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High-throughput, functional profiling of every DLBCL hotspot mutation in human germinal centre B cells

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Diffuse large B cell lymphoma (DLBCL) is a clinically and genetically heterogeneous malignancy arising from germinal centre (GC) B cells. Although genomic studies have catalogued hundreds of recurrent mutations, the functional relevance of many remains unclear, limiting progress toward precision medicine. We previously established a primary human GC B cell co-culture system that models GC microenvironment and enables high-throughput, physiologically relevant interrogation of DLBCL-associated genes and mutations. Using this platform, we performed integrated genome-scale perturbation screens to define the functional landscape of recurrent DLBCL alterations.

A genome-wide CRISPR–Cas9 loss-of-function screen in primary human GC B cells, transduced with BCL2 and BCL6 to model a pre-malignant state, identified multiple tumour suppressive pathways involving cell cycle, apoptosis, G-protein signalling and components of the aryl hydrocarbon pathway. In parallel, overexpression screening with a mutant open reading frame (ORF) library comprising 967 wild-type and DLBCL-recurrent hotspot mutations quantified the relative impact of each WT or mutant ORF on cellular fitness. Recurrent mutations conferring the strongest fitness advantages occurred in CCND3, BCL10, CARD11 and GNAI2. Functional ranking of variants within individual genes, together with a complementary tiling base-editing saturation mutagenesis, further delineated critical residues and domains. To resolve downstream transcriptional consequences, we applied single-cell perturbation transcriptional profiling across ~300,000 cells from four primary B-cell donors using a focused sub-library of 305 barcoded WT or mutant ORFs. Integration of these orthogonal datasets enabled construction of a gene regulatory network linking genetic perturbations to GC transcriptional programmes and uncovered convergent rewiring across multiple oncogenic pathways. Notably, dysregulated G-protein signalling emerged recurrently across independent screening modalities as a central axis influencing GC B cell fitness and transformation.

Together, our work establishes a comprehensive functional framework for interpreting DLBCL-associated mutations in their native cell of origin. By integrating genome-scale perturbation, mutant ORF screening, base editing and single-cell transcriptomics, we define a multilayered map of oncogenic dependencies in which G-protein signalling pathways emerge as prominent drivers of GC B cell fitness.