

# **Discovery of Genetic Variants of the Kinases that Activate Tenofovir in a Compartment-Specific Manner**

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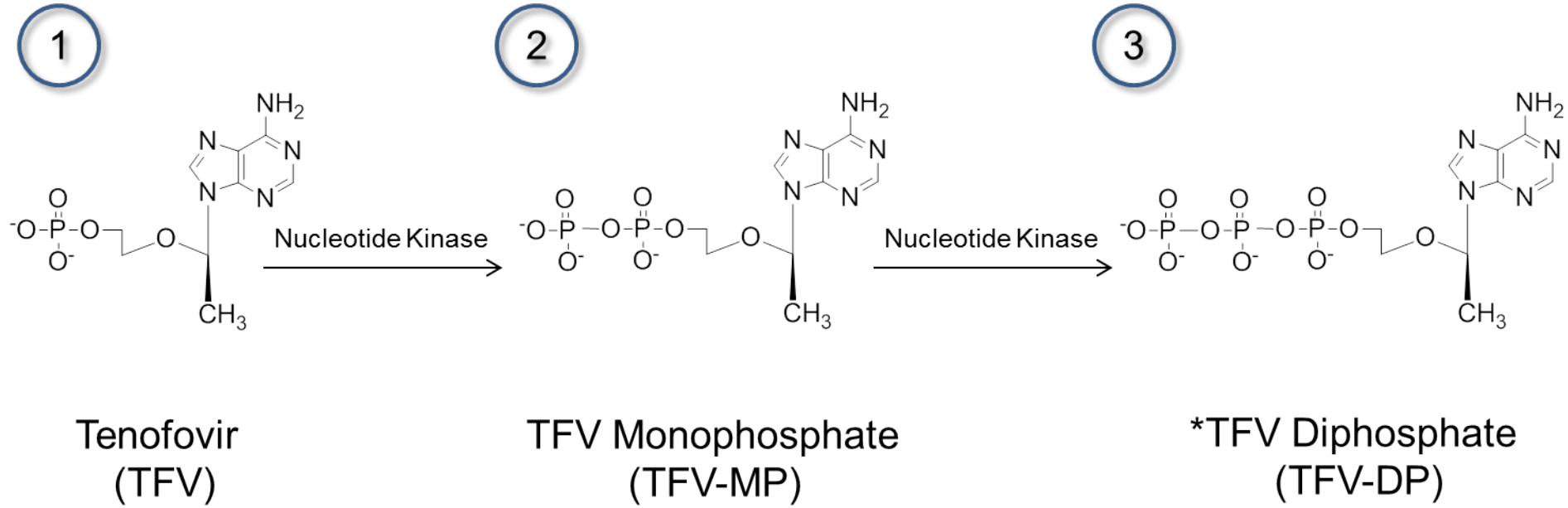
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# Tenofovir Requires Activation by Nucleotide Kinases



**Which nucleotide kinases contribute to tenofovir activation in cells and tissues at risk of HIV infection?**

# Nucleotide Mono- and Di-Phosphate Kinase Isoforms to be Investigated

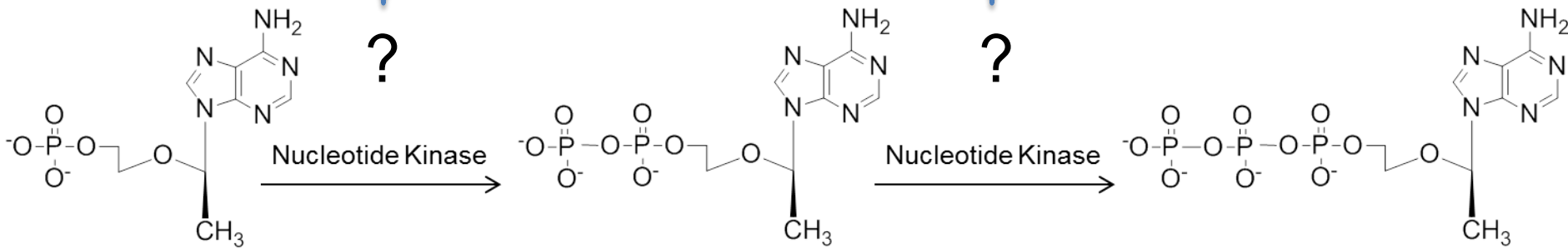
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- **Adenylate Kinases**,  $\text{ATP} + \text{AMP} \rightarrow 2 \text{ADP}$ 
  - 9 isoforms with differential tissue distributions and subcellular localizations
- **Guanylate Kinases**,  $\text{ATP} + \text{GMP} \rightarrow \text{ADP} + \text{GDP}$ 
  - 3 nucleotide phosphorylating isoforms
- **Nucleotide Diphosphate Kinases**,  $\text{NTP} + \text{NDP} \leftrightarrow \text{NDP} + \text{NTP}$ 
  - 4 enzymatically active isoforms
- **Creatine Kinases**,  $\text{ATP} + \text{Creatine} \rightarrow \text{ADP} + \text{Phosphocreatine}$ 
  - Cytosolic and mitochondrial isoforms
- **Pyruvate Kinases**,  $\text{Phosphoenolpyruvate} + \text{ADP} \rightarrow \text{Pyruvate} + \text{ATP}$ 
  - 4 isozymes result from differential splicing

# Candidate Nucleotide Kinases

Adenylate kinase 2 (AK2)  
Guanylate kinase 1 (GUK1)

Creatine kinase, muscle (CKM)  
Pyruvate kinase, muscle (PKM)  
Pyruvate kinase, liver & RBC (PKLR)



Tenofovir  
(TFV)

TFV Monophosphate  
(TFV-MP)

TFV Diphosphate  
(TFV-DP)

# siRNA Knockdown of Nucleotide Kinases

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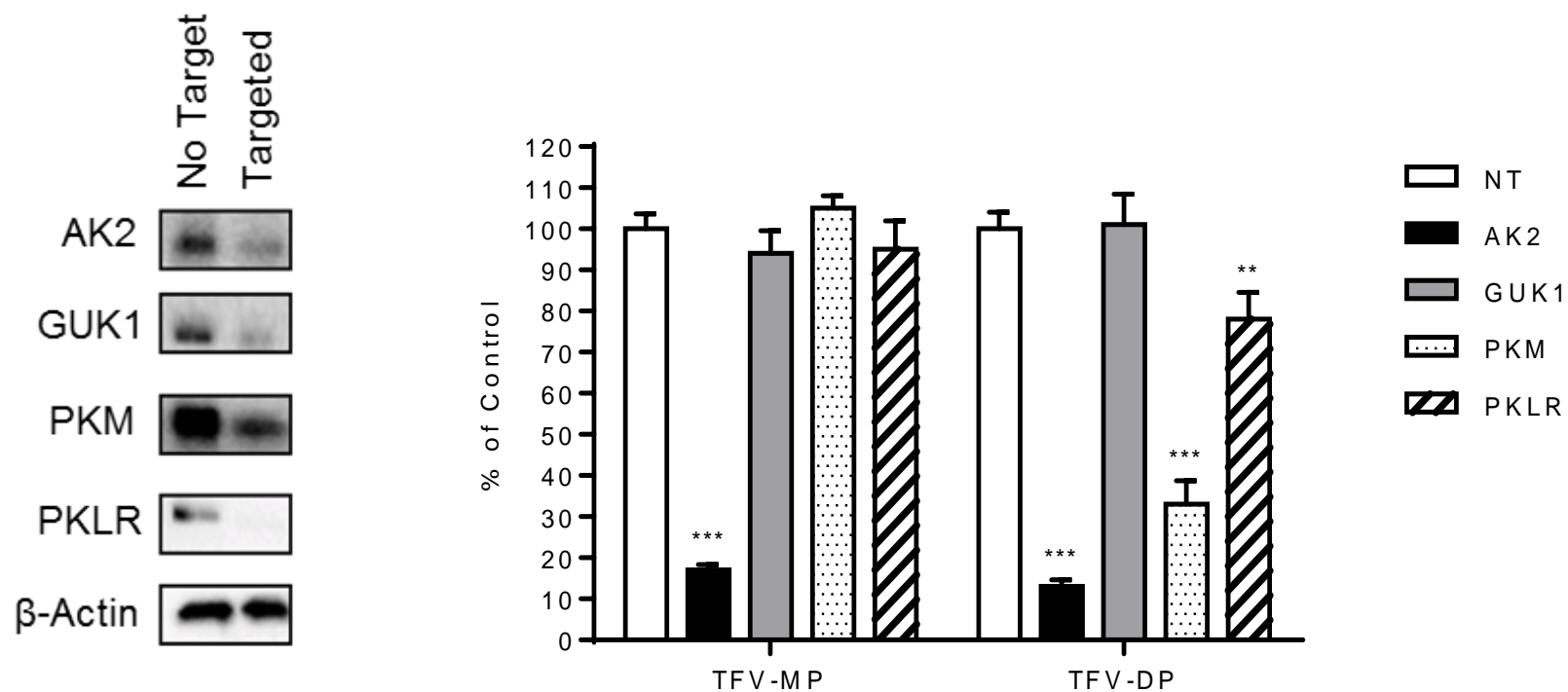
## Human Samples

- Cells and tissues:
  1. Peripheral blood mononuclear cells (PBMC)
  2. Colorectal tissue
  3. Vaginal tissue

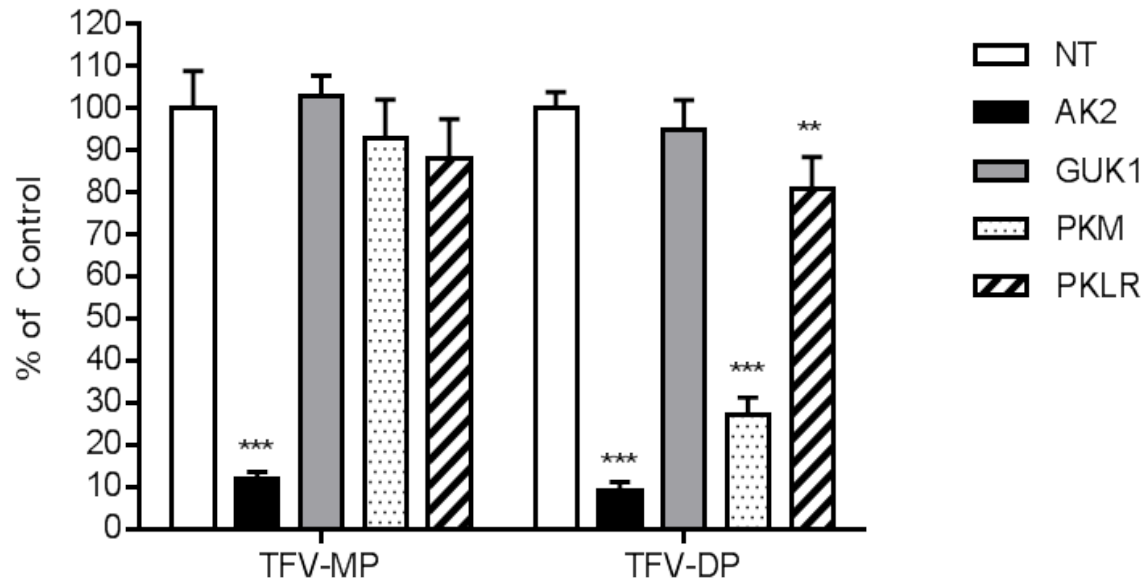
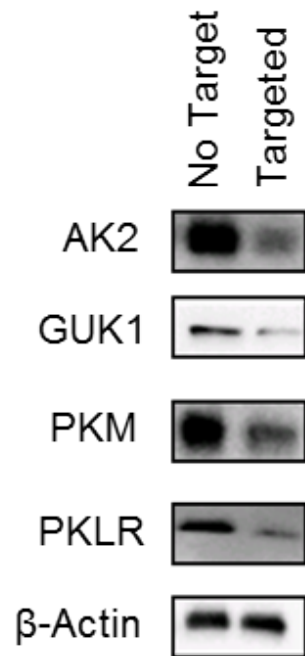
## Method

- Delivered siRNA to cells and tissues in culture
- Followed by incubation with TFV
  - Detected TFV metabolites using ultra-high performance liquid chromatography-tandem mass spectrometry

# AK2, PKM, and PKLR Contribute to Metabolite Formation in Peripheral Blood Mononuclear Cells (PBMC)

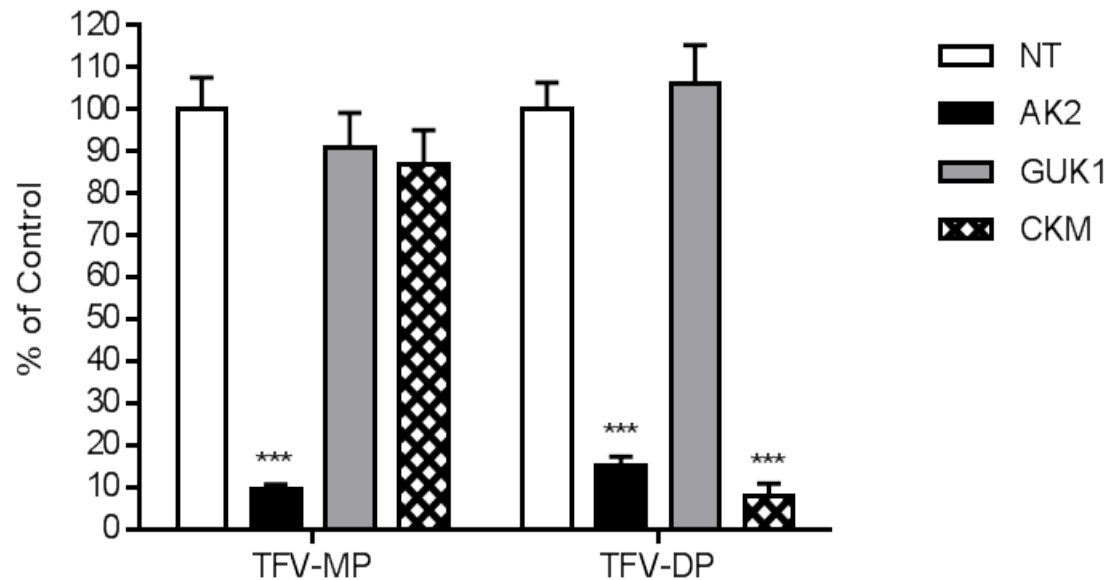
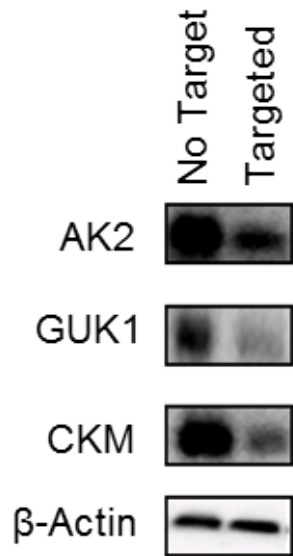


# AK2, PKM, and PKLR Contribute to Metabolite Formation in Vaginal Tissue



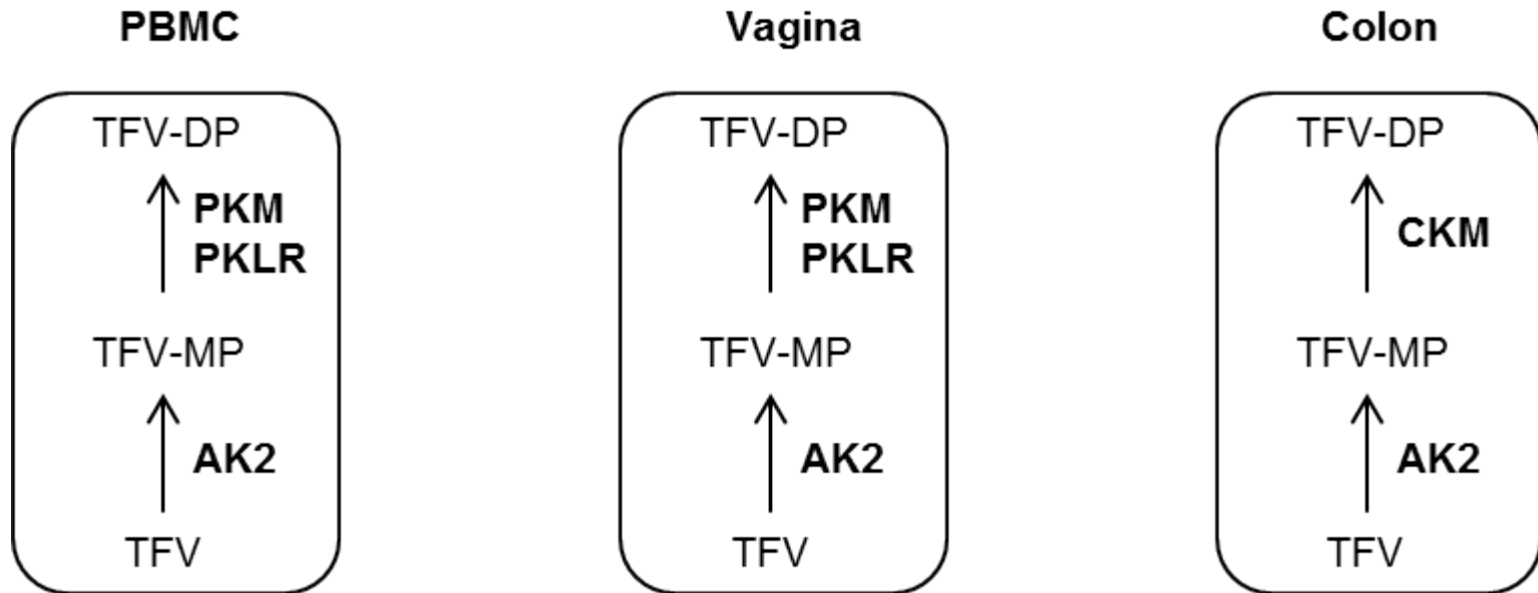


# AK2 and CKM Contribute to Metabolite Formation in Colorectal Tissue



# Tenofovir is Activated in a Tissue-Specific Manner

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**Do human genetic variants exist in the genes encoding  
these nucleotide kinases?**

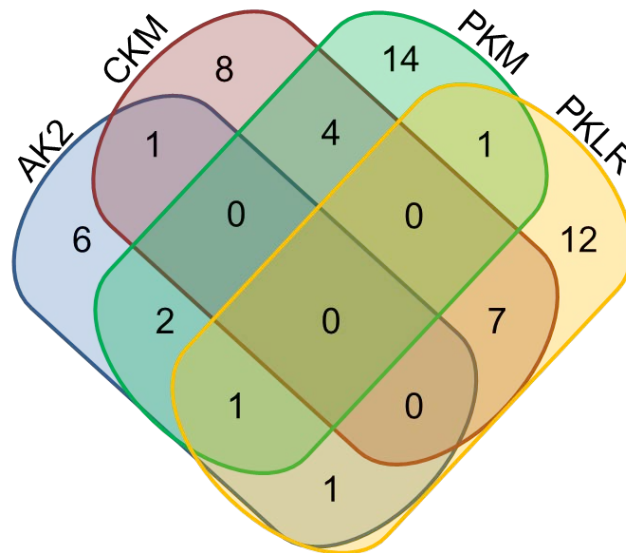
# Next-Generation Sequencing of MTN-001 Samples

## 18 *CKM* variants

- 17 participants
- 3% frequency of deleterious missense variants – 1 USA, 1 SA, 2 UGA participants

## 19 *PKM* variants

- 19 participants
- Unable to predict phenotype of missense variants



## 12 *AK2* variants

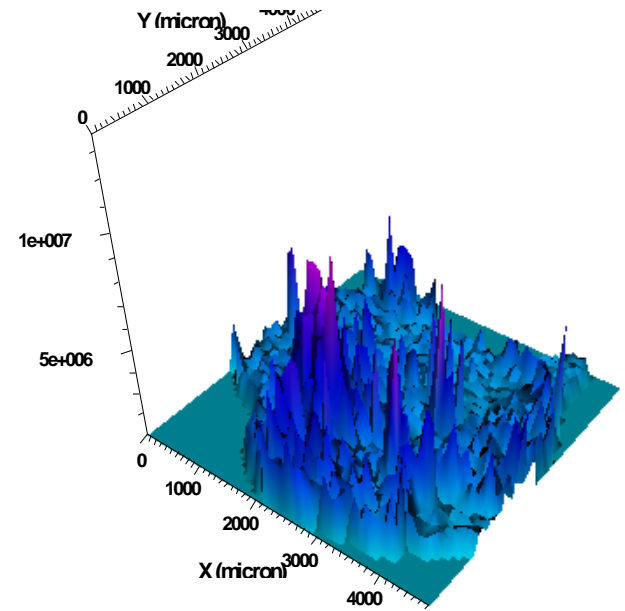
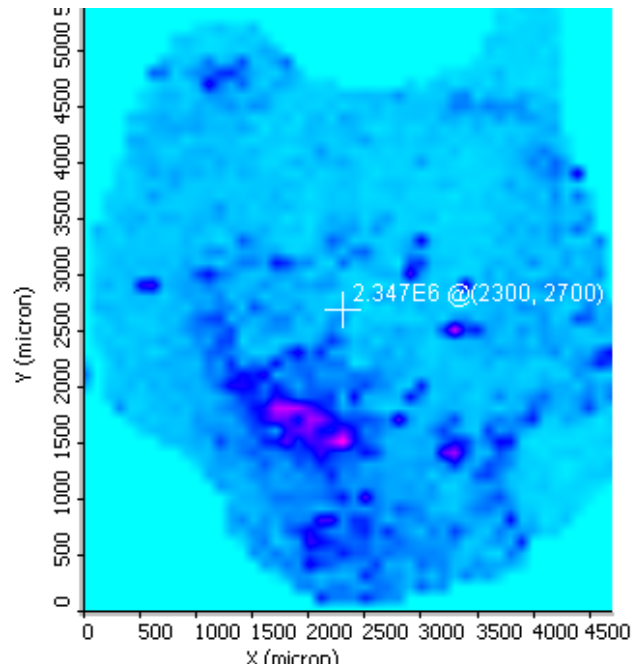
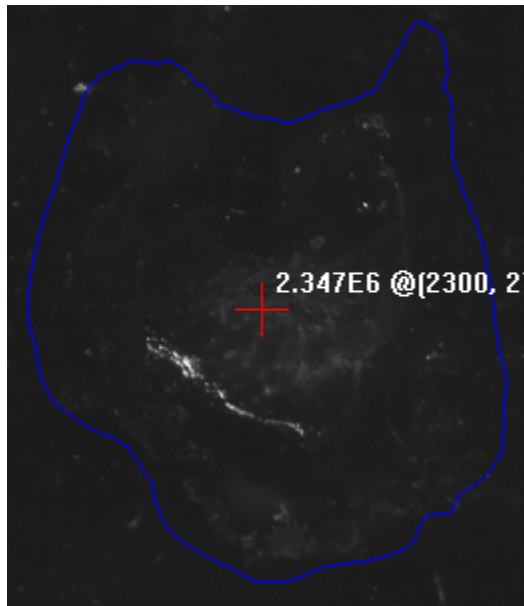
- 11 participants
- 2% frequency of deleterious missense variants – 1 USA, 2 SA participants

## 22 *PKLR* variants

- 21 participants
- 3% frequency of deleterious missense variants – 3 USA, 1 SA participants

# MALDI-MS Imaging of TFV

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# Acknowledgements

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## Lab Members:

Philip Cox

Dominique Figueroa

Carley Heck

Julie Lade

Elaine To

## Collaborator:

Craig Hendrix

## Funding:

MTN

HPTN

NIH R01GM103853