

Supplementary Material

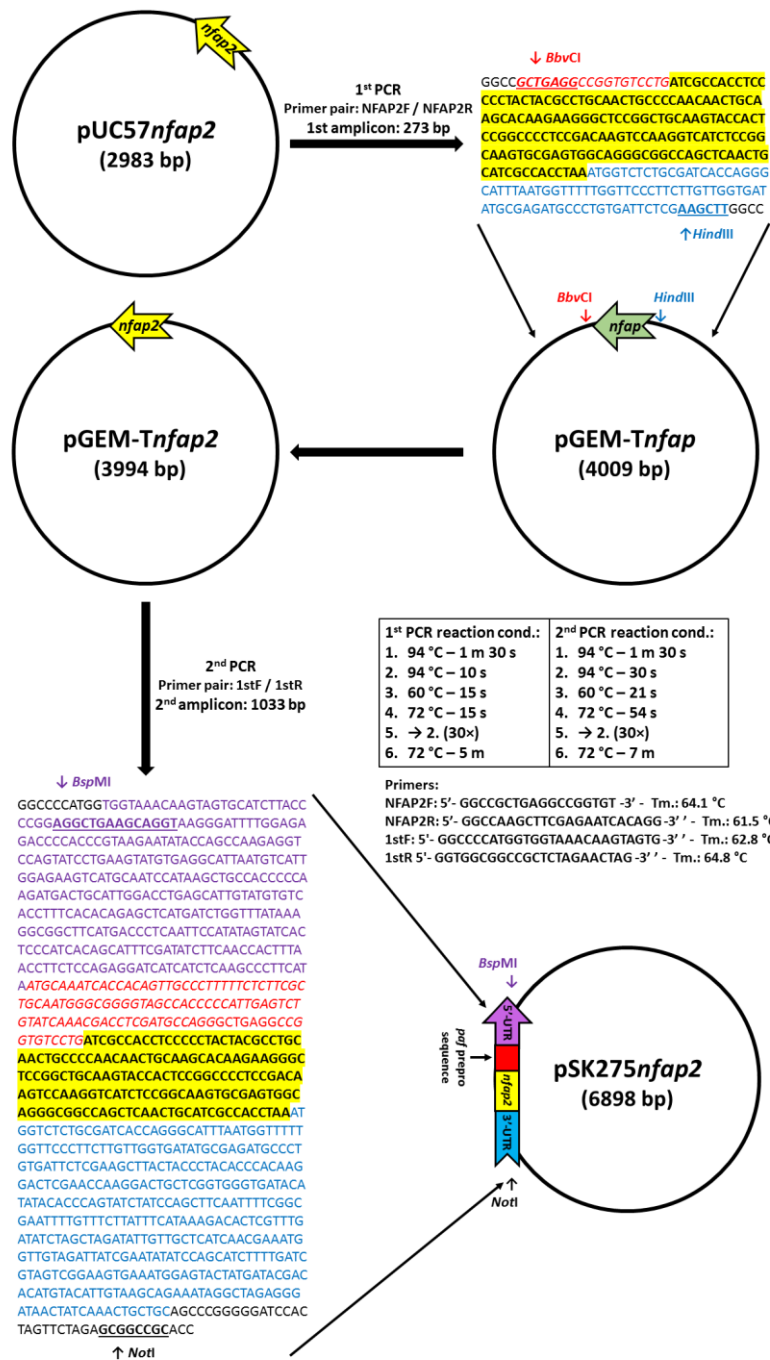
Anti-candidal activity and functional mapping of recombinant and synthetic *Neosartorya fischeri* antifungal protein 2 (NFAP2)

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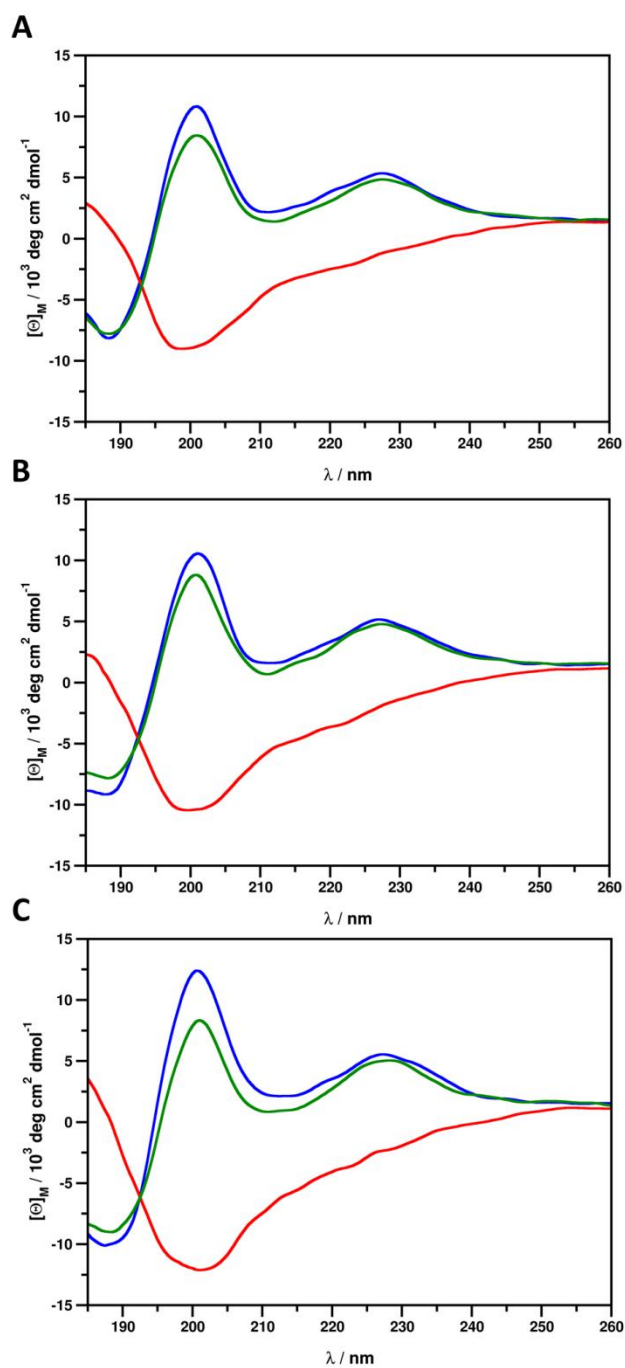
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1 Supplementary Figures and Tables

1.1 Supplementary Figures

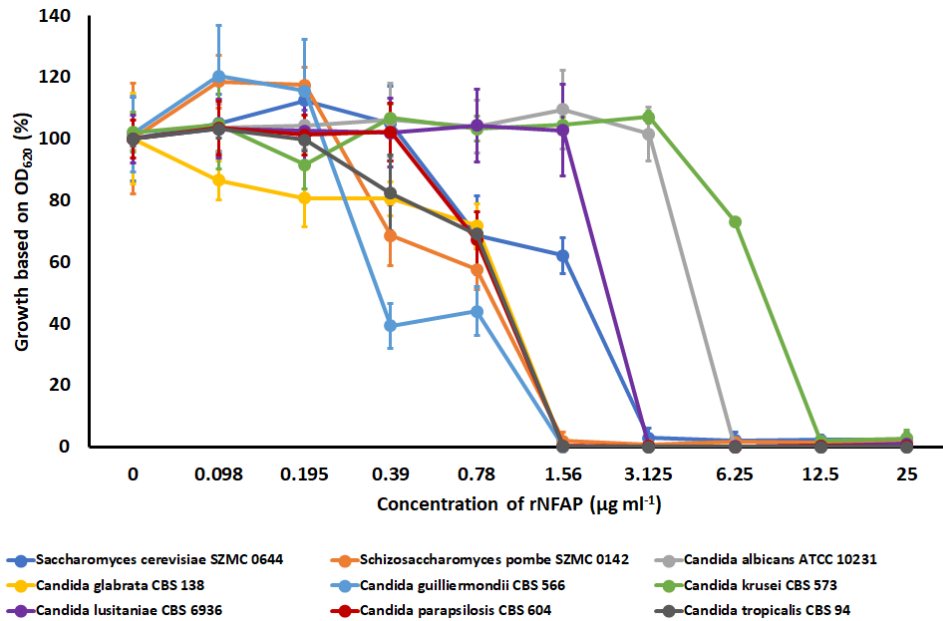


Supplementary Figure 1. Schematic representation of the cloning strategy of the NFAP2 expression vector pSK275nfap2. The mature NFAP2 coding cDNA is highlighted with yellow background, the *pac* prepro sequence indicated red letters, 5'- and 3'-UTR are shown by lilac and blue letters. The restriction sites are underlined and shown with vertical bold arrows.

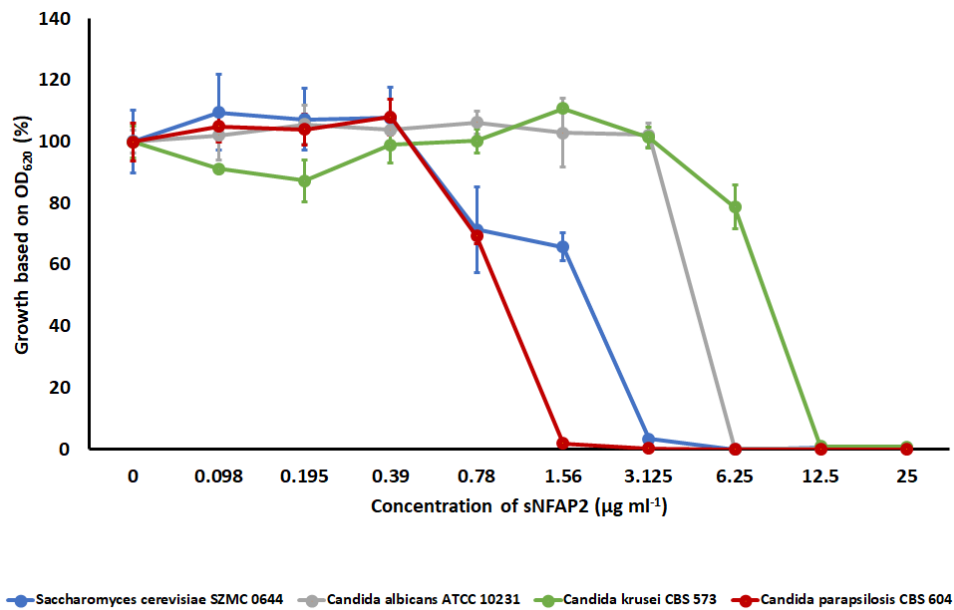


Supplementary Figure 2. ECD spectra of (A) nNFAP2, (B) rNFAP2 and (C) sNFAP2 recorded at 25 °C (blue), 95 °C (red), and after cooling and 5 minutes of equilibration at 25 °C (green). nNFAP2: native NFAP2 produced by *N. fischeri* NRRL 181, rNFAP2: recombinant NFAP2 produced by *P. chrysogenum nfap2*, sNFAP2: synthetic NFAP2.

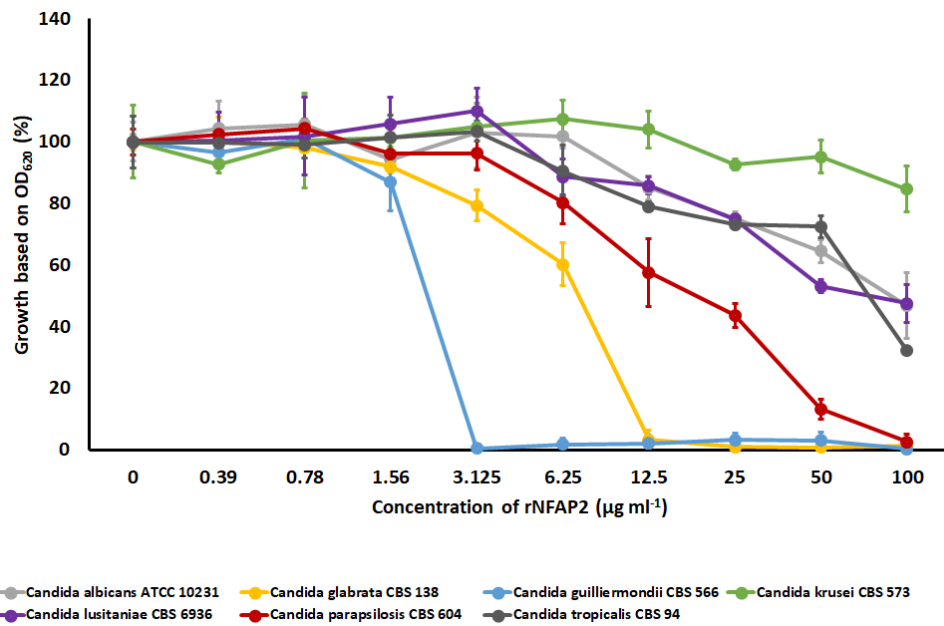
A



B



Supplementary Figure 3. Growth inhibition potential of (A) rNFAP2 and (B) sNFAP2 at different concentrations in LCM after incubation for 48 hours at 30 °C. The untreated control was defined as 100% of growth. rNFAP2: recombinant NFAP2 produced by *P. chrysogenum nfap2*, sNFAP2: synthetic NFAP2.



Supplementary Figure 4. Growth inhibition potential of rNFAP2 at different concentrations in RPMI 1640 medium after incubation for 48 hours at 35 °C. rNFAP2: recombinant *Neosartorya fischeri* antifungal protein 2. The untreated control was defined as 100% of growth. rNFAP2: recombinant NFAP2 produced by *P. chrysogenum nfap2*.

A

<i>Candida albicans</i> ATCC 10231						
rNFAP2 / FLC	100	50	25	12.5	6.25	0
1	0.25±0.36	0.59±0.83	0.67±0.95	0.34±0.47	0.84±1.18	1.17±0.95
0.5	0.42±0.59	1.68±0.71	1.01±1.42	1.51±1.18	3.43±2.72	9.11±0.35
0.25	1.51±1.18	3.43±1.07	4.62±1.55	9.46±1.30	10.55±1.42	21.52±8.17
0.125	20.85±4.38	31.49±2.13	29.23±3.67	25.29±1.90	50.92±6.40	60.44±3.12
0.0625	32.69±0.98	54.05±0.08	58.59±0.88	67.24±0.60	71.47±0.01	81.02±3.48
0	43.22±4.50	65.66±2.37	97.04±9.78	98.69±0.90	104.56±1.85	100.28±0.40

B

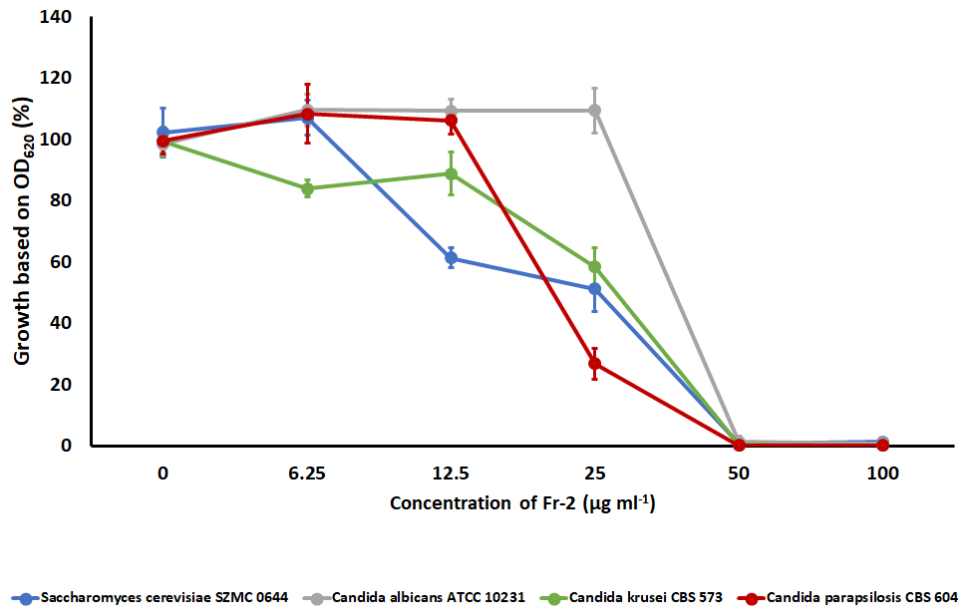
<i>Candida krusei</i> CBS 573						
rNFAP2 / FLC	100	50	25	12.5	6.25	0
64	0.65±0.61	1.85±0.15	1.09±0.00	1.09±0.31	1.74±0.31	0.54±0.46
32	3.26±3.07	15.54±3.23	19.13±2.46	27.83±0.61	33.8±1.38	66.96±4.30
16	51.09±2.46	45.48±2.83	52.5±0.15	59.67±2.31	67.04±1.41	89.24±2.00
8	107.61±4.92	107.28±0.15	107.83±2.46	94.35±9.84	100.65±1.38	100.76±2.61
4	105.96±4.18	107.07±0.15	101.41±1.38	105.54±3.54	104.78±1.23	97.17±5.23
0	100±15.99	99.24±12.14	100.11±2.31	105.33±3.23	109.13±2.15	103.59±5.07

C

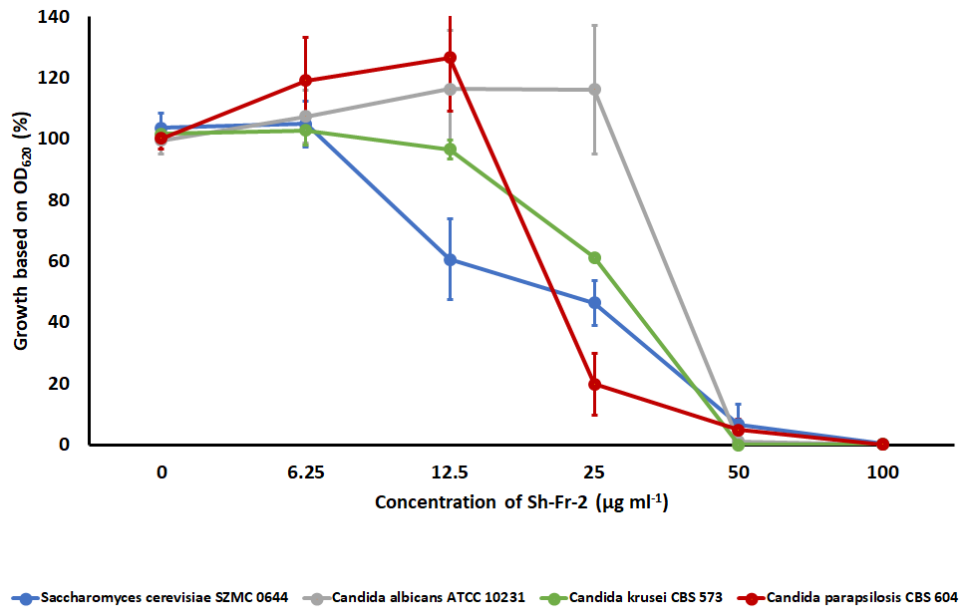
<i>Candida parapsilosis</i> CBS 604						
rNFAP2 / FLC	100	50	25	12.5	6.25	0
8	4.35±1.02	1.67±0.71	3.99±0.51	2.54±0.51	2.90±1.02	3.73±0.05
4	3.93±0.58	5.43±0.51	3.26±0.51	4.05±0.42	2.87±1.63	7.9±1.02
2	2.54±2.56	5.8±2.05	2.9±1.02	2.54±1.54	3.78±1.24	27.17±7.69
1	5.8±2.05	6.16±1.54	2.90±1.03	2.54±2.56	6.23±1.64	73.41±2.77
0.5	5.07±1.02	8.48±1.74	14.86±4.61	24.86±1.33	78.26±3.07	107.17±1.33
0	2.54±1.54	6.52±2.05	35.29±2.36	103.48±3.48	101.52±0.10	104.78±6.76

Supplementary Figure 5. Growth percentages (%) of (A) *C. albicans* ATCC 10231, (B) *C. krusei* CBS 573, and (C) *C. parapsilosis* CBS 604 in combinatorial application of rNFAP2 and fluconazole in RPMI 1640 medium after incubation for 48 hours at 35 °C. (A,B,C) Red cells indicated the total growth inhibition (growth < 5%), orange cells the reduced growth ability (growth between 5% and 90%), green cells the total growth (growth between 90% and 100%). The untreated control was defined as 100% of growth. The effective concentration combinations below the individual MICs are framed with bold line. FLC: fluconazole, rNFAP2: recombinant NFAP2 produced by *P. chrysogenum nfap2*.

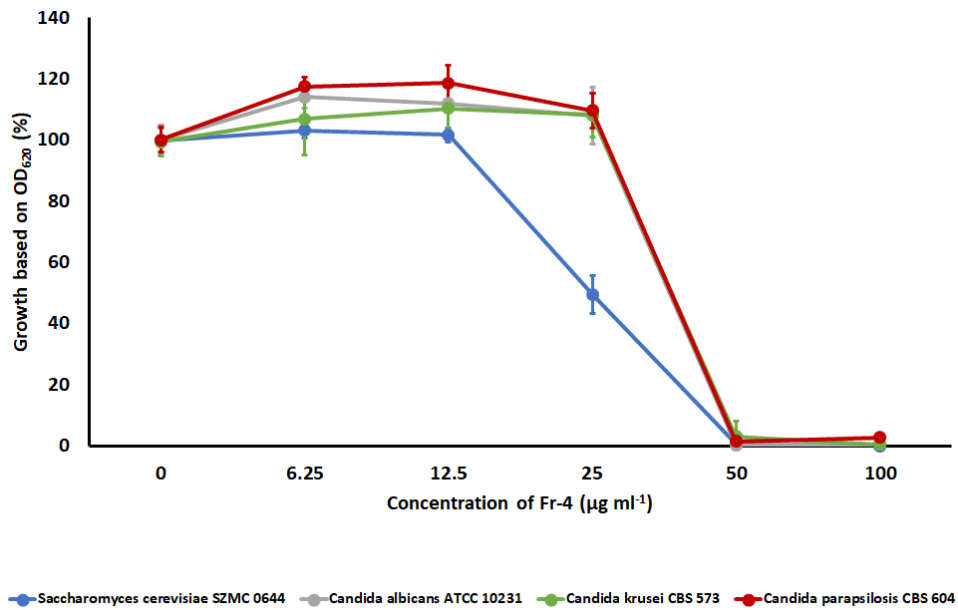
A



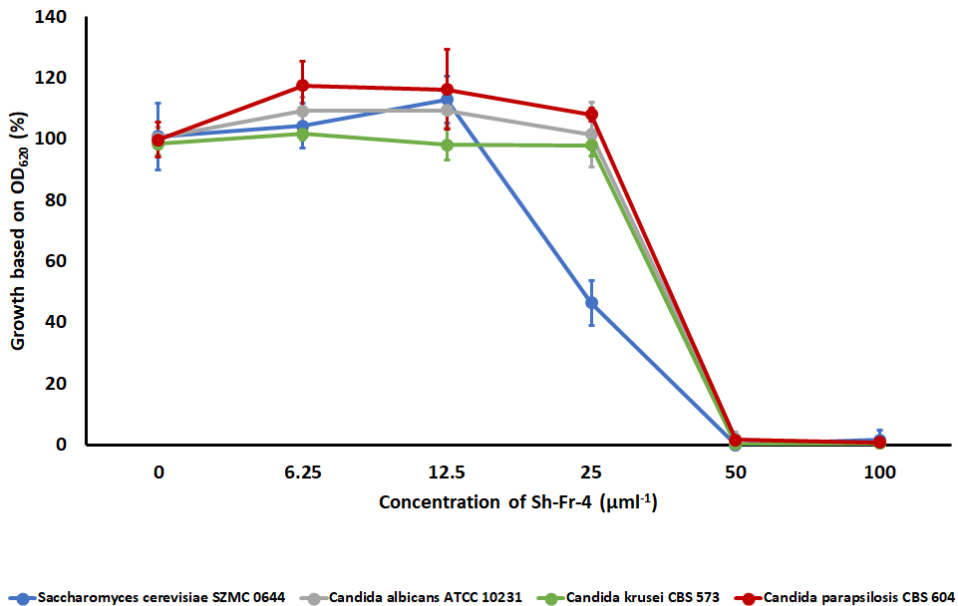
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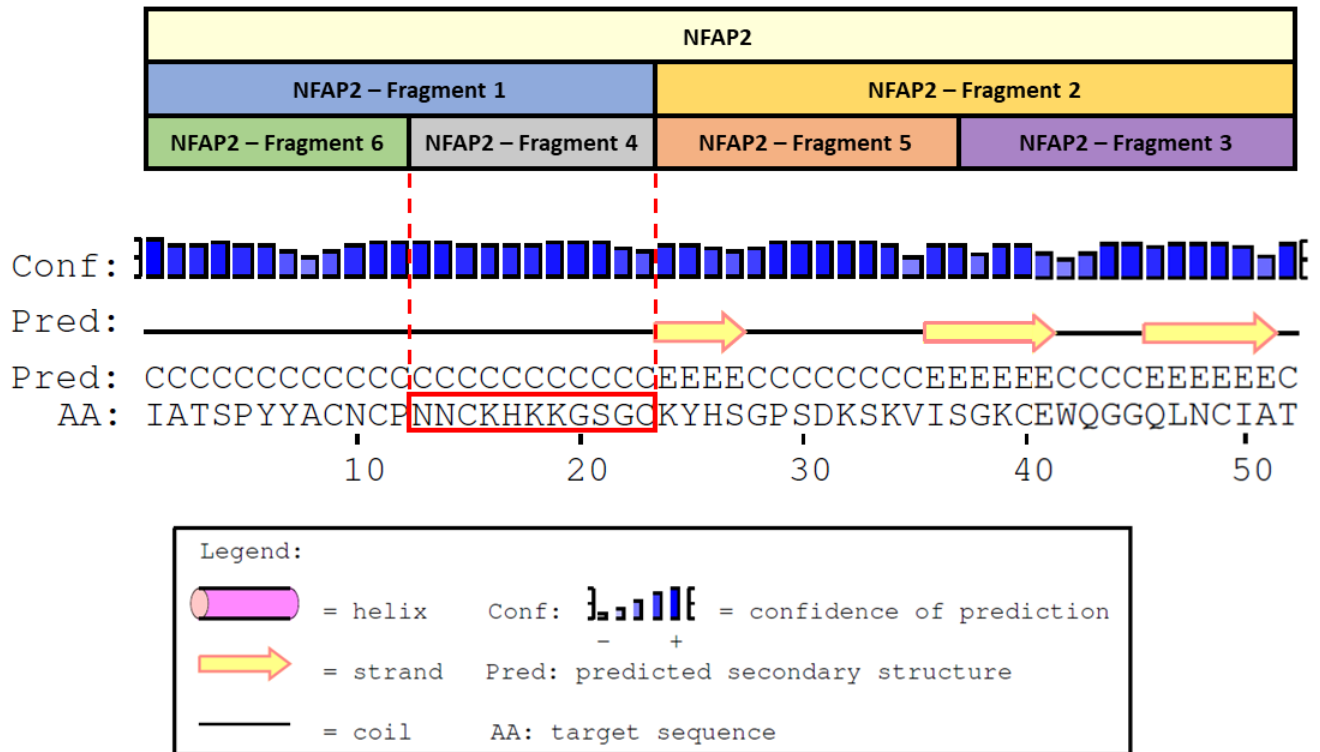
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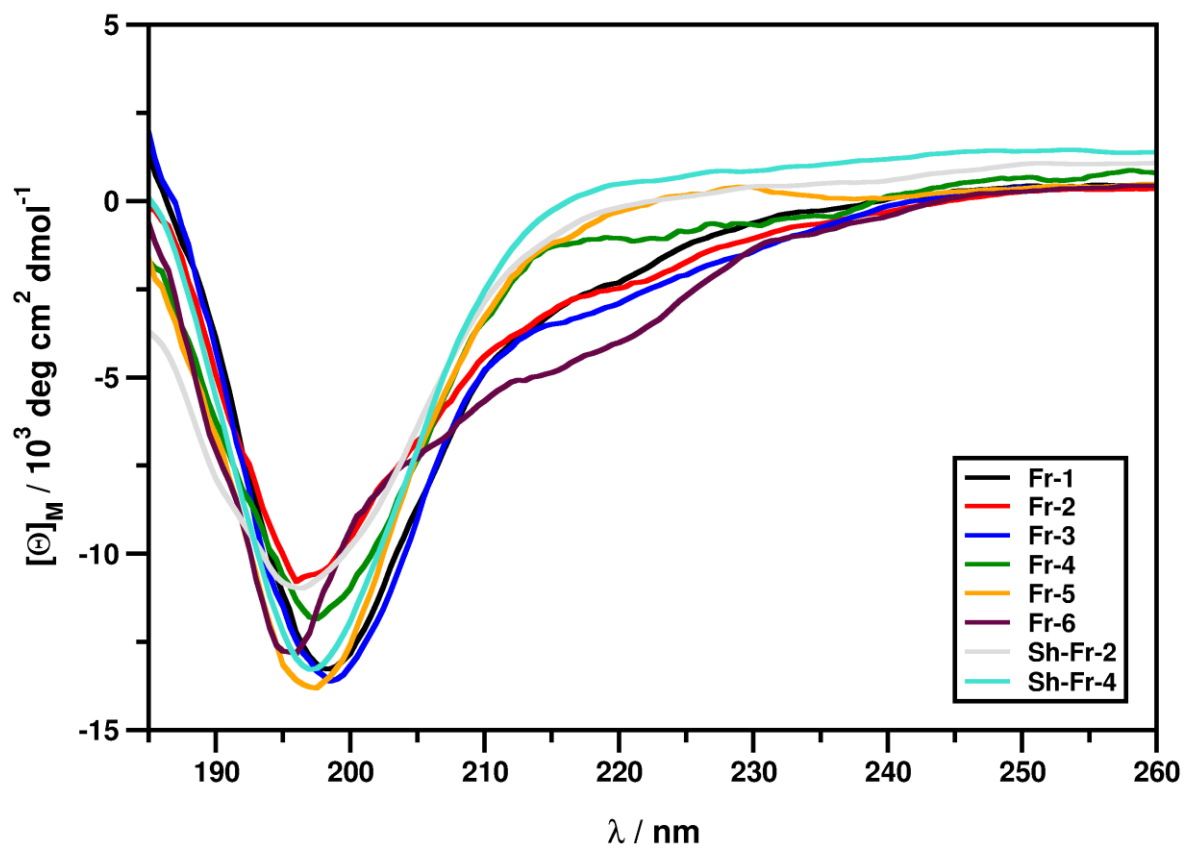
D



Supplementary Figure 6. Growth inhibition potential of (A) Fr-2, (C) Fr-4 of NFAP2 and their shuffled variants (B) Sh-Fr-2 and (D) Sh-Fr-4 at their different concentrations in LCM after incubation for 48 hours at 30 °C. Fr-2 and 4: synthetic peptide Fragment 2 and 4 of NFAP2 (Table 1); Sh-Fr-2 and 4: shuffle variants of synthetic peptide Fragments 2 and 4 of NFAP2 (Table 1).



Supplementary Figure 7. Putative secondary structure of NFAP2 by the by PSIPRED (v3.3) Protein Analysis Workbench (Buchan et al., 2013). Fragment 4 (**Table 1**), the predicted functional active part of NFAP2 is indicated in red box.



Supplementary Figure 8. ECD spectra of synthetic NFAP2 peptide fragments recorded at 25 °C. Fr-1 - 6: synthetic peptide Fragment 1 - 6 of NFAP2 (**Table 1**), Sh-Fr-2 and 4: shuffle variants of synthetic peptide Fragment 2 and 4 of NFAP2 (**Table 1**).