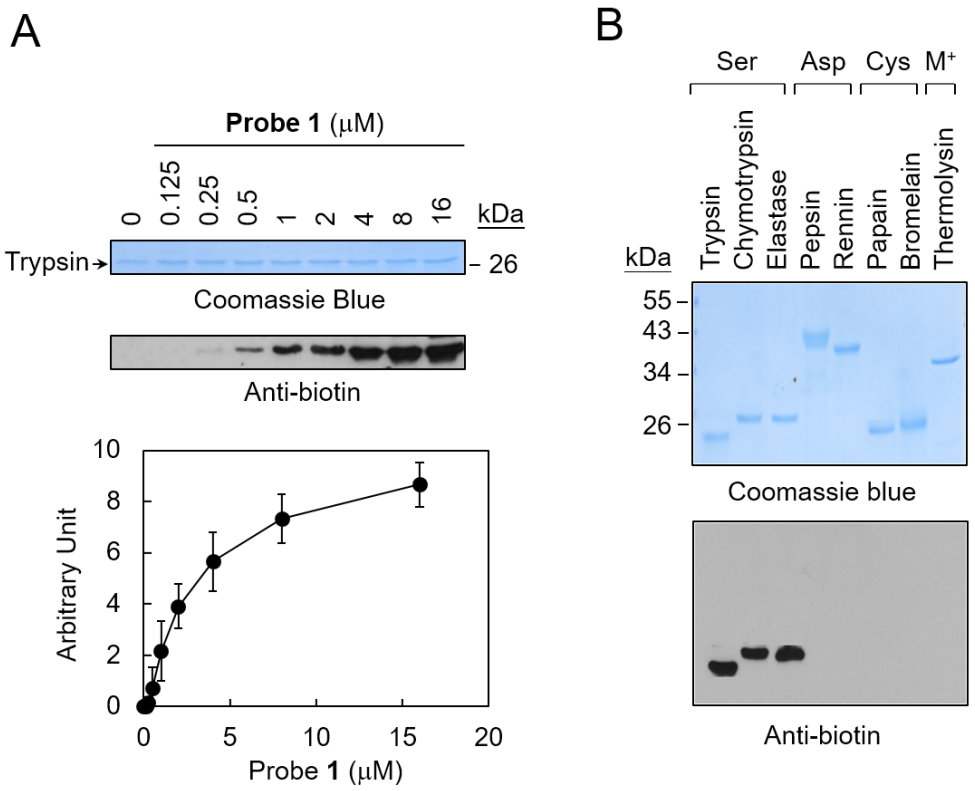

Fig. S1. Labeling property of probe **1**. **(A)** In parallel experiments, one μg each of trypsin was incubated with indicated amounts of probe **1** at 25ºC for 10 mins and separated on a 10% SDS-polyacrylamide gel. One gel was stained with Coomassie blue and the other was subjected to immunoblotting analysis using anti-biotin antibody. Quantification of the labeled proteins were plotted (bottom panels). The values were obtained from the average of three independent experiments. Error bars represent standard deviation (SD). **(B)** One μg each of the indicated proteases was labeled with probe **1** (3 μM) at 25ºC for 10 min. The labeled proteins were separated on a 10% SDS-polyacrylamide gel and then stained with Coomassie blue (top panel) followed by immunoblotting analysis using anti-biotin antibody (bottom panel).

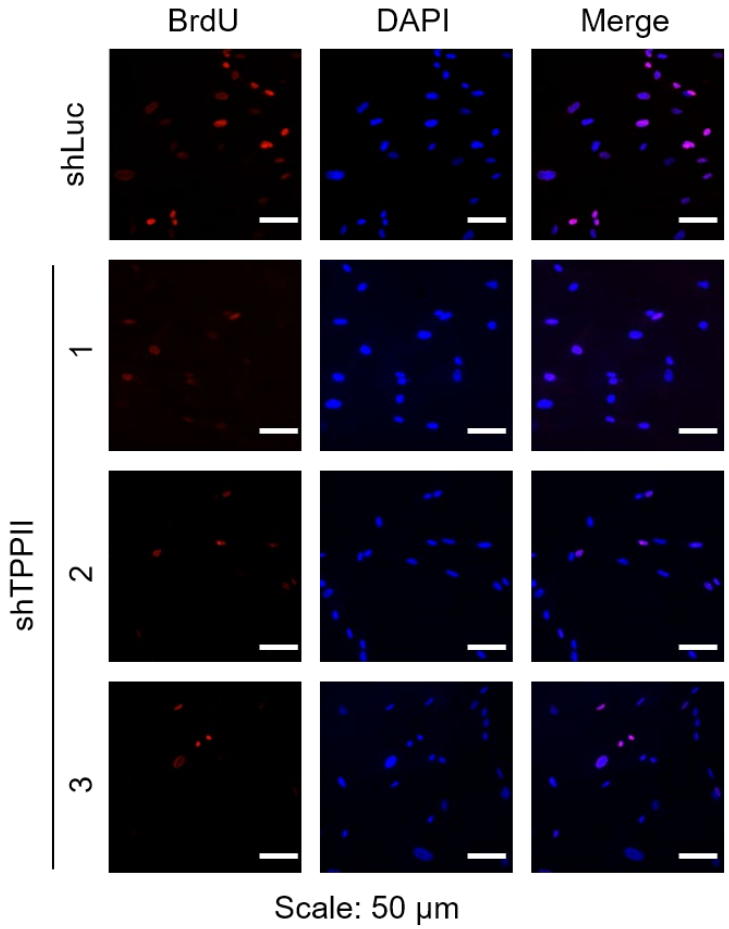

Fig. S2. IMR90 cells were infected with lentivirus carrying TPPII- or Luciferase-targeting shRNA sequences. Three days after infection, cells were re-seeded on cover slide. One day later, cells were incubated with 40 μM BrdU for 16 h, fixed, and then stained with anti-BrdU antibody (red) and DAPI (blue). The scale bar represents 50 μm.

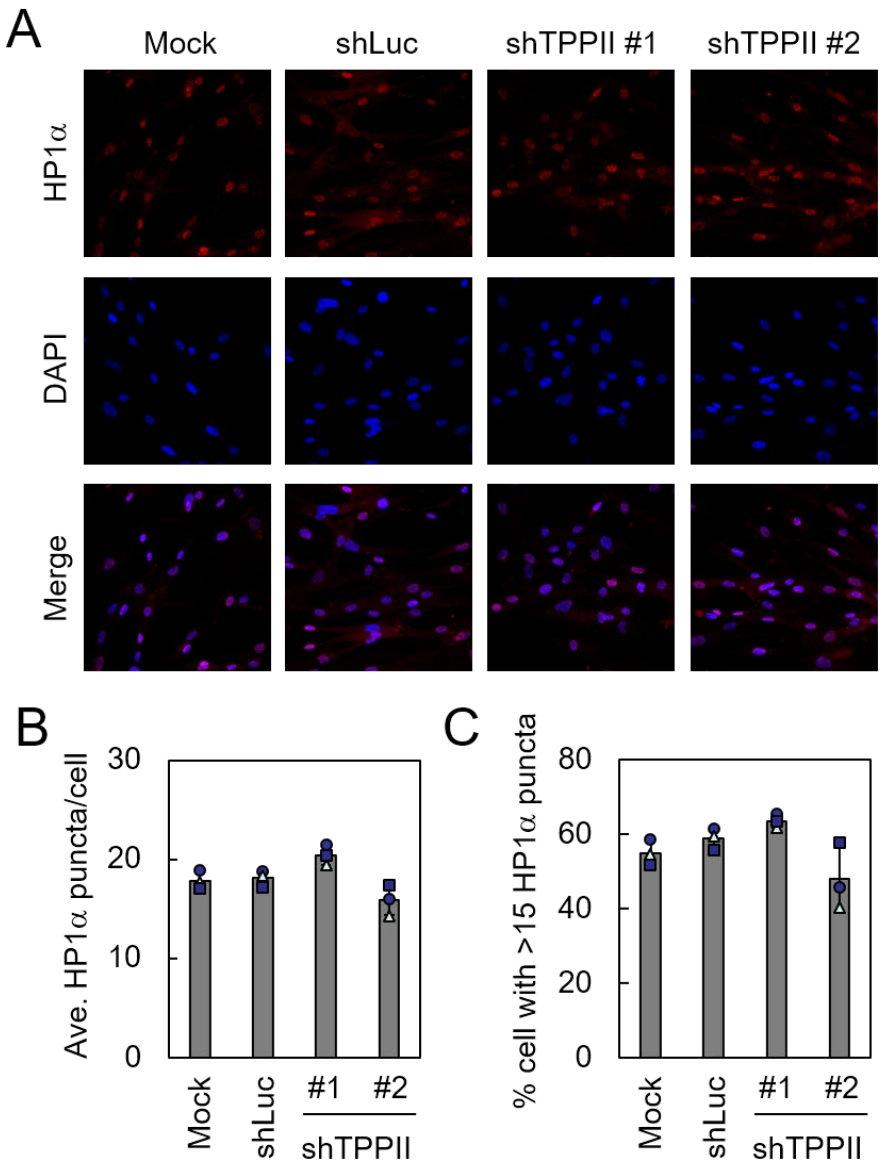

Fig. S3. IMR90 cells were infected with lentivirus carrying TPPII- or Luciferase-targeting shRNA sequences. (A) Three days after infection, cells were re-seeded on cover slide, fixed, and then stained with anti-HP1α antibody (red) and DAPI (blue). The average numbers of HP1α puncta per cell and the percentage of cells with >15 puncta were quantified in (B) and (C), respectively. Quantification results in (B, C) were conducted from the average of 3 independent experiments.

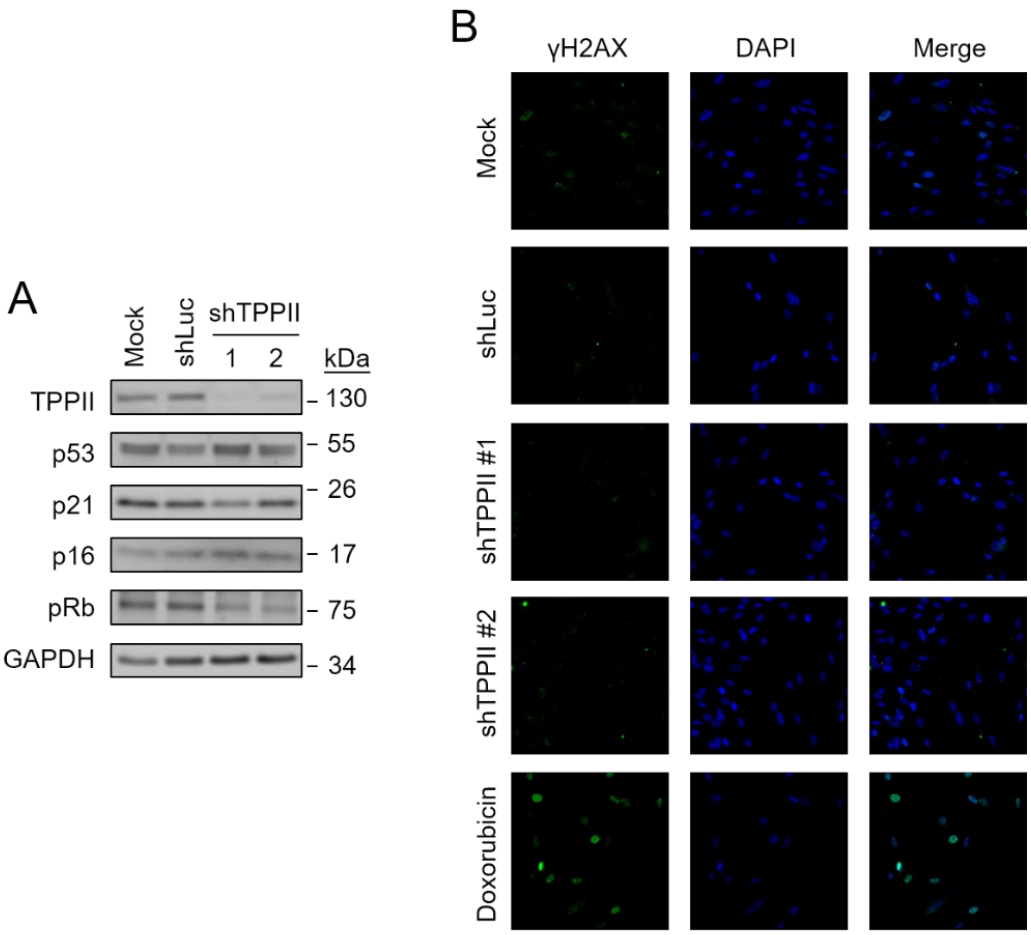

Fig. S4. IMR90 cells were infected with lentivirus carrying TPPII- or Luciferase-targeting shRNA sequences. (A) Cell extracts were prepared four days after infection and then analyzed by immunoblots using the indicated antibodies. (B) Immunofluorescence assays were also performed using anti-γH2AX antibody (green). The cell nuclei were stained with DAPI (blue).

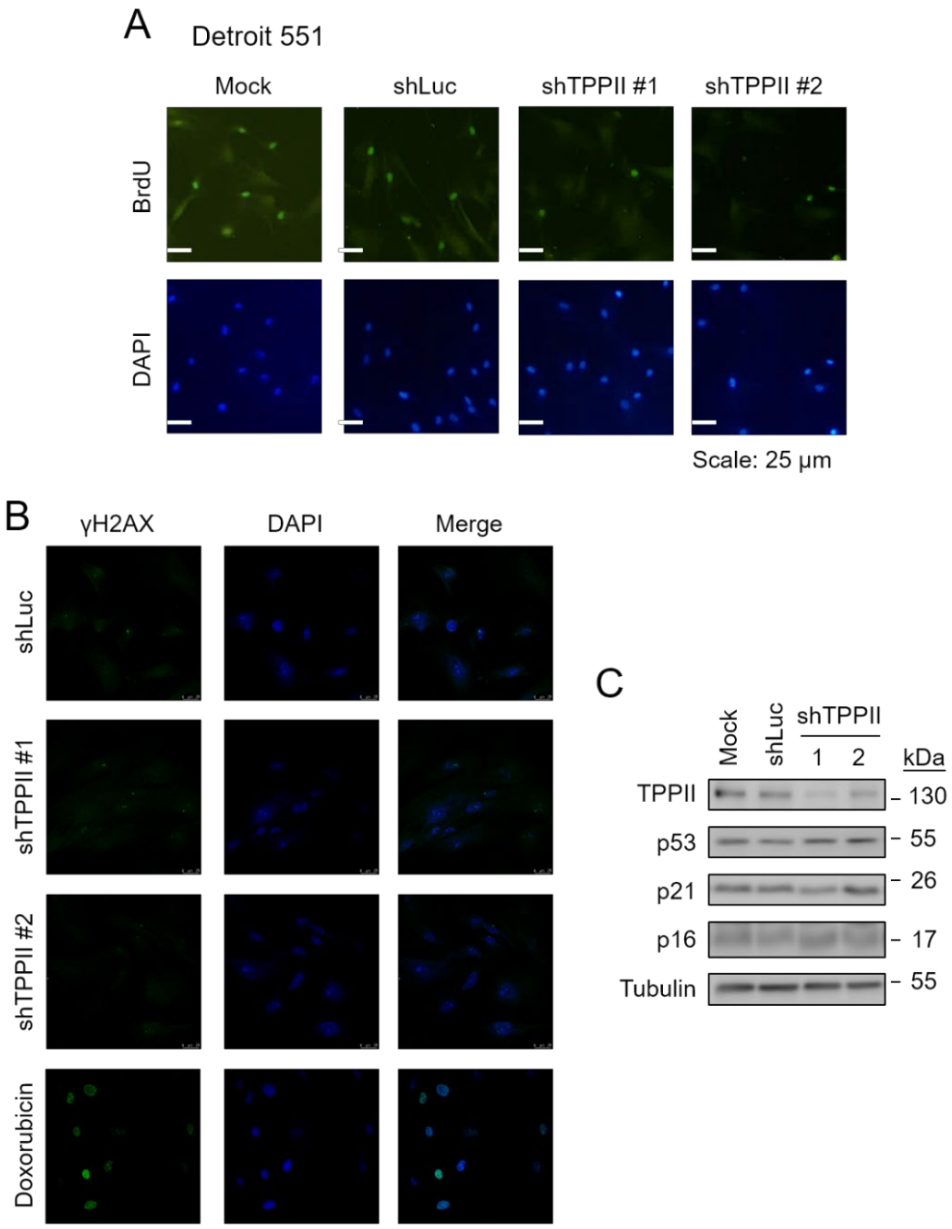

Fig. S5. Detroit551 cells were infected with lentivirus carrying TPPII- or Luciferase-targeting shRNA sequences. (A) Cells were incubated with 40μM BrdU for 16 h, fixed, and then stained with anti-BrdU antibody (green) and DAPI (blue). (B) In another experiment, cells were immunostained with anti-γH2AX antibody. (C) Cell extracts were prepared four days after infection and then analyzed by immunoblots using the indicated antibodies.

Table S1. Serine hydrolases with altered activities in senescence.

Gene	Protein Name	Ratio[1)]
Increased in senescent cells		
ACOT2	Acyl-coenzyme A thioesterase 2	100
PREP	Prolyl endopeptidase	100
LYPLA2	Lysophospholipase II/Acyl-protein thioesterase 2	17.14
ABHD14B	Abhydrolase domain-containing protein 14B	1.98
TPP1	Tripeptidyl-peptidase 1	1.92
ABHD12	Abhydrolase domain-containing 12/Monoacylglycerol lipase	1.79
LACTB	Lactamase Beta/Serine beta-lactamase-like protein LACTB	1.47
SIAE	Sialate O-acetylesterase	1.27
Decreased in senescent cells		
FASN	Fatty acid synthase	0.80
TPP2	Tripeptidyl-peptidase 2	0.78
ACOT1	Acyl-coenzyme A thioesterase 1	0.76
PNPLA6	Patatin like phospholipase domain containing 6/Neuropathy target esterase	0.75
ABHD10	Abhydrolase domain containing 10	0.73
NCEH1	Neutral cholesterol ester hydrolase 1	0.73
PPME1	Protein phosphatase methylesterase 1	0.70
LYPLA1	Lysophospholipase 1/Acyl-protein thioesterase 1	0.58
MGLL	Monoglyceride lipase	0.55
PAFAH1B3	Platelet-activating factor acetylhydrolase 1b catalytic subunit 3	0.52
ESD	Esterase D/Formylglutathione hydrolase	0.50
PAFAH2	Platelet-activating factor acetylhydrolase 2	0.49
uPA	Urokinase-type plasminogen activator	0.01

[1)] Comparison of protein abundance by senescent/young cells; 100 and 0.01 represented the detection of proteins only in senescent and proliferating cells, respectively.