Supplementary Figure Legends

Supplementary Figure 1. Transforming activity in primary and recurrent TD cells. A. Focus formation assay of the primary TD cells with (Dox+) or without (Dox-) doxycycline induction of P3F expression. B. Focus formation assay of recurrent TD subclones without doxycycline.

Supplementary Figure 2. Analysis of expression profiling data from parental, primary TD and recurrent TD cells. A. Principal component analysis of expression data corresponding to unstimulated parental cells (blue dots), doxycycline-treated primary TD cells (red dots) and unstimulated recurrent TD cells (yellow dots). B. Left and central panels; GSEA results showing the overlap of the expression profile of primary and recurrent TD cells (relative to unstimulated parental cells) with growth factor receptor and KRAS expression signatures. Right panel; GSEA results showing that the expression profiles of the recurrent CR-control relative to the recurrent CR-*FGF8* cells and Dbt/MYCN-FGF8 relative to the Dbt/MYCN-EV cells overlap with the KRAS expression signatures.

Supplementary Figure 3. Expression of P3F targets in primary and recurrent TD cells. A. Dot bar graph showing the expression of the 14 P3F target genes (represented in Figure 1C) in each recurrent TD cell line relative to the mean of the primary TD cells. This microarray data indicate that the 14 genes were significantly upregulated or downregulated in the recurrent TD cells relative to the primary TD cells. A false discovery adjusted p-value of 0.01 and a 1.5-fold change cut off were used to determine significance. B-D. *FGF8*, *PCBD1* and *DUSP6* expression in recurrent TD cells (n= 7) relative to the primary TD cells (n= 5). *FGF8*, *PCBD1* and *DUSP6* mRNA expression was assayed by real-time RT-PCR and normalized for *GAPDH* expression by the ΔΔCt method. Primary TD cells were treated with doxycycline, as indicated. A two-sided unpaired Student's *t*-test was applied to determine significant differences between the indicated groups. * $P<0.05$, Ns: not significant

Supplementary Figure 4. Cell transformation after *FGF8* knockout in recurrent TD cells (Rec 1a and Rec 2a). A. Western blot analysis of FGF8 protein expression after *FGF8* knockout in Rec 2a recurrent TD cells. B. Focus formation assay after *FGF8* knockout in Rec 2a recurrent TD cells. C-D. Western blot assay of FGF8 expression (C) and focus assay (D) of Rec 1a subclones after transduction with CR-control or CR-*FGF8* #1 constructs. E. Focus formation assay of Rec 1a subclones after transduction with CR-control or CR-*FGF8* #2 or CR-*FGF8* #3 in the presence or absence of doxycycline induction of P3F. In parts B and D, two replicates are shown for each assay.

Supplementary Figure 5. Cell migration and invasion after *FGF8* knockout in recurrent TD cells (Rec 1a). A. Growth of CR-control sc1 and CR-*FGF8* #2 sc-3 cells in the presence of hydroxyurea at the indicated concentrations. B-C. Migration and invasion assays. Before starting the experiment, both CR-control sc1 and CR-*FGF8* #2 sc-3 cells were serum-starved overnight. Cells were then seeded into the wells and hydroxyurea was added into upper and lower chambers at 0.5 mM for the CR-control sc1 cells, and at 0.25 mM for the CR-*FGF8* #2 sc-3 cells. After 48 hours, cells were fixed, stained and the optical density was measured at 560 nm. Data show the results of one representative experiment

with triplicate measurements. Two-sided unpaired Student's *t*-test was applied to determine a significant difference between the indicated conditions. ** $p < 0.01$ *** $p < 0.001$.

Supplementary Figure 6. Studies of conditioned media.
A. Quantification of the number of foci in the transformation assay shown in Figure 4A. B. Western blot analysis of FGF8 expression in CM collected from cultures of recurrent TD cells (Rec 1a) or parental cells. C. Representative images showing Dbt/MYCN/iP3F cells expressing GFP in a focus assay performed with CM derived from parental cells or recurrent TD cells (Rec 1a). Scale bars = 400 μm. D. Quantification of foci in focus assay shown in Figure 4C. E. Protein loading image (concentrated conditioned media) corresponding to lanes 1-3 of the western blot in Figure 4D. F. Quantification of foci in the focus assay shown in Figure 4E. For parts A, D, and F, the data are expressed as the mean +/- SE of 3 replicates. The two-sided unpaired Student's *t*-test was used to determine significant differences between the indicated groups (A) or between the control and test groups (D, F). ** $p < 0.01$ *** $p < 0.001$.

Supplementary Table 1. Upregulated and downregulated genes including P3F targets in primary and recurrent TD cells relative to unstimulated parental cells.