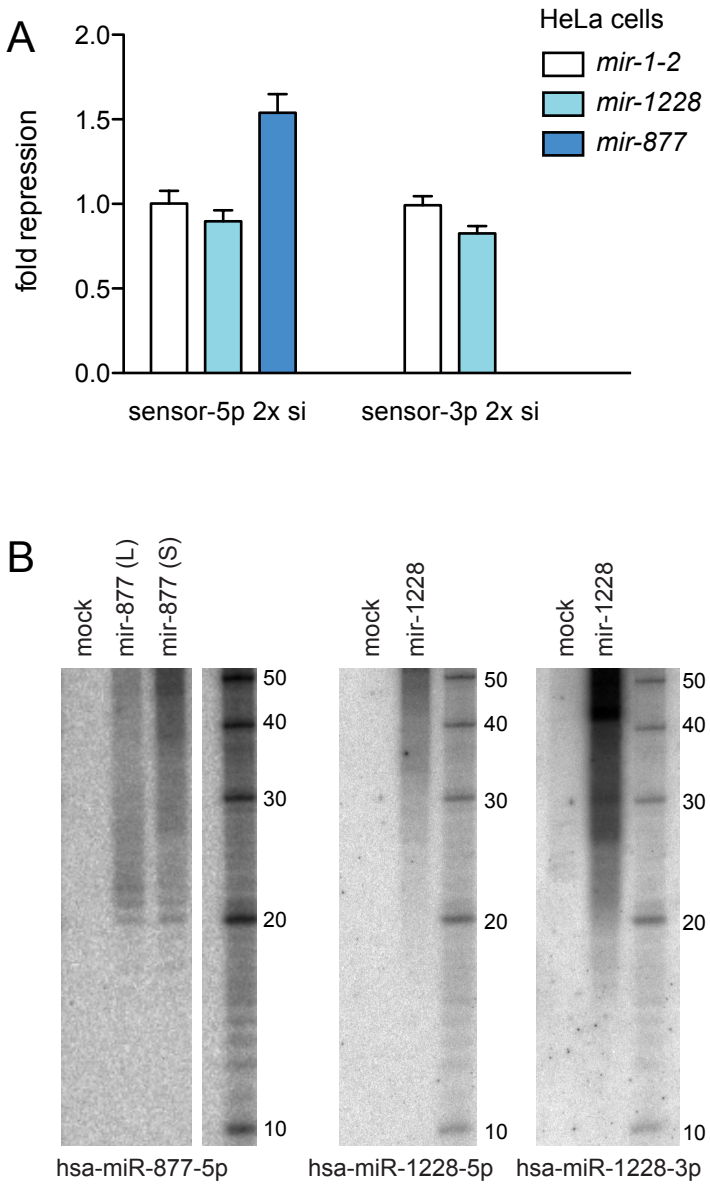

Figure S1: Activity and processing of mammalian mirtrons.

(A) Luciferase sensor tests in HeLa cells. Sensors were cloned in psiCheck2 and contained two perfect (si) sites for the cognate mature miRNA. Values were normalized to sensor activity in the presence of cotransfected mir-1-2. Only a “5p” sensor was tested for miR-877 because its processing is very asymmetric; both “5p” and “3p” sensors were tested for miR-1228. Mild sensor repression was detected only for miR-877-5p.

(B) Northern blots of HeLa cells transfected with the indicated mirtron plasmids. Small RNAs in the size range expected for mature miRNAs were observed only for miR-877-5p, but these did not appear particularly specific and also extended to a length that are not typical for miRNAs (e.g. 20 nt).

Supplementary Figure 1
Maurin and Cazalla et al

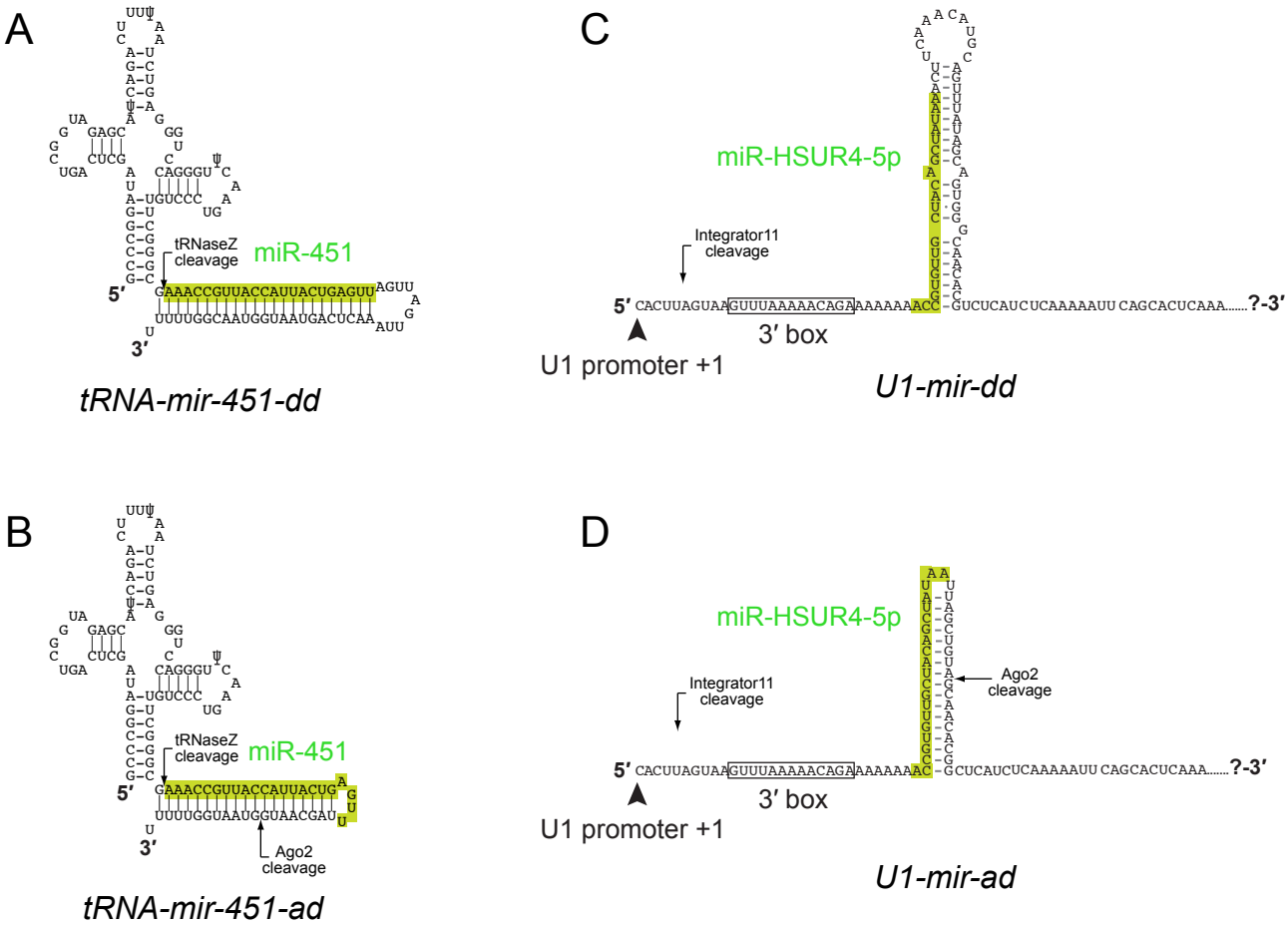

Figure S2: Constructs used to express Dicer-dependent and Ago2-dependent versions of Drosha-independent miRNAs from tRNA and snRNA backbones.

(A, B) Constructs using the $tRNA^{lys}$ backbone to express mature miR-451.
Cleavage by tRNase Z separates the tRNA and hairpin moieties, that latter of which were programmed as Dicer-dependent (dd, panel A) or Ago2-dependent (ad, panel B) lengths.

(C, D) Constructs using the U1 snRNA promoter and HSUR4 3′ box to express mature miR-HSUR4-5p.
Cleavage of the 3' box by the Integrator complex releases the hairpin moieties, which were programmed as Dicer-dependent (dd, panel C) or Ago2-dependent (ad, panel D) lengths.
The U1-mir-HSUR4-dd construct corresponds to U1-H4ΔsnRNA (Cazalla et al, Mol Cell 2011).

Supplementary Figure 2
Maurin and Cazalla et al

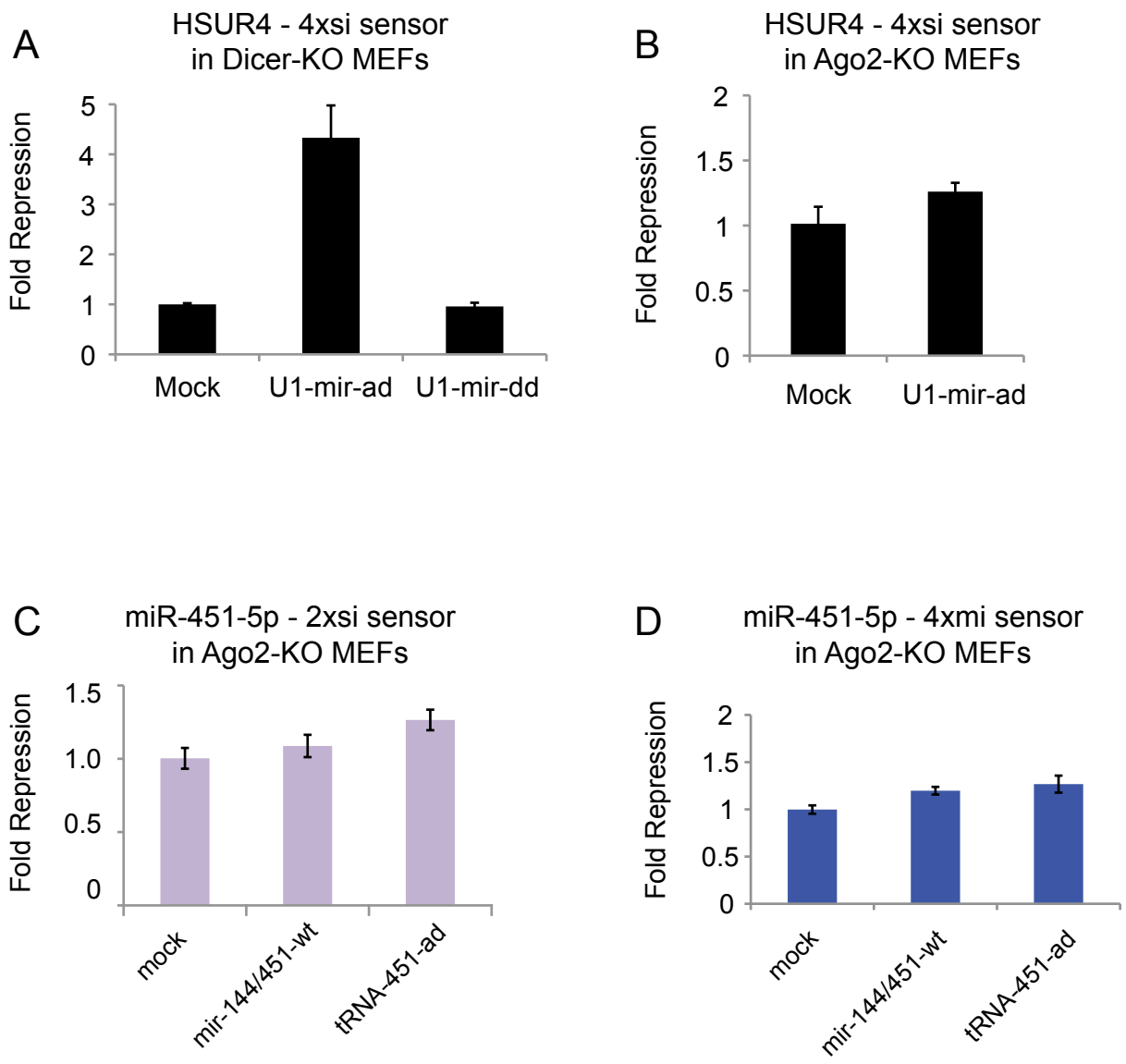

Figure S3: Genetic requirements of target regulation by RNase III-independent miRNAs

(A, B) Assays of Integrator-mediated miRNAs cognate to the HSUR4-5p sequence. Luciferase sensors contained 4 perfect antisense copies of HSUR4-5p. (A) An Ago-dependent miRNA (U1-mir-ad) is functional in Dicer-knockout (KO) mouse embryonic fibroblasts (MEFs), whereas a Dicer-dependent miRNA (U1-mir-dd) is not functional. (B) U1-mir-ad is not functional for target repression in Ago2-KO MEFs.

(C, D) Assays of RNase Z-mediated miRNAs cognate to miR-451 in Ago2-KO MEFs. Comparison is made to the well-characterized wildtype mir-144/451 construct and miR-451 sensors (Yang PNAS 2010). (C) miR-451-5p sensors containing two copies of perfect antisense target sites. Neither mir-144/451 nor tRNA-451-ad constructs are active on this sensor in Ago2-KO MEFs. (D) miR-451-5p sensors containing four copies of perfect antisense target sites. Neither mir-144/451 nor tRNA-451-ad constructs are active on this sensor in Ago2-KO MEFs. Note that these constructs are otherwise highly active in HeLa cells and Dicer-KO cells (main Figure 3).

Supplementary Figure 3
Maurin and Cazalla et al