Dear Editors,

I would like to submit the accompanying manuscript, entitled “**Deciphering Complex Traits with High-Order Genetic Analysis**”, for your consideration as a Resource in *Cell*.

It is becoming increasingly clear that most traits, including common human genetic diseases, are complex. Complex traits rely on multiple genes functioning together, and are better understood by profiling the combined effects of multiple variants. In yeast, for example, double-knockout approaches such as synthetic genetic array analysis (SGA) can uncover surprising two-gene knockout phenotypes (i.e. genetic interactions) that can point to functional dependencies.

More generally, surprising phenotypes can result from varying any number of genes, resulting in high-order genetic interactions (involving 3 or more genes). The lack of systematic studies on high-order genetic interactions fundamentally limit our ability to derive complete genotype-to-phenotype understanding. To address this gap, we developed an ‘*X*-gene’ genetic analysis (‘XGA’) that generalizes approaches such as SGA in order to consider the effects of perturbing *X* genes at a time rather than one or two.

XGA works by engineering and profiing many combinations of multiple gene variants to recover surprising combinatorial effects, which can be exploited for functional understanding. We demonstrate XGA on a family of 16 yeast ABC transporters which contains several known multidrug efflux pumps. Motivated by known interesting multi-knockout effects within this family, we generated many (>5,000) combinatorial multi-gene mutants and measured their resistance to a set of bioactive compounds (‘drugs’), yielding 85,632 genotype-to-resistance relationships. Indeed, we found many complex drug resistance interactions which would have been missed by conventional approaches.

Our study further develops a neural network model to infer genotype-to-phenotype models from these multi-perturbation effects. This modeling approach is itself a key advance, as it sidesteps many laborious and difficult processes in deriving genotype-to-phenotype understanding from complex genetic observations. Briefly, some prior knowledge of the genes being studied can be used to create a system schematic that uses genetic data to fill in the supported functions and relationships. Because the model is quantitative, we demonstrate that we can also objectively evaluate and iteratively improve it as needed.

Together, our framework addresses key difficulties in using multi-variant effects to understand complex genotype-to-phenotype relationships. We intend our overall approach to be widely applicable for many experimental organisms, and to be adapted with emerging technologies such as single-cell phenotyping approaches.

Given the present interest in combinatorial variant effects, and the general applicability of our approach towards better understanding complex traits, we believe that our manuscript will be of value to the broad readership of *Cell*.

We suggest the following reviewers:

We thank you very much for your time and consideration. Please do not hesitate to contact us if we can provide any additional information.