



**Figure S4. Overexpression of the Cas9 protein is toxic for the cell growth.**

A. The growth inhibition of the cells carrying *nmt41p*-Cas9 and *adh1p*-Cas9 plasmids was evaluated by spotting assay (left panel). The Cas9 expression driven by the *nmt41* and *adh1* promoter showed the growth inhibition. More severe inhibition was observed in the cells expressed Cas9 by the *adh1* promoter (#2 transformant cell). #1 transformant cell showed fast growth, however, lost the Cas9 expression (right panel).

B. The Cas9 expression causes the abnormal cell shape. The cells were fatten or elongated by expression of the Cas9. Scale bar indicates 10  $\mu$ m.