Supporting Information

Mergenthaler et al. 10.1073/pnas.1108225109

SI Methods

Materials. Chemicals were obtained from Sigma or Carl Roth and cell-culture media and supplements from Invitrogen or Biochrom, unless otherwise noted. Media and buffer compositions are provided as vol/vol unless otherwise noted.

Plasmids and Cloning. pCAG-MCSn1 was derived by removing LacZ from pDRIVE-CAG (Invivogen) and inserting the multiple cloning site (MCS) from pEGFP-N1 (Clontech). Bcl_{XL}, hexokinase II (HKII) and phosphoprotein enriched in astrocytes (PEA15) were PCR-amplified from a murine neuronal cDNA library and subcloned into pCAG-MCSn1. $HKII_{\Delta N}$ was generated by PCR amplification from pCAG-HKII omitting bases corresponding to the N-terminal 21-amino acid mitochondrial localization sequence. $HKII_{T473A}$ and $HKII_{S155A/S603A}$ were generated by PCR mutagenesis by overlap extension, as previously described (1). Fluorescent proteins were subcloned from pEGFP-N2 (Clontech), pRSET-B-mOrange (gift from R. Tsien, University of California, La Jolla, CA), pmCerulean-C1 (Addgene plasmid 15214) (2), or pCAG-H2b-Venus (gift from C. Harms, Charité-Universitätsmedizin Berlin, Berlin, Germany). Fusion proteins were generated with a spacer sequence. All plasmid DNA was purified (Qiagen EndoFree Plasmid Maxi Kit; Qiagen) from *Escherichia coli* (TOP-10; Invitrogen) and sequenced (LGC-Genomics).

Cell Culture. Generation of $PEA15^{-/-}$ mice used for knockout cultures has been previously described (3). MCF-7 cells were cultured in Alpha MEM (Invitrogen; 10% FCS, 2% penicillin/streptomycin) and HeLa cells in MEM (Biochrom; 10% FCS, 2 mM L-glutamine, 0.1 mM NaPyr, 1% penicillin/streptomycin).

Deferoxamine Treatment and Oxygen-Glucose Deprivation. Primary rat brain cortical neurons were treated [150 μM deferoxamine (DFO), day 8] as previously described (4). Cell cultures from the same preparations were used for mRNA analysis, Western blotting, zymography, and oxygen-glucose deprivation (OGD) 48 h after preconditioning. Neuronal survival was analyzed 24 h after OGD (BSS_0: 116 mM NaCl, 5.4 mM KCl, 0.8 mM $MgSO_4$, 1 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 10 μM glycine, 1.8 mM $CaCl_2$, 1 mM Hepes; 0.3% O_2, 5% CO_2, 37 °C, 3 h, Concept-400; Ruskinn) or OGD-controls (BSS_1: BSS_0 + 1 mM glucose; 21% O_2, 5% CO_2, 37 °C, 3 h). For analysis of survival, lactate dehydrogenase (LDH) release was measured as previously described (5) and normalized to total LDH. LDH values after OGD were subtracted from OGD-control values.

Analysis of Neuronal Survival by Cotransfection/Cocultivation. OGD was performed for 2.5–3 h, oxygen deprivation (OD; BSS_1, 0.3% O_2, 5% CO_2, 37 °C) and glucose deprivation (GD; BSS_0, 5% CO_2, 37 °C) for 5–6 h. For analysis of neuronal survival, primary cortical neurons were transfected with plasmids for eGFP or cotransfected with Bcl_{XL} and eGFP and cocultivated with mOrange-transfected neurons. In some experiments, plasmids expressing H2b-GFP or H2b-mOrange were used instead of eGFP or mOrange. Similarly, HKII, HKII mutants, and PEA15 were cotransfected with eGFP and cocultivated with mOrange-transfected neurons. Paired transfections were cocultivated for 9 d, resulting in green and red fluorescent cells in each culture well. Immediately before OGD (day 9) and 24 h after OGD, we counted the green and red fluorescent cells. In all experiments, on average 500–700 (pre-OGD) or 150–300 (post-OGD, depending on the severity of damage) cells were counted per condition per experiment on inverted epifluorescence microscopes (Leica DM IL and DMI 6000) with specific filters (Chroma). Counting was performed immediately before and 24 h after OGD, OD, or GD. Survival of transfected neurons was assessed by calculating ratios of green and red fluorescent cells.

Preparation of Total RNA and Reverse Transcription. At 0, 6, 12, 18, 24, and 48 h after DFO treatment, RNA was prepared and reverse-transcribed using random hexamers (TRIzol, SuperScript; Invitrogen).

Quantitative Real-Time PCR. Expression of HKI and HKII mRNA in each sample was normalized for RNA preparation and reverse-transcriptase reaction on the basis of its β-actin mRNA content. Similarly, expression of PEA15 from DFO samples was determined from pooled cDNAs. For β-actin, HKI and HKII, and PEA15 quantitative RT-PCR we used the LightCycler-FastStart DNA-Master SYBR-Green-I Kit (Roche) or the LightCylcer 480 Sybr.Green I Master Kit (Roche) on a LightCycler 1.5 (DFO samples) or on a LightCycler 480 (siRNA-treated samples) device (Roche). For amplification and detection, we used the LightCycler Relative Quantification Software (Roche Molecular Biochemicals). Thermal cycling conditions for LC1.5 were: 95 °C for 10 min; 30 cycles of: 95 °C for 15 s, T_A for 10 s, 72 °C for 15 s; and for LC480: 95 °C for 10 min; 45 cycles of: 95 °C for 5 s, T_A for 10 s, 72 °C for 15 s. Detection of amplification product data at T_M. T_A/T_M for HKI and HKII 68/86 °C (LC1.5) or 68/82 °C (LC480) and for PEA15 66/84 °C or 66/80 °C (LC480). After each real-time PCR, amplification products were checked by melting-curve analysis. All samples were amplified in duplicate from the same RNA preparation and the mean values of the respective crossing points (Cp) were considered. For determination of PCR efficiencies (E), we analyzed a serial dilution of β-actin, HKI and HKII, or PEA15 cDNA and calculated $E = 10^{(-1/\text{slope})}$. The relative expression of each gene part of HKI/II or PEA15 compared with β-actin mRNA expression was calculated using the respective crossing points (Cp) according to the equation $E_{(\text{gene})}{}^{-Cp(\text{gene})}/E_{(\beta\text{-actin})}{}^{-Cp(\beta\text{-actin})}$. Sequence-specific primers (Eurofins MWG Operon) were for β-actin: FWD 5′-ACCCACACTGTGCCCATCTA-3′, REV 5′-GCCACAGGATTCCATACCCA-3′; for HKI: FWD 5′-GCTGTGGTCAACGACACCGTG-3′, REV 5′-GGTCTTGACCAGGATACTGTCG-3′; for HKII: FWD 5′-TCAAACAGCTGGGTTCCACTGC-3′, REV 5′-ATCGCCTGCTTATTCACGGAGC-3′; and for PEA15: FWD 5′-ACTCTCCTTCAGGACCTGACC-3′, REV 5′-GGTTAGCTTGGTGTCCAGCTC-3′.

Western Blotting. Semidry Western blotting and chemiluminescence detection were performed after PAGE on 4–20% Tris-Hepes gels (Pierce). Primary antibodies were β-actin, HKII (Santa Cruz), PEA15 (Cell Signaling), and voltage-dependent anion channel (VDAC)-1 (Calbiochem). Secondary horseradish peroxidase-linked antibodies were used: anti-goat (Santa Cruz), anti-mouse, anti-rabbit (GE Healthcare).

Isoenzyme-Specific HK-Zymography. Cultures were washed twice with ice cold PBS, scraped, and centrifuged (300 × *g*, 5 min, 4 °C). Cells were lysed in electrophoresis buffer (see below) supplemented with 1% Nonidet P-40, 2 mM DTT, 20 mM NaF, and protease inhibitor mixture (Roche) by sonication. Total hexokinase activity was determined by measuring NADPH fluorescence at room temperature (excitation: 340 nm; emission: 425 nm; FluoroMax-P; Jobin Yvon Horiba). The reaction medium contained

100 mM Tris, 0.5 mM EDTA, 10 mM $MgCl_2$, 100 mM glucose, 2.5 mM NADP, 10 mM ATP, and 1.5 U/mL G6PDH (Carl Roth). Cellulose acetate electrophoresis (Cellogel; 57 × 140 mm, 250 µ) was performed (200 V, 40 min, room temperature) with electrophoresis buffer (36 mM Tris, 20 mM boric acid, 4 mM Na_2HPO_4, 2 mM EDTA, 100 mM glucose, pH 8.5) by loading sample volumes corresponding to equal activities of total hexokinase. Cellulose acetate sheets were overlaid with agarose (1 mm, 100 mM Tris, 0.5 mM EDTA, 10 mM $MgCl_2$, 100 mM glucose, 2.5 mM NADP, 10 mM ATP, 1.5 U/mL G6PDH) and incubated (45 min, room temperature). Hexokinase activity was detected by NADPH fluorescence (excitation: 365 nm) with a digital camera (Olympus E-330, high-pass yellow filter, transmission > 450 nm; Schneider-Kreuznach). Activities of hexokinase isoenzymes were quantified with the ImageJ gel analysis plug-in after conversion of raw images without any modifications. Separation distances of hexokinase isoenzymes were determined with rat tissue extracts from brain (HKI) and muscle (HKII).

Transfections. Neurons were transfected on a Nucleofector-II device (Amaxa, Lonza) using program O-003 (rat) or O-005 (mouse), 5 µg DNA per plasmid, 3×10^6 cells and electroporation buffer (EB) (192 mM NaCl, 9.6 mM KCl, 15 mM $MgCl_2$). Neurons were resuspended in DMEM (Biochrom) containing 3.7 g/L $NaHCO_3$, 4.5 g/L D-glucose, 10% FCS, 1% penicillin/streptomycin, 1% L-glutamine, plated (3.1×10^5 cells/cm^2). Medium was replaced with conditioned DMEM after 4–6 h and with conditioned NBM-A (Gibco, Invitrogen, containing B27, 1% penicillin/streptomycin, 0.5 mM L-glutamine, 25 mM glutamate) after 16–20 h, and partially replaced with fresh NBM-A (without glutamate) after 5 d. HeLa cells were transfected (Nucleofector-II, I-013, 2 µg DNA per plasmid, 1×10^6 cells, EB) and resuspended in culture medium after transfection. MCF-7 cells were transfected with Nanofect (Qiagen; 2 µg DNA per plasmid).

siRNA Treatment of Neurons. Primary rat cortical neurons were transfected with a plasmid for GFP or cotransfected with plasmids for GFP and PEA15. One day after transfection, culture medium was replaced with conditioned neuronal culture medium containing a siRNA pool targeted against rat HKII or a nontargeting siRNA pool at a final concentration of 1 µM, according to the manufacturer's protocol (Accell SMARTpool siRNA; Dharmacon, Thermofisher). The medium containing 1 µM siRNAs was replaced every 3 d and cell counting and OGD were performed on day 10 after transfection. After OGD, old siRNA-containing medium was added and neurons were cultured for 24 h to analyze survival. HKII and PEA15 expression was analyzed by quantitative RT-PCR (Lightcycler 480) in sister cultures treated in parallel. siRNA sequences were: rat HKII SMARTpool (E-087781; GGACCGUGUUUGAUGUUGC; CUAUGAUGACUUGUGGCUA; GGGGAAUCUUUGAAACUAA; GCAACAUUCUCAUCGAUUU); nontargeting SMARTpool (D-001910; UGGUUUACAUGUCGACUAA; UGGUUUACAUGUUUUCUGA; UGGUUUACAUGUUUUCCUA; UGGUUUACAUGUUGUGUGA).

Preparation of Mitochondria. Mitochondria from neurons were prepared by differential centrifugation as previously described (6) with modifications. Cultures were washed with PBS. All subsequent steps were performed on ice or at 4 °C. Next, 8×10^7 neurons were scraped in isolation buffer (IB) (210 mM mannitol, 70 mM sucrose, 5 mM Hepes, 1 mM EGTA, 0.5% BSA, phosphatase inhibitors). Neurons were homogenized (120 strokes; tight teflon-glass potter; 2,000 rpm, RZR 2102 Control stirrer, Carl Roth) and lysate was centrifuged (10 min, 600 × g, 4 °C) three times. Supernatant was centrifuged (10 min, 7,000 × g, 4 °C) and resulting supernatant was centrifuged (25,000 × g, 10 min, 4 °C) to obtain the cytosolic fraction in the supernatant. The pellet after 7,000 × g centrifugation containing mitochondria was resuspended (10× pellet volume, IB) and centrifuged twice (7,000 × g; 10,000 × g; 10 min each; 4 °C). This step was repeated once. Mitochondrial and cytosolic fractions were analyzed by immunoblotting with antibodies against VDAC (Calbiochem) and Akt-1 (Cell Signaling Technology).

Coimmunoprecipitation. Neurons (2×10^7, culture day 10) were washed in PBS and treated with 1% PFA (10 min, room temperature), scraped in PBS, and centrifuged (1,000 × g, 10 min, 4 °C). The pellet was resuspended in lysis buffer (LB, 10 mM Tris-HCl pH 7.5, 150 mM NaCl, 2 mM EDTA) with 15 mM 1,2-diheptanoyl-sn-glycero-3-phosphocholine (DHPC) (Avanti Polar Lipids); protease and phosphatase inhibitors; and incubated on ice for 45 min. Agarose beads (50% protein G, 50% protein A; Millipore) were washed three times in LB without DHPC. After centrifugation of neuronal lysate (20,000 × g, 10 min, 4 °C), supernatant was adjusted to 1 mL with LB without DHPC and incubated with agarose beads (30 min, 4 °C). The lysate was incubated (overnight, 4 °C) with or without 2 µg of HKII antibody (Santa Cruz). Lysates were incubated with agarose beads for 3 h at 4 °C. After incubation, beads were washed three times with lysis buffer and coimmunoprecipitation was analyzed by Western blotting.

Fluorescence Resonance Energy Transfer Measurements by Time-Correlated Single-Photon Counting Fluorescence Lifetime Imaging Microscopy. Fluorescence lifetime imaging microscopy (FLIM)-FRET was performed on an inverted Leica TCS SP5 MP (Leica Microsystems) laser-scanning microscope equipped with an integrated SPC-830 time-correlated single-photon counting (TCSPC) system (Becker & Hickl), a Chameleon Ultra Ti:Sapphire pulsed laser (Coherent) and a 63× glycerol immersion objective (HCX PL APO UV, NA = 1.3, Leica). Fluorescence-lifetime images were acquired with 820-nm dual-photon excitation and 450–490 nm emission (SPCM; Becker & Hickl) and steady-state fluorescence images with settings for mCerulean: excitation 458 nm, emission 465–490 nm; mVenus: excitation 514 nm, emission 525–560 nm (LAS AF; Leica). For FLIM measurements, MCF-7 cells were transfected with plasmid DNA encoding for HKII-mCerulean, $HKII_{T473A}$-mCerulean, PEA15-mVenus, mCerulean, or mVenus 24 h before imaging. All measurements were performed at room temperature in PBS with 20 mM glucose from at least three independent transfections (except mCerulean alone, n = 1). Numbers of analyzed cells are indicated in Fig. 4*D*.

Analysis of FLIM-FRET Measurements. TCSPC raw data were analyzed (SPCimage; Becker & Hickl) and lifetimes fit with monoexponential decays. Processed lifetime and photon intensity images were exported and average lifetimes per cell measured with ImageJ (MacBiophotonics; ImageJ distribution). FRET efficiency was calculated with the formula $E_{FRET} = 1 - (\tau_{DA}/\tau_D)$, where τ_{DA} is the mean fluorescence lifetime of FRET donor (HKII-mCerulean) in the presence of FRET acceptor (PEA15-mVenus) and τ_D is the mean lifetime of FRET donor coexpressed with mVenus (no-FRET control). For lifetime figures (Fig. 4*D*), intensity-weighted FLIM images and FLIM histograms were generated in ImageJ (plug-ins available at www.macbiophotonics.ca).

Proximity Ligation Assay. Proximity ligation assay (PLA) relies on the use of specific primary and secondary antibodies and enables the visualization of interacting proteins in cells using fluorescence detection of a localized rolling circle amplificate from the secondary antibodies (7). PLA probes anti-goat PLUS, anti-mouse MINUS, and detection reagent DUOLINK II red were obtained from Olink Bioscience. Neurons were cotransfected with plasmids encoding for HKII and PEA15 and cultured on coverslips. In total, we used three to five coverslips from different preparations/transfections. After 3–7 d in culture, neurons were exposed to OD

or GD for 135–150 min or control conditions (neurobasal medium or BSS_{10}, see above) and fixed immediately afterwards with 4% PFA in PBS for 10 min at room temperature. After permeabilization with 0.2% Triton-X100 in PBS for 10 min at room temperature, PLA was performed as described in the Duolink II protocol using goat-anti-HKII (Santa Cruz) and mouse-anti-PEA15 (Santa Cruz) primary antibodies. For controls, either the HKII or PEA15 antibody was omitted and the protocol was carried out without any further changes. Coverslips were mounted with DII mounting medium containing DAPI (Olink Bioscience). Fluorescence microscopy was performed on a Leica DMI 6000 microscope equipped with a DFC360FX CCD camera (Leica) and a HCX PL APO 40.0× NA 0.85 dry objective (Leica). Image acquisition was performed using the LAS AF software (Leica) and equal exposure settings for all images. For each coverslip, images were taken from at least 10 randomly selected fields of view. The total cell numbers per condition are indicated in Figs. S6 and S7.

Analysis of PLA. Images were exported from LAS AF as 16-bit TIF and converted to 8-bit TIF using ImageJ without applying any further adjustments. Images were loaded into Blobfinder (Uppsala University, Centre for Image Analysis, http://www.cb.uu.se/~amin/BlobFinder/) (8) and batch-processed using single-cell analysis and equal settings for all images. For statistical analysis, the resulting "PLA signals per cell" in all groups were normalized to the mean value of the control group. Representative images for each condition were prepared for figure presentation by applying brightness and contrast adjustments uniformly using ImageJ.

Induction of Apoptosis and Flow Cytometry. HeLa cells were transfected with a plasmid for GFP or cotransfected with plasmids for GFP and HKII or Bcl_{XL}. Twenty-four hours after transfection, apoptosis was induced in HeLa cells by application of 100 nM Actinomycin D (Sigma) or 75 μM Etoposide (Sigma) to the culture medium and incubation for 24 h or by submitting cultures to hypoxia (0.3% O_2, In Vivo 400; Ruskinn) for 6 h in BSS_0 and subsequent incubation in culture medium (21% O_2, 5% CO_2, 37 °C, 24 h), or for 21 h in serum and glucose-free DMEM (Invitrogen) and immediate FACS analysis. For flow cytometry, cells were trypsinized. For experiments involving actinomycin D treatment or short (6 h) hypoxia, cells were centrifuged with culture supernatants, resuspended in PBS, and stained with Annexin V-APC (eBioscience) and propidium iodide (Sigma) according the manufacturer's instructions. Samples were then analyzed by flow cytometry (FACSCalibur; BD Biosciences, equipped with a 488 nm argon laser and a 635 nm diode laser). Data were acquired and analyzed using Cellquest Pro-6.0 (BD Biosciences). Annexin V-APC and propidium iodide fluorescence was analyzed in GFP^+ cells only. A total of 5,000 GFP^+ cells were measured per sample. For experiments involving etoposide treatment and long (21 h) hypoxia, cells were centrifuged without culture supernatants and analyzed by flow cytometry. The percentage of GFP-expressing cells (mean fluorescence intensity > 10^3) before and after treatment (sister cultures) was calculated as a marker for survival and membrane integrity. All data were normalized to the percentage of GFP expression in untreated cells (controls). To compare different experiments, percentages were normalized to cells expressing GFP only [ratio = (% GFP+gene of interest)/% GFP].

Membrane-Based Split-Ubiquitin Yeast Two-Hybrid Screen. The split-ubiquitin screen using murine HKII cDNA in pDHB1 as bait and a mouse brain cDNA library (Dualsystems Biotech) as prey was carried out by Dualsystems Biotech. Screening and retesting was performed in yeast strain NMY32. Interactors were identified by sequencing library plasmids from positive clones.

Statistical Evaluation. Data are shown as mean ± SD. Statistical test were performed PASW Statistics 18.0. Box plots were generated in PASW Statistics 18.0 (SPSS) and display median, 25th and 75th percentiles within boxes and 5th and 95th percentiles with whiskers. Outliers are displayed as dots, extreme values as diamonds. Unpaired two-tailed Student's *t* test, one-way ANOVA followed by Tukey-HSD or Duncan post hoc test or Friedman's test were performed as applicable (PASW). *P* values and numbers of independent experiments are indicated in the figures.

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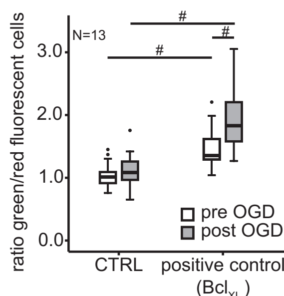


Fig. S1. Counting of control transfected neurons (expressing eGFP or mOrange only) resulted in ratios of ~1 before and after OGD, demonstrating no influence of the fluorescent proteins on neuronal survival. The increased survival of cells expressing Bcl_{XL} resulted in a larger ratio of green to red fluorescent neurons, confirming that this approach allows assessment of the effect of transfected genes on neuronal survival. N indicates number of independent experiments; $^{\#}P = 0.001$, one-way ANOVA, Duncan post hoc.

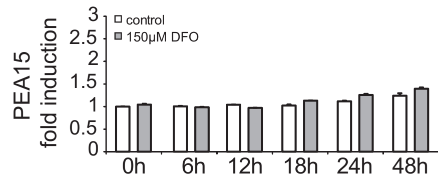


Fig. S2. PEA15 mRNA expression in primary neurons remained unaffected by hypoxia-mimicking induction of hypoxia-inducible factor (HIF)-1 with 150 μM DFO. Expression analysis was performed in pooled cDNAs from Fig.1.

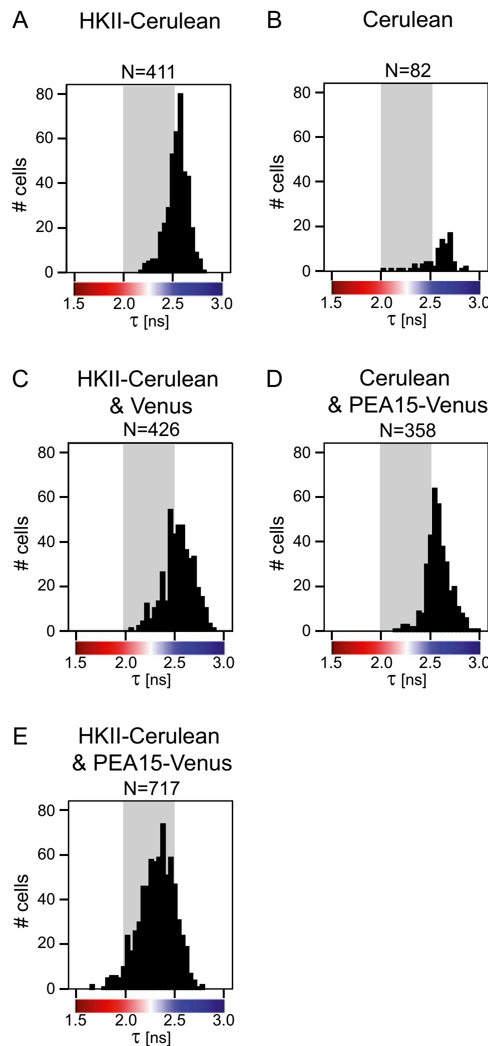


Fig. S3. Population-based fluorescence lifetime histograms. Numbers of cells imaged are indicated in each panel. Histograms demonstrate equal distribution of fluorescence lifetime of Cerulean or HKII-Cerulean under all control conditions (*A–D*) (i.e., no energy transfer). This demonstrates that neither fusion with HKII nor coexpression of Venus or PEA15-Venus significantly changes the fluorescence lifetime of Cerulean or HKII-Cerulean and that no unspecific energy transfer (i.e., FRET not because of interaction of HKII and PEA15) occurs. (*E*) Coexpression of HKII-Cerulean and Pea15-Venus decreases fluorescence lifetime of HKII-Cerulean for the entire cell population according to the parameters indicated in Fig. 4, demonstrating the range in the amount of energy transfer in all cotransfected cells. The color look-up-table (LUT) below the histograms corresponds to the FLIM-LUT used in Fig. 4.

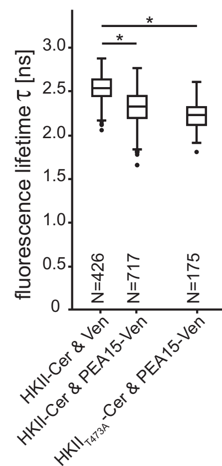


Fig. S4. Whole-cell FLIM-FRET analysis of HKII$_{T473A}$-mCerulean coexpressed with PEA15-Venus in MCF-7 cells demonstrated interaction of this non-phosphorylatable (i.e., nonmitochondrial) mutant of HKII and PEA15 in live cells. Fluorescence lifetime (τ) was significantly reduced compared with control conditions, corresponding to a FRET efficiency (E%) of 12.2%. The other two measurements (HKII-Cer & Ven; HKII-Cer & PEA15-Ven) are taken from Fig. *4D*. N represents number of cells imaged; $^*P < 0.0001$, one-way ANOVA, Tukey-HSD post hoc.

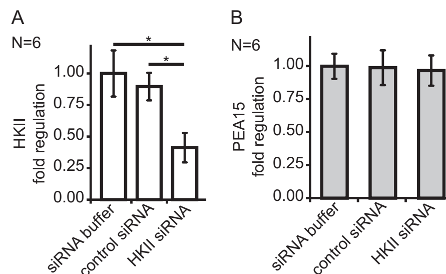
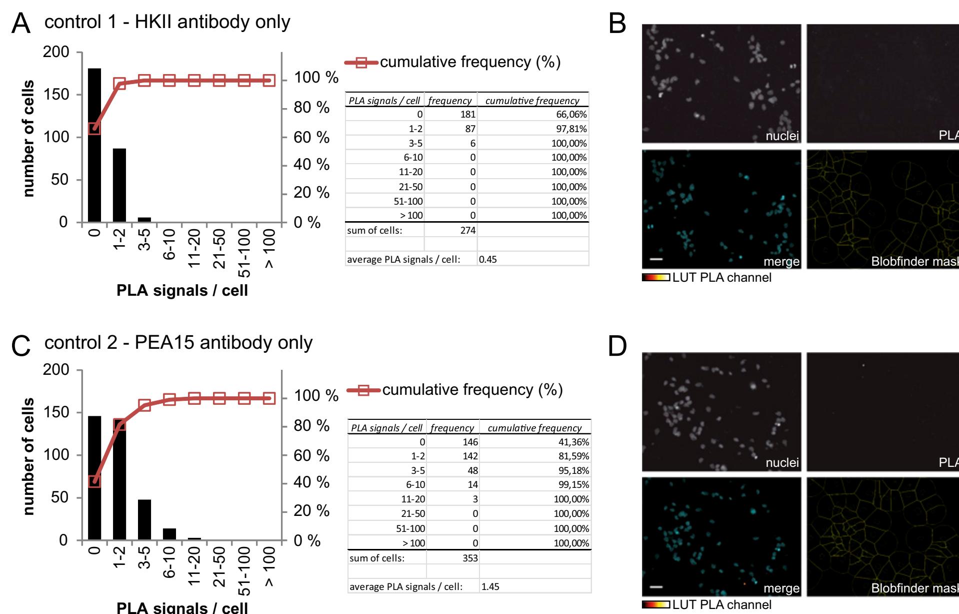


Fig. S5. (*A*) Treatment of primary cortical neurons with siRNA pools against HKII significantly reduced HKII mRNA levels to 41% ± 12 compared with untreated controls. Non targeting pools did not affect HKII expression. (*B*) PEA15 expression was not affected by control siRNA or HKII siRNA pools. N represents independent experiments from different cultures. $^*P = 0.01$, one-way ANOVA, Tukey-HSD post hoc.



PLA signals / cell	frequency	cumulative frequency
0	181	66,06%
1-2	87	97,81%
3-5	6	100,00%
6-10	0	100,00%
11-20	0	100,00%
21-50	0	100,00%
51-100	0	100,00%
> 100	0	100,00%
sum of cells:	274	
average PLA signals / cell:	0.45	

PLA signals / cell	frequency	cumulative frequency
0	146	41,36%
1-2	142	81,59%
3-5	48	95,18%
6-10	14	99,15%
11-20	3	100,00%
21-50	0	100,00%
51-100	0	100,00%
> 100	0	100,00%
sum of cells:	353	
average PLA signals / cell:	1.45	

Fig. S6. Controls for PLA: HKII primary antibody only was applied in control 1 and PEA15 primary antibody only in control 2. Histograms in *A* and *C* demonstrate that unspecific background signals are largely restricted to one to two signals per cell. (*B* and *D*) Representative images from each condition. Blobfinder mask: delineation of cells as identified by Blobfinder software. (Scale bar, 25 μm.)

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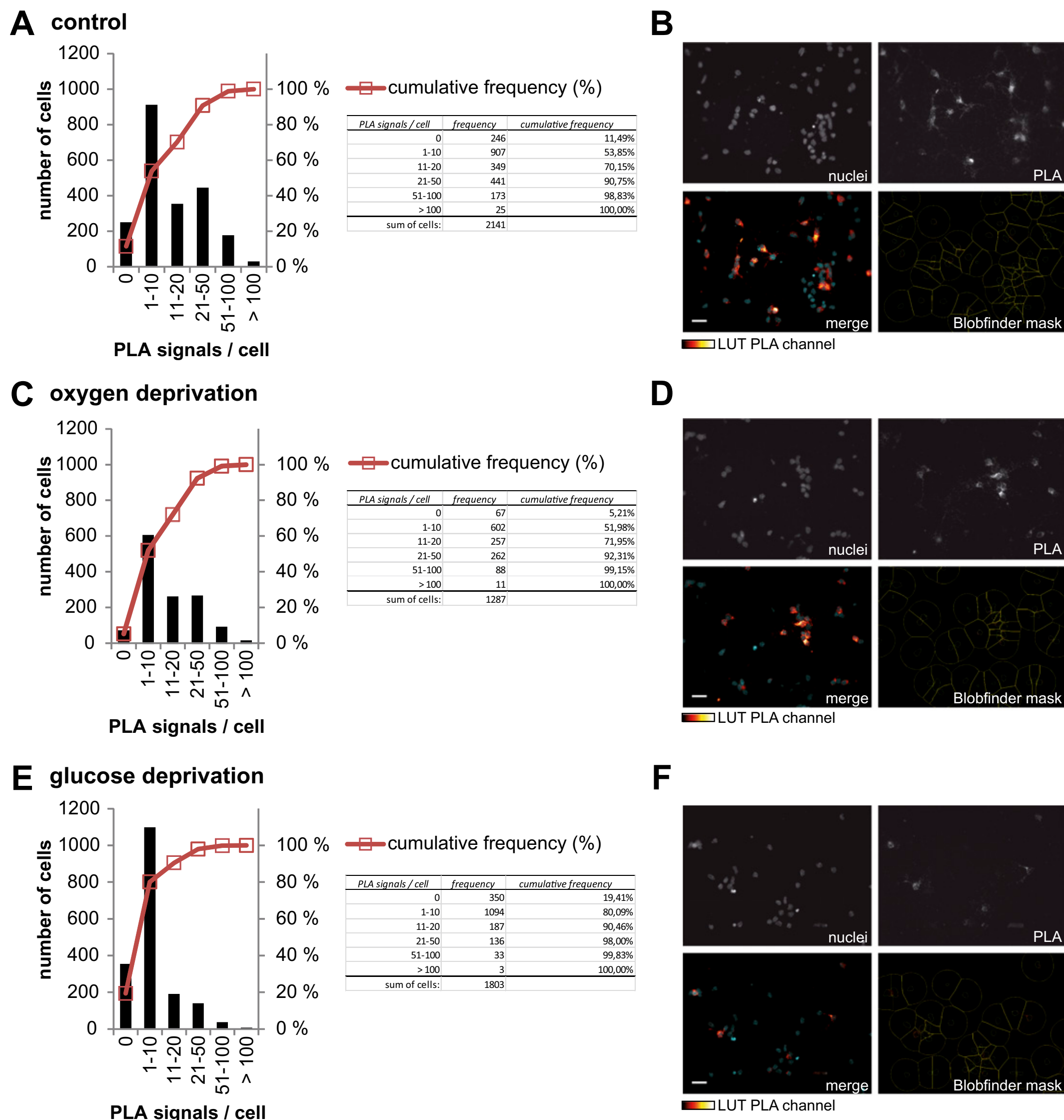

PLA signals / cell	*frequency*	*cumulative frequency*
0	246	11,49%
1-10	907	53,85%
11-20	349	70,15%
21-50	441	90,75%
51-100	173	98,83%
> 100	25	100,00%
sum of cells:	2141	

PLA signals / cell	*frequency*	*cumulative frequency*
0	67	5,21%
1-10	602	51,98%
11-20	257	71,95%
21-50	262	92,31%
51-100	88	99,15%
> 100	11	100,00%
sum of cells:	1287	

PLA signals / cell	*frequency*	*cumulative frequency*
0	350	19,41%
1-10	1094	80,09%
11-20	187	90,46%
21-50	136	98,00%
51-100	33	99,83%
> 100	3	100,00%
sum of cells:	1803	

Fig. S7. Histograms and representative images with merged PLA and nuclei (DAPI) channels from PLA experiments on transfected neurons after OD, GD, or under control conditions. (*A*, *C*, and *E*) Histograms demonstrate the distribution of PLA signals per cell, indicating a decrease of cells with a high number of PLA signals under GD. Tables indicate the number of cells per group. (*B*, *D*, and *F*) Representative images from each condition. Blobfinder mask: delineation of cells as identified by Blobfinder software. (Scale bar, 25 μm.)