Supplementary Information

Chemoproteomics-Enabled Discovery of Covalent RNF114-Based Degraders that Mimic Natural Product Function

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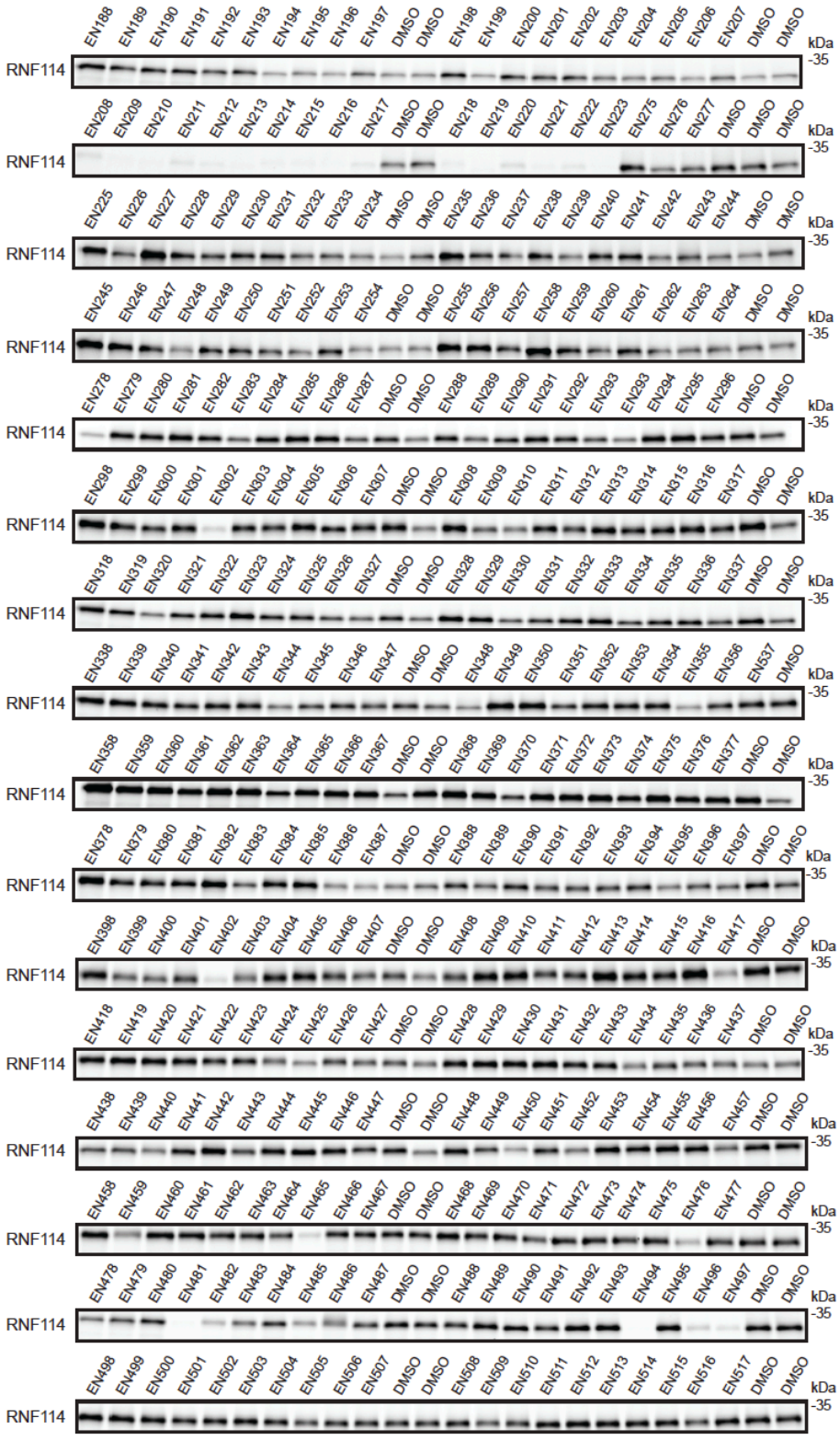

Figure S1. Related to Figure 2. Gel-based ABPP assay for screening covalent ligands against IA-rhodamine probe binding to pure RNF114 protein. Loss of fluorescence indicates covalent ligand binding to a cysteine on RNF114. DMSO vehicle or covalent ligands (50 μM) were pre-incubated with pure RNF114 protein (0.1 μg) for 30 min prior to addition of IA-rhodamine (100 nM) for 30 min at room temperature. Proteins were separated by SDS/PAGE and in-gel fluorescence was quantified. The structures of all the compounds screened can be found in **Table S1.**

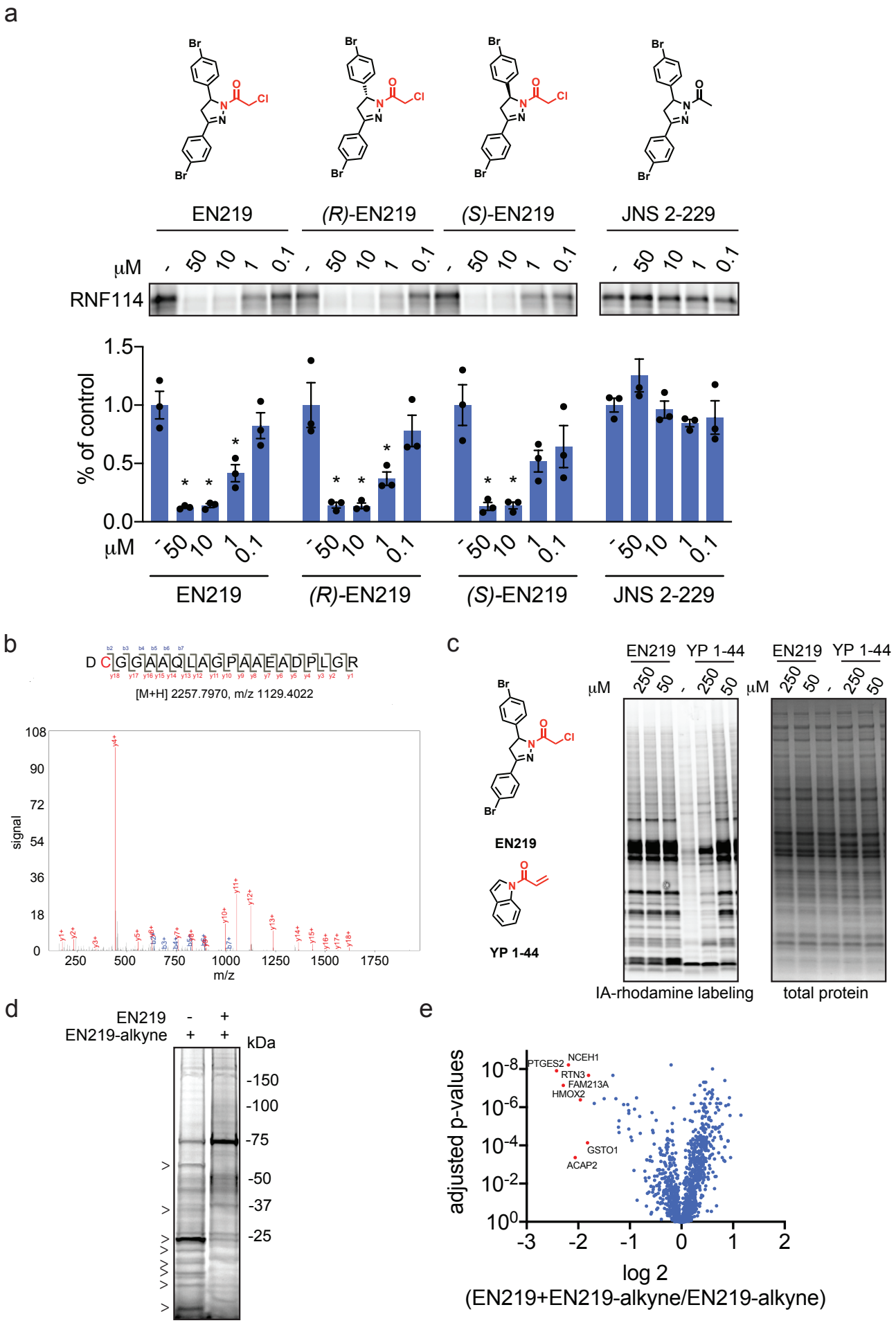
a
EN219
(R)-EN219
(S)-EN219
JNS 2-229
μM
RNF114
% of control
b
[M+H] 2257.7970, m/z 1129.4022
signal
m/z
c
EN219
YP 1-44
IA-rhodamine labeling
total protein
d
EN219
EN219-alkyne
kDa
e
adjusted p-values
log 2
(EN219+EN219-alkyne/EN219-alkyne)
PTGES2
NCEH1
RTN3
FAM213A
HMOX2
GSTO1
ACAP2

Figure S2. Related to Figure 2. EN219 reactivity with RNF114 pure protein and TMT-based quantitative proteomic profiling of EN219-alkyne-enriched targets in 231MFP cells. (a) Dose-response of racemic EN219, *(R)*-EN219, *(S)*-EN219, and non-reactive analog JNS 2-229 interactions with RNF114 by competitive gel-based ABPP. DMSO vehicle or ligands were pre-incubated with pure RNF114 protein (0.1 μg) for 30 min prior to addition of IA-rhodamine (100 nM) for 30 min at room temperature. Proteins were separated by SDS/PAGE and in-gel fluorescence was quantified. Gels shown in **(a)** are representative of n=3 biological replicates/group and bar graphs show average ± sem and individual replicate values from quantitation of IA-rhodamine labeling. Statistical significance was calculated with unpaired two-tailed Student's t-test and is expressed as *p<0.05 compared to vehicle-treated controls. **(b)** Pure RNF114 protein (10 μg, 3.89 μM) was incubated with EN219 (50 μM) for 1 h and subsequent tryptic digests of RNF114 protein were analyzed by LC-MS/MS to look for EN219 covalent modification. The Cysteine 8 highlighted in red was found to be modified. Shown is the mass spectra of the EN219 covalent adduct on the RNF114 tryptic peptide. **(c)** gel-based ABPP of EN219 and promiscuous scout ligand YP 1-44 in 231MFP breast cancer cell lysate *in vitro*. 231MFP cell lysate (20 μg) were pre-incubated with DMSO vehicle or covalent ligand for 30 min prior to IA-rhodamine labeling (1 μM) for 30 min at room temperature. Proteins were separated by SDS/PAGE and visualized by in-gel fluorescence. Shown is a representative gel from n=3 biologically independent replicates. **(d)** 231MFP cells were treated with DMSO vehicle or EN219 (35 μM) 30 min prior to treating cells with DMSO or EN219-alkyne probe (2 μM) for 90 min. Resulting cell lysates were subjected to CuAAC with rhodamine-azide and EN219-alkyne-labeled proteins were separated by SDS/PAGE and visualized by in-gel fluorescence. Proteins that show competition with EN219 are labeled with ">". Gel is a representative of n=3 biologically independent replicates/group. **(e)** 231MFP cells were treated with DMSO vehicle or EN219 (20 μM) 30 min prior to treating cells with DMSO or EN219-alkyne probe (2 μM) for 90 min. Resulting cell lysates were subjected to CuAAC with biotin-azide to append a biotin enrichment handle onto EN219-alkyne labeled proteins *ex situ*. EN219-alkyne labeled proteins were subsequently avidin-enriched, digested with trypsin, and resulting tryptic peptides from each treatment group were labeled with TMT reagents and combined and fractionated for LC-MS/MS analysis. Shown are average TMT ratios and adjusted p-values comparing EN219 pre-treated EN219-alkyne labeled groups to EN219-alkyne labeled groups to identify EN219-alkyne-labeled proteins that are competed by the parent compound EN219. Shown in red are proteins that show EN219+EN219-alkyne versus EN219-alkyne ratios <0.33 with adjusted p<0.05. The full dataset can be found in **Table S3.**

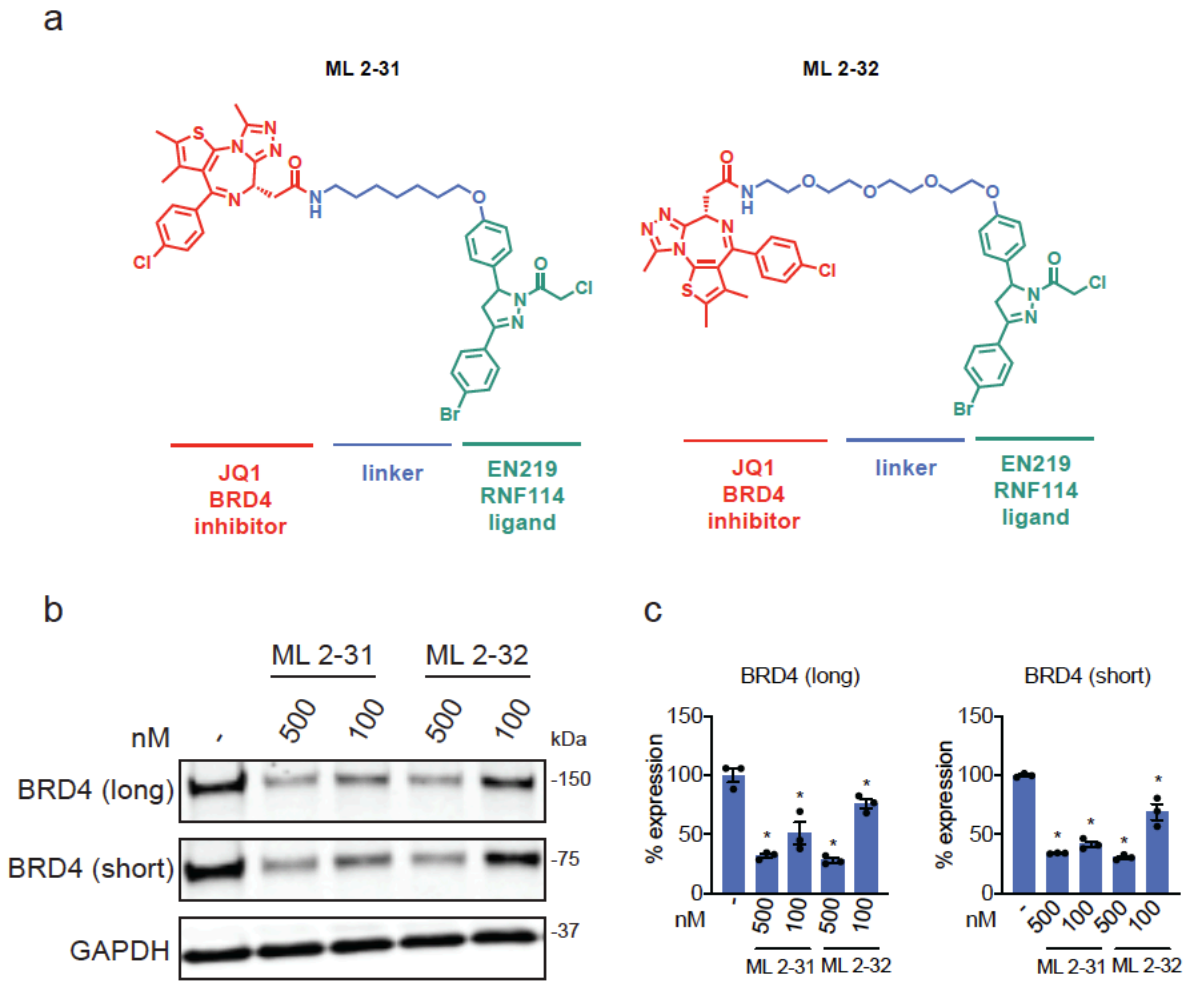

Figure S3. Related to Figure 3. EN219-based BRD4 degraders. (a) Structure of ML 2-31 and ML 2-32, EN219-based BRD4 degraders linking EN219 to BET inhibitor JQ1 with two different linkers. **(b)** Degradation of BRD4 by ML 2-31 and ML 2-32. 231MFP cells were treated with DMSO vehicle or ML 2-31 or ML 2-32 for 8 h and the long and short isoforms of BRD4 and loading control GAPDH were detected by Western blotting. **(c)** Percentage of BRD4 degradation quantified from **(b)**. Data shown in **(c)** are average and individual replicate values. Blots shown in **(b)** are representative of n=3 biological replicates/group.

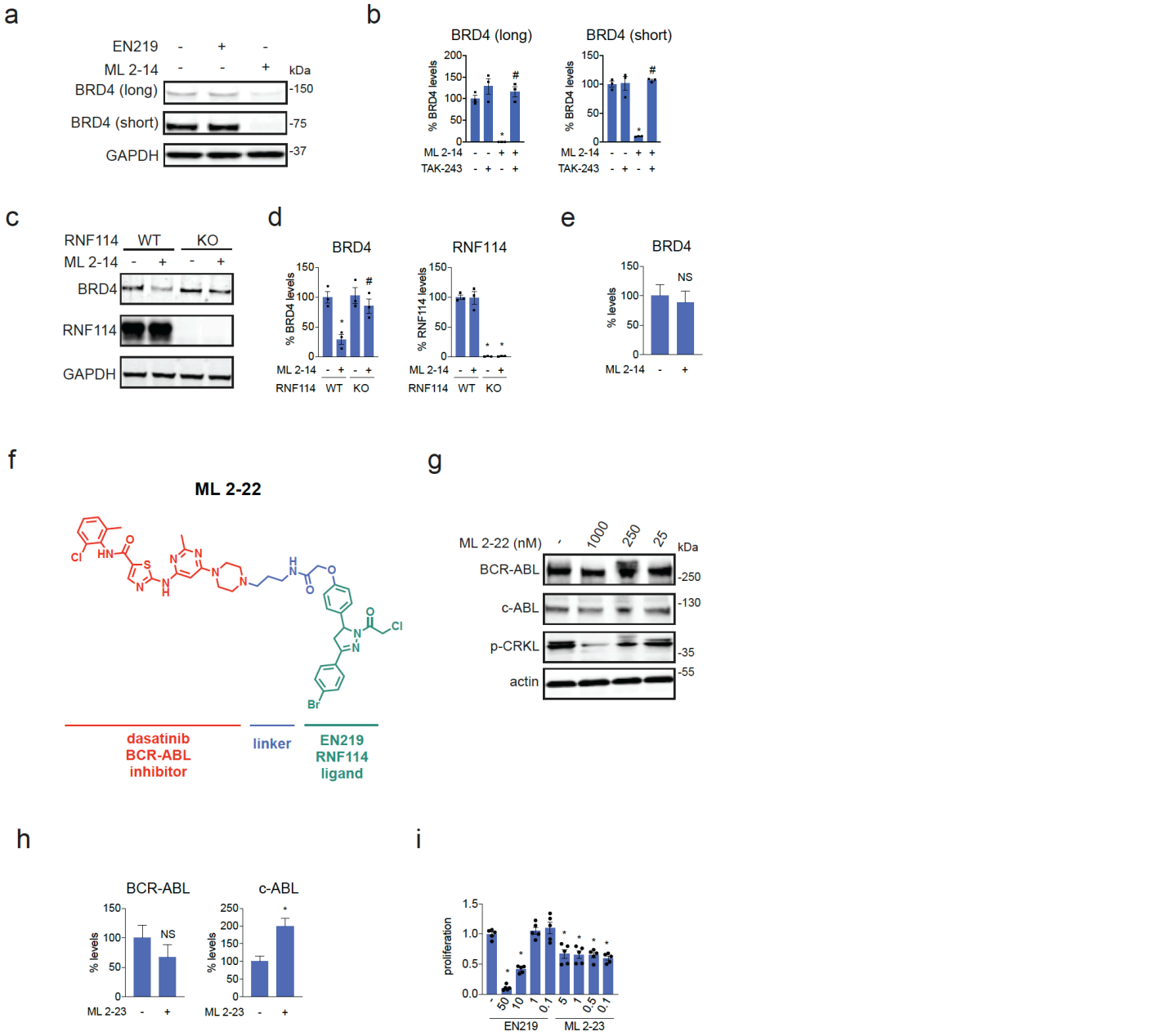

Figure. S4. Related to Figures 3 and 4. ML 2-14 and ML 2-22 mediated degradation of BRD4 and BCR-ABL, respectively. (a) BRD4 and loading control GAPDH expression in 231MFP cells treated with DMSO, EN219 (100 nM), and ML 2-14 (100 nM) for 20 h detected by Western blotting. **(b)** Quantification of data from **Figure 3f. (c)** Degradation of BRD4 (long isoform) in RNF114 wild-type (WT) or knockout (KO) HAP1 cells in a distinct separate experiment from the data shown in **Figure 3i**. RNF114 WT or KO HAP1 cells were treated with DMSO vehicle or ML 2-14 (1 μM) for 16 h and BRD4, RNF114, and loading control GAPDH levels were detected by Western blotting. **(d)** Quantification of data from **(c)**. **(e)** Quantitative PCR data of BRD4 mRNA expression in 231MFP cells treated with vehicle or ML 2-14 (100 nM, 16 h). **(f)** Structure of ML 2-22 degrader that linked EN219 to dasatinib. **(g)** BCR-ABL, c-ABL, p-CRKL, and loading control actin levels assessed by Western blot from K562 cells treated with DMSO vehicle or ML 2-22 for 20 h. **(h)** Quantitative PCR data of BCR-ABL and c-ABL mRNA expression in K562 cells treated with vehicle or ML 2-23 (5 μM, 16 h). **(i)** Cell proliferation of K562 cells treated with DMSO vehicle, EN219, or ML 2-23 for 16 h, assessed by WST reagent. Blots in **(a, c, g)** are a representative from n=3 biological replicates/group. Data shown in **(b, d, e, h, i)** are average ± sem with n=3 biological replicates/group for **(b, d, e, h)** and n=5 biological replicates/group in **(i)**. Statistical significance was calculated with unpaired two-tailed Student's t-test and is expressed as *p<0.05 compared to vehicle-treated controls or vehicle-treated wild-type controls in **(b, d, e, h, i)** and #p<0.05 compared to ML 2-14 treated groups or ML 2-14-treated wild-type groups. NS denotes not significant.