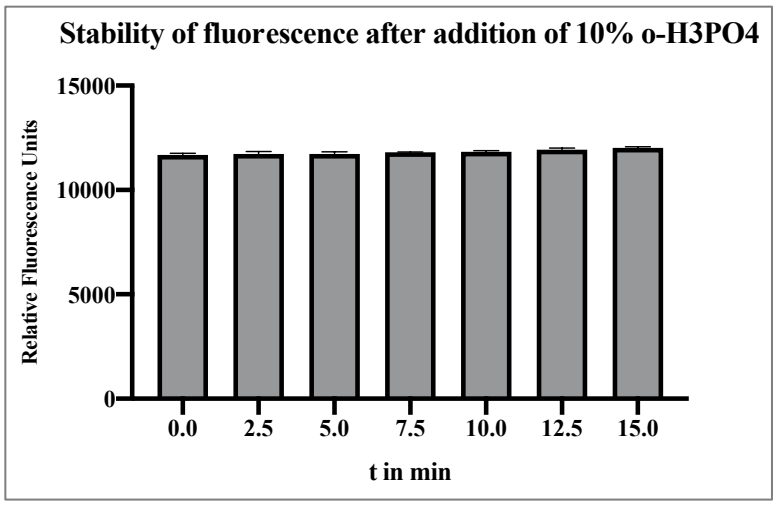


Figure S1: Stop of reaction of 4-methylumbelliferryl butyrate and CRL by addition of 10% phosphoric acid. The increase of fluorescence after 20 min after addition of the stop reagent was less than 3%. The fluorescence is therefore considered stable after stop reagent addition.

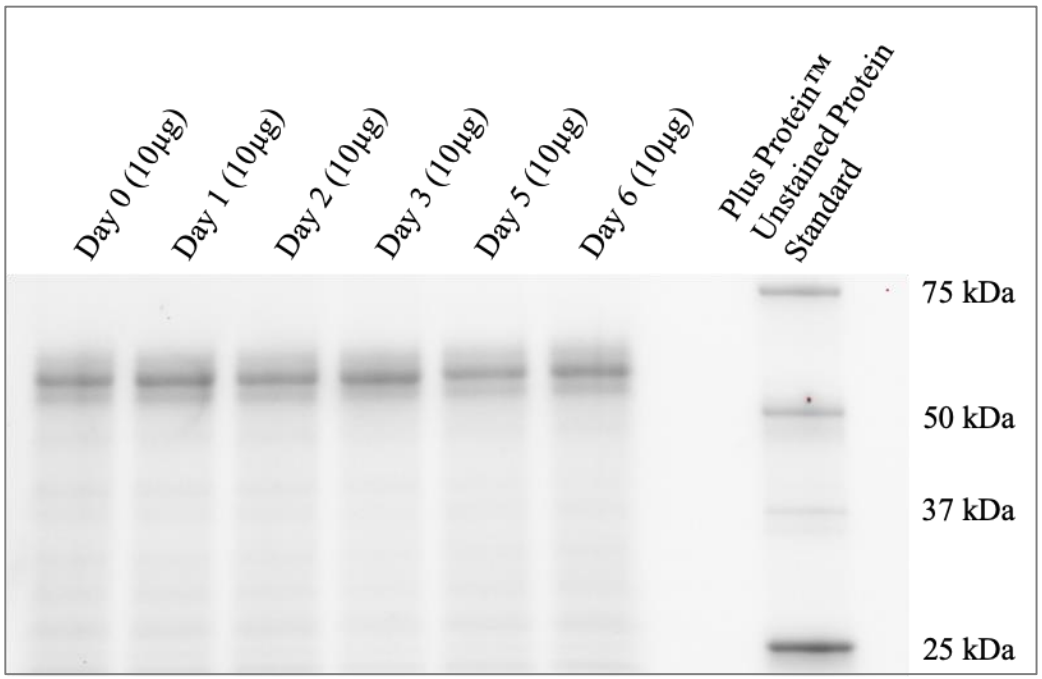


Figure S2: Stability of CRL in water throughout 6 days. The enzyme is stable showing neither an increase in bands below 60 kDa, which would indicate an increase in cleavage products, nor a reduction of the CRL band at 60 kDa.

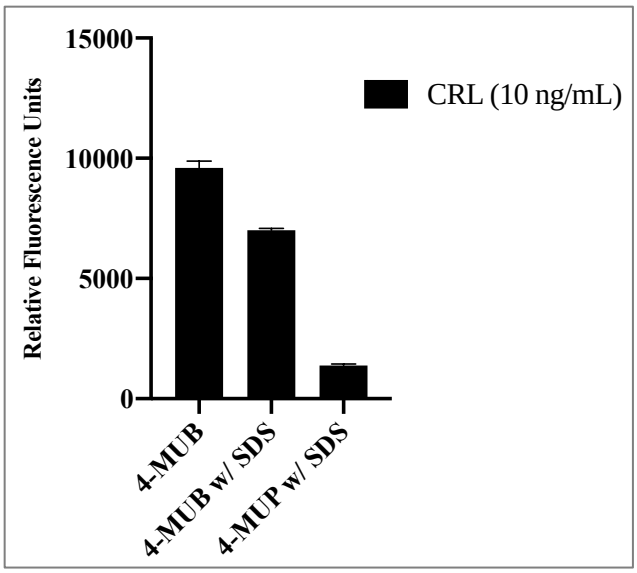
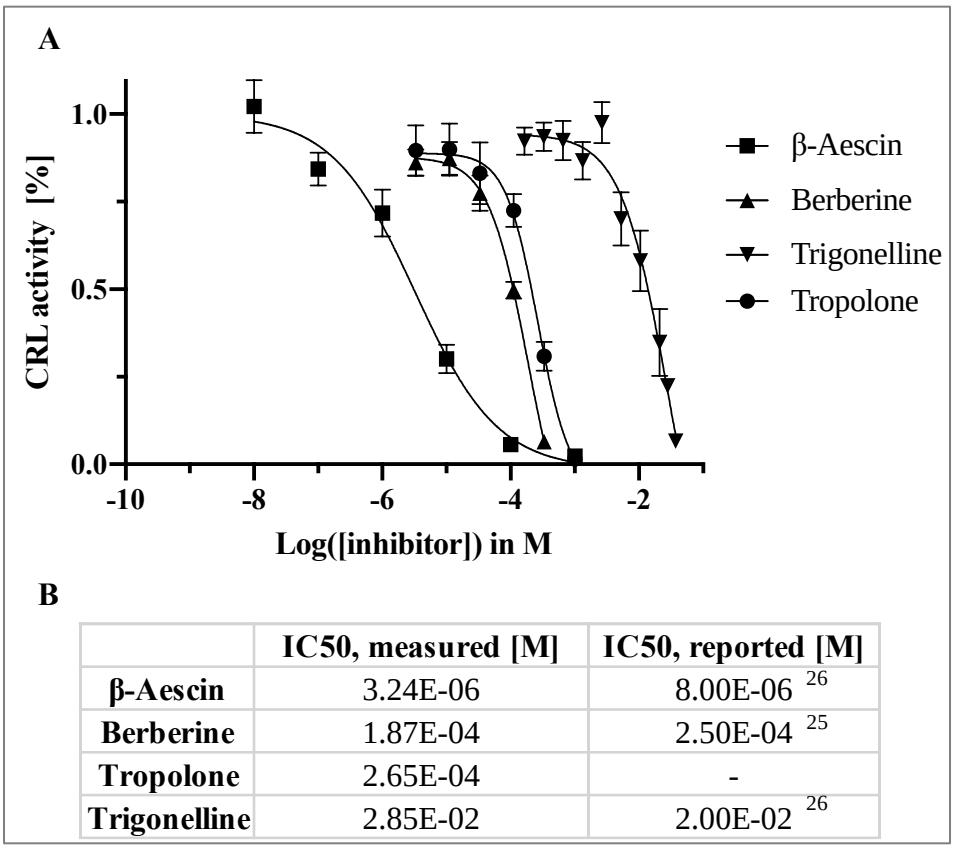
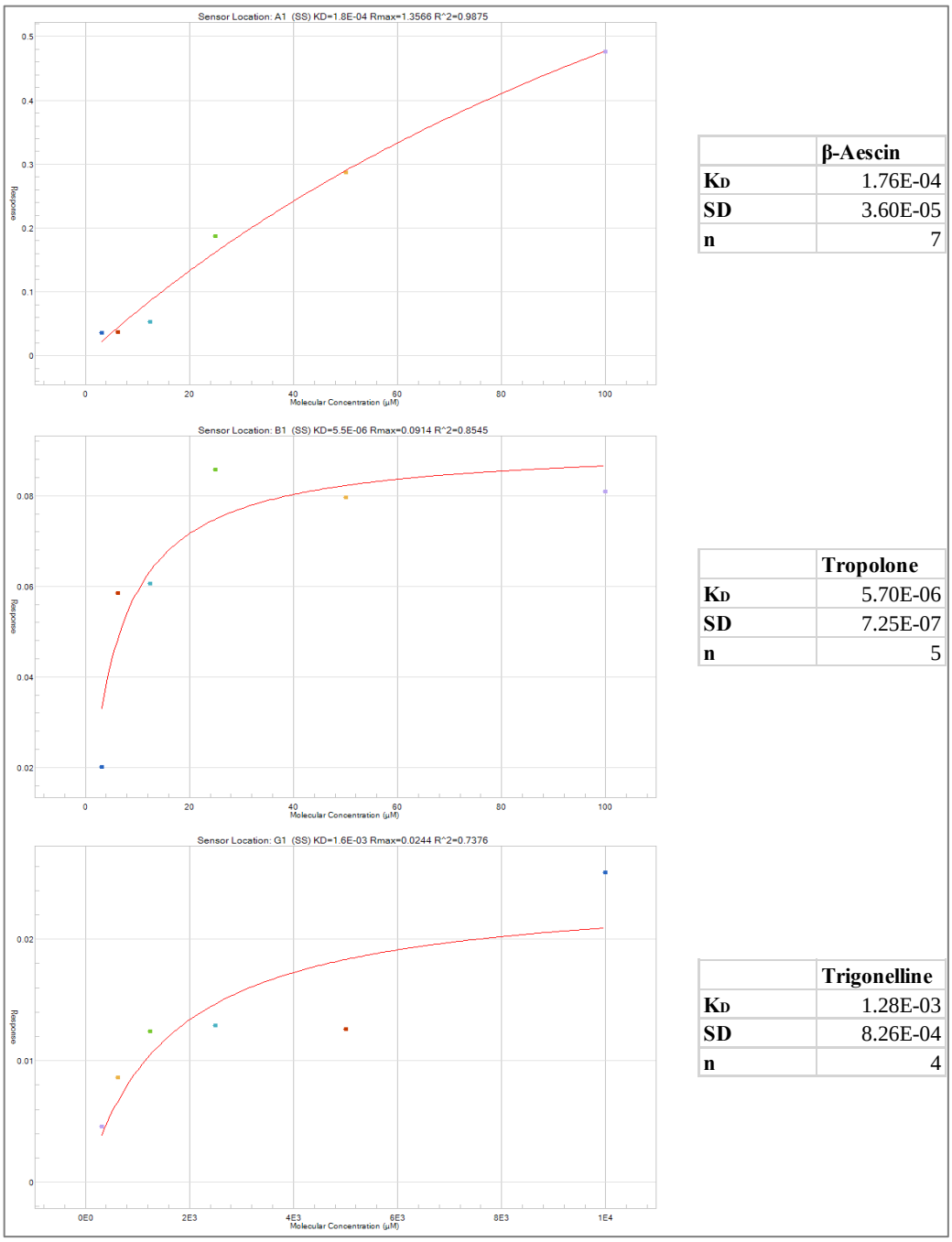


Figure S3: Direct comparison of the enzyme activity with substrate 4-MUB and 4-MUP and the impact of SDS. All data points were obtained by equalizing the assays conditions. The enzyme concentration was set to 3.3 ng/mL, while the substrate and stop reagent volume was 50 μL. This experiment indicates the great specificity difference between 4-MUP and 4-MUB, which is not solely to explain by the SDS addition, highlighting the enzyme's preference for short-chain fatty acids as reported in the literature.



	IC50, measured [M]	IC50, reported [M]
β-Aescin	3.24E-06	8.00E-06 [26]
Berberine	1.87E-04	2.50E-04 [25]
Tropolone	2.65E-04	-
Trigonelline	2.85E-02	2.00E-02 [26]

Figure S4: IC50s of Tropolone and other published inhibitors. A. Plot of varying concentrations of β-Aescin, Berberine, Tropolone and Trigonelline, and their impact on CRL activity. Tropolone and Berberine have a similar IC50 range, while β-Aescin is two orders of magnitude lower and Trigonelline two orders of magnitude higher. The CRL concentration in the assay was ten-fold lower, which allowed the measurement of weak inhibitors such as Trigonelline. The resulting IC50s are comparable to the published IC50s, which had been determined by high-performance liquid chromatography.



	β-Aescin
KD	1.76E-04
SD	3.60E-05
n	7

	Tropolone
KD	5.70E-06
SD	7.25E-07
n	5

	Trigonelline
KD	1.28E-03
SD	8.26E-04
n	4

Figure. S5: Biolayer-interferometry analysis of Tropolone in comparison to Trigonelline and β-Aescin. Tropolone shows the lowest K_D value followed by β-Aescin and Trigonelline. Although Tropolone was determined with a higher IC50 value than β-Aescin, the biolayer-interferometry shows that Tropolone's interaction with CRL is more specific.

Table S1: GCMS analysis of CRL extract from Deerland to identify extract components. Beside the primary band at 60 kDa several others occurred less intense as evident in supplementary figure S2. These bands were further investigated to assure purity of the obtained extract. Lipase 1 and 3 had the greatest coverage in all investigated bands, while lipase 2 was solely detected in the dominant band. The detected actin is an impurity, which either occurred during the dilution process or was not removed during the extraction.

Molecular weight of band	60 kDa	50 kDa	40 kDa	35 kDa
Liapse1	23	11	11	11
Lipase2	5	-	-	-
Lipase3	17	9	6	9
Lipase4	-	-	-	-
Lipase 5	-	-	-	-
Actin	1	-	-	-

Method S1: Computational determination of Tropolone as allosteric modulator of CRL. 2D structures of natural products were obtained from the ZINC database (Zbc; Zbc Leads; Zbc Drugs; Zbc frags), InterBioScreen (natural compounds) and AnalytiCon (FRGx, MEGx). 3D structures for each compound were calculated with LigPrep (Schrodinger Software). CRL's crystal structure of the open conformation of isoform CRL1 (PDB: 1CRL) was prepared with the Protein Preparation Wizard in the Schrodinger software in accordance with Paloccis et al. published appraoch[24]. Top-ranked determined allosteric binding sites were identified with SiteMap (Schrodinger Software), which required at least 15 site points per hit and described a small distinct pocket on the surface of the enzyme. The prepared ligand libraries were docked with Ligand Docking (Schrodinger Software) in the respective sites in standard and subsequently extra precision mode for ligands with a docking score below -5. The results were processed and sorted in the Canvas application (Schrodinger Software) and respective high scoring ligand Tropolone was confirmed by the in-vitro screening assay.

Method S2: K_D determination by Biolayer-Interferometry. 1mL CRL in PBS (10 mg/mL) was biotinylated with 400 nmol of EZ-link Sulfo-NHS-Biotin (ThermoScientific, Waltham, MA) for 2h at room temperature and stored at -20°C. Eight superstreptavidin-sensors (SSA, ForteBio, San Jose, CA) were coated for 10 min in 200 μL of diluted biotinylated CRL (1 mg/mL). Sensors were air-dried for 10 min and 200 μL of dilution added into separate 96-well columns. Subsequently, each dilution was measured 300s for association and 240s for dissociation in the Octet RED96e (ForteBio, San Jose, CA). K_D were determined over the different concentrations of inhibitors (Tropolone and β-Aescin: 100 μM – 3.1 μM; Trigonelline: 10 mM – 0.31 mM) for each sensor, respectively.