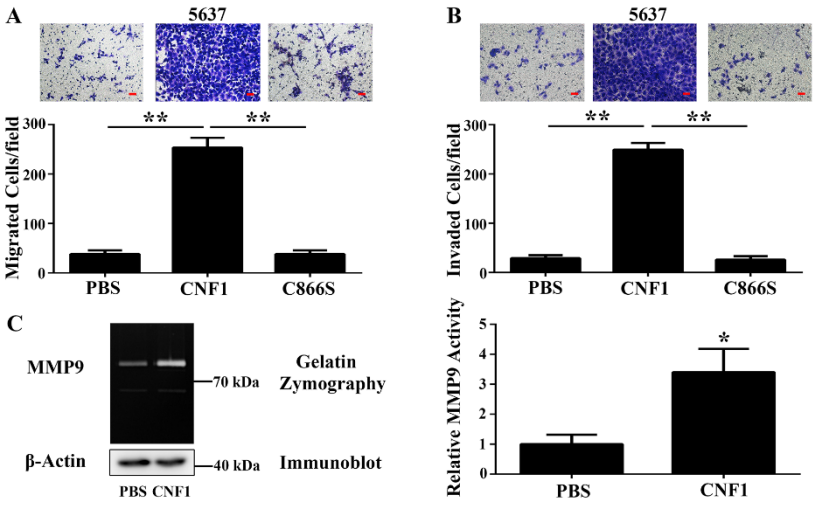
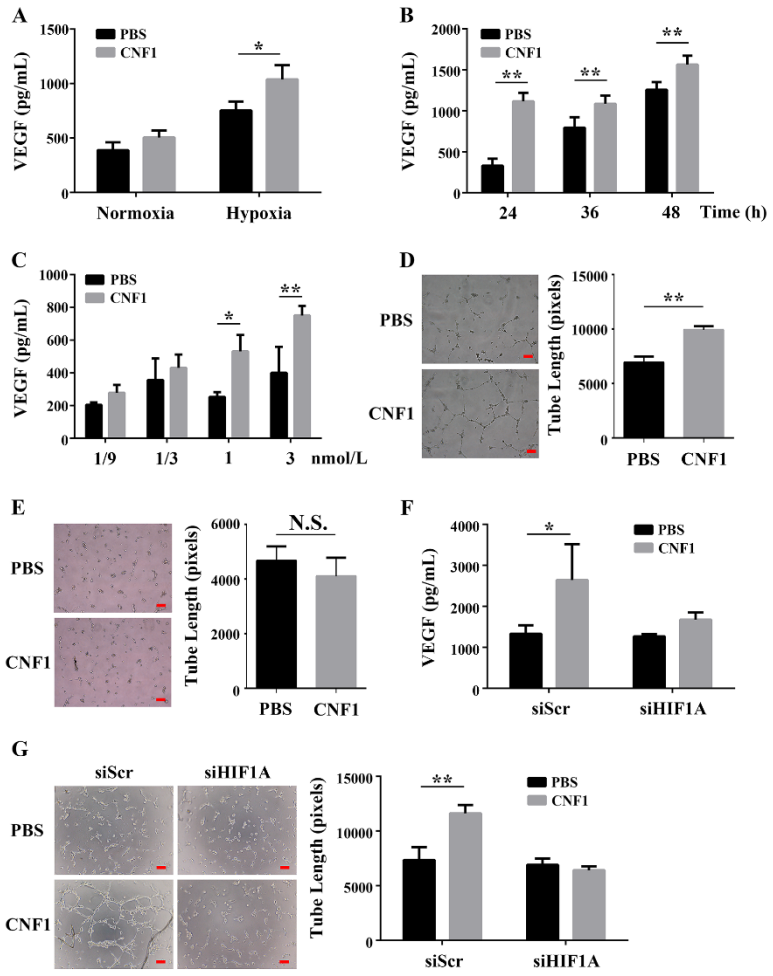
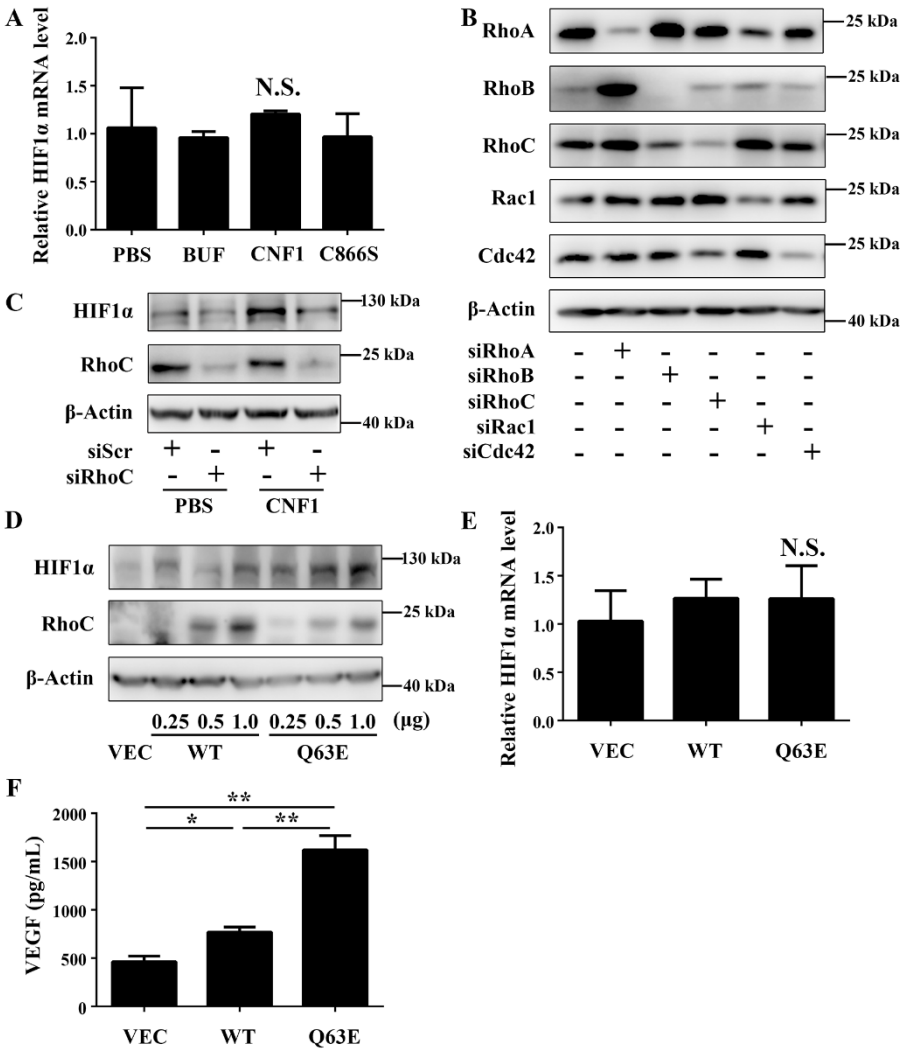


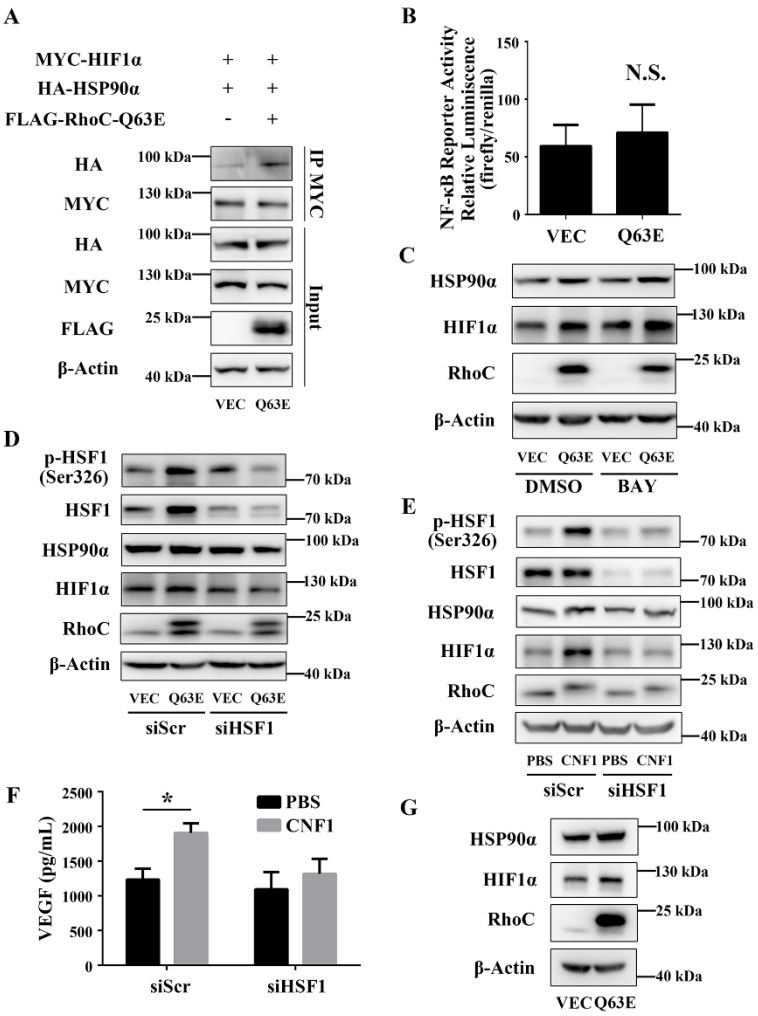
Supplementary Figure 1. CNF1 promotes the migration and invasion ability of other bladder cancer cells.
(A and B) Transwell-based migration and invasion assays of 5637 cells treated with 1 nmol/L recombinant CNF1 protein, PBS and the inactive CNF1 mutant C866S (n = 3, three independent experiments). (C) Zymography assay of MMP9 activities in UMUC3 cells treated with PBS and 1 nmol/L CNF1 (n = 3, three independent experiments). β-Actin was loaded as an internal control. Data are the mean ± SD. *$p < 0.05$, **$p < 0.01$; one-way ANOVA (A and B) or Student's *t*-test (C). Scale bar = 50 μm.



Supplementary Figure 2. CNF1 induces VEGF secretion in other bladder cancer cells and subsequently promotes angiogenesis in HUVECs. (A) The 5637 cells were incubated with CNF1 (3 nmol/L) for 24 h under normoxic or hypoxic conditions, and VEGF secretion in the culture medium was analyzed by ELISA (n = 3, three independent experiments). (B-C) Time- and concentration-dependent VEGF secretion assay of CNF1 (3 nmol/L) on 5637 cells under hypoxic conditions (n = 3, three independent experiments). (D) The 5637 cells were stimulated with CNF1 (3 nmol/L) or PBS for 36 h. The medium was collected and then applied to HUVECs for 6 h. The capillary-like structure formation in HUVECs was examined using a tube formation assay (n=3, three independent experiments). Tube formation ability was visualized and calculated by measuring the length of the tubes. (E) PBS or 3 nmol/l recombinant CNF1 protein were applied to HUVECs for 6 h. The capillary-like structure formation in HUVECs was examined using a tube formation assay (n = 3, three independent experiments). (F) 5637 cells were transfected with scrambled or HIF1A siRNA for 24 h followed by stimulation with CNF1 (3 nmol/L) or PBS for 36 h. VEGF secretion in culture medium was examined by ELISA (n = 3, three independent experiments). (G) The medium as described in (F) was applied to HUVECs for 6 h. The capillary-like structure formation in HUVECs was examined using a tube formation assay (n = 3, three independent experiments). Data are the mean ± SD. *p < 0.05, **p < 0.01, N.S. no significance; one-way ANOVA (A-C, F, G) or Student's *t*-test (D and E). Scale bar = 100 μm.



Supplementary Figure 3. The HIF1α mRNA level was not regulated by CNF1. (A) CNF1 has no effect on the mRNA level of HIF1α. qRT-PCR analysis of HIF1α in T24 cells after treatment with CNF1 (3 nmol/L) under hypoxic conditions for 24 h, with PBS, dialysis buffer and C866S treatments as controls (n=3, three independent experiments). (B) The specificity of the respective Rho GTPase siRNAs. T24 cells were transfected with scrambled or respective Rho GTPase siRNAs for 48 h, and the effects on the Rho GTPases expression were examined by western blotting. (C) 5637 cells were transfected with a scrambled control siRNA or the siRNA targeting RhoC for 24 h, followed by stimulation with PBS or CNF1 (3 nmol/L) for another 24 h under hypoxic conditions, and HIF1α expression was examined by western blotting. (D) Western blotting analysis of 293T cells transfected with vector, wild-type RhoC, or constitutively active RhoC (Q63E) under hypoxic conditions. (E) RhoC activation has no effect on HIF1α mRNA level. qRT-PCR analysis of HIF1α in T24 cells after transfection with the vector, wild-type RhoC, or Q63E under hypoxic conditions (n = 3, three independent experiments). (F) 5637 cells were transfected with vector, wild-type RhoC, or Q63E for 48 h, and VEGF secretion in the culture medium was examined by ELISA (n = 3, three independent experiments). Data are the mean ± SD. *p < 0.05, **p < 0.01, N.S. no significance; one-way ANOVA (A, E, F).



Supplementary Figure 4. RhoC does not increase HSP90α through NF-κB. (A) 293T cells were transfected with combinations of MYC-HIF1α, HA-HSP90α and FLAG-RhoC-Q63E under hypoxic conditions. Protein extracts from the transfected cells were subjected to IP with antibody against MYC and analyzed by immunoblotting with the indicated antibodies. (B) Active RhoC does not activate NF-κB. T24 cells stably expressing Q63E or the vector were transfected with NF-κB reporter gene and a Renilla luciferase control to measure the relative NF-κB activitiy (n = 3, three independent experiments). (C) Active RhoC does not increase HSP90α and HIF1α through NF-κB. Analysis of HSP90α and HIF1α of transduced T24 cells treated with the NF-κB inhibitor BAY 11-7085 (15 μmol/L), with DMSO as the control. (D) Active RhoC modulates HSP90α expression by activating HSF1. Western blotting analysis of transduced T24 cells treated with another siRNAs targeting HSF1 and a scrambled non-targeting control siRNA under hypoxic condition. (E) Western blotting analysis of 5637 cells treated by 1 nmol/L CNF1 and subject to the siRNA targeting HSF1 and scrambled non-targeting control siRNA under hypoxic conditions. (F) 5637 cells were transfected with scrambled or HSF1 siRNA for 24 h followed by stimulation with CNF1 (3 nmol/L) or PBS for 24 h, and VEGF secretion in culture medium was examined by ELISA (n = 3, three independent experiments). (G) The transduced T24 cells used for nude mice injection were confirmed *in vitro*. Western blotting analysis of T24 cells stably expressing Q63E or the vector constructed using lentiviral supernatant. Data are the mean ± SD. *$p < 0.05$, N.S. no significance; one-way ANOVA (F) or Student's *t*-test (B).

Supplementary Table 1. Strains and plasmids used in this study

Strain or plasmid	Description	Source
Bacterial strain		
11	Clinical uropathogenic *E. coli* strain	Korczak Hospital
E. coli BL21	F^- *ompT hsdS*$_B$ (r_B^- m_B^-) *gal dcm* (DE3)	Novagen, Merck-Millipore
E. coli DH5α	F^- *Φ*80*lacZ* M15 *endA recA1 hsdR* (r_k^- m_k^-) *supE44 thi-1* gyrA96 relA1_(*lacZYA-argF*) U169	Beijing Dingguo Biotechmology Development Center, Beijing, China
Plasmid		
pET-28a (+)	T7 expression vector, Kan^r	Novagen, Merck-Millipoe
pCMV-Tag2B	Mammalian Expression Vector, Kan^r	Stratagene
pLVX-EF1α-IRES-Puro	Lentivectors, Amp^r, $Puro^r$	Clontech
pLVX-IRES-Hyg	Lentivectors, Amp^r, Hyg^r	Clontech
pcDNA3.1/Myc-His B	Mammalian Expression Vector, Amp^r	Invitrogen
pQW0001	pET28a+ containing N-terminal 6 × histidine tagged *cnf1* from strain 11 containing C-terminal Myc	Our previous study
pQW0002	pET28a+ containing N-terminal 6 × histidine tagged C866S mutated *cnf1* from strain 11 containing C-terminal Myc	Our previous study
pQW0016	pCMV-Tag2B containing N-terminal FLAG tagged RhoC	This work
pQW0017	pCMV-Tag2B containing N-terminal FLAG tagged Q63E mutated RhoC	This work
pQW0018	pLVX-EF1α-IRES-Puro containing C-terminal FLAG tagged Q63E mutated RhoC	This work
pQW0019	pLVX-IRES-Hyg containing 6×Myc tag	This work
pQW0020	pLVX-IRES-Hyg containing N-terminal 6×Myc tagged HIF1α	This work
pQW0021	pcDNA3.1/Myc-HisB containing N-terminal HA tagged HSP90α	This work

Supplementary Table 2. Primers and siRNAs used in this study

Primer /siRNA	Sequence (5'-3')	Description
WQ0850	CGCGGATCCGCTGCAATCCGAAA	For cloning RhoC into pCMV-Tag2B
WQ0851	CCCAAGCTTTCAGAGAATGGGACAG	For cloning RhoC into pCMV-Tag2B
WQ1363	CCGGAATTCGCCACCATGGCTGCAATCC	For cloning RhoC into pLVX-EF1α-IRES-Puro
WQ1364	ATAAGAATGCGGCCGCTCACTTATCGTCGTCATC CTTGTAATCGAGAATGGGACAGCC	For cloning RhoC into pLVX-EF1α-IRES-Puro
WQ0561	TTTGATACTGCAGGGGAAGAGGATTA	Mutagenesis Q63E of RhoC
WQ0562	CCCCTGCAGTATCAAAAAGTCCAAGA	Mutagenesis Q63E of RhoC
WQ1452	GCTCTAGAGAGGGCGCCGGCGGCGCGAACGACA AGAAAAAGAT	For cloning HIF1A into pLVX-IRES-Hyg-6×MYC
WQ1453	CGGGATCCTCAGTTAACTTGATCCAAAGCTCTGA GTAATT	For cloning HIF1A into pLVX-IRES-Hyg-6×MYC
WQ1604	GATCGCGGCCGCGCCACCATGTACCCATACGATG TTCCAGATTACGCTCCTGAGGAAACCCAGACCCA	For cloning HSP90AA1 into pcDNA3.1/Myc-His B
WQ1605	GATCGGATCCTTAGTCTACTTCTTCCATGCGTG	For cloning HSP90AA1 into pcDNA3.1/Myc-His B
WQ1163	ATGACGAGGGCCTGGAGTGTG	RT-PCR primers for VEGF
WQ1164	CCTATGTGCTGGCCTTGGTGAG	RT-PCR primers for VEGF
WQ1245	ATCCATGTGACCATGAGGAAATG	RT-PCR primers for HIF1A
WQ1246	TCGGCTAGTTAGGGTACACTTC	RT-PCR primers for HIF1A
WQ1673	CAGATGCATTGGACAAAATCCG	RT-PCR primers for HSP90AA1
WQ1674	ATGAACGCTTTGGTCCCAGAC	RT-PCR primers for HSP90AA1
WQ1707	GCAGGCGTCGAAGAGTACG	RT-PCR primers for VHL
WQ1708	CGGACTGCGATTGCAGAAGA	RT-PCR primers for VHL
WQ1719	AGGCTGACAGGAAGACTGATG	RT-PCR primers for RWDD3
WQ1720	TGCCAGAAACCTTTTGTGTTCT	RT-PCR primers for RWDD3
WQ1675	TGTGTGGAATCAAGCACCTTC	RT-PCR primers for MAPK8
WQ1676	AGGCGTCATCATAAAACTCGTTC	RT-PCR primers for MAPK8
WQ1689	TACCTGCCCTATGCCGAGG	RT-PCR primers for SART1
WQ1690	TTCAAGCTCTTCGTCATACTTGG	RT-PCR primers for SART1
WQ1715	TTCCACTGGCAAGCCACTAT	RT-PCR primers for UBE2D1
WQ1716	CAGTCAGAGCTGGTGACCATT	RT-PCR primers for UBE2D1
WQ1695	CGGACTACACGGATCAAAATCA	RT-PCR primers for PCGF2
WQ1696	GGCGTCGATGAAGTACCCC	RT-PCR primers for PCGF2
WQ1669	ACAGGTGCTATAAGGTCATCCA	RT-PCR primers for PSEN1
WQ1670	CAGATCAGGAGTGCAACAGTAAT	RT-PCR primers for PSEN1
WQ1677	GAGGGAGAACTCCGTGTCCTA	RT-PCR primers for HDAC6
WQ1678	AATAGCCATCCATAAGACTGTGC	RT-PCR primers for HDAC6
WQ1709	GAGATGACAGCCGAGGAGATG	RT-PCR primers for FKBP4
WQ1710	GACTCGGTCCCCAATCATGG	RT-PCR primers for FKBP4
WQ1685	AAGAGCGGCAAGAAGAGTTAC	RT-PCR primers for YY1
WQ1686	CAACCACTGTCTCATGGTCAATA	RT-PCR primers for YY1
WQ1683	CGCCCTCACAAAGCCAATG	RT-PCR primers for HDAC1
WQ1684	CTGCTTGCTGTACTCCGACA	RT-PCR primers for HDAC1
WQ1691	TGACCAGGAGGCAAAACCTTC	RT-PCR primers for SUMO1
WQ1692	AATTCATTGGAACACCCTGTCTT	RT-PCR primers for SUMO1
WQ1319	AATGAGCTGCGTGTGGCT	RT-PCR primers for β-Actin
WQ1320	TAGCACAGCCTGGATAGCAA	RT-PCR primers for β-Actin

siRhoA	CCCAGAUACCGAUGUUAUATT	siRNAs for RhoA
siRhoB	ACGUCAUUCUCAUGUGCUUTT	siRNAs for RhoB
siRhoC	GAACCGGAUCAGUGCCUUUTT	siRNAs for RhoC
siRac1	GAGUCCUGCAUCAUUUGAATT	siRNAs for Rac1
siCdc42	GUGGAGUGUUCUGCACUUATT	siRNAs for Cdc42
siHIF1A	CCACCACUGAUGAAUUAAATT	siRNAs for HIF1A
1#siHSF1	CCAAGUACUUCAAGCACAATT	siRNAs for HSF1
2#siHSF1	AGGAGUGCAUGGACUCCAA	siRNAs for HSF1