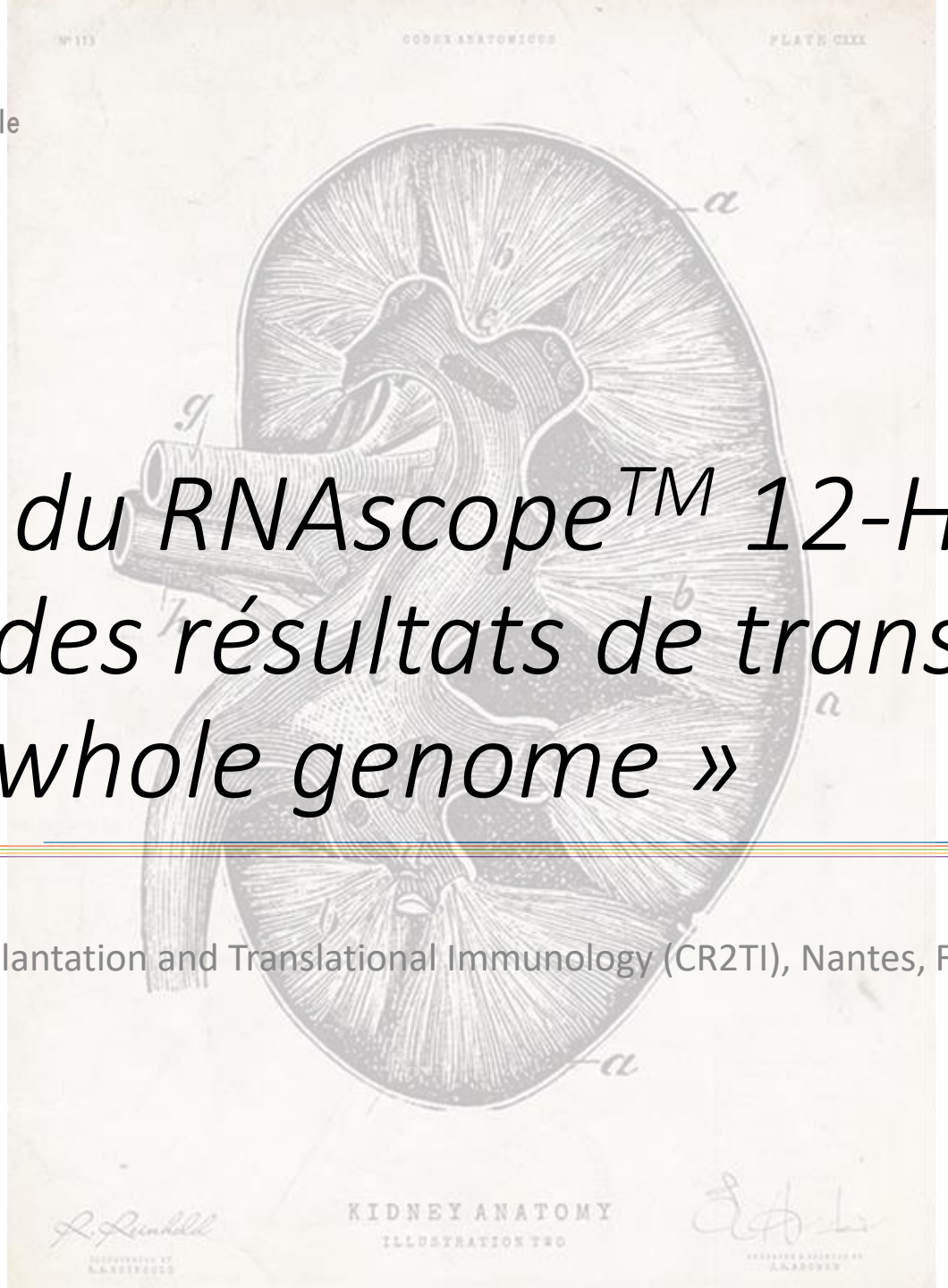
CR2TI
Centre de Recherche Translationnelle en Transplantation et Immunologie
UMR 1064




# *Utilisation du RNAscope™ 12-Hiplex pour confirmer des résultats de transcriptomique spatiale « whole genome »*

Franck HALARY, PhD

Center for Research in Transplantation and Translational Immunology (CR2TI), Nantes, France

Nov 19th, 2026

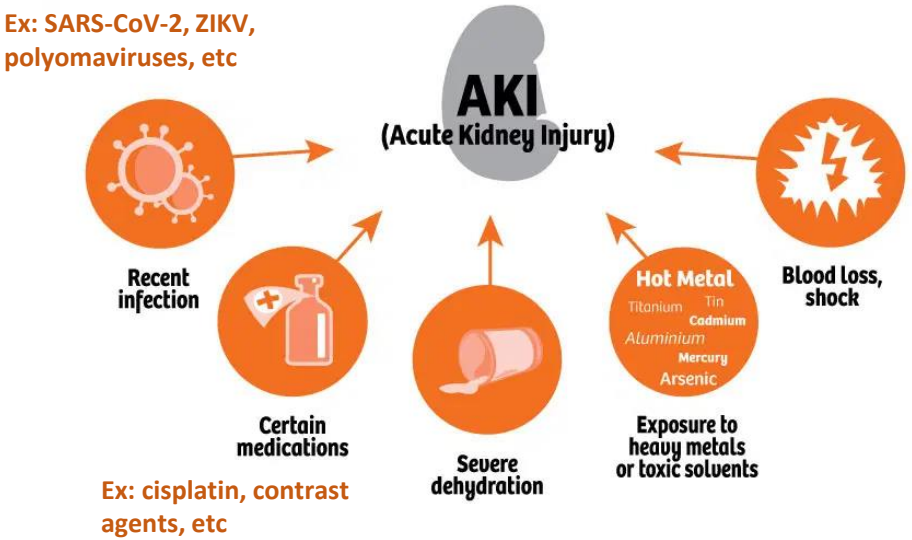
# Renal diseases: the big picture

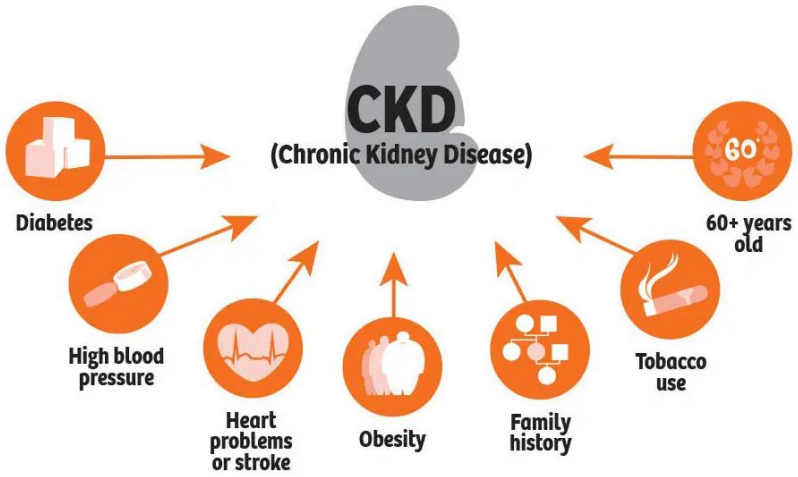
**Acute Kidney Injuries (can be reversible)**
- **Decreased blood flow, ex: NSAIDs (nephrotoxicology)**, etc.
- Direct damage to the kidney, ex: sepsis, **tubulopathies**, multiple myeloma, etc.
- Blockade of the urinary tract, ex: lithiasis, cancers, etc.

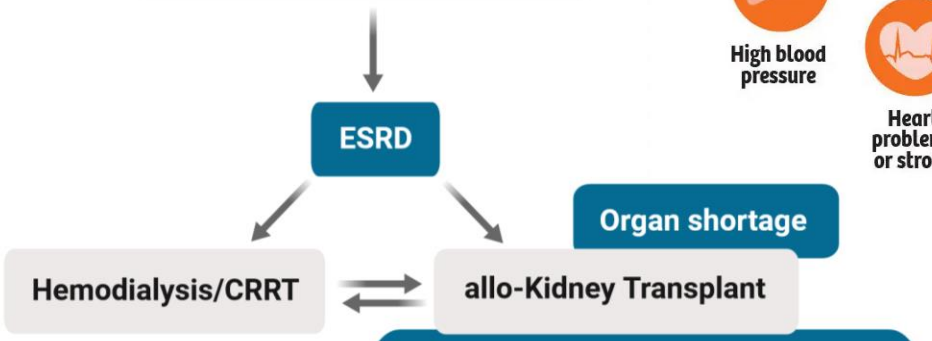
**Chronic Kidney Diseases (can lead to End-Stage Renal Diseases or ESRD)**
Glomerular damages:
- obesity,
- diabetes,
- primary glomerulopathies,
- immune diseases, etc.

**Tubular/interstitial/vessel damages:**
- **diabetes,**
- **high blood pressure,**
- **thrombotic µangiopathy, etc.**

**800M CKD patients worldwide**
**+10% /year**




**KTx complications:**
- Surgical (early, ie <1 week post KTx),
- Acute/chronic (late) rejection,
- **IS drugs side effects**, ie nephrotoxicity,
- High cholesterol, high blood pressure and cancers, ex: skin, kidney, and lymphomas,
- Viral/bacterial/fungal infections, ex: **BKPyV**, CMV due to over IS.

CR2TI UMR 1064 Centre de Recherche Translationnelle en Transplantation et Immunologie

# The BK polyomavirus

**Taxonomy & structure/genome organization:**
- Polyomaviridae family (Human Polyomavirus 1), naked, icosahedric capsid (45nm ø), dsDNA (5Kb)
- Three genomic regions : Early (LTAg/sTAg), Late (major capsid protein VP1, VP2, VP3) and agnoprotein & NCCR, "viral promoter"
- Cellular receptors: mostly *b-series* gangliosides

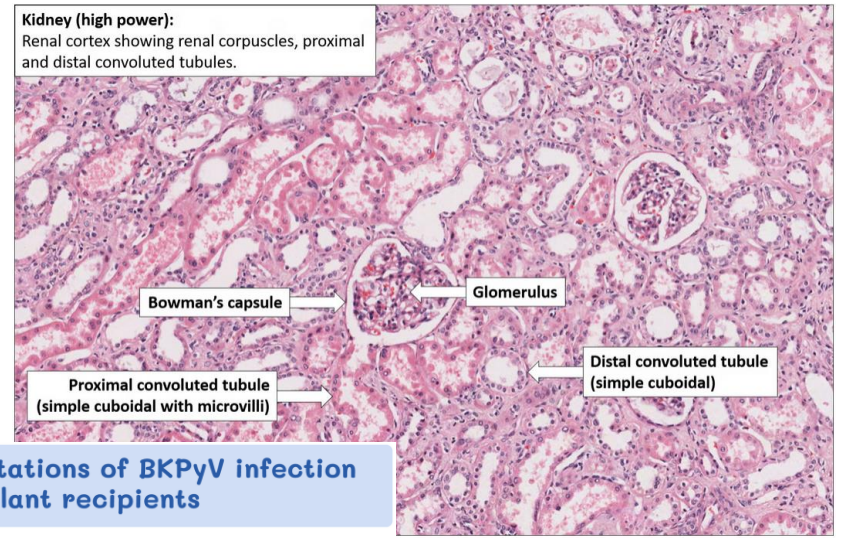
Takemoto and Mullarkey, J. Virol. 1973 ; Cubbit, Adv. Exp. Med. Biol. 2006 ; Johne et al., Arch. Virol. 2011; Low et al., J Virol 2006; Neu et al, PLoS Pathogen 2013; Pastrana et al, J Virol 2013

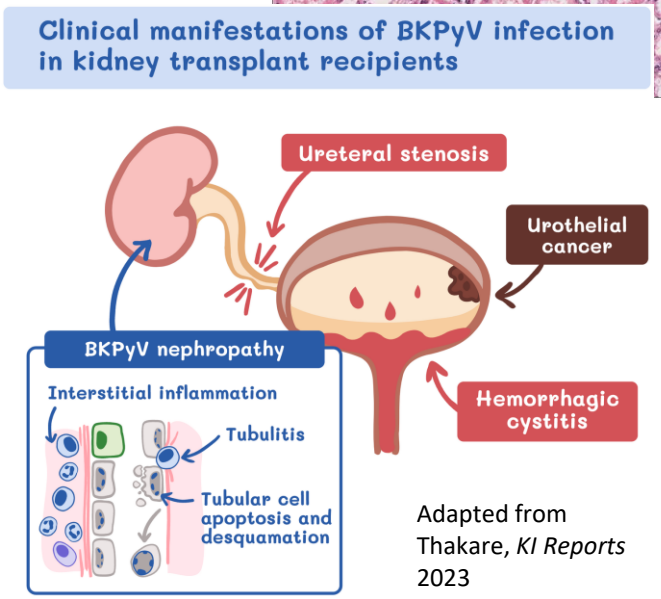
**First discovered human polyomavirus in 1971 (S. Gardner, UK):**
- Seroprevalence around >80% worldwide (>10 yrs), contamination route: upper mucosae ?
- Latency in renourinary tract.

Knowles, Adv. Exp. Med. Biol., 2006 ; Goudsmit et al., J. Med. Virol., 1982 ; Hirsch et Steiger, Lancet Inf. Dis., 2003 ; Gardner et al., The Lancet, 1971; Kean et al, PLoS Pathogens, 2009; Heritage et al., J. Med. Virol., 1981

**Reactivates in >50% of post-transplant kidney (3-9 months post-Tx):**
- 2-5% BKPyV-Associated Nephropathy (BKPyVAN)
- Main risk factors for DNAemia/BKPyVAN: Tacrolimus, Sex/age (donor), pre-transplant serum Ab level (donor) -> BKPyVAN/graft loss association (not death)
- **No approved drug (nephrotoxicity)**, IS reduction/shift

Kotton et al., Transplantation 2024; Gately et al., Kidney International Reports 2023; Nankivell, Transplantation Direct 2025; Nickeleit et al., J. Am. Soc. Nephrol. 1999; Arthur et al., NEJM 1986




Adapted from Thakare, *KI Reports* 2023

CR2TI UMR 1064 Centre de Recherche Translationnelle en Transplantation et Immunologie

# Bioprinting on-chIp micRophysiological moDels of humanized kidney tubulointErstitium (BIRDIE)

The consortium:

Maastricht University

Nantes Université

fluicell® Pioneers in Single Cell Biology

TISSUSE Emulating Human Biology

www.birdieproject.eu

This project has received funding from the European Union's Horizon 2020 FET Open programme under grant agreement No 964452

Alumni:



Coraline Chéneau
Postdoc/MPS



Kevin Riquin
Postdoc/ bioinformatics



Vivek D Ranjan



Wesley Delage



Flora Coulon
AI INSERM

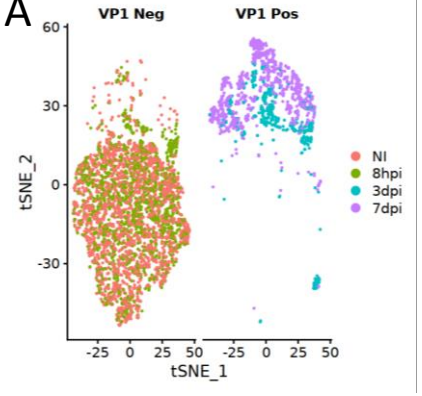


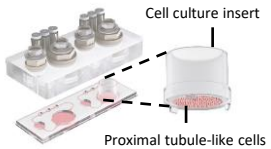

CR2TI UMR 1064 Centre de Recherche Translationnelle en Transplantation et Immunologie

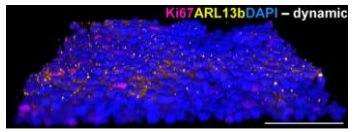
# Spatial Tx & sc-/bulk RNA-seq: cross-validation

- Analyzed samples: pre-Tx, TCMR, early and late BKPyV infections including PVANs.
- Single-cell/bulk analyses using our advanced BKV infection model on a chip.

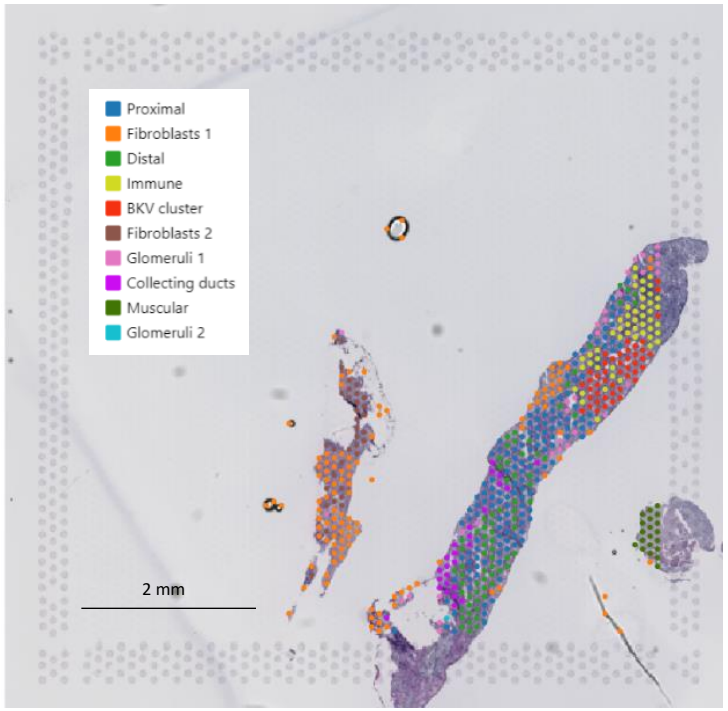
A



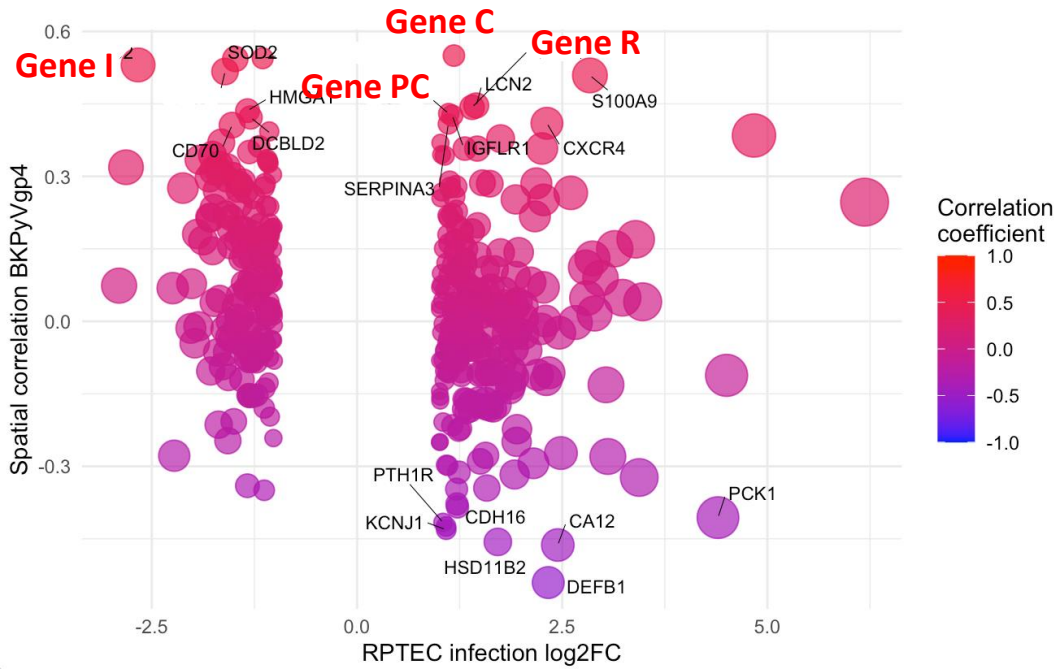




B




Reference from Lake et al., *Nature* 2023

C




**NB: gene PN expression also correlates with BKV gene expression in STx datasets.**

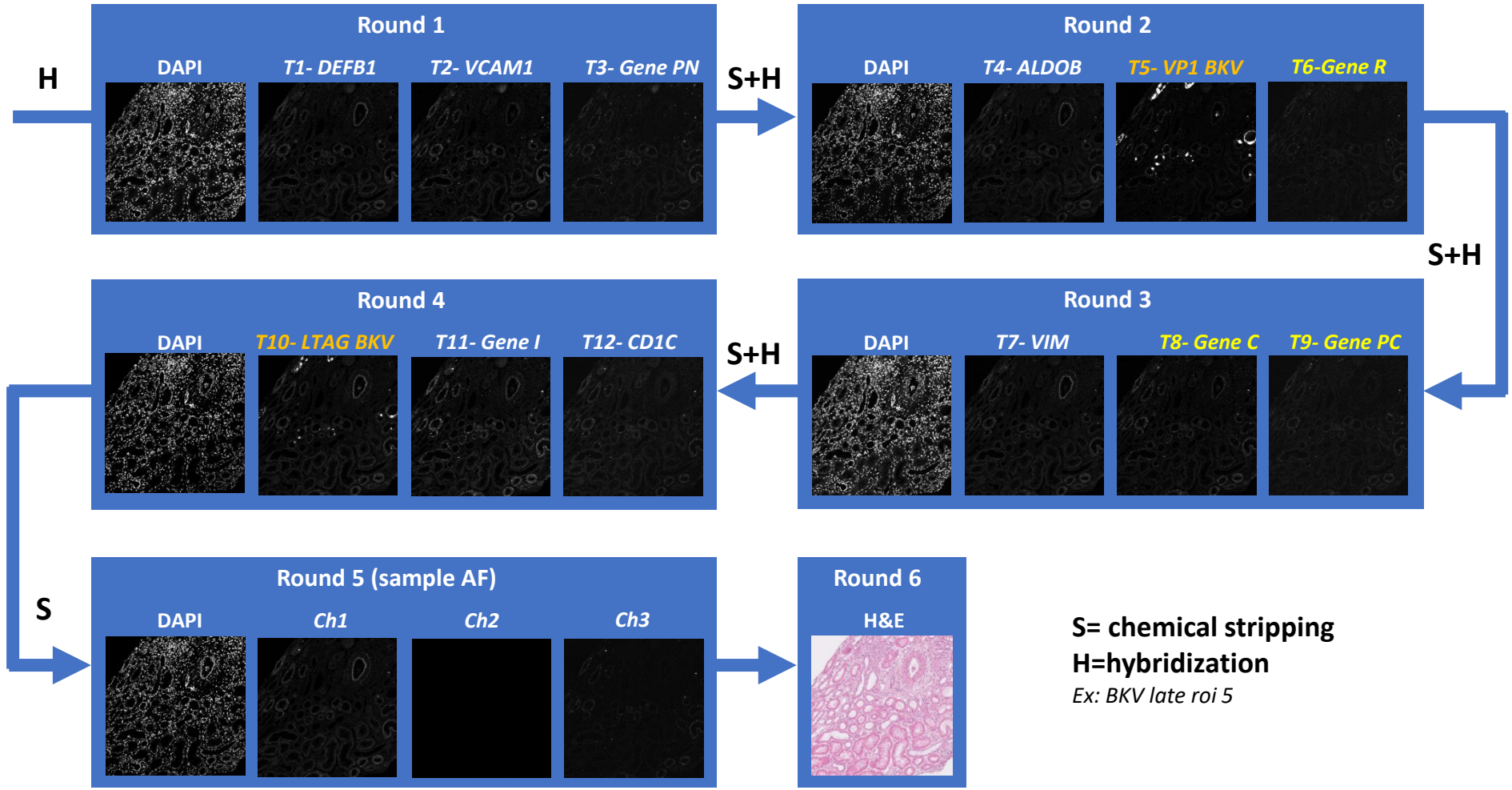
CR2TI Centre de Recherche Translationnelle en Transplantation et Immunologie UMR 1064

Datasets on the Zenodo repository

# How to locate gene expression at the sub-cellular level ?

- **Sensitivity/probe detection ?**
- **How to image (precision/resolution/alignment) ?**
- **Impact of sample fixation method ?**

| Probe Nbr | Probe name | Fluo |
|---|---|---|
| T1 | DEFB1 | AF488 |
| T2 | VCAM1 | Dylight 550 |
| T3 | Gene PN | Dylight 650 |
| T4 | ALDOB | AF488 |
| T5 | VP1 | Dylight 550 |
| T6 | Gene R | Dylight 650 |
| T7 | VIM | AF488 |
| T8 | Gene C | Dylight 550 |
| T9 | Gene PC | Dylight 650 |
| T10 | LTAG | AF488 |
| T11 | Gene I | Dylight 550 |
| T12 | CD1C | Dylight 650 |

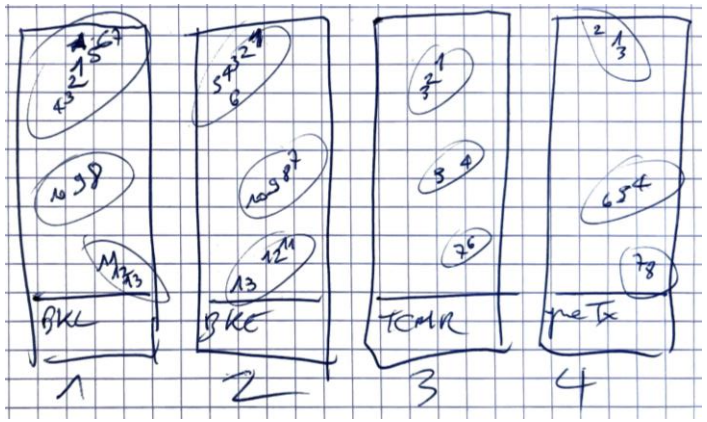


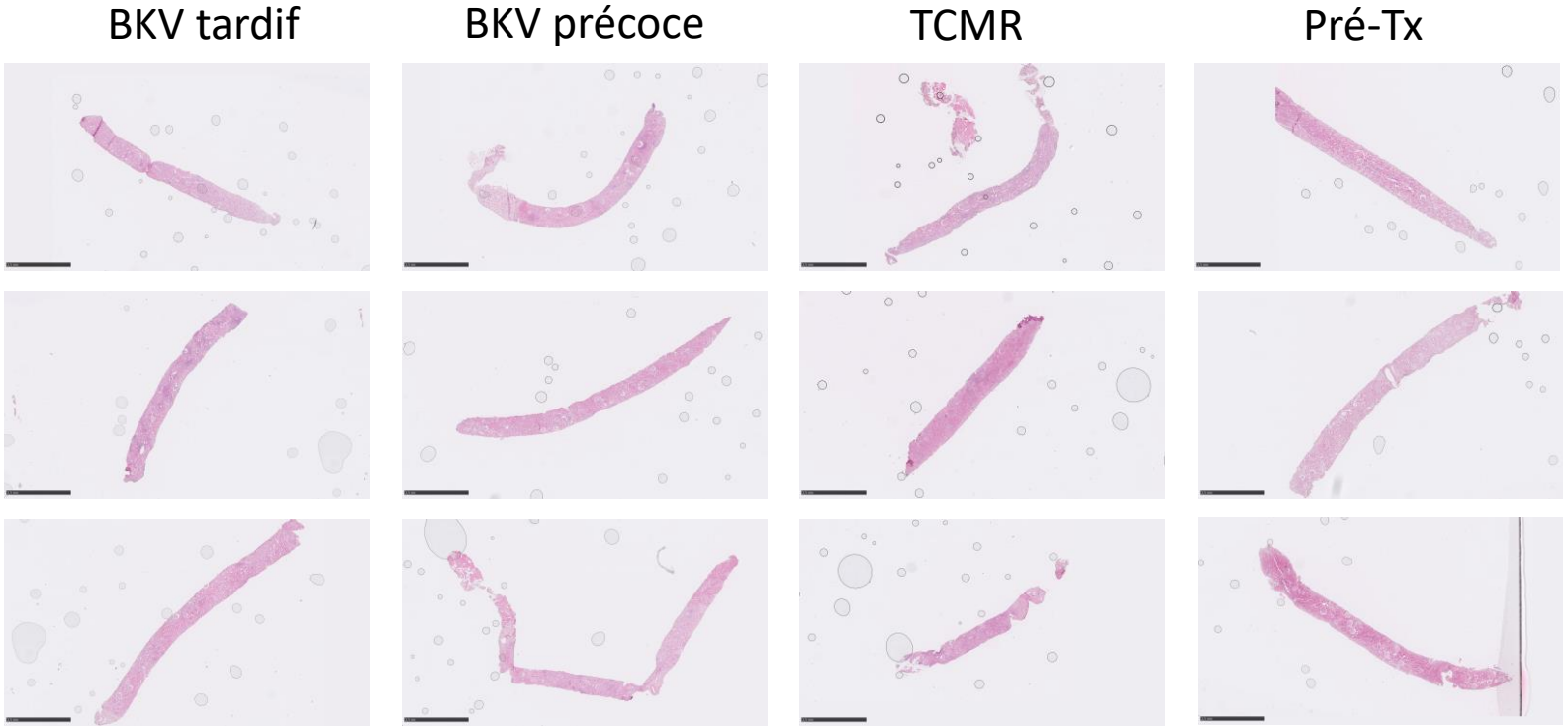

**S= chemical stripping**
**H=hybridization**
*Ex: BKV late roi 5*

CR2TI UMR 1064 Centre de Recherche Translationnelle en Transplantation et Immunologie

# Samples location on glass slides



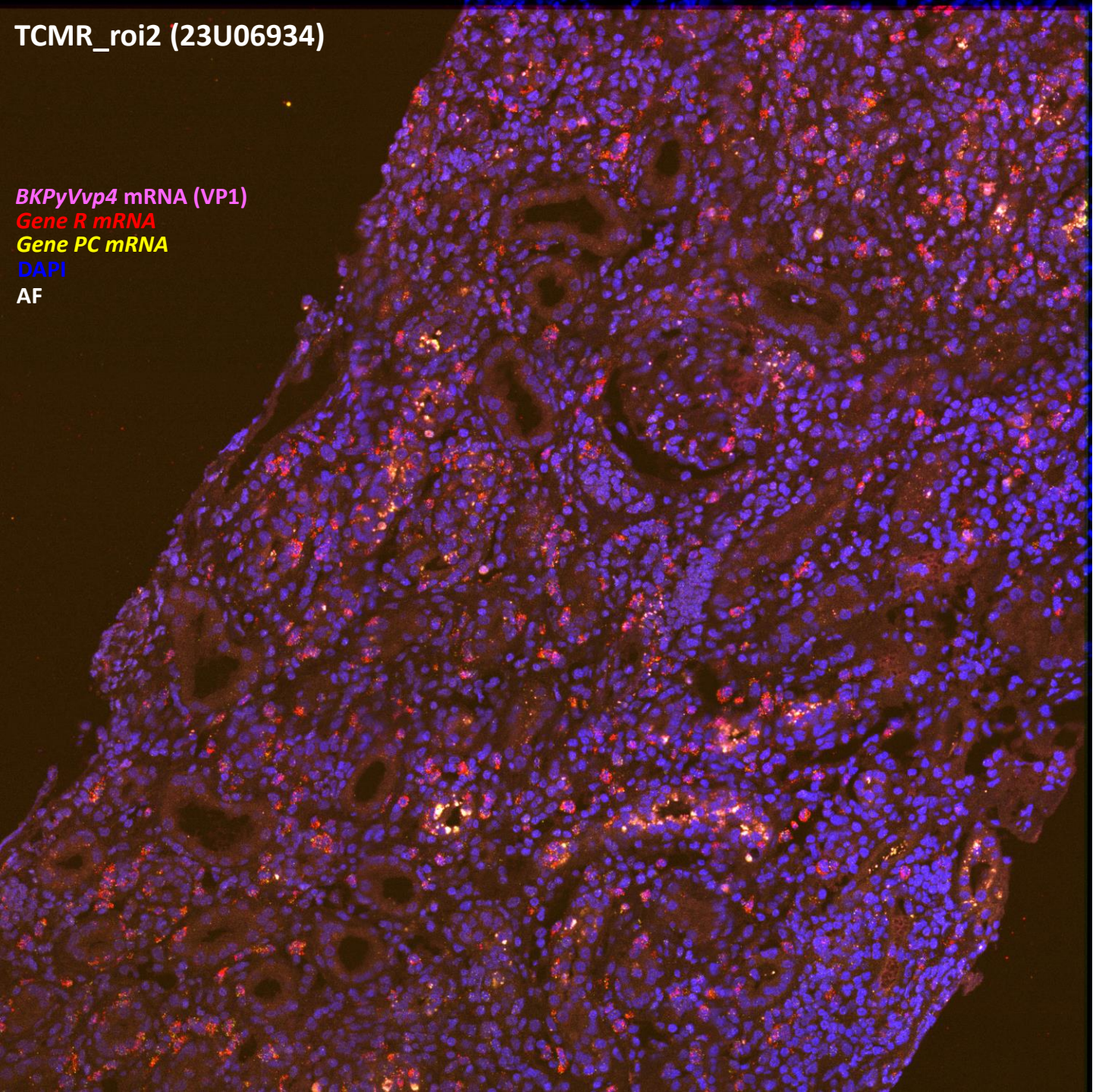



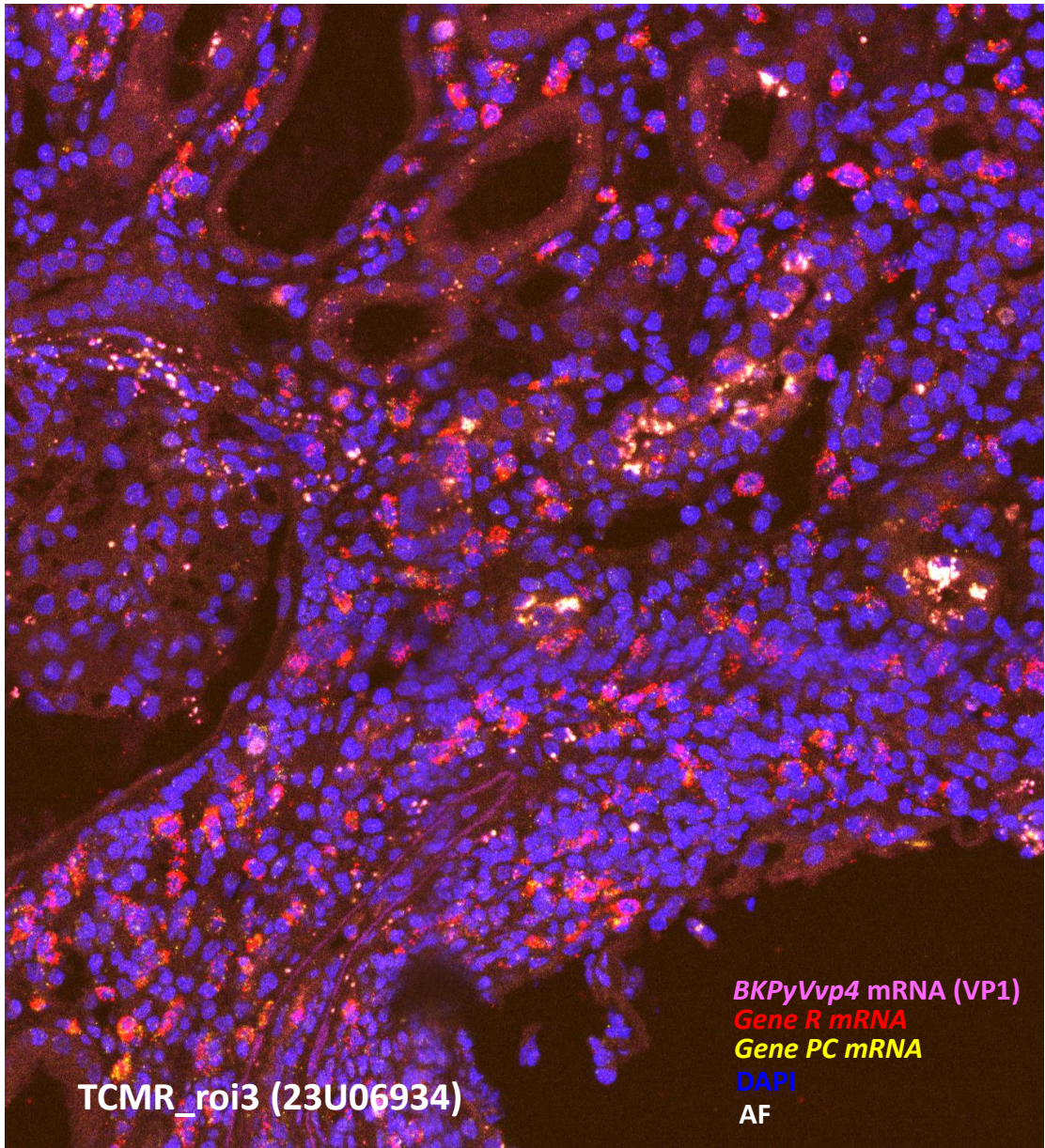



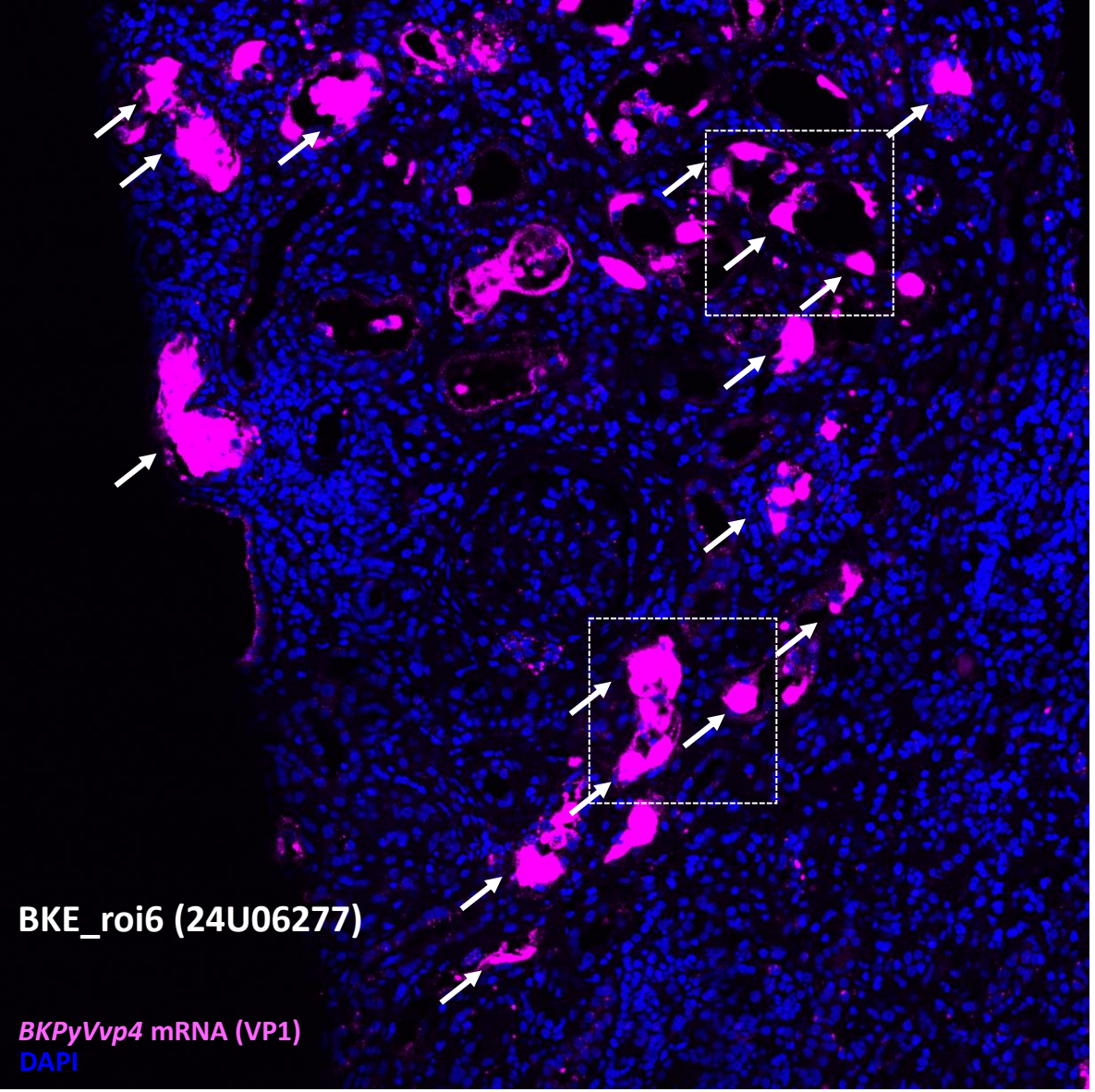

Scale bar=2.5mm

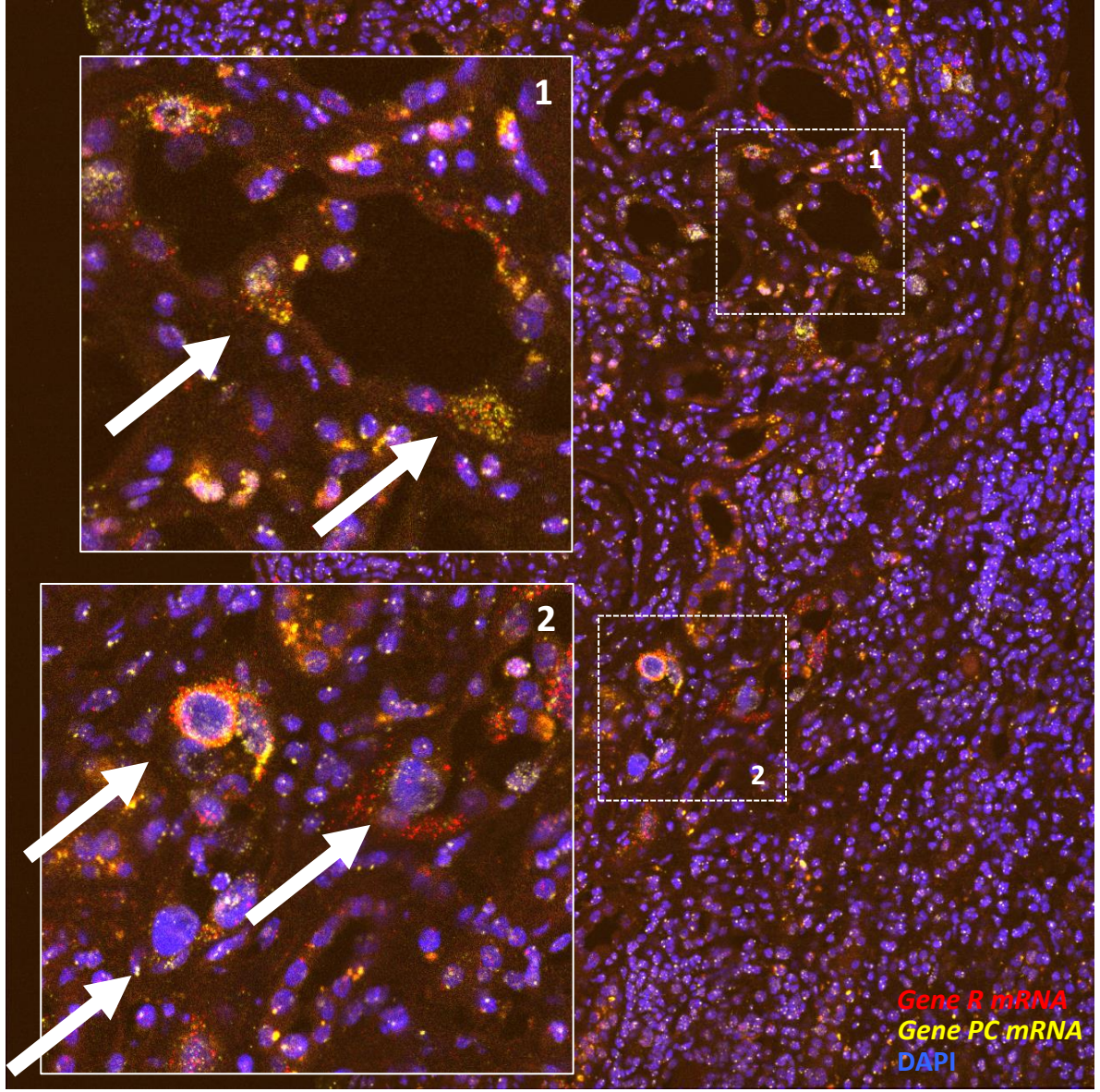
CR2TI Centre de Recherche Translationnelle en Transplantation et Immunologie UMR 1064

# Gene R/Gene PC are co-expressed by BKV-infected cells

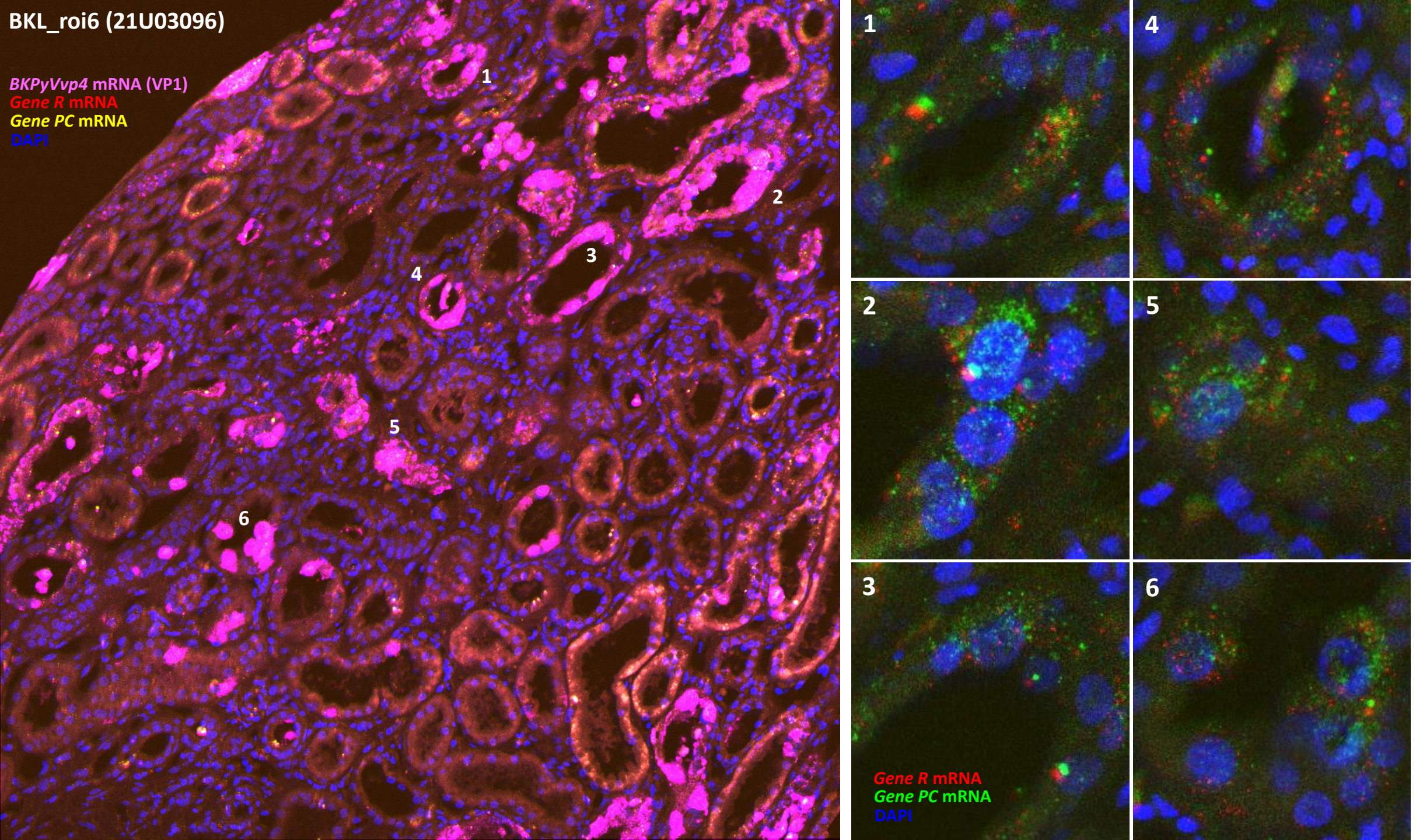


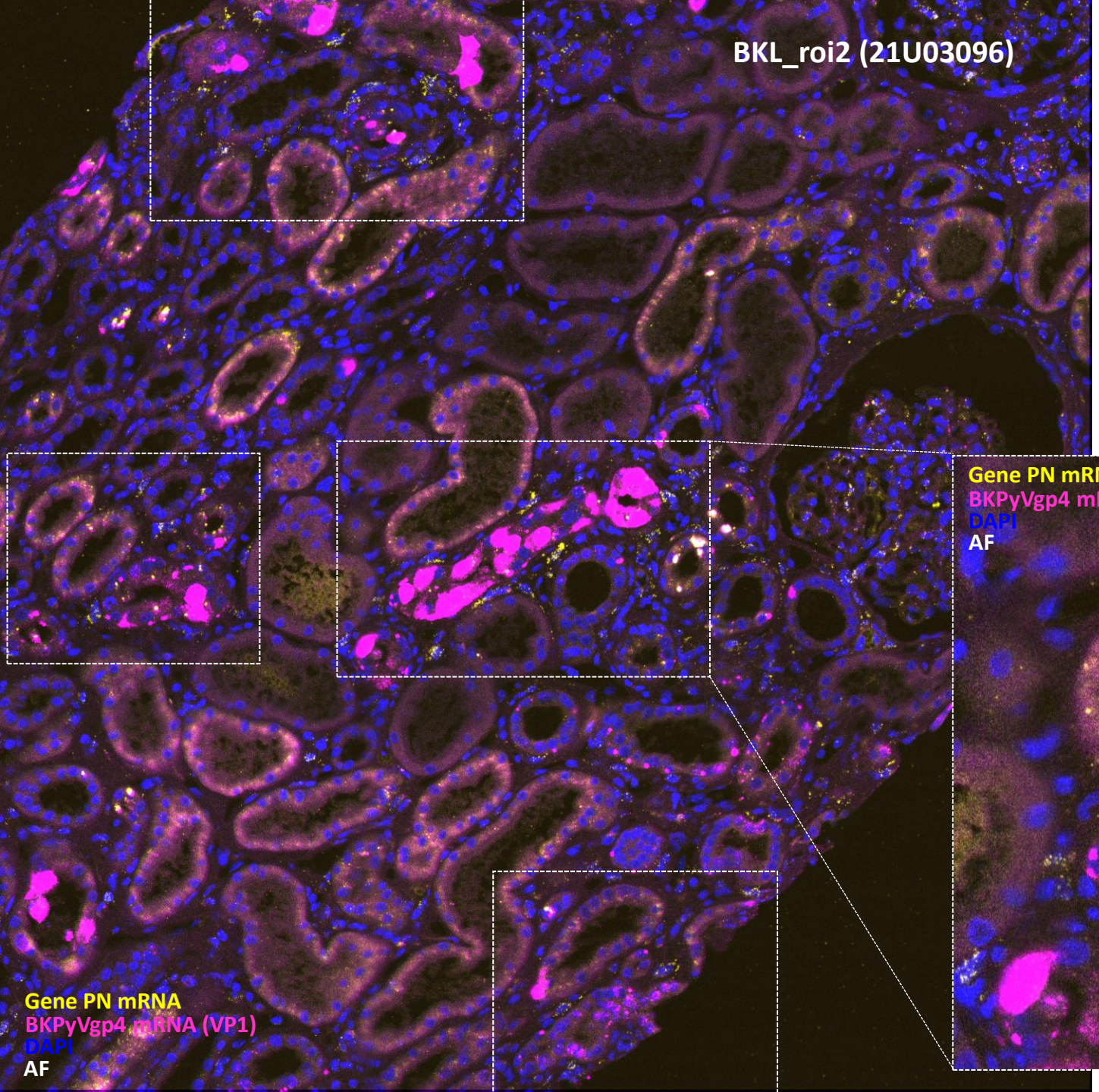


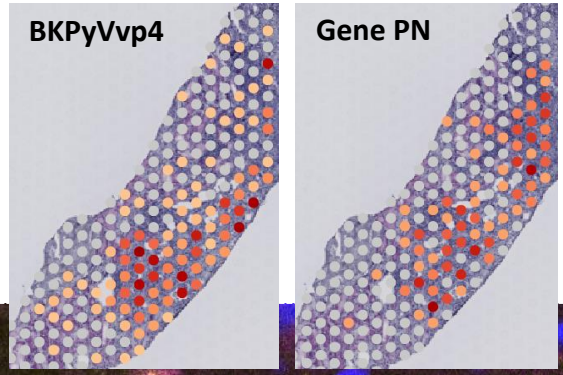



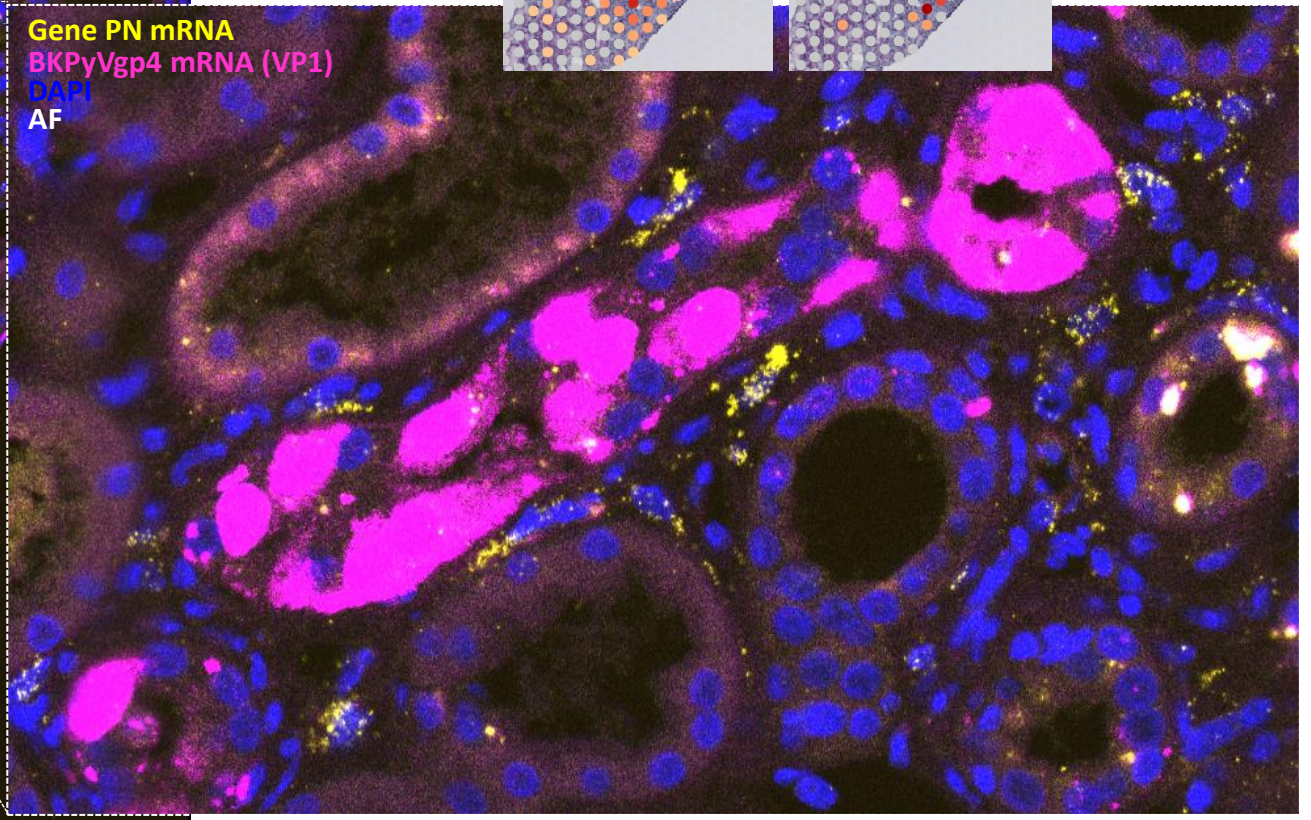

CR2TI UMR 1064 Centre de Recherche Translationnelle en Transplantation et Immunologie


BKL_roi6 (21U03096)
BKPyVvp4 mRNA (VP1)
Gene R mRNA
Gene PC mRNA
DAPI
1
2
3
4
5
6
1
4
2
5
3
6
Gene R mRNA
Gene PC mRNA
DAPI

# Gene PN is mostly upregulated in interstitial cells not in BKV-infected renal tubular cells



# Conclusions

- RNAscope™ 12-Hiplex is convenient to rapidly explore gene expression at the sub-cellular level, *i.e.*, especially for target genes with no commercialized Abs.

- Good spatial resolution (sensitivity ?) -> allowed for STx data refinement by confirming co-expression or showing distinct expression profiles within the tissue section, between newly identified cellular genes and viral genes.

- Fast custom probe design, with super strong signals for VP1 and LTAG mRNAs. Very good user experience (clear protocols).

- Main attention points: ease-of-use/result quality are dependent on the optical device to image (quality of alignment). In our hands, the LIPSI platform (MicroPicell) provided the expected results. High autofluorescence/background can negatively impact results (fixation protocols). Time consuming but straight method (manual setting) if all controls are done (control slides, probeset tests on irrelevant tissues, etc).

# Acknowledgement


CR2TI Centre de Recherche Translationnelle en Transplantation et Immunologie UMR 1064

Coraline Chéneau,
Kevin Riquin,
Flora Coulon,

Alumni:
Vivek Damodar Ranjan,
Wesley Delage,

Single Cell Facility:
Cynthia Fourgeux

Victor Gourain
Lucas Brussel
Martin Braud
Jeremie Poschmann

BIRDIE

Maastricht University

**Carlos Mota, MERLN Institute, Universiteit Maastricht**

TISSUSE Emulating Human Biology

Michelle Lechtenberg, Leopold Koenig, and Eva Maria Dehne, Berlin

fluicell Pioneers in Single Cell Biology

Gavin Jeffries and Jonas Hannestad, Göteborg

Nantes Université

CHU NANTES

Tumorothèque:
Karine AUTAIN-RENAUDIN
Cécile GIRARD

Institutions:

Nantes Université

Inserm

Funders:

European Innovation Council

anr agence nationale de la recherche

RÉGION PAYS DE LA LOIRE

Facilities/platforms:

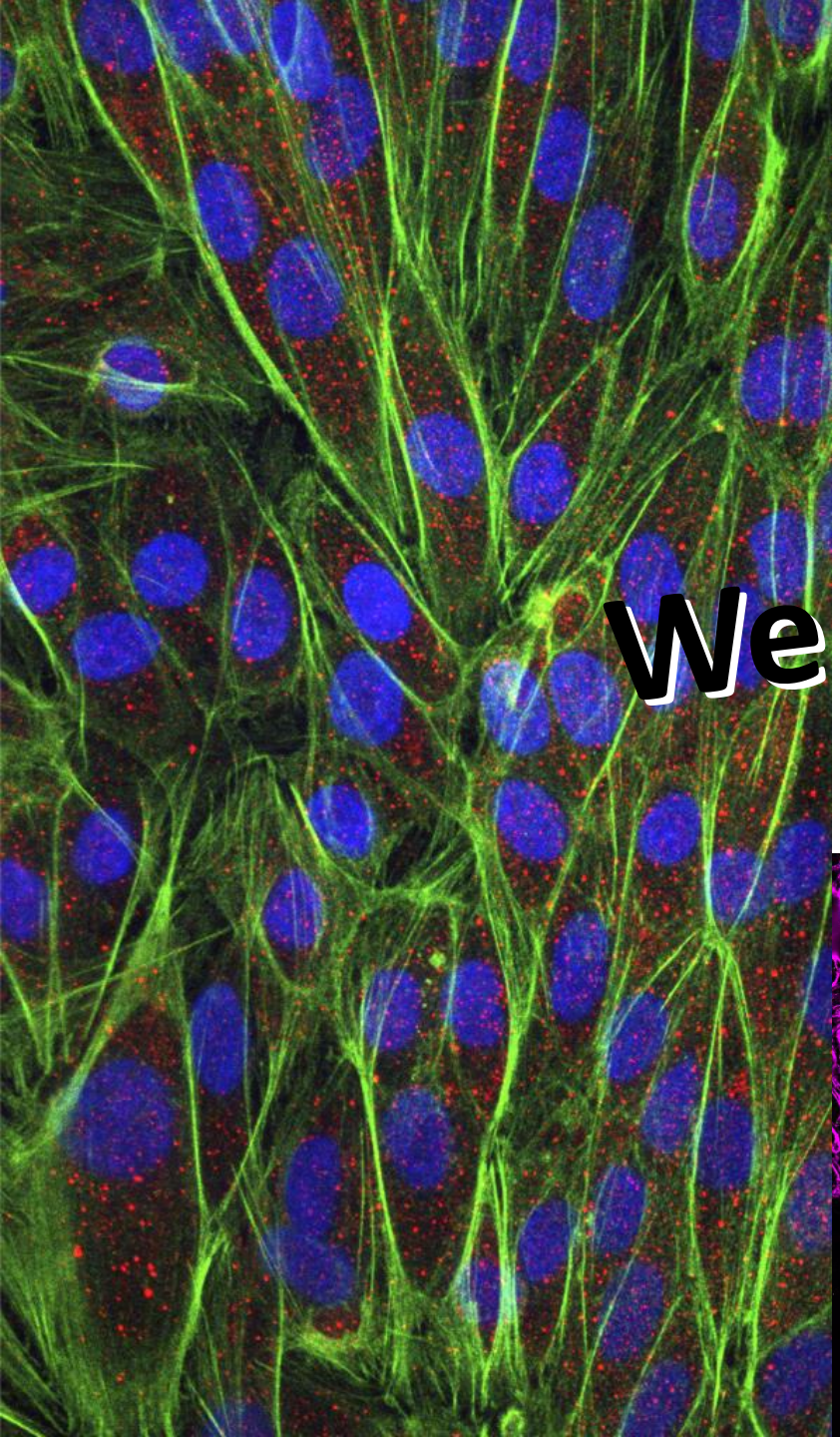
MicroPICell

Philippe Hulin,
Steven Nedellec,
Aurélie Joussaume,

Aurélie Doméné,
Perrine Paul-Gilloteaux,
Mélodie Ambroset



CR2TI Centre de Recherche Translationnelle en Transplantation et Immunologie UMR 1064



VIR-FLOW

anr agence nationale de la recherche

# We are hiring !!!

franck.halary@univ-nantes.fr or

