UKD Universitätsklinikum Düsseldorf

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# Human urine as a non-invasive source of kidney progenitor cells amenable for nephrotoxicity studies

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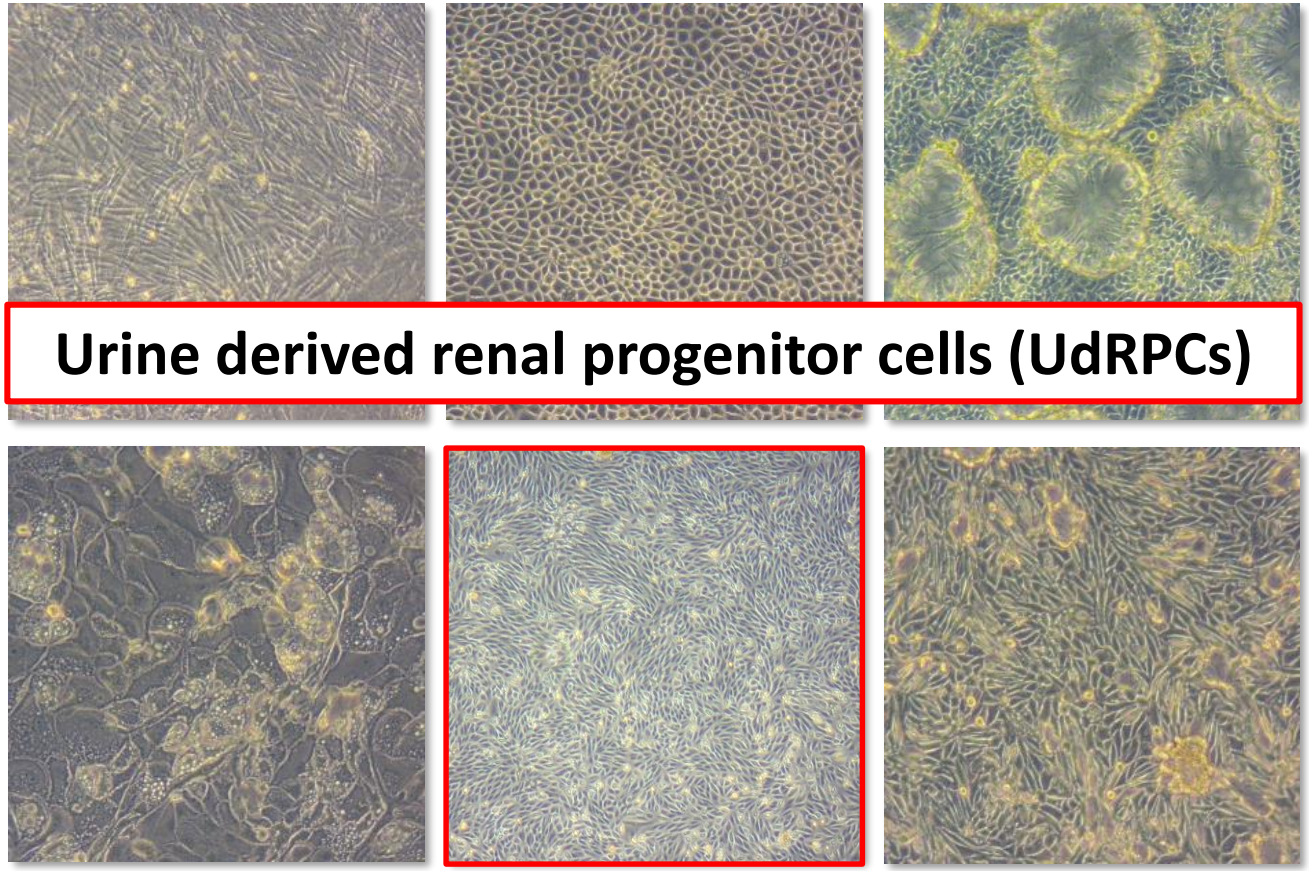
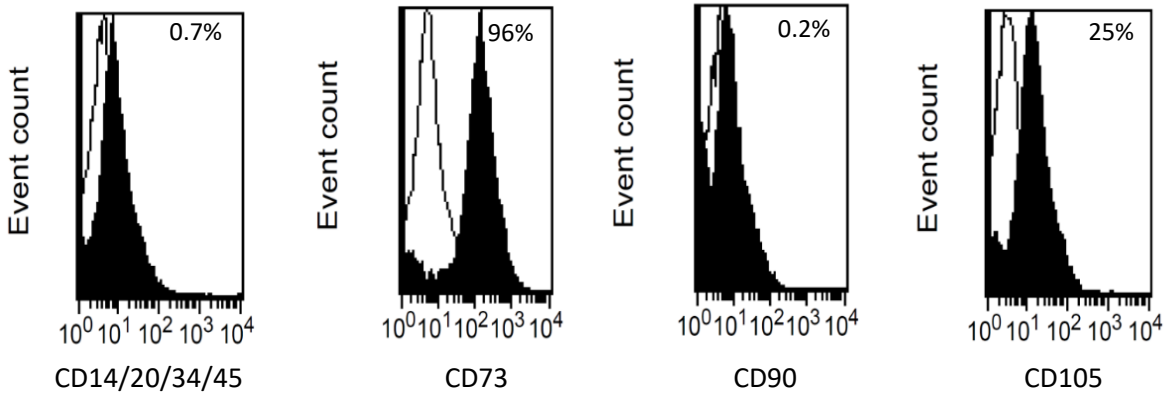
**Disclaimer: Co-founder**

# Urine consists of distinct cell types originating from the upper urinary track

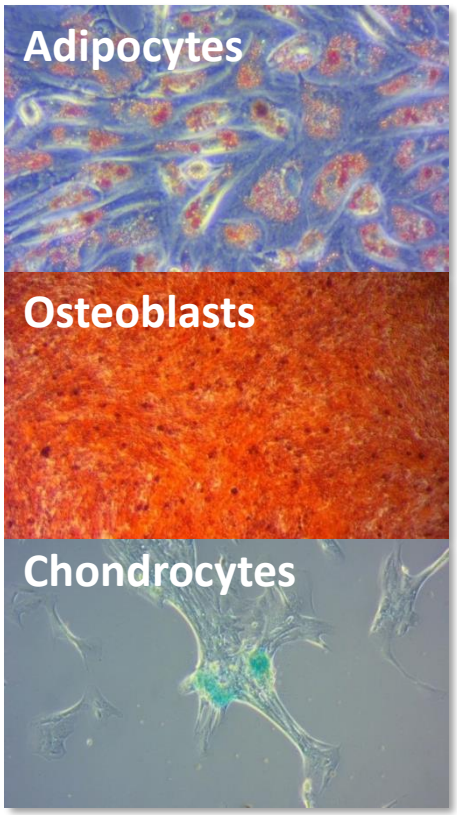


# Urine derived renal progenitor cells (UdRPCS) are MSCs

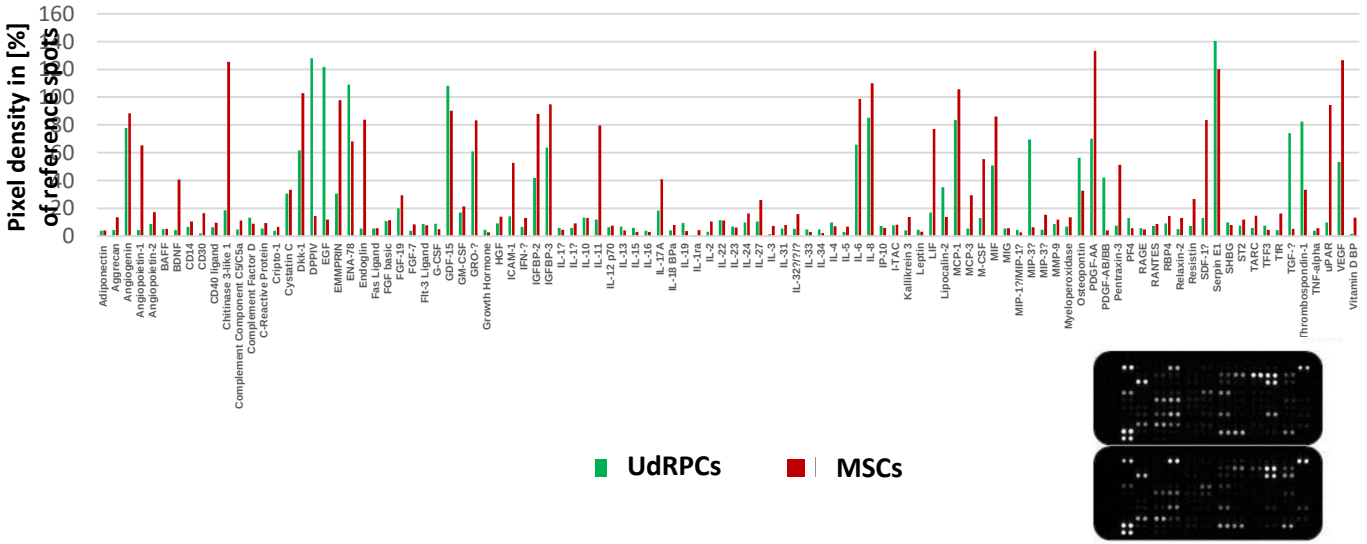
## Cell surface markers



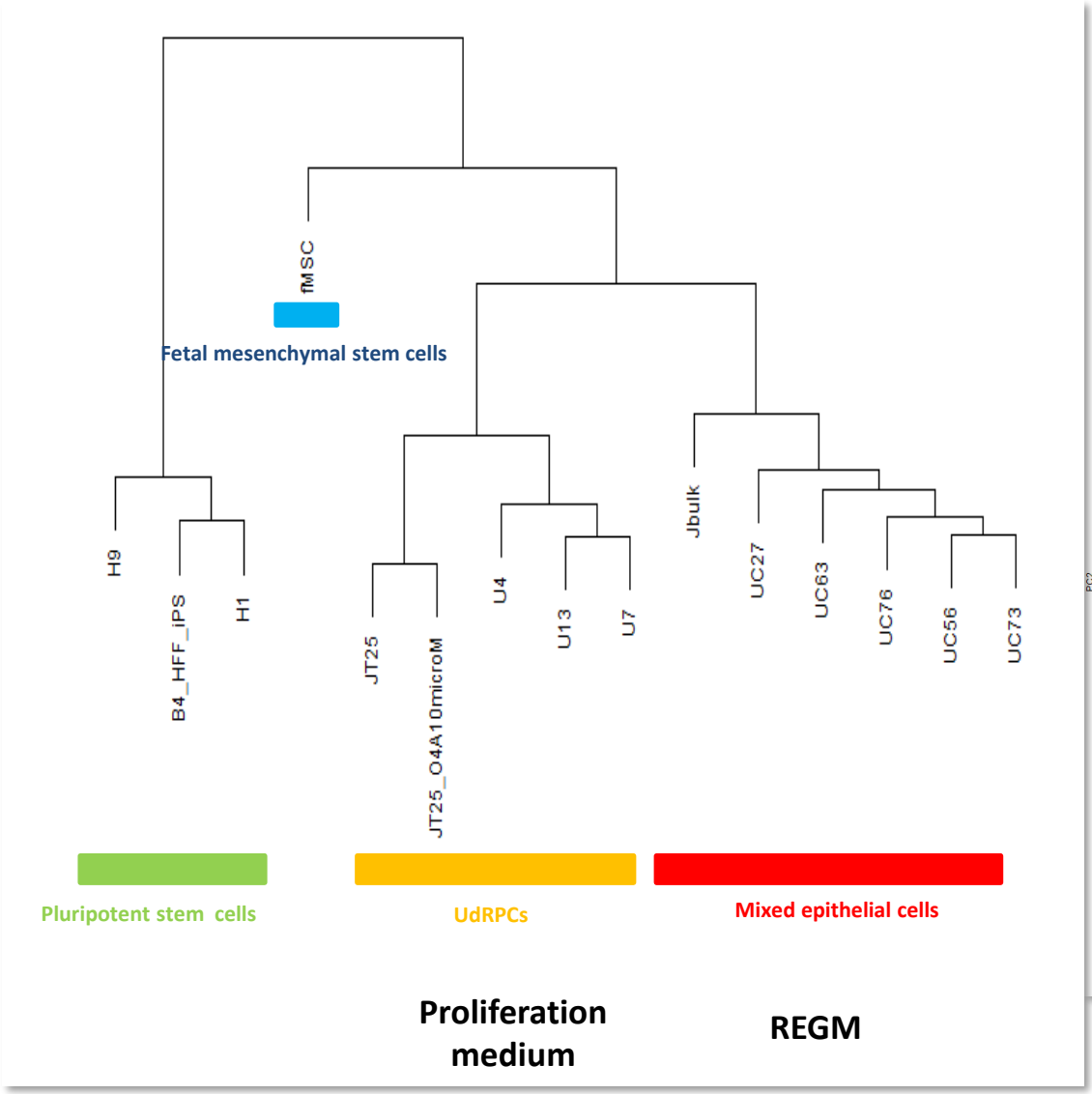

## Differentiation

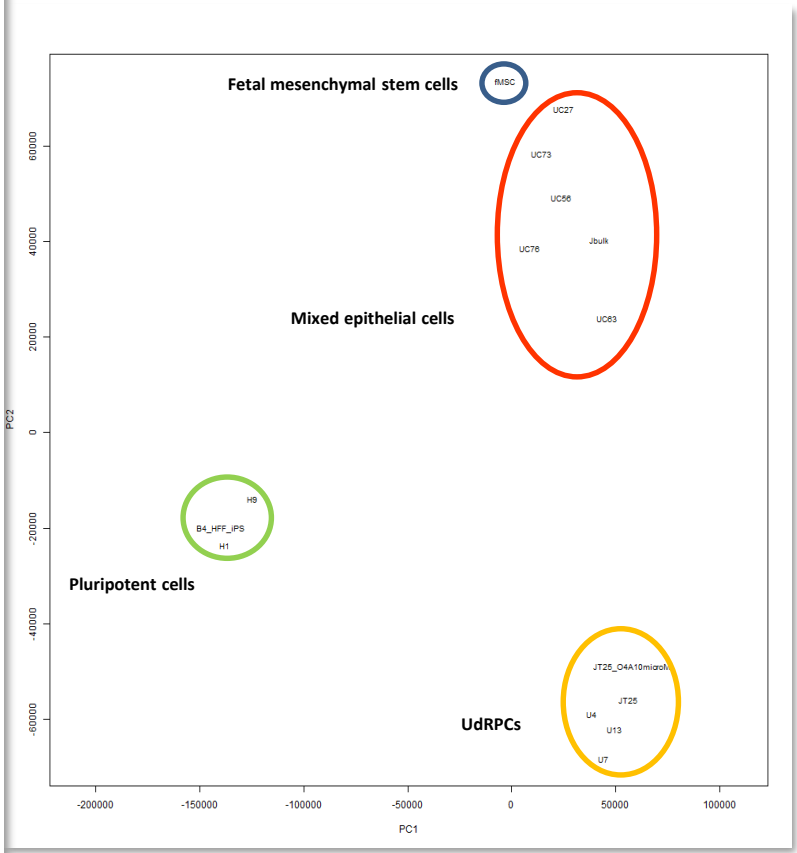



## Secretome

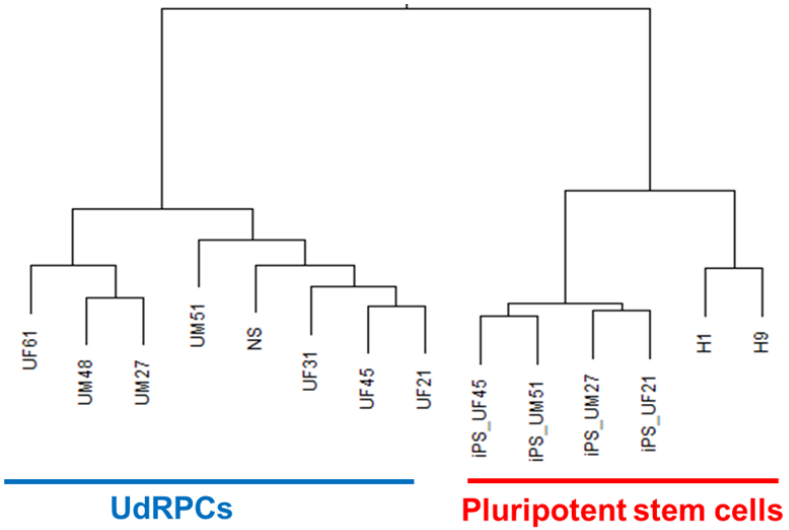


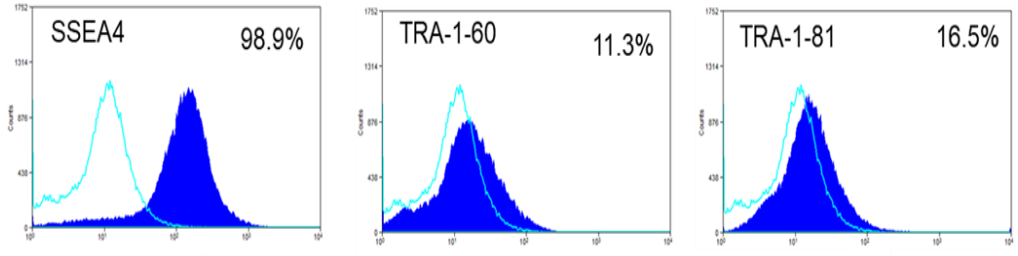
# Comparative transcriptome analysis after selection with distinct media



# UdRPCs are not pluripotent but share overlapping self-renewal pathways with pluripotent stem cells

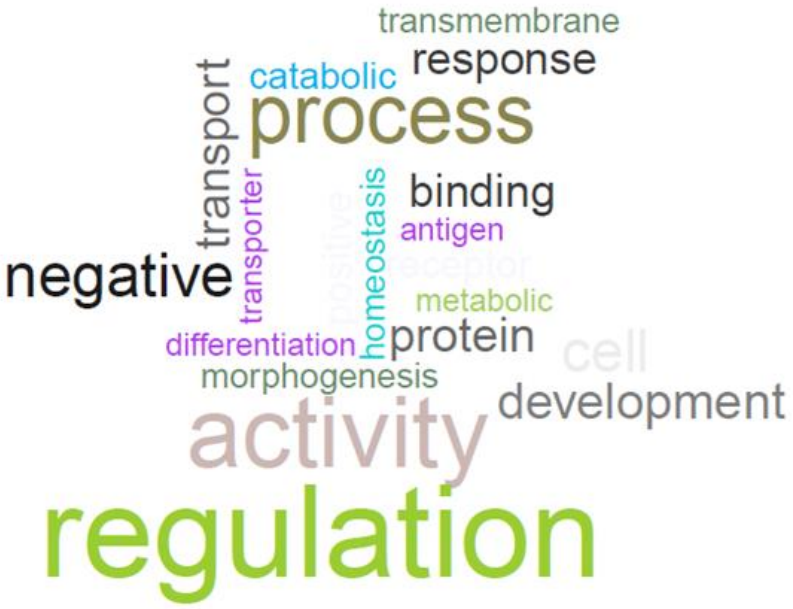


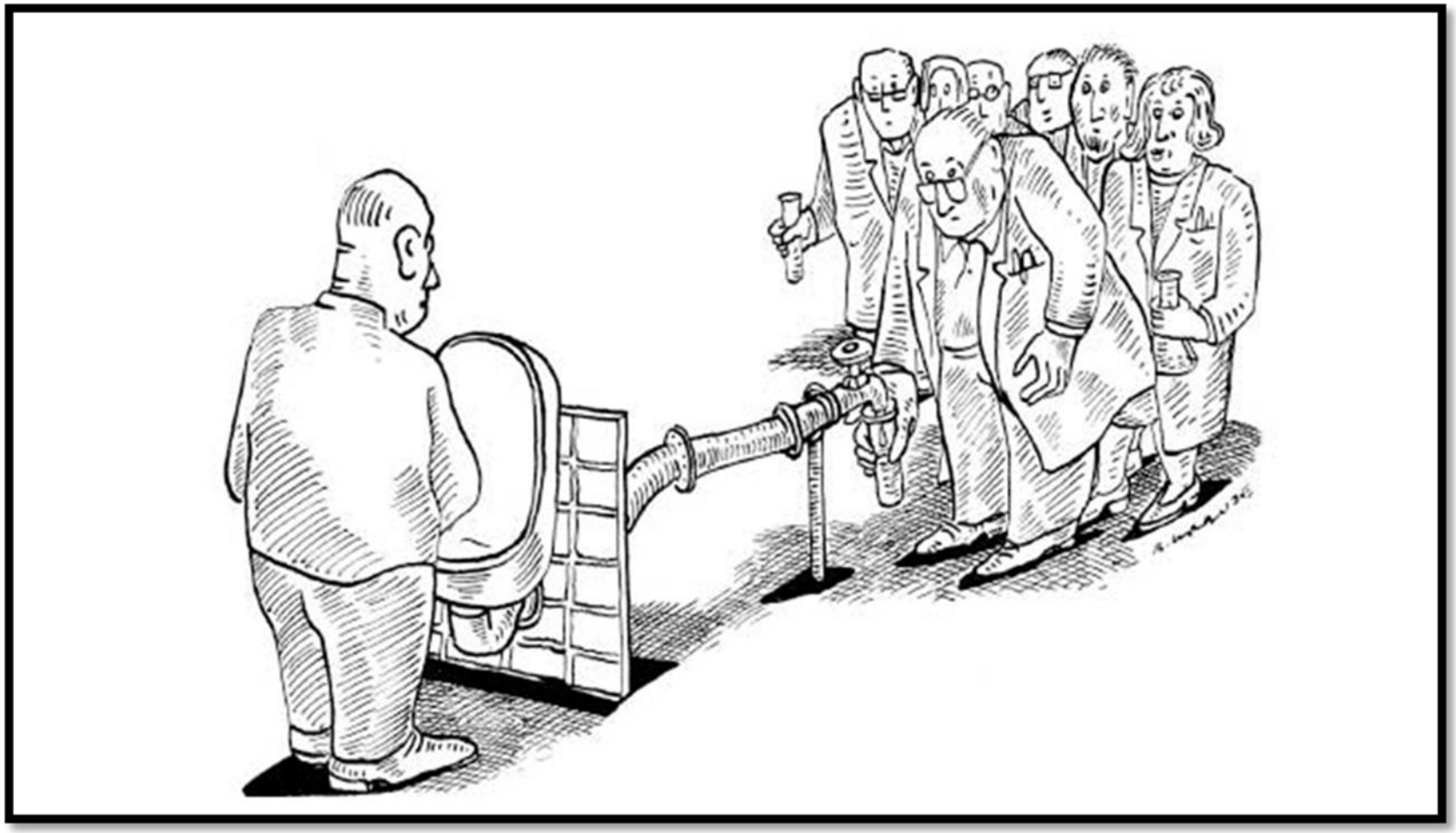




| kegg_name | p_hyper | q_hyper |
|---|---|---|
| Neurotrophin signaling pathway | 4.2817E-06 | 4.9026E-05 |
| Insulin signaling pathway | 1.8973E-05 | 0.00016711 |
| p53 signaling pathway | 7.5735E-05 | 0.00052555 |
| Notch signaling pathway | 0.00026257 | 0.00167025 |
| mTOR signaling pathway | 0.00056803 | 0.00333535 |
| Renal cell carcinoma | 0.00118158 | 0.00588219 |
| ErbB signaling pathway | 0.00211584 | 0.01009431 |
| Wnt signaling pathway | 0.00358524 | 0.01492763 |
| Focal adhesion | 0.00907485 | 0.03247096 |
| Tight junction | 0.01081839 | 0.03742005 |
| TGF-beta signaling pathway | 0.01276454 | 0.04059834 |

# Kidney-associated Gene Ontologies

| Pvalue | Term |
|---|---|
| 0.00020807 | renal system development |
| 0.00047809 | urogenital system development |
| 0.0008348 | kidney development |
| 0.00089158 | cellular component assembly involved in morphogenesis |
| 0.00295683 | system development |
| 0.00540953 | response to lipid |
| 0.00552771 | anatomical structure development |
| 0.00632622 | response to organic cyclic compound |
| 0.00912449 | organ development |
| 0.01183198 | central nervous system development |
| 0.01188333 | anatomical structure formation involved in morphogenesis |
| 0.01189563 | chemical homeostasis |
| **0.01317304** | **brain development** |
| **0.01713733** | **germ-line stem cell division** |
| **0.01713733** | **male germ-line stem cell asymmetric division** |
| **0.01713733** | **diapedesis** |
| **0.01713733** | **renal water absorption** |
| **0.01713733** | **glomerular endothelium development** |
| **0.01713733** | **germline stem cell asymmetric division** |
| **0.01718154** | **renal water homeostasis** |
| 0.0175112 | cell projection morphogenesis |
| 0.01837454 | immune system process |





# Putting the Pee in Pluripotency

The Scientist Magazine®

# Generation of integration-free neural progenitor cells from cells in human urine

Lihui Wang[1–3], Linli Wang[1,2], Wenhao Huang[1,2], Huanxing Su[1,2,7], Yanting Xue[1,2,4], Zhenghui Su[1,2,4], Baojian Liao[1,2], Haitao Wang[1,2], Xichen Bao[1,2], Dajiang Qin[1,2], Jufang He[5], Wutian Wu[6], Kwok Fai So[6], Guangjin Pan[1,2] & Duanqing Pei[1,2]

RESOURCE ARTICLE

# Urine-sample-derived human induced pluripotent stem cells as a model to study PCSK9-mediated autosomal dominant hypercholesterolemia

Karim Si-Tayeb[1,2,3,*,‡], Salam Idriss[1,2,3,*], Benoite Champon[1,2,3], Amandine Caillaud[1,2,3], Matthieu Pichelin[1,2,3,4], Lucie Arnaud[1,2,3], Patricia Lemarchand[1,2,3], Cédric Le May[1,2,3], Kazem Zibara[5] and Bertrand Cariou[1,2,3,4]

# Human Urinary Epithelial Cells as a Source of Engraftable Hepatocyte-like Cells using Stem Cell Technology

**AUTHORS**

Vanessa Sauer[1,2,4], Tatyana Tchaikovskaya[1,2], Xia Wang[1,2], Yanfeng Li[1,2], Wei Zhang[2,3], Krisztina Tar[1,2,6], Zsuzsanna Polgar[1,2], Jianqiang Ding[1,2], Chandan Guha[2,3], Ira J. Fox[5], Namita Roy-Chowdhury[1,2], Jayanta Roy-Chowdhury[1,2]

STEM CELLS®

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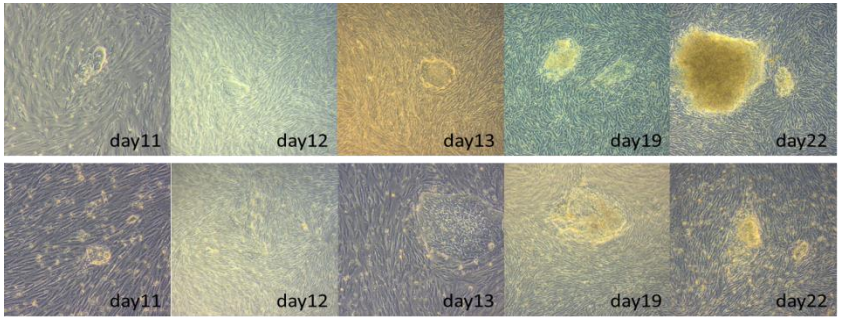
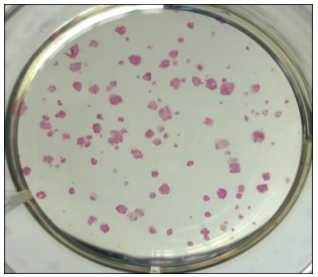
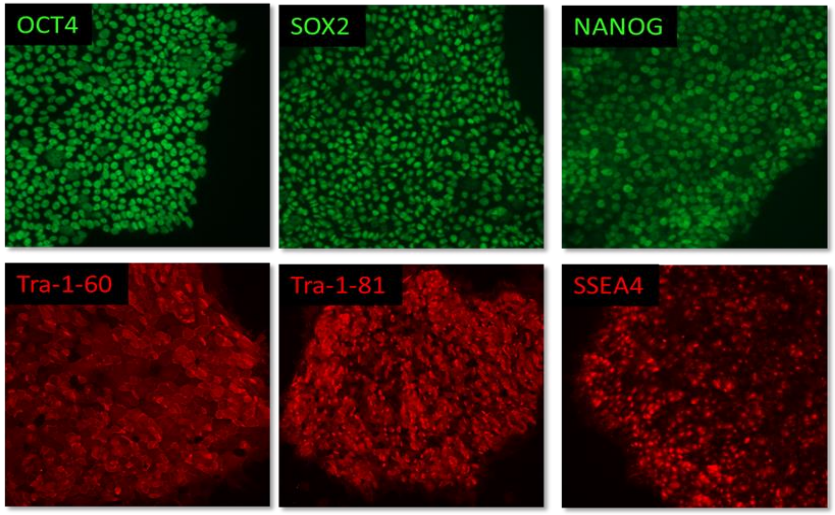
# Multipotential Differentiation of Human Urine-Derived Stem Cells: Potential for Therapeutic Applications in Urology

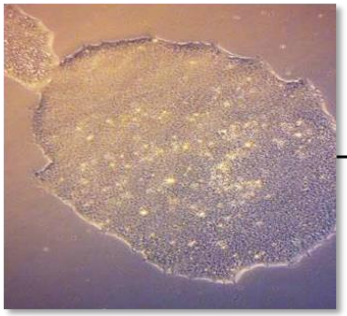
Shantaram Bharadwaj,[a] Guihua Liu,[a] Yingai Shi,[a] Rongpei Wu,[a,b] Bin Yang,[a,c] Tongchuan He,[d] Yuxin Fan,[e] Xinyan Lu,[f] Xiaobo Zhou,[g] Hong Liu,[h] Anthony Atala,[a] Jan Rohozinski,[a,i] Yuanyuan Zhang[a]

# Integration-free iPSCs can be efficiently derived using episomal-based plasmids without pathway perturbations

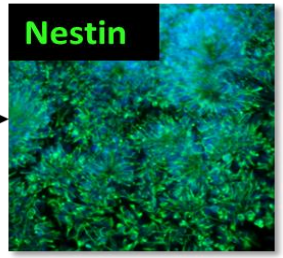


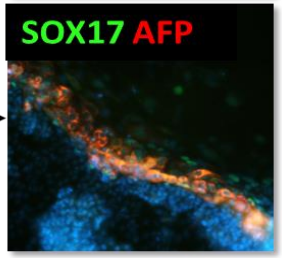


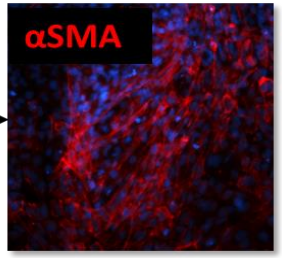
EBs




Ectoderm




Endoderm




Mesoderm

Stem Cell Research 25 (2017) 18–21

Contents lists available at ScienceDirect

Stem Cell Research

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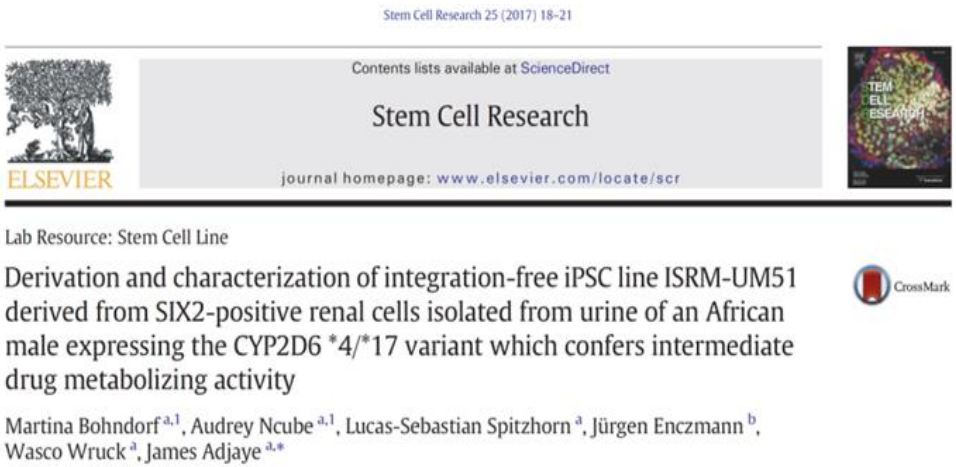
Lab Resource: Stem Cell Line

Derivation and characterization of integration-free iPSC line ISRM-UM51 derived from SIX2-positive renal cells isolated from urine of an African male expressing the CYP2D6 *4/*17 variant which confers intermediate drug metabolizing activity

Martina Bohndorf [a,1], Audrey Ncube [a,1], Lucas-Sebastian Spitzhorn [a], Jürgen Enczmann [b], Wasco Wruck [a], James Adjaye [a,*]

# Towards Personalized drug testing and development



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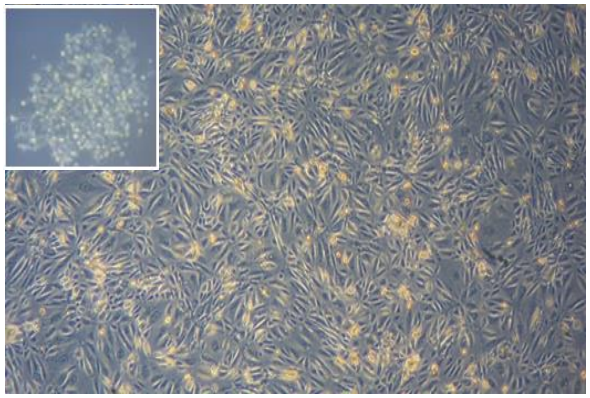
Martina Bohndorf [a,1], Audrey Ncube [a,1], Lucas-Sebastian Spitzhorn [a], Jürgen Enczmann [b], Wasco Wruck [a], James Adjaye [a,*]

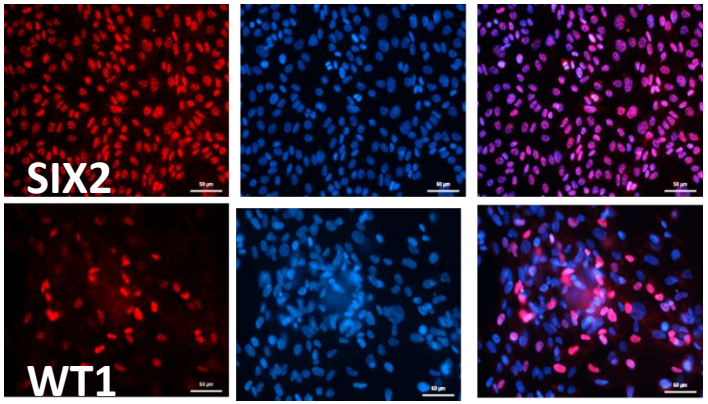
| Phenotype | Frequency |
|---|---|
| Poor Metabolizer (PM) | 5% |
| Intermediate Metabolizer (IM) | 6.9% |
| Extensive Metabolizer (EM) | 84% |
| Ultrarapid Metabolizer (UM) | 3.7% |

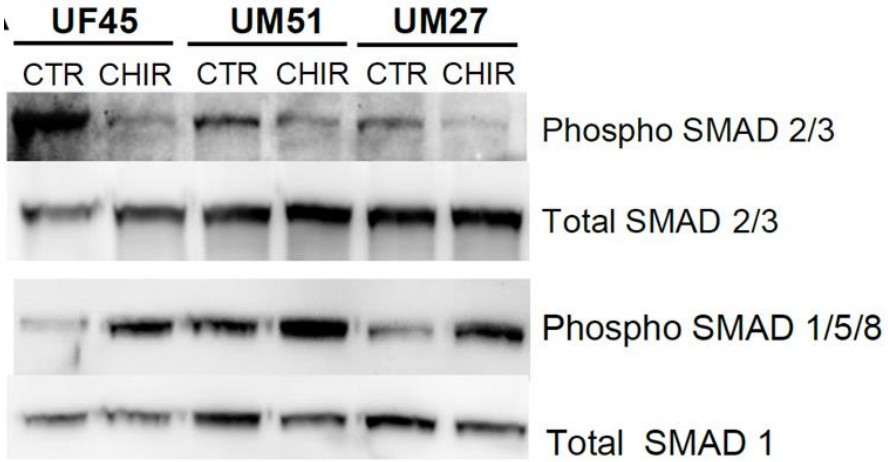
| Sample ID | Gender | Age | Ethnicity | iPSC | CYP2D6 Genotype | CYP2D6 Phenotype |
|---|---|---|---|---|---|---|
| UM48 | M | 48 | African | NO | - | - |
| UF60 | F | 60 | Caucasian | NO | - | - |
| UM27 | M | 27 | Caucasian | NO | CYP2D6*1x2/*4 | NM |
| UF27 | F | 27 | Caucasian | NO | - | - |
| UF61 | F | 61 | Caucasian | NO | - | - |
| UM51 | M | 51 | African | YES | CYP2D6*4/*17 | IM |
| UF45 | F | 45 | Caucasian | YES | CYP2D6*1/*4 | NM |
| UF31 | F | 31 | African | YES | CYP2D6*1/*41 | UM |
| UF21 | F | 21 | Caucasian | YES | CYP2D6*2/*2 | NM |
| UM54 | M | 54 | Caucasian | NO | - | - |

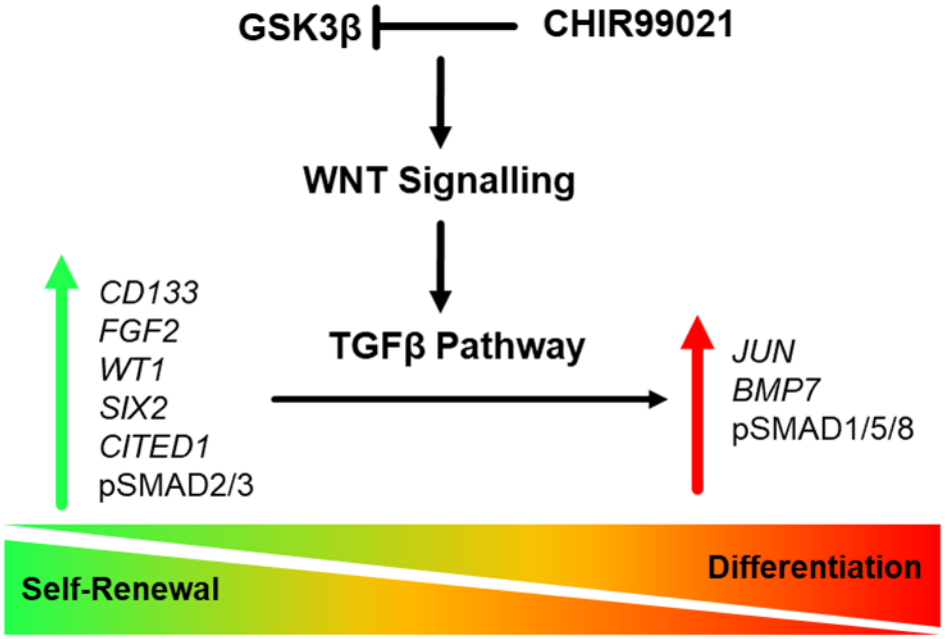
# Urine derived Renal Progenitor Cells UdRPCs

# Self-renewal in UdRPCs is driven by FGF-, WNT-, TGF-β signalling mediated by SIX2, CITED1 and WT1

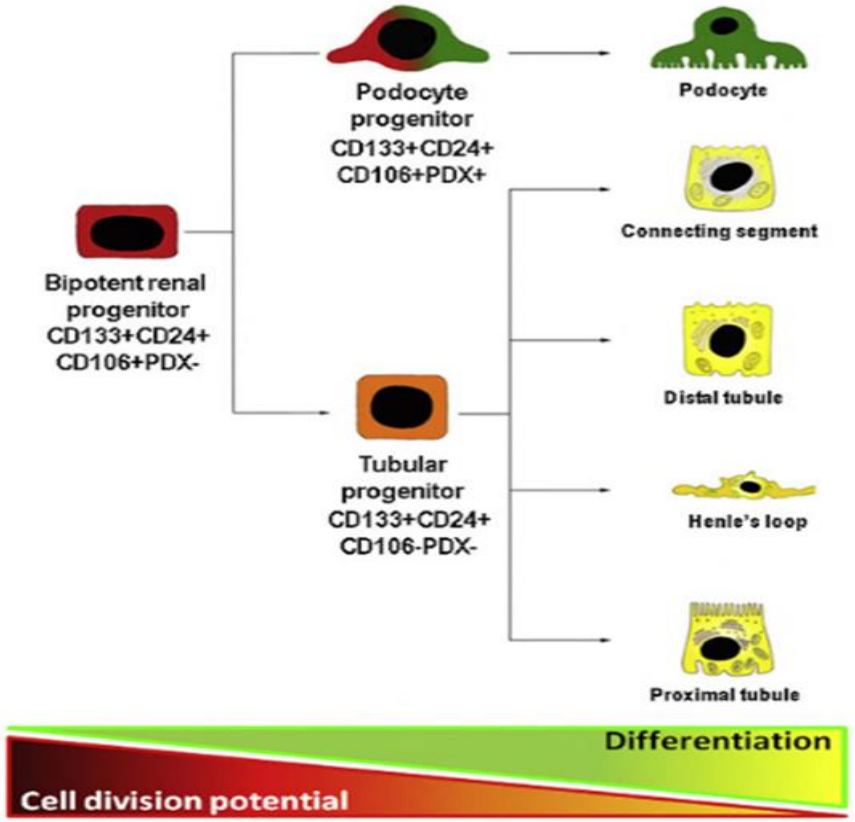


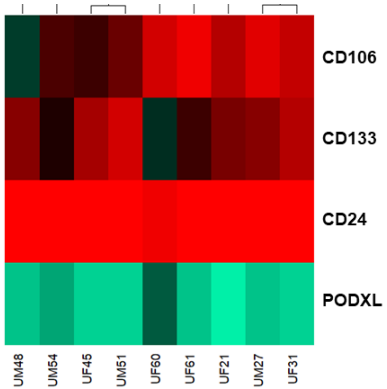


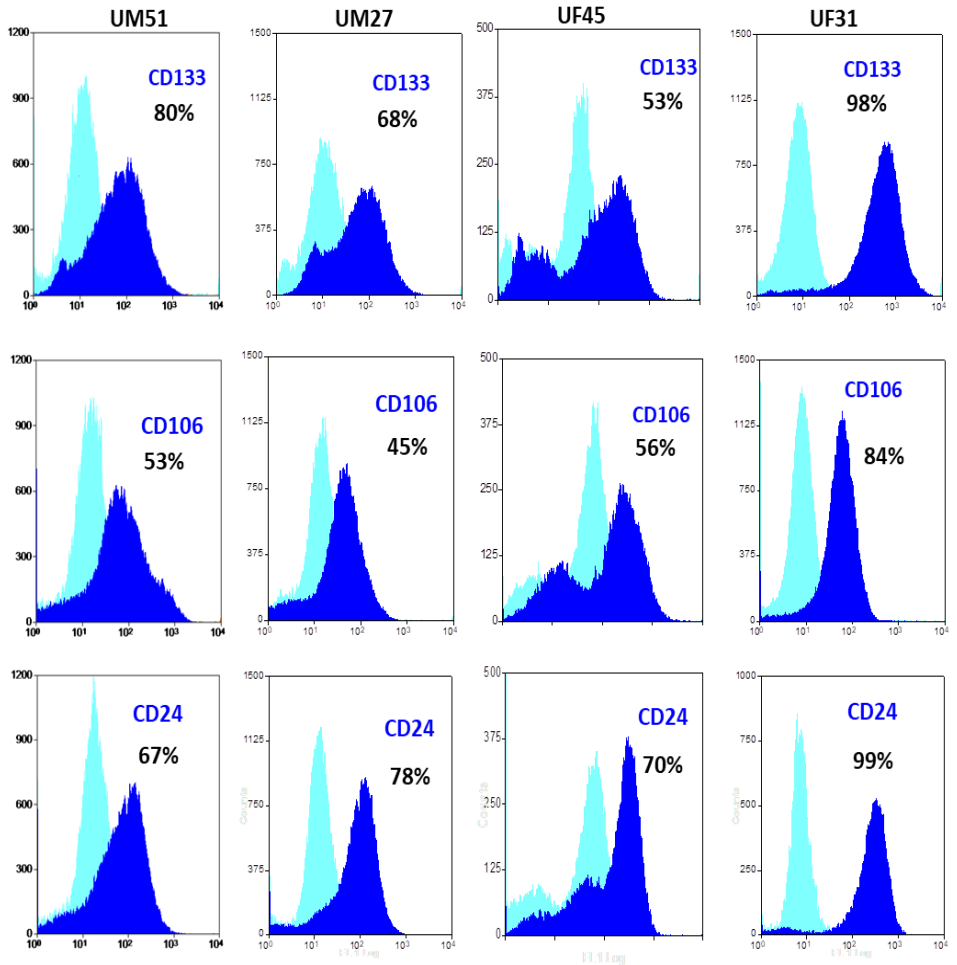



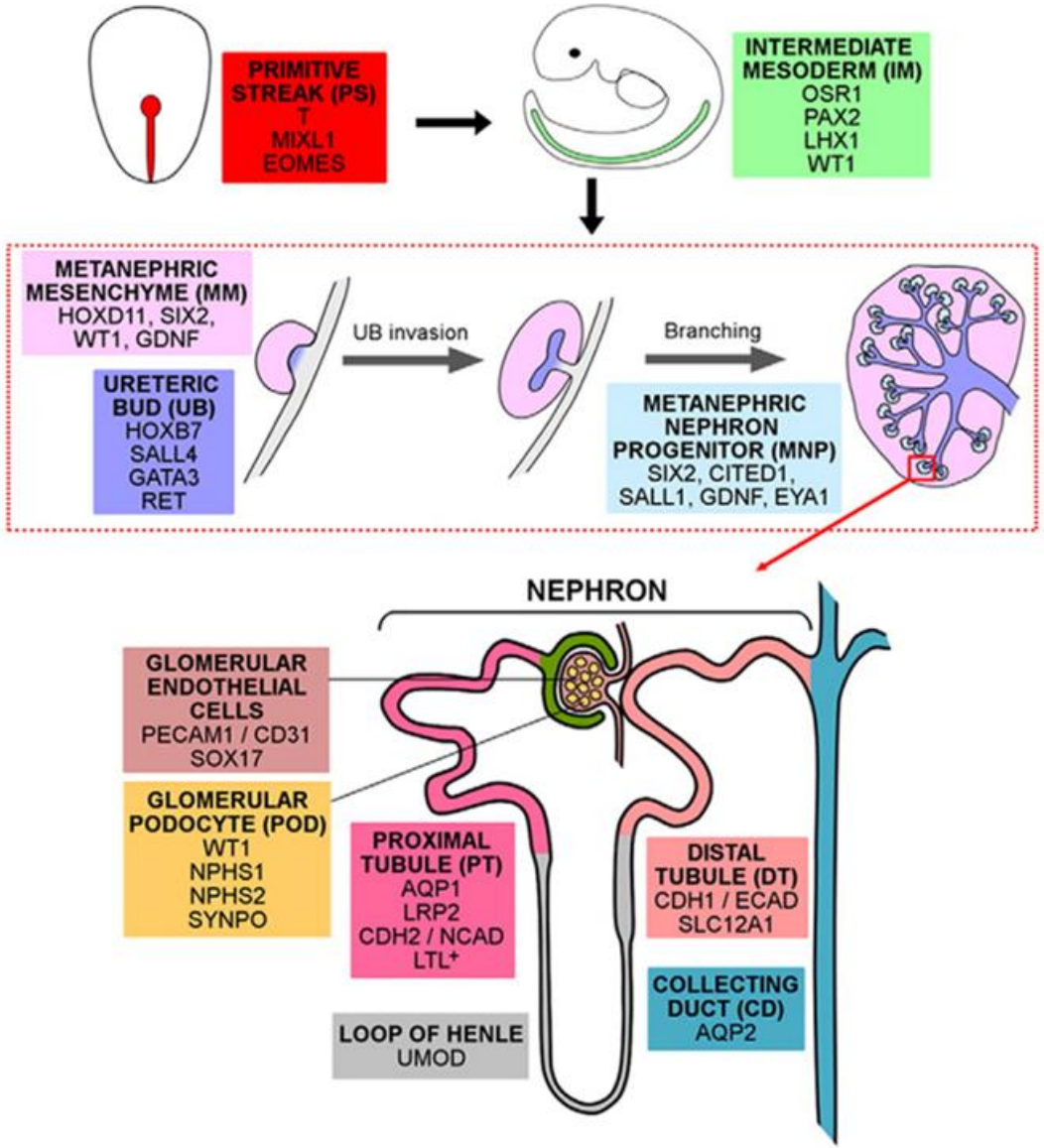
# UdRPCs are Bipotential progenitors



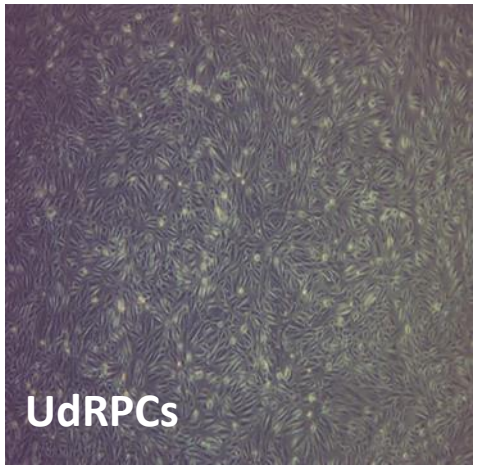



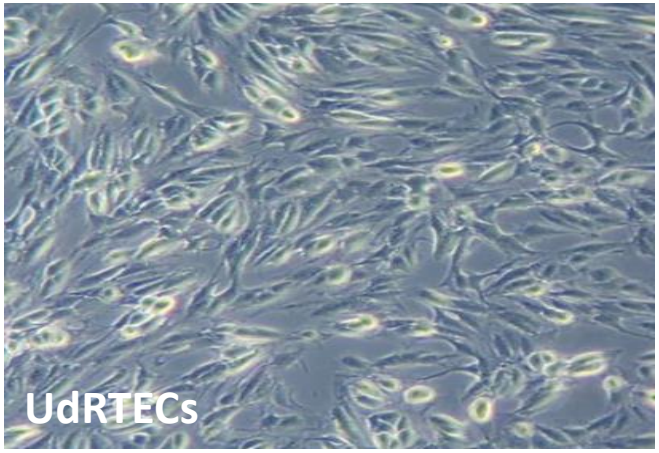
# Cell fate decisions during nephrogenesis

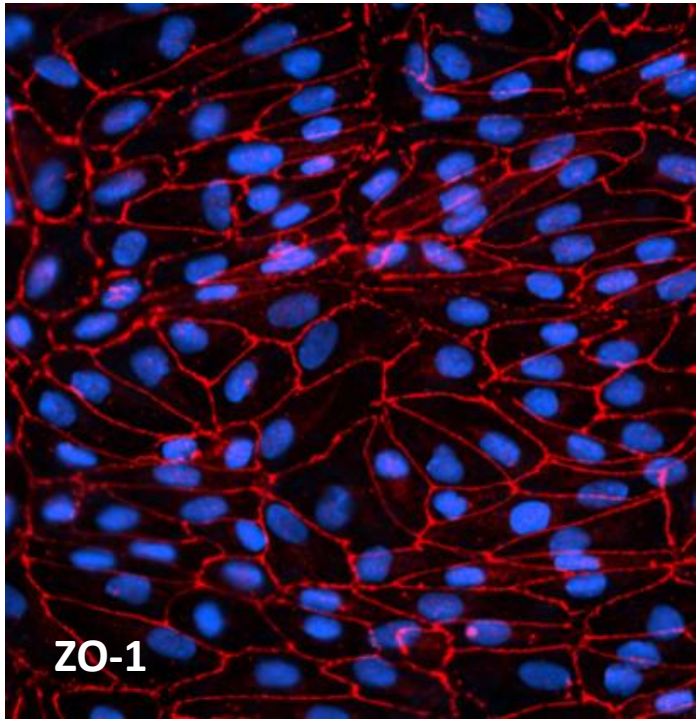


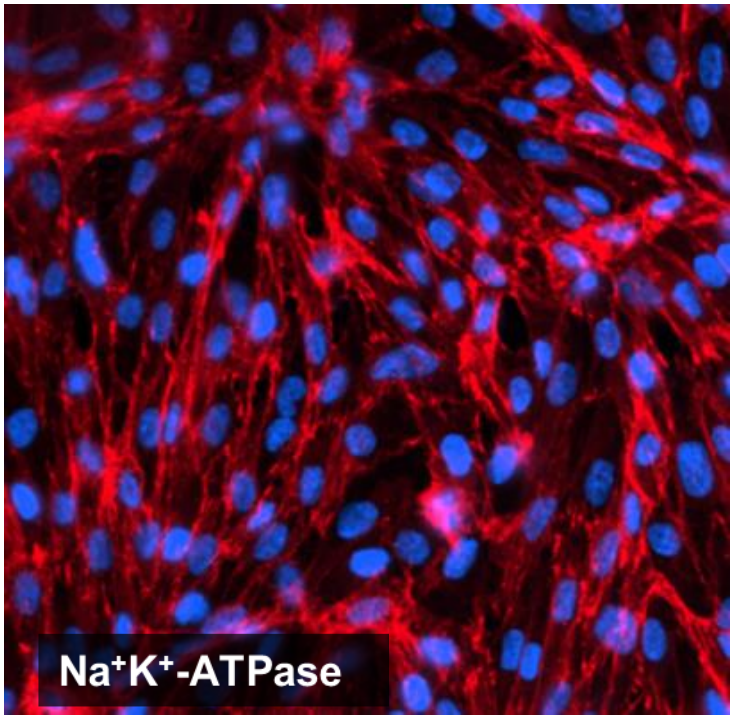
# UdRPCs derived Renal Tubular Epithelial Cells



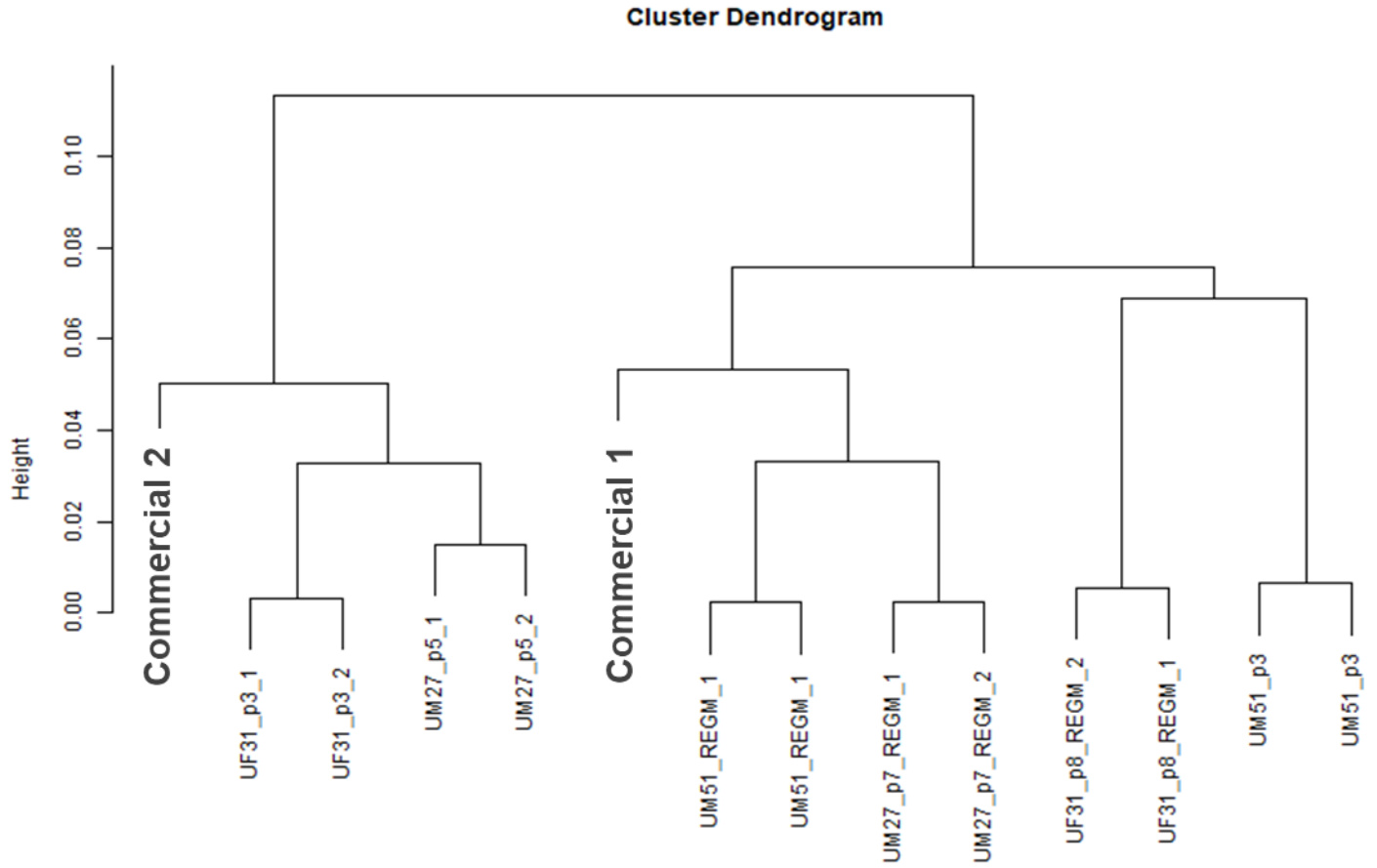

REGM



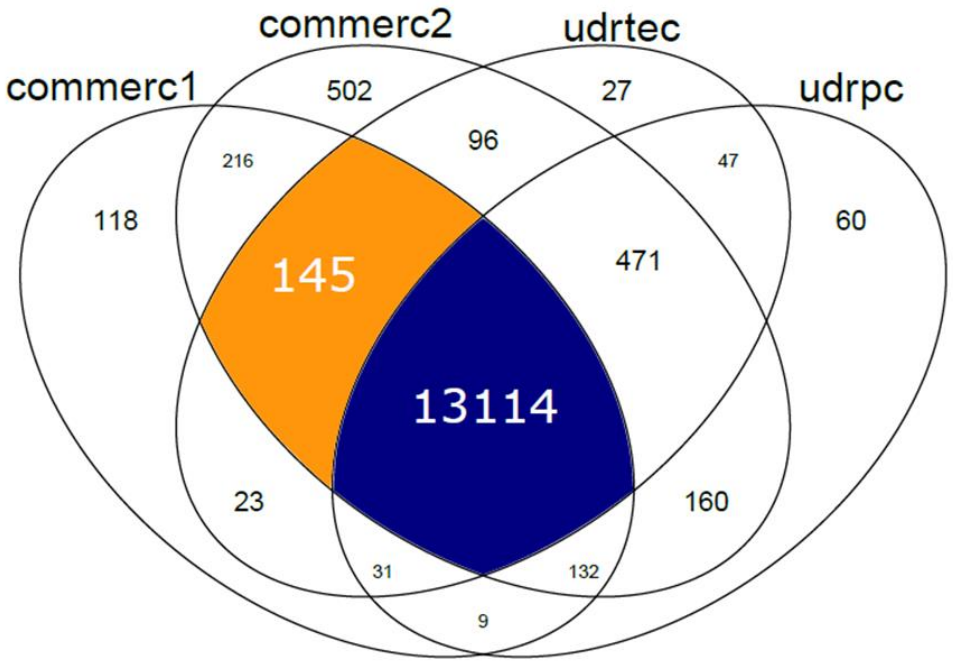



# UdRPCs- differentiated renal tubular epithelial cells (UdRTECs) are similar to kidney derived counterparts




| Sample | Commercial 1 | Commercial 2 | UdRTEC UM51 | UdRTEC UM27 | UdRTEC UF31 |
|---|---|---|---|---|---|
| Commercial 1 | 1,0000 | 0,9641 | 0,9686 | 0,9666 | 0,9522 |
| Commercial 2 | 0,9641 | 1,0000 | 0,9607 | 0,9741 | 0,9637 |
| UdRTEC UM51 | 0,9686 | 0,9607 | 1,0000 | 0,9793 | 0,9536 |
| UdRTEC UM27 | 0,9666 | 0,9741 | 0,9793 | 1,0000 | 0,9645 |
| UdRTEC UF31 | 0,9522 | 0,9637 | 0,9536 | 0,9645 | 1,0000 |

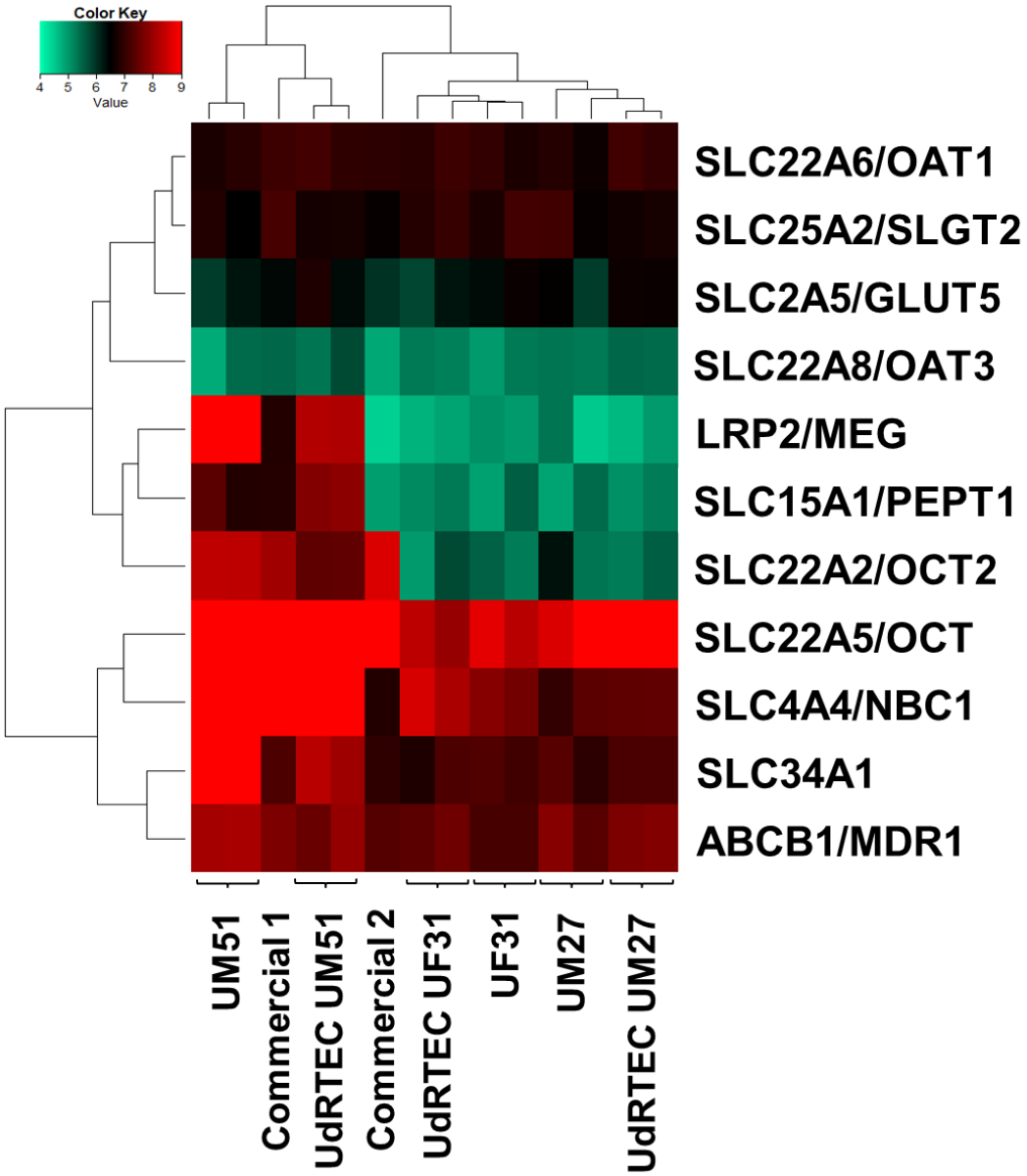
# Distinct and overlapping genes and associated biological processes



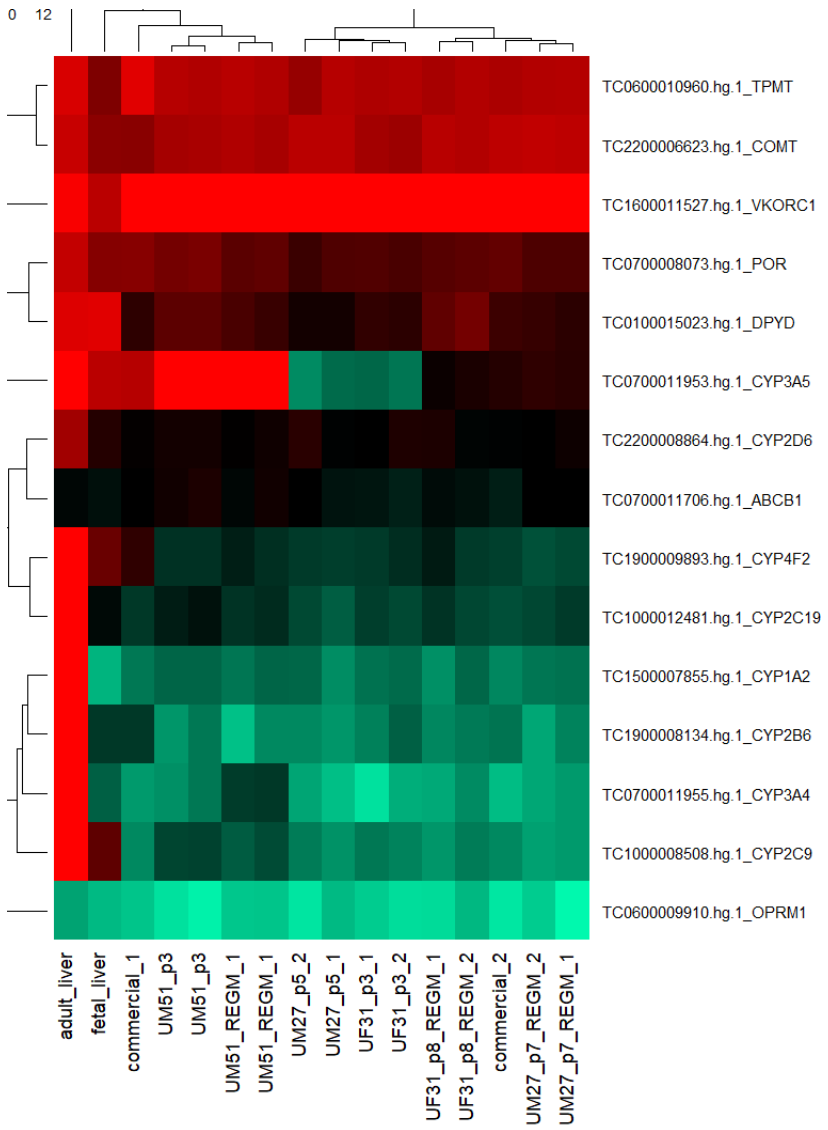

| GOID | Term |
|---|---|
| GO:0015747 | urate transport |
| GO:0046415 | urate metabolic process |
| GO:0001655 | urogenital system development |
| GO:0090087 | regulation of peptide transport |
| GO:0001822 | kidney development |
| GO:0016323 | basolateral plasma membrane |
| GO:0015143 | urate transmembrane transporter activity |
| GO:0033157 | regulation of intracellular protein transport |

| GOID | Term |
|---|---|
| GO:0006886 | intracellular protein transport |
| GO:0015031 | protein transport |
| GO:0032989 | cellular component morphogenesis |
| GO:0022604 | regulation of cell morphogenesis |
| GO:0072088 | nephron epithelium morphogenesis |
| GO:0060993 | kidney morphogenesis |
| GO:0061005 | cell dif. involved in kidney development |
| GO:0001655 | urogenital system development |
| GO:0072163 | mesonephric epithelium development |
| GO:0061333 | renal tubule morphogenesis |
| GO:0001763 | morphogenesis of a branching structure |
| GO:0072080 | nephron tubule development |
| GO:0001657 | ureteric bud development |
| GO:0016192 | vesicle-mediated transport |
| GO:0072171 | mesonephric tubule morphogenesis |
| GO:0072079 | nephron tubule formation |
| GO:0051223 | regulation of protein transport |
| GO:0003337 | MET involved in metanephros morphogenesis |
| GO:0072006 | nephron development |
| GO:0072033 | renal vesicle formation |
| GO:0072282 | metanephric nephron tubule morphogenesis |
| GO:0072087 | renal vesicle development |
| GO:0001656 | metanephros development |
| GO:0090184 | positive regulation of kidney development |
| GO:0072170 | metanephric tubule development |
| GO:0090316 | Pos. regulation of intracellular protein transport |
| GO:0061217 | regulation of mesonephros development |
| GO:0072074 | kidney mesenchyme development |
| GO:0072307 | metanephric nephron tubule epithelial cell dif. |
| GO:0001822 | kidney development |
| GO:0072273 | metanephric nephron morphogenesis |

# Overlapping expression of key kidney-associated transporters



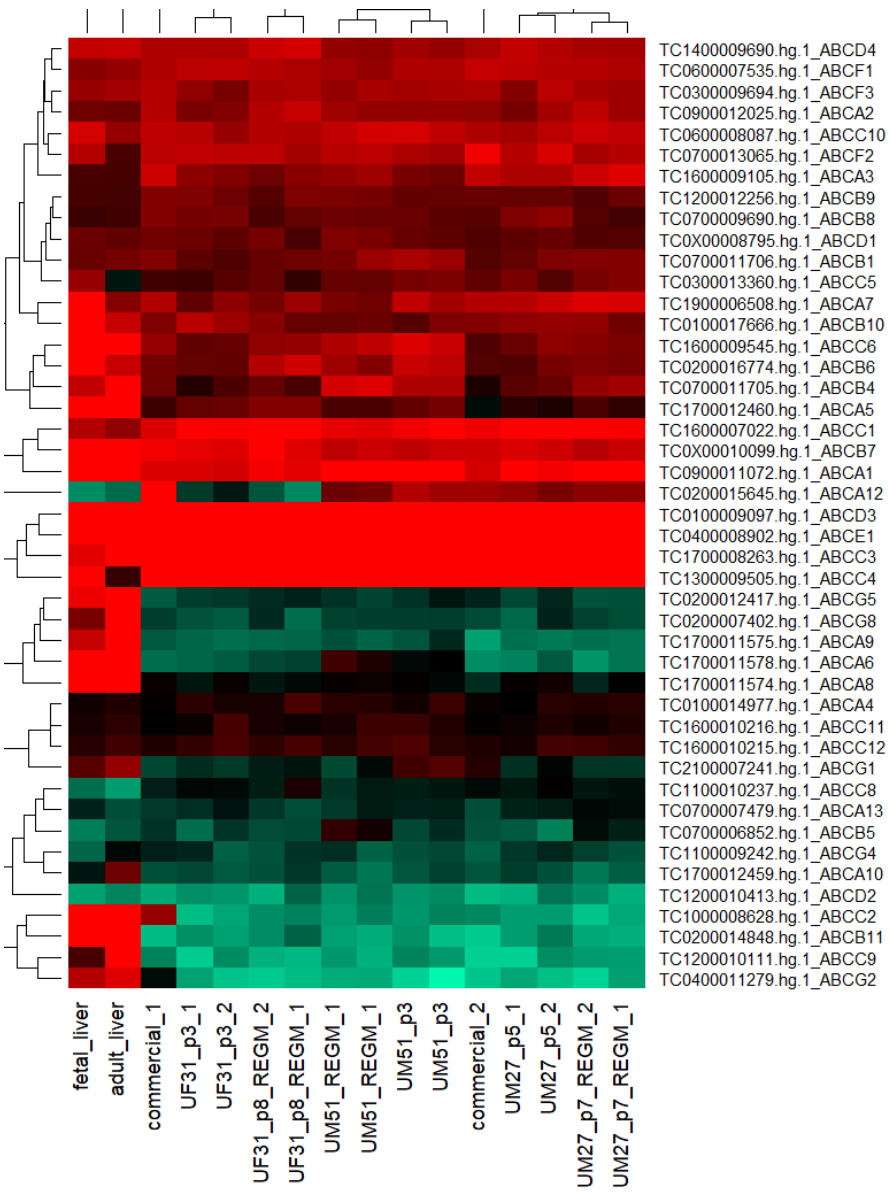
# Comparative expression of a PGx panel in liver and renal tubular epithelial cells



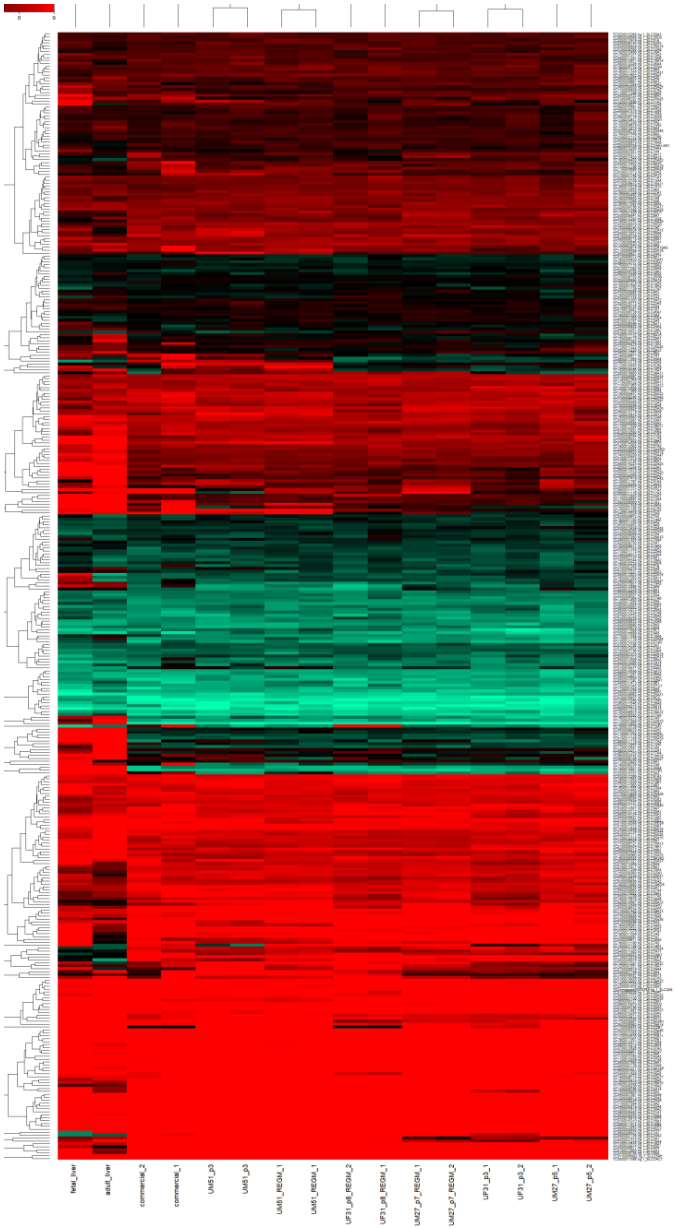

**Pharmacogenetic (PGx) testing is a type of genetic test that assesses a patient's risk of an adverse response or likelihood to respond to a given drug, informing drug selection and dosing.**

**As a pillar of the personalized medicine movement, PGx testing is anticipated to be important across all medical specialties.**

# Comparative expression of ABC transporters in liver and renal tubular epithelial cells

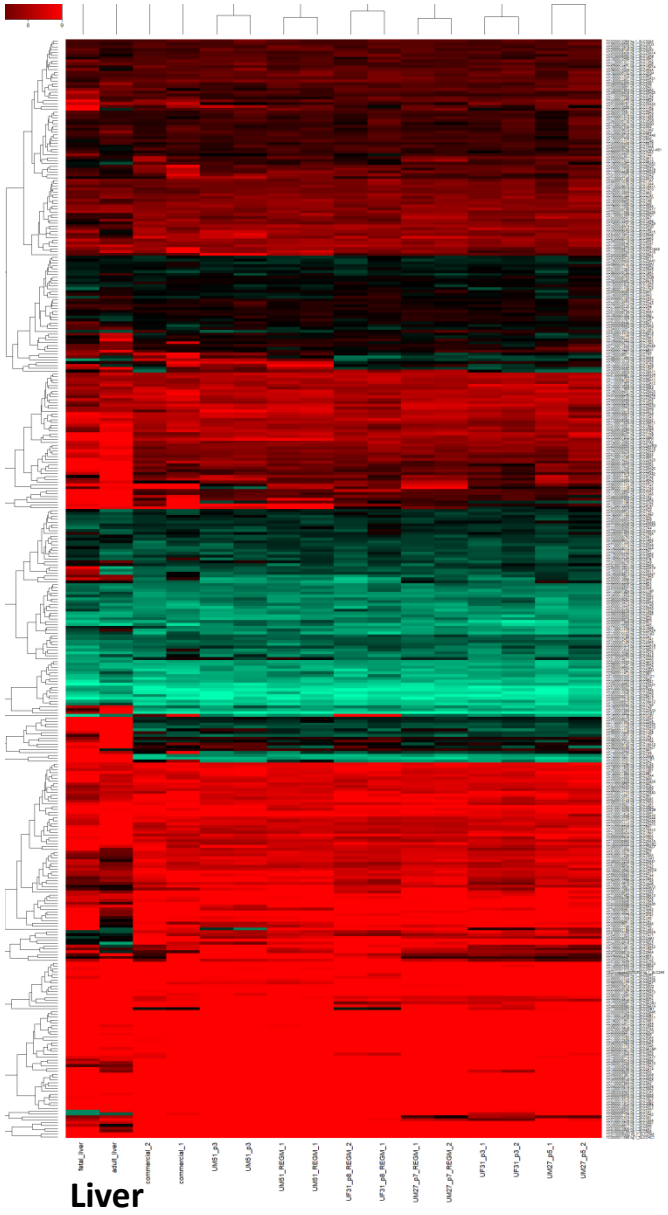


# Comparative expression of Cytochromes in liver and renal tubular epithelial cells



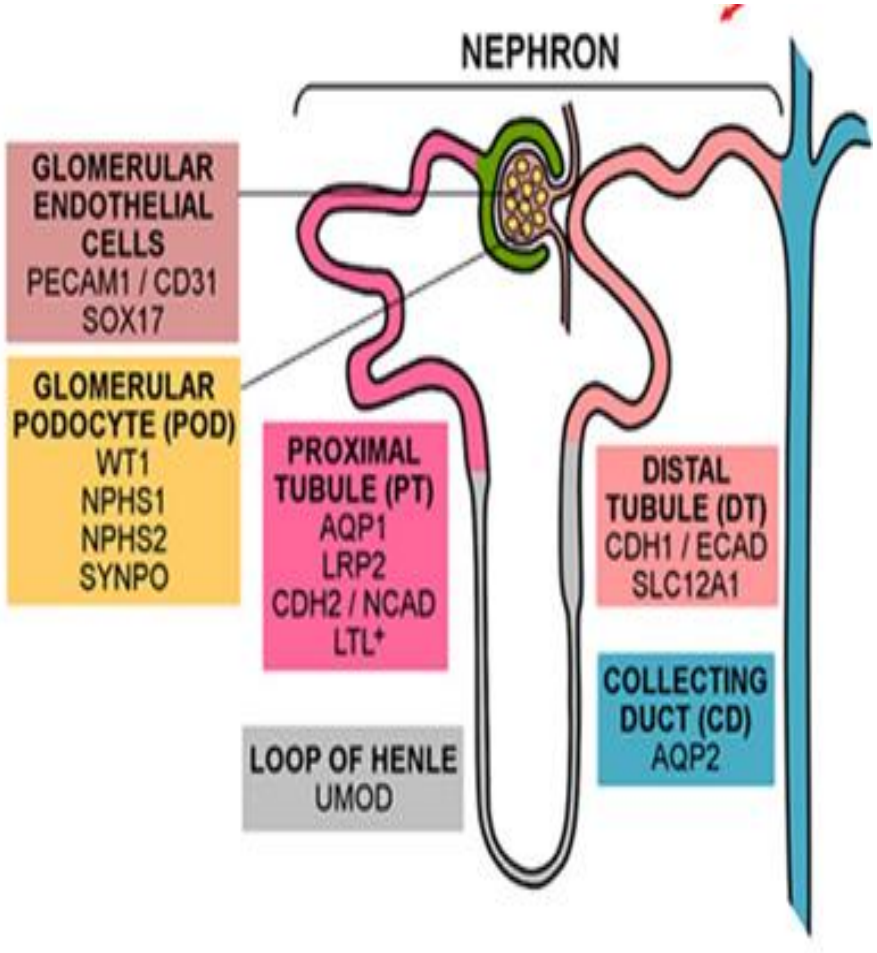

**Liver**

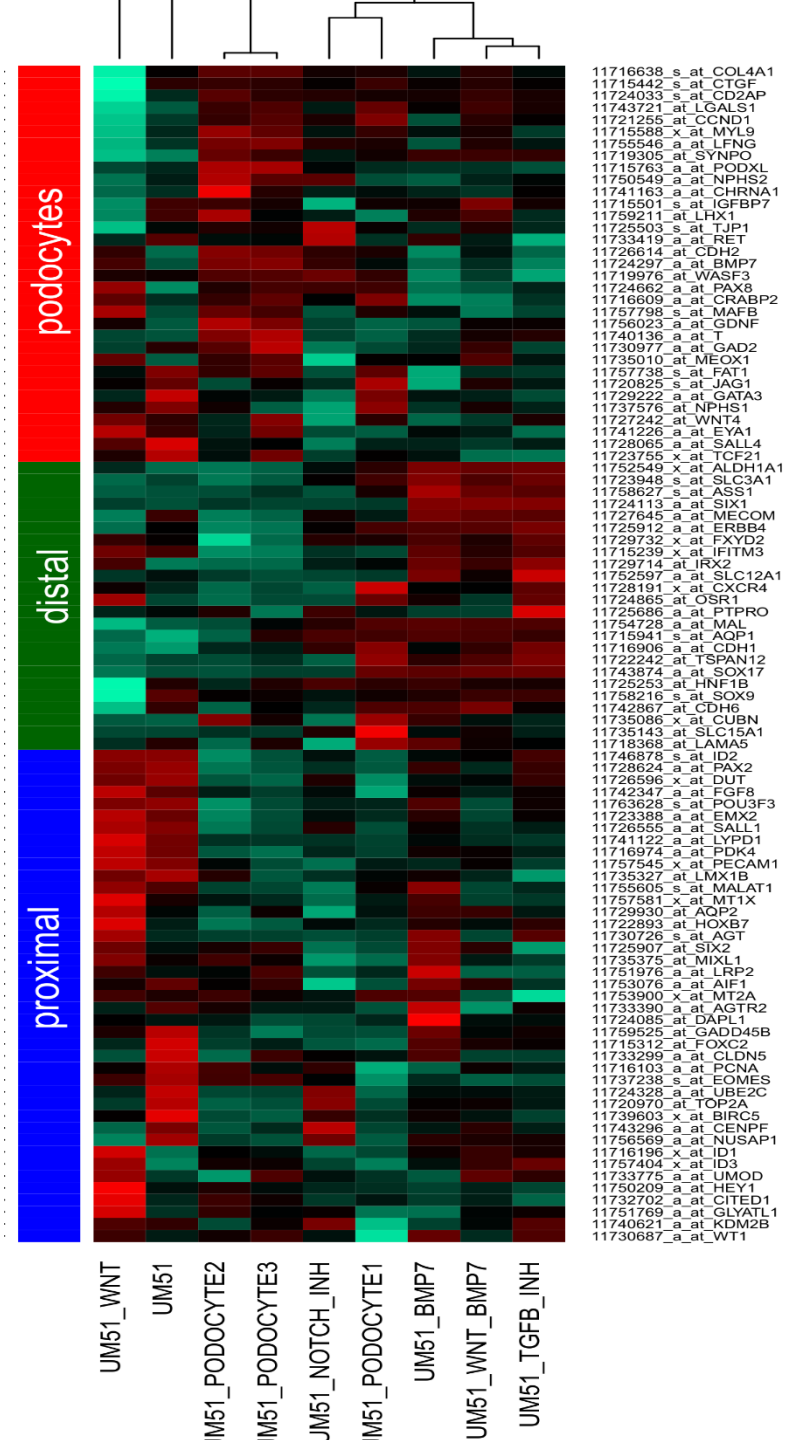
# Comparative expression of SLC transporters in liver and renal tubular epithelial cells



# UdRPCs are ideal for dissecting cell fate decisions during nephrogenesis

# UdRPCs can be easily differentiated into distinct cell types present in the kidney



- **Urine-derived renal progenitor cells (UdRPCs) from healthy adult individuals were successfully isolated from urine samples.**

- **They express the self-renewal regulating transcription factors SIX2, CITED1, WT1, as well as the bipotential renal progenitor markers CD24, CD106 and CD133 endowing them to differentiate into podocytes or renal tubular epithelial cells.**

- **UdRPCs can be efficiently reprogrammed into UdRPC-iPSCs using non-integrating episomal plasmids.**

- **Similar to MSCs, UdRPCs express Vimentin and have trilineage differentiation potential and the ability to secrete cytokines and growth factors known to support tissue regeneration and modulating the immune system.**

- **UdRPCs and kidney-derived renal tubular epithelial cells express overlapping and distinct cytochromes and transporters when compared to liver-biopsy derived hepatocytes.**

- **With our established protocols we are able to isolate renal progenitor cells from urine (UdRPCs) and thereby provide a novel tool for nephrogenesis, toxicology studies and drug development.**

**NON-INVASIVE SOURCE**

# Conclusions

- Urine derived progenitor cells ( UdRPCs) can be cultivated on plastic, gelatin and Matrigel
- UdRPCs express a number of pluripotency-associated proteins but not POU5F1/OCT4 NANOG and SOX2
- UdRPCs are not Pluripotent rather Multipotent (Osteoblasts, adipocytes and Chonrocytes)
- Induction of pluripotency is rapid and efficient compared to other somatic cell types
- UdRPCs are endowed with an epigenetic memory enabling rapid and more efficient differentiation into Renal epithelial tubular cells and Hepatocyte-like cells
- UdRPCs express a distinct set of Drug transporters not expressed in hepatocytes
- UdRPCs are an ideal cell type for studying nephrogenesis , hepatogenesis and modelling kidney and liver related diseases,
- Ideal for toxicology and drug screening

**UdRPCs should be considered for cell replacement therapies in the future**

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