Inventory of all Supplemental Items:

Figure S1

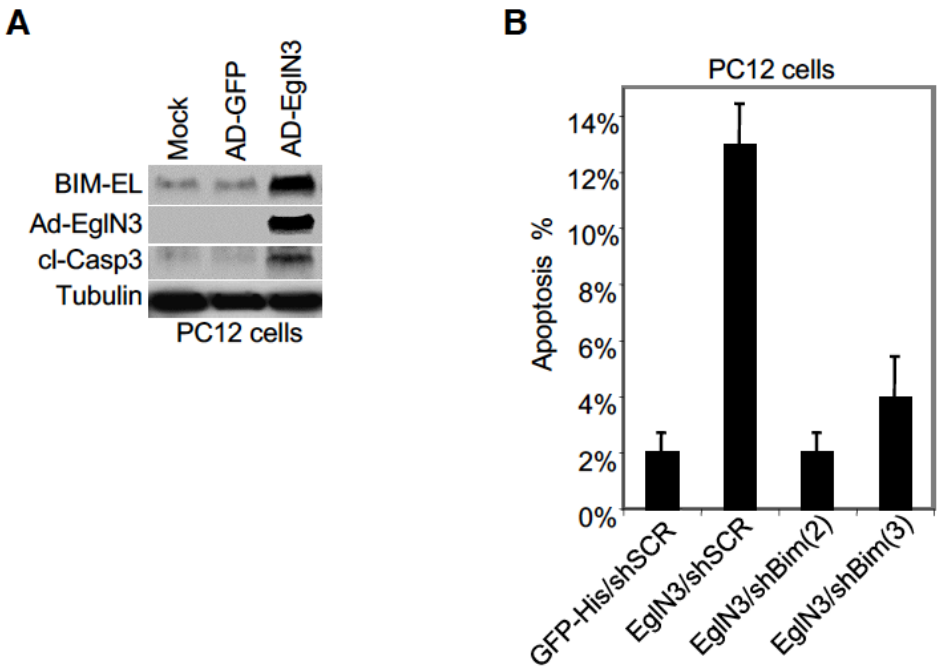
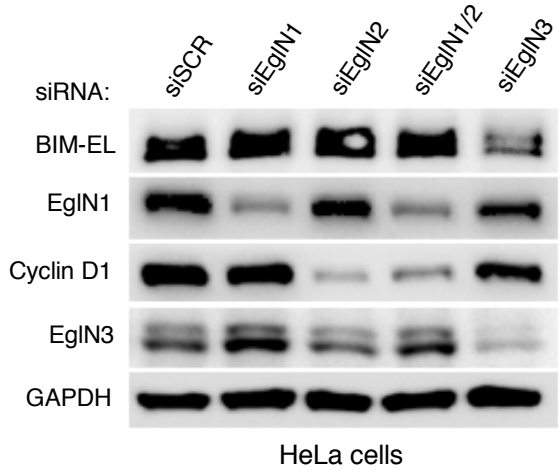

A
Mock
AD-GFP
AD-EgIN3
BIM-EL
Ad-EgIN3
cl-Casp3
Tubulin
PC12 cells
B
PC12 cells
Apoptosis %
14%
12%
10%
8%
6%
4%
2%
0%
GFP-His/shSCR
EgIN3/shSCR
EgIN3/shBim(2)
EgIN3/shBim(3)

Figure S2

A



B

Case number	Tumor type	KIF1Bb mut/wt	SDHA/B mut/wt	VHL mut	1p loss/NO 1p loss	Other mutation?
3	PCC	wt	wt	wt	NO 1p loss	
6	PCC	wt	wt	wt	NO 1p loss	
7	PCC	MUT (const)	wt	wt	1p loss	
14	PCC	wt	wt	wt	1p loss	
16	PCC	wt	wt	wt	NO 1p loss	
21	PCC	wt	wt	MUT	Not known	
25	PCC	wt	wt	MUT	1p loss	
36	PCC	wt	wt	wt	1p loss	
96	PCC	wt	SDHA MUT (const)	MUT (const)	Not known	EGLN1
108	PGL	wt	wt	MUT	NO 1p loss	

Figure S3

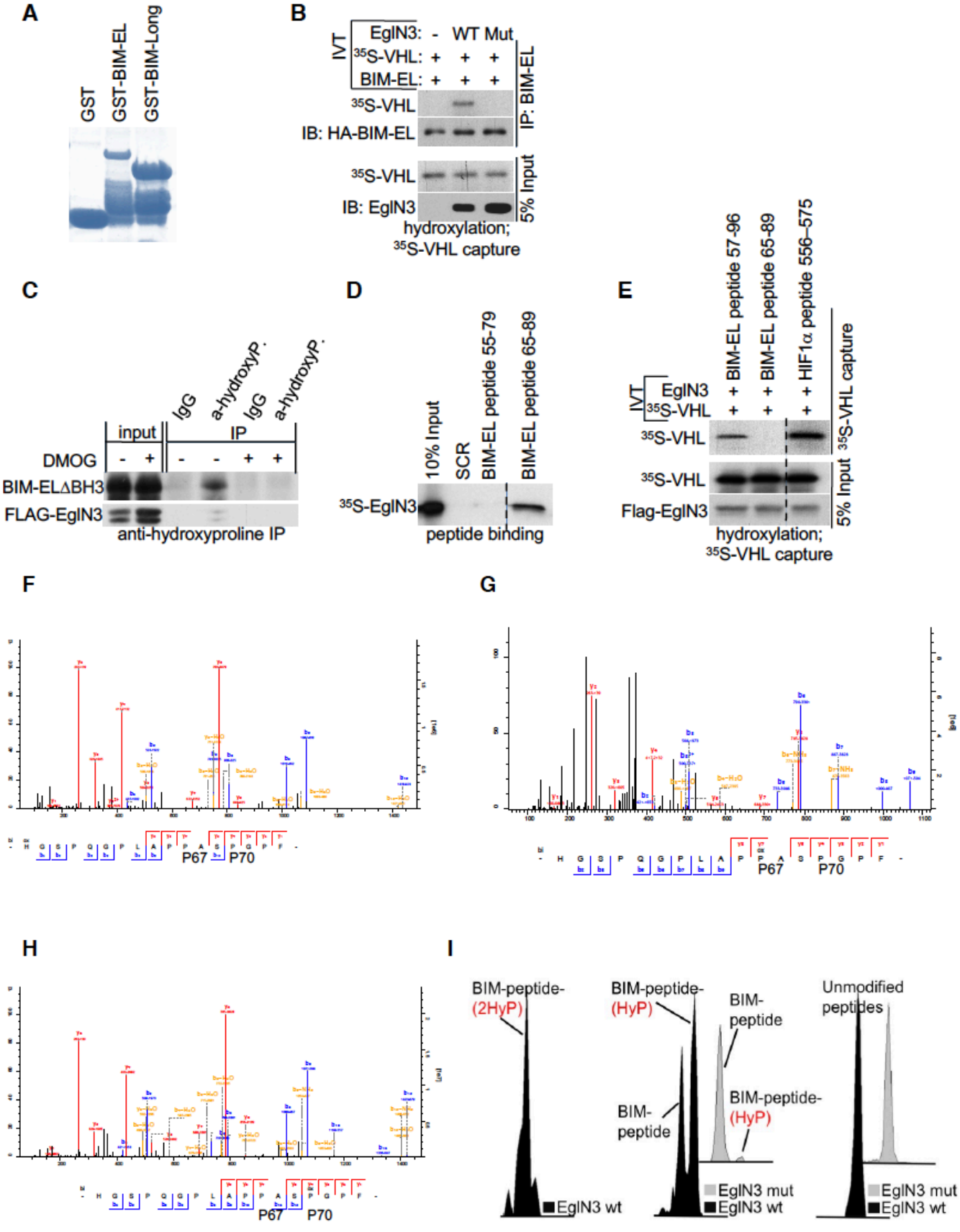
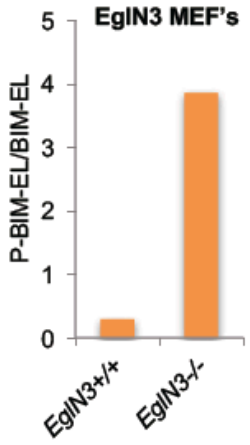

A
GST
GST-BIM-EL
GST-BIM-Long
B
IVT
EglN3: - WT Mut
35S-VHL: + + +
BIM-EL: + + +
35S-VHL
IB: HA-BIM-EL
IP: BIM-EL
35S-VHL
IB: EglN3
5% Input
hydroxylation;
35S-VHL capture
C
IgG
a-hydroxyP.
IgG
a-hydroxyP.
input
IP
DMOG - + - - + +
BIM-ELΔBH3
FLAG-EglN3
anti-hydroxyproline IP
D
10% Input
SCR
BIM-EL peptide 55-79
BIM-EL peptide 65-89
35S-EglN3
peptide binding
E
+ BIM-EL peptide 57-96
+ BIM-EL peptide 65-89
+ HIF1α peptide 556–575
IVT
EglN3 + + +
35S-VHL + + +
35S-VHL
35S-VHL
Flag-EglN3
5% Input 35S-VHL capture
hydroxylation;
35S-VHL capture
F
P67
P70
G
P67
P70
H
P67
P70
I
BIM-peptide-(2HyP)
EglN3 wt
BIM-peptide-(HyP)
BIM-peptide
BIM-peptide
BIM-peptide-(HyP)
EglN3 mut
EglN3 wt
Unmodified peptides
EglN3 mut
EglN3 wt

Figure S4

A


EglN3 MEF's
P-BIM-EL/BIM-EL
5
4
3
2
1
0
EglN3+/+
EglN3-/-

B

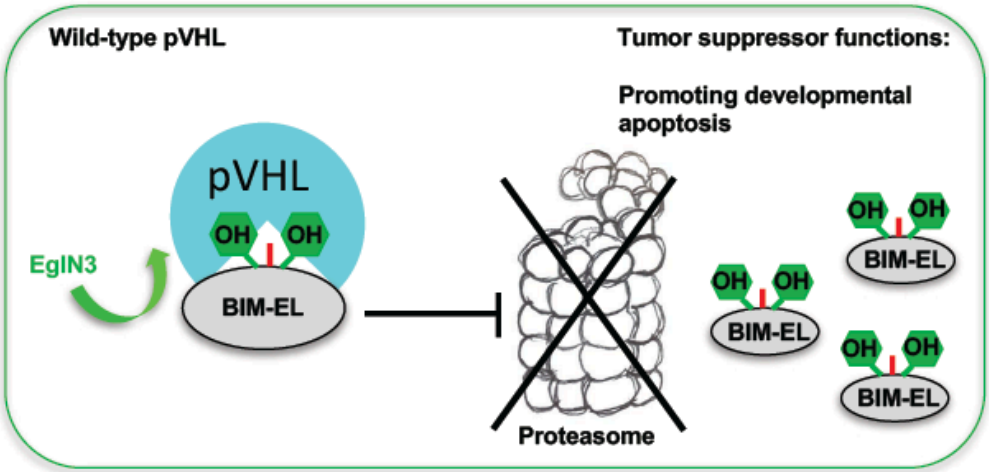
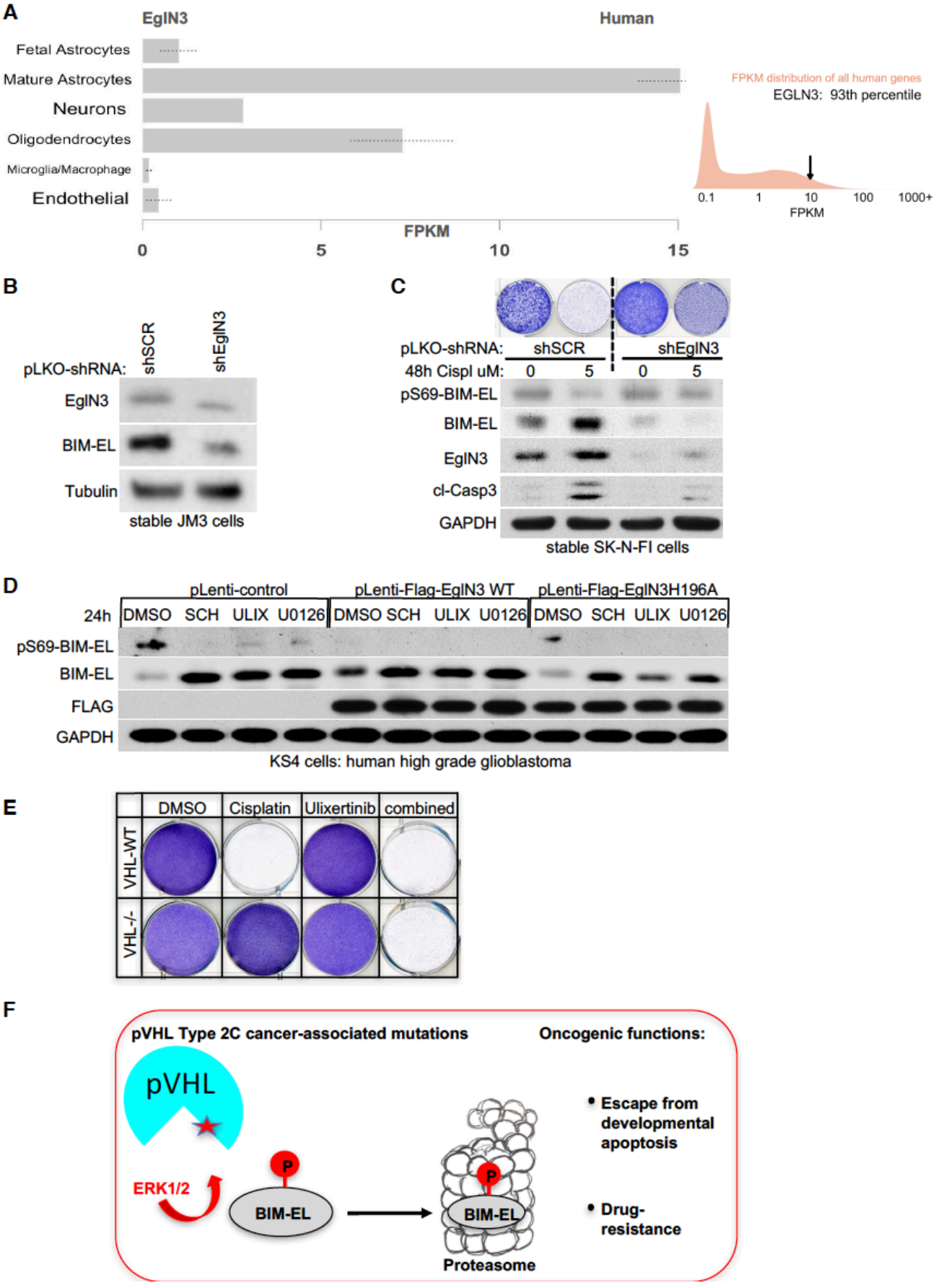

Wild-type pVHL
Tumor suppressor functions:
Promoting developmental apoptosis
pVHL
OH
OH
EglN3
BIM-EL
Proteasome
OH
OH
BIM-EL
OH
OH
BIM-EL
OH
OH
BIM-EL

Figure S5


A
EgIN3
Human
Fetal Astrocytes
Mature Astrocytes
Neurons
Oligodendrocytes
Microglia/Macrophage
Endothelial
FPKM
0
5
10
15
FPKM distribution of all human genes
EGLN3: 93th percentile
0.1
1
10
100
1000+
FPKM
B
pLKO-shRNA:
shSCR
shEgIN3
EgIN3
BIM-EL
Tubulin
stable JM3 cells
C
pLKO-shRNA:
shSCR
shEgIN3
48h Cispl uM:
0
5
0
5
pS69-BIM-EL
BIM-EL
EgIN3
cl-Casp3
GAPDH
stable SK-N-FI cells
D
pLenti-control
pLenti-Flag-EgIN3 WT
pLenti-Flag-EgIN3H196A
24h
DMSO SCH ULIX U0126
DMSO SCH ULIX U0126
DMSO SCH ULIX U0126
pS69-BIM-EL
BIM-EL
FLAG
GAPDH
KS4 cells: human high grade glioblastoma
E
DMSO
Cisplatin
Ulixertinib
combined
VHL-WT
VHL-/-
F
pVHL Type 2C cancer-associated mutations
Oncogenic functions:
pVHL
ERK1/2
P
BIM-EL
P
BIM-EL
Proteasome
• Escape from developmental apoptosis
• Drug-resistance

Supplementary Figure legends

Figure S1, related to Figure 1.

EgIN3 requires *BIM* to mediate apoptosis.

(A) Immunoblot analysis of PC12 cells transduced with adenovirus encoding EgIN3 or GFP-control. **(B)** Percentage of GFP positive PC12 cells exhibiting apoptotic changes 3 days after co-transfection to generate GFP-histone and EgIN3 along with pLKO plasmid targeting BIM (shBim) or scramble control (SCR). Data are shown as mean ±SD from two individual experiments.

Figure S2, related to Figure 2.

BIM-EL protein regulation is depending upon pVHL.

(A) Immunoblot analysis of HeLa cells transfected with siRNA targeting EgIN1, EgIN2, EgIN1/EgIN2 as pair, EgIN3 or non-targeting control (SCR) as indicated. **(B)** Table of human primary pheochromocytoma (PCC) and paraganglioma (PGL) tumors with characterized mutation status and 1p36 status were analyzed by immunoblot in Figure 2E. wt = wild-type; MUT = mutated; const. = constitutive. VHL mutations: (21) PCC VHL c.217C>T p.Gln73X; (25) PCC VHL c.193T>G p.Ser65Ala; (96) PCC VHL c.386T>C p.Leu129Pro; (108) PGL VHL c.593T>G p.Leu198Arg.

Figure S3, related to Figure 3.

BIM-EL is hydroxylated by EgIN3 at Proline 67/70 leading to VHL recognition.

(A) Protein expression of bacterially purified GST-BIM-EL and GST-BIM-Long and GST only used in Figure 2A. **(B)** Autoradiograms showing recovery of ^{35}S-labeled VHL protein bound to immunoprecipitated (IP) BIM-EL that was first subjected to hydroxylation by EgIN3 wild-

type (WT), mutant (H196A) or unprogrammed IVT (-) as indicated. ^{35}S labeling was carried out by IVT with retic lysate. EglN3 and BIM-EL were produced by cold IVT and expression and IP was verified by immunoblot (IB); – indicates unprogrammed IVT. (**C**) Immunoprecipitation using anti-hydroxyproline antibody (a-hydroxyP.) from 293T cells that were transiently transfected with plasmids encoding HA-BIM-EL apoptotic mutant (ΔBH3) and Flag-EglN3 in the presence or absence of DMOG as indicated. Immunoblots showing co-immunoprecipitation of HA-BIM-EL-ΔBH3. **(D)** Autoradiograms showing recovery of ^{35}S-labeled EglN3 protein bound to biotinylated BIM-EL-peptides, or scramble peptide (SCR) as indicated. **(E)** Autoradiograms showing recovery of ^{35}S-labeled VHL protein bound to biotinylated BIM-EL or HIF1α peptides. Prior to pull-down, peptides were subjected to hydroxylation by EglN3 produced by IVT. Expression of IVT-produced EglN3 proteins in each reaction was verified by anti-Flag immunoblot. **(F-I)** Mass spectrometry of biotinylated BIM-EL-peptide-57-96 subjected to hydroxylation assay with either EglN3 catalytic dead mutant (F) or EglN3 wild-type (G,H). Shown is the representative fragmentation peptide spectra of non-hydroxylated Biotin-HGSPQGPLAPPASPGPF (F), Biotin-HGSPQGPLAPP(ox)ASPGPF (G) and Biotin-HGSPQGPLAPPASP(ox)GPF (H). **(I)** Extracted ion chromatogram of biotinylated unmodified, oxidated, mono hydroxylated (P67 or P70) and double hydroxylated (P67 and P70) BIM peptide following an *in vitro* hydroxylation reaction with EglN3 wild-type (wt) or catalytic dead mutant (mt).

Figure S4, related to Figure 5.

VHL regulates BIM-EL protein stability depending on EglN3 enzymatic activity.

(A) Densitometric quantification of the ratio of phospho-BIM-EL to total BIM-EL of the band intensities in Figures 5C. **(B)** A schematic illustration of the regulation of BIM-EL protein

stabilization by EglN3 and VHL. Under normoxic condition, BIM-EL is hydroxylated by EglN3 on proline 67 and 70 and recognized by VHL. VHL binding in near proximity to serine 69 mask and prevents serine 69 phosphorylation, which is required for BIM-EL proteasomal degradation. Subsequently, non-phosphorylated serine residue 69 causes BIM-EL to escape proteasomal degradation, accumulate and regulate developmental apoptosis.

Figure S5, related to Figure 6.

Loss of EglN3 or VHL contributes to Cisplatin drug resistance.

(A) Screenshot of EglN3 expression from an RNA-Sequencing Transcriptome Database of glia, neurons and vascular cells of the human cerebral cortex http://web.stanford.edu/group/barres_lab/cgi-bin/geneSearch.py?geneNameIn=EglN3. **(B)** Immunoblot of human glioblastoma cells JM3 (EglN3 positive) that were stably transduced with lentivirus encoding shRNA targeting *EglN3* (shEglN3) or scramble control (shSCR). **(C)** Crystal violet staining and immunoblot analysis of SK-N-FI neuroblastoma cells stably transduced with lentivirus encoding shRNA targeting EglN3 (shEglN3) or scramble control (shSCR). Stable clones were treated once with Cisplatin (5uM). Immunoblot was performed 48h post-cisplatin treatment. For crystal violet staining, cells were maintained in culture for an additional week. **(D)** Immunoblot of KS4 cells (EglN3 negative) that were stably transduced with lentivirus encoding Flag-EglN3 wild-type (WT), catalytic dead mutant (H196A) or empty control. Cells were selected with blasticidin for 2 weeks and stable clones were respectively treated with different ERK kinase inhibitors SCH772984 (5uM), Ulixertinib (0.1uM), U0126 (10uM) for 24 hours as indicated. **(E)** Crystal violet staining of stable 786-O cells with indicated VHL status treated once with cisplatin (5uM), ERK-inhibitor Ulixertinib (0.1μM), or combination of both as indicated for 4 days. **(F)** A schematic illustration of the

regulation of BIM-EL degradation upon inhibition of oxygen sensing pathway. Under anoxic condition or EglN3 loss, BIM-EL escapes hydroxylation and VHL recognition. Additionally, under normoxia, VHL disease mutations equally prevents BIM-EL recognition. Subsequently, serine residue 69 is exposed to kinase phosphorylation. Phosphorylation of BIM-EL on Ser69 promotes proteasomal degradation. As a consequence, low BIM-EL protein abundance contributes to apoptosis resistance and drug resistance.

Supplemental Methods

Immunoblot analysis

Whole cell lysis and lysis of mouse and human tissue were performed in EBC buffer (50mM Tris at pH8.0, 120mM NaCl, 0.5% NP-40) containing phosphatase inhibitors (Catalog: 04906837001, Sigma) and protease inhibitors (Catalog: 11697498001 Roche Life Science). Samples containing equal protein amounts, as measured by Bradford assay, were immunoblotted as described previously (33). Rabbit polyclonal anti-Flag (F7425) and mouse monoclonal anti-Flag (F3165 and F1804) antibodies were purchased from Sigma. Mouse monoclonal PHD3 EglN3/PHD3 antibody is a kind gift from Peter Ratcliffe laboratory, Oxford, UK. Rabbit monoclonal BIM (C34C5) (#2933), Rabbit phospho-Bim (Ser69) (#4581), cleaved Casp3 (D175) (#9664) antibodies were purchased from Cell Signaling Technology. Rabbit polyclonal HIF1 alpha (NB100-479), rabbit polyclonal HIF-2 alpha/EPAS1 Antibody (NB100-122): were purchased from Novus Biologicals. Mouse monoclonal anti-HA antibody (#11583815001), Monoclonal Anti-α-Tubulin, Clone B-5-1-2 (T5168) were purchase from Sigma. High Sensitivity Streptavidin-HRP (#21130) that recognizes biotin was purchased from Thermo Fisher Scientific.

Apoptosis Assays

In Figure S1B, 72 hours after transient co-transfection of plasmids encoding *EglN3* and sh*BIM-EL*, apoptosis was assessed using GFP-histone to quantify apoptotic nuclei as described previously (10). In Figure 2H, polyclonal stable PC12 cells were used, that were generated by transfection with pcDNA3 plasmids encoding human HA-VHL wild type, HA-VHL-L188V, or empty control and selected with G418 (0,5mg/ml) for several weeks. EglN3

induced apoptosis in the stable clones were assessed by transiently transfecting Flag-EglN3 together with pLKOshRNA plasmids targeting rat *VHL* or non-targeting scramble control (shSCR). EglN3 induced apoptosis was assessed using GFP-histone to quantify apoptotic nuclei as described previously (10). One way ANOVA was used to determine the statistical significance using Prism software.

Viruses

Adenovirus encoding *EglN3* or *EglN3* catalytic dead mutant was a kind gift by Bob Freeman, Rochester University, USA. Adenovirus purification and titering were performed by Vector Biolabs. Adenovirus encoding GFP was purchased from Vector Biolabs. Lentiviruses encoding Flag-EglN3 and catalytic dead mutant (H196A) were generated via TOPO cloning using pLenti6.3 backbone from Invitrogen (Life Technologies).