Proteome-wide covalent ligand discovery in native biological systems

Keriann M. Backus, Bruno E. Correia, Kenneth M. Lum, Stefano Forlì, Benjamin D. Horning, Gonzalo E. González-Páez, Sandip Chatterjee, Bryan R. Lanning, John R. Teijaro, Arthur J. Olson, Dennis W. Wolan & Benjamin F. Cravatt

Small molecules are powerful tools for investigating protein function and can serve as leads for new therapeutics. Most human proteins, however, lack small-molecule ligands, and entire protein classes are considered ‘undruggable’. Fragment-based ligand discovery can identify small-molecule probes for proteins that have proven difficult to target using high-throughput screening of complex compound libraries. Although reversibly binding ligands are commonly pursued, covalent fragments provide an alternative route to small-molecule probes, including those that can access regions of proteins that are difficult to target through binding affinity alone. Here we report a quantitative analysis of cysteine-reactive small-molecule fragments screened against thousands of proteins in human proteomes and cells. Covalent ligands were identified for >700 cysteines found in both druggable proteins and proteins deficient in chemical probes, including transcription factors, adaptor/scaffolding proteins, and uncharacterized proteins. Among the atypical ligand–protein interactions discovered were compounds that react preferentially with pro- (inactive) caspases. We used these ligands to distinguish extrinsic apoptosis pathways in human cell lines versus primary human T cells, showing that the former is largely mediated by caspase-8 while the latter depends on both caspase-8 and -10. Fragment-based covalent ligand discovery provides a greatly expanded portrait of the ligandable proteome and furnishes compounds that can illuminate protein functions in native biological systems.

A major constraint of fragment-based ligand discovery (FBLD) methods is their reliance on assaying purified proteins. This aspect has restricted FBLD to proteins that can be produced in large quantities, and it accordingly remains unclear how many human proteins can be targeted by small molecules or whether these interactions can be optimized to furnish chemical probes for studying protein function in complex biological systems. We aimed to address these questions on a global scale by performing a quantitative analysis of the interactions between fragment electrophiles and thousands of cysteine residues in human proteomes and cells.

We adapted a chemical proteomic method for quantifying cysteine reactivity—termed isotopic tandem orthogonal proteolysis-activity-based protein profiling (isoTOP-ABPP)—to perform covalent FBLD in native biological systems. Lysate or intact cells are pre-treated with dimethylsulfoxide (DMSO) or an electrophilic small-molecule fragment and then exposed to a broad-spectrum cysteine-reactive probe, iodoacetamide (IA)-alkyne 1 (Fig. 1a). Proteins harbouring IA-alkyne-labelled cysteine residues from DMSO- and fragment-treated samples are then conjugated by copper-mediated azide-alkyne cycloaddition chemistry to isotopically differentiated azide-biotin tags (heavy and light, respectively), combined, enriched by streptavidin, and proteolytically digested on-bead to yield isotopic peptide pairs that are analysed by liquid chromatography–mass spectrometry (LC-MS). Quantification of MS1 chromatographic peak ratios for peptide pairs identifies fragment-competited Cys residues as those displaying high competition ratios, or R values, in DMSO/fragment comparisons.

We constructed a fragment library predominantly containing chloroacetamide or acrylamide electrophiles (Fig. 1b and Extended Data Fig. 1), which are well-characterized cysteine-reactive groups. These electrophiles were appended to structurally diverse small-molecule recognition (or binding) elements to create library members

Figure 1 | Proteome-wide screening of covalent fragments.

a. General protocol for competitive isoTOP-ABPP. Competition ratios, or R values, are measured by dividing the MS1 ion peaks for IA-alkyne (1)-labelled peptides in DMSO-treated (heavy, blue) versus fragment-treated (light, red) samples. LC/LC-MS/MS, multidimensional liquid chromatography-tandem mass spectrometry. b. General structure of electrophilic fragment library, in which the reactive (electrophilic) and binding groups are coloured green and black, respectively. c. Competitive isoTOP-ABPP analysis of the MDA-MB-231 cell proteome pre-treated with the electrophilic 3,5-di(trifluoromethyl)aniline chloroacetamide 3 and acrylamide 14 fragments, along with the non-electrophilic acrylamide analogue 17 (500μM each). Proteomic reactivity values, or liganded cysteine rates, for fragments were calculated as the percentage of total cysteines with R values ≥ 4 in DMSO/fragment (heavy/light) comparisons. d. Representative MS1 peptide ion chromatograms from competitive isoTOP-ABPP experiments marking liganded cysteines selectively targeted by one of three fragments, 3, 14 and 23.

1Department of Chemical Physiology, The Scripps Research Institute, La Jolla, California 92370, USA. 2Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, California 92370, USA. 3Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, California 92370, USA. 4Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, California 92370, USA.

*These authors contributed equally to this work.
with an average molecular weight of 284 Da. Since our goal was to probe the ligandability of cysteines in the human proteome, we screened the electrophile library at a high concentration (500 μM) similar to the compound concentrations used in FBLD experiments. A subset of the fragment library was initially assayed by competitive profiling in a human MDA-MB-231 breast cancer cell line proteome using IA-rhodamine probe, which permitted SDS–polyacrylamide gel electrophoresis (SDS–PAGE) detection of cysteine reactivity events. This experiment identified several proteins that showed reductions in IA-rhodamine labelling in the presence of one or more fragments (Extended Data Fig. 2a).

We then used competitive isoTOP-ABPP to globally map human proteins and the cysteine residues within these proteins that are targeted by fragment electrophiles. Each fragment was tested against two human cancer cell proteomes (MDA-MB-231 and Ramos cells), and most fragments were screened in duplicate against at least one of these proteomes. On average, 927 cysteines were quantified per data set, and we required that individual cysteines were quantified in at least three data sets for interpretation. On the basis of these criteria, ~6,150 cysteines from ~2,900 proteins were quantified in aggregate across all data sets with an average quantification frequency of 22 data sets per cysteine (Extended Data Fig. 2b). Fragment–competed cysteine residues, or ‘liganded’ cysteines, were defined as those showing >75% reductions in IA-alkyne labelling (R values ≥ 4). To minimize the potential for false positives, only cysteines that showed R values ≥ 4 in two or more data sets and met additional criteria for data quality control (see Supplementary Information) were considered as targets of the fragment electrophiles. The proteomic reactivity values, or liganded cysteine rates, of individual fragments were then calculated as the percentage of liganded per total quantified cysteines in isoTOP-ABPP experiments performed on that fragment.

Most fragment electrophiles showed a tempered reactivity across the human proteome, with a median liganded cysteine rate of 3.8% for the library (Extended Data Fig. 2c). Substantial differences in reactivity were observed, however, with individual electrophiles showing liganded cysteine rates of <0.1% and others displaying rates >15% (Extended Data Fig. 2c). A subset of fragments was also screened at lower concentrations (25–50 μM), which confirmed that their proteomic reactivities were concentration-dependent (Extended Data Fig. 2d). The relative reactivity of fragment electrophiles was similar in MDA-MB-231 and Ramos cell proteomes (Extended Data Fig. 2e), indicating that this parameter is an intrinsic property of the compounds. Fragments also showed consistent reactivity profiles when assayed in biological replicate experiments (Extended Data Fig. 2f). We found that the proteomic reactivity of fragment electrophiles was only marginally correlated with their glutathione addition potential, which is a commonly used surrogate assay for measurements of proteinaceous cysteine reactivity (Extended Data Fig. 2g). We attribute these differences to the impact of the recognition element of fragment electrophiles on their interactions and reactivity with proteins.

A comparison of fragments 3, 14, 17, and 23–26 provided insights into the relative proteomic reactivity of different electrophilic groups coupled to a common recognition element (3,5-bis(trifluoromethyl)phenyl group). Chloroacetamide 3 exhibited greater reactivity than acrylamide 14 (Fig. 1c), with cyanoacrylamide 23, but not more sterically congested electrophiles (24–26) exhibiting similar reactivity to 14 (Extended Data Fig. 2h). Importantly, the non-electrophilic acetamide control fragment 17 showed negligible activity in competitive isoTOP-ABPP experiments (Fig. 1c), indicating that the vast majority of detected fragment–cysteine interactions reflected covalent reactions versus non-covalent binding events. Also in support of this conclusion, ‘clickable’ alkyne analogues of 3 and 14 (compounds 19 and 18, respectively) exhibited different concentration-dependent proteome labelling profiles (19 > 18; Extended Data Fig. 2i) that mirrored the respective liganded cysteine rates of 3 and 14 determined by isoTOP-ABPP (3 > 14; Fig. 1c). Despite the greater overall proteomic reactivity of 3 relative to 14 and 23, we found clear examples of cysteines that were preferentially liganded by the latter fragments (Fig. 1d and Supplementary Table 1).

Across all isoTOP-ABPP data sets combined, 758 liganded cysteines were identified on 637 distinct proteins, which corresponded to ~12% and 22% of the total quantified cysteines and proteins, respectively (Fig. 2a and Supplementary Table 1). Only a modest fraction of the proteins harbouring liganded cysteines were found in the DrugBank database (14%; Fig. 2b), indicating that the fragment electrophiles targeted many proteins that lack small-molecule probes. Among protein targets with known covalent ligands, the fragment electrophiles frequently targeted the same cysteine residues as these known ligands (Extended Data Table 1a). For one of these targets—the protein kinase BTK—we confirmed that interaction with the drug ibrutinib could be detected by isoTOP-ABPP, which also identified a known ibrutinib off-target—MAP2K7 (ref. 20)—in Ramos cell lysates (Extended Data Fig. 3a).

DrugBank proteins with liganded cysteines mostly originated from classes that are regarded as druggable, including enzymes, channels and transporters (Fig. 2c). Non-DrugBank proteins with liganded cysteines, on the other hand, showed a broader class distribution that included proteins, such as transcription factors and adaptor/scaffolding proteins, which are considered challenging to target with small-molecule ligands (Fig. 2c). We previously found that active-site and redox-active cysteines show, in general, greater intrinsic reactivity (as measured with the IA-alkyne probe) compared with other cysteines. While this heightened reactivity appears to be a contributory factor to the ligandability of cysteines, as reflected in the high proportion of hyperreactive (and active-site and redox-active) cysteines discovered as targets of fragment electrophiles, liganded cysteines were also well represented function of their intrinsic reactivity with the IA-alkyne probe. Cysteine reactivity values (left y-axis) were taken from ref. 12, where lower ratios correspond to higher cysteine reactivity. A moving average with a step-size of 50 is shown in blue for the percentage of liganded cysteines within each reactivity bin (percentage values shown on right y-axis).
across a broad range of intrinsic reactivities and included many non-active residues (Fig. 2d, Extended Data Fig. 3b, c and Supplementary Discussion). Finally, most proteins were found to harbour a single liganded cysteine among the several cysteines that were, on average, quantified per protein by isoTOP-ABPP (Extended Data Fig. 3d, e and Supplementary Discussion).

Ligated cysteines, including those found in active and non-active sites of proteins, displayed strikingly distinct structure–activity relationships (SARs) with the fragment electrophile library (Fig. 3a, Extended Data Fig. 3f–l, Supplementary Table 1 and Supplementary Discussion). We also found that, for the majority of liganded cysteines (>60%), electrophile (IA-alkyne or fragment) reactivity was blocked by heat denaturation of the proteome, while only a modest fraction of unliganded cysteines (~20%) showed decreased IA-alkyne labelling after heat denaturation (Extended Data Fig. 3m, n and Supplementary Discussion). These results indicate that the ligand–cysteine interactions are, in general, specific, in that they depend on both the binding groups of ligands and structured sites in proteins (see Supplementary Discussion).

We next asked whether docking could predict sites of fragment electrophile reactivity. Covalent docking programs have recently been introduced to discover ligands that target pre-specified cysteines in proteins\textsuperscript{21}, here, however, we aimed to assess computationally the relative ligandability of all cysteines within a protein and match these outputs to the data acquired by isoTOP-ABPP (see Supplementary Discussion). The ranking of our computational predictions matched the experimental data for the majority of proteins investigated (that is, cases in which the top predicted ligandable cysteine matched the liganded cysteine determined by isoTOP-ABPP) (Fig. 3b, c and Extended Data Table 2). We also found that cysteines predicted to be ligandable were much more likely to have been detected by isoTOP-ABPP and exhibit heat-sensitive IA-alkyne reactivity (Extended Data Fig. 3o, p and Extended Data Table 2). These results indicate that reactive docking can provide a good overall prediction of the ligandability of cysteines.

To determine the functional impact of ligand–cysteine interactions mapped by isoTOP-ABPP, we initially selected two enzymes—the protein methyltransferase PRMT1 and the MAP3 kinase MLTK (also known as ZAK)—that possessed liganded cysteines with previously uncharacterized functions (Fig. 5a, b and Supplementary Discussion). We next evaluated proteins that possess previously uncharacterized liganded cysteines, including the nucleotide biosynthetic enzyme IMPDH2 and p53-induced phosphatase TIGAR (p53 also known as TP53). In both cases, we found that ligand–cysteine interactions affected specific functions of these proteins: regulatory nucleotide binding and catalytic activity, respectively (Extended Data Fig. 5a–g (IMPDH2), Extended Data Fig. 5h–n (TIGAR) and Supplementary Discussion). We next evaluated proteins that possess previously uncharacterized liganded cysteines, including the nucleotide biosynthetic enzyme IMPDH2 and p53-induced phosphatase TIGAR (p53 also known as TP53). In both cases, we found that ligand–cysteine interactions affected specific functions of these proteins: regulatory nucleotide binding and catalytic activity, respectively (Extended Data Fig. 5a–g (IMPDH2), Extended Data Fig. 5h–n (TIGAR) and Supplementary Discussion).

Competitive isoTOP-ABPP experiments identified distinct subsets of ligands that targeted a conserved cysteine in isocitrate dehydrogenases 1 (IDH1) and 2 (IDH2) (C269 and C308, respectively; Supplementary Table 1). IDH1 and IDH2 are mutated in a number of human cancers to produce enzyme variants with a neomorphic catalytic activity that converts isocitrate to the oncometabolite 2-hydroxyglutarate (2-HG)\textsuperscript{22}. Reversible inhibitors selective for mutant forms of IDH1 and IDH2 have been developed and are under clinical investigation for cancer treatment\textsuperscript{22}. The liganded cysteine is an active-site-proximal residue that is 13 Å from the NADPH\textsuperscript{+} molecule in a crystal structure of IDH1 (Extended Data Fig. 6a). We confirmed that fragment ligands inhibited the activity of wild-type but not a C269S mutant of IDH1, and also blocked the R132H oncogenic mutant of IDH1 both in vitro and in cells (Extended Data Fig. 6b–k and Supplementary Discussion).

Encouraged by the cellular activity displayed by IDH1 ligands, we sought to more generally assess the capacity of fragment electrophiles to modify cysteines in situ. Cells were treated with ~20 representative members of the fragment library (50–200 μM) for 2 h in situ and then harvested, lysed, and analysed by isoTOP-ABPP. The tested fragments showed a broad range of in situ reactivities that generally matched their respective activities in vitro, although some exceptional cases with greater or lesser reactivity in situ were noted (Extended Data Fig. 6f and Supplementary Table 1). These differences could reflect the impact of transport and/or metabolic pathways on the cellular concentrations of fragment electrophiles. A substantial fraction (64%) of the liganded cysteines identified in cell lysates were also sensitive to the...
same electrophilic fragments in cells (Extended Data Fig. 6m). Four fragment–cysteine interactions were observed in situ, but not in lysates, including C182 of p53, a redox-regulated residue at the dimerization interface of the DNA-binding domain25 (Extended Data Fig. 6n). These liganded cysteines may require an intact cellular environment to preserve their interactions with fragment electrophiles.

Several fragments targeted the catalytic cysteine nucleophile C360 of the protease caspase-8 (CASP8) in isoTOP-ABPP experiments performed in vitro and in situ (Extended Data Fig. 7a and Extended Data Table 1). Curiously, however, these fragments exhibited marginal to no inhibition of active CASP8 using either substrate or activity-based probe (Rho-DEVD-AOMK probe) assays (Extended Data Fig. 7b, c). This initially puzzling outcome was explained when we discovered that the electrophilic fragments selectively labelled the inactivezymogen (pro-), but not active form of CASP8 (Fig. 4a, b, Extended Data Fig. 7b–l and Supplementary Discussion). We synthesized a clickable analogue of the most potent pro-CASP8 ligand 7 (61; Fig. 4a) and found that this probe (25 μM) strongly labelled pro-CASP8, but not a pro-CASP8 C360S mutant (Fig. 4b and Extended Data Fig. 7i), and directly modified C360 of CASP8 in Jurkat cell lysates (Extended Data Fig. 8a and Supplementary Discussion). We therefore sought to address this large part due to a lack of selective, non-peptidic, and cell-active inhibitors25,26, in primary human T cells (Extended Data Fig. 8m). Interestingly, the processing of both CASP8 and the initiator caspase substrate RIP kinase was substantially inhibited in primary human T cells showed substantial resistance to 63-R at all tested concentrations and instead was completely inhibited by the dual CASP8/10 ligand 7 (Fig. 4d). We confirmed by chemical proteomics with probe 61 that 7 blocked both CASP8 and CASP10, while 63-R inhibited CASP8, but not CASP10, in primary human T cells (Extended Data Fig. 8m), indicating that CASP10 may contribute to these proteolytic events in T cells, as has been suggested by biochemical studies27. These data, taken together, support substantive functions for both CASP8 and CASP10 in primary human T cells and are consistent with genetic findings indicating that deleterious mutations in either CASP8 or CASP10 can lead to autoimmune syndromes in humans28.

By combining chemical proteomics with FBLD, we have found that the human proteome contains many ligandable cysteines. These cysteines were found in proteins not previously known to interact with small molecules, revealing that covalent chemistry can be used to expand the druggable content of the human proteome. Our results for

![Figure 4](image-url)
pro-CASP8 and the results of others for K-Ras(G12C) indicate that it is possible to improve the potency and selectivity of covalent fragment hits for protein targets, although the optimization of covalent ligands for cysteines that reside in very shallow pockets may prove more challenging. Some covalent ligands may target cysteines at non-functional sites on proteins, and, in these cases, there is potential to convert the ligands into functional probes using emergent platforms for directing liganded proteins to degradation pathways in the cells. We envision that extensions of our chemical proteomic platform could be used to discover ligands that target other nucleophilic amino acids in proteins, thereby increasing the impact covalent chemistry will have on proteome-wide ligand and drug discovery.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions B.F.C. and K.M.B. conceived of the project. K.M.B. performed MS experiments and data analysis. B.E.C. wrote software, compiled and analysed MS data. S.F. wrote software and conducted reactive docking. K.M.B. cloned, overexpressed and purified proteins, and conducted inhibition studies in vitro and in situ. S.C. cloned and purified IDH1. K.M.B., B.D.H. and B.R.L. synthesized compounds. G.E.G.-P. expressed and purified recombinant caspases and TEV protease. D.W.W. provided assistance with the caspase studies. J.R.T assisted with the T-cell studies. A.J.O. provided technical advice. K.M.B., B.E.C. and B.F.C. designed experiments and analysed data. K.M.B., B.E.C. and B.F.C. wrote the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to B.F.C. (cravatt@scripps.edu) or K.M.B. (kbackus@scripps.edu).
METHODS

Preparation of human cancer cell line proteomes. With the exception of MUM2C cells, which were provided by M. Hendrix, all cell lines were obtained from ATCC, tested negative for mycoplasma contamination, and were used without further authentication, maintaining a low passage number (<10 passages). Cell lines were grown at 37°C with 5% CO2. MDA-MB-231 (ATCC: HTB-26), HeLa (ATCC: CCL-2) and HEK-293T (ATCC: CRL-3126) cells were grown in DMEM supplemented with 10% fetal bovine serum (FBS), penicillin, streptomycin and glutamine. Jurkat A3 (ATCC: CRL-2570), Ramos (ATCC: CRL-1596) and MUM2C cells were grown in RPMI-1640 medium supplemented with 10% FBS, penicillin and streptomycin. For in vitro labelling, cells were grown to 100% confluence for MDA-MB-231 cells or until cell density reached 1.5 million cells per ml for Ramos and Jurkat cells. Cells were washed with cold PBS, scraped with cold PBS and cell pellets were isolated by centrifugation (1,400g, 3 min, 4°C), and stored at −80°C until use. Cell pellets were lysed by sonication and fractionated (100,000g, 45 min) to yield soluble and membrane fractions, which were then adjusted to a final protein concentration of 1.5 mg mL−1 for proteomics experiments and 1 mg mL−1 for gel-based ABPP experiments. The soluble lysate was prepared fresh from frozen pellets directly before each experiment. Protein concentration was determined using the Bio-Rad DC protein assay kit.

Proteomic sample preparation. IsoTOP-ABPP, stable isotope labelling by amino acids in cell culture (SILAC) and reductive dimethylation for stable isotope labelling (REDIME) samples were prepared and analysed as has been reported previously.12,23–33. For details see Supplementary Information.

In vitro covalent fragment treatment. All compounds were made up as 50 μM stock solutions in DMSO and were used at a final concentration of 500 μM. Owing to its low solubility in aqueous medium, fragment 4 was screened at a final concentration of 250 μM. Soluble lysates were adjusted to 1.5 mg mL−1 and, for each profiling sample, 0.5 μL lysate was treated with 5 μL of the 50 μM compound stock solution or 5 μL of DMSO.

In situ covalent fragment treatment. For in situ labelling, MDA-MB-231 cells were grown to 95% confluence and Ramos cells were grown to 1 million cells per ml. The media in all samples was replaced with fresh media, containing 200 μM of the indicated fragments and the cells were incubated at 37°C for 2 h, washed with cold PBS, scraped into cold PBS and harvested by centrifugation (see earlier). Fragments 2, 3, 8, 9, 10, 12, 13, 14, 21, 27, 28, 29, 31, 33, 38, 45, 51 and 56 were screened at 200 μM in situ. Fragments 4 and 11 were screened at 100 μM in situ. Fragments 2, 3, 8 and 20 were tested at 50 μM in situ.

Heat inactivation. For heat inactivation experiments, 500 μL of MDA-MB-231 soluble proteome was denatured (95°C, 10 min) and allowed to cool to ambient temperature. The denatured sample and corresponding non-denatured, native proteome (500 μL) were then each labelled with 100 μL iodoacetamide alkyne (IA-alkyne, 5 μL of 10 mM stock in DMSO) and evaluated by isoTOP-ABPP.

R value calculation and processing. The ratios of heavy/light for each unique peptide (DMSO/compound treated; isoTOP-ABPP ratios, R values) were quantified with in-house CIMAGE software12,13, using default parameters (3 MS1 acquisitions per peak and signal to noise threshold set to 2.5). Site-specific engagement of electrophilic fragments was assessed by blockade of IA-alkyne probe labelling. For profiling samples, 0.5 μL lysate was treated with 5 μL of the 50 μM compound stock solution or 5 μL of DMSO.

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In situ covalent fragment treatment. For in situ labelling, MDA-MB-231 cells were grown to 95% confluence and Ramos cells were grown to 1 million cells per ml. The media in all samples was replaced with fresh media, containing 200 μM of the indicated fragments and the cells were incubated at 37°C for 2 h, washed with cold PBS, scraped into cold PBS and harvested by centrifugation (see earlier). Fragments 2, 3, 8, 9, 10, 12, 13, 14, 21, 27, 28, 29, 31, 33, 38, 45, 51 and 56 were screened at 200 μM in situ. Fragments 4 and 11 were screened at 100 μM in situ. Fragments 2, 3, 8 and 20 were tested at 50 μM in situ.

Heat inactivation. For heat inactivation experiments, 500 μL of MDA-MB-231 soluble proteome was denatured (95°C, 10 min) and allowed to cool to ambient temperature. The denatured sample and corresponding non-denatured, native proteome (500 μL) were then each labelled with 100 μL iodoacetamide alkyne (IA-alkyne, 5 μL of 10 mM stock in DMSO) and evaluated by isoTOP-ABPP.

R value calculation and processing. The ratios of heavy/light for each unique peptide (DMSO/compound treated; isoTOP-ABPP ratios, R values) were quantified with in-house CIMAGE software12,13, using default parameters (3 MS1 acquisitions per peak and signal to noise threshold set to 2.5). Site-specific engagement of electrophilic fragments was assessed by blockade of IA-alkyne probe labelling. For profiling samples, 0.5 μL lysate was treated with 5 μL of the 50 μM compound stock solution or 5 μL of DMSO.

In situ covalent fragment treatment. For in situ labelling, MDA-MB-231 cells were grown to 95% confluence and Ramos cells were grown to 1 million cells per ml. The media in all samples was replaced with fresh media, containing 200 μM of the indicated fragments and the cells were incubated at 37°C for 2 h, washed with cold PBS, scraped into cold PBS and harvested by centrifugation (see earlier). Fragments 2, 3, 8, 9, 10, 12, 13, 14, 21, 27, 28, 29, 31, 33, 38, 45, 51 and 56 were screened at 200 μM in situ. Fragments 4 and 11 were screened at 100 μM in situ. Fragments 2, 3, 8 and 20 were tested at 50 μM in situ.

Heat inactivation. For heat inactivation experiments, 500 μL of MDA-MB-231 soluble proteome was denatured (95°C, 10 min) and allowed to cool to ambient temperature. The denatured sample and corresponding non-denatured, native proteome (500 μL) were then each labelled with 100 μL iodoacetamide alkyne (IA-alkyne, 5 μL of 10 mM stock in DMSO) and evaluated by isoTOP-ABPP.

R value calculation and processing. The ratios of heavy/light for each unique peptide (DMSO/compound treated; isoTOP-ABPP ratios, R values) were quantified with in-house CIMAGE software12,13, using default parameters (3 MS1 acquisitions per peak and signal to noise threshold set to 2.5). Site-specific engagement of electrophilic fragments was assessed by blockade of IA-alkyne probe labelling. For profiling samples, 0.5 μL lysate was treated with 5 μL of the 50 μM compound stock solution or 5 μL of DMSO.
Primary human T-cell isolation and stimulation. All studies using samples from human volunteers follow protocols approved by The Scripps Research Institute institutional review board (protocol no. IRB-15-6682). Blood from healthy donors was obtained after informed consent and peripheral blood mononuclear cells (PBMCs) were purified over Ficoll–Hypaque gradients (Sigma–Aldrich). T cells were purified via negative selection with magnetic beads (EasySep, STEMCELL). The purified T cells were washed with sterile PBS and resuspended in RPMI-1640 supplemented with PBS, penicillin, streptomycin and glutamine (2 million cells per ml) and 200,000 cells per well were seeded on non-treated tissue culture, 96-well transparent plates that had been coated with anti-CD3 (1:200, BioXcell) and anti-CD28 (1:500, Biolegend) in PBS (100 μl per well). The T cells were removed from stimulus after 3 days and cultured in complete RPMI-1640 supplemented with IL-2 (10 μg/ml), eBioscience) for 3–4 additional days.

Apoptosis assays in primary human T cells with CASP8 inhibitors. Primary human T cells were stimulated for 3 days with anti-CD3 and anti-CD28, and the cells were then washed and cultured in complete RPMI with IL-2 (10 μg/ml) for 4 additional days. For western blot analysis, 10 ml of stimulated primary human T cells (1.5 million cells per ml) in RPMI with IL-2 were then treated with the indicated compounds for 1 h before addition of Fast. (1 μl of 100 μg/ml stock solution of MegaFastLigand in water, final concentration = 10 ng/ml, Adipogen) After 3 h, cells were harvested by centrifugation, washed in PBS and lysed in cell lysis buffer (BioVision, 1067-100) with 1× complete protease inhibitor (Roche) and 40 μg of each sample were separated by SDS–PAGE on 14% polyacrylamide gels. The gels were transferred to nitrocellulose membranes and were immunoblotted overnight with the indicated antibodies. For measurements of cell viability, in triplicate for each condition, 150,000 cells (100 μl of 1.5 million cells per ml) were plated in 96-well optical-bottom plates. Fast was used at the same concentration indicated earlier with a 30 min pre-incubation with compounds at the indicated concentrations, followed by 4 h with Fast or DMSO. Twenty times compound stock solutions were made in RPMI immediately before use. Cell viability was measured with CellTiter-Glo Luminescent Cell Viability Assay (Promega) and was read on a Biotech Synergy 4 plate reader.

Western blotting. For apoptosis studies, cell pellets were resuspended in cell lysis buffer from (BioVision, 1067-100) with 1× complete protease inhibitor (Roche) and allowed to incubate on ice for 30 min before centrifugation (10 min, 16,000g). For all other studies, cell pellets were resuspended in PBS and lysed with sonication before centrifugation (10 min, 16,000g). The proteins were then resolved by SDS–PAGE and transferred to nitrocellulose membranes, blocked with 5% milk in TBST and probed with the indicated antibodies. The primary antibodies and the dilutions used are as follows: anti-PARP (Cell Signaling, 9532, 1:1,000), anti-CASP3 (Cell Signaling, 9662, 1:1,000), anti-CASP8 (cleaved form, Cell Signaling, 9746, 1:1,000), anti-CASP8 (pro-form, Cell Signaling, 4970, 1:1,000), anti-IDH1 (Cell Signaling, 3997 s, 1:500), anti-actin (Cell Signaling, 3700, 1:3,000), anti-GAPDH (Santa Cruz, sc-32323, 1:2,000), anti-Flag (Sigma Aldrich, F1804, 1:3,000), anti-CASP10 (MBL, M059-3, 1:1,000), anti-RIPK (Cell Signaling, 3493S, 1:1,000). Blots were incubated with primary antibodies overnight at 4°C with rocking and were then washed (3 × 5 min, TBST) and incubated with secondary antibodies (LICOR, IRDye 800CW or IRDye 800LT, 1:10,000) for 1 h at ambient temperature. Blots were further washed (3 × 5 min, TBST) and visualized on a LICOR Odyssey Scanner. The percentage cleavage was determined by quantifying the integrated optical density of the bands (n = 3 for STS and n = 2 for Fast), using ImageJ software. For CASP8, the 43 and 41 kDa bands were quantified together. For CASP3, the 17 kDa subunit band was quantified.

Statistical analysis. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment. No statistical methods were used to predetermine sample size. Data are shown as mean ± standard error of the mean. P values were calculated using unpaired, two-tailed Student’s t-test. P values of <0.05 were considered significant. See also Supplementary Information.
Fragment electrophiles screened by isoTOP-ABPP

Extended Data Figure 1 | Composition of fragment electrophile library and structures of additional tool compounds, click probes and fragments.
Extended Data Figure 2 | See next page for caption.
Extended Data Figure 2 | Analysis of proteomic reactivities of fragment electrophiles in human cell lysates. a, Initial analysis of the proteomic reactivity of fragments using an IA-rhodamine probe. Soluble proteome from Ramos cells was treated with the indicated fragments (500 μM each) for 1 h, followed by labelling with IA-rhodamine (1 μM, 1 h) and analysis by SDS–PAGE and in-gel fluorescence scanning. Several proteins were identified that show impaired reactivity with IA-rhodamine in the presence of one or more fragments (asterisks). Fluorescent gel shown in greyscale. b, Frequency of quantification of all cysteines across the complete set of competitive isoTOP-ABPP experiments performed with fragment electrophiles. Note that cysteines were required to have been quantified in at least three isoTOP-ABPP data sets for interpretation. c, Rank order of proteomic reactivity values (or liganded cysteine rates) of fragments calculated as the percentage of all quantified cysteines with R values ≥ 4 for each fragment. The majority of fragments were evaluated in 2–4 replicate experiments in MDA-MB-231 and/or Ramos cell lysates, and their proteomic reactivity values are reported as mean ± s.e.m. values for the replicates. d, Comparison of the proteomic reactivities of representative fragments screened at 500 versus 25 μM in cell lysates. e, Comparison of proteomic reactivity values for fragments tested in both Ramos and MDA-MB-231 lysates. f, Proteomic reactivity values of representative fragments. g, Relative GSH reactivity for representative fragment electrophiles. Consumption of GSH (125 μM) was measured using Ellman’s reagent (5 mM) after 1 h incubation with the indicated fragments (500 μM). h, Proteomic reactivity values for fragments electrophiles (500 μM) possessing different electrophilic groups attached to a common binding element. i, Concentration-dependent labelling of MDA-MB-231 soluble proteomes with acrylamide and chloroacetamide click probes detected by CuACC with a rhodamine-azide tag and analysis by SDS–PAGE and in-gel fluorescence scanning. For f and g, data represent mean values ± s.e.m. for at least three independent experiments.
Extended Data Figure 3 | See next page for caption.
Extended Data Figure 3 | Analysis of cysteines liganded by fragment electrophiles in competitive isoTOP-ABPP experiments.

a, Representative MS1 ion chromatograms for peptides containing C481 of BTK and C131 of MAP2K7, two cysteines known to be targeted by the anti-cancer drug ibrutinib. Ramos cells were treated with ibrutinib (1 μM, 1 h, red trace) or DMSO (blue trace) and evaluated by isoTOP-ABPP.
b, Total number of liganded cysteines found in the active sites and non-active sites of enzymes for which X-ray and/or NMR structures have been reported (or reported for a close homologue of the enzyme).
c, Functional categorization of liganded and unliganded cysteines based on residue annotations from the UniProt database. d, Number of liganded and quantified cysteines per protein measured by isoTOP-ABPP. Respective average values of one and three for liganded and quantified cysteines per protein were measured by isoTOP-ABPP.

e, R values for six cysteines in XPO1 quantified by isoTOP-ABPP, identifying C528 as the most liganded cysteine in this protein. Each point represents a distinct fragment–cysteine interaction quantified by isoTOP-ABPP.

f–h, Histograms depicting the percentage of fragments that are hits (R ≥ 4) for all 768 liganded cysteines (f), for liganded cysteines found in enzymes for which X-ray and/or NMR structures have been reported (or reported for a close homologue of the enzyme) (g), or for active- and non-active site cysteines in kinases (h). i, Percentage of liganded cysteines targeted only by group A (red) or B (blue) fragments or both group A and B fragments (black). Shown for all liganded cysteines, liganded cysteines in enzyme active and non-active sites, and liganded cysteines in transcription factors/regulators. For g, i, active-site cysteines were defined as those that reside < 10 Å from established active-site residues and/or bound substrates/inhibitors in enzyme structures. j, The percentage of liganded cysteines in kinases that were targeted by only group A, only group B, or both group A and B compounds. k, Heat map showing representative fragment interactions for liganded cysteines found in the active sites and non-active sites of kinases. l, Heat map showing representative fragment interactions for liganded cysteines found in transcription factors/regulators.
m, The fraction of liganded (62%; 341 of 553 quantified cysteines) and unliganded (20%; 561 of 2,870 quantified cysteines) cysteines that are sensitive to heat denaturation measured by IA-alkyne labelling (R > 3 native/heat denatured). n, Percentage of proteins identified by isoTOP-ABPP as liganded by fragments 3 and 14 and enriched by their corresponding click probes 19 and 18 that are sensitive to heat denaturation (64% (85 of 133 quantified protein targets) and 73% (19 of 26 quantified protein targets), respectively). Protein enrichment by 18 and 19 was measured by whole-protein capture of isotopically SILAC-labelled MDA-MB-231 cells using quantitative (SILAC) proteomics.

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Extended Data Figure 4 | Confirmation and functional analysis of fragment–cysteine interactions in PRMT1 and MLTK. a, Representative MS1 chromatograms for the indicated Cys-containing peptides from PRMT1 quantified in competitive isoTOP-ABPP experiments of MDA-MB-231 cell lysates, showing blockade of IA-alkyne 1 labelling of C109 by fragment 11, but not control fragment 3. b, 11, but not 3, blocked IA-rhodamine (2 μM) labelling of recombinant, purified wild-type PRMT1 (1 μM protein doped into HEK293T cell lysates). Note that a C109S PRMT1 mutant did not react with IA-rhodamine. c, Apparent IC₅₀ curve for blockade of 16 labelling of PRMT1 by 11. Cl, 95% confidence intervals. d, Effect of 11 and control fragment 3 on methylation of recombinant histone 4 by recombinant PRMT1. Shown is one representative experiment of three independent experiments that yielded similar results. e, Representative MS1 ion chromatograms for the MLTK tryptic peptide containing liganded cysteine C22 quantified by isoTOP-ABPP in MDA-MB-231 lysates treated with fragment 4 or control fragment 3 (500 μM each). f, 60, but not control fragment 3 (50 μM of each fragment), blocked labelling of recombinant MLTK kinase by a previously reported ibrutinib-derived activity probe 59 (top)²⁰. A C22A-MLTK mutant did not react with the ibrutinib probe. Anti-Flag blotting confirmed similar expression of wild-type and C22A-MLTK proteins, which were expressed as Flag-fusion proteins in HEK293T cells (bottom). g, Lysates from HEK293T cells expressing wild type or C22A MLTK treated with the indicated fragments and then an ibrutinib-derived activity probe 59 (ref. 20) at 10 μM. MLTK labelling by 59 was detected by CuAAC conjugation to a rhodamine-azide tag and analysis by SDS–PAGE and in-gel fluorescence scanning. h, Apparent IC₅₀ curve for blockade of ibrutinib probe-labelling of MLTK by 60, i, 60, but not control fragment 3 (100 μM of each fragment), inhibited the kinase activity of wild-type, but not C22A-MLTK. For c, h and i, data represent mean values ± s.e.m. for at least three independent experiments. Statistical significance was calculated with unpaired Student’s t-tests comparing DMSO- to fragment-treated samples; **P < 0.1.

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Extended Data Figure 5 | See next page for caption.
Extended Data Figure 5 | Confirmation and functional analysis of fragment-cysteine interactions in IMPDH2 and TIGAR.

a, Representative MS1 ion chromatograms for IMPDH2 tryptic peptides containing the catalytic cysteine, C331, and Bateman domain cysteine, C140, quantified by isoTOP-ABPP in cell lysates treated with the indicated fragments (500μM each).

b, Structure of human IMPDH2 (PDB accession 1NF7) (light grey) and its structurally unresolved Bateman domain modelled by I-TASSER43 (dark grey) showing the positions of C331 (red spheres), ribavirin monophosphate and C2-mycophenolic adenine dinucleotide (blue), and C140 (yellow spheres). c, Click probe 18 (25μM) labelled wild-type IMPDH2 and C331S IMPDH2, but not C140S IMPDH2 (or C140S/C331S IMPDH2). Labelling was detected by CuAAC conjugation to a rhodamine-azide reporter tag and analysis by SDS–PAGE and in-gel fluorescence scanning. Recombinant IMPDH2 wild type and mutants were expressed and purified from *Escherichia coli* and added to Jurkat lysates to a final concentration of 1μM protein.

d, Fragment reactivity with recombinant, purified IMPDH2 added to Jurkat lysates to a final concentration of 1μM protein, where reactivity was detected in competition assays using the click probe 18 (25μM). Note that 18 reacted with wild-type and C331S IMPDH2, but not C140S or C140S/C331S IMPDH2. e, Nucleotide competition of 18 (25μM) labelling of wild-type IMPDH2 added to MDA-MB-231 lysates to a final concentration of 1μM protein. f, Nucleotide competition profile for 18 labelling of recombinant wild-type IMPDH2 (500μM of each nucleotide).

g, Apparent IC_{50} curve for blockade of 18 labelling of IMPDH2 by ATP. h, Representative MS1 chromatograms for TIGAR tryptic peptides containing C114 and C161 quantified by isoTOP-ABPP in cell lysates treated with the indicated fragments (500μM each).

i, Crystal structure of TIGAR (PDB accession 3DCY) showing C114 (red spheres), C161 (yellow spheres), and inorganic phosphate (blue). j, Labelling of recombinant, purified TIGAR and mutant proteins by the IA-rhodamine (2μM) probe. TIGAR proteins were added to MDA-MB-231 lysates, to a final concentration of 2μM protein. k, 5, but not control fragment 3, blocked IA-rhodamine (2μM) labelling of recombinant, purified C161S TIGAR (2μM protein doped into Ramos cell lysates). l, Apparent IC_{50} curve for blockade of IA-rhodamine labelling of C161S TIGAR by 5. m, 5, but not control fragment 3 (100μM of each fragment) inhibited the catalytic activity of wild-type TIGAR, C161S TIGAR, but not C114S TIGAR or C114S/C161S TIGAR. n, Concentration-dependent inhibition of wild-type TIGAR by 5. Note that the C140S-TIGAR mutant was not inhibited by 5. Data represent mean values ± s.e.m. for at least three independent experiments. Statistical significance was calculated with unpaired Student’s *t*-tests comparing DMSO- to fragment-treated samples; **P < 0.01, ****P < 0.0001.
Extended Data Figure 6 | See next page for caption.
Extended Data Figure 6 | IDH1-related and general in situ activity of fragment electrophiles. a, X-ray crystal structure of IDH1 (PDB accession 3MAS) showing the position of C269 and the frequently mutated residue in cancer, R132. b, Blockade of labelling of wild-type IDH1 by representative fragment electrophiles. Recombinant, purified wild-type IDH1 was added to MDA-MB-231 lysates at a final concentration of 2 μM, treated with fragments at the indicated concentrations, followed by IA-rhodamine probe (2 μM) and analysis by SDS–PAGE and in-gel fluorescence scanning. Note that a C269S mutant of IDH1 did not label with IA-rhodamine. Reactivity of fragment and control fragment 2 with recombinant, purified wild-type IDH1 (b) or R132H IDH1 (c) added to MDA-MB-231 lysates to a final concentration of 2 or 4 μM protein, respectively. Fragment reactivity was detected in competition assays using the IA-rhodamine probe (2 μM) e, f, Apparent IC_{50} curve for blockade of labelling of IDH1 (e) and R132H IDH1 (f) by fragment 20. Note that the control fragment 2 showed much lower activity. g, Representative MS1 ion chromatograms for the IDH1 tryptic peptides containing liganded cysteine C269 and an unliganded cysteine C379 quantified by isoTOP-ABPP in MDA-MB-231 lysates treated with fragment 20 or control fragment 2 (50 μM, 2 h, in situ). i, Western blot of MUM2C cells stably overexpressing GFP (mock) or R132H-IDH1 proteins. j, Representative MS1 chromatograms for the IDH1 tryptic peptides containing liganded cysteine C269 and an unliganded cysteine C379 quantified by isoTOP-ABPP in R132H-IDH1-expressing MUM2C lysates treated with fragment 20 or control fragment 2 (50 μM, 2 h, in situ). k, Proteomic reactivity values for individual fragments are comparable in vitro and in situ. One fragment (11) marked in red showed notably lower activity in situ versus in vitro. Reactivity values were calculated as in Fig. 1c. Dashed line mark 90% prediction intervals for the comparison of in vitro and in situ proteomic reactivity values for fragment electrophiles. Blue and red circles mark fragments that fall above (or just at) or below these prediction intervals, respectively. l, Fraction of cysteines liganded in vitro that are also liganded in situ. Shown are liganded cysteine numbers for individual fragments determined in vitro and the fraction of these cysteines that were liganded by the corresponding fragments in situ. m, Representative cysteines that were selectively targeted by fragments in situ, but not in vitro. For in situ-restricted fragment–cysteine interactions, a second cysteine in the parent protein was detected with an unchanging ratio (R ≈ 1), thus controlling for potential fragment-induced changes in protein expression. For e, f, h and i, data represent mean values ± s.e.m. for at least three independent experiments. Statistical significance was calculated with unpaired Student’s t-tests comparing DMSO- to fragment-treated samples; ****P < 0.0001.
Extended Data Figure 7 | See next page for caption.
Extended Data Figure 7 | Fragment electrophiles that target pro-CASP8. a, Representative MS1 chromatograms for CASP8 tryptic peptide containing the catalytic cysteine C360 quantified by isoTOP-ABPP in cell lysates or cells treated with fragment 4 (250 μM, in vitro; 100 μM, in situ) and control fragment 21 (500 μM, in vitro; 200 μM, in situ). b, Neither 7 nor control fragment 62 (100 μM each) inhibited recombinant, purified active CASP3 and CASP8 assayed using N-acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin (DEVD-AMC) and Ac-Ile-Glu-Thr-Asp-7-amino-4-trifluoromethylcoumarin (IETD-AFC) fluorogenic substrates, respectively. DEVD-CHO (20 μM) inhibited both caspases. c, Fragment reactivity with recombinant, purified active CASP8 added to cell lysates, where reactivity was detected in competition assays using the caspase activity probe Rho-DEVD-AOMK probe (2 μM, 1 h). d, Western blot of proteomes from MDA-MB-231, Jurkat, and CASP8-null Jurkat proteomes showing that CASP8 was only found in the pro-enzyme form in these cells. e, Fragment reactivity with recombinant, purified pro-CASP8 (D374A, D384A, C409S) added to cell lysates to a final concentration of 1 μM protein, where reactivity was detected in competition assays with the IA-rhodamine probe (2 μM). f, Neither 7 nor control fragment 62 did not compete IA-rhodamine labelling of C360 of pro-CASP8. g, Apparent IC50 curve for blockade of IA-rhodamine labelling of pro-CASP8 (C409S) by 7. h, 7 (50 μM) fully competed IA-alkyne labelling of C360 of endogenous CASP8 in cell lysates as measured by isoTOP-ABPP. Representative MS1 chromatograms are shown for the C360-containing peptide of CASP8. i, Concentration-dependent reactivity of click probe 61, with recombinant, purified pro-CASP8 (D374A, D384A) added to cell lysates to a final concentration of 1 μM protein. Note that pre-treatment with 7 blocked 61 reactivity with pro-CASP8 and mutation of C360 to Ser prevented labelling of pro-CASP8 by 61 (25 μM). j, 7 (30 μM) blocked IA-alkyne labelling of C360 of pro-CASP8, but not active CASP8, as measured by isoTOP-ABPP. Recombinant pro- and active CASP8 were added to Ramos lysates at 1 μM and then treated with 7 (30 μM) followed by isoTOP-ABPP. k, Fragments 7 and 62 did not block labelling by Rho-DEVD-AOMK (2 μM) of recombinant, purified active CASP8 and active CASP3 added to MDA-MB-231 cell lysates to a final concentration of 1 μM protein. l, 7 does not inhibit active caspases. Recombinant, active caspases were added to MDA-MD-231 lysate to a final concentration of 200 nM (CASP2, 3, 6, 7) or 1 μM (CASP8, 10), treated with z-Val-Ala-Asp(OMe)-fluoromethyl ketone (z-VAD-FMK) (25 μM) or 7 (50 μM), followed by labelling with the Rho-DEVD-AOMK probe (2 μM). m, Representative MS1 chromatograms for tryptic peptides containing the catalytic cysteines of CASP8 (C360), CASP2 (C320), and CASP7 (C186) quantified by isoTOP-ABPP in Jurkat cell lysates treated with 7 or 62 (50 μM, 1 h). n, 7, but not control fragment 62, blocked extrinsic, but not intrinsic apoptosis. Jurkat cells (1.5 million cells per ml) were incubated with 7 or 62 (30 μM) or the pan-caspase inhibitor VAD-FMK (100 μM) for 30 min before addition of staurosporine (2 μM) or SuperFasLigand (100 ng ml−1). Cells were incubated for 4 h and viability was quantified with CellTiter-Glo. RLU, relative light unit. o, For cells treated as described in n, cleavage of PARP, CASP8 and CASP3 detected by western blotting as shown in o was quantified for three (STS) or two (FasL) independent experiments. Cleavage products (PARP (96 kDa), CASP8 (p43/p41, p18), and CASP3 (p17)) were visualized by western blot. p, 7 protects Jurkat cells from extrinsic, but not intrinsic apoptosis. Cleavage of PARP, CASP8 and CASP3 detected by western blotting as shown in o was quantified for three (STS) or two (FasL) independent experiments. Cleavage products (PARP (96 kDa), CASP8 (p43/p41), CASP3 (p17)) were quantified for compound treatment and the percentage cleavage relative to DMSO-treated samples was calculated. For b, g and n, data represent mean values ± s.e.m. for at least three independent experiments. For p, STS data represent mean values ± s.e.m. for three independent experiments, and FasL data represent mean values ± s.d. for two independent experiments. Statistical significance was calculated with unpaired Student’s t-tests comparing active compounds (VAD-FMK and 7) to control compound 62. **P < 0.01, ***P < 0.001, ****P < 0.0001.
Extended Data Figure 8 | CASP10 is involved in intrinsic apoptosis in primary human T cells. **a**, Representative MS1 peptide signals showing R values for caspases detected by quantitative proteomics using probe 61. ABPP-SILAC experiments. Jurkat cells (10 million cells) were treated with either DMSO (heavy cells) or the indicated compounds (light cells) for 2 h followed by probe 61 (10 μM, 1 h). **b**, 7 blocked 61 labelling of pro-CASP8 and CASP10, whereas 63-R selectively blocked probe labelling of pro-CASP8. **c**, 7, but not 63-R blocked probe labelling of pro-CASP10. Recombinant pro-CASP10 was added to MDA-MB-231 lysates to a final concentration of 300 nM, treated with the indicated compounds, and labelled with probe 61. **d**, Mutation of the catalytic cysteine C401A fully prevented labelling by 61. **e**, Neither 7 nor 63-R (25 μM each) inhibited the activity of recombinant, purified active CASP10 (500 nM), which was assayed after addition of the protein to MDA-MB-231 lysate using fluorometric Ac-Ala-Glu-Val-Asp-7-amino-4-methylcoumarin (AEVD-AMC) substrate. DEVD-CHO (20 μM) inhibited the activity of CASP10. For **d**–**f**, **h** and **j**–**k**, data represent mean values ± s.e.m. for at least three independent experiments. Statistical significance was calculated with unpaired Student’s t-tests comparing DMSO- to fragment-treated samples; **P < 0.01, ****P < 0.0001.
## Extended Data Table 1 | Ligandability of representative cysteines and proteins

### a.

| Protein | Cysteine(s) | Fragment(s) | Other cysteines quantified by isoTOP-ABPP | Previous covalent inhibitor(s) | Cysteine location | Reference |
|---------|-------------|-------------|------------------------------------------|-------------------------------|------------------|-----------|
| BTK     | C481        | 2, 3, 14, 31| C145, C337                               | Ibrutinib                     | Active site      | 44        |
| TGM2    | C277        | 12, 14, 32  | C10, C27, C230, C269, C290, C336, C370, C524, C545, C620 | 18d                           | Active Site      | 45        |
| MAP2K7  | C131        | 2, 3, 11, 14, 20, 21, 38 | C260, C280                               | Ibrutinib                     | Active Site      | 20        |
| XPO1    | C528        | 2, 3, 5, 14, 24, 31, 43, 56 | C34, C119, C164, C199, C327, C496, C723, C1070 | KPT-330                       | Non-active site  | 46        |
| CASP5   | C315        | 3, 50       | —                                        | Z-WEHD-CHO/FMK                | Active Site      | 47        |
| CASP8   | C360        | 2, 4, 11    | C236, C409                               | Z-VAD-FMK, CV8/9-AOMK         | Active Site      | 48, 49    |
| ERCC3   | C342        | 2, 3, 5, 8, 14, 21 | —                                        | Triptolide                    | Active Site      | 50        |
| PARK7 (Toxoplasma DJ-1) | C106 | 2, 9, 8, 11, 13, 43, 45, 50, 52 , C46, C53 | WRR-086 | Active Site | 51 |
| GSTO1   | C32         | 2, 13, 16, 18-22, 33, 27-30, 32-34, 36, 39, 43, 49, 50, 52, 54, 55 | C90, C192, C237 | KT53 | Active Site | 52 |
| ALDH2   | C319        | 3, 8-10, 12, 27, 28, 32, 39, 40, 43, 49, 50 | C66, C179, C386, C472 | Disulfiram | Active Site | 53 |
| CTSZ    | C92         | 4, 11, 20, 28, 32 | C89, C126, C132, C154, C170, C173, C179, C214 | Cy5DCG04 | Active Site | 54 |

### b.

| Protein | Cysteine | Fragment # | Peptide | M+H calculated (m/z) | M+H observed (m/z) | Charge |
|---------|----------|------------|---------|----------------------|--------------------|--------|
| IMPDH2  | C140     | 14         | R.HGFCGIPITDTGR.M | 715.86              | 715.86             | 2      |
| TIGAR   | C114     | 5          | R.EECPVTGPPOETLDVQK.M | 1123.97            | 1123.97             | 2      |
| IDH1    | C269     | 20         | K.SEGFIVWACK.N     | 702.84              | 702.84             | 2      |
| CASP8   | C360     | 7          | K.VFIQACQGDNYQK.G | 660.98              | 660.98             | 3      |
| CASP8*  | C360     | 61-TEV-Tag | K.VFIQACQGDNYQK.G | 1195.58 (light) and 1198.59 (heavy) | 1195.58 (light) and 1198.59 (heavy) | 2      |

a. Representative cysteines with known covalent ligands targeted by fragment electrophiles in isoTOP-ABPP experiments. b. Site of fragment labelling for recombinant proteins. Underlines mark the fragment-modified cysteines. *Measured for endogenous protein by isoTOP-ABPP using probe 61.
| Protein | PDB ID: | Most ligandable cysteine by docking | Cysteine location | Most ligandable cysteine by isoTOP-ABPP | Match | Heat Sensitive |
|---------|---------|------------------------------------|------------------|----------------------------------------|-------|---------------|
| Aldh2   | 1O05    | C319                               | Active site      | C319                                   | Yes   | Yes           |
| BTK     | 1KQP    | C481                               | Active site      | C481                                   | Yes   | ND            |
| CASP8   | 1QTN    | C360                               | Active site      | C360                                   | Yes   | Yes           |
| CCNB1   | 2GJZ    | C238                               | Non-active site  | C238                                   | Yes   | Yes           |
| CDKN3   | 1FG1    | C39                                | Non-active site  | C39                                    | Yes   | Yes           |
| CLIC4   | 2AEH    | C35                                | Non-active site  | C35                                    | Yes   | Yes           |
| DTYMK   | 1E2G    | C183                               | Non-active site  | C183                                   | Yes   | No            |
| IDH1    | 3MAP    | C269                               | Non-active site  | C269                                   | Yes   | Yes           |
| IMPDH2  | 1NF7    | C331                               | Active site      | C331, C140                             | Yes   | Yes           |
| GLRX5   | 2WUL    | C67                                | Active site      | C67                                    | Yes   | No            |
| GSTO1   | 1EEM    | C32                                | Active site      | C32                                    | Yes   | Yes           |
| NME3    | 1ZS6    | C158                               | Non-active site  | C158                                   | Yes   | Yes           |
| PKM     | 4JPG    | C423                               | Non-active site  | C423                                   | Yes   | Yes           |
| SRC     | 2SRC    | C277                               | Active Site      | C277                                   | Yes   | ND            |
| TIGAR   | 3DCY    | C114                               | Non-active site  | C114, C181                             | Yes   | Yes           |
| TXNDC   | 1WOU    | C43                                | Active site      | C43                                    | Yes   | Yes           |
| UGDH    | 3ITK    | C276                               | Active site      | C276                                   | Yes   | Yes           |
| UPP1    | 3EUF    | C162                               | Non-active site  | C162                                   | Yes   | Yes           |
| XPO1    | 3GB8    | C528                               | Non-active site  | C528                                   | Yes   | Yes           |
| CDK5    | 1UNG    | C157                               | Non-active site  | C269                                   | Second| ND            |
| EDC3    | 3DKX    | C311                               | Non-active site  | C137, C413, C499                        | Second| ND            |
| NR2F2   | 3CJW    | C213                               | Non-active site  | C326, C213(in situ)                    | Second| ND            |
| PDCD6IP | 2R02    | C231                               | Non-active site  | C90                                    | Second| ND            |
| PRMT1   | 1QRI    | C285                               | Active site      | C109                                   | Second| Yes           |
| UBE2S   | 1ZDN    | C118                               | Non-active site  | C95                                    | Second| ND            |
| FNBP1   | 2EFL    | C145                               | Non-active site  | C70                                    | No    | ND            |
| HAT1    | 2P0W    | C120                               | Non-active site  | C101                                   | No    | Yes           |
| MAPK9   | 3NPC    | C183                               | Active site      | C177                                   | No    | ND            |
| STAT1   | 1YVL    | C543                               | Non-active site  | C492, C255                             | No    | ND            |

Shows the most ligandable cysteine predicted by reactive docking. Match indicates whether the top cysteine by docking matches that identified by isoTOP-ABPP. Heat sensitive column indicates whether the top cysteine identified by covalent docking is sensitive to heat denaturation. ND, not detected.