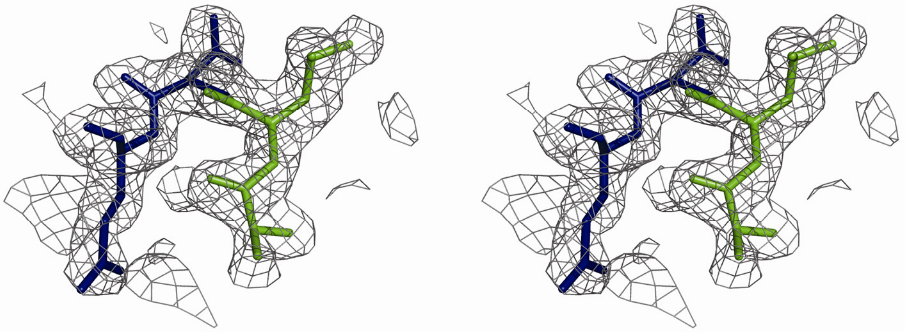


Fig. S1. Quality of Cif experimental phasing by single wavelength anomalous diffraction. The selenomethionine Cif-WT model is shown in stereo, together with the experimental density-modified single wavelength anomalous diffraction map, contoured at 1σ. Partial electron density for neighboring residues is shown in this image, although for clarity the corresponding model is not. The figure shows the carboxy-terminal boundary between the cap (green) and core (blue) domains at a kink in helix 11 that separates α11a and α11b (see Fig. 3).

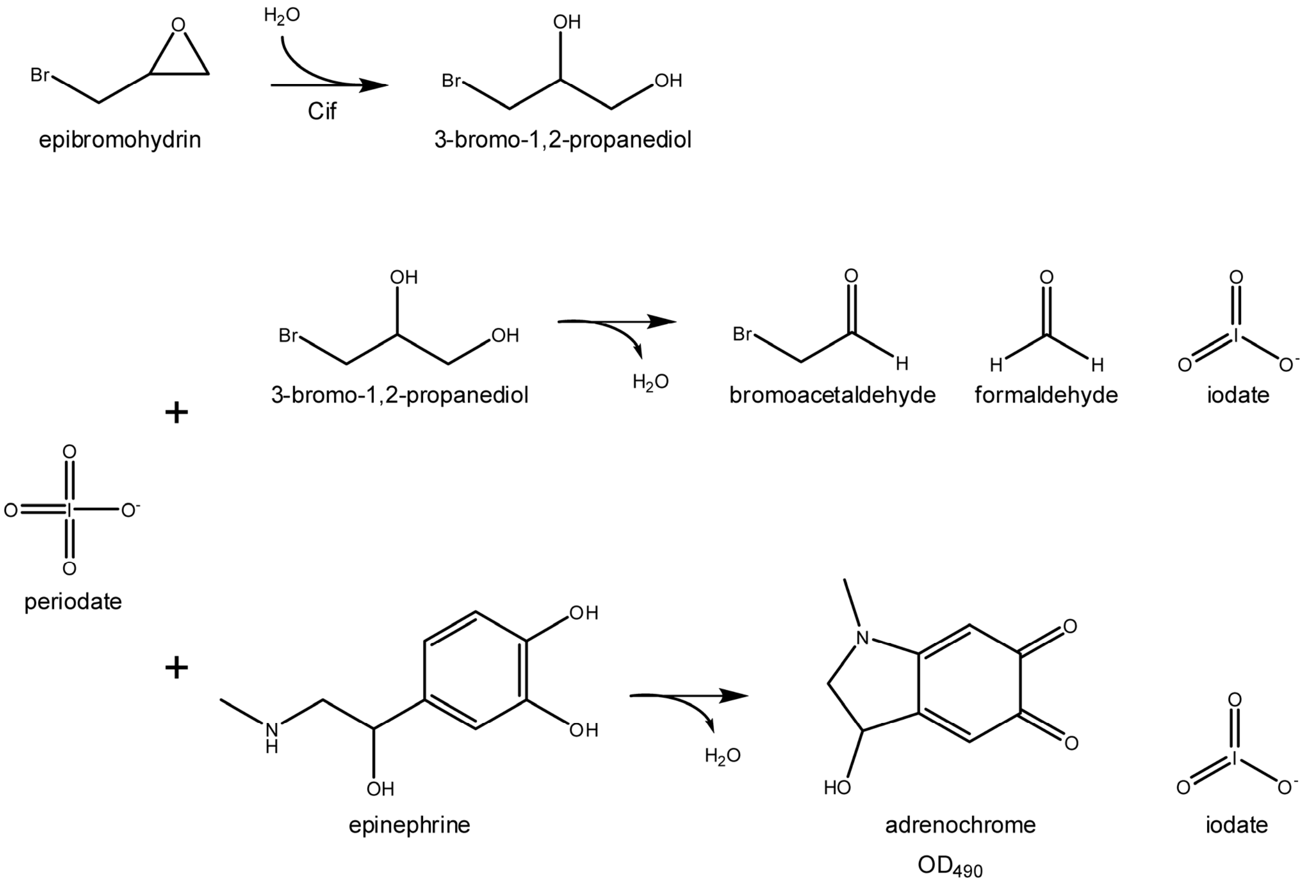


Fig. S2. Chemical reactions of the assay for epibromohydrin hydrolysis. The product of epibromohydrin (EBH) hydrolysis is 3-bromo-1,2-propanediol (BPD, top reaction), which interacts with and reduces periodate (middle reaction). Residual periodate is quantitated based on its reaction with epinephrine, which yields adrenochrome (bottom reaction) and can thus be quantitated by OD_{490}.

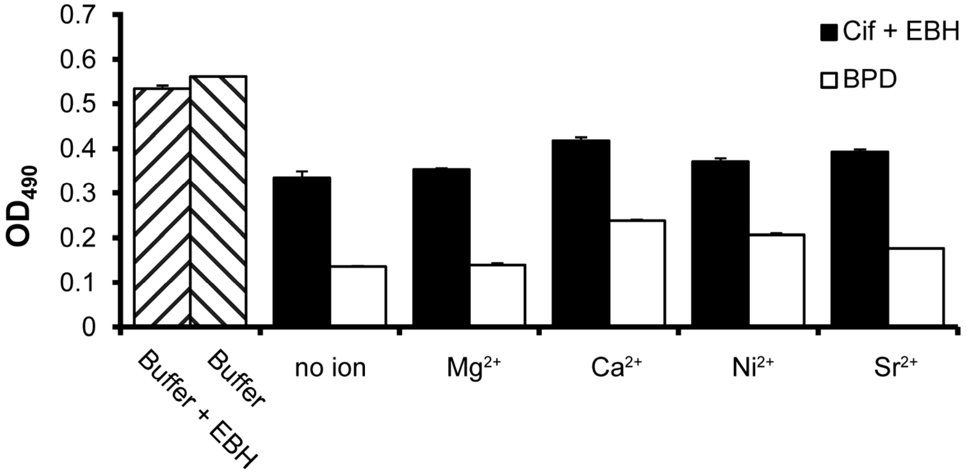


Fig. S3. Divalent cations are not significant modulators of Cif epoxide hydrolase activity. Hydrolysis of 10 mM EBH by 10 µM Cif-WT for 15 min at 37°C was assayed in the presence of a panel of 1 mM divalent cations, delivered as a chloride salts. Additionally, the effect of the same panel of cations was tested for interference with the assay's ability to accurately detect the EBH hydrolysis product 3-bromo-1,2-propanediol (BPD). The data are shown as direct absorbance measurements, and as such the OD$_{490}$ is inversely related to the quantity of BPD produced (see Fig. S2). This assay was carried out with less Cif protein and for a shorter time period than previous experiments in order to optimize the detection of any changes in enzyme activity using a single time point.

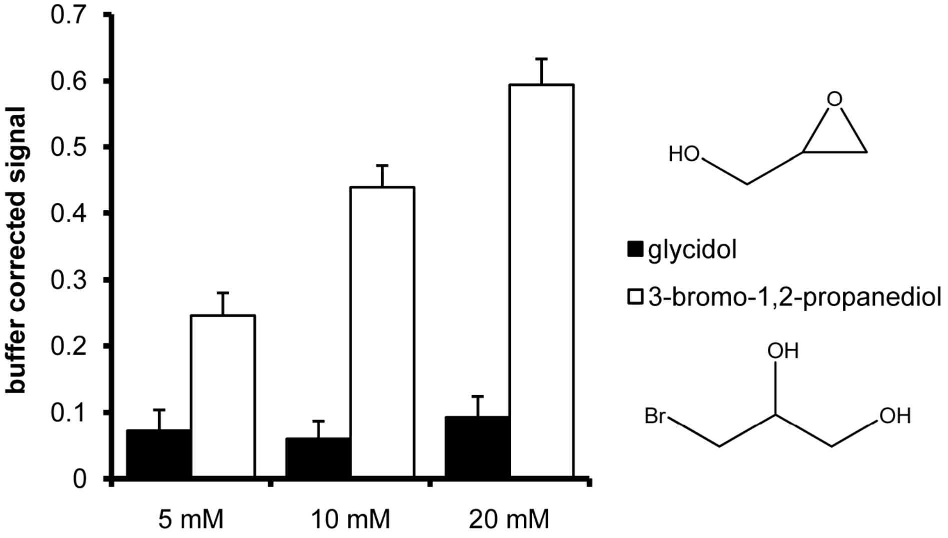
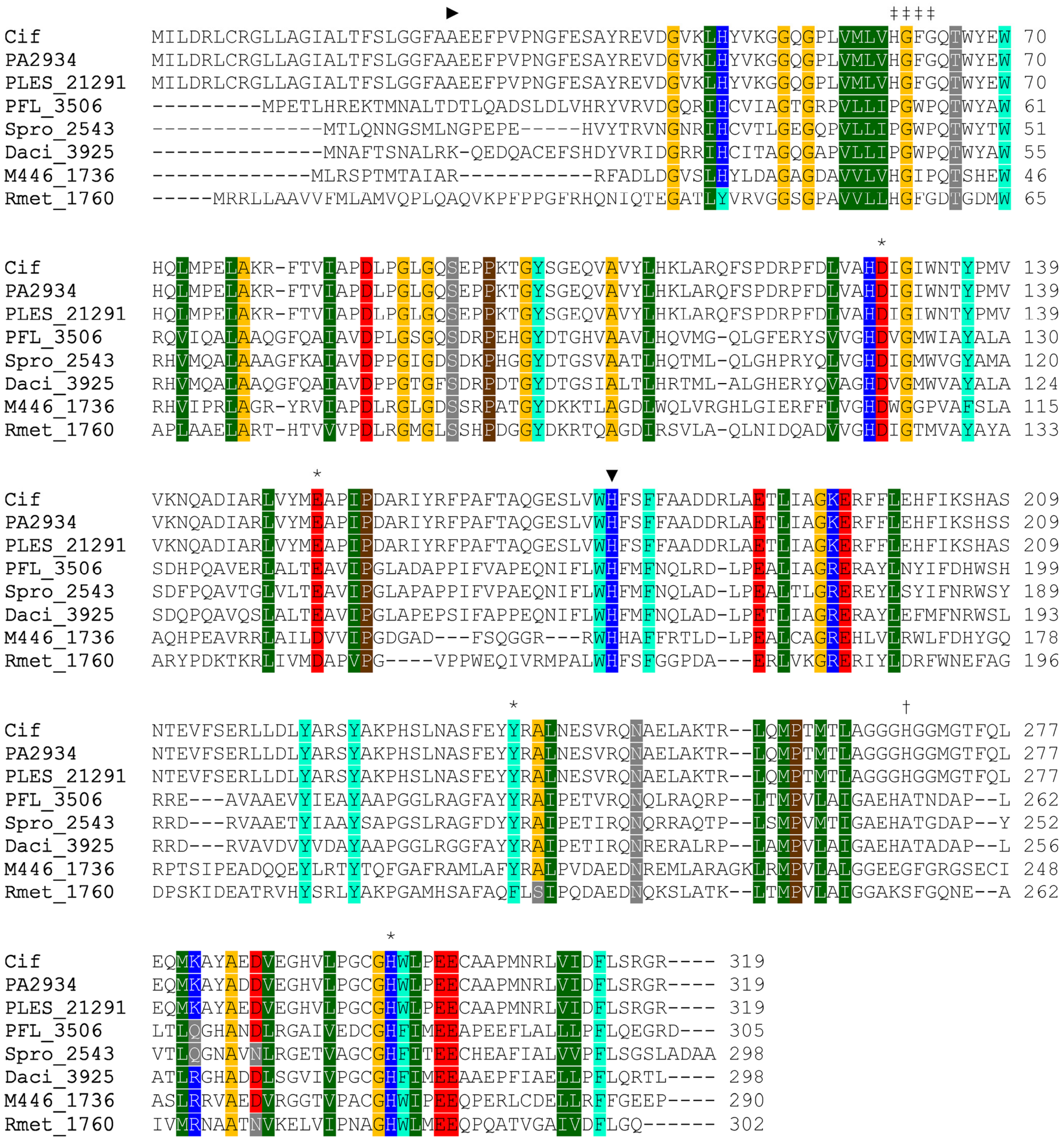


Fig. S4. EH activity is responsible for the EBH/adrenochrome assay signal. A dose response for the products of EBH epoxide hydrolysis and debromination was determined using the same procedure as the enzyme assay. Glycidol, which would be the product of haloalkane dehalogenase (HLD) activity on EBH, was not detected by this assay, while the EH product 3-bromo-1,2-propanediol (BPD) yielded a dose dependant signal. In this assay, the signal reported for the samples is subtracted from the buffer signal (the maximum signal possible), and as such a high signal is representative of a low OD_{490} at the level of raw data (see Fig. S2). Note that the adrenochrome response to BPD is no longer in the linear range at 20 mM.



```
                                      ▶                                    ‡‡‡‡
Cif           MILDRLCRGLLAGIALTFSLGGFAAEEFPVPNGFESAYREVDGVKLHYVKGGQGPLVMLVHGFGQTWYEW 70
PA2934        MILDRLCRGLLAGIALTFSLGGFAAEEFPVPNGFESAYREVDGVKLHYVKGGQGPLVMLVHGFGQTWYEW 70
PLES_21291    MILDRLCRGLLAGIALTFSLGGFAAEEFPVPNGFESAYREVDGVKLHYVKGGQGPLVMLVHGFGQTWYEW 70
PFL_3506      ---------MPETLHREKTMNALTDTLQADSLDLVHRYVRVDGQRIHCVIAGTGRPVLLIPGWPQTWYAW 61
Spro_2543     --------------MTLQNNGSMLNGPEPE-----HVYTRVNGNRIHCVTLGEGQPVLLIPGWPQTWYTW 51
Daci_3925     --------------MNAFTSNALRK-QEDQACEFSHDYVRIDGRRIHCITAGQGAPVLLIPGWPQTWYAW 55
M446_1736     -------------MLRSPTMTAIAR-----------RFADLDGVSLHYLDAGAGDAVVLVHGIPQTSHEW 46
Rmet_1760     -----MRRLLAAVVFMLAMVQPLQAQVKPFPPGFRHQNIQTEGATLYVRVGGSGPAVVLLHGFGDTGDMW 65

                                                                         *
Cif           HQLMPELAKR-FTVIAPDLPGLGQSEPPKTGYSGEQVAVYLHKLARQFSPDRPFDLVAHDIGIWNTYPMV 139
PA2934        HQLMPELAKR-FTVIAPDLPGLGQSEPPKTGYSGEQVAVYLHKLARQFSPDRPFDLVAHDIGIWNTYPMV 139
PLES_21291    HQLMPELAKR-FTVIAPDLPGLGQSEPPKTGYSGEQVAVYLHKLARQFSPDRPFDLVAHDIGIWNTYPMV 139
PFL_3506      RQVIQALAAQGFQAIAVDPLGSGQSDRPEHGYDTGHVAAVLHQVMG-QLGFERYSVVGHDVGMWIAYALA 130
Spro_2543     RHVMQALAAAGFKAIAVDPPGIGDSDKPHGGYDTGSVAATLHQTML-QLGHPRYQLVGHDIGMWVGYAMA 120
Daci_3925     RHVMQALAAQGFQAIAVDPPGTGFSDRPDTGYDTGSIALTLHRTML-ALGHERYQVAGHDVGMWVAYALA 124
M446_1736     RHVIPRLAGR-YRVIAPDLRGLGDSSRPATGYDKKTLAGDLWQLVRGHLGIERFFLVGHDWGGPVAFSLA 115
Rmet_1760     APLAAELART-HTVVVPDLRGMGLSSHPDGGYDKRTQAGDIRSVLA-QLNIDQADVVGHDIGTMVAYAYA 133

                           *                       ▼
Cif           VKNQADIARLVYMEAPIPDARIYRFPAFTAQGESLVWHFSFFAADDRLAETLIAGKERFFLEHFIKSHAS 209
PA2934        VKNQADIARLVYMEAPIPDARIYRFPAFTAQGESLVWHFSFFAADDRLAETLIAGKERFFLEHFIKSHSS 209
PLES_21291    VKNQADIARLVYMEAPIPDARIYRFPAFTAQGESLVWHFSFFAADDRLAETLIAGKERFFLEHFIKSHAS 209
PFL_3506      SDHPQAVERLALTEAVIPGLADAPPIFVAPEQNIFLWHFMFNQLRD-LPEALIAGRERAYLNYIFDHWSH 199
Spro_2543     SDFPQAVTGLVLTEAVIPGLAPAPPIFVPAEQNIFLWHFMFNQLAD-LPEALTLGREREYLSYIFNRWSY 189
Daci_3925     SDQPQAVQSLALTEAVIPGLAPEPSIFAPPEQNIFLWHFMFNQLAD-LPETLIAGRERAYLEFMFNRWSL 193
M446_1736     AQHPEAVRRLAILDVVIPGDGAD---FSQGGR---RWHHAFFRTLD-LPEALCAGREHLVLRWLFDHYGQ 178
Rmet_1760     ARYPDKTKRLIVMDAPVPG----VPPWEQIVRMPALWHFSFGGPDA---ERLVKGRERIYLDRFWNEFAG 196

                                             *                               †
Cif           NTEVFSERLLDLYARSYAKPHSLNASFEYYRALNESVRQNAELAKTR--LQMPTMTLAGGGHGGMGTFQL 277
PA2934        NTEVFSERLLDLYARSYAKPHSLNASFEYYRALNESVRQNAELAKTR--LQMPTMTLAGGGHGGMGTFQL 277
PLES_21291    NTEVFSERLLDLYARSYAKPHSLNASFEYYRALNESVRQNAELAKTR--LQMPTMTLAGGGHGGMGTFQL 277
PFL_3506      RRE---AVAAEVYIEAYAAPGGLRAGFAYYRAIPETVRQNQLRAQRP--LTMPVLAIGAEHATNDAP--L 262
Spro_2543     RRD---RVAAETYIAAYSAPGSLRAGFDYYRAIPETIRQNQRRAQTP--LSMPVMTIGAEHATGDAP--Y 252
Daci_3925     RRD---RVAVDVYVDAYAAPGGLRGGFAYYRAIPETIRQNRERALRP--LAMPVLAIGAEHATADAP--L 256
M446_1736     RPTSIPEADQQEYLRTYTQFGAFRAMLAFYRALPVDAEDNREMLARAGKLRMPVLALGGEEGFGRGSECI 248
Rmet_1760     DPSKIDEATRVHYSRLYAKPGAMHSAFAQFLSIPQDAEDNQKSLATK--LTMPVLAIGGAKSFGQNE--A 262

                                 *
Cif           EQMKAYAEDVEGHVLPGCGHWLPEECAAPMNRLVIDFLSRGR---- 319
PA2934        EQMKAYADDVEGHVLPGCGHWLPEECAAPMNRLVIDFLSRGR---- 319
PLES_21291    EQMKAYAEDVEGHVLPGCGHWLPEECAAPMNRLVIDFLSRGR---- 319
PFL_3506      LTLQGHANDLRGAIVEDCGHFIMEEAPEEFLALLPFLQEGRD--- 305
Spro_2543     VTLQGNAVNLRGETVAGCGHFITEECHEAFIALVVPFLSGSLADAA 298
Daci_3925     ATLRGHADDLSGVIVPGCGHFIMEEAAEPFIAELLPFLQRTL---- 298
M446_1736     ASLRRVAEDVRGGTVPACGHWIPEEQPERLCDELLRFFGEEP---- 290
Rmet_1760     IVMRNAATNVKELVIPNAGHWLMEEQPQATVGAIVDFLGQ------ 302
```

Fig. S5. Cif alignment with homologous proteins. The sequences of Cif and several homologous protein sequences from related *Pseudomonas* strains and species were aligned using ClustalW. Protein sequences compared with Cif (PA14_26090) are named according to the Kyoto Encyclopedia of Genes and Genomes. The replacement of a canonical active site Tyr with a His (His177 in Cif) and perturbations in the HGxP motif are not features unique to Cif. Amino acids are colored as follows: small side chains are orange (Gly and Ala), Pro is brown, Cys is maroon, polar side chains are grey (Ser, Thr, Asn and Gln), acidic side chains are red (Asp and Glu), basic side chains are blue (His, Arg and Lys), non-polar side chains are green (Ile, Leu, Met and Val), and aromatic side chains are cyan (Phe, Tyr and Trp). ►first residue of the mature Cif protein; ‡HGxP motif; *Active site residues; ▼His177 of Cif; †Residue His269 of Cif

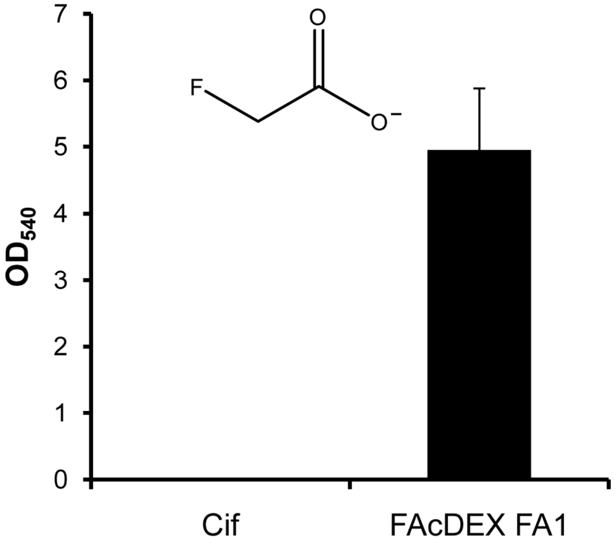


Fig. S6. Lack of detectable haloacetate dehalogenase activity for Cif. 10 µM enzyme was incubated with 10 mM fluoroacetate at 30°C for 30 min in 100 mM glycine (pH 9). Data shown is the buffer subtracted signal, and the fluoroacetate dehalogenase from *Burkholderia* sp. Strain FA1 (FAc-DEX FA1) signal is a calculated OD reading, accounting for the 1:10 dilution in concentrated H_2SO_4 necessary to prevent saturation of the optical density as measured by the spectrophotometer.

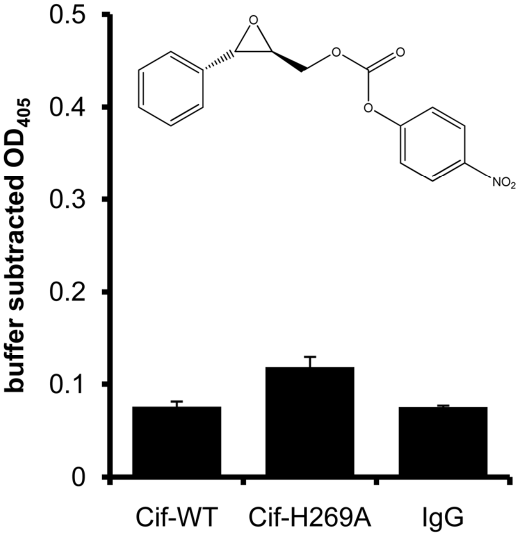


Fig. S7. S-NEPC is not a substrate for Cif. 100 µg of protein (Cif-WT or Cif-H269A) was incubated with 100 µM S-NEPC at 37°C for 60 min in 500 mM NaCl, 20 mM Tris (pH 8.5) in a 200 µL volume. Data shown is the buffer subtracted signal. 100 µg of bovine serum IgG (Sigma) was used as a negative protein control.