Design of sgRNA libraries for CRISPRn screens

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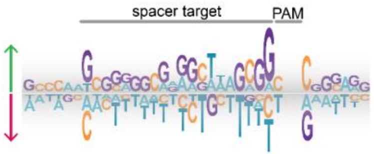
Lukas Badertscher, PhD | VP R&D

2024

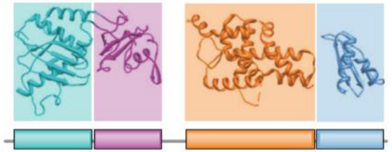
The sgRNA design algorithm for CRISPRn screens

Training dataset of ~46,000 sgRNAs from published and in-house data sources

Target Sequence



Protein organization

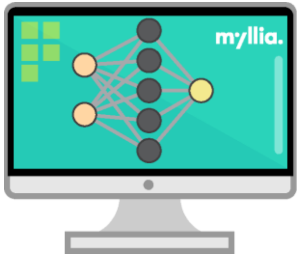


Evolutionary conservation

```
MAKNKLRGPKS------RNVFHIASQKNFKAKNKAKPVTTNLKKINI
MAKNKLKGQKS------RNVFHIASHKTFKAKNKAKPVTTNLKKINI
MGKNKQKGKKQ------PNVFQVAN-KQSKPKTKAKPVKSSLKHINT
MAKSKGKGQKQKTTKKQNVFHVANSKSVKAKNKAKPVKTSLKKIHG
*.*.* :* *.       ***::*. *  *.*.*****.:.**:*:
```

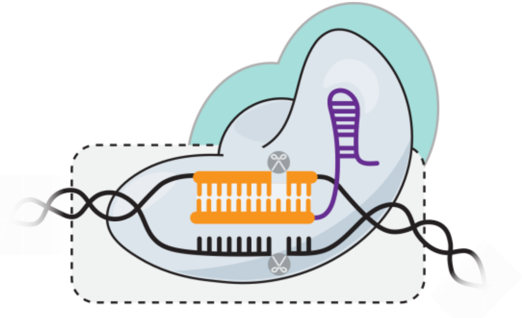
… and additional features

Convolutional neural network model



● Myllia (4) ✂✂✂✂

CRISPRn (indel formation)

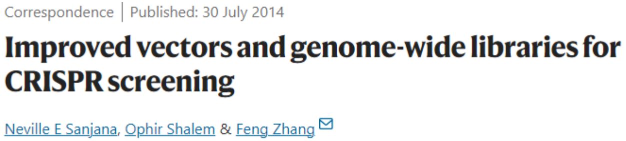


Experimentally validate the sgRNA activity on both fitness & non-fitness phenotypes

Commonly used genome-scale sgRNA libraries for CRISPRn screens

myllia.

● **GeCKO_v2** ✂✂✂✂✂✂



Correspondence | Published: 30 July 2014

Improved vectors and genome-wide libraries for CRISPR screening

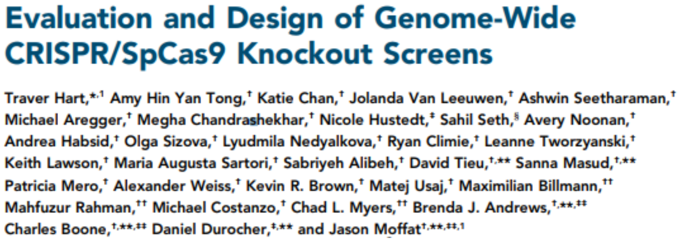
Neville E Sanjana, Ophir Shalem & Feng Zhang ✉

● **Behan** ✂✂✂✂✂

Prioritization of cancer therapeutic targets using CRISPR–Cas9 screens

Fiona M. Behan[1,2,12], Francesco Iorio[1,2,3,12], Gabriele Picco[1,12], Emanuel Gonçalves[1], Charlotte M. Beaver[1], Giorgia Migliardi[4,5], Rita Santos[6], Yanhua Rao[7], Francesco Sassi[4], Marika Pinnelli[4,5], Rizwan Ansari[1], Sarah Harper[1], David Adam Jackson[1], Rebecca McRae[1], Rachel Pooley[1], Piers Wilkinson[1], Dieudonne van der Meer[1], David Dow[2,6], Carolyn Buser-Doepner[2,7], Andrea Bertotti[4,5], Livio Trusolino[4,5], Euan A. Stronach[2,6], Julio Saez-Rodriguez[2,3,8,9,10], Kosuke Yusa[1,2,11,13*] & Mathew J. Garnett[1,2,13*]

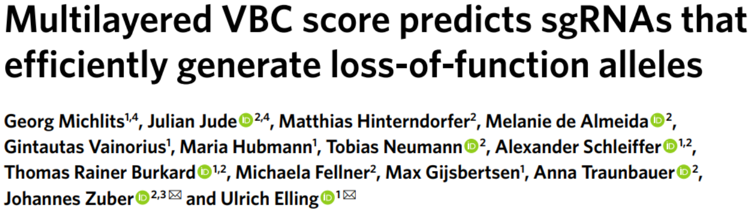
● **TKO_v3** ✂✂✂✂



Evaluation and Design of Genome-Wide CRISPR/SpCas9 Knockout Screens

Traver Hart,[*,1] Amy Hin Yan Tong,[†] Katie Chan,[†] Jolanda Van Leeuwen,[†] Ashwin Seetharaman,[†] Michael Aregger,[†] Megha Chandrashekhar,[†] Nicole Hustedt,[‡] Sahil Seth,[§] Avery Noonan,[†] Andrea Habsid,[†] Olga Sizova,[†] Lyudmila Nedyalkova,[†] Ryan Climie,[†] Leanne Tworzyanski,[†] Keith Lawson,[†] Maria Augusta Sartori,[†] Sabriyeh Alibeh,[†] David Tieu,[†,**] Sanna Masud,[†,**] Patricia Mero,[†] Alexander Weiss,[†] Kevin R. Brown,[†] Matej Usaj,[†] Maximilian Billmann,[††] Mahfuzur Rahman,[††] Michael Costanzo,[†] Chad L. Myers,[††] Brenda J. Andrews,[†,**,‡‡] Charles Boone,[†,**,‡‡] Daniel Durocher,[‡,**] and Jason Moffat[†,**,‡‡,1]

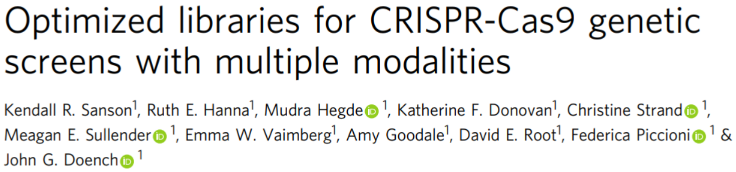
● **VBC** ✂✂✂✂



Multilayered VBC score predicts sgRNAs that efficiently generate loss-of-function alleles

Georg Michlits[1,4], Julian Jude[2,4], Matthias Hinterndorfer[2], Melanie de Almeida[2], Gintautas Vainorius[1], Maria Hubmann[1], Tobias Neumann[2], Alexander Schleiffer[1,2], Thomas Rainer Burkard[1,2], Michaela Fellner[2], Max Gijsbertsen[1], Anna Traunbauer[2], Johannes Zuber[2,3] ✉ and Ulrich Elling[1] ✉

● **Brunello** ✂✂✂✂



Optimized libraries for CRISPR-Cas9 genetic screens with multiple modalities

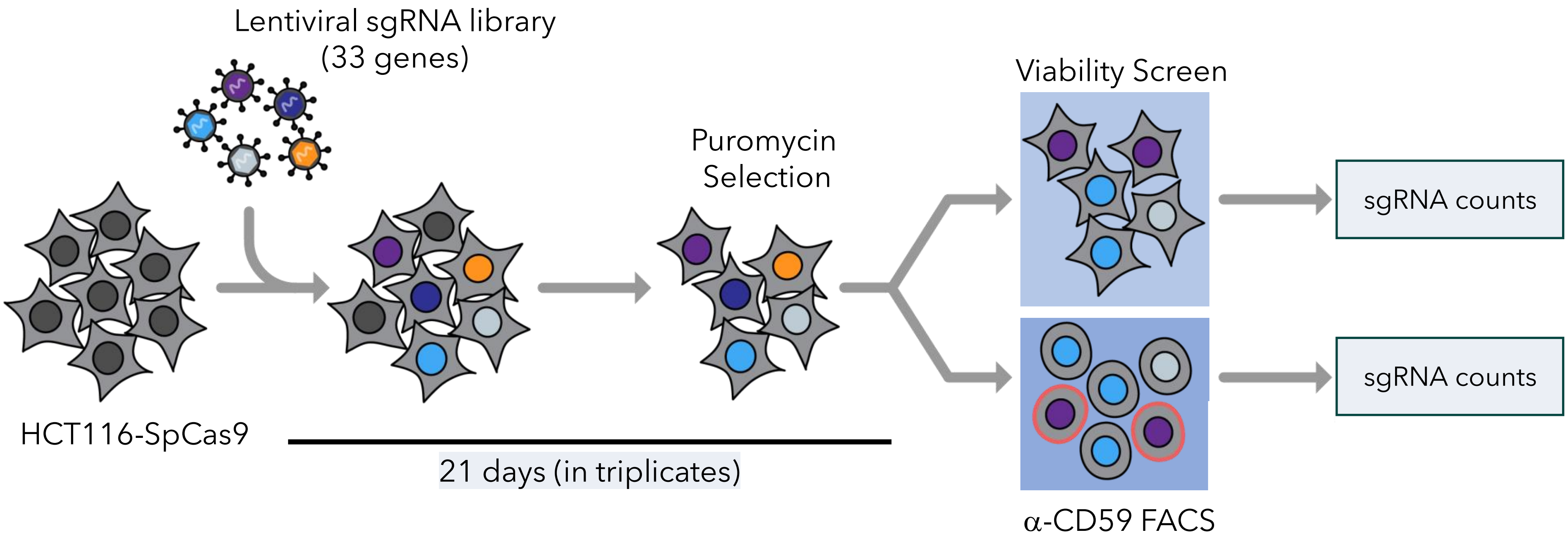
Kendall R. Sanson[1], Ruth E. Hanna[1], Mudra Hegde[1], Katherine F. Donovan[1], Christine Strand[1], Meagan E. Sullender[1], Emma W. Vaimberg[1], Amy Goodale[1], David E. Root[1], Federica Piccioni[1] & John G. Doench[1]

● **MinLibCas9** ✂✂

Minimal genome-wide human CRISPR-Cas9 library

Emanuel Gonçalves[1], Mark Thomas[1], Fiona M. Behan[1], Gabriele Picco[1], Clare Pacini[1,2], Felicity Allen[1], Alessandro Vinceti[3], Mamta Sharma[1], David A. Jackson[1], Stacey Price[1], Charlotte M. Beaver[1], Oliver Dovey David Parry-Smith[1], Francesco Iorio[1,3], Leopold Parts[1,4], Kosuke Yusa[5] and Mathew J. Garnett[1*]

CRISPR screens to target essential and non-essential genes



Lentiviral sgRNA library
(33 genes)

HCT116-SpCas9

Puromycin
Selection

21 days (in triplicates)

Viability Screen

α-CD59 FACS

sgRNA counts

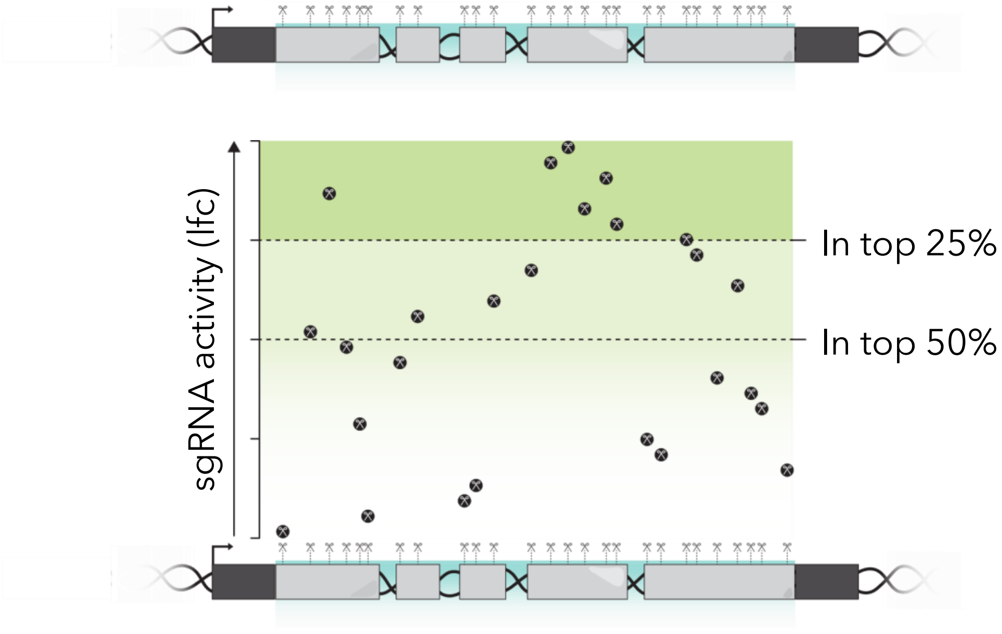
sgRNA counts

Dropout screen based on essentiality
- 24 genes, ~50 sgRNAs/gene

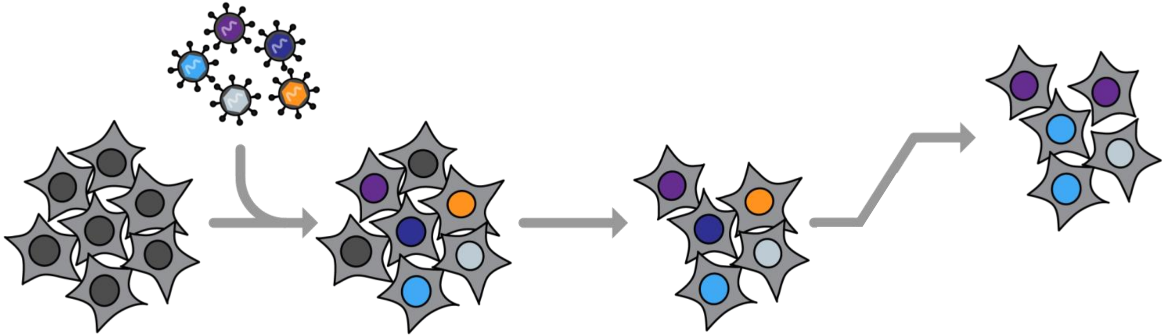
CD59 FACS readout
- 9 genes, ~50 sgRNAs/gene

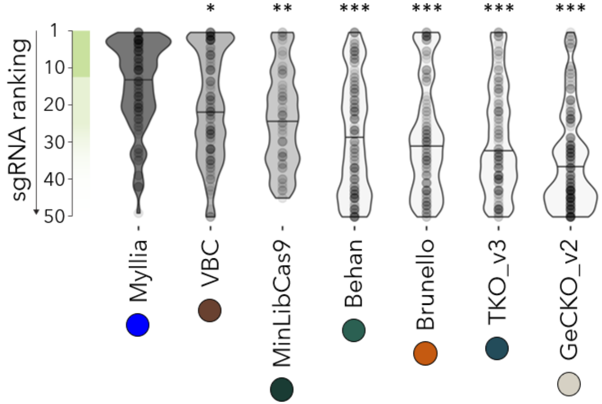
Ranking sgRNA activities based on the observed phenotypes

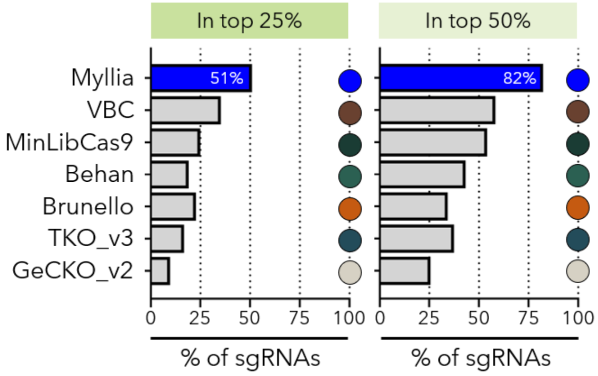
~ 50 sgRNAs against each gene to cover the CDS



Viability phenotype to assess sgRNA activities



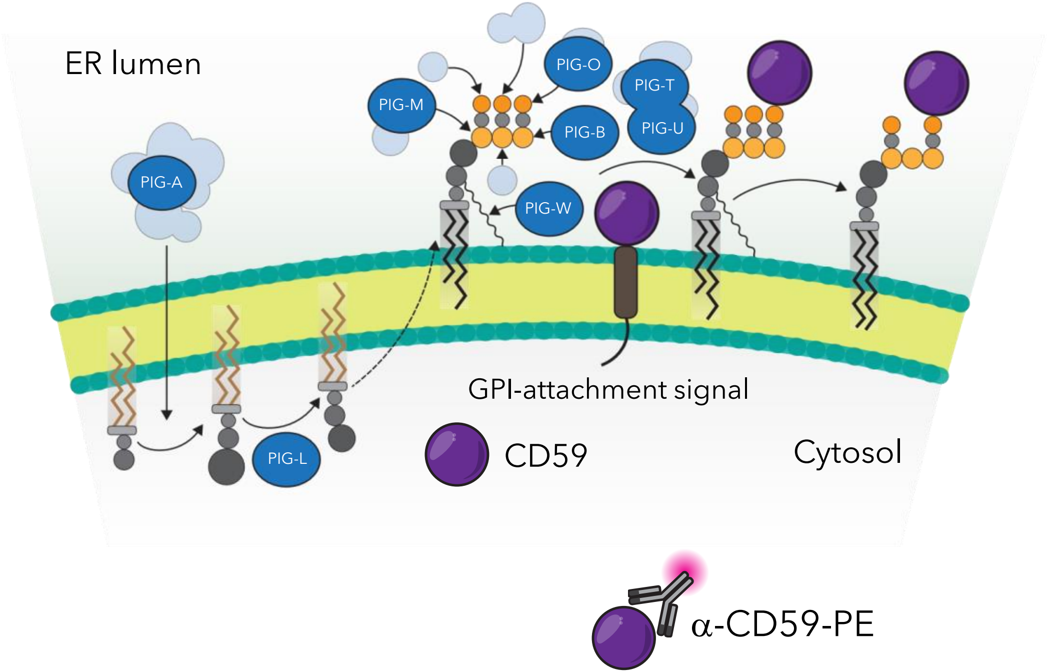




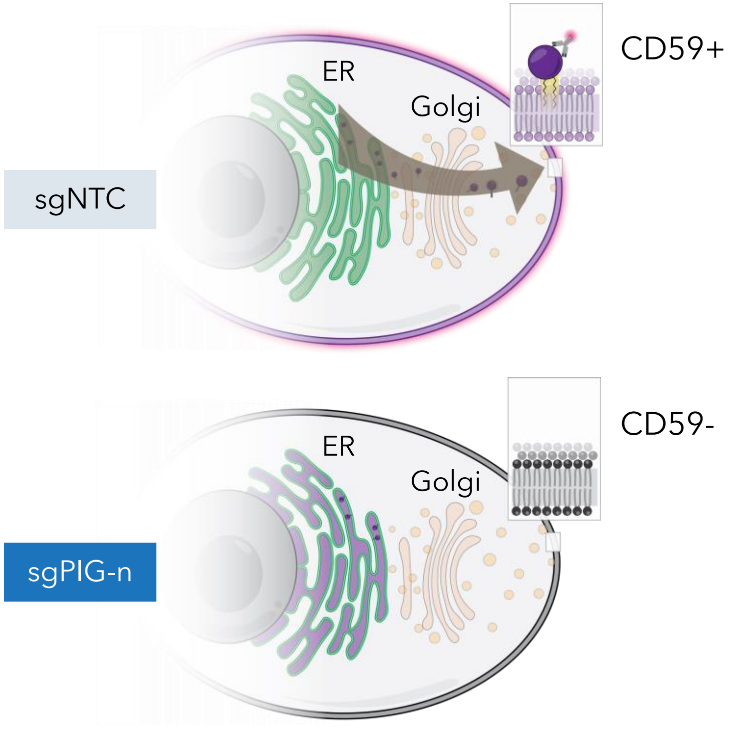
* = significant difference to Myllia, U-test on normalized LFC
*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001

FACS-based CRISPR screen to monitor CD59 localization

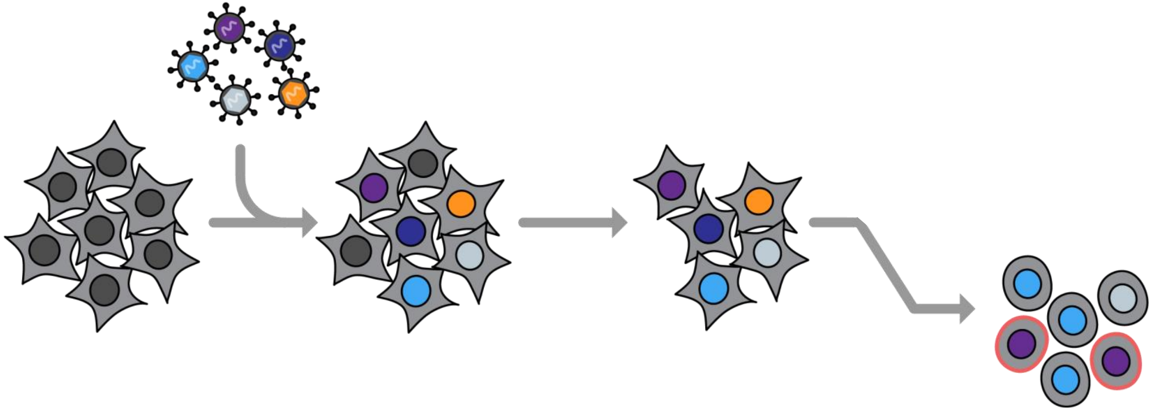
Biosynthesis of GPI in the endoplasmic reticulum

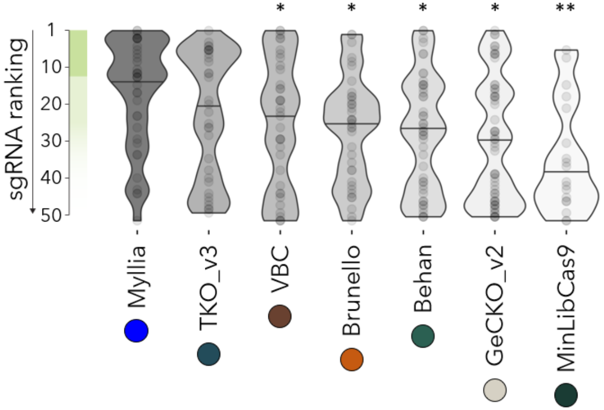


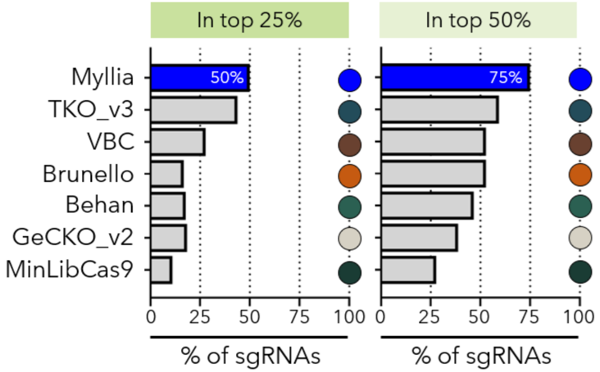
Maturation of GPI-anchored proteins



CD59 localization to evaluate sgRNAs for targeting non-essential genes







* = significant difference to Myllia, U-test on normalized LFC
*$p \leq 0.05$, **$p \leq 0.01$,

Conclusions

- Myllia´s sgRNA design algorithm selects highly active sgRNA sequences for targeting both essential and non-essential genes and appears to be “superior” to other publicly available libraries

- Comparable observations have been made in: A benchmark comparison of CRISPRn guide RNA design algorithms and generation of small single and dual-targeting libraries to boost screening efficiency (Lukasiak et al., 2024 bioRxiv)

- We will expand the screening campaign to target additional non-essential genes that may help further improve sgRNA design and increase editing rates

- CRISPRi libraries will be evaluated using a similar workflow

Acknowledgements

Evaluation of sgRNAs
Anatoly Vasilyev
Nicole Untermoser
Sumit Pawar
Adam Krejci
Lukas Badertscher

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Myllia Biotechnology strives to perform next-generation CRISPR screening workflows utilizing cancer cell lines and primary human T cells.

Contact us to discuss your CRISPR projects in the space of cancer immunotherapy and immuno-oncology!

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