

Supplemental Information

Supplemental Figures

Figure S1.

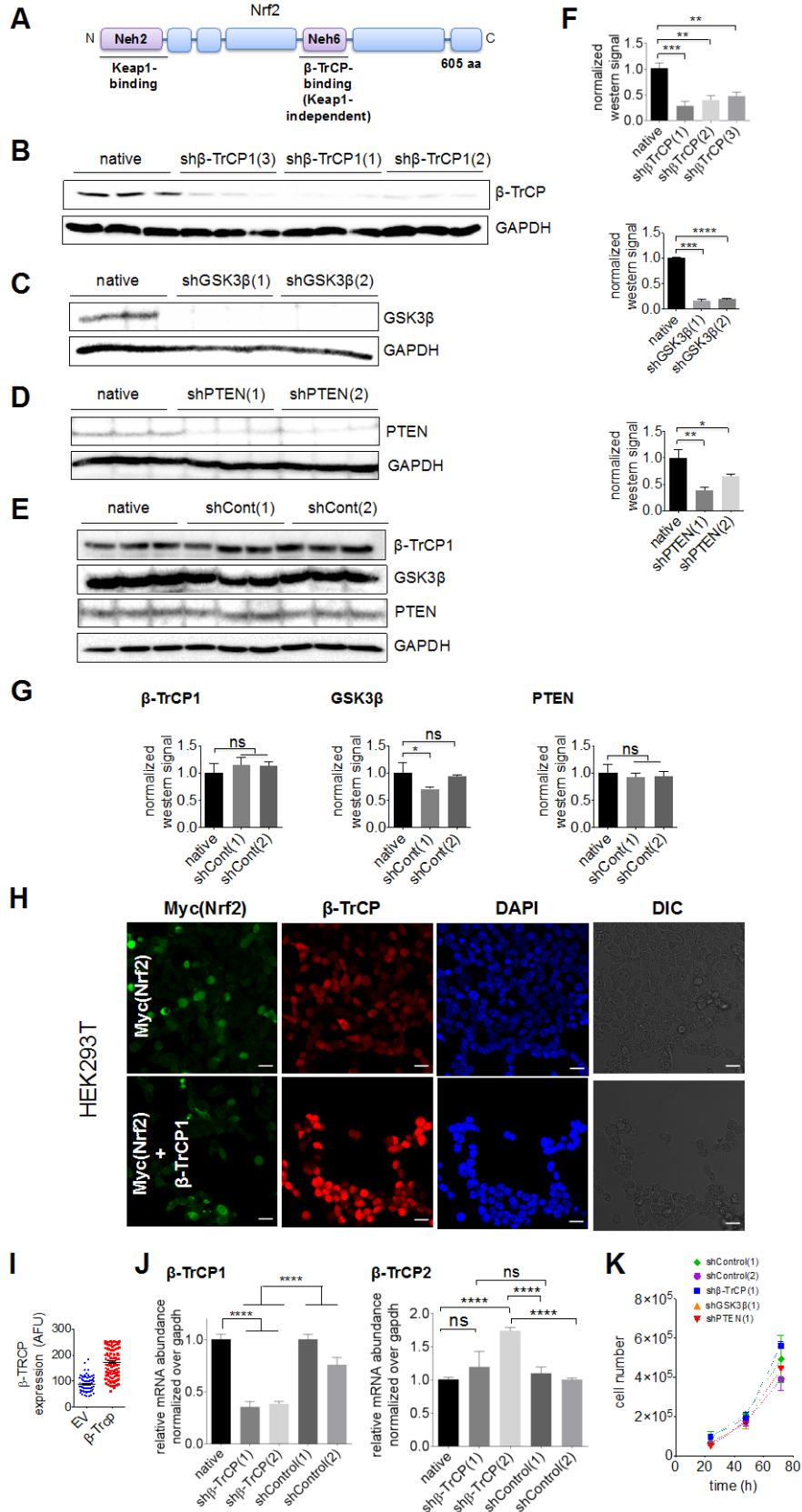


Figure S1. Validation of reduced protein-levels in knockdown cells in which postulated redox-sensitive regulators of the antioxidant response depleted by lentiviral shRNA, Related to Figure 1B and Figure 2: (A) Domain structure of human Nrf2 [modeled on (Hayes and Dinkova-Kostova, 2014)]. Neh2 domain binds Keap1 and Neh6 domain is required for the β -TrCP-mediated Nrf2 degradation. Western blot analyses of protein expression in knockdown lines versus respective scrambled controls. (B-D) Knockdown of β -TrCP1, GSK3 β , and PTEN via lentiviral-induced shRNA delivery is efficient: (E) whereas no appreciable knockdowns were observed in cells transduced with control shRNAs. (F-G) Quantitation of western blot data: representative blots in B, C, and D (F); and in E (G). Errors indicate s.d. (N > 3). Student's t-test was performed to determine significance. * p < 0.01, ** p < 0.001, *** p < 0.001, and **** p < 0.0001. (H) Overexpressed β -TrCP1 is largely nuclear. Representative images showing HEK293T cells transiently transfected with pFN21a- β -TrCP1 and pCDNA3-myc-Nrf2 and analyzed by immunofluorescence (IF) imaging (using primary antibody: D13F10, Cell Signaling, Table S1). Scale bar, 20 μ m. (I) Quantification (N > 40, s.e.m.). AFU, arbitrary fluorescence units. EV, empty vector (see Table S2 and SI "plasmids" section for details). Transient transfection of mammalian cells with plasmid encoding β -TrCP1 results in selective increase of β -TrCP signal in the nucleus (note: antibody detects both β -TrCP-homologs), suggesting that β -TrCP1 is preferentially nuclear localized in agreement with the previous reports (Cuadrado, 2015; Davis, et al., 2002; Lassot, et al., 2001; Seo, et al., 2009;). Myc-Nrf2 expression serves as a readout for effects of Nrf2 protein levels upon β -TrCP modulation. (J) qRT-PCR analyses of relative mRNA abundance levels of β -TrCP1 and β -TrCP2 in indicated knockdown lines. sh β -TrCP hairpin plasmids selectively knocked down β -TrCP1 mRNA levels. β -TrCP2 gene transcript levels are unchanged for one line, but slightly upregulated in another. (K) The knockdown lines display comparable growth rates to non-targeted shRNA. Viability analysis was carried out by AlamarBlue[®] assays. Cell counting was performed by Countess II (Invitrogen). Errors designate s.d. [N = 6; two independent biological replicates are shown with 3 technical replicates in each set].

Figure S2.

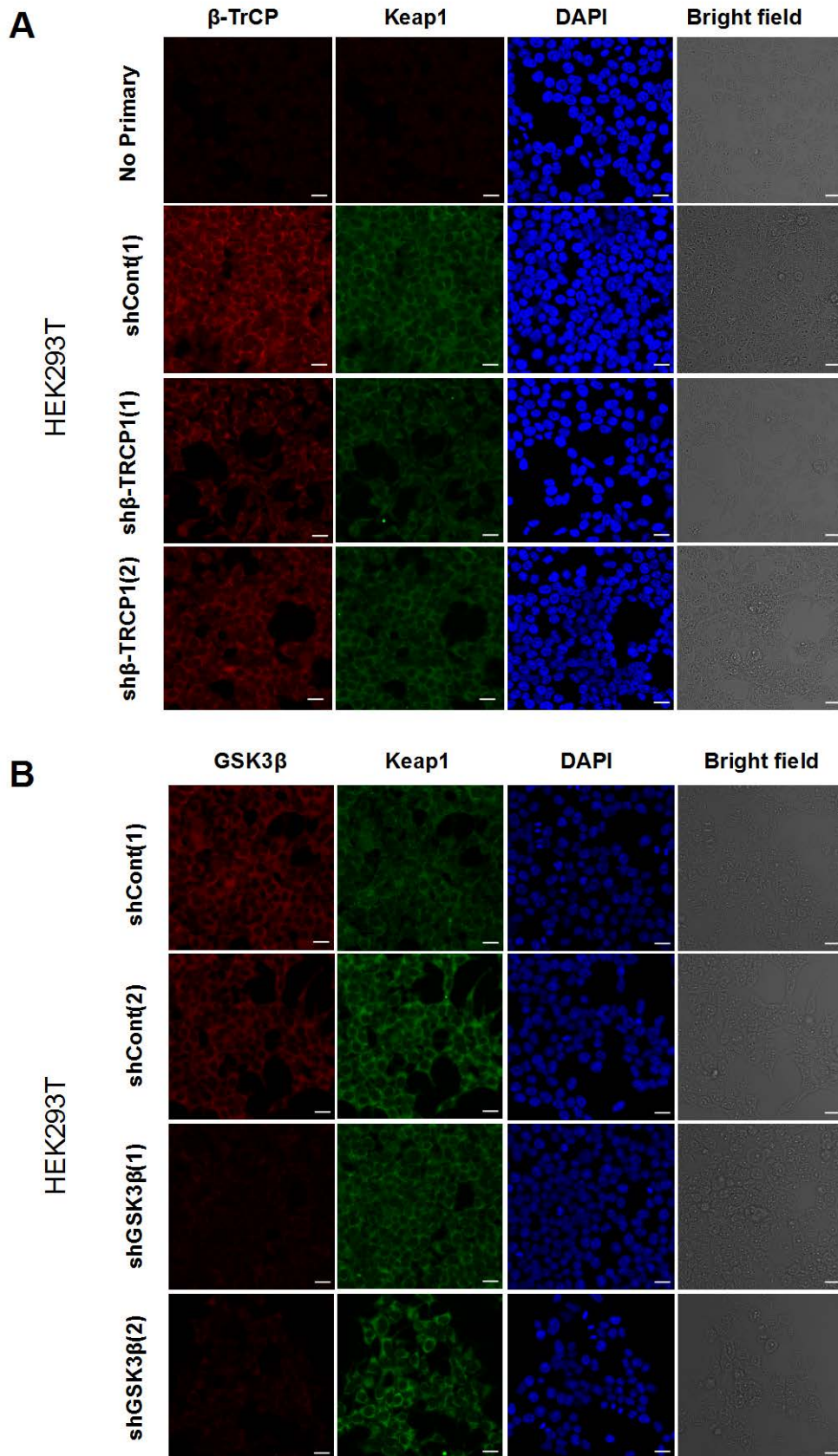


Figure S2. Efficiency of β -TrCP1 knockdown assessed by immunofluorescence imaging, Related to Figure 2 and Figure S1: Representative images showing reduced protein levels of nuclear β -TrCP (**A**) and GSK3 β (**B**) in the respective knockdown lines compared to control shRNAs. The expression levels of Keap1 do not change in these cell lines. Scale bar, 20 μ m.

Figure S3.

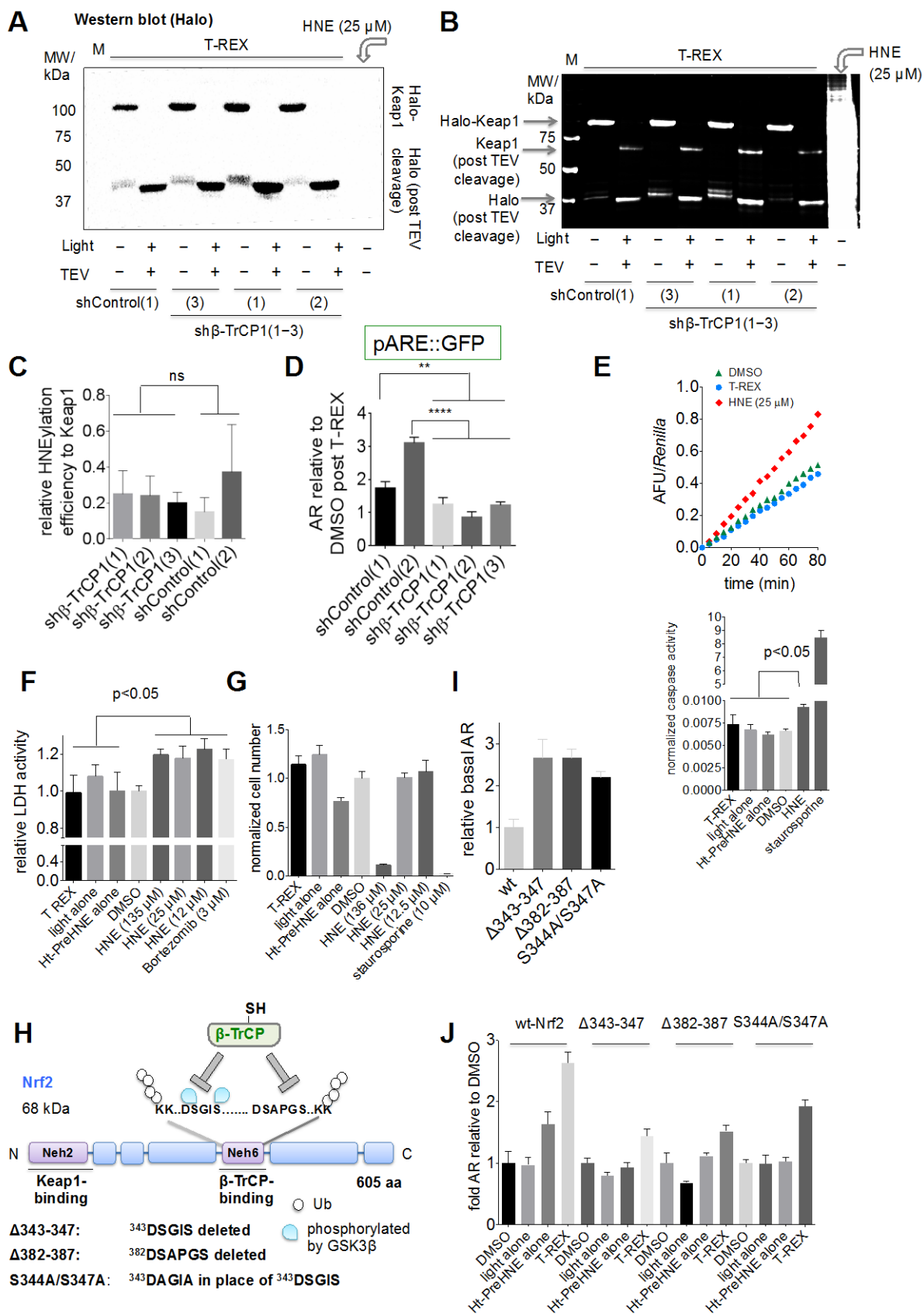


Figure S3. The differential AR upregulation upon Keap1-specific-redox modification in β -TRCP1 knock down line is independent of Nrf2- β -TRCP1 interaction, Related to Figure 2 and 3A. Western blot (A) and the corresponding in-gel fluorescence (B) analyses show that T-REX-targeted HNEylation of Keap1 remains functional in β -TrCP1-knockdown lines ectopically expressing Halo-Keap1. TEV protease cleavage post cell lysis enables separation of the Halo and Keap1 and quantitation of HNEylation efficiency on Keap1 (Lin, et al., 2015; Parvez, et al., 2016). Whole-cell HNE treatment (20 min) of shControl cells (with no Halo-Keap1 transfection) under otherwise identical conditions results in non-specific HNEylation of the proteome (last lane from left). M marks molecular weight ladder. Varying intensity bands at ~37 kDa represent adventitious cleavage of the TEV-linker during lysis, which varies stochastically (but *not* in a manner that can explain T-REX-specific AR-hypomorphism in these lines because (1) delivery (that requires fusion between Keap1 and Halo) is equal in all lines, and (2) the knockdown lines do not show a consistent gain or loss in this band). (C) Gel-based targeting efficiency estimation as previously reported (N = 6, s.d.) (Lin, et al., 2015; Parvez, et al., 2016). (D) Independent flow-cytometry-based validation of the results in **Figure 2C** using a reporter plasmid expressing GFP under the Antioxidant Response Element (ARE) promoter. HEK293T cells expressing the indicated shRNAs were transfected with Halo-Keap1 and Nrf2, and pARE::GFP (1:1:1). T-REX-assisted Keap1-HNEylation-specific AR upregulation was ablated in three different sh β -TrCP1-knockdown lines, but is operative in shControl lines (N=5, s.e.m.). (E-G) T-REX sidesteps toxicity-related issues caused by whole-cell HNE bathing. (E) Measurements of caspase-3/7 activity in HEK293T cells using hydrolysis of AC-DEVD-AMC (Durriveau, et al., 1998). *Bottom*: Raw traces showing increase in AMC (AFU/*Renilla*) as a function of time (no increase in signal is observed in absence of lysate). *Bottom*: quantification of plots in N>3. AFU, arbitrary fluorescence units. (F) Native HEK293T cells were subjected to the indicated conditions and after 18 h media was removed, and assayed for LDH activity as detailed in materials and methods (N>3). (G) Cells were grown in 48-well plates and subject to the indicated conditions. After 18 h, AlamarBlue[®] was added and left for 2.5 h. After this time, number of cells was measured by fluorescence. (H) β -TrCP1 domain structure. Nrf2 repression by β -TrCP: DSGIS and DSAPGS motifs in the Neh6 domain recruit SCF ^{β -TRCP}, allowing ubiquitylation of Lysine-residues bordering these motifs [modeled on (Hayes and Dinkova-Kostova, 2014)]. Ser344/347-phosphorylation at the DSGIS motif by GSK3 β creates a phosphodegron, enhancing this suppression. Three Nrf2- β -TrCP1-interaction mutants; the numbers in superscript designate starting residue number. Ub, ubiquitin; aa, amino acid. (I) Basal AR levels in non-stimulated HEK293T cells expressing the Nrf2- β -TrCP1-binding-mutants are higher as a consequence of the loss of β -TrCP-mediated Nrf2 suppression. (J) AR fold-upregulation [measured by luciferase reporter assays (inset, Figure 2C)] after T-REX-mediated Keap1-targeted HNEylation in HEK293T cell lines transfected with either Nrf2-wt or the three Nrf2- β -TrCP1-binding-mutants (Figure S3H), separately. See also Figure 3A for the same analysis using an independent readout and cell line. Data are presented as Mean \pm s.d. Each bar graph are from n>3 independent biological replicates. Student's t-test was performed to determine significance. * p <0.01, ** p <0.001, *** p <0.001, and **** p <0.0001.

Figure S4.

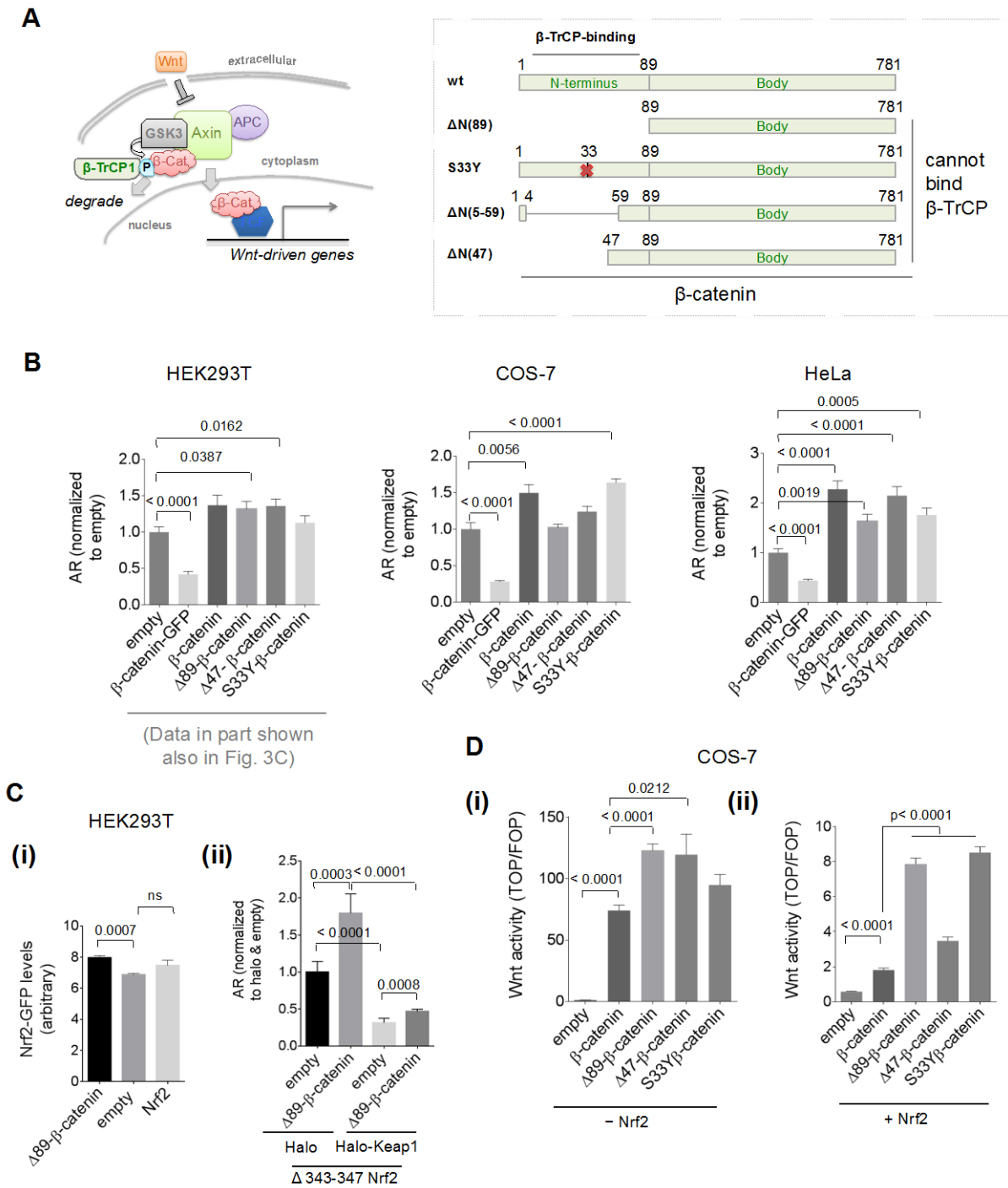


Figure S4. Regulation of AR by β -catenin is independent of its N-terminus, Related to Figure 3: (A) Schematic of the canonical Wnt signaling pathway. Inset: β -catenin-wt and N-terminal-deletion- or -point-mutants used in this study. (B) β -catenin expression upregulates AR (unless fused to GFP). Indicated cell line was transfected with ARE::firefly luciferase, CMV::renilla luciferase, and the indicated plasmid, and after 48 h, AR was measured using luminescence. Also see Fig. 3C. (C) (i) HEK293T cells were transfected with Nrf2-GFP and the indicated plasmid, and Nrf2-GFP expression was detected by measuring GFP by Flow cytometry. (ii) HEK293T cells were transfected with ARE::firefly luciferase, CMV::renilla luciferase, and the indicated plasmids, and AR was measured after 48 h. (D) COS-7 cells were transfected with TOP or FOP, CMV::renilla luciferase (see inset, Fig. 4A), the indicated plasmids and either (i) empty vector, or (ii) Nrf2. After 48 h, Wnt activity was assessed by luminescence. Data is presented as Mean \pm s.e.m. Student's t-test was performed to determine significance. * p <0.01, ** p <0.001, *** p <0.001, and **** p <0.0001.

Figure S5.

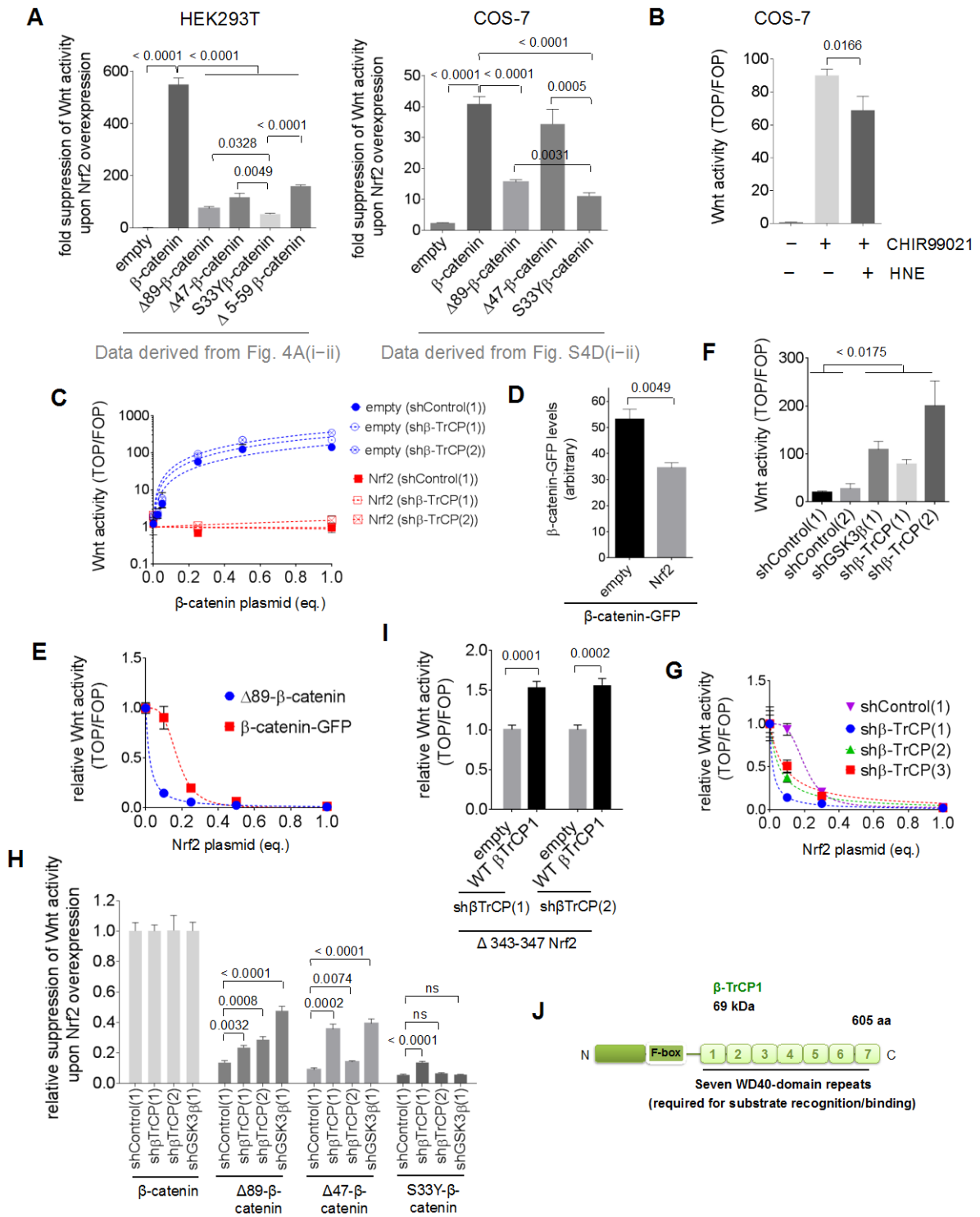


Figure S5. Regulation of β -catenin by AR depends on the N-terminus of β -catenin, Related to Figures 4 and 5. (A) The indicated cell line was transfected with TOP or FOP, CMV::renilla luciferase, the indicated plasmids and either Nrf2 or empty vector (1 equivalent). After 48 h, the fold suppression in Wnt activity caused by Nrf2 transfection was calculated by luminescence. Also see Fig. 4A and 4D. (B) COS-7 cells were transfected with TOP or FOP, CMV::renilla luciferase, empty vector, and after 24 h, were treated with either DMSO, CHIR99021 (10 μ M) or HNE (50 μ M) for 18 h. (C) The indicated HEK293T line was transfected with TOP or FOP, CMV::renilla luciferase, and varying amounts of wt- β -catenin plasmid (balanced against an empty vector). This mix was supplemented with either empty vector or Nrf2, and Wnt activity was measured after 48 h. (D) HEK293T cells were transfected with either empty vector or Nrf2 and equal amounts of β -catenin-GFP. After 48 h, GFP levels were assessed by flow cytometry. (E) HEK293T cells were transfected with TOP or FOP, CMV::renilla luciferase, the indicated β -catenin construct and varying amounts of Nrf2 (balanced against an empty vector). After 48 h, Wnt signaling was assessed by luminescence. (F) HEK293T cell lines expressing the indicated shRNA, were transfected with TOP or FOP, CMV::renilla luciferase, and empty vector. After 48 h, Wnt signaling was assessed by luminescence. (G) The indicated HEK293T line expressing the indicated shRNA was transfected with TOP or FOP, CMV::renilla luciferase, wt- β -catenin-GFP, and varying amounts of Nrf2 plasmid (balanced by an empty vector). After 48 h, Wnt signaling was assessed by luminescence. (H) The indicated HEK293T line was transfected with TOP or FOP, CMV::renilla luciferase, the indicated β -catenin construct, and 1 equivalent of either empty vector or Nrf2 plasmid. After 48 h, Wnt signaling in empty-vector- or Nrf2-transfected cells was assessed. This is expressed as fold-suppression in Wnt activity relative to that observed for wt- β -catenin for each knockdown line; i.e., a lower number means *less* suppression by Nrf2. (I) HEK293T cells expressing the indicated shRNA were transfected with TOP or FOP, CMV::renilla luciferase, 0.05 equivalents of β -TrCP1-binding-defective (Δ 343-347)-Nrf2-mutant (Fig. S3H) plasmid, 0.95 equivalents of empty vector, and either empty vector or wt-(mouse)- β -TrCP1. After 48 h, Wnt signaling was assessed by luminescence. (J) Domain structure of β -TrCP1. Data are presented as Mean \pm s.e.m. Student's t-test was performed to determine significance. * p <0.01, ** p <0.001, *** p <0.001, and **** p <0.0001.

Figure S6.

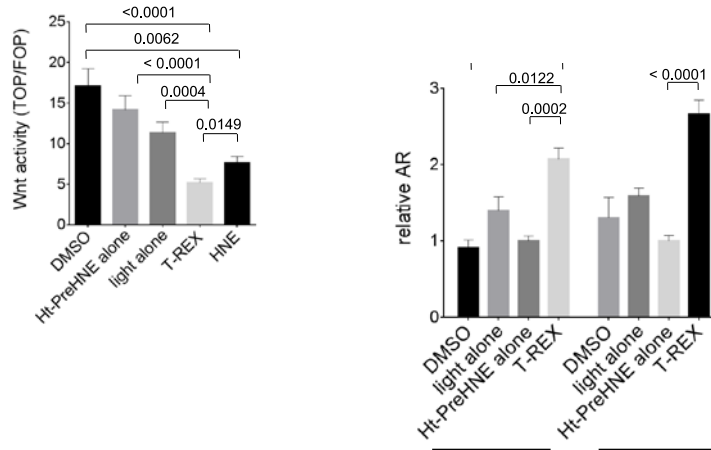


Figure S6. Wnt upregulation suppresses T-REX-mediated Keap1-alkylation-specific AR response, Related to Figure 4: (A) HEK293T cells were transfected with Halo-Keap1, Myc-Nrf2, TOP or FOP, and CMV::renilla luciferase. After 24 h, cells were exposed to T-REX conditions or indicated controls, and Wnt signaling was assessed after 18 h. (B) Wnt upregulation induced by small-molecule CHIR99021 (10 μ M) suppresses T-REX-assisted Keap1-HNEylation-specific AR. HEK293T cells were transfected with Halo-Keap1, Myc-Nrf2, ARE::firefly luiferase, and CMV::renilla luciferase. After 24 h, cells were exposed to the indicated conditions. Post light exposure, or at an equivalent point in time for controls, cells were treated with either CHIR99021 (10 μ M) or DMSO. After 18 h, AR was measured by luminescence. Each bar/point corresponds to $N > 3$. Data is presented as Mean \pm s.d. Student's t-test was performed to determine significance. * $p < 0.01$, ** $p < 0.001$, *** $p < 0.001$, and **** $p < 0.0001$.

Supplemental Tables

Table S1. Antibodies. Related to Figure 1, Figures S1 and S2, and STAR Methods section.

Antibody	Catalog No.; Supplier	Dilution	Use
Rabbit monoclonal anti-GSK3 β primary	12456S (clone D5C5Z); Cell Signaling	1:1000 1:200	WB IF
Rabbit monoclonal anti- β TRCP primary	11984 (clone D12C8); Cell Signaling	1:500 1:100	WB IF
Rabbit monoclonal anti-PTEN primary	Ab32199 (clone Y184); Abcam	1:1000	WB
Mouse monoclonal anti-Keap1 primary	Ab119403 (clone 1B4); Abcam	1:2000	WB
Mouse monoclonal anti-c-myc primary	MMS-150P (clone 9E10); Covance MMS-150P (clone 9E10); BioLegend	1:2000 1:500	WB
Rabbit polyclonal anti-Nrf2 primary	Ab137550; Abcam (Note: predicted MW of Nrf2 is still debated: see, for instance, Lau et al., 2013 <i>Antioxid Redox Signal</i> 18, 91–93)	1:1000	WB
Rabbit monoclonal anti-Tubullin primary	2125S (clone 11H10); Cell Signaling	1:5000	WB
Rabbit polyclonal anti-HaloTag [®] primary	9281; Promega	1:1000 1:200	WB IF
Rabbit polyclonal Anti-GFP primary	sc-8334; Santa Cruz Biotechnology	1:1000	WB
Monoclonal anti-gapdh-peroxidase	G9295; Sigma	1:30000	WB
Anti-mouse-HRP secondary	Ab6789; Abcam	1:7000	WB
Anti-rabbit-HRP secondary	Ab97051; Abcam	1:7000	WB
Anti-goat-HRP secondary	Ab97100; Abcam	1:7000	WB
Goat anti-mouse-FITC	1010-02, SouthernBiotec	1:1000	IF
Goat anti-rabbit Alexa-488	A11008, Invitrogen	1:1000	IF
Goat anti-rabbit Alexa-647	AB150063, AbCam	1:1000	IF

WB = western blot; IF = immunofluorescence.

Table S2. Oligonucleotides used for qRT-PCR experiments and gene cloning. Related to Figure 2, Figures S1 and S4, and STAR Methods section.

Primers for qRT-PCR	Sequence
β-TrCP1	Fwd-5'-gaggatagtcagtgggcct-3' Rev-5'-tccacaagggtccgtagaca-3'
β-TrCP2	Fwd-5'-aggtctttgtggctaggctg-3' Rev-5'-ccattacttatctggagacatctga-3'
Lef1	Fwd-5'-cttccatgtccaggtttccc-3' Rev-5'-tgtcagtggtgggatgtcc-3'
Tcf7	Fwd-5'-acatgcagctatacccaggc-3' Rev-5'-tccgggtaagtaccgaatgc-3'
c-myc	Fwd-5'-gtagtggaaaaccagcagcc-3' Rev-5'-agaaataggctgcaccgag-3'

Cloning primers	
pFN21A_HaloTag [®] -Keap1	Step1: 5'-gagcgataacgcgatgccatgcagccagatcccaggc-3' (forward primer) 5'-ggatccccgggtaccgagcccgaattcgtttaaacacaggtacagttctgctggtcaatc-3' (reverse primer)
	Step 2: 5'-gagattccggcgagccaaccactgaggatctgtactttcagagcgataacgcgatcgcc-3' (forward-extender primer) 5'-tgtagcagccggatcagcttgcctgcaggctgactc tagaggatccccgggtacc-3' (reverse-extender primer)
pFN21A_β-TrCP1 (without the HaloTag [®])	Step1: 5'-cactatagggctagcaaaagccaccatggaccggccgaggcg-3' (forward primer) 5'-atcatgtctgctcgaagcgg-3' (reverse primer)
	Step 2: 5'-aattacagctctaaggctagagtattaatacactcactatagggctagcaaaagccacc-3' (forward-extender primer) 5'-cattctagttgtggtttgtccaaactcatcaatgtatcttatcatgtctgctcgaagcgg-3' (reverse-extender primer)
“empty” pFN21a vector (control plasmid for β-TrCP1)	5'-aggctagagtattaatacactcactatagggctagcaaaagccactggaccggccgag-3' (forward primer) 5'-ctcggccgggtccaagtggctttgtagccctatagtgatcgatgctgattaatacttagcct-3' (reverse primer)
ARE::GFP reporter plasmid	Step1: 5'-caatccggtactgttgtaagccaccatggtgagcaagggcgag-3' (forward primer) 5'-cgccccgactctagagtcgcccctcactgtacagctcgtccatg-3' (reverse primer)
	Step 2: 5'-gagggtatataatggaagctcgaactccagcttggaatccggtactgttgtaaacg-3' (forward primer) 5'-ctcatcaatgtatcttatcatgtctcgaagcggccggccccgactctagagtcg-3' (reverse primer)

Primers used to generate β-catenin mutant	
$\Delta N(5-59)$ - β -catenin	Step 1: 5'-ccgagctcggatccccagcgtggacaatggctactcaatcccaagtctgtatgagtggg-3' (forward primer) 5'-caaaggtgcatgattgcgg-3' (reverse primer)
	Step 2: 5'-cgaaattaatcagactcactatagggagaccaagcttggtagcagctcggatccccag-3' (forward primer) 5'-actgaactagtcgtggaatggcaccctgctcacgcaaaggtgcatgattgcgg-3' (reverse primer)

Primers used to amplify β-catenin and β-TrCP1 from genomic DNA	
β -catenin	Region 1: 5'- cctgtcccctgagggtattg -3' (forward primer) 5'- gaaggcagctctcgtaatagcc -3' (reverse primer)
	Region 2: 5'- accatacaactgtttgaaaatccag -3' (forward primer) 5'- gatgtgcacgaacaagcaactg -3' (reverse primer)
β -TrCP1	Region 1: 5'- cctcccgtttctccttag -3' (forward primer) 5'- atctggattgttacggtatttaggc -3' (reverse primer)
	Region 2: 5'- gcagacagctatggagagtatag -3' (forward primer) 5'- gtgaagcttgaattaaaaactgc -3' (reverse primer)
	Region 3: 5'- cctctgtgaatagaaaagacggtcc -3' (forward primer) 5'- cagctccacatttctctcc -3' (reverse primer)

Primers used to sequence genomic DNA	
β -catenin	β -catenin 1: 5'-gaaacatgcagttgtaaac-3' β -catenin 2: 5'-actgcctcaaatttagct-3' β -catenin 3: 5'-tgtctcttcgtcatctgac-3' β -catenin 4: 5'-ggctcctgtgaactgc-3' β -catenin rev seq: 5'-tgcgatgcaatttcctc-3' β -catenin rev seq1: 5'-ggaacatagcagctcgtaccct-3' β -catenin rev seq2: 5'-catcctctcctcaggattgcc-3' β -catenin rev seq3: 5'-ctgtagtcaactggcagcaacag-3' β -catenin 5: 5'-cagtgcgttagctggtggg-3'
β -TrCP1	Region 1: 1btcrpfs: 5'-gggattggatcatattgctg-3' 1btcrpfs2: 5'-gcacttgcgtttcaataatgg-3' btcrp1s1: 5'-acttatctaactcggctgtgg-3'

	btreplsf1 5'-gcctgaccaacatggtgaaac-3' btreplsf2 5'-tctcttacaggcgagtaggg-3' btreplsf3 5'-ctaagcattatttctagaaagtaagtgaag-3' 1btrecpf1: 5'-cggcctggcaccacaaagg-3' 1btrecpf2: 5'-tgcgcctgagaggtgagagag-3' 1btrecpr1: 5'-cctgggcaagtatgaggtca-3' 1btrecprint: 5'-gcatttcacctgtattacatcccac-3' 1btrecpintf: 5'-gtgggatgta aatacagggtg aaatgc-3'
	Region 2: 2btrecpf1: 5'-ctggcagctagagtgggtgc-3' 2btrecpr1: 5'-ctcccttaggcatttgtagcc-3' 2btrecprs1: 5'-ggaggccatctgtgggtc-3' 2btrecprs2: 5'-cagacttgatggtcctg-3' 2btrecpfs1: 5'-ctgtgtgggacatggctctg-3'
	Region 3: btrepl3f1: 5'-ggccctttagttactgacatttaac-3' btrepl3f2: 5'-acttgcccatattcaagcag-3' btrepl3f3: 5'-ccttaatggttcagtctgtttc-3' btrepl3f4: 5'-cttgaattgctctgaaaacgtacag-3' btrepl3f5: 5'-ctttaatccgtgtattaccttcc-3'

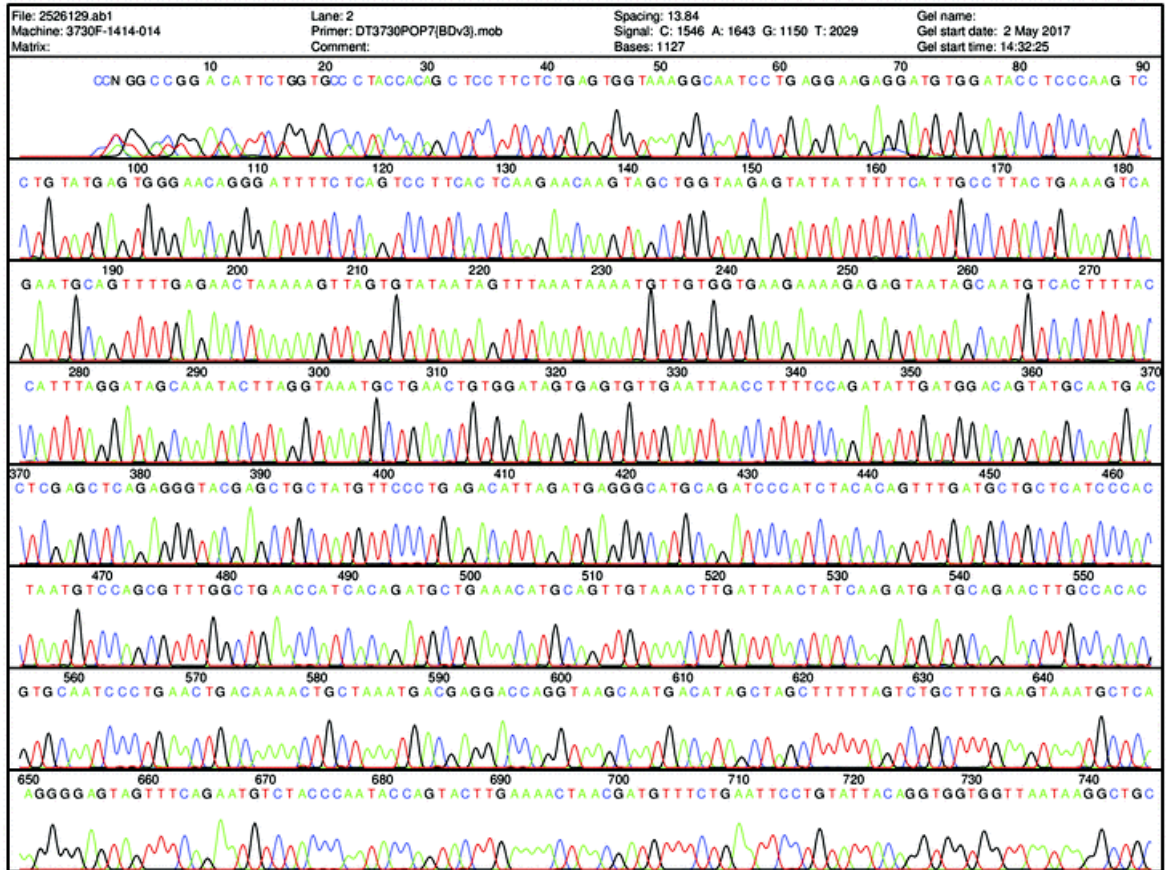
Table S3. Summary of results from sequencing of genomic DNA of β -catenin and β TrCP1 genes in HEK293T, HeLa, and COS-7 cell lines. Related to STAR Methods ‘Verification of genomic DNA sequences’.

See next 2 pages

Cell line	Gene	Protein ID	Coverage protein residues	Locus	Coverage (mismatch, relative to human)	Note
HEK293T	β -catenin	AAL89457.1	1-300	Human; Ch3; NG 013302.2	30691-31380 (0) 30515-29548 (0) 30189-29584 (0) 30168-31264 (0)	Kozak intact Introns intact
HeLa	β -catenin	AAL89457.1	1-299	Human; Ch3; NG 013302.2	30703-31380 (0) 29586-30517 (0) 29584-30189 (0) 30168-31319 (0)	Kozak intact
COS-7	β -catenin	AAL89457.1	1-80; 166-299	Human; Ch3; NG 013302.2	30691-31380 (4%) 29598-30523 (3%) 29584-30185 (2%) 29706-30353 (10%)	Kozak intact Best alignment with <i>Macaca fascicularis</i> LT160001.1
HEK293T	β TrCP1	Q9Y297.1	1-16; 249-281; 282-236; 366-435; 448-489; 489-526;	Human Ch10; NG009234.1	181953-182296 182726-183716 183492-184438 (read low quality; 19% mismatch/gaps) 184823-185663 185451-189289 186222-187122 186995-187835	
HeLa	β TrCP1	Q9Y297.1	280-326; 366-448; 449-489; 489-526	Human Ch10; NG009234.1	181953-182288 182726-183709 183479-184426	1 bp mismatch 183669 T to G Read low quality

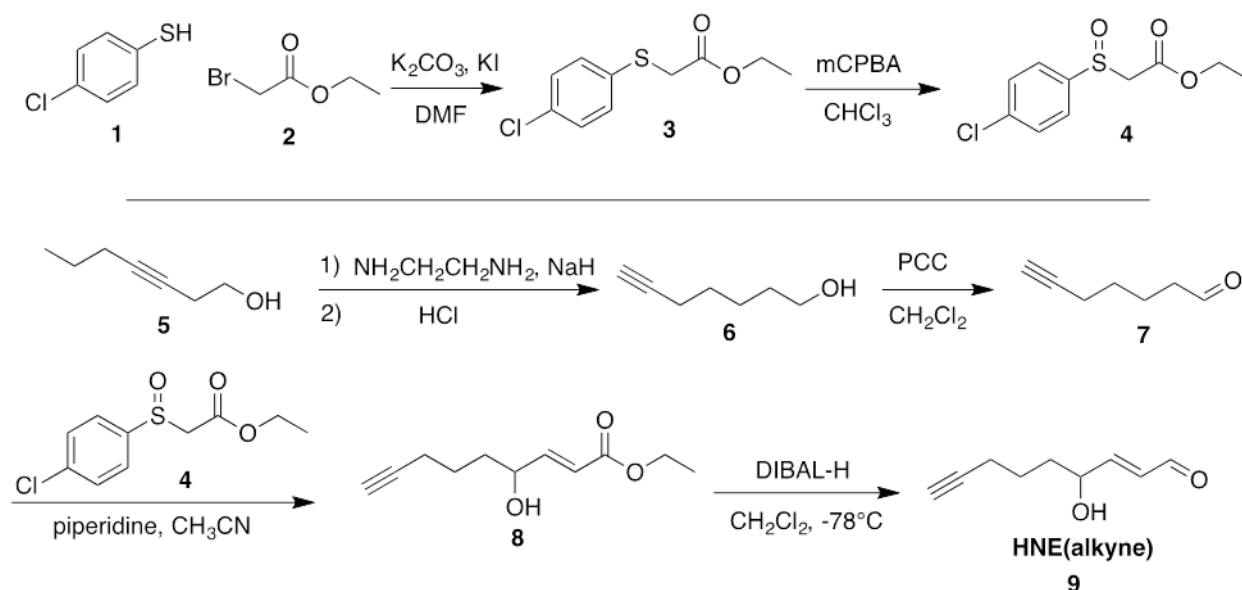
					184880-185720 185451-186289 186222-187122 186995-187955	
COS-7	β TrCP1	Q9Y297.1	249-280; 280-326; 327-366; 366-420; 457-489.	Human Ch10; NG009234.1	181952-183065 (12%) 182726-183684 (9%) 183479-184460 184839-185675 (9%) 185451-186406 (8%) 186225-187053 (9%)	(30 base pair insertion)

Table S4: Raw Sanger Sequencing Trace. 500 ng PCR amplified β -catenin genomic DNA from HEK293T cells was mixed with 1 μ L 3.2 μ M β -catenin ‘sequence 5’ (see Table S2) in a total of 18 μ L milliQ-water. The sample was sent to Cornell University sequencing facility. Raw Sanger sequencing trace is shown. **Related to STAR Methods ‘Verification of genomic DNA sequences’.**



Reagents for Chemical Synthesis. Unless otherwise noted, all reactions were carried out in oven-dried glassware under an atmosphere of nitrogen and stirred magnetically. All chemical reagents were from either Fisher or Sigma in highest available purity. Tetrahydrofuran (THF) and ether were purified by distillation from sodium/benzophenone. Acetonitrile (CH_3CN) and dichloromethane (CH_2Cl_2) were distilled from CaH_2 . When specified, samples were concentrated using a rotary-evaporator attached to a diaphragm pump followed by removal of residual solvent using a vacuum pump. Analytical thin layer chromatography (TLC) was performed using silica gel 60 F0254 pre-coated glass plates (0.25 mm). TLC plates were analyzed by short wave UV illumination or permanganate stain. ^1H and ^{13}C NMR were obtained on a Varian INOVA 400 MHz spectrometer in CDCl_3 . Ozonolysis was performed on Welsbach ozonator per manufacturer's instructions. The NMR spectra of HNE alkyne and HtpreHNE alkyne have been reported previously (Fang, et al., 2013).

Scheme S1: Synthesis of HNE alkyne



4-chlorothiophenol **1** (7.5 g, 51.86 mmol, 1 eq.) and K_2CO_3 (10.75 g, 77.79 mmol, 1.5 eq.) was added to 30 mL dry dimethylformamide at room temperature and stirred for 30 minutes. To this solution was further added ethyl bromoacetate **2** (6.90 mL, 62.23 mmol, 1.2 eq.) and KI (2.58 g, 15.56 mmol, 0.3 eq.); the reaction was then allowed to stir overnight at room temperature. The reaction was then quenched with brine and the product was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 , filtered, and concentrated in vacuo to yield the sulfate **3** as a clear liquid, which was carried on without purification (13.0 g, 100% yield): 1H NMR (300 MHz) δ 1.23 (3H, t, $J = 7.1$ Hz), 3.60 (2H, s), 4.16 (2H, q, $J = 7.1$ Hz), 7.27 (2H, m), 7.36 (2H, m).

Sulfate **3** (14.3 g, 61.99 mmol, 1 eq.) was dissolved in chloroform and cooled to $0^\circ C$. 3-chloroperoxybenzoic acid (77%, 13.9 g, 61.99 mmol, 1 eq.) was slowly added into the solution, and the reaction was allowed to stir overnight while the reaction temperature was raised naturally to room temperature. The reaction was then quenched with saturated aqueous $NaHCO_3$, extracted with chloroform, washed, dried, and concentrated. The resulting product was purified by flash chromatography with Hexanes:EtOAc (5:1 v/v) to yield the sulfoxide **4** as a yellowish liquid (13.0 g, 100% yield): 1H NMR (400 MHz) δ 1.24 (3H, t, $J = 7.1$ Hz), 3.67 (1H, d, $J = 13.7$ Hz), 3.85 (1H, d, $J = 13.7$ Hz), 4.17 (2H, q, $J = 7.1$ Hz), 7.53 (2H, m), 7.64 (2H, m).

NaH (60% in mineral oil, 4.14 g, 103 mmol, 4.2 eq.) was added to ethylenediamine (40 mL, 591 mmol, 24 eq.) in a 500 mL round bottom flask at $0^\circ C$ under an inert atmosphere and stirred at room temperature for 1 hour. The reaction was then heated at $60^\circ C$ for another hour. The mixture was then cooled to $45^\circ C$ and 3-heptyn-1-ol **5** (3.0 mL, 24.6 mmol, 1 eq.) was added. The reaction was heated to $60^\circ C$ for 1 hour, then cooled to $0^\circ C$, and quenched with 1N HCl (~30 mL). The reaction was extracted with Et_2O , and the organic was washed, dried, filtered, and concentrated in vacuo to yield the alcohol **6** as a yellow oil, which was carried on without

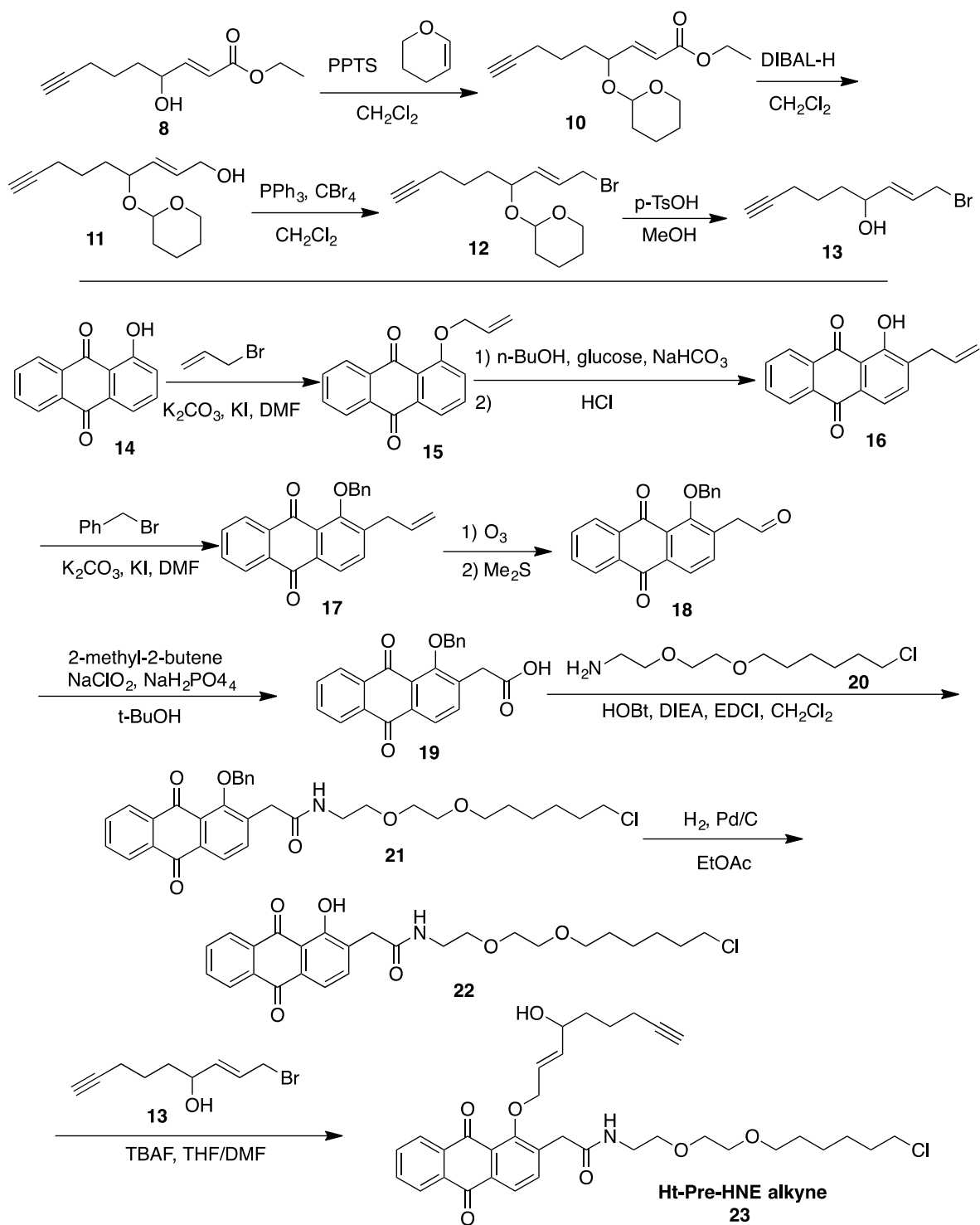
purification (2.25 g, 82% yield): $^1\text{H-NMR}$ (400 MHz) δ 1.13-1.69 (4H, m), 1.95 (1H, t, $J = 2.6$ Hz), 2.21 (2H, td, $J = 2.6, 6.8$ Hz), 3.66 (2H, t, $J = 6.4$ Hz).

PCC (8.62 g, 40.1 mmol, 2 eq.) was mixed with roughly an equal volume of Celite in 20 mL CH_2Cl_2 and stirred for 30 minutes. Alcohol **6** (2.25 g, 20.06 mmol, 1 eq.) was then dissolved in 20 mL CH_2Cl_2 , which was then added to the reaction and stirred for 3 hours at room temperature. The mixture was then filtered through Celite. The filtrate was concentrated, re-dissolved in Et_2O , and filtered again through a silica plug in a 600 mL fritted funnel with ~100 g silica, using Et_2O as a running solvent. The filtrate was concentrated in vacuo at room temperature to yield the aldehyde **7** as a light yellow volatile oil, which was carried on without further purification (1.2 g, 54% yield): $^1\text{H-NMR}$ (400 MHz) δ 1.53-1.62 (2H, m), 1.73-1.82 (2H, m), 1.96 (1H, t, $J = 2.9$ Hz), 2.23 (2H, td, $J = 2.8, 7.1$ Hz), 2.48 (2H, td, $J = 1.7, 7.3$ Hz), 9.78 (1H, t, $J = 1.7$ Hz).

Sulfoxide **4** (1.93 g, 9.08 mmol, 1 eq.) was dissolved in CH_3CN (40 mL), and piperidine (1.79 mL, 18.16 mmol, 2 eq.) and aldehyde **7** (1.2 g, 10.9 mmol, 1.2 eq.) were added to the mixture. The reaction was stirred overnight at room temperature followed by quenching with aqueous NH_4Cl . The acetonitrile was evaporated off, and the remaining mixture was extracted with CH_2Cl_2 . The organic layer was washed, dried, filtered, and concentrated in vacuo. The residue was purified via flash chromatography with Hexanes:EtOAc (10:1 v/v) to yield the ester **8** as a yellow oil (0.83 g, 62% yield): $^1\text{H-NMR}$ (400 MHz) δ 1.30 (3H, t, $J = 7.1$ Hz), 1.58-1.82 (4H, m), 1.97 (1H, t, $J = 2.7$ Hz), 2.25 (2H, td, $J = 2.5, 6.6$ Hz), 4.21 (2H, q, $J = 7.1$ Hz), 4.37 (1H, m), 6.05 (1H, dd, $J = 1.7, 15.7$ Hz), 6.95 (1H, dd, $J = 4.9, 15.7$ Hz).

Ester **8** (0.4 g, 2.04 mmol) was dissolved in CH_2Cl_2 (20 mL) and the reaction was cooled to -78 °C. DIBAL-H (1.0 M in hexanes, 4.0 mL, 4.0 mmol, 1.3 eq.) was added dropwise to the mixture. After stirring 1 hour at the same temperature, the reaction was quenched with saturated potassium sodium tartrate tetrahydrate aqueous solution and extracted with Et_2O . The organic layer was washed with brine, dried over anhydrous Na_2SO_4 , and concentrated in vacuo. The residue was purified via flash chromatography with Hexanes:EtOAc (8:1 v/v) to yield the desired HNE alkyne **9** (0.035 g, 11% yield): $^1\text{H-NMR}$ (400 MHz) δ 1.58-1.88 (4H, m), 2.00 (1H, t, $J = 2.7$ Hz), 2.27 (2H, td, $J = 2.7, 6.7$ Hz), 2.71 (1H, br), 4.45-4.55 (1H, m), 6.32 (1H, ddd, $J = 1.6, 7.0, 15.6$ Hz), 6.86 (1H, dd, $J = 4.5, 15.7$ Hz), 9.57 (1H, d, $J = 7.9$ Hz). $^{13}\text{C-NMR}$ (101 MHz) δ 18.17, 24.01, 35.15, 69.08, 70.45, 83.78, 130.67, 159.18, 193.83.

Scheme S2: Synthesis of Ht-PreHNE alkyne



Ester **8** (0.65 g, 3.31 mmol, 1 eq.) was dissolved in 20 mL CH₂Cl₂, and 3,4-dihydropyran (1.8 mL, 19.9 mmol, 6 eq.) and pyridinium *p*-toluenesulfonate (0.17 g, 0.66 mmol, 0.2 eq.) were added under an inert atmosphere. The reaction was stirred at room temperature overnight, and then quenched with saturated aqueous NaHCO₃ and extracted with CH₂Cl₂. The organic layer was washed, dried, and concentrated in vacuo to yield the THP-protected ester **10** as a colorless liquid, which was carried on without purification (0.8 g, 86% yield): ¹H NMR (300 MHz) δ 1.14-1.92 (13H, m), 1.93-1.99 (1H, m), 2.11-2.36 (2H, m), 3.40-3.57 (2H, m), 3.75-3.97 (2H, m), 4.20 (2H, q, *J* = 7.1 Hz), 4.28-4.40 (1H, m), 4.51-4.79 (1H, m), 6.01 (1H, dd, *J* = 15.7, 1.4 Hz), 6.95 (1H, dd, *J* = 15.7, 5.3 Hz).

THP protected ester **10** (1.1 g, 3.92 mmol, 1 eq.) was dissolved in 20 mL CH₂Cl₂ and cooled to 0°C. DIBAL-H (1.0 M in hexanes, 8.0 mL, 8.0 mmol, 2 eq.) was added dropwise, and the reaction was allowed to stir for 90 minutes. The reaction was quenched with saturated aqueous potassium sodium tartrate tetrahydrate and extracted with CH₂Cl₂. The organic layer was washed, dried, and concentrated in vacuo to yield the alcohol **11**, which was carried on without purification (0.9 g, 95% yield): ¹H NMR (300 MHz) δ 1.95 (1H, m), 2.22 (2H, td, *J* = 7.1, 2.6 Hz), 3.35-3.59 (2H, m), 3.78- 3.95 (2H, m), 4.04-4.32 (6H, m), 4.65 (1H, t, *J* = 3.4 Hz), 4.70 (1H, m), 4.93 (1H, m), 5.54 (1H, ddt, *J* = 15.6, 7.9, 1.2 Hz) 5.78 (1H, m), 5.85 (1H, m).

Allylic alcohol **11** (0.9 g, 3.78 mmol, 1 eq.) was dissolved in 20 mL CH₂Cl₂ and cooled to 0°C, upon which carbon tetrabromide (1.31 g, 3.97 mmol, 1.05 eq.) and triphenylphosphine (1.14 g, 4.34 mmol, 1.15 eq.) were added. The reaction was allowed to stir for 20 minutes, and subsequently quenched with aqueous NaHCO₃ and extracted with CH₂Cl₂. The organic layer was washed, dried, and concentrated in vacuo. The residue was purified three times by trituration with Et₂O, with the triphenylphosphine oxide precipitate being filtered out each time. The remaining filtrate was concentrated in vacuo to yield the bromide **12** (0.6 g, 53% yield): ¹H NMR (400 MHz) δ 1.95 (1H, t, *J* = 2.7 Hz), 2.23 (2H, m), 3.50 (2H, m), 3.85 (2H, t, *J* = 9.9 Hz), 3.96 (2H, t, *J* = 7.4 Hz), 4.15 (2H, dt, *J* = 7.1, 6.7 Hz), 4.62 (1H, t, *J* = 3.3 Hz), 5.60 (1H, dd, *J* = 15.1, 7.4 Hz), 5.85 (2H, m).

Allylic bromide **12** (0.56 g, 2.0 mmol, 1 eq.) and *p*-toluenesulfonic acid (0.15 g, 0.80 mmol, 0.4 eq.) were dissolved in 25 mL methanol, and the reaction was allowed to stir overnight at room temperature. The reaction was quenched with saturated aqueous NaHCO₃ and the product was extracted with ethyl acetate. The organic layer was washed, dried, concentrated in vacuo, and purified via flash chromatography with Hexanes:EtOAc (5:1 v/v) to yield the deprotected allylic bromide **13** (0.25 g, 58% yield): ¹H NMR (400 MHz) δ 1.51-1.71 (4H, m), 1.98 (1H, t, *J* = 2.6 Hz), 2.20-2.35 (3H, m), 3.97 (2H, d, *J* = 7.4 Hz), 4.17 (1H, q, *J* = 5.7 Hz), 5.75-5.86 (1H, m), 5.91 (1H, dtd, *J* = 14.7, 7.3, 1.0 Hz).

1-hydroxyanthraquinone **14** (2.0 g, 8.92 mmol, 1 eq.) and K₂CO₃ (3.7 g, 26.76 mmol, 3 eq.) were dissolved in 40 mL dimethylformamide under an inert atmosphere and stirred for 30 minutes. Allyl bromide (1.16 mL, 13.38 mmol, 1.5 eq.) and KI (0.592 g, 3.57 mmol, 0.4 eq.) were then added, and the reaction was heated to 45°C with an oil bath and allowed to stir for 4 hours, after which the heat was turned off and the reaction was allowed to stir overnight until completion. The reaction was quenched with brine and extracted with ethyl acetate. The organic layer was washed, dried, and concentrated in vacuo to yield the allyl ether **15** as an orange solid, which was carried on without purification (2.23 g, 95% yield): ¹H NMR (300 MHz) δ 4.79 (2H, dt, *J* = 5.0, 1.7 Hz),

5.41 (1H, ddt, $J = 10.6, 1.5, 1.5$ Hz), 5.69 (ddt, $J = 17.3, 1.7, 1.7$ Hz), 6.16 (1H, ddt, $J = 17.2, 10.6, 4.8$ Hz), 7.34 (1H, dd, $J = 8.4, 1.1$ Hz), 7.74 (3H, m), 7.98 (1H, dd, $J = 7.7, 1.1$ Hz), 8.27 (2H, m).

Allylated hydroxyanthraquinone **15** (2.23 g, 8.43 mmol, 1 eq.) was dissolved in 40 mL *n*-butanol under an inert atmosphere. Glucose (7.60 g, 42.2 mmol, 5 eq.) was added to the mixture, which was then heated to 80°C for 10 minutes. NaHCO₃ was subsequently added to the reaction, which was then heated to 130°C for 90 minutes until complete. The reaction was then neutralized with 1N HCl and cooled slowly to 0°C, causing the alcohol **16** to crystallize out of the organic layer. This product was filtered out as a yellow-orange solid and carried on without further purification (2.12 g, 92% yield): ¹H NMR (300 MHz) δ 3.54 (2H, d, $J = 6.7$ Hz), 5.14 (1H, q, $J = 1.5$ Hz), 5.16-5.23 (1H, m), 5.88-6.14 (1H, m), 7.57 (1H, dp, $J = 7.7, 0.6$ Hz), 7.73-8.00 (3H, m), 8.10-8.47 (2H, m), 13.02 (1H, q, $J = 0.6$ Hz).

Hydroxyanthraquinone **16** (1.3 g, 4.92 mmol, 1 eq.) was dissolved in 30 mL DMF, and K₂CO₃ (4.08 g, 29.51 mmol, 6 eq.), benzyl bromide (1.76 mL, 14.76 mmol, 3 eq.), and KI (0.245 g, 1.48 mmol, 0.3 eq.) were added. The resulting mixture was stirred at 65°C for 1 hour, and then cooled to room temperature. The reaction was diluted with water and extracted with EtOAc. The organic extracts were washed sequentially with water, brine, and 1N HCl, then dried and concentrated in vacuo. The residue was recrystallized in refluxed hexanes to provide the benzyl-protected ether **17** as an orange solid (1.3 g, 75% yield): ¹H NMR (400 MHz) δ 3.50 (2H, dt, $J = 6.6, 1.5$ Hz), 5.05 (2H, s), 5.09-5.29 (2H, m), 5.92 (1H, ddt, $J = 16.7, 10.1, 6.6$ Hz), 7.27-7.50 (6H, m), 7.69-7.78 (3H, m), 8.20-8.42 (2H, m).

All further reactions were done under dim light and appropriately protected from strong light due to light sensitivity.

Benzylated hydroxyanthraquinone **17** (1.3 g, 3.67 mmol) was dissolved in 30 mL CH₂Cl₂ and cooled to -78°C. O₃ was bubbled through the solution for 15 minutes, and then Me₂S (10 mL) was added. The reaction mixture was allowed to warm up to room temperature naturally while stirring. The mixture was then concentrated in vacuo, and the residue was diluted with EtOAc and washed with water. The organic layer was dried, concentrated in vacuo, and recrystallized with refluxed hexanes to afford aldehyde **18** as a yellow solid (0.9 g, 81% yield): ¹H NMR (400 MHz) δ 4.71 (2H, d, $J = 5.5$ Hz), 4.93 (2H, m), 7.27-7.50 (5H, m), 7.62 (1H, d, $J = 7.9$ Hz), 7.71-7.88 (2H, m), 8.17-8.40 (3H, m), 9.66 (1H, s).

Aldehyde **18** (0.65 g, 1.82 mmol, 1 eq.) and 2-methyl-2-butene (20 mL) were dissolved in *t*-BuOH (40 mL) and cooled to 0°C. NaH₂PO₄•H₂O (1.76 g, 12.77 mmol, 7 eq.) and NaClO₂ (80%, 1.85 g, 16.41 mmol, 9 eq.) were dissolved in water (20 mL) and added to the above solution dropwise. The resulting mixture was allowed to warm up to room temperature and stirred overnight. The reaction was quenched with 0.1 N HCl (150 mL) and extracted with EtOAc. The organic extracts were washed, dried, filtered, and concentrated in vacuo. The residue was recrystallized in refluxed hexanes to yield the carboxylic acid **19** as a yellow solid (0.45 g, 67% yield): ¹H NMR (400 MHz) 4.99 (2H, s), 7.31-7.42 (3H, m), 7.53 (2H, d, $J = 7.2$ Hz), 7.68 (1H, d, $J = 7.9$ Hz), 7.73-7.86 (2H, m), 8.20 (1H, d, $J = 8.0$ Hz), 8.25-8.36 (2H, m).

Acid **19** (0.45 g, 1.21 mmol, 1 eq.) and 2-(2-(6-chlorohexyloxy)ethoxy)ethanamine **20** (0.27 g, 1.21 mmol, 1 eq.) were dissolved in 15 mL CH₂Cl₂ and cooled to 0°C. HOBt (hydrate with 20% H₂O, 0.245 g, 1.45 mmol, 1.2 eq.), DIEA (0.631 mL, 3.63 mmol, 3 eq.), and EDCI (0.324 g, 1.69 mmol, 1.4 eq.) were sequentially added. The reaction was warmed to room temperature naturally and stirred overnight. The reaction was then quenched with water and extracted with CH₂Cl₂. The organic layers were washed, dried, concentrated in vacuo, and purified via flash chromatography using Hexanes:EtOAc (1:2 v/v) to yield amide **21** as a yellow solid (0.62 g, 86% yield): ¹H NMR (300 MHz) δ 1.31-1.62 (4H, m), 1.62-1.79 (2H, m), 3.27-3.57 (14H, m), 5.00 (2H, s), 6.27 (1H, br), 7.26-7.51 (3H, m), 7.54-7.67 (2H, m), 7.76-7.85 (3H, m), 8.17 (1H, t, *J* = 7.6 Hz), 8.28 (2H, ddd, *J* = 7.3, 6.8, 2.4 Hz).

Amide **21** (0.30 g, 0.519 mmol, 1 eq.) was dissolved in EtOAc (30 mL) in a three-necked round-bottom flask and 10% Pd/C (51.9 mg) was added. The solution was degassed and refilled with hydrogen gas (1 atm) at room temperature. The resulting mixture was stirred for 1 hour. The reaction mixture was then filtered through Celite and concentrated in vacuo to yield the phenol **22** as a yellow solid, which was carried on without purification (0.18 g, 72% yield): ¹H NMR (400 MHz) δ 1.47-1.64 (6H, m), 1.71-1.82 (2H, m), 3.26-3.73 (14H, m), 6.49 (1H, br), 7.46-7.76 (1H, m), 7.78-8.02 (3H, m), 8.22-8.42 (2H, m), 13.30 (1H, s).

Hydroxyanthraquinone **22** (0.1 g, 0.2 mmol, 1 eq.) and TBAF (0.112 g, 0.4 mmol, 2 eq.) were dissolved in THF (2 mL) and DMF (2 mL). Bromide **13** (0.177 g, 0.8 mmol, 4 eq.) was added and the resulting mixture was stirred at room temperature for 7.5 hours. The reaction was quenched with water and extracted with EtOAc. The organic layer was concentrated in vacuo, and the residue was purified via flash chromatography using Hexanes:EtOAc (1:3 v/v) to afford product Ht-Pre-HNE alkyne **23** as a yellow solid (0.069 g, 55% yield). The ¹H and ¹³C NMR data of the purified product were identical to those previously reported. (Fang, et al., 2013; Parvez, et al., 2015; Parvez, et al., 2016)