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Supplementary Materials for

pNaKtide inhibits Na/K-ATPase reactive oxygen species amplification and attenuates adipogenesis

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The PDF file includes:

RESULTS

Figure S1: pNaKtide distribution in 3T3L1 cells and adipose tissue.

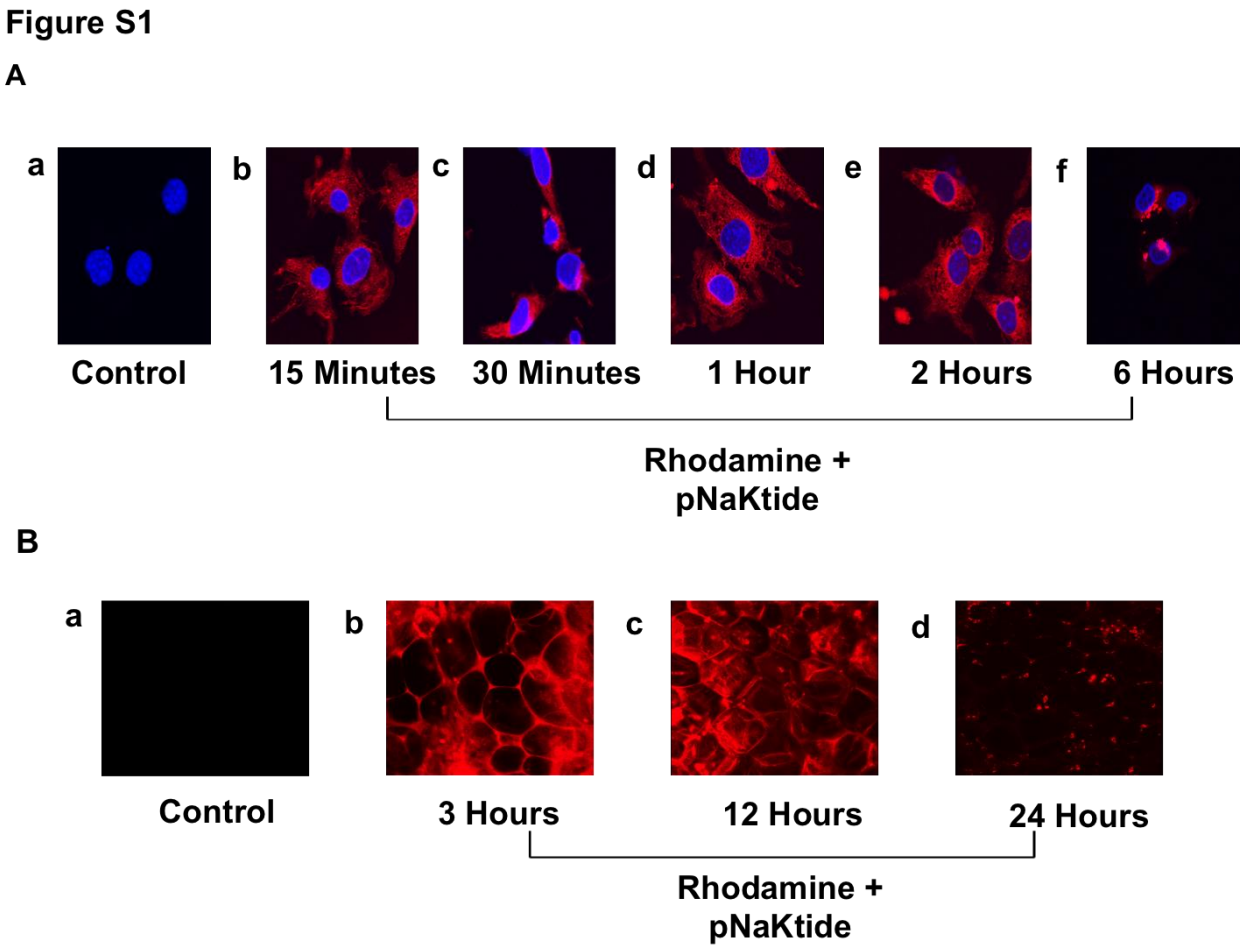
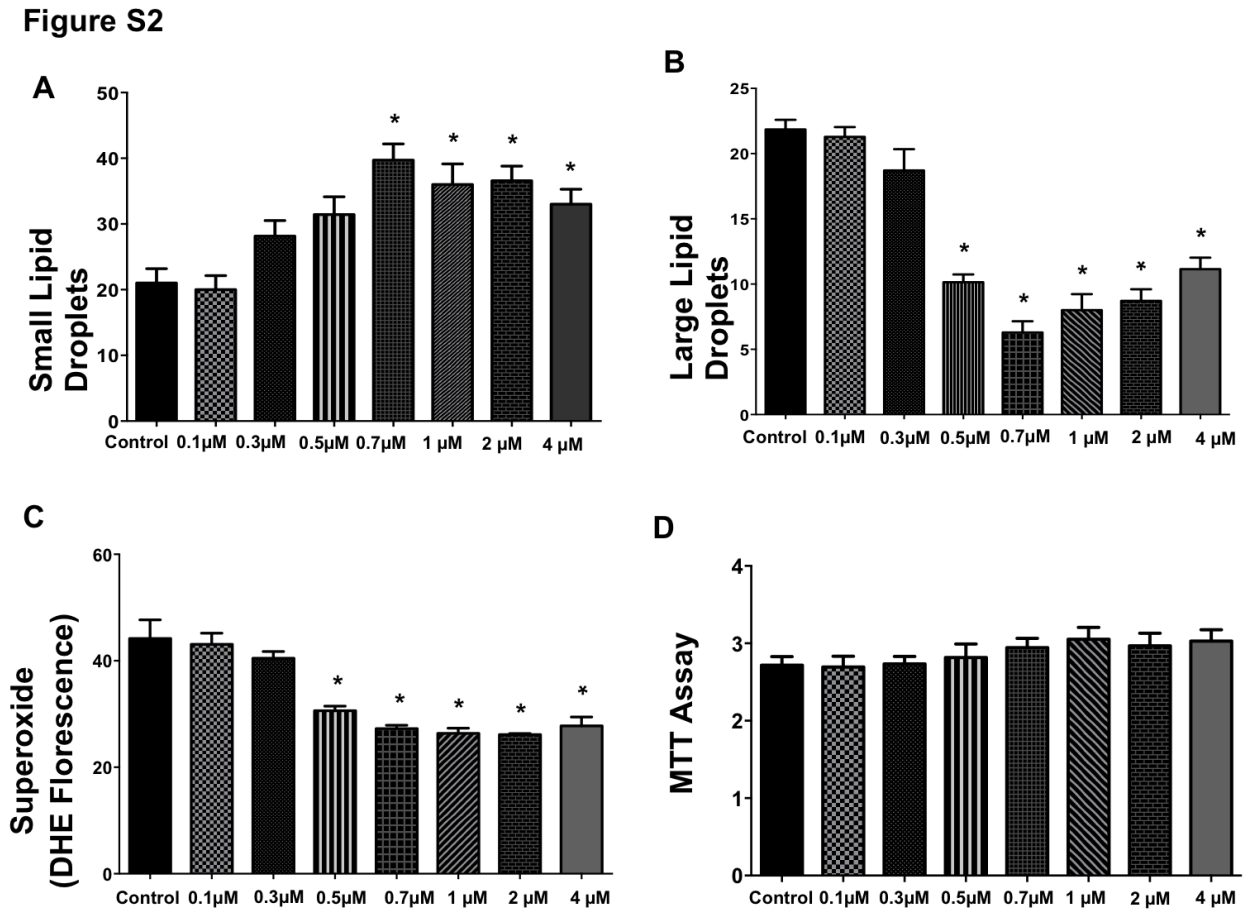


Figure S1: (A) rhodamine-labeled pNaKtide was added to the culture medium as described in the method section. The drug was efficiently distributed in 3T3L1 cells and the maximum distribution was seen after 2 hours of incubation (n=4). **(B)** To study whether pNaKtide was distributed in adipose tissue by IP injections, rhodamine-labeled

pNaKtide was injected in mice. The rhodamine-labeled pNaKtide was efficiently distributed in adipose tissue (n=4).

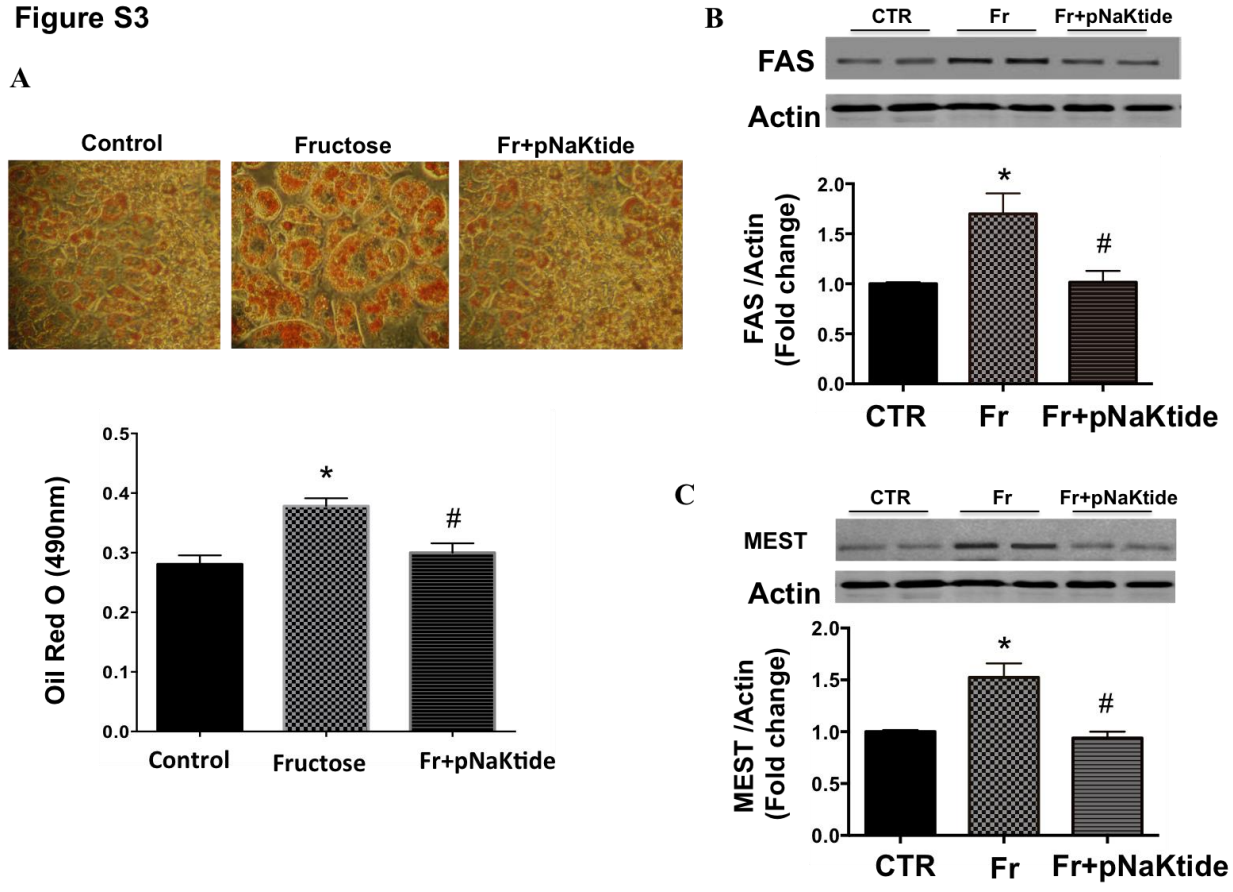
Figure S2: pNaKtide decreased large lipid droplets and oxidative stress and increased small lipid droplets in 3T3L1 adipocytes:



In order to examine the effect of pNaKtide on lipid droplets size, superoxide levels and cell toxicity 3T3L1 cells were exposed to different concentrations of pNaKtide for 7 days. **(A)** Number of small lipid droplets from Oil Red O stained 3T3L3 cells with increasing

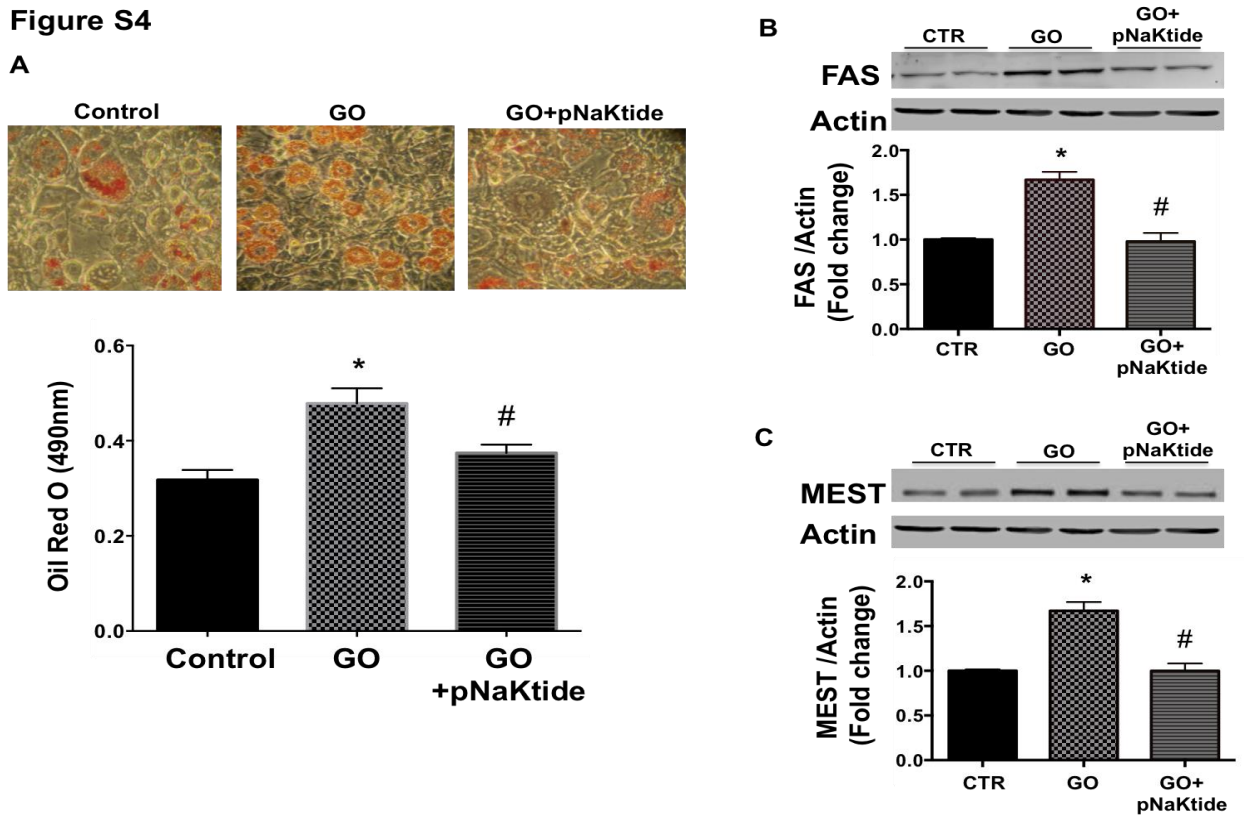
concentrations (0.1, 0.3, 0.5, 0.7, 1, 2, and 4 μM) of pNaKtide, magnifications: 40x (n=6); **(B)** number of large lipid droplets from Oil Red O stained 3T3L1 cells with increasing concentrations of pNaKtide, magnifications: 40x (n=6). *, $p < 0.05$ *vs.* control. **(C)** Superoxide measurement (marker of oxidative stress) in 3T3L1 adipocytes. * p <0.05 vs CTR. **(D)** MTT assay to study the cytotoxicity at increasing concentrations of pNaKtide. The pNaKtide treatment had no cytotoxic effects at concentrations up to 4 μM. Results are means ± SEM of six independent treatments.

Figure S3: pNaKtide decreased lipid accumulation and adipogenic markers in 3T3L1 adipocytes exposed to fructose treatment:



0.7 μM of pNaKtide was determined to be the optimal concentration for inhibiting adipogenesis in 3T3L1 cells. Therefore 3T3L1 cells were treated with fructose, a potent inducer of oxidative stress (500 μM concentration) with and without pNaKtide (0.7μM) every day for 7 days. **(A)** Effect of pNaKtide on adipogenesis in mouse 3T3L1 cells. Adipogenesis was measured as the relative absorbance of Oil Red O at day 7 after inducing adipogenesis as described in the materials and methods section. * $p<0.05$ vs. control, # $p<0.05$ vs. Fr. **(B)** Western blot and densitometry analysis of FAS levels. Data are shown as mean band density normalized to β-actin, Results are means ± SE, n=6, * $p<0.05$ vs. control, # $p<0.05$ vs. Fr. **(C)** Western blot and densitometry analysis of MEST levels. Data are shown as mean band density normalized to β-actin, Results are means ± SE, n=6, * $p<0.05$ vs. control, # $p<0.05$ vs. Fr.

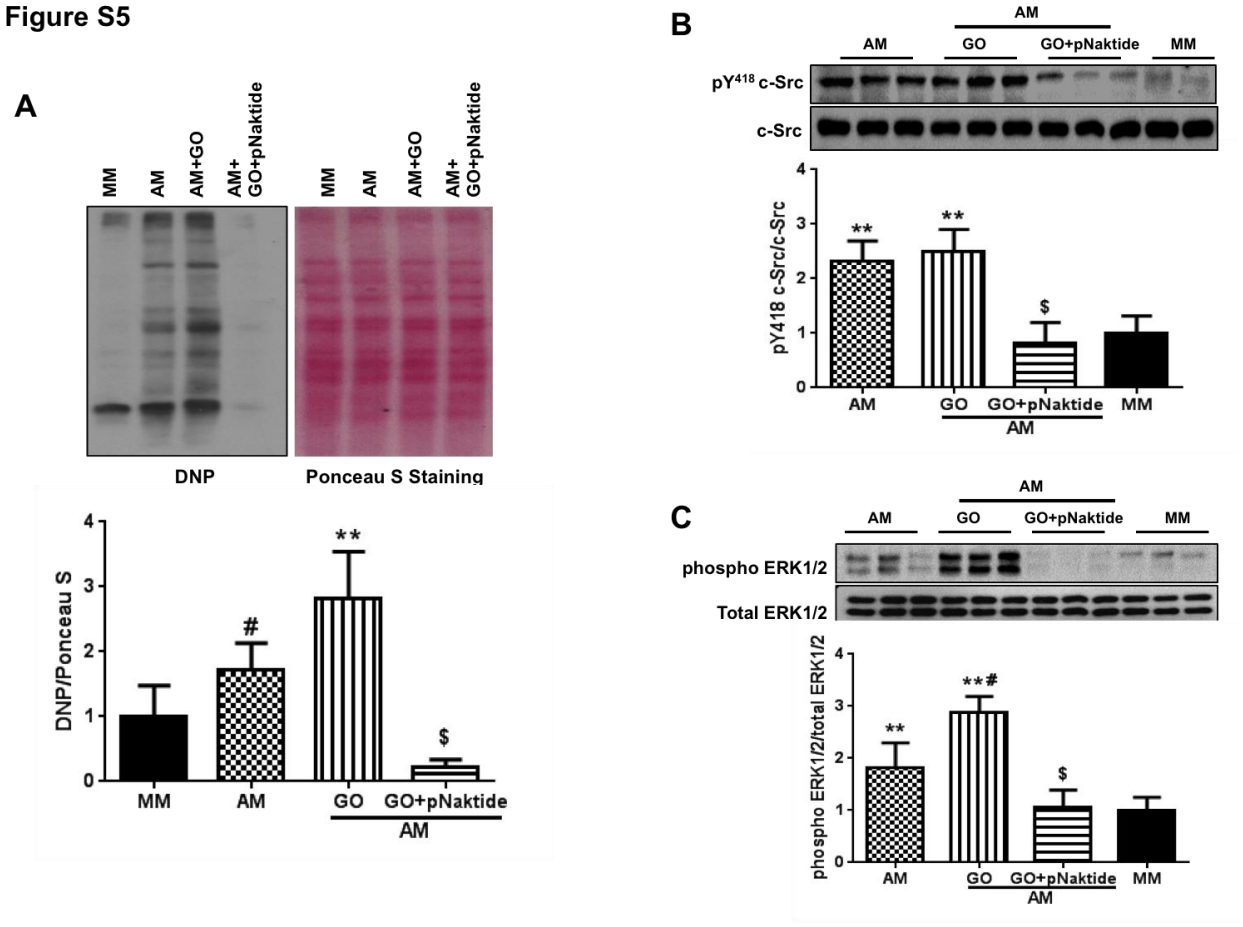
Figure S4: pNaKtide decreased lipid accumulation and adipogenic markers in 3T3L1 adipocytes exposed to glucose oxidase treatment:



To further confirm our hypothesis that pNaKtide decreases oxidative stress, 3T3L1 cells were treated with glucose oxidase, another known inducer of oxidative stress (3 mU/L concentration as determined by concentration curve (data not shown)) with and without pNaKtide (0.7μM) every day for 7 days. (**A)** Effect of pNaKtide on adipogenesis in mouse 3T3L1 cells. Adipogenesis was measured as the relative absorbance of Oil Red O at day 7 after inducing adipogenesis as described in the materials and methods section. * $p<0.05$ vs. control, # $p<0.05$ vs. GO. (**B)** Western blot and densitometry analysis of FAS levels. Data are shown as mean band density normalized to β-actin,

Results are means ± SE, n=6, *p<0.05 vs. control, # p<0.05 vs. GO. (**C)** Western blot and densitometry analysis of MEST level. Data are shown as mean band density normalized to β-actin, Results are means ± SE, n=6, *p<0.05 vs. control, # p<0.05 vs. GO.

Figure S5: pNaKtide decreased carbonylation and phosphorylation of Src and ERK in 3T3L1 adipocytes exposed to glucose oxidase treatment:



Whole cell lysates were prepared with Nonidet P-40 buffer and western blot analysis was performed to determine protein carbonylation **(A)**, activation of c-Src **(B)** and activation of ERK1/2 **(C)**. MM, maintenance medium; AM, adipogenic medium; GO, glucose oxidase. Results were expressed as Means ± SD, N=4-6/group, # $p<0.05$ vs. MM, ** $p<0.01$ vs. MM, $ $p\leq0.01$ vs. AM and AM+GO, & $p<0.01$ vs. AM.