Supplementary Information for

**Epigenetic reactivation of LC3B induces melanoma cell death and predicts response to immunotherapy**

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**This file includes:**

Supplementary Tables S1 to S7

**Supplementary Table S1. Melanoma cell lines used in this study including identified genetic alterations in important genes.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Cell**  **Lines** | **Stage** | **Primary site** | **BRAF** | **NF1** | **CDKN2A** | **FGFR2** | **MC1R** | **Nras** | **p14ARF** | **PTEN** | **p53** |
| A04 | 4 | non-CSD | wt | wt | HD | A648T | V60L +/- | wt | HD | wt | wt |
| C092 | 3 | occult | wt | likely wt | wt | wt | wt | wt | n/a | wt | wt |
| C006 | 3 | non-CSD | wt | likely wt | n/a | n/a | wt | Q61L | wt | n/a | wt |
| C013 | 3 | non-CSD | wt | wt | wt | wt | R151C +/-  D294H +/- | Q61L | wt | 380G>A  Gly127Glu | P278S |
| C008 | 3 | occult | wt | splice | wt | wt | wt | wt | n/a | wt | wt |
| D22 | 4 | n/a | wt | R440X | wt | R759stop;  D530N | D294H -/- | wt | wt | wt | E287K |
| D05 | 4 | non-CSD | V600E | likely wt | LOH+  [frameshift] | wt | R151C +/- | wt | wt | wt | wt |
| D14 | 4 | non-CSD | V600E | likely wt | LOH+  P114L | wt | wt | wt | wt | HD | G266E |
| D20 | 4 | n/a | V600E | likely wt | wt | n/a | wt | wt | wt | HD | R248Q |

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Cell**  **Lines** | **Rb** | **TERT**  **(% mutant)** | **MEK** | **BCL2L12** | **MAP3K9** | **FLRT2** | **HDAC7** | **HDAC9** | **ERBB4** | **MET** |
| A04 | wt | wt | wt | wt | wt | wt | wt | wt | wt | wt |
| C092 | wt | wt | wt | wt | wt | n/a | wt | wt | wt | T922I |
| C006 | wt | wt | wt | wt | wt | R486Q | n/a | n/a | n/a | n/a |
| C013 | R455X | 138CC>TT | wt | wt | wt | wt | wt | wt | wt | wt |
| C008 | n/a | -146C/T | wt | F17F | R160C | n/a | wt | E982A | R1067Q | E75K  R242K |
| D22 | n/a | -124 C/T | P124L | wt | wt | E102K | n/a | n/a | n/a | n/a |
| D05 | wt | -124C/T | wt | F17F | P263L | wt | wt | wt | wt | wt |
| D14 | wt | -124 T/T | wt | wt | Y646C | wt | E892K | wt | wt | wt |
| D20 | n/a | -146 T/T | wt | wt | wt | wt | n/a | n/a | n/a | n/a |

**Supplementary Table S2. Antibodies used in this study.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Antibodies** | **Company** | **Species** | **Dilution** | **Catalog #** | **RRID** |
| **Westerns** |  |  |  |  |  |
| **Primaries** |  |  |  |  |  |
| G9a | Cell Signaling | Rabbit | 1:1000 | 3306S |  |
| LC3B | Abcam | Rabbit | 1:500 | ab48394 |  |
| P62 | Cell Signaling | Rabbit | 1:1000 | 8025S |  |
| H3K9me2 | Abcam | Rabbit | 1:700 | ab1220 |  |
| Cleaved PARP | BD Biosciences | Mouse | 1:1000 | 552596 |  |
| Total H3 | Abcam | Rabbit | 1:1000 | ab1791 |  |
| Tubulin | Abcam | Rabbit | 1:5000 | ab6046 |  |
| **Secondaries** |  |  |  |  |  |
| Anti-rabbit HRP | Cell Signaling |  | 1:10000 | 7074S |  |
| Anti-mouse HRP | Cell Signaling |  | 1:10000 | 7076S | AB\_330924 |
| **ChIP** |  |  |  |  |  |
| G9a | Abcam | Rabbit |  | ab40542 | AB\_731483 |
| H3K9me2 | Abcam | Rabbit |  | ab1220 |  |
| RNA polymerase II | Abcam | Rabbit |  | ab817 |  |
| IgG control | Abcam | Rabbit |  | ab37415 |  |
| **IHC** |  |  |  |  |  |
| G9a | Abcam | Rabbit | 1:8000 | ab40542 |  |
| LC3B | Cell Signaling | Rabbit | 1:14000 | 3868S |  |
| SOX10 | Santa Cruz | Goat | 1:600 | sc-17342 | AB\_2195374 |
| PD-L1 | Abcam | Rabbit | 1:1000 | ab213480 | AB\_2773715 |
| CD8a | eBioscience | Rat | 1:100 | 14-0081-82 |  |
| **IF** |  |  |  |  |  |
| G9a | Santa Cruz | Mouse |  | sc-515726 |  |
| LC3B | Abcam | Rabbit |  | ab48394 |  |
| ABCB5 | Abcam | Goat |  | ab77549 |  |
| **Secondaries** |  |  |  |  |  |
| Anti-mouse AF 488 | Life Technologies | Donkey |  | A21206 |  |
| Anti-rabbit AF 568 | Life Technologies | Donkey |  | A10042 |  |
| Anti-goat AF 633 | Life Technologies | Donkey |  | A21082 |  |
| Anti-rat AF 647 | Life Technologies | Chicken |  | A21247 |  |

**Supplementary Table S3. Summary of the baseline characteristics of patients in the different datasets.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **TCGA dataset** | **Hugo et al dataset** | **TMA** |
| **Number of Patients** | 469 | 27 | 40 |
| **Sex** |  |  |  |
| Male | 289 | 19 | 26 |
| Female | 180 | 8 | 13 |
| NA | 0 | 0 | 1 |
| **Age (yrs)** |  |  |  |
| Median (range) | 58 (48-71) | 61 (19-84) | 69 (32-88) |
| **Tumour Stage** |  |  |  |
| In situ (stage 0) | 7 |  |  |
| Low stage (stage I or II) | 235 |  |  |
| High stage (stage III or IV) | 191 | 27 | 40 |
| NA | 36 |  |  |
| **Mutation status** |  |  |  |
| BRAF | 189 | 14 | 14 |
| NRAS | 98 | 6 | 8 |
| NF1 | 47 | 6 |  |

**Supplementary Table S4. Primers used in this study including sequences.**

|  |  |  |
| --- | --- | --- |
| **Primers** | **Use** | **Sequence** |
| ChIP h*MAP1LC3B* F | ChIP | AGGAGATACAAGGGAAGTGGCT |
| ChIP h*MAP1LC3B* R | ChIP | TTGAAGGTCTTCTCCGACGGCAT |
| ChIP h*MAP1LC3B* 5 kb F | ChIP | ATCTTGGCTCACTGCAACCT |
| ChIP h*MAP1LC3B* 5 kb R | ChIP | GTTTGCGTCCTTTCCCTGTA |
| RT h*MAP1LC3B* F | qPCR | GATGTCCGACTTATTCGAGAGC |
| RT h*MAP1LC3B* R | qPCR | TTGAGCTGTAAGCGCCTTCTA |
| RT h*HPRT* F | qPCR | TGCAGACTTTGCTTTCCTTGGTCAGG |
| RT h*HPRT* R | qPCR | CCAACACTTCGTGGGGTCCTTTTCA |
| RT h*CCL2* F | qPCR | GAGAGGCTGAGACTAACCCAGA |
| RT h*CCL2* R | qPCR | ATCACAGCTTCTTTGGGACACT |
| RT h*PTGS2* F | qPCR | GAATGGGGTGATGAGCAGTT |
| RT h*PTGS2* R | qPCR | CAGAAGGGCAGGATACAGC |
| RT h*TNFAIP3* F | qPCR | CGTCCAGGTTCCAGAACACCATTC |
| RT h*TNFAIP3* R | qPCR | TGCGCTGGCTCCTATCTCAGTTG |
| RT h*IRF-1* F | qPCR | GAGGAGGTGAAAGACCAGAGCA |
| RT h*IRF-1* R | qPCR | TAGCATCTCGGCTGGACTTCGA |
| RT h*ICAM1* F | qPCR | GGCCGGCCAGCTTATACAC |
| RT h*ICAM1* R | qPCR | TAGACACTTGAGCTCGGGCA |
| RT h*SOD2* F | qPCR | GGCCTACGTGAACAACCTGAA |
| RT h*SOD2* R | qPCR | CTGTAACATCTCCCTTGGCCA |
| RT h*CD274* F | qPCR | AAATGGAACCTGGCGAAAGC |
| RT h*CD274* R | qPCR | GATGAGCCCCTCAGGCATTT |
| RT m*MAP1LC3B* F | qPCR | GTCCTGGACAAGACCAAGTTCC |
| RT m*MAP1LC3B* R | qPCR | CCATTCACCAGGAGGAAGAAGG |
| RT m*CD274* F | qPCR | GCTCCAAAGGACTTGTACGTG |
| RT m*CD274* R | qPCR | TGATCTGAAGGGCAGCATTTC |
| RT m*IFNG* F | qPCR | CAGCAACAGCAAGGCGAAAAAGG |
| RT m*IFNG* R | qPCR | TTTCCGCTTCCTGAGGCTGGAT |
| RT m*IRF-1* F | qPCR | ATGCCAATCACTCGAATGCG |
| RT m*IRF-1* R | qPCR | TTGTATCGGCCTGTGTGAATG |
| RT m*HPRT* F | qPCR | CACAGGACTAGAACACCTGC |
| RT m*HPRT* R | qPCR | GCTGGTGAAAAGGACCTCT |

**Supplementary Table S5. The expression of *EHMT2* (G9a), *MAP1LC3B* and their combination as independent prognostic indicators.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Covariate** | **Overall survival** | | |  | **Relapse-free survival** | | |
| **HR**\* | **95%CI**\* | ***P*** |  | **HR** | **95%CI** | ***P*** |
| *EHMT2* (quartiles) | 1.215 | 1.053 - 1.401 | ***0.008*** |  | 1.343 | 1.056 - 1.707 | ***0.0167*** |
| Stage | 1.392 | 1.231 - 1.574 | ***<0.0001*** |  | 1.410 | 1.127 - 1.764 | ***0.0028*** |
| Male vs. Female | 1.136 | 0.816 - 1.582 | 0.4536 |  | 1.419 | 0.802 - 2.509 | 0.2315 |
| BRAF mut vs. WT | 1.447 | 0.717 - 2.918 | 0.3047 |  | 0.925 | 0.187 - 4.569 | 0.9239 |
| NRAS mut vs. WT | 1.472 | 0.736 - 2.944 | 0.2767 |  | 1.002 | 0.200 - 5.038 | 0.9977 |
| NF1 mut vs. WT | 1.484 | 0.815 - 2.702 | 0.1995 |  | 0.407 | 0.085 - 1.956 | 0.2641 |
| WT vs. any mutation | 1.459 | 0.673 - 3.160 | 0.3411 |  | 0.724 | 0.131 - 3.998 | 0.7124 |
|  |  |  |  |  |  |  |  |
| **Covariate** | **Overall survival** | | |  | **Relapse-free survival** | | |
| **HR** | **95%CI** | ***P*** |  | **HR** | **95%CI** | ***P*** |
| *MAP1LC3B* (quartiles) | 1.006 | 0.875 - 1.156 | 0.9392 |  | 1.262 | 1.000 - 1.593 | 0.0514 |
| Stage | 1.379 | 1.220 - 1.558 | ***<0.0001*** |  | 1.435 | 1.148 - 1.794 | ***0.0016*** |
| Male vs. Female | 1.113 | 0.799 - 1.551 | 0.5302 |  | 1.416 | 0.801 - 2.503 | 0.2342 |
| BRAF mut vs. WT | 1.424 | 0.701 - 2.892 | 0.3308 |  | 0.983 | 0.198 - 4.881 | 0.9837 |
| NRAS mut vs. WT | 1.636 | 0.815 - 3.282 | 0.1684 |  | 1.354 | 0.272 - 6.731 | 0.7124 |
| NF1 mut vs. WT | 1.524 | 0.838 - 2.770 | 0.1695 |  | 0.522 | 0.110 - 2.471 | 0.4146 |
| WT vs. any mutation | 1.571 | 0.721 - 3.426 | 0.2582 |  | 1.060 | 0.192 - 5.838 | 0.9473 |
|  |  |  |  |  |  |  |  |
| **Covariate** | **Overall survival** | | |  | **Relapse-free survival** | | |
| **HR** | **95%CI** | ***P*** |  | **HR** | **95%CI** | ***P*** |
| *EHMT2/MAP1LC3B* | 1.266 | 1.099 - 1.457 | ***0.0011*** |  | 1.286 | 1.018 - 1.625 | ***0.0362*** |
| Stage | 1.365 | 1.208 - 1.542 | ***<0.0001*** |  | 1.368 | 1.091 - 1.715 | ***0.0069*** |
| Male vs. Female | 1.141 | 0.819 - 1.589 | 0.4393 |  | 1.385 | 0.784 - 2.444 | 0.2643 |
| BRAF mut vs. WT | 1.402 | 0.694 - 2.833 | 0.3484 |  | 0.951 | 0.185 - 4.873 | 0.9518 |
| NRAS mut vs. WT | 1.466 | 0.733 - 2.931 | 0.2814 |  | 1.127 | 0.218 - 5.836 | 0.8873 |
| NF1 mut vs. WT | 1.441 | 0.794 - 2.614 | 0.2318 |  | 0.458 | 0.099 - 2.132 | 0.3223 |
| WT vs. any mutation | 1.478 | 0.683 - 3.202 | 0.3241 |  | 0.827 | 0.146 - 4.686 | 0.8309 |
|  |  |  |  |  |  |  |  |

**Supplementary Table S6. Univariate and multivariate survival analysis of LC3B expression in immunotherapy-treated metastatic melanoma.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **1. Survival** | **Univariate HR (95% CI)** | ***P*** |  | **Multivariate *P*** |
| %LC3B+ cells (>18.5% *vs* ≤18.5%) | 0.1166 (0.0151-0.9040) | ***0.0407*** |  | *0.0763* |
| LC3B intensity (>753.31 *vs* ≤753.31) | 0.3125 (0.0680-1.4365) | *0.137* |  | *0.3154* |
| PDL1 status (pos. *vs* neg.) | 0.2201 (0.0470-1.0311) | ***0.056*** |  | *0.1533* |
| Age (> 65 *vs* ≤ 65) | 0.7421 (0.2277-2.4191) | *0.6226* |  | *0.384* |
| Sex (female *vs* male) | 0.7090 (0.1892-2.6574) | *0.6118* |  | *0.7349* |
| Stage (M1c *vs* others) | 1.5448 (0.3220-7.4107) | *0.5886* |  | *0.8102* |
| LDH (pos. *vs* neg.) | 2.7019 (0.7667-9.5218) | *0.1239* |  | *0.2478* |
| Mutation Status (mut. *vs* WT) | 2.0688 (0.5380-7.9557) | *0.2926* |  | *0.8806* |
| **2. Response (CR/PR)** | **Univariate HR (95% CI)** | ***P*** |  | **Multivariate *P*** |
| %LC3B+ cells (>18.5% *vs* ≤18.5%) | 0.0925 (0.0121-0.7071) | ***0.0225*** |  | ***0.0228*** |
| LC3B intensity (>753.31 *vs* ≤753.31) | 0.2519 (0.0562-1.1293) | *0.0731* |  | *0.169* |
| PDL1 status (pos. *vs* neg.) | 0.0699 (0.0090-0.5415) | ***0.0113*** |  | ***0.0326*** |
| Age (> 65 *vs* ≤ 65) | 0.4188 (0.1294-1.3555) | *0.1485* |  | *0.5023* |
| Sex (female *vs* male) | 0.5335 (0.1456-1.9552) | *0.3455* |  | *0.3776* |
| Stage (M1c *vs* others) | 1.0033 (0.2655-3.7915) | *0.9962* |  | *0.3306* |
| LDH (pos. *vs* neg.) | 2.0110 (0.6088-6.6432) | *0.2543* |  | *0.5859* |
| Mutation Status (mut. *vs* WT) | 4.9531 (1.0879-22.551) | ***0.0396*** |  | *0.4217* |
| **3. Progression** | **Univariate HR (95% CI)** | ***P*** |  | **Multivariate *P*** |
| %LC3B+ cells (>18.5% *vs* ≤18.5%) | 0.2836 (0.0941-0.8547) | ***0.0259*** |  | ***0.0502*** |
| LC3B intensity (>753.31 *vs* ≤753.31) | 0.4773 (0.1724-1.3213) | *0.1567* |  | *0.3605* |
| PDL1 status (pos. *vs* neg.) | 0.1794 (0.0512-0.6284) | ***0.0075*** |  | ***0.0195*** |
| Age (> 65 *vs* ≤ 65) | 0.6682 (0.2718-1.6428) | *0.3822* |  | *0.4745* |
| Sex (female *vs* male) | 1.2676 (0.5164-3.1117) | *0.6066* |  | *0.5679* |
| Stage (M1c *vs* others) | 1.7176 (0.4875-6.0520) | *0.4023* |  | *0.8333* |
| LDH (pos. *vs* neg.) | 2.7610 (0.9997-7.6252) | *0.0512* |  | ***0.0523*** |
| Mutation Status (mut. *vs* WT) | 3.7468 (1.2361-11.357) | ***0.0202*** |  | *0.7226* |
| **4. Acquired Resistance** | **Univariate HR (95% CI)** | ***P*** |  | **Multivariate *P*** |
| %LC3B+ cells (>18.5% *vs* ≤18.5%) | 0.3073 (0.0834-1.1319) | ***0.0776*** |  | *0.9423* |
| LC3B intensity (>753.31 *vs* ≤753.31) | 0.6108 (0.1848-2.0187) | *0.4213* |  | *0.3937* |
| PDL1 status (pos. *vs* neg.) | 0.1794 (0.0512-0.6284) | ***0.0275*** |  | ***0.0376*** |
| Age (> 65 *vs* ≤ 65) | 0.9717 (0.3087-3.0585) | *0.9611* |  | *0.9039* |
| Sex (female *vs* male) | 1.6237 (0.5403-4.8790) | *0.3903* |  | *0.7000* |
| Stage (M1c *vs* others) | 1.5659 (0.3276-7.4836) | *0.5761* |  | *0.6981* |
| LDH (pos. *vs* neg.) | 1.2534 (0.2486-6.3191) | *0.7854* |  | *0.1811* |
| Mutation Status (mut. *vs* WT) | 2.0392 (0.5986-6.9466) | *0.2569* |  | *0.5914* |

**Supplementary Table S7. Gene Set Enrichment Analysis showing the top pathways identified from the D05 RNA-seq expression data.**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **GS** | **GS DETAILS** | **SIZE** | **ES** | **NES** | **NOM p-val** | **FDR q-val** | **FWER p-val** | **RANK AT MAX** | **LEADING EDGE** |
| **follow link to MSigDB** |
| 1 | [HALLMARK\_TNFA\_SIGNALING\_VIA\_NFKB](http://www.gsea-msigdb.org/gsea/msigdb/cards/HALLMARK_TNFA_SIGNALING_VIA_NFKB) | 40 | 0.7 | 2.21 | 0 | 0 | 0 | 211 | tags=98%, list=33%, signal=137% |
| 2 | [HALLMARK\_INTERFERON\_GAMMA\_ RESPONSE](http://www.gsea-msigdb.org/gsea/msigdb/cards/HALLMARK_INTERFERON_GAMMA_RESPONSE) | 18 | 0.69 | 2.01 | 0.01 | 0.01 | 0.027 | 65 | tags=50%, list=10%, signal=54% |
| 3 | [HALLMARK\_EPITHELIAL\_MESENCHYMAL\_ TRANSITION](http://www.gsea-msigdb.org/gsea/msigdb/cards/HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION) | 15 | 0.7 | 1.94 | 0.01 | 0.02 | 0.064 | 7 | tags=20%, list=1%, signal=20% |
| 4 | [HALLMARK\_MTORC1\_SIGNALING](http://www.gsea-msigdb.org/gsea/msigdb/cards/HALLMARK_MTORC1_SIGNALING) | 22 | 0.64 | 1.89 | 0.01 | 0.02 | 0.083 | 222 | tags=91%, list=35%, signal=135% |
| 5 | [HALLMARK\_CHOLESTEROL\_HOMEOSTASIS](http://www.gsea-msigdb.org/gsea/msigdb/cards/HALLMARK_CHOLESTEROL_HOMEOSTASIS) | 19 | 0.64 | 1.84 | 0.02 | 0.03 | 0.122 | 230 | tags=95%, list=36%, signal=144% |
| 6 | [HALLMARK\_UV\_RESPONSE\_UP](http://www.gsea-msigdb.org/gsea/msigdb/cards/HALLMARK_UV_RESPONSE_UP) | 16 | 0.57 | 1.61 | 0.04 | 0.05 | 0.251 | 196 | tags=69%, list=31%, signal=97% |
| 7 | [HALLMARK\_HYPOXIA](http://www.gsea-msigdb.org/gsea/msigdb/cards/HALLMARK_HYPOXIA) | 16 | 0.52 | 1.45 | 0.07 | 0.08 | 0.421 | 209 | tags=63%, list=33%, signal=91% |
| 8 | [HALLMARK\_P53\_PATHWAY](http://www.gsea-msigdb.org/gsea/msigdb/cards/HALLMARK_P53_PATHWAY) | 16 | 0.47 | 1.31 | 0.14 | 0.14 | 0.676 | 211 | tags=56%, list=33%, signal=82% |

GS: Gene set; ES: Enrichment Score; NES: Normalized Enrichment Score; NOM: Nominal; FDR: False-discovery rate; FWER: Familywise error rate.