SUPPLEMENTARY INFORMATION

Supplementary Tables

	development stages	E14.5	E15.5	E16.5	E18.5	new born	weaning
observed ratios	wt	5	6	6	3	16	13
	het	6	7	3	17	39	27
	mut	8	6	4	9	10	0
	total	19	19	13	29	65	40
	p values	0.172	0.518	0.112	0.188	0.156634	**0.0013**

Table1: Ago2 allele segregation analysis from Ago2 catalytically inactive heterozygous intercrosses

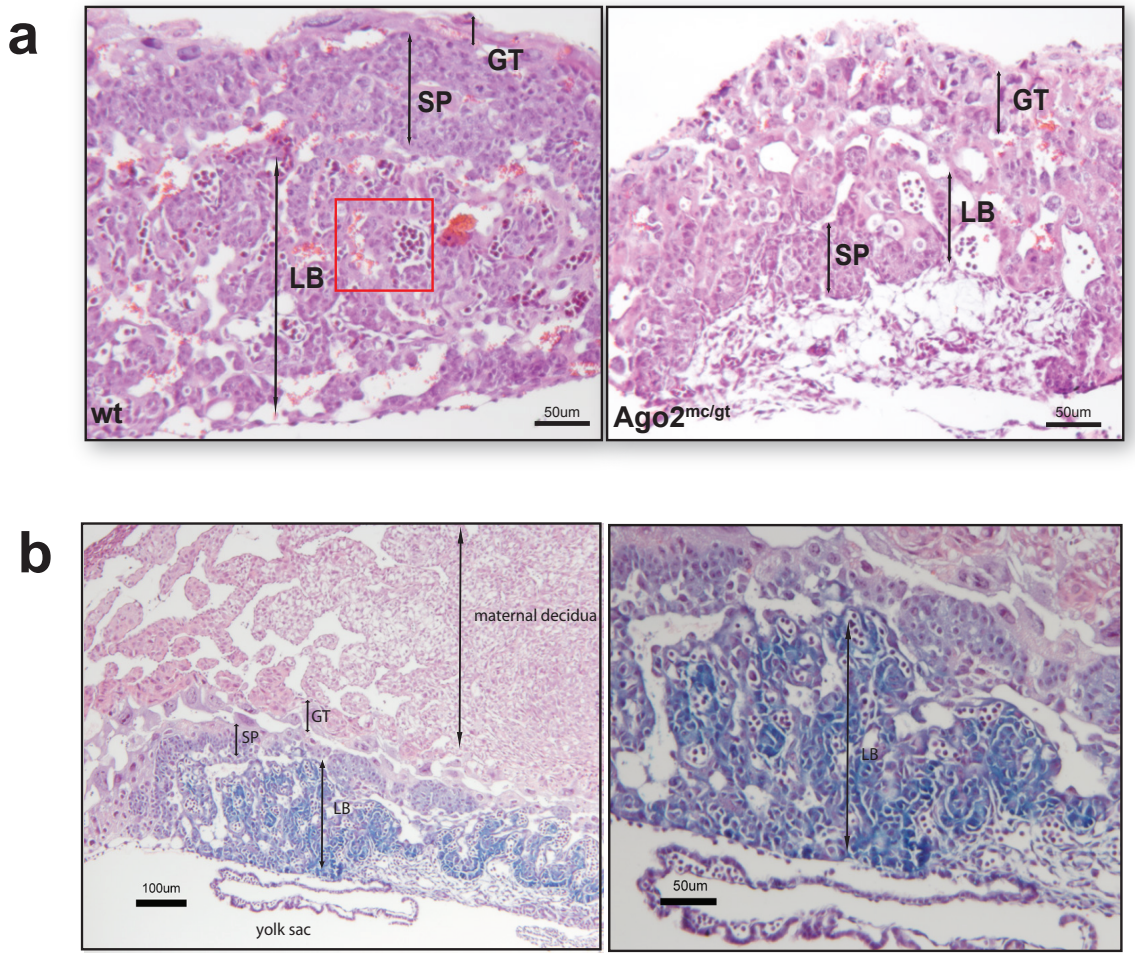

Figure S1. Labyrinthine layer defect in the Ago2 mutant mice

a. Histological analysis of wild type placenta left and mutant placenta right. Ago2 mutant embryos have a reduced labyrinthine layer. (LB: Labyrinth, SP: spongioblast, GT: giant trophoblast). Red rectangle depicts the site of nutrient exchange between the maternal blood islands and the fetal blood (nucleated red blood cells) **b.** Cross section of lacZ stained E9.5 placenta from embryos heterozygous for the Ago2gt allele. Left panel: low magnification capturing the three layers of the placenta and the maternal decidua. Right panel: higher magnification capturing the labyrinth layer where Ago2 is highly expressed.

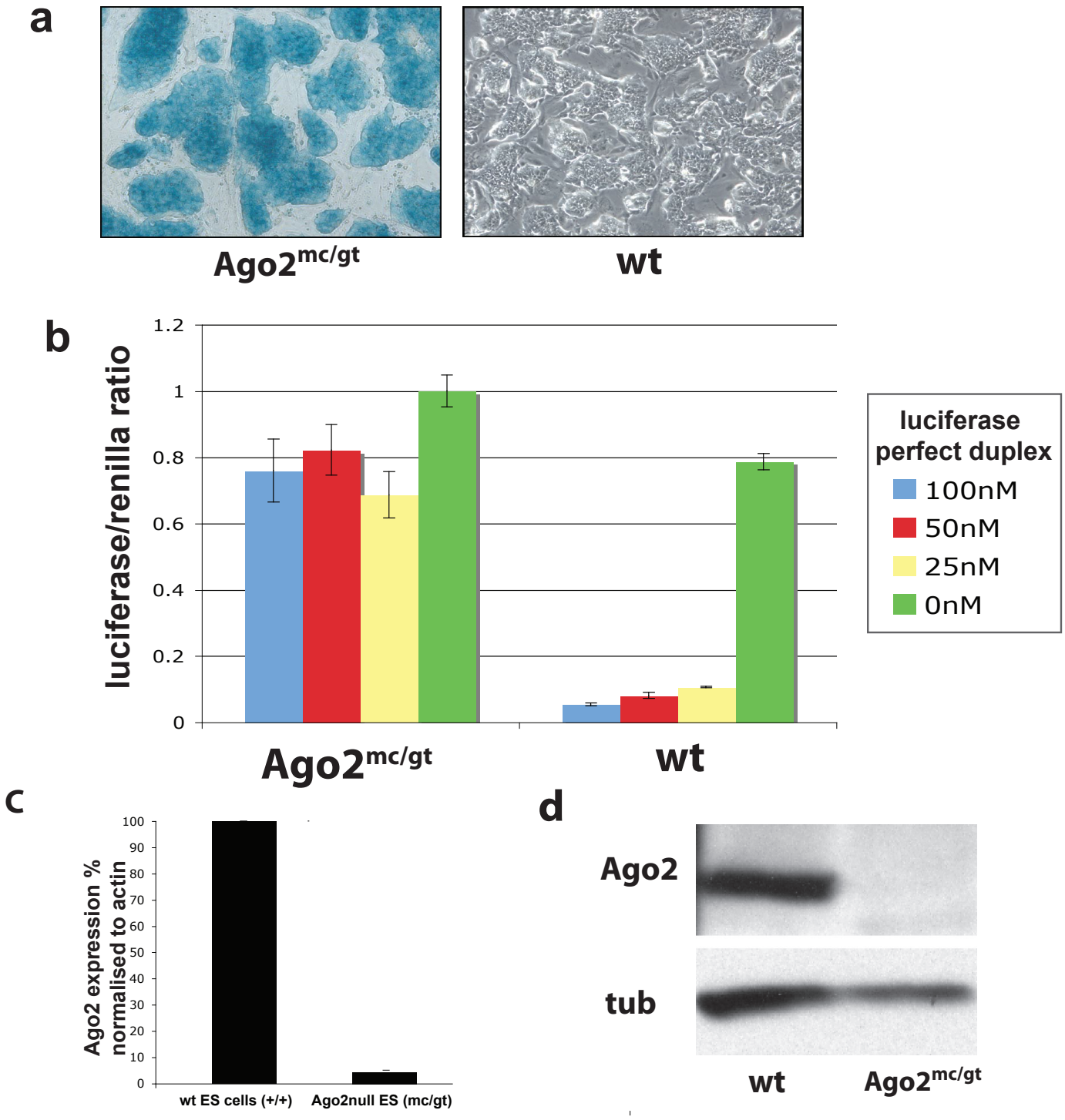

Figure S2. Characterization of Ago2 null ES cells

a. Ago2 null ES cells derived from blastocyts (left), wt ES cells as a control for the reporter staining (left). **b.** Dual luciferase siRNA silencing assay: Ago2 null cells fail to mount an siRNA mediated silencing response. Here, a perfectly matched luciferase siRNA duplex (Dharmacon) is titrated. Data are the mean of three technical replicates ±SD. **c.** Semi-quantitative RT-PCR showing depletion of Ago2 message in the Ago2 null ES cell line. **d.** Western blot analysis: Ago2 protein is not detected in the Ago2 null ES cells.

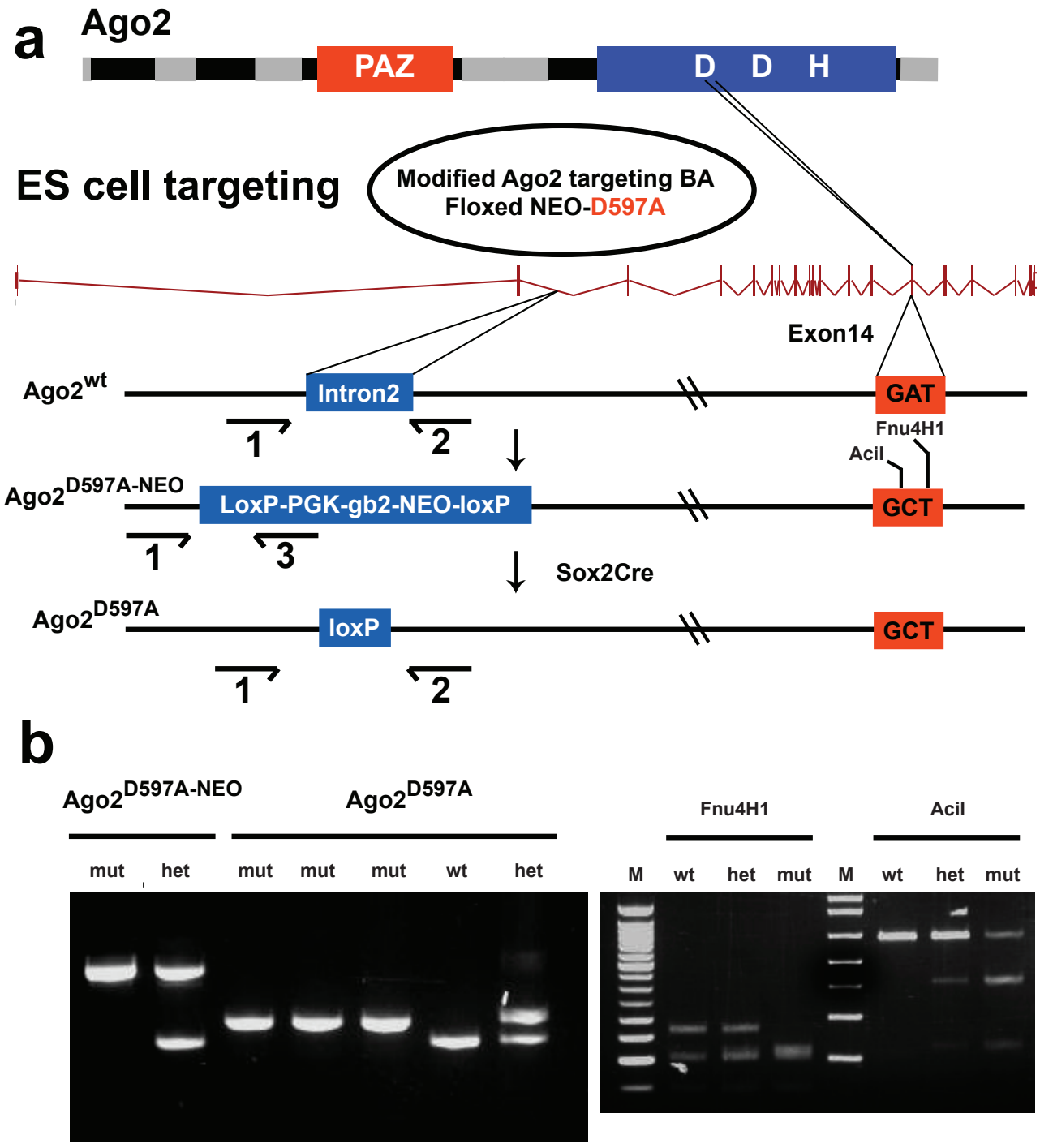

Figure S3. Schematic of the Ago2 catalytically inactive knock-in allele
a. Ago2 catalytically inactive knock-in allele targeting strategy. **b.** Genotyping strategy using either the selection cassette (left gel) or restriction fragment length polymorphism created by the point mutation (right gel).

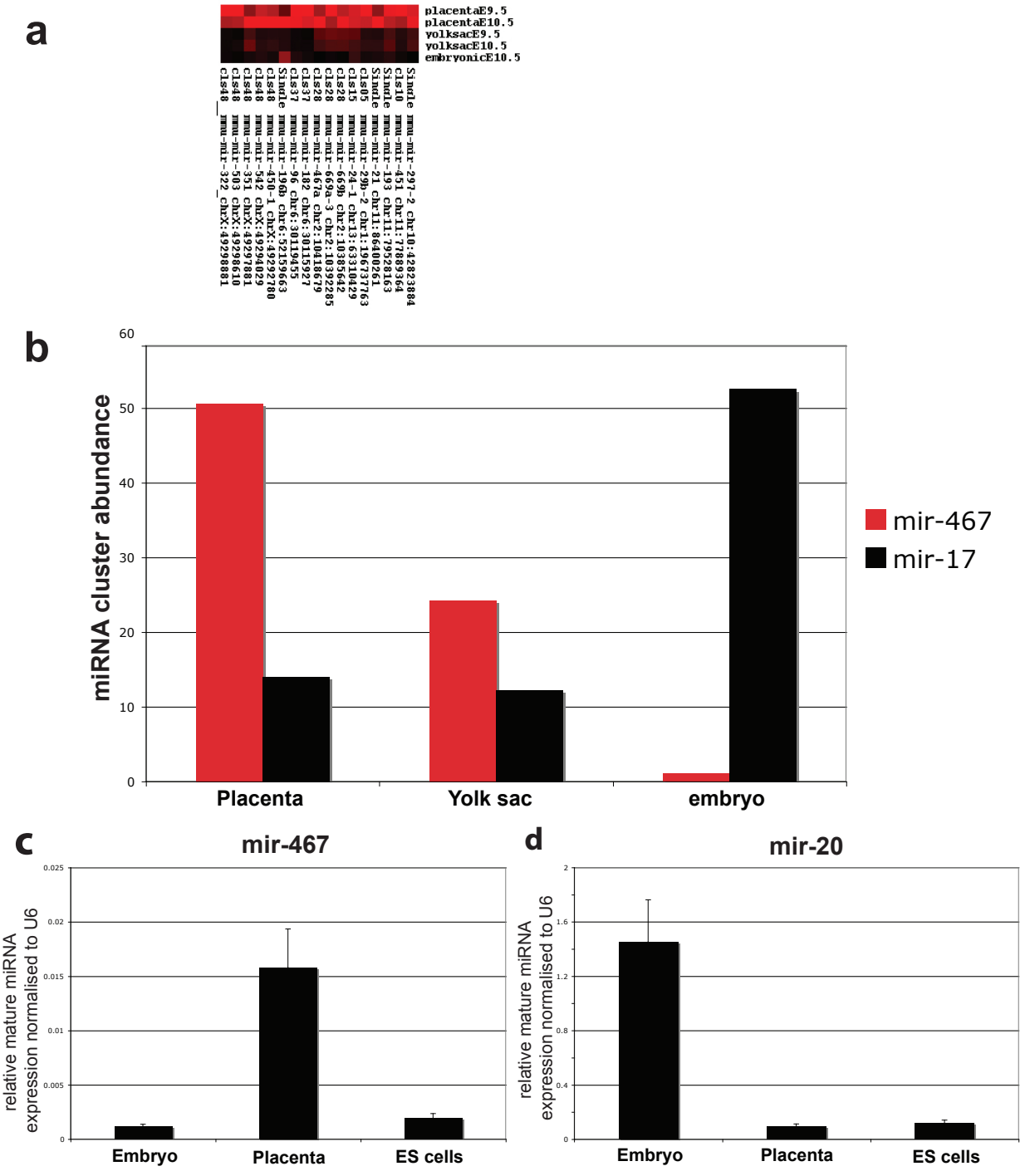

Figure S4. Extraembryonic-specific miRNAs

a. miRNA expression profile in placenta, yolk sac, embryo from left to right. (extraembryonic profiles are taken at two time points during midgestation (E9.5 and E10.5) each miRNA is classified as singleton or as a member of a cluster, mirbase annotation and chromosomal location. **b.** mir-467 is a placental specific miRNA cluster representing half of the miRNA population in the placenta as represented by total reads of miRNA from sequencing miRNA libraries.mir-467 abundance in the placenta is compared to mir-17 (an embryonic specific miRNA cluster). **c.** Q-PCR validation of mir-467a and mir-20 members of the mir-467 and mir-17 clusters respectively.

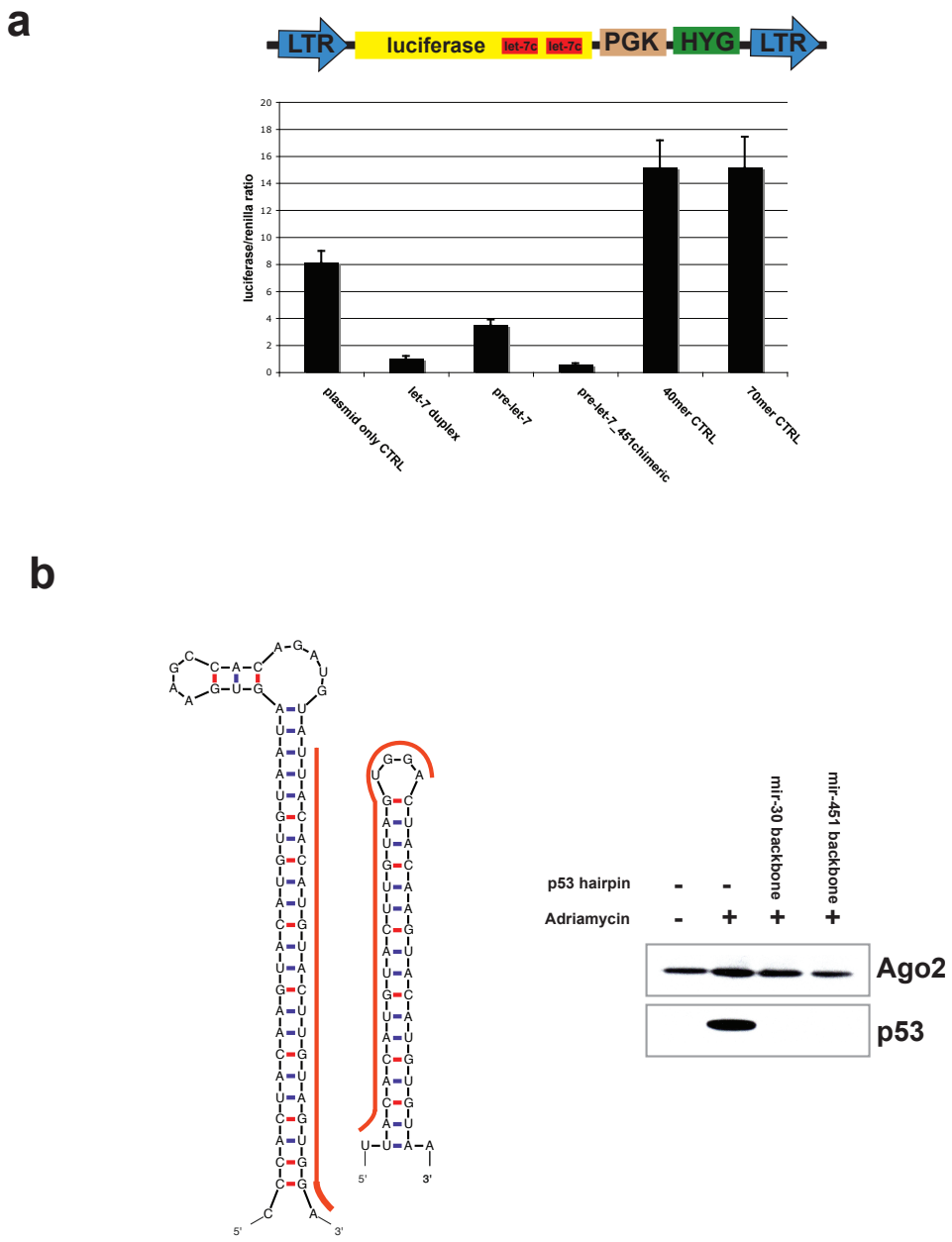

Figure S5. mir-451 mimics

a. Dual luciferase assay reporting mature let-7 activity. Top: Schematic of let-7 MSCV-luciferase reporter construct containing two perfectly matching let-7c sites. Bottom: Histogram showing luminescence values of luciferase/renilla ratios. Data are the mean of three technical replicates ± SD. **b.** left panel: schematic of p53 hairpin design in the mir-30 backbone or following the mir-451 fold. Right panel: Western blot analysis showing p53 knowdown in ES cells upon transfection of p53 hairpins and induction of p53 with adriamycin.

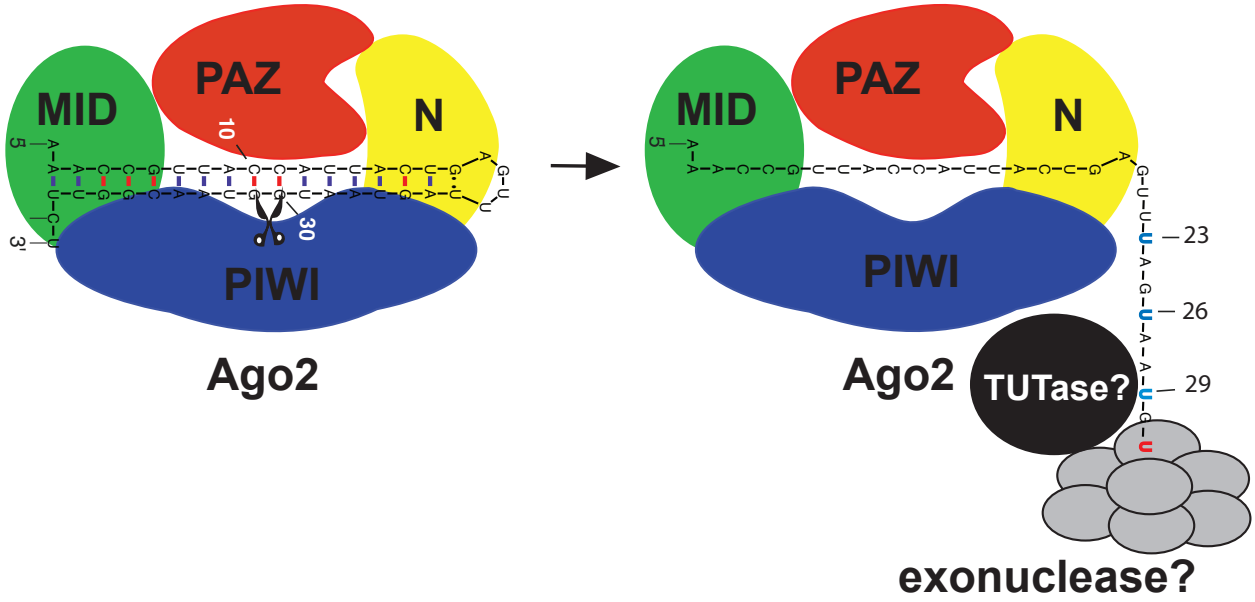

Figure S6. Hypothetical Model for mir-451 loading and 3'end trimming.
After Ago2 catalysis we envision cycles of uridine transfer mediated by TUTases and exonucleolytic trimming. The domains of the Ago2 protein are color coded: Amino terminal domain (yellow), PAZ domain (Red), MID domain (green) and PIWI domain (blue). mir-451 is drawn here according to its predicted secondary structure. Scissors denote Ago2 mediated catalysis. mir-451 cleavage products are drawn post Ago2 catalysis.

a

20n t AAACCGUUACCAUUACUGAG
21n t AAACCGUUACCAUUACUGAGU
22n t AAACCGUUACCAUUACUGAGUU
23n t AAACCGUUACCAUUACUGAGUUUU
24n t AAACCGUUACCAUUACUGAGUUUAU
25n t AAACCGUUACCAUUACUGAGUUUAGU
26n t AAACCGUUACCAUUACUGAGUUUAGUU
27n t AAACCGUUACCAUUACUGAGUUUAGUAU
28n t AAACCGUUACCAUUACUGAGUUUAGUAAU
29n t AAACCGUUACCAUUACUGAGUUUAGUAAUU
30n t AAACCGUUACCAUUACUGAGUUUAGUAAUGU

b

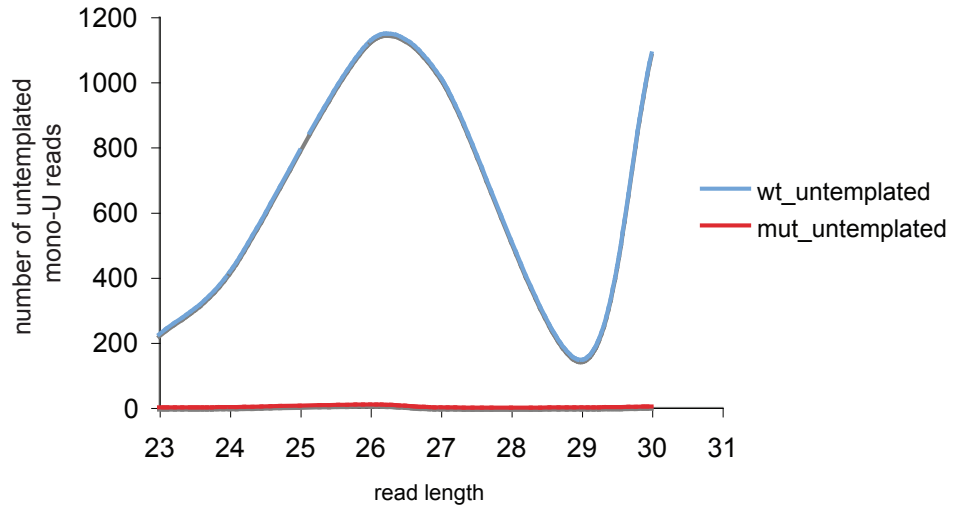

Figure S7. Analysis of untemplated U transferred to the 3'ends of mir-451
a. untemplated mono-Us at the 3'end of mir-451. Endogenous Us in blue and untemplated Us in red.
b. endogenous mir-451 read length distribution representing abundance of untemplated mono-U non mapping reads only. See (Fig. 5) for non-modified endogenous reads.

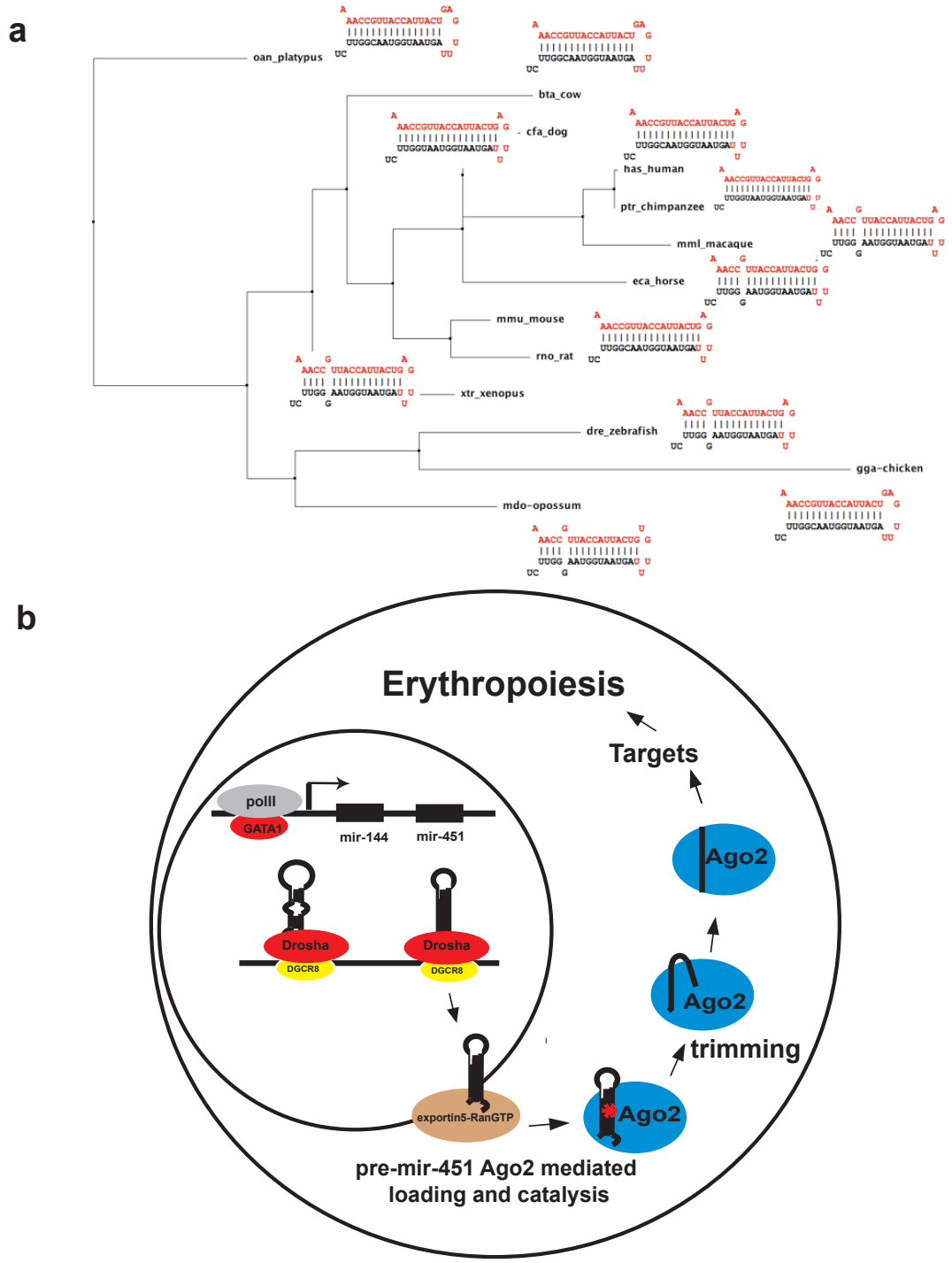

Figure S8. A conserved miRNA biogenesis pathway that depends on Ago2 catalysis
a. A neighbor-joining tree constructed using Jalview program depicting mir-451 hairpin conservation in vertebrates. Mouse and rat hairpins are identical. The guide strand is shown in red. Sequences of mir-451 were extracted from mirbase. **b.** mir-451 bicitronic cluster under the control of GATA-1 transcription factor. The mir-451 primary transcript is processed by Drosha/DGCR8 complex in the nucleus then possibly exported to the cytoplasm where the pre-mir451 is loaded onto Argonaute2 which then cleaves its passenger arm to generate the mature species of mir-451 that target genes to regulate erythropoiesis.