

Supplementary Information Text

Interestingly, we found that stimulation of KP lung adenocarcinoma cells in 3-dimensional culture conditions with R-spondin alone was more efficient in promoting the formation of proliferating spheroids, as well as overall proliferation, compared to exogenously provided Wnt or even the combination of R-spondin and Wnt (Figure 1b; Extended Data Figure 1a, b, e, h). These findings are surprising, given that the combination of R-spondin and Wnt resulted in significantly stronger Wnt pathway activation than either R-spondin or Wnt alone (Extended Data Figure 1e, h). That R-spondin alone provided most efficient proliferation and spheroid growth may suggest that endogenous Wnt gradients established by cancer cells themselves within the context of a 3-dimensional tumour is critical for driving self-renewal, and that providing exogenous Wnt can perturb these gradients. In contrast, exogenous Rspo can reinforce endogenous Wnt signaling output in the Lgr5/Lgr4 positive subpopulation without disrupting endogenous Wnt gradients. In other words, it may be more efficient for tumour growth to activate Wnt signaling in a subpopulation of cancer cells rather than globally.