SUPPLEMENTARY INFORMATION

Exploring the Target Scope of KEAP1 E3 Ligase-based PROTACs

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Supplementary Figures

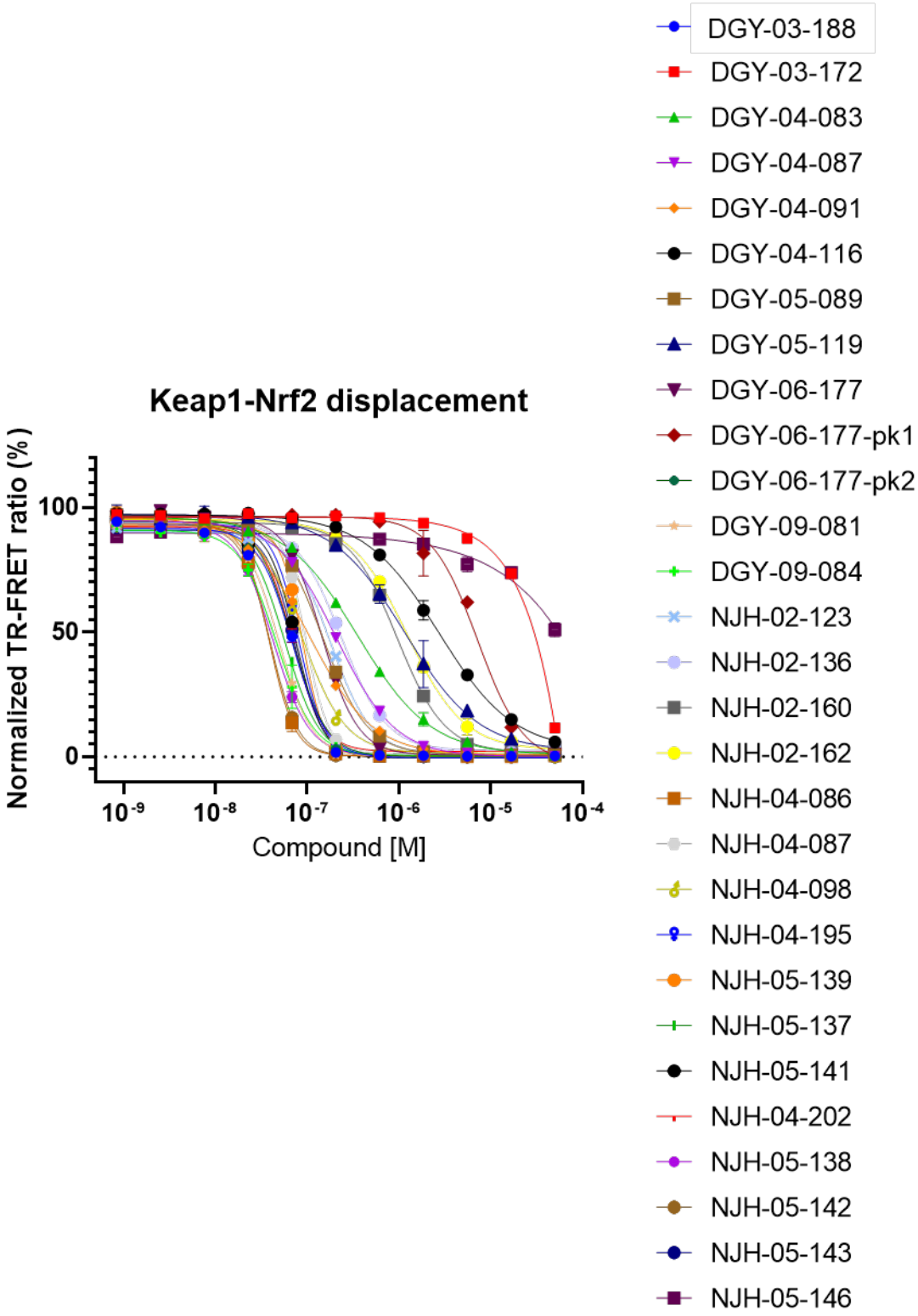

Figure S1. KEAP1-NRF2 displacement binding assay (related to Figure 1 and Figure 3). Preassembled 0.1 μM FITC-labelled NRF2 peptide and 0.1 μM biotinylated KEAP1 were treated with an increasing concentration of NRF2 peptide, and the signal was measured by TR-FRET. Lines represent standard four-parameter log-logistic curve fit. Data are presented as the mean ± SEM with at least three technical replicates. The figure is one representation from two independent experiments.

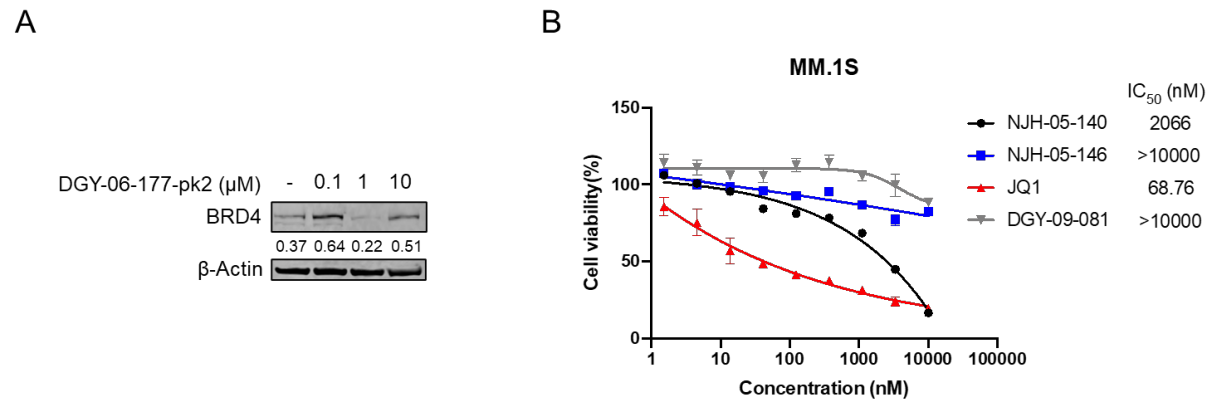

Figure S2. Validation of lead molecule DGY-06-177-pk2 (related to Figure 2 and figure 4). **A**. OVCAR8 cells were treated for 16 h with 0.1 μM, 1 μM or 10 μM of indicated compound. Representative blots from two independent experiments are shown. The relative intensity of each band (BRD4 normalized to β-actin) is shown under each band. **B.** Cells were treated with the indicated compounds for 72 h. Cell viability was assessed with CellTiter-Glo. Data are presented as the mean ± SEM with three technical replicates. The figure is one representation from two independent experiments (NJH-05-140 is another batch of DGY-06-177-pk2).

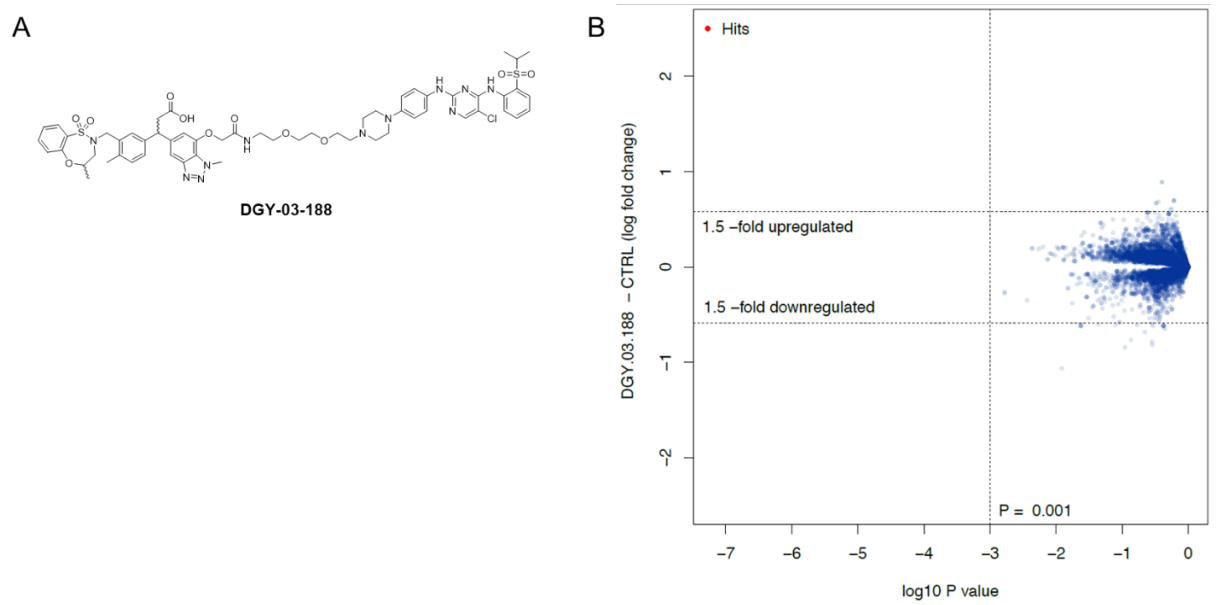

Figure S3. Quantitative proteomics analysis of DGY-03-188 in HEK293T cells (related to Figure 5). A. Chemical structure of DGY-03-188 **B**. Quantitative proteomics profiles of HEK293T cells treated for 10 h with 0.5 μM of DGY-03-188.

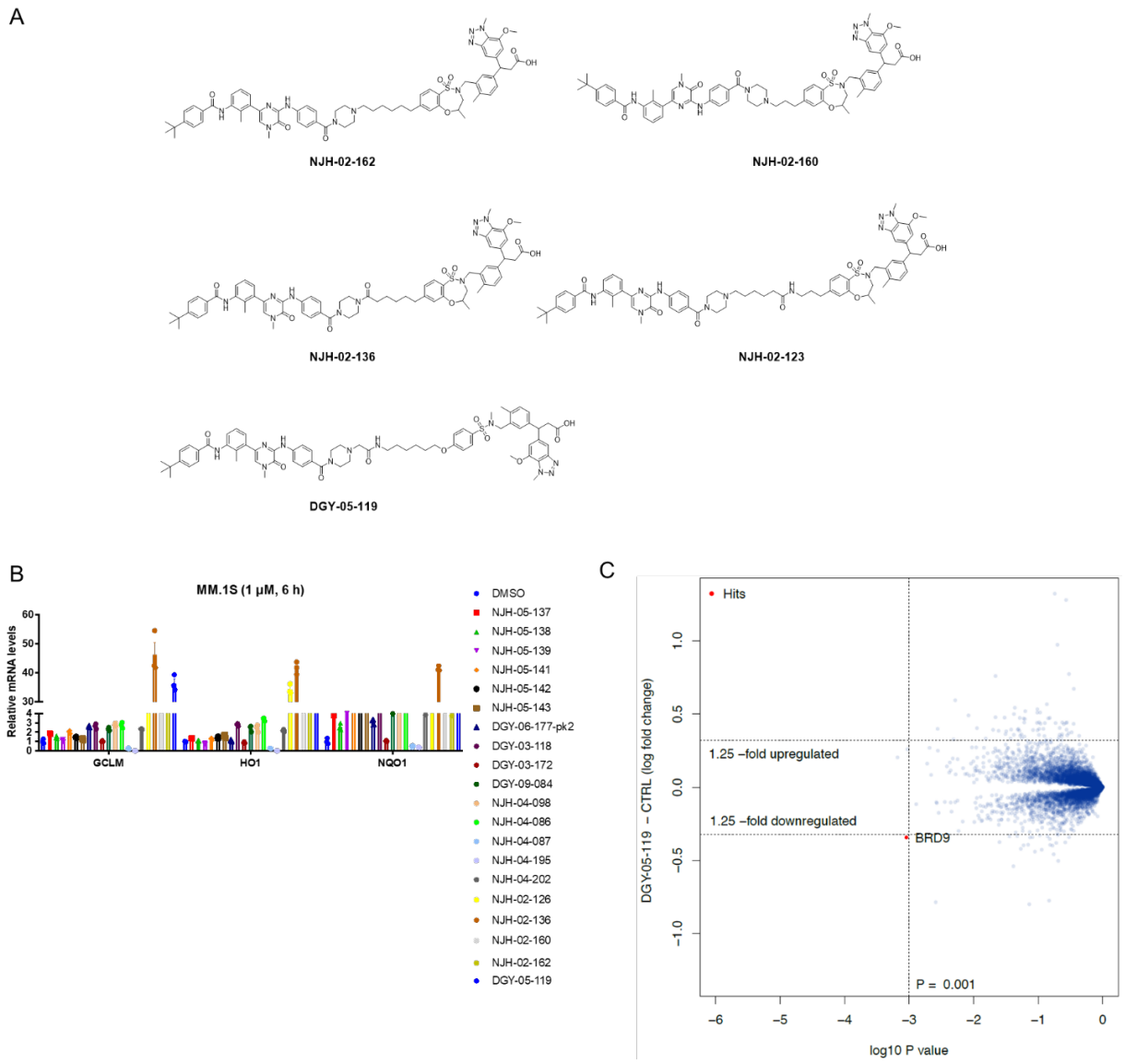

Figure S4. Screening and evaluation of BTK degraders (related to Figure 6). A. Structures of KEAP1-BTK compounds. **B**. qPCR of the indicated gene expression level following the indicated compounds treatment in MM.1S cells. Data are presented as the mean ± SEM with three technical replicates. Representative figure from two independent experiments is shown. **C.** Quantitative proteomics profiles of HEK293T cells treated for 6 h with 5 μM of DGY-05-119.

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C. Quantitative proteomics profiles of HEK293T cells treated for 6 h with 5 μM of DGY-05-119.

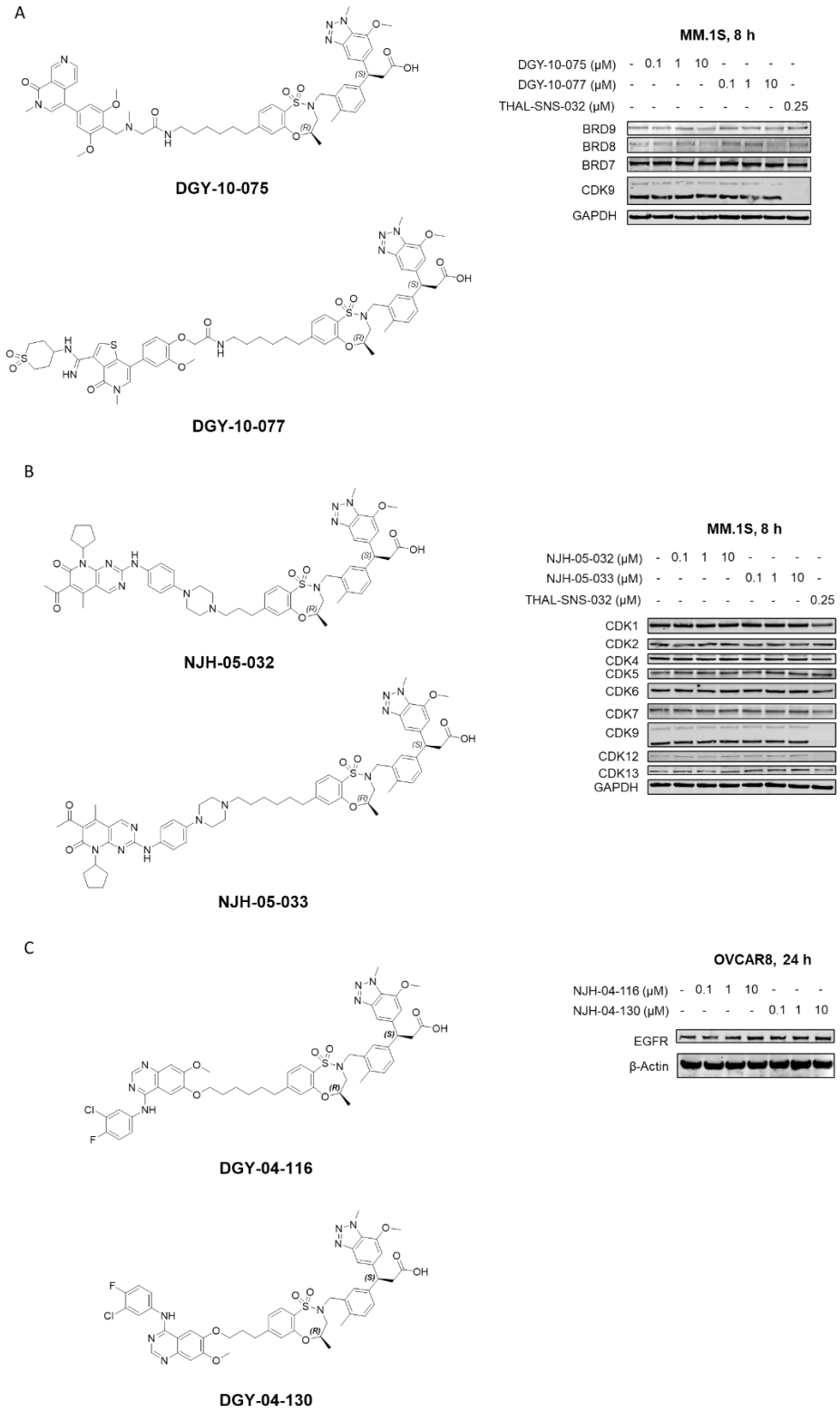

Figure S5. Screening of BRD9, CDK4/6, and EGFR degraders (related to Figure 6). A. Immunoblot analysis of protein abundant of BRD7/8/9 in MM.1S cells. MM.1S cells were treated for 8 h with 0.1 μM, 1 μM or 10 μM of indicated compounds. Representative blots from two independent experiments are shown. The relative intensity of each band (BRD7/8/9 normalized to β-actin) is shown under each band. **B.** Immunoblot analysis of protein abundant of CDKs in MM.1S cells. MM.1S cells were treated for 8 h with 0.1 μM, 1 μM or 10 μM of indicated compounds. THAL-SNS-032 were used as positive control. Representative blots from two independent experiments are shown. The relative intensity of each band (CDK1/2/4/5/6/7/9/12/13 normalized to β-actin) is shown under each band. **C.** Immunoblot analysis of protein abundant of EGFR in OVCAR8 cells. OVCAR8 cells were treated for 24 h with 0.1 μM, 1 μM or 10 μM of indicated compounds. Representative blots from two independent experiments are shown. The relative intensity of each band (EGFR normalized to β-actin) is shown under each band.

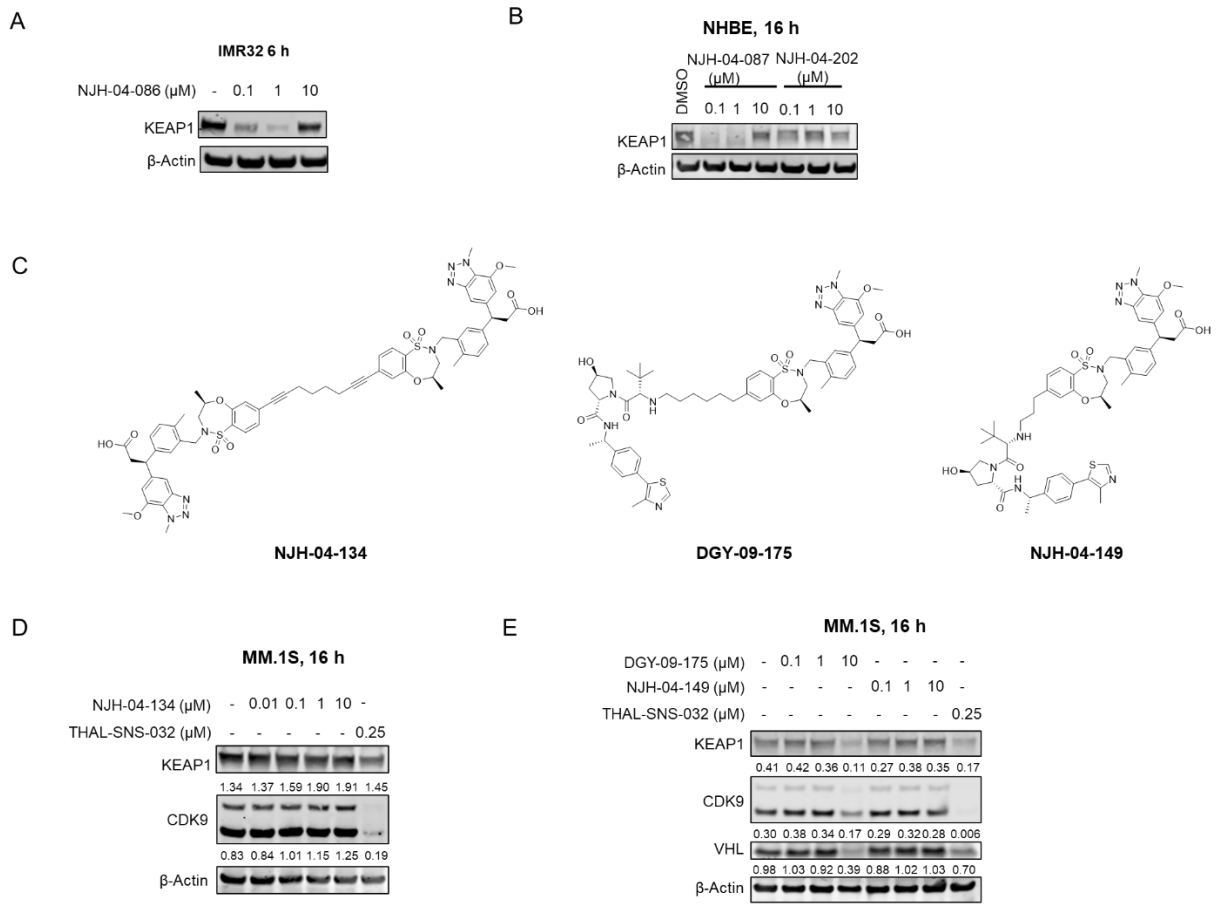

Figure S6. Screening of different E3 ligase degrader (related to Figure 7). A. Immunoblot analysis of protein abundant of KEAP1 in IMR32 cells. IMR32 cells were treated for 6 h with 0.1 μM, 1 μM or 10 μM of NJH-04-086. Representative blots from two independent experiments are shown. The relative intensity of each band (KEAP1 normalized to β-actin) is shown under each band. **B.** Immunoblot analysis of protein abundant of KEAP1 in NHBE cells. NHBE cells were treated for 16 h with 0.1 μM, 1 μM or 10 μM of indicated compounds. Representative blots from two independent experiments are shown. The relative intensity of each band (KEAP1 normalized to β-actin) is shown under each band. **C**. Structure of NJH-04-134, DGY-09-175 and NJH-04-149. **D.** Immunoblot analysis of protein abundant of KEAP1 and CDK9 in MM.1S cells. MM.1S cells

were treated for 16 h with 0.1 μM, 1 μM or 10 μM of indicated compounds. THAL-SNS-032 were used as positive control. Representative blots from two independent experiments are shown. The relative intensity of each band (KEAP1 and CDK9 normalized to β-actin) is shown under each band. **E.** Immunoblot analysis of protein abundant of KEAP1 and CDK9 in MM.1S cells. MM.1S cells were treated for 16 h with 0.1 μM, 1 μM or 10 μM of indicated compounds. THAL-SNS-032 were used as positive control. Representative blots from two independent experiments are shown. The relative intensity of each band (KEAP1, CDK9 and VHL normalized to β-actin) is shown under each band.

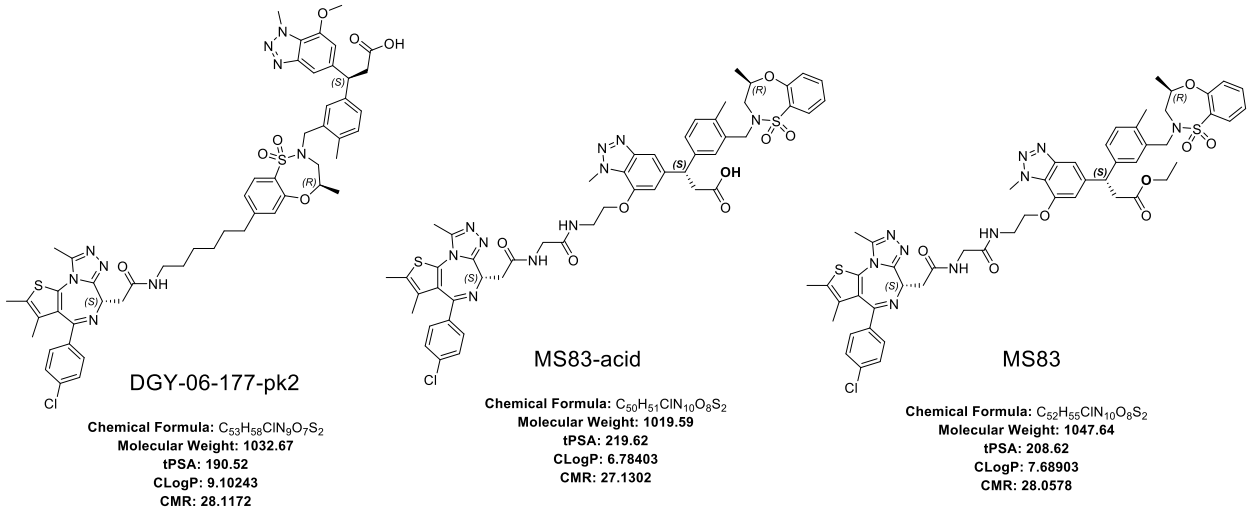

Figure S7. Comparison of DGY-06-177-pk2, MS83-acid and MS83 (related to Figure 2 and Figure 3). Structures and some predicted properties of DGY-06-177-pk2, MS83-acid and MS83.

Supplementary Tables

Table S1. IC_{50} values of KEAP1-NRF2 displacement TR-FRET binding assay. Data are presented as the mean ± SD with at least three technical replicates. (related to Figure 1 and Figure 3)

Compound	IC50 (nM)
DGY-03-188	69.08
DGY-03-172	> 10000
DGY-04-083	356.4
DGY-04-087	210.9
DGY-04-091	106
DGY-04-116	2721
DGY-05-089	146.8
DGY-05-119	1165
DGY-06-177	144.8
DGY-06-177-pk1	7185
DGY-06-177-pk2	68.31
DGY-09-081	49.11
DGY-09-084	46.59

NJH-02-123	181.1
NJH-02-136	235
NJH-02-160	954.5
NJH-02-162	1235
NJH-04-086	38.6
NJH-04-087	95.47
NJH-04-098	88.18
NJH-04-195	81.18
NJH-04-202	68.68
NJH-05-137	56.8
NJH-05-138	42.45
NJH-05-139	89.16
NJH-05-141	75.73
NJH-05-142	39.4
NJH-05-143	73.31
NJH-05-146	> 10000