

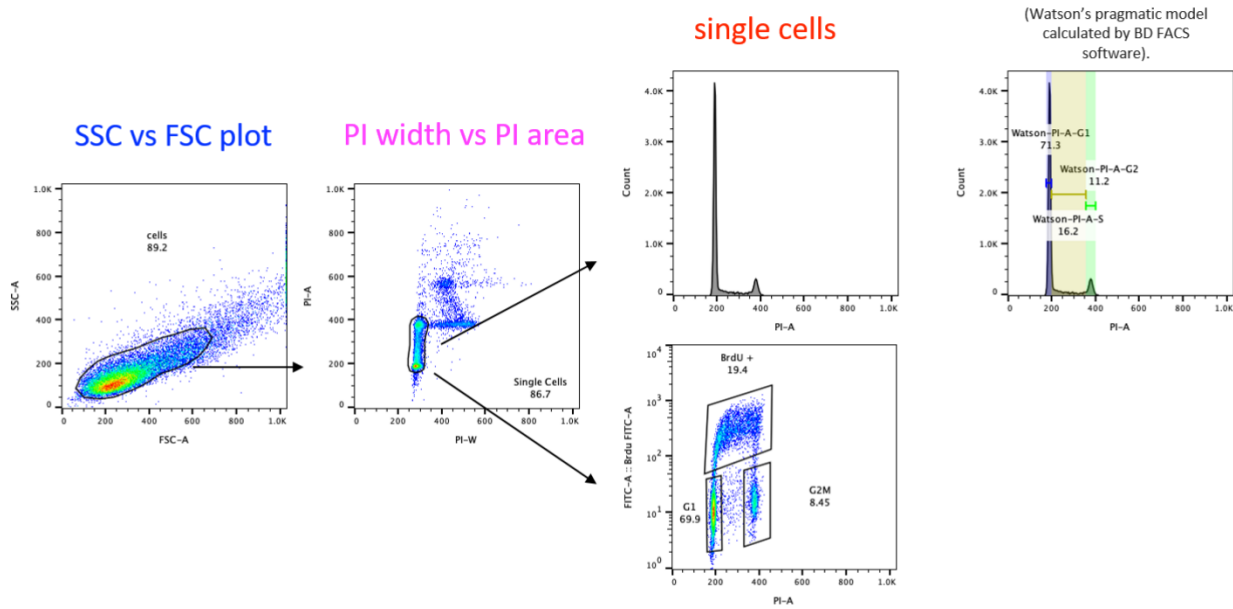
Supplementary Methods

A broader approach of pathway enrichment analysis was performed by using EnrichR¹. In R². All differentially expressed genes (FDR < 0.05) were used in the analysis. In addition to the KEGG database⁰, annotated gene sets from the GO Biological Process database^{3,4}, the WikiPathways database⁵, and the MSigDB Hallmark database⁶ were assessed for gene enrichment. The R package, *GeneSetCluster*⁷, was used to calculate the distance between enriched gene sets based on common differentially expressed genes contained within each gene set and that contribute to its individual enrichment significance. The top 100 gene sets, enriched in TTP488-treated orthotopic primary tumors and lung metastasis versus DMSO control, respectively, were plotted by unsupervised clustering using the R package, *heatmap*. Clustered enriched pathways were summarized manually.

Supplementary References

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- 4) Gene Ontology Consortium. The Gene Ontology resource: enriching a GOld mine. *Nucleic Acids Res.* 2021 Jan 8;49(D1):D325-D334. doi: 10.1093/nar/gkaa1113.
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- 8) Ewing, E., Planell-Picola, N., Jagodic, M. *et al.* GeneSetCluster: a tool for summarizing and integrating gene-set analysis results. *BMC Bioinformatics* **21**, 443 (2020).

Supplementary Figure 2



Supplementary Figure 2. Gating strategy used in cell cycle analysis of 4175 cells by flow cytometry. A standard gating strategy was used for the flow cytometric analysis of cell cycle. Debris was excluded on SSC vs FSC plot, and doublets were discriminated by plotting PI width vs PI area. 2N DNA content was arbitrarily set at 200 on the PI-area axis. Cell cycle profile of viable single cells was plotted as a histogram (PI-area), and cell cycle phase distribution was analyzed. Similarly, incorporation of BrdU (FITC) in viable single cells was plotted vs DNA content (PI- area) to simultaneously assess cell cycle distribution and BrdU incorporation (BrdU-FITC area vs PI area) on this population.

Source Files

Figure 6C

