Supplementary Discussion

Explanation for the apparent stronger negative selection of frameshift mutations when occurring in functionally important domains

In the deep sequencing analysis of CRISPR mutations shown in Figure 2i-k, we observed that frameshift mutations underwent negative selection when induced at any of the three sgRNA locations (BD1 or non-BD1 sites). However, the severity of negative selection is significantly less when targeting outside of BD1. The reasons for this are not immediately obvious, since it would be expected that truncating Brd4 at any of these 3 N-terminal sites should eliminate most of the full-length protein. However, it is important to consider the diploid nature of these cells. Each cell in the population will acquire a random CRISPR mutation on each copy of the *Brd4* gene. As depicted in Supplementary Figure 6, pairing of a frameshift mutation with an in-frame variant will likely prevent negative selection from occurring to the same severity as when a cell is homozygous for frameshift mutations. Hence, the functionality of in-frame variants will influence the negative selection behavior of frameshift mutations. Since in-frame mutations in a domain appear to lack functionality, it would be expected that frameshift mutations would more strongly deplete when targeted via CRISPR to a domain region.

Another potential explanation for these differential effects would be that different lengths of a truncated protein might retain varying levels of functionality or could potentially have differing degrees of dominant negative effects. It is also possible that varying levels of nonsense-mediated decay could influence the phenotypic consequences of these different frameshift mutations. It also a possibility that some of the frameshift mutations occurring at 5' exons could be 'rescued' by the use of an alternative start codon, which could restore expression of a nearly full-length protein. It is also worth emphasizing the findings in Supplementary Figure 5, where we observe a degree of variability in the frequency of in-frame mutations for certain sgRNAs. This reflects a degree of bias in the outcome of CRISPR mutagenesis, which can favor the formation of certain mutations. This variation in the frequency of in-frame mutations would also be expected to contribute to the variable severity of negative selection. Certain sgRNA sequence features may favor the formation of frameshift mutations[4, 5]. As a final consideration in this analysis, variation in the overall efficiency of CRISPR mutagenesis can also influence the ratios of the different genotypes.

Using deep sequencing-based measurements of mutation abundance to rule out off-target effects when validating dependencies identified from CRISPR screens.

We noted that the deep sequencing-based measurement of mutation abundance provided a useful means of excluding off-target effects, which has been a confounding variable in negative selection screens. Mutations induced by the *Brd4* sgRNA e3.1 exhibit a categorical separation of allele functionality for the in-frame (functional) and frameshift (non-functional) mutations (Fig. 2i). This pattern would not have occurred if negative selection was attributed due to mutagenesis of an off-target site, which would instead display a random pattern of negative selection when comparing frameshift and in-frame Brd4 variants. The consistency of this pattern across 75 distinct *Brd4* mutations provides strong evidence that the *Brd4* open reading frame encodes an essential protein in RN2c cells. Hence, performing a deep sequencing analysis of mutation abundance outside of critical domain can be useful for validating that a gene is essential.

Supplementary References

1. Shalem, O. et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science* **343**, 84-87 (2014).
2. Koike-Yusa, H., Li, Y., Tan, E.P., Velasco-Herrera Mdel, C. & Yusa, K. Genome-wide recessive genetic screening in mammalian cells with a lentiviral CRISPR-guide RNA library. *Nat Biotechnol* **32**, 267-273 (2014).
3. Dow, L.E. et al. Inducible in vivo genome editing with CRISPR-Cas9. *Nat Biotechnol* **33**, 390-394 (2015).
4. Doench, J.G. et al. Rational design of highly active sgRNAs for CRISPR-Cas9-mediated gene inactivation. *Nat Biotechnol* **32**, 1262-1267 (2014).
5. Wang, T., Wei, J.J., Sabatini, D.M. & Lander, E.S. Genetic screens in human cells using the CRISPR-Cas9 system. *Science* **343**, 80-84 (2014).