**Supporting Information**

Quantitative proteomic analysis of small and large extracellular vesicles (EVs) reveals enrichment of adhesion proteins in small EVs.

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Supporting Figures



Supporting Tables

**Supporting Figures**

**Figure S1. Selected enrichment plots from Gene Set Enrichment Analysis representing proteins upregulated in SEVs.**

Eight of the 51 significantly upregulated gene sets with proteins that show a striking enrichment in SEVs. The top portion of each plot shows the running enrichment score (ES) for the gene set. Each of these plots show a distinct peak at the beginning. The lower portion of each plot shows the proteins associated with the gene set and how they ranked in the ranked list, represented as black lines. There was an abundance of proteins near the enrichment peak. The red to blue bar corresponding to the log2 fold ratio of proteins in the SEVs over the LEVs, with red indicating an elevated level in exosome. The Normalized Enrichment Scores (NES) and False Discovery Rates (FDR) for each of these plots are shown in the upper right corner.

**Figure S2. Selected enrichment plots from Gene Set Enrichment Analysis representing proteins upregulated in LEVs.**

Eight of the 52 significantly upregulated gene sets with proteins that show an enrichment in LEVs. The top portion of each plot shows the running enrichment score (ES) for the gene set. Each of these plots show a distinct peak at the end of the plot. The lower portion of each plot shows the proteins associated with the gene set and how they ranked in the ranked list, represented as black lines. There was an abundance of proteins near the enrichment peak. The red to blue bar corresponding to the log2 fold ratio of proteins in the SEVs over the LEVs, with blue indicating an elevated level in LEVs. The Normalized Enrichment Scores (NES) and False Discovery Rates (FDR) for each of these plots are shown in the lower left corner.

**Figure S3. Representative Ponceau-stained Western blot membranes.**

A. Ponceau-stained Western blot 6% and 10% membranes of LEVs and SEVs from DKs8 and HT1080 cells. B. Ponceau-stained Western blot 7% and 10% membranes of SEVs from DKs8 shScramb. and shARRDC1-KD cells. C. Ponceau-stained Western blot 7% and 12.5% membranes of SEVs from DKs8 shScramb. and shRab27a-KD cells.

**Figure S4. Western blot analysis of Rab27a KD SEVs.**

A. Western blot analysis of DKs8 shScramb. and shRab27a-KD TCLs for Rab27a and Beta actin. B. Representative nanoparticle tracking traces of SEVs from DKs8 shScramb. and

shRab27a-KD cells. C. Quantitation of SEVs numbers from DKs8 shScramb. and shRab27a-KD cells determined in nanoparticle tracking analysis (n=3). D. Western blot analysis of DKs8 shScramb. and shRab27a-KD SEVs assessing the levels of EphA2, EphB1, EphB4, Integrin $\alpha$3, Integrin $\beta$8, Thrombospondin-1, ARRDC1, Claudin 3, Rab27a and Hsp70. DKs8 shScramb. and shRab27a-KD SEVs were loaded at equal protein concentration and equal volume of resuspended vesicles. E. Quantitation of Western blots from 3 independent experiments * $p < 0.05$; ** $p < 0.01$ paired *t* test comparisons of the band intensities of DKs8 shScramb., shRab27a-KD SEVs.

**Figure S1. Selected enrichment plots from Gene Set Enrichment Analysis representing proteins upregulated in SEVs.**

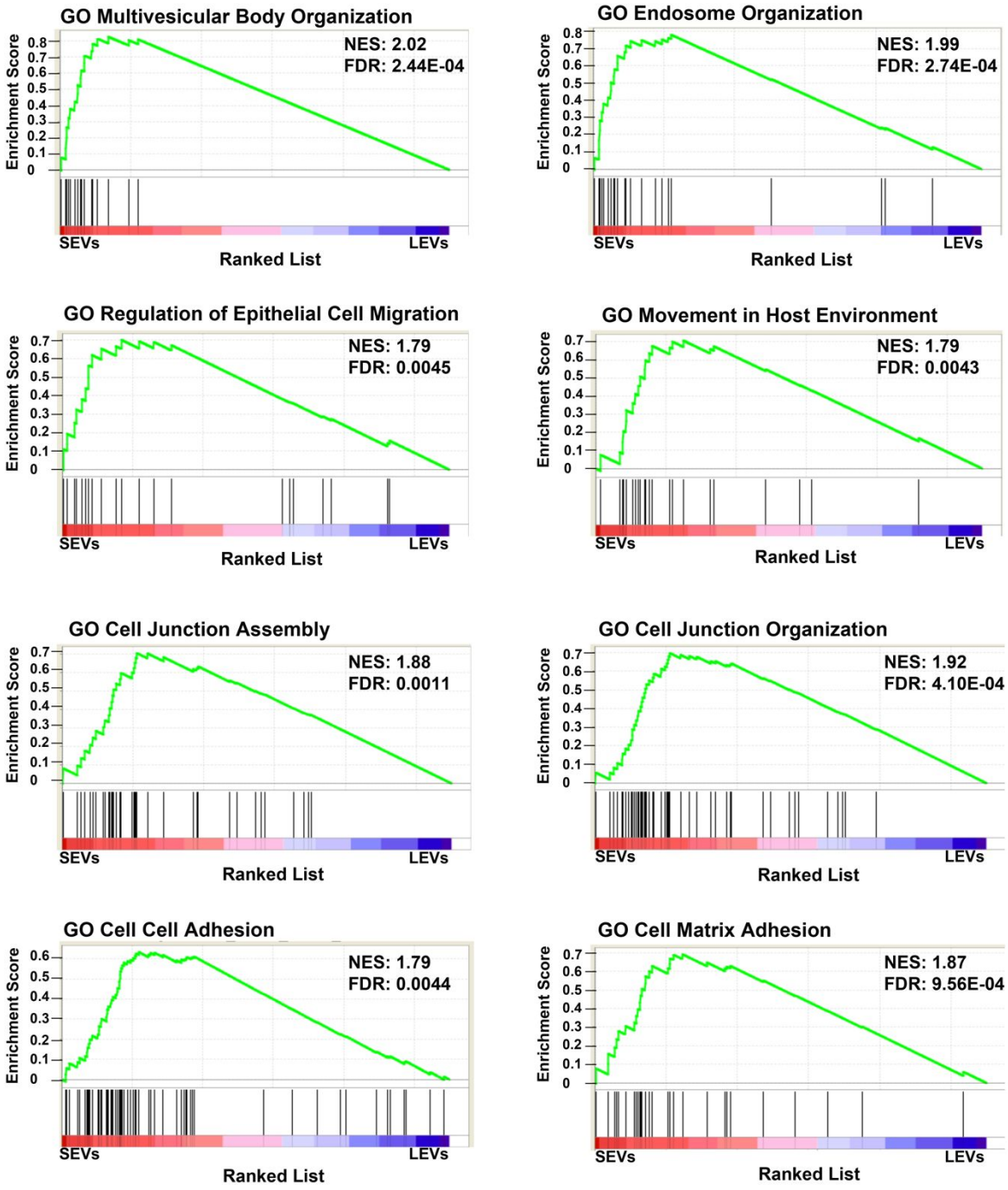


**Figure S2. Selected enrichment plots from Gene Set Enrichment Analysis representing proteins upregulated in LEVs.**

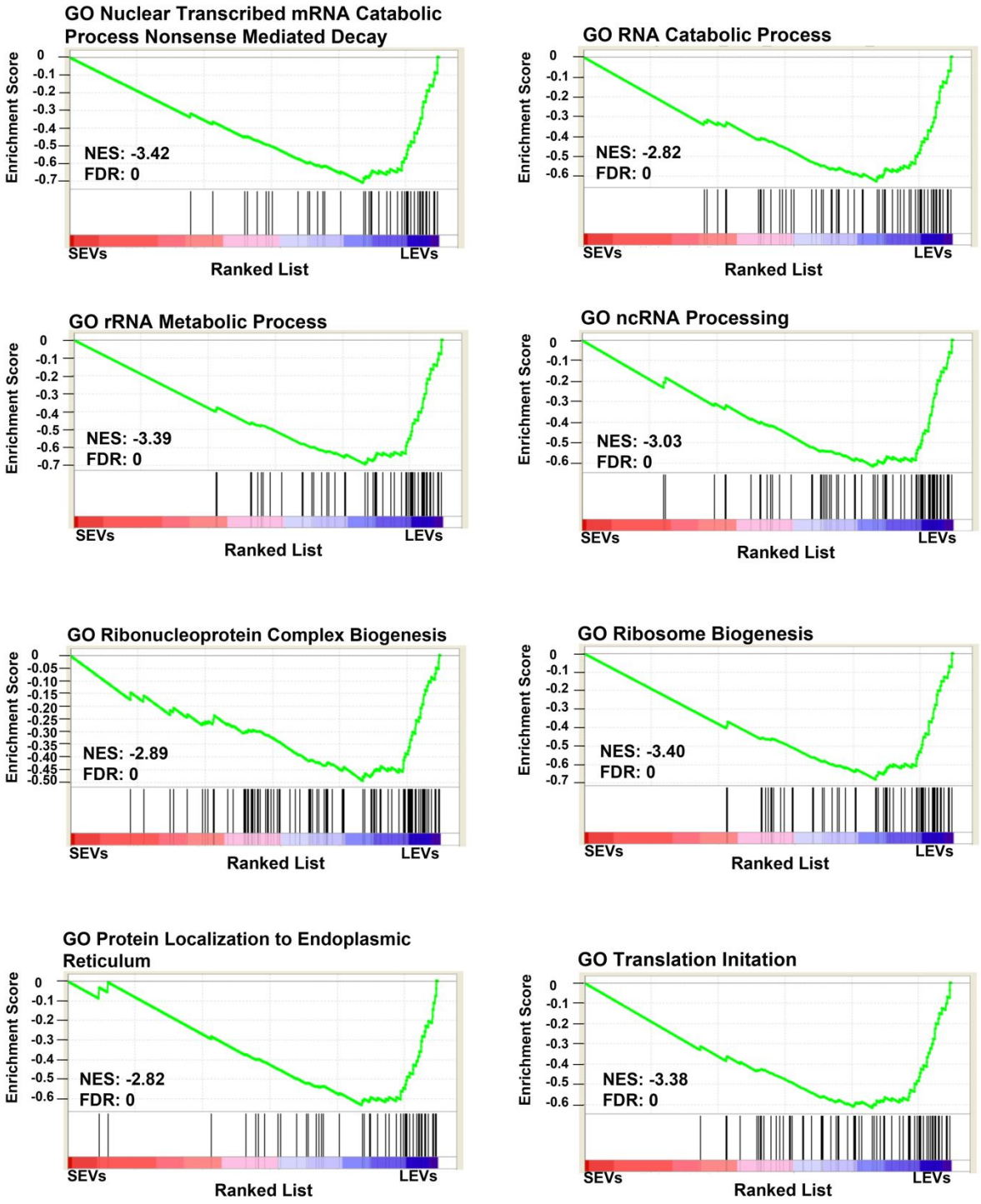


**Figure S3. Representative Ponceau-stained Western blot membranes.**

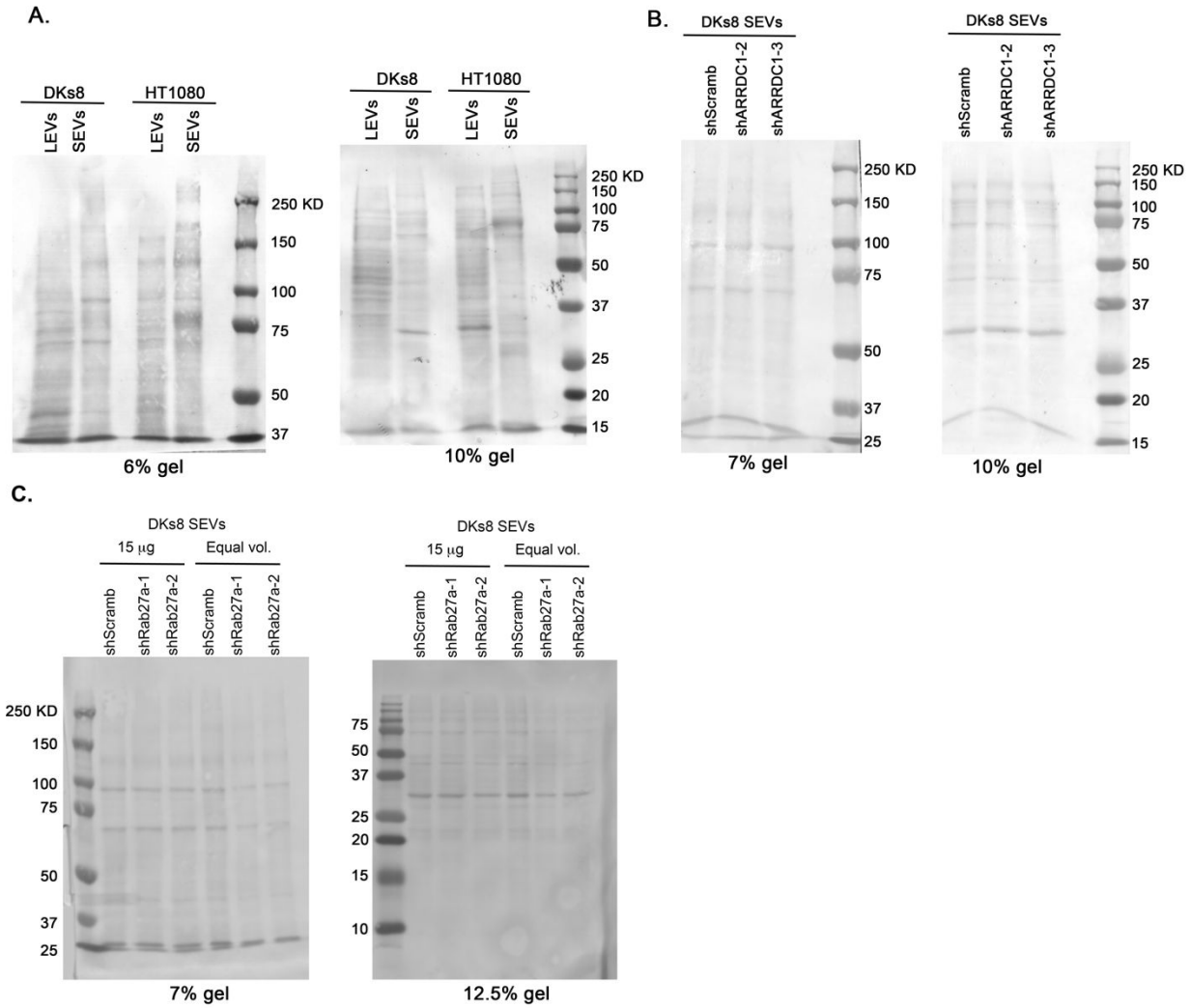
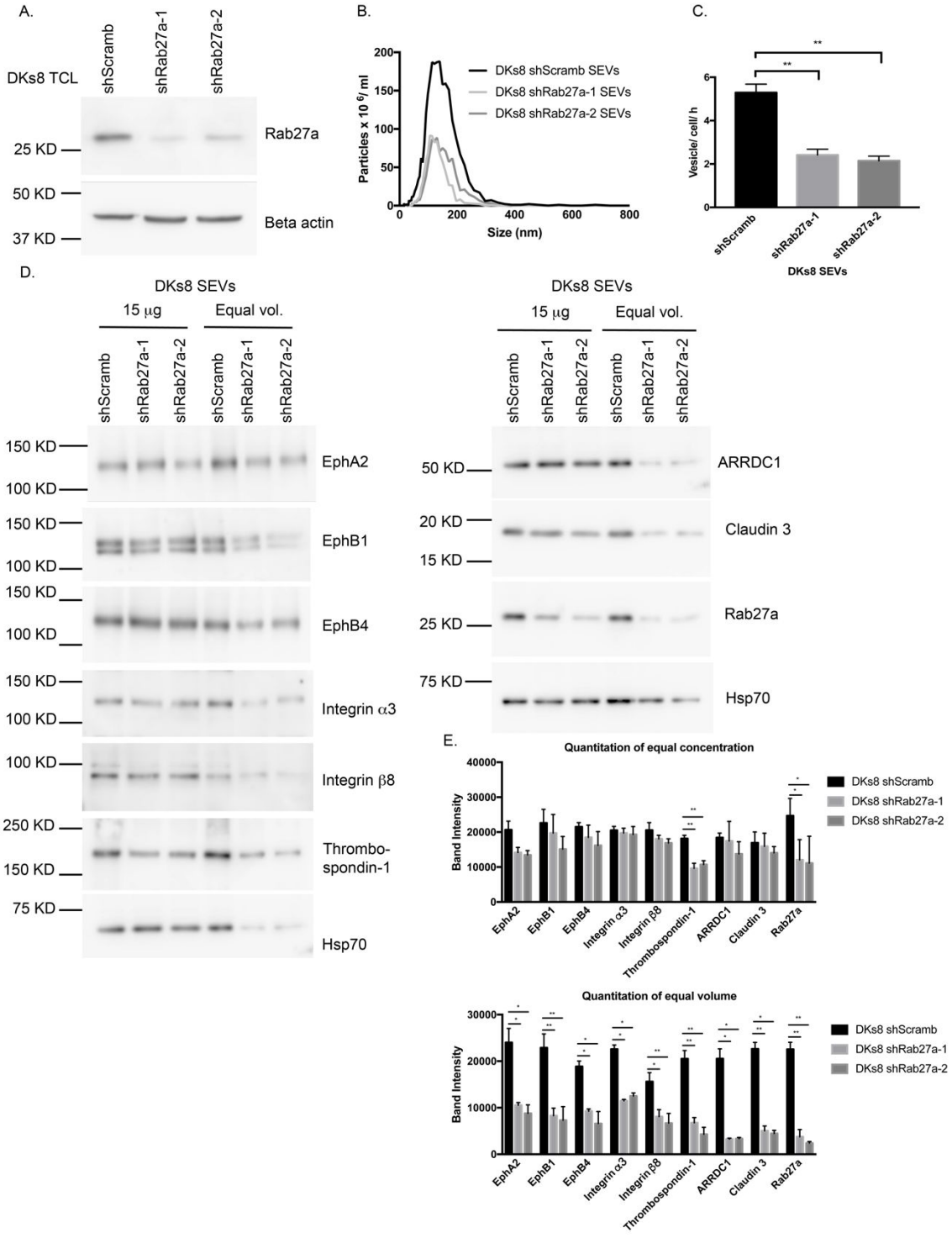


**Figure S4. Western blot analysis of Rab27a KD SEVs.**



**Supporting Tables**

**Table S1. All the proteins identified in iTRAQ experiments.**
Sheet 1- Proteins Identified in iTRAQ experiment 1; Sheet 2- Proteins Identified in iTRAQ experiment 2; Sheet 3- Proteins Identified in iTRAQ experiment 3; Sheet 4- The commonly identified proteins in all three iTRAQ Replicates; Sheet 5- The commonly identified proteins that showed an adjusted *P* value of < 0.01 in Limma analysis; Sheet 6- The proteins that showed an adjusted *P* value of < 0.01 and at least 2 fold enrichment in SEVs; Sheet 7- The proteins that showed an adjusted *P* value of < 0.01 and at least 2 fold enrichment in LEVs.

**Table S2. Complete list of GSEA categories for proteins enriched in SEVs and LEVs.**
Sheet 1- Top 51 gene sets for upregulated proteins in SEVs; Sheet 2- Top 52 gene sets for upregulated proteins in LEVs.

**Table S3. Categorization of proteins enriched in SEVs and LEVs.**
Sheet 1- Categorization of proteins enriched in SEVs (at least 4-fold change, *P* value < 0.01); Sheet 2- Categorization of proteins enriched in LEVs (at least 2-fold change, *P* value < 0.01).
d in LEVs (at least 2-fold change, *P* value < 0.01).