

SUPPLEMENTARY MATERIAL

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The polyglutamylase activity of TLL4 is negatively regulated by the NIMA family kinase NEK5

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Supplementary methods:

Reverse Transcriptase PCR (RT-PCR)

RNA from Hek293T and hNEK5 depleted in Hek293T was extracted with TRIzol® (Invitrogen) and reverse transcribed utilizing SuperScript® III Reverse Transcriptase (Invitrogen). Quantitative RT-PCR reactions were performed following manufacture's instructions for Taq High Fidelity (Invitrogen). Specific pairs of primer for NEK5 were sense 5' GGAATTCGAGAATCTTATTCCCAAAT ATTT 3' and antisense 5' AATCTCGAGGTCGACTCACATCAGGATGAGCAC 3', and for α -tubulin were sense 5' GGATCCAAGCTTAGATGCGT GAGTGCATCTCCATCC 3' and antisense 5' CTGCAGGTCGACTTAGTATT CCTCTCCTTCTTCCTC 3. Different amplification cycles were analyzed with 30, 35 and 40 cycles and samples were resolved on 1% Agarose gel stained with Ethidium Bromide.

In vitro Polyglutamylation Assay

Control or silenced cells were transfected with TTLL4 or TTLL5. The cell extracts were used for the determination of polyglutamylase activities as previously described ^(16,53). Briefly, cells were lysed in MEM buffer (50 mM MES/NaOH, pH 6.8, 2 mM EGTA, 1 mM MgCl₂) complemented with 0.2% NP-40 and protease inhibitors. The cell extract was incubated with the reaction mixture (50mM Tris-HCl [pH 9.0], 400 mM ATP, 2.4 mM MgCl₂, 500 μM DTT, 4,6 μM taxol, 8 mM.L-[3H]-glutamate (45–55 Ci/mmol, GE Healthcare) and 0.2 mg/ml tubulin and then were incubated at 30 °C for 2 hr. Tubulins were prepared from adult mouse brains as described (8). The samples were separated in a SDS-PAGE gel, blotted onto nitrocellulose membrane, and stained with Ponceau S. Quantification of the radioactivity incorporated was performed by scintillation counting, as described before ⁽⁵³⁾.

SUPPLEMENTARY FIGURES E FIGURE LEGENDS

1 80

TTLL4	RRLLRWKMST	VTPNIVKQTI	GRSHF-KISK	RNDDWLGCW-	GHHMKSPGFR	SIREHQKLNH	FPGSFQIGRK	DRLWRNLSRM
TTLL5	RYHLSYKIVR	TDSRLVRSIL	TAHGFEHVHP	SSTDYNLMT	GSHLKPFLLR	TLSEAQKVNH	FPRSYELTRK	DRLYKNIIRM
TTLL7	KGIITANVAG	TKFEIVRLVI	DEMGFMKTPD	EDETSNLIWC	DAAVQQEKIT	DLQNYQRINH	FPGMGEICRK	DFLARNMTKM
Consensus	r.l.l.k...	t...iVr..i	...gF.k...	...d.nl.W.	g.h.k....r	.l.#.Qk.NH	FPgs.#i.RK	DrL.rN..rM

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81
TLL4  QSRFGKKEFS FFPQSFILPQ DSKLLRKAW E S----SSRQK WIVKPPASAR GIGIQVIHKW SQLPKRRPLL VQRYLHKPYL
TLL5  QHTHGFKAFH ILPQTFLLPA EYAEFCNSY- S----KDRGP WIVKPVASSR GRGVYLINNP NQISLEENIL VSRYINNPLL
TLL7  IKSRPM-DYT FVPRTWIFPS EYTQFQNYVK ELKKKRKQKT FIVKPANGAM GHGISLIRNG DKVPSQDHLI VQEYIEKPFL
Consensus q...g.k.% f.Pqtfilp #y..f.n... s.....r.. wIVKP.asar G.G!.lI.n. .q.p....ll VqrYi.kP.L

161
TLL4  ISGSKFDLRI YVYVTSYDPL RIYLFSDGLV RFASCKY-SP SMKSLSNKFM HLTNYSVNKK NTEYQANAD- ETACQGHKWA
TLL5  IDDFKFDVRL YVLVTSYDPL VIYLYEEGLA RFATVRY-DQ GSKNIRNQFM HLTNYSVNKK SGDYVSCDDP EVEDYGNKWS
TLL7  MEGYKFDLRI YILVTSCDPL KIFLYHDGLV RMGTEKYIPP NESNLTQLYM HLTNYSVNKH NERFERN--- ETEDKGSKRS
Consensus i.g.KFDlri Y!lVTSyDPL .I%L%.#GLv Rfat.ky..p ..knl.#.%M HLTNYSVNKK n...%..n.d. Eted.G.Kws

241
TLL4  LKALWNYLSQ KGINSDAIWE KIKDVVVKTI ISSEPYVTNL LKLYVR---- -RPYSHEL F GFDIMLDENL KPWVLEVNIS
TLL5  MSAMLRYLKQ EGKDTTALMA HVEDLIIKTI ISAELAIATA CKTFVP---- -HRSSCFELY GFDVLIDNTL KPWLLEVNLS
TLL7  IKWFTEFLQA NQHDTVTFWS DISELVVKTL IVAEPHVLHA YRMCRPGQPP GSESVCFEVL GFDILLDRKL KPWLLEINRA
Consensus .ka...%L.q .g.#.ta.w. .!.#l!!Kti IsaEp.!...a .k..vp.... ...sscfel. GFD!$lD..L KPWLLE!N.s

321
TLL4  PSLHSNSPLD ISIKGQMIRD LLNLAGFVLP NMEDIIS
TLL5  PSLACDAPLD LKIKASMISD MFTVVGFVCQ DPAQRTS
TLL7  PSFGTDQKID YDVKRGVLLN ALKLLNIRTS DKRKNLA
Consensus PSl...#.plD ..!K..mi.# .l.l.gfv.. #.....s

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Supplementary Figure 1: Alignment of TLL domain of TLL4, TLL5 and TLL7. The TLL domain of TLL4 (599 – 942 a.a.), TLL5 (62 – 407 a.a.) and TLL7 (38 – 390 a.a.) was made using the Multalin version 5.4.1. Some of residues identified as phosphorylated in TLL4 is conserved between TLL4, TLL5 and TLL7. The residues phosphorylated in TLL4 and its correspondent residues for TLL5 and TLL7 are bolded and underlined. Consensus symbols: ! is anyone of IV; \$ is anyone of LM; % is anyone of FY; # is anyone of NDQEBZ.

1 80

TLL4_mouse MASAGTEHYS IGLRRGNSFK QRHPSGTVSA SPSEKPSEVK VWSQAHQQVK PIWKLEKKHV GTLSAGLGTS FLGVPSQPAY
TLL4_human MASAGTQHYS IGLRQKNSFK QSGPSGTVPA TPPEKPSEGR VWpQAHQQVK PIWKLEKKQV ETLsAGLGpG LLGVPPQPAY
Consensus MASAGT#HYS IGLRrgNSFK QrgPSGTVpa sPpEKpSEgr VWpQAHQQVK PIWKLEKKqV eTLsAGLGpg lLGVPpQPAY

81 160

TLL4_mouse FLCpSTLCSS GTTAVIAGHS NPCYLQSLPN LFSNTLLYRR TNVRQKPYQQ LESFCLRSSP SEKRsfSLPQ KGLPVSVTAN
TLL4_human FFCpSTLCSS GTTAVIAGHS SSCYLHSLPD LFNSTLLYRR SsyRQKPYQQ LESFCLRSSP SEKspFSLPQ KSLPVSLTAN
Consensus FLCpSTLCSS GTTAVIAGHS npcYLqSLP# LFnnTLLYRR snyRQKPYQQ LESFCLRSSP SEKrpFSLPQ KglPVSlTAN

161 240

TLL4_mouse KATSSTVFPM AQPmATSPtD PYLSLAAAGE NPSRkSLASA ISGKIAsPLS --YKpMLNNN SFMRPNSTKV PLSQATDGLK
TLL4_human KATSSMVFsm AQPmAsSStE PYLCLAAAGE NPSGKSLASA ISGKIpsPLS SsyKpMLNNN SFMwPNSTpV PLLQTtQGLK
Consensus KATSSmVFpM AQPmAsSpT# PYLclAAAGE NPSrkSLASA ISGKIAsPLS ..YKpMLNNN SFMrPNSTkv PllQAt#GLK

241 320

TLL4_mouse PVSSPKIQPV SWHHSgGTGD CVPQPGDHKV PQNIATVLDd VTAPITPSIP STLNIStASV TSSQCSQSNF RMEaHP--CG
TLL4_human PVsPPKIQPV SWHHSgGTGD CAPQpVDHKV PKSIGtVPAD ASAHIALStA SshDTStTSV ASSWYNrNNL AMRAEPLSCA
Consensus PVSpPKIQPV SWHHSgGTGD CapQpGDHKV PqniAtVldd asAhIalsia Ssh#iStasV assqcnrnNl aMrAeP..Ca

321 400

TLL4_mouse LDENpDSQSA TKEVHFTEAV RKLAEKGLek MPRQGYQFEQ ACfVNpSFQW GLLNRSRRWk P-LMGQRFPQ EDIGLDSAIL
TLL4_human LDDSSDSQDP TKEIRfTEAV RKLtARGFek MPRQGCQLEQ SSfLNpSFQW NVLNRSRRWk PPAVNQQFPQ EDAGSVRRVL
Consensus LD#npDSQda TKE!rfTEAV RKLaarGLEK MPRQGCQLEQ acfLNpSFQW nllNRSRRWk P.amnQrFPQ EDaGldra!L

401 480

TLL4_mouse PGTSdTLGLD STVFCTKRIS IHLLASHVHG LNPSPACGSa VDPQVLGEDR APVPPSSLQP LGVAEVATRL SSVHLDQPGK
TLL4_human PGASdTLGLD nTVFCTKRIS IHLLASHaSG LNHNPACESV IDSSAFGEgK APGPPFP-QT LGIANVATRL SSIQLGQSEK
Consensus PGASdTLGLD nTVFCTKRIS IHLLASHahG LNhnPACeSa !DpqalGEDr APgPPfp.Qp LG!A#VATRL SS!qLdQpeK

481 560

TLL4_mouse E-PEEAkDLN SCTKGGGSAT DLQPNQVEPE DTEDELGDGL EDSCSHDENE EEeGDSECSs LSVVSPSESv ALISRNCVDL
TLL4_human ERPEEAReLD SsDRDISSAT DLQPDQAEtE DTEeELVDGL EDCCSRDENE EEeGDSECSs LSAVSPSESv AMISRSCMEI
Consensus E.PEEAr#L# ScdrdggSAT DLQP#QaEpe DTE#ELgDGL EDcCSrDENE EEeGDSECSs LSAvSPSESv A\$ISRnCm#i

561 640

TLL4_mouse MSKSLPNHEK VVRPALIYSL FPNVTPTIYF GTRDERVEKL PWEQRLLLRW KMSTVTPNIV KQTIGRSHFK ISKRNDDDLWLG
TLL4_human LTKPLSNHEK VVRPALIYSL FPNVPPTIYF GTRDERVEKL PWEQRKLLRW KMSTVTPNIV KQTIGRSHFK ISKRNDDDLWLG
Consensus \$skpLpNHEK VVRPALIYSL FPNVpPTIYF GTRDERVEKL PWEQRrLLRW KMSTVTPNIV KQTIGRSHFK ISKRNDDDLWLG

641 720

TLL4_mouse CWGHMKSPG FRSIREHQKL NHFPGSFQIG RKDRLWRNLS RMQSRFGKKE FSFFPQSFIL PQDSKLLRKA WESSSRQKWI
TLL4_human CWGHMKSPS FRSIREHQKL NHFPGSFQIG RKDRLWRNLS RMQSRFGKKE FSFFPQSFIL PQDAKLLRKA WESSSRQKWI
Consensus CWGHMKSPg FRSIREHQKL NHFPGSFQIG RKDRLWRNLS RMQSRFGKKE FSFFPQSFIL PQDaKLLRKA WESSSRQKWI

721 800

TLL4_mouse VKPPASARGI GIQVIHKWSQ LPKRRPLLQV RYLHKPYLIS GSKFDLRIYV YVTSYDPLRI YLFSGLVRF ASCKYSPSMK
TLL4_human VKPPASARGI GIQVIHKWSQ LPKRRPLLQV RYLHKPYLIS GSKFDLRIYV YVTSYDPLRI YLFSGLVRF ASCKYSPSMK
Consensus VKPPASARGI GIQVIHKWSQ LPKRRPLLQV RYLHKPYLIS GSKFDLRIYV YVTSYDPLRI YLFSGLVRF ASCKYSPSMK

801 880

TLL4_mouse SLSNKFHMLT NYSVNKKNT YQANADETAC QGHKWALKAL WNYLSQKGIN SDAIWEKIKD VVVKTIISSE PYVTNLLKLY
TLL4_human SLGNKFHMLT NYSVNKKNA YQANADEMAC QGHKWALKAL WNYLSQKGVN SDAIWEKIKD VVVKTIISSE PYVTSLLKMY
Consensus SLgNKFHMLT NYSVNKKNA YQANADEmAC QGHKWALKAL WNYLSQKG!N SDAIWEKIKD VVVKTIISSE PYVTnLLK\$Y

881 960

TLL4_mouse VRRPYSCHEL FGFDIMLDEN LKPWVLEVNI SPSLHNSSPL DISIKQMIR DLLNLAGFVL PNMEDIISS SSPSSSSGSS
TLL4_human VRRPYSCHEL FGFDIMLDEN LKPWVLEVNI SPSLHSSSPL DISIKQMIR DLLNLAGFVL PNAEDIISS --PSSCSST
Consensus VRRPYSCHEL FGFDIMLDEN LKPWVLEVNI SPSLHNSSPL DISIKQMIR DLLNLAGFVL PNAEDIISS . . .PSScSgSs

961 1040

TLL4_mouse TSLPSSPRDK CQMTPEHFTA QMKMKAYYLT QKIPDQDFYA SVLDVLTDPDD VRVLVEMEDE FSRRGQFERI FPSRISSRYL
TLL4_human TSLPTSPGDK CRMAPEHVTA QMKMKAYYLT QKIPDQDFYA SVLDVLTDPDD VRILVEMEDE FSRRGQFERI FPSHISSRYL
Consensus TSLPsSPrDK CrMAPEHFTA QMKMKAYYLT QKIPDQDFYA SVLDVLTDPDD VR!LVEMEDE FSRRGQFERI FPSrISSRYL

1041 1120

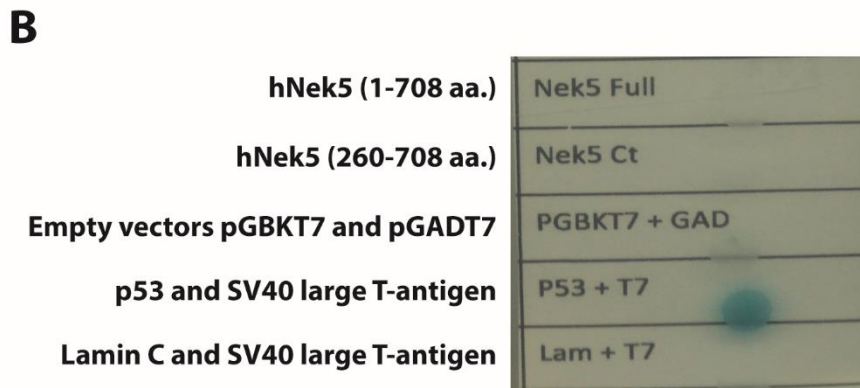
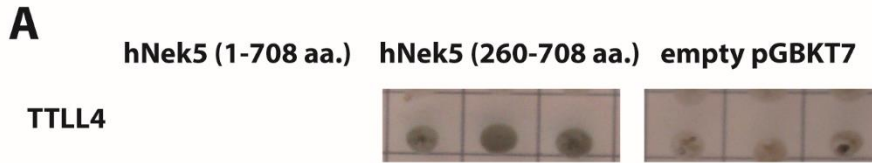
TLL4_mouse RFFEQPRYFN ILTTQWEQKY HGNKLGVDL LRNWYKGFH TGIVSDSAPL WSLPTSLMTT SKGDGTPNSA SKSR-----K
TLL4_human RFFEQPRYFN ILTTQWEQKY HGNKLGVDL LRSWCYKGFH MGVVSDSAPV WSLPTSLMTT SKDDVILNAF SKSETSKLGK

Consensus RFFEQPRYFN ILTTQWEQKY HGnKLKGVDL LRnWCYKGFH mG!VSDSAPl WSLPTSL\$Ti SKdDgilNaa SKSr.....K

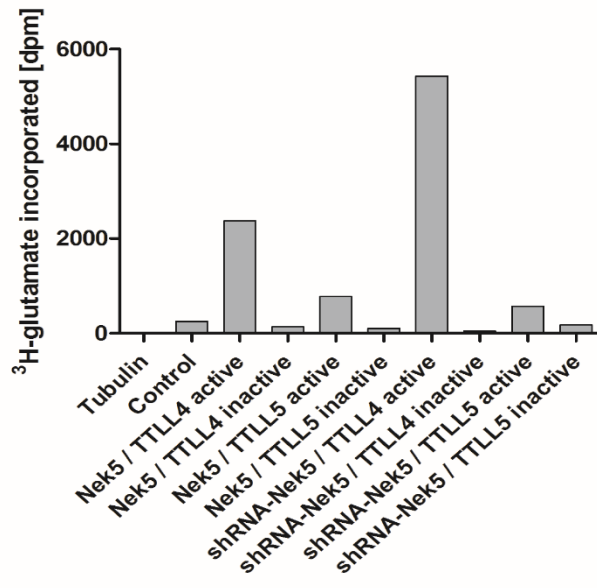
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1121 1200
TLL4_mouse KSASEGTTLS SEDRSTPKSK KSQAGLSPIS RKTLSRSNE NTSKQSKRST PGLPVLKYSQ QSSRLSAASA SQSVTDSRLT
TLL4_human QSSCEVSLLL SEDGTPKSK KTQAGLSPYP QKPSSSKDSE DTSKEPSLST QTLPVIKCSG QTSRLSASST FQSISDS-LL
Consensus qSacEgslLl SEDrsTPKSK KsQAGLSPIp rKplSSrdnE #TSK#pkrST qgLPViKcSG QsRLSAaSa fQS!sDS.Ll
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1201 1204
TLL4_mouse AVSS
TLL4_human AVSP
Consensus AVSp
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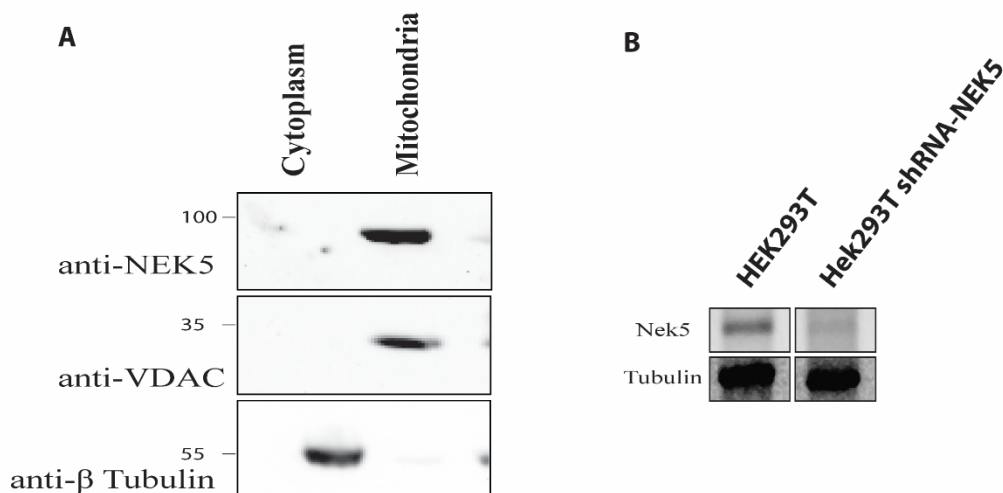
Supplementary Figure 2: Alignment of TLL4 from human and mouse. The alignment of the full sequence of TLL4 from human and mouse was made using the Multalin version 5.4.1. The residues identified as phosphorylated in TLL4 from mouse are conserved with human sequence and are bolded and underlined. Consensus symbols: ! is anyone of IV; \$ is anyone of LM; % is anyone of FY; # is anyone of NDQEBZ.



Supplementary Figure 3: Yeast Two-Hybrid controls. **A)** The interaction of TTL4 with the C-terminus. **B)** Positive controls: pGBKT7-53 and pGADT7-T encode fusions between Gal4 DNA-BD and murine p53 and Gal4 DNA-AD and SV40 large T-antigen ^(54, 55). Negative controls: pGBKT7-Lam encodes fusion of Gal4 DNA-BD and the human Lamin C and provides a control for interaction between unrelated proteins ^(56, 57). Auto-activation controls: NEK5 full-length (1-708 a.a.) and NEK5 C-terminal (260-708 a.a.) encode fusion of Gal4 DNA-BD to check its ability to activate transcription on its own.



Supplementary Figure 4: In vitro experiment for polyglutamylase activity. Tubulins from mouse brain were subjected to in vitro polyglutamylation assays with extracts from NEK5 and shRNA-NEK5 cells expressing the respective TTLL-EYFP proteins.



Supplementary Figure 5: Cell fractionating controls and silencing of NEK5 in Hek293T. A) Hek293T cells were submitted to subcellular fractionation and the

proteins analyzed by Western blot. VDAC and Tubulin were used as mitochondrial and cytoplasmic fraction marker respectively. **B)** HEK293T cells were silenced for NEK5 (shNEK5) using NEK5 shRNA (h) Lentiviral Particles (Santa Cruz Biotechnology, Inc.). The expression of mRNA was analyzed by RT-PCR using the PCR pairs of primer sequences for NEK5 (Sense: 5'-GGATCCAAGCTTAGATGCGTGAGTGCATCTCCATCC-3') and for α -tubulin (Sense: 5'-CTGCAGGTCGACTTAGTATTCCTCTCCTTCTTCCTC-3').

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