Expanded View Figures

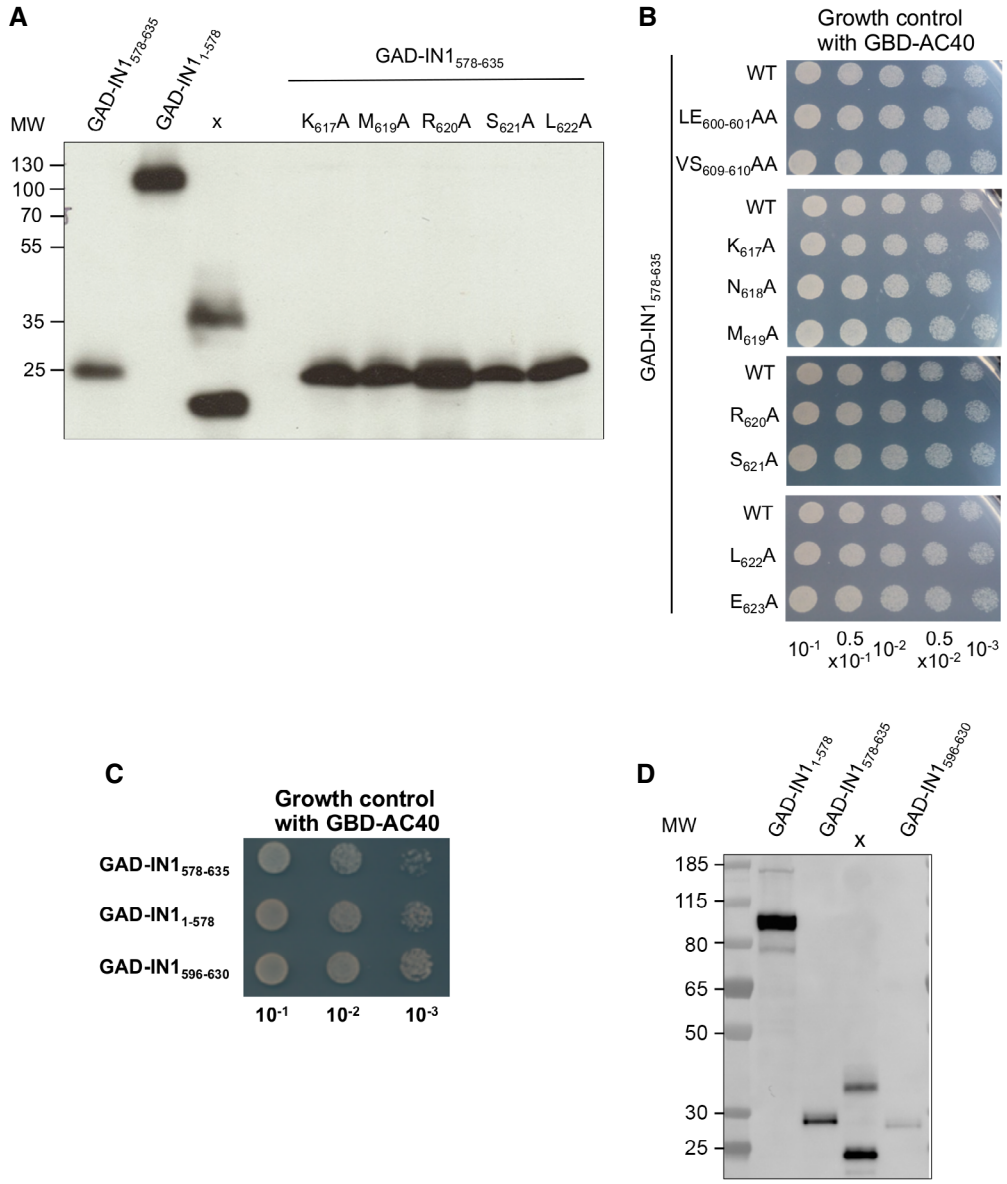

Figure EV1. Mutations in the IN1 bNLS linker sequence abolish the interaction with AC40.

A Validation of the expression of GAD-IN1 fusion proteins for the two-hybrid assay shown in Fig 1B. Whole-cell extract samples were prepared for immunoblotting from 1 DO_{600} of the cell culture by TCA precipitation. GAD-IN1 fusion proteins were detected by Western blot with anti-HA antibody (12CA5, Roche). All constructs harbor an HA-tag that is present between the GAD and IN1 sequences in the original pACTII vector. Expected sizes of the proteins are 26 kDa for GAD-$IN1_{578–635}$ and GAD-$IN1_{578–635}$ mutants and 82 kDa for GAD-$IN1_{1–578}$. "x", non-relevant sample.

B Growth control of cell cultures corresponding to the two-hybrid assay shown in Fig 1B. Twofold serial dilutions of cell cultures, starting from 10^{-1}, were plated on DO-Leu-Trp plates to check for growth.

C Growth control of cell cultures corresponding to the two-hybrid assay shown in Fig 1C. Twofold serial dilutions of cell cultures, starting from 10^{-1}, were plated on DO-Leu-Trp plates to check for growth.

D Validation of the expression of GAD-IN1 fusion proteins for the two-hybrid assay shown in Fig 1C. Whole-cell extract samples were prepared for immunoblotting from 1 DO_{600} of the cell culture by TCA precipitation. GAD-IN1 proteins were detected by Western blot with anti-GAD antibody (Santa Cruz Biotechnology). Expected sizes of the proteins are 82 kDa for GAD-$IN1_{1–578}$, 26 kDa for GAD-$IN1_{578–635}$, and 24 kDa for GAD-$IN1_{596–630}$. "x", non-relevant sample.

Source data are available online for this figure.

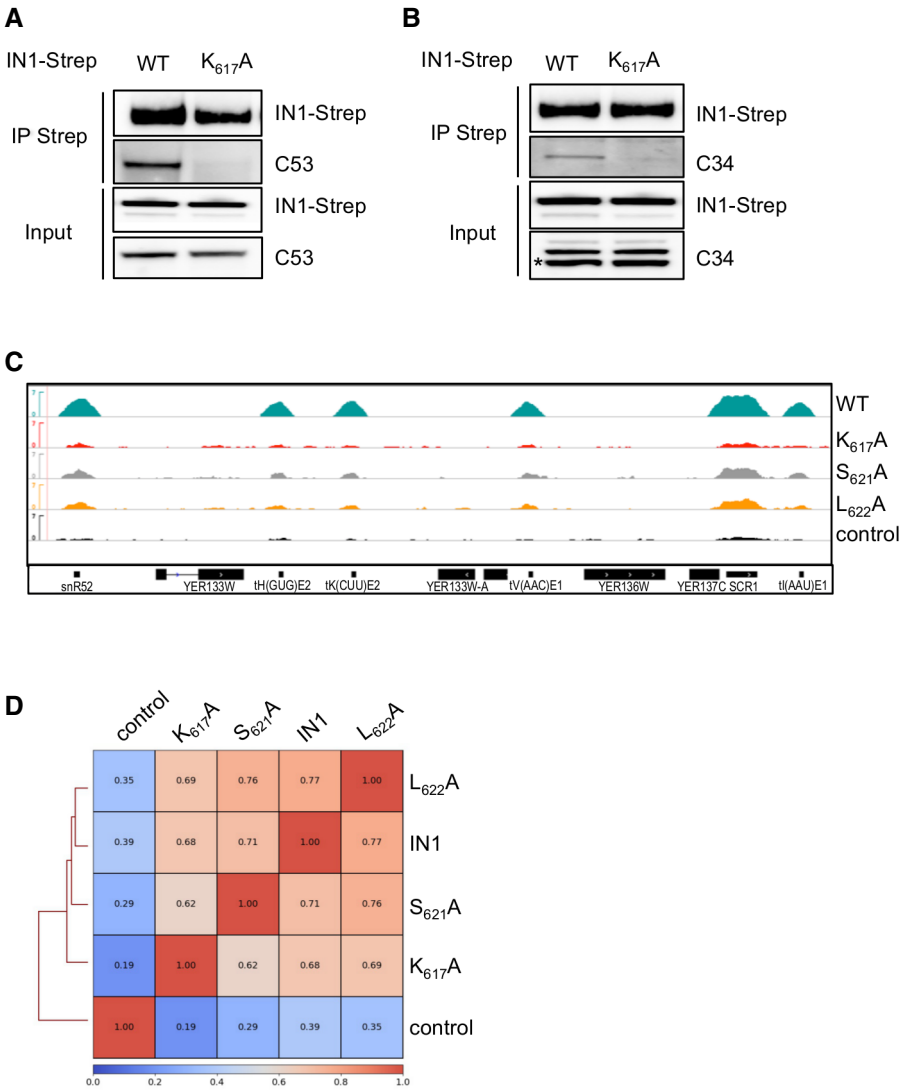

Figure EV2. AC40 recruits IN1 at Pol I- and Pol III-transcribed genes.

A, B Co-immunoprecipitation of endogenous Pol III subunits (C53 and C34) using ectopically expressed Ty1 integrase (IN1-Strep) as bait. Panels A and B were two separate lysates from two separate co-IPs. Expected sizes are 53 kDa for C53, 34 kDa for C34 and 82 kDa for IN1-Strep (WT and $K_{617}A$ mutant). *; C34.

C Genome browser visualization of different IN1-HA occupancy for chromosome V (coordinates chrV:431129..443275). Control is an anti-HA immunoprecipitation of chromatin extracts in cells expressing IN1-Strep. The region contains *tH(GUG)E2*, *tK(CUU)E2*, *tV(ACC)E1*, *tI(AAU)E1*, *SNR52*, and *SCR1*, all transcribed by Pol III.

D Pearson correlation heatmap at *tDNAs* between the following ChIP-seq: IN1 WT, $K_{617}A$, $S_{621}A$, $L_{622}A$ and control.

Source data are available online for this figure.

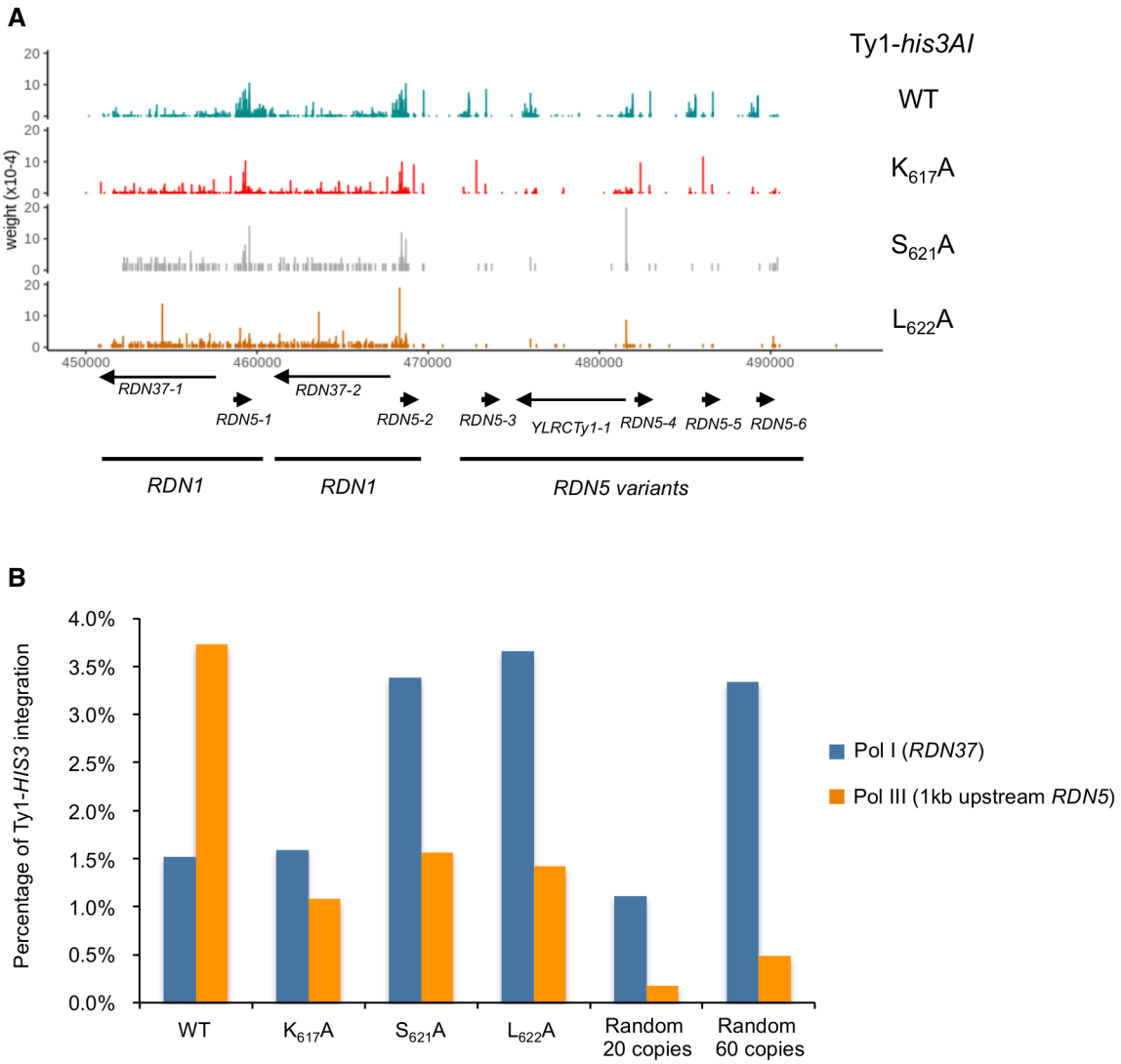

Figure EV3. Ty1 integration profile at the *RDN1* repeats.

A Visualization of WT and IN1 mutant Ty1-*HIS3* insertions into the *RDN1* repeats and adjacent *RDN5* variants.

B Quantification of Ty1-*HIS3* insertions events (WT and mutants) at *RDN37* (Pol I-transcribed gene) and in a 1 kb window upstream of *RDN5* (Pol III-transcribed gene). Control cases are 1×10^5 Ty1 random insertions in artificial yeast genomes composed of 20 or 60 *RDN1* repeats. Of note, less *de novo* Ty1 insertion events were recovered for the $S_{621}A$ and $L_{622}A$ mutants than for WT and $K_{617}A$ mutants.

Figure EV4. IN1 targeting domain directs Ty5 integration at Pol III-transcribed genes.

A–D (A and C) Growth control of cell cultures corresponding to the two-hybrid assay shown in Fig 5A. Ten-fold serial dilutions of cell cultures, starting from 10^{-1}, were plated on DO-Leu-Trp plates to check for growth (B and D). Validation of the expression of GAD-IN1 and GAD-IN5 fusion proteins for the two-hybrid assay shown in Fig 5A. Whole-cell extract samples were prepared for immunoblotting as for Fig EV1A. Expected sizes of the proteins are 25 kDa for GAD-$IN1_{578-635}$ and GAD-$IN1_{578-635}$ mutants and 82 kDa for GAD-$IN1_{1-578}$, around 100 kDa for GAD-IN5 constructions.

E Genome browser visualization of HA-IN1 or HA-IN5 occupancy at the *RDN1* locus encoding ribosomal RNA genes. Control is an anti-HA immunoprecipitation of chromatin extracts in cells expressing IN1-Strep. Values obtained from ChIP-seq analysis have been normalized for each condition (WT and mutant IN5) to input and adjusted in log2 RPKM.

F Pearson correlation heatmap at *tDNA*s between the following ChIP-seq: IN1, IN5, $IN5_{\Delta TD5}$, $IN5_{\Delta TD5+bNLS}$, and control.

G Retrotransposition frequency, shown on a log scale of p*GAL1*-Ty5*his3AI* and p*GAL1*-Ty5*his3AI* ($IN5_{\Delta TD5+bNLS}$) in an *spt3-101* (LV47) or *rad52*Δ strain (LV172). Values are mean ± SD, $n = 2$ experiments, each performed with four independent colonies.

Source data are available online for this figure.

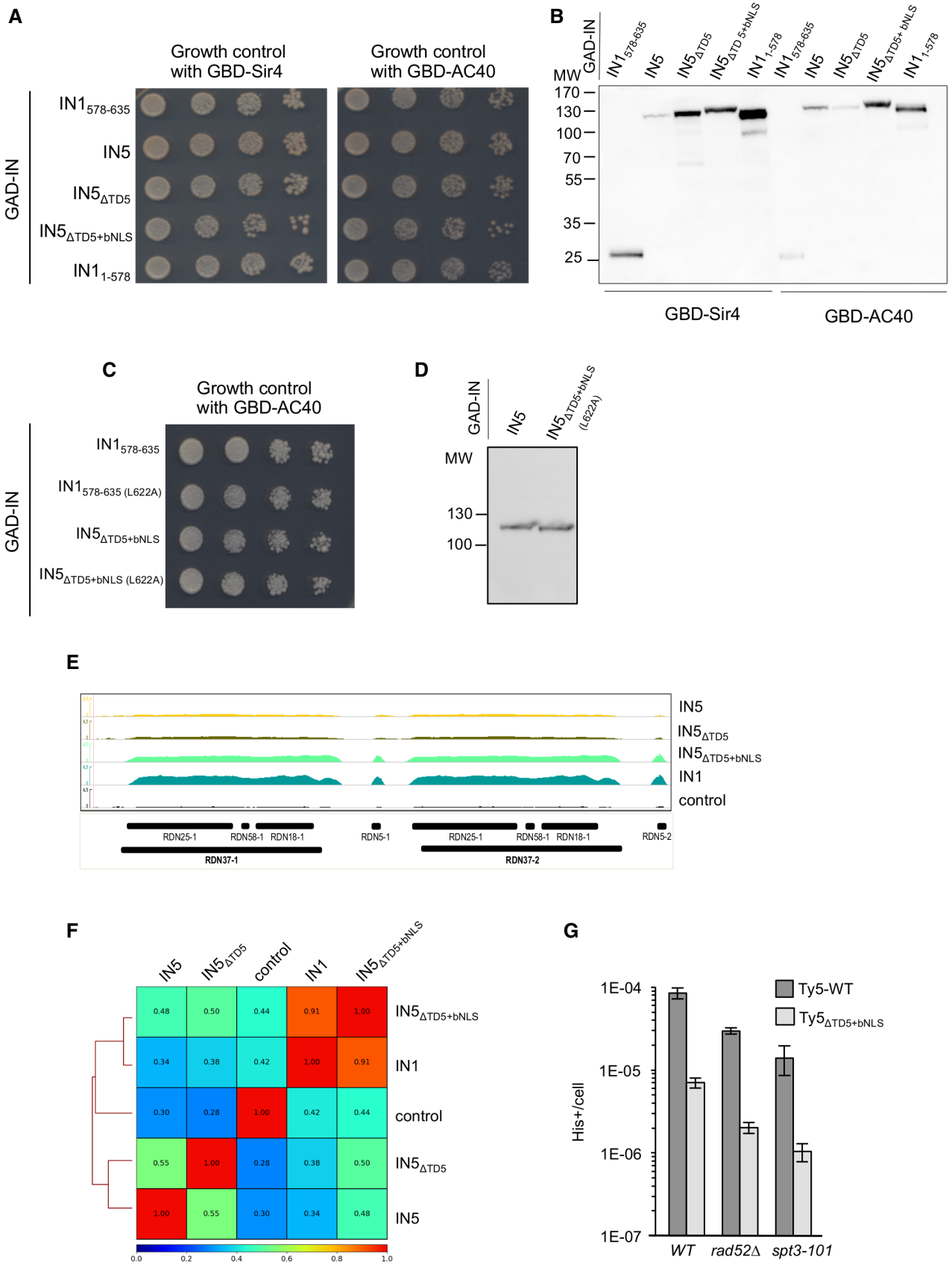
A
Growth control with GBD-Sir4
Growth control with GBD-AC40
GAD-IN
IN1578-635
IN5
IN5ΔTD5
IN5ΔTD5+bNLS
IN11-578
B
GAD-IN
MW
170
130
100
70
55
35
25
GBD-Sir4
GBD-AC40
C
Growth control with GBD-AC40
IN1578-635
IN1578-635 (L622A)
IN5ΔTD5+bNLS
IN5ΔTD5+bNLS (L622A)
D
GAD-IN
IN5
IN5ΔTD5+bNLS (L622A)
MW
130
100
E
IN5
IN5ΔTD5
IN5ΔTD5+bNLS
IN1
control
RDN25-1
RDN58-1
RDN18-1
RDN5-1
RDN37-1
RDN37-2
RDN5-2
F
IN5
IN5ΔTD5
control
IN1
IN5ΔTD5+bNLS
0.48 0.50 0.44 0.91 1.00
0.34 0.38 0.42 1.00 0.91
0.30 0.28 1.00 0.42 0.44
0.55 1.00 0.28 0.38 0.50
1.00 0.55 0.30 0.34 0.48
0.0 0.2 0.4 0.6 0.8 1.0
G
Ty5-WT
Ty5ΔTD5+bNLS
His+/cell
1E-04
1E-05
1E-06
1E-07
WT
rad52Δ
spt3-101

Figure EV4.