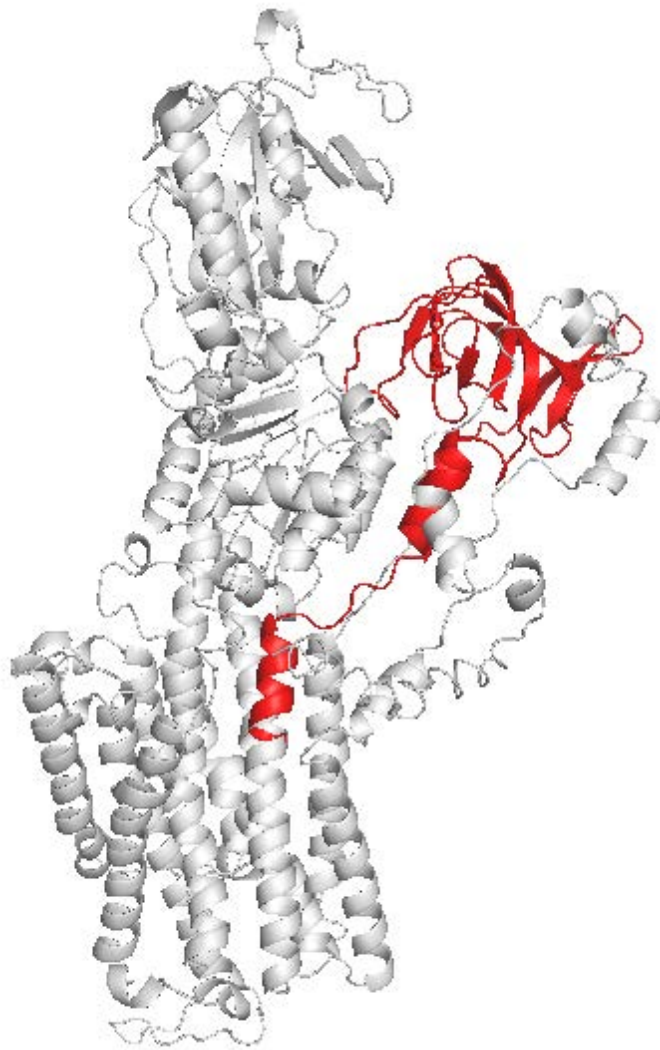


# The Non-Gastric H<sup>+</sup>/K<sup>+</sup> ATPase (ATP12A) Is Expressed in Mammalian Spermatozoa

## Supplementary Material



**Figure S1.** The 3D comparative model of bovine ATP12A is reported in white cartoon representation. Red cartoon indicates the localization of residues 171-306, which form the target region of the employed A62134 EpiGentek antibody.

Homo.sapiens.ATP12A.i1.NP_001667	171	NIMSSFNKMI	PQALVIR	DSSEKKT	IPSEQLVVG	DIVEVKGGDQ	IPADIRVLS	SSQGRV	DNSSLT	GESE	QPR	243
Homo.sapiens.ATP12A.i2.NP_001172014	171	NIMSSFNKMI	PQALVIR	DSSEKKT	IPSEQLVVG	DIVEVKGGDQ	IPADIRVLS	SSQGRV	DNSSLT	GESE	QPR	243
Bos.taurus.ATP12A.XP_002691916	169	NIMSSFRKMI	PQALVIR	DSSEKKT	IPADQLVVG	DIVEIKGGDRVP	ADVRIL	STQGGK	VDNSSVT	GESE	QARS	241
Bubalus.bubalis.ATP12A.XP_006046156	170	NIMSSFRKMI	PQALVIR	DSSEKKT	IPADQLVVG	DIVEIKGGDRVP	ADVRIL	STQGGK	VDNSSVT	GESE	QARS	242
Ovis.aries.ATP12A.XP_004012362	191	NIMSSFHKMI	PQALVIR	DSSEKKT	IPADQLVVG	DIVEIKGGDRVP	ADIRIL	STQGGK	VDNSSVT	GESE	QARC	263
Homo.sapiens.ATP4A.NP_000695	167	NIIASFKNLVP	QQATVIR	GGDKFQ	INADQLVVG	DLVEIKGGDRVP	ADIRIL	LAAGGK	VDNSSLT	GESE	QTRS	239
Bos.taurus.ATP4A.NP_001137561	166	NIIASFKNLVP	QQATVIR	GGDKFQ	INADQLVVG	DLVEIKGGDRVP	ADIRIL	LAAGGK	VDNSSLT	GESE	QTRS	238
Bubalus.bubalis.ATP4A.XP_006068866	166	NIIASFKNLVP	QQATVIR	GGDKFQ	INADQLVVG	DLVEIKGGDRVP	ADIRIL	LAAGGK	VDNSSLT	GESE	QTRS	238
O.aries.ATP4A.XP_027833250.1	145	NIIASFKNLVP	QQATVIR	GGDKFQ	INADQLVVG	DLVEIKGGDRVP	ADIRIL	LAAGGK	VDNSSLT	GESE	QTRS	217
Homo.sapiens.ATP1A1.NP_000692	156	KIMESFKNMVP	QQALVIR	NGEKM	INAEVVVVG	DLVEIKGGDRIP	ADLRIL	SAHGCK	VDNSSLT	GESE	QTRS	228
Bos.taurus.ATP1A1.NP_001070266	154	KIMESFKNMVP	QQALVIR	NGEKM	INAEVVVVG	DLVEIKGGDRIP	ADLRIL	SAHGCK	VDNSSLT	GESE	QTRS	226
B.bubalis.ATP1A1.XP_006058746.1	154	KIMESFKNMVP	QQALVIR	NGEKM	INAEVVVVG	DLVEIKGGDRIP	ADLRIL	SAHGCK	VDNSSLT	GESE	QTRS	226
Ovis.aries.ATP1A1.XP_027824133	125	KIMESFKNMVP	QQALVIR	NGEKM	INAEVVVVG	DLVEIKGGDRIP	ADLRIL	SAHGCK	VDNSSLT	GESE	QTRS	197
Homo.sapiens.ATP1A2.NP_000693	154	KIMDSFKNMVP	QQALVIR	NGEKM	INAEVVVVG	DLVEIKGGDRVP	ADLRIL	SSHGCK	VDNSSLT	GESE	QTRS	226
Bos.taurus.ATP1A2.NP_001074993.1	154	KIMDSFKNMVP	QQALVIR	NGEKM	INAEVVVVG	DLVEIKGGDRVP	ADLRIL	SSHGCK	VDNSSLT	GESE	QTRS	226
B.bubalis.ATP1A2.XP_006060545.1	154	KIMDSFKNMVP	QQALVIR	NGEKM	INAEVVVVG	DLVEIKGGDRVP	ADLRIL	SSHGCK	VDNSSLT	GESE	QTRS	226
Ovis.aries.ATP1A2.XP_004002710	154	KIMDSFKNMVP	QQALVIR	NGEKM	INAEVVVVG	DLVEIKGGDRVP	ADLRIL	SSHGCK	VDNSSLT	GESE	QTRS	226
Homo.sapiens.ATP1A3.NP_689509	146	KIMESFKNMVP	QQALVIR	REGEKMQV	INAEVVVVG	DLVEIKGGDRVP	ADLRIL	SAHGCK	VDNSSLT	GESE	QTRS	218
Bos.taurus.ATP1A3.XP_024834878.1	160	KIMESFKNMVP	QQALVIR	REGEKMQV	INAEVVVVG	DLVEIKGGDRVP	ADLRIL	SAHGCK	VDNSSLT	GESE	QTRS	232
B.bubalis.ATP1A3.XP_006041318.1	160	KIMESFKNMVP	QQALVIR	REGEKMQV	INAEVVVVG	DLVEIKGGDRVP	ADLRIL	SAHGCK	VDNSSLT	GESE	QTRS	232
O.aries.ATP1A3.XP_027834157.1	147	KIMESFKNMVP	QQALVIR	REGEKMQV	INAEVVVVG	DLVEIKGGDRVP	ADLRIL	SAHGCK	VDNSSLT	GESE	QTRS	219
Homo.sapiens.ATP1A4.NP_653300	164	KIMESFKNMVP	QQALVIR	RGGEKMQV	INAEVVVVG	DLVEIKGGDRVP	ADLRIL	SAHGCK	VDNSSLT	GESE	QTRS	236
Bos.taurus.ATP1A4.NP_001137575	165	KIMESFKNMVP	QQALVIR	RGGEKMQV	INAEVVVVG	DLVEIKGGDRIP	ADLRIL	SSQGGK	VDNSSLT	GESE	QTRS	237
B.bubalis.ATP1A4.XP_006060547.1	165	KIMESFKNMVP	QQALVIR	RGGEKMQV	INAEVVVVG	DLVEIKAGDRIP	ADLRIL	SSQGGK	VDNSSLT	GESE	QTRS	237
O.aries.ATP1A4.XP_014947816.1	165	KIMESFKNMVP	QQALVIR	RGGEKMQV	INAEVVVVG	DLVEIKGGDRIP	ADLRIL	SSHGCK	VDNSSLT	GESE	QTRS	237

Homo.sapiens.ATP12A.i1.NP_001667	244	SEFTHENPLE	TKNICFY	STTCL	EG-----	IVTGMVINT	GDRTII	GHIASLAS	GVGNE	KTP	IAIEIEHF	306
Homo.sapiens.ATP12A.i2.NP_001172014	244	SEFTHENPLE	TKNICFY	STTCL	EG-----	IVTGMVINT	GDRTII	GHIASLAS	GVGNE	KTP	IAIEIEHF	312
Bos.taurus.ATP12A.XP_002691916	242	CEFTHESPLE	TKNIAFF	STTCL	EG-----	TATGMVINT	GDRTII	GQIASLAS	GVKDL	KTP	IAIEIEHF	304
Bubalus.bubalis.ATP12A.XP_006046156	243	CEFTHESPLE	TKNIAFF	STTCL	EG-----	TATGMVINT	GDRTII	GQIASLAS	GVKDL	KTP	IAIEIEHF	305
Ovis.aries.ATP12A.XP_004012362	264	CEFTHESPLE	TKNIAFF	STTCL	EG-----	TATGMVINT	GDRTII	GQIASLAS	GVKDL	KTP	IAIEIEHF	326
Homo.sapiens.ATP4A.NP_000695	240	PECTHESPLE	TRNIAFF	STMC	LEG-----	TVQGLVVNT	GDRTII	IGRIASLAS	GVGNE	KTP	IAIEIEHF	302
Bos.taurus.ATP4A.NP_001137561	239	PECTHESPLE	TRNIAFF	STMC	LEG-----	TAQGLVVNT	GDRTII	IGRIASLAS	GVGNE	KTP	IAIEIEHF	301
Bubalus.bubalis.ATP4A.XP_006068866	239	PECTHESPLE	TRNIAFF	STMC	LEG-----	TAQGLVVNT	GDRTII	IGRIASLAS	GVGNE	KTP	IAIEIEHF	301
O.aries.ATP4A.XP_027833250.1	218	PDCTHESPLE	TRNIAFF	STMC	LEG-----	TAQGLVVNT	GDRTII	IGRIASLAS	GVGNE	KTP	IAIEIEHF	289
Homo.sapiens.ATP1A1.NP_000692	229	PDFTNENPLE	TRNIAFF	STNC	VEG-----	TARGIVVYT	GDRTVMGR	IATLAS	GLEG	GGT	PTAAIEIEHF	291
Bos.taurus.ATP1A1.NP_001070266	227	PDFTNENPLE	TRNIAFF	STNC	VEG-----	TARGIVVYT	GDRTVMGR	IATLAS	GLEG	GGT	PTAAIEIEHF	289
B.bubalis.ATP1A1.XP_006058746.1	227	PDFTNENPLE	TRNIAFF	STNC	VEG-----	TARGIVVYT	GDRTVMGR	IATLAS	GLEG	GGT	PTAAIEIEHF	289
Ovis.aries.ATP1A1.XP_027824133	198	PDFTNENPLE	TRNIAFF	STNC	VEG-----	TARGIVVYT	GDRTVMGR	IATLAS	GLEG	GGT	PTAAIEIEHF	260
Homo.sapiens.ATP1A2.NP_000693	227	PEFTHENPLE	TRNICFF	STNC	VEG-----	TARGIVVIAT	GDRTVMGR	IATLAS	GLEV	GRTP	IAIEIEHF	289
Bos.taurus.ATP1A2.NP_001074993.1	227	PEFTHENPLE	TRNICFF	STNC	VEG-----	TARGIVVIAT	GDRTVMGR	IATLAS	GLEV	GRTP	IAIEIEHF	289
B.bubalis.ATP1A2.XP_006060545.1	227	PEFTHENPLE	TRNICFF	STNC	VEG-----	TARGIVVIAT	GDRTVMGR	IATLAS	GLEV	GRTP	IAIEIEHF	289
Ovis.aries.ATP1A2.XP_004002710	227	PEFTHENPLE	TRNICFF	STNC	VEG-----	TARGIVVIAT	GDRTVMGR	IATLAS	GLEV	GRTP	IAIEIEHF	289
Homo.sapiens.ATP1A3.NP_689509	219	PDCTHDNPLE	TRNITFF	STNC	VEG-----	TARGVVVAT	GDRTVMGR	IATLAS	GLEV	GGT	PTAAIEIEHF	281
Bos.taurus.ATP1A3.XP_024834878.1	233	PDCTHDNPLE	TRNITFF	STNC	VEG-----	TARGVVVAT	GDRTVMGR	IATLAS	GLEV	GGT	PTAAIEIEHF	295
B.bubalis.ATP1A3.XP_006041318.1	233	PDCTHDNPLE	TRNITFF	STNC	VEG-----	TARGVVVAT	GDRTVMGR	IATLAS	GLEV	GGT	PTAAIEIEHF	295
O.aries.ATP1A3.XP_027834157.1	220	PDCTHDNPLE	TRNITFF	STNC	VEG-----	TARGVVVAT	GDRTVMGR	IATLAS	GLEV	GGT	PTAAIEIEHF	282
Homo.sapiens.ATP1A4.NP_653300	237	PDFTNENPLE	TRNICFF	STNC	VEG-----	TARGIVVIAT	GDSTVMGR	IASLTS	GLAV	GGT	PTAAIEIEHF	299
Bos.taurus.ATP1A4.NP_001137575	238	TEFTNENPLE	TQNICFF	STNC	VEG-----	SAQGIIVVIAT	GDSTVMGR	IASLTS	GLAV	GGT	PTAAIEIEHF	300
B.bubalis.ATP1A4.XP_006060547.1	238	TEFTNENPLE	TQNICFF	STNC	VEG-----	SAQGIIVVIAT	GDSTVMGR	IASLTS	GLAV	GGT	PTAAIEIEHF	300
O.aries.ATP1A4.XP_014947816.1	238	TEFTNENPLE	TQNICFF	STNC	VEG-----	SAQGIIVVIAT	GDSTVMGR	IASLTS	GLV	VGGT	PTAAIEIEHF	300

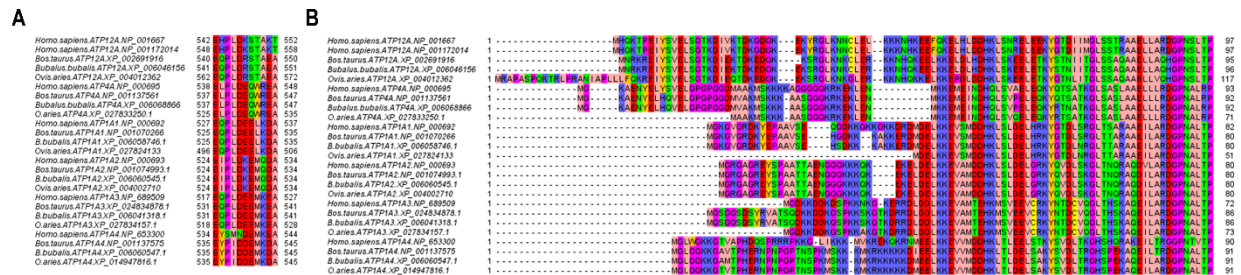
**Figure S2.** MSA of residues from the different sampled ATP1A1-4, ATP12A and ATP4A sequences, aligned with 171-306 residues from the bovine ATP12A, forming the target region of the employed A62134 EpiGentek antibody.



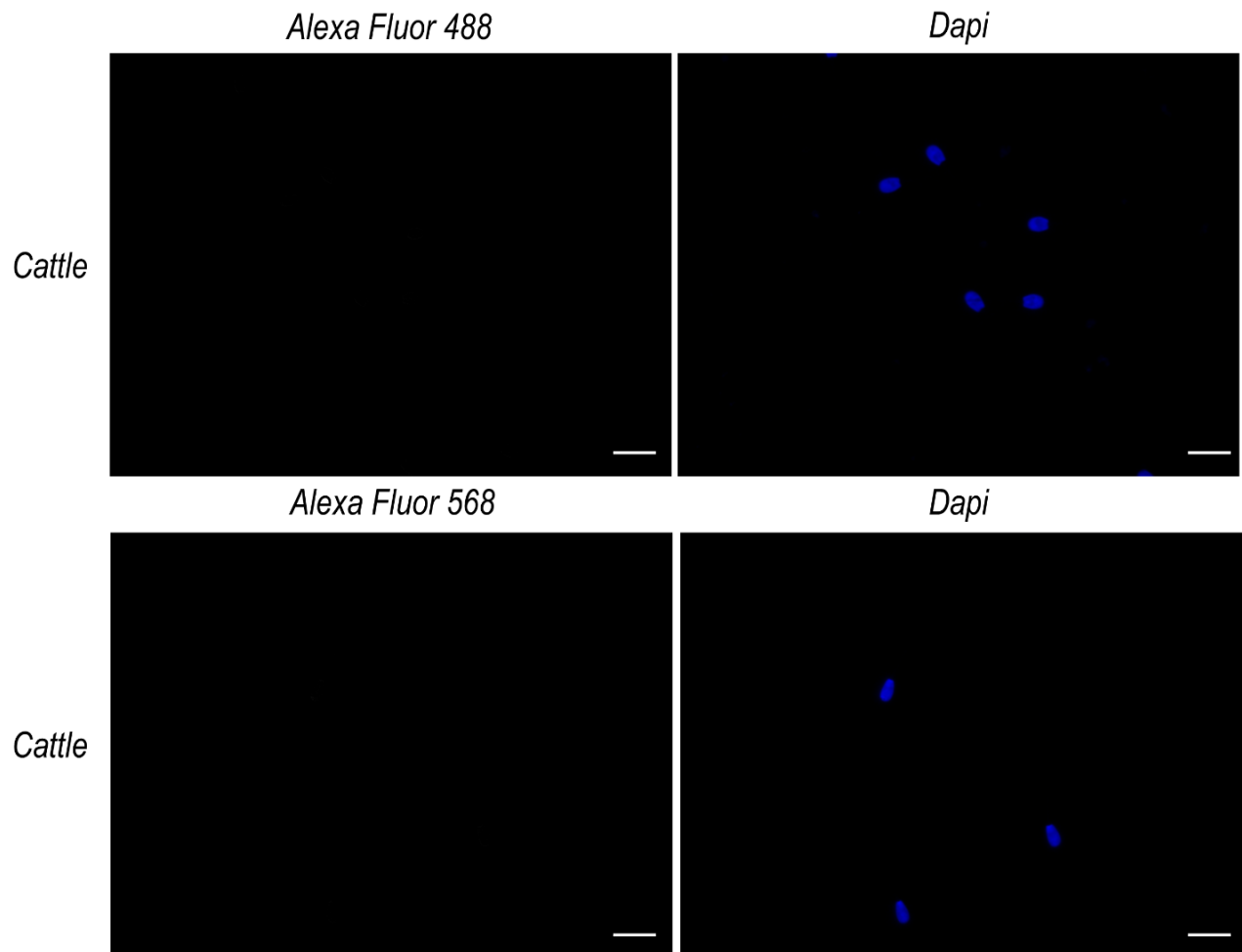
**Figure S3.** The 3D crystallized structure of bovine ATP1A1 (white cartoon representation) in complex with ATP1B1 (black cartoon representation) is reported. Red cartoon indicates the localization of residues 64-241, which form the target region of the employed CSB-PA002326LA01HU antibody.

*H.sapiens.ATP1B1.NP\_001668.1* 64 **K**P**T****I****Y****D****D****R****V****A****P****P****G****L****T****O****I****P****D****I****O****K****T** --- **E****I****S****F****R****P****N****O****P****K****S****Y****E****A****Y****V****L****N****I****V****R****F****L****E****K****Y****D****S****A****K****D****D****M****I****F****E****D****C****G****N****V****S****E****L****K****D****R****G****E****F****N****N****E****G****E****R****K****V****C****R****F****L** 153  
*B.taurus.ATP1B1.pdbj4xE5B* 64 **K**P**T****I****Y****D****D****R****V****A****P****P****G****L****T****O****I****P****D****I****O****K****T** --- **E****I****A****F****R****P****N****O****P****K****S****Y****M****T****Y****V****D****N****I****D****N****F****L****K****K****Y****R****S****A****K****D****D****M****I****F****E****D****C****G****N****V****S****E****L****K****D****R****G****E****F****N****N****E****G****E****R****K****V****C****R****F****L** 153  
*B.bubalis.ATP1B1.XP\_006054394.1* 64 **K**P**T****I****Y****D****D****R****V****A****P****P****G****L****T****O****I****P****D****I****O****K****T** --- **E****I****A****F****R****P****N****O****P****K****S****Y****M****T****Y****V****D****N****I****D****N****F****L****K****K****Y****R****S****A****K****D****D****M****I****F****E****D****C****G****N****V****S****E****L****K****D****R****G****E****F****N****N****E****G****E****R****K****V****C****R****F****L** 153  
*O.aries.ATP1B1.NP\_001009796.1* 64 **K**P**T****I****Y****D****D****R****V****A****P****P****G****L****T****O****I****P****D****I****O****K****T** --- **E****I****A****F****R****P****N****O****P****K****S****Y****M****T****Y****V****D****N****I****D****N****F****L****K****K****Y****R****S****A****K****D****D****M****I****F****E****D****C****G****N****V****S****E****L****K****D****R****G****E****F****N****N****E****G****E****R****K****V****C****R****F****L** 153  
*H.sapiens.ATP1B2.NP\_001669.3* 69 **H****T****P****K****Y****D****D****R****L****A****T****P****G****L****M****I****R****P****K** - **T****E****N** - **L****D****V****I****V****N****S****D****T****S****W****D****D****H****V****K****L****N****K****F****L****E****P****Y****N****D****S****I****A****G****K****N** - - - **D****V****C****R****P****G****R****Y****E****E****P****D****N****G****V****L****N****Y****P****K****R****A****C****D****F****N****R** 154  
*B.taurus.ATP1B2.NP\_777102.1* 69 **H****T****P****K****Y****D****D****R****L****A****T****P****G****L****M****I****R****P****K** - **T****E****N** - **L****D****V****I****V****N****S****D****T****S****W****D****D****H****V****K****L****N****K****F****L****E****P****Y****N****D****S****I****A****G****K****N** - - - **D****V****C****R****P****G****R****Y****E****E****P****D****N****G****V****L****N****Y****P****K****R****A****C****D****F****N****R** 154  
*B.bubalis.ATP1B2.NP\_001277856.1* 69 **H****T****P****K****Y****D****D****R****L****A****T****P****G****L****M****I****R****P****K** - **T****E****N** - **L****D****V****I****V****N****S****D****T****S****W****D****D****H****V****K****L****N****K****F****L****E****P****Y****N****D****S****I****A****G****K****N** - - - **D****V****C****R****P****G****R****Y****E****E****P****D****N****G****V****L****N****Y****P****K****R****A****C****D****F****N****R** 154  
*O.aries.ATP1B2.XP\_004012719.1* 69 **H****T****P****K****Y****D****D****R****L****A****T****P****G****L****M****I****R****P****K** - **T****E****N** - **L****D****V****I****V****N****S****D****T****S****W****D****D****H****V****K****L****N****K****F****L****E****P****Y****N****D****S****I****A****G****K****N** - - - **D****V****C****R****P****G****R****Y****E****E****P****D****N****G****V****L****N****Y****P****K****R****A****C****D****F****N****R** 154  
*H.sapiens.ATP1B3.NP\_001670.1* 68 **V****P****K****Y****R****D****I****P****S****P****G****L****M****V****F****K****P****V****S****A** - **L****E****V****T****F****S****R****D****P****T****S****Y****A****G****I****E****D****L****K****K****F****L****K****P****Y** - **T****L****E****E****O****K****N****L** - - **T****V****C****P****G****A****L****F****E****E** - **K****G****P****V** - - **Y****V****A****C****D****F****L** 148  
*B.taurus.ATP1B3.NP\_001030470.1* 68 **V****P****K****Y****R****D****I****P****S****P****G****L****M****V****F****K****P****V****S****A** - **L****D****F****S****F****S****L****D****A****S****Y****G****G****I****D****D****L****K****K****F****L****K****P****Y** - **G****L****E****E****O****K****N****L** - - **T****D****C****T****G****T****F****F****E****E** - **K****G****P****E** - - **Y****T****A****C****D****F****L** 148  
*B.bubalis.ATP1B3.XP\_006078090.1* 68 **V****P****K****Y****R****D****I****P****S****P****G****L****M****V****F****K****P****V****S****A** - **L****D****F****S****F****S****L****D****A****S****Y****G****G****I****D****D****L****K****K****F****L****K****P****Y** - **G****L****E****E****O****K****N****L** - - **T****D****C****T****G****T****F****F****E****E** - **K****G****P****E** - - **Y****T****A****C****D****F****L** 148  
*O.aries.ATP1B3.XP\_011957641.1* 68 **V****P****K****Y****R****D****I****P****S****P****G****L****M****V****F****K****P****V****S****A** - **L****D****F****S****F****S****L****D****A****S****Y****G****G****I****D****D****L****K****K****F****L****K****P****Y** - **G****L****E****E****O****K****N****L** - - **T****D****C****T****G****T****F****F****E****E** - **K****G****P****E** - - **Y****T****A****C****D****F****L** 148  
*H.sapiens.ATP1B4.NP\_001135919.1* 141 **Y****I****P****T****F****T****E****R****V****K****P****P****G****V****M****I****R****P****F****A****H****S** - - - **L****N****F****N****F****N****S****E****P****D****T****W****H****Y****V****I****S****L****N****G****F****L****G****G****Y****N****D****S****L****D****E****E****M****N** - - - **V****D****C****P****P****G****Q****Y****F****I****D** - - **G****N****E****D****E****D****K****K****A****C****D****F****K** 224  
*B.taurus.ATP1B4.NP\_036201.1* 137 **Y****I****P****T****F****T****E****R****V****K****P****P****G****V****M****I****R****P****F****A****H****S** - - - **L****N****F****N****F****N****S****E****P****D****T****W****H****Y****V****I****S****L****N****G****F****L****G****G****Y****N****D****S****L****D****E****E****M****N** - - - **V****D****C****P****P****G****Q****Y****F****I****D** - - **G****N****E****D****E****D****K****K****A****C****D****F****K** 220  
*B.taurus.ATP1B4.XP\_005227518.1* 135 **Y****M****P****T****F****T****E****R****V****K****P****P****G****V****M****I****R****P****F****A****H****S** - - - **L****N****F****N****F****N****S****E****P****D****T****W****H****Y****V****I****S****L****N****G****F****L****G****G****Y****N****D****S****L****D****E****E****M****N** - - - **V****D****C****P****P****G****Q****Y****F****I****D** - - **G****N****E****D****E****D****K****K****A****C****D****F****K** 218  
*B.bubalis.ATP1B4.XP\_006048859.1* 135 **Y****M****P****T****F****T****E****R****V****K****P****P****G****V****M****I****R****P****F****A****H****S** - - - **L****N****F****N****F****N****S****E****P****D****T****W****H****Y****V****I****S****L****N****G****F****L****G****G****Y****N****D****S****L****D****E****E****M****N** - - - **V****D****C****P****P****G****Q****Y****F****I****D** - - **G****N****E****D****E****D****K****K****A****C****D****F****K** 218  
*O.aries.ATP1B4.XP\_011962738.1* 136 **Y****M****P****T****F****T****E****R****V****K****P****P****G****V****M****I****R****P****F****A****H****S** - - - **L****N****F****N****F****N****S****E****P****D****T****W****H****Y****V****I****S****L****N****G****F****L****G****G****Y****N****D****S****L****D****E****E****M****N** - - - **V****D****C****P****P****G****Q****Y****F****I****D** - - **G****N****E****D****E****D****K****K****A****C****D****F****K** 219  
*H.sapiens.ATP4B.NP\_000696.1* 69 **Y****T****P****D****Y****D****D****Q****L****R****S****P****G****V****T****L****R****P****D****T****Y****G****D****K****G****L****D** **I****S****Y****N****V****S****D****N****R****T****W****A****G****L****T****O****L****H****A****F****L****A****G****Y****S****P****A****A****E****D****N** - - - **I****N****C****T****S****E****Q****Y****F****F****O****E****R****F****L****A****P****N****H****T****K****F****S****C****K****F****T****A** 156  
*B.taurus.ATP4B.NP\_001193069.1* 69 **Y****T****P****D****Y****D****D****Q****L****R****S****P****G****V****T****L****R****P****D****T****Y****G****D****K****G****L****D** **I****S****Y****N****V****S****D****N****R****T****W****A****G****L****T****O****L****H****A****F****L****A****G****Y****S****P****A****A****E****D****N** - - - **I****N****C****T****S****E****Q****Y****F****F****O****E****R****F****L****A****P****N****H****T****K****F****S****C****K****F****T****A** 156  
*B.bubalis.ATP4B.XP\_006076675.1* 69 **Y****T****P****D****Y****D****D****Q****L****R****S****P****G****V****T****L****R****P****D****T****Y****G****D****K****G****L****D** **I****S****Y****N****V****S****D****N****R****T****W****A****G****L****T****O****L****H****A****F****L****A****G****Y****S****P****A****A****E****D****N** - - - **I****N****C****T****S****E****Q****Y****F****F****O****E****R****F****L****A****P****N****H****T****K****F****S****C****K****F****T****A** 156  
*O.aries.ATP4B.XP\_027829708.1* 69 **Y****T****P****D****Y****D****D****Q****L****R****S****P****G****V****T****L****R****P****D****T****Y****G****D****K****G****L****D** **I****S****Y****N****V****S****D****N****R****T****W****A****G****L****T****O****L****H****A****F****L****A****G****Y****S****P****A****A****E****D****N** - - - **I****N****C****T****S****E****Q****Y****F****F****O****E****R****F****L****A****P****N****H****T****K****F****S****C****K****F****T****A** 156

*H.sapiens.ATP1B1.NP\_001668.1* 154 **S****W****L****G****N****C****S****G****L****N****D****E****T** - **Y****G****Y****K****E****G****K****P****C****V****I****I****K****L****N****R****V****L****G****F****K****P****P****K****N****E****S****L****E****T****Y****P****V****M****K****Y****N****P****Y****V****L****P****V****D****C****T****G****K****R** - **D****E****D****K****E****K****V****G****S****I****E****Y****F****G****L****G****S****N****P****G****F****F****L****O** 241  
*B.taurus.ATP1B1.pdbj4xE5B* 154 **S****W****L****G****N****C****S****G****L****N****D****E****T** - **Y****G****Y****K****D****G****K****P****C****V****I****I****K****L****N****R****V****L****G****F****K****P****P****K****N****E****S****L****E****T****Y****P****V****M****K****Y****N****P****Y****V****L****P****V****D****C****T****G****K****R** - **D****E****D****K****E****K****V****G****S****I****E****Y****F****G****L****G****S****N****P****G****F****F****L****O** 241  
*B.bubalis.ATP1B1.XP\_006054394.1* 154 **S****W****L****G****N****C****S****G****L****N****D****E****T** - **Y****G****Y****K****D****G****K****P****C****V****I****I****K****L****N****R****V****L****G****F****K****P****P****K****N****E****S****L****E****T****Y****P****V****M****K****Y****N****P****Y****V****L****P****V****D****C****T****G****K****R** - **D****E****D****K****E****K****V****G****S****I****E****Y****F****G****L****G****S****N****P****G****F****F****L****O** 241  
*O.aries.ATP1B1.NP\_001009796.1* 154 **S****W****L****G****N****C****S****G****L****N****D****E****T** - **Y****G****Y****K****E****G****K****P****C****V****I****I****K****L****N****R****V****L****G****F****K****P****P****K****N****E****S****L****E****T****Y****P****V****M****K****Y****N****P****Y****V****L****P****V****D****C****T****G****K****R** - **D****E****D****K****E****K****V****G****S****I****E****Y****F****G****L****G****S****N****P****G****F****F****L****O** 241  
*H.sapiens.ATP1B2.NP\_001669.3* 155 **T****O****L****G****N****C****S****G****I****D****S****T****H****Y****G****S****T****G****D****P****C****V****F****I****K****M****N****R****V****I****N****F****Y****A****G** - - - - - **A****N****O****S****M****N****V****T****C****A****G****K****R** - **D****E****D****A****E****N****L****G****N****F****V****M****F****P****A****N****G** - - **N****I****D****L****M** 226  
*B.taurus.ATP1B2.NP\_777102.1* 155 **T****O****L****G****N****C****S****G****I****D****S****T****H****Y****G****S****T****G****D****P****C****V****F****I****K****M****N****R****V****I****N****F****Y****A****G** - - - - - **A****N****O****S****M****N****V****T****C****V****G****K****R** - **D****E****D****A****E****N****L****G****N****F****V****M****F****P****A****N****G** - - **N****I****D****L****I** 226  
*B.bubalis.ATP1B2.NP\_001277856.1* 155 **T****O****L****G****N****C****S****G****I****D****S**



**Figure S5. (A)** MSA of residues from the different sampled ATP1A1-4, ATP12A and ATP4A sequences, aligned with 496-506 residues from lamb ATP1A1, forming the target region of the employed MA3-929 Invitrogen antibody. **(B)** MSA of residues from the different sampled ATP1A1-4, ATP12A and ATP4A sequences, aligned with 496-506 residues from the lamb ATP1A1, forming the target region of the employed MA3-929 Invitrogen antibody.



**Figure S6.** Negative controls sperms were incubated with either anti-rabbit or anti-mouse IgG secondary antibodies conjugated to Alexa Fluor 488 (upper panels) or Alexa Fluor 568 (lower panels), respectively. Nuclei were counterstained with DAPI (blue). Scale bar: 20  $\mu$ m.