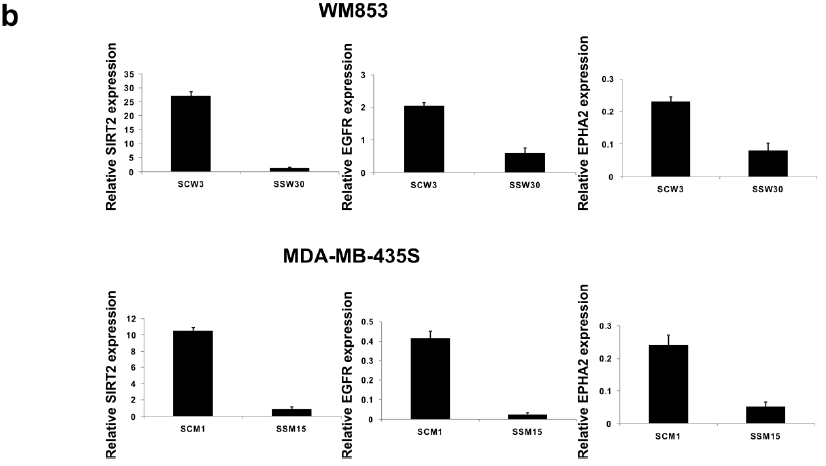
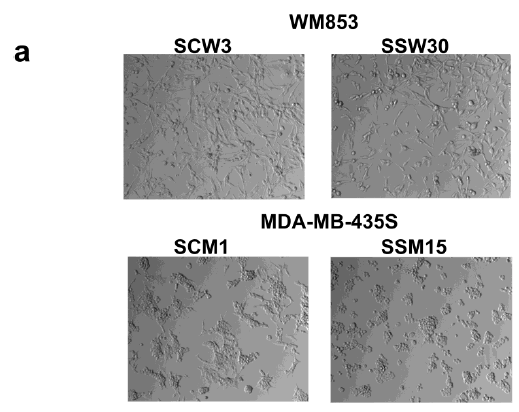
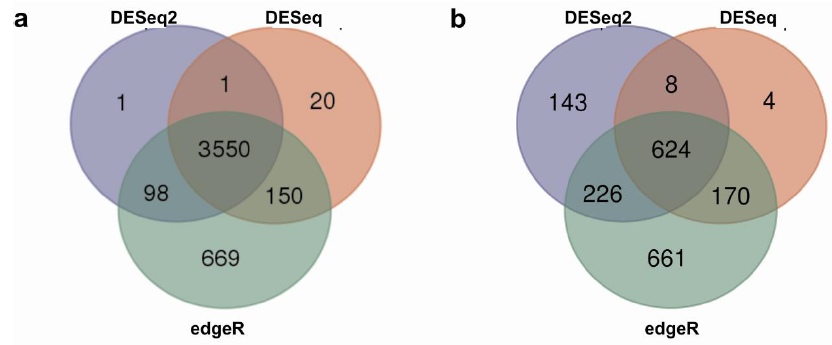
Supplementary Materials: SIRT2 Contributes to the Resistance of Melanoma Cells to the Multikinase Inhibitor Dasatinib

Iwona Karwaciak, Anna Sałkowska, Kaja Karaś, Marta Sobalska-Kwapis, Aurelia Walczak-Drzewiecka, Łukasz Pułaski, Dominik Strapagiel, Jarosław Dastych and Marcin Ratajewski

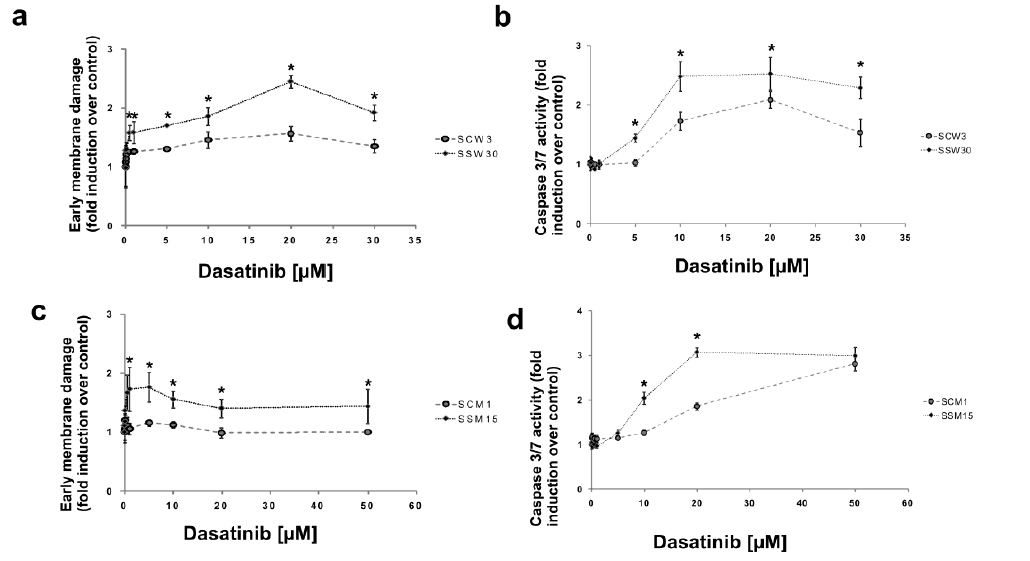
Supplementary Figures



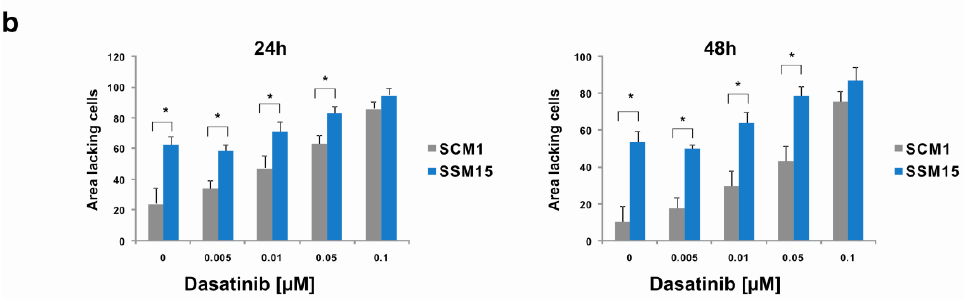
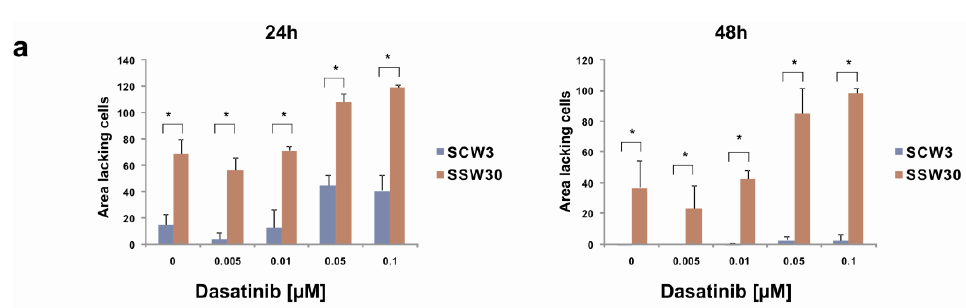
**Figure S1.** Phenotypes of the *SIRT2*-deficient melanoma cell lines.(**a**) Phenotypes of SCW3 and SSW30 clones of WM853 cells and SCM1 and SSM15 clones of MDA-MB-435S (photo from an optical microscope). 10 × 10 magnification. (**b**) *SIRT2*, *EGFR* and *EPHA2* expression in SCW3 and SSW30 clones of WM853 cells and SCM1 and SSM15 clones of MDA-MB-435S, as determined using quantitative PCR and normalized to the expression of the housekeeping gene *RPL13A*, mean ± SD, *n* = 3.



**Figure S2.** Venn diagrams to compare the identified DEGs from the different expression analysis tools and designate the shared DEGs. (**a**) WM853 SCW3 and WM853 SSW30 cells. (**b**) MDA-MB-435S SCM1 and MDA-MB-435S SSM15 cells.



**Figure S3.** Individual cytotoxic effects of dasatinib on melanoma cell lines. (**a**) Effect of dasatinib on early membrane damage in SCW3 and SSW30 clones; mean ± SD, *n* = 6. \* Indicates a statistically significant difference at *p* < 0.05. (**b**) Effect of dasatinib on apoptosis in SCW3 and SSW30 clones as measured by increased caspase 3/7 activity; mean ± SD, *n* = 6. \* Indicates a statistically significant difference at *p* < 0.05. (**c**) Effect of dasatinib on early membrane damage in SCM1 and SSM15 clones; mean ± SD, *n* = 6. \* Indicates a statistically significant difference at *p* < 0.05. (**d**) Effect of dasatinib on apoptosis in SCM1 and SSM15 clones as measured by increased caspase 3/7 activity; mean ± SD, *n* = 6. \* Indicates a statistically significant difference at *p* < 0.05.



**Figure S4.** Computer-generated area lacking values to illustrate the in vitro scratch assay from Figure 3. (**a**) Results of the scratch assay of the WM853 SCW3 and WM853 SSW30 after 24 and 48 h. (**b**) Results of the scratch assay of the MDA-MB-435S SCM1 and MDA-MB-435S SSM15 after 24 and 48 h. The data represent the mean ± SD, (*n* = 4). \* Indicates a statistically significant difference at *p* < 0.05.

Supplementary Tables

**Table S1.** Gene ontology (biological process) term results from the PANTHER overrepresentation test for significantly differentially expressed (DE) genes regulated in both melanoma cells analyzed. GOID: Gene Ontology term identifier; number of genes: the number of genes mapped to a specific GO term; *p*-value: the expected value calculated by Fisher’s exact test with false discovery rate multiple test correction (*p* < 0.05) for overrepresentation of selected DE genes in the GO category.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Cells** | | **WM853** (**Clones SCW3 and SSW30**) | | **MDA-MB-435S** (**Clones SCM1 and SSM15**) | |
| **GO ID** | **GO Biological Process Term** | **Number of Genes** | ***p*-Value** | **Number of Genes** | ***p*-Value** |
| GO:0007155 | cell adhesion | 189 | 4.61 × 10-11 | 74 | 6.77 × 10-18 |
| GO:0098609 | cell-cell adhesion | 92 | 9.56 × 10-05 | 35 | 7.67 × 10-08 |
| GO:0030155 | regulation of cell adhesion | 128 | 1.41 × 10-06 | 39 | 2.15 × 10-06 |
| GO:0031589 | cell-substrate adhesion | 39 | 6.31 × 10-04 | 17 | 3.60 × 10-06 |
| GO:0016477 | cell migration | 171 | 2.83 × 10-07 | 63 | 2.91 × 10-12 |
| GO:0048870 | cell motility | 181 | 2.66 × 10-06 | 66 | 1.25 × 10-11 |
| GO:2000145 | regulation of cell motility | 191 | 1.77 × 10-13 | 49 | 2.50 × 10-07 |
| GO:0030334 | regulation of cell migration | 187 | 6.67 × 10-15 | 46 | 6.02 × 10-07 |
| GO:0008283 | cell proliferation | 125 | 2.89 × 10-05 | 37 | 2.31 × 10-05 |
| GO:0042127 | regulation of cell proliferation | 315 | 1.96 × 10-15 | 63 | 5.06 × 10-04 |
| GO:0010941 | regulation of cell death | 296 | 1.10 × 10-09 | 66 | 4.79 × 10-04 |
| GO:0045595 | regulation of cell differentiation | 349 | 1.74 × 10-18 | 89 | 3.15 × 10-10 |
| GO:0030154 | cell differentiation | 640 | 2.01 × 10-22 | 139 | 1.89 × 10-07 |
| GO:0048583 | regulation of response to stimulus | 683 | 1.40 × 10-15 | 170 | 7.63 × 10-11 |
| GO:0009605 | response to external stimulus | 375 | 3.09 × 10-15 | 86 | 1.68 × 10-06 |
| GO:0010033 | response to organic substance | 517 | 2.73 × 10-18 | 113 | 2.13 × 10-06 |
| GO:0050896 | response to stimulus | 1209 | 2.13 × 10-15 | 261 | 6.74 × 10-06 |
| GO:0009719 | response to endogenous stimulus | 252 | 1.35 × 10-08 | 59 | 2.26 × 10-04 |

**Table S2.** Expression of selected genes in A375 melanoma cells after treatment with the SIRT2 inhibitor thiomyristoyl. Cells were treated with 50 μM thiomyristoyl for 48 h and then were subjected for RNA extraction. Expression of the cognate mRNA was determined using quantitative PCR and normalized to that of the housekeeping gene RPL13A, mean ± SD, *n* = 3. \* Indicates a statistically significant difference at *p* < 0.05.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene** | | **Control** | | **Thiomyristoyl** |
| *EGFR* | 1.40 ± 0.14 | | 0.84 ± 0.17 \* | |
| *EPHA2* | 1.65 ± 0.13 | | 1.26 ± 0.07 \* | |
| *ITGA1* | 21.8 ± 1.2 | | 3.6 ± 1.1 \* | |
| *SDC2* | 11.7 ± 0.80 | | 5.1 ± 0.8 \* | |
| *GAK* | 1.92 ± 0.22 | | 1.76 ± 0.29 | |
| *LYN* | 0.639 ± 0.049 | | 0.517 ± 0.080 | |
| *DDR1* | 1.22 ± 0.15 | | 0.62 ± 0.09 \* | |
| *EPHB1* | 0.479 ± 0.048 | | 0.219 ± 0.032 \* | |

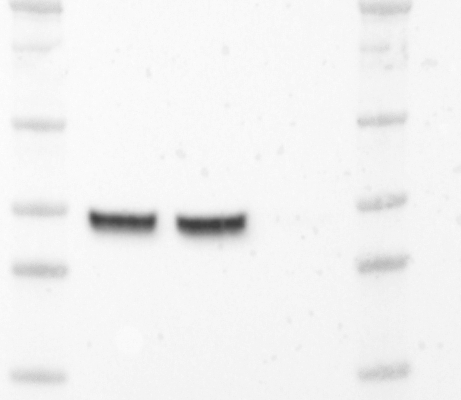
DataSet S1–S3: please find at supplementary files

Original Scans of Figures 1, 5 and 6

Original scans of Figures 1

SIRT2

Size marker, MDA-MB-435S, SCM1, SSM15, size marker



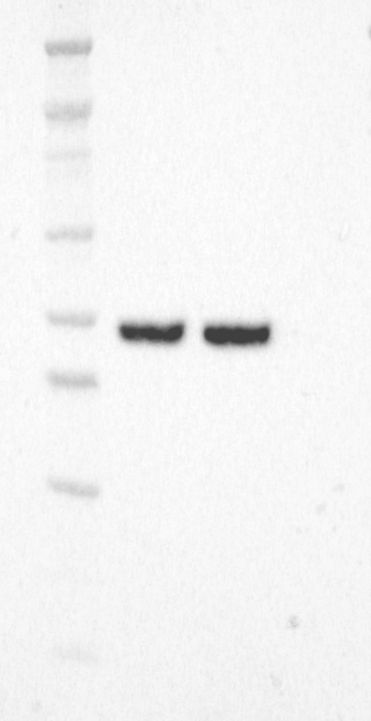
β-actin

size marker, MDA-MB-435S, SCM1, SSM15, size marker



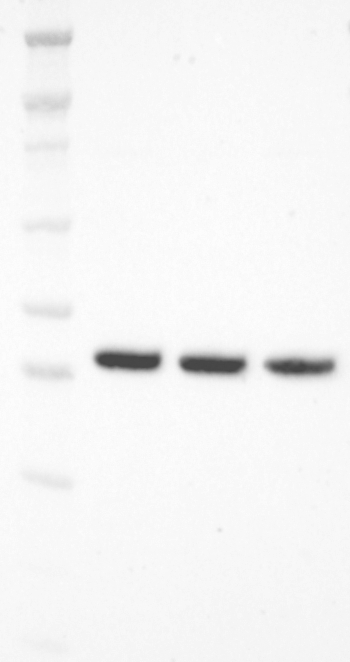
SIRT2

Size marker, WM853, SCW3, SSW30



β-actin

Size marker, WM853, SCW3, SSW30



Original scans of Figures 5

SCM1 pEGFR

SCM1 ctrl; SCM1 EGF 50 ng/mL10 min; SCM1 EGF 50 ng/mL30 min; SCM1 EGF 200 ng/mL10 min

pEGFR cst3777

SSM15 pEGFR

SSM15 ctrl; SSM15 EGF 50 ng/mL10 min; SSM15 EGF 50 ng/mL30 min; SSM15 EGF 200 ng/mL10 min

pEGFR cst3777

SCM1 EGFR

SCM1 ctrl; SCM1 EGF 50 ng/mL10 min; SCM1 EGF 50 ng/mL30 min; SCM1 EGF 200 ng/mL10 min

EGFR MDA zmod

SSM15 EGFR

SSM15 ctrl; SSM15 EGF 50 ng/mL10 min; SSM15 EGF 50 ng/mL30 min; SSM15 EGF 200 ng/mL10 min

EGFR MDA zmod

SCM1 β actin

SCM1 ctrl; SCM1 EGF 50 ng/mL10 min; SCM1 EGF 50 ng/mL30 min; SCM1 EGF 200 ng/mL10 min

beta aktyna ab8227

SSM15 β actin

SSM15 ctrl; SSM15 EGF 50 ng/mL10 min; SSM15 EGF 50 ng/mL30 min; SSM15 EGF 200 ng/mL10 min

beta aktyna ab8227

SCW3 pEGFR SCW3 ctrl; SCW3 EGF 50 ng/mL10 min; SCW3 EGF 50 ng/mL30 min; SCW3 EGF 200 ng/mL10 min

pEGFR cst3777 WM

SSW30 pEGFR

SSW30 ctrl; SSW30 EGF 50 ng/mL10 min; SSW30 EGF 50 ng/mL30 min; SSW30 EGF 200 ng/mL10 min

pEGFR cst3777 WM

SCW3 EGFR

SCW3 ctrl; SCW3 EGF 50 ng/mL10 min; SCW3 EGF 50 ng/mL30 min; SCW3 EGF 200 ng/mL10 min

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SSW30 EGFR

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EGFR cst2232 WM

SCW3 β actin

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beta aktyna ab8227

SSW30 β actin

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beta aktyna ab8227

pEPHA2 (TYR588)

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pEPHA2 cst12677 supernova 4 min mod2

MDA pEPHA2 cst12677_modyfikacja 2MDA pEPHA2 cst12677

pEPHA2 cst6347

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pEPHA2 cst6347 MDA

MDA pEPHA2 cst6347MDA pEPHA2 cst6347

β actin ab8227

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MDA beta aktyna ab8227

MDA beta aktyna ab8227MDA beta aktyna ab8227

pEPHA2 cst12677

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pEPHA2 cst12677 supernova 2min

WM pEPHA2 cst12677 WM pEPHA2 cst12677

β actin ab8227

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beta aktyna WM

WM beta aktyna ab8227 WM beta aktyna ab8227

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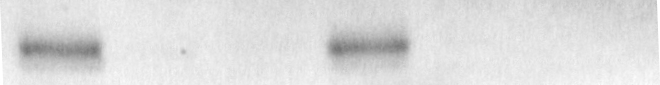
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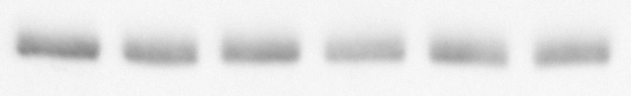
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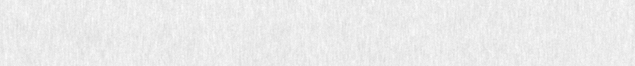
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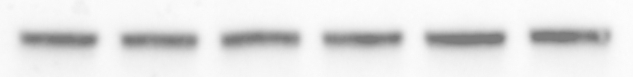
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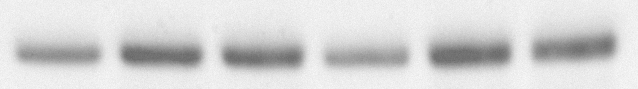
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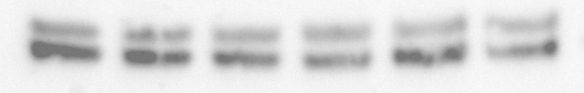
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Β-actin ab8227

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WM853

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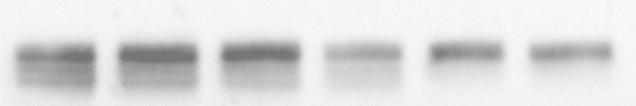
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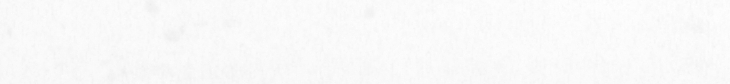
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EGFR cst2232

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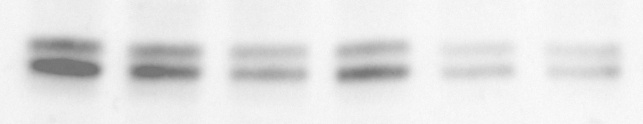
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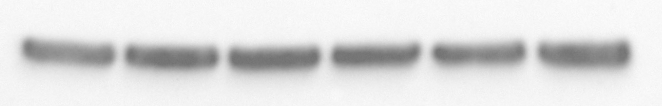
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Β-actin ab8227

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