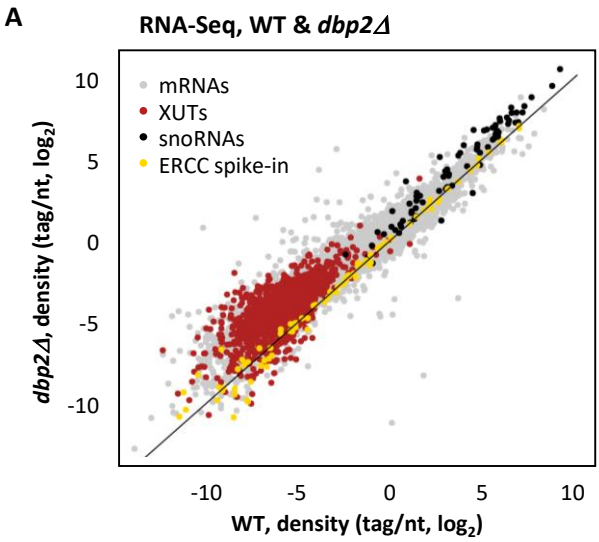
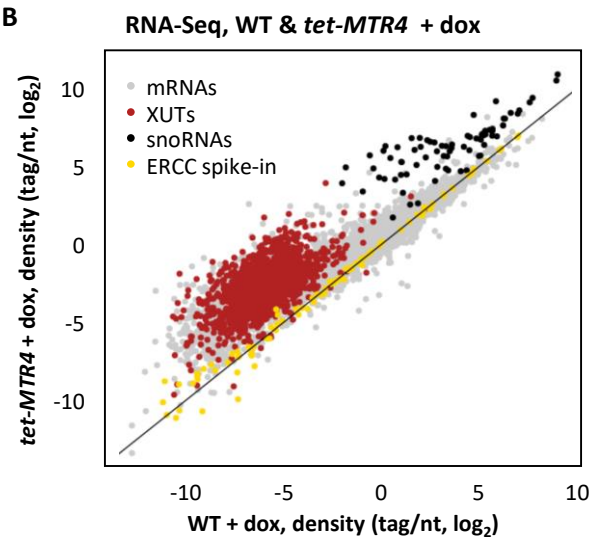
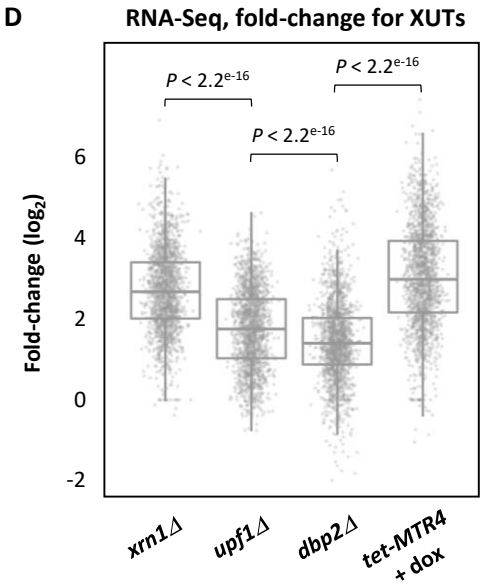


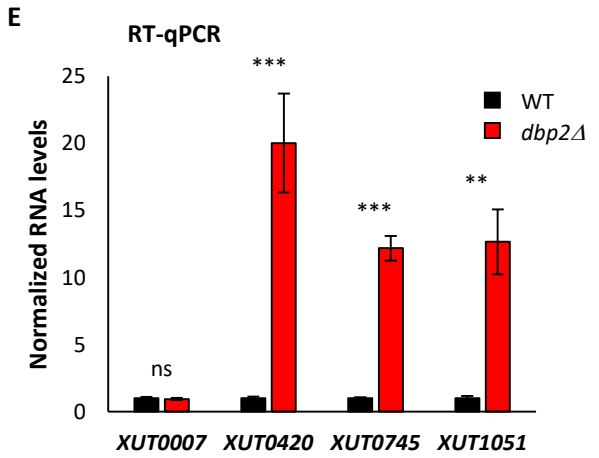


C

RNA-Seq,
Mean fold-change (log_2)

	Dbp2	Mtr4
mRNAs	0.9	1.12
XUTs	1.44	3.03
CUTs/NUTs	1.34	4.16
snoRNAs	0.83	2.71





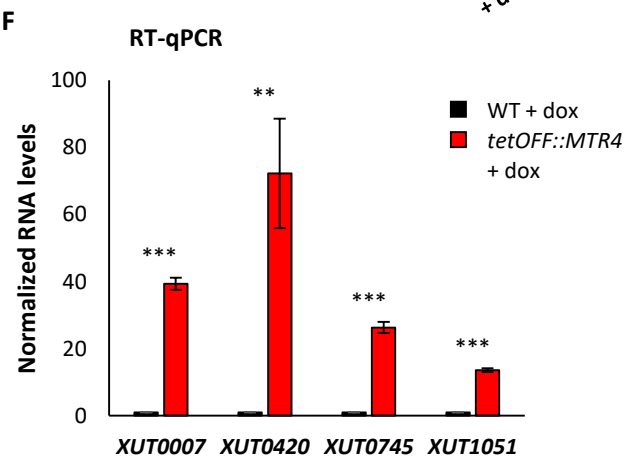


Figure S1. Identification of Dbp2- and Mtr4-sensitive XUTs.

A. Total RNA-Seq was performed using total RNA extracted from two biological replicates of exponentially growing YAM1 (WT) and YAM2627 (*dbp2Δ*) cells. The data are presented as densities (tag/nt, log_2 scale) for mRNAs (grey), XUTs (red) and snoRNAs (black) upon normalization on the ERCC spike-in (yellow). **B.** Same as above using YAM115 (WT) and YAM997 (*tetOFF::MTR4*) cells grown in the same conditions and treated for 6 hours with 10 µg/ml doxycycline (dox). **C.** Mean fold-change (log_2) for mRNAs, XUTs, CUTs/NUTs and snoRNAs upon inactivation of Dbp2 or depletion of Mtr4. **D.** Box-plot showing the sensitivity of XUTs to Xrn1, Upf1, Dbp2 and Mtr4. The data are presented as the expression fold-change (log_2) between each mutant and its isogenic WT control. Data for the *upf1Δ* mutant were previously described (Wery et al., 2016). *P*-values were obtained upon two-sided Wilcoxon rank-sum test (adjusted for multiple testing with the Benjamini–Hochberg procedure). **E.** YAM1 (WT, black) and YAM2627 (*dbp2Δ*, red) were grown to mid-log phase, at 30°C, in YPD medium. After total RNA extraction, the levels of *XUT007*, *XUT0420*, *XUT0745* and *XUT1051* were assessed by strand-specific RT-qPCR, and then normalized on the *PMA1* mRNA (unaffected housekeeping gene). The normalized level of each XUT in the WT condition was set to 1. Mean and SD values were calculated from three independent biological replicates. ** $P < 0.01$; *** $P < 0.001$; ns, not significant upon t-test. **F.** Same as above using YAM115 (WT, black) and YAM997 (*tetOFF::MTR4*, red) cells grown in the same conditions and then treated for 6 hours with 10 µg/ml doxycycline (dox).

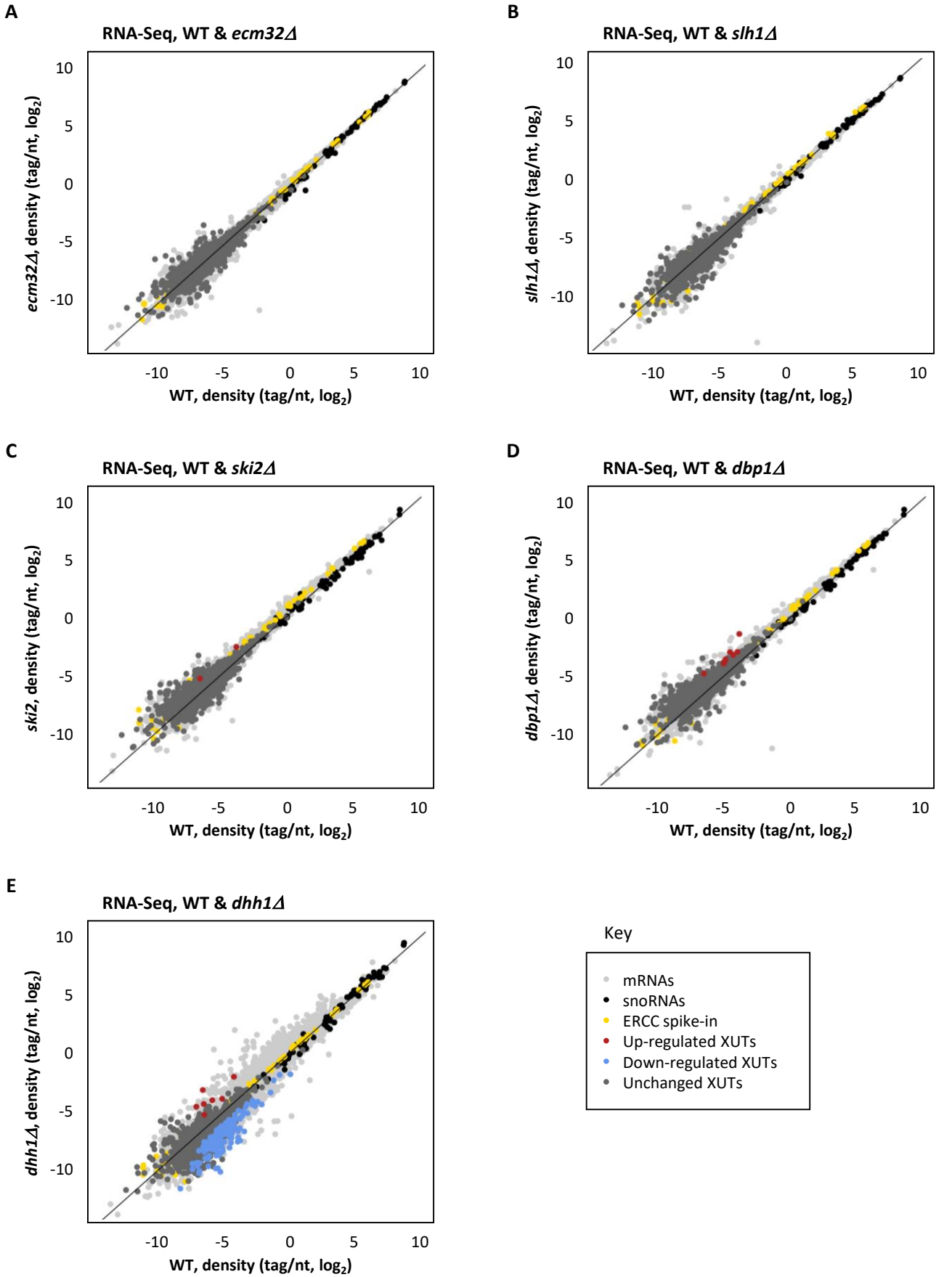


Figure S2. XUTs do not globally accumulate upon inactivation of the Ecm32, Ski2, Slh1, Dbp1 and Dhh1 RNA helicases.
A. Total RNA-Seq was performed using total RNA extracted from two biological replicates of exponentially growing YAM1 (WT) and YAM2834 (*ecm32Δ*) cells. The data are presented as densities (tag/nt, log_2 scale) for mRNAs (light grey), XUTs (dark grey when unaffected), snoRNAs (black) and the ERCC spike-in (yellow). **B.** Same as above using YAM2835 (*slh1Δ*) cells. **C.** Same as above using YAM2628 (*ski2Δ*) cells. The red dots represent the 2 up-regulated XUTs (*ski2Δ*/WT > 2 and adjusted *P*-value < 0.05). **D.** Same as above using YAM2630 (*dbp1Δ*) cells. The red dots represent the 8 up-regulated XUTs (*dbp1Δ*/WT > 2 and adjusted *P*-value < 0.05). **E.** Same as above using YAM2632 (*dhh1Δ*) cells. The red and blue dots represent the 7 up-regulated XUTs (*dhh1Δ*/WT > 2 and adjusted *P*-value < 0.05) and the 185 down-regulated XUTs (*dhh1Δ*/WT < 0.5 and adjusted *P*-value < 0.05), respectively.

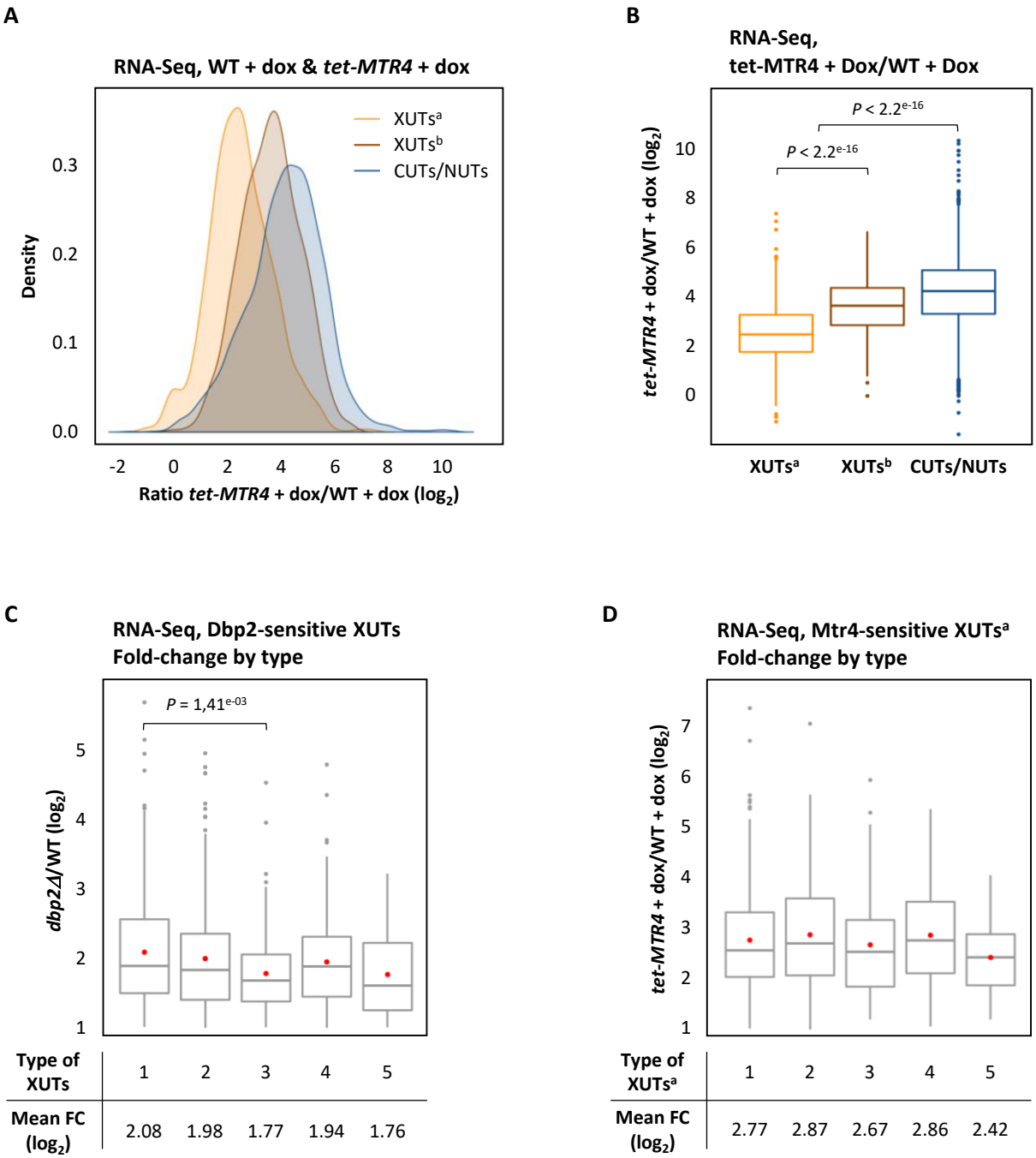


Figure S3. Dbp2 and Mtr4 target XUTs independently of their configuration to protein-coding genes.
A. XUTs were separated into two subsets according the overlap (≥ 1 nt) or not with a CUT or a NUT. The sensitivity to Mtr4 of each subset is presented as a density-plot of the expression fold-change upon 6 hours of Mtr4 depletion (ratio of tag densities, $\log_2$ scale). The orange, brown and blue curves represent the Mtr4-sensitivity of XUTs[a] (no overlap with a CUT/NUT), XUTs[b] (≥ 1 nt overlap with a CUT/NUT) and CUTs/NUTs, respectively. **B.** Box-plot showing the Mtr4-sensitivity of XUTs[a], XUTs[b] and CUTs/NUTs. The data are presented as the expression fold-change ($\log_2$ scale) for each subset of transcript upon Mtr4 depletion. *P*-values were obtained upon two-sided Wilcoxon rank-sum test (adjusted for multiple testing with the Benjamini–Hochberg procedure). **C.** Sensitivity to Dbp2 for the five types of XUTs, defined according to their configuration relative to sense mRNAs (see Figure 3A). The data are represented as a box-plot of the expression fold-change ($\log_2$ scale) for each type of XUTs in the *dbp2Δ* mutant relative to the isogenic WT control. The red point indicates the mean-fold change for each type of XUTs (also indicated below the plot). The indicated *P*-value was obtained upon two-sided Wilcoxon rank-sum test (adjusted for multiple testing with the Benjamini–Hochberg procedure). The *P*-values for all other comparisons were > 0.05 (not significant). **D.** Same as above for the Mtr4-sensitive XUTs[a] subset. The *P*-values (obtained as above) for all other comparisons were > 0.05 (not significant).

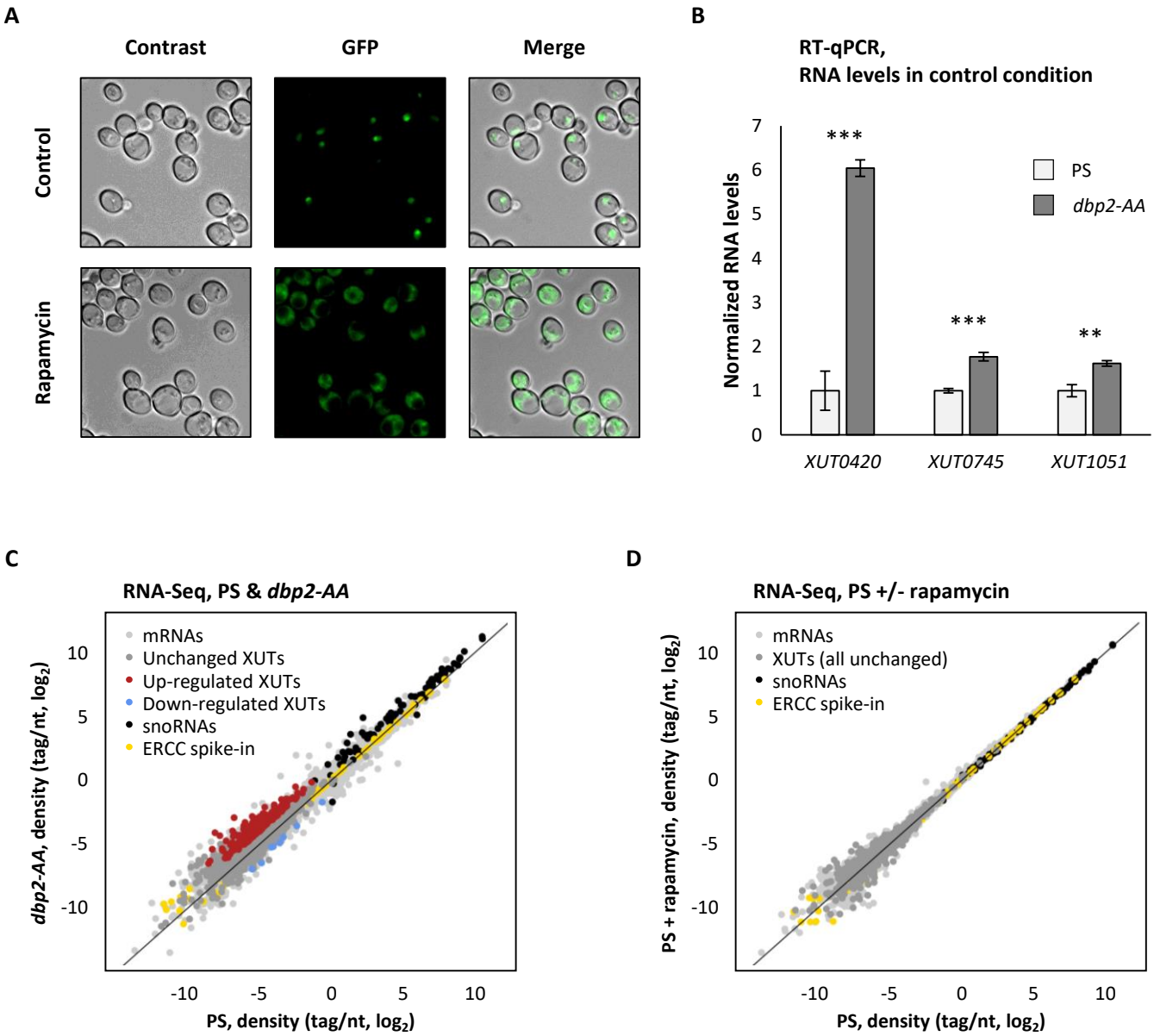


Figure S4. XUTs are insensitive to Dbp2 nuclear depletion.

A. Rapamycin-dependent nuclear depletion of Dbp2-FRB-GFP using anchor away (Dbp2-AA). Dbp2-FRB-GFP was detected on living YAM2673 cells by fluorescent microscopy before (upper row) and after 60 min (bottom row) of treatment with rapamycin. **B.** YAM2672 (parental strain, PS) and YAM2673 (*dbp2-AA*) cells were grown to mid-log phase, at 30°C, in YPD medium. After total RNA extraction, the levels of *XUT0420*, *XUT0745* and *XUT1051* were assessed by strand-specific RT-qPCR, and then normalized on the *PMA1* mRNA (unaffected housekeeping gene). The normalized level of each XUT in the PS condition was set to 1. Mean and SD values were calculated from three independent biological replicates. ** $P < 0.01$; *** $P < 0.001$ upon t-test. **C.** Total RNA-Seq was performed using the same RNA extracts as above. The data are presented as densities (tag/nt, $\log_2$ scale) for mRNAs (light grey), unaffected XUTs (dark grey), up-regulated XUTs (red), down-regulated XUTs (blue), snoRNAs (black) and the ERCC spike-in (yellow). **D.** Same as C using total RNA extracted from YAM2672 (PS) cells grown as above and then treated or not for 60 min with rapamycin (1 µg/ml, final concentration).