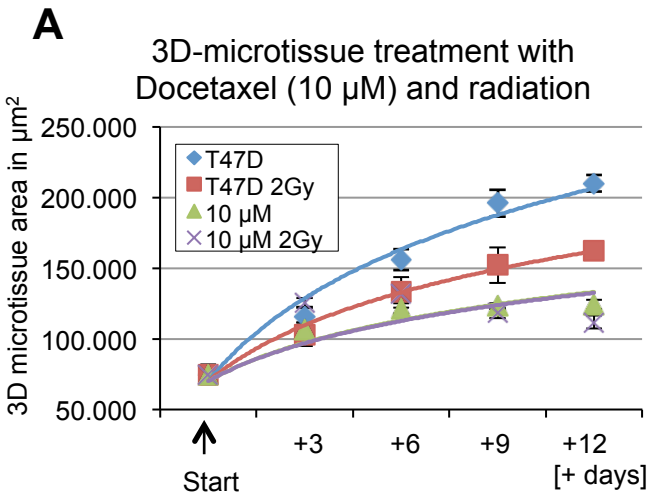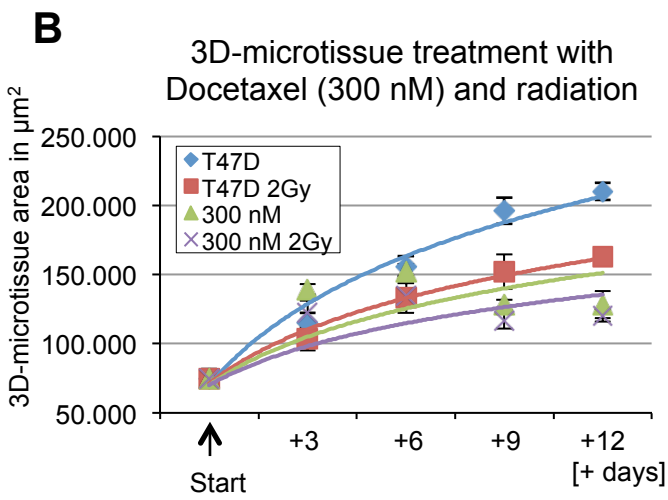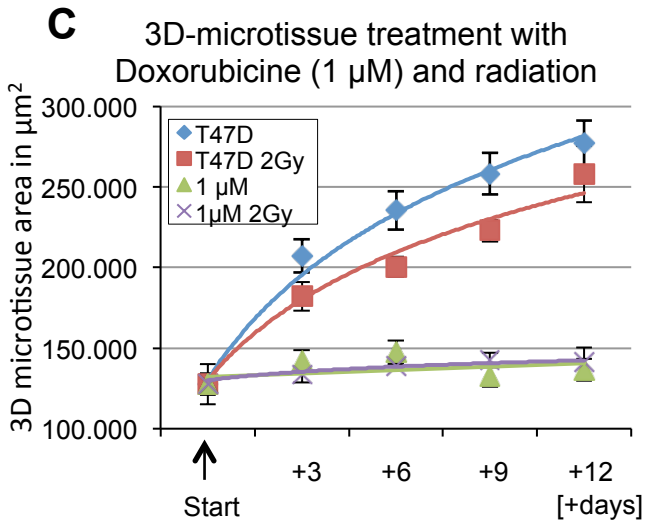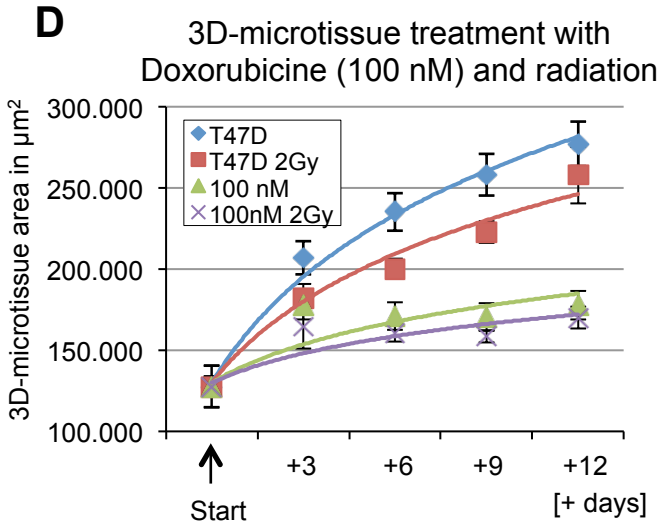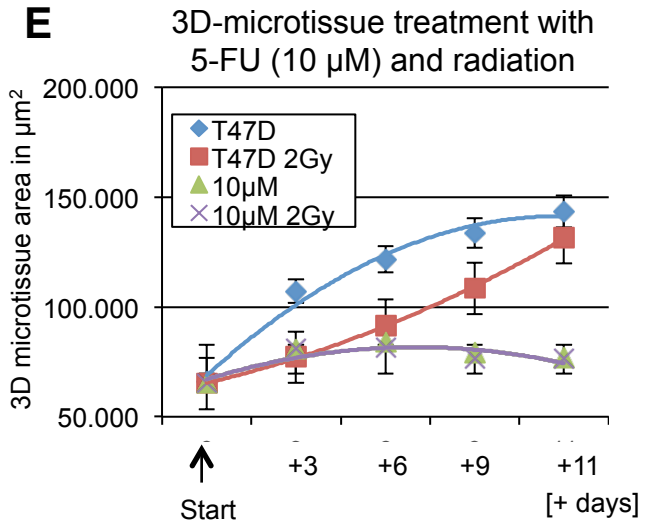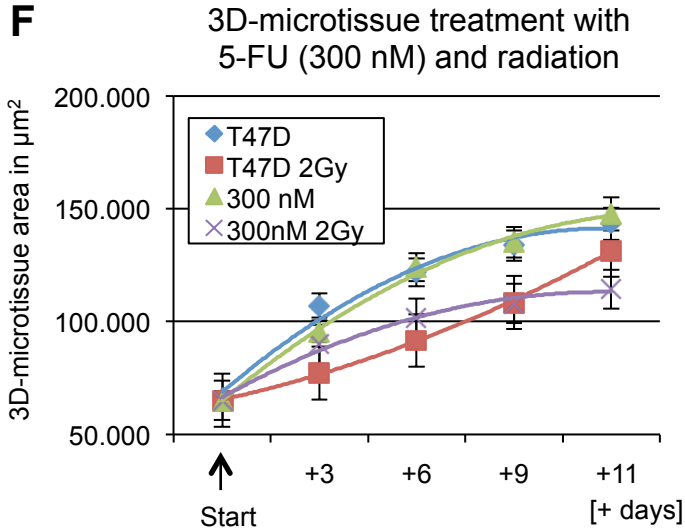Additional File 3