Electronic Supplementary Material (ESI) for Metallomics.

Copper complexes of synthetic peptides mimicking neurotrophin-3 enhance neurite outgrowth and CREB phosphorylation

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Table 1. Protonation constant ($\log\beta_{qr}$) for the L_qH_r species and pK values (calculated as $\log\beta$ difference between L_qH_r and L_qH_{r-1}) for NT3(1-13), NT3(1-5) and AcNT3(5-13) (T= 298 K and I = 0.1 M KNO_3).[a]

	$\log\beta$ **NT3(1-13)**	$\log\beta$ **NT3(1-5)**	$\log\beta$ **AcNT3(5-13)**
LH	9.75 (3)	10.39 (6)	10.07 (3)
LH_2	19.23 (3)	20.00 (8)	19.48 (3)
LH_3	26.66 (3)	27.44 (1)	25.92 (5)
LH_4	33.23 (3)	33.6 (1)	30.25 (6)
LH_5	39.11 (3)	37.5 (1)	-
LH_6	43.37 (4)	-	-
LH_7	46.92 (4)	-	-
pK Tyr/Lys	9.75	10.39	10.07
pK Tyr/Lys	9.48	9.61	9.41
pK NH_2	7.43	7.44	-
pK His	6.57	6.21	6.44
pK His	5.88	-	-
pK Glu	4.26	3.83	4.33
pK Glu	3.55	-	-

[a] Standard deviations (3σ values) are given in parentheses. Charges are omitted for clarity.

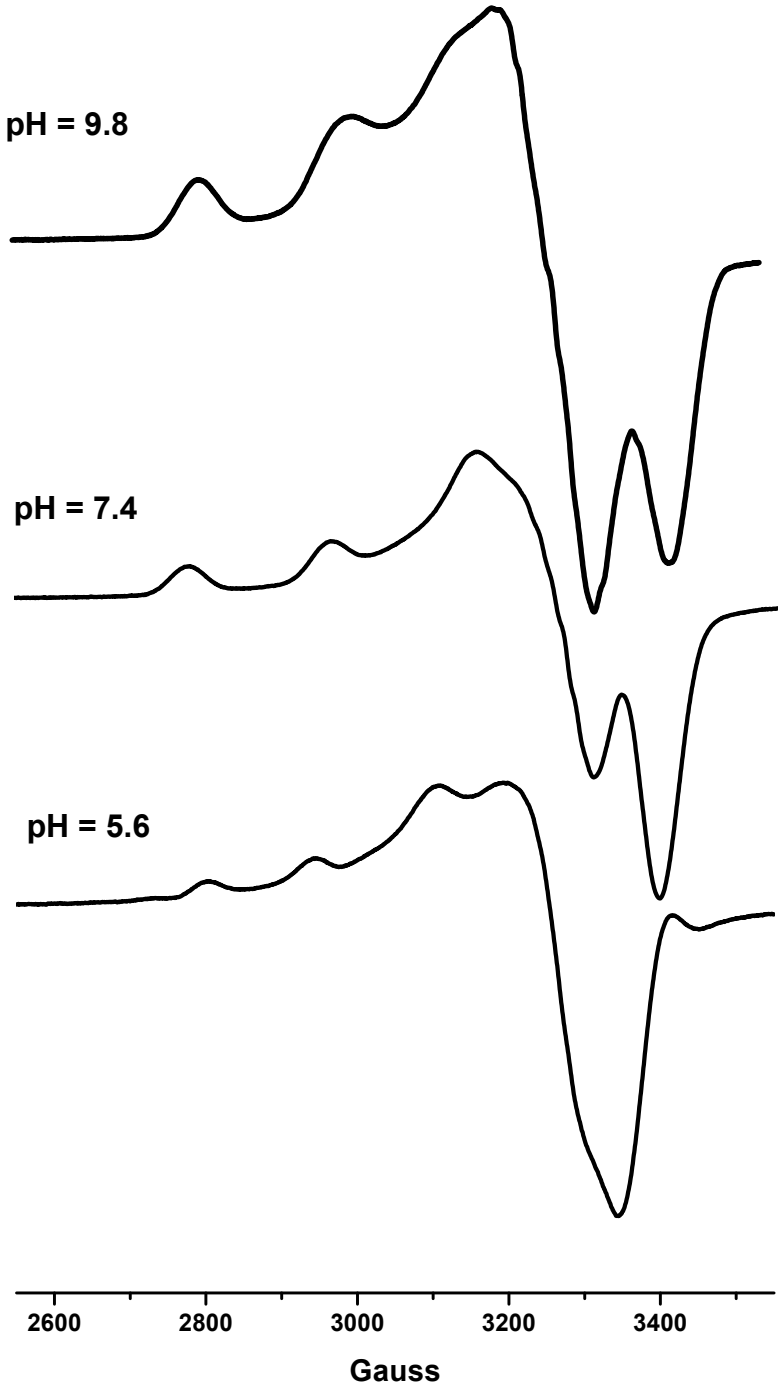

Figure S1. EPR spectra of Cu-NT3(1-5) at 1:1 molar ratio. [Cu^{2+}]=1x 10^{-3} M

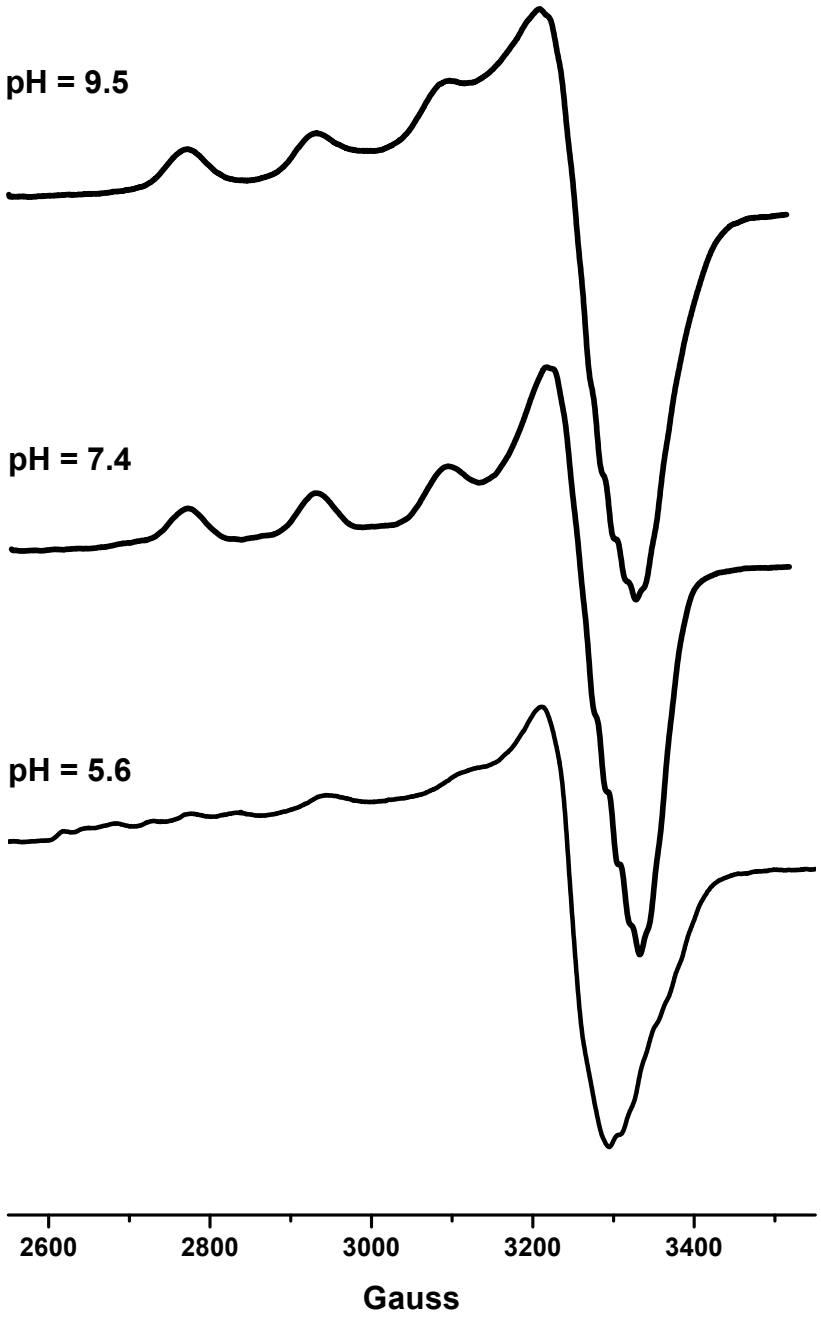

Figure S2. EPR spectra of Cu-AcNT3(5-13) at 1:1 molar ratio. [Cu^{2+}]= 1x 10^{-3} M

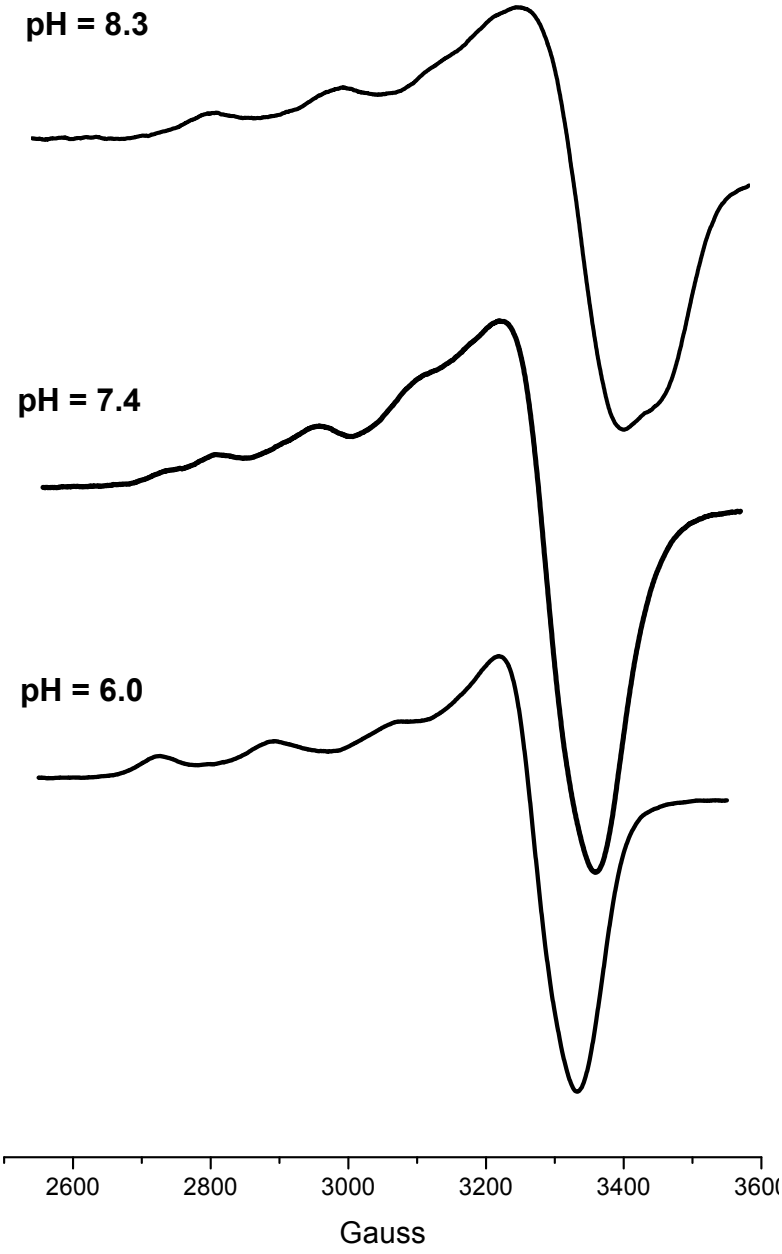

Figure S3. EPR spectra of Cu-NT3(1-13) at 1:1 molar ratio. [Cu^{2+}]= 1x 10^{-3} M

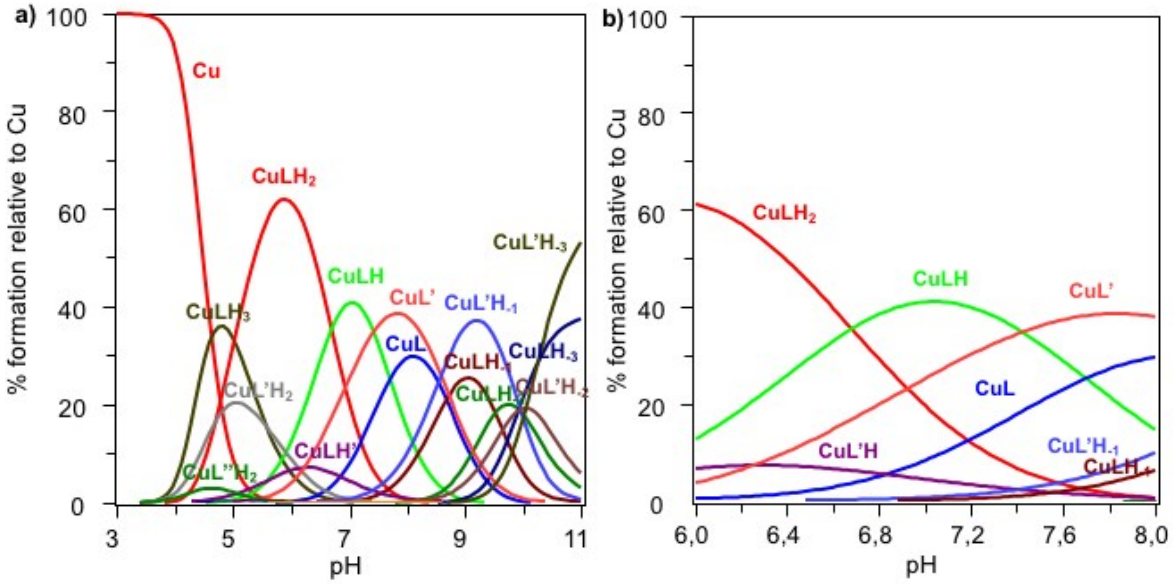

Figure S4. Species distribution diagram for the complexes formed with L = NT3(1-13); L' = NT3(1-5); L'' = AcNT3(5-13), [Cu(II)] = [L] = [L'] = [L'']= 1X10^{-3} M; a) pH range 3-11; b) enlargement pH range 6-8.

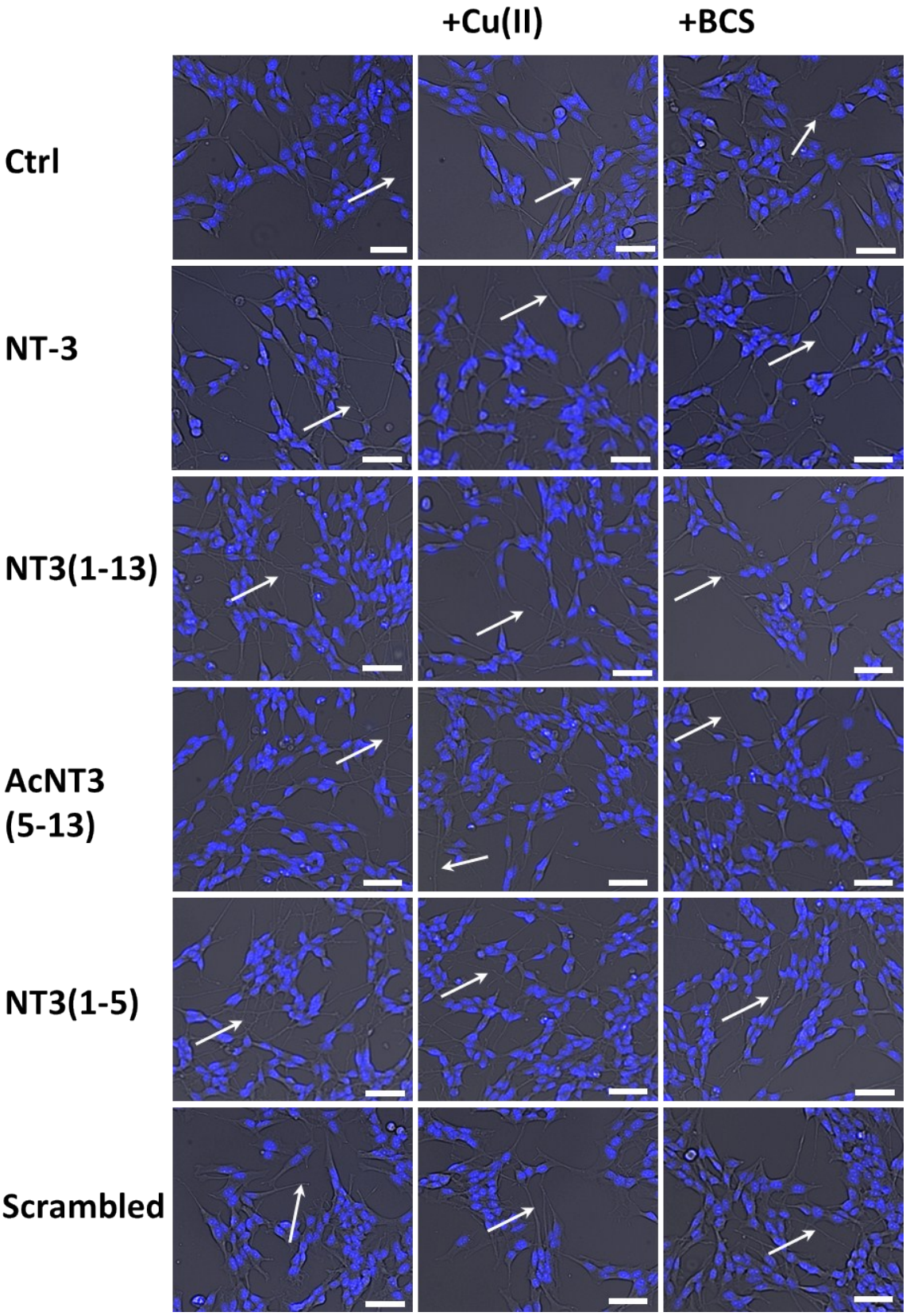

Figure S5. Phase contrast micrographs of SH-SY5Y cells untreated (a) and 72 hrs treated (in 0.5 % of FBS) with 50 ng/mL NT-3, 100 μM **NT3(1-13)**, 100 μM Ac**NT3(5-13)**, 100 μM **NT3(1-5)**, 100 μM **scrambled peptide (Sc)**, in presence of copper(II) ions or BCS. The white arrows point to the neurite outgrowth. Scale bar = 100 μm.

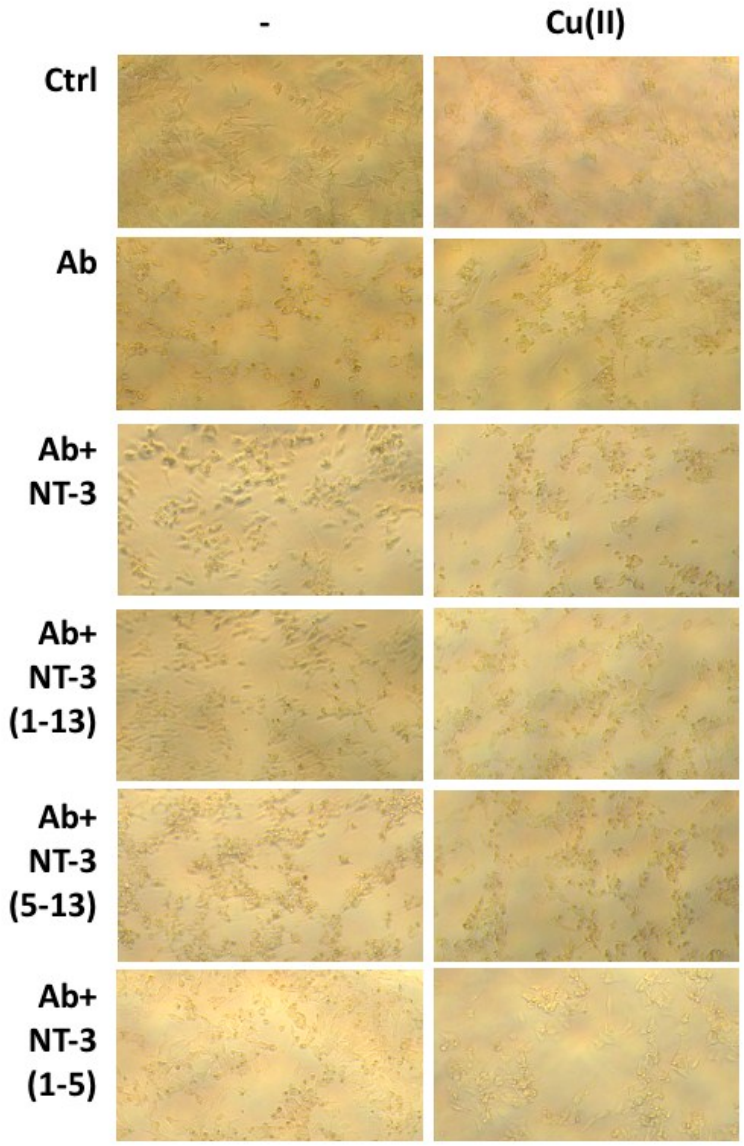

Figure S6. Phase contrast microscopy pictures of SH-SY5Y cells following a 1h of pre-treatment with anti-Trk antibody and after a 72 hrs of treatment with NT-3 whole protein or NT3 peptides in the presence or the absence of Cu(II). Original magnification ×20. a) Control; b) Anti-TrK (Ab); c) Ab + NT-3; d) Ab + NT3(1-13); e) Ab + AcNT3(5-13); f) Ab + NT3(1-5)

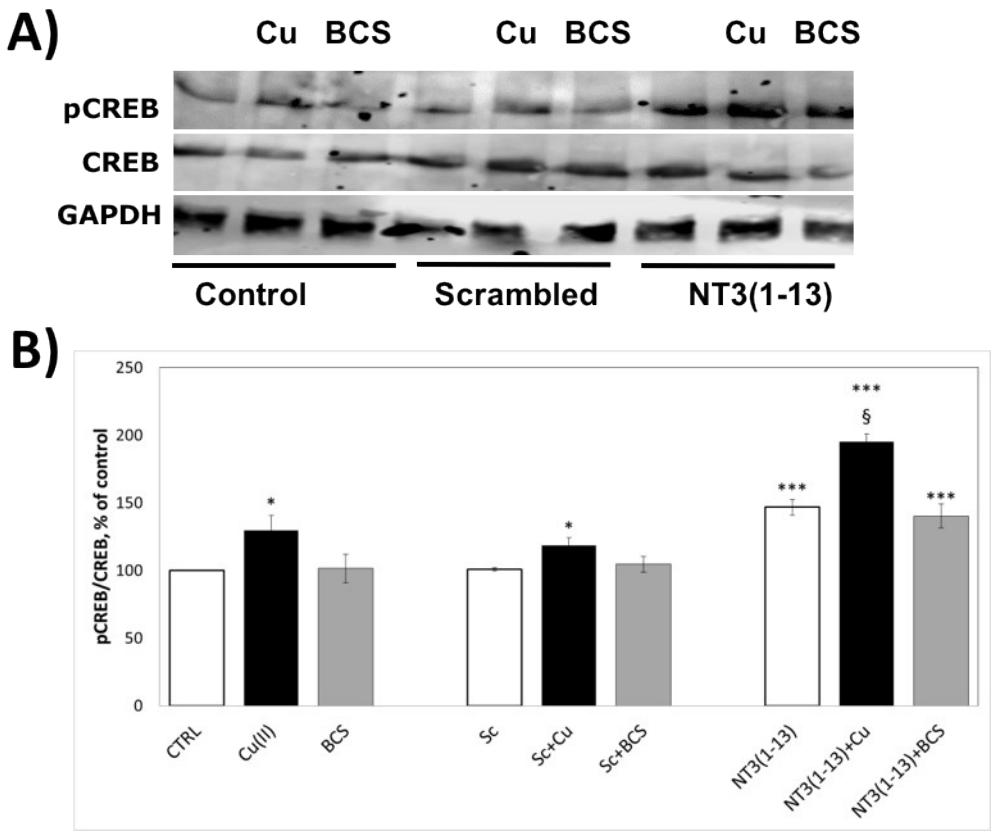

Figure S7. Representative western blotting (a) and densitometric analyses (b) of pCREB in neuroblastoma cell lysates. Cells were treated in the complete medium with 0.5 % of FBS for 30 min with 100 µM of scrambled or NT3(1-13) peptides in the absence (O) or presence of 10 µM of copper (O) or 50 µM BCS (O). For experiment with BCS, medium was 24 hrs pre-treated with BCS. Values are expressed as mean ± SEM over at least three independent experiments. (* = $p \leq 0.05$ vs negative control, *** = $p \leq 0.001$ vs. negative control, § = $p \leq 0.05$ vs. NT3(1-13)).