Primers for Chromatin Immunoprecipitation (ChIP) Assays

ChIP assays were performed as described in the materials and methods section. The two additional pairs of primers for the proximal regions of the mouse Pim1 promoter used in chromatin immunoprecipitation (ChIP), both of which failed to show enrichment of that gene segment by IP with anti-HOXA9 antibody, are listed below:

**Pair #1**: forward, 5' ‐ GGAGTCTCTGCGCATCTTTTA ‐ 3' (-1402 to –1383), reverse, 5'-CGAGTCGCTTTTACATCTTTTG-3'(-1136 to –1116);
**Pair #2**: forward, 5'-CACACCTTGCTACGGCTCCAG-3' (-481 to –462), reverse, 5'-CCAACCAGAGACGCCTTAT-3' (-184 to –165).

Quantitative real-time PCR (Q RT-PCR) analysis

PCR amplification was performed in a 10-µl final volume containing 1× SYBR Green PCR buffer, 2 mM magnesium chloride, 0.5 mM dNTP mix with dUTP, 8 ng of each primer, 0.1 U AmpErase UNG, 0.25 U AmpliTaq Gold, and 1 µl of cDNA templates (cDNA equivalent of 50 cells per reaction) using 50°C for 2 min and 95°C for 10 min, followed by 45 cycles at 95°C for 15 s and 60°C for 1 min. Primers were designed using Primer Express software (Applied Biosystems, Foster City, California). Each amplification was performed in triplicate. The primer sequences were as follows:

**HOXA9:**
Human - forward, 5'‐AAAACAATGCTGGAATGAGAGCG-3', reverse, 5'-TGGTGTTTTGTAAGGGGGCACC-3'
Mouse - forward, 5'-TGGCCGAACCCCG-3', reverse, 5'-CACAAACACCGCCGC-3'

**Pim1:**
Human - forward, 5'-TGCACTGCTTGCTTCT-3', reverse, 5'-TAATTATCCTATATACACAGTCAGCAATGC-3'
Mouse - forward, 5'-TGCTCTCTGGCCTCCCAACA-3', reverse, 5'-GGACCTGGAGTCTGGAATGAGTT-3'

**Control genes:**
Mouse GAPDH - forward, 5' TGC ACC ACC AAC TGC TTA G reverse, 3' GAT GCA GGG ATG ATG TTC
Human GAPDH primers were purchased from Applied Biosystems and used at a final concentration of 250 nM.

Mean Red Blood Cell Volume

Peripheral blood from wild-type and mutant adult animals was analyzed using an automatic Hemavet® analyzer (Drew Scientific, Oxford, CT):

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>MCV</th>
<th>Standard Deviation</th>
<th>S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A9+/+</td>
<td>18</td>
<td>46.0*</td>
<td>1.4</td>
<td>0.3</td>
</tr>
<tr>
<td>A9/-</td>
<td>19</td>
<td>44.9*</td>
<td>1.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Difference:</td>
<td>1.1</td>
<td>*P = .014</td>
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