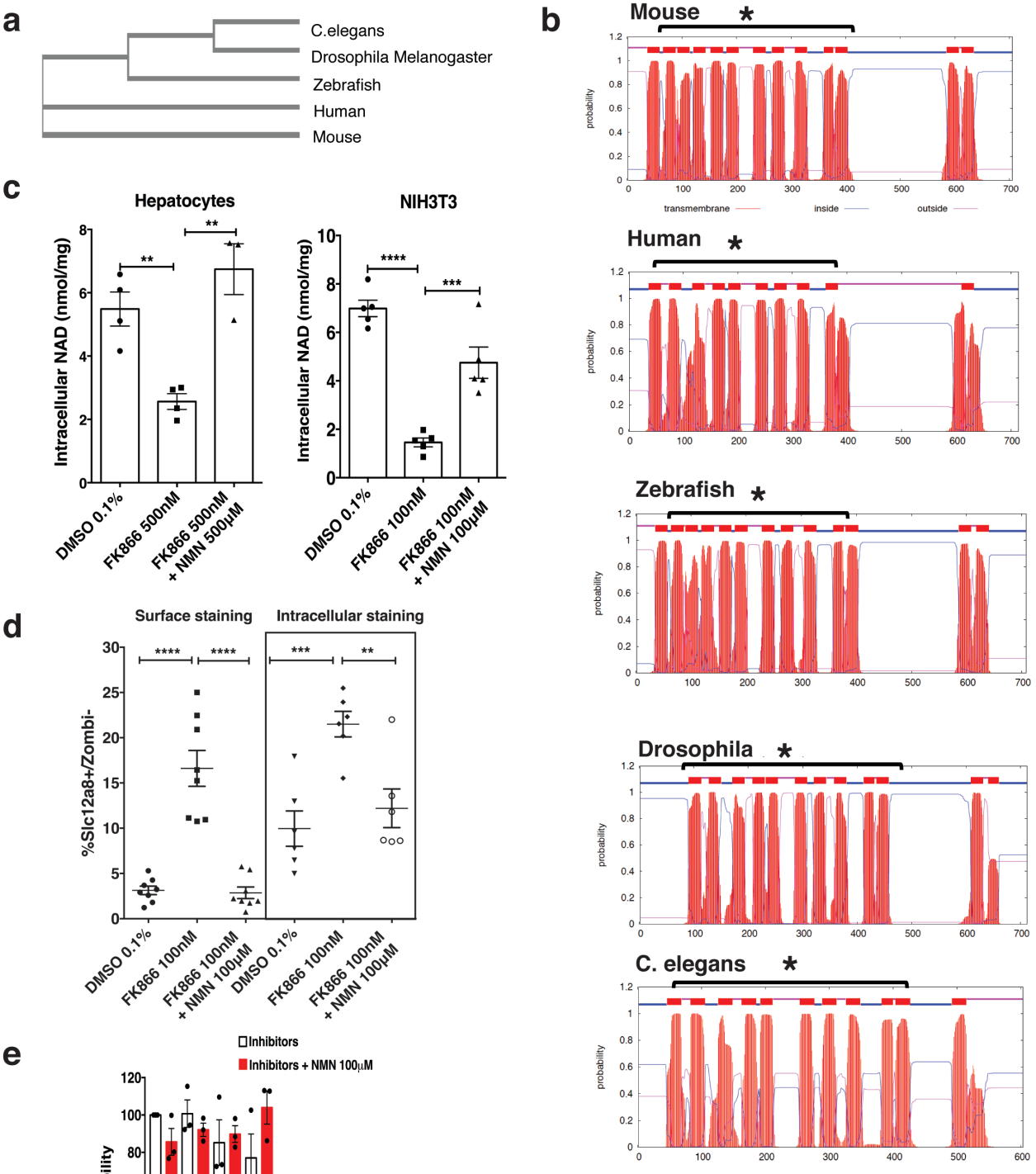
Supplementary Fig.1



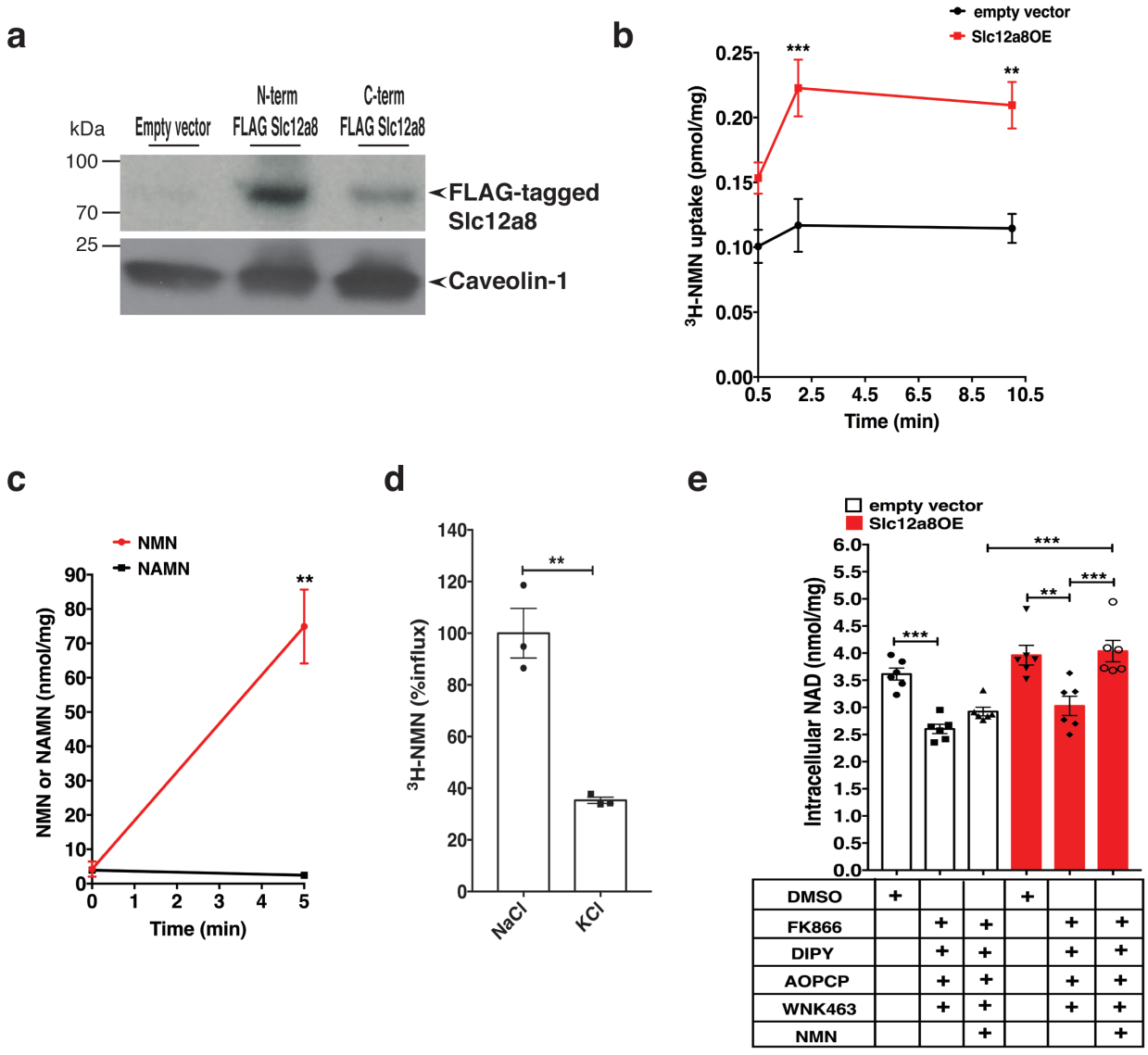
f

	AMP 100μM	AMP 100μM +AOPCP 500μM
	Adenosine (nmol/mg/min)	Adenosine (nmol/mg/min)
Hepatocytes	1.009 ± 0.193	0.026 ± 0.016

Supplementary Figure 1.

a, A cladogram of Slc12a8 homologs. The cladogram was produced using the Clustal Omega program. **b,** Predicted transmembrane domains of Slc12a8 homologs. Potential transmembrane domains were predicted using the TMHMM program (ExPASy). Asterisks indicate predicted transmembrane domains conserved throughout all Slc12a8 homologs. **c,** Intracellular NAD^+ content in mouse primary hepatocytes and NIH3T3 fibroblasts treated with DMSO, FK866 alone, and FK866 plus NMN (n=4 mice for hepatocytes treated with DMSO and FK866, and n=3 mice for hepatocytes treated with FK866 and NMN; n=5 biologically independent samples for NIH3T3 cells; analyzed using ANOVA with Tukey's test; hepatocytes, DMSO vs. FK866 **p=0.0083, FK866 vs. FK866+NMN **p=0.0015; NIH3T3 DMSO vs. FK866 **** p<0.0001, FK866 vs. FK866+NMN ***p=0.0005). **d,** Surface and intracellular Slc12a8 protein expression levels were measured in NIH3T3 cells treated with FK866 and FK866 plus NMN for 48h by flow cytometry analysis. Percentage of positive NIH3T3 cells for Slc12a8 staining (Slc12a8+) was calculated among cells negatively selected for a marker of apoptosis (Zombi-) (n=8 biologically independent samples for surface staining, and n=6 biologically independent samples for intracellular staining; analyzed by unpaired two-sided *t*-test; Surface staining, DMSO vs. FK866 and FK866 vs. FK866+NMN **** p<0.0001; Intracellular staining, DMSO vs. FK866 ***p=0.0002, FK866 vs. FK866+NMN **p=0.0032). **e,** AOPCP inhibits 5'-nucleotidase activity in mouse primary hepatocytes. Mouse primary hepatocytes were incubated with AMP or AMP plus AOPCP. The generation of extracellular adenosine was measured at different time points (0, 1, 5, 15, and 30 min) by HPLC (n=3 mice). **f,** The viability of mouse primary hepatocytes was evaluated when treated with 500 μM AOPCP, 20 μM dipyridamole and 500 nM FK866 or these inhibitors plus 100 μM NMN at the indicated time points (n=3 mice). All values are presented as mean ± SEM.

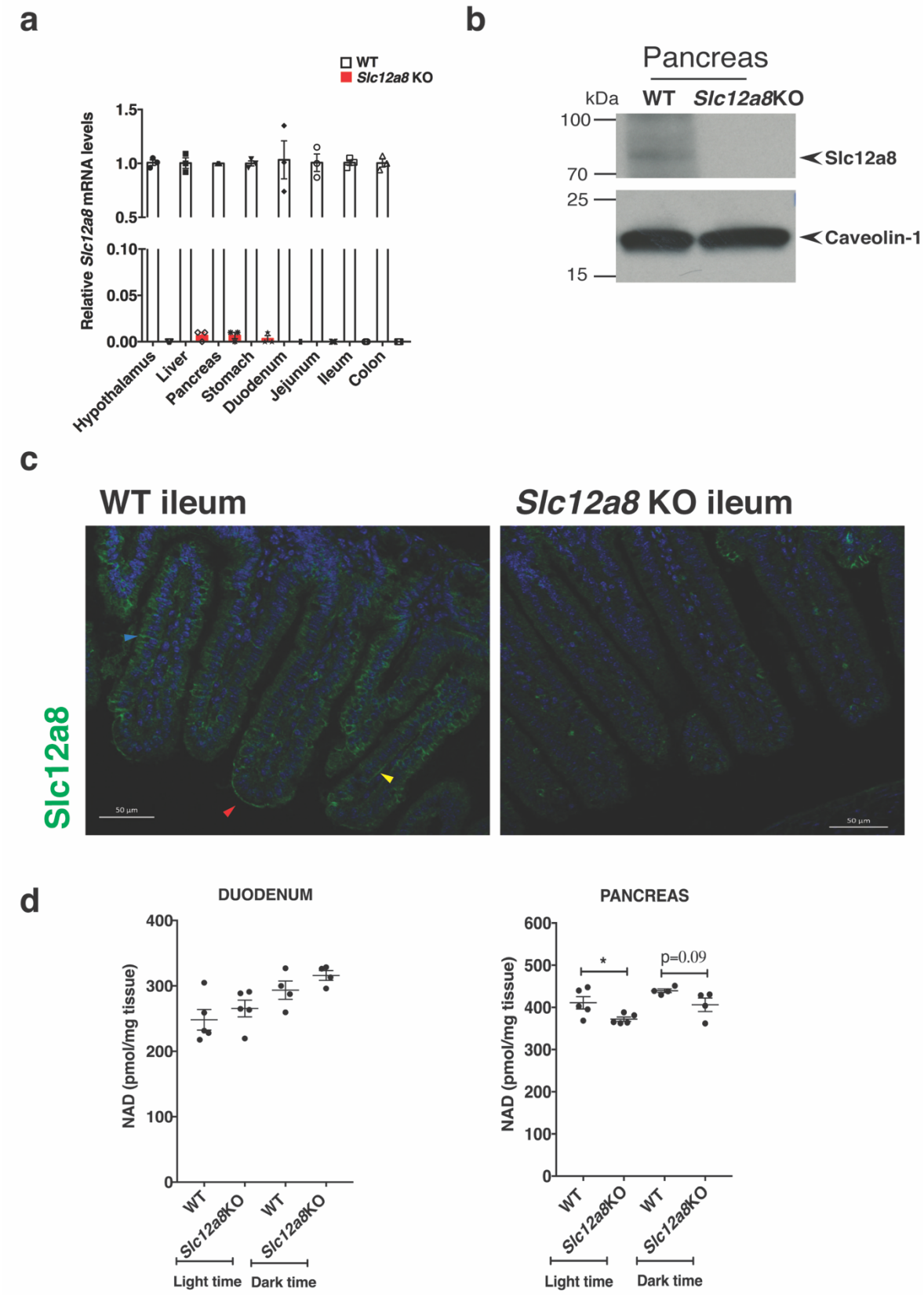
Supplementary Fig.2



Supplementary Figure 2.

a, The N- and C-terminally FLAG-tagged Slc12a8 proteins were detected in plasma membrane fractions from control (empty vector), N-terminally FLAG-tagged Slc12a8-OE (N-term FLAG Slc12a8), and C-terminally FLAG-tagged Slc12a8-OE (C-term FLAG Slc12a8) NIH3T3 cells (n=1 independent experiment). **b,** Uptake of ^{3}H-NMN (22 nM, 25°C) in proteoliposomes produced from plasma membrane fractions of control (empty vector) and Slc12a8-OE NIH3T3 cells in transport buffer (n=4 biologically independent samples; analyzed by one-way ANOVA with Sidak's multiple comparisons test, **p=0.0023, ***p=0.0008,). **c,** Uptake of NMN or NaMN (5mM, 25°C) measured by HPLC in proteoliposomes produced from plasma membrane fractions of Slc12a8-OE NIH3T3 cells in transport buffer (n=3 biologically independent samples; analyzed by unpaired two-sided *t*-test, **p=0.0025). **d,** Potassium dependency of NMN uptake by Slc12a8. Sodium ion (Na^+) was replaced with an equimolar concentration of potassium ion (K^+) (n=3 biologically independent samples, analyzed by unpaired two-sided *t*-test, **p=0.0026). **e,** Intracellular NAD^+ content was measured by preincubating cells with 2 μM dipyridamole, 500 μM AOPCP, 100 nM FK866, and 10 μM WNK463, a specific inhibitor for the WNK kinase that regulates the activity of the cation-chloride cotransporters, for 1h and then with the same inhibitors and 100 μM NMN for an additional 1h (n=6 biologically independent samples; analyzed by ANOVA with Tukey's test; empty vector DMSO vs. inhibitors ***p=0.0004; Slc12a8OE, DMSO vs. inhibitors **p=0.0012, inhibitors vs. inhibitors+NMN ***p=0.0005; empty vector, inhibitors+NMN vs. Slc12a8OE, inhibitors+NMN ***p=0.0001). All values are presented as mean ± SEM.

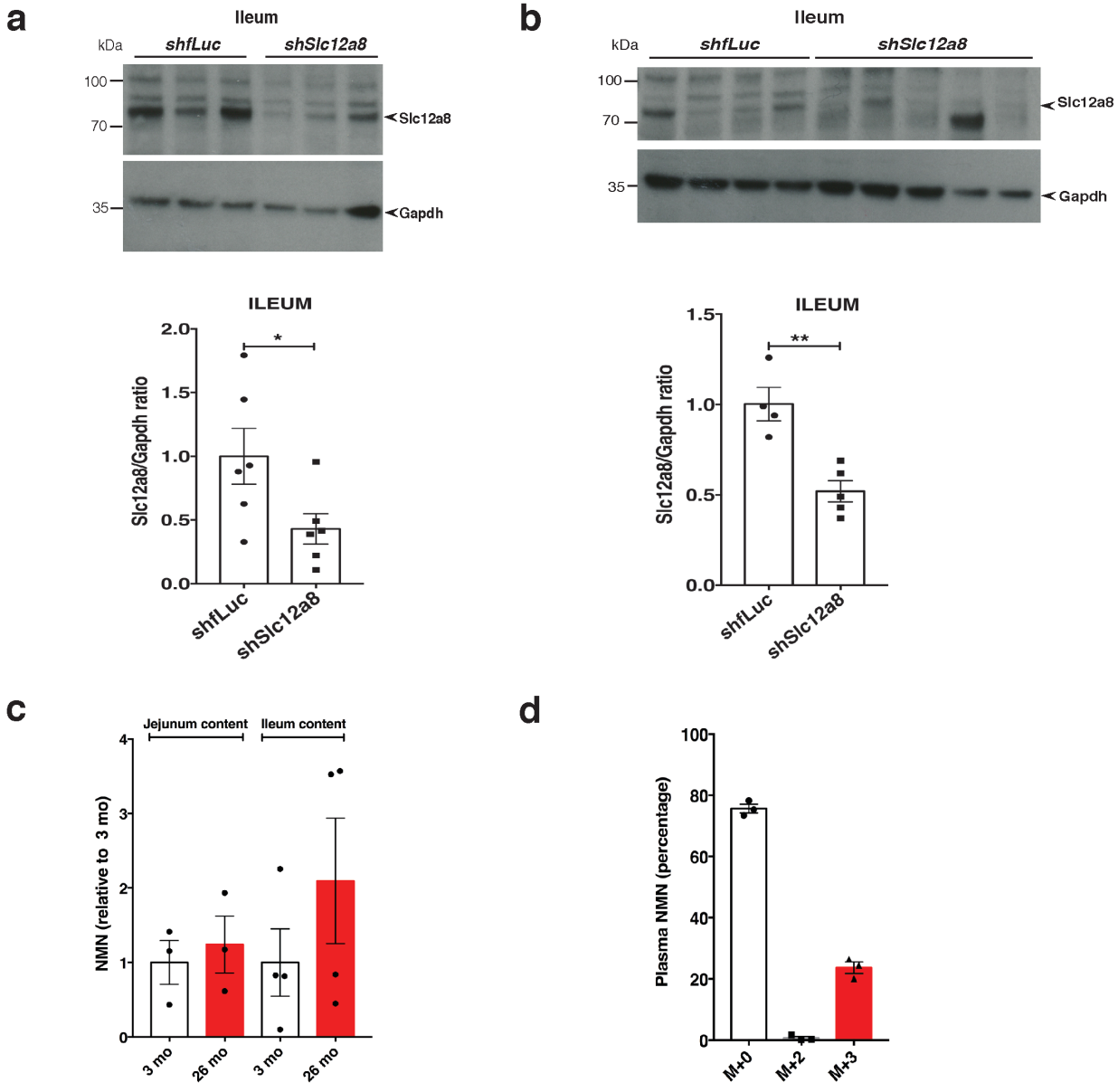
Supplementary Fig. 3



Supplementary Figure 3.

a, Relative full-length *Slc12a8* mRNA levels in different tissues from *Slc12a8* knockout (*Slc12a8*KO) mice and control wild-type littermates (WT) (n=3 mice, males at 2-3 months of age). **b,** Western blotting of Slc12a8 and caveolin-1 in whole tissue lysates of the pancreata of *Slc12a8*KO mice and WT littermates (n=3 mice). **c**, Immunostaining of Slc12a8 (green) in the ileum from 10 month-old *Slc12a8*KO female mice and WT littermates. Nuclei were counterstained with DAPI. Red, blue, and yellow arrowheads indicate apical, lateral and basal membranes, respectively. Scale bars: 50 μm (n=3 mice). **d,** NAD^+ levels measured by HPLC in the duodenum and pancreas from *Slc12a8*KO mice and WT littermates, collected during light time (9-10 am) or during dark time (9-10 pm) (n=5 mice for the light time, and n=4 mice for the dark time, females at 8-10 months of age; analyzed by unpaired two-sided *t*-test; *p=0.0378). All values are presented as mean ± SEM.

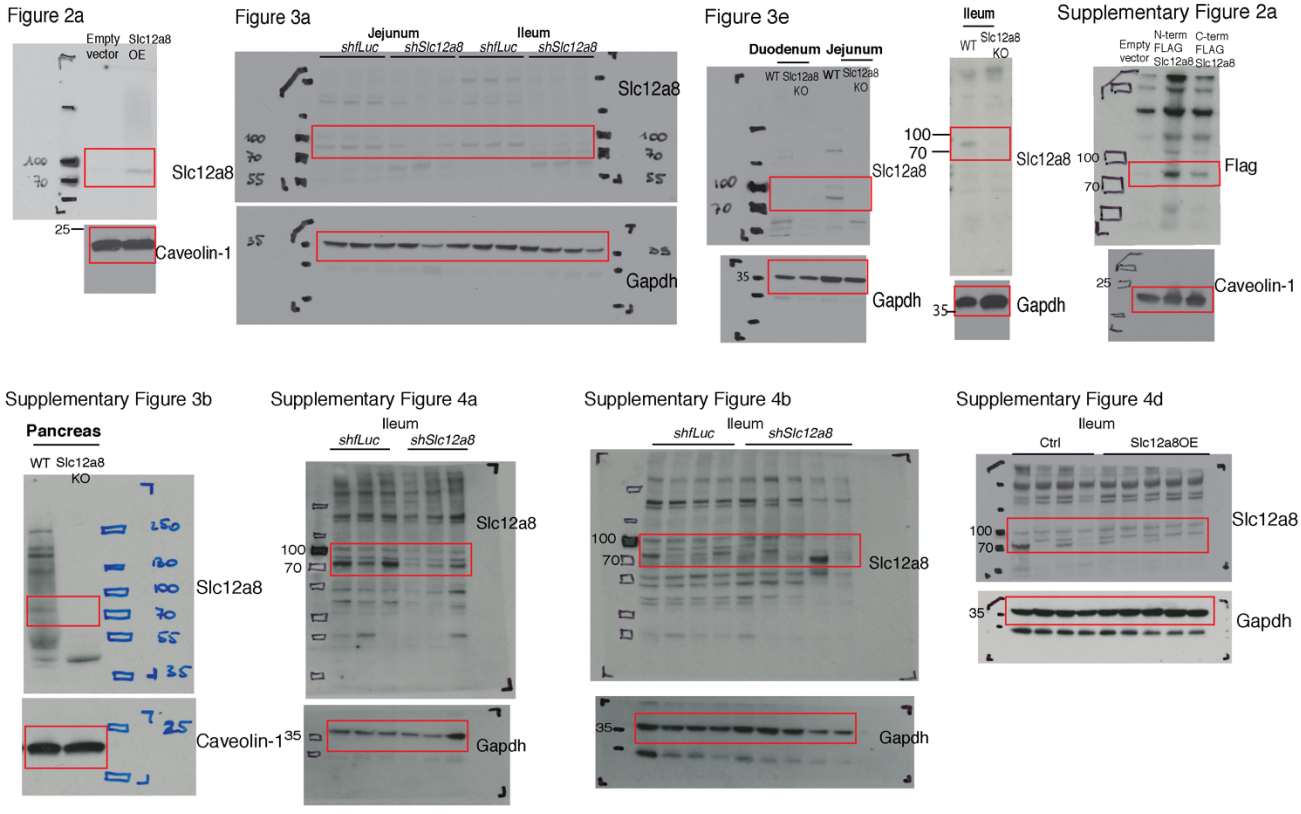
Supplementary Fig. 4



Supplementary Figure 4.

a, b, Western blotting of Slc12a8 and Gapdh in ileal lysates of 2 month-old (**a**) or 24 month-old (**b**) control (*shfLuc*) and intestinal *Slc12a8*-KD (*shSlc12a8*) B6 female mice. Representative Western blots are shown (upper panels), and bar graphs show relative Slc12a8 protein levels normalized to those in the ilea of control mice (down panels) (n=3 mice repeated independently twice, for each 2 month-old control and intestinal *Slc12a8*-KD mice, and n=4 mice for 24 month-old control and 5 mice for 24 month-old intestinal *Slc12a8*-KD mice repeated independently twice; analyzed by unpaired two-sided *t*-test; **a,** 2 mo *shfLuc* vs. *shSlc12a8* *p=0.0455; **b,** 24 mo *shfLuc* vs. shSlc12a8 **p=0.0026). The arrowhead indicates the bands of the full-length Slc12a8 protein. **c,** Relative luminal NMN levels measured by mass spectrometry in the jejuna and ilea from 3 month-old (3 mo) and 26 month-old (26 mo) B6 female mice (n=3 mice for the jejunum and n=4 mice for the ileum). **d,** Western blotting of Slc12a8 and Gapdh in ileal extracts of 2 month-old control (Ctrl) and intestinal *Slc12a8*-overexpressing (Slc12a8OE) B6 female mice. A representative Western blot is shown (left panel), and bar graphs show relative Slc12a8 protein levels normalized to those in the control ilea (right panels) (n=4 mice, analyzed by unpaired two-sided *t*-test, *p=0.0488). **e,** Ileal NAD^+ levels in 2 month-old control (*Ctrl*) and intestinal *Slc12a8*-overexpressing (*Slc12a8* OE) B6 female mice (n=4 mice, analyzed by unpaired two-sided *t*-test). **f,** Detection of doubly labeled, isotopic NMN (O18-D-NMN) in mouse plasma at 5 min after oral gavage by extracting plasma samples freshly without freezing/thawing. O18-D-NMN (M+3), O18-NMN (M+2), and NMN (M+0) were detected in mouse plasma by mass spectrometry (n=3 mice). All values are presented as mean ± SEM.

Supplementary Fig. 5



Supplementary Figure 5.

Uncropped images of Western blotting results are shown. Red boxes indicate cropped images shown in main figures. The molecular weights (kDa) of size markers are shown, as indicated.