## Supplementary methods

### *Cell culture*

All cell lines were expanded in RPMI 1640 medium (Invitrogen) containing 10% fetal bovine serum (FBS, Gibco), glutamine (Gibco), and penicillin/streptomycin. Before experiments, CCRCC cells were cultured in this medium until they reached confluence, and then for an additional 1-2 days in the same medium without added serum (changed daily and with or without added inhibitors). pCMVR and pBABE infected populations were selected for 14 days and 4 days with G418 (1 mg/ml) and puromycin (2 μg/ml) respectively, this resulted in significant cell death and selection of stable populations which were then expanded. The 9x HRE VEGF reporter has been described elsewhere (supplementary reference 1).

### *Small Interfering RNA Transfection, qPCR and ChIP*

siRNA reverse transfections were performed in p6-well plates, each well containing $5x10^6$ suspended cells and 5 μl LipofectAMINE 2000 (Invitrogen). Oligo concentration was 100 nM unless otherwise indicated. After transfection, cells were allowed to grow for 1-2 days with 10% FBS and then without serum for 1-2 more days before analysis.

ChIP was performed as follows. DNA-protein complexes were cross-linked by adding 0.75% formaldehyde, followed by blocking with glycine. Cells were washed twice with cold PBS and lysed on ice using FA buffer (50 mM HEPES-KOH pH7.5, 140 mM NaCl, 1 mM EDTA pH8., 0, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, and protease inhibitors). Chromatin samples were sonicated on ice, diluted in RIPA buffer (50 mM Tris-HCl pH8, 150 mM NaCl, 2 mM EDTA pH8, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS and protease inhibitors), centrifuged, and supernatants precleared with protein A sepharose beads pretreated with salmon sperm (Sigma). Antibodies were incubated overnight with the samples on a rotator at $4^oC$. Fresh protein A sepharose beads with salmon sperm were added afterwards, incubated for 2 hours, and washed at room temperature three times with wash buffer (0.1% SDS, 1% Triton X-100, 2 mM

EDTA, 20 mM Tris-HCl pH 8, 150 mM NaCl) and once with final wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8, 500 mM NaCl ). DNA was eluted with elution buffer (1% SDS; 100mM NaHCO3), reverse cross-linked and purified by chloroform/phenol extraction.

***siRNA target sequences and qPCR/ChIP primers***

siRNA:

For the firefly luciferase gene see (http://www.rockefeller.edu/labheads/tuschl/)

HIF-1α oligo A 5`- CTGATGACCAGCAACTTGA

HIF-1α oligo B 5`- CAATCAAGAAGTTGCATTA

HIF-1α oligo C 5`- CGTTGTGAGTGGTATTATT

HIF-2α oligo A 5`- CAGCATCTTTGATAGCAGT

HIF-2α oligo B 5`- GCGACAGCTGGAGTATGAA

HIF-2α oligo C 5`- GCAAATGTACCCAATGATA

NEDD9 oligo A 5`- CACCCAAGAACAAGAGGTA

NEDD9 oligo B 5`- GGAAAGGGATGGTGTTTAT

qPCR:

VEGF-F 5’-TGCCAAGTGGTCCCAG

VEGF-R 5’-GTGAGGTTTGATCCGC

AurkA-F 5’-TTCAGGACCTGTTAAGGCTAC

AurkA-R 5’-TCTGCTTCTGATTCTGAACC

NEDD9-isoform 1-F 5’-CCTCCTTCTCATACCACTCA

NEDD9-isoform 1-R 5’-GCGGGATGTCATACACC

NEDD9-isoform 2-F 5’-CCAGAGAGATGGGCAAGTGT

NEDD9-isoform 2-R 5’-CAAGCCTCCAAACTCAGGAC

p130CAS-F 5’-AACCCCACTGACAAGACCAG

p130CAS-L 5’-TCATAGTCCTCCATCCAGCC

Claudin1-F 5’-GCTTTAAATCGCGGCGCCCAG

Claudin1-R 5’-CTCTGGGTCGGGGTTGGGGTC

ChIP:

EPO-F 5’-CAGGCGTCCTGCCCCTGCT

EPO-R 5’-CAGCCCGCGAGTACTCACCGTG

NEDD9-1-F 5’-GGAAGGGCTTTGAATCATTGTCCTCCTT

NEDD9-1-R 5’-CCTTGCTTCTCCTCATTTGTCCTTAGAGCA

NEDD9-2-F 5’-CTCAGGGACAGCTATTGTCTGGATTCAC

NEDD9-2-R 5’-CAGTCTCTCTCCAGCGAAGCACAGAAC

NEDD9-3-F 5’-CCAGGCTCATCAAACAGTGGGCAT

NEDD9-3-R 5’-GTCACACATATTGAGCGACCTTCAGTGG

NEDD9-4-F 5’-CCACTGAAGGTCGCTCAATATGTGTGA

NEDD9-4-R 5’-TGTCAGTCTGCAAGGAGCTGGAAGAC

## Supplementary Figure Legends

***Supplementary Figure 1-***

Western blot using the indicated antibodies and lysates from *VHL*-defective RCC10 cells transfected with siRNA oligos (oligo pairs B and C) for HIF-1α, HIF-2α and the firefly luciferase gene (control).

**Supplementary Figure 2-**

(**A-C**) The number of nuclei stained with DAPI (3 experiments in duplicate and 8 fields per coverslip were analyzed) and number of cells stained with BrDU (3 experiments in duplicate and 8 fields per coverslip) were counted to assess changes in proliferation in the indicated cell lines and treatments. (**D**) Percentage of BrdU positive cells among all migrated cells (stained with DAPI); the results correspond to 3 experiments in duplicate (4 fields per coverslip).

## Supplementary references

1. Aragones J, Jones DR, Martin S, San Juan MA, Alfranca A, Vidal F, Vara A, Merida I, Landazuri MO: Evidence for the involvement of diacylglycerol kinase in the activation of hypoxia-inducible transcription factor 1 by low oxygen tension. *J Biol Chem* 276: 10548-55, 2001

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NEDD9-isoform 1-R 5’-GCGGGATGTCATACACC

NEDD9-isoform 2-F 5’-CCAGAGAGATGGGCAAGTGT

NEDD9-isoform 2-R 5’-CAAGCCTCCAAACTCAGGAC

p130CAS-F 5’-AACCCCACTGACAAGACCAG

p130CAS-L 5’-TCATAGTCCTCCATCCAGCC

Claudin1-F 5'-GCTTTAAATCGCGGCGCCCAG

Claudin1-R 5'-CTCTGGGTCGGGGTTGGGGTC

ChIP:

EPO-F 5'-CAGGCGTCCTGCCCCTGCT

EPO-R 5'-CAGCCCGCGAGTACTCACCGTG

NEDD9-1-F 5'-GGAAGGGCTTTGAATCATTGTCCTCCTT

NEDD9-1-R 5'-CCTTGCTTCTCCTCATTTGTCCTTAGAGCA

NEDD9-2-F 5'-CTCAGGGACAGCTATTGTCTGGATTCAC

NEDD9-2-R 5'-CAGTCTCTCTCCAGCGAAGCACAGAAC

NEDD9-3-F 5'-CCAGGCTCATCAAACAGTGGGCAT

NEDD9-3-R 5'-GTCACACATATTGAGCGACCTTCAGTGG

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NEDD9-4-R 5'-TGTCAGTCTGCAAGGAGCTGGAAGAC

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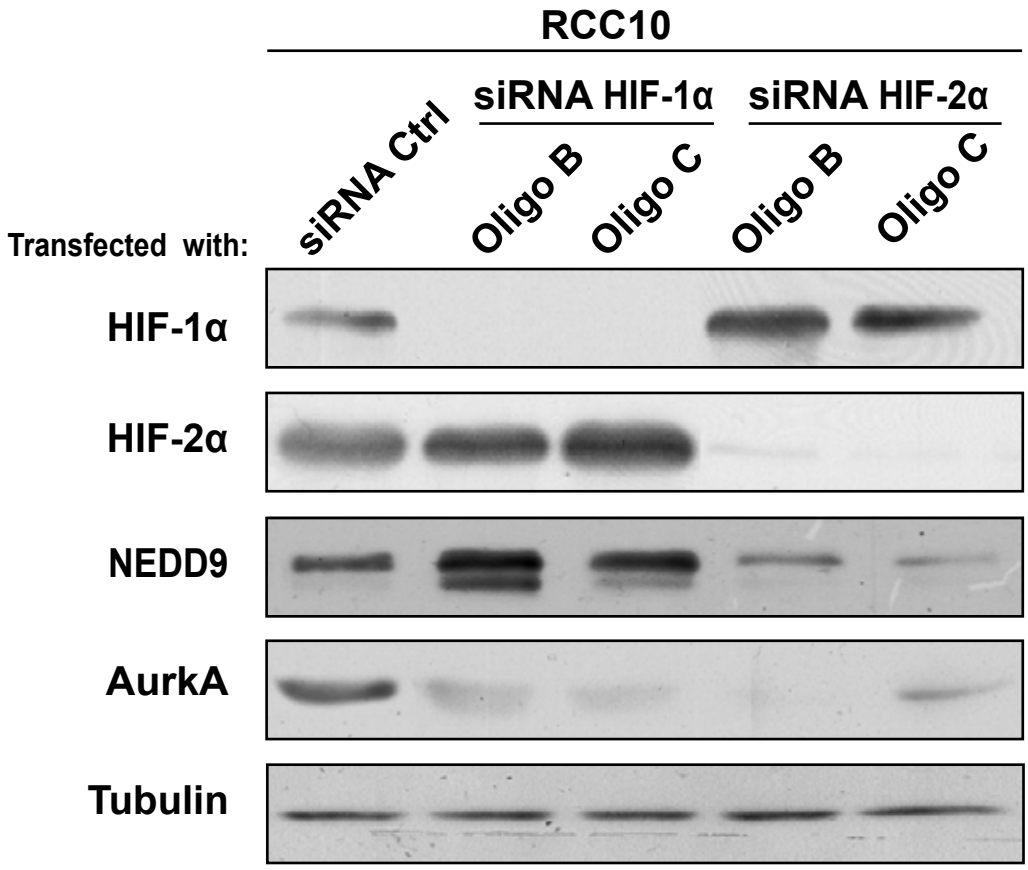
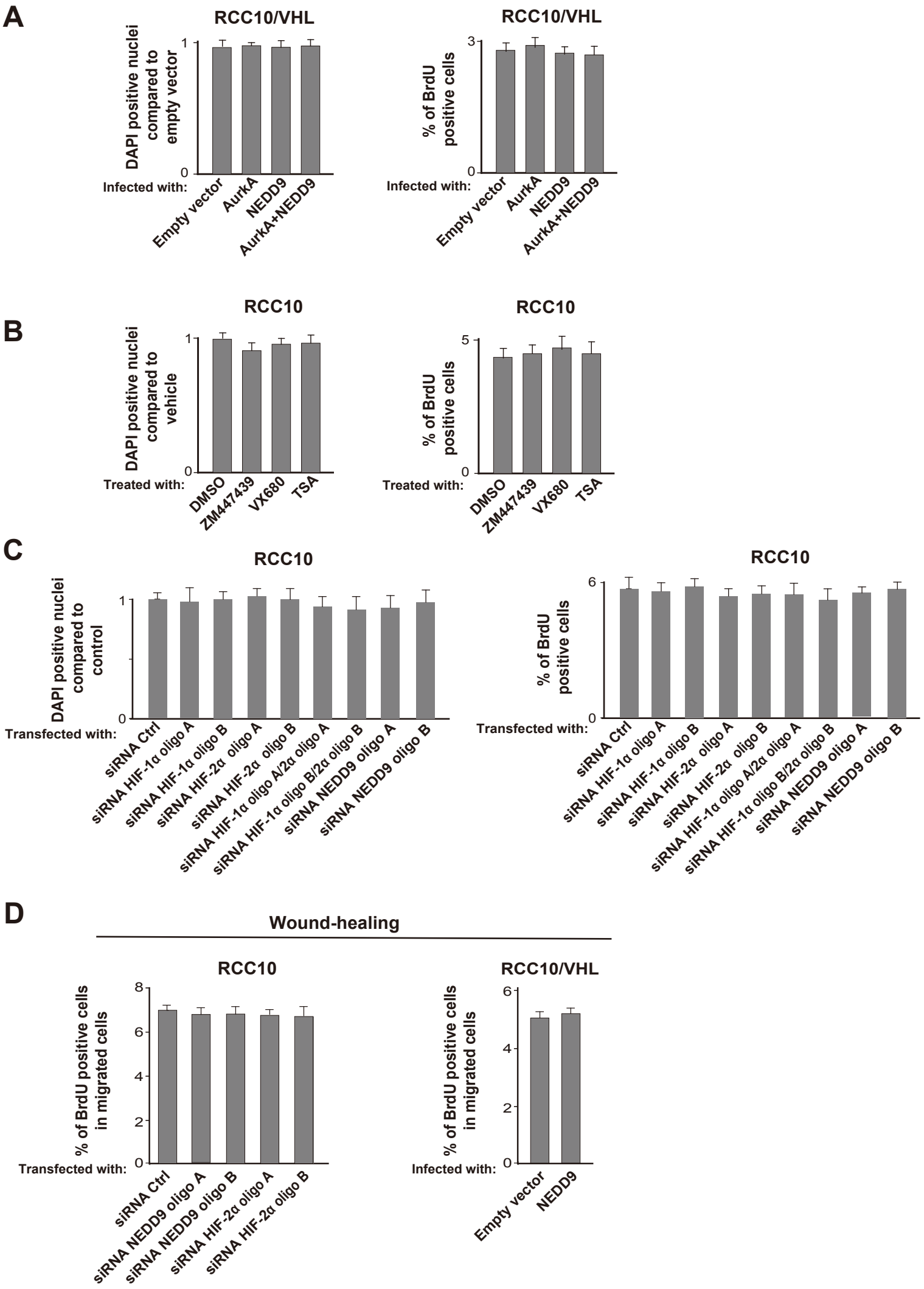
## Suppl. Fig. 1


RCC10
siRNA Ctrl
siRNA HIF-1α
siRNA HIF-2α
Oligo B
Oligo C
Oligo B
Oligo C
Transfected with:
HIF-1α
HIF-2α
NEDD9
AurkA
Tubulin

# Suppl. Fig. 2


A
RCC10/VHL
DAPI positive nuclei compared to empty vector
1
0
Infected with:
Empty vector
AurkA
NEDD9
AurkA+NEDD9
RCC10/VHL
% of BrdU positive cells
3
0
Infected with:
Empty vector
AurkA
NEDD9
AurkA+NEDD9
B
RCC10
DAPI positive nuclei compared to vehicle
1
0
Treated with:
DMSO
ZM447439
VX680
TSA
RCC10
% of BrdU positive cells
5
0
Treated with:
DMSO
ZM447439
VX680
TSA
C
RCC10
DAPI positive nuclei compared to control
1
0
Transfected with:
siRNA Ctrl
siRNA HIF-1α oligo A
siRNA HIF-1α oligo B
siRNA HIF-2α oligo A
siRNA HIF-2α oligo B
siRNA HIF-1α oligo A/2α oligo A
siRNA HIF-1α oligo B/2α oligo B
siRNA NEDD9 oligo A
siRNA NEDD9 oligo B
RCC10
% of BrdU positive cells
6
0
Transfected with:
siRNA Ctrl
siRNA HIF-1α oligo A
siRNA HIF-1α oligo B
siRNA HIF-2α oligo A
siRNA HIF-2α oligo B
siRNA HIF-1α oligo A/2α oligo A
siRNA HIF-1α oligo B/2α oligo B
siRNA NEDD9 oligo A
siRNA NEDD9 oligo B
D
Wound-healing
RCC10
% of BrdU positive cells in migrated cells
8
6
4
2
0
Transfected with:
siRNA Ctrl
siRNA NEDD9 oligo A
siRNA NEDD9 oligo B
siRNA HIF-2α oligo A
siRNA HIF-2α oligo B
RCC10/VHL
% of BrdU positive cells in migrated cells
6
4
2
0
Infected with:
Empty vector
NEDD9