

Differential HIF2 α protein expression in human carotid body and adrenal medulla under physiologic and tumorigenic conditions

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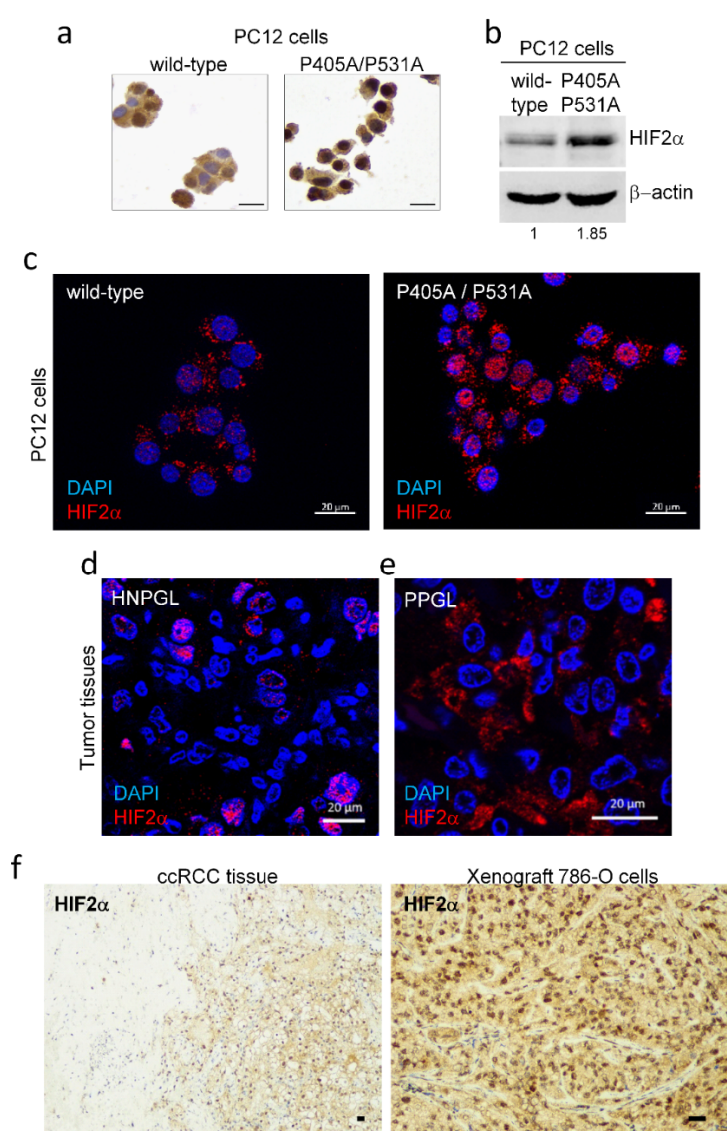


Figure S1. Analysis of the specificity of HIF2 α antibody. (a–c) PC12 cells were transiently transfected with plasmids expressing wild-type HIF2 α or normoxically stable P405A/P531A mutant HIF2 α . Expression of HIF2 α was then analyzed by immunocytochemistry (counterstained with hematoxylin) (a), western blot (b) or immunofluorescence (c). Numbers below western blot represent the relative expression of HIF2 α . As shown in panels a and c, higher expression levels of nuclear HIF2 α was detected in cells transfected with normoxically stable P405A/P531A mutant HIF2 α than with wild-type HIF2 α . (d–e). Representative images of HIF2 α immunofluorescence in HNPGL (d) and PPGL tumor tissues (e). (f) Representative images of HIF2 α immunohistochemistry in a clear cell renal cell carcinoma (ccRCC) tissue or a mouse xenograft produced by inoculation of the *VHL*-mutant ccRCC-derived 786-O cells. Note that HIF2 α is detected at the cell nuclei of HNPGL and ccRCC samples but not in PPGL which showed cytoplasmic perinuclear staining. Scale bars, 20 μ m (c, d, e), 50 μ m (a), 100 μ m (f).

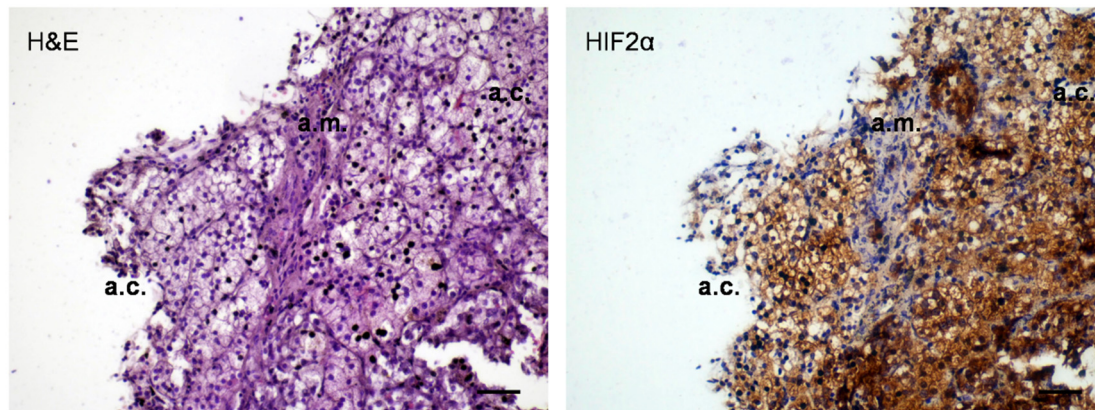
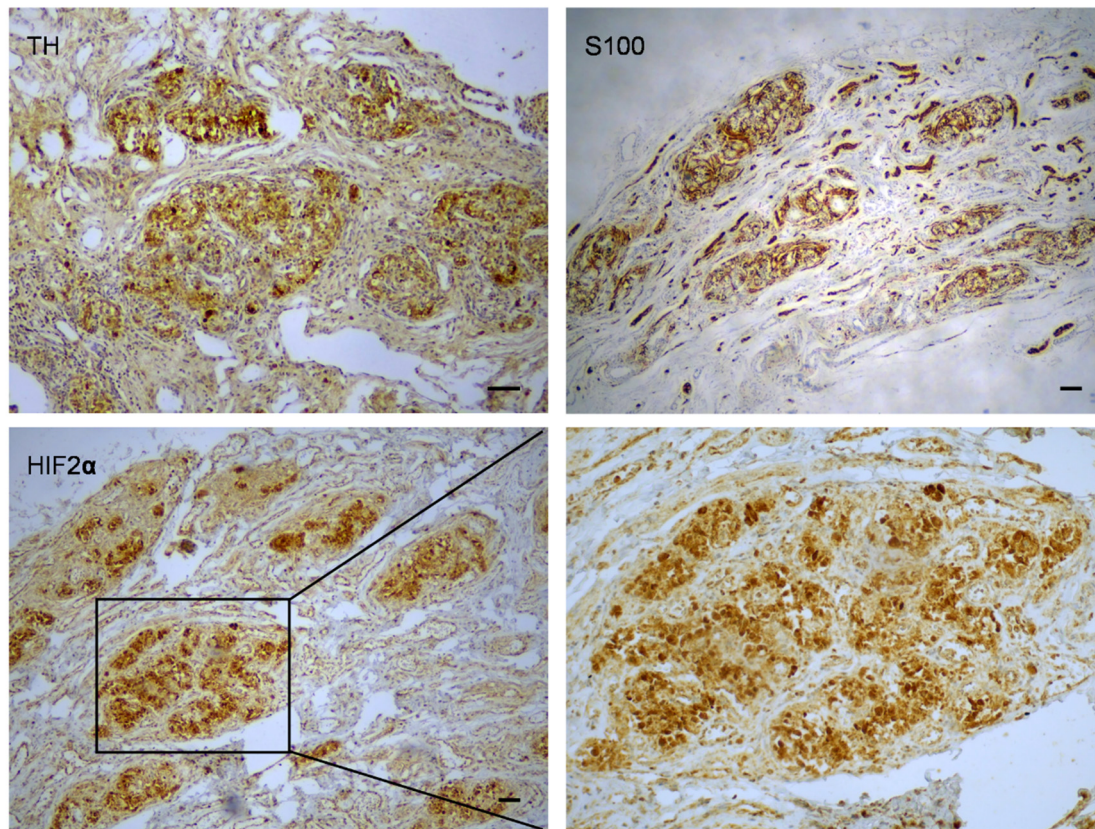
a**non-cancerous adrenal gland****b****non-cancerous carotid bodies**

Figure S2. HIF2 α immunostaining of human adrenal gland and carotid body. (a) Representative images of hematoxylin-eosin (H&E) staining and HIF2 α immunohistochemistry performed in non-cancerous human adrenal medulla. a.c., adrenal cortex; a.m., adrenal medulla. (b) Representative TH, S100 and HIF2 α immunostainings in a non-tumoral human carotid body. Scale bars, 50 μ m.

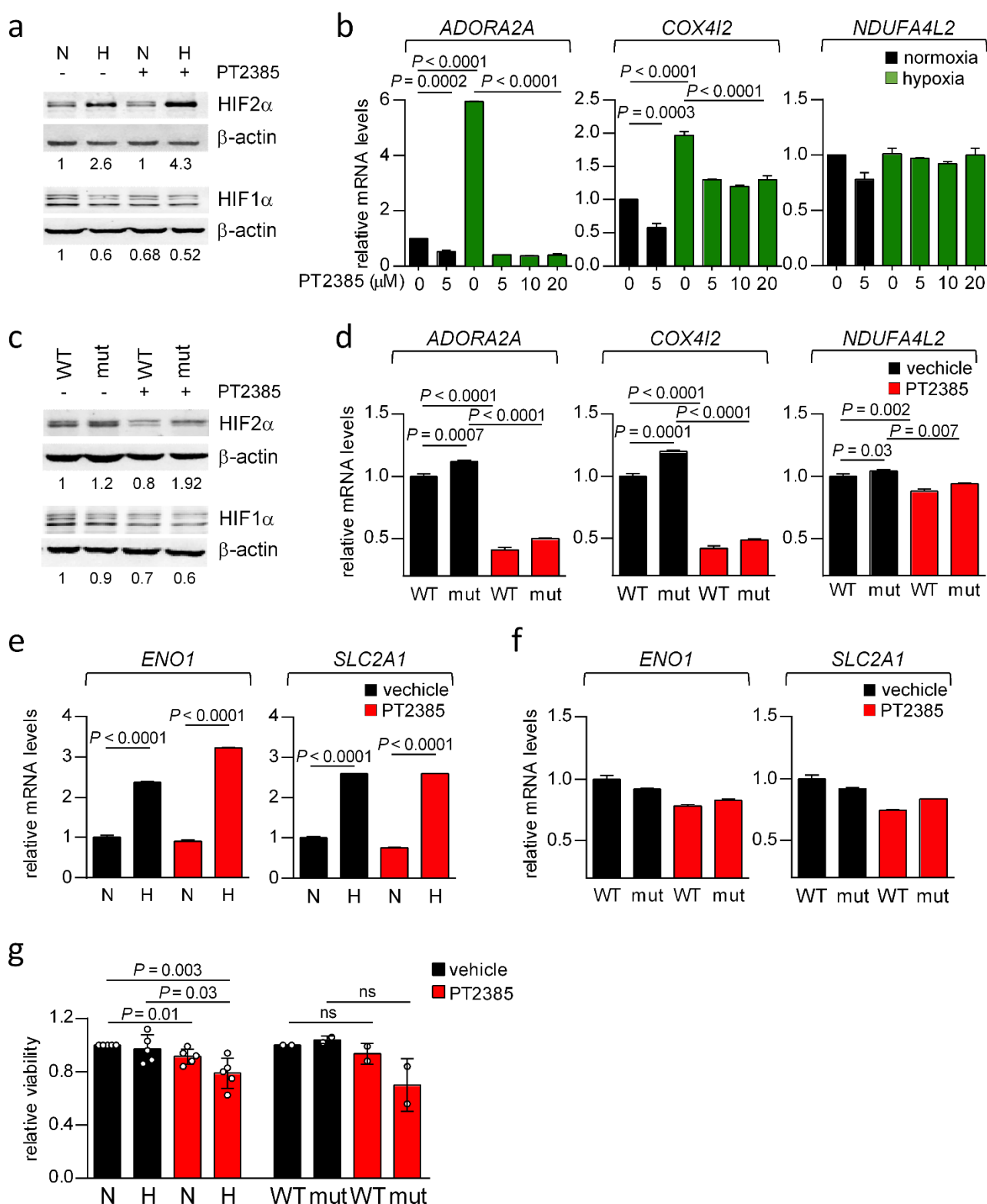


Figure S3. Induction of *ADORA2A*, *COX4I2*, and *NDUFA4L2* expression by hypoxia is HIF2α-dependent in PC12 cells. (a–f). Representative HIF2α and HIF1α immunoblots (a, c) and mRNA levels of the indicated genes (d, e, f) in PC12 cells exposed (H) or not (N) to hypoxia (1% O₂) for 36 h, or transiently transfected with plasmids encoding wild-type HIF2α (WT) or P405A/P531A mutant HIF2α (mut). PT2385 (or vehicle: 0.05% DMSO) was added to the cell culture at 5 μM final concentration. Time of exposure to PT2385 was 30 h. β-actin was used to assess equal loading. Numbers below immunoblots represent the relative expression levels of each protein. (b). Relative mRNA levels of the indicated genes in PC12 cells exposed or not to hypoxia (1% oxygen) for 36 h in the absence or presence of the indicated concentrations of PT2385. (g). Cell viability was determined by CellTiter 96® AQueous One Solution Cell Proliferation Assay in PC12 cells treated or not with 5 μM PT2385 for 30h. PT-2385 treatment was performed under normoxic (N) or hypoxic (H, 1% oxygen) conditions or in cells transfected with plasmids encoding wild-type (WT) HIF2α or P405A/P531A mutant (mut) HIF2α. ns: not significant. Uncropped Western Blots can be found at supplementary original images.

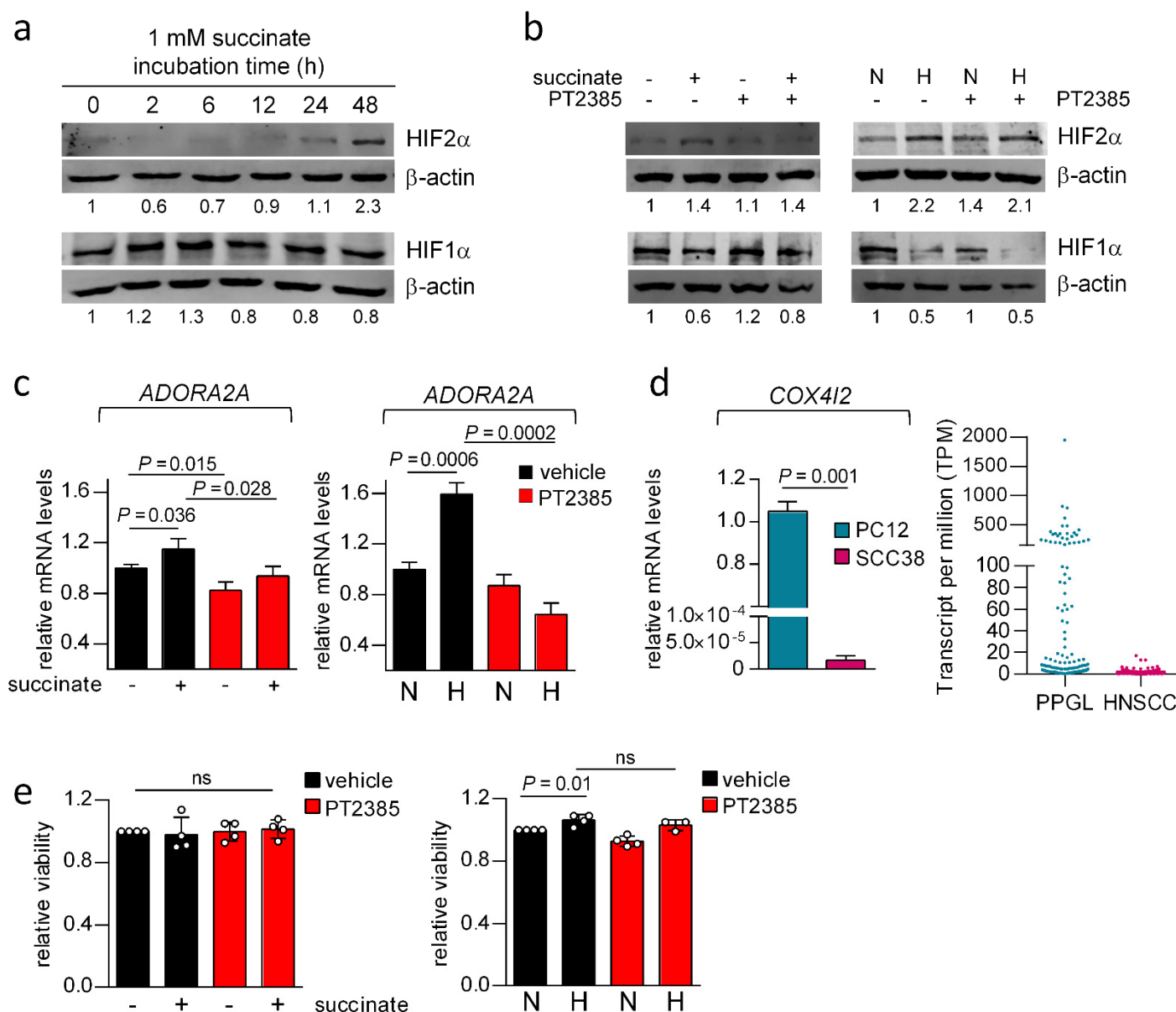


Figure S4. Induction of HIF2α expression by succinate in cells derived from squamous cell carcinoma. (a) Representative immunoblots of HIF2α and HIF1α in SCC38 cells treated with 1 mM succinate for the indicated time (h). (b, c, e) SCC38 cells were preincubated with 1 mM succinate (or vehicle) for 12 h or exposed or not (N) to 1% O₂ (H) for 6 h before the addition of 5 μM PT2385 (or vehicle: 0.05% DMSO) and subsequent incubation for 36 h. A representative immunoblot showing HIF2α and HIF1α protein levels in the different conditions is shown in panel b. β-actin was used to assess equal loading. Numbers represents the relative expression of HIF2α and HIF1α. (c) Relative mRNA levels of *ADORA2A* in SCC38 cells. (d) Relative mRNA levels of *COX4I2* in PC12 and SCC38 cells showing the almost negligible levels of this gene in SCC38 cells as compared with PC12 cells (left panel). *COX4I2* expression levels were also evaluated in tumors using the TCGA database of head and neck squamous cell carcinoma (HNSCC) and PPGL (right panel). As shown, *COX4I2* gene is highly expressed in PPGL but not detected in most HNSCC. Therefore, *COX4I2* gene expression could not be evaluated in SCC38 cells. (e). Cell viability was determined by MTS assay in SCC38 cells treated as indicated. . Uncropped Western Blots can be found at supplementary original images.

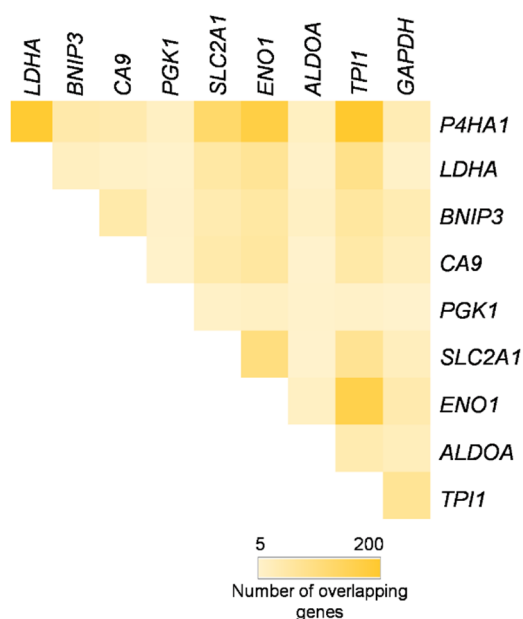


Figure S5. Matrix of all pairwise intersections. Minimal triangular matrix showing pairwise intersections of the 10 gene sets obtained through the analysis of positive correlations with *LDHA*, *BNIP3*, *CA9*, *PGK1*, *SLC2A1*, *ENO1*, *ALDOA*, *TPI1* and *GAPDH*.

Table S1. Clinical data of patients.

		Primary tumor type	
		PPGL	HNPGL
Tumor location			
	extradrenal	9	
	adrenal	14	
	carotid body		15
	vagal PGL*		3
	jugulo-tympanic PGL*		13
	parapharynx		1
	metastasis	6	1
Gender			
	female	9	24
	male	14	8
Age at diagnosis (years): mean (range)		41.7 (14-83)	46.7 (25-73)
Metastatic disease			
	no	9	31
	yes	14	1
Genotype			
	<i>SDHB</i>	8	4
	<i>SDHD</i>	0	11
	<i>SDHC</i> (epimutation)	0	1
	<i>VHL</i>	1	1
	<i>RET</i>	1	0
	<i>NF1</i>	1	0
	<i>SDH/VHL/EPAS1</i> WT	12	15

*paraganglia

Table S2. Correlation matrix for 10 HIF-related genes in PPGL.



Supp_Table
S2.xlsx

Table S3. List of genes included in the HIF-PPGL signature and GO/pathway enrichment.



Supp_Table
S3.xlsx

Table S4. Genes overlapping in HIF-PPGL and other hypoxia-related signatures.



Supp_Table
S4.xlsx

Table S5. List of genes, contained in the HIF-PPGL signature, that are commonly upregulated in *VHL* and *SDH/EPAS1*-PPGL. GO/pathway enrichment.



Suppl Table
S5.xlsx

Table S6. List of genes, contained in the HIF-PPGL signature, that are upregulated in *VHL*-PPGL. GO/pathway enrichment.



Suppl Table
S6.xlsx

Table S7. Association of HIF2 α immunostaining and genetic/clinical data.

	HIF2 α immunostaining					
	PPGL			HNPGL		
	positive	negative	<i>P</i>	positive	negative	<i>P</i>
<i>SDH/VHL</i> mutated						
yes	9	0	0.048	17	0	0.212
no	8	6		13	2	
Tumor location						
extradrenal	7	2	1			
adrenal	10	4				
carotid body				15	0	0.374
vagal bulb				3	0	
jugulo-tympanic bulb				11	2	
parapharynx				1	0	
Age at diagnosis						
<45 years	7	4	0.181	17	0	0.212
>45 years	8	1		13	2	
Gender						
female	5	4	0.132	22	2	0.556
male	12	2		8	0	
Metastatic disease						
no	6	1	0.382	29	2	0.938
yes	11	5		1	0	

Table S8. Association of HIF1 α and HIF2 α expression.

HIF2 α		HIF1 α		<i>P</i>
		positive	negative	
PPGL (n = 21)	positive	2	14	0.228
	negative	2	3	
HNPGL (n = 30)	positive	17	11	0.179
	negative	0	2	

Table S9. List of genes positively correlated with *EPAS1* in PPGL and GO/pathway enrichment.



Suppl Table
S9.xlsx