

## **Pinched-flow Fractionation-based Extracellular Vesicle Isolation by ExoFAST and Its Comparison with Commercial Technologies**

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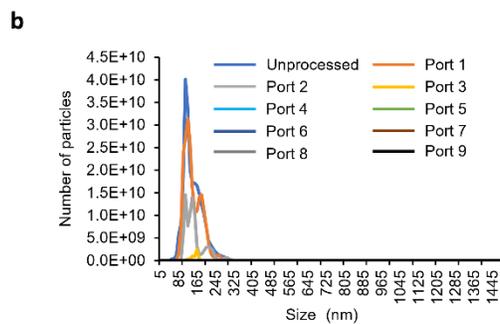
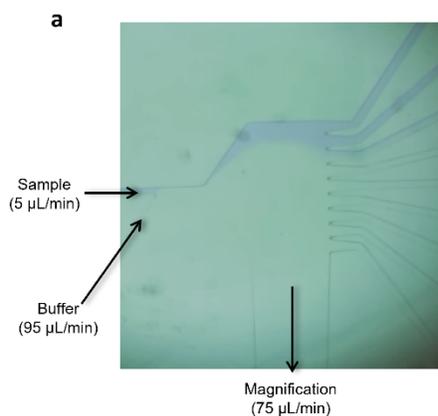
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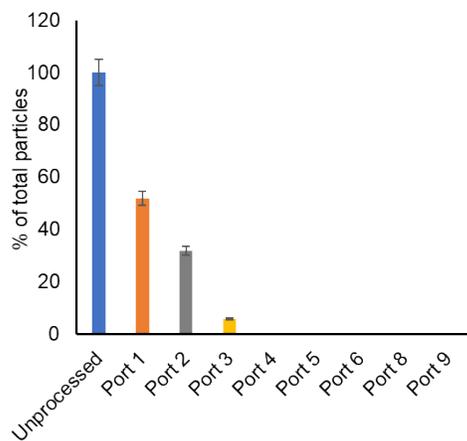
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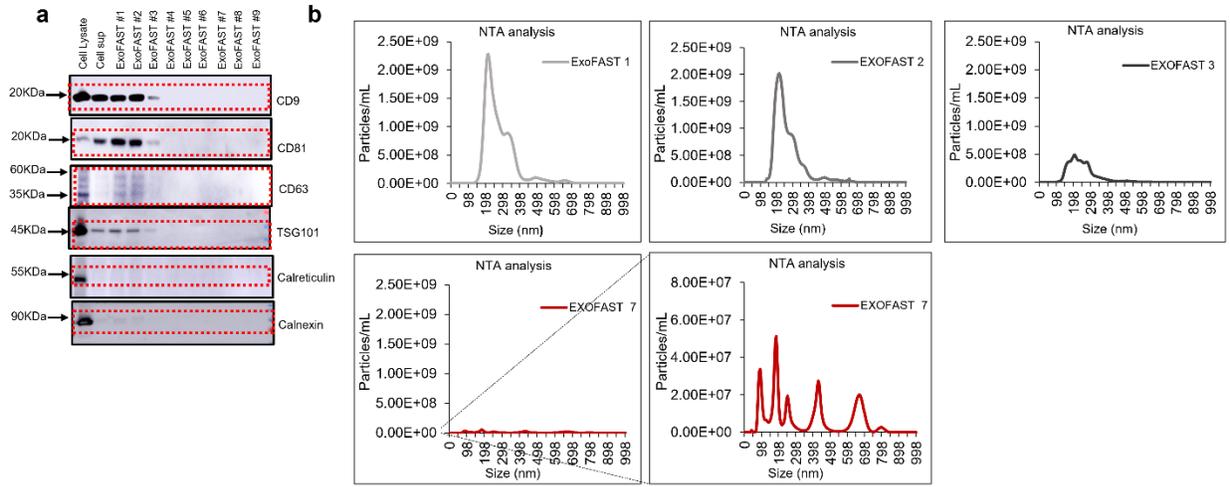
**c**



**Suppl Fig. 1 Flow control test and separation of the nano particle by bead test using ExoFAST device (a)**

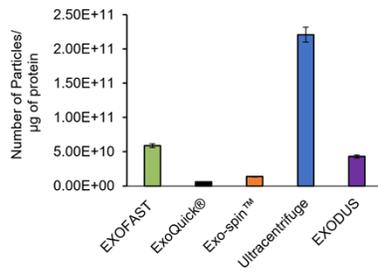
Optimal separation for samples were indicated: Sample flow rate of 5  $\mu\text{L}/\text{min}$ , buffer flow rate of 95  $\mu\text{L}/\text{min}$  and magnification of 75  $\mu\text{L}/\text{min}$ . (b) Confirmation of separation of the nano particle into the first 3 outlet channels from 1-9 using 100 nm polystyrene beads. Blue line represents the unprocessed sample. Port 1 to Port 9 represent each of the outlet channels in the chip. (c) Percentage recovery of particles in the outlet ports 1 to 9, in comparison with the unprocessed sample.

**Supplementary Figure 2**

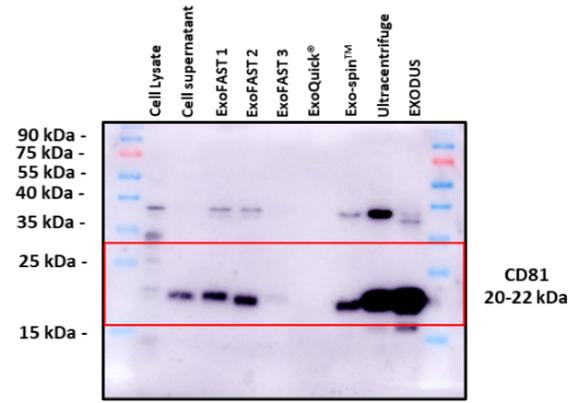
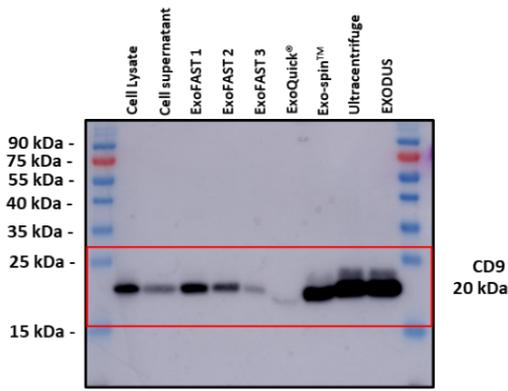


**Suppl Fig. 2 Western blotting of EV-specific proteins and NTA analysis from the outlet channels (1-9) of the ExoFAST device (a) Presence of EV-proteins; CD9, CD81, CD63 and TSG101 in the first three outlet channels of the ExoFAST device and absence of apoptotic-body by markers calnexin and calreticulin. (b) Particles within the 200 nm size range in outlet channels (1-3, and 7) of the ExoFAST device.**

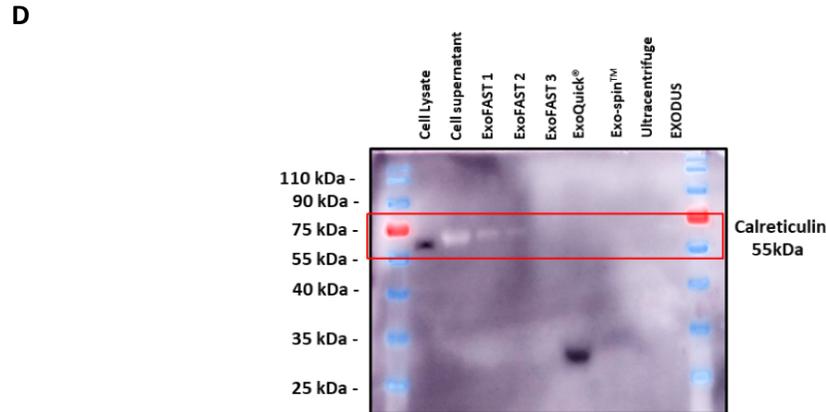
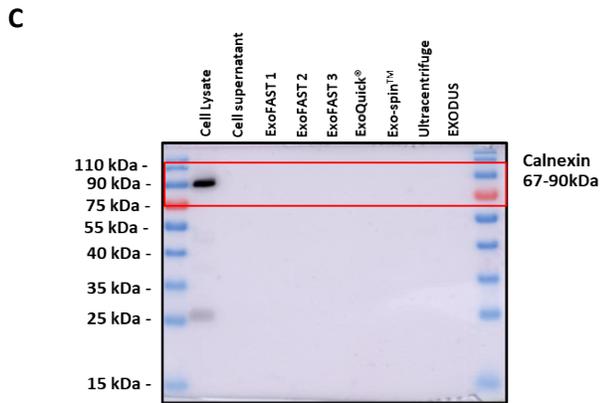
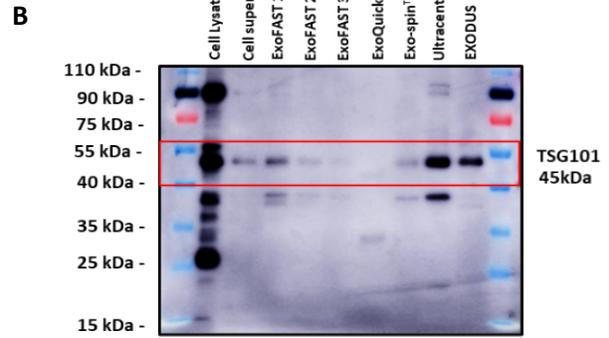
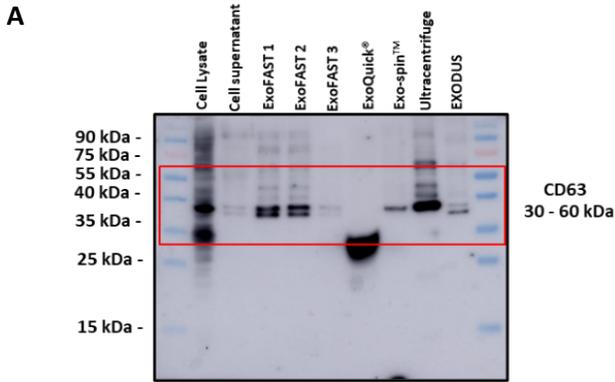
### Supplementary Figure 3



**Suppl Fig. 3 Yield of particles per unit of protein for different exosome isolation methods.** Ultracentrifuge and ExoFAST yielded the high-purity and highest particle to protein ratio.



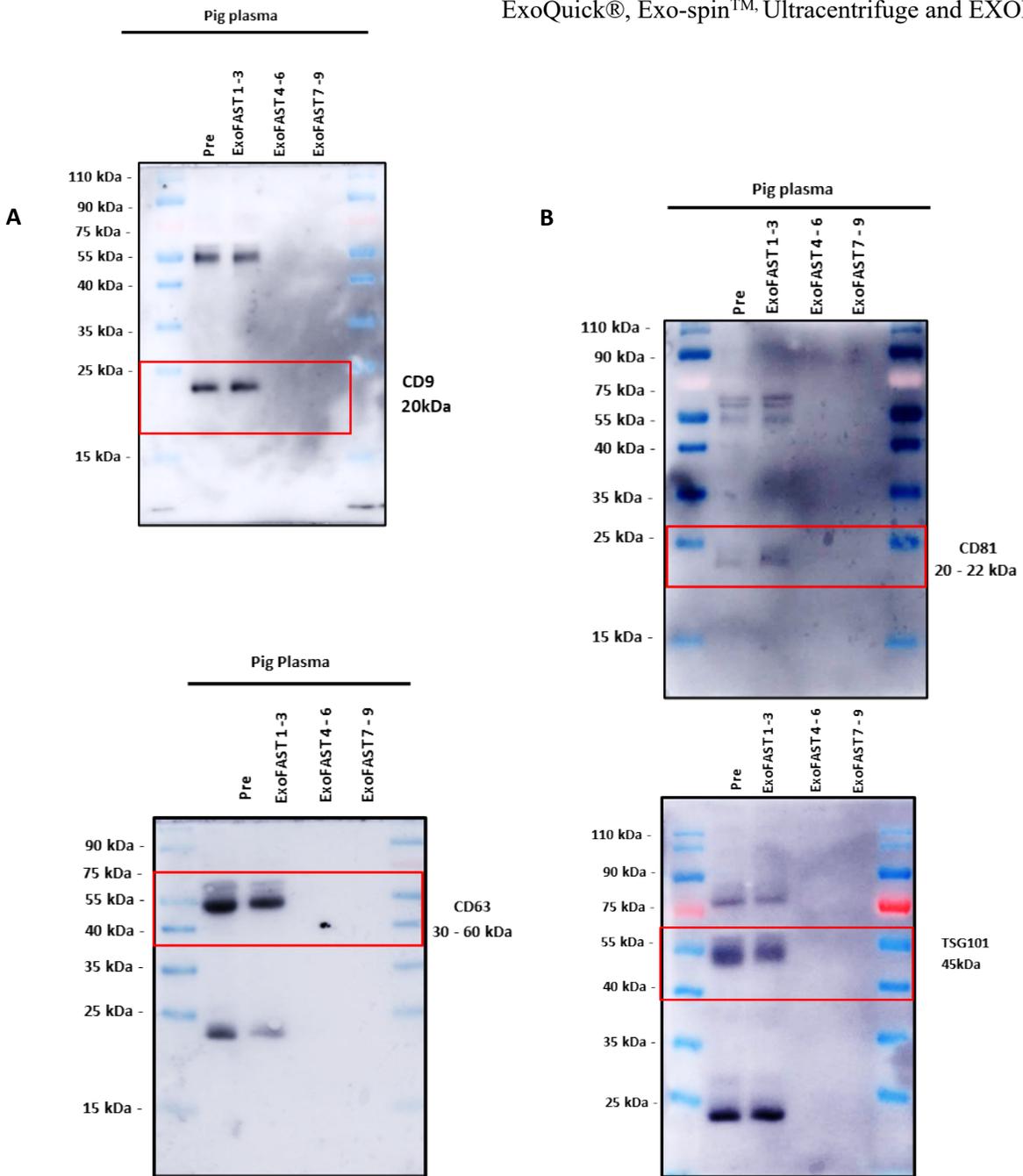
Supplementary Figure 4



**E**

**F**

**Suppl Fig. 4 Western blot membranes of EV-specific proteins from Figure 5 (A) CD9 (B) CD81 (C) CD63 (D) TSG101 (E) Calnexin (F) Calreticulin, in cell lysate, cell supernatant, ExoFAST channels 1-3, ExoQuick®, Exo-spin™, Ultracentrifuge and EXODUS.**

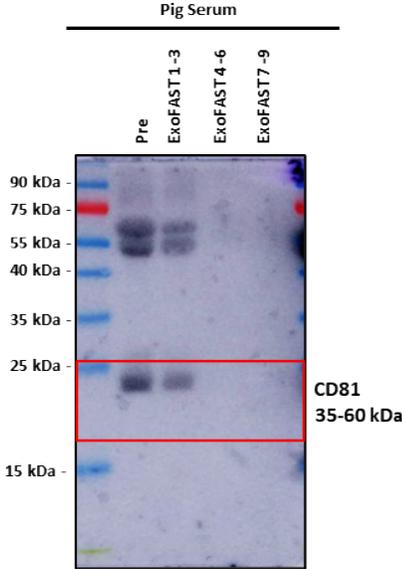


**Supplementary Figure 5**

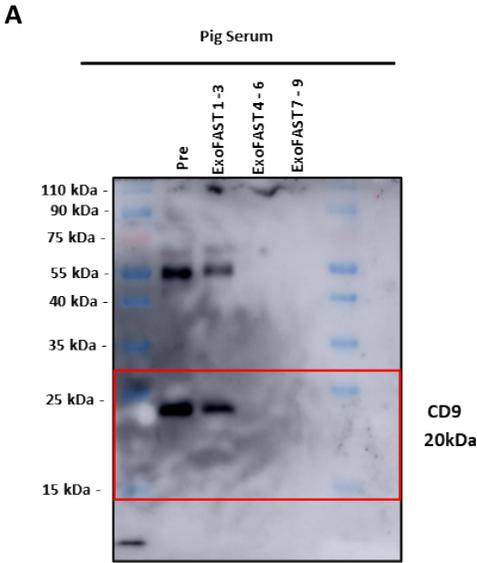
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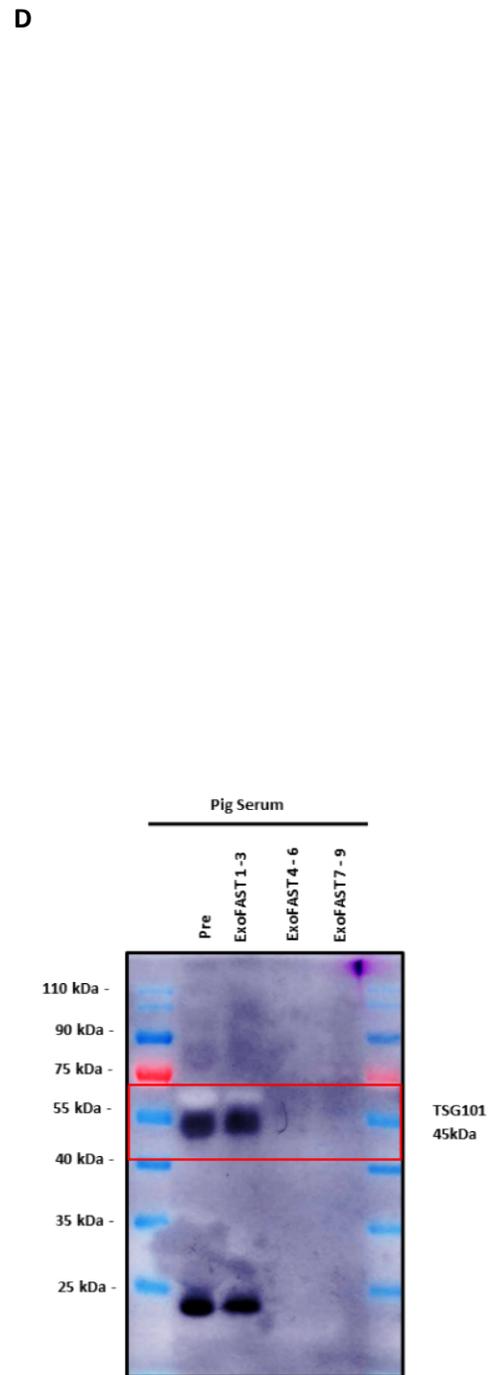
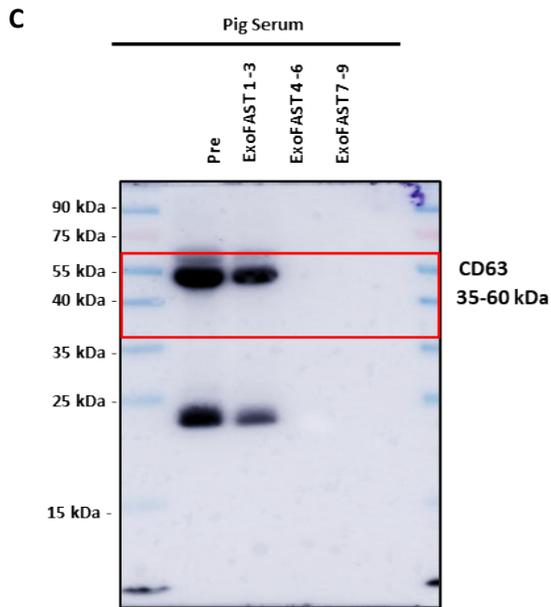
**Suppl Fig. 5 Western blot membranes of EV-specific proteins from pig plasma samples, Fig 6 (A) CD9 (B) CD81 (C) CD63 (D) TSG101 isolated using ExoFAST. Unenriched exosome samples and pooled exosome samples from channel 1-3, 4-6 and 7-9 are shown.**



**Supplementary Figure 6**

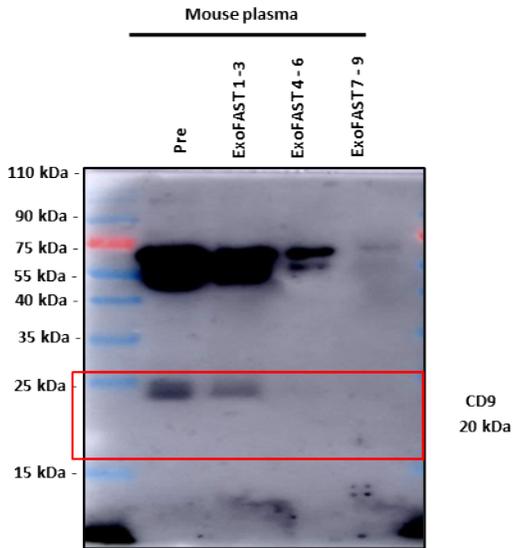
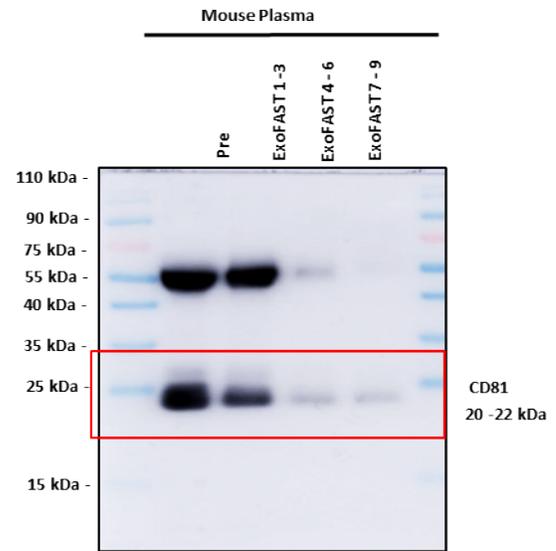
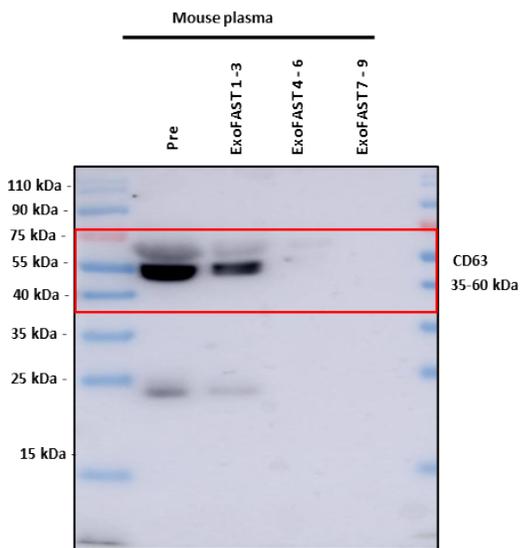
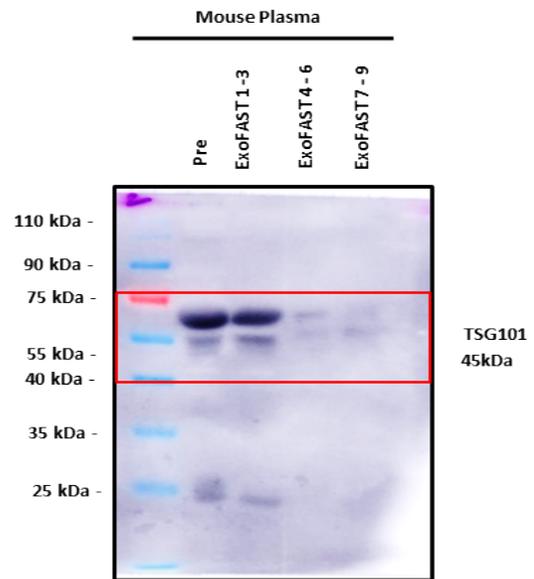


**B**



**Suppl Fig. 6 Western blot membranes of EV-specific proteins from pig serum samples, Fig 6 (A) CD9 (B) CD81 (C) CD63 (D) TSG101 isolated using ExoFAST. Unenriched exosome samples and pooled exosome samples from channel 1-3, 4-6 and 7-9 are shown.**

**Supplementary Figure 7**

**A****B****C****D**

**Suppl Fig. 7 Western blot membranes of EV-specific proteins from mouse serum samples, Fig 6 (A) CD9 (B) CD81 (C) CD63 (D) TSG101 isolated using ExoFAST. Unenriched exosome samples and pooled exosome samples from channel 1-3, 4-6 and 7-9 are shown.**

