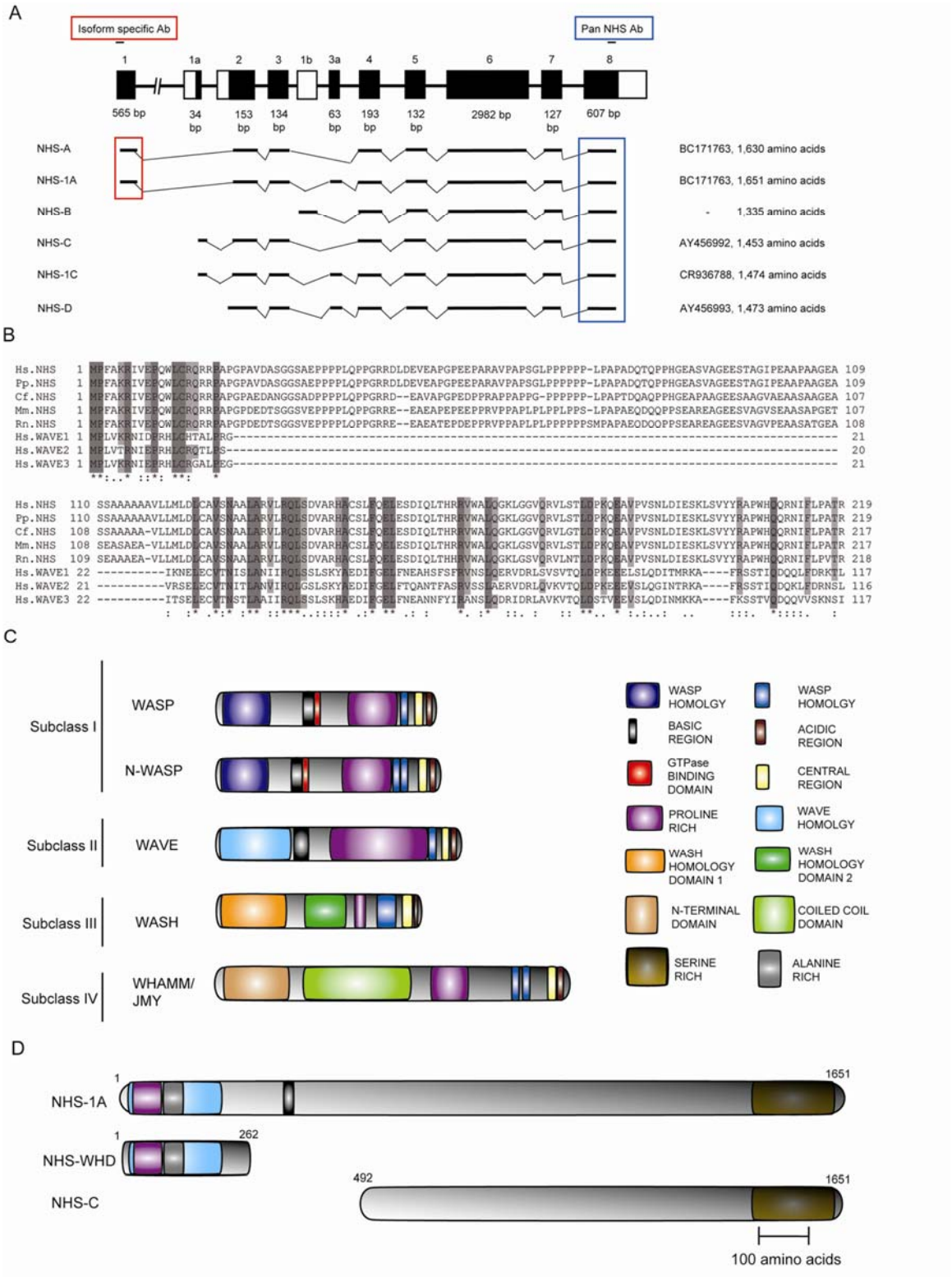
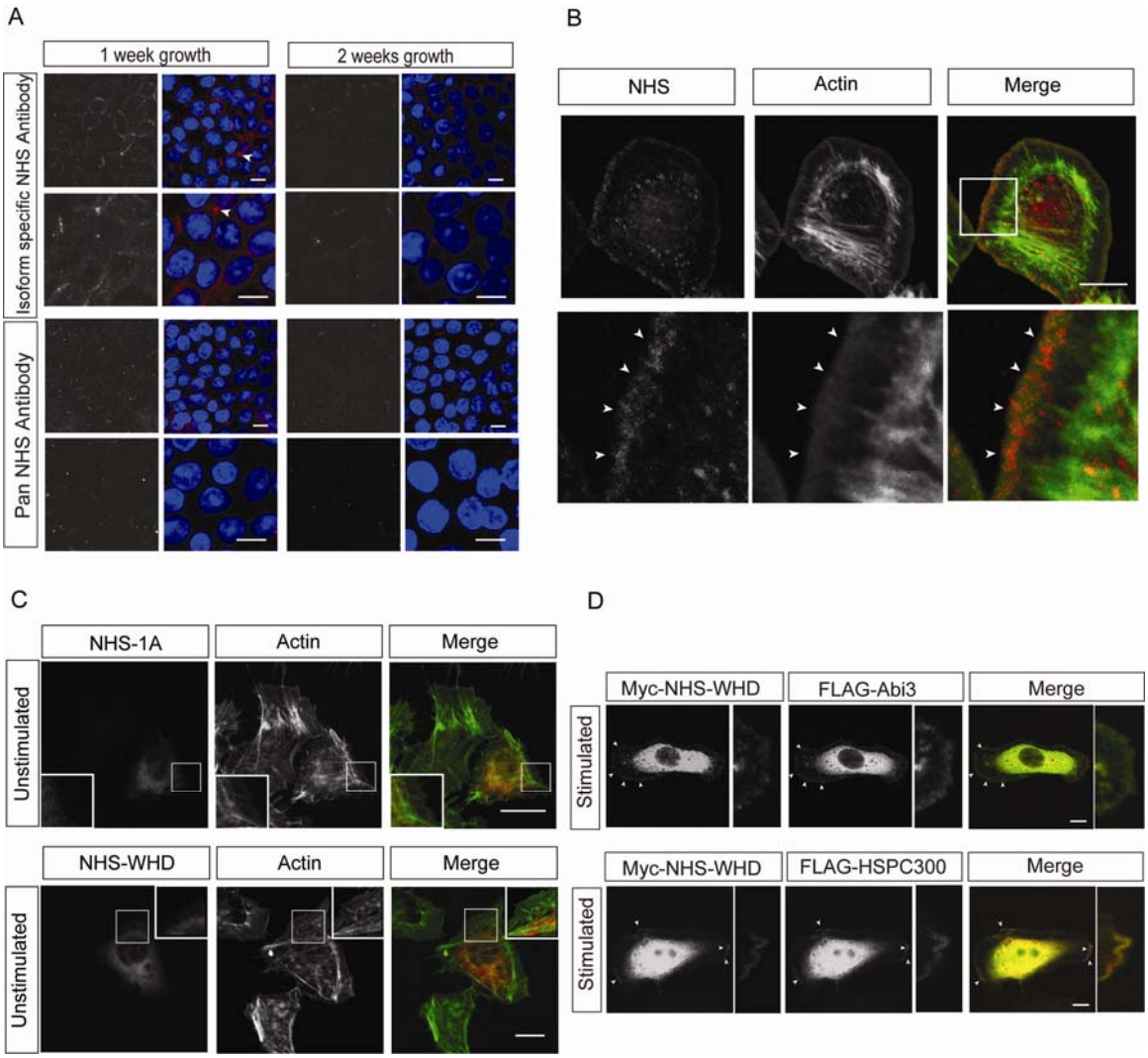
# The Nance-Horan Syndrome protein encodes a functional WAVE Homology Domain (WHD) and is important for co-ordinating actin remodelling and maintaining cell morphology

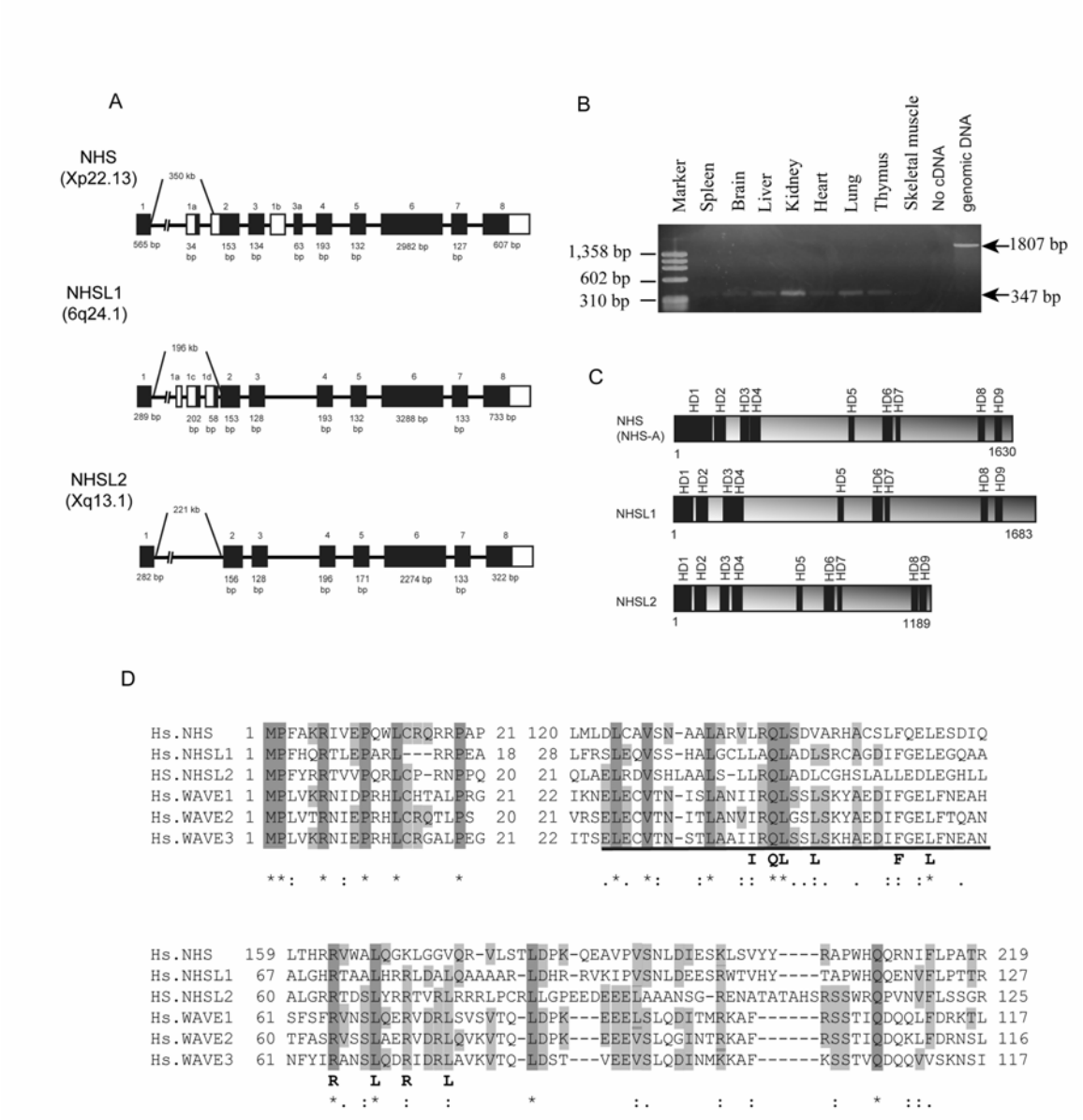


**Supplementary Figure 1. The NHS protein contains a WAVE homology domain (WHD) at its conserved N-terminus. (A)** Genomic structure of the NHS gene depicting isoforms**.** Accession numbers and predicted number of amino acids for each isoform are given on the right. Anti-peptide antibodies generated against the N- and C-termini (isoform specific NHS-A/NHS1A and pan-NHS antibodies respectively) are highlighted showing isoforms detected. **(B)** The NHS protein contains a WAVE homology domain (WHD) at its N-terminus which is conserved across species**.** Alignment of NHS protein across mammalian species with the human WAVE proteins. Note the NHS WHD is interrupted by a poly proline domain. Asterisks denote conserved residues in mammalian WAVEs, Drosophila SCAR, Dictyostelium SCAR, and mammalian NHS. Hs, Homo sapiens; Pp, Pongo pygmaeus; Cf, Canis familaris; Mm, Mus musculus; Rn, Rattus norvegicus. **(C)** Domain organisation of the WASP protein family**.** All members of the WASP family have a conserved modular structure. Members of the WASP family are subdivided (Class I-IV) depending on their N-terminal sequence **(D)** Domain organisation of the NHS protein. NHS contains a WAVE homology domain, basic and proline-rich regions common to the WASP family. The NHS-WHD and NHS-C constructs generated are also depicted.

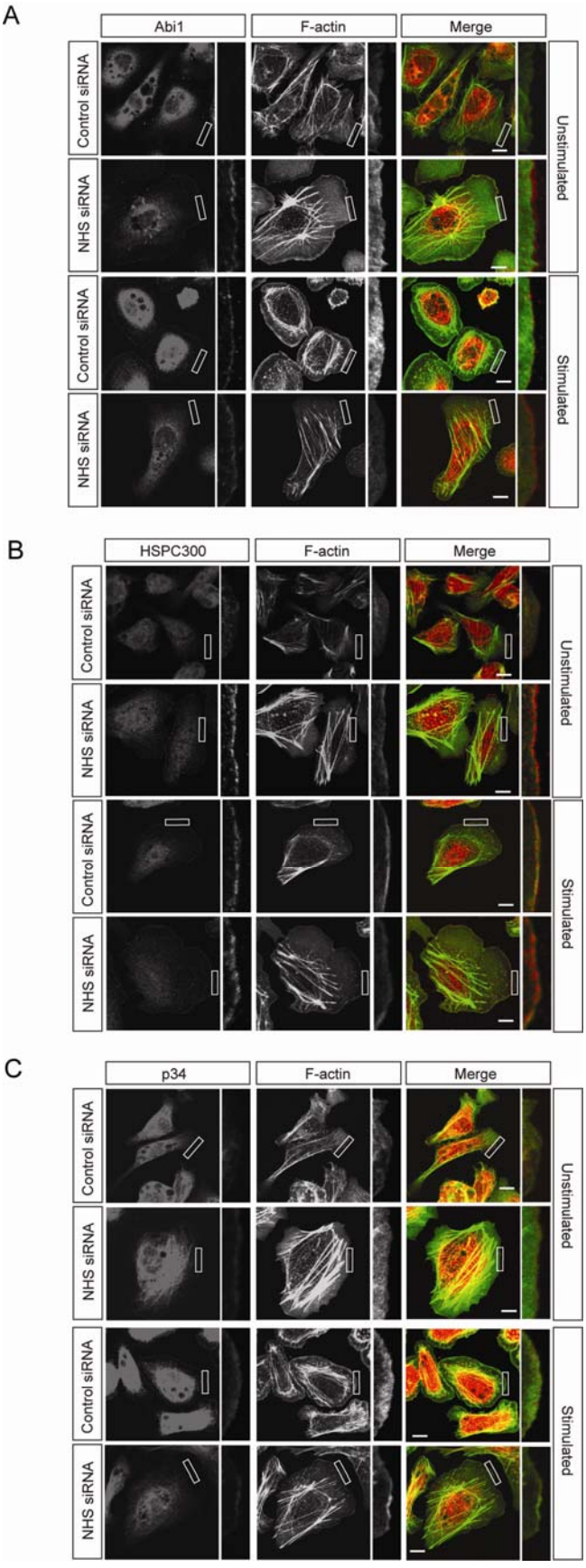



**Supplementary Figure 2. Endogenous and ectopic expression of NHS. (A)** Endogenous NHS (red) expression and localisation to sites of cell-cell contact in Caco-2 cells reduced as the cells differentiated over 2 weeks. NHS was detected by an N-terminal isoform specific antibody and a C-terminal pan NHS antibody, as indicated. Lower panel for each antibody staining is a higher magnification. Nuclei are counterstained with DAPI. Scale bar 10 μm. **(B)** Endogenous NHS localised to the leading edge at the 1 minute transient. Cells stimulated with EGF for 1 minute revealed localisation of endogenous NHS at the leading edge of the lamellipod (arrowheads) detected with the pan NHS C-terminal antibody (red). Cells were counterstained with F-actin (green). Scale bar 10 μm. **(C)** Expression of NHS constructs (red) in unstimulated

cells**.** Ectopic expression of NHS-1A or NHS-WHD did not reveal localisation at the cell periphery in the absence of EGF. Cells were counterstained with F-actin (green). Scale bar 10 μm. **(D)** Co-localisation of NHS-WHD (red) with Abi3 (green) or HSPC300 (green) at the leading edge of lamellipodia (arrowheads) in EGF stimulated cells.



**Supplementary Figure 3. The *NHS* gene is a founder member of a new gene family.** **(A)** Genomic structure of the *NHS* gene family: *NHS*, *NHSL1*, and *NHSL2*. Shaded boxes indicate open reading frames (ORF), with sizes of coding regions given below. Sizes of the large intron 1 of each gene are shown. The genomic structures share a striking resemblance. **(B)** Expression analysis of *NHSL2* using a human fetal cDNA panel. Primers were designed to amplify exon 6 – 8 of the *NHSL2* gene. **(C)** Schematic representation of the regions of homology between the NHS protein family. Positions of homology domains 1-9 (HD1-9) within NHS, NHSL1, and NHSL2 are shown. **(D)** Alignment of the N-termini of the human NHS protein family (homology domain 1) with the N-termini of the human WAVE proteins. Asterisks denote conserved residues. Residues conserved across all the NHS and WAVE family members are shaded in dark grey, residues partly conserved between an NHS protein family member and at least one WAVE protein are highlighted in light grey. Letters in bold beneath the alignment denote conserved residues in mammalian WAVEs, Drosophila SCAR, and Dictyostelium SCAR. The Abi-binding domain is underlined.


A
Abi1
F-actin
Merge
Control siRNA
NHS siRNA
Control siRNA
NHS siRNA
Unstimulated
Stimulated
B
HSPC300
F-actin
Merge
Control siRNA
NHS siRNA
Control siRNA
NHS siRNA
Unstimulated
Stimulated
C
p34
F-actin
Merge
Control siRNA
NHS siRNA
Control siRNA
NHS siRNA
Unstimulated
Stimulated

**Supplementary Figure 4. NHS affects the cellular response to EGF stimulation.** Knockdown of NHS resulted in lamellipodia formation in the absence of EGF (3 minute stimulation). Removal of NHS also resulted in consistent localisation of endogenous **(A)** Abi1 (red), **(B)** HSPC300 (red), and **(C)** Arp2/3 (red) at the edges of MTLn3 cells in the absence of EGF. Cells were counterstained for F-actin (green). Scale bar 10 μm.