**Supplemental Material to:**

**Heterodimerization of AML1/ETO with CBF is required for leukemogenesis but not for myeloproliferation**

Verena Thiel1#, Benedetto Daniele Giaimo2#, Peggy Schwarz1, Karin Soller3, Virag Vas3, Marek Bartkuhn4, Tamara J. Blätte5, Konstanze Döhner5, Lars Bullinger5, Tilman Borggrefe2, Hartmut Geiger3, 6\*, Franz Oswald1\*

1University Medical Center Ulm, Center for Internal Medicine, Department of Internal Medicine I, University of Ulm, 89081 Ulm, Germany

2Institute of Biochemistry, University of Giessen, 35392 Giessen, Germany

3Institute of Molecular Medicine, University Ulm, 89081 Ulm, Germany

4Institute for Genetics, University of Giessen, 35392 Giessen, Germany

5University Medical Center Ulm, Center for Internal Medicine, Department of Internal Medicine III, University of Ulm, 89081 Ulm, Germany

6Division of Experimental Hematology and Cancer Biology, CCHMC, Cincinnati, USA

#these authors contributed equally to this work

\*To whom correspondence should be addressed: Franz Oswald, University Medical Center Ulm, Center for Internal Medicine, Department of Internal Medicine I, University of Ulm, Albert-Einstein-Allee 23, 89081 Ulm, Germany, email: franz.oswald@uni-ulm.de, Tel.: ++49-731-50044544; Fax: ++49-731-50044502

Correspondence may also be addressed to: Hartmut Geiger, Institute of Molecular Medicine, University Ulm, James Franck-Ring 11c, 89081 Ulm, Germany, email: hartmut.geiger@uni-ulm.de, Tel.: ++49-731-5057650; Fax: ++49-731-5057651

**Files in this Data supplement:**

Supplementary Figure Legends 1, 2, 3, 4, 5, 6 and 7

Supplementary Materials and Methods

Supplementary references

Supplementary Table 1

**Supplementary Figure Legends**

**Supplementary Figure 1.** Expression of alternative Exone 9a in primary human AML cases with t(8;12). (A) Integrated genome viewer snapshot (IGV; http://software.broadinstitute.org/software/igv/) of RNAseq reads for a 10 kb region of *RUNX1T1* [chr8:92,987,992-92,998,705 (hg19); x-axis] demonstrating the expression of an alternative exon 9a (indicated by red box) in three different t(8;21) positive primary AML cases (three upper lines, indicated by red bar), whereas three cytogenetically normal (CN) AML cases with a *NPM1* mutation (*NPM1*mut) showed no alternative exon 9a reads (the y-axis displays the read coverage at a given position). (B) Normalized expression of *RUNX1T1* in t(8;21) positive AML cases (n=10) versus inv(16) positive AML (n=11) and *NPM1*mut AML (n=12) displayed as read counts per million; t(8;21) positive cases display on average approx. 50 reads/million sequenced in comparison to almost no reads in the non-t(8;21) cases. (C) Bar plot demonstrating the average number (#) of reads per base for exons 9 and 9a (grey bar) with the black bar indicating the number of reads for the alternative exon 9a. On average the proportion of alternative exon 9a to normal exon 9 was 24% across the n=10 t(8;21) cases. (D, E) Expression of Runt and Runt NT (D) as well as AE9a and AE9aNT (E) proteins used in Fig. 1C was verified by Western blotting using an anti-Flag antibody. HEK-293 cells were transfected with plasmids encoding for of Flag-tagged Runt, Runt NT, AE9a or AE9aNT. Lysates from untransfected cells served as a negative control.

**Supplementary Figure 2.** Subcellular localization of AE9a-GFP constructs used for coimmunoprecipitation experiments and confocal laser scanning microscopy. N-terminally Flag-tagged AE9a-GFP proteins (Flag-AE9a-GFP, Flag-AE9aNT-GFP) and N-terminally untagged AE9a proteins (AE9a-GFP, AE9aNT-GFP) predominantly localize to the nucleus. HeLa cells were analyzed by fluorescence microscopy 24 h post-transfection. Scale bar, 10 µm.

**Supplementary Figure 3.** (A) The SPOC domain of SHARP interacts with wildtype AE9a (AE9a, lane 4, upper), and double mutant AE9a (AE9aNT, lane 5, upper) in co-immunoprecipitation experiments. Input controls are shown in the middle (AE9a and AE9aNT) and the lower panel (SHARP SPOC-domain) panels. HEK293 cells were transfected with the indicated expression constructs for Flag-tagged SPOC domain of SHARP and GFP-tagged AE9a or AE9aNT, respectively. The asterisk denotes the light chain of the antibody used for immunoprecipitation. (B) AE9a mediated activation of RBPJ-SPOC-dependent transcription decreases in a dose dependent manner after expression of the wild type but not CBF binding defective Runt domain of AML1 (Runt wt and Runt NT, respectively) in HeLa cells. Activity of AE9a was tested in luciferase assays using the RBPJ-dependent reporter construct pGA891/6 and RBP-SPOC fusion protein together with AE9a and increasing amounts of Runt wt or Runt NT, respectively. Mean values ± SD (error bars) based on at least three independent experiments are shown ([ns] not significant, [\*\*] P < 0.01, [\*\*\*] P < 0.001, unpaired Student’s *t*-test). (C) CBF is expressed in HeLa cells. Western blotting was performed with the indicated amount of HeLa cell lysates using an anti CBF antibody. (D) RBP-J is enriched at the Notch-responsive elements of Notch target genes. The occupancy of RBP-J was analyzed by ChIP-qPCR at the Notch-responsive elements of *Hey1* (-0.8kb), *Hes1* (+0.6kb) and *Nrarp* (-3kb) Notch target genes in HoxB4 cells. *Nrarp +1.9kb* was used as negative control. Shown is the mean ± SD of a representative experiment measured three times ([\*\*] P < 0.01, [\*\*\*] P < 0.001, unpaired Student’s *t*-test). (E) Schematic representation of the Notch-responsive elements (red boxes) of *Hey1* (-0.8kb), *Hes1* (+0.6kb) and *Nrarp* (-3kb) Notch target genes analyzed in Fig. S3D.

**Supplementary Figure 4.** (A)Schematic overview of the *in vivo* experiments. C57BL/6 Ly5.2 (BL6) mice were injected with 5-FU. After 3 days, bone marrow (BM) cells were harvested and retrovirally transduced either with IRES-GFP (control), AE9a-IRES-GFP or AE9aNT-IRES-GFP. Sorted GFP+ cells were tested in colony forming cell assays. In a second approach, unsorted bone marrow cells were transplanted into lethally irradiated (11 Gy) C57BL/6 Ly5.1 (BoyJ) mice. (B) Western blotting showing the expression of AE9a and AE9aNT after retroviral transduction with the indicated constructs. AE9a and AE9aNT were detected by using an anti-AML1 antibody whereas an anti-GFP was used to detect GFP as loading control.

**Supplementary Figure 5.** Engraftment and GFP expression in transplanted mice.Peripheral blood (PB) was analyzed by FACS at the indicated time points post-transplant for (A) level of engraftment of transplanted bone marrow cells by assessing the percentage of Ly5.1- cells and (B) for GFP expression in the Ly5.1- cell population in mice transplanted with either GFP (control, green), AE9a (red) or AE9aNT (blue). Mean values based on the number of animals.

**Supplementary Figure 6.** (A) White blood cell count (WBC) increased in AE9a but not in AE9aNT mice over time. WBC was monitored at given time points from peripheral blood (PB) in mice transplanted either with GFP (control, green), AE9a (red) or AE9a NT (blue). Mean values based on numbers of animals. (B) Expression of *AE9a* and *AE9aNT* in BM 214 days post transplantation. (C) Notch target genes are de-repressed in BM from AE9a but not AE9aNT transduced mice. Total RNA from BM (214 days post-transplantation) of IRES-GFP, AE9a or AE9aNT mice was isolated, reverse transcribed in cDNA and analyzed via qPCR using primers for *Hey1, Hes1, Nrarp, Igf1r, Gpr56 or -actin*. Data were normalized to the housekeeping genes *Hypoxanthine-guanine phosphoribosyltransferase* (HPRT) in (B) and *Glyceraldehyde-3-Phosphate-Dehydrogenase* (GAPDH) in (C). nd, not detectable.Mean values ± SD (error bars) of at least five mice per cohort are shown, ([ns] not significant, [\*] P < 0.05, [\*\*\*] P < 0.001, unpaired Student’s *t*-test.

**Supplementary Figure 7.** AE is enriched at RBP-J-bound regions of *Hes1* (A) and *Hey1* (B). Publically available ChIP-Seq data were analyzed to identify the regions bound by Notch-1 and RBP-J. Subsequently, the Notch-1/RBP-J-bound regions were analyzed for the occupancy of AE in MCF7 or K562 cells. Coverage vectors of publicly available ChIP-Seq experiments for Notch1 and RBP-J (blue - in CUTLL1 cells) as wells as AE (green - in MCF7 and K562 cells) along with identified binding site intervals (rectangles) are shown. Grey shaded area indicates genomic regions characterized by overlapping Notch1/RBP-J/AE signals.

**Supplementary Materials and Methods:**

Retroviral infection of HoxB4 cells

Retroviral infection of HoxB4 cells was achieved as follows: Briefly, 5 x 106 *PhoenixT*M cells were plated in a 19 cm dish and incubated for 16-24 h at 37°C. The day after, 20 g of plasmid DNA were added to 860 l of sterile H2O and 120 l of 2 M CaCl2 were pipetted into the DNA solution and briefly vortexed. To obtain small precipitates, the DNA solution was added dropwise to 1 ml of 2x HBS (50 mM HEPES pH 7.05, 10 mM KCl, 12 mM Glucose, 280 mM NaCl, 1.5 mM Na2HPO4) while vortexing and the resulting solution was incubated 20 min at room temperature. In the meantime, *PhoenixTM* cells were incubated with 25 M of chloroquine (Sigma-Aldrich C6628-100G) for 10 minutes at room temperature. The DNA solution was added to the cells and after at least 6 h of incubation at 37°C the medium was replaced. 24 h after transfection, the retrovirus containing supernatant was filtered and supplemented with polybrene (Sigma-Aldrich H9268) at a final concentration of 2 g/ml. The retroviral suspension was added to the HoxB4 cells and the cell/retroviral suspension was centrifuged 45 min at 1800 rpm at 37°C. After centrifugation, the medium was replaced with fresh one. The infection was repeated four times over two days. Cells were grown in culture, analyzed by FACS and sorted.

Retroviral gene transfer and bone marrow transplantation

Three days after injection of 5-FU (135 mg/kg of body weight, i.p.), femora and tibiae were isolated from 4-6 weeks old C57BL/6 mice and flushed. Mononuclear BM cells were isolated by low-density centrifugation on Histopaque (Sigma-Aldrich, #1083). Cells at a density of 1.7-2x107 cells/well were pre-stimulated in non-tissue culture 6-well dishes for 2 days in IMDM medium (Lonza) supplemented with 10% FBS (HyClone, Thermo Scientific), 100 ng/ml mSCF, 100 ng/ml TPO, 100 ng/ml G-CSF (Prospec, Tany TechnoGene Ltd.), 1% penicillin/streptomycin and 2 mM glutamine. Transduction was performed on RetroNectin-coated (9.5 mg/cm2; TaKaRa) non-tissue culture dishes. Virus preloading was carried out with retrovirus containing supernatants by centrifugation (40 min, at 4° C, 1300 g). The pre-stimulated BM cells were then transduced twice with fresh thawed retrovirus containing supernatants supplemented with cytokines (100 ng/ml mSCF, TPO, G-CSF) overnight at a MOI of 0.8 ± 0.37 among our experiments. After 2 days, transduction efficiency was measured by flow cytometry. In three independent experiments, the graft contained: 24.0%, 26.1%, 8.1% AE9a GFP+ cells; 37.1%, 42.8%, 9.7% AE9aNT GFP+ cells; and 22.1%, 23.2%, 23% GFP+ control cells. Recipient B6.SJL-*Ptprca Pep3b*/BoyJ mice were irradiated with 11 Gy and approximately 106 cells/recipient were transplanted by retro-orbital injection.

Flow cytometry analysis of PB, BM and spleen samples

PB was incubated with anti CD45.1/Ly5.1 (PE conjugated monoclonal mouse anti-mouse IgG, A20, BD Pharmingen), anti-CD45R/B220 (APC conjugated monoclonal rat anti-mouse IgG, RA3-6B2, BD Pharmingen), anti-CD11b (Alexafluor-700 conjugated monoclonal rat anti-mouse IgG, M1/70, eBioscience), anti-CD3e (PE-Cy7 conjugated monoclonal hamster anti-mouse IgG, 145-2C11, eBioscinece), anti-Ly-6G/Ly-6C (Gr-1) (eFluor450 conjugated monoclonal rat anti-mouse IgG, RB6-8C5, eBioscinece) and anti-CD117/c-kit (PerCP-Cy5.5 conjugated monoclonal rat anti-mouse IgG, 2B8, BD Pharmingen) for 20 min on ice.

For end point in-depth analysis, mice were sacrified and BM and spleen cells were isolated. For stem/progenitor cell measurement and in order to exclude Lin+ cells, mononuclear cells (Histopaque, Sigma-Aldrich, #1083) were incubated with a cocktail of biotinylated antibodies directed against specific surface molecules: anti-CD11b (clone M1/70), anti-B220 (clone RA3-6B2), anti-CD5 (clone 53-7.3) anti-Gr-1 (clone RB6-8C5), anti-Ter119, as well as anti-CD8a (Clone 53-6.7) and Streptavidin-APC-Cy7 as secondary antibody (all from BD Pharmingen). Cells were subsequently incubated with anti-Sca-1-PE-Cy7 (clone D7, eBioscience), anti-c-kit-APC (clone 2B8, eBioscience), anti-CD127/IL7Rα (clone A7R34, eBioscience), anti CD16/CD32 (FcγR, clone 2.4G2, eBioscience), Streptavidin-APC-Cy7 (BD Pharmingen). For lymphoid and myeloid progenitors, the following marker combinations were used: CMPs: Lin-, IL7R-, Sca-1-, c-Kit+, FcγRlow, CD34+. GMPs: Lin-, IL7R-, Sca-1-, c-Kit+, FcR+, CD34+; MEPs: Lin-, IL7R-, Sca-1-, c-Kit+, FcR-, CD34- and CLPs: Lin-, Sca-1low, c-Kitlow, IL7R+.

Western Blotting

To resolve proteins, SDS-polyacrylamide gels were used in concentrations varying from 7.5 to 12% acrylamide, depending on the size of the proteins. After separation via SDS-PAGE, proteins were electrophoretically transferred to PVDF membranes (Millipore) for 1 h at 250 mA using a Tris-glycine buffer system. Membranes were blocked with 3% milk powder in PBS-T (0.1% Tween-20 in PBS) and incubated with selected antibodies accordingly to manufacturer’s instructions.

The following antibodies were used: anti-Flag (M5, Sigma-Aldrich, #F4042; secondary antibody peroxidase conjugated sheep anti-mouse IgG, NA931V, GE healthcare), anti-runx1 (goat polyclonal IgG raised against the N-terminus of AML1, sc-8563, Santa Cruz; secondary antibody peroxidase-conjugated rabbit anti-goat IgG, Jackson ImmunoResearch), PEBP2/CBF (rabbit polyclonal IgG raised against amino acids 1-182 of full-length PEBP2, sc-20693, Santa Cruz; secondary antibody peroxidase-conjugated donkey anti-rabbit IgG, GE Healthcare), anti-GFP (7.1/13.1, mouse monoclonal IgG, Roche, secondary antibody NA931V, GE healthcare).

Chromatin Immunoprecipitation (ChIP) and qPCR

HoxB4 cells were washed twice in PBS and fixed for 1 h at room temperature in 10 mM dimethyladipimate (DMA) in PBS. Subsequently, cells were washed once in PBS and fixed for 30 min at room temperature in 1% formaldehyde (FMA). The reaction was blocked with 1/8 volume of 1 M glycine pH 8.0. Cells were washed twice with PBS and resuspended in SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl pH 8.1). After 10 min of incubation on ice, the cell suspension was sonicated using a sonication device (Covaris System S220 AFA) and the lysate was centrifuged 10 min at 13000 rpm at 4°C. The chromatin was diluted with ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl pH 8.1, 167 mM NaCl) and pre-cleared with 30 µl/ml nProtein A Sepharose 4 Fast Flow [(GE Healthcare); pre-saturated with salmon sperm DNA (Invitrogen)] for 30 minutes at 4°C. After centrifugation, 1% of chromatin was collected as input control and the rest of the chromatin was aliquoted and incubated overnight with the desired antibody [IgG (Diagenode, kch-504-250), RBPJ (abcam, ab25949)]. Antibodies were immobilized with 40 µl nProtein A Sepharose 4 Fast Flow (pre-saturated with Salmon Sperm DNA) for 1 h at 4°C with shaking. Beads were washed with low salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8.1, 150 mM NaCl), high salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8.1, 500 mM NaCl), LiCl wash buffer (0.25 M LiCl, 1% IGEPAL-CA630, 1 mM EDTA, 10 mM Tris-HCl pH 8.1) and TE buffer (10 mM Tris-HCl, 1 mM EDTA pH 8.0). Chromatin was eluted from beads with Elution Buffer (1% SDS, 0.1 M NaHCO3) and crosslinks were reverted at 65°C over night in presence of 180 mM NaCl. Samples were treated with Proteinase K for 1 h at 45°C and the DNA was purified by phenol/chloroform extraction. After precipitation overnight at -20°C in presence of 10 µg of yeast tRNA, 40 µg glycogen and 500 µl 2-propanol, the DNA was washed with 70% EtOH. The DNA was dried, dissolved in TE pH 8.0 and analyzed by qPCR. qPCR reactions were assembled with Absolute QPCR ROX Mix (Thermo Scientific AB-1139), gene-specific oligonucleotides and double-dye probes (see Supplementary Table 1) and finally analyzed using the StepOnePlusTM Real-Time PCR System (Applied Biosystem).

Patients

Mononuclear cells enriched from bone marrow of 33 AML patients were used for RNA-seq analysis. All patients participated in the AMLSG Bio Registry study (NTC 01252485) and gave their informed consent for both bio banking of leukemia samples and molecular analysis of the samples according to the Declaration of Helsinki.

RNA-sequencing

RNA was extracted from patient samples using the AllPrep DNA/RNA Mini Kit (Qiagen) and RNA quality was assessed using a BioAnalyzer 2100 (Agilent). Libraries were prepared from 1µg of total RNA using the TruSeq Stranded Total RNA Kit with Ribo-Zero Human/Mouse/Rat (Illumina) according to the manufacturer’s instructions. The pooled RNA libraries were sequenced on an Illumina HiSeq2000 to obtain 100 bp paired-end reads. RNA-Seq reads were aligned to the human reference genome (hg19) and quantified using the stranded option of STAR v.2.4.2a. Furthermore, the DESeq2 package31 was used to obtain normalized expression values.

Analysis of ChIP-Seq data

ChIP-Seq reads were downloaded from NCBI’s Gene Expression Omnibus and converted to fastq format using the SRA toolkit version 2.3.5. Reads were subsequently aligned to a precompiled hg18 reference index with BOWTIE with *-k* option set to 1 1 2. Unambiguously mapped and unique reads were kept for subsequent generation of binding profiles and calling of peaks was done using MACS v1.41 3 at default settings. Input reads were used as control when available. All downstream analyses were done in R/BioConductor (http://www.bioconductor.org) 4. Browser snap shots were generated with Gviz 5. GEO data sets used in this study: GSM732904 (Notch1), GSM732906 (RBP-J) and GSM732907 (Input DNA) from CUTLL1 cells (from GSE29600 6); GSM726983 (AE) in MCF7 cells and GSM726986 (AE) in K562 cells (from GSE23730 7).

Fluorescence microscopy

Confocal microscopy: cell imaging was performed by plating HeLa cells at a concentration of 1x105 cells/cm2 on chamber coverslips (Nunc). 16 h later, cells were transfected with 300-400 ng of expression plasmids using the Nanofectin transfection reagent (PAA). 24 h post-transfection, cells were fixed and pictures were taken using a confocal laser scanning microscope LSM710 (Zeiss) equipped with a 40x oil immersion objective. Images were acquired in sequential scan mode with airy pinholes as single confocal sections: CH1 ex: 488 laser line, em: 505-535nm, CH2 ex: 561 laser line, 571-620nm. Images were processed and analyzed using ImageJ software (NIH, USA). Epifluorescence microscopy: pictures were taken using a fluorescence microscope (IX71, Olympus) equipped with a digital camera (C4742, Hamamatsu) and a 100-W mercury lamp (HBO 103W/2, Osram). The following filter sets were used: green channel (ex: HQ470/40, em: HQ525/50), blue channel: (ex: HQ360/40, em: HQ457/50).

Giemsa staining

Blood smears and cells from Mouse colony forming cell assays were stained with Giemsa (Merck, #1.09204.0500) following standard procedures.

**Supplementary references:**

1. Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* 2009; **10**(3)**:** R25.

2. Langmead B. Aligning short sequencing reads with Bowtie. *Curr Protoc Bioinformatics* 2010 Dec; **Chapter 11:** Unit 11 17.

3. Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE*, et al.* Model-based analysis of ChIP-Seq (MACS). *Genome Biol* 2008; **9**(9)**:** R137.

4. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S*, et al.* Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol* 2004; **5**(10)**:** R80.

5. Hahne F, Ivanek R. Visualizing Genomic Data Using Gviz and Bioconductor. *Methods Mol Biol* 2016; **1418:** 335-351.

6. Wang H, Zou J, Zhao B, Johannsen E, Ashworth T, Wong H*, et al.* Genome-wide analysis reveals conserved and divergent features of Notch1/RBPJ binding in human and murine T-lymphoblastic leukemia cells. *Proc Natl Acad Sci U S A* 2011 Sep 06; **108**(36)**:** 14908-14913.

7. Martens JH, Mandoli A, Simmer F, Wierenga BJ, Saeed S, Singh AA*, et al.* ERG and FLI1 binding sites demarcate targets for aberrant epigenetic regulation by AML1-ETO in acute myeloid leukemia. *Blood* 2012 Nov 08; **120**(19)**:** 4038-4048.

|  |  |  |
| --- | --- | --- |
| **Oligonucleotide** | **Sequence** |  |
| *real time PCR* |  | Probe |
| beta actin\_F | 5´-GGATGCAGAAGGAGATTACTGC-3´ | 63 |
| beta actin\_R | 5´-CCACCGATCCACACAGAGTA-3´ |  |
| AE\_F | 5´-CACAAACCCACCGCAAGT-3´ | 21 |
| AE\_R | 5´-AGATTGCGTCTTCACATCCA-3´ |  |
| Hey1\_F | 5´-CATGAAGAGAGCTCACCCAGA-3´ | 17 |
| Hey1\_R | 5´-CGCCGAACTCAAGTTTCC-3´ |  |
| Hes1\_F | 5´-ACACCGGACAAACCAAAGAC-3´ | 99 |
| Hes1\_R | 5´-CGCCTCTTCTCCATGATAGG-3´ |  |
| Nrarp\_F | 5´-GCTACACATCGCCGCTTT-3´ | 49 |
| Nrarp\_R | 5´-TTGGCCTTGGTGATGAGATA-3´ |  |
| Igf1r\_F | 5´-TCTGCCCAATGGTAACTTGA-3´ | 45 |
| Igf1r\_R | 5´-ATCGGCGTACTTTCTGATGG-3´ |  |
| Gpr56\_F | 5´-TGGCTTGTGTCTTCACTATTGC-3´ | 1 |
| Gpr56\_R | 5´-GGACTTTGATGGTGTAGTCACG-3´ |  |
| Csf1r\_F | 5´-CAGCAATGATGTTGGCACA-3´ | 49 |
| Csf1r\_R | 5´-TCAAGTTTAAGTAGGCACTCTCCA-3´ |  |
| Mcm2\_F | 5´-TTGGTGATGGCATGGAGAG-3´ | 17 |
| Mcm2\_R | 5´-AGCTGCCTCTCTCTGACTGG-3´ |  |
| Vegfc\_F | 5´-TGCCAGCAACATTACCACAG-3´ | 27 |
| Vegfc\_R | 5´-GGCACATGTAGTTATTCCACACA-3´ |  |
| TBP\_F | 5´-GGGGAGCTGTGATGTGAAGT-3´ | 97 |
| TBP\_R | 5´-CCAGGAAATAATTCTGGCTCAT-3´ |  |
| GAPDH\_F | 5´- GGGTTCCTATAAATACGGACTGC -3´ | 68 |
| GAPDH\_R | 5´- CCATTTTGTCTACGGGACGA -3´ |  |
| HPRT | Quanti Tect Assay, QT00166768 (Qiagen) |  |
| *ChIP* |  | Probe |
| Hes1 +0.6kb\_F | 5’-TTTCCCAGGCACAAAGAACT-3’ | 40 |
| Hes1 +0.6kb\_R | 5’-TAGTGCTGGCGGGGTAAG-3’ |  |
| Hey1 -0.8kb\_F | 5’-GCAGCATCCCTTGAGTCTCT-3’ | 49 |
| Hey1 -0.8kb\_R | 5’-GGGTCCTACAGGAACAGTCG-3’ |  |
| Nrarp -3kb\_F | 5’-CCTATCCTCTCTTCTACCAGGTGT-3’ | 66 |
| Nrarp -3kb\_R | 5’-TGGGAAAGAGGAGAGTGTTTCT-3’ |  |
| Nrarp +1.9kb\_F | 5’-GGGCTGCTGTGCTCTTTC-3’ | 60 |
| Nrarp +1.9kb\_R | 5’-CATAATAGGGCCTTGGAGCA-3’ |  |

**Supplementary Table 1.** Oligonucleotides designed for real time PCR and ChIP