

Fig. S1. Targeting of the neomycin reporter gene to the endogenous *Gata6* locus. (A) A schematic of the strategy for targeting a promoterless neomycin with a polyA signal at the start codon of endogenous *Gata6* gene. The first targeting step introduces a floxed puromycin selection cassette, which is then removed using a transient expression of Cre to create the final reporter cell line. Positions of the 5', 3' and Neo Southern probes are shown as are the locations of *Bgl*II and *Spe*I restriction sites, which are used for the Southern blot strategies. The positions of the qRT-PCR primers, to identify the reporter specific transcript, are indicated. (B) Phosphorimager scans of Southern blots to show gene targeting. Probes, restriction sites and expected sizes for each allele are detailed. WT, E14 genomic DNA; C1, first targeted line including the floxed puromycin cassette; G4, final targeted line after removal of puromycin cassette.

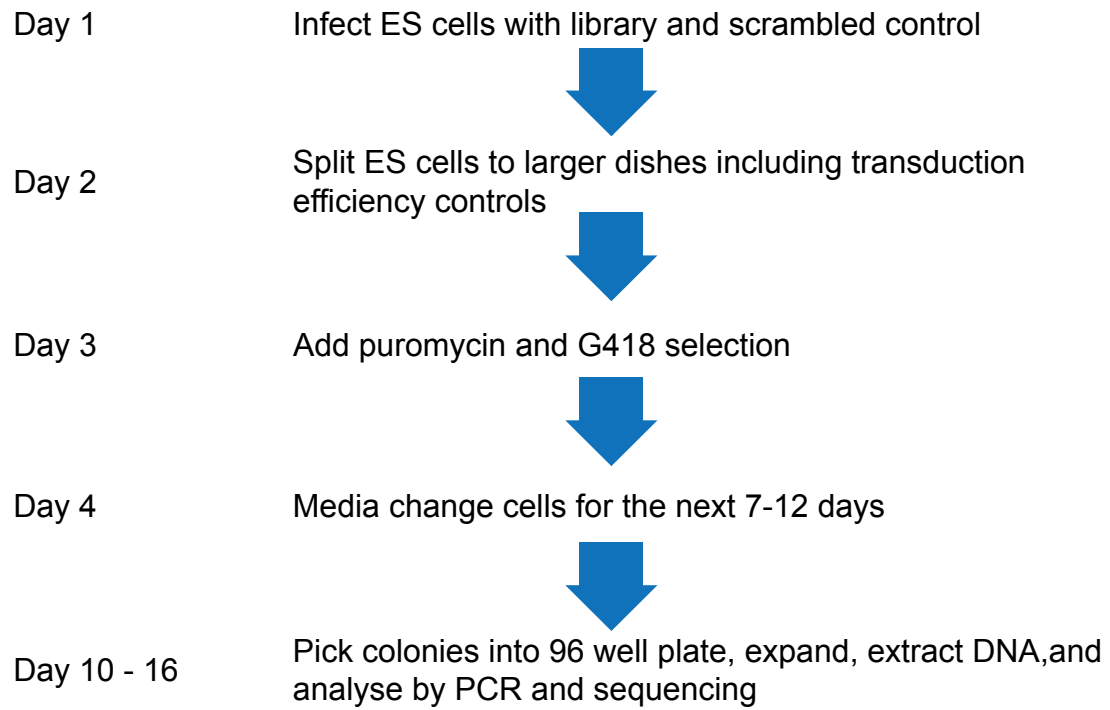


Fig. S2. Schematic overview of the screening process.

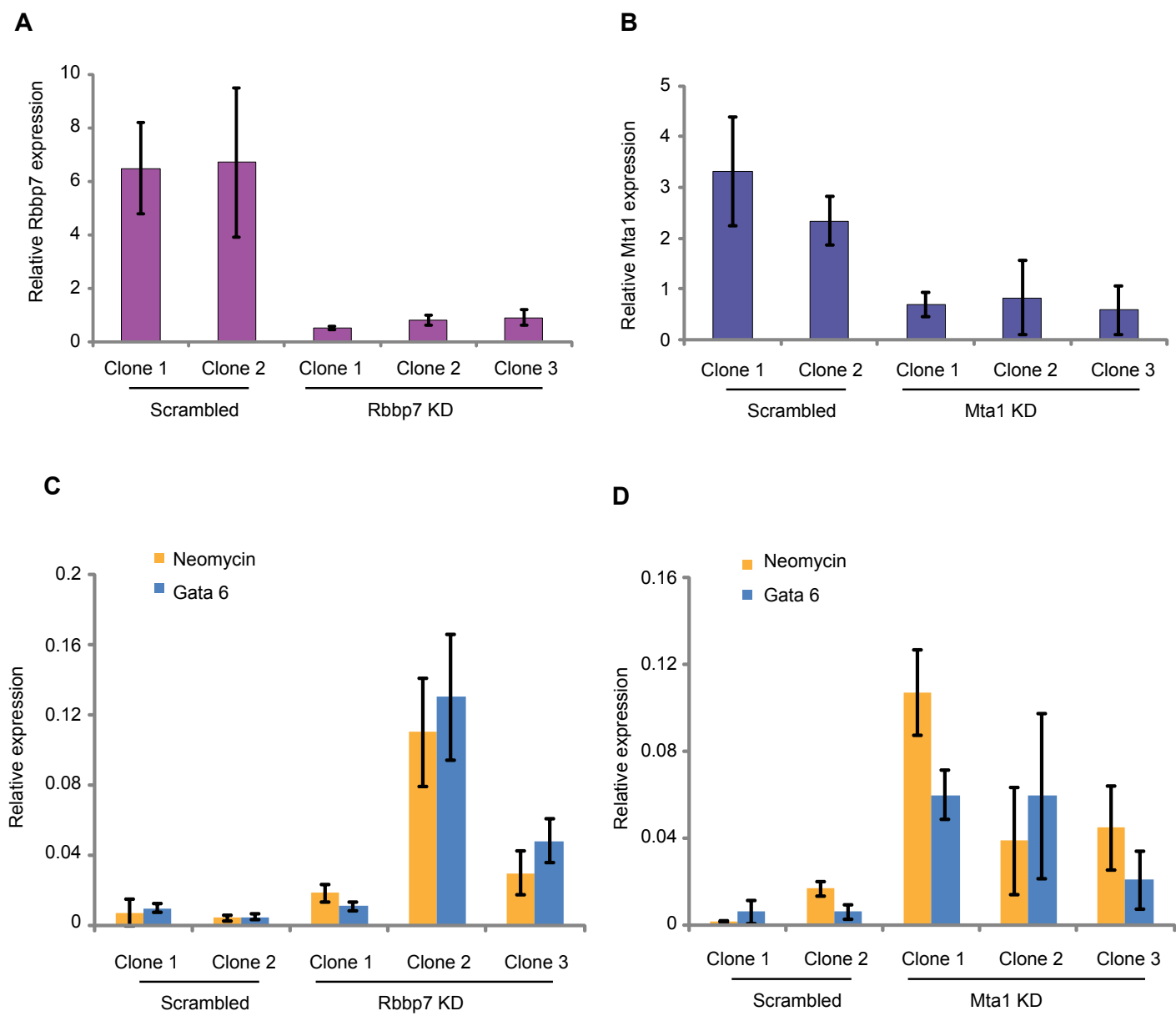


Fig. S3. Validation of *Rbbp7* and *Mta1* hits. (A) *Rbbp7* expression analysis of clones containing scrambled or shRNA targeting *Rbbp7*. (B) *Mta1* expression analysis of clones containing scrambled or shRNA targeting *Mta1*. (C) Neomycin and *Gata6* expression of clones shown in A. (D) Neomycin and *Gata6* expression of clones shown in B. Error bars represent 95% confidence intervals of three technical replicates.

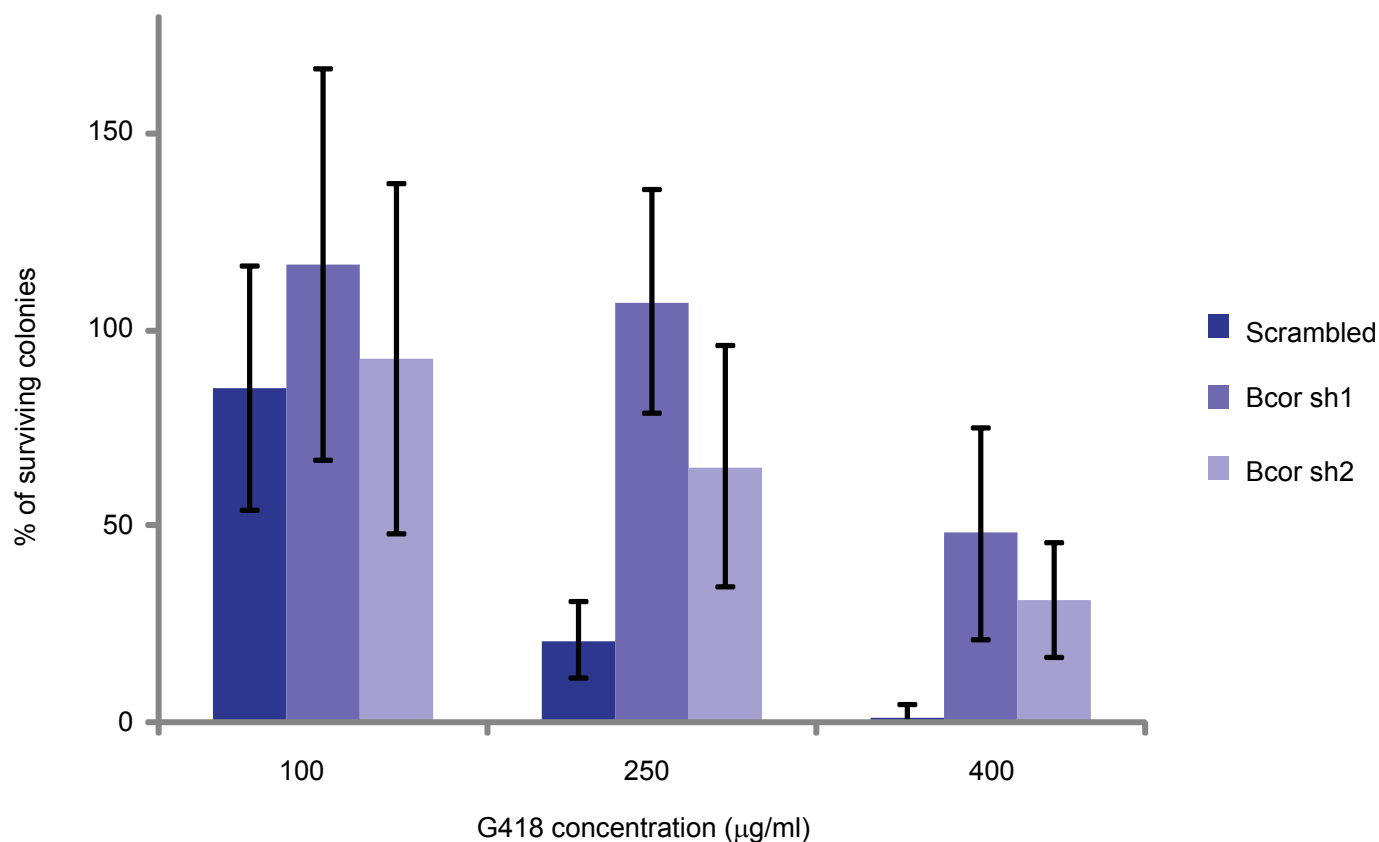


Fig. S4. Colony-counting experiment after Bcor knockdown. Number of colonies surviving at three concentrations of G418 (100, 250 and 400 µg/ml) shown as a percentage of number of colonies surviving without G418, in cells stably expressing either a scrambled control shRNA or a Bcor knockdown shRNA (Bcor sh1 and Bcor sh2). Error bars show 95% confidence intervals of three biological replicates.

Table S1. Primer and oligonucleotide sequences.

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Table S2. Full results of the screen. List of genes (symbols and full names) together with the sequence of the hairpin identified in the screen. The library comes as ten pools for ease of screening. The list of genes is arranged in pool numbers and screening was carried out using each pool individually. The shRNA hairpins for each gene are within a single pool, but there are no specific categories of genes within each pool.

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Table S3. Gene functional classification of screen hits. List of screen hits analysed by DAVID using the gene functional classification tool.

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Table S4. Functional annotation of screen hits. List of screen hits analysed by DAVID using the functional annotation tools. Category: the database from which the term is found. Term: specific name that is enriched. Count: number of genes included in that term and as a percentage of total. %: percentage of hits from the screen which fall into that term. *P* value. Genes: list of genes included in the term. List total: Total number of screen hits represented in that category. Pop hits: Number of genes in that category. Pop total: total number of genes. Fold enrichment: enrichment over what would be expected by chance. Bonferroni and Benjamini-Hochberg: *P* value adjusted for multiple testing correction. FDR: false discovery rate.

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