Supplementary Methods

Patients and samples Diagnosis of pheochromocytoma and/or paraganglioma was histologically confirmed in every case. Heredity status was defined by a mutation in one of the susceptibility genes (PSGs), *RET, VHL, SDHB, SDHC, SDHD, TMEM127, MAX* and *KIF1Bϐ* genes, as previously described, or by clinical features in case of neurofibromatosis type 1(Qin, et al. 2010). A total of 239 pheochromocytomas or paragangliomas were included in this study. Seventy-two samples were defined as hereditary, based on the presence of a germline mutation in *RET* (n=18), *VHL* (n=12), *NF1* (n=9), *SDHB* (n=9), *SDHC* (n=1), *SDHD* (n=11), *TMEM127* (n=9), *MAX* (n=1), or *KIF1Bϐ* (n=2). The remaining 167 samples were considered sporadic cases due to negative PSG screening. In addition, a set of 107 normal samples of matched ethnic background to the patients were used as controls. Data available from the Human1000 genome project and SNPdb were used as additional references for detected variants.

Reagents-Antibodies targeting Glut1, from Novus Biologicals, cleaved caspase 3, from Cell Signaling Technology; HA- from Covance, beta-actin antibody, cyclohexamide, polybrene, horse serum, collagen type IV, oligonucleotides, from Sigma-Aldrich; HRP-labeled anti-mouse and anti-rabbit secondary antibodies from BioRad; Lipofectamine 2000, DMEM, fetal calf serum, horse serum penicillin, streptomycin, trypsin, from Life Technologies, HotStart Taq, dideoxynucleotides and buffers from 5Prime.

Sanger sequencing- Primers flanking exons and exon-intron boundaries of each of *HIF2A* 16 exons and HIF1A exons 9, 12 and 16 (sites of three regulatory hydroxylation domains) were designed based on NCBI Build 37.3 and used for PCRs and Sanger sequencing at the UTHSCSA Nucleic Acid Sequencing Core and Beckman Genomics. Sequence traces were analyzed with the Mutation Surveyor Software (Softgenetics). Primer sequences and PCR conditions are available upon request.

Quantitative Real-time PCR (qRT-PCR)-Target gene expression was measured by real time-quantitative PCR (qRT-PCR) with SYBR green fluorescence (Applied Biosystems). Samples were prepared in triplicate using the StepOne Plus Cycler from Applied Biosystems and the TATA-box binding protein (TBP) or ubiquitin C (UBC) genes were used as reference (RNA data) and the albumin gene (DNA copy number) for calculation as previously reported(Qin et al. 2010). Primer sequences for human and rat oligonucleotides are available upon request.

Microarray-based expression profiling-We used normalized transcription data from a collection of 126 pheochromocytomas that were generated using Affymetrix U133 microarrays, previously published and available under GEO accession number GSE28416 and GSE199877(Dahia, et al. 2005; Qin et al. 2010). Two *HIF2A* mutant tumors (37 and 208) were available in this cohort and were used for comparisons with profiles from representative tumors from each of the well-established hereditary pheochromocytoma clusters: Cluster 1 containing *VHL-, SDHB-, SDHD*- mutants and Cluster 2, with *TMEM127, RET-, NF1- or KIF1Bβ*-mutated tumors. Comparisons were performed using normalized data of probes representing HIF-associated expression profiling, as described in the text. The strength of the differential expression was measured by t-test. Significance was set at $P < 0.05$.

Clones and constructs- A HA-HIF2alpha-pBabe-puro construct containing the full-length HIF2A coding sequence, generated by Dr. Willam Kaelin's laboratory(Kondo, et al. 2003), was obtained from Addgene. Site-direct mutagenesis with Phusion polymerase (Thermo Scientific) was used to generate each of the HIF2A 531, 532, and 71 mutants identified in pheochromocytomas. Mutagenized oligonucleotides were obtained from Sigma Genosys. A HA-tagged full-length VHL construct was cloned into the MSCV-GFP retroviral vector.

Cell Culture and Transfections HEK293 and 786-0 were cultured in DMEM and 10% fetal bovine serum supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin. Undifferentiated PC12 cells were cultured on collagen-coated dishes in RPMI, 10% horse serum, 5% fetal calf serum and antibiotics. For hypoxia experiments cells were maintained in a hypoxia chamber set to 1% O2 (InVivo200, Ruskinn) for 16 hour prior to harvesting. Retroviral transduction was carried out as previously described(Qin et al. 2010). Viral supernatants collected at 48 and 72h and used to transduce 786-0 parental cells, HEK293 and rat PC12 cell lines in the presence of polybrene 8μg/ml. Puromycin-resistant pools or clones were used for the experiments described in the text. MSCV-HA-VHL constructs were sorted by flow cytometry based on GFP expression(Qin et al. 2010).

NGF differentiation and withdrawal-PC12 cells were exposed to RPMI with 1% horse serum and NGF 50ng/ml (Harlan) for 5-7days(Lee, et al. 2005). For NGF withdrawal, cells were washed with serum-free medium followed by incubation in NGF-free medium containing 1% horse serum for 24h. Live PC12 cells were imaged with the Micron System Software (Western Scientific), and harvested for RNA or whole cell lysates. Apoptosis was measured by cleaved caspase 3 immunoblots and gene expression was analyzed by qRT-PCR of the genes indicated in the text.

Soft agar colony forming assay-Colony assay was performed with HEK293 stably expressing wild-type or HIF2A mutants, as previously described(Schlisio, et al. 2008).

Nude mouse xenografts-Nude mouse xenograft assays were performed as described(Rai, et al. 2010). Cells stably expressing empty vector, wild-type HIF2α, 531 and 532 mutants were trypsinized, resuspended in PBS and injected ($5x10^6$) subcutaneously into the flank of 5-week-old nude mice (Harlan) previously irradiated with 300 cGy. Six or 7 mice were included per cell type. Caliper measurements were obtained every 3 days and animals were sacrificed after 28 days. Tumors were dissected, and weights recorded. Two additional cohorts with 12 mice per group were performed. For these groups,

animals that developed tumors were sacrificed when tumor reached maximum size ($1cm^3$) following UTHSCSA-IACUC approved protocols.

References

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