Supplementary Information

Chemically Labeled ThUBD permits Rapid and Super-Sensitive Imaging of Polyubiquitination Signal

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Figure S1. The TUF-WB method relies on the quality of the GST antibody.
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Figure TOC.
ImageJ macro used to process and analyze the images obtained in this work.

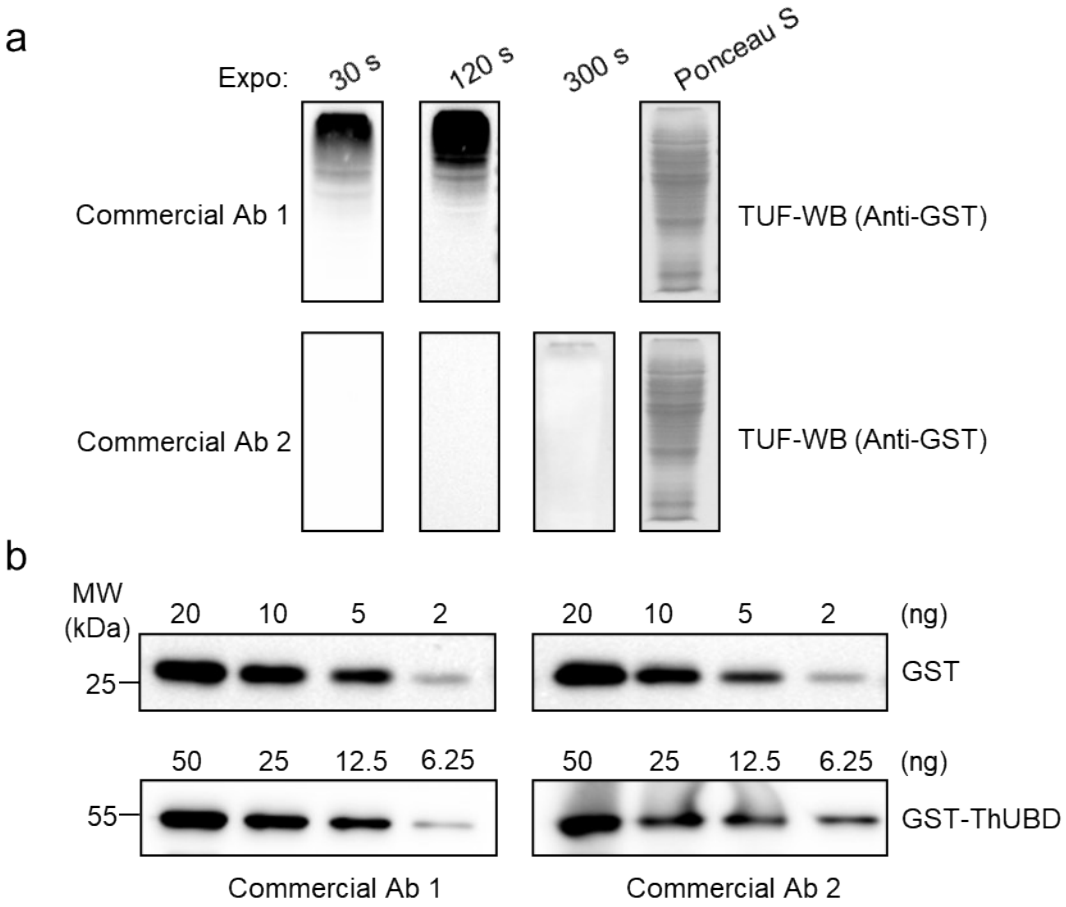

Figure S1. The TUF-WB method relies on the quality of the GST antibody.

(a) Two different commercial anti-GST antibodies were used to detect equal amounts of 293T whole cell lysates in the TUF-WB method.

(b) GST and GST-fused ThUBD were probed with two different commercial anti-GST antibodies.

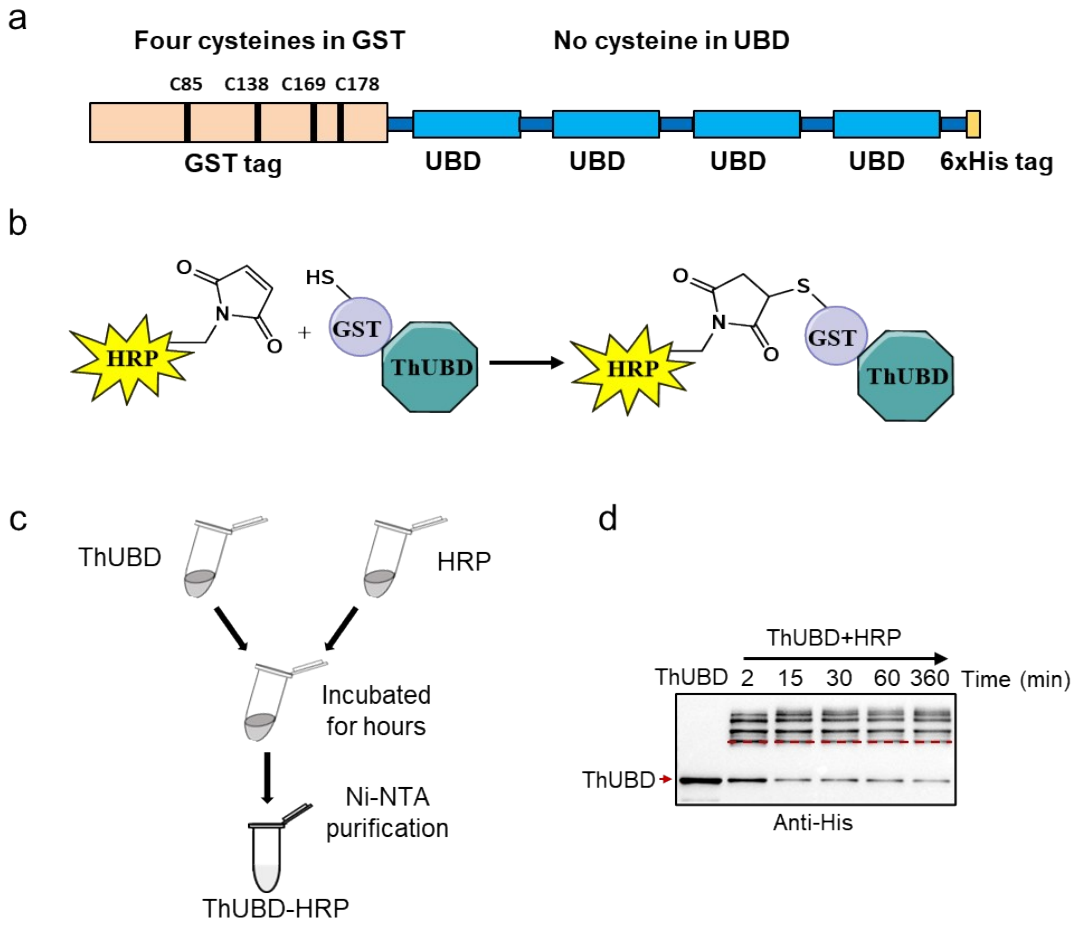

Figure S2. Reaction scheme for conjugation of Maleimide activated HRP to -SH of GST tag on the ThUBD.

(a) The structure diagram of the ThUBD. One GST molecular contains four cysteine residues which can react with one Maleimide Activated HRP. The ThUBD itself has no cysteine residue to be labeled.

(b) Schematic diagram of GST-ThUBD and HRP reaction.

(c) The workflow of HRP conjugated to ThUBD and then purified by Ni-NTA.

(d) The unlabeled ThUBD and HRP conjugated ThUBD (mix) in different reaction time were detected by anti-His antibody. The His tag was located on the C-terminal of ThUBD.

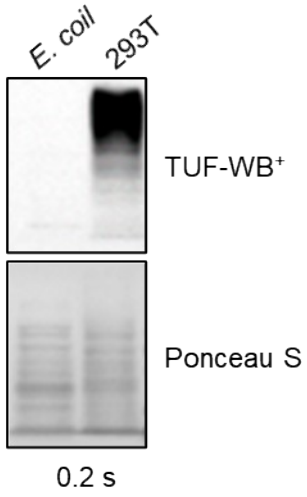

Figure S3. The whole cell lysates from *E. coli* and 293T cells were detected by TUF-WB^{+}. Samples were loaded in an equal amount (20 μg). Ponceau S staining was set as loading control. The exposure time was 0.2s.

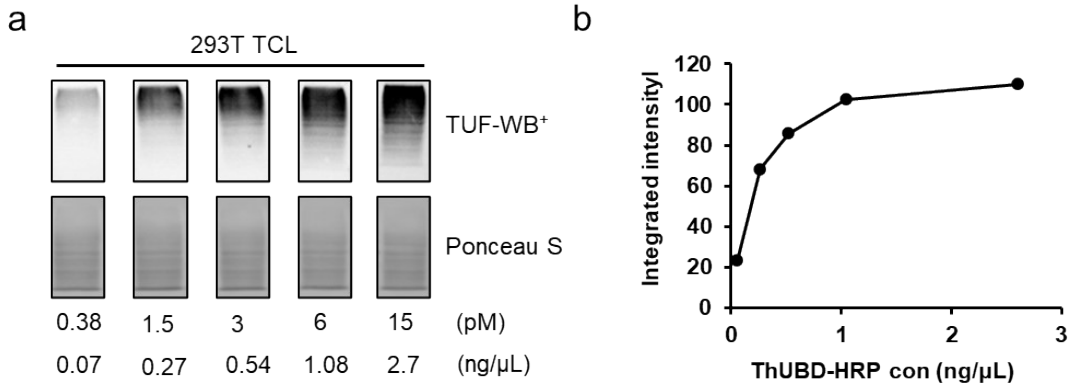

Figure S4. Optimizing the concentration of HRP-labeled ThUBD used in the TUF-WB$^+$ method.

(a) The membranes were incubated with different concentrations of ThUBD-HRP, ranging from 0.38 pM to 15 pM.

(b) The saturation curve generated based on the signal intensity detected in panel a.

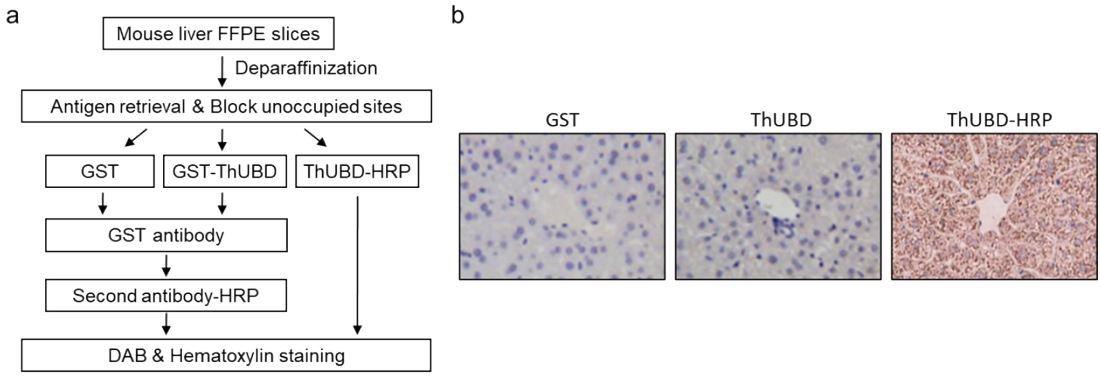

Figure S5. Immunohistochemical analysis of FFPE samples.

(a) Workflow of the three probes for the polyubiquitination signal detection in the FFPE samples.

(b) GST, ThUBD and ThUBD-HRP probe were used to detect polyubiquitination signal in FFPE samples.

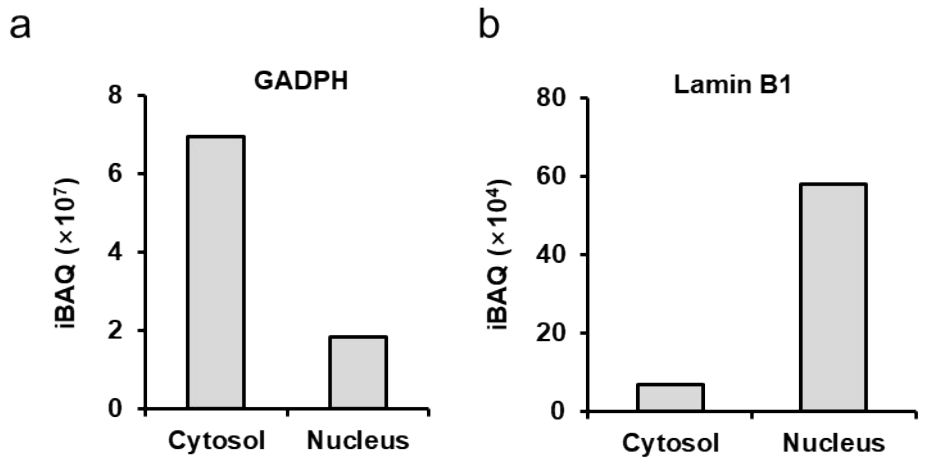

Figure S6. Quantification analysis of GAPDH (a) and Lamin B1 (b) protein in fractionated cytosolic and nuclear proteins in mouse liver.

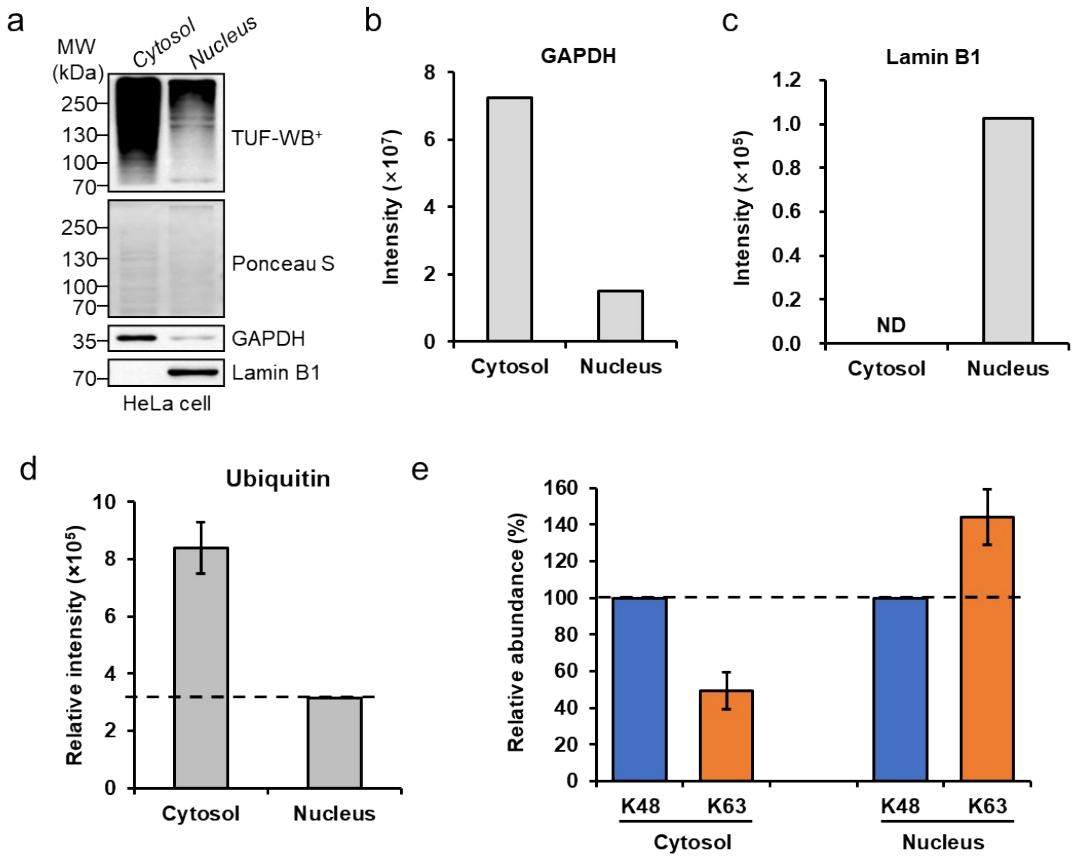

Figure S7. The ubiquitination signal of cytosol and nuclear proteins in HeLa cell.

(a) TUF-WB$^+$ method detect ubiquitin signal in fractionated cytosol and nucleus from HeLa cell. The cytosol marker GAPDH and the nucleus marker Lamin B1 were used as control.

(b&c) Quantification analysis of GAPDH and Lamin B1 protein in fractionated cytosolic and nuclear proteins in HeLa cells.

(d) Ubiquitin signal intensity in cytosol and nucleus detected by LC-MS/MS.

(e) Signal intensity of K48 chain and K63 chain in cytosol and nucleus in HeLa cells quantified by LC-MS/MS. Signal intensity of K48 chain detected in cytosol and nucleus separately was used as control.

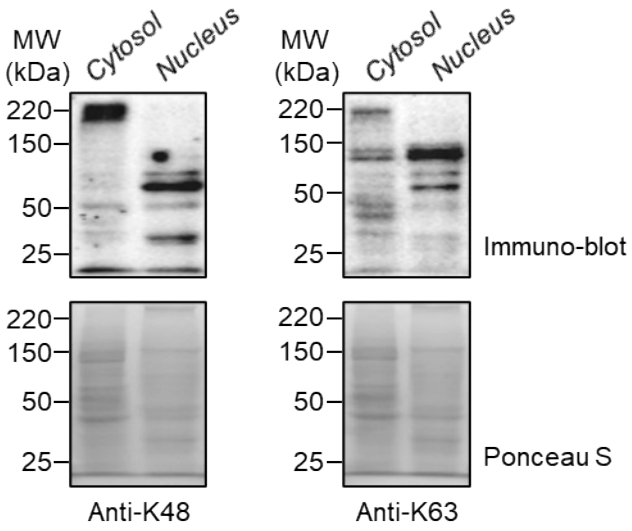

Figure S8. Western blot analysis K48 and K63 ubiquitin chains in cytosol and nucleus fractions in HeLa cell. Anti-K48 chain antibody and anti-K63 chain antibody was used in parallel.

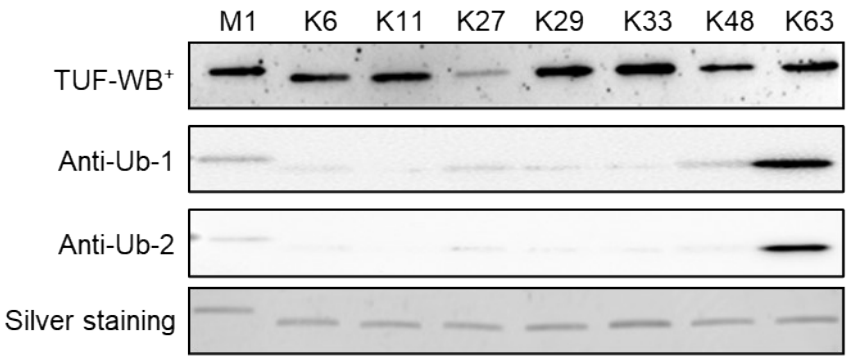

Figure S9. Comparison of TUF-WB$^+$ and anti-ubiquitin antibody (Anti-Ub-1 and Anti-Ub-2) for the detection of eight types of ubiquitin chains. The silver staining gel served as a loading control.