

## SUPPLEMENTARY MATERIALS

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"The gene network and knowledge base on human thermoregulation"

### Supplementary Material S1. Input terms entered in the search query for genes associated with thermoregulation in the Gene Ontology resource, Entrez Gene and ANDSystem

Database	Input terms	Number of genes found
Gene Ontology	Sensory perception of cold stimulus	334
	Cold acclimation	
	Response to cold	
	Cold-induced thermogenesis	
	Trehalose metabolism in response to cold stress	
	Cellular response to cold	
	Regulation of cold-induced thermogenesis	
	Positive regulation of cold-induced thermogenesis	
	Negative regulation of cold-induced thermogenesis	
	Detection of cold stimulus involved in thermoception	
	Thermoception	
	Detection of temperature stimulus	
	Regulation of cellular response to heat	
	Positive regulation of cellular response to heat	
	Negative regulation of cellular response to heat	
	Cellular response to heat	
	Response to heat	
	Heat acclimation	
	Trehalose metabolism in response to heat stress	
	Cellular heat acclimation	
	mRNA export from nucleus in response to heat stress	
	Heat generation	
	Heat dissipation	
	Regulation of heat dissipation	
	Negative regulation of heat generation	
	Positive regulation of heat generation	
	Negative regulation of heat dissipation	
	Positive regulation of heat dissipation	
	Regulation of heat generation	
	Regulation of mRNA export from nucleus in response to heat stress	
	Trehalose biosynthesis in response to heat stress	
	Trehalose catabolism in response to heat stress	
	Thermomorphogenesis	
Thermoception		
Detection of temperature stimulus involved in sensory perception		
Cold-induced thermogenesis		
Adaptive thermogenesis		
Temperature-gated ion channel activity		
Temperature-gated cation channel activity		
Detection of hot stimulus involved in thermoception		
Circadian temperature homeostasis		
Temperature homeostasis		
Entrez Gene	Thermoregulation	43
ANDSystem	Positive regulation of cold-induced thermogenesis	253
	Negative regulation of cold-induced thermogenesis	
	Response to cold	
	Detection of temperature stimulus involved in sensory perception of pain	
	Cellular response to heat	
Response to heat		
Detection of temperature stimulus involved in sensory perception of pain		

## Supplementary Material S2

### Materials and methods

**Building gene network.** The gene network was built using the ANDSystem software and information system developed at the IC&G SB RAS (Ivanisenko V.A. et al., 2019; Ivanisenko T.V. et al., 2024). The Query Wizard tool of the ANDVisio tool (a software component of the ANDSystem) was used. As the first step, we built a network involving all the genes associated with thermoregulation and the proteins encoded by them, choosing *Homo sapiens* as the type of organism. This network included all types of interactions, except for the type of interactions called “associations”. As the second step, a list of proteins encoded by genes associated with thermoregulation was submitted to the Query Wizard tool, and a search for microRNAs regulating expression of these proteins was conducted. This search identified regulatory interactions using data imported into ANDSystem from miRTarBase, a database containing experimentally validated microRNA–mRNA interactions (Cui et al., 2025). Next, the two networks were merged using the ANDVisio tool.

**Analysis of evolutionary characteristics of genes.** The analysis of the evolutionary characteristics of genes was carried out using the PAI (Phylostratigraphic Age Index) and DI (Divergence Index). Both sets of data are available in the PAI and DI fields of the *Genes\_evol* table within the *Termo\_Reg\_Human 1.0* Knowledge Base. The phylostratigraphic age index calculated using the Orthoweb system (Ivanov et al., 2024) was suggested in our previous studies (Mustafin et al., 2017). It is a numerical scale (numbers from 1 to 16) reflecting the evolutionary age of a gene, conceptually similar to the phylostratigraphic levels used in previous phylostratigraphic studies (Domazet-Loso et al., 2007; Domazet-Loso et al., 2008; Zhang et al., 2019). The PAI value corresponds to the conditional distance from the root of the phylogenetic tree for the taxon at which the divergence of the studied species occurred with the most distant related taxon in which the homolog of the gene in question was found. The later in the course of evolution the ancestor form of the studied gene is detected, the greater is the PAI value of this gene. The PAI values were calculated using the Orthoweb program (Ivanov et al., 2024) based on the KEGG SSDB (Sequence Similarity DataBase), taking into account the sequences of homologous genes that are 50 % or more identical to the human gene under consideration (Mustafin et al., 2017, 2021). Thus, the PAI values were calculated for 19,504 human protein-coding genes.

The DI values were also calculated in the Orthoweb program (Ivanov et al., 2024) based on a comparison of protein-coding regions of human genes with regions of homologous genes of closely related organisms from the hominid family (*Pan troglodytes* (chimpanzee), *Pan paniscus* (pygmy chimpanzee), *Gorilla gorilla gorilla* (western lowland gorilla), *Pongo abelii* (Sumatran orangutan)), as described in the publication (Mustafin et al., 2021). Thus, the DI index values were calculated for 19,504 human protein-coding genes.

The probability of obtaining a given number of genes with a certain value of the PAI or DI evolutionary metrics was estimated using the chi-square criterion.

### References

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**Supplementary Material S3.** The references to scientific publications confirming interactions between genes and proteins shown in Figure 2b

Object 1, regulator	Object 2, regulated	Reference
CEBPB (protein)	PPARG (gene)	Ahmed M., Lai T.H., Hwang J.S., Zada S., Pham T.M., Kim D.R. Transcriptional regulation of autophagy genes via stage-specific activation of CEBPB and PPARG during adipogenesis: a systematic study using public gene expression and transcription factor binding datasets. <i>Cells</i> . 2019;8(11):1321. doi 10.3390/cells8111321
EGR1 (protein)	PPARG (gene)	Nebbaki S.S., El Mansouri F.E., Benderdour M., Martel-Pelletier J., Pelletier J.P., Fahmi H. Egr-1 mediates the suppressive effect of IL-1 on PPARG expression in human OA chondrocytes. <i>Arthritis Res Ther</i> . 2012;14(Suppl. 1):P12. doi 10.1186/ar3613
IL1B (protein)	PPARG (gene)	Nebbaki S.S., El Mansouri F.E., Benderdour M., Martel-Pelletier J., Pelletier J.P., Fahmi H. Egr-1 mediates the suppressive effect of IL-1 on PPARG expression in human OA chondrocytes. <i>Arthritis Res Ther</i> . 2012;14(Suppl. 1):P12. doi 10.1186/ar3613
IL4 (protein)	PPARG (gene)	Szanto A., Balint B.L., Nagy Z.S., Barta E., Dezso B., Pap A., Szeles L., Poliska S., Oros M., Evans R.M., Barak Y., Schwabe J., Nagy L. STAT6 transcription factor is a facilitator of the nuclear receptor PPAR $\gamma$ -regulated gene expression in macrophages and dendritic cells. <i>Immunity</i> . 2010;33(5):699-712. doi 10.1016/j.immuni.2010.11.009
LEP (protein)	PPARG (gene)	Wu K., Tan X.Y., Xu Y.H., Chen G.H., Zhuo M.Q. Functional analysis of promoters of genes in lipid metabolism and their transcriptional response to STAT3 under leptin signals. <i>Genes (Basel)</i> . 2018;9(7):334. doi 10.3390/genes9070334
LIPL (protein)	PPARG (gene)	Zhao W.S., Hu S.L., Yu K., Wang H., Wang W., Loo J., Luo J. Lipoprotein lipase, tissue expression and effects on genes related to fatty acid synthesis in goat mammary epithelial cells. <i>Int J Mol Sci</i> . 2014;15(12):22757-22771. doi 10.3390/ijms151222757
MECP2 (protein)	PPARG (gene)	Mann J., Chu D.C., Maxwell A., Oakley F., Zhu N.L., Tsukamoto H., Mann D.A. MeCP2 controls an epigenetic pathway that promotes myofibroblast transdifferentiation and fibrosis. <i>Gastroenterology</i> . 2010;138(2):705-714.e1-4. doi 10.1053/j.gastro.2009.10.002
PRGC1 (protein)	PPARG (gene)	Jiménez-Flores L.M., López-Briones S., Macías-Cervantes M.H., Ramírez-Emiliano J., Pérez-Vázquez V. A PPAR $\gamma$ , NF- $\kappa$ B and AMPK-dependent mechanism may be involved in the beneficial effects of curcumin in the diabetic db/db mice liver. <i>Molecules</i> . 2014;19(6):8289-8302. doi 10.3390/molecules19068289
TNFA (protein)	PPARG (gene)	McPhie M.L., Wang A., Molin S., Herzinger T. Lichen planopilaris induced by infliximab: a case report. <i>SAGE Open Med Case Rep</i> . 2020;8:2050313X20901967. doi 10.1177/2050313X20901967
WN10B (protein)	PPARG (gene)	Ross S.E., Hemati N., Longo K.A., Bennett C.N., Lucas P.C., Erickson R.L., MacDougald O.A. Inhibition of adipogenesis by Wnt signaling. <i>Science</i> . 2000;289(5481):950-953. doi 10.1126/science.289.5481.950
ZN423 (protein)	PPARG (gene)	Zhang J., Cai B., Ma M., Luo W., Zhang Z., Zhang X., Nie Q. <i>ALDH1A1</i> inhibits chicken preadipocytes' proliferation and differentiation via the PPAR $\gamma$ pathway <i>in vitro</i> and <i>in vivo</i> . <i>Int J Mol Sci</i> . 2020;21(9):3150. doi 10.3390/ijms21093150
PPARG (protein)	ARNTL (gene)	Tal Y., Chapnik N., Froy O. Non-obesogenic doses of palmitate disrupt circadian metabolism in adipocytes. <i>Adipocyte</i> . 2019;8(1):392-400. doi 10.1080/21623945.2019.1698791
PPARG (protein)	DDIT3 (gene)	Sabatino L., Fucci A., Pancione M., Colantuoni V. PPARG epigenetic deregulation and its role in colorectal tumorigenesis. <i>PPAR Res</i> . 2012;2012(1):687492. doi 10.1155/2012/687492
PPARG (protein)	FABP4 (gene)	Hua T.N.M., Kim M.K., Vo V.T.A., Choi J.W., Choi J.H., Kim H.W., Cha S.K., Park K.S., Jeong Y. Inhibition of oncogenic Src induces FABP4-mediated lipolysis via PPAR $\gamma$ activation exerting cancer growth suppression. <i>EBioMedicine</i> . 2019;41:134-145. doi 10.1016/j.ebiom.2019.02.015
PPARG (protein)	LCN2 (gene)	Kundu P., Ling T.W., Korecka A., Li Y., D'Arienzo R., Bunte R.M., Berger T., Arulampalam V., Chambon P., Mak T.W., Wahli W., Pettersson S. Absence of intestinal PPAR $\gamma$ aggravates acute infectious colitis in mice through a lipocalin-2-dependent pathway. <i>PLoS Pathog</i> . 2014;10(1):e1003887. doi 10.1371/journal.ppat.1003887
PPARG (protein)	LIPE (gene)	Deng T., Shan S., Li P.P., Shen Z.F., Lu X.P., Cheng J., Ning Z.Q. Peroxisome proliferator-activated receptor- $\gamma$ transcriptionally up-regulates hormone-sensitive lipase via the involvement of specificity protein-1. <i>Endocrinology</i> . 2006;147(2):875-884. doi 10.1210/en.2005-0623
PPARG (protein)	PNPLA2 (gene)	Kershaw E.E., Schupp M., Guan H.P., Gardner N.P., Lazar M.A., Flier J.S. PPAR $\gamma$ regulates adipose triglyceride lipase in adipocytes <i>in vitro</i> and <i>in vivo</i> . <i>Am J Physiol Endocrinol Metab</i> . 2007;293(6):E1736-E1745. doi 10.1152/ajpendo.00122.2007
PPARG (protein)	SIRT6 (gene)	Zuo Y., Huang L., Enkhjargal B., Xu W., Umut O., Travis Z.D., Zhang G., Tang J., Liu F., Zhang J.H. Activation of retinoid X receptor by bexarotene attenuates neuroinflammation via PPAR $\gamma$ /SIRT6/FoxO3a pathway after subarachnoid hemorrhage in rats. <i>J Neuroinflammation</i> . 2019;16(1):47. doi 10.1186/s12974-019-1432-5

**Supplementary Material S3 (end)**

Object 1, regulator	Object 2, regulated	Reference
PPARG (protein)	SLC27A1 (gene)	Briot A., Decaunes P., Volat F., Belles C., Coupaye M., Ledoux S., Bouloumié A. Senescence alters PPAR $\gamma$ (Peroxisome Proliferator-Activated Receptor Gamma)-dependent fatty acid handling in human adipose tissue microvascular endothelial cells and favors inflammation. <i>Arterioscler Thromb Vasc Biol.</i> 2018;38(5):1134-1146. doi 10.1161/ATVBAHA.118.310797
PPARG (protein)	STAT3 (gene)	Ju K.D., Lim J.W., Kim H. Peroxisome proliferator-activated receptor- $\gamma$ inhibits the activation of STAT3 in Cerulein-stimulated pancreatic acinar cells. <i>J Cancer Prev.</i> 2017;22(3):189-194. doi 10.15430/JCP.2017.22.3.189
PPARG (protein)	UCP1 (gene)	Yang X., Xiao X., Wang H., Li Y., Wang L., Li G., Deng S. Renoprotective effect of Danhong injection on streptozotocin-induced diabetic rats through a peroxisome proliferator-activated receptor $\gamma$ mediated pathway. <i>Evid Based Complement Alternat Med.</i> 2018;2018:3450141. doi 10.1155/2018/3450141
PPARG (protein)	VEGFA (gene)	Rapp J., Kiss E., Meggyes M., Szabo-Meleg E., Feller D., Smuk G., Laszlo T., Sarosi V., Molnar T.F., Kvell K., Pongracz J.E. Increased Wnt5a in squamous cell lung carcinoma inhibits endothelial cell motility. <i>BMC Cancer.</i> 2016;16(1):915. doi 10.1186/s12885-016-2943-4

**Supplementary Material S4.** The difference between the observed number of genes from the *Thermoregulation\_467* set having PAI = 1 and the expected one, calculated based on data for all human protein-coding genes (the set *allCDS\_19,504*)

Set or subset of genes	Set of genes		
	<i>allCDS_19,504</i>	<i>Thermoregulation_467</i>	
	Observed number	Expected number	Observed number
All genes from the set	19,504	467	467
Genes having PAI = 1	6,350	$467 * 6,350 / 19,504 = 152.04$	176
Genes having PAI > 1	13,154	$467 * 13,154 / 19,504 = 314.96$	291
<i>p</i> -value (chi-square test)			$p < 0.05$

**Supplementary Material S5.** The difference between the observed number of genes from the *Thermoregulation\_467* set having PAI = 6 and the expected one, calculated based on data for all human protein-coding genes (the set *allCDS\_19,504*)

Set or subset of genes	Set of genes		
	<i>allCDS_19,504</i>	<i>Thermoregulation_467</i>	
	Observed number	Expected number	Observed number
All genes from the set	19,504	467	467
Genes having PAI = 6	3,203	$467 * 3,203 / 19,504 = 76.69$	100
Genes having PAI $\neq$ 6	16,301	$467 * 16,301 / 19,504 = 390.30$	367
<i>p</i> -value (chi-square test)			$p < 0.01$

**Supplementary Material S6.** Functional analysis for 176 genes having PAI = 1 using DAVID according to the GOTERM\_BP\_Direct dictionary. Five GO terms associated with the highest number of genes and having FDR < 0.05 are presented

GO term	Number of genes (percentage of the total number)	FDR
GO:0045944~positive regulation of transcription by RNA polymerase II	43 (24.6 %)	1.5E-11
GO:0120162~positive regulation of cold-induced thermogenesis	39 (22.3 %)	2.4E-49
GO:0000122~negative regulation of transcription by RNA polymerase II	32 (18.3 %)	8.0E-8
GO:0006357~regulation of transcription by RNA polymerase II	31 (17.7 %)	0.01
GO:0034605~cellular response to heat	22 (12.6 %)	1.1E-25

**Supplementary Material S7.** Functional analysis for 100 genes having PAI = 6 using DAVID according to the GOTERM\_BP\_Direct dictionary. Five GO terms associated with the highest number of genes and having FDR < 0.05 are presented

GO term	Number of genes (percentage of the total number)	FDR
GO:0120162~positive regulation of cold-induced thermogenesis	21 (20.8 %)	1.7-23
GO:0007204~positive regulation of cytosolic calcium ion concentration	14 (13.9 %)	4.7E-10
GO:0007186~G protein-coupled receptor signaling pathway	14 (13.9 %)	0.03
GO:0008284~positive regulation of cell population proliferation	12 (11.9 %)	0.004
GO:0120163~negative regulation of cold-induced thermogenesis	10 (9.9 %)	1.3E-9