Supplementary files

Temperature down-shift modifies expression of UPR-ERAD-related genes and enhances production of a chimeric fusion protein in CHO cells

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| Symbol | Gene name                  | Accession no.* | Primer   | Sequence (5’-3’)                      |
|--------|----------------------------|----------------|----------|--------------------------------------|
| gapdh  | Glyceraldehyde-3-phosphate dehydrogenase | NM_001244854.2 | Forward  | GCCAAGAGGGTCATCATCTCT                |
|        |                            |                | Reverse  | CTTTCCAACATGGCCAAAGTT                |
| hEPO-Fc|                            |                | Forward  | AGTGCTGAGAGACAGGCTC                 |
|        |                            |                | Reverse  | CAGGAAGTGAGATACACCCG                |
| Ern1/IRE1 | ER to nucleus signaling 1  | XM_027436202.1 | Forward  | AAGGCTGCCAGTTGCTCAC                  |
|        |                            |                | Reverse  | ACCGGAAGACCTACAGCTA                 |
| Atf6   | Activating transcription factor 6 | XM_003506878.4 | Forward  | TGCTCATGGGTTTCCAACA                 |
|        |                            |                | Reverse  | TCATGCTCTGACCCACACAC                |
| Eif2ak3/PE | Eukaryotic translation initiation factor 2 alpha | XM_027437608.1 | Forward  | ACCCTCACTTTTTGCCAGGA                |
| RK     | initiation factor 2 alpha   |                | Reverse  | GCAGATGGTGCTAAGTGCCTA               |
| Xbp1s  | X-box binding protein 1     | NM_001244049.1 | Forward  | CTCGCTTGGGAATGGATGTG                |
|        |                            |                | Reverse  | GTAGACCTCTGCGGAGTCC                |
| Grp78/BiP | Heat shock protein family A (Hsp70) member 5 | NM_001246739.2 | Forward  | TCAGCCAATTATACACAACTC                |
|        |                            |                | Reverse  | ACAGCTTCTGACGATCTAGT                |
| Grp94  | Heat shock protein 90 beta  | XM_003505850.4 | Forward  | AGGAAAACCGGGAAGCAACA               |
|        | family member 1             |                | Reverse  | GCCCCGTGGTGATGCTTGTG               |
| Ero1α  | ER oxidoreductase 1 alpha   | XM_007651327.3 | Forward  | CGCTGCTTCTGTCAGTGTGT               |
|        |                            |                | Reverse  | GCCTTCTGGTTTCTCACT                  |
| Trib3  | Tribbles pseudokinase 3     | XM_007652053.3 | Forward  | TCTCCCTTCCAGACTACGC                |
|        |                            |                | Reverse  | AACCATACAGCCCAACTCG                |
| Ddit3/CHO | DNA damage inducible transcript 3 | XM_007648093.3 | Forward  | CACCATACCTGAAAGCAGAA               |
| P      |                            |                | Reverse  | ACCTCCTGAGATCCTCATA                |
| Atf5   | Activating transcription factor 5 | XM_003510884.3 | Forward  | TCCTAGTCTGCCCATACCA               |
|        |                            |                | Reverse  | CAGGTTGCCCCCTTACAT                 |
| Atf4   | Activating transcription factor 4 | NM_001246812.1 | Forward  | CAGGTTGCCCCCTTACAT                 |
|        |                            |                | Reverse  | CAGGTTGCCCCCTTACAT                 |
| Edem3  | ER degradation enhancing alpha-mannosidase like protein 3 | XM_007641575.3 | Forward  | CACCTCAAAATGGGCAGT                 |
|        |                            |                | Reverse  | AGATCCAGCCCACACTG                 |
| Sels/VIMP | Selenoprotein S              | NM_001256848.1 | Forward  | CCAGCTACGGCTGCTACATC               |
|        |                            |                | Reverse  | GCCTGCTGCTACAGCTAC                 |
| Syvn1  | Synoviolin 1                | XM_016963602.2 | Forward  | GTCTGCGCAACATACACACA               |
|        |                            |                | Reverse  | CGGAGGCTTTCAGCTTCA                |
*Transcript accession number according to CHO-K1 genome assembly released in 2018 (Rupp et al. 2018).

**Figure S1.** Relationship between ΔCt values for the recombinant hEPO-Fc gene expression and cell specific productivity \( (q_{hEPO-Fc}) \) in batch (circles) and fed-batch (squares) cultures at 37°C (black) and 32°C (grey). The ΔCt values correspond to the difference of Ct values of hEPO-Fc and gapdh (housekeeping gene). High abundance of the recombinant gene results in low Ct values for hEPO-Fc and therefore, low ΔCt values indicate higher expression of the hEPO-Fc. Each ΔCt and qP values represent a biological replicate for batch and fed-batch cultures, both at 37°C and 32°C.
Table S2. Statistical analysis of the mRNA expression levels of UPR gene targets

|                  | BC32 vs BC37 | FB32 vs FB37 |
|------------------|--------------|--------------|
|                  | t-test       | Adjusted p-value | t-test       | Adjusted p-value |
| em1/IRE1         |              |                |              |                |
| D3               | 2.452        | 0.071         | 0.996        | 0.365          |
| D6               | 0.974        | 0.385         | 0.61         | 0.948          |
| D9               | n.r.         | n.r.          | 1.067        | 0.34           |
| atf6             |              |                |              |                |
| D3               | 0.361        | 0.811         | 0.115        | 0.913          |
| D6               | 0.804        | 0.715         | 1.16         | 0.672          |
| D9               | n.r.         | n.r.          | 0.112        | 0.936          |
| eil2ak/PERK      |              |                |              |                |
| D3               | 1.363        | 0.214         | 1.013        | 0.356          |
| D6               | 0.134        | 0.989         | 1.071        | 0.374          |
| D9               | n.r.         | n.r.          | 0.976        | 0.246          |
| xbp1             |              |                |              |                |
| D3               | 0.770        | 0.484         | 0.513        | 0.634          |
| D6               | 6.418        | **0.003**     | 6.048        | **0.004**      |
| D9               | n.r.         | n.r.          | 3.317        | **0.029**      |
| grp78/BIP        |              |                |              |                |
| D3               | 2.887        | **0.044**     | 2.190        | 0.093          |
| D6               | 3.564        | **0.024**     | 5.171        | **0.006**      |
| D9               | n.r.         | n.r.          | 3.623        | **0.022**      |
| grp94            |              |                |              |                |
| D3               | 0.034        | 0.974         | 1.759        | 0.153          |
| D6               | 2.341        | 0.079         | 5.081        | **0.007**      |
| D9               | n.r.         | n.r.          | 3.799        | **0.019**      |
| ero1α            |              |                |              |                |
| D3               | 1.224        | 0.288         | 1.061        | 0.348          |
| D6               | 3.442        | **0.026**     | 3.111        | **0.035**      |
| D9               | n.r.         | n.r.          | 1.914        | 0.128          |
| trib3            |              |                |              |                |
| D3               | 0.311        | 0.771         | 0.237        | 0.824          |
| D6               | 3.201        | **0.032**     | 7.452        | **0.001**      |
| D9               | n.r.         | n.r.          | 3.594        | **0.022**      |
| ddit3/CHOP       |              |                |              |                |
| D3               | 0.038        | 0.971         | 1.651        | 0.174          |
| D6               | 5.310        | **0.006**     | 6.466        | **0.003**      |
| D9               | n.r.         | n.r.          | 3.124        | **0.035**      |
| atf5             |              |                |              |                |
| D3               | 1.665        | 0.171         | 2.802        | **0.048**      |
| D6               | 2.788        | **0.049**     | 1.877        | 0.133          |
| D9               | n.r.         | n.r.          | 3.409        | **0.027**      |
| Atf4             |              |                |              |                |
| D3               | 10.370       | **0.000**     | 0.016        | 0.988          |
|   | D6  | D9  | D3  | D6  | D9  | D3  | D6  | D9  |
|---|-----|-----|-----|-----|-----|-----|-----|-----|
| edem3 | 0.459 | 0.699 | 0.288 | 0.787 | n.r. | n.r. | 1.089 | 0.337 |
| sels  | 3.704 | 0.021 | 2.363 | 0.077 | 14.311 | 0.000 | 2.403 | 0.074 |
| herpud1 | 3.645 | 0.022 | 1.342 | 0.251 | 183.4 | 0.000 | 2885 | 0.000 |
| syvn1  | 2.931 | 0.042 | 0.038 | 0.971 | 666.1 | 0.000 | 0.134 | 0.899 |
|       | 61.21 | 0.000 | 1.784 | 0.149 | 9.553 | 0.000 | 1.431 | 0.251 |

n.r.: non-reported

Highlighted values represent values significantly different.

Figure S2. Ct values of the housekeeping genes *gapdh* and *β-actin* of the CHO-hEPO-Fc cell line in batch (BC) and fed-batch (FB) cultures, both at 37°C and 32°C. This figure illustrates that the mRNA expression of the internal standard (*gapdh*) gene was similar among all culture conditions.
Figure 3S. Relationship between the mRNA expression of UPR-related targets and hEPO-Fc production (left) and cell specific productivity (right) in low temperature cultures. *Xbp1s* (black), *grp78* (red), *grp94* (blue), *ero1a* (white), *trib3* (dark grey), *ddit3* (light grey). The mRNA expression of upregulated gene targets (i.e., *xbp1s*, *ddit3*, *att5*, *grp78*, *grp94*, *ero1a* and *trib3*) were plotted against the relative hEPO-Fc production (i.e., titre at 32°C vs titre at 37°C) and the relative cell specific productivity (i.e., $q_{\text{EPO-FC}}$ at 32°C vs $q_{\text{EPO-FC}}$ at 37°C) for day 3, 6 and 9 (only for FB cultures). Pearson’s correlations were calculated considering each gene target and the relative titer or $q_{\text{EPO-FC}}$ corresponding to the specific time point and culture mode.
Figure 4S. PNGase treatment to hEPO-Fc. Western blot indicated medium sample from day 6 of batch cultures at 37°C (Lane 1), purified hEPO-Fc using protein A agarose beads (Lane 2) and PNGase treated hEPO-Fc (Lane 3). PNGase treatment was performed by incubating overnight 5 units of enzyme at 37°C.