

## **Supplemental Material to:**

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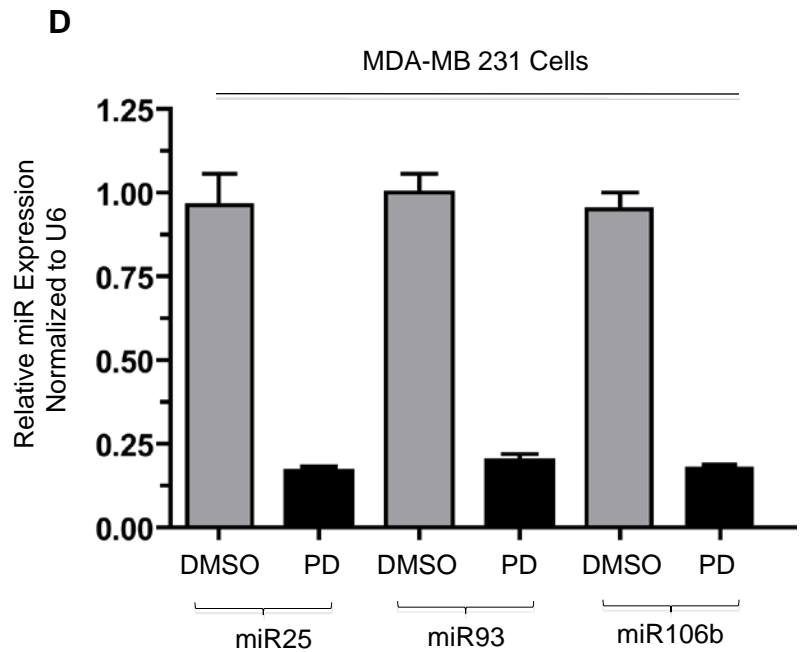
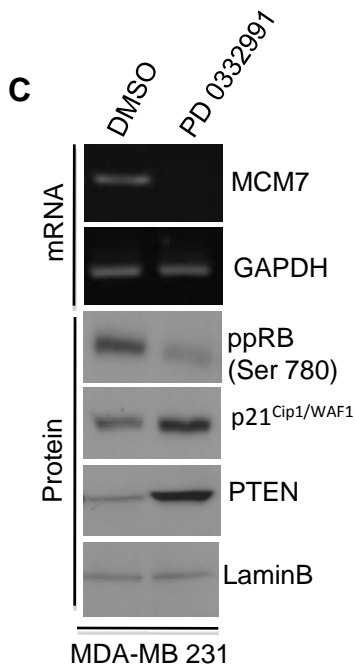
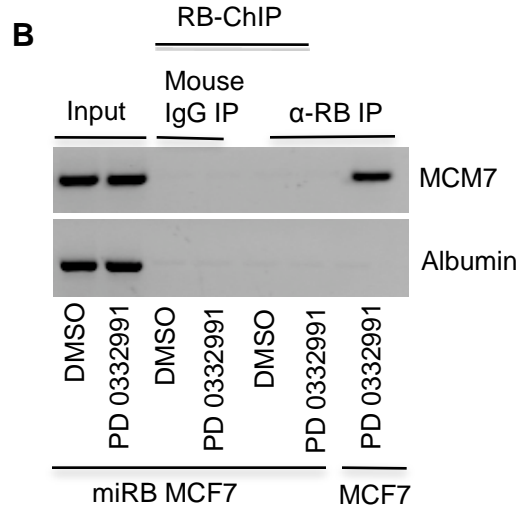
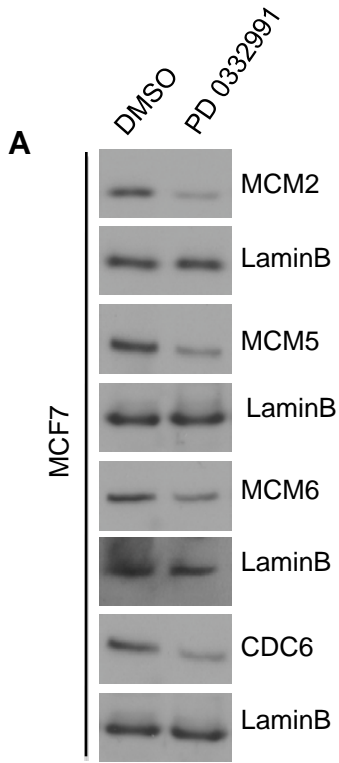
**Regulation of miR106b cluster through the RB-pathway:  
Mechanism and functional targets**

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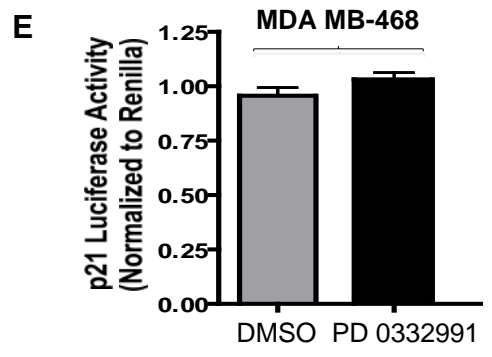
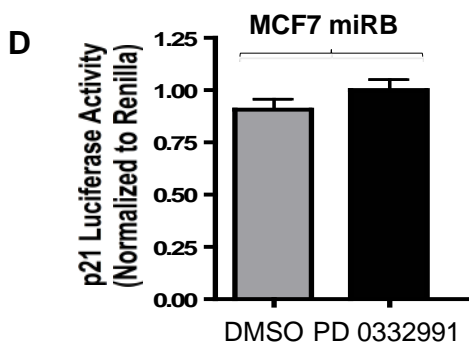
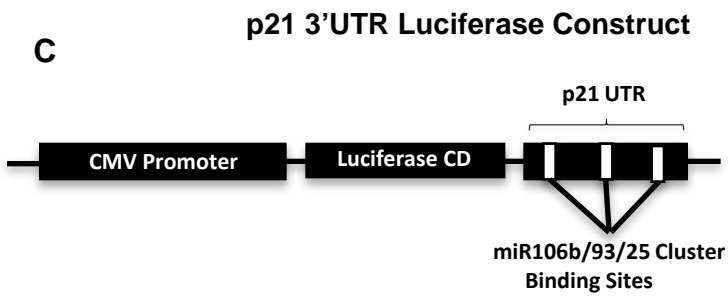
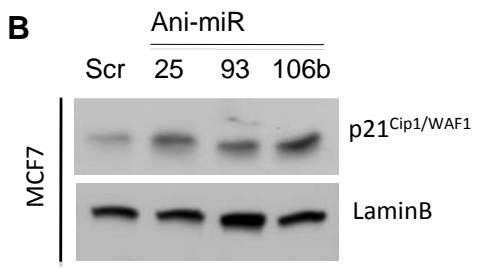
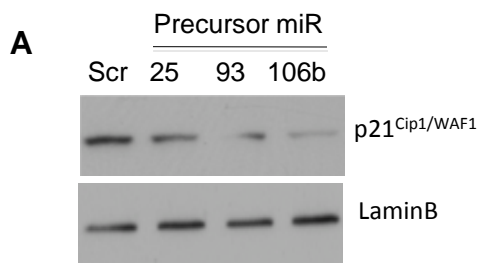
# Supplemental Figure 1



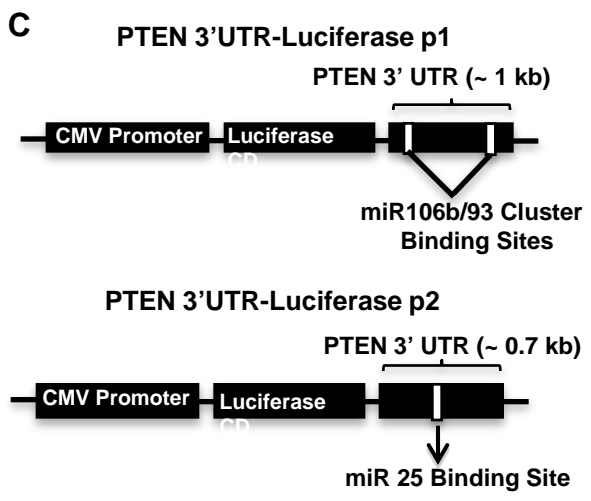
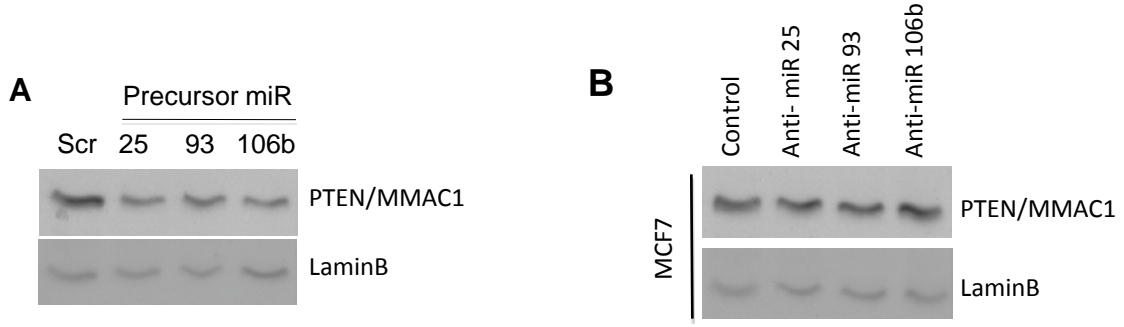
## Supplemental Figure. 2

microRNA	Gene Accession #	Homology (%)			Seed Sequence	Predicted mRNA Targets
		H	M	R		
miR-25	MI0000082	100	100	98	N	SLC 12A5,MAP2K4,ATP2B4,NF1B,NRK1AURKA,SP1,E2F3,USF-2,DDIT4,PTEN and p21
miR-93	MI0000095	100	96	94	Y	FIBIN,NRMD6,CHD9,MARK4,PTEN and 21
miR-106B	MI0000734	100	96	96	Y	ATL3,USP32,MYCL1,PTEN , RB and p21
miR-99b	MI0000746	100	100	100	N	CTDSPL,IGF1R,NXF1 and SMARCD1
miR-324-3p	MIMAT0000761	100	100	100	N	CCNM,SAP18,RPA2,AKT2,TET2,STAGE2,RXRB,MEF2D,CALN1,LAD and ACTN4
miR-324-5p	MIMAT0000762	100	100	100	N	SEPT3,SMAD2,RUNX1,CUL5,SRF,SMARCD1,SOX,NFAT5 andCLCN4
miR-494	MI0003134	100	100	99	N	AR1D4B,IKBKAP,MAP4,PTEN,NOVA1 and MAP4
miR-671	MI0003760	100	100	99	N	FAM54B,GNAO1,TOMM40,BRCA1,SLC3OA6,LIN9 ST85IA5,CFL2,KRT9 and SUMF2

# Supplemental Figure 3

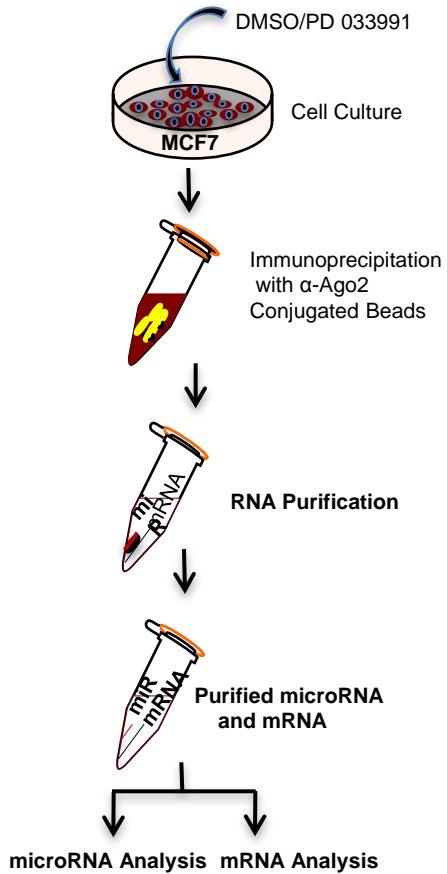


# Supplemental Figure 4

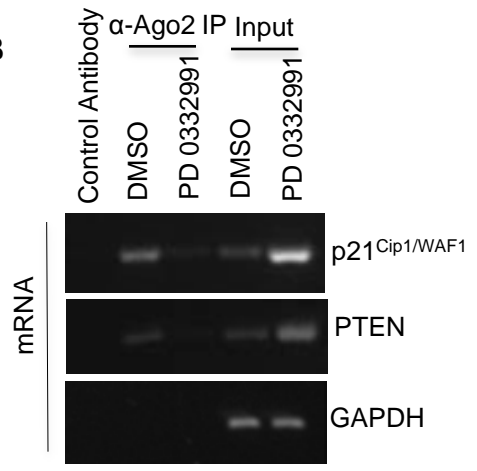


# Supplemental Figure 5

## A RNA IP Schematics



## B



**Supplemental Information:**

**Supplemental Table1.** Primers used for RNA/DNA manipulation in the study.

**Supplemental Figure1. Analysis of DNA replication complex gene regulation and RB CHIP in MCF7 and miRB MCF7 Cells.** (A) Immunoblotting of MCM2, MCM4, MCM6, CDC6 and loading control LaminB. (B) pRB CHIP on RB/E2F consensus binding site I of MCM7 promoter in response DMSO or PD 0332991 in RB deficient miRB cells. (C) Semi-quantitative RT-PCR analysis of MCM7, GAPDH mRNA, immunoblot analysis of RB, p21, PTEN and LaminB proteins in MDA-MB 231 cells in response to PD 0332991. (D) QRT-PCR analysis of of miR 25,93 and 106b microRNAs in MDA-MB 231 cells in response to DMSO or PD 0332991. Each data point is a mean  $\pm$  SD from 3 or more independent experiments. For all experiments,  $p < 0.001$ -  $p < 0.05$  were considered as significant.

**Supplemental Figure 2.** Table shows the predicted homology, seed sequence and differentially regulated microRNAs and their target mRNAs.

**Supplemental Figure 3. Analysis of p21 regulation in response to CDK4/6 inhibitor and miR106b-cluster.** (A) Immunoblotting of p21 in precursor miR106b-cluster expressed MCF7 cells and loading control LaminB. (B) Immunoblotting of p21 in anti-miR miR106b-cluster expressed MCF7 cells. (C) Schematic representation of p21 3'UTR luciferase construct. (D) p21 3' luciferase activity in miRB MCF7 cells in response to PD 0332991. (E) p21 3' luciferase activity in MDA-MB 468 cells in response to PD 0332991.

**Supplemental Figure 4. Analysis of PTEN/MMAC1 regulation in response to miR and**

**anti-miR106B-cluster.** (A) Immunoblotting of PTEN in ectopically expressed precursor miR106b-cluster in MCF7 cells. (B) Immunoblotting of PTEN in anti-miR miR106b-cluster expressing MCF7 cells. (C) Schematic representation of PTEN 3'UTR luciferase construct (p1 and p2).

**Supplemental Figure5.** (A) Schematic representation of stepwise RNA immunoprecipitation procedure. (B) Agarose gel picture of semi-quantitative RT-PCR analysis of p21, PTEN and loading control GAPDH mRNA from RNA immunoprecipitates in MCF7 cells in response to CDk4/6 inhibitor.



