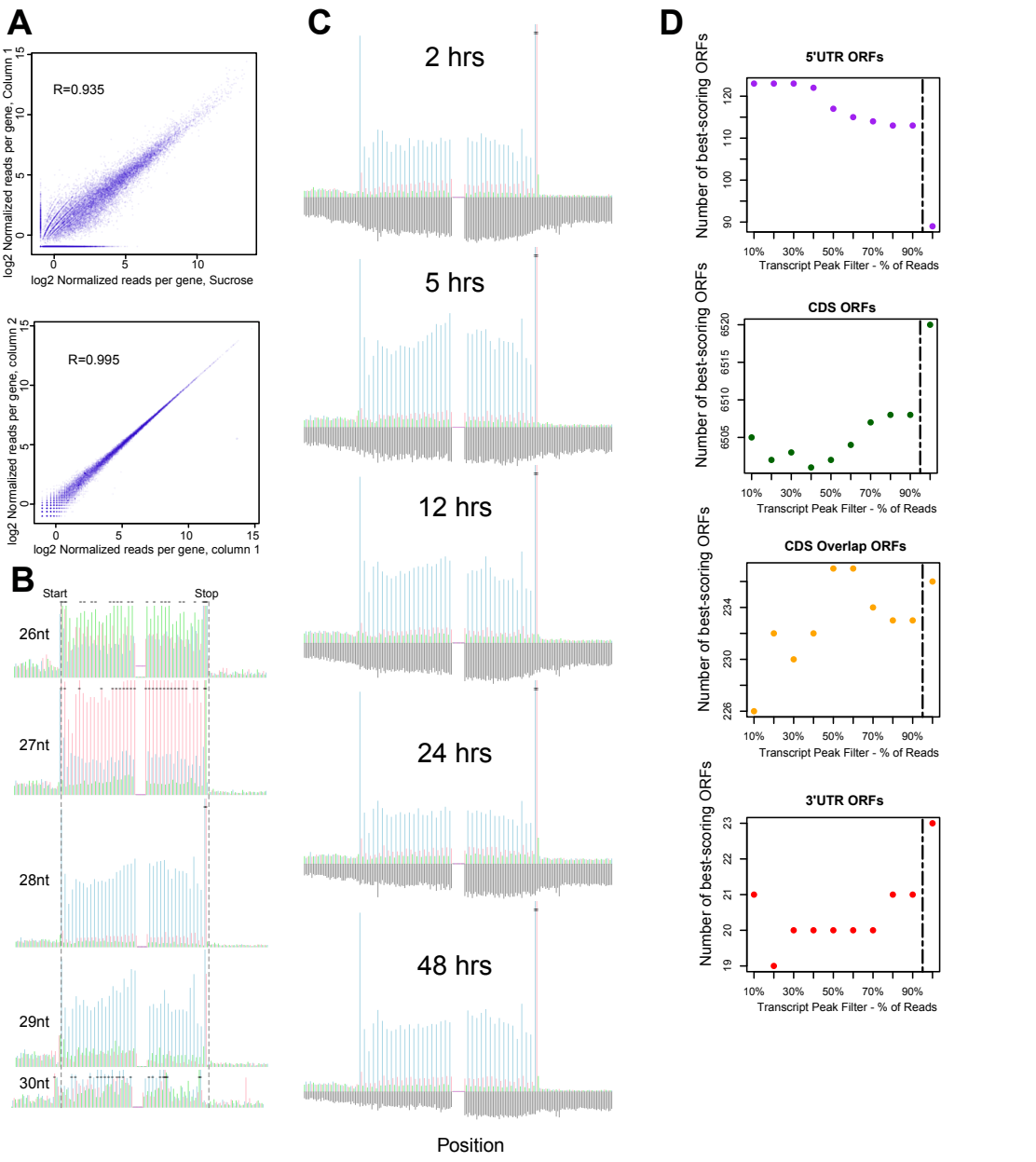
Bazzini et al., Figure S1



Figure

S1. Ribosome profiling in Zebrafish embryos.

Previously published zebrafish ribosome profiling data in zebrafish were generated by ribosome purification using sucrose cushion separation (Bazzini et al., 2012). Here, we have purified ribosomes using a chromatography column separation approach for the first time in zebrafish; this shows a strong correlation with ribosome profiling generated using the sucrose cushion approach. This new approach speeds up the protocol and can minimize RNA degradation. Biological replicates using

chromatography columns also correlate strongly, showing the high reproducibility of our experiments (Supplementary Fig. 1). Taking all RPFs with different sizes together (26 to 31), we observed a strong bias in reads aligning to frame 1. To identify the size classes with the most defined phasing, a metaplot analysis was generated for each RPF size (26 to 31), showing that the 28 and 29 nt RPFs represent a higher degree of phasing and make up a majority of the recovered reads (Supplementary Fig. 1, Supplementary Table 1). Similar features were observed at all time points (Supplementary Fig. 1).
(A) Biplots comparing log normalized reads per gene between biological replicates, purifying the ribosomes by sucrose cushion vs. chromatography column (Pearson's R=0.93) and between biological replicates purifying the ribosomes with chromatography columns (Pearson's R=0.99).
(B) Relative read density of ribosome protected fragments (RPFs) from 26 to 31 nt and input reads at 5 hours post fertilization mapped to a composite RefSeq transcript. Note that 28 & 29 nt RPFs are both highly enriched in position 1.
(C) Similar metagene plot comparing phasing of 28 & 29 nt RPFs at each time point
(D) Number of ORFs in 5'UTR, annotated CDS, overlapping the annotated CDS and 3'UTR that scored highest in RefSeq transcripts at different filter levels for the most abundant position (% of total reads in ORF that occupy that position). The point to the right of the dashed line indicates the number of best-scoring ORFs without any peak filter.