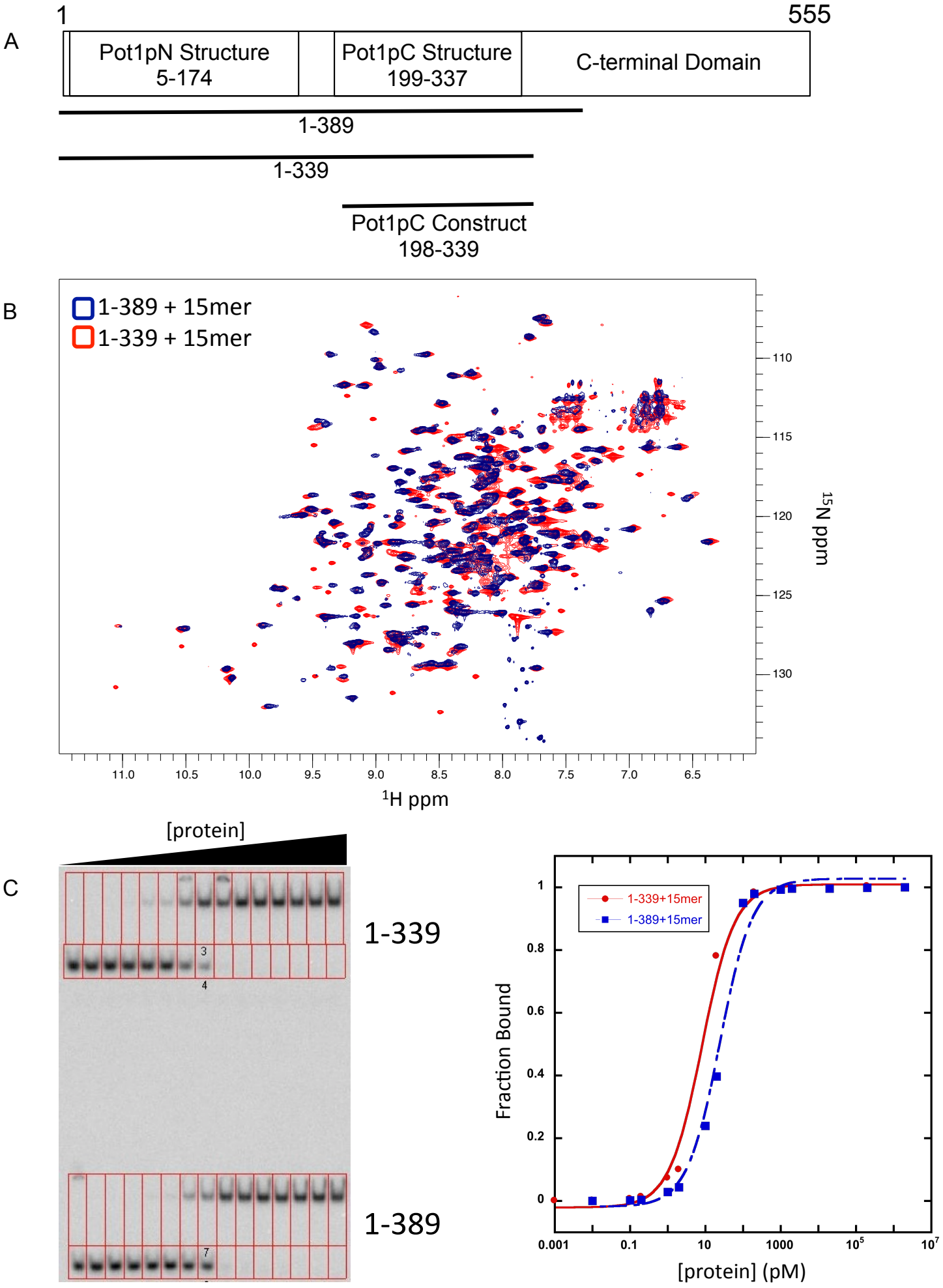
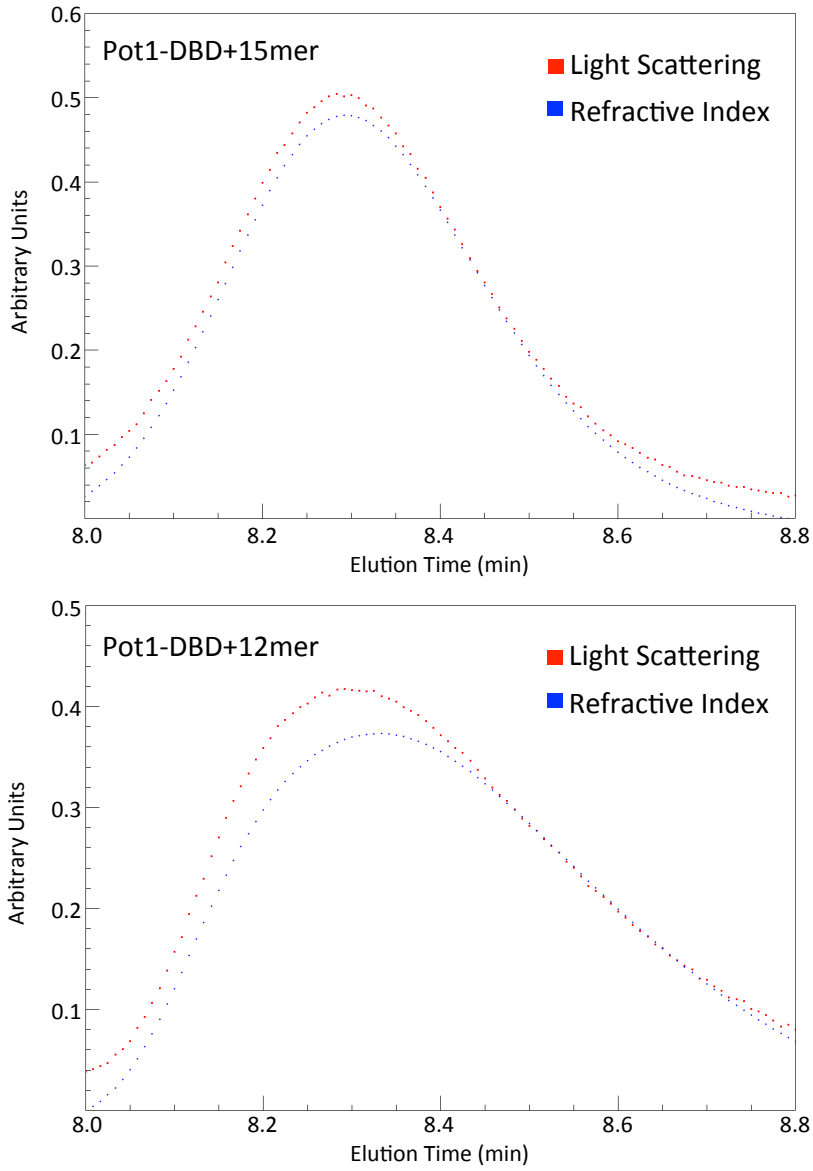
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure Legends

Supplementary Figure 1. Characterization of the new 1-339 Pot1-DBD construct. A) The domain map of Pot1 shows the location of the new construct relative to previous studied constructs (1-3). B) The ^{15}N -HSQC spectrum of 1-339 closely resembles that of 1-389, suggesting the same global fold. Weak peaks are due to chitin-binding domain contamination. C) Representative EMSA and fitted curves from EMSA data illustrate the similarity in DNA-binding activity between constructs.

Supplementary Figure 2. The offset refractive index and light scattering traces of Pot1-DBD+12mer indicate mixture of species not seen in the Pot1-DBD+15mer complex. Conditions are the same as Figure 3B (34 μM Pot1-DBD, 20 mM Tris-HCl pH 8.0, 400 mM NaCl).

Supplementary Tables

Supplementary Table 1. Shifted residues from Figure 1C

Shifted >0.5 ppm	Split Peaks	Unassigned in Pot1-DBD only	Unassigned in pN and DBD	Unassigned in pC and DBD
Val6	Ile207	Thr41	Ser8	Ser200
Ile7	Asp269	Gly47	Gly23	Phe201
Phe52	Thr292	Ile48	Glu24	Arg212
Arg118	Lys293	Cys76	Phe27	Asp226
Gln120	Glu301	Leu105	Leu36	Lys227
Ser202		Ile110	Gln37	Asp235
Leu203		Val290	Lys38	Tyr248
His212		Arg291	Lys39	Thr249
Glu270			Asn40	Ser250
His271			Val49	Ser251
Ile294			Lys50	Ser252
Leu304			Ser55	Arg255
Ser315			Glu64	Gly256
			Trp65	Asn277
			Tyr70	His296
			Trp72	His305
			Asp73	Gly306
			Asp77	Asp307
			Thr78	Ser308
			Ser79	Ala309
			Ser80	Lys310
			Ile81	Arg311
			Gly82	Tyr312
			Leu83	Asn313
			Gln84	Met314
			Lys90	
			Asn93	
			Gly102	

Shifted >0.5 ppm	Split Peaks	Unassigned in Pot1-DBD only	Unassigned in pN and DBD	Unassigned in pC and DBD
			Gln103	
			His108	
			Gln109	
			Arg116	
			Asp117	
			Gln126	
			Phe127	
			Asp134	
			Phe135	
			Ser136	
			Asn138	
			Ser139	
			Lys140	
			Asp141	
			Thr142	
			Leu143	
			Cys144	
			Gln146	
			Met148	
			Lys153	
			Asp156	
			Lys157	
			Lys167	
			Ile168	
			Trp169	
			Asp170	
			Glu171	
			Gln172	
			Thr173	
			Asn174	

Supplementary Table 2. Binding affinities for Pot1-DBD constructs tested

Protein Construct	15mer K_D (pM)[a]	Fold change[b]	12mer K_D (pM)[a]	Fold change[c]
Pot1-DBD_WT	4 ± 1	-	5 ± 1	-
Pot1-DBD_Y224A	3.2 ± 0.7	0.88	5.9 ± 0.5	1.3
Pot1-DBD_linkerdel	1.3 ± 0.5	0.37	3.2 ± 0.7	0.70

[a] Apparent K_D values are averaged from triplicate EMSA experiments. Representative data are shown in Figures 2 and 5.
[b] Fold change is relative to the Pot1-DBD_WT+15mer affinity
[c] Fold change is relative to the Pot1-DBD_WT+12mer affinity

Supplementary References

1. Lei, M., Podell, E.R., Baumann, P. and Cech, T.R. (2003) DNA self-recognition in the structure of Pot1 bound to telomeric single-stranded DNA. *Nature*, **426**, 198–203.

2. Dickey, T.H., McKercher, M.A. and Wuttke, D.S. (2013) Nonspecific Recognition Is Achieved in Pot1pC through the Use of Multiple Binding Modes. *Structure*, **21**, 121–132.

3. Croy, J.E., Podell, E.R. and Wuttke, D.S. (2006) A new model for Schizosaccharomyces pombe telomere recognition: the telomeric single-stranded DNA-binding activity of Pot11-389. *J. Mol. Biol.*, **361**, 80–93.