Independent studies demonstrate the presence of Dicer in exosomes

1. **Nat Cell Biol. 2019** Jul; 21(7): 856–866. doi: 10.1038/s41556-019-0345-y

Identification of **Dicer** and **AGO2** in breast cancer vesicles.

Same exosomes used as in the Cancer Cell 2014 manuscript (**MDA-MB-231**).

Demonstration of **pre-miR21 in exosomes**.

Demonstration of **precursor microRNAs processing in**

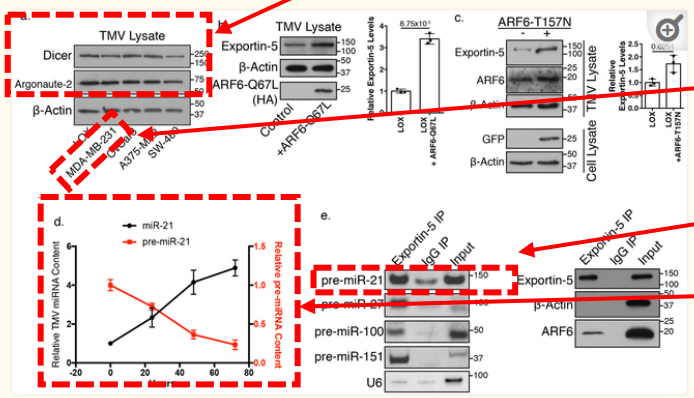
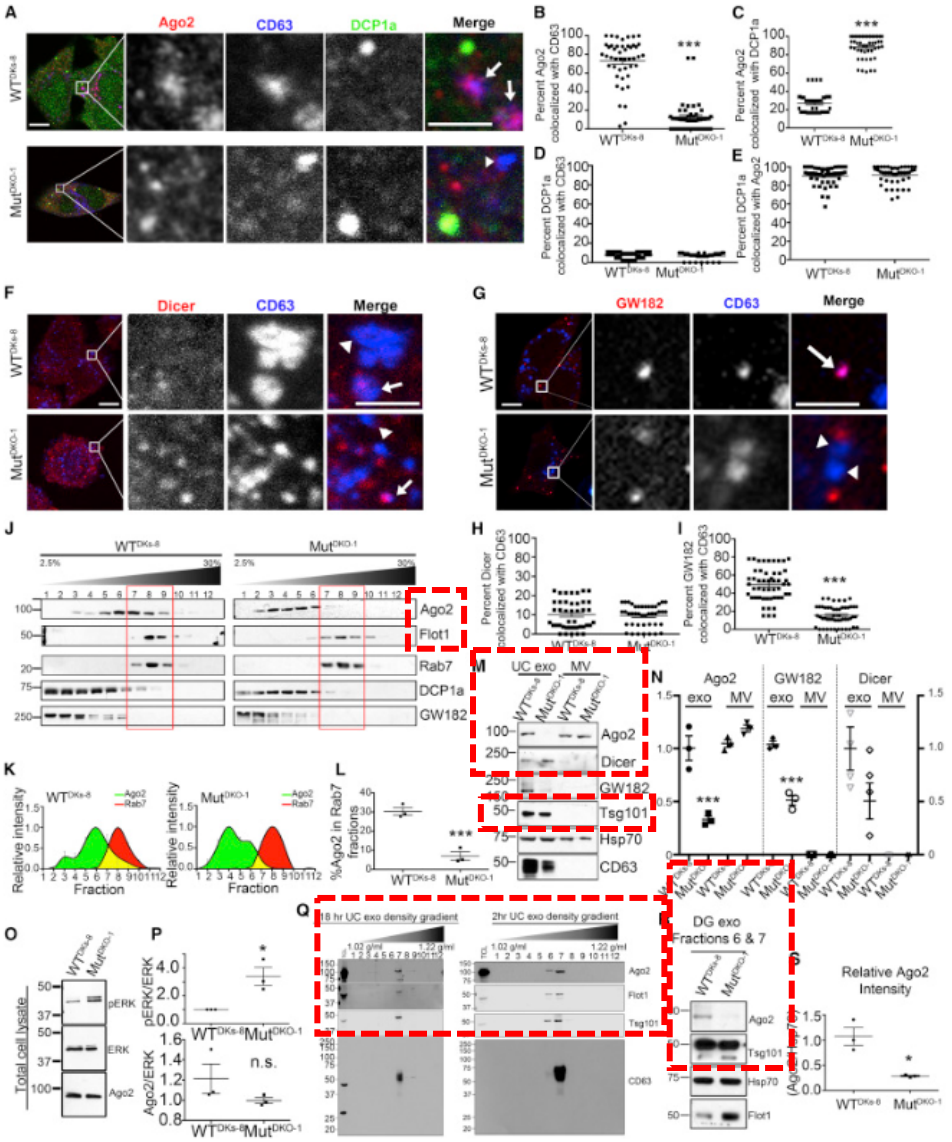


Figure 3:

pre-miRNA processing machinery is contained in shed TMVs.

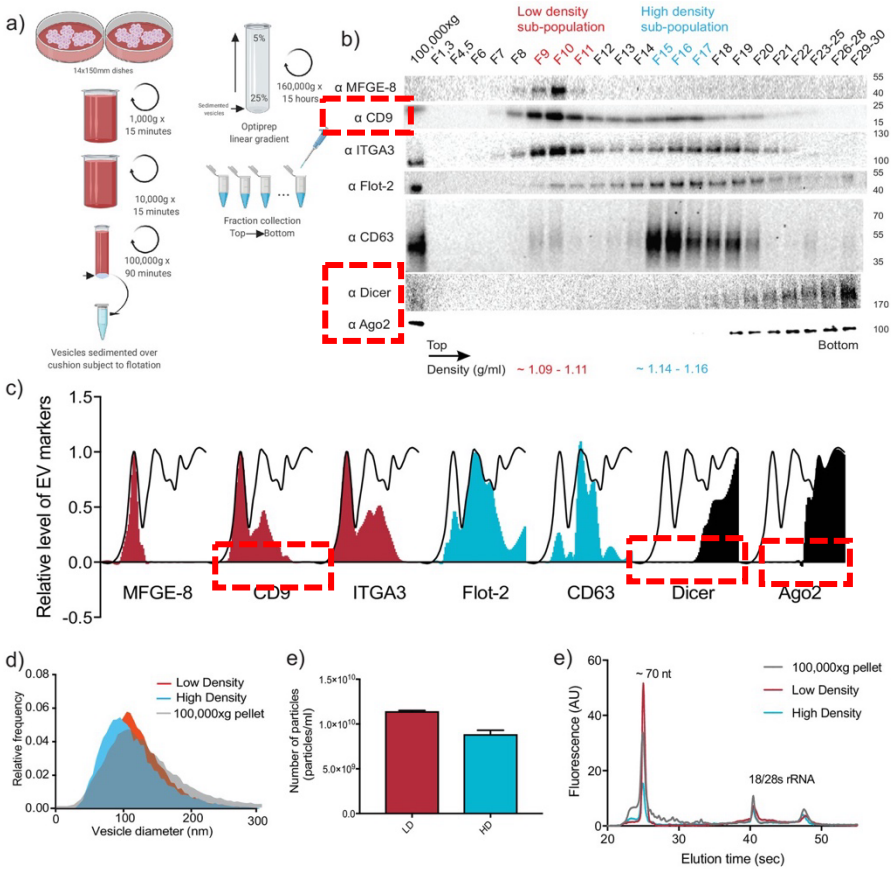
a. TMVs from multiple invasive tumor cell lines were analyzed for the inclusion of pre-miRNA processing proteins Dicer and Argonaute-2 by western blotting. N=4 biologically independent samples. **b.** Western blot analysis of lysate generated from equal number of (1×10^8) TMVs released by parental melanoma cells compared to those expressing constitutively active ARF6 reveals an enrichment of Exportin-5 content within TMVs when ARF6 is activated. Data presented as mean±SD (N=3 biologically independent experiments). P-value determined by unpaired two-tailed t-test. P-value <0.05 was considered significant. **c.** Western blot analysis of lysate generated from equal number of (1×10^8) TMVs released by parental melanoma cells compared to those transfected with fast-cycling ARF6-T157N confirms an enrichment of Exportin-5 content within TMVs when ARF6 is activated. Data presented as mean±SD (N=3 biologically independent experiments). P-value determined by unpaired two-tailed t-test. p-value <0.05 was considered significant. **d.** RNA extracted from equal numbers (5×10^6) of isolated TMVs maintained in cell-free conditions at 37°C for the times indicated was analyzed by qRT-PCR. The relative amounts of pre-miR21 and mature miR-21 were measured as described in methods. Data presented as mean±SD from N=3 biologically independent experiments. **e.** TMVs were isolated from invasive melanoma cells and endogenous Exportin-5 precipitated from 200 µg of input TMV protein. Co-precipitating RNA was examined by RT-PCR, and co-precipitating proteins examined by western blotting. Representative data from N=3 biologically independent samples shown. Unprocessed blot images shown in Supplemental Image 7. Statistical Source in Supplementary Table 1.

3. **Cell Rep. 2016** May 3;15(5):978-987. doi: 10.1016/j.celrep.2016.03.085.



Demonstrates presence of **AGO2**, **Flotilin 1**, **Dicer**, and **TSG101** in exosomes.

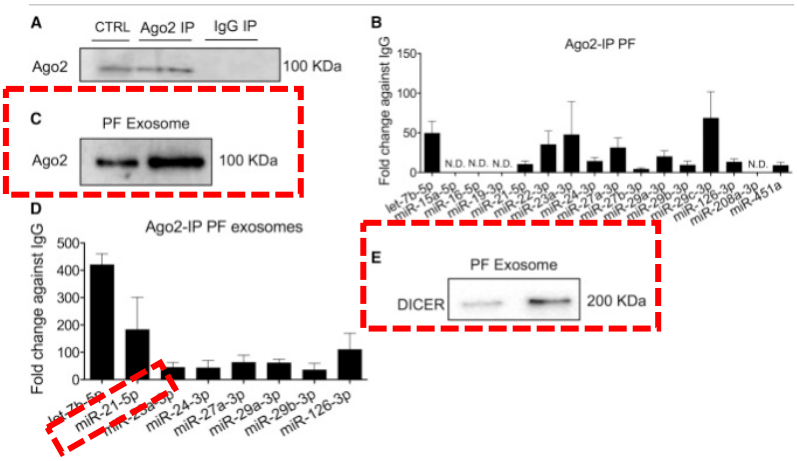
4. **eLife 2019** 8:e47544 Morayma M et al… Randy Schekman. Distinct mechanisms of microRNA sorting into cancer cell-derived extracellular vesicle subtypes.



Demonstrates presence of **CD9**, **Dicer**, and **AGO2** in exosomes.

Two biochemically distinct sEV sub-populations are released by MDA-MB-231 cells.

5. **Mol Ther. 2017** Mar 1;25(3):679-693. doi: 10.1016/j.ymthe.2016.12.022.



Demonstrates presence of **AGO-2, Dicer, and miR-21** in exosomes.

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Figure4. PF Exosomes Contain DICER and AGO-2 Protein

(A) Validation by immunoblotting of AGO-2 immunoprecipitation (IP) performed on human PF samples using AGO-2 antibody. Mouse non-specific IgG antibody was used as control for the IP. ECs were used as positive control (CTRL). (B) The miRNA expression after AGO-2 IP is presented as fold enrichment relative to IgG; mean+ SEM; n= 5. (C) AGO-2 in exosomes enriched from PF samples (representative western blot images). (D) AGO-2 IP was performed on exosomes enriched from PF samples. miRNA expression is expressed as fold enrichment in the AGO-2 IP relative to IgG; mean+ SEM; n= 2. (E) Representative western blot images of DICER protein incorporated in the exosomes.

7. **J Biol Chem. 2013** Jul 5; 288(27): 20014–20033 doi: 10.1074/jbc.M112.438895

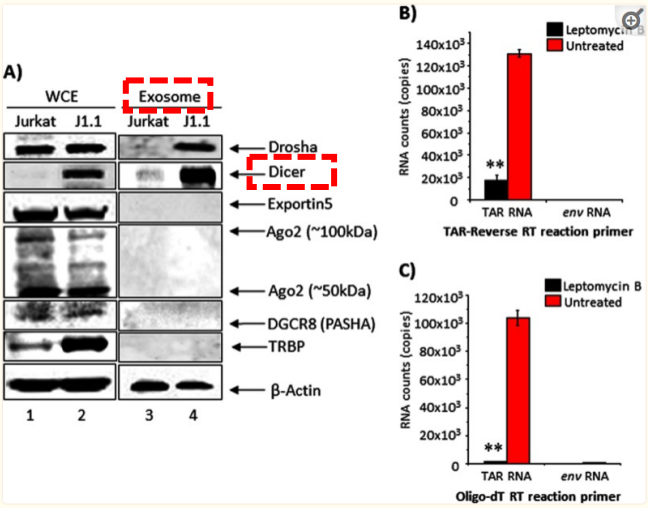


FIGURE 5.

J1.1-derived exosomes contain components of the RNAi machinery. *A*, Jurkat- and J1.1-derived whole cell extracts and exosomes were separated on a 4–20% Tris-glycine gel and analyzed by Western blot using antibodies against Dicer, Drosha, exportin, Ago2, DGCR8, TRBP, and β-actin. Total RNA isolated from J1.1-derived exosomes with and without leptomycin B treatment (10 nM) was subjected to qRT-PCR with TAR-reverse primer (*B*) and oligo(dT) primers (*C*). cDNA was then quantified by SYBR Green real time PCR with the primer sets specific for HIV-1 TAR and env sequences. *Error bars* show the standard deviation from three independent RNA preparations. *Double asterisk* indicates $p \leq 0.01$.

Demonstrates presence of **Dicer** in exosomes.

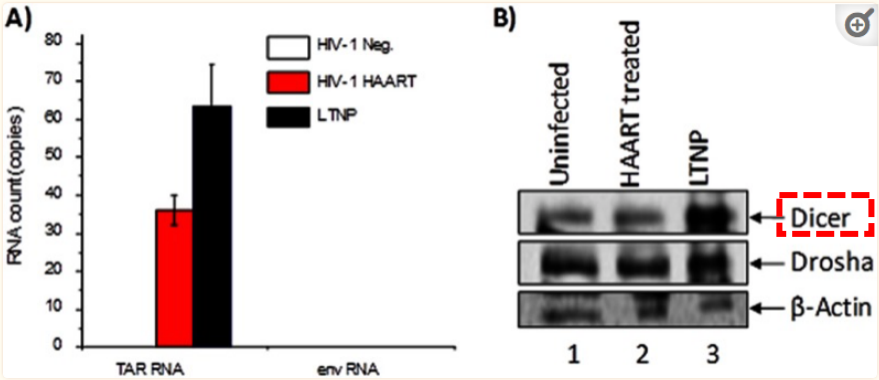
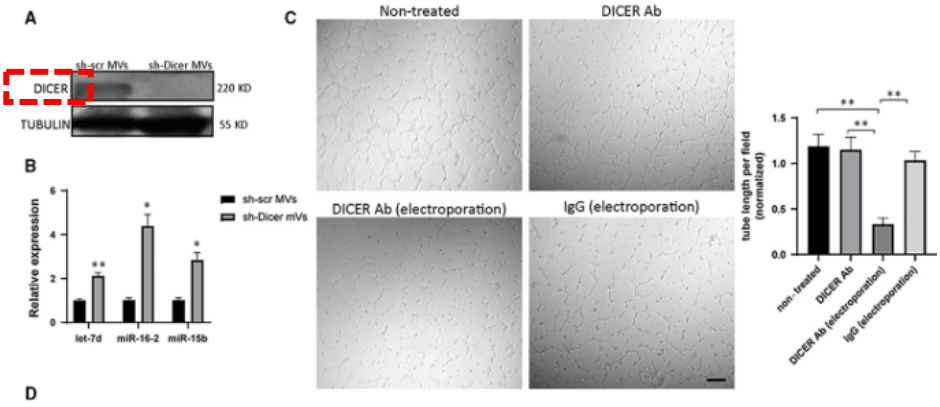


FIGURE 10.

TAR RNA, Dicer, and Drosha can be detected in serum exosomes. *A*, exosomes were isolated from pooled sera obtained from uninfected (control), HAART-treated, and LTNP HIV-1-infected patient groups and analyzed by qRT-PCR with TAR- and env-specific primers. Results are presented as a mean of three independent measurements ± S.D. *B*, serum exosomes were analyzed by Western blot using antibodies against Dicer, Drosha, and β-actin. The ExoQuick-purified material was diluted by a 1:10 ratio (TNE-50 + 0.1% Nonidet P-40), passed through a Sephadex G-10 spin column, and analyzed by Western blot.

Demonstrates presence of **Dicer** in exosomes.

8. **J Cell Mol Med. 2020** May;24(9):4915-4930. doi: 10.1111/jcmm.14917



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