

Supplementary Information

Control of intracellular pH and bicarbonate by CO₂ diffusion into human sperm

Elena Grahn^{1§}, Svenja V. Kaufmann^{2§}, Malika Askarova¹, Momchil Ninov^{2,3}, Luisa M. Welp^{2,3}, Thomas K. Berger^{1,4}, Henning Urlaub^{2,3}, and U. Benjamin Kaupp^{1,5}

¹Max Planck Institute for Neurobiology of Behavior–caesar, Molecular Sensory Systems, Ludwig-Erhard-Allee 2, 53175 Bonn, Germany; ²Max Planck Institute for Multidisciplinary Sciences, Bioanalytical Mass Spectrometry, Am Fassberg 11, 37077 Göttingen, Germany; ³University Medical Center Göttingen, Institute of Clinical Chemistry, Bioanalytics, Robert-Koch-Strasse 40, 37075 Göttingen, Germany; ⁴Department of Neurophysiology, Institute of Physiology and Pathophysiology, Philipps-University Marburg, Deutschhausstrasse 1-2, 35037 Marburg, Germany; ⁵Life & Medical Sciences Institute (LIMES), University Bonn, Carl-Troll-Strasse 31, 53115 Bonn, Germany

§E.G. and S.V.K. contributed equally to this work.

Send correspondence to: Thomas K. Berger
Department of Neurophysiology, Institute of Physiology and Pathophysiology, Philipps-University Marburg
Deutschhausstrasse 1-2
35037 Marburg, Germany.
Tel.: ++496421-28-62398, Fax: ++496421-28-62306
e-mail: thomas.berger@uni-marburg.de

U. Benjamin Kaupp
LIMES Institute, University Bonn
Carl-Troll-Strasse 31
53175 Bonn, Germany
Tel.: ++49228-9656-100, Fax: ++49228-9656-9273
e-mail: ubkaupp@uni-bonn.de

Henning Urlaub
Max-Planck-Institute for Multidisciplinary Sciences
Bioanalytical Mass Spectrometry Group
Am Fassberg 11
37077 Göttingen, Germany
Tel.: ++49551 201 1060 (Office)/-1500 (Lab)
email: henning.urlaub@mpinat.mpg.de

Supplementary Note 1. Summary of reports on alkalization during capacitation.

Several reports have been cited for sperm alkalization during capacitating conditions (Supplementary Table 6)¹⁻¹¹. Meizel & Deamer studied acrosomal rather than cytosolic pH; changes in pH_i during capacitation were not examined¹. Babcock and colleagues studied pH_i increase by NH₄Cl or changes in [K⁺]; neither capacitation was studied nor HCO₃⁻ was employed or mentioned². Parrish and colleagues studied the effect of heparin on alkalization³. Both pH_o and pH_i increased during 5 h incubation. The mechanism by which heparin changes pH_i is not known. Zeng and colleagues report an increase of ΔpH_i = 0.2 after HCO₃⁻ addition⁴; spontaneous recovery from alkalization does not involve Na⁺/H⁺ exchange or other ion-dependent transport pathways. Vredenburg-Wilberg & Parrish observed ΔpH_i of 0.2 upon heparin treatment for 5 h (ref. 5). Nakanishi and colleagues studied acrosomal pH_i during capacitation⁶. Demarco and colleagues measured ΔpH_i = 0.15 upon addition of 5 mM HCO₃⁻ (ref. 7). Finally, Carlson and colleagues did not observe a pH_i change upon HCO₃⁻ treatment¹¹. In summary, three out of nine studies reported ΔpH_i of 0.1-0.2 upon capacitation; one reported a larger increase (ΔpH_i = 0.75) that was related to CFTR activity⁸; one study reported no change in pH_i (ref. 11). We suggest that during lengthy incubations in an open system, CO₂ leaves the solution and, thereby, shifts the CO₂/HCO₃⁻/H⁺ equilibrium to the left, and the solution alkalizes.

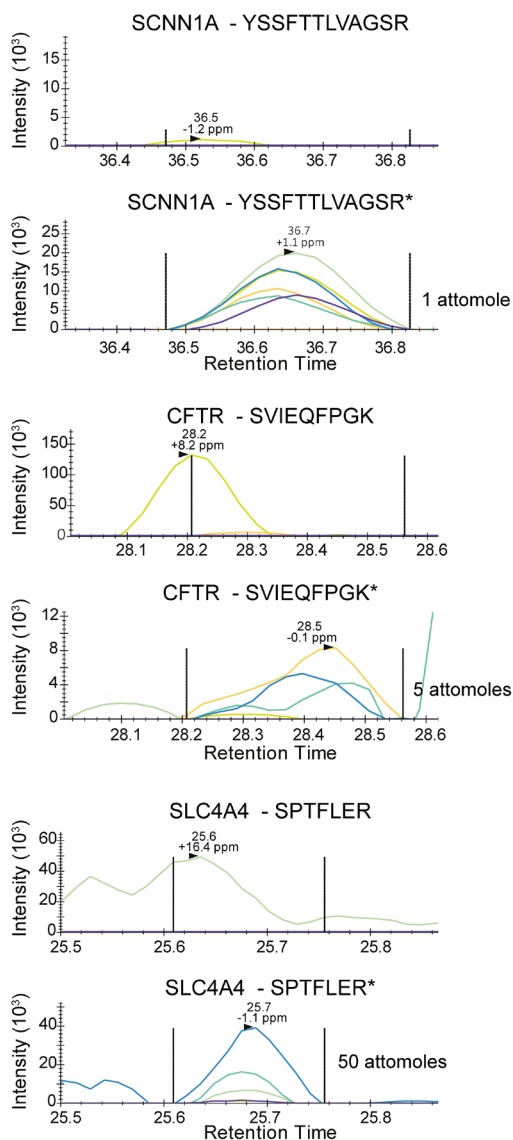
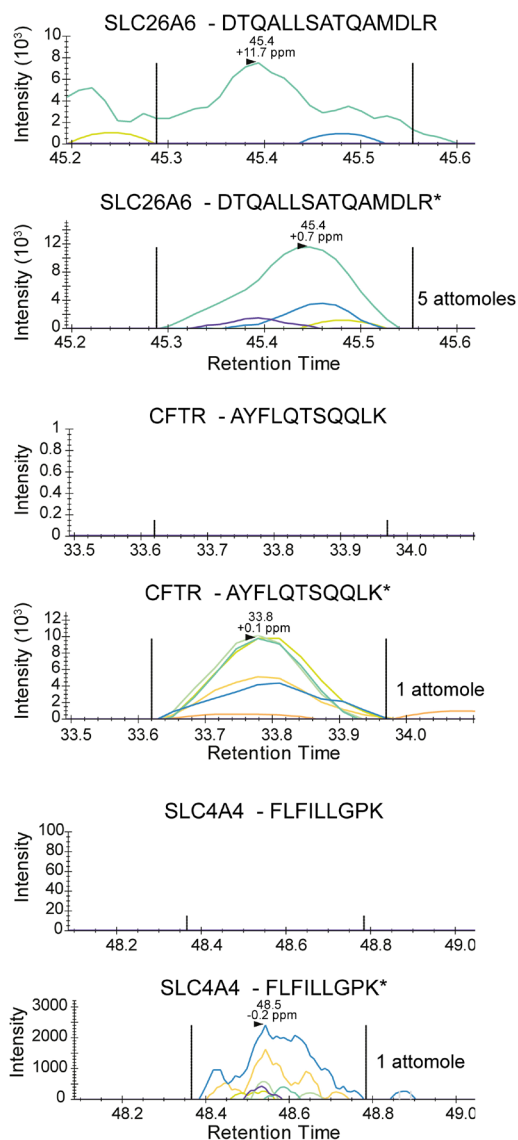
Supplementary Note 2. Kinetics of CO₂ diffusion across membranes.

We calculated the kinetics of CO₂ diffusion across cell membranes. The mechanisms of CO₂/HCO₃⁻ transport are controversial¹²⁻¹⁴, but for the following estimate irrelevant. If only CO₂ diffusion contributes, $\tau = V/(A P_{\text{CO}_2})$, wherein V is the cell volume, A is the membrane surface, and P_{CO₂} is the membrane permeability. Values for P_{CO₂} vary between 1.5x10⁻³ cm s⁻¹ and 0.33 cm s⁻¹ (ref 14). Taking the smallest P_{CO₂}, for a flagellum (radius r = 0.125 μm; length l = 50 μm) and a spherical CHO cell (r = 10 μm), τ becomes 25 ms and 333 ms, respectively. Larger P_{CO₂} values yield even faster transport rates. Thus, the kinetics of cell acidification are not rate-limited by CO₂ diffusion across the membrane. Rather, the rate constants of the CO₂ + H₂O → HCO₃⁻ + H⁺ reaction determine the rate of k the changes in pH_i. In the absence of carbonic anhydrase (CA), k = 0.037 s⁻¹ or τ = 27 s (ref. 15). In conclusion, the large difference of τ_{off} of ΔpH_i between human sperm, CHO cells, and HEK293 cells must be caused by different CA inventories.

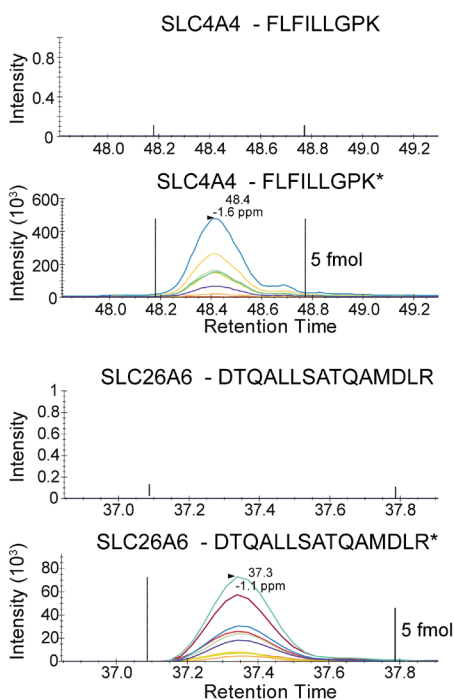
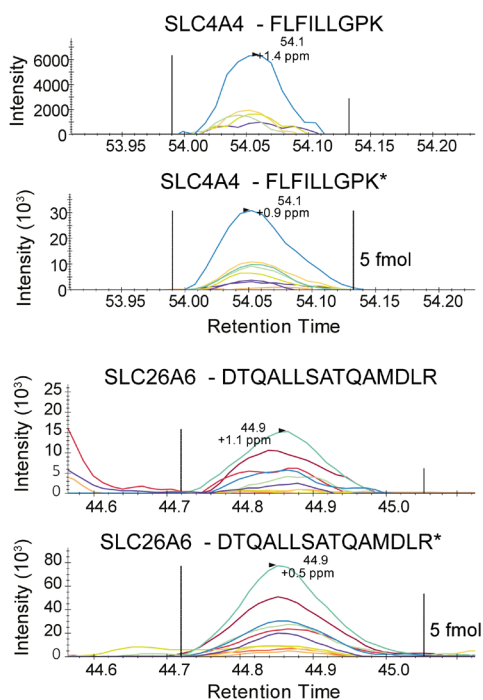
Supplementary References

- 1 Meizel, S. & Deamer, D. W. The pH of the hamster sperm acrosome. *J. Histochem. Cytochem.* **26**, 98-105 (1978).
- 2 Babcock, D. F., Rufo, G. A., Jr. & Lardy, H. A. Potassium-dependent increases in cytosolic pH stimulate metabolism and motility of mammalian sperm. *Proc. Natl. Acad. Sci. USA* **80**, 1327-1331 (1983).
- 3 Parrish, J. J., Susko-Parrish, J. L. & First, N. L. Capacitation of bovine sperm by heparin: inhibitory effect of glucose and role of intracellular pH. *Biol. Reprod.* **41**, 683-699 (1989).
- 4 Zeng, Y., Oberdorf, J. A. & Florman, H. M. pH regulation in mouse sperm: identification of Na⁺, Cl⁻, and HCO₃⁻-dependent and arylaminobenzoate-dependent regulatory mechanisms and characterization of their roles in sperm capacitation. *Dev. Biol.* **173**, 510-520 (1996).
- 5 Vredenburg-Wilberg, W. L. & Parrish, J. J. Intracellular pH of bovine sperm increases during capacitation. *Mol. Reprod. Dev.* **40**, 490-502 (1995).
- 6 Nakanishi, T., Ikawa, M., Yamada, S., Toshimori, K. & Okabe, M. Alkalinization of acrosome measured by GFP as a pH indicator and its relation to sperm capacitation. *Dev. Biol.* **237**, 222-231 (2001).
- 7 Demarco, I. A. *et al.* Involvement of a Na⁺/HCO₃⁻ cotransporter in mouse sperm capacitation. *J. Biol. Chem.* **278**, 7001-7009 (2003).
- 8 Xu, W. M. *et al.* Cystic fibrosis transmembrane conductance regulator is vital to sperm fertilizing capacity and male fertility. *Proc. Natl. Acad. Sci. USA* **104**, 9816-9821 (2007).
- 9 Cross, N. L. & Razy-Faulkner, P. Control of human sperm intracellular pH by cholesterol and its relationship to the response of the acrosome to progesterone. *Biol. Reprod.* **56**, 1169-1174 (1997).
- 10 Lopez-Gonzalez, I. *et al.* Membrane hyperpolarization during human sperm capacitation. *Mol. Hum. Reprod.* **20**, 619-629 (2014).
- 11 Carlson, A. E., Hille, B. & Babcock, D. F. External Ca²⁺ acts upstream of adenylyl cyclase SACY in the bicarbonate signaled activation of sperm motility. *Dev. Biol.* **312**, 183-192 (2007).
- 12 Missner, A. & Pohl, P. 110 years of the Meyer-Overton rule: predicting membrane permeability of gases and other small compounds. *Chem. Phys. Chem.* **10**, 1405-1414 (2009).
- 13 Boron, W. F., Endeward, V., Gros, G., Musa-Aziz, R. & Pohl, P. Intrinsic CO₂ permeability of cell membranes and potential biological relevance of CO₂ channels. *Chem. Phys. Chem.* **12**, 1017-1019 (2011).
- 14 Endeward, V., Arias-Hidalgo, M., Al-Samir, S. & Gros, G. CO₂ Permeability of Biological Membranes and Role of CO₂ Channels. *Membranes (Basel)* **7** (2017).
- 15 Roughton, F. J. W. The Kinetics and Rapid Thermochemistry of Carbonic Acid. *J. Am. Chem. Soc.* **11**, 2930-2934 (1941).

a



b

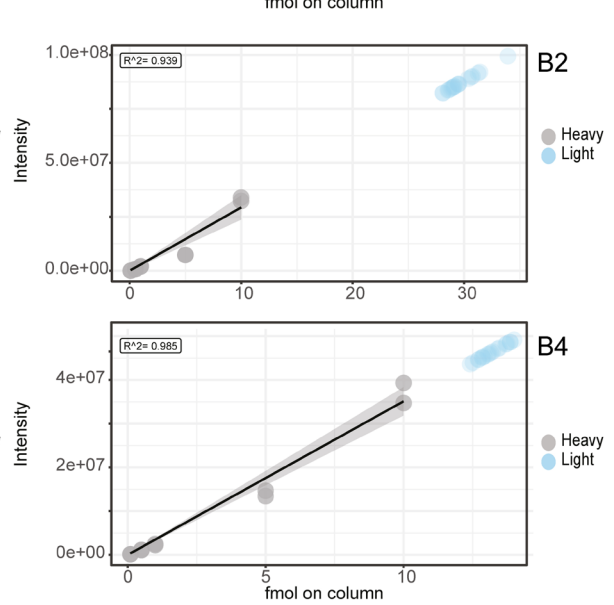
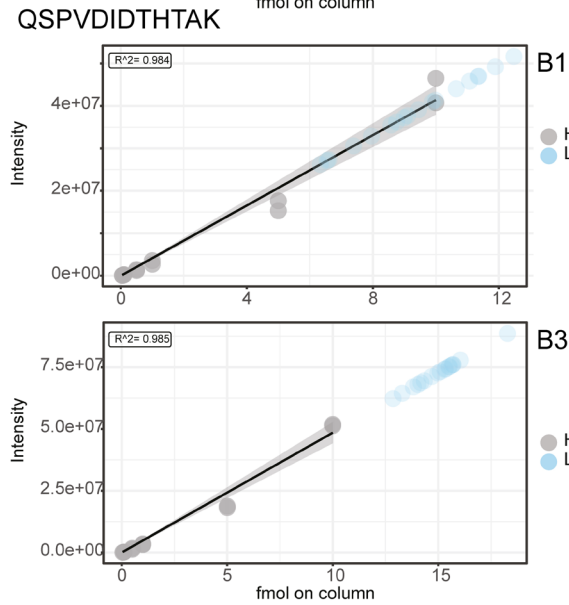
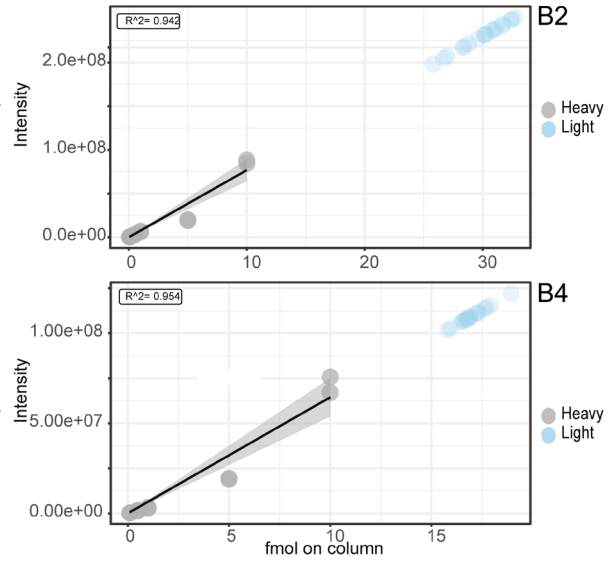
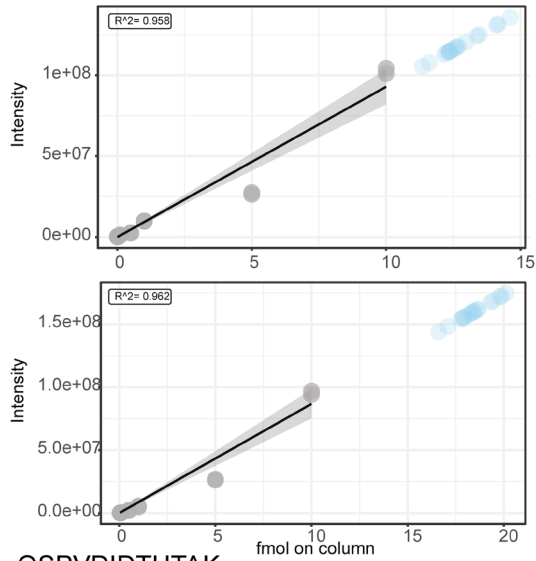


Supplementary Figure 1. MS signal of lowest LOD values and positive controls.

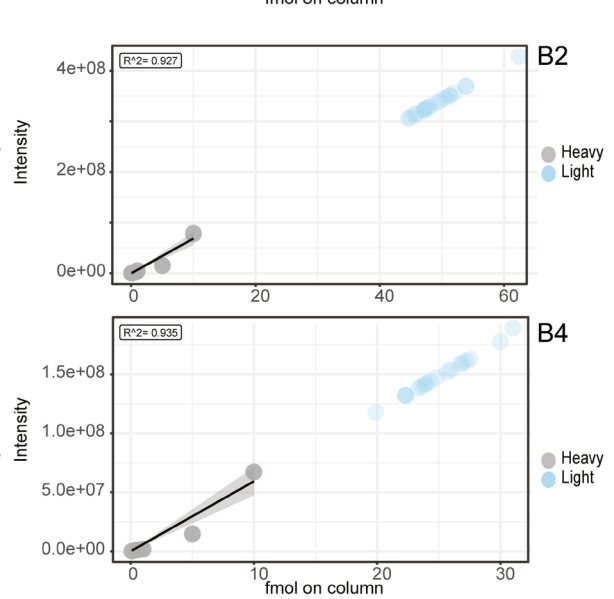
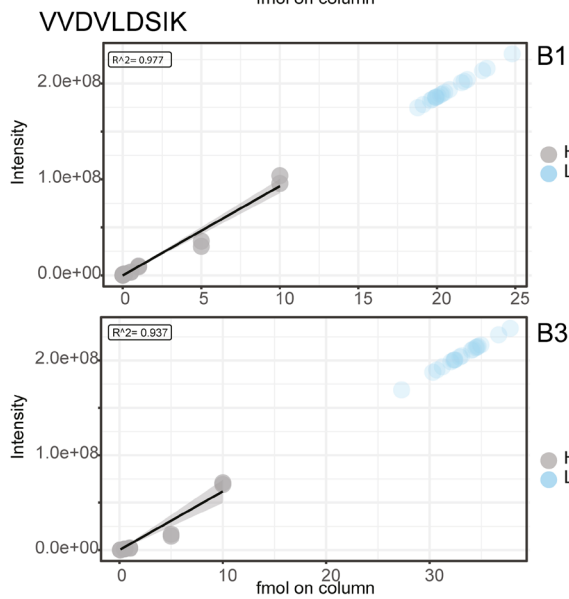
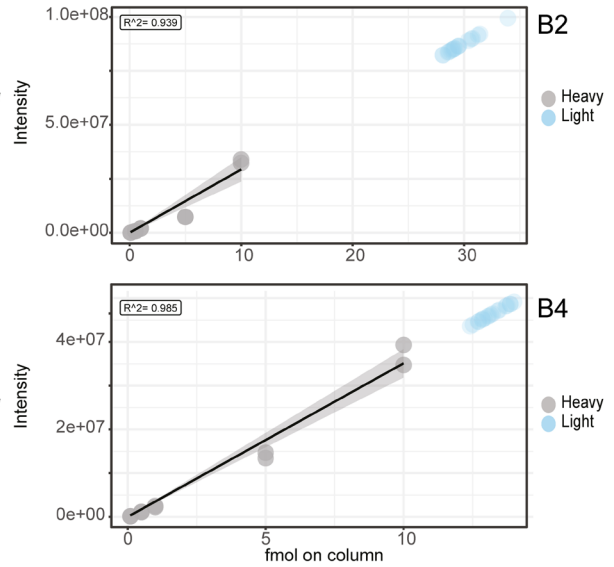
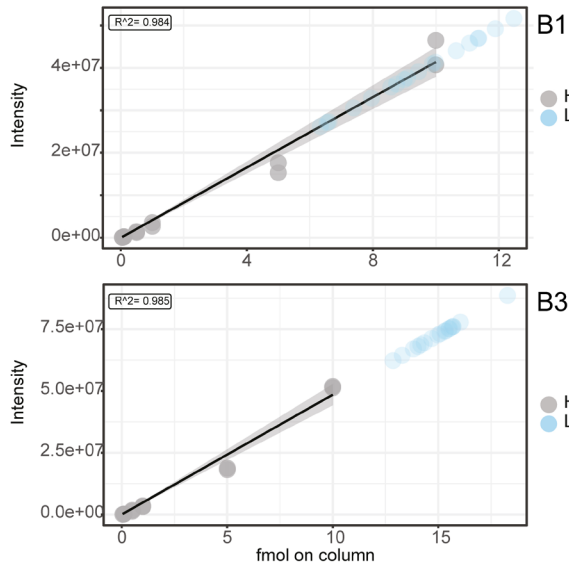
(a) Transition channels of the most sensitive PRM analysis of all used peptides corresponding to SCNN1A, CFTR, SLC4A4, and SLC26A6. Peptide sequences are given; asterisks denote the ^{13}C - and ^{15}N -labelled amino acids in standard peptides. Upper panels: No specific PRM signals detected for endogenous peptides derived from 90,000 sperm cells; lower panels: PRM signals detected from 1 attomole (5 attomoles for DTQALLSATQAMDRL (SLC26A6) and 50 attomoles for SPTFLER (SLC4A4)) of standard peptide spiked into endogenous peptides derived from 90,000 sperm cells. Transitions of the different fragment ions are color-coded. **(b)** Transition channels of PRM analysis of heavy peptides spiked into HEK293 cell peptides as positive control. Upper panel: On the left, 5 fmol of standard peptide FLFILLGPK (SLC4A4) spiked into 1 μg of HEK293 cell peptides, signal for standard and endogenous peptide can be detected; on the right, 5 fmol of this standard peptide measured without matrix, no endogenous signal can be detected. Lower panel: On the left, 5 fmol of standard peptide DTQALLSATQAMDRL (SLC26A6) spiked into 1 μg of HEK293 peptides, signal for standard and endogenous signal can be detected; on the right, 5 fmol of this peptide measured without matrix, no endogenous signal can be detected.

a Calibration curves for quantification of carbonic anhydrase 2.

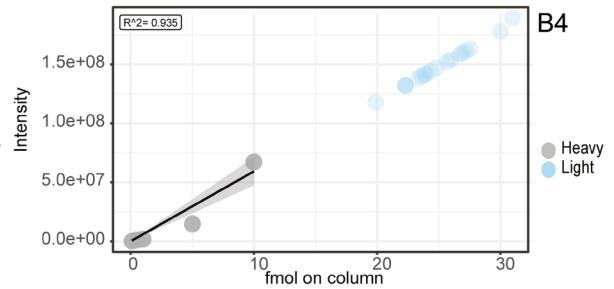
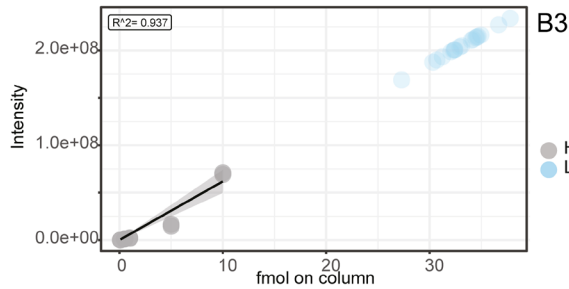
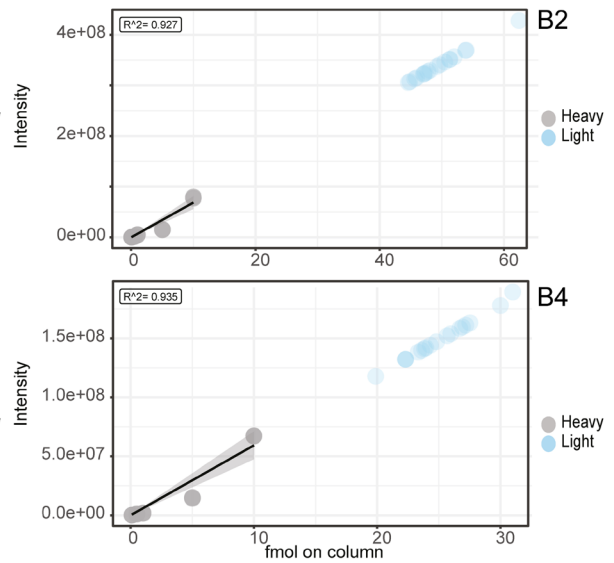
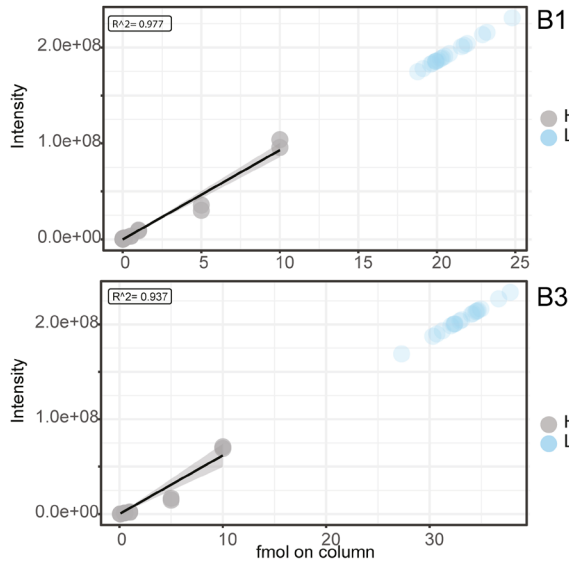
EPISVSSEQVLK



QSPVDIDHTAK

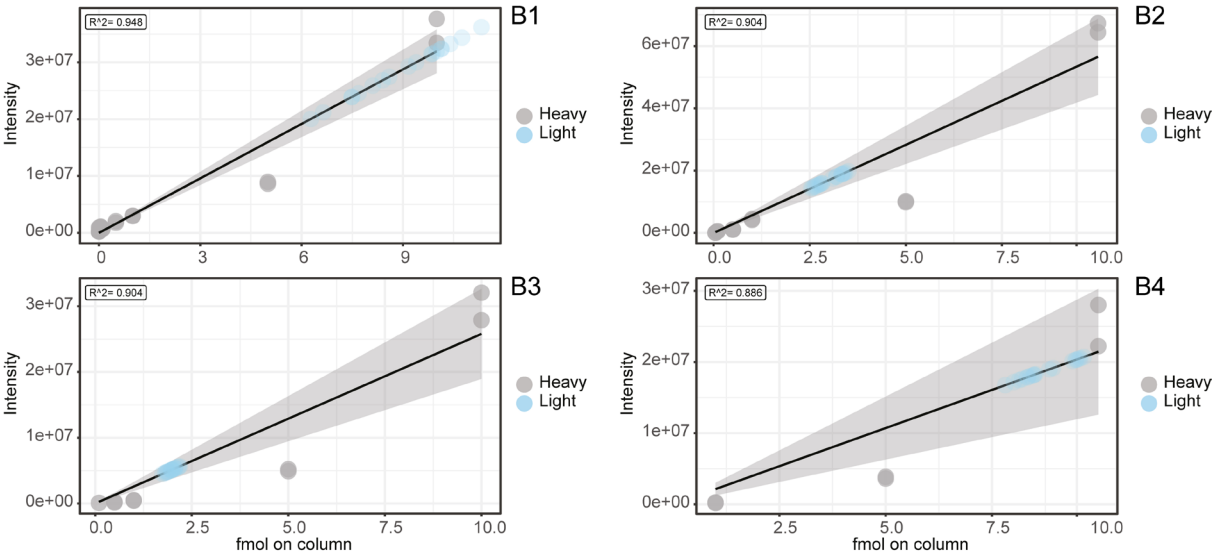


VVDVLDSIK

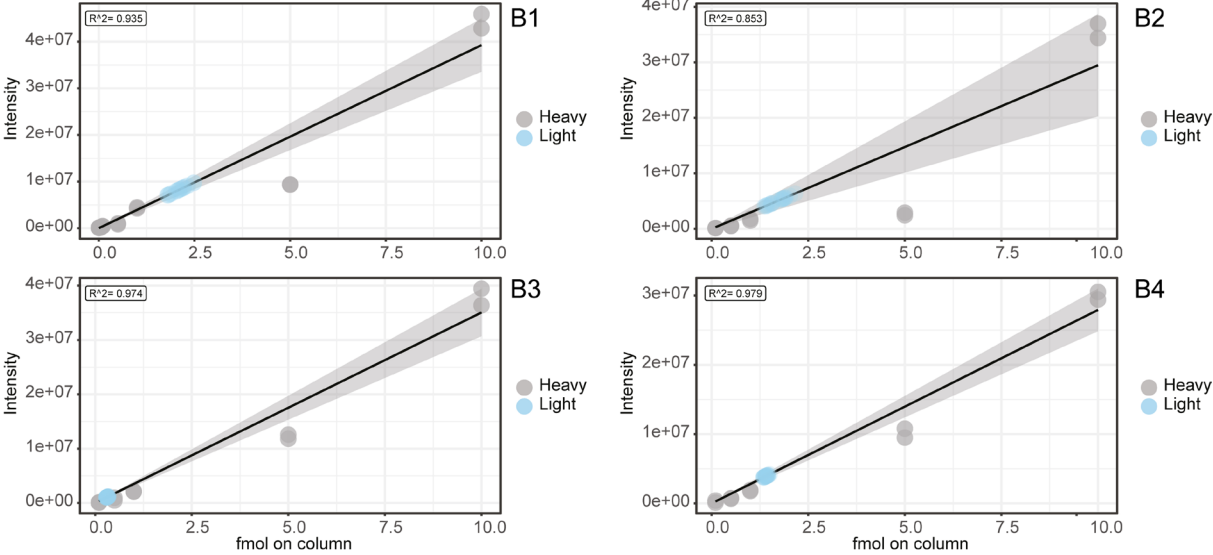


b Calibration curves for quantification of chloride anion exchanger SLC26A3.

LIDAVGFSPLR

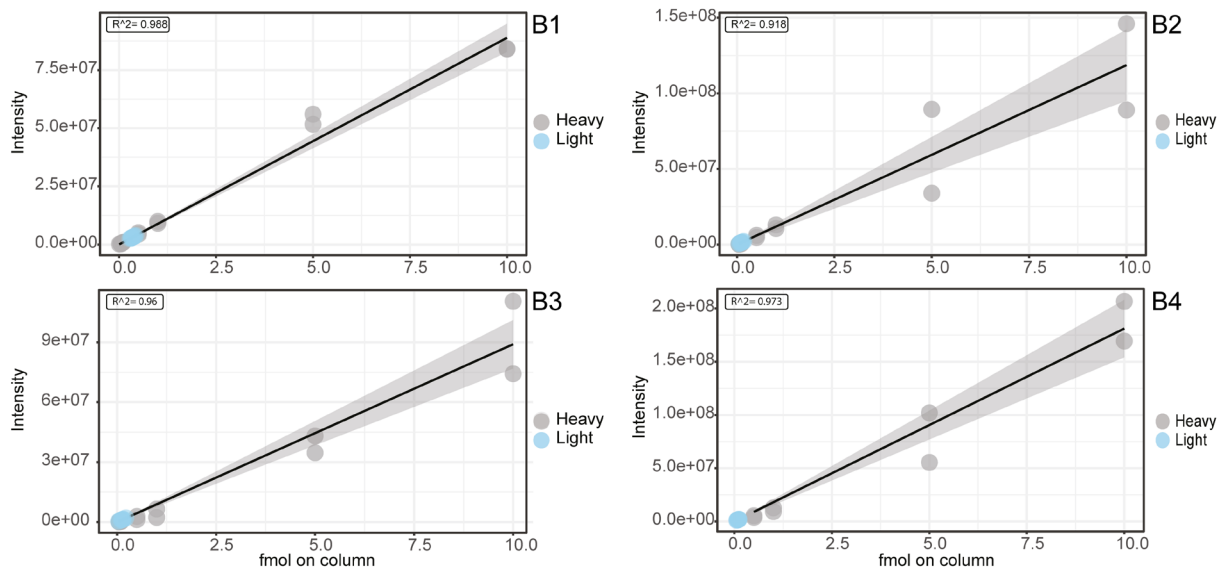


YEFFDGEVK

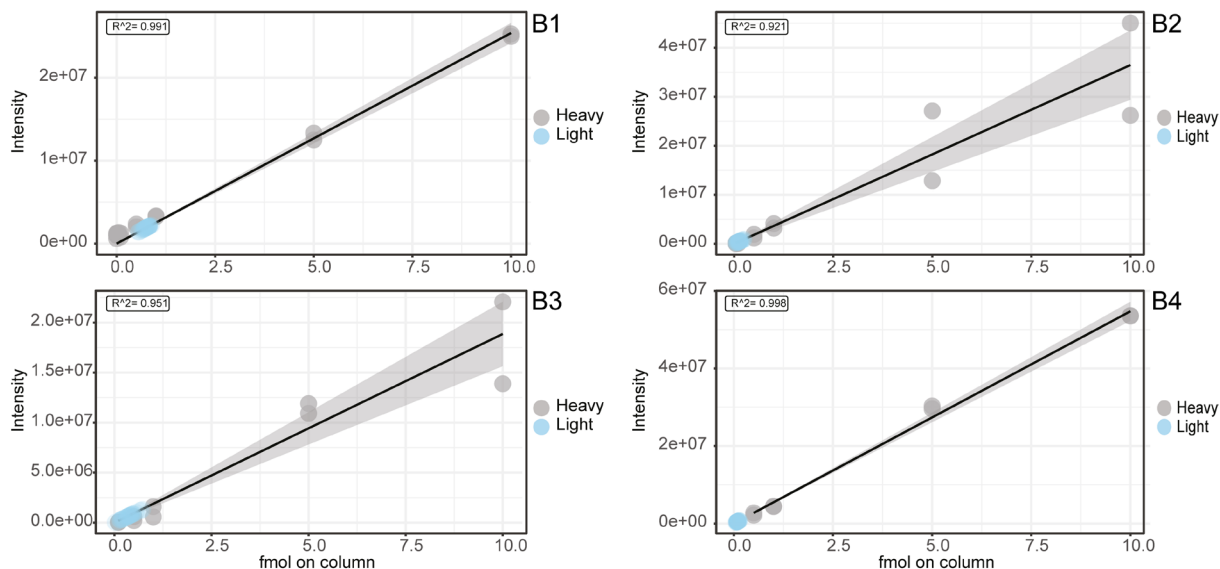


c Calibration curves for quantification of CatSper1.

SLIHDAPGPAASR

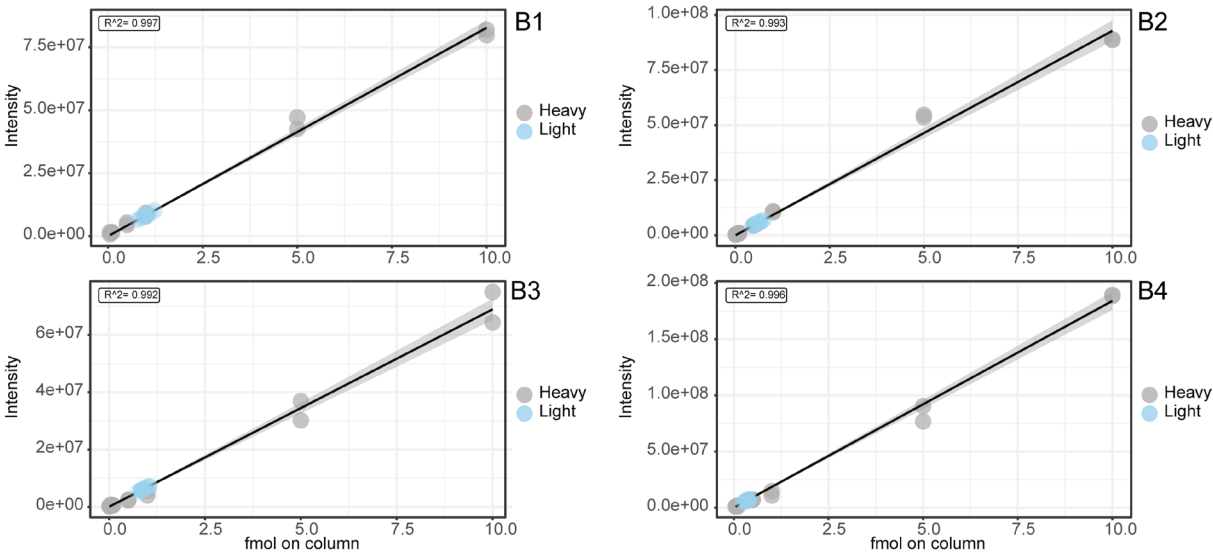


SYGEDYHDELQR



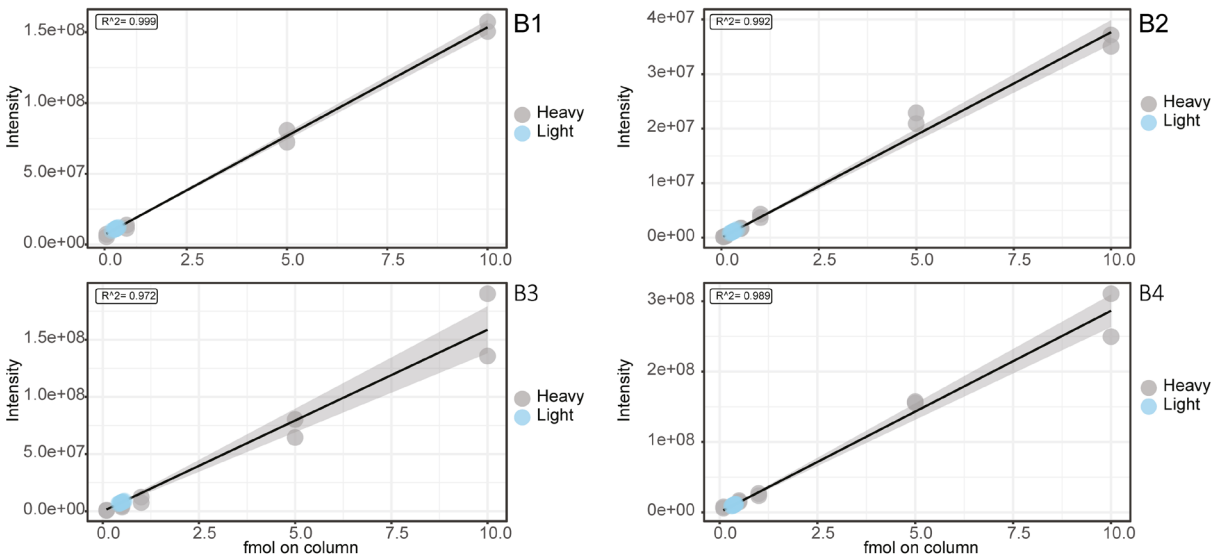
d Calibration curves for quantification of CatSper2.

LQYNLEER

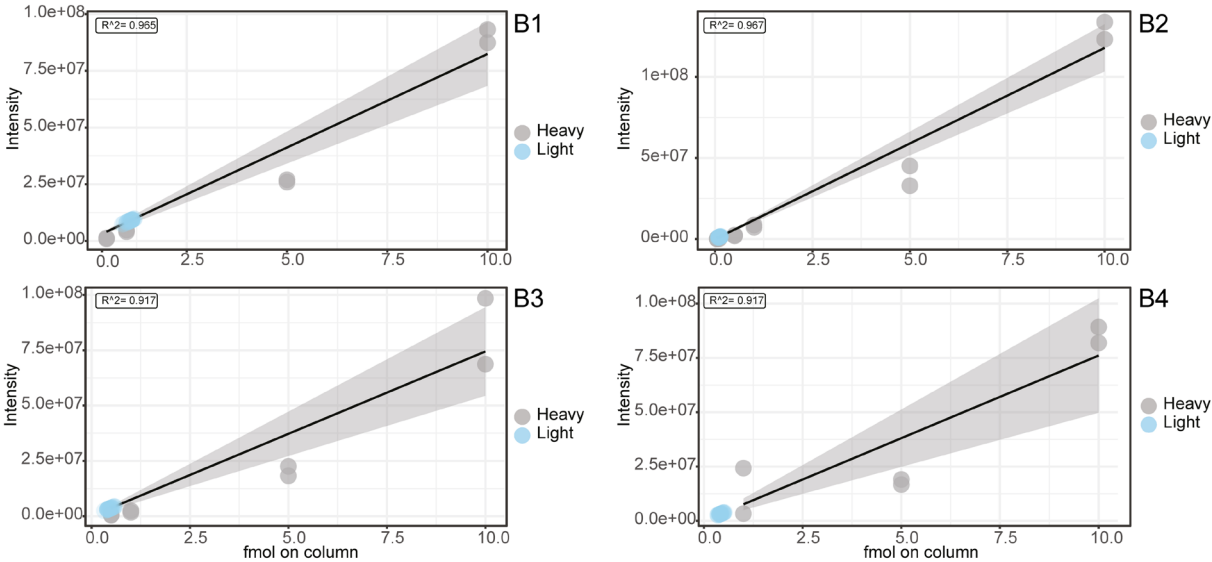


e Calibration curves for quantification of CatSper3.

LIGYSQGIR

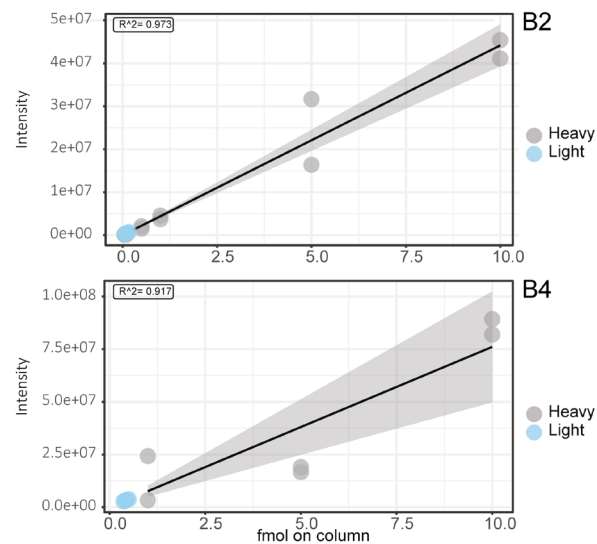
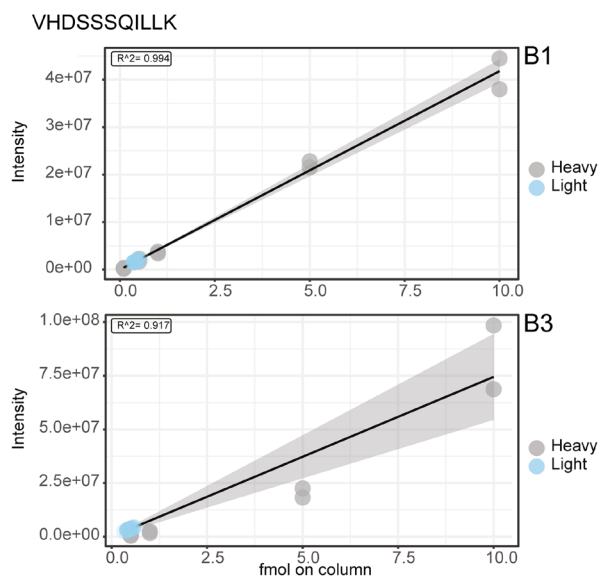
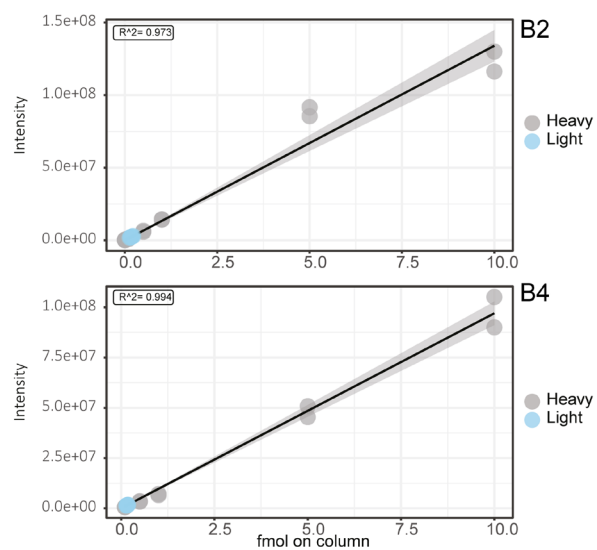
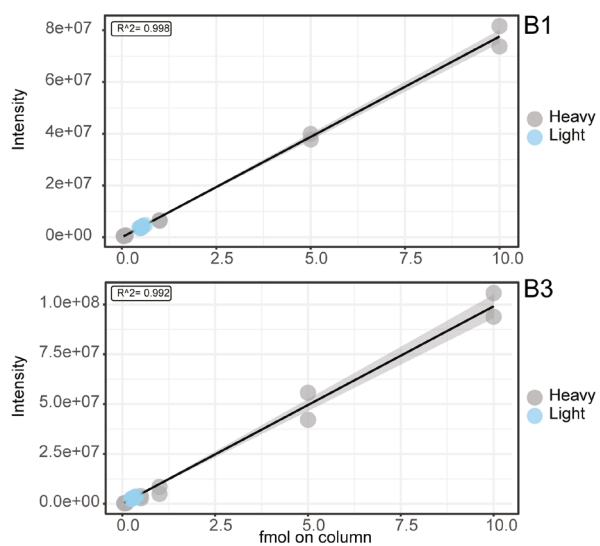


TVASVLLR

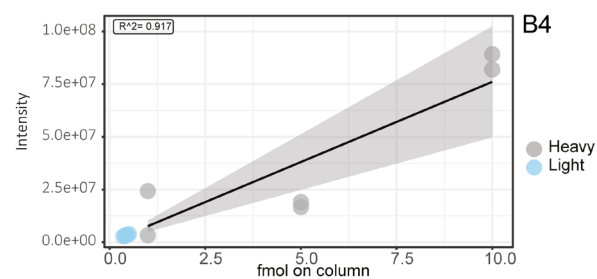
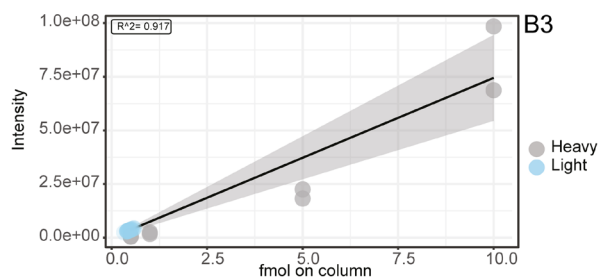
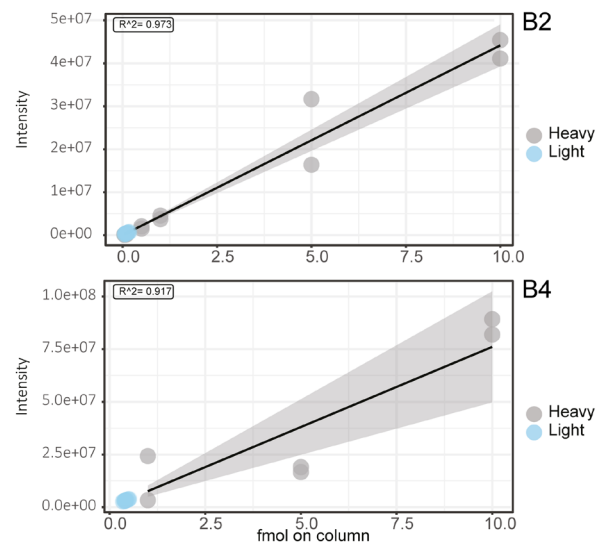
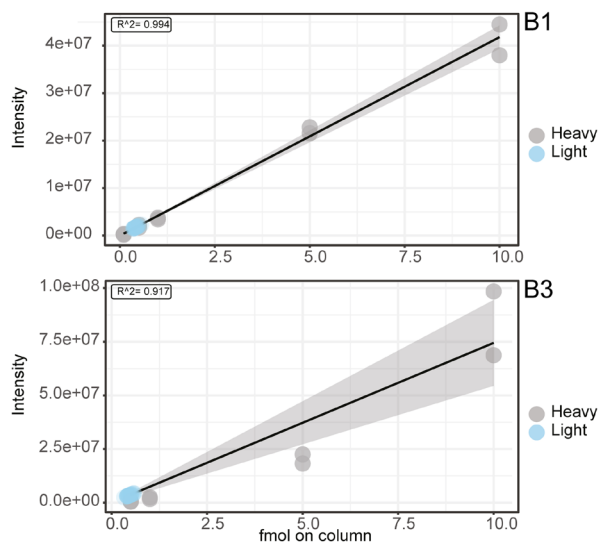


f Calibration curves for quantification of CatSper4.

FNQQESEVLNR

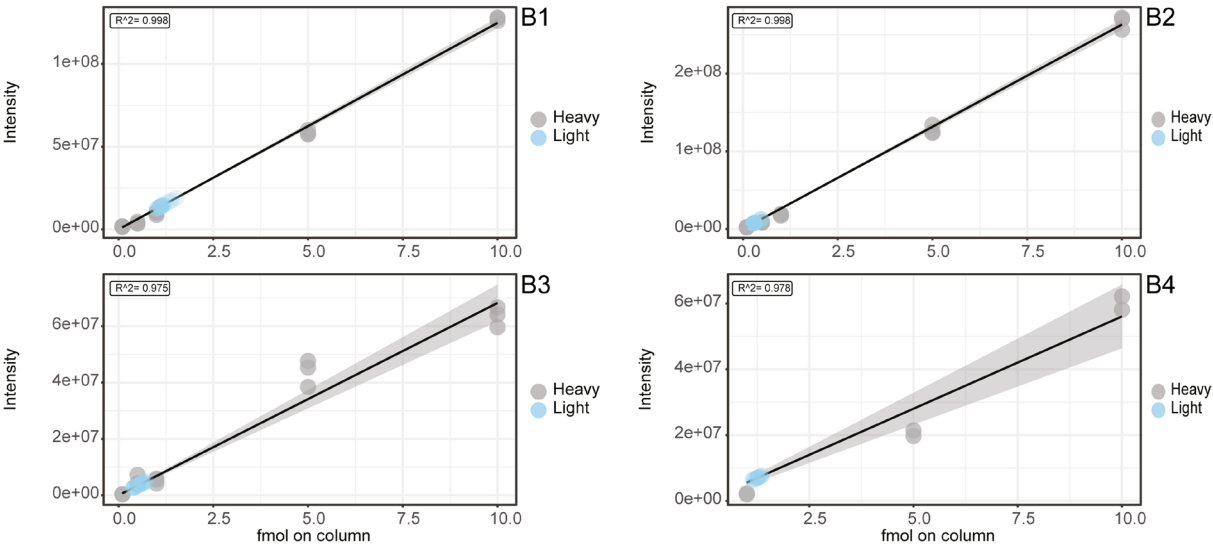


VHDSSSQILLK

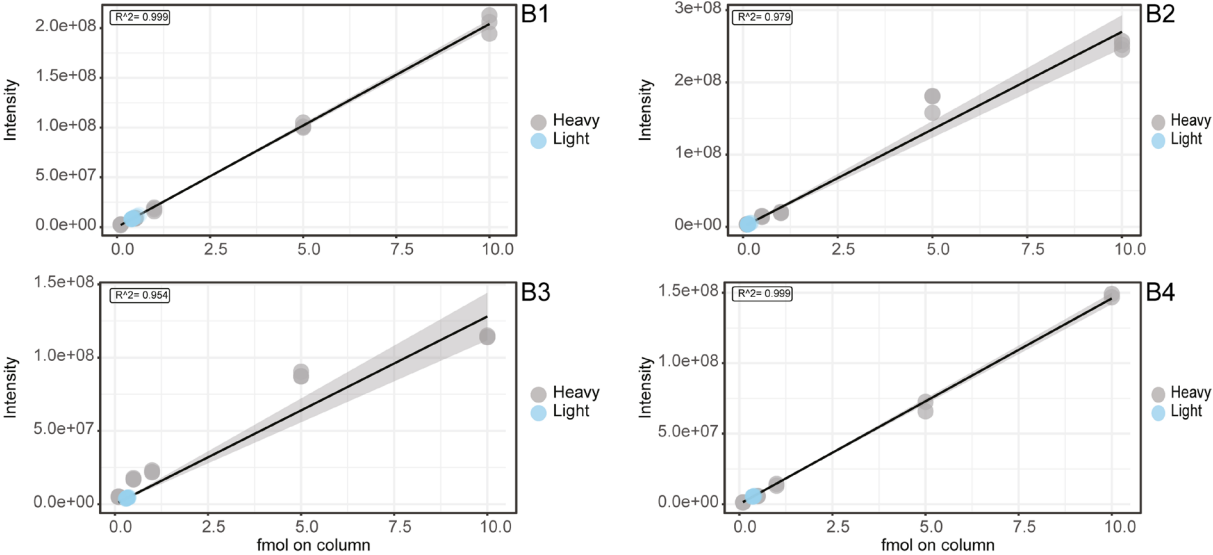


g Calibration curves for quantification of CatSperB.

GFPQENEIIK

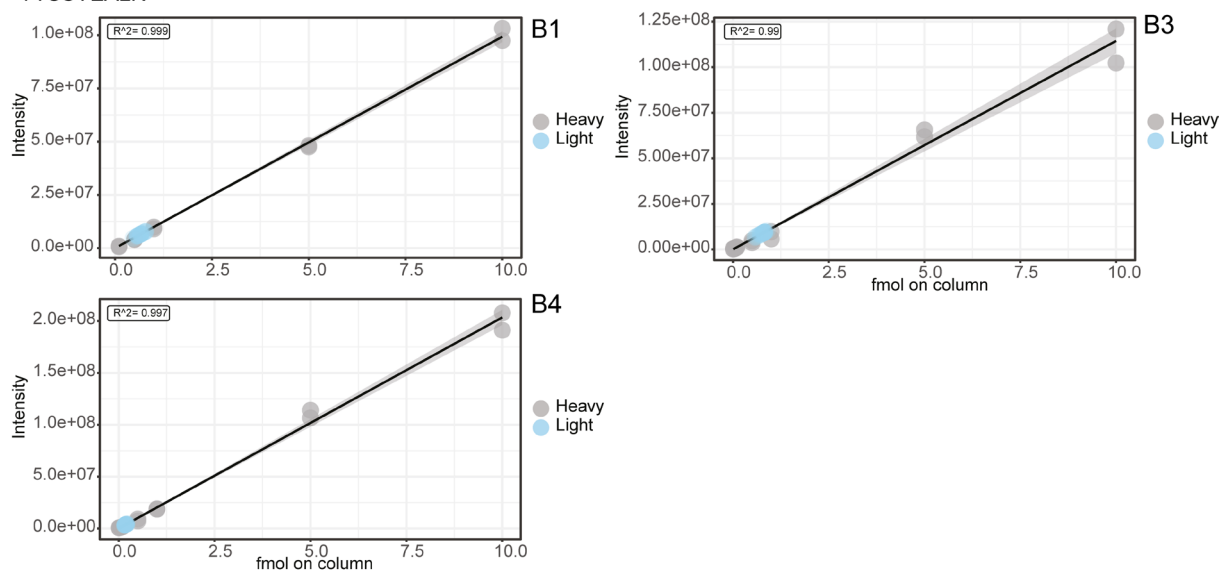


FEASGPPTAFGNSR



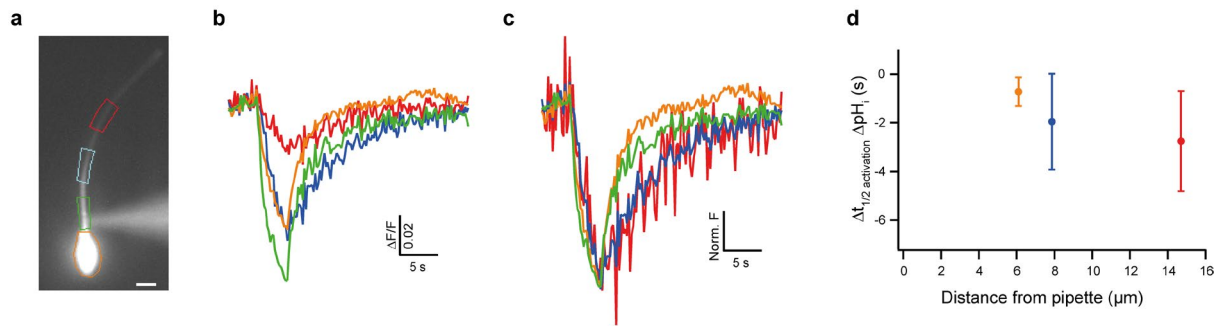
h Calibration curves for quantification of Slo3.

YTSSYEALK



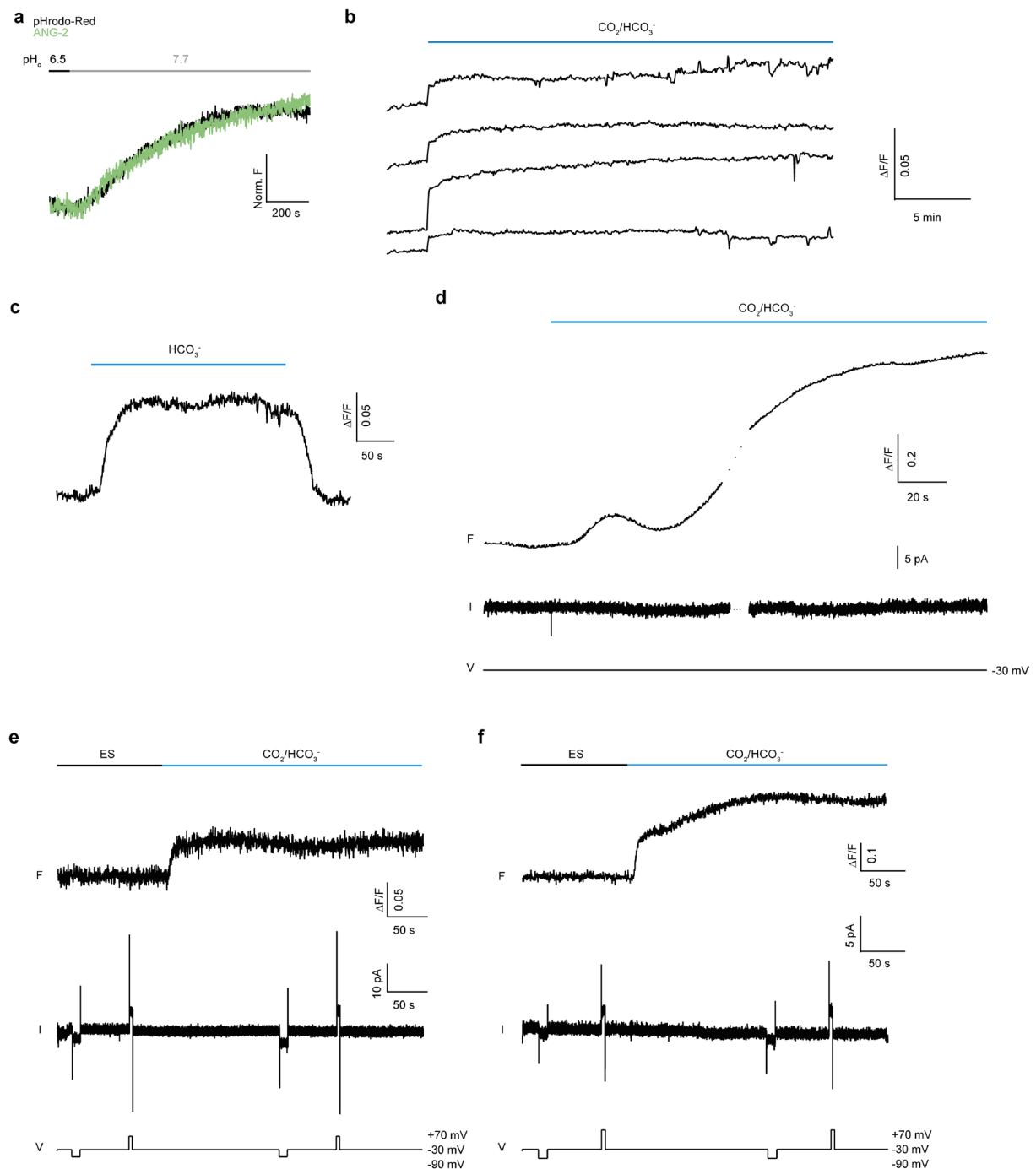
Supplementary Figure 2. Calibration curves for all quantified proteins.

(a-h) Calibration curves generated for quantification of CA2, SLC26A3, CatSper1-4, CatSperB, and Slo3. Calibration curves from all biological replicates and for all peptides of the respective proteins are shown. X-axis shows fmol of peptide on column and y-axis shows the signal intensity. Grey data points correspond to signal generated by standard peptide, turquoise data points correspond to the signals generated by endogenous peptides. R² value for each calibration curve is given in the inset. Shaded grey area indicates confidence interval of the linear regression. Source data is provided as a source data file.



Supplementary Figure 3. PCF recordings of human sperm. Comparison of changes in pH_i in different compartments of the cell.

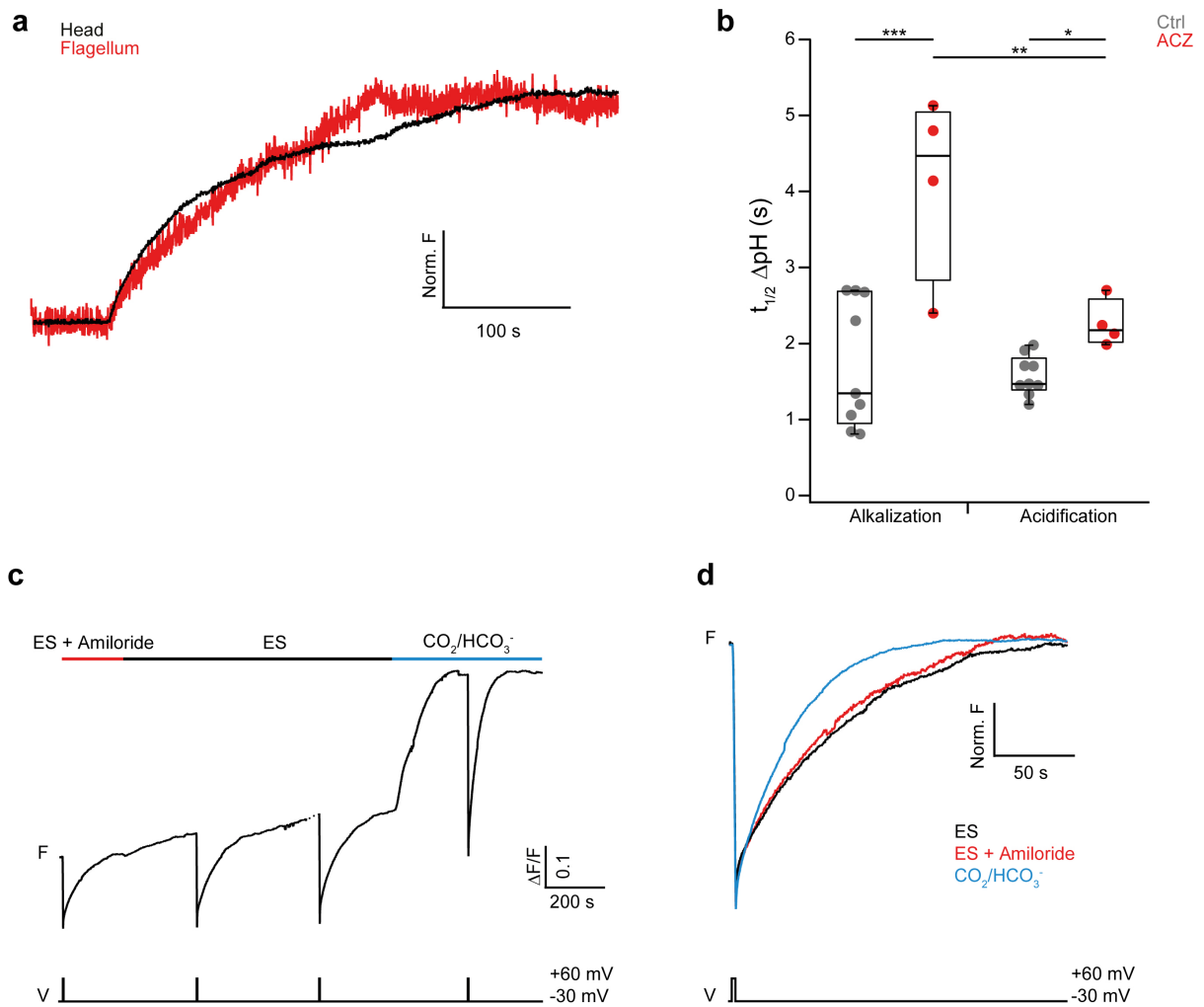
(a) pHrodo-Red-loaded human sperm with attached patch pipette and manually chosen ROIs. Scale bar 3 μm. (b) Alkalization induced by a +70-mV depolarization step and subsequent H_v1 activation during PCF recording in presence of CO₂/HCO₃⁻ (5%/25 mM). Superposition of fluorescent signal of different ROIs. (c) Normalized superposition of fluorescent signals from (b). (d) Difference of t_{1/2} of H_v1-induced alkalization activation from respective ROIs compared to the ROI at the pipette (green, n_{cells} = 3, data points and error bars show mean ± SD). Source data is provided as a source data file.



Supplementary Figure 4. Additional experiments testing for Na⁺/H⁺ exchange and behavior of sperm exposed to CO₂/HCO₃⁻.

(a) Superposition of the fluorescence change of pHrodo-Red (black) and ANG-2 (green) in a sperm cell upon switching from pH_o6.5 to pH_o7.7. **(b)** Long-term recording of pH_i with pHrodo Red-loaded sperm before and during superfusion with CO₂/HCO₃⁻ (5%/25 mM).

Traces from four individual sperm cells. **(c)** Fluorescence change of pHrodo-Red loaded sperm during exposure to 25 mM HCO_3^- without additional CO_2 gassing. **(d)** PCF recording showing changes in pH_i (pHrodo-Red 12.5 μM) (F) and currents (I) upon superfusion of sperm with $\text{CO}_2/\text{HCO}_3^-$ (5%/25 mM). Cell was held at $V_m = -30$ mV. The gap was caused by the software delay between the start of two subsequent recordings. **(e)** PCF recording from sperm of a wt mouse during superfusion with ES (black bar) and $\text{CO}_2/\text{HCO}_3^-$ (blue bar). Changes in pH_i (upper) and currents (lower). V_m was first stepped from a holding potential of -30 mV to -90 mV to activate putative voltage-gated SLC9C1, followed by a step from -30 mV to +70 mV to activate H_v1 . **(f)** Similar recordings from sperm of an *SLC9C1*^{-/-} mouse.

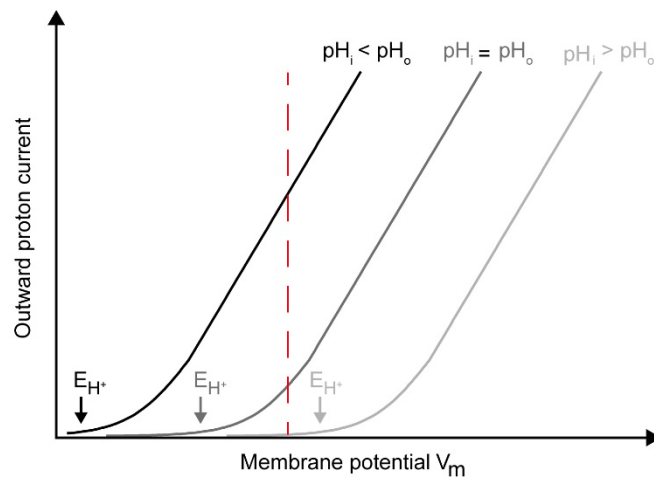


Supplementary Figure 5. Additional PCF experiments on human sperm and CHO cells expressing hHv1.

(a) Time course of fluorescent change during solution exchange between pipette and sperm head (black) and flagellum (red) measured by PCF after break-in. The pipette was filled with 12.5 μM of pHrodo Red-maleimide. The holding potential V_m was -30 mV. **(b)** Half times $t_{1/2}$ of Hv1-mediated alkalization and subsequent acidification of a single sperm cell in absence (grey) and in presence of ACZ (100 μM , red; $n_{\text{control}} = 9$; $n_{\text{ACZ}} = 4$). Two-factor ANOVA followed by post-hoc Tukey's test for multiple comparison: Ctrl_{Alk} vs. ACZ_{Alk} $p < 0.0001$; Ctrl_{Acid} vs. ACZ_{Acid} $p = 0.4$; Ctrl_{Alk} vs. Ctrl_{Acid} $p = 0.9$; ACZ_{Alk} vs. ACZ_{acid} $p = 0.006$. **(c)** Changes in pH_i recorded by patch-clamp fluorometry in a CHO cell expressing hHv1. The cell

was sequentially superfused with ES + amiloride, ES, and finally ES + CO₂/HCO₃⁻ (5%/25 mM). The pH_i response was elicited by stepping V_m from -30 mV to +60 mV. **(d)**

Superposition of the normalized pH_i response in ES (black), ES + amiloride (500 μM) (red), and ES + CO₂/HCO₃⁻ (blue). Box plots represent 25%, 50% (median), and 75% quartiles, with whiskers displaying minimum and maximum data points. Source data is provided as a source data file.



Supplementary Figure 6. Control of H_v1 activity by V_m and ΔpH_{tm} across the membrane.

Scheme illustrating the control of H_v1 by ΔpH_{tm} across the membrane and V_m. The interrupted line (red) illustrates that proton currents via H_v1 can be activated without a change in V_m.

Supplementary Table 1. Summary of proteomic studies.

Author	Method	Preparation	Detected proteins
Wang et al., 2013	LC-MS/MS	60% Percoll gradient centrifugation	4675 sperm proteins; 227 testis-specific
Baker et al., 2013	LC-MS/MS	15-75% Percoll gradient fractionation + head/tail separation by sonification & Percoll centrifugation	1429
Gu et al., 2011	LC-MS/MS	Swim-up	1019
Amaral et al., 2014	LC-MS/MS	50% Percoll centrifugation + leucocyte removal with Dynabeads + tail isolation by sonification & sucrose gradient centrifugation	1049

Supplementary Table 2: Signaling proteins identified in human sperm by MS

genes	encoded proteins	Uniprot entry	Wang et al.	B1	B2	B3
<i>ABHD2</i>	Monoacylglycerol lipase	P08910	+	+	+	+
<i>ADCY10</i>	Soluble adenylate cyclase	Q96PN6	+	+	+	+
<i>AKAP3</i>	A-Kinase anchor protein 3	Q75969	+	+	+	+
<i>AKAP4</i>	A-Kinase anchor protein 4	Q5JQC9	+	+	+	+
<i>ATP1A4</i>	Na ⁺ /K ⁺ -ATPase alpha-4	Q13733	+	+	+	+
<i>ATP2B1</i>	Plasma membrane Ca ²⁺ -transporting ATPase	P20020	+	+	+	+
<i>ATP2B3</i>	Plasma membrane Ca ²⁺ -transporting ATPase	Q16720	-	+	+	+
<i>ATP2B4</i>	Plasma membrane Ca ²⁺ -transporting ATPase	P23634	+	+	+	+
<i>CA 2</i>	Carbonic anhydrase 2	P00918	+	+	+	+
<i>CA 4</i>	Carbonic anhydrase 4	P22748	+	+	+	+
<i>CATSPER 1</i>	Sperm-specific Ca ²⁺ channel 1	Q8NEC5	+	+	-	+
<i>CATSPER 2</i>	Sperm-specific Ca ²⁺ channel 2	Q96P56	+	+	+	+
<i>CATSPER 3</i>	Sperm-specific Ca ²⁺ channel 3	Q86XQ3	-	+	+	+
<i>CATSPER 4</i>	Sperm-specific Ca ²⁺ channel 4	Q7RTX7	+	+	+	+
<i>CATSPER B</i>	Sperm-specific Ca ²⁺ channel beta	Q9H7T0	+	+	+	+
<i>CATSPER G</i>	Sperm-specific Ca ²⁺ channel gamma	Q6ZRH7	+	+	+	+
<i>CATSPER D</i>	Sperm-specific Ca ²⁺ channel delta	Q86XM0	+	+	+	+
<i>CATSPER E</i>	Sperm-specific Ca ²⁺ channel epsilon	Q5SY80	+	+	+	+
<i>CATSPER Z</i>	Sperm-specific Ca ²⁺ channel zeta	Q9NTU4	+	+	-	-
<i>EFCAB9</i>	Ca ²⁺ -binding protein	A8MZ26	+	+	+	+
<i>HVCN1</i>	H _v 1 channel	Q96D96	+	+	+	+
<i>KCNU1</i>	Slo3 K ⁺ channel	A8MYU2	+	+	+	+
<i>LRRC52</i>	Auxiliary subunit of Slo3	Q8N7C0	+	+	+	+
<i>PDE10A</i>	cAMP-specific phosphodiesterase	Q9Y233	+	+	+	+
<i>PPP1CC</i>	Sperm-specific phosphatase	P36873		+	+	+
<i>PRKACA</i>	cAMP-dependent protein kinase catalytic subunit alpha	P17612	+	+	+	+
<i>PRKARIA</i>	cAMP-dependent protein kinase type I regulatory subunit alpha	P10644	+	+	+	+
<i>PRKAR2A</i>	cAMP-dependent protein kinase type II regulatory subunit alpha	P13861	+	+	+	+
<i>PRKAR1B</i>	cAMP-dependent protein kinase type I regulatory subunit beta	P31321	+	+	+	+
<i>SLC9C1</i>	Na ⁺ /H ⁺ exchange	Q4G0N8		+	-	+
<i>SLC9C2</i>	Na ⁺ /H ⁺ exchange	Q5TAH2	- ¹	+	+	+
<i>SLC9B1</i>	Na ⁺ /H ⁺ exchange	Q4ZJ14	-	+	+	+

<i>SLC9B2</i>	Na ⁺ /H ⁺ exchange	Q86UD5	-	+	+	+
<i>SLC26A3</i>	Cl ⁻ /HCO ₃ ⁻ transport	P40879	-	+	+	+
<i>SLC26A8</i>	Cl ⁻ , oxalate, SO ₄ ²⁻ transport	Q96RN1	+	+	+	+
<i>SLC30A1</i>	Zn ²⁺ transport	Q9Y6M5	+	+	+	+
<i>SLC30A9</i>	Zn ²⁺ transport	Q6PML9	-	+	+	+
<i>SLCO6A1</i>	organic anion transport	Q86UG4	+	+	+	+
<i>TMEM249</i>	CatSper auxiliary subunit	Q2WGI8	+	+	+	+

¹Two peptides were detected in 1 from 3 measurements. The peptide sequences do not completely agree with the SLC9C1 sequence (86%). Probably SLC9C2 was detected.

Supplementary Table 3: Diverse other sperm-specific proteins identified in human sperm by MS

genes	encoded proteins	Uniprot entry	Wang et al.	B1	B2	B3
<i>ACR</i>	Acrosin	P10323	+	+	+	+
<i>CRISP1</i>	Cystein-rich secretory protein 1	P54107	+	+	+	+
<i>DNAH17</i>	Dynein heavy chain 17 axonemal	Q9UFH2	+	+	+	+
<i>GAPDH S</i>	Sperm-specific glyceraldehyde-3-phosphate dehydrogenase	Q14556	+	+	+	+
<i>IZUMO1</i>	Izumo sperm-egg fusion protein 1	Q8IYV9	+	+	+	+
<i>IZUMO2</i>	Izumo sperm-egg fusion protein 2	Q6UXV1	+	+	+	+
<i>IZUMO3</i>	Izumo sperm-egg fusion protein 3	Q5VZ72	+	+	+	+
<i>IZUMO4</i>	Izumo sperm-egg fusion protein 4	Q1ZYL8	+	+	+	+
<i>LDHC</i>	L-Lactate dehydrogenase c	P07864	+	+	+	+
<i>ODF1</i>	Outer dense fiber protein 1	Q14990	+	+	+	+
<i>ODF2</i>	Outer dense fiber protein 2	Q5BJF6	+	+	+	+
<i>ODF3</i>	Outer dense fiber protein 3A	Q96PU9	+	+	+	+
<i>ODF3B</i>	Outer dense fiber protein 3B	A8MYP8	+	+	+	+
<i>PPP3R2</i>	Calcineurin subunit B type 2	Q96LZ3	+	+	+	+
<i>PLCZ1</i>	Phospholipase C zeta 1	Q86YW0	+	+	+	+
<i>SPACA1</i>	Sperm acrosome-associated protein 1	Q9HBV2	+	+	+	+
<i>SPACA3</i>	Sperm acrosome-associated protein 3	Q8IXA5	+	+	+	+
<i>SPACA4</i>	Sperm acrosome-associated protein 4	Q8TDM5	+	+	+	+
<i>SPACA5</i>	Sperm acrosome-associated protein 5	Q96QH8	+	+	+	+
<i>SPACA6</i>	Sperm acrosome-associated protein 6	W5XKT8	-	+	+	+
<i>SPACA7</i>	Sperm acrosome-associated protein 7	Q96KW9	+	+	+	+
<i>SPACA9</i>	Sperm acrosome-associated protein 9	Q96E40	-	+	+	+

Supplementary Table 4: Signaling proteins that were <i>not</i> detected in human sperm by MS			
genes	encoded proteins	Uniprot entry	Wang et al.
<i>CFTR</i>	Cystic fibrosis transmembrane conductance	P13569	-
<i>KCNMA1</i>	K ⁺ channel Slo1	Q12791	-
<i>KCNMB1</i>	K ⁺ channel Slo1 beta subunit 1	Q16558	-
<i>KCNMB2</i>	K ⁺ channel Slo1 beta subunit 2	Q9Y691	-
<i>KCNMB3</i>	K ⁺ channel Slo1 beta subunit 3	Q9NPA1	-
<i>KCNMB4</i>	K ⁺ channel Slo1 beta subunit 4	Q86W47	-
<i>LRRC26</i>	K ⁺ channel Slo1 gamma subunit 1	Q2I0M4	-
<i>LRRC38</i>	K ⁺ channel Slo1 gamma subunit 4	Q5VT99	-
<i>LRRC55</i>	K ⁺ channel Slo1 gamma subunit 3	Q6ZSA7	-
<i>SCNN1A</i>	Na ⁺ channel ENaC subunit A	P37088	-
<i>SCNN1B</i>	Na ⁺ channel ENaC subunit B	P51168	-
<i>SCNN1G</i>	Na ⁺ channel ENaC subunit G	P51170	-
<i>SLC4A1</i>	Cl ⁻ /HCO ₃ ⁻ transport	P02730	+ ¹
<i>SLC4A2</i>	Cl ⁻ /HCO ₃ ⁻ transport	P04920	-
<i>SLC4A3</i>	Cl ⁻ /HCO ₃ ⁻ transport	P48751	-
<i>SLC4A4</i>	Na ⁺ /HCO ₃ ⁻ co-transport	Q9Y6R1	-
<i>SLC4A5</i>	Na ⁺ /HCO ₃ ⁻ co-transport	Q9BY07	-
<i>SLC4A7</i>	Na ⁺ /HCO ₃ ⁻ co-transport	Q9Y6M7	-
<i>SLC4A8</i>	Na ⁺ /HCO ₃ ⁻ co-transport	Q2Y0W8	-
<i>SLC4A9</i>	anion exchange; protein 4	Q96Q91	-
<i>SLC4A10</i>	Na ⁺ -driven Cl ⁻ /HCO ₃ ⁻ transport	Q6U841	-
<i>SLC4A11</i>	Na ⁺ /HCO ₃ ⁻ transporter-like	Q8NBS3	-
<i>SLC8A1</i>	Na ⁺ /Ca ²⁺ exchange 1	P32418	-
<i>SLC8A2</i>	Na ⁺ /Ca ²⁺ exchange 2	Q9UPR5	-
<i>SLC8A3</i>	Na ⁺ /Ca ²⁺ exchange 3	P57103	-
<i>SLC22A14</i>	organic cation transport	Q9Y267	-
<i>SLC24A2</i>	Na ⁺ /Ca ²⁺ -K ⁺ exchange	Q94I40	-
<i>SLC24A4</i>	Na ⁺ /Ca ²⁺ -K ⁺ exchange	Q8NFF2	-
<i>SLC26A1</i>	SO ₄ ²⁻ transport	Q9H2B4	-
<i>SLC26A2</i>	SO ₄ ²⁻ transport	P50443	-
<i>SLC26A4</i>	Na ⁺ -independent Cl ⁻ /I ⁻ transport	Q43511	-
<i>SLC26A5</i>	prestin	P58743	-
<i>SLC26A6</i>	organic/inorganic anion transport	Q9BXS9	-
<i>SLC26A7</i>	Na ⁺ -independent anion HCO ₃ ⁻ transport	Q8TE54	-
<i>SLC26A9</i>	Cl ⁻ /HCO ₃ ⁻ exchange; HCO ₃ ⁻ transport	Q7LBE3	-
<i>SLC26A10</i>	Cl ⁻ /HCO ₃ ⁻ exchange	Q8NG04	-
<i>SLC26A11</i>	Na ⁺ -independent SO ₄ ²⁻ transport	Q86WA9	-

¹SLC4A1 is a house-keeping HCO₃⁻ transporter that is abundantly expressed in many cell types. A positive result may be due to small contaminations.

Supplementary Table 5: List of standard peptides	
Protein	Standard peptide sequence
Carbonic anhydrase 2	EPISVSSEQVLK
	QSPVDIDHTAK
	VVDVLDSIK
Chloride anion exchanger	LIDAVGFSPLR
	YEFFDGEVK
Cystic fibrosis transmembrane conductance regulator	AYFLQTSQQLK
	SPFTHLVTSLK
Amiloride-sensitive sodium channel subunit alpha	KPCSVTSYQLSAGYSR
	YSSFTTLVAGSR
Electrogenic sodium bicarbonate cotransporter 1	SPTFLER
	FLFILLGPK
Solute carrier family 26A, member 6	CGVDVDFLISQK
	DTQALLSATQAMDLR
CatSperB	GFPQENEIHK
	FEASGPPTAFGNSR
CatSper1	SLIHDAPGPAASR
	SYGEDYHDELQR
CatSper2	LQYNLEER
CatSper3	LIQYSQGIR
	TVASVLLR
CatSper4	FNQEQESEVLNR
	VHDSSSQILLK
Potassium channel subfamily U member 1	QHITVPSVK
	YTSSYEALK

Supplementary Table 6. Summary of studies that examined changes in pH_i under capacitating conditions ¹				
Authors	Species	Agent (mM)/system²	ΔpH	pH indicator
Meizel und Deamer (1978)	Hamster	none	acrosome	9-AA ³ ¹³ C-methylamine
Babcock et al. (1983)	Bovine	none	n.d.	CF ⁴
Parrish et al. (1989)	Bovine	Heparin	0.4	CF ⁴
Vredenburg-Willberg und Parrish (1995)	Bovine	Heparin	0.22 ⁵	BCECF ⁶
Zeng et al. (1996)	Mouse	HCO_3^- (22.5)/o.s.	0.19 ⁷	BCECF
Nakanishi et al. (2001)	Mouse	HCO_3^- (25)/o.s.	acrosome	EGFP ⁸
Demarco et al. (2003)	Mouse	HCO_3^- (5)/o.s.	0.15	BCECF
Xu et al. (2007)	Mouse	HCO_3^- (25) ⁹	0.75	BCECF
Carlson et al. (2007)	Mouse	HCO_3^- (15)/o.s.	no change	BCECF
Cross and Razy-Faulkner (1997)	Human	HCO_3^- (25)/o.s.	0.14	BCECF
Lopez-Gonzalez et al. (2014)	Human	HCO_3^- (15)/o.s.	n.d. ¹⁰	BCECF

¹Studies that are cited in the literature as showing changes in pH_i under capacitating conditions; in fact, several of these studies do not use capacitating conditions involving HCO_3^- . ²o.s., open system; ³9-AA, 9-aminoacridine; ⁴CF, carboxyfluorescein; ⁵ pH_i was constant for 3 h and then increased within 2 h; ⁶BCECF, 2'-7'-bis(carboxyethyl)-5(6)-carboxyfluorescein, ⁷capacitation was completed in < 2 h; ⁸EGFP, pH-sensitive enhanced green fluorescent protein; ⁹conditions were not clear from description, ¹⁰ ΔpH_i was not determined; only a subpopulation of sperm displayed a shift in flow cytometry.