

Expanded View Figures

Figure EV1. Effects of silent mutations on gene expression, cell growth, and monomeric-to-filamentous actin ratio, related to Fig 2.

In this expanded view figure, the shape of the dots is conserved from Fig 2 and allows to identify the strains on the different graphs (circles for Sc, squares for ScNI, triangles for Sc[Ca], inverted triangles for Sc[Sp], diamonds for Sc[At] and crosses for nonviable strains). The color of the dots indicates the percentage of identity of the nucleotide sequences to the actin gene of *S. cerevisiae*, ranging from 100% (blue) to 75% (orange).

- A Actin expression levels, relative to wild-type, as a function of nucleotide conservation, showing that increased number of silent mutations lowers actin expression. Data are presented as mean \pm SD ($n = 4$ for Sc, $n = 8$ for ScNI; $n = 12$ for Sc[Ca], Sc[Sp] and Sc[At]; 2 biological replicates with $n/2$ technical replicates each). Pearson correlation coefficient r is considered nonsignificant if $P > 0.05$.
- B Differential gene expression of Sc[Ca], Sc[Sp] and Sc[At] strains compared to ScNI strain. Y-axis represents the adjusted P -value of FDR (False Discovery Rate) calculated with Benjamini and Hochberg method, and X-axis fold-changes. Red dots highlight proteins of interest (actin and 32 regulatory proteins) and grey dots represent all the other proteins identified by RNA-seq.
- C Growth constant as a function of nucleotide identity, showing a threshold of nucleotide conservation ($78\% < id < 82\%$) below which growth rates drastically reduce. Data are presented as mean \pm SD ($n = 6$ for Sc, $n = 3$ for ScNI, Sc[Ca], Sc[Sp] and Sc[At]; technical replicates). Pearson correlation coefficient r is considered nonsignificant if $P > 0.05$.
- D Evaluation of monomeric-to-filamentous actin ratios. Data are presented as mean \pm SD ($n = 12$ for Sc and ScNI and 4 for Sc[Ca], Sc[Sp] and Sc[At]; 2 biological replicates with $n/2$ technical replicates each). (Brown–Forsythe and Welch ANOVA tests, with Dunnett's T3 multiple comparisons tests).

Source data are available online for this figure.

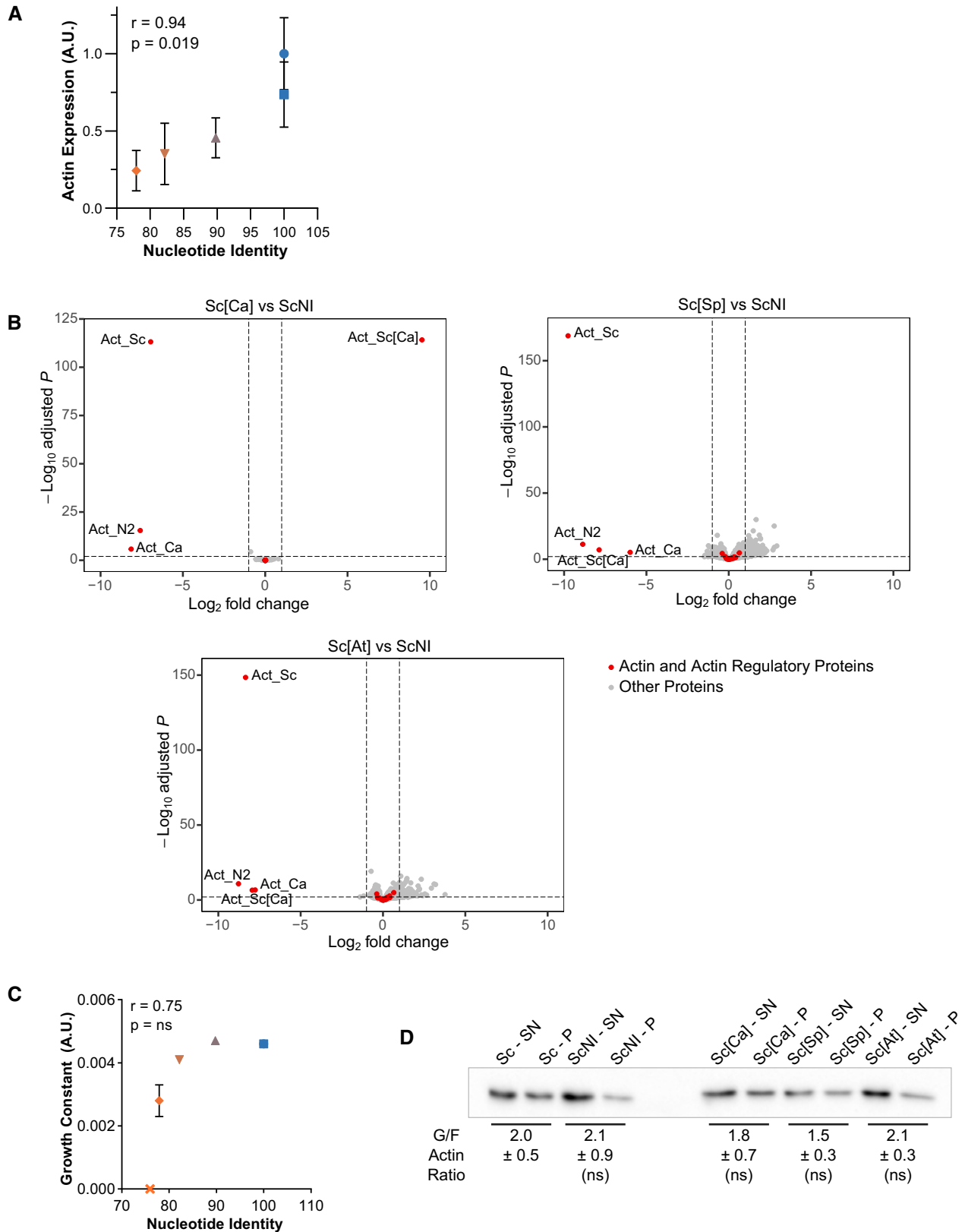


Figure EV1.

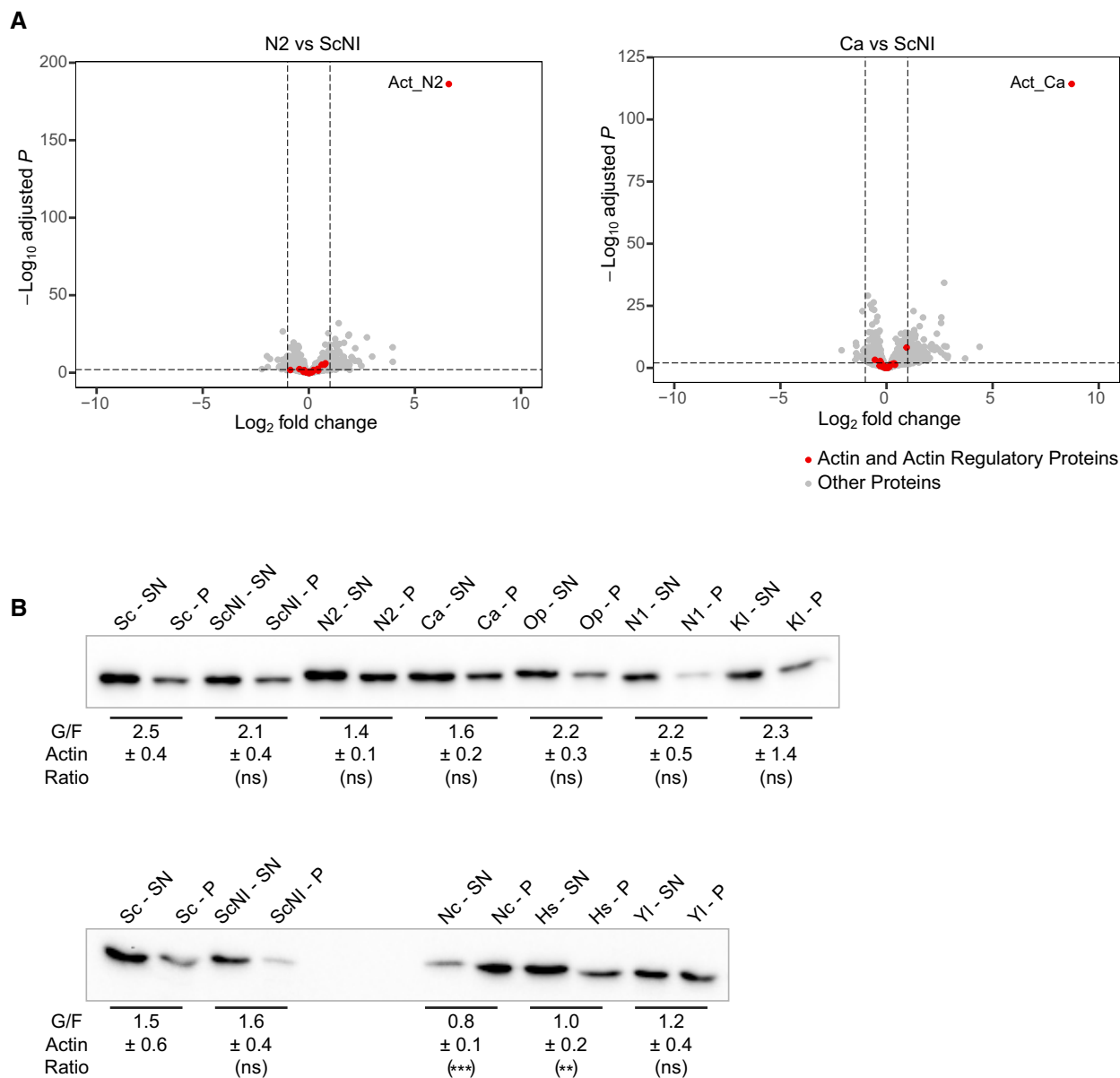


Figure EV2. Effects of swapping actin for different variants on gene expression and monomeric-to-filamentous actin ratio, related to Fig 3.

A Differential gene expression of Ca and N2 strains compared to ScNI strain. Y-axis represents the adjusted *P*-value of FDR (False Discovery Rate) calculated with Benjamini and Hochberg method, and X-axis fold-changes. Red dots highlight proteins of interest (actin and 32 regulatory proteins) and grey dots represent all the other proteins identified by RNA-seq.

B Evaluation of monomeric-to-filamentous actin ratios. Control strains and strains expressing the most (resp. the least) conserved actin variants are analyzed in the upper (resp. the lower) western blot. Data are presented as mean ± SD (*n* = 12 for Sc and ScNI and *n* = 4 for all other conditions; 2 biological replicates with *n*/2 technical replicates each). ***P* < 0.01, ****P* < 0.001 (Brown-Forsythe and Welch ANOVA tests, with Dunnett's T3 multiple comparisons tests).

Source data are available online for this figure.

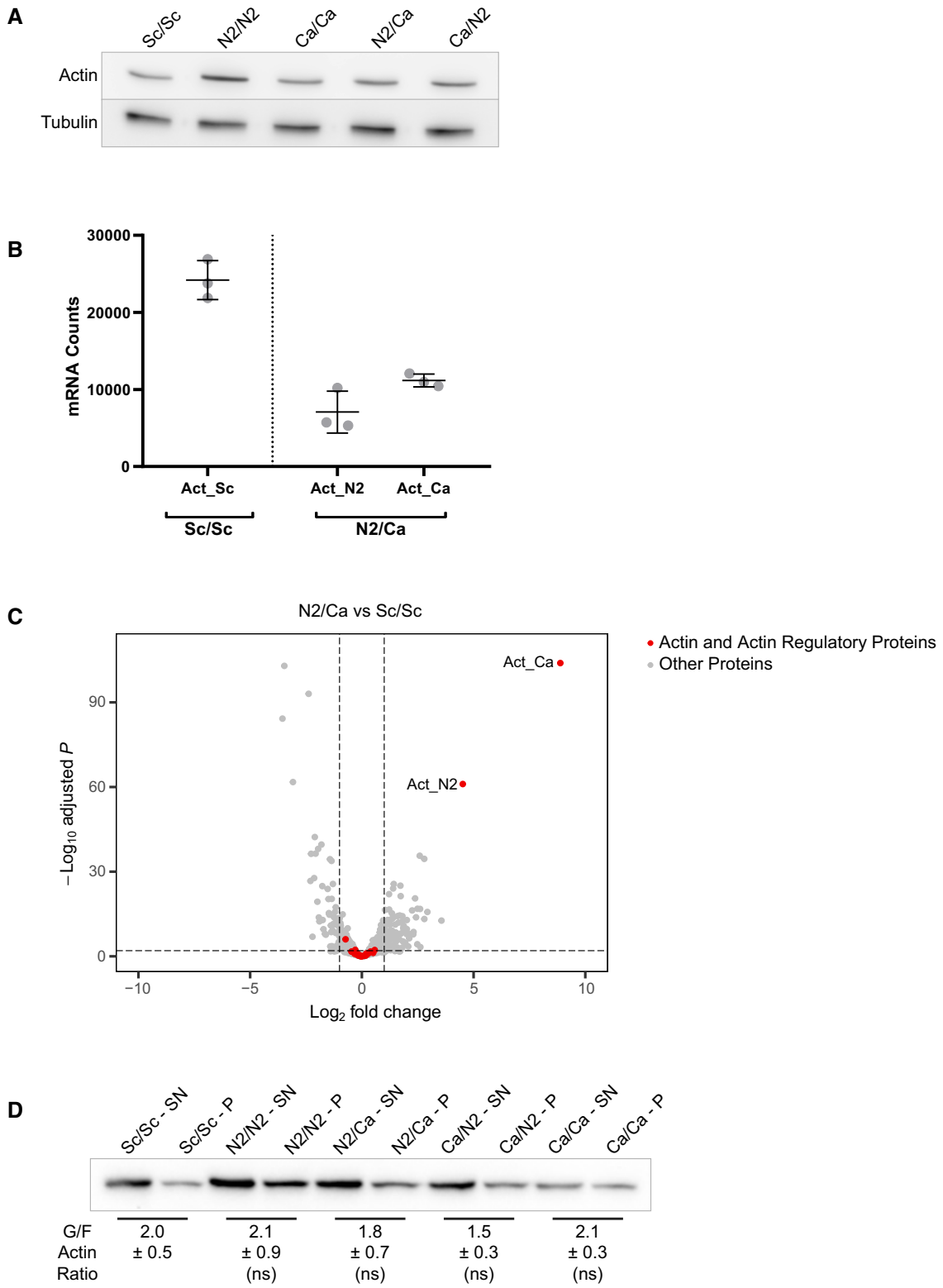


Figure EV3.

Figure EV3. Effect of a dual expression of actins on protein expression and monomeric-to-filamentous actin ratio, related to Fig 6.

- A Total actin expression levels shown by western blotting for diploid strains expressing one or two actin variants, with tubulin (Tub1p) as a loading control.
- B Comparison of actin gene expression levels in Sc/Sc and Ca/N2 strains. Data are presented as mean \pm SD ($n = 3$ for all conditions; biological replicates).
- C Differential gene expression of N2/Ca strain compared to Sc/Sc strain. Y-axis represents the adjusted P -value of FDR (False Discovery Rate) calculated with Benjamini and Hochberg method, and X-axis fold-changes. Red dots highlight proteins of interest (actin and 32 regulatory proteins) and grey dots represent all the other proteins identified by RNA-seq.
- D Evaluation of monomeric-to-filamentous actin ratios. Data are presented as mean \pm SD ($n = 4$ for all conditions; 2 biological replicates with 2 technical replicates each). (Brown–Forsythe and Welch ANOVA tests, with Dunnett's T3 multiple comparisons tests).

Source data are available online for this figure.