

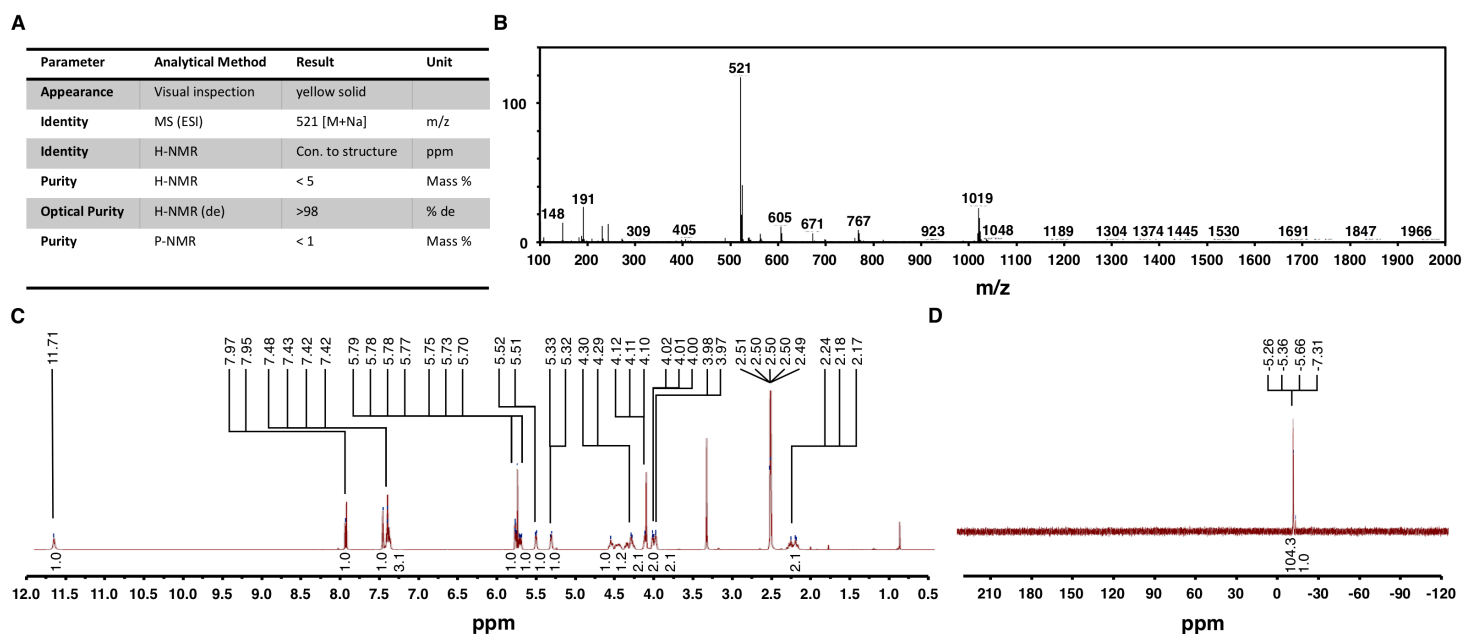
Cell Reports, Volume 30

Supplemental Information

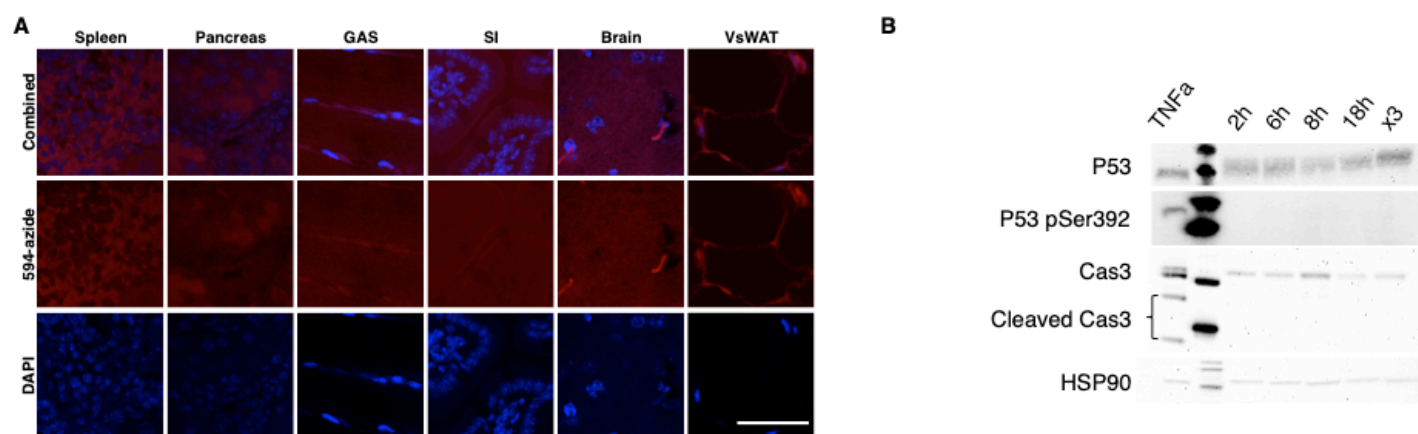
**iTAG-RNA Isolates Cell-Specific Transcriptional
Responses to Environmental Stimuli
and Identifies an RNA-Based Endocrine Axis**

Jonatan Darr, Archana Tomar, Maximilian Lassi, Raffaele Gerlini, Lucia Berti, Annette Hering, Fabienne Scheid, Martin Hrabě de Angelis, Michael Witting, and Raffaele Teperino

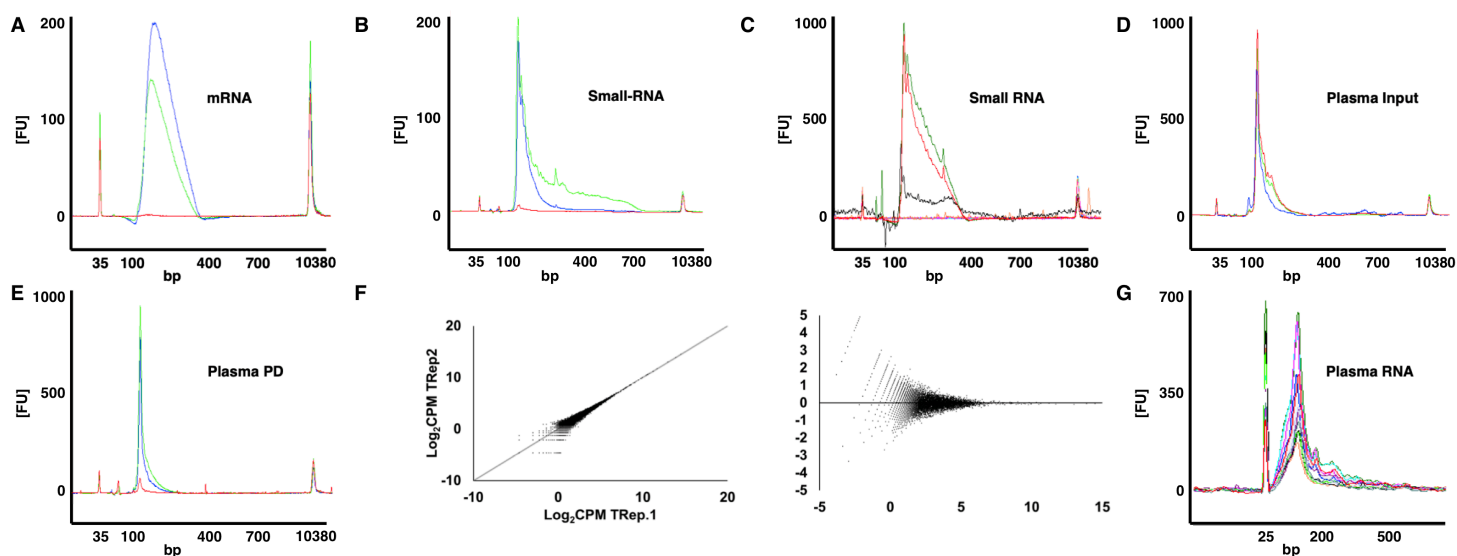
Supplementary Figures and Legends



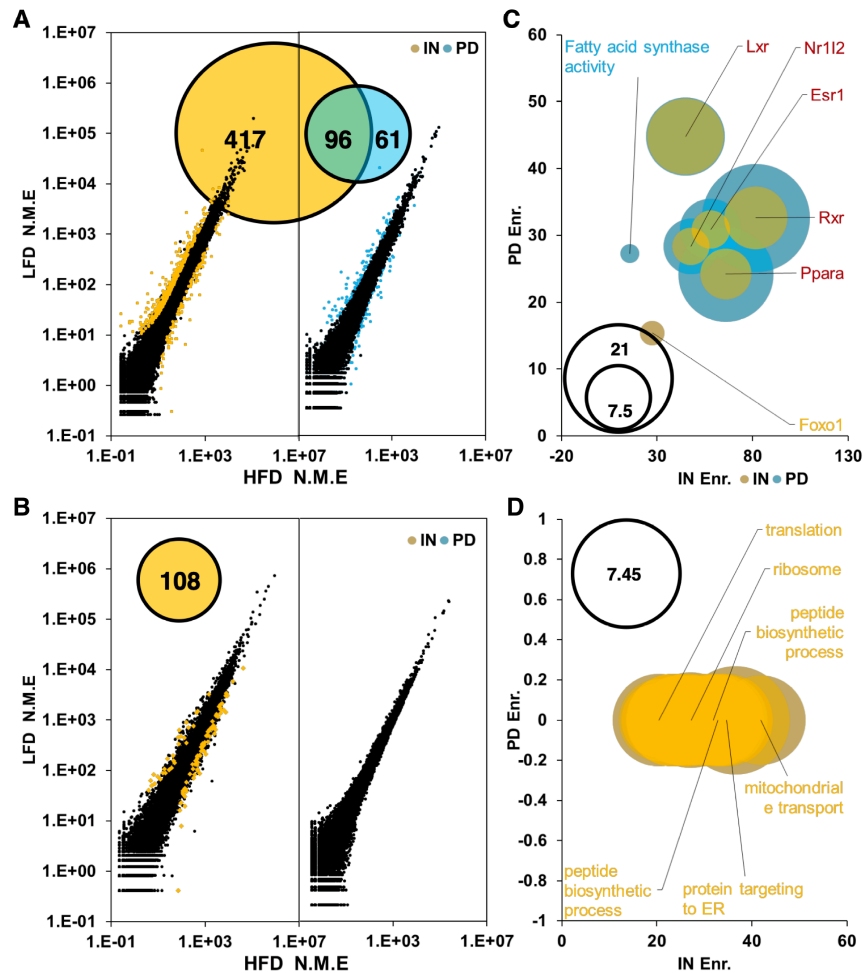
Supplementary Figure 1. Quality assessment of HD5EU synthesis. Related to figure 1 a) Summary table listing all examined parameters and methodologies used to validate the structural conformity of the molecule and the purity of the product. **b)** MS Base Peak at 520.95 corresponding to $M+Na^+$ and at 1019 for $2M+Na^+$. **c)** 1H -NMR conformity to structure. 513 MHz. DMSO as solvent. **d)** ^{31}P -NMR measurement for purity. 202.46 MHz. DMSO as solvent.



Supplementary Figure 2. Negative controls for in-vivo tissue staining. Related to figure 2 a) Click-it staining in tissues collected from saline treated animals. Scale Bar = 50 μ M. **b)** W.B. validating HD5EU safety. Lack of p53 activation and downstream Caspase cleavage at multiple time points following administrations of HD5EU and following consecutive administration of HD5EU. MEFs treated with TNFa for 16 hours serve as positive controls for the western blot staining.



Supplementary Figure 3. Specificity and reproducibility of RNA pull-down, library construction and sequencing. Related to figure 4 **A)** Bio-analyzer plot for mRNA Pull-down libraries. Blue - HD5EU labeled liver, Green - HD5EU labeled kidney, Red - saline treated liver. **B)** Bio-analyzer plot for small RNA pull-down libraries. Blue – HD5EU labeled liver, Green - HD5EU labeled kidney, Red – Saline treated liver. **C)** Consistent failures in library generation from small RNA (<200bp) pull-down in multiple tissue following 2h HD5EU treatment (Testis = orange, vsWAT = pink and blue = Spleen). Input RNA generates expected library amplicons (Black, red and green). **D)** Bio-analyzer plots for small RNA input libraries from plasma. **E)** Bio-analyzer plots for small RNA pull-down libraries from plasma following multiple injections. **F)** Scatter plot and MA plot for two technical replicates from HD5EU labeled hepatocyte samples. Pearson correlation coefficient = 0.95. **G)** Plasma ccfRNAs' size distribution.



Supplementary Figure 4. Dietary intake effects on liver and kidney transcriptional programs. Related to figure 4. A-B) Venn diagram demonstrating the overlap between differentially expressed genes in input mRNA (orange) and pull-down mRNA (Blue) in liver (A) and kidney (B) with the corresponding scatterplot. HFD normalized mean expression on the X-axis, LFD normalized mean expression on the Y-axis. Bubble size proportional to $-\log_{10}$ of adj. PV. **C-D)** GO and gene set enrichment analysis for dietary induced differentially expressed protein coding genes identified in pull-down (blue) and input (orange) liver (C) and kidney (D) libraries. Bubble size proportional to $-\log_{10}$ of adj. PV.