APPEAL project
(Antivirus Pandemic Preparedness EuropeAn pLatform):
Identification of broad-spectrum antivirals targeting cellular factors

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INTRODUCTION

The development of new antivirals, particularly against emerging and re-emerging viruses prioritized by the World Health Organization (WHO)[1], is a **major public health priority.**

Viruses rely on **host dependency factors (HDFs)** for infection, while **host-restriction factors (HRFs)** limit viral replication. HDFs and HRFs could be targeted or induced for the development of **host-directed antiviral therapies,** which represent a promising strategy for the development of **broad-spectrum antivirals**, notably because they reduce the risk of emergence of viral resistant mutants.

However, identifying relevant host targets remains **challenging** due to the **variability and limited reproducibility** of genome-wide high-throughput screens, as observed in studies on SARS-CoV-2[2] and influenza A virus.

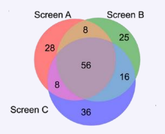
OBJECTIVES

The APPEAL project proposes an approach integrating multidisciplinary and interconnected expertise aimed at :

- Developing a **bioinformatics** and **experimental pipeline** to identify and prioritize host genes involved in the replication of the pathogenic viruses of concern.
- Validating these candidate genes in **physiologically relevant cellular models** from healthy donors.
- **Identifying** and/or **repurposing** molecules that target these host factors or their associated pathways, to support the development of **novel antiviral strategies.**

MACHINE LEARNING

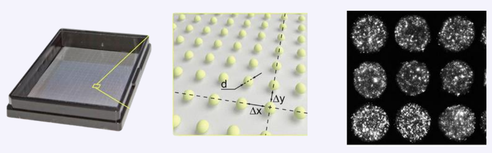
Identification of **consistent patterns** across heterogeneous screening data by integrating diverse omics information from virus-infected cells



Generation of a comprehensive list of ~2000 HDFs/HRFs candidates

HIGH-DENSITY CELL ARRAYS (HD-CA)

High-throughput screening platform using **microscopy-based phenotypic analysis** assays[2]

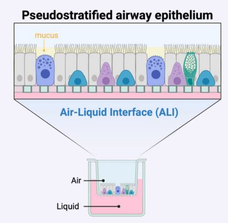


- Ability to "spot" **up to 24,576 sgRNAs/siRNA** for *in-situ* transfection of overlaid cells of model
- Cost- and time-effective

CRISPR screens on HD-CA for the selection of promising HDFs/HRFs candidates

3D PRIMARY CELL MODELS

Physiologically relevant cellular models for the characterization of cell-pathogen interactions and **better prediction** of compounds efficiency and toxicity



(e.g., primary human airway epithelia cultured at the Air-liquid interface (ALI))

HDF/HRF and drug candidates validation on relevant models, bypassing misleading results often obtained from cancer cell lines

FINAL COMPOUND ASSESMENT

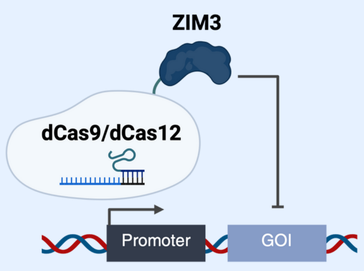
Candidate compound is selected for **preclinical** and **clinical** evaluation as a **repurposed drug**

Better preparedness for future pandemics, reinforcing the world's capacity to respond to health crises

OPTIMIZATION OF CRISPR-CAS TECHNOLOGIES FOR HDF/HRF SCREENING IN PRIMARY CELLS

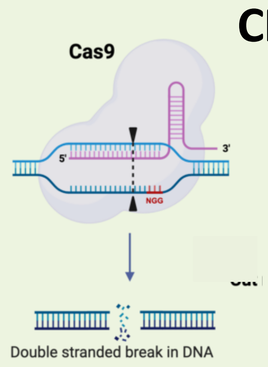
TOOL : DEVELOPMENT OF AN **ALL-IN-ONE PLATFORM** FOR GENOME EDITING

CRISPR INTERFERENCE (CRISPRi)

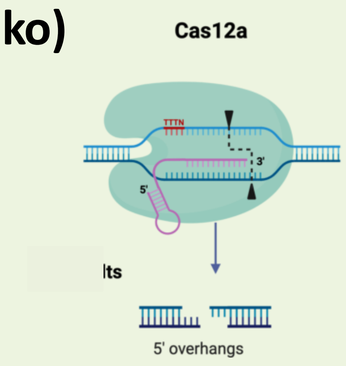


*Cas protein-fused to transcriptional repressor KRAB domains **(ZIM3)***

CRISPR KNOCK-OUT (CRISPR ko)



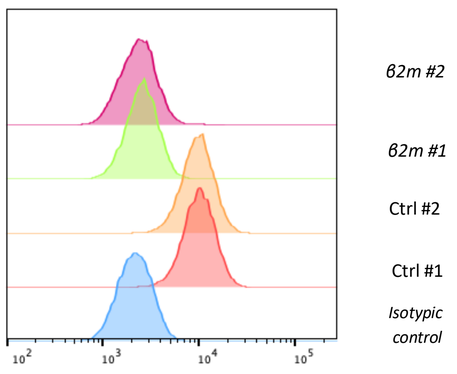
Generation of double-stranded DNA breaks



dCas9-based

*ZIM3-dCas9 **(DNase inactive)***

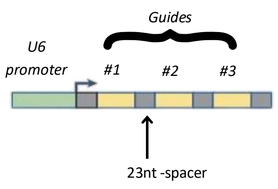
*Individual CRIPSRi populations in A549 cells using **our novel all-in-one platform pLentiCRISPRv3-dCas9-ZIM3***
Surface expression of ß2m was analyzed 6 days post transduction by flow cytometry

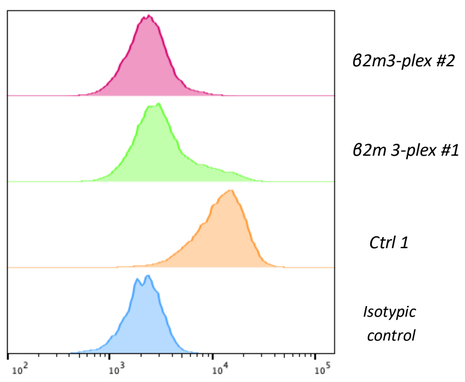


dCas12a-based

*ZIM3-denCas12A R1226A **(DNase-slow)***

Enables **multiplexed gene editing**4 through its ability to **process its own gRNA**

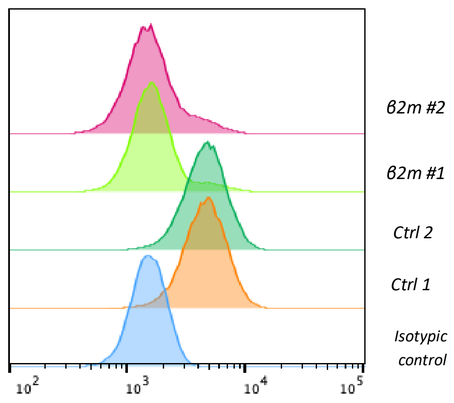




*Individual CRIPSRi populations in A549 cells using **pLentiCRISPRv3-ZIM3-denCas12a.***
Surface expression of ß2m was analyzed 6 days post transduction by flow cytometry

Cas9-based

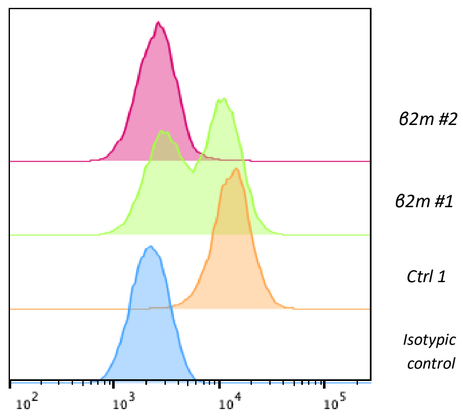
DNase active



*Individual KO populations in A549 cells s using **pLentiCRISPRv3-Cas9.***
Surface expression of ß2m was analyzed 6 days post transduction by flow cytometry

Cas12a-based

DNase active



*Individual KO populations in A549 cells s using **pLentiCRISPRv3-Cas12a.***
Surface expression of ß2m was analyzed 6 days post transduction by flow cytometry

NEXT STEPS

- Genetic modification of **Human Airway Epithelial (HAE-ALI)** with the all-in-one editing tools.
- Performing HD-CA to narrow down the HDFs/HRFs candidates list.

(1) WHO. Prioritizing Diseases for Research and Development in Emergency Contexts. (2025)

(2) Rebendenne, A., Roy, P., Bonaventure, B. et al. Bidirectional genome-wide CRISPR screens reveal host factors regulating SARS-CoV-2, MERS-CoV and seasonal HCoVs. Nat Genet 54, 1090–1102 (2022).

(3) Starkuviene V, Kallenberger SM, Beil N, et al. High-Density Cell Arrays for Genome-Scale Phenotypic Screening. SLAS Discov. 2019;24(3):274-283. doi:10.1177/2472555218818757

(4) Hsiung, C.CS., Wilson, C.M., Sambold, N.A. et al. Engineered CRISPR-Cas12a for higher-order combinatorial chromatin perturbations. Nat Biotechnol 43, 369–383 (2025). doi.org/10.1038/s41587-024-02224-0

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