Quantification of Mal-d-GFP enrichment in Act5C promoter region (A) and peak 1 (B) by qPCR. Genomic DNA precipitated from tub mal-d 3xGFP; mal-d^{17} / mal-d^{17} and OR+ ovary extracts was used as a template. For input, 1% of the total sample was used, mock is a sample immuno-precipitated without antibody, tub mal-d 3xGFP and OR+ were precipitated using GFP beads. Data show the mean ± s.d based on three independent biological replicates.
Supplemental Figure S2: Functional ontology profile of ChIP-seq and expression profile data.

(A) Functional annotation using the Gene Score Resampling (GSR) method implemented in the ErmineJ software (version 3.0.2) [1] using the default settings. Briefly, peak scores as reported by MACS or q-values of the gene expression analysis were used to compute p-values for each Gene Ontology (GO) set of the GO domains Biological Process and the Cellular Compartment. Multifunction corrected q-values were used to identify significantly enriched GO categories of the unique bound genes (n=155) at a false discovery rate (FDR) < 10% and q-val < 0.1 (A). Unadjusted p-values of <0.05 were used for the down regulated genes (n=95) in order to account for the lower enrichment in specific GO categories (B). Color coded bar indicates the % of bound (A) and % of down regulated (B) associated to every category.

(B) Downregulated genes

Supplemental Figure S3: Actin5C expression in FACS sorted migratory cells

Quantification of Actin 5C primary and mature transcripts levels in FACS sorted cells (method below) by qPCR: wild type (slbo-Gal4, UAS-10xGFP/+), mal-d mutant (slbo-Gal4,mal-dΔ7/mal-dΔ7,UAS-PH-GFP) and mal-d mutant with re-expression of Actin 5C (EP-Actin5C/+; slbo-Gal4, mal-dΔ7/mal-dΔ7, UAS-PH-GFP). Data shows mean ± standard deviation from three biological replicates.

Materials and Methods: Fluorescence-Activated Cell Sorting (FACS)
Fifty ovaries from 2-4 day old female (slbo-Gal4, UAS-10xGFP), pre-incubated for 20 hours on rich food were dissected in Schneider’s medium [Gibco] with 5 mg/ml insulin [Sigma], dissociated at 37°C for 15 min in 0.5 ml PBS, 0.05% Trypsin-EDTA, 7 µg/ml collagenase [Sigma], filtered through a 62 µm nylon mesh and collected in 0.5 ml 10% fetal calf serum. FACS was performed on a BD FACSaria Ilu SORP. A 100 µm sort setup nozzle was used at a 20 psi sheath pressure for 103 events per second. GFP positive cells were detected using a 488nm diode attenuated to 20mW and recovered. RNA was prepared using TRIzol [Ambion] according to manufacturers instructions.
Supplemental Figure S4: Control experiments in β-actin expressing cell lines

(A) Quantification of β-actin transgene induction in TetR + β-Actin and TetR+ Flag β-Actin by qPCR. RNA samples were extracted from cells after Tetracycline induction (see online methods). Data show the mean ± s.d based on three independent biological replicates. (B) Quantification of distance moved under the agar (see figure 4) of TetR + β-Actin and TetR+ Flag β-Actin in the presence of Tetracycline (Tet). Data show