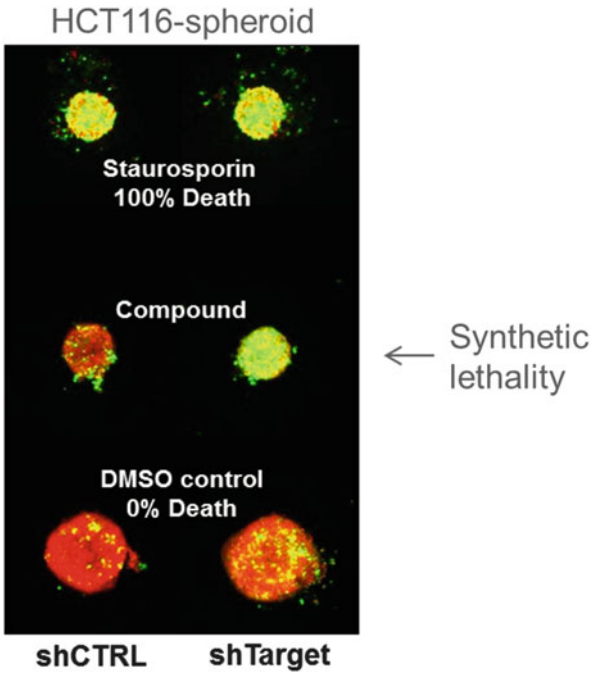

Fig. 4 3D tumor spheroids represent a cell-based model that mimic the situation in a real tumor more appropriately. *Green color* indicates dead cells (Sytox Green assay counterstained by Hoechst). Spheroids targeted by shRNAs only (cells at the bottom) do not show an increased cell death (negative control), while the combination of a specific compound and the targeting shRNA causes death of the cells inside the spheroid (synthetic lethality). Spheroids treated with very high concentrations of staurosporine (multikinase inhibitor that blocks ATP binding) do not survive independent of additional shRNA treatment (positive control, cells at the top). shCTRL is a nontargeting (so-called scrambled) control (figure generously provided by Patrick Steigemann and Gerrit Erdmann, Bayer Pharma AG)

cellular DNA and detection of either the unique shRNA sequence or a barcode, which can also be introduced into the DNA as part of the shRNA construct. As an alternative, also microarray technique can be used to measure abundance of each shRNA. If the amount of shRNA at the start of the experiment is higher than at the end, then this indicates that those cells who had the shRNA introduced into their genome either died or at least grew slower than the control (i.e., an shRNA that does not target a gene/nontargeting vector). This approach is named dropout screening accordingly.

An alternative or additional way to read out pooled screens is by FACS sorting. Cells that, for example, can be engineered to have a reporter gene construct integrated that translates into a fluorescent protein, which then can be sorted for. Subsequent NGS analysis allows for the identification of target genes whose knockdown contributed to activation of the reporter gene.