Supplementary Figure Lgends:

Supplementary Figure S1: (A) Nucleotide sequence of exons (red) and introns (blue) retained in the human *a-raf*$_{short}$ mRNA and corresponding amino acid sequence. Asterisks in intron 1 and 3 indicate splicing. Shaded areas represent amino acid sequences lacking in the generic A-Raf protein. Red boxes indicate G-rich regions. PTS; pre-terminal stop-codon. **(B)** Alignment of all four known human A-Raf isoforms. Shown are protein sequences where red letters indicate amino acids derived from intronic sequences which are exclusively found in the A-Raf$_{short}$ isoform.

Supplementary Figure S2: Schematic overview of *a-raf* isoforms. (A) Shown are the exon/intron structures of mRNAs and corresponding proteins of the so far known different *a-raf* isoforms. Boxes indicate exons, lines indcate introns. E: exon; i: intron; UTR: untranslated region. **(B)** Functional domains of *a-raf* isoforms. The numbers indicate amino acid residues of human A-Raf. CR: conserved region; RBD: Ras binding domain; CRD: Cysteine rich domain; S/T Rich: Serine/Threonine rich domain

Supplementary Figure S3: A-Raf$_{short}$ acts upstream of Raf. (A) NIH3T3 fibroblasts were tranfected with an oncogenic viral Raf (vRaf) construct (1µg). Cells were co-tranfected with A-Raf$_{short}$ as indicated (1µg). After two weeks in culture foci were stained with GIEMSA and scored. **(B)** Numbers indicate average number of foci per microgram of DNA (vRaf construct). Error bars represent standard deviation of at least three independent experiments. n.s.; not significant. **A-Raf$_{short}$, like daRaf1 and daRaf2, inhibits Ras-induced cellular tranformation. (C)** NIH3T3 fibroblasts were tranfected with hyperactive Ras constructs (100ng). Cells were co-tranfected with either A-Raf$_{short}$, daRaf1, or daRaf2 as indicated (1µg).

After two weeks in culture foci were stained with GIEMSA and scored. **(D)** Numbers indicate average number of foci per microgram of DNA (Ras constructs). Error bars represent standard deviation of at least three independent experiments. * $p \leq 0.05$. ** $p \leq 0.01$.

Supplementary Figure S4: The A-Raf$_{short}$ isoform expression is induced by cell density.
(A) HeLa cells were grown to medium conluency (< 60%) or full confluency (>90%) and grown in DMEM supplemented with increasing concentrations of FCS as indicated. Lysates were immunoblotted for expression of A-Raf$_{short}$, and tubulin as loading control. Shown are the representative results from three independent experiments. **(B)** HeLa cells were grown to increasing condluency (left panel) and lysates were immunoblotted for expression of A-Raf$_{short}$, A-Raf$_{WT}$, c-Myc, hnRNPH, pERK1/2 and ERK1/2 as loading control (right panel). Shown are the representative results from three independent experiments. The protein expression levels of A-Raf$_{wt}$ and A-Raf$_{short}$ were quantified using ImageJ (upper right). All values were standardized using ERK1/2. Shown are relative ODs. **(C) hnNRP H is not regulated by the MAPK pathway.** Hela cells were treated with the ERK1/2 inhibitor UO 126 for a timecourse of 12 hours as indicated. Lysates were immunoblotted for expression of A-Raf$_{short}$, hnRNPH, pERK1/2 and ERK1/2 as loading control.

Supplementary Figure S5: Human HNRNPH1 genomic locus and binding of c-Myc. (A)
DNA sequences and annotation data for c-Myc binding (human genome assembly Mar. 2006, NCBI36/hg18) were retrieved from the Genome Browsers of the University of California Santa Cruz (1). We analyzed the genomic locus of the human HNRNPH1 gene (coordinates: chr5:178,971,093-178,987,247) and used the c-Myc ChIP annotation provided in UCSC table 'ENCODE Open Chromatin, Duke/UNC/UT' showing density graphs for the following cell

lines (2): GM12878 (lymphoblastoid cell line), H1-hESC (Human Embryonic Stem Cells), HeLa-S3 (cervical carcinoma cell line), HepG2 (liver carcinoma cell line), HUVEC (Human Umbilical Vein Endothelial Cell line), K562 (leukemia cell line). ChIP-seq results are shown as number of DNA fragments obtained at each position relative to the genomic average. **(B)** Positions of the PCR amplicons used for c-Myc ChIP analysis.

Supplementary Figure S6: Protein expression of A-Raf$_{wt}$, and A-Raf$_{short}$ in HNSCC and normal adjacent tissues. (A) The expression of protein was assessed by immunoblotting in normal mucosa (n=3) and carcinoma tissues (n=3) of the head and neck area. ERK1/2 levels were determined as a loading control. **(B)** Relative quantified expression of A-Raf isoforms. Shown are dotplots where asterisks and dots indicate the median and individual (respectively), relative expressions levels.

Supplemental Experimental Procedures

siRNA sequences

The following siRNA sequences were used:

hnRNPH: 5'-GGAGCUGGCUUUGAGAGGA dTdT-3'
A-Raf$_{WT}$:   Silencer Validated siRNA 151 (Ambion, Austin, US)
A-Raf$_{short}$ (1):   5'-AAUGGCAGGGGCUGUGGAUGGdTdT-3'
A-Raf$_{short}$ (2):   5'-AACUCUGGGAUCAAAAGGGUGdTdT-3'
A-Raf$_{short}$ (3):   5'-AAUCAGGACUGCUGUGUGGUCdTdT-3'
A-Raf$_{short}$ (4):   5'-AUGUUGGGGGUGUCCUUGACdTdT-3'
c-Myc: ON-TARGETplus SMARTpool Human MYC (Dharmacon)

Semi-quantitative PCR

Cell lines and human tissues were analysed by semi-quantitative PCR for the expression of

the following human mRNAs *c-myc, hnrnph, a-raf$_{WT/short}$, a-raf$_{WT}$, a-raf$_{short}$, daraf1, daraf2,*

beta-actin, gapdh, and mapk. PCR conditions were denaturing at 95 °C for 30 seconds,

annealing for 30 seconds, extension at 72 °C for 30 seconds).

The following primers were used; the amplicon size of the PCR product given in brackets:

hnRNPH (385bp):   5'-AAAATGGGGCTCAAGGTATTCG-3'
                          5'-GCTATTTCCTGTGAAGCAAACTGC-3'

a-raf$_{WT/short}$:   5'-ATGGAGCCACCACGGGGC-3'
(*a-raf$_{short}$* 372bp; *a-raf$_{WT}$* 210bp):   5'-CGTCTTTCGTCCCTTGATGAGTC-3'

a-raf$_{short}$ (161bp):   5'- CGGTGGTGAGTCATGGAAGC-3'
                          5'- GTATGTGCAGATGTAGGGGTCC-3'

hdaraf1/2:   5'-CATGGAGCCACCACGGGG-3'
(*daraf1* 578bp, *daraf2* 695bp)   5'-TTCAGCTAAGGCATCCCTAC-3'

c-myc (236bp):   5'- TCCGTCCTCGGATTCTCTGC-3'
                          5'-CCAGTGGGCTGTGAGGAGGT-3'

gapdh (258bp):   5'-TGTCGCTCTTGAAGTCAGAGGAGA-3'
                          5'-AGAACATCATCCCTGCCTCTACTG-3'

mapk1 (233bp):   5'- CCTTCCAACCTGCTGCTCAACAC-3'
                          5'- GGAAAGATGGGCCTGTTAGAAAGC-3'

beta–actin (174bp):   5'- AGCCTCGCCTTTGCCGA-3'

5'- CTGGTGCCTGGGGCG-3'

Immunoblot analysis

Protein lysates or immunoprecipitates were resolved by SDS-PAGE (10-15%) and blotted on PVDF membrane (Millipore, Bedford, US). Protein visualisation was performed using the following antibodies in combination with HRP-conjugated secondary antibodies and the ECL system (GE-Healthcare, Munich, Germany): Polyclonal rabbit anti-human hnRNPH antibody (Bethyl Laboratories, Montgomery, US), polyclonal goat anti-human A-Raf antibody sc-408-G (Santa Cruz, Santa Cruz, US), polyclonal rabbit anti-human MST2 antibody (Stratagene, La Jolla, US), monoclonal mouse anti-human tubulin antibody sc-8035 (Santa Cruz, Santa Cruz, US), monoclonal mouse anti-human Flag antibody F3165 (Sigma, Taufkirchen, Germany), monoclonal mouse anti-HA tag antibody 3F10 (Roche Diagnostics, Mannheim, Germany), monoclonal mouse anti-human Ras antibody sc-29 (Santa Cruz, Santa Cruz, US), polyclonal rabbit anti-human B-Raf antibody sc-166 (Santa Cruz, Santa Cruz, US), monoclonal rabbit anti-human MST2 (N-term.) antibody 1943-1 (Epitomics Inc., Burliname, US), polyclonal rabbit anti-human mitogen acivated protein kinase (ERK1, ERK2) antibody M-5670 (Sigma, Taufkirchen, Germany), monoclonal mouse anti-human MAP kinase, activated (Diphosphorylated ERK-1&2) antibody M-8159 (Sigma, Taufkirchen, Germany), polyclonal rabbit anti-human caspase-3 antibody sc-7148 (Santa Cruz, Santa Cruz, US), monoclonal rabbit anti-human caspase-3 antibody (8G10) sc-9665 (Santa Cruz, Santa Cruz, US), monoclonal mouse anti-human PARP antibody (Becton Dickinson, Franklin Lakes, US), monoclonal mouse anti-human c-Myc antibody (9E10) sc-40 (Santa Cruz, Santa Cruz, US). The polyclonal anti-human antibody against endogenous A-Raf$_{short}$ was raised in rabbits using a peptide of the N-terminus of A-Raf (EPPRGPPANGAEPS) (Eurogentec Ltd, Southampton,

UK). In order to demonstrate antibody specificity, the above-mentioned peptide was added as competitor at a final concentration of 50μg peptide/μg antibody.

Chromatin Immunoprecipitation (ChIP)

HeLa cells were serum-starved for 18 hrs, stimulated with 10nM EGF for 20 min as indicated and fixed in 1% formaldehyde for 10 min. ChIP assays were performed using the SimpleChIP Enzymatic Chromatin IP Kit (Agarose Beads) (Cell Signaling, Danvers, MA) according to the manufacturer's instructions. We used the rabbit antibody against c-Myc (D84C12, Cell Signaling, Danvers, MA) and rabbit IgGs as a negative control. The purified DNA from cross-linked cells was dissolved in 50μl TE buffer; 1μl was used for PCR. As an input we used 2% chromatin before immunoprecipitation. DNA sequences were analysed by quantitative real-time PCRs (SYBR Green) in triplicate using the ABI 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) with the following thermal cycling conditions: Initial denaturation (10min, 95°C) followed by 45 cycles of denaturation (15sec, 95°C) and annealing/extension (1min, 60°C). Dissociation curves were analyzed to ensure PCR quality and to monitor primer dimers. The following primer sets were used to cover regions of the hnRNP H genomic locus as depicted in Figures 5 and S5:

PCR1:   5'-ATCTTAGGTTTTGGGCAGTGCTGC-3'
           5'-TGCATCTCCCAGAGCAGAATTGGT-3'

PCR2:   5'-TAAGGGGAAGTGGAGGCACACA-3'
           5'-GCGCCGCCTGTCTTCCCTTTAT-3'

PCR3:   5'-AACCCAAGCGTGTAATAATCCGCC-3'
           5'-CTGCGCAACCTAAATAAGGTCCCTT-3'

PCR4:   5'-TAGCTTGGTAACACTGATCCCCTTT-3'
           5'-AACATACTTCGTTGCGAGCTGTTTT-3'

Fold-change was obtained based on critical threshold (C_T) measurements calculating the signal-to-noise ratio normalized to the input. In detail, the amount of genomic DNA precipitated with specific antibody was calculated compared to the total input DNA used for each immunoprecipitation in the following way: $\Delta C_T = C_T$(input DNA)$-C_T$(specific antibody), where C_T are the mean threshold cycles performed in triplicates. Finally, $\Delta\Delta C_T$ was calculated using $\Delta\Delta C_T = \Delta C_T$ (c-Myc antibody)$-C_T$(IgG control antibody) and linearly transformed ($2^{\Delta\Delta C_T}$) for each amplicon and sample. Error bars represent standard deviations.

Sequence and annotation data

DNA sequences and annotation data for c-Myc binding (human genome assembly Mar. 2006, NCBI36/hg18) were retrieved from the Genome Browsers of the University of California Santa Cruz (1). We analyzed the genomic locus of the human HNRNPH1 gene (coordinates: chr5: 178,971,093-178,987,247) and used the c-Myc ChIP annotation provided in UCSC table 'ENCODE Open Chromatin, Duke/UNC/UT' showing density graphs (2).

Supplemetal References

1.   Karolchik D, Baertsch R, Diekhans M, et al. The UCSC Genome Browser Database. Nucleic Acids Res 2003; 31: 51-4.
2.   Birney E, Stamatoyannopoulos JA, Dutta A, et al. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature 2007; 447: 799-816.