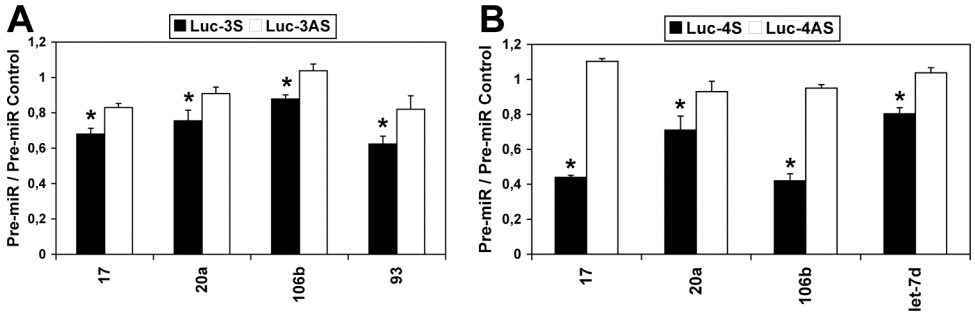
Supplementary Table 1: *miR-17-92* miRNA target sites in human *GAM* 3'-UTR whose sequence is 100% conserved in *Canis familiaris*, *Mus musculus* and *Rattus Norvegicus*.

MiRNA target sites	*miR-92a-1, miR-92-a-2, miR-92b, miR-25*	*miR-17*, *miR-20a, miR-20b, miR-93, miR-106a, miR-106b*	*miR-17*, *miR-20a, miR-20b, miR-93, miR-106a, miR-106b*
Sequence match	GUGCAAUA	GCACUUUA	GCACUUUA
Position in *GAM* 3'-UTR	229-236	2302-2309	2683-2690
Nt changed in mutants	CA-GUU--	CG-GAA--	C-U-AAA-

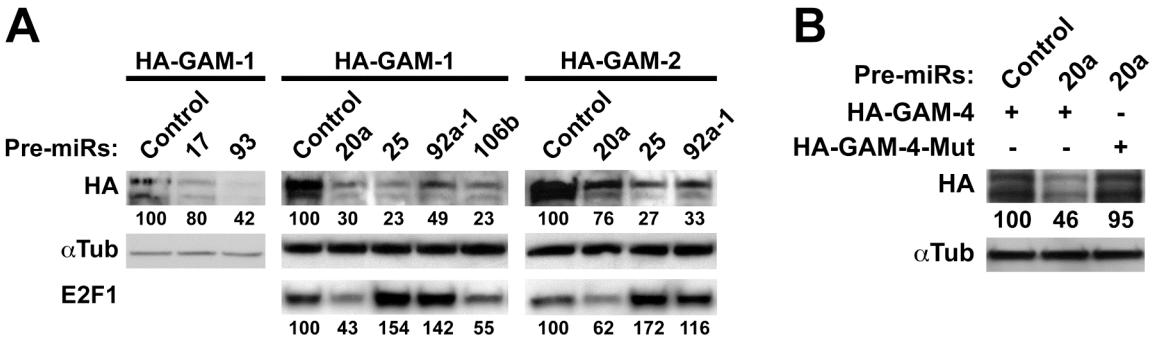
Supplementary Table 2: *let-7* miRNA target sites in human *GAM* 3'-UTR whose sequence is 100% conserved in *Canis familiaris*, *Mus musculus* and *Rattus Norvegicus*.

let-7 target sites	1	2	3
Sequence match	CUACCUC	CUACCUC	CUACCUC
Position in *GAM* 3'-UTR	499-505	598-604	2589-2595
Nt changed in mutants	GAU-GAG	GA-GGA-	G-U-G-G

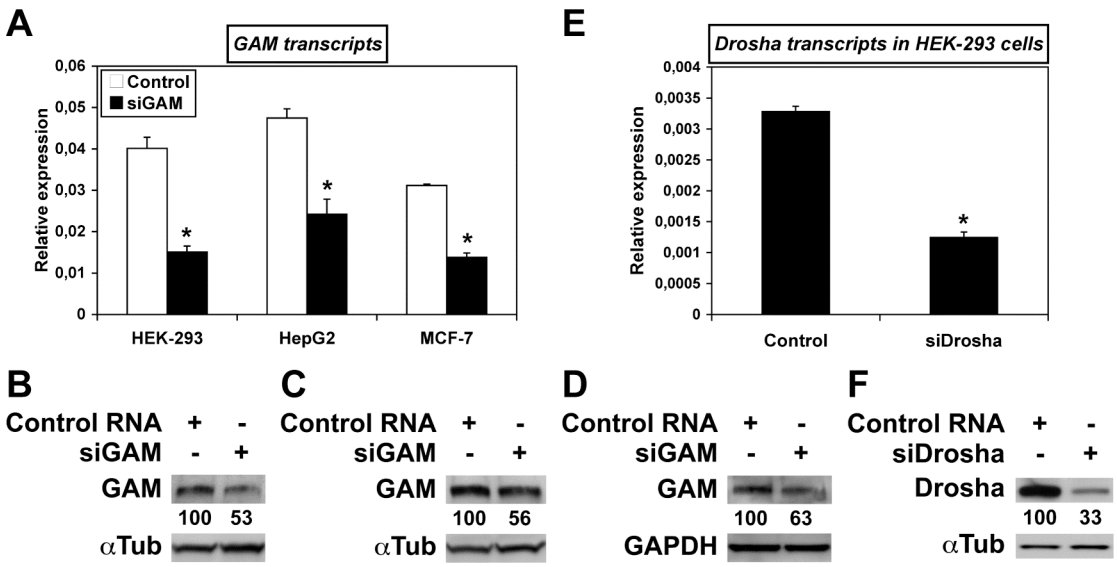


Supplementary Figure 1: The two *miR-17/20a* and the third *let-7* sites in *GAM* 3'-UTR are respectively targeted by *miR-17-92* and *let-7* miRNAs. Luciferase assays were done with *Luc-3* (A) or *Luc-4* (B) as indicated, both in sense (*S*) and antisense (*AS*)

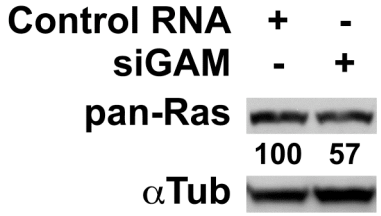
orientation. Bars show the ratios of the *Firefly* luciferase (normalized to *Renilla* luciferase) activity measured following transfection with the indicated pre-miRNAs to that obtained following transfection with the pre-miR Control for the same construct. Values represent the mean ± standard deviation (n = 4). *, Significantly different from pre-miR Control, $P < 0.01$.



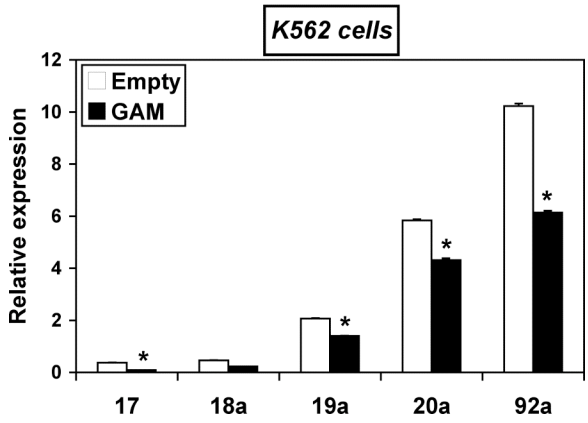
Supplementary Figure 2: While *miR-17-92* miRNAs decrease HA-GAM levels, *miR-92a-1* and its ortholog *miR-25* upregulate E2F1. A,B. HEK-293 cells were transfected with either *HA-GAM-1* or *HA-GAM-2* (A) or *HA-GAM-4* or *HA-GAM-4-Mut* (a construct containing a mutated *miR-17/20a* target site) (B), along with a pre-miR-Control or the indicated pre-miRNAs. Western blots were first analyzed with anti-HA antibody and then reprobed with the indicated antibodies.



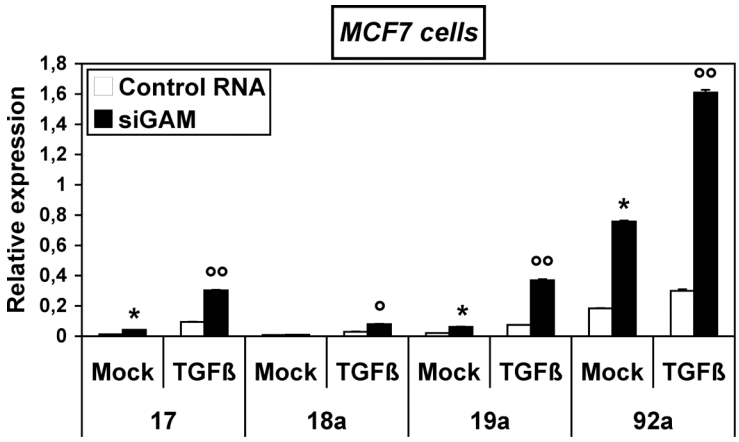
Supplementary Figure 3: Validation of siRNAs directed against *GAM* (*siGAM*) and *Drosha* (*siDrosha*) transcripts. The downregulation of *GAM* transcripts following transfection with *siGAM* (A) and the downregulation of *Drosha* transcripts following transfection with *siDrosha* (E) where determined by qRT-PCR. Values represent the mean ± standard deviation (n = 3). *, Significantly different from Control RNA, $P < 0.0001$. The respective effects of *siGAM* on GAM levels in HEK-293 (B), HepG2 (C) and MCF-7 cells (D) and those of *siDrosha* on Drosha levels in HEK-293 cells (F) were determined by Western Blotting.



Supplementary Figure 4: *siGAM* decreases Ras levels. The effects of *siGAM* on Ras factors in HEK-293 cells were determined by Western Blotting using a pan-Ras antibody.

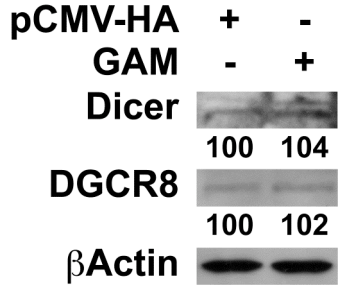


Supplementary Figure 5: GAM downregulates *miR-17-92* miRNAs in K562 lymphoid cells. The relative levels of *miR-17-92* miRNAs in K562 cells transfected with either *pCMV-HA* (Empty) or *pCMV-HA-GAM* (GAM) were determined by qRT-PCR. Values represent the mean ± standard deviation (n = 3). *, Significantly different from Empty control vector, $P < 0.05$.

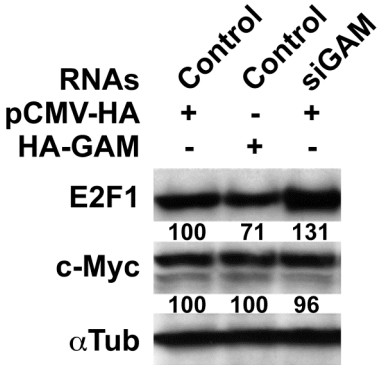


Supplementary Figure 6: Both GAM and TGFβ have differential yet opposite effects on *miR-17-92* miRNA levels. MCF7 cells were transfected with either a control RNA or *siGAM*. 34 hours later, they were either mock-treated or treated with TGFβ as indicated. The relative levels of the indicated miRNAs were determined by qRT-PCR. Values represent the mean ± standard deviation (n = 3). *, Significantly different from mock-treated Control

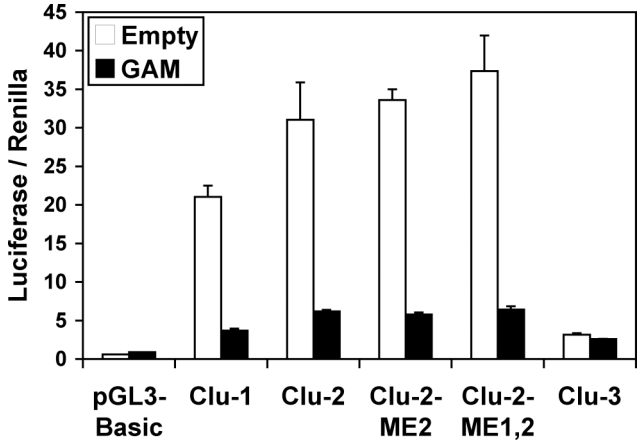
RNA, $P < 0.0005$. o and oo, Significantly different from TGFβ-treated Control RNA, o, $P < 0.005$, oo, $P < 0.0005$.



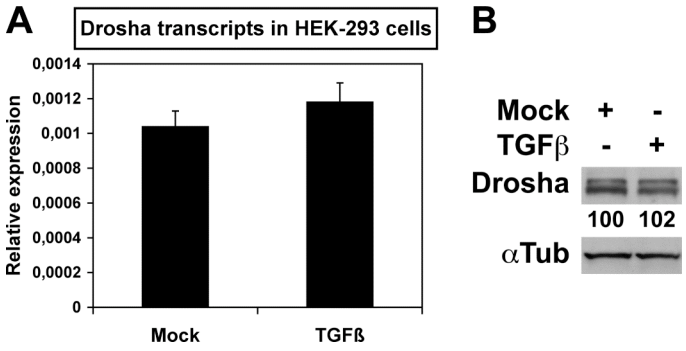
Supplementary Figure 7: *GAM* overexpression does not change the levels of endogenous Dicer and DGCR8. The effects of HA-GAM on Dicer and DGCR8 levels in HEK-293 cells were analyzed by Western blotting.



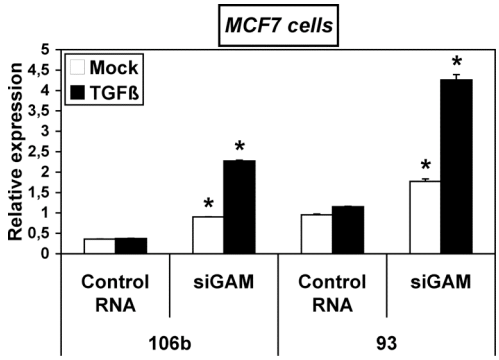
Supplementary Figure 8: *GAM* overexpression or depletion does not change the levels of endogenous c-Myc. The effects of HA-GAM and *siGAM* on E2F1 and c-Myc levels in HEK-293 cells were analyzed by Western blotting.



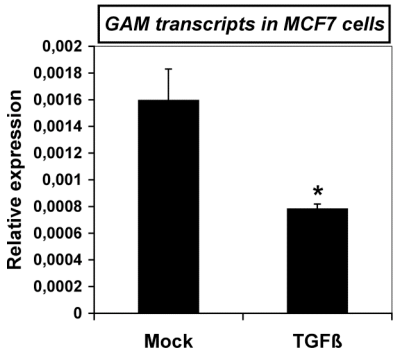
Supplementary Figure 9: Mutating *E2F* binding sites *1* and *2* neither impairs the expression of *Clu-2* construct nor decreases GAM repressing activity. MCF7 cells were transfected with the empty *pGL3-Basic* vector, the *Clu-1* to *Clu-3* constructs or *Clu-2* with *E2F* site *2* (*Clu-2ME2*) or *E2F* sites *1* and *2* (*Clu-2ME1,2*) mutated as indicated, along with the empty *pCMV-HA* vector or a construct expressing *GAM*. The *Firefly* luciferase activity was measured 48 hours after transfection and then normalized to the *Renilla* luciferase activity. Values represent the mean ± standard deviation (n = 4).



Supplementary Figure 10: TGFβ does not change *Drosha* transcript nor Drosha protein levels. The effects of TGFβ on *Drosha* transcript (A) and Drosha protein levels (B) were respectively analyzed by qRT-PCR and Western blotting. In A, values represent the mean ± standard deviation (n = 3) and are not significantly different.



Supplementary Figure 11: GAM also opposes the upregulation of miRNAs of the *miR-106b-93-25* cluster by TGFβ. The levels of *miR-106b* and *miR-93* in MCF7 cells transfected with a control RNA or *siGAM* and then mock-treated or treated with TGFβ were determined by qRT-PCR. Values represent the mean ± standard deviation (n = 3). *, Significantly different from mock-treated Control RNA, $P < 0.05$.



Supplementary Figure 12: TGFβ downregulates *GAM* transcript levels in MCF7 cells. The levels of *GAM* transcripts in MCF7 cells either mock-treated or treated with TGFβ were determined by qRT-PCR. *, Significantly different from mock treatment, $P < 0.01$.