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

Analytical data of the final product:

$^1$H NMR (500MHz, CDCl$_3$, Me$_4$Si) 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.


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 \( \lambda = 596 \) 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 (MCal= 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 \( \lambda = 596 \) 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_{\text{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 1 ml/ 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_{\text{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_{\text{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. Successful 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\(\Delta\)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.