Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

Release of extracellular vesicles triggered by low-intensity pulsed ultrasound immediate and delayed reactions

Yiwen Zheng,‡[a] Mengyao Liu,‡[a] Qian Yu,[a] Rui Wang,[a] Yijing Yao,[a] and Lixin Jiang*[a]*

a. Department of Medical Ultrasound, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200127, China

‡ These authors contributed equally to this work as senior authors.

***Corresponding author: Yijing Yao, E-mail: 598641050@qq.com; Lixin Jiang, E-mail: jinger_28@sina.com**

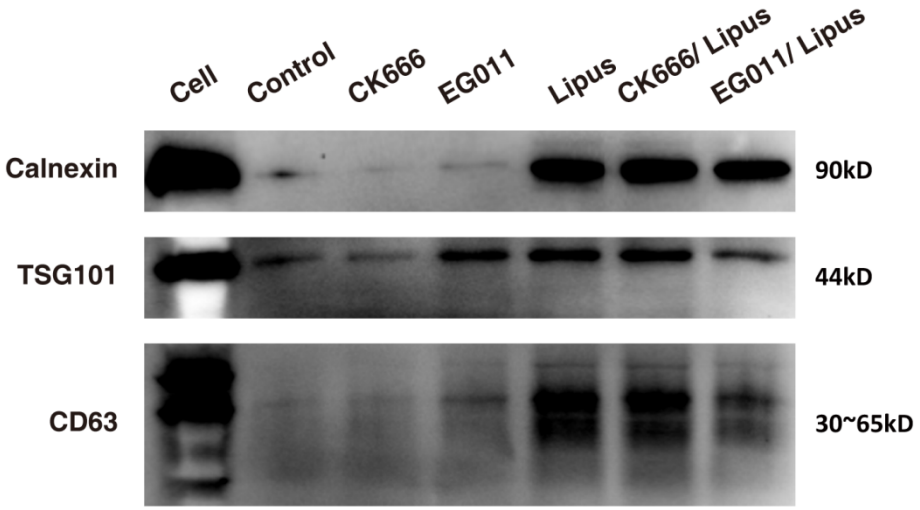

Figure S1. Lipus promoted the release of large extracellular vesicles.

After replacing the culture medium with fresh medium containing CK666 or EG011, the cells were treated with Lipus and then cultured for another 24 hours. The cell culture supernatant was aspirated. After removing the cells and cell debris, the 10,000 g centrifugation step was skipped, and the pellet containing the large vesicles was obtained by ultracentrifugation. Immunoblotting of ultracentrifugation pellet for EVs proteins CD63, TSG101, Calnexin was performed. Excessive sample loading on the cell lysate protein lane resulted in band overexposure but had no effect on the results. Calnexin, TSG101, and CD63 protein levels were significantly higher in the three LIPUS-treated groups. Calnexin, an endoplasmic reticulum marker, has been reported to be highly positive for large vesicles but weakly positive or negative for small vesicles (exosomes) in general.

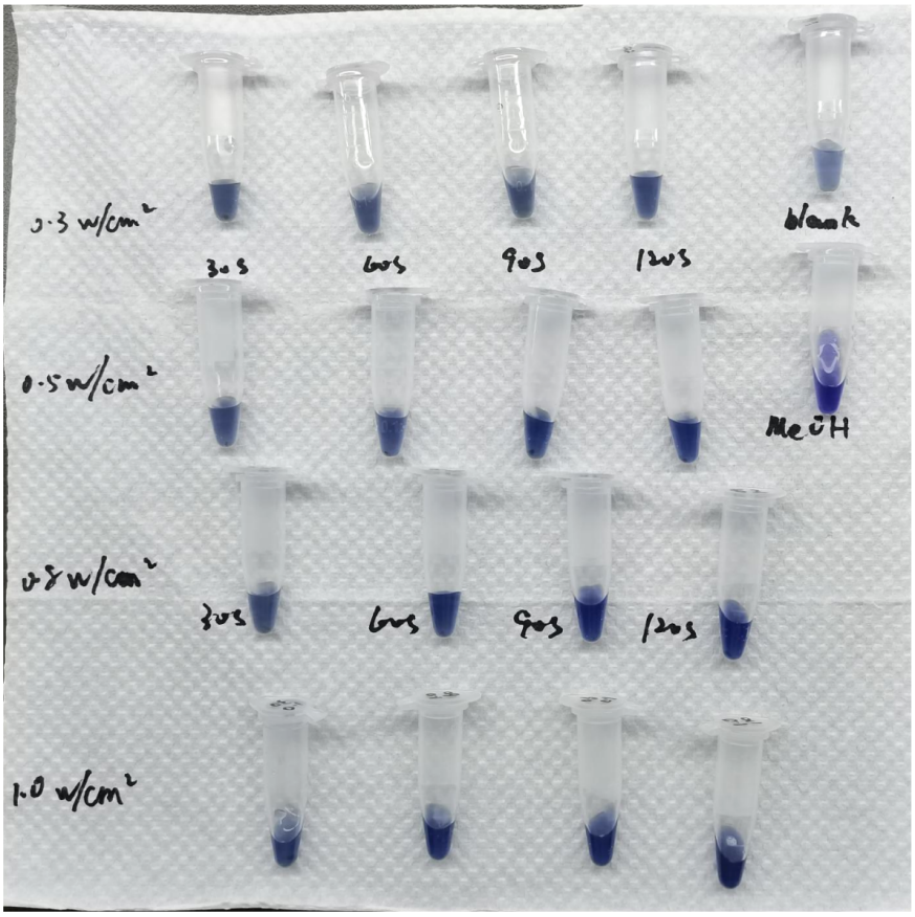

Figure S2. Lipus' destructive effect on BHQ-2-loaded liposomes was time and sound intensity dependent.

A total of 110 μl liposome suspension was added to 1.5 ml centrifuge tubes and stimulated for 30s, 60s, 90s, and 120s at 0.3 w/cm^2, 0.5 w/cm^2, 0.8 w/cm^2, and 1.0 w/cm^2, respectively. BHQ-2 was released from the liposomes as the liposomes were destroyed, and the color of the suspension changed. In the upper right corner, there is a negative control of untreated liposomes, as well as a positive control in which the liposomes were completely dissolved with methanol.

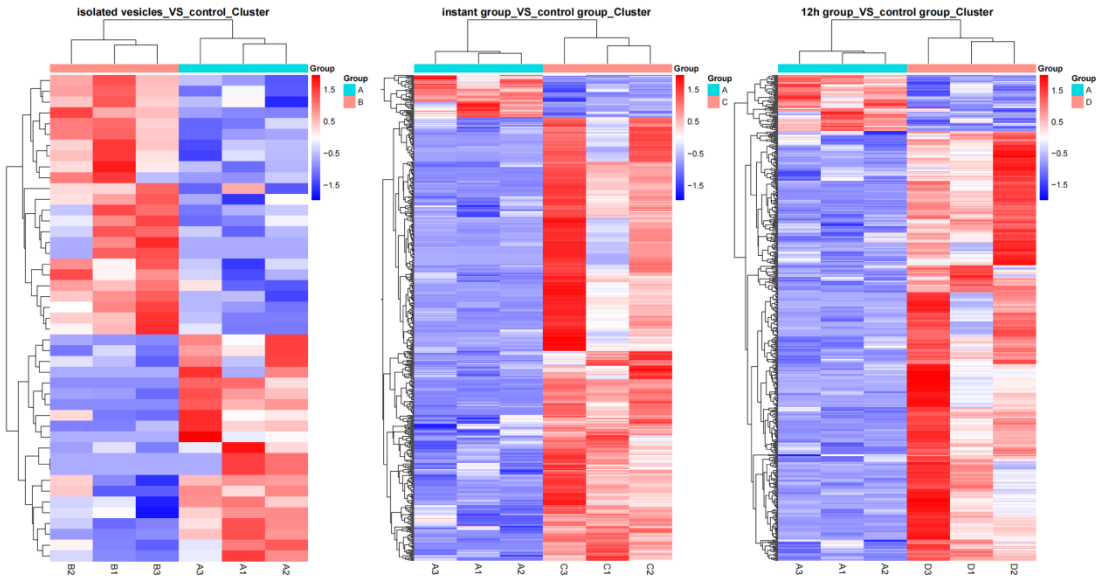

Figure S3. Hierarchical clustering analysis of the differentially expressed proteins

Hierarchical clustering analysis of the differentially expressed proteins in the isolated vesicles treated alone by Lipus compared to the control group (left panel), vesicles isolated from the cell culture system immediately after treatment compared to the control group (middle panel), vesicles isolated from the cell culture system 12 hours after treatment compared to the control group (right panel). In the color bar, red represents high expression, and blue represents low expression. n = 3 per group.

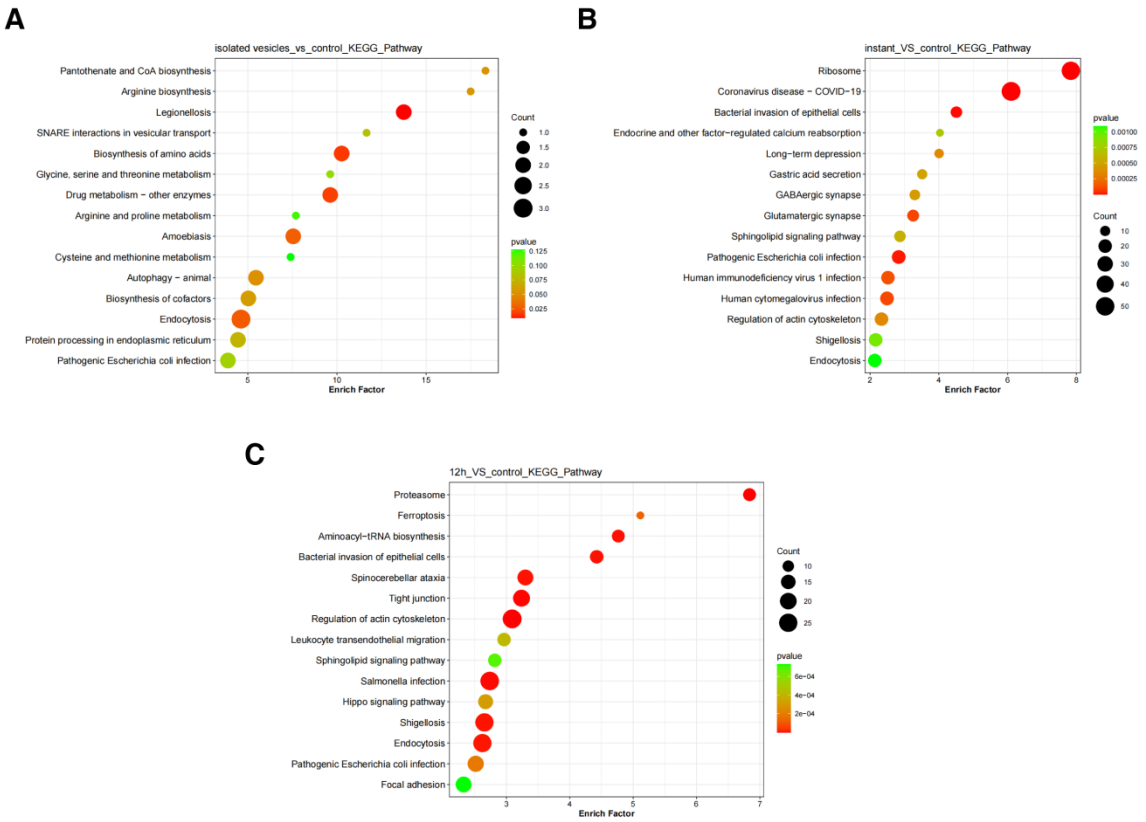

Figure S4. KEGG analysis

Significantly differentially regulated genes were compared between vesicles isolated immediately after treatment, 12 hours later after treatment, isolated vesicles exposed to Lipus outside the culture system and the control group. The x-axis represents the enrichment factor. The y-axis represents KEGG-enriched terms. The number of genes under a given term is indicated by the size of the dots. The adjusted P-value is represented by the color of the dots.

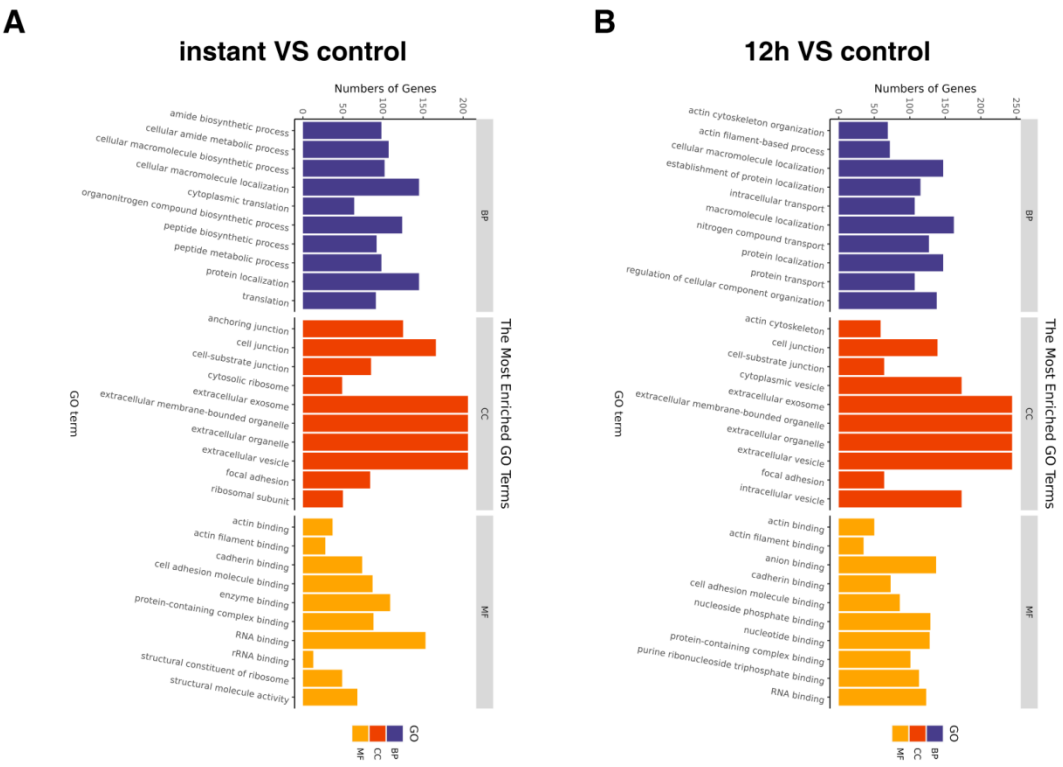

Figure S5. Gene Ontology term enrichment analysis of differentially expressed genes.

Go analysis of DEGs in Lipus treatment groups compared with control group. The 10 items with the lowest p-value were chosen for the bar chart. The vertical axis is Numbers of Count (the number of genes matched to this item), and the horizontal axis is Entry. The figure depicts information pertaining to molecular function (MF), cellular components (CC), and biological processes (BP).