

BCL6* – regulated by AhR/ARNT and wild-type *MEF2B* – drives expression of germinal center markers *MYBL1* and *LMO2

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SUPPLEMENTAL METHODS

Gene expression analyses

RNA was prepared using the RNeasy Mini kit including the RNase Free DNase Set (Qiagen). TaqMan probes (Applied Biosystems) were used to quantify human *AhR* (Hs00169233_m1), *ARNT* (Hs01121918_m1), *BCL6* (Hs00153368_m1), *CGGBP1* (Hs00383191_m1), *CHMP2B* (Hs01045897_m1), *CTXN3* (Hs00416961_m1), *CYP1A1* (Hs01054797_g1), *GAPDH* (Hs02758991_g1), *ITM2B* (Hs00222753_m1), *LMO2* (Hs00153473_m1), *MEF2B* (Hs04188747_m1), *MYBL1* (Hs00277143_m1), *RB1* (Hs01078066_m1), *SOCS1* (Hs00705164s1) and *SOCS2* (Hs00919620_m1) expression levels with *TATA box binding protein (TBP)* as endogenous control. Relative expression levels were calculated using the $\Delta\Delta\text{Ct}$ -method.

Preparation of recombinant retroviral supernatants and retroviral transduction

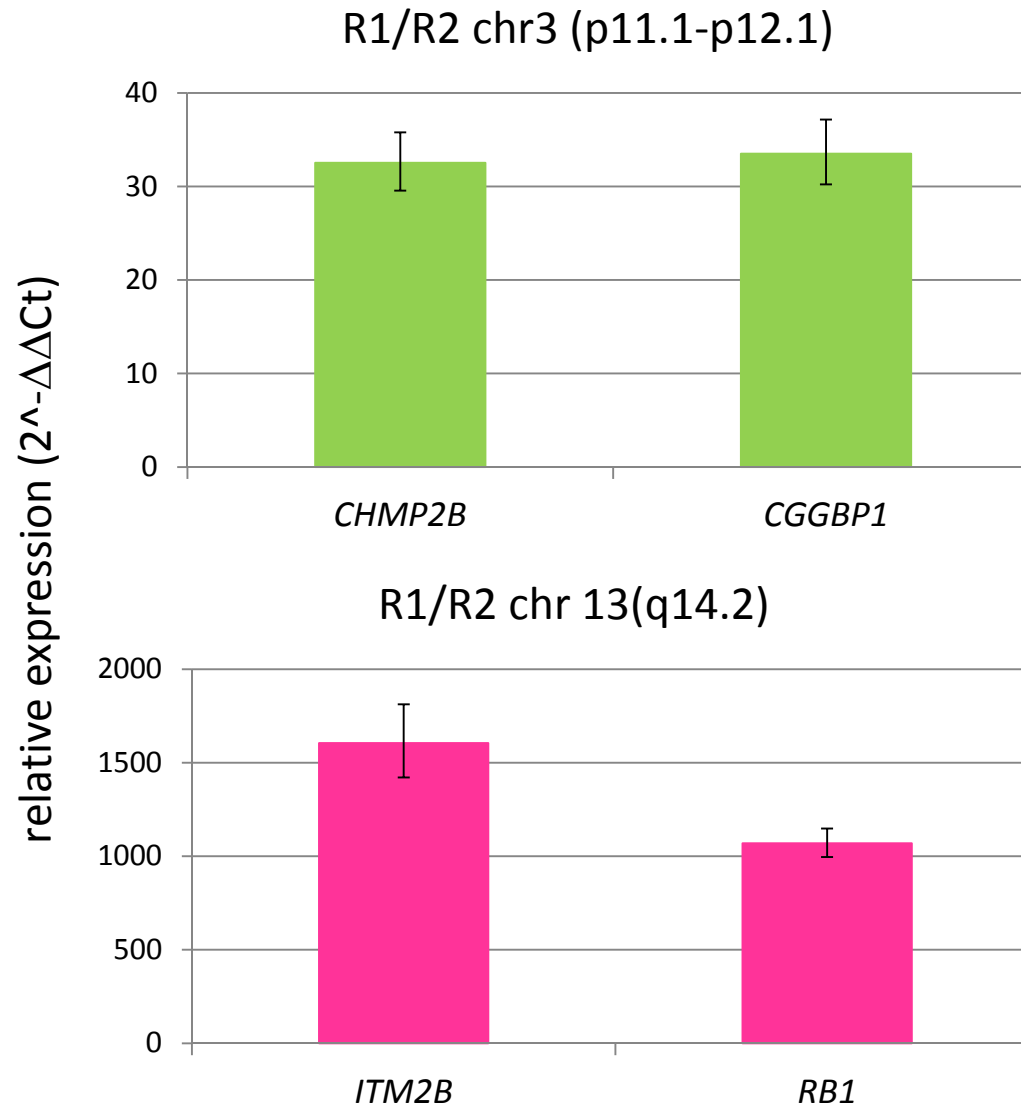
Retroviral supernatants were generated by calcium phosphate co-transfection of 293T cells using MSCV-BCL6-IRES-GFP (obtained from Addgene, Cambridge, MA, USA (<http://www.addgene.org>) or MSCV-IRES-GFP (vector control), M57 for gag/pol and pMD.g. Medium was replaced after 12 h by RPMI 1649 medium + 10% fetal calf serum for an additional 12 h. Retroviral supernatant was collected in RPMI 1640, twice within 24 h, pooled, cleared by low-speed centrifugation and filtered through a 0.45-mm filter. Viral supernatants were centrifuged for 10 to 16 h at 10,000 rpm at 10°C for concentration of viral particles.

Each construct was transduced into 5×10^5 U-2932 R2 cells at a multiplicity of infection (MOI) of about 1. Cells were cultivated for 4 d and GFP+ cells were sorted by a BD FACS Aria (Becton Dickinson). RNA of sorted cells was isolated 10 d after sorting using TRIzol reagent (Invitrogen, Karlsruhe, Germany).

Whole exome sequencing

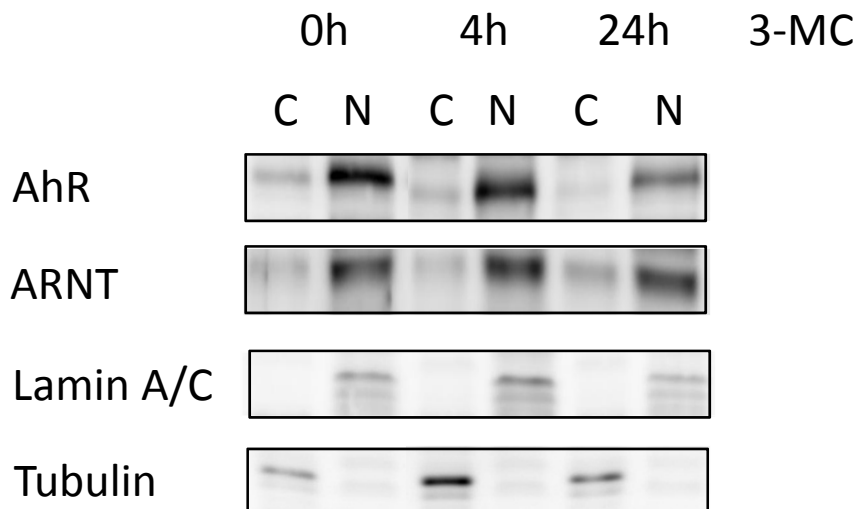
Paired-end exome (SureSelect XT Human All Exon V5 + UTR, Agilent, Santa Clara, CA, USA) sequencing of U29332R1 and U2932R2 on HiSeq2500 (Illumina, San Diego, CA, USA) resulted in 44 and 53 million reads resulting in 119 and 143 mean coverage, respectively. After data preprocessing (STAR 2.4.0b, samtools 0.1.19, picard 1.121) variants were identified via GATK (3.2.2) tools and mutation effects revealed via Ensembl VEP.

Suppl. Fig. 1: Subclone-specific gene expression

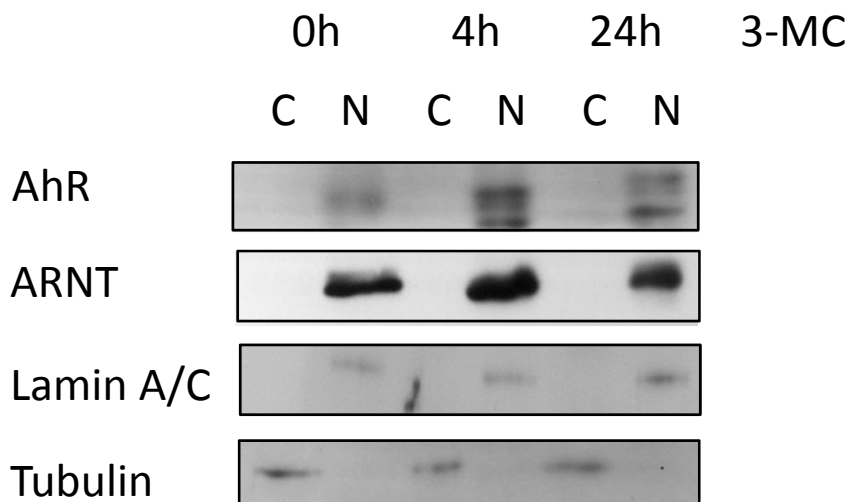


Suppl. Fig. 2: Nuclear localization of AhR/ARNT

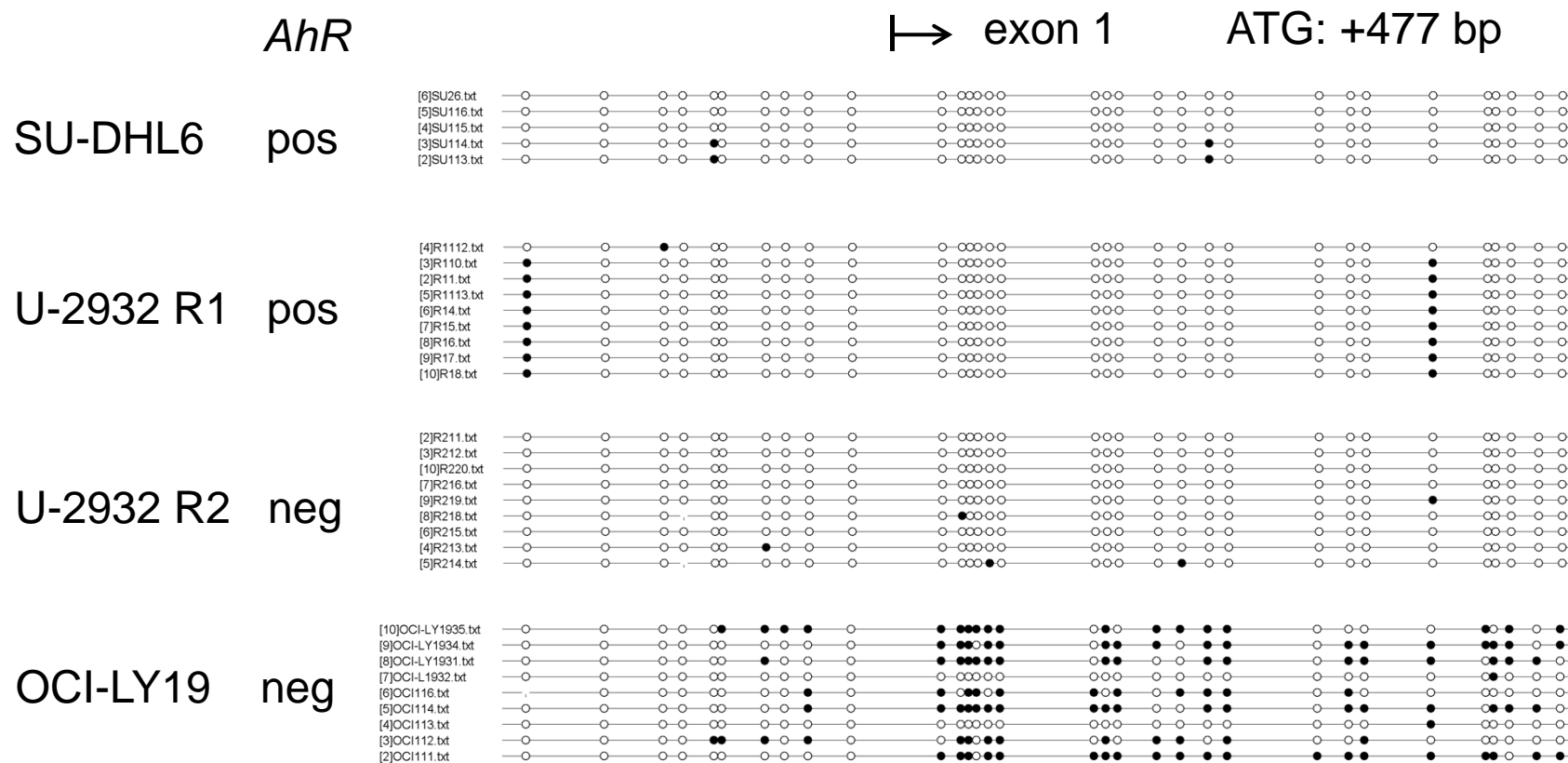
U-2932 R1



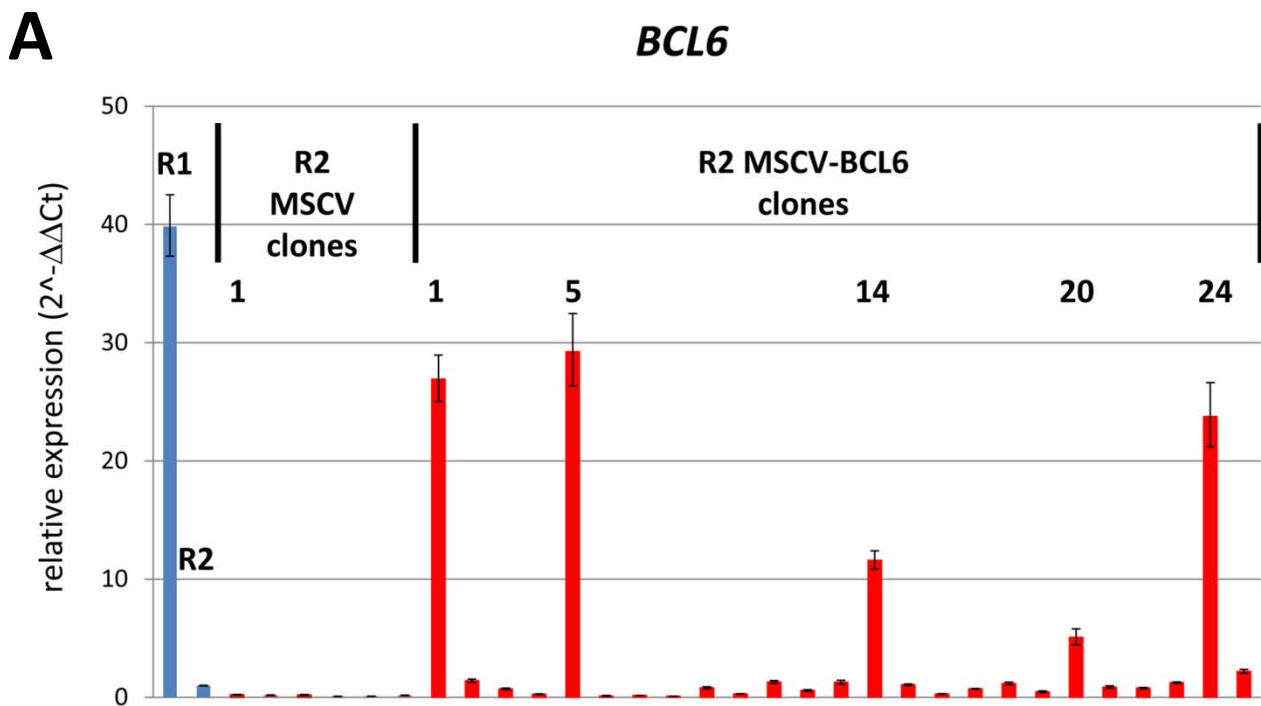
U-2932 R2



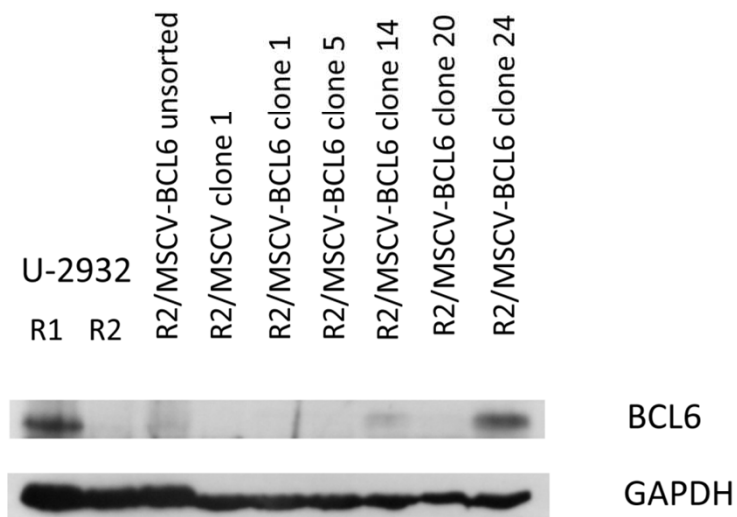
Suppl. Fig. 3: Bisulfite sequencing of the *AhR* promoter



Suppl. Fig. 5: *BCL6* mRNA (A) and protein (B) expression



B



Supplementary Table 1

Primer sequences for real-time PCR

| | | |
|---------------|-----|---|
| <i>ABL1</i> | FRW | 5'-GTG GCC AGT GGA GAT AAC AC-3' |
| | REV | 5'-CGC AAT TCC CAG ATT TCT ATC-3' |
| <i>CDCP1</i> | FRW | 5'-GTC TCC GGC TTC AGC ATT G-3' |
| | REV | 5'-ACC TAA TCA GAT AGC AGG GAG-3' |
| <i>CGGBP1</i> | FRW | 5'-ATT ACT TCC AGA ATG AAC TGT TGT T-3' |
| | REV | 5'-GCT GTT ACT ACA AAT CGC TCC-3' |
| <i>CHMP2B</i> | FRW | 5'-CTA TCT ATA TTT GAT GTG TTC CCT T-3' |
| | REV | 5'-GTG GAT CCA TCT TCT TGT TAA CT-3' |
| <i>EPHA6</i> | FRW | 5'-CTC TTC TGA AAT GTA AGT GTT CG-3' |
| | REV | 5'-CGC CCA CTA CAG ACT TCT C-3' |
| <i>FLT1</i> | FRW | 5'-TCT GAC CTG TGA AGC AAC AGT-3' |
| | REV | 5'-TAC AGA GCA CTT CGG CTT ATG-3' |
| <i>ITM2B</i> | FRW | 5'-ACC TGT CTC ATG CTG AAT TGC-3' |
| | REV | 5'-CCA CAG TAG TAC ACG TCA TCT-3' |
| <i>KPNA3</i> | FRW | 5'-AGT GGT CCA ATT GAG TGC TGT-3' |
| | REV | 5'-CCA GGT AAC ACA ACT GAG GAA-3' |
| <i>RB1</i> | FRW | 5'-CTT GGA CTT GTA ACA TCT AAT GG-3' |
| | REV | 5'-CCT CCC TCC ACA GTC TCA A-3' |

PCR was performed for 40 cycles with 60°C annealing temperature.

Supplemental Table 2 Statistics

```
#####  
#  
Statistics referring to Figure 4  
#  
#####  
#  
# fishers exact test  
  
stat.fig4a ← matrix(c(8, 5, 0, 10),  
  nrow = 2,  
  dimnames = list(AhR = c("AhR pos", "AhR neg"),  
    MEF2B = c("MEF2B pos", "MEF2B neg")))  
stat.fig4a  
  
fisher.test(stat.fig4a, alternative = "greater")  
  
# #  
# Fisher's Exact Test for Count Data  
#  
# data: stat.fig4a  
# p-value = 0.002625  
# alternative hypothesis: true odds ratio is greater than 1  
# 95 percent confidence interval:  
# 2.872487      Inf  
# sample estimates:  
# odds ratio  
#      Inf  
  
stat.fig4b ← matrix(c(12, 1, 1, 9),  
  nrow = 2,  
  dimnames = list(  
    MEF2B = c("MEF2B pos", "MEF2B neg"),  
    BCL6 = c("BCL6 pos", "BCL6 neg")))  
stat.fig4b  
  
fisher.test(stat.fig4b, alternative = "greater")  
  
##  
# Fisher's Exact Test for Count Data  
#  
# data: stat.fig4b  
# p-value = 0.0001145
```

```
# alternative hypothesis: true odds ratio is greater than 1
# 95 percent confidence interval:
# 5.951049      Inf
# sample estimates:
# odds ratio
# 67.95602
#
```

Statistics referring to Supp. Table 3:

```
#####
```

```
#
```

```
# McNemar's chi-squared test for symmetry of rows and columns
```

```
#
```

```
#####
```

```
M ← as.table(rbind(c(9, 2), c(15, 34)))
```

```
dimnames(M) ← list(ploidy = c("hyper (>2n)", "hypo (<2n)"),  
  expression=c("high", "low"))
```

```
chisq.test(M)
```

```
#
```

```
# Pearson's Chi-squared test with Yates' continuity correction
```

```
#
```

```
# data: M
```

```
# X-squared = 7.7968, df = 1, p-value = 0.005234
```

```
mcnemar.test(M)
```

```
#
```

```
# McNemar's Chi-squared test with continuity correction
```

```
#
```

```
# data: M
```

```
# McNemar's chi-squared = 8.4706, df = 1, p-value = 0.003609
```

```
#
```


Supplementary Table 4

Correlation *BCL6* and *BCL6* target gene expression in B-NHL cell lines

| Probe set ID | Gene symbol | FARAGE | Karpas-1106P | U-2940 | U-2932 R1 | U-2932 R2 | NU-DHL1 | OCI-LY19 | SU-DHL8 | mean |
|--------------|-----------------|--------|--------------|--------|-----------|-----------|---------|----------|---------|-------|
| 203140_at | <i>BCL6</i> | 4.21 | 4.60 | 3.75 | 4.38 | -1.08 | 0.69 | 0.47 | -1.54 | 1.93 |
| 205681_at | <i>BCL2A1</i> | 5.01 | 2.93 | 3.98 | 4.17 | -1.45 | -5.00 | -4.79 | -3.75 | 0.14 |
| 228434_at | <i>BTNL9</i> | 3.81 | 3.04 | 2.01 | 1.11 | -3.08 | 0.00 | -1.60 | -4.97 | 0.04 |
| 1552448_a_at | <i>C8orf12</i> | -4.36 | -1.93 | -4.69 | 2.51 | -3.69 | -4.32 | -4.79 | -5.01 | -3.28 |
| 228592_at | <i>MS4A1</i> | 4.92 | 5.91 | 5.22 | 5.05 | 3.59 | 3.47 | 1.20 | 0.07 | 3.68 |
| 204581_at | <i>CD22</i> | 1.69 | 1.75 | 0.69 | 1.15 | -2.14 | 1.50 | 0.52 | -1.43 | 0.47 |
| 206150_at | <i>CD27</i> | 0.97 | 0.02 | 0.40 | 2.36 | -0.75 | -1.22 | -0.03 | -3.11 | -0.17 |
| 205692_s_at | <i>CD38</i> | 0.47 | -0.02 | -2.71 | 0.77 | -1.21 | -0.03 | 0.26 | 3.20 | 0.09 |
| 200985_s_at | <i>CD59</i> | 1.96 | 1.47 | 1.28 | 1.98 | -2.03 | -0.11 | -0.60 | -1.43 | 0.32 |
| 231391_at | <i>CTXN3</i> | -4.29 | -4.82 | -5.10 | 1.34 | -4.30 | -4.91 | -5.14 | -4.90 | -4.01 |
| 204720_s_at | <i>DNAJC6</i> | 0.30 | 1.25 | -0.05 | 0.98 | -3.58 | -1.48 | -0.37 | -1.12 | -0.51 |
| 219517_at | <i>ELL3</i> | 2.79 | 4.06 | 2.84 | 4.11 | 0.19 | -1.26 | -1.22 | -0.06 | 1.43 |
| 220161_s_at | <i>EPB41L4B</i> | -3.41 | 2.67 | 1.44 | 0.95 | -2.68 | -3.70 | -3.18 | -4.18 | -1.51 |
| 212288_at | <i>FNBP1</i> | 3.35 | 4.76 | 3.02 | 3.30 | -2.47 | 3.39 | 1.92 | 0.63 | 2.24 |
| 206018_at | <i>FOXP1</i> | -4.51 | -4.19 | -4.69 | 2.79 | -3.81 | -4.03 | -5.02 | -4.86 | -3.54 |
| 204875_s_at | <i>GMDS</i> | 1.38 | -1.42 | -1.46 | 1.80 | -1.00 | -0.20 | 0.96 | -0.34 | -0.03 |
| 203394_s_at | <i>HES1</i> | -2.36 | -4.44 | -4.69 | 0.34 | -3.75 | 0.22 | -3.50 | -5.04 | -2.90 |
| 208937_s_at | <i>ID1</i> | -3.23 | -3.71 | -2.96 | 2.10 | -1.77 | -3.32 | -4.39 | -3.43 | -2.59 |
| 209291_at | <i>ID4</i> | -4.94 | -4.48 | -4.46 | 1.06 | -4.56 | -2.96 | -4.23 | -4.58 | -3.65 |
| 218656_s_at | <i>LHFP</i> | 0.00 | 1.32 | -3.18 | 0.70 | -3.47 | -4.91 | -0.53 | -4.41 | -1.81 |
| 217892_s_at | <i>LIMA1</i> | 1.86 | 2.35 | 2.10 | 1.14 | -2.64 | 0.09 | -0.97 | -2.53 | 0.17 |
| 204249_s_at | <i>LMO2</i> | 4.46 | 3.99 | 3.77 | 2.06 | -3.47 | 0.34 | 3.59 | -2.51 | 1.53 |
| 1559942_at | <i>MDFIC</i> | 0.53 | -0.15 | 1.61 | 1.83 | -1.45 | -1.22 | -5.14 | -3.07 | -0.88 |
| 238576_at | <i>MOCOS</i> | -4.80 | -3.84 | -3.70 | -1.96 | -4.82 | -3.41 | -4.64 | -4.74 | -3.99 |
| 213906_at | <i>MYBL1</i> | 0.83 | 2.45 | 1.63 | 1.16 | -5.12 | -4.09 | -1.80 | -0.26 | -0.65 |
| 1552531_a_at | <i>NLRP11</i> | -2.92 | -0.33 | -2.96 | 3.30 | -2.06 | -2.22 | -3.59 | -1.44 | -1.53 |
| 209621_s_at | <i>PDLIM3</i> | -3.47 | -2.44 | -4.35 | 2.33 | -3.17 | -3.46 | -3.14 | -4.36 | -2.76 |
| 205174_s_at | <i>QPCT</i> | 2.55 | 3.12 | 3.03 | 2.88 | -3.52 | -3.75 | -3.69 | -4.36 | -0.47 |
| 1568752_s_at | <i>RGS13</i> | 2.94 | 2.56 | 3.05 | 3.11 | -5.39 | -2.79 | -4.08 | 1.06 | 0.06 |
| 208456_s_at | <i>RRAS2</i> | 1.18 | 2.02 | 0.94 | 1.84 | -2.11 | 0.11 | -2.97 | -0.86 | 0.02 |
| 212560_at | <i>SORL1</i> | 3.73 | 3.19 | 1.71 | 4.32 | -2.54 | -0.60 | 2.58 | -4.10 | 1.04 |
| 243198_at | <i>TEX9</i> | 1.90 | 0.10 | -0.02 | 0.04 | -3.81 | -4.81 | -3.42 | -2.30 | -1.54 |
| 217979_at | <i>TSPAN13</i> | 2.93 | -0.50 | -4.01 | 2.28 | -3.36 | 3.11 | 3.96 | 3.47 | 0.99 |

LEGENDS SUPPLEMENTAL FILES

Supplemental Figure 1 - Subclone-specific gene expression. Quantitative RT-PCR was performed to test mRNA levels in the U-2932 subclones R1 and R2. U-2932 R2 was used as reference. Note that structural aberrations on chr 3 and chr 13 correlate with the R1-restricted overexpression of target genes: U-2932 subclone R1 has 32x higher mRNA expression levels of *CHMP2B* and *CGGBP1* than R2, *ITM2B* and *RB1* is >1000x fold higher expressed in R1 than in R2.

Supplemental Figure 2 - Nuclear localization of AhR/ARNT. AhR localized in the nucleus of U-2932 R1 and R2 cells, without stimulation with the ligand 3-methylcholantrene (3-MC) (2 μ M, 0 h – 24 h). Note that AhR expression in R2 was extremely low, detectable only after long exposure. C: cytoplasm; N: nucleus. Protein expression was determined by Western blot analysis.

Supplemental Figure 3 – Bisulfite sequencing of the *AhR* promoter. Parts of the AhR promoter region were sequenced after bisulfite conversion of DNA in the two *AhR*-positive (SU-DHL6, U-2932 R1) and *AhR* negative (U-2932 R2, OCI-LY19) cell lines. CpGs are represented as open dots (unmethylated) or filled dots (methylated).

Supplemental Figure 4 - Expression of AhR, ARNT, MEF2B and BCL6 in DLBCL cell lines. Protein expression was determined by Western blot analysis. GAPDH was used as endogenous control.

Supplemental Figure 5 - Expression of *BCL6* in U-2932 R1, R2, and in R2 clones transfected with MSCV and MSCV-BCL6. A) *BCL6* mRNA and B) protein

expression was determined using qRT-PCR and Western blot analysis. Note that only a minority of MSCV-BCL6-IRES-GFP clones expressed *BCL6*, although all clones were GFP-positive. After cryopreservation, recloning of R2-MSCV clone 24 was necessary to obtain BCL6 positive subclones.

Supplemental Table 1 - Primers for quantitative genomic PCR. PCR was performed for 40 cycles with 60°C annealing temperature.

Supplemental Table 2 – Statistical evaluation of results. R-programmed statistical analysis.

Supplemental Table 3 - Genes with >10x expression differences between R1 and R2. mRNA expression was determined by microarray analysis. Colored: Genes showing numerical disparities. Green: true negative (34); yellow: true positive (9); red: false positive (2); blue: false negative (5); grey: not included in statistical analysis because neither R1 nor R2 were diploid. Numerical differences between the two subclones predict differences in gene expression (sensitivity 0.64, specificity 0.94, accuracy 0.78). McNemar's chi-squared test with continuity correction rejected non-correlation of the two parameters with a p-value of 0.0036 compared to 0.05 as level of significance.

Supplemental Table 4 – Positive *BCL6* target genes. Expression array analysis reveals a positive correlation between the expression of *BCL6* and positive candidate target genes of Fig. 5 (sensitivity 0.77, specificity 0.83, accuracy 0.80). Red: expression above average; green: expression below average.