Title: Peptide Ligation by a Reversible and reusable C-Terminal Thiol Handle

Author: Yi-An Lu¹ and James P. Tam^{1,2}

¹ Department of Biomedical Research, Scripps Florida, Jupiter, FL 33458

² School of Biological Sciences, Nanyang Technological University, Singapore 637551

e-mail: jamestam@scripps.edu

S1. The Sequences and HPLC data of peptide **1**, peptide **2**

	Sequence	HPLC Rt(min)
	Peptide 1 (-OCH ₂ CH ₂ SH)	
1a	AEVSYG	16.52*
1b	SAEVSYG	17.00*
1c	GYGGFLG	24.11
1d	GYGGFLG **	
	Peptide 2 (-SCH ₂ CH ₂ CONH ₂)	
2a	Ac-GASLRRSFGG	17.86*
2b	FTQGVGNPVG	13.54
2c	VPDTYIGASLRRSFGG	17.45

* HPLC linear gradient 0-85% B/30 min. Buffer A:0.045% TFA/ H₂O; B:60% CH₃CN/0.04% TFA/ H₂O. Other peptides HPLC linear gradient was 10-90% B/30 min. **mercaptopropylester

S2. HPLC and MS data of peptide di-ester **3**

Peptide 3	HPLC Rt (min)	MS [M+H] ⁺	Cal.
3a	19.27*	714.91	715.74
3b	19.20*	1804.01	1802.71
3c	22.89	1760.01	1759.11
3d	20.91	1686.92	1685.54
3e	20.82	1701.46	1700.63
3f	17.84	2362.11	2361.46

* HPLC linear gradient 0-85% B/30 min.
Buffer A:0.045% TFA/ H₂O; B:60% CH₃CN/0.04% TFA/ H₂O.
Other peptides HPLC linear gradient was 10-90% B/30 min.

S3. HPLC and MS data of peptide **4**

Peptide 4	HPLC Rt (min)	MS [M+H] ⁺	Cal.
4a	20.15*	1715.21	1715.42
4b	20.57*	1804.32	1802.76
4c	26.34	1761.03	1759.11
4d	22.74	1686.32	1685.54
4e	24.84	1701.45	1700.63

* HPLC linear gradient 0-85% B/30 min.
Buffer A:0.045% TFA/ H₂O; B:60% CH₃CN/0.04% TFA/ H₂O