Deep multiplexed single-cell imaging reveals that cell phenotypic state pre-tunes oncogenic signalling characteristics, including signalling noise

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Isogenic cancer cells display pronounced signalling heterogeneity upon growth factor or cytokine stimulation; a major barrier to effective therapy widely attributed to stochastic molecular noise. Understanding whether this variability is instead pre-determined by pre-existing phenotypic states requires simultaneous quantification of cell identity and signalling response across large molecular panels at single-cell resolution - a challenge beyond the reach of conventional approaches.

Here, we apply 50-plex cyclic immunofluorescence (cycIF) imaging to capture >1,300 quantitative features per cell, spanning 28 markers of cell state and 22 markers of signalling response across >43,000 A549 (NSCLC) cells stimulated with five oncogenic ligands (EGF, TGFβ, TNFα, IL6, IFNγ) over five timepoints. This deep single-cell phenotyping, including subcellular protein relocalisation measurements accessible only through imaging, enabled construction of a stimulation-invariant phenotypic manifold resolving epithelial-to-mesenchymal (E/M) cell states, against which signalling responses could be systematically compared across four dimensions: magnitude, kinetics, subcellular routing, and variability.

E/M state emerged as the dominant organiser of signalling response across all stimuli, far exceeding the influence of cell cycle phase. Strikingly, a subset of cell states exhibited selectively elevated within-state signalling variability that correlated with a multi-molecular stress signature - a relationship we validated by showing that pharmacological stress induction can, in specific combinations of stress context and signalling pathway, amplify signalling noise.

These findings, enabled by the combinatorial depth uniquely afforded by highly multiplexed imaging, establish tumour cells as state-dependent information-processing units and suggest that selective noise amplification in stressed cells may constitute a bet-hedging strategy diversifying responses under therapeutic pressure.