

## Supplementary Materials

### **Glycosylated extracellular vesicles drive a metabolic interplay between gastric cancer cells and adipocytes**

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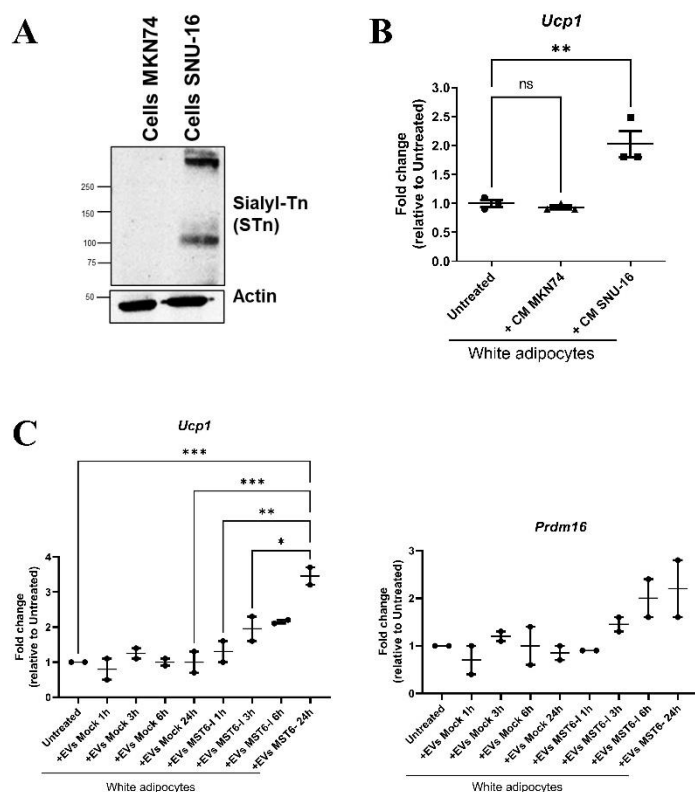
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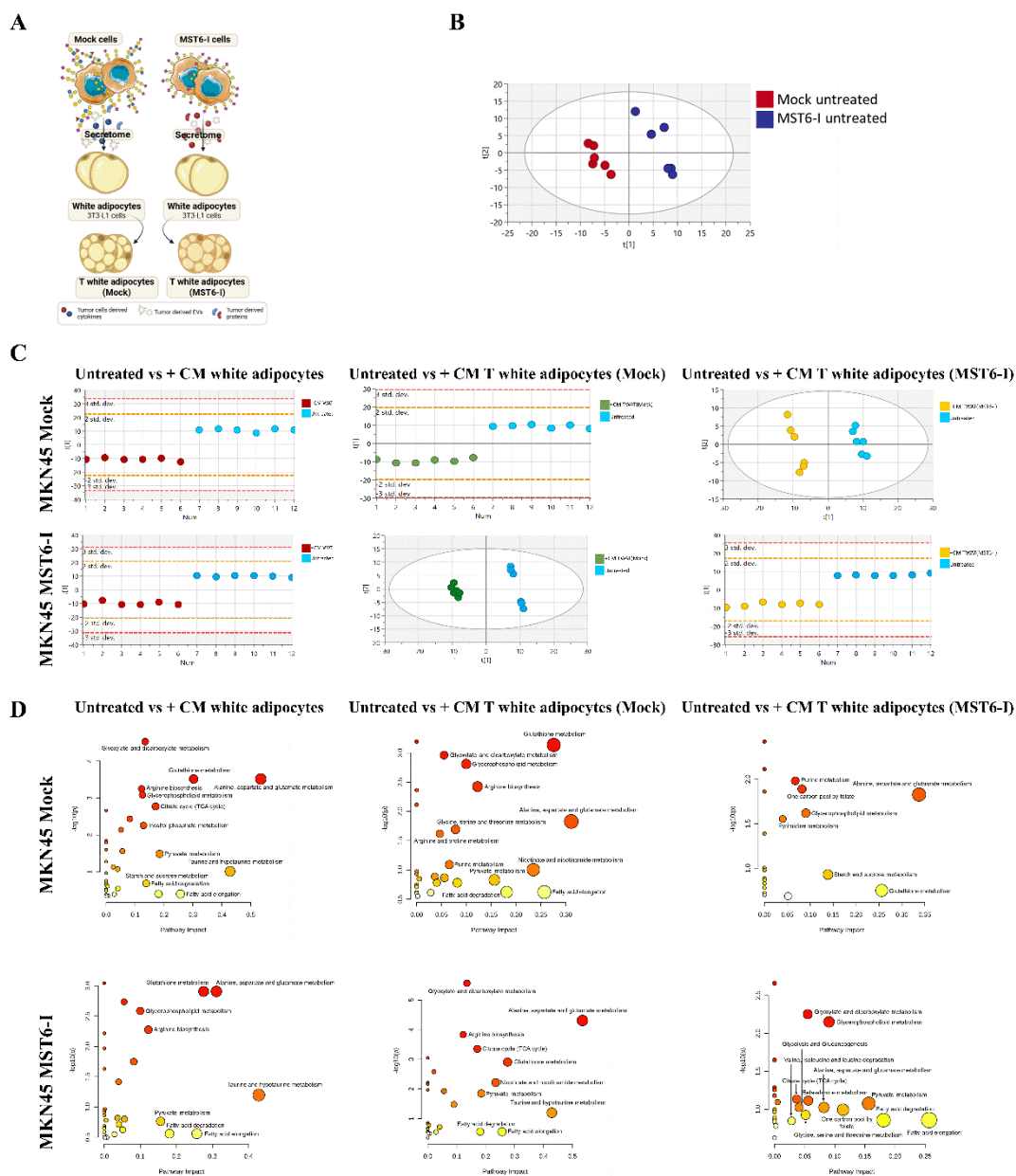


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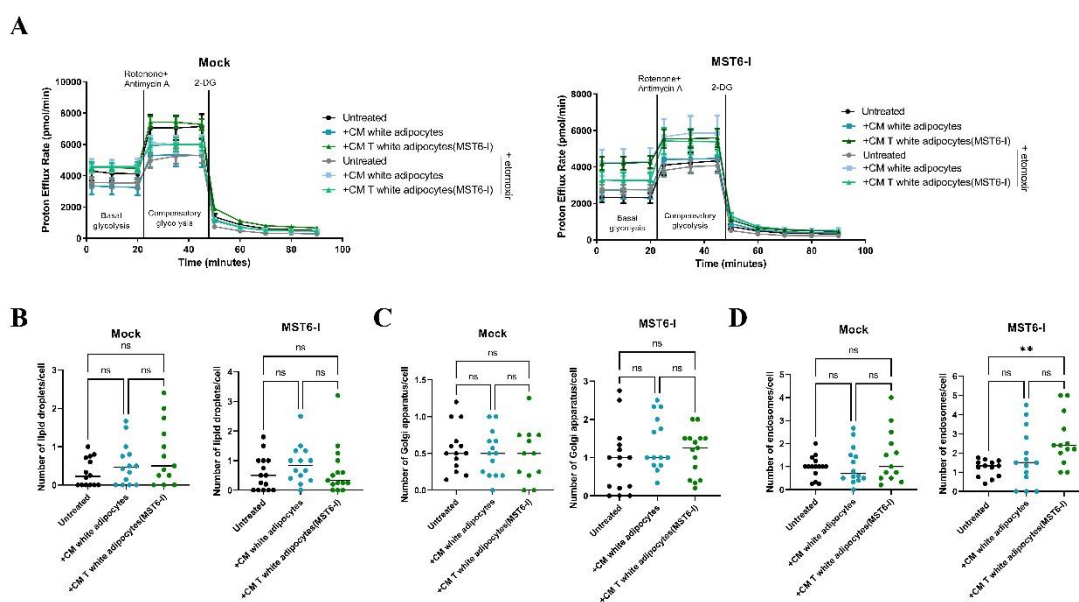


**Supplementary Figure 1.** Secretome and EVs derived from STn-positive gastric cancer cells induce metabolic reprogramming in 3T3-L1 adipocytes. (A) Western blot analysis of the STn antigen in SNU-16 and MKN74 cells. One biological replicate was performed. (B) RT-qPCR analysis of *Ucp1* expression in adipocytes treated with the secretome/conditioned medium (CM) of gastric cancer cells that express (SNU-16) or do not express (MKN74) the STn antigen. Results are shown as mean  $\pm$  SEM. One-way ANOVA was used for statistical analysis (C) RT-qPCR analysis of *Ucp1* (left panel) and *Prdm16* (right panel) expression in adipocytes exposed to 5  $\mu$ g of Mock and MST6-I EVs at different time points (1 hour, 3 hours, 6 hours, and 24 hours). Results are shown as mean  $\pm$  SEM. One-way ANOVA was used for statistical analysis; unless otherwise indicated, differences were not significant (ns). Biological replicates are represented as individual data points. ns: p-value > 0.05; \*: p-value  $\leq$  0.05; \*\*: p-value  $\leq$  0.01; \*\*\*: p-value  $\leq$  0.001.



**Supplementary Figure 2.** Pathway analysis of the metabolic reprogramming of gastric cancer cells after treatment with the adipocyte-derived secretome. (A) Schematic representation of the experimental workflow illustrating the generation of transdifferentiated (beige-like) adipocytes by treating cells with secretome/conditioned medium (CM) from Mock (TWAT(Mock)) or MST6-I (TWAT(MST6-I)) cells. Created with BioRender.com. (B) Partial least squares-discriminant analysis (PLS-DA) score plots of untreated Mock and MST6-I cells (ESI+ mode, Pareto scaling). Significant metabolic features were identified with  $VIP > 1$  and  $|p(\text{corr})| > 0.85$ . (C) PLS-DA score plots of Mock and MST6-I cells treated with secretome/CM from different adipocyte

types compared with untreated cells (ESI+ mode, Pareto scaling). Significant metabolic features were identified with  $VIP > 1$  and  $|p(\text{corr})| > 0.85$ . (D) Pathway enrichment analysis indicating pathway impact (circle size) and statistical significance (color intensity), with more intense red indicating a lower p-value.



**Supplementary Figure 3.** The adipocyte-derived secretome induces a metabolic shift and organelle remodeling in STn-positive gastric cancer cells. (A) Seahorse analysis of the Proton Efflux Rate (PER) in Mock (left panel) and MST6-I (right panel) cells, untreated or treated with secretome/conditioned medium (CM) from white and transdifferentiated/beige-like adipocytes (TWAT(MST6-I)), with or without etomoxir. (B-D) Quantification of the number of lipid droplets (B), Golgi apparatus (C), and endosomes (D) per cell in Mock and MST6-I cells after treatment with secretome/CM from white or transdifferentiated/beige-like (TWAT(MST6-I)) adipocytes, based on TEM imaging. Results are shown as mean  $\pm$  SEM, with statistical significance determined by One-way ANOVA. ns: p-value  $> 0.05$ ; \*\*: p-value  $\leq 0.01$ .