Methods

S.2.1 Modification of DNSP-11 (R9K) for ^{125}I-labeling

MN9D cells were cultured in DHEM and supplemented with 10% Fetal Bovine Serum (FBS, Hyclone), 50 U/ml penicillin and streptomycin. For protection studies, cells were plated on 24-well poly-D-lysine in DHEM with 1% (v/v) penicillin-streptomycin. The cells were grown at 37 ^{o}C in 5% CO_2. The dUTP nick-end labeling (TUNEL) assay was performed as previously reported [10]. MN9D cells were administered STS (1 μM) and treated with either the original and/or modified (R9K) DNSP-11 peptide (10 nM). The percent from control was determined by the ratio of apoptotic versus total cells.

Results

S3.1. Modification of DNSP-11 (R9K) for ^{125}I-labeling

To facilitate iodination of DNSP-11 for our tracer studies, a conservative substitution (Arg to Lys) was introduced at the 9th position of the original sequences. To determine if modifying the original sequence of DNSP-11 for iodination via the Bolton-Hunter method would alter the normal biological activity of the peptide; protection of the modified peptide against STS, a nonselective protein kinase inhibitor and cytotoxin, was compared with DNSP-11. At 12 hours, MN9D cells treated with 1 μM of STS, were assessed for cytotoxicity by TUNEL staining. MN9D cells treated with either DNSP-11 or the modified peptide sequence (R9K) showed protection against STS induced cytotoxicity. More importantly, there was no difference between DNSP-11 and the modified sequence's ability to protect against STS induced cytotoxicity, indicating that the normal biological activity of the peptide was preserved (Fig. S1).

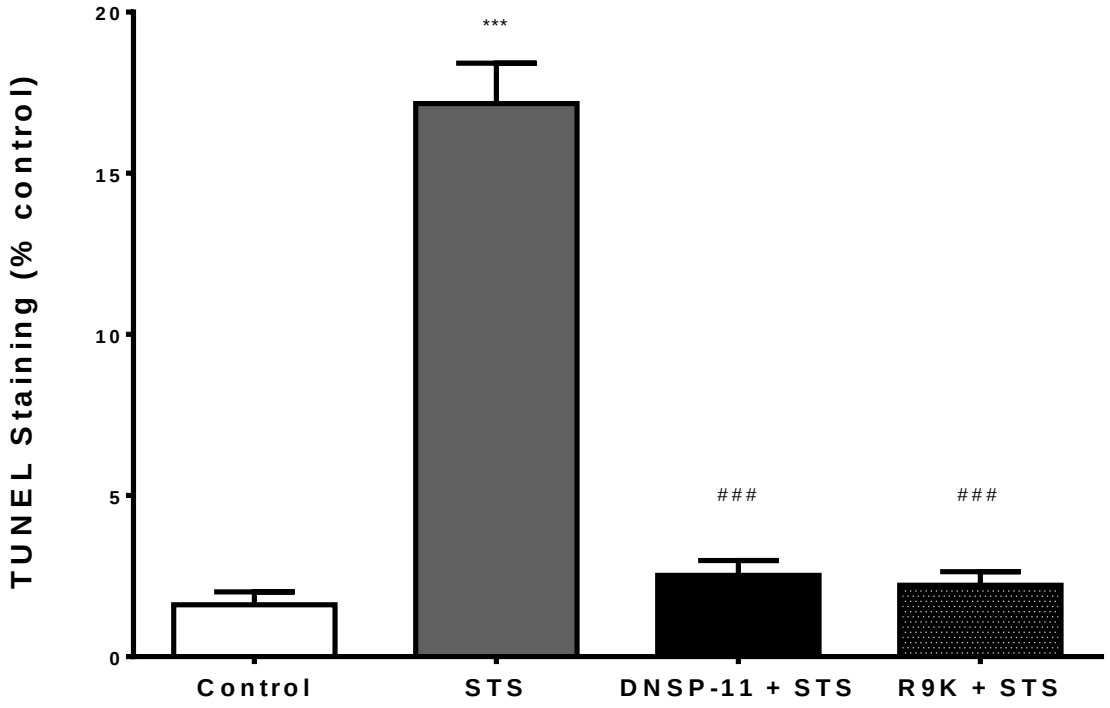

Figure S1: *Modified DNSP-11 sequence (R9K) for tracer studies.*
Tunnel staining in MN9D cells suggests that at 10nM the original and modified DNSP-11 (Arg-9 for Lys) protects against 1uM of staurosporine (STS) at 12 hours. One-way ANOVA with Tukey's multiple comparison. Data presented as mean $\pm$ sem ***$p<0.0001$ compared to control and ### $p<0.0001$ compared to STS.