Supporting Information

Intracellular protein delivery system using a target-specific repebody and translocation domain of bacterial exotoxin

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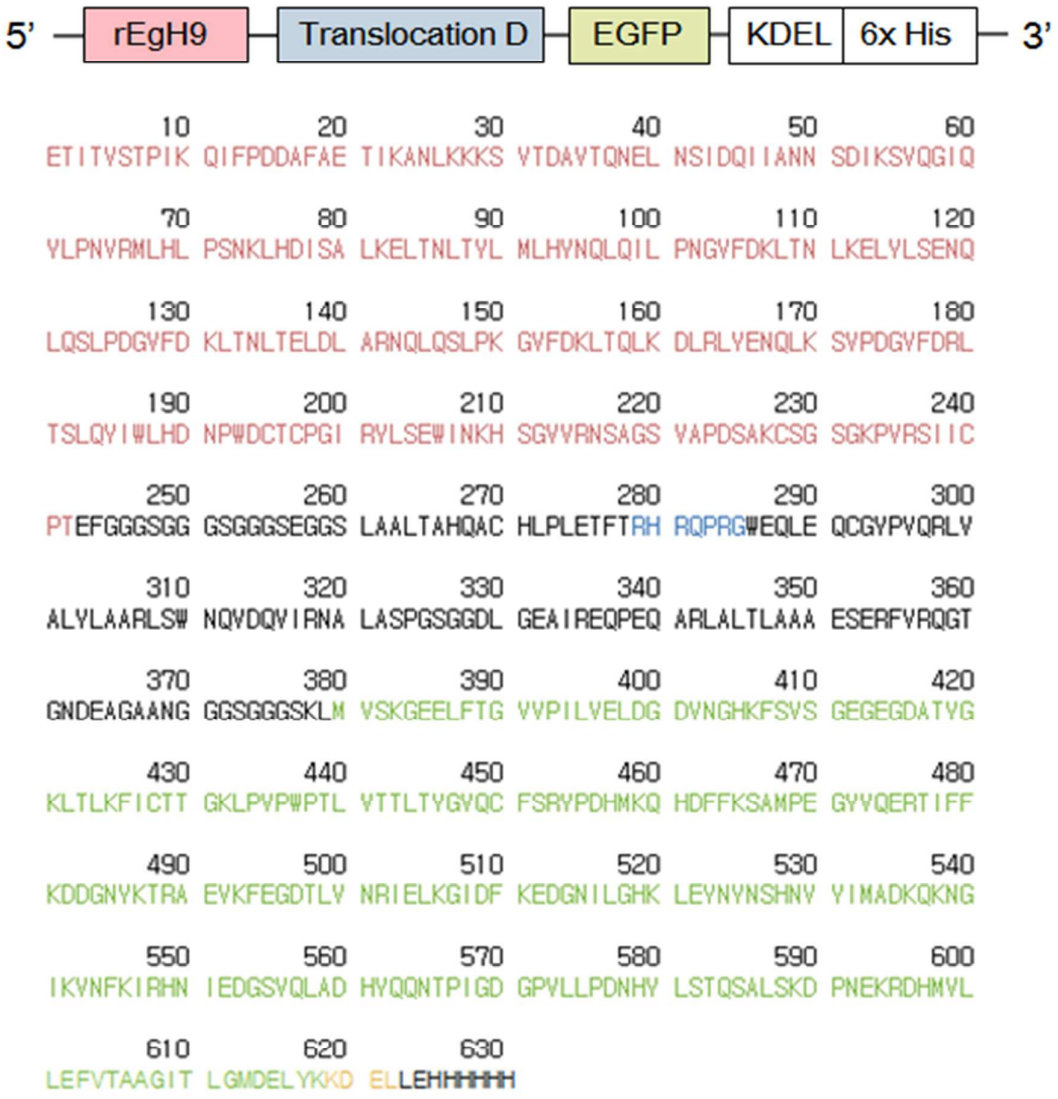
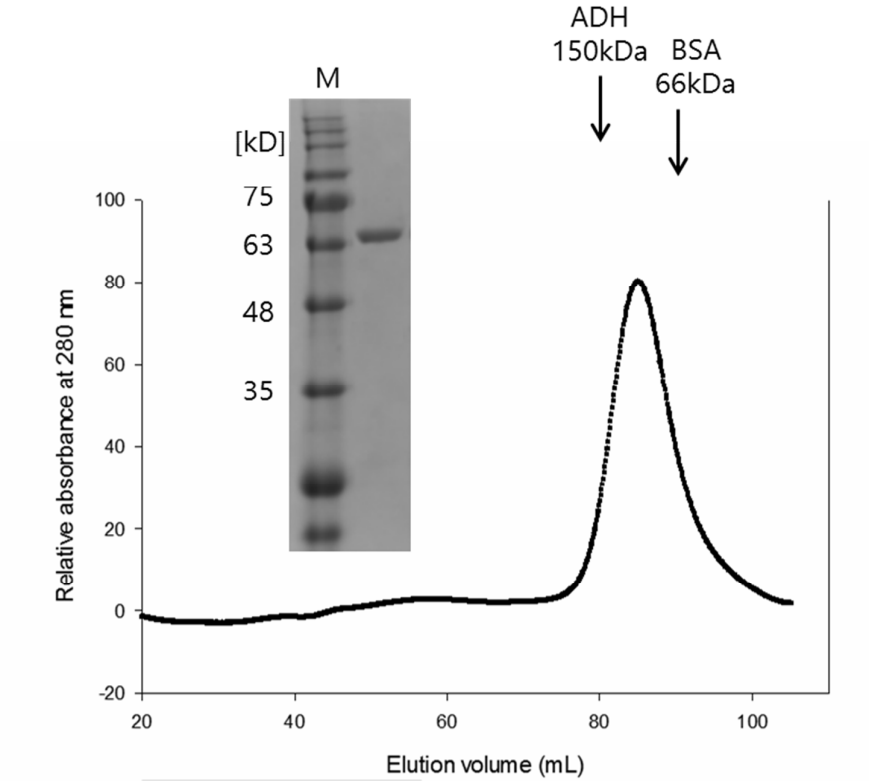
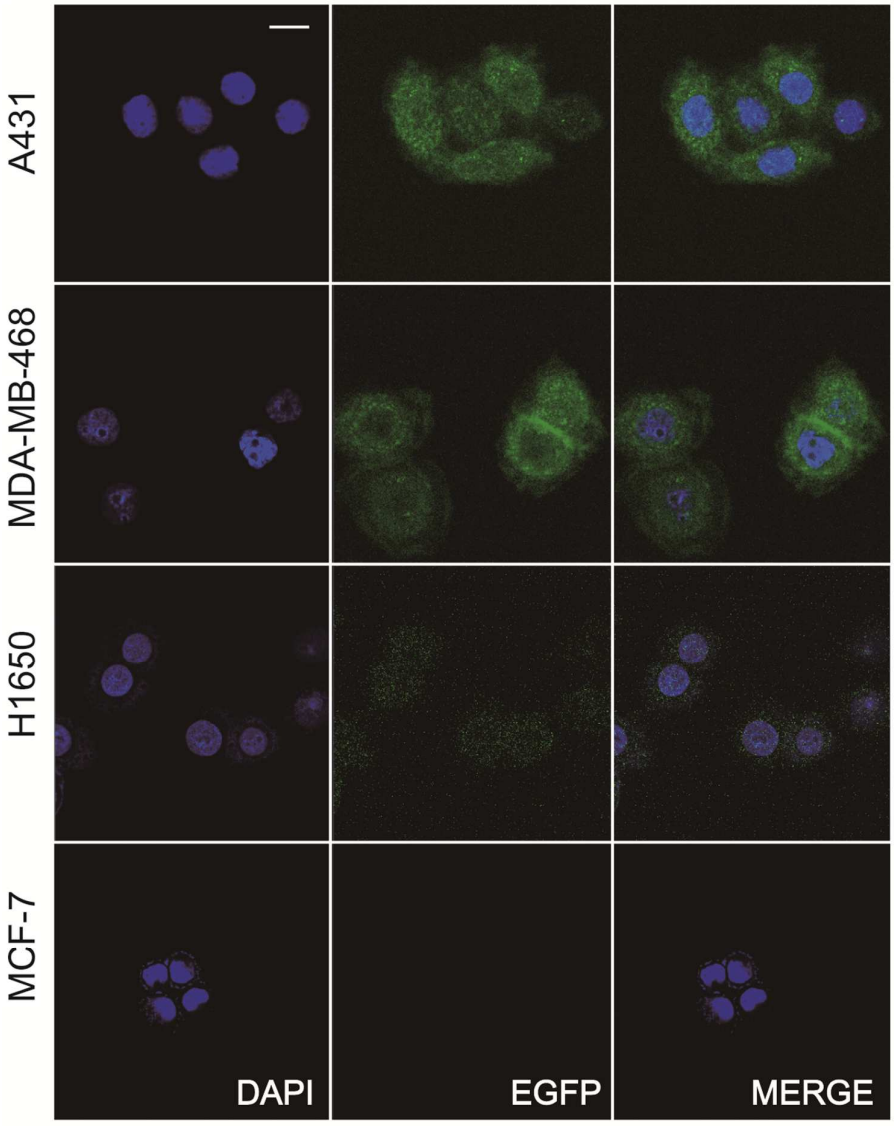


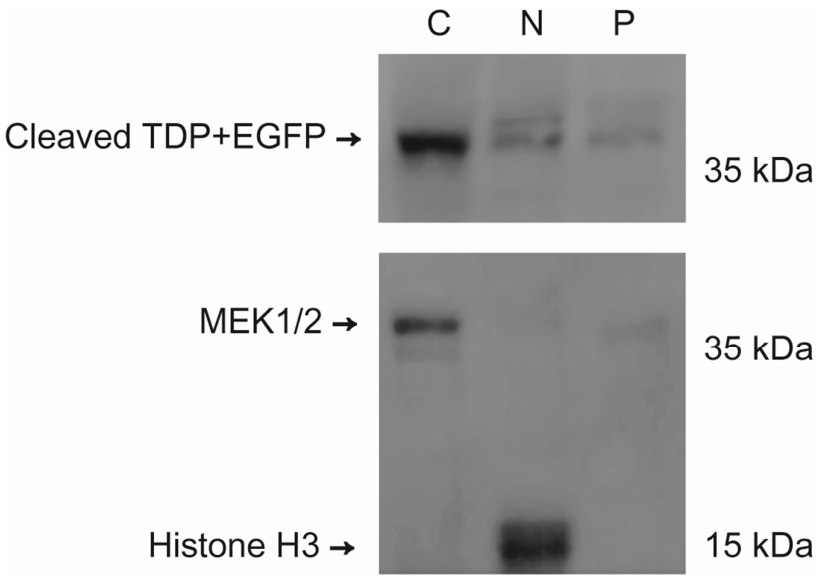
Supplementary Figure S1. Schematic representation and the complete amino acid sequence of Rb-TDP-EGFP. His-tag was added to the C-terminus for purification. The construct was cloned into pET21a vector using NdeI and XhoI enzyme sites.



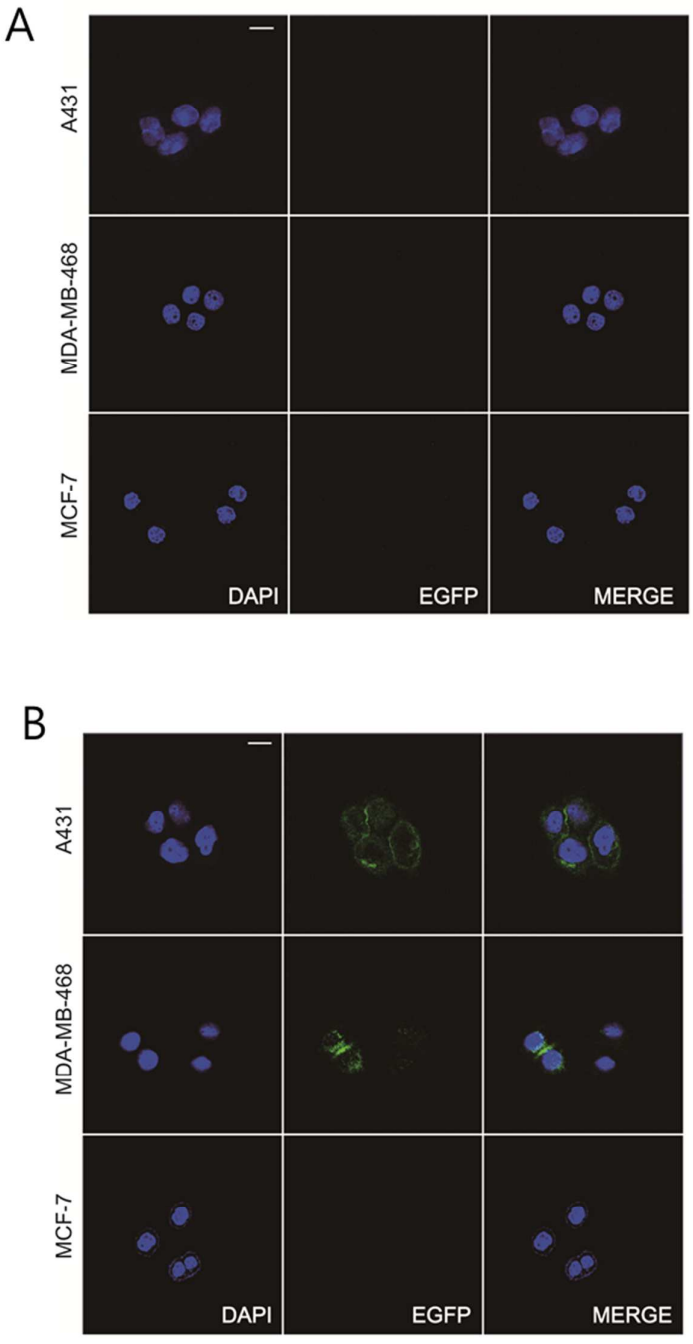
Supplementary Figure S2. **Size-exclusion chromatograms and SDS-PAGE analysis for Rb-TDP-EGFP**. Elution profile of Rb-TDP-EGFP was analyzed by size-exclusion chromatography. The protein was loaded on a Superdex 200 column and the elution profiles were traced by the relative absorbance at 280 nm. The determined molar masses are given by the horizontal traces at the corresponding protein peaks. Arrows indicate the elution volumes of the standard marker proteins. ADH, alcohol dehydrogenase (150 kDa); BSA, bovine serum albumin (66 kDa). M stands for a marker.



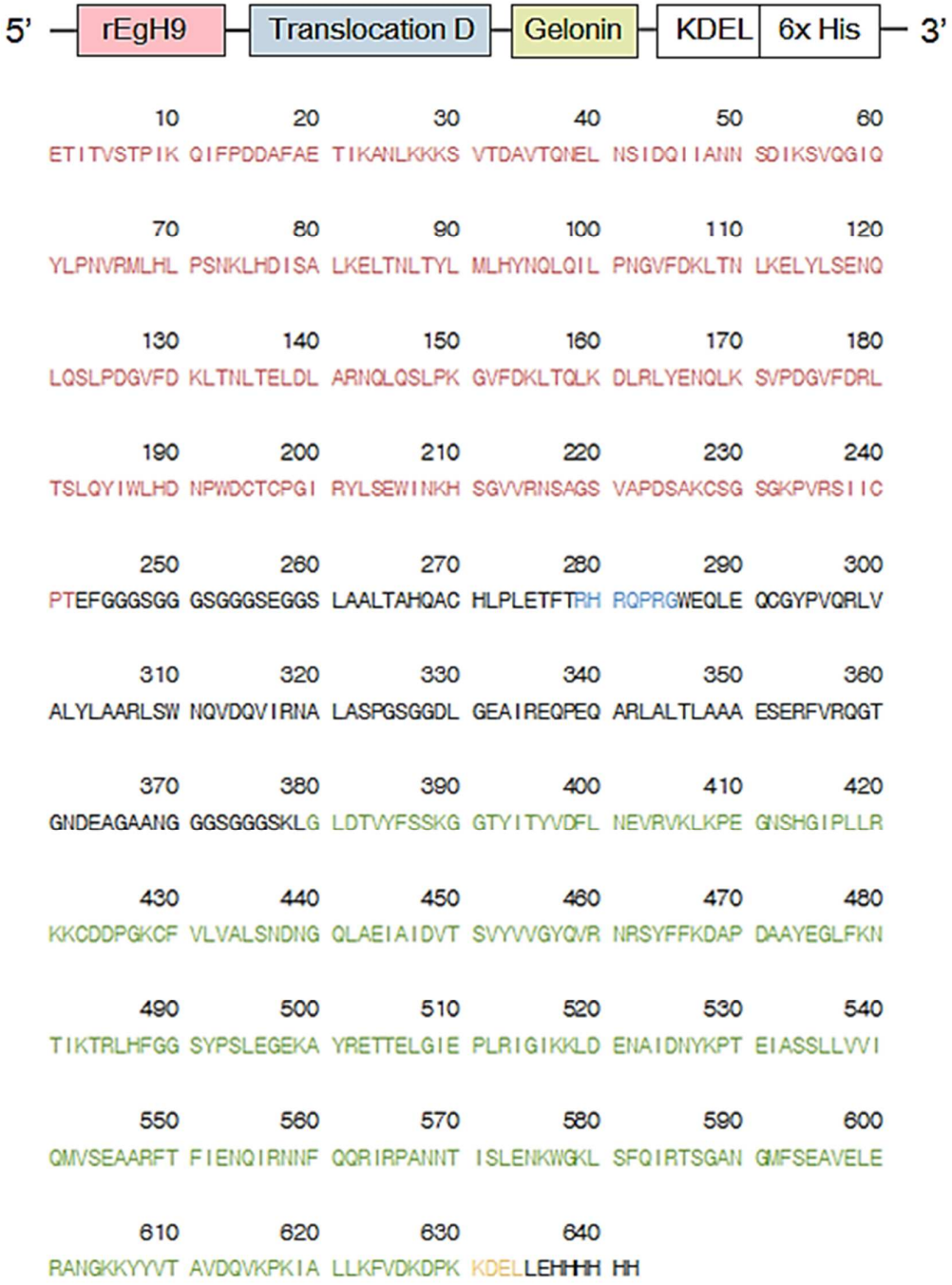
Supplementary Figure S3. Receptor-specific intracellular delivery of EGFP. A431, MDA-MB-468 and H1650 cells were incubated with 50 nM of Rb-TDP-EGFP for 6 hr. Scale bar = 20 μm.



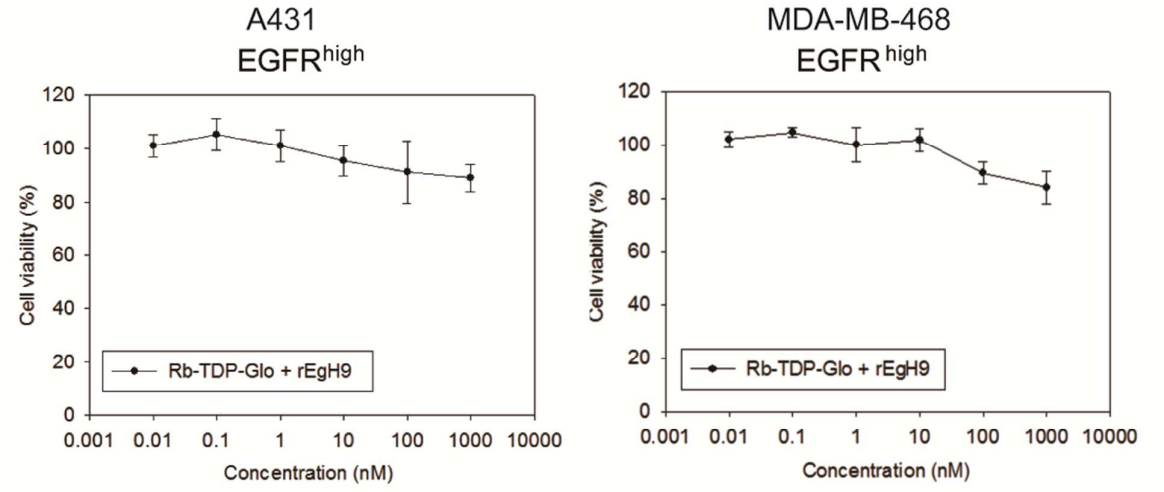
Supplementary Figure S4. Subcellular fractionation of the cells after treatment with Rb-TDP-EGFP. A431 cells were treated with 1 μM of Rb-TDP-EGFP for 6 hr followed by subcellular fractionation and western blot analysis using corresponding antibodies. MEK1/2 and Histone H3 were used as cytosolic and nucleus markers, respectively. C, cytosolic fraction; N, nuclear fraction; P, pellet.



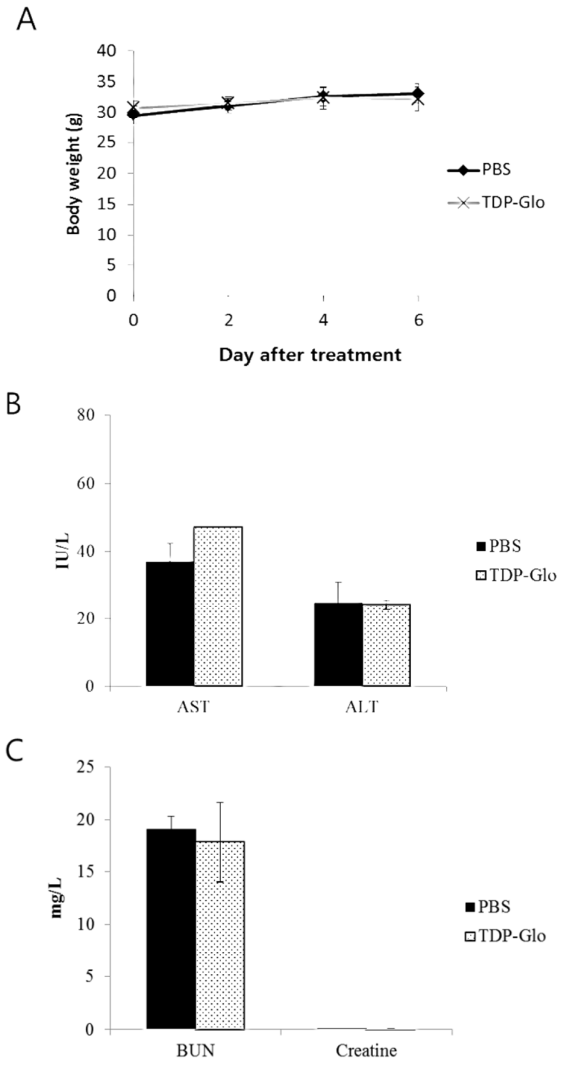
Supplementary Figure S5. Translocation of EGFP using the delivery platform with off-target repebody or without translocation domain. (A) Delivery platform with off-target repebody (Rb_{off}-TDP-EGFP). (B) Delivery platform without translocation domain (Rb-EGFP). A431, MDA-MB-468 and MCF-7 cells were incubated with 1 μM of each delivery platform for 6 hr followed by fluorescence imaging using confocal microscope. Scale bar = 20 μm.



Supplementary Figure S6. Schematic representation and the complete amino acid sequence of Repebody-TDP-Glo. His-tag was added to the C-terminus for purification. The construct was cloned into pET21a vector using NdeI and XhoI enzyme sites.



Supplementary Figure S7. Receptor-specific cytotoxicity of Rb-TDP-Glo. Pre-incubation with free EGFR-specific repebody (1 μM) prevented the cytotoxicity of gelonin in EGFR-positive cell lines.



Supplementary Figure S8. ***In vivo*** **toxicity of TDP-Glo.** (A) Change in body weight of mice after injection of TDP-Glo. PBS was used as a control. (B) Hepatotoxicity and (C) nephrotoxicity of TDP-Glo. Changes in the levels of aspartate and alanine aminotransferase (ALT and AST), creatine and blood urea nitrogen (BUN) were assayed on day 7 after injection of TDP-Glo. Each mouse was injected with 100 μl of 27 μM TDP-Glo through the tail vein Differences in the mean hepatotoxicity and nephrotoxicity of each group were not significant. Values are presented as means ± S.D. of 4 mice per group.

Supplementary methods

In vitro cytotoxicity

Respective cells were seeded in a 96-well plate (SPL) at a density of 5×10^3 cells/well, and the medium was changed to a serum-free medium containing each proteins. Cells were pre-incubated with free repebody (rEgH9, 1 μM) for 4 h before treatment with repebody-TDP-Gelonin. Different concentrations of the repebody-TDP-Gelonin were added to each well, followed by incubation for 3 days, and the serum-free medium supplemented with a 10% (v/v) CCK-8 reagent (Dojindo) was added to the wells and incubated for 2 hr at 37 °C. The cell viability was determined by measurement of $OD_{450\ nm}$ using an Infinite M200 plate reader (Tecan).

Subcellular fractionation

About 10^6 cells were seeded in a dish with 60 mm in diameter followed by treatment with 1 μM of Rb-TDP-EGFP for 6 hr. Following a washing with DPBS, cells were lysed using 500 μl of fractionation buffer (250 mM Sucrose, 20 mM HEPES (pH 7.4), 10 mM KCl, 1.5 mM MgCl2, 1 mM EDTA, 1 mM EGTA, 1mM DTT, and protease inhibitor cocktail). The lysate was passed through a 25 Ga needle 10 times and left on ice for 20 min. The nuclear pellet was collected by centrifugation at 3000 rpm for 5 min and resuspended in nuclear lysis buffer (10% glycerol and 0.1% SDS in PBS-T). The resuspension solution was used as a nuclear fraction. The supernatant was centrifuged at 8,000 rpm for 10 min, and the resulting solution was used as a cytosolic fraction, and the pellet was resuspended in the same buffer as used for the nuclei. The antibodies used in western blot were: GFP, sc-9996 (Santa-cruz); histone H3, #1791 (abcam); MEK1/2, #9124 (cell signaling).

Determination of toxicity in mice

The animals were divided into 2 groups of 6 weeks male ICR mice (n=4). Each group of animals was given injections with 27 μM of TDP-Glo (100ul per mouse) through the tail vein. The body weight was monitored 3 times per week. Hepatotoxicity and nephrotoxicity were investigated at 1 week after injection of TDP-Glo by measuring serum AST, ALT, BUN, and creatine.