Extracellular vesicle ITGAM and ITGB2 mediate severe acute pancreatitis-related acute lung injury

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Supporting Information.

The Supporting Information is available free of charge at .

The distribution and effect of SAP-EV on lung tissue in vivo (Figure S1- S6). Figure S7. Western blot analysis and graphs of ITGAM and ITGB2 expression on serum EVs isolated from clinically healthy negative control subjects (NC) and patients with mild acute pancreatitis (MAP) and severe acute pancreatitis (SAP). Figure S8. The role of S100A9 gene on SAP-EV in acute lung injury (Supporting figures).

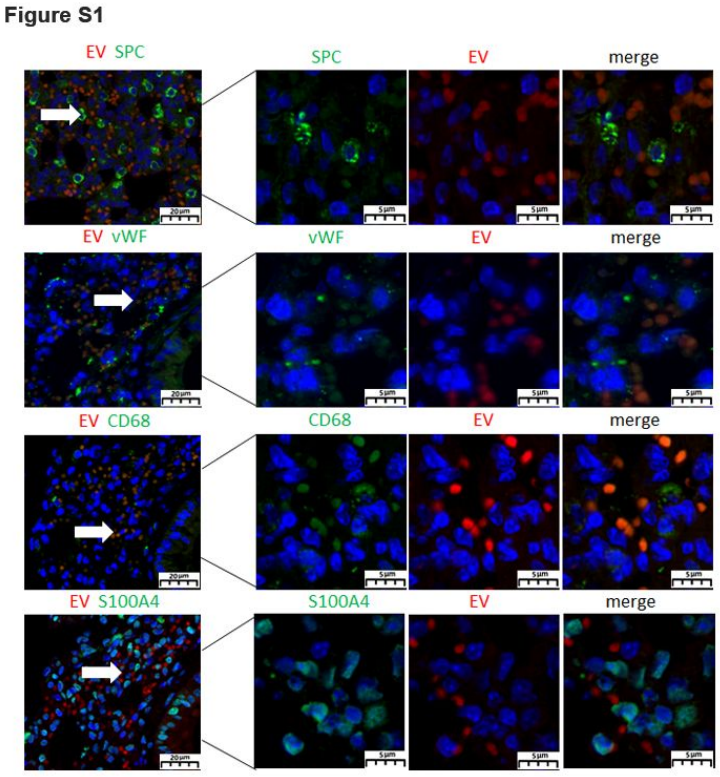

Figure S1. After SAP-EV fractions were injected into SC mice the labeled SAP-EV were found to colocalize with pulmonary microvascular endothelial cells

(vWF-positive) and macrophages (CD68-postive), but not epithelial cells (SPC-positive) and fibroblasts (S100A4-positive).

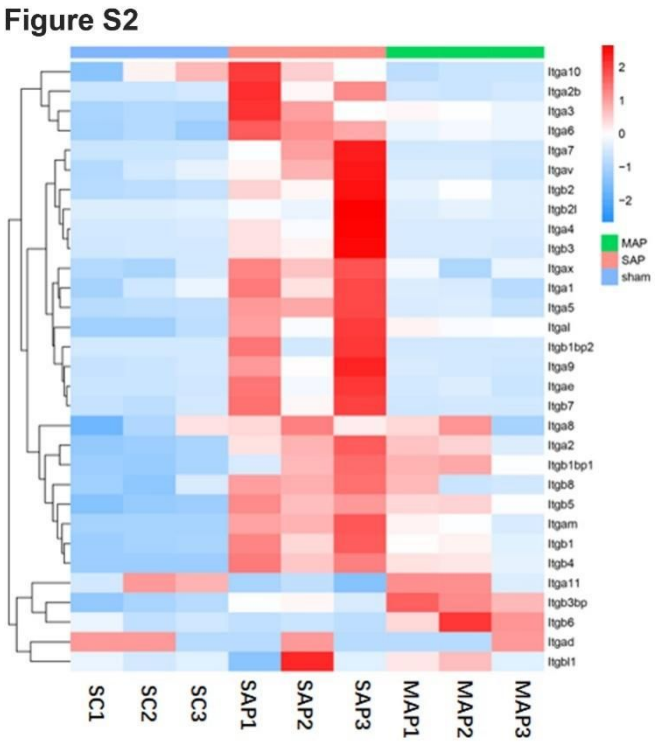

Figure S2. Heat map of mRNA-sequence analysis of integrins expressed in pancreatic tissue of SC, SAP, and MAP mice.

Figure S3

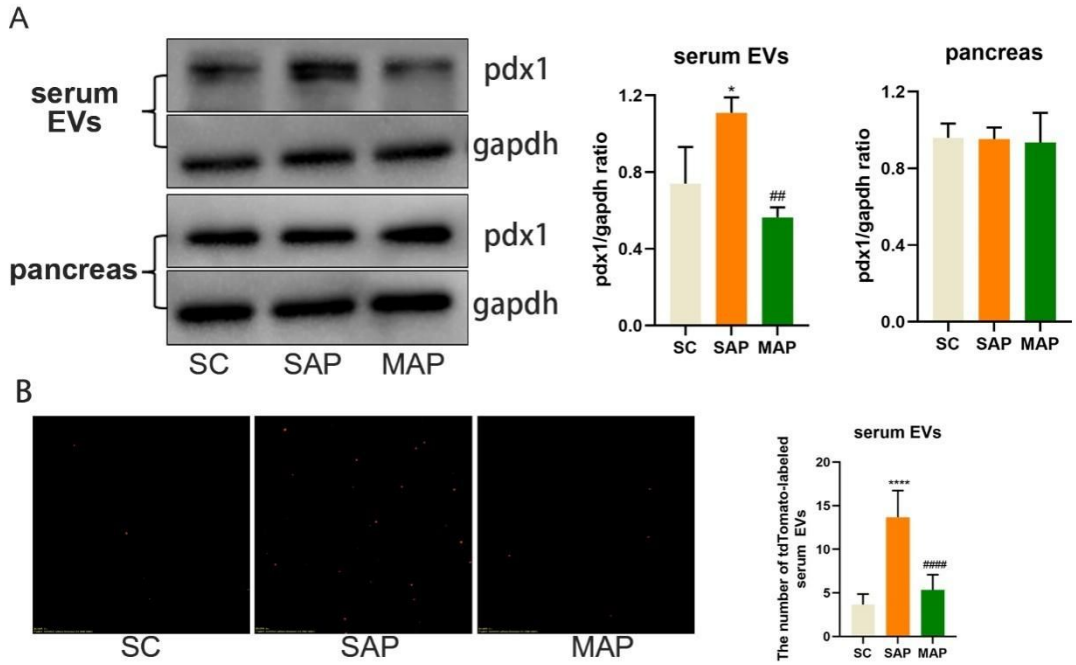

Figure S3. Serum EV fractions of SAP mice contain more pancreas-derived EVs than those of SC or MAP mice. (**A**) Western blot analysis and graphs of pdx1 and gapdh in serum EVs and pancreas tissue of SC, SAP, and MAP pdx-cre tdTomato transgenic mice (n = 6/group; $^{*}p < 0.05$ *vs* SC, $^{\#\#}p < 0.01$ *vs* SAP). (**B**) Representative images and graphs of serum EV red fluorescent tdTomato signal in EV fractions of SC, SAP, and MAP transgenic mice (n = 6/group; $^{****}p < 0.0001$ vs SC, $^{\#\#\#\#}p < 0.0001$ *vs* SAP).

Figure S4

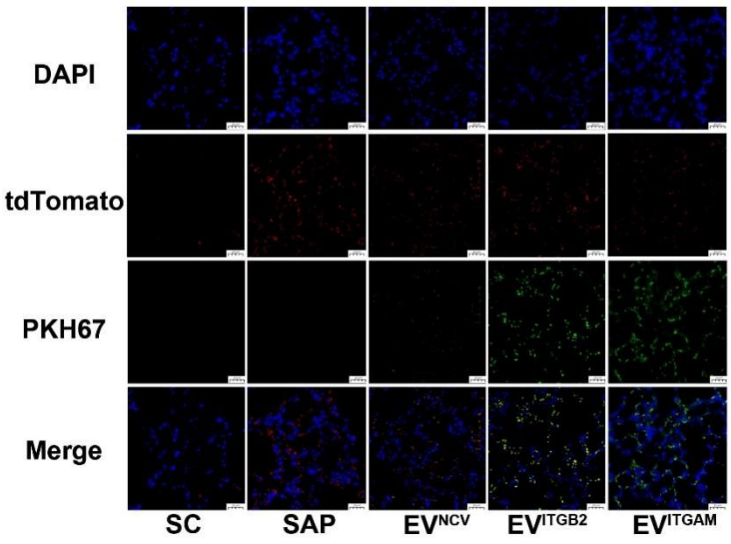

Figure S4. Distribution of tdTomato-labeled pancreatic SAP-EVs and PKH67-labeled EV^{NCV}, EV^{ITGB2}, and EV^{ITGAM} fractions in lung tissue histology sections of SC and SAP mice injected with or without EV^{NCV}, EV^{ITGB2}, and EV^{ITGAM} fractions (scale bar = 20 μm).

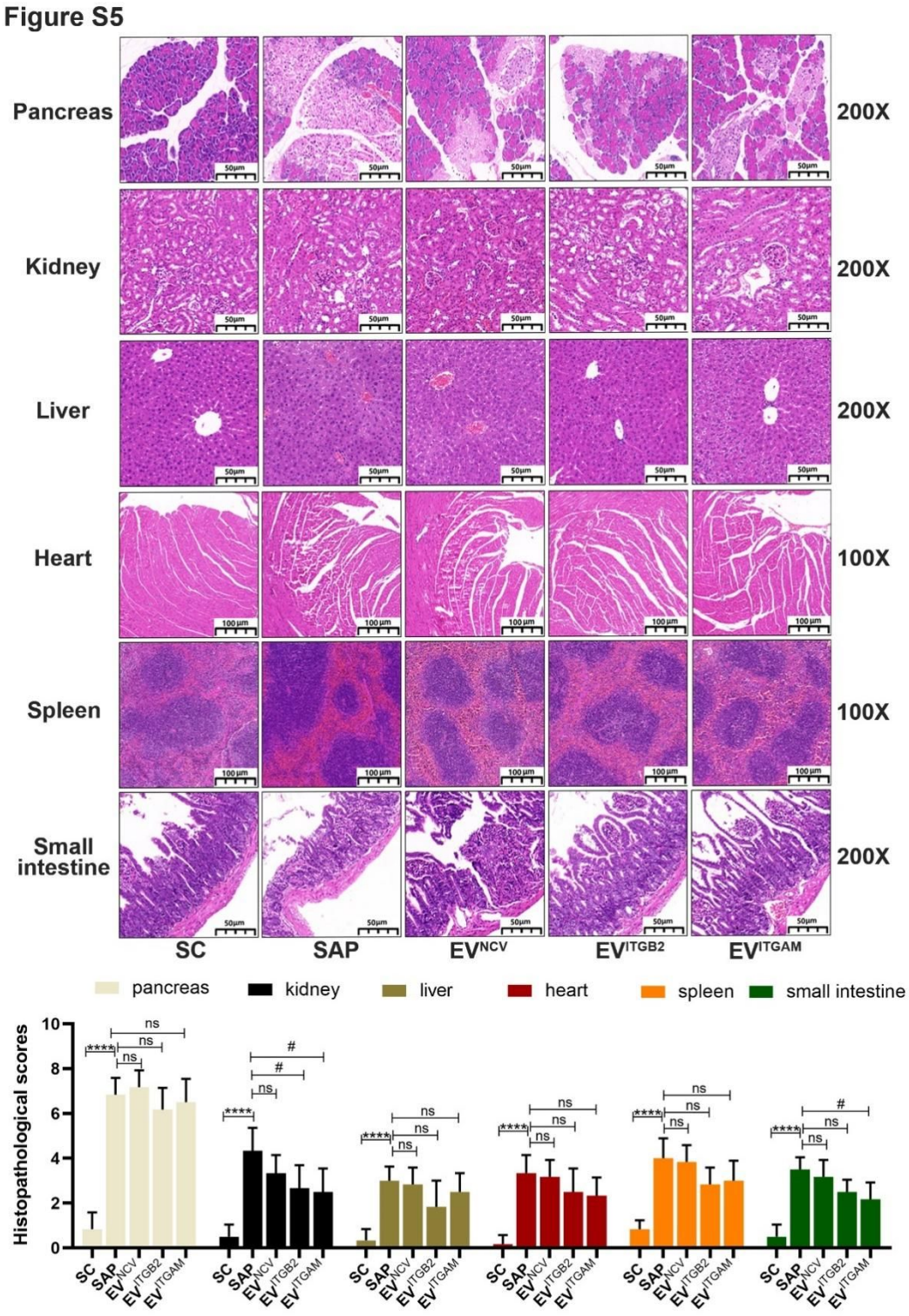

Figure S5. Representative pancreas, kidney, liver, spleen, small intestine histologic sections of SC, SAP, and EV-treated SAP mice and their pathology scores (n = 6;

scale bar = 50 μm in pancreas, kidney, liver, small intestine, scale bar = 100 μm in heart and spleen, ****$p < 0.0001$ vs SC, $^{\#}p < 0.05$ vs SAP).

Figure S6

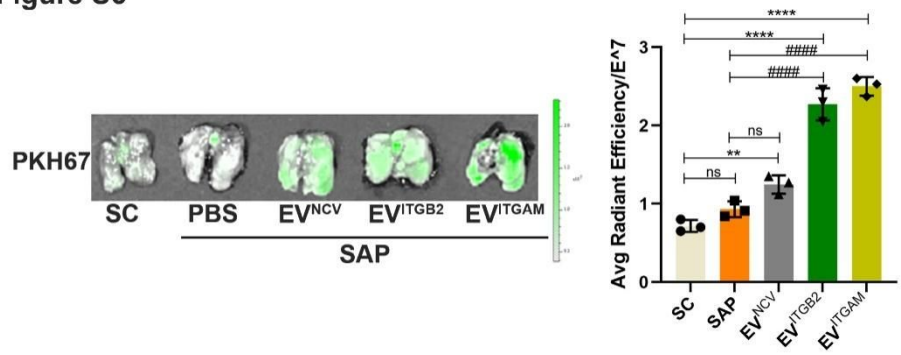

Figure S6. Relative pulmonary accumulation of PKH67-labeled EV^{ITGAM}, EV^{ITGB2}, and EV^{NCV} in SAP mice, where SC and PBS indicate fluorescent signal in non-injected SC mice and PBS-injected SAP mice that serve as negative controls (**$p < 0.01$, ****$p < 0.0001$ vs SC, $^{\#\#\#\#}p < 0.0001$ vs SAP).

Figure S7

Figure S7. Western blot analysis and graphs of ITGAM and ITGB2 expression on serum EVs isolated from clinically healthy negative control subjects (NC) and patients with mild acute pancreatitis (MAP) and severe acute pancreatitis (SAP) (n = 6/group; *$p < 0.05$, ****$p < 0.0001$ vs NC, $^{\#\#}p < 0.05$ vs MAP). The experiment was approved by the Ethics Committee on Biomedical Research, West China Hospital of Sichuan University (Permit No. 2021(1041).

Figure S8

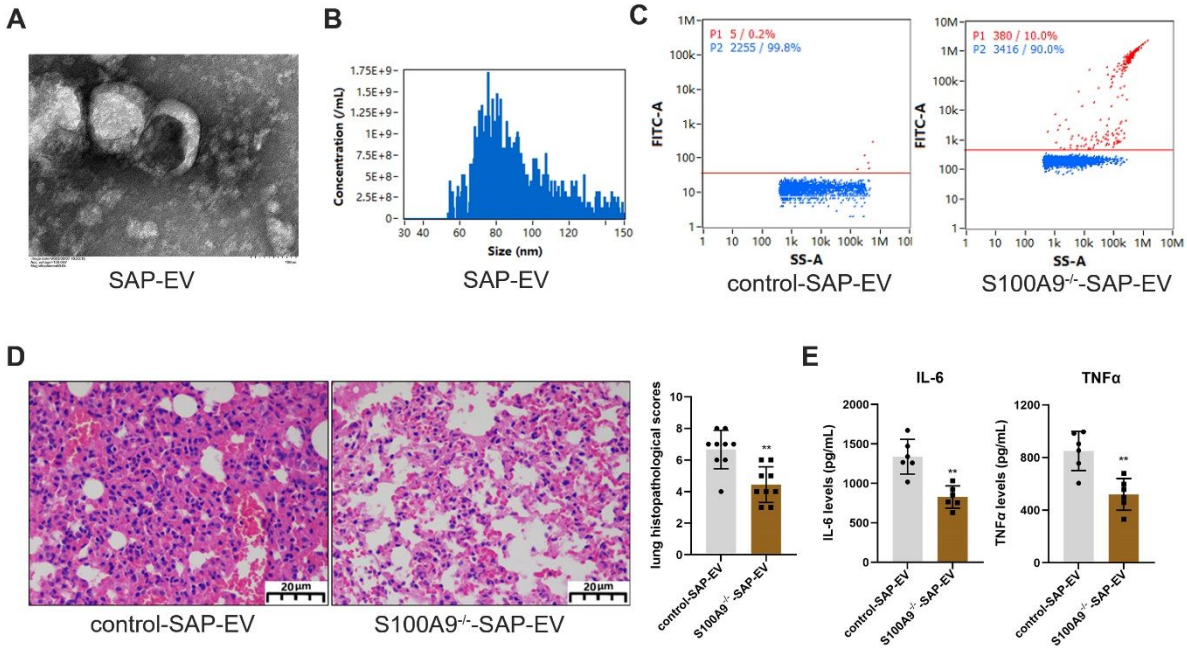

Figure S8. The role of S100A9 gene on SAP-EV in acute lung injury. (**A**) Transmission electron micrographs of SAP-EV. (**B**) Size distribution and mean diameter of SAP-EV determined by nano-particle tracking analysis. (**C**) After co-incubation of SAP-EV with FITC-carrying siRNA-S100A9 for 24h, the FITC fluorescence of SAP-EV was detected by nanoflow cytometry to determine siRNA transfection efficiency. (**D**) Lung histology and pathology scores of healthy mice after control-SAP-EV and S100A9$^{-/-}$-SAP-EV injection by the tail vein (n = 9/group; scale bar = 20 μm, $^{**}p < 0.01$ vs control-SAP-EV). (**E**) Lung IL-6 and TNFα expression after control-SAP-EV and S100A9$^{-/-}$-SAP-EV injection (n = 6/group; $^{**}p < 0.01$ vs control-SAP-EV).