Legends to Supplementary Figures

Supplementary Figure-S1. Levels of the nine members of the *let-7* family in pediatric malignant-GCTs. A) Levels were determined in 20 samples from set-1 with matched microRNA and mRNA profiling. Values were determined from microarray data and were normalized to the mean of the three normal gonadal control samples. B) Linear-regression analysis of each individual *let-7* family member versus *LIN28* levels for the set-1 samples. The data are color-coded by sample type, as in the key.

Supplementary Figure-S2. PCR quantification of *let-7e*, *LIN28* and *LIN28B* in malignant-GCTs. The graphs show levels of *let-7e* versus levels of *LIN28* (left) and *LIN28B* (right). All values were determined by qRT-PCR in sample set-3. Correlation *p*-values were determined by linear-regression. The data are color-coded by sample type, as in the key.

Supplementary Figure-S3. *Sylamer* analysis of up-regulated genes in pediatric and adult malignant-GCTs. *Sylamer* landscape plots for SCR words corresponding to the common seed of the nine *let-7* microRNA family members, in pediatric malignant-GCTs (set-1; top panel) and adult malignant-GCTs (set-2; lower panel). Log_{10}-transformed and sign-adjusted enrichment *p*-values for each SCR word, relative to *p*-values of all other words, are plotted on the *y*-axis, against the ranked gene list on the *x*-axis (orientated with down-regulated genes to the left and up-regulated genes to the right). A negative *y*-axis deflection on the right-hand side of the plot therefore signifies SCR enrichment in up-

regulated genes. For each comparison, the left-hand plot shows data for the hexamer complementary to the core 2-7nt component of the common seed-region, the central plot the two heptamers (1-7nt; 2-8nt), and the right-hand plot the octamer (1-8nt). The single summed significance score and *p*-value for all four SCR words in each comparison are given in each left-hand plot.

Supplementary Figure-S4. Correlations between levels of *let-7* and mRNA targets. In each graph, the *x*-axis shows median levels of the nine *let-7* family members, while the *y*-axis shows levels of each mRNA. Data were obtained from microarray analyses of the 20 pediatric malignant-GCTs from set-1 with matched microRNA and mRNA profiling. Correlation *p*-values were determined by linear-regression. Samples are color-coded, as in the key.

Supplementary Figure-S5. PCR quantification of *HMGA2* in pediatric malignant-GCTs. The graphs show: A) *HMGA2* levels in the 32 samples from set-3; B) mean expression levels of *HMGA2* in GCTs of different histologic types from set-3; and C) linear-regression analysis of *HMGA2* versus *let-7e* levels. CL = cell-line. Error-bars=SEM. Samples are color-coded, as in the key.

Supplementary Figure-S6. PCR validation of expression of selected *let-7* mRNA targets in pediatric malignant-GCTs. The graphs show mean qRT-PCR expression the 32 samples from set-3. The left-hand column shows mRNAs over-expressed in malignant-GCTs of particular histologic subtypes, compared to the control samples used, while the

right-hand column shows mRNAs that were over-expressed in all malignant GCT subtypes. Error-bars=SEM. Samples are color-coded, as in the key.

Supplementary Figure-S7. Effects of *LIN28* depletion in malignant-GCT cells. A) Western blots showing LIN28 protein expression in 2102Ep on d1, d2, d6 and d7 following *LIN28* depletion, compared with NTC-treated cells. For corresponding western blots for d3-d5, see Figure-3A. By densitometry, LIN28 protein levels were 30-40% of those in NTC-treated cells on d1 and d2 and <10% on d3 to d7. B) Western blots showing LIN28 expression on d4 following *LIN28* depletion in TCam2 (left), 1411H (centre) and GCT44 (right), compared with NTC-treated cells. C) The graph plots LIN28 protein *vs.* transcript levels for all four malignant-GCT cell-lines on d4 following *LIN28* depletion. All values were compared with NTC-treated cells. D) Relationship between LIN28 protein levels and cell growth in all four malignant-GCT cell-lines on d4 following *LIN28* depletion. All values were compared with NTC-treated cells. NTC=non-targeting-control siRNA, kd=knockdown. Protein levels were quantified by densitometry. Correlation *p*-values were determined by linear-regression. Error-bars=SEM.

Supplementary Figure-S8. Independent confirmation of specific effects of *LIN28* depletion in malignant-GCT cells. A) *LIN28* and *LIN28B* levels in 2102Ep cells on d4 following transfection with two independent *LIN28* siRNAs (Hs_LIN28_7, left; Hs_LIN28_8, right) at 66.7nM, as measured by qRT-PCR. Treatment with each siRNA resulted in significant depletion of *LIN28*, but not *LIN28B*. NTC = Qiagen AllStars non-targeting-control siRNA (catalogue number 1027280), at 66.7nM; kd=knockdown. B) Cell numbers on d4, relative to

d0, in the experiments shown in A). Treatment with each siRNA resulted in significantly reduced cell numbers compared with NTC-treated cells. C) Levels of *let-7e* on d4 in the experiments shown in A) and B). Treatment with each siRNA resulted in significantly increased *let-7e* levels compared with NTC-treated cells. Error-bars=SEM.

Supplementary Figure-S9. Effects of *let-7e* mimic in malignant-GCT cells. A) Levels of four *let-7* mRNA targets (*MYCN*, *AURKB*, *LIN28* and *LIN28B*) at d1-d3 following transfection of 2102Ep with *let-7e* mimic, referenced to cells treated with mimic negative control (MNC). B) Mean expression of each mRNA over d1-d3 versus the number of *let-7* SCRs in the 3'UTR of each gene. The correlation *p*-value was determined by linear-regression. C) Depiction of the *let-7e* SCR in the *LIN28* 3'UTR. *Let-7e* is used as a representative *let-7* family member. The 2-7nt seed and the corresponding SCR sequence are underlined.

Supplementary Figure-S10. Relationships between *MYCN* and *C-MYC* versus *LIN28* and *LIN28B* in malignant-GCTs. A) Microarray levels of *CMYC* (left) and *MYCN* (right) versus *LIN28* in pediatric samples (upper panel; set-1) and adult samples (lower panel; set-2). B) qRT-PCR levels of *MYCN* versus *LIN28* (left) and *LIN28B* (right), as determined in sample set-3. All correlation *p*-values were determined by linear-regression. Samples are color-coded, as in the key.

Supplementary Figure-S11. *MYCN* depletion in malignant-GCTs. Cell numbers and qRT-PCR expression levels of *MYCN*, *LIN28 and LIN28B* on d1 and d2 following *MYCN* depletion

in 2102Ep (left) and TCam2 (right), referenced to NTC-treated cells. Error-bars=SEM. *=$p<0.05$;**=$p<0.005$;***=$p\leq0.0001$. *MYCN* depletion was also confirmed at the protein level by Western blot in 2102Ep cells on both d1 and d2 following *MYCN* depletion. Levels were reduced to 60.2% and 60.7%, respectively (data not shown).

Supplementary Figure-S12. Schematic of the *LIN28*/*let-7* axis in malignant-GCTs. In the nuclei of malignant-GCT cells, LIN28 binds the stem-loop of *pri-let-7*, preventing processing by Drosha, most likely resulting in rapid degradation of primary transcripts. The *pre-let-7* that is generated is exported from the nucleus by Exportin 5. Cytoplasmic LIN28 also binds the stem-loop (10) and recruits the terminal-uridyl-transferase enzyme ZCCHC11 (10,11), resulting in 3' uridylation of *pre-let-7*, preventing subsequent processing by Dicer and targeting the *pre-let-7* for degradation (12). In malignant-GCTs, the high LIN28 levels are also likely to prevent KSRP binding to the stem-loop of both *pri-let-7* and *pre-let-7* molecules (13), preventing the promotion of *let-7* maturation. Consequently, low levels of all mature *let-7* family members are observed in malignant-GCTs. This is likely to contribute to increased expression of pro-malignant genes, including *MYCN* and *LIN28*. Accordingly, double-negative feedback is likely to contribute to the maintenance of high levels of *LIN28* in malignant-GCT cells. In addition, malignant-GCTs show down-regulation of other microRNAs (17) that regulate *LIN28* via 3'UTR binding sites, e.g. miR-9, miR-30 family, miR-125 and miR-181 (14-16), providing a likely further potential contribution to high *LIN28* levels.

Legends to Supplementary Tables

Supplementary Table-S1. Clinico-pathological data for sample set-3. These samples were principally used as a largely independent set for qRT-PCR validation of mRNA microarray profiling data. Of the 26 clinical specimens in the set, 23 were from pediatric patients (<16y). Nineteen were gonadal and seven extragonadal.

Supplementary Table-S2. Primers used for mRNA qRT-PCR.

Supplementary Table-S3. Expression of *let-7* family microRNAs in pediatric malignant-GCTs. Data are from our previous analysis of sample set-1 (17). Fold-change is versus 14 non-malignant control samples (teratomas and normal gonadal controls). The microRNAs are ranked by adjusted *p*-value. The common 2-7nt seed sequence is underlined.

Supplementary Table-S4. *Let-7* mRNA targets identified in combined microarray analysis of pediatric and adult malignant-GCTs. The 27 genes are ranked by fold-change in our microarray analysis of pediatric malignant-GCTs (sample set-1). The adult tumors were those in sample set-2. The linear-regression *p*-value was derived for the twenty set-1 samples with matched microRNA and mRNA profiles. The 16 genes selected for further analysis are highlighted in grey.

Supplementary Table-S5. The six *let-7* mRNA targets selected following microarray and qRT-PCR analysis. The linear-regression *p*-value was derived from qRT-PCR analysis of *let-7e* and target gene levels in sample set-3.

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