

Supplementary material to:

**EFFECT OF GLUCOSE AND INSULIN SUPPLEMENTATION ON
THE ISOLATION OF PRIMARY HUMAN HEPATOCYTES**

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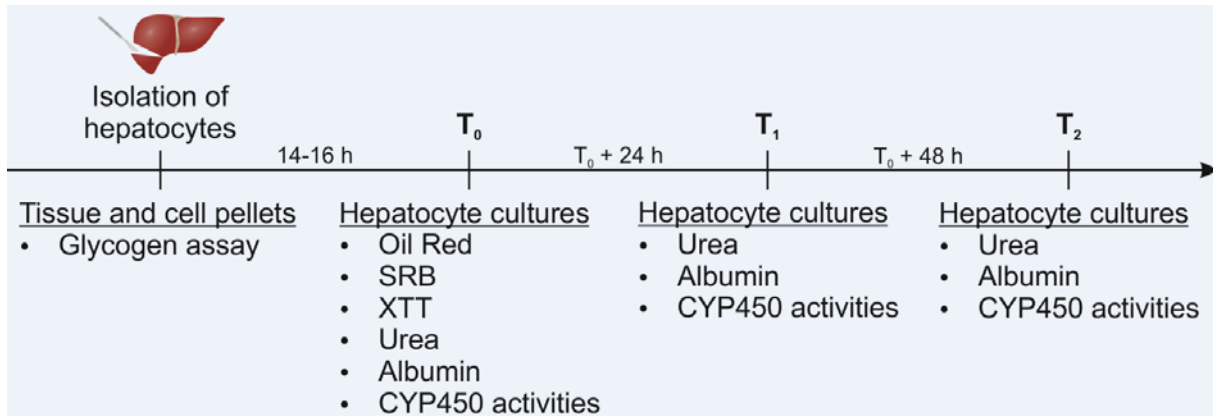
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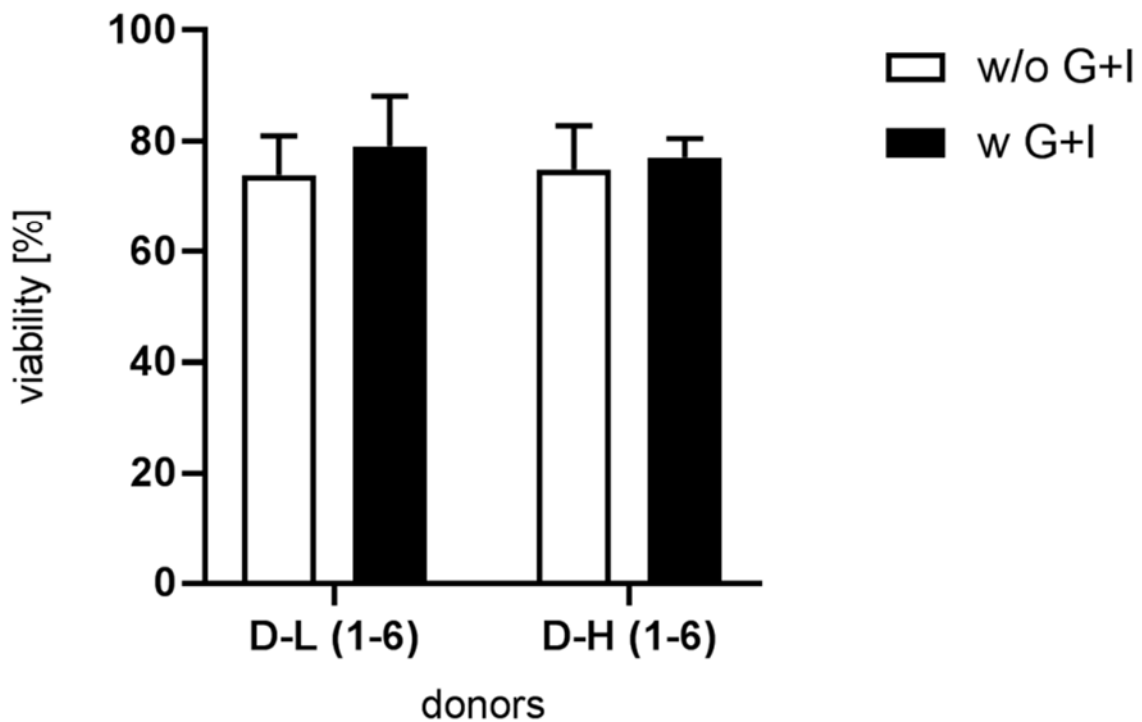
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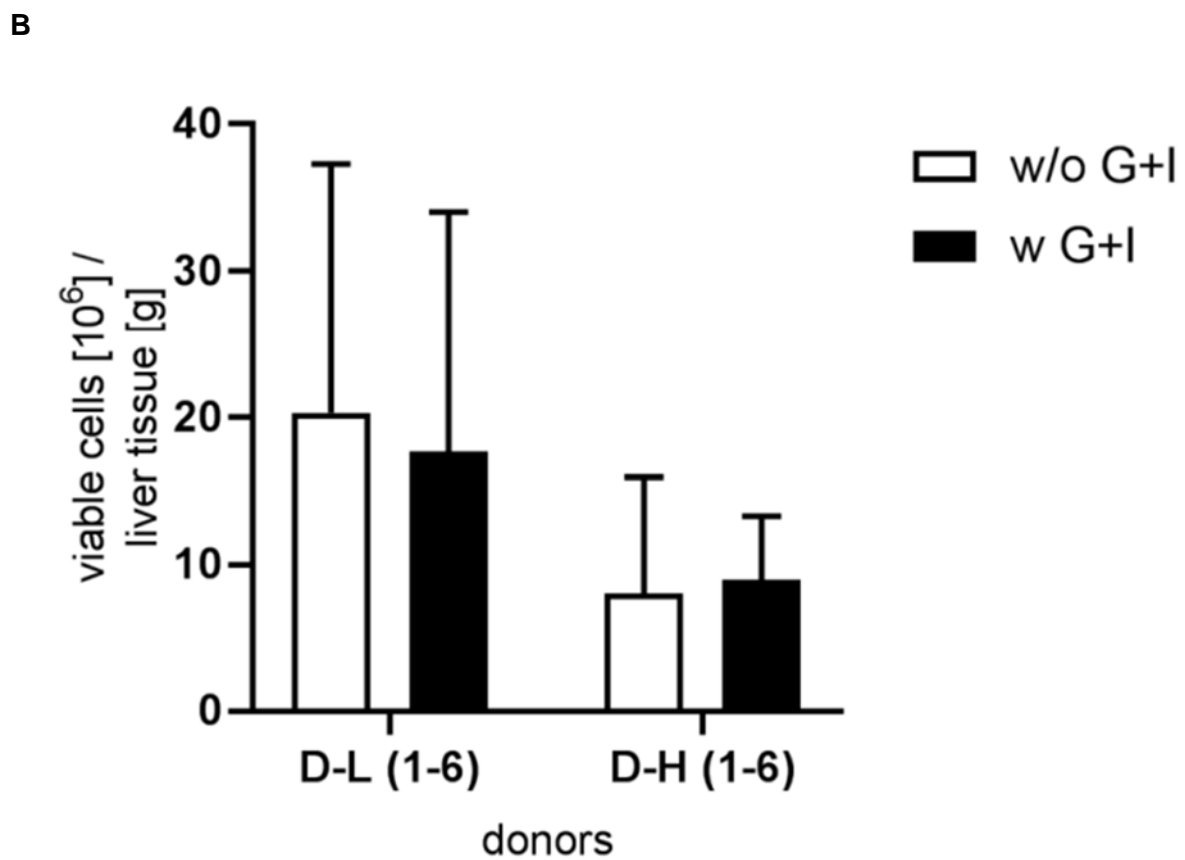
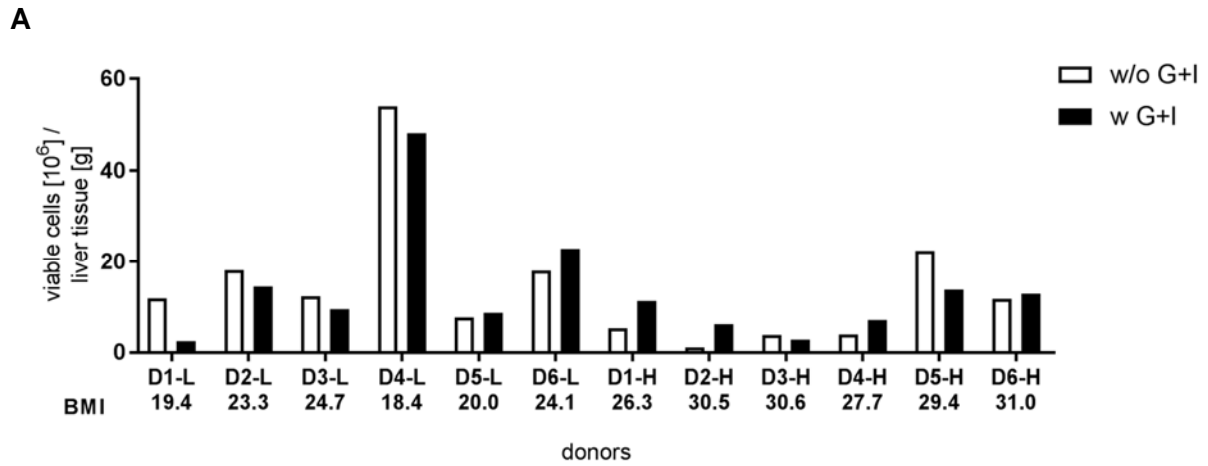
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Supplementary Figure 1: Timeline of performed experiments. Tissue and freshly isolated PHH were used for glycogen assay. The cultured PHH were used for indicated assays after designated cultivation terms.

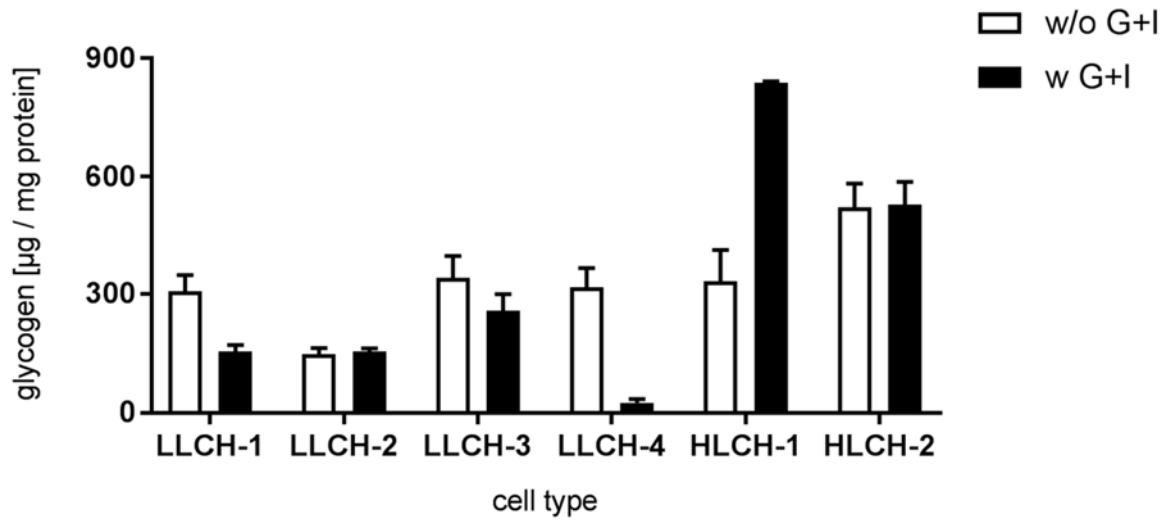


Supplementary Figure 2: Viability of freshly isolated hepatocytes. Supplementation of perfusion solution I with glucose and insulin seems to have no effect on viability of PHHs, directly measured after cell isolation. Data are plotted as mean \pm standard deviation (N=6).

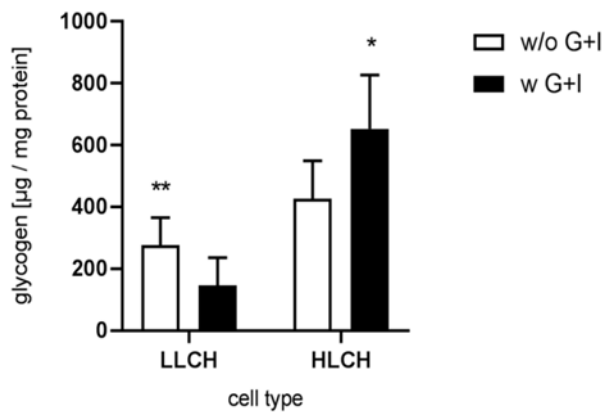


Supplementary Figure 3: Cell yield of freshly isolated hepatocytes. Supplementation of perfusion solution I with glucose and insulin seems to have no effect on cell yield of PHH, directly measured after cell isolation (**A**). There was no statistically significant interaction between BMI and supplementation of perfusion solution I (Two-way ANOVA, $F(1, 20) = 0,1197$, $p = 0,7330$). Data are presented as mean \pm SD, plotted as replicates ($N=6$) (**B**).

A



B



Supplementary Figure 4: Glycogen content of hepatocytes. Supplementation of perfusion solution I with glucose and insulin seems to have no effect on glycogen content of PHH. Glycogen content was directly measured after cell isolation. Data are normalized on protein content measured via BCA. There was a significant main effect for interaction (Two-way ANOVA, $F(1, 31) = 19,96$, $p < 0,0001$) and BMI (Two-way ANOVA, $F(1, 31) = 67,68$, $p < 0,0001$). Data are presented as mean \pm SD, plotted as technical replicates ($n = 3$) (A). Data are presented as mean \pm SD, plotted as LLCH-group ($N = 4$, $n = 12$) and HLCH-group ($N = 2$, $n = 6$) (B). Single groups were analyzed by unpaired T-Test with Welch's correction (* $p = 0,0447$, ** $p = 0,0016$).

Supplementary Table 1: Summary of all donor data of liver tissues used for this study, including liver tissues used for glycogen analysis only and liver tissue used for PHH isolation. Donor data include sex, age, medical history and body mass index (BMI). Samples were grouped according to BMI as low (L) with BMI < 25 or high (H) with BMI ≥ 25.

| Donor | Sex | Age | Disease | Secondary diagnosis | BMI |
|-------|--------|-----|---------------------------------|--|------|
| D1-L | male | 28 | echinococcosis | slight portal fibrosis, nicotine consumption | 19.4 |
| D2-L | female | 77 | colorectal liver metastasis | diabetes mellitus, steatosis (< 5 %), slight portal fibrosis, necrosis | 23.3 |
| D3-L | male | 44 | echinococcosis | none | 24.7 |
| D4-L | female | 46 | bile duct cyst | diabetes mellitus | 18.4 |
| D5-L | female | 79 | intrahepatic cholangiocarcinoma | none | 20.0 |
| D6-L | female | 74 | intrahepatic cholangiocarcinoma | none | 24.1 |
| D7-L | female | 36 | adenoma | none | 23.0 |
| D8-L | female | 27 | unclear liver tumor | slight portal fibrosis | 23.8 |
| D9-L | female | 42 | hemangioma | steatosis (25 %) | 24.4 |
| D10-L | male | 49 | liver metastasis | slight steatosis | 24.8 |
| D1-H | female | 73 | colorectal liver metastasis | steatosis (25 %), slight portal fibrosis, necrosis | 26.3 |
| D2-H | female | 44 | adenoma | arterial hypertension | 30.5 |
| D3-H | male | 55 | hemangioma | non-alcoholic fatty liver disease (15-45 %), slight portal fibrosis | 30.6 |
| D4-H | male | 60 | Klatskin tumor | nicotine and alcohol consumption | 27.7 |
| D5-H | male | 80 | hepatocellular carcinoma | diabetes mellitus type 2, steatosis (10 %), slight fibrosis | 29.4 |
| D6-H | male | 77 | hepatocellular carcinoma | diabetes mellitus type 2, steatosis, nicotine consumption | 31.0 |
| D7-H | male | 72 | liver metastasis | steatosis (40 %), slight fibrosis | 25.0 |
| D8-H | female | 38 | Klatskin tumor | biliary fibrosis | 26.0 |
| D9-H | male | 76 | hepatocellular carcinoma | steatosis | 29.0 |
| D10-H | male | 54 | colorectal liver metastasis | steatosis (40 %) | 29.5 |

Supplementary Table 2: List of substrates, concentrations, detected fluorescent metabolites and catalyzing enzymes for (a) phase I assays and (b) phase II assays.

(a) substrates phase I assays

| Substrate | | Concentration | Fluorescent metabolite | | CYP enzyme |
|-----------|--|---------------|------------------------|---------------------------------------|--------------------------|
| BFC | 7-Butyloxy-4-(trifluoromethyl)coumarin | 100 μ M | HFC | 7-Hydroxy-4-(trifluoromethyl)coumarin | 1A2, 3A4, 2B6, 2C19, 2C9 |
| EFC | 7-Ethoxy-4-(trifluoromethyl)coumarin | 40 μ M | HFC | 7-Hydroxy-4-(trifluoromethyl)coumarin | 2B6, 2E1, 2C19 |
| MFC | 7-Methoxy-4-(trifluoromethyl)coumarin | 40 μ M | HFC | 7-Hydroxy-4-(trifluoromethyl)coumarin | 2E1, 2C9 |
| 7-EC | 7-Ethoxycoumarin | 50 μ M | UMB | Umbelliferone | 1A2, 2E1 |
| C | Coumarin | 100 μ M | UMB | Umbelliferone | 2A6 |
| 7-ER | 7-Ethoxyresorufin | 12.5 μ M | RESO | Resorufin | 1A2, 1A1 |
| DBF | Dibenzylfluorescein | 20 μ M | FLUO | Fluorescein | 2C9, 2C8 |

(b) substrates phase II assays

| Substrate | | Concentration |
|-----------|---------------------------------------|---------------|
| HFC | 7-Hydroxy-4-(trifluoromethyl)coumarin | 25 μ M |
| UMB | Umbelliferone | 20 μ M |
| RESO | Resorufin | 10 μ M |
| MCB | Monochlorobimane | 80 μ M |
| 4MU | 4-Methylumbelliferone | 100 μ M |

Supplementary Table 3: Determination of (a) phase I and (b) phase II enzyme activity of donors with low (L) BMI. Enzyme activity of PHH was determined after initial adherence (T_0) and additional 24 h (T_1) and 48 h (T_2). Data are presented as means \pm average absolute deviation in nMol/mg protein/h.

(a) phase I enzyme activity

| Substrate | Time | D4-L | | D5-L | | D6-L | |
|-----------|-------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| | | w/o G+I | w G+I | w/o G+I | w G+I | w/o G+I | w G+I |
| BFC | T_0 | 4.868 \pm 0.416 | 4.137 \pm 0.388 | 1.608 \pm 0.078 | 2.639 \pm 0.078 | 1.229 \pm 0.055 | 1.089 \pm 0.035 |
| | T_1 | 3.211 \pm 0.467 | 5.128 \pm 1.048 | 0.233 \pm 0.004 | 0.746 \pm 0.004 | 0 \pm 0 | 0 \pm 0 |
| | T_2 | 2.566 \pm 0.092 | 3.460 \pm 0.698 | 0.008 \pm 0.008 | 1.262 \pm 0.311 | 0 \pm 0 | 0.015 \pm 0.015 |
| EFC | T_0 | 6.219 \pm 0.960 | 6.052 \pm 0.180 | 1.216 \pm 0.138 | 1.019 \pm 0.015 | 0.402 \pm 0.324 | 0.703 \pm 0.008 |
| | T_1 | 1.743 \pm 0.067 | 2.345 \pm 0.168 | 0.208 \pm 0.007 | 0.629 \pm 0.045 | 0.093 \pm 0.011 | 0.105 \pm 0.057 |
| | T_2 | 1.247 \pm 0.067 | 1.053 \pm 0.090 | 0.291 \pm 0.137 | 1.123 \pm 0.161 | 0.084 \pm 0.020 | 0.220 \pm 0.003 |
| MFC | T_0 | | | 1.843 \pm 0.143 | 1.486 \pm 0.052 | 1.017 \pm 0.169 | 1.180 \pm 0.078 |
| | T_1 | | | 0.281 \pm 0.090 | 0.804 \pm 0.070 | 0.439 \pm 0.005 | 0.590 \pm 0.003 |
| | T_2 | | | 0.156 \pm 0.128 | 0.813 \pm 0.001 | 0.099 \pm 0.006 | 0.337 \pm 0.014 |
| 7-EC | T_0 | 1.739 \pm 0.080 | 1.279 \pm 0.101 | 0.195 \pm 0.097 | 0.288 \pm 0.144 | 0.346 \pm 0.009 | 0.330 \pm 0.165 |
| | T_1 | 0.454 \pm 0.200 | 0.702 \pm 0.193 | 0.260 \pm 0.000 | 0.352 \pm 0.003 | 0.332 \pm 0.166 | 0.682 \pm 0.341 |
| | T_2 | 0.382 \pm 0.086 | 0.218 \pm 0.190 | 0 \pm 0 | 0.339 \pm 0.041 | 0.485 \pm 0.078 | 0.199 \pm 0.038 |
| C | T_0 | 5.789 \pm 0.605 | 5.996 \pm 0.465 | 1.332 \pm 0.126 | 1.790 \pm 0.182 | 0.597 \pm 0.035 | 1.185 \pm 0.170 |
| | T_1 | 1.192 \pm 0.307 | 3.777 \pm 0.418 | 0.270 \pm 0.046 | 1.180 \pm 0.325 | 0.118 \pm 0.001 | 0.315 \pm 0.046 |
| | T_2 | 2.246 \pm 0.342 | 1.996 \pm 0.091 | 0.129 \pm 0.010 | 0.380 \pm 0.074 | 0.023 \pm 0.023 | 0.031 \pm 0.018 |
| 7-ER | T_0 | 0.515 \pm 0.009 | 0.438 \pm 0.012 | 0 \pm 0 | 0.061 \pm 0.000 | 0.202 \pm 0.003 | 0.137 \pm 0.005 |
| | T_1 | 0.235 \pm 0.074 | 0.695 \pm 0.187 | 0.053 \pm 0.005 | 0.090 \pm 0.006 | 0.119 \pm 0.035 | 0.260 \pm 0.004 |
| | T_2 | 0.418 \pm 0.027 | 0.365 \pm 0.019 | 0.209 \pm 0.007 | 0.136 \pm 0.014 | 0.058 \pm 0.000 | 0.163 \pm 0.008 |
| DBF | T_0 | 8.193 \pm 0.504 | 8.074 \pm 0.747 | 3.378 \pm 1.082 | 2.342 \pm 0.198 | 0.784 \pm 0.008 | 1.086 \pm 0.102 |
| | T_1 | 4.617 \pm 1.006 | 6.378 \pm 0.293 | 0.577 \pm 0.048 | 1.309 \pm 0.140 | 0.381 \pm 0.027 | 0.490 \pm 0.062 |
| | T_2 | 5.017 \pm 0.609 | 5.366 \pm 0.642 | 0.270 \pm 0.090 | 1.213 \pm 0.357 | 0.021 \pm 0.001 | 0.141 \pm 0.035 |

(b) phase II enzyme activity

| Substrate | Time | D4-L | | D5-L | | D6-L | |
|-----------|----------------|-----------------|------------------|-----------------|-----------------|----------------|------------------|
| | | w/o G+I | w G+I | w/o G+I | w G+I | w/o G+I | w G+I |
| HFC | T ₀ | 56.019 ± 0.930 | 51.052 ± 5.632 | 33.932 ± 4.962 | 34.169 ± 5.721 | 24.821 ± 1.575 | 23.549 ± 2.303 |
| | T ₁ | 28.917 ± 1.116 | 39.305 ± 1.651 | 3.88 ± 0.018 | 41.121 ± 11.119 | 21.387 ± 0.059 | 25.983 ± 1.745 |
| | T ₂ | 28.582 ± 0.928 | 27.152 ± 1.363 | 5.617 ± 0.406 | 36.984 ± 7.660 | 4.736 ± 0.313 | 16.757 ± 0.480 |
| UMB | T ₀ | 32.339 ± 2.077 | 27.527 ± 1.449 | 18.291 ± 3.775 | 23.103 ± 4.731 | 31.751 ± 2.024 | 31.194 ± 1.737 |
| | T ₁ | 19.708 ± 3.324 | 30.979 ± 4.129 | 9.063 ± 3.114 | 24.658 ± 7.260 | 16.608 ± 1.288 | 23.877 ± 2.863 |
| | T ₂ | 26.022 ± 1.073 | 20.051 ± 0.712 | 15.818 ± 0.953 | 35.967 ± 0.313 | 8.952 ± 1.499 | 19.736 ± 0.582 |
| RESO | T ₀ | 6.314 ± 0.148 | 6.767 ± 0.285 | 2.396 ± 0.699 | 3.387 ± 0.060 | 2.945 ± 0.331 | 4.010 ± 0.247 |
| | T ₁ | 5.362 ± 0.738 | 7.745 ± 0.392 | 2.847 ± 0.268 | 4.718 ± 0.482 | 4.257 ± 0.629 | 4.312 ± 0.351 |
| | T ₂ | 7.038 ± 0.873 | 6.928 ± 0.264 | 0.469 ± 1.211 | 6.344 ± 0.456 | 1.115 ± 0.329 | 2.521 ± 0.007 |
| MCB | T ₀ | 23.241 ± 3.928 | 26.325 ± 1.319 | 7.668 ± 0.917 | 6.325 ± 0.072 | 6.025 ± 0.007 | 5.619 ± 0.731 |
| | T ₁ | 30.458 ± 4.285 | 51.857 ± 3.130 | 1.127 ± 0.252 | 4.219 ± 0.039 | 4.110 ± 0.510 | 4.79 ± 0.212 |
| | T ₂ | 32.759 ± 1.547 | 33.44 ± 3.129 | 1.566 ± 0.618 | 17.376 ± 0.093 | 0.001 ± 0.001 | 12.092 ± 0.056 |
| 4MU | T ₀ | 37.382 ± 7.091 | 35.698 ± 1.516 | 55.590 ± 11.842 | 97.411 ± 6.606 | 47.041 ± 0.406 | 111.313 ± 12.220 |
| | T ₁ | 104.69 ± 8.264 | 230.128 ± 24.386 | 0 ± 0 | 69.985 ± 15.523 | 0 ± 0 | 46.983 ± 27.485 |
| | T ₂ | 177.751 ± 8.354 | 170.956 ± 7.366 | 0 ± 0 | 33.845 ± 14.911 | 22.44 ± 8.914 | 50.491 ± 5.281 |

Supplementary Table 4: Determination of (a) phase I and (b) phase II enzyme activity of donors with high (H) BMI. Enzyme activity of PHH was determined after initial adherence (T₀) and additional 24 h (T₁) and 48 h (T₂). Data are presented as means ± average absolute deviation in nMol/mg protein/h.

(a) phase I enzyme activity

| Substrate | Time | D4-H | | D5-H | | D6-H | |
|-----------|----------------|----------------|----------------|---------------|---------------|---------------|---------------|
| | | w/o G+I | w G+I | w/o G+I | w G+I | w/o G+I | w G+I |
| BFC | T ₀ | 1.280 ± 0.090 | 1.179 ± 0.144 | 1.044 ± 0.040 | 1.030 ± 0.042 | 5.408 ± 0.563 | 4.753 ± 0.688 |
| | T ₁ | 0.733 ± 0.018 | 0 ± 0 | 0.611 ± 0.007 | 0.505 ± 0.010 | 2.974 ± 0.089 | 3.004 ± 0.000 |
| | T ₂ | 0.958 ± 0.002 | 0.625 ± 0.218 | 0.460 ± 0.051 | 0.483 ± 0.079 | 1.510 ± 0.180 | 1.984 ± 0.105 |
| EFC | T ₀ | 1.327 ± 0.416 | 1.627 ± 0.207 | 0.778 ± 0.039 | 0.924 ± 0.009 | 2.537 ± 0.009 | 1.612 ± 0.074 |
| | T ₁ | 0.876 ± 0.006 | 0.519 ± 0.013 | 0.629 ± 0.004 | 0.557 ± 0.096 | 1.491 ± 0.017 | 1.043 ± 0.034 |
| | T ₂ | 0.526 ± 0.059 | 0.420 ± 0.146 | 0.391 ± 0.037 | 0.635 ± 0.019 | 0.766 ± 0.040 | 0.878 ± 0.016 |
| MFC | T ₀ | 2.338 ± 0.110 | 1.655 ± 0.060 | 1.366 ± 0.006 | 1.371 ± 0.004 | 5.875 ± 0.403 | 4.063 ± 0.242 |
| | T ₁ | 1.032 ± 0.218 | 0.693 ± 0.160 | 0.863 ± 0.033 | 0.598 ± 0.066 | 3.162 ± 0.158 | 2.500 ± 0.048 |
| | T ₂ | 0.638 ± 0.001 | 0 ± 0 | 0.806 ± 0.013 | 0.826 ± 0.040 | 1.710 ± 0.087 | 1.837 ± 0.109 |
| 7-EC | T ₀ | 1.363 ± 0.028 | 0 ± 0 | 0.741 ± 0.076 | 0.342 ± 0.075 | 0.022 ± 0.022 | 0.004 ± 0.004 |
| | T ₁ | 0.828 ± 0.032 | 0.337 ± 0.337 | 0.406 ± 0.123 | 0.344 ± 0.027 | 0.098 ± 0.036 | 0.192 ± 0.012 |
| | T ₂ | 0.256 ± 0.128 | 0.015 ± 0.546 | 0.249 ± 0.249 | 0.618 ± 0.062 | 0.087 ± 0.006 | 0.095 ± 0.010 |
| C | T ₀ | 14.738 ± 0.634 | 10.965 ± 0.904 | 2.935 ± 0.051 | 3.049 ± 0.027 | 3.244 ± 0.102 | 2.105 ± 0.025 |
| | T ₁ | 6.223 ± 0.587 | 0.868 ± 0.057 | 0.844 ± 0.031 | 0.860 ± 0.017 | 1.271 ± 0.079 | 1.036 ± 0.033 |
| | T ₂ | 2.610 ± 0.210 | 0.244 ± 0.065 | 0.566 ± 0.066 | 0.994 ± 0.069 | 0.519 ± 0.022 | 0.557 ± 0.179 |
| 7-ER | T ₀ | 0.201 ± 0.015 | 0.248 ± 0.034 | 0.111 ± 0.006 | 0.122 ± 0.001 | 0.899 ± 0.021 | 0.675 ± 0.012 |
| | T ₁ | 0.110 ± 0.009 | 0.090 ± 0.010 | 0.212 ± 0.017 | 0.125 ± 0.006 | 0.350 ± 0.011 | 0.268 ± 0.008 |
| | T ₂ | 0.183 ± 0.013 | 0.466 ± 0.020 | 0.402 ± 0.003 | 0.213 ± 0.022 | 0.123 ± 0.006 | 0.113 ± 0.013 |
| DBF | T ₀ | 0.658 ± 0.132 | 0.334 ± 0.105 | 0.515 ± 0.012 | 0.498 ± 0.025 | 0.913 ± 0.090 | 0.805 ± 0.066 |
| | T ₁ | 0.327 ± 0.035 | 0.097 ± 0.025 | 0.298 ± 0.019 | 0.275 ± 0.004 | 0.548 ± 0.029 | 0.429 ± 0.045 |
| | T ₂ | 0.610 ± 0.032 | 0.720 ± 0.014 | 0.177 ± 0.006 | 0.161 ± 0.000 | 0.351 ± 0.007 | 0.354 ± 0.003 |

(b) phase II enzyme activity

| Substrate | Time | D4-H | | D5-H | | D6-H | |
|-----------|----------------|-----------------|-----------------|-----------------|-----------------|----------------|----------------|
| | | w/o G+I | w G+I | w/o G+I | w G+I | w/o G+I | w G+I |
| HFC | T ₀ | 35.212 ± 0.524 | 23.768 ± 0.309 | 22.628 ± 0.014 | 15.986 ± 0.450 | 22.210 ± 0.593 | 24.839 ± 1.044 |
| | T ₁ | 27.029 ± 0.674 | 26.422 ± 3.414 | 25.221 ± 1.104 | 12.527 ± 0.132 | 20.269 ± 0.414 | 24.214 ± 1.339 |
| | T ₂ | 19.534 ± 0.359 | 21.247 ± 2.049 | 27.557 ± 0.960 | 16.803 ± 1.557 | 13.959 ± 0.143 | 19.787 ± 0.021 |
| UMB | T ₀ | 26.612 ± 2.020 | 40.934 ± 5.624 | 22.717 ± 0.241 | 15.584 ± 0.155 | 19.059 ± 0.378 | 20.496 ± 0.952 |
| | T ₁ | 22.641 ± 0.162 | 21.744 ± 0.726 | 21.766 ± 0.070 | 14.303 ± 0.020 | 16.883 ± 0.839 | 19.079 ± 0.496 |
| | T ₂ | 19.427 ± 0.304 | 36.163 ± 4.999 | 25.437 ± 0.776 | 11.201 ± 0.171 | 27.202 ± 0.759 | 30.262 ± 0.084 |
| RESO | T ₀ | 4.082 ± 0.677 | 5.741 ± 1.433 | 6.348 ± 0.334 | 5.996 ± 0.549 | 3.652 ± 0.446 | 1.685 ± 0.046 |
| | T ₁ | 3.901 ± 0.129 | 0.718 ± 0.274 | 6.345 ± 0.102 | 4.122 ± 0.274 | 5.545 ± 0.135 | 2.449 ± 0.251 |
| | T ₂ | 5.179 ± 0.019 | 10.358 ± 1.746 | 4.858 ± 0.031 | 4.631 ± 0.507 | 3.632 ± 0.113 | 3.179 ± 0.139 |
| MCB | T ₀ | 11.229 ± 0.560 | 6.340 ± 1.529 | 0.780 ± 0.157 | 1.016 ± 0.105 | 2.144 ± 0.168 | 1.747 ± 0.127 |
| | T ₁ | 2.828 ± 0.252 | 1.779 ± 0.205 | 3.541 ± 0.287 | 1.353 ± 0.177 | 1.554 ± 0.358 | 1.016 ± 0.047 |
| | T ₂ | 2.080 ± 0.137 | 3.109 ± 0.451 | 2.789 ± 0.491 | 2.532 ± 0.131 | 2.836 ± 0.026 | 4.076 ± 0.350 |
| 4MU | T ₀ | 52.787 ± 5.512 | 21.920 ± 21.92 | 21.488 ± 3.707 | 33.705 ± 0.180 | 53.682 ± 6.488 | 52.762 ± 1.375 |
| | T ₁ | 74.535 ± 14.348 | 23.250 ± 41.224 | 65.632 ± 7.488 | 61.335 ± 1.150 | 44.759 ± 2.329 | 49.265 ± 6.165 |
| | T ₂ | 103.601 ± 0.753 | 0 ± 0 | 121.177 ± 2.058 | 81.467 ± 11.288 | 55.456 ± 4.064 | 65.258 ± 3.422 |