



Figure S5. Removal of all three PPxY motifs slightly improves Rod1-promoted adaptation.

(A) Cultures of a GEV derivative of the protease-deficient strain BJ5459 expressing GST-Rod1 or the indicated GST-Rod1 mutant were grown to mid-exponential phase. Protein expression was induced with β -estradiol and the cells were harvested by centrifugation and ruptured by vigorous vortex mixed with glass beads. GST-fusions in the resulting extracts were captured by binding to glutathione-agarose beads. The bound proteins were resolved by SDS-PAGE and analyzed by immunoblotting with the indicated antibodies. (B) The adaptation-promoting capacity of GST-Rod1 or variants lacking Rsp5-binding sites, a PANA PAAA double mutant (PPxY-less) and a VPSY PANA VASA PAAA triple mutant (V/PPxY-less). (C) Expression of the α -arrestin variants in the cells in (B) was assessed as described in Fig. 1D.