EXPANDING THE TOOL BOX-GENE DELIVERY TO THE KIDNEY

Indiana University O'Brien Center Imaging Course

2017

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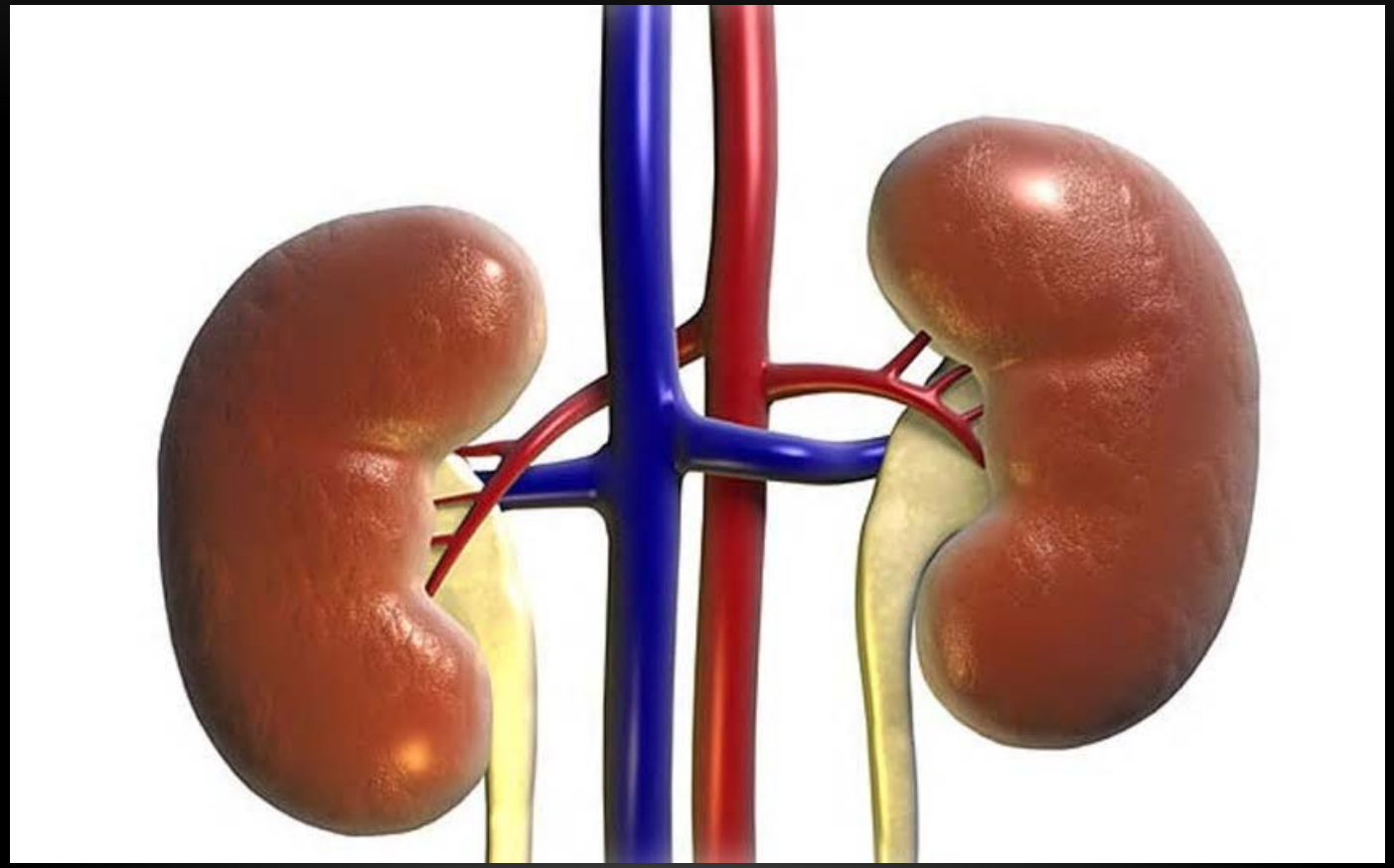
EXPANDING THE TOOL BOX-GENE DELIVERY TO THE KIDNEY

- Background-History of gene delivery to the kidney.
- Routes of gene delivery (rats)
  - Hydrodynamic delivery
  - Subcapsular injection
- What about mice?
  - Ultrasound guided injection
  - Renal pedicle injection
  - Hydrodynamic delivery

- Consideration of gene delivery vectors
  - AAV
  - Adenovirus
  - Lentivirus
  - Baculovirus (yep it can be used-don't recommend it).
- New adenovirus vectors
- Conclusions and Questions

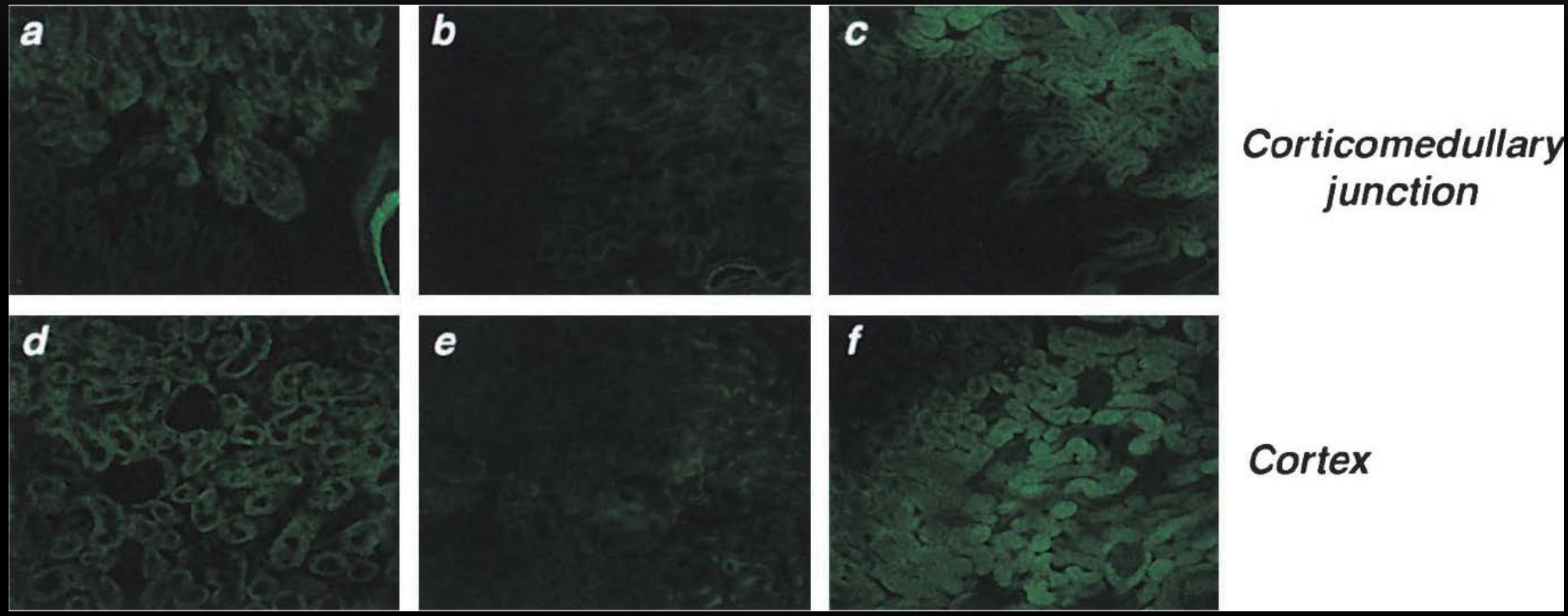
GENERAL WAYS TO DELIVER ANYTHING TO THE KIDNEY



GENE DELIVERY TO THE KIDNEY

- Moulier et al, 1994-Adenoviral-mediated gene transfer to renal tubular cells in vivo, KI
  - L kidney exposed, aorta clamp under the superior mesenteric artery and above the inferior mesenteric artery. 30 gauge needle, 1 x 1010 pfu/ml, flow rate 1-2 ml min/also given retrograde via ureter.
- Zhu et al, 1996, KI-adenovirus delivered either by renal artery infusion or retrograde via ureter.

- Gusella et al, In vivo gene transfer to kidney by lentiviral vector, 2002.
- Retrograde via ureter

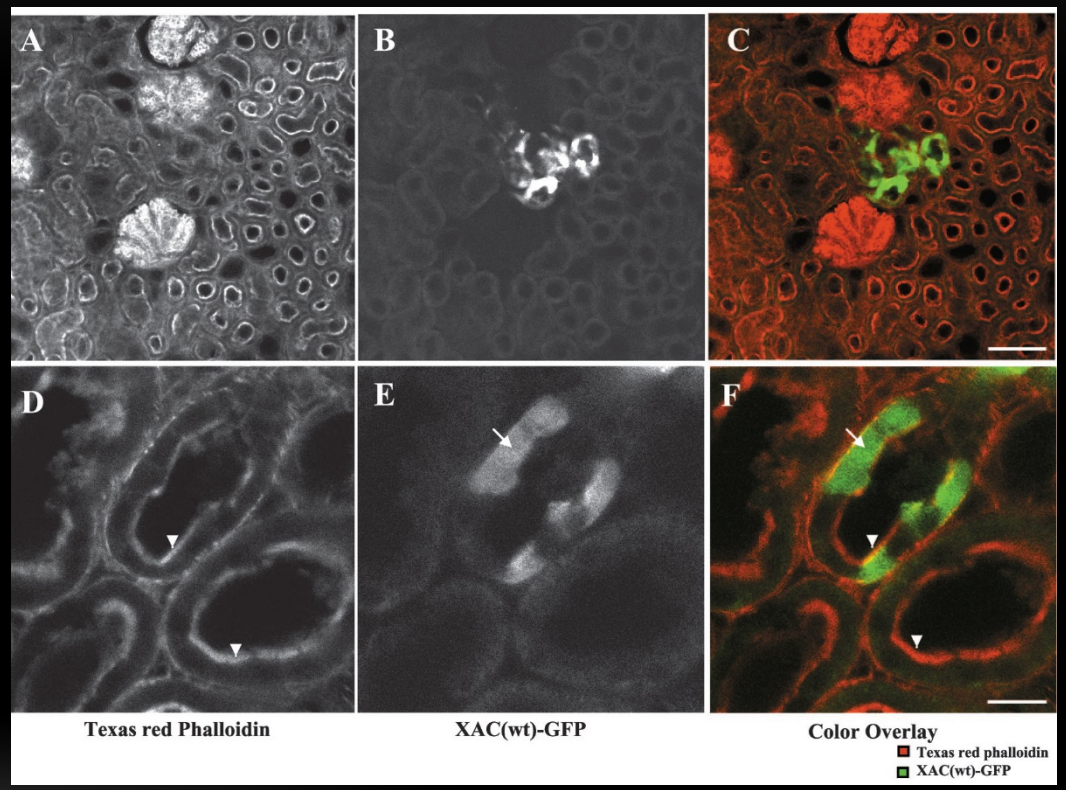


MICRO-INJECTION INTO THE VASCULAR WELL POINTS ON THE KIDNEY SURFACE OR DIRECTLY INTO RENAL TUBULES

- Tanner et al, AJP-renal, F638, 2005
- Adenovirus injection 3-5 E 11 pfu/ml diluted 1: 100 in

PBS and injected using viscous oil to create a localized plug resulting in “stopped flow” conditions. Used to inject renal tubules.

- Alternatively look for “well points” on the kidney surface (similar to a sprinkler head) and injected into that vascular space.



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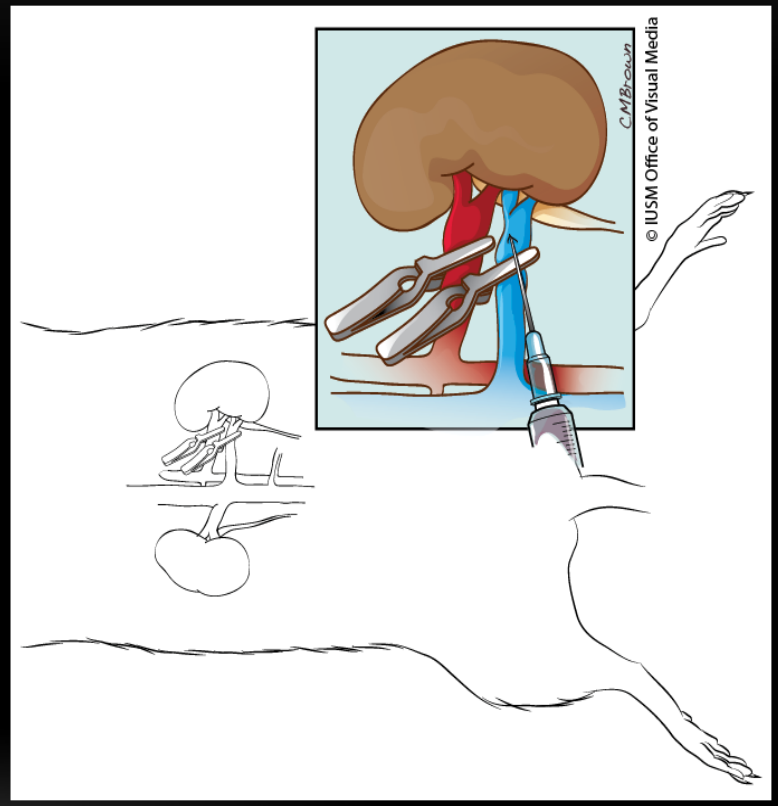
HYDRODYNAMIC DELIVERY

- Transgene Delivery
  - Plasmid DNA
  - Adenovirus
  - Baculovirus

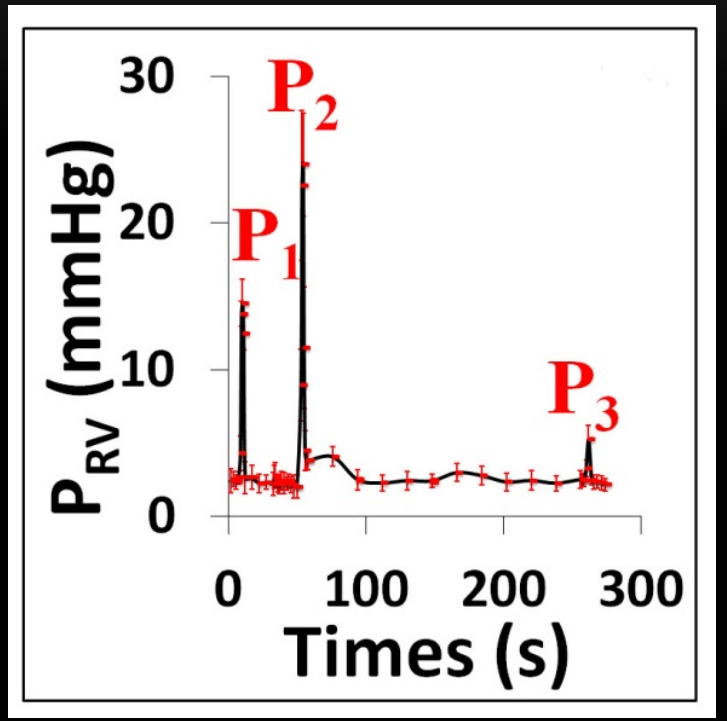
- Hydrodynamic delivery of high molecular weight molecules
- Theory based on fact that capillary endothelium and parenchymal cells are closely associated.
- Gene delivery uses a hydrodynamic force generated by a pressurized injection of a large volume of DNA solution into the blood vessel to permeabilize the capillary endothelium and generate pores in the plasma membrane of the surrounding parenchyma cells.
- First demonstrated as a tail vein injection using a bolus of physiologic saline equivalent to 8-10% of the body weight.
  - Retrograde, rapid venous injection.
  - Avoid the glomerular barrier (Kelley et al., AJP-Renal Physiol. 276: F1-9, 1999)
  - Take advantage of fenestrated venous beds (Maruyama et al., Human Gene Ther. 13: 455-468, 2002)



Current Data
Hydrodynamic Pressure Pulse

HYDRODYNAMIC DELIVERY

-Fluid is delivered via the renal vein
-Volume of fluid injected is 50% of the total organ volume.
-This volume is injected back toward the organ within a 30 second time span.
-Flow through the renal vein is occluded for an additional 3 minutes
-Injection dynamics first developed to optimize gene delivery in the kidney.



INJECTION RATE

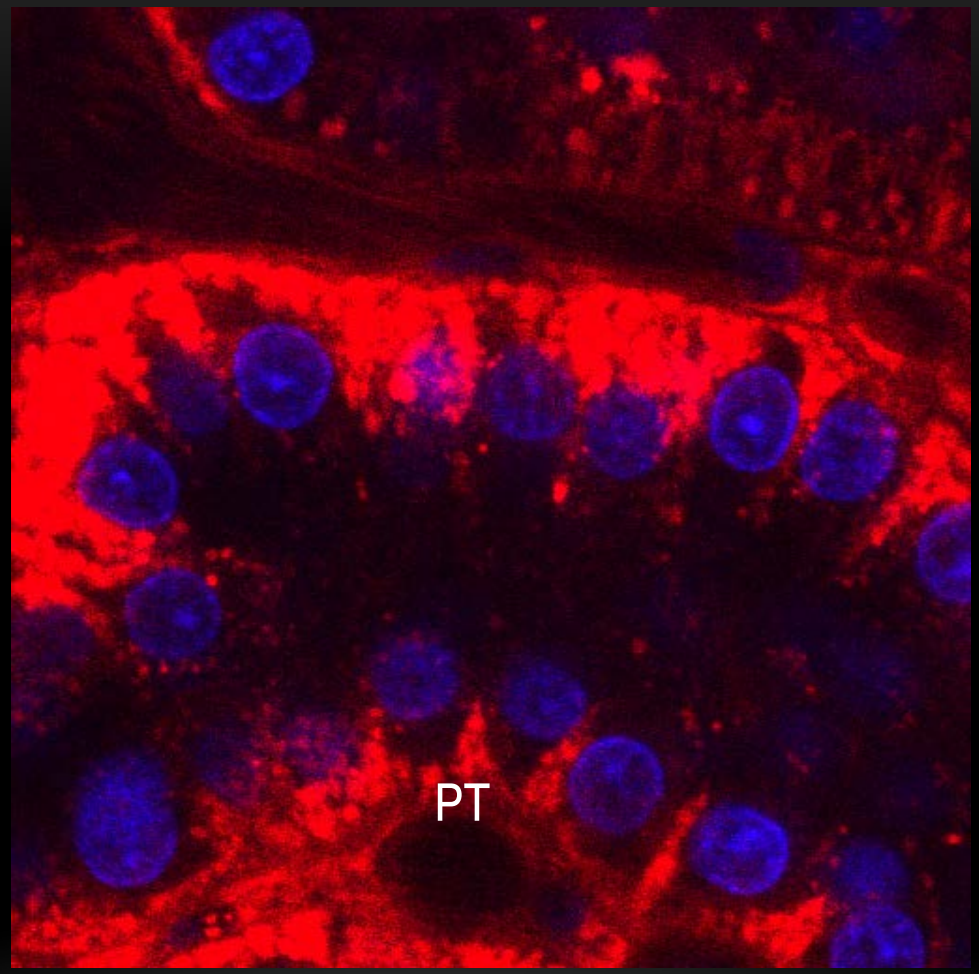
- Inject at a rate of 3-5 ml /second
- Firm pressure during delivery
- Volume 0.5 ml per kidney

Avoid Kidney Blowout-First Described on FOX TV!

DETAILS OF THE INJECTION

How to practice hydrodynamic delivery

INTRAVITAL DATA:
LARGE (150 KDa) MOLECULAR WEIGHT TRITC DEXTRANS:
(20X OBJ); 30 msec/frame
Hoechst 33342 –blue nuclei

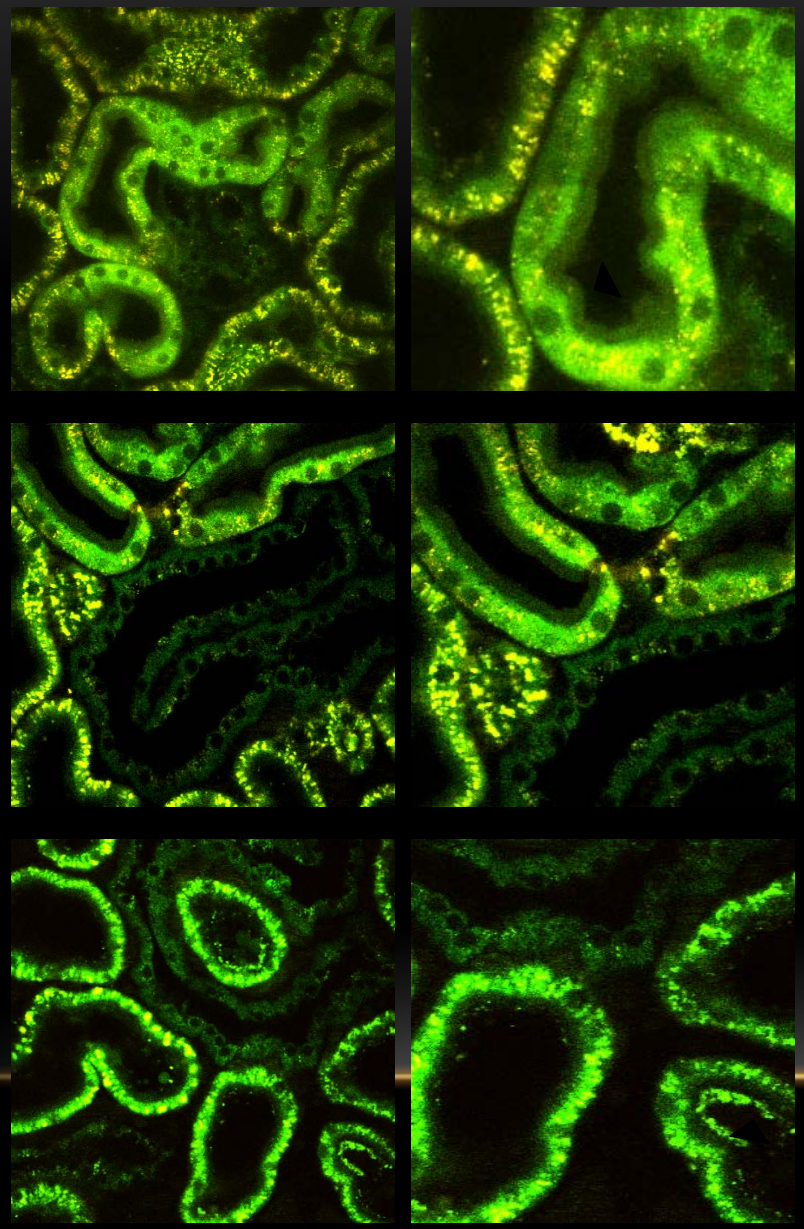


Expression Time-Course: Hydrodynamic-based GFP-Actin Plasmid Expression in Live Animals

3 days post injection

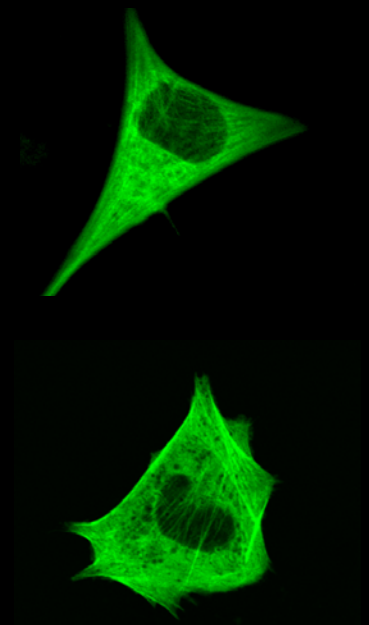
14 days post injection

28 days post injection



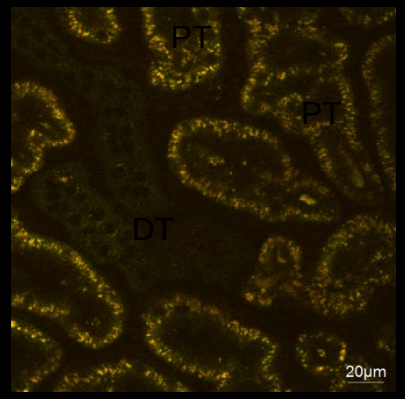
GFP appeared to aggregate over time

S3 cells incubated with the same plasmids
(1 day after incubation)

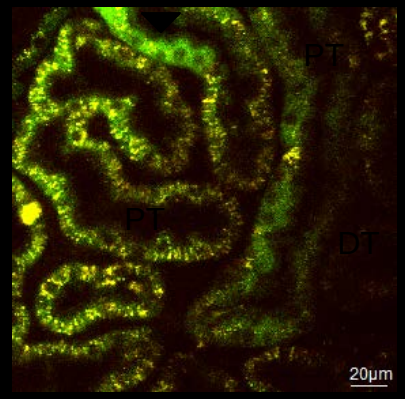


Time-dependent Actin-GFP Adenovirus Transgene Expression

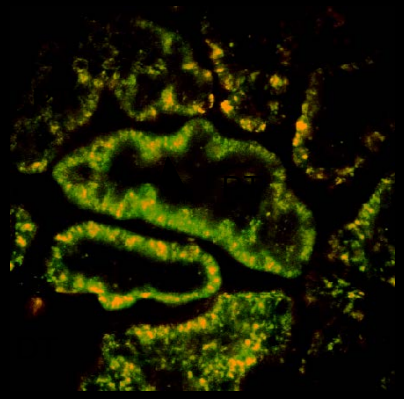
Day 0
No Injection
Tissue
Autofluorescence



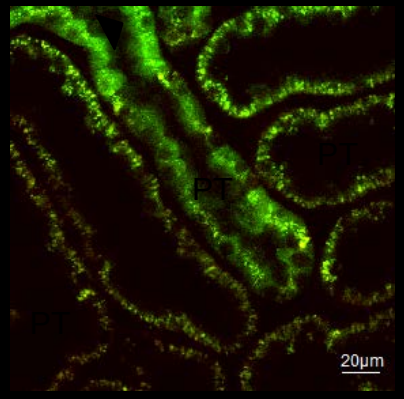
3 Days Post
Injection of
Actin-GFP
Adenovirus



7 Days Post
Injection of
Actin-GFP
Adenovirus



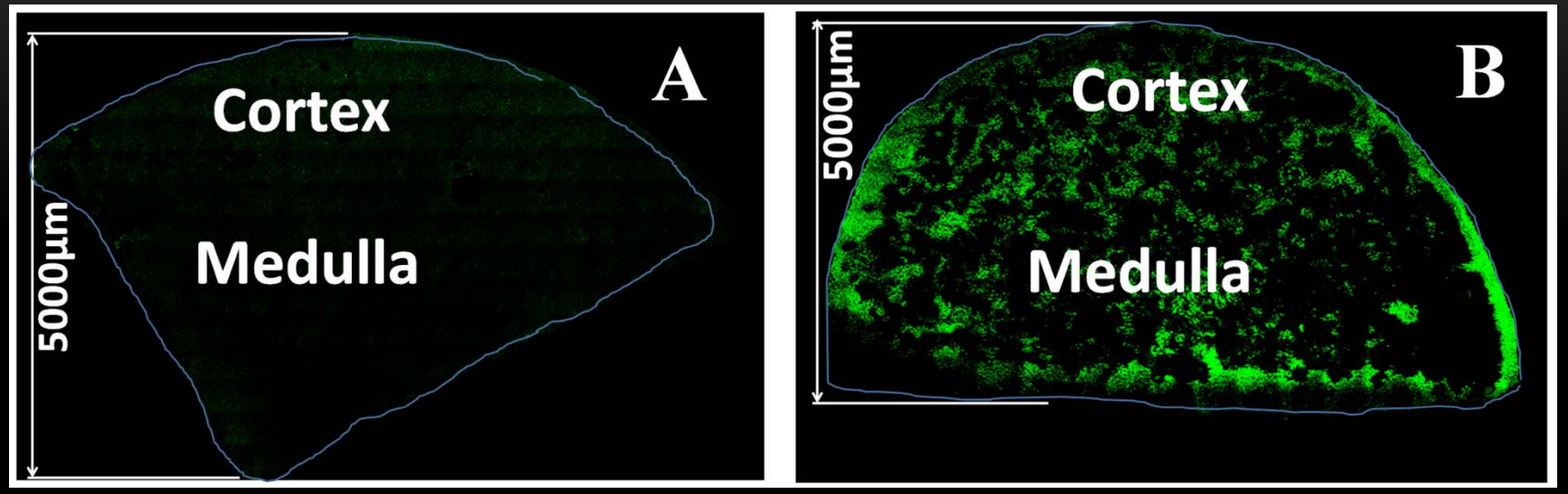
14 Days Post
Injection of
Actin-GFP
Adenovirus



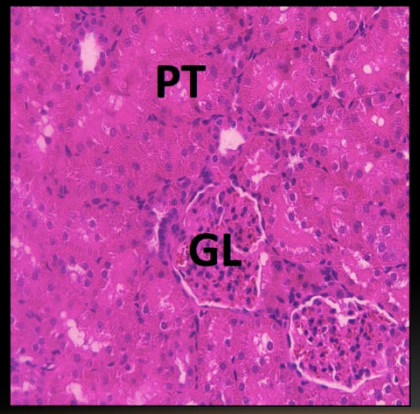
TRANSGENE DISTRIBUTION THROUGHOUT THE KIDNEY

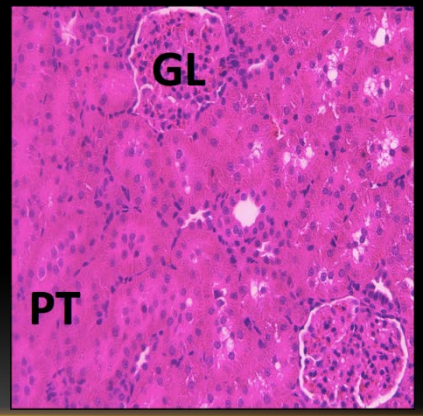
SALINE

GREEN FLUORESCENT PROTEINS

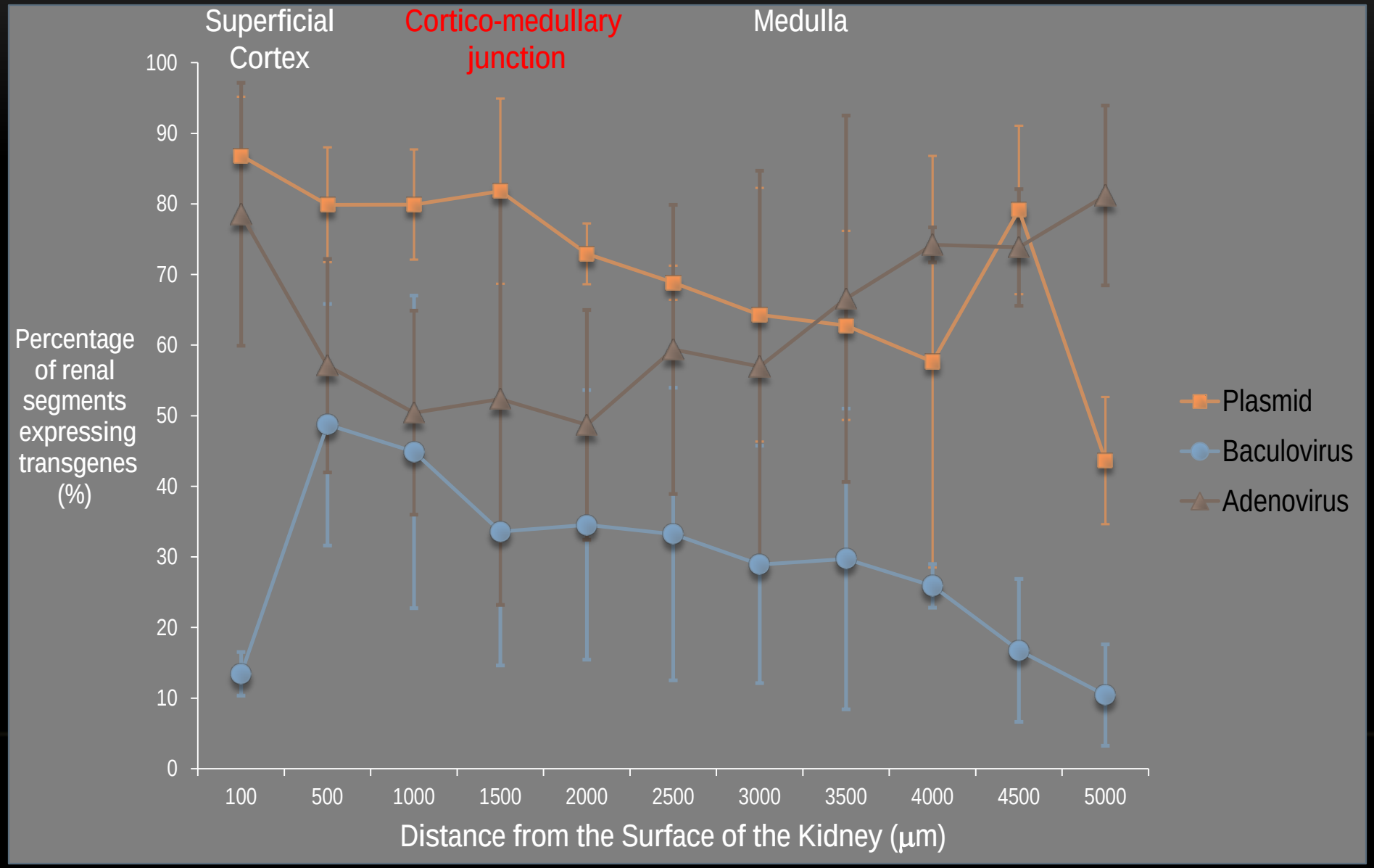


H and E Staining

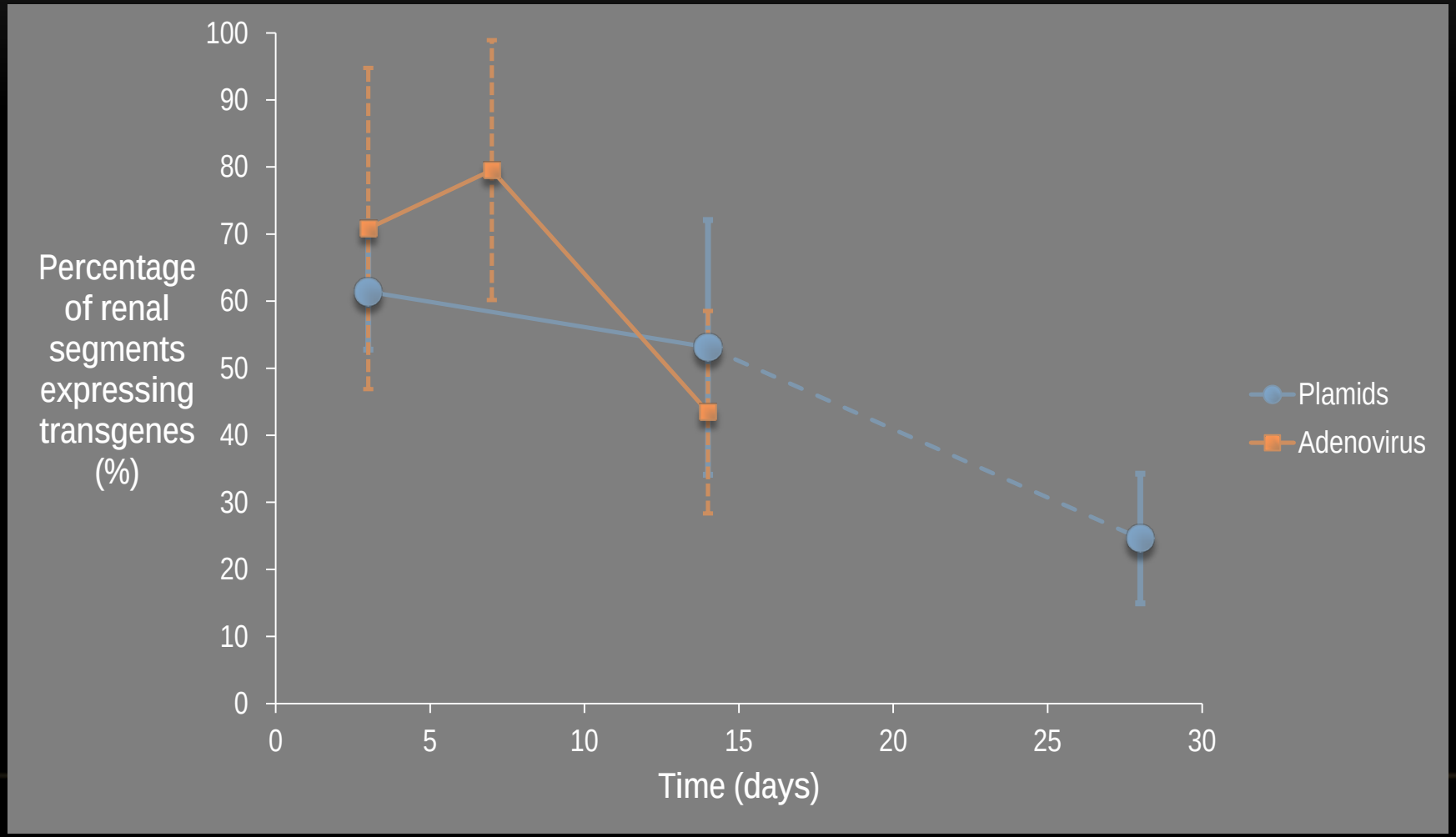




PLASMID AND ADENOVIRUS PROVIDE EFFICIENT LEVELS OF FLOURESCENT PROTEIN EXPRESSION



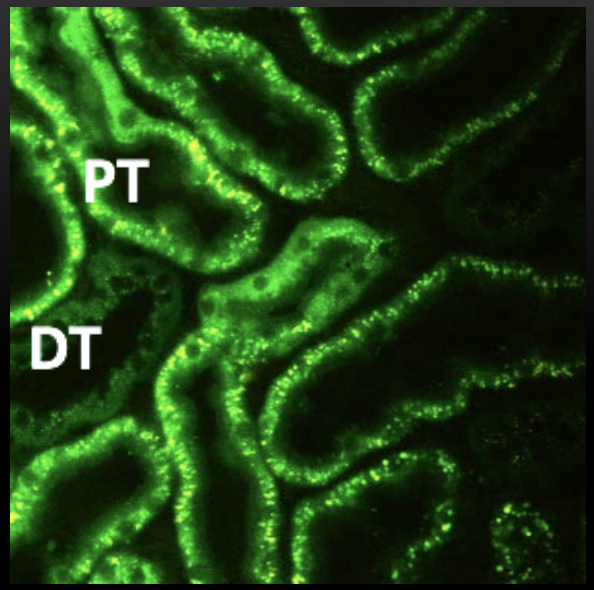
PLASMID AND ADENOVIRUS TRANSGENE EXPRESSION VERSUS TIME



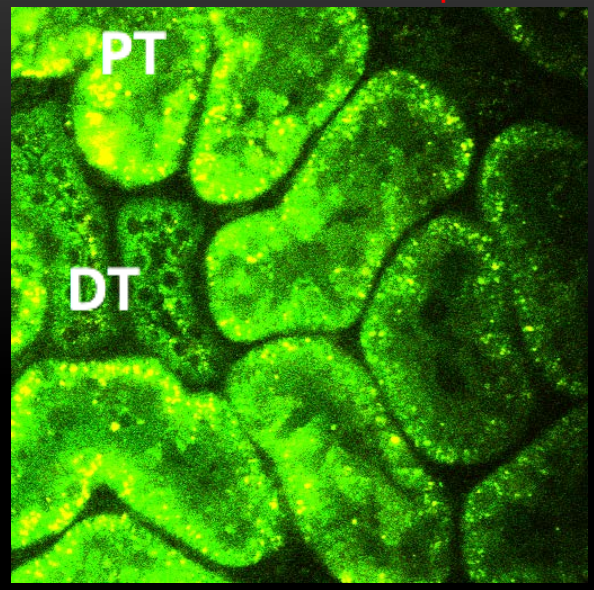
Hydrodynamic delivery:

Allows us the to track live change during ischemia-reperfusion injury

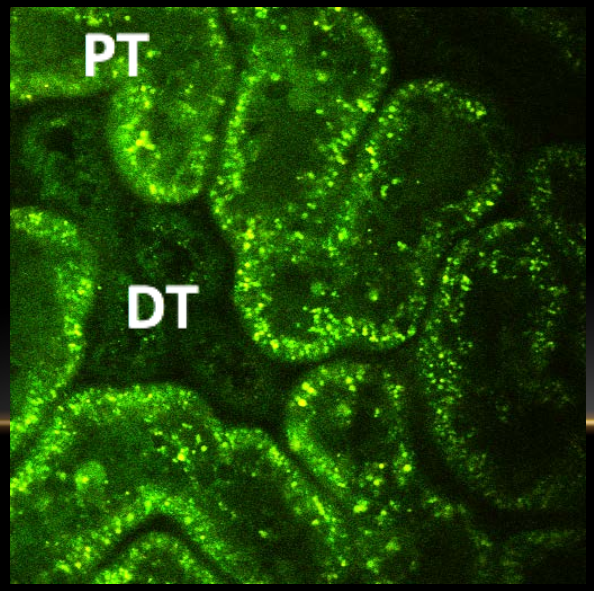
Before Injury



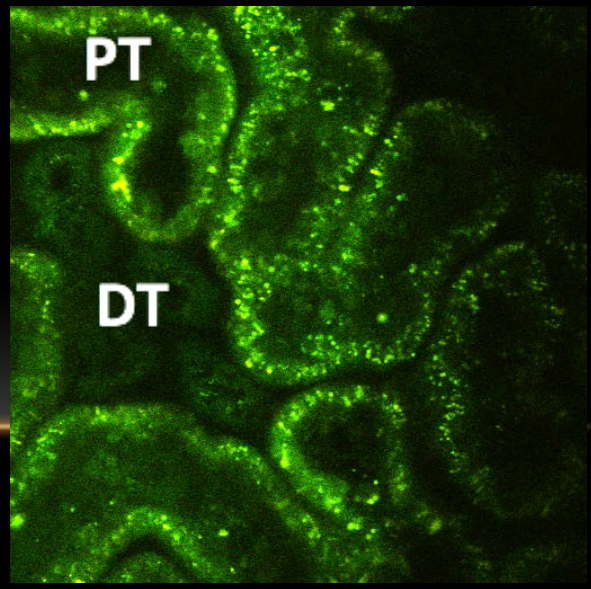
10 mins after ischemia-reperfusion



40 mins after ischemia-reperfusion



50 mins after ischemia-reperfusion



HYDRODYNAMIC GENE DELIVERY

- Expression in all nephron segments (no nephron segment specific promoters used)
- Procedure takes 30 minutes.
- Amount of plasmid DNA required-3 ug/g total body weight.
  - i.e for a 250 g rat this amounts to 750 ug DNA
  - 7.5 x 10E14 DNA molecules delivered
- Adenovirus-10E6 to 10E7 viral particles delivered for efficient expression
  - Therefore adenovirus is vastly more efficient

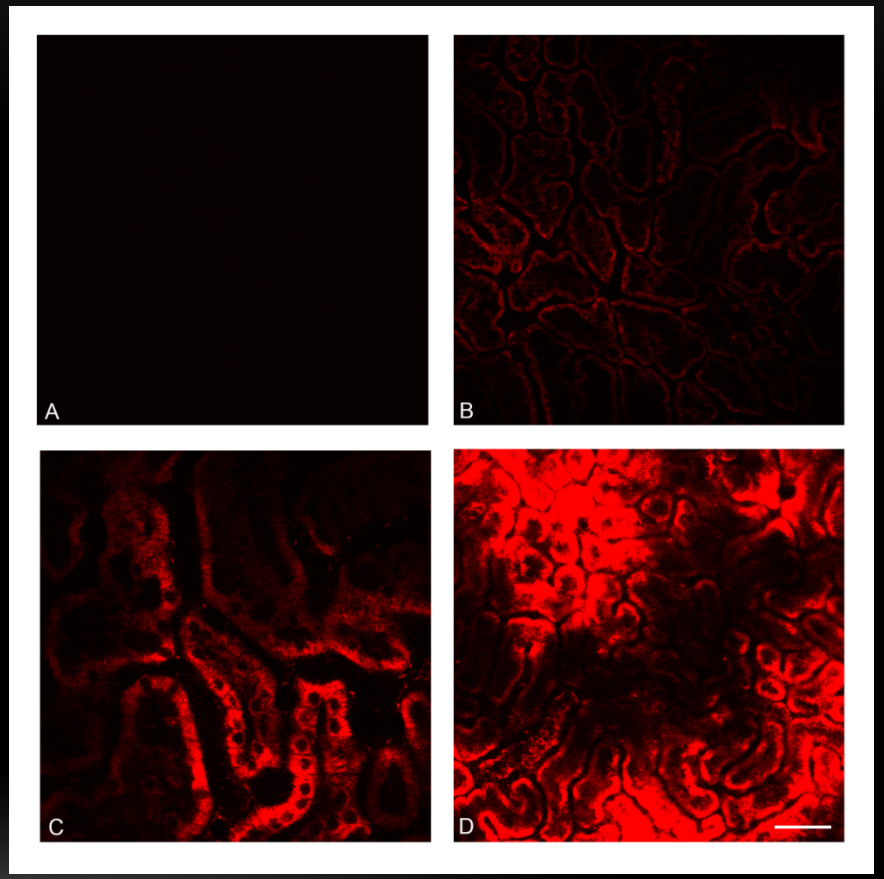
HYDRODYNAMIC GENE DELIVERY CAN CHANGE WHOLE ORGAN FUNCTION

- Gene target-IDH2
- Preliminary data suggests that protein can be used to protect cells from ischemia/reperfusion injury.

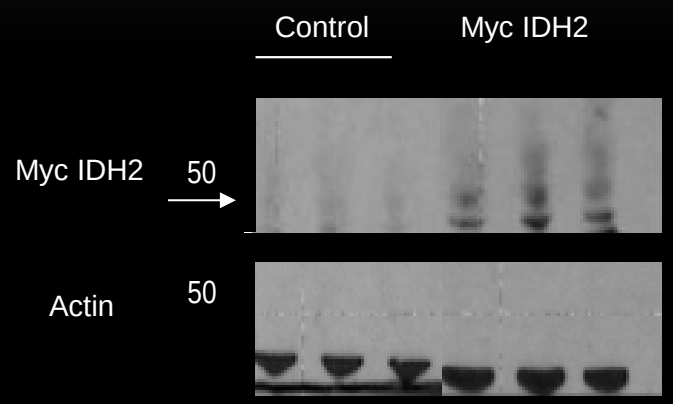
- Experimental Protocol
  - Day (-7) +gene delivery versus saline delivery via hydrodynamic delivery
  - Day 0, Right nephrectomy, contralateral 35 minutes of ischemia via pedicle cross clamp.
  - Day 1, measure serum creatinine

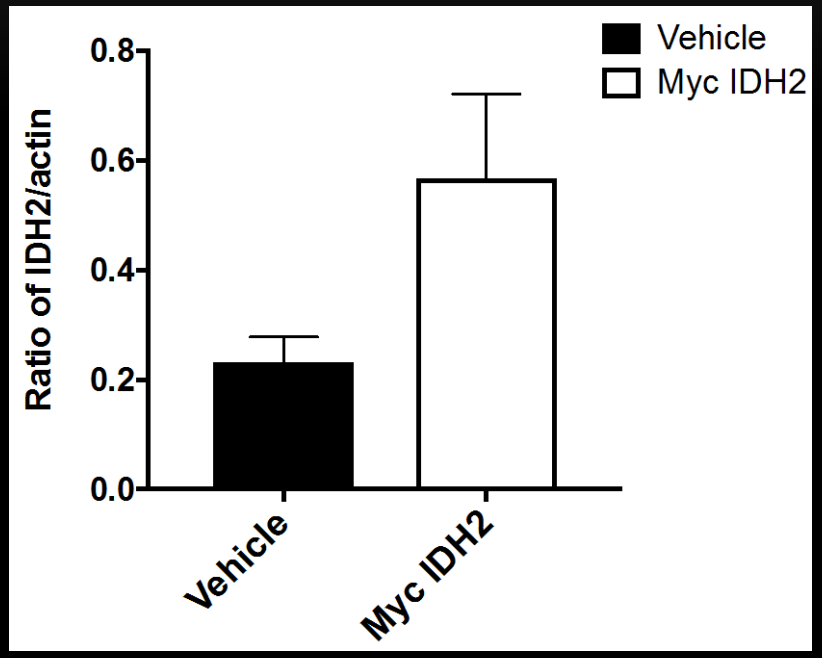
HYDRODYNAMIC DELIVERY OF IDH2 OR SULT 1C2 INCREASES MITOCHONDRIA POTENTIAL

- Images from live animal kidneys labeled with TMRM
- A: Control
- B: Ischemia preconditioning
- C: Sult1C2 transgene delivery
- D: IDH2 transgene delivery



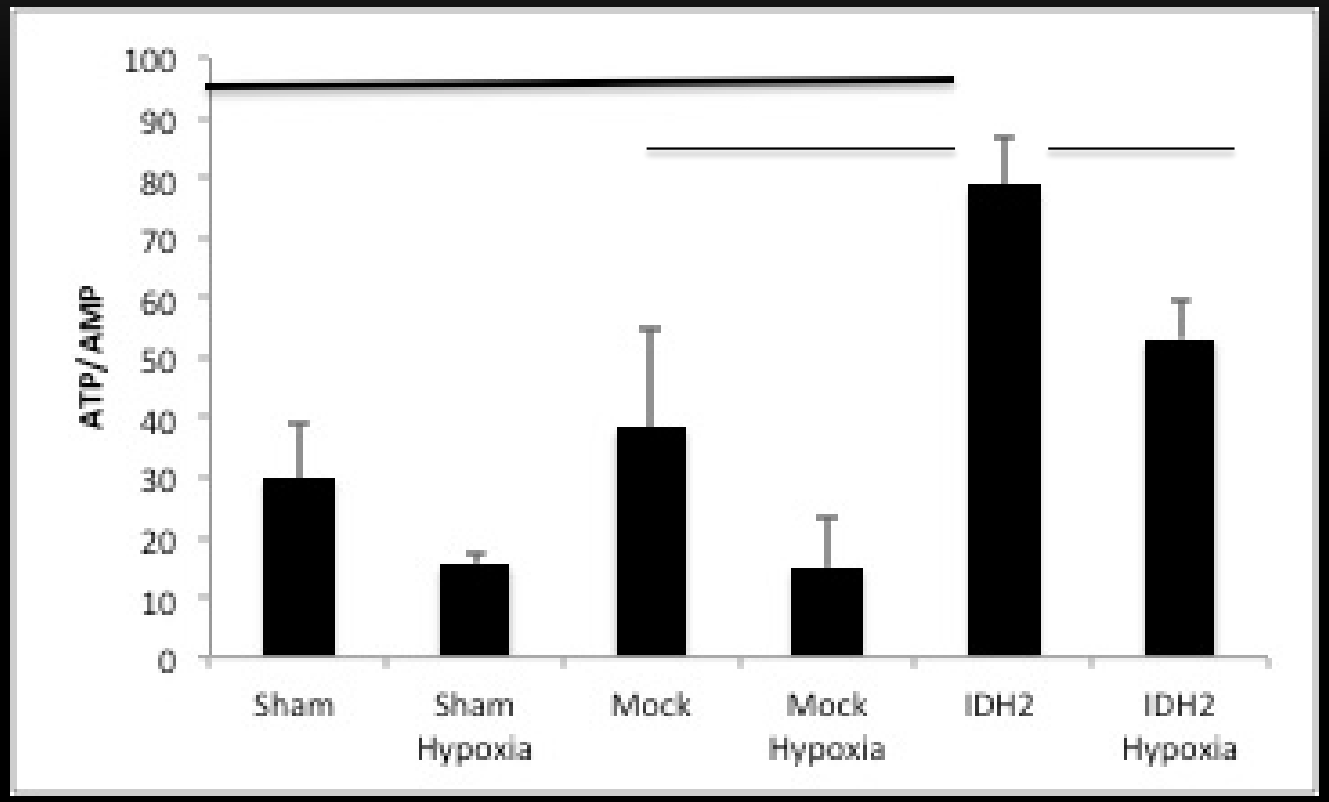
DOES IDH2 LOCALIZE TO THE MITOCHONDRIA?





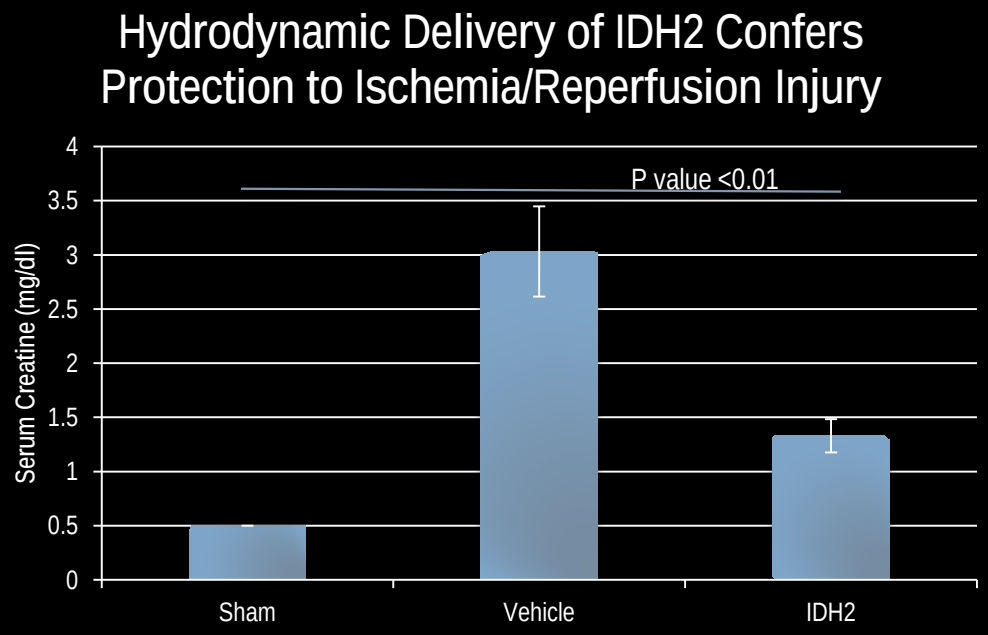
Western Blot of isolated mitochondrial lysates. Mitochondria were isolated using using a PBI shredder (homogenization) and mitochondrial isolation buffer, followed by high speed centrifugation. 20 ug of protein were loaded into each well.

PRIOR DELIVERY OF IDH2 IMPROVES ATP LEVELS AFTER I/R INJURY



*,$,# p<0.05

GENE THERAPY CONFERS ORGAN-WIDE RESISTANCE TO ISCHEMIA/REPERFUSION INJURY



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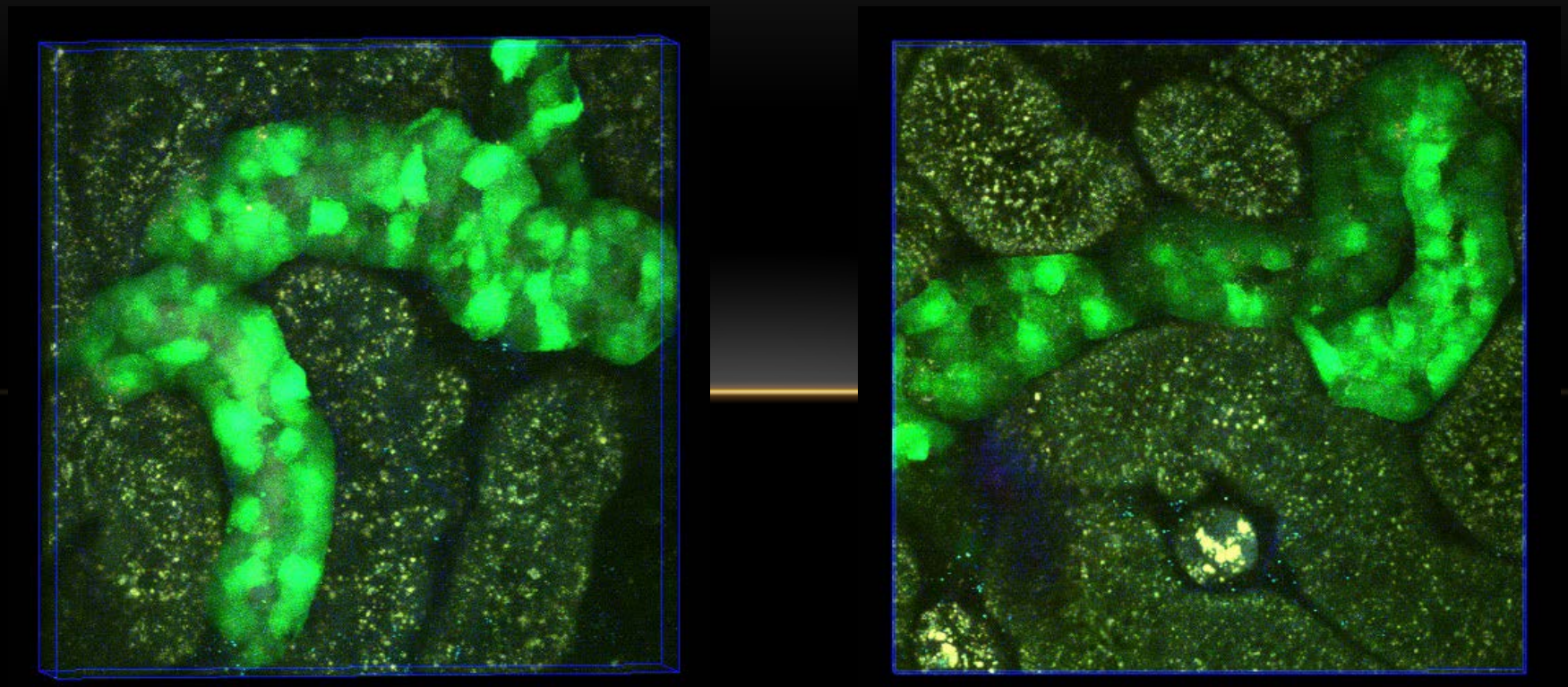
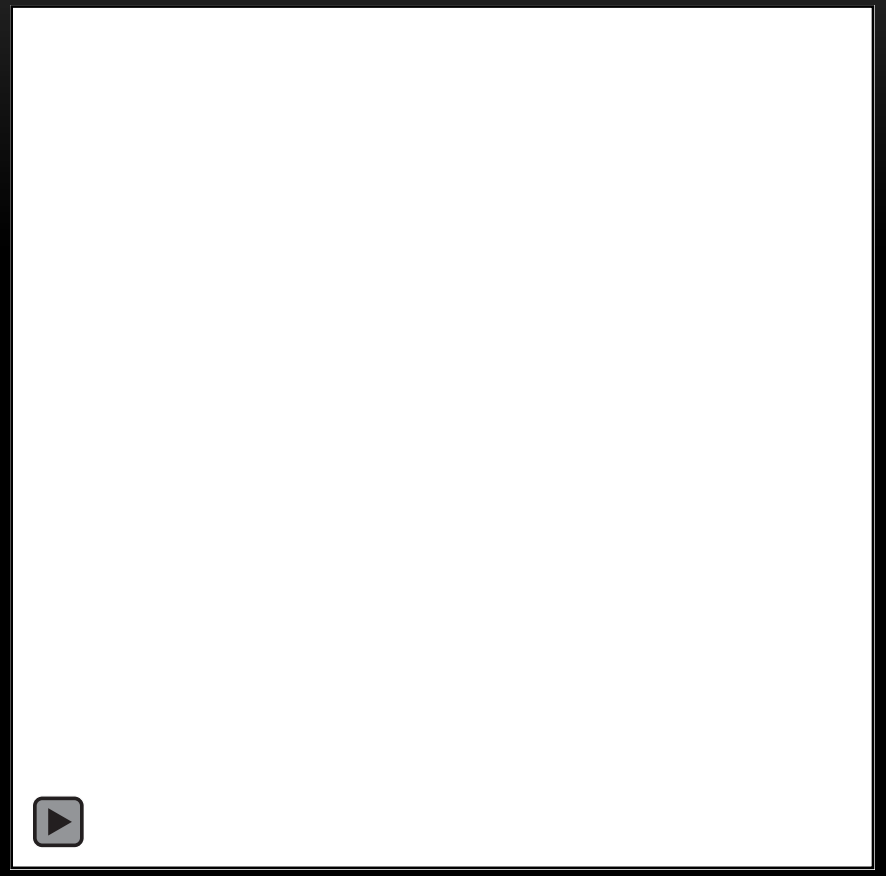
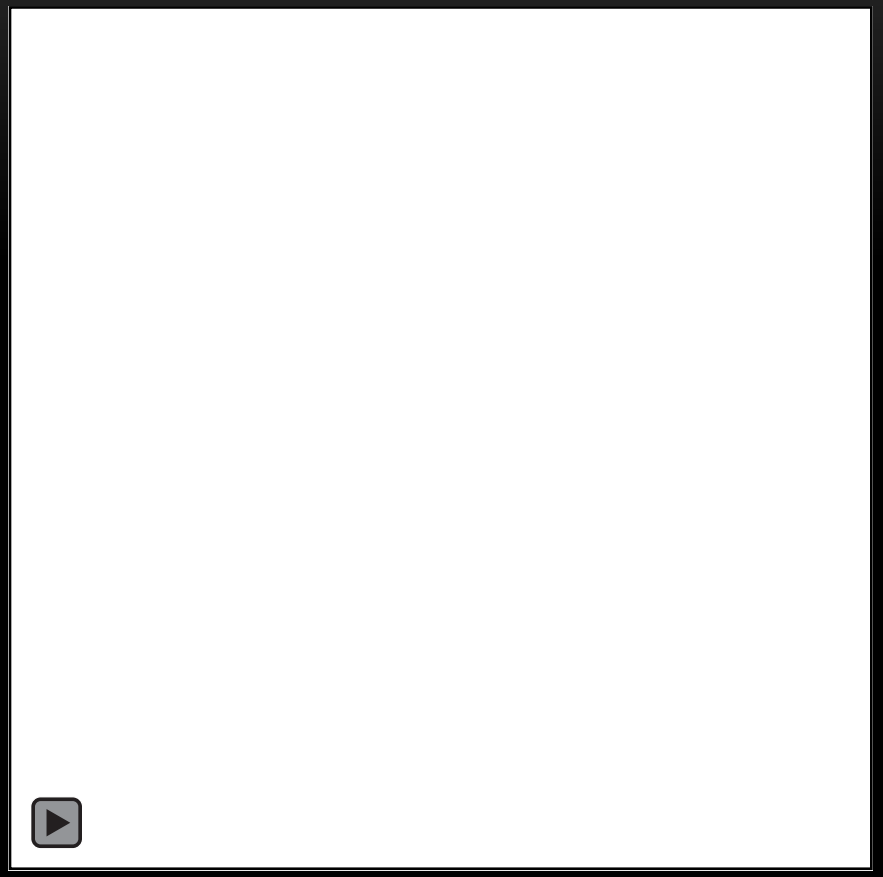
Gene transfer by subcapsular injection of adeno-associated virus (AAV9)



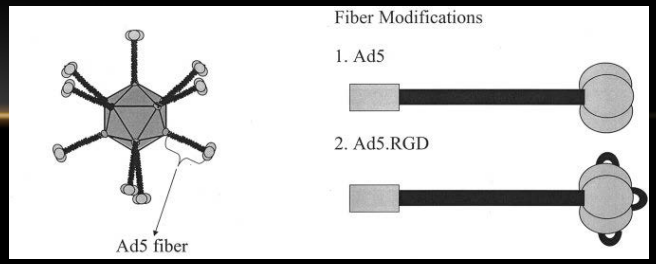
George Rhodes and Xiao Xiao
UNC

GENE TRANSFER BY SUBCAPSULAR INJECTION OF ADENO-RGD-GFP





4 days after subcapsular injection of adeno-RGD-GFP



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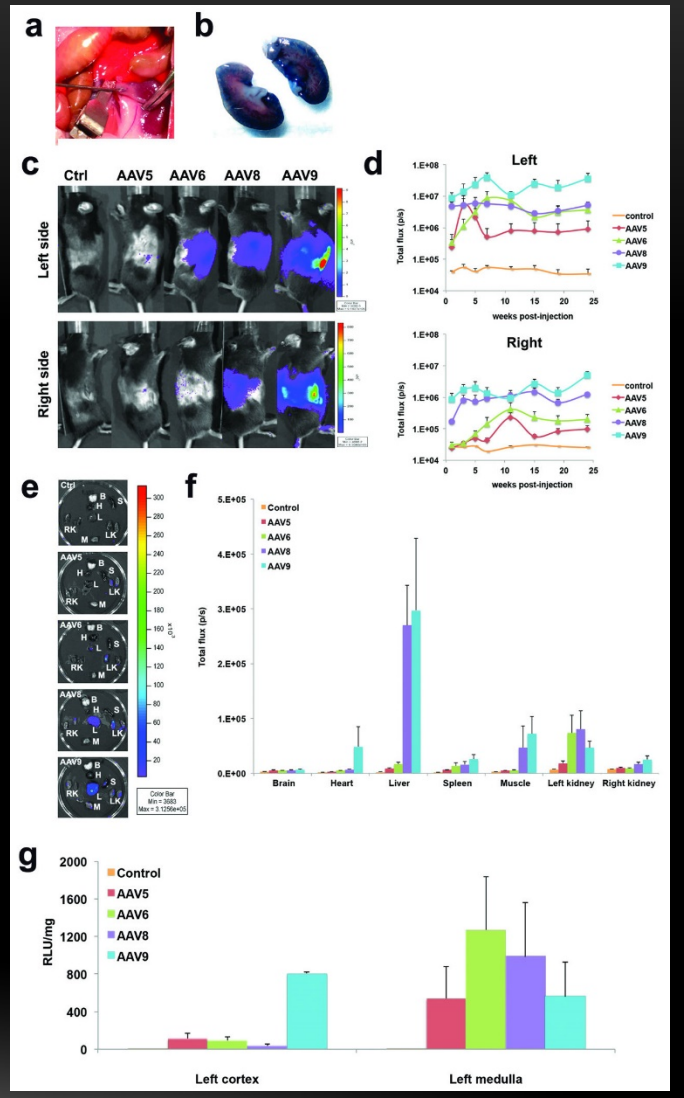
WHAT ABOUT MICE?

- Ultrasound guided lentivirus injection into the kidney
  - Espana-Agusti et al., Scientific Reports, Minimally invasive, lentiviral based method for the rapid and sustained genetic manipulation of renal tubules, 2015.
  - Luciferase construct with strawberry fluorescent protein.
  - Transgene assayed by bioluminescence, cortico-medullary strawberry expression observed
- Renal Pedicle Injection
- Kim et al., KI, Kidney-specific reconstitution of the A1 adenosine receptor in A1 adenosine receptor knockout mice reduces renal ischemia-reperfusion injury, 75(8), 809-823, 2009
  - Kidney exteriorized, lentivirus injected into the renal pedicle.
  - Extensive cortico-medullary expression of GFP marker.
  - Conferred protection against I/R injury.

WHAT ABOUT MICE

- Hydrodynamic Delivery-retrograde through the renal vein.
- Rocca, et al, rAAV9 combined with renal vein injection is optimal for kidney-targeted gene delivery: conclusion of a comparative study, Gene Ther., 21(6), 618-628, 2014.
- Hydrodynamic delivery with clamped renal pedicle, maintain clamp for 15 minutes



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COMPARISON OF GENE DELIVERY VECTORS

- Plasmid DNA
  - Least toxicity if the plasmid preparation is endotoxin free.
  - Gene expression requires large amounts of DNA
- Lentivirus
  - Neurotropic virus-requires more fastidious biosafety for recombinant virus production.
  - Requires stable integration, this can have unanticipated effects depending on the integration site.
- AAV
  - Less immune response than adenovirus, AAV9 seems to give better expression in kidneys.
  - More difficult to make recombinant virus

COMPARISON OF GENE DELIVERY VECTORS

- Adenovirus
  - Can cause a localized immune response.
  - Easier to make recombinant virus
  - Modifying the stalk protein with RGD sequence improves infectivity..
  - Can express two recombinant adenovirus in a cell!
  - Need to measure PFU’s or infectious titer.

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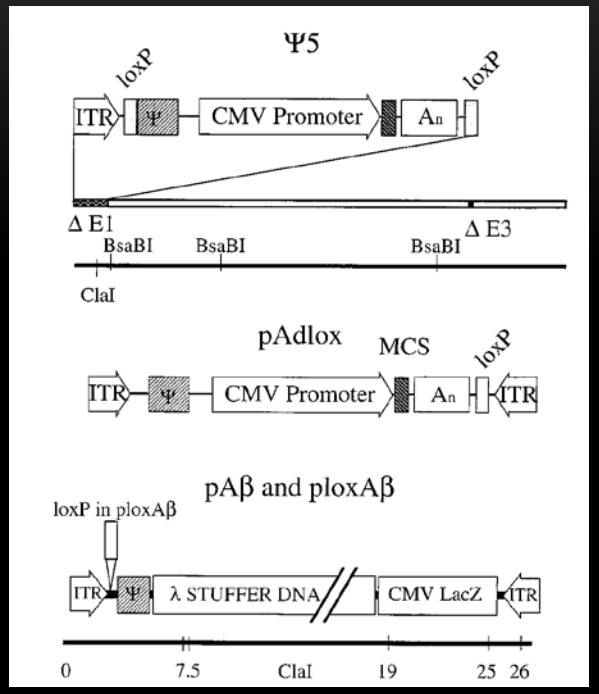
Modified Ψ–5 Adenovirus and Efficiency of Adenovirus Production



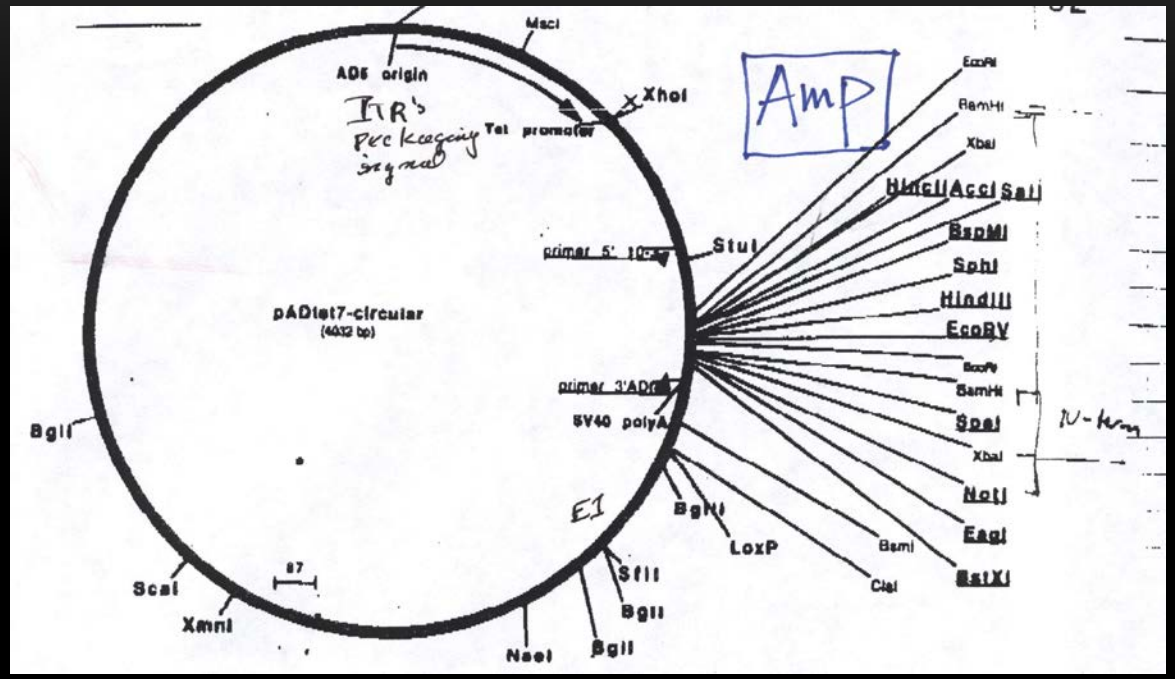


TABLE 1. Comparisons of Cre-*lox*- and homology-driven recombination and of viral and plasmid DNAs as sources of the virus backbone

Shuttle vector	Mode of recombination	Type of donor DNA		% *lacZ*-positive cells in FDG assay			
		Viral	Plasmid	3 days	7 days	10 days	14 days
pAdlox, cut	CRE-*lox*	Ψ5		1.7	100		
pAdCMV B, cut	Homology	Ψ5		0.2	100		
	Homology		pBHG10	0	0	0.65	100
pAdCMV B	Homology		pBHG10	0	0	0	0

Optimization of Adenovirus-Conversion to a Gateway Acceptor

Gateway Cloning

Cloning method developed in the 1990's

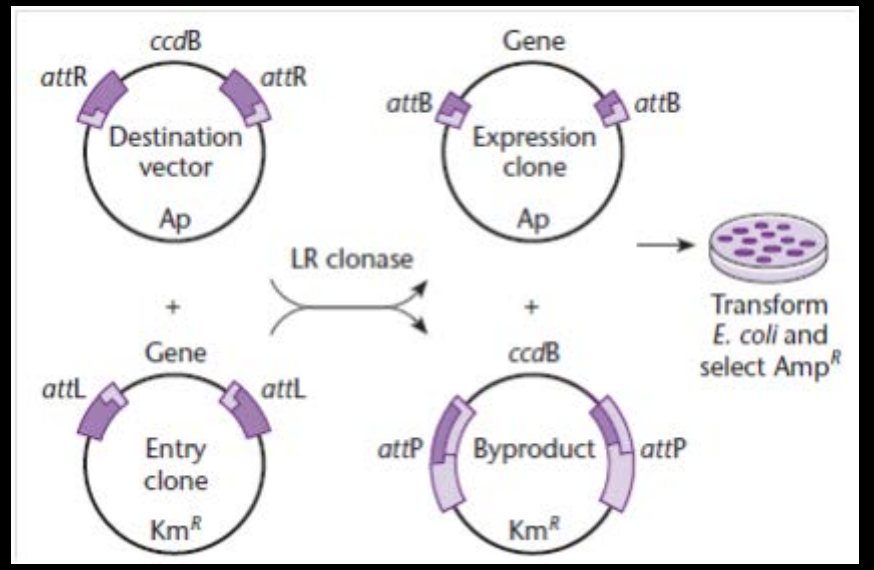
Supported by the NIH Mammalian Gene Collection

-The collection has 17,592 non-redundant full-length human genes

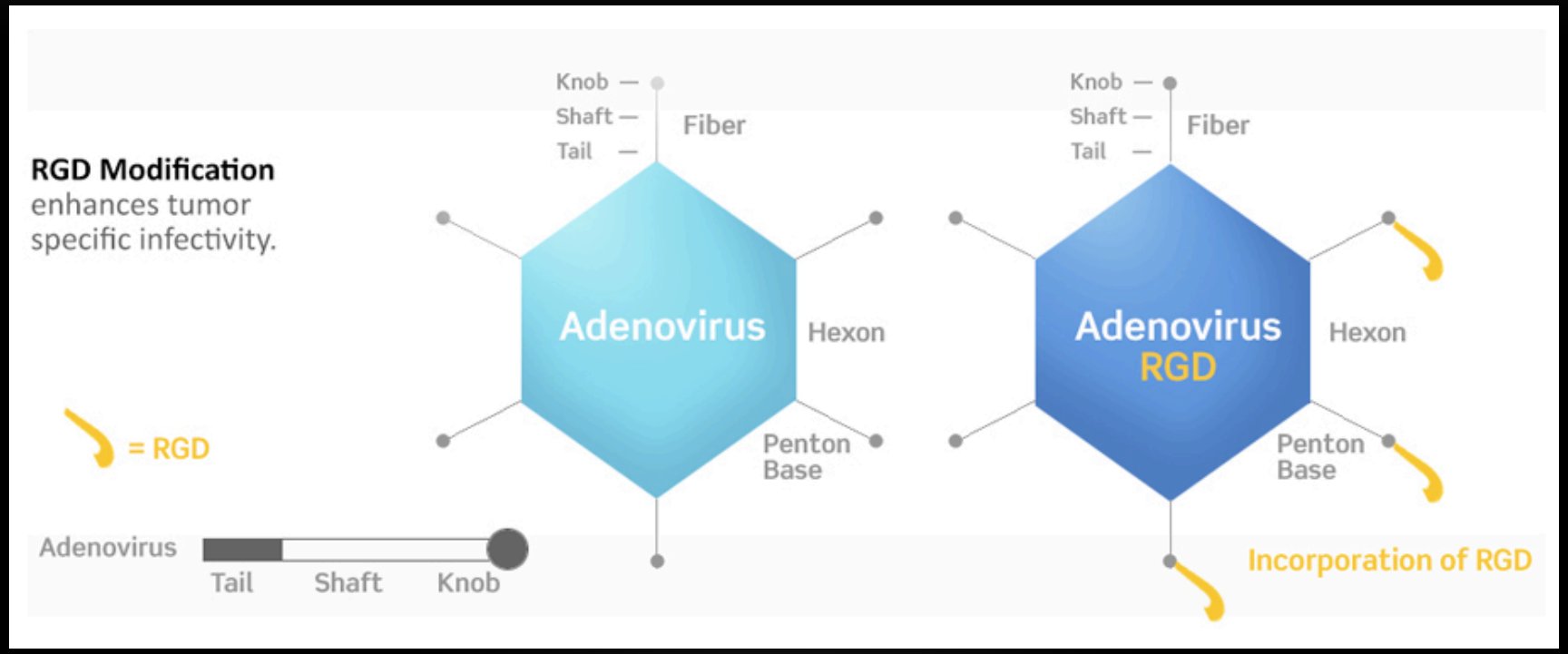
17,701 non-redundant full-length mouse genes

6486 non-redundant full-length rat genes

Invitrogen offers many of these genes with GFP or myc tagged constructs.



MODIFYING ADENOVIRUS TO EXPAND ITS TROPISM



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SUMMARY AND NEXT STEPS

- Both hydrodynamic delivery and subcapsular delivery result in gene expression
  - Subcapsular delivery yields strong localized expression
- Both AAV and Adenovirus work well for gene transfer
  - Modified RGD stalk adenovirus works better than normal adenovirus

- Adenovirus is easier to produce
- When given at the appropriate MOI little inflammatory response is observed
- Gives long lasting expression
- More than one adenovirus can infect cells-therefore two transgenes can be expressed
- Recombinant adenovirus production can be problematic-can it be optimized?

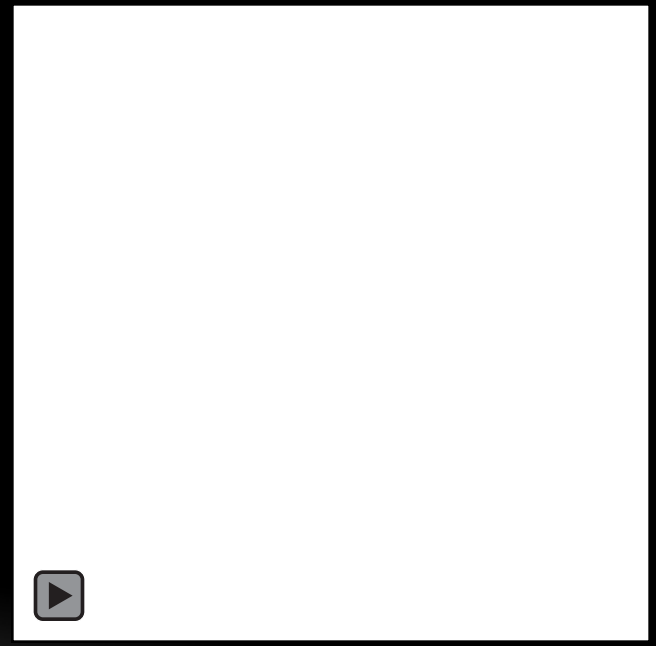
Acknowledgements

Peter Corridon
Alex Kolb
George Rhodes
Ken Dunn
Simon Atkinson
David Basile
Bruce Molitoris

Wei min Xu
Jason Collett
Purvi Molhatra

NIDDK: *P30 DK 079312-01*

Glomerulus movie made by Sherry Clendenon



QUESTIONS?