Lens aquaporin-5 inserts into bovine fiber cell plasma membranes via unconventional protein secretion

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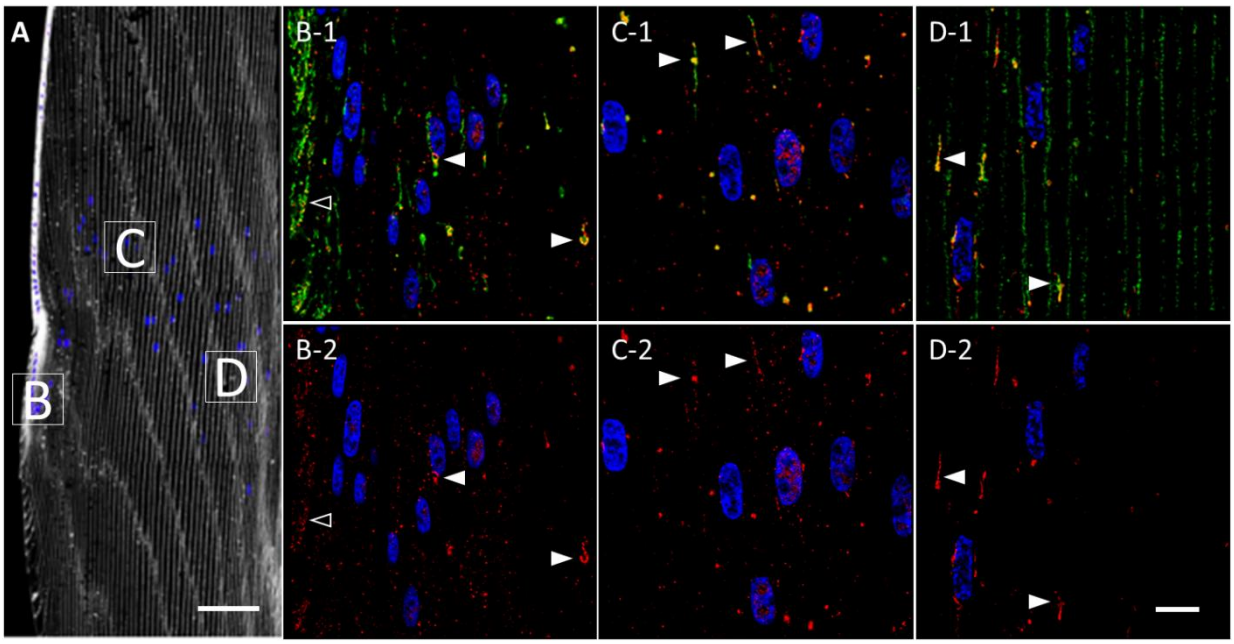
SUPPLEMENTARY DATA



S1. Cytochrome c oxidase subunit IV (COX IV) and AQP5 are *co-expressed* molecular markers for the same cluster of cytoplasmic vesicles in the bovine lens cortex.

A*.** A low-magnification representative image of the bovine lens bow region with plasma membranes (white***) and cellular nuclei (***blue***) labeled by WGA and DAPI, respectively. The *peripheral outer cortex* (i.e. includes *lens modiolus*), *medial outer cortex*, and *outer cortex-inner cortex transitional region* are demarcated by ***B***, ***C***, and ***D***, respectively, and correspond spatially with the images in ***B*-*D***.

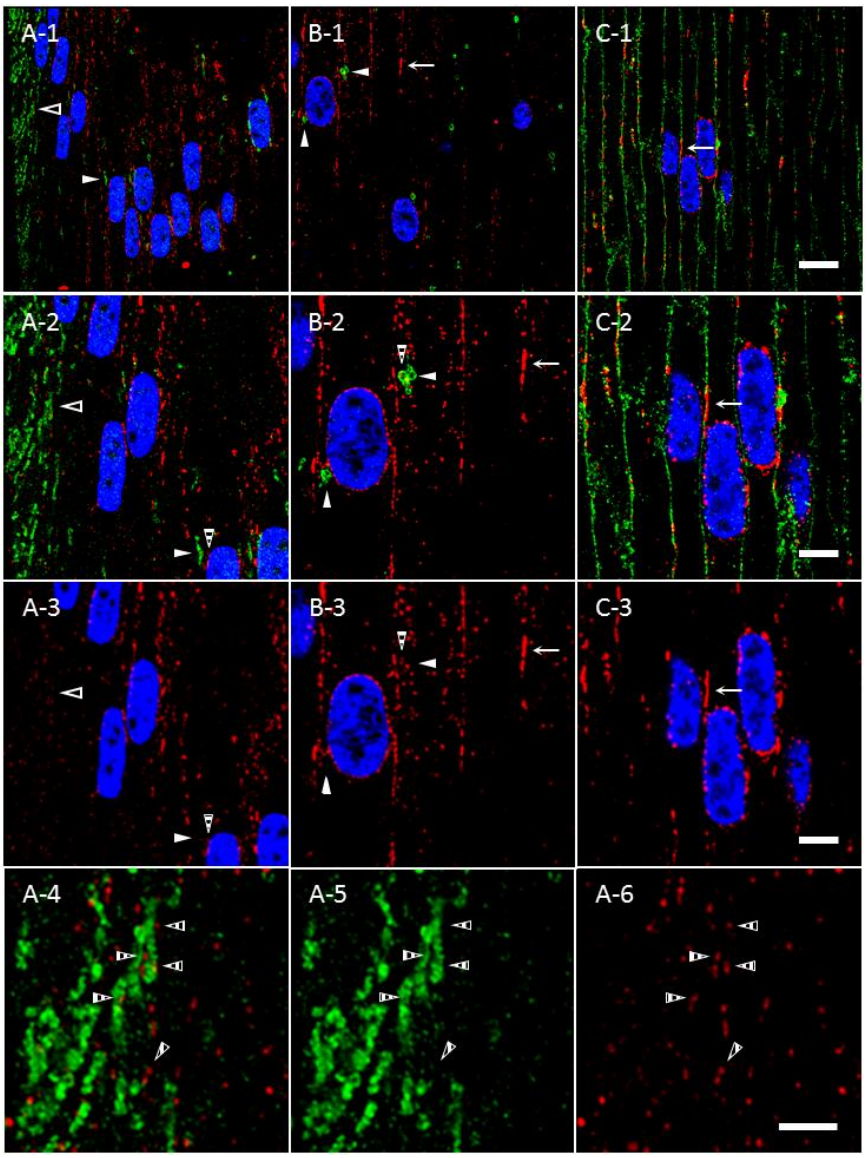
B-1 – ***D-1*.** High-magnification confocal images of *AQP5* immunolabeling (***green***) and *cytochrome c oxidase subunit IV* (*COX IV*) immunolabeling (***red***) in the *peripheral outer cortex* (**A**), *medial outer cortex*

(**B**), and *outer cortex-inner cortex transitional region* (**C**) of the bovine lens as demarcated in **Figure S1A**.

Tubular (***A***, *open arrowheads*) and spheroidal, tubular (**B** and **C**; *closed arrowheads*) AQP5-containing cytoplasmic vesicles and COX IV-containing mitochondria colocalize in the outer cortex of bovine lenses.

B-2 – ***D-2*****.** Replicate images of ***A-1***, ***B-1***, and ***C-1*** with COX IV immunolabeling and DAPI labeling only displayed.

Scale bars represent 100 µm (***A***) and 10 µm (***B***, ***C***, and ***D***).



S2. AQP5-containing cytoplasmic vesicles and calnexin-containing endoplasmic reticula fail to overlap in the bovine lens cortex.

A-1 – ***C-1*****.** High-magnification confocal images of *AQP5* immunolabeling (***green***) and *calnexin* labeling (***red***) in the *peripheral outer cortex* (***A-1***), *medial outer cortex* (***B-1***), and *outer cortex-inner cortex transitional region* (***C-1***) of the bovine lens (demarcated in **Figure 3A**). Calnexin is an integral membrane protein in the endoplasmic reticulum (ER). In the *peripheral outer cortex* and *medial outer cortex*, calnexin is cytoplasmically expressed in irregular, punctate vesicles or tubular, plaque-

like ER compartments apposed to fiber cell plasma membranes. Calnexin is also expressed in the perinuclear ER (***B-1***, ***B-2***, ***C-1***, and ***C-2***). By the outer cortex-inner cortex transitional region, calnexin expression is primarily localized to the perinuclear ER and tubular ER apposed to fiber cell plasma membranes (***B*** and ***C***, arrows).

AQP5-containing cytoplasmic vesicles lack significant calnexin expression. However, calnexin-containing ER form multiple appositions (***A-3***, ***A-4***, ***A-5***, ***A-6***, ***B-3***, and ***B-4,*** *striped arrowheads*) with tubular (***A***, *open arrowheads*) and spheroidal, tubular (***A*** and ***B***; *closed arrowheads*) AQP5-containing cytoplasmic vesicles.

A-2 – ***C-2*.** Enlarged images of the AQP5-containing cytoplasmic vesicles demarcated by the *arrowheads* in ***A-1*** and ***B-1*** and of the calnexin-containing tubular ER compartment demarcated by the *arrows* in ***B-1*** and ***C-1***.

A-3 – ***C-3*.** Replicate images of ***A-2***, ***B-2***, and ***C-2*** with calnexin immunolabeling and DAPI labeling only displayed.

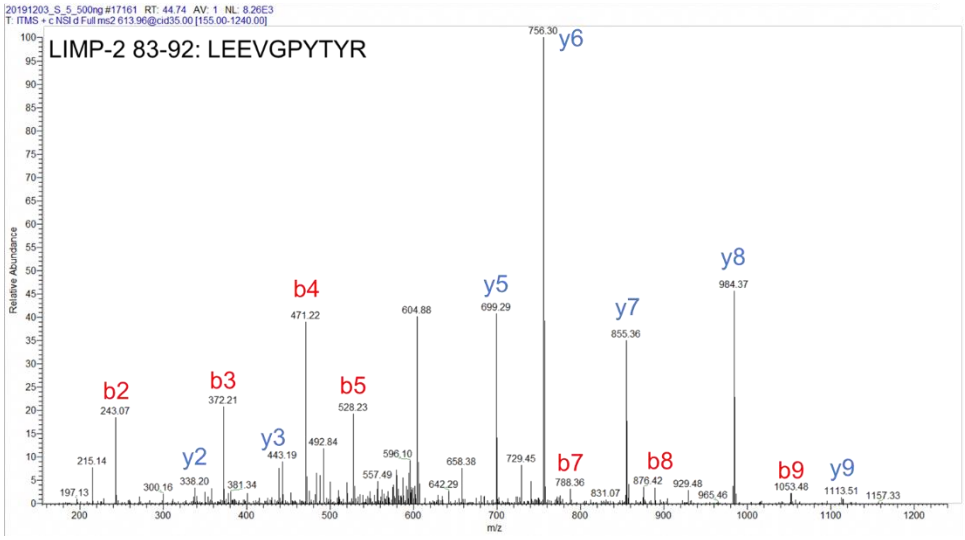
A-4*.** Enlarged image of tubular AQP5-containing cytoplasmic vesicles demarcated by the *open arrowhead* in ***A-2. These vesicles typically form several appositions with calnexin-containing ER (*striped arrowheads*)

A-5*.** A replicate image of ***A-4 with AQP5 immunolabeling only displayed.

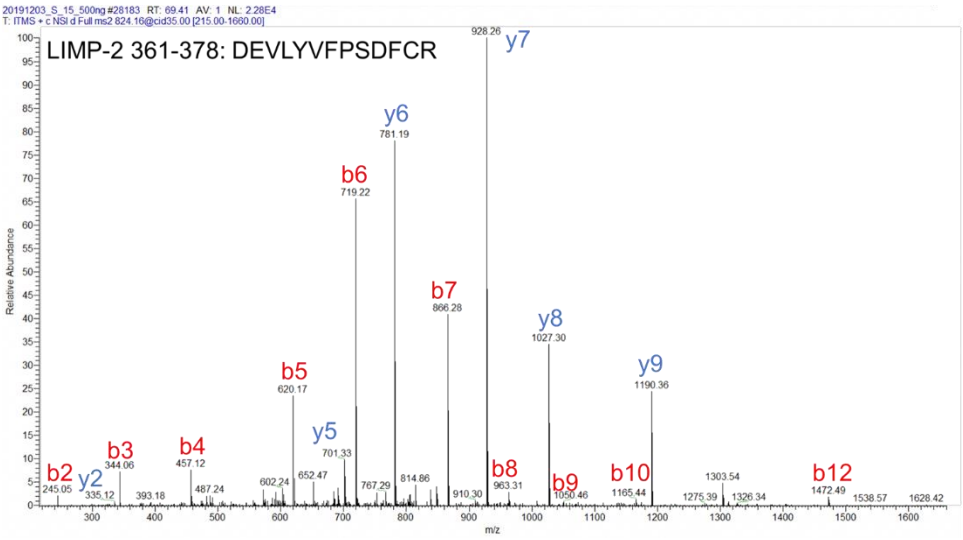
A-6*.** A replicate image of ***A-4 with calnexin immunolabeling only displayed.

Scale bars represent 10 µm (***A-1***, ***B-1***, and ***C-1***), 5 µm (***A-2***, ***A-3***, ***B-2***, ***B-3***, ***C-2***, and ***C-3***), and 2.5 µm (***A-4***, ***A-5***, and ***A-6***).

A.



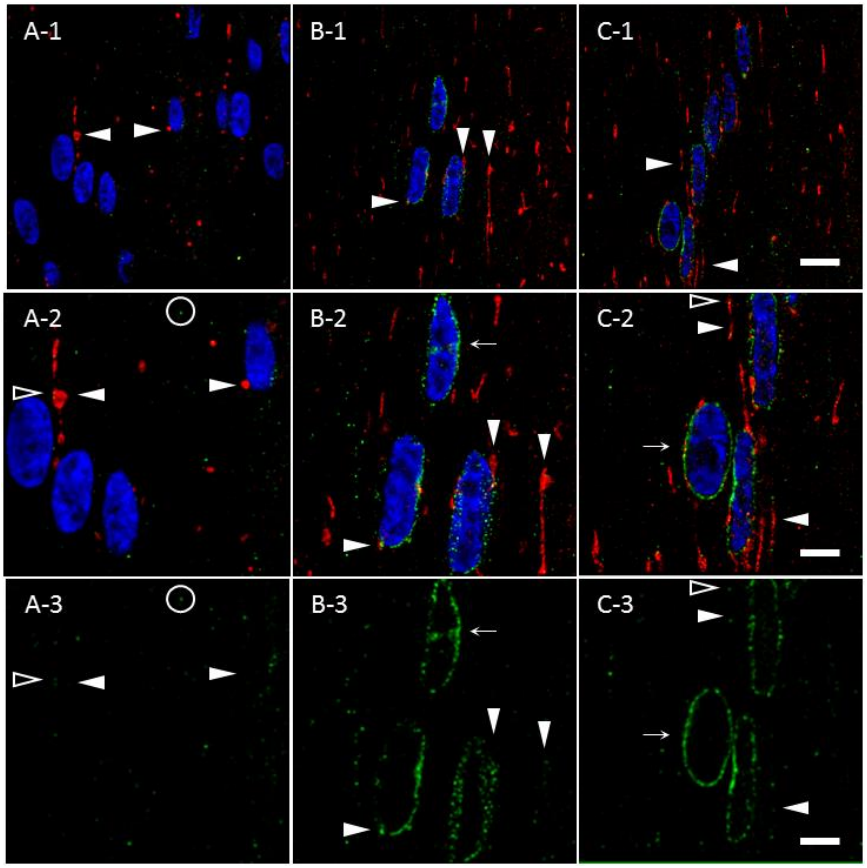
B.



S3. Tandem mass spectra identifying LIMP-2 in bovine lens cortical fiber cells.

A. High mass resolution CID spectrum of doubly-charged LIMP-2 peptide 82-93, m/z 824.16, acquired on a Thermo Fisher Velos Pro linear ion trap. The observed mass corresponds to one carbamidomethylated cysteine residue on the peptide (b12, y2). The peptide amino acid sequence is included above.

B. High mass resolution CID spectrum of doubly-charged LIMP-2 peptide 361-378, m/z 824.16, acquired on a Thermo Fisher Velos Pro linear ion trap. The observed mass corresponds to one carbamidomethylated cysteine residue on the peptide (b12, y2). The peptide amino acid sequence is included above.



S4. TOMM20-containing cytoplasmic vesicles lack significant Sec22β expression in bovine lens cortical fiber cells.

A-1 – ***C-1*.** High-magnification images of *Sec22β* immunolabeling (***green***) and *TOMM20* immunolabeling (***red***) in the *peripheral outer cortex* (**A**), *medial outer cortex* (**B**), and *outer cortex-inner cortex transitional region* (**C**) as demarcated in **Figure 3A**.

TOMM20-containing cytoplasmic vesicles (**closed arrowheads**) are expressed throughout the outer cortex. Initial *Sec22β* expression in the *peripheral outer cortex* (**A**) is weak but strengthens within the *medial outer cortex* (**B**) and remains consistent within the rest of the outer cortex in **C**. Throughout the outer cortex, *Sec22β* is initially expressed within diffuse, punctate cytoplasmic vesicles (**A**, *hollow circle*) that tangibly but insignificantly colocalize with TOMM20-containing cytoplasmic vesicles (***A-2***, ***A-3***, ***C-2***, and ***C-3***; *open arrowheads*). Excluding the lens modiolus, *Sec22β* is also ubiquitously expressed within the cellular nucleus-contiguous rough endoplasmic reticulum (***B-2***, ***B-3***, ***C-2***, and ***C-3***; *closed arrows*).

A-2 – ***C-2*.** Enlarged images of the TOMM20-containing cytoplasmic vesicles demarcated by arrowheads in ***A-1***, ***B-2,*** and ***C-1***.

A-3 – ***C-3*.** Replicate images of ***A-2*** – ***C-2*** depicting *Sec22β* immunolabeling and DAPI labeling only.

Scale bars denote 10 µm (***A-1*** – ***C-1***) and 5 µm (***A-2* – *C-2*** and ***A-3* – *C-3***).