Figure S1 - Related to Figure 1

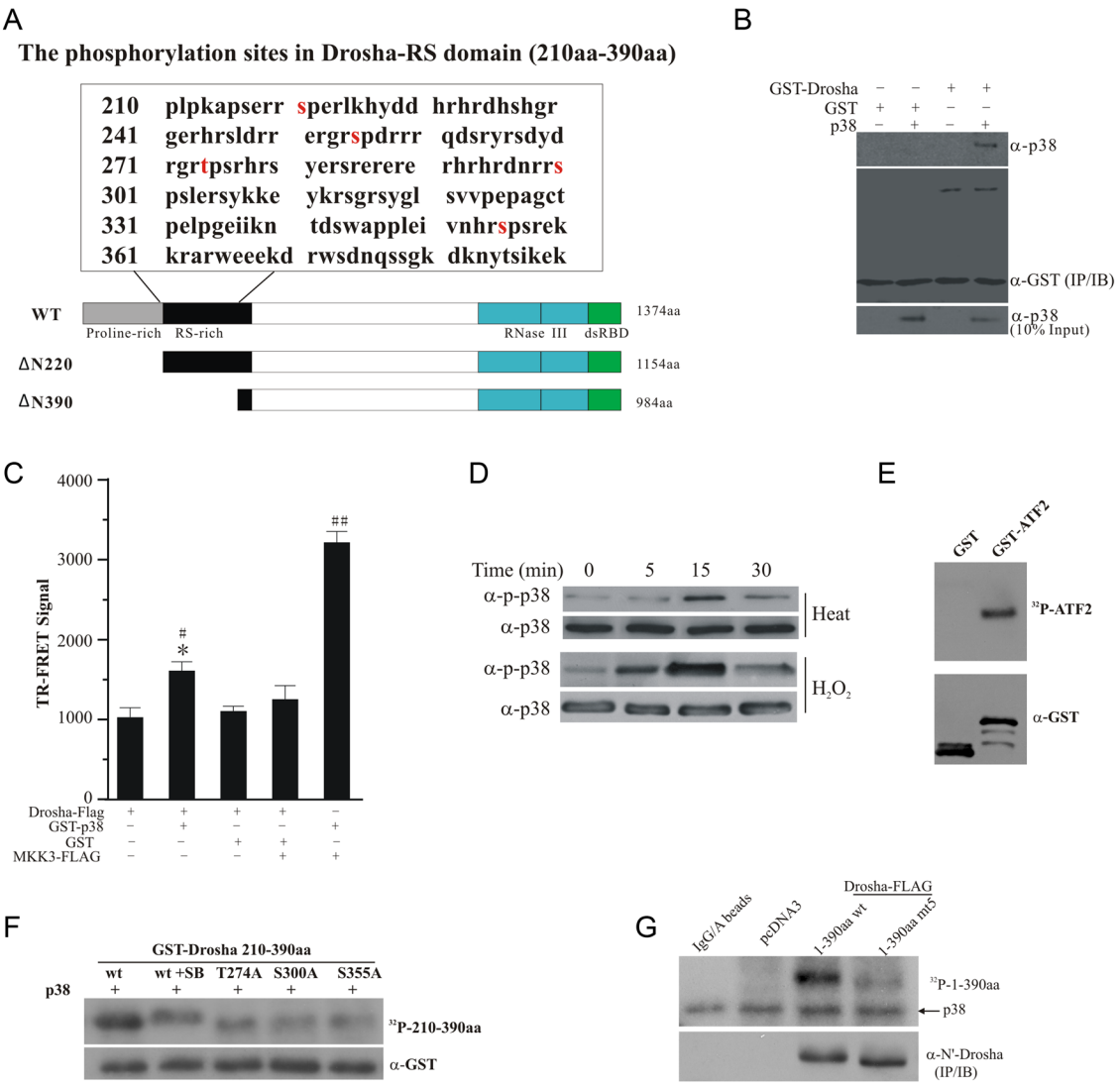

Figure S1 - Related to Figure 1

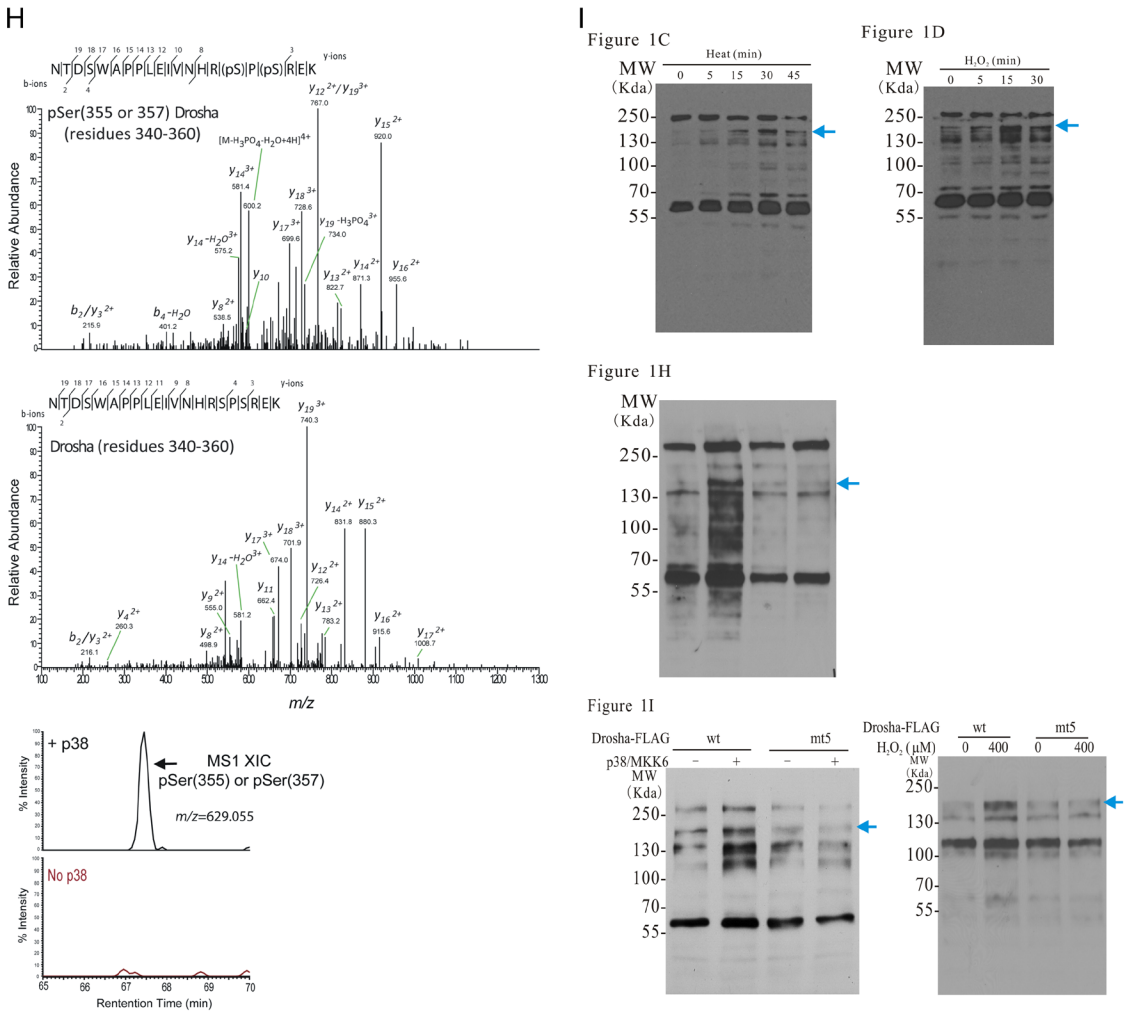

Figure S1 - Phosphorylation of Drosha RS-rich domain by p38 MAPK. (A) The domain structure and phosphorylation sites in the RS-rich domain of Drosha. The proline-directed serine and threonine sites are highlighted in red. The bottom panel shows the structure of the two Drosha truncation mutants used in Figure 1E. (B) Interaction between Drosha and p38 by GST pull-down assay. His-tag p38 (100 ng, Sino Biological) was incubated with purified GST (500 ng) or GST-Drosha(aa210-390, 500 ng) beads for 2 h at 4 °C. The proteins were analyzed by western blotting with anti-p38 and anti-GST antibodies. (C) Controls for interaction between Drosha and p38 by TR-FRET. HEK293 cells were transfected with various plasmids carrying Drosha-Flag, GST-p38, GST, and MKK3-FLAG. Overexpressed proteins from cell lysates were collected for TR-FRET signal assay as desecribed in Figure 1. The TR-FRET signal was expressed as ratio

(A665/A615 × 10^4). The data were analyzed and expressed as mean TR-FRET signal with SD from triplicate samples (*p <0.05 vs Drosha-Flag alone group; $^{\#}p$ < 0.05 or $^{\#\#}p$ < 0.01 vs GST-p38 alone group; ANONA with Student Newman Kuels Test). (D) Stress-induced increase in phosphorylated p38 MAPK. HEK293T cells were treated with heat (45^0C) or H_2O_2 (400 μM) for the indicated period of time and cellular lysates were analyzed by immunoblot for phosphorylated (activated) and total p38 MAPK. (E) Phosphorylation of GST-ATF2 but not GST by p38 MAPK *in vitro*. Commercially purified GST-ATF2 or control GST were incubated with purified active p38 MAPK in a kinase reaction buffer containing γ-^{32}P-ATP. The results were analyzed by electrophoresis and autoradiograph (top panel). The membrane was immunoblotted using an anti-GST antibody (bottom panel). (F) Phosphorylation of Drosha mutants by p38 MAPK. Purified wild and mutant GST-Drosha 210-390aa were incubated with purified p38 MAPK in kinase assay (top panel, autoradiograph). The same membrane was blotted with an anti-GST antibody (bottom panel) [wild type (wt); wt + SB 203580 (SB, 1 μM); mutants: T274A (site 3), S300A (site 4), and S355A (site 5)]. (G) Phosphorylation of Drosha 1-390aa by p38 MAPK. Drosha 1-390aa-FLAG (wt and mt5) was immunoprecipitated from HEK293 cells with anti-N' terminal Drosha antibody and phosphorylated by p38 MAPK *in vitro*. (H) Recombinant GST-Drosha aa210-390 was incubated with or without recombinant p38 MAPK and peptides were examined by LC-MS/MS following LysC digestion. **Top two panels:** representative Drosha quadruply charged (M+4H) phosphopeptideMS/MS spectrumdisplaying precursor neutral loss peak (*m/z* 600.2*)* of phosphoric acid (upper panel). A mass shift of 80 Da on the assigned *y*-ions series in the modified MS/MS spectrum is observed compared to the unmodified peptide spectrum (bottom panel). **Bottom two panels:** Representative extracted ion chromatograms (measured as the percentage intensity using ± 20 ppm mass tolerance) for phosphopeptide (*m/z* = 629.055) in the p38 treated and untreated Drosha samples. *X*-axis indicates the retention time when the peptide eluted from the LC column. Peptide intensities were normalized to the sample containing p38 kinase (in black). (I) Full blots of anti-p-Ser-substrates for Figure 1 panels as indicated. Arrow indicates the position of Drosha.

Figure S2 - Related to Figure 2

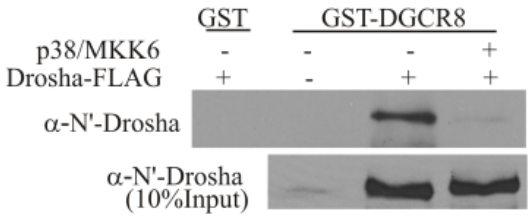

Figure S2 - Phosphorylation-reduced change in binding between Drosha and DGCR8. Purified GST-DGCR8 was incubated with lysates transfected as indicated in pull-down assay. The levels of bound Drosha (top panel) and Drosha input (bottom panel) were blotted.

Figure S3 - Related to Figure 3

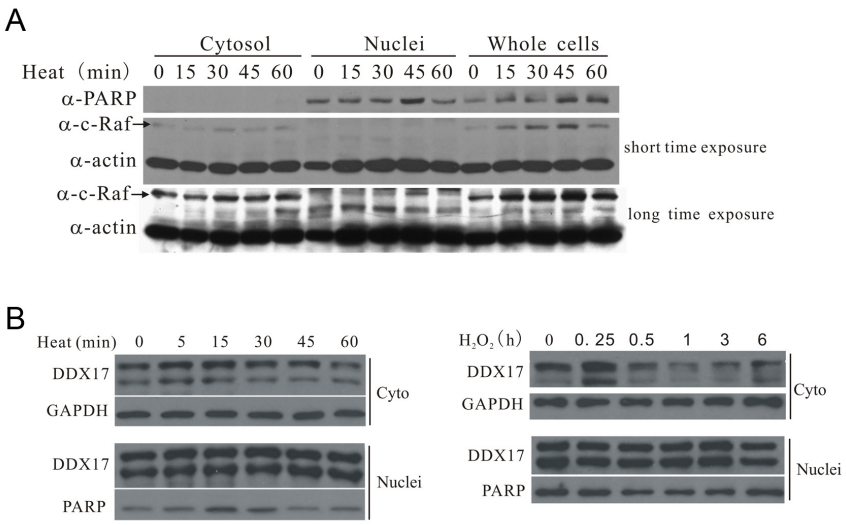

Figure S3 - Stress-induced phosphorylation-dependent nuclear export of Drosha. (A) The quality control for cross contamination of cytoplasmic and nuclear fractions. HEK297 cells were exposed to heat (45°C) for the indicated time. Whole cell lysates and cytoplasmic as well as nuclear fractions were prepared and blotted for Drosha. C-Raf and PARP were as cytoplasmic and nuclear markers, respectivly. (B) Different patterns of protein translocation from nuclei to cytosol following heat or H_2O_2 stress. HEK293 cells were treated with heat (45^{o}C) or H_2O_2 (400 μM) at different time periods indicated. Cytosol and nuclear fractionas were blotted with DDX17 antibody. GAPDH and PARP were used as cytosol and nuclear markers, respectively.

Figure S4 - Related to Figure 4

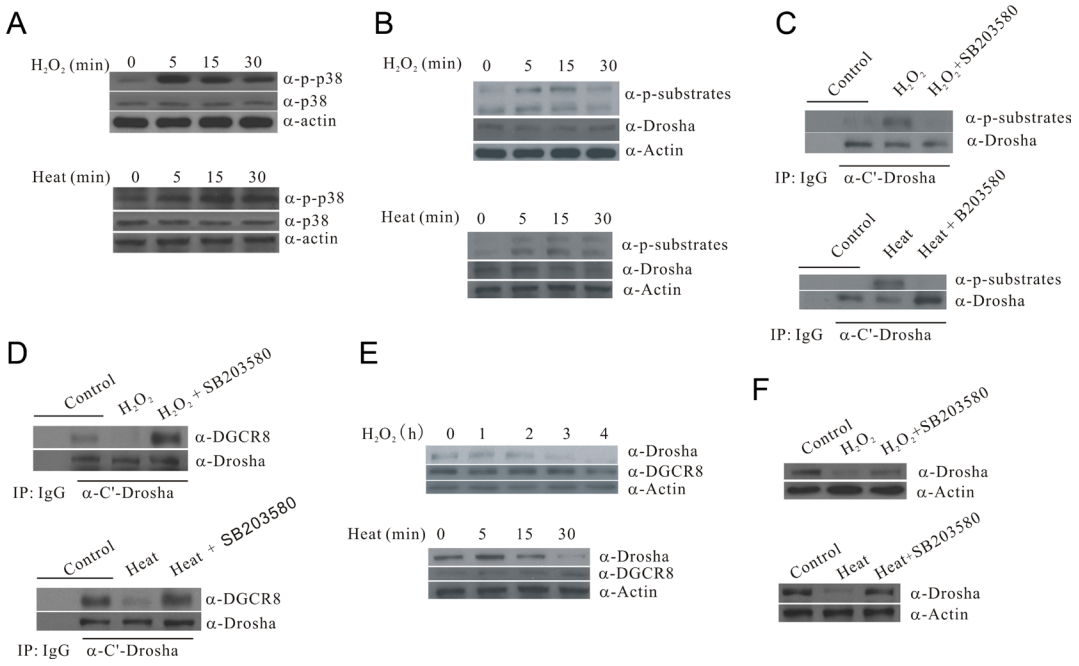

Figure S4 - p38 MAPK-mediated phosphorylation and degradation of Drosha in primary cortical neurons. **(A)** H_2O_2- and heat-induced phosphorylation of p38 MAPK in primary cortical neurons. Primary cortical neurons at 12 DIV (days in vitro) were treated with heat (45^0C) or H_2O_2 (100 μM) for the indicated time. The levels of phosphorylated and total p38 MAPK were blotted. **(B)** H_2O_2-and heat-induced phosphorylation of Drosha in primary cortical neurons. Primary cortical neurons at 12 DIV were treated with heat (45^0C) or H_2O_2 (100 μM). The levels of phosphorylated Drosha were blotted. **(C)** p38-mediated phosphorylation of Drosha in primary cortical neurons. Cortical neurons were treated with vehicle or SB203580 (10 μM) for 30 min prior to H_2O_2 (100 μM, 10 min) or heat (45^0C, 15 min) treatment. Immunoprecipitated Drosha from lysates was blotted for phosphorylation with the anti-phospho Ser antibody. **(D)** Stress-induced inhibition of Drosha and DGCR8 interaction in primary cortical neurons. Cortical neurons were treated as described in (B). Drosha was immunoprecipitated from the lysates and the precipitates were blotted for DGCR8. **(E)** Stress-induced change in Drosha level in parimary cortical neurons. Primary cortical neurons at 12 DIV were treated with H_2O_2 (100 μM) or heat (45^0C) as indicated. At different time points, Drosha and DGCR8 levels were blotted. **(F)** Effect of inhibition of p38 MAPK on Stress-induced degradation of Drosha in primary cortical neurons. Drosha levels from primary cortical neurons after stress (H_2O_2 at 100 μM for 3 h; heat at 45^0C for 30 min) with or without 30 min pretreatment of SB203580 were blotted.

Figure S5- Related to Figure 6

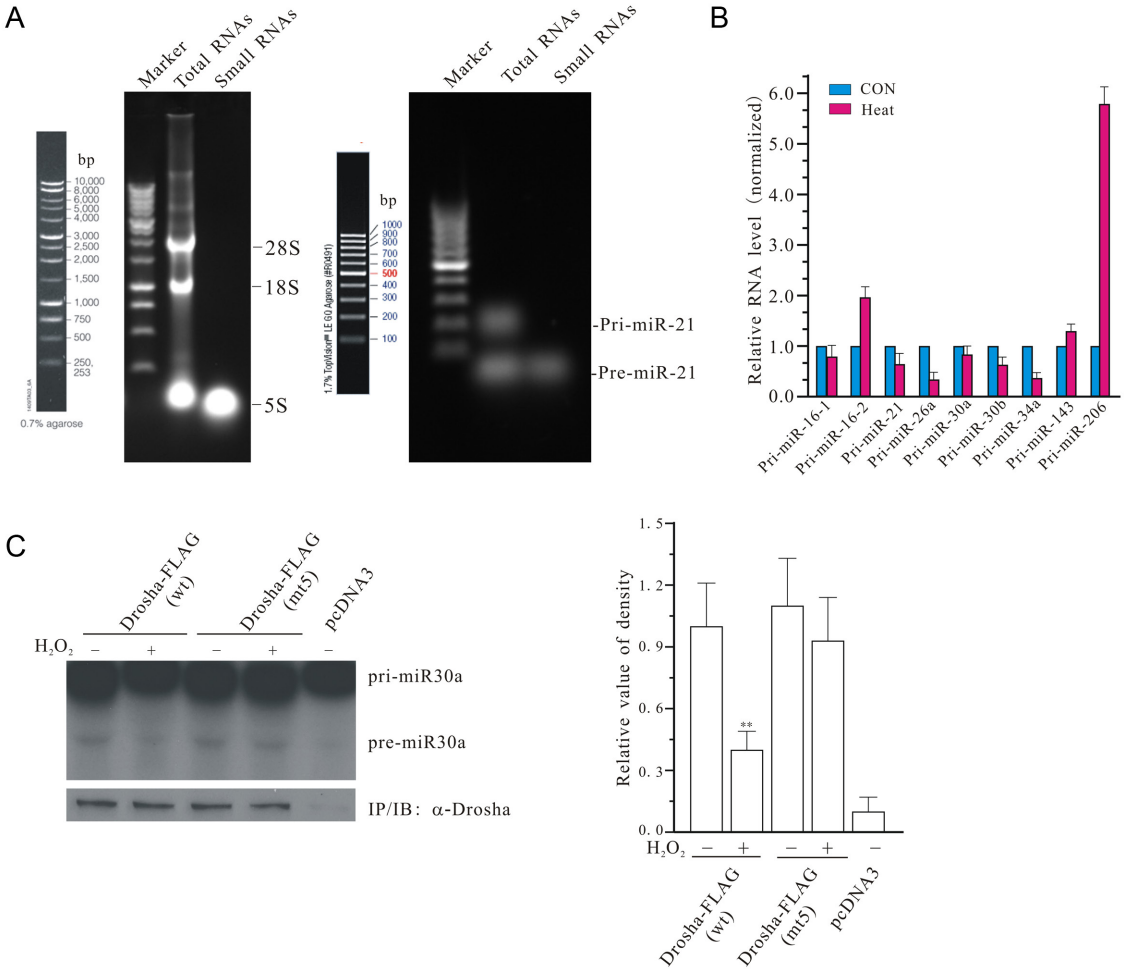

Figure S5 - Stress- and p38 MAPK-induced loss of Drosha function. (A) Assessment of total and small RNA preparations. Left, image of electropheresis shows that 1 μg of total RNAs purified with TRIzol reagent (Invitrogen) or small RNAs purified with PureLink miRNA isolation kit (Ambion, K1570-01). Right, image of electropheresis shows results of qRT-PCR using RNA sampels purified with TRIzol reagent (Invitrogen) or PureLink miRNA isolation kit (Ambion, K1570-01). (B) Analysis of endogenous pri-miRNAs following stress by qRT-PCR. Levels of various pri-miRNAs in the total RNAs isolated by TRIzol from HEK293 cells treated as described in Figure 6A were quantified by qRT-PCR. (C) Resistance to H_2O_2-induced inhibition of pri-miR-30a processing by mt5 Drosha. HEK293 cells transfected as indicated were exposed to H_2O_2 (400 μM for 12 h). Comparable amounts of Drosha-FLAG were immunoprecipitated from the lysates and assessed for pri-miR-30a conversion as described in Figure 6B (right graph is quantification of three independent experiments. $^{**}p < 0.01$ vs Drosha-FLAG without H_2O_2).

Figure S6- Related to Figure 7

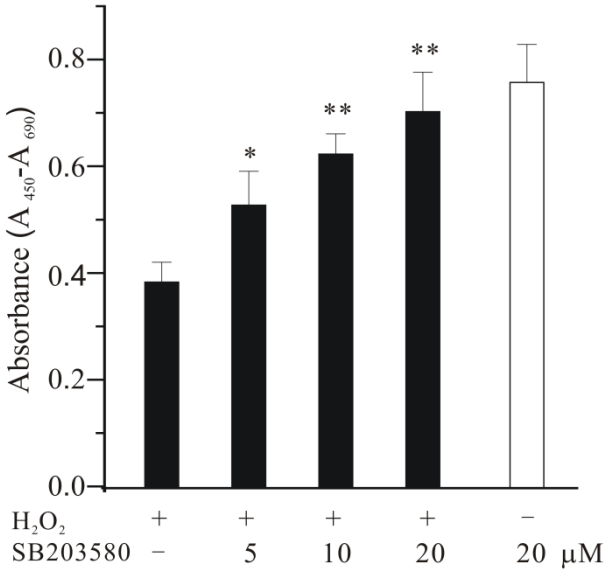

Figure S6 - H_2O_2-induced p38 MAPK dependent apoptosis in HEK293T cells. HEK293T cells were exposed to H_2O_2 (400 μM) for 24 hr with or without treatment of SB230580 (added 30 min before H_2O_2). Cell viability was measured by WST-1 assay (mean ± SEM, **p*<0.05, ***p*<0.01, ANOVA and Dunnett's test).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Drosha Phosphorylation Analysis by Proteomics with MS/MS

Immunoprecipitated samples were prepared as previously described(Xu et al., 2009). Peptides were ionized with 2.0 kV electrospray ionization voltage from a nano-ESI source (Thermo) on a hybrid LTQ XL Orbitrap mass spectrometer (Thermo). Data dependent acquisition of centroid MS spectra at 30,000 resolution and MS/MS spectra were obtained in the LTQ following collision induced dissociation. The SageN Sorcerer SEQUEST 4.3 algorithm was used to search and match MS/MS spectra to a target-decoy human refseq database (Xu et al., 2009). The MS/MS spectra of the matched Drosha phosphopeptide was manually validated as described previously(Herskowitz et al., 2010). The spectrum corresponding to ser355 on Drosha was also manually examined for signature phosphate neutral losses (-24.5 m/z) for quadruply charged peptides.

Primary Neuron Cultures

Culture of primary cortical neurons from Long Evans rats at embryonic day 18 was carried out as described previously (Mao and Wiedmann, 1999). Briefly, Cortical neurons were digested with trypsin and plated on poly-L-lysine-coated plates with neurobasal medium (Invitrogen) containing 2% B27 (Invitrogen) and 0.5 mM glutamine (Cellgro). Cortical neurons were treated at 15 days in vitro or culture time specifically indicated.

qRT-PCR Assay

Primers used to determine pre-miRNAs are listed in the following table:

pre-miRNA	Forward primer	Reverse primer
pre-miR-30a	5'-CGACTGTAAACATCCTCGAC	5'- GCAAACATCCGACTGAAAGCC
pre-miR-30b	5'-CATGTAAACATCCTACACTCAGCT	5'-ATCCACCTCCCAGCCAAT
pre-miR-21	5'-TGTCGGGTAGCTTATCAGAC	5'-TGTCAGACAGCCCATCGACT
pre-miR-34a	5'-TGGCAGTGTCTTAGCTGGTTG	5'-GGCAGTATACTTGCTGATTGCTT
pre-miR-16-1	5'-GCAGCACGTAAATATTGGCGT	5'-CAGCAGCACAGTTAATACTGGAGA
pre-miR-16-2	5'-GCACGTAAATATTGGCGTAGT	5'-AAGCAGCACAGTAATATTGGTG
pre-miR-26a	5'-TTCAAGTAATCCAGGATAGGCTGT	5'-TGCAAGTAACCAAGAATAGGCC
pre-miR-103	5'-GCTTCTTTACAGTGCTGCCT	5'-TTCATAGCCCTGTACAATGCT
pre-miR-143	5'-TGAGGTGCAGTGCTGCATC	5'-GCTACAGTGCTTCATCTCAGACTC
pre-miR-145	5'-GTCCAGTTTTCCCAGGAATC	5'-AGAACAGTATTTCCAGGAAT
pre-miR-206	5'-ACATGCTTCTTTATATCCCCA	5'-AAACCACACACTTCCTTACATTC
5S rRNA	5'-TACGGCCATACCACCCTGA	5'-GGCGGTCTCCCATCCAA

Primers used to determine pri-miRNAs are listed in the following table:

pri-miRNA	Forward primer	Reverse primer
pri-miR-30a	5’-GTTGCCTGCACATCTTGGAA	5’-CCGACTGAAAGCCCATCTGT
pri-miR-30b	5’-GTGAATGCTGTGCCTGTTC	5’-GCCTCTGTATACTATTCTTGCCA
pri-miR-21	5’-TTTTGTTTTGCTTGGGAGGA	5’-AGCAGACAGTCAGGCAGGAT-3′
pri-miR-34a	5’-CAACCAGCTAAGACACTGCCAA	5’-CCTCCTGCATCCTTTCTTTCCT
pri-miR-16-1	5’-CCTCTAATGCTGCATAAGCT	5’-CCAGTATTAACTGTGCTGCT
pri-miR-16-2	5’-CGTTTTATGTTTGGATGAACTG	5’-CGCCAATATTTACGTGCTG
pri-miR-26a	5’-AATGAAGCCACAGGAGCCA	5’-TGCACAGCCTATCCTGGATTA
pri-miR-103	5’-TACTTGAATCCAGCCACAGCC	5’-TCATGACCTGGACAGACTGTCC
pri-miR-143	5’-GTGCTGCATCTCTGGTCAGTTG	5’-AGCACTTACCACTTCCAGGCTG
pri-miR-145	5’-GGGATTCCTGGAAATACTGT	5’-CCTCTTACCTCCAGGGACAG
pri-miR-206	5’-AAGGAAGTGTGTGGTTTCGGC	5’-TGGCACAAAGCCCTGATGA
β-*actin*	5’-TCACCCACACTGTGCCCATCTACGA	5’-CAGCGGAACCGCTCATTGCCAATGG

Northern Blot Assay

Probes used to detect miRNAs or pre-miRNA 30a are listed in the following table:

miRNA	Probe sequences
miR-30a	5’-CTTCCAGTCGAGGATGTTTACA
miR-26a	5’-AGCCTATCCTGGATTACTTGAA
miR-34a	5’-ACAACCAGCTAAGACACTGCCA
pre-miR-30a	5’-GCCCATCTGTGGCTTCACAG

SUPPLEMENTAL REFERENCES:

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Mao, Z., and Wiedmann, M. (1999). Calcineurin enhances MEF2 DNA binding activity in calcium-dependent survival of cerebellar granule neurons. J Biol Chem *274*, 31102-31107.

Xu, P., Duong, D.M., and Peng, J. (2009). Systematical optimization of reverse-phase chromatography for shotgun proteomics. J Proteome Res *8*, 3944-3950.