***Appendix***

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**Appendix Table S1. Lysis buffer composition.**

| | |
|---|---|
| 6.6 mM Trizma-acetate pH 7.5 | Carl Roth, Karlsruhe, Germany |
| 6.6 mM Magnesium-acetate | Carl Roth, Karlsruhe, Germany |
| 33.3 mM potassium-acetate | Carl Roth, Karlsruhe, Germany |
| 0.44% Tween | Carl Roth, Karlsruhe, Germany |
| 0.44% IGEPAL CA-630 | Carl Roth, Karlsruhe, Germany |
| 0.10 unit of Proteinase K | 800U/ml, New England Biolabs, Ipswich, MA, US |
| diluted in PBS | |

**Appendix Table S2. PCR 1, PCR 2 and SAP preparation mixes.**

| **PCR#1** | µL | |
|---|---|---|
| water | 14.04 | Qiagen, Hilden, Germany |
| 10x pcr buffer | 2 | Agena Bioscience, San Diego, CA, USA |
| mgcl2 | 1.6 | Agena Bioscience, San Diego, CA, USA |
| dNTP mix | 0.1 | Agena Bioscience, San Diego, CA, USA |
| pcr primer p01 | 0.12 | Agena Bioscience, San Diego, CA, USA |
| pcr enzyme | 0.64 | Agena Bioscience, San Diego, CA, USA |
| ung enzyme | 0.5 | Agena Bioscience, San Diego, CA, USA |
| Volume | 19 | |
| Lysed single cell (input) | 3 | |
| Total | 22 | |

| **PCR#2** | µL | |
|---|---|---|
| water | 17.32 | Qiagen, Hilden, Germany |
| 10x pcr buffer | 4 | Agena Bioscience, San Diego, CA, USA |
| mgcl2 | 3.2 | Agena Bioscience, San Diego, CA, USA |
| dNTP-dUTP mix | 0.2 | Agena Bioscience, San Diego, CA, USA |
| pcr primer p01 | 8 | Agena Bioscience, San Diego, CA, USA |
| pcr enzyme | 1.28 | Agena Bioscience, San Diego, CA, USA |
| ung enzyme | 0 | Agena Bioscience, San Diego, CA, USA |
| Volume | 34 | |
| PCR#1 product (input) | 6 | |
| Total | 40 | |

*PCR#2 was performed in duplicate for patient samples; both reactions were pooled for analysing all variants covered by the panels.*

| **SAP** | µL | |
|---|---|---|
| Water | 12.24 | Qiagen, Hilden, Germany |
| SAP buffer | 1.36 | Agena Bioscience, San Diego, CA, USA |
| SAP enzyme | 2.4 | Agena Bioscience, San Diego, CA, USA |
| Volume | 16 | |

**Appendix Figure S1. Recapitulative figure for the mutations detected among the different types of samples (tissues, cfDNA, CTC) for the 33 melanoma patients at the beginning of the new line of treatment.**

On the top of the panel, patient ID is indicated with the type of sample analyzed. We distinguished samples obtained before initiation of treatment from those obtained during treatment. 'Tissue UltraSEEK' stands for the analysis of gDNA from a tumor tissue sample with UltraSEEK® melanoma panel. The assays with positive values on leukocytes validation set are marked with an asterisk.

SDHD.Mut3 stands for SDHD mutation Chr11:111, 957,544 C>T.