Using Systems Biology Approaches to Analyze the GUDMAP Gene Expression Database to Improve Understanding of Lower Urinary Tract Development and Disease

Bruce Aronow
Cincinnati Children's Hospital Medical Center
College of Medicine
University of Cincinnati

The GUDMAP Project: goals, strategies, & parts!

Overall Goal: To Define the Molecular and Microstructural Basis of Genitourinary Systems Development

Developmental Stages | Anatomical Structures

(built an ontology of anatomical structures and stages!)

Renal | Lower Urinary Tract | Repro-Gonadal

GeneChips: > 260 micro-tissue samples obtained via laser capture, FACS, or micro dissection

***in situ* hybridization**: >2700 genes/-specific whole mount and high resolution ISH

Data Mining Goals of the GUDMAP Resource

enable users to identify and characterize

- genes
- anatomic structures
- pathways and processes

*that are **critical for development and function of GU structures and functions***

http://gudmap.cchmc.org
http://www.gudmap.org

National Institutes of Health

Harvard

Columbia University Medical Center

The University of Edinburgh

Vanderbilt University

University of Cincinnati

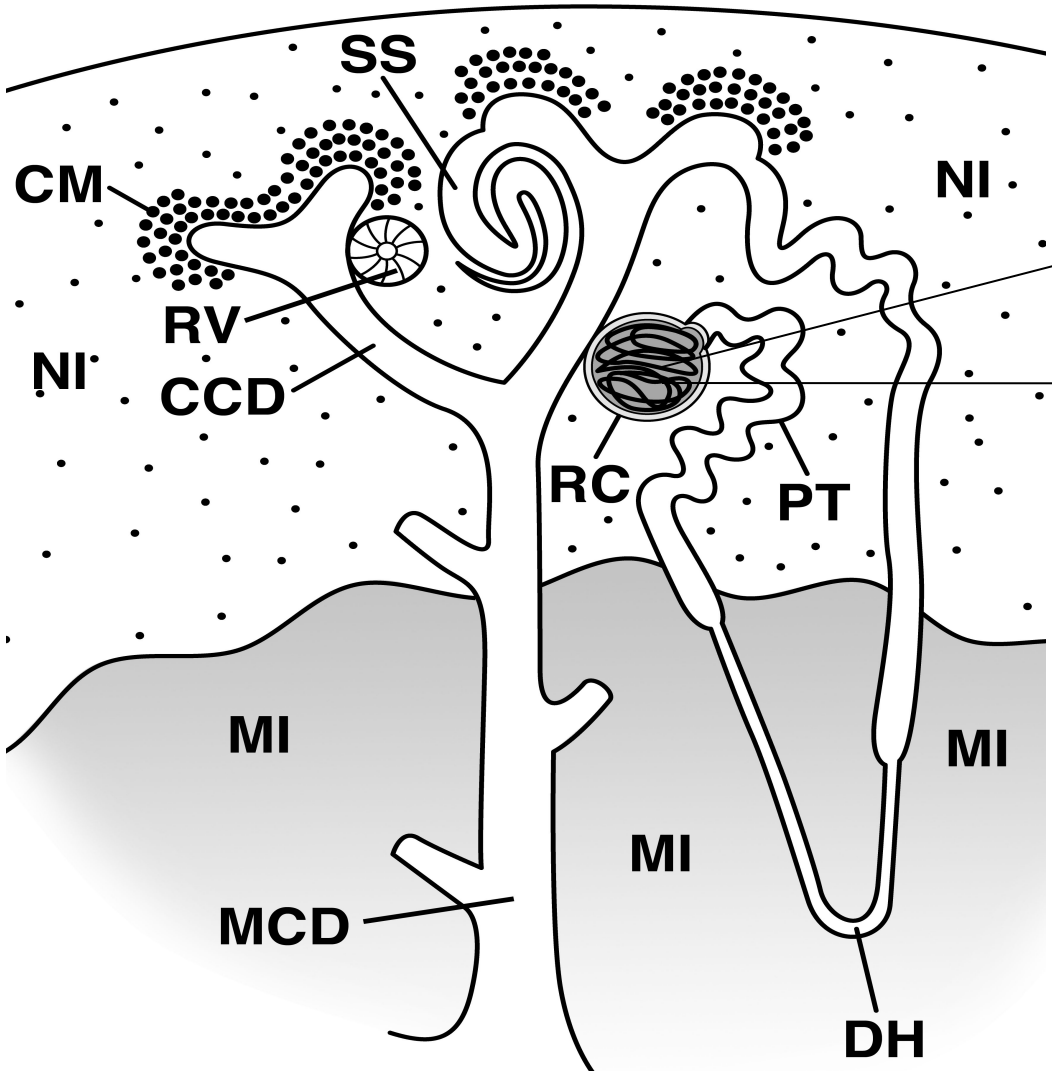
Cincinnati Children's Hospital Medical Center

The University Of Queensland Australia

Towards a Functional Molecular Anatomical Map of the Developing Kidney
Final Samples for 430 plus 2 Atlas:

N	name	samp	method
E11.5	MetanephMesenchym	MM	LCM
E15.5	Cap Mesenchyme-Six2	CM	Trypsinization/FACS
E12.5	RV	RV	LCM
E15.5	SS	SS	LCM
E11.5	UretBud	UB	LCM
E15.5	PodocytMafB	Pod-MafB	Trypsinization/FACS
E15.5	InterstitMeis1	Mesang	Trypsinization/FACS
E15.5	RenalCorpusc	RC_glom	LCM
E15.5	Endothelial Cells Flk1	Endothel	Trypsinization/FACS
E15.5	Proximal Tubules	PT	LCM
E15.5	ColDuctCortic	CCD	LCM
E15.5	ColDuctMedul	CUB	LCM
E15.5	HenleAnlag	Hen	LCM
E15.5	UretTip	UretT	LCM
E15.5	MedulInterstit	MI	LCM
E15.5	UretSmooMusc	USM	LCM
E15.5	Urothelium	UT	LCM

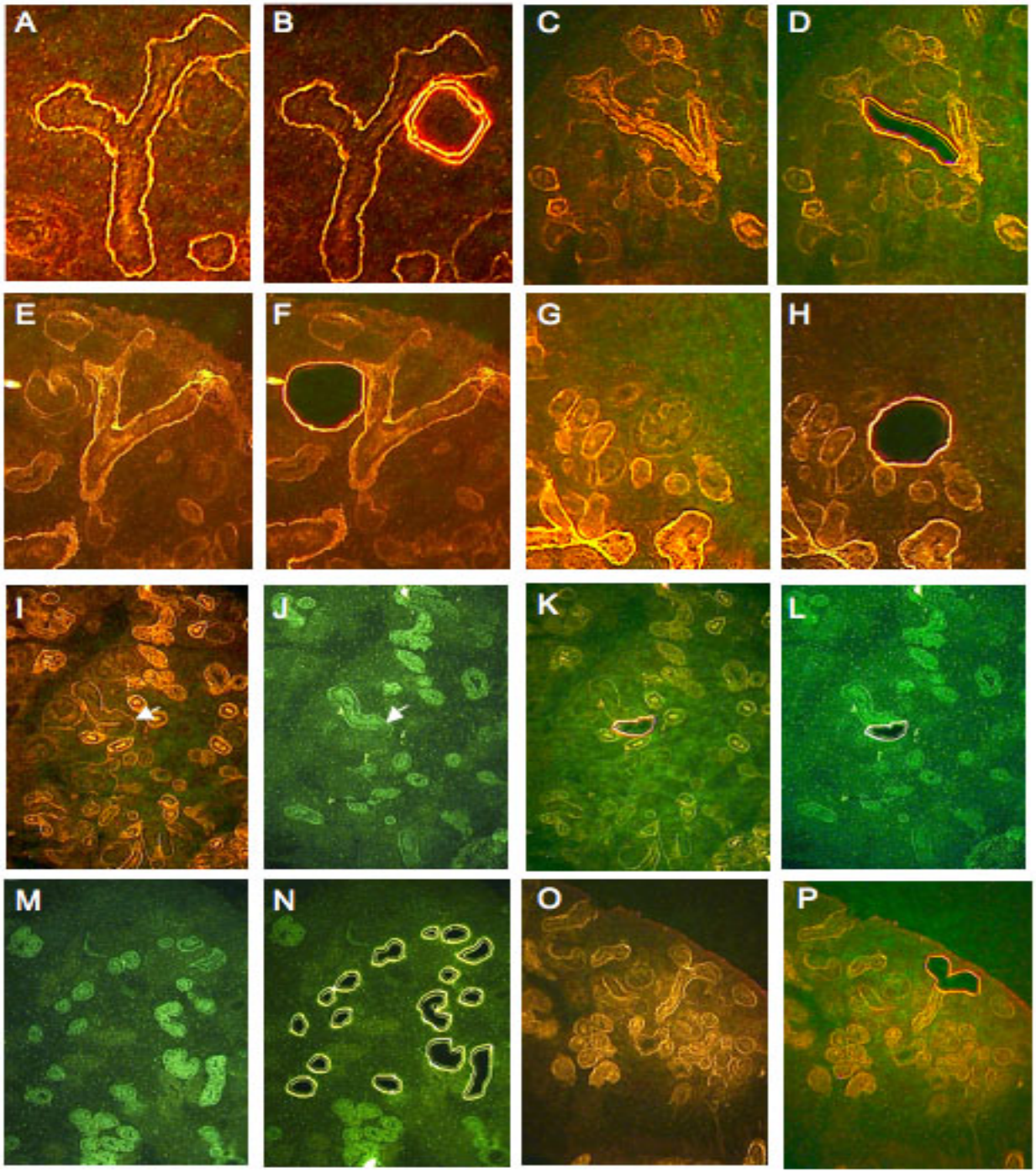
Sampled Compartments

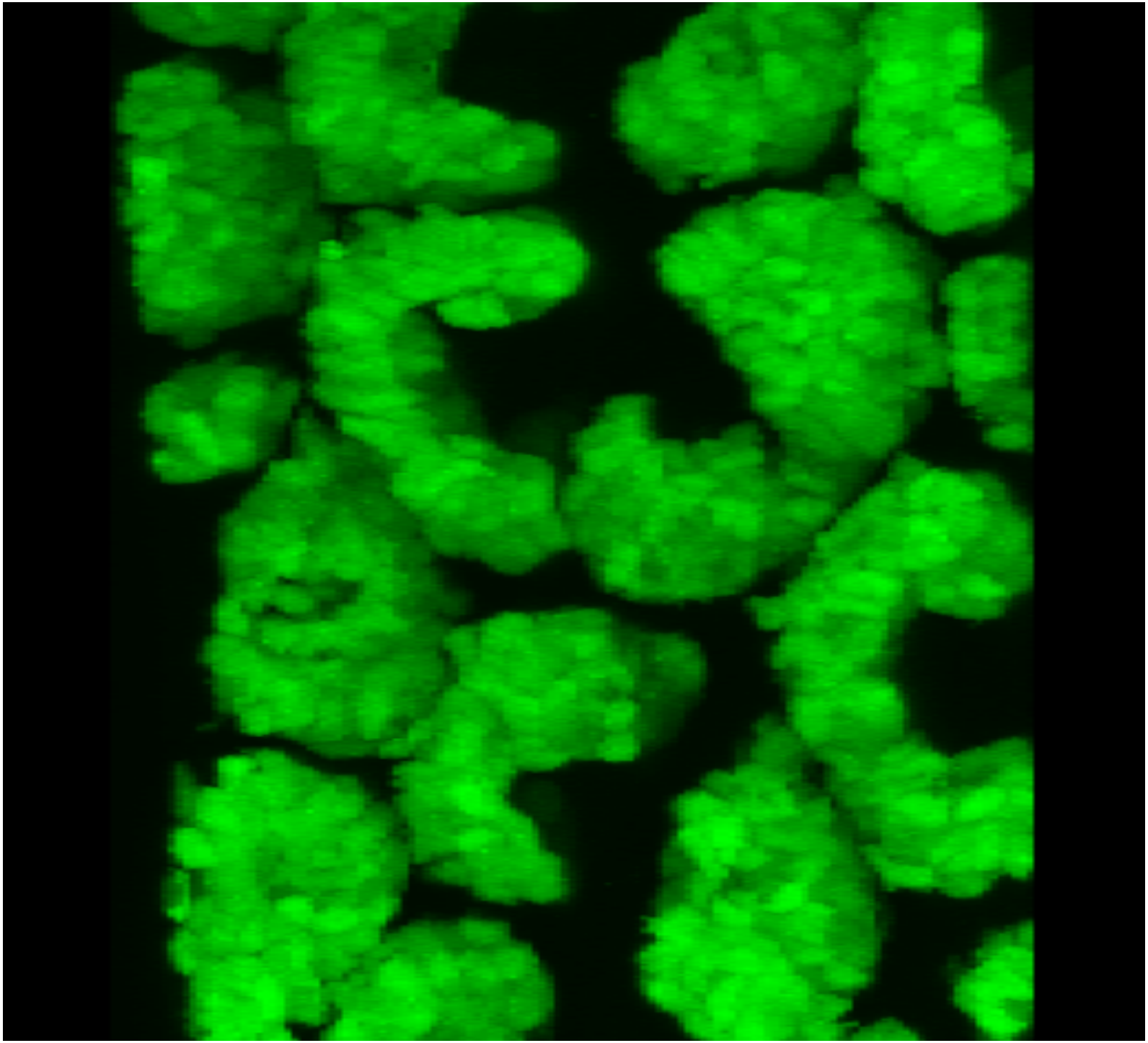


Meis1- Mesang

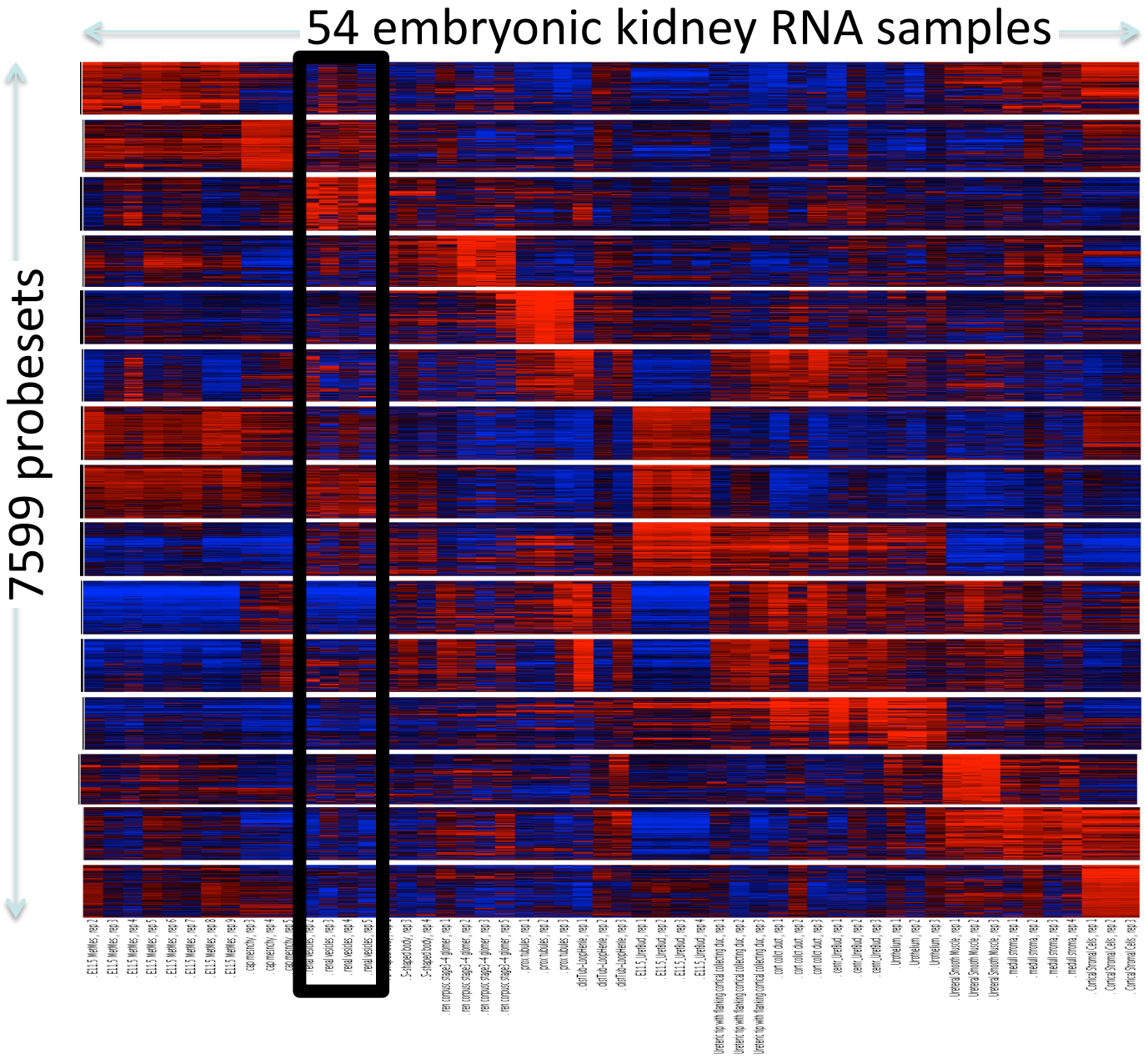
Podocytes
MafB-GFP

Flk1- Endoth


A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P



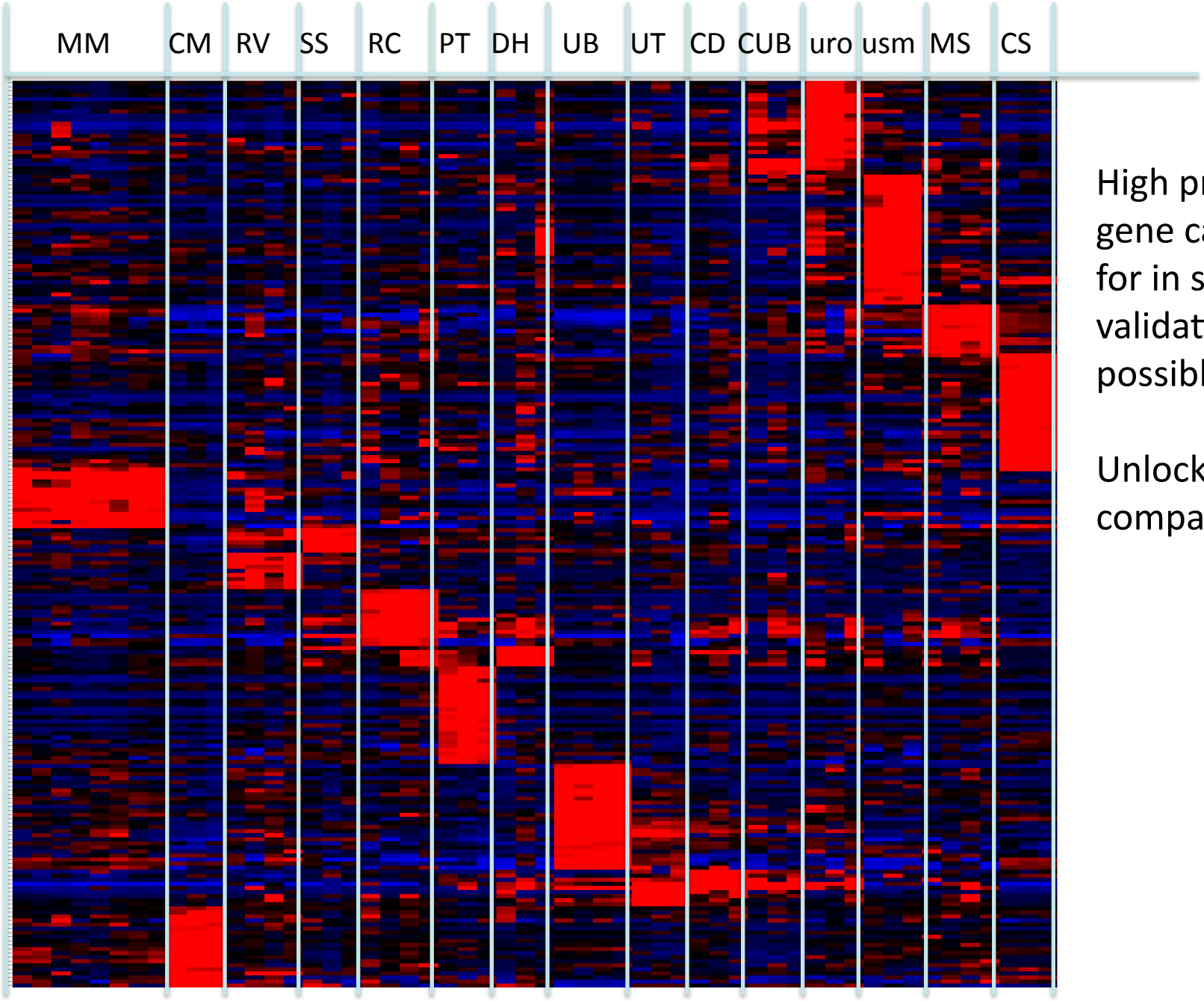
Functional Dissection of Expression Patterns in the Developing Kidney



cluster

A: 378ps C2
B: 374ps C3
C: 526ps C10
D: 521ps C13
E: 640ps C12
F: 416ps C15
G: 642ps C7
H: 667ps C4
I: 540ps C1
J: 651ps C6
K: 576ps C5
L: 411ps C14
M: 343ps C11
N: 441ps C8
O: 473ps C9

Gudmap by Compartment : top 223 candidate compartment markers set

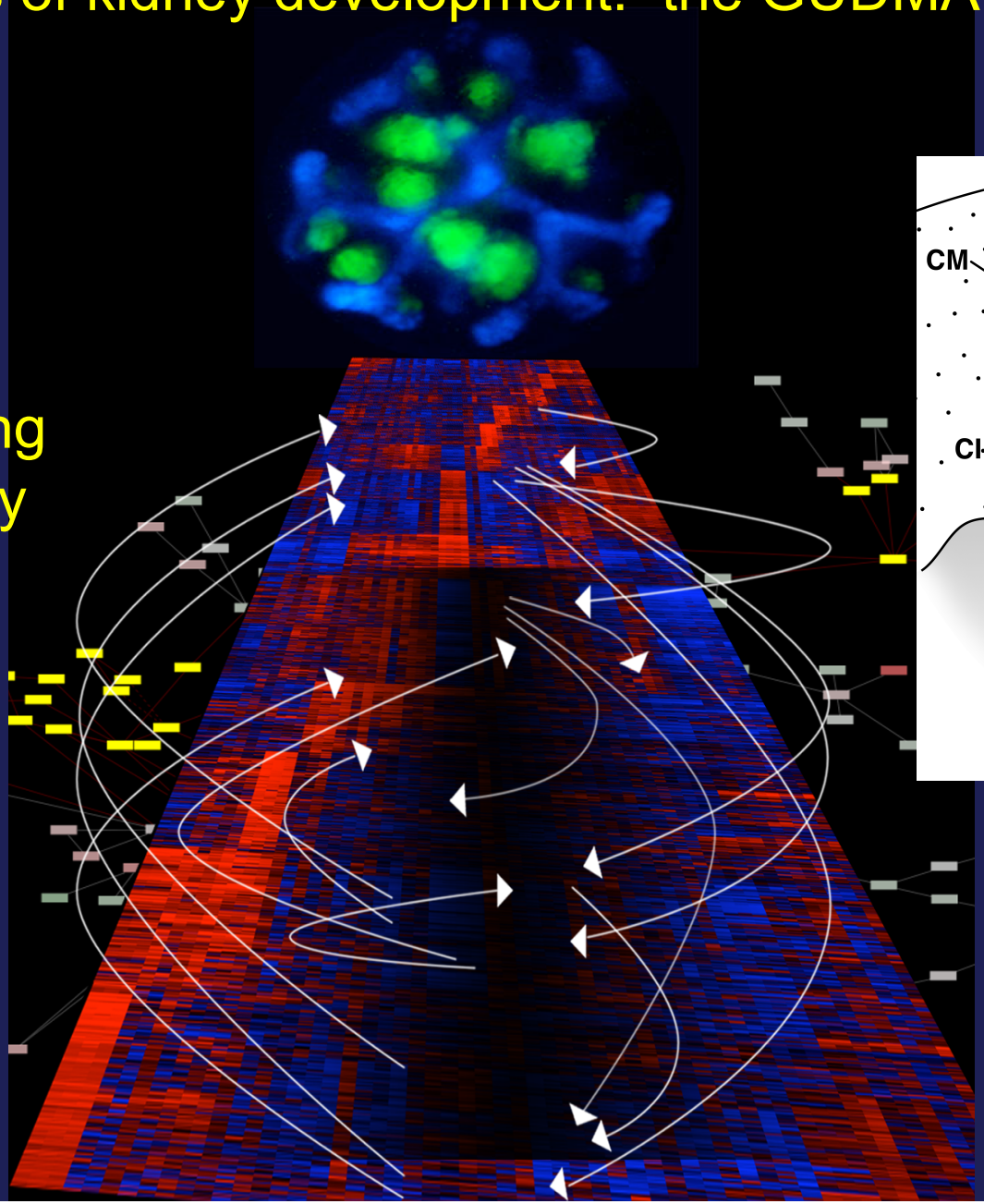


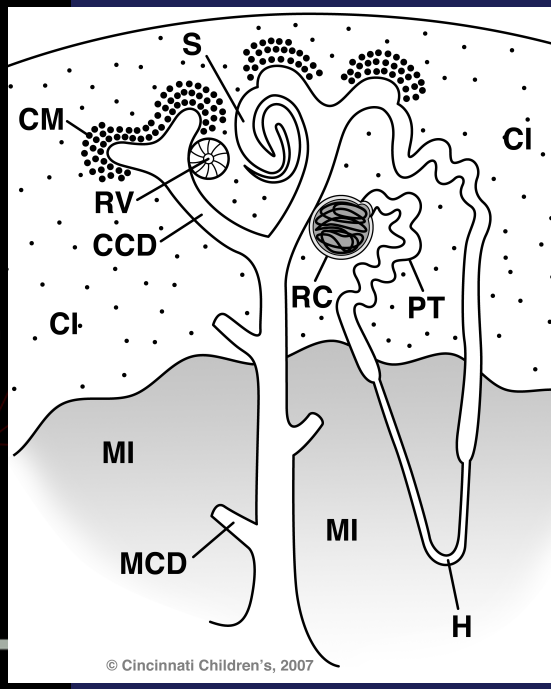
High priority gene candidates for in situ validation, possible KI/KO,

Unlock new compartments?

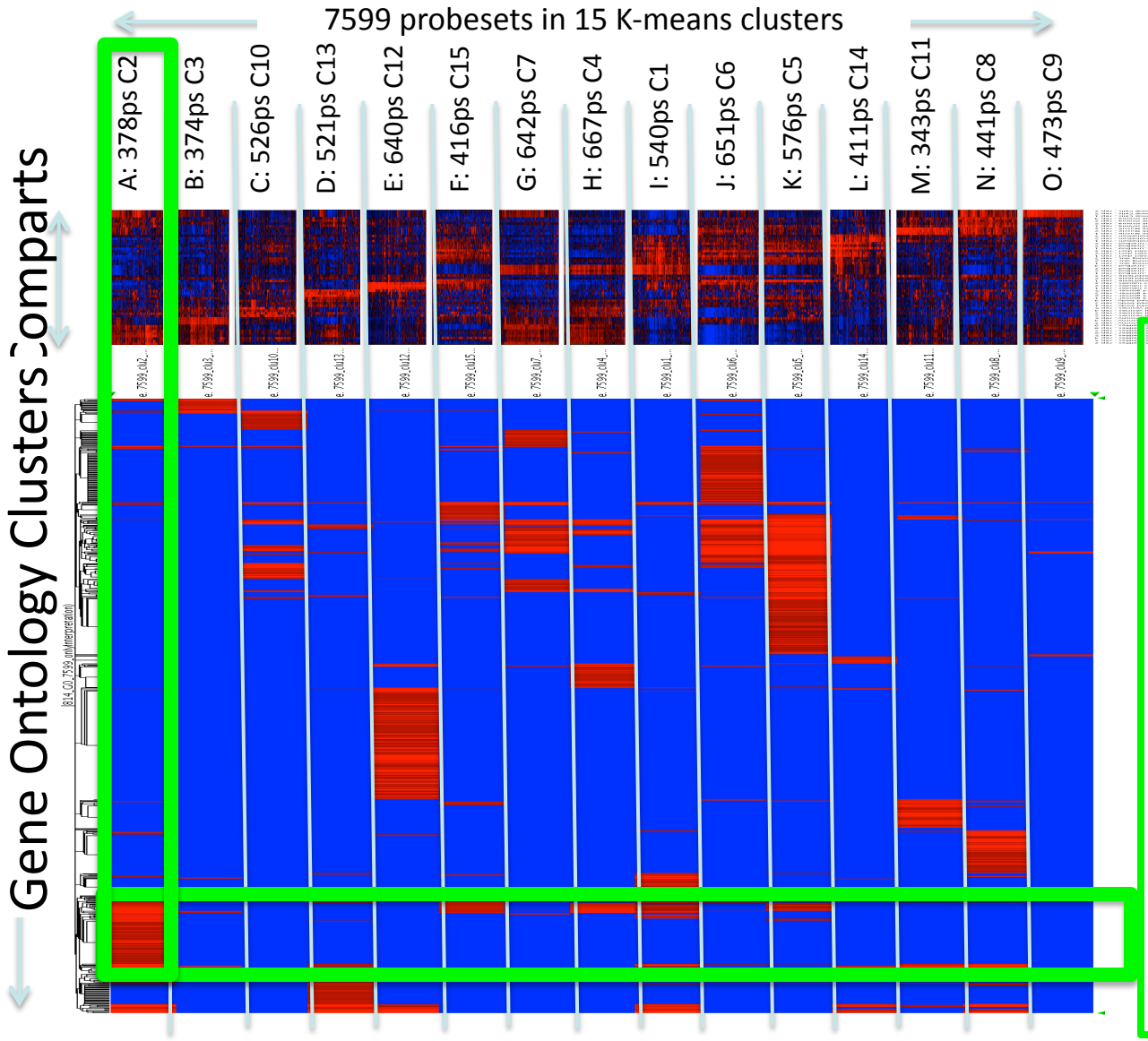
Undertaking a holistic approach to understanding molecular mechanisms of kidney development: the GUDMAP project

the developing mouse kidney at E15.5





Identifying Critical Biological Processes and Pathways active in developing kidney compartments: enrichment analysis of large-scale gene patterns

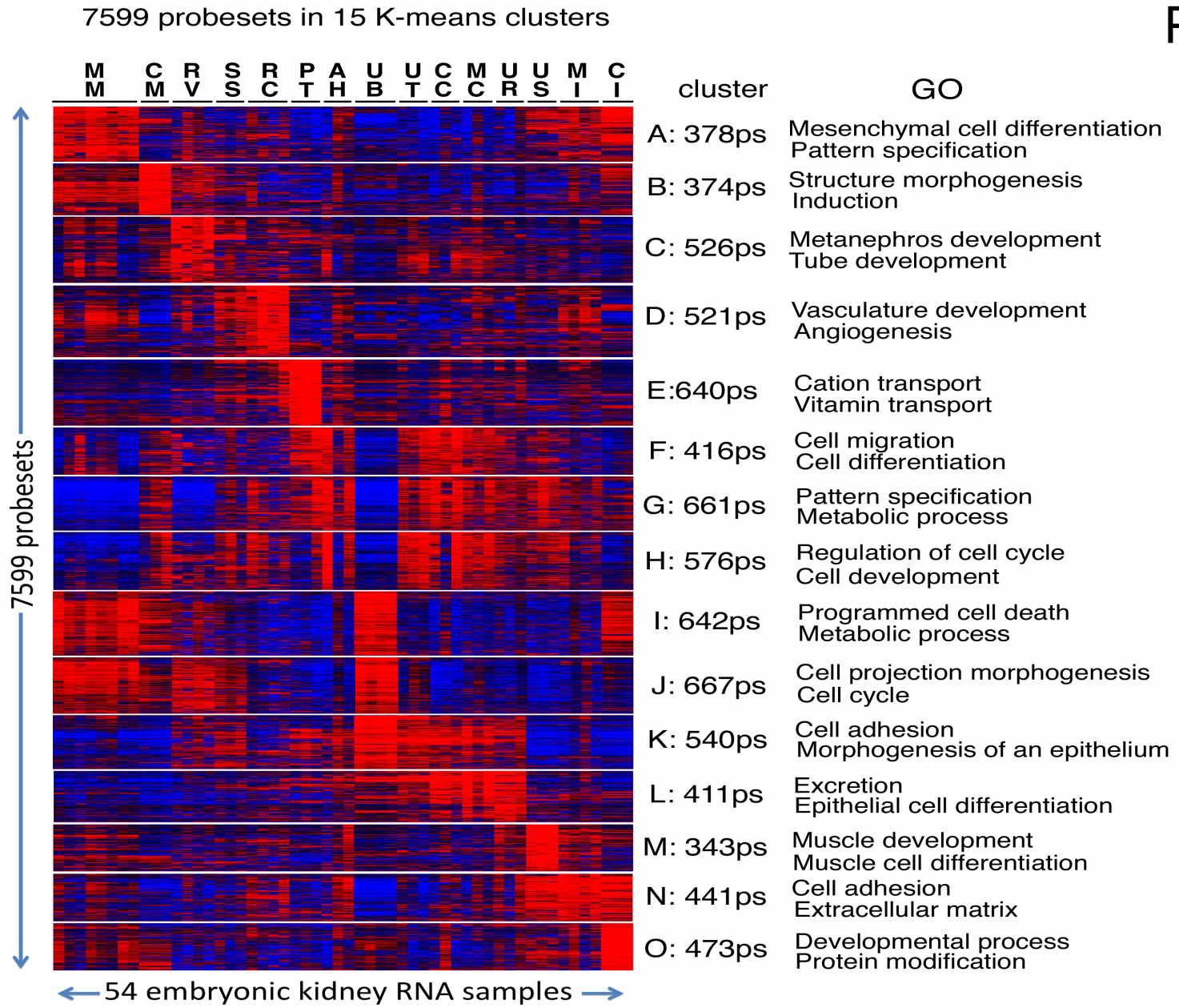


Example: understanding cluster C2; a CS/MM pattern

C2 _extracellular matrix; biological adhesion; mesenchymal cell differentiation; neural crest cell development; tube development

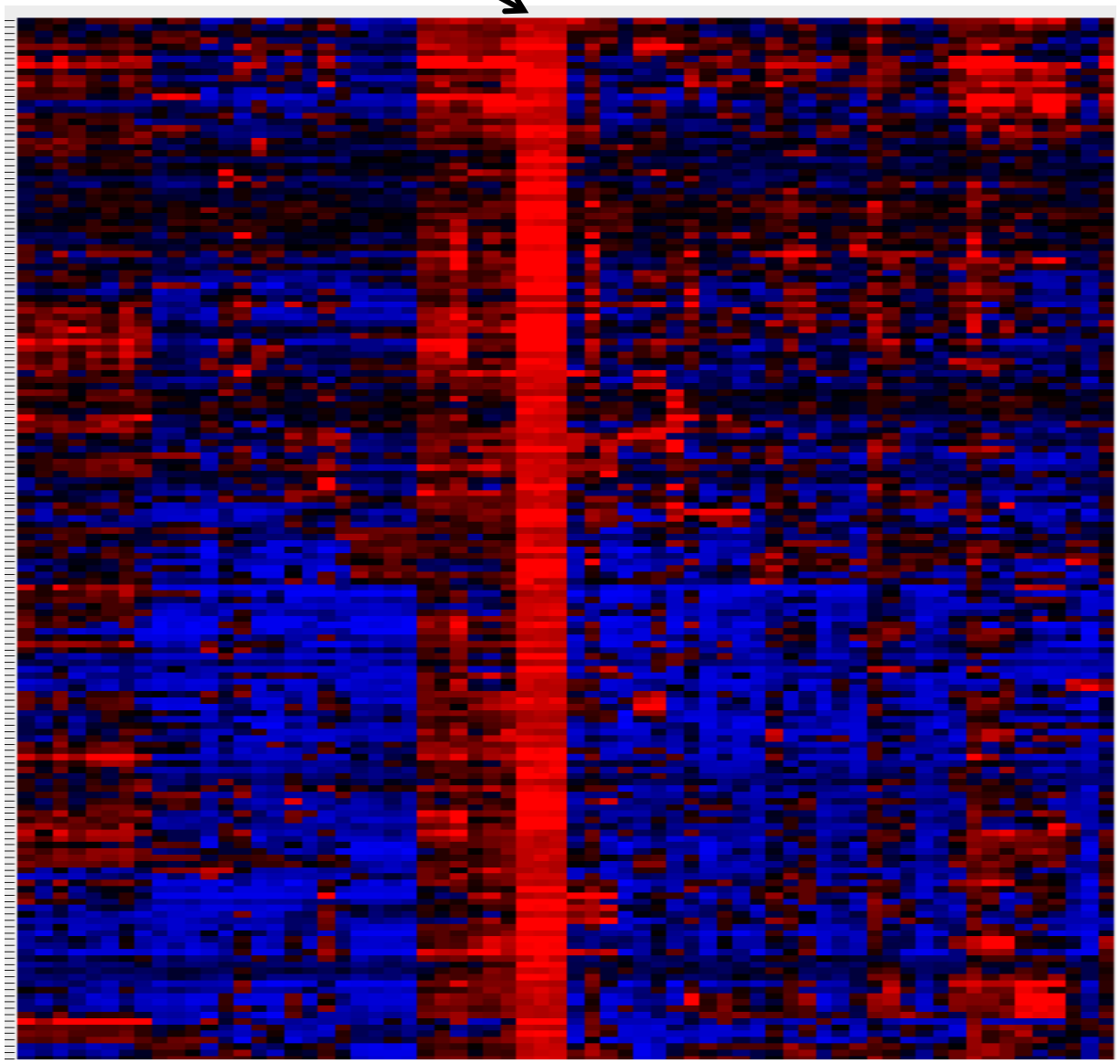
e.g. EDN3, TWIST1, LAMA4, RTN4, NRP2, GLI2, NR2F2, SLIT3, CHL1, FOXD1, GFRA3, CXCL12, TGFB2, UNC5C, RELN, TEK, NTN1, NRXN1, CCK, CDH2, PPAP2B, ISL1

Filtering Approach– “Top 1000 probesets per compartment”



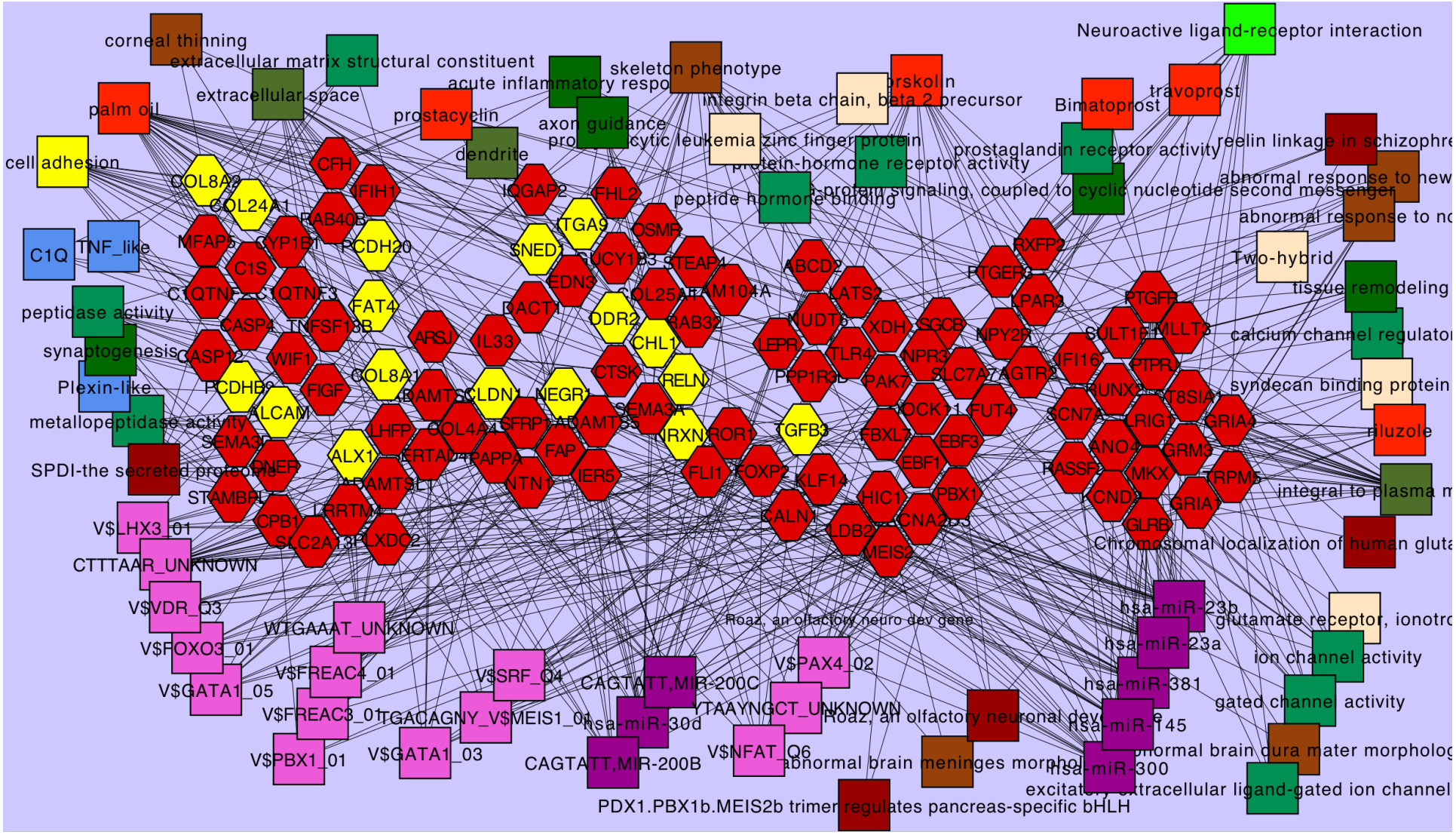
Pool, cluster, enrichment analysis

Example of following the Systems Biology Approach: analyze the strong cluster of genes observed to be expressed largely exclusively in Meis1-GFP derived glomerular mesangial cells

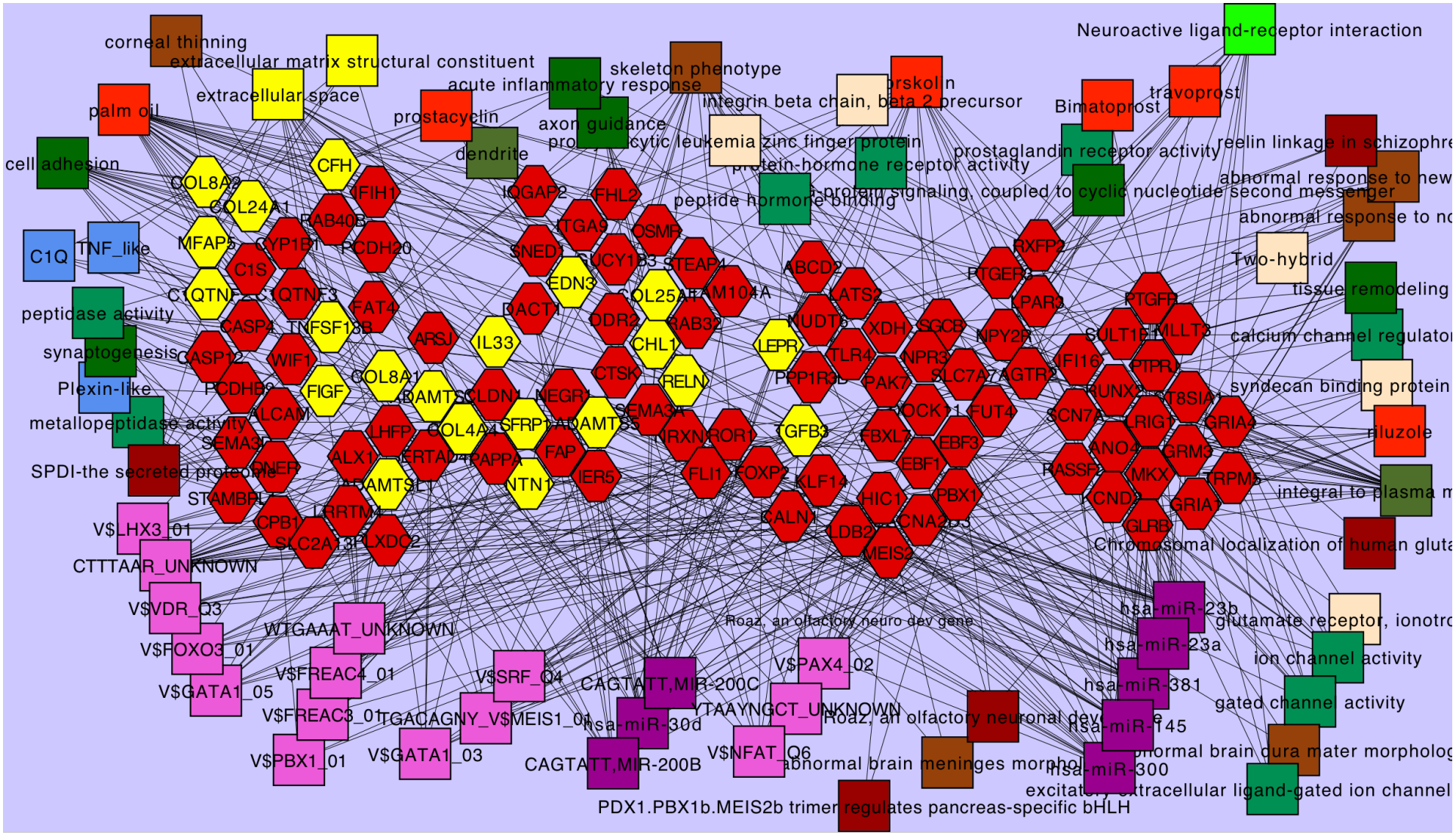


166 genes/ transcripts with high-level and compartment specific expression in Meis1-GFP cells FACS sorted from semi-purified glomeruli

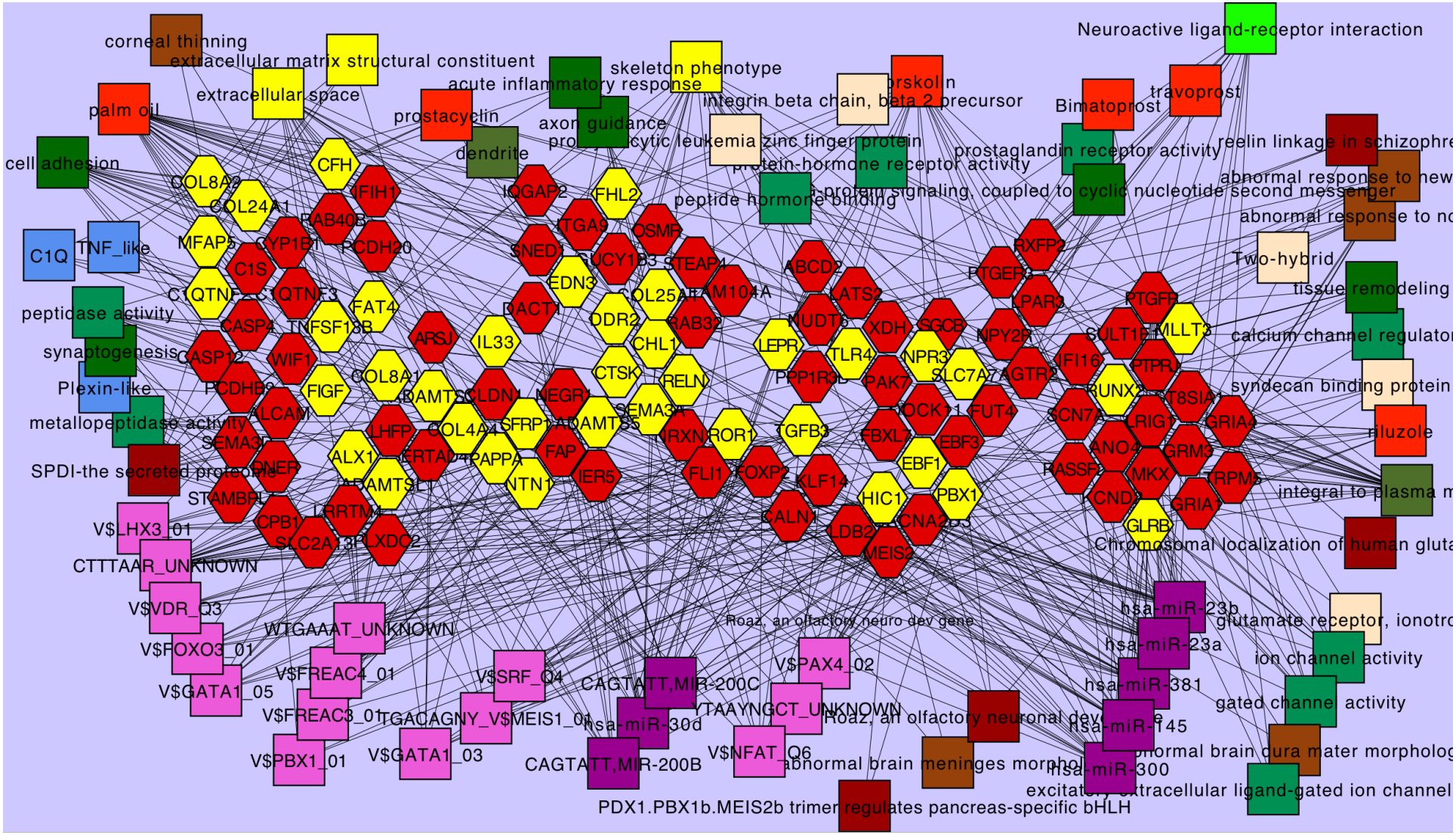
Knowledge network-based analysis of genes observed to be specifically expressed in glomerular mesangial cells



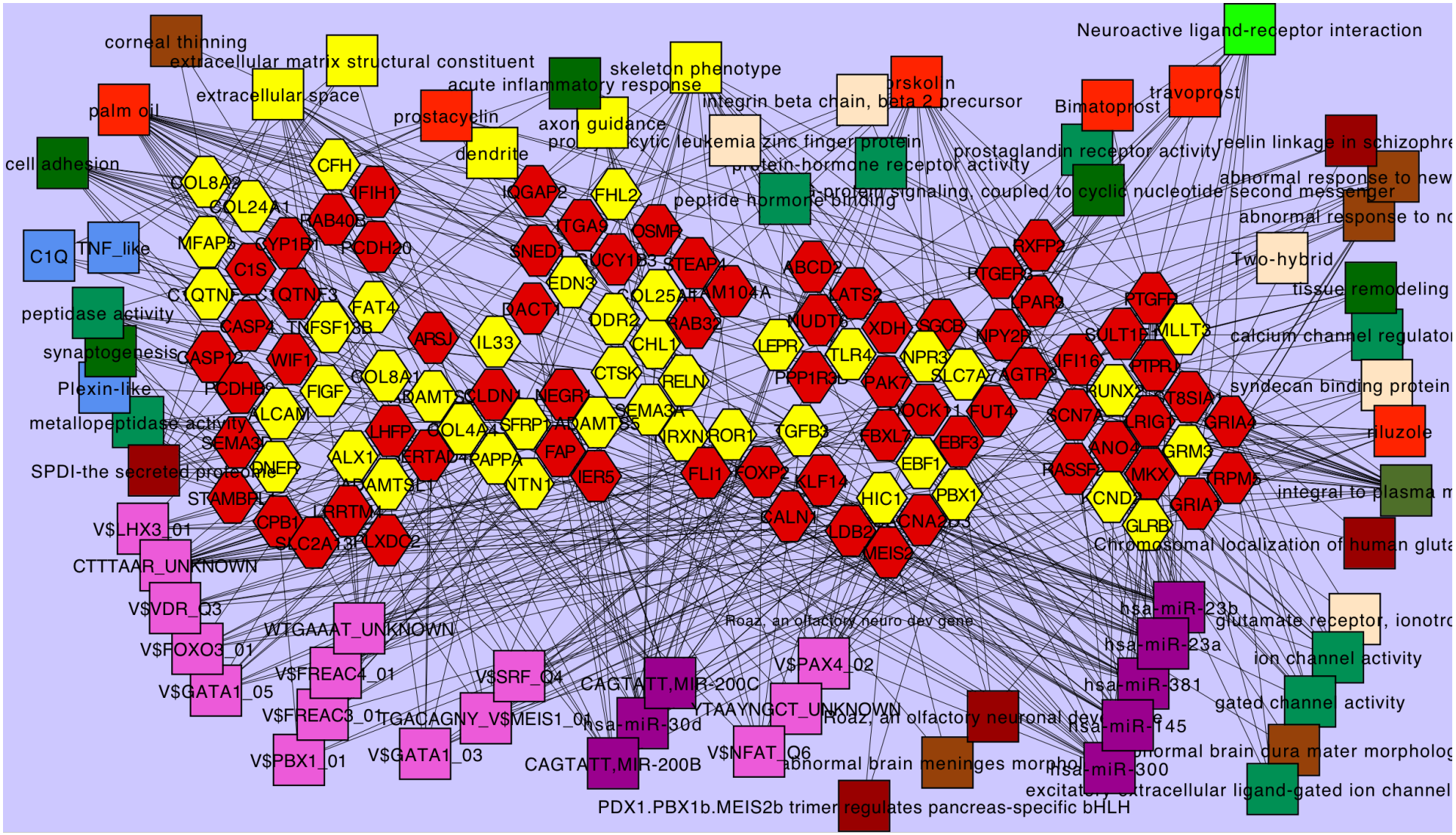
Knowledge network-based analysis of genes observed to be specifically expressed in glomerular mesangial cells



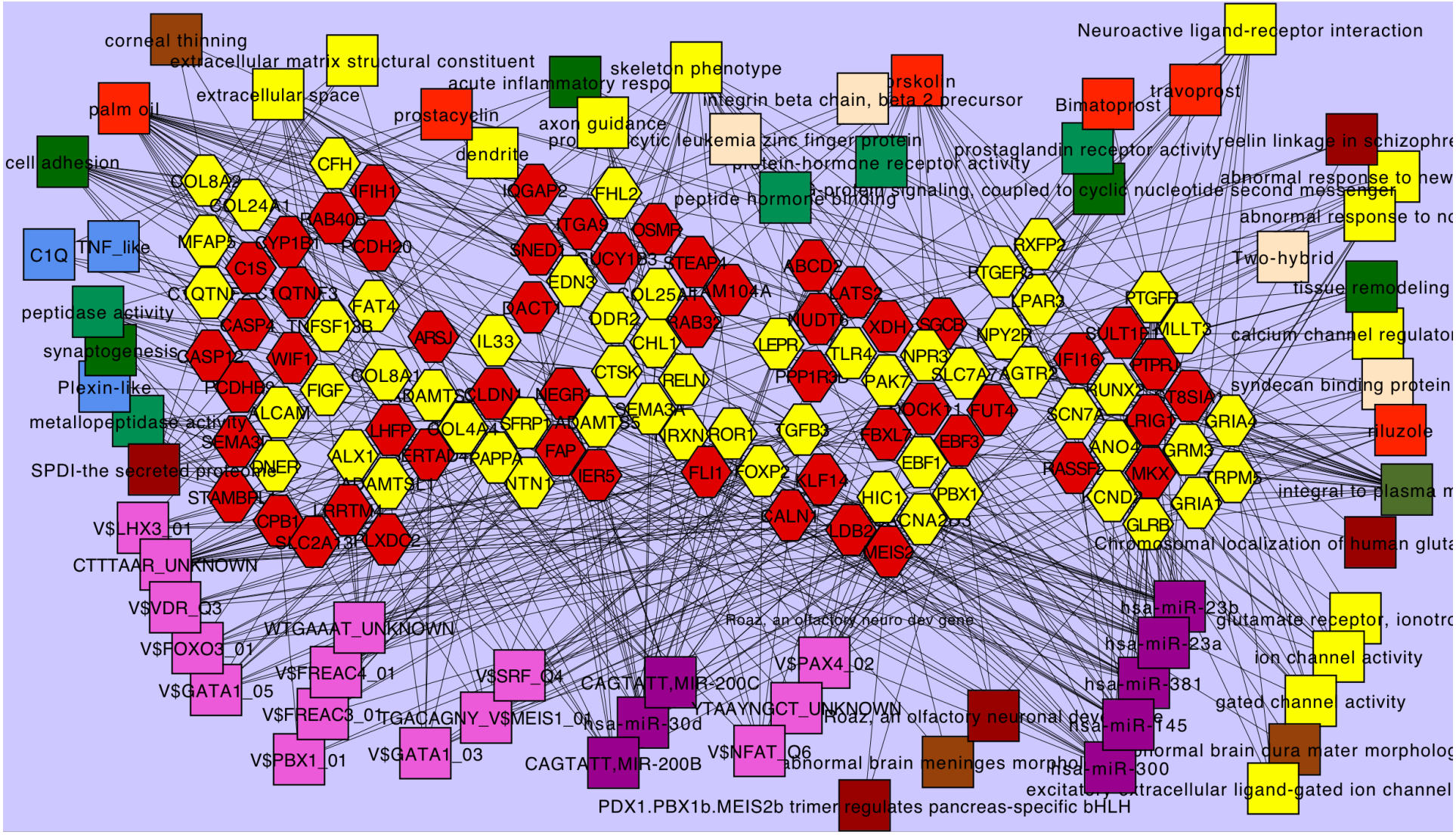
Knowledge network-based analysis of genes observed to be specifically expressed in glomerular mesangial cells



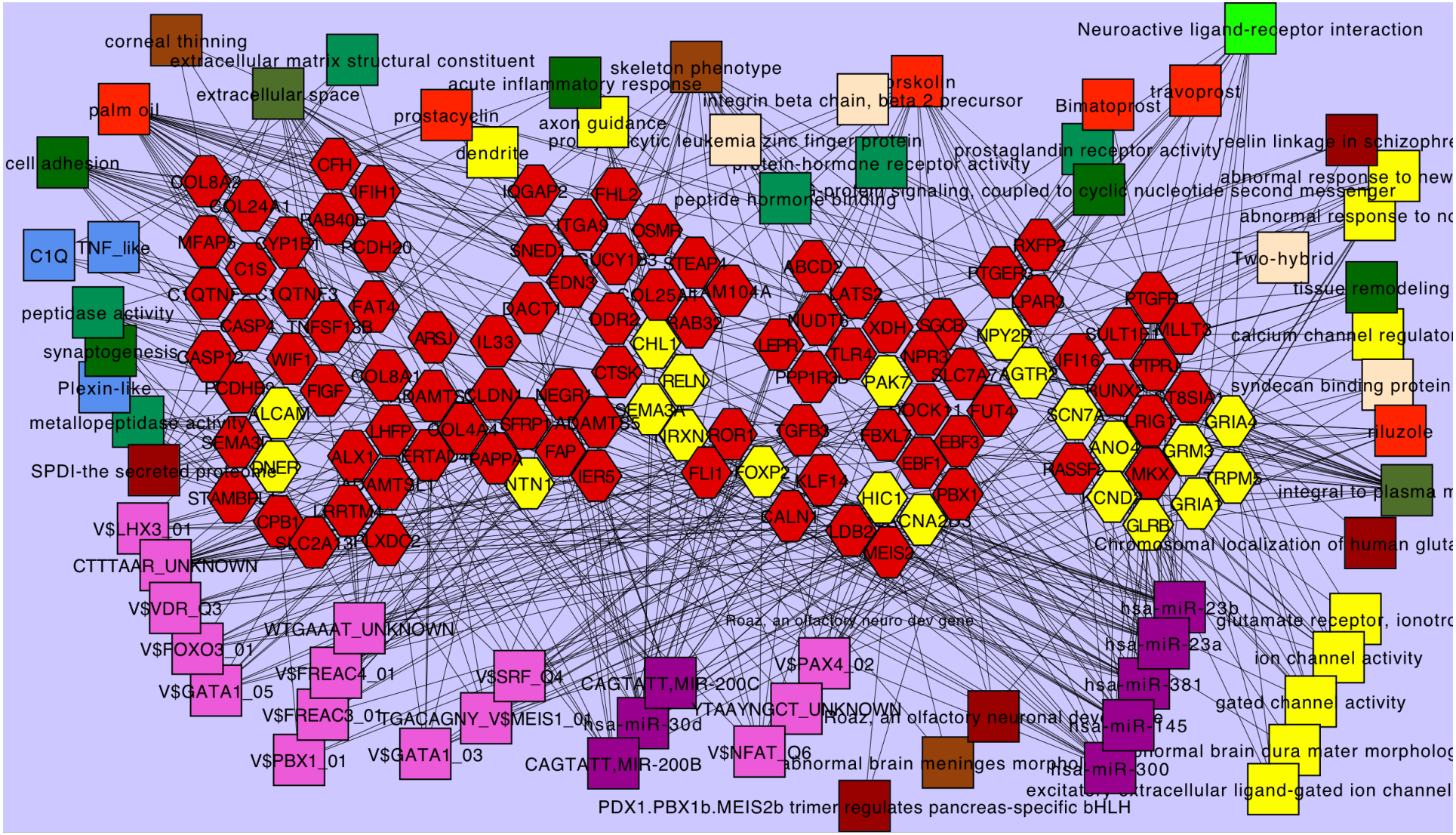
Knowledge network-based analysis of genes observed to be specifically expressed in glomerular mesangial cells



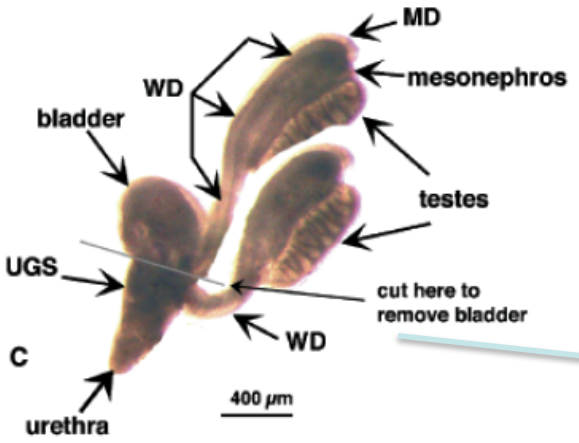
Network knowledge analysis of genes observed to be specifically expressed in glomerular mesangial cells



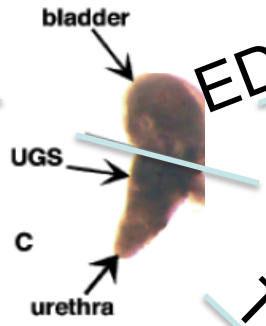
Network knowledge analysis of genes observed to be specifically expressed in glomerular mesangial cells



Isolation of Mesenchymal and Epithelial Compartments of E13 Mouse Bladder



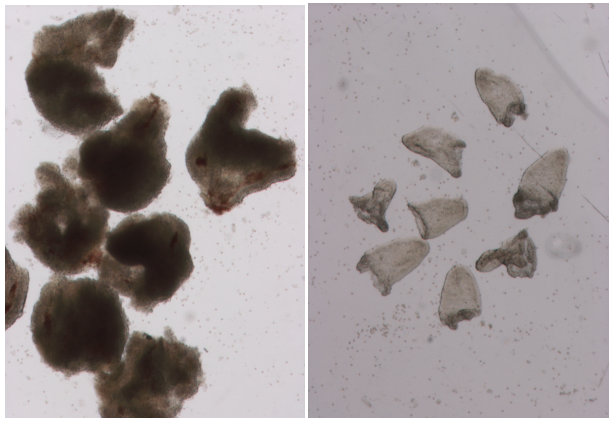
Isolate bladder and urethra. Remove Wolffian Ducts, Ureters, and Umbilical Arteries.



EDTA/Tyrodes

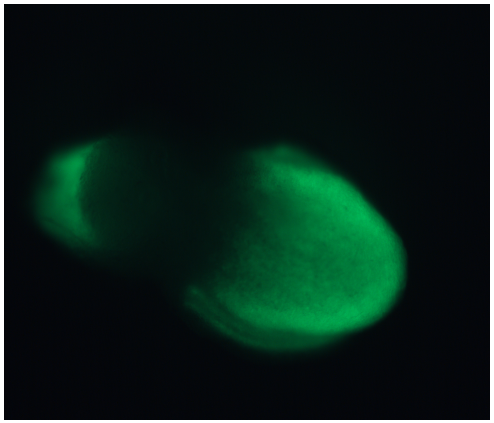
Bladder Mesenchyme

Epithelium



Trypsin/Tyrodes

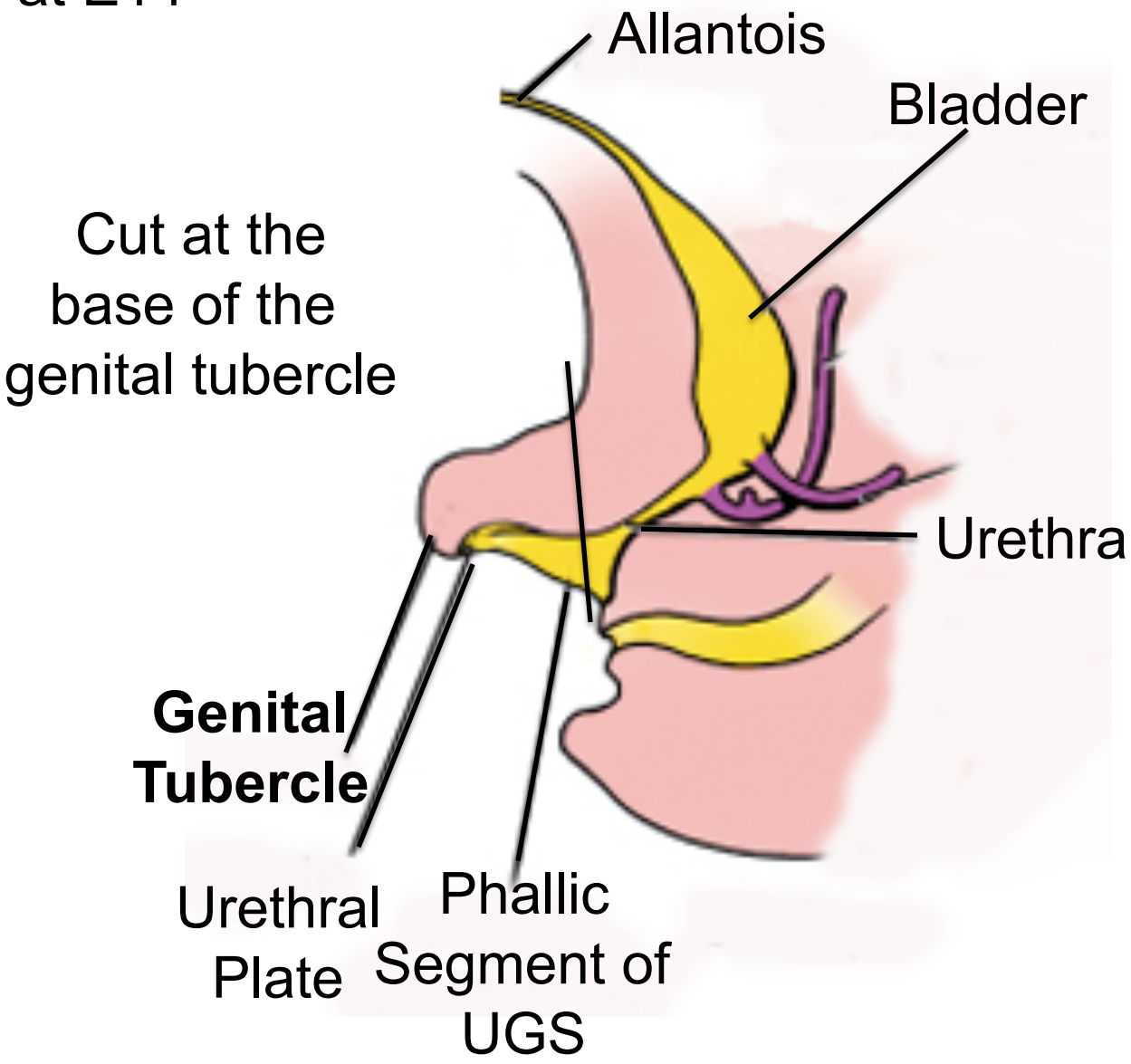
Dissection/
Trypsin/FACS



Bladder Neck
&
Urethra
Mesenchyme

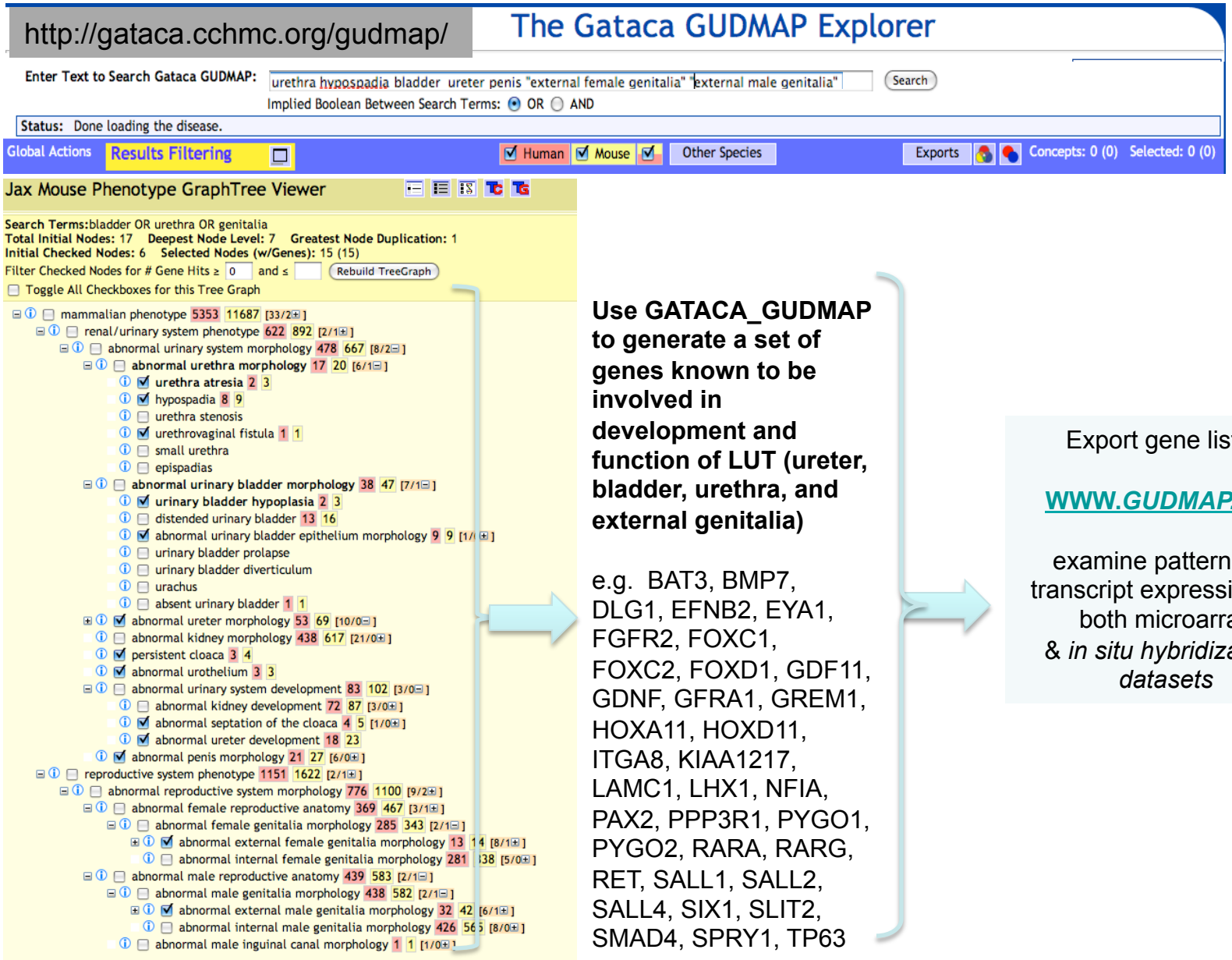
Epithelium

Analysis of Gene Expression in the Genital Tubercle at E14



GUDMAP-GATACA: a Genome-Phenome Web Tool to Explore the Molecular Basis of Structures, Functions, and Disorders of the Urogenital Organs and Systems

http://gataca.cchmc.org/gudmap/



Use GATACA_GUDMAP to generate a set of genes known to be involved in development and function of LUT (ureter, bladder, urethra, and external genitalia)

e.g. BAT3, BMP7, DLG1, EFNB2, EYA1, FGFR2, FOXC1, FOXC2, FOXD1, GDF11, GDNF, GFRA1, GREM1, HOXA11, HOXD11, ITGA8, KIAA1217, LAMC1, LHX1, NFIA, PAX2, PPP3R1, PYGO1, PYGO2, RARA, RARG, RET, SALL1, SALL2, SALL4, SIX1, SLIT2, SMAD4, SPRY1, TP63

Export gene list to

WWW.GUDMAP.ORG

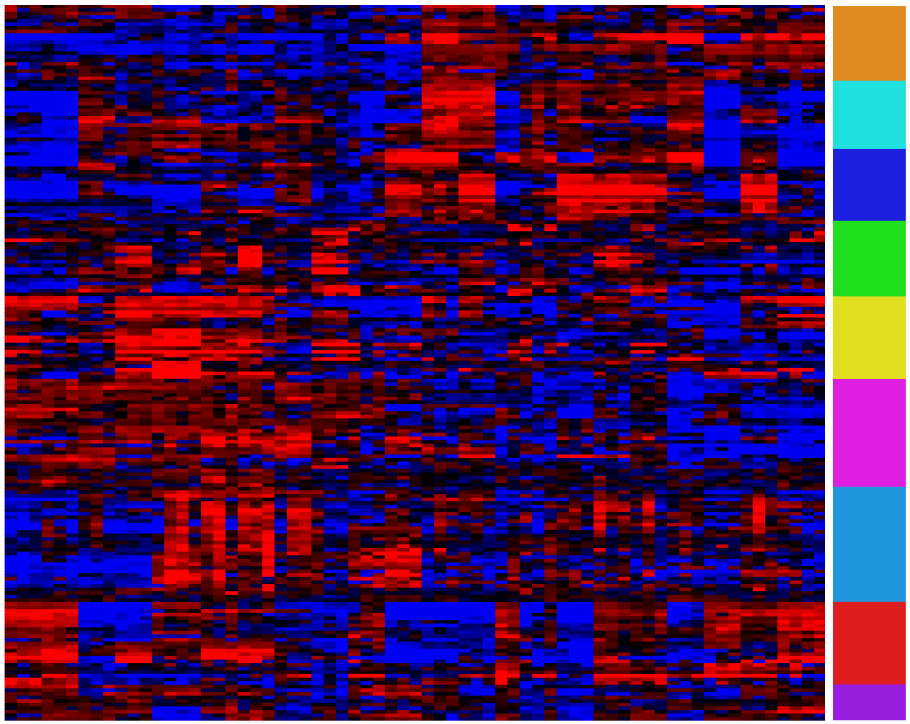
examine patterns of transcript expression in both microarray & *in situ hybridization datasets*

LUT "*Diseasome*" Genes Analyzed using the GUDMAP Microarray Database

Step 1. identify genes and good probes known to be critical for development of structure and function of the lower urogenital system

Step 2. derive gene expression clusters over the GUDMAP ***LUT*** expression database

Step 3. Analyze identified clusters for shared features and co-functions with based on other knowledge e.g. pathways, functions, cis elements, microRNA target sequences



SLC9A6,VANGL1,NIPA1, SGSH,AGA,NAGLU,MCOLN1,ANXA4, ADAMTS1 **Abnormal bladder morphology, incontinence, hydronephrosis**

BMP4,EVC2,FGFR1,PITX2,FBN1,HSPG2: **Hypospadias**, **Bladder disorders, Cryptorchidism, Abnormal cartilage, artery morphology**

KCNMA1,GATA5,ATP2A2,MYH11,PCSK5,BMP5: **Abnormal urination, abnormal ureter, penis morphology**

PDGFB,SLC7A9,CHRNA3, LHX1,FOXC2 SERPINC1,NHLH2, WT1, CYP11A1, SP8 **: Abnormal morphology: bladder, ureter, vas deferens, hypospadias**

EYA1,NIPBL,SALL4,SALL1,SIX1,FOXD2,HTR3A,FOXD1,GDNF: **Vesicoureteral reflux, short ureter, Hyposadia, abnormal digits and limbs, cryptorchism**

FGFR2,AR,EPHB3,EPHB2 FRAS1,FREM2,JARID1C,CUL4B,PHF6 CHD7,DKC1,GLI3, SALL2,RET,ITGA8,PYGO1,GFRA1,RARB,SLIT2 : **Hypospadias, Small penis, Decreased testicular size, Vaginal atresia, Abnormal ureter morphology,**

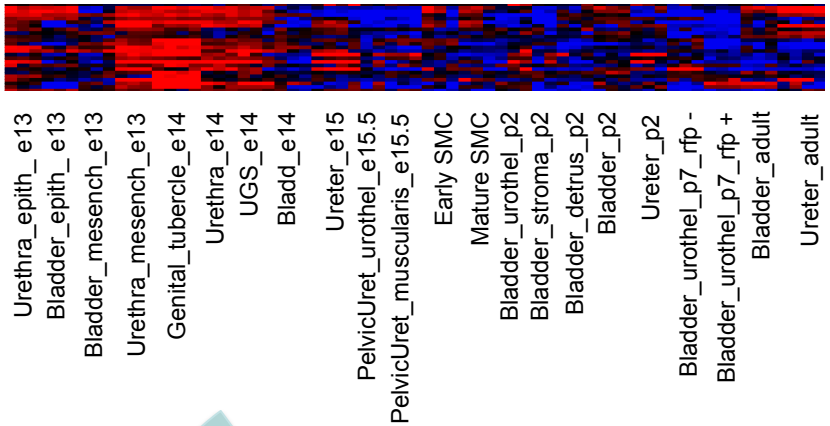
TBX3,JARID1C,PHF6,PTPN11, SGSH, T, NOTCH2, NOTCH3, PTEN, INHBA **Small penis, anal atresia, abnormal vulva, abnormal clitoris**

RET,ITGA6,GFRA1,UPK3A,UPK2, ITGB4, FGFR3, LAMC2, TP63: **Abnormal bladder, ureter, uterus morphology, abnormal skin, teeth**

FGFR2,MKS1,TBX3,HOXA13, HOXA10: **Hand-foot-genital syndrome**, **abnormal bladder, ureter, urethra, uterus, vagina, penis morphology, abnormal skin, teeth**

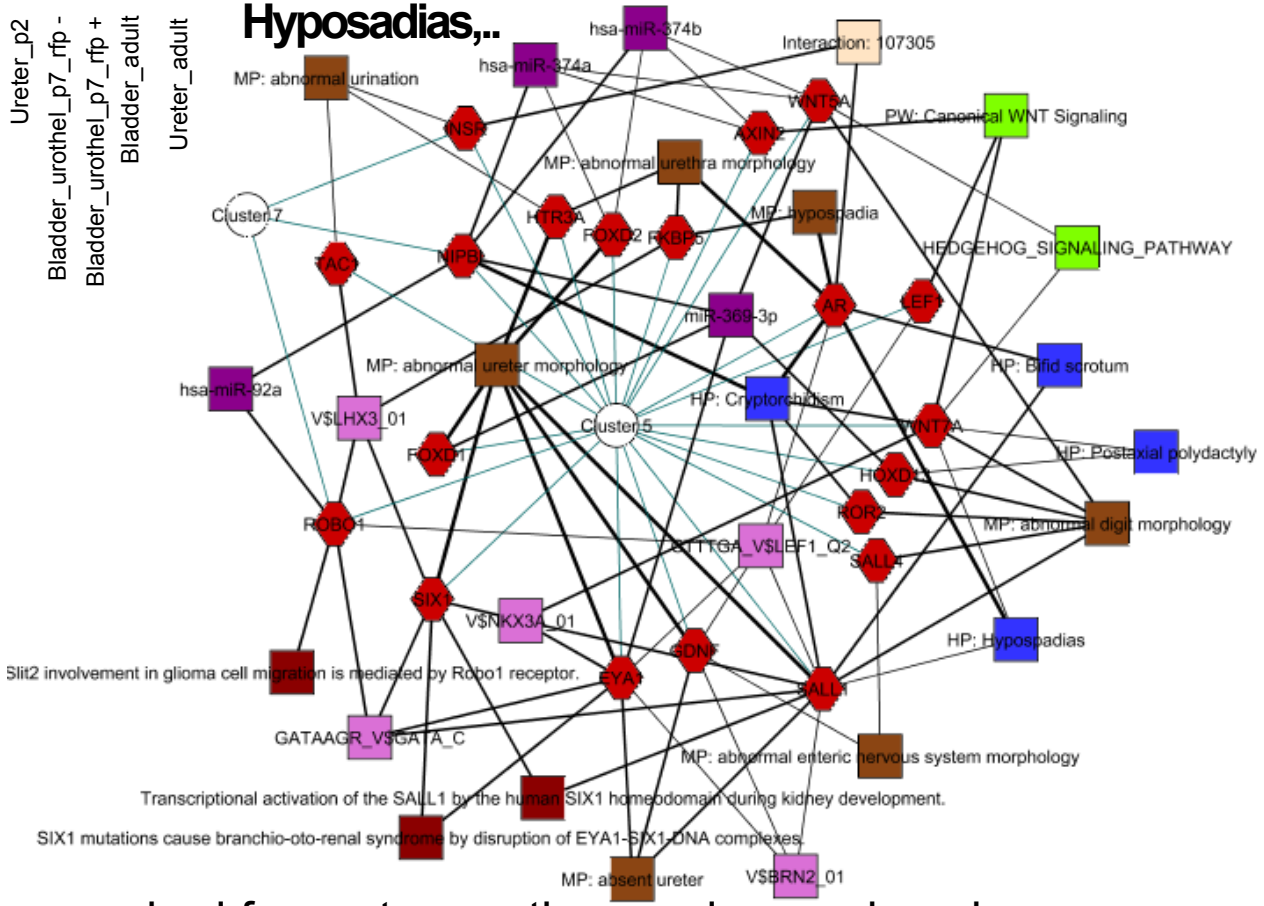
LUT "*Diseasome*" Genes Analyzed using the GUDMAP Microarray Database

c5 Gene expression pattern clusters of genes known to be critical for development of structure and function of the lower urogenital system as analyzed over the GUDMAP LUT expression database



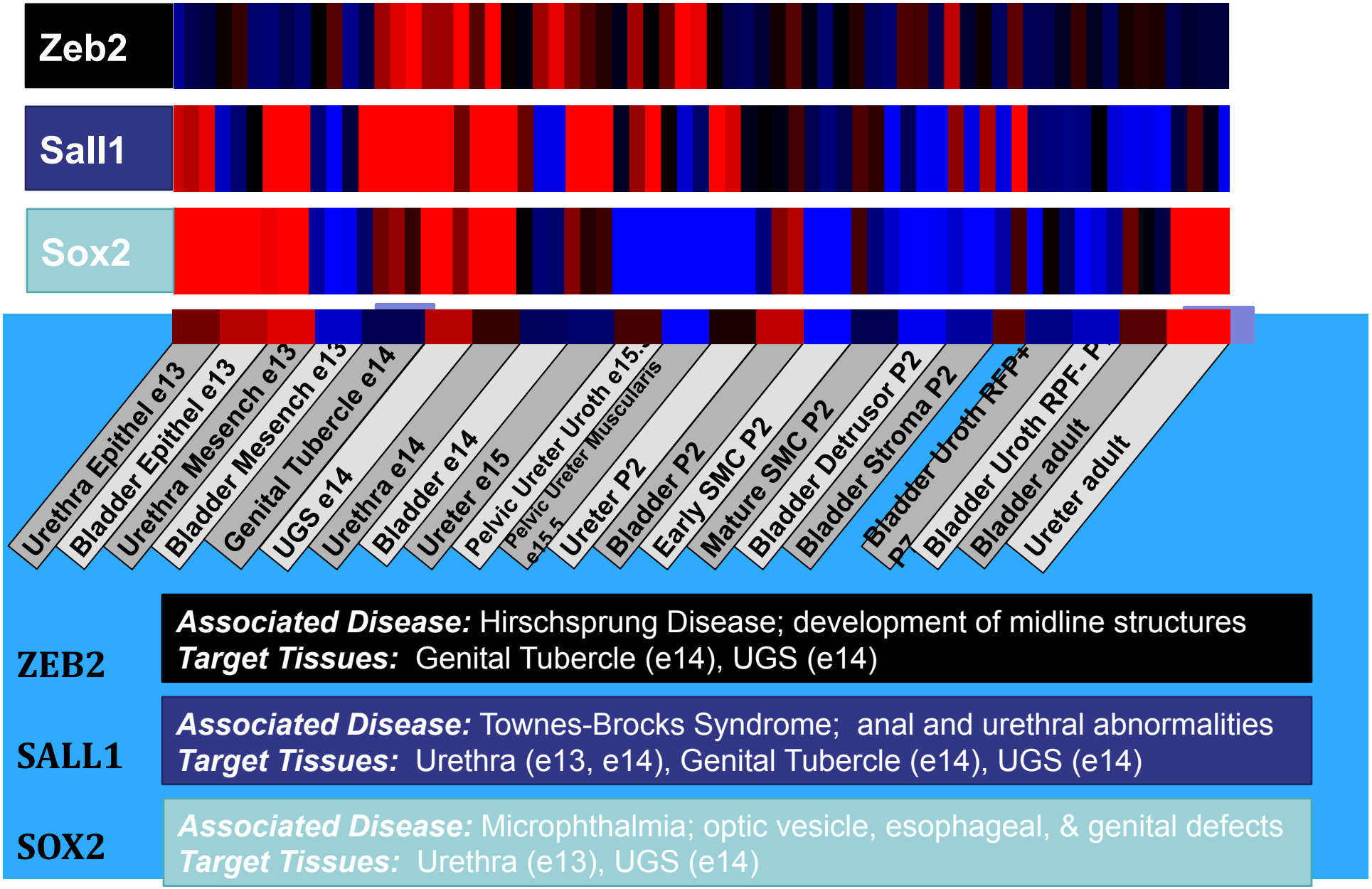
EYA1,NIPBL,SALL4,SALL1,SIX1,FOXD2,HTR3A,FOXD1,GDNF: **Vesicoureteral reflux, short ureter, Hyposadias,..**

Gene Cluster 5: Network/ Interactome-based Enrichment Analysis



Cluster C5 is enriched in genes required for ureter, urethra, and normal penis development and contains a densely connected set of genes known to participate in Hedgehog and WNT signaling, and appendage and digit development

Identifying **hypospadias-associated pathways**: reference genes from each LUT diseasome cluster (Clusters 7, 9, 11)

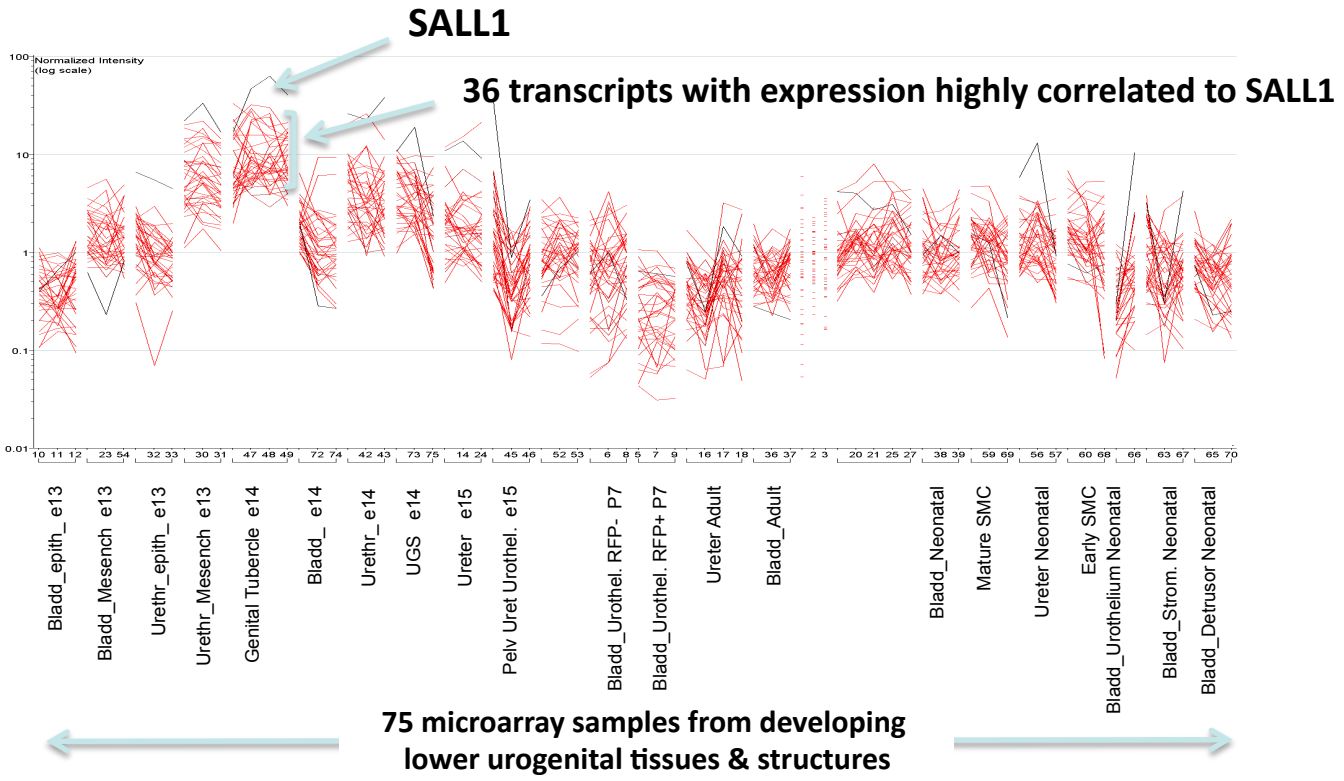


Using the Gudmap Database to Extend Knowledge and to Make Novel Hypotheses of Critical Genes for Genito-Urinary Tract Developmental Based on Syn-Expression Gene Set Analysis

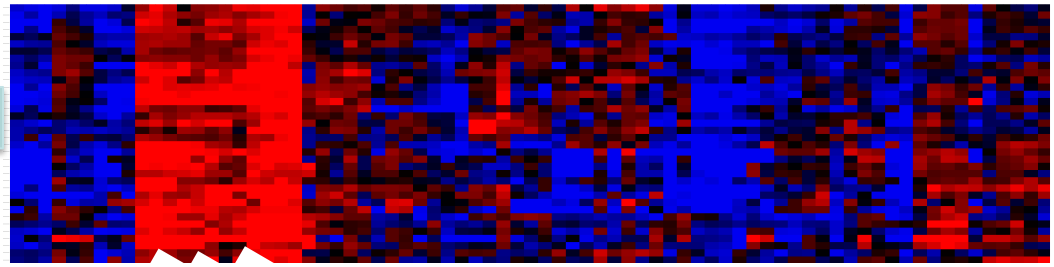
Example Approach:

Step 1. Start from a known key gene: i.e. causes human disorder in a relevant structure (e.g. Sall1)
Step 2. Identify good probe(s) for selected key gene
Step 3. Perform microarray dataset analysis to identify a "syn-expressed geneset" across a set of relevant samples
Step 4. Prioritize identified genes for evaluation of co-function with key gene, based on other knowledge e.g. pathways, functions

Example: **SALL1** --*Towne-Brockes syndrome:* anal and urethral abnormalities; mouse: expression in urethral mesenchyme, caudal hindgut, genital tubercle expression)

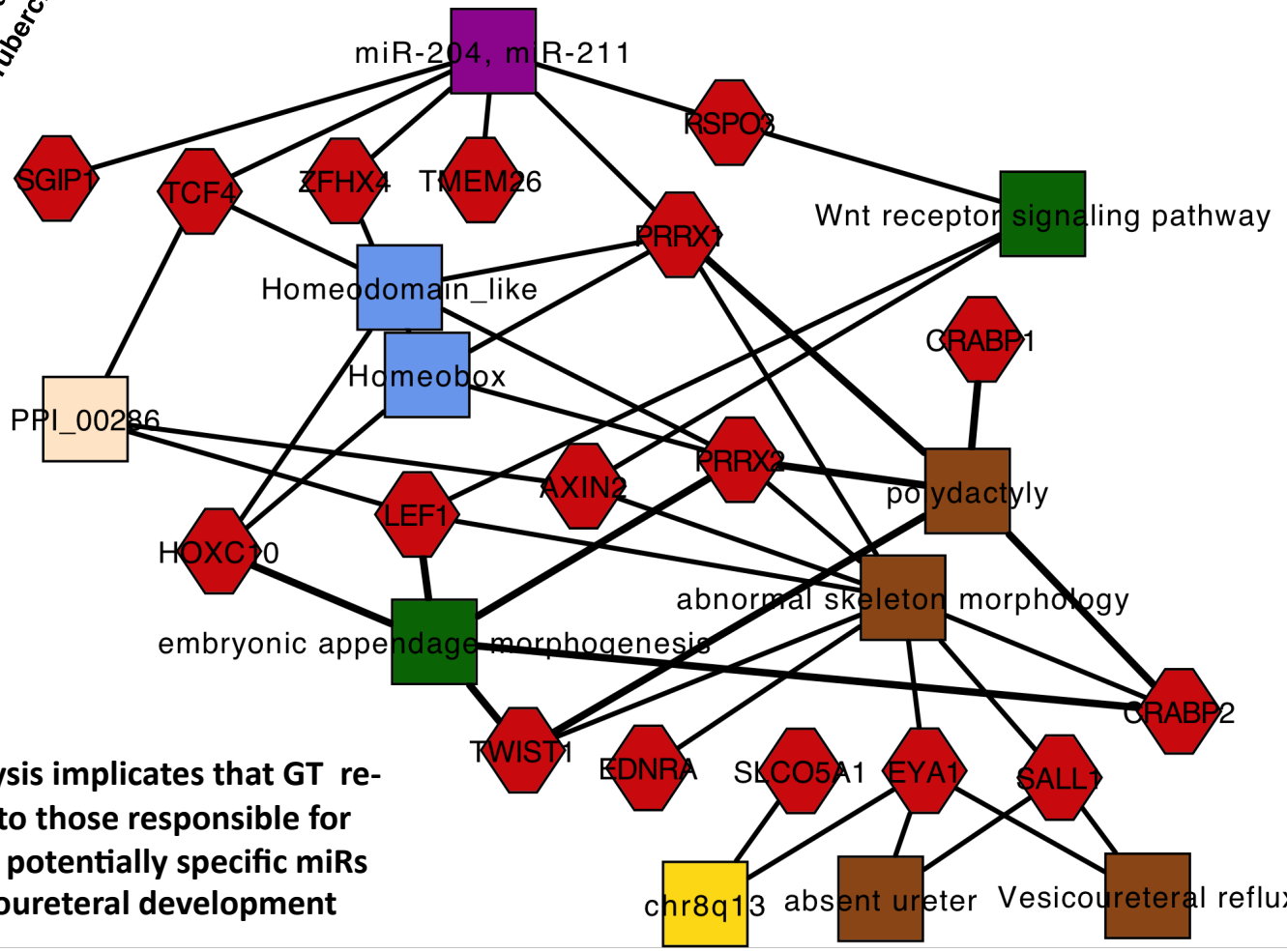


From GUDMAP array data: Sall1-correlated expression pattern



Pattern-match based identification of 36 genes

Sall1-correlated Gene Cluster: Network/ Interactome-based Enrichment Analysis



Sall1- correlated gene cluster analysis implicates that GT re-uses a Wnt/Hox program similar to those responsible for skeletal/limb morphogenesis, and potentially specific miRs may be used in ureter and vesicoureteral development

Diseasome Concept—use high-resolution expression data to expand known disease genes with observed genes that fit into categories of importance for development of organ-specific structure and function

Known Genes & Pathways

1: Define training set by making a list of all known kidney development-critical genes

2: Map to high quality array probesets

3: Cluster and analyze to identify gene Eigen patterns

5: Per pattern, use ISH from GUDMAP DB to define syn-expressing genesets

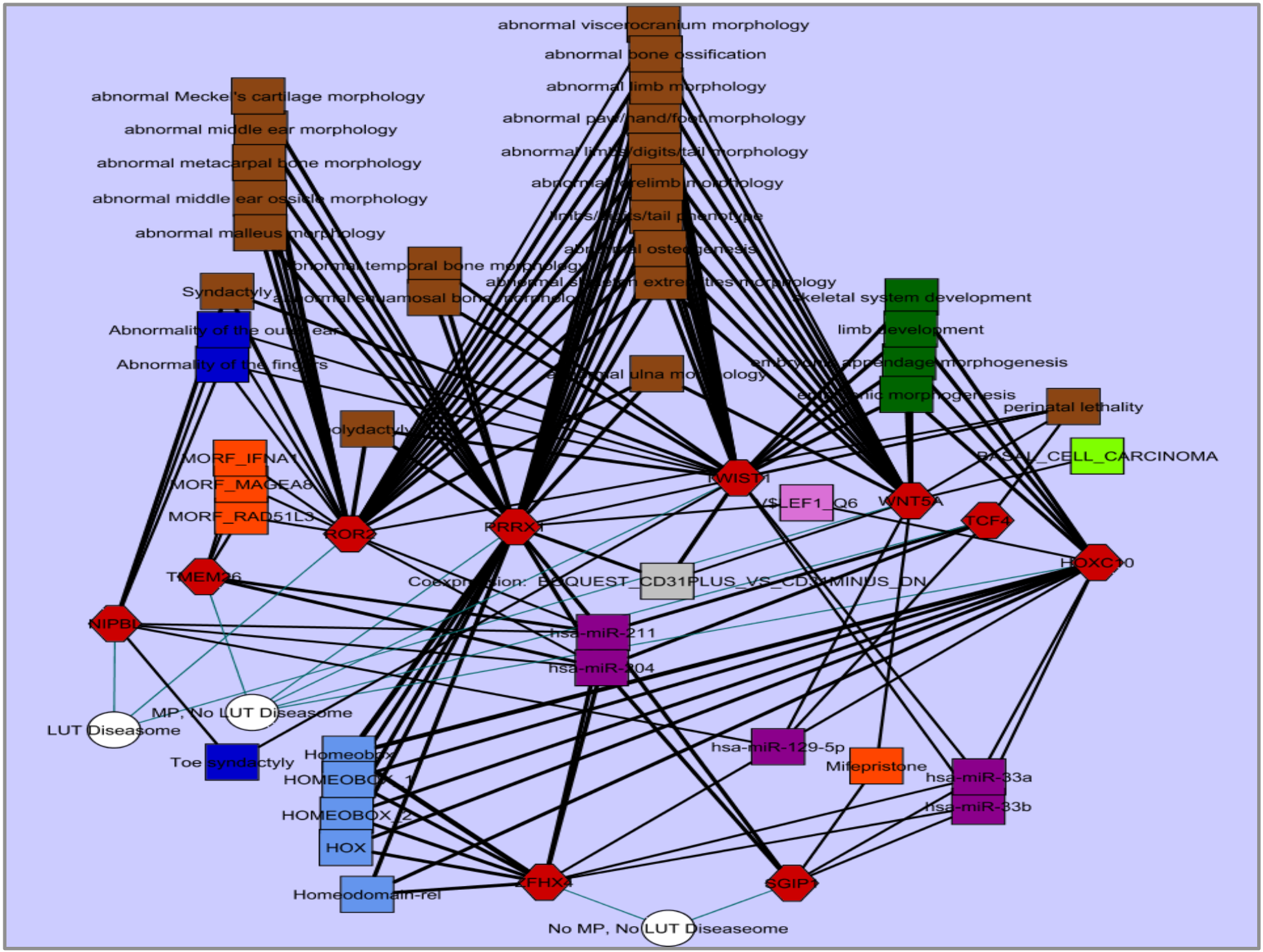
---

6: For each syn-expression gene pattern, expand geneset using microarray data (forms a candidate geneset)

8: Prioritize test set based on functional enrichment relative to training genes, pathways, function, etc..

9: Validate high ranking genes to expand molecular knowledge of developmental disease pathways.

New Genes & New/ Deeper Understanding of Pathways


abnormal viscerocranium morphology
abnormal bone ossification
abnormal limb morphology
abnormal paw/hand/foot morphology
abnormal limbs/digits/tail morphology
abnormal forelimb morphology
limbs/digits/tail phenotype
abnormal osteogenesis
abnormal skeletal system extremities morphology
abnormal Meckel's cartilage morphology
abnormal middle ear morphology
abnormal metacarpal bone morphology
abnormal middle ear ossicle morphology
abnormal malleus morphology
abnormal temporal bone morphology
abnormal squamosal bone morphology
abnormal ulna morphology
skeletal system development
limb development
embryonic appendage morphogenesis
epithelial morphogenesis
perinatal lethality
Syndactyly
Abnormality of the outer ear
Abnormality of the fingers
Polydactyly
MORF_IFNA1
MORF_MAGEA8
MORF_RAD51L3
ROR2
PRRX1
TWIST1
MYL_EF1_Q6
WNT5A
TCF4
BASAL_CELL_CARCINOMA
HOXC10
TMEM26
Coexpression: BIOQUEST_CD31PLUS_VS_CD31MINUS_DN
NIPBL
hsa-miR-211
hsa-miR-204
MP; No LUT Diseasome
LUT Diseasome
Toe syndactyly
Homeobox
HOMEOBOX_1
HOMEOBOX_2
HOX
Homeodomain-rel
hsa-miR-129-5p
Mifepristone
hsa-miR-33a
hsa-miR-33b
ZFHX4
SGIP1
No MP, No LUT Diseaseome

The GUMAP Kidney and Lower Urinary Team

Steve Potter
Eric Brunskill
Larry Patterson

Cincinnati Children's Hospital Medical Center

University of Cincinnati

Anil Jegga
Julia Jones
Jeremy Aronow
Vivek Kaimal
Scott Tabar
Eric Bardes

Andy McMahon
Todd Valerius
Jing Lu

Harvard

James Lessard
Alfor Lewis
John Szucik

Jamie Davies
Jane Armstrong
Jane Brennan
Chris Armit

The University of Edinburgh

Duncan Davidson
Derek Houghton
Mehran Sharghi
Xingjun Pi
Simon Harding

MRC Human Genetics Unit

The University Of Queensland
Australia

Melissa Little
Sean Grimmond
Kylie Georgas
Bree Rumballe

Vanderbilt University

Michelle Southard-Smith

Cathy Mendelsohn

Columbia University Medical Center

National Institutes of Health

Betsy Wilder
Deborah Hoshizaki