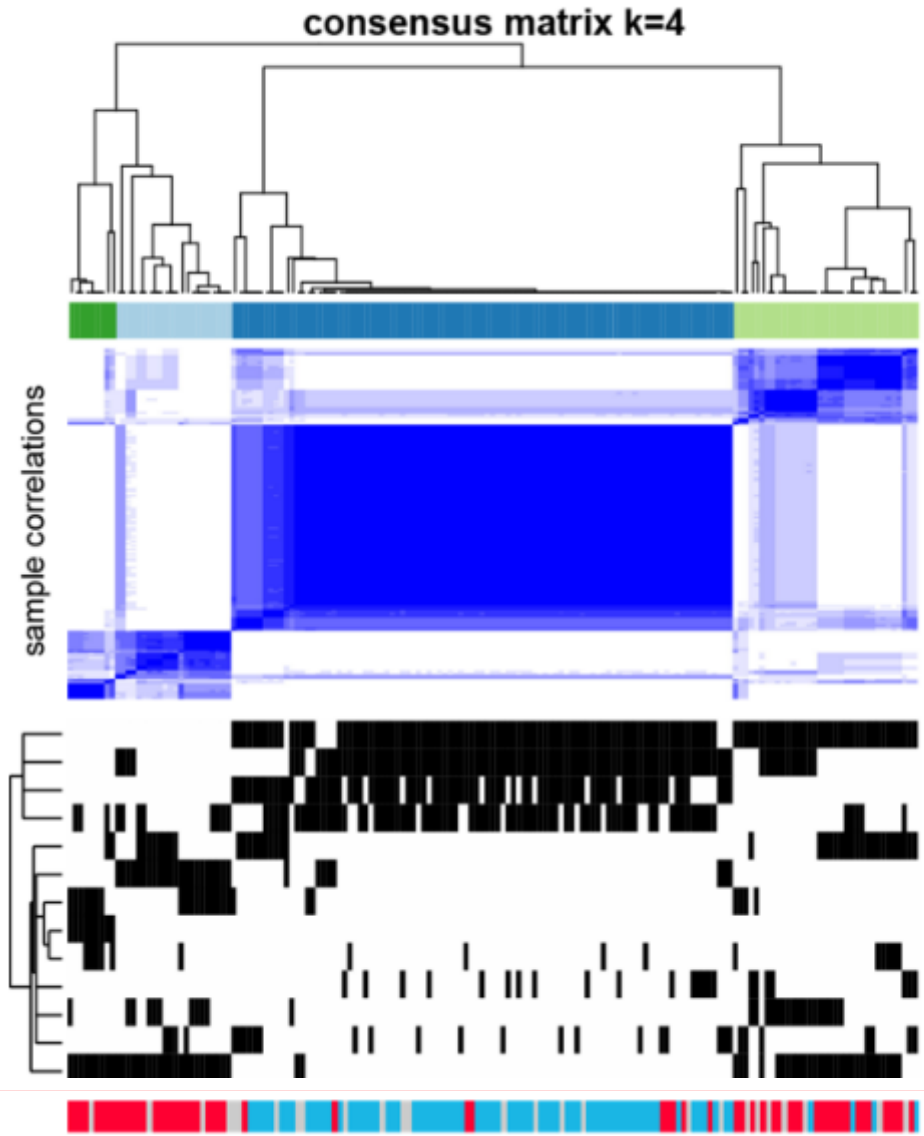
TCGA KIRP manuscript status

September 15, 2014

Please email chris.ricketts@nih.gov with any corrections and suggestions.

Cluster of Cluster Analysis (COCA)



clusters (k=4)

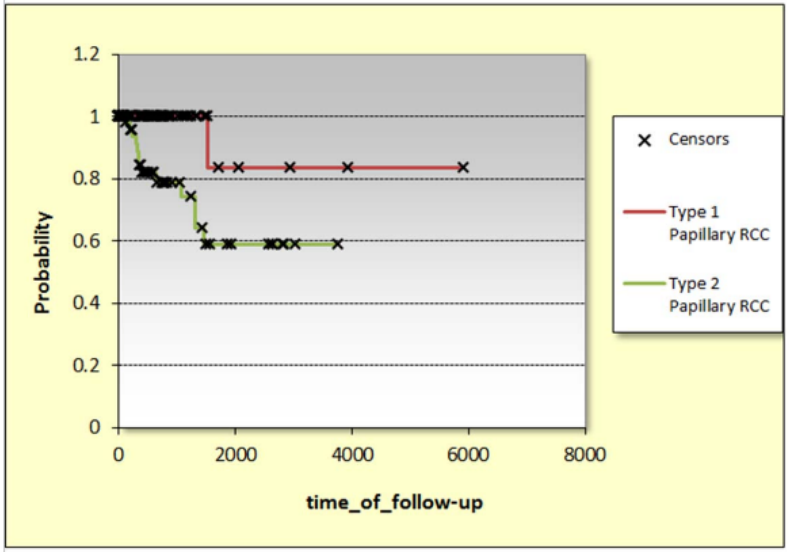
- 1 **Pap. Type 2, Poor Surv.** (T2-86%, Unc-14%)
- 2 **Mostly Pap. T1** (T1-75%, T2-9%, Unc-16%)
- 3 **Mostly Pap. T2, Good Surv.** (T1-11%, T2-69%, Unc-20%)
- 4 **Pap. Type 2, CIMP** (T2-89%, Unc-11%)

Unsupervised clustering clearly separates tumors by type 1/type 2 status

Cluster of Cluster Analysis (COCA)

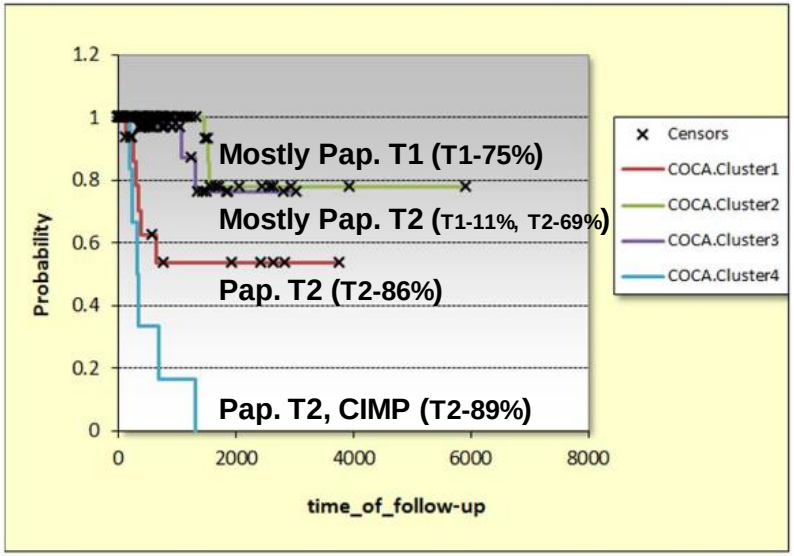
Survival differences, type status by pathology

Chi-square	Degrees of Freedom	P
10.51900121	1	0.001181533



Survival differences, COCA k=4

Chi-square	Degrees of Freedom	P
77.05613781	3	1.31325E-16

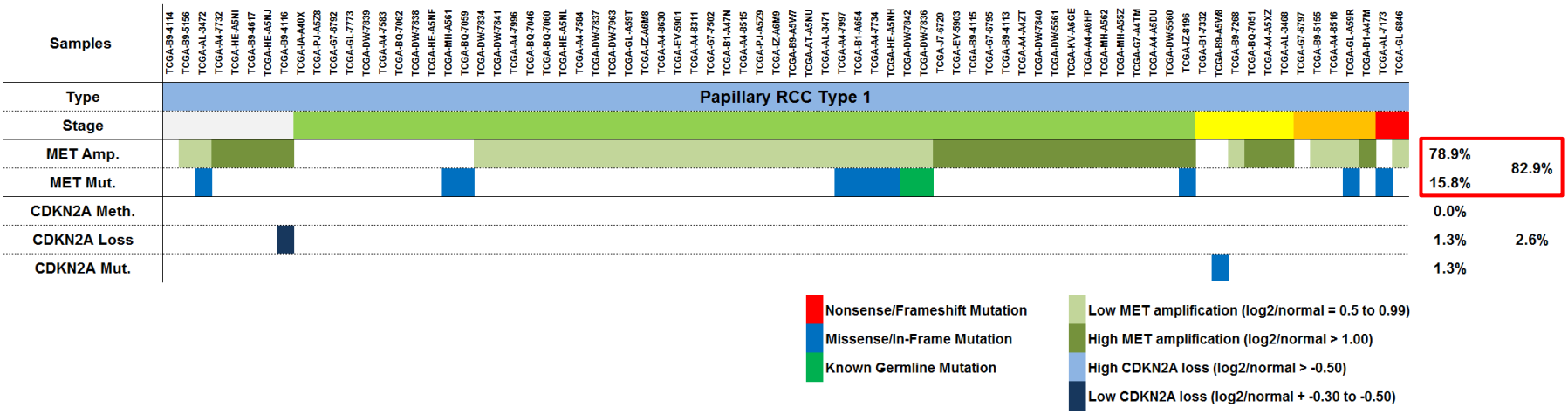


Breakdown of COCA data

This analysis provides several key points that can be expanded upon:

1. The type 1 papillary cancer is a relatively homogeneous group with good survival rates, compared to type 2 papillary cancer that has multiple sub-groupings with varied survival rates. (Slides 2 and 3)
2. That type 1 papillary cancer is associated with alterations to the MET gene such as activating mutations, copy number amplifications, miRNA alterations and novel splice versions of the gene. (Slides 5 and 6)
3. That a specific subset of type 2 papillary cancer exists that exhibits a global methylation phenotype that is associated with high stage, very poor survival, specific methylation of *CDKN2A* and mutation of the *FH* gene. (Slide 7)
4. That the remaining type 2 papillary cancers are split into two groups by COCA that demonstrate dramatically different survival rates.
5. That the poorer survival type 2 papillary cancers are associated with higher stage, *SETD2* mutations and increased activation of the NRF2 pathway. (Slide 8 and 9)

Type 1 Papillary Cancer and the *MET* Gene

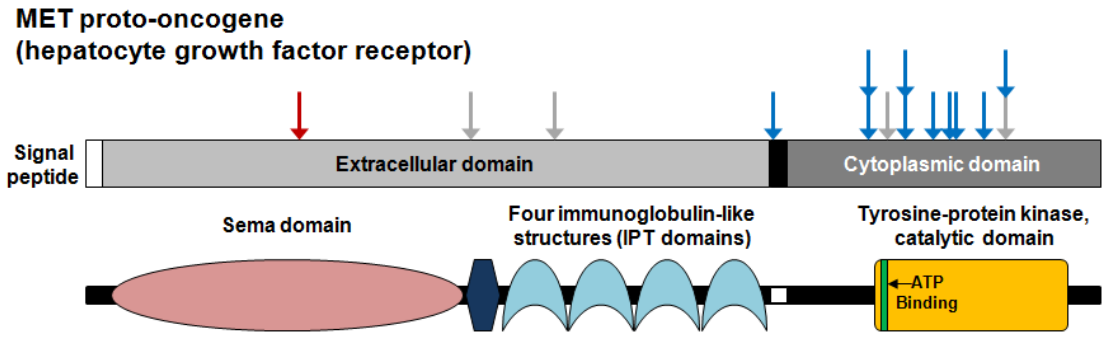


We could produce a figure that reinforces the strong association of *MET* alterations with type 1 papillary cancer.

- A large proportion of the type 1 pap. cancers demonstrate amplification of the *MET* gene (see above) including amplifications of more than a single copy and this correlates to higher *MET* expression (Slide 6 figure B).
- All the tumors with activating mutations in the tyrosine kinase domain of *MET* occur in type 1 pap. tumors (Slide 6 figure A).
- Lower levels of miR-34a-5p correlate to a degree with increased *MET* expression (Slide 6 figure C).
- Novel splice versions of *MET* were observed in type 1 pap. cancers that may represent a new pathway for increased MET protein activation.

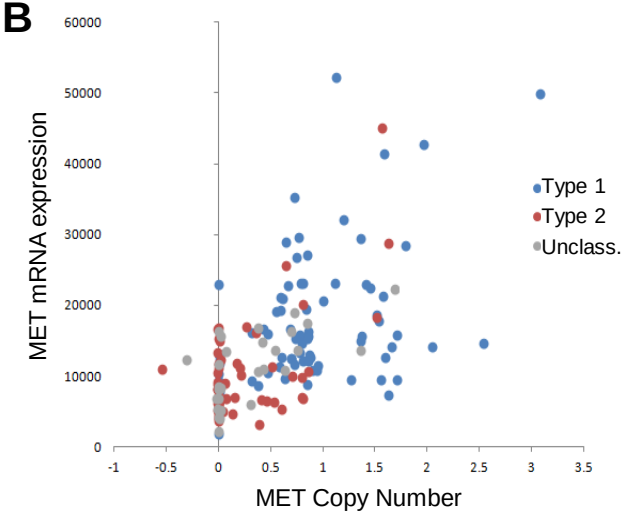
Type 1 Papillary Cancer and the *MET* Gene

A

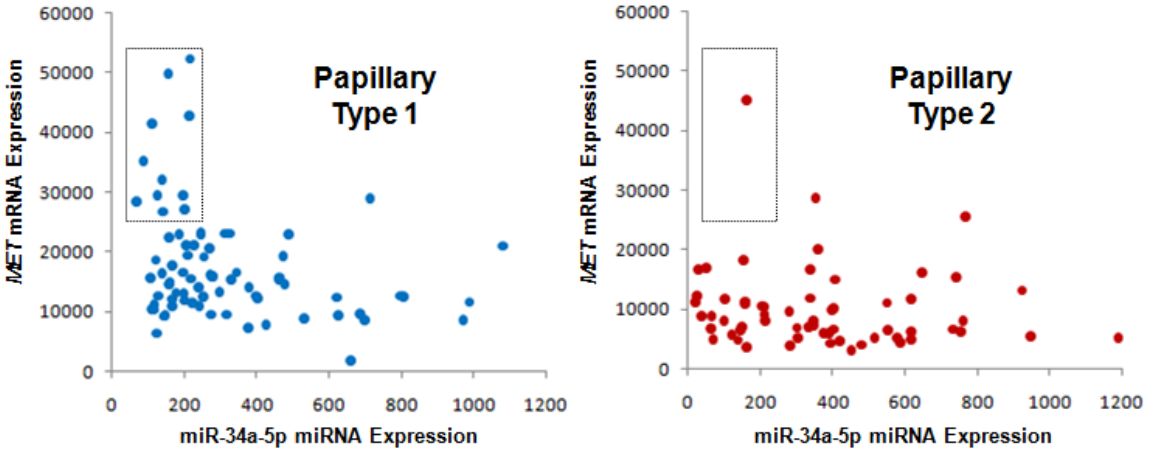


	Gene	Mutation	Type
TCGA-BQ-5882	MET	p.L296P	Type 2
TCGA-B9-4117	MET	p.C541G	Unclass.
TCGA-IA-A40U	MET	p.I639L	Unclass.
TCGA-HE-A5NH	MET	p.V956delinsVVSISTALLLLGF	Type 1
TCGA-HE-A5NH	MET	p.F970V	Type 1
TCGA-AL-3472	MET	p.V1088E	Type 1
TCGA-GL-A59R	MET	p.V1088R	Type 1
TCGA-DZ-6132	MET	p.V1110I	Unclass.
TCGA-A4-7997	MET	p.H1112Y	Type 1
TCGA-AL-7173	MET	p.H1112Y	Type 1
TCGA-IZ-8196	MET	p.F1218I	Type 1
TCGA-BQ-7059	MET	p.D1246H	Type 1
TCGA-B1-A654	MET	p.Y1248H	Type 1
TCGA-A4-7734	MET	p.S1254R	Type 1
TCGA-DZ-6135	MET	p.M1268T	Unclass.
TCGA-MH-A561	MET	p.M1268T	Type 1

B



C



A – MET gene mutations cluster in the Tyrosine Kinase Domain and are associated with Pap. Type 1

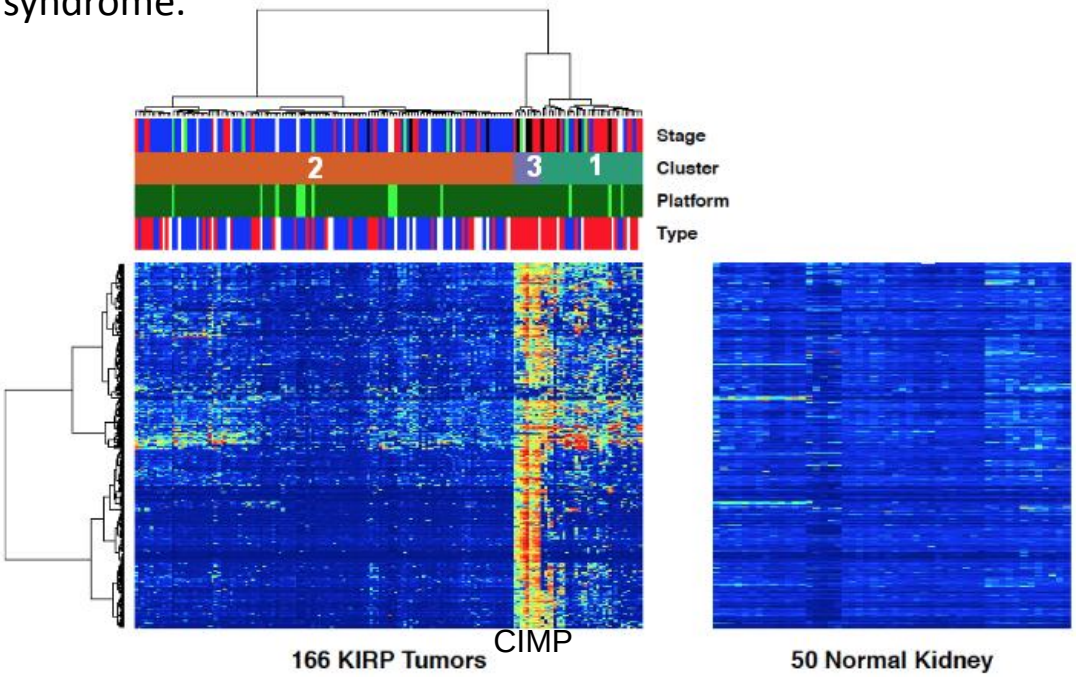
B – Higher MET copy number correlates with MET mRNA expression.

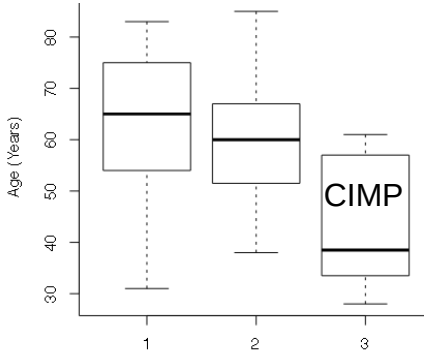
C – Some evidence that lower expression levels of miR-34a-5p correlate with high MET mRNA expression in Pap. Type 1

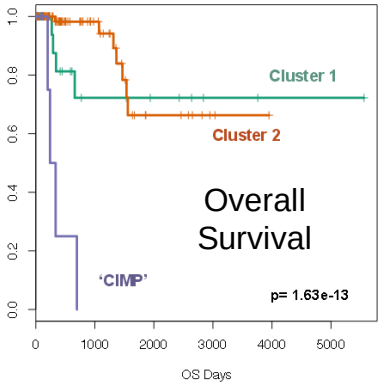
CIMP-associated Type 2 Papillary Cancer

This should represent a major feature of the paper as a hitherto unknown subtype of sporadic type 2 papillary cancer that is defined by the presence of the CpG island methylator phenotype (CIMP).

- This cluster only represents type 2 papillary and unclassified tumors.
- The tumors are associated with high state and very poor survival and present with a significantly younger age of onset.
- These tumors are associated with specific methylation of the CDKN2A gene and germline and/or somatic mutation of the *FH* gene. The germline mutated samples represent patients with HLRCC, but the samples without germline mutations could represent the sporadic form of this hereditary syndrome.





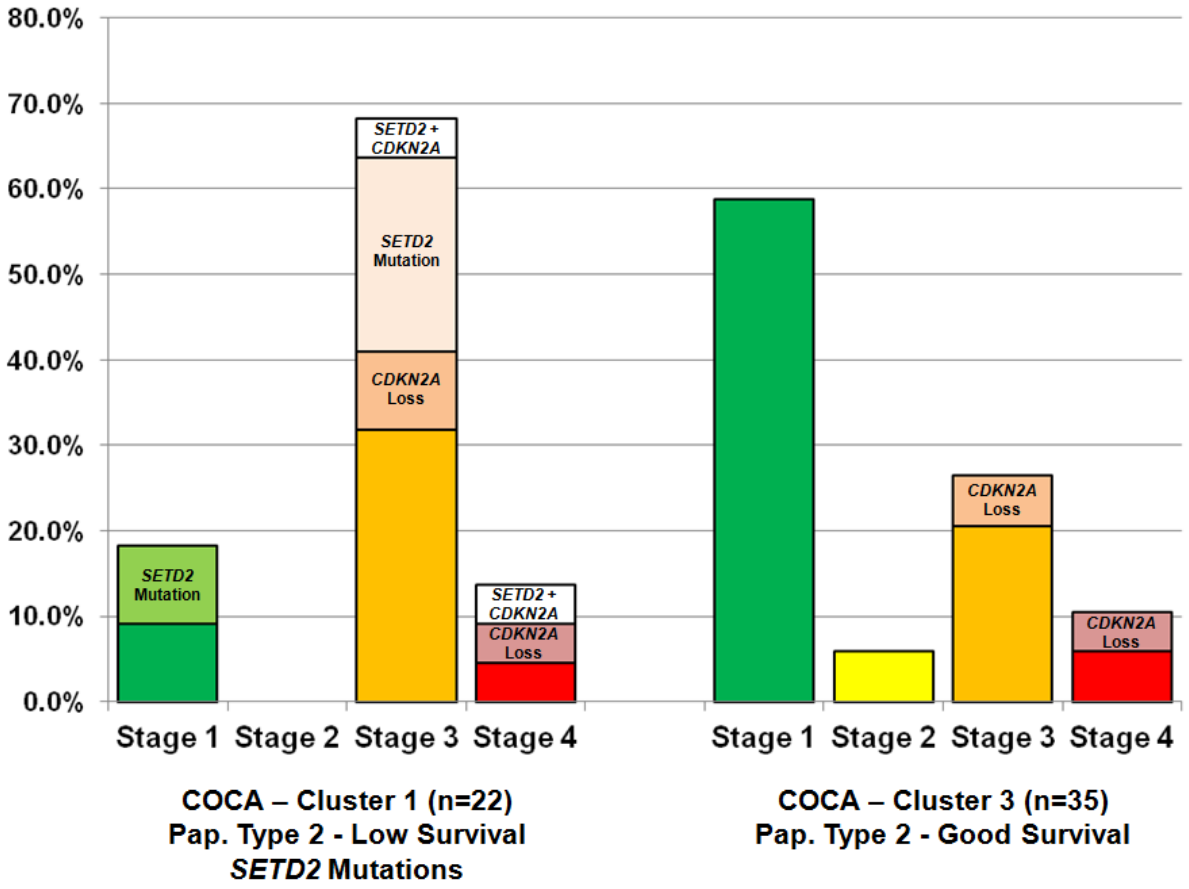


Subtypes of Type 2 Papillary Cancer

We could highlight that this integrated analysis can separate out two specific groups of type 2 papillary cancer, one with low survival and one with good survival.

The poor survival group is associated with higher stage and *SETD2* mutation.

Additionally the activation of the NRF2 pathway appears increased in this group. (Slide 9)

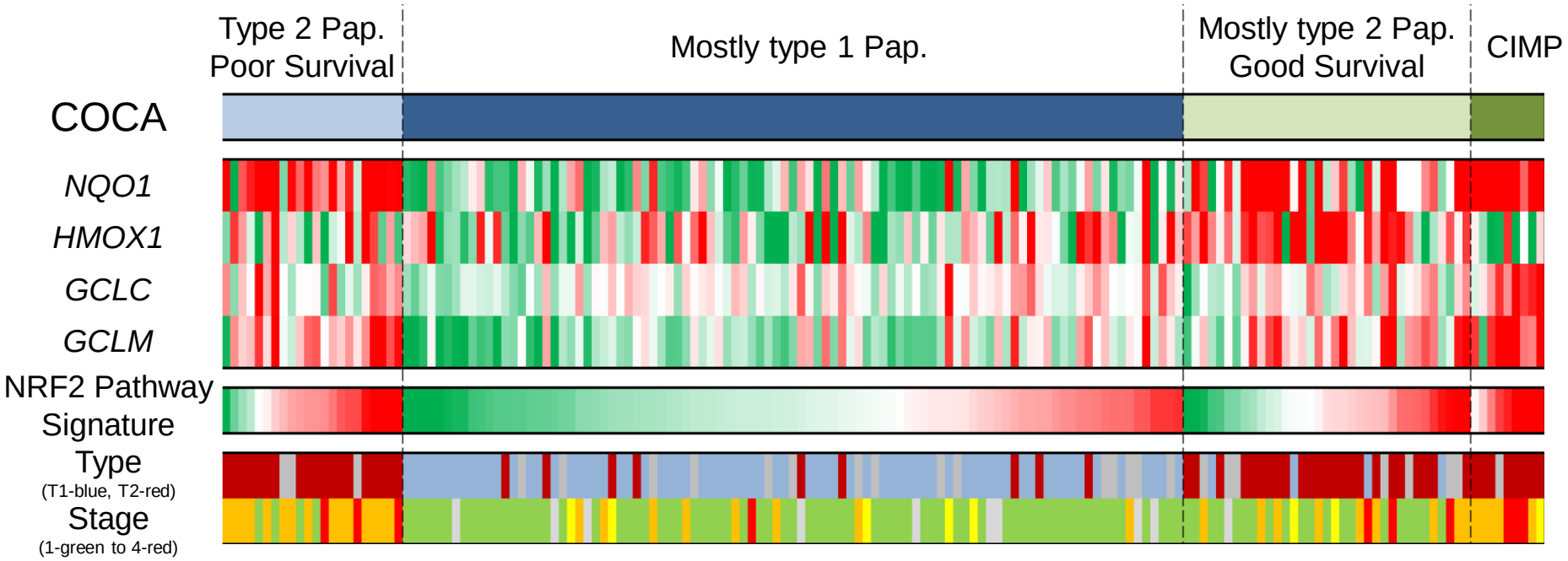


Subtypes of Type 2 Papillary Cancer

The NRF2 pathway is represented either by 4 transcription gene targets (NQO1, HMOX1, GCLC, GCLM) or the NRF2 pathway signature provided by Chad.

The CIMP group and the poor survival type 2 papillary group have notably high levels of expression of the NRF2 pathway genes (red represented increased expression). Although high levels of expression can be seen in members of other groups.

Other features of these groups should be investigated?

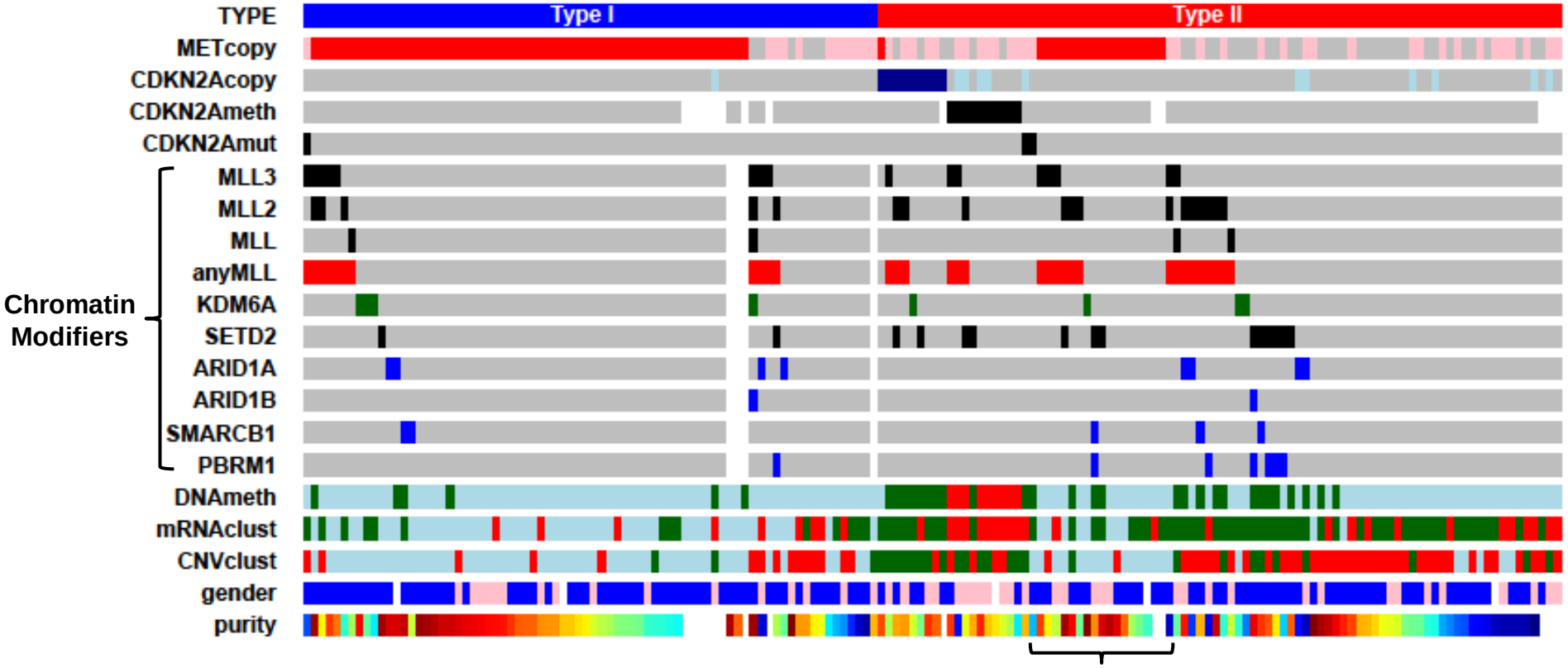


Other Potential Key Points

1. Currently we only have 2 SMGs (*MET* and *KDM6A*) using MutSigCV, do we want to look at other versions of MutSig (after Firehose run) and what do we want to say about *KDM6A*. Should we investigate mutations in other chromatin modifying genes selected by biological relevance? (Slide 11)
2. Copy number analysis highlighted loss of chromosome 9p, including *CDKN2A*, and this is associated with type 2 papillary cancer and poor survival (as does *CDKN2A* hypermethylation) and could represent some of the disparity between type 1 and type 2 survival . (Slide 12)
3. Analysis of the mRNA alone demonstrates a relatively clear separation of type 1 and type 2 papillary cancers with the most noticeable difference between the two types being activation of the NRF2 pathway in Type 2 papillary cancer. (Slides 13-15)
4. Analysis of the miRNA alone also demonstrates a relatively clear separation of type 1 and type 2 papillary cancers and highlights specific miRNAs. (Slide 16)
5. New data from the RPPA analysis?
6. Other additional data?

Other Potential Key Points

Alterations in a range of chromatin modifying genes could be assessed as KDM6A was one of the few SMGs identified and mutations in chromatin modifying genes seem to be common in urological cancers. Initial analysis by Hui Shen is shown below.

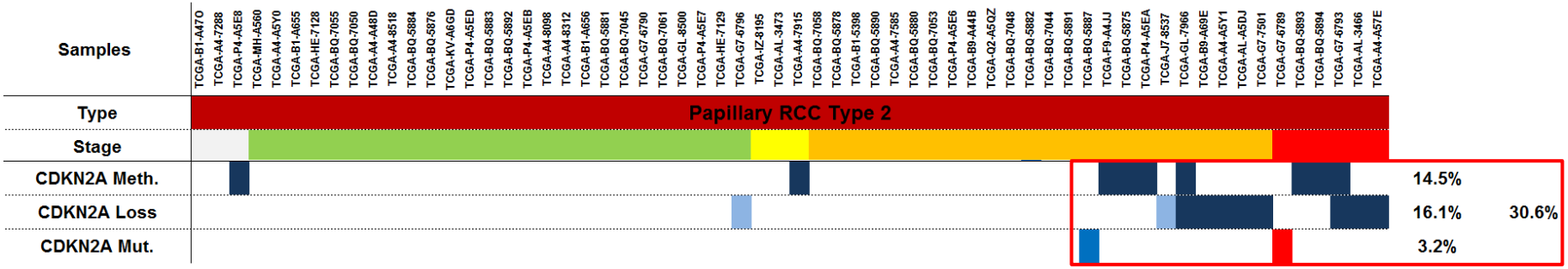


Overall Genomic Analysis – Hui Shen

Type 2 samples that have profiles similar to Type 1 tumors

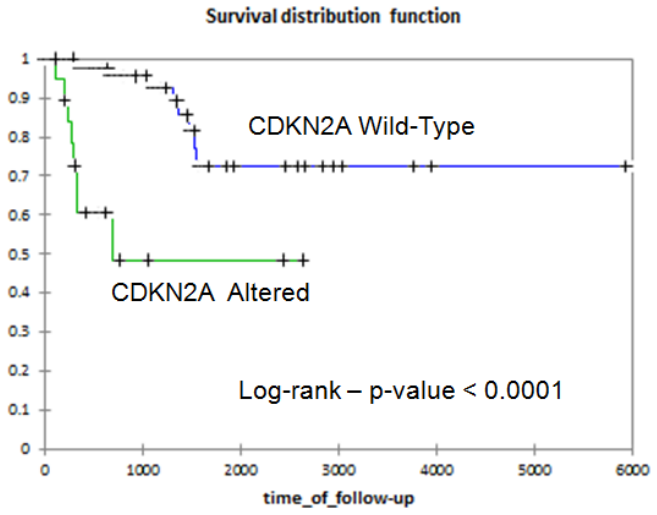
CDKN2A and Type 2 Papillary Cancer

Alterations of the CDKN2A gene either by chromosomal loss, hypermethylation or mutation associate with high stage type 2 papillary cancers and associate with poorer survival when present in any of the papillary tumors.



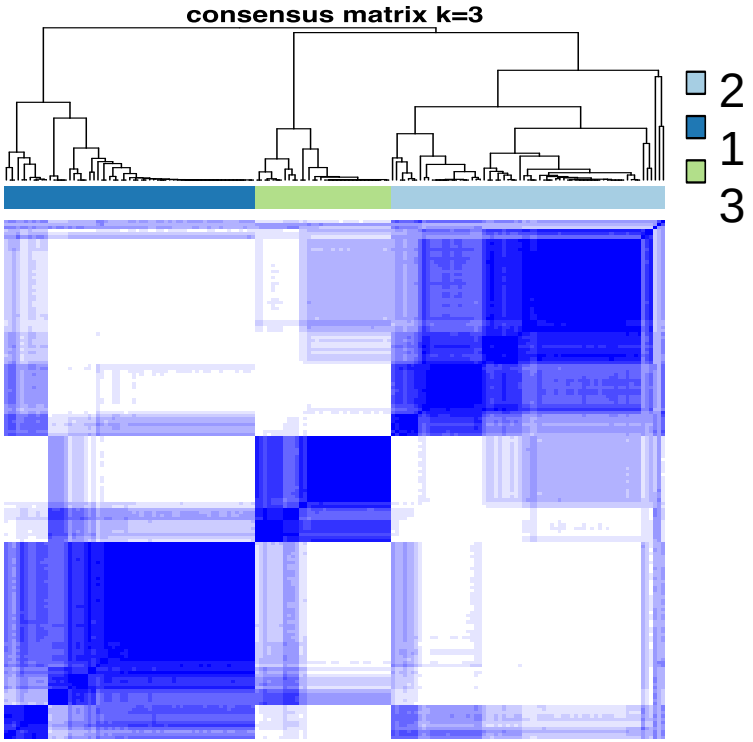
CDKN2A alterations associate with poorer survival

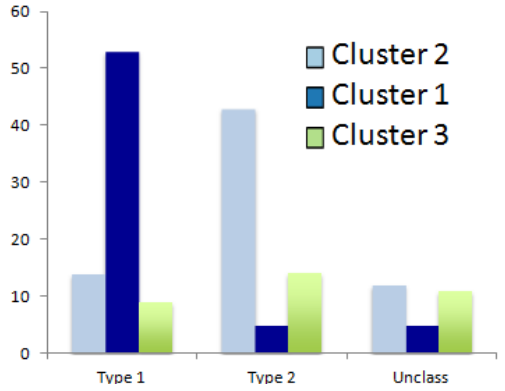
Note: All the CIMP samples are in the CDKN2A altered group.

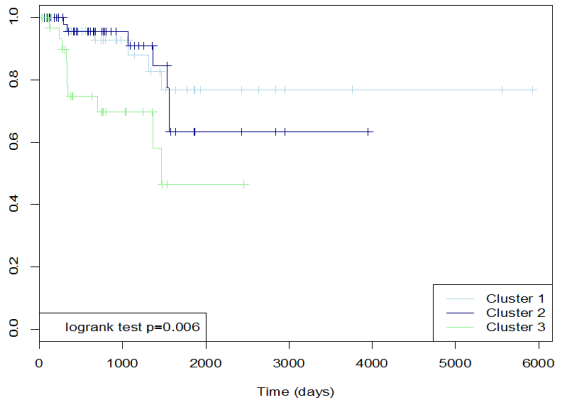


RNASeq Analysis

- The normals and the tumors cluster differently.
- The data produces three main clusters that largely segregate the samples by histological type with cluster 1 consisting largely of Type 1 and cluster 2 consisting largely of Type 2.







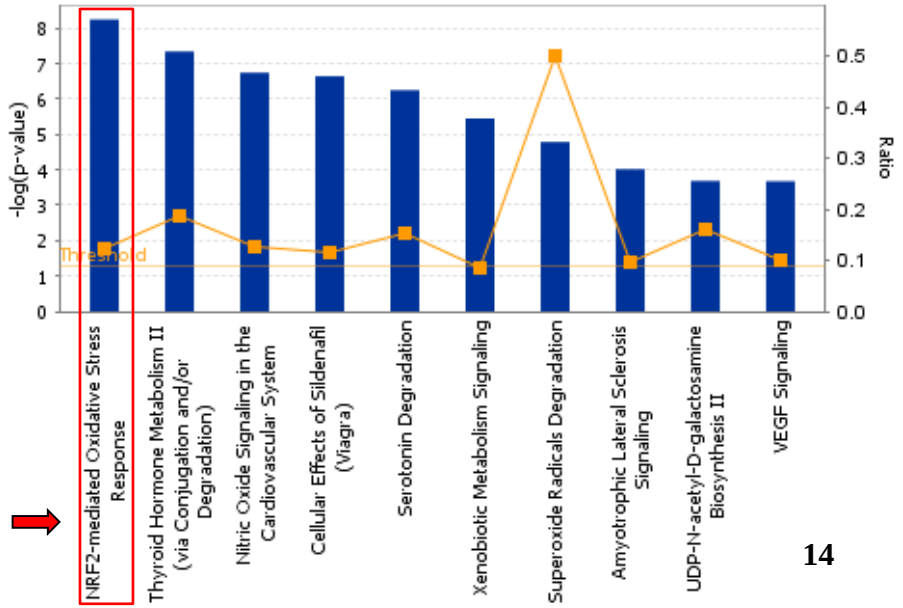
Pathway Analysis

- Due to the relatively distinct split between type 1 and type 2 by unsupervised clustering the expression profiles of these two groups were directly compared by Chad.

- Using the Ingenuity Software, the type 2 samples show a significant increase in the NRF2/anti-oxidative stress pathway. This pathway is active in a small percentage of type 1 and interestingly is usually associated with mutation of this pathway. This seems to be a more general signal in type 2.

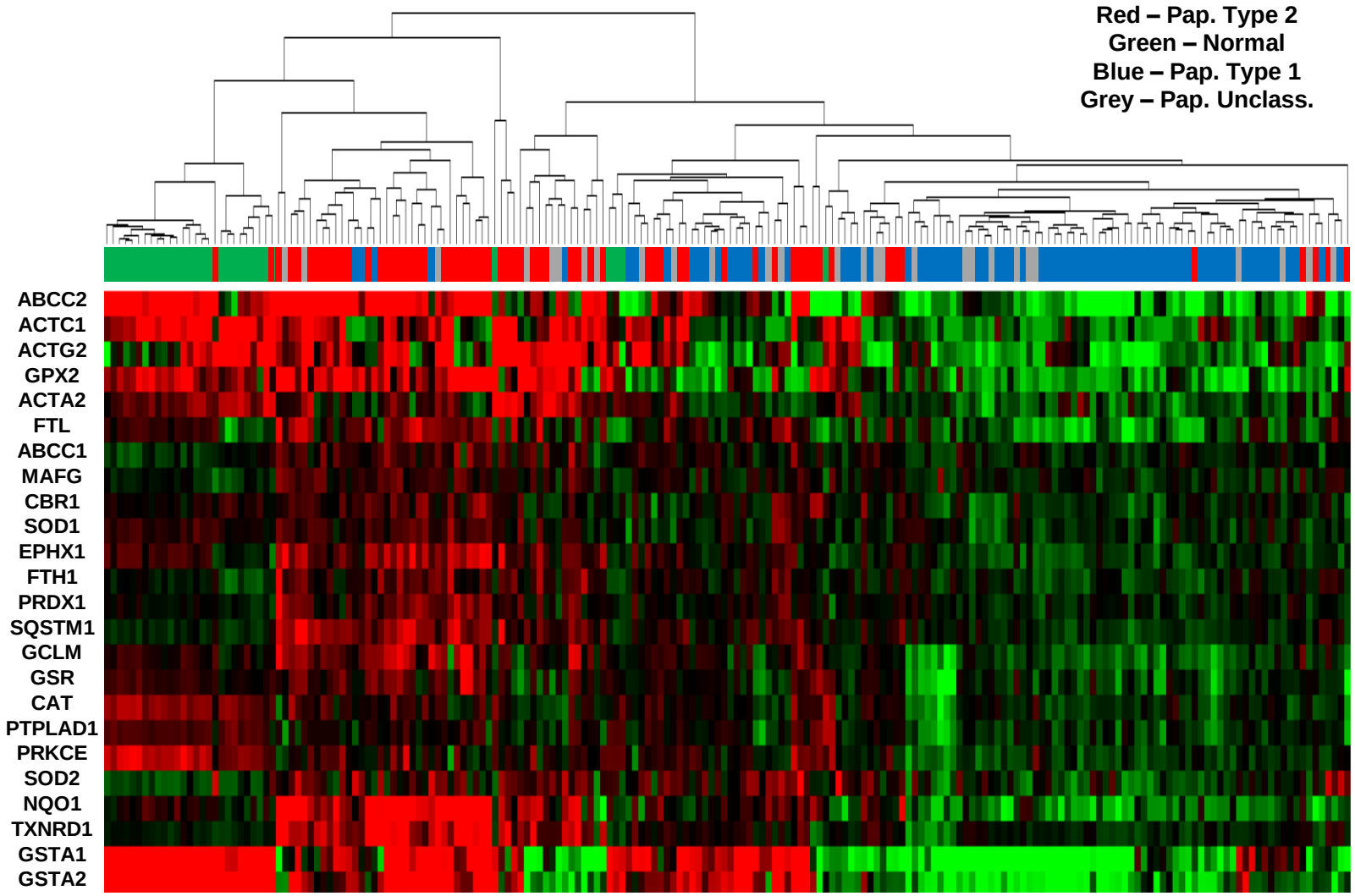
	Gene	Fold Change (type2/type1)	t-test	
1	ISPD	0.283	1.633E-24	higher_type1
2	DYNC2LI1	0.367	4.573E-24	higher_type1
3	SOSTDC1	0.022	4.253E-23	higher_type1
4	FHL1	0.103	1.634E-21	higher_type1
5	CLIC6	0.065	2.723E-21	higher_type1
6	KRT7	0.055	3.416E-21	higher_type1
7	PLEKHH2	0.146	1.106E-20	higher_type1
8	FLJ32063	0.140	1.226E-19	higher_type1
9	SATB2	0.280	5.39E-19	higher_type1
10	CRLS1	0.451	1.248E-18	higher_type1
11	DKFZP586I1420	0.484	1.32E-18	higher_type1
12	UPK1B	0.044	2.058E-18	higher_type1
13	CXCL6	0.048	2.927E-18	higher_type1
14	SUN1	0.473	4.349E-18	higher_type1
15	PRICKLE1	0.293	4.543E-18	higher_type1
16	C7orf63	0.318	1.006E-17	higher_type1
17	SLC34A2	0.038	1.685E-17	higher_type1
18	LNP1	0.222	1.699E-17	higher_type1
19	TLE4	0.489	2.722E-17	higher_type1
20	WNT5A	0.245	3.55E-17	higher_type1

	Gene	Fold Change (type2/type1)	t-test	
1	UGT1A9	37.95385632	2.344E-20	higher_type2
2	SQSTM1	2.633120723	6.601E-20	higher_type2
3	STC2	8.042719994	9.917E-19	higher_type2
4	TSKU	4.789653552	1.117E-17	higher_type2
5	NQO1	6.981863065	4.489E-17	higher_type2
6	UGT1A6	18.54004763	5.872E-17	higher_type2
7	EPHX1	2.714940899	3.797E-16	higher_type2
8	DYSF	4.305096668	1.026E-15	higher_type2
9	EIF2S3	1.590437916	1.131E-15	higher_type2
10	GNPDA1	1.992205253	1.631E-15	higher_type2
11	UBXN8	1.733226102	1.711E-15	higher_type2
12	SAMD5	6.87688618	2.037E-15	higher_type2
13	GCLM	3.116611062	2.191E-15	higher_type2
14	ADRA1B	4.063412897	9.662E-15	higher_type2
15	C1orf43	1.540495907	1.06E-14	higher_type2
16	CMBL	2.823525413	1.434E-14	higher_type2
17	TYRO3	3.675081544	1.441E-14	higher_type2
18	SLC5A2	10.33492548	1.662E-14	higher_type2
19	RSL1D1	1.581474866	2.77E-14	higher_type2
20	ME1	5.621819926	2.876E-14	higher_type2



Top Upstream Regulator was also *NFE2L2*

Pap. Type 2 – NRF2 Pathway



miRNA Analysis

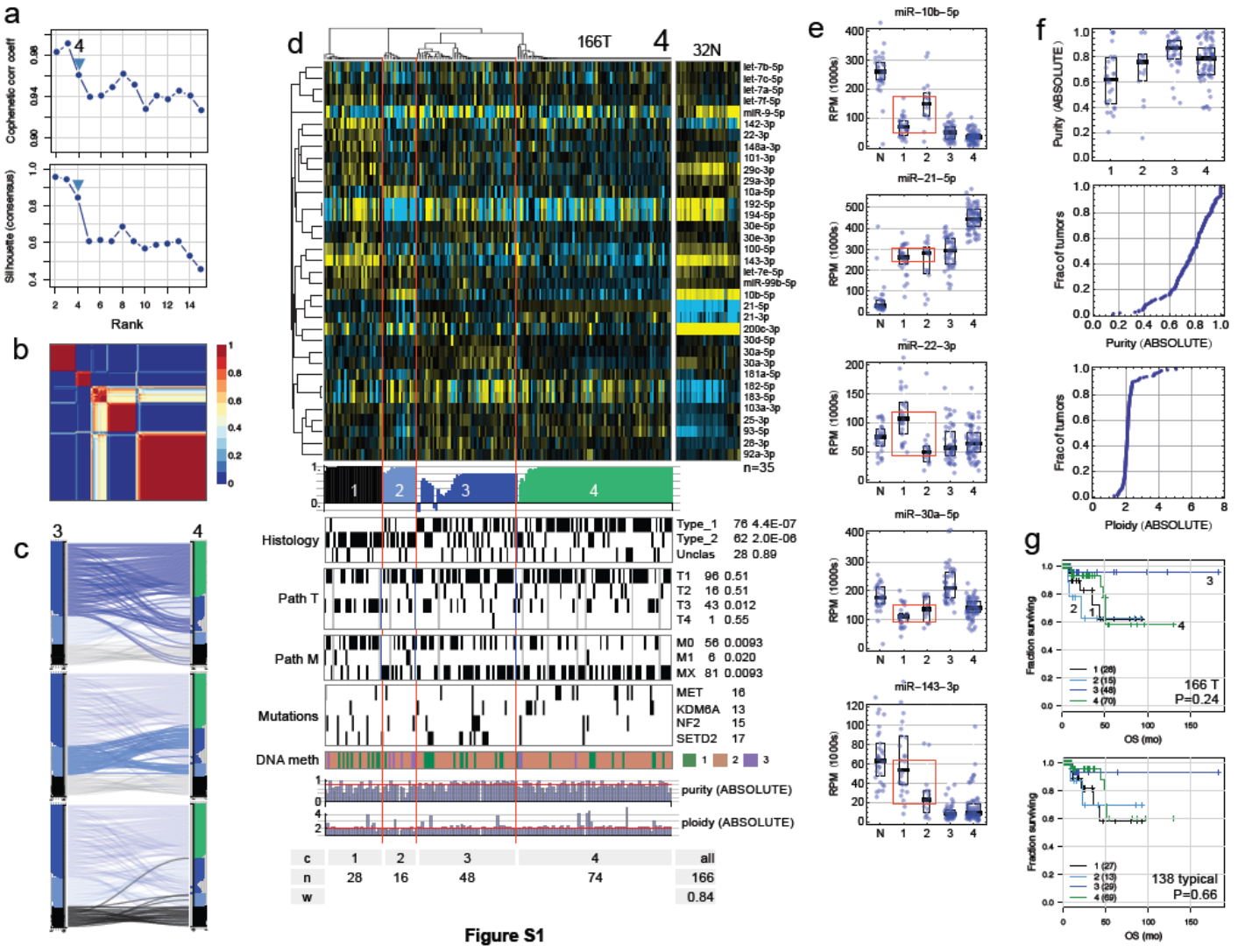


Figure S1

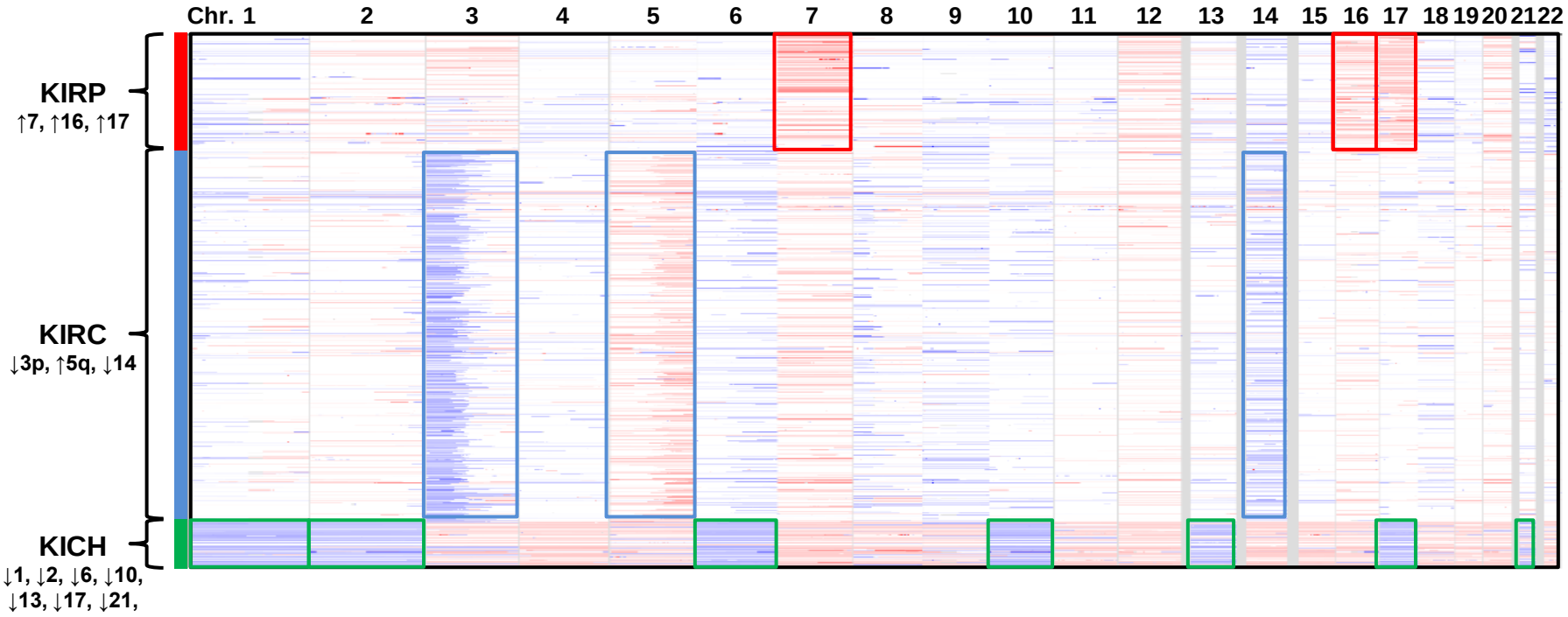
Papillary RCC type can be resolved into 4 clusters with cluster 1 consisting mostly of Pap. Type 2 samples and cluster 4 consisting of mostly Pap. Type 1 samples.

Supplementary Data Points

1. The clear differences between the three major types of kidney cancer, clear cell, chromophobe and papillary can be easily defined by their chromosomal copy number profiles. (Slide 18)
2. mRNA profile analysis demonstrates that the likely tissue of origin for papillary cancer is the proximal tubule similar to that of clear cell cancers but different to chromophobe cancer. (Slide 19)
3. miRNA analysis can also separate out the three types of kidney cancer with chromophobe kidney cancer represented by a single cluster while 4 main clusters represent the majority of clear cell cancers with a small amount of overlap of clear cell cancers in the papillary cancer cluster. (Slides 20 and 21)
4. Analysis of the metabolic pathways of glucose usage shows that the CIMP group demonstrates a similar Warburg-like profile to that observed in high stage clear cell tumors. Additionally the type 1 and 2 papillary tumors largely demonstrate differing metabolic profiles. (Slide 22)

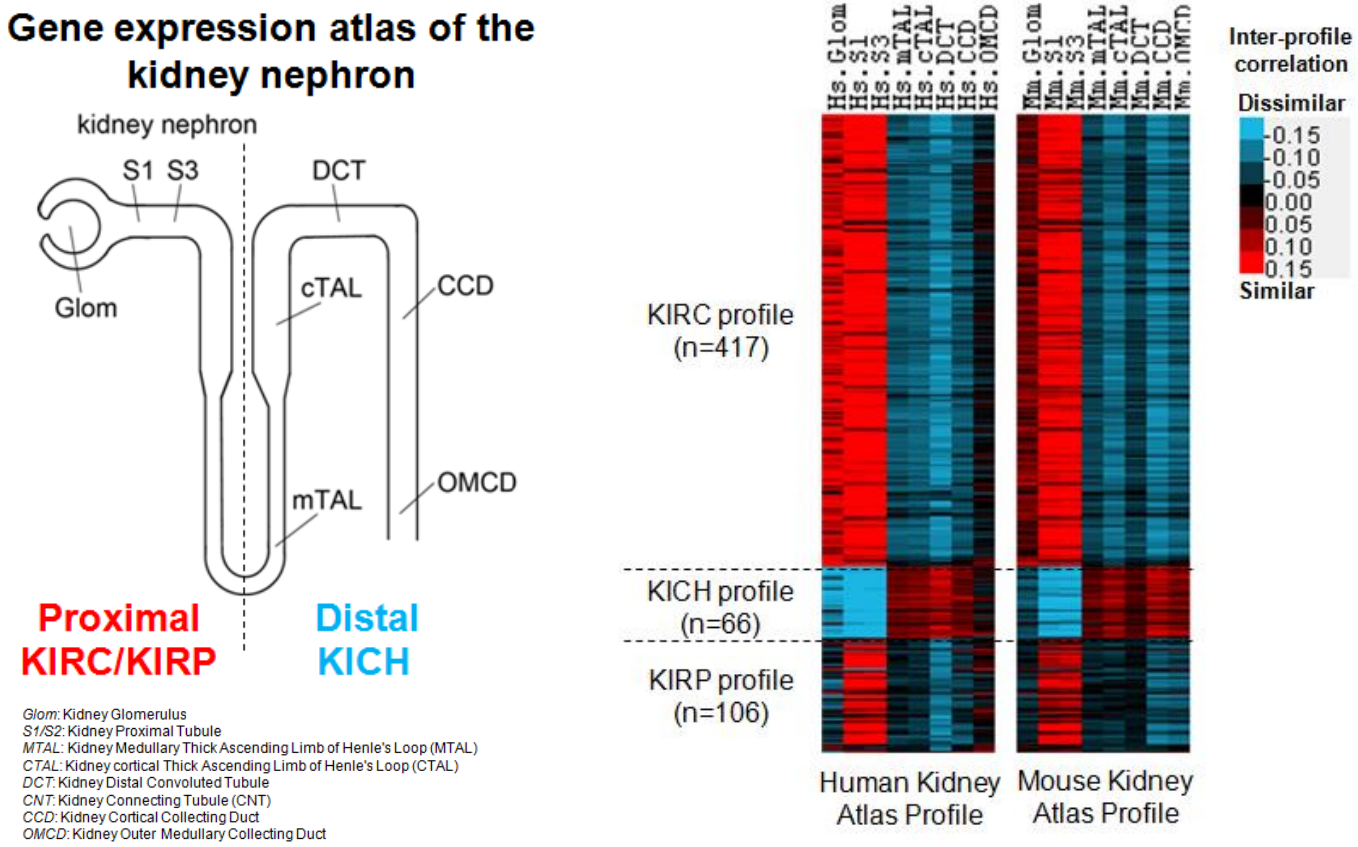
Chromosomal Copy Number Suppl.

- Copy number profiles very different between kidney cancer histological subtypes (KIRP vs KICH vs KIRC) and to some extent can distinguish Type 1 KIRP (MET gain) from Type 2 KIRP (CDKN2A loss).



Cell of Origin Suppl.

This data has now been updated to include all samples and will be added to supplementary as it nicely demonstrates what was already predicted.



miRNA Analysis Suppl. 1

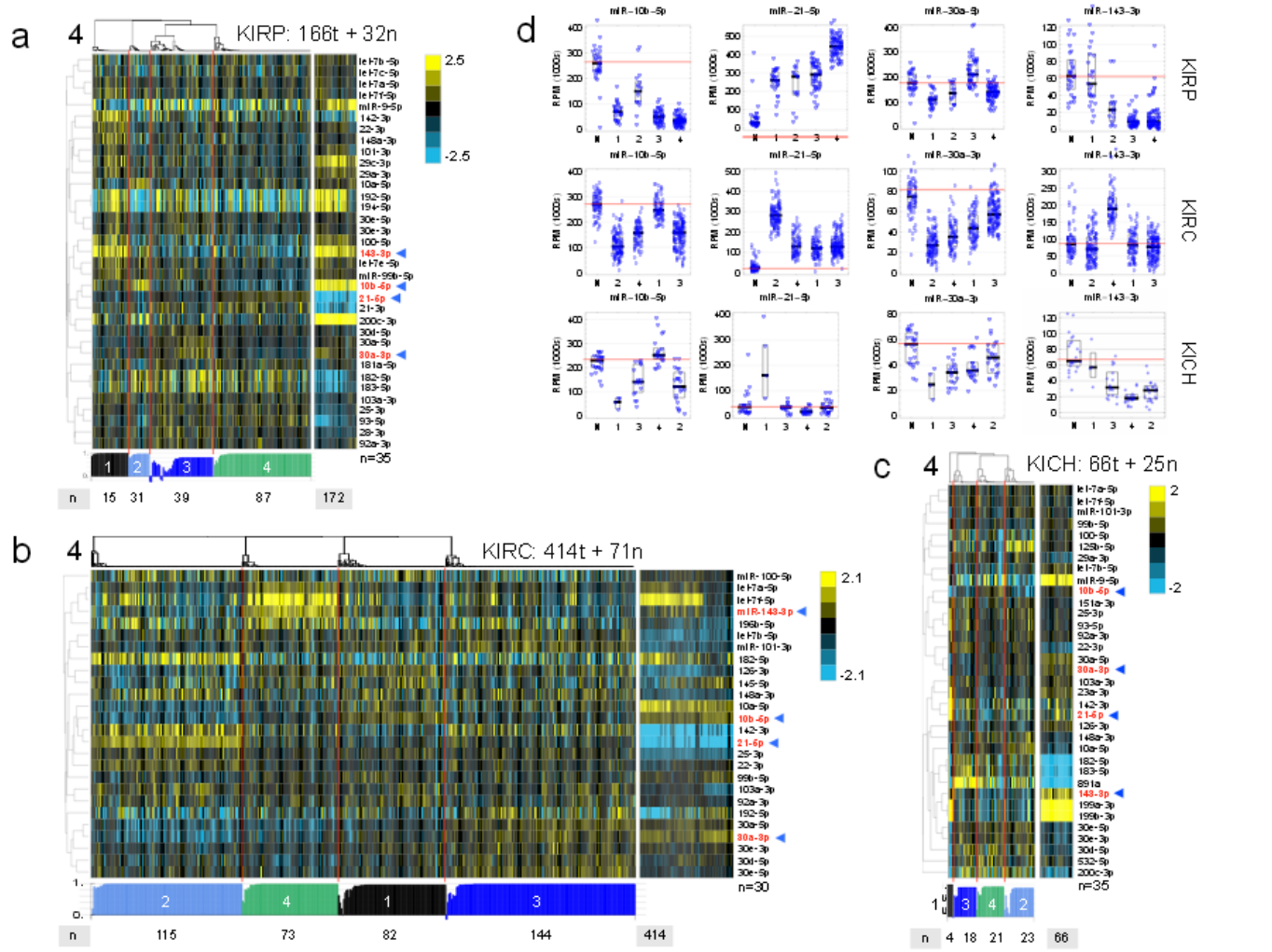


Figure S4

Each kidney type can be resolved into 4 clusters that have distinct marker miRNAs

miRNA Analysis Suppl. 2

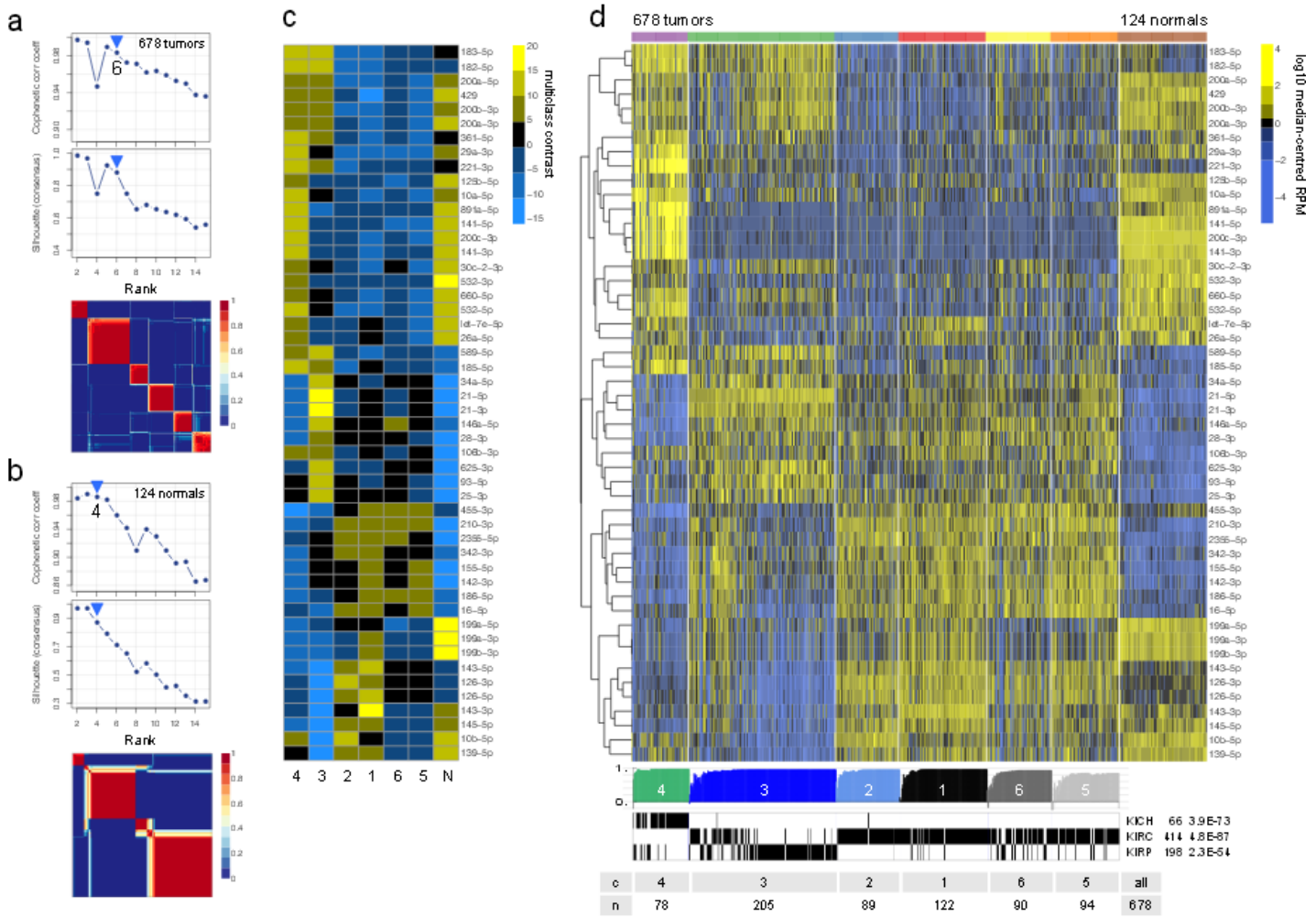


Figure S5

The miRNA analysis demonstrates that each type shows a relatively distinct miRNA profile with the exception of some over between papillary and a subgroup of clear cell tumors

Metabolic Pathway Analysis Suppl.