Electronic Supplementary Information

for

Phosphorylated 5-ethynyl-2'-deoxyuridine for advanced DNA labeling

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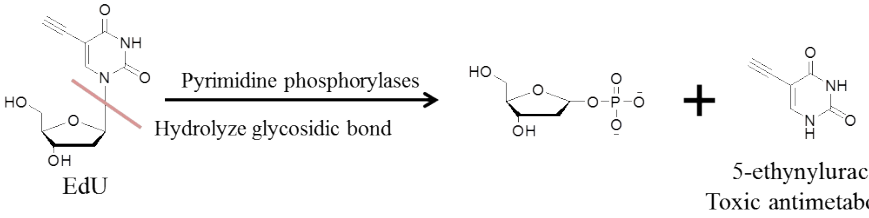
1. Glycosidic bond hydrolysis by pyrimidine phosphorylases generates 5-ethynyluracil, itself a toxic antimetabolite



2. EdUMP which is generated from EdU inhibits dTMP synthesis

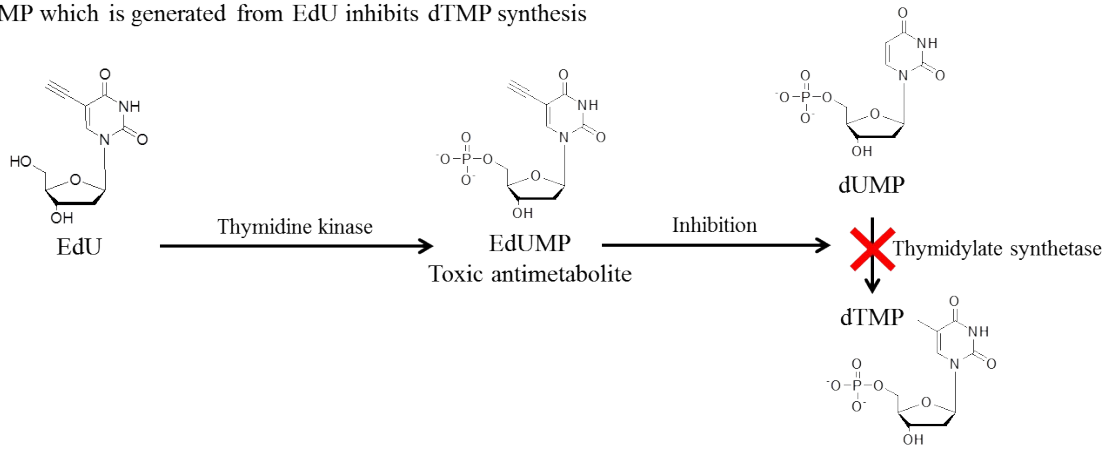


Figure S1. Cytotoxic pathways of EdU.

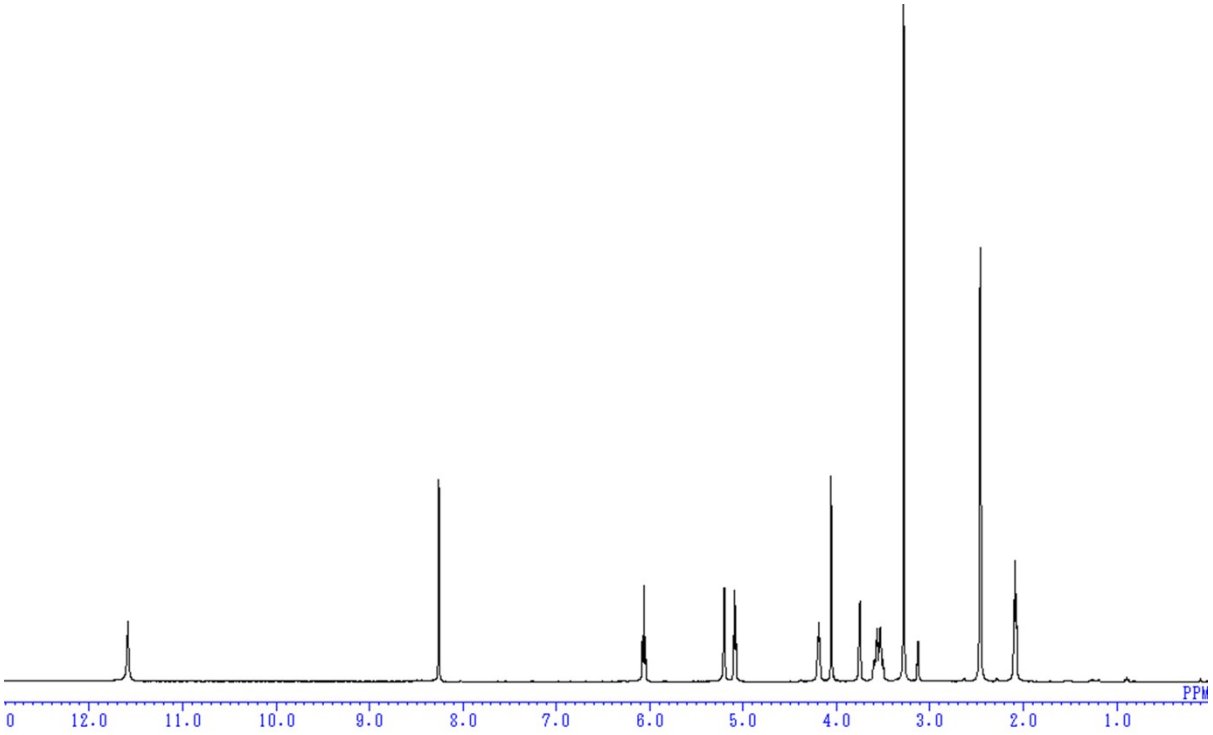


Figure S2. ^{1}H NMR spectrum (400 MHz) of EdU(1) in DMSO at 25 °C.

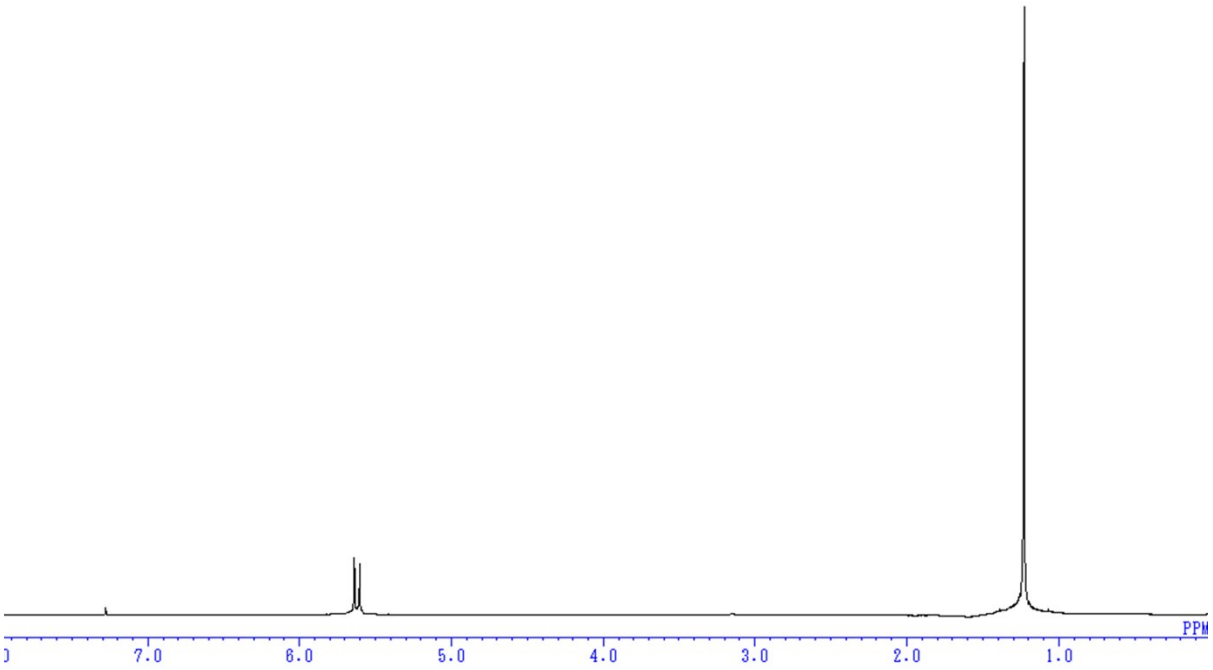


Figure S3. ^{1}H NMR spectrum (400 MHz) of chlorobis(POM) phosphate(2) in $CDCl_3$ at 25 °C.

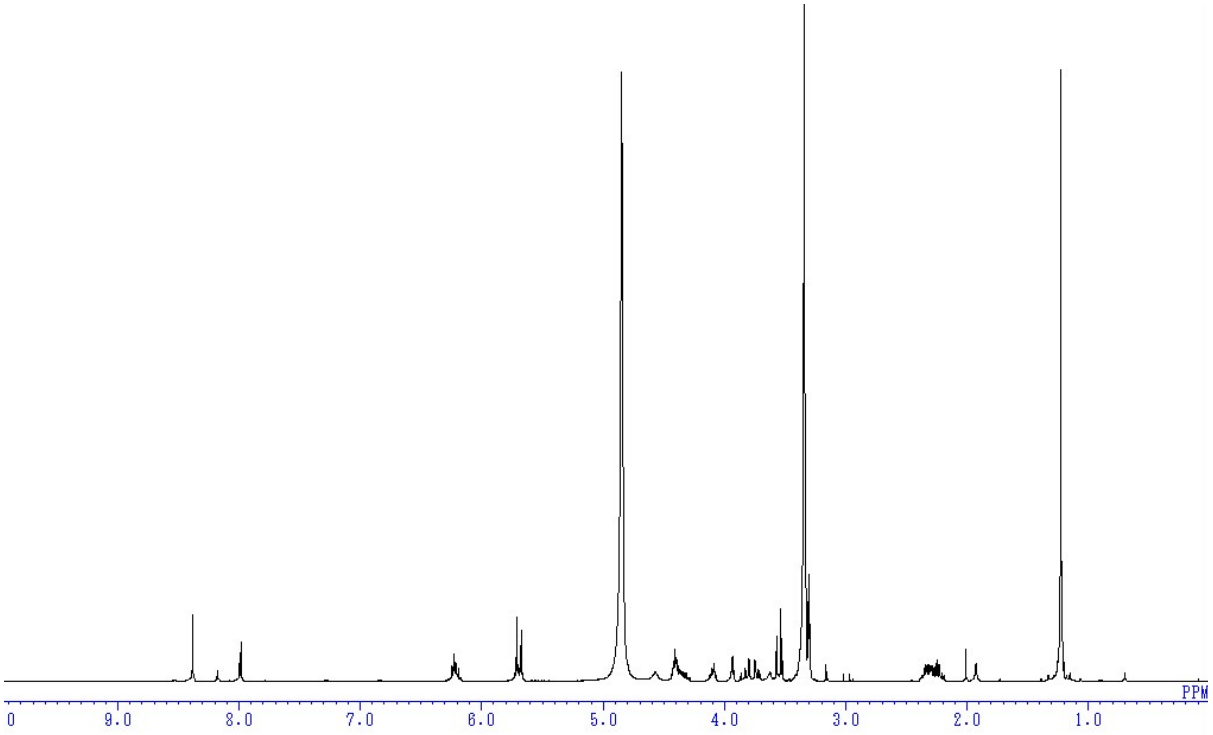


Figure S4. ^{1}H NMR spectrum (400 MHz) of PEdU(3) in CD_3OD at 25 °C.

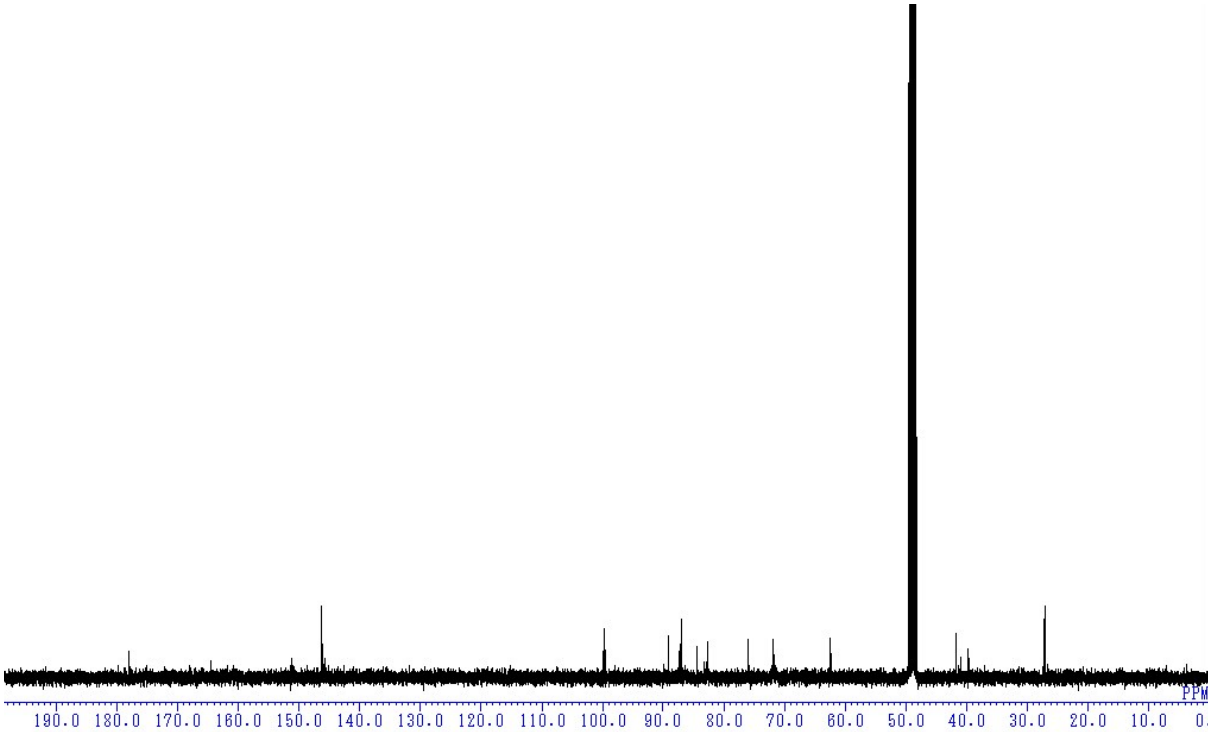


Figure S5. ^{13}C NMR spectrum (400 MHz) of PEdU(3) in CD_3OD at 25 °C.

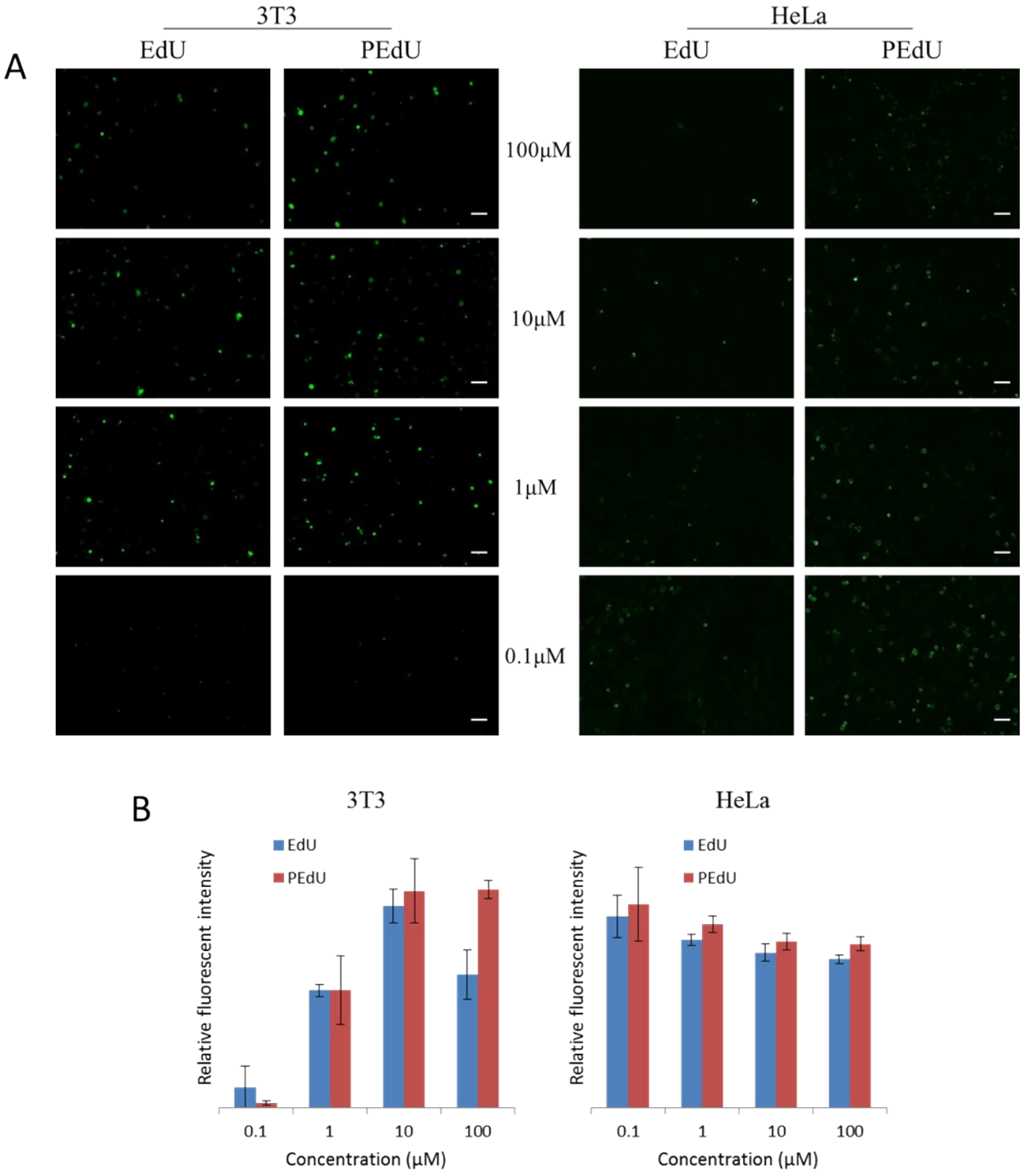


Figure S6. Metabolic labeling of DNA in 3T3 and HeLa cells at various concentrations (0.1–100 μM) of EdU or PEdU. After 72 h, incorporated DNA was stained with Alexa Fluor 488 azide (A). Scale bar: 50 μm. The relative fluorescence intensity was measured using image analysis software (B). n=4

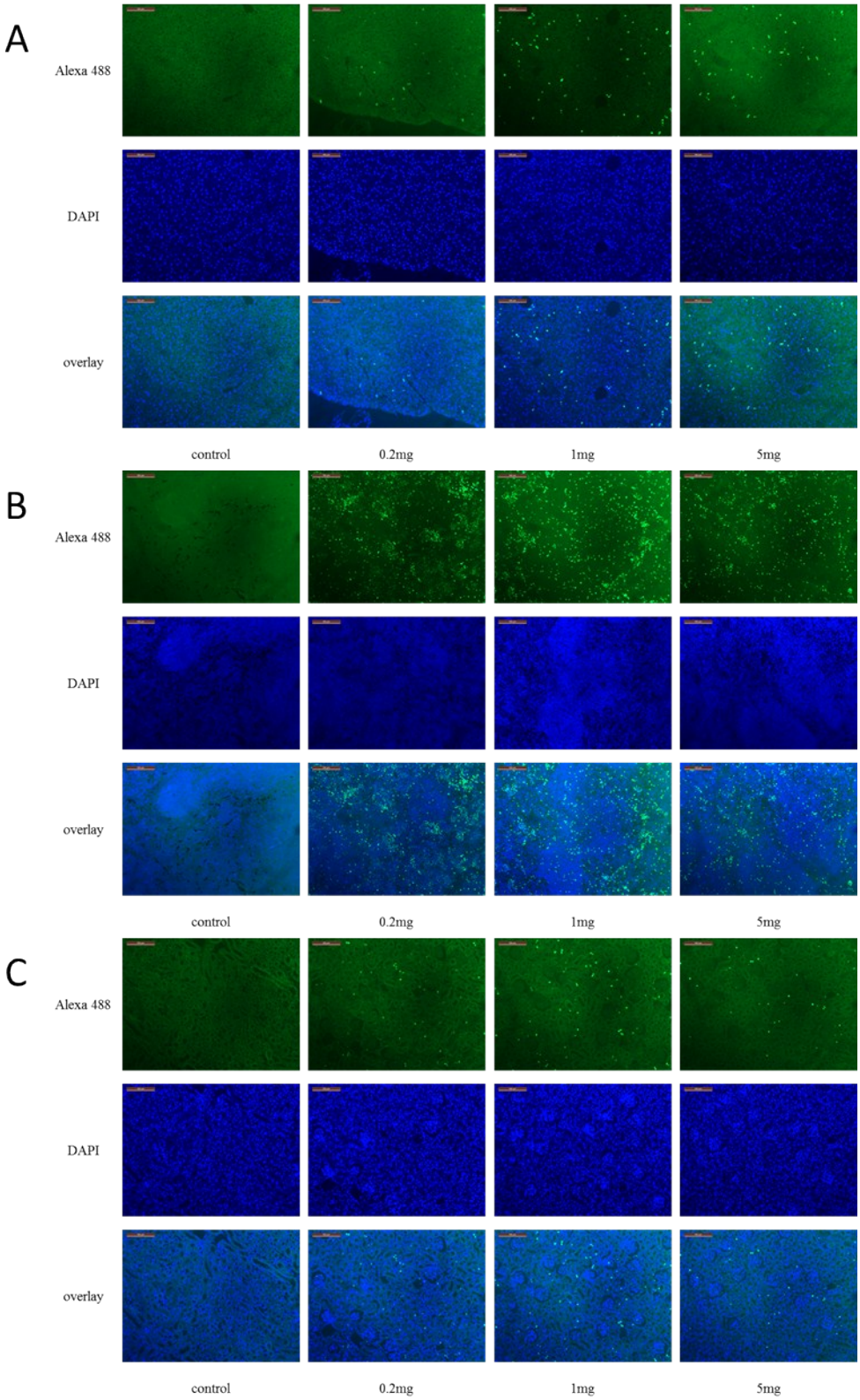


Figure S7. Mice were injected i.p. with 0.2, 1 or 5 mg PEdU. After **24** h, livers (A), spleens (B) and kidneys (C) were harvested. Sections from the organs were fixed and stained with Alexa Fluor 488 azide. Total DNA was stained with DAPI. Scale bar: 100 µm.

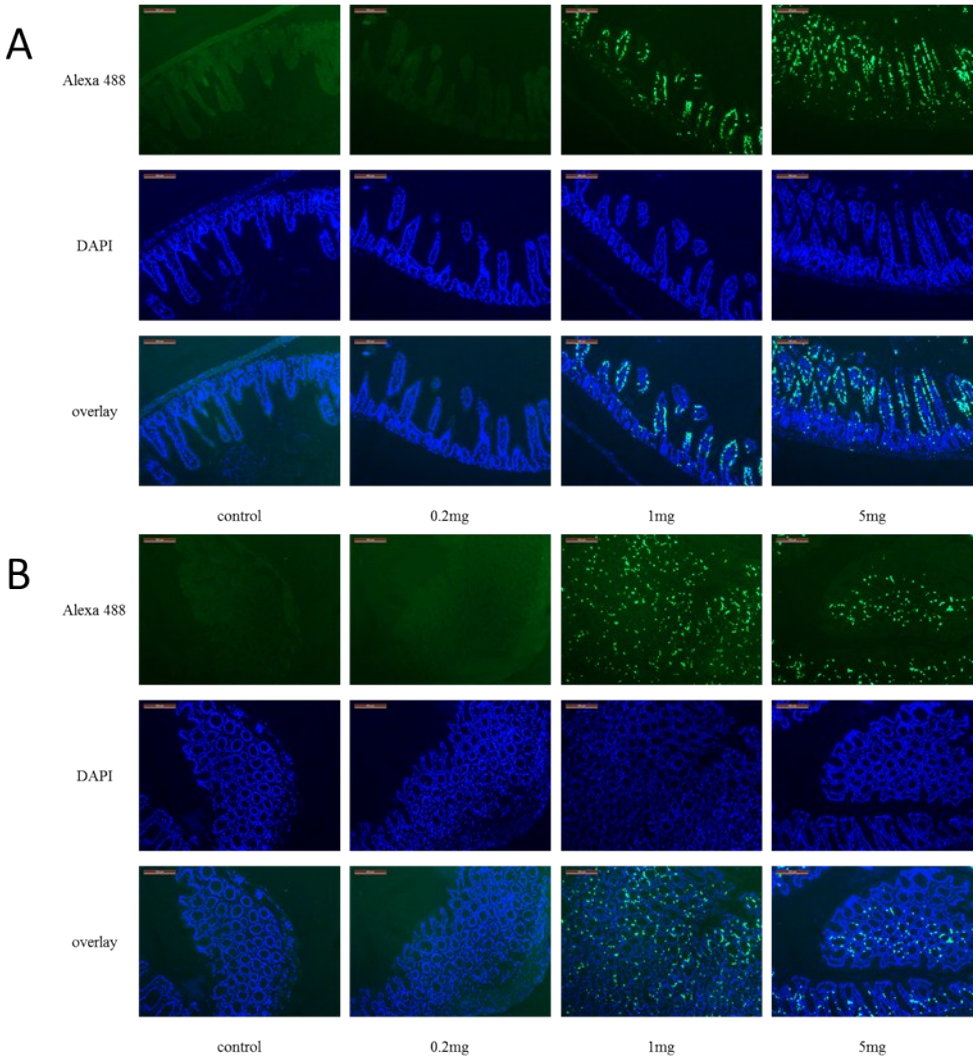


Figure S8. Mice were injected i.p. with 0.2, 1 or 5 mg PEdU. After **48** h, ileums (A) and colons (B) were harvested. Sections from the organs were fixed and stained with Alexa Fluor 488 azide. Total DNA was stained with DAPI. Scale bar: 100 µm.

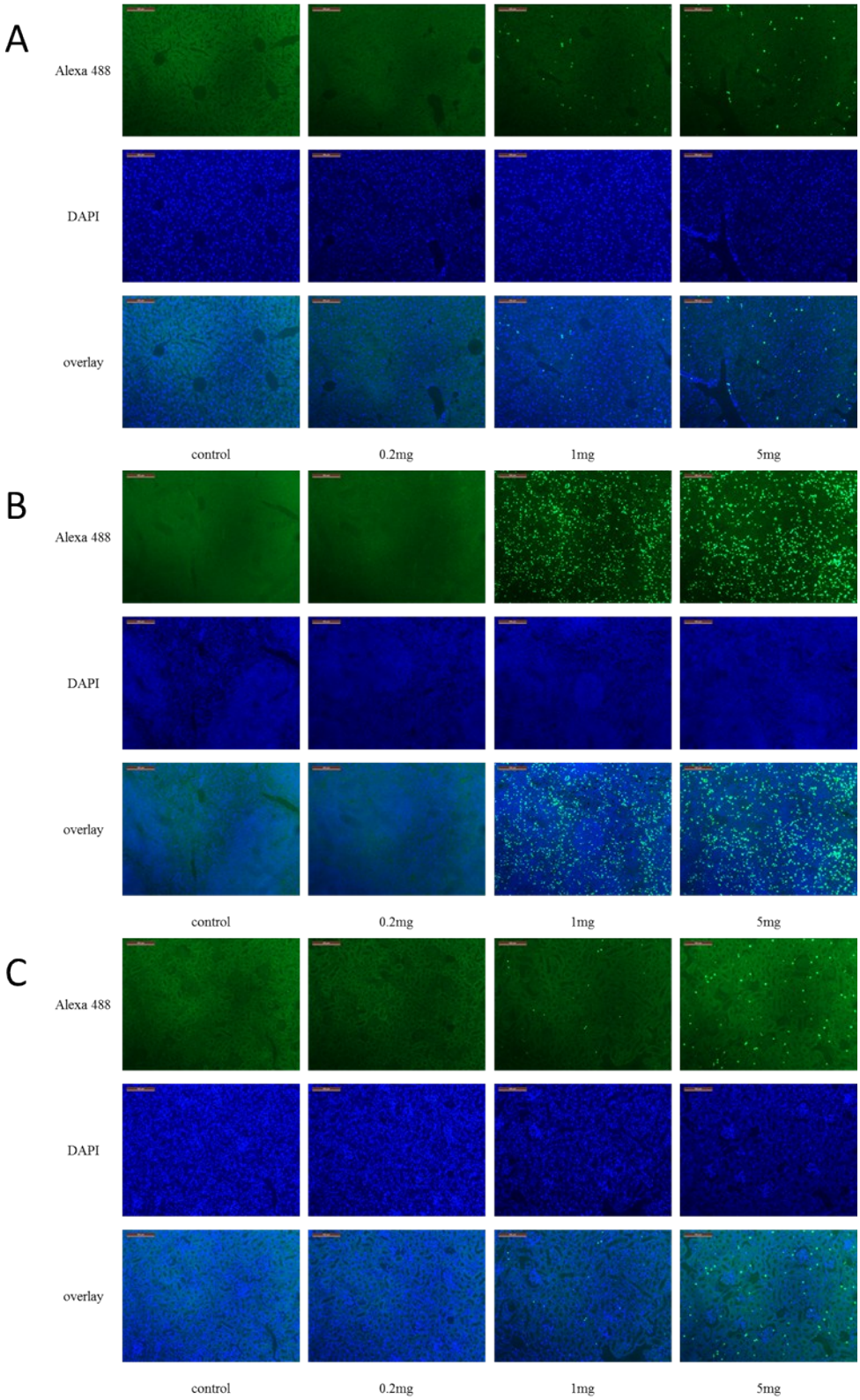


Figure S9. Mice were injected i.p. with 0.2, 1 or 5 mg PEdU. After **48** h, livers (A), spleens (B) and kidneys (C) were harvested. Sections from the organs were fixed and stained with Alexa Fluor 488 azide. Total DNA was stained with DAPI. Scale bar: 100 µm.