



**Figure S5.** Southern blot analysis and phenotype of *S. macrospora* SMAC\_06770 deletion strains. **A.** Overview of the SMAC\_06770 locus in the wild type and deletion strains. Three introns within SMAC\_06770 are indicated as white boxes. The regions encoding the conserved GT\_ALG11\_like domain are indicated in yellow above the SMAC\_06770 gene locus. Restriction enzyme sites of enzymes used in B as well as probes for Southern blot analysis are indicated. Due to the close proximity of the next gene downstream of SMAC\_06770, the 3' flank of the deletion construct included part of the 3' end of the SMAC\_06770 coding region. **B.** Southern blot analysis of deletion strains. Strains T180.2.2 and T180.5.9 are primary transformants, the other four strains are single spore isolates derived from T180.2.2 (S156862, S156865) and T180.5.9 (S156867, S156868). Bands of the expected sizes for hybridization with the SMAC\_06770 deletion cassette after digestion with *XhoI* can be seen in the wild type, the recipient strain  $\Delta$ ku70, and six  $\Delta$ SMAC\_06770 transformants (wild type and  $\Delta$ ku70 1.8 kb and 3.1 kb, SMAC\_06770 deletion strains 4.5 kb). The band around 6 kb seen in the  $\Delta$ ku70 strain and the SMAC\_06770 deletion strains stems from the PtrpC promoter used in both Nourseothricin resistance cassette for the *ku70* deletion (Pöggeler and Kück 2006) as well as in the Hygromycin resistance cassette for the SMAC\_06770 deletion. The expected bands can also be seen after digestion with *XbaI* and probing with a fragment of the SMAC\_06770 gene, namely 3 kb in the wild type and  $\Delta$ ku70, whereas no band is seen in the SMAC\_06770 deletion strains. **C.** Phenotypic analysis of SMAC\_06770 deletion strains. The wild type,  $\Delta$ ku70, and the six  $\Delta$ SMAC\_06770 strains shown in B were grown for 14 d on corn meal medium at 25 °C in constant light. As expected, the wild type and the  $\Delta$ ku70 strain have grown completely across the petri dish and form fruiting bodies (black dots). The deletion strains grow very slowly (mycelial diameter after 14 d < 3 cm), and they do not form fruiting bodies.