

A genetic variant in *SLC30A2* causes breast dysfunction during lactation by inducing ER stress, oxidative stress and epithelial barrier defects

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SUPPLEMENTARY INFORMATION

Supplementary Table S1. Macronutrient concentrations in breast milk from women harboring wild-type ZnT2 (T²⁸⁸) or the ZnT2 variant (S²⁸⁸).

Macronutrient (protein, lactose and fat) concentrations were measured in breast milk from women harboring wild-type ZnT2 (T²⁸⁸) or the ZnT2 variant with a threonine to serine substitution (S²⁸⁸). Protein (g/L) was measured by Dumas analysis, lactose (g/dL) was measured using Lactose Assay kit, and fat (%) was determined by creatatocrit. Data represent mean concentration \pm SD from n=4-5 samples/group; p-value obtained by Student's t-test.

Supplementary Figure S1. Confirmation of ER-ZAPCY1 sensor localization in cultured MECs.

Representative confocal images of the ER Zn sensor, ER-ZAPCY1 (green) and the ER marker, calnexin (red). Merged images (yellow) illustrate co-localization of ER-ZAPCY1 and calnexin in MECs. Nuclei were counterstained with DAPI (blue). Note co-localization of ER-ZAPCY1 and calnexin, validating this ER sensor in our cell model.

Supplementary Figure S2. Gel and immunoblots relating to Figure 1 are shown.

- (a) Full-length zymography gel of MMP-2 activity.
- (b) Membrane was cut in half prior to immunoblot to probe for Mucin-4 (top) and 4-HNE (bottom).
- (c) Membrane was cut in half prior to immunoblot to probe for Endoplasmin.
- (d) Replicate gel stained with Coomassie Blue.

Supplementary Figure S3. Immunoblots relating to Figure 2 are shown.

- (a) Full-length blot probed for Endoplasmin.
- (b) Membrane was cut in half and re-probed for β -actin.

Supplementary Figure S4. Immunoblots relating to Figure 3 are shown.

- (a) Full-length blot probed for p-STAT3.
- (b) Membrane was cut in half to re-probe for total STAT3 (top) and β -actin (bottom).

Supplementary Figure S5. Immunoblots relating to Figure 4 are shown.

(a) Full-length blot probed for ZO-1.

(b) Membrane stained with Ponceau.

Supplementary Figure S6. Immunoblots relating to Figure 5 are shown.

(a) Full-length blot probed for p-serine.

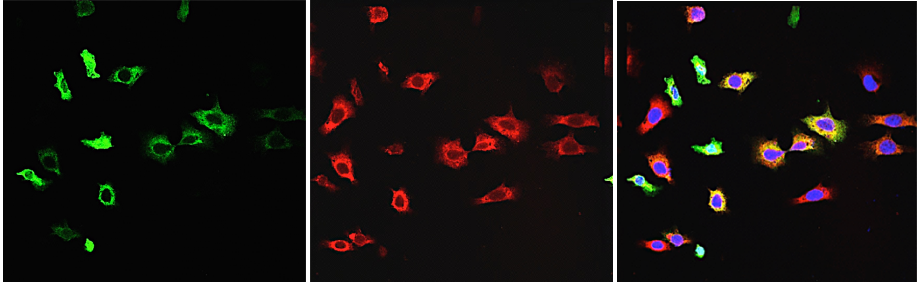
(b) Full-length blot probed for HA.

Supplementary Table S1

Macronutrients**	T ²⁸⁸	S ²⁸⁸	p-value
Protein (g/L)	17.2 ± 3.2	21.2 ± 4.2	0.1232
Lactose (g/dL)	7.1 ± 1.0	7.2 ± 2.4	0.9215
Fat (%)	8.1 ± 3.3	7.7 ± 1.8	0.8486

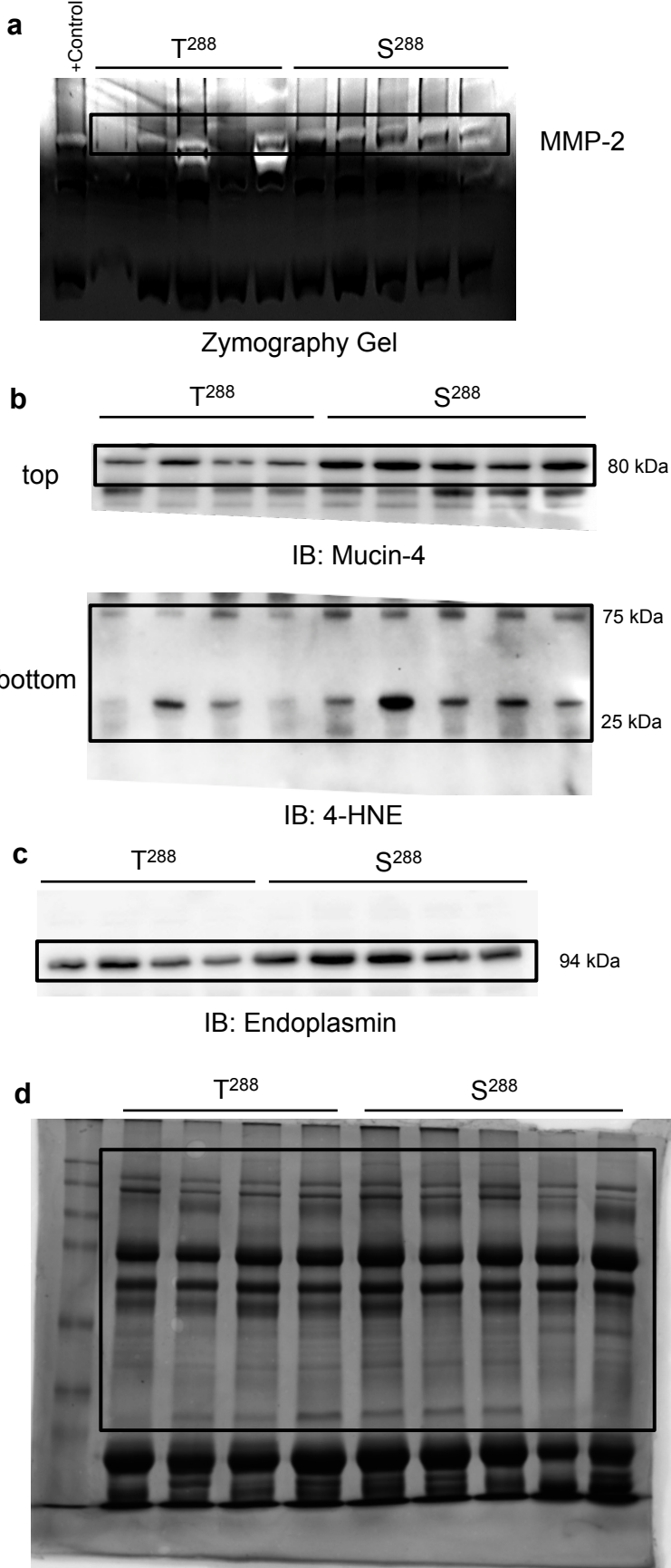
**Units are different for each macronutrient.

Supplementary Figure S1

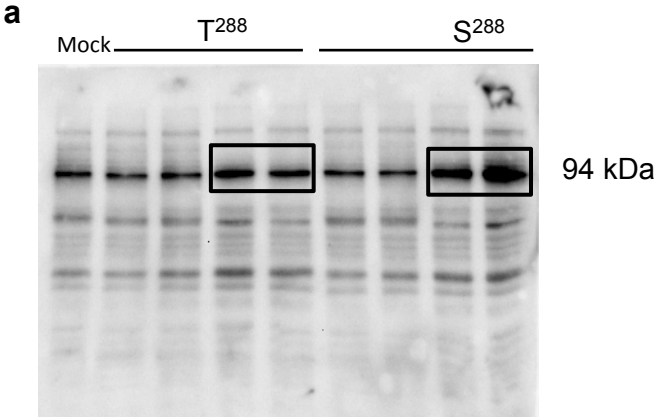


ER-ZapCY1 / Calnexin / DAPI / Merge

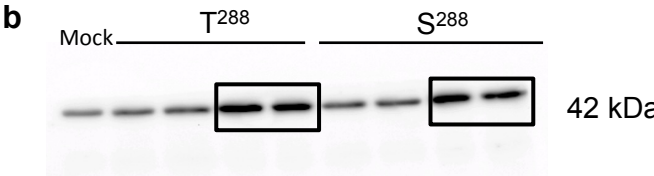
Supplementary Figure S2



Supplementary Figure S3

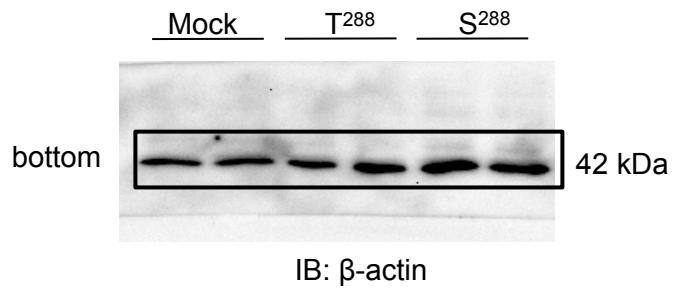
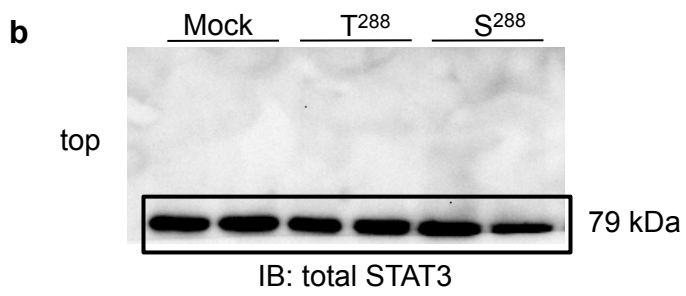
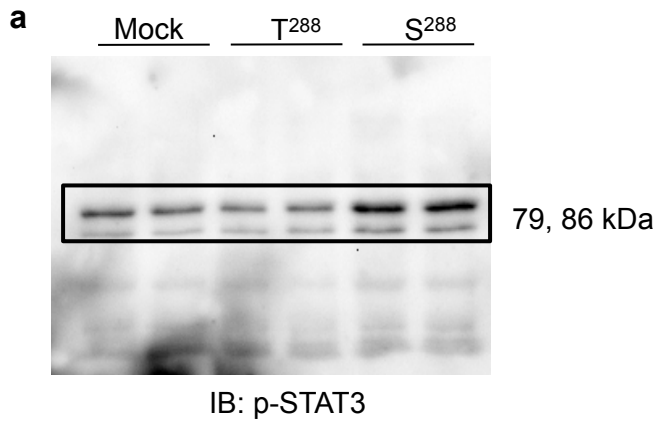


IB: Endoplasmic reticulum chaperone protein

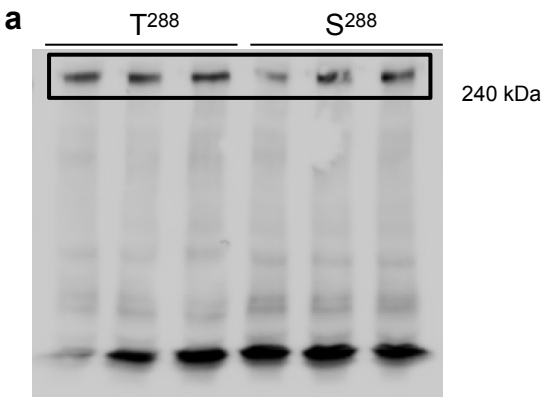


IB: β -actin

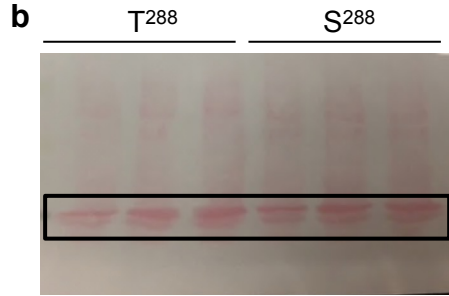
Supplementary Figure S4



Supplementary Figure S5

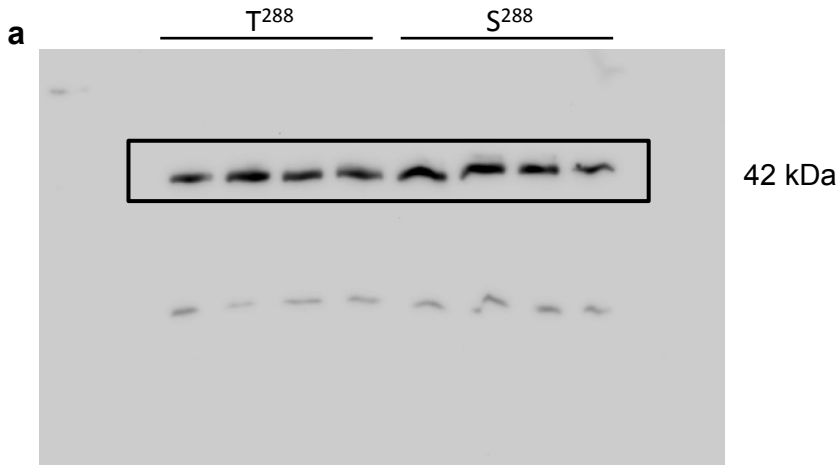


IB: ZO-1

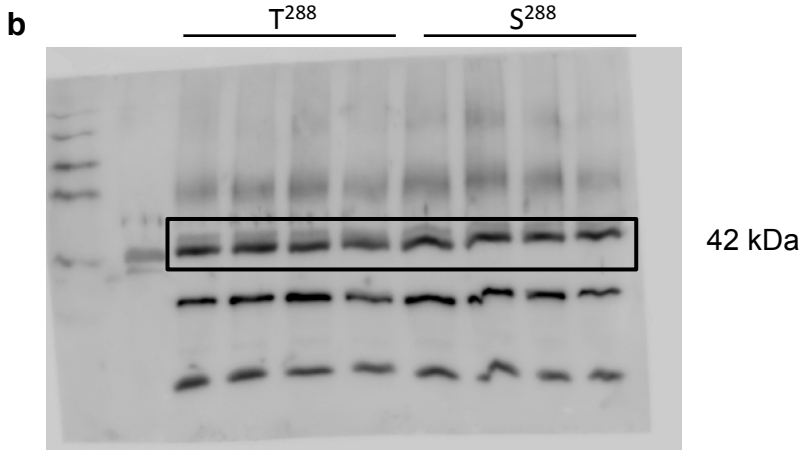


Ponceau Stain

Supplementary Figure S6



IB: p-serine



IB: HA