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A CRISPRi/a platform in human iPSC-derived microglia uncovers regulators of disease states

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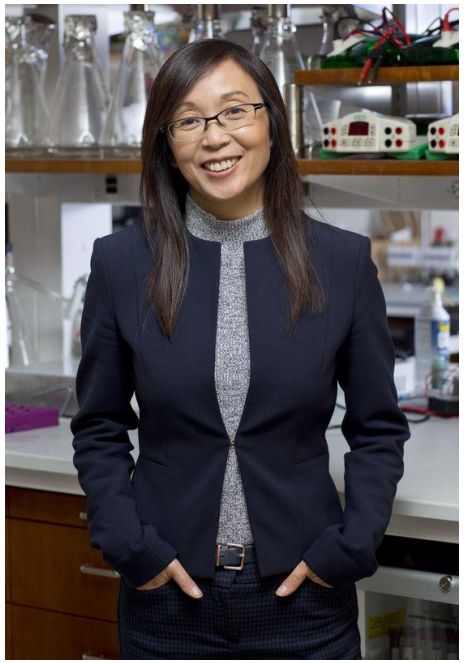

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CRISPRbrain

Data Commons for functional genomics screens in differentiated human cell types

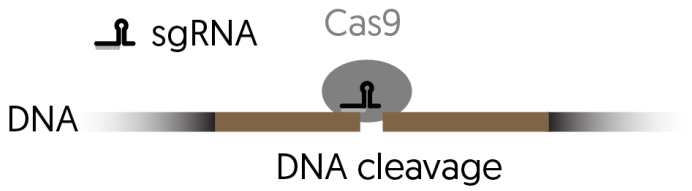
We have pioneered a **CRISPR**-based functional genomics platform in human **iPSC**-derived neurons, glia and 3D assembloids, which enables genome-wide modifier screens of disease-relevant cell biology in patient-derived cells.

(https://kampmannlab.ucsf.edu/welcome-kampmann-lab)

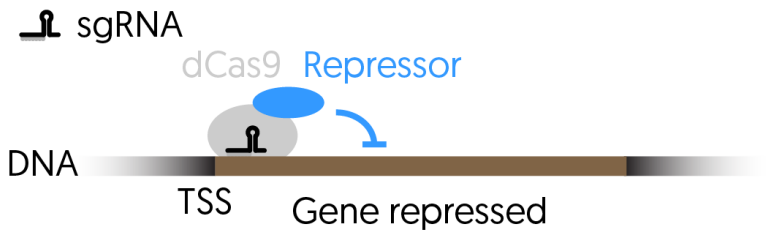
背景　CRISPR-based functional genomics とは

CRISPR-based Perturbation of Genes

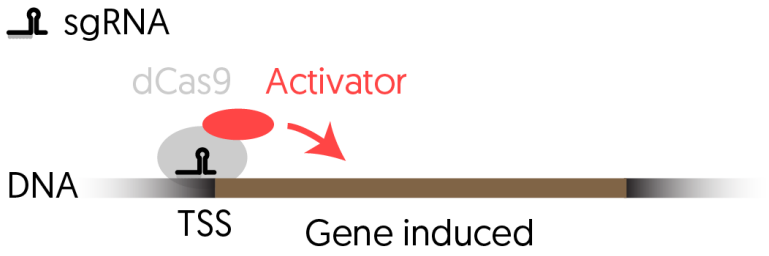
CRISPRn



CRISPR interference (CRISPRi)

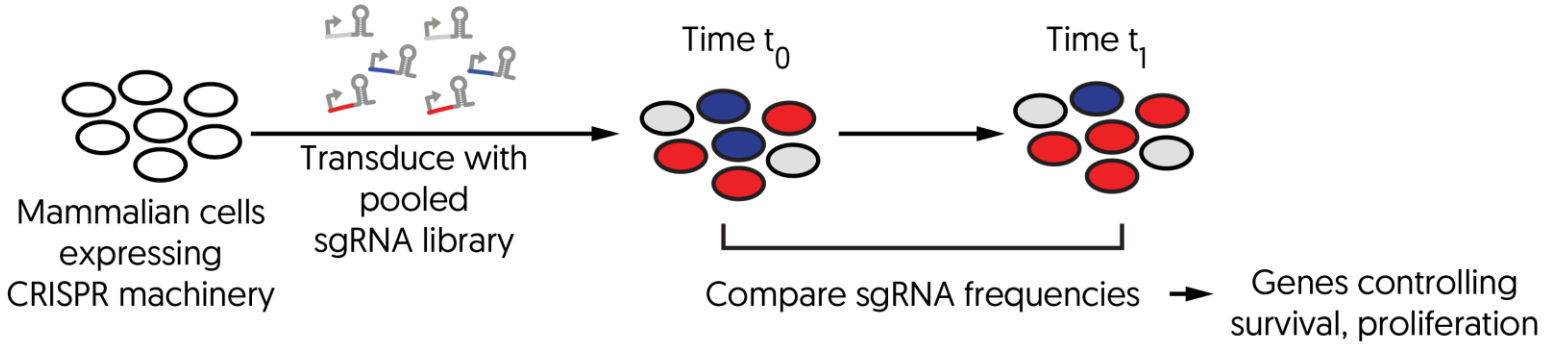


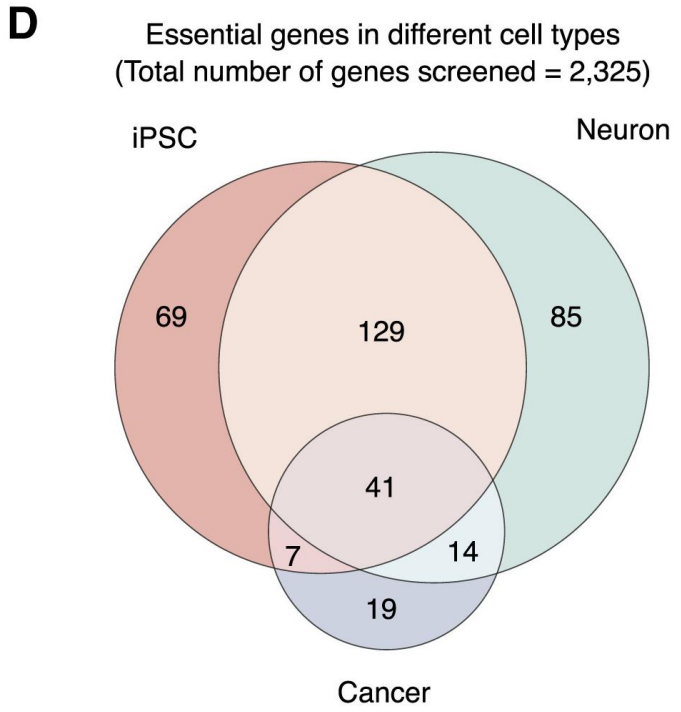
CRISPR activation (CRISPRa)



(https://crisprbrain.org/background/)

Survival Screening





“Survival screens uncover genes essential for neurons, but not iPSCs or cancer cells”

(Tian et al., 2019)

概要

Microglia are emerging as key drivers of neurological diseases. However, we lack a systematic understanding of the underlying mechanisms. Here, we present a screening platform to systematically elucidate functional consequences of genetic perturbations in human induced pluripotent stem cell-derived microglia. We developed an efficient 8-day protocol for the generation of microglia-like cells based on the inducible expression of six transcription factors. We established inducible CRISPR interference and activation in this system and conducted three screens targeting the 'druggable genome'. These screens uncovered genes controlling microglia survival, activation and phagocytosis, including neurodegeneration-associated genes. A screen with single-cell RNA sequencing as the readout revealed that these microglia adopt a spectrum of states mirroring those observed in human brains and identified regulators of these states. A disease-associated state characterized by osteopontin (SPP1) expression was selectively depleted by colony-stimulating factor-1 (CSF1R) inhibition. Thus, our platform can systematically uncover regulators of microglial states, enabling their functional characterization and therapeutic targeting.

Fig. 1, 2

CRISPRi/aを適用可能な、新しいiPSC分化プロトコルを開発し、ミクログリア様細胞（iTF-Microglia）を作成した。

Fig. 3

iTF-MicrogliaにCRISPRi/aを適用し、機能ゲノミクス解析の実験モデルを確立した。

Fig. 4, 5

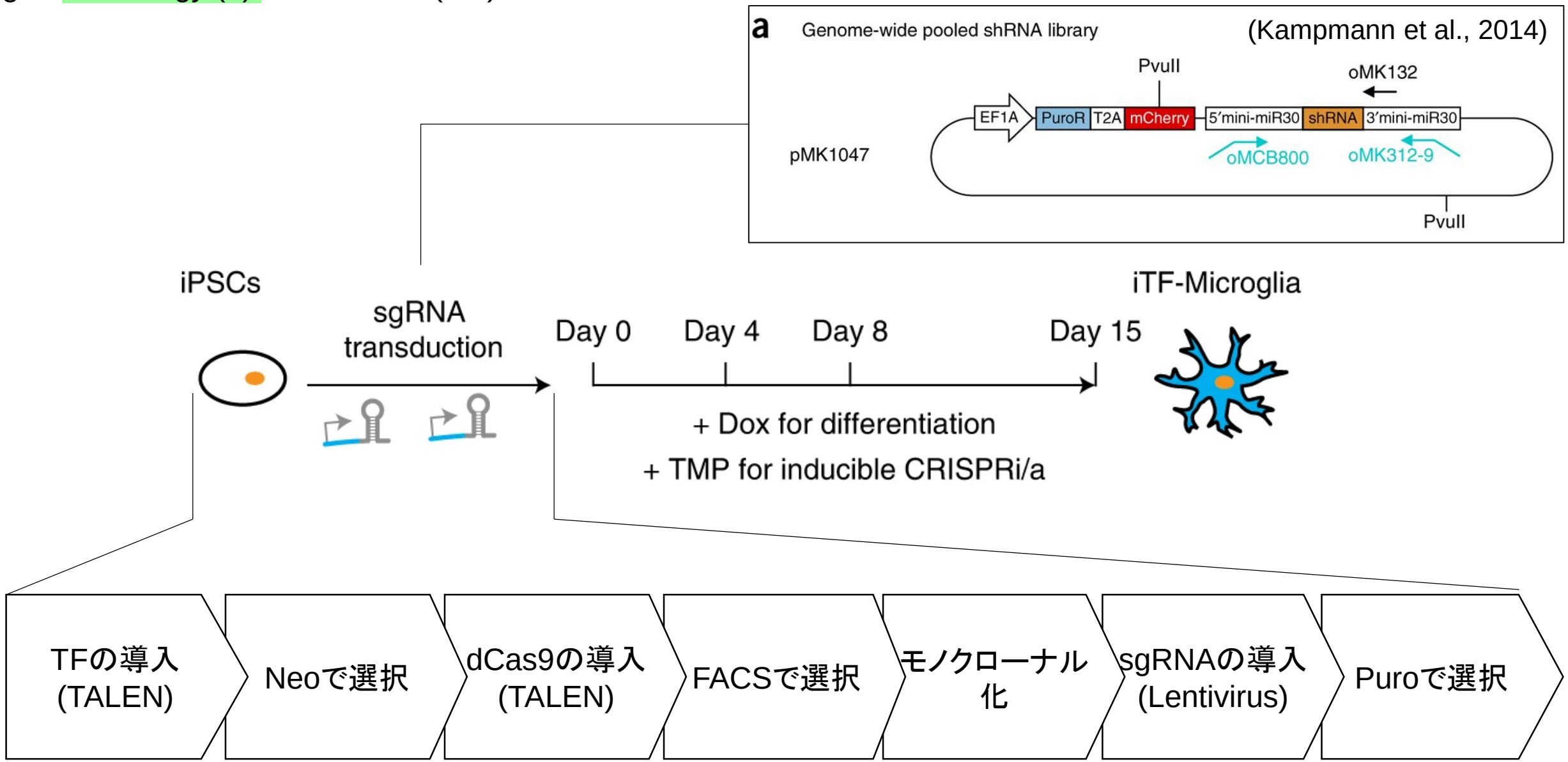
3種のスクリーニングにより、ミクログリアの①生存②炎症反応③食作用に重要な遺伝子を同定した。

Fig. 6, 7

CRISPRiとscRNA-seqによるトランスクリプトーム解析を組み合わせて、疾患関連ミクログリアの制御因子を同定した。

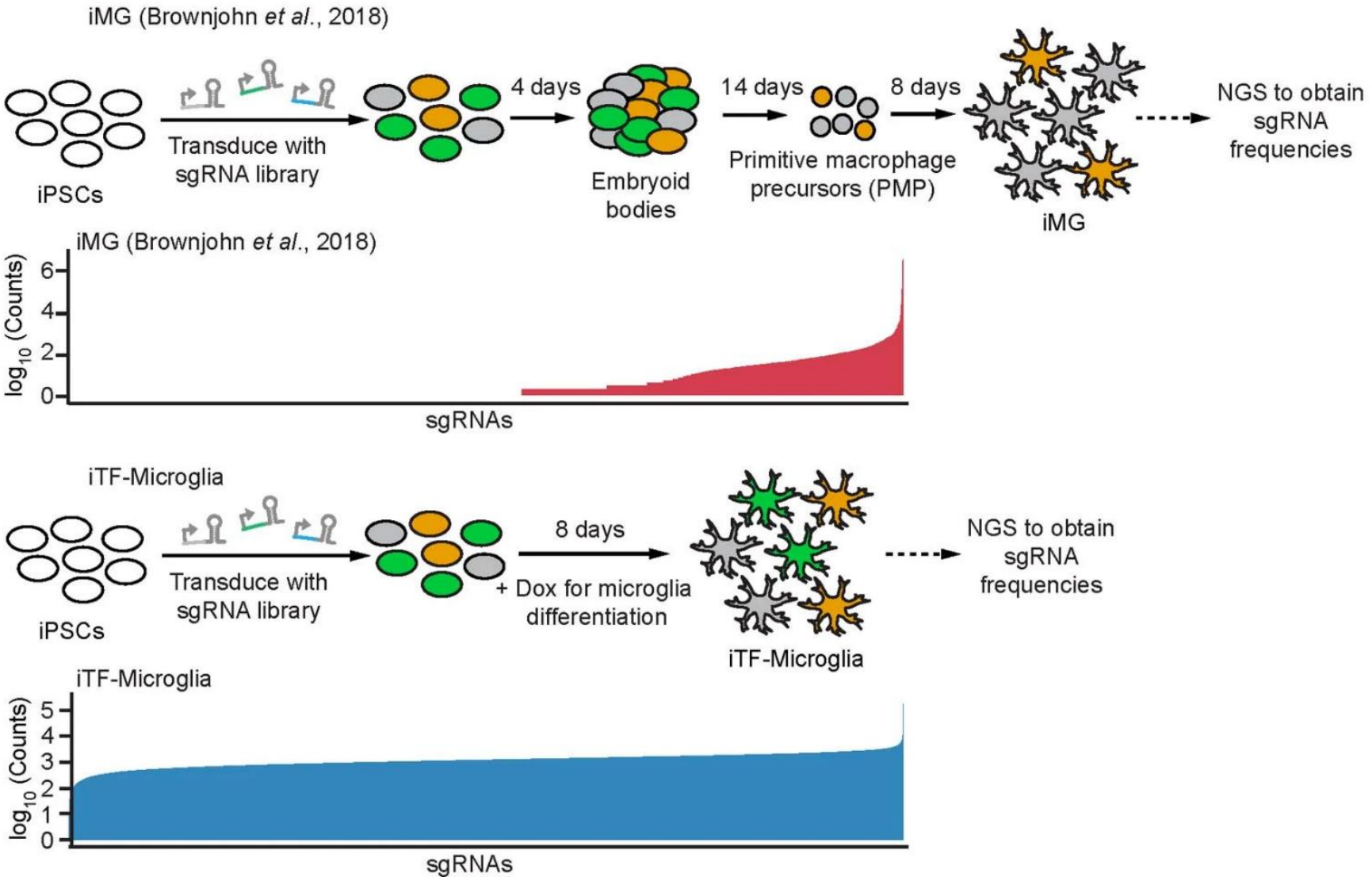
iTF-Microglia × CRISPR-based functional genomicsのタイムライン

Fig. 3 ①Strategy (a) ②Validation (b-d)



既存の分化プロトコルでは、分化の過程でsgRNAが脱落してしまう。

Fig. S1d

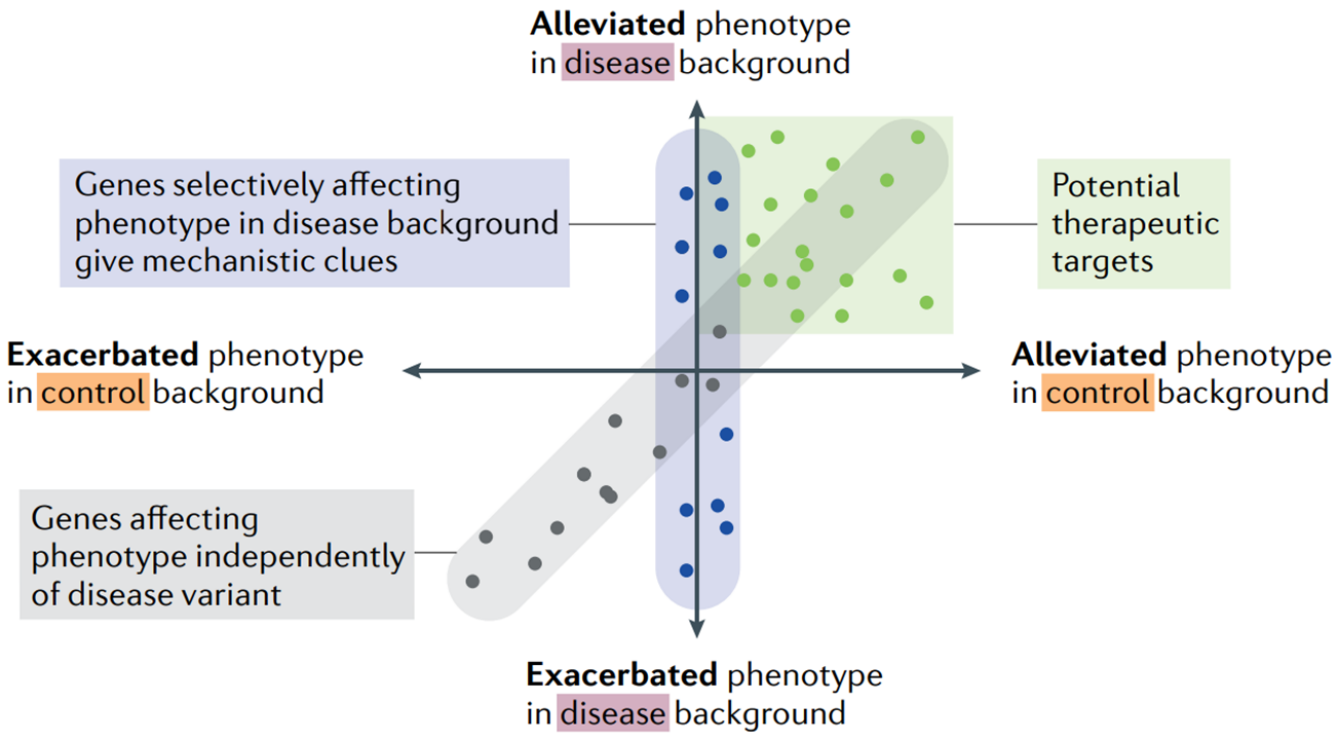


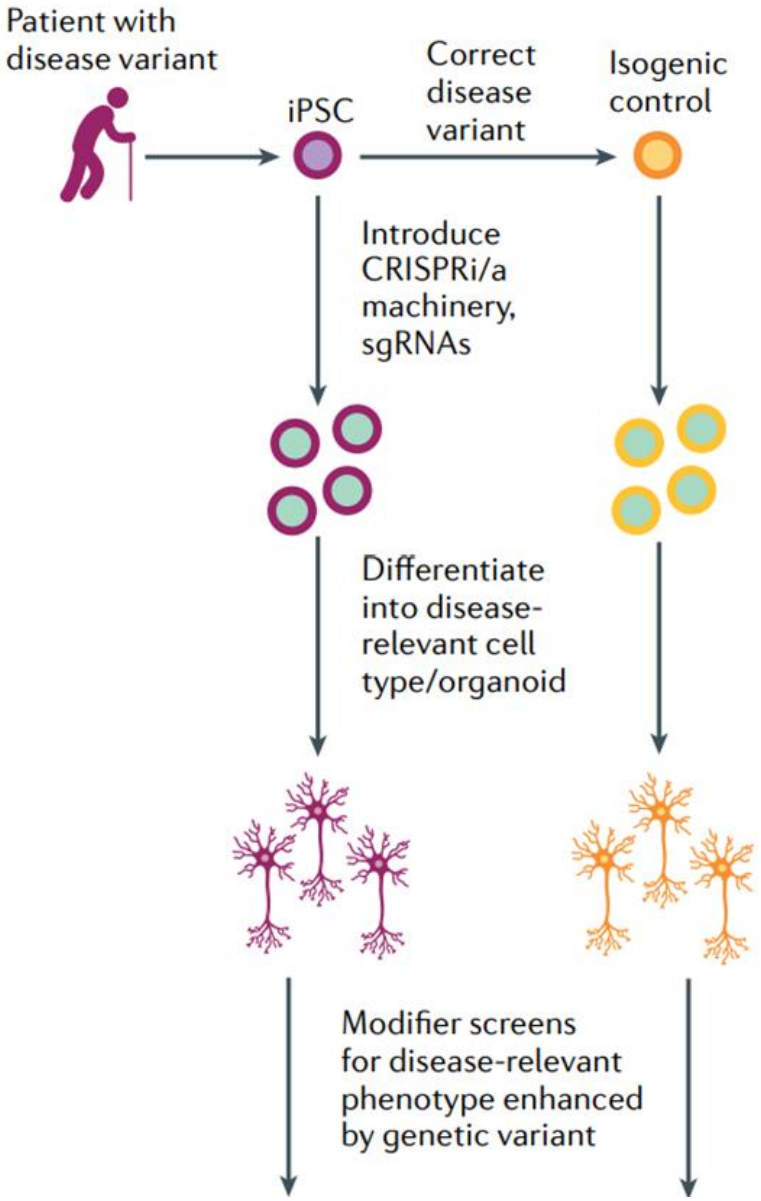
sgRNA recovery after transduction with a pooled sgRNA library in iPSCs and differentiation with two different iPSC-Microglia protocols. Strategy for the infection of iPSCs with an sgRNA library with 13,025 elements and timepoint of sgRNA recovery in iPSC-Microglia with the actual recovered counts of sgRNAs after next-generation-sequencing (NGS) from the protocol from Brownjohn et al.18 (Top) and iTF-Microglia (Bottom).

Discussion

iTF-MicrogliaのCRISPR-based functional genomics platformを活用することで、ミクログリアの様々な表現型と遺伝子の関係性について、さらなる理解の得られることが期待される

- ニューロンとの共培養、脳オルガノイドへの組み込み
- 患者由来iPS細胞
- マウスへの移植によるin vivo実験





（Kampmann, 2020)