

# Transcriptomic Analysis Reveals Systemic Effects of Heart Xenotransplantation: Implications for Multi-Organ Function and Safety

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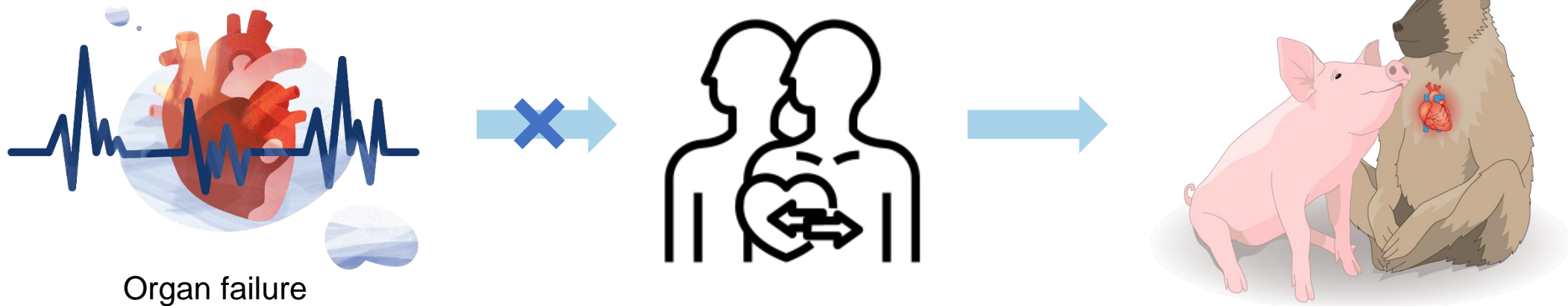


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## Xenotransplantation

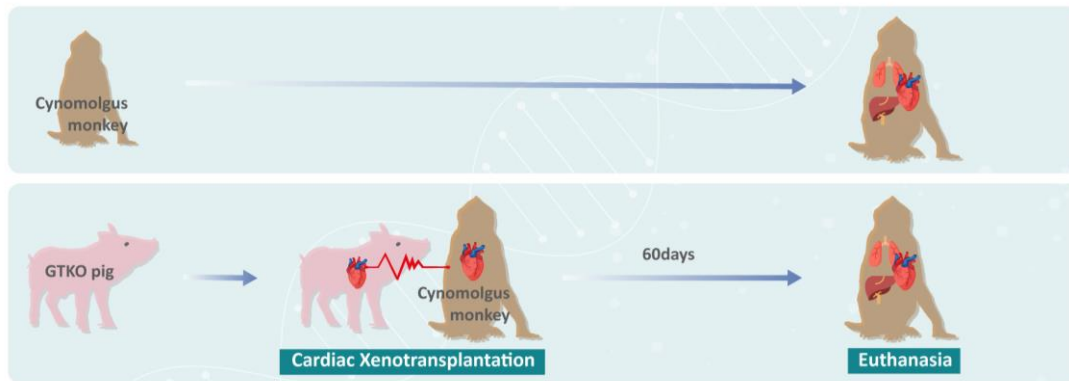
- Procedure that involves the transplantation, implantation or infusion into a recipient of either live cells, tissues, or organs from other animal source
- Genetically engineered pig hearts are a potential alternative for heart failure due to limited human donors



**To elucidate the transcriptomic mechanisms occurring in other tissues subsequent to pig-to-monkey xenotransplantation.**

## 1. Animal and experimental design

Days	-7	35	8	14	21	28	35	42	49	95	
POD		POD0								POD60	Phenotype analysis



(Hetero heart xenotransplantation)

## 2. RNA-seq

- Library & sequencing

- RNA extraction from 24 samples (WH [3], WA [3], WLi [3], WLu [3], XTH [3], XTA [3], XTLi [3], XTLu [3])
- cDNA libraries were constructed with the TruSeq Stranded Total RNA LT Sample Prep Kit
- 150 bp paired-end sequencing by Illumina HiSeq X Ten

Wile heart (Not xenotransplanted heart)



- Raw data processing

- Quality check (FastQC-0.11.8)
- Trimming (Trimmomatic-0.39)
- Mapping / Sorting (HISAT2-2.1.0 / SAMtools-1.9)
- Counting (featureCounts (Subread-1.6.3))

- Annotation

- Macaca fascicularis 5.0.101

## 3. Differentially expressed gene (DEG) analysis

- Normalization: The gene expression level relative abundances were obtained as the trimming means of M values (TMM) (edgeR-3.22.5)
- Cutoff: absolute  $\log_2$  fold change  $\geq 1$  and FDR  $< 0.05$

## 4. Functional annotation

- Gene Ontology (GO) database
- Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database

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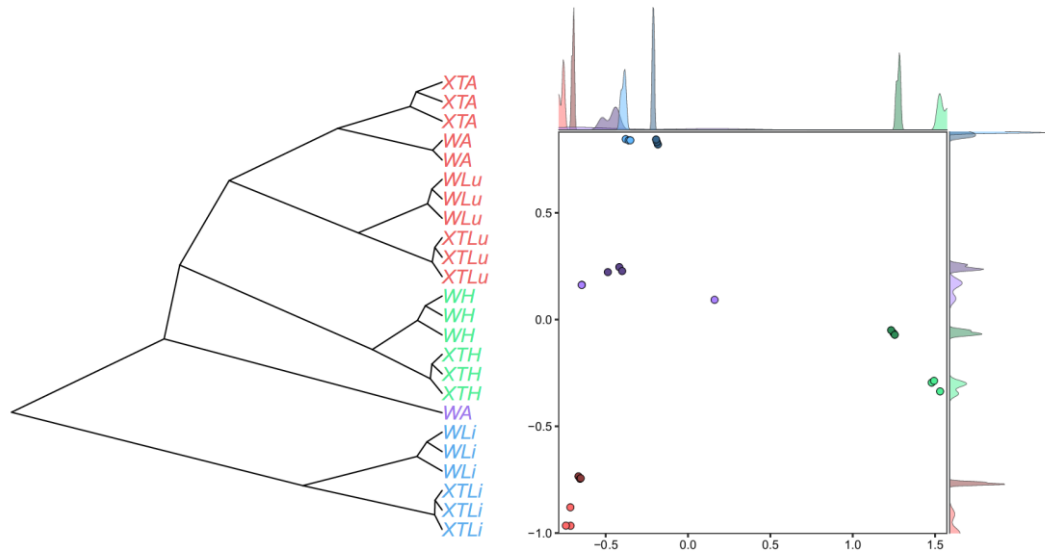


Fig 1. Cluster and multi-dimensional scaling (MDS) plot.

**Liver and lung had relatively higher changes after heart xenotransplantation**

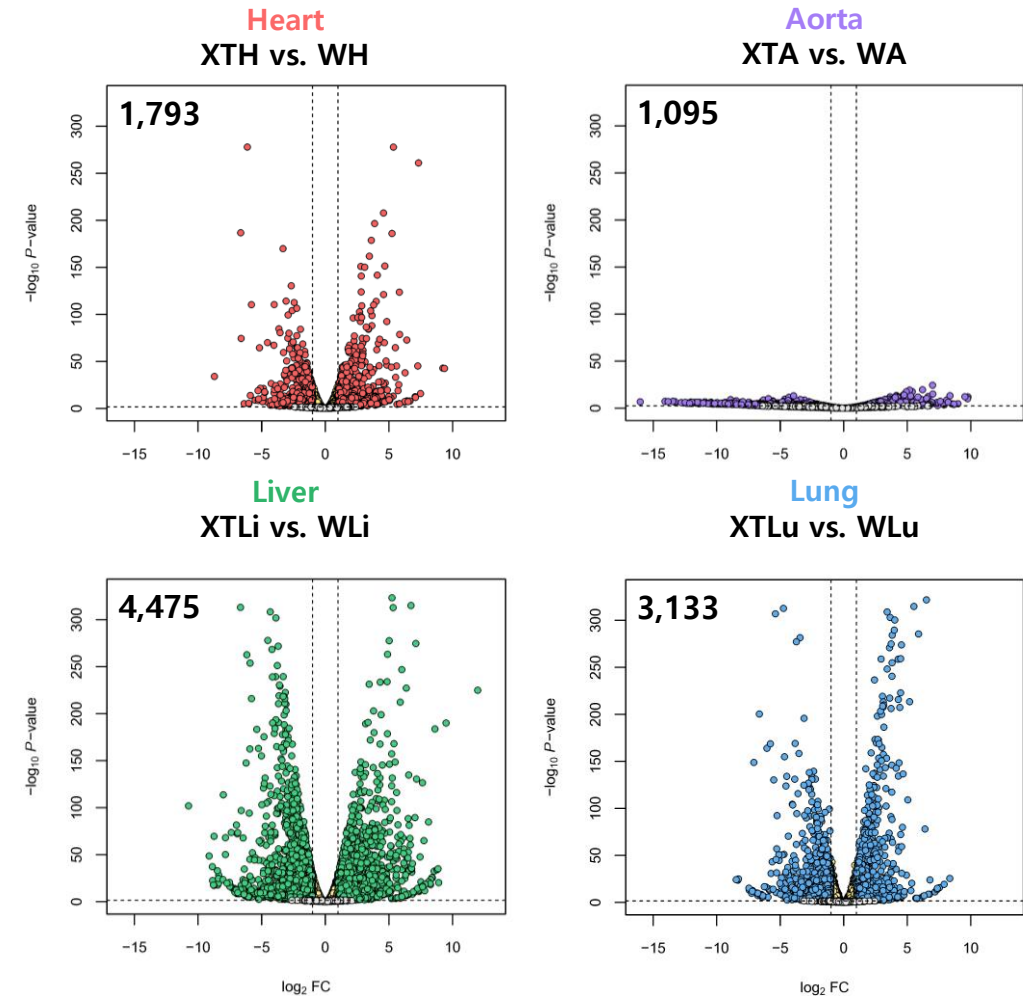


Fig 2. Volcano plots. The number of DEGs are indicated top left corner.

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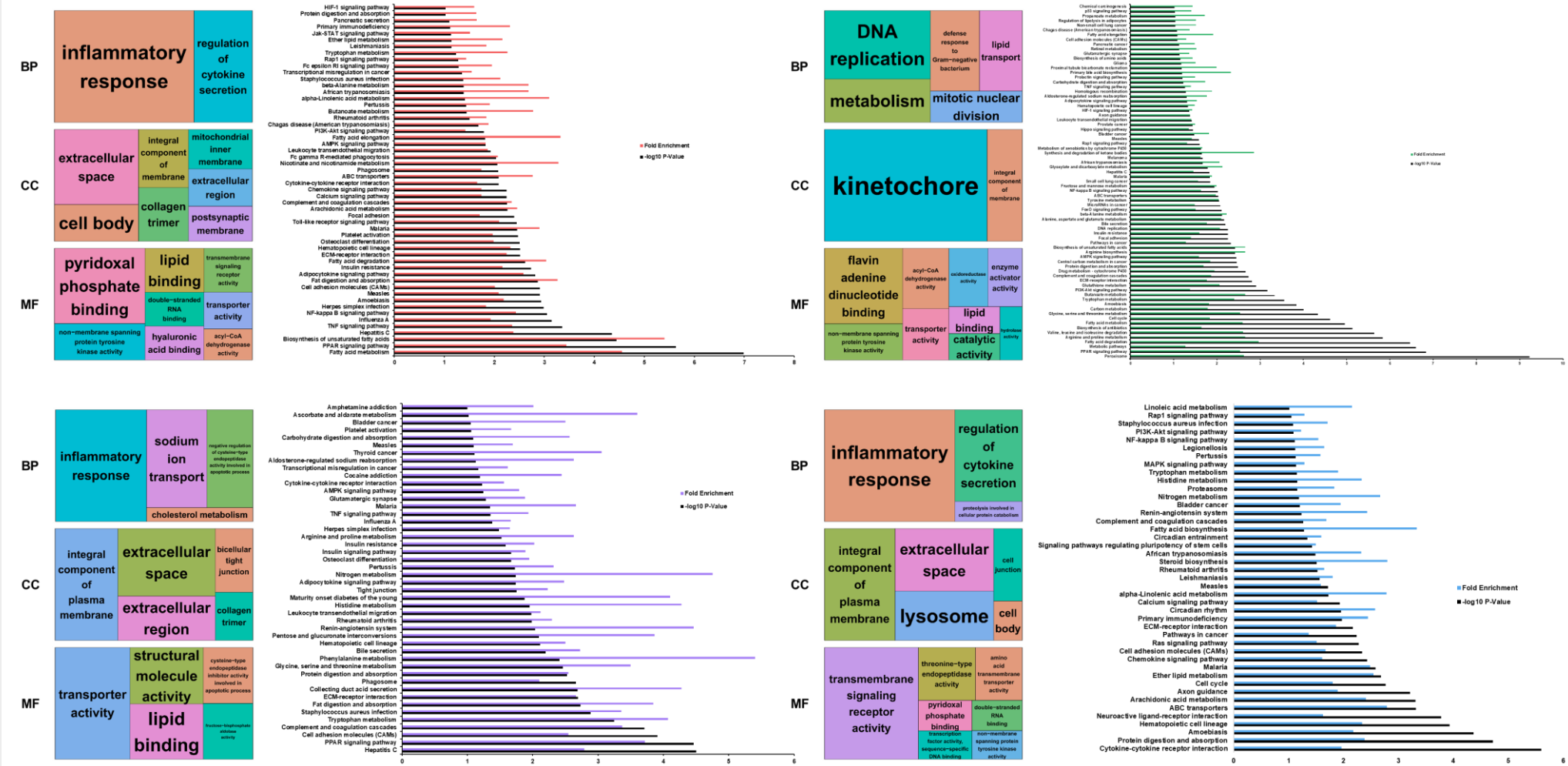


Fig 3. Functional annotation in heart (red), aorta (purple), liver (green), and lung (blue) samples

Heart xenotransplantation affects other organs such as normal heart, aorta, liver, and lung.

Liver and lung showed more severe effects, such as side effects, than others.