Supplemental Information

Central IGF-1 protects against features of cognitive and sensorimotor decline with aging in male mice

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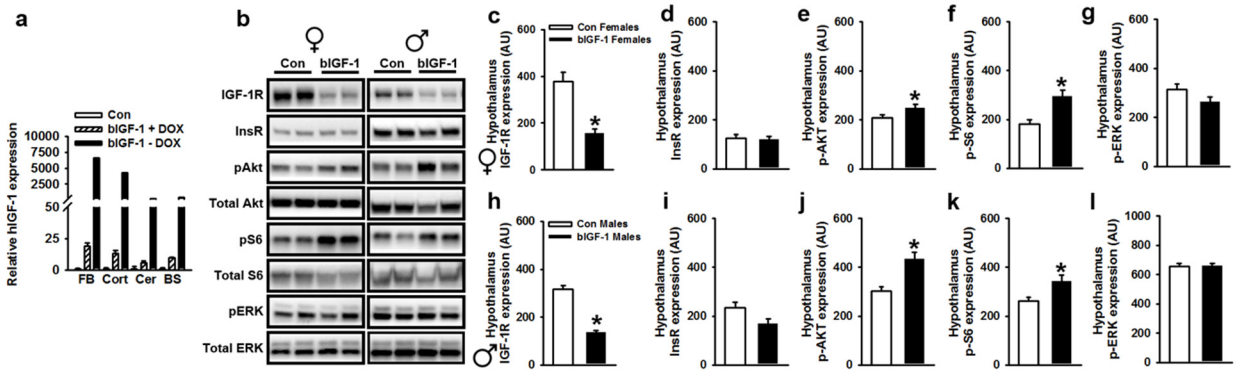
Supplementary Table 1 RT-PCR primers.

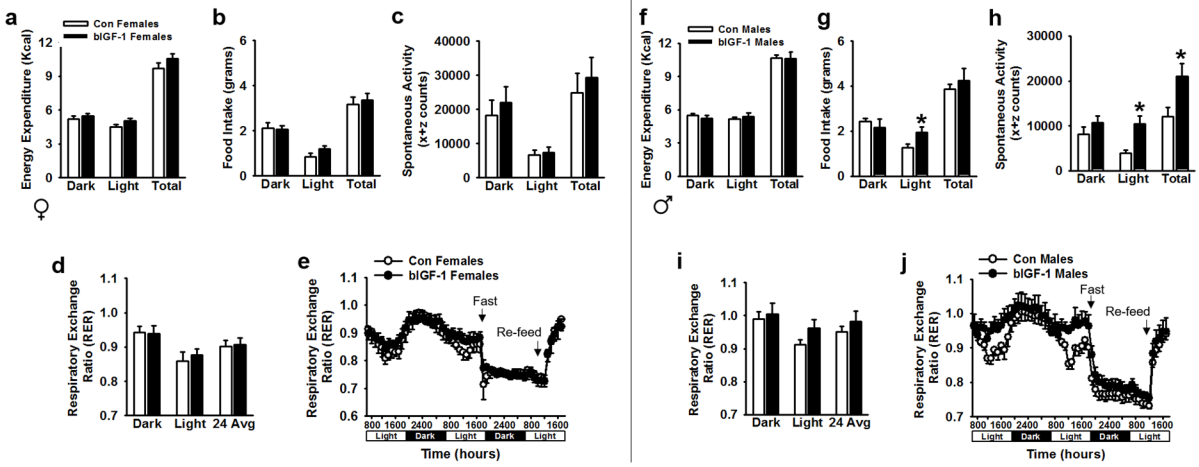
Gene	Primer Pair	Primer sequence
Cyclophylin A	*PPIA* F	5'-GCGTCTSCTTCGAGCTGTT-3'
	PPIA R	5'-RAAGTCACCACCCTGGCA-3'
Human *IGF-1*	*hIGF-1* F	5'-CCGGAGCTGTGATCTAAGGA-3'
	hIGF-1 R	5'- CCTGCACTCCCTCTACTTGC-3'
Mouse *IGF-1*	*mIGF-1* F	5'-AAATCAGCAGCCTTCCAACTC-3'
	mIGF-1 R	5'-GCACTTCCTCTACTTGTGTTCTT-3'
IGF-1R	*IGF-1R* F	5'-CCACCCTCCTTGTCCACG-3'
	IGF-1R R	5'-GGGCCCACAGATTTCTCCAC-3'
InsR	*InsR* F	5'-TCAAGACCAGACCCGAAGATT-3'
	InsR R	5'-TCTCGAAGATAACCAGGGCATAG-3'
IL-6	*IL-6* F	5'-AGTTGCCTTCTTGGGACTGA-3'
	IL-6 R	5'-TCCACGATTTCCCAGAGAAC-3'
IL-1β	*IL-1β* F	5'-GCCCATCCTCTGTGACTCAT-3'
	IL-1β R	5'-AGGCCACAGGTATTTTGTCG-3'
IL-23	*IL-23* F	5'-GACTCAGCCAACTCCTCCAG-3'
	IL-23 R	5'-GGCACTAAGGGCTCAGTCAG-3'
TNFα	*TNFα* F	5'-ATGAGAAGTTCCCAAATGGC-3'
	TNFα R	5'-CTCCACTTGGTGGTTTGCTA-3'
Gclm	*Gclm* F	5'-CTTCGCCTCCGATTGAAGATG-3'
	Gclm R	5'-AAAGGCAGTCAAATCTGGTGG-3'
NQO1	*NQO1* F	5'-TATCCTTCCGAGTCATCTCTAGCA-3'
	NQO1 R	5'-TCTGCAGCTTCCAGCTCCTTG-3'
Txn1	*Txn1* F	5'-CATGCCGACCTTCCAGTTTTA-3'
	Txn1 R	5'-TTTCCTTGTTAGCACCGGAGA-3'
GPX2	*GPX2* F	5'-GCCTCAAGTATGTCCGACCTG-3'
	GPX2 R	5'-GGAGAACGGGTCATCATAAGGG-3'
HMOX1	*HMOX1* F	5'-GATAGAGCGCAACAAGCAGAA-3'
	HMOX1 R	5'-CAGTGAGGCCCATACCAGAAG-3'

Supplementary Figure 1 Characterization of transgene expression in brain and IGF-1 signaling in hypothalamus.



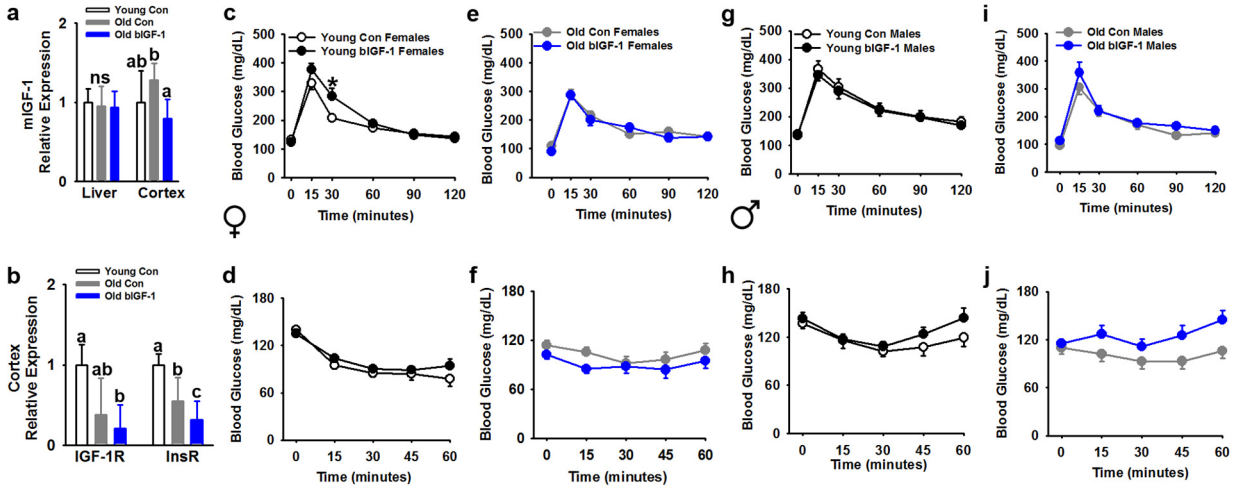
(**a**) Control animals and bIGF-1 animals on DOX diet showed little to no expression, compared to animals off the DOX diet, which greatly overexpress IGF-1 in different regions of the brain (*n*=3 Controls, *n*=3 bIGF-1 + DOX, *n*=3 bIGF-1 - DOX). FB = Forebrain. Cort = Cortex**.** Cer = Cerebellum. BS = Brain Stem. (**b-l**) bIGF-1 mice showed a decrease in IGF-1R expression, while they showed an increase in pAkt and pS6 expression, and no significant change in pERK or InsR expression was seen (*n*=9 Control females, *n*=13 bIGF-1 females; *n*=13 Control males, *n*=11 bIGF-1 males). Logarithmically transformed data from male and female mice was analyzed using a one-way ANOVA. Bars represent mean±SE.* Significantly different from controls, $P \leq 0.05$.

Supplementary Figure 2 Effect of chronic IGF-1 expression in brain on energy balance.



(**a-e**) In female mice, energy expenditure, food intake, spontaneous activity and substrate utilization were similar in bIGF-1 and control animals (*n*=8 Control females, *n*=8 bIGF-1 females). (**f-j**) Likewise, bIGF-1 male mice showed no significant differences in energy expenditure or total substrate utilization. However, males demonstrated a slight increase in Respiratory Exchange Ratio (RER) during the light phase, which was accompanied by an increase in food intake and spontaneous activity (*n*=8 Control males, *n*=7 bIGF-1 males). Bars and lines represent mean±SE. *Significantly different from controls, $P \leq 0.05$.

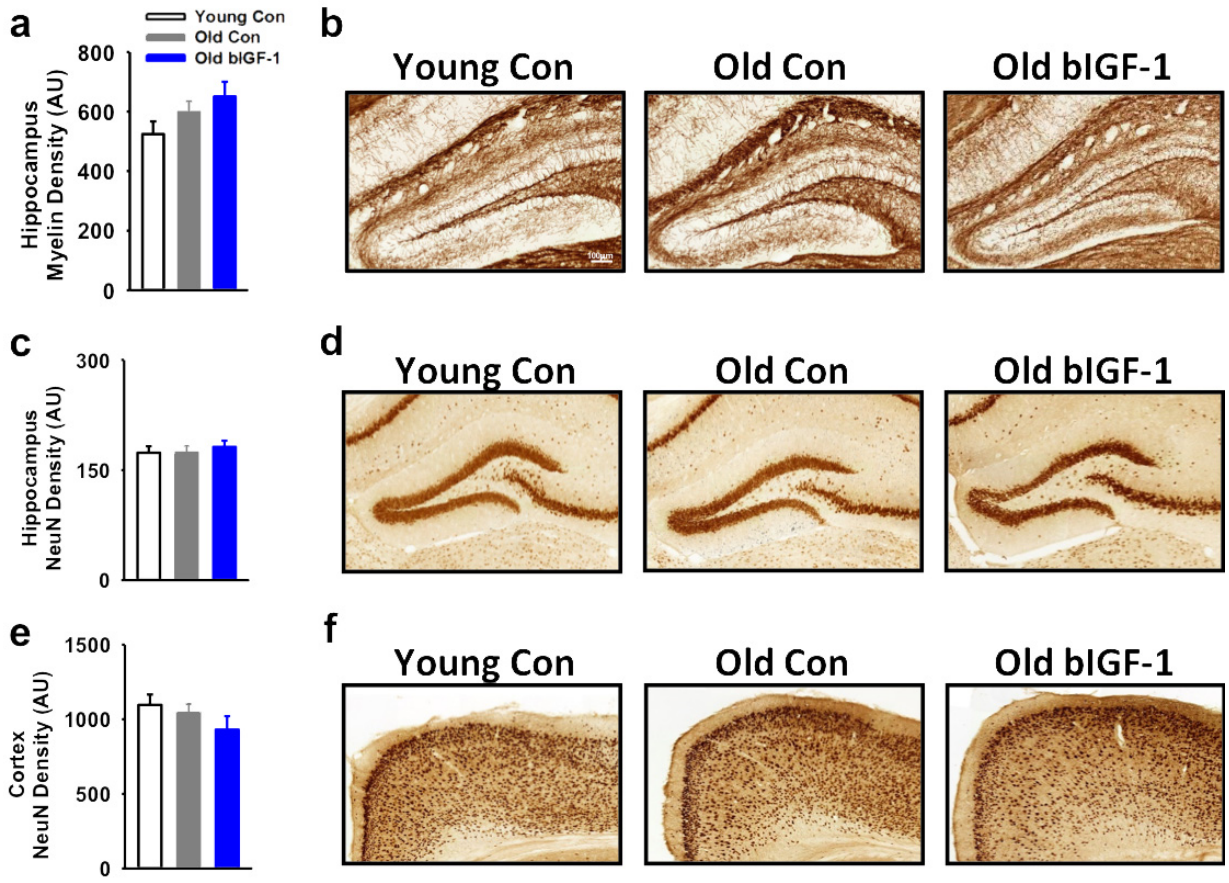
Supplementary Figure 3 Effect of brain IGF-1 overexpression on glucose metabolism and expression of endogenous regulators of IGF-1 signaling with aging.



(**a**) There was no difference in endogenous expression of IGF-1 in liver with age or IGF-1 overexpression. However, a reduction in mIGF-1 expression in cortex of old bIGF-1 mice was observed when compared to age-matched controls [Young Controls (*n*=16 total; *n*=8 females, *n*=8 males), Old Controls (*n*=17 total; *n*=9 females, *n*=8 males), Old bIGF-1 (*n*=17 total; *n*=9 females, *n*=8 males)]. (**b**) IGF-1R and InsR expression in cortex tended to be reduced with age, which was further reduced by IGF-1 overexpression [Young Controls (*n*=16 total; *n*=8 females, *n*=8 males), Old Controls (*n*=17 total; *n*=9 females, *n*=8 males), Old bIGF-1 (*n*=17 total; *n*=9 females, *n*=8 males)]. (**c-d**) Female mice showed a slight difference in glucose tolerance at one point, while no difference was seen in the insulin tolerance test between groups (*n*=12 Young Control females, *n*=11 Young bIGF-1 females) (**e-f**) Glucose tolerance test and insulin tolerance test showed no differences between groups in old females (*n*=12 Old Control females, *n*=12 Old bIGF-1 females). (**g-h**) Young male mice showed no differences in neither glucose or insulin tolerance tests (*n*=11 Young Control males, *n*=15 Young bIGF-1 males). (**i-j**) No difference was seen in old males during glucose or insulin tolerance tests (*n*=14 Old Control males, *n*=11 Old

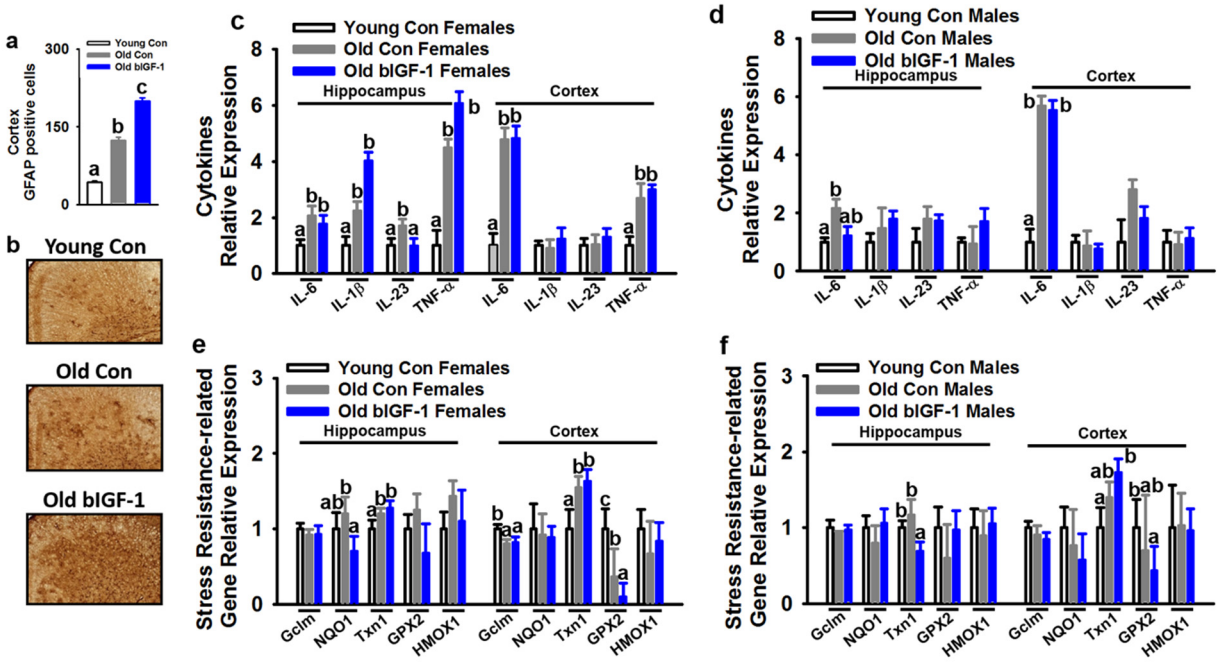
bIGF-1 males). Bars represent mean±SE. Different letters denote a significant difference between groups, $P \leq 0.05$. *Significantly different from controls, $P \leq 0.05$.

Supplementary Figure 4 Effect of aging and IGF-1 overexpression on myelin and neuronal density.



(**a-b**) In hippocampus, no statistical differences were observed among groups for myelin density. [Young Controls (*n*= 16 total; *n*=8 females, *n*=8 males), Old Controls (*n*=17 total; *n*=9 females, *n*=8 males), Old bIGF-1 (*n*=13 total; *n*=6 females, *n*=7 males)]. (**c-f**) Likewise, neuronal density, as determined by NeuN staining, was not different in hippocampus or cortex between groups [Young Controls (*n*= 16 total; *n*=8 females, *n*=8 males), Old Controls (*n*=17 total; *n*=9 females, *n*=8 males), Old bIGF-1 (*n*=13 Old bIGF-1; *n*=6 females, *n*=7 males)]. Bars represent mean±SE.

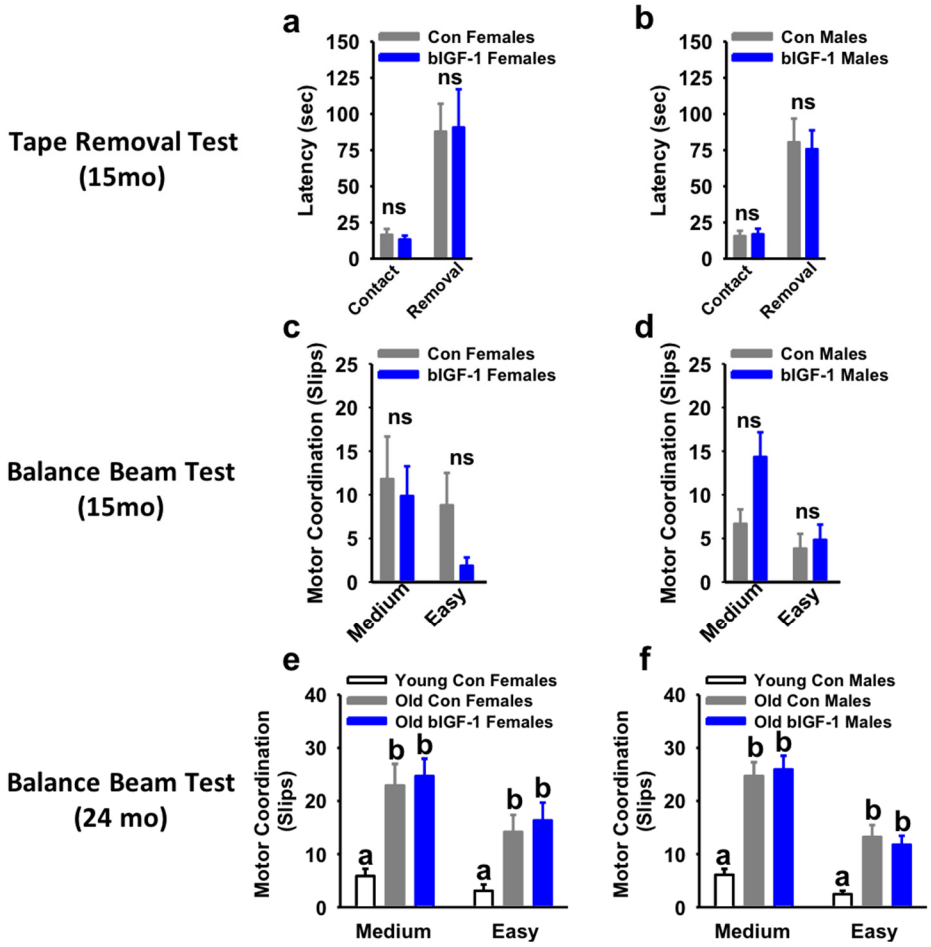
Supplementary Figure 5 Effect of IGF-1 on brain inflammatory markers and stress resistance genes with age.



(**a-b**) HET3 mice showed an age-related increase in astrocytic cells in cortex, which was exacerbated by bIGF-1 overexpression [Young Controls (*n*= 16 total; *n*=8 females, *n*=8 males), Old Controls (*n*=17 total; *n*=9 females, *n*=8 males), Old bIGF-1 (*n*=13 total; *n*=6 females, *n*=7 males)]. (**c-d**) Females and males showed an age-related *IL-6* in cortex, regardless of overexpression. *IL-23* significantly increases with age in female hippocampus and IGF-1 overexpression reduces this age-related change, while no change is seen in cortex or in either tissue in males (*n*=8 Young Control females, *n*=9 Old Control females, *n*=9 Old bIGF-1 females, *n*=8 Young Control males, *n*=8 Old Control males, *n*=8 Old bIGF-1 males). (**e-f**) Nrf2 target gene relative expression was also evaluated in hippocampus and cortex. Females had a marked age-related increase in *Txn1* in both tissues, which was not affected by IGF-1 overexpression, while *Txn1* expression in hippocampus of Old bIGF-1 males was significantly reduced. There was little to no change in *Gclm, NQO1, GPX2 and HMOX1* (*n*=8 Young Control females, *n*=9 Old Control females, *n*=9 Old bIGF-1 females, *n*=8 Young Control males, *n*=8 Old Control males, *n*=8 Old

bIGF-1 males). Bars represent mean±SE. Different letters denote a significant difference between groups, $P \leq 0.05$.

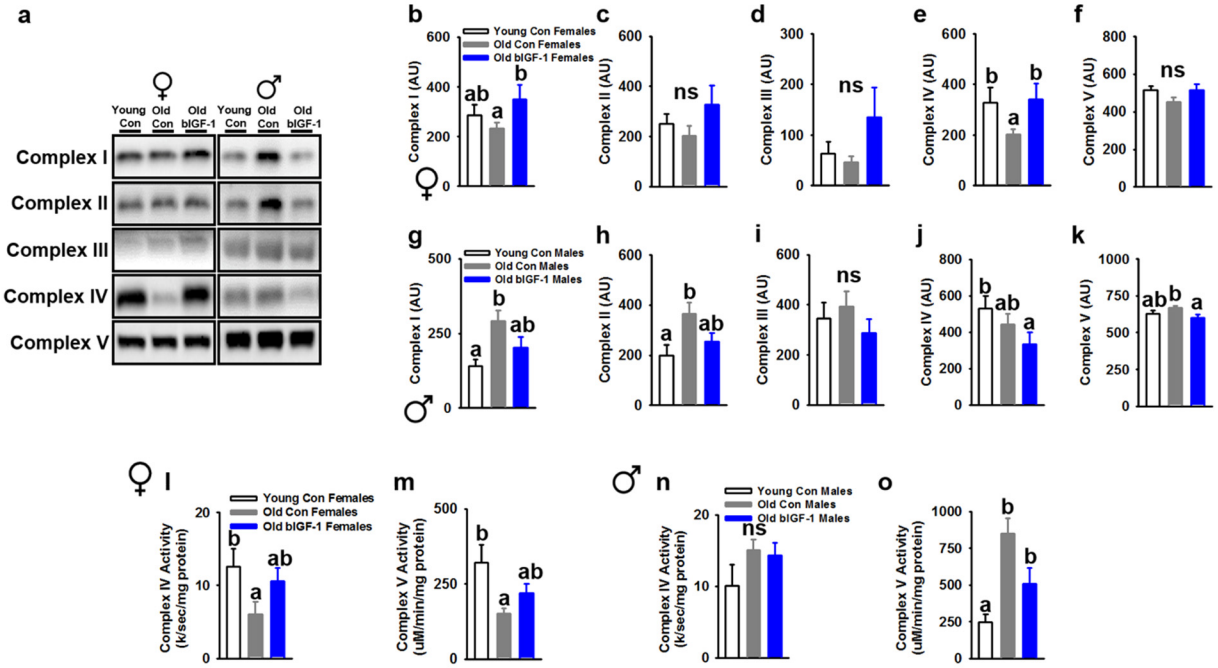
Supplementary Figure 6 Effect of chronic brain IGF-1 overexpression on fine and gross motor coordination.



(**a-d**) At 15mo of age, there was no difference in fine motor coordination by the tape removal test or in gross motor coordination, assessed by the balance beam test, between groups or sexes (*n*=5 Control females, *n*=7 bIGF-1 females, *n*=7 Control males, *n*=7 bIGF-1 males). (**e-f**) At 24mo of age, animals showed an age-related decline in gross motor coordination, when compared to Young (6mo old) Controls, regardless of IGF-1 overexpression (*n*=12 Young Control females, *n*=11 Old Control females, *n*=11 Old bIGF-1 females; *n*=11 Young Control males, *n*=13 Old Control males, *n*=13 Old bIGF-1 males). Logarithmically transformed data was analyzed by one-

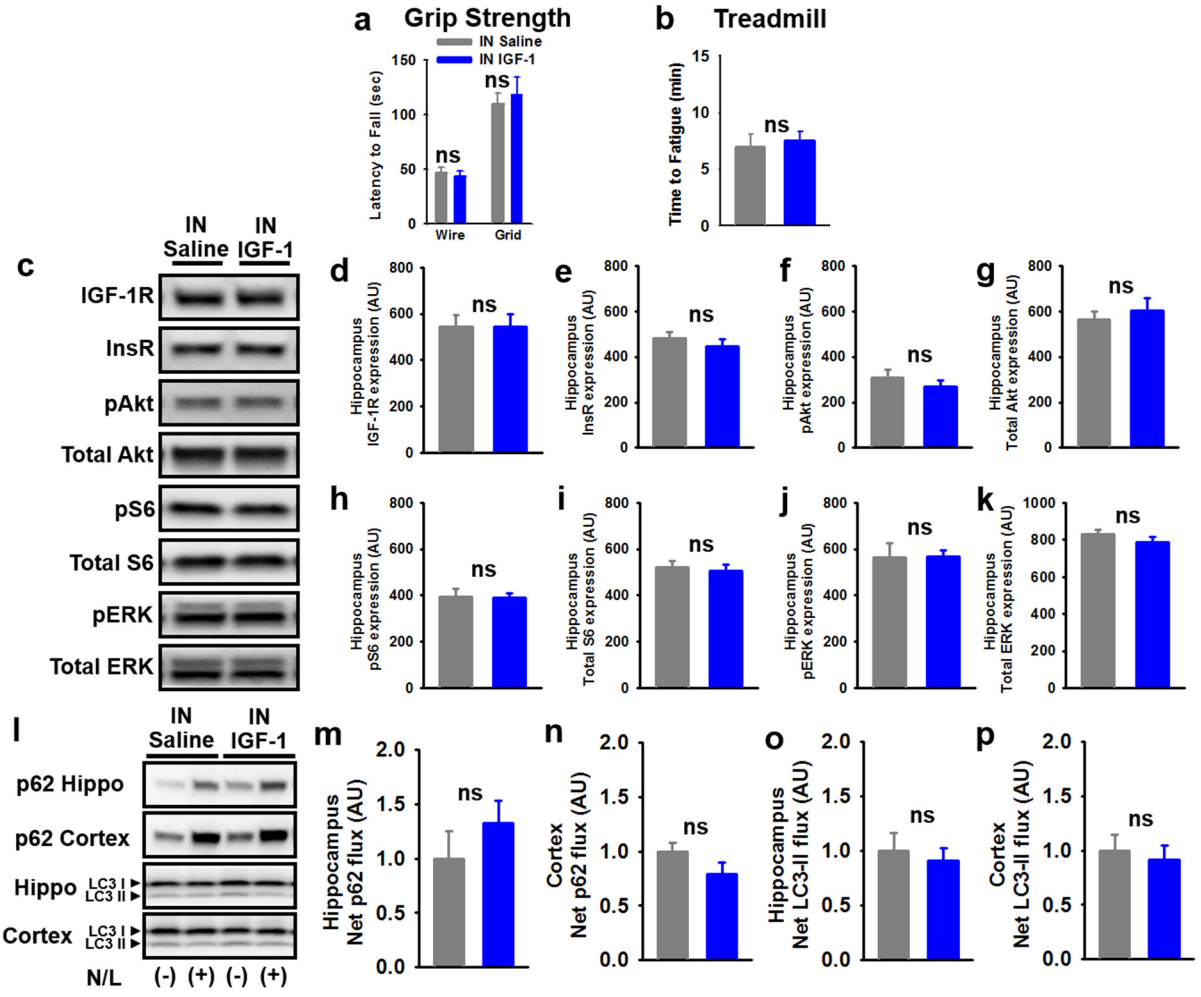
way ANOVA. Bars represent mean±SE. ns = not significant. Letters indicate a significant difference between groups, $P \leq 0.05$.

Supplementary Figure 7 Effect of chronic brain IGF-1 overexpression on mitochondrial electron transport chain content and related activities.



(**a-k**) Mitochondrial content expression levels in muscle, including Complex I, II, III, IV and V (*n*=8 Young Control females, *n*=9 Old Control females, *n*=6 Old bIGF-1 females, *n*=7 Young Control males, *n*=8 Old Control males, *n*=8 Old bIGF-1 males) Females showed an age-related decline in Complex IV, which is rescued by IGF-1 overexpression, and Complex I is increased in Old bIGF-1 females compared to age-matched controls. Males show an age-related increase in Complex I and II that is slightly preserved in Old bIGF-1 male mice. (**l-m**) Complex IV and V activities decreased with age in control females, with a slight preservation in bIGF-1 females (*n*=8 Young Control females, *n*=9 Old Control females, *n*=8 Old bIGF-1 females). (**n-o**) In males, Complex IV activity did not change with age or IGF-1 overexpression, while Complex V activity increased with age and overexpression (*n*=8 Young Control males, *n*=8 Old Control males, *n*=8 Old bIGF-1 males). Logarithmically transformed data was analyzed by one-way ANOVA. Bars represent mean±SE. ns = not significant. Letters indicate a significant difference between groups, $P \leq 0.05$.

Supplementary Figure 8 Effect of short-term intranasal IGF-1 treatment in old male mice on performance, signaling and related markers.



(**a-b**) There were no differences in wire grip strength, inverted grid grip strength or endurance on the treadmill between IN IGF-1-treated and saline-treated groups (*n*=13 IN Saline, *n*=15 IN IGF-1). (**c-k**) There were no differences in hippocampus in downstream signaling protein expression, including IGF-1R and InsR, pAKT, pS6, pERK, total AKT, total S6 or total ERK, between groups (*n*=5 IN Saline, *n*=6 IN IGF-1). (**l-p**) The net autophagy flux, assessed by p62 and LC3 II expression levels, both in hippocampus and cortex showed no differences between groups (*n*=5 IN Saline, *n*=6 IN IGF-1). Logarithmically transformed data was analyzed by one-way ANOVA. N/L=NH_4Cl/Leupeptin. ns=not significant. Bars represent mean±SE.