Supporting Information

Photocontrol of STAT6 dimerization and translocation

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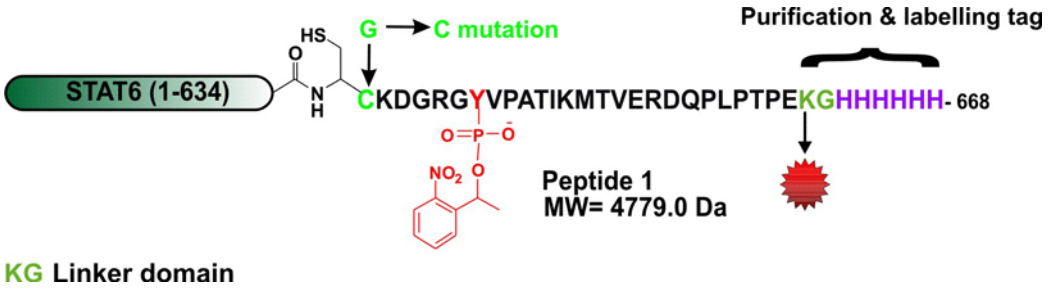

Figure S1: Cy5 labeled caged phosphorylated STAT6 prepared by expressed protein ligation of recombinantly produced STAT6(1-634) and synthetic peptides comprising amino acids 635-668.

Synthesis of caged phosphotyrosine

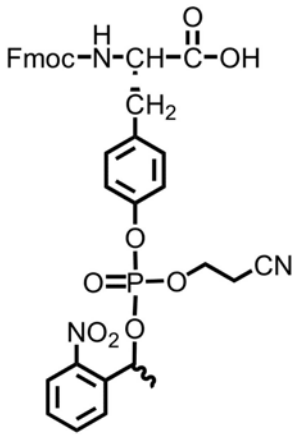

Figure S2: N-α- Fmoc-phospho(1-nitrophenylethyl-2-cyanoethyl)-L-tyrosine

Synthesis of caged phosphotyrosine was carried out as previously described by Rothman *et al.*[1, 2] Analytical data of the final product:

^{1}H NMR (500MHz, $CDCl_3$, Me_4Si) δ (ppm): 8.4 (br, s, 1 H); 7.8 (d, J_{HH}= 8 Hz, 1 H); 7.6 (m, 3 H); 7.5-7.4 (m, 3 H); 7.3-7.2 (m, 3 H); 7.17-7.19 (m, 2 H); 6.9 (m, 4 H); 6.1 (m, 1 H); 5.4 (m, 1 H); 4.52 (s, 1 H) ; 4.34 (s, 1 H); 4.25 (s, 2 H); 4.0 (s, 2 H); 3.0 (m, 2 H); 2.50 (m, 2 H); 1.64 (dd, J_{HH}= 2 Hz, 6 Hz, 3 H). **^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm):** 177.8, 156.0, 149.1, 146.9, 143.9, 141.5, 136.8, 134.2, 134.1, 131.1, 129.2, 127.9, 125.2, 124.7, 120.2, 120.15, 120.1, 116.2, 74.3, 67.2, 63.0, 54.9, 47.3, 37.2, 24.3, 20.9. **^{31}P NMR (400 MHz, $CDCl_3$) δ (ppm):** -7.74. **ESI-MS:** $[M+Na]^+_{obs}$: 708.13, $[M+H]^+_{cal}$: 685.13. **Yield:** 329 mg (50%)

Synthesis of peptides (1-7)

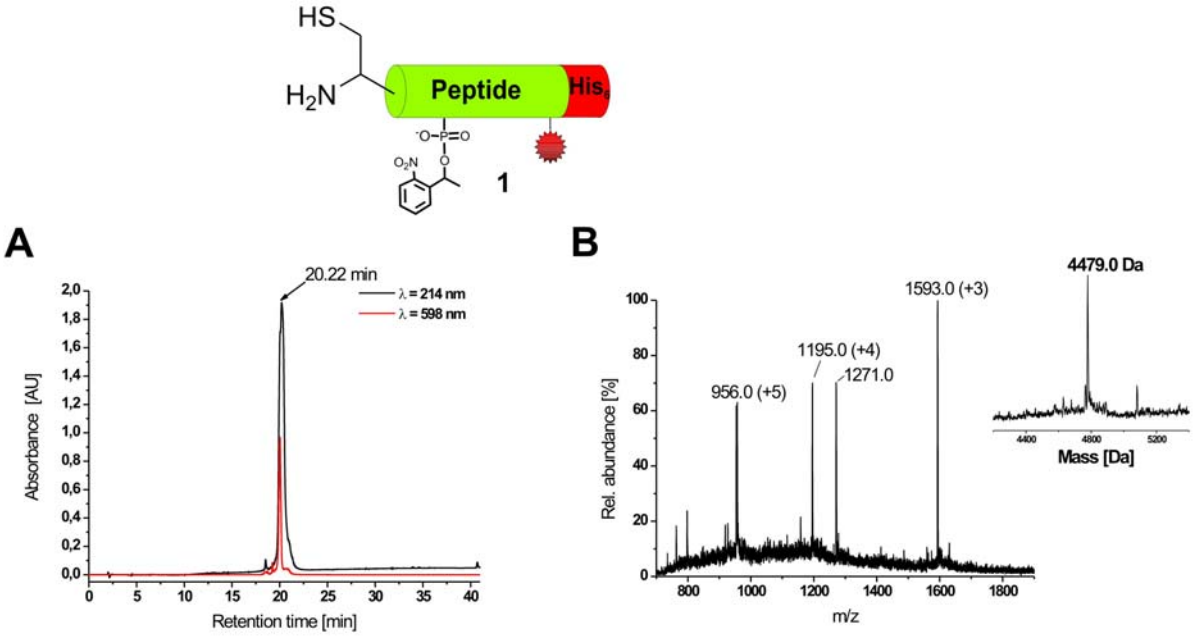

Figure S3: A) Single peak of purified peptide **1** was eluted at a retention time of 20.2 min from analytical C4-RP-HPLC by using a gradient of buffer B from 5% (v/v) to 65% (v/v) in buffer A over 30 min with a flow rate of 1 ml/min. Cy5 signal was detected at λ= 596 nm. **B)** ESI-MS of purified peptide 1. MS signals at 956.0 Da, 1195.0 Da, and 1593.0 Da correspond to the desired mass of this peptide (M_{cal}= 4779.0 Da).

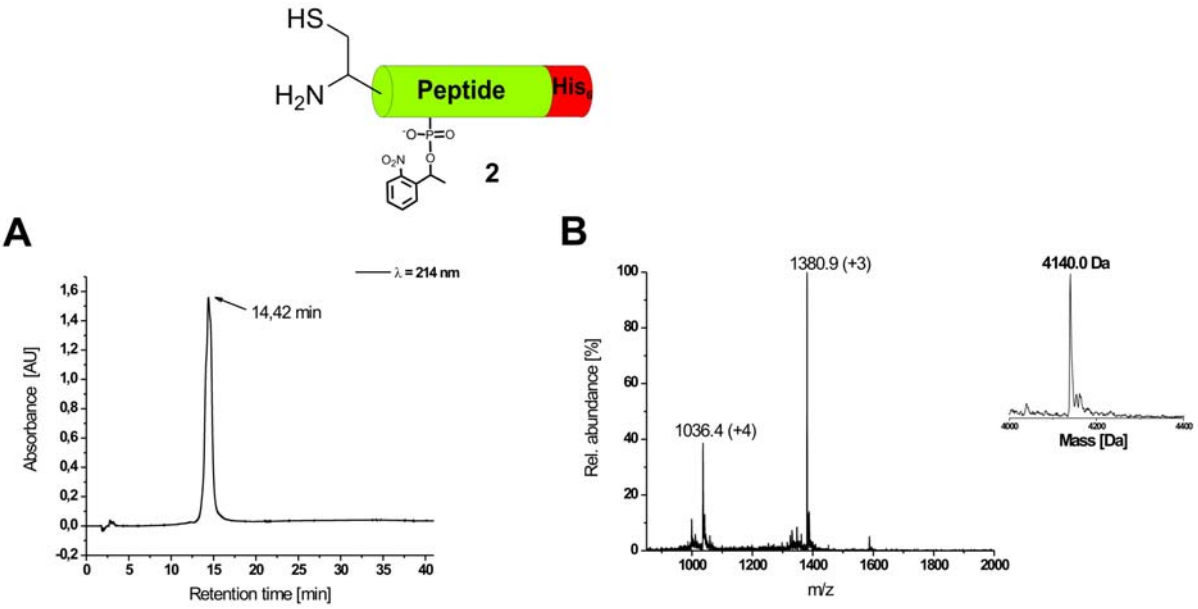

Figure S4: A) Single peak corresponding to purified peptide **2** was eluted at a retention time of 14.42 min from analytical C4-RP-HPLC by using buffer B from 5% (v/v) to 65% (v/v) in buffer A over 40 min with a flow rate of 1 ml/min. **B)** ESI-MS of purified peptide **2**. MS signals at 1036.4 Da, and 1380.9 Da, correspond to the desired mass of this peptide (Mcal= 4140.0 Da).

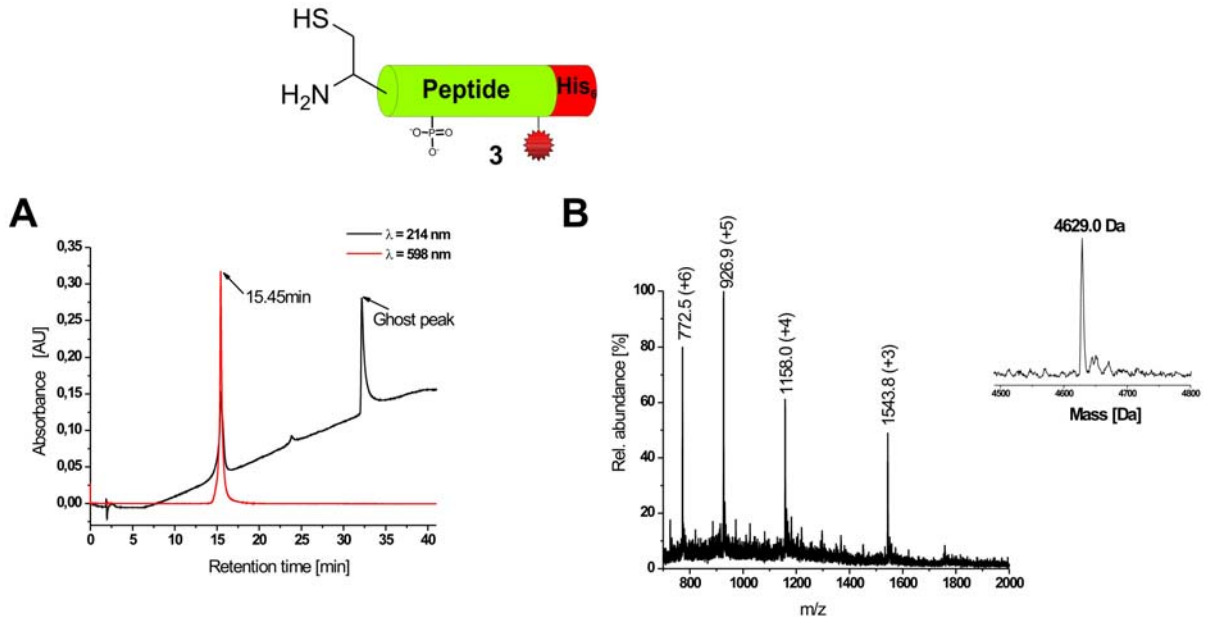

Figure S5: A) Single peak of purified peptide **3** was eluted at a retention time of 15.45 min from analytical C4-RP-HPLC by using buffer B from 5% (v/v) to 65% (v/v) in buffer A over 40 min with a flow rate of 1 ml/min. Cy5 signal was detected at λ= 596 nm. **B)** ESI-MS of purified peptide **3**. MS signals at 772.5.Da, 926.9 Da, 1158.0 Da and 1543.8 Da correspond to the desired mass of this peptide (M_{cal}= 4629.0 Da).

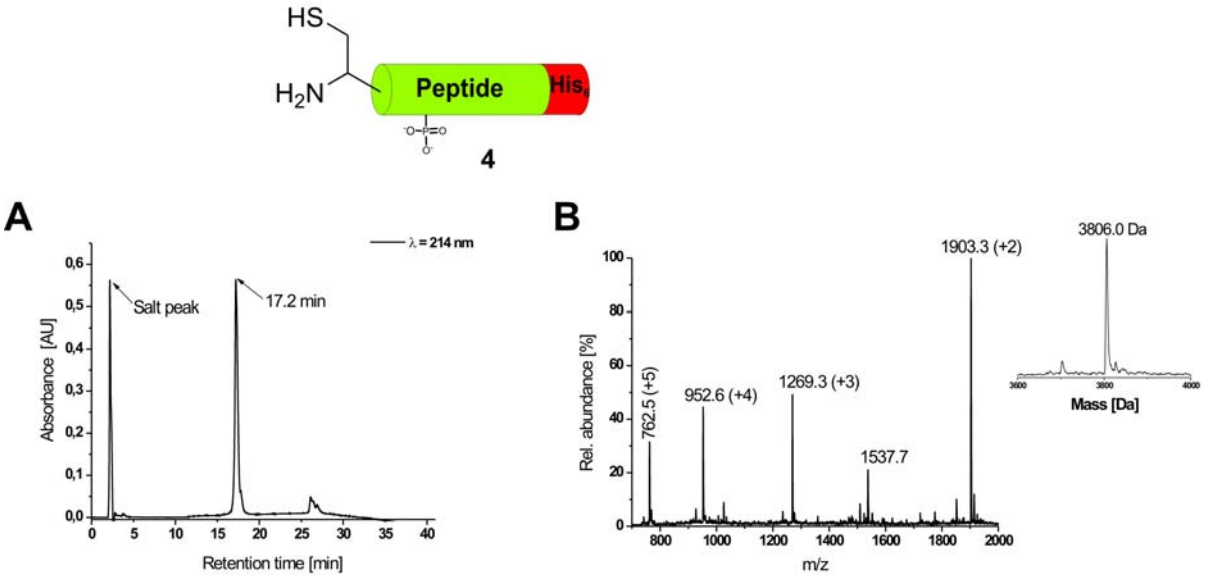

Figure S6: A) Single peak of purified peptide **4** was eluted at a retention time of 17.2 min from analytical C4-RP-HPLC by using buffer B from 5% (v/v) to 65% (v/v) in buffer A over 40 min with a flow

rate of 1 ml/ min. **B)** ESI-MS of purified peptide **4**. MS signals at 762.5.Da, 952.6 Da, 1269.3 Da and 1903.3 Da correspond to the desired mass of this peptide (M_{cal}= 3806.0 Da).

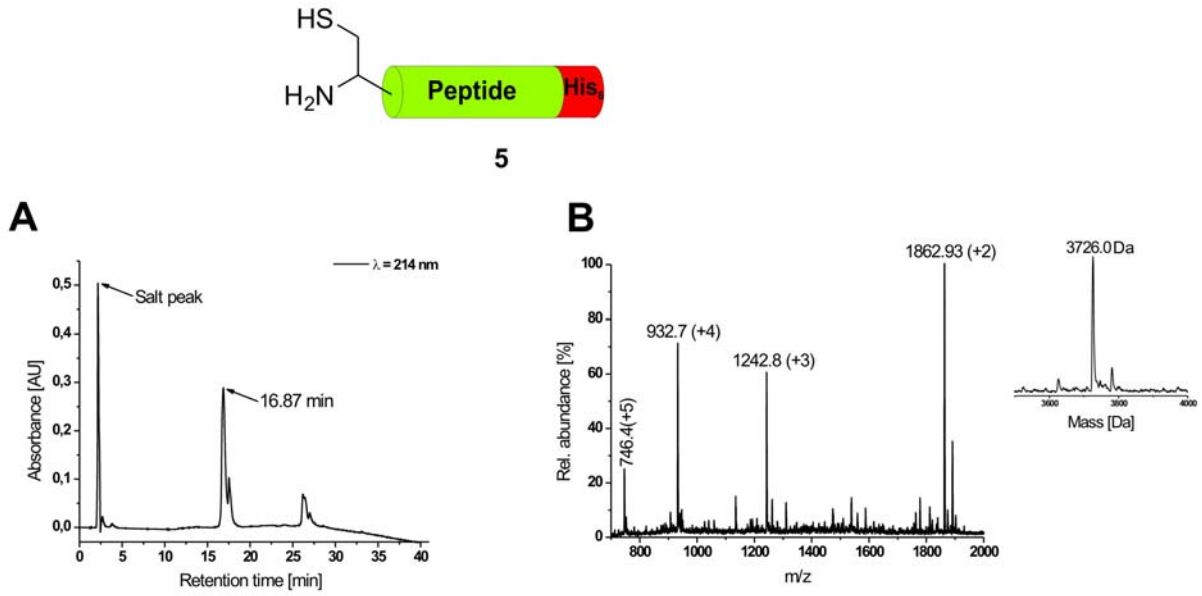

Figure S7: A) Single peak of purified peptide **5** was eluted at a retention time of 16.87 min from analytical C4-RP-HPLC by using buffer B from 5% (v/v) to 65% (v/v) in buffer A over 40 min with a flow rate of 1ml/ min. **B)** ESI-MS of purified peptide **5**. MS signals at 746.4.Da, 932.7 Da, 1242.8 Da and 1862.9 Da correspond to the desired mass of this peptide (M_{cal}= 3726.0 Da).

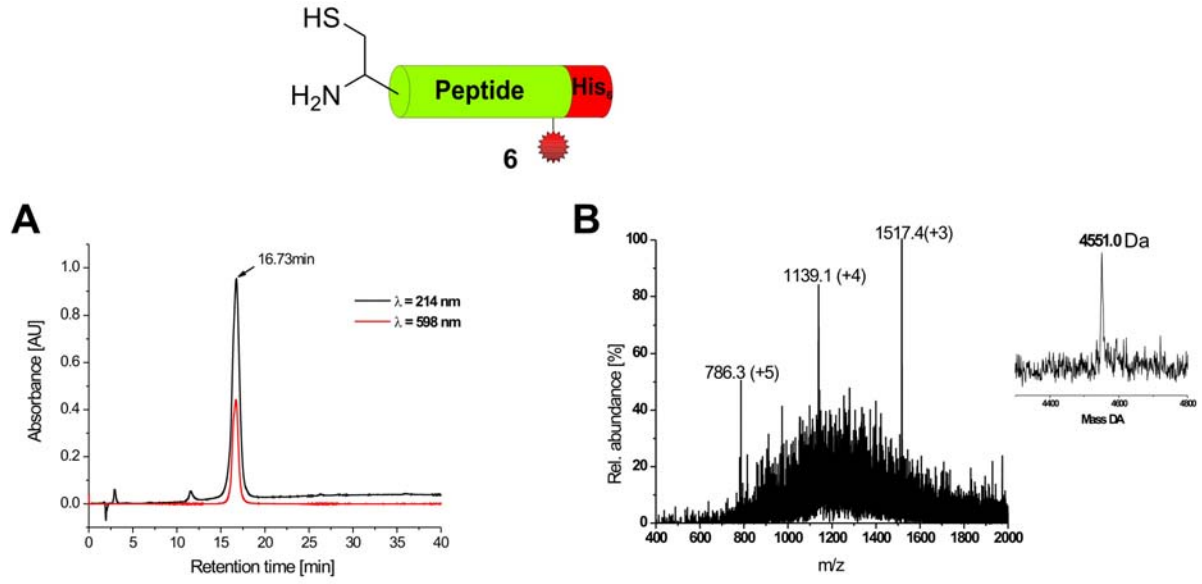

Figure S8: A) Single peak of purified peptide **6** was eluted at a retention time of 17.2 min from analytical C4-RP-HPLC by using buffer B from 5% (v/v) to 65% (v/v) in buffer A over 40 min with a flow rate of 1 ml/min .**B)** ESI-MS of purified peptide **6**. MS signals at 786.3 Da, 1139.1 Da and 1517.4 Da corresponds to the desired mass of this peptide (M_{cal}= 4551.0 Da).

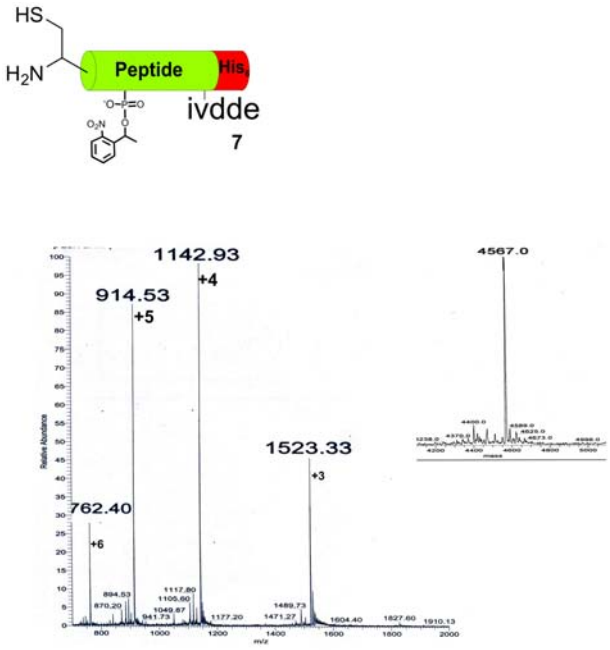

Figure S9: Peptide **7** was the precursor of peptides **1** and **2.** No HPLC purification was necessary here since ESI-MS measurements indicated a purity sufficient for initial testing of NPE removal with UV light. ESI-MS signals 762.4 Da, 914.53 Da, 1142.03 Da and 1523.33 Da which corresponds to the desired mass of this peptide (M_{cal}= 4567.0 Da).

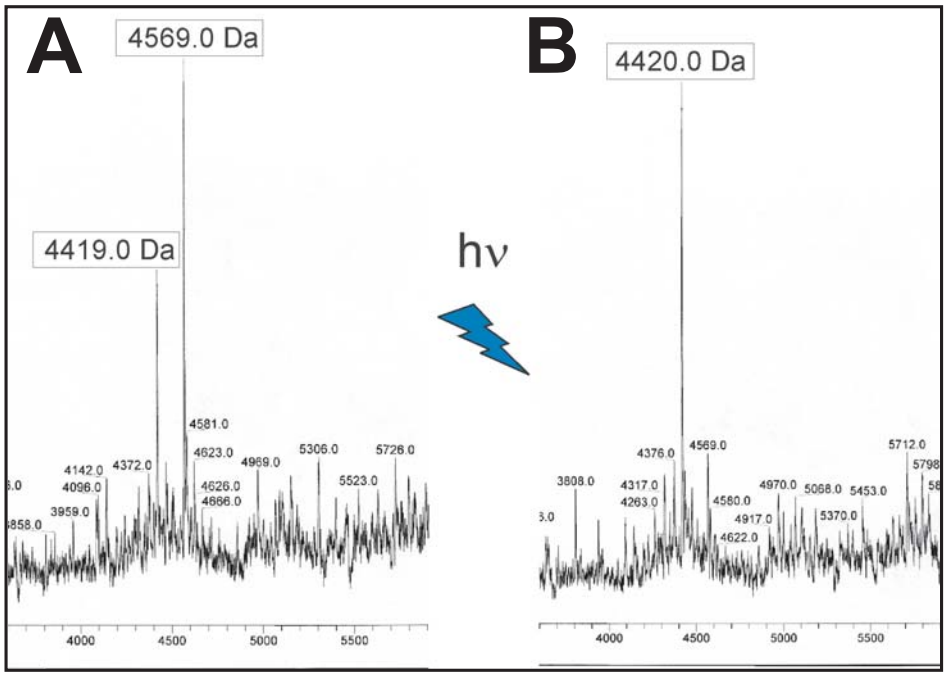

Figure S10: Decaging of peptide **7. A)** Decaging of peptide **7** for 5 min with 365 nm light from a Xenon lamp. The NPE group is only partially released. **B)** Peptide **7** after 20 min of irradiation at 365 nm. The NPE group is quantitatively removed.

Preparation of semisynthetic STAT6 variants

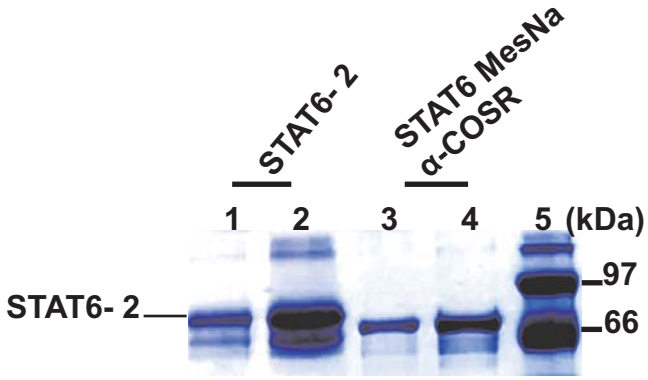

Figure S11: Semisynthesis of STAT6-**2** monitored by SDS-PAGE
Lanes 1&2: Ligation between STAT6(1-634)-thioester and peptide **2**; lanes 3&4: STAT6(1-634)-thioester without peptide **2**; lane 5: MMW.

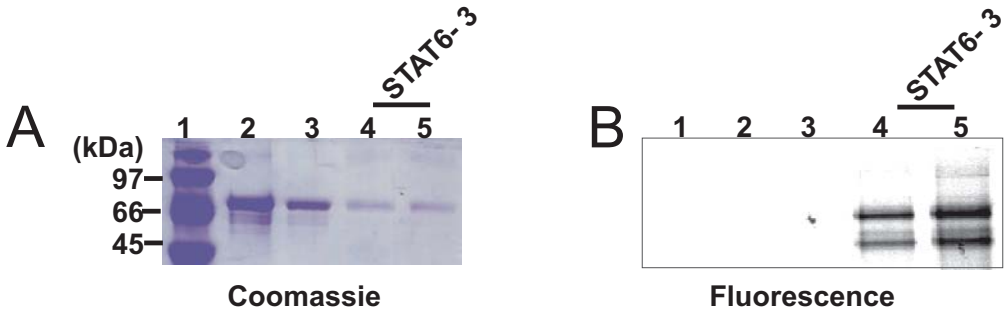

Figure S12: Semisynthesis of STAT6-**3** monitored by SDS-PAGE
A) Lanes 2&3: STAT6(1-634)-thioester; lanes 4&5: Ligation of STAT6(1-634)-thioester with peptide **3.** lane 1: MWM. **B)** Fluorescence scanning of this SDS-gel at ex: 635 nm, em: 670 nm. Successfull ligation between STAT6(1-634)-thioester and peptide-**3** is confirmed by the occurrence of fluorescent bands in lanes 4 and 5.

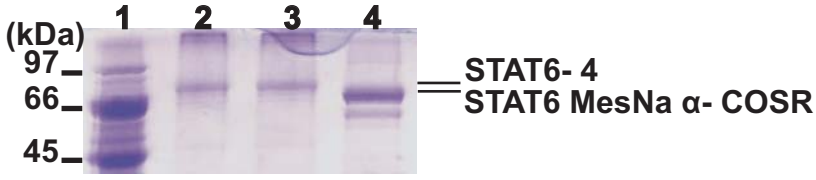

Figure S13: Semisynthesis of STAT6-**4** monitored by SDS-PAGE
Lanes 2&3: Ligation product of STAT6(1-634)-thioester with peptide **4**; lane 2: STAT6(1-634)-thioester; lane 1: MWM.

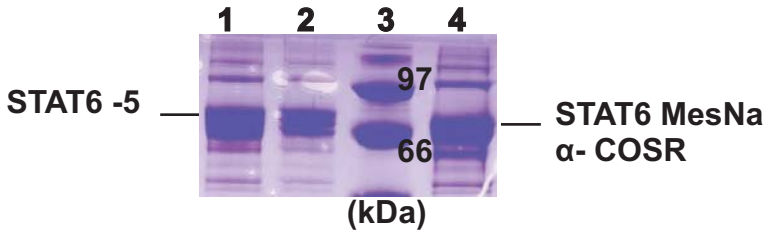

Figure S14: Semisynthesis of STAT6-**5** monitored by SDS-PAGE
Lanes 1&2: Ligation reaction of STAT6(1-634)-thioester with peptide **5**; lane 3: MWM; lane 4: STAT6(1-634)-thioester.

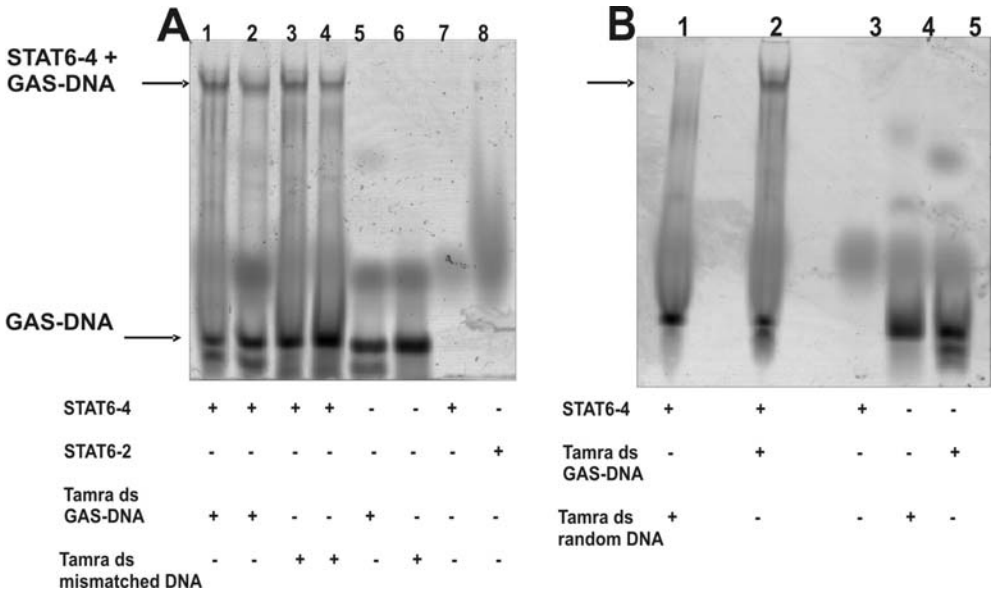

Figure S15: A) Fluorescence scanning at ex: 510 nm, em: 575 nm shows binding of STAT6-**4** with Tamra-dsGAS-DNA (lanes 1 and 2) indicated by an arrow, STAT6-**4** in complex with Tamra-labeled mismatch-dsDNA (lanes 3 and 4), Tamra-labeled dsGAS-DNA (lane 5) indicated by an arrow, Tamra-labeled mismatch-dsDNA (lane 6), STAT6-**4** (lane 7) and STAT6-**2** (lane 8). **B)** Fluorescence scans at ex: 510 nm, em: 575 nm show no binding of STAT6-**4** with Tamra-labeled random dsDNA (lane 1); binding is observed for STAT6-**4** with Tamra-labeled dsGAS-DNA (lane 2), STAT6-**4** (lane 3), Tamra-labeled random dsDNA (lane 4) and Tamra-dsGAS-DNA (lane 5).

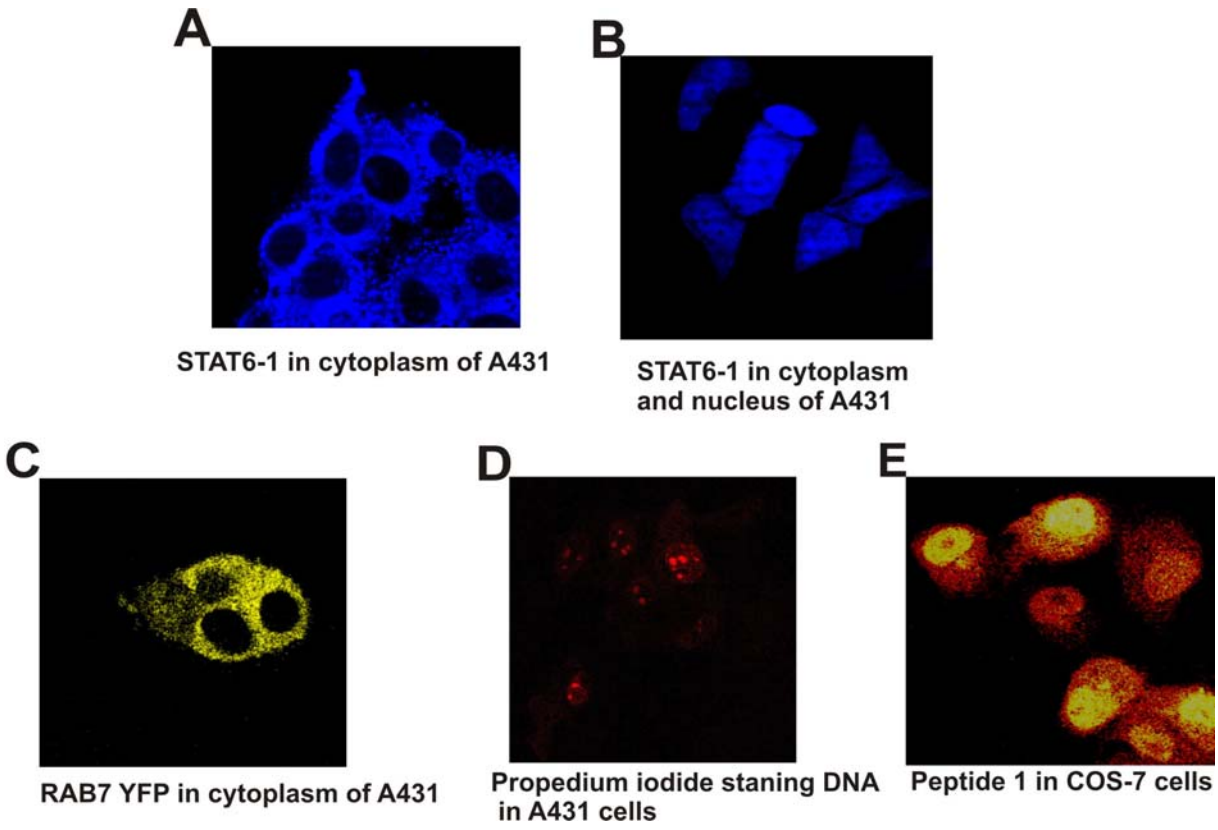

Figure S16: Microinjection of STAT6-**1** into A431 cells

A) STAT6-**1** is microinjected in A431 cells and Cy5 fluorescence indicated its localization in the cytoplasm. **B**) STAT6-**1** is microinjected in A431 cells and Cy5 fluorescence shows the localization in the cytoplasm and nucleus after extended incubation times. **C)** Localization of microinjected Rab∆7-YFP in cytoplasm (measured at YFP absorption ex: 500 nm and em: 535 nm). **D)** Microinjected A431 cells with Propidium iodide. **E)** Peptide **1** microinjected in COS cells and Cy5 absorption at ex: 635 nm and em: 670 nm. Peptide **1** is found in the nucleus as well in the cytoplasm.

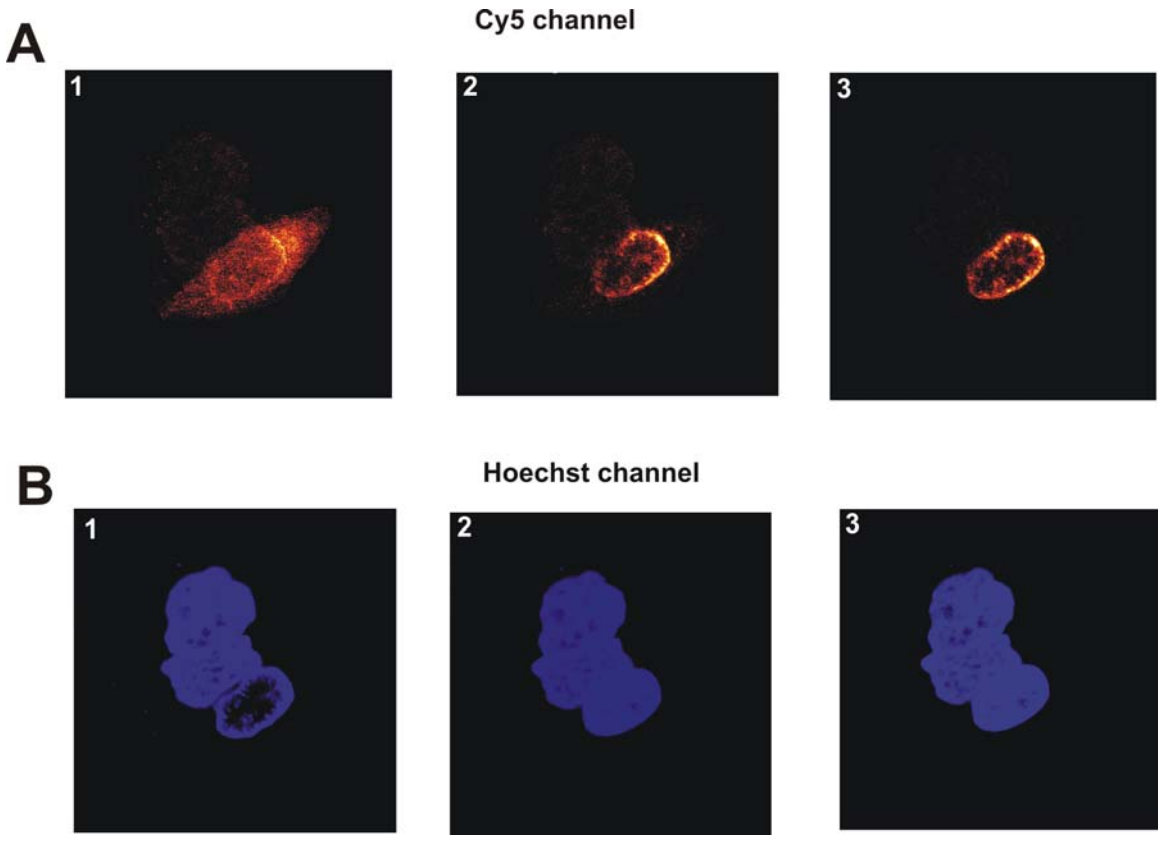

Figure S17: A) Cy5 channel shows microinjected STAT6-**1** in the cytoplasm of MDCK cells prior to UV laser flash (A 1), upon irradiation with UV light of 405 nm from a diode laser for 1 second, STAT6-**1** quickly migrates into the nucleus (A 2-3). **B)** Hoechst 33445 channel showing three nuclei stained with Hoechst 33445.

1. D. M. Rothman, M. E. Vazquez, E. M. Vogel and B. Imperiali, *Organic Letters*, 2002/7/30, **4**, 2865-2868.
2. D. M. Rothman, M. E. Vazquez, E. M. Vogel and B. Imperiali, *Journal of Organic Chemistry*, 2003/8/22, **68**, 6795-6798.