We performed a phosphoproteomic analysis of breast cancer-derived exosomes to provide insight into the molecular and cellular regulatory mechanisms important for breast cancer tumor progression and metastasis [1]. We examined three cell line models for breast cancer: MCF10A (non-malignant), MCF7 (estrogen and progesterone receptor-positive, metastatic), and MDA-MB-231 (triple-negative, highly metastatic). To obtain a comprehensive overview of the exosome phosphoproteome derived from each cell line, effective phosphopeptide enrichment techniques IMAC and TiO2, followed by LC-MS/MS, were performed. Among 855 distinct phosphoproteins, we validated four enzymes associated with cancer and present only in exosomes isolated from MCF7 and MDA-MB-231 cell lines: ATP citrate lyase (ACLY), phosphofructokinase-M (PFKM), sirtuin-1 (SIRT1), and sirtuin-6 (SIRT6). With the exception of PFKM, the specific activity of these enzymes was significantly higher in MDA-MB-231 when compared with MCF10A-derived exosomes. This study demonstrates that exosomes contain functional metabolic enzymes that could be further explored for their potential use in early breast cancer diagnostic and therapeutic applications.