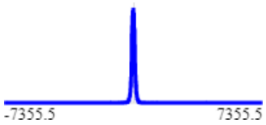
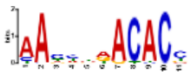
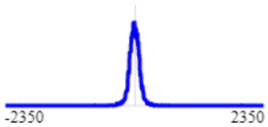
Supplemental Figure 1A

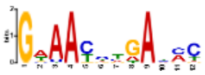
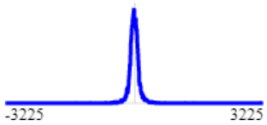
SIX1 tumor only peaks motif discovery

			p-value	E-value	Sites			Distribution
1-GWAACYTGAK			4.7e-071	2.1e-069	6932 (71.0%)	↧	⇢	

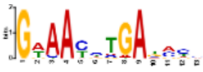
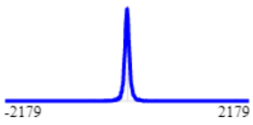
SIX1-Q177R only peaks motif discovery

1-AAYHHRACACC			1.7e-040	7.6e-039	3944 (41.6%)	↧	⇢	

Shared Tumor peaks motif discovery

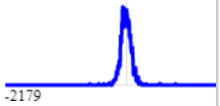
1-GWAAYYTGABMC			5.6e-072	3.2e-070	5999 (75.1%)	↧	⇢	

Wk17hFK SIX1 ChIP-seq motif discovery

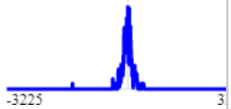
1-GWAAYYTGAKMYB			1.3e-110	6.6e-109	6178 (64.2%)	↧	⇢	

Supplemental Figure 1B

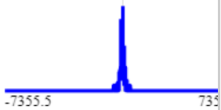
Wk17hFK peaks – MEIS1 motif enrichment

Logo	Database	ID	Alt ID	*P*-value	*E*-value	Q-value	TP	FP	Enrichment Ratio	Score Threshold	Positional Distribution
	MA1639.1	MA1639.1	MEIS1	1.47e-15	1.47e-15	1.47e-15	973 / 8666 (11.2%)	655 / 8666 (7.6%)	1.48	9.73	

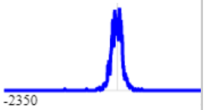
Shared Tumor peaks – MEIS1 motif enrichment

Logo	Database	ID	Alt ID	*P*-value	*E*-value	Q-value	TP	FP	Enrichment Ratio	Score Threshold	Positional Distribution
	MA1639.1	MA1639.1	MEIS1	4.11e-14	4.11e-14	4.11e-14	251 / 7186 (3.5%)	110 / 7186 (1.5%)	2.27	13.42	

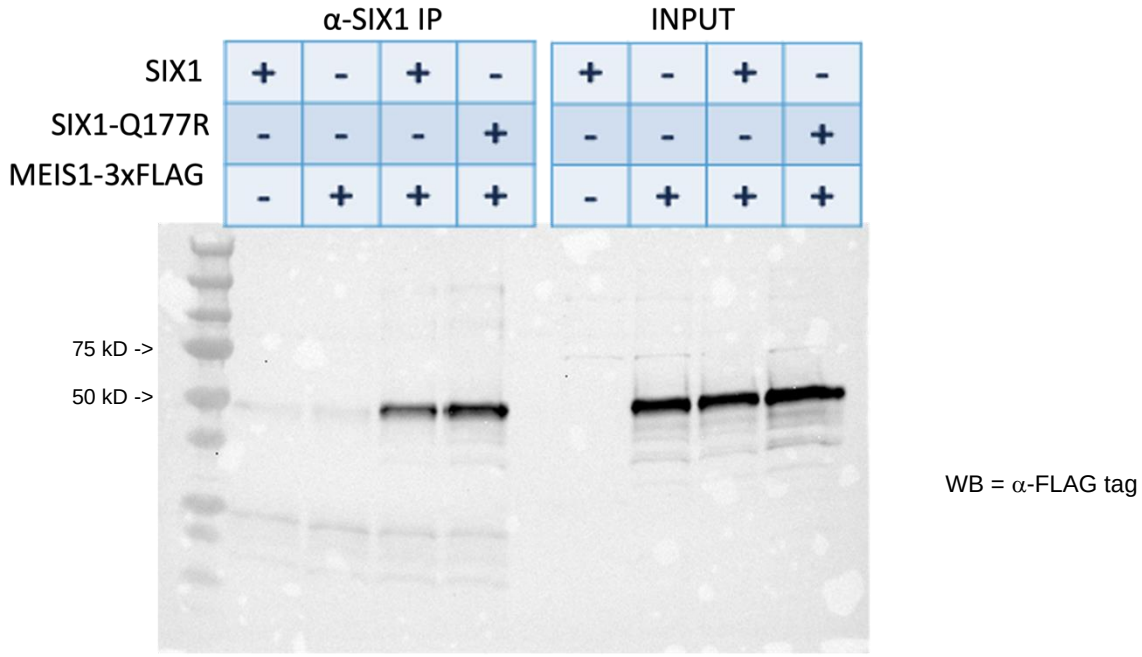
SIX1 tumor only peaks – MEIS1 motif enrichment

Logo	Database	ID	Alt ID	*P*-value	*E*-value	Q-value	TP	FP	Enrichment Ratio	Score Threshold	Positional Distribution
	MA1639.1	MA1639.1	MEIS1	1.61e-2	1.61e-2	1.61e-2	219 / 8784 (2.5%)	174 / 8784 (2.0%)	1.26	12.57	

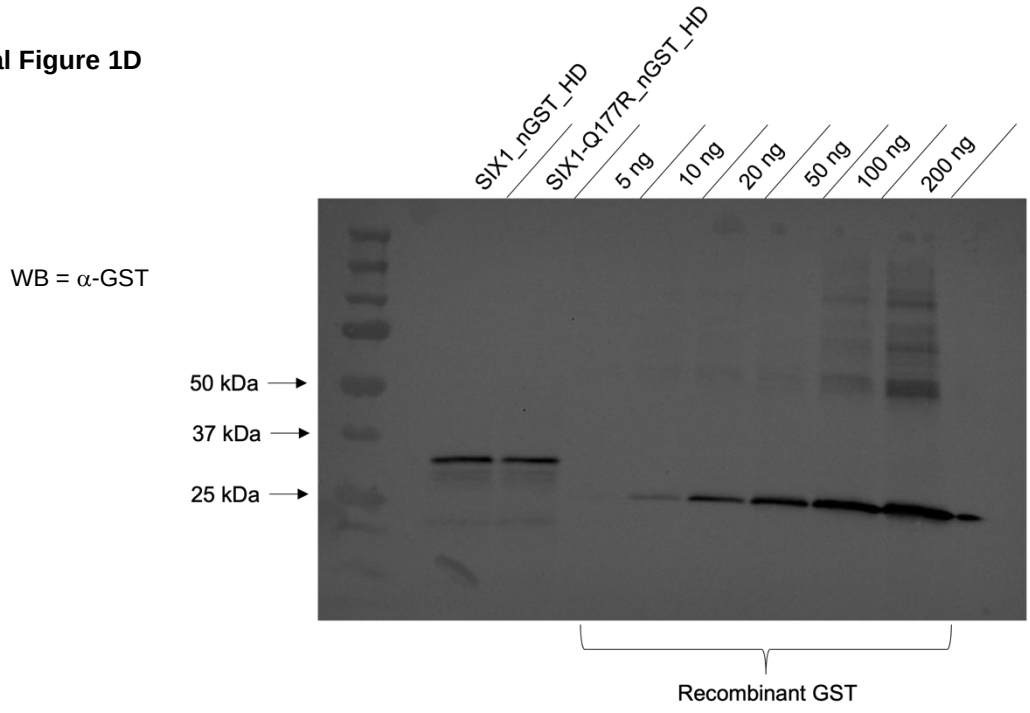
Q177R tumor only peaks – MEIS1 motif enrichment

Logo	Database	ID	Alt ID	*P*-value	*E*-value	Q-value	TP	FP	Enrichment Ratio	Score Threshold	Positional Distribution
	MA1639.1	MA1639.1	MEIS1	2.23e-40	2.23e-40	2.23e-40	1018 / 8535 (11.9%)	505 / 8535 (5.9%)	2.01	10.47	

Supplemental Figure 1C

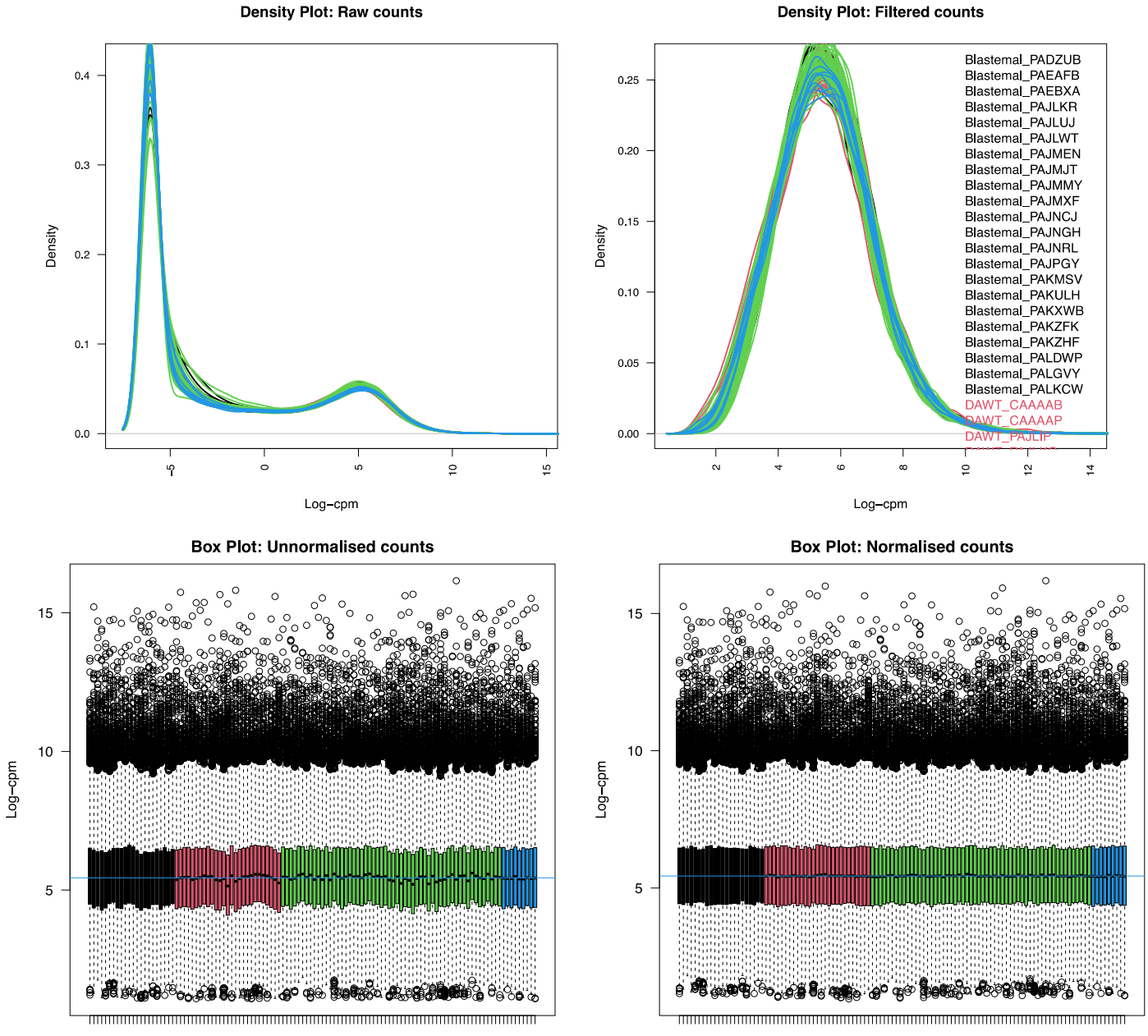


Supplemental Figure 1D

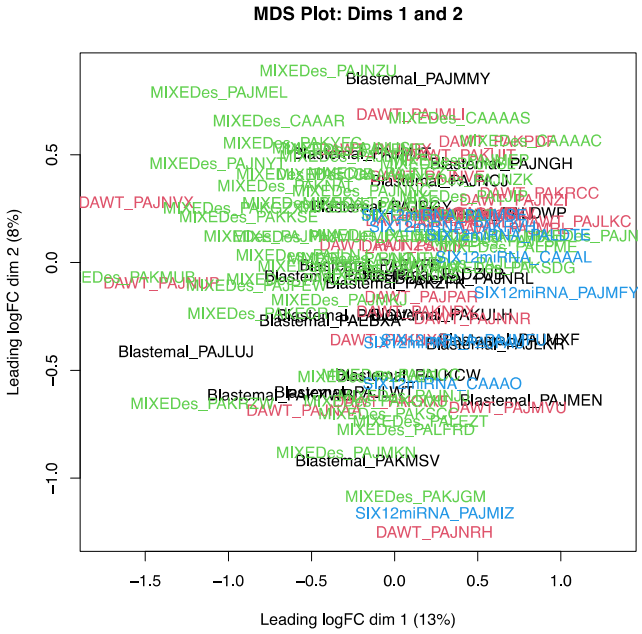


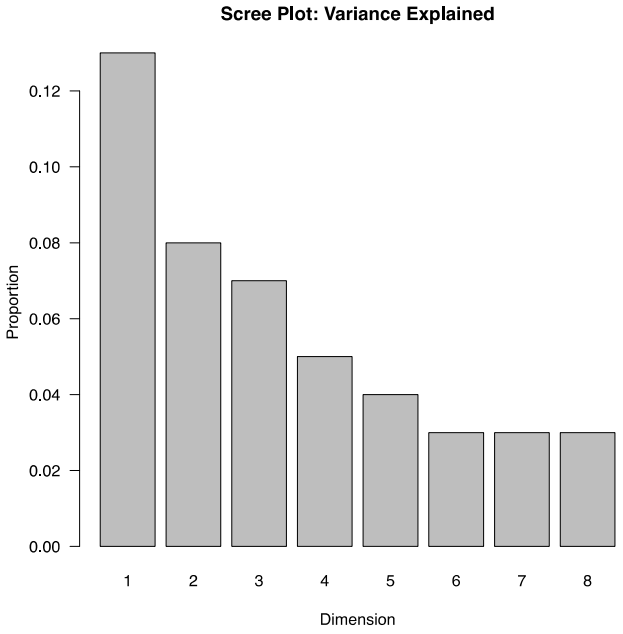
Supplemental Figure 1: **A)** Motif discovery output from STREME tool. Peak sequences used had been derived from after a first round of motif discovery using STREME followed by use of the FIMO tool to extract sequences containing SIX1-like motif. **B)** Motif enrichment output from SEA tool using same peak sequences used in panel A and searching for enrichment of MEIS1 DNA binding motif (JASPAR #MA1639.1). **C)** Western Blot using α-FLAG tag antibody following SIX1 immunoprecipitation and SDS-PAGE. **D)** Western Blot using α-GST antibody that was used to quantify concentration of *in vitro* transcribed/translated SIX1_nGST_HD and SIX1-Q177R_nGST_HD protein fragments alongside dilution series of recombinant GST protein.

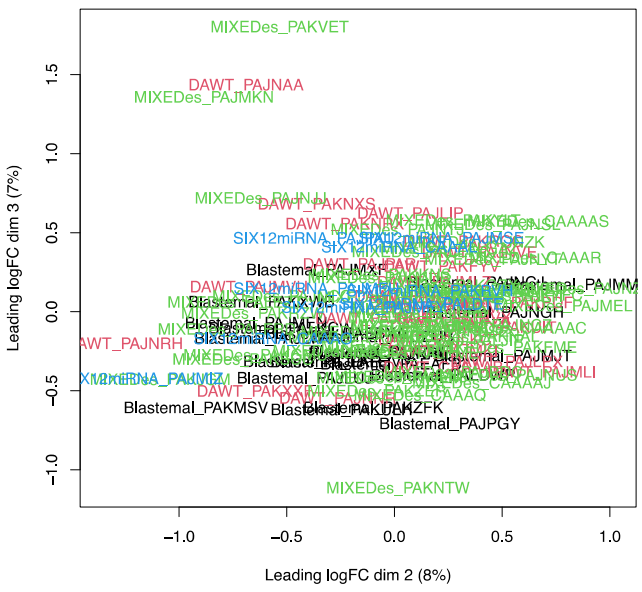
Supplemental Figure 2

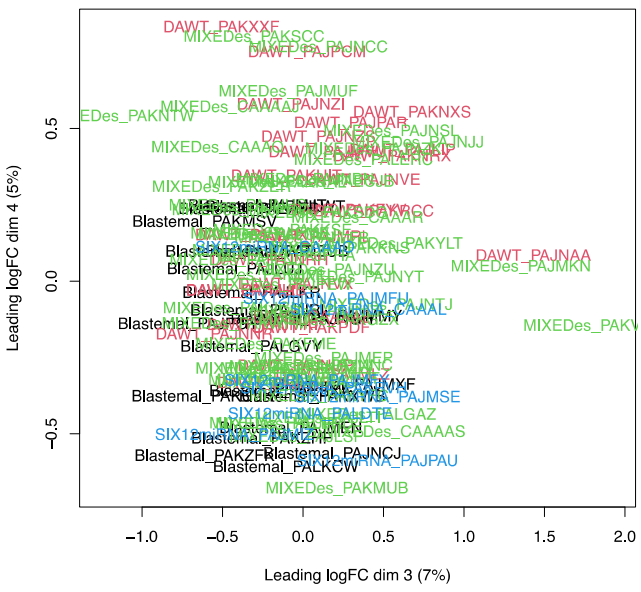


Supplemental Figure 2 continued

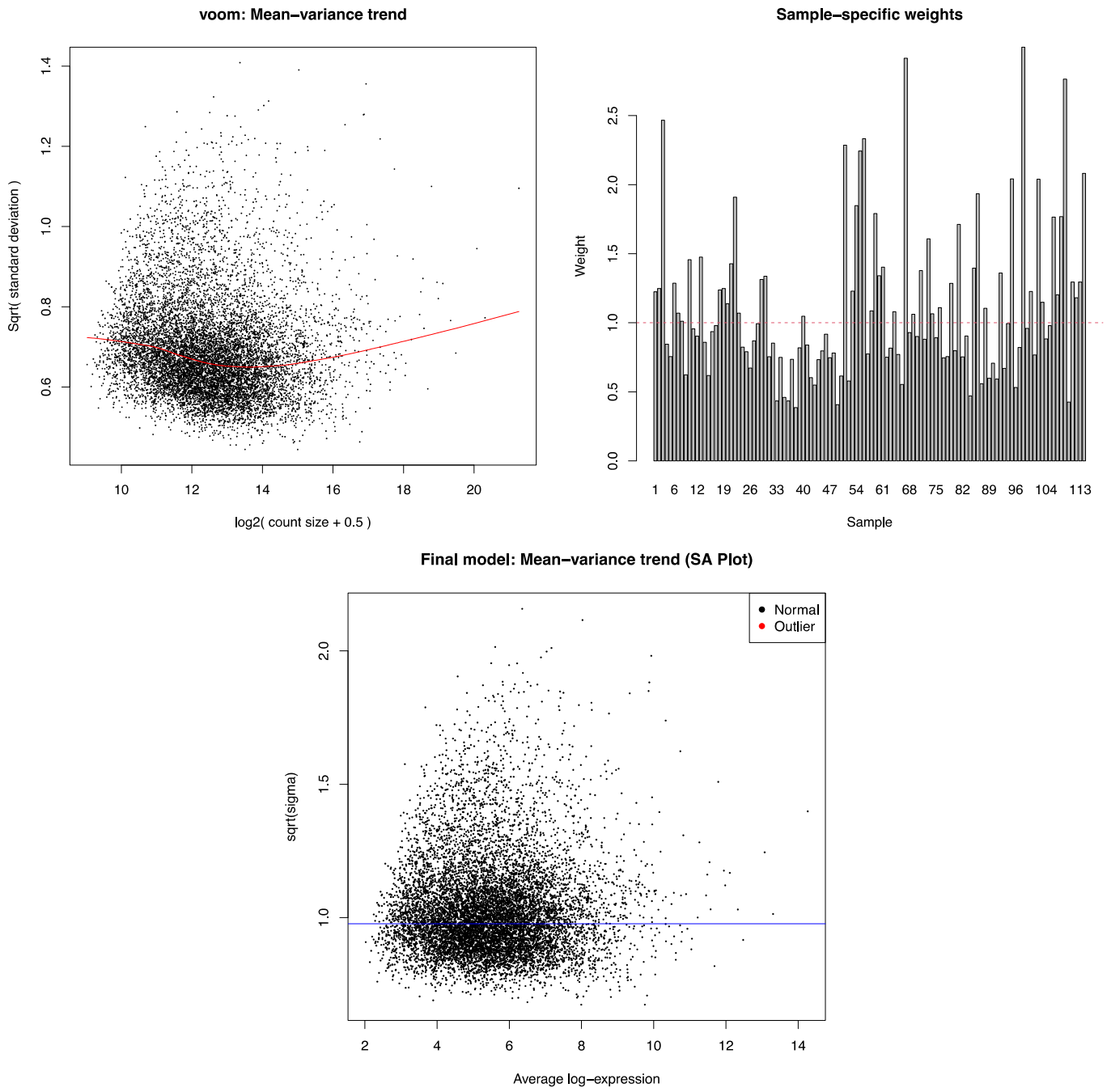






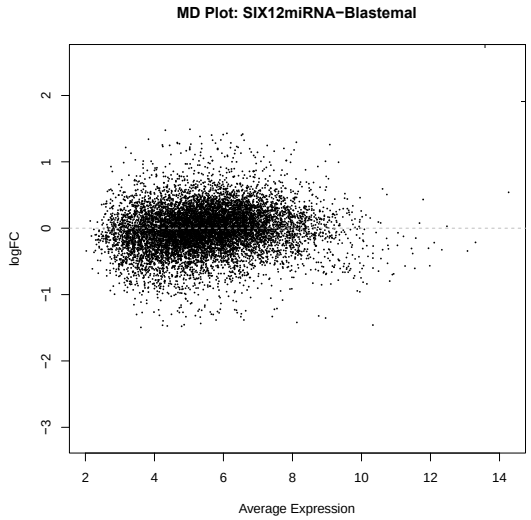


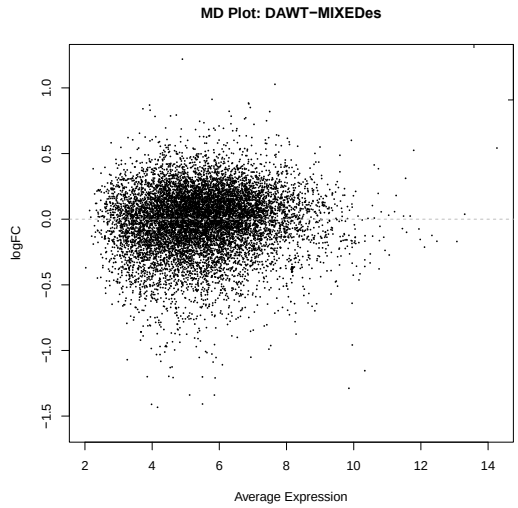
Supplemental Figure 2 continued

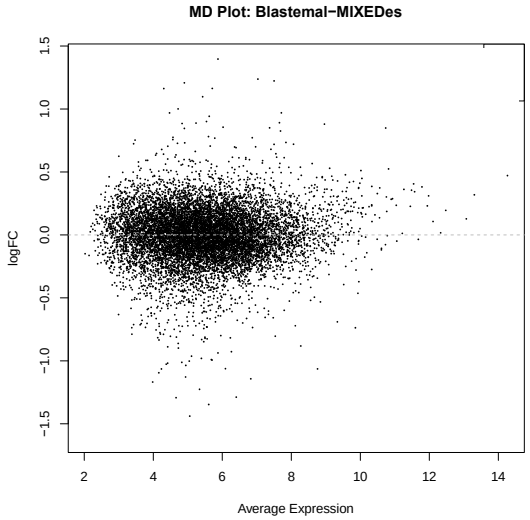


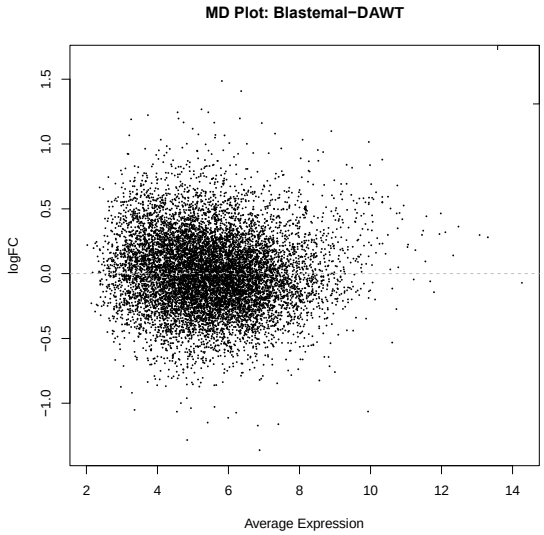
Supplemental Figure 2: Various plots/charts of metrics from limma-voom differential gene expression analysis.

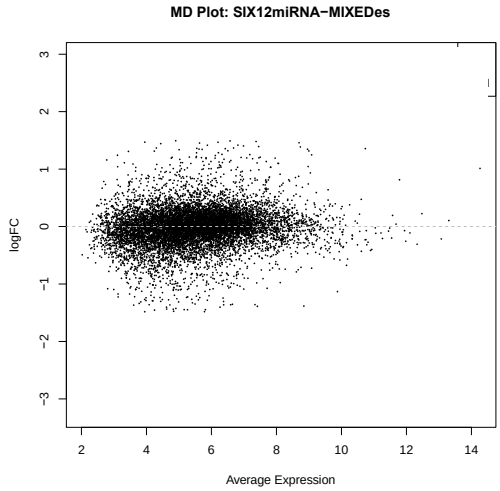
Supplemental Figure 3A

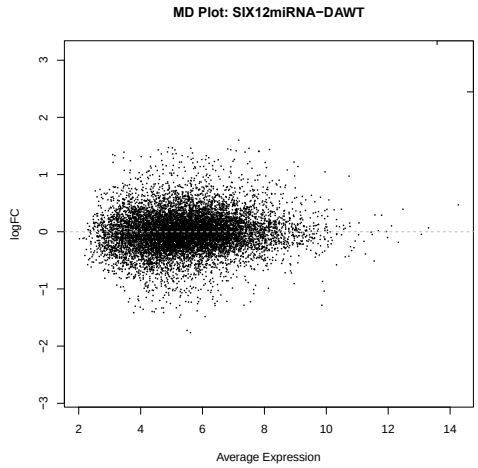






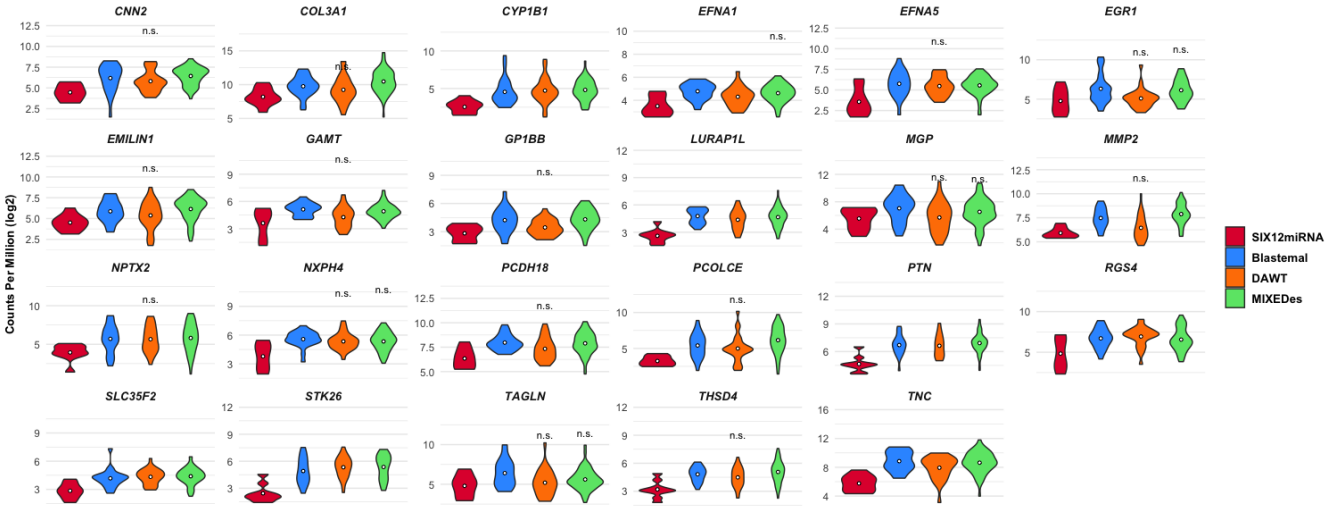






Supplemental Figure 3B

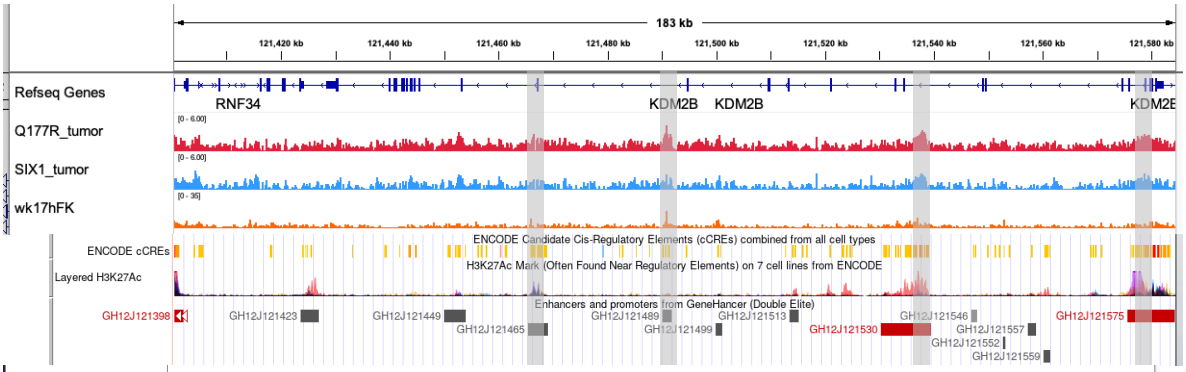
Downregulated in SIX1/2miRNA vs Blastemal (log2 FC > |1.5|, adj p < 0.05)



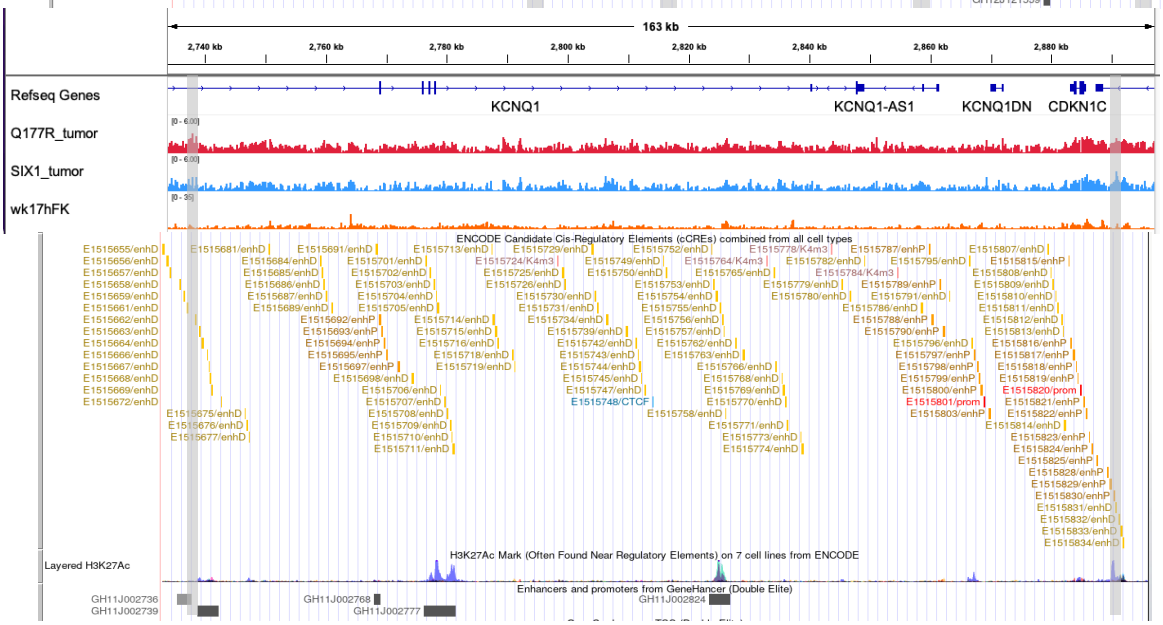
Supplemental Figure 3: **A)** MD plots from results of limma-voom differential gene expression analysis. Red dots indicate genes with > 1.5 log2 fold change and adj p-value < 0.05, blue dots indicate genes with < -1.5 log2 fold change and adj p-value < 0.05, Average Expression = log2 CPM. **B)** Violin plots showing the distributions of log2 counts per million (CPM) of the indicated gene set across tumor groups. Dot within each group plot represents the mean. Unless indicated by n.s. (not significant), the log2 fold change of that gene in the SIX1/2miRNA group was > |1.5| and adjusted p-value < 0.05 with respect to that tumor group.

Supplementary Figure 4A

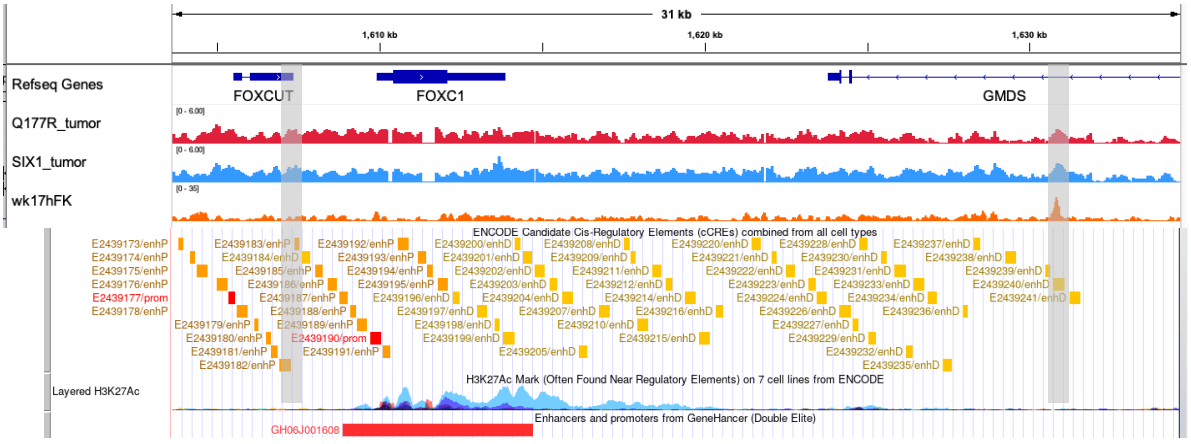
KDM2B



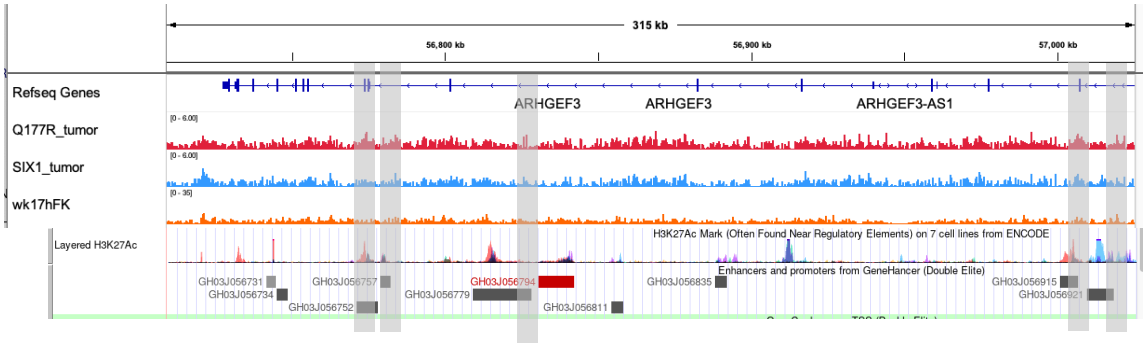
CDKN1C



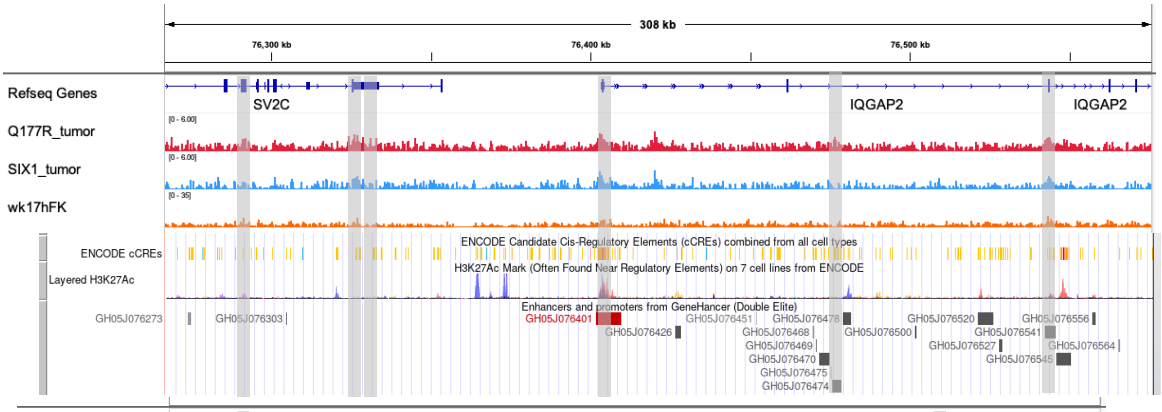
FOXC1



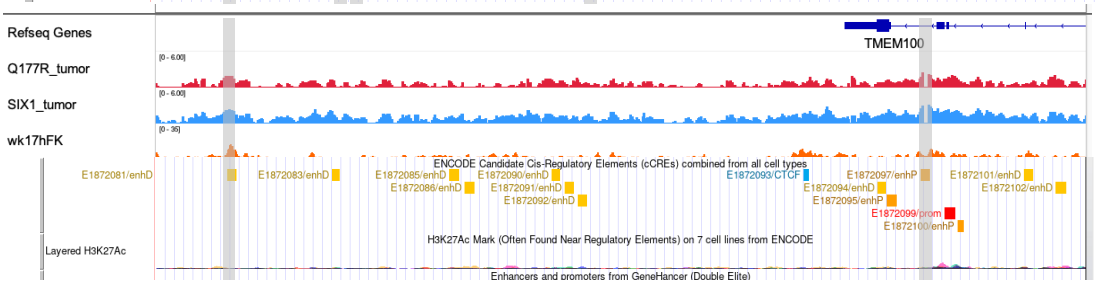
ARHGEF3



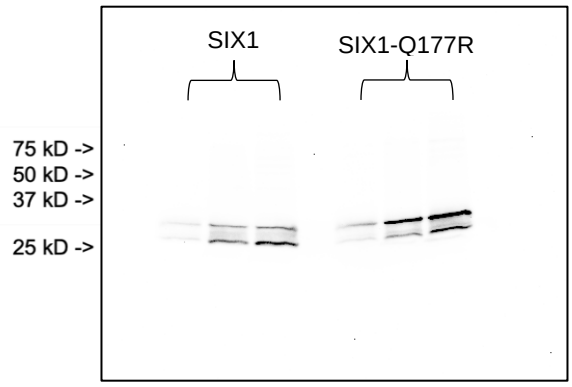
IQGAP2



TMEM100



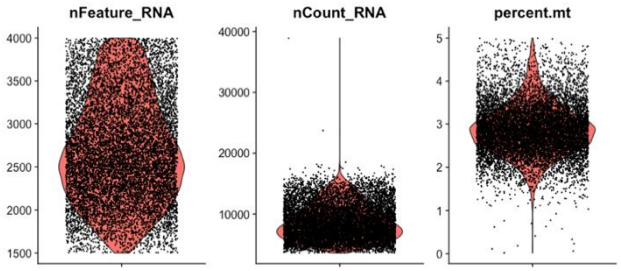
Supplemental Figure 4B



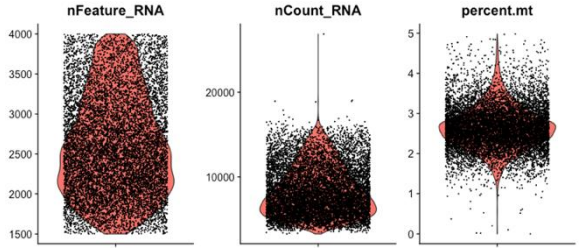
Supplemental Figure 4: **A)** IGV browser snapshots showing Q177R tumor, SIX1 tumor, and wk17hFK SIX1 ChIP-seq peak tracks, grey-shaded bars indicate locations of SIX1-Q177R called peaks. Below IGV browser snapshots are corresponding UCSC genome browser snapshots showing ENCODE candidate cis-regulatory elements, layered H3K27Ac, and GeneHancer predicted enhancer and promoter tracks. **B)** Western blot using α-SIX1 antibody following SDS-PAGE of increasing concentrations of purified SIX1 and SIX1-Q177R.

Supplemental Figure 5A

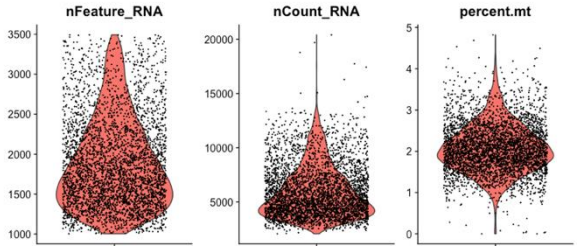
Lindstrom Wk 14 kidney 1



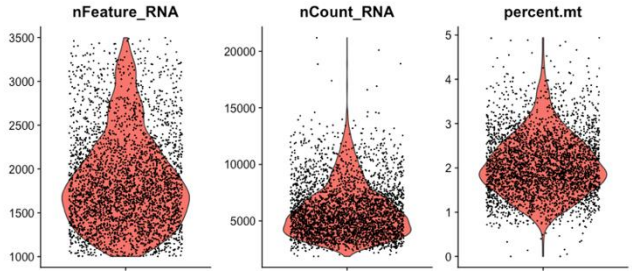
Lindtsrom Wk 14 kidney 2

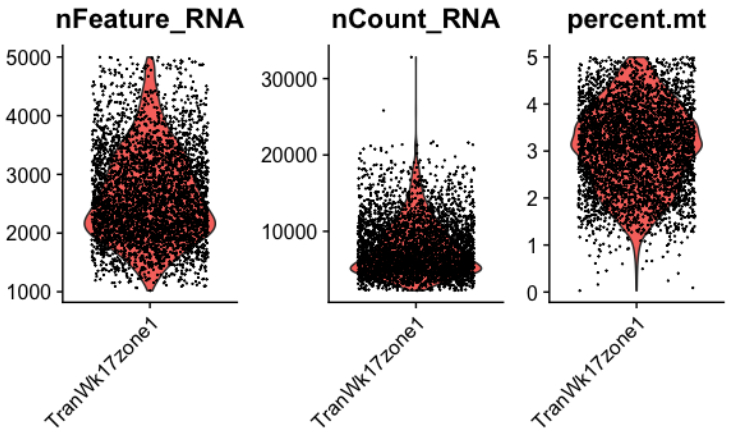


Lindstrom Wk 17 kidney 1

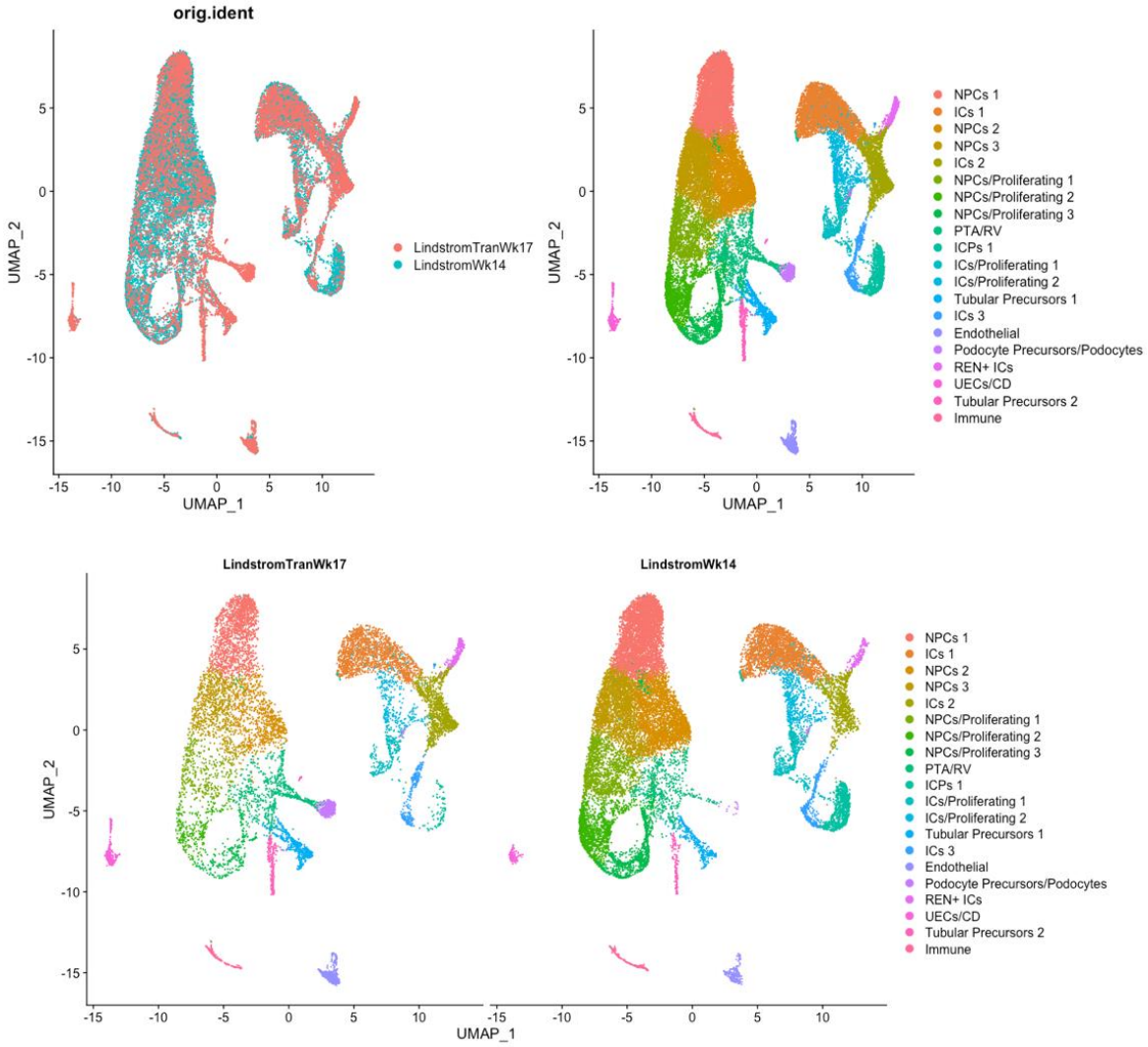


Lindtsrom Wk 17 kidney 2

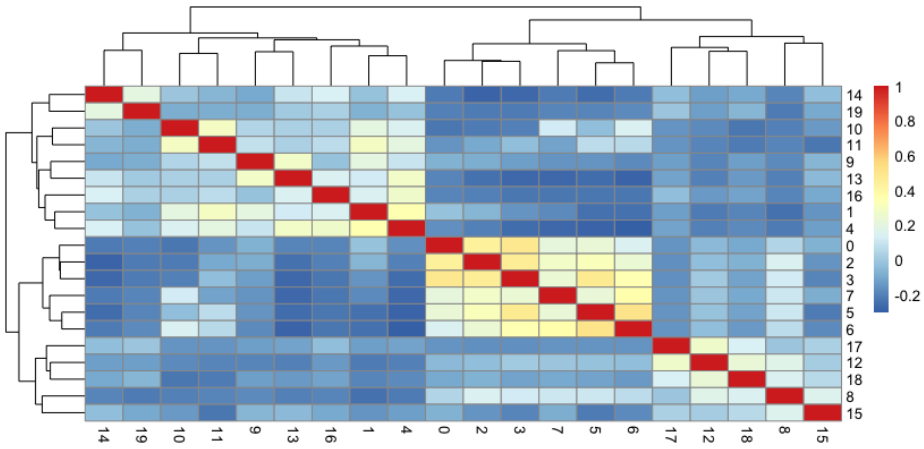




Supplemental Figure 5B



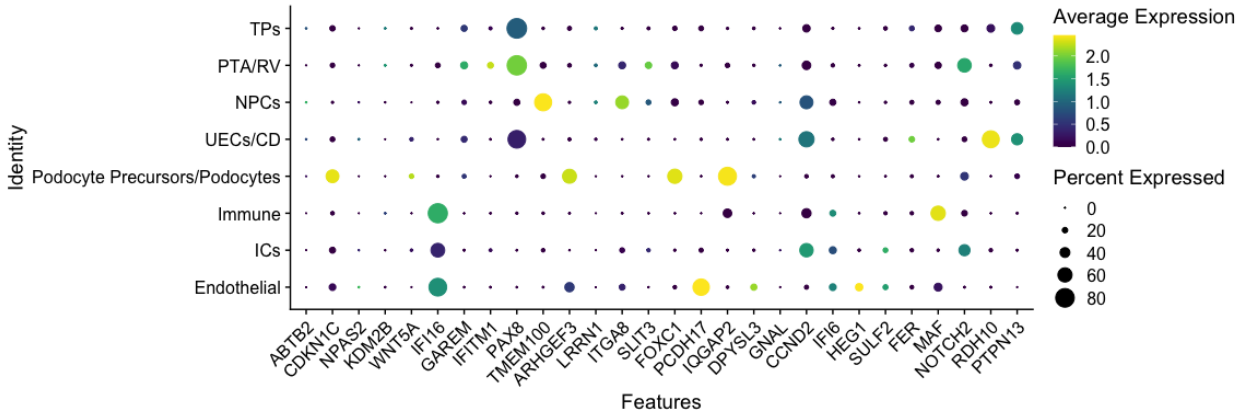
Supplementary Figure 5C



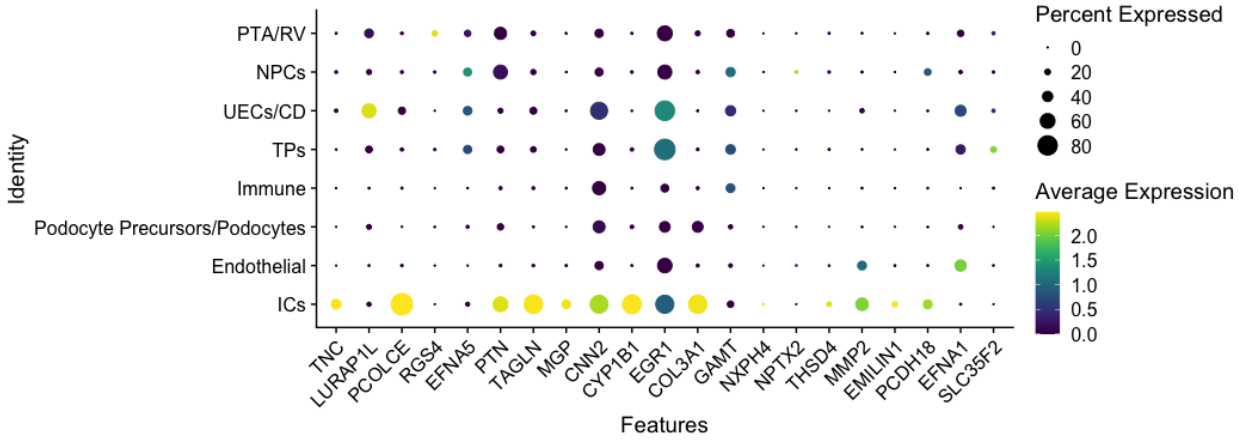
Supplementary Figure 5: **A)** Plots of QC metrics for cells in each of the indicated single cell RNA-seq datasets after data processing. **B)** UMAP plots showing 20 clusters generated from initial UMAP dimensional reduction and the location of cells within each cluster from each dataset grouping. **C)** Heatmap from unsupervised hierarchical clustering using Pearson's correlation coefficients derived from scaled average expression values of cells within each of the indicated clusters. Cluster number corresponds to UMAP plots above, numbered from top-bottom 0-19.

Supplementary Figure 6

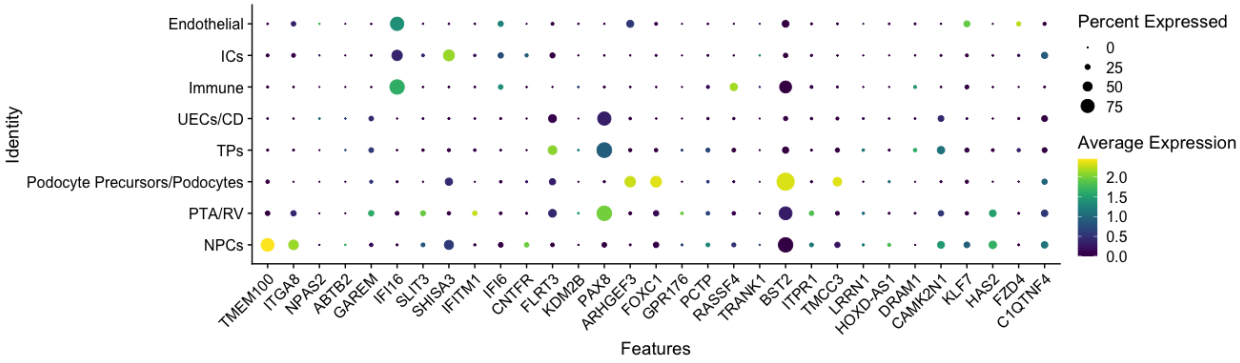
Upregulated in SIX1/2miRNA vs. Blastemal (log2 FC > 1.5, adj p <0.05)



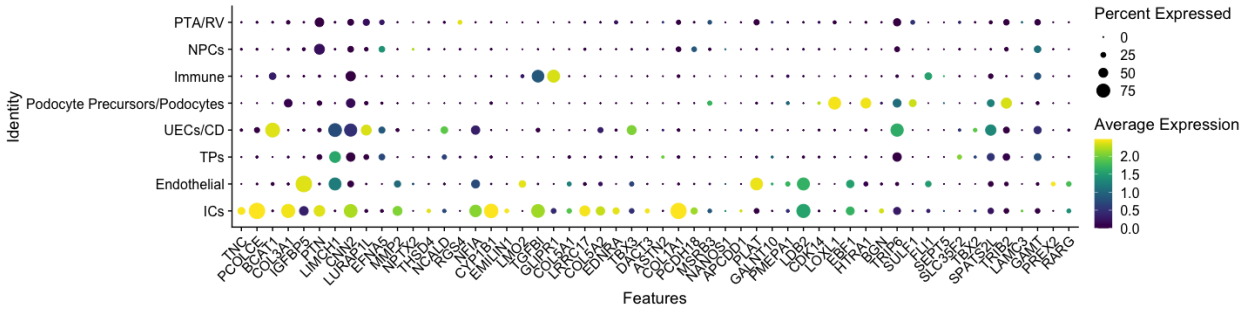
Downregulated in SIX1/2miRNA vs. Blastemal (log2 FC > |1.5|, adj p <0.05)



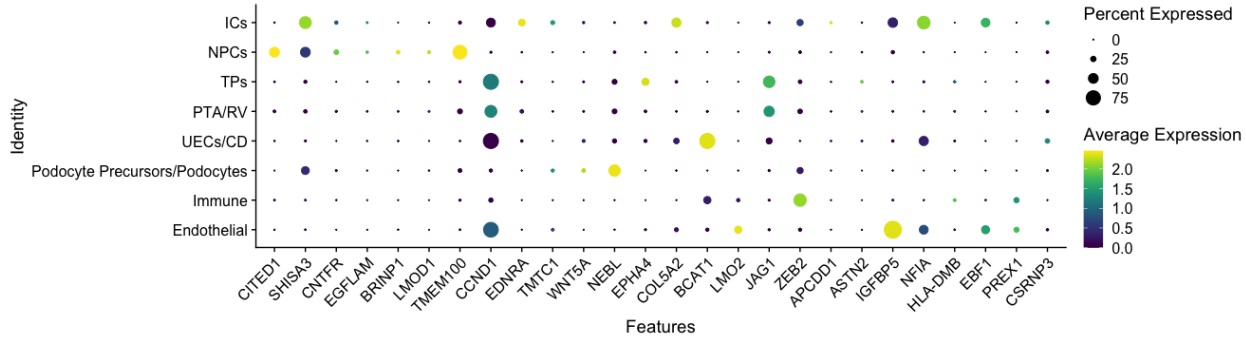
Upregulated in SIX1/2miRNA vs. MIXED/ES (log2 FC > 1.5, adj p <0.05)



Downregulated in SIX1/2miRNA vs. MIXED/ES (log2 FC > |1.5|, adj p < 0.05)



Upregulated and Downregulated in Blastemal vs. MIXED/ES (log2 FC > |1|, adj p < 0.05)



Supplemental Figure 6: Dot plots of indicated gene sets displaying the scaled average expression z-score value for each gene on the x-axis and the percent of cells expressing that gene within the cell clusters shown on the y-axis.

Supplemental Table 1: TARGET Wilms tumor sample IDs and corresponding group assignments used for differential gene expression analysis

Sample ID	***Group***	***Sample ID***	***Group***
PADZUB	Blastemal	**PAJLTH**	MIXED/ES
PAEAFB	Blastemal	**PAJLTI**	MIXED/ES
PAEBXA	Blastemal	**PAJMEL**	MIXED/ES
PAJLKR	Blastemal	**PAJMEP**	MIXED/ES
PAJLUJ	Blastemal	**PAJMKJ**	MIXED/ES

PAJLWT	Blastemal	**PAJMKN**	MIXED/ES
PAJMEN	Blastemal	**PAJMUF**	MIXED/ES
PAJMJT	Blastemal	**PAJNBN**	MIXED/ES
PAJMMY	Blastemal	**PAJNCC**	MIXED/ES
PAJMXF	Blastemal	**PAJNCZ**	MIXED/ES
PAJNCJ	Blastemal	**PAJNJJ**	MIXED/ES
PAJNGH	Blastemal	**PAJNLT**	MIXED/ES
PAJNRL	Blastemal	**PAJNNC**	MIXED/ES
PAJPGY	Blastemal	**PAJNSL**	MIXED/ES
PAKMSV	Blastemal	**PAJNTJ**	MIXED/ES
PAKULH	Blastemal	**PAJNUS**	MIXED/ES
PAKXWB	Blastemal	**PAJNYT**	MIXED/ES
PAKZFK	Blastemal	**PAJNZK**	MIXED/ES
PAKZHF	Blastemal	**PAJNZU**	MIXED/ES
PALDWP	Blastemal	**PAJPDC**	MIXED/ES
PALGVY	Blastemal	**PAJPEW**	MIXED/ES
PALKCW	Blastemal	**PAJPHA**	MIXED/ES
CAAAAB	DAWT	**PAKECR**	MIXED/ES
CAAAAP	DAWT	**PAKFME**	MIXED/ES
PAJLIP	DAWT	**PAKGMU**	MIXED/ES
PAJLKC	DAWT	**PAKGZX**	MIXED/ES
PAJLPX	DAWT	**PAKJGM**	MIXED/ES
PAJMKI	DAWT	**PAKKNS**	MIXED/ES
PAJMLI	DAWT	**PAKKSE**	MIXED/ES
PAJMLZ	DAWT	**PAKMUB**	MIXED/ES
PAJMRL	DAWT	**PAKNAL**	MIXED/ES

PAJMVU	DAWT	**PAKNTW**	MIXED/ES
PAJNAA	DAWT	**PAKRZW**	MIXED/ES
PAJNNR	DAWT	**PAKSCC**	MIXED/ES
PAJNRH	DAWT	**PAKSDG**	MIXED/ES
PAJNUP	DAWT	**PAKVET**	MIXED/ES
PAJNVE	DAWT	**PAKWPM**	MIXED/ES
PAJNVX	DAWT	**PAKYFC**	MIXED/ES
PAJNZI	DAWT	**PAKYLT**	MIXED/ES
PAJNZS	DAWT	**PAKZER**	MIXED/ES
PAJPAR	DAWT	**PALERC**	MIXED/ES
PAJPCM	DAWT	**PALEZT**	MIXED/ES
PAKFYV	DAWT	**PALFME**	MIXED/ES
PAKNRX	DAWT	**PALFRD**	MIXED/ES
PAKNXS	DAWT	**PALGAZ**	MIXED/ES
PAKPDF	DAWT	**PALGLU**	MIXED/ES
PAKRCC	DAWT	**PALJIP**	MIXED/ES
PAKUIT	DAWT	**PALLFB**	MIXED/ES
PAKXXF	DAWT	**CAAAL**	SIX1/2miRNA
CAAAAC	MIXED/ES	**CAAAO**	SIX1/2miRNA
CAAAAJ	MIXED/ES	**PAJMFU**	SIX1/2miRNA
CAAAAS	MIXED/ES	**PAJMFY**	SIX1/2miRNA
CAAAQ	MIXED/ES	**PAJMIZ**	SIX1/2miRNA
CAAAR	MIXED/ES	**PAJMSE**	SIX1/2miRNA
PAECJB	MIXED/ES	**PAJPAU**	SIX1/2miRNA
PAJLNJ	MIXED/ES	**PAKRVH**	SIX1/2miRNA
PAJLSP	MIXED/ES	**PALDTE**	SIX1/2miRNA

Supplemental Table 2: Oligo and primer sequences used in cloning, luciferase assays, EMSAs, and PBMs

>SIX1_enhancer_Luciferase assay
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>WNT5A_promoter_Luciferase assay
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>WNT5A_distal1_Luciferase assay
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>MEIS1-3xFLAG_Rev primer for cloning
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>SIX1_gBlock for protein purification and luciferase assay
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>SIX1-Q177R_gBlock for protein purification and luciferase assay
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>EYA1-2xHA_Rev primer for cloning
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>SIX1_nGST_HD for PBM

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