## SUPPLEMENTARY FIGURE LEGENDS

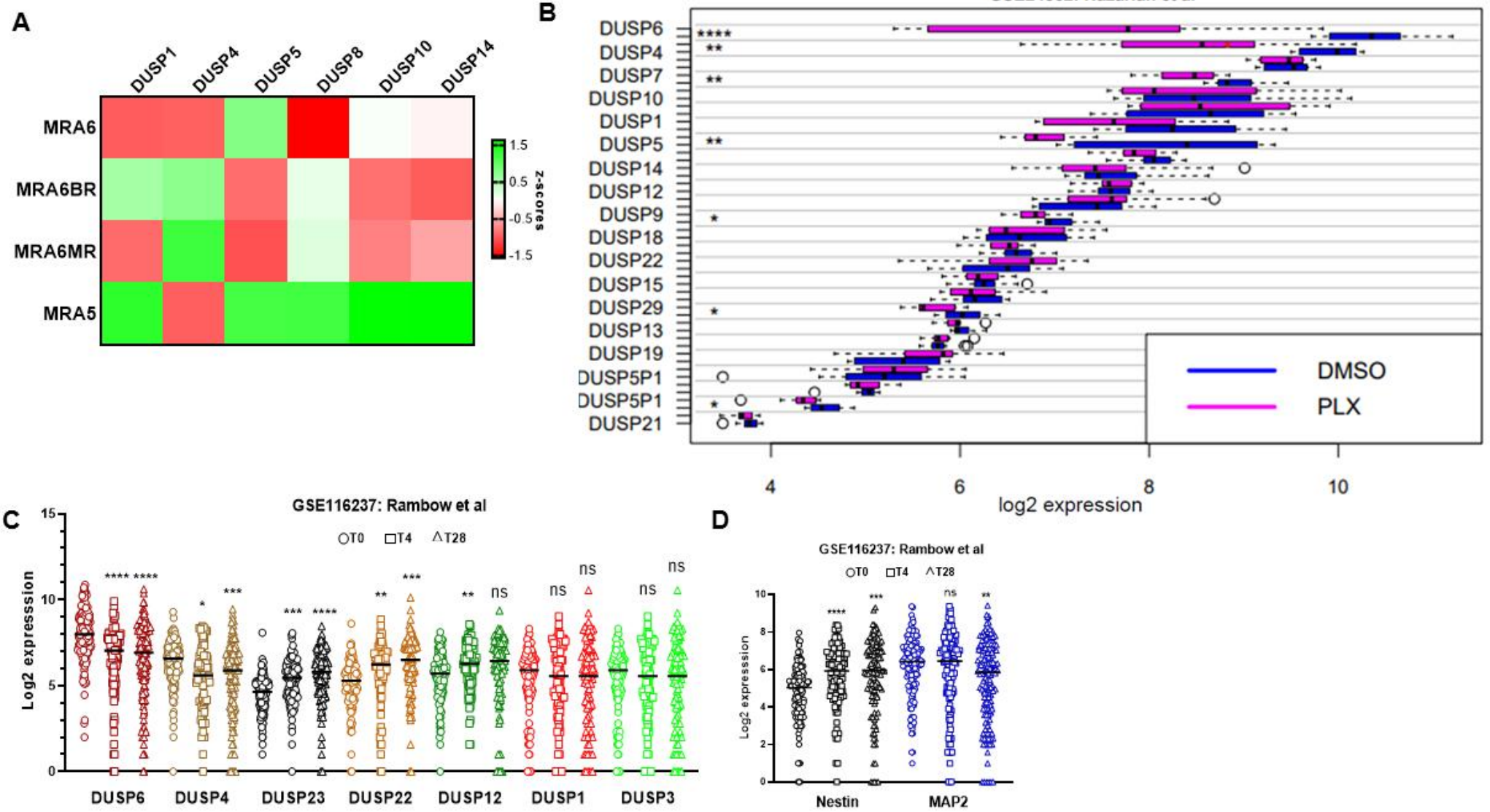
**Supple Figure S1: Gene Expression analysis of DUSPs in publicly available datasets (A)** RNA sequencing for DUSP genes in MAPKi-resistant (MRA5, MRA6BR, and MRA6MR) and MAPKi-sensitive (MRA6) BRAFV600E mutant cells. For six DUSP genes, z-scores were computed. The differential expression of all six DUSPs is represented in the form of a heatmap, demonstrating their differential effect of expression from MAPKi-sensitive to MAPKi-resistant cells. **(B)** GEO dataset GSE24862 consists of parental and resistant sub-lines of melanoma cell lines treated or untreated with PLX4032 Log base 2 transformed expression (Affymetrix Human Gene 1.0 ST Array) is shown for 18 DUSP genes and for 12 DMSO treated and 12 PLX treated samples. Genes are ordered from top to bottom by fold change (difference in average log expression between two treatment groups). The observed expression changes are statistically significant (Benjamini-Hochberg adjusted p-value < 0.01) for DUSP4,5,6,7, according to the Wilcoxon rank sum test. **(C)** GSE116237 datasets consists of single cell RNA and single cell DNA sequencing from a PDX melanoma model before and on treatment (BRAF&MEK inhibitors)**.** Log base 2 transformed expression (Affymetrix Human Gene 1.0 ST Array) is shown for 7 DUSP genes. Genes are ordered from left to right by fold change [difference in average log expression between untreated (T0) vs four-day treatment (T4) vs 28-day treatment (T28)]. The observed expression changes are statistically significant (Benjamini-Hochberg adjusted p-value < 0.01) according to the Wilcoxon rank sum test.

**Supple Figure 2: Gene expression analysis of DUSPs in the genetically matched cell lines established from same patients.** RNA sequencing differential expression data for DUSP genes in genetically matched primary (**P**) WM115 and lymph node metastatic cell lines WM239A (**L1**), WM266-4 (**L2**) and WM165-1 (**L3**)**.** For all twenty DUSP genes, z-scores were computed. The

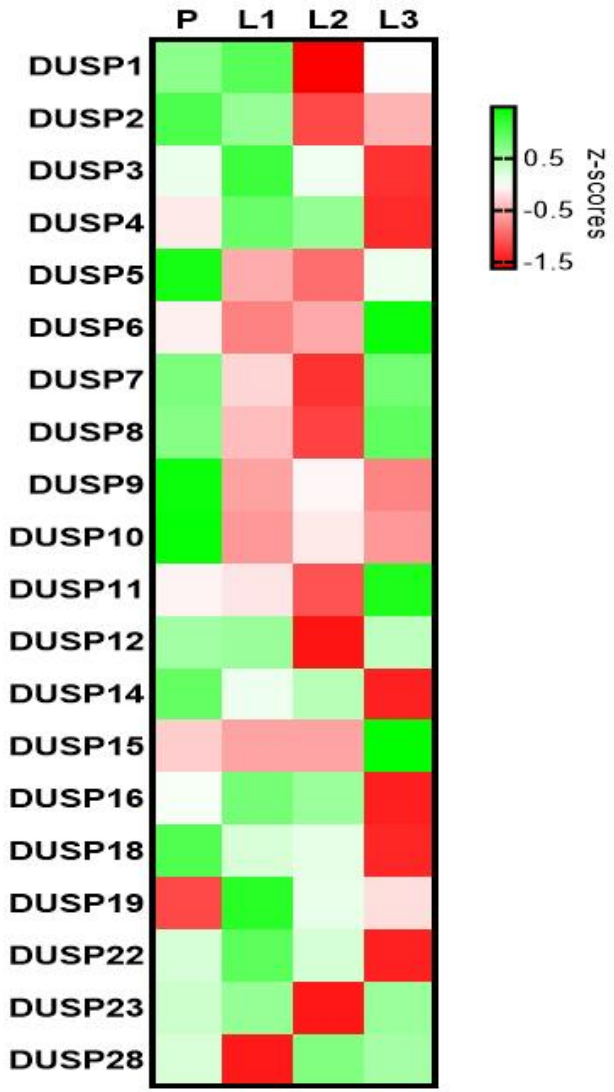
differential expression of all six DUSPs is represented in the form of a heatmap, demonstrating their differential effect of expression from **P** to **L3** cells.

**Supple Figure 3: Effect of DUSP1 KD and DUSP8 KD on sensitivity of melanoma cells to MAPKi. (A)** MAPKi-resistant MRA5, MRA6BR, MRA6MR and MAPKi-sensitive MRA6 and MAPKi-resistant MRA6BR and MRA6MR cells were transfected with DUSP1 and DUSP8 siRNAs. Cells were trypsinized 24h post-transfection and 5000 cells were seeded in 5-6 replicate wells in 96-well plates and treated for 48h with DMSO or 2.5µM PLX4032, AZD6244 or 10µM PLX4032, AZD6244 (MRA5 and MRA6 cells). or combination of PLX-4032+AZD-6244 (MRA5, MRA6, MRA6BR and MRA6MR cells). All datas are shown as mean ± SD and analyzed using Student's t-test. P values: * denotes $P \leq 0.05$, ** $\leq 0.01$; *** $\leq 0.001$ and **** $\leq 0.0001$.

# Supplementary Figure 1



## Supplementary Figure 2



# Supplementary Figure 3

**A**

| Cell Lines | siRNA Transfection | Comparison | p-value |
|---|---|---|---|
| MRA5 | Control | DMSO vs BRAFi | **** |
| | | DMSO vs MEKi | **** |
| | DUSP1 | DMSO vs BRAFi | **** |
| | | DMSO vs MEKi | **** |
| | DUSP8 | DMSO vs BRAFi | **** |
| | | DMSO vs MEKi | **** |
| MRA6 | Control | DMSO vs BRAFi | **** |
| | | DMSO vs MEKi | **** |
| | DUSP1 | DMSO vs BRAFi | **** |
| | | DMSO vs MEKi | **** |
| | DUSP8 | DMSO vs BRAFi | **** |
| | | DMSO vs MEKi | **** |
| MRA6BR | Control | DMSO vs BRAFi | **** |
| | | DMSO vs MEKi | **** |
| | DUSP1 | DMSO vs BRAFi | **** |
| | | DMSO vs MEKi | **** |
| | DUSP8 | DMSO vs BRAFi | **** |
| | | DMSO vs MEKi | **** |
| MRA6MR | Control | DMSO vs BRAFi | **** |
| | | DMSO vs MEKi | **** |
| | DUSP1 | DMSO vs BRAFi | **** |
| | | DMSO vs MEKi | **** |
| | DUSP8 | DMSO vs BRAFi | **** |
| | | DMSO vs MEKi | **** |

**B**

| Cell Lines | siRNA transfection | Comparison | p-value |
|---|---|---|---|
| MRA5 | Control | DMSO vs BRAFi+MEKi | **** |
| | DUSP1 | DMSO vs BRAFi+MEKi | **** |
| | DUSP8 | DMSO vs BRAFi+MEKi | **** |
| MRA6 | Control | DMSO vs BRAFi+MEKi | **** |
| | DUSP1 | DMSO vs BRAFi+MEKi | **** |
| | DUSP8 | DMSO vs BRAFi+MEKi | **** |
| MRA6BR | Control | DMSO vs BRAFi+MEKi | **** |
| | DUSP1 | DMSO vs BRAFi+MEKi | **** |
| | DUSP8 | DMSO vs BRAFi+MEKi | **** |
| MRA6MR | Control | DMSO vs BRAFi+MEKi | **** |
| | DUSP1 | DMSO vs BRAFi+MEKi | **** |
| | DUSP8 | DMSO vs BRAFi+MEKi | **** |

**Table 1: Melanoma cell lines used in this study**

| Cell Line | Stage | Stage/Level | Mutations | | | | Homozygous Loss |
|---|---|---|---|---|---|---|---|
| | | | BRAF | PTEN | N-Ras | c-KIT | |
| WM115 | VGP | Level III | V600D | Hemizygous Deletion | WT | WT | CDKN2A, CDKN2B |
| WM266-4 | Metastasis | Level III | V600D | Hemizygous Deletion | WT | WT | CDKN2A, CDKN2B |
| WM239A | Metastasis | Level III | V600D | Hemizygous Deletion | WT | WT | CDKN2A, CDKN2B |
| WM165-1 | Metastasis | Level III | V600D | Hemizygous Deletion | WT | WT | CDKN2B |
| MRA-5 | Metastasis | Stage IV | V600E | Absent | ND | ND | ND |
| MRA-6 | Metastasis | Stage IV | V600E | Absent | ND | ND | ND |

Data for the cell lines highlighted in light blue were obtained from Rockland Immunochemicals Inc., Limerick, PA. MRA series of cell lines were established in-house by Dr. Mark Albertini and the gene mutation data were obtained targeted sequencing and western blotting.

RGP: radial growth phase; VGP: vertical growth phase; Stage: AJCC stage; Level: Clark's level; WT: wild type; Absent: protein not detected by immunoblotting assay

**Table 2: Sources and dilutions of antibodies used in this study**

| Antibodies | Catalogue # | Dilution | Source | Vendor |
|---|---|---|---|---|
| DUSP1/MKP1 | ab236501 | 1:750 | Mouse | Abcam |
| DUSP3 | ab125077 | 1:750 | Rabbit | |
| DUSP8 | ab198175 | 1:750 | Rabbit | |
| DUSP9 | ab194355 | 1:750 | Rabbit | |
| Nestin | 33475S | 1:1000 | Mouse | Cell Signaling Technology |
| MAP2 | 8707S | 1:1000 | Rabbit | |
| GFAP | 3670S | 1:1000 | Mouse | |
| phospho-p38 MAPK (Thr180/Tyr182) | 4511S | 1:1000 | Rabbit | |
| p38 MAPK | 8690S | 1:1000 | Rabbit | |
| p44/42 Map Kinase (Erk1/2) | 4696S | 1:1000 | Mouse | |
| Phospho-p44/42 MAPK (Erk1/2) | 4370 | 1:1000 | Rabbit | |
| phospho-SAPK/JNK (Thr183/Tyr185) | 9255S | 1:750 | Mouse | |
| JNK1 | 3708S | 1:1000 | Mouse | |
| GAPDH | 6000-1-Ig | 1:1500 | Mouse | Proteintech |
| Anti-Rabbit HRP-conjugated | NA934 | 1:2000 | Donkey | GE Healthcare |
| Anti-Mouse HRP-conjugated | NA931 | 1:2000 | Goat | |

**Table S3: List of all DUSPs genes with the potential druggable targets using TCDA (The Cancer Druggable Gene Atlas)**

| Genes | Functional class | PubTator score | Target development /druggability level (TDL) | Tractability (smallmolecule) | Tractability (antibody) |
|---|---|---|---|---|---|
| BRAF | Kinase | 99% | Tclin | Clinical precedence | Predicted Tractable-High confidence |
| DUSP1 | Enzyme | 90% | Tchem | Discovery precedence | - |
| DUSP2 | Enzyme | 86% | Tbio | Predicted Tractable | - |
| DUSP3 | Enzyme | 79% | Tchem | Discovery precedence | |
| DUSP4 | Enzyme | 71% | Tbio | NA | - |
| DUSP5 | Enzyme | 62% | Tbio | Predicted Tractable | - |
| DUSP6 | Enzyme | 80% | Tbio | Discovery precedence | - |
| DUSP7 | Enzyme | 43% | Tbio | NA | |
| DUSP8 | Enzyme | 48% | Tbio | NA | - |
| DUSP9 | Enzyme | 47% | Tbio | NA | |
| DUSP10 | Enzyme | 56% | Tbio | NA | |
| DUSP11 | Enzyme | 47% | Tbio | NA | |
| DUSP12 | Enzyme | 49% | Tbio | NA | - |
| DUSP14 | Enzyme | 33% | Tbio | NA | - |
| DUSP15 | Enzyme | 33% | Tbio | Discovery precedence | Predicted Tractable |
| DUSP16 | Enzyme | 51% | Tbio | NA | - |
| DUSP18 | Enzyme | 34% | Tbio | NA | - |
| DUSP19 | Enzyme | 27% | Tdark | NA | - |
| DUSP20 | Enzyme | - | - | - | - |
| DUSP22 | Enzyme | 30% | Tbio | Discovery precedence | Predicted Tractable |
| DUSP23 | Enzyme | 51% | Tbio | Discovery precedence | - |
| DUSP28 | Enzyme | 25% | Tbio | NA | |

Tclin:- Potential druggable genes were targeted by approved drugs

Tchem:- small molecules that satisfy the activity thresholds

Tbio/Tdark: biological functions were still unknown

http://fcgportal.org/TCDA/gene_detail.php