SUPPLEMENTARY INFORMATION

Supplementary Table 1: X-ray structure determination

Data collection	
Space group	$C222_1$
Unit cell dimensions [Å]	a = 38.7, b = 92.8, c = 86.8
Wavelength [Å]	0.8726
Resolution range [Å] [a]	33.04-2.05 (2.16-2.05)
Unique reflections	9,762 (987)
Multiplicity [a]	3.8 (3.8)
Mean $I/\sigma_{(I)}$ [a]	7.8 (3.5)
Wilson B-factor [$Å^2$]	32.6
Completeness [%] [a]	96.5 (98.0)
Half-set correlation ($CC_{1/2}$)	0.99 (0.88)
R_{sym} [b] [%]	10.8 (30.3)
Refinement	
Number of protein atoms	1181
Number of other atoms	43
R_{cryst}[%] [c,d]	22.4
R_{free} [%] [e]	24.6
R.m.s.d. bond lengths [Å]	0.016
R.m.s.d. angles [°]	1.33
Mean B-factors [$Å^2$]	43.6
Protein	43.2

Solvent	43.1
Ramachandran plot: Favored regions [%]	100
Clash score (Chen et al., 2010)	2.1
Residue segments interpreted	A: 220-294 B: 217-293
Residues with incomplete side chains	A: K233, N235, D236, R240 B: R217, K248, K265, K272, K273, R293

[a] Values in parentheses refer to the highest resolution shell.

[b] R_{sym} (I) = $\Sigma_{hkl}\Sigma_i$ | $I_{hkl,\,i}$ - $\langle I_{hkl,\,i}\rangle$ | / $\Sigma_{hkl}\Sigma_i$ | $I_{hkl,\,i}$ | where $\langle I_{hkl,\,i}\rangle$ is the mean intensity of symmetry-related measurements of the reflections.

[c] $R_{cryst} = \Sigma_{hkl} | F_{obs} - F_{calc}| / \Sigma_{hkl} | F_{obs} |$

[d] R_{free} is as R_{cryst} but calculated over 9 % of data that were excluded from the refinement process.

[e] RMSD is the root-mean square deviation from ideal geometry.

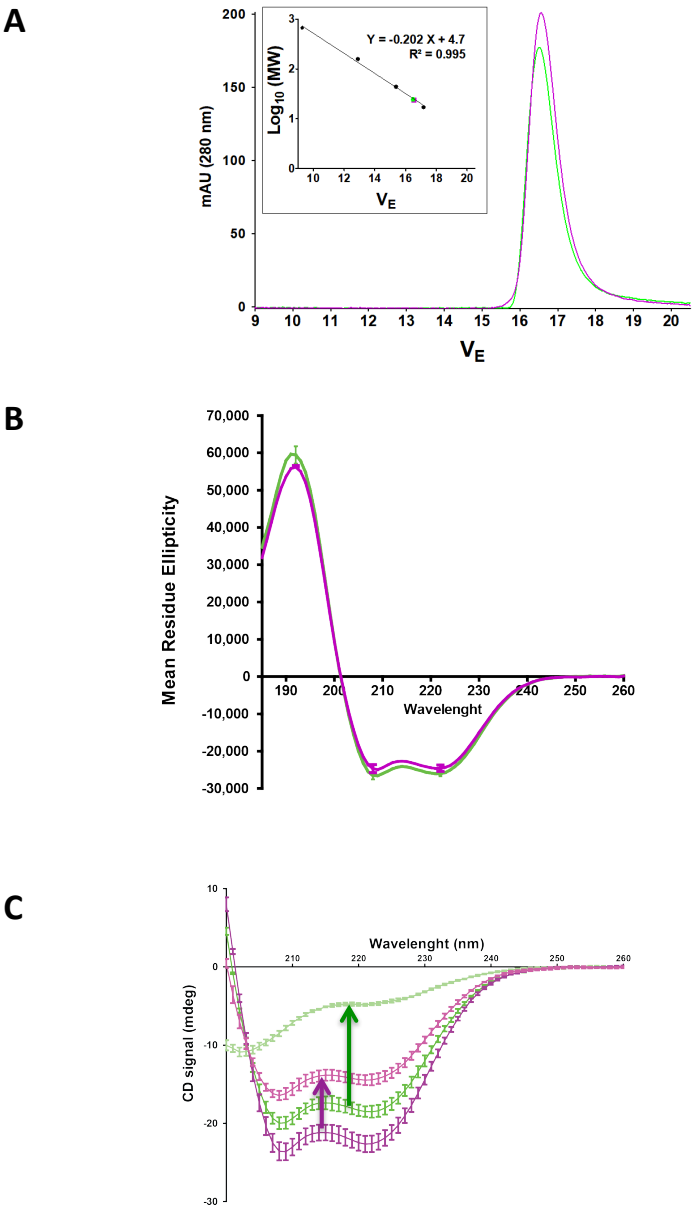
Supplementary Table 2: Complete searches for validated MITF-related structural targets

Query	Group represent-tative	Rank of represent-tative	TM Query cover [%]	TM Target cover [%]	TM Alignment length	RMS [Å]	TM Sequence identity
4ATH	4ATH	1	100,0	100,0	162	0,0	100,0
	6FX5	2	83,2	92,4	145	2,0	55,2
	6G1L	13	75,7	77,6	128	1,5	96,9
	1R05	13	75,6	70,4	141	2,7	15,6
	1NKP	20	71,4	67,6	134	2,8	20,9
	5EYO	29	77,0	71,7	137	2,2	16,1
	5I4Z	35	69,5	76,1	124	2,2	24,2
	1AN2	51	68,2	64,3	121	2,2	18,2
	1AM9	59	67,7	35,1	118	2,0	34,7
	5GNJ	78	66,5	73,8	116	2,0	19,0
	4ATI	84	66,2	83,8	110	1,2	96,4
	1HLO	124	64,0	67,8	111	1,9	19,8
	1UKL	182	61,9	10,0	107	1,8	28,0
	4ATK	194	61,2	82,6	102	1,2	94,1
	2YPB	309	56,8	65,3	99	2,0	25,3
	3U5V	501	54,0	70,5	94	2,0	26,6
	2YPA	606	56,3	30,8	97	1,8	24,7
	4F3L	637	52,0	13,6	90	1,9	30,0
	4H10	660	51,6	68,0	87	1,5	31,0
	5Y7Y	676	51,3	17,9	87	1,5	26,4
6G1L	6G1L	1	100,0	100,0	158	0,0	100,0
	5EYO	3	86,5	78,6	146	1,9	23,3
	1AM9	5	85,6	43,2	143	1,7	36,4
	1NKP	7	83,7	77,3	142	1,9	26,8
	1HLO	8	83,3	86,1	141	1,9	24,1
	1AN2	10	80,6	74,0	139	2,2	24,5
	4ATH	14	77,6	75,7	128	1,5	96,9
	2YPB	16	75,7	84,8	126	1,7	26,2
	2YPA	17	75,5	40,3	126	1,7	26,2
	5GNJ	18	74,7	80,8	126	1,9	25,4
	4ATK	19	74,3	97,9	120	1,1	98,3
	4ATI	20	74,1	91,4	127	2,1	70,9
	6FX5	21	72,0	78,0	120	1,6	67,0
	1R05	21	70,7	64,2	124	2,3	21,0
	4H10	22	69,5	89,3	115	1,6	32,2
	1MDY	23	69,2	84,2	114	1,4	22,8
	5I4Z	42	66,2	70,6	116	2,3	25,9
	4ZPK	67	64,9	17,4	112	2,2	26,8
	2QL2	77	64,1	44,1	105	1,3	25,7

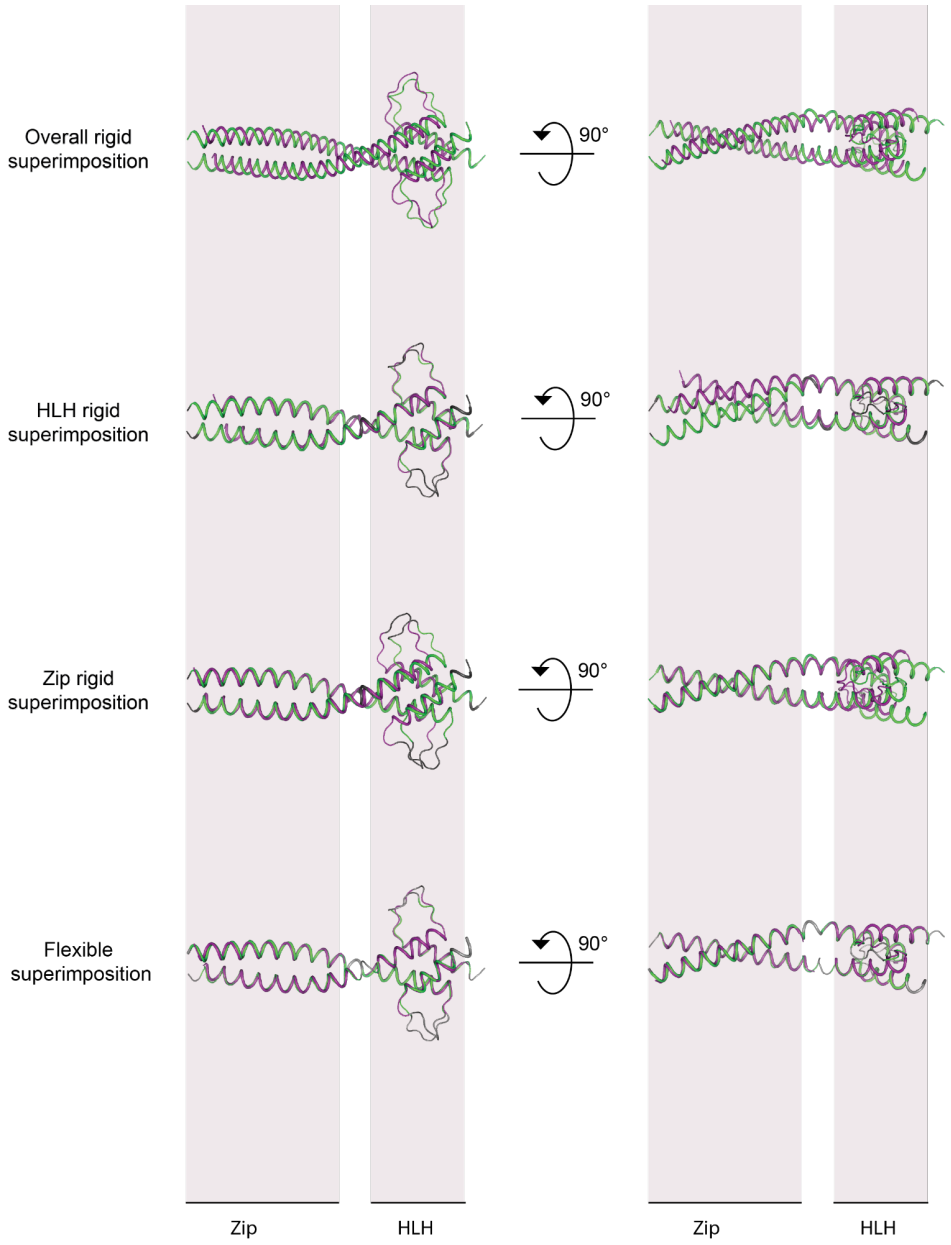
	2QL2	89	63,4	87,2	104	1,4	26,0
	1UKL	93	62,9	10,0	105	1,7	28,6
	4ZPR	104	62,0	19,1	103	1,6	29,1
	3U5V	113	60,7	77,3	100	1,5	32,0
	2MH3	117	60,4	68,2	106	2,3	37,7
	5NJ8	118	60,4	27,3	101	1,7	33,7
	5SY7	121	59,8	17,1	101	1,9	30,7
	1A0A	123	59,6	74,8	100	1,8	35,0
	1AN4	129	59,3	72,1	105	2,5	41,9
	5NJ8	131	59,0	30,1	99	1,8	31,3
	4F3L	142	57,5	14,6	96	1,7	35,4
	5Y7Y	227	55,8	19,0	94	1,8	30,9
	5V0L	247	55,1	25,5	94	2,0	35,1
	5SY5	347	51,4	4,9	87	2,0	33,3
	3N50	466	47,4	24,9	82	2,2	22,0
	3N50	467	47,4	8,4	82	2,2	22,0
	2LFH	491	46,7	54,3	80	2,0	27,5
	4ZQD	642	44,2	13,1	77	2,2	27,3
6FX5	6FX5	1	100,0	100,0	146	0,0	100
	4ATH	2	92,4	83,2	145	2,0	55,2
	1R05	5	84,5	70,9	137	2,4	22,6
	1NKP	8	82,9	70,8	131	2,0	21,4
	5I4Z	9	81,8	80,7	137	2,7	22,6
	1AN2	19	78,2	66,4	131	2,8	22,1
	6G1L	20	78,0	72,0	120	1,6	67
	5GNJ	53	76,2	76,2	122	2,2	18,9
	1AM9	62	75,1	35,0	116	1,8	33,6
	1UKL	114	69,9	10,2	108	1,7	35,2
	1HLO	120	69,5	66,4	107	1,6	19,6
	4ATI	153	68,0	77,6	103	1,4	70,9
	4ATK	355	61,7	75,0	93	1,3	82,8
	2YPB	372	58,6	60,6	91	1,8	28,6
	2YPA	485	59,6	29,4	93	1,9	28
	4H10	613	55,9	66,4	88	2,0	33
	1MDY	670	53,8	60,4	84	1,9	26,2
	3U5V	711	53,6	63,1	83	1,7	30,1

Caption Supplementary Table 2: Validated targets for 4ATH, 6GIL and 6FX5 (for further details see Materials & Methods Section. Extracted values from TopSearch Tables **(1)**: MITF structures, red; related HLH-bZip homo-dimers, green; related HLH-bZip hetero-dimers, blue (cf. Table 2). All remaining targets are in black.

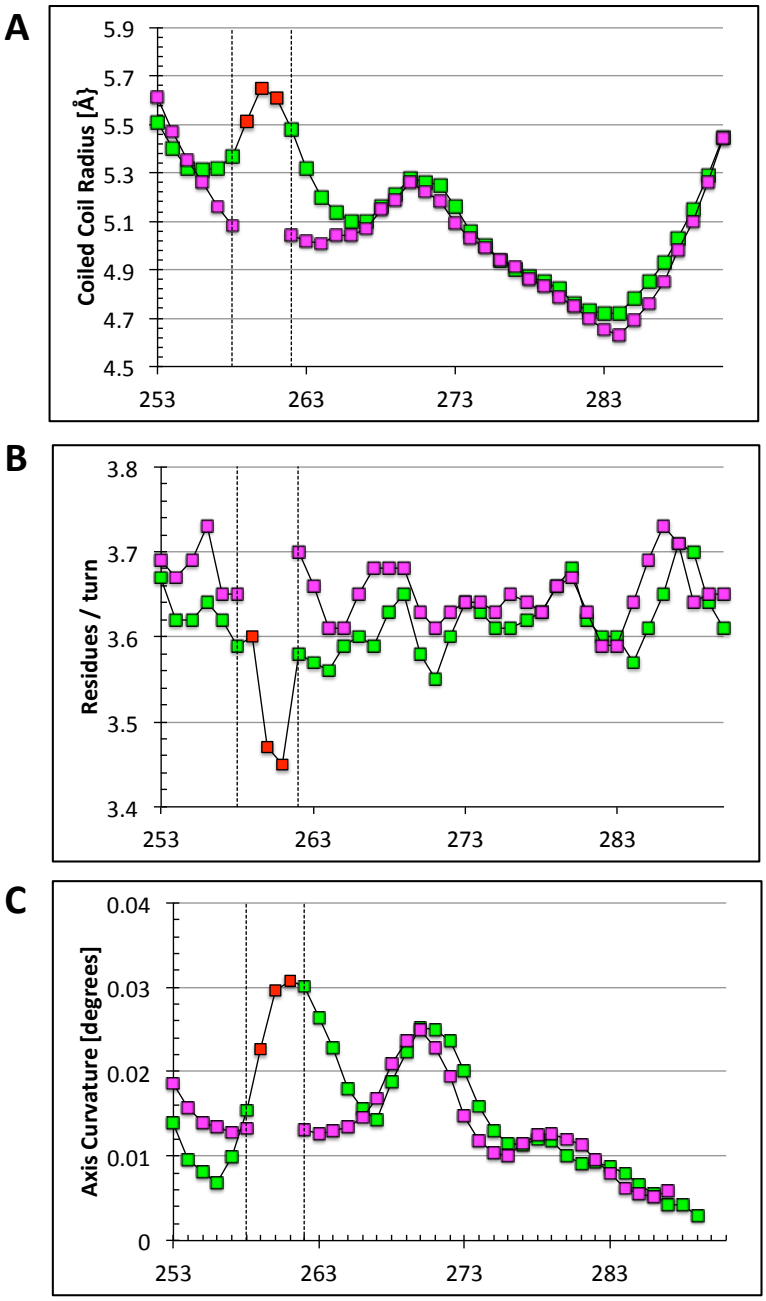
SUPPLEMENTARY FIGURES



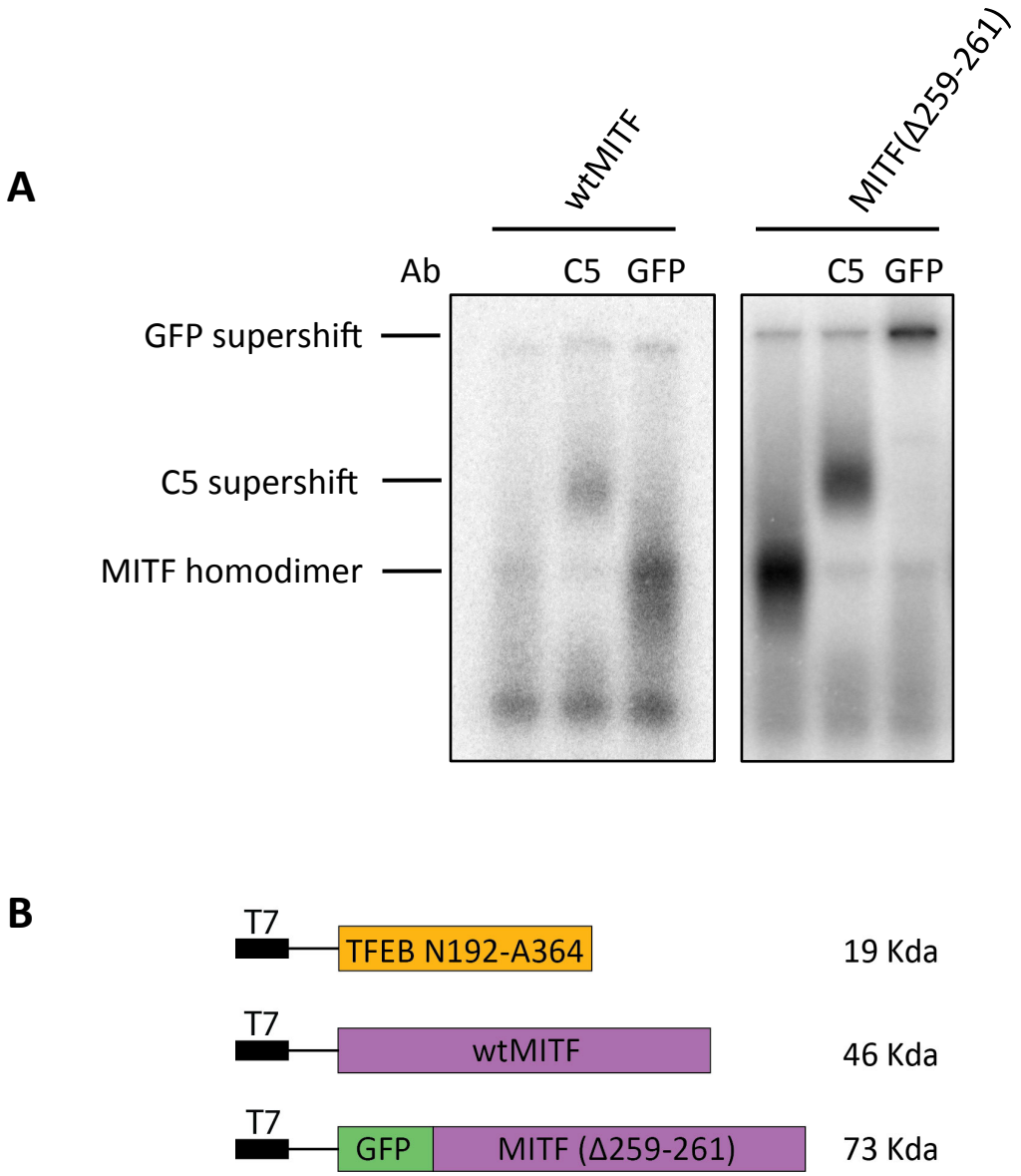
Supplementary Figure 1: Biophysical characterization of MITF variants. A) Gel filtration data of MITF(*wt*) and MITF(Δ)**.** According to the column calibration, the elution volumes correspond to molecular weight of 24 kDa for MITF, consistent with a homodimeric species. **B)** CD spectra of MITF (wt) and MITF (Δ); C) CD spectra focusing on the wavelength window 200-260 nm of MITF(*wt*) and MITF(Δ) before and after Proteinase K digestion, indicated by arrows. Color codes are as in **Figure 1.**



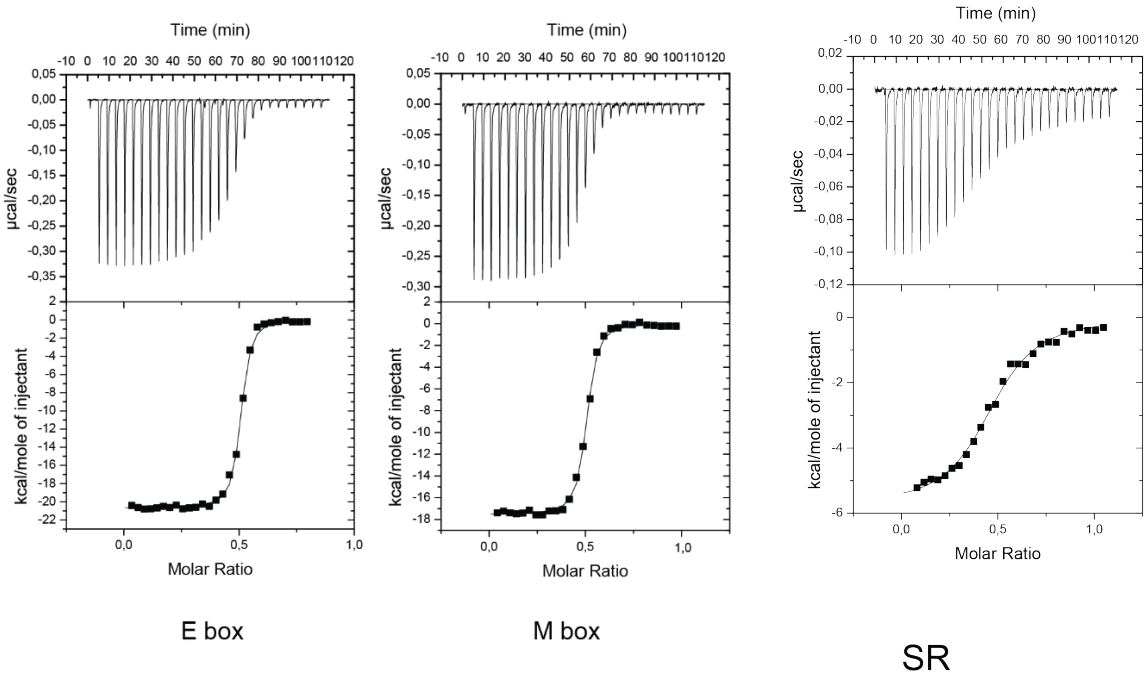
Supplementary Figure 2: Overall and segmental superimpositions of MITF(Δ) and MITF(*wt*). The analysis revealed the presence of two conserved subdomains, one constituted of the HLH domain interrupted by few flexible amino acids in the loop region and the other one by the leucine zipper**)**. Independent alignments of these rigid bodies from the two MITF variants result in r.m.s.d. of 0.57 Å on 72 aligned residues for the HLH and 0.67 Å on 63 aligned residues for the zipper. Color codes are as in **Figure 1.**



Supplementary Figure 3: Comparison of coiled coil parameters in MITF(Δ) and MITF(*wt*). A) Coiled coil radius, B) residues/turn; C) axis curvature. Geometric effects caused by the stammer insert are propagated by different degrees towards the C-terminus of the structure and sequence. Color codes are as in **Figure 1.** The stammer segment is indicated by a vertical dashed line. The stammer residues in MITF(*wt*) are in red. Residue numbers are indicated.



Supplementary Figure 4: EMSA control data. A) EMSA showing the specific supershifts of MITF(*wt*) and GFP-tagged MITF (Δ) with the anti-MITF (C5) antibody, and anti-GFP antibodies, respectively. (B) Scheme of the constructs utilized for the *in vitro* translation of the proteins assayed in the EMSAs.



Supplementary Figure 5: MITF(Δ)/DNA interactions measured by ITC. Upper panels, raw titration data; lower panel, integrated data fits.

References

1. Wiederstein, M., Gruber, M., Frank, K., Melo, F. and Sippl, M.J. (2014) Structure-based characterization of multiprotein complexes. *Structure*, **22**, 1063-1070.