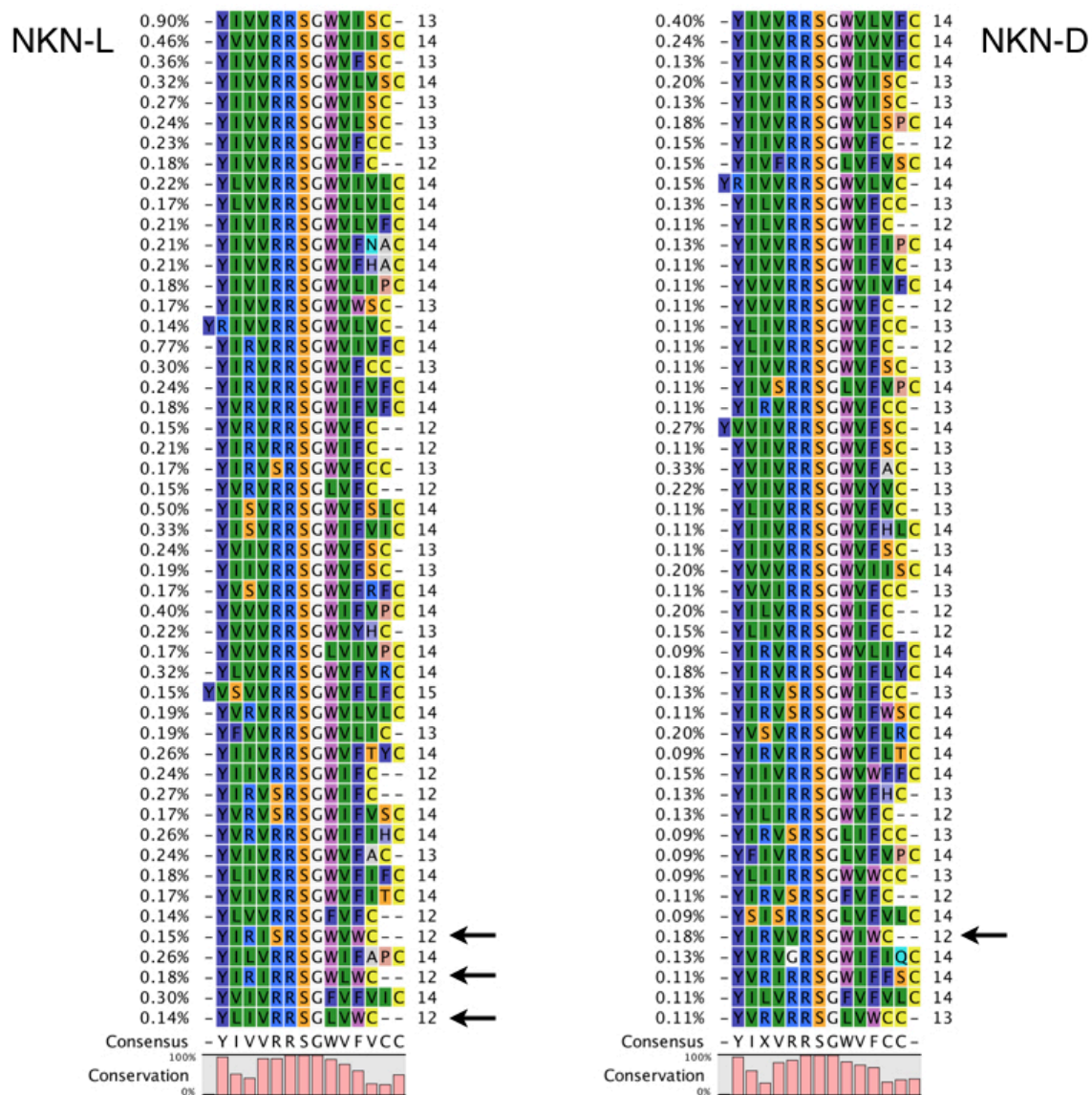
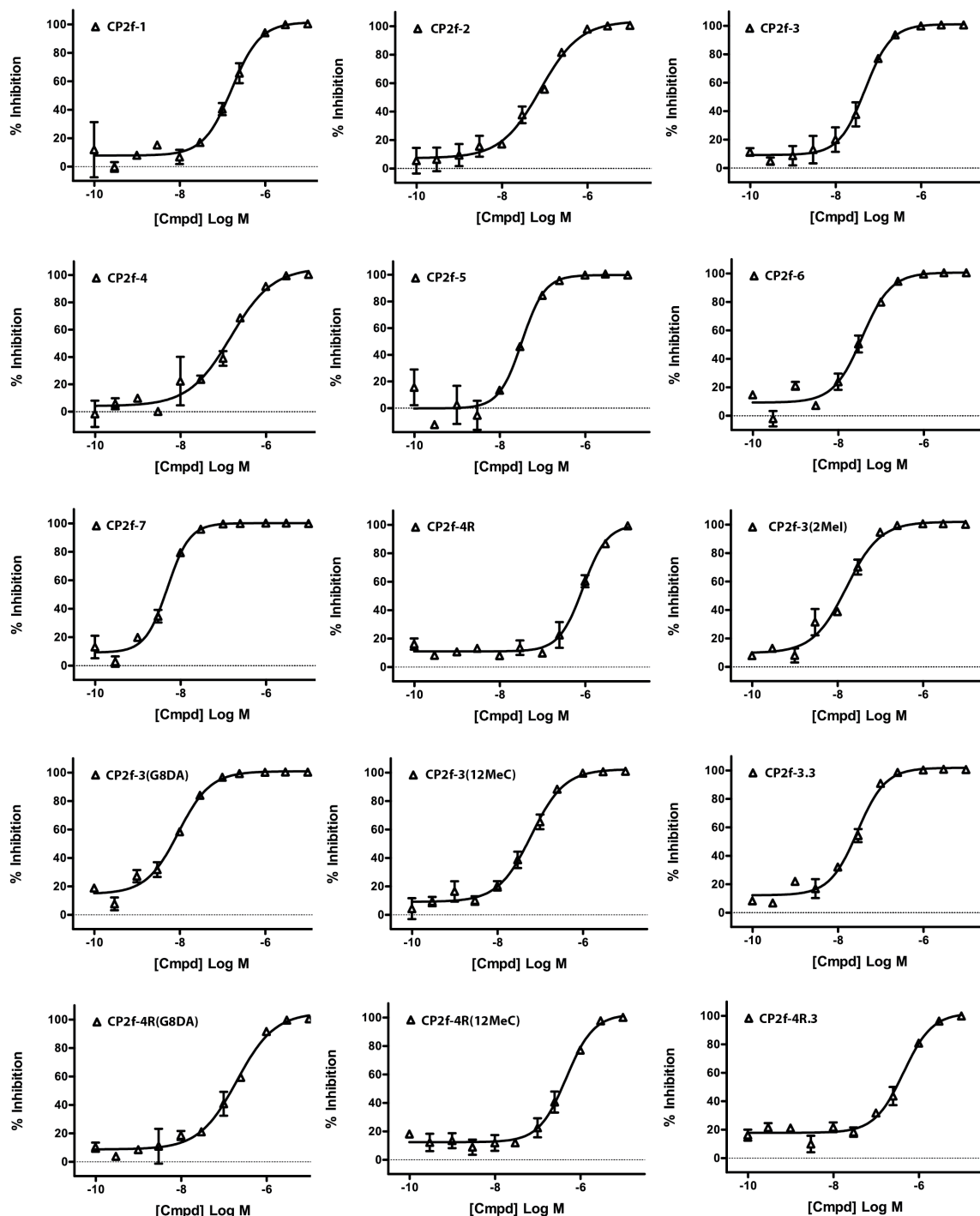


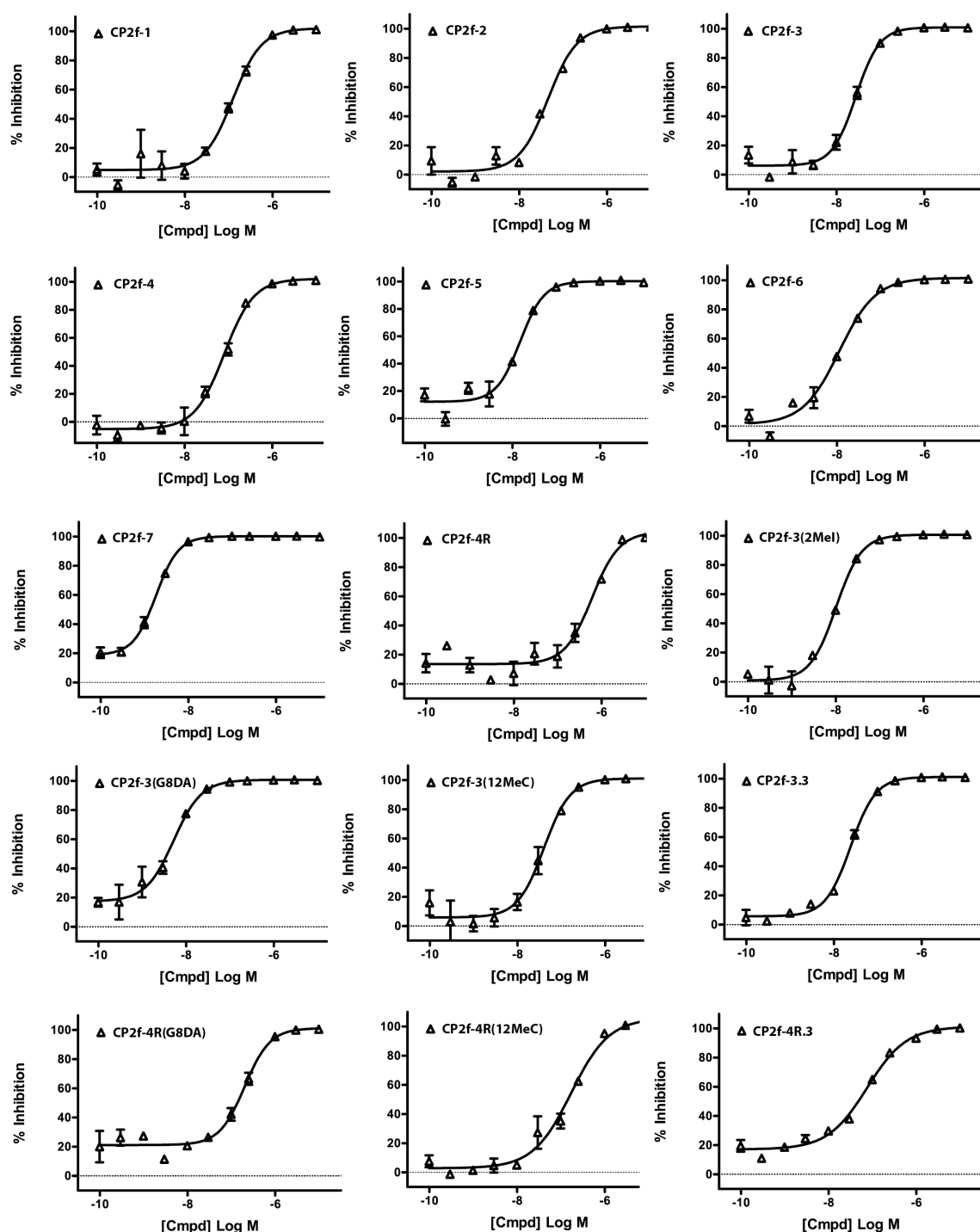
Supplementary Figure 1 – Alignment of 50 most enriched unique peptide sequences following 5 rounds of selection with NNK libraries. Percentage values on the left indicated fraction of final library, numbers on the right the length of the peptide. Arrows indicate sequences chosen for further study.



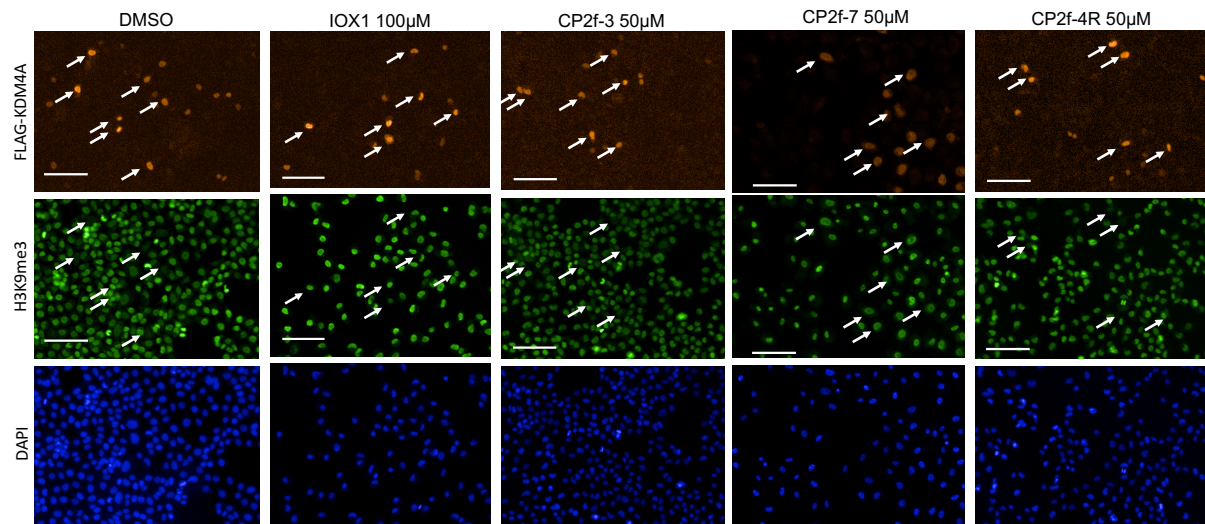
Supplementary Figure 2 – Alignment of 50 most enriched unique peptide sequences following 5 rounds of selection with NKN libraries. Percentage values on the left indicated fraction of final library, numbers on the right the length of the peptide. Arrows indicate sequences chosen for further study.



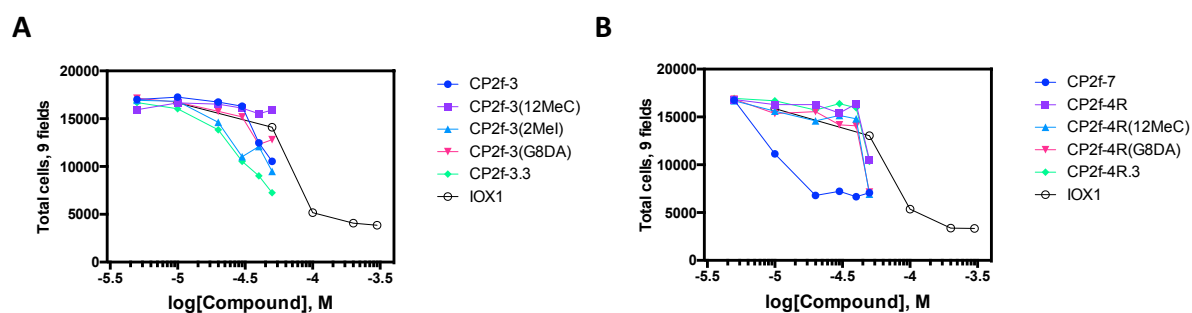
Supplementary Figure 3 – In vitro profiling of macrocyclic peptide inhibitors against KDM4A using AlphaScreen catalytic turnover assay. A representative set of IC_{50} data is shown. IC_{50} data were determined from an 11-point titration curve and the data plotted in GraphPad prism version 5. Each concentration point is the mean \pm SD of two determinations.



Supplementary Figure 4 – In vitro profiling of macrocyclic peptide inhibitors against KDM4C using AlphaScreen catalytic turnover assay. A representative set of IC₅₀ data is shown. IC₅₀ data were determined from an 11-point titration curve and the data plotted in GraphPad prism version 5. Each concentration point is the mean \pm SD of two determinations.



Supplementary Figure 5 – Immunofluorescence analysis of the effect of inhibitors on the cellular activity of KDM4A. HeLa cells were transiently transfected with FLAG-tagged full length KDM4A, and treated with inhibitors (in 0.5% DMSO) for 24 hrs. Select images of cells stained with DAPI nuclear stain (blue), H3K9me3 antibody (green), and Flag-tag antibody (red) are shown; scale bar 100 μm



Supplementary Figure 6 – Relative numbers of inhibitor dosed cells in the assay. (A,B)

Total number of cells in 9 fields based on DAPI staining for the IF assay in Figure 4.

Reduction in cell numbers indicate cytotoxicity of the inhibitors.

Supplementary Table 1 – Peptide mass spectrometric (MALDI-TOF) data.

	[M+H] calculated	[M+H] observed
CP2f-1	1459.76	1459.86
CP2f-2	1534.85	1534.74
CP2f-3	1647.98	1647.85
CP2f-4	1503.92	1503.80
CP2f-5	1564.89	1564.76
CP2f-6	1576.80	1576.90
CP2f-7	1870.90	1870.01
CP2f-4R	1601.84	1601.94
CP2f-3(2MeI)	1661.86	1661.86
CP2f-3(G8DA)	1661.86	1661.85
CP2f-3(12MeC)	1661.86	1661.32
CP2f-3.3	1689.89	1689.84
CP2f-4R(G8DA)	1615.85	1615.93
CP2f-4R(12MeC)	1615.85	1615.93
CP2f-4R.3	1629.87	1629.88
CP2f-1-Fluorescein	1947.94	1948.52
CP2f-2-Fluorescein	2023.93	2023.60
CP2f-3-Fluorescein	2136.02	2136.53
CP2f-4-Fluorescein	1992.99	1992.76
CP2f-5-Fluorescein	2052.94	2053.28
CP2f-6-Fluorescein	2065.97	2065.72
CP2f-7-Fluorescein	2358.08	2358.74
CP2f-4R-Fluorescein	2090.01	2090.70
CP2f-3(2MeI)-Fluorescein	2148.02	2148.07
CP2f-3(G8DA)-Fluorescein	2148.02	2148.12
CP2f-3(12MeC)-Fluorescein	2148.02	2148.10
CP2f-3.3-Fluorescein	2177.05	2177.08
CP2f-4R(G8DA)-Fluorescein	2103.01	2103.08
CP2f-4R(12MeC)-Fluorescein	2103.01	2103.08
CP2f-4R.3-Fluorescein	2118.05	2118.17
CP2.3-fluorescein	2382.00	2382.70

Supplementary Table 2. – **KDM4A inhibition and binding by selected macrocyclic peptides.** Single peptide concentration screening at 300nM was carried out using BLI. Apparent K_d values are indicated. n.a. – no binding

	KDM4A IC50 (nM)	KDM4A BLI K_d^{app} (nM)
CP2	41 ± 22	29.8 ¹
CP2f-1	82 ± 40	49 ± 5
CP2f-2	41 ± 20	21 ± 1
CP2f-3	23 ± 10	42 ± 1
CP2f-4	81 ± 30	173 ± 81
CP2f-5	31 ± 10	16 ± 0.5
CP2f-6	43 ± 20	3.7 ± 0.3
CP2f-7	6 ± 2	2.6 ± 0.1
CP2f-4R	615 ± 160	>300
CP2f-3(2MeI)	11 ± 3	14 ± 1
CP2f-3(G8DA)	15 ± 4	3.1 ± 0.6
CP2f-3(12MeC)	34 ± 14	8.3 ± 1.2
CP2f-3.3	17 ± 6	23 ± 1
CP2f-4R(G8DA)	208 ± 50	14 ± 2
CP2f-4R(12MeC)	373 ± 87	9 ± 3
CP2F-4R.3	269 ± 90	n.a.

Supplementary Methods:

Bio-layer interferometry (BLI)

BLI experiments were performed on an OctetRed 384 machine (ForteBio) as described¹ with minor modifications. Biotinylated KDM4A was made up in BLI buffer (50 mM Tris, 150 mM NaCl, 0.05% Tween-20, 1 mM TCEP, pH 7.5) to a final concentration of 250 nM, and loaded onto streptavidin-functionalised biosensors at 25 °C. CP2f peptides (in DMSO) were diluted into BLI buffer to a final peptide concentration of 300 nM, and a DMSO concentration of 1%. The protein-loaded sensors were first allowed to equilibrate in BLI buffer containing 1% DMSO, followed by immersion into 300 nM CP2f peptide solutions in BLI buffer to allow association of peptide to protein, followed by a dissociation step, facilitated by immersion of the sensors into 1% DMSO-supplemented BLI buffer alone. Un-loaded streptavidin sensors were used as a reference to subtract the interaction of CP2f peptides to streptavidin alone. Additionally, a blank sample (1% DMSO in BLI buffer only) was included as an additional control in order to correct for signal drift over the duration of the experiment.

Association and dissociation kinetics data was fit using the ForteBio Data Analysis software (v9.0.0.4), and dissociation constants (K_d^{app}) calculated.

Supplementary References

1. A. Kawamura, M. Munzel, T. Kojima, C. Yapp, B. Bhushan, Y. Goto, A. Tumber, T. Katoh, O.N. King, T. Passioura, L.J. Walport, S.B. Hatch, S. Madden, S. Muller, P.E. Brennan, R. Chowdhury, R.J. Hopkinson, H. Suga, C.J. Schofield. *Nature communications*. 2017; 8:14773.