Supplemental material

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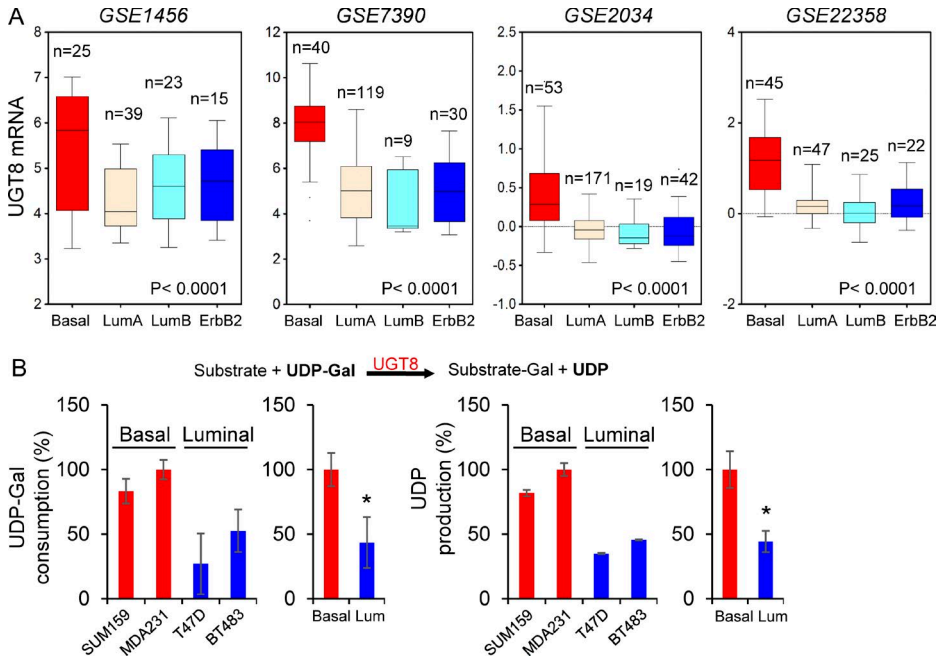

Figure S1. **Expression and activity of UGT8 is up-regulated in BLBC. (A)** Box plots indicated *UGT8* mRNA expression in different subtypes of breast cancer from multiple gene expression datasets (GSE1456, GSE7390, GSE2034, and GSE22358). Comparisons were analyzed by one-way ANOVA. **(B)** In vitro activity assay of UGT8 was performed by mixing UDP-galactose, substrate, and lysate of breast cancer cell lines that contained two luminal and two BLBC cell lines. UDP-galactose consumption and UDP production were tested by HPLC system. The percentage of UDP-galactose consumption and UDP production was shown in the bar graph (mean ± SD in three separate experiments). *, $P < 0.05$ by Student's *t* test.

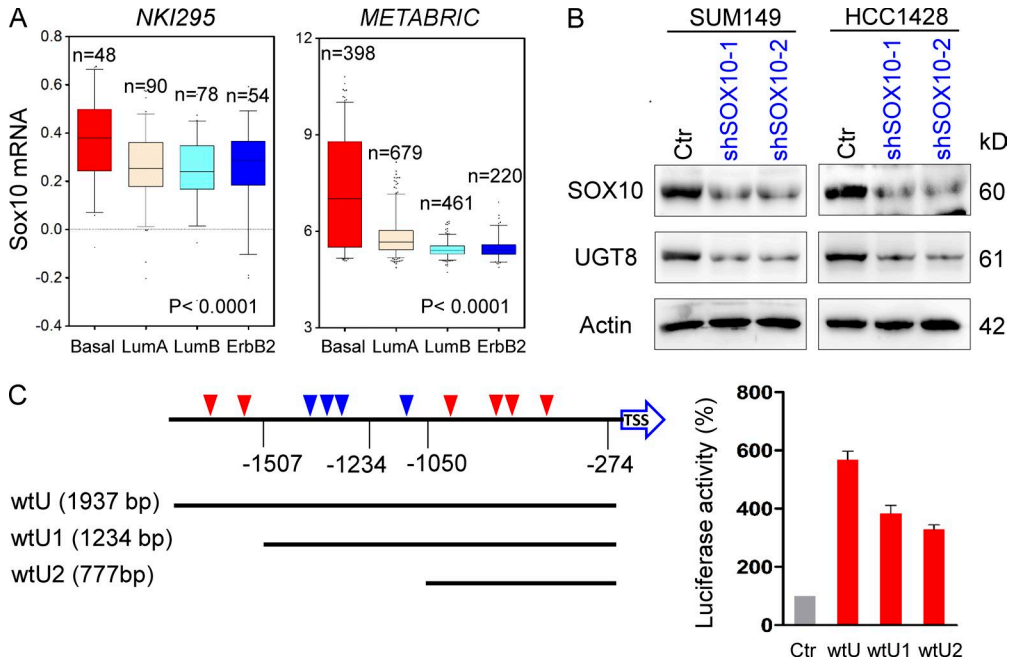

Figure S2. **UGT8 is a target of Sox10. (A)** Box plots indicated *Sox10* mRNA expression in different subtypes of breast cancer from NKI295 and METABRIC datasets. Comparisons were analyzed by one-way ANOVA. **(B)** Expression of UGT8 and Sox10 was analyzed by Western blotting in SUM149 and HCC1428 cells with empty vector or knockdown of Sox10 expression. **(C)** Schematic diagram showing 10 positions of potential Sox10-binding motifs in UGT8 promoter. Deletion constructs of UGT8 promoter luciferase were shown (left). UGT8 promoter luciferase constructs (wtU, wtU1, and wtU2) were coexpressed with empty vector or Sox10-expressing vector in HEK-293T cells. Luciferase activities were determined and normalized (mean ± SD in three separate experiments; right).

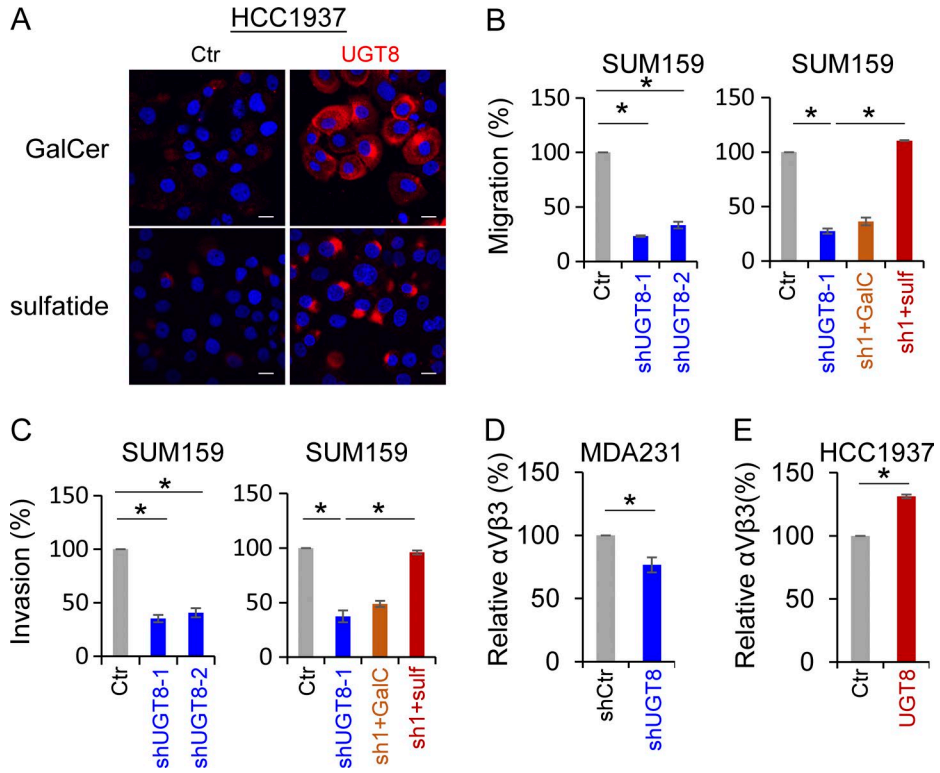

Figure S3. **UGT8 promotes GalCer and sulfatide production and migration and invasion of breast cancer cells. (A)** Expression of GalCer and sulfatide was analyzed by immunofluorescent staining in HCC1937 cells with stable empty vector or UGT8 expression. Nuclei were visualized with DAPI (blue). Bars, 20 µm. **(B and C)** Migratory ability (B) and invasiveness (C) of SUM159 cells with stable empty vector or knockdown of UGT8 expression (left) as well as shUGT8-expressing SUM159 cells treated with or without GalCer (2 µM) or sulfatide (2 µM; right) were analyzed. The percentage of migratory and invasive cells was shown in the bar graph (mean ± SD in three separate experiments). **(D and E)** The level of αVβ3 was analyzed by flow cytometry in MDA-MB231 cells with stable empty vector or knockdown of UGT8 expression (D) as well as HCC1937 cells with stable empty vector or UGT8 expression (E). Data are presented as a percentage of the control (mean ± SD in three separate experiments). *, $P < 0.05$ by Student's *t* test.

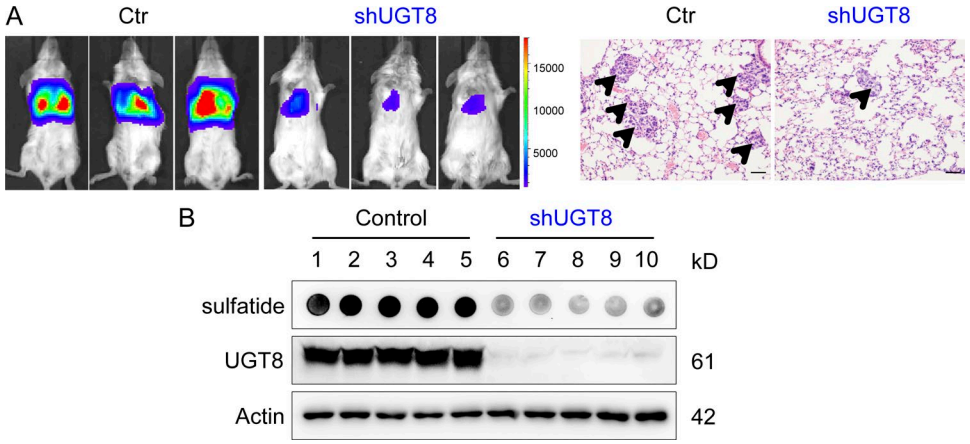

Figure S4. **Knockdown of UGT8 expression inhibits metastasis of breast cancer cells. (A)** MDA-MB231 cells with stable empty vector or knockdown of UGT8 expression were injected into SCID mice via the tail vein. After 4 wk, the development of lung metastases was monitored using bioluminescence imaging and quantified by measuring photon flux. Three representative mice from each group were shown (left). Lung metastatic nodules were examined in paraffin-embedded sections stained with hematoxylin and eosin (right). Bars, 100 µm. **(B)** Expression of sulfatide and UGT8 was analyzed in metastatic nodules removed from two group mice in A.