

Using high-throughput promoter reporter assays to examine regulatory pathways: Hypoxia case study

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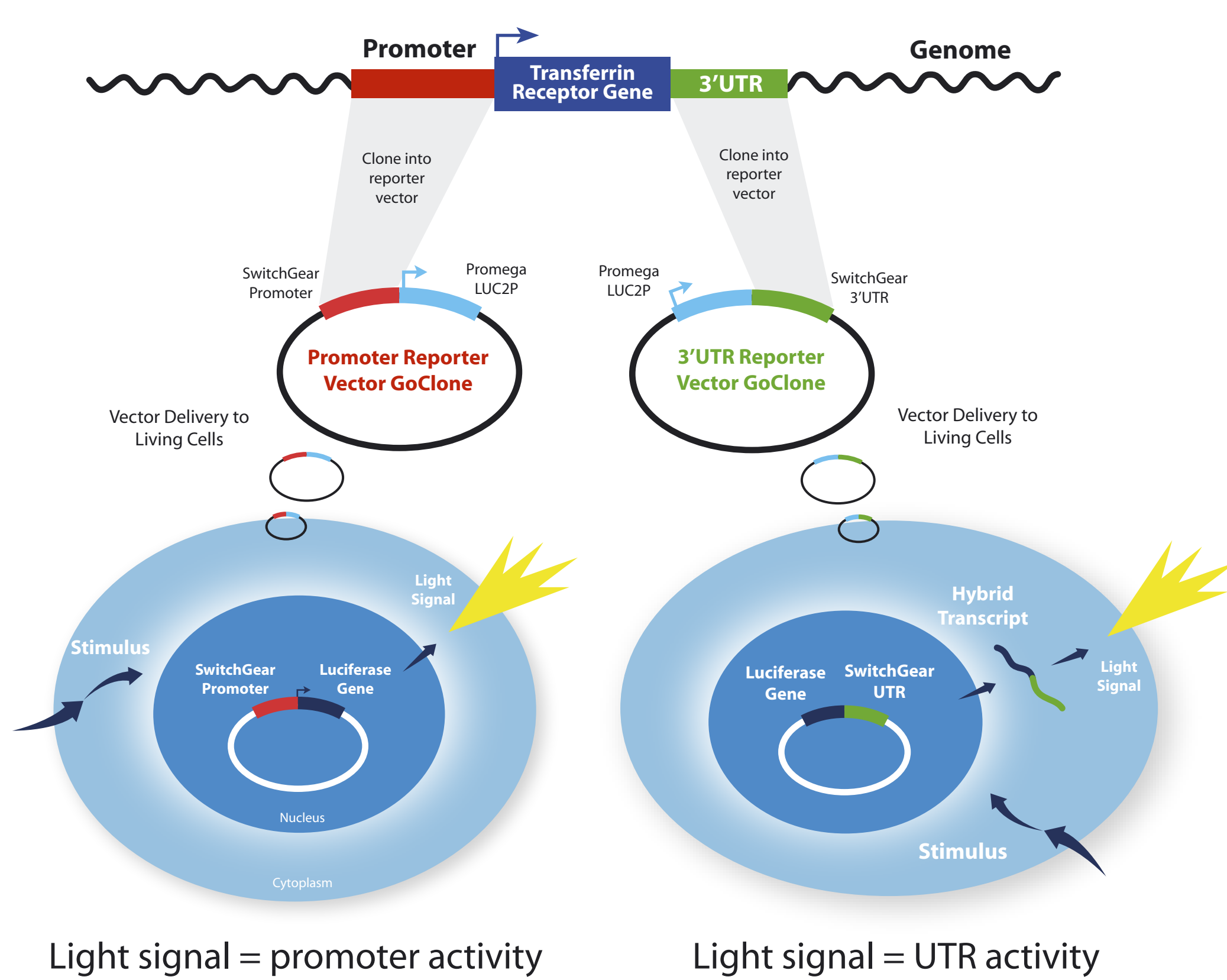
INTRODUCTION

Recent studies have highlighted the benefit of conducting genome-wide expression studies and transcription factor binding in parallel. After generating these descriptive data sets, a number of questions remain. Which genomic elements are responsible for transcript level changes? What is the effect of a transcription factor binding event?

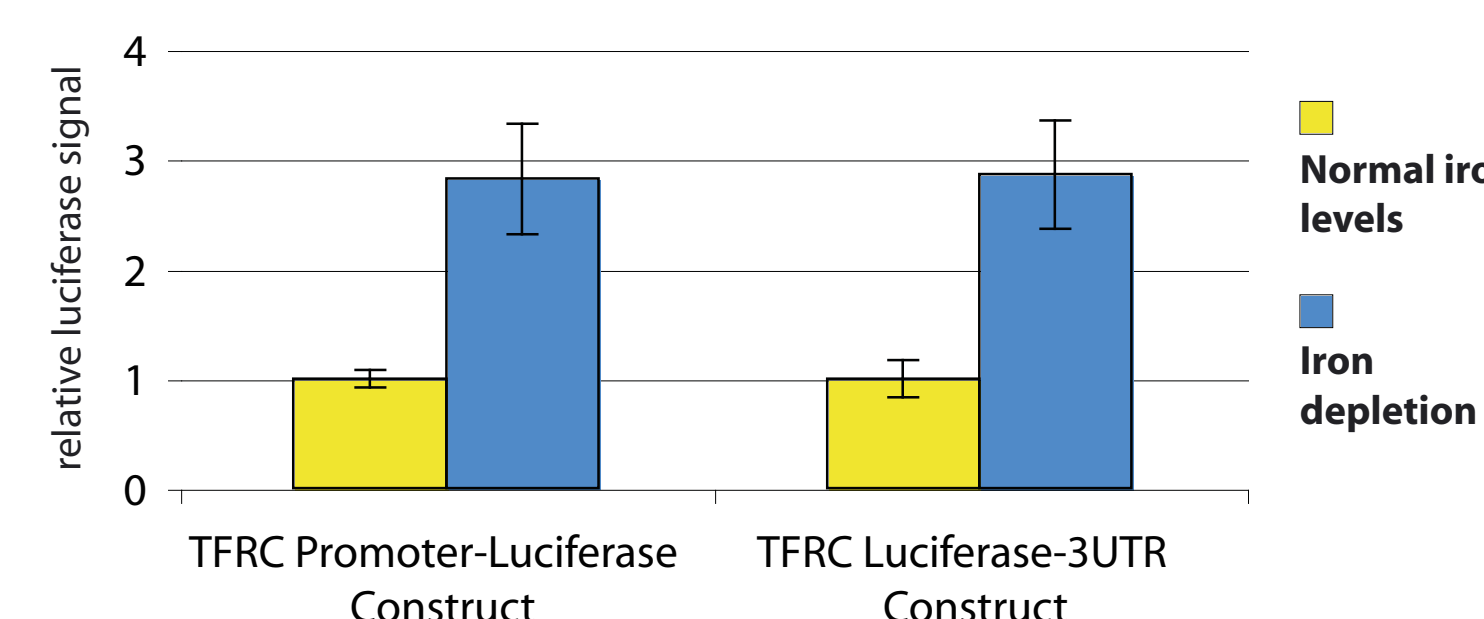
Using functional reporter assays to study the behavior of genomic elements in living cells can help answer many of these questions. To enable these studies in a high-throughput format, we have created a genome-wide library of human promoters and UTRs in a luciferase-based reporter system GoClones. In this study we applied this resource and approach to the hypoxia regulatory pathway.

GOALS

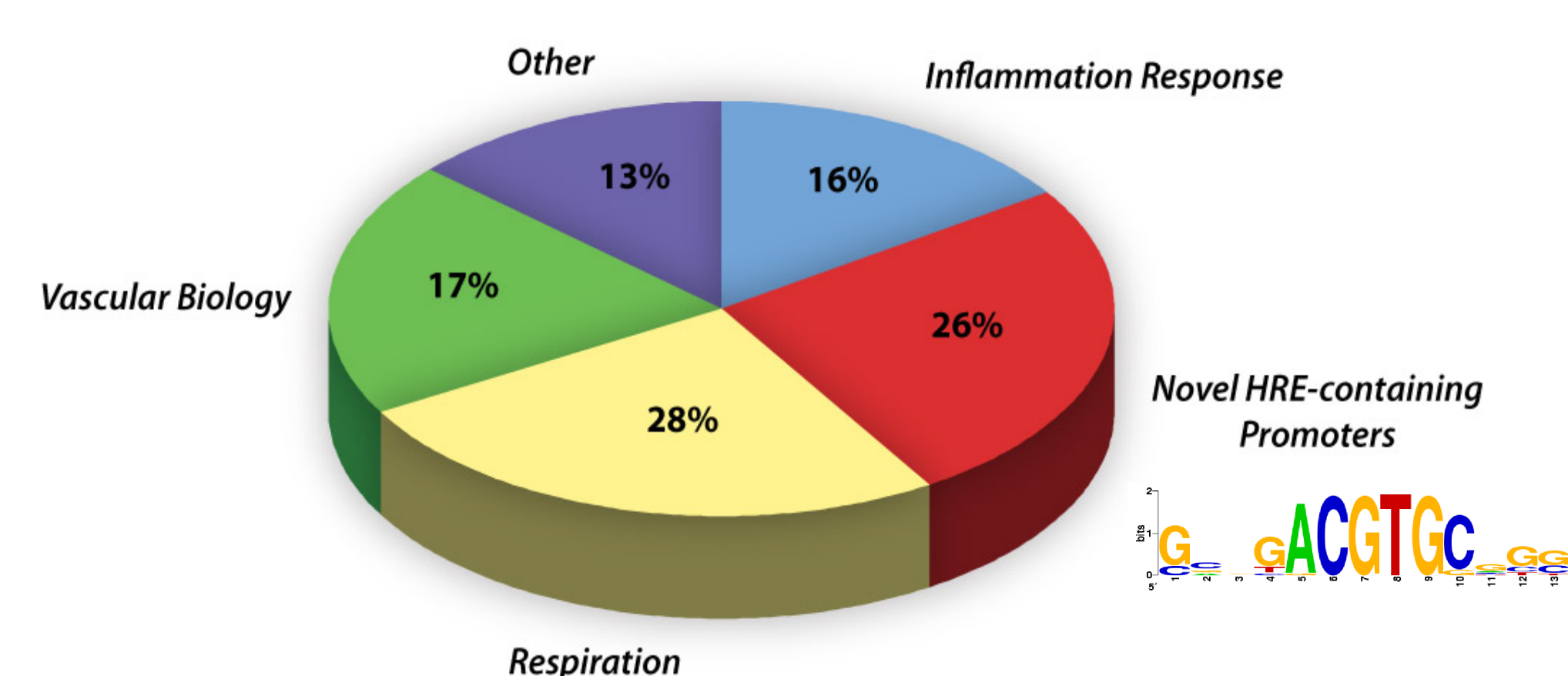
- ▶ Assemble a panel of known and potentially novel hypoxia-related promoters
- ▶ Measure the function of this panel in a variety of different conditions and cell types
- ▶ Measure the effects of naturally occurring sequence variants
- ▶ Compare to other hypoxia-related data types



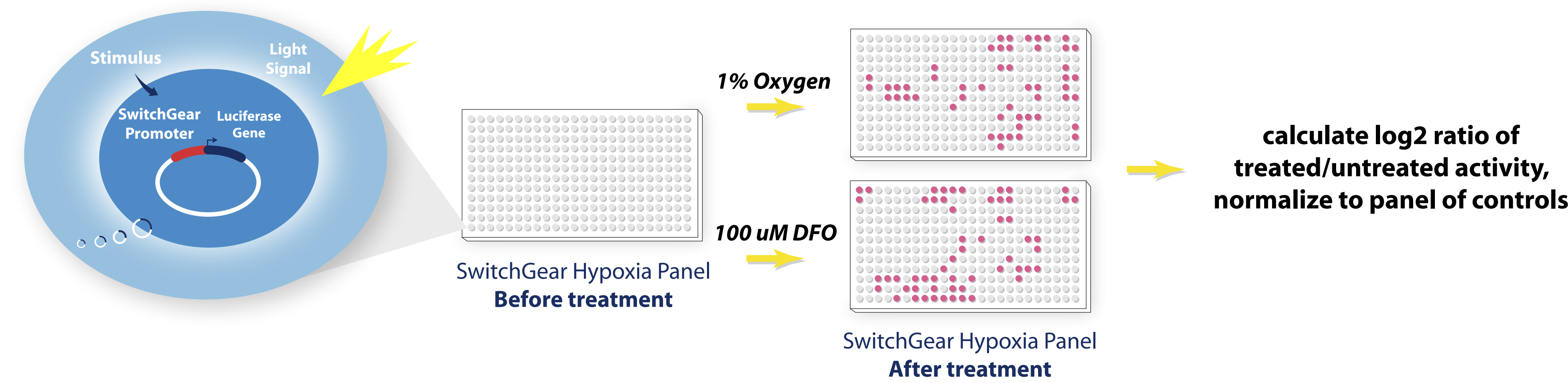
Promoter and 3'UTR elements contribute equally to a 10-fold induction of the TFRC mRNA by a hypoxia pathway inducing compound (DFO)



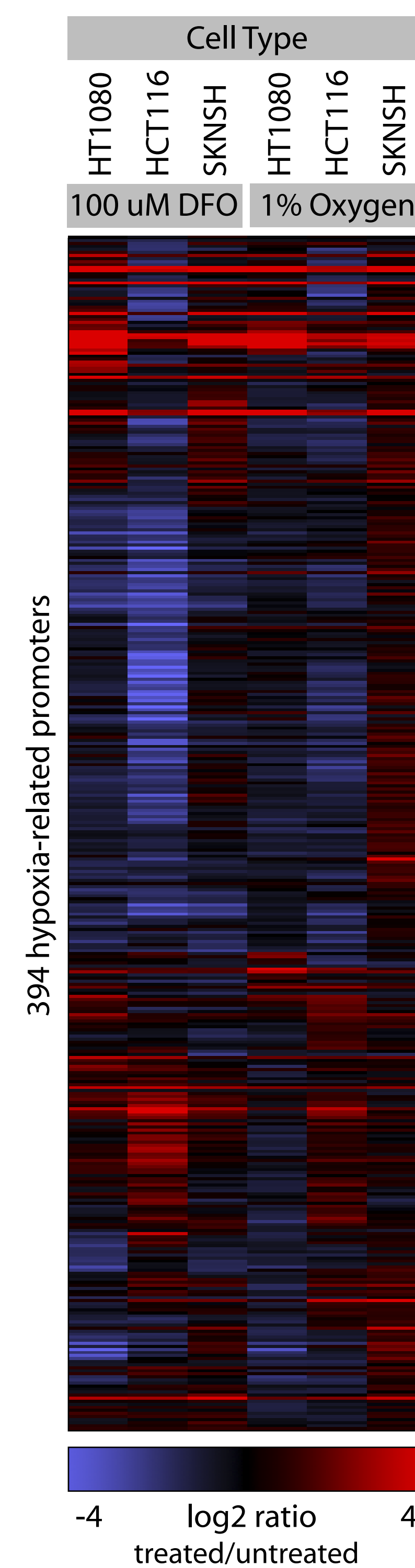
Composition of hypoxia promoter pathway panel (394 promoters)



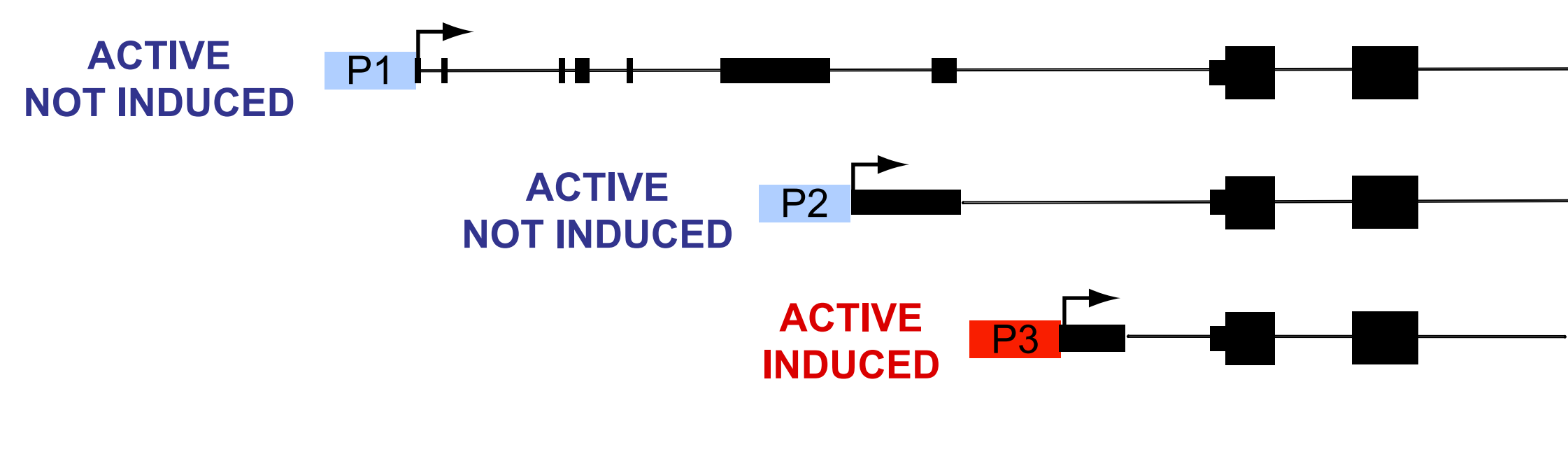
EXPERIMENTAL DESIGN



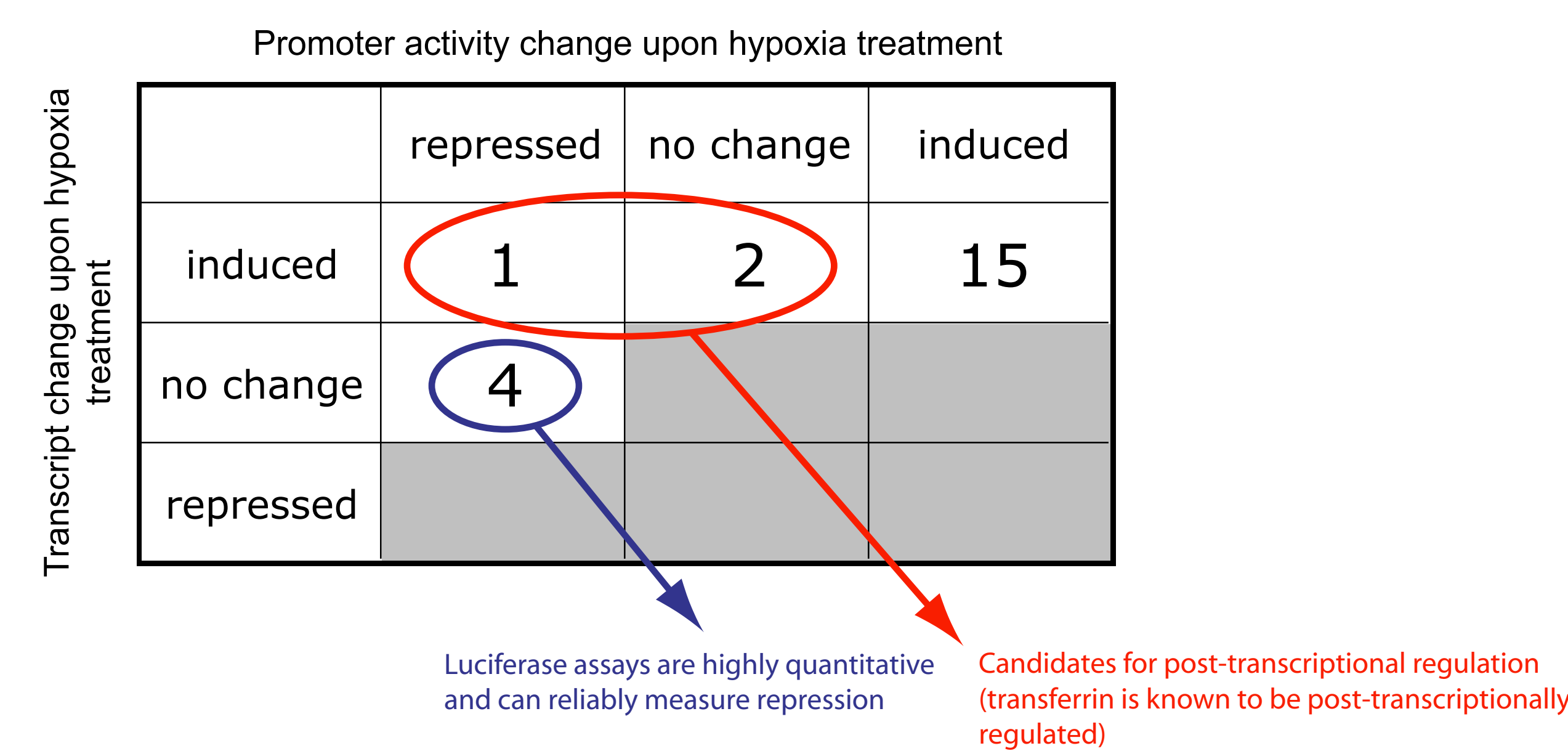
RESULTS



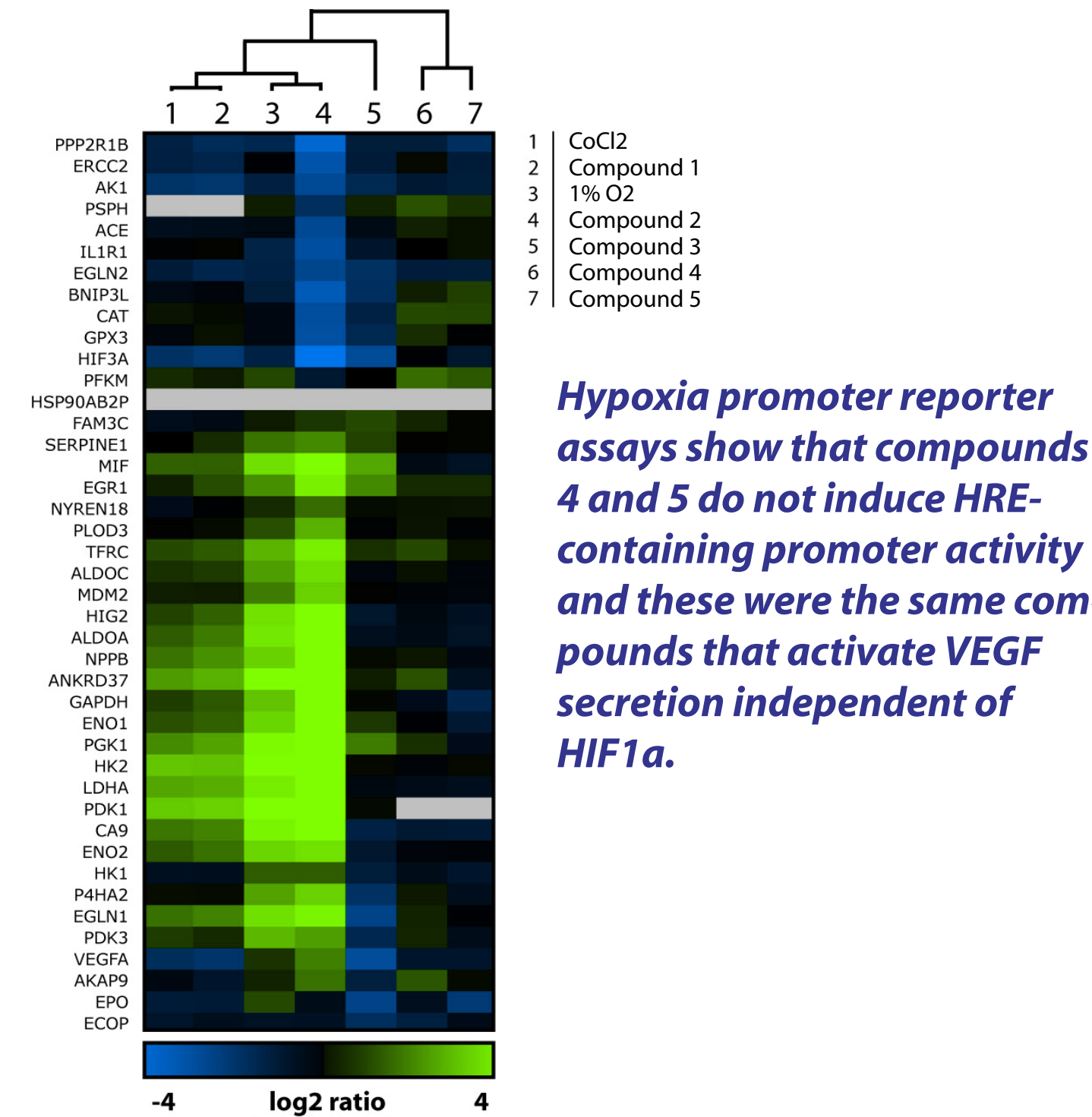
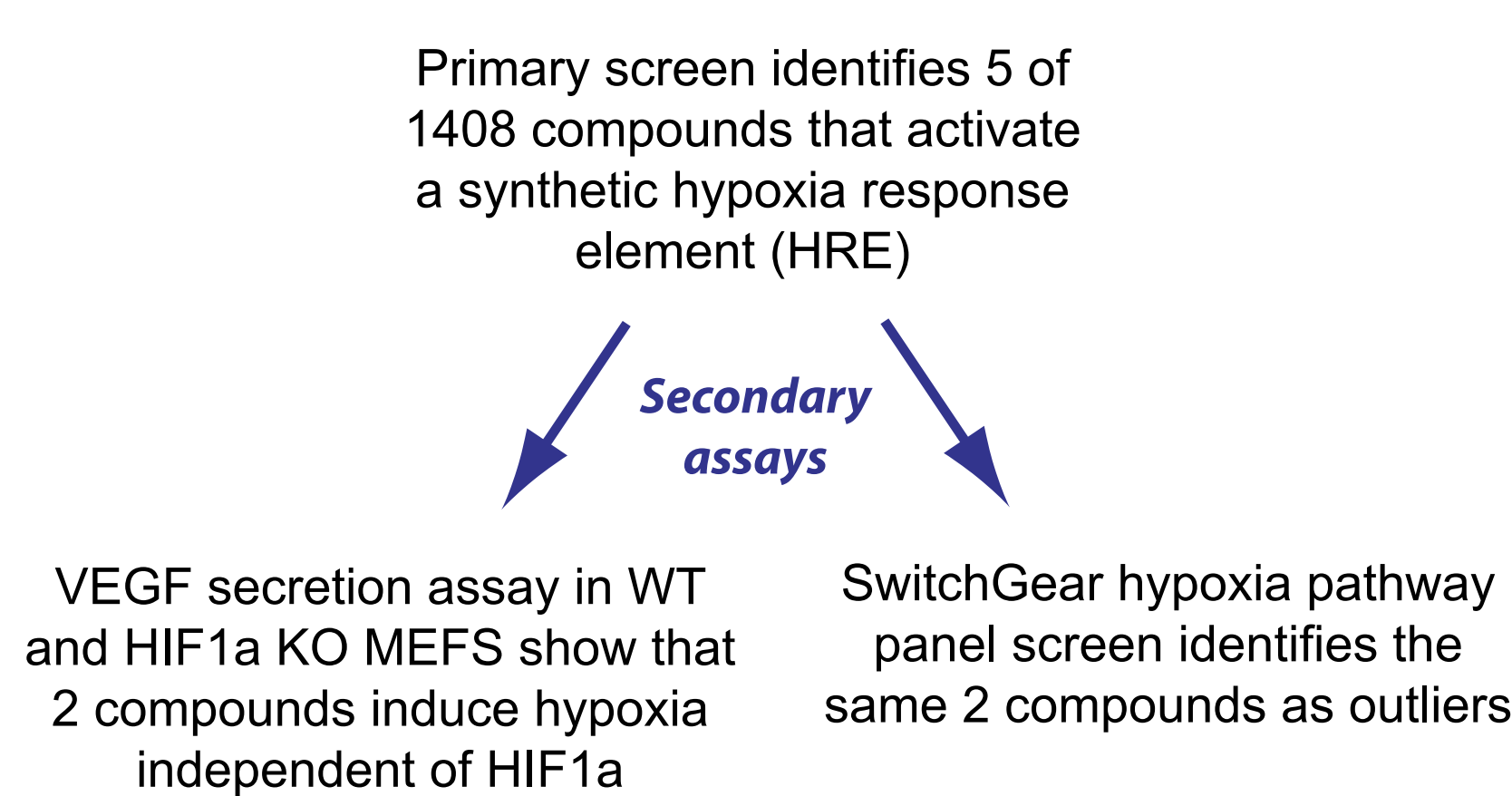
Alternative promoters play a role in hypoxia regulation for many loci - ALDOA is one example



Promoter activities for 22 known hypoxia responsive genes correlate well with existing expression data (Sonna et al. (2003) Physiol Genomics 12:195-207)

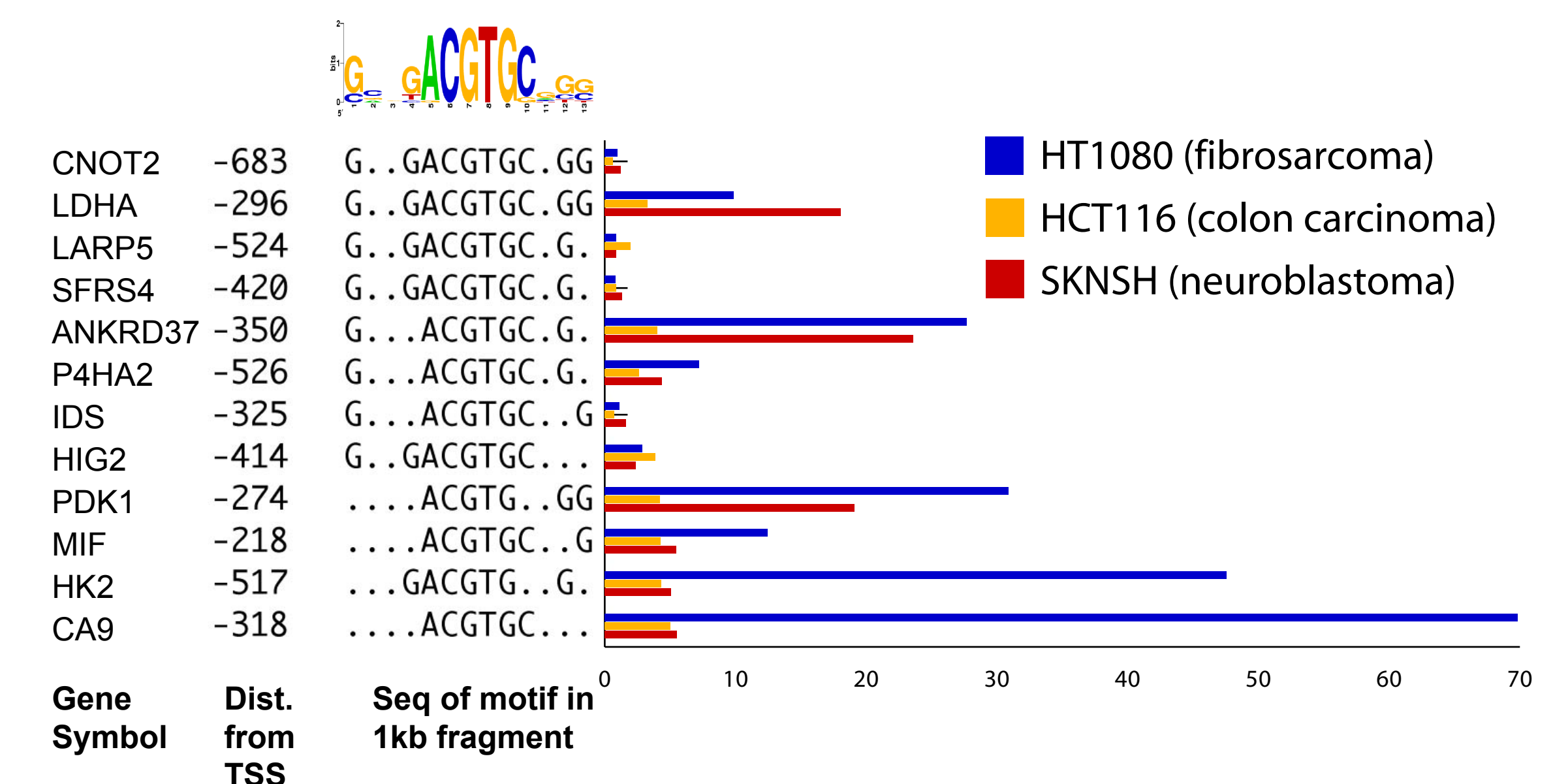


Screening a toxicology panel for HIF1a-dependent hypoxia inducers: VEGF secretion assay and promoter activation patterns identify the same top candidates (Xia et al. manuscript submitted)

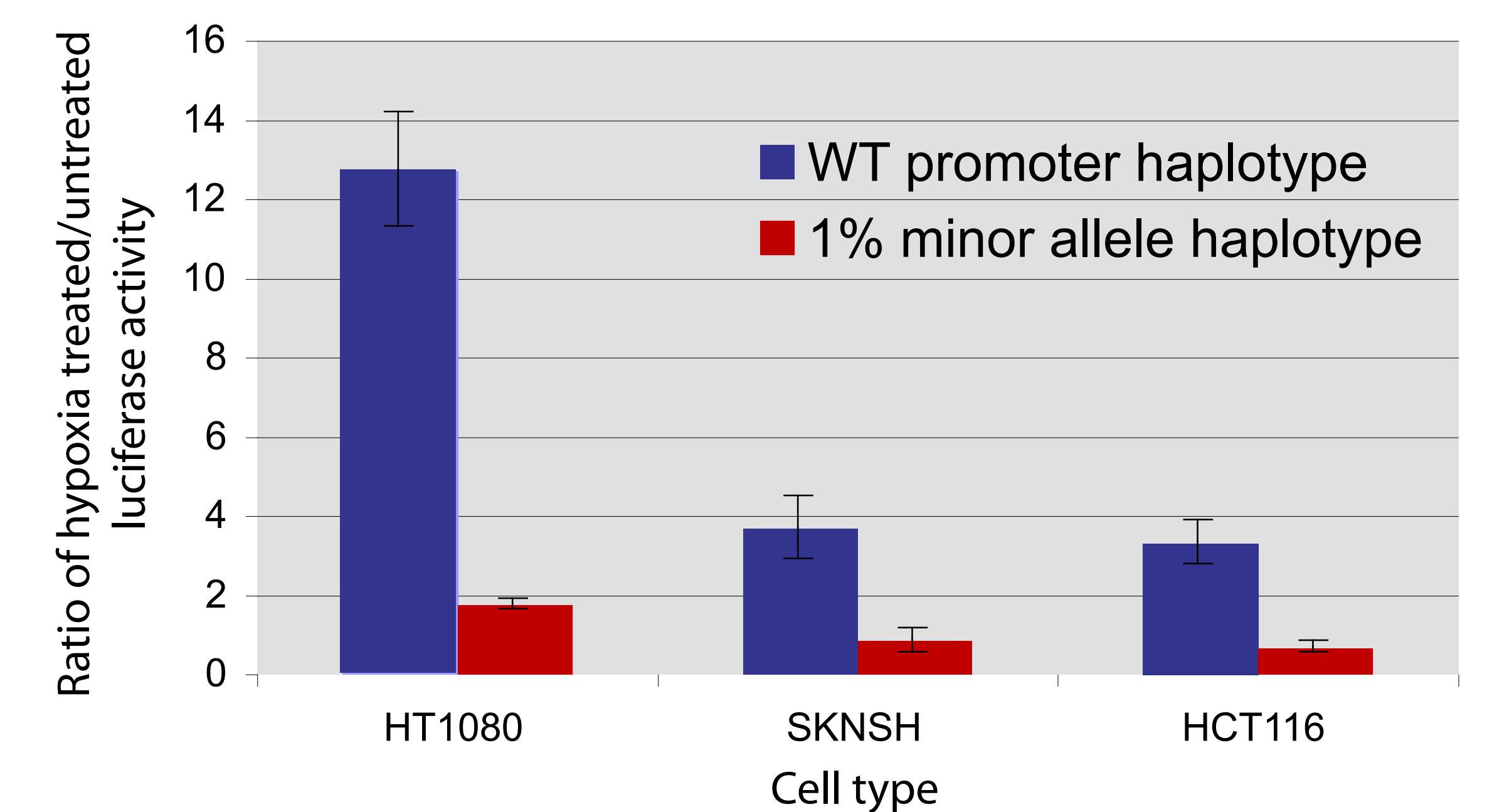


RESULTS

HIF1a motif occurrence does not predict functional activity



High-throughput reporter assays can be used to measure sequence variant effects on regulatory element function



CONCLUSIONS

We have gained new insight into the regulation of the human hypoxia pathway by mapping and measuring the behavior of hypoxia-responsive genomic elements and overlaying this information on existing datasets.

ONGOING WORK

- ▶ Confirming changes in endogenous transcript levels by qRT-PCR
- ▶ Extending the scope of the study to include 3'UTR-reporter assays
- ▶ Applying the same library resources and strategies to many other human regulatory pathways

ADDITIONAL RESOURCES

Data on the genome-wide regulatory element library resource can be found at:

www.switchdb.com

SwitchDB is an open-access database of human promoters and 3'UTRs.