

Supporting Information

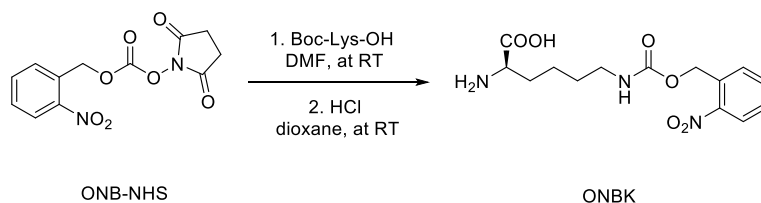
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I. List of Primers.

- P1 5'-GGAGATATACATATGGACTCCATCCAGGCTGAGGAG -3'
- P2 5'-CACGAGGAAGGTCCCTCT-3'
- P3 5'-AGAGGGACCTTCCTCGTGTAGGAAAGTGAGACCACGA-3'
- P4 5'-AGTTATTGCTCAGCGGTGGCAG-3'
- P5 5'-AGAGGGACCTTCCTCGTGAAAGAAAGTGAGACCACGA-3'
- P6 5'-GTGCGAGAAAGTGAGACCGTGAAAGGTGCCTACGCGCTCTCAGTGTCTGACTTC-3'
- P7 5'-GAAGTCAGACACTGAGAGCGCGTAGGCACCTTTCACGGTCTCACTTTCTCGCAC-3'
- P8 5'-CCTCAACGTGAAGCACTACCTGATCCGCAAGCTGGACAGC-3'
- P9 5'-GCTGTCCAGCTTGCGGATCAGGTAGTGCTTACGTTGAGG-3'

II. Synthesis of ONBK



2-Nitrobenzyl-N-succinimidyl carbonate (ONB-NHS) was synthesized by following a reported procedure.¹ To the solution of ONB-NHS (688mg, 2.34 mmol) in dimethylformamide (11.7 mL) was added N α -(*tert*-butoxycarbonyl)-L-lysine (634mg, 2.57 mmol). After the mixture was stirred at room temperature overnight, 40 mL of ethyl acetate was added. The resulting mixture was washed with water and brine (three times) to remove DMF. The organic layer was concentrated under vacuum. Purification by silica-gel flash chromatography (1% acetic acid and 33% to 66% ethyl acetate in hexane as gradient) afforded Boc-protected ONBK as a white solid (1.221 g). The Boc-protected ONBK was then dissolved in 4 mL of 1,4-dioxane and 10 mL of 4 N HCl solution in 1,4-dioxane was added dropwise. The reaction was stirred at room temperature for 3 hours. The white solid precipitation was collected by vacuum filtration and washed with diethyl ether. ONBK (0.772 g) was obtained as a white powder in 99% yield from ONB-NHS. ¹H-NMR (300 MHz, DMSO-d₆): δ ppm: 13.76 (bs, 1 H), 8.35 (bs, 2 H), 8.11 (d, $J = 8.1$ Hz, 1 H), 7.81 (m, 1 H), 7.67-7.58 (m, 2 H), 7.47 (s, 1 H), 5.36 (s, 2 H), 3.85 (m, 1 H), 2.99 (m, 2 H), 1.79-1.77 (m, 2 H), 1.5-1.20 (m, 2 H). ¹³C-NMR (400 MHz, DMSO-d₆): δ ppm: 170.9, 155.5, 147.1, 134.0, 132.9, 128.8, 138.7, 124.7, 66.3, 61.8, 51.7, 29.5, 28.7, 21.5. The NMR spectra can be found in Fig. S4 and Fig. S5. MS (LCQ): calcd for C₁₄H₂₀N₃O₆ ([M+H]⁺), 326.3; Found, 326.3.

III. Additional data

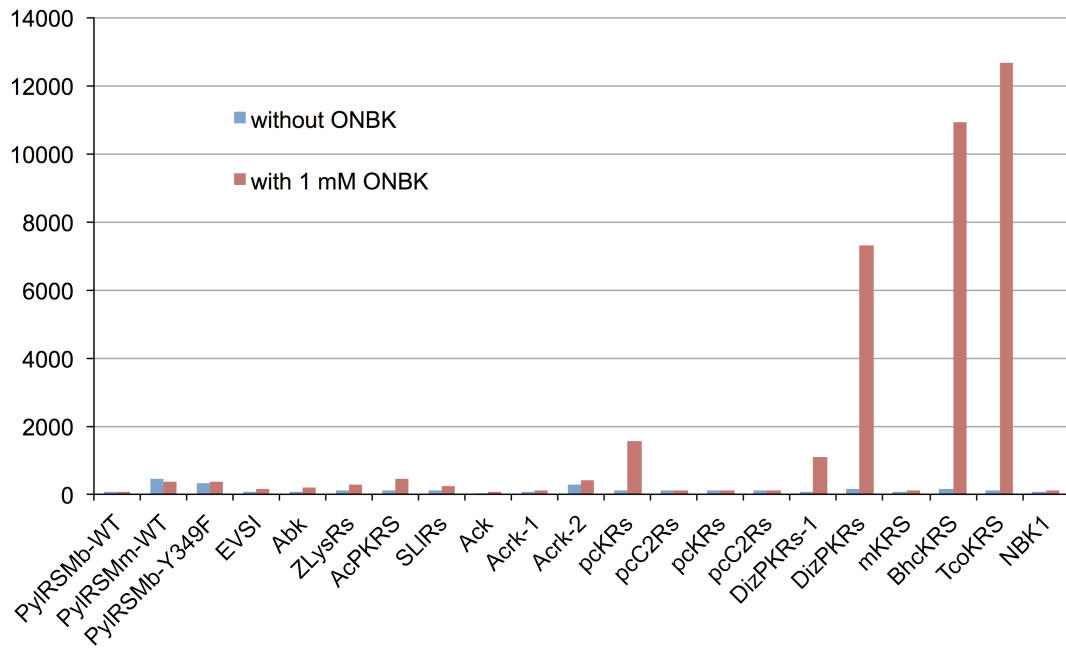


Fig. S1 GFP_{UV} fluorescence assays.

Fluorescence intensity was normalized to cell growth.

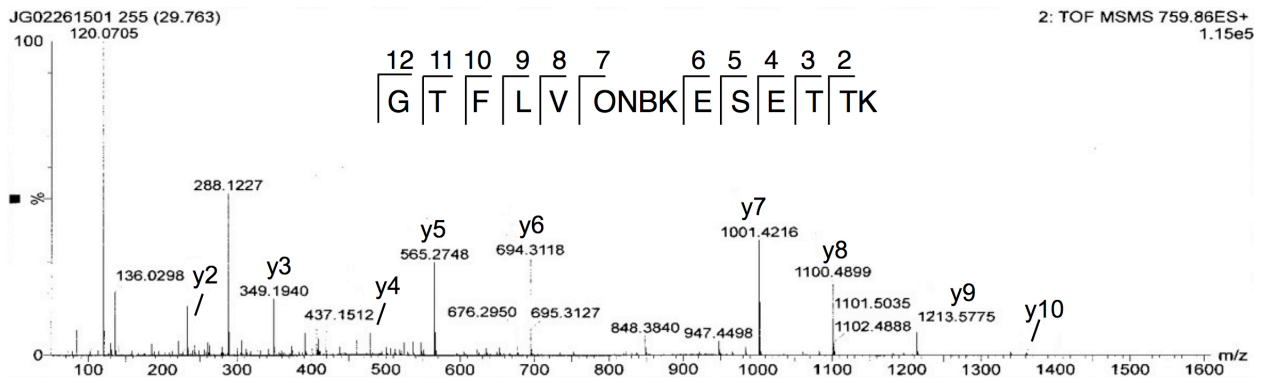
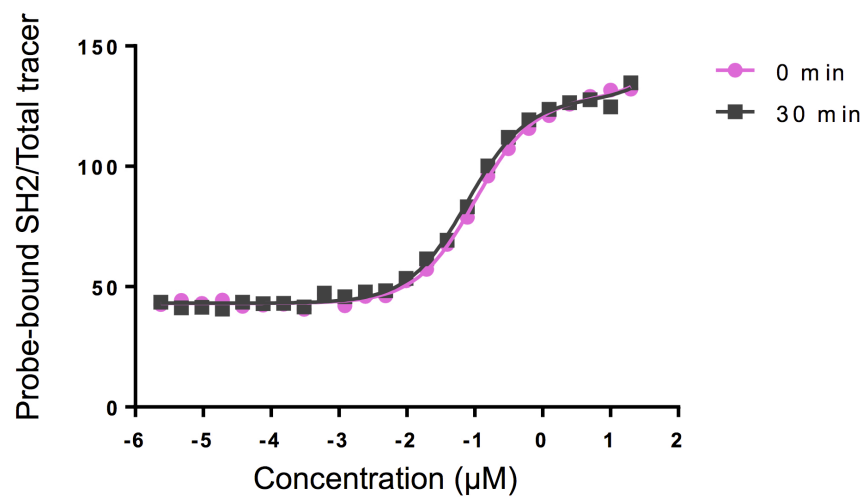


Fig. S2 Mass spectrometry analysis of SH2 domain mutant containing ONBK at position 35. The y ions are marked in the spectrum. The amino acid sequence of the peptide fragment, GTFLV-**ONBK**-ESETTK, from SH2 domain mutant containing ONBK is shown on top.



| | 0 min | 30 min |
|-------------------------|-------|--------|
| K_D (μM) | 0.117 | 0.099 |

Fig. S3 Recognition of the phosphopeptide probe by the SH2-Arg35Lys mutant with and without 30 minutes of UV irradiation.

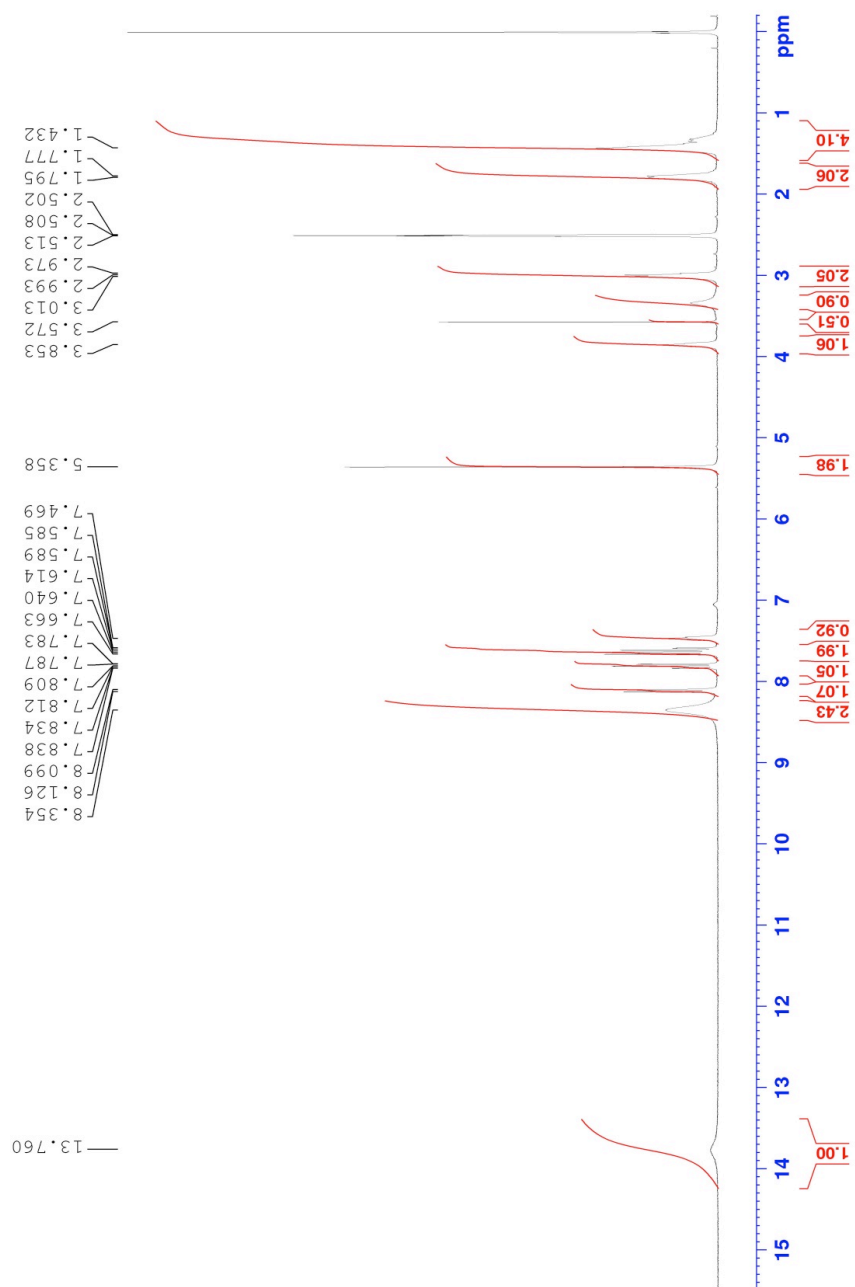


Fig. S4 ^1H NMR of ONBK.

Chemical shifts are reported (in parts per million) relative to internal tetramethylsilane ($\delta = 0.0$ ppm) with DMSO-d_6 as solvent.

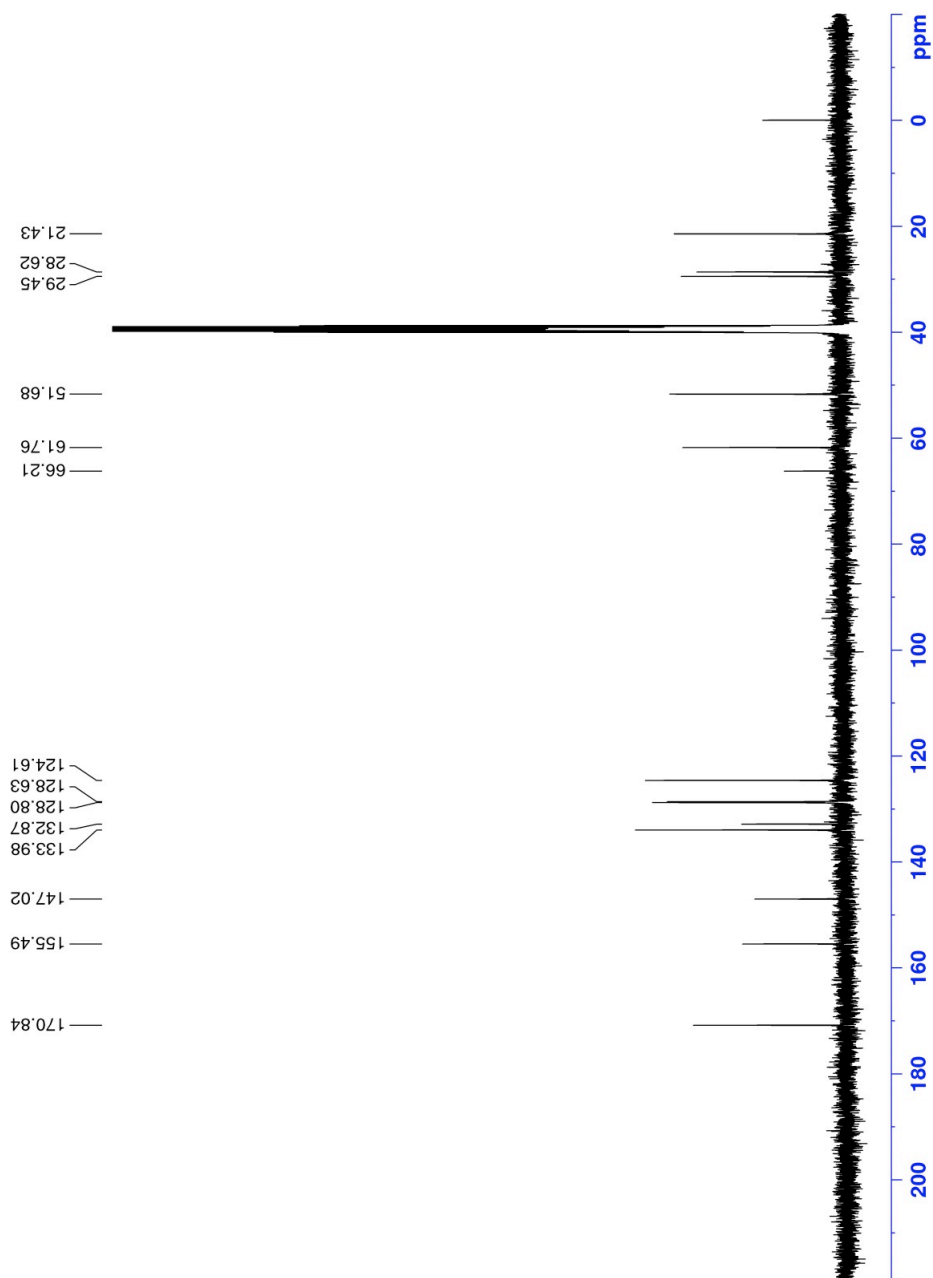


Fig. S5 ^{13}C NMR of ONBK.

Chemical shifts are reported (in parts per million) relative to internal tetramethylsilane ($\delta = 0.0$ ppm) with DMSO-d_6 as solvent.

IV. Reference

1. Y. Kawada, T. Kodama, K. Miyashita, T. Imanishi and S. Obika, *Organic & biomolecular chemistry*, 2012, **10**, 5102-5108.