Supplementary Figures

Supplementary Figure S1: Akt3 is specific for ESC survival

R1-ESCs cultured in 20%FBS plus LIF (FBS/LIF) or 2i/LIF medium were treated with lentiviral shCtl, shAkt1, shAkt2, shAkt1 plus -2 (shAkt1/2), shAkt3, or shAkt1, -2, plus -3 (shAkt1/2/3) for 3 days (Bar = 250 μm).
Supplementary Figure S2: Akt3 is specific for ESC Proliferation

**S2A.** R1-ESCs expressing pMCs vector control (Ctl/R1) or KD-Akt3 (KD-Akt3/R1) were seeded in 12-well-plates and grown in 2i/LIF medium on day 0, and cell number counted 3 days later.

**S2B.** R1-ESCs were seeded in 12-well-plates and grown in 2i/LIF medium on day 0, cultured with 0, 20, or 40 μM LY294002 on day 2 for 24 h, and subjected to Annexin V apoptosis analysis on day 3 (**P < 0.01, n = 2).

**S2C.** R1-ESCs were seeded in 12-well-plates and grown in 2i/LIF medium on day 0, cultured with 0, 20, or 40 μM LY294002 on day 2 for 24 h, and subjected to cell cycle analysis on day 3 (**P < 0.01, n = 2).
Supplementary Figure S3: Akt3 specifically regulates p53 protein expression

S3

Left panel: Western-blotting of ESCs treated by shCtl or shAkt1 plus shAkt2 (shAkt1/2). Right panel: Western-blotting of ESCs treated by either shCtl or shAkt1 plus shAkt2 and shAkt3 (shAkt1/2/3).
Supplementary Figure S4: p53 is important in Akt3 mediated ESC survival

S4A. qRT-PCR analysis of RNAs from MEFs or R1-ESCs stably expressing lentiviral shCtl or shp53. All values were normalized to GAPDH and relative to the expression of shCtl cells.

S4B. shp53/R1 and shCtl/R1-ESC lines treated with lentiviral shCtl or shAkt3 for 3 days and cultured in 2i/LIF medium (Bar = 250 μm).

S4C. ESCs as described in (S7) were incubated with Annexin V-FITC (Annex) and PI for 30 min and analyzed by flow cytometry. Percentages of live, early, and late apoptotic cells are shown (*P < 0.05, n = 2).

S4D. qRT-PCR analysis of RNAs from R1-ESCs stably expressing lentiviral shCtl or different shp21 constructs (-a and -b). All values were normalized to GAPDH and relative to the expression of shCtl ESCs.
**Supplementary Table S1: Sequences for shRNA Constructs**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’–3’)</th>
<th>Accession Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>shAkt1</td>
<td>GCACATCAAGATAACGGACTT</td>
<td>NM_009652.3</td>
</tr>
<tr>
<td>shAkt2</td>
<td>GCTCATTCTTATGGAGGAGAT</td>
<td>NM_001110208.1</td>
</tr>
<tr>
<td>shAkt3a</td>
<td>GCTCATTCATAGGCTATAAGG</td>
<td>NM_011785.3</td>
</tr>
<tr>
<td>shAkt3b</td>
<td>GGATGCGTCTACAACCCCATCA</td>
<td>NM_011785.3</td>
</tr>
<tr>
<td>shAkt3d</td>
<td>GCTTAGGGTTCTGGCAATTGA</td>
<td>NM_011785.3</td>
</tr>
</tbody>
</table>

**Supplementary Table S2: Primers for real time qRT-PCR**

Click here to Download Table S2