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Supplementary information

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Saturation genome editing maps the functional spectrum of pathogenic *VHL* alleles

In the format provided by the authors and unedited

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Supplementary Tables provided separately:

Supplementary Note 1. Optimizing the SGE protocol to assay *VHL*.

We substantially optimized SGE to enable accurate measurement of functional effects of *VHL* SNVs. Sequencing of initial libraries revealed two regions of exon 1 to have skewed variant distributions at sites of repetitive, GC-rich sequence (**Extended Data Fig. 2a-e**). Additional synonymous mutations were therefore engineered in these regions, resulting in improved library uniformity (**Extended Data Fig. 2f-l**).

For four exonic regions initially assayed using normal HAP1 culture media, only modest growth defects were observed for expected LoF variants (**Fig. 1e, Extended Data Fig. 3**). HAP1 cells can revert to diploidy with prolonged culture[64], a phenomenon that could weaken recessive effects measured in multiplex. Recently, 10-deacetyl-baccatin-III (DAB) was identified via small molecule screening to select for haploid cells[32]. Therefore, we next performed SGE for all *VHL* regions in media containing 2.5 μM DAB. This led to a substantial improvement in dynamic range (**Fig. 1f, Extended Data Fig. 3**). In exon 2, for example, the median function score of nonsense and canonical splice site SNVs dropped 4-fold, from -0.62 to -2.49. Across all SGE regions assayed with and without DAB, there were 39.3% more significantly depleted SNVs identified in DAB-treated cells. Therefore, we used only data from SGE experiments performed with DAB to calculate final function scores.

Supplementary References

64. Beigl, T. B., Kjosås, I., Seljeseth, E., Glomnes, N. & Aksnes, H. Efficient and crucial quality control of HAP1 cell ploidy status. *Biol. Open* **9**, (2020).