Legends to Supplementary Figures

Supplementary Figure S1. IR status in human HCC tumours.

qPCR evaluation of total IR mRNA *(A)*, IR-A mRNA *(B)* and IR-A:IR-B ratio *(C)* in 43 human paired HCC (T) / non tumour (NT) liver tissue samples (collection #2). *(D)* The induction of IR-tot, IR-B, IR-A and IR-A:IR-B ratio was evaluated by qPCR in T *vs* NT liver tissue from collections #1 and #2. *(E)* The induction of the IR-A:IR-B ratio was evaluated by qPCR in T *vs* NT liver tissue from collections #1 and #2 and classified according to the etiology/charateristics of the liver disease. MS, metabolic syndrome.

Statistical analysis: T *versus* NT: Wilcoxon test for paired values, $^{**}p < 0.01$, $^{***}p < 0.001$.

Supplementary Figure S2. EGFR-dependent signalling increases IR-A mRNA splicing in HCC cells.

(A) PLC/PRF5 cells were treated for 24 h with inhibitors against EGFR (gefitinib, 2.5 μM), MEK (U0126, 5 μM), PI3K (LY294002, 10 μM), AKT (Inh AKT VIII, 5 μM), mTOR (rapamycin, 1 μM), JNK (SP600125, 5 μM) or STAT3 (WP1066, 1 μM), and the IR-A:IR-B ratio was evaluated by quantitative (*left*) and qualitative (*right*) PCR. *(B)* Effect of treatment duration with gefitinib (2.5 μM) on the IR-A:IR-B ratio in PLC/PRF5 cells. *(C)* PLC/PRF5 (*upper*), Hep3B (*middle*) and HuH7 (*lower*) cells were transiently transfected with 100 nM of control siRNA (siCont), EGFR siRNA (siEGFR) or amphiregulin siRNA w(siAR). After 72 h, EGFR and AR mRNA levels as well as the IR-A:IR-B ratio were analysed by quantitative and/or qualitative PCR. (*D*) Effect of treatment duration with U0126 (5 μM) on the IR-A:IR-B ratio in PLC/PRF5 cells. Statistical analysis: n = 3-6 in duplicate; Student's *t*-test; $^{**}p < 0.01$, $^{***}p < 0.001$.

Supplementary Figure S3: Basal and EGFR-activated ERK levels in HCC cell lines.

HCC cell lines cultured for 24 h in serum-free medium were stimulated for 10 min with amphiregulin (50 ng/ml) and analysed by Western blotting for phosphorylated and total ERK levels. Blots are representative of three experiments.

Supplementary Figure S4: CUGBP1, hnRNPH, hnRNPA2B1 and S2/ASF mRNA levels are controlled by EGFR in HuH7 cells.

(A) HuH7 cells were transiently transfected with 100 nM of EGFR siRNA or control siRNA for 72 h. CUGBP1, hnRNPH, hnRNPA1, hnRNPA2B1, hnRNPF and S2/ASF mRNA levels were analysed by qPCR. (*B*) HuH7 cells were treated for 24 h with EGFR ligands (AR, EGF) and splicing factor expression was analysed by qPCR. Statistical analysis: n = 3 in duplicate; Student's *t*-test; $*p < 0.05$, $**p < 0.01$.

Supplementary Figure S5: The blockage of EGFR signalling does not alter the subcellular localization of splicing factors in HCC cells

Representative immunofluorescence analysis of the subcellular localization of CUGBP1, hnRNPH, hnRNPA2B1, hnRNPA1 ans SF2/ASF (green) in the presence or absence of gefitinib (2.5 μM) or U0126 (5 μM). Nuclei are counterstained with DAPI (blue).

Supplementary Figure S6: EGFR-dependent signalling increases IR-A mRNA splicing in colon cancer cell lines.

SW40 (*A*) and LoVo (*B*) colon cancer cell lines were transiently transfected with 100 nM of control siRNA (siCont), amphiregulin siRNA (siAR) or EGFR siRNA (siEGFR) and treated with or without gefitinib (2.5 μM) or U0126 (5 μM). After 72 h, AR and EGFR mRNA levels

as well as the IR-A:IR-B ratio were analysed by qPCR. Statistical analysis: n = 3-6 in duplicate; Student's *t*-test; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.