Committee Meeting #2

Karin Isaev
Supervisor: Dr. Juri Reimand
September 19th, 2017

Outline

Thesis: Genomic characterization of clinically relevant lncRNAs in multiple cancer types

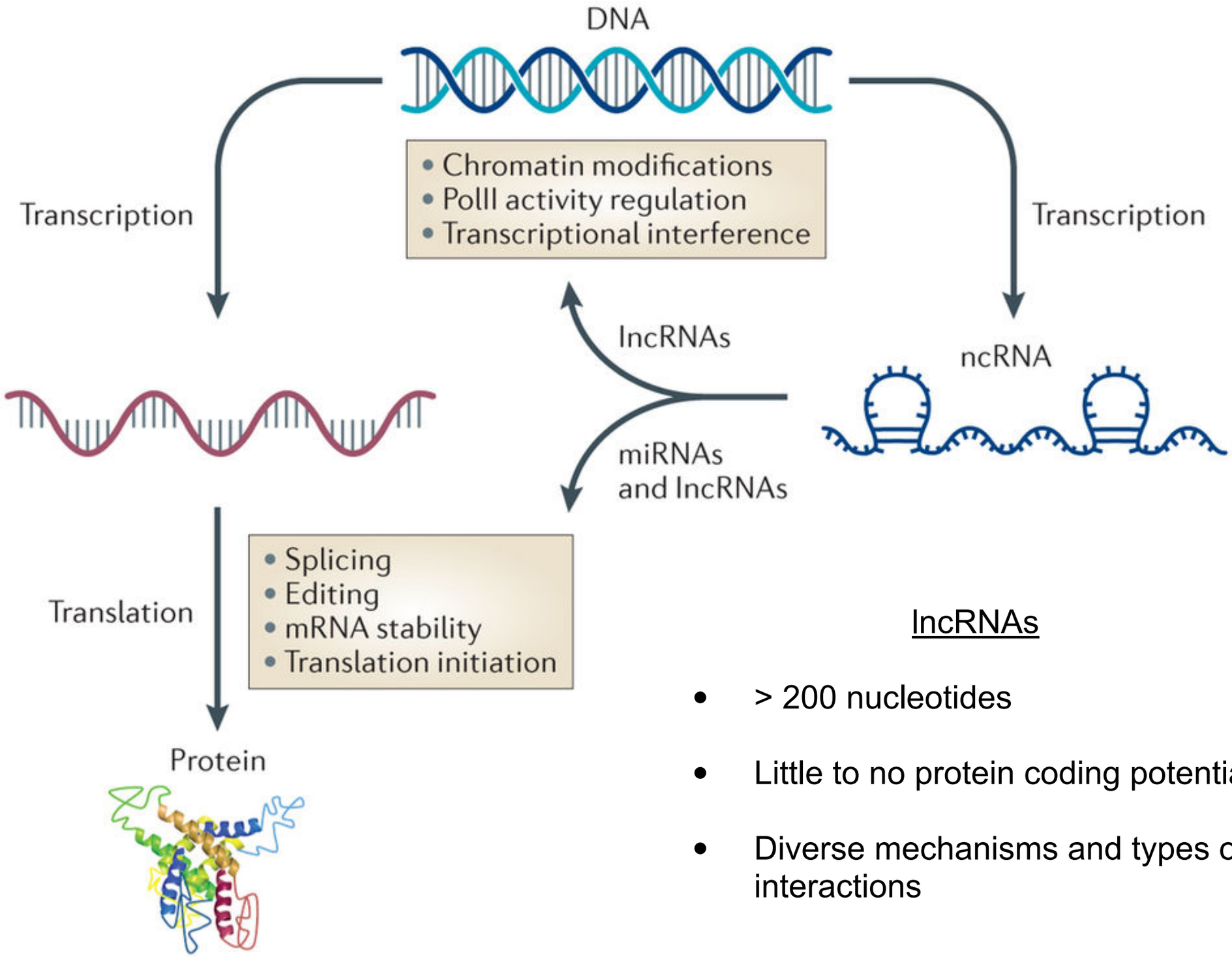
1. Review of lncRNAs
2. Summary of the last SCM
3. Summary of progress since last meeting
4. Future directions

Outline

Thesis: Genomic characterization of clinically relevant lncRNAs in multiple cancer types

1. **Review of lncRNAs**
2. Summary of the last SCM
3. Summary of progress since last meeting
4. Future directions

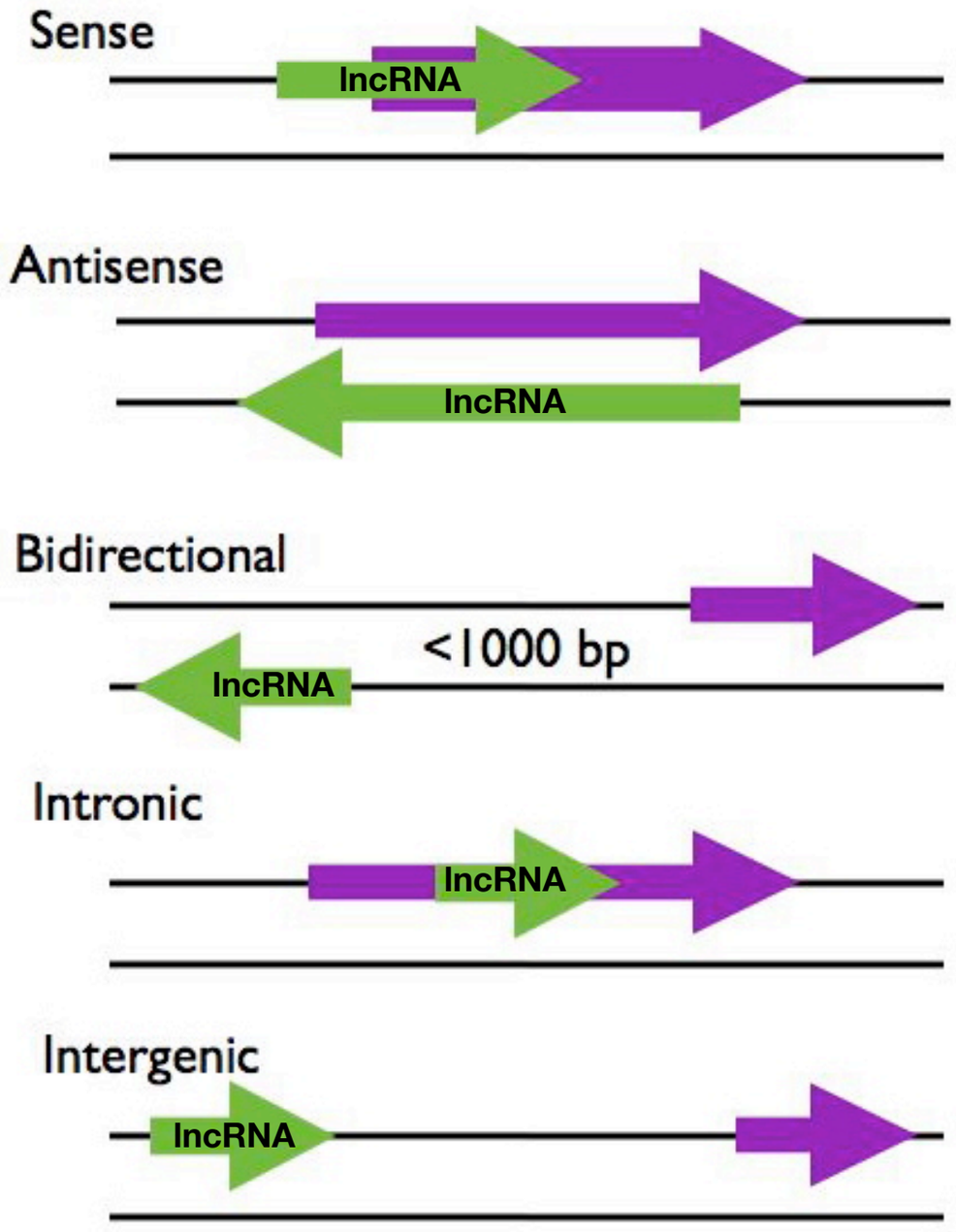
lncRNAs



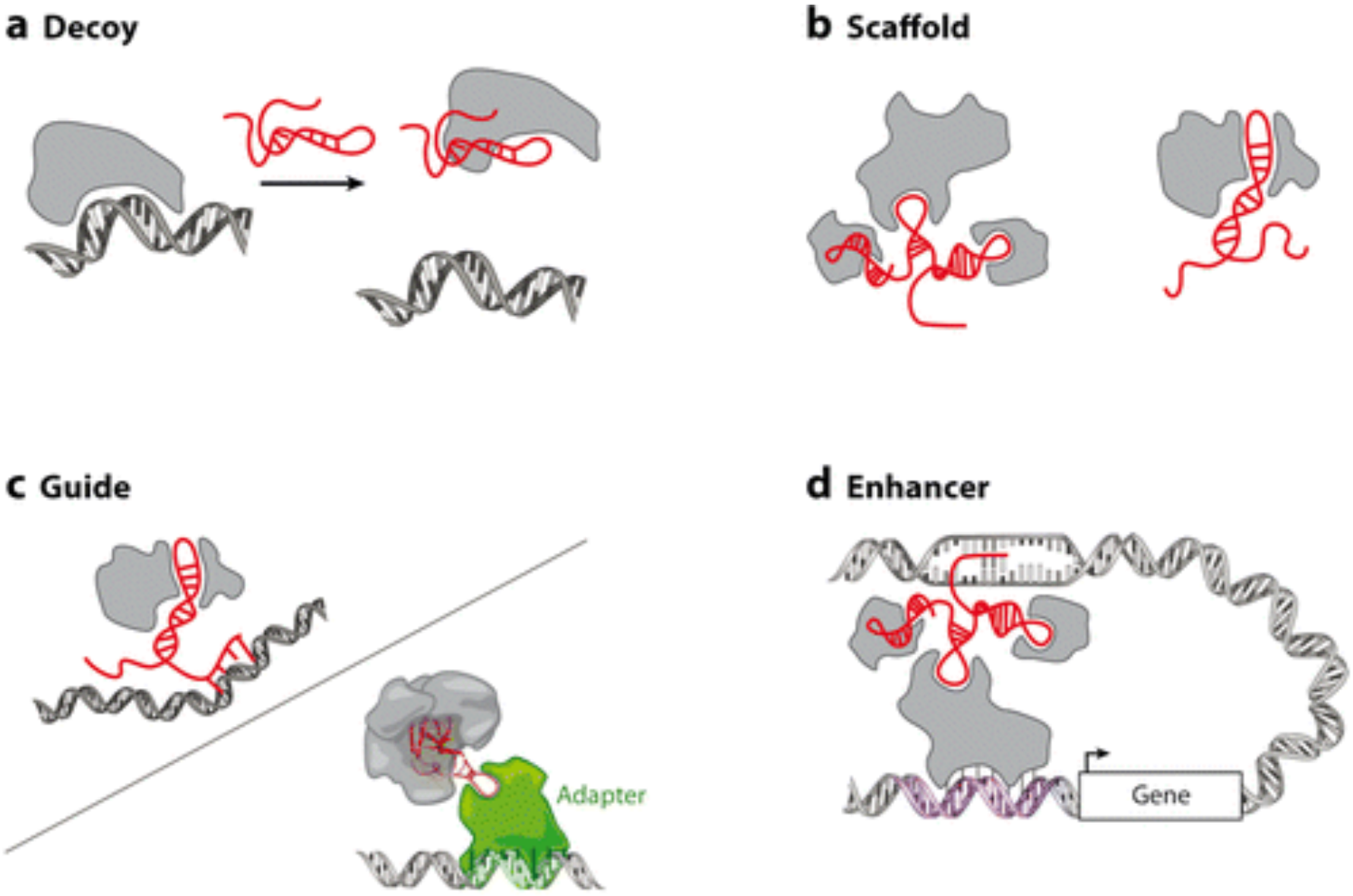
<u>lncRNAs</u>

- > 200 nucleotides
- Little to no protein coding potential
- Diverse mechanisms and types of interactions

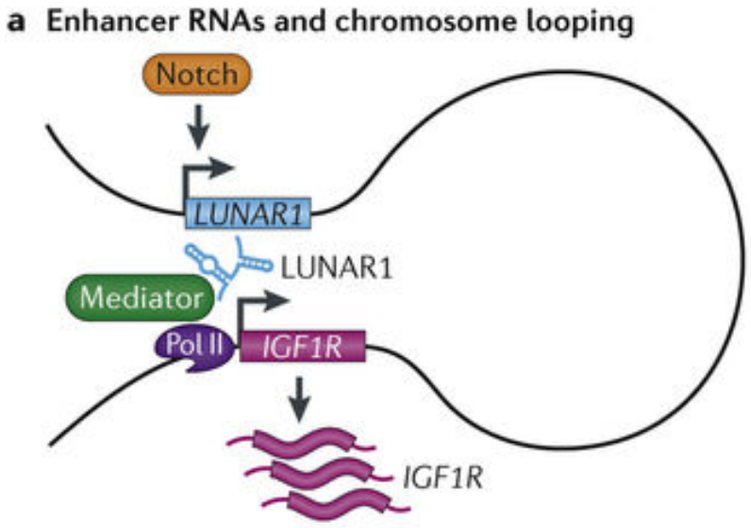
lncRNA type related to location of protein coding genes

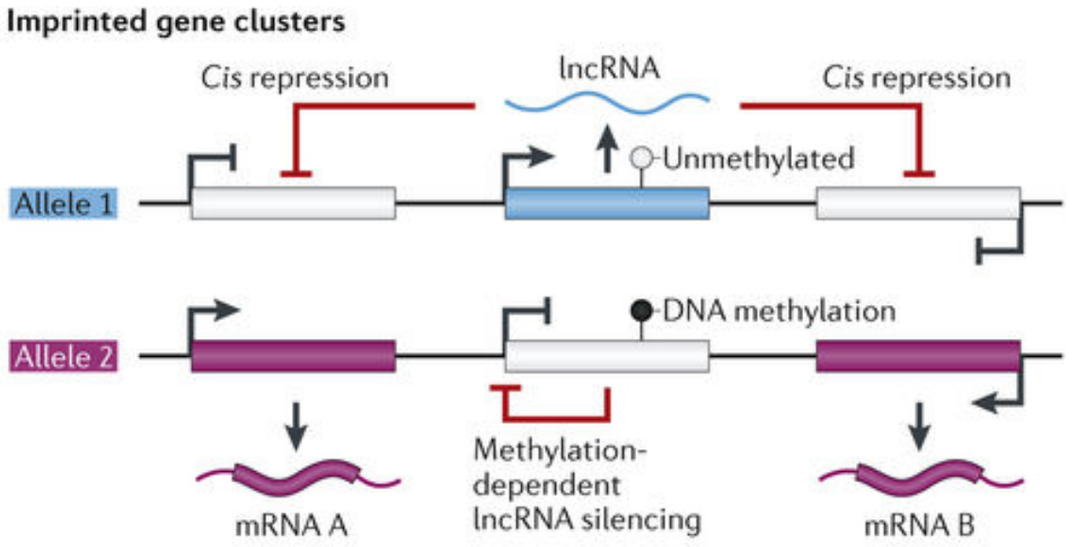


Examples of known lncRNA functions

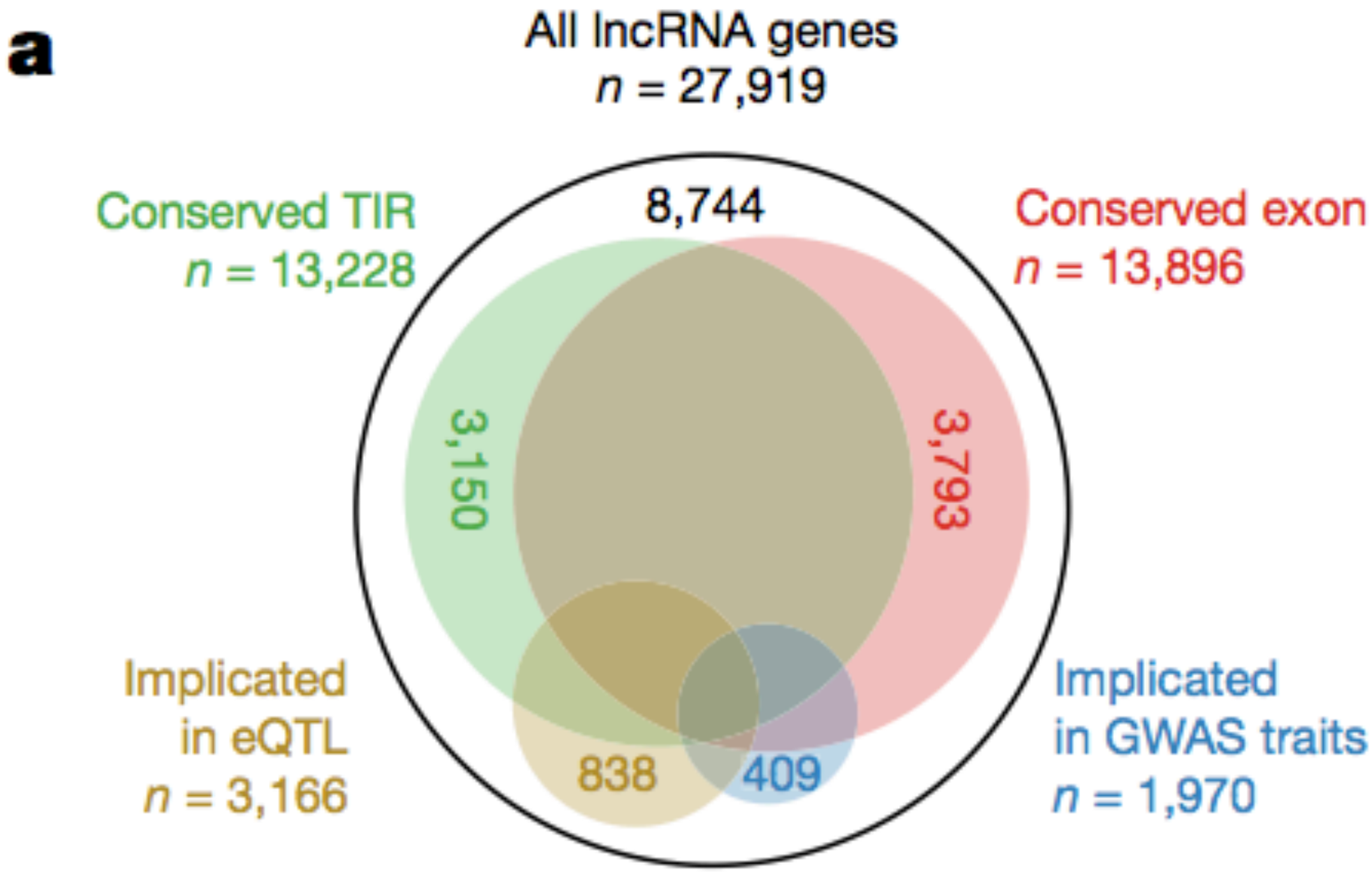


Examples of known lncRNA functions





~20,000 predicted functional lncRNAs in 2017



Outline

Thesis: Genomic characterization of clinically relevant lncRNAs in multiple cancer types

1. Review of lncRNAs
2. **Summary of the last SCM**
3. Summary of progress since last meeting
4. Future directions

Summary of the last SCM

Project aims:

- Using the PCAWG dataset, lncRNA candidates will be identified in multiple cancer types through associating their gene expression with patient survival outcomes.
    - Rationale: lncRNAs are expressed in a tissue specific manner and are often differentially expressed between tumour and matched normal samples
- Through further analysis including, differential expression, co-expression and pathway enrichment analysis, these lncRNA candidates will be associated with predicted cellular roles relevant to cancer progression in these patients.
- Cancer specific filtration of candidate lncRNAs
- Overcoming low abundance and possibility of noise

Literature based:

- Understand lncRNAs and protein coding potential
    - Open Reading Frames
- lncRNA in relation to protein coding genes
    - Overcoming possible noise
- lncRNA FANTOM CAGE-Seq data
    - Expanded study and database

Summary of progress

1. Identified high expressing cancer specific lncRNAs through several filtration steps across multiple cancer types.

2. Confirmed cancer specific expression through comparing the distributions of each candidate lncRNA's expression among the cancer types studied

3. Identified cancer specific high expressing lncRNAs associated with patient survival outcome using the Cox Proportional Hazards model
    - 42 lncRNAs in 4 cancer types
    - 7 lncRNAs with adjusted p-value < 0.1 in 2 caner types (Tier 1)
    - 35 lncRNAs with p-value < 0.05 in 4 cancer types (Tier 2)

4. Assessed tumour versus normal tissue lncRNA expression by comparing ranked values of expression using match tissue data from GTEX

5. 21/42 lncRNAs were predicted to be either tumour suppressive or oncogenic based on sign of hazard ratio and difference in expression between normal and tumour tissues

6. 2 candidates in Ovarian cancer were further studied to predict regulatory roles that may lead patients from the same cancer type to develop worse prognosis

Summary of progress

1. **Identified high expressing cancer specific lncRNAs through several filtration steps across multiple cancer types.**

2. Confirmed cancer specific expression through comparing the distributions of each candidate lncRNA's expression among the cancer types studied

3. Identified cancer specific high expressing lncRNAs associated with patient survival outcome using the Cox Proportional Hazards model

    - 42 lncRNAs in 4 cancer types
    - 7 lncRNAs with adjusted p-value < 0.1 in 2 caner types (Tier 1)
    - 35 lncRNAs with p-value < 0.05 in 4 cancer types (Tier 2)

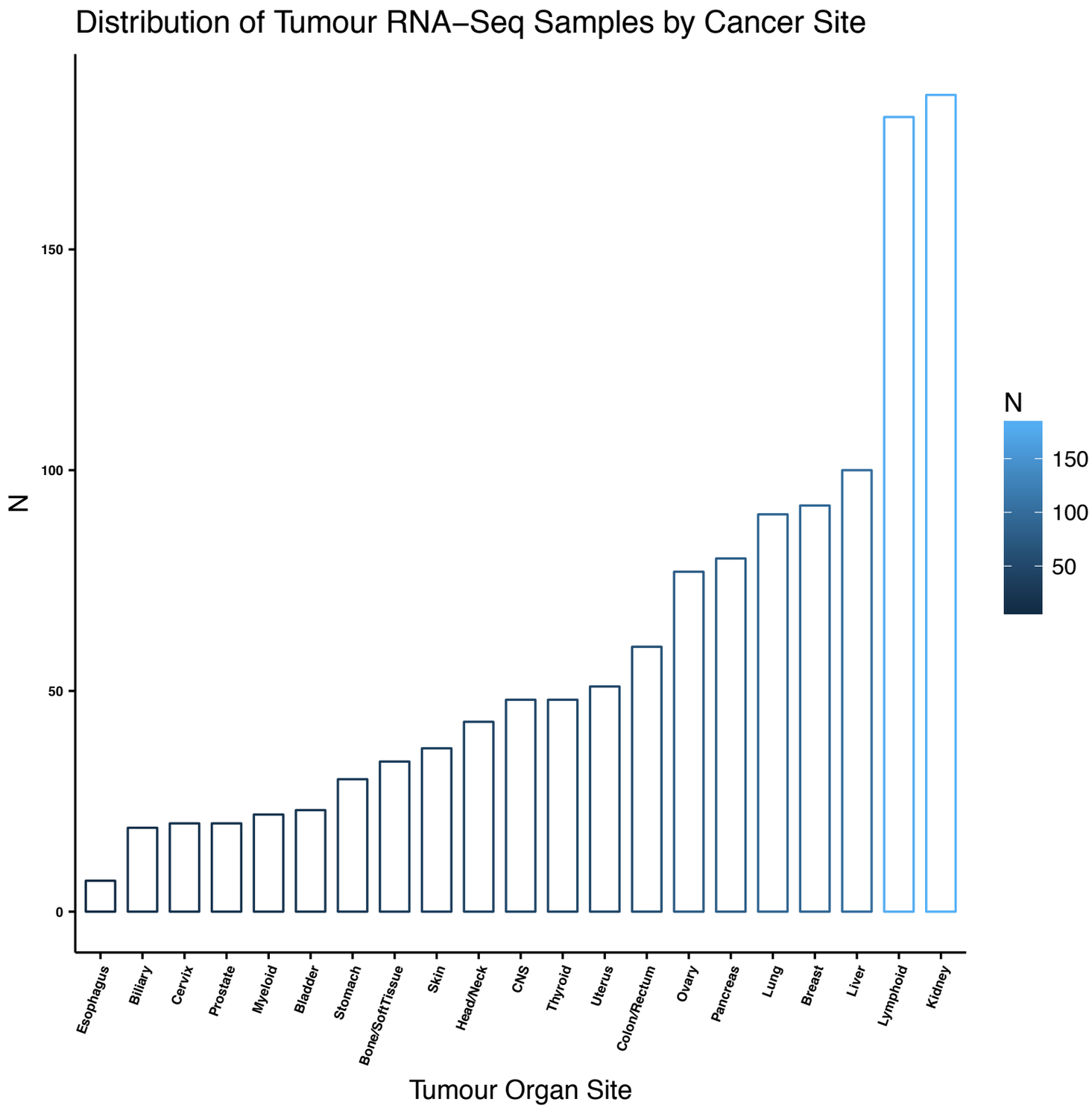
4. Assessed tumour versus normal tissue lncRNA expression by comparing ranked values of expression using match tissue data from GTEX

5. 21/42 lncRNAs were predicted to be either tumour suppressive or oncogenic based on sign of hazard ratio and difference in expression between normal and tumour tissues

6. 2 candidates in Ovarian cancer were further studied to predict regulatory roles that may lead patients from the same cancer type to develop worse prognosis

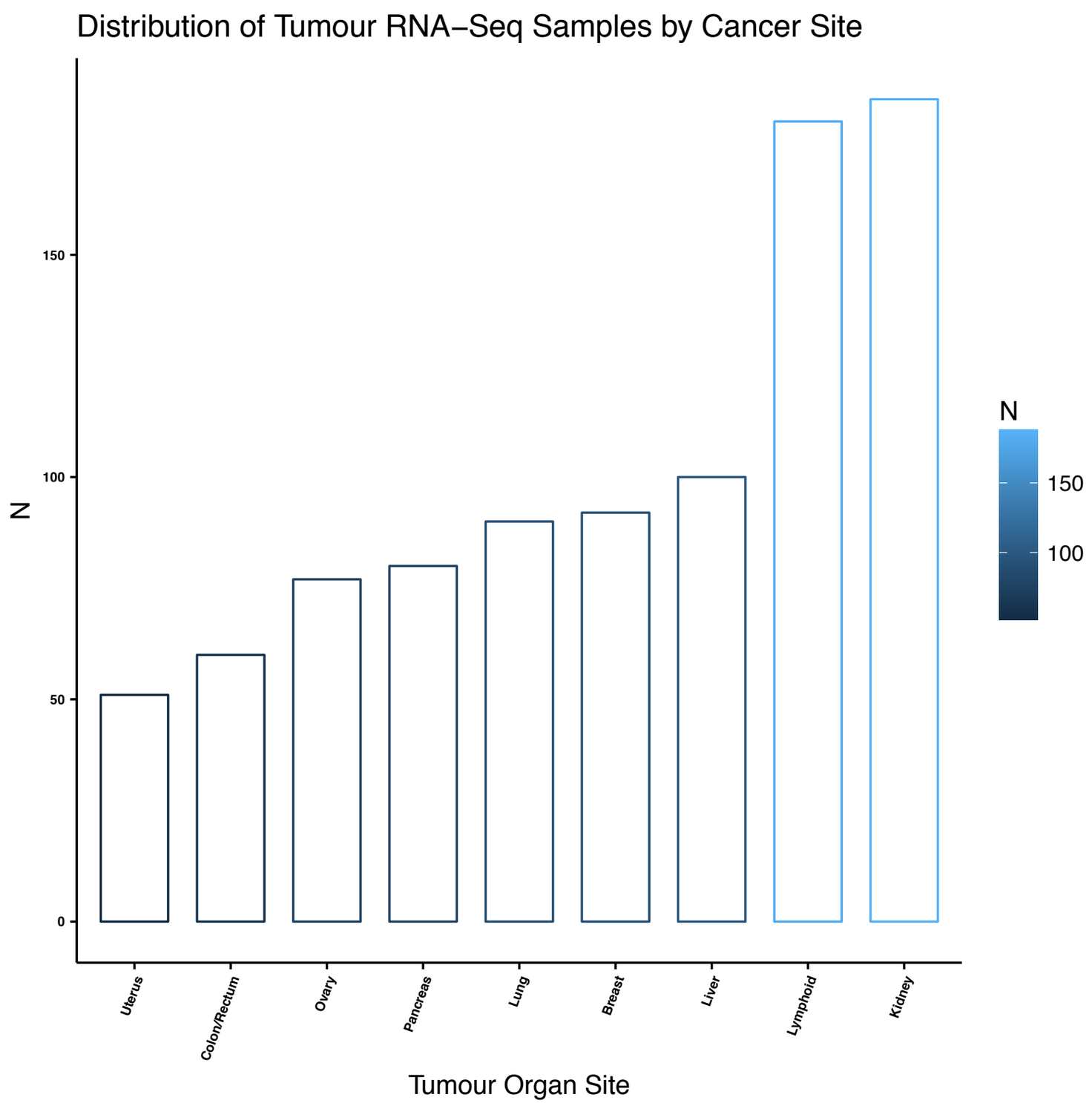
1. Identifying cancer specific lncRNAs

n= 1,267 PCAWG patients with tumour (161 of which also have normal tissue samples) RNA-Seq data from the following cancer types:



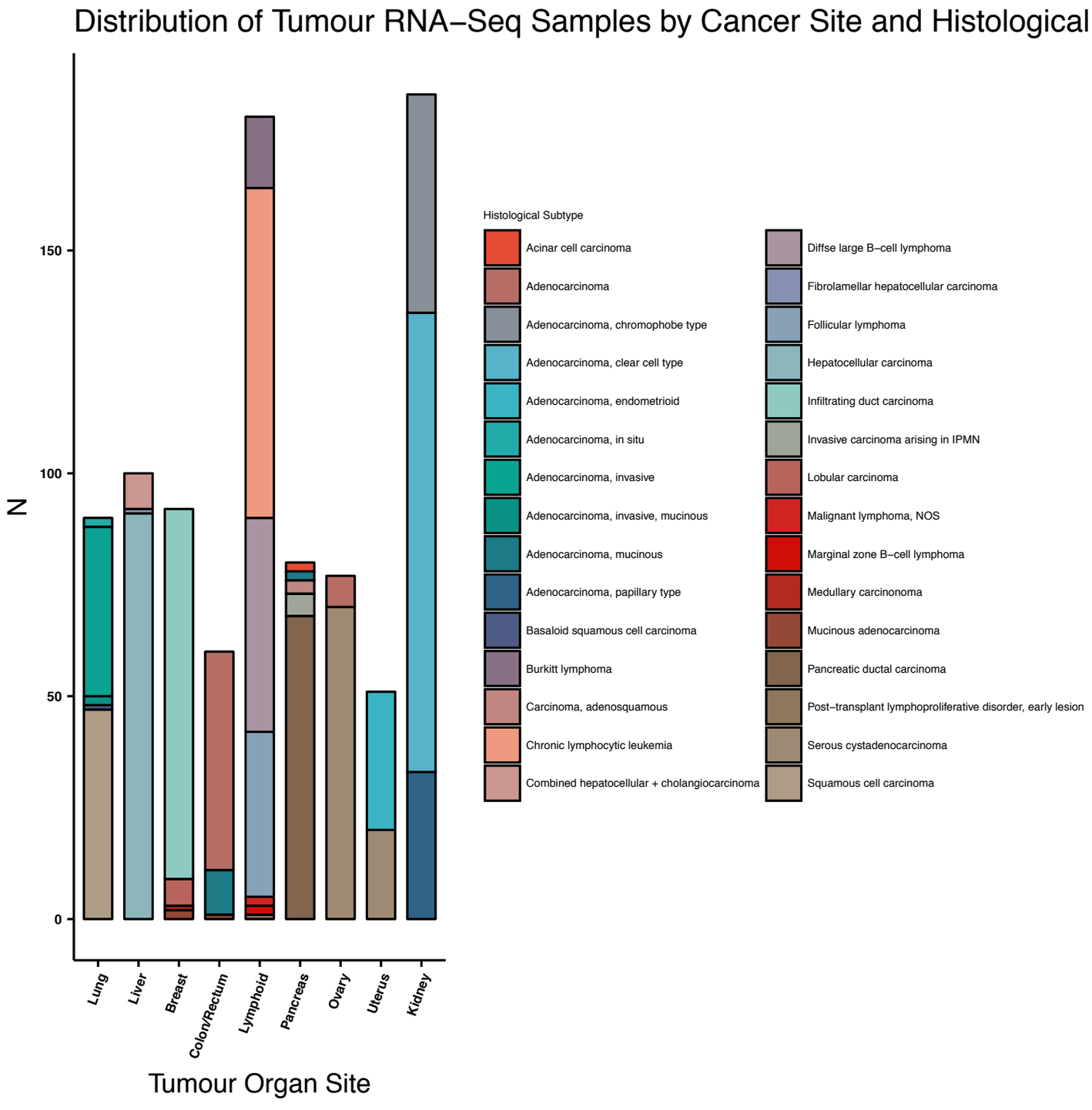
1. Identifying cancer specific lncRNAs

Cancers with at least 50 patient samples:



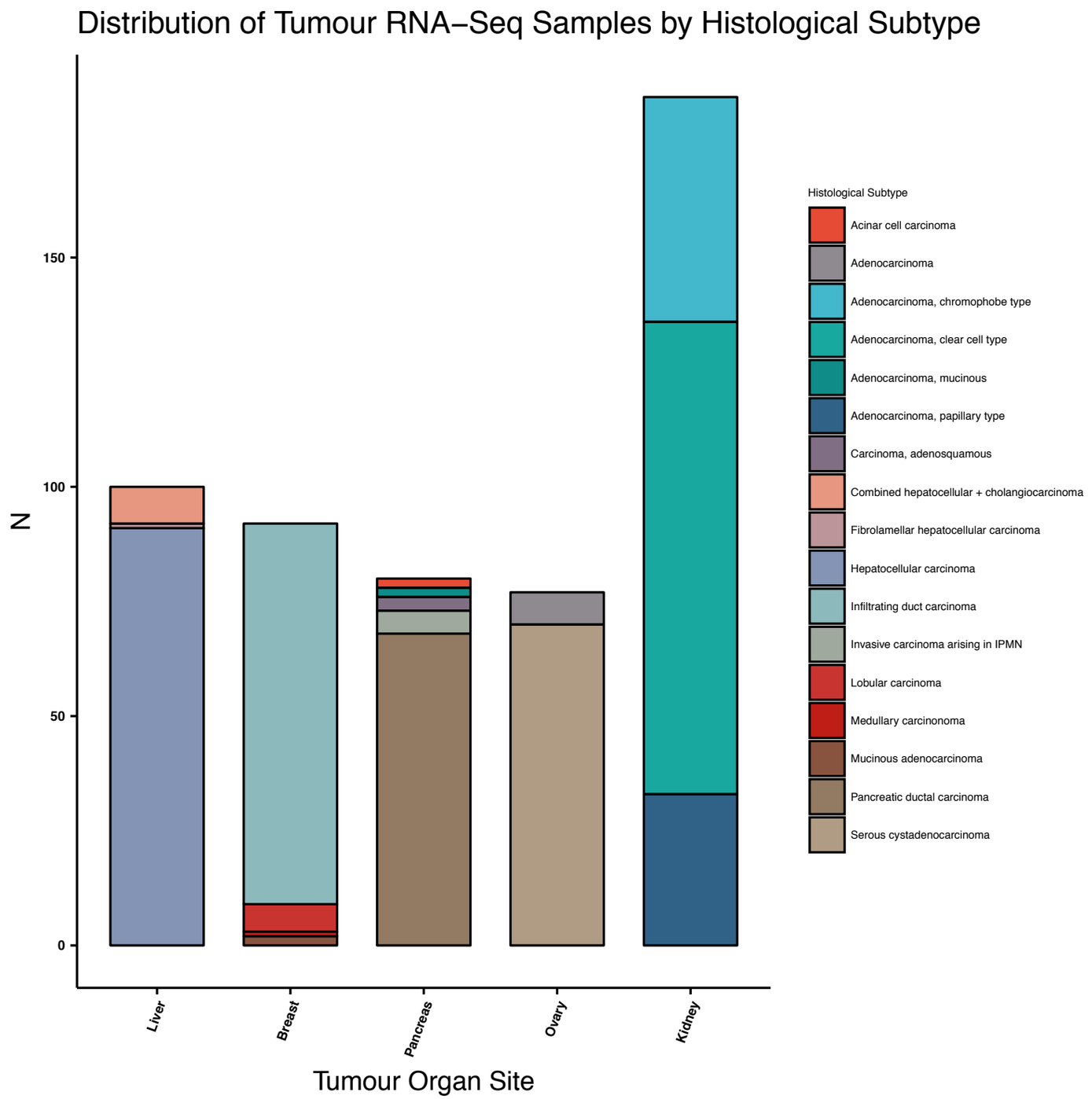
1. Identifying cancer specific lncRNAs

How many histological subtypes within each of these cancers and what are the subtypes?

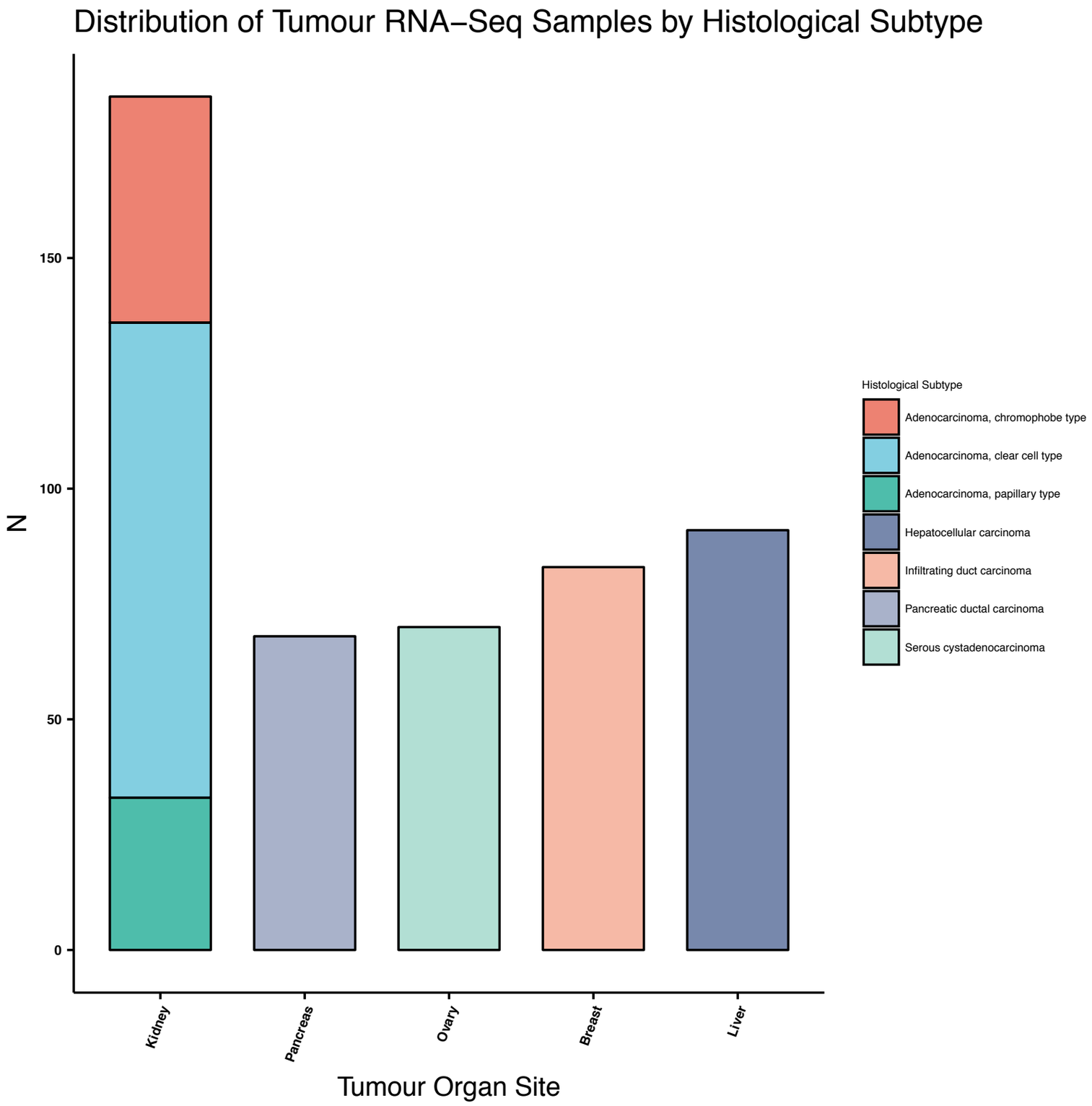


1. Identifying cancer specific lncRNAs

Ideally, the cohort will include an equal distribution of different subtypes or even better if cancer type has one histological subtype with many patients:

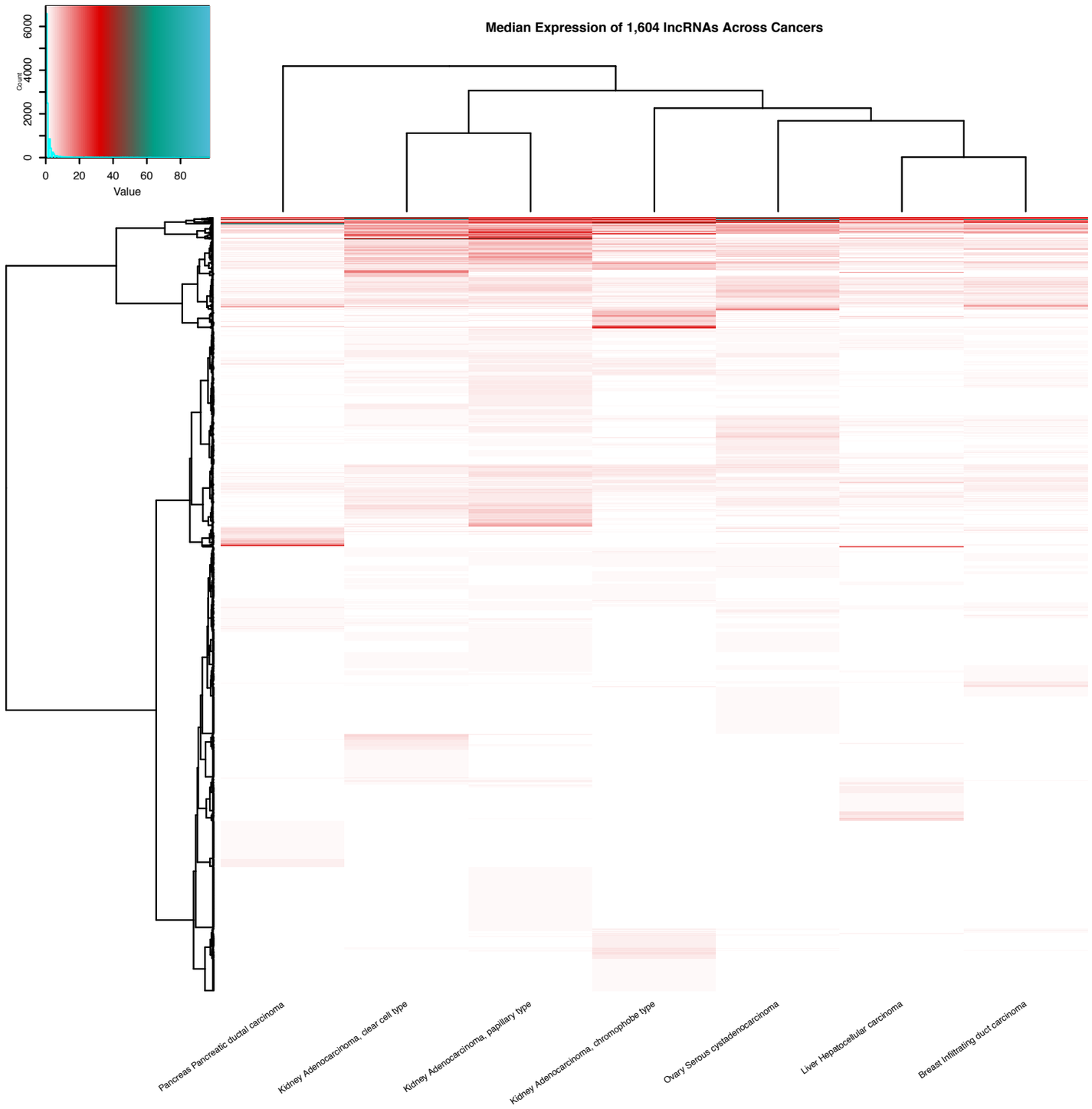


1. Identifying cancer specific lncRNAs



1. Identifying cancer specific lncRNAs

- 12,598 lncRNAs with RNA-Seq data
- Remove genes who had median of 0 in every cancer type
- Floored the median FPKM values
- Removed genes who had median of 0 in every cancer type (removing those who originally had medians slightly greater than 0 before flooring)
- End up with 1,604 lncRNAs plotted in the heat map



*** Add Histogram**

1. Identifying cancer specific lncRNAs

12,598 Ensemble lncRNAs in PCAWG RNA-Seq dataset

↓

Selected only those labelled as antisense or intergenic

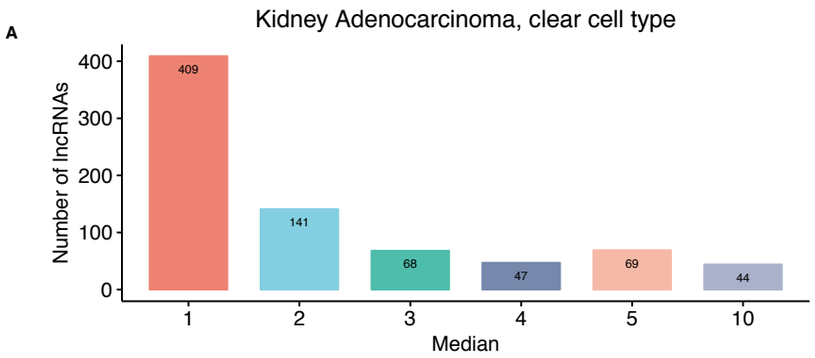
* Add how many this was

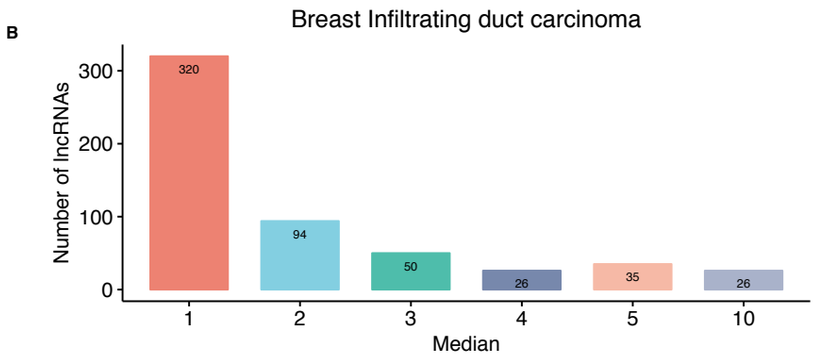
↓

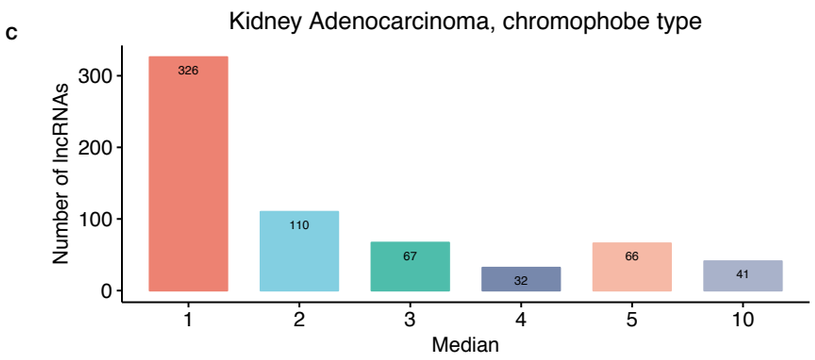
5,607 also in FANTOM5

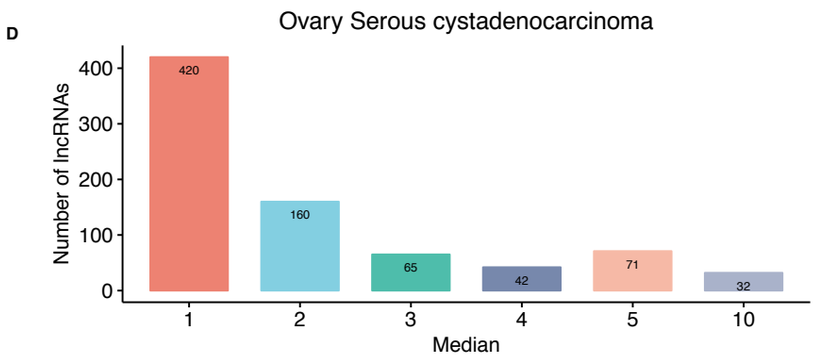
*** Add Histogram**

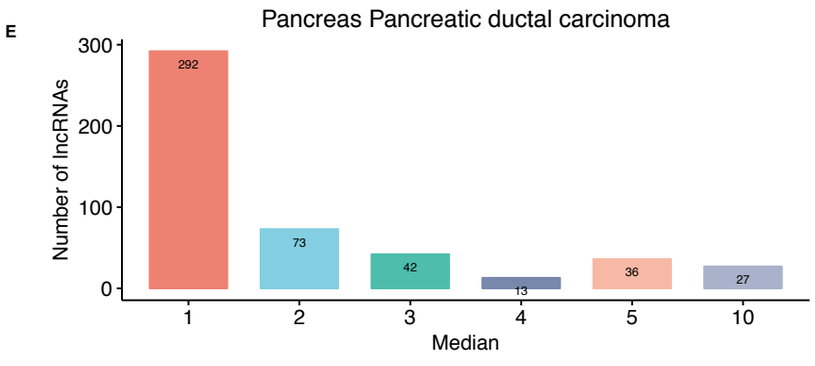
1. Identifying cancer specific lncRNAs

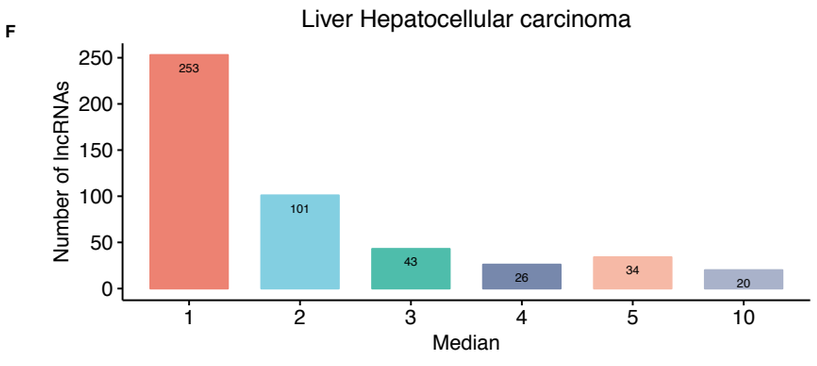


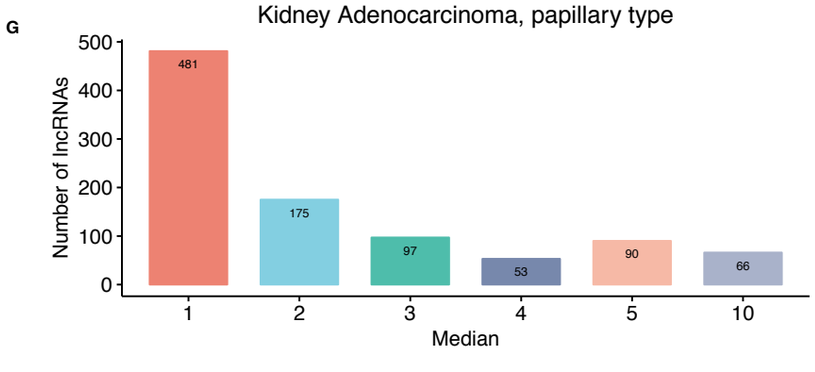












Goal: establish reasonable median cutoff

1. Identifying cancer specific lncRNAs

- 12,598 Ensemble lncRNAs in RNA-Seq dataset
- 5,607 also in FANTOM
- **215 unique lncRNAs selected for study**
  - Minimum median expression of 5 FPKM in at least one cancer type

	Cancer	High Expressing lncRNAs
1	Liver Hepatocellular carcinoma	45
2	Breast Infiltrating duct carcinoma	47
3	Pancreas Pancreatic ductal carcinoma	49
4	Kidney Adenocarcinoma, chromophobe type	76
5	Ovary Serous cystadenocarcinoma	83
6	Kidney Adenocarcinoma, clear cell type	84
7	Kidney Adenocarcinoma, papillary type	109

*** Convert to barplot, show how many overlap with each other**

Summary of progress

1. Identified high expressing cancer specific lncRNAs through several filtration steps across multiple cancer types.

2. **Confirmed cancer specific expression of lncRNAs that are highly expressed in only one cancer type**

3. Identified cancer specific high expressing lncRNAs associated with patient survival outcome using the Cox Proportional Hazards model

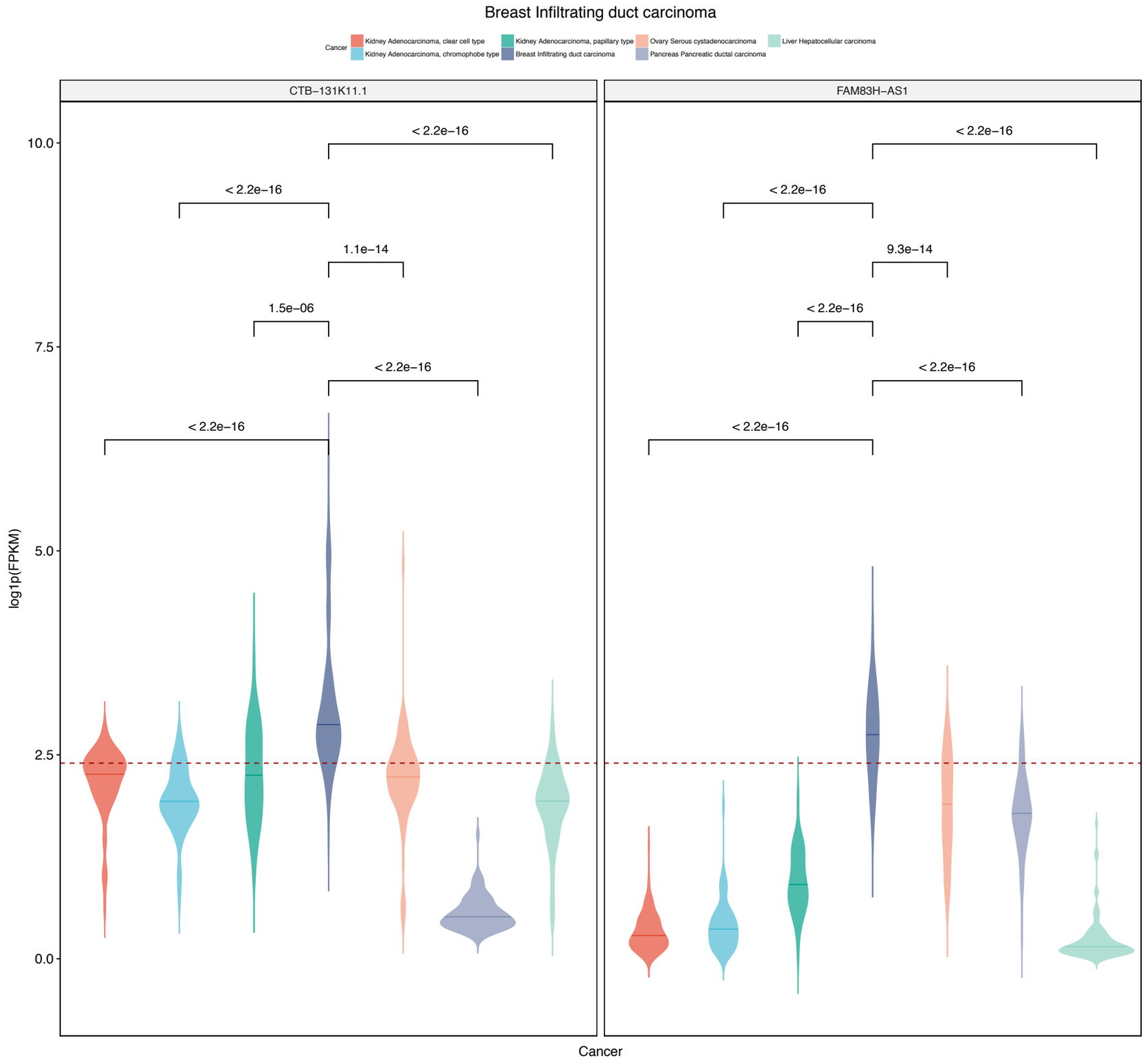
    - 42 lncRNAs in 4 cancer types
    - 7 lncRNAs with adjusted p-value < 0.1 in 2 caner types (Tier 1)
    - 35 lncRNAs with p-value < 0.05 in 4 cancer types (Tier 2)

4. Assessed tumour versus normal tissue lncRNA expression by comparing ranked values of expression using match tissue data from GTEX

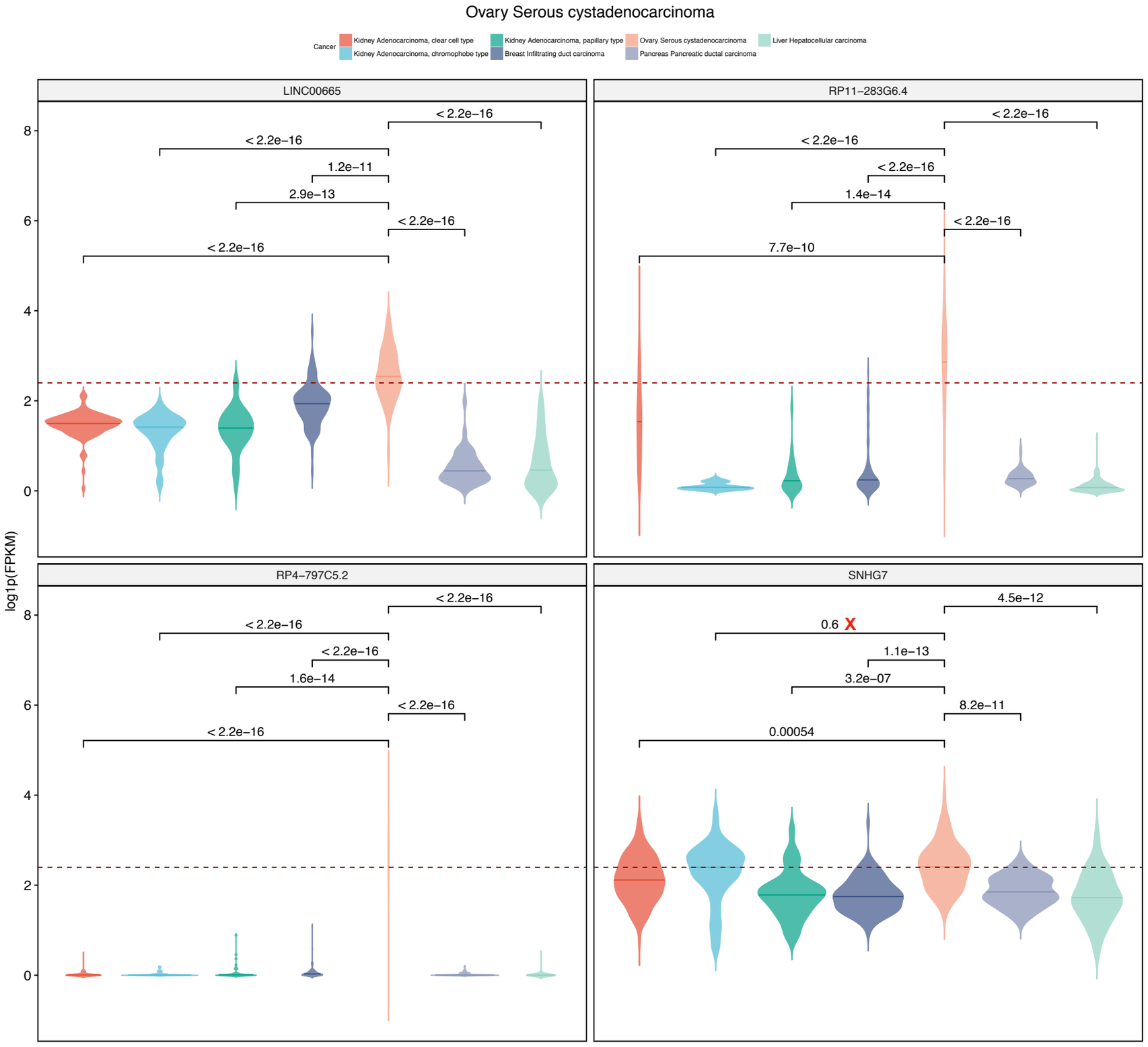
5. 21/42 lncRNAs were predicted to be either tumour suppressive or oncogenic based on sign of hazard ratio and difference in expression between normal and tumour tissues

6. 2 candidates in Ovarian cancer were further studied to predict regulatory roles that may lead patients from the same cancer type to develop worse prognosis

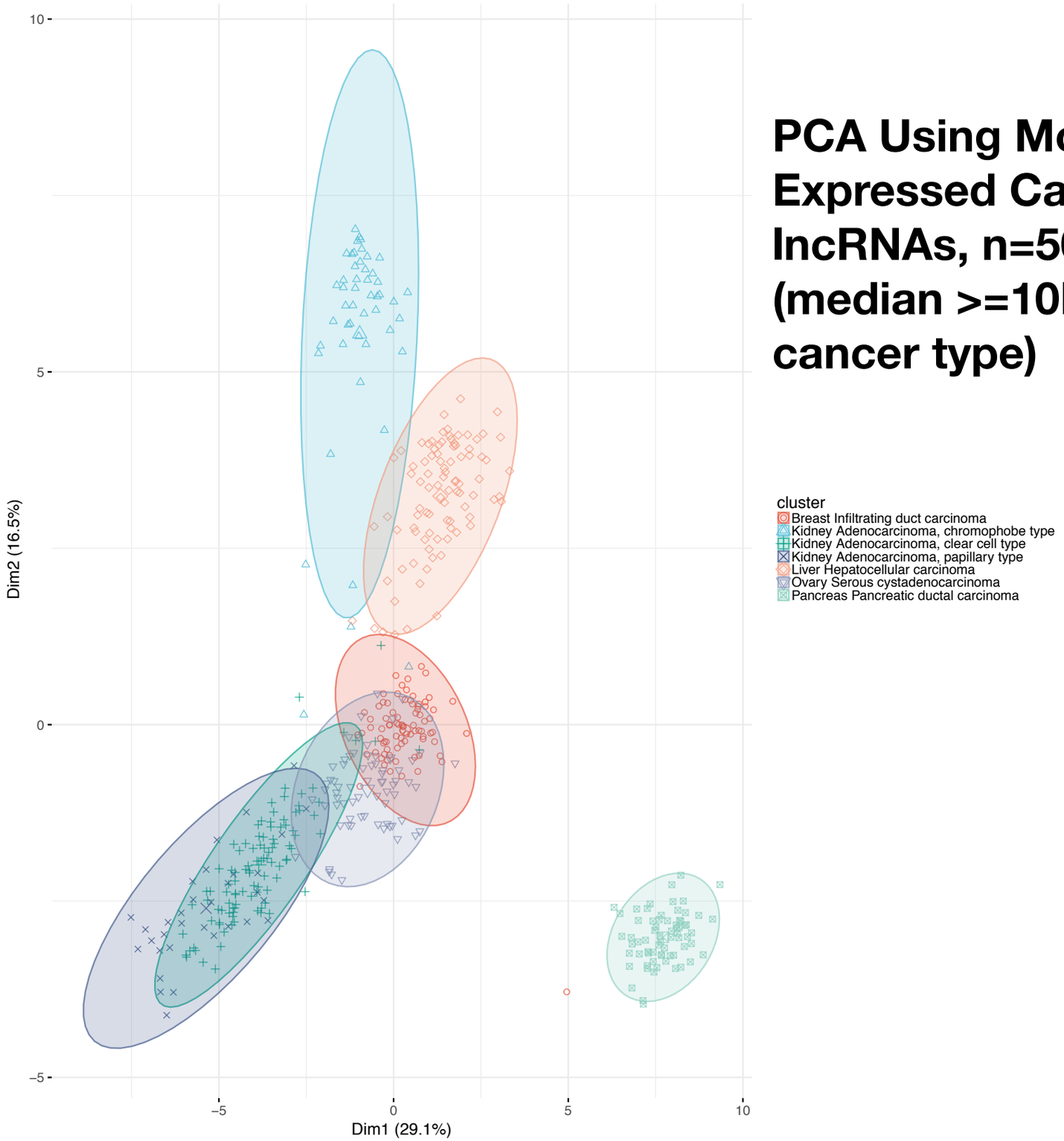
2. Confirming lncRNA Cancer Specificity



2. Confirming lncRNA Cancer Specificity



2. Confirming lncRNA Cancer Specificity



PCA Using Most Highly Expressed Cancer Specific lncRNAs, n=50 (median >=10FPKM in only one cancer type)

Summary of progress

1. Identified high expressing cancer specific lncRNAs through several filtration steps across multiple cancer types.

2. Confirmed cancer specific expression through comparing the distributions of each candidate lncRNA's expression among the cancer types studied

3. **Identified cancer specific lncRNAs associated with patient survival outcome using the Cox Proportional Hazards model**
    - 42 lncRNAs in 4 cancer types
    - 7 lncRNAs with adjusted p-value < 0.1 in 2 caner types (Tier 1)
    - 35 lncRNAs with p-value < 0.05 in 4 cancer types (Tier 2)

4. Assessed tumour versus normal tissue lncRNA expression by comparing ranked values of expression using match tissue data from GTEX

5. 21/42 lncRNAs were predicted to be either tumour suppressive or oncogenic based on sign of hazard ratio and difference in expression between normal and tumour tissues

6. 2 candidates in Ovarian cancer were further studied to predict regulatory roles that may lead patients from the same cancer type to develop worse prognosis

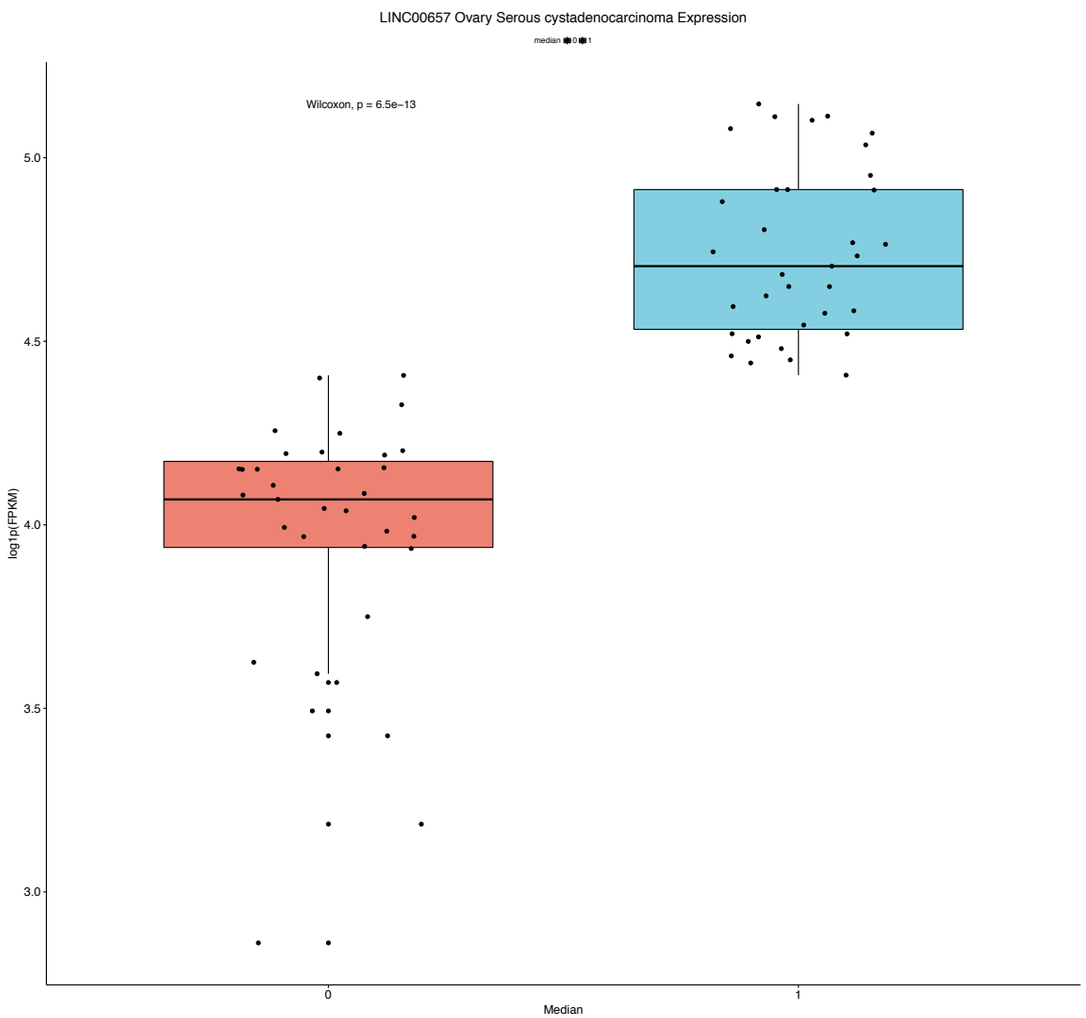
3. Identified lncRNAs associated with patient survival outcome using the Cox Proportional Hazards model

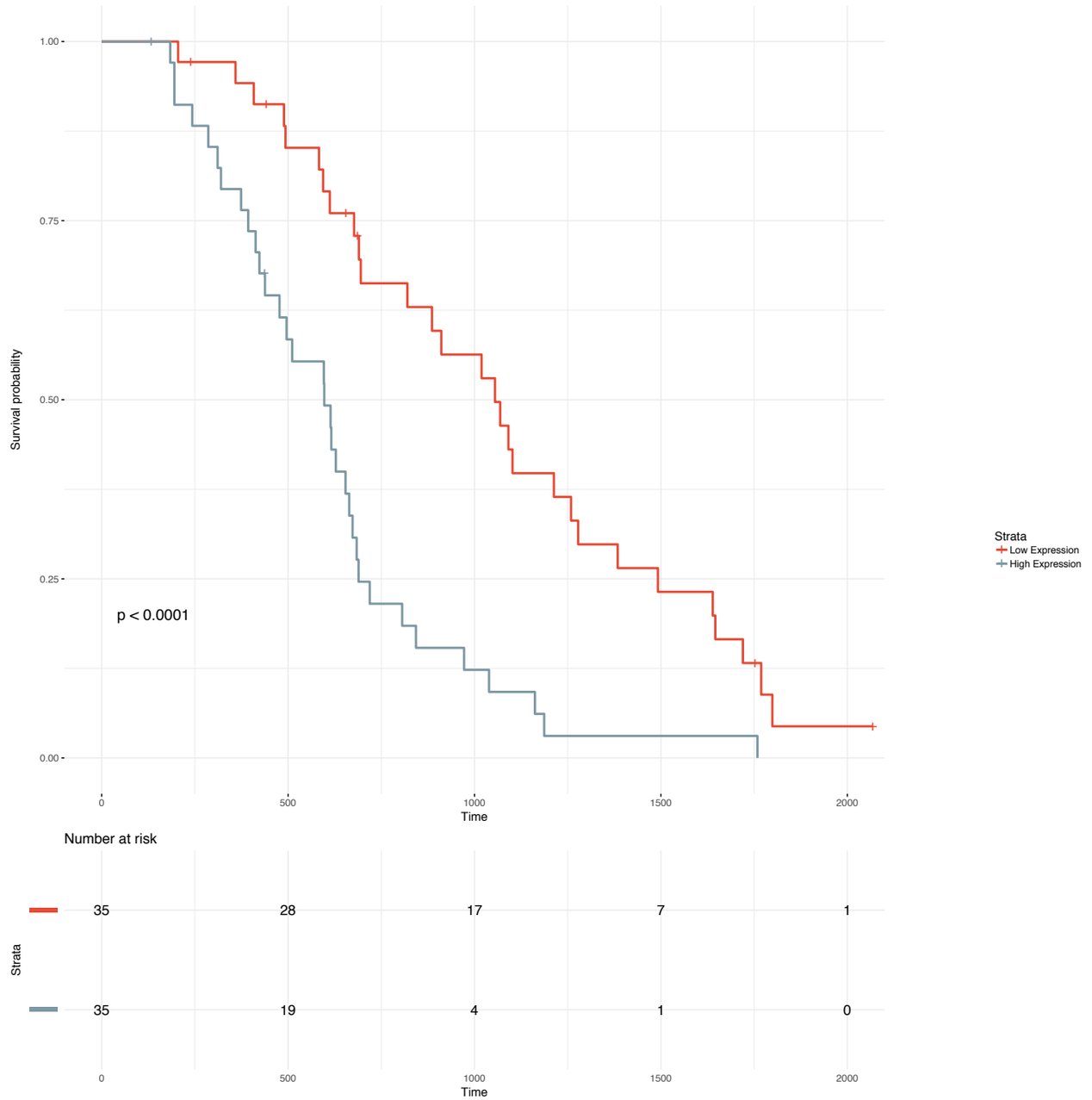
- Median expression of lncRNA used to dichotomize patients into high and low groups
- Predictor of survival is survival tag:
  - 1 = high
  - 0 = low
- res.cox <- coxph(Surv(time, status) ~ median, data = df)

	gene	coef	HR	pval	canc	fdr
1	LINC00657	1.09089497046119	2.97693715151224	6.714862e−05	Ovary Serous cystadenocarcinoma	0.01103476
2	LINC00665	−1.23789638086889	0.28999361285883	4.933441e−05	Ovary Serous cystadenocarcinoma	0.01103476
3	ZNF503−AS2	−1.21096375798916	0.29791002786222	5.909242e−05	Ovary Serous cystadenocarcinoma	0.01103476
4	AC009336.24	−0.915329978890643	0.400384485861799	6.039664e−04	Ovary Serous cystadenocarcinoma	0.05014560
5	RP11−622K12.1	−0.969070407483862	0.379435594700213	6.102913e−04	Ovary Serous cystadenocarcinoma	0.05014560
6	AP000355.2	−1.50935541867795	0.221052418307288	4.777307e−04	Liver Hepatocellular carcinoma	0.05014560
7	RP11−622A1.2	−1.42689280391215	0.240053658412211	1.416813e−03	Liver Hepatocellular carcinoma	0.09978412
8	SNHG15	0.778032349402381	2.17718411047022	3.557492e−03	Ovary Serous cystadenocarcinoma	0.19487153
9	RP11−220I1.1	−1.31154573241281	0.269403308950893	3.371553e−03	Liver Hepatocellular carcinoma	0.19487153
10	SNHG12	0.764311259067298	2.14751478385111	4.537974e−03	Ovary Serous cystadenocarcinoma	0.22372214
11	SNHG1	0.743189814071518	2.10263183341979	5.168054e−03	Ovary Serous cystadenocarcinoma	0.23162277
12	GS1−251I9.4	0.725427456398557	2.06561387114612	6.281176e−03	Ovary Serous cystadenocarcinoma	0.23892283
13	RP11−295G20.2	1.23237659225222	3.42937007343585	6.300196e−03	Liver Hepatocellular carcinoma	0.23892283
14	OSER1−AS1	0.699393073114752	2.01253087763027	9.189207e−03	Ovary Serous cystadenocarcinoma	0.31979542
15	RP11−157P1.4	0.66631704328976	1.94705318673016	9.730084e−03	Ovary Serous cystadenocarcinoma	0.31979542
16	LINC00493	1.07830444424117	2.93969091314685	1.054080e−02	Liver Hepatocellular carcinoma	0.32478839
17	U47924.27	0.680113191614456	1.97410117128317	1.176238e−02	Ovary Serous cystadenocarcinoma	0.34110901
18	AC006126.4	−0.918822841885361	0.398988437230102	1.473127e−02	Kidney Adenocarcinoma, clear cell type	0.36995216
19	NEAT1	0.642552733080203	1.90132827320059	1.365801e−02	Ovary Serous cystadenocarcinoma	0.36995216
20	MMP24−AS1	0.998979579568135	2.71550945287663	1.500820e−02	Liver Hepatocellular carcinoma	0.36995216
21	FGD5−AS1	−0.866125022806513	0.420578126385491	1.824250e−02	Kidney Adenocarcinoma, clear cell type	0.40879774
22	ADORA2A−AS1	−1.0238468792094	0.359210439705118	1.798022e−02	Liver Hepatocellular carcinoma	0.40879774
23	RP11−49I11.1	0.979396006633756	2.66284741394427	1.917262e−02	Liver Hepatocellular carcinoma	0.41096102
24	RP11−304L19.5	−0.727854754389591	0.48294391292074	2.111194e−02	Pancreas Pancreatic ductal carcinoma	0.43367449
25	MMP24−AS1	0.825276522328797	2.28251184356216	2.453876e−02	Kidney Adenocarcinoma, clear cell type	0.45015634
26	NCBP2−AS2	0.601706131619117	1.82523022838774	2.465359e−02	Ovary Serous cystadenocarcinoma	0.45015634
27	NEAT1	−0.931193692129404	0.394083015701056	2.327743e−02	Liver Hepatocellular carcinoma	0.45015634
28	CTB−131K11.1	0.579258152521435	1.78471395398422	2.992787e−02	Ovary Serous cystadenocarcinoma	0.48644962
29	DANCR	−0.569578402465635	0.56576391309669	2.961290e−02	Ovary Serous cystadenocarcinoma	0.48644962
30	FAM83H−AS1	−0.572351978944779	0.564196897737599	2.971973e−02	Ovary Serous cystadenocarcinoma	0.48644962
31	DANCR	0.910329458260571	2.48514114880777	3.058811e−02	Liver Hepatocellular carcinoma	0.48644962
32	RP11−834C11.4	−0.563198488147974	0.569384977143017	3.520396e−02	Ovary Serous cystadenocarcinoma	0.52126585
33	RPPH1	−0.639134723510223	0.527748875230572	3.412066e−02	Pancreas Pancreatic ductal carcinoma	0.52126585
34	RP11−139H15.1	0.895710166661311	2.44907442303575	3.594937e−02	Liver Hepatocellular carcinoma	0.52126585
35	CTC−503J8.6	−0.551357810106765	0.576166953702487	3.954573e−02	Ovary Serous cystadenocarcinoma	0.53743244
36	MFI2−AS1	0.539284764289622	1.7147799516152	3.840014e−02	Ovary Serous cystadenocarcinoma	0.53743244
37	RP11−469A15.2	−0.881443557345828	0.414184580728924	4.033469e−02	Liver Hepatocellular carcinoma	0.53743244
38	HOXD−AS1	−0.521580642937459	0.593581565558328	4.176834e−02	Ovary Serous cystadenocarcinoma	0.54188927
39	SNHG7	0.734356702478046	2.08414083853393	4.533632e−02	Kidney Adenocarcinoma, clear cell type	0.55960215
40	RP11−834C11.4	−0.600714941409506	0.548419408156069	4.563384e−02	Pancreas Pancreatic ductal carcinoma	0.55960215
41	DBH−AS1	−0.83141909496413	0.435430929832266	4.653892e−02	Liver Hepatocellular carcinoma	0.55960215
42	SNHG8	−0.599508861430317	0.549081244858215	4.976660e−02	Pancreas Pancreatic ductal carcinoma	0.58416513

3. Identified lncRNAs associated with patient survival outcome using the Cox Proportional Hazards model

Top Hit: LINC00657 in Ovarian Cancer with Hazard Ratio of 2.97





Summary of progress

1. Identified high expressing cancer specific lncRNAs through several filtration steps across multiple cancer types.

2. Confirmed cancer specific expression through comparing the distributions of each candidate lncRNA's expression among the cancer types studied

3. Identified cancer specific high expressing lncRNAs associated with patient survival outcome using the Cox Proportional Hazards model
    - 42 lncRNAs in 4 cancer types
    - 7 lncRNAs with adjusted p-value < 0.1 in 2 caner types (Tier 1)
    - 35 lncRNAs with p-value < 0.05 in 4 cancer types (Tier 2)

4. **Assessed tumour versus normal tissue lncRNA expression by comparing ranked values of expression using tissue data from GTEX**

5. 21/42 lncRNAs were predicted to be either tumour suppressive or oncogenic based on sign of hazard ratio and difference in expression between normal and tumour tissues

6. 2 candidates in Ovarian cancer were further studied to predict regulatory roles that may lead patients from the same cancer type to develop worse prognosis

Summary of progress

1. Identified high expressing cancer specific lncRNAs through several filtration steps across multiple cancer types.

2. Confirmed cancer specific expression through comparing the distributions of each candidate lncRNA's expression among the cancer types studied

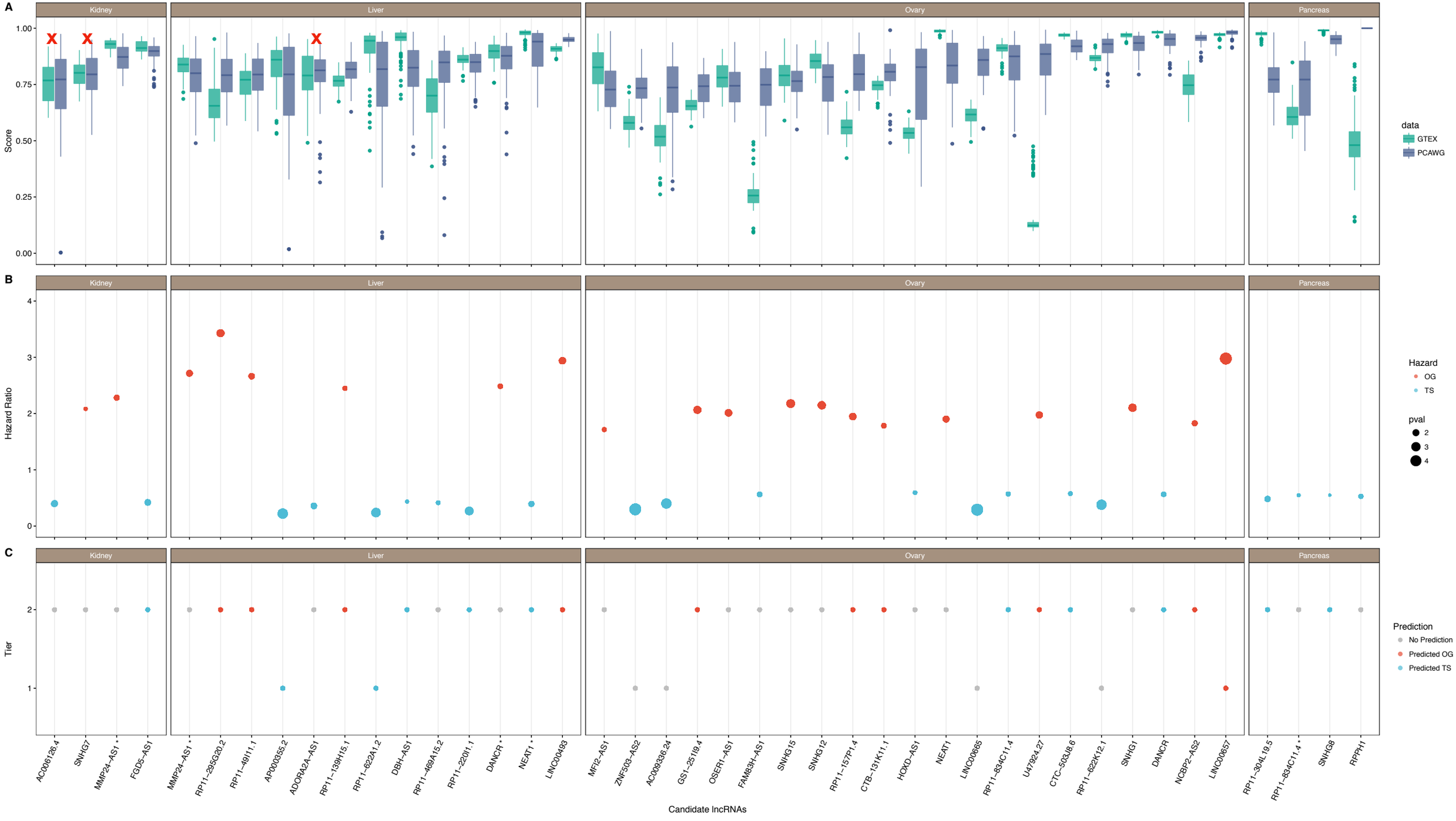
3. Identified cancer specific high expressing lncRNAs associated with patient survival outcome using the Cox Proportional Hazards model
    - 42 lncRNAs in 4 cancer types
    - 7 lncRNAs with adjusted p-value < 0.1 in 2 caner types (Tier 1)
    - 35 lncRNAs with p-value < 0.05 in 4 cancer types (Tier 2)

4. Assessed tumour versus normal tissue lncRNA expression by comparing ranked values of expression using tissue data from GTEX

5. **21/42 lncRNAs were predicted to be either tumour suppressive or oncogenic based on sign of hazard ratio and difference in expression between normal and tumour tissues**

6. 2 candidates in Ovarian cancer were further studied to predict regulatory roles that may lead patients from the same cancer type to develop worse prognosis

4. Prediction of tumour suppressive or oncogenic properties



Summary of progress

1. Identified high expressing cancer specific lncRNAs through several filtration steps across multiple cancer types.

2. Confirmed cancer specific expression through comparing the distributions of each candidate lncRNA's expression among the cancer types studied

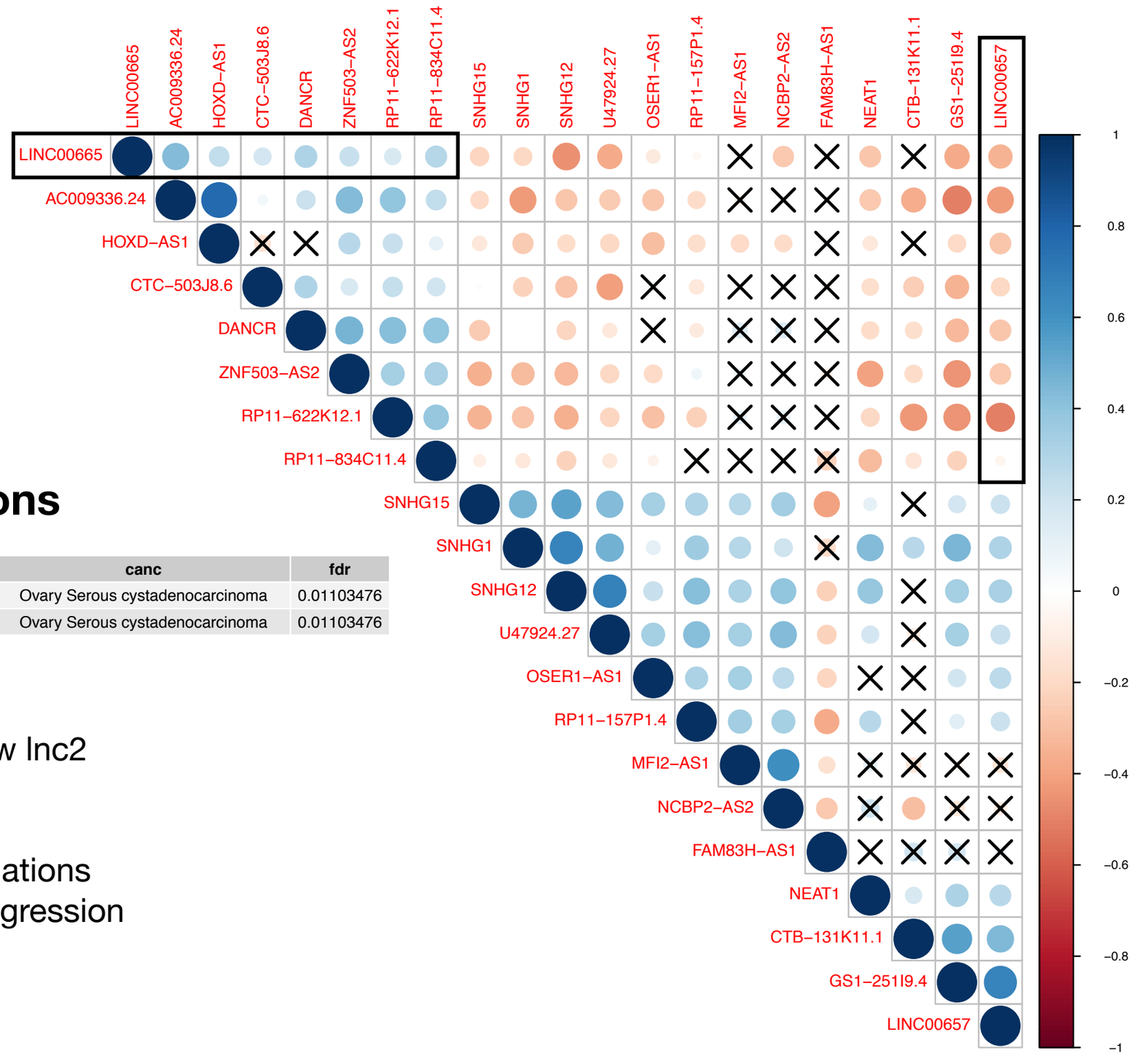
3. Identified cancer specific high expressing lncRNAs associated with patient survival outcome using the Cox Proportional Hazards model
    - 42 lncRNAs in 4 cancer types
    - 7 lncRNAs with adjusted p-value < 0.1 in 2 caner types (Tier 1)
    - 35 lncRNAs with p-value < 0.05 in 4 cancer types (Tier 2)

4. Assessed tumour versus normal tissue lncRNA expression by comparing ranked values of expression using tissue data from GTEX

5. 21/42 lncRNAs were predicted to be either tumour suppressive or oncogenic based on sign of hazard ratio and difference in expression between normal and tumour tissues

6. **2 candidates in Ovarian cancer were further studied to predict regulatory roles that may lead patients from the same cancer type to develop worse prognosis**

6. Further analysis of top 2 candidates in Ovarian Cancer

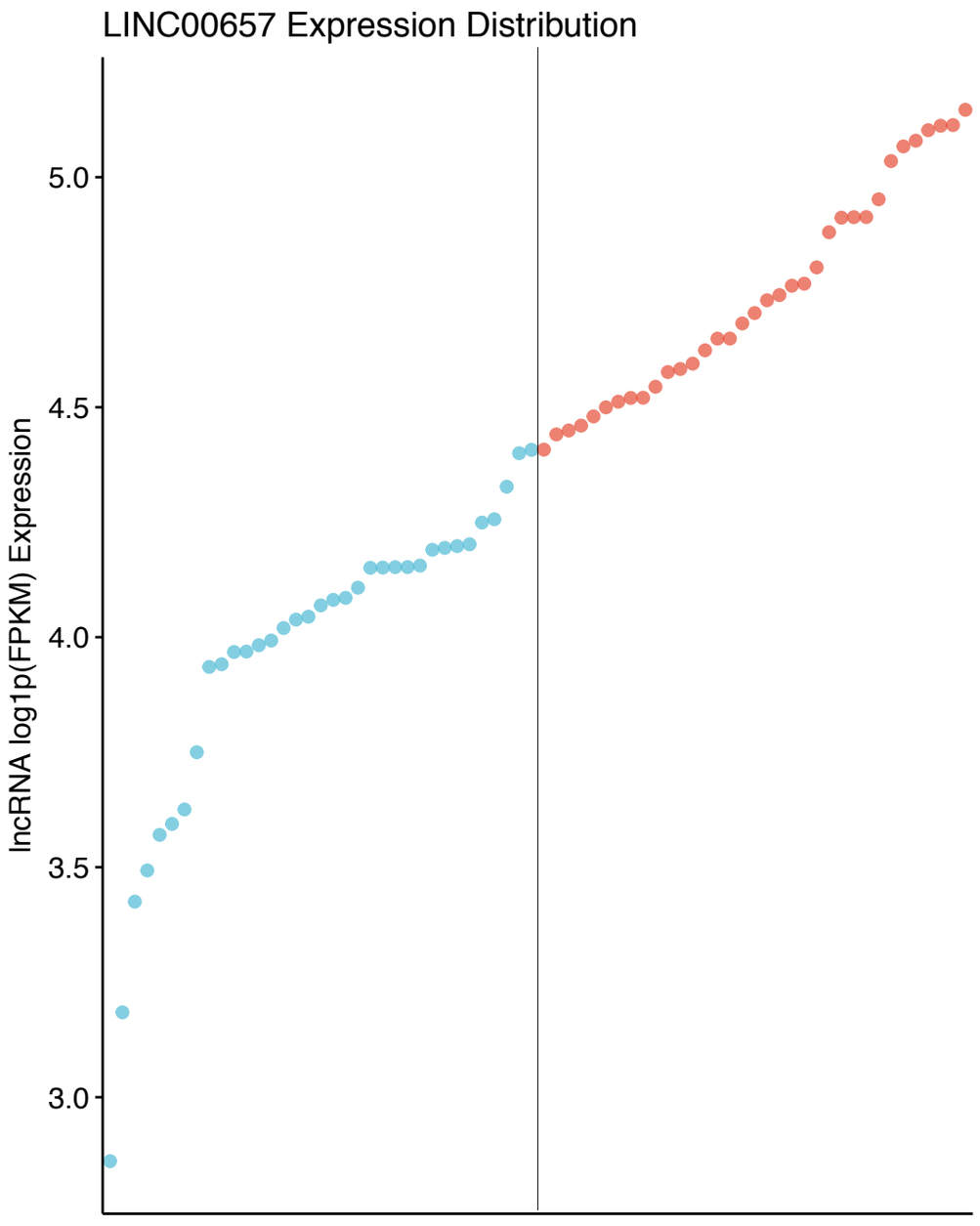


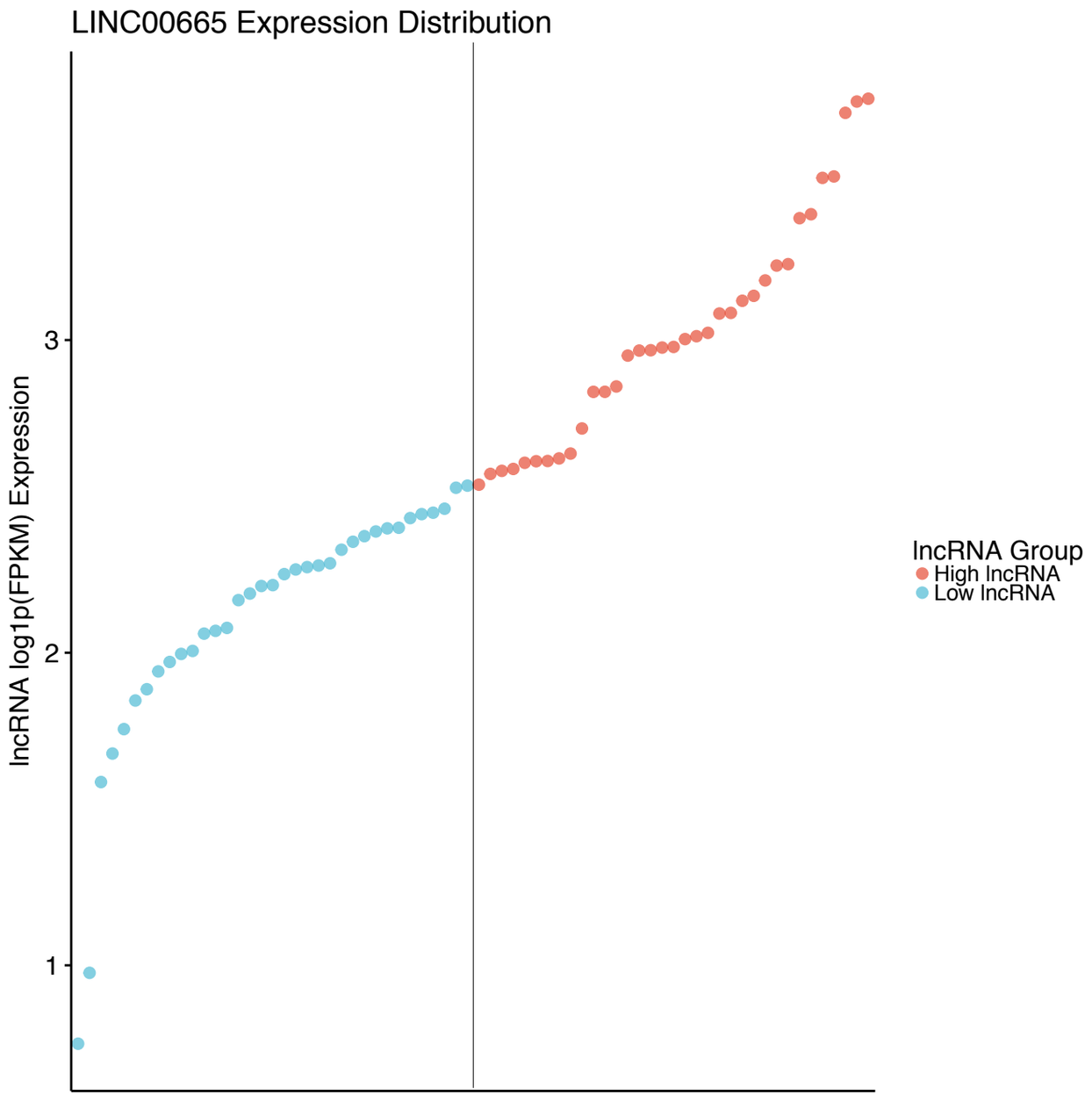
Ovarian Cancer Candidate lnc-lnc Correlations

	gene	coef	HR	pval	canc	fdr
1	LINC00657	1.09089497046119	2.97693715151224	6.714862e−05	Ovary Serous cystadenocarcinoma	0.01103476
2	LINC00665	−1.23789638086889	0.28999361285883	4.933441e−05	Ovary Serous cystadenocarcinoma	0.01103476

* Apply FDR to spearman p-values
* 29 individuals have high lnc1 and low lnc2
  * 9 have high lnc1 and lnc2
  * 9 have low lnc1 and low lnc2
* Show LINC00665/LINC00657 Correlations
* Univariate versus multivariate cox regression

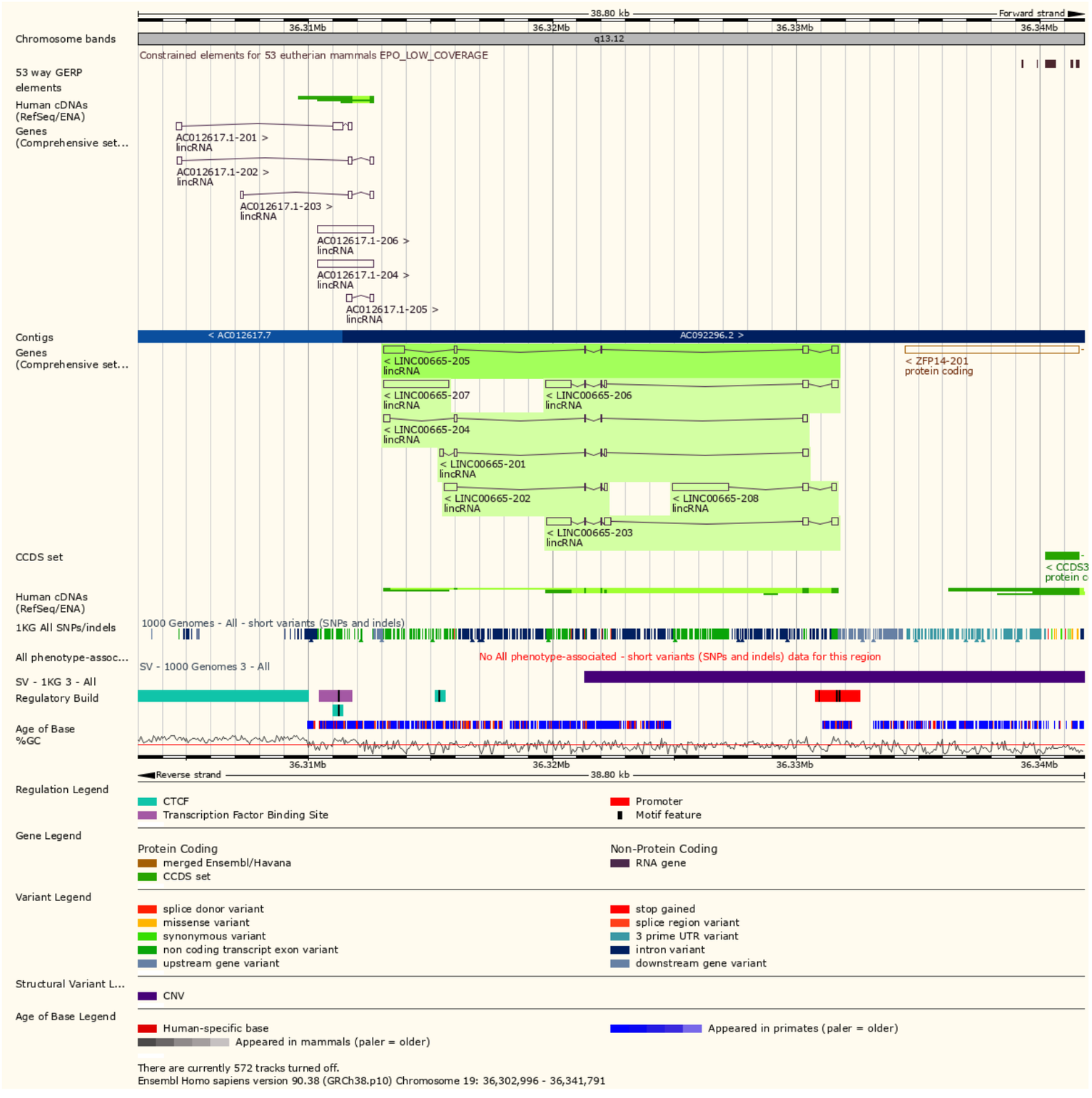
6. Further analysis of top 2 candidates in Ovarian Cancer





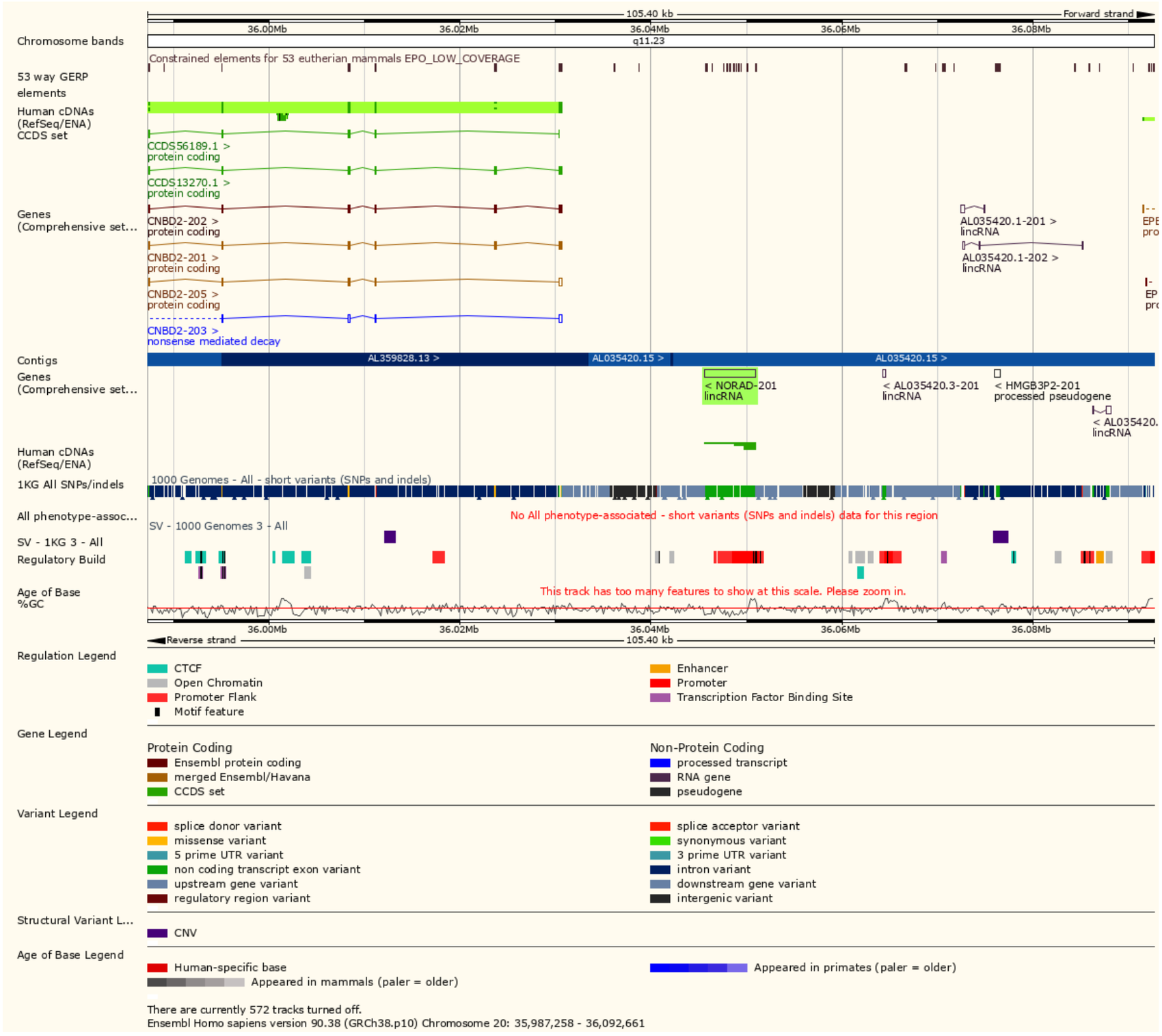
6. LINC00665

Chr 19



6. LINC00657

Chr 20



6. Further analysis of top 2 candidates in Ovarian Cancer

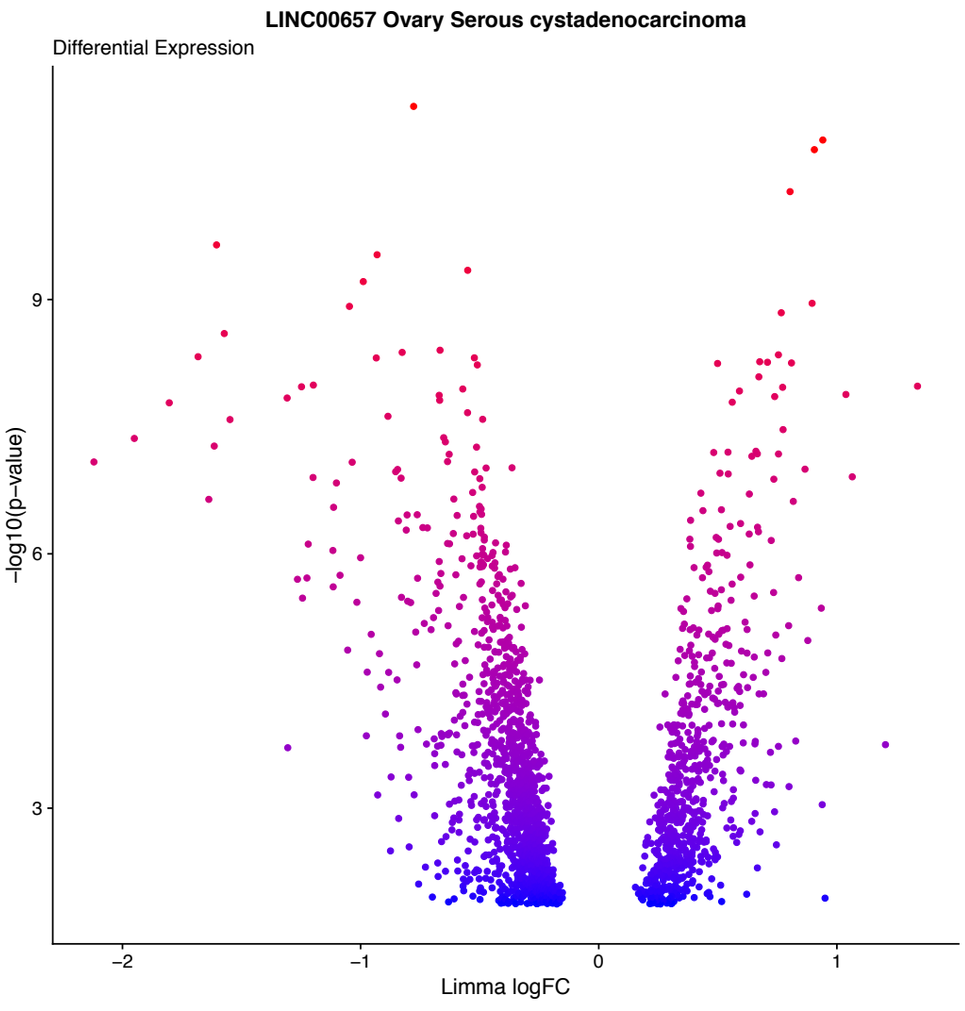
- Dichotomize patients based on median lncRNA expression
- Differential expression analysis of protein coding genes between high and low lncRNA expressing patients
    - 1,837 PCGs differentially expressed between high and low LINC00657 patients
    - 641 PCGs differentially expressed between high and low LINC00665 patients
- Visualize
- Pathway enrichment analysis to identify pathways differentially expressed between the two groups

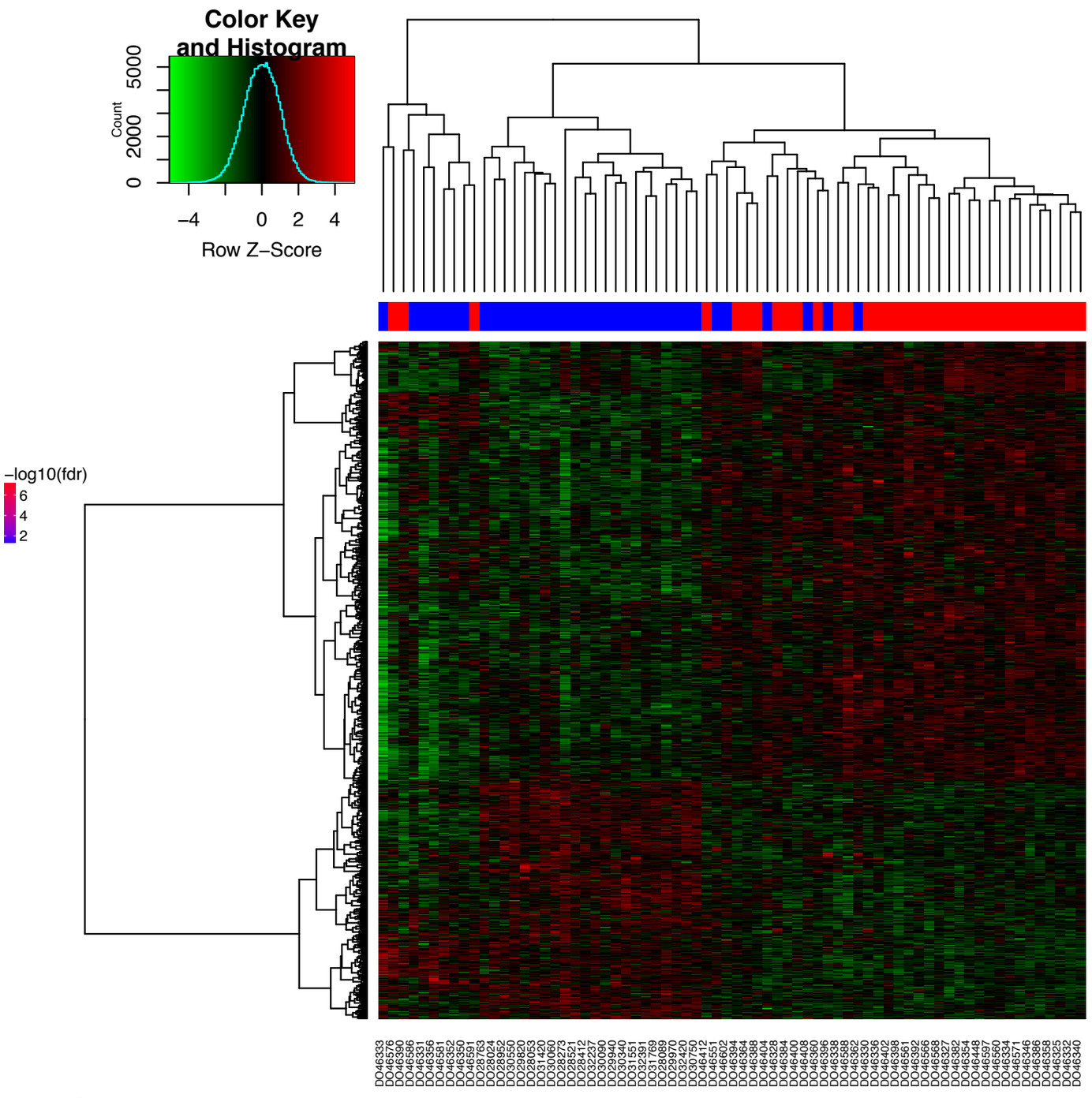
6. Further analysis of top 2 candidates in Ovarian Cancer

- Dichotomize patients based on median lncRNA expression
- Differential expression analysis of protein coding genes between high and low lncRNA expressing patients
    - 1,837 PCGs differentially expressed between high and low LINC00657 patients
    - 641 PCGs differentially expressed between high and low LINC00657 patients
- **Visualize**
- Pathway enrichment analysis to identify pathways differentially expressed between the two groups

6. Further analysis of top 2 candidates in Ovarian Cancer

LINC00657

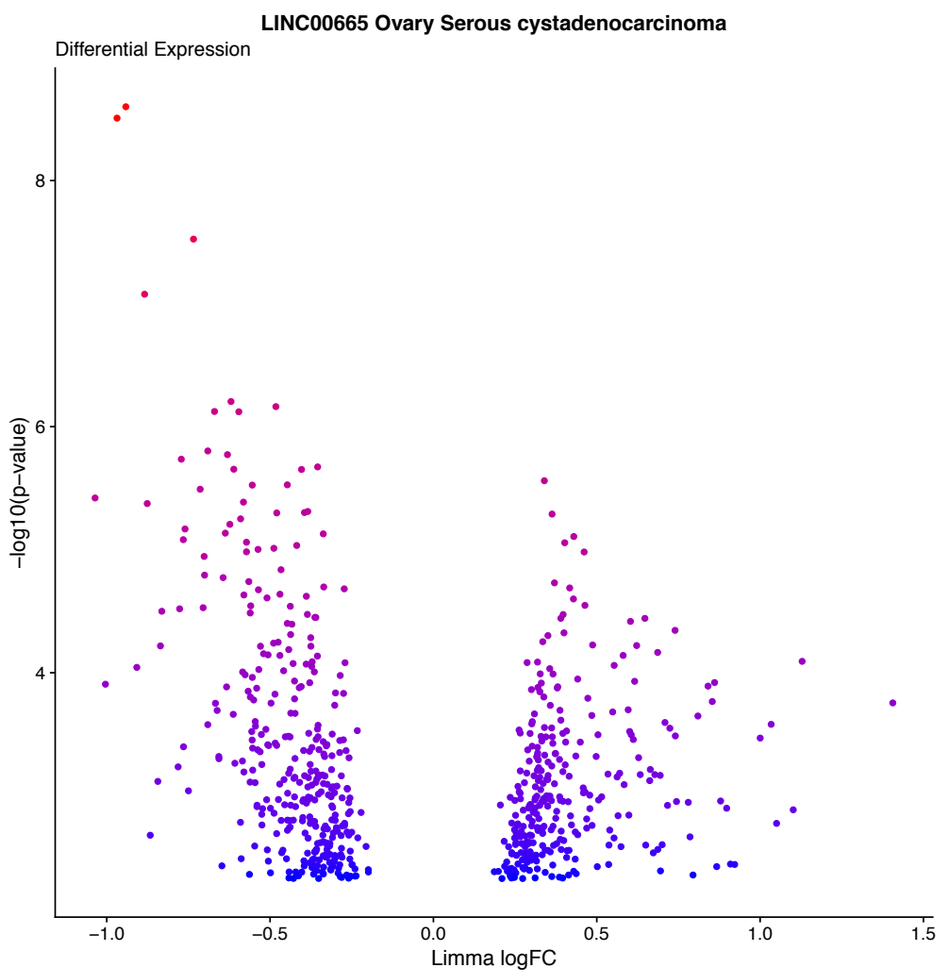


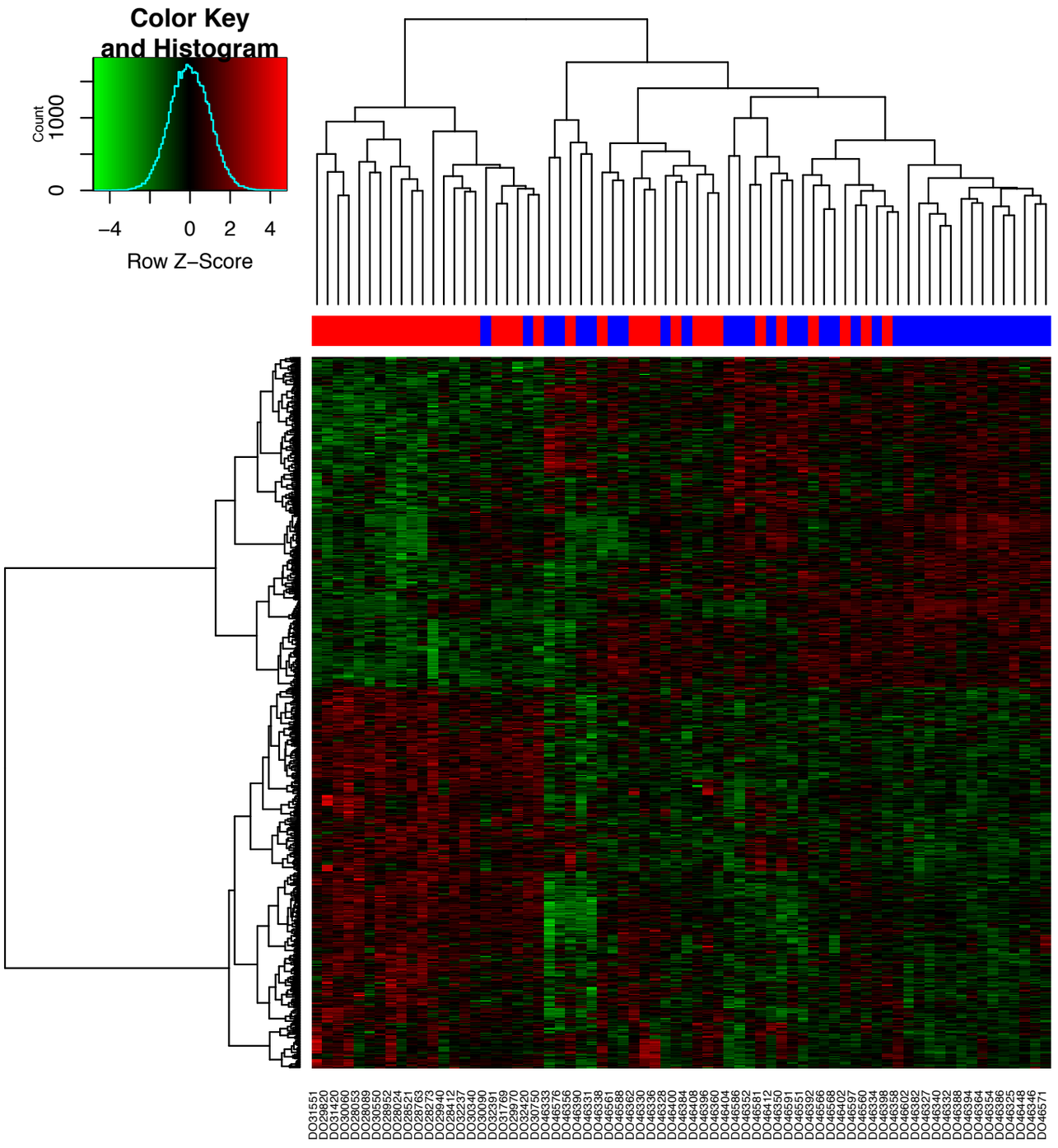


* Add legends to heatmap
* Add labels to most significant points

6. Further analysis of top 2 candidates in Ovarian Cancer

LINC00665



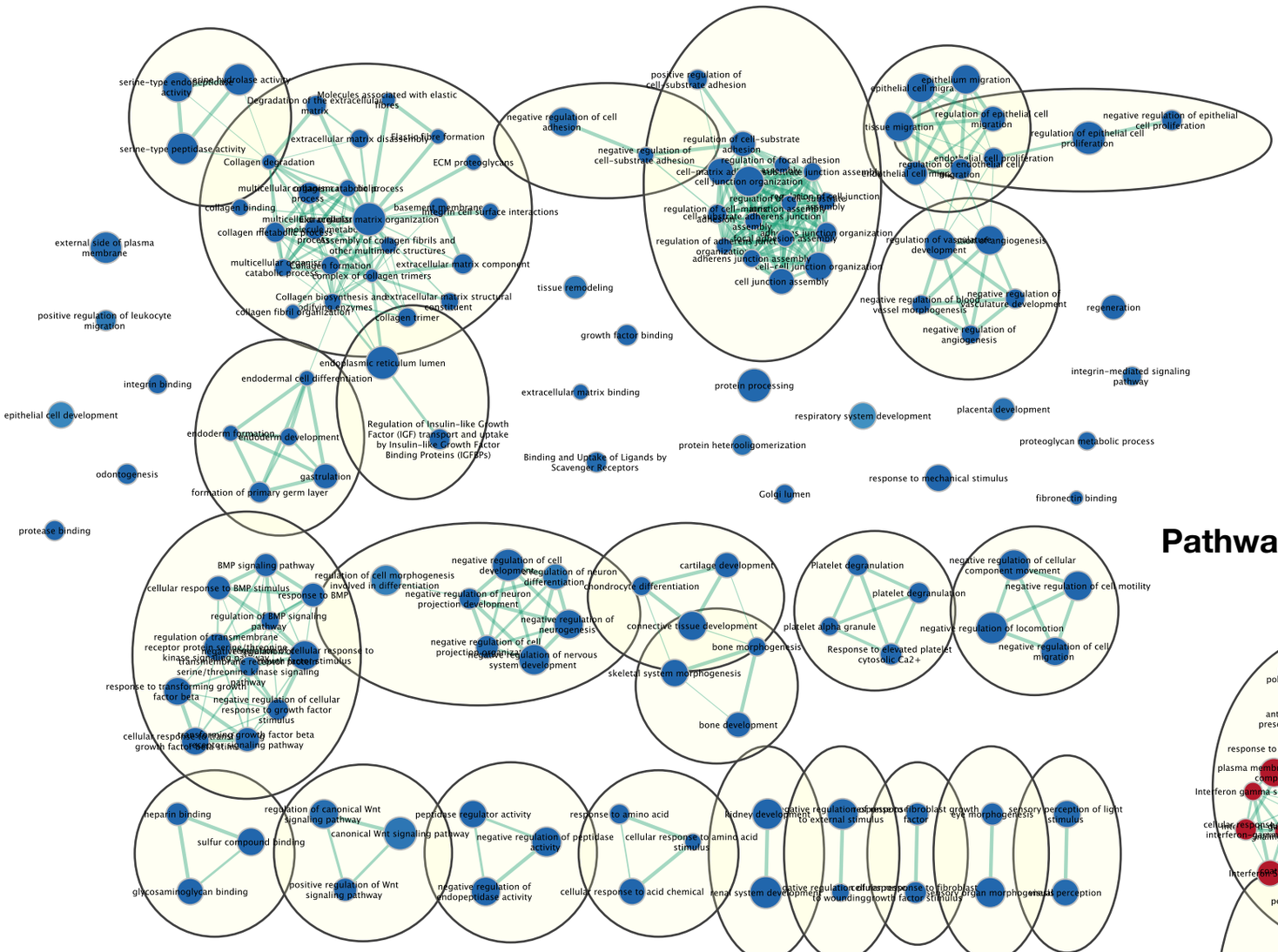


* Add legends to heatmap

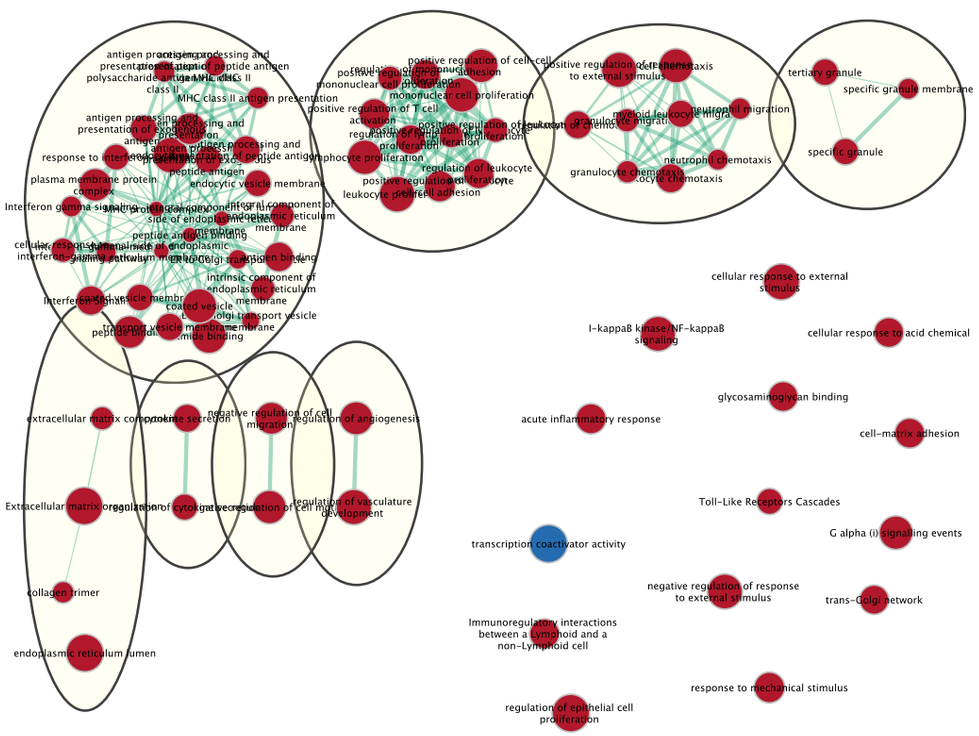
6. Further analysis of top 2 candidates in Ovarian Cancer

- Dichotomize patients based on median lncRNA expression
- Differential expression analysis of protein coding genes between high and low lncRNA expressing patients
    - 1,837 PCGs differentially expressed between high and low LINC00657 patients
    - 641 PCGs differentially expressed between high and low LINC00657 patients
- Visualize
- **Pathway enrichment analysis to identify pathways differentially expressed between the two groups**

6. Further analysis of top 2 candidates in Ovarian Cancer



Pathways enriched by PCGs expressed in patients with high LINC00657 expression



Pathways enriched by PCGs expressed in patients with low LINC00665 expression

* Use all significantly co-expressed PCGs not just top 200

* Combined maps in one screen to see pathways affected by each genes versus those that are unique to each lncRNA

Summary of progress

1. Identified high expressing cancer specific lncRNAs through several filtration steps across multiple cancer types.

2. Confirmed cancer specific expression through comparing the distributions of each candidate lncRNA's expression among the cancer types studied

3. Identified cancer specific high expressing lncRNAs associated with patient survival outcome using the Cox Proportional Hazards model
    - 42 lncRNAs in 4 cancer types
    - 7 lncRNAs with adjusted p-value < 0.1 in 2 caner types (Tier 1)
    - 35 lncRNAs with p-value < 0.05 in 4 cancer types (Tier 2)

4. Assessed tumour versus normal tissue lncRNA expression by comparing ranked values of expression using tissue data from GTEX

5. 21/42 lncRNAs were predicted to be either tumour suppressive or oncogenic based on sign of hazard ratio and difference in expression between normal and tumour tissues

6. 2 candidates in Ovarian cancer were further studied to predict regulatory roles that may lead patients from the same cancer type to develop worse prognosis

Future Directions

Future Directions

1. Validation of survival associated lncRNAs in additional datasets
    - TCGA datasets
    - Thorough analysis of specific cancer cohorts available including Japanese liver cancer cohort and Toronto Ovarian cancer cohort
    - Improve biomarker discovery pipeline through additional data integration and analysis

Future Directions

1. Validation of survival associated lncRNAs in additional datasets

    - TCGA datasets

    - Thorough analysis of specific cancer cohorts available including Japanese liver cancer cohort and Toronto Ovarian cancer cohort

    - Improve biomarker discovery pipeline through additional data integration and analysis

2. In depth analysis of high confidence candidate lncRNAs to predict cellular mechanisms that could be experimentally validated

    - Predict interactions through co-expression analysis with improved predictors and assess permutations

    - Predictors will include copy number aberrations, promoter methylation, presence of TAD boundaries, single nucleotide variants in lncRNA promoter or predicted enhancer regions and integration of cancer specific properties such as driver mutation status

    - Integrate available protein level data to evaluate protein-protein co-expression as well as lncRNA-co-expression to build a lncRNA-protein regulatory network

Future Directions

1. Validation of survival associated lncRNAs in additional datasets

    - TCGA datasets

    - Thorough analysis of specific cancer cohorts available including Japanese liver cancer cohort and Toronto Ovarian cancer cohort

    - Improve biomarker discovery pipeline through additional data integration and analysis

2. In depth analysis of high confidence candidate lncRNAs to predict cellular mechanisms that could be experimentally validated

    - Predict interactions through co-expression analysis with improved predictors and assess permutations

    - Predictors will include copy number aberrations, promoter methylation, presence of TAD boundaries, single nucleotide variants in lncRNA promoter or predicted enhancer regions and integration of cancer specific properties such as driver mutation status

    - Integrate available protein level data to evaluate protein-protein co-expression as well as lncRNA-co-expression to build a lncRNA-protein regulatory network

3. Apply analysis to normal tissues available in GTEX to predict differential co-expression of key pathways between normal and tumour tissues