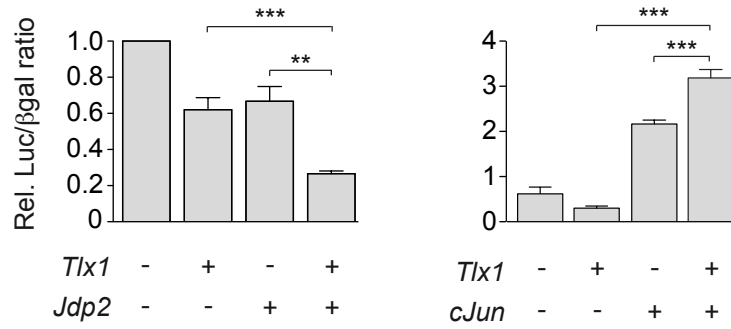


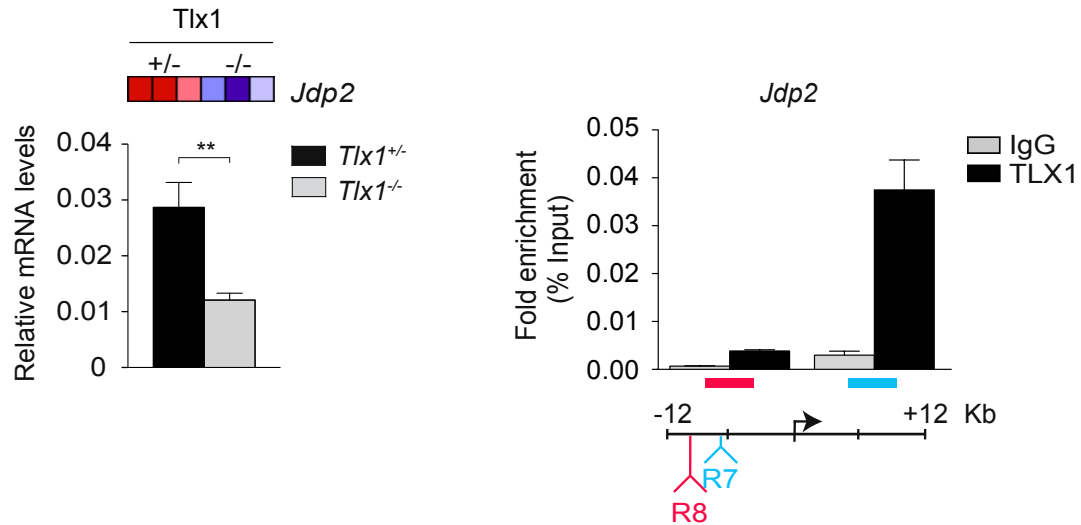
**Figure S1. Gene ontology analysis**

Selected gene ontology terms associated with the gene expression data showed significant differences in the expression of genes connected with developmental processes including spleen development. The color of each node illustrates its significance and is defined in the scale bar, which displays the false discovery rate on a log10 scale.

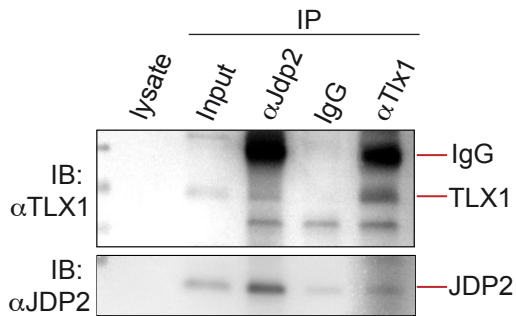
**A**



**B**



**C**

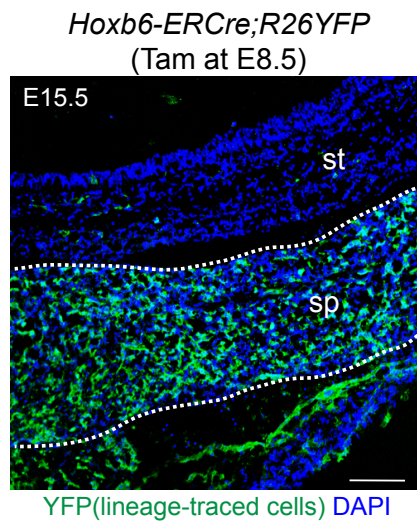


**Figure S2. TLX1 cooperates with the AP1 family member JDP2**

(A) Luciferase activity of an AP1-Luc reporter system was assessed at 48 hrs in NIH3T3 cells transiently transfected with *Tlx1* in combination with *Jdp2* or *cJun* expression vectors. The means of triplicates ± SD are shown, \*\*p<0.01 \*\*\*p<0.001(2-tailed Student’s t test). Data are representative of one out of three independent experiments.

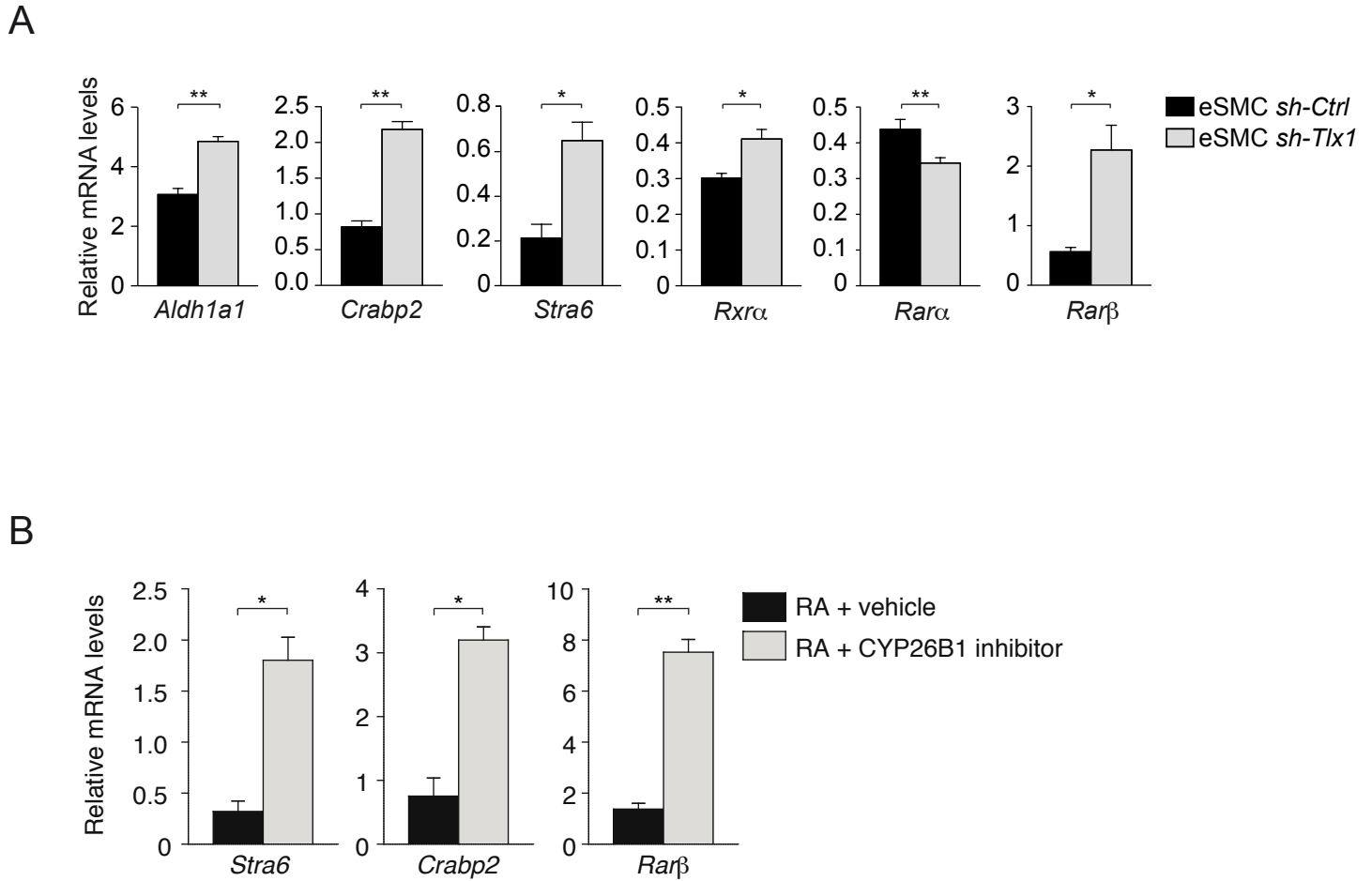
(B) Heat map and validation of *Jdp2* expression by qPCRs in E13.5 *Tlx1*<sup>+/-</sup> and *Tlx1*<sup>-/-</sup> embryonic spleens. The means of triplicates ± SD are shown, \*\*p<0.01 (2-tailed Student’s t test). Data are representative of two independent experiments. ChIP-qPCR analysis of TLX1 binding to the *Jdp2* locus. Negative (R7) and positive (R8 and R9) regions are indicated relative to the gene locus. Data are normalized to the amplification of the input chromatin. Data are representative of one out of two different experiments.

(C) Protein extracts from 293T cells transiently expressing JDP2 and TLX1 were immunoprecipitated (IP) and immunoblotted (IB) with anti-JDP2, anti-TLX1 or control antibodies respectively. Data are representative of one out of two different independent experiments.



**Figure S3. Contribution of the LPM to the developing splenic mesenchyme**

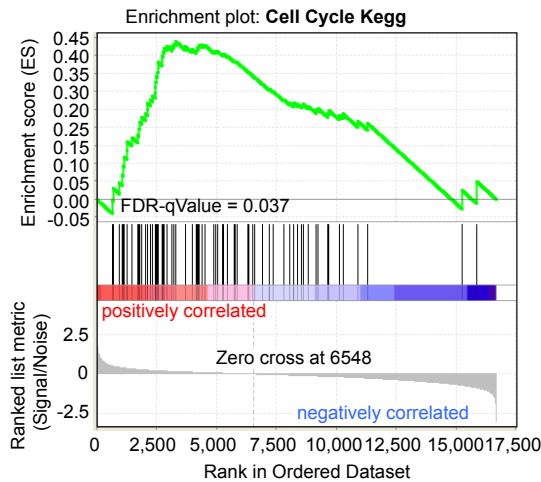
Representative confocal images of E15.5 spleen sagittal sections from *HoxB6-ERCre;R26YFP* embryos injected with tamoxifen at E8.5 and stained with anti-YFP antibody to show the contribution of LPM cells of the *Hoxb6*<sup>+</sup> lineage (green, lineage-traced cells) to the developing spleen. Scale bars represent 100  $\mu$ m. Data are representative of one embryo out of 3 embryos analyzed. st, stomach; sp, spleen.



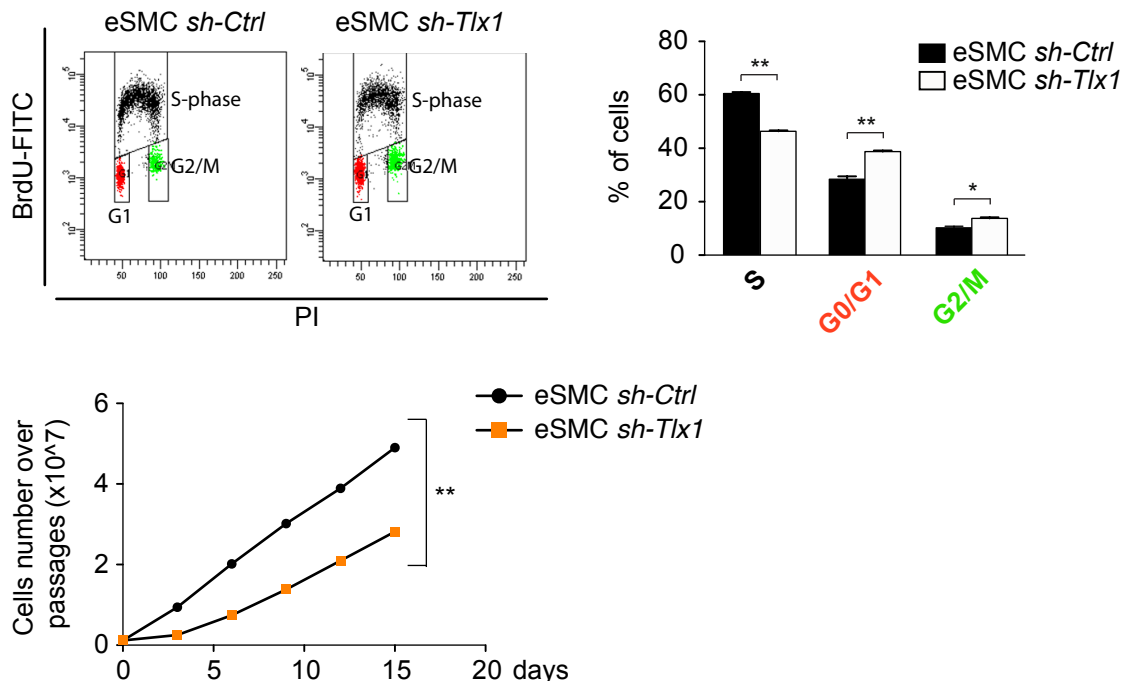
**Figure S4. Loss of CYP26B1 activity causes deregulation of the RA signaling pathway**

Expression of *Stra6*, *Crabp2* and *Rarb* by qPCR in eSMCs cultured for 24hrs in the presence of 1 $\mu$ M atRA + vehicle or 1 $\mu$ M atRA + 1 $\mu$ M R116010 (a Cyp26b1 inhibitor). The means of triplicates  $\pm$  SD are shown, \*\*\*p<0.001 (2-tailed Student's t test). Data are representative of one out of three independent experiments.

A



B



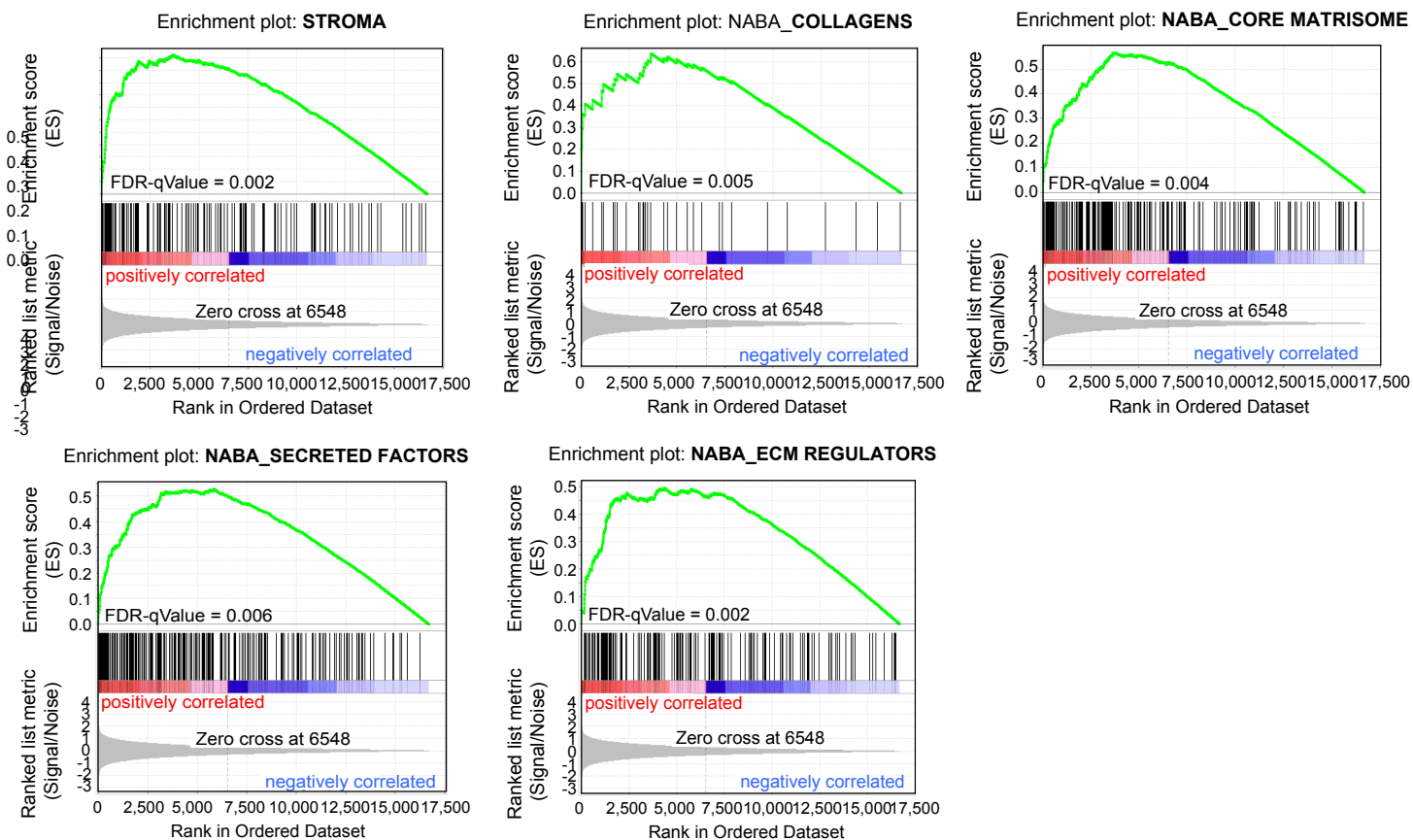
### Figure S5. Loss of *Tlx1* is associated with cell-cycle defects and reduced proliferation

A) GSEA enrichment plots of differentially expressed genes belonging to the Kegg-Cell Cycle pathway associated with loss of *Tlx1*.

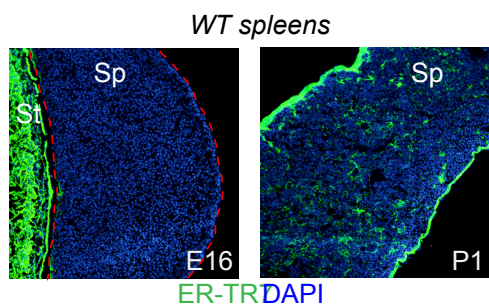
B) BrdU analysis of asynchronous eSMCs line expressing *sh-Tlx1* or *sh-Ctrl* (upper panels). The means of triplicates  $\pm$  SD are shown, \* $p < 0.05$  \*\* $p < 0.001$  (2-tailed Student's t test). Data are representative of two independent silencing experiments. Growth curve analysis of *sh-Tlx1* (—■—) or *sh-Ctrl* (—●—) eSMCs (lower panel). The means of triplicates  $\pm$  SD are shown, \*\* $p < 0.01$  (2-way Anova). Data are representative of one out of three independent silencing experiments.

C) Validation of RA-associated genes by qPCR was performed in *sh-Ctrl* or *sh-Tlx1* eSMCs. The means of triplicates  $\pm$  SD are shown, \* $p < 0.05$  \*\* $p < 0.01$  (2-tailed Student's t test). Data are representative of one out of three independent silencing experiments.

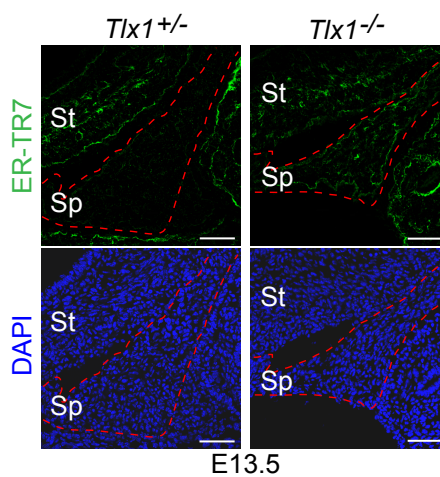
A



B



C



D

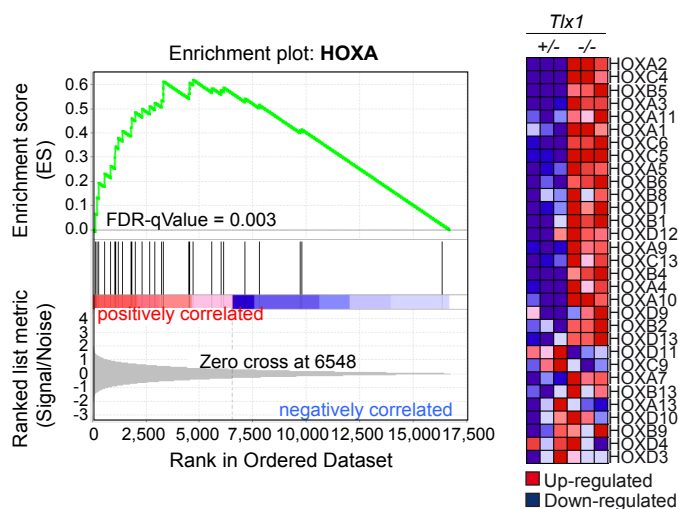


Figure S6. Lenti et al. 2016

**Figure S6. Loss of *Tlx1* promotes cellular differentiation**

(A) GSEA enrichment plots of differentially expressed genes of lymphoid stromal cells and ECM networks during differentiation associated with loss of *Tlx1*.

(B-C) Confocal images of E16 and P1 (B) and E13.5 (C) spleen sagittal sections stained with anti-ER-TR7 antibody (green). Nuclei are visualized by DAPI staining (blue). Dashed red lines indicated the developing spleen. Scale bars represent 50 $\mu$ m. St, stomach; sp, spleen. Data are representative of one embryo out of three analyzed for each genotype.

(D) GSEA enrichment plots and heat maps of differentially expressed genes belonging to the HOXA signature associated with loss of. The bar-code plot indicates the position of the genes on the expression data rank-sorted by its association with *Tlx1* mutants, with red and blue colors indicating over- and under-expression in the RNA.

## Supplemental Tables

**Supplemental Table 1.** AP-1 sequence in RA-associated genes deregulated in GSEA analysis

GENE NAME	GENE ID	CHROMOSOME	START	END	N. OF AP-1 SITES
Rxrg	NM_009107	chr1	169508492	169528592	5
Aldh3a1	NM_007436	chr11	61002243	61022343	7
Rara	NM_009024	chr11	98779009	98799109	6
Rara	NM_001177302	chr11	98779031	98799131	6
Rara	NM_001177303	chr11	98781024	98801124	5
Rarb	NM_011243	chr14	17407624	17427724	8
Crabp2	NM_007759	chr3	87732614	87752714	3
Adh5	NM_007410	chr3	138086127	138106227	4
Aldh1a3	NM_053080	chr7	73572263	73592363	5
Crabp1	NM_013496	chr9	54592614	54612714	8
Stra6	NM_001162475	chr9	57956894	57976994	2
Stra6	NM_009291	chr9	57957155	57977255	2
Stra6	NM_001162476	chr9	57961881	57981981	1
Stra6	NM_001162479	chr9	57962471	57982571	1
Rbp1	NM_011254	chr9	98303379	98323479	2
Rbp2	NM_009034	chr9	98370955	98391055	5
Rarg	NM_001042727	chr15	102076683	102096783	4
Rarg	NM_011244	chr15	102087814	102107914	2
Rxrb	NM_011306	chr17	34148756	34168856	4
Rxrb	NM_001205214	chr17	34148756	34168856	4
Rxrb	NM_001205215	chr17	34149294	34169394	4
Rbp4	NM_001159487	chr19	38199711	38219811	6
Rxra	NM_011305	chr2	27512720	27532820	3
Adh7	NM_009626	chr3	137860736	137880836	4
Aldh1a2	NM_009022	chr9	71043595	71063695	2
Rara	NM_001176528	chr11	98801784	98821884	2
Aldh1a1	NM_013467	chr19	20656471	20676571	1



**Supplemental Table 2.** Sequence of Specific Primers and Probes Used for qPCR Analysis  
(Probe number corresponds to Universal Probe Library – Roche)

<b>Gene</b>	<b>Forward Sequence</b>	<b>Reverse Sequence</b>	<b>Probe N°</b>
<i>Tlx1</i>	CGGGTGTCACAACCTCA	CCGGTTCTGATAGGGGTGA	#58
<i>Cyp26b1</i>	ACATCCACCGCAACAAGC	GGGCAGGTAGCTCTCAAGTG	#41
<i>Raldh1</i>	GCTGAACAAGCTGGCTGAC	CCATTGAGTGCCTCCATTGTA	#84
<i>Stra6</i>	GGAAGCCTGCAAGGTGAAT	CCATGCTCCAGCTCTTCTTC	#71
<i>Rpb2</i>	AGTGGGTGGAGGGAGACAA	TGAACACTTGTCGGCACAC	#1
<i>Crabp2</i>	TTGAGGAAATGCTAAAAGCTCTG	TCCTGTTTGATCTCGACTGCT	#75
<i>RARalpha</i>	AGACACGCAGACGGGTTG	GAGGATGCCACTCCCAGA	#83
<i>RARbeta</i>	CACCGGCATACTGCTCAA	CAAACGAAGCAGGGCTTG	#63
<i>RARgamma</i>	TTTCCACCAGGTCCCTCAC	CTGTCCAGTGGGTTTCCAAG	#104
<i>RXRalpha</i>	AGGGGTGGCTGTGGGTAG	TCTCCCTCCCTTCTTGAGTC	#26
<i>Jdp2</i>	AAGAAGGAACGCACAGAGTTTC	CGTCTTCAGCTCTGCGTTC	#19
<i>Desmin</i>	GCCACCTACCGGAAGCTACT	GCAGAGAAGGTCTGGATAGGAA	#15
<i>Vegf-A</i>	ACTGGACCCTGGCTTTACTG	TCTGCTCTCCTTCTGTCGTG	#22
<i>Laminin B1</i>	TTGCGTGTGTTTGTGATCCT	ATCCAGAGGCACAGTCATCA	#50
<i>Collagen4a1</i>	TTAAAGGACTCCAGGGACCAC	CCCCTGAGCCTGTACAC	#56
<i>Nkx2-5</i>	CGCCTTTCTCAGTCAAAGACA	CAGACAGGTCCCCAGACG	#1
<i>Sfl</i>	CCAGGAGTTCGTCTGTCTCAA	GGCTGTGGTTGTTTCAGGAAT	#41
<i>Rpl13a</i>	CCCTCCACCCTATGACAAGA	GTAGGCTTCAGCCGAACAAC	#108

**Supplemental Table 3.** Sequence of Specific Primers and Probes Used for qChIP Analysis  
(Probe number corresponds to Universal Probe Library – Roche)

<b>Gene</b>	<b>Forward Sequence</b>	<b>Reverse Sequence</b>	<b>Probe N°</b>
<i>CYP26b1 (R1)</i>	AGACGGGCTCTTTCTGTGAA	GTCACCCAGTTCGCCAAA	#25
<i>CYP26b1 (R2)</i>	AGCGCCATCTCTTCGACA	GATGGGGTATGGGCAGTG	#5
<i>RXRα (R3)</i>	GCAAGGGAGAGCAGCACTT	GGAGTTTGCTGGGTGCTTC	#2
<i>RXRα (R4)</i>	GTCATGTTCCCACTCCAGAAC	CCCAGGAAAGGGCAGGTA	#67
<i>RARα (R5)</i>	GGAGCTGGGCAGTAGTGAGA	GGCTTCAGTACTCAGGCTTCA	#110
<i>RARα (R6)</i>	GGTGGGCTGATAGGCTCTG	GGAACCCACCAAACCAAAAT	#67
<i>JDP2 (R7)</i>	TGGGTCAGACTCTTTTCATGTG	GCCAGTCTTCCCTAAGATTTGAT	#49
<i>JDP2 (R8)</i>	CAATGTGGAGACTCCCTCAAC	GTCAGCCAGTGTGTGCTGTT	#25





**Supplemental sequence 3. *RARα* 5' flanking region (from -9500bp to -3947bp)**

-9500bp      tctttgcagattgcctagccctccactccactccatctcacctagacagaaggtaacctgactgaaaaagtg  
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 ggactttgctccactccctataaaggagcccaggtctcctccaggtgcaggacacactgggtgcccaagggttt  
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 agtgtgtgagttcaatcctacatctgcacctctgcaccgacaaactgtaagac

**RARa (R5) positive region**

**RARa (R6) negative region**

**AP-1 site**

**Supplemental sequence 4. *JDP2* 5' flanking region (from -9300bp to ATG)**

-9300bp      gagaccagcctggggtaccaagaggctgtgtcccctggcctcccaatggggtgggctctcat  
gtgtgagatcctgaggccagccagagaggctctcttacaggagggcattaatttcagccccactgtcagaag  
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-8721bp      tcctcccttcataaacatttactgaaataactttctgctgatggtgttttaagacctgcagcagaaaagg-----  
-----  
-6260bp      ----  
tgactaacttaactgtgcaccatgagaaagcagttccaacggctcctgcatttagtctcacatcaggggtgagt  
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-----  
-300bp      ----  
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0bp            ctctcgggagccgCGCGGGCGCGCCGCGGACCGCA**ATG**

**JDP2 (R7) positive region**

**JDP2 (R8) negative region**

**AP-1 site**

