Development of a genetic screening approach for mechanism-of-action studies of small molecule inhibitors of ciliary signalling

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Cell-to-cell communication, through direct cell contact or secreted ligands, is crucial for development of multicellular organisms [1]. This is exemplified by the mammalian Hedgehog (Hh) pathway, which is activated through the morphogen Sonic Hedgehog, and relies on a specialized organelle, the primary cilium, for signal transduction. Dysregulation of the Hh pathway, through mutations in pathway components or structural and functional defects in the cilium, results in disease development, including cancer [2]. As such, small molecules targeting the Hh pathway are exploited as anti-cancer drugs, and as tools to uncover signaling biology. While discovery of various molecules through phenotypic screening campaigns has been successful, the identification of their cellular target and mechanism-of-action has proven challenging. In this study, we aim to fill this gap through the generation of chemo-genetic profiles of known inhibitors, and their application as a prediction tool for the mechanism of action of novel compounds. Using a focused library of sgRNAs targeting important Hh signaling genes [3], we identified genetic signatures that allow the distinction of upstream and downstream pathway inhibitors. Two genes, *Mosmo* and *Paxip1*, have been selected for follow-up studies with phenotypic screen hits, expanding the toolbox of methods that allow us to decipher their mechanism of action.

References:

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