Supporting Information

Type II Epithelial-mesenchymal transition upregulates protein N-glycosylation to maintain proteostasis and extracellular matrix production

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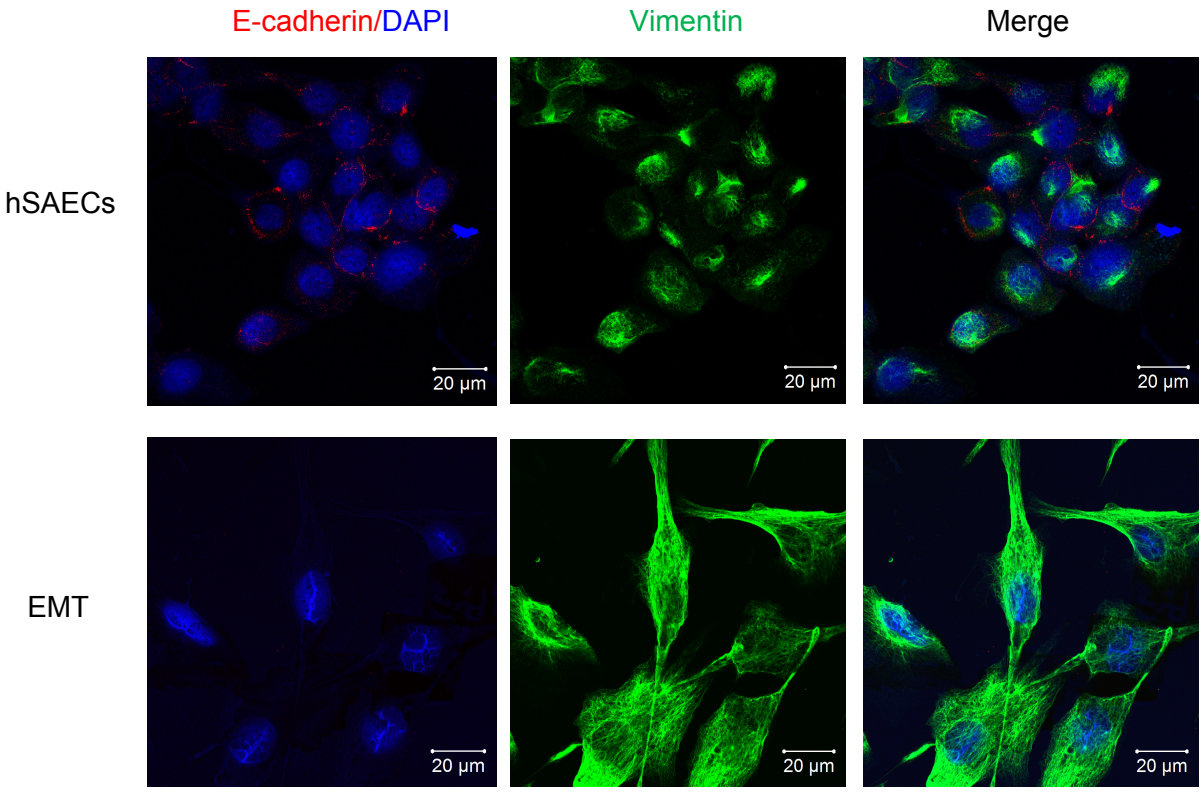
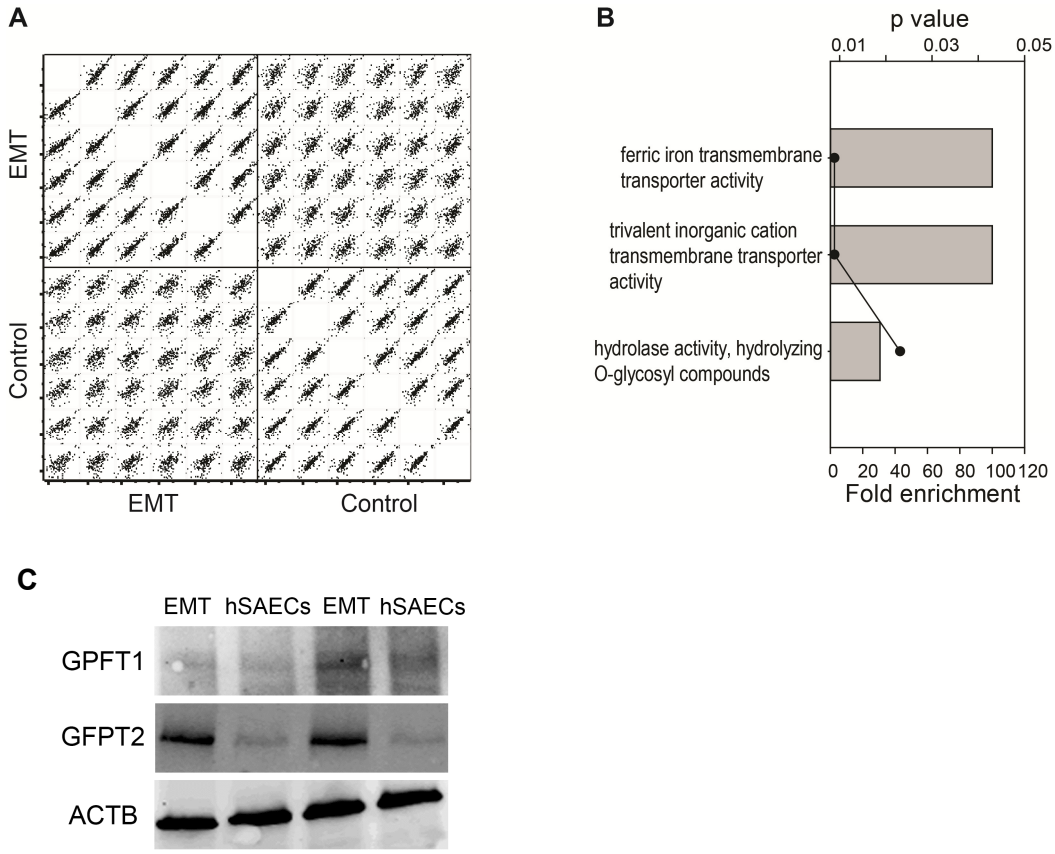
Supporting component	Caption
Supplemental Table S1	The primers for RT-PCR
Supplemental table S2	The parameters for LC-SRM-MS analysis.
Supplemental table S3	The parameters for LC-MS/MS label-free quantification and MaxQuant analysis.
Supplemental table S4	2D annotation enrichment analysis of transcriptome and proteome data
Supplemental table S5	N-glycosylated peptides identified and quantified in hSAECs and EMT-hSAECs
Supplemental table S6	The proteins identified from the secretome of hSAECs and EMT-hSAECs in the presence or absence of tunicamycin.
Supplemental table S7	The proteins identified from the secretome of hSAECs and EMT-hSAECs in the presence or absence of STF-083010.
Supplemental Figure S1	Immunostaining of markers of epithelial cells and mesenchymal cells
Supplemental Figure S2	TGFβ induced EMT alters protein N-glycosylation.
Supplemental Figure S3	Label-free quantification of the secretome of hSAECs and EMT-hSAECs in the presence or absence of tunicamycin.
Supplemental Figure S4	The effect of GlcNAc supplement on the EMT process and secretion of ECM.
Supplemental figure S5	Label-free quantification of the secretome of hSAECs and EMT-hSAECs in the presence or absence of STF-083010.
Supplemental Figure S6	Activation of UPR-XBP1 and HBP during the course of EMT.
Supplemental Figure S7	The effect of XBP1 splicing inhibitor on the EMT process.
Supplemental Figure S8	The effect of Brefeldin A (BFA) on the secretion of ECMs and the turnover of fibronectin

Supplemental Figure S1



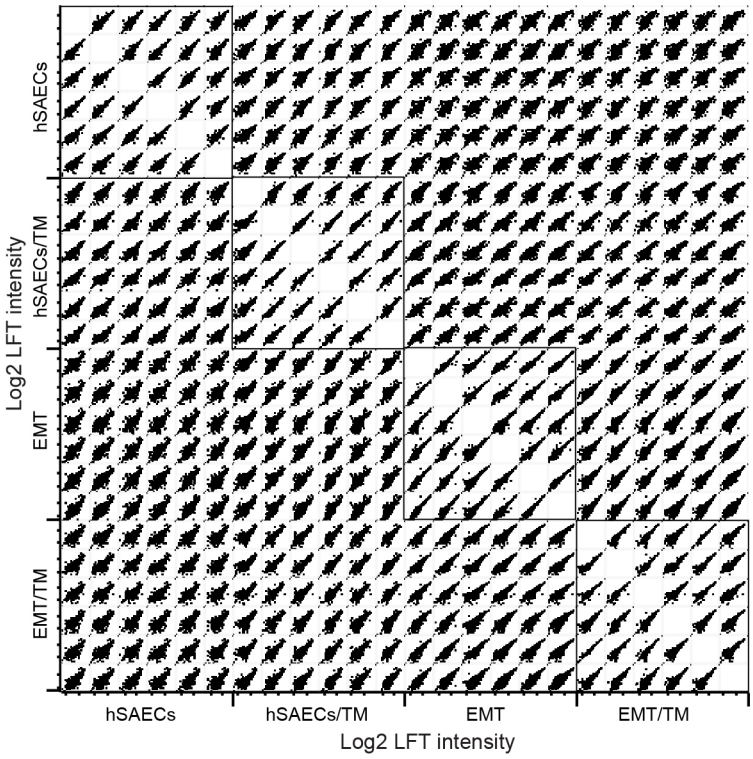
Supplemental Figure S1. Immunostaining of markers of epithelial cells and mesenchymal cells. The fixed hSAECs (upper panel) and EMT-hSAECs (lower panel) were stained with DAPI for nuclei (blue), E-cadherin (red) and vimentin (green) (magnify x63).

Supplemental Figure S2.



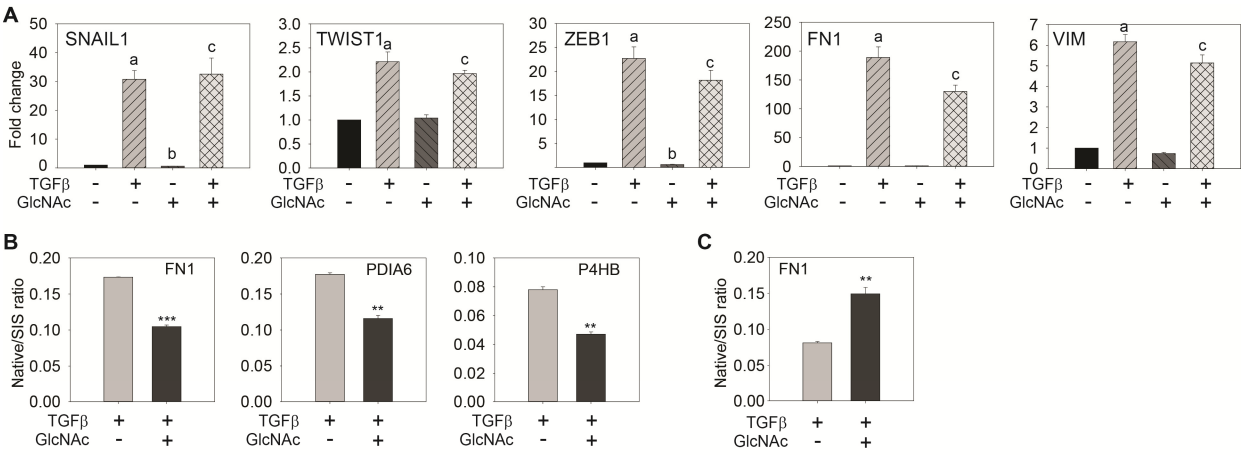
Supplemental Figure S2. TGFβ induced EMT alters protein N-glycosylation. The hSAECs were treated with TGFβ for 14 days. (**A**) Multiple scatter plots of the MS intensity of N-glycosylated peptides identified from EMT-hSAECs and control SAECs (six replicates for each sample). (**B**) GO molecular function enrichment analysis for the proteins which N-glycosylation levels were down-regulated by EMT (P value < 0.05 with Bonferroni correction for multiple testing). Each annotation is displayed by fold enrichment (bar) and p-value (scatter plot). (C) Western blot of GFPT1 and GFPT2 in the cell lysate of hSAECs and EMT-hSAECs.

Supplemental Figure S3



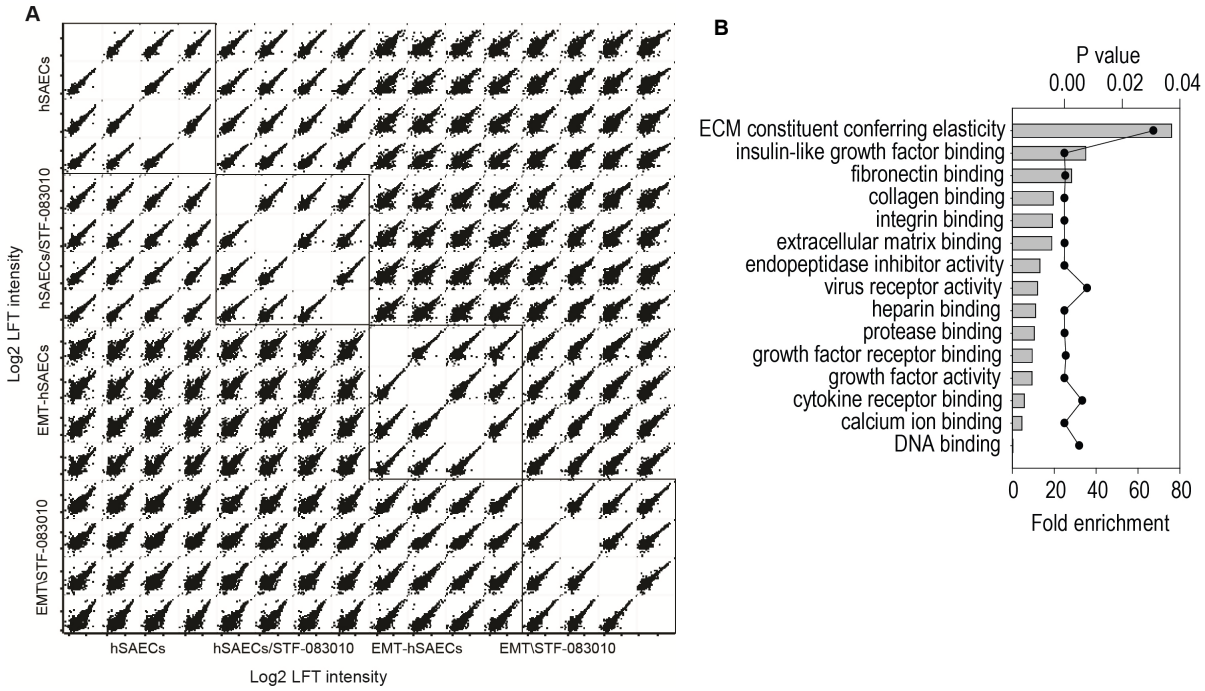
Supplemental Figure S3. Label-free quantification of the secretome of hSAECs and EMT-hSAECs in the presence or absence of tunicamycin. Multiple scatter plots of the LFQ intensity of the proteins quantified in each biological and technical replicates.

Supplemental Figure S4



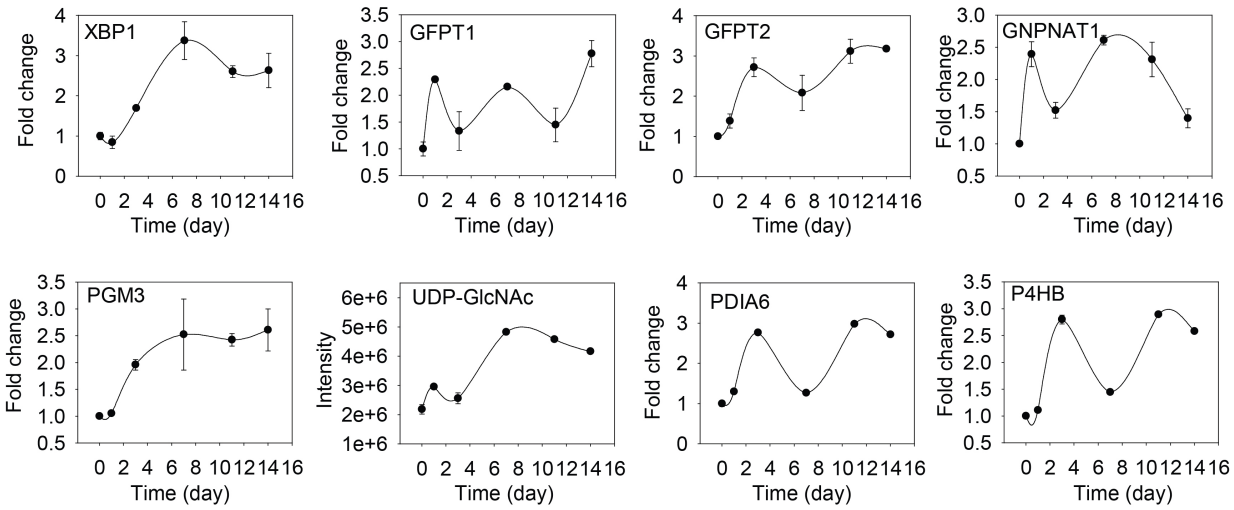
Supplemental Figure S4. The effect of GlcNAc supplement on the EMT process and secretion of ECM. (**A**) RT-PCR analysis of gene expression of EMT transcription regulators (SNAIL1, TWIST1, and ZEB1) and EMT markers (FN1 and VIM). P-value <0.05 in the following comparison: a, EMT vs hSAECs; b, hSAECs/GlcNAc vs. hSAECs; and c, EMT/GlcNAc vs. EMT. (**B**) The protein expression of FN1, PDIA6, and P4HB in the cell lysate of EMT-hSAECs ± GlcNAc. (**C**) The abundance of FN1 in the cell culture medium of EMT-hSAECs ± GlcNAc. Error bar, standard error. **, p-value of t test is below 0.05; ***, p-value of t test is below 0.001.

Supplemental Figure S5



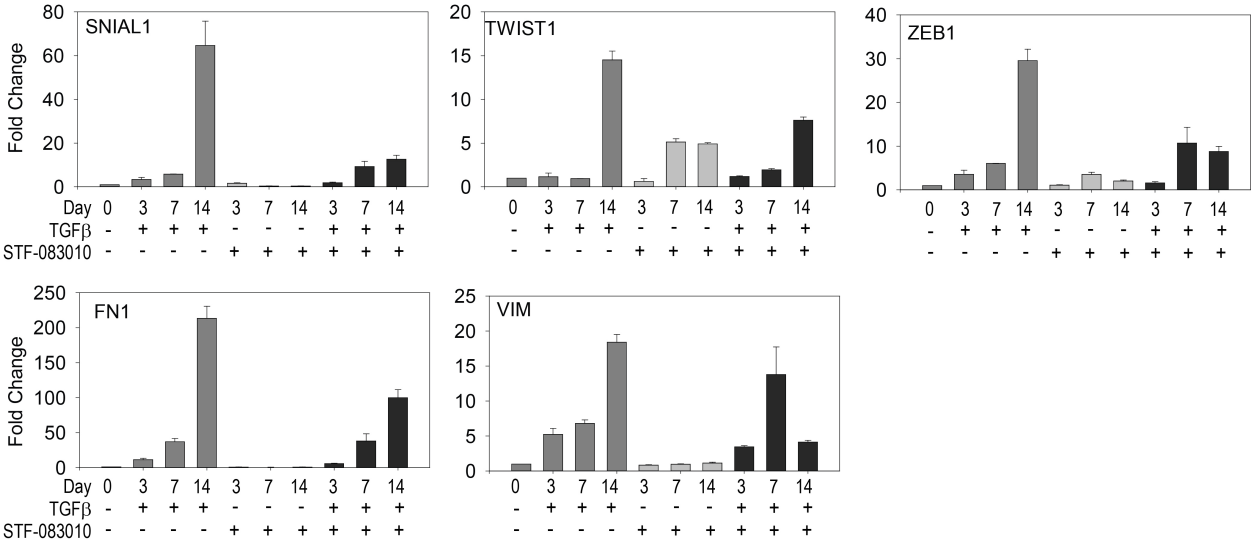
Supplemental Figure S5. Label-free quantification of the secretome of hSAECs and EMT-hSAECs in the presence or absence of STF-083010. (A) Multiple scatter plots of the LFQ intensity of the proteins quantified in each biological and technical replicates. (B) GO molecular function enrichment analysis of proteins which secretion was up-regulated by EMT, but down-regulated by STF-083010 (Cluster 3 in the Figure 5D).

Supplemental Figure S6



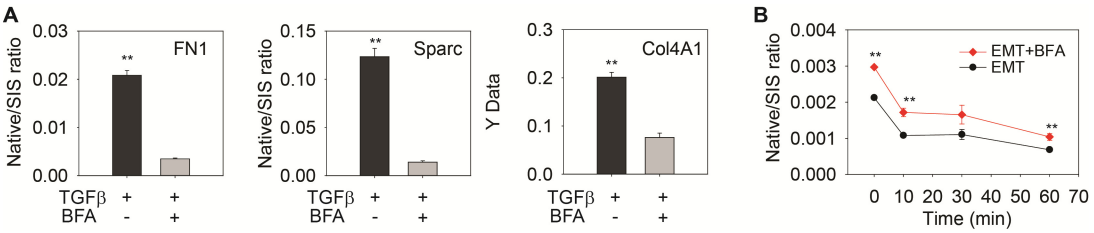
Supplemental Figure S6. Activation of UPR-XBP1 and HBP during the course of EMT. hSAECs were treated with TGFβ for 0, 1, 3, 7, 11, and 14 d. The protein expression of GFPT1, GFPT2, GNPNAT1, PGM3, PDIA6, P4HB, and XPB1 were measured by SID-SRM-MS. The intracellular level of UDP-GlcNAc during this time course was measured by SRM-MS. Error bar, standard error.

Supplemental Figure S7



Supplemental Figure S7. The effect of XBP1 splicing inhibitor on the EMT process. RT-PCR analysis of EMT transcription regulators (SNAIL1, TWIST1, and ZEB1) and EMT markers (FN1 and VIM).

Supplemental Figure S8



Supplemental Figure S8. The effect of Brefeldin A (BFA) on the secretion of ECMs and the turnover of fibronectin. (**A**) The abundance of ECMs (FN1, Sparc, and Col4A) in the cell culture medium of EMT-hSAECs ± BFA. (**B**) The turnover of FN1 in EMT-hSAECs treated with cycloheximide in the presence or absence of BFA. Error bar, standard error. **, p-value of t test is below 0.05; ***, p-value of t test is below 0.001.