Figure S1. Trp[150] and Trp[153] of Rspo2 are C-mannosylated in A549 cells. MS/MS spectra of the double-charged un-, mono-, and di-mannosylated [146]EVGHWSEWGTCSR[158] peptides (*m/z*=796.33, 877.36 and 958.39, respectively). The indicated y-ions were detected as single-charged ions. Un-mannosylated- (top), only Trp[150]-mannosylated- (middle), and both Trp[150]- and Trp[153]-mannosylated- (bottom) peptides were observed. Trp, tryptophan; Rspo2, Rspo2, R-spondin2; MS/MS, tandem mass spectroscopy.

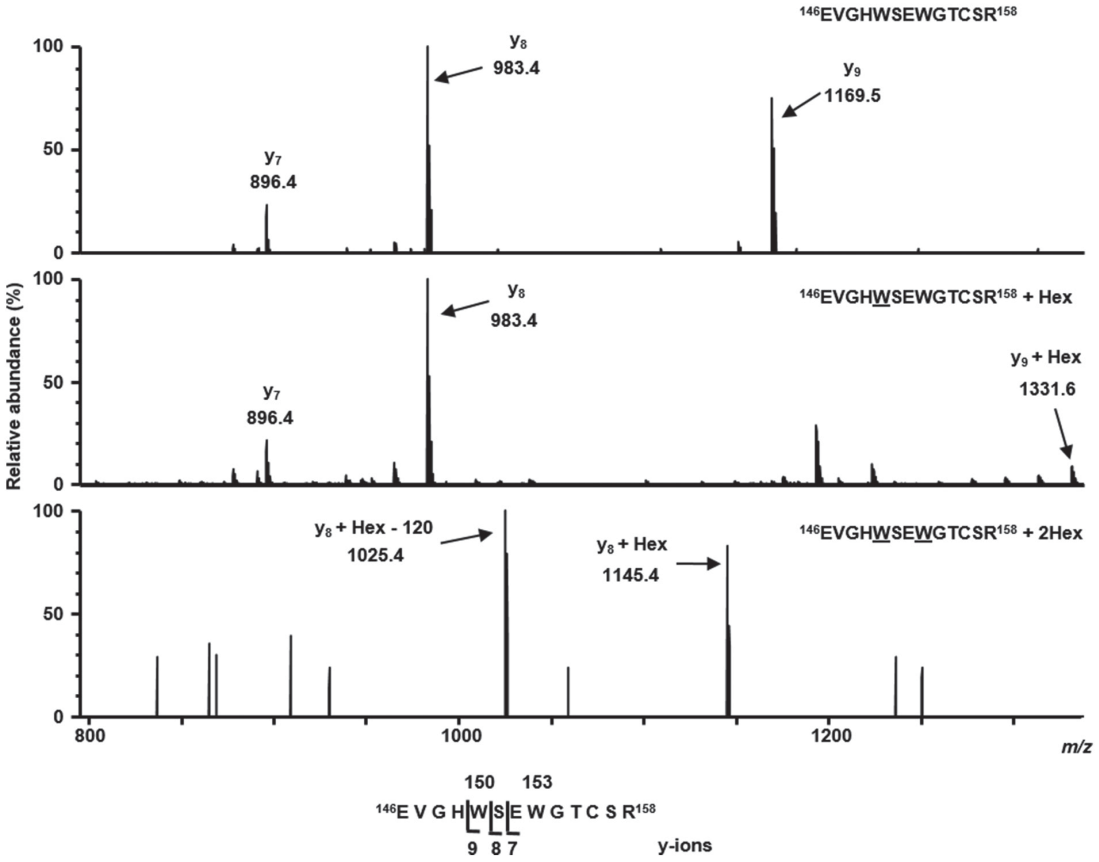

Figure S2. Effect of *C*-mannosylation at Trp^{150} on the agonistic activity of Rspo2. Parental HT1080 cells were transfected with pCI-neo, Rspo2/wt-MH, Rspo2/2WA-MH and Rspo2/W150A-MH. At 72 h post-transfection, cells were selected with 400 μg/ml G418 for 10 days. Each cell line was transfected with TOPFlash or FOPFlash in the presence of 30% L-Wnt3a cell-conditioned medium. After 24 h, luciferase activities were measured and normalized to *Renilla* luciferase. *P<0.05 compared with neo; $^{\#}$P<0.05 compared with wt. Trp, tryptophan; Rspo2, R-spondin2; wt, wild-type; ns, not significant.