Supplementary Online Material

Inventory

The Supplementary Material contains 8 Figures and their Legends. Figures S1 and S2 relate to the introduction in the main text and relate to design of palladin peptides. Figure S3, S4, S5 and S6 relate to HSQC results for titration and assignment of Act-EF34. Figure S7 relates to PRE results and Figure S8 relates to relaxation data.

Supplemental Figures

Supplemental Figure Legends

Supplementary Figure 1. Alignment of the α-actinin interaction regions for human palladin family members. Residues 229-280 of palladin, 816-870 of myopalladin, and 83-137 of myotilin were aligned using ClustalW[1]. Stars depict identical residues in all three family members, double dots for identity between two family members, and single dots depict conservative changes. A highly homologous 17 amino-acid segment can be identified and is underlined. The bold proline residue was identified as an exonic point mutation (P239S) associated with familial pancreatic cancer[2].

Supplemental Figure 2. Domain organization of palladin and α-actinin. Schematic representation of palladin (isoform #4, 72 kDa) and α-actinin (94 kDa), highlighting the domain organization and sites of interaction: PR2, proline-rich region 2; red line represents the P239S, Family-X mutation site within the actinin-binding site in yellow (229-280); Ig,

immunoglobulin domains; ABD, actin binding domain; R1-4, spectrin-like repeats; EF 1-4, EF hand domains of the C-terminal calmodulin (CaM)-like domain.

Supplemental Figure 3. NMR Titration of ^{15}N-α-actinin EF34 with palladin peptides. (A) Overlay of ^{15}N-HSQC spectra of ^{15}N-labeled Act2-EF34 (black) and in complex with unlabeled palladin peptides; WT (magenta) or Family X mutant (cyan). (B). Titration of Act-EF34 with increasing concentration of WT palladin peptide, represented by colored peaks. Inset show two specific peaks, Ile 24 which does not shift and Asp 36 which undergoes large chemical shift perturbation upon binding palladin peptide.

Supplemental Figure 4. ^{15}N-HSQC spectrum of Act-EF34 at pH 6.6 and 27 °C, showing amid backbone resonance assignments(from Yanghan Au Thesis, A. Pastore permission). Solid horizontal lines indicate side-chain amide protons of asparagine and glutamine residues, and the arrow indicate assignment of "second species" peaks.

Supplemental Figure 5. NMR Titration of ^{15}N-Act-EF34 with palladin family member peptides. Overlay of ^{15}N-HSQC spectra of ^{15}N-labeled apo Act2-EF34 (black) and in complex with unlabeled palladin (green), myopalladin (red), or myotilin (blue) peptides.

Supplementary Figure 6. NMR Titration of ^{15}N-Act-EF34 with mutant palladin peptide. Overlay of ^{15}N-HSQC spectra of ^{15}N-labeled Act2-EF34 (black) and in complex with unlabeled mutant L(1,4)N palladin peptide.

Supplemental Figure 7. Paramagnetic relaxation broadening of Act-EF34 by MTSL-palladin peptide. Bars graph of intensity difference between ^{15}N-HSQC cross-peaks of 15N-Act-EF34 in the presence of saturating amounts of palladin peptide labeled with oxidized versus reduced spin label MTSL. Amide protons in closest proximity to spin label experience the greatest effect of paramagnetic broadening.

Supplemental Figure 8. T_1 and T_2 relaxation data for apo and WT palladin-bound Act-EF34. Open circles correspond to apo Act-EF34 and closed circles are WT-bound Act-EF34. Small overall increase in T_2 relaxation rates for bound Act-EF34 with a few notable exceptions (7, 11, and 42).

```
Palladin        PATRFGHGQTPAAFLSALLPSQPPPAAVNALGLPKGVT---PAGFPKKASRTARI
Myopalladin     PVSPTSRIQNPVAFLSSVLPSLPAIPPTNAMGLPRSAPSMPSQGLAKKNTKSPQP
Myotilin        QRVTTTYNQSPASFLSSILPSQPDYNSSKIPSAMDSNYQQSSAGQPINAKPSQTA
                       *.*.:***::***  *   .:  .   .     . * . : . :
```

Fig. S1

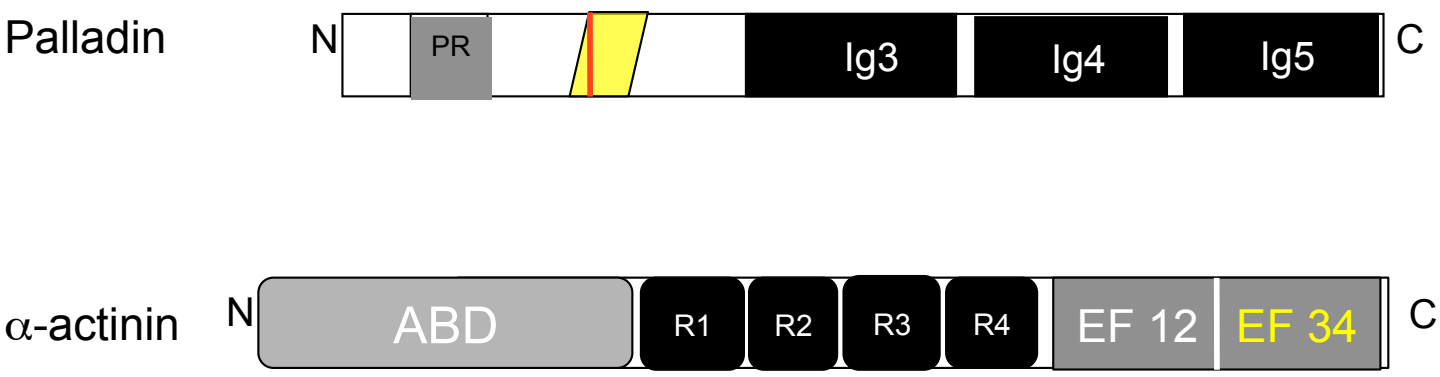
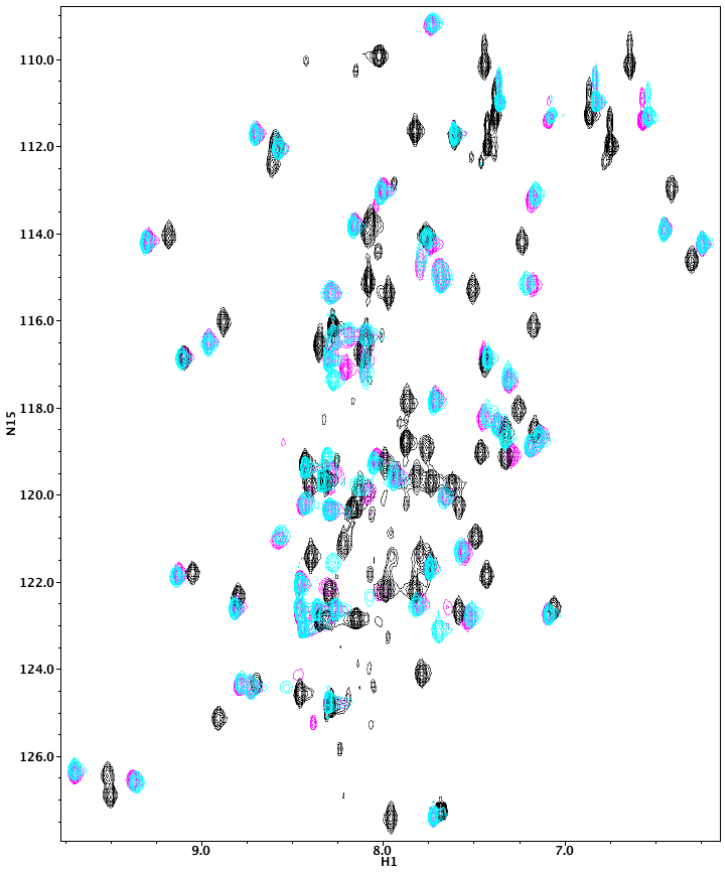

Palladin
N
PR
Ig3
Ig4
Ig5
C
α-actinin
N
ABD
R1
R2
R3
R4
EF 12
EF 34
C

Fig. S2

A


110.0
112.0
114.0
116.0
118.0
120.0
122.0
124.0
126.0
N15
9.0
8.0
7.0
H1

B

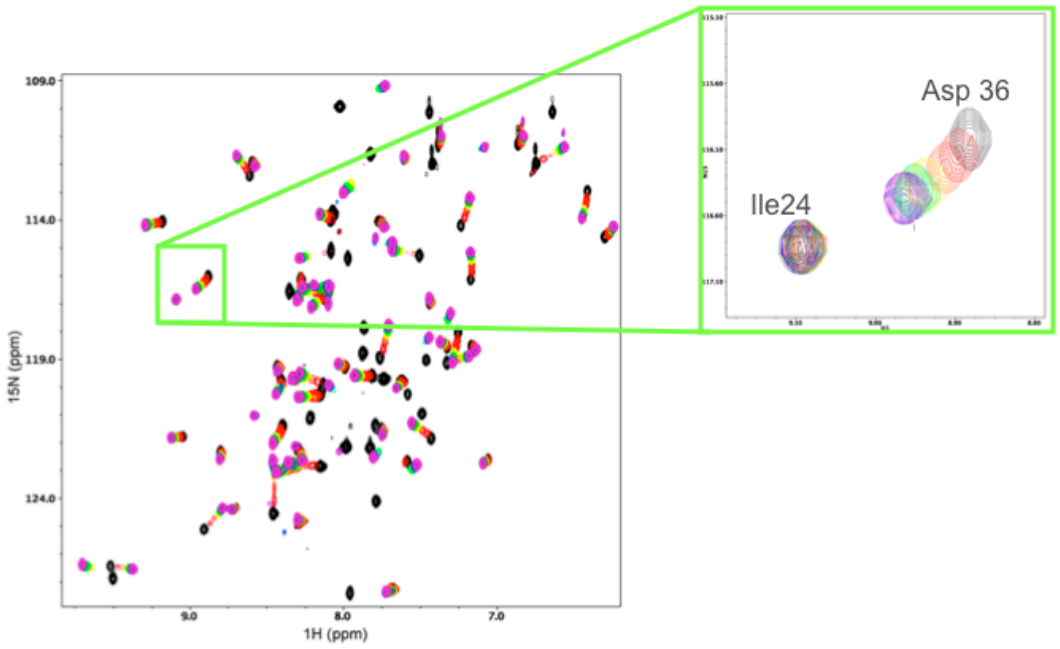

109.0
114.0
119.0
124.0
15N (ppm)
9.0
8.0
7.0
1H (ppm)
Asp 36
Ile24

Fig. S3

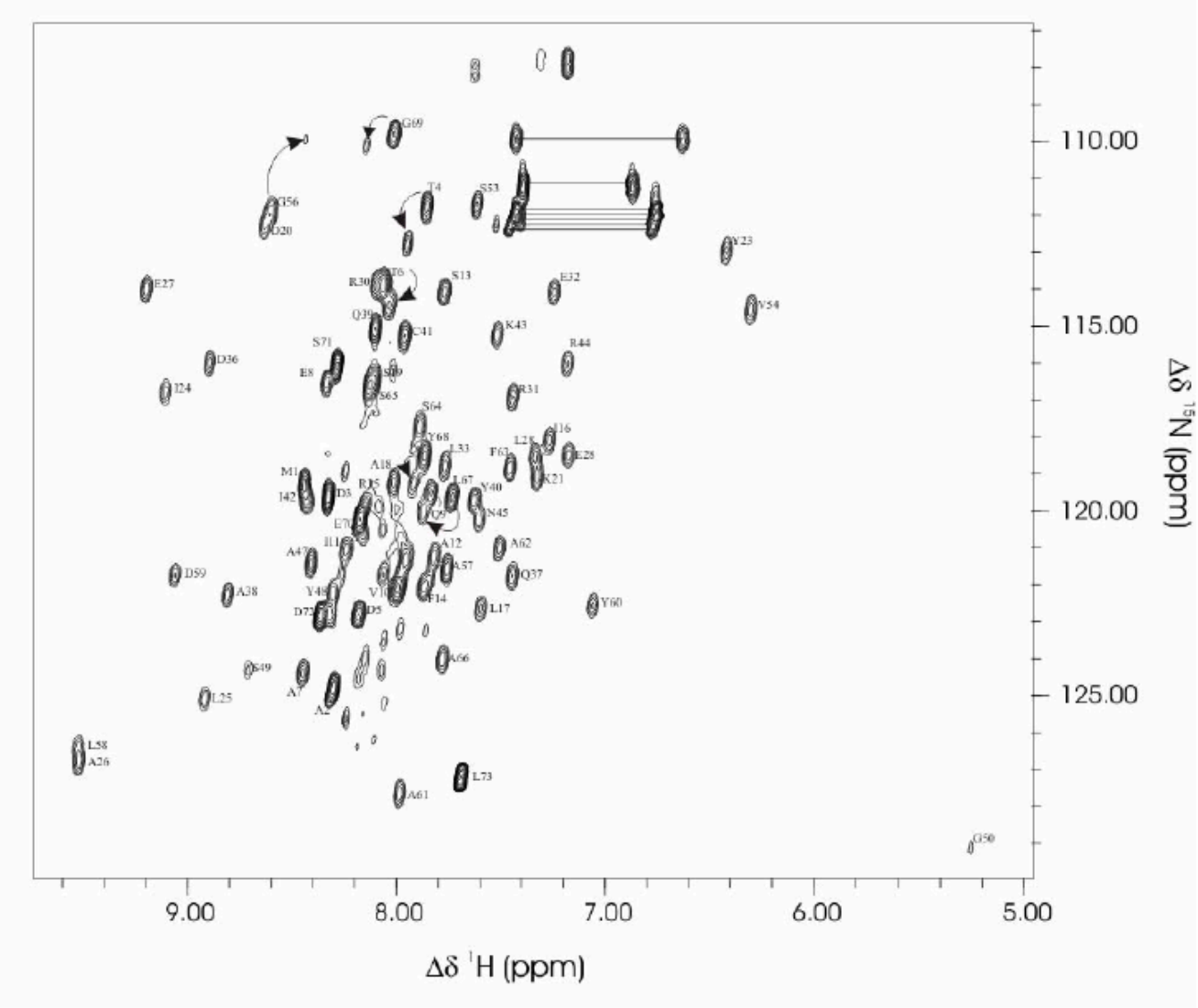

110.00
115.00
120.00
125.00
Δδ ^{15}N (ppm)
9.00
8.00
7.00
6.00
5.00
Δδ ^{1}H (ppm)

Fig. S4

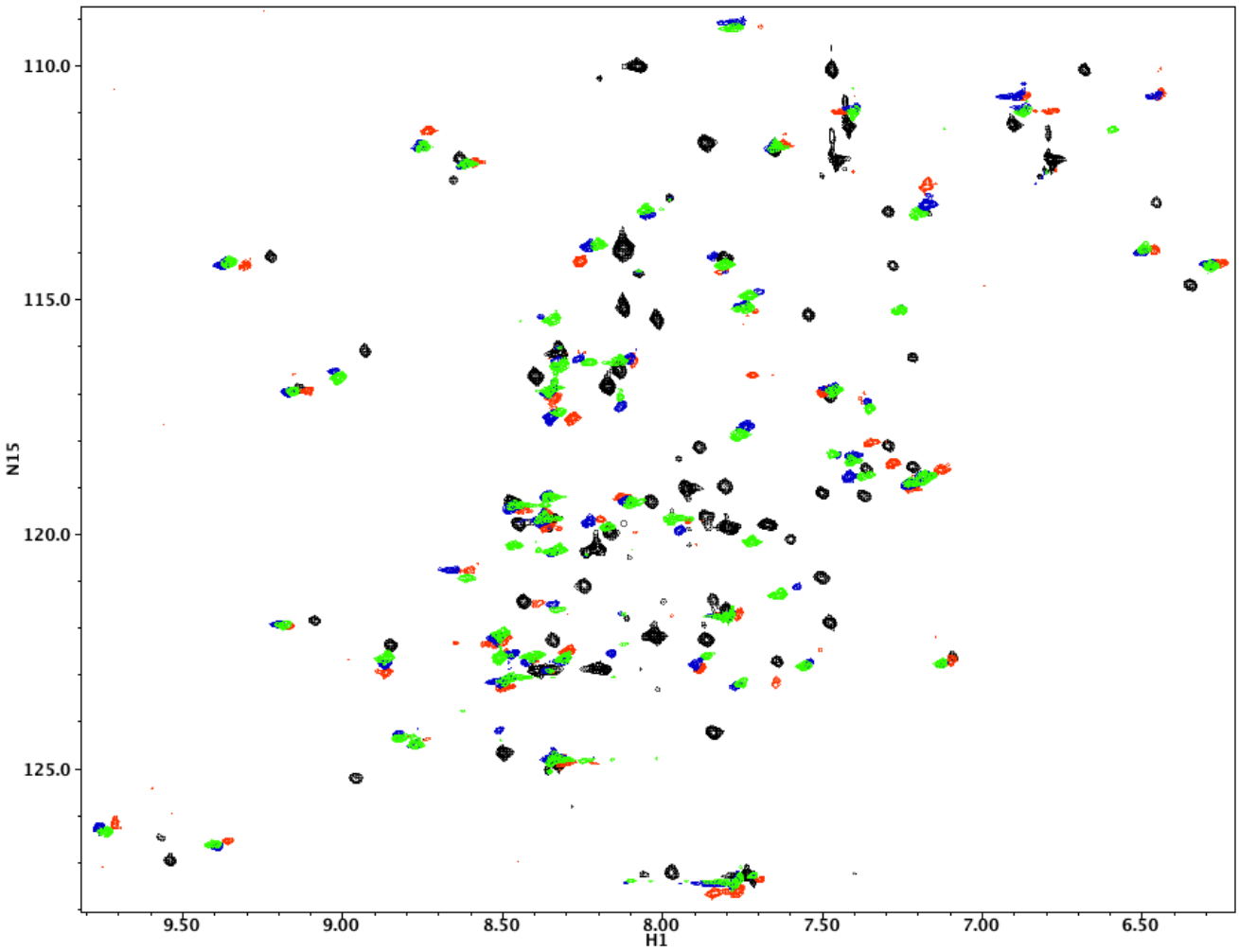


Fig. S5

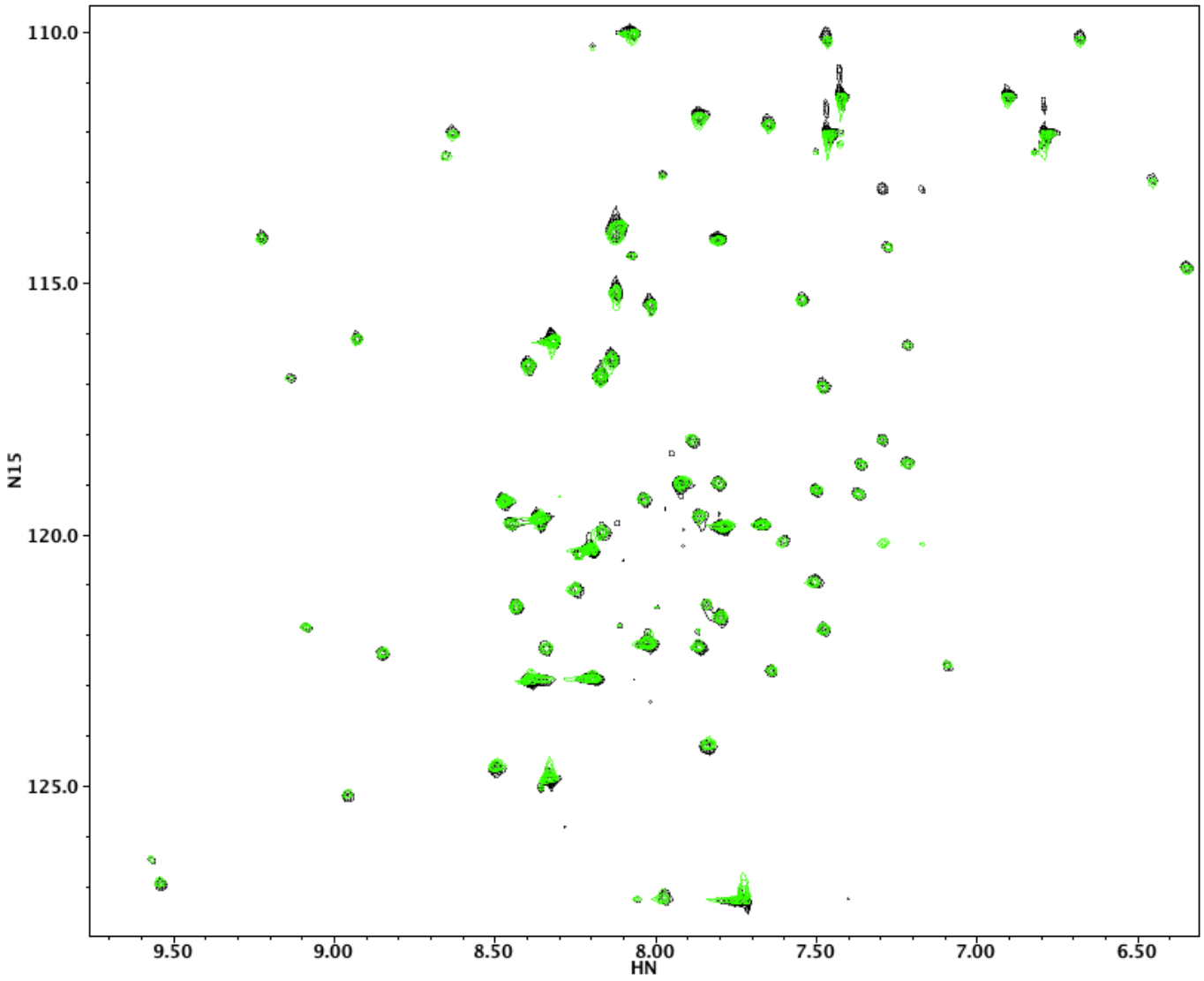


Fig. S6

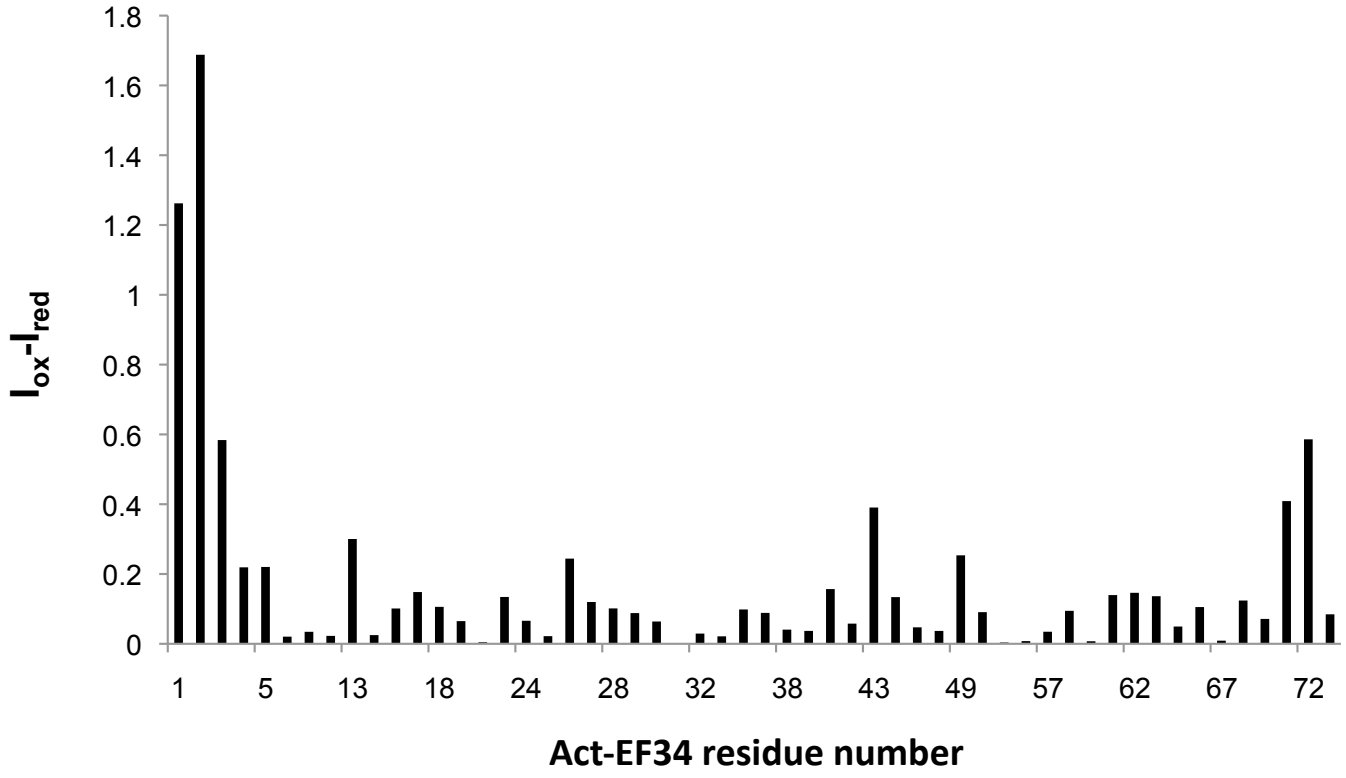

1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
I_{ox}-I_{red}
1
5
13
18
24
28
32
38
43
49
57
62
67
72
Act-EF34 residue number

Fig. S7

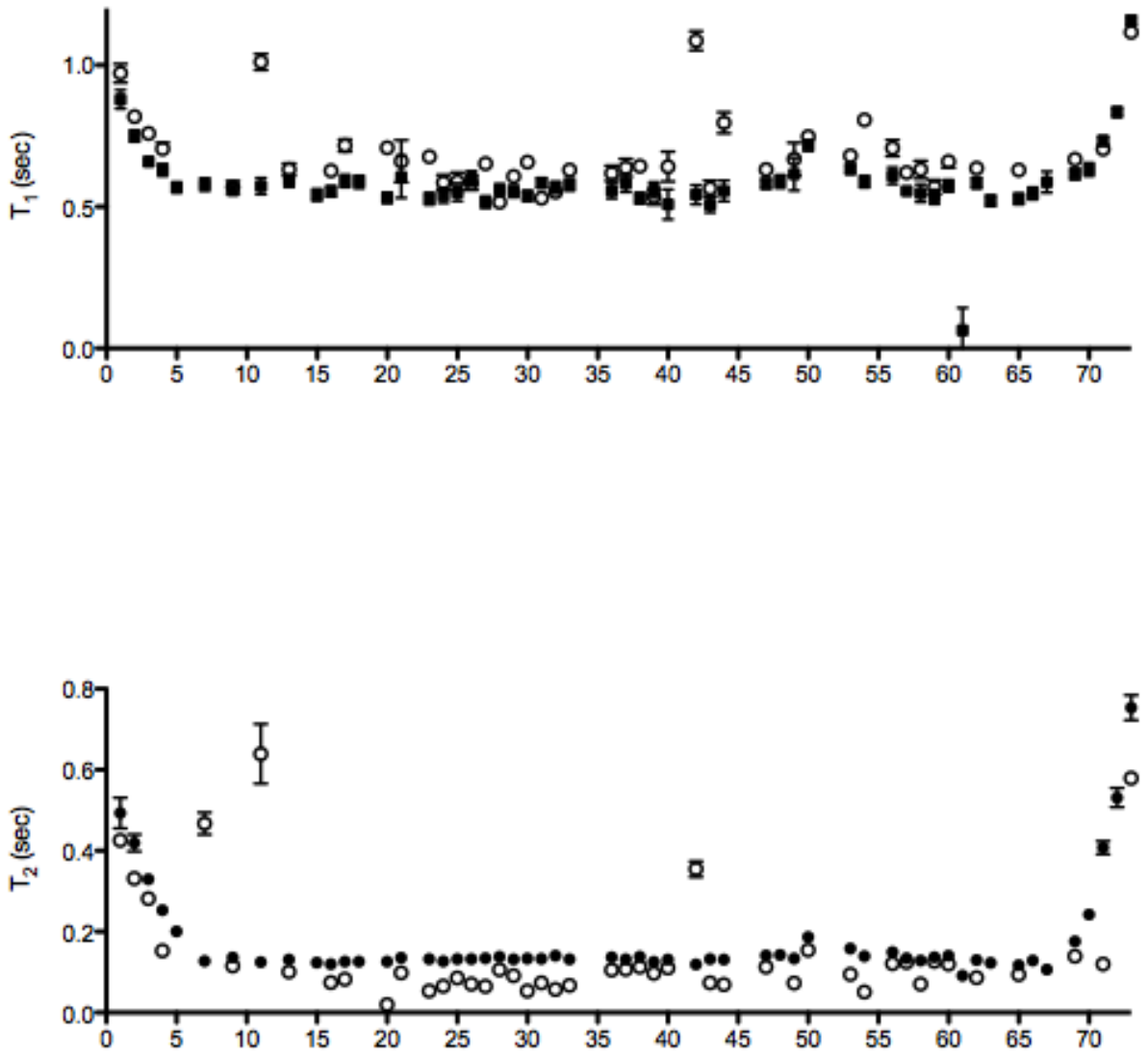

T1 (sec)
1.0
0.5
0.0
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
T2 (sec)
0.8
0.6
0.4
0.2
0.0

Fig. S8