**LanCLs have *C-*glutathionylation activity with potential to trap the eliminylome**

**Table of content in ORA-data**

1. **Figure 3**. **A strategy for chemical generation of eliminated MEK1 proteins.**

Figure 3.B. Predicted side-chain accessibility of sites with / without ‘masking’ nucleotide and Mg (II) by use of Naccess (File: CellCoraDataFig3B.docx)

1. **Figure 5. LanCL proteins catalyze non-canonical glutathionylation of dehydroamino acid containing proteins (File: CellCoraDataFig5.xlsx)**
2. Figure 5.C. Time course of GSH addition to MEK1-Dha218 catalyzed by wild-type or mutant LanCL1/2.
3. Figure 5.D. Pseudo-single substrate Michaelis-Menten plot in the presence of wt-LanCL1 or wt-LanCL2 (see also in Table 1 for processed data)
4. **Eliminylome Analysis (File: CellCoraData\_eliminylomeSurvey\_SI\_Table.xlsx)**
5. **Key Resources Table 2. Kinetic parameters for phosphorylation of protein ERK by MEK1 variants (see also in Figure S6d for ESI-MS progress curves; Folder: CellCoraDataFigS6d)**
6. ESI-MS progress curves (file: CellCoraFigS6d.xlsx)
7. LC-MS spectra of related MEK1 variants (Folder: CellCoraDataFigS6d\_LC-MS\_related MEK1variants)
8. **Key Resources Table 4. Results of MEK1 variants treated with different bisalkylating/elimination agents in the absence of additives at 37 °C (Folder: CellCoraDataKey Resources Table 4\_LCMS)**
9. **Key Resources Table 5. Results of MEK1 treated with different bis-alkylating/ elimination agents in the presence of ADP/Mg2+ at 37 oC (Folder: CellCoraDataKey Resources Table 5\_LCMS)**
   * + 1. **Figure S2. Scoping and Controlling Chemical Elimination of MEK1. Related to Figure 3.**

Figure S2.c. Thermal shifts, ΔTm, determined by differential scanning fluorimetry (DSF) of MEK1C222 in the presence or absence of ATP, ATPγS, ADP and MgCl2 (Folder: CellCoreDataKeyResourcesFigS2c\_Thermal\_shift)

1. **Figure S5. Construction of MEK1-Dha218Dha222. Related to Figure 4.**

FigureS5.f. Comparative circular dichroism measurement of MEK1-Cs and MEK1-Dhas (File: CellCoraDataFigS5f.xlsx).

1. **Figure S6. Kinase activity assay of MEK1. Related to Figure 5 and Key Resources Table 2 (Folder: CellCoraDataFigS6d)**
2. Figure S6.d. ESI-MS progress curves allowing time course analysis of ERK1 by MEK1 variants (see also Key Resources Table 2 for processed data, file: CellCoraFigS6d.xlsx)
3. LC-MS spectra of related MEK1 variants (Folder: CellCoraDataFigS6d\_LC-MS\_related MEK1variants)
4. **Figure S7. LanCL proteins catalyze *C-*glutathionylation of MEK-Dha and ERK1-Dha. Related to Figure 5 and Table 1 (Folder: CEllCoraDataFigS7).**
5. Figure S7.A. End-point assays of LanCL1 or LanCL2 with His6-MEK1-Dha218 as the substrate for glutathionylation (File: CellCoraDataFigS7.xlsx)
6. Figure S7.B. top insert: Time-dependent GS addition to ERK1-Dha202 catalyzed by wild type or mutant LanCL proteins (File: CellCoraDataFigS7.xlsx)
7. Related LC-MS and Tandem MS/MS data of ERK1C202, ERK1C82C202, ERK1Dha202, and ERK1Dha82Dha202 (Folder:CellCoraDataFigS7\_ERK1\_LCMS\_ MSMS)
8. **Figure S8. LanCL proteins add GSH to dehydroamino acid-containing proteins and peptides. Related to Figures 5 and 6 (Folder: CellCoraDataFigS8A).**
9. Figure S8.A. LanCL2 adds GSH to GST-3XFlag-ERK2-Dhb185pY187 generated using the pThr lyase OspF (File: CellCoraDataFigS8A.xlsx)
10. Related LC-MS of GST-3XFLAG-ERK2, GST-3XFLAG-ERK2pT185pY187, GST-3XFLAG-ERK2Dhb185pY187 (File: CellCoraDataFigS8A\_GST-Flag-ERK2\_ LCMS.png)

**Extra raw data mentioned in this paper.**

1. Tandem MS/MS spectra related to Figure 4, Key Resources Table 4 and Table 5 (Folder: CellCoraData\_Tandem MSMS\_Fig4\_Key Resources Table4\_Table5)