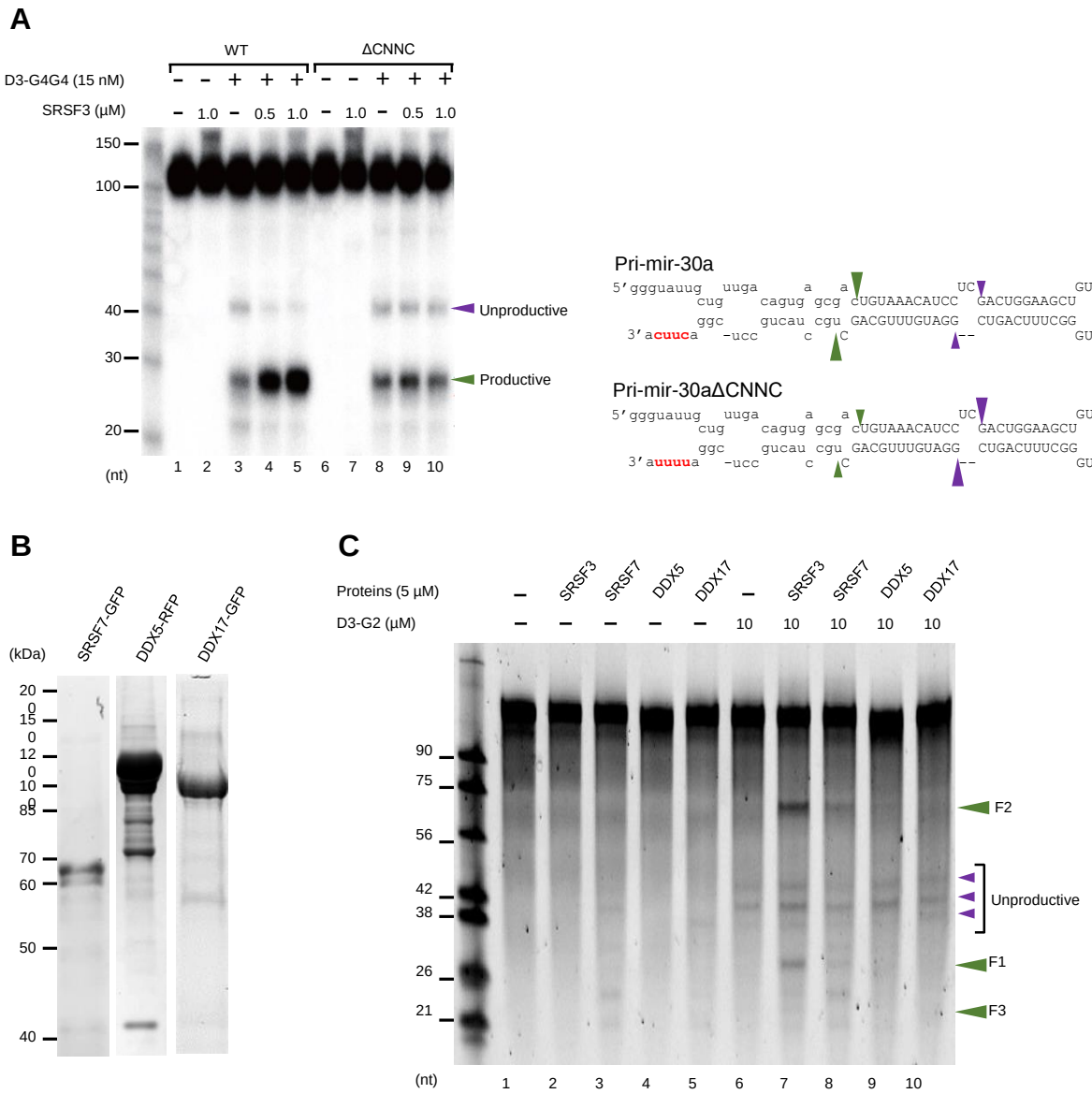
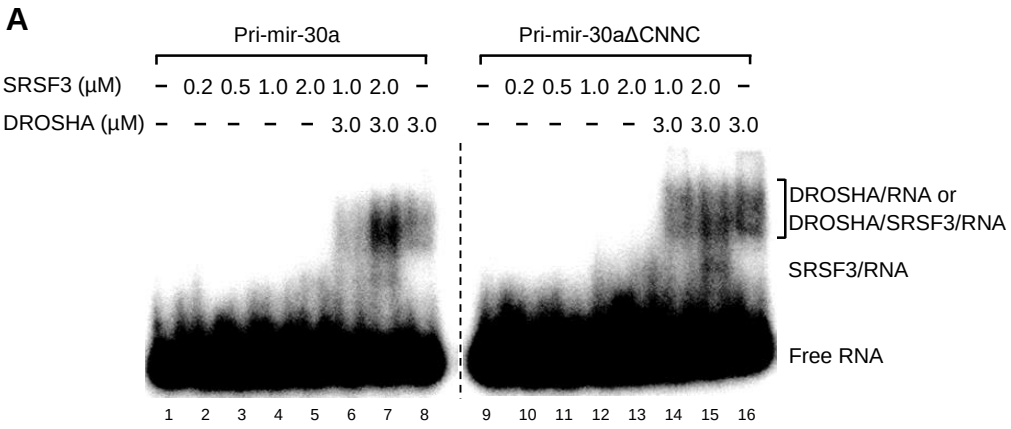
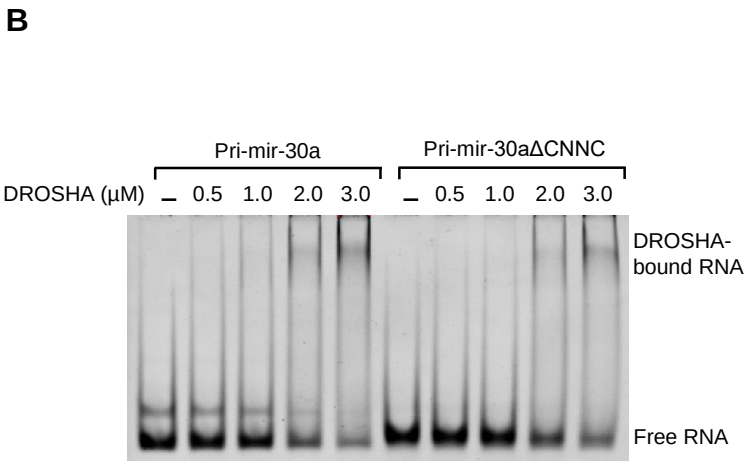


Figure S1. (A) SRSF3 recruits DROSHA to the CNNC-containing basal junction. Processing of the pri-mir-30a substrates by D3-G4G4 and SRSF3 without GFP tag. The end-labeled pri-mir-30a substrates were incubated with the indicated proteins for 1 h under the conditions described in Materials and Methods. The productive and unproductive products are indicated by the green and purple arrowheads, respectively. (B) The proteins were analyzed by SDS-PAGE. (C) The effect of SRSF7 (9G8), DDX5, and DDX17 on pri-miRNA processing by D3-G2. The pri-mir-30a substrates (0.5 μM) were incubated with the indicated proteins for 1 h under the conditions described in Materials and Methods. The three products, a 5′-fragment, pre-miRNA, and a 3′-fragment were named as F1, F2, and F3, respectively.





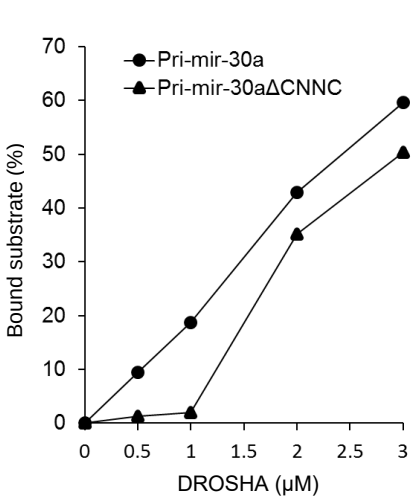


Figure S2. SRSF3 and DROSHA (D3-G2) form a complex with pri-miRNA. (A) The electrophoretic mobility shift assay (EMSA) experiments were carried out with the pri-mir-30a substrates. The increasing amounts of the indicated proteins were incubated with the radiolabeled pri-mir-30a wild-type (WT) or ΔCNNC substrate in 20 μl of the buffer containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 10% glycerol, 0.2 mg/ml BSA, 1 mM DTT and 2 mM EDTA. The reaction mixtures were incubated on ice for 1 h and supplemented with 4 μl of the 6X sample buffer containing 0.01% (w/v) bromophenol blue, 60% (v/v) glycerol. 10 μl of each sample was loaded on 6% native PAGE and run at 4^{o}C for 1 h. (B) The EMSA experiments were carried out with the pri-mir-30a substrates and DROSHA similarly as described in (A). The increasing amounts of the indicated proteins (0.5, 1, 2, 3 μM) were incubated with 0.5 μM of the pri-mir-30a WT or ΔCNNC substrate. (C) The amount (%) of the DROSHA-RNA complexes formed was plotted against the amount of DROSHA. The values of the graph were the average of the three independent experiments.

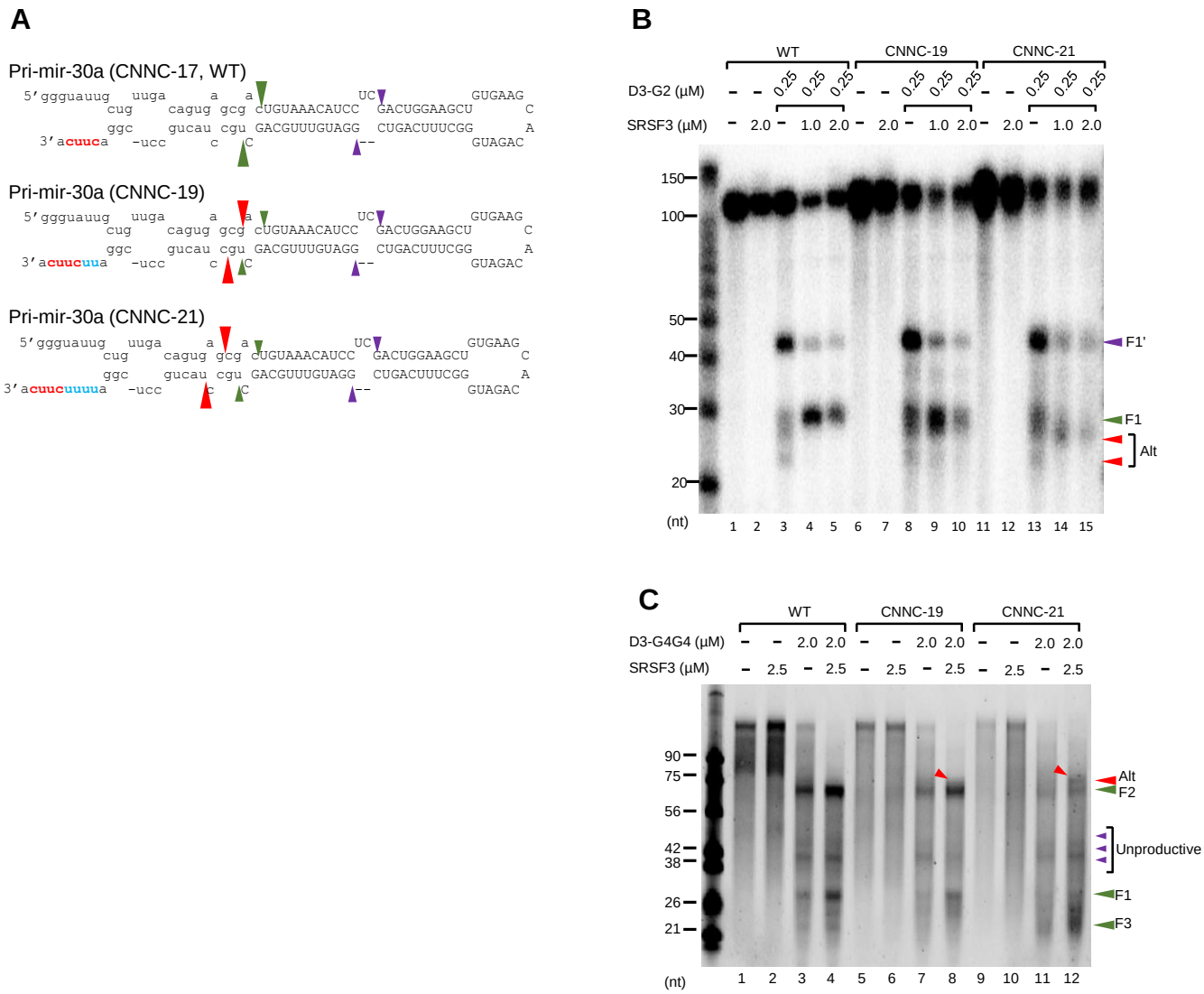


Figure S3. SRSF3 stimulates the productive activity of DROSHA in a CNNC position-dependent manner. (A) The pri-mir-30a substrate. The uppercase letters represent the pre-miRNA region. The CNNC motif and the added nucleotides are highlighted in red and blue, respectively. The green, purple, and red arrowheads indicate the productive, unproductive, and alternative cleavage sites of DROSHA, respectively. (B) Processing of the pri-mir-30a substrates by D3-G2 and SRSF3. The end-labeled pri-mir-30a substrates were incubated with the indicated proteins for 1 h under the conditions described in Materials and Methods. F1 and F1′ are the productive and unproductive products, respectively. The alternative products are indicated with the red arrowheads. (C) Processing of the pri-mir-30a substrates by D3-G4G4 and SRSF3. The pri-mir-30a substrates (5 μM) were incubated with the indicated proteins for 1 h under the conditions described in Materials and Methods. The productive, unproductive, and alternative products are indicated with the green, purple, and red arrowheads, respectively.