**iScience, Volume *27***

## Supplemental information

# Tracking fructose 1,6-bisphosphate dynamics in liver cancer cells using a fluorescent biosensor

**Israel Pérez-Chávez, John N. Koberstein, Julia Malo Pueyo, Eduardo H. Gilglioni, Didier Vertommen, Nicolas Baeyens, Daria Ezeriņa, Esteban N. Gurzov, and Joris Messens**

**Data S1. HYlight/HYlight Null aminoacid and codon-optimized DNA sequence for its usage in HepG2, HLE, and Huh6 cell lines.** Related to figure 1.

Color Legend
CggR 96-180
Linkers
cpGFP
CggR 181-340
EcoRI/XhoI
BamHI/HindIII
HYlight Null Mutation (T152E)

Amino Acid Sequence:
MGSKDVLGLTLLEKTLKERLNLKDAIIVSGDSDQSPWVKKEMGRAAVACMKKRFSGKNI
VAVTGGTTIEAVAEMMTPDSKNRELLFVPARGGLGEPPYNVFIMADKQKNGIKANFKIR
HNIEDGGVQLAYHYQQNTPIGDGPVLLPDNHYLSVQSKLSKDPNEKRDHMVLLEFVTA
AGITLGMDELYKGGTGGSMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATY
GKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYIQERTI
FFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNFNKEDVKNQANTIC
AHMAEKASGTYRLLFVPGQLSQGAYSSIIEEPSVKEVLNTIKSASMLVHGIGEAKTMAQ
RRNTPLEDLKKIDDNDAVTEAFGYYFNADGEVVHKVHSVGMQLDDIDAIPDIIAVAGGSS
KAEAIEAYFKKPRNTVLVTDEGAAKKLLRDESG*

DNA Sequence:
GAATTCGGATCCATGGGCAGCAAAGACGTGCTGGGGCTGACCCTGCTGGAAAAAACCCTGA
AAGAACGCCTGAATCTGAAAGACGCCATTATCGTTAGCGGCGATAGCGACCAAAGCCCATG
GGTCAAAAAAGAAATGGGCCGCGCTGCTGTTGCTTGTATGAAGAAACGCTTTAGCGGCAAG
AATATCGTGGCCGTTACTGGGGGGACTACCATTGAAGCCGTGGCCGAGATGATGACCCCAG
ATAGCAAAAATCGCGAACTGCTGTTTGTGCCAGCTCGCGGCGGTCTGGGGGAACCTCCTTA
TAACGTCTTTATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCC
ACAACATCGAGGACGGCGGCGTGCAGCTGGCCTATCACTACCAGCAGAACACCCCTATCGG
CGACGGCCCTGTGCTGCTGCCTGACAACCACTACCTGAGCGTGCAGAGCAAACTGAGCAAA
GACCCTAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATC
ACTCTGGGCATGGACGAGCTGTACAAGGGCGGCACCGGCGGGAGCATGGTGAGCAAGGG
CGAGGAGCTGTTCACCGGGGTGGTGCCTATCCTGGTCGAGCTGGACGGCGACGTGAACGG
CCACAAGTTCAGCGTGAGCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCT
GAAGTTCATCTGCACCACCGGCAAGCTGCCTGTGCCTTGGCCTACCCTGGTGACCACCCTG
ACCTACGGCGTGCAGTGCTTCAGCCGCTACCCTGACCACATGAAGCAGCACGACTTCTTCA
AGAGCGCCATGCCTGAAGGCTACATTCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAA
CTATAAGACCCGCGCTGAGGTTAAGTTCGAGGGCGACACTCTGGTTAACCGCATCGAGCTG
AAGGGCATCGACTTCAAGGAGAGACGGCAACATCCTGGGCCATAAGCTGGAATATAACTTC
AACAAGGAGGATGTGAAGAATCAAGCTAATACCATTTGTGCACATATGGCCGAGAAAGCCAG
CGGTACCTATCGCCTGCTGTTTGTCCCTGGTCAGCTGAGCCAAGGTGCATACAGCAGCATT
ATCGAAGAACCTAGCGTCAAAGAAGTCCTGAATACCATCAAAAGCGCTAGCATGCTGGTCCA
TGGGATTGGGGAAGCAAAAACTATGGCTCAGCGCCGCAATACCCCACTGGAAGACCTGAAA
AAGATCGATGATAATGATGCCGTGACCGAAGCCTTTGGCTACTATTTTAATGCTGATGGTGA
GGTGGTGCATAAAGTGCATAGCGTGGGTATGCAACTGGATGATATTGATGCCATCCCTGATA
TTATTGCAGTCGCAGGTGGGAGCAGCAAGGCAGAGGCAATTGAGGCATATTTTAAGAAACC
ACGCAATACTGTTCTGGTCACTGATGAGGGTGCTGCCAAAAAGCTGCTGCGCGATGAAAGC
GGCTAAAAGCTTCTCGAG

**Fig. S1.**

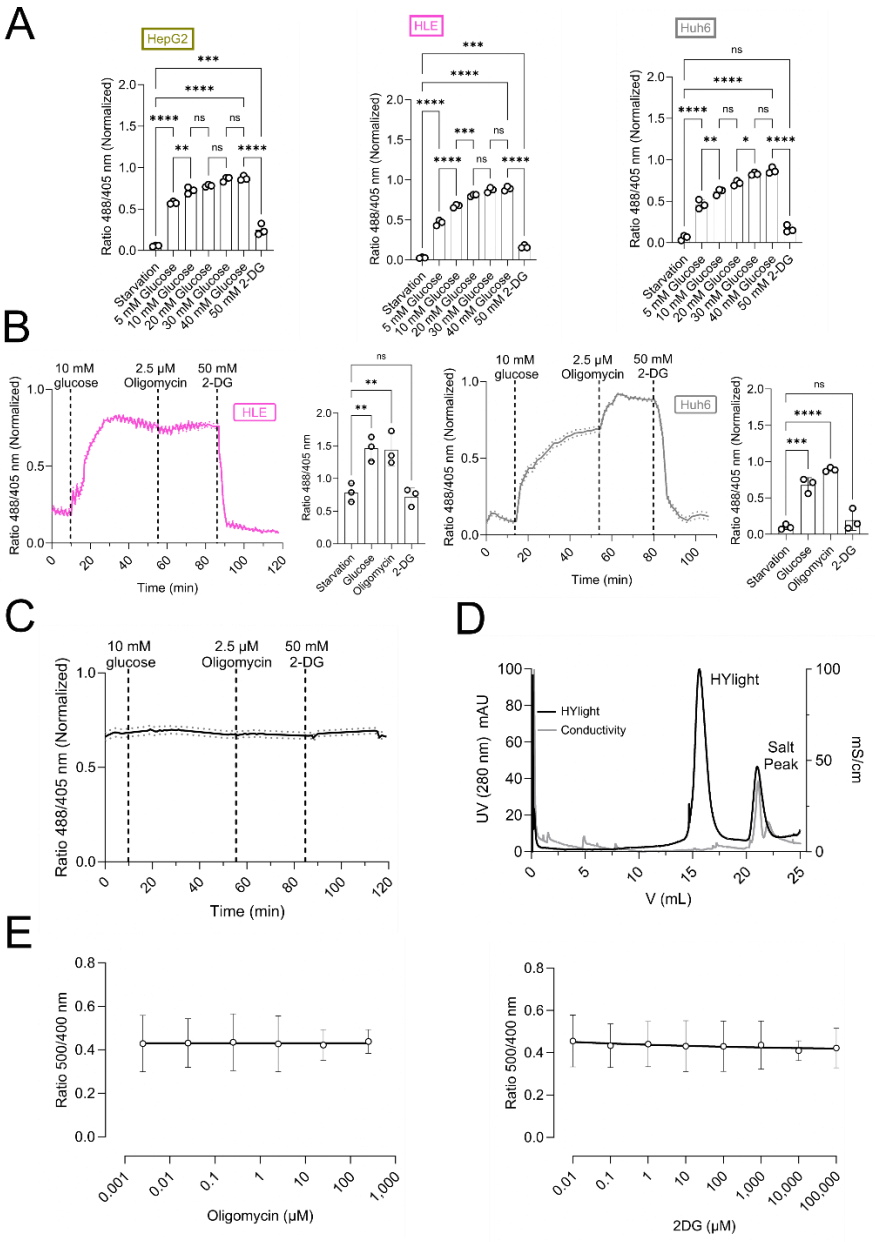


**Figure S1. HYlight response varies across human liver cell lines. Related to Figure 1. A.** Averaged fluorescence ratio upon compound addition in HepG2, HLE and Huh6 cells. Data are shown as mean ± SD. **B.** Averaged min-max normalized ratio change of HYlight in HLE and Huh6 cells after 1 h of glucose starvation and following the addition of glucose, oligomycin, and 2-deoxy-D-glucose (2-DG). Data are the mean ± SEM. Averaged min-max normalized fluorescence ratio upon compound addition. Data are shown as mean ± SD. n=3 with approximately 50 cells per replicate. **C.** No change of the ratio of HYlight Null in Hep G2 cells after 1 h of glucose starvation and following the addition of glucose, oligomycin, and 2-DG. Data are mean ± SEM. n=3 Approximately 50 cells per replicate. **D.** Size exclusion chromatography (SEC) on Superdex 200 10/300 of HYlight which elutes as a single peak indicating a monomeric state. **E.** No change in fluorescence ratio as a function of oligomycin and 2-DG concentration using purified recombinantly expressed HYlight. Data are shown as mean ± SD. n=3. ns: No significant; *<0.05; **<0.005; ***<0.0005; ****<0.00005. Ordinary One-Way ANOVA Šídák´s Multiple Comparisons Test. Related to figure 1.

**Fig. S2.**

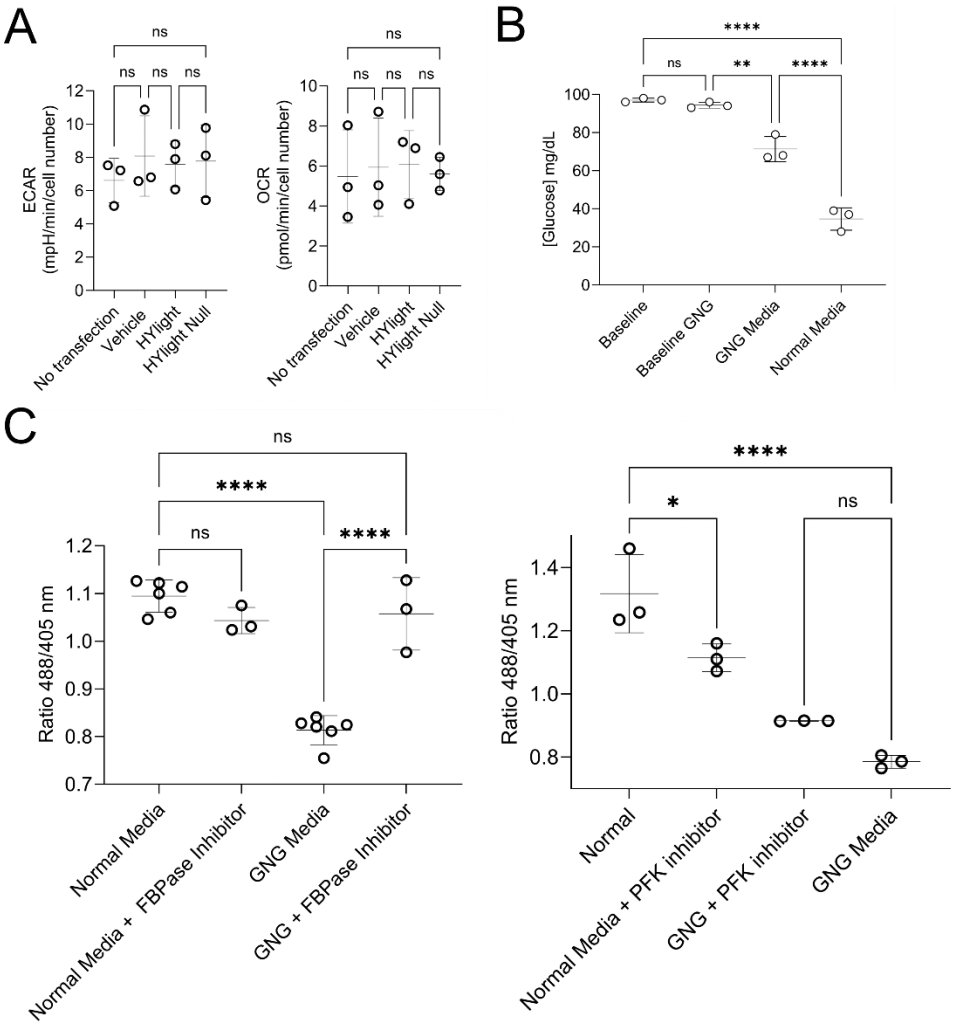


**Figure S2. HYlight does not affect fructose 1,6-bisphosphate (FBP) levels in liver cells, and its response is exclusively linked to glycolysis. Related to Figure 2. A.** ECAR and OCR differences at averaged glucose addition. ECAR is expressed in milli-pH units per minute and normalized to cell number. OCR is expressed in picomoles of oxygen per minute and normalized to cell number. Mean ± SD. n=3 **B.** Comparison of media glucose content after 48 h between gluconeogenic media (GNG) and normal media (DMEM GlutaMAX™) in HepG2 cells. Data are shown as mean ± SD. n=3 **C.** Averaged change in the fluorescence ratio of HYlight in HepG2 liver cancer cells after glucose addition. Comparison between gluconeogenic media (GNG) and normal media (DMEM GlutaMAX™) in the presence or absence of either 20 μM FBP1 inhibitor or 20 μM PFK inhibitor. Mean ± SD. n=3, with minimum 50 cells per replicate. ns: Not significant; *<0.05; **<0.005; ***<0.0005; ****<0.00005. Ordinary One-Way ANOVA Šídák´s Multiple Comparisons Test. Related to figure 2.

**Fig. S3.**

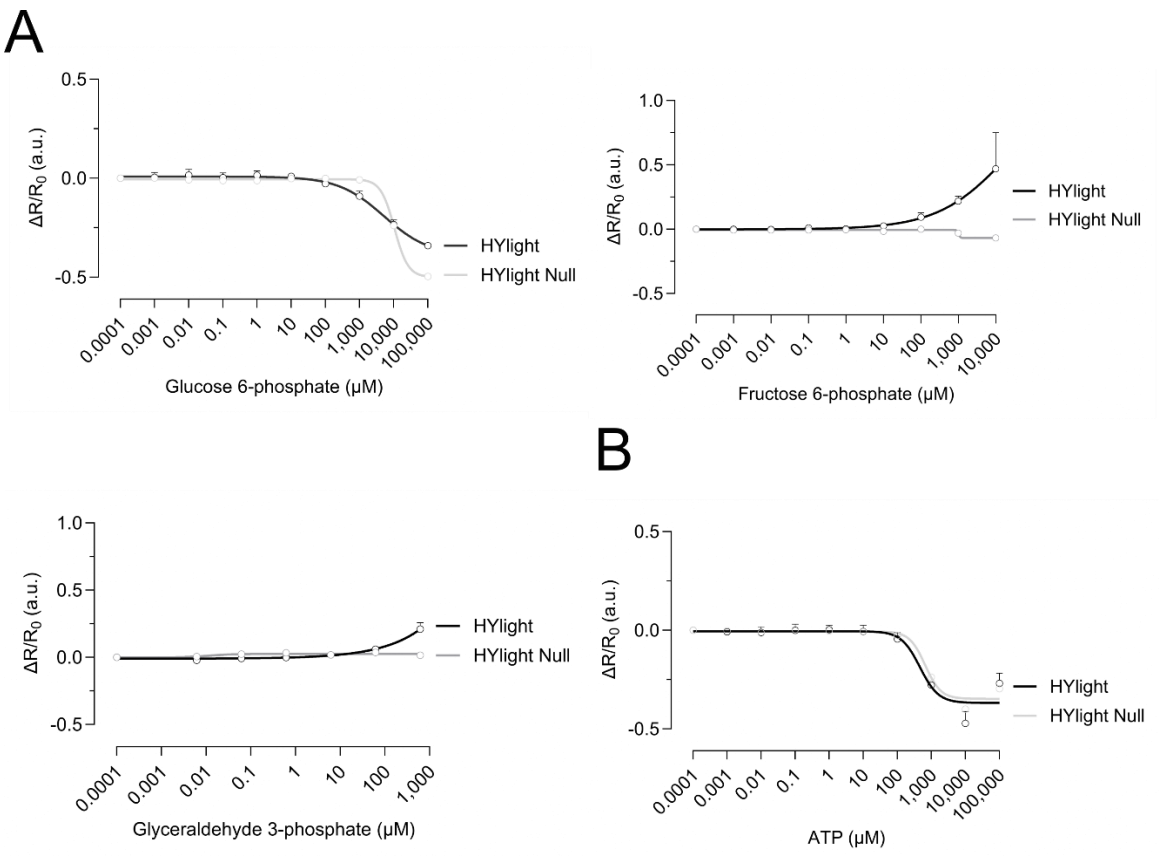


**Figure S3. Dihydroxyacetone phosphate binds HYlight. Related to Figure 4. A.** Change of the fluorescence ratio in HYlight and HYlight Null relative to 0 μM as a function of glucose 6-phosphate, fructose 6-phosphate, and glyceraldehyde 3-phosphate concentrations. **B.** Change of the fluorescence ratio in HYlight and HYlight Null relative to 0 μM as a function of ATP concentrations. SEC: Size Exclusion Chromatography Buffer: 50 mM Tris pH 7.5; 125 mM NaCl. R=Ratio 500 nm/400 nm. $\Delta R/R_0=(R_x-R_0)/R_0$. The data are shown as the mean ± SD. n=3 for each concentration. Related to figure 4.

**Fig. S4.**

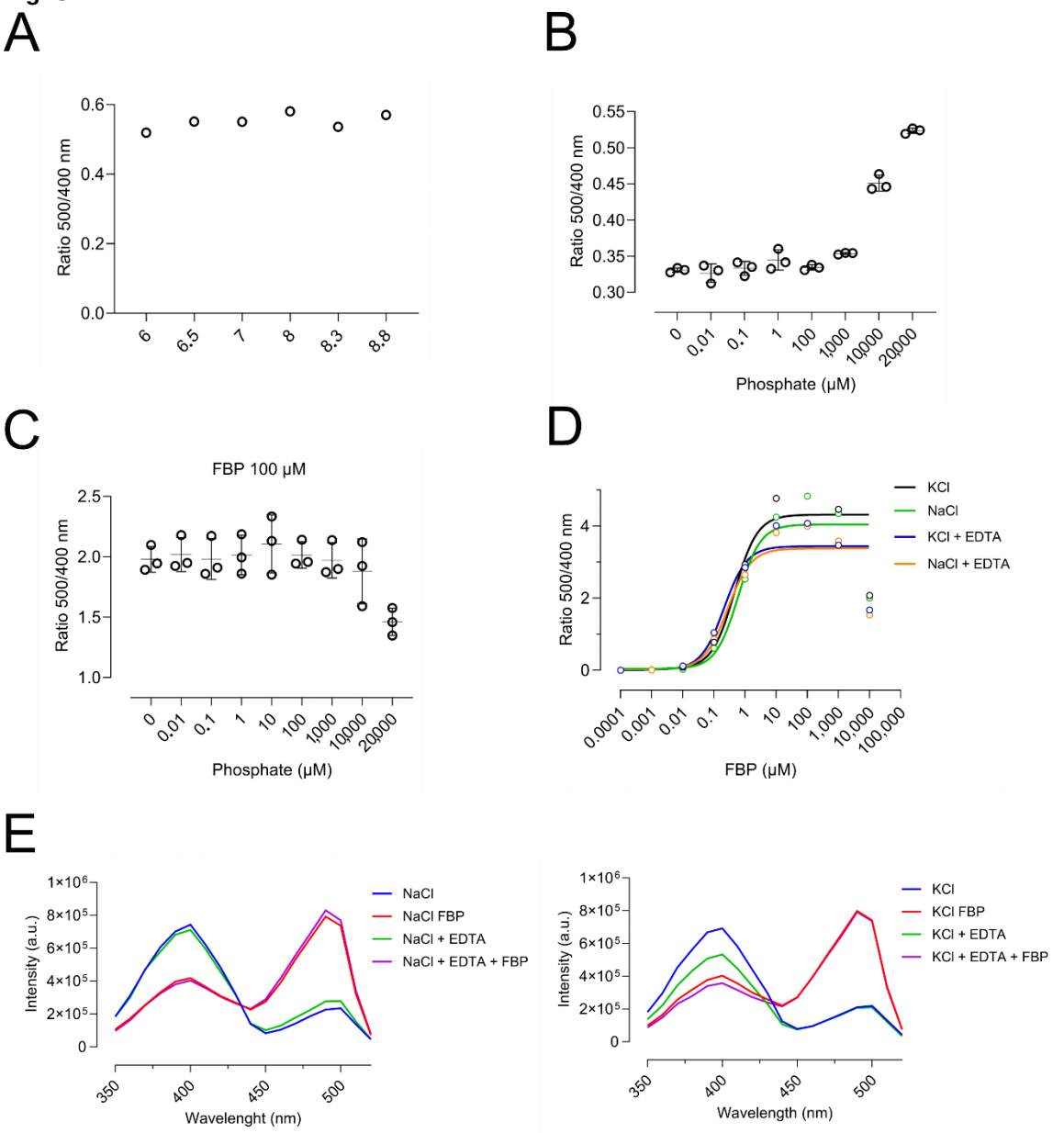


**Figure S4. HYlight is sensitive to phosphorous compounds but not to metal binding. Related to Figure 6. A.** Change of the fluorescence ratio in HYlight Null at different pH values. **B.** Fluorescence ratio change of HYlight at different phosphate concentrations **C.** Fluorescence ratio change of HYlight at different phosphate concentrations in the presence of 100 µM of fructose 1,6-bisphosphate (FBP). **D.** Relative change of the fluorescence ratio in HYlight as a function of different FBP concentrations in the presence of 150 mM NaCl, 150 mM KCL, and/or 0.1 mM EDTA. **E.** Excitation spectra of HYlight in the presence of 150 mM NaCl, 150 mM KCL, and/or 0.1 mM EDTA and 100 µM FBP. Data are shown as mean ± SD. n=3 for each experiment. Related to figure 6.

**Fig. S5.**

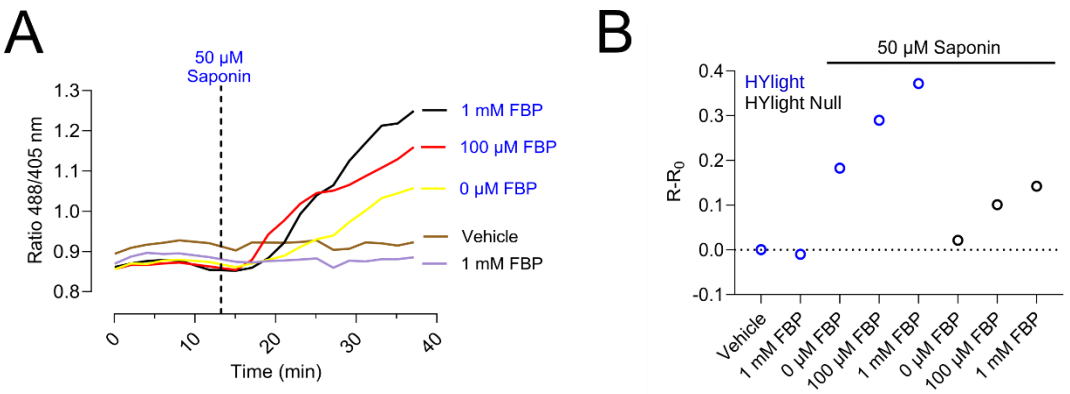


**Figure S5. Saponin permeabilizes the cellular membrane allowing the entry of fructose 1,6-bisphosphate (FBP) in HepG2 cells. Related to Figure 7. A.** Change of the fluorescence ratio of HYlight in HepG2 liver cancer cells after 1 h of glucose starvation and sequential addition of 50 µM Saponin and various concentrations of FBP. **B.** Maximum fluorescence ratio of HYlight and HYlight Null in HepG2 liver cancer cells after 1 hour of glucose starvation and addition of 50 µM saponin and various concentrations of FBP. $R\text{-}R_0$=Ratio 488/405 nm - Minimum Ratio 488/405 nm. n=1, with at least 50 cells per condition. Related to figure 7.

**Table S1.** Fructose 1,6-bisphosphate concentrations in cells. Related to figure 7.

| FBP (μM) | Treatment | Cell Type | Organism | Method | Source |
|---|---|---|---|---|---|
| 1520 (Absolute) | - | Kidney (iBMK) | *M. musculus* | Mass Spectrometry | [1] |
| 31 | - | Human Erythrocyte | *H. sapiens* | - | [2] |
| 1 (Free in liver) | - | Liver | *R. rattus* | - | [3] |
| 50 (Maximum) | - | Liver | - | - | [4] |
| 50 | - | Liver | *R. rattus* | - | [5] |
| 8 (Enzyme site) | - | Liver | *R. rattus* | Enzymatic Assay | [6] |
| 100±50 | 5 mM Glucose | Liver | *R. norvegicus* | Enzymatic Assay | [7] |
| 25000±7600 | 5 mM Glucose | Liver Hepatoma (AS-30D) | *R. norvegicus* | Enzymatic Assay | [7] |
| 23±2 | No treatment | Liver Hepatoma (HepG2) | *H. sapiens* | Mass Spectrometry | Current Article |
| 36±3 | 10 mM Glucose | Liver Hepatoma (HepG2) | *H. sapiens* | Mass Spectrometry | Current Article |
| 43±3 | 2.5 μM Oligomycin | Liver Hepatoma (HepG2) | *H. sapiens* | Mass Spectrometry | Current Article |
| 11±1 | 50 mM 2-DG | Liver Hepatoma (HepG2) | *H. sapiens* | Mass Spectrometry | Current Article |

## SI References


1. Park, J.O., Rubin, S.A., Xu, Y.F., Amador-Noguez, D., Fan, J., Shlomi, T., and Rabinowitz, J.D. (2016). Metabolite concentrations, fluxes and free energies imply efficient enzyme usage. Nat Chem Biol *12*, 482-489. 10.1038/nchembio.2077.
2. Maskow, T., and von Stockar, U. (2005). How reliable are thermodynamic feasibility statements of biochemical pathways? Biotechnol Bioeng *92*, 223-230. 10.1002/bit.20572.
3. Tornheim, K., and Lowenstein, J.M. (1976). Control of phosphofructokinase from rat skeletal muscle. Effects of fructose diphosphate, AMP, ATP, and citrate. J Biol Chem *251*, 7322-7328.
4. Van Schaftingen, E., Jett, M.F., Hue, L., and Hers, H.G. (1981). Control of liver 6-phosphofructokinase by fructose 2,6-bisphosphate and other effectors. Proc Natl Acad Sci U S A *78*, 3483-3486. 10.1073/pnas.78.6.3483.
5. Boscá, L., and Corredor, C. (1984). Is phosphofructokinase the rate-limiting step of glycolysis? Trends in Biochemical Sciences *9*, 372-373. https://doi.org/10.1016/0968-0004(84)90214-7.
6. Albe, K.R., Butler, M.H., and Wright, B.E. (1990). Cellular concentrations of enzymes and their substrates. Journal of Theoretical Biology *143*, 163-195. https://doi.org/10.1016/S0022-5193(05)80266-8.
7. Marín-Hernández, A., Rodríguez-Enríquez, S., Vital-González, P.A., Flores-Rodríguez, F.L., Macías-Silva, M., Sosa-Garrocho, M., and Moreno-Sánchez, R. (2006). Determining and understanding the control of glycolysis in fast-growth tumor cells. Flux control by an over-expressed but strongly product-inhibited hexokinase. Febs j *273*, 1975-1988. 10.1111/j.1742-4658.2006.05214.x.