Dear Editors,

We are submitting the accompanying manuscript, entitled “**High-Order Genetic Analysis Enables Systematic Functional Dissection of a Complex Trait”**, for your consideration as a Resource in *Cell*.

It is becoming increasingly clear that most traits, including common human genetic diseases, are complex. Genetic analysis of complex traits, which rely on multiple genes functioning together, requires phenotypic study of multiple variant combinations. Combinatorial genetic analysis can be done exhaustively for two-gene combinations, for example in yeast, where two-gene synthetic genetic array analysis (SGA) has revealed genetic interactions and derived a global functional relationship network.

However, many functional relationships are only revealed by higher-order analysis of variation in three or more genes. The lack of systematic studies on high-order genetic interactions has fundamentally limited our ability to map from genotype to phenotype and thus model pathway function.

To address this gap, we implemented an ‘*X*-gene’ genetic analysis (‘XGA’). XGA works by engineering and profiling many combinations of multiple gene variants to recover surprising combinatorial effects, which can be exploited for functional understanding. We demonstrate XGA on a complete set of the 16 yeast ABC transporters (efflux pumps) which have been implicated in multidrug resistance. We generated a set of >5,000 strains carrying different knockout combinations of multi-gene mutants. Using a combination of next-generation barcode-sequencing technologies, every strain was genotyped and phenotyped for resistance to a set of 16 bioactive compounds (‘drugs’), yielding 85,632 genotype-to-resistance relationships.

XGA analysis revealed many complex drug resistance interactions amongst ABC transporters which would have been missed by one- and two-gene approaches. We could use a neural network to objectively model functional influence relationships from XGA data, including many examples of efflux action in parallel and (surprisingly) six pairs of ABC transporters for which one repressed the other’s activity. Further neural network modeling

and follow-up experimental supported a model in which this repression is happening via both direct and indirect influence mechanisms.

Given the current pressing need to better understand complex disease, and the potential general applicability of our approach towards other traits and other model organisms, we believe that our manuscript will benefit the broad readership of *Cell*.

We appreciate your time and consideration. Please feel free to contact us for discussion.