

Background

- Prostate cancer (PCa) and bladder cancer (BCa) are both genetically diverse.
- Next-generation sequencing technologies have identified numerous genetic alterations associated with both cancers.
- The complex and dynamic nature of cancer **heterogeneity** leads to a challenge in clinical practice during the stratification of patients into low-risk and high-risk treatment.
- Different cell and tissue types have different responses to genetic oncogenic drivers.
- It is important to investigate the common and **tissue-specific genetic alterations** in both cancer types for development of effective treatments.
- In this study, we have selected 14 tumor suppressor and oncogenes that are recurrently altered in PCa and BCa (Figure 1) and will characterize PCa and BCa tissue-specific responses to these oncogenic factors.

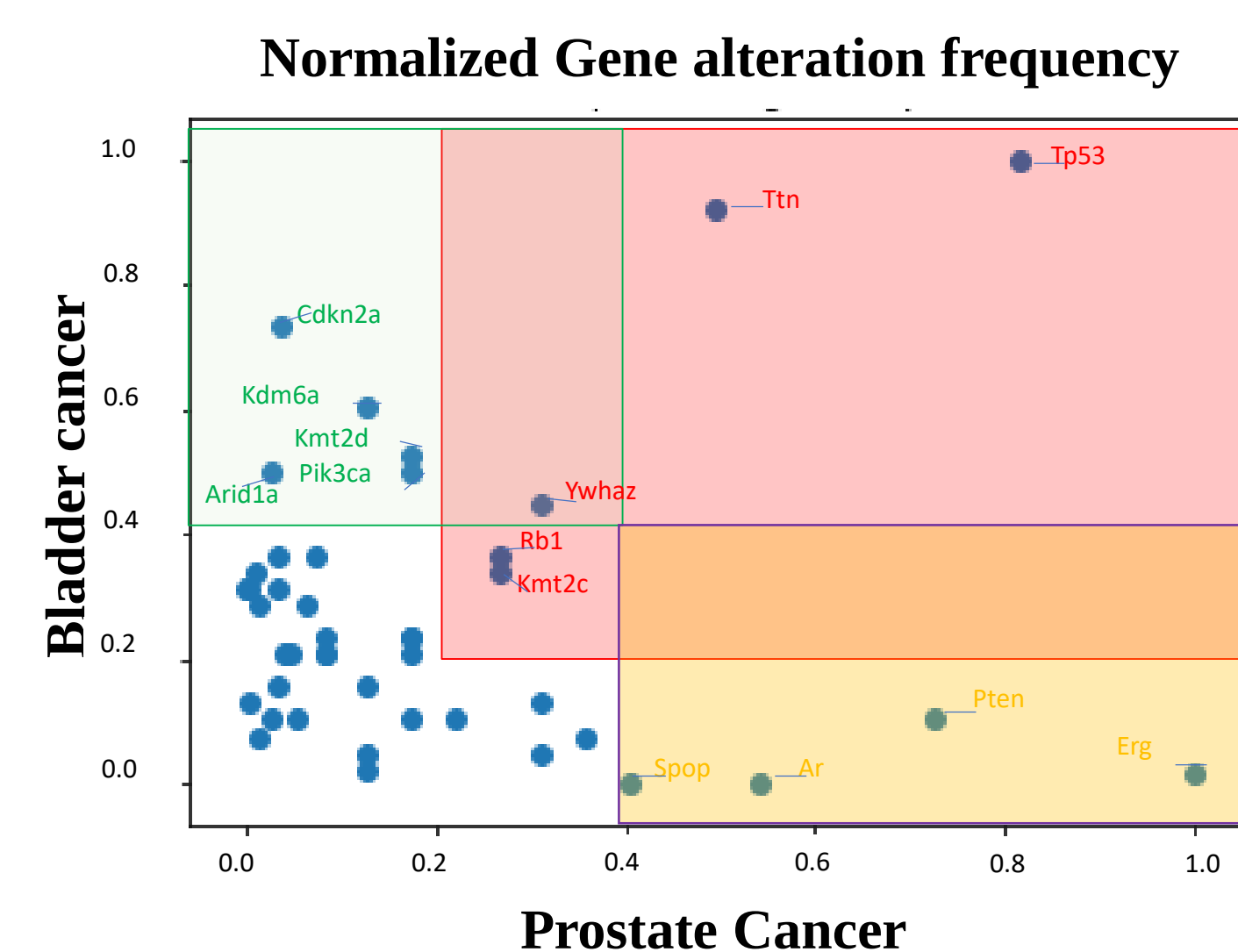
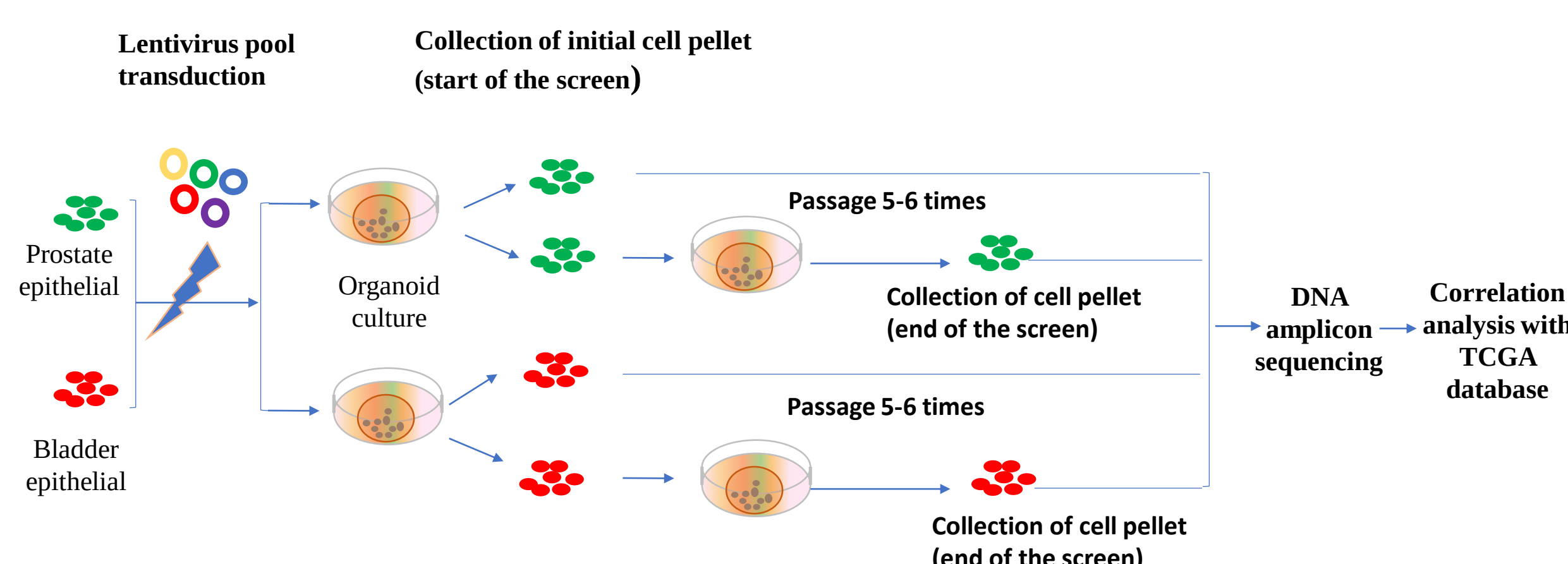


Figure 1: Recurrently mutated genes associated with either PCa or BCa or both.

Methods

Identify tissue-specific genetic alterations that modulate cellular fitness in the process of mouse prostate epithelial and bladder urothelial organoids transformation



Methods

Generation of barcoded lentiviral constructs

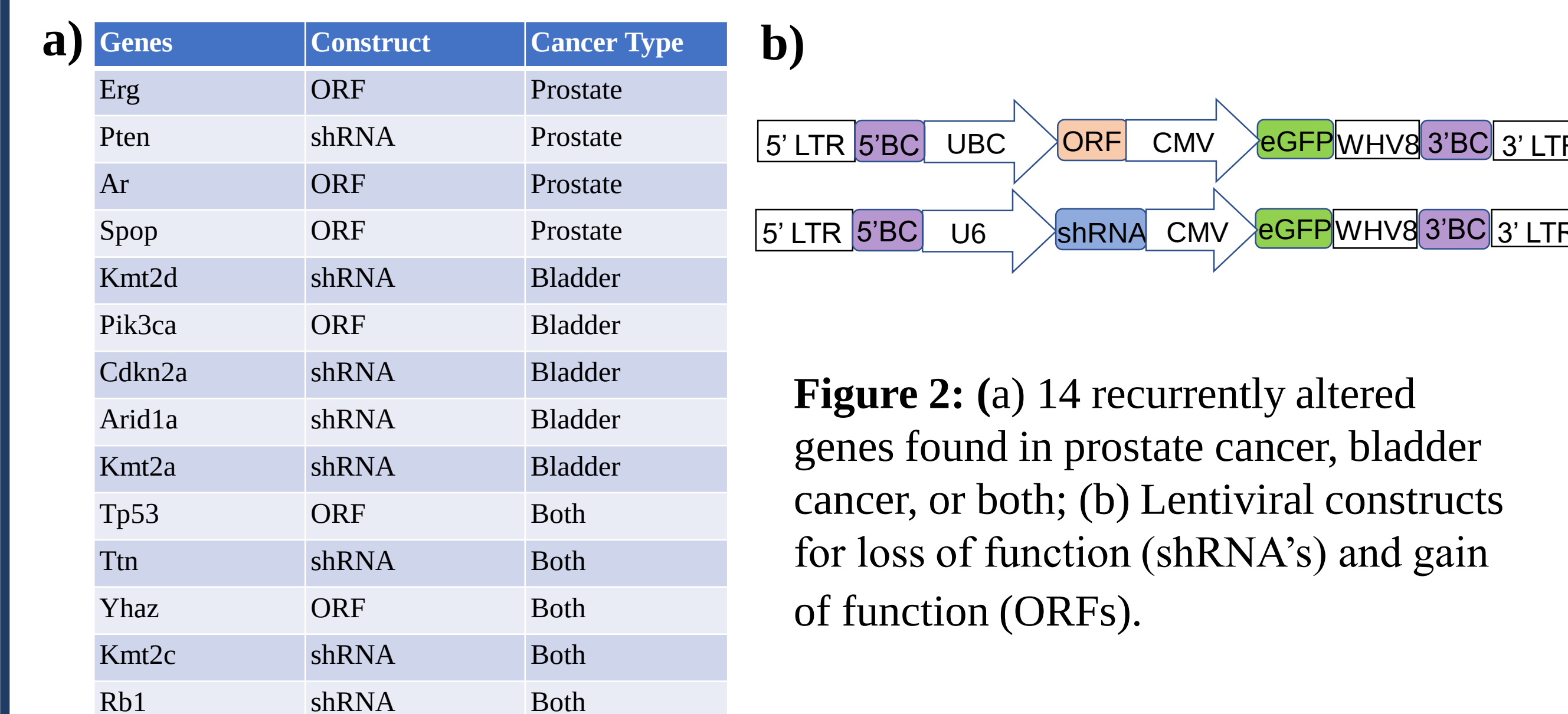
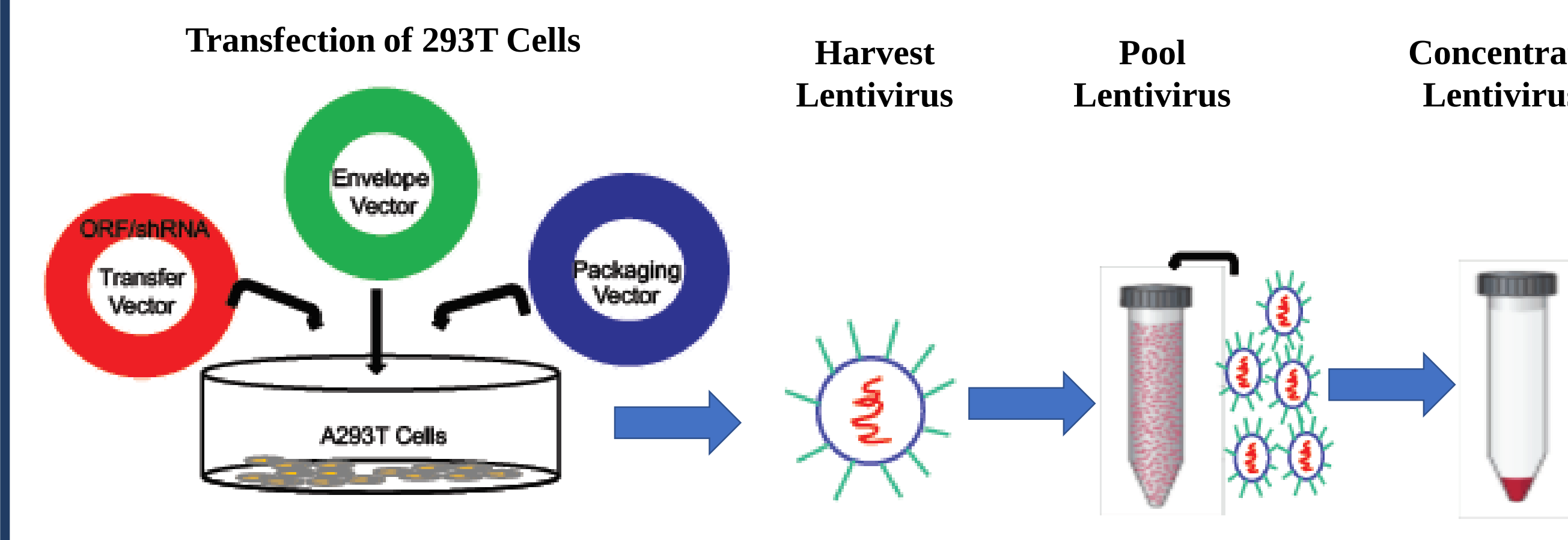


Figure 2: (a) 14 recurrently altered genes found in prostate cancer, bladder cancer, or both; (b) Lentiviral constructs for loss of function (shRNA's) and gain of function (ORF's).

Generation of lentiviral library



Results

Transfection of 293T cells with lentiviral constructs

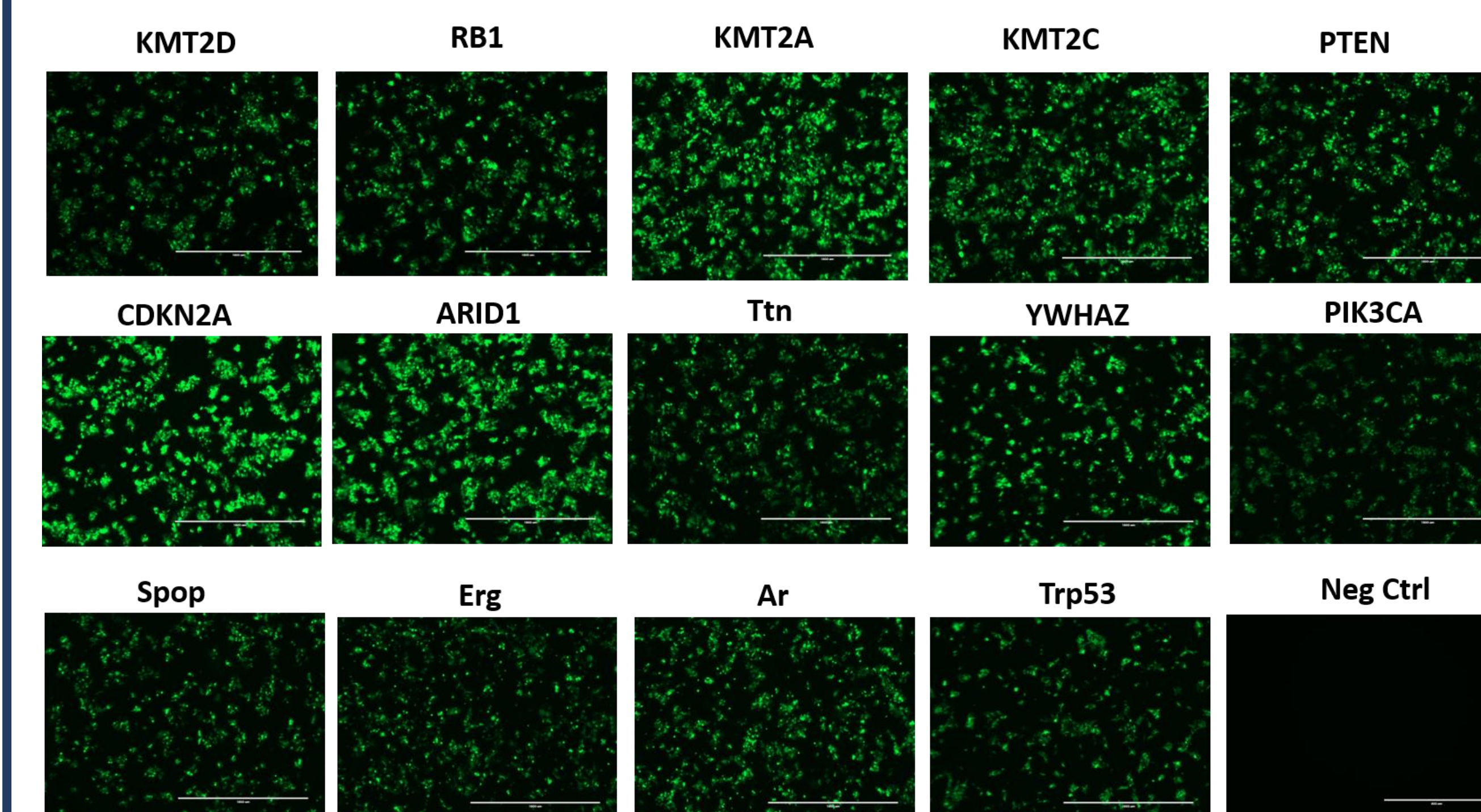


Figure 3: Transfection of 293T cells, monitored by GFP fluorescence.

Results

Transduction of mouse prostate organoids with pooled lentivirus

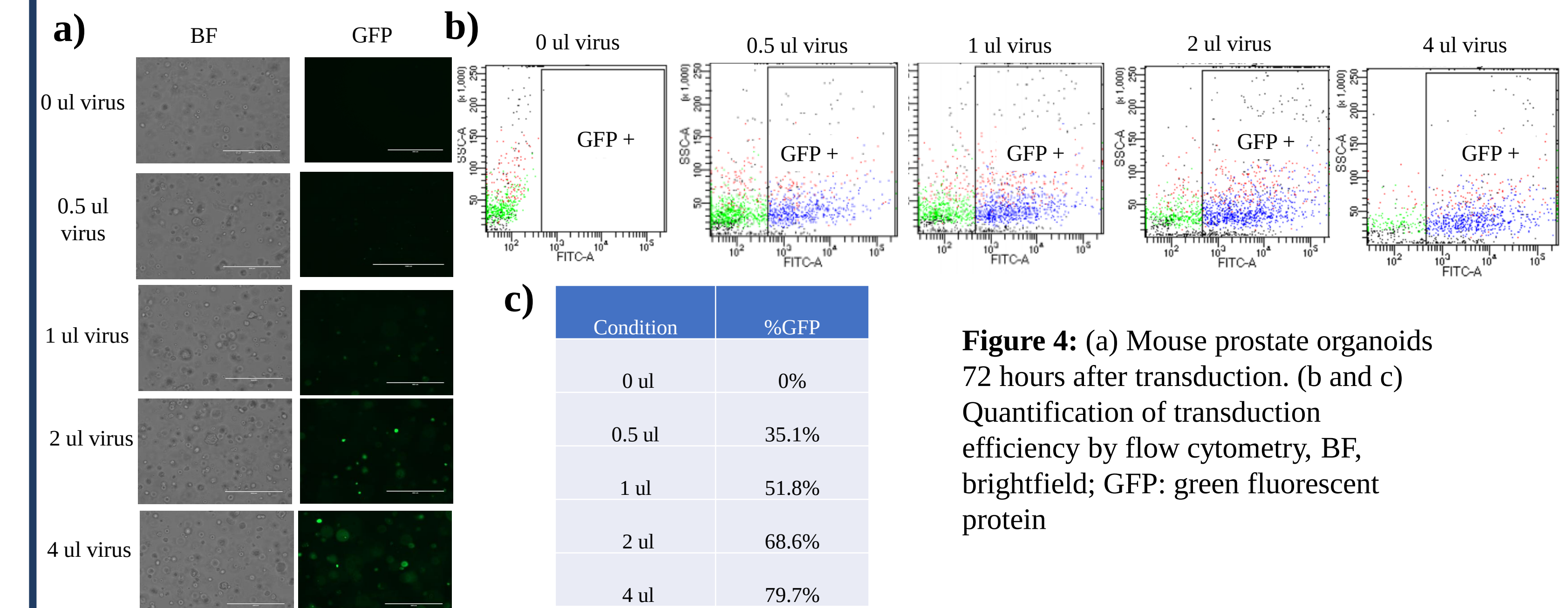


Figure 4: (a) Mouse prostate organoids 72 hours after transduction. (b and c) Quantification of transduction efficiency by flow cytometry, BF, brightfield; GFP: green fluorescent protein

Transduction of mouse bladder organoids with pooled lentivirus

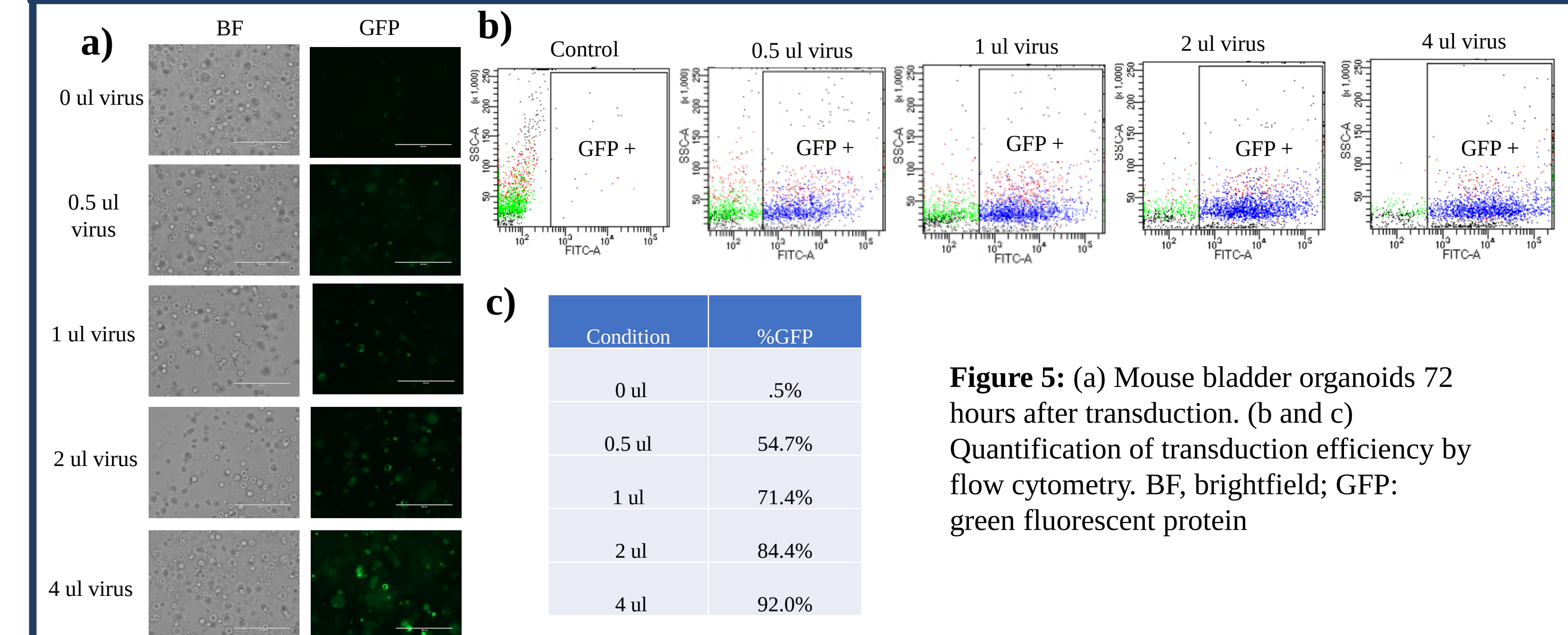


Figure 5: (a) Mouse bladder organoids 72 hours after transduction. (b and c) Quantification of transduction efficiency by flow cytometry. BF, brightfield; GFP: green fluorescent protein

Discussion and Future Plan

- We have generated a lentiviral pool encoding 14 recurrently mutated genes in either PCa or BCa or both.
- The titer of pooled lentivirus was determined by transducing mouse prostate and bladder epithelial organoids followed by flow cytometry.
- We will examine lentiviral representation in the pool by bulk DNA amplicon sequencing.
- The optimized lentiviral pool will be used for tissue-type specific assay.

Acknowledgements

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