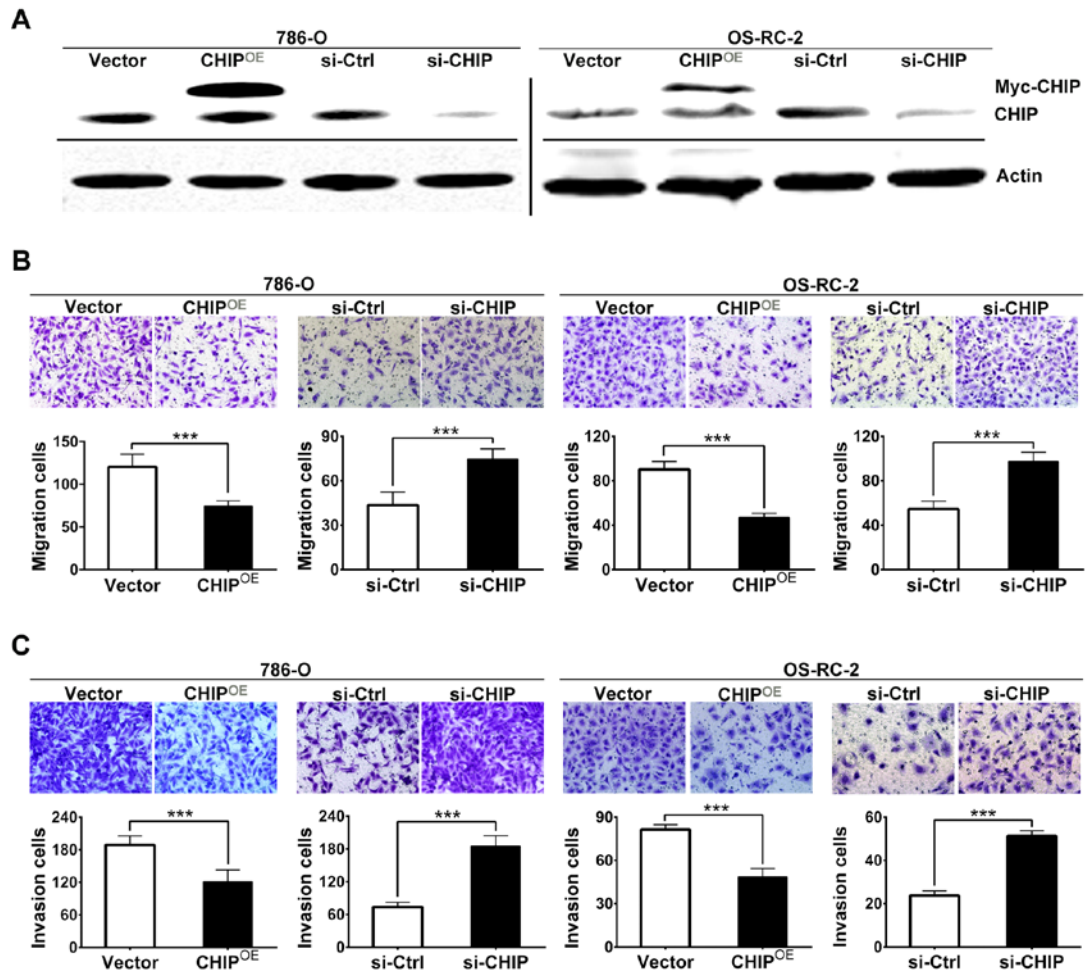
Supplementary Methods S4:

Gelatin zymography: Transfected 786-O and OS-RC-2 cells ( $1 \times 10^6$ ) were cultured in 6-well plate with fresh complete medium for 24 h, and the medium was collected and centrifuged to remove any cell debris before its use as a conditioned medium. 15  $\mu$ g of the proteins was loaded in non-reducing conditions on a 10% polyacrylamide gel containing 0.1% gelatin (Sigma, St. Louis, USA). After electrophoresis, gels were soaked in 2.5% Triton X-100 for 45 minutes with single change of detergent solution. Gels were incubated for 36 h at 37°C in substrate buffer (50 mM Tris-HCl, pH 7.5, 5 mM CaCl<sub>2</sub>, and 0.02% NaN<sub>3</sub>), stained with 0.05% Coomassie brilliant blue G-250 (Sigma, St. Louis, USA), and destained in 10% acetic acid and 20% methanol. Gels were then scanned on the Odyssey Two-Color Infrared Imaging System (LI-COR Biotechnology, Lincoln, Nebraska, USA).

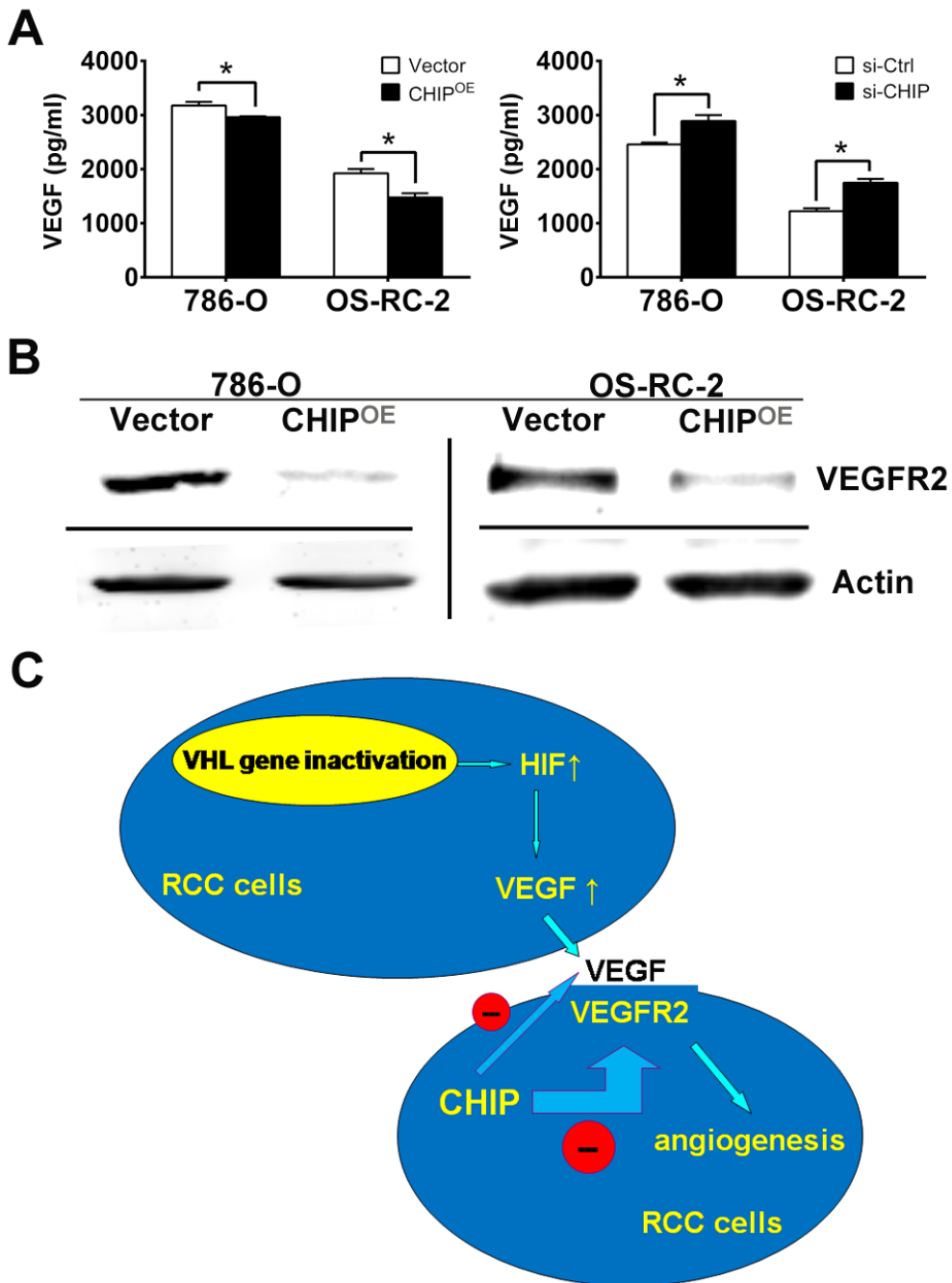
Revised Supplementary Fig. S5: CHIP did not affect the protein expression of HIF-1 $\alpha$ . Western blot analysis of the protein levels of HIF-1 $\alpha$  and Actin after CHIP overexpression or knockdown for both 786-O and OS-RC-2 cell lines.



Revised Fig. 3



Revised Fig. 5



Unprocessed original scans for all of the blots:

All image acquisition tools: Odyssey Two-Color Infrared Imaging System

(LI-COR Biotechnology, Lincoln, Nebraska, USA)

All image processing software packages: Adobe Photoshop CS5.0

Figure 3

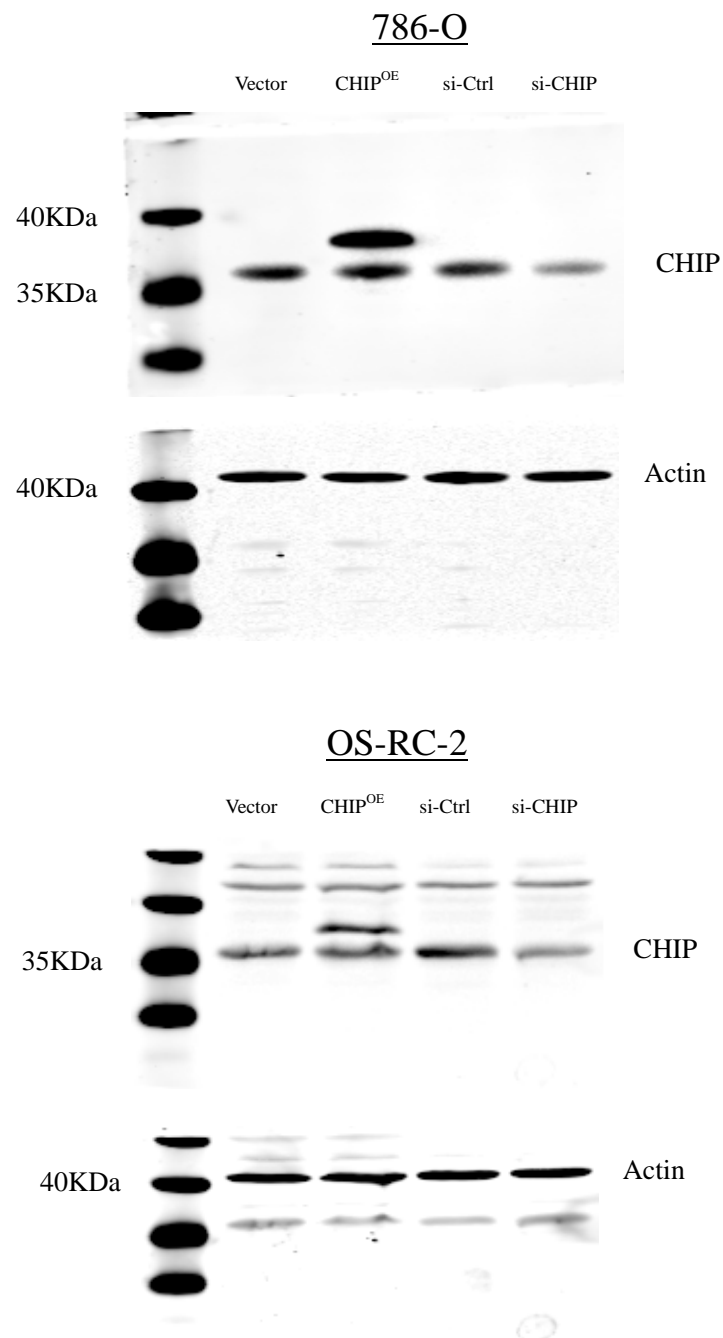


Figure 5

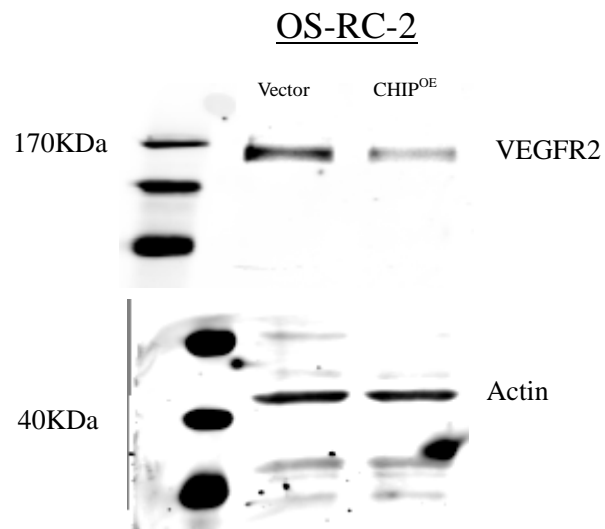
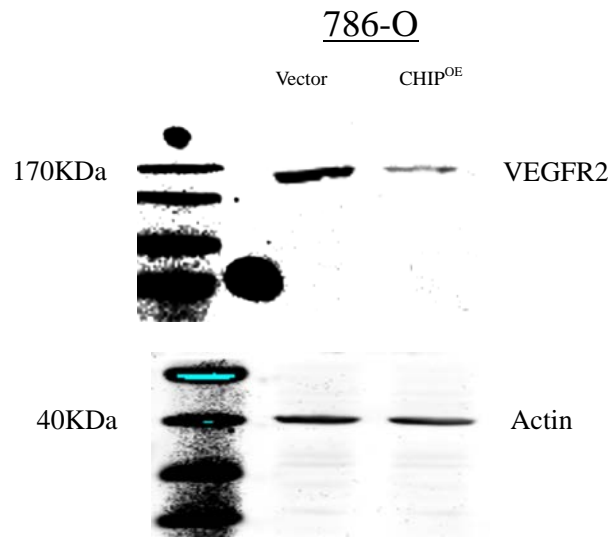
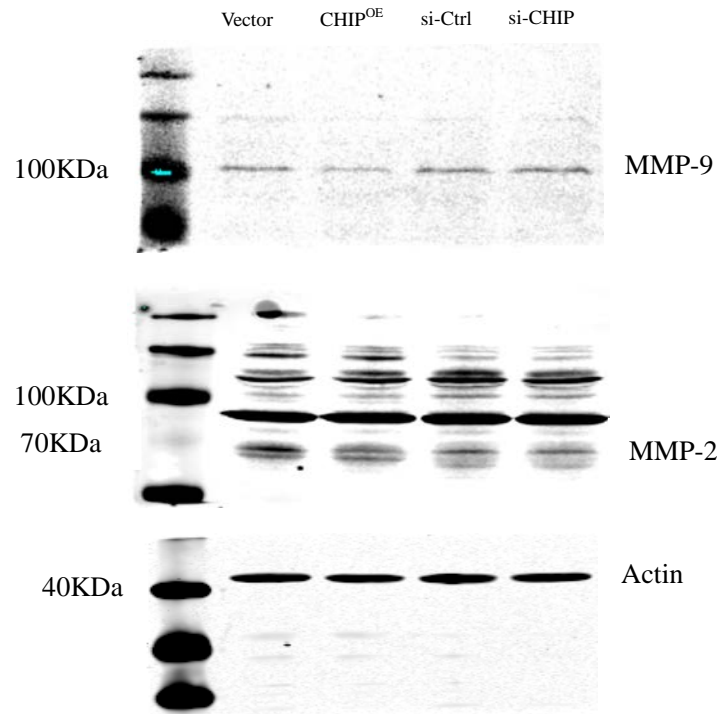


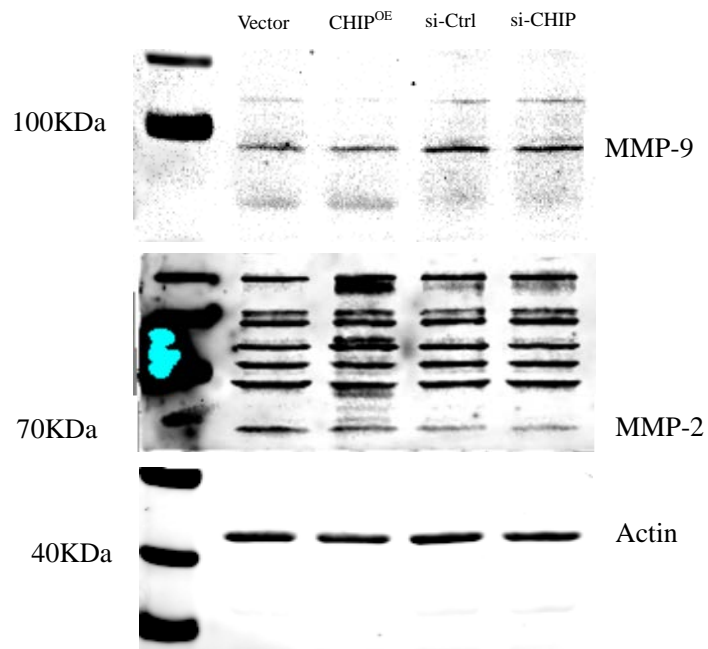


Figure S3

786-O



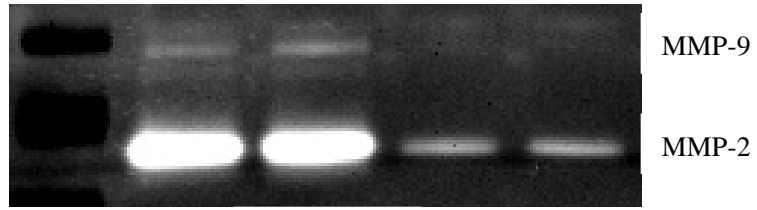
OS-RC-2



786-O

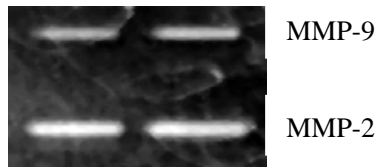
OS-RC-2

Vector      CHIP<sup>OE</sup>      Vector      CHIP<sup>OE</sup>



786-O

si-Ctrl      si-CHIP



OS-RC-2

si-Ctrl      si-CHIP

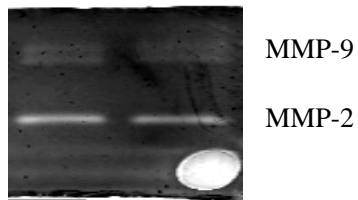


Figure S5

