Table W1. Primer Sequences.

Cloning		
Target	Forward	Reverse
YFP	5′-gtgtgtaagcttaccatggtgagcaagggcgaggagc	5′-acacacggatccgagcttcttgtacagctcgtccatgc
Drosha ΔE31	5′-ccttggcggacctttggaatgagttcattttgaatcag	5′-ctgattcaaaatgaactcattccaaaaggtccgccaagg
Drosha ΔE31-32	5′-ccttggcggacctttggaatgactctgcagacagtgg	5′-ccactgtctgcagagtcattccaaaaggtccgccaagg
Drosha ΔE30-32	5′-catgaaggacacttaactgactctgcagacagtgg	5′-ccactgtctgcagagtcagttaagtgtccttcatg
Flag deletion to obtain HA-DGCR8	5′-ctgatcgccgccgccatgtacccttatgacgtgc	5′-gcacgtcataagggtacatggcggcggcgatcag
Drosha E1045Q	5′-ggccaattgttttcaagcgttaatagg	5′-cctattaacgcttgaaaacaattggcc
Drosha E1222Q	5′-ggcggacctttgcaatcatttattgcagcg	5′-cgctgcaataaatgattgcaaaaggtccgcc
Specific RT for Mature miRNAs		
Target	Stem-loop Primer	
miR-17	5′-gtcgtatccagtgcagggtccgaggtattcgcactggatacgacctacct	
miR-25	5′-gtcgtatccagtgcagggtccgaggtattcgcactggatacgactcagac	
PCR		
Target	Forward	Reverse
Drosha E29-35	5′-ccacaatcagagaatggaattcc	5′-gacaacagtcacagttactgagc
Quantitative PCR		
Target	Forward	Reverse
WT Drosha	5′-gcttctttccacgattgaaagag	5′-ccattgctgctcccatttcc
Drosha ΔE31	5′-ggcggacctttttggaatgag	5′-ccattgctgctcccatttcc
Drosha ΔE31-32	5′-gcggacctttttggaatgactc	5′-ccattgctgctcccatttcc
Drosha ΔE30-32	5′-atcatgaaggacacttaactgac	5′-ccattgctgctcccatttcc
Endogenous Drosha	5′-gtgacatatccaggcggaac	5′-gaagcagcctcagattttgg
Endogenous DGCR8 (5′UTR)	5′-actcgcttagtcgccagtca	5′-ggccacattgctcttttcat
pri-let-7a-3	5′-accaagaccgactgcccttt	5′-ctctgtccaccgcagatatt
pri-miR-17	5′-acatcaccttgtaaaactgaagattg	5′-aaaaagcactcaacatcagcag
pri-miR-25	5′-ggtcgcctactcacaaaacag	5′-ctcacaggacagctgaactcc
pri-miR-16-2	5′-tgttttcatcatcagatgttcgt	5′-agttgctgtatccctgtcacac
miR-17	5′-gtcgtatccagtgcagggtccgaggtattcgcactggatacgacctacct	5′-gtgcagggtccgaggt
miR-25	5′-gtcgtatccagtgcagggtccgaggtattcgcactggatacgactcagac	5′-gtgcagggtccgaggt
Cyclophilin A	5′-gtcaaccccaccgtgttctt	5′-ctgctgtctttgggaccttgt
RPLP0	5′-ggcgacctggaagtccaact	5′-ccatcagcaccacagccttc
Pre-45S rRNA	5′-cggtcgtgtgtgggttgact	5′-ctccttccttccgaggcaga
$tRNA^{Lys}$	5′-cccgaacagggacttgaac	5′-gcccggatagctcagtcg
Primary MiRNA Processing		
Target	t7_Forward	Reverse
pri-miR-15b~16-2	5′-taatacgactcactatagggctaggttggatgaatccta	5′-aatacaaacaattgataaaatag

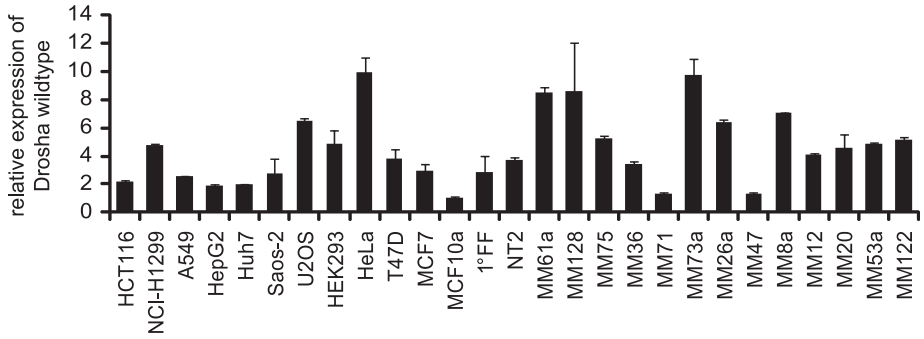

Figure W1. Drosha wild-type expression in different cell lines. Relative expression levels of wild-type Drosha mRNA were quantified by quantitative PCR using primers specific for the wild-type sequence. Values are normalized to RPLP0 levels. One representative data set is shown; error bars, SEM.

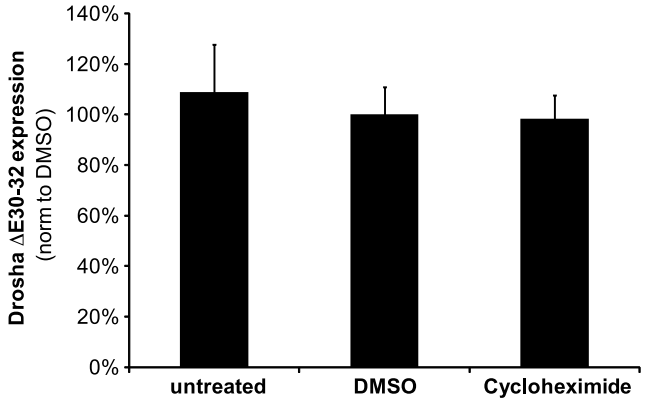

Figure W2. Drosha ΔE30-32 splice variant is not subject to NMD. Ma-Mel-71 melanoma cells were treated with cycloheximide to block the initial round of translation required for NMD. The expression of the Drosha ΔE30-32 splice variant as quantified by quantitative RT-PCR in comparison to wild-type Drosha remained unchanged when NMD was blocked, indicating that it is not subject to NMD. Depicted is the average of three experiments, with error bars representing SEM.

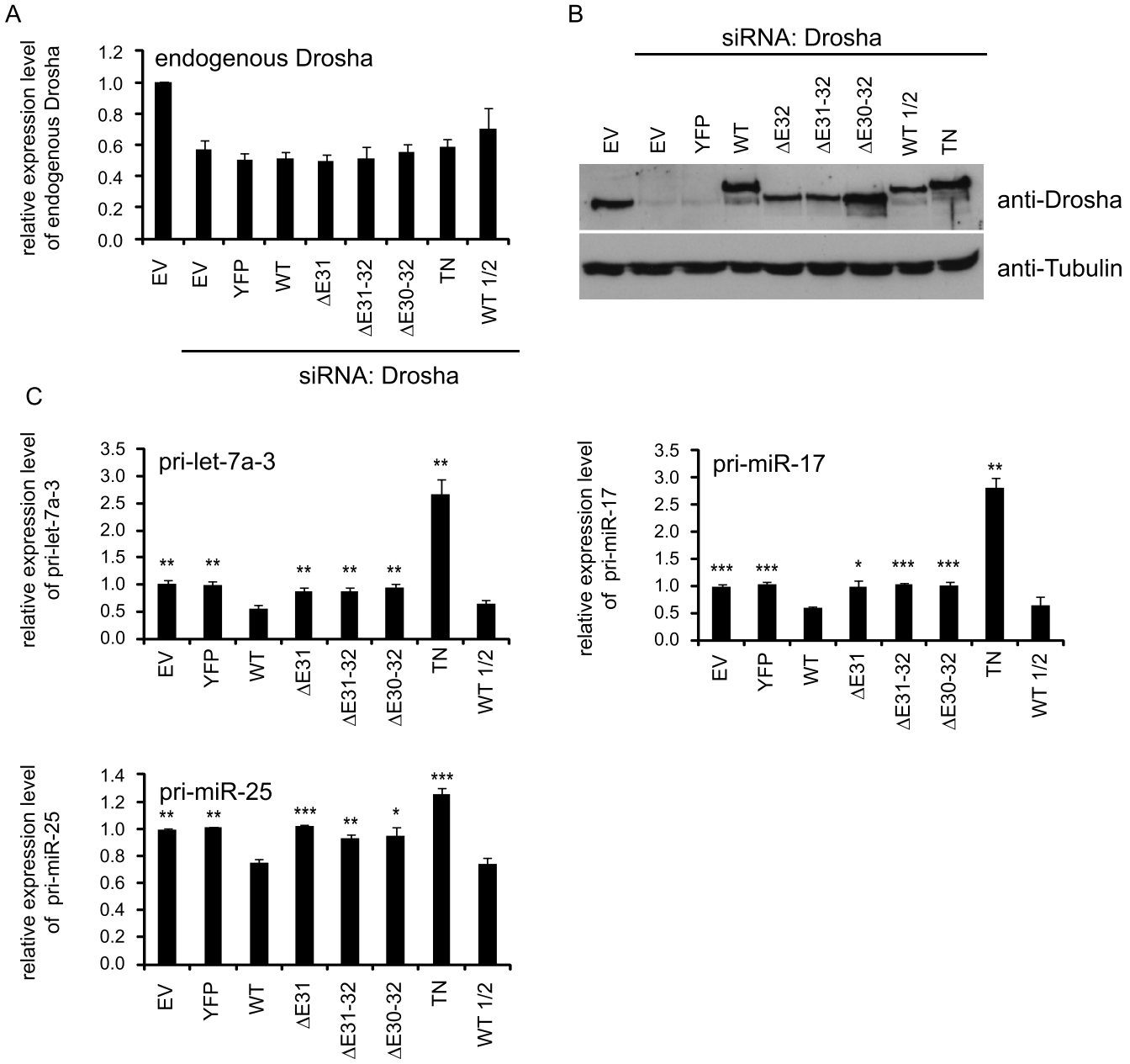

Figure W3. Drosha splice variants exhibit no pri-miRNA processing activity. HEK293 cells depleted of endogenous Drosha using an siRNA against the 3′UTR of Drosha were transfected with HA-DGCR8 and siRNA-resistant Drosha constructs as indicated. WT 1/2 indicates that half the amount of Drosha WT plasmid was used for transfection. EV indicates empty vector; WT, wild-type. (A) Endogenous Drosha levels were determined by quantitative RT-PCR to verify knockdown efficiency. (B) Protein expression levels of endogenous and ectopic Drosha were analyzed by Western blot. (C) Quantification of pri-miRNA levels by quantitative RT-PCR normalized to cyclophilin mRNA levels. $n = 3$. Error bars, SEM. Asterisks denote statistically significant differences: $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ (unpaired t test).