Full images of gels in Manuscript SC-EDG-09-2024-006493

1. PAGE analysis of the formation of UBLE.

At first, we used PAGE analysis to confirm the formation of the UBLE as shown in Fig. 2b. The unmodified raw images are provided and marked with a red wireframe below.

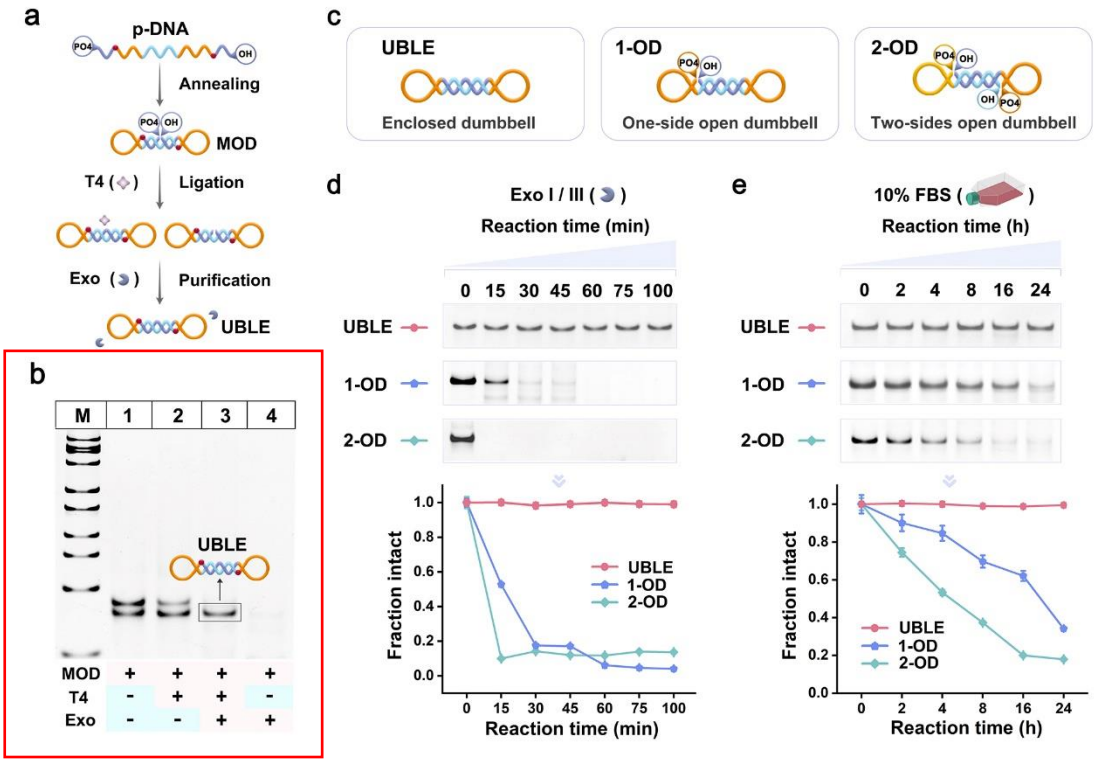
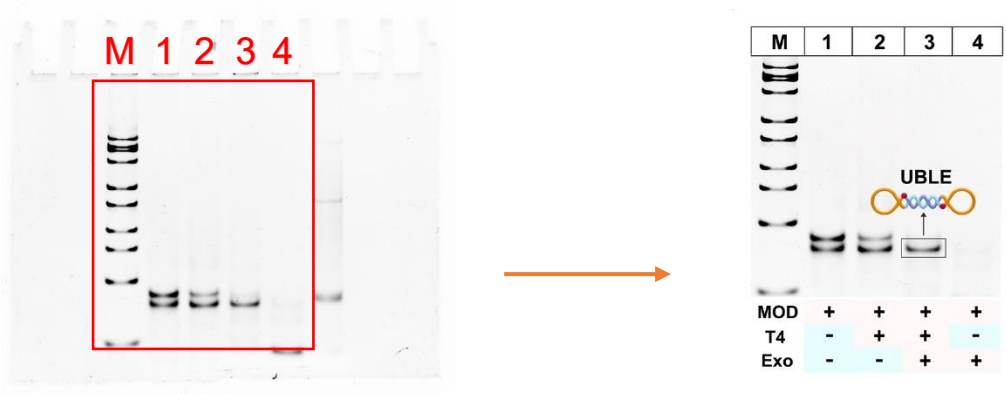


Fig. 2 Preparation and biostability characterization of UBLE. (a) Schematic illustration of the formation of UBLE through ligation by T4 and purification with Exo I/III. (b) PAGE analysis of the formation of UBLE. Lane 1, MOD; Lane 2, MOD + T4; Lane 3, MOD + T4 + Exo I/III (equal to UBLE); Lane 4, MOD + Exo I/III. (c) Schematic design of UBLE (without open sides) and two dumbbell probes including 1-OD (with one open side) and 2-OD (with two open sides). (d, e) Resistance comparison of UBLE, 1-OD, and 2-OD treated with Exo I/III (d) and 10% FBS (e) at different time points, respectively.

Fig. 2b: PAGE analysis of the formation of UBLE.

Raw image **Used data**



The left image is the raw image of the formation of UBLE and the right image is glorified and used in our manuscript. It can be seen that there is a residue of Lane 4 in the raw image (left), we speculate that it is a residual product after the action of exonuclease. According to the results of Lanes 3 and 4, it had no significant impact on the experimental results. Therefore, considering the simplification, we only presented data to illustrate the disappearance of MOD. If possible, we can replace the complete experimental results in the manuscript.

2. PAGE analysis of the resistance ability of UBLE in Exo I/III

We used PAGE analysis to evaluate the resistance ability of the UBLE, 1-OD, and 2-OD in Exo I/III as shown in Fig. 2d. The unmodified raw images are provided and marked with a red wireframe below.

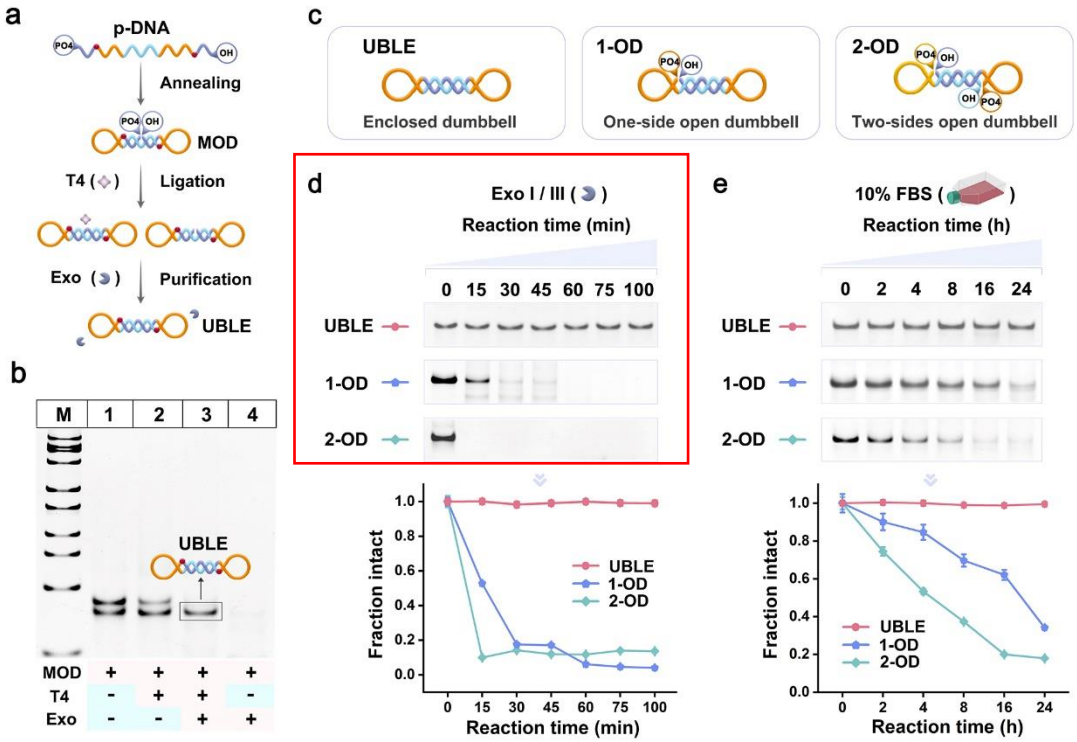


Fig. 2 Preparation and biostability characterization of UBLE. (a) Schematic illustration of the formation of UBLE through ligation by T4 and purification with Exo I/III. (b) PAGE analysis of the formation of
UBLE. Lane 1, MOD; Lane 2, MOD + T4; Lane 3, MOD + T4 + Exo I/III (equal to UBLE); Lane 4, MOD + Exo I/III. (c) Schematic design of UBLE (without open sides) and two dumbbell probes including 1-OD (with one open side) and 2-OD (with two open sides). (d, e) Resistance comparison of UBLE, 1-OD, and 2-OD treated with Exo I/III (d) and 10% FBS (e) at different time points, respectively.

Fig. 2d: Resistance comparison of different probes in Exo I/III.

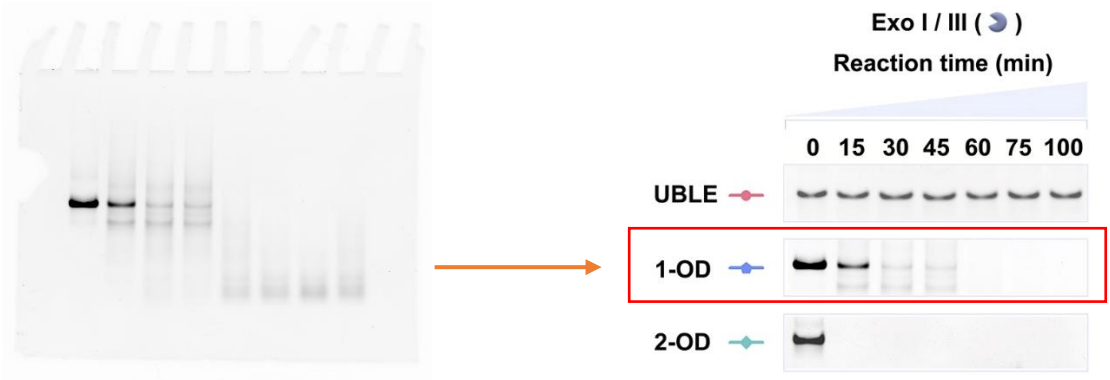
UBLE:

Raw image **Used data**

The left image is the raw image of the resistance study of UBLE in Exo I/III and the right image is glorified and used in our manuscript.

1-OD:

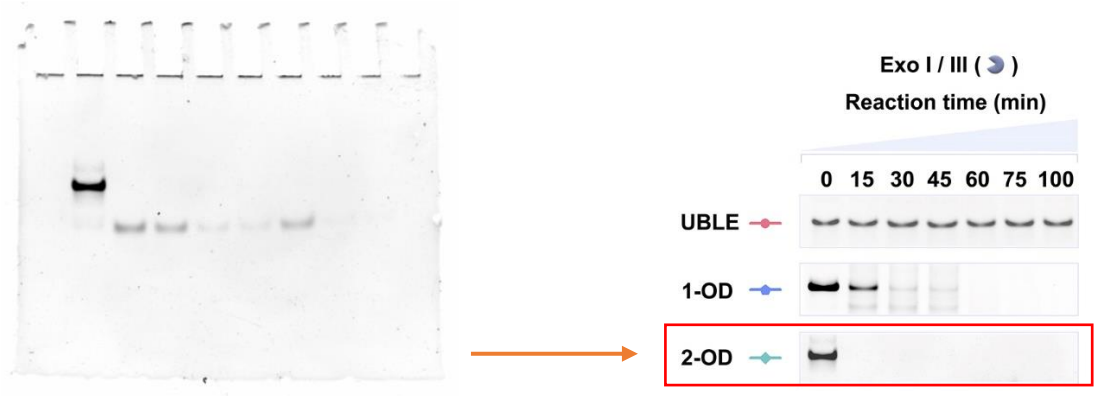
Raw image **Used data**



The left image is the raw image of the resistance study of 1-OD in Exo I/III and the right image is glorified and used in our manuscript.

2-OD:

Raw image **Used data**



The left image is the raw image of the resistance study of 2-OD in Exo I/III and the right image is glorified and used in our manuscript.

3. PAGE analysis of the resistance ability of UBLE in FBS

We used PAGE analysis to evaluate the resistance ability of the UBLE, 1-OD, and 2-OD in FBS as shown in Fig. 2e. The unmodified raw images are provided and marked with a red wireframe below.

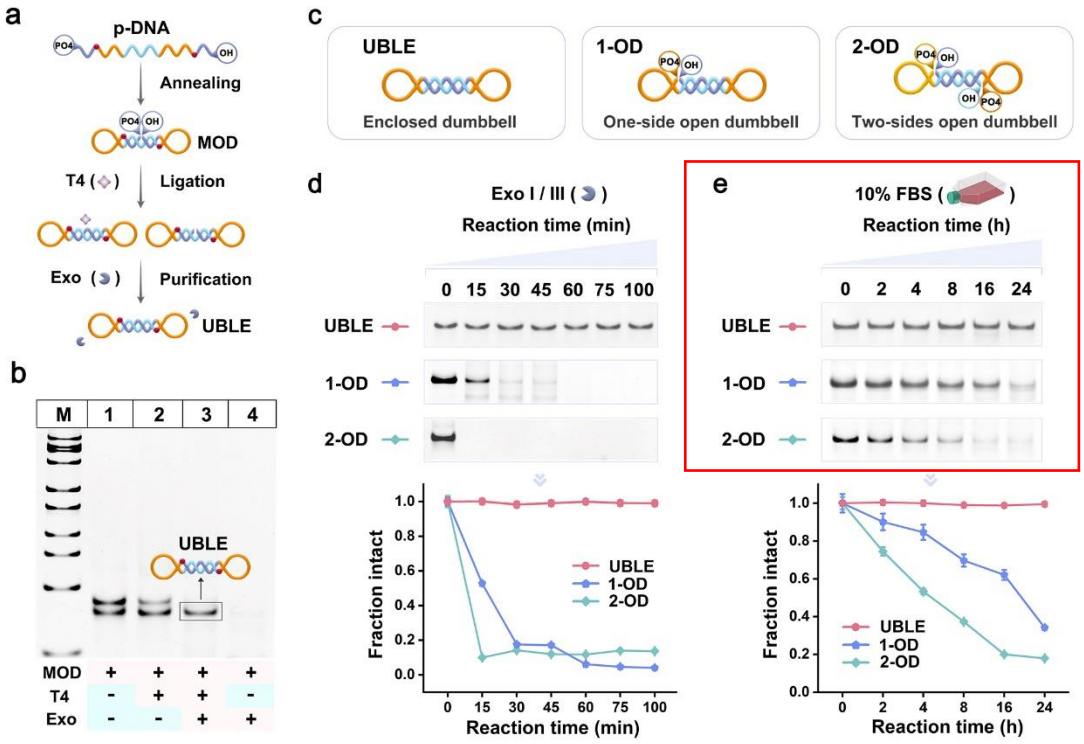
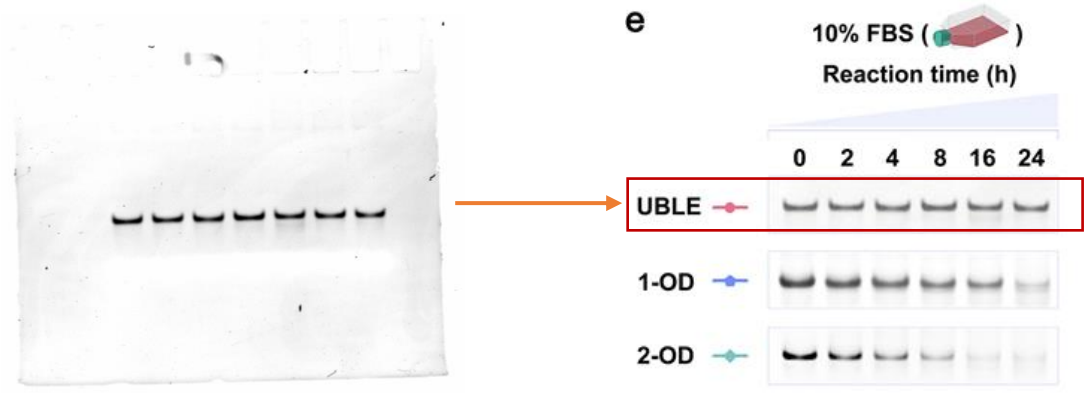


Fig. 2 Preparation and biostability characterization of UBLE. (a) Schematic illustration of the formation of UBLE through ligation by T4 and purification with Exo I/III. (b) PAGE analysis of the formation of UBLE. Lane 1, MOD; Lane 2, MOD + T4; Lane 3, MOD + T4 + Exo I/III (equal to UBLE); Lane 4, MOD + Exo I/III. (c) Schematic design of UBLE (without open sides) and two dumbbell probes including 1-OD (with one open side) and 2-OD (with two open sides). (d, e) Resistance comparison of UBLE, 1-OD, and 2-OD treated with Exo I/III (d) and 10% FBS (e) at different time points, respectively.

Fig. 2e: Resistance comparison of different probes in FBS.

UBLE:

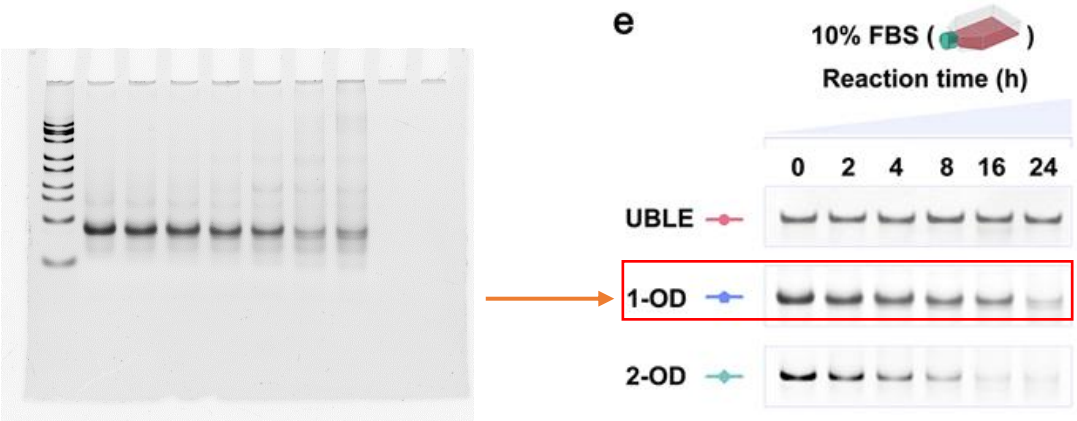
Raw image **Used data**



The left image is the raw image of the resistance study of UBLE in FBS and the right image is glorified and used in our manuscript.

1-OD:

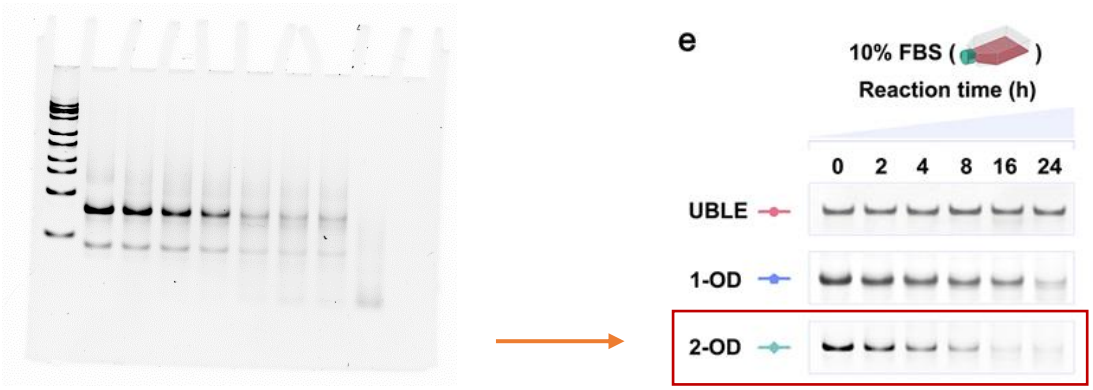
Raw image **Used data**



The left image is the raw image of the resistance study of 1-OD in FBS and the right image is glorified and used in our manuscript.

2-OD:

Raw image **Used data**



The left image is the raw image of the resistance study of 2-OD in FBS and the right image is glorified and used in our manuscript.

4. PAGE analysis of the self-assembly of UBLE.

We performed PAGE analysis to investigate the activation of UBLE for long dsDNA self-assembly in Fig. 3b. The unmodified raw images are provided and marked with a red wireframe below.

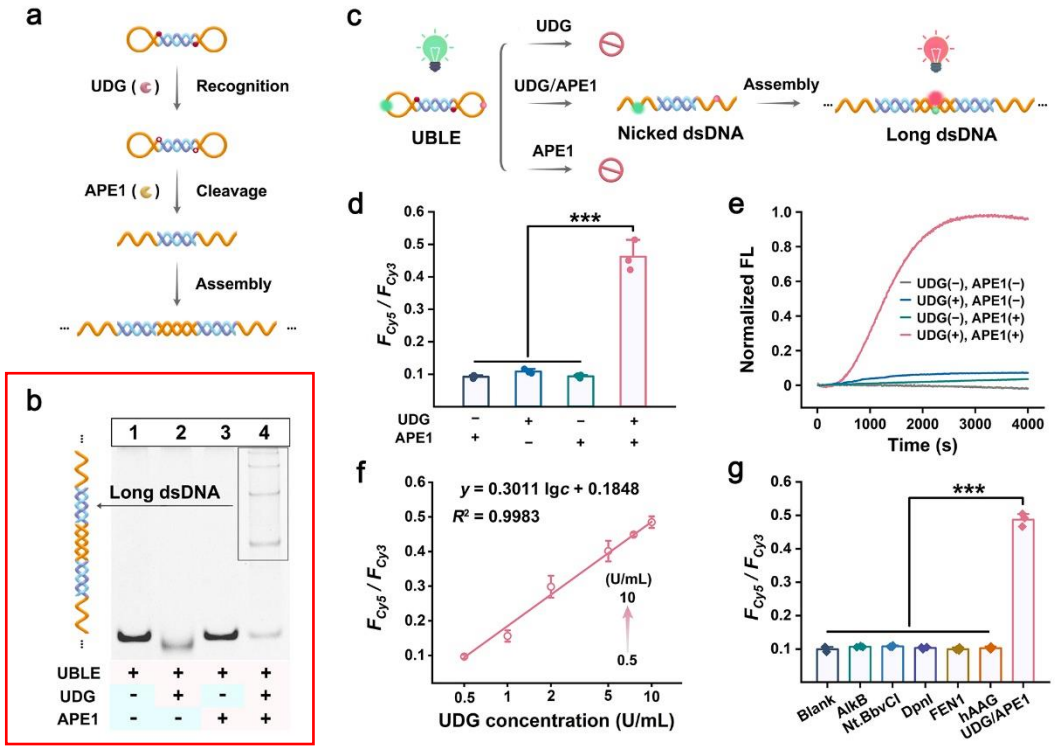
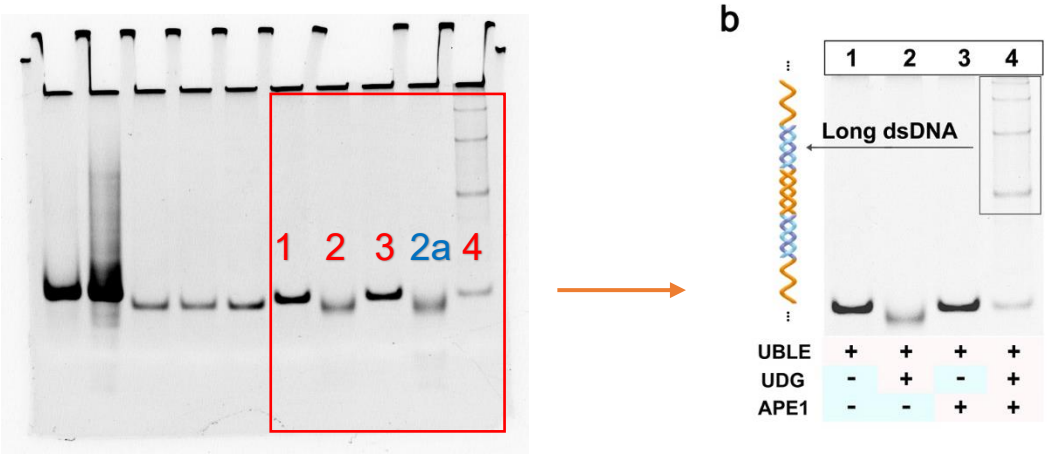


Fig. 3 Selective activation of UBLE by repair enzymes of UDG and APE1. (a) Schematic illustrating the orthogonal recognition of UDG and APE1 on UBLE to trigger long dsDNA self-assembly. (b) PAGE analysis of long dsDNA formation by DNA lesion-gated self-assembly of UBLE. Lane 1, UBLE; Lane 2, UBLE + UDG; Lane 3, UBLE + APE1; Lane 4, UBLE + UDG + APE1. (c) Schematic illustration of the FRET response to DNA lesion-gated long dsDNA formation. (d, e) FRET response (d) and kinetic analysis (e) of UBLE in the presence of UDG and APE1 alone or together. (f) Calibration curves for quantifying different concentrations of UDG on UBLE (where the concentration of APE1 is consistent with UDG concentration). (g) FRET response analysis of UBLE in response to various interfering enzymes.

Fig. 3b: PAGE analysis of the self-assembly of UBLE

Raw image **Used data**



The left image is the raw image of the self-assembly of UBLE and the right image is glorified and used in our manuscript. It could be found that a redundant lane (Lane 2a) appears in the raw image. In fact, Lane 2a is a duplicate band of Lane 2 because of the repeat addition. We thought this would not interfere with the experimental results, so, we discarded the Lane 2a for data analysis in order to simplify the data. This doesn't seem rigorous. If possible, we can provide a more rigorous experimental results in the manuscript.

Full images of gels in Supplementary information

Additionally, we also performed PAGE analysis to evaluate the resistance ability of the UBLE compared to different hairpins (SP and HP) in Exo I/III and FBS respectively in the supplementary information (Fig. S5). The unmodified raw images are provided and marked with a red wireframe below.

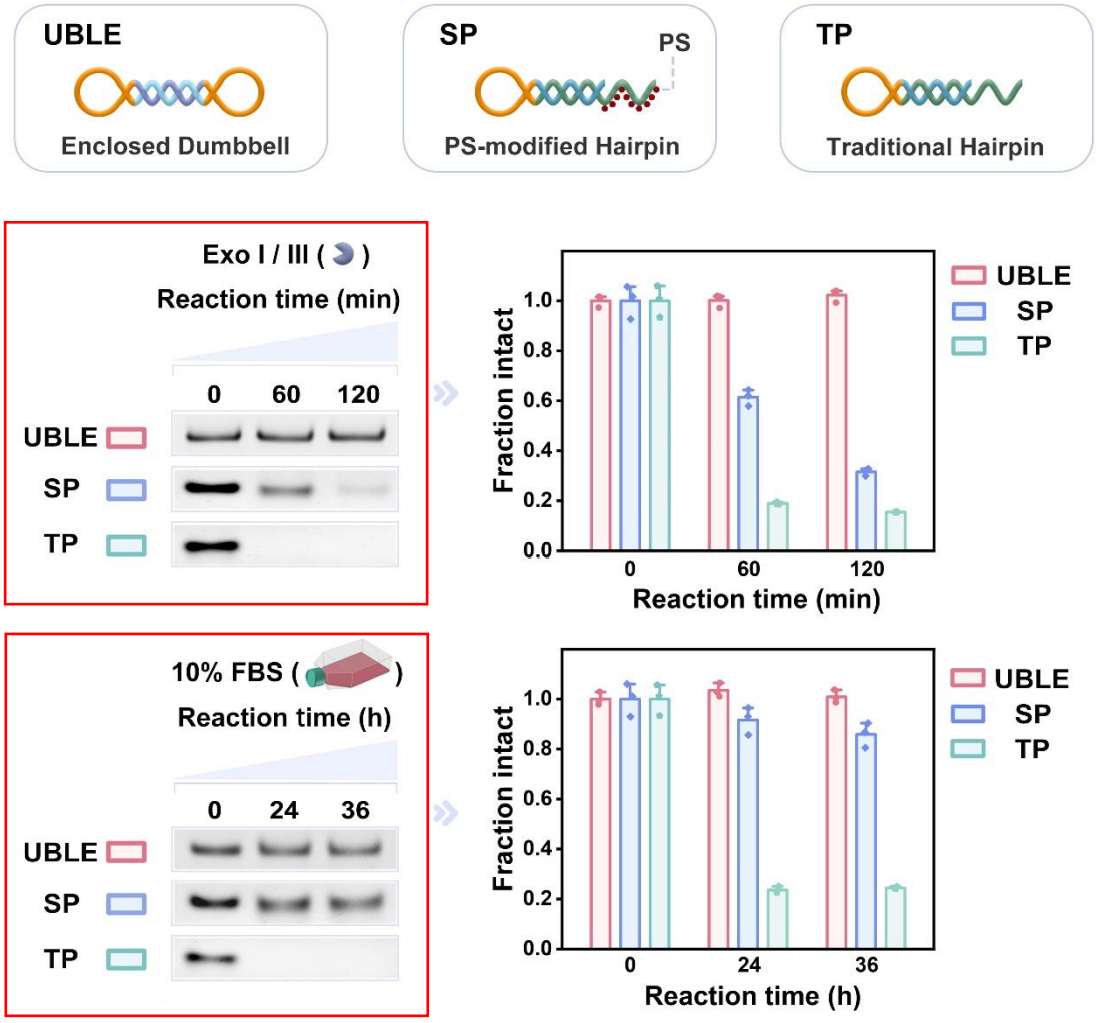


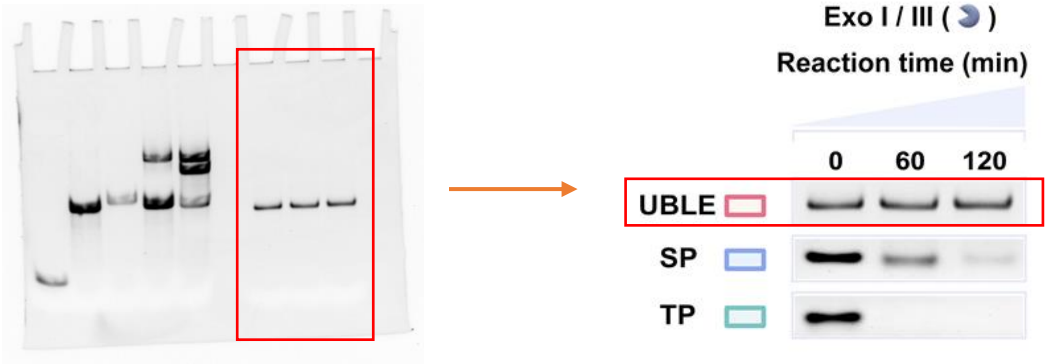
Fig. S5. Resistance comparison of UBLE, SP, and TP by treated with Exo I/III or 10% FBS at different time points.

Fig. S5: Resistance comparison in Exo I/III or FBS.

(1) in Exo I/III:

UBLE:

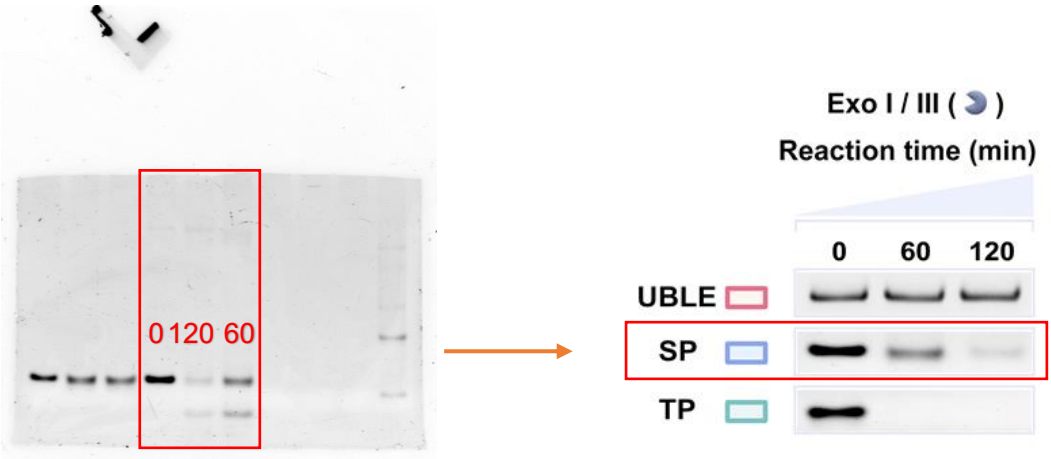
Raw image **Used data**



The left image is the raw image of the resistance of UBLE in Exo I/III and the right image is glorified and used in our manuscript.

SP:

Raw image **Used data**

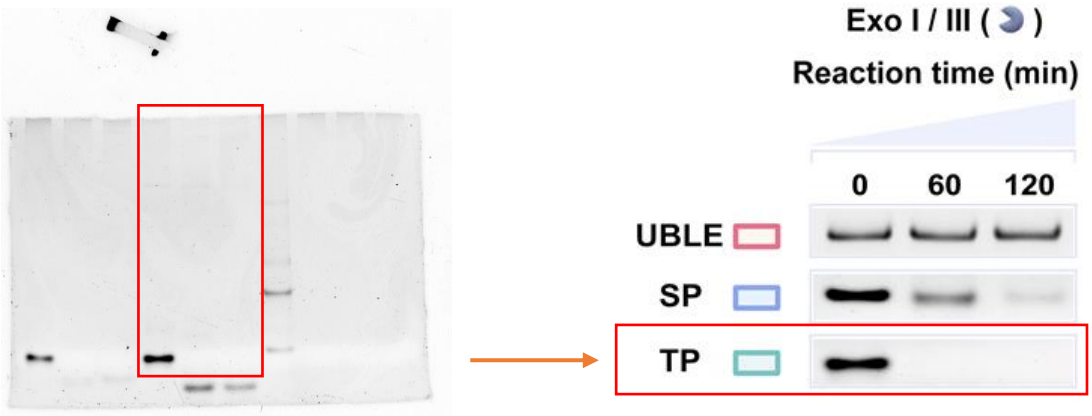


The left image is the raw image of the resistance of SP in Exo I/III and the right image is glorified and used in our manuscript. However, there is a sequential-exchange in the experimental results from raw

image. After careful consideration of the experimental results, we think it does not affect the conclusion of the experiment. So, we only change its order in our used data for more intuitive analysis, and do not conduct secondary experiment. This is due to our inadequate thinking and we will be more cautious in the subsequent process.

TP:

Raw image **Used data**

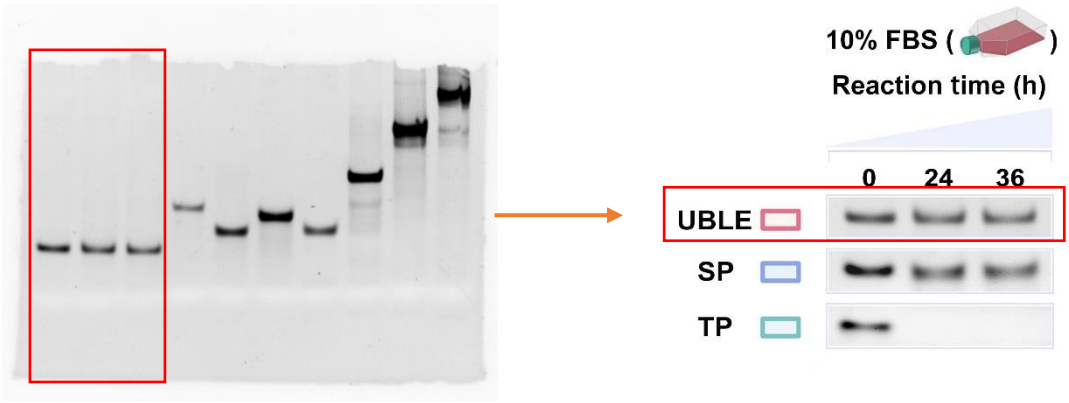


The left image is the raw image of the resistance of TP in Exo I/III and the right image is glorified and used in our manuscript.

(2) In FBS:

UBLE:

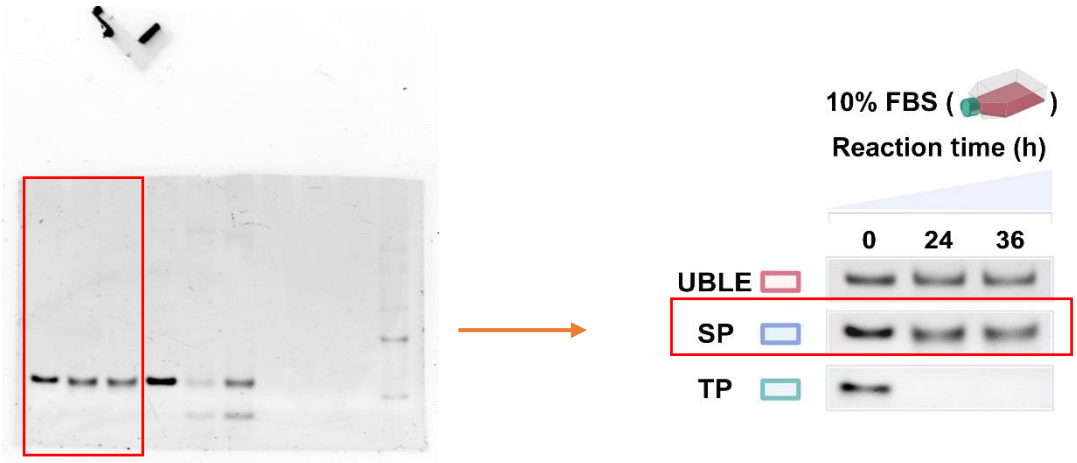
Raw image **Used data**



The left image is the raw image of the resistance of UBLE in FBS and the right image is glorified and used in our manuscript.

SP:

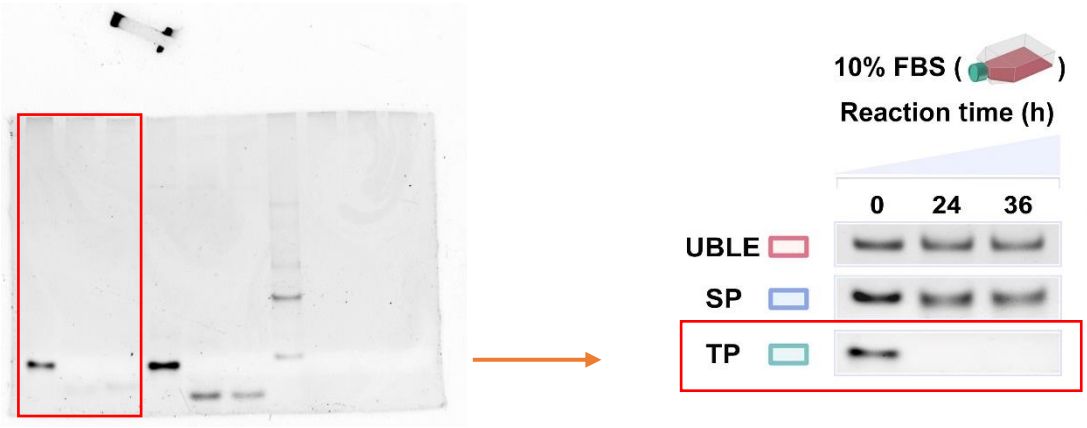
Raw image **Used data**



The left image is the raw image of the resistance of SP in FBS and the right image is glorified and used in our manuscript.

TP:

Raw image **Used data**



The left image is the raw image of the resistance of TP in FBS and the right image is glorified and used in our manuscript.