**Supplementary Materials**

**Fluid shear stress induces a shift from glycolytic to amino acid pathway in human trophoblasts**

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**Methods**

Calculation of flow rates for dyne/cm2

𝜏 = 4 ∗ µ ∗ 𝑄 / (𝑟 ∗ 𝑝𝑖 ∗ h 2 ∗ 𝑡𝑢𝑏𝑒 𝑟𝑎𝑑𝑖𝑢𝑠)

𝜏 = dyne/cm²

µ = viscosity of the medium

Q = flow rate in ml/min

r = inner radius of the flow chamber

h = height of the chamber

**Tables**

**Supplementary Table 1**

Differential gene expression between static vs 3 ml/min is provided online in a separate excel sheet (Supplemental Table 1\_DGA)

Supplementary Table 2

*Baseline characteristics of CTRL and IUGR cases (Cohort Graz, used for protein- and qPCR analysis)*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  | *CTRL* | *IUGR* |
| *n* |  |  | 5 | 5 |
| *Birthmode* | Prim.CS | n(%) | 80% | 100% |
| *Fetal sex* | m | n(%) | 40 | 40 |
|  | f | n(%) | 60 | 60 |
| *Fetal* | gestational age | days mean(SD) | 254,2 (9,26) | 249 (13,8) |
|  | placental weight | g mean(SD) | 468 (64,65) | 420 (72,93) |
|  | fetal weight | g mean(SD) | 2607,6 (294) | 1983 (549,63) |
|  | fetal length | cm mean(SD) | 48,36 (1,89) | 46,4 (2,07) |
|  | weight percentile | <3. Perc. (%) | 0 | 80 |
| *Doppler data* | A. umbilicalis | PI mean (SD) | 0.934 | 1,132 (0,37) |
|  | A. uterina left | PI mean (SD) | - | 1,038 (0,64) |
|  | A. uterina right | PI mean (SD) | - | 1,25 (0,67) |
| *Maternal* | BMI pregnancy | kg/m2mean(SD) | 28,24 (4,21) | 28.29 (4.61) |
|  | weight pregnancy | kg mean(SD) | 62,6 (5,77) | 64.4 (13.45) |
|  | weight at delivery | kg mean(SD) | 78,13 (8,25) | 74.02 (13.13) |

Supplementary Table 3

*Baseline characteristics of CTRL and IUGR cases (Cohort Munich, used for immunofluorescence)*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  | CTRL | IUGR |
| n |  |  | 18 | 20 |
| Birthmode | Prim.CS | n(%) | 83,33 | 80 |
| Fetal sex | m | n(%) | 44,44 | 55 |
|  | f | n(%) | 55,56 | 45 |
| Fetal | gestational age | days mean(SD) | 252,67 (25,66) | 234,7 (41,17) |
|  | placental weight | g mean(SD) | 417,50 (84,46) | 289,3 (99,16) |
|  | fetal weight | g mean(SD) | 2785,61 (718,63) | 1694,3 (1013,44) |
|  | fetal length | cm mean(SD) |  |  |
|  | weight percentile | mean (SD) |  |  |
| Doppler data | A. umbilicalis | PI mean (SD) |  |  |
|  | A. uterina left | PI mean (SD) |  |  |
|  | A. uterina right | PI mean (SD) |  |  |
| Maternal | BMI pregnancy | kg/m2mean(SD) |  |  |
|  | weight pregnancy | kg mean(SD) |  |  |
|  | weight at delivery | kg mean(SD) |  |  |

Supplementary Table 4

*Baseline characteristics of CTRL and IUGR cases (Cohort Berlin, used for qPCR)*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  | CTRL | IUGR |
| n |  |  | 9 | 4 |
| Birthmode | CS | n(%) | 88,89 | 50 |
| Fetal sex | m | n(%) | 66,67 | 50 |
|  | f | n(%) | 33,33 | 50 |
| Fetal | gestational age | days mean(SD) | 265,56 (14,50) | 272,25 (8,42) |
|  | placental weight | g mean(SD) | 648,33 (253,63) | 405 (73,26) |
|  | fetal weight | g mean(SD) | 3292,78 (701,55) | 2485 (237,42) |
|  | fetal length | cm mean(SD) | 50,33 (5,19) | 47,75 (0,96) |
|  | weight percentile | <3. Perc. (%) | 0 | 100 |
| Doppler data | A. umbilicalis | PI mean (SD) | 0,86 (0,14) | 1,27 (0,15) |
|  | A. uterina left | PI mean (SD) | - | - |
|  | A. uterina right | PI mean (SD) | - | - |
| Maternal | BMI pregnancy | kg/m2mean(SD) | 27,93 (5,51) | 31,94 (8,31) |
|  | weight pregnancy | kg mean(SD) | 63,62 (12,68) | 72,55 (9,51) |
|  | weight at delivery | kg mean(SD) | 77,44 (14,05) | 84,08 (13,75) |

**Supplementary Table 5**

Primer sequences

|  |  |  |
| --- | --- | --- |
| Primer | Sequence (5’ -> 3’) | Product length (bp) |
| *SLC2A1* forward | TGTCTTCTATTACTCCACGAGCA |  |
| *SLC2A1* reverse | CTCCACCACAAACAGCGAC | 121 |
| *HK2* forward | CATCTGCTTGCCTACTTCTTCAC |  |
| *HK2* reverse | ACTCTCCGTGTTCTGTCCCA | 99 |
| *PFKP* forward | CCCTTCGACATCAGGGATCTG |  |
| *PFKP* reverse | GCAGCTCTCATTTCTGAGCAC | 226 |
| *PGK1* forward | GGTCCTGAAAGCAGCAAGAAGT |  |
| *PGK1* reverse | GGCAGTGTCTCCACCACCTAT | 99 |
| *PDHX* forward | TGCTGACTCTGTAAAGGCTCT |  |
| *PDHX* reverse | TCGATGCCAAACATCCCCAA | 183 |
| *GOT1* forward | CTCAAGGAGAAGCGGGTAGGAG |  |
| *GOT1* reverse | AGCAGCGGAAAACACAGCATT | 168 |
| *GOT2* forward | CTTGAAGAGTGGCCGGTTTG |  |
| *GOT2* reverse | GGCAGAAAGACATCTCGGCT |  |
| *SLC7A8* forward | AGGCTGGAACTTTCTGAATTACG |  |
| *SLC7A8* reverse | ACATAAGCGACATTGGCAAAGA | 126 |
| *ACTB* forward | AAAGACCTGTACGCCAACAC |  |
| *ACTB* reverse | GTCATACTCCTGCTTGCTGAT | 219 |
| *TBP* forward | TGA CCC AGC ATC ACT GTT TC |  |
| *TBP* reverse | CCA GCA CAC TCT TCT CAG CA | 76 |
| *B2M* forward | GAT GAG TAT GCC TGC CGT GT |  |
| *B2M* reverse | TGT CTC GAT CCC ACT TAA CTA TCT | 70 |
| *HPRT1* forward | GAA AGG GTG TTT ATT CCT CAT |  |
| *HPRT1* reverse | CCA GCA GGT CAG CAA AGA ATT | 142 |

**Supplementary Table 6**

Antibodies used for immunoblotting

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Antibody | Company | Clon | Species | Dilution | Incubation time | Temp. |  |
| GLUT1 | Abcam | EPR3915 | mAb Rabbit | 1:10.000 | 1h | RT |  |
| GOT1 | Thermo Fisher | GT1255 | mAb Mouse | 1:10.000 | o/n | 4°C |  |
| GOT2 | Merck | 3E9 | mAb Mouse | 1:2.000 | o/n | 4°C |  |
| Vincullin | Invitrogen |  | pAb Rabbit | 1:1.000 | o/n | 4°C |  |
| Cyclophilin B | Cell Signaling | D1V5J | mAb Rabbit | 1:2.000 | o/n | 4°C |  |
| β-HCG | Thermo Fisher |  | mAB Rabbit | 1:50 | o/n | 4°C |  |

**Supplementary Table 7**

Primary antibodies for immunofluorescence staining

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Antibody | Company | Clon | Species | Dilution | AG retrival\* |
| GLUT1 | Abcam | EPR3915 | mAb Rabbit | 1:500 | pH9 |
| GOT1 | Thermo Fisher | GT1255 | mAB Mouse | 1:500 | pH9 |
| GOT2 | Merck | 3E9 | mAB Mouse | 1:500 | pH9 |
| CK7 | Acris | RCK105 | mAb Rabbit | 1:500 | pH9 |
| CK7 | DB Biotech | RCK105 | mAb Mouse | 1:500 | pH9 |
| cleaved Caspase 3 | Abcam | 5A1E | mAB Rabbit | 1:200 | pH9 |
| E-Cadherine | Abcam | 4A2 | mAB Mouse | 1:200 | pH9 |

\*Antigenretrival

**Supplementary Table 8**

Primers for in situ hybridization

|  |  |  |  |
| --- | --- | --- | --- |
| Primers | Sequences (5´ - 3´) | Primers | Sequences (5´ - 3´) |
| ACTB\_LNA | C+GG+GC+GG+CG+GATCGGCAAAG | **RV\_GOT2\_19** | GGTTGTCTCTGTTTCCT | |
| RV\_GOT1\_1 | TTGAAGACCAGGACAG | **RV\_GOT2\_20** | AGAGGCTGAAGACAGAA | |
| RV\_GOT1\_2 | GAAGTCGGCAGTGAG | **RV\_GOT2\_21** | TCTGTGTGAAGCTCT | |
| RV\_GOT1\_3 | GGCAAAACCCAGGGA | **RV\_GOT2\_22** | CCCAACTGGAGAAAC | |
| RV\_GOT1\_4 | CTCCACTTTCTTCACTAC | **RV\_GOT2\_23** | AACCGGGCAGAGACAA | |
| RV\_GOT1\_5 | GGTGTGTTCTTGTTGTTTGT | **RV\_GOT2\_24** | CAACAAAGGGAGGAGG | |
| RV\_GOT1\_6 | GGTGAGGACACATAGA | **RV\_GOT2\_25** | AGTGGGTGAAGCCTGAA | |
| RV\_GOT1\_7 | TCCAATCCTCTCTTCTC | **RV\_GOT2\_26** | AGATGGTGGTTCTTT | |
| RV\_GOT1\_8 | ATCATTCAGGAAGCCC |
| RV\_GOT1\_9 | GGTGCTTCATGACAG |
| RV\_GOT1\_10 | AAGGGGAACAGAAAC |
| RV\_GOT1\_11 | CACAGAAGAACTCGAA |
| RV\_GOT1\_12 | TTGGAGAAGGACTGG |
| RV\_GOT1\_13 | GTAGAGCCCGAAGTT |
| RV\_GOT1\_14 | CTCAGGTTCTTTTCCA |
| RV\_GOT1\_15 | AAAGGACTTGCAGGA |
| RV\_GOT1\_16 | GGGTTAGAGAGGGTG |
| RV\_GOT1\_17 | GTCCATTCCTCAAAGA |
| RV\_GOT1\_18 | TTTGATCAGTGATGTGG |
| RV\_GOT1\_19 | TGAAGCTGAACATGCC |
| RV\_GOT1\_20 | AAATTCGTCTCAAGGG |
| RV\_GOT1\_21 | CTCTAATCCCAGTCTC |
| RV\_GOT1\_22 | ATTTGCTTTGACCTCC |
| RV\_GOT2\_1 | GAATGGGATCTGGAGG |
| RV\_GOT2\_2 | AAAGGCTTCAGTGAC |
| RV\_GOT2\_3 | GCTATTGGTGTCCCTC |
| RV\_GOT2\_4 | CAGGTTGGTTTGGGC |
| RV\_GOT2\_5 | TGGGTGTGTGGTTTC |
| RV\_GOT2\_6 | GCCAGCATCCCTGAA |
| RV\_GOT2\_7 | AAATATCCTCCACAGC |
| RV\_GOT2\_8 | CACTCTGCTCTGGTATTTTT |
| RV\_GOT2\_9 | CAGGCATGCAGAAGAAG |
| RV\_GOT2\_10 | TTCCTTTTCTTCACCAC |
| RV\_GOT2\_11 | CAAAGAACGCAAAGAG |
| RV\_GOT2\_12 | CCTACACGCTCACCA |
| RV\_GOT2\_13 | TGCAGACCATAGTGAAG |
| RV\_GOT2\_14 | GCCATGACTTTCACT |
| RV\_GOT2\_15 | TGCCAATGATGCGGT |
| RV\_GOT2\_16 | TGAAACAGAACATGCC |
| RV\_GOT2\_17 | CGCTCCACCTGTTCA |
| RV\_GOT2\_18 | CGGCCATCTTTTGTCATGT |

+: the following base is LNA (locked nucleic acid) modified

Supplementary Table 9

*Padlock probes for in situ hybridization*

|  |  |
| --- | --- |
| Padlock probes | Sequences (5´ - 3´) |
| plp\_GOT1\_1 | TTCCGCAGGCCCAGC**CCTCAATGCTGCTGCTGTACTACTGCGTCTATTTAGTGGAGCC**TAGAGCATCTGACGCTACAGTCTTTGCCGAGG |
| plp\_GOT1\_2 | GGATGACTGCCATCC**CCTCAATGCTGCTGCTGTACTACTGCGTCTATTTAGTGGAGCC**TAGAGCATCTGACGCTAGGGAGCATATCGCAC |
| plp\_GOT1\_3 | CGTTGGTACAATGGA**CCTCAATGCTGCTGCTGTACTACTGCGTCTATTTAGTGGAGCC**TAGAGCATCTGACGCTAGCTGATTTCTTAGCG |
| plp\_GOT1\_4 | TACTGGGATGCAGAG**CCTCAATGCTGCTGCTGTACTACTGCGTCTATTTAGTGGAGCC**TAGAGCATCTGACGCTAATTCGGTCCTATCGC |
| plp\_GOT1\_5 | GGAGCAGTGGAAGCA**CCTCAATGCTGCTGCTGTACTACTGCGTCTATTTAGTGGAGCC**TAGAGCATCTGACGCTAGATTGACCCAACTCC |
| plp\_GOT1\_6 | TTGTGTCTGAAGGCT**CCTCAATGCTGCTGCTGTACTACTGCGTCTATTTAGTGGAGCC**TAGAGCATCTGACGCTAGGGCCATTCGCTATT |
| plp\_GOT1\_7 | TCTGACTGTGGTTGG**CCTCAATGCTGCTGCTGTACTACTGCGTCTATTTAGTGGAGCC**TAGAGCATCTGACGCTATGAGAGAGTCGGGAA |
| plp\_GOT1\_8 | ATTGTGGCCAGCACC**CCTCAATGCTGCTGCTGTACTACTGCGTCTATTTAGTGGAGCC**TAGAGCATCTGACGCTAGCCCAGGGAGCACGA |
| plp\_GOT1\_9 | CCTGGGACCTGGAAC**CCTCAATGCTGCTGCTGTACTACTGCGTCTATTTAGTGGAGCC**TAGAGCATCTGACGCTAGAAGCCCTCAAAACC |
| plp\_GOT1\_10 | CGGCCCCACATGAAG**CCTCAATGCTGCTGCTGTACTACTGCGTCTATTTAGTGGAGCC**TAGAGCATCTGACGCTAAGCCTCTGTTTAAAC |
| plp\_GOT2\_1 | ATGTGGAAATGGGAC**CAGTGAATGCGAGTCCGTCTTGCGTCTATTTAGTGGAGCC**TTGATATAGCGTATTCGCGGCTCCTGGTGGACCC |
| plp\_GOT2\_2 | TGTCTTTCTGCCCAA**CAGTGAATGCGAGTCCGTCTTGCGTCTATTTAGTGGAGCC**TTGATATAGCGTATTCGCGTAAGTTCAGCCGAGA |
| plp\_GOT2\_3 | GACTTCACAGGCGCT**CAGTGAATGCGAGTCCGTCTTGCGTCTATTTAGTGGAGCC**TTGATATAGCGTATTCGCGAAGACTTGCGGTTTT |
| plp\_GOT2\_4 | GTGGAAGGAAATAGC**CAGTGAATGCGAGTCCGTCTTGCGTCTATTTAGTGGAGCC**TTGATATAGCGTATTCGCGCCCGCGTCCGGAACA |
| plp\_GOT2\_5 | GCCAAGAACATGGGC**CAGTGAATGCGAGTCCGTCTTGCGTCTATTTAGTGGAGCC**TTGATATAGCGTATTCGCGCTCTGCCAATCATAT |
| plp\_GOT2\_6 | ACAATGGCTGCAAGA**CAGTGAATGCGAGTCCGTCTTGCGTCTATTTAGTGGAGCC**TTGATATAGCGTATTCGCGCCCAGATTTGCGAAA |
| plp\_GOT2\_7 | GACCAAATTGGCATG**CAGTGAATGCGAGTCCGTCTTGCGTCTATTTAGTGGAGCC**TTGATATAGCGTATTCGCGTGGCAACACATCACC |
| plp\_GOT2\_8 | TCCCTGGTGCGAGGA**CAGTGAATGCGAGTCCGTCTTGCGTCTATTTAGTGGAGCC**TTGATATAGCGTATTCGCGGGTCACCAAGTAATG |
| plp\_GOT2\_9 | GAATGATAGTGCAAG**CAGTGAATGCGAGTCCGTCTTGCGTCTATTTAGTGGAGCC**TTGATATAGCGTATTCGCGTCTCGGCATCCTCTT |
| plp\_GOT2\_10 | TTTGCGTGCTGCTAG**CAGTGAATGCGAGTCCGTCTTGCGTCTATTTAGTGGAGCC**TTGATATAGCGTATTCGCGGAGGATATTCTAGGC |
| plp\_ACTB | AGCCTCGCCTTTGCC**TCTACGAGTTTGCAGTCACGTGCGTCTATTTAGTGGAGCC**GGTTGCTACGATGACTCACGCCCCGCGAGCACAG |

Padlock probes were 5’-phosphorylated. underlined: target complement sequence

**Supplementary Table 10**

*Detection Oligos in situ hybridization*

|  |  |
| --- | --- |
| Detection probes | Sequences (5´ - 3´) |
| D1\_Atto425 | Atto425-CAGTGAATGCGAGTCCGTCT |
| D2\_Atto488 | Atto488-TCTACGAGTTTGCAGTCACG |
| D3\_TexasRed | TexasRed-CCTCAATGCTGCTGCTGTACTAC |
| D4\_CY7 | CY7-TGCGTCTATTTAGTGGAGCC |

The detection probes were 5´conjugated with fluorophores (Atto425, Atto488, TexasRed, CY7 are fluorescent labels)

**Supplementary Figures**

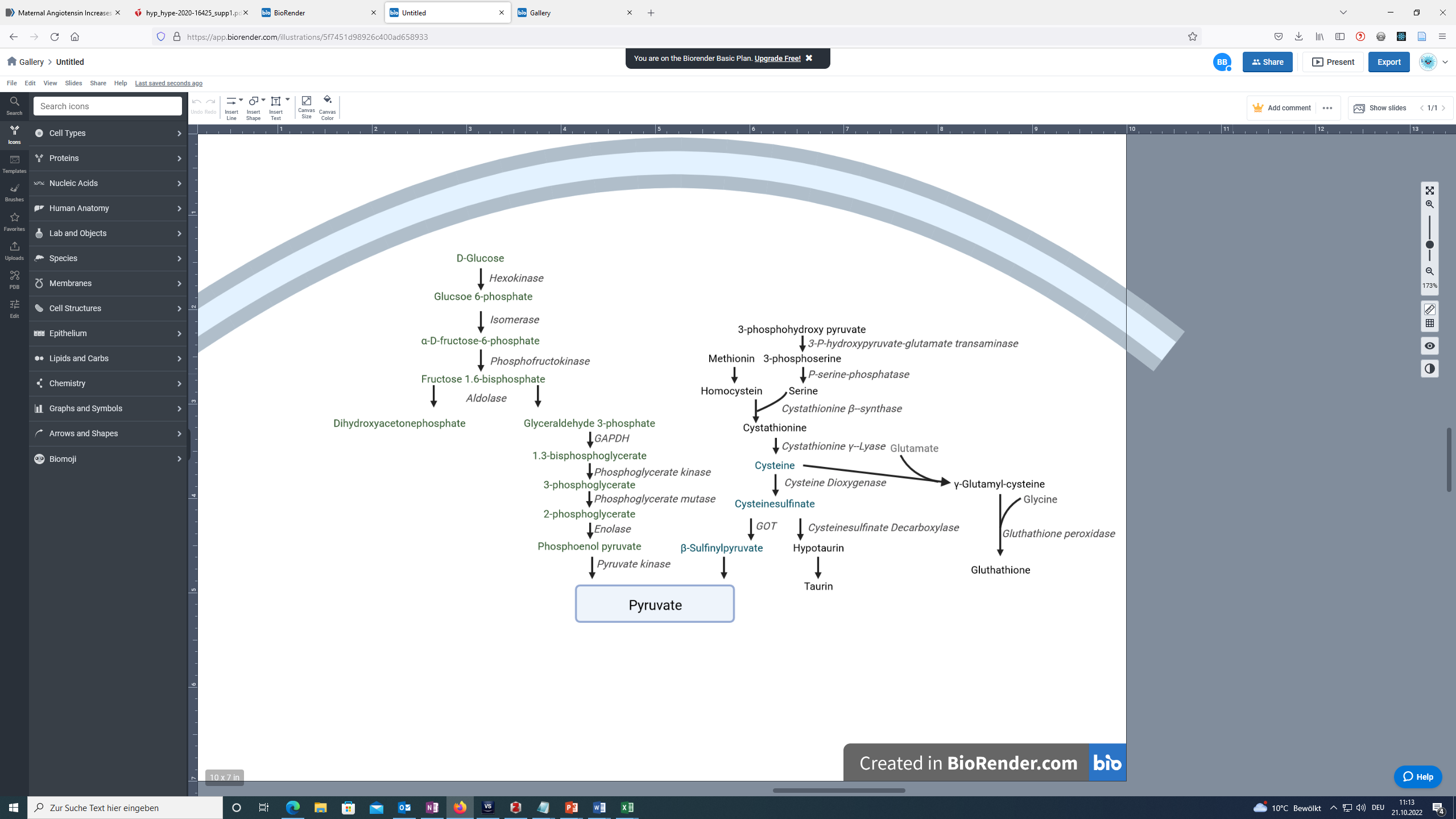
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**Supplementary Figure 1**

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**Supplementary Figure 2**



**Supplementary Figure 3**

**Supplementary Figure 4**



**Supplementary Figure 5**

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**Supplementary Figure 6**

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**Supplementary Figure 7**

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**Supplementary Figure 8**

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