Supplementary

Table S1. Experimental design: 20 patient samples run on 10 2D-PAGE gels; samples were labeled randomly with Cy3 and Cy5, and a pooled sample stained with Cy2 was used as an internal standard.

Gel	Cy3	Cy5	Cy2
1	1	11	Pooled sample
	(Euthyroid)	(Hypothyroid)	
2	12	2	Pooled sample
	(Hypothyroid)	(Euthyroid)	
3	3	13	Pooled sample
	(Euthyroid)	(Hypothyroid)	
4	14	4	Pooled sample
	(Hypothyroid)	(Euthyroid)	
5	5	15	Pooled sample
	(Hypothyroid)	(Euthyroid)	
6	16	6	Pooled sample
	(Euthyroid)	(Hypothyroid)	
7	7	17	Pooled sample
	(Hypothyroid)	(Euthyroid)	
8	18	8	Pooled sample
	(Euthyroid)	(Hypothyroid)	
9	9	19	Pooled sample
	(Hypothyroid)	(Euthyroid)	
10	20	10	Pooled sample
	(Hypothyroid)	(Euthyroid)	

Table S2. Canonical pathways and network Pathways obtained from IPA functional analysis.

INGENUITY PATHWAY ANALYSIS Analysis Name: Hypothyroid for IPA - 2016-10-27 12:08 PM Analysis Creation Date: 2016-10-27 Build version: 400896M Content version: 28820210 (Release Date: 2016-09-24) **Analysis Settings** Reference set: Ingenuity Knowledge Base (Genes Only) Relationship to include: Direct and Indirect Does not Include Endogenous Chemicals Optional Analyses: My Pathways My List ID Associated Network Functions Score 1 Neurological Disease, Immunological Disease, Metabolic Disease 2 Cell-To-Cell Signaling and Interaction, Cellular Compromise, Cellular Function and Maintenance Top Canonical Pathways Name Overlap p-value 5.3 % 9/169 Acute Phase Response Signaling 2.35E-17 Complement System 3.91E-09 10.8 % 4/37 LXR/RXR Activation 4.36E-09 4.1 % 5/121 FXR/RXR Activation 5.35E-09 4.0 % 5/126 Coagulation System 1.74E-04 5.7 % 2/35

Figure S1: Power calculation for determination of the minimum number of required biological variants for 2-DIGE analysis. The power curve was used to calculate the sample size required to find significant difference with a fold-change of \geq 1.5 between two paired groups at 80% power and *p*-value \leq 0.05.

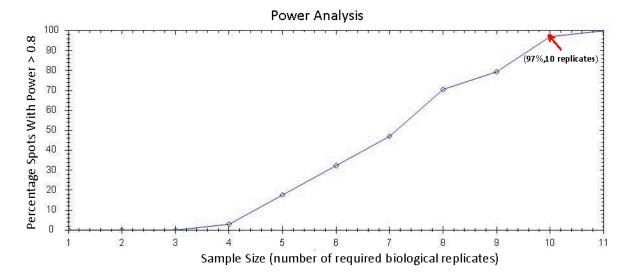


Figure S2: Representative focussing of the 13 protein spots between the euthyroid and hypothyroid samples on the 2D-DIGE gel images and their expression between the two groups.

