Supporting Information

Covalent and Non-Covalent Targeting of Tcf4/β-Catenin Strand Interface with β-Hairpin Mimics

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Table of Contents

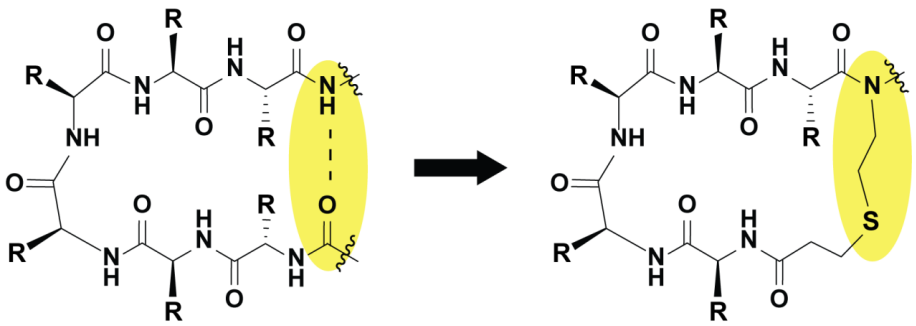
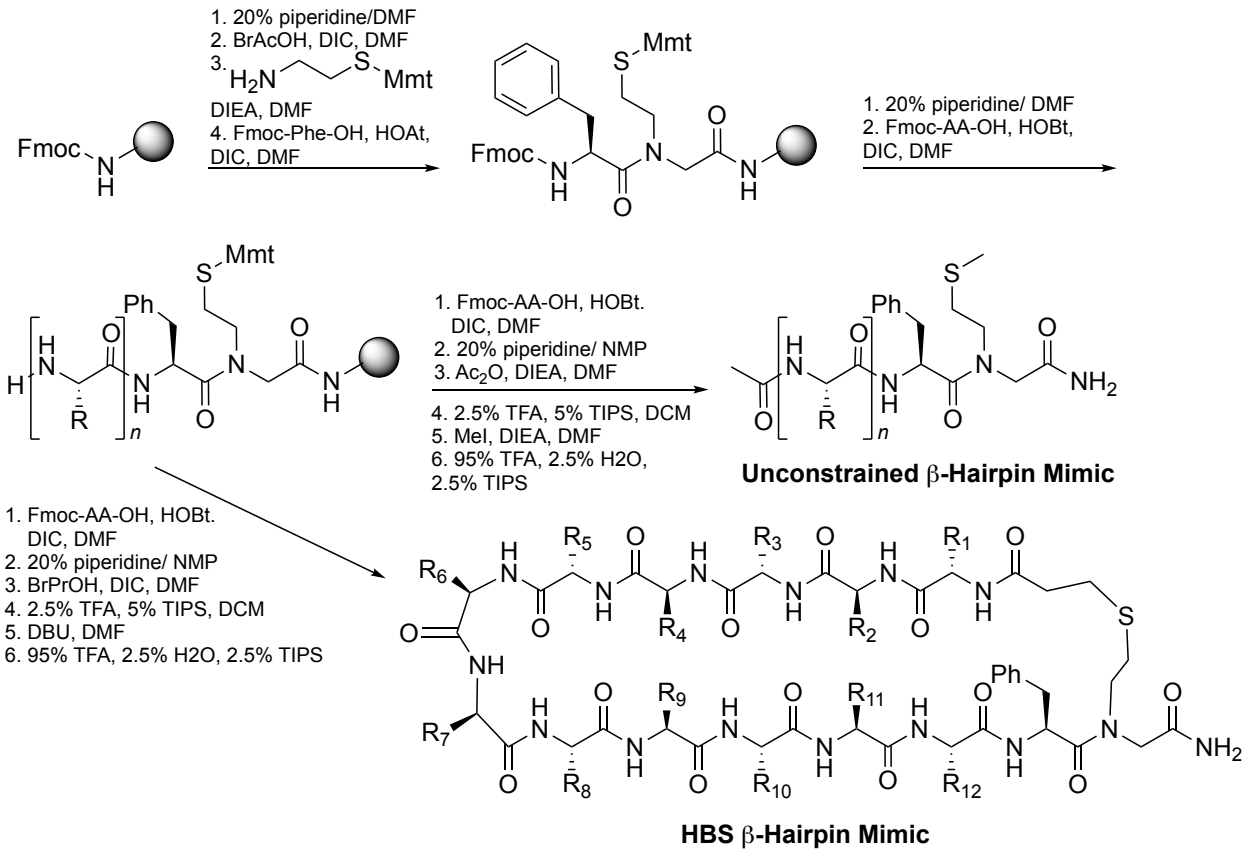
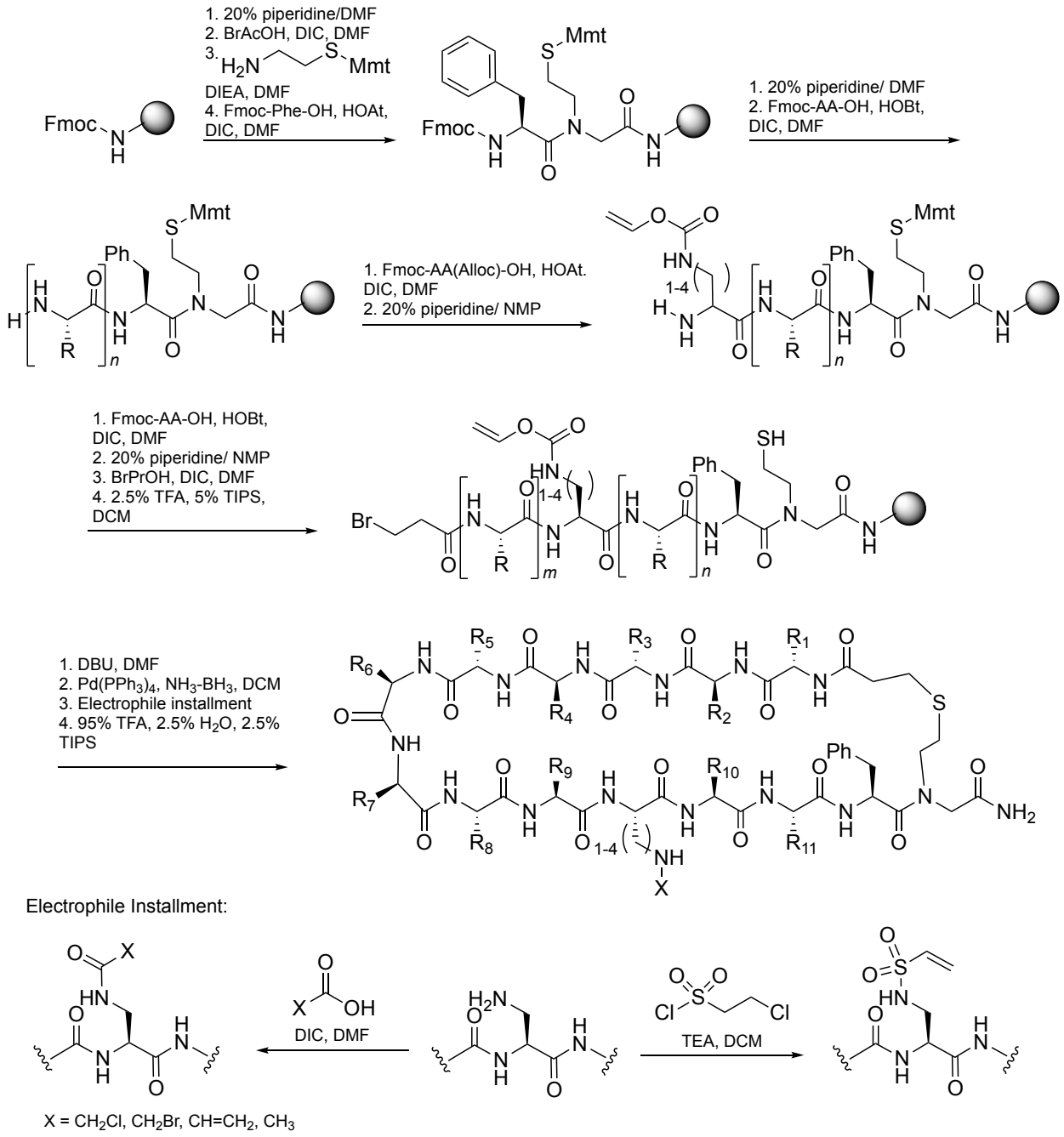


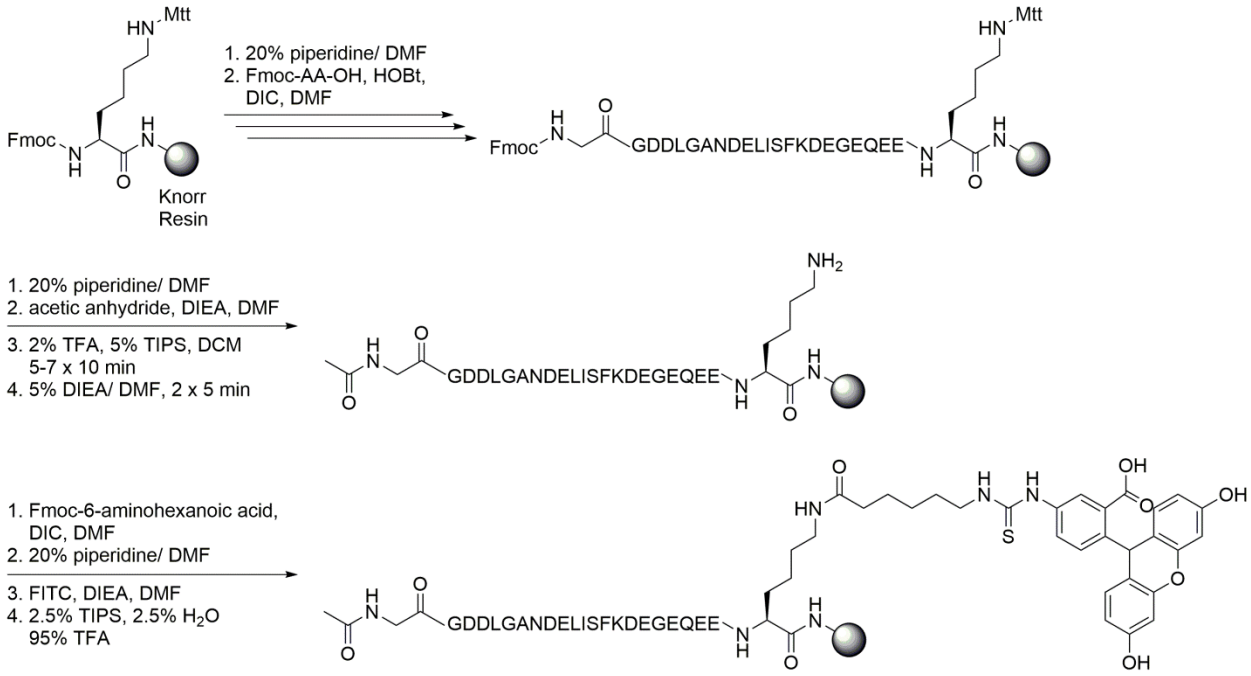
Figure S1. Hydrogen bond surrogate (HBS) approach to stabilize β-hairpin structure. The HBS approach replaces an intrachain hydrogen bond with an isosteric covalent linkage (highlighted in yellow).



Scheme S1. General synthetic scheme of HBS and unconstrained β-hairpin peptidomimetics.



Scheme S2. General synthetic scheme of covalent HBS β-hairpin peptidomimetics.



Scheme S3. Synthetic scheme of fluorescent Tcf4 probe, Tcf4^{FL}.

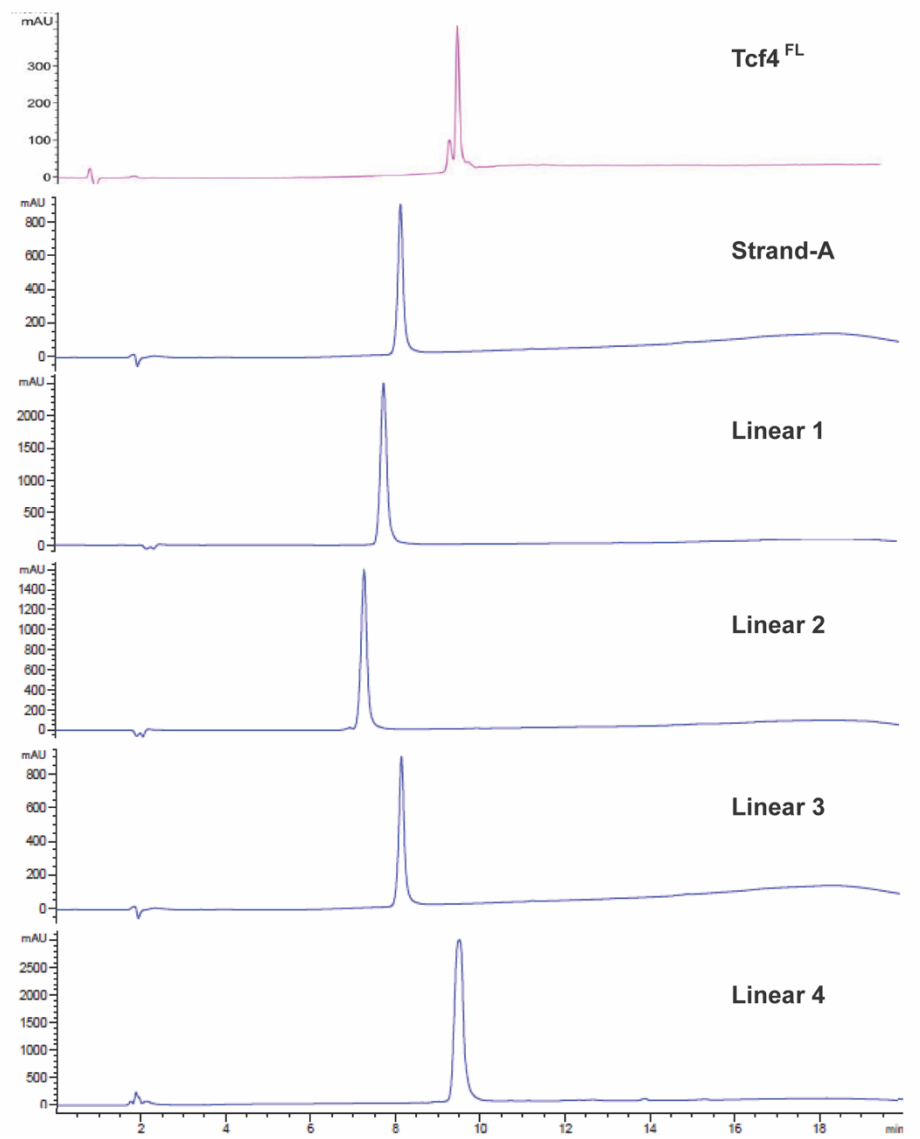
Peptide Characterization

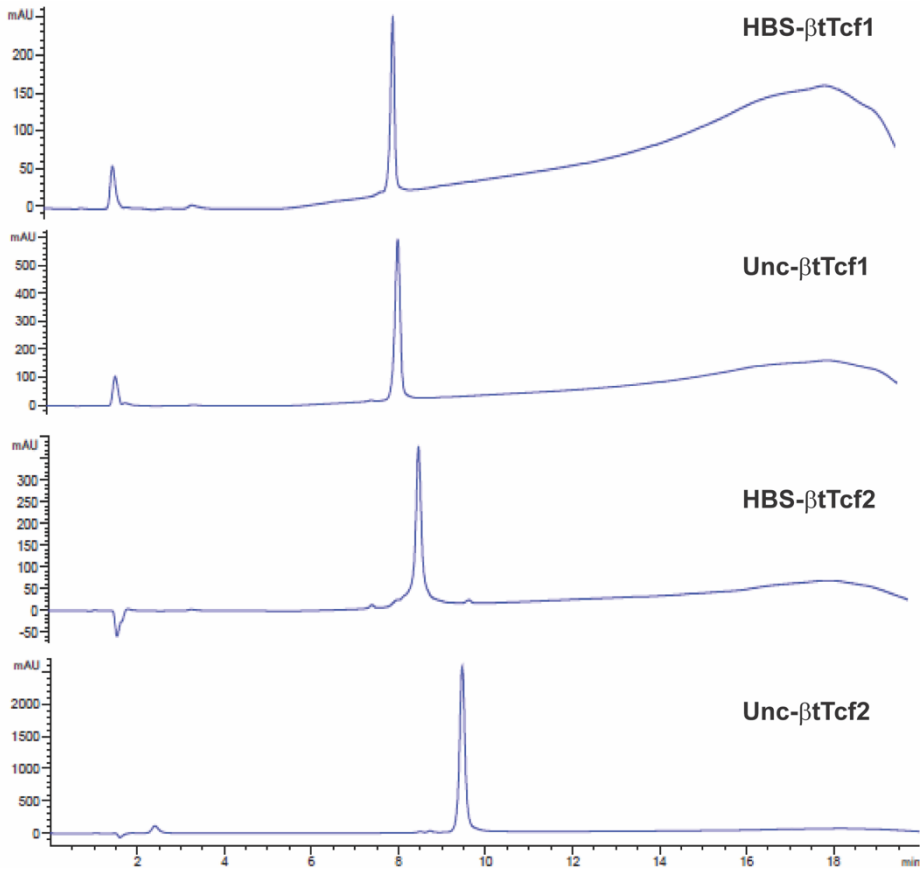
Table S1. Mass spectroscopic characterization of HBS and control peptides.

Compound	Sequence	L18 Modification	Exact Mass Calculated	Observed $[M+H]^+$
Tcf4^{FL}	Ac-GGDDLGANDELISF KDEGEQEEK(6AH-FITC)-NH_2	N/A	3039.3	3040.5
Strand-A	Ac-ANDELITF-NH_2	N/A	962.5	963.6
Linear 1	Ac-DLGANDEDap(Ac)ISF-NH_2	Dap + acetyl	1248.6	1249.7
Linear 2	Ac-DLGANDEDap(Ac)IS-NH_2	Dap + acetyl	1014.5	1015.7
Linear 3	Ac-ANDEDap(Ac)ISF-NH_2	Dap + acetyl	963.4	964.2
Linear 4	Ac-DLGANDDap(Ac)LISF-NH_2	N/A	1266.6	1267.7
HBS-βtTcf1	XTQTRApGDELITFG*	N/A	1617.8	1618.6
Unc-βtTcf1	Ac-TQTRApGDELITFG^O	N/A	1619.8	1620.9
HBS-βtTcf2	XTWTRAGNDELITFG*	N/A	1692.8	1693.9
Unc-βtTcf2	Ac-TWTRAGNDELITFG^O	N/A	1694.8	1695.7
βtCov1	XTQTRApGDEDap(ClAc)ITFG*	Dap + chloroacetyl	1666.7	1669.5
βtCov2	XTWTRAGNDEDap(ClAc)ITFG*	Dap + chloroacetyl	1741.7	1744.7
βtCov3	XTQTRAGNDEDap(ClAc)ITFG*	Dap + chloroacetyl	1683.7	1685.2
βtCov4	XTWTRApGDEDap(ClAc)ITFG*	Dap + chloroacetyl	1724.7	1726.2
βtCon1	XTQTRApGDEDap(Ac)ITFG*	Dap + acetyl	1632.8	1634.2
βtCon2	XTWTRAGNDEDap(Ac)ITFG*	Dap + acetyl	1707.8	1709.2
βtCon3	XTQTRAGNDEDap(Ac)ITFG*	Dap + acetyl	1649.7	1650.7
βtCon4	XTWTRApGDEDap(Ac)ITFG*	Dap + acetyl	1690.8	1691.9
βtCov1A	XTQTRApGDEDap(Acryl)ITFG*	Dap + acrylamide	1644.8	1646.1
βtCov1B	XTQTRApGDEDap(BrAc)ITFG*	Dap + bromoacetyl	1710.7	1712.9
βtCov1C	XTQTRApGDEDap(VSA)ISFG*	Dap + vinylsulfonamide	1680.7	1681.8
βtDab1	XTQTRApGDEDab(ClAc)ISFG*	Dab + chloroacetyl	1680.7	1682.0
βtOrn1	XTQTRApGDEOrn(ClAc)ISFG*	Orn + chloroacetyl	1694.7	1696.1
βtLys1	XTQTRApGDEK(ClAc)ISFG*	K + chloroacetyl	1708.8	1710.2

X denotes a residue derived from propionic acid, used to form the HBS macrocyclic linkage; G* denotes N-2-mercaptoethyl-Gly; G^o denotes S-methyl-2-mercaptoethyl-N-Gly; ClAc denotes chloroacetyl group; Acryl denotes acryloyl group; BrAc denotes bromoacetyl group; Ac denotes acetyl group.

Analytical Traces

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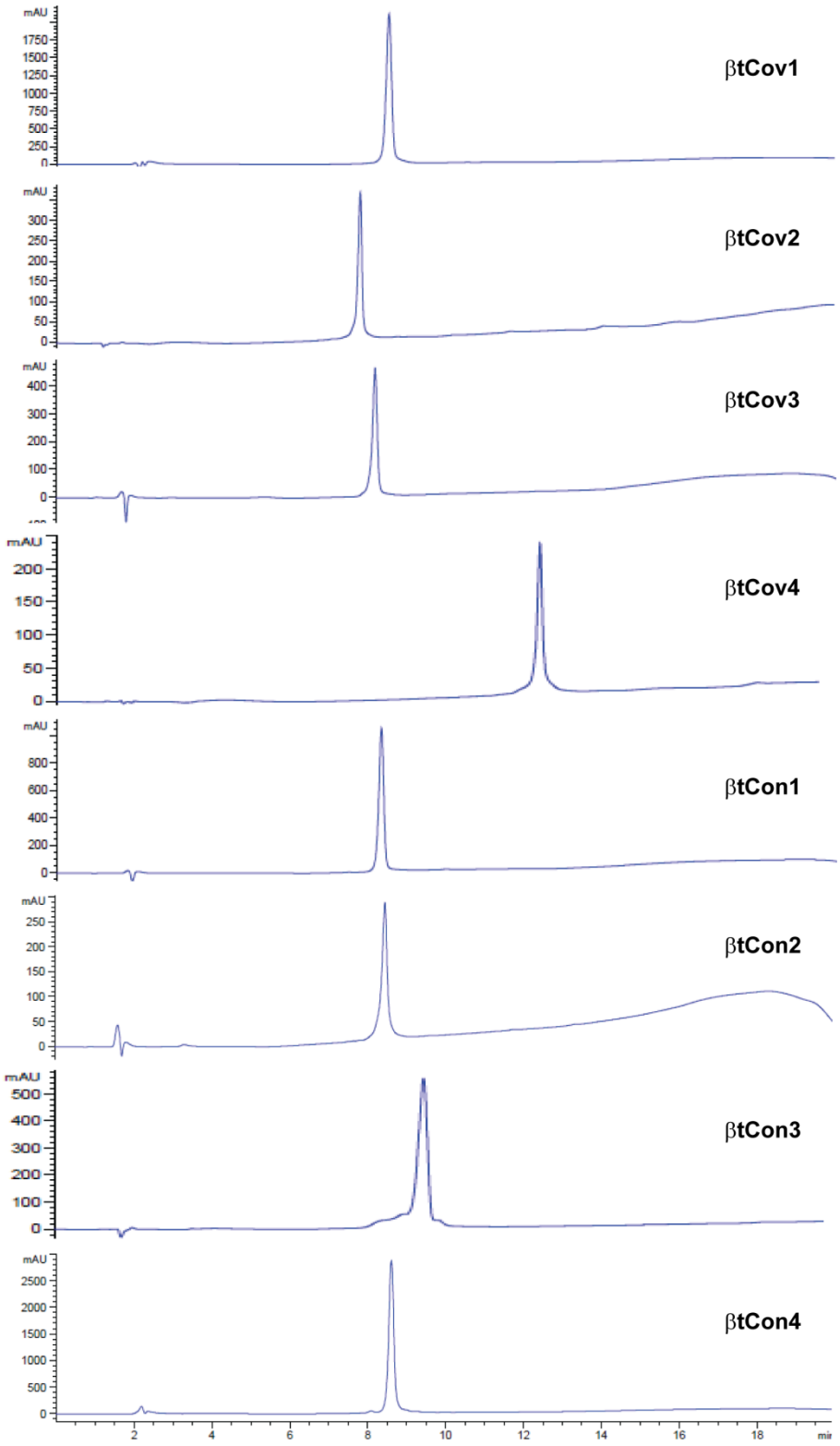
HBS-βtTcf2
Unc-βtTcf2
mAU
min


mAU
βtCov1
βtCov2
βtCov3
βtCov4
βtCon1
βtCon2
βtCon3
βtCon4
2
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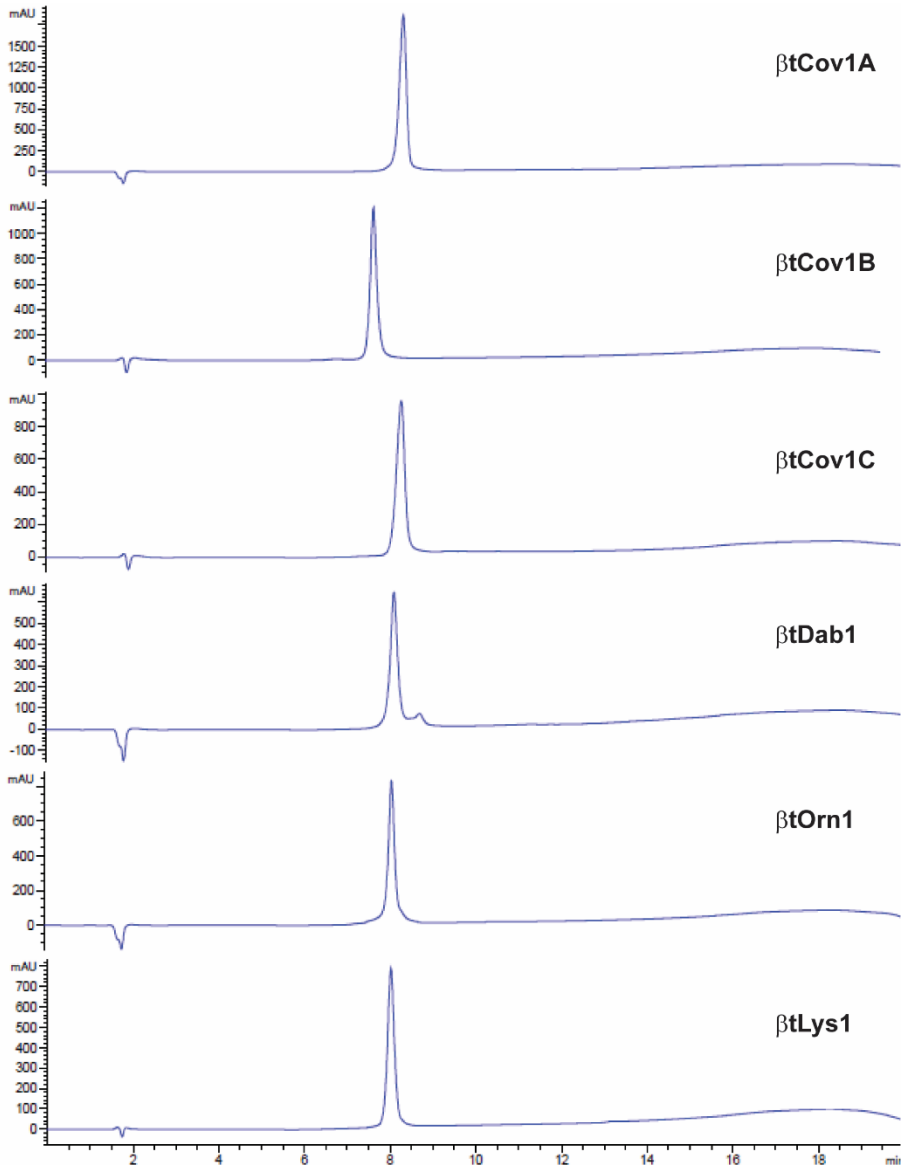

Figure S2. Analytical traces of fluorescent probe, HBS β-hairpins, and related peptides. Traces were collected using a 5-95% acetonitrile: H2O gradient over 12 minutes with UV detection at 220 nm.

Fluorescence Polarization – Direct Binding

$K_d = (R_T \times (1-F_{SB}) + L_{ST} \times F_{SB}^2)/F_{SB}-L_{ST}$ (S1)

R_T = total concentration of β-Catenin

L_{ST} = total concentration of fluorescent peptide

F_{SB} = Fraction of bound fluorescent peptide

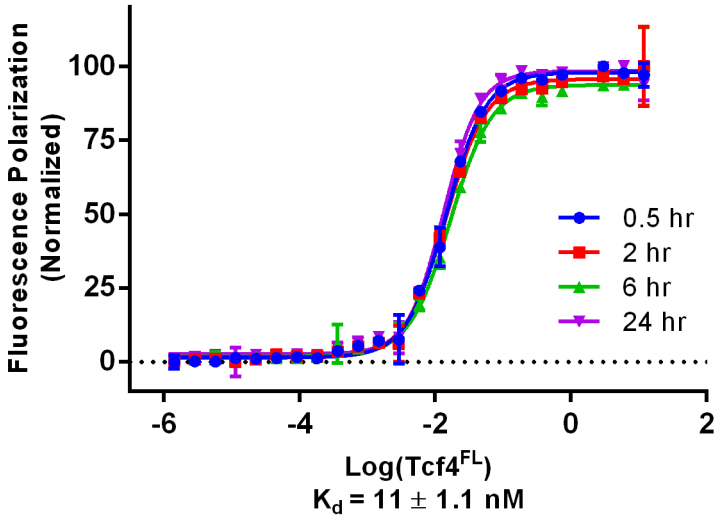

Figure S3. Direct FP binding curves between the armadillo domain β-catenin and $Tcf4^{FL}$ monitored across 24 hours.

Fluorescence Polarization – Competitive Inhibition

$K_i = K_d \times F_{SB} \times ((L_T/(L_{ST} \times F_{SB2} – (K_d + L_{ST} + R_T) \times F_{SB} + R_T)) – 1/(1 – F_{SB}))$ (S2)

K_d = K_d of fluorescent probe

R_T = total concentration of β-catenin protein

L_{ST} = total concentration of fluorescent probe

F_{SB} = fraction of bound inhibitors (at IC_{50})

L_T = total concentration of inhibitor (IC_{50})

Table S2. Inhibitory constants of HBS β-hairpins and related peptides calculated using FP data collected at 6 hours of incubation. Error shown represents 95% confidence interval.

Peptide	Sequence	K_i (μM)
HBS-βtTcf1	XTQTRApGDELITFG*	33 ± 9.2
Unc-βtTcf1	Ac-TQTRApGDELITFG^{O}	265 ± 195
HBS-βtTcf2	XTWTRAGNDELITFG*	18 ± 4.0
Unc-βtTcf2	Ac-TWTRAGNDELITFG^{O}	205 ± 99
βtCov1	XTQTRApGDEDap(ClAc)ITFG*	6.0 ± 1.8
βtCov2	XTWTRAGNDEDap(ClAc)ITFG*	16 ± 3.0
βtCov3	XTQTRAGNDEDap(ClAc)ITFG*	34 ± 15
βtCov4	XTWTRApGDEDap(ClAc)ITFG*	31 ± 8.7
βtCon1	XTQTRApGDEDap(Ac)ITFG*	27 ± 5.1
βtCon2	XTWTRAGNDEDap(Ac)ITFG*	4.7 ± 4.0
βtCon3	XTQTRAGNDEDap(Ac)ITFG*	93 ± 80
βtCon4	XTWTRApGDEDap(Ac)ITFG*	6.3 ± 2.3
βtCov1A	XTQTRApGDEDap(Acryl)ITFG*	48 ± 21
βtCov1B	XTQTRApGDEDap(BrAc)ITFG*	4.0 ± 0.5
βtCov1C	XTQTRApGDEDap(VSA)ISFG*	11 ± 2.6
βtDab1	XTQTRApGDEDab(ClAc)ISFG*	9.1 ± 3.4
βtOrn1	XTQTRApGDEOrn(ClAc)ISFG*	13 ± 3.3
βtLys1	XTQTRApGDEK(ClAc)ISFG*	12 ± 2.6

X denotes a residue derived from propionic acid, used to form the HBS macrocyclic linkage; G* denotes N-2-mercaptoethyl-Gly; G^{o} denotes S-methyl-2-mercaptoethyl-N-Gly; ClAc denotes chloroacetyl group; Acryl denotes acryloyl group; BrAc denotes bromoacetyl group; Ac denotes acetyl group.

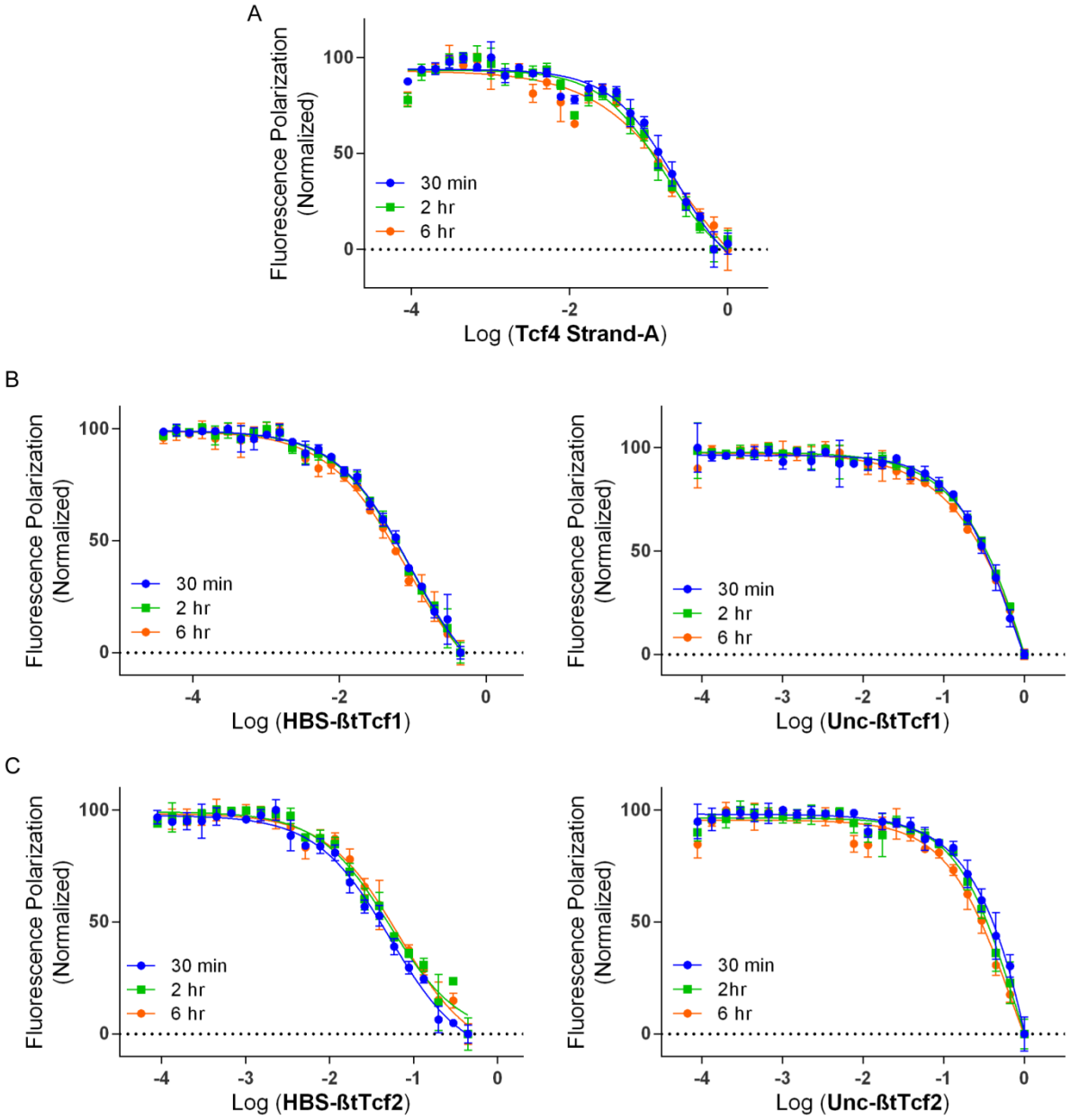

Figure S4. Time-monitored competitive FP binding curves for minimal binding sequence **Strand-A** (A) and non-covalent HBS and unconstrained β-hairpin mimics **HBS/Unc-βtTcf1** (B) and **HBS/Unc-βtTcf2** (C). Data points are fit to a 1:1 binding model (solid curved lines).

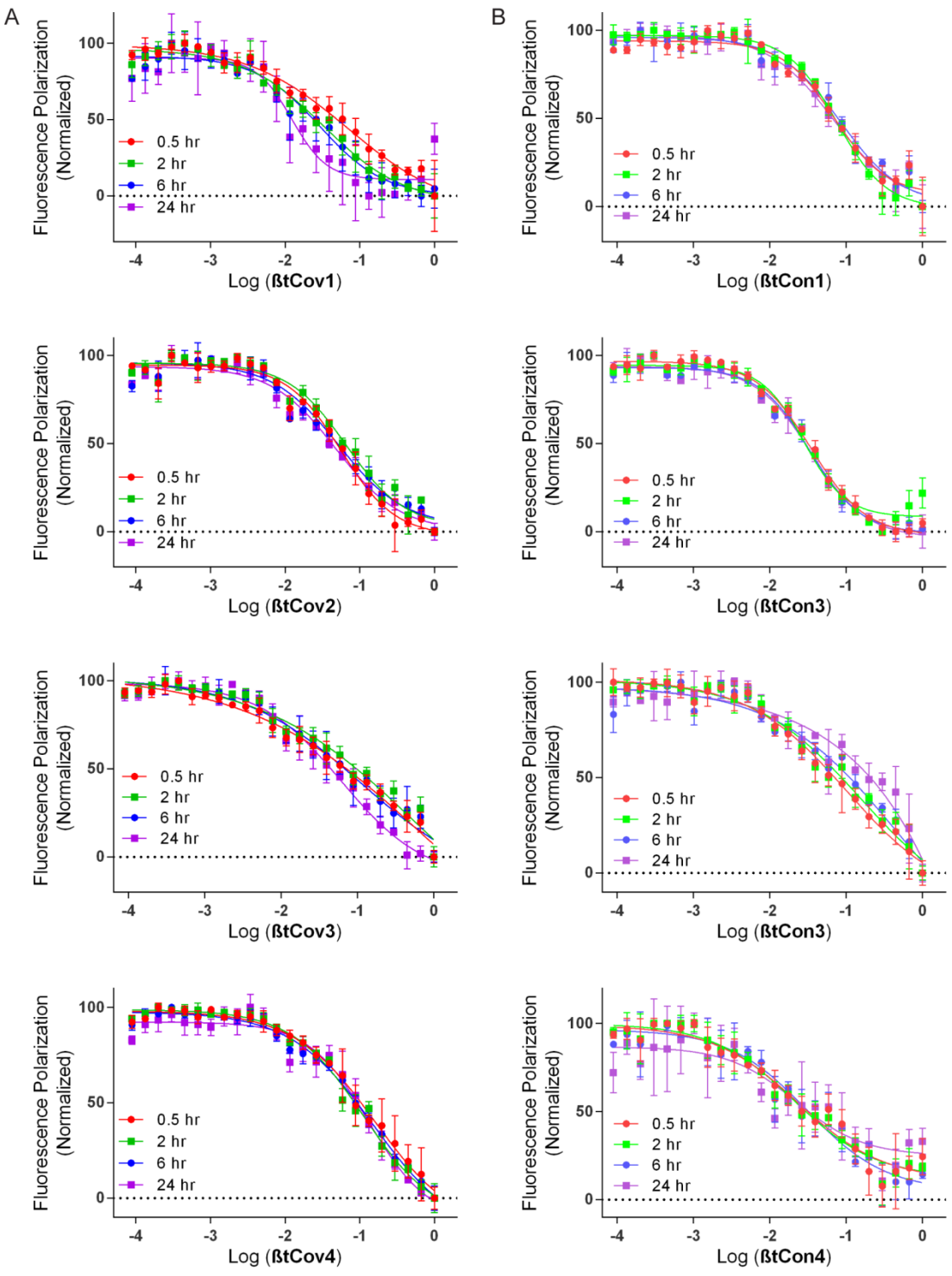

Figure S5. Time-monitored competitive FP binding curves for covalent HBS β-hairpin mimics (A) and non-covalent acetylated control peptides (B). Data points are fit to a 1:1 binding model (solid curved lines).

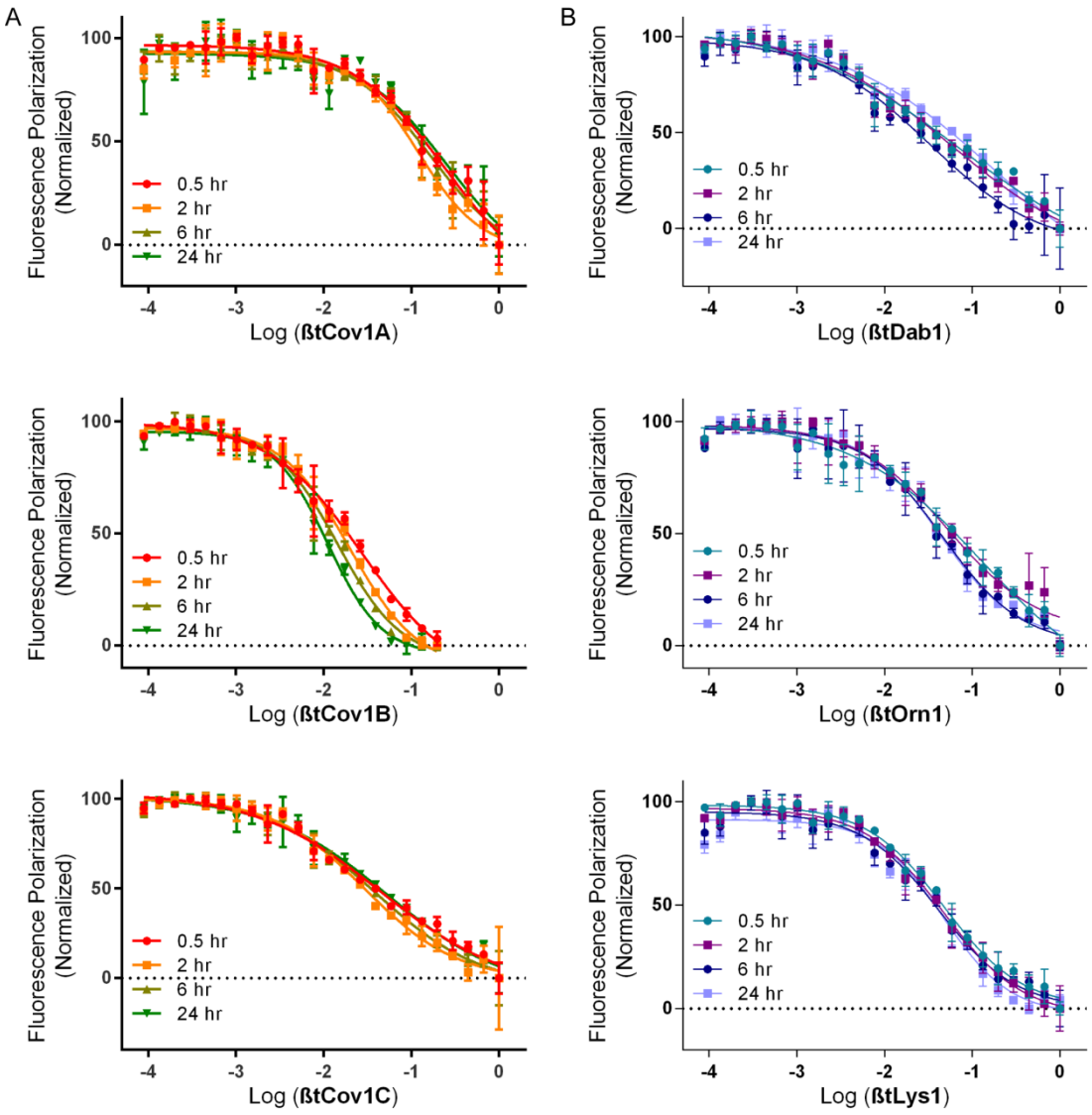

Figure S6. Time-monitored competitive FP binding curves for covalent HBS **βtCov1** electrophile variants (A) and variants with increasing side chain length at the site of chloroacetamide installment (B). Data points are fit to a 1:1 binding model (solid curved lines).

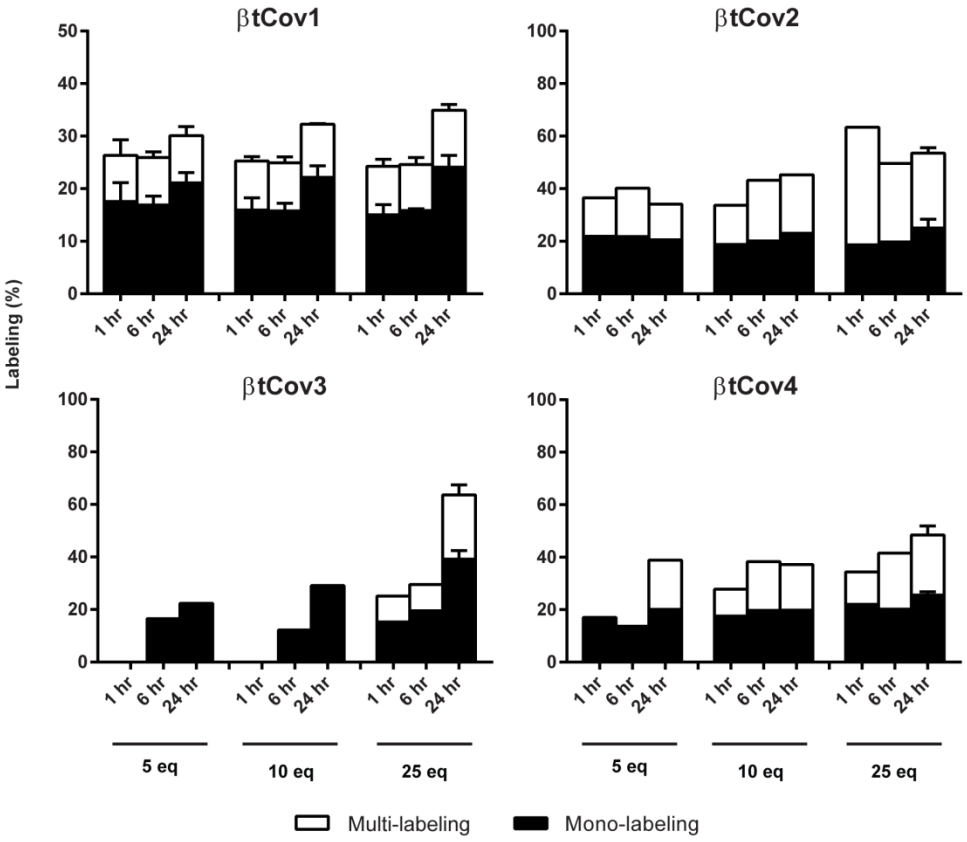

Figure S7. Time and concentration-dependent labeling of β-catenin armadillo domain by **βtCov1-4**.

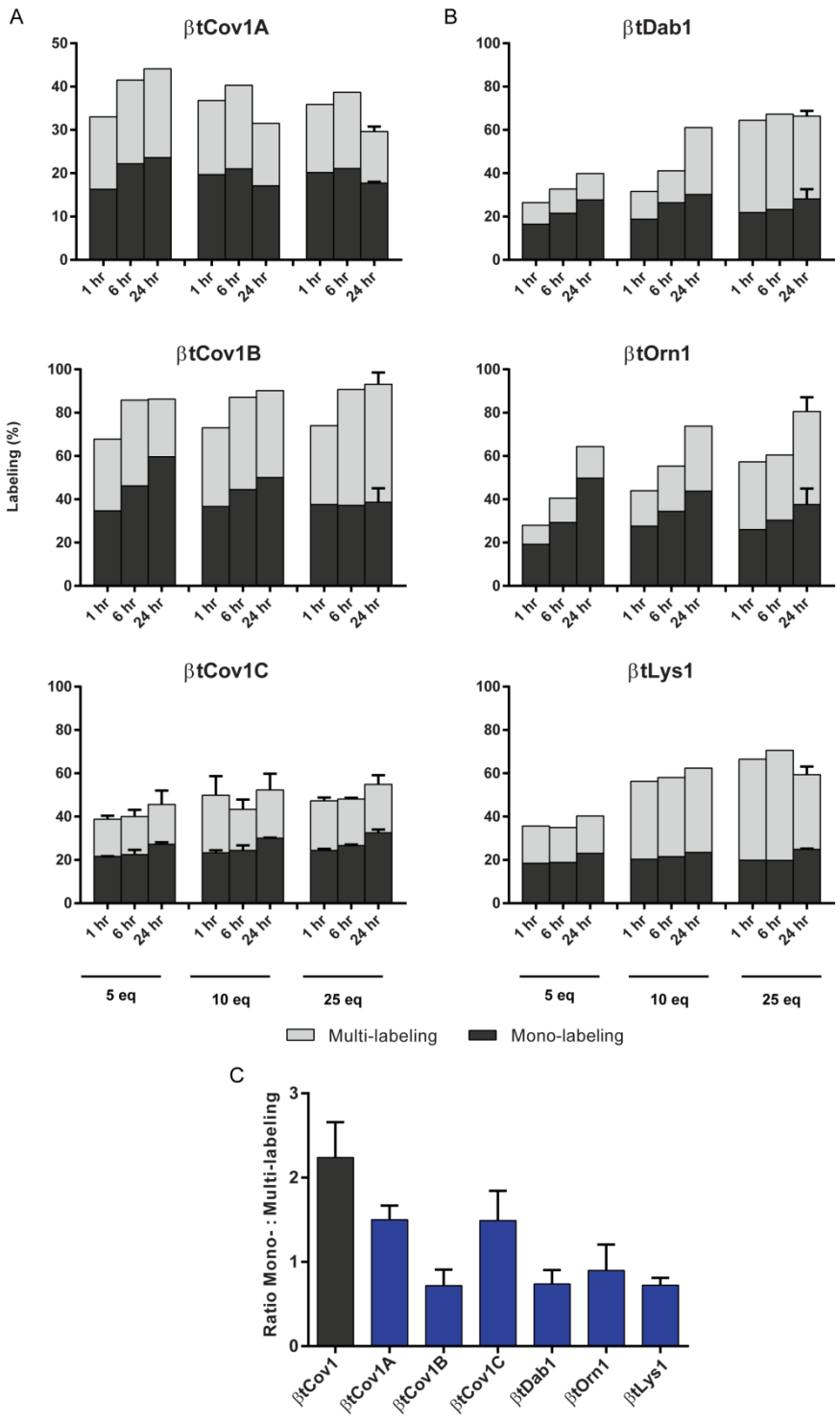

Figure S8. Time and concentration-dependent labeling of β-catenin armadillo domain by **βtCov1** electrophile variants (A) and side chain length variants at the site of chloroacetamide installment (B). Ratio of mono- to multi-labeling of variant mimics relative to **βtCov1** (C).

Design of Strand A

Table S3. Inhibitory constants of **Strand-A** and related peptides. Error shown represents 95% confidence interval.

Peptide	Sequence*	K_i (μM)
Linear 1	Ac-DLGANDEDap(Ac)ISF-NH_2	16 ± 4.0
Linear 2	Ac-DLGANDEDap(Ac)IS-NH_2	> 100
Linear 3	Ac-ANDEDap(Ac)ISF-NH_2	~ 50
Linear 4	Ac-DLGANDDap(Ac)LISF-NH_2	> 100
Strand-A	Ac-ANDELITF-NH_2	57 ± 18

*Ac = acetyl group.

To identify a suitable starting point for the sequence of the binding strand of our HBS β-hairpins we evaluated various linear peptide derivatives of the extended region of Tcf4. The first peptide evaluated, **Linear 1**, incorporated amino acid residues D11 through F21, and demonstrated an inhibitory constant of 16 μM. We truncated the C-terminal F21 amino acid residue to produce **Linear 2** which showed no measurable binding affinity. This observation underscores the importance of the hydrophobic interactions of this region of Tcf4 and corroborates the finding that these residues (especially I19 and F21) contribute significantly to binding.[1-3] Starting from **Linear 1** again, we truncated the N-terminal amino acid residues D11, L12, and G13 to produce **Linear 3** which showed approximately 3-fold weaker binding affinity than **Linear 1** with an inhibitory constant of ~50 μM. Further truncation of the N-terminus of the sequence beyond amino acid residue A14 yielded peptides with no measurable binding affinity. **Linear 3** served as the design start point for **Strand-A**, which demonstrated comparable binding affinity. In **Strand-A** we chose to mutate S20T to promote extended β-strand dihedral angles. We also chose to evaluate the effect of changing the position of the Dap residue, mutating E17 instead of L18 in **Linear 4** in attempt to engage C466 of β-catenin instead of C429. This peptide showed no measurable binding affinity and this observation suggests that peptides with Dap in position L18 interact more favorably with β-catenin, possibly because the salt bridge interaction between E17 and K508 of β-catenin remains intact.[4]

Supplementary References

[1] Fasolini, M., Wu, X., Flocco, M., Trosset, J.-Y., Oppermann, U., and Knapp, S. (2003) Hot Spots in Tcf4 for the Interaction with β-Catenin, *J. Biol. Chem. 278*, 21092-21098.

[2] Poy, F., Lepourcelet, M., Shivdasani, R. A., and Eck, M. J. (2001) Structure of a human Tcf4-beta-catenin complex, *Nat. Struct. Biol. 8*, 1053-1057.

[3] Von Kries, J. P., Winbeck, G., Asbrand, C., Schwarz-Romond, T., Sochnikova, N., Dell'Oro, A., Behrens, J., and Birchmeier, W. (2000) Hot spots in B-catenin for interactions with LEF-1, conductin and APC, *Nat. Struct. Biol. 7*, 800-807.

[4] Gail, R., Frank, R., and Wittinghofer, A. (2005) Systematic peptide array-based delineation of the differential beta-catenin interaction with Tcf4, E-cadherin, and adenomatous polyposis coli, *J. Biol. Chem. 280*, 7107-7117.