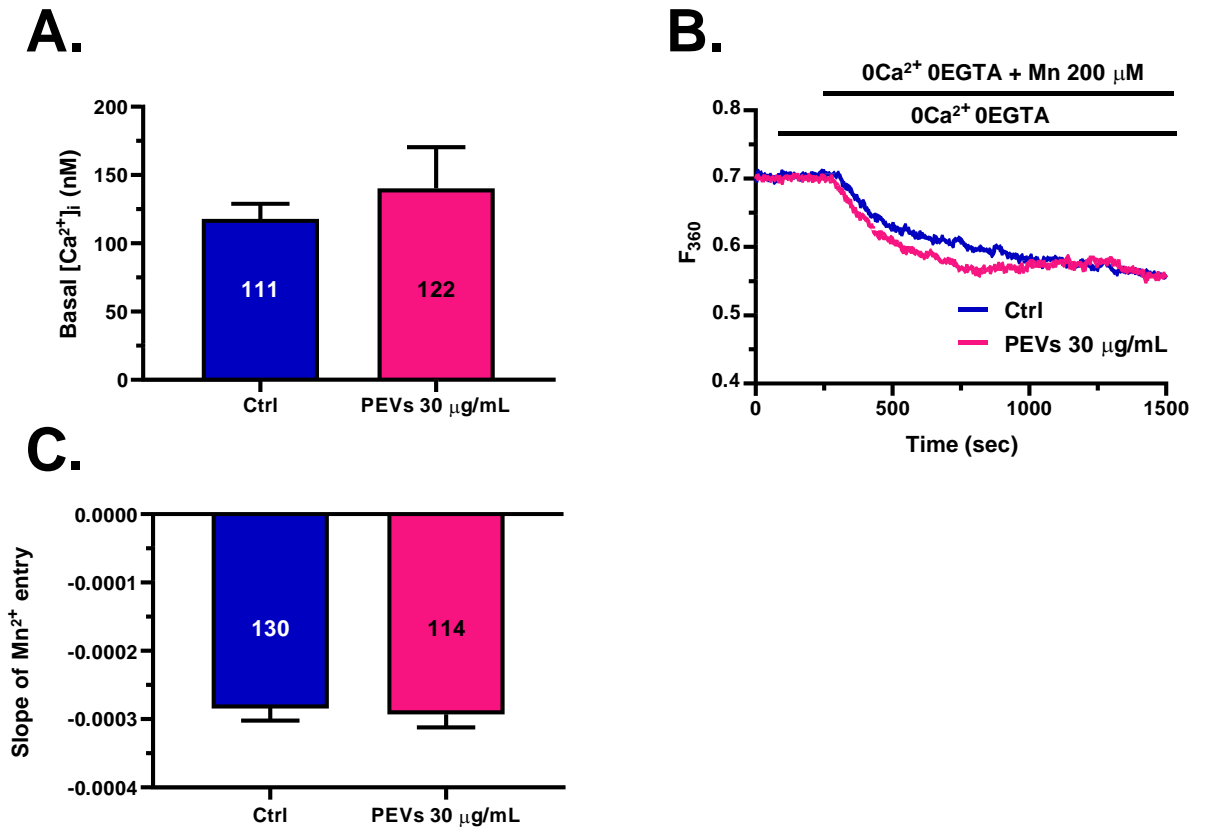
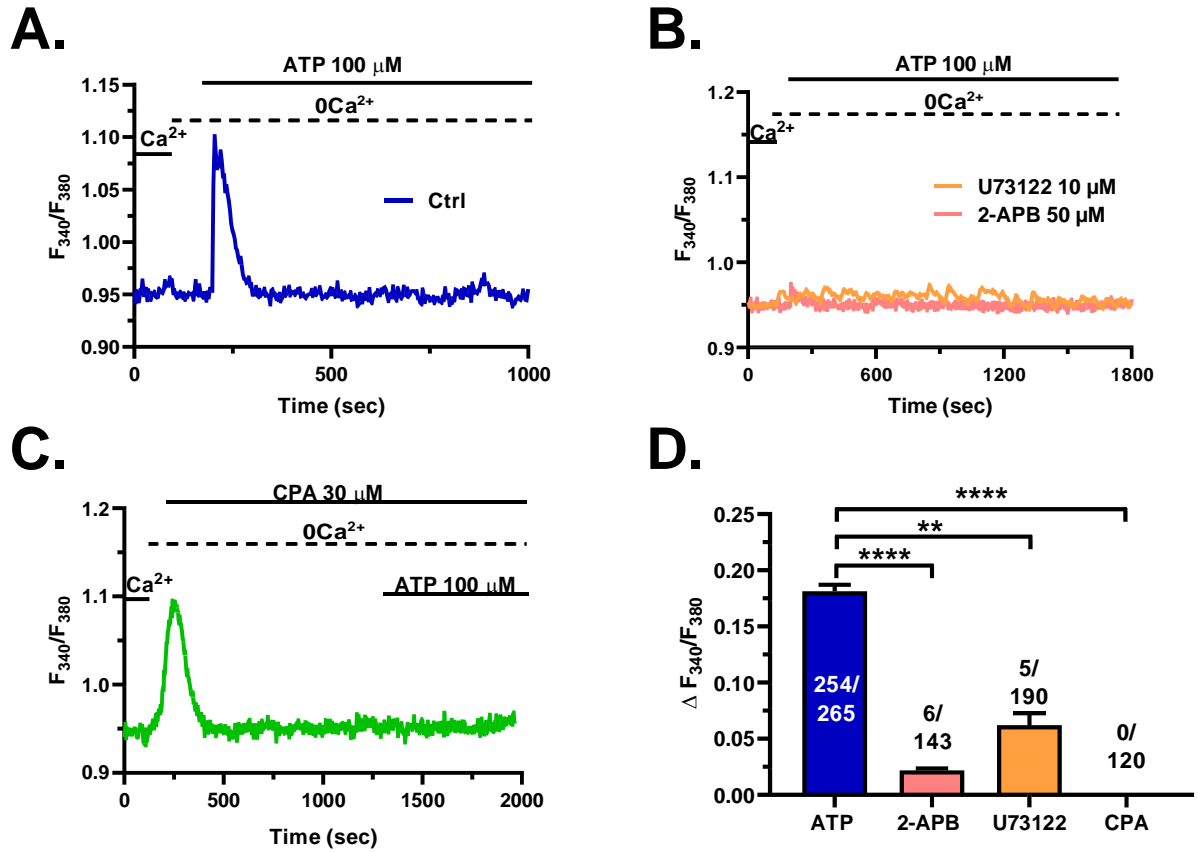


## Supplementary Information



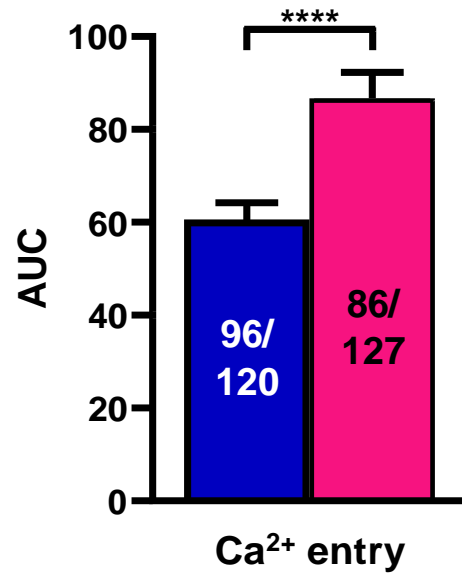
**Figure 1. Long-term exposure of MDA-MB-231 cells with PEVs did not affect basal  $[Ca^{2+}]_i$  and constitutive  $Ca^{2+}$  entry.** **A.** Evaluation of resting  $[Ca^{2+}]_i$  did not reveal any difference between control (Ctrl) cells and cells treated with PEVs (30  $\mu\text{g/mL}$ , 24 h). Basal was evaluated by using the Grynkiewicz equation, as shown elsewhere [35]. **B.** Resting  $Ca^{2+}$  entry in MDA-MB-231 cells was evaluated by using the  $Mn^{2+}$ -quenching technique, as described in Materials and methods. The extracellular physiological solution was first replaced with a 0Ca<sup>2+</sup> solution and then 200  $\mu\text{M}$   $Mn^{2+}$  was added to cause an immediate decay in Fura-2 fluorescence, which is consistent with the occurrence of constitutive  $Ca^{2+}$  entry. Resting  $Mn^{2+}$  influx in MDA-MB-231 cells was not impaired by the treatment with PEVs (30  $\mu\text{g/mL}$ , 24 h). The rate of fluorescence decay for each individual tracing was calculated as the slope of a linear regression. **C.** Mean  $\pm$  SEM of the quenching rate of Fura-2 fluorescence induced by  $Mn^{2+}$  addition in control (Ctrl) and treated cells.





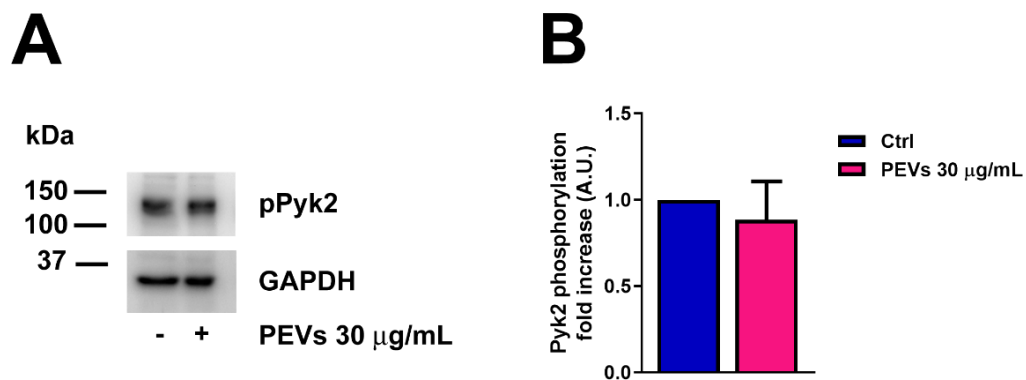
**Figure S2. InsP $_3$ -dependent ER  $\text{Ca}^{2+}$  mobilization supports ATP-induced intracellular  $\text{Ca}^{2+}$  release in MDA-MB-231 cells.** **A.** Intracellular  $\text{Ca}^{2+}$  release evoked by ATP (100  $\mu\text{M}$ ) in untreated (Ctrl) MDA-MB-231 cells in the absence of extracellular  $\text{Ca}^{2+}$  ( $0\text{Ca}^{2+}$ ). **B.** ATP-evoked intracellular  $\text{Ca}^{2+}$  release was largely suppressed by blocking PLC $\beta$  activity with U73122 (10  $\mu\text{M}$ , 30 min) and inhibiting InsP $_3$ Rs with 2-APB (50  $\mu\text{M}$ , 30 min). **C.** ATP-evoked intracellular  $\text{Ca}^{2+}$  release was abrogated upon depletion of the ER  $\text{Ca}^{2+}$  pool with CPA (30  $\mu\text{M}$ ) under  $0\text{Ca}^{2+}$  conditions. **D.** Mean  $\pm$  SE of the amplitude of ATP-evoked endogenous  $\text{Ca}^{2+}$  release under the designated treatments. \*\*\*\* indicate  $p < 0.0001$ , \*\* indicate  $p < 0.01$ .





**Figure S3. Further evidence that long-term exposure of MDA-MB-231 cells with PEVs increases FBS-induced extracellular  $\text{Ca}^{2+}$  entry.** Measurement of the AUC (mean $\pm$ SE) confirmed that pre-treatment of MDA-MB-231 cells with PEVs (30  $\mu\text{g}/\text{mL}$ , 24 h) significantly increased 20% FBS-induced extracellular  $\text{Ca}^{2+}$  entry. \*\*\*\* indicate  $p < 0.0001$ .





**Figure S4. Long-term exposure of MDA-MB-231 cells with PEVs did not increase Pyk2 phosphorylation.** Phosphorylation of Pyk2 (**A-B**) in MDA-MB-231 cells incubated with PEVs (30 µg/mL, 24 h) or left untreated. Representative immunoblots are reported in **A** for phospho-Pyk2 where GAPDH staining is for equal loading control. The quantification of the results is shown in **B** for phospho-pPyk2 as the mean  $\pm$  SD of three experiments.



**Table S1. Primers used for real-time qPCRs.**

Target gene	Primer name	Primer sequence (5'–3')	Amplicon length (bp)
SERCA2B	SERCA2b_F1	ATGCCATTGTTCTGAAGCCTC	136
	SERCA2b_R1	TCACCTTCCACTCTGTCCAG	
SERCA3	SERCA3_F1	TCCGCCTCATCGAGATCAAG	135
	SERCA3_R1	GCATGTTCTTCTTGTCCTGGT	
InsP <sub>3</sub> R1	ITPR1_F	GGAGTTTCAGCCCTCAGTGG	101
	ITPR1_R	CTTCAGGCACAGAGACCAGG	
InsP <sub>3</sub> R2	ITPR2_F	CACTCTGGGAAATAGAGGTGGTT	124
	ITPR2_R	TCAGGATTAAGCTCTGCAGCTA	
InsP <sub>3</sub> R3	ITPR3_F1	GCTTCATCAGCACTTTGGGG	104
	ITPR3_R1	CTTGAAGAGGCAGTCACGGA	
STIM1	STIM1_F1	GCCCTGTGGCTCCTCTG	130
	STIM1_R1	TTCGGCAAACTCTGCTGC	
Orai1	ORAI1_F	GCTTCGCCATGGTGGCAAT	127
	ORAI1_R	ATCATGAGCGCAAACAGGTG	
Actin beta <sup>1</sup>	ACTB_alt1_F	GGACCTGACTGACTACCTCAT	107
	ACTB_alt1_R	CGTAGCACAGCTTCTCCTTAAT	
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH_alt_F	CGGGAAACTGTGGCGTGATGG	120
	GAPDH_alt_R	GCCATGCCAGTGAGCTTCCC	



