Supporting Information

Targeting the Src homology 2 (SH2) domain of signal transducer and activator of transcription 6 (STAT6) with cell-permeable, phosphatase-stable phosphopeptide mimics potently inhibits activation and transcriptional activity.

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Table S1. Characterization of phosphopeptides assayed for binding to STAT6.

#	Phosphopeptide	(M+H) Calcd	(M+H) Found	HPLC RT (min)[a]	Yield (mg)
1	Ac-pTyr-Lys-Pro-Phe-Gln-Asp-Leu-Ile-NH_2	1144.5444	1144.5484	25.4	91
2	Ac-pTyr-Lys-Pro-Phe-Gln-Asp-Leu-NH_2	1031.4603	1031.4668	21.4	48
3	Ac-pTyr-Lys-Pro-Phe-Gln-Asp-NH_2	918.3763	918.3787	16.4	75
4	Ac-pTyr-Lys-Pro-Phe-Gln-NH_2	803.3493	803.3534	16.1	50
5	Ac-pTyr-Lys-Pro-Phe-NH_2	675.2907	675.2950	17.17	63
6	Ac-pTyr-Lys-Pro-NH_2	528.2223	528.2234	9.33	28
7	Ac-pTy-Tle-Pro-Phe-NH_2	660.2798	660.2880	21.73	46
8	Ac-pTy-Nle-Pro-Phe-NH_2	660.2798	660.2863	24.07	31
9	Ac-pTy-Ala-Pro-Phe-NH_2	618.2329	618.2415	18.40	39
10	$PhCH_2CH_2CO$-pTyr-Lys-Pro-Phe-NH_2	765.3377	765.3385	24.16	63
11	$PhCH_2CO$-pTyr-Lys-Pro-Phe-NH_2	751.3220	751.3241	22.52	58
12	PhCO-pTyr-Lys-Pro-Phe-NH_2	737.3064	737.3081	21.66	71
13	PhCH=CHCO-pTyr-Lys-Pro-Phe-NH_2	763.3220	763.3239	24.92	42
14	pCinn-Lys-Pro-Phe-NH_2	616.2536	616.2521	17.9	49
15	p-Indole-Lys-Pro-Phe-NH_2	629.2489	629.2490	18.17	32
16	p-Benzofuran-Lys-Pro-Phe-NH_2	630.2329	630.2324	18.04	71

[a] Peptides were tested for purity by reverse phase HPLC on a Waters Alliance HPLC using a Phenomenex Luna C18 (2) 5μM 2.1 x 250 mm column. A gradient of 0-40% MeCN/30 min at a flow rate of 0.4 mL/min was used with both mobile phases containing 0.1% TFA. Elution was monitored at 230 nm, 254 nm, and 275 nm.

Table S2. Characterization of phosphate analogs of **17**.

	Sequence	Yield mg	% Yield	HRMS (M+H) Calcd	HRMS (M+H) Found	HPLC RT (min)[a]
18a	pCinn-Tle-Pro-N(4-I-Ph)(Ph)	35	47	732.1336	732.1333	22.5
19a	βMpCinn-Tle-Pro-N(4-I-Ph)(Ph)	39	53	746.1492	746.1458	22.8
20	pInd-Tle-Pro-N(4-I-Ph)(Ph)	61	72	745.1288	745.1273	22.13
18b	pCinn-Tle-Pro-NPh_2	23	39	606.2369	606.2350	18.4
18c	pCinn-Tle-Pro-N(Me)(Ph)	41	27	544.22	544.20	26.7
18d	pCinn-Tle-Pro-NHPh	34	22	530.21	530.25	30.3
19b	βMpCinn-Tle-Pro-NPh_2	31	51	620.2526	620.2510	19.46
19c	βMpCinn-Tle-Pro-N(Me)(Ph)	29	56	558.2369	558.2373	15.13
19d	βMpCinn-Tle-Pro-NHPh	25	48	544.2213	544.2181	17.2
19e	βMpCinn-Tle-Pro-$NHCH_2Ph$	27	51	558.2369	558.2336	16.01
19f	βMpCinn-Tle-Pro-$NHCH_2CH_2Ph$	31	53	572.2526	572.2526	16.57
19g	βMpCinn-Tle-Pro-N(Me)(C_6H_{11})	37	67	564.2839	564.2842	17.5
19h	βMpCinn-Tle-Pro-$N(CH_3)_2$	30	61	496.2213	496.2191	10.7
19i	βMpCinn-Tle-Pro-$N(C_2H_5)_2$	27	52	524.2526	524.2502	12.00
21	βMpCinn-Tle-Sar-N(Me)(Ph)	22	42	532.2213	532.2186	15.92
22	βMpCinn-Tle-Ala-N(Me)(Ph)	31	59	532.2213	532.2184	15.15
23	βMpCinn-Tle-N(Me)Ala-N(Me)(Ph)	30	55	546.2369	546.2189	24.4
24	βMpCinn-Tle-pyrrolidine-2-CH=CHPh	22	40	527.2311	527.2312	20.47
25	βMpCinn-Tle-pyrrolidine-2-$CH=CHCH_2CH_2Ph$	19	35	555.2624	555.2867	24.52
26	βMpCinn-Tle-pyrrolidine-2-$CH_2CH_2CH_2CH_2Ph$	16	30	557.2780	557.2827	25.52
27	βMpCinn-Tle-pyrrolidine-2-$CH_2N(Me)Ph$	44	31	544.25	544.35	28.14

[a] Peptides were tested for purity by reverse phase HPLC on a Waters Alliance HPLC using a Phenomenex Luna C18 (2) 5μM 2.1 x 250 mm column. A gradient of 0-40% MeCN/30 min at a flow rate of 0.4 mL/min was used with both mobile phases containing 0.1% TFA. Elution was monitored at 230 nm, 254 nm, and 275 nm.

Table S3. Yields and characterization of prodrug analogs of **1**.

#	Structure	Yield (mg)	% Yield	(M+H) Calcd	(M+H) Found	HPLC RT (min)[a]
17	F2PmCinn(POM_2)-Tle-Pro-N(4-I-Ph)(Ph)	64	66	994.2716	994.2753	35.03
28	F2PmCinn(POM_2)-Tle-Pro-NPh_2	63	58	868.3750	868.3753	35.6
29	F2PmCinn(POM_2)-Tle-Pro-N(Me)(Ph)	56	53	806.3593	806.3578	31.2
30	F2PmCinn(POM_2)-Tle-Pro-NHPh	49	38	792.3437	792.3463	33.2
31	βMF2PmCinn(POM_2)-Tle-Pro-N(Me)Ph	56	53	820.3750	820.3746	34.73
32	βMF2PmCinn(POM_2)-Tle-Pro-N(Me)(C_6H_5)	48	45	826.4219	826.3944	33.77

[a] Prodrugs were tested for purity by reverse phase HPLC on a Waters Alliance HPLC using a Phenomenex Luna C18 (2) 5μM 2.1 x 250 mm column. A gradient of 10-80% MeCN/30 min at a flow rate of 0.4 mL/min was used. Elution was monitored at 230 nm, 254 nm, and 275 nm.

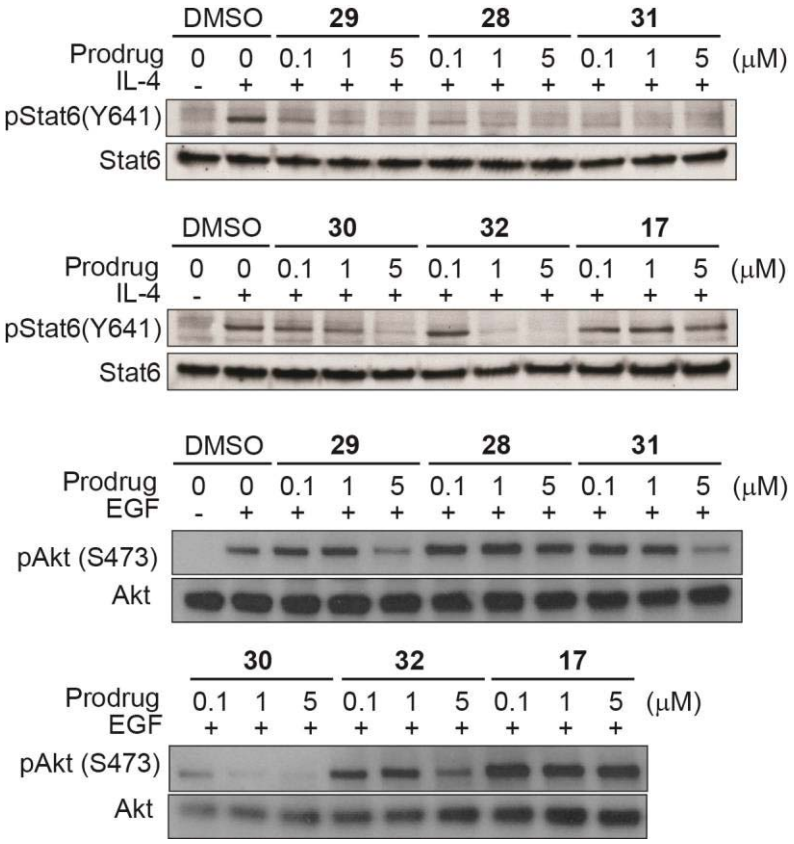

Figure S1. Inhibition of STAT6 and Akt phosphorylation in MDA-MB-468 breast cancer cells. Cells were serum starved overnight and were treated with prodrugs for 2 h. IL-4 or EGF were added and total and phosphoprotein levels were determined by western blot after 1 hr.

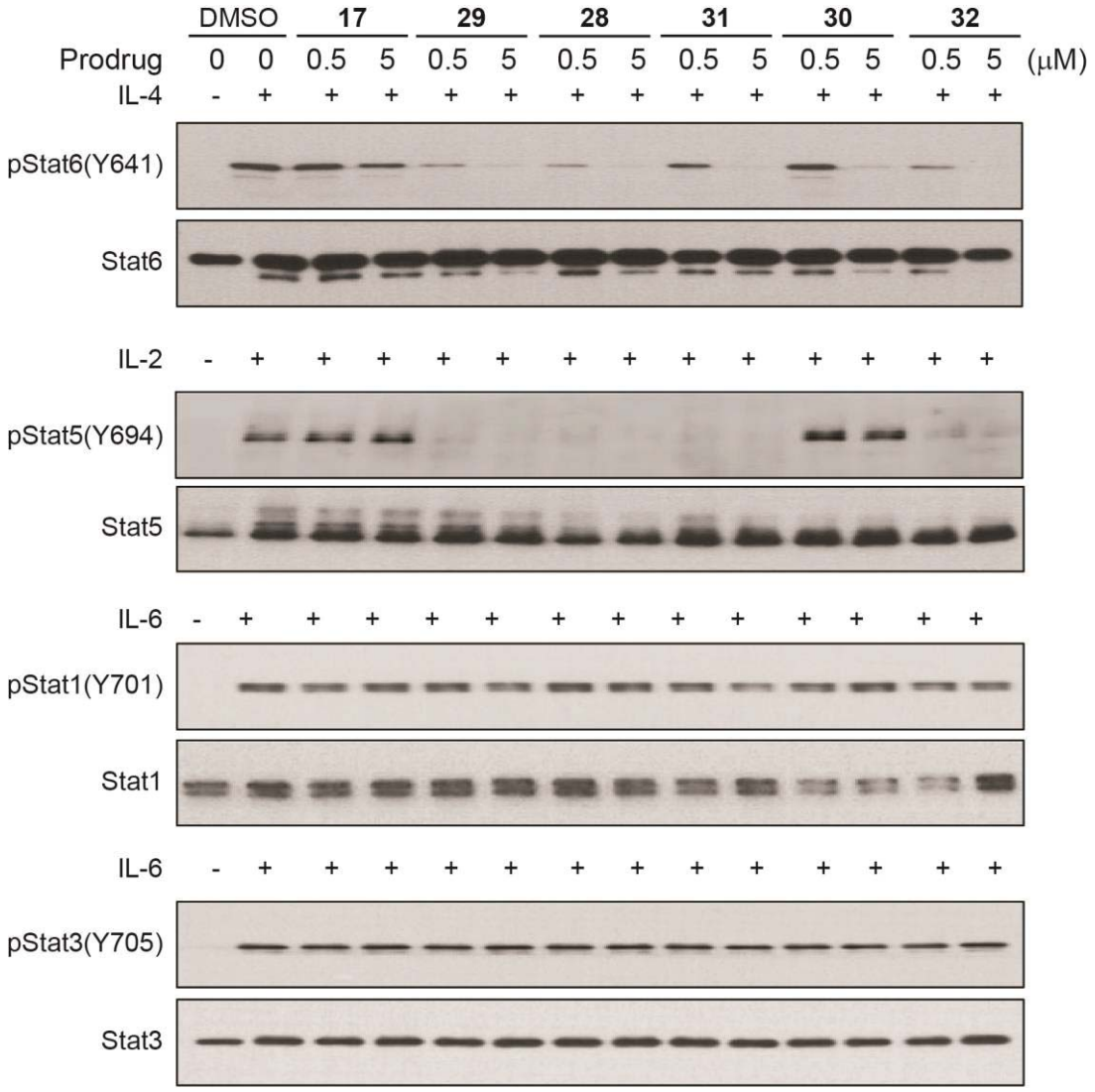

Figure S2. Inhibition of STAT proteins in $CD4^+$ T lymphocytes from C57BL/6J mice. $CD4^+$ T cells isolated from C57BL/6J mice were pre-treated with STAT6 inhibitors for 2 hours and activated with plate-bound anti-CD3/anti-CD28 in the presence or absence of indicated cytokines for 15 minutes. The whole cell lysate was subjected to western blot assay to detect the levels of phosphorylated and total STAT6, STAT5, STAT1 and STAT3.

NMR of Compound **19c**

^{1}H NMR ($CDCl_3$, 500 MHz)

Trans :*cis* based on integration of the Tle CHα, Pro CHα, and Cinn CHα resonances

Trans isomer, 83%

	αNH	αCH	βCH2	γCH2	δCH2	Arom	NCH3	NPh
Cinn		6.50	2.42 (CH3)			7.17, 7.49		
Tle	7.97	4.59		1.01				
Pro		4.19	1.97, 1.76	1.96, 1.73	3.76, 3.63			
NMePh							3.15	7.3-7.5

NOE

Cis isomer, 17%

	αNH	αCH	βCH2	γCH2	δCH2	Arom	NCH3	NPh
Cinn		6.58	under DMSO			7.19, 7.55		
Tle	7.91	4.38		0.72				
Pro		4.27	2.10, 1.97	1.90, 1.74	3.51, 3.34			
NMePh							3.17	7.3-7.5

NOE TleCHα-ProCHα, minor isomer, *cis* Tle-Pro

^{13}C NMR (DMSO-d_6, 150 MHz):

δ 16.4, 24.7, 26.0, 26.5, 29.0, 34.9, 35.7, 37.1, 47.9, 55.1, 56.8, 119.7, 119.8, 119.9, 127.1, 127.6,

NMR Characterization of **31**

^{1}H NMR ($CDCl_3$, 600 MHz)

Trans :*cis* ratio based on integration of the Tle NH, Tle CHα, Cinn CHα and NCH3 resonances

Trans isomer,90%

	αNH	αCH	βCH2	γCH2	δCH2	Arom	NCH3	NPh	CH2	tBu
Cinn		6.02	2.50 (CH3)			7.58, 7.47				
Tle	6.39	4.78		1.12						
Pro		4.38	1.85	2.04, 1.75	3.87, 3.73					
NMePh							3.27	7.3-7.6		
POM										1.22
NOE										

Cis isomer, 10%

	αNH	αCH	βCH2	γCH2	δCH2	Arom	NCH3	NPh
Cinn		6.11	2.52 (CH3)			7.53, 7.58		
Tle	6.30	4.43		1.12				
Pro		4.36	2.06	2.09, 1.79	3.80, 3.50			
NMePh							3.31	7.3-7.6
POM								1.22

^{13}C NMR ($CDCl_3$, 150 MHz) δ:

17.6, 25.2, 26.6, 26.8, 29.6, 36.2, 37.7, 38.7, 48.8, 56.7, 57.7, 82.4, 121.2, 126.4, 126.5, 127.7, 127.9, 128.0, 129.8, 143.4, 145.5, 149.9, 165.9, 169.8, 171.7, 176.6

Resonances from the *cis* isomer were not distinguishable from those of the *trans* .