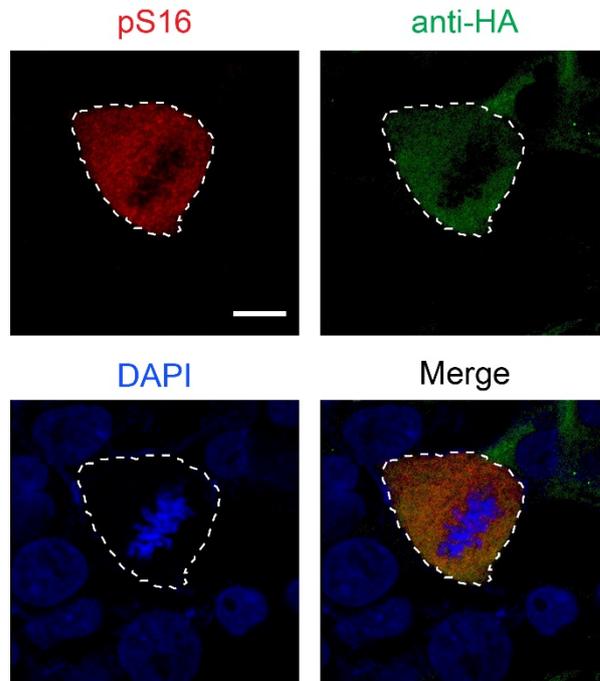


# Supplementary Figures

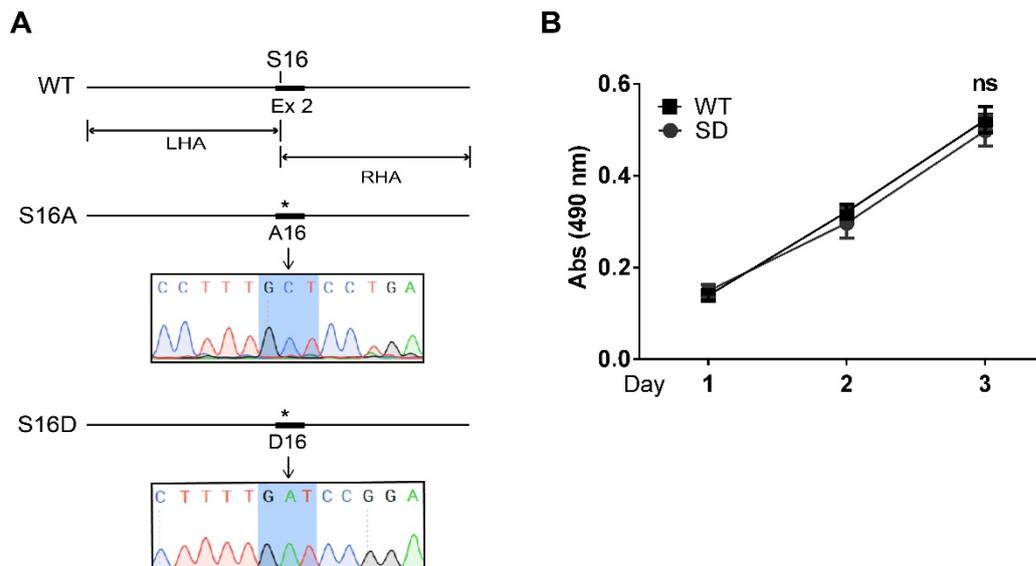
## Figure S1

293A +  $\alpha$ 5-TBHA



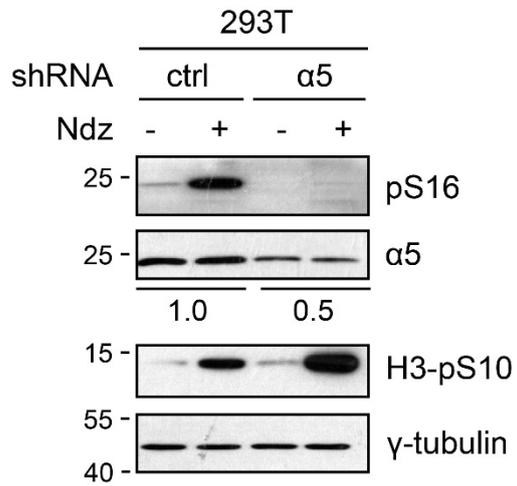
**Figure S1:** Immunofluorescence staining of  $\alpha$ 5-pS16. Asynchronous 293A cells transfected with  $\alpha$ 5-TBHA were immunostained with anti-pS16 and anti-HA antibodies and imaged with a confocal microscope. Scale bar = 10  $\mu$ m. The contour of the only mitotic cell in this eye-field is outlined. Note that a neighboring interphase cell transfected with  $\alpha$ 5-TBHA was negative for pS16.

## Figure S2



**Figure S2:** CRISPR/Cas9-mediated knock-in of  $\alpha 5$ -S16 mutations. **(A)** Gene editing strategy for knock-in. Thick black bar represents exon 2 (Ex 2) of the PSMA5 gene where Ser16 is located. LHA and RHA stand for left/right homology arm in the repair donor template. Allele-specific sequencing results of the mutated sites are shown. **(B)** MTS assay was performed on WT and S16D (heterozygous) cells as in Fig. 3E.

### Figure S3



**Figure S3:** 293T cells were infected with lentiviruses expressing a control or  $\alpha 5$ -targeting shRNA. Partial knockdown of endogenous  $\alpha 5$  was confirmed by western blot. Cells were treated with Ndz and pS16 was analyzed.