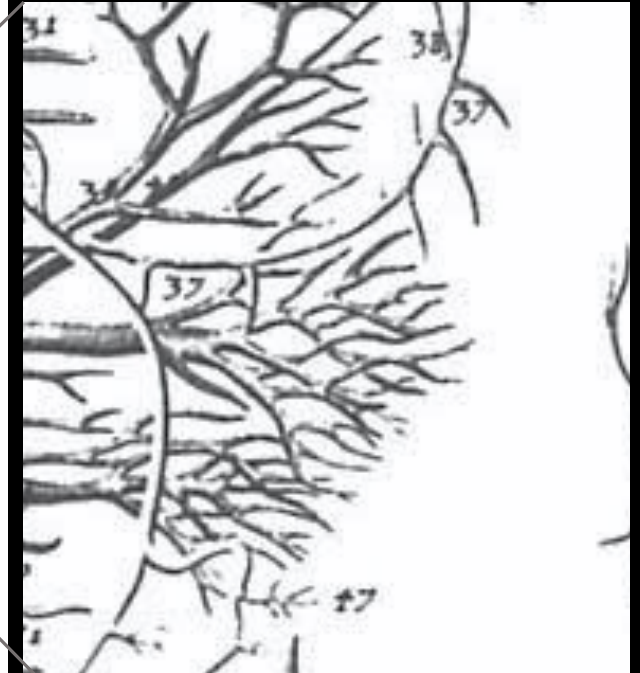
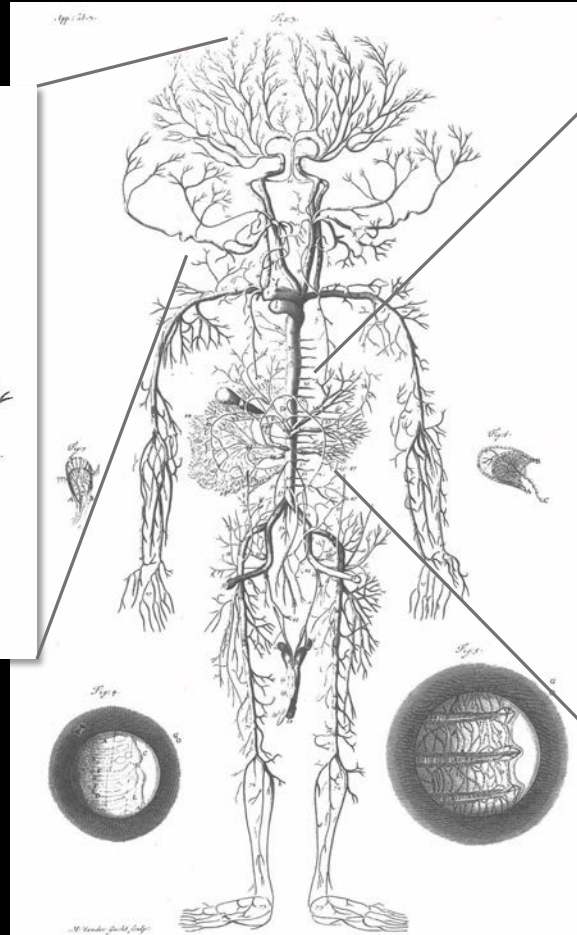
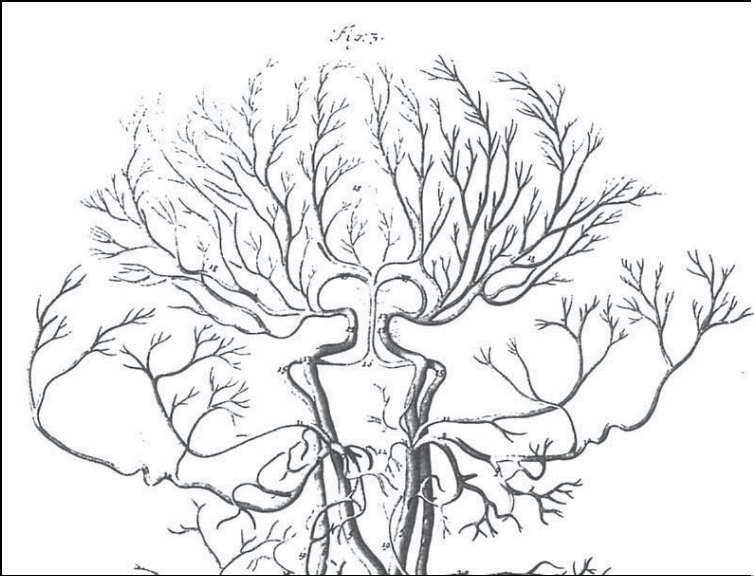


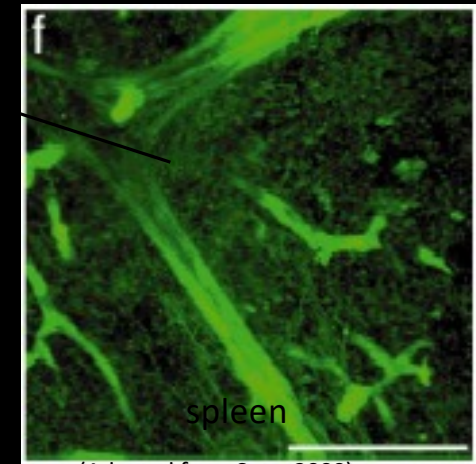
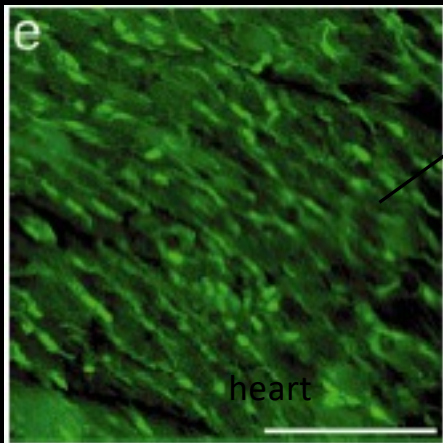
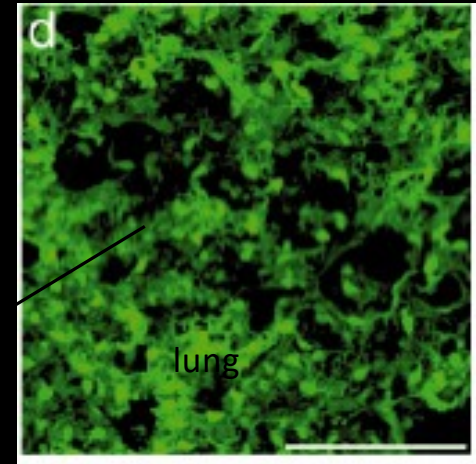
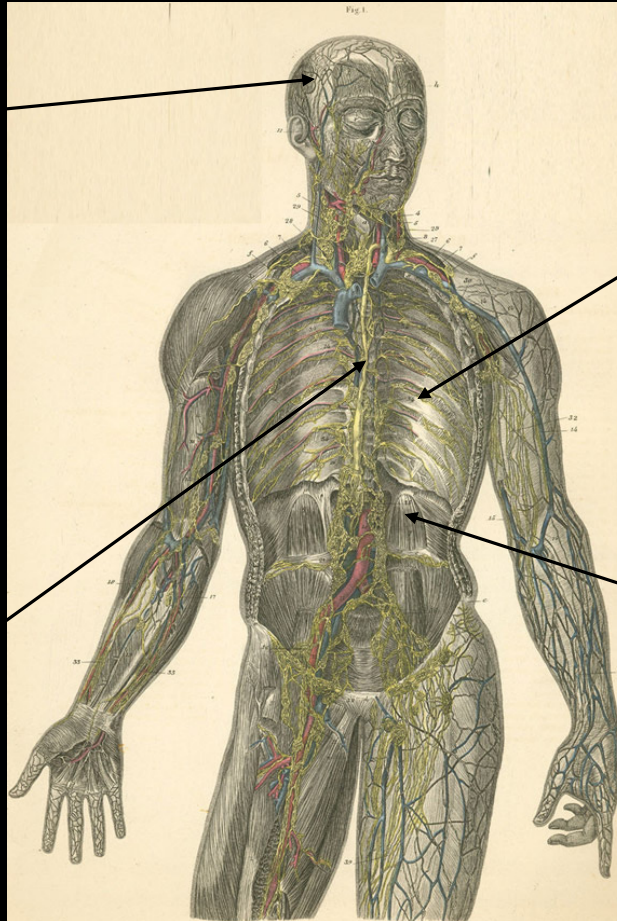
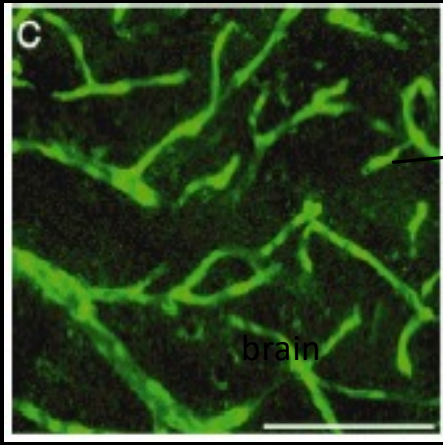
The Vasculome – Mapping Knowledge about Human Vasculature Across Body Scales



Ondine Cleaver, Dept Molecular Biology
Center for Regenerative Science and Medicine (CRSM),
UT Southwestern Medical Center

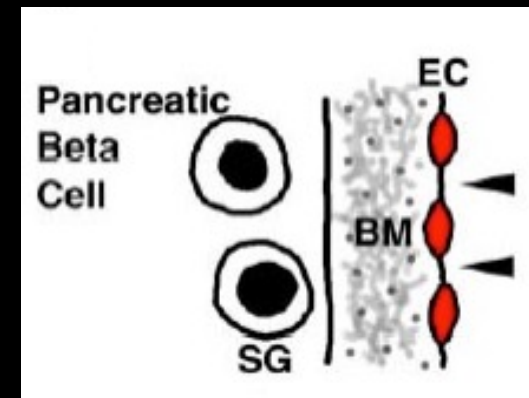
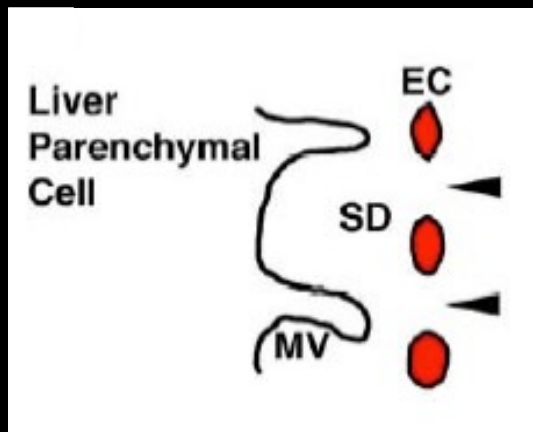
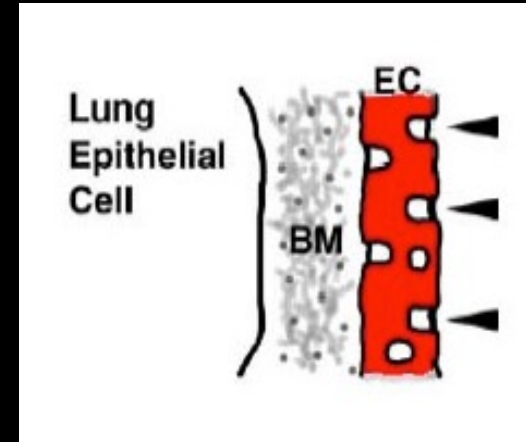
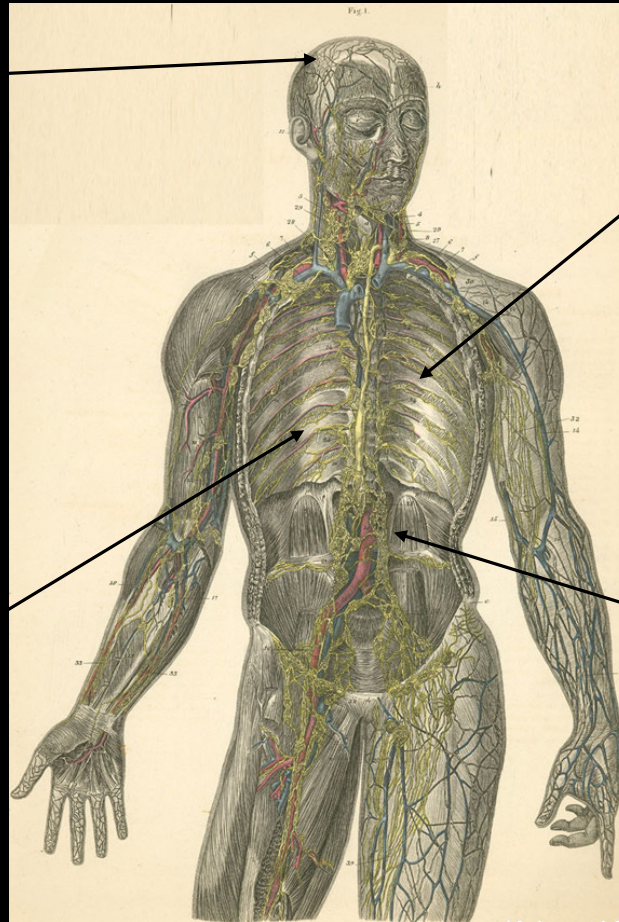
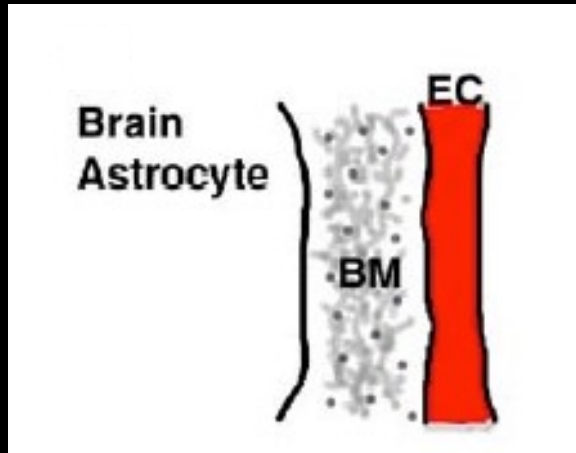
Mapping the Vasculome, NAVBO
August 31st, 2021

EC heterogeneity: Anatomy - vessel size and density



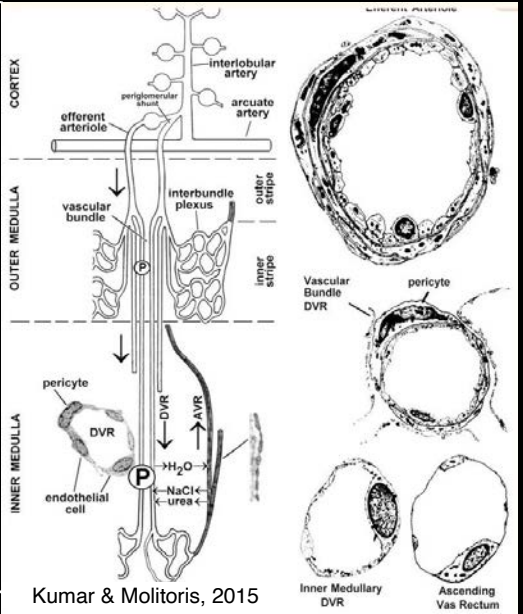
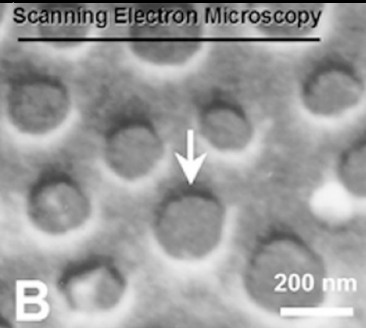
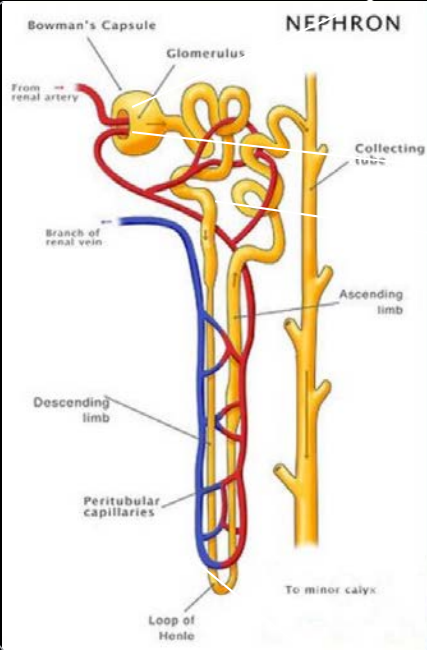
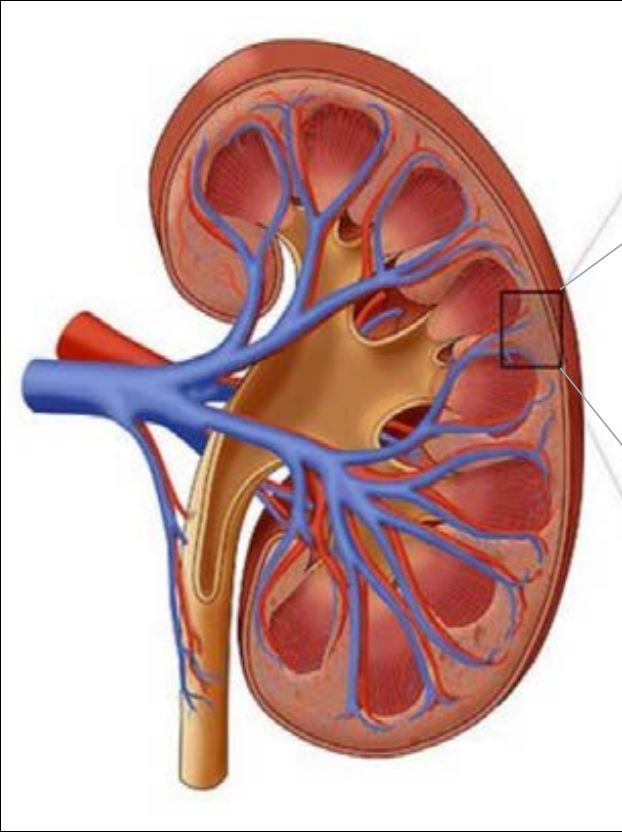
(Adapted from Sato, 2000)

EC heterogeneity: cellular ultrastructure



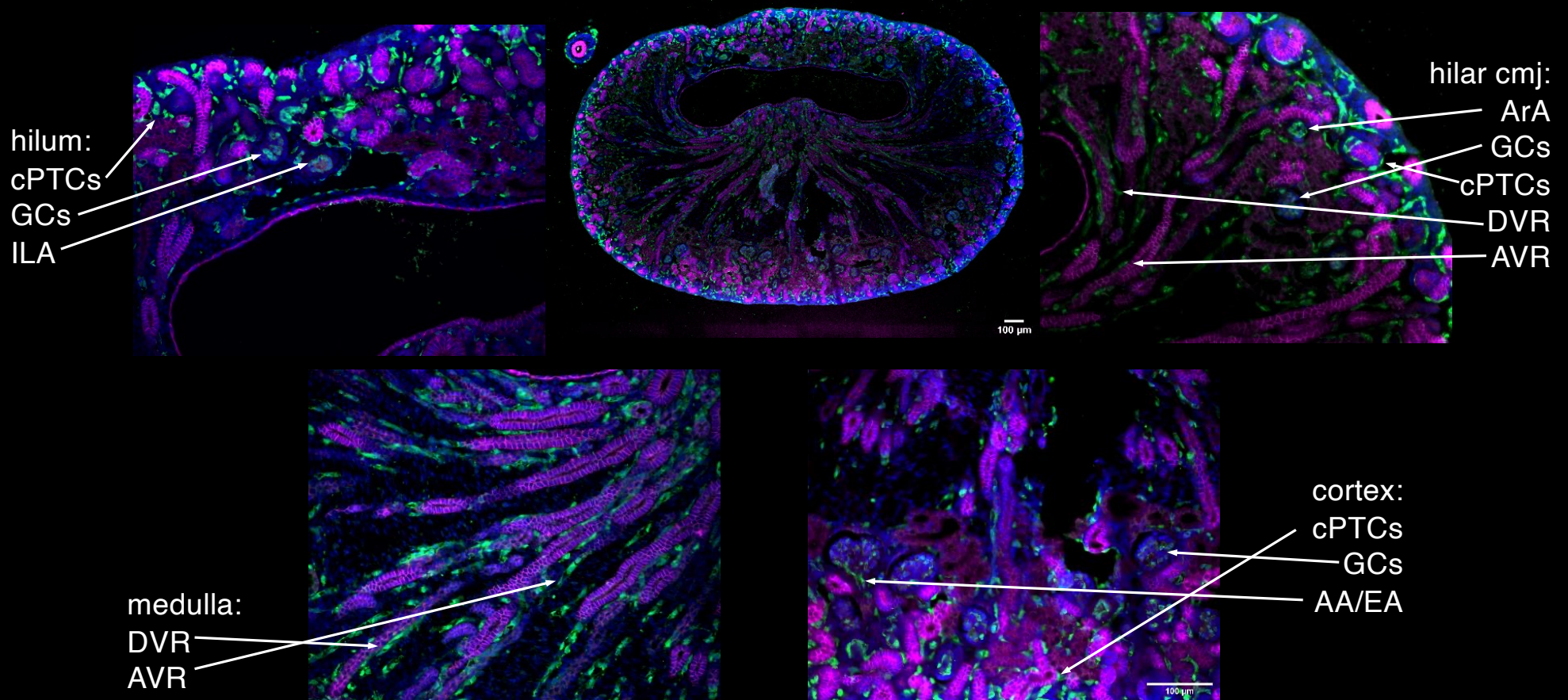
(Adapted from Nikolova and Lammert, 2003)

Organ 3D anatomy can be exceedingly complex



3D architecture is critical to function

Identification of vessel subtypes in the kidney



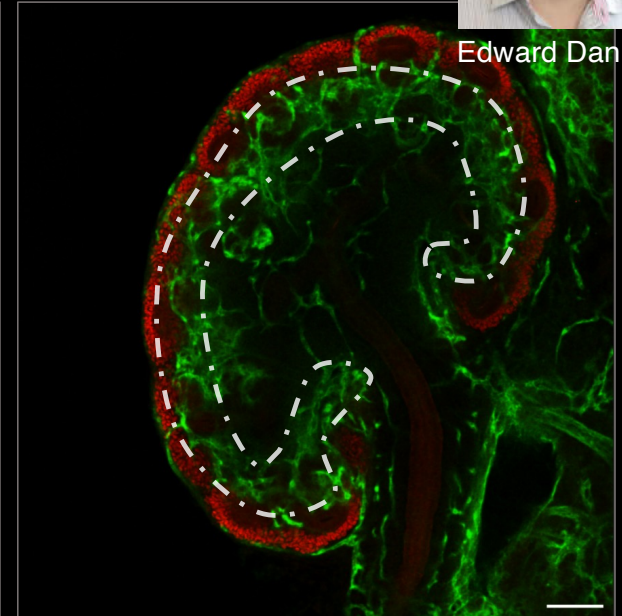
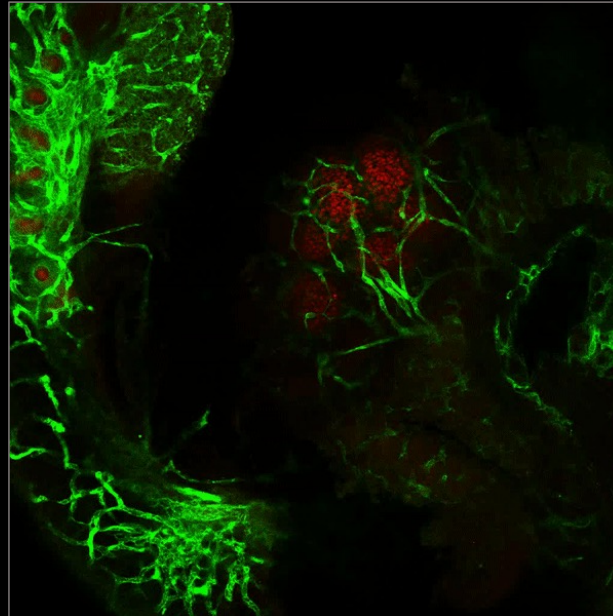
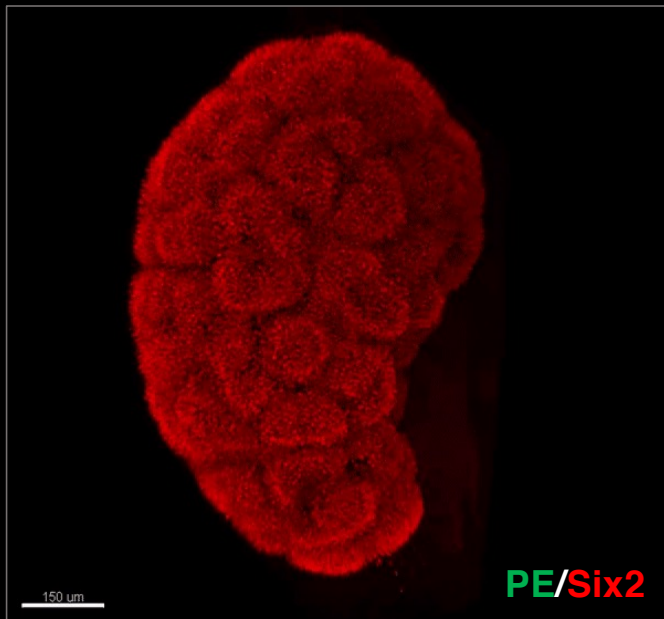
E18.5 Flk1-eGFP kidney eGFP Ecad DAPI IF

Endothelial cells form dense plexus next to progenitor cells (red) during kidney development



Edward Daniel

E13.5

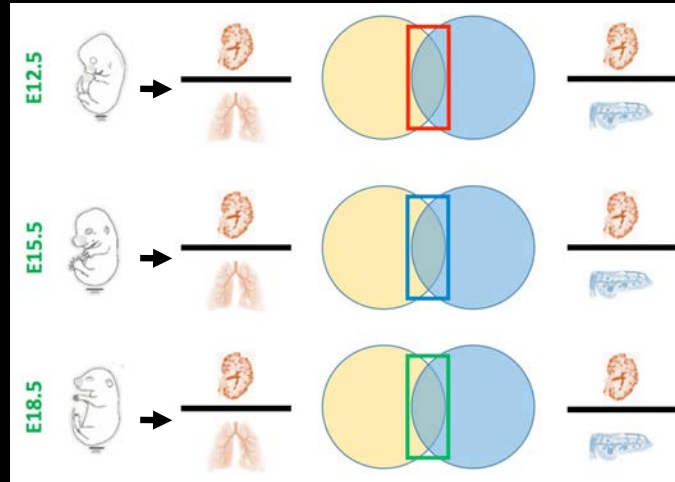
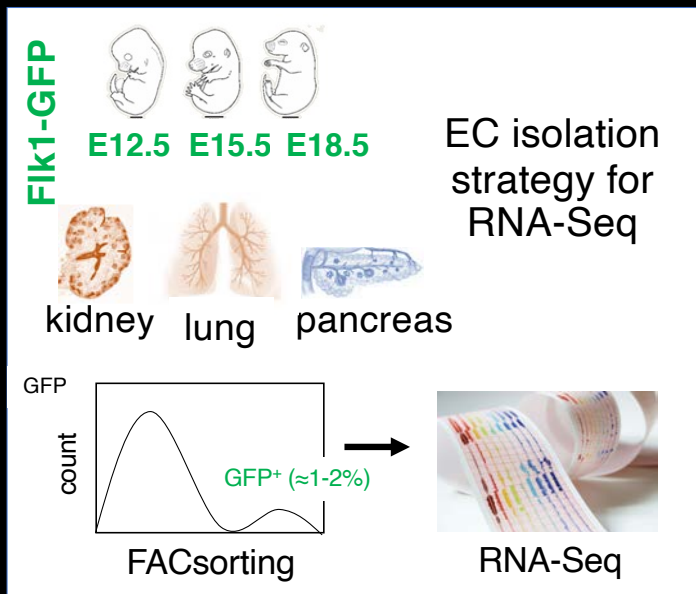


Identify kidney-enriched EC genes

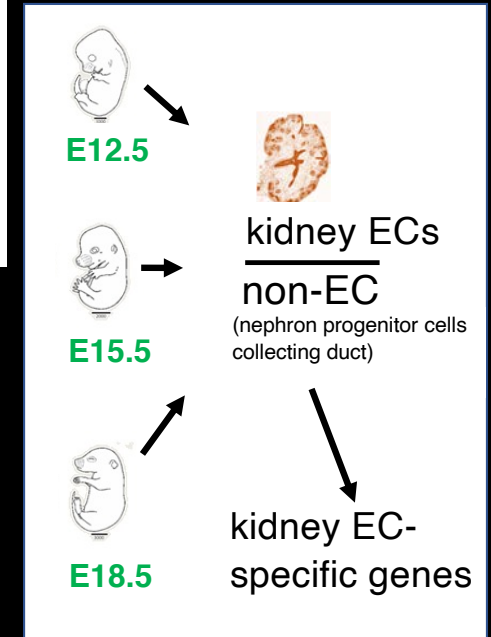
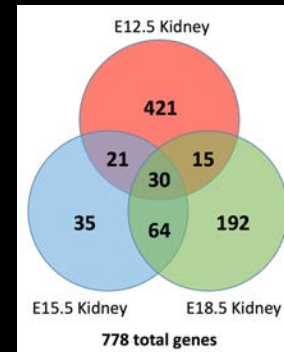
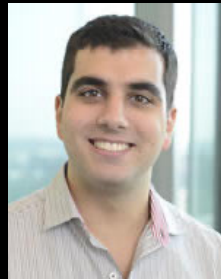
Berfin Azizoglu



Chris Chaney
(Carroll lab)

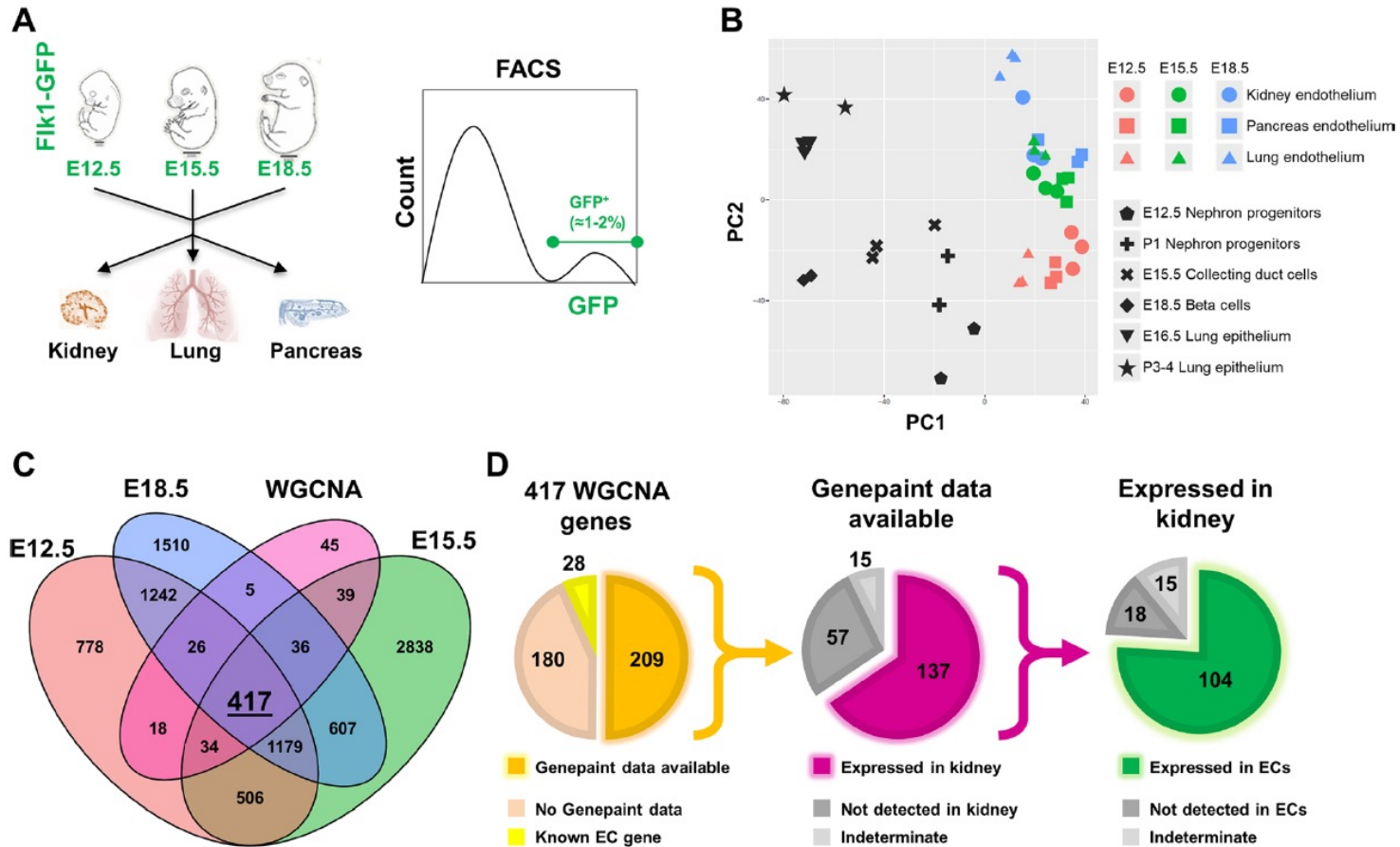


Edward



Transcriptional profiling of ECs by EC isolation, RNAseq & comparison

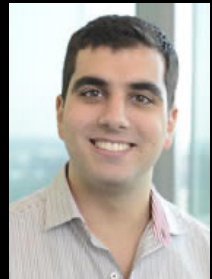
We isolated and analyzed the kidney endothelium



Chris Chaney (Carroll lab)



Edward Daniel

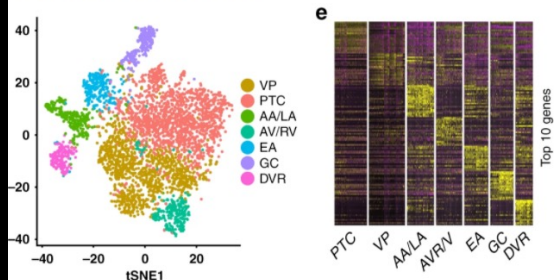


Recent papers on kidney vasculature

Molecular determinants of nephron vascular specialization in the kidney

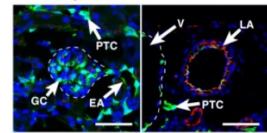
David M. Barry , Elizabeth A. McMillan, Balvir Kumar, Raphael Lis, Tuo Zhang, Tyler Lu, Edward Daniel, Masataka Yokoyama, Jesus M. Gomez-Saliner, Angara Sureshbabu, Ondine Cleaver, Annarita Di Lorenzo, Mary E. Choi, Jenny Xiang, David Redmond, Sina Y. Rabbany, Thangamani Muthukumar & Shahin Rafii 

Nature Communications 10, Article number: 5705 (2019) | [Cite this article](#)

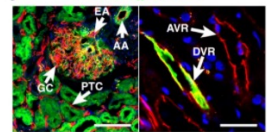


Less than 5% show expression by ISH

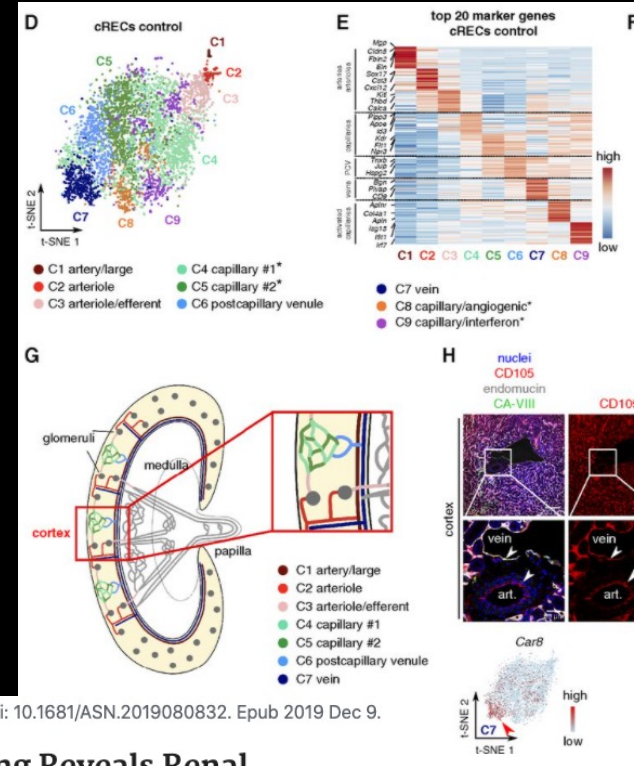
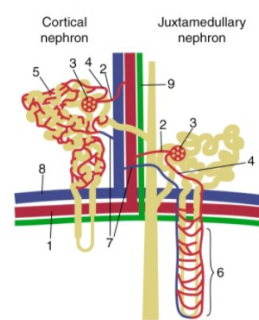
h *Gja5/Fik1-eGFP/DAPI*



j *Cdh5/Aqp1/DAPI*



o Populations identified



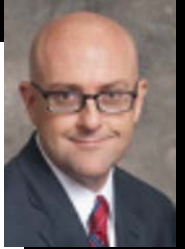
> *J Am Soc Nephrol.* 2020 Jan;31(1):118-138. doi: 10.1681/ASN.2019080832. Epub 2019 Dec 9.

Single-Cell RNA Sequencing Reveals Renal Endothelium Heterogeneity and Metabolic Adaptation to Water Deprivation

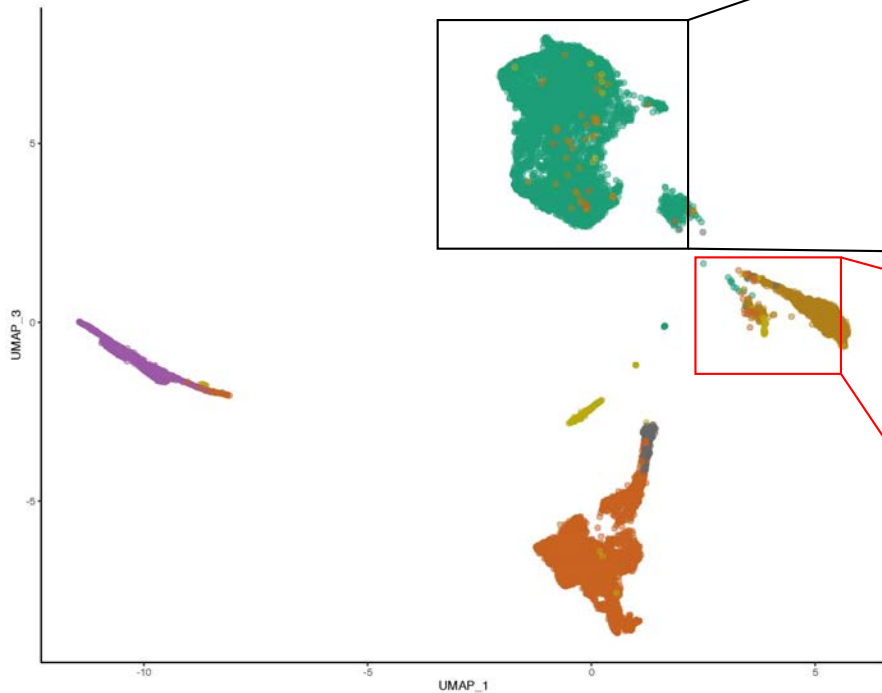
Sébastien J Dumas^{1 2}, Elda Meta^{1 2}, Mila Borri^{1 2}, Jermaine Goveia^{1 2}, Katerina Rohlenova^{1 2}, Nadine V Conchinha^{1 2}, Kim Falkenberg^{1 2}, Laure-Anne Teuwen^{1 2}, Laura de Rooij^{1 2}, Joanna Kalucka^{1 2}, Rongyuan Chen³, Shawez Khan^{1 2}, Federico Taverna^{1 2}, Weisi Lu³, Magdalena Parys^{1 2}, Carla De Legher^{1 2}, Stefan Vinckier^{1 2}, Tobias K Karakach^{1 2}, Luc Schoonjans^{1 2 3}, Lin Lin^{4 5}, Lars Bolund^{4 5}, Mieke Dewerchin^{1 2}, Guy Eelen^{1 2}, Ton J Rabelink⁶, Xuri Li⁷, Yonglun Luo^{8 5 9 10}, Peter Carmeliet^{11 2 3}

The kidney stroma (which includes ECs) is very heterogeneous

Chris Chaney
(Carroll lab)

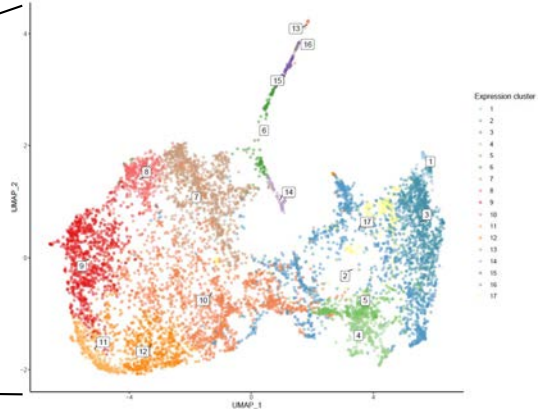


Cell clusters in E18.5 mouse kidney

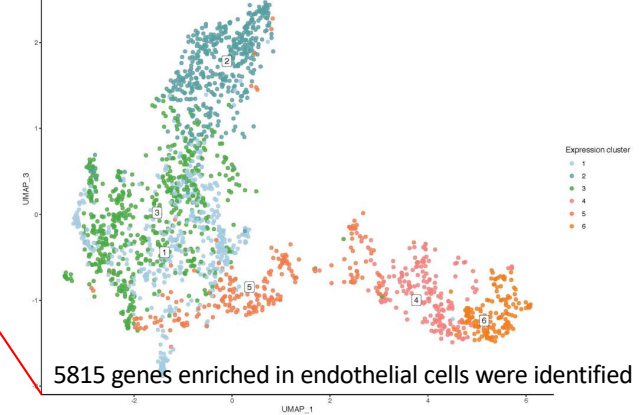


- Cell type
- Endothelium
 - Epithelium
 - Erythrocyte
 - Interstitial
 - Leukocyte
 - Podocyte

Interstitial re-clustered



Endothelium re-clustered



Data sourcing and analysis pipeline



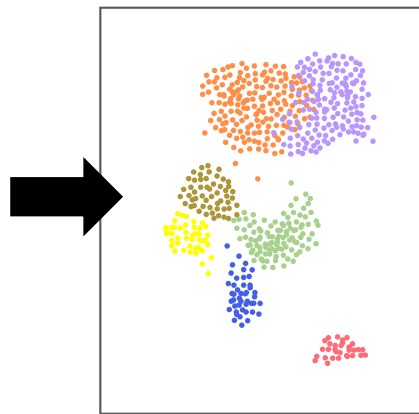
Annie Ryan



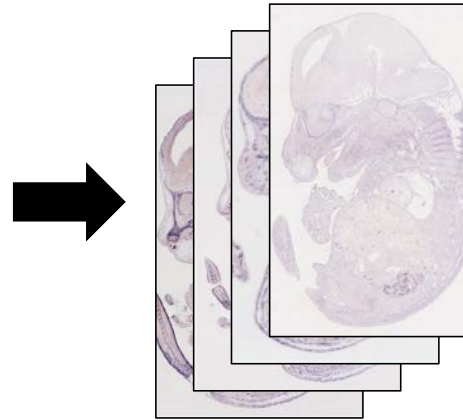
Neha Ahuja

RNA seq data
compilation

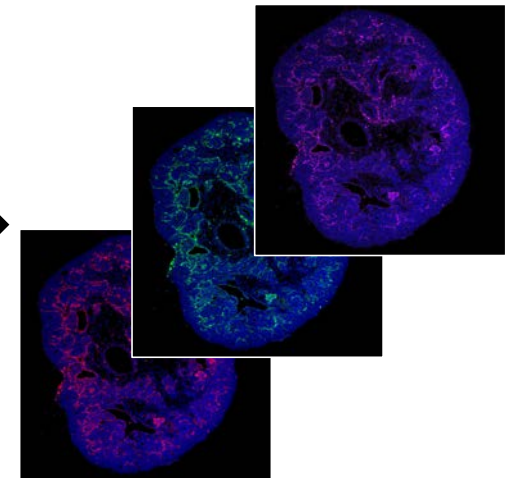
Kidney ECs



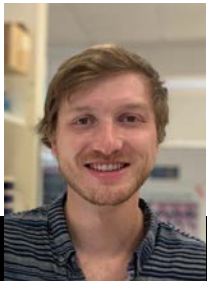
in silico analysis



Genepaint,
online database review
and our in situs



FISH Validation



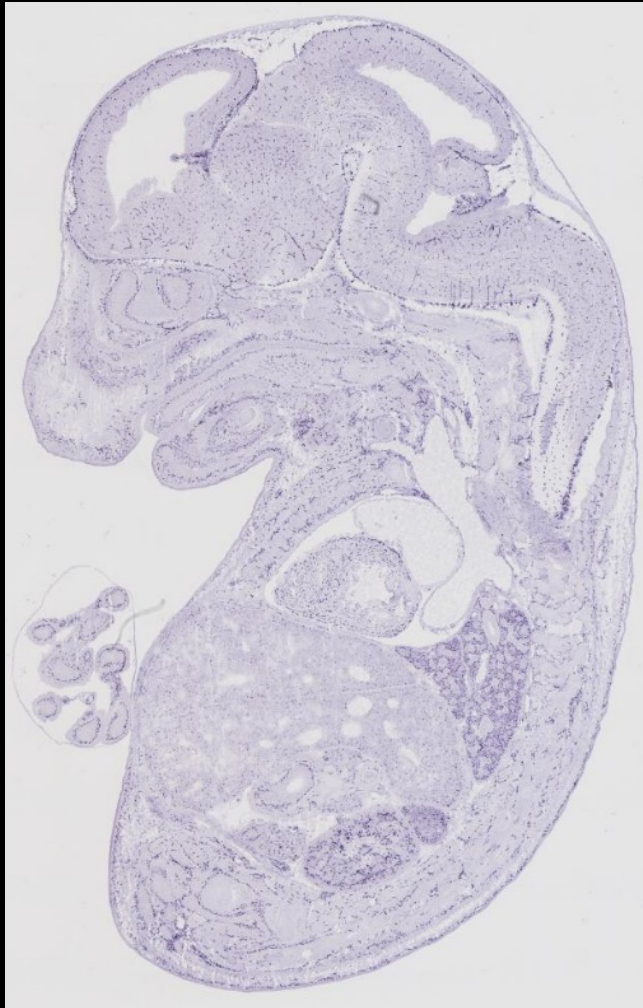
Max Hiltabidle



Peter Luo

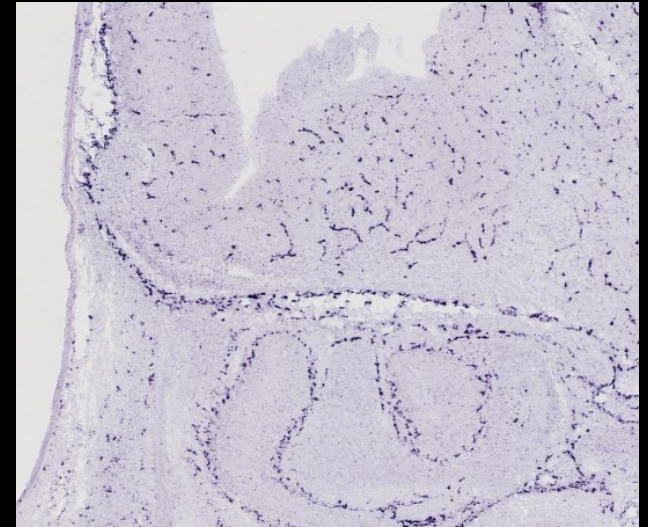
In situ hybridization

Whole embryo

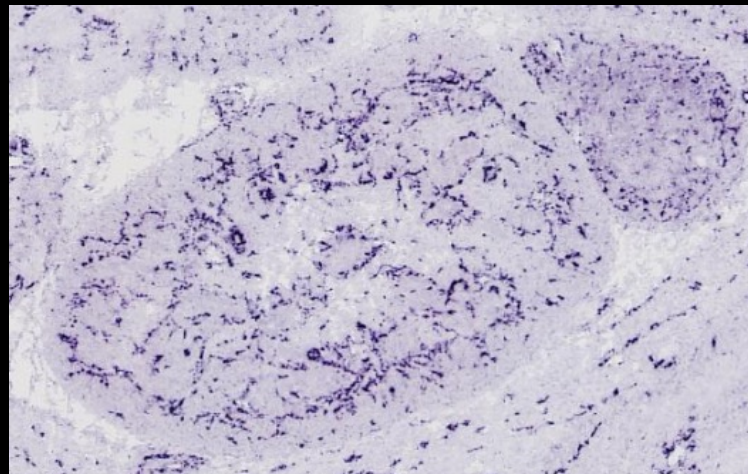


Probe for VEGFR2

Head



Kidney



Closeup



We must VALIDATE our gene lists

Search: - T1 x Genepaint x Single Cell x Tabula Mur x Gene: RAS x KIT | Result x KIT | Result x https://cell x A single-cell x REC dehyd x ISH Data x

proteinatlas.org/search

THE HUMAN PROTEIN ATLAS

MENU HELP NEWS

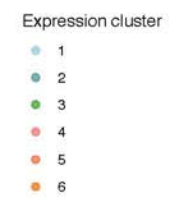
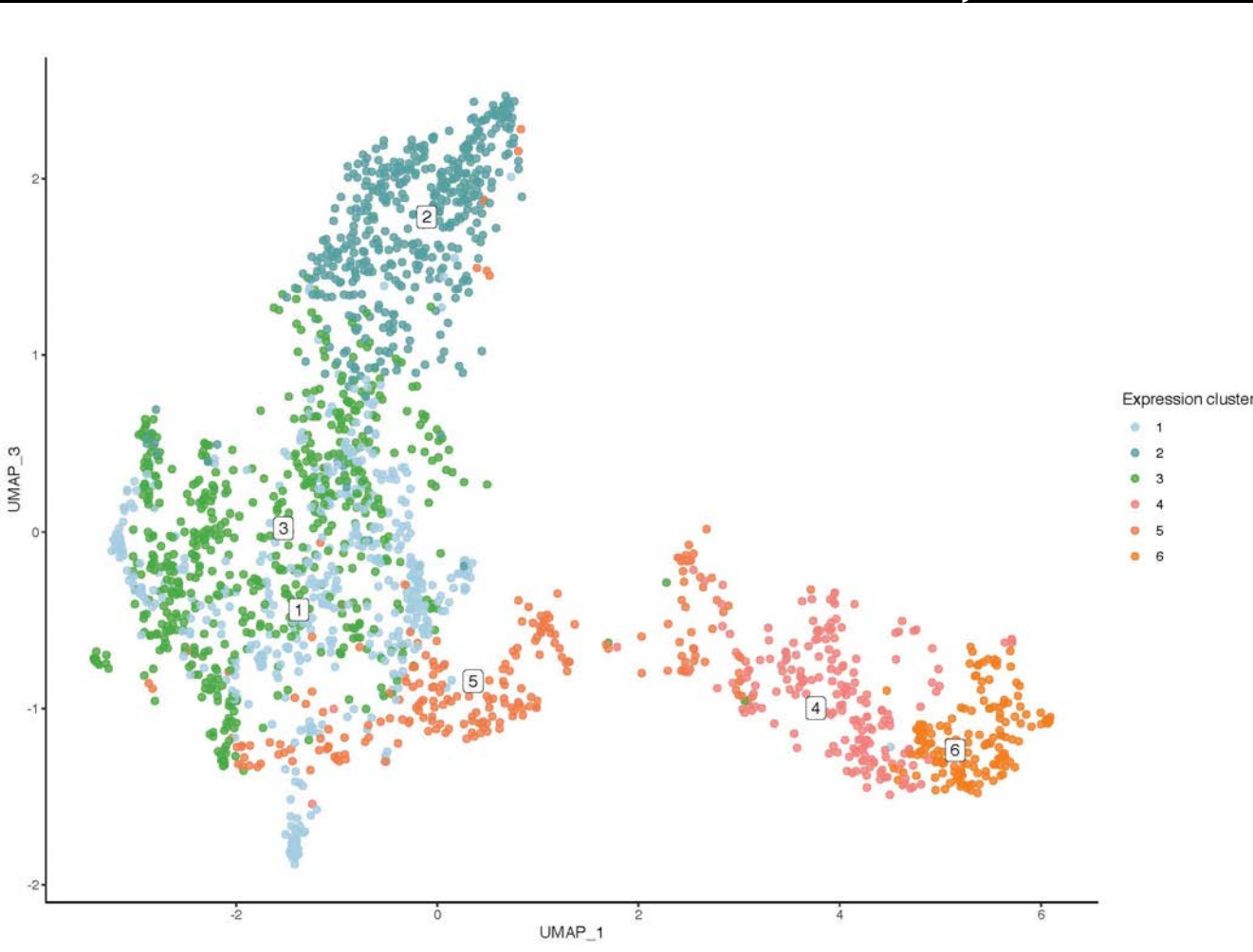
19670 GENES FOUND¹

Show / hide columns v Download: XML | RDF | TSV | JSON | Custom TSV/JSON v Page 1 of 394 | next >

Gene ¹	Gene description ¹	Evidence ¹ x	Tissue ¹	Cell type ¹	Pathology ¹	Brain ¹	Blood ¹	Cell ¹
A1BG	Alpha-1-B glycoprotein	■		RNA		RNA	RNA	RNA
A1CF	APOBEC1 complementation factor	■		RNA		RNA	RNA	
A2M	Alpha-2-macroglobulin	■		RNA		RNA	RNA	RNA
A2ML1	Alpha-2-macroglobulin like 1	■		RNA		RNA	RNA	RNA
A3GALT2	Alpha 1,3-galactosyltransferase 2	■	RNA	RNA	RNA	RNA	RNA	RNA
A4GALT	Alpha 1,4-galactosyltransferase (P blood group)	■	RNA	RNA	RNA	RNA	RNA	
A4GNT	Alpha-1,4-N-acetylglucosaminyltransferase	■		RNA		RNA	RNA	RNA
AAAS	Aladin WD repeat nucleoporin	■		RNA		RNA	RNA	

How much of what we find is useful, or even real?

A		
	cluster.1	
2	Gpc3	dark all over
3	Col1a2	valve, kidney stro
4	Col3a1	kidney strom
5	Lsp1	kidney strom, all
6	Tcf21	kidney strom
7	Col1a1	valve, kidney stro
8	Mdk	all over, kidney e
9	Col6a1	valve, all over
10	Ptn	valve, lots of plac
11	Meg3	dark all over
12	Mfap4	valve,
13	Tpm2	low
14	Acta2	dark all over
15	Lgals1	valve, lots of plac
16	Vstm4	low
17	Ncam1	gloms
18	Cdh11	lots of places
19	Hoxd11	low, ubiquitous
20	Basp1	nig, microglia by l
21	Ogn	kidney strom, EC,
22	Serping1	kidney capsule,
23	6330403K	Valve
24	Ifitm1	thymus, B-pericyt
25	Lhfp	low, B-pericyte,
26	Myl9	aorta, B-SMC
27	Cfh	kidney strom, all
28	Col14a1	all over, B-smc, TI
29	Alcam	B-fibroblast
30	Deaf1	nig, TM-epithel



H		
		ut+NT, B-EC
		rer, artery ECs, B-smc+aEC, TM-EC
		1 kidney arteries, TM-EC
		rer, B-all over
		3-EC, gumap-epithelium
		lood?, B-EC, TM-EC, gumap-EC
		: EC specific!, TM-EC
		rer, B-EC, TM-all over
		/artery EC, many places, gumap-epi
		rer
		all over, brain EC, B-EC+pericyte, TM-
		bone, B-EC+SMC
		rer
		t/brain, gut ECs, B-all over
		kidney epithelium, TM-EC
		rer, B-EC, TM-EC+epithe, gumap-Glon
		Ecs, all over (by TM also)
		rer
		rer, B-EC, Allen-all over
		ey epith, liver, TM-EC+epithelium
		rer
		all over
		rer, B-EC/astrocyte, TM all over
		: EC, neural tube, TM-EC+fibroblast
		pericyte

Gene validation

ISH, FISH, IF

Tabula Muris

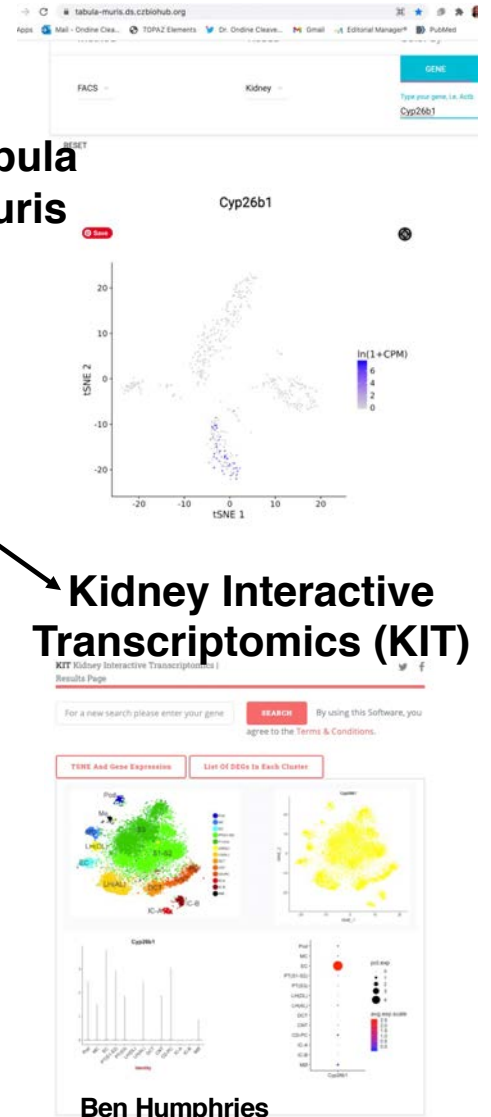
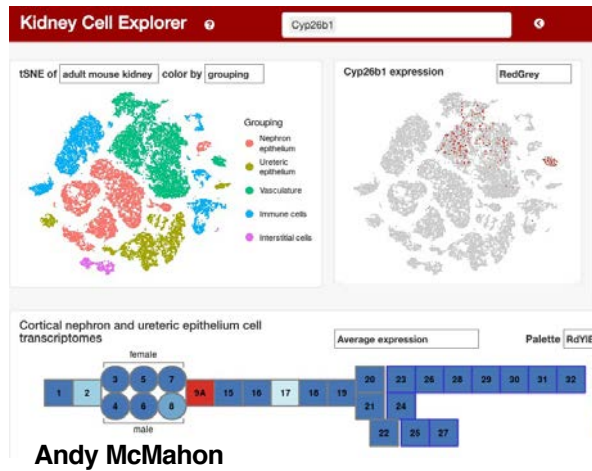
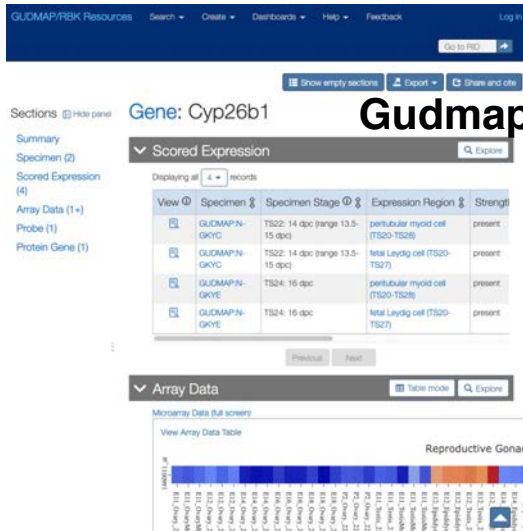
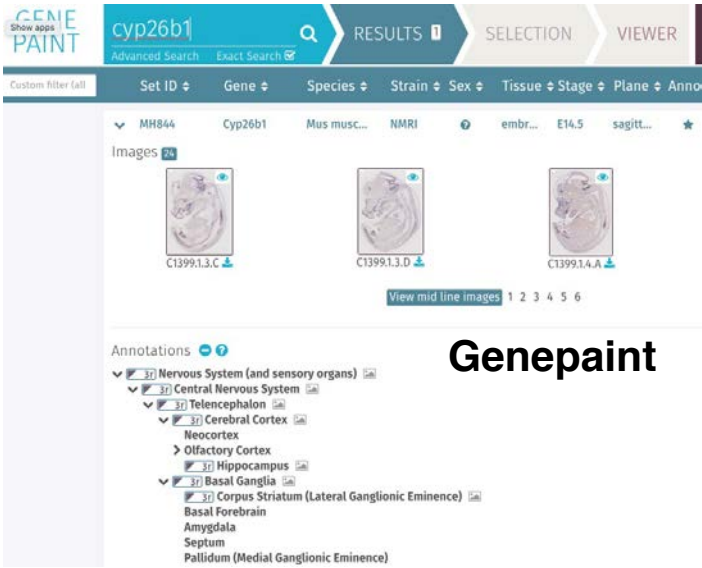
Gene of interest

Kidney Cell Explorer (KCE)

Kidney Interactive Transcriptomics (KIT)

Genepaint

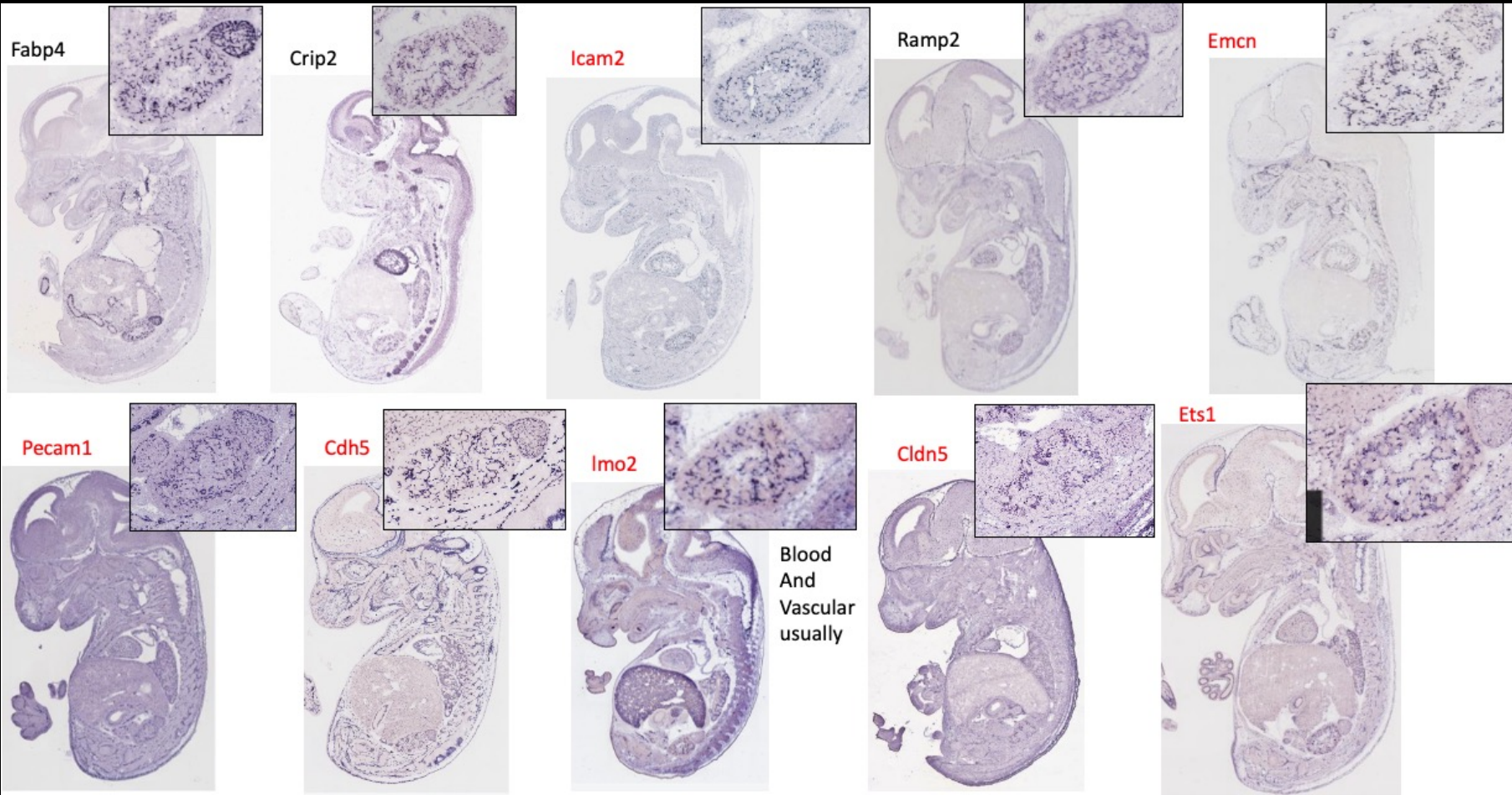
Gudmap/RBK



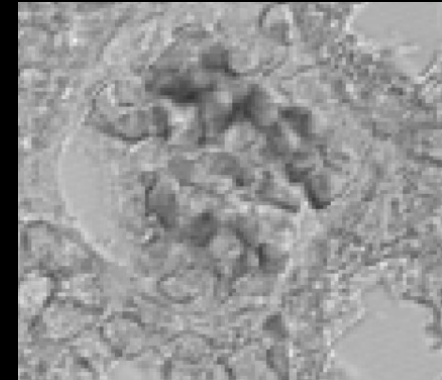
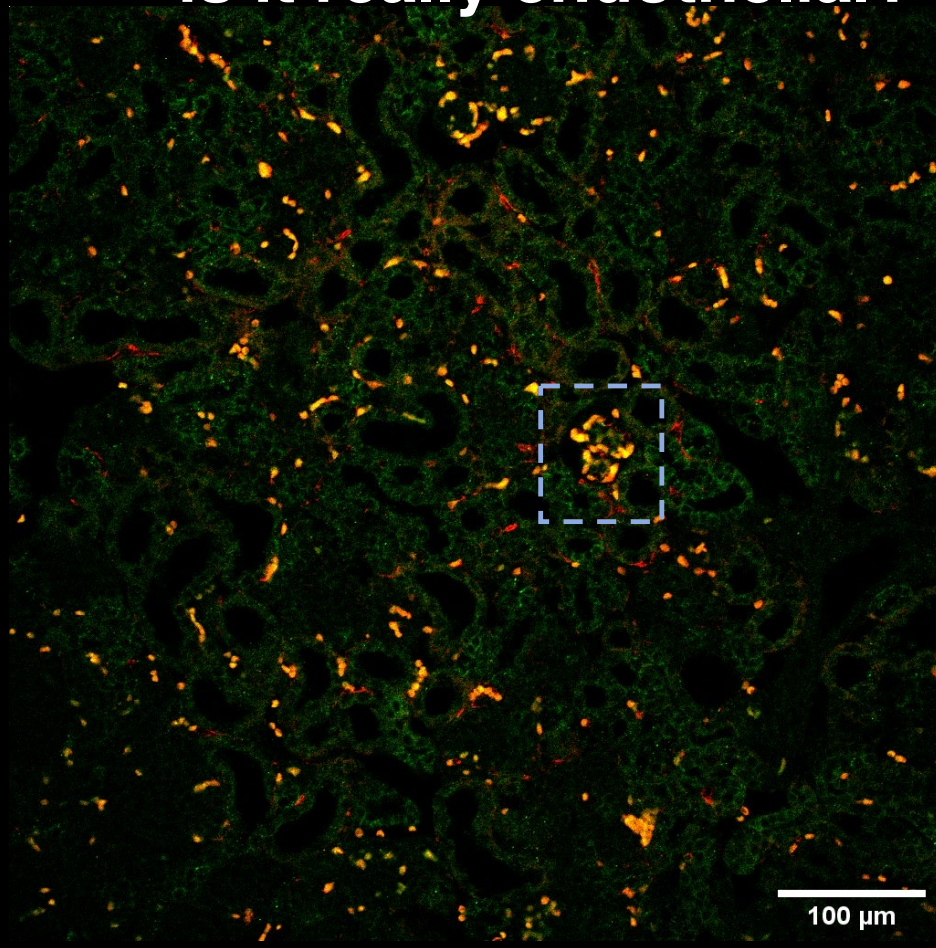
Transcriptomic Index based on multiple website analysis

	A	B	C	D	E	F	G	H	I	J	K	L
1	Gene	genepaint	KEC	KIT	Gudmap	Betsholtz	Tabula Muris	Allen Brain A	Human Protein Atla	Single Cell Mouse		Index
2	Ramp2	2	1	2.7	2.1	1	2	1	1	2		1.64
3	Plvap	2.8	1	1	1.1	2.9	1	na		2.4	1.4	1.71
4	Igf2r	2.5	2	1.8	2.5	2.9	2.5			2.7	2.7	2.45
5	Ppfbp1	2.9	1.5	2	2.5	1.8	1			2.5	2.7	2.11
6	Rnf130	na	2.5	2.9	2.5	2.5	2.6			2.8	2.6	2.63
7	Tm6sf1	na	2.5	2.3	3	2.1	2.7			2.8	2	2.49
8	Lpp	2.5	2.7	2.8	2.3	2.7	1.8			2.8	2.3	2.49
9	Ifit1	3	1	1.3	2.6	1.8	1.3			2.5	3	2.06
10	Ctso	2.9	2.1	2.7	2.6	2.2	2			2.7	2.8	2.5
11	Lfng	na	2.1	1.3	2.2	2.6	2.5			2.8	2.7	2.31
12	Itpr1	3	2.8	2.9	2.8	2.8	2.7			2.8	2.6	2.8
13	Stx3	3.5	2.7	2.8	2.9	1	2.9			2.9	2.8	2.69
14	Stox2	2.9	1.8	2.9	2.5	2.7	2			2.5	2	2.19
15	Filip1	2.9	2.5	1.5	2.5	2.1	1			2.8	2.2	2.19
16	Oasl2	4	1.5	1.3	na	1	2		na		2	1.97
17	Plau	2.8	2.8	2.8	2.1	3	2.6			2.9	1.5	2.56
18	Kank3	3.5	1.3	1.3	1.1	1	1			1.3	1.1	1.45
19	Tmem252	3.5	2.1	2.8	4	1	na			4	1.4	2.69
20	Herpud1	2.8	2.3	2.8	2.7	2.3	2.5			2.8	2.8	2.63
21	Gbp7	4	1	1.3	na	1	1			2.6	2	1.84
22	Tgm2	3.7	1.3	2.3	2.1	1.8	1.7			2.5	2.2	2.2
23	Tmem37	3.7	2.9	2.9	2.4	2.8	2.9			2.9	1.3	2.73
24	Exoc3l4	2.2	1	2.9	3	2.9	1			3	1	2.13
25	Mapk3	2.7	1.7	2.2	2.2	2.2	1.8			2.8	1.3	2.11
26	Kras	na	2.5	2.9	2.4	1.2	2.6			2.6	2.4	2.37
27	Picalm	2.7	2.6	2.9	2	2.5	2.3			2.3	2.7	2.5
28	Lama5	3.5	2.6	2.9	2.3	2.5	1.4			2.5	2.6	2.53
29	Fabp4	2	1	1.1	2.7	2.8	1			2.6	2.6	1.98
30	Grasp	na	1.7	1.1	2.1	1	1.2			2.4	1.4	1.56
31	Agri	3	2.5	2.9	2	2	1.5			2.8	2.6	2.41
32	Ankrd37	na	2	2.6	2.7	1	2.6			2.8	2.6	2.33

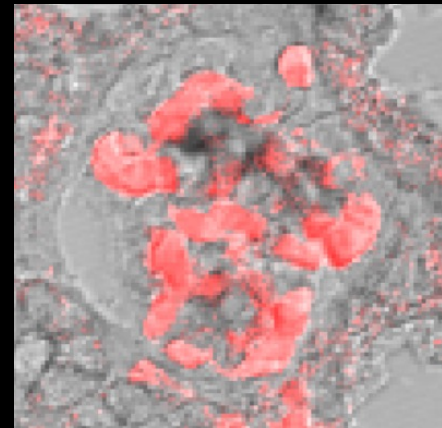
Lots of known and novel EC genes



Even if appears to be a nice vascular signal,
is it really endothelial?



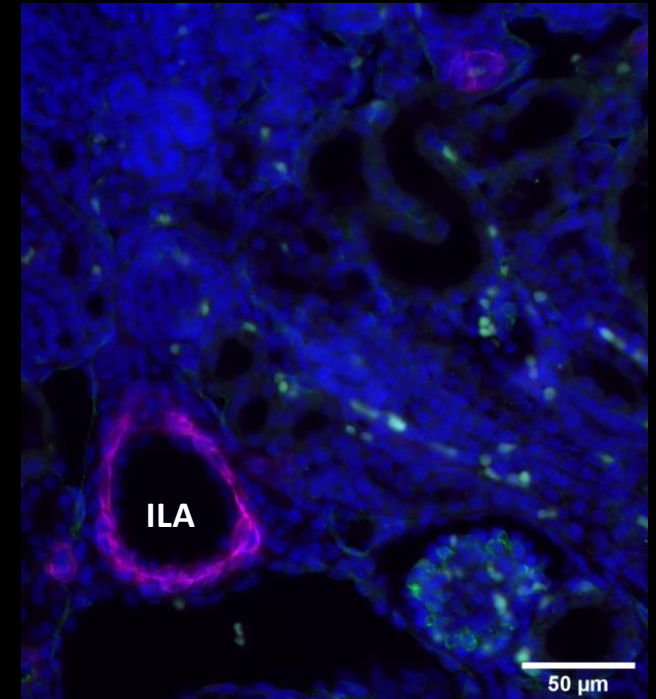
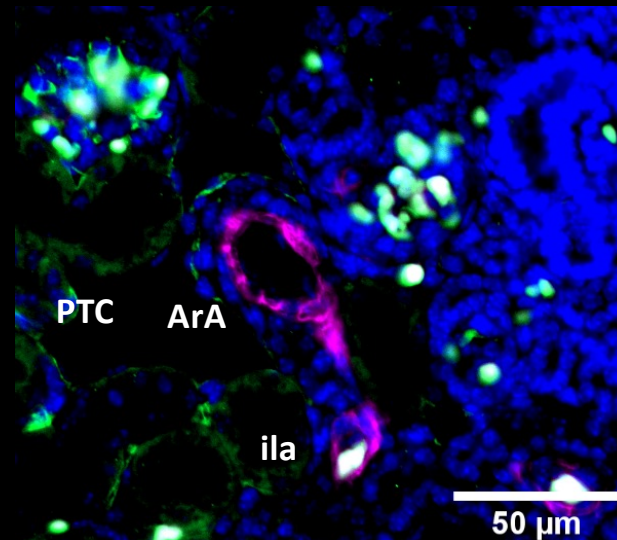
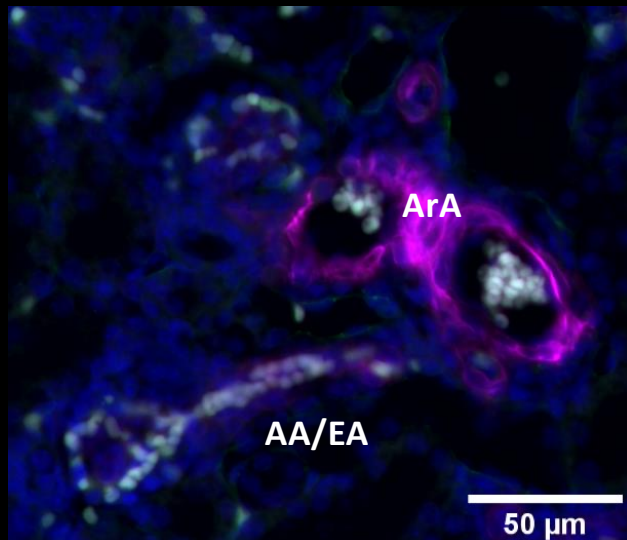
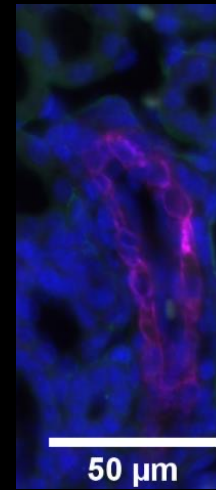
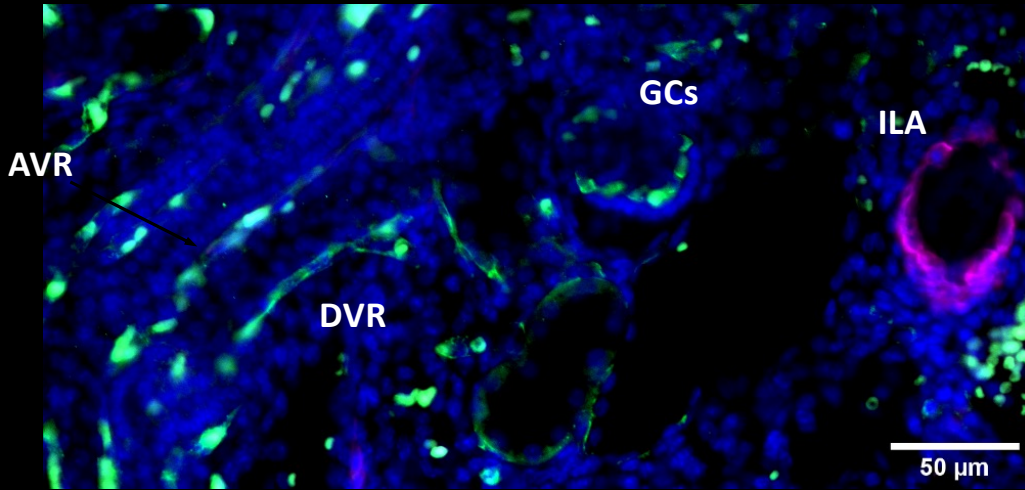
ISH



Ab

Ecad endothelial over Epas1 ISH (20x)

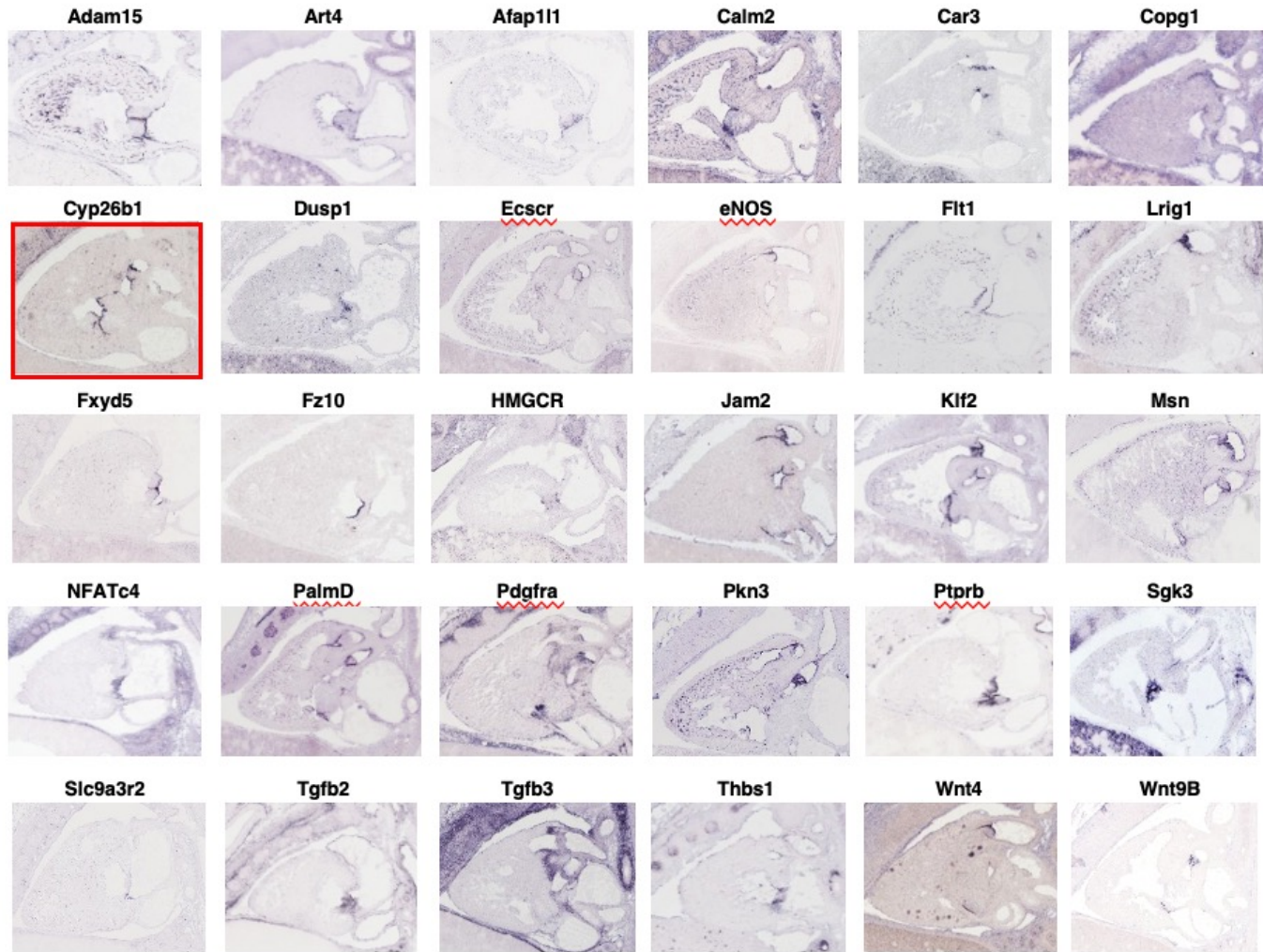
Epas1 is found in arterial endothelial cells



E18.5 WT kidney PIEE Epas1 FISH DAPI IF

Heterogeneity of the valve vasculature

Valve endothelial expression



Papers on single cell RNAseq in ECs

Original Paper | [Open Access](#) | Published: 01 June 2021

Endothelial cell plasticity at the single-cell level

[Alessandra Pasut](#), [Lisa M. Becker](#), [Anne Cuypers](#) & [Peter Carmeliet](#) ✉

Angiogenesis **24**, 311–326 (2021) | [Cite this article](#)

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Cell Reports

Volume 26, Issue 7, 12 February 2019, Pages 1934–1950.e5



Resource

Single-Cell Transcriptome Analysis Maps the Developmental Track of the Human Heart

Yueli Cui^{1,3,6}, Yuxuan Zheng^{1,3,5,6}, Xixi Liu^{1,2,3,4,6}, Liying Yan^{1,2,4}, Xiaoying Fan^{1,3}, Jun Yong^{1,2,4}, Yuqiong Hu^{1,3,5}, Ji Dong^{1,3}, Qingqing Li^{1,3}, Xinglong Wu^{1,3,5}, Shuai Gao^{1,3}, Jingyun Li^{1,3,5}, Lu Wen^{1,3}, Jie Qiao^{1,2,4,5,6,8}, Fuchou Tang^{1,3,5,7,8}

PLoS One. 2010; 5(8): e12034.

PMCID: PMC2919381

Published online 2010 Aug 10. doi: [10.1371/journal.pone.0012034](https://doi.org/10.1371/journal.pone.0012034)

PMID: [20706631](https://pubmed.ncbi.nlm.nih.gov/20706631/)

Gene Expression Programs of Mouse Endothelial Cells in Kidney Development and Disease

[Eric W. Brunskill](#) and [S. Steven Potter](#) *

RESEARCH ARTICLE | 14 JUNE 2019

Single cell expression analysis reveals anatomical and cell cycle-dependent transcriptional shifts during heart development **FREE**

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Development (2019) 146 (12): dev173476.

[Comparative Study](#) > *Cancer Cell*. 2007 Jun;11(6):539–54. doi: [10.1016/j.ccr.2007.04.017](https://doi.org/10.1016/j.ccr.2007.04.017).

Genes that distinguish physiological and pathological angiogenesis

[Steven Seaman](#)¹, [Janine Stevens](#), [Mi Young Yang](#), [Daniel Logsdon](#), [Cari Graff-Cherry](#), [Brad St Croix](#)

Affiliations + expand

PMID: 17560335 PMCID: PMC2039723 DOI: [10.1016/j.ccr.2007.04.017](https://doi.org/10.1016/j.ccr.2007.04.017)

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J Am Soc Nephrol. 2018 Aug; 29(8): 2060–2068.

PMCID: PMC6065081

Published online 2018 May 24. doi: [10.1681/ASN.2018030238](https://doi.org/10.1681/ASN.2018030238)

PMID: [29794128](https://pubmed.ncbi.nlm.nih.gov/29794128/)

A Single-Cell Transcriptome Atlas of the Mouse Glomerulus

[Nikos Karaiskos](#)¹, [Mahdieh Rahmatollahi](#)², [Anastasiya Boltengagen](#)¹, [Haiyue Liu](#)¹, [Martin Hoehne](#)², [Markus Rinschen](#)^{2,3}, [Bernhard Schermer](#)^{2,4,5}, [Thomas Benzing](#)^{2,4,5}, [Nikolaus Rajewsky](#)¹, [Christine Kocks](#)¹, [Martin Kann](#)² and [Roman-Ulrich Müller](#)^{2,4,5}

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The Vasculome

“Mapping knowledge about human vasculature across body scales”

NAVBO Zoom panel on Tuesday August 31st, 1-2:30pm

WHY?



Ondine Cleaver, PhD
UT Southwestern Medical Center

WHAT?



Zorina Galis, PhD
Chief of Vascular Biology &
Hypertension, NIH NHLBI
HuBMAP

HOW?

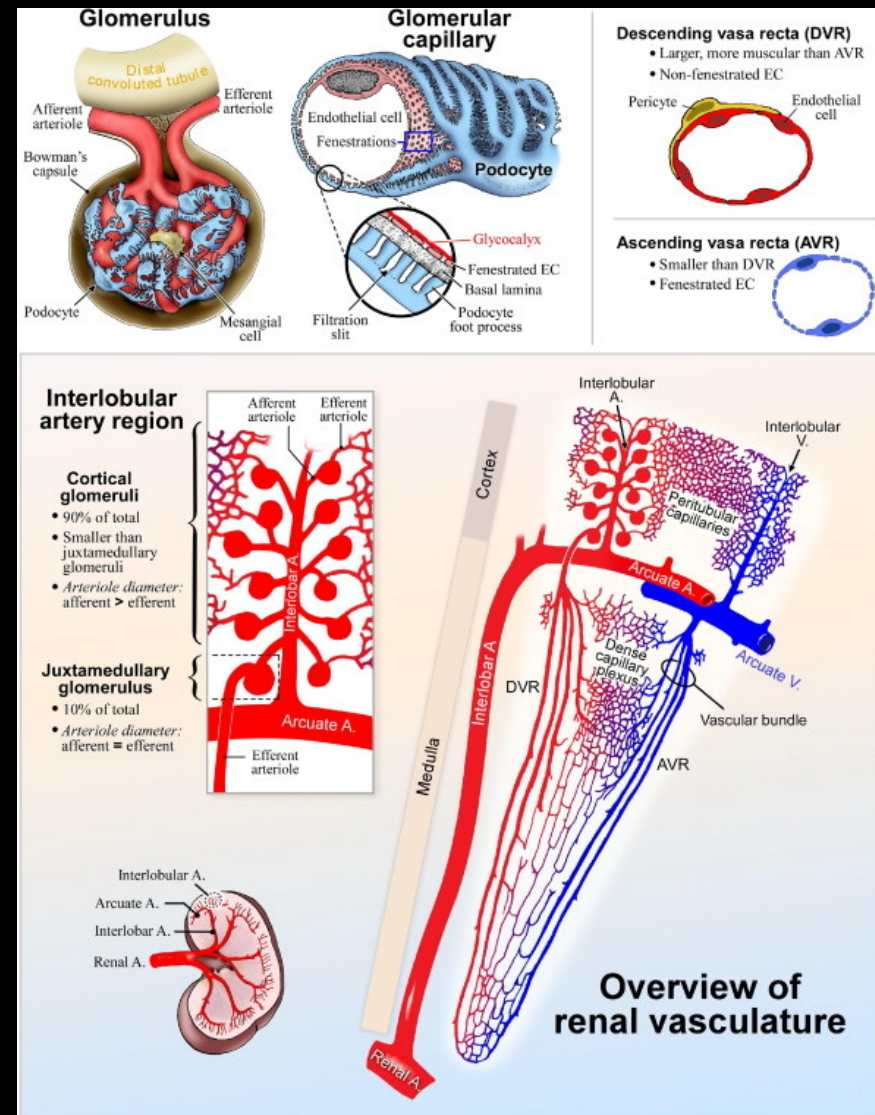


Griffin Weber, MD PhD
Biomedical Informatics
Harvard Medical School
VCCF & HuBMAP

Point being

Each of us is generating lots of EC data 😊

Let's assemble it to generate a full map of the kidney vasculature!



Acknowledgements



Neha Ahuja



Annie Ryan



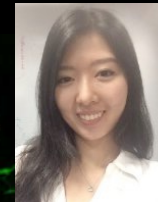
Max Hiltabidle



Haley Barlow



Stryder Meadows



Yeon Koo



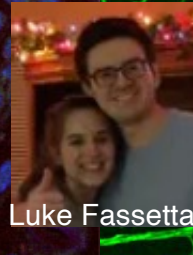
Xiaowu Gu



Mitzy Cowdin



Edward Daniel



Luke Fassetta



Funding

RBK

NIH- NHLBI

NIH-NIDDK

JDRF

Past

BCBC – SPEED

CPRIT

March of Dimes

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ACS



Ke Xu



Aiy Villasenor

Other Lab members-

SophieVoss

Past:

Yadanar Htike

Stryder Meadows

Diana Chong

Stephen Fu

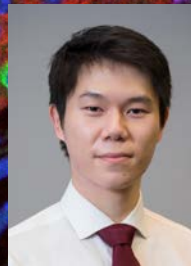
Lyndsay Ratliff

Simon Lee

Matthew Hensley etc



James Runnels



Peter Luo



Berfin Azizoglu



Leilani MartySantos

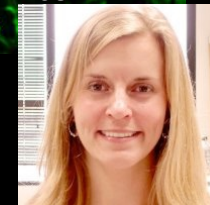
Collaborators and Reagents

Thomas Carroll - UTSW

Denise Marciano – UTSW

Jennifer Lewis - Harvard

RBK and Dept Molecular Biology – so many people



Caitlin Braitsch



David Barry