

Supplemental Figure S3. Consequences of Cic inactivation in MEFs.

- (A) Proliferation of $Cic^{+/+}$ (open circles) or $Cic^{\Delta 2-6/\Delta 2-6}$ (closed circles) MEFs for the indicated time. Three independent MEF cultures were used for each genotype. Data represent mean \pm SD.
- (*B*) Western blot analysis of Cic protein expression in $Cic^{+/+}$, $Cic^{+/\Delta 2-6}$ and $Cic^{\Delta 2-6/\Delta 2-6}$ MEFs. GAPDH expression levels served as a loading control.
- (C) Relative expression levels of Etv1, Etv4 and Etv5 mRNAs in $Cic^{+/+}$ (open bars), $Cic^{+/\Delta 2-6}$ (red bars) and $Cic^{\Delta 2-6/\Delta 2-6}$ MEFs (closed bars). β -Actin expression levels were used for normalization. Data represent mean \pm SD.
- (*D*) Western blot analysis of p-Akt, Akt, p-Erk1/2 and Erk1/2 protein expression in $Cic^{+/+}$ and $Cic^{\Delta 2-6/\Delta 2-6}$ MEFs. GAPDH expression levels served as a loading control. Three independent MEF cultures were used for each genotype.

- (*E*) Focus formation using $Cic^{+/+}$ or $Cic^{\Delta 2-6/\Delta 2-6}$ MEFs stably infected with empty retroviruses or retroviruses expressing H-Ras^{G12V}, adenoviral E1A or H-Ras^{G12V}+E1A. Focus formation was scored after 14 days.
- (F) Colony formation in soft agar using $Cic^{+/+}$ or $Cic^{\Delta 2-6/\Delta 2-6}$ MEFs stably infected with empty retroviruses or retroviruses expressing H-Ras^{G12V}, adenoviral E1A or H-Ras^{G12V}+E1A. Colony formation was scored after 14 days.
- (*G*) Colony formation using *H-Ras*^{-/-};*N-Ras*^{-/-};*K-Ras*^{lox/lox} MEFs stably transduced with an empty vector (negative control), a vector expressing an shRNA against p53 (*shp53-A*, positive control), or *H-Ras*^{-/-};*N-Ras*^{-/-};*K-Ras*^{lox/lox};*Cic*^{lox/lox} MEFs infected with adenoviruses expressing GFP or the Cre recombinase. Colony formation is shown as the ratio of colonies that formed after Adeno-Cre (Rasless) vs. Adeno-GFP (K-Ras^{lox}) infection.