

Discovery of pancreastatin inhibitor PSTi8 for the treatment of insulin resistance and diabetes: studies in rodent models of diabetes mellitus

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Supplementary Table 1: Peptide secondary structure analysis by circular dichroism using the K2D3 software

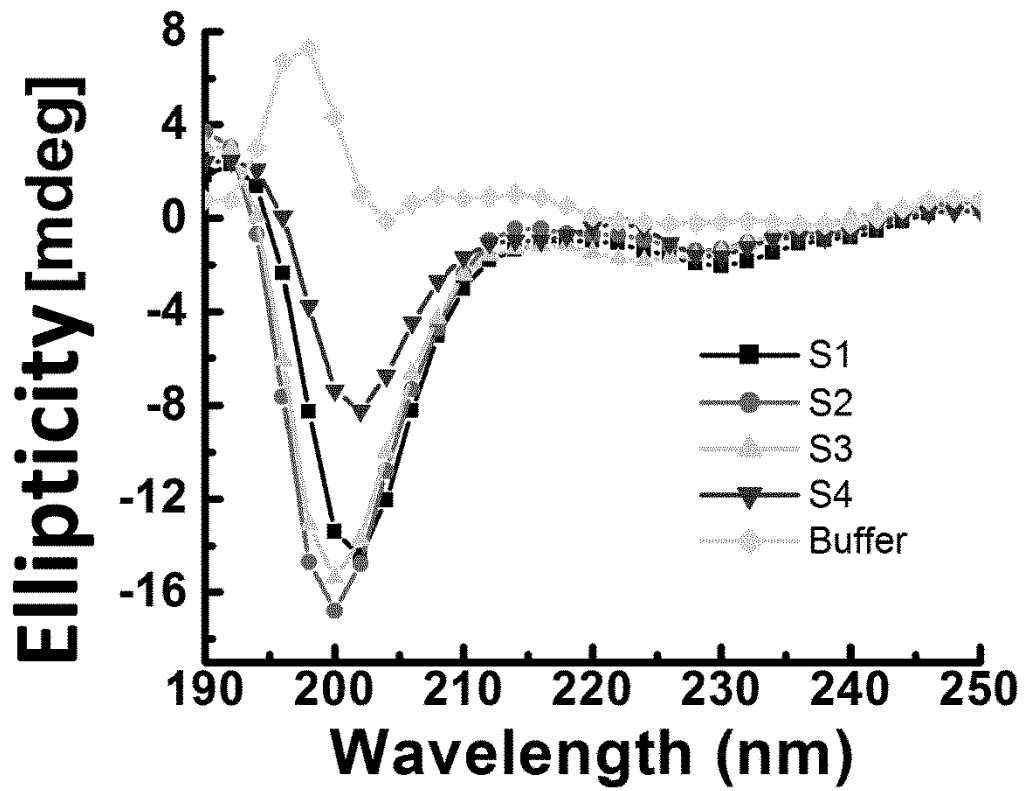
| Peptide Name | Molecular Weight (kDa) | Alpha helix (%) | Beta sheet (%) | Anti-PST activity |
|--------------|------------------------|-----------------|----------------|-------------------|
| S1/PST | 3.28 | 8.82 | 16.54 | |
| S2/PSTi8 | 2.427 | 9.07 | 16.52 | +++ |
| S3/PSTi7 | 2.624 | 12.06 | 15.41 | + |
| S4/PSTi2 | 2.685 | 2.07 | 17.62 | - |

Supplementary Table 2. List of PST inhibitor peptides sequence

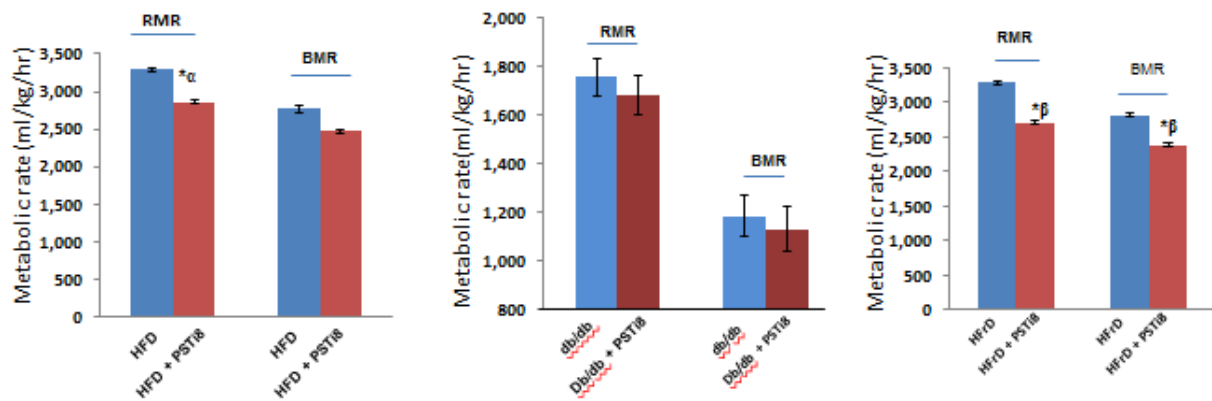
| Name | Peptide Sequence |
|----------|--|
| PSTi1 | GKGEQEHSQQKEEEEEMAVVPQGLFRG, 27 aa, Amidation (C-Terminal) |
| PSTi2 | GEQEHSQQKEEEEEMAVVPQGLFRG, 25 aa, Amidation (C-Terminal) |
| PSTi3 | QEHSQQKEEEEEMAVVPQGLFRG, 23 aa, Amidation (C-Terminal) |
| PSTi4 | HSQQKEEEEEMAVVPQGLFRG, 21 aa, Amidation (C-Terminal) |
| PSTi5 | PEGKGEQEHSQQKEEEEEMAVVPQGLF, 27 aa, Amidation (C-Terminal) |
| PSTi6 | PEGKGEQEHSQQKEEEEEMAVVPQG, 25 aa, Amidation (C-Terminal) |
| PSTi7 | PEGKGEQEHSQQKEEEEEMAVVP, 23 aa, Amidation (C-Terminal) |
| PSTi8 | PEGKGEQEHSQQKEEEEEMAV, 21 aa, Amidation (C-Terminal) |
| RI-PSTi7 | pvvameeeekqqsheqeGkGep, 23 aa, Amidation (C-Terminal) |
| RI-PSTi8 | vameeeekqqsheqeGkGep, 21 aa, Amidation (C-Terminal) |

Supplementary Table 3. Primer sequence of related genes used for RT qPCR

| Gene name | Forward primers | Reverse primers |
|------------------------------|-------------------------|-------------------------|
| <i>hPfk1</i> | GAAGTCAAACCTGAATGTGTC | CCTCTTGTAGGCAGTAAGTC |
| <i>hHk</i> | CACCTGTGAGGTTGGACTCA | CCACCATCTCCACGTTCTTC |
| <i>hPepck</i> | AGATGGAGGAAGAGGGCATC | GTGAGAGCCAACCAGCAGTT |
| <i>hG6pase</i> | ACTGGTTCAACCTCGTCTT | CGAAAGATAGCGAGAGTAGA |
| <i>hPpara</i> | CCGCAATGGACCATGTAAC | CAGCTCTAGCATGGCCTTTT |
| <i>hCpt1a</i> | CACCTCCGTAGCTGACTCG | CGTGAAC TGAAAGGCCACA |
| <i>hSrebp1c</i> | GTGGGCACTGAGGCAAAG | GACAGCAGTGCGCAGACTTA |
| <i>hScd1</i> | CCTAGAAGCTGAGAACTGGTGA | ACATCATCAGCAAGCCAGGT |
| <i>hGapdh</i> | AGCCACATCGCTCAGACAC | AATACGACCAAATCCGTTGACT |
| <i>hβactin</i> | CTACGTCGCCCTGGACTTCGAGC | GATGGAGCCGCCGATCCACACGG |
| <i>mUcp2</i> | GTTCTCTGTCTCGTCTTGC | GGCCTTGAAACCAACCA |
| <i>mPgc1a</i> | AGCCGTGACCACTGACAACGAG | CTGCATGGTTCTGAGTGCTAAG |
| <i>mFoxo1</i> | CTTCAAGGATAAGGGCGACA | GACAGATTGTGGCGAATTGA |
| <i>mAkt1</i> | TCGTGTGGCAGGATGTGTAT | ACCTGGTGTTCAGTCTCAGAGG |
| <i>mPparβ</i> | TCCAGAAGAAGAACCGCAACA | GGATAGCGTTGTGCGACATG |
| <i>mPepck</i> | ATGTGTGGGCGATGACATT | AACCCGTTTTCTGGGTTGAT |
| <i>mG6pase</i> | CATGGGCGCAGCAGGTGTATACT | CAAGGTAGATCCGGGACAGACAG |
| <i>mPfk1</i> | GGAAGCCAAATGGGACTGT | CGCACTACCGATGATGGTC |
| <i>mPk</i> | GTGGAGGCTTCCTTCAAGTG | AGGTCCGGTAGCGAGACAGAA |
| <i>mPparγ</i> | CAGGCCGAGAAGGAGAAGCT | GGCTCGCAGATCAGCAGACT |
| <i>mSrebp1c</i> | GGTTTTGAACGACATCGAAGA | CGGGAAGTCACTGTCTTGGT |
| <i>mFas</i> | CGGAAACTTCAGGAAATGTCC | TCAGAGACGTGTCACTCCTGG |
| <i>mGapdh</i> | GTGTCCGTCGTGGATCTGA | CCTGCTTACCACCTTCTTG |
| <i>mβactin</i> | CCTCACCTCCCAAAGC | GTGGACTCAGGGCATGGA |
| <i>hPyruvate carboxylase</i> | GACTGTTTCGGAACCTTCAGCAT | GAGGCAGCAGCGGTAGAG |

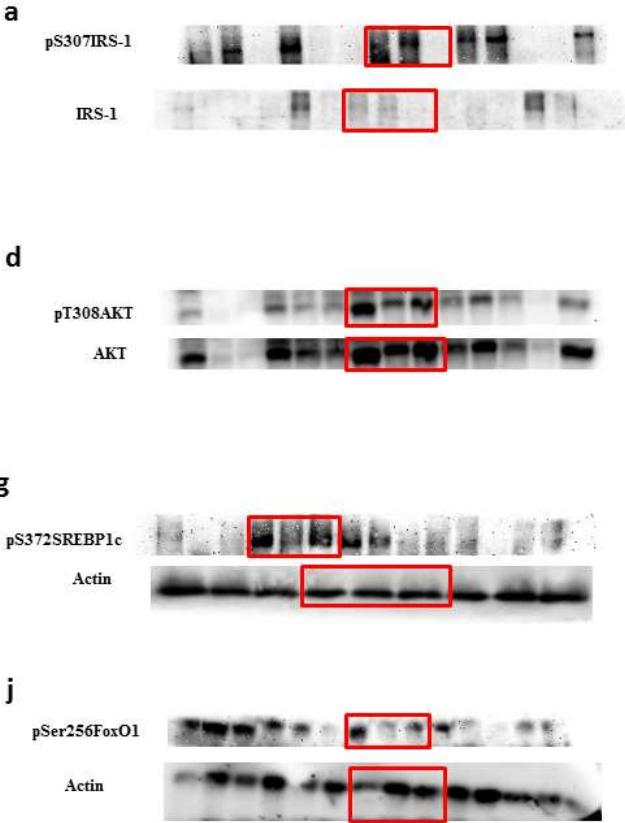


Supplementary Figure 1. Determination of secondary structures of PST and PST inhibitors in aqueous environment using circular dichroism (CD). S1: PST, S2: PSTi8, S3: PSTi7, S4: PSTi2.

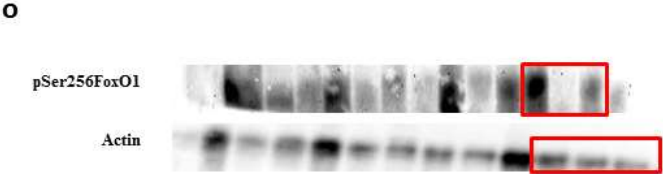
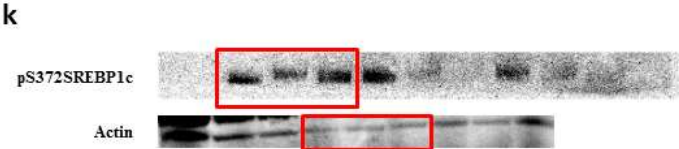
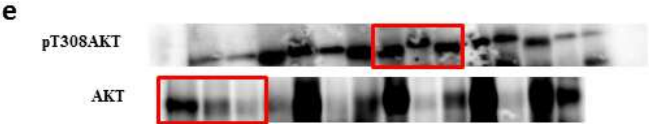
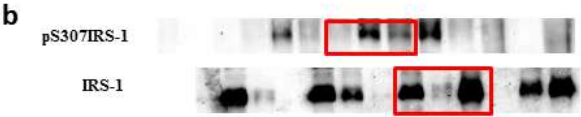


Supplementary Figure 2 Measurement of Basal metabolic rate (BMR) and resting metabolic rate (RMR) in HFD, HFrD-fed and db/db and its PSTi8 treated mice. α , HFD vs HFD +PSTi8; β , HFrD vs HFrD + PSTi8. *, $p < 0.05$, error bar \pm s.e.m of mean.

Supplementary information for figure 5 a, d, g and j (unedited full blots)



Supplementary information for figure 5 b, e, k and o (unedited full blots)



Supplementary information for figure 5 c, f, i and l (unedited full blots)

