Supplemental Methods

Primers used for qRT-PCR:

rat Ttn, sense 5’-AAGCCAAGAAACAGGAACCA-3’

rat Ttn, antisense 5’-TGCAATAGCCTTTCCATCCT-3’;

rat 18s, sense 5’-GTGGAGCGATTTGTCTGGTT-3’

rat 18s, antisense 5’-CGCTGAGCCAGTCAGTGTAG-3’.

Primers used for 5’ RACE:

Gene specific antisense primers used for the 5’ RACE study:

outer 5’- GCTGACTGAAGTTGGGTGGT-3’

inner 5’- TGGCTCTCAGGGAGTATCGT-3’.

Figure Legends

Figure S1. A: Mfold was used to generate the mRNA structure of titin's 5'-UTR. The red arrow depicts the portion of the stem-loop that was targeted for mutagenesis. **B:** NRVM were transiently transfected with pGL3 control, 5'-UTR, or combined 5'-UTR-Luc-3'-UTR reporter for 24hrs and cells were lysed and assayed for luciferase activity. Data presented as Mean±SEM (n=3); *p<0.05 vs Control. Groups were compared by ANOVA.

Figure 2S. A: NRVM were transiently transfected with pGL3 control or Ttn 5' UTR for 24 hours. NRVM were either unpaced, paced at 30 V, 2 Hz, 10ms for 24hrs, or treated with 3μM blebbistatin. Cells were lysed and assayed for luciferase activities. Data presented as Mean±SEM (n=3-4). **B:** NRVM were transiently transfected with reporter constructs as above and were either treated with vehicle or 20ng/mL NRG-1 for 24 hours, and cells lysed and assayed for luciferase activity. Data presented as Mean±SEM, (n=3). **C:** NRVM were transiently transfected with reporter constructs as above and were treated with vehicle or 200μM PMA, and cells lysed and assayed for luciferase activity. Data presented as Mean±SEM, (n=3). * p<0.05 vs control, groups were analyzed by ANOVA.