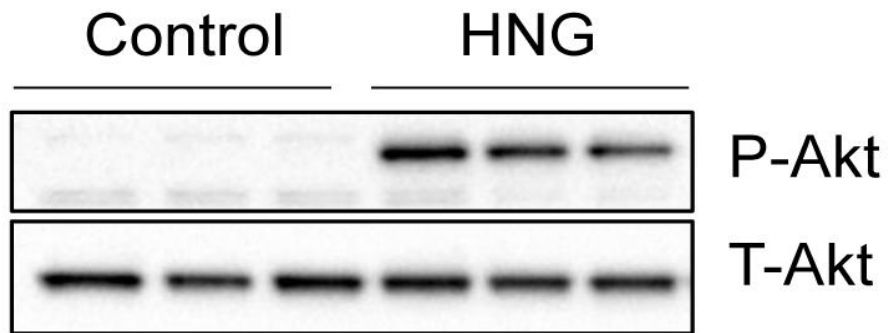


# The mitochondrial-derived peptide humanin activates the ERK1/2, AKT, and STAT3 signaling pathways and has age-dependent signaling differences in the hippocampus

## Supplementary Material

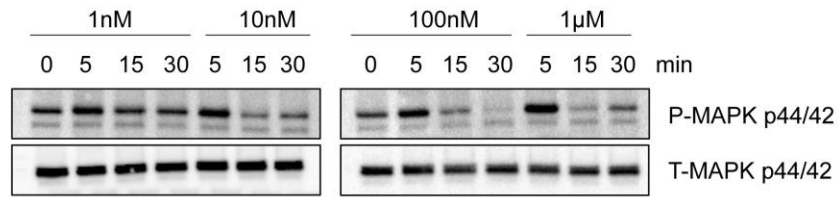


Supplemental Figure 1: Top canonical pathways to which the humanin-targeted proteins are associated. (A) The high-ranking categories are displayed along the x axis in decreasing order of significance. The y axis displays the  $-\log(p)$  values. The orange points connected by a line represent the ratio. Based on the z-score, the orange indicates activation, blue inactivation, white have a z-score at or very closed to 0, and gray bars indicate pathways where no prediction can currently be made. (B) A list of genes associated with IGF-1 signaling.

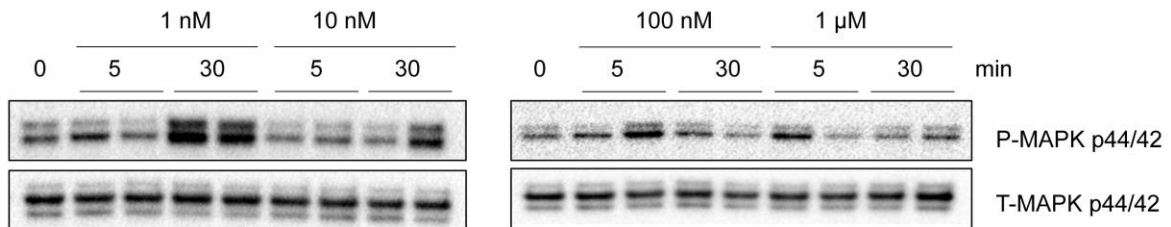


Supplemental Figure 2: HNG increases AKT (Thr<sup>308</sup>) phosphorylation in SH-SY5Y cells. Western blot analysis for phospho- AKT at Thr<sup>308</sup> and total-AKT. Cells were treated with 100 $\mu$ M HNG for 30min.

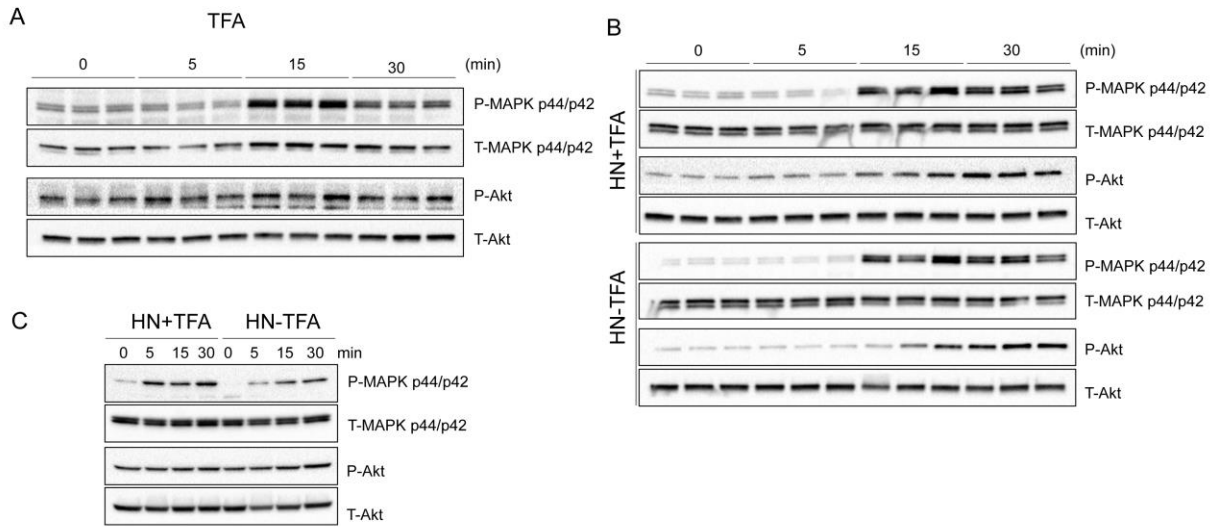
A



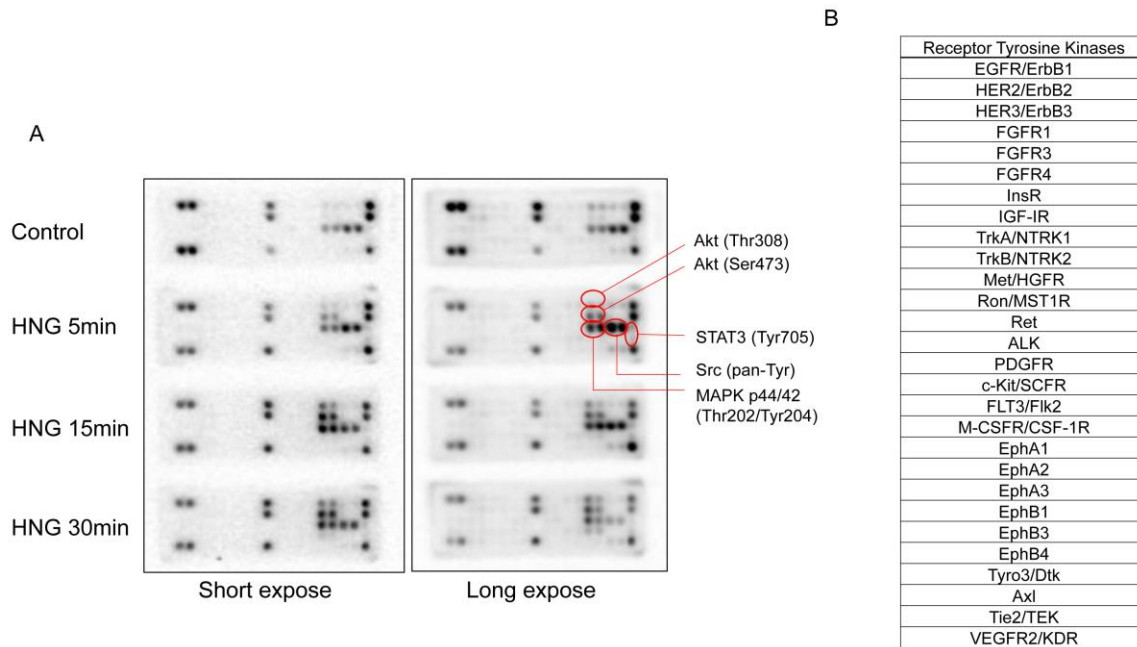
B



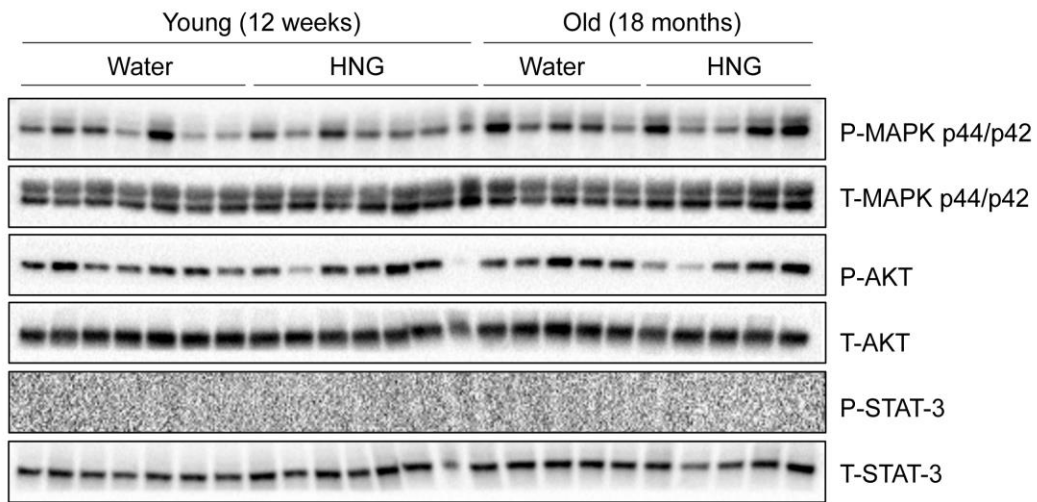
Supplemental Figure 3: HNG increases MAPK p44/42 (ERK 1/2) phosphorylation from 1nM to 100μM. Western blot analysis for phospho- and total-ERK 1/2 in (A) HEK293 and (B) SH-SY5Y cells. Cells were treated with 1nM, 10nM, 100nM, and 1μM HNG for the indicated time periods.



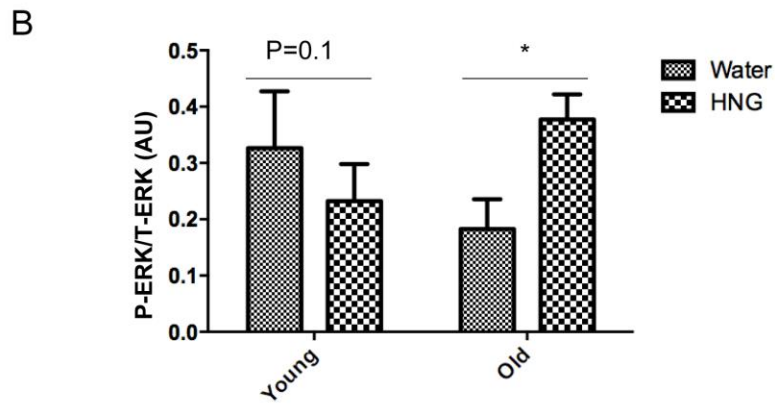
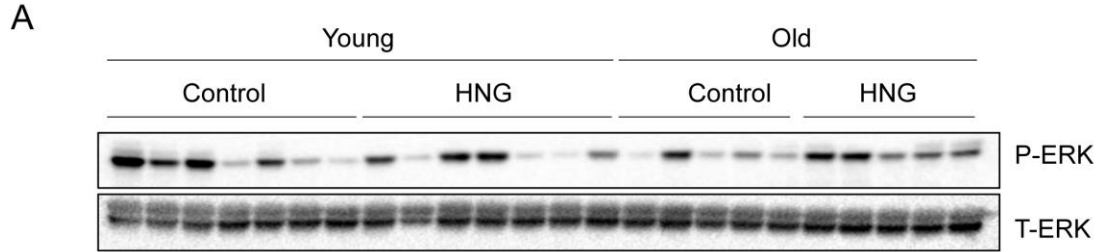
Supplemental Figure 4: Trifluoroacetic acid (TFA) increases MAPK p44/42 (ERK 1/2) phosphorylation. (A) Western blot analysis for phospho- and total-AKT and ERK in SH-SY5Y cells. Cells were treated with 5 $\mu$ M TFA for the indicated time periods. Western blot analysis for phospho- and total-AKT and ERK in (B) SH-SY5Y and (C) HEK293 cells. Cells were treated with 100  $\mu$ M Humanin with TFA (HN+TFA) and 100  $\mu$ M Humanin without TFA (HN-TFA) for the indicated time periods.



Supplemental Figure 5: No evidence for humanin signaling mediated by RTK. (A) SH-SY5Y cells were treated with 100 $\mu$ M HNG for the indicated time periods. The PathScan RTK Signaling Antibody Array images were captured using Bio-Rad ChemiDoc XRS<sup>+</sup> imager, with 20 second (short) and 90 second (long) exposure times. (B) A list of RTKs in the antibody array.



Supplemental Figure 6: No evidence for humanin-mediated AKT, ERK or STAT3 signaling in the hypothalamus. Western blot analysis for phospho- and total-AKT, ERK, and STAT3 in hypothalamus of 12-week-old mice and 18-month-old mice.



Supplemental Figure 7: HNG increases ERK phosphorylation in the hippocampus of old mice. (A) Western blot analysis for phospho- and total-ERK in hippocampus of 12-week-old mice and 18-month-old mice in the same gel. (B) Quantitation of normalized ERK activation in response to HNG. Total protein was used to normalize protein loading. Data are reported as mean  $\pm$  SEM (n= 7 for 12-week-old mice, n=5 for 18-month-old mice). \*p<0.05, ns, not significant.