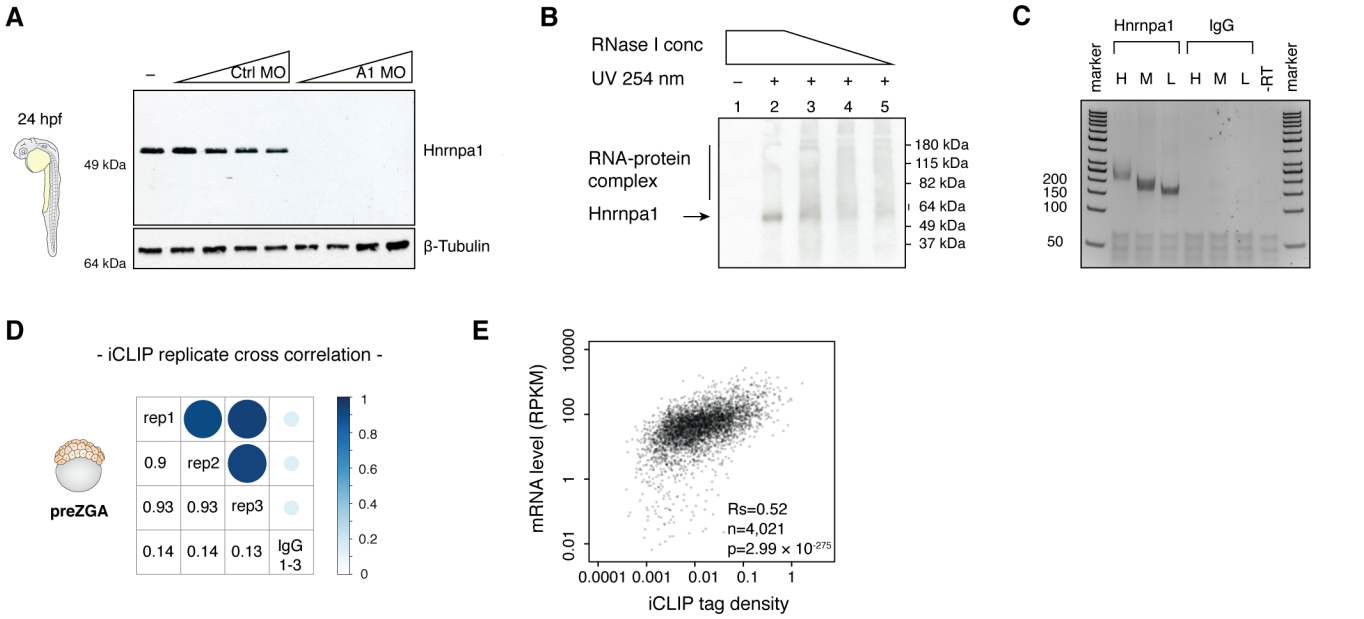
Despic_Supplemental_Fig_S2



Supplemental Figure S2 (related to Figure 2). Hnrnpa1 iCLIP optimization and preZGA Hnrnpa1 iCLIP statistics. (A) Western blots showing the specific reactivity of the monoclonal α-Hnrnpa1 antibody with endogenous zebrafish Hnrnpa1 protein. 1 cell zebrafish embryos were injected with an increasing concentration of control and Hnrnpa1 morpholinos (Ctrl and A1 MOs, respectively; see Supplemental Methods) and total protein extract from 24 hpf embryos probed with Hnrnpa1 antibody. The lack of protein detection in morphant samples indicates that the antibody specifically recognizes endogenous zebrafish Hnrnpa1 protein. β-Tubulin served as a loading control. **(B)** Autoradiograph of immunopurified Hnrnpa1-RNA complexes after different RNase treatments (lanes 2-5: 10 µl of 1:50, 1:500, 1:1,000, 1:1,500 RNase I stock dilutions). **(C)** Representative size-selected and PCR amplified iCLIP cDNA library. H, M, L: high, medium, low fractions. (**D**) Cross-correlation between preZGA Hnrnpa1 and summed IgG (control) replicates. Pearson correlation coefficient is shown, color-coded and proportional to the size of the displayed circles. **(E)** Scatterplot showing high correlation between iCLIP tag density and mRNA expression. Based on this finding, iCLIP enrichment (see Fig. 2F and Fig. 3B) was calculated as iCLIP tag density normalized to mRNA expression level.