**Supplementary analysis of paired E64-treated and untreated *P. falciparum* 3D7 schizonts.**

**Methods**

In order to compare the effect of E64 on parasite transcriptomes, schizont-stage parasites were incubated with 10 µM E64 (or PBS control) for 5.5 hours in the presence of uninfected erythrocytes. Schizonts were purified as in the main materials and methods, with the exception that untreated cultures were prepared in three times the volume of E64-treated cultures, to ensure that untreated cultures contained sufficient remaining schizont-stage material for RNA-seq, following parasite egress over the course of the treatment. Following the treatment, schizonts were percoll purified and RNA was extracted and sequenced in accordance with main materials and methods.

**Analysis summary**

Log2 FPKM values for samples and replicates were all strongly positively correlated by both Spearman and Pearson correlations (Correlations Summary 1) with significant p-values (Correlations Summary 2)

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| Correlations Summary 1Spearman and Pearson correlations of E64-treated and untreated replicate. |
|  | **Rep1-E64** | **Rep1-Untreated** | **Rep2-E64** | **Rep2-Untreated** | **Rep3-E64** | **Rep3-Untreated** | **Rep4-E64** | **Rep4-Untreated** |
| Rep1-E64 |  | 1 | 0.952381 | 0.97619 | 0.952381 | 0.928571 | 0.904762 | 0.97619 | **Spearman** |
| Rep1-Untreated | 0.997363 |  | 0.952381 | 0.97619 | 0.952381 | 0.928571 | 0.904762 | 0.97619 |
| Rep2-E64 | 0.824789 | 0.811063 |  | 0.97619 | 0.952381 | 0.857143 | 0.880952 | 0.928571 |
| Rep2-Untreated | 0.900262 | 0.891954 | 0.979414 |  | 0.97619 | 0.904762 | 0.928571 | 0.952381 |
| Rep3-E64 | 0.991939 | 0.989546 | 0.86838 | 0.932429 |  | 0.952381 | 0.97619 | 0.97619 |
| Rep3-Untreated | 0.990177 | 0.995798 | 0.784867 | 0.875363 | 0.984077 |  | 0.97619 | 0.97619 |
| Rep4-E64 | 0.911197 | 0.903819 | 0.908583 | 0.942909 | 0.950347 | 0.908216 |  | 0.952381 |
| Rep4-Untreated | 0.912057 | 0.910578 | 0.940235 | 0.974183 | 0.947738 | 0.911399 | 0.983432 |  |
|  | **Pearson** |  |

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| Correlations Summary 2Spearman and Pearson p-values for correlations in Table 1. |
|  | **Rep1-E64** | **Rep1-Untreated** | **Rep2-E64** | **Rep2-Untreated** | **Rep3-E64** | **Rep3-Untreated** | **Rep4-E64** | **Rep4-Untreated** |
| Rep1-E64 |  | 0.00E+00 | 2.60E-04 | 3.31E-05 | 0.00E+00 | 6.79E-04 | 8.63E-04 | 0.00E+00 | **Spearman** |
| Rep1-Untreated | 1.62E-07 |  | 2.60E-04 | 3.31E-05 | 0.00E+00 | 6.79E-04 | 8.63E-04 | 0.00E+00 |
| Rep2-E64 | 2.49E-04 | 5.41E-04 |  | 3.31E-05 | 2.60E-04 | 1.38E-02 | 1.49E-02 | 2.60E-04 |
| Rep2-Untreated | 2.95E-05 | 6.41E-05 | 3.83E-06 |  | 3.31E-05 | 7.47E-03 | 6.53E-03 | 3.31E-05 |
| Rep3-E64 | 9.21E-07 | 4.15E-06 | 1.12E-04 | 2.09E-05 |  | 6.79E-04 | 8.63E-04 | 0.00E+00 |
| Rep3-Untreated | 6.50E-06 | 5.82E-07 | 1.17E-03 | 1.70E-04 | 1.24E-05 |  | 6.55E-05 | 6.79E-04 |
| Rep4-E64 | 3.33E-05 | 6.53E-05 | 5.42E-04 | 1.71E-04 | 1.98E-06 | 2.89E-05 |  | 0.00E+00 |
| Rep4-Untreated | 1.55E-05 | 1.46E-05 | 1.43E-04 | 1.42E-05 | 2.85E-06 | 5.18E-06 | 6.06E-06 |  |
|  | **Pearson** |  |

Differential gene expression analysis was conducted using DEseq2 with the design formula design = ~ Treatment + Replicate.

Only a single gene, PF3D7\_0220700, was differentially expressed by a fold change greater than ±log2 2 and an adjusted p-value < 0.01. This sub-telomeric gene encodes an exported protein of unknown function from the ‘hyp9 family.