Myc Phosphorylation in the bHLH-LZ Inhibits Binding to Max and DNA
Supplementary Information

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1 Protein constructs

MycWT bHLH-LZ
MHHHHHHENLYFQGSNVKRRTHNVLERQRRNELKRSFFALRDQIPELENNEKAPKVVILK
KATAYILSVQAEEQKLISEEDLLRKRREQLKHKLEQLRNS

Max bHLH-LZ
MHHHHHHENLYFQGSMDKRAHHNALERKRRDHIKDSFHSLRDSVPSLQGEKASRAQILDK
ATEYIQYMRRKNHTHQQDIDDLKRQNALLEQQVRALGG

Myc S373D/T400D bHLH-LZ
MHHHHHHENLYFQGSNVKRRTHNVLERQRRNELKRDFFALRDQIPELENNEKAPKVVILK
KADAYILSVQAEEQKLISEEDLLRKRREQLKHKLEQLRNS

Myc S373E/T400E bHLH-LZ
MHHHHHHENLYFQGSNVKRRTHNVLERQRRNELKREFFALRDQIPELENNEKAPKVVILK
KAEAYILSVQAEEQKLISEEDLLRKRREQLKHKLEQLRNS

Myc S373D bHLH-LZ
MHHHHHHENLYFQGSNVKRRTHNVLERQRRNELKRDFFALRDQIPELENNEKAPKVVILK
KATAYILSVQAEEQKLISEEDLLRKRREQLKHKLEQLRNS

Myc T400D bHLH-LZ
MHHHHHHENLYFQGSNVKRRTHNVLERQRRNELKRSFFALRDQIPELENNEKAPKVVILK
KADAYILSVQAEEQKLISEEDLLRKRREQLKHKLEQLRNS

Omomyc
MHHHHHHENLYFQGSNVKRRTHNVLERQRRNELKRSFFALRDQIPELENNEKAPKVVILK
KATAYILSVQAETQKLISEIDLLRKQNEQLKHKLEQLRNS

2 E-box DNA

5'-AGTTGACCACGTGGTCTGGG-3'
5'-CCCAGACCACGTGGTCAACT-3'

3 Supplementary Tables and Figures

phosphorylation site	phospho peptide seq.	exp. mass	theor. mass
pT358	RRTHNVLER	1259.624	1259.647
pS373	RSFFALR	975.469	974.492
pT400	KATAYILSVQAEEQK	1757.860	1757.883
pY402	KATAYILSVQAEEQK	1757.860	1757.883
pS405	ATAYILSVQAEEQK	1629.763	1629.788
pS437	LEQLRNS	928.422	938.445

Table S1: Phosphorylation sites identified using MS with corresponding peptides including the monoisotopic experimental and theoretical masses. Corresponding MSMS spectra and Mascot (Matrix Science) match are highlighted in Figures S1-S3.

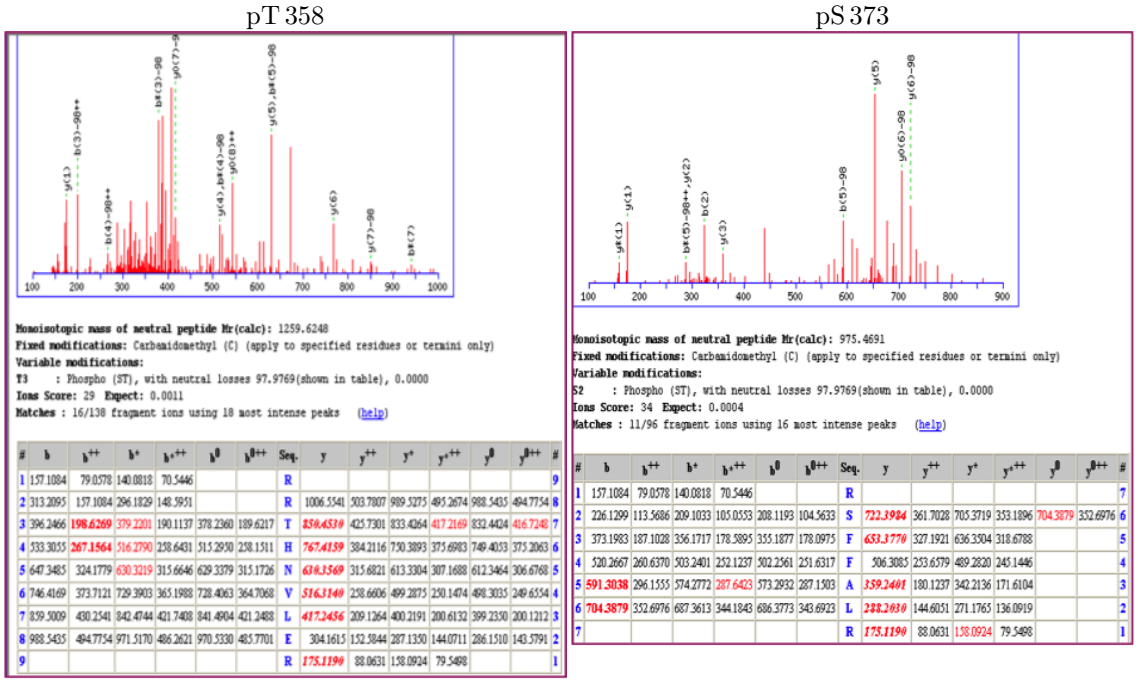
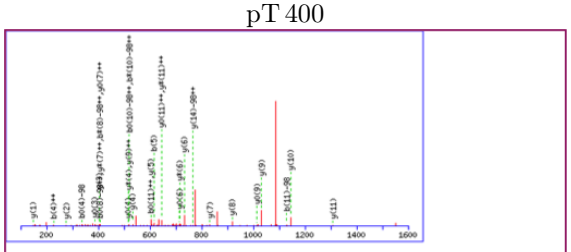


Figure S1: Output of the MSMS search results from Mascot (Matrix Science). Identified peptide sequence The table shows theoretical b and y MSMS ion masses of the corresponding peptide (center column) fragments. MS spectra and match of experimetal and theoretical fragment ions for each peptide are highlighted in red. The matched ions confirm the identity of the peptides containing phosphorylated T358 and S373.

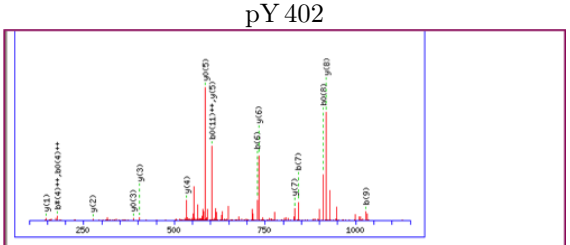
pT 400



Monoisotopic mass of neutral peptide Mr(calc): 1757.8600
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
T3 : Phospho (ST), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 35 **Expect:** 0.00029
Matches : 32/244 fragment ions using 69 most intense peaks (help)

#	b	b++	b*	b*++	b0	b0++	Seq.	y	y++	y*	y*++	y0	y0++	#
1	129.1022	65.0548	112.0757	56.5415			K							15
2	200.1394	100.5733	183.1128	92.0600			A	1630.7724	815.8898	1613.7458	807.3766	1612.7618	806.8846	14
3	381.1534	191.0803	364.1268	182.5670	363.1428	182.0750	T	1559.7353	780.3713	1542.7087	771.8580	1541.7247	771.3660	13
4	452.1905	226.5989	435.1639	218.0856	434.1799	217.5936	A	1378.7213	689.8643	1361.6947	681.3510	1360.7107	680.8590	12
5	615.2538	308.1305	598.2273	299.6173	597.2432	299.1253	Y	*1307.6842*	654.3457	1290.6576	645.8324	1289.6736	645.3404	11
6	728.3379	364.6726	711.3113	356.1593	710.3273	355.6673	I	*1144.6203*	572.8141	1127.5943	564.3008	1126.6103	563.8088	10
7	841.4219	421.2146	824.3954	412.7013	823.4114	412.2093	L	*1031.5363*	516.2720	1014.5102	507.7587	1013.5262	507.2667	9
8	928.4540	464.7306	911.4274	456.2173	910.4434	455.7253	S	*918.4527*	459.7300	901.4262	451.2167	900.4421	450.7247	8
9	1027.5224	514.2648	1010.4958	505.7516	1009.5118	505.2595	V	*831.4207*	416.2140	814.3941	407.7007	813.4101	407.2087	7
10	1155.5810	578.2941	1138.5544	569.7808	1137.5704	569.2888	Q	*732.3523*	366.6798	715.3257	358.1665	714.3417	357.6745	6
11	1226.6181	613.8127	1209.5915	605.2994	1208.6075	604.8074	A	*604.2917*	302.6505	587.2671	294.1372	586.2831	293.6452	5
12	1355.6607	678.3340	1338.6341	669.8207	1337.6501	669.3287	E	*533.2566*	267.1319	516.2300	258.6186	515.2460	258.1266	4
13	1484.7033	742.8553	1467.6767	734.3420	1466.6927	733.8500	E	*404.2140*	202.6106	387.1874	194.0974	386.2034	193.6053	3
14	1612.7618	806.8846	1595.7353	798.3713	1594.7513	797.8793	Q	*275.1714*	138.0893	258.1448	129.5761			2
15							K	*147.1128*	74.0600	130.0863	65.5468			1

pY 402

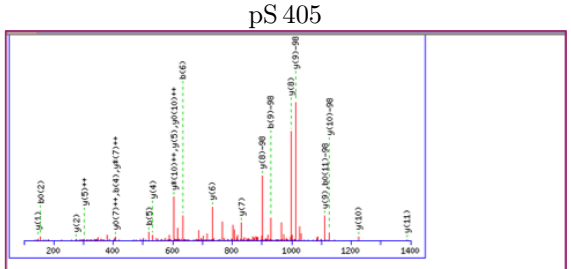


Monoisotopic mass of neutral peptide Mr(calc): 1757.8600
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
Y5 : Phospho (Y)
Ions Score: 68 **Expect:** 1.7e-07
Matches : 17/160 fragment ions using 19 most intense peaks (help)

#	b	b++	b*	b*++	b0	b0++	Seq.	y	y++	y*	y*++	y0	y0++	#
1	129.1022	65.0548	112.0757	56.5415			K							15
2	200.1394	100.5733	183.1128	92.0600			A	1630.7724	815.8898	1613.7458	807.3766	1612.7618	806.8846	14
3	301.1870	151.0972	284.1605	142.5839	283.1765	142.0919	T	1559.7353	780.3713	1542.7087	771.8580	1541.7247	771.3660	13
4	372.2241	186.6157	355.1976	178.1024	354.2136	177.6104	A	1458.6876	729.8474	1441.6611	721.3342	1440.6770	720.8422	12
5	615.2538	308.1305	598.2273	299.6173	597.2432	299.1253	Y	1387.6505	694.3289	1370.6239	685.8156	1369.6399	685.3236	11
6	*728.3379*	364.6726	711.3113	356.1593	710.3273	355.6673	I	1144.6208	572.8141	1127.5943	564.3008	1126.6103	563.8088	10
7	*841.4219*	421.2146	824.3954	412.7013	823.4114	412.2093	L	1031.5368	516.2720	1014.5102	507.7587	1013.5262	507.2667	9
8	928.4540	464.7306	911.4274	456.2173	910.4434	455.7253	S	*918.4527*	459.7300	901.4262	451.2167	900.4421	450.7247	8
9	*1027.5224*	514.2648	1010.4958	505.7516	1009.5118	505.2595	V	*831.4207*	416.2140	814.3941	407.7007	813.4101	407.2087	7
10	1155.5810	578.2941	1138.5544	569.7808	1137.5704	569.2888	Q	*732.3523*	366.6798	715.3257	358.1665	714.3417	357.6745	6
11	1226.6181	613.8127	1209.5915	605.2994	1208.6075	604.8074	A	*604.2917*	302.6505	587.2671	294.1372	586.2831	293.6452	5
12	1355.6607	678.3340	1338.6341	669.8207	1337.6501	669.3287	E	*533.2566*	267.1319	516.2300	258.6186	515.2460	258.1266	4
13	1484.7033	742.8553	1467.6767	734.3420	1466.6927	733.8500	E	*404.2140*	202.6106	387.1874	194.0974	386.2034	193.6053	3
14	1612.7618	806.8846	1595.7353	798.3713	1594.7513	797.8793	Q	*275.1714*	138.0893	258.1448	129.5761			2
15							K	*147.1128*	74.0600	130.0863	65.5468			1

Figure S2: MSMS search results from MASCOT for T400 and Y402 phosphorylation. See Figure S1 for further details.

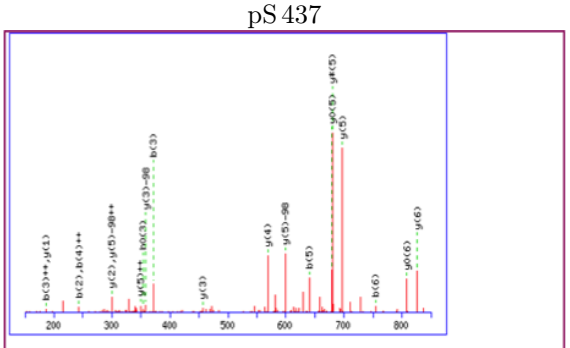
pS 405



Monoisotopic mass of neutral peptide Mr(calc): 1629.7651
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
S7 : Phospho (ST), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 76 **Expect:** 2.5e-08
Matches : 24/208 fragment ions using 24 most intense peaks (help)

#	b	b++	b*	b*++	b0	b0++	Seq.	y	y++	y*	y*++	y0	y0++	#
1	72.0444	36.5258					A							14
2	173.0921	87.0497			155.0815	78.0444	T	1461.7584	731.3828	1444.7318	722.8696	1443.7478	722.3775	13
3	244.1292	122.5682			226.1186	113.5629	A	1360.7107	680.8590	1343.6842	672.3457	1342.7001	671.8537	12
4	*407.1925*	204.0999			389.1819	195.0946	Y	1289.6736	645.3404	1272.6470	636.8272	1271.6630	636.3352	11
5	*520.2766*	260.6419			502.2660	251.6366	I	*1126.6103*	563.8088	1109.5837	555.2955	1108.5997	554.8035	10
6	*633.3606*	317.1840			615.3501	308.1787	L	*1013.5262*	507.2667	996.4997	498.7535	995.5156	498.2615	9
7	702.3821	351.6947			684.3715	342.6894	S	*906.4421*	453.7247	883.4156	442.2114	882.4316	441.7194	8
8	801.4505	401.2289			783.4400	392.2236	V	*831.4207*	416.2140	814.3941	407.7007	813.4101	407.2087	7
9	*929.5091*	465.2582	912.4825	456.7449	911.4985	456.2529	Q	*732.3523*	366.6798	715.3257	358.1665	714.3417	357.6745	6
10	1000.5462	500.7767	983.5197	492.2635	982.5356	491.7715	A	*604.2917*	302.6505	587.2671	294.1372	586.2831	293.6452	5
11	1129.5888	565.2980	1112.5623	556.7848	*1111.5782*	556.2928	E	*533.2566*	267.1319	516.2300	258.6186	515.2460	258.1266	4
12	1258.6314	629.8193	1241.6048	621.3061	1240.6208	620.8141	E	404.2140	202.6106	387.1874	194.0974	386.2034	193.6053	3
13	1386.6900	693.8486	1369.6634	685.3354	1368.6794	684.8433	Q	*275.1714*	138.0893	258.1448	129.5761			2
14							K	*147.1128*	74.0600	130.0863	65.5468			1

pS 437



Monoisotopic mass of neutral peptide Mr(calc): 938.4222
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
S7 : Phospho (ST), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 48 **Expect:** 1.6e-05
Matches : 20/98 fragment ions using 21 most intense peaks (help)

#	b	b++	b*	b*++	b0	b0++	Seq.	y	y++	y*	y*++	y0	y0++	#
1	114.0913	57.5493					L							7
2	*243.1339*	122.0706			225.1234	113.0653	E	*826.3455*	413.6764	809.3189	405.1631	808.3349	404.6711	6
3	*371.1925*	*186.0999*	354.1660	177.5866	353.1819	177.0946	Q	*697.3029*	349.1551	680.2763	340.6418	679.2923	340.1498	5
4	484.2766	*242.6419*	467.2500	234.1287	466.2660	233.6366	L	*569.2443*	285.1258	552.2178	276.6125	551.2337	276.1205	4
5	*640.3777*	320.6925	623.3511	312.1792	622.3671	311.6872	R	*456.1602*	228.5838	439.1337	220.0705	438.1497	219.5785	3
6	*754.4206*	377.7139	737.3941	369.2007	736.4100	368.7087	N	*300.8591*	150.9332	283.0326	142.0199	282.0486	141.5279	2
7							S	*186.0162*	93.5117			168.0056	84.5065	1

Figure S3: MSMS search results from MASCOT for S3405 and S437 phosphorylation. See Figure S1 for further details.

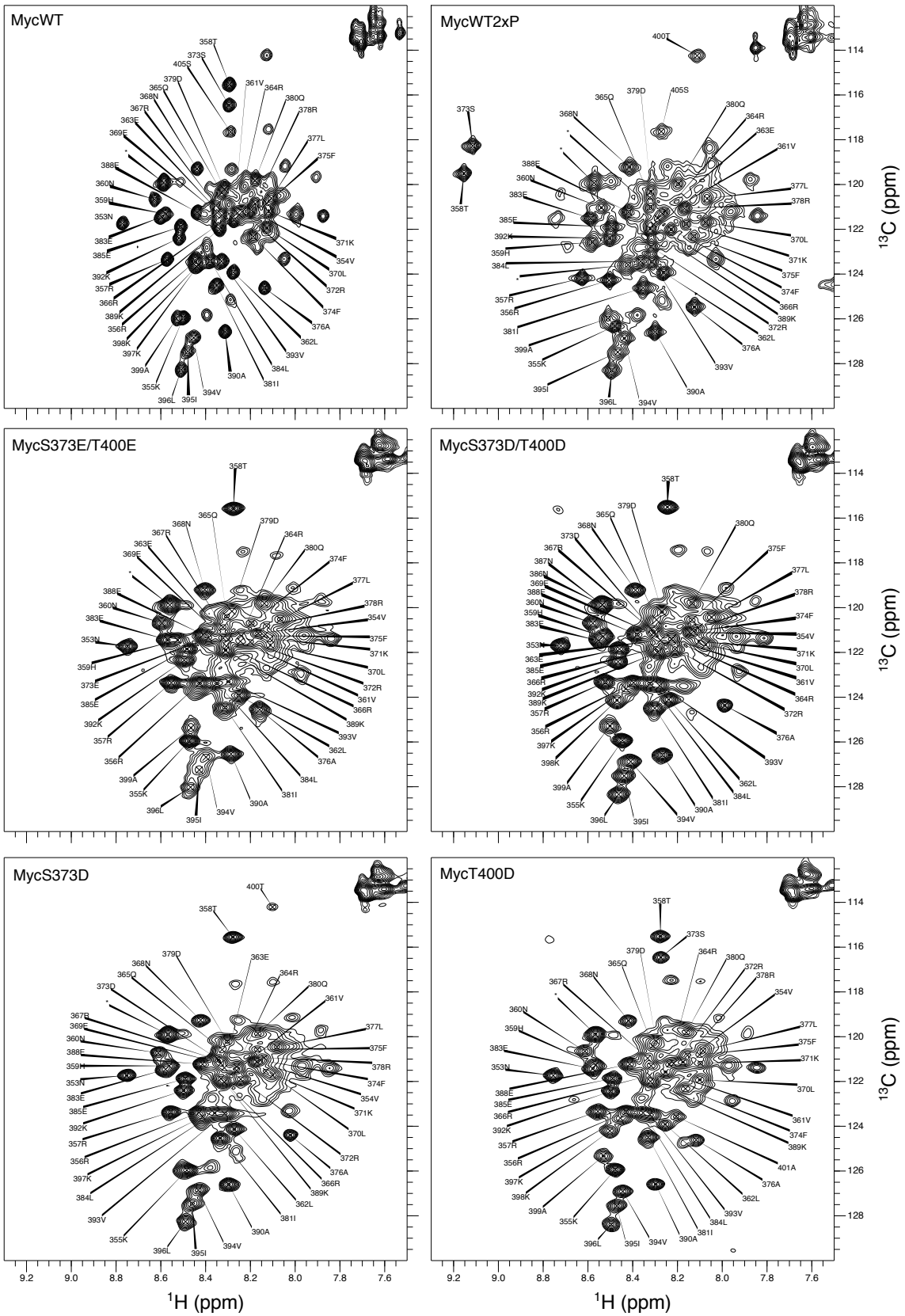


Figure S4: NMR spectra with assignment and corresponding BMRB entry ID codes of Myc_{WT} (BMRB: 27414), Myc_{WT}-2P (BMRB: 27422), $Myc_{S373E/T400E}$ (BMRB: 27419), $Myc_{S373D/T400D}$ (BMRB: 27416), Myc_{S373D} (BMRB: 27418), and Myc_{T400D} (BMRB: 27421). Minor differences between the spectra of mutants and WT (main text Fig. 1) indicate no effect of the mutations on the structure of Myc bHLH-LZ.

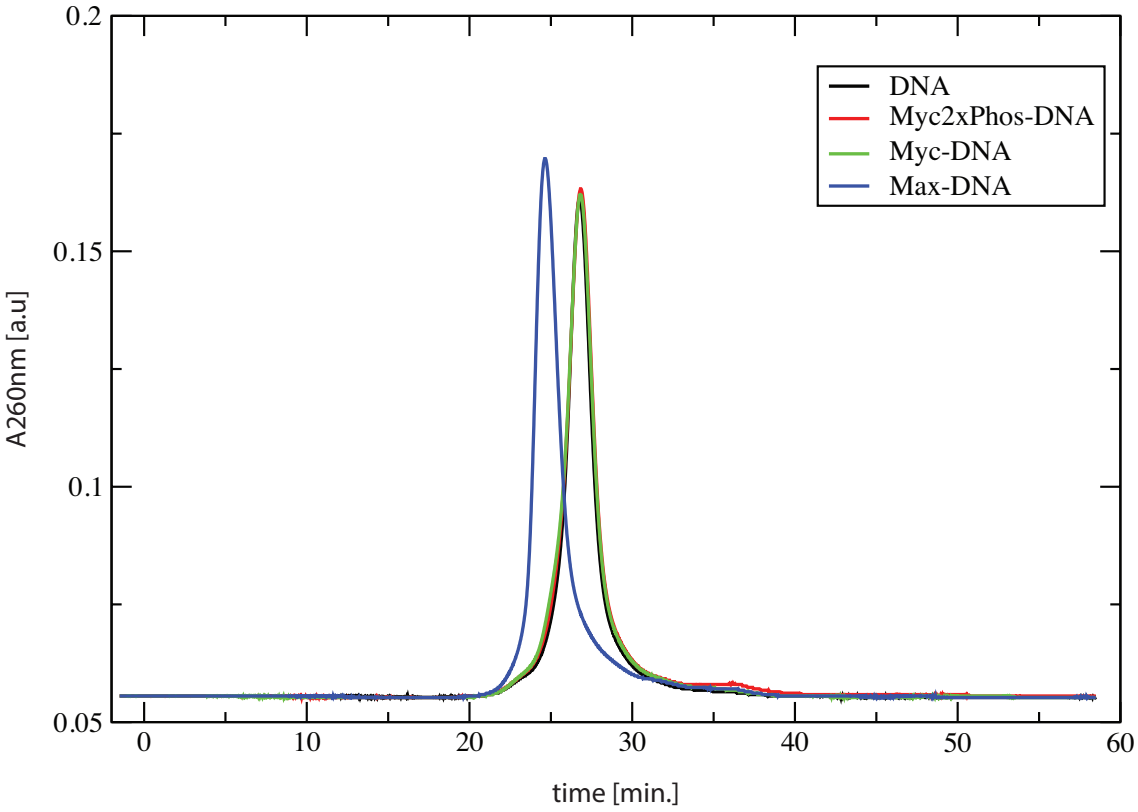


Figure S5: Gel filtration DNA shift assay of MycWT, MycWT 2xPhos, and Max. Max (blue) homodimerizes and binds to DNA, shifting the peak of DNA to a shorter elution time relative to the DNA control (black). In contrast to Max (blue), MycWT (green) and MycWT 2xPhos (red) are not able to form stable complex with DNA.

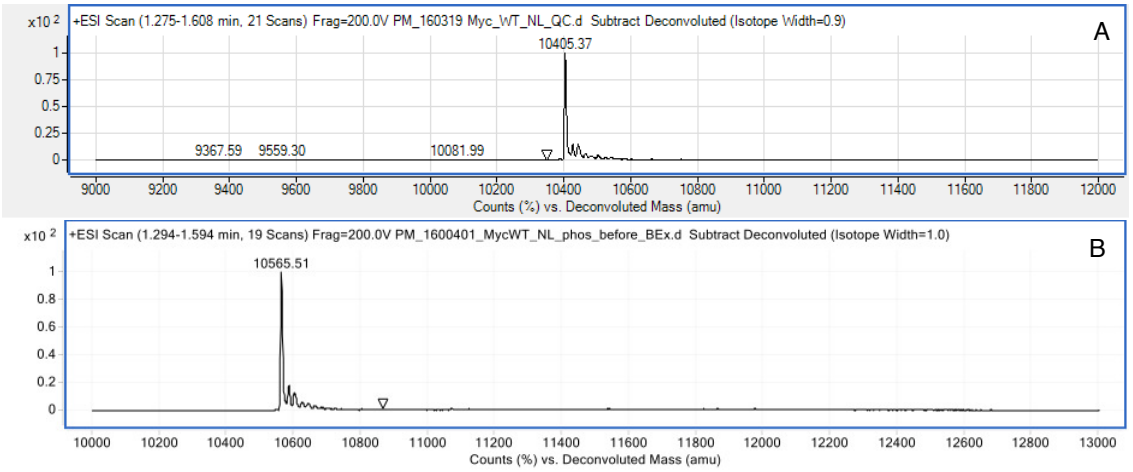


Figure S6: MS quality control of (A) Myc_{WT} before phosphorylation and (B) Myc_{WT}-2P after the phosphorylation by PAK2 and purification on ResS cation exchange. Difference of 160 Da indicate two additional phospho groups on Myc_{WT}.

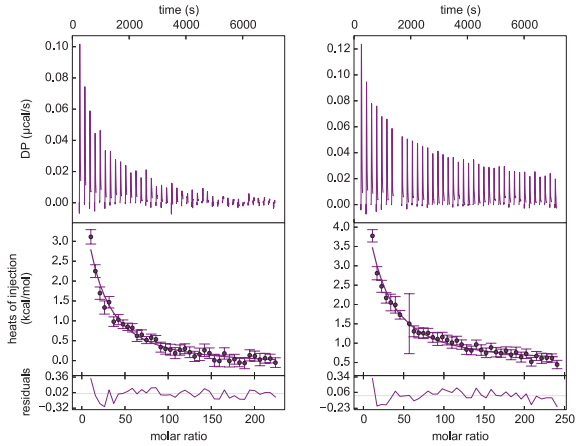


Figure S7: ITC injection heats, including fit and fit residuals for Max dissociation. Max bHLH-LZ (160μM) was titrated into the buffer as described in experimental procedures.

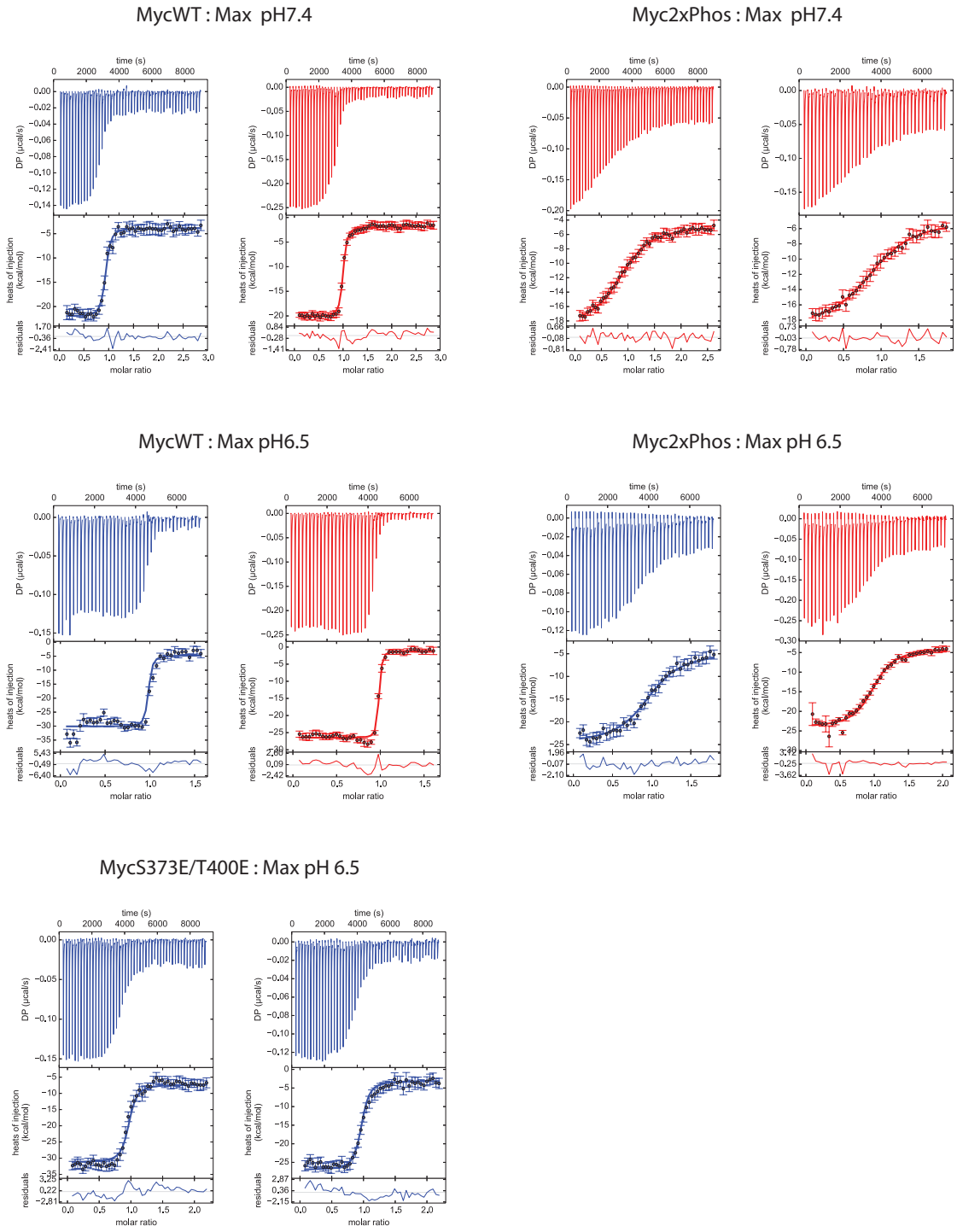


Figure S8: ITC injection heats, including fit and fit residuals for MycWT and MycWT 2xPhos at pH 7.4 and MycWT, MycWT 2xPhos, and MycS373E/T400E at pH 6.5. Data were obtained at 2μM Max and 20μM titrant (red) and 4μM Max and 40μM titrant (blue). Data at different concentrations were fitted simultaneously to obtain binding constant and thermodynamic information.

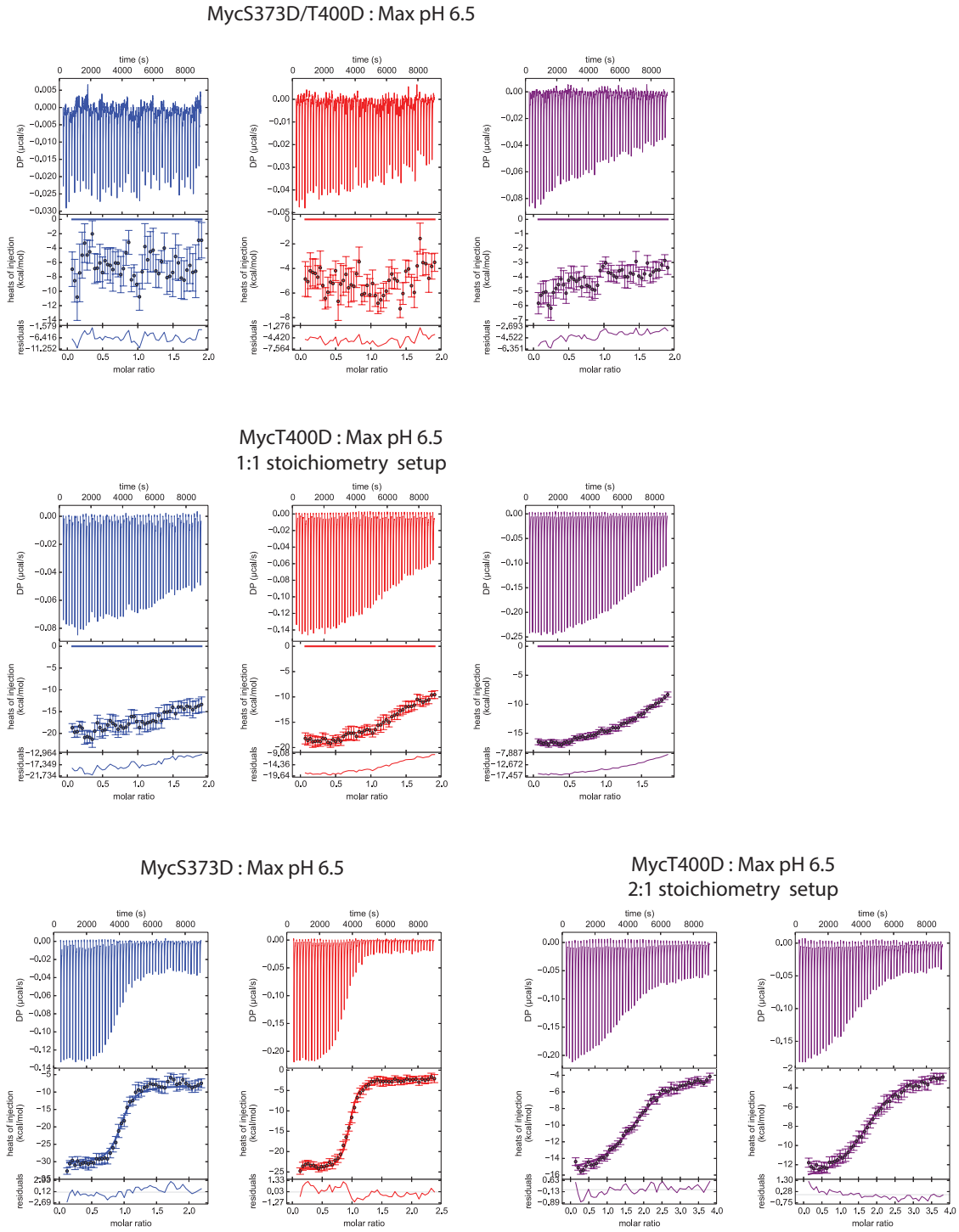


Figure S9: ITC injection heats, including fit and fit residuals for MycS373D/T400D, MycT400D and Myc373D at ITC setup for expected 1:1 stoichiometry followed by MycT400D at ITC setup for 2:1 stoichiometry.. Data were obtained at 2μM Max and 20μM titrant (red), 4μM Max and 40μM titrant (blue), and 8μM Max and 80μM titrant (magenta). Data at different concentrations were fitted simultaneously to obtain binding constant and thermodynamic information.

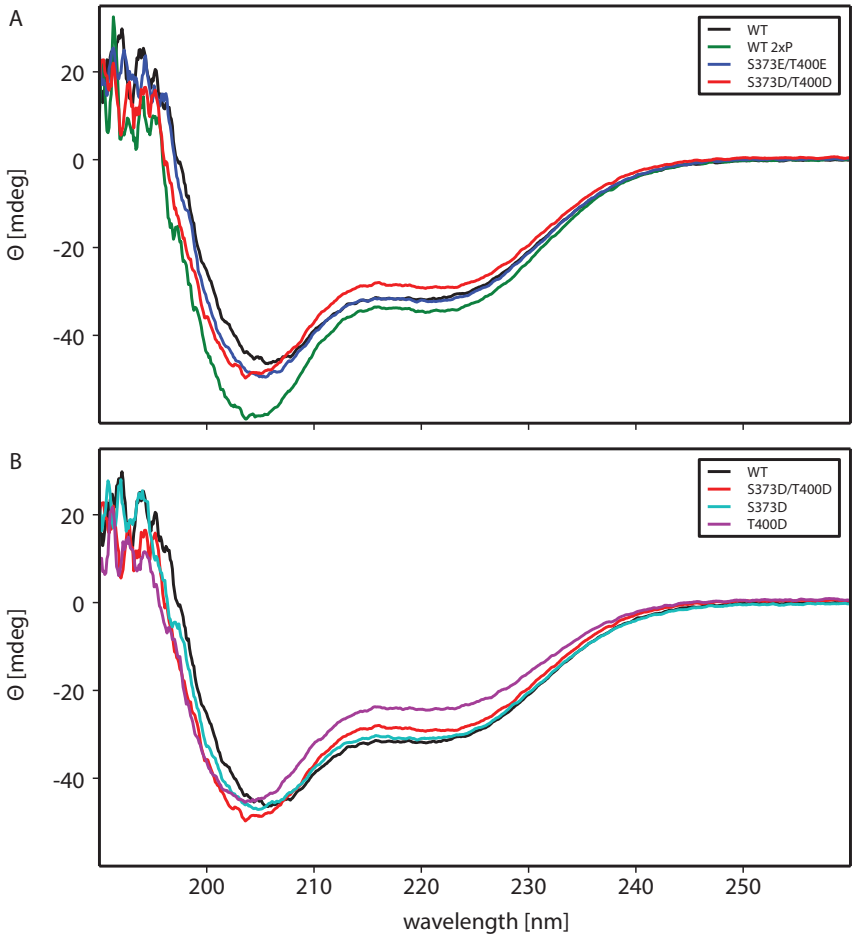


Figure S10: Raw circular dichroism spectra of MycWT (black), MycWT 2xPhos (green), Myc S373D/T400D (red), Myc S373E/T400E (blue), Myc T400D (magenta), and Myc T373D (cyan). Θ minimum at 205nm indicate presence of a random coil mixed with α-helix. The presence of α-helix charecterized by Θ at 222nm.