Exercise Attenuates Obesity-Related Cognitive and Sleep-Circadian Dysfunctions by Attenuating Neuroinflammation via JAK/STAT in Sex and Age Specific Manner

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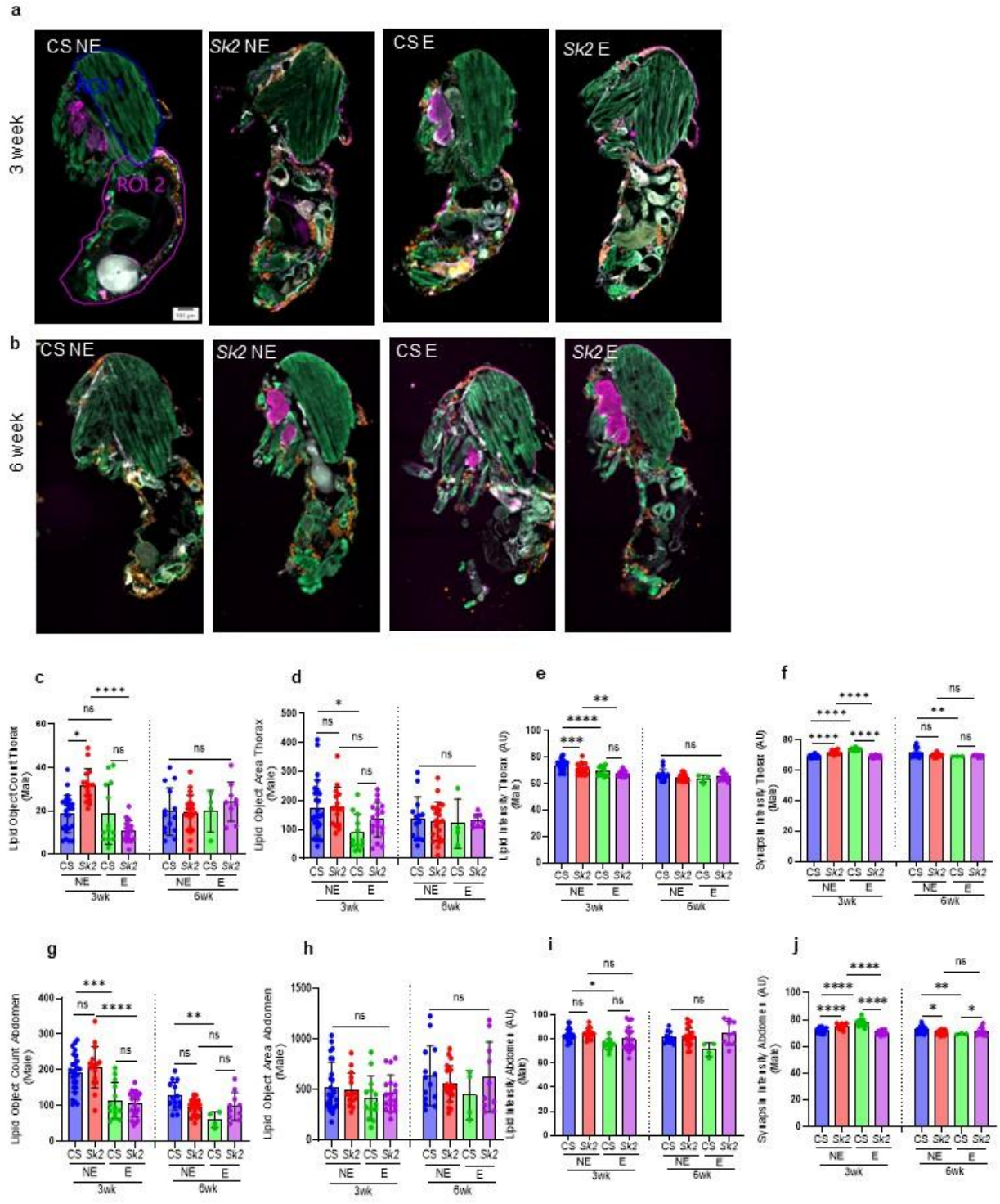
Supplementary Information

Supplementary Figures 1-5

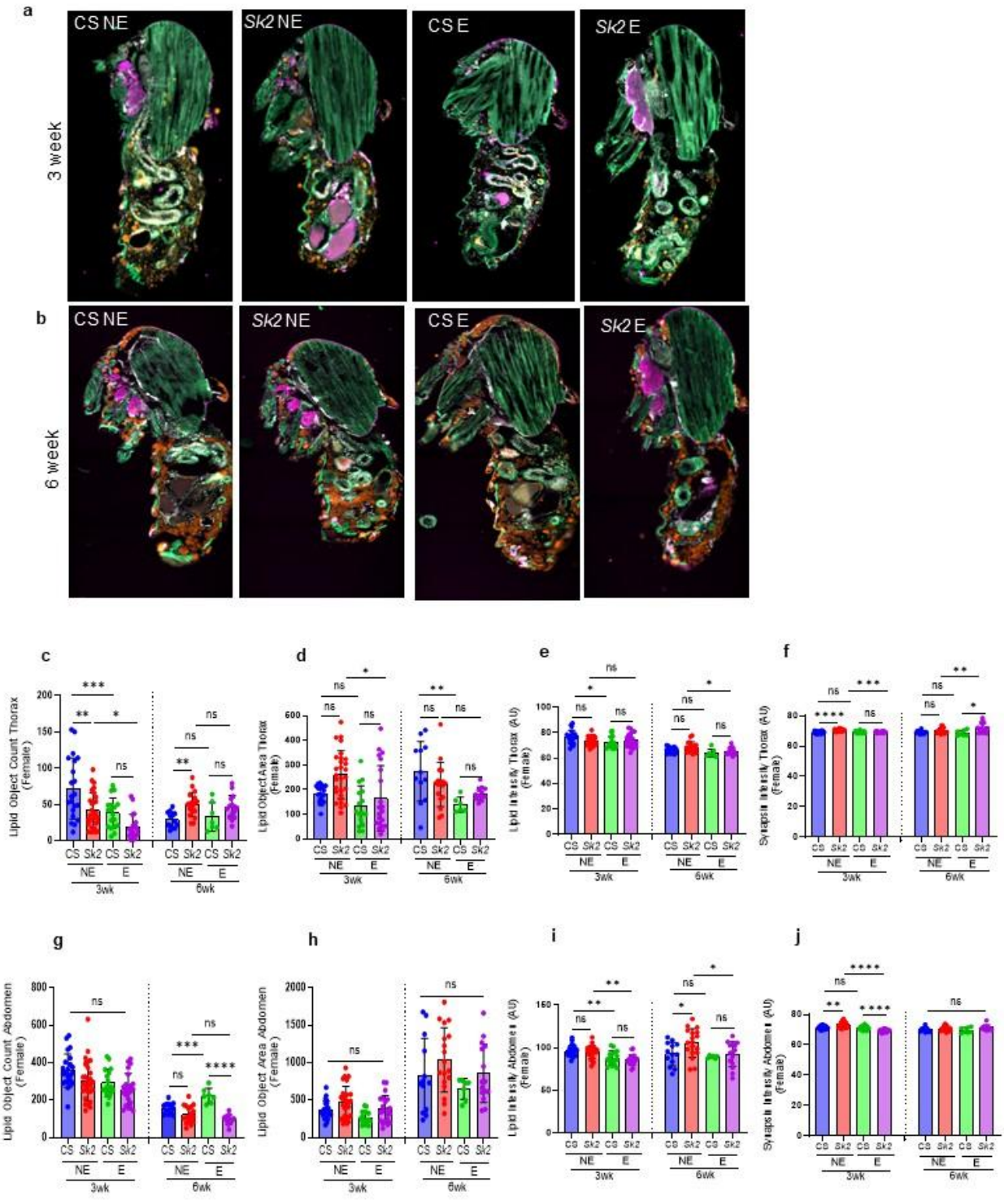
Supplementary Fig. 1



Supplementary Fig. 1 Effects of exercise on thorax and abdomen morphology in male *CS* and *Sk2* groups at 3 and 6 weeks.

(a, b) Representative images showing lipid accumulation (orange), phalloidin staining (green), synapsin (purple, indicating synaptic loss and neurodegeneration), and nuclei (DAPI, white) in the thorax and abdomen of male and female *Drosophila* from CS (control) and *Sk2* mutant models. Quantification of lipid object count/ area **(c, d, g, h),** lipid and synapsin intensity **(e, f, l, j)** in the thorax and abdomen of male flies. Exercise significantly reduced obesity-associated lipid accumulation in both the thorax and abdomen of 3-week-old male *Drosophila*. Compared to age-matched controls, *Sk2* mutant flies exhibited significantly higher lipid accumulation and synapsin intensity, indicating synaptic dysfunction, along with decreased lipid intensity in the thorax and no change in the abdomen. Exercise intervention led to a notable reduction in lipid content and synapsin levels, suggesting improved lipid metabolism and synaptic health. Images are representative of three independent experiments. Statistical analysis was performed using two-way ANOVA with Tukey's multiple comparisons test. (ns = $p > 0.05$,
* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.0001$). Data are shown as Mean ± SD.

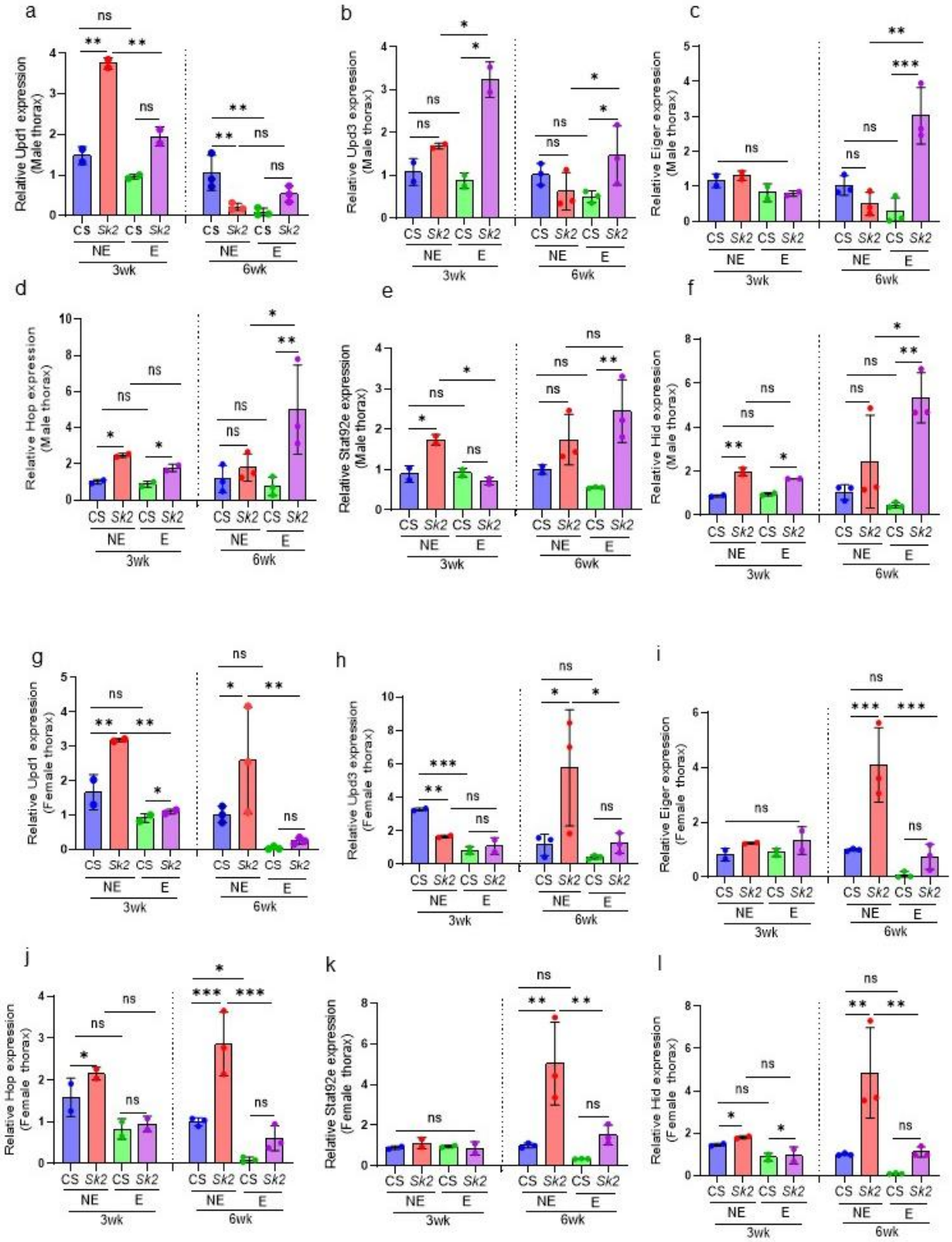
Supplementary Fig. 2



Supplementary Fig. 2 Effects of exercise on thorax and abdomen morphology in female CS and Sk2 groups at 3 and 6 weeks.

(a, b) Representative images showing lipid accumulation (orange), phalloidin staining (green), synapsin (purple, indicating synaptic loss and neurodegeneration), and nuclei (DAPI, white) in the thorax and abdomen of male and female Drosophila from CS (control) and Sk2 mutant models. Quantification of lipid object count/ area (c, d, g, h), lipid and synapsin intensity (e, f, I, j) in the thorax and abdomen of male flies. Exercise significantly reduced obesity-associated lipid accumulation in the thorax of 3-week-old male Drosophila. Compared to age-matched controls, Sk2 mutant flies exhibited lower lipid accumulation and higher synapsin intensity, indicating synaptic dysfunction, along with decreased lipid intensity in the thorax and in the abdomen. Exercise intervention led to a notable reduction in lipid content and synapsin levels, suggesting improved lipid metabolism and synaptic health. Images are representative of three independent experiments. Statistical analysis was performed using two-way ANOVA with Tukey's multiple comparisons test. (ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.0001$). Data are shown as Mean ± SD.

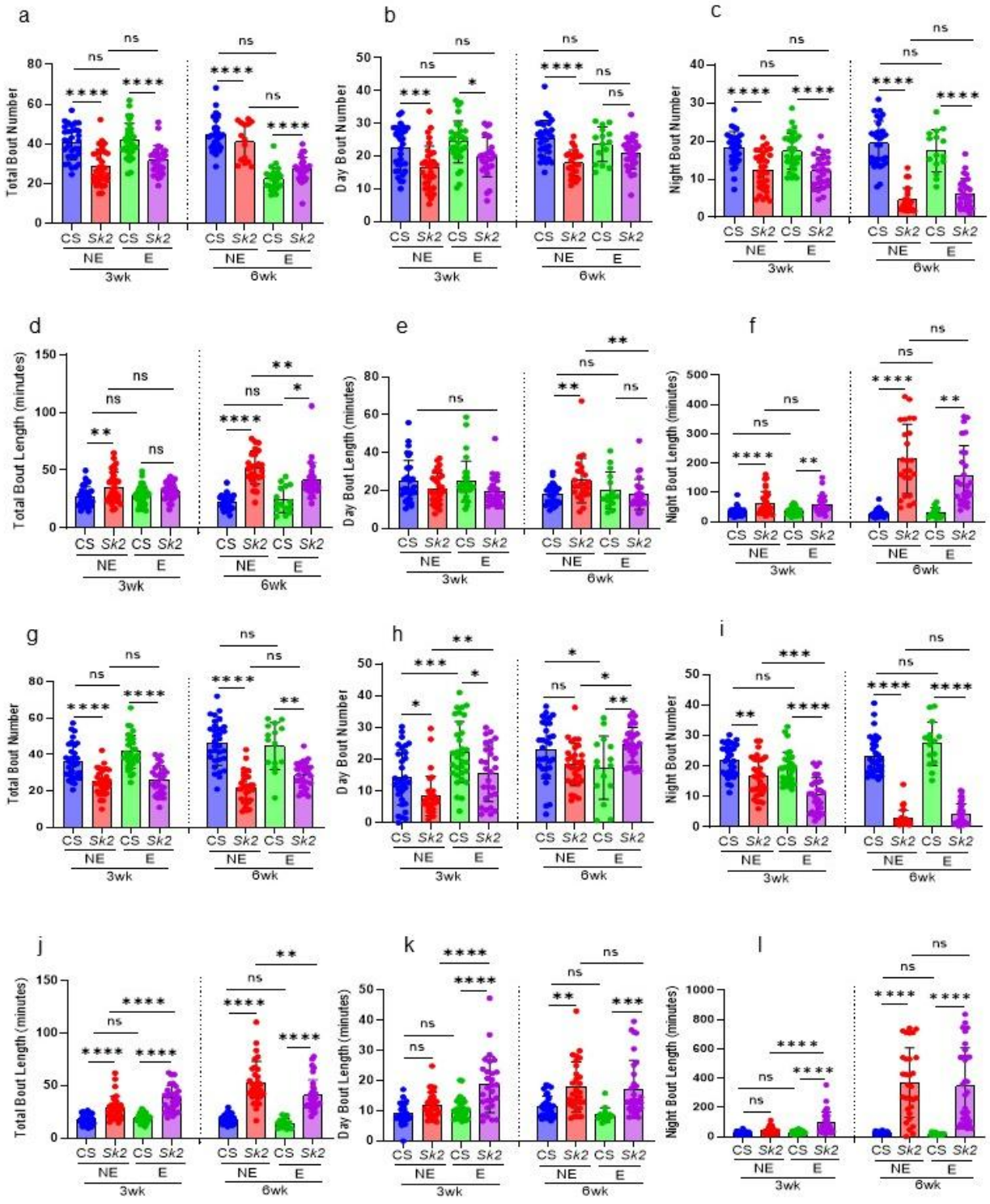
Supplementary Fig. 3



Supplementary Fig. 3 Effects of exercise on cytokine, signaling, and apoptosis-related gene expression in female and male thorax in CS and Sk2 groups at 3 and 6 weeks.

qPCR analysis in male thorax (a-f) further revealed elevated expression of inflammatory cytokines (Upd1, and Upd3) and their signaling components (Hop, Stat), along with the pro-inflammatory marker Eiger in Sk2 mutant brains at 3 and 6 weeks. Exercise (E) strongly down-regulated Upd1 their signaling components (Hop, Stat), along with the pro-inflammatory marker Eiger, which is not changed in Sk2 mutant thorax at 3 and but increasing significantly with exercise at 6 weeks, indicating activation of JAK/STAT signaling. Conversely, pro-apoptotic gene hid showed marked reductions with exercise, particularly at 6 weeks, suggesting suppression of apoptotic pathways. Overall, exercise enhanced cytokine signaling and reduced apoptosis-related gene expression, with effects more pronounced in Sk2 females at 6 weeks. qPCR analysis in female thorax (g-l) further revealed elevated expression of inflammatory cytokines (Upd1, and Upd3) and their signaling components (Hop, Stat), along with the pro-inflammatory marker Eiger in Sk2 mutant brains at 3 and 6 weeks. Pro-apoptotic gene Hid were also changed, indicating that obesity promotes both inflammation and apoptosis. Exercise significantly downregulated these genes in Sk2 mutants, suggesting a protective effect on brain health. Importantly, exercise had no impact on gene expression in control flies. Statistical analysis was performed using two-way ANOVA with Tukey's multiple comparisons test (ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.0001$). Quantitative analyses are presented as mean ± SD with individual data points.

Supplementary Fig. 4



Supplementary Fig. 4 Effects of exercise on sleep bouts and bout length in CS and *Sk2* male *Drosophila* at 3 and 6 weeks. Obesity alters bout length and number in both male **(a-f)**

and female **(g-l)** *Drosophila*. Obese flies displayed increased total and night-time bout lengths, along with reduced bout numbers compared to controls at 3 and 6 weeks. These effects were more prominent in females. *Sk2* mutant flies without exercise (Non-Exercised, NE) showed significantly longer bout lengths and fewer bouts during both day and night. Structured exercise (Exercised, E) partially rescued these deficits, leading to improvements in total, daytime, and nighttime bout lengths, as well as in bout numbers of effects that were more pronounced in females. Data were analyzed using two-way ANOVA followed by Tukey's multiple comparisons test (n = 32 flies per group). Error bars represent mean ± SD. Significance: ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.0001$.

Supplementary Fig. 5

Summary Table		3 week	6 week	3 week	6 week	3 week	6 week	3 week	6 week
		Genotype *Sk2* NE vs CS NE	Genotype *Sk2* NE vs CS NE	Effect of exercise CS NE vs CS E		*Sk2* E vs CS E		*Sk2* E vs *Sk2* NE	
		♂ *Sk2* ♀	♂ *Sk2* ♀	♂ *Sk2* ♀	♂ *Sk2* ♀	♂ *Sk2* ♀	♂ *Sk2* ♀	♂ *Sk2* ♀	♂ *Sk2* ♀
Sleep pattern	Total Sleep	Down*/ NS	Down*/ NS	Up*/Up*	Up*/Up*	Down*/NS	Down*/NS	NS/Up*	NS/Up*
	Day Sleep	Down*/ NS	Down*/ NS	Up*/Up*	Up*/Up*	Down*/NS	Down*/NS	NS/Up*	NS/Up*
	Night Sleep	Up*/ Up*	Up*/ Up*	NS/Up*	NS/Up*	NS/NS	NS/NS	NS/NS	NS/NS
	Total Activity	NS/ Up*	NS/ Up*	Down/NS*	Down/NS*	Up*/NS	Up*/NS	NS/Down*	NS/Down*
	Day Activity	Up*/ Up*	Up*/ Up*	Down/NS*	Down/NS*	Up*/NS	Up*/NS	Down*/Down*	Down*/Down*
	Night Activity	NS/Down*	NS/Down*	NS/Down*	NS/Down*	NS/NS	NS/NS	NS/NS	NS/NS
	Memory impairment	Memory Impairment	Memory Impairment	Learning	Learning	Learning	Learning	Learning	Learning
Lipid accumulation	Head lipid object count	NS/NS*	NS/NS*	Up*/NS*	Up*/NS*	Down*/Up*	Down*/Up*	Down*/NS	Down*/NS
	Head lipid object area	Down*/NS	Down*/NS	Down*/NS	Down*/NS	Up*/Up*	Up*/Up*	Up*/NS	Up*/NS
	Head lipid Intensity	NS/NS	NS/NS	NS/NS	NS/NS	Up*/Up*	Up*/Up*	NS/NS	NS/NS
	Synapsin	Up*/NS	Up*/NS	Up*/NS	Up*/NS	Down*/NS	Down*/NS	Down*/NS	Down*/NS
Gene expression	Upd1	Up*/Up*	Up*/Up*	NS/Up*	NS/Up*	Down*/NS	Down*/NS	NS/Down*	NS/Down*
	Upd2	Down*/Up*	Down*/Up*	Up*/Up*	Up*/Up*	Up*/NS	Up*/NS	NS/Down*	NS/Down*
	Upd3	Up*/Up*	Up*/Up*	Up*/NS	Up*/NS	NS/NS	NS/NS	NS/NS	NS/NS
	Stat92e	NS/Up*	NS/Up*	NS/NS	NS/NS	Up*/Up*	Up*/Up*	Up*/Down*	Up*/Down*
	Hop	Up*/Up*	Up*/Up*	NS/Down*	NS/Down*	Up*/NS	Up*/NS	NS/Down*	NS/Down*
	Eiger	Up*/Up*	Up*/Up*	NS/Up*	NS/Up*	NS/Down*	NS/Down*	Down*/Down*	Down*/Down*
	Hid	NS/Up*	NS/Up*	NS/NS	NS/NS	NS/Up*	NS/Up*	NS/Down*	NS/Down*
	Reaper	Down*/Up*	Down*/Up*	NS/NS	NS/NS	Down*/Up*	Down*/Up*	NS/Down*	NS/Down*

Genotype* Sk2 compared to CS, Effect of exercise CS compared to CS genotype, Effect of exercise Sk2 compared to CS exercise, Genotype Sk2 compared to Sk2 exercise

Supplementary Fig. 5. Summary of sleep, memory, lipid accumulation, and gene expression changes associated with Sk2 genotype and exercise across age. Summary table showing the direction of change (Up, Down, or NS; with statistical significance indicated by *) for multiple phenotypic and molecular parameters. Comparisons include Sk2 NE vs CS genotype, effect of exercise (CS E vs CS), and exercise in Sk2 background (Sk2 E vs Sk2 NE) at 3-week and 6-week time points. Sleep parameters include total sleep, day/night sleep, sleep activity, and night activity. Memory outcomes distinguish impairment versus learning. Lipid accumulation measures include head lipid count, area, and intensity, along with synapsin levels. Gene expression analysis covers inflammatory and signaling-related genes (Upd1, Upd2, Upd3, Stat92e, Hop, Eiger, Hid, and Reaper). Color coding indicates upregulation (red), downregulation (blue), or no significant change (NS), providing an integrated overview of age-, genotype-, and exercise-dependent effects.