Supplementary Material Section S7:

Materials and methods of qRT-PCR experiments, results in the training set, validation set and circuits elements, measured in the entire patient cohort, and luciferase assay procedures and results.

This session is divided in four subparts:

a) Materials and methods of qRT-PCR experiments
b) qRT-PCR data analyses including borderline samples
c) qRT-PCR data analyses excluding borderline samples
d) luciferase assay procedures and results.

Sub-session A: Materials and methods of qRT-PCR experiments and primers list for qRT-PCR experiments.

Mature miRNA and gene expression levels were examined by quantitative reverse transcription-PCR (qRT-PCR) using Sybr Green protocol (Qiagen) on an Applied Biosystems 7900HT (Ambion-ABI). Primer pair sequences are listed here below. Experiments were run in triplicate, using 384-well reaction plates in an automatic liquid handling station (epMotion 5075LH; Eppendorf, Milano, Italy). Raw data was generated with SDS Relative Quantification software (version 2.3; Ambion-ABI), data were normalised using the geometric mean of the four independent housekeeping controls (for miRNAs: RNU6B, SNORD61, SNORD72, SNORD68; for genes: ACTB, B2M, PPIA e HPRT1). Two-sided student's t-test and Wilcoxon test were used to verify among groups mean differences, p-value<0.05 was considered statistically significant.

Table S7.1: Primer Pair sequences, Genebank accession number and annealing temperature (Ta) of genes analysed by qRT-PCR. Fw, forward; Rv, reverse.

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Sub-session B: miRNAs and genes qRT-PCR expression values analysis including borderline samples

Table S7.2: miRNA qRT-PCR analysis in training and validation sets. The logarithmic fold change of class 1 average expression value versus class 2 average expression value (log₂(FC)) of selected miRNAs. P-values lower than 0.05 were considered significant. Mucinous (Muc), clear cell (Cc), serous (Ser), endometrioid (End).

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Fig. S7.1: qRT-PCR validations in training set. qRT-PCR expression values for miR192, miR-194, miR-30a, BMI1, CIAO1, PSME3, CUL4A, PTGER3, E2F3. * means p<0.05; ** means p<0.01; *** means p<0.001.
Fig. 7.2: qRT-PCR validations in validation set. qRT-PCR expression values for miR192, miR-194, miR-30a and miR-30a*, BMI1, CIAO1, PSME3, CUL4A, PTGER3, E2F3.
Table S7.3: qRT-PCR analysis of selected genes in training and validation set. The logarithmic fold change of class 1 average expression value versus class 2 average
expression value (log$_2$(FC)) is reported. P-values lower than 0.05 were considered significant. Mucinous (Muc), clear cell (Cc), serous (Ser), endometrioid (End).

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<td>2.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Muc vs. Ser</td>
<td>-4.21</td>
<td>7.56E-15</td>
<td>1.84</td>
</tr>
<tr>
<td>miR-194/192</td>
<td>MAPK4A</td>
<td>Muc vs. Cc</td>
<td>-0.32</td>
<td>0.5711</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Muc vs. End</td>
<td>-0.80</td>
<td>0.1324</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Muc vs. Ser</td>
<td>-0.83</td>
<td>0.1226</td>
<td>0.67</td>
</tr>
<tr>
<td>miR-194</td>
<td>UBEV2V2</td>
<td>Muc vs. Cc</td>
<td>-0.92</td>
<td>0.1273</td>
<td>0.95</td>
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<td></td>
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<td>Muc vs. End</td>
<td>0.01</td>
<td>0.9836</td>
<td>0.47</td>
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<td></td>
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<td>Muc vs. Ser</td>
<td>-1.05</td>
<td>0.0882</td>
<td>0.57</td>
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<tr>
<td>hsa-miR-192</td>
<td>APOLIA</td>
<td>Muc vs. Cc</td>
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<td>2.80E-08</td>
<td>2.71</td>
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<tr>
<td></td>
<td></td>
<td>Muc vs. End</td>
<td>-3.12</td>
<td>3.43E-05</td>
<td>1.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Muc vs. Ser</td>
<td>-3.44</td>
<td>3.74E-05</td>
<td>0.07</td>
</tr>
<tr>
<td>hsa-miR-192</td>
<td>PTGER3</td>
<td>Muc vs. Cc</td>
<td>-4.32</td>
<td>2.44E-12</td>
<td>3.04</td>
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<tr>
<td></td>
<td></td>
<td>Muc vs. End</td>
<td>-4.44</td>
<td>3.45E-14</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Muc vs. Ser</td>
<td>-5.07</td>
<td>8.67E-15</td>
<td>3.14</td>
</tr>
</tbody>
</table>

**Table S7.4:** Logarithmic qRT-PCR expression values of the four EOC subtype markers in the two cohorts of patients defined as training set and the validation set.
Mucinous (Muc), clear cell (Cc), serous (Ser), endometrioid (End), Interquantile range (IQR).

Table S7.5: Logarithmic qRT-PCR expression values of the four EOC subtype marker putative targets in the training and validation set. Mucinous (Muc), clear cell (Cc), serous (Ser), endometrioid (End), Interquantile range (IQR).

<table>
<thead>
<tr>
<th>miRNA (putative regulator)</th>
<th>miRNA (putative target)</th>
<th>Muc (IQR (Q1-Q3))</th>
<th>End (Q1-Q3)</th>
<th>Cc (Q1-Q3)</th>
<th>Ser (Q1-Q3)</th>
<th>Muc (IQR (Q1-Q3))</th>
<th>End (Q1-Q3)</th>
<th>Cc (Q1-Q3)</th>
<th>Ser (Q1-Q3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-194</td>
<td>BMI-1</td>
<td>4.03 (0.07 - 4.1)</td>
<td>3.43 (5.23 - 8.66)</td>
<td>2.88 (4.47 - 7.35)</td>
<td>2.78 (4.98 - 7.77)</td>
<td>4.07 (0.82 - 4.89)</td>
<td>4.67 (5.77 - 10.44)</td>
<td>4 (4.8 - 8.81)</td>
<td>3.27 (5.41 - 8.68)</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>2.30</td>
<td>3.20</td>
<td>5.98</td>
<td>6.19</td>
<td>2.65</td>
<td>7.60</td>
<td>6.45</td>
<td>7.01</td>
</tr>
<tr>
<td>miR-194/192</td>
<td>CIAO1</td>
<td>3.39 (2.93 - 6.32)</td>
<td>2.42 (6.31 - 8.73)</td>
<td>1.25 (6.66 - 7.91)</td>
<td>1.84 (7 - 8.84)</td>
<td>1.34 (3.64 - 4.99)</td>
<td>2.03 (5.02 - 7.05)</td>
<td>1.34 (6.85 - 8.18)</td>
<td>2.45 (4.49 - 6.95)</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>4.74</td>
<td>7.63</td>
<td>7.28</td>
<td>7.83</td>
<td>4.15</td>
<td>5.95</td>
<td>7.47</td>
<td>5.79</td>
</tr>
<tr>
<td>miR-194</td>
<td>PSME3</td>
<td>3.2 (4.31 - 7.52)</td>
<td>3.26 (7.7 - 10.96)</td>
<td>3.02 (8.21 - 11.23)</td>
<td>2.62 (8.52 - 11.14)</td>
<td>2.51 (5 - 7.51)</td>
<td>2.24 (7.18 - 9.41)</td>
<td>1.09 (9.4 - 10.49)</td>
<td>2.33 (6.39 - 8.72)</td>
</tr>
<tr>
<td>miR-194</td>
<td>CUL4A</td>
<td>2.77 (3.14 - 5.91)</td>
<td>3.4 (6.39 - 9.8)</td>
<td>3.34 (5.81 - 9.14)</td>
<td>3.77 (6.62 - 10.39)</td>
<td>1.02 (5.16 - 6.18)</td>
<td>2.27 (6.9 - 9.17)</td>
<td>2.23 (7.61 - 9.84)</td>
<td>3.61 (5.57 - 9.17)</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>4.36</td>
<td>7.98</td>
<td>7.50</td>
<td>8.57</td>
<td>5.79</td>
<td>8.14</td>
<td>8.81</td>
<td>7.63</td>
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### miR-194/192 MAPK4A

<table>
<thead>
<tr>
<th>IQR (Q1-Q3)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.42 (5.7 - 8.13)</td>
<td>6.96</td>
</tr>
<tr>
<td>3.57 (6.38 - 9.95)</td>
<td>7.76</td>
</tr>
<tr>
<td>2.4 (6.1 - 8.51)</td>
<td>7.28</td>
</tr>
<tr>
<td>4.03 (5.75 - 9.79)</td>
<td>7.79</td>
</tr>
<tr>
<td>3.81 (3.12 - 6.93)</td>
<td>4.33</td>
</tr>
<tr>
<td>2.08 (4.49 - 6.57)</td>
<td>5.36</td>
</tr>
<tr>
<td>3.12 (4.07 - 7.2)</td>
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<tr>
<td>2.3 (3.58 - 5.87)</td>
<td>5.00</td>
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### miR-194 UBEV2V2

<table>
<thead>
<tr>
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<th>Mean</th>
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</thead>
<tbody>
<tr>
<td>4.05 (4.58 - 8.62)</td>
<td>7.13</td>
</tr>
<tr>
<td>5.16 (4.78 - 9.94)</td>
<td>7.12</td>
</tr>
<tr>
<td>3.59 (6.32 - 9.91)</td>
<td>8.05</td>
</tr>
<tr>
<td>4.4 (6.51 - 10.91)</td>
<td>8.18</td>
</tr>
<tr>
<td>2.07 (6.11 - 8.18)</td>
<td>6.75</td>
</tr>
<tr>
<td>1.91 (6.27 - 8.46)</td>
<td>7.22</td>
</tr>
<tr>
<td>3.12 (6.11 - 8.51)</td>
<td>7.70</td>
</tr>
<tr>
<td>2.3 (3.58 - 5.87)</td>
<td>6.18</td>
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### miR-194 PTGER3

<table>
<thead>
<tr>
<th>IQR (Q1-Q3)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.78 (1.22 - 3.01)</td>
<td>1.98</td>
</tr>
<tr>
<td>3.41 (4.7 - 8.12)</td>
<td>6.42</td>
</tr>
<tr>
<td>2.25 (4.88 - 7.13)</td>
<td>6.29</td>
</tr>
<tr>
<td>4.1 (5.04 - 9.14)</td>
<td>7.04</td>
</tr>
<tr>
<td>1.21 (3.4 - 4.61)</td>
<td>3.80</td>
</tr>
<tr>
<td>1.68 (6.59 - 8.28)</td>
<td>7.44</td>
</tr>
<tr>
<td>1.42 (5.91 - 7.34)</td>
<td>6.85</td>
</tr>
<tr>
<td>2.75 (5.94 - 8.69)</td>
<td>6.94</td>
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### miR-192 APOL1A

<table>
<thead>
<tr>
<th>IQR (Q1-Q3)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.14 (4.12 - 8.26)</td>
<td>5.59</td>
</tr>
<tr>
<td>3.14 (6.96 - 10.1)</td>
<td>8.72</td>
</tr>
<tr>
<td>3.29 (9.16 - 12.45)</td>
<td>10.34</td>
</tr>
<tr>
<td>3.54 (7.49 - 11.04)</td>
<td>9.03</td>
</tr>
<tr>
<td>3.34 (3.81 - 7.15)</td>
<td>6.38</td>
</tr>
<tr>
<td>4.4 (5.91 - 10.31)</td>
<td>8.25</td>
</tr>
<tr>
<td>1.1 (8.71 - 9.81)</td>
<td>9.09</td>
</tr>
<tr>
<td>3.65 (4.9 - 8.55)</td>
<td>6.32</td>
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### miR-30a E2F3

<table>
<thead>
<tr>
<th>IQR (Q1-Q3)</th>
<th>Mean</th>
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<tr>
<td>2.17 (8.68 - 10.86)</td>
<td>9.77</td>
</tr>
<tr>
<td>2.88 (8.72 - 11.6)</td>
<td>9.95</td>
</tr>
<tr>
<td>1.8 (4.79 - 6.59)</td>
<td>5.74</td>
</tr>
<tr>
<td>3.01 (7.94 - 10.95)</td>
<td>9.38</td>
</tr>
<tr>
<td>4.64 (7.41 - 12.05)</td>
<td>9.43</td>
</tr>
<tr>
<td>3.7 (8.16 - 11.86)</td>
<td>9.91</td>
</tr>
<tr>
<td>1.73 (5.17 - 6.9)</td>
<td>5.61</td>
</tr>
<tr>
<td>2.9 (8.42 - 11.32)</td>
<td>9.67</td>
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</tbody>
</table>

### miR-30a ZNF64

<table>
<thead>
<tr>
<th>IQR (Q1-Q3)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.36 (8.63 - 10.99)</td>
<td>9.41</td>
</tr>
<tr>
<td>3.18 (8.06 - 11.24)</td>
<td>9.37</td>
</tr>
<tr>
<td>3.05 (7.74 - 10.78)</td>
<td>8.72</td>
</tr>
<tr>
<td>2.57 (9.1 - 11.67)</td>
<td>10.29</td>
</tr>
<tr>
<td>2.31 (8.69 - 11)</td>
<td>9.91</td>
</tr>
<tr>
<td>1.49 (10.26 - 11.75)</td>
<td>10.91</td>
</tr>
<tr>
<td>1.9 (8.97 - 10.87)</td>
<td>9.91</td>
</tr>
<tr>
<td>2.27 (9.87 - 12.14)</td>
<td>10.92</td>
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</table>

### Table S7.6: MDM2 and CDKN2A qRT-PCR measurements for all patients.

<table>
<thead>
<tr>
<th>Class comparison</th>
<th>log2(FC)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDM2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muc vs End</td>
<td>-4.30</td>
<td>1.69E-21</td>
</tr>
<tr>
<td>Muc vs Ser</td>
<td>-4.07</td>
<td>3.70E-19</td>
</tr>
<tr>
<td>Muc vs Cc</td>
<td>-4.26</td>
<td>2.41E-12</td>
</tr>
<tr>
<td><strong>CDKN2A</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muc vs End</td>
<td>3.67</td>
<td>1.57E-13</td>
</tr>
<tr>
<td>Muc vs Ser</td>
<td>4.48</td>
<td>2.09E-16</td>
</tr>
<tr>
<td>Muc vs Cc</td>
<td>5.65</td>
<td>3.86E-14</td>
</tr>
</tbody>
</table>
Sub-session C: gene and miRNA qRT-PCR expression value analysis excluding borderline samples

Table S7.7: qRT-PCR analysis of miRNAs in training and validation set. The logarithmic fold change of class 1 average expression value versus class 2 average expression value (log₂(FC)) of selected miRNAs. P-values<0.05 were considered significant. Mucinous (Muc), clear cell (Cc), serous (Ser), endometrioid (End).

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Class comparisons</th>
<th>Training set</th>
<th>Validation set</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>log₂(FC)</td>
<td>p-value</td>
<td>log₂(FC)</td>
</tr>
<tr>
<td>Mucinous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsa-miR-192</td>
<td>Muc vs. Cc</td>
<td>8.508973</td>
<td>2.42E-15</td>
</tr>
<tr>
<td></td>
<td>Muc vs. End</td>
<td>6.777230</td>
<td>7.03E-11</td>
</tr>
<tr>
<td></td>
<td>Muc vs. Ser</td>
<td>8.535057</td>
<td>8.82E-17</td>
</tr>
<tr>
<td>hsa-miR-194</td>
<td>Muc vs. Cc</td>
<td>5.802759</td>
<td>5.62E-08</td>
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<tr>
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<td>Muc vs. End</td>
<td>5.225740</td>
<td>2.93E-08</td>
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<tr>
<td></td>
<td>Muc vs. Ser</td>
<td>7.017255</td>
<td>4.90E-13</td>
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<tr>
<td>hsa-miR-30a</td>
<td>Cc vs. End</td>
<td>5.246976</td>
<td>1.67E-19</td>
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<tr>
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<td>Cc vs. Muc</td>
<td>5.380313</td>
<td>1.99E-15</td>
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<tr>
<td></td>
<td>Cc vs. Ser</td>
<td>5.888193</td>
<td>3.24E-19</td>
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<tr>
<td>hsa-miR-30a*</td>
<td>Cc vs. End</td>
<td>5.513418</td>
<td>2.48E-09</td>
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<td>Cc vs. Muc</td>
<td>6.481336</td>
<td>6.36E-10</td>
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<tr>
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<td>Cc vs. Ser</td>
<td>7.723055</td>
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Table S7.8: qRT-PCR measurements in all patients (training and validation set).

<table>
<thead>
<tr>
<th>Class comparison</th>
<th>log₂(FC)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDM2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muc vs. End</td>
<td>-4.019541012</td>
<td>7.85E-19</td>
</tr>
<tr>
<td>Muc vs. Ser</td>
<td>-3.841076385</td>
<td>3.48E-15</td>
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<tr>
<td>Muc vs. Cc</td>
<td>-3.951486255</td>
<td>4.60E-11</td>
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<tr>
<td>CDKN2A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muc vs. End</td>
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<td>PSME3</td>
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<td>1.35E-09</td>
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<tr>
<td>Muc vs. Cc</td>
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<tr>
<td>CUL4A</td>
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<td>Muc vs. Cc</td>
<td>-2.915366796</td>
<td>2.28E-08</td>
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</tbody>
</table>
Sub-session D: Materials and methods of luciferase assay procedures and results.

PSME3, CUL4A and BMI1 3’UTR containing predicted miRNA binding site for miR-194, were amplified by PCR using Advantage2 polymerase Mix (Clontech, Palo Alto, CA US) and cloned into pmirGLO or psiCHECK-2 reporter plasmid (Promega, Milano, Italy), immediately downstream of the reporter luciferase gene, thus forming pmirGLO-CUL4 (pCUL4), pmirGLO-PSME3 (pPSME3) and psiCHECK-BMI1 (pBMI1). Using primers pair sequences reported in S7, PCR amplification conditions were: 95°C for 1 min (1 cycle); 95°C for 30 sec, Ta for 1 min, 68°C for 2 min (40 cycles), and a final extension step at 68°C for 1 min. Human embryonic kidney (HEK) 293 cells, originally obtained from American Type Culture Collection, were routinely grown at 37°C, 5% CO2 in DMEM medium (Life Technologies, Milano Italy) supplemented with 10% fetal bovine serum (Lonza, Milano, Italy) and 1% L-Glutamine 200mM (Biowest, Milano, Italy). Sub-confluent HEK 293 cells, seeded in 96-well reaction plates, were co-transfected with the above described plasmids (10 ng) and with 2 pmol of synthesized, double-stranded miRNA-194 (miScript miRNA Mimic, Qiagen) or siRNA scramble (AllStar Negative Control siRNA, Qiagen) using Lipofectamine 2000 (Life Technologies). Twenty-four hours after transfection, firefly and renilla luciferase activity was measured consecutively in a microplate reader (Tecan Infinite®M200, Milano, Italy) using the Dual Luciferase Assay System (Promega). Co-traasfection of psiCHECK-ZEB1 (pZEB1), created subcloning the 3’UTR of the ZEB1 gene in the psiCHECK-2 vector, along with synthetic miR-200c (miScript miRNA Mimic, Qiagen) was used as positive control. Empty vectors were transfected as negative controls. Each sample was assayed in six replicates and repeated twice.

![Figure S7.4: Dual-luciferase assay of HEK 293 cell line transfected with luciferase constructs containing the 3’UTR sequence of genes predicted to be regulated by miR-194 (pCUL4A, pPSME3, pBMI1), together with a synthetic miR-194 (synth miRNA) or with a control siRNA with scrambled sequence (scramble ctr). Empty vectors (pGLO and psiCHECK-2) were used as negative controls. Co-transfection of miR-200c with vector containing the 3’UTR of ZEB1 was used as a positive control. Data](image-url)
represent mean and s.e.m of six experiments. Significance was assessed by Student's t test. * $p < 0.05$; ** $p < 0.001$. 