

## Supplementary Information

### Cytidine deaminase deficiency in tumor cells is associated with sensitivity to a naphthol derivative and a decrease in oncometabolite levels

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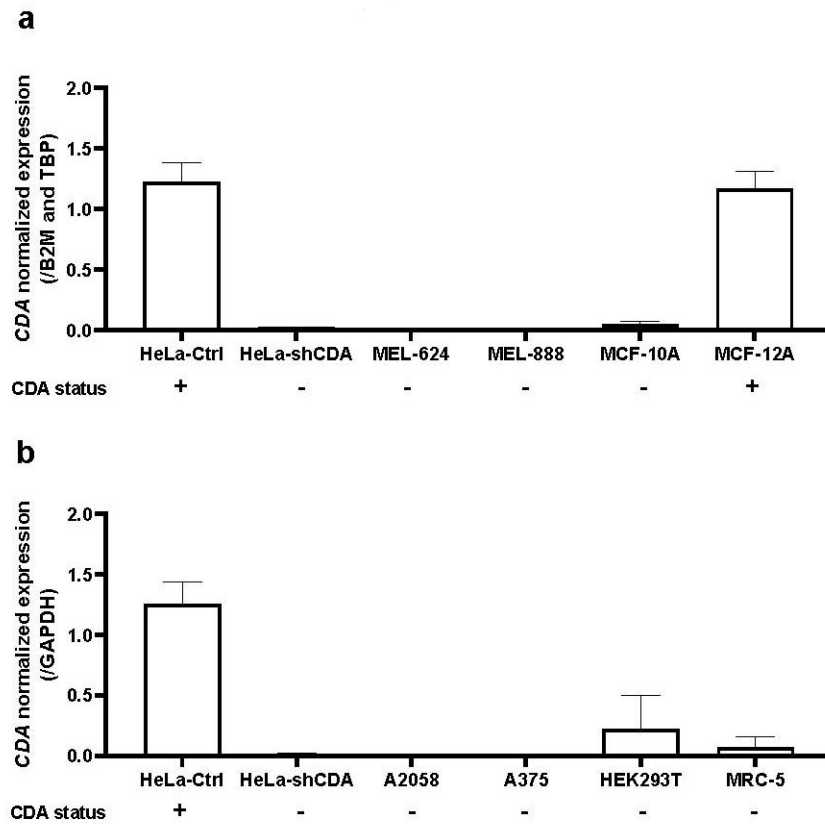
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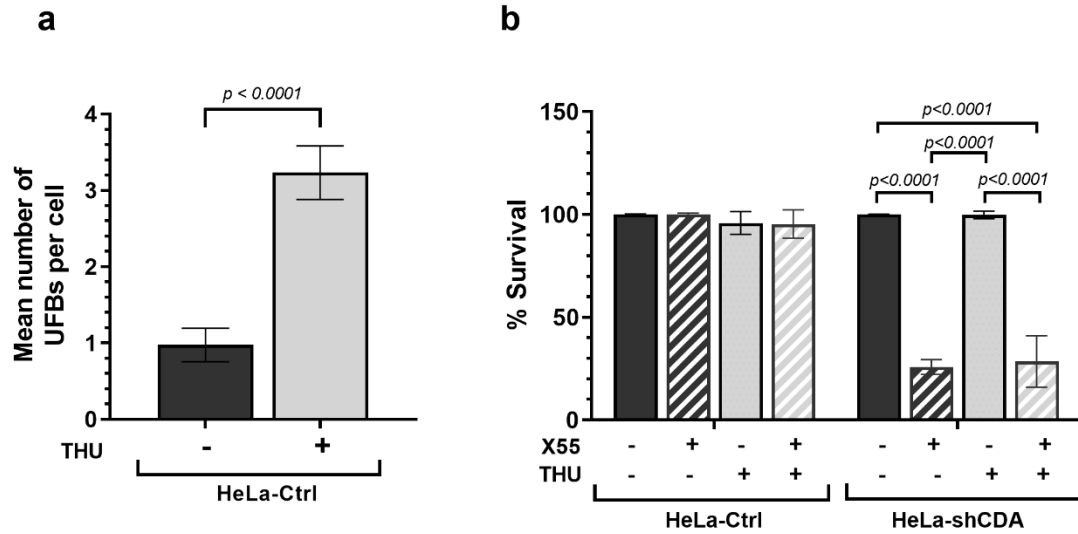
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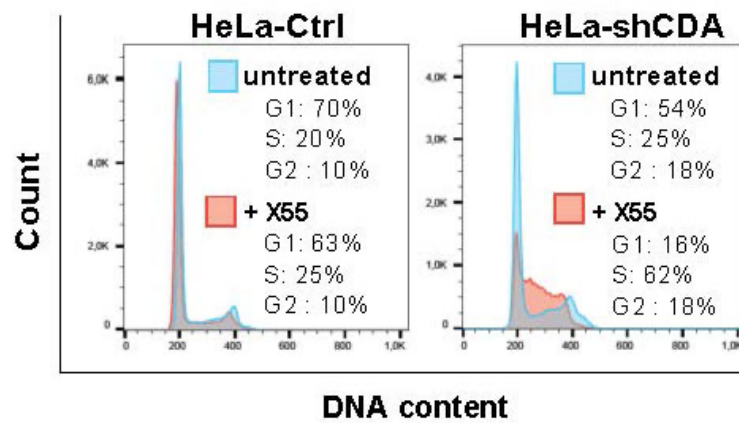
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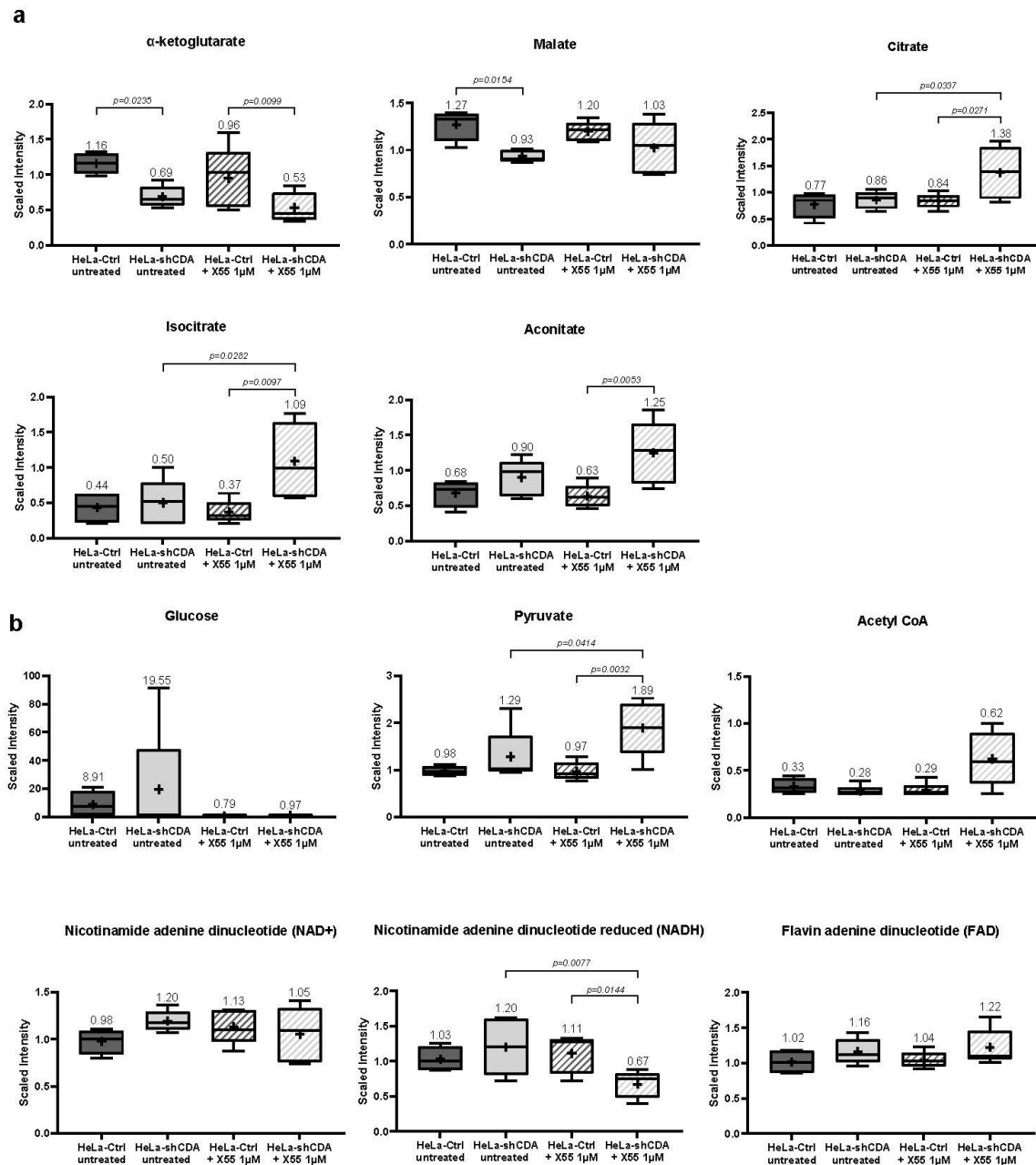
**Supplementary Figure 1.** CDA expression status of the cell lines not presented by Mameri et al. (2017) [1]. **(a)** *CDA* levels were monitored by RT-qPCR in HeLa-Ctrl and HeLa-shCDA (as controls) and in MEL-624, MEL-888, MCF10A and MCF-12A cells. *B2M* and *TBP* were used as housekeeping genes for normalization. The error bars represent means  $\pm$  SD of three independent experiments (except for MCF12A, 2 independent experiments). **(b)** *CDA* levels were monitored by RT-qPCR in HeLa-Ctrl and HeLa-shCDA (as controls) and in A2058, A375, HEK293T and MRC5 cells. *GAPDH* was used as housekeeping gene for normalization. The error bars represent means  $\pm$  SD for of three independent experiments (except for A2058 and HEK293T, 2 independent experiments).



**Supplementary Figure 2.** CDA activity is dispensable for X55 cytotoxicity in CDA-depleted HeLa cells. **a** Mean number of UFBs per cell in HeLa control cells (HeLa-Ctrl) left untreated (black bars) or treated for 96 h (2x48 h) with 100  $\mu$ M THU (gray bars). Error bars represent means  $\pm$  SD for two independent experiments. The significance of differences was assessed in Mann-Whitney tests.  $P < 0.05$  was considered statistically significant. **b** HeLa control cells (HeLa-Ctrl) and CDA-depleted HeLa cells (HeLa-shCDA) were left untreated (black bars) or were subjected to pretreatment for 96 h (2x48 h) with 100  $\mu$ M THU (gray bars). They were then left untreated or were treated with 1  $\mu$ M X55 for 24 h (hatched bars). Error bars represent means  $\pm$  SD for three independent experiments. The significance of differences was assessed by two-way ANOVA.  $P < 0.05$  was considered statistically significant.

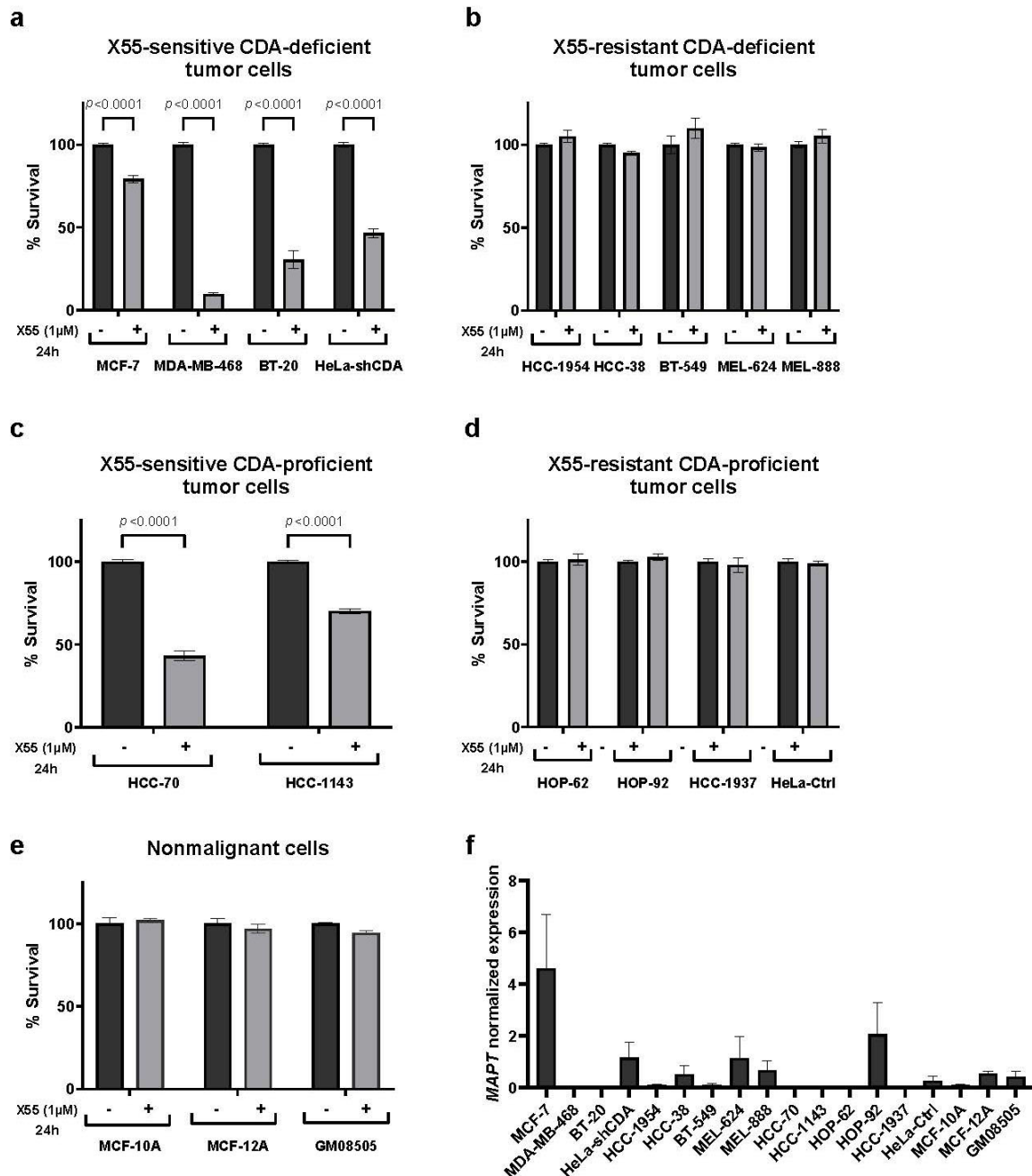


**Supplementary Figure 3.** Representative cell cycle distribution for HeLa-Ctrl cells (left panel) and CDA-depleted HeLa cells (right panel), left untreated (blue) or treated for 24 hours with 1  $\mu$ M X55 (red). The percentages of cells in the G1, S and G2 phases are indicated.



**Supplementary Figure 4.** Abundance of TCA cycle metabolites and key players of this pathway in CDA-depleted HeLa cells and in control HeLa cells. Measurement of the levels of citrate, isocitrate, aconitate,  $\alpha$ -ketoglutarate, and malate **(a)** and of glucose, pyruvate, acetyl-CoA, NAD<sup>+</sup>, NADH, and FAD **(b)** in HeLa-Ctrl cells (dark gray) and CDA-depleted HeLa cells (light gray) left untreated (block-shaded) or treated for 24 hours with 1  $\mu$ M X55 (hatched). The error bars represent means  $\pm$  SD for four or five independent experiments. The significance of

differences was assessed by two-way ANOVA.  $P < 0.05$  was considered statistically significant.



**Supplementary Figure 5.** X55 sensitivity of CDA-deficient cells and CDA-proficient cells. **a**, **b**, **c**, **d**, **e** X55-sensitive CDA-deficient cells (**a**), X55-resistant CDA-deficient cells (**b**), X55-sensitive CDA-proficient cells (**c**), X55-resistant CDA-proficient cells (**d**), nonmalignant cells (**e**), left untreated (black bars) or treated with 1 μM X55 for 24 h (gray bars) and the percentage

of cells surviving. The significance of differences was assessed by two-way ANOVA. The error bars represent means  $\pm$ SD for at least three independent experiments.  $P < 0.05$  was considered statistically significant. **f** Relative basal *MAPT* mRNA levels in all cell lines tested. *MAPT* expression levels were monitored by RT-qPCR. *B2M* and *TBP* were used as housekeeping genes for normalization. The error bars represent means  $\pm$  SD for at least two independent experiments.

**Supplementary Table 1.** Raw metabolomic data. Values are normalized in terms of raw area counts (Metabolon Inc.). The shaded column corresponds to the sample from the control HeLa group (without X55 treatment) that was considered to be an outlier and was excluded from the statistical analysis. *Provided as a separate file (.xlsx)*

**Supplementary Table 2.** List of metabolites for which levels were significantly deregulated in CDA-depleted HeLa cells relative to control HeLa cells. The significance of differences in metabolite levels was assessed by ANOVA ( $P < 0.05$ ). Downregulated metabolites are shown in green, and upregulated metabolites are shown in red.

*Provided as a separate file (.doc)*

**Supplementary Table 3.** List of the metabolites with levels significantly deregulated by X55 treatment in control HeLa cells (49) and CDA-depleted HeLa cells (270). The significance of differences in metabolite levels was assessed by ANOVA ( $P < 0.05$ ). Upregulated metabolites are shown in red, and downregulated metabolites are shown in green. The names of the 17 metabolites deregulated in both cells in response to X55 are indicated in bold.

*Provided as a separate file (.doc)*

**Supplementary Table 4.** List of 125 metabolites with levels significantly deregulated in both untreated CDA-depleted HeLa cells and in X55-treated CDA-depleted HeLa cells relative to untreated HeLa-Ctrl or HeLa-shCDA cells, respectively. The significance of differences in metabolite levels was assessed by ANOVA ( $P < 0.05$ ). The metabolites upregulated in both sets of conditions (55) are shown in red, and those downregulated in both sets of conditions (21) are shown in green. The metabolites deregulated in opposite ways (49) are shown in bold.

*Provided as a separate file (.doc)*

**Supplementary Material 1.** Growth conditions for the cell lines used in this study

|                                | Cell line  | Medium                                                                                                                                                 |
|--------------------------------|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Cervical cancer</b>         | HeLa-Ctrl  | DMEM + 1% glutamine + 1% penicillin/streptomycin + 10% FBS                                                                                             |
|                                | HeLa-shCDA |                                                                                                                                                        |
| <b>Breast cancer</b>           | MCF-7      | MEM (Eagle) +1% glutamine+ 1% penicillin/streptomycin + 10% FBS + 1.5 g/L sodium bicarbonate + 0.1 mM non-essential amino acids + 1 mM sodium pyruvate |
|                                | BT-20      |                                                                                                                                                        |
|                                | MDA-MB-468 | RPMI-1640 + 1% glutamine + 1% penicillin/streptomycin + 10% FBS                                                                                        |
|                                | ZR75-1     |                                                                                                                                                        |
|                                | T47D       |                                                                                                                                                        |
|                                | HCC-1428   |                                                                                                                                                        |
|                                | HCC-1187   | RPMI-1640 + 1% glutamine + 1% penicillin/streptomycin + 10% FBS + 1.5 g/L sodium bicarbonate + 10 mM HEPES + 1 mM sodium pyruvate                      |
|                                | HCC-38     |                                                                                                                                                        |
|                                | Hs578T     | DMEM + 1% glutamine + 1% penicillin/streptomycin + 10% FBS                                                                                             |
|                                | HCC-1954   | RPMI-1640 + 1% glutamine + 1% penicillin/streptomycin + 10% FBS                                                                                        |
|                                | BT-474     | RPMI-1640 + 1% glutamine + 1.5g/l sodium bicarbonate + 1% penicillin/streptomycin + 10% FBS                                                            |
|                                | BT-549     | RPMI-1640 + 1% glutamine + 1% penicillin/streptomycin + 10% FBS + 1.5 g/L sodium bicarbonate + 10 mM HEPES + 1 mM sodium pyruvate                      |
|                                | HCC-1143   |                                                                                                                                                        |
|                                | HCC-70     | L15 + 1% glutamine + 1% penicillin/streptomycin + 10% FBS without CO <sub>2</sub>                                                                      |
|                                | MDA-MB-436 |                                                                                                                                                        |
|                                | MDA-MB-231 | DMEM + 1% glutamine + 1% penicillin/streptomycin + 10% FBS                                                                                             |
|                                | HCC-1937   | RPMI-1640 + 1% glutamine + 1% penicillin/streptomycin + 10% FBS + 1.5 g/L sodium bicarbonate + 10 mM HEPES + 1 mM sodium pyruvate                      |
| <b>Melanoma</b>                | A2058      | MEM (Eagle) + 1% glutamine + 1% penicillin/streptomycin + 10% FBS                                                                                      |
|                                | A375       | DMEM + 1% glutamine + 1% penicillin/streptomycin + 10% FBS                                                                                             |
|                                | MEL888     |                                                                                                                                                        |
|                                | MEL624     |                                                                                                                                                        |
| <b>Lung cancer</b>             | H23        | RPMI-1640 + 1% glutamine + 1% penicillin/streptomycin + 10% FBS                                                                                        |
|                                | H522       |                                                                                                                                                        |
|                                | HOP-92     |                                                                                                                                                        |
|                                | HOP-62     |                                                                                                                                                        |
| <b>Ovarian cancer</b>          | IGROV-1    |                                                                                                                                                        |
|                                | SKOV-3     |                                                                                                                                                        |
| <b>Nonmalignant cell lines</b> | MRC-5      | MEM(Eagle) +1% glutamine + 1% penicillin/streptomycin + 10% FBS + 0.1 mM non-essential amino acids                                                     |
|                                | HEK293T    | DMEM + 1% glutamine + 1% penicillin/streptomycin + 10% FBS                                                                                             |
|                                | GM08505    |                                                                                                                                                        |
|                                | MCF-10A    | DMEM/F12 + 5% horse serum + 20 ng/mL EGF + 100 ng/mL cholera toxin + 0.01 mg/mL insulin + 500 ng/mL hydrocortisone                                     |
|                                | MCF-12A    |                                                                                                                                                        |

## Supplementary Material 2. Primer sequences for RT-qPCR

| qPCR primers |   | Sequence 5' to 3'        | Reference             |
|--------------|---|--------------------------|-----------------------|
| <i>MAPT</i>  | F | GCTCATTAGGCAACATCCATC    | Bou Samra et al. [2]  |
|              | R | GTCAGCTTGTGGGTTTCAATC    |                       |
| <i>CDA</i>   | F | CCCTACAGTCACTTTCCTG      | Chabosseau et al. [3] |
|              | R | CGGGTAGCAGGCATTTTCTA     |                       |
| <i>B2M</i>   | F | CGCTCCGTGGCCTTAGC        | Kellouche et al. [4]  |
|              | R | GAGTACGCTGGATAGCCTCCA    |                       |
| <i>TBP</i>   | F | TGCACAGGAGCCAAGAGTGAA    | Pasmant et al. [5]    |
|              | R | CACATCACAGCTCCCCACCA     |                       |
| <i>GAPDH</i> | F | GAAATCCCATCACCATCTTCCAGG | West et al. [6]       |
|              | R | GAGCCCCAGCCTTCTCCATG     |                       |

## References

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