Inducible Gene Expression to Drive Cell Differentiation in In Vitro Renal Tubule Cells

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INTRODUCTION

- Scarcity of organ donations decreases treatment options for patients with chronic kidney disease
- Novel bioartificial kidneys present new solutions for organ failure and require using *in vitro* cells for development
- Renal epithelial cells are specialized for reabsorption and secretion in the kidney
- *In vitro* proximal tubule epithelial cells have a de-differentiated transcriptional profile compared to *in vivo* counterparts
- Overexpression of LKB1 and STRADα improve differentiation in cultured intestinal epithelial cells[1]
- Liver Kinase B1, LKB1, is a tumor suppressor gene that aids in cell metabolism and polarity
- STE-20 Related Adaptor Protein, STRADα, regulates the localization of LKB1[2]

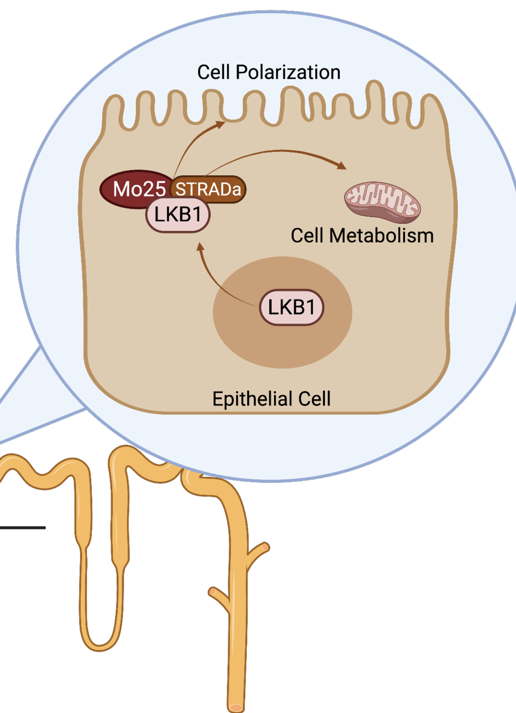


Figure 1. LKB1 and STRADα form a protein complex to aid in cell differentiation mechanisms such as polarization and metabolism

We aim to study how changing the expression of LKB1 and STRADα influences *in vitro* renal cell differentiation and their transcriptional profile.

METHODS

- Human renal proximal tubule epithelial cells (RPTECs) were cultured on standard 6-well polystyrene plates
- Cells were transfected with *piggyBac (PB)* transposase + CuO-STRADα or CuO-LKB1 vector
- RPTECs were cultured in a 1:1 ratio of DMEM/F12 media with 5.5mM glucose and supplemented with EGF, insulin, hydrocortisone, T3, 0.5% FBS, and ascorbic acid[5]

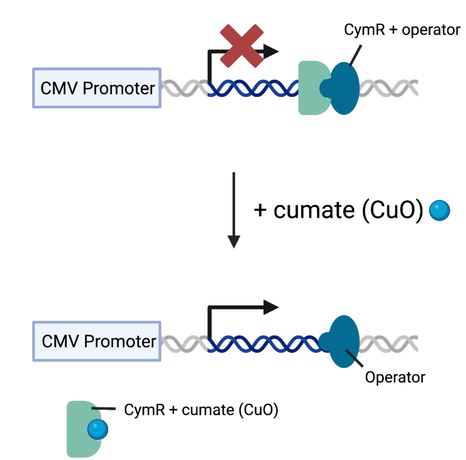


Figure 2. The cumate gene-switch system allows for controllable transcription[3]

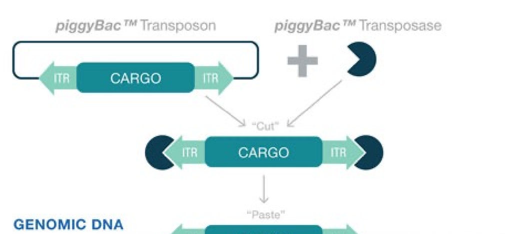
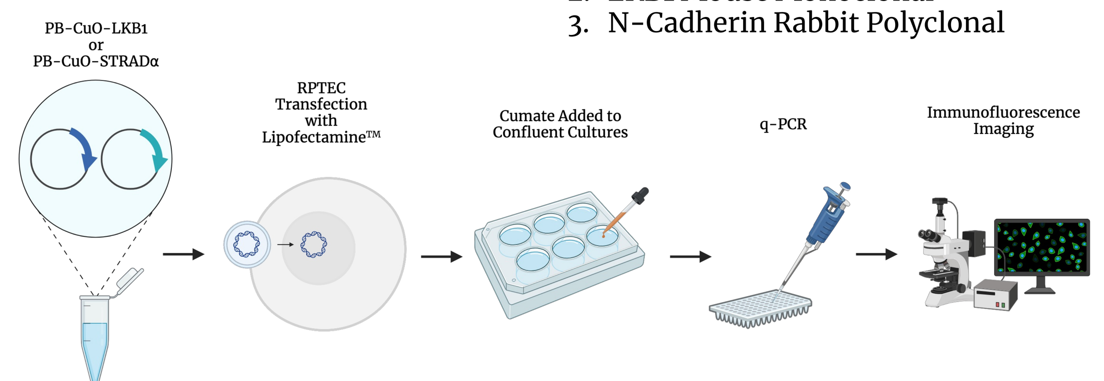


Figure 3. *piggyBac* transposon/transposase accurately inserts desired gene into cell's genome[4]

- Once cells reached confluence, 30 ug/mL cumate (CuO) was added to half the wells to induce transposon expression
- Quantitative real-time PCR (RT-qPCR) was used to assess gene expression[5]
- We assessed whether the genes were translated to proteins by indirect immunofluorescence[5]:
  1. STRADα (LYK5) Rabbit Polyclonal
  2. LKB1 Mouse Monoclonal
  3. N-Cadherin Rabbit Polyclonal



RESULTS

Inducible Gene Expression and LKB1 Localization

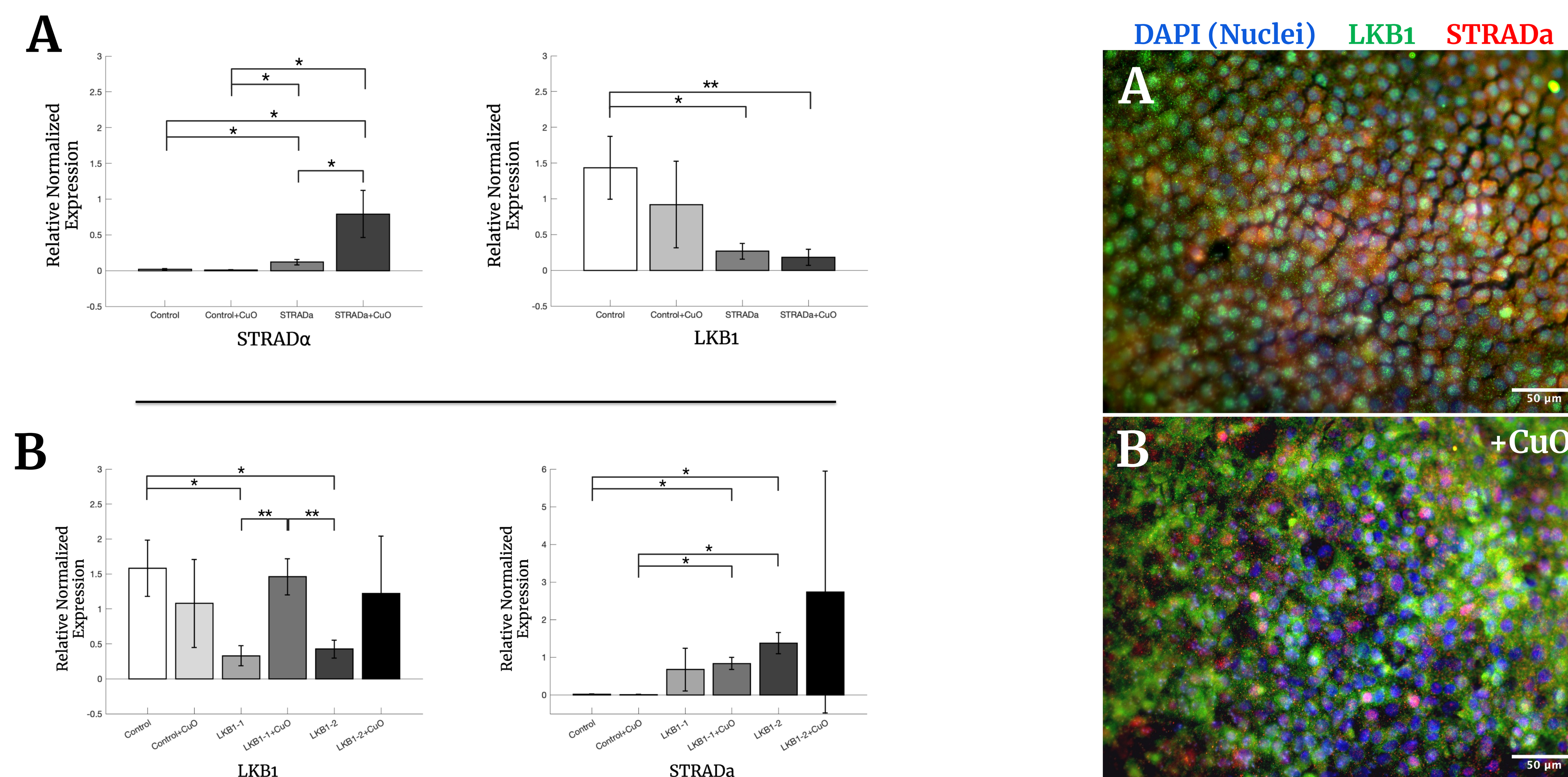


Figure 4. Confirmation of gene expression induction of STRADα and LKB1 in cells transfected with *PiggyBac* - CuO vector. A) STRADα expression significantly increased for STRADα-transfected cells in the presence of cumate, LKB1 expression decreases **B)** LKB1 expression significantly induced in the presence of cumate amongst the first clone, LKB1-1; Expression of STRADα significantly increases in transfected CuO-LKB1 cells. *Expression levels are normalized to GAPDH transcription and presented as mean +/- SD. *p<0.05, **p<0.01*

Figure 5. Localization of LKB1 by STRADα in the presence of cumate. A) LKB1 within the nuclei of the transposon-transfected STRADα cells, no cumate added. **B)** LKB1 amongst cell membranes and cytoplasm in the presence of cumate.

Genetic Variation and *In Vivo*-like Transcriptional Profiles Amongst Clones

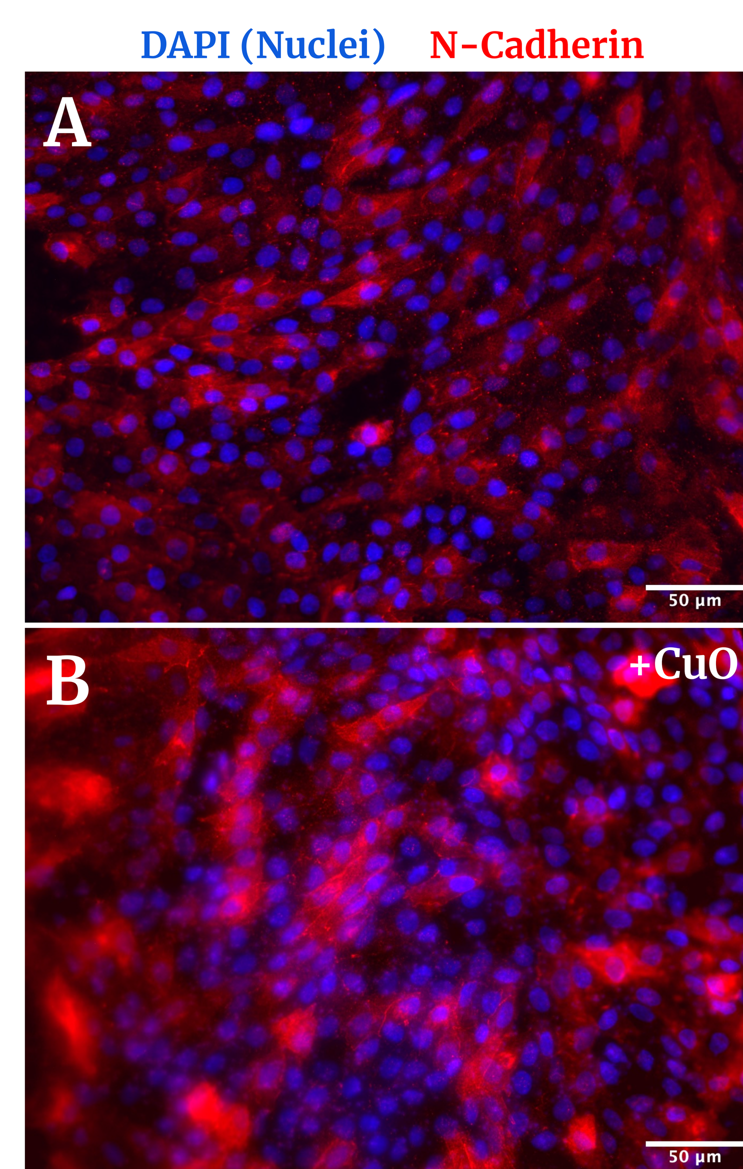


Figure 6. *In vivo* **proximal tubule biomarkers in transfected clones. A)** Appearance of N-Cadherin (abundant protein in proximal tubule cells *in vivo*) along the cell membrane in cultures **B)** Significant N-Cadherin presence despite cumate addition.

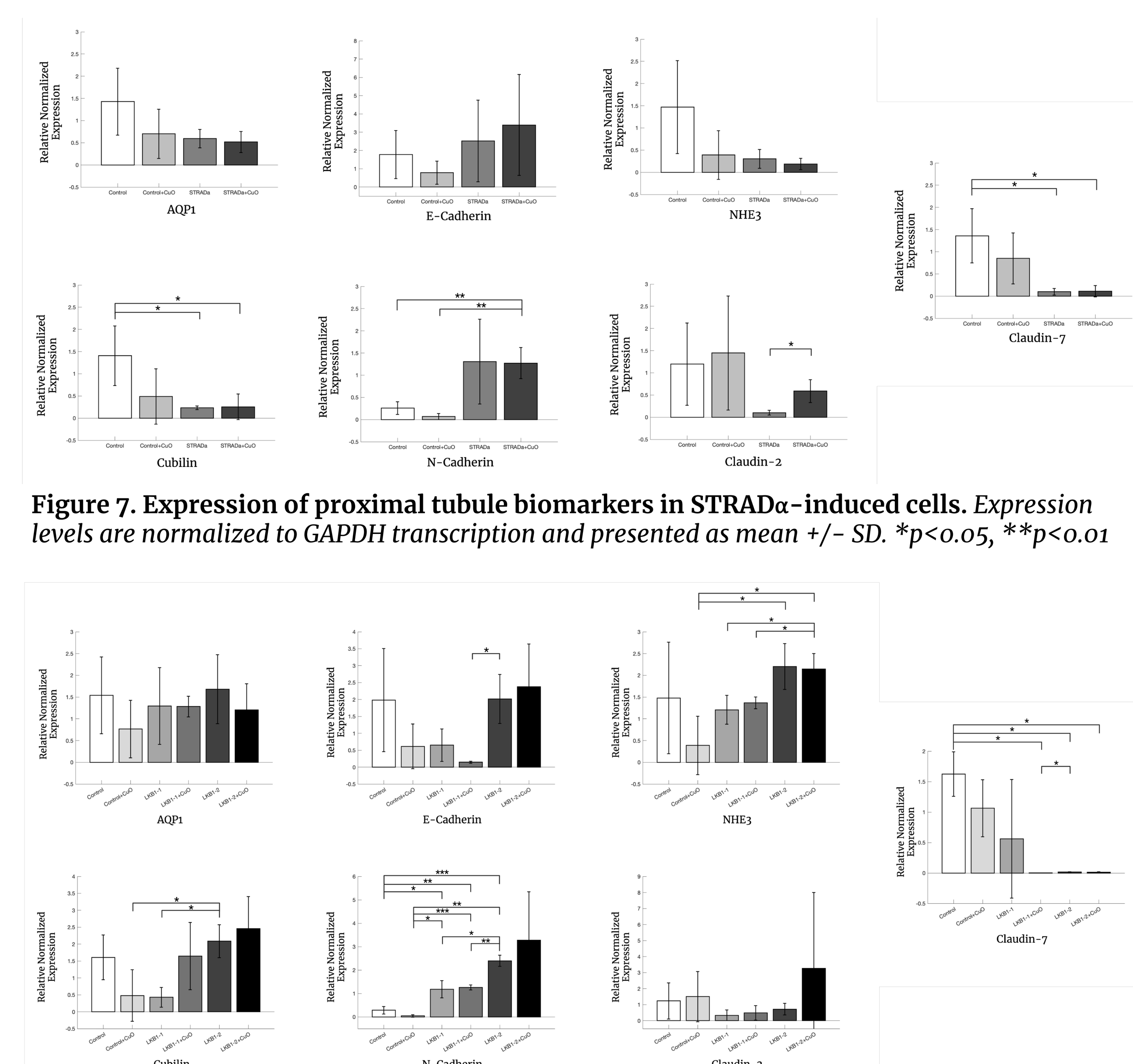


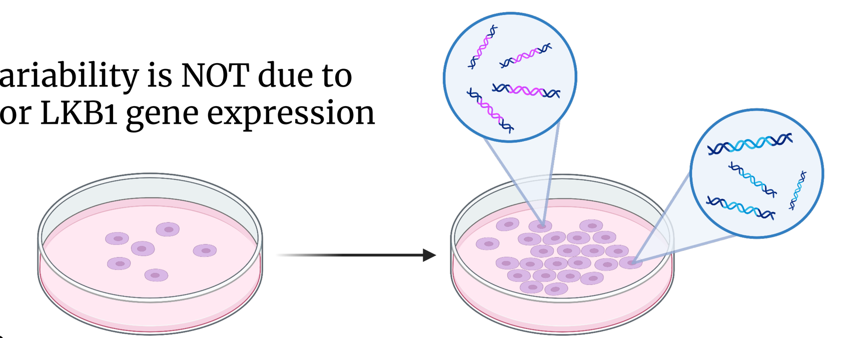
Figure 7. Expression of proximal tubule biomarkers in STRADα-induced cells. *Expression levels are normalized to GAPDH transcription and presented as mean +/- SD. *p<0.05, **p<0.01*

Figure 8. Expression of proximal tubule biomarkers in LKB1-induced clones LKB1-1 and LKB1-2. *Expression levels are normalized to GAPDH transcription and presented as mean +/- SD. *p<0.05, **p<0.01, ***p<0.001*

DISCUSSION

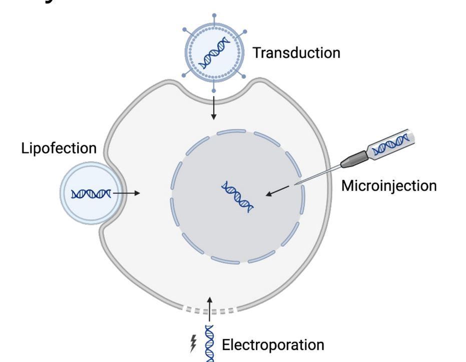
Conclusion

- RPTECs contain significant levels of transcriptional variability amongst clones
- The high genetic variability is NOT due to inducible STRADα or LKB1 gene expression
- The STRADα and LKB1 genes were significantly inducible with the addition of cumate



Future Directions

- Begin trying to isolate different clones from cell cultures and start new independent cultures for each clone and assess the genetic variability
- Test each culture and perform assays for water and ion transport
- Investigate other mechanistic pathways by which cell differentiation can occur
- Create new inducible vectors with other genes to test their impact on cell differentiation *in vitro*
- Explore more efficient transfection methods such as electroporation



REFERENCES

[1] Baas, A. F., Kuipers, J., van der Wel, N. N., Batlle, E., Koerten, H. K., Peters, P. J., & Clevers, H. C. (2004). Complete polarization of single intestinal epithelial cells upon activation of LKB1 by STRAD. *Cell*, *116*(3), 457-466.

[2] Baas, A. F., Boudeau, J., Sapkota, G. P., Smit, L., Medema, R., Morrice, N. A., ... & Clevers, H. C. (2003). Activation of the tumour suppressor kinase LKB1 by the STE20-like pseudokinase STRAD. *The EMBO journal*, 22(12), 3062-3072.

[3] Mullick, A., Xu, Y., Warren, R., Koutroumanis, M., Guilbault, C., Broussau, S., ... & Massie, B. (2006). The cumate gene-switch: a system for regulated expression in mammalian cells. *BMC biotechnology*, 6(1), 1-18..

[4] Allison, D. W. (2023, June 5). *Faster, higher-titer bioproduction*. GEN. https://www.genengnews.com/resources/faster-higher-titer-bioproduction/

[5] Love, H. D., Ao, M., Jorgensen, S., Swearingen, L., Ferrell, N., Evans, R., ... & Fissell, W. H. (2019). Substrate elasticity governs differentiation of renal tubule cells in prolonged culture. *Tissue Engineering Part A*, 25(13-14), 1013-1022.

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