# Generation of proliferating mouse hepatocytes (upcyte® mouse hepatocytes)

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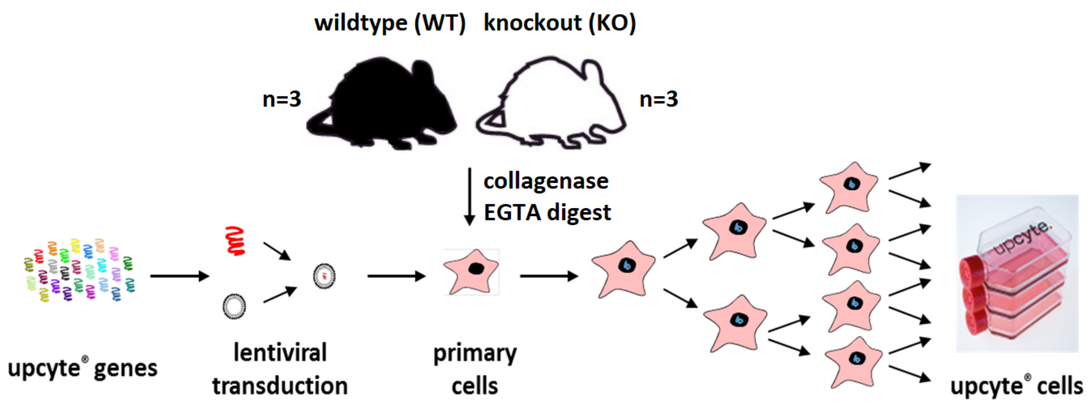
## INTRODUCTION

The concern about the use of laboratory animals is increasing and leads to the support of alternative test methods. upcyte® technologies GmbH contributes to the 3R concept (Reduce, Replace, Refine) through the development of alternatives to avoid the use of animals. Laboratory mice are frequently used for gene knockout studies *in vivo* to evaluate the influence on the organism in total. Additionally isolated mouse cells are an appropriate tool for gene knockout studies on a cellular level. However, the use of primary mouse cells is hampered by e.g. short culture longevity, the limited quantity of cells that can be isolated from one mouse and the lack of proliferation capacity. For experiments on a cellular level, these disadvantages result in repetitive cell isolations and the use of more laboratory mice and thus contradict the concept of the 3Rs.

Since we have successfully generated several human upcyte® cells (e.g. upcyte® hepatocytes, upcyte® liver sinusoidal & microvascular endothelial cells), the feasibility of the upcyte® technology on other species is of interest. Here, we show for the first time whether the transduction of proliferation-inducing genes could extend the lifespan of primary mouse hepatocytes without losing their primary characteristics. For this purpose, primary mouse hepatocytes from three wildtype (WT) and three knockout (KO) C57BL/6 mice were isolated and subsequently transduced with upcyte® proliferation genes.

## RESULTS

### Generation of upcyte® mouse hepatocytes

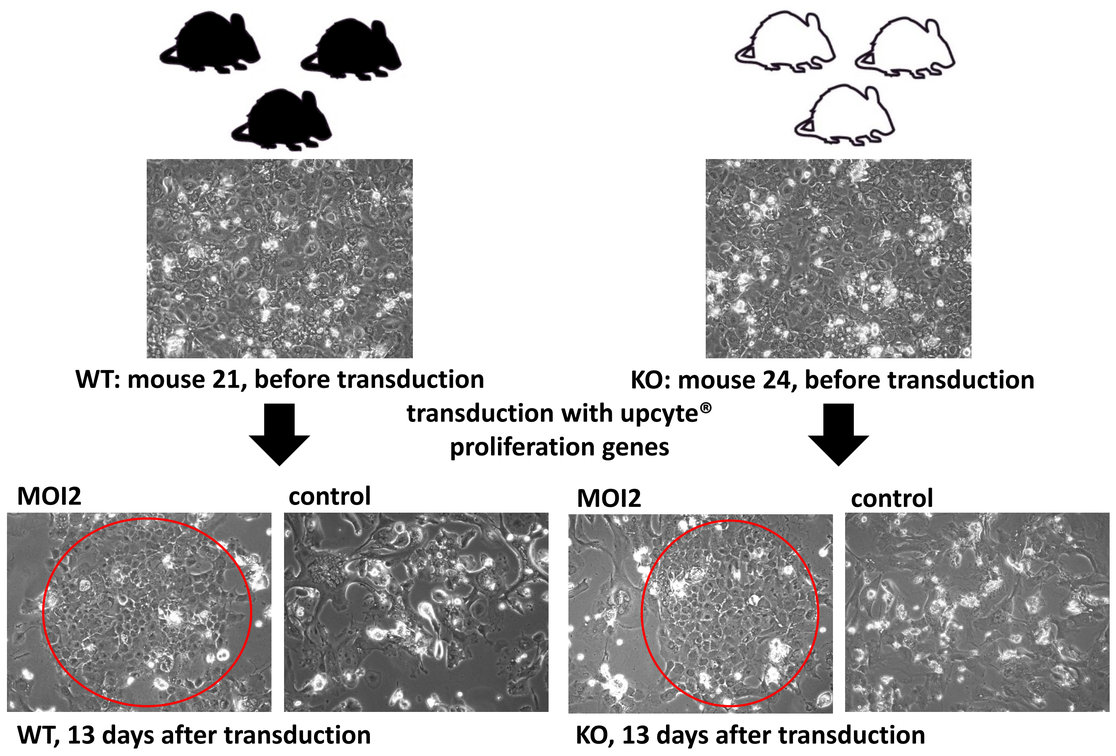



**Transduction of primary cells with a defined cocktail of lentiviral vectors**

Previously, a novel technique was reported which enabled the generation of non-transformed proliferating liver cells from primary human hepatocytes which maintained an adult phenotype, so-called “upcyte® cells”. The upcyte® technology was tested for feasibility on primary mouse hepatocytes. For this purpose three **wildtype (WT)** and three **knockout (KO) C57BL/6 mice** were used for isolation of hepatocytes. upcyte® cells start to grow from primary cells after transduction with a defined cocktail of lentiviral vectors carrying proliferation inducing genes. upcyte® cells were expanded and for each mouse a master cell bank (MCBs) was generated. After generation of MCBs, mouse hepatocytes were analyzed for their morphology and mouse hepatic markers.

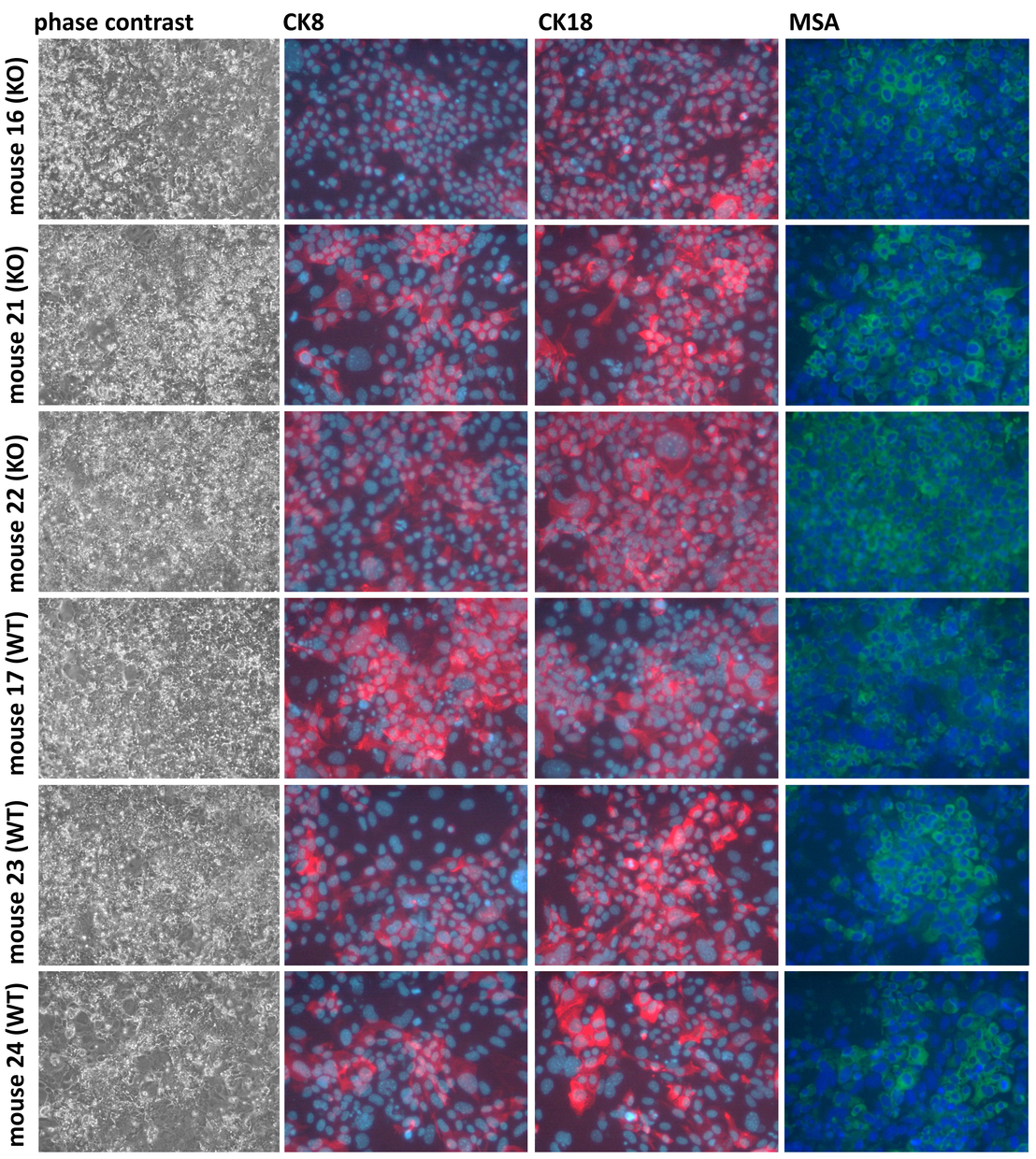
### Isolation and upcyting of mouse hepatocytes

Murine hepatocytes were isolated from three wildtype (mouse 16, 21 and 22) and three knockout (mouse 17, 23 and 24) C57/BL6 mice using a two-step collagenase perfusion technique as described previously (Li *et al.* 2010, Methods Mol Biol.; 633: 185-96). Briefly, mice were anaesthetized by intraperitoneal injection of ketamine and xylazine. Livers were perfused with EGTA and collagenase via the vena cava inferior and the portal vein. Digested liver tissues were manually dissociated and filtered through a cell strainer. Resulting cells were counted and seeded sub-confluent, to allow additional growth, on collagen coated cell culture vessels, in hepatocyte growth medium. After 24 h isolation of mouse hepatocytes, cells were transduced at multiplicity of infection (MOI) 1 and 2.




Isolated mouse hepatocytes maintained their typical cobblestone morphology and a confluent monolayer before transduction. Primary cells were transduced and cells were monitored for proliferating spots of hepatocytes. After 13 days proliferating cells were visible (red circle), whereas only senescent cells where found in untransduced control wells. For all six mice proliferating upcyte® cells were found. Wildtype mouse 21 and for knockout mouse 24 are exemplary shown.

### Morphology and mouse hepatic markers




All six upcyte® mouse hepatocytes were analysed for their morphology and for the expression of mouse hepatocyte marker proteins. upcyte® mouse hepatocytes were seeded at confluence of 150,000 cells/cm² (“confluent”). Cultures seeded at high densities displayed a primary hepatocyte-like morphology. The expression of marker proteins were analysed by fluorescence staining for **cytokeratin 8, CK8 (red, left panel), cytokeratin 18, CK18 (red, mid panel), mouse serum albumin, MSA (green, right panel)** and counter-stained against **DAPI (blue)**. They are characterized by expression of the basal marker CK8 and CK18 and identified as mouse hepatocytes by expression of MSA. Identical expression of marker proteins were verifiable for wildtype and knockout upcyte® cells.

## SUMMARY & CONCLUSION

In conclusion, the upcyte® technology can be used to generate proliferating mouse hepatocytes from wildtype and knockout mice, while retaining their phenotype when cultured at confluence. The resulting cells called “upcyte® mouse hepatocytes” express hepatocyte markers such as CK8, CK18 and MSA. Thus, the upcyte® technology can contribute to the 3Rs concept and provide a suitable tool for knockout studies on a cellular level.

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