


WESTERN BLOT STUDIES OF NUCLEAR RECEPTOR PROTEIN EXPRESSION USING NHDF-C ADULT CELLS



NHDF-C adult cell line culture and treatment

NHDF adult cells were cultured in Fibroblast Growth medium supplemented with Fibroblast Growth factor (recombinant human) (1ng/ml), Insulin (recombinant human) (5µg/ml), fetal calf serum (0.02ml/ml), 100units/ml penicillin G, and 100µg/ml streptomycin at 37°C, 5% CO₂ incubator.. 80% confluence in culture flask. The cells were then conventionally subcultured and counted using Hemocytometer. The cell count was adjusted to 5x10⁶ cells/2ml. 2ml of cell suspension is added to each dish in P35dishes and incubated until the cells reach 80% confluence. NHDF-c adult cells were then treated with various concentrations of test sample . Post incubation, the cells were harvested for isolation of protein using RIPA buffer (Sigma; R0-278)

Isolation of protein using RIPA buffer

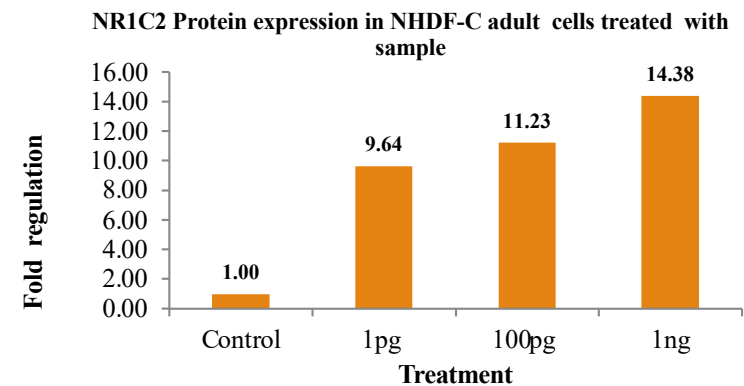
1. The cells, post harvesting, were washed twice using 1XPBS.
 2. The cell pellets were gently suspended in 500 μ l of RIPA buffer with 1X Protease Inhibitor (Sigma; P-8340)
 3. The cells were incubated for 30mins by gentle mixing every 5mins.
 4. Post incubation, the cells were centrifuged at 10,000rpm for 12-15 minutes.
 5. The protein lysates in the supernatant was transferred to fresh sterile tubes and stored in -20°C until further use.
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SDS-PAGE and Western Blot procedure

1. 100ug protein sample from each cell lysate was mixed with 5X loading dye and heated for 6 min at 98⁰C.
2. Protein samples were loaded and separated on 12% SDS-PAGE gel using Mini protean Tetra cell (Bio-Rad).
3. Methanol activated 0.45uM PVDF membrane was pre-wet in transfer buffer for 10 min @ RT.
4. Protein transfer was done for 10 min in Turbo Transblot (Bio-Rad) apparatus.
5. Blot was blocked in 5% BSA + TBST for 1 hr @RT.
6. Blot was incubated with 1⁰ Ab @ dilution: 1: 1000 for overnight @ 4⁰C on shaker.
7. Washed 3 times with TBST for 5min @ RT
8. Blot was incubated with 2⁰ Ab (Anti-Rabbit HRP- IgG) @ dilution 1: 1000 for 1hr @ RT.
9. Washed 3 times with TBST for 5min @ RT
10. Blot was rinsed with ECL reagent (two component system) for 1 min in dark and image was captured with 40 sec exposure in Chemidoc MP imaging system (Bio-Rad).

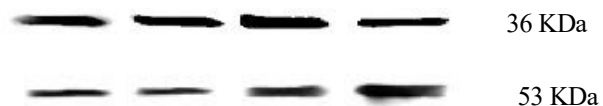
Western Blot for the expression of GAPDH, NR1C2 in NHDF-C adult cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR1C2		
Control	22820.53	1237.30	0.05	1.00
1pg	22293.17	11657.66	0.52	9.64
100pg	28700.34	17471.60	0.61	11.23
1ng	25945.03	20223.57	0.78	14.38

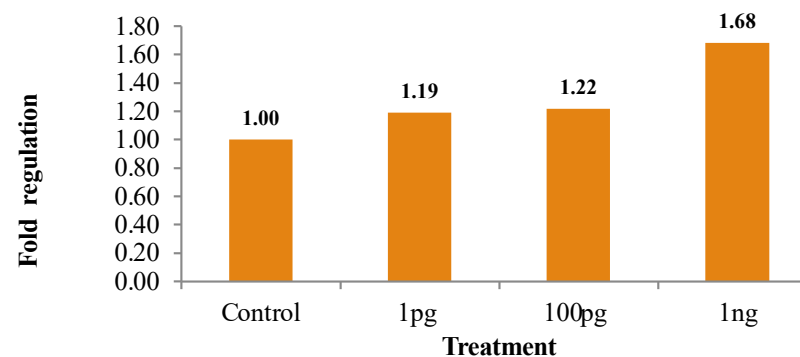


Western Blot for the expression of GAPDH, NR1C3 in NHDF-C adult cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR1C3		
Control	22820.539	10991.35	0.48	1.00
1pg	22293.175	12767.64	0.57	1.19
100pg	28700.347	16807.47	0.59	1.22
1ng	25945.033	20998.23	0.81	1.68

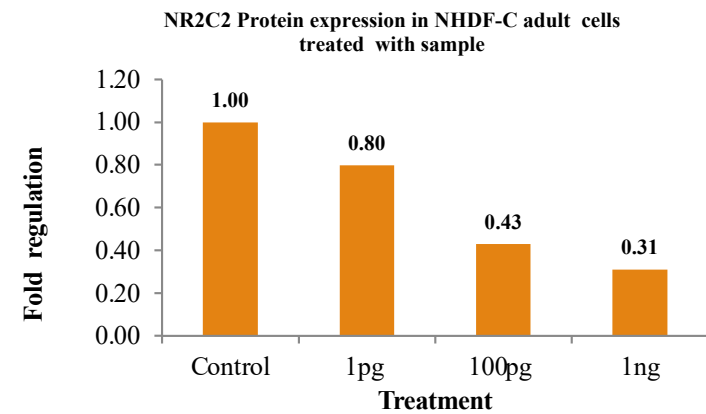


NR1C3 Protein expression in NHDF-C adult cells treated with sample



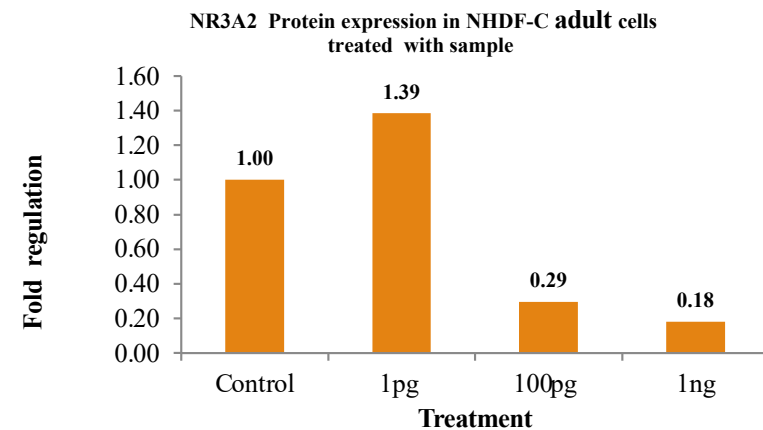
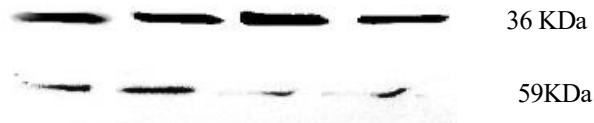
Western Blot for the expression of GAPDH, NR2C2 in NHDF-C adult cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR2C2		
Control	22820.54	28230.95	1.24	1.00
1pg	22293.18	22043.05	0.99	0.80
100pg	28700.35	15214.65	0.53	0.43
1ng	25945.03	9948.49	0.38	0.31



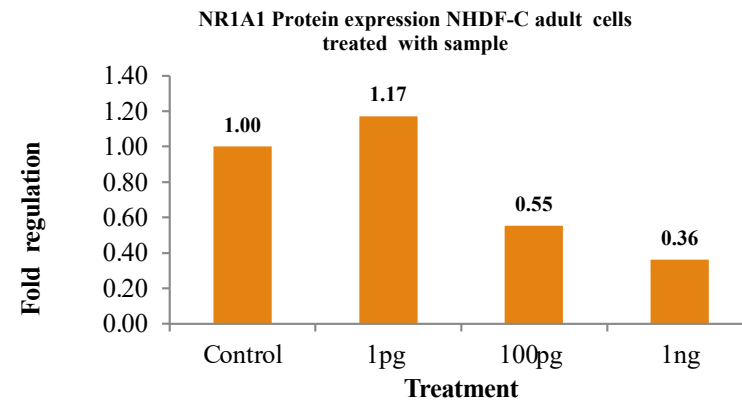
Western Blot for the expression of GAPDH, NR3A2 in NHDF-C adult cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR3A2		
Control	22820.54	10769.95	0.47	1.00
1pg	22293.18	14579.34	0.65	1.39
100pg	28700.35	3979.67	0.14	0.29
1ng	25945.03	2219.62	0.09	0.18



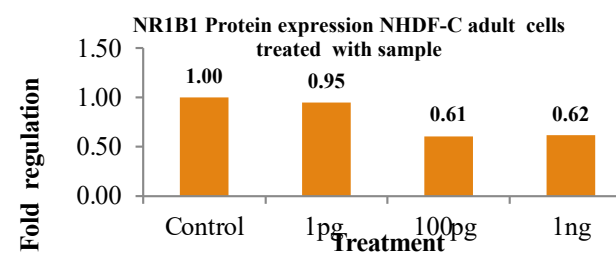
Western Blot for the expression of GAPDH, NR1A1 in NHDF-C adult cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR1A1		
Control	22820.54	19172.58	0.84	1.00
1pg	22293.18	21950.24	0.98	1.17
100pg	28700.35	13377.97	0.47	0.55
1ng	25945.03	7885.74	0.30	0.36



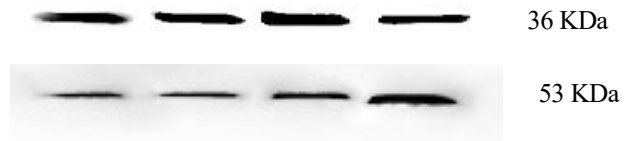
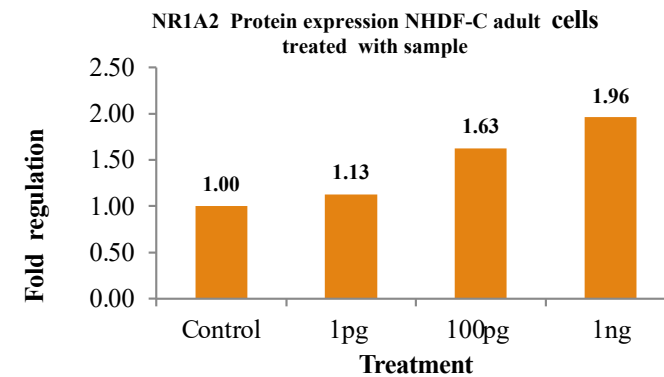
Western Blot for the expression of GAPDH, NR1B1 in NHDF-C adult cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR1B1		
Control	22820.54	13916.26	0.61	1.00
1pg	22293.18	12865.87	0.58	0.95
100pg	28700.35	10604.48	0.37	0.61
1ng	25945.03	9730.74	0.38	0.62



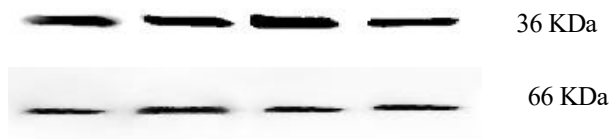
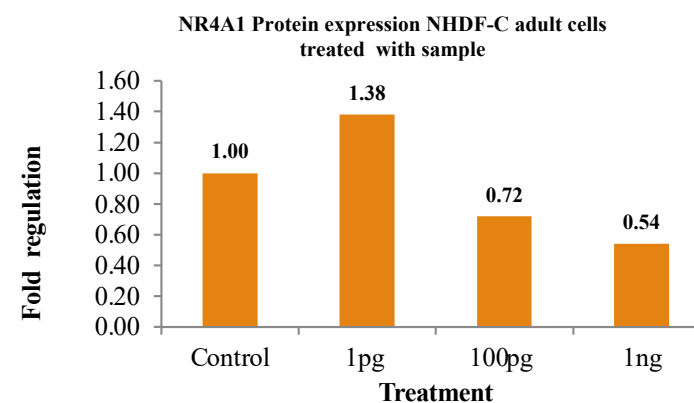
Western Blot for the expression of GAPDH, NR1A2 in NHDF-C adult cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR1A2		
Control	20820.539	8655.095	0.42	1.00
1pg	22293.175	10444.338	0.47	1.13
100pg	28700.347	19401.823	0.68	1.63
1ng	25945.033	21137.622	0.81	1.96



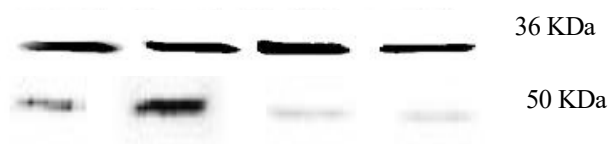
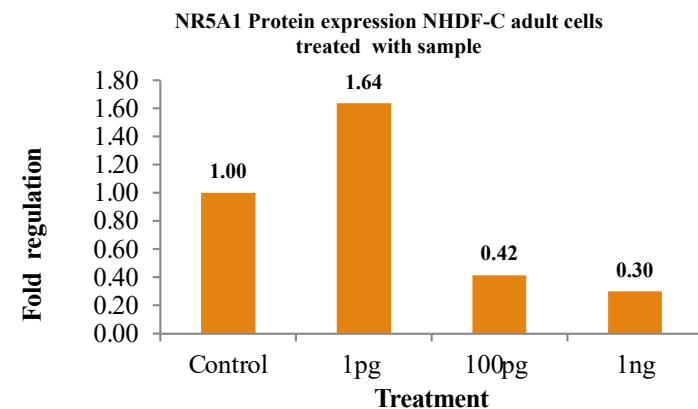
Western Blot for the expression of GAPDH, NR4A1 in NHDF-C adult cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR4A1		
Control	22820.539	13565.752	0.59	1.00
1pg	22293.175	18292.673	0.82	1.38
100pg	28700.347	12268.924	0.43	0.72
1ng	25945.033	8367.51	0.32	0.54



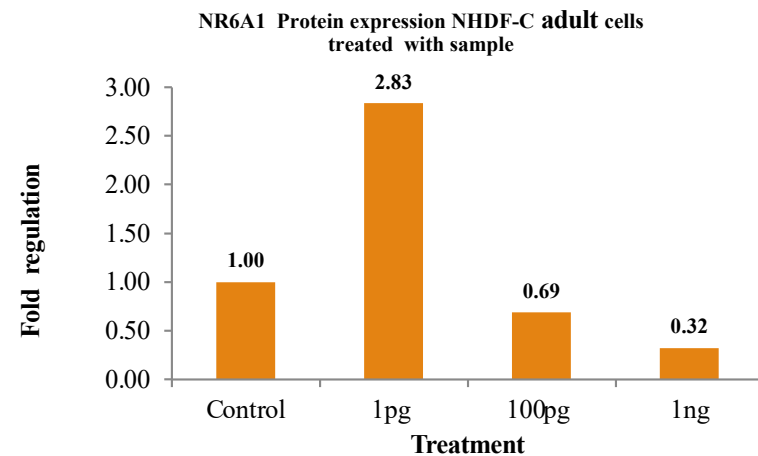
Western Blot for the expression of GAPDH, NR5A1 in NHDF-C adult cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR5A1		
Control	22820.539	11452.024	0.50	1.00
1pg	22293.175	18312.439	0.82	1.64
100pg	28700.347	5996.439	0.21	0.42
1ng	25945.033	3912.589	0.15	0.30



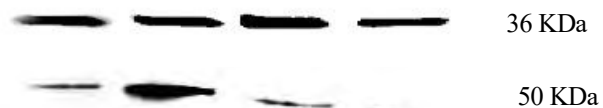
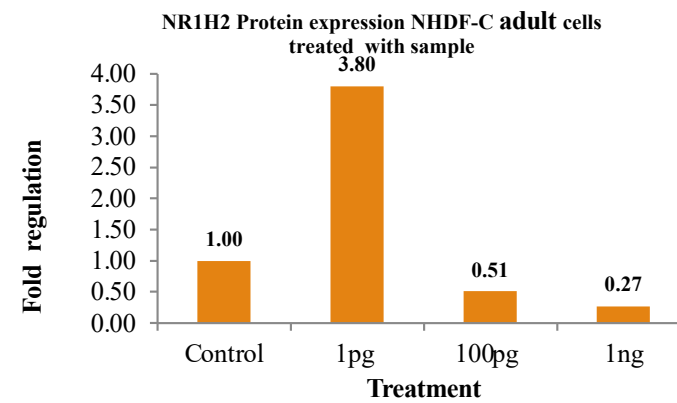
Western Blot for the expression of GAPDH, NR6A1 in NHDF-C adult cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR6A1		
Control	22820.54	5504.79	0.24	1.00
1pg	22293.18	15244.24	0.68	2.83
100pg	28700.35	4754.36	0.17	0.69
1ng	25945.03	2020.06	0.08	0.32



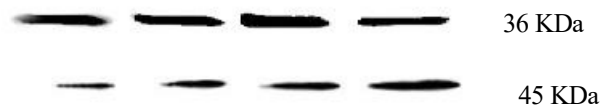
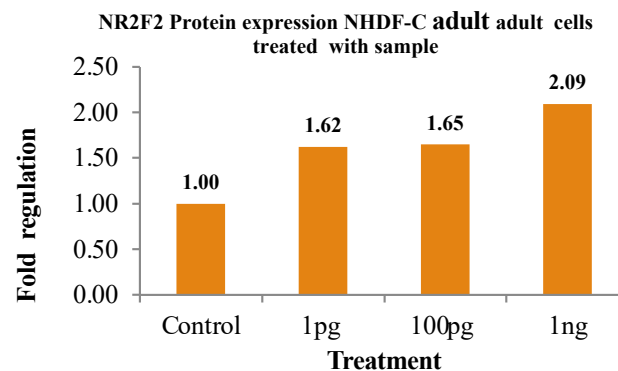
Western Blot for the expression of GAPDH, NR1H2 in NHDF-C adult cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR1H2		
Control	22820.54	7250.95	0.32	1.00
1pg	22293.18	26925.39	1.21	3.80
100pg	28700.35	4662.08	0.16	0.51
1ng	25945.03	2230.72	0.09	0.27



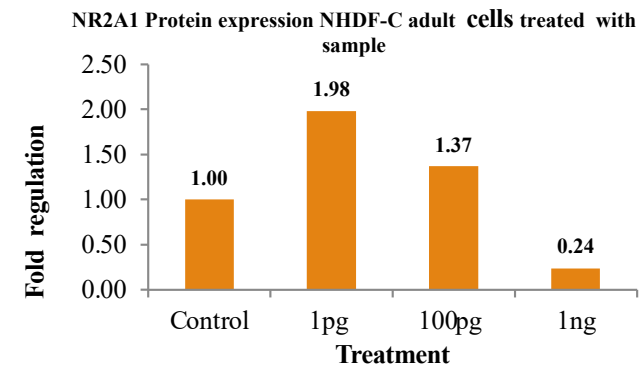
Western Blot for the expression of GAPDH, NR2F2 in NHDF-C adult cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR2F2		
Control	22820.54	9422.47	0.41	1.00
1pg	22293.18	14911.53	0.67	1.62
100pg	28700.35	19535.00	0.68	1.65
1ng	25945.03	22409.83	0.86	2.09



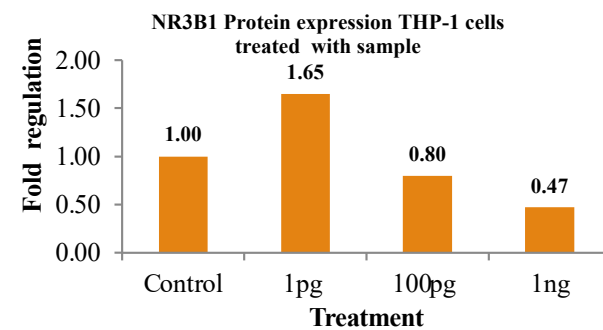
Western Blot for the expression of GAPDH, NR2A1 in NHDF-C adult cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR2A1		
Control	22820.54	9291.64	0.41	1.00
1pg	22293.18	17992.71	0.81	1.98
100pg	28700.35	15998.49	0.56	1.37
1ng	25945.03	2517.00	0.10	0.24



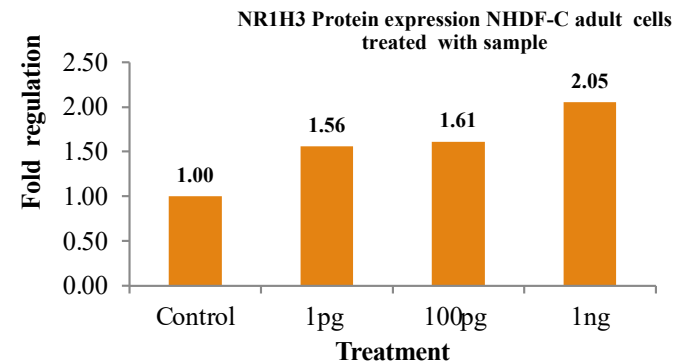
Western Blot for the expression of GAPDH, NR3B1 in NHDF-C adult cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR3B1		
Control	22820.54	12915.58	0.57	1.00
1pg	22293.18	19610.22	0.88	1.55
100pg	28700.35	12404.46	0.43	0.76
1ng	25945.03	8469.36	0.33	0.58



Western Blot for the expression of GAPDH, NR1H3 in NHDF-C adult cells

Samples	Band Intensity Of Protein		Normalised	Relative Protein Expression
	GAPDH	NR1H3		
Control	22820.54	9546.79	0.42	1.00
1pg	22293.18	14521.74	0.65	1.56
100pg	28700.35	19312.46	0.67	1.61
1ng	25945.03	22259.95	0.86	2.05



Conclusion

NHDF-C adult cells were treated with sample **Metadichol[®]** at 1pg/ml, 100pg/ml, and 1ng/ml. The relative expression of target genes was quantified using GAPDH for normalization. **Metadichol[®]** showed a dose dependent increase in NR1C1 ,NR1H3 ,NR2F2 and NR1A2 expression. Rest of protein Dose dependent down regulation was observed in 1ng/ml. NR2C1 ,NR1D1 and NR2F6 not expressed in NHDF-C adult cells. Only 15nucelar receptors were expressed. We did not detect the rest as some of them likely depend on co factors that are not used in the assay