Supplementary Figure 1

(a) Western blot analysis showing the protein expression levels of OVOL1 in different cell lines: + (MCF7), + (HCT116wt), and - (HeLa). The protein amount is relative to MCF7.

(b) Immunohistochemistry (IHC) images and H Score analysis for OVOL1 expression in + (MCF7), + (HCT116wt), and - (HeLa) conditions. Image analysis mark-up and H Score bars are presented.

(c) Staining images with and without primary antibody or IgG from rabbit serum. + (OVOL1) shows positive staining, - (No primary Antibody) shows no staining, and - (IgG from rabbit serum) shows negative staining.
Supplementary Figure 2

a

- (HeLa)  + (MCF7)

EPHX3

- (HeLa)  + (MCF7)

B-act

- (HeLa)  + (MCF7)

Protein amount relative to MCF7

b

IHC

- (HeLa)  + (MCF7)

Image analysis Mark-up

EPHX3

H Score

0 50 100 150

0.0 0.5 1.0 1.5

0 50 100 150

0 0.5 1.0 1.5

0 50 100 150

0 0.5 1.0 1.5

C

+ (EPHX3)

- (No primary Antibody)

+ (EPHX3)

- (IgG from rabbit serum)

+ (EPHX3)

- (IgG from rabbit serum)
Supplementary Figure 3

(a) Western blot analysis showing protein bands for OLIG3 and B-actin. The bands are visualized for samples labeled + (HeLa) and - (HT29).

(b) IHC images of HT29 and HeLa cell lines. The images show the distribution of OLIG3 protein. The H Score analysis indicates the staining intensity and distribution for each cell line.

(c) Microscopy images illustrate the localization of OLIG3 protein in different conditions. The images show OLIG3 (+) staining, absence of primary antibody (-), and IgG from rabbit serum (-).
Supplementary Figure 4

a

AKT3

Protein expression

Mel-ST  Mel-STR  WM266-4  LOX-IMVI  Malme-3M

b

IHC

AKT3

Protein expression

MALME-3M  1205Lu  WM266-4

No primary antibody

C

+ (AKT3)  - (No primary Antibody)
Supplementary Figure 5

(a) Western blot analysis showing Protein expression for TFAP2B and B-actin across different cell lines.

(b) Immunohistochemical staining for TFAP2B in various melanoma cell lines.

(c) Protein expression levels of TFAP2B in different cell lines, including MEL-ST, WM35, WM266-4, WM793, WM115, WM3248, WM164, A375, LOX-IMVI, WM266-4, MALME-3M, SK-MEL-5, SK-MEL-28, M14, and 1205Lu.
Supplementary Figure 6

Nevus

Primary melanoma

Metastasis

OVOL1

EPHX3
Supplementary Figure 7

OLIG3 - Nevus

OLIG3 – Primary melanoma

OLIG3 – Metastasis

AKT3 – Primary melanoma

TFAP2B – Primary melanoma
Supplementary Figure 8

(a) IHC Image analysis Mark-up

H score

28.67

117.21

156.65

(b) Density

Intensity

OVLG1

EPHX3

OLG3

AKT3

TFAP2B
Supplementary Figure 9

- **AKT3**
  - Pearson $r$: 0.74
  - P value (two-tailed): $< 0.0001$

- **EPHX3**
  - Pearson $r$: 0.81
  - P value (two-tailed): $< 0.0001$

- **GJB2**
  - Pearson $r$: 0.71
  - P value (two-tailed): $< 0.0001$

- **HOXA9**
  - Pearson $r$: 0.79
  - P value (two-tailed): $< 0.0001$

- **MEOX2**
  - Pearson $r$: 0.70
  - P value (two-tailed): $< 0.0001$

- **RBP1**
  - Pearson $r$: 0.84
  - P value (two-tailed): $< 0.0001$

- **SERPINE2**
  - Pearson $r$: 0.72
  - P value (two-tailed): $< 0.0001$

- **TBC1D16**
  - Pearson $r$: 0.72
  - P value (two-tailed): $< 0.0001$

- **TFAP2B**
  - Pearson $r$: 0.68
  - P value (two-tailed): $< 0.0001$

- **TWIST1**
  - Pearson $r$: 0.70
  - P value (two-tailed): $< 0.0001$
Supplementary Figure 10

**a**

**PON3**

- UM (n = 36)
  - Log Rank; p-value = 0.001
  - HR (95% CI): 4.79 (1.72 – 13.39)
- M (n = 35)
- UM (n = 36)
  - Log Rank; p-value = 0.12
  - HR (95% CI): 2.01 (0.82 – 4.93)
- M (n = 22)

**OLIG3**

- UM (n = 36)
  - Log Rank; p-value = 0.02
  - HR (95% CI): 2.80 (1.13 – 6.94)

**MEOX2**

- UM (n = 35)
  - Log Rank; p-value = 0.001
  - HR (95% CI): 4.79 (1.72 – 13.39)

**b**

**PON3 and Breslow**

- LB and UM (n = 38)
- LB and M (n = 17)
- HB and UM (n = 5)
- HB and M (n = 5)
- NU and UM (n = 31)
- NU and M (n = 13)

**PON3 and ulceration**

- U and UM (n = 12)
- U and M (n = 9)

**PON3 and TILs**

- U and TILs (n = 28)
- M and TILs (n = 13)
- M and No (n = 9)

Log Rank; p-value = 0.0002

Patients features (N= 65)

<table>
<thead>
<tr>
<th>Feature</th>
<th>Benefit</th>
<th>Risk</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>1.21 (0.50 – 2.96); p = 0.66</td>
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<td></td>
</tr>
<tr>
<td>Age (&gt; vs &lt; 50 years)</td>
<td>0.74 (0.36 – 1.60); p = 0.75</td>
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<tr>
<td>TILs (absence/weak vs non-brisk/brisk)</td>
<td>0.29 (0.10 – 0.83); p = 0.022***</td>
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<tr>
<td>Breslow (≤ 4 vs &gt; 4 mm)</td>
<td>9.38 (2.78 – 31.62); p = 0.0003***</td>
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<tr>
<td>Ulceration (negative vs positive)</td>
<td>3.23 (1.21 – 8.65); p = 0.025*</td>
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<tr>
<td>Clark index (III vs II-IV-V)</td>
<td>3.42 (0.78 – 14.98); p = 0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PON3 hypermethylation</td>
<td>2.77 (1.06 – 7.26); p = 0.038*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Log Rank; p-value = 0.041
HR (95%CI): 3.99 (0.94 – 16.88); p-value = 0.059

M (n = 170)
UM (n = 53 )
Supplementary Figure 13

(a) Log Rank; p-value = 0.4920
AKT3-Low (n=78)
AKT3-High (n=71)

Log Rank; p-value = 0.7142
EPHX3-Low (n=71)
EPHX3-High (n=74)

Log Rank; p-value = 0.8049
OLIG3-Low (n=73)
OLIG3-High (n=67)

Log Rank; p-value = 0.7696
TFAP2B-Low (n=58)
TFAP2B-High (n=86)

(b) Log Rank; p-value = 0.3964
AKT3-Low (n=79)
AKT3-High (n=66)

Log Rank; p-value = 0.7432
EPHX3-High (n=70)
EPHX3-Low (n=71)

Log Rank; p-value = 0.2063
OLIG3-Low (n=72)
OLIG3-High (n=64)

Log Rank; p-value = 0.2302
TFAP2B-High (n=86)
TFAP2B-Low (n=55)