Supplementary information

Supplementary Material and Methods

Plasmid construction The transposable element vectors for inducible expression of RFP-FUS^{wt} and EGFP-FUS^{R521C} and EGFP-FUS^{P525L} were derived from the enhanced piggyBac (ePiggyBac) vector epB-Bsd-TRE described in Rosa and Brivanlou, 2011. Briefly, a cassette encoding for the rtTA-Advanced protein (Clontech) was fused to the Puromycin or Blasticidin resistance coding sequences through a T2A self-cleavage peptide element, and put under the control of the ubiquitous pUbc promoter in the epB-Bsd-TRE vector. The resulting plasmids (epB-Puro-TT and epB-Bsd-TT) hold on the opposite direction the tetracycline-responsive promoter element (TRE), followed by a short multicloning site. Therefore both elements of the TET-ON system are present in the same vector. The RFP and EGFP coding sequences, devoid of the stop codon, were then inserted in the epB-Puro-TT and epB-Bsd-TT plasmids, respectively, generating the epB-Puro-TT-RFP and epB-Bsd-TT-EGFP. Finally, the coding sequences of FUS, wild type or mutated, were cloned in frame with the fluorescent proteins, generating the epB-Puro-TT-RFP-FUS^{wt}, epB-Bsd-TT-EGFP-FUS^{R521C} and epB-Bsd-TT-EGFP- FUS^{P525L}.

Drosha *in vitro* processing. *In vitro* processing assay was carried out as previously described (Lee and Kim, 2007). Pri-miRNA substrates were prepared by *in vitro* transcription, using T7 RNA polymerase (Promega), from PCR amplified templates (oligonucleotides are listed in Table II), in the presence of [α-32P]UTP (Perkin-elmer). 100'000 cpm of each pri-miRNA transcript were incubated with 15 mg of SK-N-BE nuclear extract cells at 37°C for 90 minutes.

Supplementary references

Lee Y, Kim VN (2007) *In vitro* and in vivo assays for the activity of Drosha complex. *Methods Enzymol* **427**: 89-106

Rosa A, Brivanlou AH (2011) A regulatory circuitry comprised of miR-302 and the transcription factors OCT4 and NR2F2 regulates human embryonic stem cell differentiation. EMBO J **30**: 237-248

Supplementary Figure Legends

Figure S1. (**A**) miRNA and protein levels during SK-N-BE cells differentiation. SK-N-BE cells were induced to differentiate with retinoic acid (RA) and incubated for the indicated times (0, 1, 3, 6 and 10 days). miR-9, miR-125b and miR-132 were analyzed by Northern blot using corresponding specific oligonucleotides. 5.8S rRNA was used as internal control. The histogram indicates the relative levels normalized for the 5.8S signal. Lower panel: Western blot analysis of N-Myc and FUS proteins at the same time points. (**B**) miRNA profiling in SK-N-BE and HeLa cells treated with anti-FUS siRNA (siFUS) or with AllStars Negative Control siRNA (siScr). SK-N-BE cells were cultured in RA for 6 days. Pie charts and tables showing the percentage of miRNA derelegulated more than 0,2 fold.

Figure S2. FUS interference. (**A**) Levels of neuronal-specific miRNAs in RA-treated SK-N-BE cells in two different sets of RNAi experiments where the residual FUS levels were 45% and 20% respectively. AllStars Negative Control siRNA (siScr) were utilized as control. Left panels: Western blot analysis of FUS and GAPDH proteins. Relative quantification (RQ) of FUS versus GAPDH is shown with respect to the siScr condition set to a value of 1. Right panels: histograms of miRNA levels analysed by qPCR normalized for the snoRNA-U25 internal control. The values are

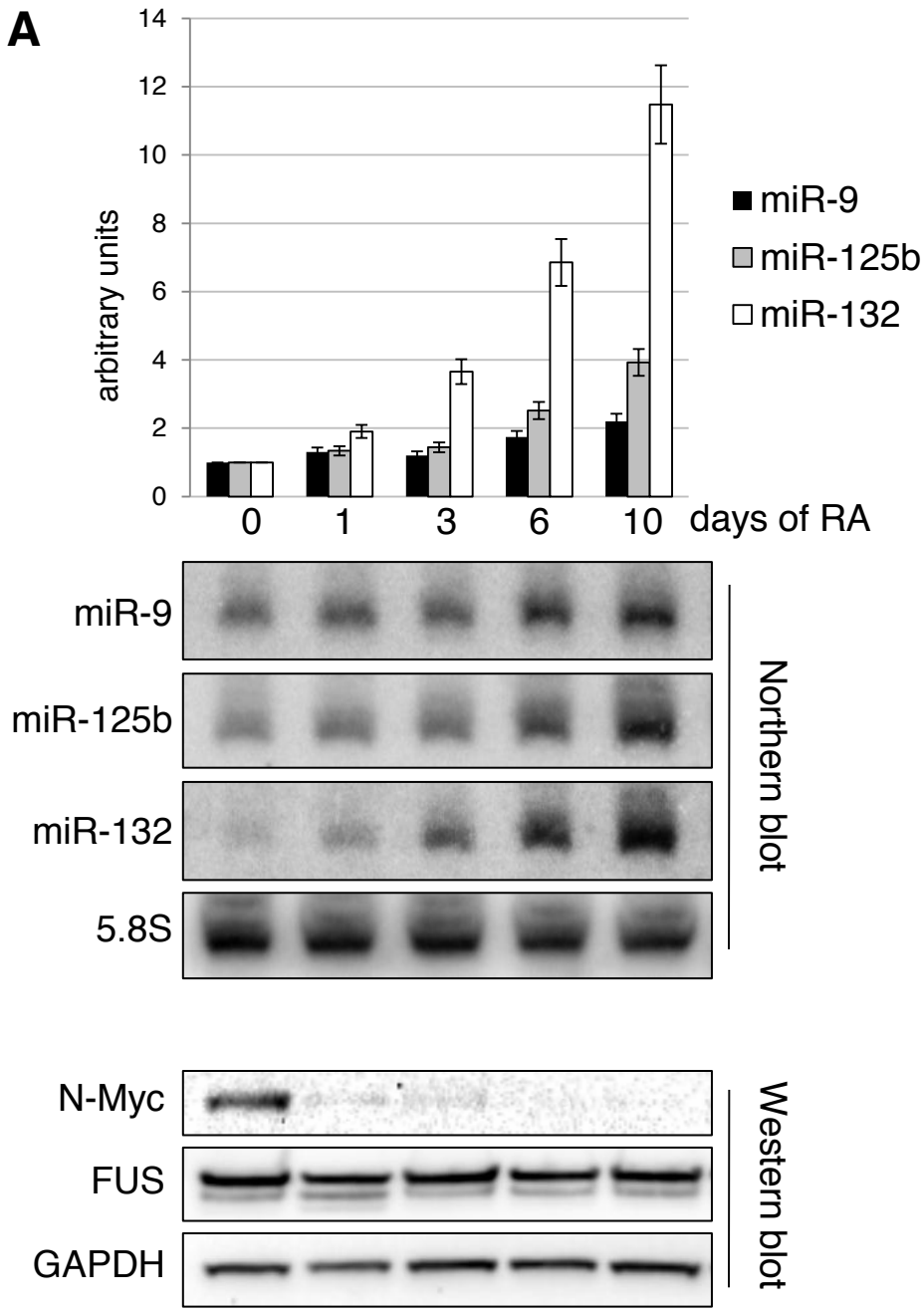
the average from 3 independent experiments and are expressed with respect to the siScr condition set to a value of 1. (**B**) SK-N-BE cells were treated with siRNAs against the 3'UTR of FUS (siFUS-3') or scrambled siRNA (siScr) and maintained in retinoic acid (RA) for 6 days. miRNA levels were analyzed by RT-qPCR. The histogram represents the average of 3 different measurements. miRNA levels were normalized for the snoRNA-U25 internal control.

Figure S3. Coomassie staining showing the quality of purified recombinant GST-FUS^{WT} and GST-FUS^{R521C}. Different amounts of BSA protein are used as quantity control. Marker of molecular weight is also shown.

Figure S4. Intracellular localization of wild type and mutated FUS proteins. (**A**) Schematic representation of the epB-Puro-TT-RFP-FUS^{wt}, epB-Bsd-TT-EGFP-FUS^{R521C} and epB-Bsd-TT-EGFP-FUS^{P525L} constructs. Triangles indicate the 5' and 3' Terminal Repeats (TR) of the epiggyBac vector. (**B**) SK-N-BE cells were co-transfected with epB-Puro-TT-RFP-FUS^{wt} and epB-Bsd-TT-EGFP-FUS^{R521C} (top panels) or with epB-Puro-TT-RFP-FUS^{wt} and epB-Bsd-TT-EGFP-FUS^{P525L} (bottom panels), together with a plasmid encoding for the epiggyBac transposase. After selection with 1μg/ml Puromycin and 10 μg/ml Blasticidin, stably transfected cells were induced with 0,2μg/ml Doxycyclin for 3 days. Images were taken with a Zeiss Axio Observer A1 fluorescence microscope at 20X magnification. Scale bar=20 μm.

Figure S5. FUS depletion does not affect Drosha-mediated processing *in vitro*. *In vitro* processing with ^{32}P-UTP labelled pri-miR-9-2, pri-miR-125-b and pri-miR-132 using nuclear extracts from SK-N-BE cells treated either with AllStars Negative Control siRNA (siScr) or siRNA against FUS (siFUS). The mock samples with no extract are used as controls.

Figure S1


A
14
12
10
8
6
4
2
0
arbitrary units
miR-9
miR-125b
miR-132
0
1
3
6
10
days of RA
miR-9
miR-125b
miR-132
5.8S
Northern blot
N-Myc
FUS
GAPDH
Western blot

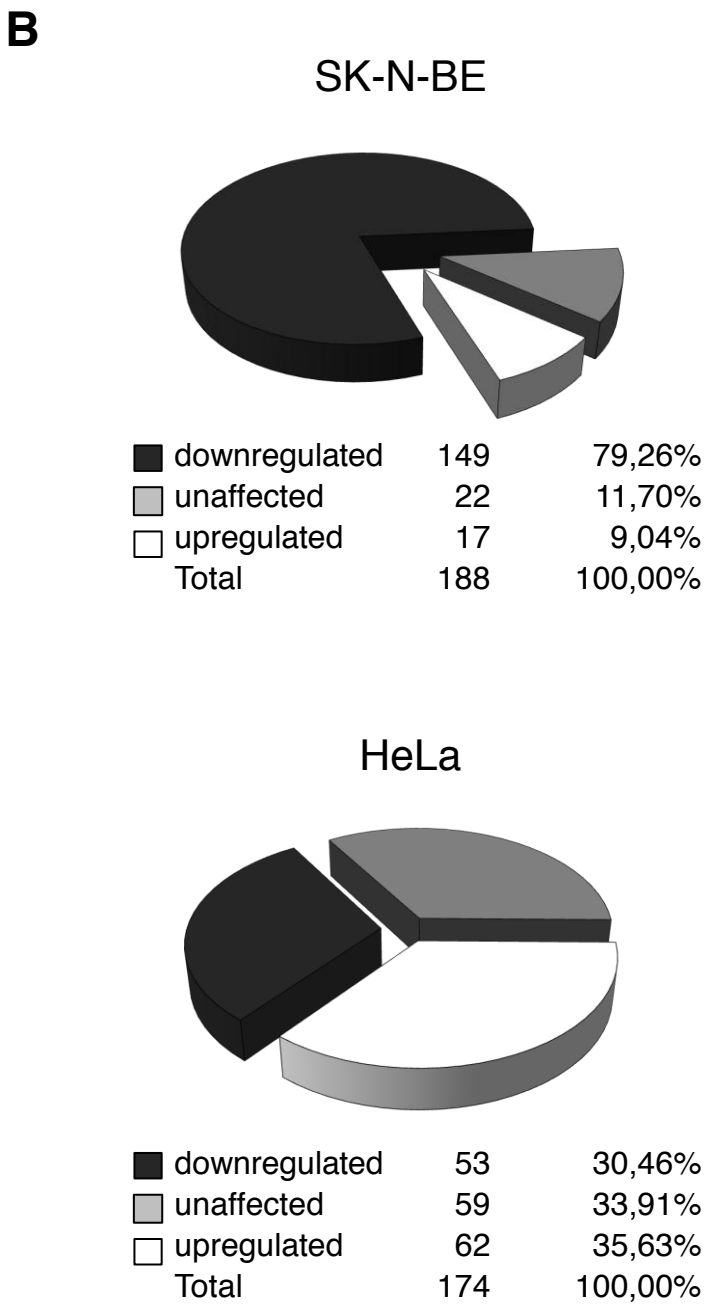

B
SK-N-BE
downregulated 149 79,26%
unaffected 22 11,70%
upregulated 17 9,04%
Total 188 100,00%
HeLa
downregulated 53 30,46%
unaffected 59 33,91%
upregulated 62 35,63%
Total 174 100,00%

Figure S2

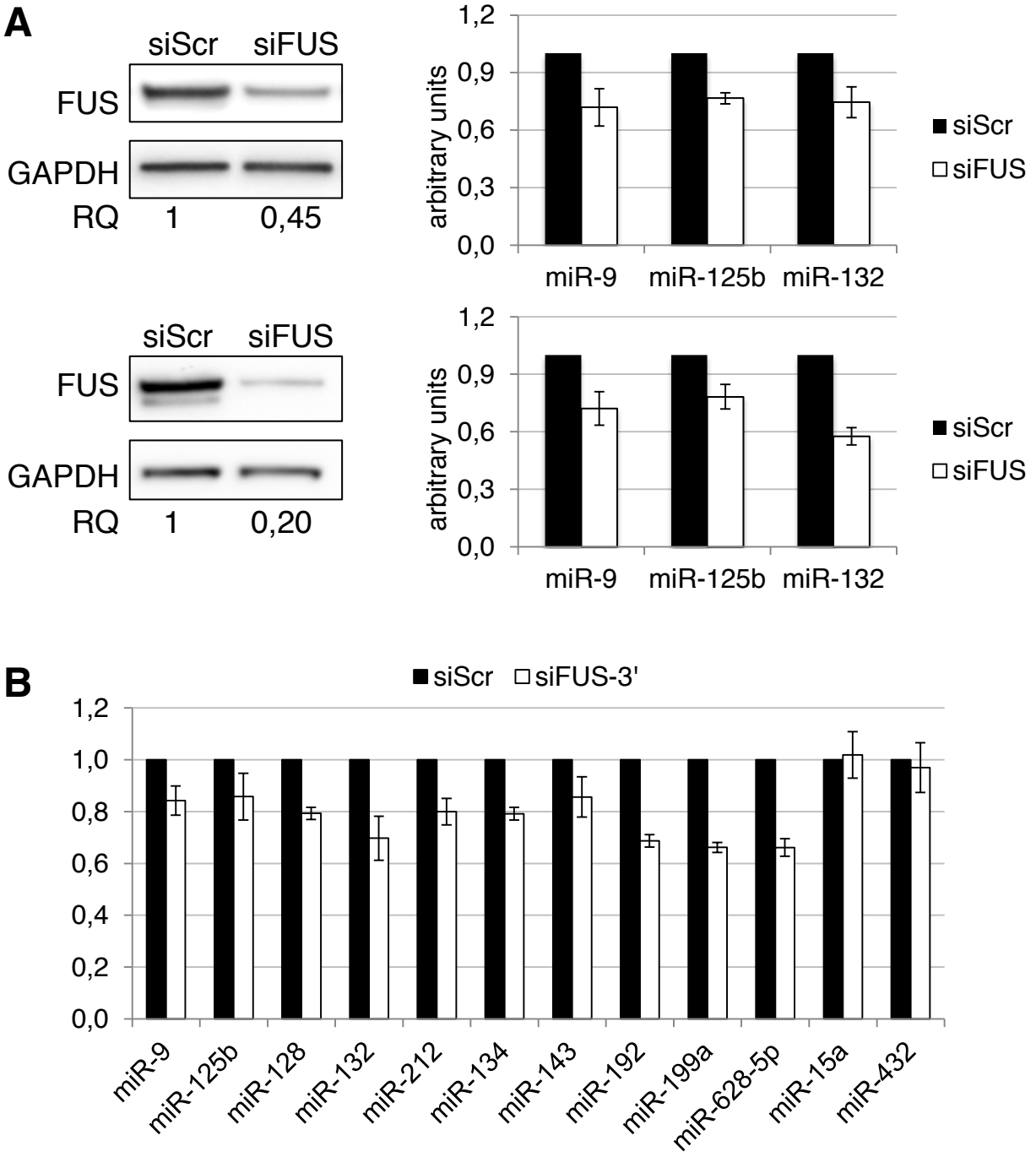

A
siScr
siFUS
FUS
GAPDH
RQ 1 0,45
siScr
siFUS
FUS
GAPDH
RQ 1 0,20
arbitrary units
1,2
0,9
0,6
0,3
0,0
miR-9
miR-125b
miR-132
siScr
siFUS
arbitrary units
1,2
0,9
0,6
0,3
0,0
miR-9
miR-125b
miR-132
siScr
siFUS
B
siScr
siFUS-3'
1,2
1,0
0,8
0,6
0,4
0,2
0,0
miR-9
miR-125b
miR-128
miR-132
miR-212
miR-134
miR-143
miR-192
miR-199a
miR-628-5p
miR-15a
miR-432

Figure S3

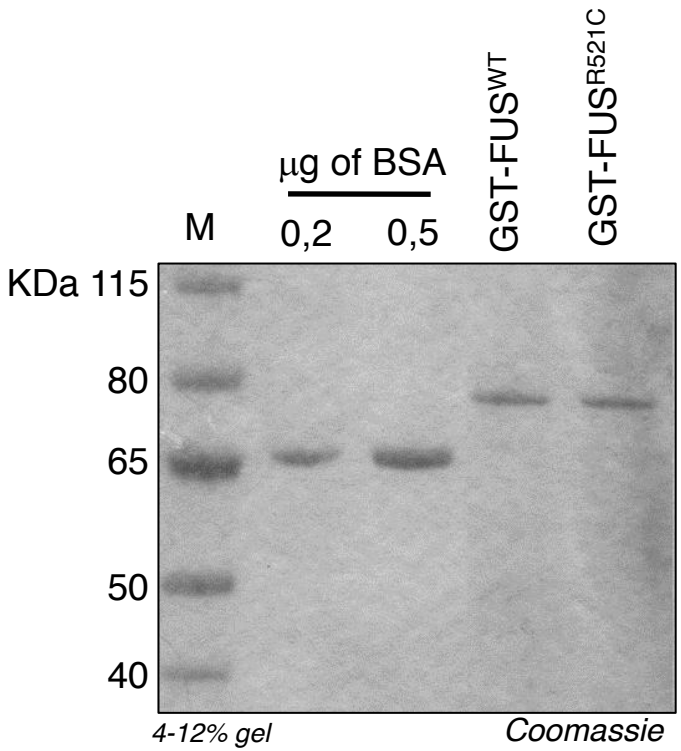

μg of BSA
M
0,2
0,5
GST-FUS^WT
GST-FUS^R521C
KDa 115
80
65
50
40
4-12% gel
Coomassie

Figure S4

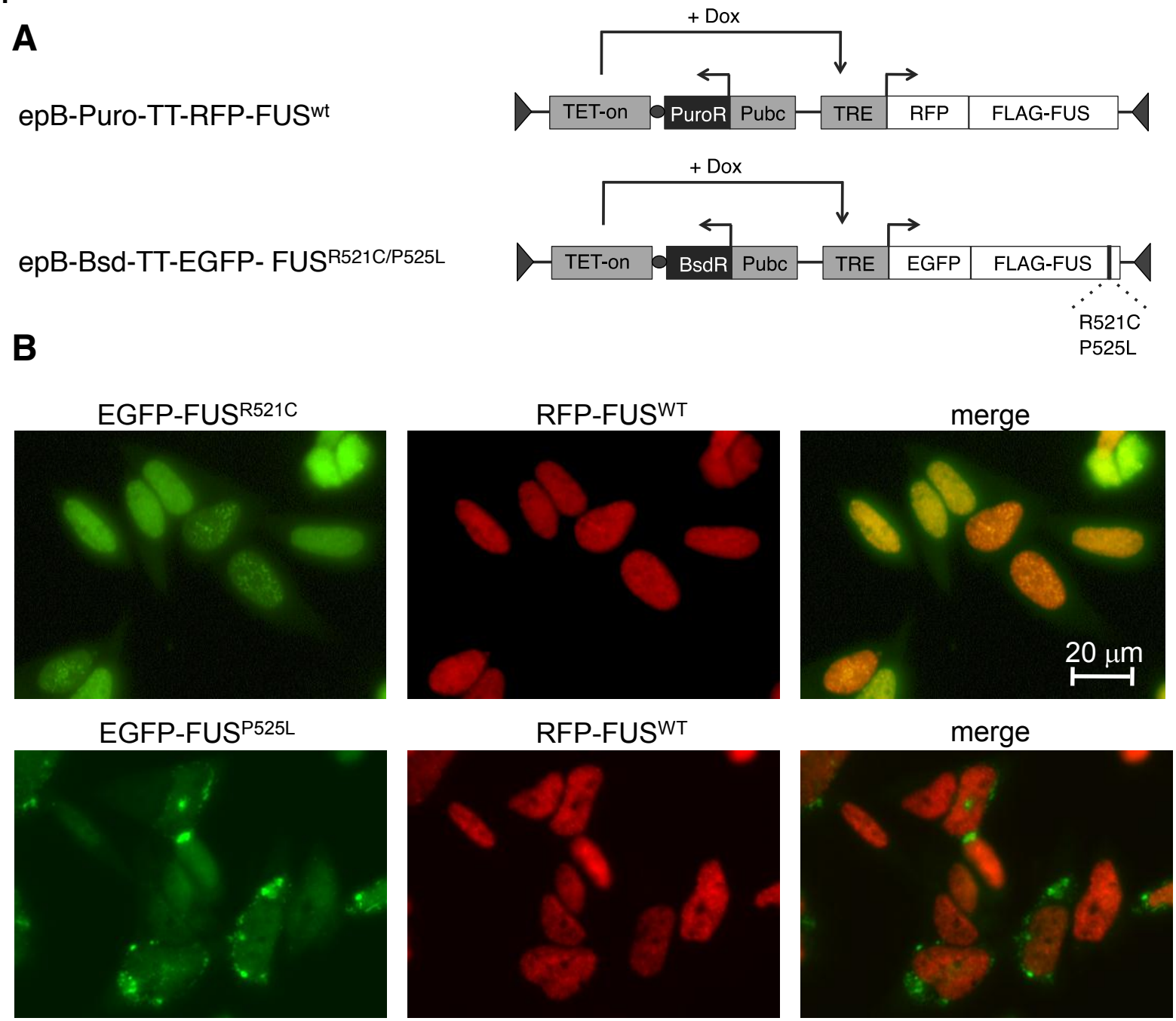
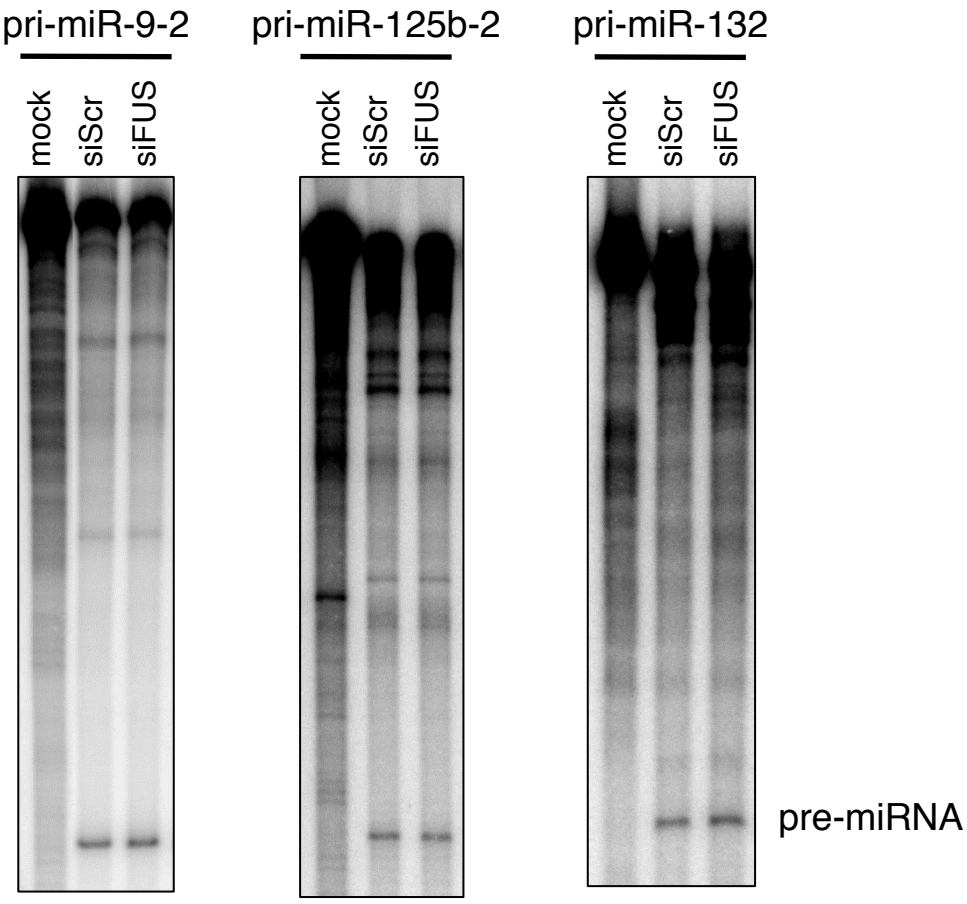

A
epB-Puro-TT-RFP-FUSwt
+ Dox
TET-on
PuroR
Pubc
TRE
RFP
FLAG-FUS
epB-Bsd-TT-EGFP- FUSR521C/P525L
+ Dox
TET-on
BsdR
Pubc
TRE
EGFP
FLAG-FUS
R521C
P525L
B
EGFP-FUSR521C
RFP-FUSWT
merge
20 μm
EGFP-FUSP525L
RFP-FUSWT
merge

Figure S5


pri-miR-9-2
pri-miR-125b-2
pri-miR-132
mock
siScr
siFUS
mock
siScr
siFUS
mock
siScr
siFUS
pre-miRNA