

# **SUPPLEMENTAL MATERIAL**

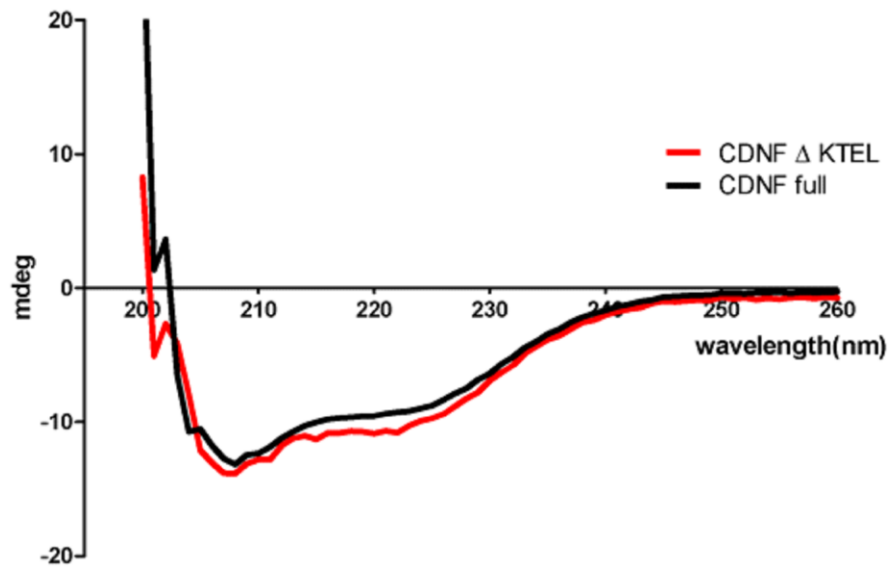
**Table S1. Left ventricular developed pressure of isolated perfused rat hearts in mmHg.**

<b>Groups</b>	<b>Time</b>	<b>Rat hearts LVDP [mmHg]</b>	
<b>Control (n=5)</b>	baseline	103.1	± 8.9
<b>preCDNF (n=5)</b>	baseline	97.9	± 14.3
	after intervention	102.5	± 11.2
<b>postCDNF(n=5)</b>	baseline	101.9	± 18.2
<b>preCDNF + Wortmannin (n=5)</b>	baseline	95.7	± 8.3
	after intervention	88.3	± 10.2
<b>preCDNF + Chelerythine (n=5)</b>	baseline	105.1	± 11.1
	after intervention	92.6	± 12.6
<b>preCDNF + Rothlerin (n=5)</b>	baseline	100.8	± 8.7
	after intervention	91.4	± 6.9
<b>preCDNF + AG490 (n=5)</b>	baseline	115.4	± 14.6
	after intervention	95.9	± 11.8
<b>postCDNF+ Wortmannin (n=5)</b>	baseline	111.5	± 10.7
	after intervention	89.2	± 14.3
<b>preCDNF + TRPQTEL(n=5)</b>	baseline	102.3	± 15.1
	after intervention	96.1	± 7.2
<b>preCDNF + THPKTEL(n=5)</b>	baseline	97.6	± 8.1
	after intervention	91.7	± 13.4
<b>preCDNF + DRATSAL(n=5)</b>	baseline	99.3	± 13.2
	after intervention	102.4	± 6.9
<b>PreCDNF + aβ-CDNF(n=5)</b>	baseline	107.4	± 10.4
	after intervention	98.3	± 8.2
<b>PreCDNF-ΔKTEL (n=5)</b>	baseline	112.3	± 6.9
	after intervention	110.9	± 6.7
<b>No-I/R (n=6) (mitochondria assay)</b>	baseline	101.6	± 6.9
<b>I/R (n=6) (mitochondria assay)</b>	baseline	104.2	± 8.8
	ischemia 5 min	0.0	± 0.0
	ischemia 30 min	0.0	± 0.0
	reperfusion 10 min	14.1	± 16.3
<b>preCDNF (n=6) (mitochondria assay)</b>	baseline	110.8	± 7.5
	after intervention	105.3	± 15.9
	ischemia 5 min	0.0	± 0.0
	ischemia 30 min	0.0	± 0.0
	reperfusion 10 min	43.9	± 7.6 *

<b>PostCDNF (n=6)</b> <b>(mitochondria assay)</b>	baseline	99.8	± 10.9
	after intervention	100.2	± 8.3
	ischemia 5 min	0.0	± 0.0
	ischemia 30 min	0.0	± 0.0
	reperfusion 10 min	33.7	± 11.4
<b>preCDNF +Wortmannin</b> <b>(n=6) (mitochondria assay)</b>	baseline	113.3	± 11.6
	after intervention	102.0	± 6.1
	ischemia 5 min	0.0	± 0.0
	ischemia 30 min	0.0	± 0.0
	reperfusion 10 min	15.9	± 14.3 <sup>&amp;</sup>
<b>postCDNF+Wortmannin</b> <b>(n=6) (mitochondria assay)</b>	baseline	102.1	± 19.7
	after intervention	93.5	± 15.4
	ischemia 5 min	0.0	± 0.0
	ischemia 30 min	0.0	± 0.0
	reperfusion 10 min	11.3	± 12.5 <sup>\$</sup>

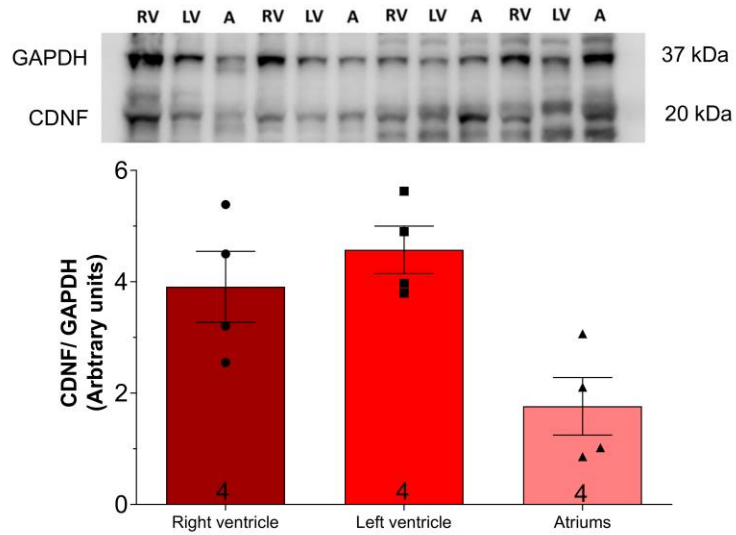
Developed left ventricular pressure (LVDP) was calculated as the difference between the systolic and the end-diastolic pressure. LVDP values (mmHg) were measured at different time points: at end of stabilization period (baseline), **after the intervention and** at 5 and 30 min of ischemia, and at 10 min of reperfusion (**for mitochondria assay only**). Mean ± SEM. \* $P < 0.05$  vs I/R, &  $< 0.05$  vs preCDNF,  $^{\$}P < 0.05$  vs postCDNF with two-way ANOVA followed by Bonferroni post-hoc tests for LVDP and LVEDP analysis.

Figure S1. CDNF $\Delta$ KTEL presents the same secondary structure of CDNF.



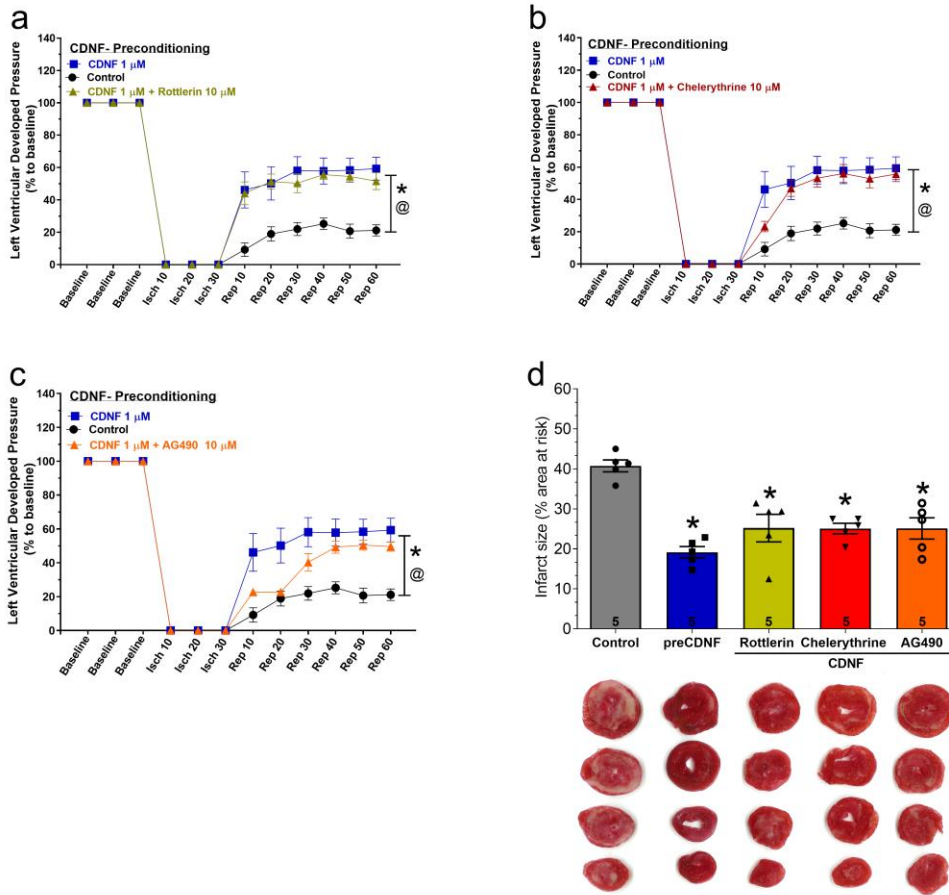
Circular dichroism spectra of both proteins at 10  $\mu$ M show the typical profile of alpha-helix rich proteins.

**Figure S2. Endogenous CDNF expression in rat heart chambers.**



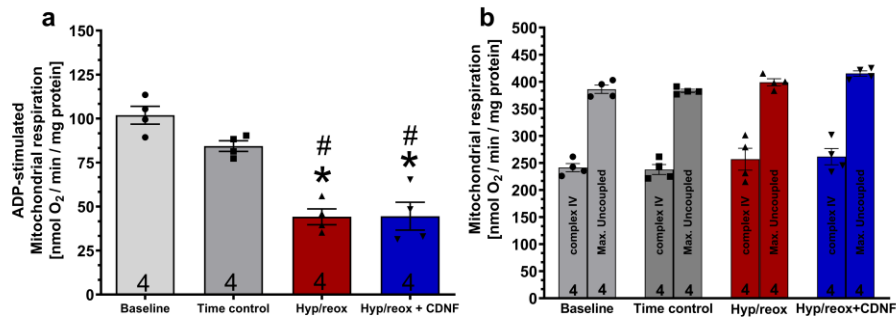
Extracts from different heart compartments (50 $\mu$ g protein) were fractionated by SDS-PAGE followed by immunoblotting for CDNF and GAPDH. The levels of CDNF were estimated by normalizing the intensity of the CDNF band to the GAPDH band as shown in the graph. Data are means  $\pm$  S.E.M. Number in each column is *n* of hearts. RV= right ventricle; LV = left ventricle; A = atria. Fresh untreated hearts were used in these experiments. No statistical differences between the groups with one-way ANOVA followed by Bonferroni post-hoc tests.

**Figure S3. The cardioprotective activity of CDNF is not prevented by rottlerin (a), chelerythrine (b), or AG490 (c).**



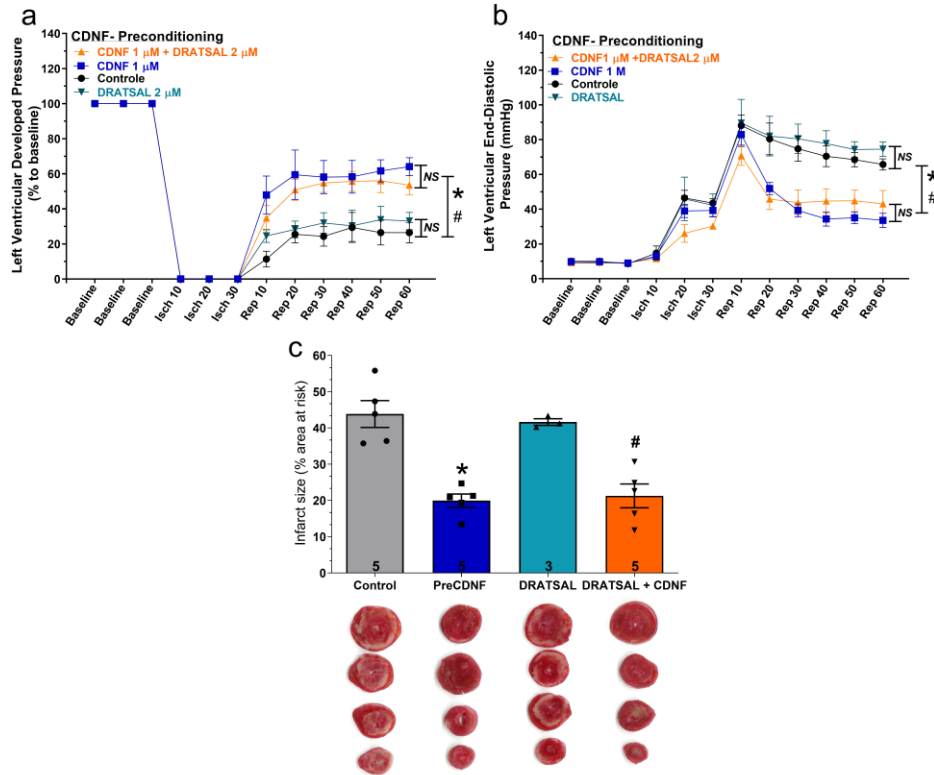
Time courses of left ventricular developed pressure (LVDP) during I/R protocol (30 min of global ischemia and 60 min of reperfusion) or when the hearts were subjected to the previous perfusion with CDNF (1 $\mu$ mol/L/5 min - preconditioning) or with CDNF+inhibitor (5 min before I/R). Controls (circles), CDNF treatment (squares) and CDNF+inhibitor (triangles). (d) Rottlerin, chelerythrine, and AG490 do not counteract the protective effect of CDNF in reducing the infarct area of hearts subjected to I/R. Representative cross-section images of TCC-stained ventricles hearts subjected to I/R in the absence or in the presence of CDNF and peptides. Number in each column is *n* of hearts. The data were expressed as means  $\pm$  S.E.M. @P<0.01 vs. control; \*P<0.01 vs. preCDNF. With one-way ANOVA followed by Bonferroni post-hoc tests for infarct area analysis and two-way ANOVA followed by Bonferroni post-hoc tests for LVDP and LVEDP analysis.

**Figure S4. CDNF does not protect isolated mitochondria from hypoxia/reoxygenation.**



(a) ADP-Stimulated complex I respiration and (b) Complex IV-induced respiration with TMPD and ascorbate, and maximal uncoupled oxygen uptake with FCCP. The mitochondria were isolated from naive rat hearts and then subjected to hypoxia/reoxygenation in the absence or in the presence of CDNF (1  $\mu$ mol/L). Groups: Baseline; Time control =10 min of mitochondria incubation in the chamber before the experiment; Hyp/reox=10min of hypoxia followed by reoxygenation; Hyp/reox+CDNF=CDNF incubation (1  $\mu$ mol/L) before Hyp/reox. The data were expressed as means  $\pm$  S.E.M. Number in each bar is *n* of hearts. \*P<0.05 vs. time control; #P<0.05 vs. baseline with one-way ANOVA followed by Bonferroni post-hoc tests.

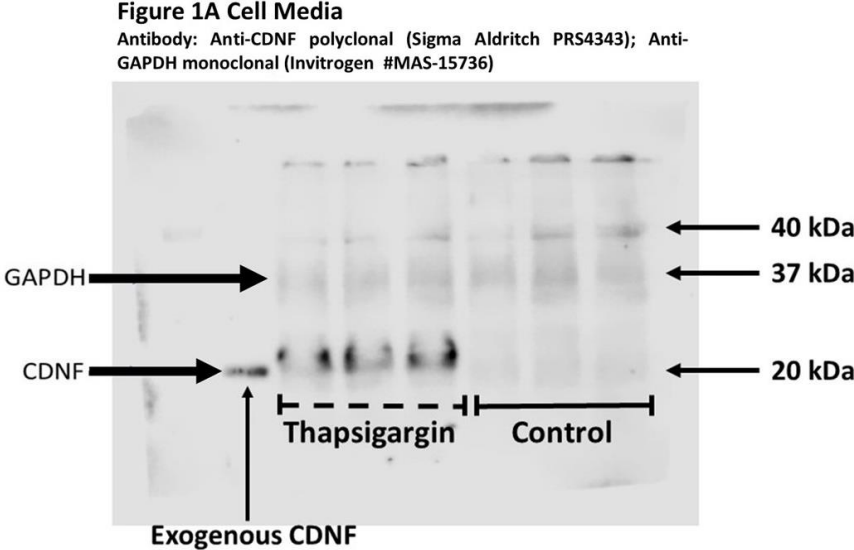
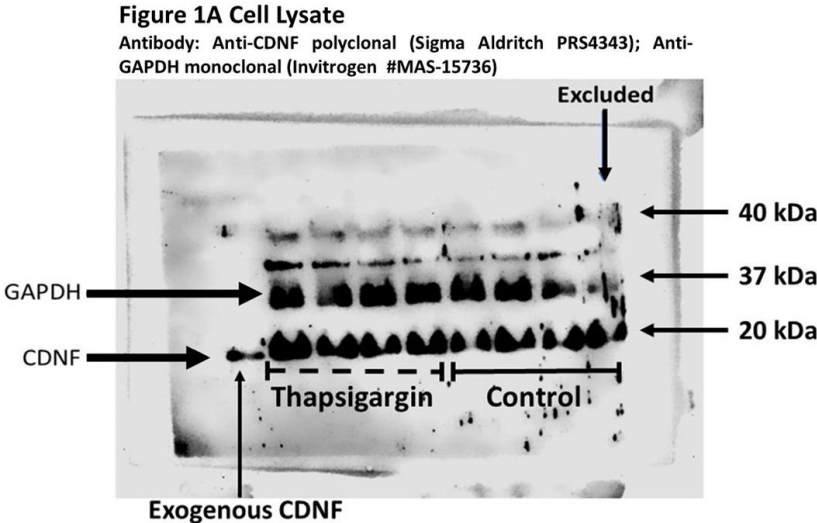
**Figure S5. The cardioprotective effect of exoCDNF is not blocked by the scrambled peptide DRATSAL.**



Time course of (a) left ventricular developed pressure (LVDP) and (b) left ventricular end-diastolic pressure (LVEDP) during I/R protocol (30 min of global ischemia and 60 min of reperfusion). As indicated by the different tracings, CDNF (1 μmol/L), peptide (DRATSAL, 2 μmol/L), or CDNF (1 μmol/L)+DRATSAL (2 μmol/L) were perfused before ischemia (5 min). Control (circles), CDNF (squares), DRATSAL alone (inverted triangles) and CDNF+DRATSAL (triangles). (c) DRATSAL does not block the decrease in the infarct area induced by CDNF after I/R. Representative cross-sections of TTC-stained ventricles. The data were expressed as means ± S.E.M. The number of hearts used in each experiment is shown inside the bars. \*P < 0.01 control vs CDNF; #P < 0.001 control vs. CDNF + DRATSAL with one-way ANOVA followed by Bonferroni post-hoc tests for infarct area analysis and two-way ANOVA followed by Bonferroni post-hoc tests for LVDP and LVEDP analysis.

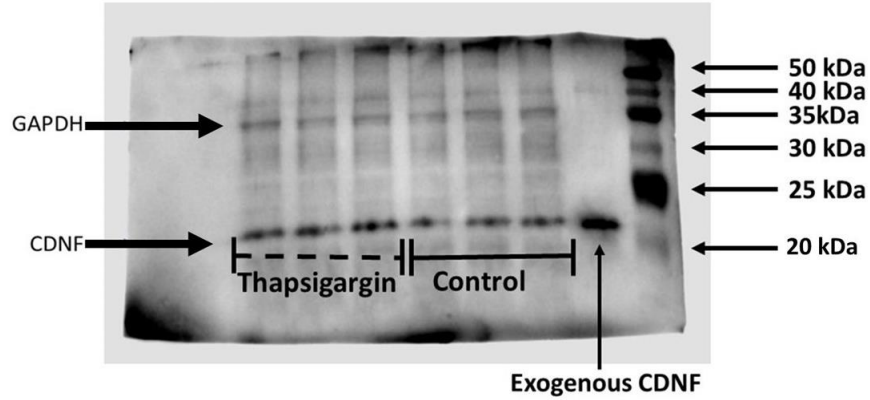


Figure S6. Full Western Blot PVDF membrane photo.



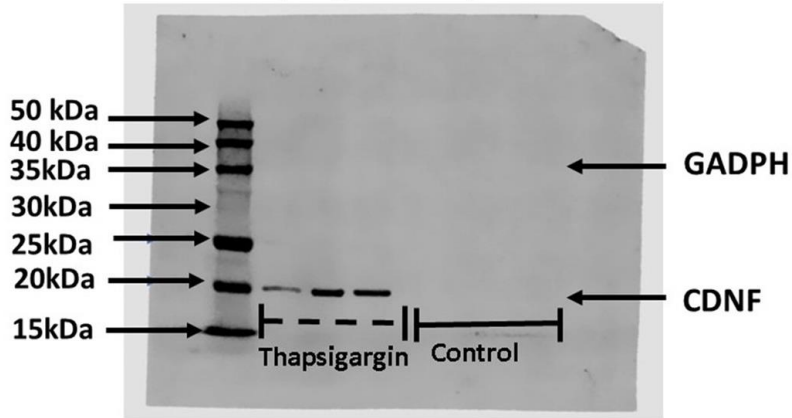
**Figure 1B Cell Lysate**

Antibody: Anti-CDNF polyclonal (Sigma Aldrich PRS4343); Anti-GAPDH monoclonal (Invitrogen #MAS-15736)



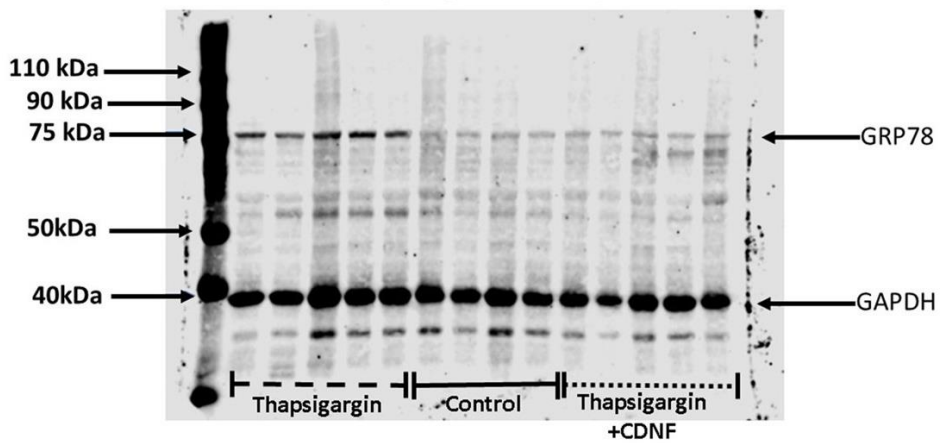
**Figure 1B Cell Media**

Antibody: Anti-CDNF polyclonal (Sigma Aldrich PRS4343); Anti-GAPDH monoclonal (Invitrogen #MAS-15736)



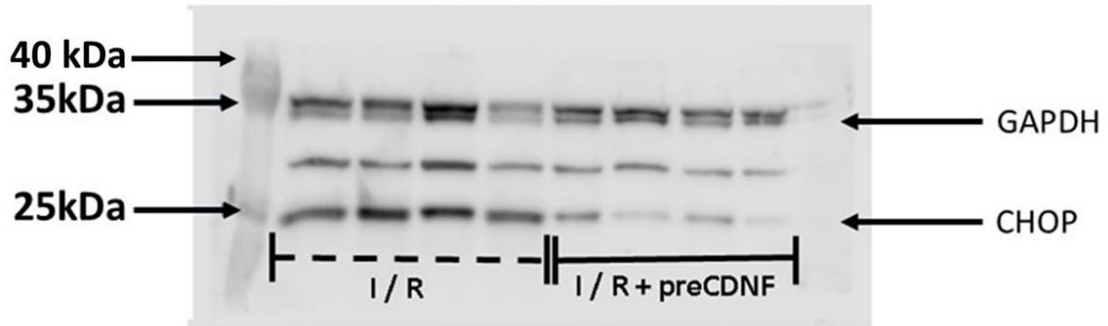
**Figure 1C**

Antibody: Anti-GRP78 polyclonal (Santa Cruz Biotechnology SC33575); Anti-GAPDH monoclonal (Invitrogen #MAS-15736)



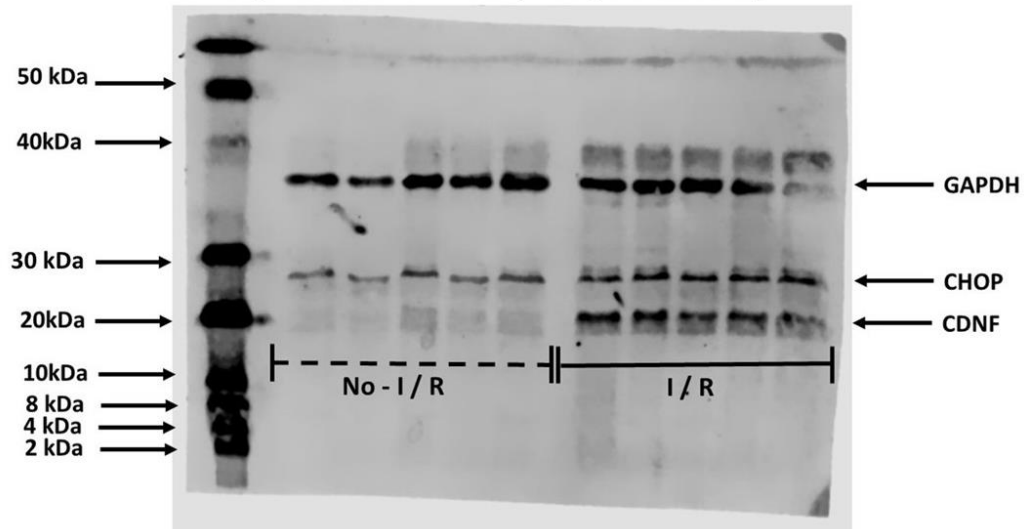
**Figure 1D**

Antibody: Anti-CHOP monoclonal (Santa Cruz Biotechnology Sc-166682); Anti-GAPDH monoclonal (Invitrogen #MAS-15736)



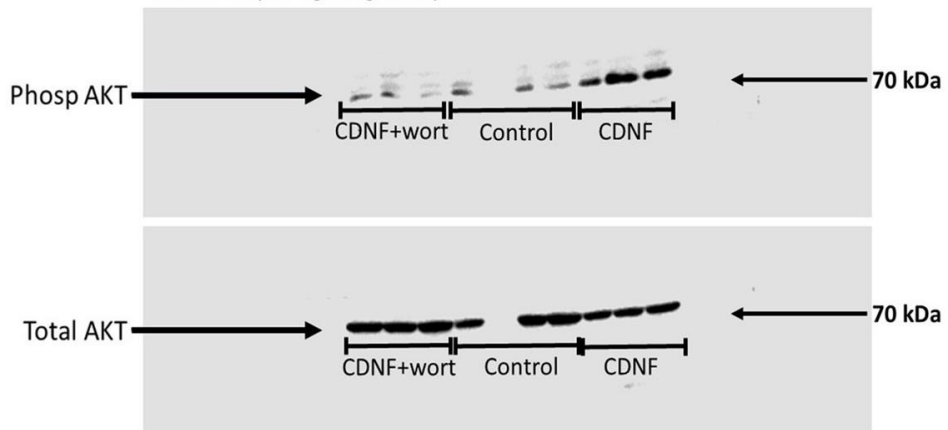
**Figure 1D**

Antibody: Anti-CDNF polyclonal (Sigma Aldrich PRS4343); Anti-CHOP monoclonal (Santa Cruz Biotechnology Sc-166682); Anti-GAPDH monoclonal (Invitrogen #MAS-15736)



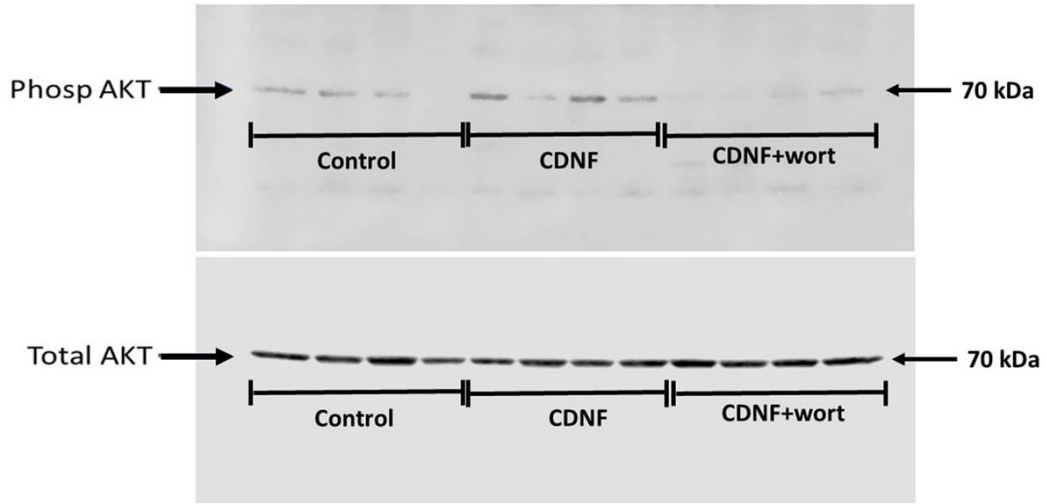
**Figure 4A**

Antibody: Anti-Phosp-AKT monoclonal (Cell signaling #4058); Anti-Total-AKT monoclonal (Cell signaling #4691)



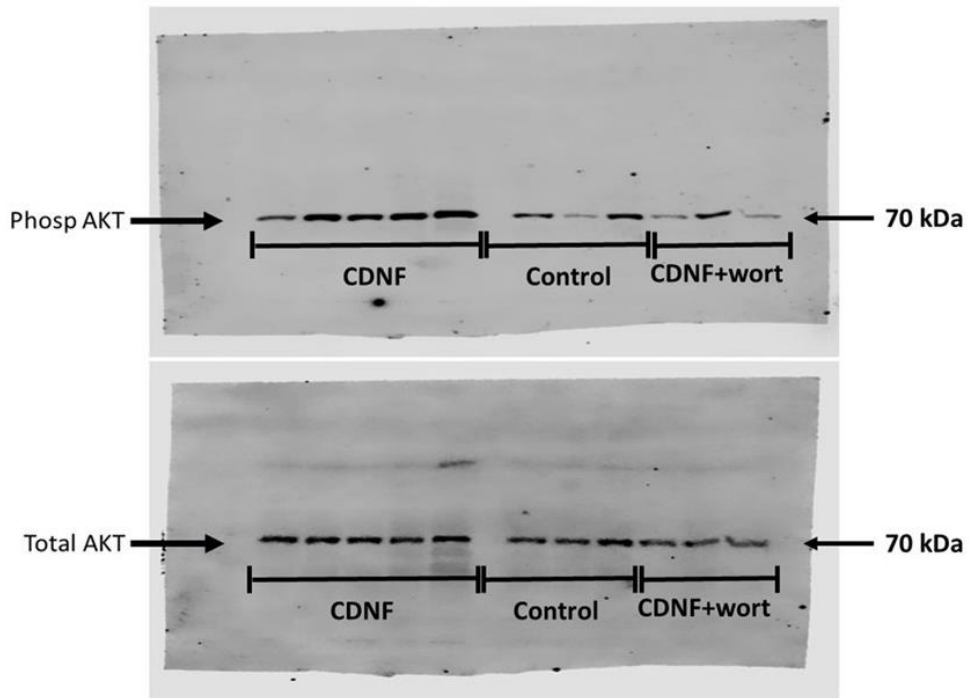
**Figure 4B**

Antibody: Anti-Phosp-AKT monoclonal (Cell signaling #4058); Anti-Total-AKT monoclonal (Cell signaling #4691)



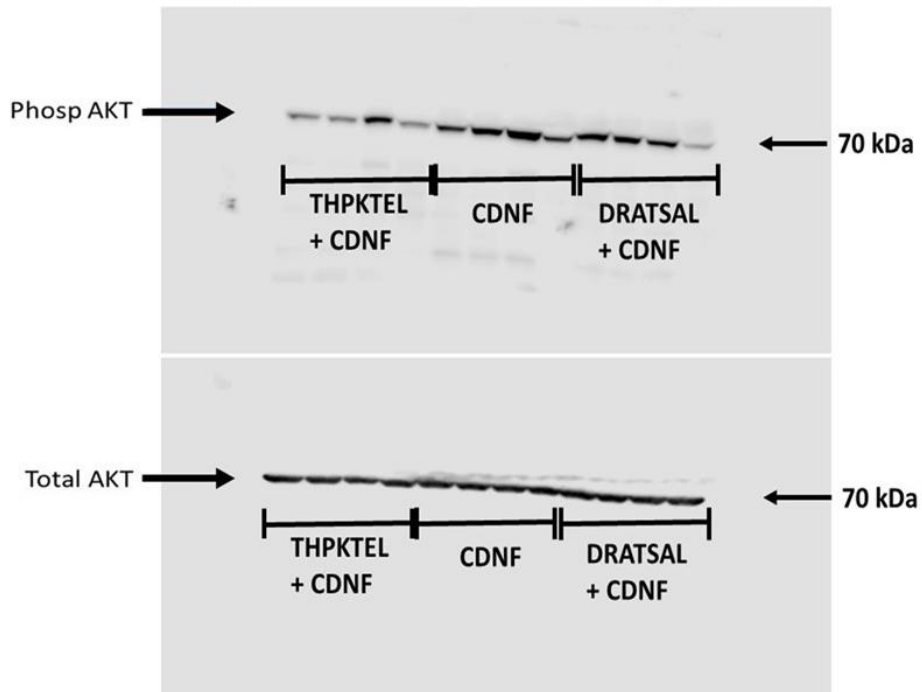
**Figure 4C**

Antibody: Anti-Phosp-AKT monoclonal (Cell signaling #4058); Anti-Total-AKT monoclonal (Cell signaling #4691)



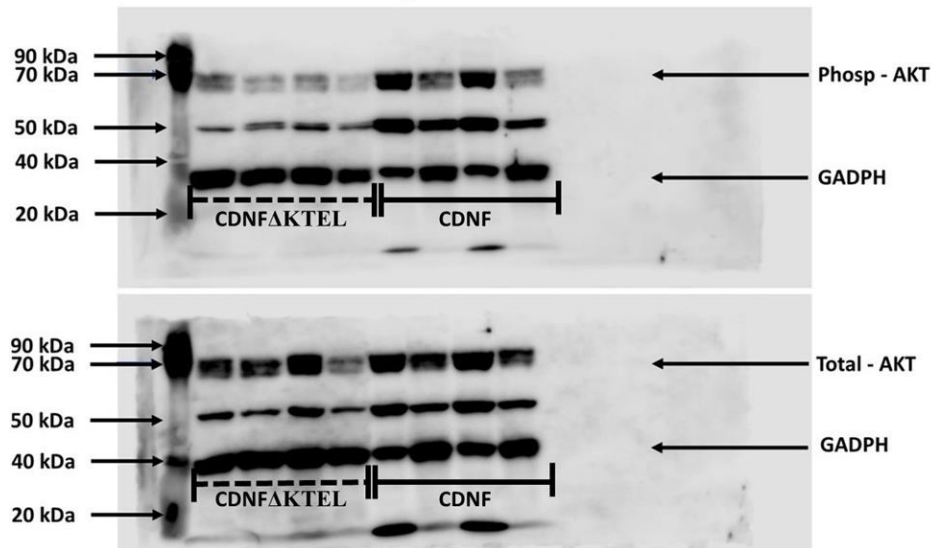
**Figure 4D**

Antibody: Anti-Phosp-AKT monoclonal (Cell signaling #4058); Anti-Total-AKT monoclonal (Cell signaling #4691)



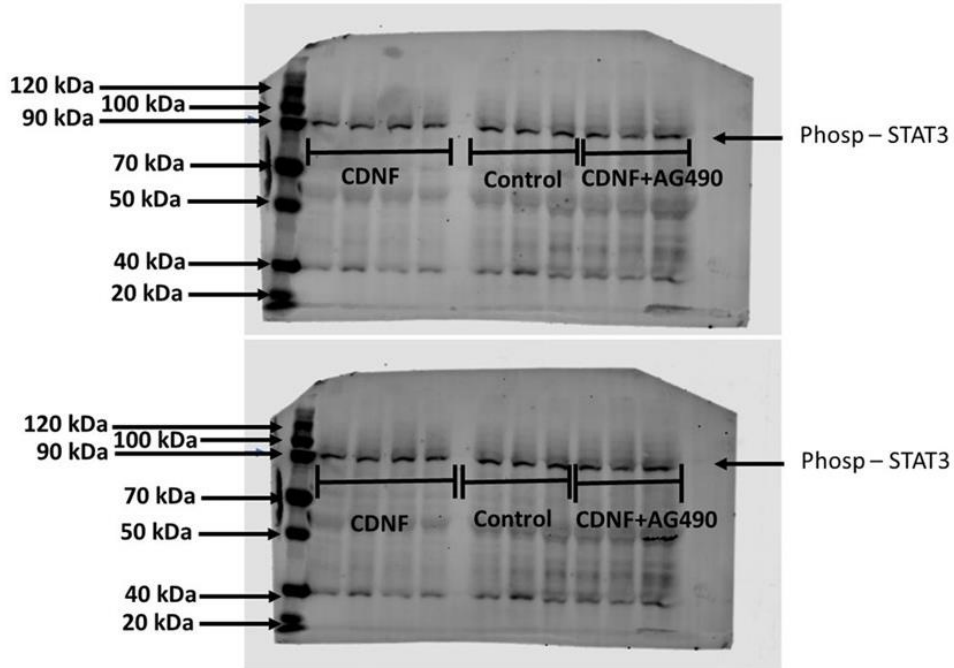
**Figure 4E**

Antibody: Anti-Phosp-AKT monoclonal (Cell signaling #4058); Anti-Total-AKT monoclonal (Cell signaling #4691); Anti-GAPDH monoclonal (Invitrogen #MAS-15736)



### Figure 4F - STAT 3

Antibody: Anti-Phosp-STAT3 monoclonal (Cell Signaling #9145); Anti-Total-STAT3 monoclonal (Cell Signaling #12640)



### Figure 4 F - PKCe

Antibody: Anti-Phosp-PKCe Polyclonal (abcam (ab63387)); Anti-Total-PKCe Polyclonal (abcam (ab233292))

