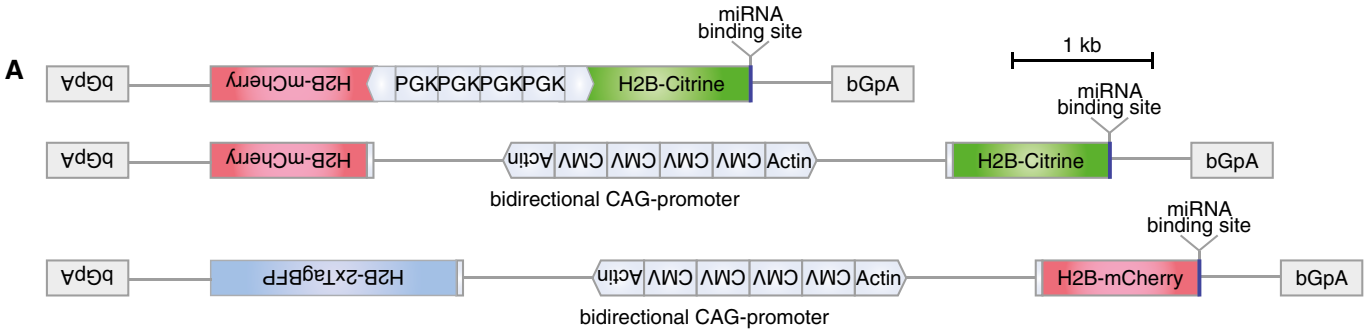
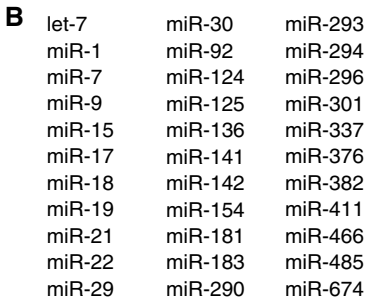
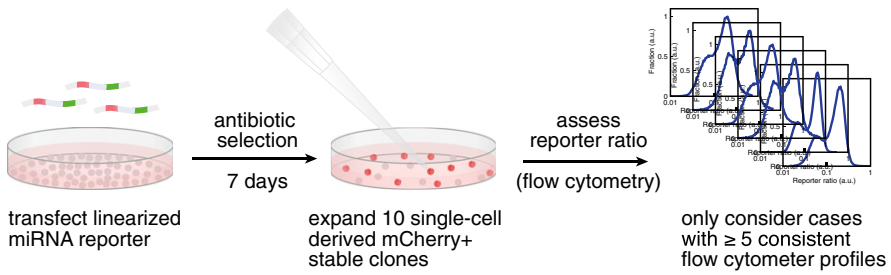
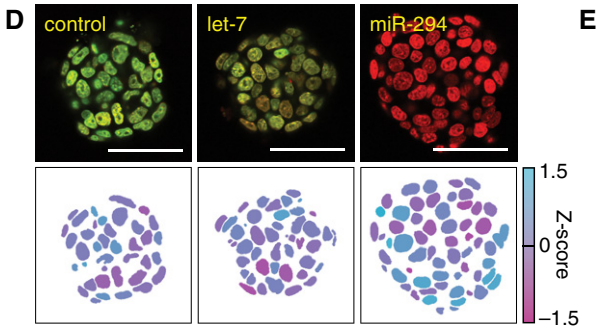
Expanded View Figures









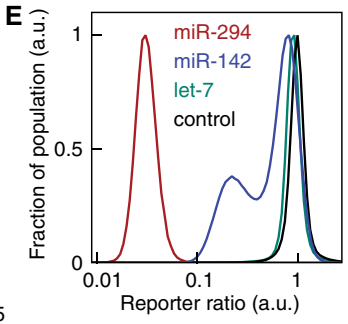


Figure EV1. Single-cell miRNA activity reporter.

A A synthetic bidirectional promoter drove the expression of H2B-Cherry as normalizer to control for transcriptional noise and H2B-Citrine as detector of miRNA activity with a target sequence for the respective miRNA 11 bp downstream of its stop codon. Four enhancer elements of the mouse phosphoglycerate kinase *Pgk1* gene (PGK) promoter were inserted between two back-to-back arranged minimal PGK-promoter fragments to create a bidirectional PGK promoter (upper panel). The bidirectional CAG-promoter was constructed by placing four CMV immediate-early enhancer elements between two back-to-back arranged fragments of the promoter, first exon and partial intron of chicken *β-actin* gene fused to the splice acceptor of the rabbit *β-globin* gene (lower panel). A positive selection cassette was included (not depicted). Intronic sequences are represented as lines. bGpA: rabbit *β-globin* genomic fragment containing the polyadenylation signal. For use in the *Rex1*-dGFP knockin mESC line, we constructed an activity reporter based on a bidirectional CAG-promoter driving the expression of H2B-2xTagBFP as normalizer and H2B-Cherry as detector.

B List of candidate miRNAs used in this screen.

C Experimental scheme for the generation and screening of clonal mESC lines stably expressing miRNA activity reporters.

D Reporter signal in single cell-derived mESC colonies and corresponding Z-score of the reporter ratio for a non-targeted control, a let-7a-5p and a miR-294-3p activity reporter. Scale bar: 50 μm.

E Reporter ratio distribution in mESCs stably expressing the activity reporters for miR-294-3p (red line), miR-142-3p (blue line), let-7a-5p (green line) and a non-targeted control (black line).

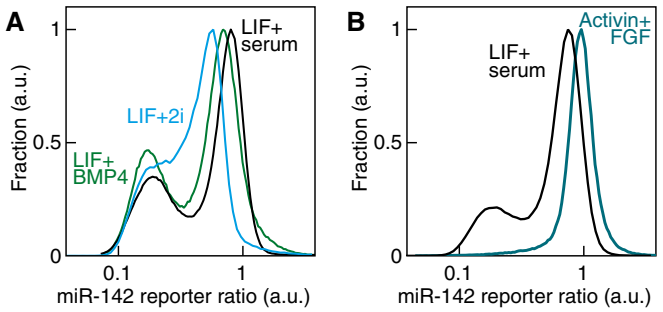


Figure EV2. miR-142 activity reporter in different pluripotency-sustaining media.

A Bimodal miR-142 expression in naïve LIF-dependent pluripotency conditions. Distribution of the miR-142-3p reporter ratio in mESCs cultured in 10 ng/ml LIF supplemented with serum (LIF+serum, black line) or 10 ng/ml BMP4 (LIF+BMP4, green line) or in 1 μM PD0325901 + 3 μM CHIR99021 (LIF+2i, blue line).

B miR-142-3p reporter ratio distribution in primed pluripotency conditions (12 ng/ml FGF2 and 20 ng/ml Activin A, blue line) compared to naïve LIF-dependent pluripotency conditions (LIF+serum, black line).

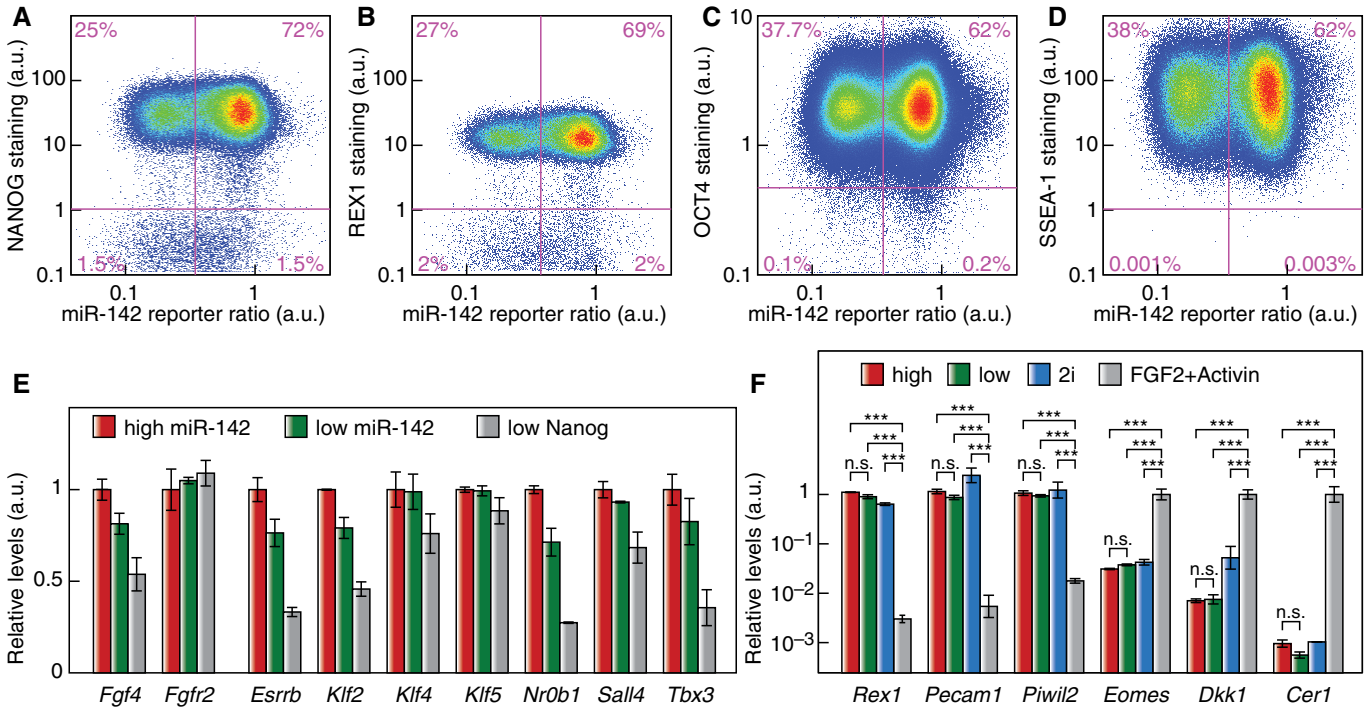


Figure EV3. "High" and "low" miR-142 mESCs express pluripotency markers at equal levels and do not express epiblast stem cell markers.

A–C Protein expression levels of the pluripotency markers NANOG (A), REX1 (B) and OCT4 (C) in single mESCs expressing the miR-142 activity reporter.

D Expression levels of the pluripotency marker SSEA-1 in single mESCs expressing the miR-142 activity reporter.

E mRNA expression levels of additional pluripotency markers in FACS-purified "high" and "low" miR-142 state mESCs as well as cells with low Nanog expression (n = 2; data represented as mean ± SEM).

F mRNA expression levels of mESC and epiblast stem cell markers in "high" and "low" miR-142 state mESCs as well as mESCs maintained in "2i" and epiblast stem cells maintained in primed pluripotency conditions (FGF2 + Activin) (n = 2; n.s.: not significant, ***P < 0.001, two-sided t-test). Data represented as mean ± SEM.

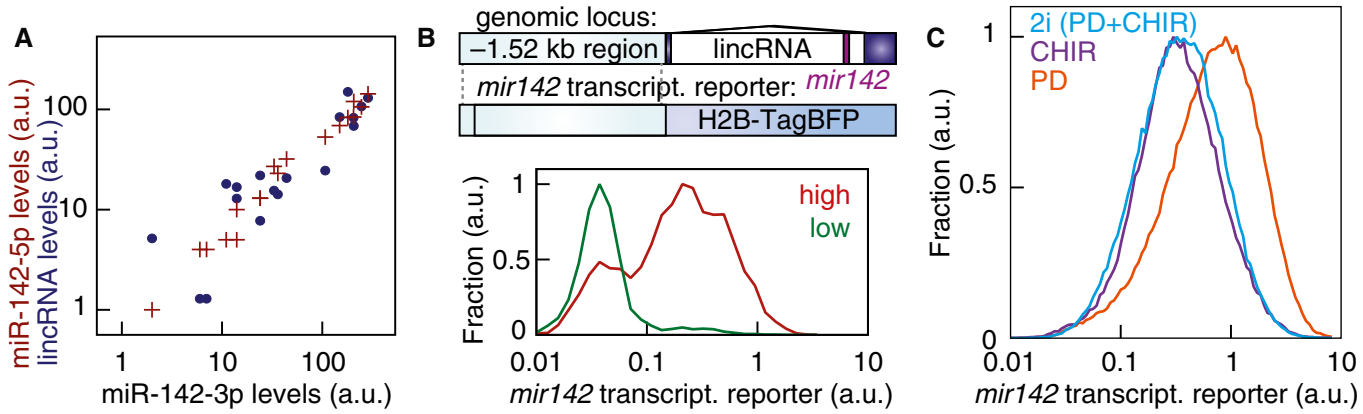


Figure EV4. *mir142* transcriptional reporter.

A Comparison between miR-142-3p, miR-142-5p and *mir142*-hosting lincRNA expression levels measured by deep sequencing across 18 matched mRNA-miRNA libraries. Levels of miR-142-3p were well-correlated with the levels of miR-142-5p and of the *mir142*-hosting lincRNA (r = 0.989, $P = 10^{-14}$; r = 0.881, $P = 10^{-6}$, respectively).

B Design of the fluorescent *mir142* transcriptional reporter and *mir142* transcriptional reporter signal in "high" and "low" miR-142 mESCs in double transgenic mESC lines expressing both the miR-142 activity and the *mir142* transcriptional reporters. The *mir142* transcriptional reporter was not expressed beyond background autofluorescence levels in the majority of "low" miR-142 mESCs but was expressed in the majority of "high" miR-142 mESCs confirming transcription as the source of the bimodal regulation of *mir142* expression.

C Distribution of *mir142* transcriptional reporter expression in the presence of 1 μM PD0325901 (PD, orange line) or 3 μM CHIR99021 (CHIR, purple line) or the combination of both inhibitors ("2i", blue line). GSK-3 inhibition counteracted the effects of ERK-inhibition on the *mir142* transcriptional reporter expression.

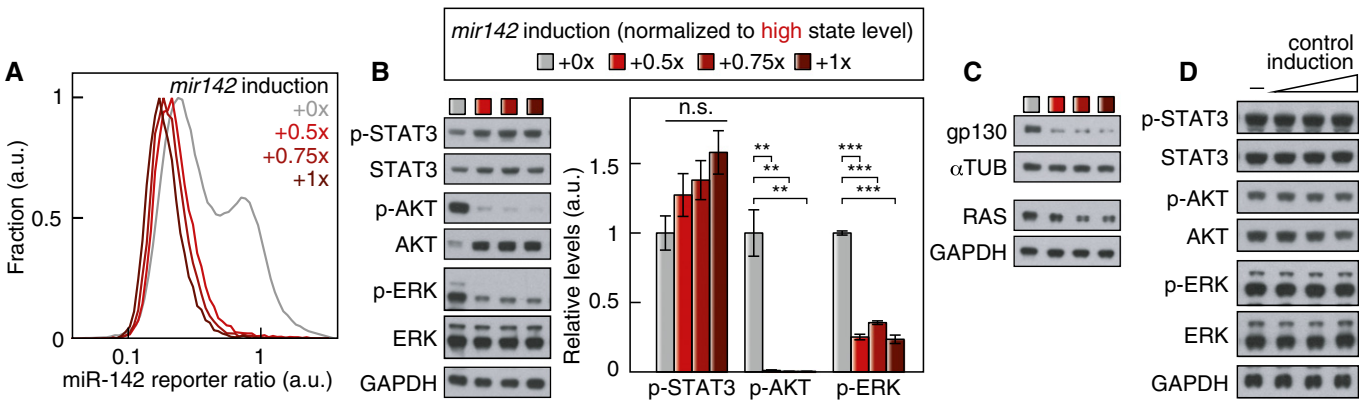


Figure EV5. Effect of miR-142 gain-of-function on the activation of the STAT3, AKT and ERK signaling pathways.

A Distribution of miR-142 reporter ratio at the three *mir142* induction levels used to assay the pathway activation status.

B Activation status of ERK, AKT, STAT3 upon *mir142* induction in mESCs and quantification (n = 3; $^{**}P < 0.01$, $^{***}P < 0.001$, two-sided t-test; error bars represent SEM). Induction levels are color-coded according to the boxed legend.

C gp130 and RAS protein levels upon *mir142* induction in mESCs. *mir142* induction levels are color-coded according to the boxed legend in (B).

D Activation status of ERK, AKT and STAT3 and their total levels upon induction of a control construct in mESCs.