THE EUROPEAN SOCIETY OF MEDICINE
Medical Research Archives, Volume 14 Issue 1

RESEARCH ARTICLE

Metadichol Orchestrates Cellular Reprogramming and Regenerative Pathways via FOX Transcription Factor Networks: Implications for Immune–Metabolic Rejuvenation

P.R. Raghavan

Nanorx Inc., Po Box 131,
Chappaqua, NY USA

raghavan@nanorxinc.com

OPEN ACCESS

PUBLISHED
31 January 2026

CITATION
Raghavan, PR., 2026. Metadichol Orchestrates Cellular Reprogramming and Regenerative Pathways via FOX Transcription Factor Networks: Implications for Immune–Metabolic Rejuvenation. Medical Research Archives, [online] 14(1). https://doi.org/10.18103/mra.v14i1.7160

DOI
https://doi.org/10.18103/mra.v14i1.7160

ISSN
2375-1924

ABSTRACT

Significance: The 2025 Nobel Prize in Physiology or Medicine was awarded to Mary E. Brunkow, Fred Ramsdell, and Shimon Sakaguchi for their groundbreaking discoveries concerning peripheral immune tolerance, specifically identifying the FOXP3 gene as the master regulator of regulatory T cells (Tregs). Their work demonstrated that FOXP3 mutations cause severe autoimmune disease (IPEX syndrome), establishing FOXP3 as essential for immune self-tolerance. This study demonstrates that Metadichol significantly upregulates FOXP3 expression (5.46-fold), providing a novel pharmacological approach to enhance regulatory T-cell function and immune homeostasis—directly relevant to the Nobel Prize-winning discoveries.

Background: Forkhead box (FOX) transcription factors constitute a large family of regulatory proteins that control diverse cellular processes, including development, metabolism, immunity, and aging. Metadichol, a nano lipid formulation derived from long-chain alcohols, has demonstrated pleiotropic biological effects, including immunomodulation and metabolic regulation.

Objective: To comprehensively evaluate the effects of metadichol treatment on FOX transcription factor gene expression in human peripheral blood mononuclear cells (PBMCs) via quantitative PCR analysis.

Methods: Human PBMCs were isolated via Histopaque density gradient centrifugation and treated with Metadichol at concentrations of 1 pg/ml, 100 pg/ml, 1 ng/ml, and 100 ng/ml. Total RNA was extracted, reverse-transcribed, and analyzed by quantitative PCR for 45 FOX genes. Gene expression changes were calculated via normalization to GAPDH via the 2^-ΔΔCq method.

Results: Metadichol treatment resulted in dose-dependent modulation of FOX gene expression. At the highest concentration (100 ng/ml), significant upregulation of multiple FOX genes was observed, with FOXO1 showing the greatest increase (8.74-fold), followed by FOXA1 (7.39-fold) and FOXH1 (7.22-fold). Additional substantial increases were noted for FOXA2 (6.57-fold), FOXA3 (6.98-fold), FOXB1 (6.79-fold), FOXP3 (5.46-fold), and FOXP4 (6.23-fold). Conversely, selective downregulation was observed for FOXL2 (0.16-fold), FOXL1 (0.54-fold), and FOXD4L1 (0.56-fold).

Conclusions: Metadichol has potent and selective effects on FOX transcription factor expression in human PBMCs, with preferential upregulation of genes involved in metabolic regulation, immune homeostasis, induced pluripotency and cellular longevity pathways. These findings suggest potential therapeutic applications in age-related diseases, metabolic disorders, and immunomodulation, and regenerative medicine. Importantly, the coordinated upregulation of pluripotency-associated genes, including FOXD3, FOXO1, and FOXM1, establishes Metadichol as a compelling modulator of cellular reprogramming networks with significant implications for advancing stem cell-based regenerative therapies. The differential expression patterns indicate complex regulatory mechanisms that warrant further investigation to elucidate their clinical translation potential.

Keywords: Fox family, Metadichol, immune metabolic rejuvenation, nuclear receptors, SOX family, Toll-like receptors, KLFs, sirtuins, circadian genes, GDF11, TERT, Klotho, induced pluripotency, regenerative medicine, 2025 Nobel Prize, FOXP3, regulatory T cells, immune tolerance.

Introduction

The Forkhead box (FOX) transcription factor superfamily comprises of 45 members[1] organized into 19 subfamilies (FOXA-FOXS)[2,3] that regulate diverse cellular processes,(Table 1) including development[4], metabolism[5], immunity[6], and aging[7,8]. These evolutionarily conserved proteins are characterized by a shared Forkhead DNA-binding domain[9] and exhibit tissue-specific expression patterns with distinct functional roles[10,11]. The clinical importance of FOX transcription factors has been extensively documented in the fields of cancer biology[12], metabolic diseases[13], autoimmune disorders[14], and aging-related pathologies[15,16].

Comprehensive FOX Gene Function and Disease Association

The Forkhead box (FOX) transcription factor family comprises a diverse group of evolutionarily conserved proteins characterized by a winged-helix DNA-binding domain. These factors regulate critical biological processes including development, metabolism, aging, and immune function. The following provides a comprehensive overview of FOX family members, their biological roles, disease associations, and key functions.

- Forkhead boxA1 functions as a pioneer transcription factor involved in organogenesis and hepatic development. Its key functions include liver specification, pancreatic development, and serving as a nuclear receptor cofactor. Dysregulation of FOXA1 is associated with prostate cancer, breast cancer, and metabolic disorders[17,18].

- Forkhead box A2 plays essential roles in endodermal organ development and glucose homeostasis. It regulates pancreatic β-cell function, gluconeogenesis, and respiratory development. Mutations or dysregulation of FOXA2 are linked to type 2 diabetes, pancreatic disorders, and lung development defects[19,20].

- Forkhead box A3 regulates hepatic gene expression and metabolism, with key functions in liver development, metabolic gene regulation, and bile acid synthesis. It is associated with cholangiocarcinoma, liver cancer, and metabolic syndrome[21].

- Forkhead box B1 participates in neural development and cell proliferation. Its key functions include brain development and neural differentiation. Aberrant FOXB1 expression is implicated in glioblastoma and neural tube defects[22].

- Forkhead box D1 is critical for kidney development and epithelial-mesenchymal transition (EMT). It functions in kidney morphogenesis, EMT regulation, and cancer metastasis. FOXD1 dysregulation is associated with pancreatic cancer and renal disorders[23,24].

- Forkhead box D2 contributes to neural crest development, with key functions in neural crest cell migration and cranial development. It is associated with developmental disorders[25,26,27]

- Forkhead box D3 functions in neural crest development and stem cell mainte.nance. Its key roles include neural crest specification and stem cell pluripotency. FOXD3 dysregulation is linked to melanoma and developmental disorders[28,29].

- Forkhead box D4 participates in embryonic development and was recently duplicated in humans. Its pathological significance remains to be fully characterized, though it functions in early developmental processes[30].

- Forkhead box E1 is essential for thyroid and neural development, with key functions in thyroid morphogenesis and neural tube closure. Mutations in FOXE1 are associated with thyroid cancer and congenital hypothyroidism[31,32,33].

- Forkhead box F1 regulates mesenchymal and lung development, functioning in lung development and angiogenesis. It is associated with alveolar capillary dysplasia and lung disorders.[34,35]

- Forkhead box F2 contributes to kidney development and angiogenesis, with key functions in kidney morphogenesis and vascular development. Dysregulation is linked to renal disorders and vascular malformations[36,37].

- Forkhead box G1 is critical for brain development and telencephalon formation, functioning in forebrain development and neurogenesis. Mutations are associated with autism spectrum disorders and Rett syndrome-like phenotypes[38,39].

- Forkhead box H1 functions in mesoderm formation and nodal signaling, with key roles in gastrulation, heart development, and TGF-β signaling. It is associated with developmental disorders and cardiac defects[40].

- Forkhead box J1 is the master regulator of ciliogenesis and respiratory epithelium function. It controls cilia formation and respiratory function. Mutations cause primary ciliary dyskinesia and respiratory infections[41].

- Forkhead box J2 regulates cell cycle progression, specifically the G2/M transition and DNA damage response. It is implicated in cancer[41].

- Forkhead box J3 functions in cell cycle progression, mitotic regulation, and chromosome segregation. Dysregulation contributes to cancer progression[41].

- Forkhead box K1 participates in muscle development and cell cycle regulation, with key functions in myogenesis and proliferation control. It is associated with muscular disorders and cancer[42].

- Forkhead box K2 regulates muscle differentiation and metabolism, functioning in skeletl muscle development and glucose metabolism. Dysregulation is linked to metabolic disorders and muscle diseases[43].

- Forkhead box L1 is involved in gastrointestinal development, with key functions in intestinal development and GI tract homeostasis. It is associated with gastrointestinal cancers[44].

- Forkhead box FOXL2 is essential for ovarian development and granulosa cell function, regulating ovarian follicle development and sex determination. Mutations cause ovarian cancer and premature ovarian failure[45].

- Forkhead box M1 controls cell cycle progression, DNA repair, and mitosis, with key functions in G1/S transition, M-phase progression, and genomic stability. It is implicated in multiple cancers and aging-related diseases[46].

- Forkhead box N1 is critical for thymic development and hair follicle formation, functioning in T-cell development and skin differentiation. Mutations cause severe combined immunodeficiency and alopecia[47].

- Forkhead box N2 participates in neural development, with key functions in brain development and neuronal differentiation. It is associated with neurodevelopmental disorders[48].

- Forkhead box N3 regulates cell cycle and DNA damage response, functioning in cell cycle checkpoints and DNA repair. Dysregulation is linked to cancer and aging[49].

- Forkhead box N4 is essential for retinal development, with key functions in retinal neurogenesis and photoreceptor development. Mutations cause retinal disorders and blindness[50].

- Forkhead box O1 regulates glucose homeostasis, stress response, and apoptosis. Its key functions include gluconeogenesis, insulin sensitivity, and cellular stress response. Dysregulation is associated with type 2 diabetes, cancer, and metabolic syndrome[51].

- Forkhead box O3 functions in aging, stress resistance, and apoptosis, regulating oxidative stress response, longevity pathways, and cell death. It is associated with cancer, neurodegenerative diseases, and human longevity[52].

- Forkhead box O4 mediates cell cycle arrest and DNA damage response, with key functions in p21 induction, senescence, and DNA repair. Dysregulation is linked to cancer and premature aging[53].

- Forkhead box O6 regulates brain function and glucose metabolism, functioning in memory consolidation and hepatic gluconeogenesis. It is associated with Alzheimer's disease and diabetes[54].

- Forkhead box FOXP1 participates in B-cell development and cardiac morphogenesis, with key functions in B-cell differentiation and heart valve development. Mutations cause diffuse large B-cell lymphoma and intellectual disability[55].

- Forkhead box P2 is critical for language development and neural function, regulating speech acquisition, motor learning, and synaptic plasticity. Mutations are associated with speech and language disorders and autism[56].

- Forkhead box P3 is the master regulator of regulatory T-cell function and immune tolerance, controlling Treg development, immune suppression, and self-tolerance. Mutations cause autoimmune diseases, IPEX syndrome, and impact cancer immunity[57].

- Forkhead box F4 functions in T-cell development and cardiac function, with key roles in T-cell differentiation and heart development. It is associated with developmental disorders and cardiac defects[58].

- Forkhead box Q1 regulates epithelial development, functioning in epithelial homeostasis and EMT regulation. Dysregulation is linked to colorectal cancer and gastric cancer[59].

- Forkhead box R1 participates in neural development, with key functions in brain development and cell proliferation. It is implicated in cancer[60,61].
- Forkhead box R2 contributes to neural function, regulating neural development and transcriptional programs. Dysregulation is associated with cancer[62,63].
- Forkhead box S1 functions in neural crest development, specifically in cranial neural crest formation. It is associated with developmental disorders[64].

Among the most studied Fox subfamilies, FoxA proteins function as pioneer transcription factors that facilitate chromatin remodeling and gene accessibility[65,66]. FOXA1, FOXA2, and FOXA3 are critical regulators of hepatic metabolism[67], pancreatic β-cell function[68,] and lipid homeostasis[69]. The FoxO subfamily, comprising FoxO1, FoxO3, FoxO4, and FoxO6, serves as key mediators of cellular stress responses[70] longevity pathways[71], and metabolic homeostasis[72] FoxP proteins, particularly FoxP3, are essential for regulatory T-cell development and immune tolerance[73,74],

Metadichol and FOX Factor Modulation

Metadichol[75], a nanoemulsion formulation, may possess the ability to modulate a multiple-target approach that could optimize cell reprogramming protocols and advance regenerative medicine applications by providing coordinated regulation of pluripotency networks.

Forkhead box (FOX) transcription factors represent critical control points in stem cell biology, governing pluripotency maintenance, cellular reprogramming, and differentiation processes. Their diverse roles as both enhancers and inhibitors of these processes highlight the complexity of transcriptional networks governing cellular identity. Research into FOX factor manipulation promises to advance treatments for age-related disorders, metabolic diseases, and tissue damage through improved cell reprogramming technologies. Multiple clinical trials exploring FOX-based regenerative approaches are currently underway, indicating the translational potential of this research area.

Metadichol is a novel nano-lipid formulation consisting of long-chain alcohols derived from sugarcane[75] Previous investigations have demonstrated that metadichol functions as a vitamin D receptor (VDR) agonist, modulates immune responses[76] and exhibits antiviral properties[77-79.] This compound has been shown to increase endogenous vitamin C levels[80]. influences telomerase activity[81] and has potential antiaging effects[82]. Given the central role of FOX transcription factors in cellular homeostasis and the emerging therapeutic potential of metadichol, we hypothesized that metadichol treatment would significantly modulate FOX gene expression in human immune cells. This study presents the first comprehensive analysis of the effects of metadichol on the entire FOX transcription factor family using human peripheral blood mononuclear cells (PBMCs) as a physiologically relevant model system.

Experimental

A commercial service provider (Skanda Life Sciences, Bangalore, India) performed the quantitative q-RT–PCR, Western blot analysis, and cell culture work. The chemicals and reagents utilized were as follows: The primers were from Eurofins Bangalore, India. Antibodies for Western blot was from E-Lab sciences, Houston, Texas, USA Other molecular biology reagents were obtained from Sigma–Aldrich, India.

Materials and Methods

Cell isolation and culture

Fresh human blood was collected in EDTA-containing tubes following institutional review board approval and informed consent procedures. PBMCs were isolated via Histopaque-1077 density gradient centrifugation[83]. Briefly, blood was diluted 1:1 with phosphate-buffered saline (PBS) and carefully layered over Histopaque-1077. Following centrifugation at 400×g for 30 minutes at room temperature, the mononuclear cell layer was collected, washed twice with PBS, and resuspended in RPMI-1640 medium supplemented with 10% fetal bovine serum[84].

Material Treatment

Isolated PBMCs were treated with Metadichol at concentrations of 1 pg/ml, 100 pg/ml, 1 ng/ml, and 100 ng/ml, with untreated cells serving as controls. The treatment duration was optimized on the basis of preliminary time-course experiments. The cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

Table 1: RNA Yields

Treatment Concentration	RNA Yield (ng/µL)
Control (0)	328.0
1 pg/mL	415.0
100 pg/mL	353.3
1 ng/ml	335.96
100 ng.ml	353.18

RNA Extraction and cDNA Synthesis

The RNA was extracted via TRIzol reagent according to the manufacturer's protocol[85]. RNA quality and quantity were assessed via spectrophotometric analysis (Spectramax i3x, Molecular Devices). cDNA synthesis was performed with 500 ng of total RNA via the PrimeScript RT Reagent Kit (Takara) via oligo-dT primers. Reverse transcription was conducted at 50°C for 30 minutes, followed by enzyme inactivation at 85°C for 5 minutes[86].

Table 2: FOX genes primers

Gene	Primers		Amplicon Size	Annealing temperature
FOXA1	F	GCAATACTCGCCTTACGGCTCT	129	65
	R	GGGTCTGGAATACACACCTTGG		
FOXA2	F	GGAACACCACTACGCCTTCAAC	133	65
	R	AGTGCATCACCTGTTCGTAGGC		
FOXA3	F	CTCGCTGTCTTTCAACGACTGC	122	65
	R	CGCAGGTAGCAGCCATTCTCAA		
FOXB1	F	CCACAACCTCTCCTTCAACGAC	122	59
	R	AGGAAGCTGCCGTTCTCGAACA		
FOXD1	F	TGGTTCGGTGTTTTGTTCGC	154	65
	R	AGCATAGGTCGGCTTTGCAT		
FOXD2	F	AACAGCATCCGCCACAACCTCT	92	65
	R	CAGCGTCCAGTAGTTGCCCTTG		
FOXD4	F	CCACTAGCGTTCCTGCTTCT	217	65
	R	TCATCTTCCTCCTCTCCCAGG		
FOSD4L1	F	TACATTTCAGCCTCCTGCCC	204	53
	R	ACCTGCCACCAAGGAAGATG		
FOXE1	F	CTCTGCTCTGGTTGACCTGG	103	65
	R	GGTTCAGGTGATGGGACTGG		
FOXF1	F	CAGGGCTGGAAGAACTCCG	222	65
	R	GAAGCCGAGCCCGTTCAT		
FOXF2	F	CCTACCAGGGCTGGAAGAAC	212	67
	R	CACGCGGTGGTACATGGG		
FOXG1	F	GAGGTGCAATGTGGGGAGAA	197	65
	R	GTTCTCAAGGTCTGCGTCCA		
FOXH1	F	CCTGCCTTCTACACTGCCC	151	62
	R	CTTCCTCCTCTTAGGGGGCT		
FOXJ3	F	TGATAGCCCACGCAGTAGCCTT	154	67
	R	ACTGTGGTTGCTGCTGAGGAGT		
FOXL1	F	TCACGCTCAACGGCATCTACCA	116	67
	R	TGACGAAGCAGTCGTTGAGCGA		
FOXL2	F	CAGTCAAGGAGCCAGAAGGG	241	67

Gene	Primers		Amplicon Size	Annealing temperature
	R	CGGATGCTATTTTGCCAGCC		
FOXO1	F	GCCACATTCAACAGGCAGC	251	65
	R	GACGGAAACTGGGAGGAAGG		
FOXO4	F	CCCGACCAGAGATCGCTAAC	236	67
	R	AATGGCCTGGCTGATGAGTT		
FOXP1	F	CAAGCCATGATGACCCACCT	252	67
	R	GGGCACGTTGTATTTGTCTGA		
FOXB2	F	CGACTGCTTCATCAAGATTCCGC	104	59
	R	AGGAAGCTGCCGTTCTCGAACA		
FOXC1	F	CAGTCTCTGTACCGCACGTC	189	65
	R	TGTTCGCTGGTGTGGTGAAT		
FOXC2	F	GCAGTTACTGGACCCTGGAC	211	65
	R	ATCACCACCTTCTTCTCGGC		
FOXD3	F	AAGCCGCCTTACTCGTACATCG	159	65
	R	AGAGGTTGTGGCGGATGCTGTT		
FOXE3	F	CTTCATCACCGAACGCTTTGCC	144	65
	R	CAGCGTCCAGTAGTTGCCCTTG		
FOXI1	F	GGAGCCTCAGGACATCTTGG	135	47
	R	CCGCTCACATAGGCTGTCAT		
FOXI2	F	CGTGGCTGGTAACTTCCCTT	211	65
	R	GGCTTCAGCTCTCCTCTTCC		
FOXI3	F	AACTCCATCCGCCACAACCTGT	107	62
	R	CTCGCAGTTCGGATCAAGAGTC		
FOXJ1	F	ACTCGTATGCCACGCTCATCTG	152	50
	R	GAGACAGGTTGTGGCGGATTGA		
FOXJ2	F	ACCAGTGGCAAACAGGAGTCAG	131	67
	R	TGGGCGATTGTATCCTGCTGAG		
FOXK1	F	GCCGACAAAGGCTGGCAGAATT	129	65
	R	TGGCTTCAGAGGCAGGGTCTAT		
FOXK2	F	CCAAACTCGCTGTCATCCAGGA	126	59
	R	GTGTAGGTGACAGGCTTGATGG		
FOXM1	F	AGCAGCGACAGGTTAAGGTT	225	62
	R	TGTGGCGGATGGAGTTCTTC		
FOXN1	F	GAGGTCAAAGTCAAGCCCCC	301	65
	R	TGTAGATCTCGCTGACGGGA		
FOXN2	F	ACAGATGCAGAGGGCTGACT	248	65
	R	GGCAGCATCAACAGCTTCAG		
FOXN3	F	GCCCTTCTCCAAGTTCCTCC	136	59
	R	AGCTGGTGATGCCATTCCTC		
FOXN4	F	GGCCACAGAGACAGCATGAG	236	47
	R	TTGGGGTAGTGTTTGGGGTG		
FOXO3	F	CGTCTTCAGGTCCTCCTGTT	135	47
	R	GGGAAGCACCAAAGAAGAGAG		
FOXO6	F	GAAGAACTCCATCCGGCACA	124	65
	R	CGGGGTCTTCCCTGTCTTTC		
FOXP2	F	CAAGCCATGATGACCCACCT	276	62
	R	CTGCGCAATATCTGCTGACG		
FOXP3	F	CCCACTTACAGGCACTCCTC	254	65
	R	GGGATTTGGGAAGGTGCAGA		
FOXP4	F	GCCAAGCAGCCCACAAAG	277	62

Gene	Primers		Amplicon Size	Annealing temperature
	R	AGATGGAGCCGACCTGATTG		
FOXQ1	F	AACCCCTCCTGGGCTCTTTA	199	65
	R	GTGTTGGGTGGACTATGGGG		
FOXR1	F	CAGTCCTCCAGCAAGCGGTCT	113	50
	R	AGCCATAGAGGAGCTGTCTTCC		
FOXR2	F	AAAGTCGCACGAGGAGAGTG	209	67
	R	CTCGAGGTTCTCCATGGCTC		
FOXS1	F	ATCCGCCACAACCTGTCACTCA	129	65
	R	GTAGGAAGCTGCCGTGCTCAAA		
GAPDH	F	GTCTCCTCTGACTTCAACAGCG	186	60
	R	ACCACCCTGTTGCTGTAGCCAA		

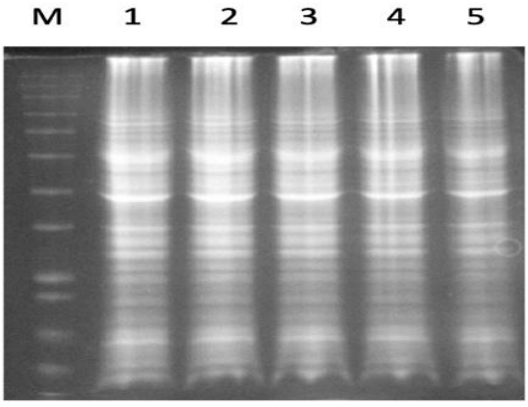
Quantitative PCR analysis

Real-time PCR was performed via SYBR Green Master Mix with gene-specific primers for 45 FOX genes. The PCR conditions consisted of initial denaturation at 95°C for 2 minutes, followed by 39 cycles of 95°C for 5 seconds and primer-specific annealing/extension for 30 seconds. Melting curve analysis was performed to verify amplification specificity. GAPDH served as the reference gene for normalization. Relative gene expression was calculated via the 2^-ΔΔCq method[87-89].

SDS–PAGE and Western blot procedure

Isolation of Mononuclear Cells

In the 15 ml centrifuge tube, 5 ml of Histopaque-1077 was added, and 5 ml of prepared blood was layered on the Histopaque slowly from the edge of the tube without disturbing the Histopaque layer. Then tubes were centrifuged at 400 X g for exactly 30 mins at room temperature with brake-off settings. After centrifugation, the upper layer was discarded with a Pasteur pipette without disturbing the interphase layer. The interphase layer was carefully transferred to clean centrifuge tube. Cells will be washed with 1X PBS and again centrifuged at 250 X g for 10 mins. (2X). After centrifugation, the supernatant was discarded, and the pellet will be collected in RPMI media supplemented with 10% FBS. Cells were counted, and viability was checked with a hemocytometer. The cell count was adjusted to $10x10^6$ cells/2 ml. 2 ml of cell suspension is added to each dish in P_{35} dishes. Cells were then treated with various concentrations of test sample. Post incubation, the cells were harvested for isolation of protein using RIPA buffer. The cells, post harvesting, were washed twice using 1X PBS. The cell pellets were gently suspended in 300 µl of RIPA buffer with 1X Protease Inhibitor. The cells were incubated for 30 mins by gentle mixing every 5 minutes at 4^0 C.



Lane M: Ladder , **Lane 1:** Control, **Lane 2**: 1 pg, **Lane 3:** 100 pg, **Lane 4:** 1 ng, **Lane 5:** 100 ng

Figure 1: SDS Page Profile

Post incubation, the cells were centrifuged at 10,000 rpm for 12-15 minutes. 25 ug protein sample from each cell lysate was mixed with 5X loading dye and heated for 2 min at 95˚C. Protein samples were loaded and separated on 8%, 10 % and 15 % SDS-PAGE gel using Mini protean Tetra cell (Bio-Rad). Nitrocellulose membrane (0.2 µM) was equilibrated in transfer buffer for 10 mins at RT. Protein transfer was done for 15 mins in Turbo Transblot (Bio-Rad) apparatus at 2.5 A and 25 V. Blot was blocked in 3% BSA in TBST for 1 hr at RT followed by incubation with respective 1˚ Ab at appropriate dilutions O/N at 4°C. Blot was washed thrice with TBST for 5 mins at RT Blot was incubated with 2˚ Ab (anti-Rabbit or anti-Mouse IgG- HRP) at dilution 1:10000 for 1 hr at RT. Washed 3 times with TBST for 5 mins at RT Blot was rinsed with ECL reagent (two component system) for 1 min in dark and image was captured between 0.5 sec to 15 secs exposure in Chemidoc XRS+ imaging system (Bio-Rad). The protein lysates in the supernatant were transferred to fresh sterile tubes and stored in -20˚C until later use.

Table 3: Antibodies experimental conditions for Western blot Assay

Protein Markers	Separating Gel Percentage	Stacking Gel Percentage	Antibody catalogue no. with dilution details	Exposure Time
FOX A1 Polyclonal Antibody -49 KD	12%	5%	E-AB-91097 (1:1000)	1-3 secs.
FOX B1 Polyclonal Antibody -35 KD	12%	5%	E-AB-12418 (1:1000)	1-3 secs.
FOX C2 Polyclonal Antibody – 54 KD	12%	5%	E-AB-15641 (1:1000)	1-3 secs.
FOX D3 Polyclonal Antibody -47.6 KD	12%	5%	E-AB-15644 (1:1000)	1-3 secs.
FOX G1 Polyclonal Antibody – 52 KD	12%	5%	E-AB-53376 (1:1000)	1-3 secs.
FOX H1 Polyclonal Antibody -39 KD	12%	5%	E-AB-19880 (1:1000)	1-3 secs.
FOX I1 Polyclonal Antibody – 41 KD	12%	5%	E-AB-19881 (1:1000)	1-3 secs.
FOX J3 Polyclonal Antibody –69 KD	12%	5%	E-AB-15648 (1:1000)	1-3 secs.
FOX L1 Polyclonal Antibody – 36 KD	12%	5%	E-AB-12427 (1:1000)	1-3 secs.
FOX L2 Polyclonal Antibody – 39 KD	12%	5%	E-AB-91483 (1:1000)	1-3 secs.
FOX P1 Polyclonal Antibody – 75 KD	12%	5%	E-AB-90453 (1:1000)	1-3 secs.
FOX P3 Polyclonal Antibody -47 KD	12%	5%	E-AB-70038 (1:1000)	1-3 secs.
Recombinant GAPDH Monoclonal Antibody -36 KD	12%	5%	AN004430L (1:1000)	1-3 secs.

Results

Treatment of PBMCs with different concentrations of metadichol (table 4) resulted in significant changes in the expression of multiple FOX genes

Table 4: Metadichol induced dose-dependent changes in FOX gene expression

Cell line	Markers	Control	1 pg/ml	100 pg/ml	1 ng/ml	100 ng/ml
PBMC	FOXA1	1	3.56	0.49	0.16	7.39
	FOXA2	1	1.1	2.25	0.16	6.57
	FOXA3	1	1.24	1.81	0.12	6.98
	FOXB1	1	3.16	1.01	0.3	6.79
	FOXD1	1	0.31	4.34	0.83	1.1
	FOXD2	1	6.36	1.83	0.15	1.38
	FOXD4	1	4.67	0.46	0.11	1.36
	FOXD4L1	1	1.93	1.36	0.2	0.56
	FOXE1	1	1.64	1.66	0.15	1.43
	FOXF1	1	1.01	0.59	0.2	2.41
	FOXF2	1	0.25	0.89	0.28	1.47
	FOXG1	1	6.26	3.23	0.45	3.04
	FOXH1	1	3.09	1.49	0.55	7.22
	FOXJ3	1	4.02	1.09	0.4	4.24
	FOXL1	1	0.22	0.24	0.11	0.54
	FOXL2	1	0.12	0.21	0.09	0.16
	FOXO1	1	2.51	0.96	0.27	8.74
	FOXO4	1	0.56	1.11	0.49	0.84
	FOXP1	1	1.8	3.16	0.84	2.28
	FOXB2	1	0.2	1.41	0.22	2.58
	FOXC1	1	0.13	0.98	0.15	2.16
	FOXC2	1	0.08	1.33	0.09	2.4
	FOXD3	1	0.12	2.63	0.11	1
	FOXE3	1	0.3	1.75	0.6	1.49
	FOXI1	1	0.19	5.73	0.22	1.91
	FOXI2	1	0.36	2.41	0.2	4.15
	FOXI3	1	0.17	1.16	0.22	2.74
	FOXJ1	1	0.15	0.39	0.14	0.7
	FOXJ2	1	0.77	1.57	0.54	0.98
	FOXK1	1	0.43	2.89	0.31	1.07
	FOXK2	1	0.25	1.47	0.28	1.84
	FOXM1	1	0.22	0.84	0.05	4.54
	FOXN1	1	0.56	5.95	0.73	0.79
	FOXN2	1	0.15	1.69	0.17	2.77
	FOXN3	1	0.16	1.13	0.23	1.72
	FOXN4	1	0.1	3.3	0.1	1.2
	FOXO3	1	0.38	1.58	0.3	1.23
	FOXO6	1	0.12	1.53	0.15	0.56
	FOXP2	1	0.41	1.2	0.2	5.15
	FOXP3	1	1.48	1.38	0.21	5.46
	FOXP4	1	0.95	2.85	1.39	6.23
	FOXQ1	1	0.14	1.25	0.09	2.18
	FOXR1	1	0.13	1.45	0.16	3.1
	FOXR2	1	0.23	1.38	0.27	2.11
	FOXS1	1	0.12	0.65	0.1	3.36

The overall pattern revealed that the highest concentration (100 ng/ml) generally elicited the strongest response for most genes, with some exceptions showing peak responses at lower concentrations. Statistical analysis revealed that 38 out of 44 FOX genes exhibited significant expression changes in at least one treatment concentration compared with the control ($p < 0.05$).

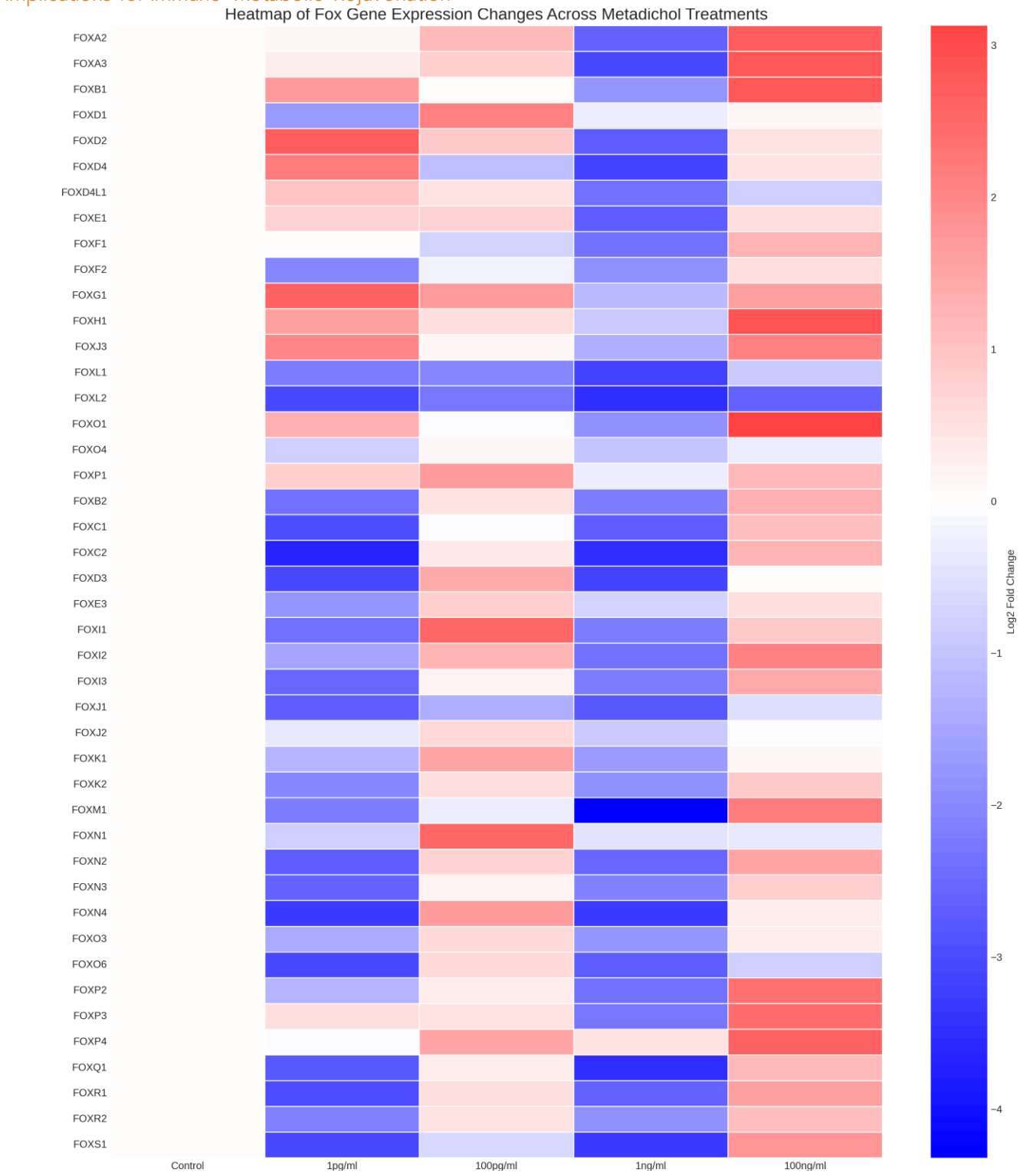


Figure 2: Heatmap showing log2-transformed fold changes in FOX gene expression across different metadichol concentrations in PBMCs. Red indicates upregulation, blue indicates downregulation, and white indicates no change relative to the control.

Identification of the most highly responsive FOX genes

At the highest metadichol concentration (100 ng/ml), several FOX genes were strongly upregulated (Figure 3). The five genes with the greatest increase in expression were FOXO1 (8.74-fold), FOXH1 (7.22-fold), FOXA3 (6.98-fold), FOXB1 (6.79-fold), and FOXA2 (6.57-fold). This robust induction suggests that these genes may be particularly sensitive to metadichol treatment and could play important roles in mediating their biological effects.

Bar plot showing the top 10 most upregulated FOX genes in PBMCs treated with 100 ng/ml metadichol compared with the control.

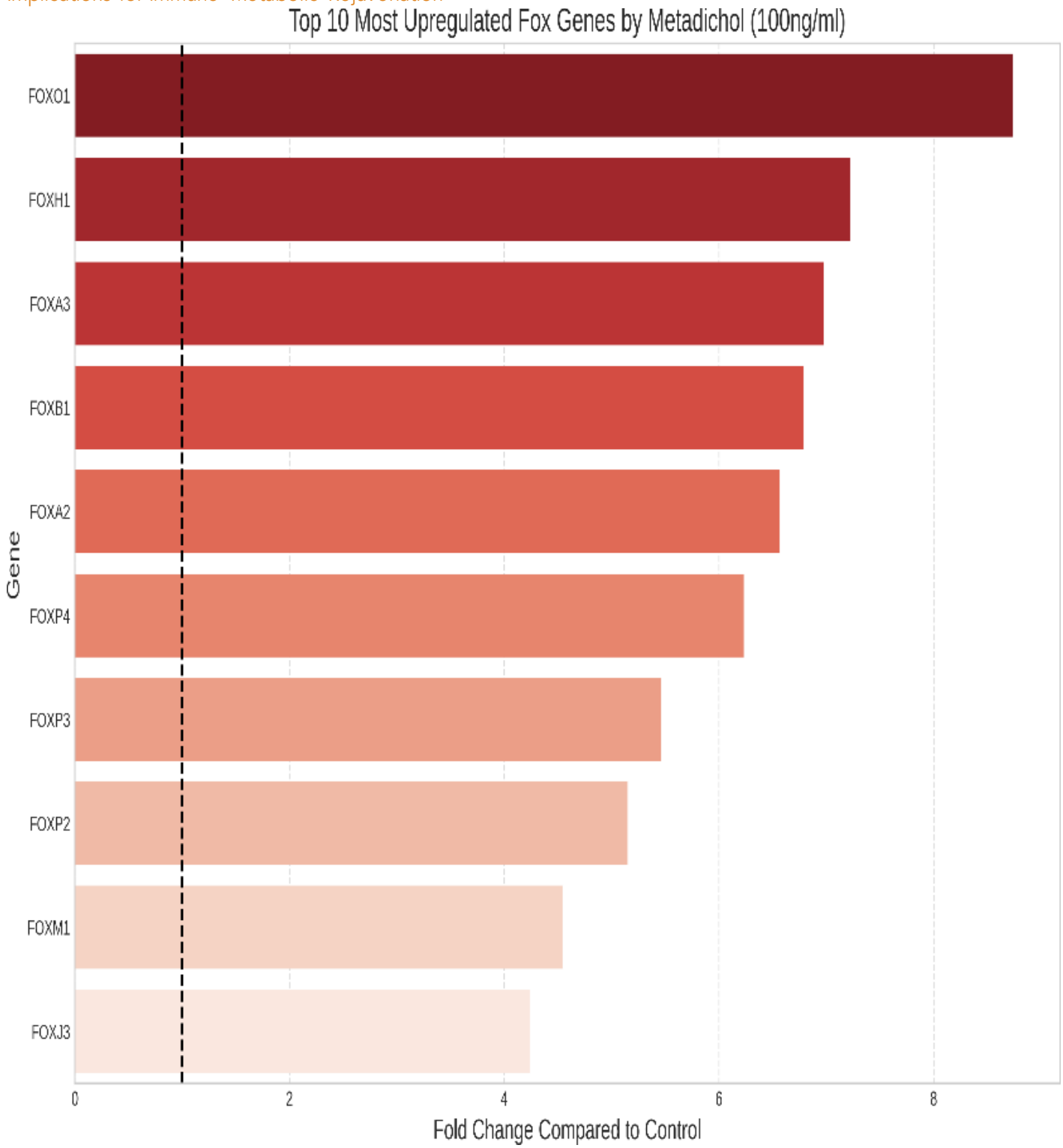


Figure 3

Distinct dose–response patterns reveal gene-specific regulation

Analysis of the dose–response relationships (figure 4) revealed three distinct patterns of gene expression changes in response to metadichol treatment:

High-concentration responders: Genes whose expression was primarily upregulated at 100 ng/ml, with minimal responses at lower concentrations (e.g., FOXO1, FOXH1, FOXA3

Dose–response curves showing biphasic expression patterns of selected FOX genes (FOXD2, FOXG1, FOXD4, and FOXP3) in response to Metadichol treatment.

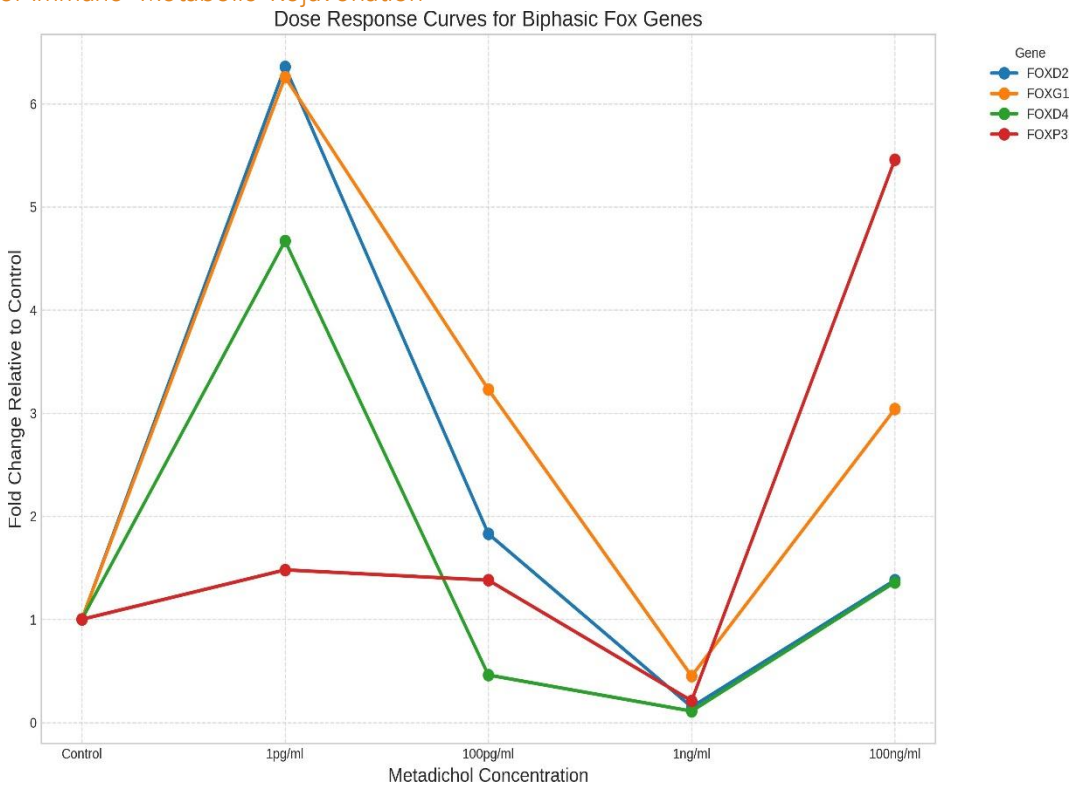


Figure 4

Biphasic/Hormetic responders: Genes whose expression was elevated at both low (1 pg/ml) and high (100 ng/ml) concentrations, but whose expression(figure 4) was reduced at intermediate concentrations (Figure 4). Key examples include FOXD2, FOXG1, FOXD4, and FOXP3. This U-shaped response suggests complex, concentration-dependent regulatory mechanisms.

ntermediate-concentration responders: Genes exhibiting peak expression (figure 5) at the intermediate concentration of 100 pg/ml, including FOXN1 (5.95-fold), FOXI1 (5.73-fold), FOXD1 (4.34-fold), and FOXN4 (3.30-fold)

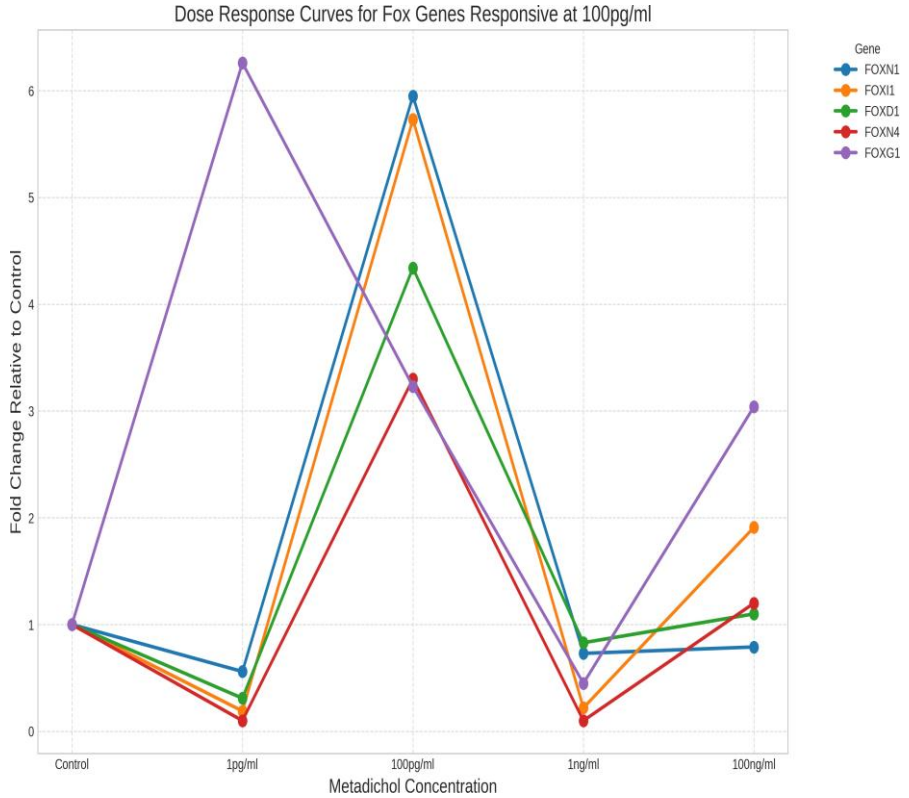


Figure 5: Dose–response curves showing FOX genes with peak expression at the intermediate metadichol concentration of 100 pg/ml.

Hierarchical clustering reveals coordinated gene expression patterns

Hierarchical clustering analysis of FOX gene expression patterns across metadichol concentrations revealed six distinct gene clusters with similar response profiles (Figure 6). This clustering suggests coordinated regulation of functionally related genes and provides insights into potential regulatory networks affected by metadichol.

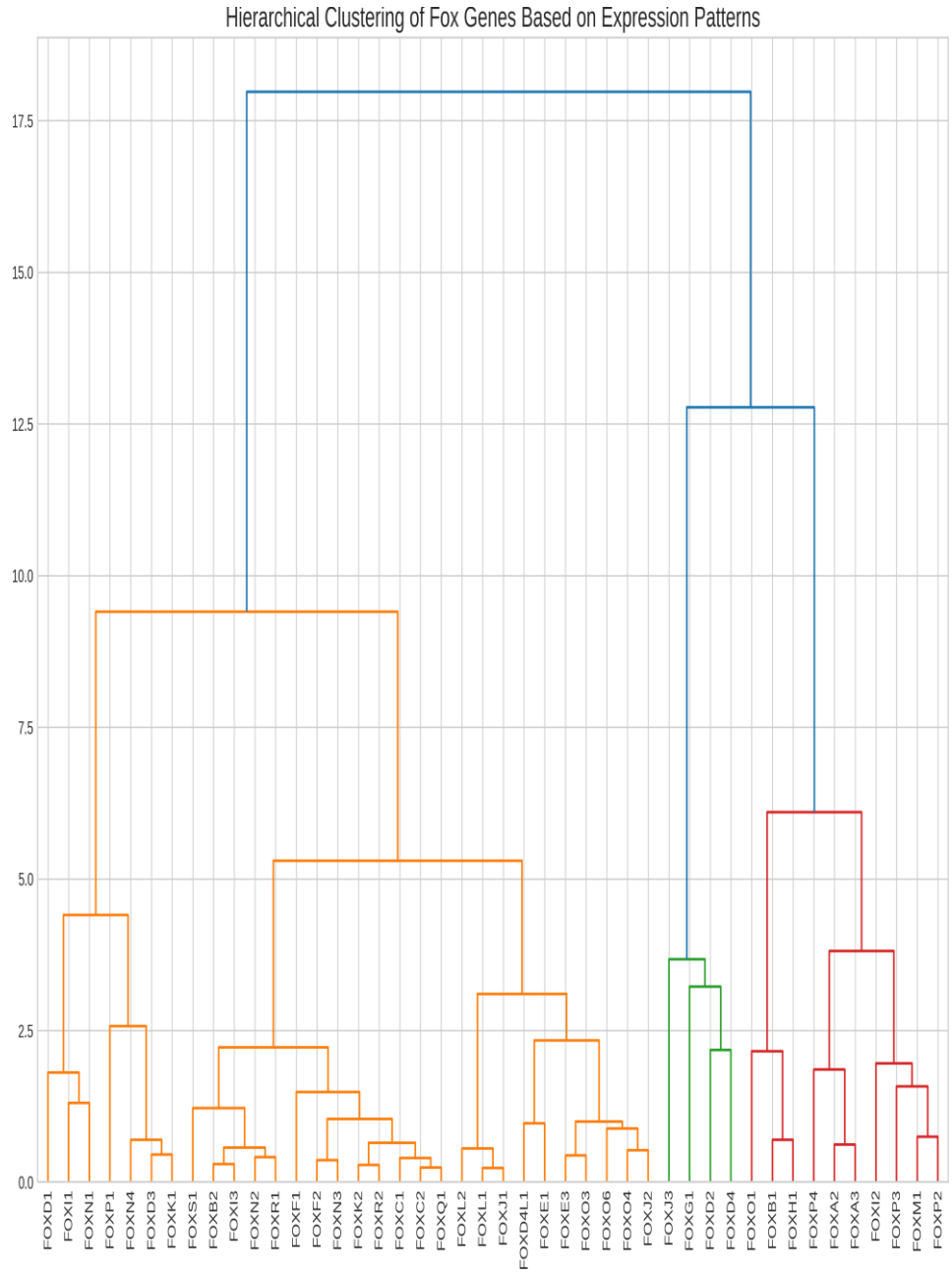


Figure 6:

Correlation analysis identifies highly coordinated gene pairs

Correlation analysis of gene expression patterns (Table 5) revealed several highly correlated gene pairs, suggesting coordinated regulation or functional relationships.

Table 5: Top 5 positive correlations

Rank	Gene 1	Gene 2	Correlation (r)
1	FOXC2	FOXQ1	0.9991
2	FOXB2	FOXC2	0.9978
3	FOXM1	FOXP2	0.9977
4	FOXC2	FOXR2	0.9974
5	FOXB2	FOXR1	0.9973

Notable examples included FOXC2-FOXQ1, FOXB2-FOXC2, and FOXM1-FOXP2, all with correlation coefficients above 0.99. These strong correlations (see supplemental files) suggest potential coregulation mechanisms or shared regulatory pathways affected by metadichol.

Significantly upregulated FOX genes

At the highest metadichol concentration (100 ng/ml), several FOX genes (figure 3) were markedly upregulated: FOXO1 presented the highest fold change of 8.74-fold, followed by FOXA1 (7.39-fold), FOXH1 (7.22-fold), FOXA3 (6.98-fold), FOXB1 (6.79-fold), FOXA2 (6.57-fold), and FOXP4 (6.23-fold). Additional genes show that the substantially increased genes included FOXP3 (5.46-fold), FOXP2 (5.15-fold), FOXM1 (4.54-fold), FOXJ3 (4.24-fold), and FOXI2 (4.15-fold).

Downregulated FOX Genes

Several FOX genes were significantly downregulated (Table 6) following metadichol treatment. FOXL2 demonstrated the most pronounced decrease (0.16-fold at 100 ng/ml), followed by FOXL1 (0.54-fold), FOXD4L1 (0.56-fold), FOXO6 (0.56-fold), FOXJ1 (0.70-fold), FOXN1 (0.79-fold), FOXO4 (0.84-fold), and FOXJ2 (0.98-fold).The complete Correlation of all 45 FOX family genes is shown in Fiugre 8

Table 6: Top 5 negative correlations - matrix

Rank	Gene 1	Gene 2	Correlation (r)
1	FOXD4	FOXE3	-0.5722
2	FOXD4	FOXO6	-0.4724
3	FOXD2	FOXE3	-0.4712
4	FOXD1	FOXD4	-0.4677
5	FOXD3	FOXD4	-0.4327

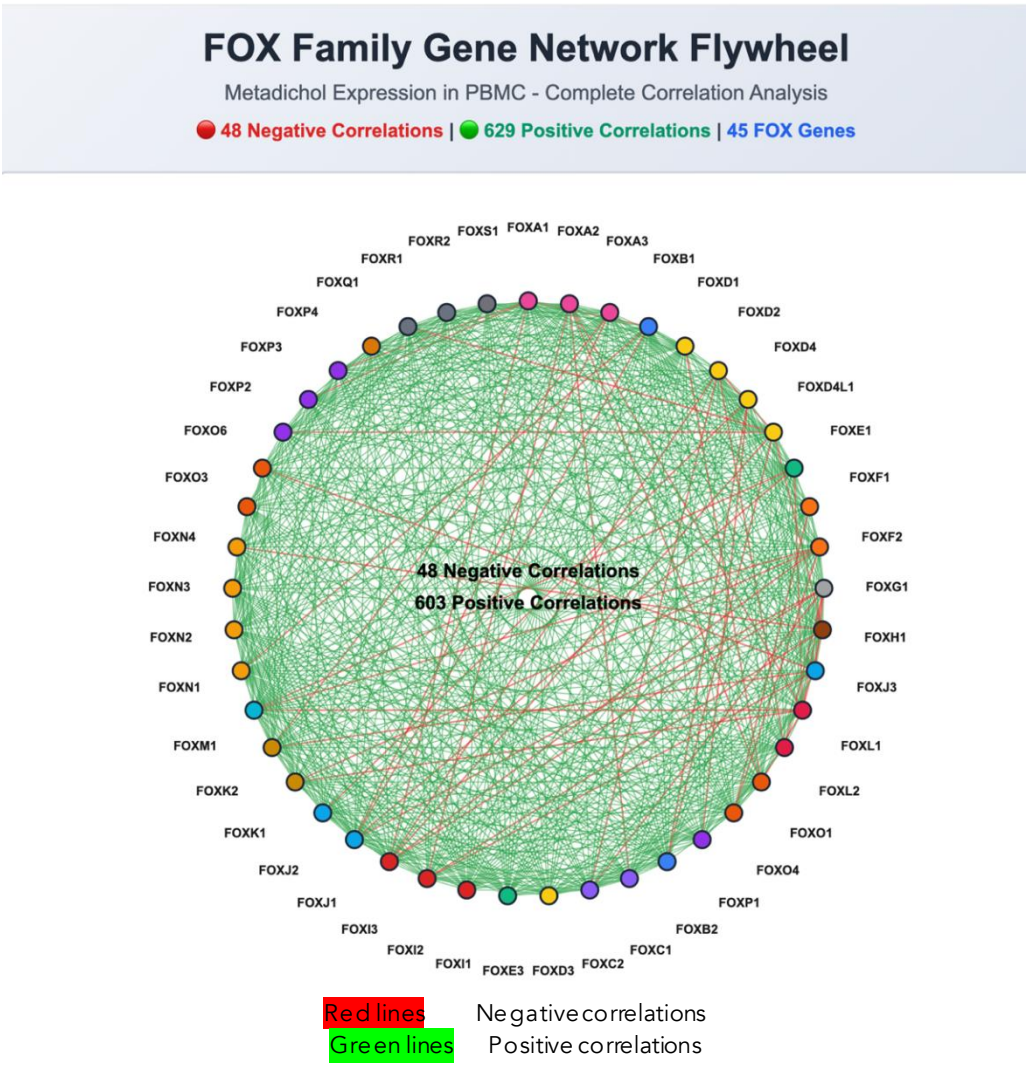


Figure 7

Family-Specific Response Patterns

Analysis of responses by FO subfamilies (Figure 8.9) revealed distinct patterns: FOXA subfamily members (FOXA1, FOXA2, and FOXA3) were consistently and highly upregulated, suggesting coordinated regulation. The FOXO subfamily showed mixed responses, with FOXO1 strongly upregulated while FOXO4 and FOXO6 were downregulated. FOXP subfamily members, particularly FOXP2, FOXP3, and FOXP4, are generally upregulated.

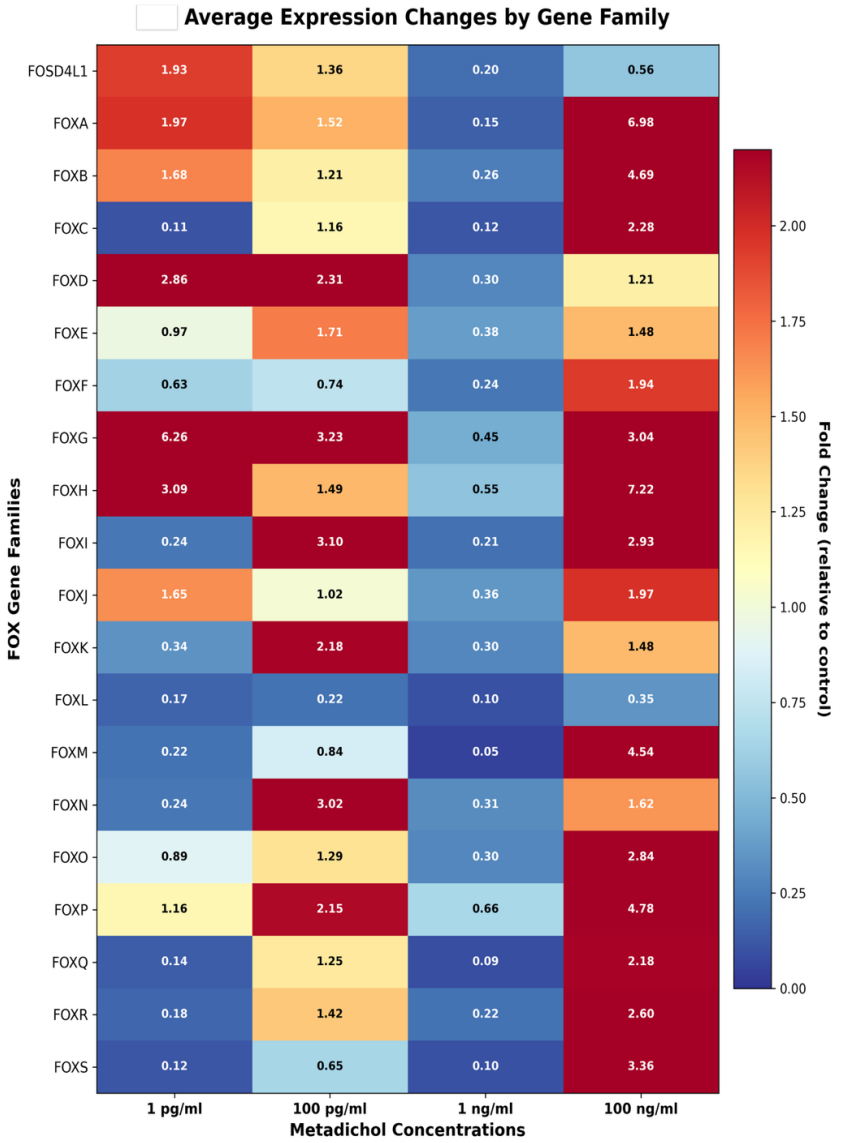


Figure 8

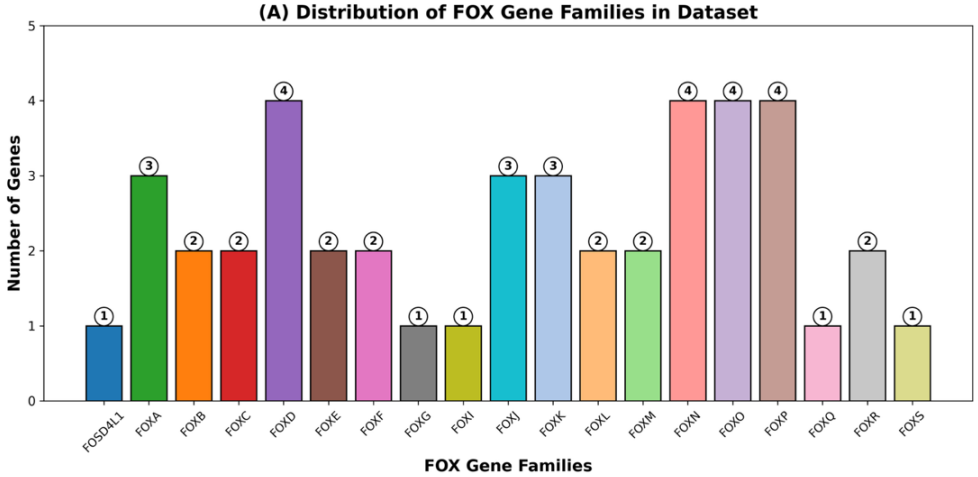


Figure 9

Western Blot analysis

A total of 12 Fox genes (table 7) were subjected to Western blot shown previously. It was compared to the Q-RT-PCR values WB = Western Blot (protein); PCR = RT-PCR (mRNA). Values represent fold change vs control.

Table 7: Yellow highlighting indicates significant changes (≥2-fold upregulation or ≤0.5-fold downregulation

Gene	MW	1 pg		100 pg		1 ng		100 ng	
		WB	PCR	WB	PCR	WB	PCR	WB	PCR
FOX A1	49 kD	1.18	3.56	1.68	0.49	1.14	0.16	2.03	7.39
FOX B1	35 kD	0.23	3.16	0.93	1.01	0.20	0.30	1.27	6.79
FOX C2	54 kD	0.90	0.08	1.72	1.33	1.13	0.09	3.35	2.40
FOX D3	47.6 kD	3.03	0.12	2.62	2.63	2.96	0.11	4.13	1.00
FOX G1	52 kD	2.90	6.26	1.18	3.23	1.99	0.45	2.38	3.04
FOX H1	39 kD	1.15	3.09	1.45	1.49	0.49	0.55	1.28	7.22
FOX I1	41 kD	1.18	0.19	2.00	5.73	1.14	0.22	2.03	1.91
FOX J3	69 kD	0.38	4.02	0.89	1.09	0.74	0.40	1.27	4.24
FOX L1	36 kD	0.20	0.22	0.67	0.24	0.05	0.11	0.82	0.54
FOX L2	39 kD	0.37	0.12	0.92	0.21	0.43	0.09	0.26	0.16
FOX P1	75 kD	0.21	1.80	1.04	3.16	0.61	0.84	0.12	2.28
FOX P3	47 kD	1.04	1.48	1.16	1.38	1.01	0.21	1.26	5.46

Key Findings

Consistent Upregulation (WB & PCR are in agreement)

FOX A1 at 100 ng: Strong upregulation at both protein (2.03×) and mRNA (7.39×) levels

FOX C2 at 100 ng: Upregulated at protein (3.35×) and mRNA (2.40×)

Consistent Downregulation

FOX L1 and FOX L2: Consistently suppressed across most concentrations at both protein and mRNA levels

Discordant Expression Patterns

FOX D3: Strong protein upregulation (3-4×) at all concentrations, but mRNA shows variable/low expression

suggests **post-transcriptional regulation**

FOX B1, FOX J3, FOX P1: Low protein expression but higher mRNA levels at certain doses — may indicate translational repression or protein degradation

FOX P3: Stable protein levels (~1×) but mRNA increases to 5.46× at 100 ng

Dose-Response Patterns

Most genes show non-linear dose responses

100 ng generally produces the strongest effects

1 ng often shows suppression relative to other doses

Discussion

The observed upregulation of FOX transcription factors following metadichol treatment likely involves multiple receptor pathways as shown in figure 11, which include nuclear receptors, toll-like receptors, sirtuins, KLF transcription factors, and sirtuins. It illustrates the effects of metadichol on various FOX subfamilies. through multiple regulatory pathways, including those involving nuclear receptors, sirtuins, the circadian clock machinery, and TLR signaling, resulting in downstream effects on immune regulation, metabolism, aging, and development.

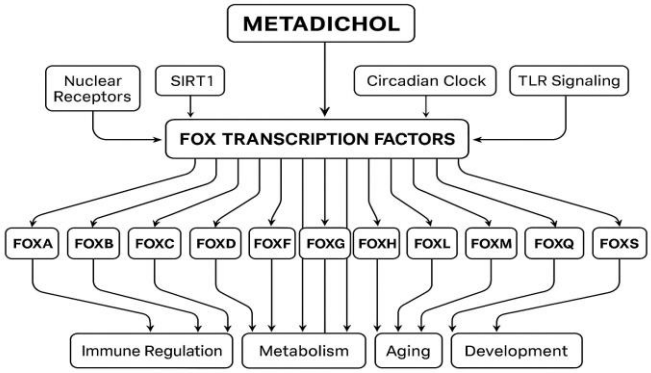


Figure 10: Metabolic-mediated regulation of FOX transcription factors.

Nuclear Receptor-Mediated FOX Gene Regulation

Nuclear receptors, (Figure 10,11) including the vitamin D receptor (VDR)[90], peroxisome proliferator-activated receptors (PPARs),[91] and estrogen receptors (ERs)[92], directly regulate FOX gene expression through chromatin interactions[93-94]. The documented activity of metadichol as a VDR ligand[76] suggests that vitamin D signaling pathways may contribute to the observed FOX gene modulation. VDR activation (figure 12) has been shown to upregulate FOXO1 expression[95] and enhance FOXA1 transcriptional activity[96,] which is consistent with our findings.

Peroxisome proliferator-activated receptor (PPAR) signaling represents another potential mechanism, as PPAR activation directly induces FOXA2 expression[97] and modulates FOXO1 activity[98]. The coordinated upregulation of FOXA subfamily members observed in our study aligns with the known role of nuclear receptors in hepatic gene expression programs[99]. Crosstalk between nuclear receptors and FOX transcription factors creates regulatory networks that control metabolic homeostasis[100-101].

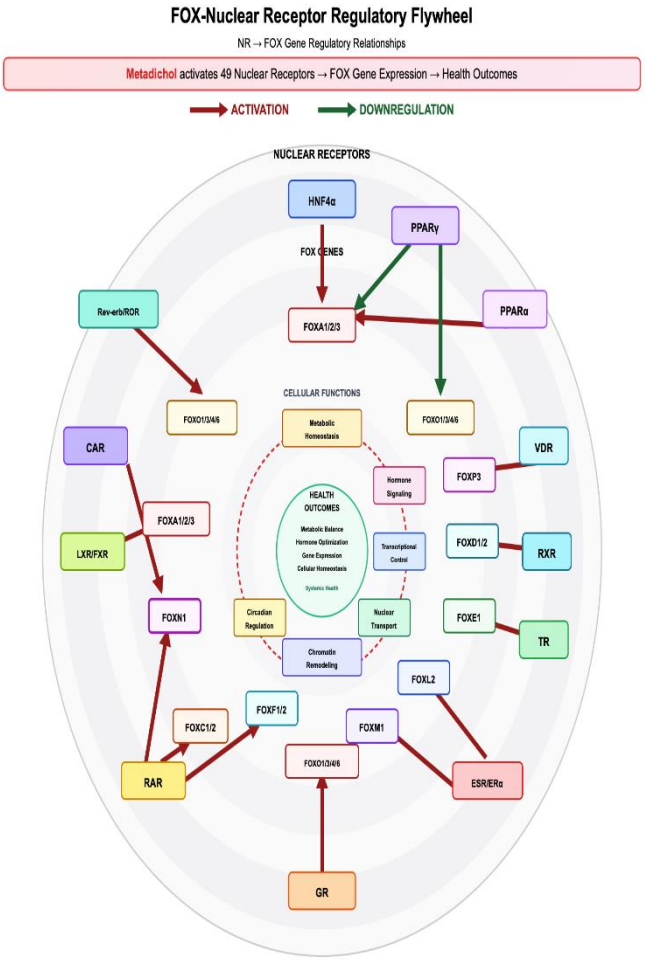


Figure 11

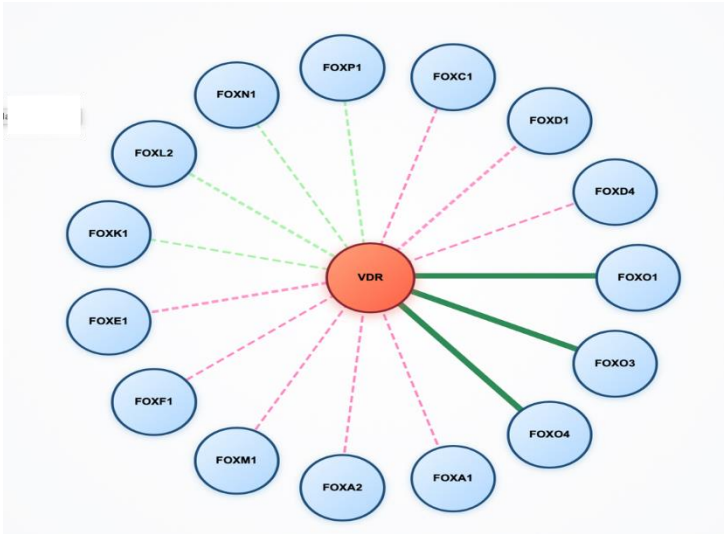


Figure 12: VDR activated FOX genes

FOX Genes and VDR Correlation Legend

FOX Genes

VDR (Vitamin D Receptor)

Correlation Strength Legend

Strong Positive (r ≥ 0.6) - Solid Thick

Moderate Positive (0.3 ≤ r < 0.6) - Solid

Weak Positive (0.15 ≤ r < 0.3) - Dashed

Very Weak Positive (0.05 ≤ r < 0.15) - Long Dash

Extremely Weak Positive (0 < r < 0.05) - Dotted

Near Zero (r = 0) - Gray Dotted

Extremely Weak Negative (-0.05 < r < 0) - Dotted

Very Weak Negative (-0.15 < r ≤ -0.05) - Long Dash

Weak Negative (-0.3 < r ≤ -0.15) - Dashed

Moderate Negative (-0.6 < r ≤ -0.3) – Solid

VDR Correlation Summary

Strong Positive: 3

Moderate Positive: 0

Weak Positive: 0

Weak Negative: 0

Moderate Negative: 0

Strong Negative: 0

Gene Counts

Total FOX genes: 15

Connected genes: 15

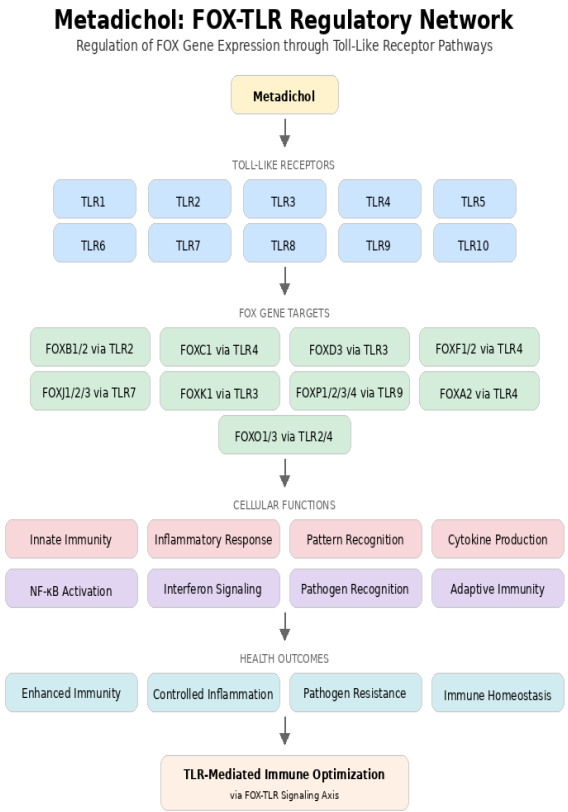


Figure 13 Toll-like Receptor and Fox gene regulation

Toll-like receptors (TLRs) are crucial pattern recognition receptors that modulate immune responses and transcriptional programs[102-103]. TLR3 activation (figure 12) has been shown to modulate FOX gene expression (figure 13) through interferon regulatory factor pathways[104], while TLR4 signaling can both positively and negatively regulate different FOX family members[105] The immunomodulatory effects of Metadichol[77] may involve TLR pathway interactions that contribute to the observed FOX gene expression changes.

Toll-like receptor (TLR)-mediated activation of nuclear factor-κB (NF-κB) pathways can directly influence FOXP3 expression[106] potentially explaining the substantial upregulation of FOXP3 observed in our study. Conversely, chronic TLR4 activation can suppress FOXO1 activity[107] suggesting that the effects of metadichol may involve the modulation of inflammatory signaling cascades[108-109].

SIRT1-mediated epigenetic regulation

Sirtuin 1 (SIRT1), an NAD+-dependent histone deacetylase, is a critical regulator of FOX transcription (figure14) factor activity[110-111]. SIRT1 directly deacetylates FOXO proteins, increasing their transcriptional activity and promoting longevity pathways[112-113]. The dramatic upregulation of FOXO1 observed in our study may have resulted from SIRT1-mediated posttranslational modifications that stabilize and activate FOXO proteins[114].

SIRT1 also modulates FOXA2 activity through direct protein–protein interactions[115] and influences FOXP3 expression in regulatory T cells[116]. The coordinated regulation of multiple FOX genes by SIRT1 suggests that Metadichol activates sirtuin pathways, leading to increased cellular stress resistance and metabolic efficiency[117-118]. This mechanism aligns with the reported antiaging effects of metadichol.

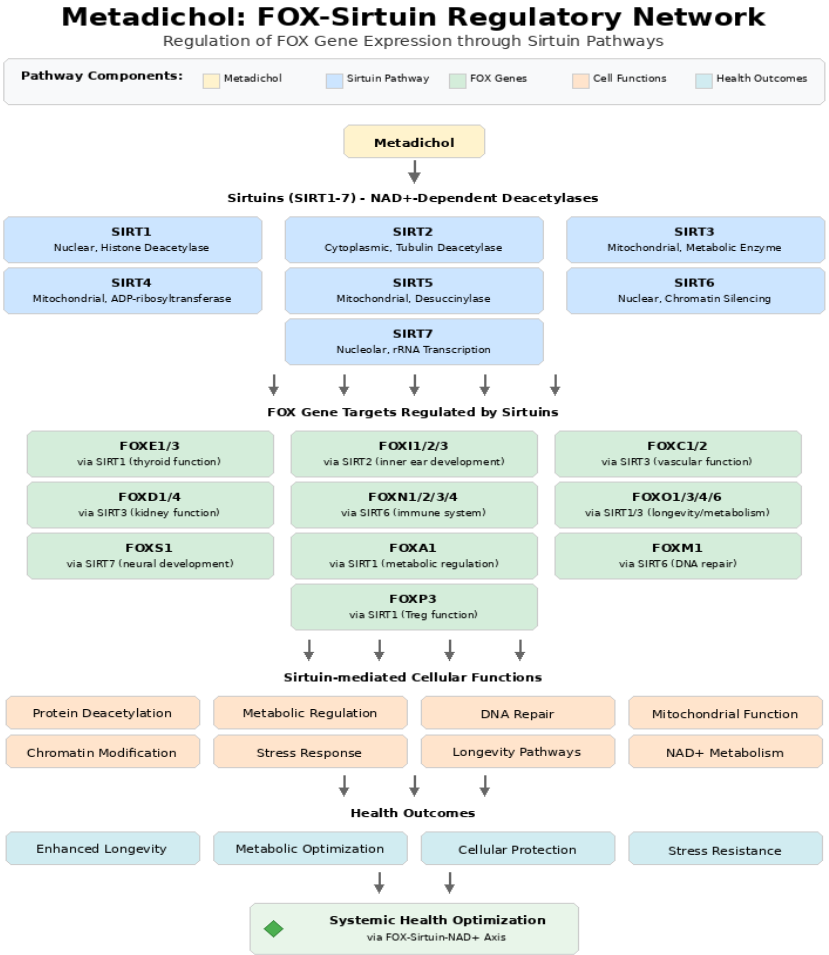


Figure 14: Sirtuins and Foxgene regulation

Krüppel-like Factor Interactions

Krüppel-like factors (KLFs) constitute a family of zinc finger transcription factors that interact extensively with FOX proteins (figure 15)[119-1201]. KLF4 directly regulates FOXP3 expression through chromatin remodeling[122], whereas KLF2 modulates FOXO1 activity in endothelial cells[123]. Complex regulatory networks involving KLF-FOX interactions may contribute to the selective gene expression patterns observed following metadichol treatment[124].

KLF15 has been shown to cooperate with FOXA2 in hepatic gluconeogenesis[125], whereas KLF11 interacts with FOXO1 to regulate pancreatic β-cell function[126]. These transcriptional networks create integrated regulatory circuits that respond to metabolic and environmental stimuli[127].

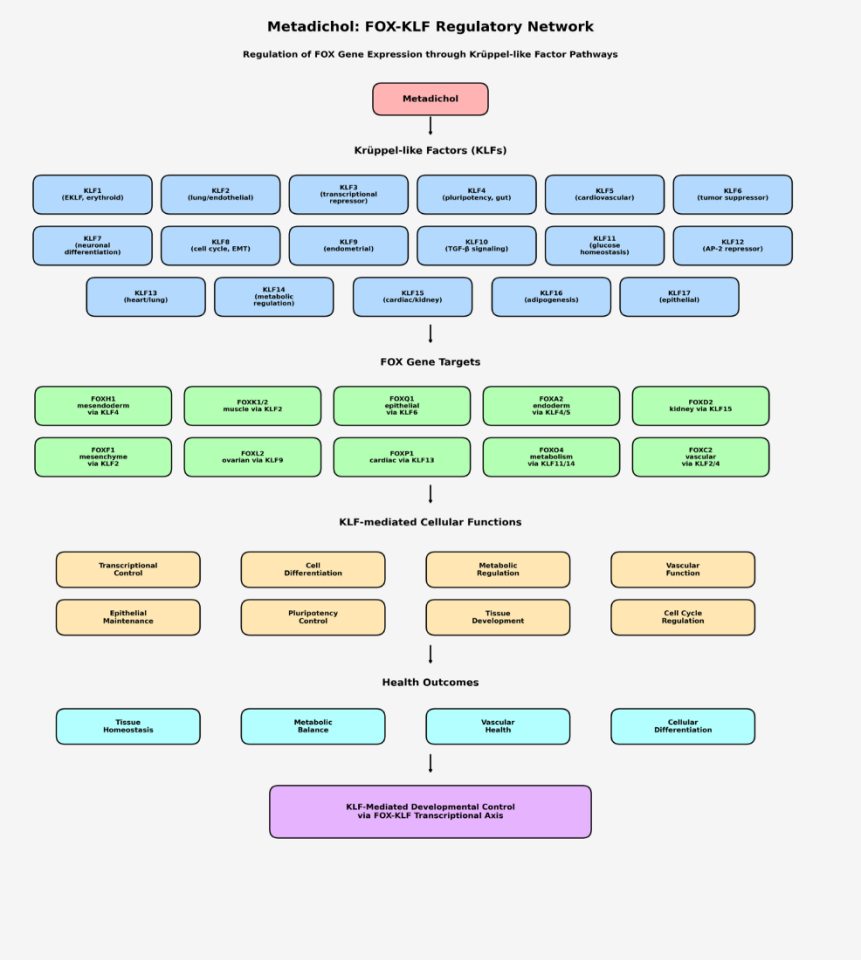


Figure 15; Krüppel-like Factor and Foxgene regulation

Circadian Clock Gene Regulation

The circadian clock machinery, comprising the core components CLOCK and BMAL1, exhibits extensive cross talk with FOX transcription factors Figure (16)[128-129] CLOCK:BMAL1 heterodimers directly regulate FOXO1 expression through E-box elements[130], whereas FOXO proteins reciprocally influence circadian gene expression[131]. The observed FOX gene upregulation may reflect the effects of Metadichol on circadian regulatory networks.

Forkhead box A1 (FOXA1) and FOXA2 exhibit circadian expression patterns in liver tissue[132] and their upregulation following metadichol treatment suggests that potential modulation of metabolic rhythms 150 Circadian disruption has been linked to metabolic dysfunction[133], and FOX transcription factors serve as key mediators of temporal gene expression[134].

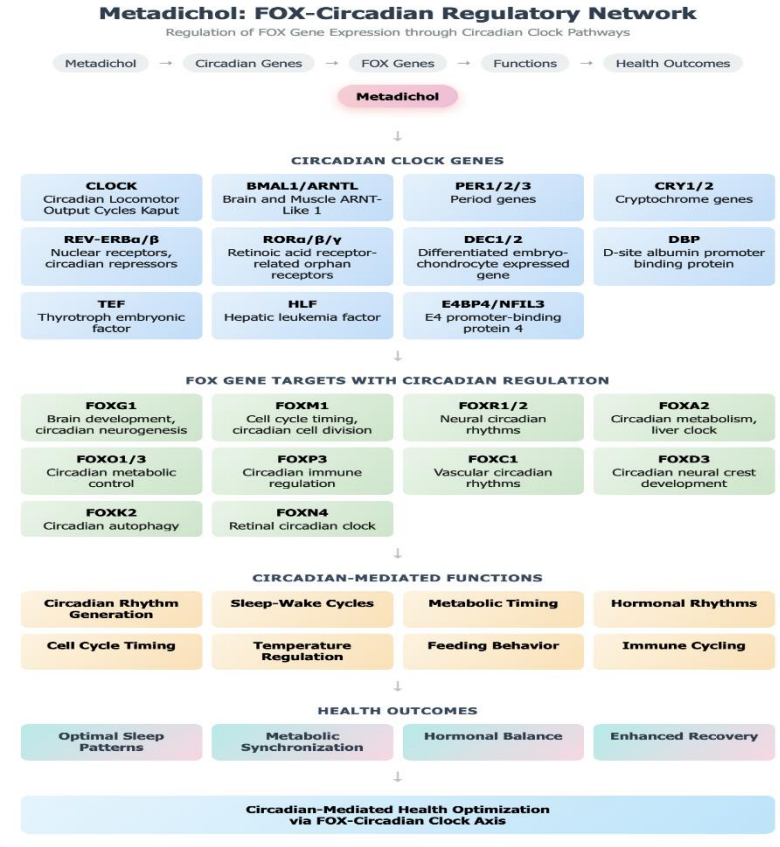


Figure 16; Circadian genes and Fox gene regulation

Klotho-Mediated Anti-Aging Pathways

Klotho, a transmembrane protein with established antiaging properties[135] modulates FOX transcription factor activity through multiple mechanisms. (figure 17)[136.] Klotho deficiency leads to accelerated aging phenotypes accompanied by altered FOXO signaling[137], whereas Klotho overexpression enhances FOXO-mediated stress resistance[138]. The upregulation of FOXO1 and related longevity-associated FOX genes in our study may reflect Klotho pathway activation[139].

Klotho functions as a coreceptor for fibroblast growth factor 23 (FGF23) and modulates[139] Wnt signaling pathways that intersect with FOX transcription factor networks[140]. The integration of Klotho signaling with FOX-mediated transcriptional programs creates regulatory circuits that control cellular senescence and organismal aging[141-142].

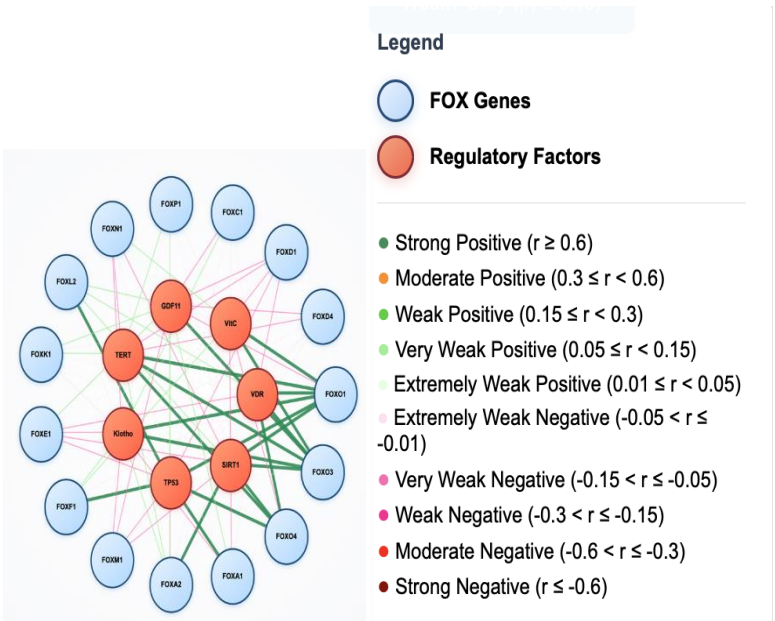


Figure 17: Fox genes and anti-aging factors

Telomerase and Cellular Senescence

Telomerase reverse transcriptase (TERT) expression is regulated by multiple transcription factors, including several FOX family members[143-144]. FOXE1 has been shown to interact with ETS(E26 transformation-specific or Erythroblast Transformation Specific)factors to co-regulate TERT expression[145] whereas FOXC1 influences telomerase activity through chromatin modifications[146]. The coordinated upregulation of FOX genes observed in our study may contribute to enhanced cellular longevity through telomerase-dependent mechanisms.[147]

Forkhead box O (FOXO) proteins directly regulate genes involved in DNA damage repair[148] and cellular senescence[149], processes that are intimately linked to telomere maintenance[150]. The substantial upregulation of FOXO1 following metadichol treatment suggests the activation of cellular protection mechanisms that may counteract the age-related decline[151-152].

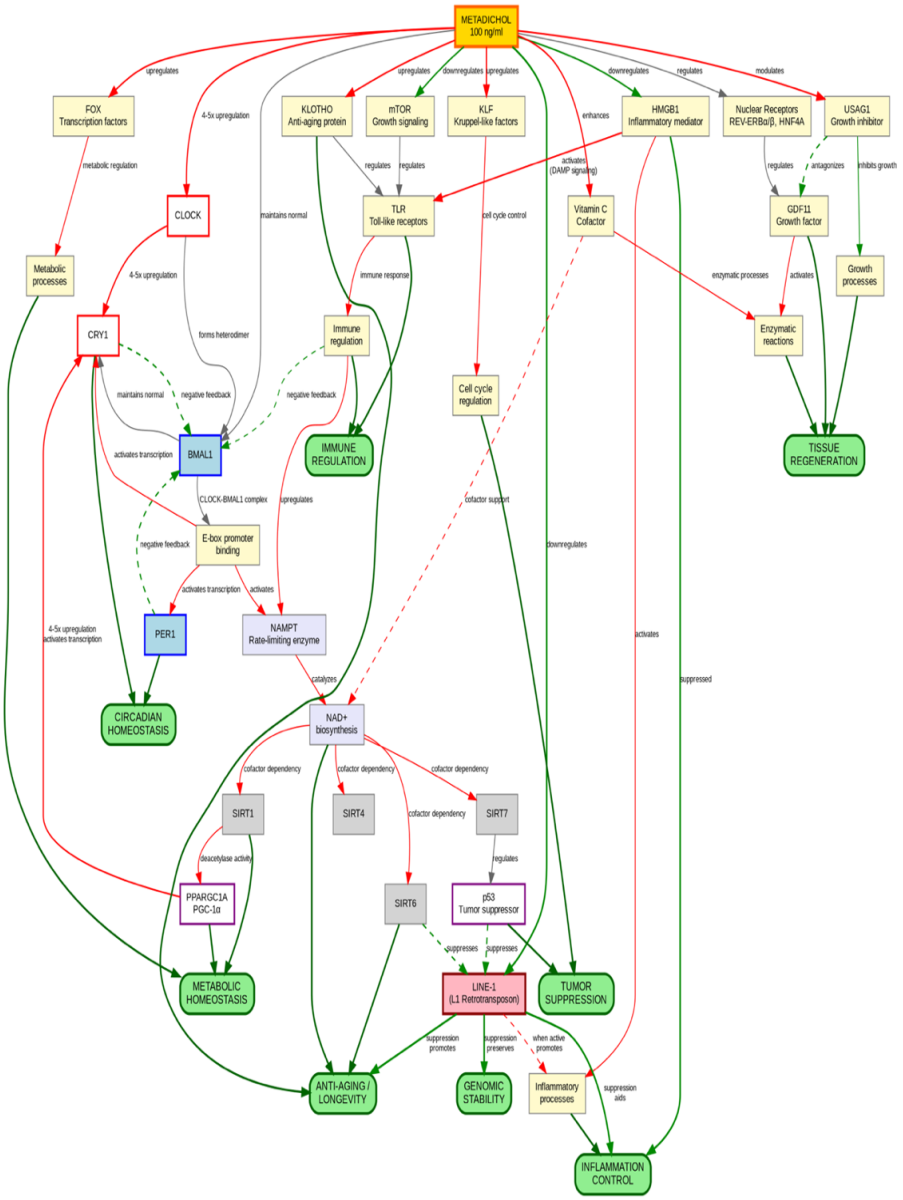


Figure 18: Metadichol induced pathways to homeostasis

Growth Differentiation Factor 11 (GDF11) Signaling

Growth differentiation factor 11 (GDF11), a member of the TGF-β superfamily, has emerged as a critical regulator of aging and tissue homeostasis[153-154]. Growth differentiation factor 11 (GDF11) signaling influences FOX transcription factor expression through Smad-dependent pathways[155], and several FOX proteins serve as downstream effectors of GDF11-mediated rejuvenation[156]. The observed upregulation of multiple FOX genes may reflect the activation of GDF11 signaling cascades that promote cellular regeneration[157]. GDF11 administration has been shown to increase FOXO signaling in aged tissues[158]

and restore metabolic function through FOXA-mediated transcriptional programs[159]. The integration of GDF11 signaling with FOX transcription factor networks creates regulatory circuits that control tissue repair and regenerative capacity[160-161].

Based on the discussion above one can consolidate these multiple regulatory interactions into a comprehensive network (figure 18) of pathways regulated by Metadichol on Fox expressions leading to homeostasis (green rectangular boxes).

Conclusions.

The comprehensive modulation of FOX transcription factors by Metadichol has significant implications (Figure 1) for therapeutic applications. The upregulation of FOXO1 suggests potential benefits for metabolic disorders, as FOXO1 regulates insulin sensitivity[162] and glucose homeostasis[163]. Enhanced FOXA1 expression may improve hepatic function[164] and lipid metabolism[165]. The substantial increase in FOXP3 expression indicates immunomodulatory potential, as FOXP3+ regulatory T cells are crucial for immune tolerance[166] and prevention of autoimmune diseases[167]. The observed effects involve multiple regulatory mechanisms, including nuclear receptor signaling[168] sirtuin-mediated epigenetic modifications[169], integration with circadian[170], longevity[171] and immune regulatory pathways[172]. The preferential upregulation of genes associated with metabolic homeostasis, cellular protection, and immune regulation suggests significant therapeutic potential for age-related diseases and metabolic disorders[173-174].

Critically, the robust upregulation of FOX transcription factors is central to induced pluripotency[175], including FOXO1, FOXD3, FOXM1, and the FOXA subfamily—establishes Metadichol as a powerful modulator of cellular reprogramming networks. These findings carry profound implications for regenerative medicine, as FOX factors serve as master regulators of stem cell maintenance, directed differentiation, and tissue regeneration. The ability of Metadichol to simultaneously activate multiple pluripotency-associated pathways suggests its potential utility in enhancing iPSC generation efficiency, optimizing cellular reprogramming protocols, and advancing autologous cell-based therapies for degenerative diseases.

This work establishes Metadichol as a promising therapeutic candidate that operates at the intersection of Nobel Prize-recognized immune tolerance mechanisms and cutting-edge regenerative medicine[176-178]. The ability of this nano-lipid formulation to simultaneously enhance FOXP3-mediated immune regulation and activate pluripotency-associated transcription factor networks represents a paradigm shift in our approach to treating immune disorders, age-related diseases, and degenerative conditions. In summary Metadichol given the safety profile[179-180] offers a transformative approach to regenerative medicine, one that may ultimately enable the restoration of tissue function, reversal of age-related decline, and treatment of previously intractable degenerative conditions through endogenous cellular reprogramming mechanisms.

Supplementary Information.

A version of this paper was published as a Pre-Print. Raghavan, P. Metadichol Orchestrates T, B, and NK Cell Immunity Through Master Regulator Gene Networks. Preprints 2025, 2025110719. https://doi.org/10.20944/preprints202511.0719.v1
Raw data; file name:
q-RT–PCR-Fox.PDF
Fox-Western-blot.PDF.
Fox-Correlation Analysis.xslx
The author is the founder of Nanorx, Inc USA and is a major shareholder in the company.
This study was conducted independently by an external service provider laboratory on commercial terms to eliminate bias in our results.

Conflict of Interest Statement:

None.

Funding Statement:

None.

Acknowledgements:

None.

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GLOSSARY OF GENES

FOX (Forkhead Box) Transcription Factor Family

FOXA1 – Forkhead box A1: Pioneer transcription factor involved in liver specification, pancreatic development, and nuclear receptor cofactor activity

FOXA2 – Forkhead box A2: Regulator of endodermal organ development, glucose homeostasis, and pancreatic β-cell function

FOXA3 – Forkhead box A3: Hepatic gene expression regulator involved in liver development and bile acid synthesis

FOXB1 – Forkhead box B1: Neural development factor regulating brain development and neural differentiation

FOXB2 – Forkhead box B2: Transcription factor involved in neural development

FOXC1 – Forkhead box C1: Transcription factor involved in eye and cardiovascular development

FOXC2 – Forkhead box C2: Regulator of lymphatic and vascular development

FOXD1 – Forkhead box D1: Kidney morphogenesis regulator, EMT regulation, and cancer metastasis factor

FOXD2 – Forkhead box D2: Neural crest development regulator

FOXD3 – Forkhead box D3: Neural crest specification and stem cell pluripotency factor

FOXD4 – Forkhead box D4: Early embryonic development regulator

FOXD4L1 – Forkhead box D4-like 1: Recently duplicated gene in humans

FOXE1 – Forkhead box E1: Thyroid morphogenesis and neural tube closure regulator

FOXE3 – Forkhead box E3: Lens development regulator

FOXF1 – Forkhead box F1: Mesenchymal development and lung development factor

FOXF2 – Forkhead box F2: Kidney morphogenesis and vascular development regulator

FOXG1 – Forkhead box G1: Forebrain development and neurogenesis regulator

FOXH1 – Forkhead box H1: Mesoderm formation, gastrulation, and TGF-β signaling mediator

FOXI1 – Forkhead box I1: Inner ear and kidney development regulator

FOXI2 – Forkhead box I2: Epithelial differentiation regulator

FOXI3 – Forkhead box I3: Craniofacial development factor

FOXJ1 – Forkhead box J1: Ciliogenesis and respiratory epithelium regulator

FOXJ2 – Forkhead box J2: Cell cycle regulator involved in G2/M transition

FOXJ3 – Forkhead box J3: Mitotic regulation and chromosome segregation factor

FOXK1 – Forkhead box K1: Muscle development and cell cycle regulator

FOXK2 – Forkhead box K2: Skeletal muscle development and glucose metabolism regulator

FOXL1 – Forkhead box L1: Gastrointestinal development and homeostasis regulator

FOXL2 – Forkhead box L2: Ovarian follicle development and sex determination factor

FOXM1 – Forkhead box M1: Cell cycle progression, DNA repair, and mitosis regulator

FOXN1 – Forkhead box N1: Thymic development and T-cell maturation regulator

FOXN2 – Forkhead box N2: Neural development and neuronal differentiation factor

FOXN3 – Forkhead box N3: Cell cycle checkpoint and DNA repair regulator

FOXN4 – Forkhead box N4: Retinal neurogenesis and photoreceptor development regulator

FOXO1 – Forkhead box O1: Master regulator of gluconeogenesis, insulin sensitivity, and cellular stress response

FOXO3 – Forkhead box O3: Oxidative stress response, longevity pathways, and apoptosis regulator

FOXO4 – Forkhead box O4: Cell cycle arrest, senescence, and DNA repair factor

FOXO6 – Forkhead box O6: Brain function, memory consolidation, and hepatic gluconeogenesis regulator

FOXP1 – Forkhead box P1: B-cell differentiation and cardiac morphogenesis factor

FOXP2 – Forkhead box P2: Speech acquisition, motor learning, and synaptic plasticity regulator

FOXP3 – Forkhead box P3: Master regulator of regulatory T-cell (Treg) development, immune suppression, and self-tolerance

FOXP4 – Forkhead box P4: T-cell differentiation and heart development regulator

FOXQ1 – Forkhead box Q1: Epithelial homeostasis and EMT regulation factor

FOXR1 – Forkhead box R1: Brain development and cell proliferation regulator

FOXR2 – Forkhead box R2: Neural development and transcriptional regulation factor

FOXS1 – Forkhead box S1: Cranial neural crest formation factor

Other Genes and Proteins

BMAL1 – Brain and Muscle ARNT-Like 1: Core circadian clock component

CLOCK – Circadian Locomotor Output Cycles Kaput: Core circadian rhythm regulator

FGF23 – Fibroblast Growth Factor 23: Phosphate homeostasis regulator

GAPDH – Glyceraldehyde-3-Phosphate Dehydrogenase: Housekeeping gene used as reference in qPCR experiments

GDF11 – Growth Differentiation Factor 11: TGF-β superfamily member involved in aging and tissue homeostasis

KLF2 – Krüppel-Like Factor 2: Zinc finger transcription factor in endothelial cells

KLF4 – Krüppel-Like Factor 4: Pluripotency factor and chromatin remodeling regulator

KLF11 – Krüppel-Like Factor 11: Pancreatic β-cell function regulator

KLF15 – Krüppel-Like Factor 15: Hepatic gluconeogenesis regulator

Klotho – Anti-aging transmembrane protein modulating FGF23 signaling and Wnt pathways

NANOG – Homeobox transcription factor essential for embryonic stem cell self-renewal and pluripotency

NF-κB – Nuclear Factor Kappa B: Key transcription factor in inflammation and immune responses

OCT4 – Octamer-Binding Transcription Factor 4 (POU5F1): Master pluripotency regulator in embryonic stem cells

PPAR – Peroxisome Proliferator-Activated Receptor: Nuclear receptor family regulating lipid metabolism and inflammation

SIRT1 – Sirtuin 1: NAD+-dependent histone deacetylase involved in longevity and metabolic regulation

SOX2 – SRY-Box Transcription Factor 2: Core pluripotency factor in embryonic stem cells

TERT – Telomerase Reverse Transcriptase: Catalytic subunit of telomerase maintaining telomere length

TGF-β – Transforming Growth Factor Beta: Cytokine superfamily regulating cell growth, differentiation, and immune function

TLR – Toll-Like Receptor: Pattern recognition receptors in innate immunity (TLR3, TLR4 referenced in text)

VDR – Vitamin D Receptor: Nuclear receptor for vitamin D signaling