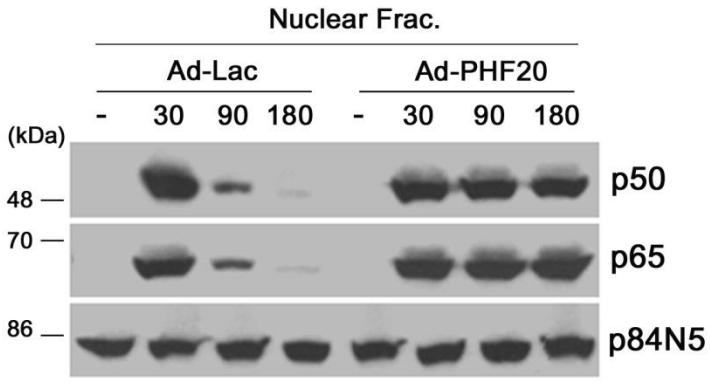
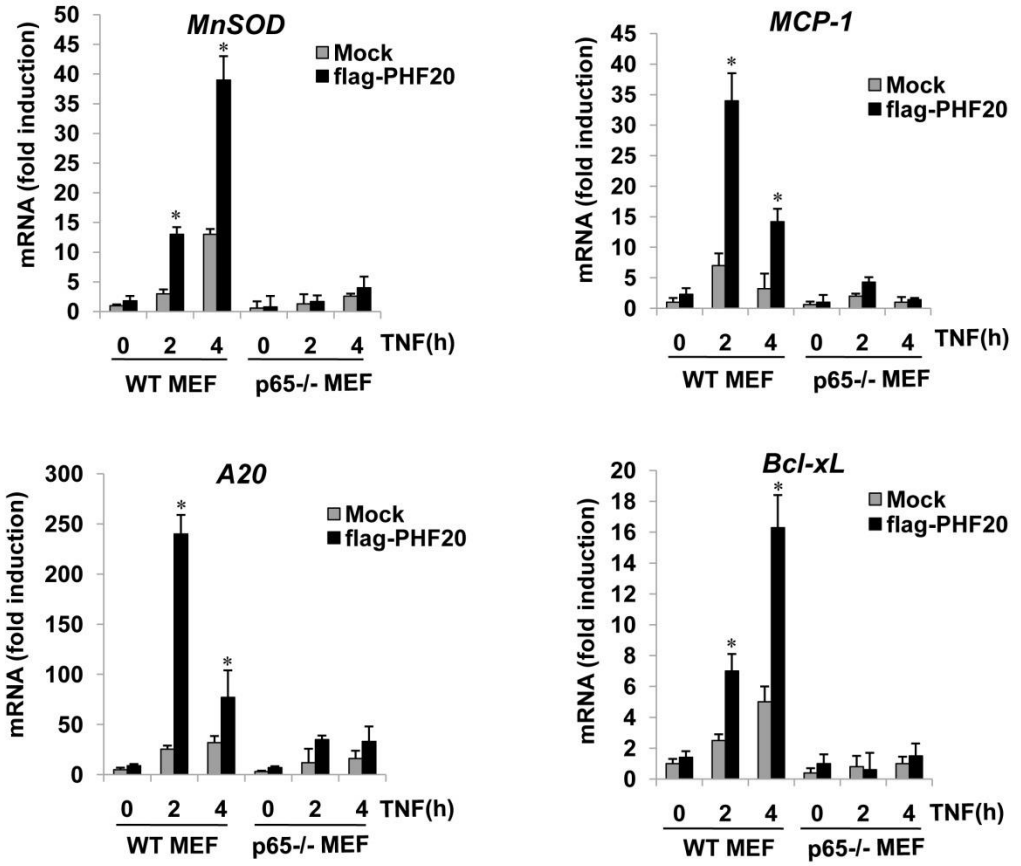
Supplementary Figure S1



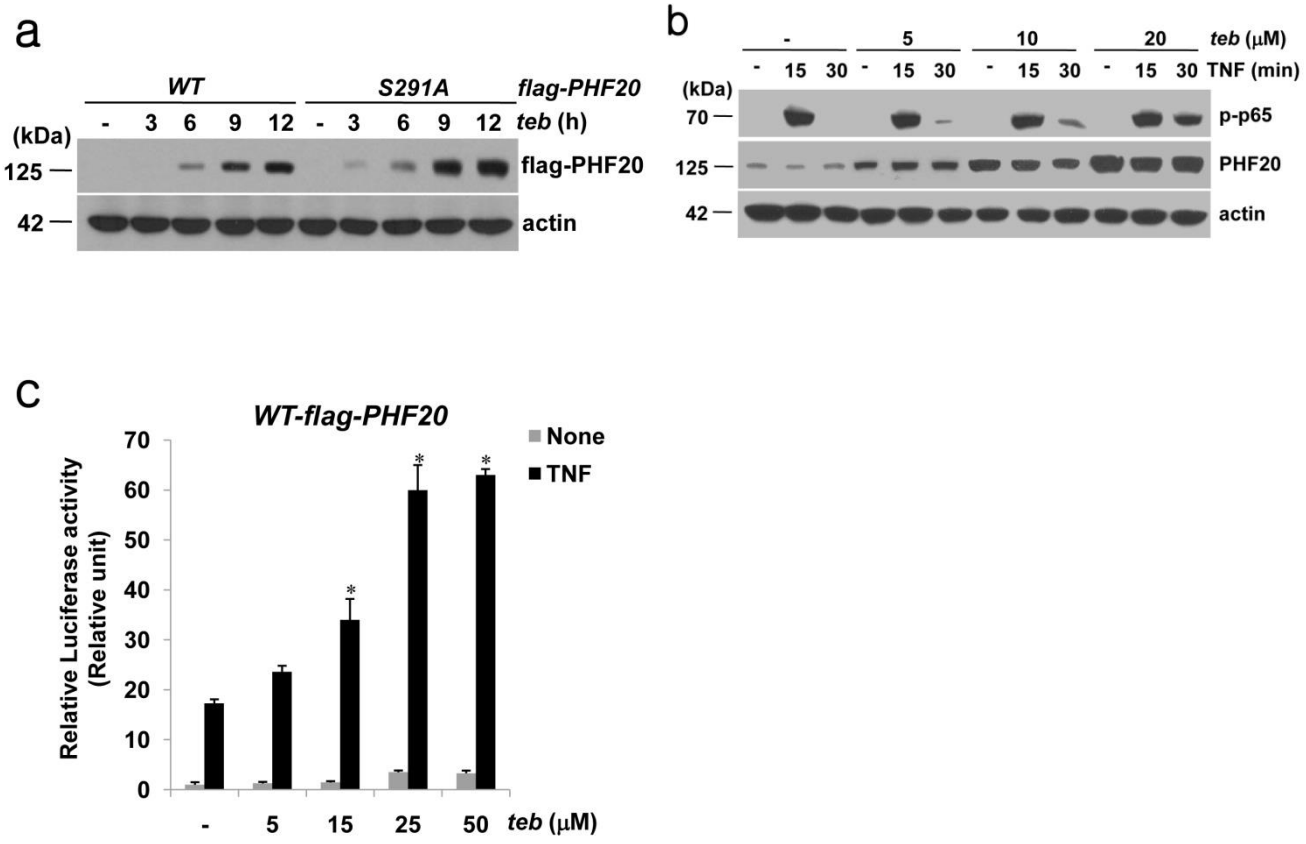
Supplementary Figure S1:Persistent nuclear retention of p65/p50 heterodimer upon TNF treatment by PHF20 overexpresion. HeLa cells were infected with either rAd-La c or rAd-PHF20 for 24 h, and then treated with TNF for indicated times. Nuclear fractions were obtained as described in Methods, and immunoblott was performed with indicated antibodies.

Supplementary Figure S2



Supplementary Figure S2:TNF-induced genes whose expression is enhanced by PHF20 are p65-dependent. Wild type and p65-/- MEFs were infected with either rAd-La c or rAd-PHF20 as described in Supplementary figure S1. Total RNA was prepared at the indicated times after TNF treatment, and mRNA expression levels of MnSOD, MCP-1, A20 and Bcl-xL were measured by real-time PCR. . Each columns shows mean ± S.E. of at least three independent experiments. *$P<0.05$, compared with mock-transfected cells (Student's *t*-test)

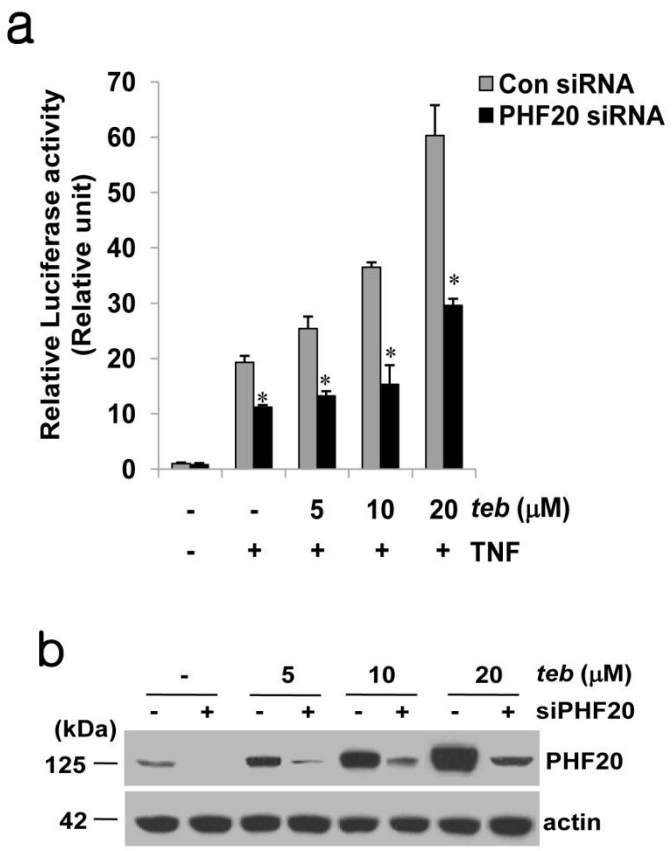
Supplementary Figure S3



Supplementary Figure S3: Expression and correlation of PHF20 with NF-κB activity and phosphorylation levels of p65 in *teb*-inducible cells. (a) HEK293 cells with stable *teb*-inducible expression plasmids of wild-type of PHF20 (WT) or mutant of PHF20 (S291A) were stimulated with tebufenozide (*teb*, 25μM) for indicated times. Whole cell lysates were immunoblotted with indicated antibodies.**(b)** *Teb*-on PHF20 HEK293 cells were treated with the different concentrations of *teb* for 12 h, followed by TNF (15 ng/ml) for indicated times. Whole cell lysates were immunoblotted with indicated antibodies as in (a). **(c)** After transient transfection of NF-κB responsive luciferase reporter plasmid in *Teb*-on PHF20 HEK293 cells, cells were treated with *teb* as in (b). Luciferase assays were performed as described in Methods. Each columns shows mean ± S.E. of at least three

independent experiments. *$P < 0.05$, compared with TNF/DMSO-treated *teb*-on PHF20 HEK293 cells (Student's *t*-test)

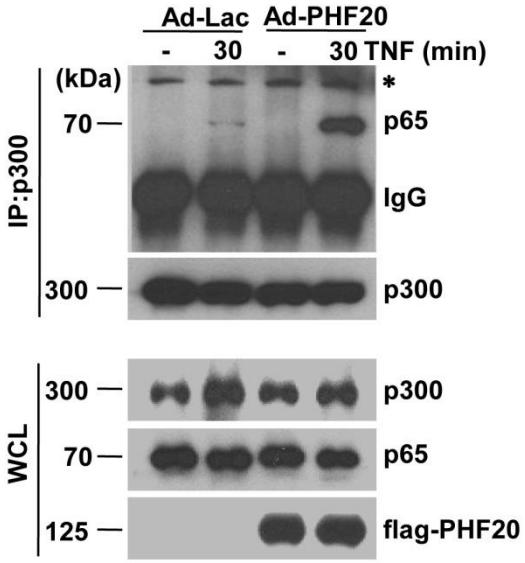
Supplementary Figure S4



Supplementary Figure S4:The effect of silencing PHF20 on the TNF-induced NF-κB activation depends on the PHF20 expression level.(a) To generate stable cells harbor different levels of PHF20 expression, *Teb*-on PHF20 HEK293 cells were treated with the indicated concentrations of *teb.* After 12 h, cells were transiently transfected with either scramble siRNA(150 pmol) or PHF20siRNA (150 pmol) along with p2xNF-κB-Luc and pRSV-β-gal for 24 h, and then further treated with TNF (15 ng/ml) for 6h. Luciferase assays were performed as described in Methods, and the activity of each sample was normalized according to β-galactosidase activity. Each columns shows mean ± S.E. of at least three independent experiments. *$P<0.05$, compared with control siRNA-transfected

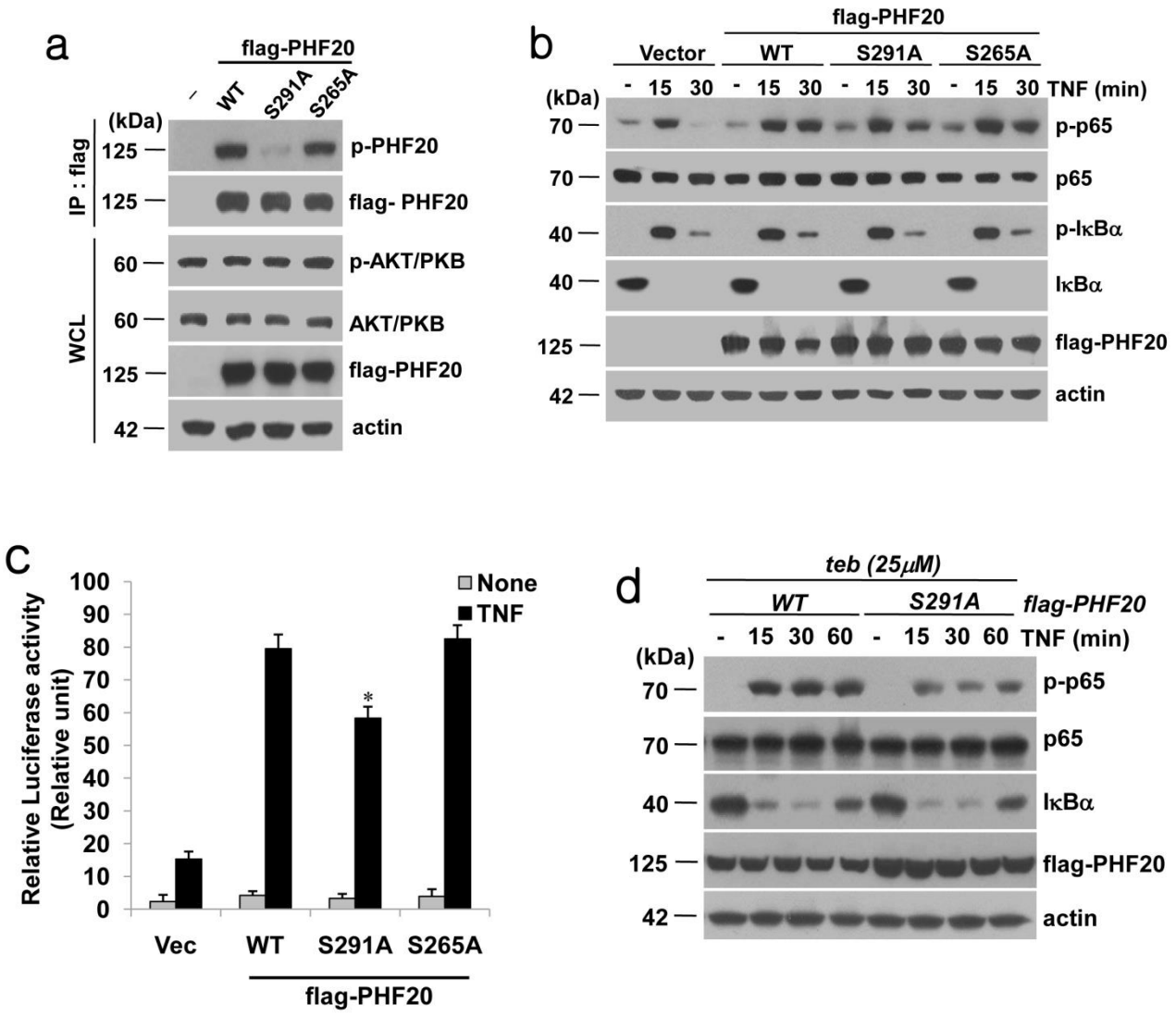
group (Student's *t*-test). **(b)** Expression levels of PHF20 in each samples were confirmed by immunoblotting with anti-PHF20 antibody.

Supplementary Figure S5



Supplementary Figure S5:PHF20 enhances TNF-induced association between endogenous p65 and p300. HeLa cells were infected with either rAd-Lac or rAd-PHF20 for24 h, and then cells were treated with TNF for 30 min. After whole cell extracts from each sample were subjected to immunoprecipitation with anti-p300 antibody, immunoprecipitatedp65 was detected by immunoblotting with anti-p65 antibody (top first row).Whole cell lysates were also immunoblotted with indicated antibodies (bottom). The asterisk indicates nonspecific bands for anti-p300 antibody.

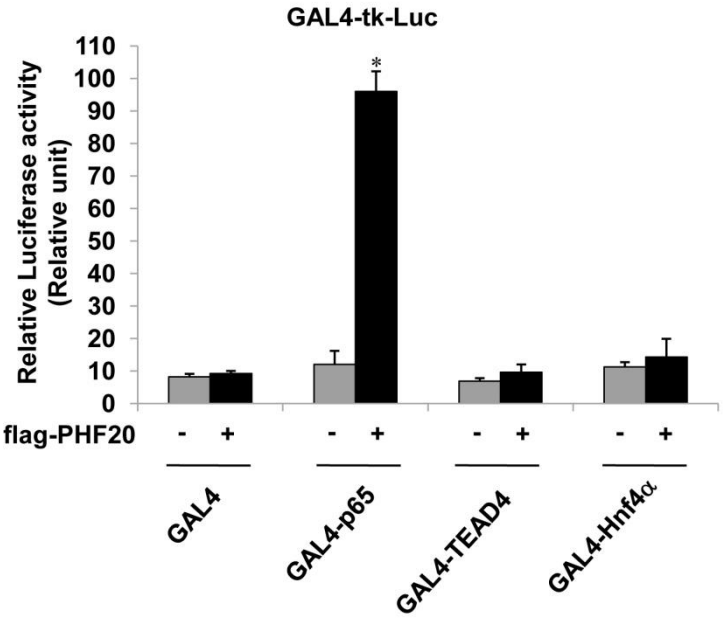
Supplementary Figure S6



Supplementary Figure S6: Phosphorylation at Ser291 partially functions for PHF20-mediated persistant p65 phosphorylation after TNF treatment.(a) HEK293 cells were transfected with indicated various forms of flag-tagged PHF20 for 48h and subjected to immunoprecipitation with anti-flag antibody. Immunoprecipitates and WCL were analyzed by immunoblotting as indicated. **(b)**HEK293 cells were transfected as in (a), and then

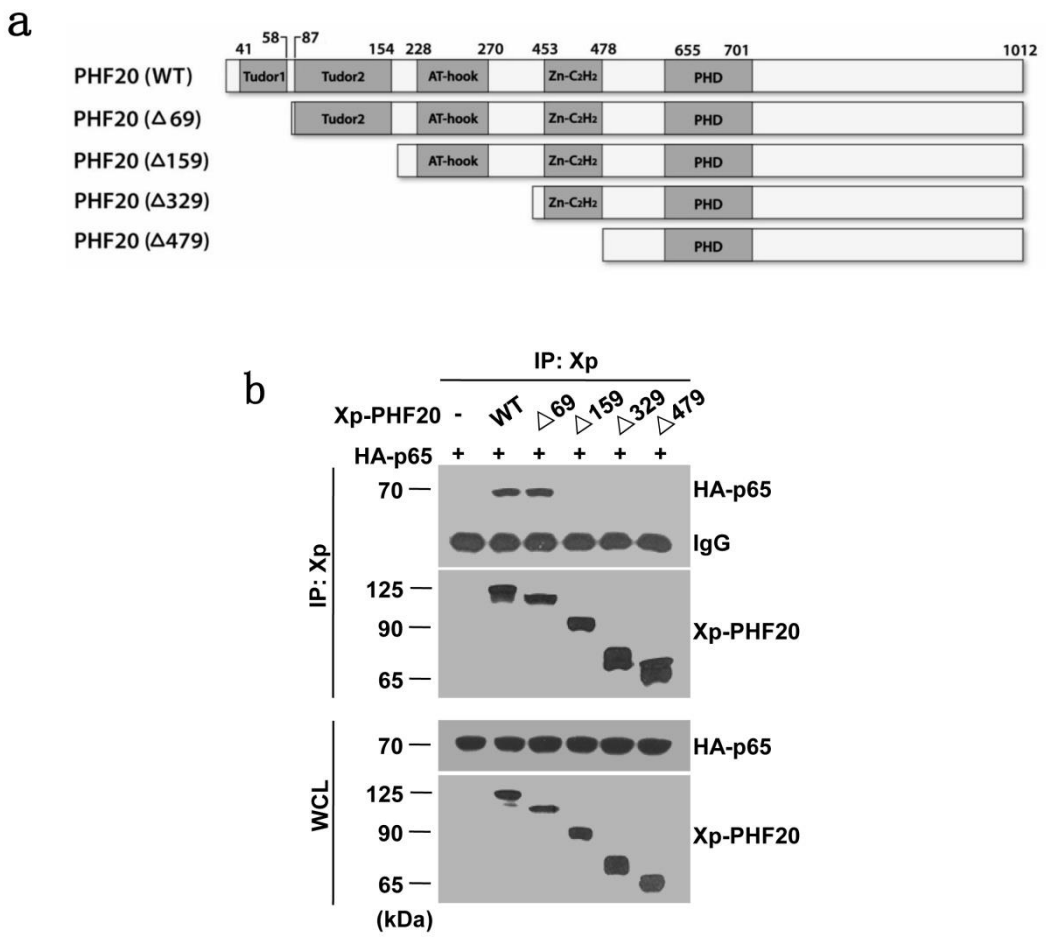
treated TNF (15 ng/ml) for indicated times. WCL were analyzed by immunoblotting as indicated. **(c)**HEK293 cells were co-transfected with indicated various forms of flag-tagged PHF20 along with NF-κBresponsiveluciferase reporter plasmid as in (c), and then treated TNF (15 ng/ml) for 6h. Luciferase assays were performed as described in Methods. *$P<0.05$, compared with TNF-treated group in wild-type PHF20-overexpressed cells (mean ± S.E. of triplicates, Student's *t*-test). **(d)***Teb*-on WT and mutant (S291A) PHF20 HEK293 cells were incubated with *teb,* (25μM) for 12h, and then further treated with TNF for indicated times. WCL were analyzed by immunoblotting as indicated.

Supplementary Figure S7



Supplementary Figure S7:PHF20 selectively enhances p65-mediated transcriptional activity.HEK293 cells were co-transfected with GAL4-tk-Luc and indicated GAL4 expression constructs with or without flag-PHF20 plasmid for24 h. Luciferase assays were performed as described in Methods. *$P<0.05$, compared with GAL4-p65 only-transfected group (mean ± S.E. of triplicates, Student's *t*-test).

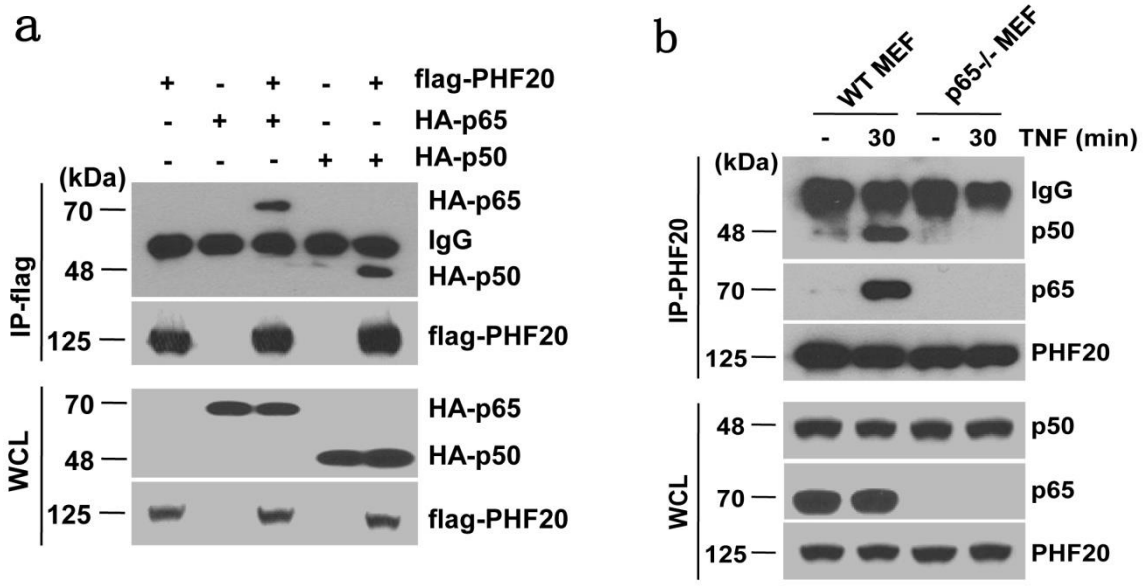
Supplementary Figure S8



Supplementary Figure S8:PHF20 interacts with p65 via its tudor 2 domain.(a) Deletion mutants of PHF20 used in domain mapping experiments. Numbers in parentheses indicate amino acids included in construct. **(b)** HEK293 cells were cotransfected with plasmids expressing HA-tagged p65 and Xp-tagged various PHF20 mutants. PHF20-p65 interaction was determined by immunoprecipation with anti-Xp antibody and followed by immunoblotting with the indicated antibodies (top first row). The efficacy of immunoprecipitants (top second row) and expression levels of the

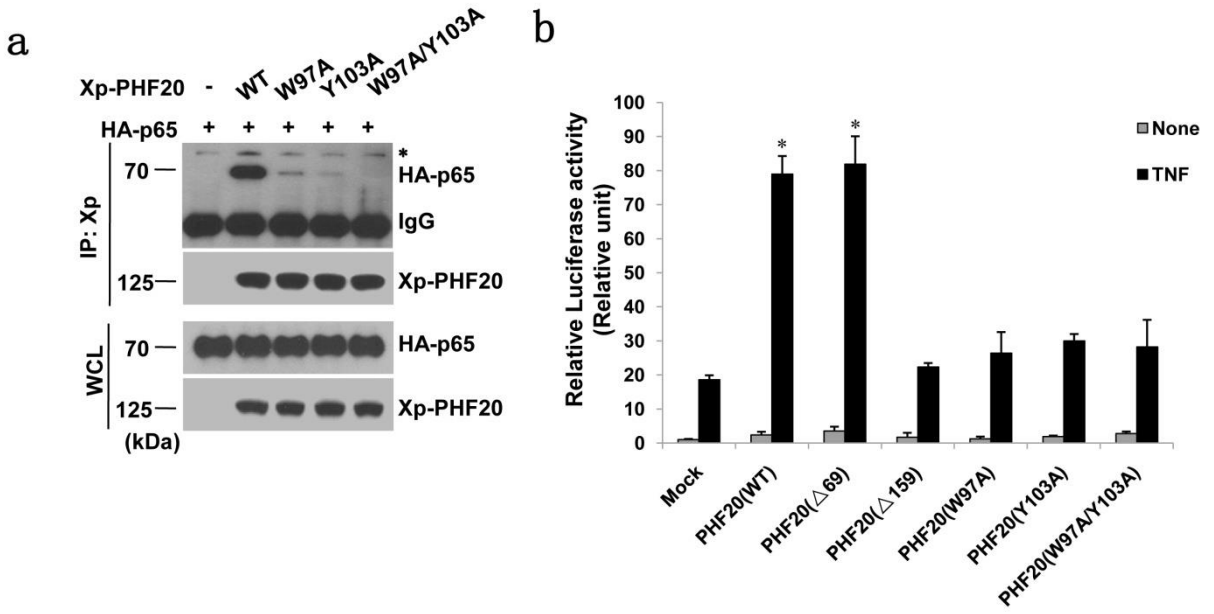
transfected plasmidsin the whole cell lysates (bottom) were analyzed by immunoblotting with anti-HA and anti-Xp antibodies, respectively.

Supplementary Figure S9



Supplementary Figure S9:PHF20 interacts with p65/p50 heterodimer through the selective association with p65. (a)HEK293 cells were co-transfected with flag-tagged PH F20 and HA-tagged p65 or p50 plasmids for 24h, as indicated. Afterimmunoprecipitation withanti- f l a g antibody, immunoprecipitants were detected by immunoblotting with indicated antibodies. The efficiency of immunoprecipitants andexpression levels of thetransfected plasmids were analyzed by immunoblotting with the indicated antibodies. **(b)** Wild type and p65-/- MEFs were treated with TNF (15 ng/ml) for 30 min. Cells were lysed and immunoprecipitated with anti-PHF20 antibody and immunoblotting was performed with antibodies against p50, p65 and PHF20.

Supplementary Figure S10



Supplementary Figure S10:The methyllysine binding cage in PHF20 Tudor 2 domain plays a critical role for the upregulation of NF-κB activity through its interaction with p65.(a) HEK293 cells were co-transfected with HA-tagged p65 along with Xp-tagged wild type and indicated mutants of full length PHF20 plasmids for 24h, as indicated. Afterimmunoprecipitation withanti-Xp antibody, immunoprecipitants were detected by immunoblotting with indicated antibodies (top). The efficiency of immunoprecipitants andexpression levels of thetransfected plasmids were analyzed by immunoblotting with the indicated antibodies (bottom). The asterisk indicates nonspecific bands for anti-HA antibody. **(b)**HEK293 cells were co-transfected with indicated wild type and PHF20 mutants along with p2xNF-κB-Luc and pRSV-β-gal. Luciferase assays were performed as described inMethods, and the activity of each sample was normalizedaccording to β-galactosidase activity. **P*< 0.05, compared with TNF-treated group in mock-transfected cells (mean ± S.E. of triplicates, Student's *t*-test).

Supplementary Table S1

SupplementaryTable S1. Sequences of primers for quantitative real-time PCR used in this study

Gene Name	**Sense**	**Antisense**
TNF	5'-CGGACTCCGCAAAGTCTAAG-3'	5'-ACGGCATGGATCTCAAAGAC-3'
MnSOD	5'-AACGCGCAGATCATGCA-3'	5'-CTCCCAGTTGATTACATTC-3'
ICAM-1	5'-AGACCTTAGCGCGGTGTAGA-3'	5'-AGTAGCAGAGGAGCTCAGCG-3'
MCP-1	5'-TCCCAAAGAAGCTGTGATCTTCA-3'	5'-TGCTTGTCCAGGTGGTCCAT-3'
Bcl-xL	5'-GAGGCAGGCGACGAGTTTGAA-3'	5'-GGGGTGGGAGGGTAGAGTGGA-3'
CCL-20	5'-TGATGTCAGTGCTGCTACTC-3'	5'-ATGTCACAGCCTTCATTGGC-3'
A20	5'-GCACACTGTGTTTCATCG-3'	5'-GGCATACATTGCTTGAAC-3'
xIAP	5'-ACGGATCTTTACTTTTGGGAC-3'	5'-CACCCTGGATACCATTTAGCAT-3'
PHF20	5'-GGGAAAGTGAAAGCATTGGA-3'	5'-ATCTGGGTTGGTGGTCACAT-3'

Supplementary Methods

Plasmids and adenovirus construction. Xp-tagged and flag-tagged full length PHF20 was prepared by amplifying the cDNA library in-frame with corresponding primers into pcDNA 3.1C (Invitrogen). Plasmids for flag-p65 WT and mutant forms (K218A, K221A and K218/221A) were prepared as previouslydescribed[32]. Gal4-p65plasmid encoding full-length p65 subunit of NF-κB fused to the Gal4 DNA-binding domain.Myc-p300 was from Addgene (NO.30489).GAL4-TEAD4 and GAL4-Hnf4αwas provided by Minho Shong (Chungnam National University, Daejeon, Korea). HA-HDAC3 was provided by Kyoon Eon Kim (Chungnam National University, Daejeon, Korea).Flag-PP2Ac was provided by Daniel Krappmann(German Research Center for EnvironmentalHealth,Neuherberg, Germany). Flag-PP1 was provided by ZengqiangYuan(Chinese Academy of Sciences, Beijing,China).Flag-WIP1 was from VinayTergaonkar (National Universityof Singapore, Singapore). Flag-PP4 was provided by Jong-Ik Hwang(Seoul National University, Seoul, Korea). Adenoviral expression vector for wild type PHF20 and LacZ wasprepared by using Adenoviral Expression Kit (Invitrogen) according tothe manufacturer' instruction. Deletion mutants (PHF20-△69, -△159, -△329, and -△479) were generated by standard PCR methods. The point mutants at Ser 265 (PHF20-S265A), Ser 291 (PHF20-S291A), Trp 97 (PHF20-W97A) and Tyr 103 (PHF20-Y103A) were created byusing the QuickchangeTM site-directed mutagenesis kit (Stratagene)with pENTR-Flag-PHF20 orpcDNA 3.1-Xp-PHF20 using wild-type as template. All constructs were amplifiedby PureHelixTMFast-n-Pure Plasmid Kit (NanoHelix, South Korea) andconfirmed by automated DNA sequencing. Sequences of the mutagenicoligonueotides are available upon request.

Cell culture, adenovirus infection and transfection.HEK293 cells stably expressing the IL-1 receptor and toll-like receptor4 (293/IL-1R/TLR4) were kindly donated by Dr. Xiaoxia Li (Lerner Research Institute, Cleveland Clinic Foundation, USA). Wild-type (WT) and p65 null(p65-/-) MEFs cells were provided by Dr. Zheng-gang Liu. HeLa cells, D54 cells and HEK293 cells were obtained from ATCC (Manassas, VA, USA). All of the cell lines were maintained in Dulbecco's modifiedEagle's medium (DMEM; Invitrogen) supplementedwith 10% fetal bovine serum, 2 mmol/l of glutamine,100 units/ml of penicillin and 100 mg/ml of streptomycin ina 5% CO_2 atmosphere at 37°C. For the knockdown experiments, cells were transfected with 50 pmol of human PHF20 (Santa Cruz, SC-76117)- or human PP2Ac (Santa Cruz, SC-43509)-specificsiRNAusing Lipofectamine2000 (Invitrogen Life Technologies), following themanufacturer's protocol. Alternatively, HeLa were infected with recombinant virus with the titers of 200 PFU per cells obtained PHF20- or LacZ-pAd/PL-DEST-transfected 293A Phoenix packaging cells.