Supplementary Information File

Ferreira JN, et al. 2024

Table S1. List of primary and secondary antibodies and respective dilutions. AF - Alexa Fluor™, HRP - horseradish peroxidase-conjugated

Antibody	Source	Catalog No.	Dilution
Rabbit monoclonal anti-AQP-5 IgG	Abcam	Ab92320	1:100
Rabbit polyclonal anti-KRT5 IgG	Abcam	Ab53121	1:100
Rabbit monoclonal anti-KRT14 IgG	Abcam	Ab181595	1:100
Mouse monoclonal anti-KRT19 IgG	Novus biologicals	NBP142238	1:100
Rabbit polyclonal anti-NKCC1 IgG	Abcam	Ab59791	1:100
Mouse monoclonal anti-GAPDH IgG	Santa Cruz	sc-365062	1:100
AF 594 goat anti-rabbit IgG	Abcam	Ab150080	1:200
AF 488 goat anti-rabbit IgG	Abcam	Ab150077	1:200
AF 488 goat anti-mouse IgG	Abcam	Ab150113	1:200
Goat anti-rabbit IgG, (H+L) HRP	Invitrogen	1TFS-AB-626120	1:3000
Goat anti-mouse IgG, (H+L) HRP	Invitrogen	1TFS-AB-626520	1:2000

Table S2. List of media, solutions and reagents used for LG primary cell isolation and organoid culture. Please see reference 14 for more details.

Washing buffer	Final concentration	Volume (mL)
1XPBS	Not applicable	86.67
1XPBSPenicillin/Streptomycin (100%)	10%v/v	10
Bovine resum albumin (30%)	1%v/v	3.33
Total		100

Enzymatic dissociation buffer	Final concentration	Volume (mL)
1XPBS	Not applicable	1.763
1XPBSPenicillin/Streptomycin (100%)	1%v/v	0.020
Bovine resum albumin (30%)	1%v/v	0.067
Calcium chloride solution (50 mM)	1.25 mM	0.050
Collagenase II (40 mg/mL)	1 mg/mL	0.050
Hyaluronidase	1 mg/mL	0.050
Total		2

Basal media	Final concentration	Volume (mL)
DMEM/F12	Not applicable	98
L-Glutamine (100 mM)	1 mM	1
Penicillin/Streptomycin (100%)	1% v/v	1
Total		100

Expansion media (EM)	Final concentration	Volume (mL)
Basal media	Not applicable	94.85
Fetal bovine serum (100%)	5%v/v	5
EGF (20 µg/mL)	20 ng/mL	0.1
Total		100

Epithelial enrichment media (EEM)	Final concentration	Volume (mL)
Define Keratinocyte SFM	Not applicable	99.80
EGF (20 µg/mL)	20 ng/mL	0.1
FGF-10 (100 µg/mL)	50 ng/mL	0.05
FGF-7 (100 µg/mL)	50 ng/mL	0.05
Total		100

Table S3. Statistical analysis output from Z-score heatmap displayed in Fig. 2E comparing native LG tissue biopsies (NLG) with the LG organoid (LGO) experimental group (n=3/group). * *p*-value < 0.05

Gene	Adjusted *p*-value	Gene	Adjusted *p*-value
BMP4	0.2381	*NFYA**	0.0351
BMPR1A	0.1462	*NLK*	0.5193
BMPR2	0.8510	*NRAS*	0.6973
CD44	0.9681	*PAFAH1B1*	0.8893
CDH1	0.4199	*PCGF6**	0.0209
CTBP1	0.8850	*POU5F1*	0.4099
*CTNND1**	0.0097	*PPARD*	0.4860
*DLX5**	0.0026	*PPP2CA*	0.1836
DVL3	0.8616	*PPP2R1A*	0.9474
*EPAS1**	0.0008	*PPP2R1B*	0.3794
*FGF2**	0.0009	*PPP2R2A*	0.1023
FOXD3	0.6509	*PPP2R5B*	0.9569
FZD1	0.2743	*PPP2R5C*	0.3583
FZD4	0.2950	*PPP2R5E*	0.2591
*FZD7**	0.0125	*PRKCH*	0.0532
*ID2**	0.0037	*PRKCI*	0.4339
*ID4**	0.0059	*RAF1*	0.1154
IL6ST	0.1073	*RHOA*	0.5371
JARID2	0.0959	*SMAD9*	0.0780
KLF4	0.4105	*SMARCAD1*	0.8749
LEF1	0.5548	*SOX2**	0.0331
LIF	0.5794	*STAT3*	0.2041
MAP2K4	0.1699	*UTF1*	0.1274
MAP3K7	0.9328	*VIM*	0.3848
MAPK11	0.7663	*WNT10B*	0.4816
*MAPK14**	0.0499	*WNT3**	0.0405
MEIS1	0.0537	*WNT3A*	0.3986
*MYC**	0.0019	*ZFHX3*	0.1915

Table S4. Statistical analysis output from plotted Z-scores displayed in Fig. 3E comparing the LG organoid (LGO) with the senescence-induced LGO treated with etoposide (LGO+Eto) experimental groups (n=3/group). *p-value < 0.05

Gene	Adjusted p-value
*CASP3**	0.0276
CASP9	0.4140
*SFN**	0.0004
*TP53**	0.0071
*CDC20**	0.0350
*UBE2C**	0.0273
*UBE2D1**	0.0003
*CCNA2**	0.0006
*CCNB1**	0.0085

Table S5. Statistical analysis output from plotted Z-scores displayed in Fig. 4G comparing the senescence-induced etoposide-treated LG organoid (LGO+Eto) with the etoposide-treated LG organoid transfected with Box A plasmid (LGO+Eto+BoxA) experimental groups (n=3/group). *p-value < 0.05

Gene	Adjusted p-value
*HMGB1**	0.00130
*CASP3**	0.04650
CASP9	0.96983
*SFN**	0.00038
*TP53**	0.01698
*TUBA1C**	0.04881
*VIM**	0.00171
*CDC20**	0.01467
*UBE2C**	0.01330
*UBE2D1**	0.00693
*CCNA2**	0.00005
*CCNB1**	0.00156

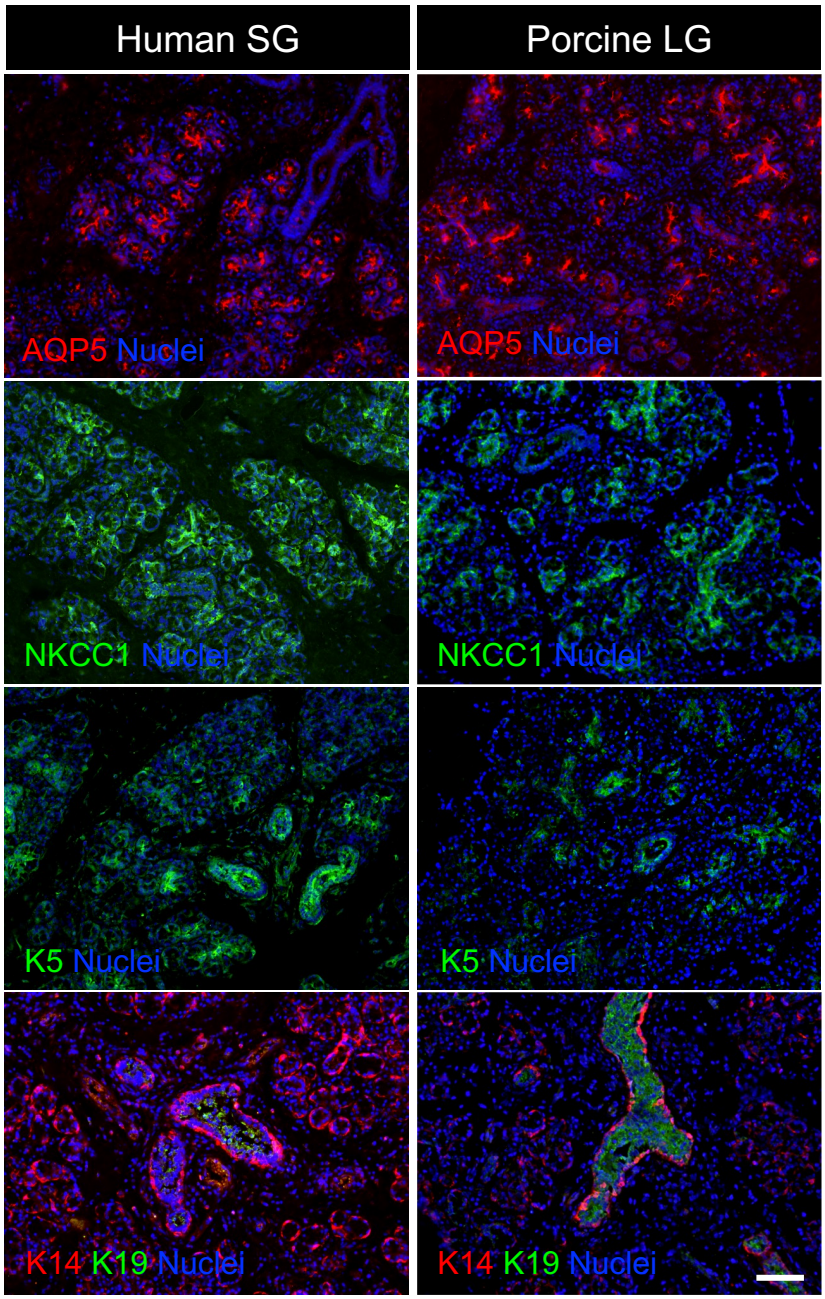

Figure S1. Primary antibody validation and characterization for LG-like protein markers. SG – salivary gland. LG – lacrimal gland. Scale bar: 200 μm.

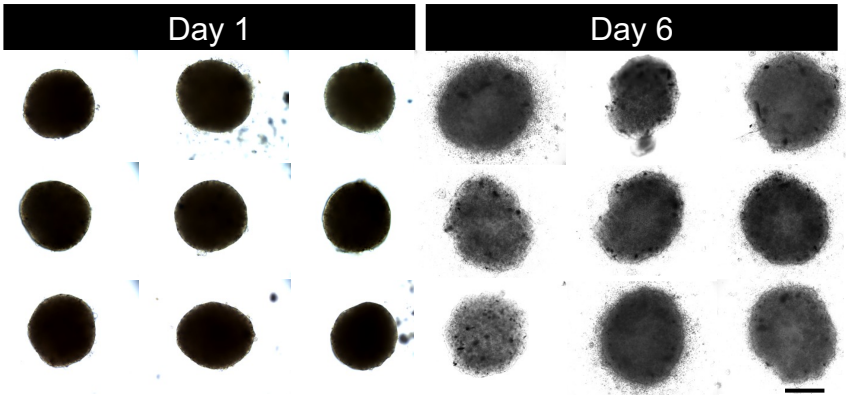

Figure S2. Brightfield micrographs showing consistent biofabrication of LG spheroids by M3DB at 1 and 6 days of culture. Scale bar: 200 μm.

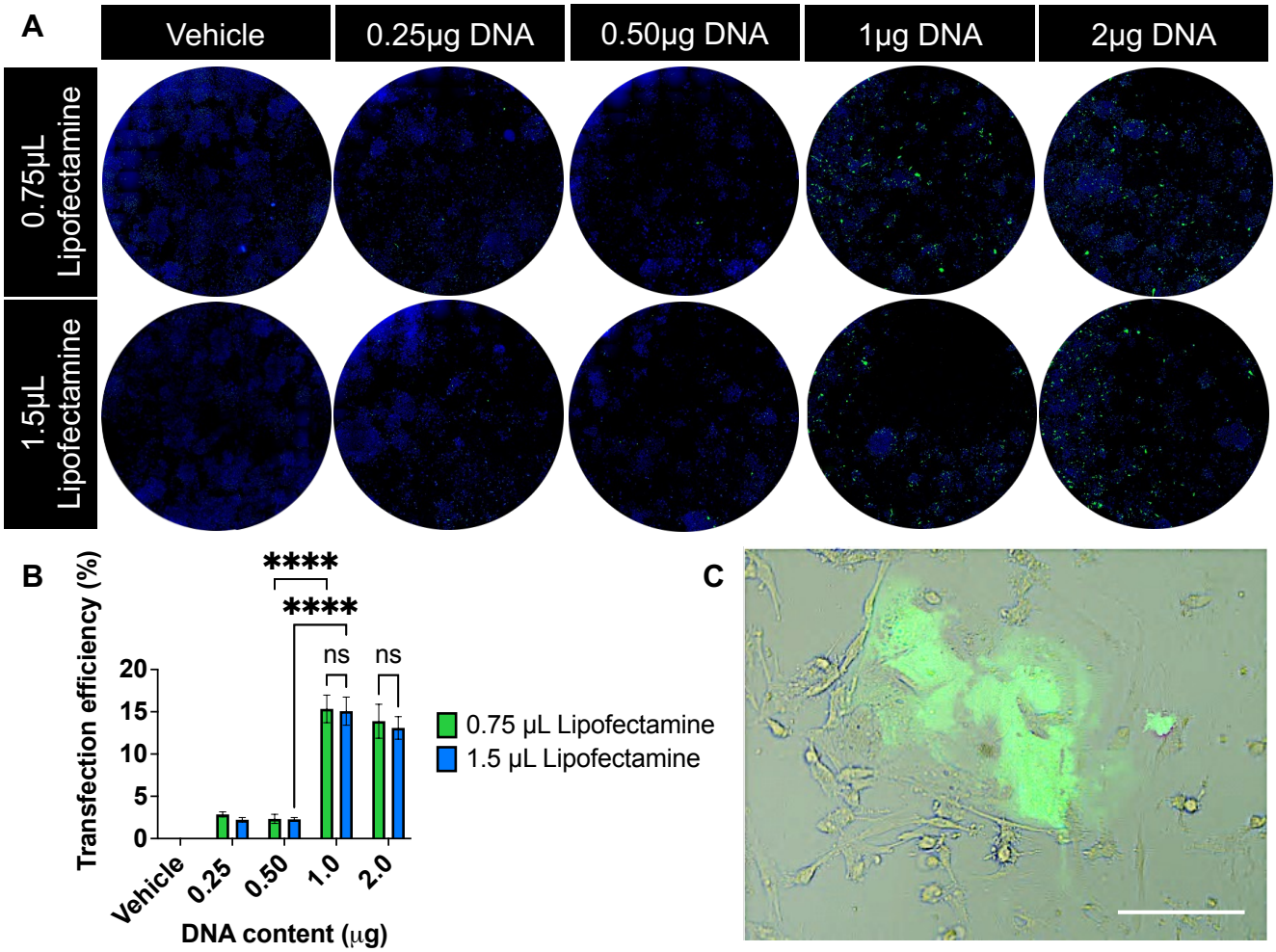

Figure S3. Plasmid transfection efficiency of Box A-GFP plasmid in differentiated LG cells. Epithelial enriched porcine LG cells cultured as a monolayer and were transfected with Box A plasmid at various of DNA content:Lipofectamine ratios. **A** The transfection efficiency was determined by observing GFP signal under a fluorescence microscope. **B** Transfected cells were quantified by automated cytometry using Countess 3 Automated Cell Counter and results were plotted as mean ±S.D. ****p<0.0001 when compared with non-transfected cells or untreated control by a two-way ANOVA (n=3). LG cells treated with only Lipofectamine was recognized as a vehicle control. **C** Representative fluorescence micrograph displaying the Box A-GFP-expressed in epithelial-like LG cells. Scale bar: 100 µm.

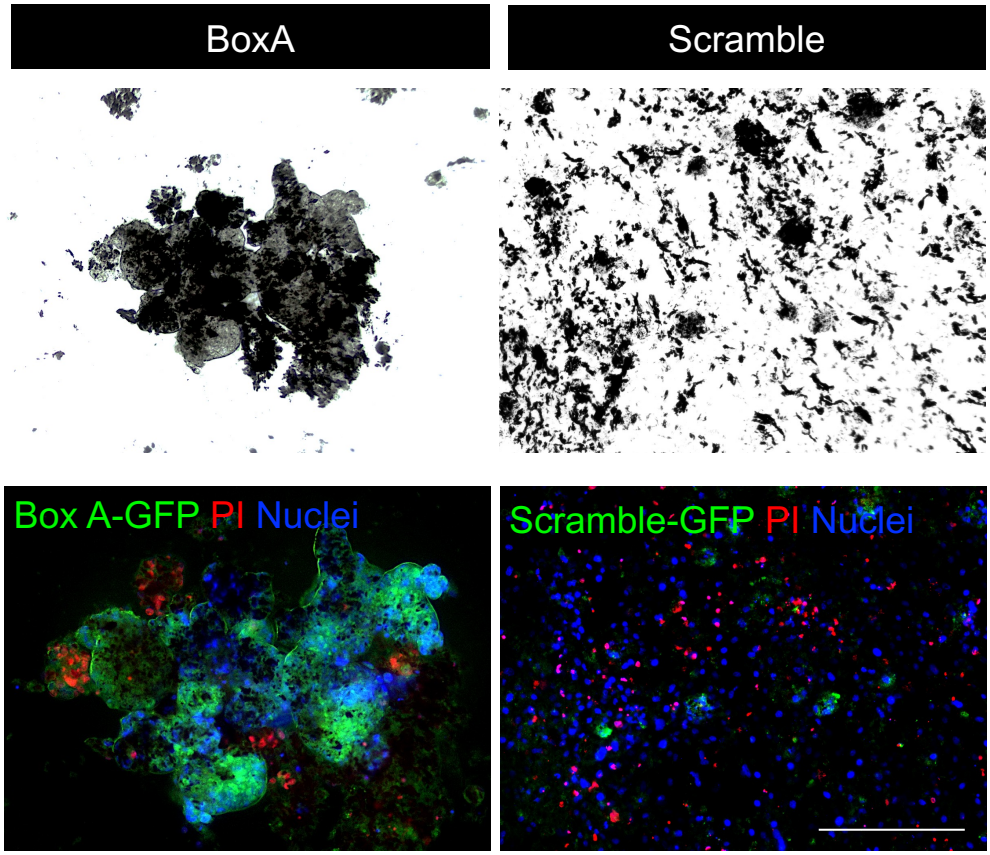

Figure S4. Cell viability assessment by propidium iodide (PI) staining after LG cell transfection in monolayer cultures with Box A-GFP and Scramble-GFP plasmids. Scale bar: 200 μm.

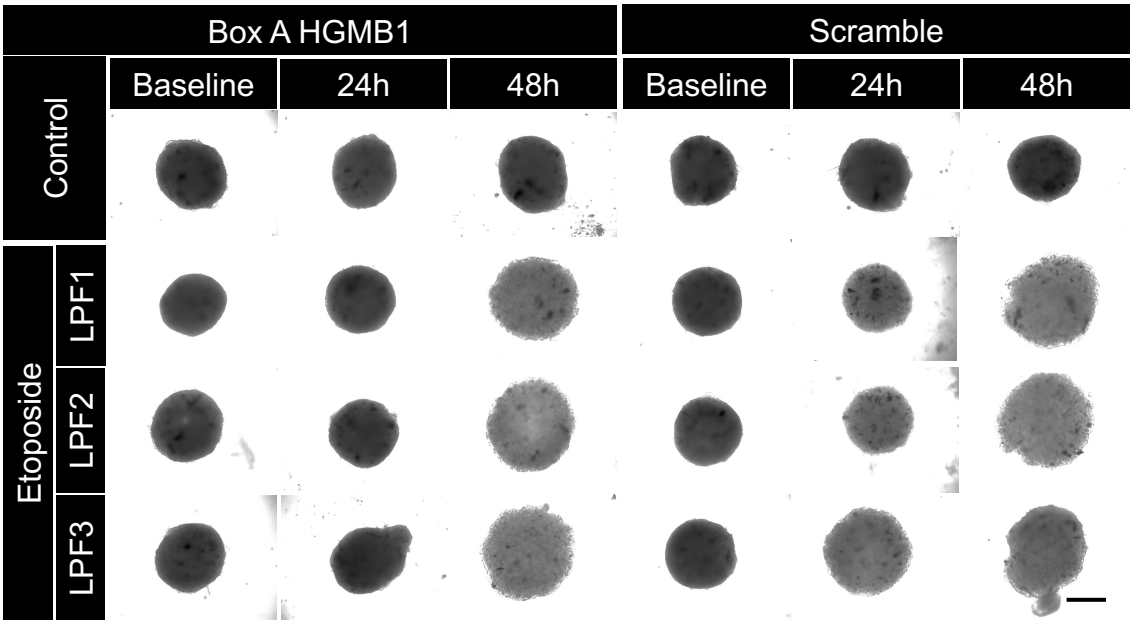

Figure S5. Brightfield micrographs showing the morphology of organoids assembled by magnetic bioprinting of primary LG cells up to 48h of culture after gene therapy (Box A HMGB1 or Scramble) and senescence induction by 10 μM of etoposide. Scale bar: 200 μm.

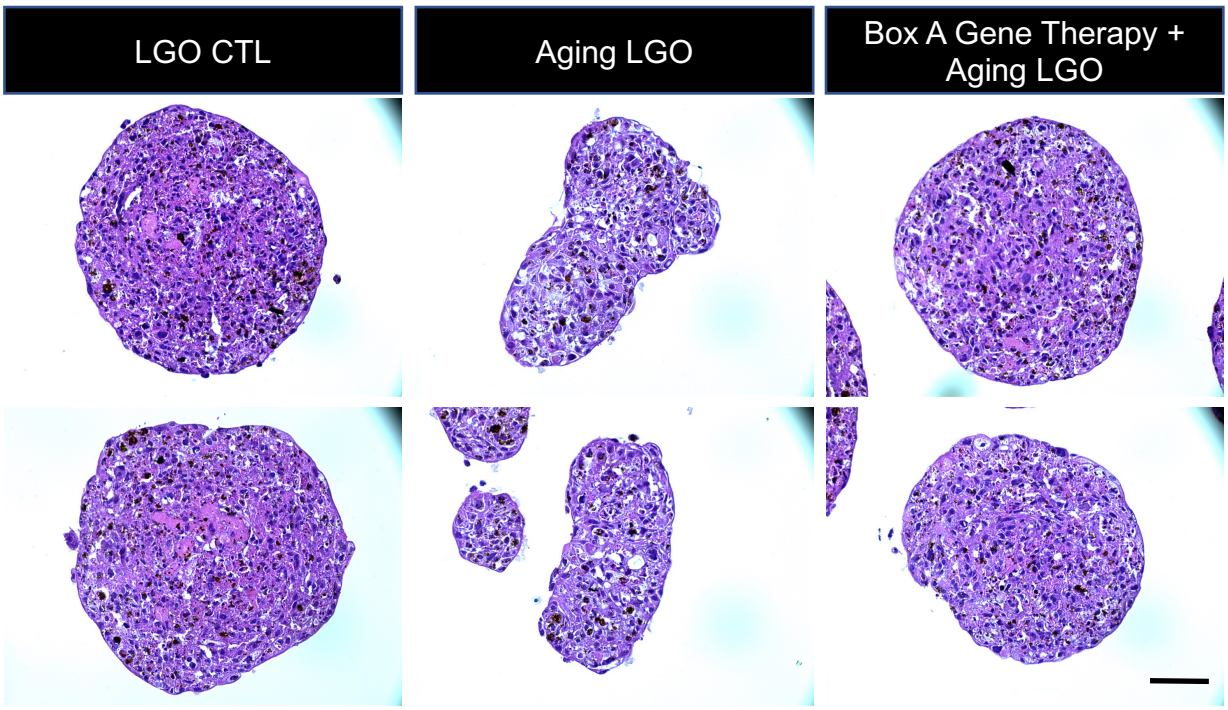

Figure S6. Brightfield micrographs after H&E staining for histological comparison and showing the morphology of the epithelial parenchyma with epithelial cells and matrix deposition in healthy LG organoids (LGO) and in LGO after aging induction without and with Box A gene therapy. The senescence-associated pathogenesis phenotype can be observed in the aging LGO without gene therapy. Scale bar: 50 µm.

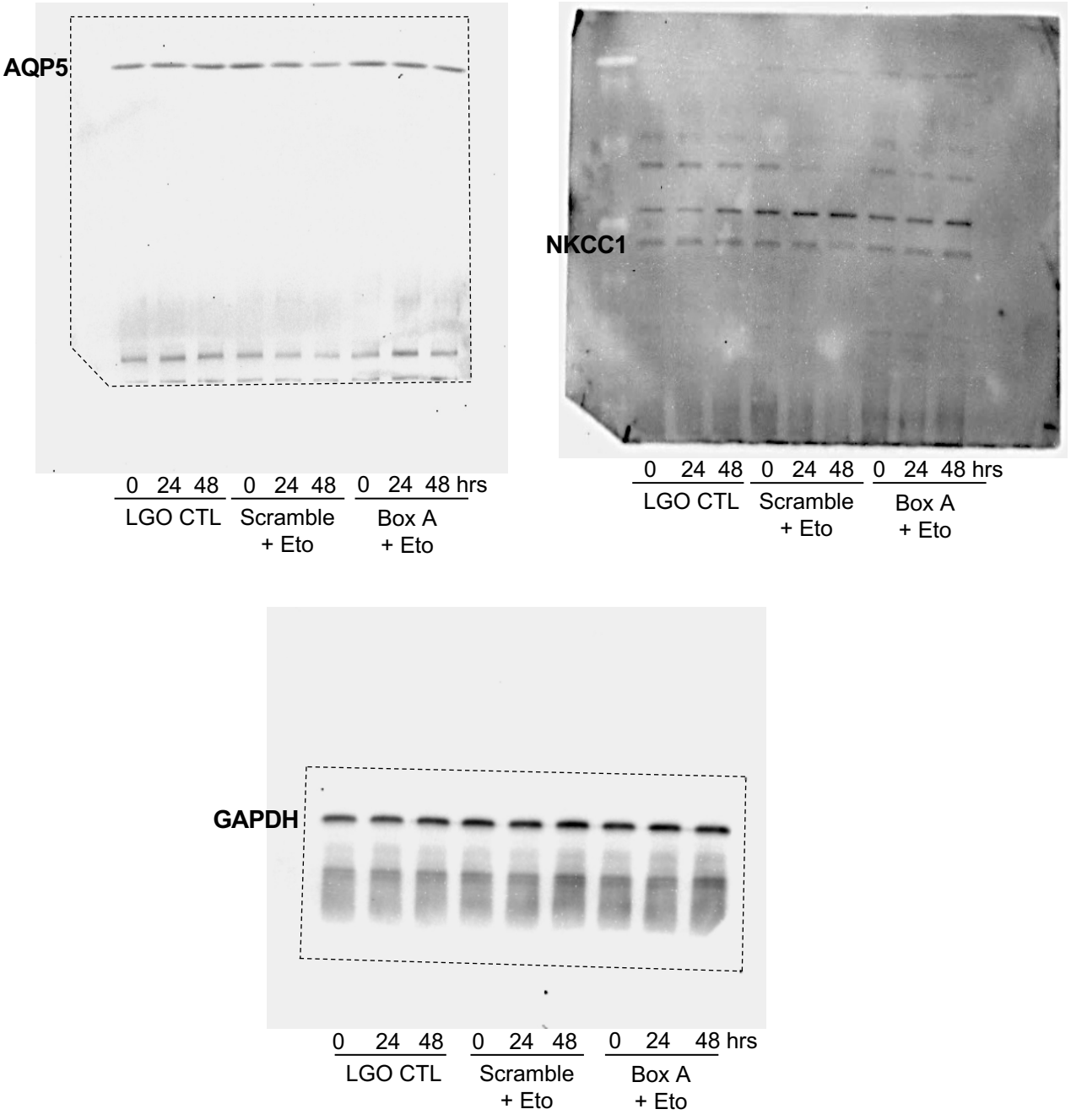

Figure S7. Full-length western blots used to produce Figure 5F. These images are the original and unprocessed versions. The top left blot for AQP5 and the lower one for GAPDH were cut prior to hybridization with antibodies, and a black dashed line was added to demarcate the membrane edges of the blots. Blot for NKCC1 has membrane edges visible. Predicted band sizes for: AQP5 – 28 kDa; NKCC1 – 130-180 kDa depending on glycosylation level; GAPDH - 37 kDa.