## SUPPLEMENTARY MATERIAL

corresponding to:

Analysis of *NUAK1* and *NUAK2* expression during early chick development reveals specific patterns in the developing head

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gNUAK1    MEGAEAAACGSSSSPAQPRCSSGGEAAAAAMEEPLGEAASPGVAATAAGPRKQQGVKRHH  60
hNUAK1    MEGAAAPVAG--DRPDLGLGA-PG--------SPREAVA--GATAA-LEPRKPHGVKRHH
mNUAK1    MEGAAVSAAG--DGPAVETGL-PG--------SPLEAVA--GATAAFVEPRKPHGVKRHH
gNUAK2    MERAAVGFGG----------------------TSLVE----GFIKSPRPLMKKQAVKRHH
hNUAK2    MESLVFARRSGPTPSAAELA------------RPLAE----GLIKSPKPLMKKQAVKRHH
mNUAK2_A  MESVALLQRPSQAPSASALASESA--------RPLAD----GLIKSPKPLMKKQAVKRHH
mNUAK2_B  MESVALLQRPSQAPSASALASESA--------RPLAD----GLIKSPKPLMKKQAVKRHH
          **      .       :      .    *  :  * * :.*****

gNUAK1    HKHNLKHRYELQETLGKGTYGKVKRAIERFSGRVVAIKSIRKDKIKDEQDMVHIRREIEI  120
hNUAK1    HKHNLKHRYELQETLGKGTYGKVKRATERFSGRVVAIKSIRKDKIKDEQDMVHIRREIEI
mNUAK1    HKHNLKHRYELQETLGKGTYGKVKRATERFSGRVVAIKSIRKDKIKDELDMVHIRREIEI
gNUAK2    HKHNLKHRYFLETLGKGTYGKVKRARER-SGRLVAIKSIRKDRIKDEQDLIHIRREIEI
hNUAK2    HKHNLRHRYEFLETLGKGTYGKVKRARES-SGRLVAIKSIRKDKIKDEQDLMHIRREIEI
mNUAK2_A  HKHNLRHRYEFLETLGKGTYGKVKRARES-SGRLVAIKSIRKDKIKDEQDLLHIRREIEI
mNUAK2_B  HKHNLRHRYEFLETLGKGTYGKVKRARES-SGRLVAIKSIRKDKIKDEQDLLHIRREIEI
          ****.****: ********** .*  **.************.**** .**:*****

gNUAK1    MSSLSHPHITIYE--------VFENKDKIVIIMEYASKGELYDYISERRRLSERETRHF  180
hNUAK1    MSSLNHPHIISIYE--------VFENKDKIVIIMEYASKGELYDYISERRRLSERETRHF
mNUAK1    MSSLNHPHIISIYE--------VFENKDKIVIIMEYASKGELYDYISERRRLSERETRHF
gNUAK2    MSSLNHPHILAVHE--------VFENSSKIVIVMEYASKGELYDYISEROLSEQEARHF
hNUAK2    MSSLNHPHILAIHE--------VFENSSKIVIVMEYASRGDLYDYISERQQLSEREARHF
mNUAK2_A  MSSLNHPHILAIHEVGRSRLVTVFENSKIVIVMEYASRGDLYDYISERPRLSERDARHF
mNUAK2_B  MSSLNHPHILAIHE--------VFENSSKIVIVMEYASRGDLYDYISERPRLSERDARHF
          ****.****:::*        ****..*:**:*******.*:****:.::***

gNUAK1    FRQIVSAVHYCHKNGVVHRDLKLENILLDDNFNIKIADFGLSNLYHKDKFLQTFCGSPLY  240
hNUAK1    FRQIVSAVHYCHKNGVVHRDLKLENILLDDNCNIKIADFGLSNLYQKDKFLQTFCGSPLY
mNUAK1    FRQIVSAVHYCHKNGVVHRDLKLENILLDDNCNIKIADFGLSNLYQKDKFLQTFCGSPLY
gNUAK2    FRQVVSAVYYCHKNGIVHRDLKLENILLDANGNIKIADFGLSNVYQQDKFLQHYCGSPLY
hNUAK2    FRQIVSAVHYCHQNRVVHRDLKLENILLDANGNIKIADFGLSNLYHQGKFLQHYCGSPLY
mNUAK2_A  FRQIVSALHYCHQNGIVHRDLKLENILLDANGNIKIADFGLSNLYHKGKFLQHFCGSPLY
mNUAK2_B  FRQIVSALHYCHQNGIVHRDLKLENILLDANGNIKIADFGLSNLYHKGKFLQHFCGSPLY
          ***:***::**:*  *:*************..*******:***.*.*:..:*****.*****

gNUAK1    ASPEIVNGRPYRGPEVDSWALGVLLYTLVYGTMPFDGFDHKNLIRQISSGEYREPTQTSD  300
hNUAK1    ASPEIVNGRPYRGPEVDSWALGVLLYTLVYGTMPFDGFDHKNLIRQISSGEYREPTQPSD
mNUAK1    ASPEIVNGRPYRGPEVDSWALGVLLYTLIYGTMPFDGFDHKNLIRQISSGEYREPTQPSD
gNUAK2    ASPEIINGRPYKGPEVDSWSLGVLLYILVHGTMPFDGHDYKTLVKQITSGDYREPTKLSD
hNUAK2    ASPEIVNGKPYTGPEVDSWSLGVLLYIILVHGTMPFDGHDHKILVKQISNGAYREPPKPSD
mNUAK2_A  ASPEIVNGKPYVGPEVDSWSLGVLLYIILVHGTMPFDGQDHKTLVKQISNGAYREPPKPSD
mNUAK2_B  ASPEIVNGKPYVGPEVDSWSLGVLLYIILVHGTMPFDGQDHKTLVKQISNGAYREPPKPSD
          *****:**.** ******:***** *:.********* * *:.**.:* ****.: **

gNUAK1    ARGLIRWMLMVNPERRATIEDIANHWWVNWGYKSSVCDCDAMRDSESPL----LARFIDW  360
hNUAK1    ARGLIRWMLMVNPDRRATIEDIANHWWVNWGYKSSVCDCDALHDSESPL----LARIIDW
mNUAK1    ARGLIRWMLMVNPDRRATIEDIANHWWVNWGYKSSVCDCDALPDSESPL----LARIIDW
gNUAK2    ACGLIRWLLMVNPTRRATLEDIATHWWVNWGYRVPVGEQELLRESSESP----LATVAEW
hNUAK2    ACGLIRWLLMVNPTRRATLEDVASHWWVNWGYATRVGEQEAPHEGGHPGSDSARASMADW
mNUAK2_A  ACGLIRWLLMVNPTRRATLEDVASHWWVNWGYTTGVGEQEALREGGHPSGDFGRASMADW
mNUAK2_B  ACGLIRWLLMVNPTRRATLEDVASHWWVNWGYTTGVGEQEALREGGHPSGDFGRASMADW
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gNUAK1    HHRSTGLQPETDTKMKCLSKPKGPE-----VTLERQRSLKKSKKENDIAQSIQEG----G  420
hNUAK1    HHRSTGLQADTEAKMKGLAKPTTSE-----VMLERQRSLKKSKKENDFAQSGQDA----V
mNUAK1    HHRSTGLQAEAEAKMKGLAKPGASE-----VVLERQRSLKKSKKENDFPQSGQDS----V
gNUAK2    LRRSSRPLFENGSKVRCFLKQHIPG-----VALERQRSLKKSKKENDVSHALQEGAA--A
hNUAK2    LRRSSRPLLENGAKVCSFFKQHAPGGGSTTPGLERQHSLKKSRKENDMAQSLHSDTADDT
mNUAK2_A  LRRSSRPLLENGAKVCSFFKQHVPGGGSTVPGLERQHSLKKSRKENDMAQNLQGDPAEDT
mNUAK2_B  LRRSSRPLLENGAKVCSFFKQHVPGGGSTVPGLERQHSLKKSRKENDMAQNLQGDPAEDT
          .**: :   :*:  :  *       .    **** .*****.****..:  :

gNUAK1    AENASKPTSKRPKGILKKRSNSEHRSHSAGFIEGVVSPVLPSAFKLEQELCRTGVAIKTV  480
hNUAK1    PESPSKLSSKRPKGILKKRSNSEHRSHSTGFIEGVVGPALPSTFKMEQDLCRTGVLLPSS
mNUAK1    PESPSKLSSKRPKGILKKRSNSEHRSHSTGFIEGIVSPALPSPFKMEQDLCRTAIPLPSS
gNUAK2    PENPSKSILKRPKGILKKRNSCE-------------------QKVP---IPLPTA
hNUAK2    AHRPGKSNLKLPKGILKKVSASAEGV-------------------QEDPPELSPIPAS
mNUAK2_A  SSRPGKSSLKLPKGILKKSSTSSGEV-------------------QEDPQELRPVPDT
mNUAK2_B  SSRPGKSSLKLPKGILKKSSTSSGEV-------------------QEDPQELRPVPDT
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gNUAK1    VEGEVAGKYGTKQSSLMPKKGILKKTQQRESGYYSSPERSESSELLDNNEETVNSDTSPG  540
hNUAK1    PEAEVPGKLSPKQSATMPKKGILKKTQQRESGYYSSPERSESSELLDSNDVMGSSIPSPS
mNUAK1    PEADMSGKLSLKQSATMPKKGILKKTQQRESGYYSSPERSESSELLDSNDVVISGGLSSP
gNUAK2    PGGDGVV------SVAVPKKGILKKPKRESGYYSSLECCESGDVLDAGSLDLEGNVFAD
hNUAK2    PGQAAP---------LLPKKGILKPRQRESGYYSSPEPSESGELLDAGDVFVSGDPKEQ
mNUAK2_A  PGQPVPA------VSLLPRKGILKSRQRESGYYSSPEPSESGELLDASDVFVSGDPVEQ
mNUAK2_B  PGQPVPA------VSLLPRKGILKSRQRESGYYSSPEPSESGELLDASDVFVSGDPVEQ
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gNUAK1    VTEPSRNGSYSHSCRRKGILKHNGKYSTSSTESALISPDTPTLEAMEEVVLPGDALPRSY  600
hNUAK1    PPDPARVTSHSLSCRRKGILKHSSKYSAGTMDPALVSPEMPTLESLSEPGVPAEGLSRSY
mNUAK1    PPDPARGTSHSLSCRRKGILKHSSRYSDGGTDPALTRPEMPTLESLSPPGVPSDGISRSY
gNUAK2    SP-TAERGPPVLPSSRKGILKHSSKYTSGAEPHSPPRQSFGCFDEVSLPKAPLAPRPR--
hNUAK2    KP-PQASG---LLLHRKGILKLNGKFSQTALELAAPT-TFGSLDELAPPR-PLARASR--
mNUAK2_A  KS-PQASG---LLLHRKGILKLNGKFSRTALEGTTPS-TFGSLDQLASSH-PAARPSR--
mNUAK2_B  KS-PQASG---LLLHRKGILKLNGKFSRTALEGTTPS-TFGSLDQLASSH-PAARPSR--
          .   .    ****** ...::   :          :   ::  :   *   .*

gNUAK1    SRPSSVISDDSILSSDSFDLLDLQENRPN--RQRIRSCVSAENFLQIQD--FEGLQNHPR  660
hNUAK1    SRPSSVISDDSVLSSDSFDLLDLQENRPA--RQRIRSCVSAENFLQIQD--FEGLQNRPR
mNUAK1    SRPSSIISDDSVLSSDSFDLLELQENRPA--RQRIRSCVSAENFLQLQD--FETPHNRPR
gNUAK2    --PPSAVSEDSILSTESFEQLDLPRPVPPGRSGAMRGCVSADSLLLLEEEEEVDEGRRLR
hNUAK2    --PSGAVSEDSILSSESFDQLDLPERLPE---PPLRGCVSVDNLTGLEEPPSEGPGSCLR
mNUAK2_A  --PSGAVSEDSILSSESFDQLDLPERLPE---TPLRGCVSVDNLRGLEQPPSEG----LK
mNUAK2_B  --PSGAVSEDSILSSESFDQLDLPERLPE---TPLRGCVSVDNLRGLEQPPSEG----LK
          *.. :*:**:**::**: *:*  . *     :*.***.::.  :::

gNUAK1    PQYLKRYRNRLGDSSFSLLADMDDVTQVYKKALEICNKLN  700
hNUAK1    PQYLKRYRNRLADSSFSLLTDMDDVTQVYKQALEICSKLN
mNUAK1    PQY----LKRLADSSFSLLTDMDDVTQVYKQALEICSKLN
gNUAK2    RWTVTHCPTALGESRFS-LESCDNVTEVHHRRAVAISMKLS
hNUAK2    RWR----QDFLGDSCFS-LTDCQEVTATYRQALRVCSKLT
mNUAK2_A  RWW----QESLGDSCFS-LTDCQEVTAAYRQALGICSKLS
mNUAK2_B  RWW----QESLGDSCFS-LTDCQEVTAAYRQALGICSKLS
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Supplementary Fig. S1. Amino acid sequence alignment for NUAK1 and NUAK2 as gNUAK1 [Gallus gallus]: XP_416310.2, hNUAK1 [Homo sapiens]: NP_055655.1, mNUAK1 [Mus musculus]: NP_001004363.1, gNUAK2 [Gallus gallus]: XP_417962.2, hNUAK2 [Homo sapiens]: NP_112214.1, mNUAK2 isoform A [Mus musculus]: NP_001181954.1 and mNUAK2 isoform B [Mus musculus]: NP_083054.1. Positions with a single, fully conserved residue (41.1 %) are indicated by an asterisk (*); conservative substitutions are indicated by dots under the sequences. Two dots (:) (13.3 %) indicates conservative substitution within a strong amino acid group, score greater than 0.5; one dot (.) (6.3 %) indicates conservative substitution within a weak group, score 0.5 or less. The ATP binding sites in the NUAK catalytic domain are shown in green and blue, the domains of NUAK1 and NUAK2 activated by LKB1 phosphorylation (T loops) are shown in yellow and blue, with the phosphorylation sites shown in black. The conserved KKAR sequence which corresponds to the nuclear localization signal (NLS) is indicated in grey. The conserved RQRIRS, AKT phosphorylation motif is indicated in red, with the putative phosphorylation site, Ser, shown in black.
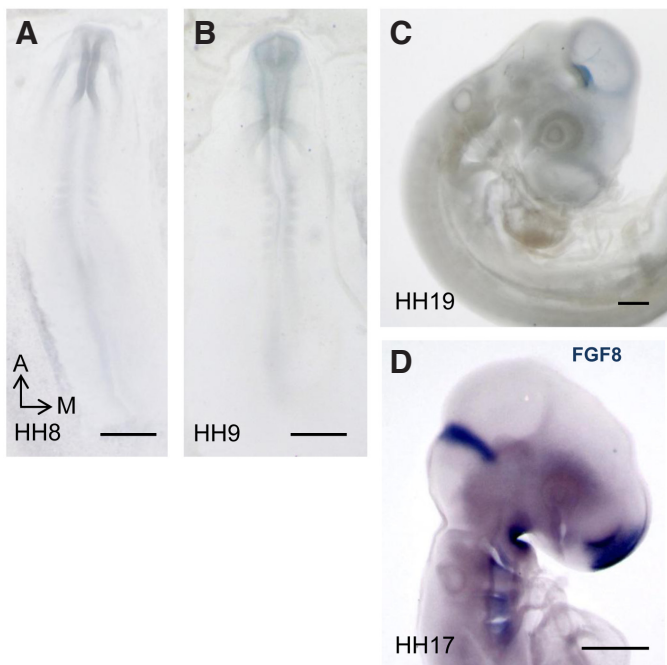
Supplementary Fig. S2. Non-specific labeling control. *Whole-mount* In situ *Hybridization was performed on chick embryos at stages HH8, 9 and 19, using* NUAK2 *sense mRNA probe for negative staining* **(A-C)**, *and at stage HH17 using* FGF8 *anti-sense mRNA probe for positive staining* **(D)**. *Abbreviations: A-M, anterior-medial. Scale bar, 500 μm.*