

Supplementary Figure 1: Knockdown of AROS depletes immunofluorescent signal in all subcellular compartments:

A) Immunofluorescent staining for AROS (green) and nucleolin (red) in MCF7 cells either treated with mock siRNA (above) or depleted of AROS by RNAi (below). Merge images are shown with the DNA stain Hoechst (blue). Bar is 10 μ m. B) Western blots confirming the extent of knockdown of AROS by RNAi in parallel to the images in A) and Figure 1B). β -tubulin is used as a loading control.



Supplementary Figure 2: Ribosomal RNA regulation in HEK293 cells:

A) Northern blotting for pre-rRNA and mature rRNA species following RNAi in HEK293 cells as detailed in Figure 1B for HCT116 cells. Lamin AC RNAi is used as a control knockdown with no effect on rRNA processing. B) Pixel density quantification using Image J software of entire lanes for Mock, AROS siRNA and RPS19 siRNA treatments from (A).



Supplementary Figure 3: AROS is similar to small ribosomal subunit proteins in multiple cell lines:

A) Purified protein from post-nuclear fractionation of MCF7 cells were analysed for protein content by SDS-PAGE and western blotting against AROS and ribosomal proteins. * indicates a non-specific band and an arrow indicates RPL3 protein. B) AROS protein localisation following treatment for 5 minutes with translation initiation inhibitor Harringtonine ($2\mu g/mL$) in MCF7 cells. Purified protein was separated and analysed as either sub-polysomal (S, Subs) or polysomal (P, Polys). Inset traces shown loss of polysomes following Harringtonine treatment – traces are not to scale. C) HCT116 cells were treated with 100µg/mL cycloheximide treatment of for indicated times and purified whole cell protein analysed by SDS-PAGE and western blot. P53 protein depletion is used as a control for cycloheximide efficacy and β -tubulin as a loading control.

Supplementary Figure 3



Supplementary Figure 4: Ribosomal protein regulation in further cell lines:

A) Western blotting for AROS and ribosomal proteins from the 40S and 60S subunits following RNAi in HEK293 cells. Lamin AC protein knockdown is used a negative control with no effect on ribosomal protein abundance, and β -tubulin as a loading control. B) Western blotting for AROS and ribosomal protein abundance following RNAi in MCF7 cells. β -tubulin is used as a loading control.

Supplementary Figure 4



Supplementary Figure 5: Knockdown of AROS does not influence phosphorylation of the eIF4F complex:

Lysates as in Figure 7B were immunoblotted for total and phosphorylated regulatory eIF4E from the eIF4F complex and its suppressive binding partner 4E-BP1. β -tubulin is used as a loading control.