METHODS:

Myograph studies

After removing the adhering connective tissue, distal saphenous artery was cut into segments of ~ 2 mm in length and vessel functions were measured as previously described (1-4). Briefly, each segment was mounted in a Multi Myograph System (Danish Myo Technology). The organ chamber was filled with 6 ml of physiological salt solution buffer (130 mM NaCl, 4.7 mM KCl, 1.6 mM $CaCl_2$, 1.17 mM $MgSO_4$, 1.18 mM KH_2PO_4, 14.9 mM $NaHCO_3$, 0.026 mM EDTA and 5.5 mM glucose, pH 7.4), which was constantly bubbled with 95% O_2/5% CO_2 and maintained at 37°C. Each ring was stretched initially to an optimal tension (5 mN) and then allowed to stabilize at baseline tone. After pre-constriction with phenylephrine (2 μM), vasodilatory responses to various concentrations of acetylcholine (Ach) were recorded.

Measurement of serum insulin, FFA and NO levels

Serum insulin concentrations were measured using a rat insulin ELISA assay kit. Serum FFA concentrations were measured using a HR Series NEFA-HR (2) kit, which is based on an in vitro enzymatic method for the quantitative determination of non-esterified fatty acids. Serum NO levels were quantified using the 280i Nitric Oxide Analyzer (GE Analytical Instruments). In brief, ice-cold ethanol was added into plasma samples at a ratio of 2:1. The mixture was kept at 0°C for 30 min and then centrifuged at 16,000 g for 5 min. The supernatant was then used for NO analysis based on a gas phase chemiluminescent reaction between NO and ozone, as described (5).

References:

1. Zhao L, Fu Z, Wu J, Aylor KW, Barrett EJ, Cao W, Liu Z: Globular adiponectin ameliorates metabolic insulin resistance via AMPK-mediated restoration of microvascular insulin responses. The Journal of Physiology 2015;593:4067-4079

2. Zhao L, Fu Z, Wu J, Aylor Kevin W, Barrett Eugene J, Cao W, Liu Z: Inflammation-induced microvascular insulin resistance is an early event in diet-induced obesity. Clinical Science 2015;129:1025-1036

3. Fu Z, Zhao L, Chai W, Dong Z, Cao W, Liu Z: Ranolazine recruits muscle microvasculature and enhances insulin action in rats. The Journal of Physiology 2013;591:5235-5249

4. Fu Z, Zhao L, Aylor KW, Carey RM, Barrett EJ, Liu Z: Angiotensin-(1–7) recruits muscle microvasculature and enhances insulin's metabolic action via Mas receptor. Hypertension 2014;63:1219-1227

5. Chai W, Fu Z, Aylor KW, Barrett EJ, Liu Z: Liraglutide prevents microvascular insulin resistance and preserves muscle capillary density in high-fat diet-fed rats. Am J Physiol Endocrinol Metab 2016;311:E640-E648

ANTIBODY AND ASSAY KITS MANUFACTURERS AND RRIDS:

Goat anti-mouse/rat CD31/PECAM-1 antibody: R&D Systems, MN; RRID: AB_2161028

Alexa Fluor Plus 647 donkey anti-goat secondary antibody: Thermo Fisher Scientific, MA; RRID:AB_2762840

Rabbit anti-mouse/rat CD68 antibody: Abcam, Cambridge, UK; RRID:AB_2922954

Alexa Fluor 488 goat anti-rabbit secondary antibody: Thermo Fisher Scientific, MA; RRID:AB_143165

Rat insulin ELISA assay kit: ALPCO, Salem, NH; RRID: AB_2820242

HR Series NEFA-HR (2) kit: FUJIFILM Wako Pure Chemical Corporation, Japan; RRID:SCR_021034

Primary antibodies against total eNOS (RRID:AB_329863), phospho-eNOS (Ser^{1177}) (RRID:AB_329837), total Akt (RRID:AB_32982), phospho-Akt (Ser^{473}) (RRID:AB_2315049), total ERK1/2 (RRID:AB_390779), phospho-ERK (Thr^{202}/Tyr^{204}) (RRID:AB_331646), total AMPKα (RRID:AB_10622186), phospho-AMPKα (Thr^{172}) (RRID:AB_331250), and β-actin (RRID:AB_330288) were from Cell Signaling Technology (Danvers, MA). Primary antibody against VEGF (RRID:AB_922745)was from Novus Biologicals (Centennial CO), anti-VEGF receptor 1 antibody (RRID:AB_778798) and anti-VEGF receptor 2 antibody (RRID:AB_2132349) were from Abcam (Cambridge, UK).

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure1. Body weight and its relationships with fat mass, and microvascular or metabolic insulin responses. A) Final body weight. B) Correlation between body weight and epididymal fat mass. C) Correlation between body weight and MBV at 30 min of insulin clamp. D) Correlation between body weight and MBV at 60 min of insulin clamp. E) Correlation between body weight and MBV at 120 min of insulin clamp. F) Correlation between body weight and steady-state GIRs. All by simple linear regression analysis. Lir – Liraglutide. Ex – Exercise.

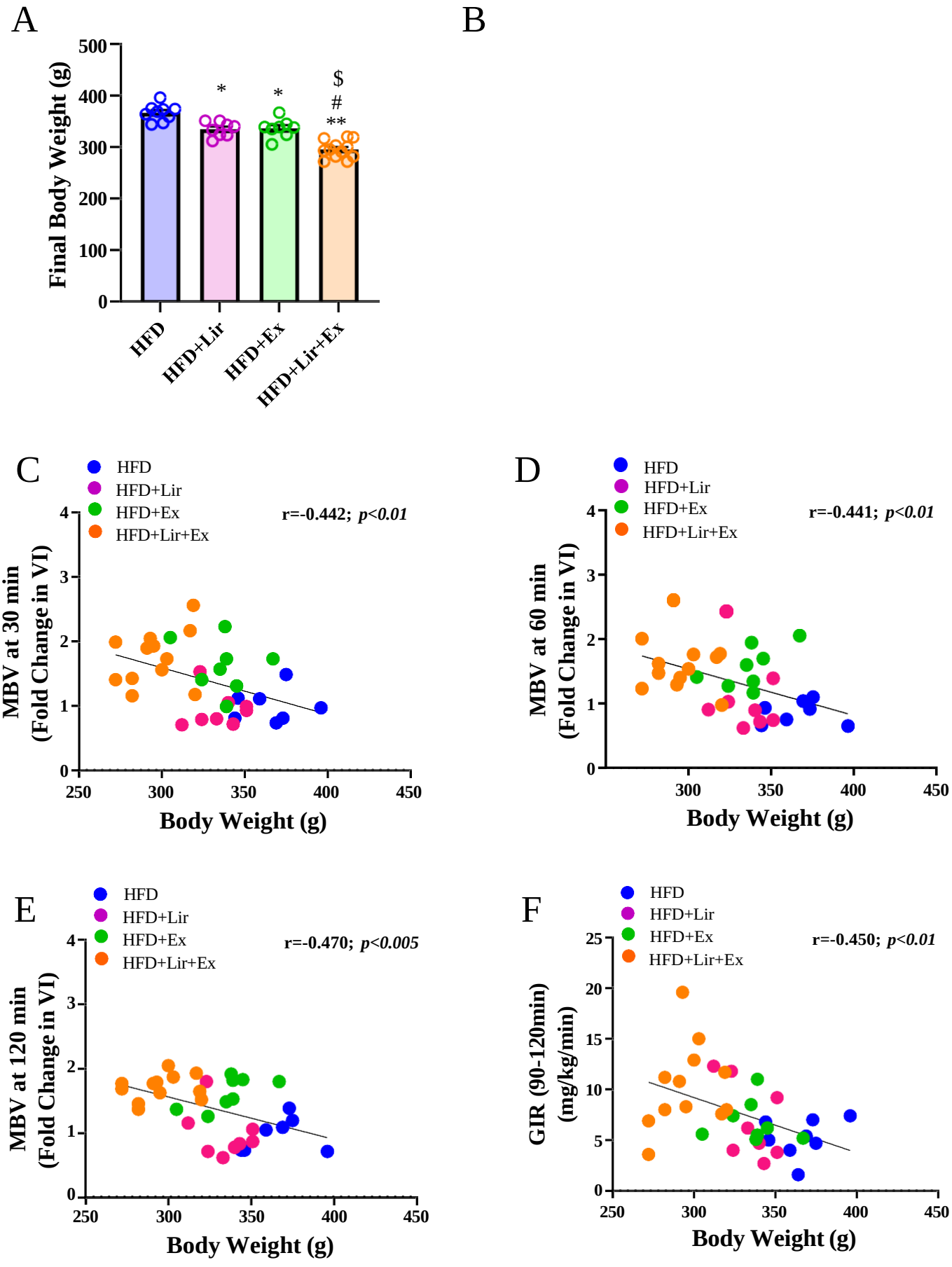
Supplemental Figure 2. Correlation between MBF and body adiposity at 30 min (A), 60 min (B) and 120 min (C) of insulin clamp. Lir – Liraglutide. Ex – Exercise.

Supplemental Figure 3. Effects of exercise, with or without liraglutide, on insulin signalling intermediates and AMPK phosphorylation in aorta. A) Representative Western blot images of phosphorylated and total proteins in aorta. B) Akt phosphorylation (Ser473). C) eNOS phosphorylation (Ser1177). D) ERK1/2 phosphorylation (Thr202/Tyr204). E) AMPK phosphorylation (Thr172). n= 4 per group. Values are mean ± SEM. * $p<0.05$, ** $p<0.01$ vs. HFD alone group. ANOVA with Tukey's post-hoc analysis. Lir – Liraglutide. Ex – Exercise.

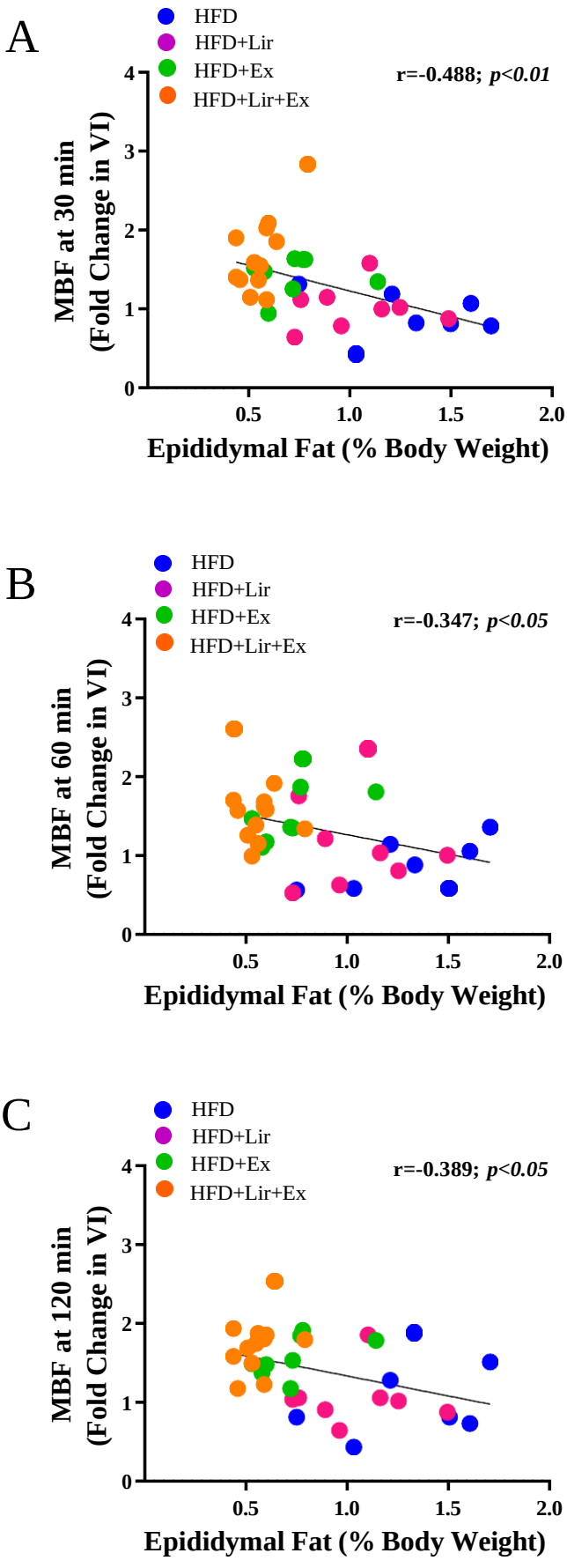
Supplemental Figure 4. Effects of exercise, with or without liraglutide, on insulin signalling intermediates, AMPK phosphorylation, and VEGF and its receptor proteins expression in skeletal muscle. A) Representative Western blot images of phosphorylated and total proteins in skeletal muscle. B) Akt phosphorylation (Ser473). C) ERK1/2 phosphorylation (Thr202/Tyr204). D) AMPK phosphorylation (Thr172). E) VEGF expression. F) VEGF receptor 1 (VEGFR1)

expression. G) VEGF receptor 2 (VEGFR2). n= 4 per group. Values are mean ± SEM. ANOVA with Tukey's post-hoc analysis. Lir – Liraglutide. Ex – Exercise.

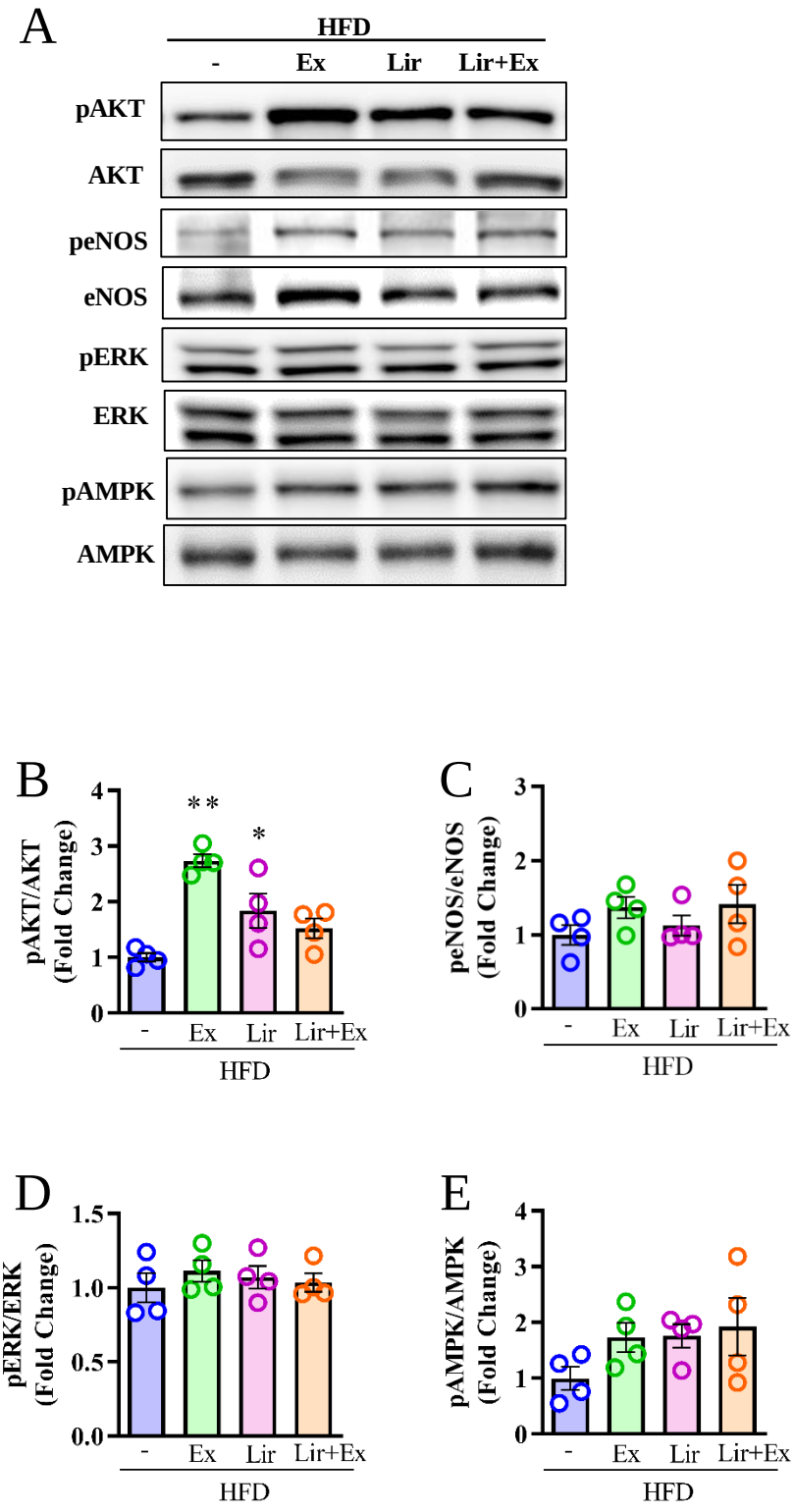
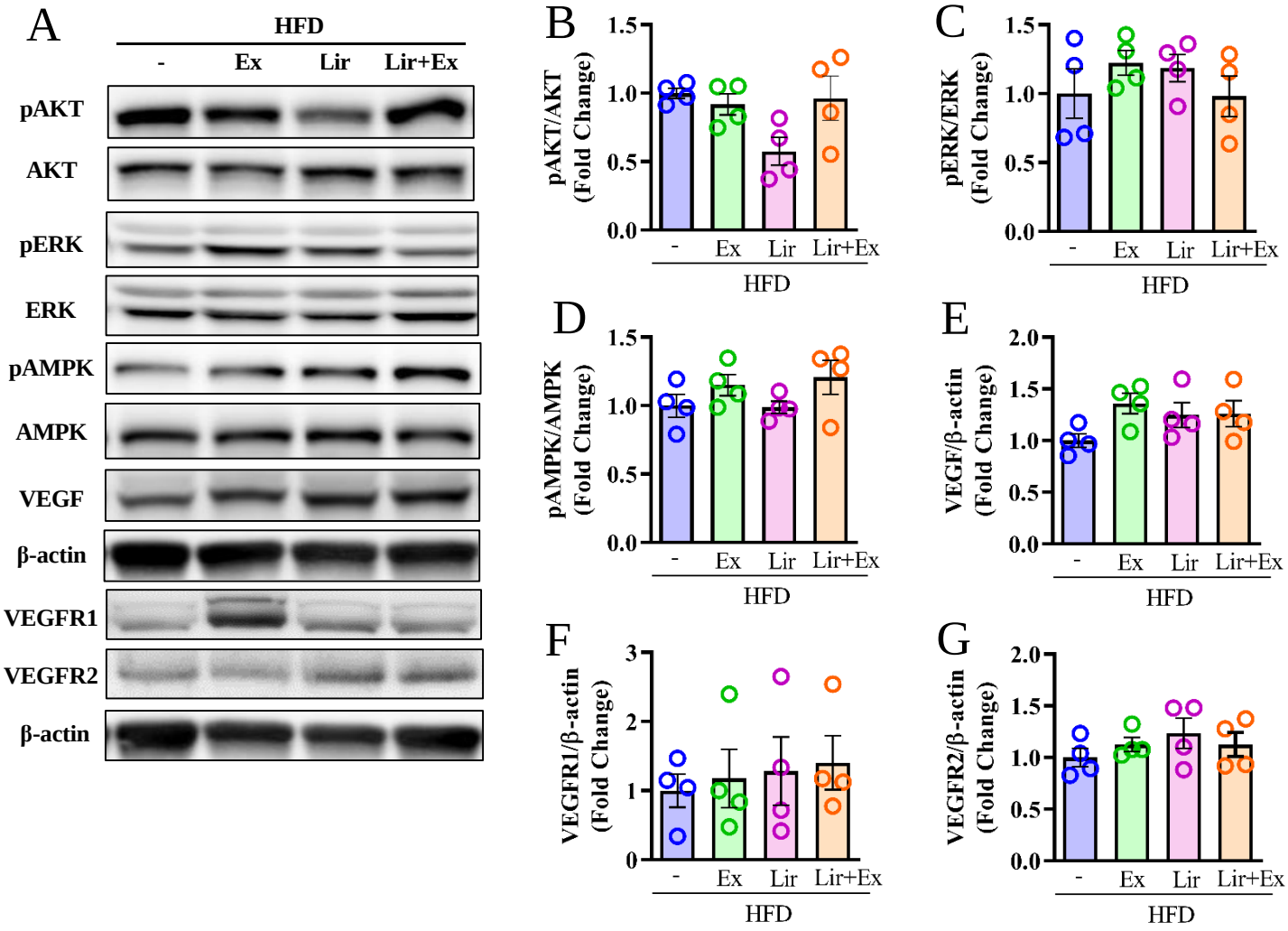
Supplemental Fig.1



Supplemental Fig. 2



Supplemental Fig. 3



Supplemental Fig. 4