Supplementary Materials

The Epithelial to Mesenchymal Transition Promotes Glutamine Independence by Suppressing *GLS2* Expression

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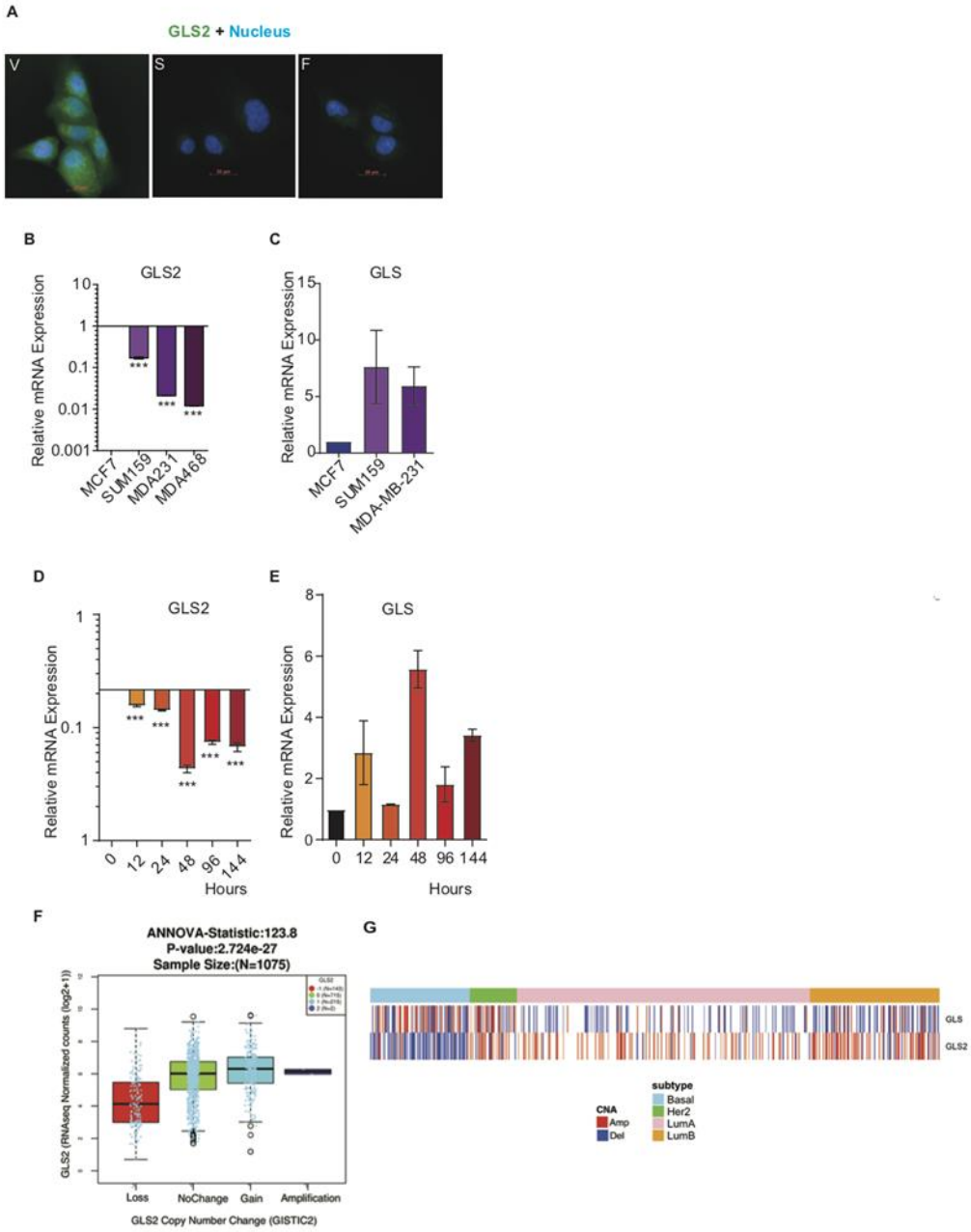


Figure S1. GLS and GLS2 are inversely associated in multiple models of EMT-induced cell lines. (**A**) Representative immunofluorescent images of HMLER-vector (V), HMLER-SNAIL (S), and HMLER-FOXC2 (F) cells stained with human GLS2 antibody (green) co-stained with Dapi (blue). Scale bar: 20

μm. (**B**) GLS2 mRNA expression in MCF7 (n = 3), SUM159 (n = 3), MDA231 (n = 3), and MDA468 (n = 3) cells. (**C**) RT-PCR of GLS mRNA in MCF7, SUM159, and MDA-MB231 (n = 3). (**D**) GLS2 mRNA levels in MCF10A cells treated with 5 ng/mL of TGFβ for 12, 24, 48, 96, and 144 hours (n = 3). (**E**) RT-PCR of GLS mRNA in MCF10A cells treated with 5 ng/mL of TGFβ for 0, 12, 24, 48, 96, and 144 hours (n = 3). (**F**) Copy number change of GLS2 in breast cancer patient samples. Deletion in one copy (-1) of GLS2 gene is observed in 143 patients in the TCGA Breast cancer cohort. (**G**) Amplifications (red, copy number gain, +1 and amplification, +2) and deletions (blue, copy number loss, −1 and deletion, −2) of GLS and GLS2 by PAM50 subtype.The data are reported as means +/− SD; NS indicates $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

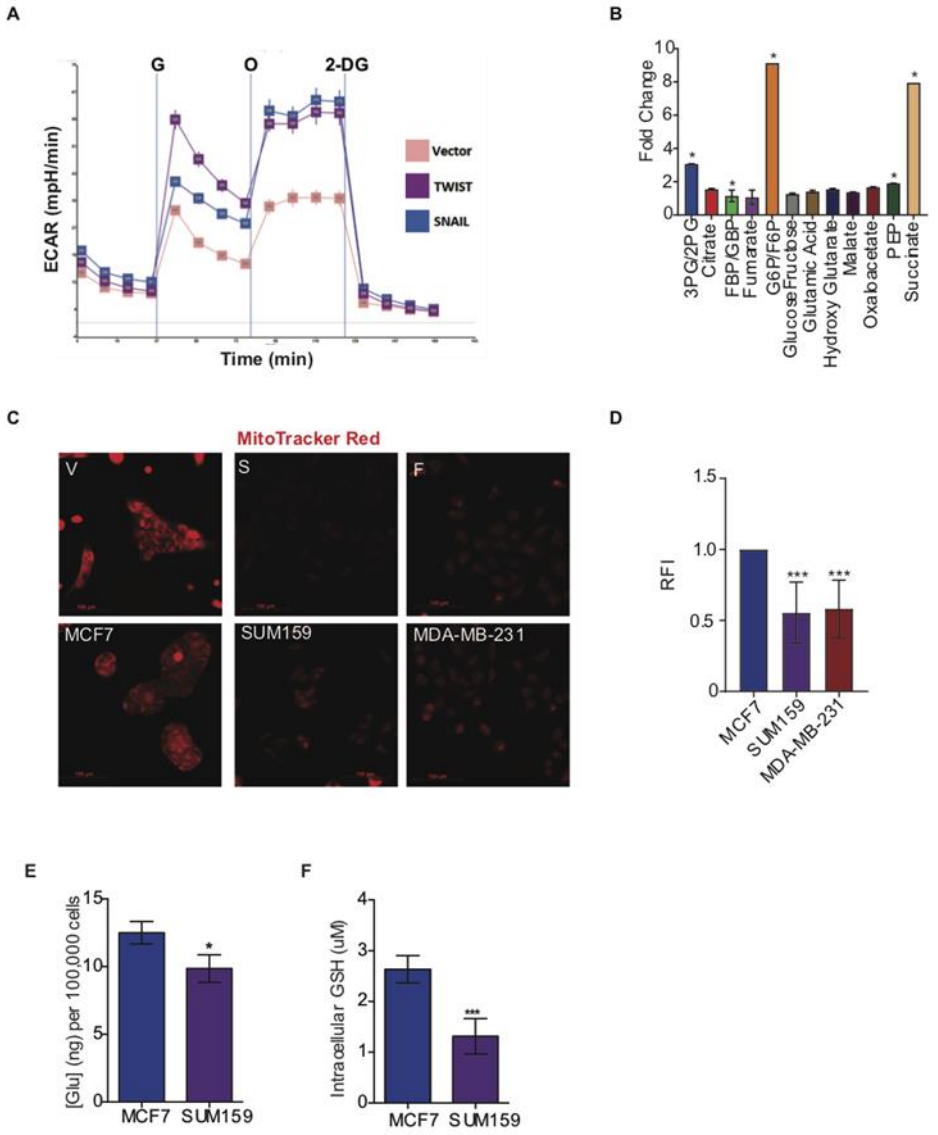


Figure S2. Cells induced to undergo EMT exhibit metabolic reprogramming. (**A**) Extracellular acidification rate (ECAR) over time with the addition of glucose (G), oligomycin (O), and 2-deoxyglucose (2-DG) in HMLE-SNAIL (n = 5) and HMLE-TWIST (n = 5) cells compared to epithelial HMLE-GFP (n = 5) control cells measured using the Seahorse XFe96 Analyzer. (**B**) TCA cycle and glycolysis metabolites were quantified by mass spectrometry in HMLER-FOXC2 (n = 3) and HMLER-vector (n = 4) cells. Plotted is the fold change. (**C**) Representative immunofluorescent images taken at 20× magnification of MitoTracker Red (red) staining of HMLER-vector, HMLER-SNAIL, HMLER-FOXC2, and breast cancer cell lines MCF7, SUM159, and MDA-MB-231. (**D**) Quantification of relative fluorescence intensity (RFI) from MitoTracker Red staining in MCF7 (n = 100), SUM159 (n = 100), and MDA-MB-231 (n = 100) cells calculated with ImageJ software. (**E**) Quantification of intracellular

glutamate in MCF7 (n = 3) and SUM159 (n = 3) cells. (**F**) Quantification of intracellular GSH in MCF7 (n = 5) and SUM159 cells (n = 5). The data are reported as means +/− SD; NS indicates $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

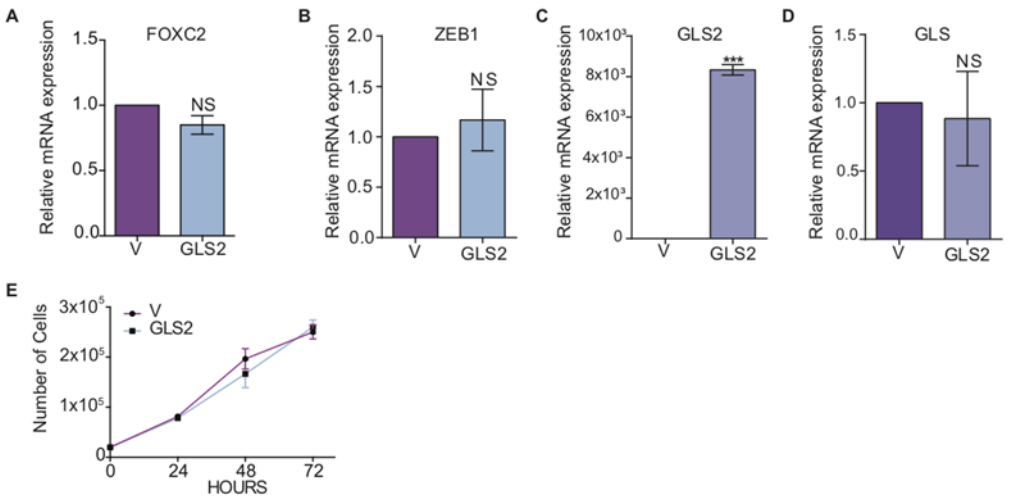
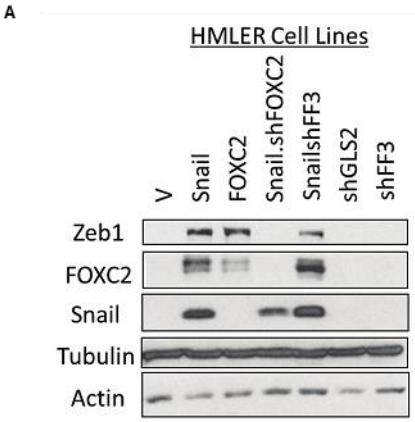
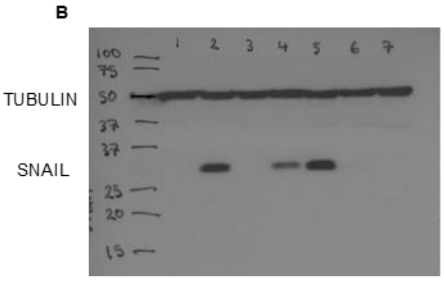


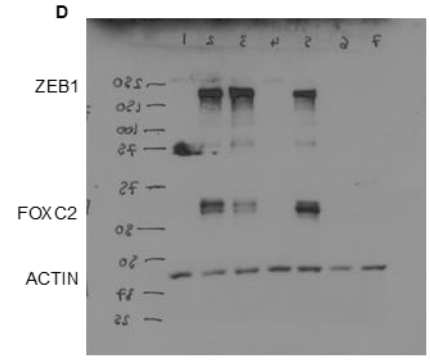
Figure S3. FOXC2 expression does not change after GLS2 over-expression. (**A**)RT-PCR of FOXC2 mRNA in SUM159-vector (V) and SUM159 over-expressing GLS2 (GLS2) (n = 3). (**B**) RT-PCR analysis of ZEB1 mRNA in SUM159-vector (V) and SUM159-GLS2 (GLS2) cells (n = 3). (**C**)RT-PCR analysis of GLS2 mRNA in HMLER-SNAIL-vector (V) and HMLER-SNAIL-GLS2 (GLS2) cells (n = 3). (**D**) RT-PCR analysis of GLS mRNA in HMLER-SNAIL-vector (V) and HMLER-SNAIL-GLS2 (GLS2) cells (n = 3). (**E**) Proliferation measured by number of cells counted at 0, 24, 48, 72 hours (n = 3). The data are reported as mean +/− SD (NS $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).





C

LANE	SNAIL	TUBULIN	RATIO
1	15.61	163.63	0.10
2	146.59	158.73	0.92
3	10.99	158.69	0.07
4	86.36	168.72	0.51
5	163.66	165.19	0.99
6	11.45	156.52	0.07
7	6.61	158.36	0.04



E

LANE	FOXC2	ACTIN	RATIO
1	1.17	36.88	0.03
2	63.39	19.84	3.20
3	25.89	30.35	0.85
4	0.83	33.91	0.02
5	75.86	38.45	1.97
6	-0.04	17.06	0.00
7	0.13	32.21	0.00

F

LANE	ZEB1	ACTIN	RATIO
1	-0.83	36.88	-0.02
2	78.80	19.84	3.97
3	85.01	30.35	2.80
4	-0.82	33.91	-0.02
5	62.00	38.45	1.61
6	-0.77	17.06	-0.04
7	-0.82	32.21	-0.03

Figure S4. Protein expression of Zeb1, FOXC2, Snail, Tubulin, and Actin in HMLER cell lines. (**A**) Western blot of HMLER-V, HMLER-Snail, HMLER-FOXC2, HMLER-SNAIL-shFOXC2, HMLER-SNAIL-shFF3 (vector control), HMLERshGLS2, and HMLERshFF3 (vector control) (**B**) Scan of full blot with Tubulin and Snail proteins (**C**) Quantification of relative Snail and tubulin protein levels and the protein: loading control ratio analyzed by ImageJ. (**D**) Scan of full blot with ZEB1, FOXC2 and Actin proteins (**E**) Quantification of relative FOXC2 and Actin protein levels and the protein: loading control ratio analyzed by ImageJ. (**F**) Quantification of relative ZEB1 and Actin protein levels and the protein: loading control ratio analyzed by ImageJ.

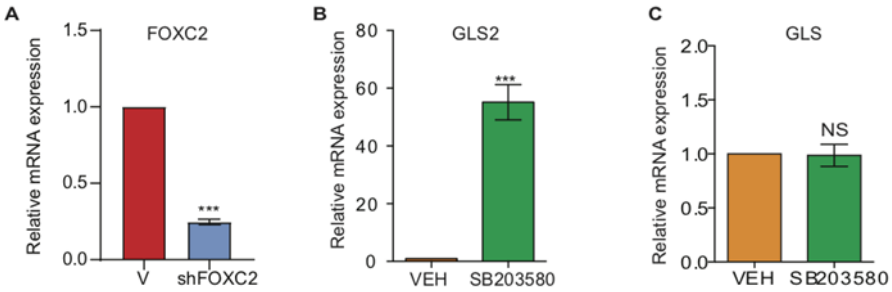


Figure S5. Expression of GLS2 inversely correlated with FOXC2. (**A**) RT-PCR analysis of FOXC2 mRNA expression in HMLER-SNAIL-vector (V) and HMLER-SNAILshFOXC2 (shFOXC2) cells (n = 3). (**B**) RT-PCR analysis of GLS2 mRNA expression in HMLER-FOXC2 cells treated with vehicle (VEH) or with 20 μM SB203580 for 24 hours (n = 3). (**C**) RT-PCR analysis of GLS mRNA expression in HMLER-FOXC2 cells with vehicle and SB203580 treatment for 24 hours (n = 3). The data are reported as mean +/− SD (NS $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).