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# Supplementary Materials: New Intracellular Peptide Derived from Hemoglobin Alpha Chain Induces Glucose Uptake and Reduces Blood Glycemia

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**Table S1.** Primers sequences.

| Gene | Sequence | Amplicon (bp) | Accession number |
|---|---|---|---|
| Peroxisome Proliferator Activated Receptor Gamma (PPAR-γ ) | Fwd: CATAAAGTCCTTCCCGCTGA Rev: GAAACTGGCACCCTT-GAAAA | 102 | NM_001127330.2 |
| Peroxisome Proliferator Activated Receptor Alpha (PPAR-α) | Fwd: TGCAATTCGCTTTGGAA-GAA Rev: CTTGCCCAGAGATTT-GAGGT | 118 | NM_011144.6 |
| Aldolase A, Fructose-bisphos-phate (AldoA) | Fwd: GAGCTGTCTGACATCGCTCA Rev: TCTCGTGGAAGAGGATCACC | 204 | NM_001177307.1 |
| Lipoprotein Lipase (LPL) | Fwd: GTCTGGCTGACACTG-GACAAA Rev: CCCAC-TTTCAAACACCCAAA | 122 | NM_008509.2 |
| Phosphoglycerate Mutase 2 (Pgam2) | Fwd: TGGAATGAGGAGATCG-CACC Rev: ATTCCAGTGGG-CAGGTTCAG | 143 | NM_018870.3 |
| Myosin, Light Chain 1 (MYL-1) | Fwd: GGAGGCATTTCTCCTGTTTG Rev: CCTGGTCCTTGTTGTTGGAG | 133 | NM_021285.3 |
| Small Muscle Protein X-linked (SPMXc) | Fwd: CAGCCTCCCAGAAGGAAAG Rev: ACTGTTCACCTTTGGGGACA | 113 | NM_001252591.2 |
| cAMP Responsive Element Bind-ing Protein 1 (Creb1) | Fwd: GGTGCCAAGGATTGAA-GAAG Rev: GTACCCCATCCGTAC-CATTG | 110 | NM_133828.2 |
| Cytochrome c Oxidase Subunit IV Isoform 1 (COX4i1) | Fwd: CGCTGAAGGAGAAGGA-GAAG Rev: GGATGGGGCCATACACATAG | 77 | NM_009941.3 |
| Troponin I, skeletal, fast 2 (TNNI2) | Fwd: TGCAAACAACTG-CATGCGAA Rev: TTGAACTT-GCCCCTCAGGTC | 131 | NM_009405.3 |
| Troponin I, skeletal, fast 3 (TNNT3) | Fwd: CCCCAGCCTTTCTCAGACTC | 106 | NM_001163664.1 |

| | | | |
|---|---|---|---|
| | Rev: TTGGGCCTCCTCTTCCTCTT | | |
| Insulin-like Growth Factor 1 (IGF1) | Fwd: TGTACTTCAGAA-GCGATGGGG Rev: AGAGGTGTGAAGAC-GACATGA | 112 | NM_010512.5 |
| Solute Carrier Family 2 - Slc2a4 (GLUT4) | Fwd: CCAACAGCTCTCAGGCATCA Rev: CAGCTCCTATGGTGGCG-TAG | 98 | NM_001359114.1 |
| Glyceraldehyde 3-phosphate de-hydrogenase (GAPDH) | Fwd: GTGCAG-TGCCAGCCTCGTCC Rev: CAGGCGCCCAATAC-GGCCAA | 75 | BC_085275 |

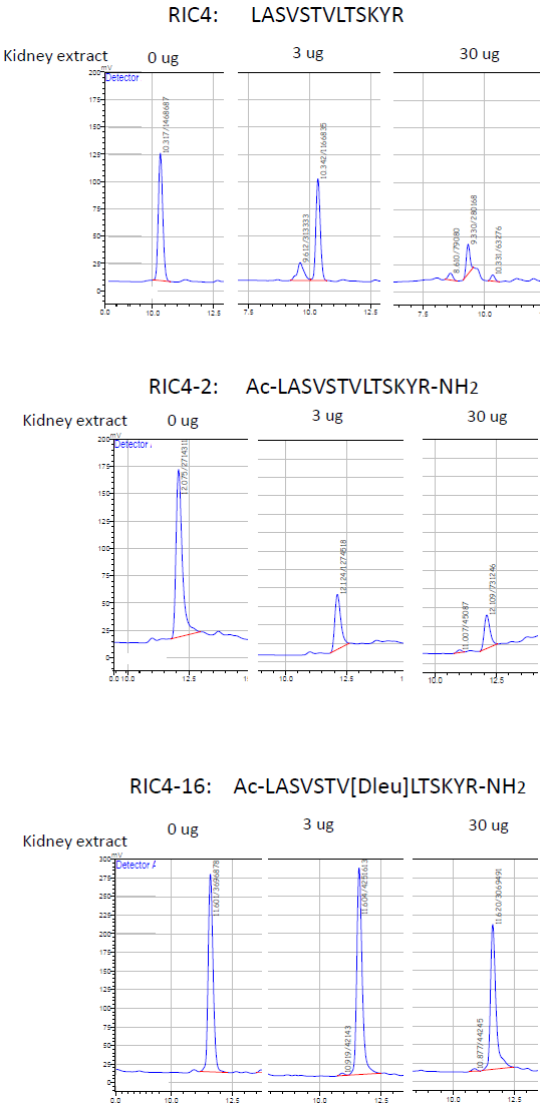


**Figure S1.** Typical experiments to evaluate the enzymatic stability of Ric4 and derivatives on kidney tissue extracts. In order to determine if the peptides investigated herein were substrates of tissue peptidases, 50 µM of each peptide was individually incubated for 20 min in the presence of increasing crude kidney tissue extracts (0 µg, 3 µg or 30 µg, as indicated), in a final volume of 250 µl of 0,025 M Tris-HCl, containing 0.125 M NaCl (TBS). Peptide hydrolyses were analyzed by reverse phase liquid chromatography (HPLC) using a C18 µBond-pak column (4.6 x 250 mm; Millipore Corp.) with a linear gradient of 5-65% acetonitrile in 0.1 % TFA for 20 min at a flow rate of 1 mL/min,

and absorbance monitored at a wavelength of 214 nm, as previously described [8,36,37]. Cleaved peptide bonds were identified by mass spectrometry sequencing after isolating the cleavage fragments manually after HPLC (data not shown). Note the greater stability of the Ric4-derivative Ac-LASVSTV[DLeu]TSKYR-NH2 (Ric4-16) compared to Ac-LASVSTVLTSKYR-NH2; Ric4-2 (Ric4-2) or LASVSTVLTSKYR (Ric4).