



Figure S3. Immunoblot analysis of the Cas9 protein expressed by the *nmt1*- and *nmt41* promoter.

Cas9 protein was expressed by the *nmt1*- and *nmt41* promoter of a gRNA-Cas9 vector (pAH236 (gRNA + *nmt1p*-Cas9-*LEU2*), pAH237 (gRNA + *nmt41p*-Cas9-*LEU2*), pAH242 (gRNA + *nmt1p*-Cas9-*ura4⁺*), and pAH243 (gRNA + *nmt41p*-Cas9-*ura4⁺*)). Two independent transformants carrying the vectors were cultured in EMM selective medium without thiamine to induce the expression of Cas9 protein and analyzed by immunoblotting using anti-Flag antibody. Cells carrying empty vector (pSLF273) were analyzed as a negative control (-). Cdc2 (α -PSTAIR) was used as a loading control. Arrow: full-length Cas9 protein. Arrowheads: degraded Cas9 proteins.