



Figure S1. Construction of strains used to show the function of ZafA in regulating gene expression through binding to the ZR motifs *in vivo*. Schematic representation of the construction of the derivative uridine-uracil-prototrophic *PyrG*⁺ *A. fumigatus* strains AFZR0 and AFZR1 that harbored the coding sequence of the firefly luciferase (*luc*, blue arrow) and green fluorescent protein (*gfp*, green arrow) under the control of the divergent *asf2-zrfC* promoter (abbreviated as *PzrfC*). The three ZR motifs present in *PzrfC* (red boxes) had been inactivated by site directed mutagenesis in the mutant version of this promoter (*PzrfC*^{ZR123}). EcoRI-EcoRI DNA fragments of 7286 bp excised from plasmids pPYRGQ191 and pPYRGQ193, which carried respectively the [*luc* ← *PzrfC*^{wt} → *gfp*] and [*luc* ← *PzrfC*^{ZR123} → *gfp*] constructs, were used to transform the CEA17 uridine-uracil-auxotrophic *pyrG1* strain to generate respectively the AFZR0 and AFZR1 strains. Both strains harboured the DNA fragment of interest inserted at the *pyrG* locus between their AFUA_2G08360 (*pyrG*) and AFUA_2G08350 loci, as verified by PCR analyses using the pairs of oligonucleotides JA197/JA505, JA197/JA463 and JA298/JA505.