Parallel reverse genetic screening in mutant human cells using transcriptomics

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Abstract

Question:

Reverse genetic screens have driven gene annotation and target discovery in model organisms. However, many disease-relevant genotypes and phenotypes cannot be studied in lower organisms.

Research methods:

Here, we establish a reverse genetic approach based on highly robust and sensitive multiplexed RNA sequencing of mutant human cells. We conduct 10 parallel screens using a collection of engineered haploid isogenic cell lines with knockouts covering tyrosine kinases and identify known and unexpected effects on signaling pathways.

- Introduction
- Materials and Methods
- Results
- Discussion

Introduction

- Forward and reverse genetic approaches have both been crucial for elucidating fundamental biological processes as well as identifying therapeutic targets.

- One of the hurdles associated with large-scale reverse genetics in human cells is the technical challenge to generate large sets of individual, targeted mutants.

- In this work, we exploit advances in parallel sequencing and genome editing to revisit reverse genetics in human cells.

Materials and Methods

- Cell lines
- Reagents and stimulation of cells
- RNA sequencing
- Quantitative real-time PCR
- Western blotting
- RNA-seq data processing and alignment
- Expression analysis

Results

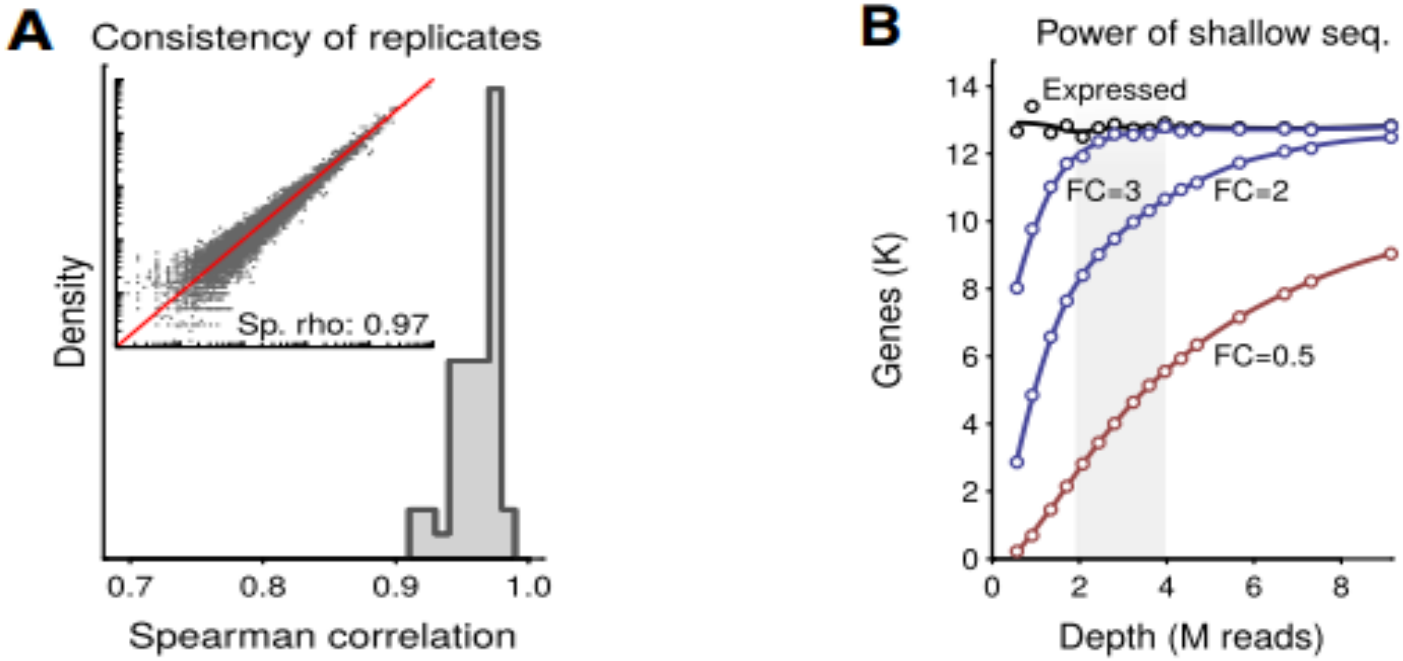


Fig A:Spearman correlations between replicates of expression profiles in HAP1 cells measured by shallow RNA-seq.

Fig B:Data-based modeling of the effect of sequencing depth on gene expression analysis.

Results

Result 1.

Expression profiles of replicate samples were strongly correlated, indicating robust and consistent performance of the assay (Fig 1A).

Modeling of sequencing depth showed that measuring ~1 million reads per sample was sufficient to identify nearly all the ~12,000 genes expressed in HAP1 cells (Fig 1B).

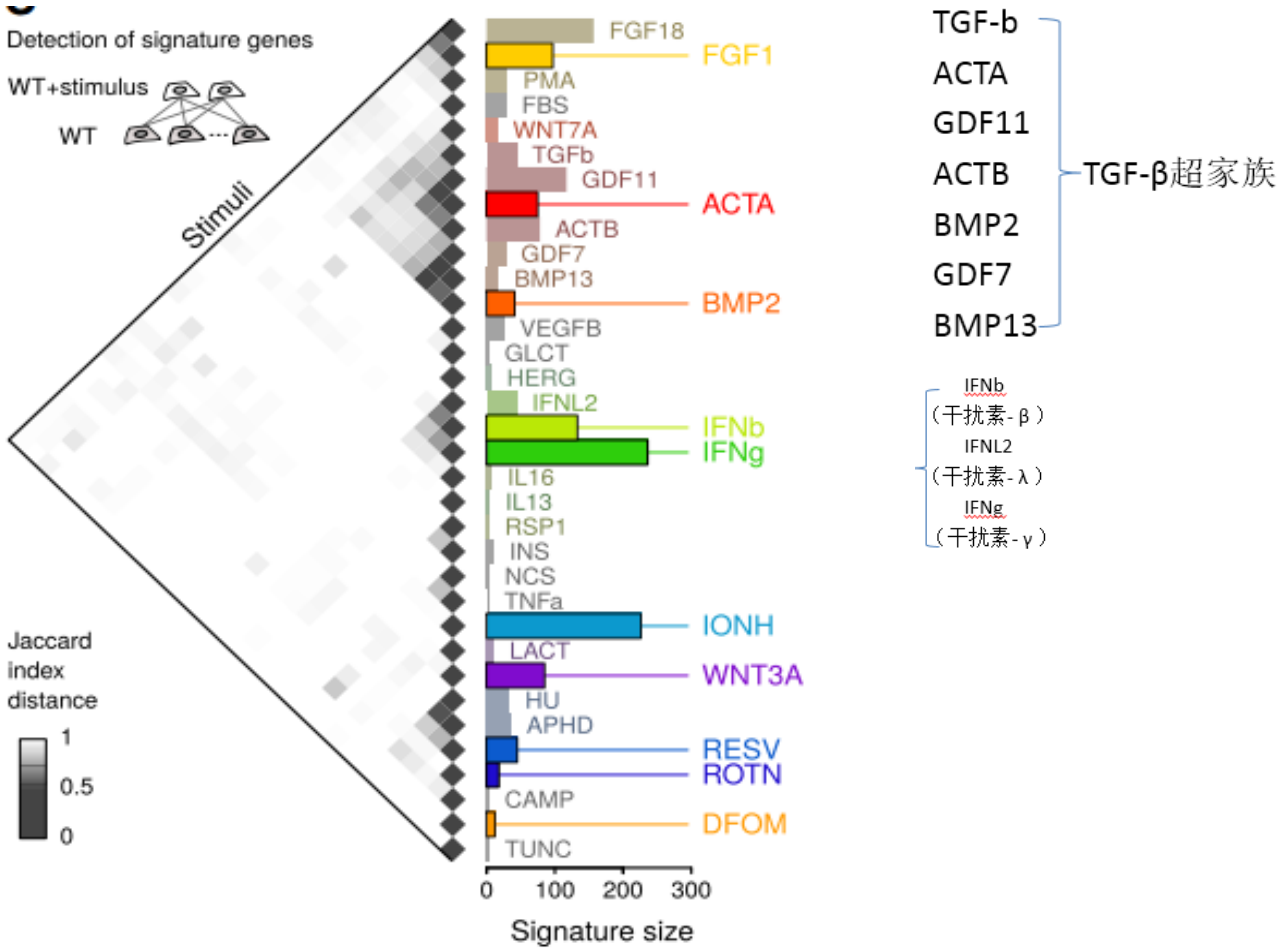
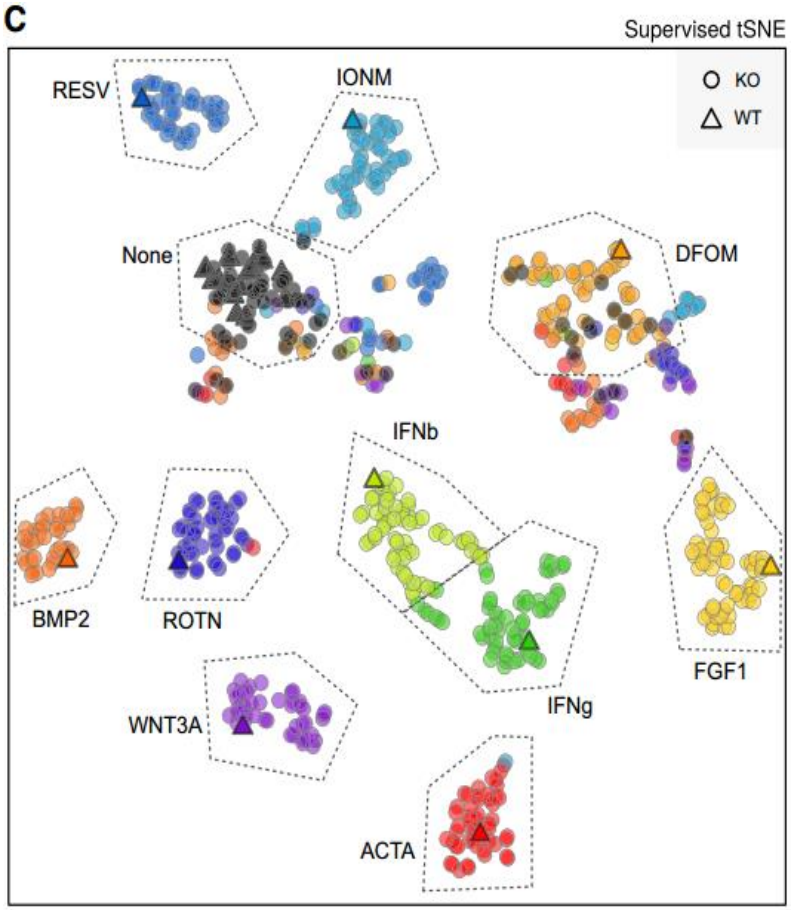


Fig: Clustering of signature gene sets from polypeptide and small molecule stimulations.

Result 2.

Although related signatures (e.g., interferons) contained genes in common, they also contained gene subsets known to be specific to the respective stimuli. This indicates that the resolution of shallow RNA sequencing can capture not only broad responses to perturbations, but can reveal nuances of signaling cascades as well.



10 stimuli from the benchmarking experiment based on signature size and diversity for parallel screening.

Fig C: Supervised Stochastic Neighbour Embedding (tSNE) clustering of all stimulated and unstimulated HAP1 wild-type and knockout cell lines.

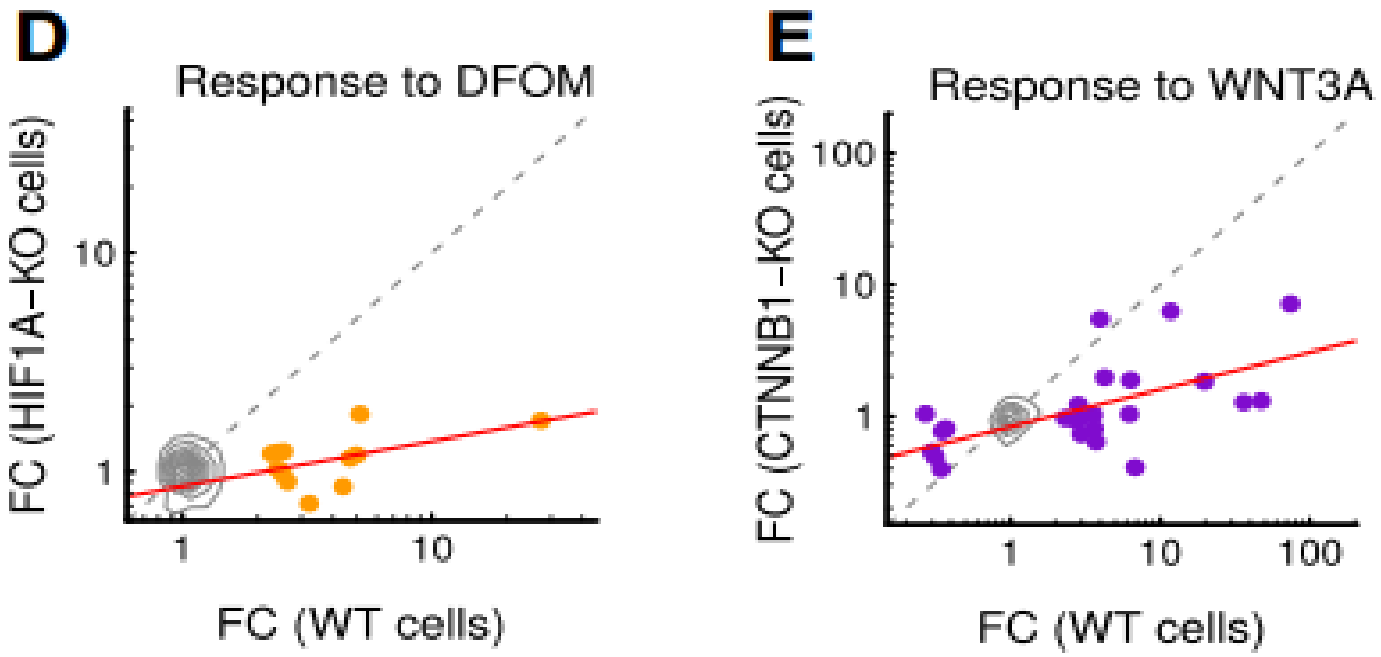


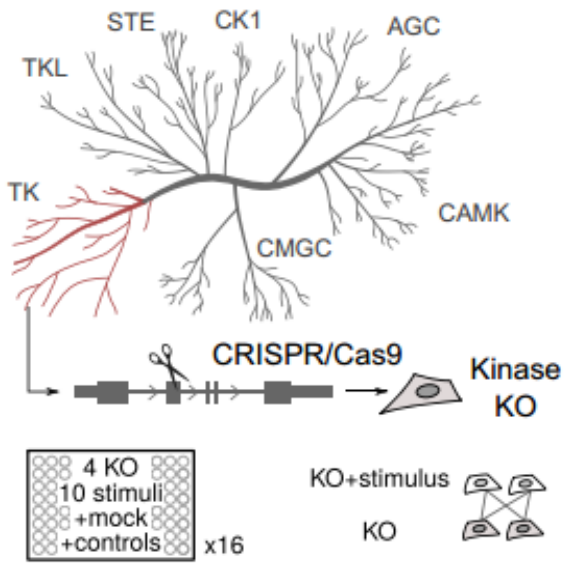
Fig D:Comparison of expression profiles of wild-type cells and HIF1A-KO cells in response to DFOM stimulation.

Fig E:Same as in (D), except for WNT3A stimulus in CTNNB1-KO cells.

Result 3.

Genes upregulated by DFOM and WNT3A were strongly reduced in the HIF1A and CTNNB1 mutants, respectively (Fig 1D and E),which validated that the previously defined signatures can be exploited to functionally annotate genes using mutant cell lines.

Parallel reverse genetic screening of kinase knockout cells.



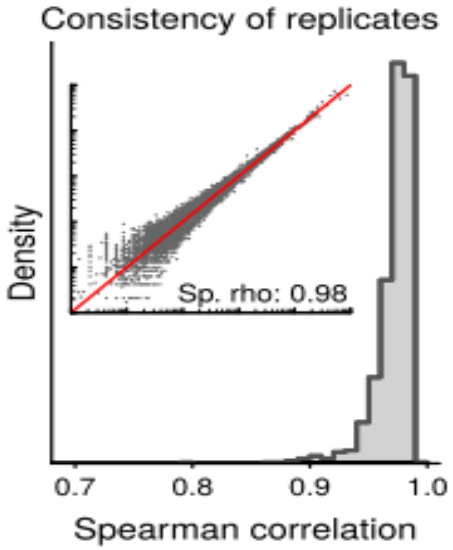


Fig 1:On top, cartoon illustrating the assembly of a collection of HAP1 knockouts using CRISPR/Cas9 technology. At bottom, scheme for screening design showing that individual kinase KO cells are measured along all relevant controls.

Fig 2:Spearman correlations between replicates of stimulated and unstimulated wild-type and knockout cells in the transcriptomic screen of 16 96-well plates.

Used residuals to score individual cell lines' responses to each stimulus. This revealed several knockout-specific signaling dependencies.

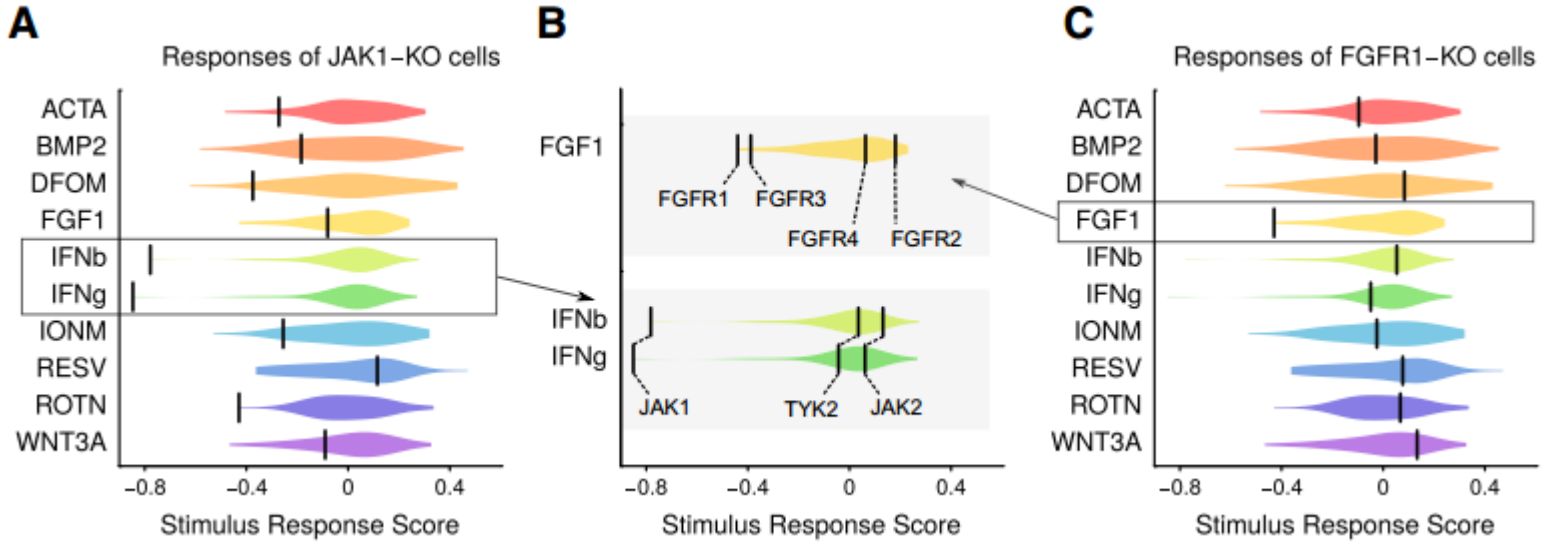


Fig A: Responses of JAK1-KO cells to the ten selected stimuli.

Fig B:Showing detailed view of responses to FGF1 and IFNb/IFNg stimulation of selected knockout cells. Bars indicate labeled mutants of FGFR and JAK family members.

Fig C:Responses of FGFR1-KO cells to the ten selected stimuli.

Result 4.

This finding is surprising as these three JAK family members have been reported to contribute to a transcriptional response upon stimulation with type I or type II interferons (Rane & Reddy, 2000). Our results confirm a critical role for JAK1 in interferon signaling and suggest a distinct function of this kinase compared to the other two family members, at least in HAP1 cells.

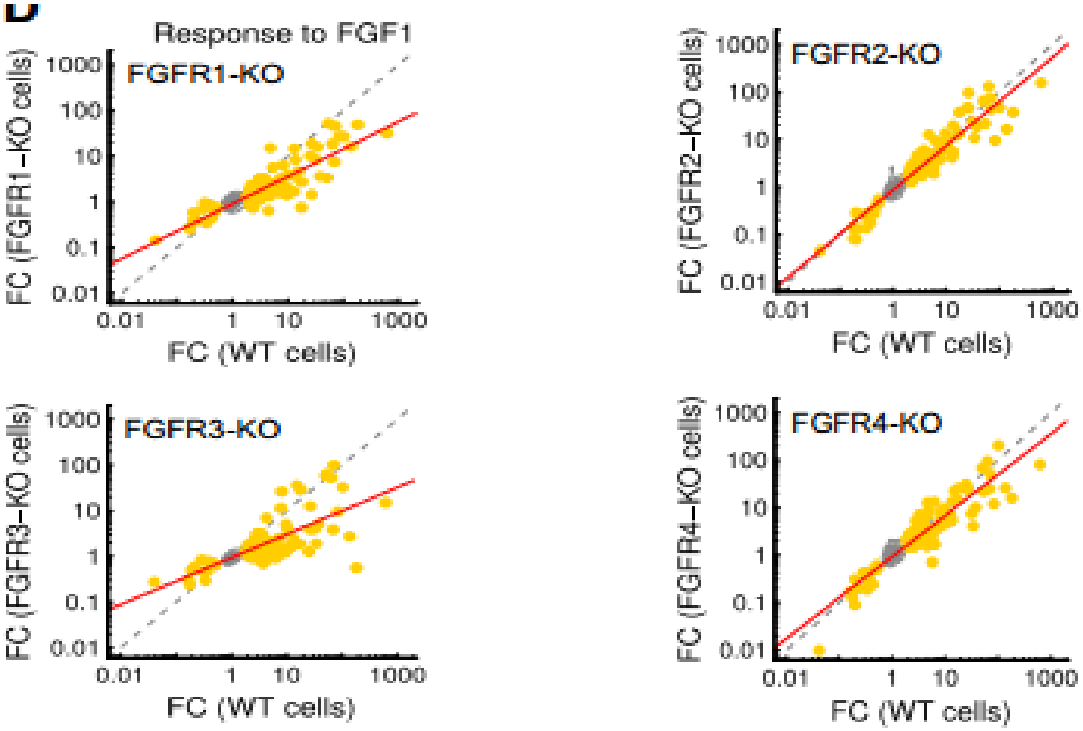


Fig: Comparison of response signatures in wild-type and FGFR-KO mutant cells.

Result 5.

Studying the signature genes in more detail, we further noted that loss of FGFR1 had a uniform effect on FGF1 signaling. In contrast, in FGFR3 knockout cells, the attenuation was less uniform. These observations highlight the complexity of FGF1 signaling and illustrate how the profiling platform can spark new hypotheses even for well-studied pathways.

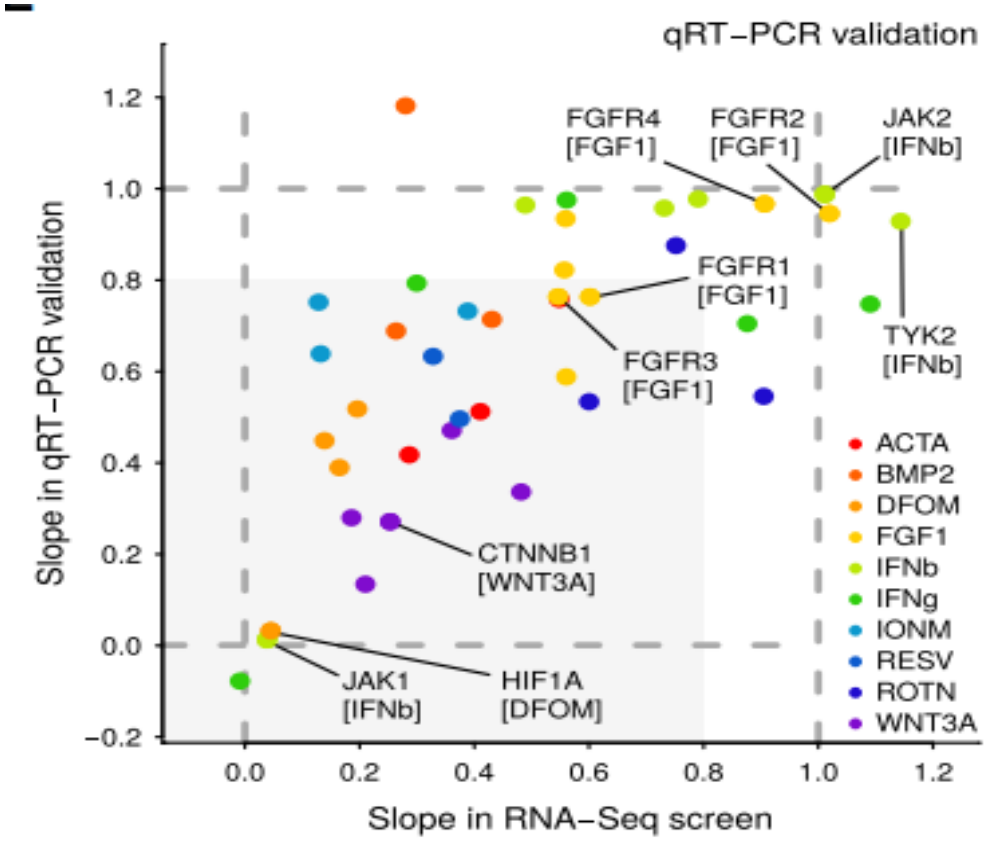


Fig: Comparison of stimulus response as measured by RNA-seq and qRT–PCR.

Result 6.

Many other gene–stimulus combinations also resulted in subtle reductions in signaling strength.

These experiments also confirmed another observation that some mutant clones show aberrations in more than one signaling pathway .

Discussion

contributions：

- Present an approach for parallel reverse genetics of mutant human cells based on shallow RNA sequencing, and demonstrate its suitability for studying cellular perturbations.
- Anticipate that the strategy of transcriptional screening of mutant cells is generic and can be applied to study many other cellular systems provided relevant reference/control signatures are measured.
- The presented strategy can be deployed to address a multitude of biological questions beyond the study of full knockout mutants.

limitations ：

- The resolution of shallow RNA-seq is not as high as obtained from deeper sequencing protocols.
- Furthermore, cellular changes that do not affect gene transcription, or only very transiently, cannot be quantified using this method.
- Envisioned applications include hit validation and targeted hypothesis testing that are difficult to tackle through forward genetics.

个人总结

（1）用一句话概括此研究的主要结论或创新点。

此研究提供了可扩展的方法将基因型与人类细胞中的表型关联的概念证明，其具有广泛的应用。

（2）此研究对你有何启发。

先在酵母中发现转录图谱以将基因型连接到表型，证明转录图谱是反向遗传学的合适测定方法，然后应用基于扰动的类似策略来研究人类细胞。启发我们可以根据生物界共用一套遗传密码子的规律，来更好的利用微生物的一些特性和研究方法应用于人类细胞的研究。

（3）此研究还存在哪些问题可以改进。

预想的应用包括命中验证和目标假设测试，难以通过正向遗传学。

谢谢！