Supplemental Materials

Sulfated Glycosaminoglycans Mediate Prion-like Behavior of p53 Aggregates

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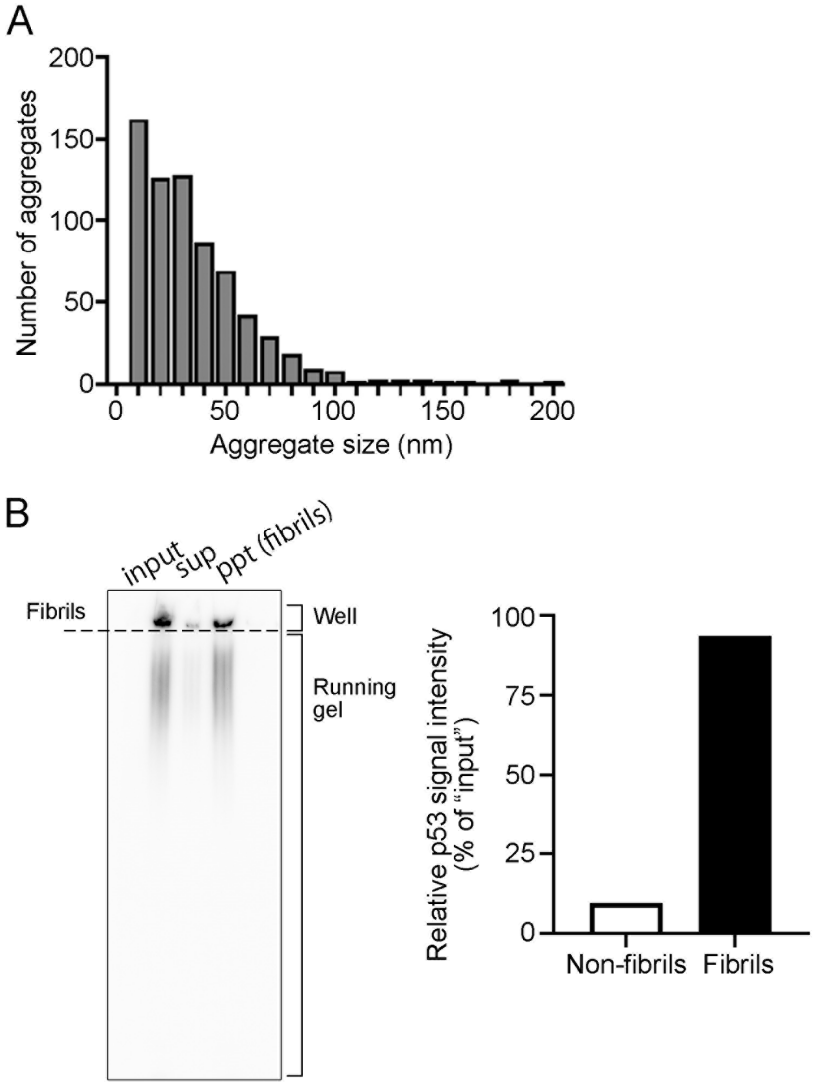


Figure S1. Size distribution and fibril content of the recombinant p53 fibrils.
(A) Size of each assemblies in the atomic force microscopy (AFM) image in Figure 1 was calculated by using ImageJ. (B) Fibril content of the recombinant p53 fibril preparation. Recombinant p53 fibrils were prepared as described in Materials and Methods. One hundred µL of the preparation was centrifuged at 20,000 × g for 1 h at 4 ° C. The supernatant (sup) and pellet (ppt, fibril fraction) were dissolved in 3M urea, and the proteins of each fraction were subjected to native-PAGE with a 5–20 % gel followed by Western blotting with the E26 anti-p53 antibody. The graph shows the percentages of p53 signals of fibrils and non-fibril fractions as determined by densitometric analysis with the Image J software. Some portions of p53 proteins in the supernatant failed to enter the running gel, which indicated that the supernatant contained p53 fibrils that are not precipitated by centrifugation. Taken together, the fibril content of the preparation was determined as 93.7 %.

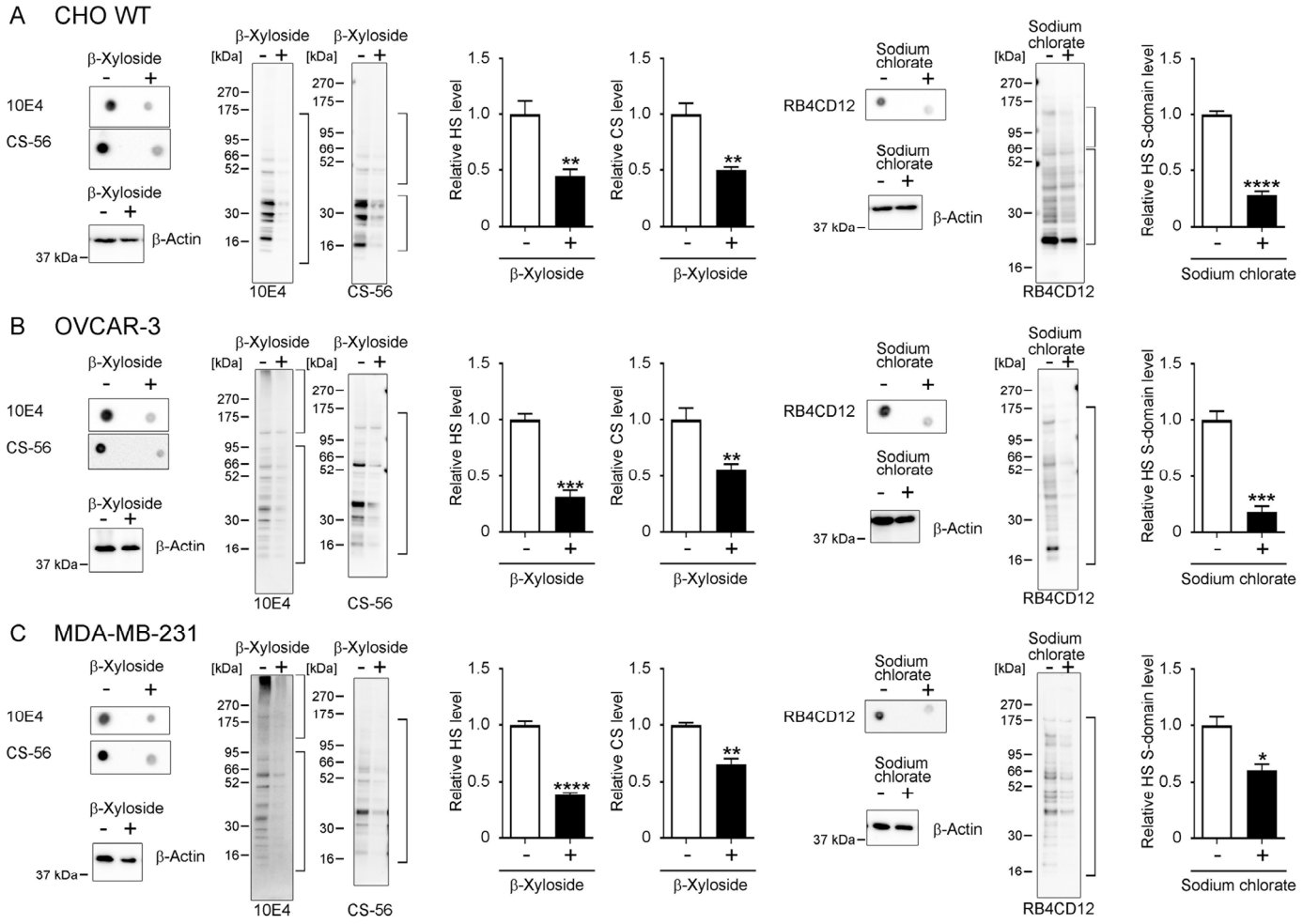
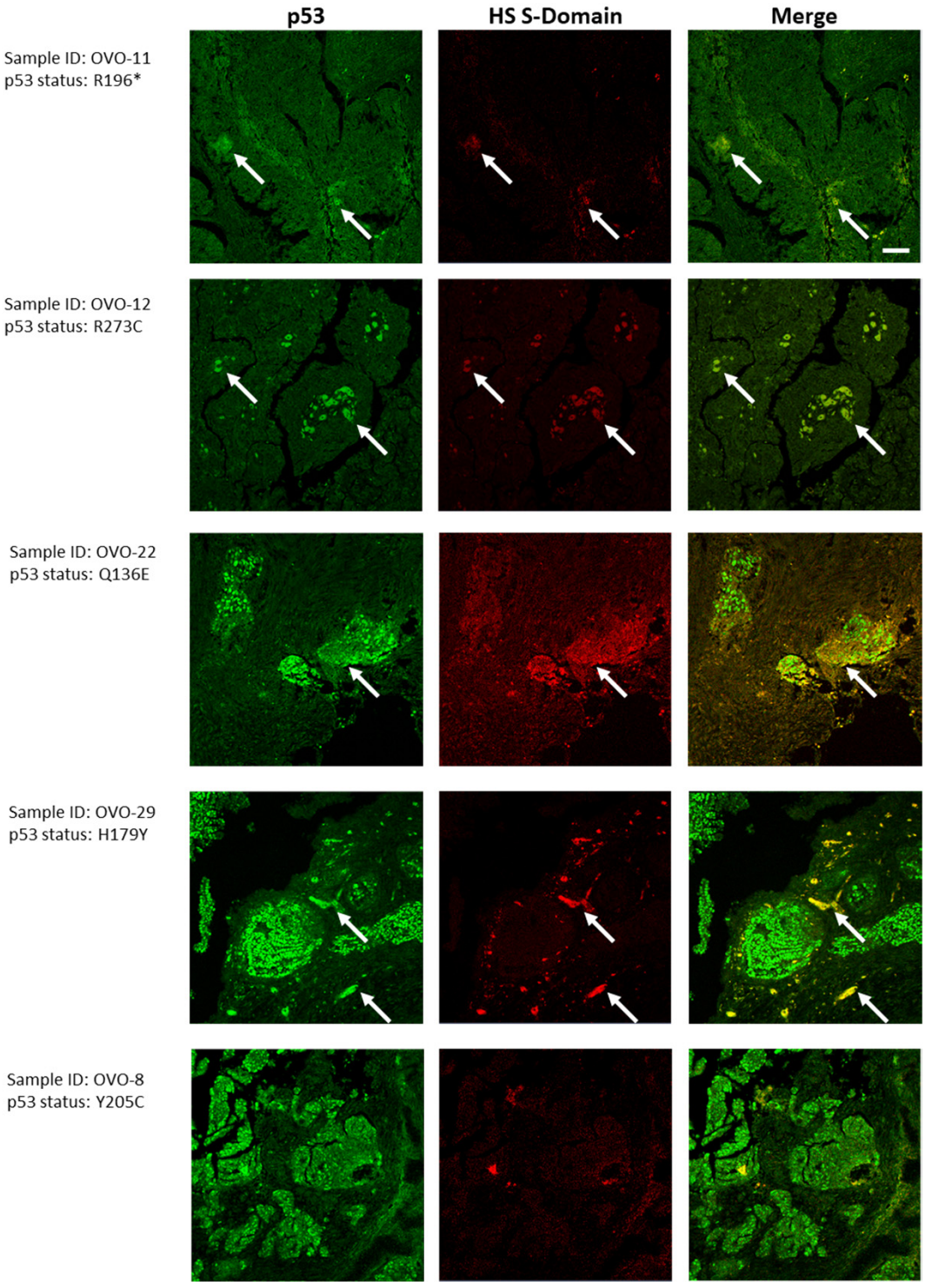


Figure S2. Effects of pretreatment with β-xyloside or sodium chlorate on HS, CS, and HS S-domains.
(A) CHO, (B) OVCAR-3, and (C) MDA-MB-231 cells were cultured and treated with b-xyloside (2.5 mM) or sodium chlorate (100 mM) in OPTI-MEM for 24 h, after which whole cell lysates were prepared by means of TCA precipitation. Aliquots (0.75 µL) were spotted on a nitrocellulose membrane. After being dried, the membrane was incubated with the 10E4 anti-HS antibody, the CS-56 anti-CS antibody, or the RB4CD12 anti-HS S-domain antibody, followed by a horseradish peroxidase-conjugated monoclonal anti-mouse IgM or anti-VSV antibody. The bound antibodies were visualized with ImmunoStar LD and examined by using a LuminoGraph image analyzer. Western blotting was performed for the whole cell lysates. Brackets show notable band intensities different between samples with or without treatment. ß-Actin was used as a loading control. Immunoreactivities against HS, CS, and HS S-domains were quantitated by dot blotting.


p53
HS S-Domain
Merge
Sample ID: OVO-11
p53 status: R196*
Sample ID: OVO-12
p53 status: R273C
Sample ID: OVO-22
p53 status: Q136E
Sample ID: OVO-29
p53 status: H179Y
Sample ID: OVO-8
p53 status: Y205C

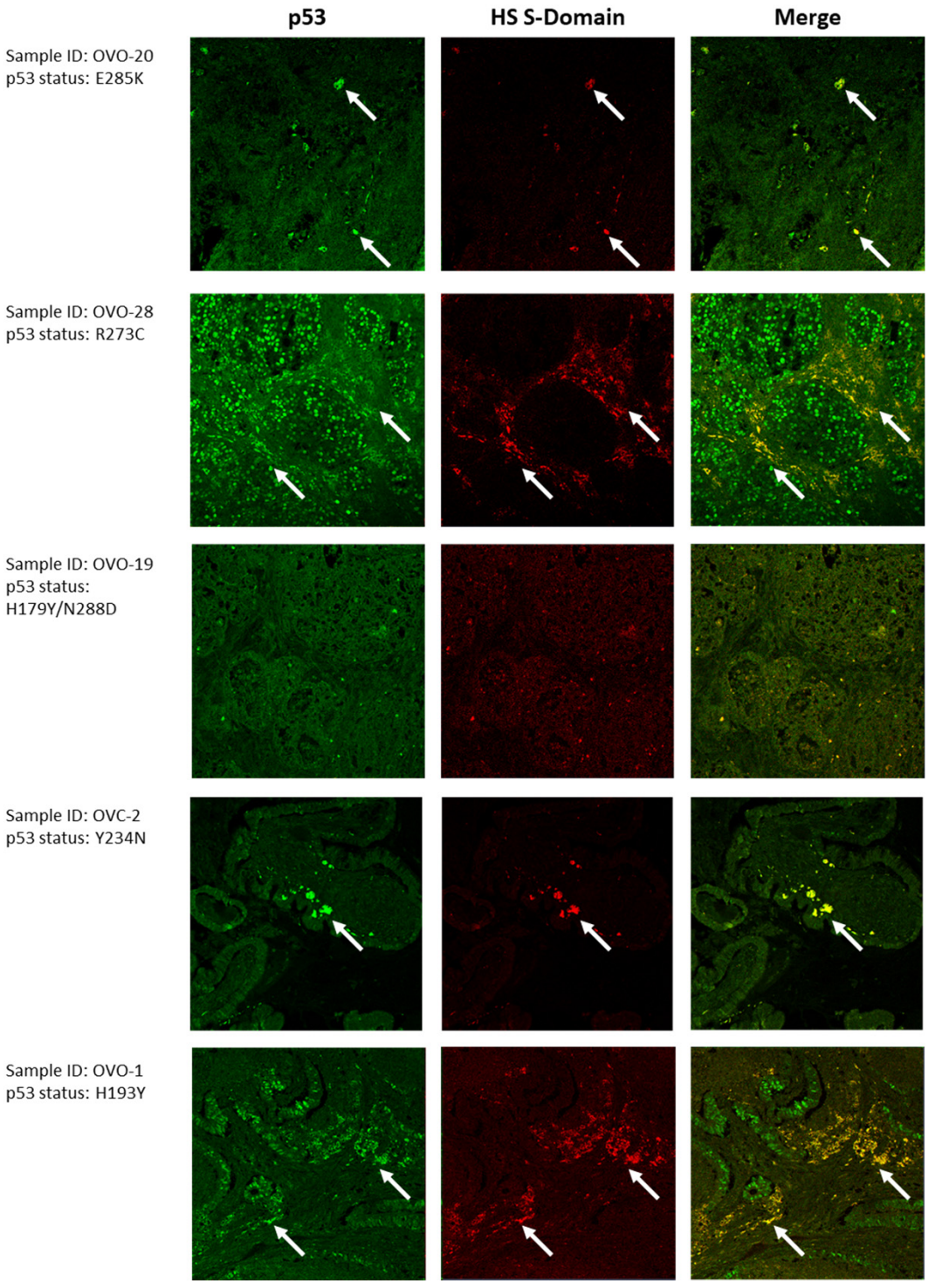


Figure S3. p53 deposits showed some positive results for RB4CD12 HS S-domain staining.
Sections of ovarian cancer tissues were stained with the RB4CD12 anti-HS S-domain antibody (red) and the DO-1 anti-p53 antibody (green). Arrows indicate co-localization of p53 deposits and HS S-domains. Scale bar, 50 µm.

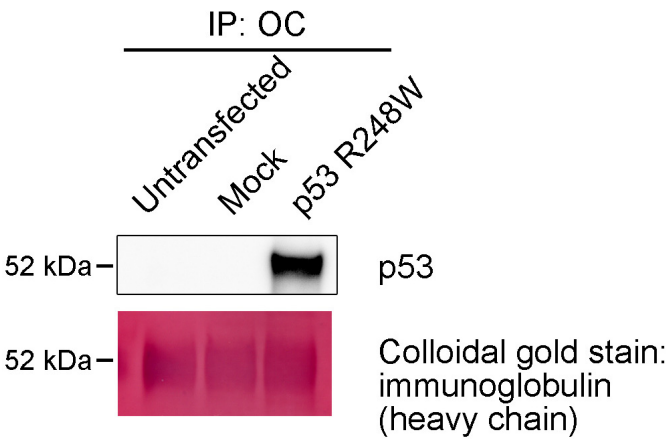


Figure S4. p53 R248W-transfectant PC-3 cells extracellularly released OC-positive p53 aggregates.
p53-null PC-3 cells were transfected with pCMV-Neo-Bam (mock) or pCMV-Neo-Bam p53 R248W. After 24 h from the transfection, culture media were collected and protein aggregates were immunoprecipitated with the OC anti-amyloid antibody. p53 protein levels in the immunoprecipitates were analyzed by means of Western blotting with the DO-1 anti-p53 antibody.