Supplementary

Table S1. Primers used for RT-qPCR

PROX1 (Prospero Homeobox 1)	5′ – CCAGCTCCAATATGCTGAAGACCTA – 3′ 5′ – CATCGTTGATGGCTTGACGTG – 3′
MMP-1 (Matrix Metallopeptidase 1)	5' –CTGTCCCTGAACAGCCCAGTACTTA– 3' 5' –CTGGCCACAACTGCCAAATG– 3'
FGF2 (Fibroblast Growth Factor 2)	5′ - GGCTTCTTCCTGCGCATCCA – 3′ 5′ – GCTCTTAGCAGACATTGGAAGA – 3′
MMP-3 (Matrix Metallopeptidase 3)	GAAATGAGGTACGAGCTGGATACC– 3′ 5′ –ATGGCTGCATCGATTTTCCT– 3′
NUDT6 (Nudix Hydrolase 6)	5′ –GGCGAGCTGGACAGATTC– 3′ 5′ –GCAGCAGGGGCAATAAATCG– 3′
BAIAP2 (BAI1 Associated Protein 2)	5′ –AAGTCCACAGGCAGATCCAG– 3′ 5′ –GCCTTTGCTCCTTTGCTCAG– 3′
VEGFC (Vascular Endothelial Growth Factor C)	5′ –GCCACGGCTTATGCAAGCAAAGAT– 3′ 5′ –AGTTGAGGTTGGCCTGTTCTCTGT– 3′
ANGPT1 (Angiopoietin 1)	5′ –GAAGGGAACCGAGCCTATTC– 3′ 5′ –AGCATCAAACCACCATCCTC– 3′
KDR (Kinase Insert Domain Receptor)	5′ –AGGAGAGCGTGTCTTTGTGG– 3′ 5′ –GCCTGTCTTCAGTTCCCCTC– 3′
VEGFA (Vascular Endothelial Growth Factor A)	5′ –CTTGCCTTGCTGCTCTACCT– 3′ 5′ –AAGATGTCCACCAGGGTCTC– 3′
PLAT (Plasminogen Activator, Tissue Type)	5′ –AGGAGAGCGTGTCTTTGTGG– 3′ 5′ –GCCTGTCTTCAGTTCCCCTC– 3′
MDK (Midkine)	5′ –CCTGCAACTGGAAGAAGGAG– 3′ 5′ -- CTTTCCCTTCCCTTTCTTGG– 3′
ADAMTS9 (ADAM Metallopeptidase With Thrombospondin Type 1 Motif 9)	5′ –ACGAAAAACCTGCCGTAATG– 3′ 5′ –TCAGAGTCTCCATGCACCAG– 3′
TIMP3 (TIMP Metallopeptidase Inhibitor 3)	5′ –CTGACAGGTCGCGTCTATGA– 3′ 5′ –AGTCACAAAGCAAGGCAGGT– 3′
ACTB (Beta Actin)	5′ – GCCGAGGACTTTGATTGC – 3′ 5′– CTGTGTGGACTTGGGAGAG – 3′

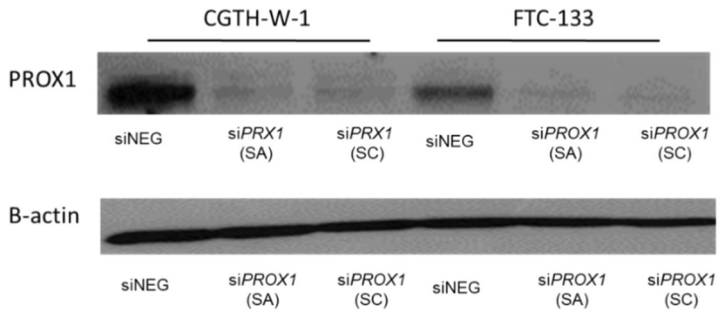

Figure S1. Efficient silencing of PROX1 in CGTH-W-1 and FTC-133 cells. Western blotting analysis shows a decrease in PROX1 protein level by targeting with siRNAs purchased from Santa Cruz (SC) and Sigma-Aldrich (SA) in both CGTH-W-1 and FTC-133 cell line. Beta-actin was used as a loading control of protein lysates.

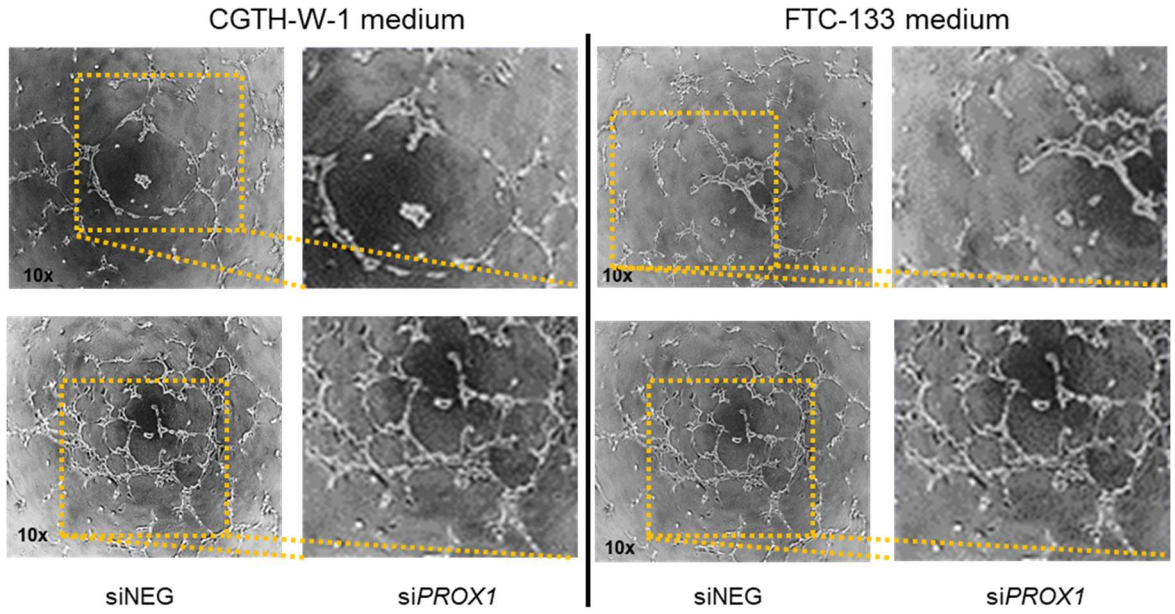

Figure S2. The tube formation assay. The silencing of PROX1 in CGTH-W-1 and FTC-133 cells enhances the angiogenesis *in vitro* of endothelial cells. HUVECs were cultured in 96-well plates coated with a semi-solid Matrigel. Cells were cultivated in conditioned medium collected from CGTH-W-1 and FTC-133 cells after silencing of PROX1 (with siRNA (SC)) and compared to HUVECs in medium collected from cells treated with siNEG. The vascularization effect was assessed under a light microscope after 5 h incubation; original magnifications: 10x.

Table S2. The list of angiogenic genes, which are significantly changed in the CGTH-W-1 cell line after the PROX1 knockdown using siPROX1from Sigma-Aldrich (SA) and Santa Cruz (SC). In the table, the upregulated genes are colored red, whereas the downregulated genes are colored green.

gene symbol	**gene name**	***p* value**	**fold change (SA)**	***p* value**	**fold change (SC)**
MMP1	matrix metallopeptidase 1 (interstitial collagenase)	0,0162	4,0250	0,0001	2,3912

FGF2	fibroblast growth factor 2 (basic)	0,0000	2,2940	0,8264	0,9647
MMP3	matrix metallopeptidase 3 (stromelysin 1, progelatinase)	0,0007	2,0390	0,0004	1,7780
IL24	interleukin 24	0,0065	1,9427	0,0031	1,7074
MCTS1	malignant T cell amplified sequence 1	0,0138	1,9280	0,0227	1,5566
LGALS1	lectin, galactoside-binding, soluble, 1	0,0000	1,7967	0,0105	1,4510
BRMS1	breast cancer metastasis suppressor 1	0,0006	1,7679	0,0174	1,4405
DKK1	dickkopf homolog 1 (Xenopus laevis)	0,0038	1,7620	0,0027	1,6146
KRT18	keratin 18	0,0047	1,6500	0,0050	1,5256
PTTG1	pituitary tumor-transforming 1	0,0002	1,6245	0,0077	1,5131
TK1	thymidine kinase 1, soluble	0,0009	1,6210	0,0119	1,3597
PCNA	proliferating cell nuclear antigen	0,0000	1,6041	0,0010	1,5668
NUDT6	nudix (nucleoside diphosphate linked moiety X)-type motif 6	0,0262	1,5930	0,7389	1,0610
EP300	E1A binding protein p300	0,0494	1,5706	0,0001	1,9584
MTOR	mechanistic target of rapamycin (serine/threonine kinase)	0,0012	1,5385	0,0043	1,4173
BAIAP2	BAI1-associated protein 2	0,0029	1,5350	0,0010	1,5799
HRAS	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	0,0378	1,5336	0,0563	1,3573
MCAM	melanoma cell adhesion molecule	0,0031	1,5174	0,0001	1,8458
NRG1	neuregulin 1	0,0174	1,5150	0,0001	2,4956
KISS1	KiSS-1 metastasis-suppressor	0,0246	1,5076	0,0076	1,6321
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog	0,0023	1,4850	0,0048	1,4279
CDKN3	cyclin-dependent kinase inhibitor 3	0,0128	1,4600	0,0262	1,3771
VEGFC	vascular endothelial growth factor C	0,0083	1,4300	0,0572	1,2478
ANGPT1	angiopoietin 1	0,0463	1,3855	0,8629	0,9194
BDKRB1	bradykinin receptor B1	0,0429	1,3589	0,0130	0,7567
PTK2	PTK2 protein tyrosine kinase 2	0,0126	1,3504	0,0057	1,4274
PLAUR	plasminogen activator, urokinase receptor	0,0397	1,3303	0,0195	0,7450
ID1	inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	0,0033	1,3029	0,0001	1,6678
ADAMTS9	ADAM metallopeptidase with thrombospondin type 1 motif, 9	0,2651	0,7955	0,4425	0,6736
CTNNA1	catenin (cadherin-associated protein), alpha 1, 102kDa	0,0480	0,7880	0,0346	0,7813

NRP2	neuropilin 2	0,0460	0,7516	0,0061	0,6989
HMGA1	high mobility group AT-hook 1	0,0373	0,7420	0,0052	0,6404
POFUT1	protein O-fucosyltransferase 1	0,0386	0,7291	0,1076	0,8194
PLXNA1	plexin A1	0,0217	0,7124	0,0013	0,6437
MTA1	metastasis associated 1	0,0152	0,7050	0,0871	0,8059
CXCL12	chemokine (C-X-C motif) ligand 12	0,0005	0,6937	0,0018	0,6906
MDK	midkine (neurite growth-promoting factor 2)	0,0032	0,6890	0,0635	0,7583
THBS1	thrombospondin 1	0,0051	0,6840	0,0559	0,5449
SFRP1	secreted frizzled-related protein 1	0,0012	0,6694	0,1859	0,7198
MVP	major vault protein	0,0052	0,6658	0,0010	0,6055
FN1	fibronectin 1	0,0002	0,6577	0,0365	1,3113
HTATIP2	HIV-1 Tat interactive protein 2, 30kDa	0,0557	0,6468	0,0589	0,6058
COL18A1	collagen, type XVIII, alpha 1	0,0127	0,6138	0,0104	0,6892
CLIC4	chloride intracellular channel 4	0,0000	0,6108	0,0000	0,5256
FOSL1	FOS-like antigen 1	0,0000	0,5829	0,0422	0,6727
PTEN	phosphatase and tensin homolog	0,0451	0,5769	0,0001	0,4454
POSTN	periostin, osteoblast specific factor	0,0299	0,5662	0,0259	0,6744
EGR1	early growth response 1	0,0075	0,5382	0,0008	0,5469
SDC2	syndecan 2	0,0003	0,5366	0,0000	0,5178
TIMP3	TIMP metallopeptidase inhibitor 3	0,0000	0,5332	0,0161	0,7486
AHR	aryl hydrocarbon receptor	0,0000	0,5176	0,0531	0,7561
IGFBP7	insulin-like growth factor binding protein 7	0,0029	0,4848	0,0028	0,5675
FTH1	ferritin, heavy polypeptide 1	0,0000	0,4347	0,0024	0,7104
LPAR1	lysophosphatidic acid receptor 1	0,0000	0,4239	0,0061	0,6825
PLAT	plasminogen activator, tissue	0,0000	0,3460	0,0001	0,3460

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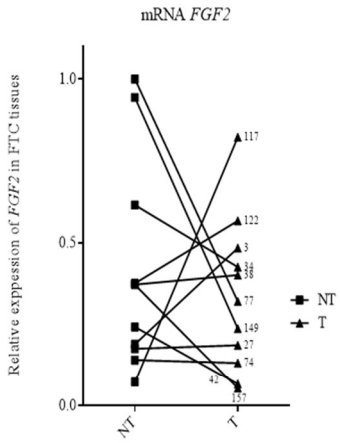
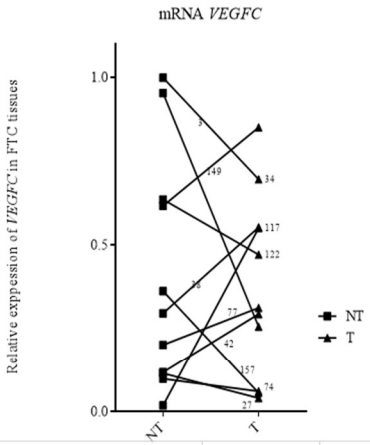


Relative expression of *FGF2* in FTC tissues	Non-tumoral	Tumoral
77	1,000	0,321
74	0,142	0,132
34	0,616	0,426
38	0,373	0,401
27	0,176	0,187
42	0,243	0,067
117	0,072	0,822
3	0,190	0,484
122	0,376	0,567
149	0,944	0,238
157	0,372	0,054



Relative expression of VEGFC in FTC tissues	Non-tumoral	Tumoral
77	0,202	0,313
74	0,099	0,061
34	1,000	0,697
38	0,296	0,554
27	0,115	0,041
42	0,119	0,294
117	0,019	0,551
3	0,954	0,257
122	0,637	0,472
149	0,617	0,852
157	0,363	0,057

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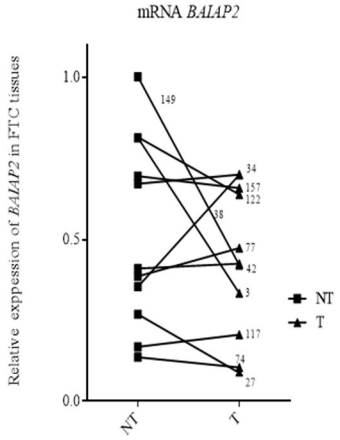

Relative expression of *BAIAP* in FTC tissues	Non-tumoral	Tumoral
77	0,387	0,474
74	0,138	0,107
34	0,672	0,700
38	0,355	0,699
27	0,271	0,091
42	0,411	0,426
117	0,170	0,208
3	0,813	0,335
122	0,815	0,639
149	1,001	0,422
157	0,695	0,658

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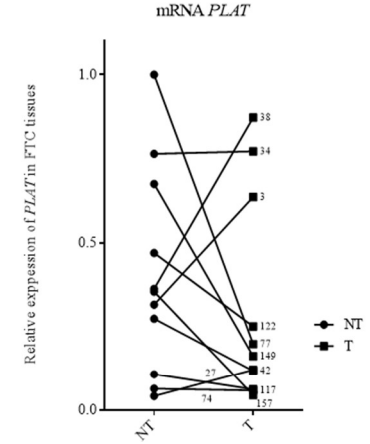

Relative expression of *PLAT* in FTC tissues	Non-tumoral	Tumoral
77	1,000	0,198
74	0,107	0,062
34	0,764	0,772
38	0,363	0,872
27	0,042	0,121
42	0,274	0,118
117	0,064	0,059
3	0,316	0,636
122	0,470	0,251
149	0,675	0,162
157	0,354	0,045

Figure S3. Angiogenic factors: (a) *FGF2*, (b) *VEGFC*, (c) *BAIAP2*, (d) *PLAT* are differentially expressed in FTCs and normal thyroid tissues. The relative expression level of mRNAs in FTC tissues and non-tumoral tissues were analyzed by RT-qPCR and normalized against the housekeeping gene: *ACTB*.

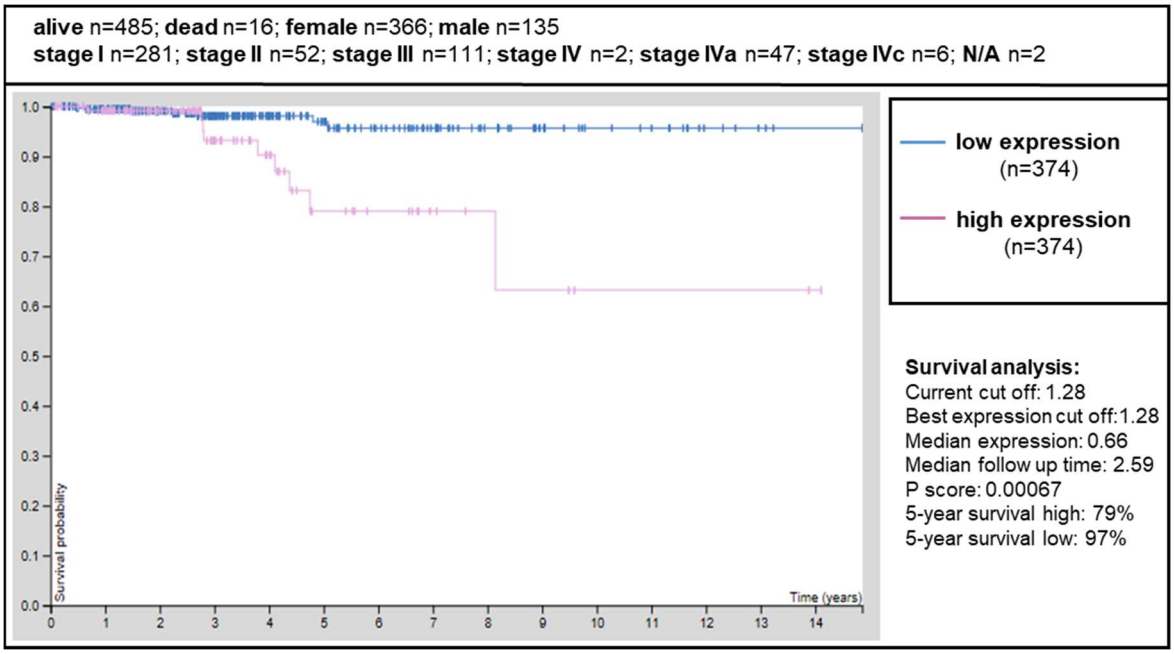

Figure S4. Correlation of PROX1 expression with the survival of patients with PTC. Data analysis obtained using The Human Protein Atlas https://www.proteinatlas.org/ENSG00000117707-PROX1/pathology/thyroid+cancer.

*P score: log-rank *p* value for Kaplan-Meier plot showing results from analysis of correlation between mRNA expression level and patient survival

*5-year survival for patients with higher / lower expression than the expression cutoff.