SUPPLEMENTARY INFORMATION FOR:

A fragment-based electrophile-first approach to target histidine with aryl-fluorosulfates: application to hMcl-1

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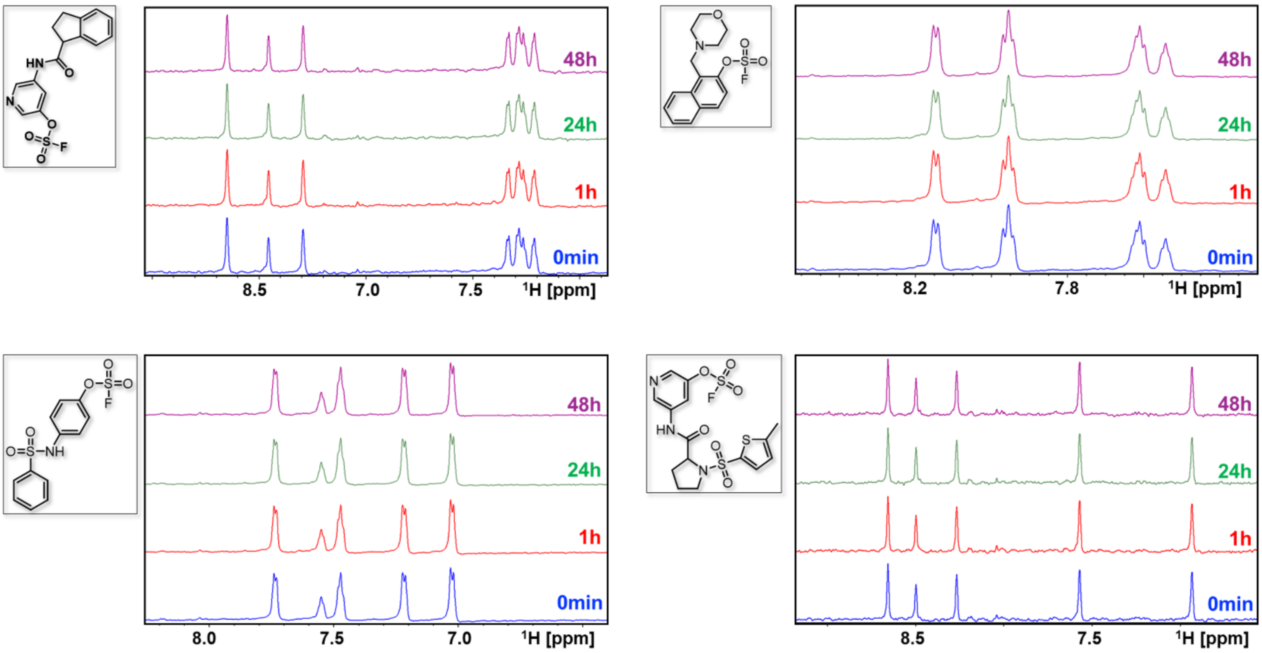
Supplementary information contains:

Supplementary Figures S1 – S9

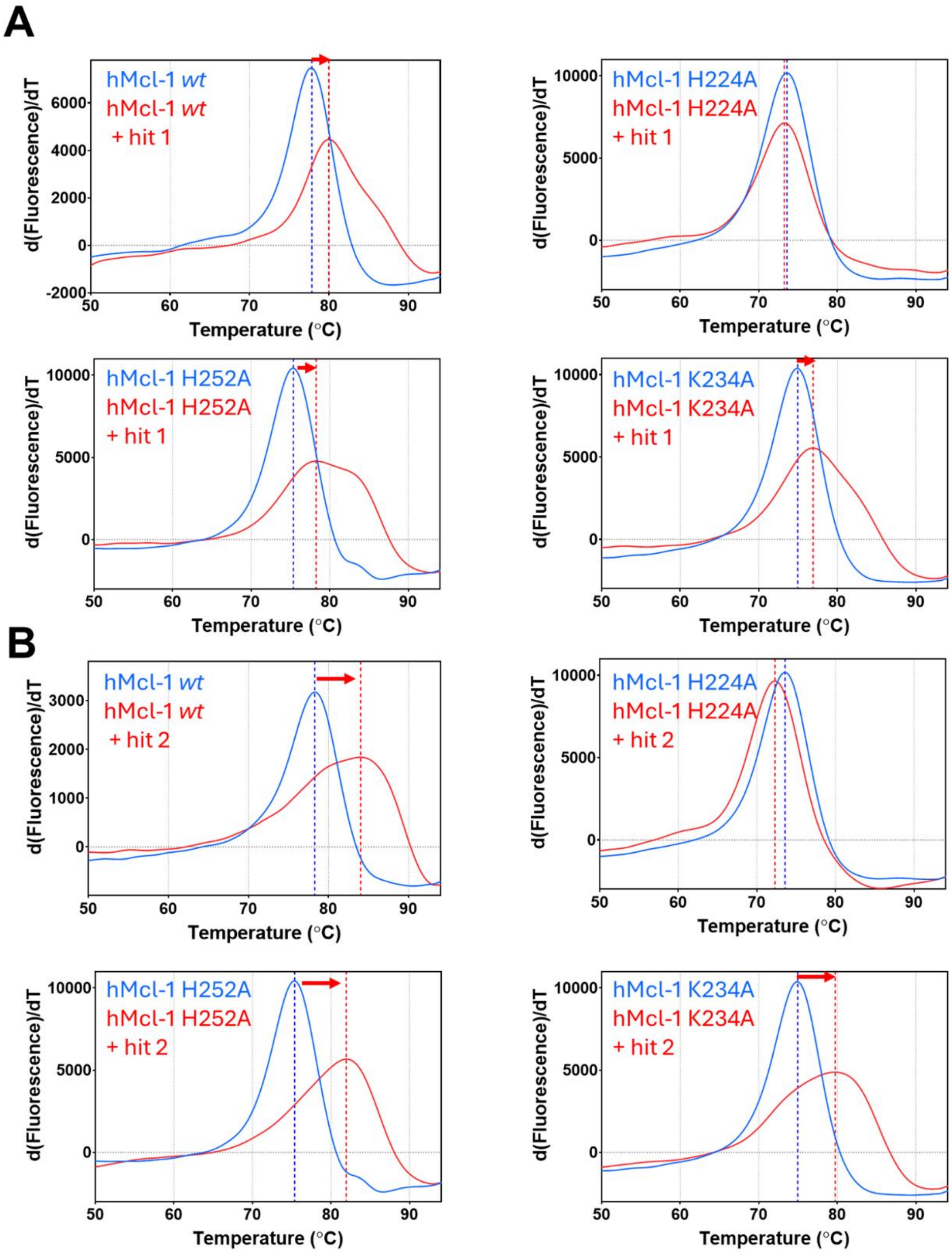
Supplementary Tables S1 – S2

SUPPLEMENTARY FIGURES

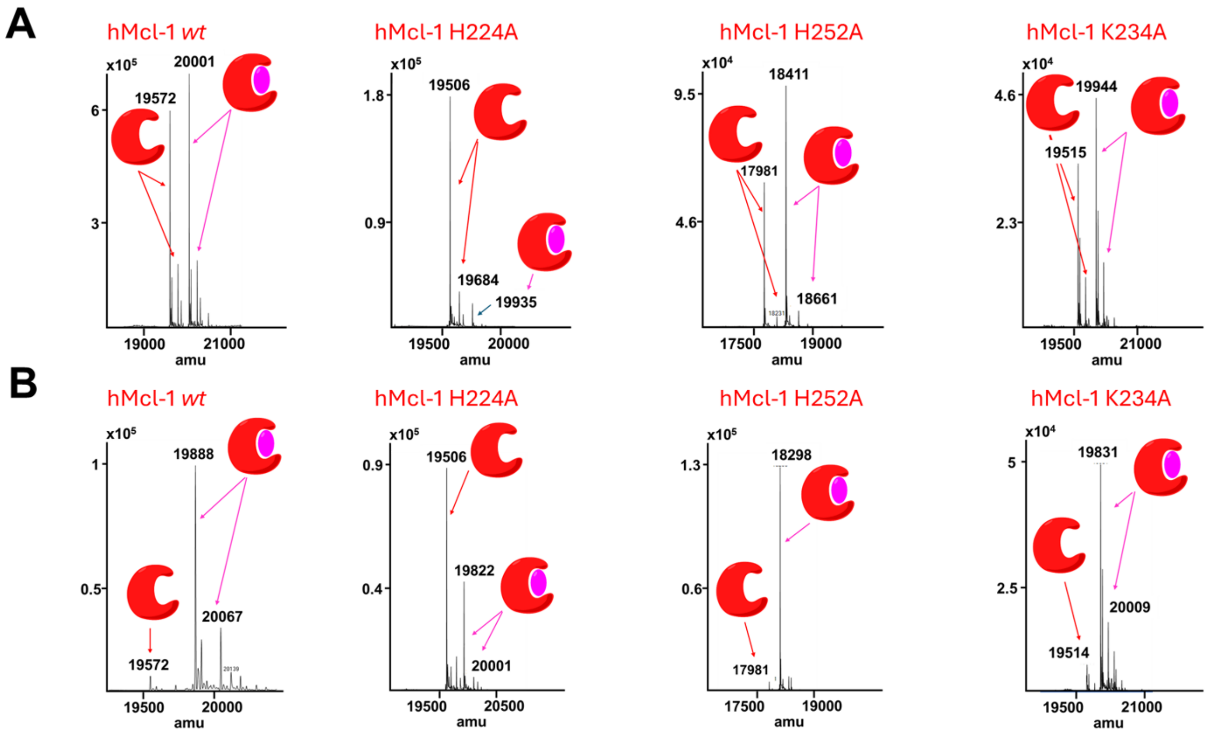
Supplementary Figure S1: Stability of fluorosulfates tested with 1D ^{1}H NMR spectra collected at different time points (0 min, 1 h, 24 h, 48 h).



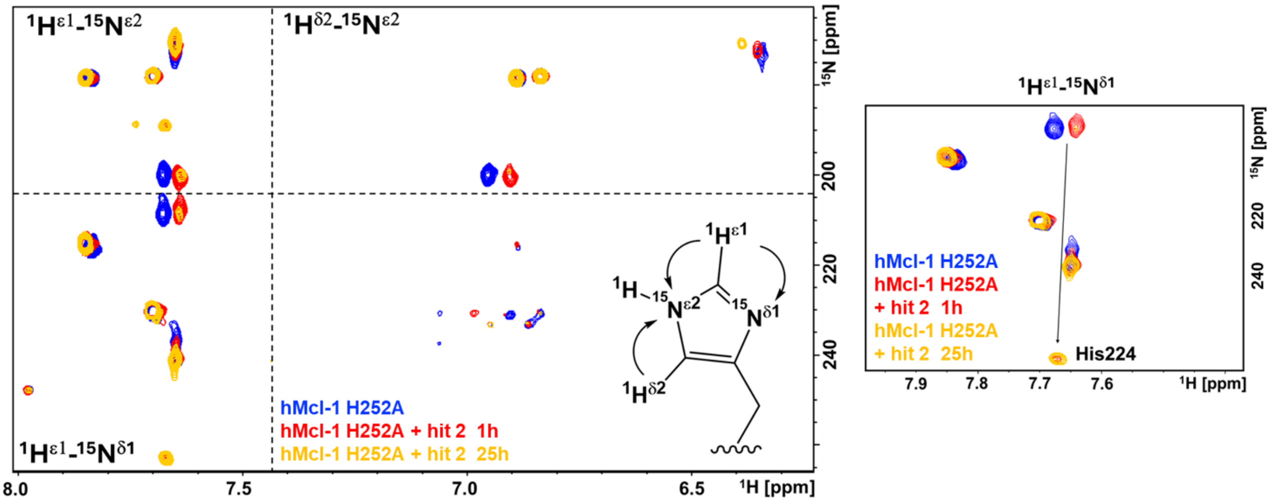
Supplementary Figure S2: Thermal denaturation curves for hMcl-1(172-323) or mutant proteins as indicated, collected in absence and presence of fragment hit **1** or fragment hit **2**. **A)** ΔTm (*wt*-hMcl-1) = 2.11 ± 0.01; ΔTm (hMcl-1 H224A) = -0.34 ± 0.09; ΔTm (hMcl-1 H252A) = 2.90 ± 0.17; ΔTm (hMcl-1 K234A) = 1.96 ± 0.17. **B)** ΔTm (*wt*-hMcl-1) = 3.97 ± 0.17; ΔTm (hMcl-1 H224A) = -1.28 ± 0.17; ΔTm (hMcl-1 H252A) = 6.57 ± 0.26; ΔTm (hMcl-1 K234A) = 4.77 ± 0.26.



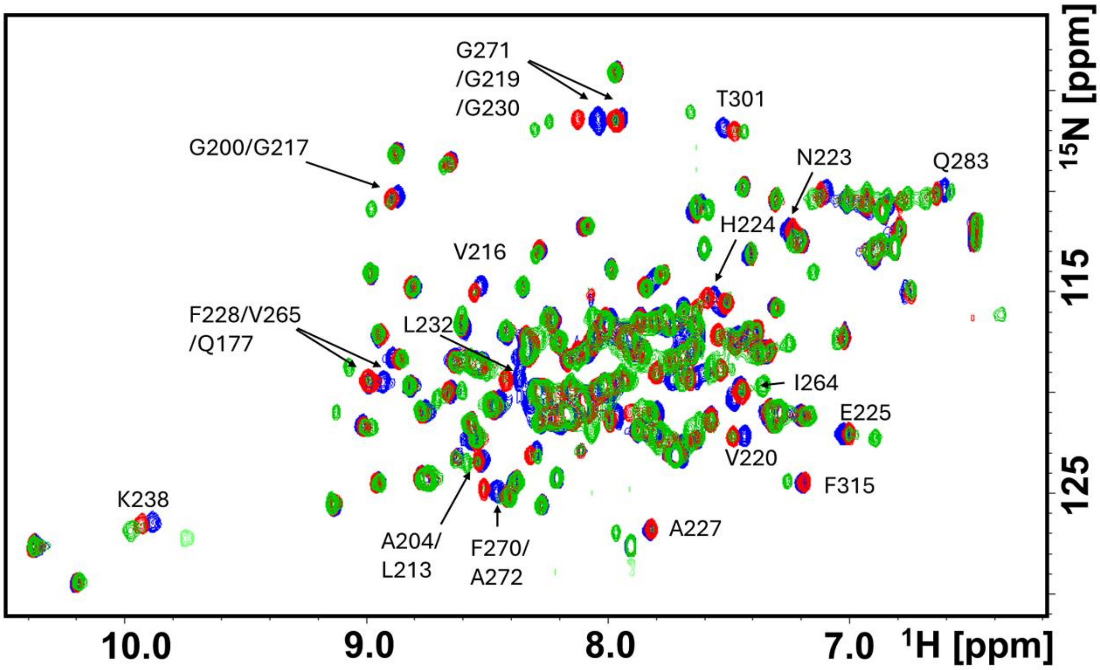
Supplementary Figure S3: Mass spectrometry analyses of hMcl-1(171-323) and mutants as indicated collected in presence of fragment hit **1** (A) or fragment hit **2** (B).



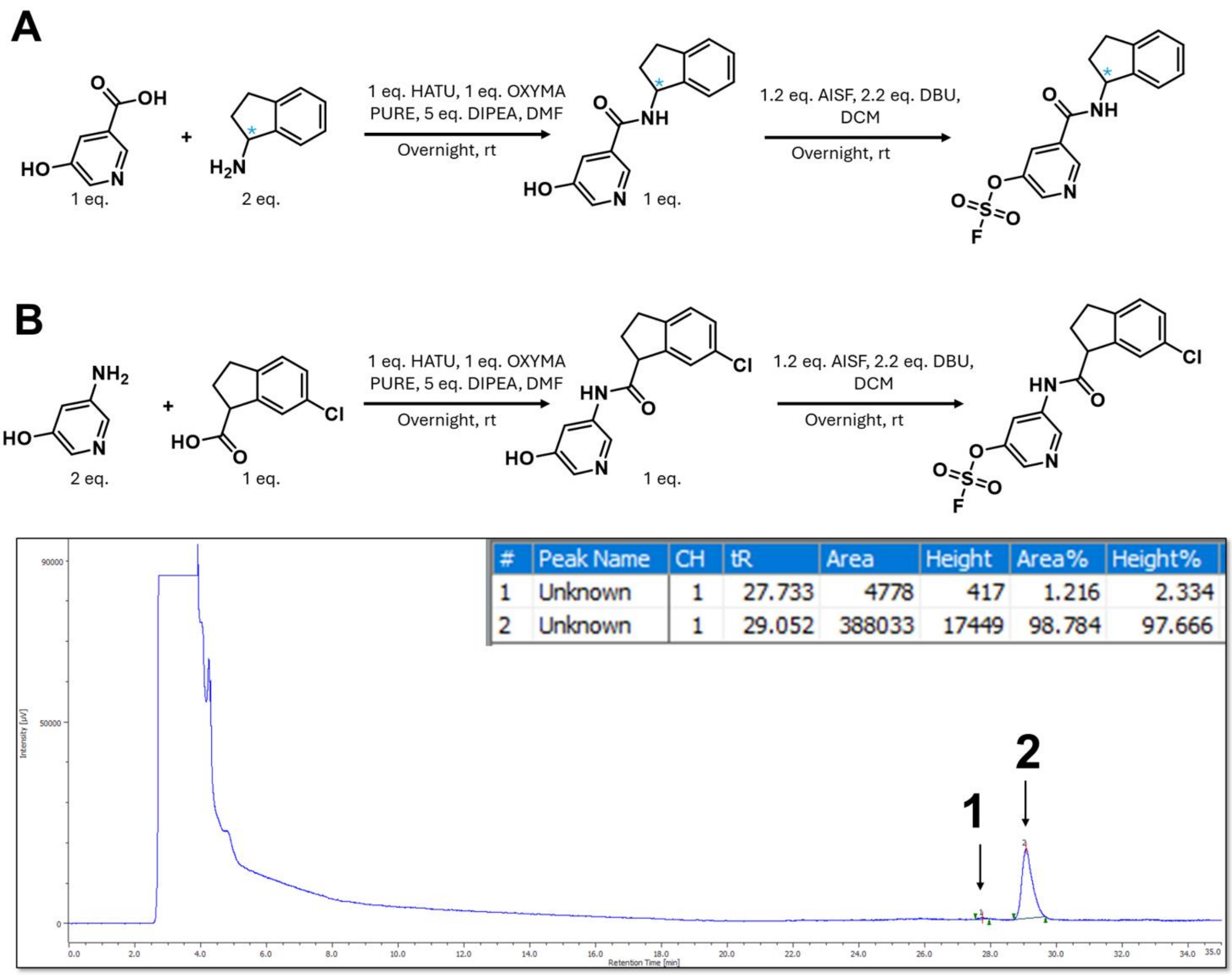
Supplementary Figure S4: Heteronuclear long range [^{15}N,^{1}H] NMR correlation spectra for His side chains detection. The spectra for hMcl-1(172-323) His252Ala, collected in absence (blue) or presence of fragment hit **2** after various incubation times (red, 1 h; yellow, 25 h) are superimposed. On the right, a zoomed region highlighting the chemical shift perturbations at His 224 is displayed.



Supplementary Figure S5: Backbone 2D [^{15}N,^{1}H] correlation spectra for ^{15}N-hMcl-1(172-323) (50 μM) collected in absence (blue) or presence of fragment hit **2** (1 mM) after various incubation times (red, 1 h; green, 25 h). Resonance assignments are also reported.

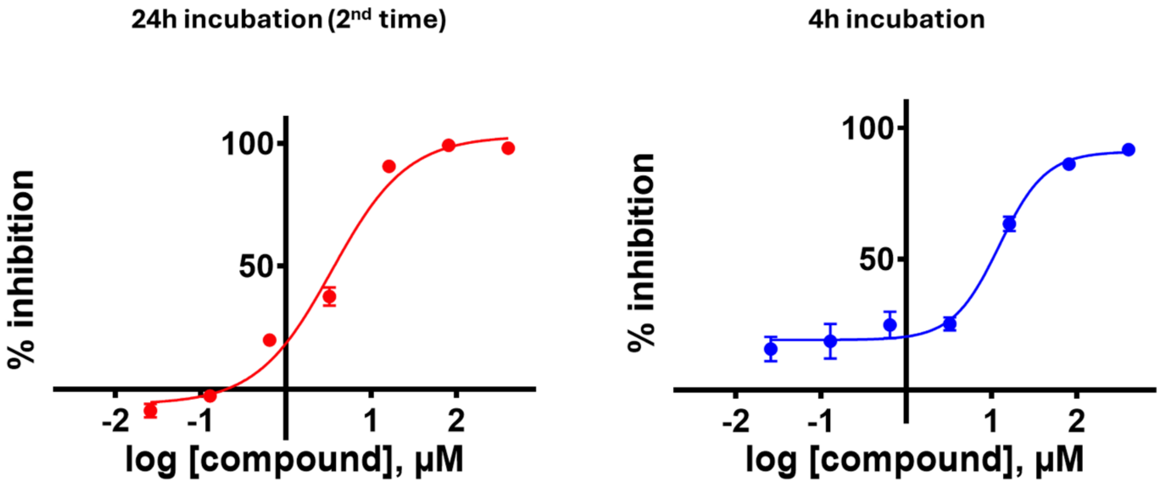


Supplementary Figure S6: Synthetic route used to obtain fragment hit **2** and its analogs reported in **Figure 6**. The purity of **165D9** by HPLC is also reported (purity > 98%).

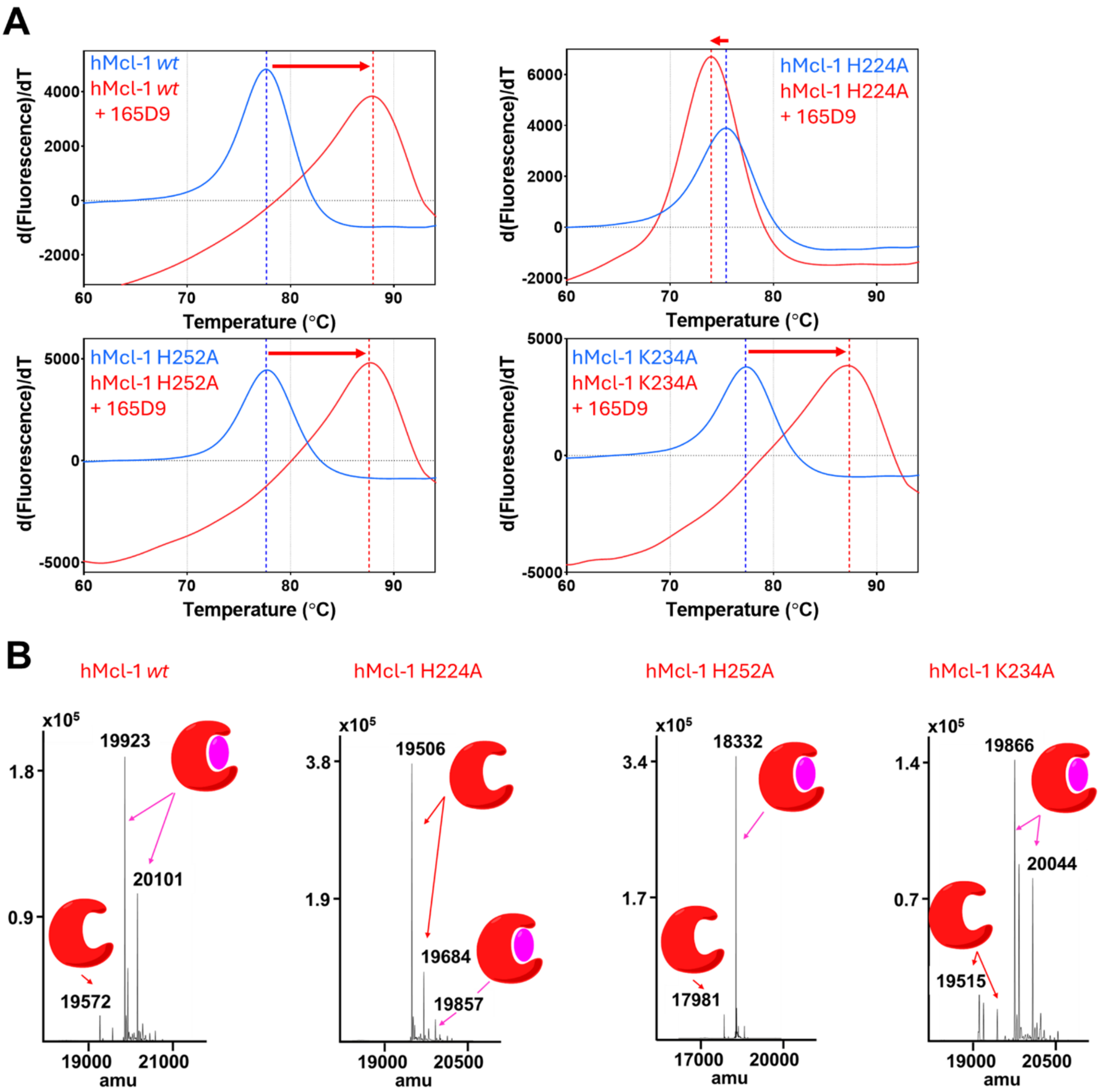


#	Peak Name	CH	tR	Area	Height	Area%	Height%
1	Unknown	1	27.733	4778	417	1.216	2.334
2	Unknown	1	29.052	388033	17449	98.784	97.666

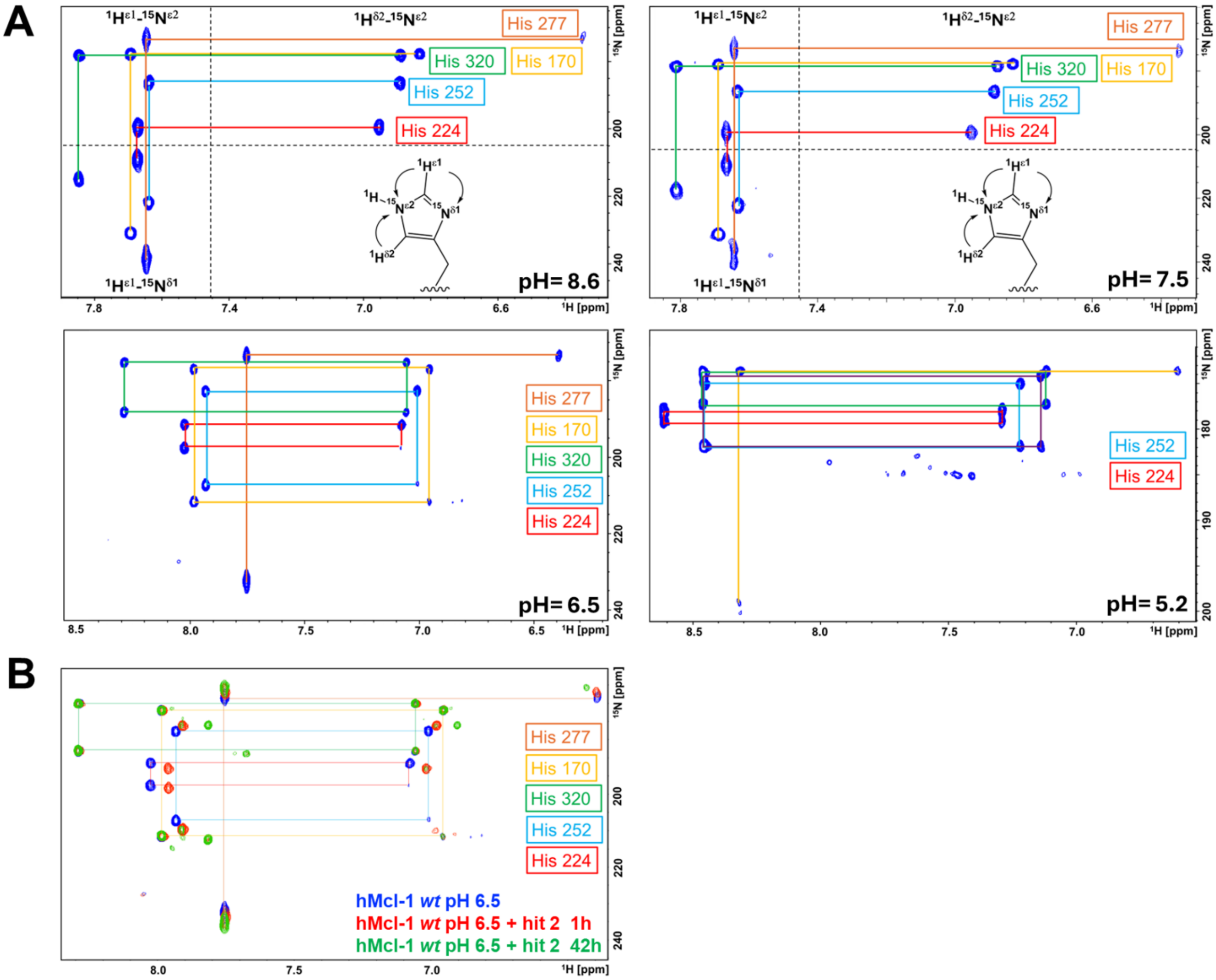
Supplementary Figure S7: Dose-response DELFIA inhibition curves with compound **165D9** after 24 h incubation (IC$_{50}$ ~ 3.0 μM; this experiment is an independent replicate of the data reported in **Figure 6D** that resulted in an IC$_{50}$ value of ~ 2.5 μM) or 4 h incubation (IC$_{50}$ ~ 12 μM).



Supplementary Figure S8: A) Thermal denaturation curves for hMcl-1(172-323) or its mutants as indicated, collected in absence (blue) or presence of (250 µM) **165D9**, after 8 h incubation. ΔTm (*wt*-hMcl-1) = 10.23 ± 0.05; ΔTm (hMcl-1 H224A) = -1.15 ± 0.04; ΔTm (hMcl-1 H252A) = 10.44 ± 0.06; ΔTm (hMcl-1 K234A) = 9.96 ± 0.20. **B**) Mass spectrometry analyses of hMcl-1(172-323) or mutants as indicated (each at 10 µM), collected in presence of **165D9** (250 µM) after 8 h incubation.



Supplementary Figure S9: **A**) 2D long-range [^{15}N, ^{1}H] correlation spectra for His side chains of ^{15}N-hMcl-1(172-323) (50 μM) collected at various pH values, as indicated. **B**) 2D long-range [^{15}N,^{1}H] correlation spectra for His side chains of hMcl-1(172-323) (50 μM) collected at pH= 6.5 in absence (blue) or presence of fragment hit **2** (1 mM) after various incubation times (red, 1 h; green, 42 h).



SUPPLEMENTARY TABLES

Supplementary Table S1: Mass spectrometry data of hMcl-1(172-323) and its mutants collected in absence or presence of various agents as indicated. Molecular masses are measured and analyzed using an Agilent 6545 QTOF LC/MS mass spectrometer.

ID	Calcd [M]	Obs. (m/z)
hMcl-1 *wt*	19572.16	[M] = 19572 [M] +178 = 19750
hMcl-1 *wt* + hit 1	20001.66	[M] = 20001 [M+H]$^+$ +178 = 20180
hMcl-1 *wt* + hit 2	19888.5	[M] = 19888 [M+H]$^+$ +178 = 20067
hMcl-1 *wt* + 165D9	19922.2	[M+H]$^+$ = 19923 [M+H]$^+$ +178 = 20101

ID	Calcd [M]	Obs. (m/z)
hMcl-1 H224A	19506.09	[M] = 19506 [M] +178 = 19684
hMcl-1 H224A + hit 1	19935.59	[M] = 19935
hMcl-1 H224A + hit 2	19822.4	[M] = 19822 [M+H]$^+$ +178 = 20001
hMcl-1 H224A + 165D9	19856.11	[M+H]$^+$ = 19857

ID	Calcd [M]	Obs. (m/z)
^{15}N hMcl-1 H252A (no His tag)	17981	[M] = 17981 [M] +178 = 18159
^{15}N hMcl-1 H252A (no His tag) + hit 1	18410.5	[M+H]$^+$ = 18411 [M+H]$^+$ +178 = 18661
^{15}N hMcl-1 H252A (no His tag) + hit 2	18297.34	[M+H]$^+$ = 18298 [M+H]$^+$ +178 = 20001
^{15}N hMcl-1 H252A (no His tag) + 165D9	18331.02	[M+H]$^+$ = 18332

ID	Calcd [M]	Obs. (m/z)
hMcl-1 K234A	19515.06	[M] = 19515 [M] +178 = 19693
hMcl-1 K234A + hit 1	19944.56	[M] = 19944 [M+H]$^+$ +178 = 20123
hMcl-1 K234A + hit 2	19831.4	[M] = 19831 [M] +178 = 20009
hMcl-1 K234A + 165D9	19865.08	[M+H]$^+$ = 19866 [M+H]$^+$ +178 = 20044

Supplementary Table S2: Data collection and refinement statistics for the complex between hMcl-1(172-323) and fragment hit **2**.

	hMcl-1(172-323)/hit2
Data collection	
Beamline	DLS I-04
Wavelength	0.7460
Resolution range (Å)	39.03 - 1.82 (1.885 - 1.82)
Space group	P 1 21 1
Unit cell	41.556, 40.135, 42.052 (90, 111.868, 90)
$R_{merge}{}^{a}$	0.08596 (4.649)
Total reflections	11680 (834)
Mean I/sigma(I)	12.29 (0.30)
Completeness (%)	96.96 (73.39)
Wilson B-factor	28.06
Multiplicity	7.0 (7.3)
Refinement	
Resolution	39.03 - 1.82 (1.885 - 1.82)
R_{work}	0.2536 (0.4440)
R_{free}	0.2869 (0.4703)
Unique reflections	11327 (833)
Number of non-hydrogen atoms	1227
macromolecules	1196
ligands	22
solvent	9
RMS bonds (Å)	0.009
RMS angles (°)	1.25
Ramachandran favored (%)	92.57
Ramachandran allowed (%)	7.43
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	0.78

Clashscore	9.52
Average B-factor	49.39
macromolecules	49.41
ligands	46.97
solvent	52.37

Statistics for the highest-resolution shell are shown in parentheses.