Cell Reports, Volume *40*

Supplemental information

Genetic- and diet-induced ω-3 fatty acid enrichment enhances TRPV4-mediated vasodilation in mice

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Supplementary data

Supplementary Figure S1

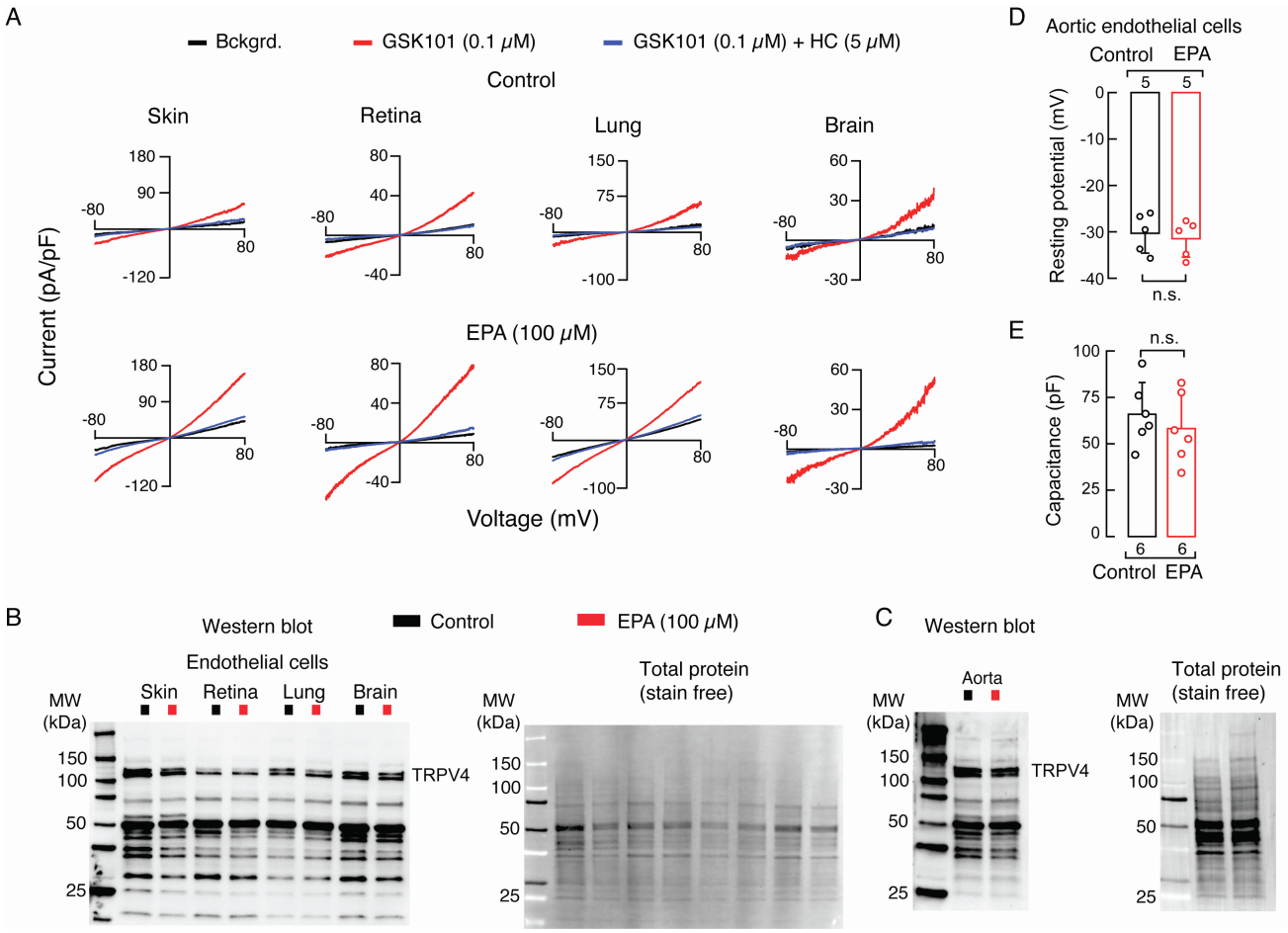

Figure S1. Related to Figure 1. EPA supplementation enhances TRPV4 activity in human microvascular endothelial cells from the skin, lung, brain, and retina.
(A) Representative current-voltage relationships determined by whole-cell patch-clamp recordings of control (top) and EPA (bottom)-treated human vascular endothelial cells, from skin, retina, lung, and brain, challenged with GSK101 (0.1 μM, TRPV4 agonist) and GSK101 (0.1 μM) + HC067047 (HC, TRPV4 antagonist; 5 μM). Bckgrd. indicates background currents. **(B)** Left: Representative western blots (anti-TRPV4) of the membrane fractions of control and EPA (100 μM)-treated endothelial cells from skin, retina, lung, and brain. Right: Representative Stain-Free™ gels (Bio-Rad) of the total chemically labeled proteins of the membrane fractions from control and EPA (100 μM)-treated endothelial cells from skin, retina, lung, and brain. **(C)** Left: Representative western blots (anti-TRPV4) of the membrane fractions of control and EPA (100 μM)-treated endothelial cells from aorta. Right: Representative Stain-Free™ gels (Bio-Rad) of the total chemically labeled proteins of the membrane fractions from control and EPA (100 μM)-treated endothelial cells from aorta. **(D)** Membrane potential values were recorded just after the whole-cell configuration was achieved in control and EPA (100 μM)-treated aortic endothelial cells. Two-tailed unpaired *t*-test. **(E)** Membrane capacitance of control and EPA (100 μM)-treated aortic endothelial cells. Two-tailed unpaired *t*-test. n.s. indicates values not significantly different from the control. n is indicated in each panel.

Supplementary Figure S2

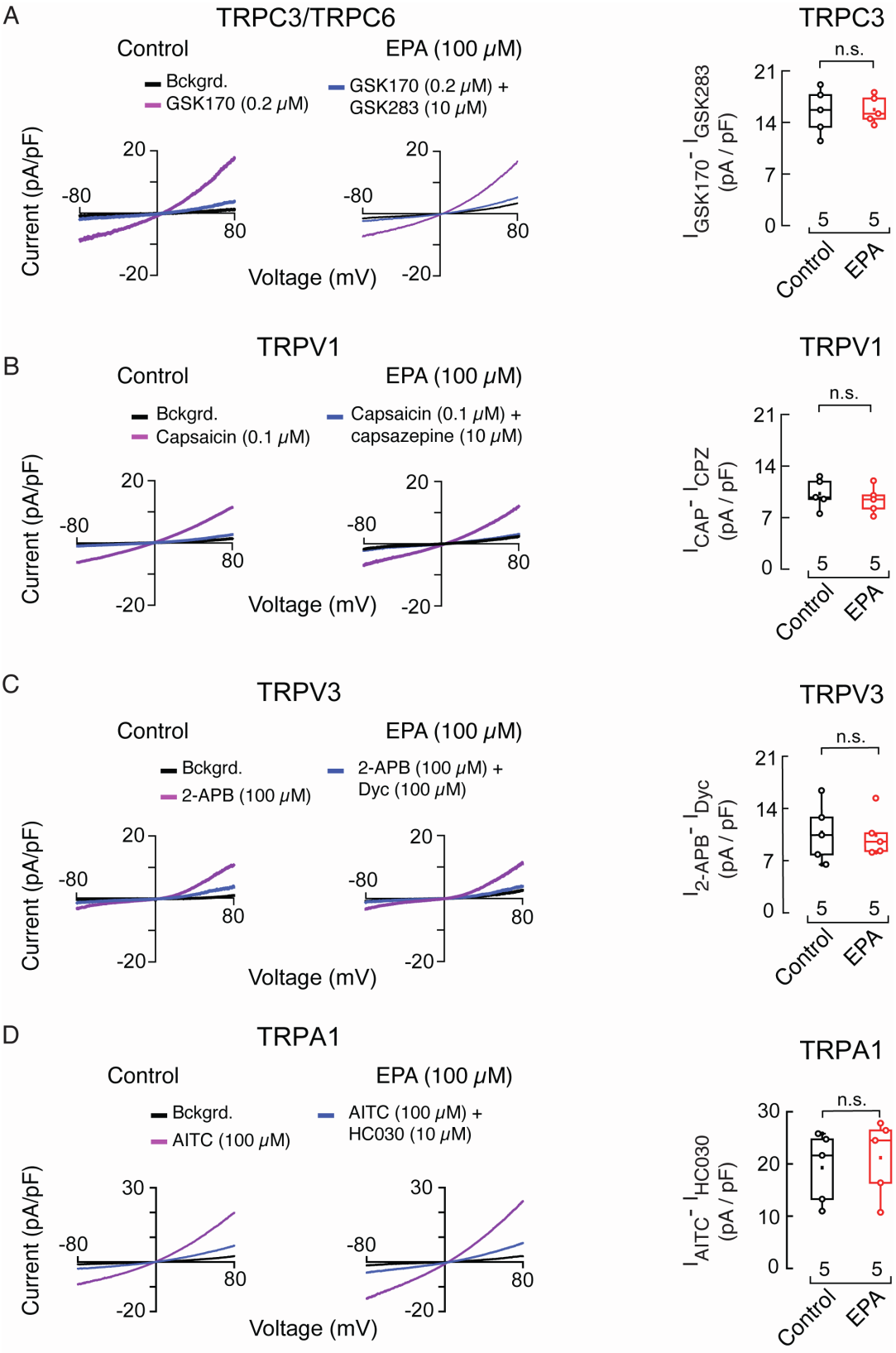

Figure S2. Related to Figure 1. Effect of EPA supplementation on other TRP channels.
(A) Representative current-voltage relationships determined by whole-cell patch-clamp recordings of control and EPA (100 μM)-treated aortic endothelial cells challenged with GSK170 (GSK1702934A; TRPC3/TRPC6 agonist; 0.2 μM) and GSK170 (0.2 μM) + GSK283 (GSK2833503A; TRPC3/TRPC6 antagonist 10 μM). Bckgrd. indicates background currents. Boxplots show mean (square), median (bisecting line), bounds of box (75^{th} to 25^{th} percentiles), outlier range with 1.5 coefficient (whiskers), and data points of TRPC3 currents ((I_{GSK170} - I_{GSK283}) pA/pF) obtained by whole-cell patch clamp recordings (+80 mV) of control and EPA-treated endothelial cells from aorta. **(B)** Representative current-voltage relationships, determined by whole-cell patch-clamp recordings, of control and EPA (100 μM)-treated aortic endothelial cells challenged with capsaicin (CAP; TRPV1 agonist; 0.1 μM) and CAP (0.1 μM) + capsazepine (CPZ; TRPV1 antagonist 10 μM). Bckgrd. indicates background currents. Boxplots show mean (square), median (bisecting line), bounds of box (75^{th} to 25^{th} percentiles), outlier range with 1.5 coefficient (whiskers), and data points of TRPV1 currents ((I_{CAP} - I_{CPZ}) pA/pF) obtained by whole-cell patch clamp recordings (+80 mV) of control and EPA-treated endothelial cells from aorta. **(C)** Representative current-voltage relationships, determined by whole-cell patch-clamp recordings, of control and EPA (100 μM)-treated aortic endothelial cells challenged with 2-aminoethoxydiphenyl borate (2-APB; TRPV3 agonist; 100 μM) and 2-APB (100 μM) + dyclonine (Dyc; TRPV3 antagonist 100 μM). Bckgrd. indicates background currents. Boxplots show mean (square), median (bisecting line), bounds of box (75^{th} to 25^{th} percentiles), outlier range with 1.5 coefficient (whiskers), and data points of TRPV3 currents (($I_{2\text{-}APB}$ - I_{Dyc}) pA/pF) obtained by whole-cell patch clamp recordings (+80 mV) of control and EPA-treated endothelial cells from aorta. **(D)** Representative current-voltage relationships, determined by whole-cell patch-clamp recordings, of control and EPA (100 μM)-treated aortic endothelial cells challenged with allyl isothiocyanate (AITC; TRPA1 agonist; 100 μM) and AITC (100 μM) + HC030 (HC030031; TRPA1 antagonist 10 μM). Bckgrd. indicates background currents. Boxplots show mean (square), median (bisecting line), bounds of box (75^{th} to 25^{th} percentiles), outlier range with 1.5 coefficient (whiskers), and data points of TRPA1 currents ((I_{AITC} – I_{HC030}) pA/pF) obtained by whole-cell patch clamp recordings (+80 mV) of control and EPA-treated endothelial cells from aorta. Two-tailed unpaired *t*-test between control and supplemented cells. n is indicated in each panel. n.s. means not significantly different.

Supplementary Figure 3

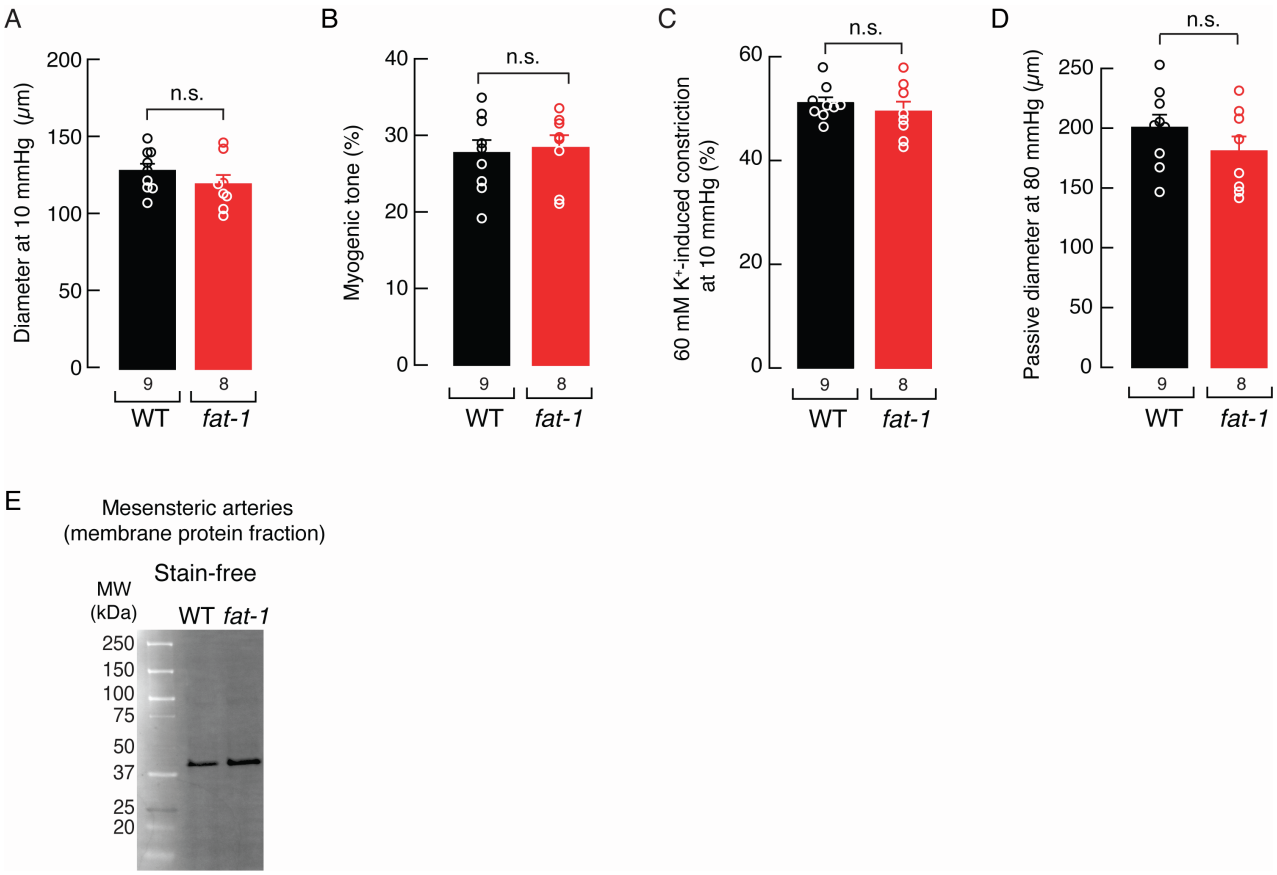

Figure S3. Related to Figure 3. *fat-1* mesenteric arteries feature normal physical parameters. Bar graphs show mean ± SEM for active diameter at 10 mm Hg **(A)**, myogenic tone **(B)**, 60 mM K^+-induced constriction at 10 mmHg **(C),** and passive diameter at 80 mmHg **(D)**, in mesenteric arteries of WT and *fat-1* mice. n is denoted below the bars. Two-tailed unpaired *t*-test. n.s. indicates values not significantly different from WT. **(E)** Representative Stain-Free™ gel (Bio-Rad) of the total chemically labeled proteins of the membrane fractions from the mesenteric arteries of WT and *fat-1* mice.

Supplementary Figure 4

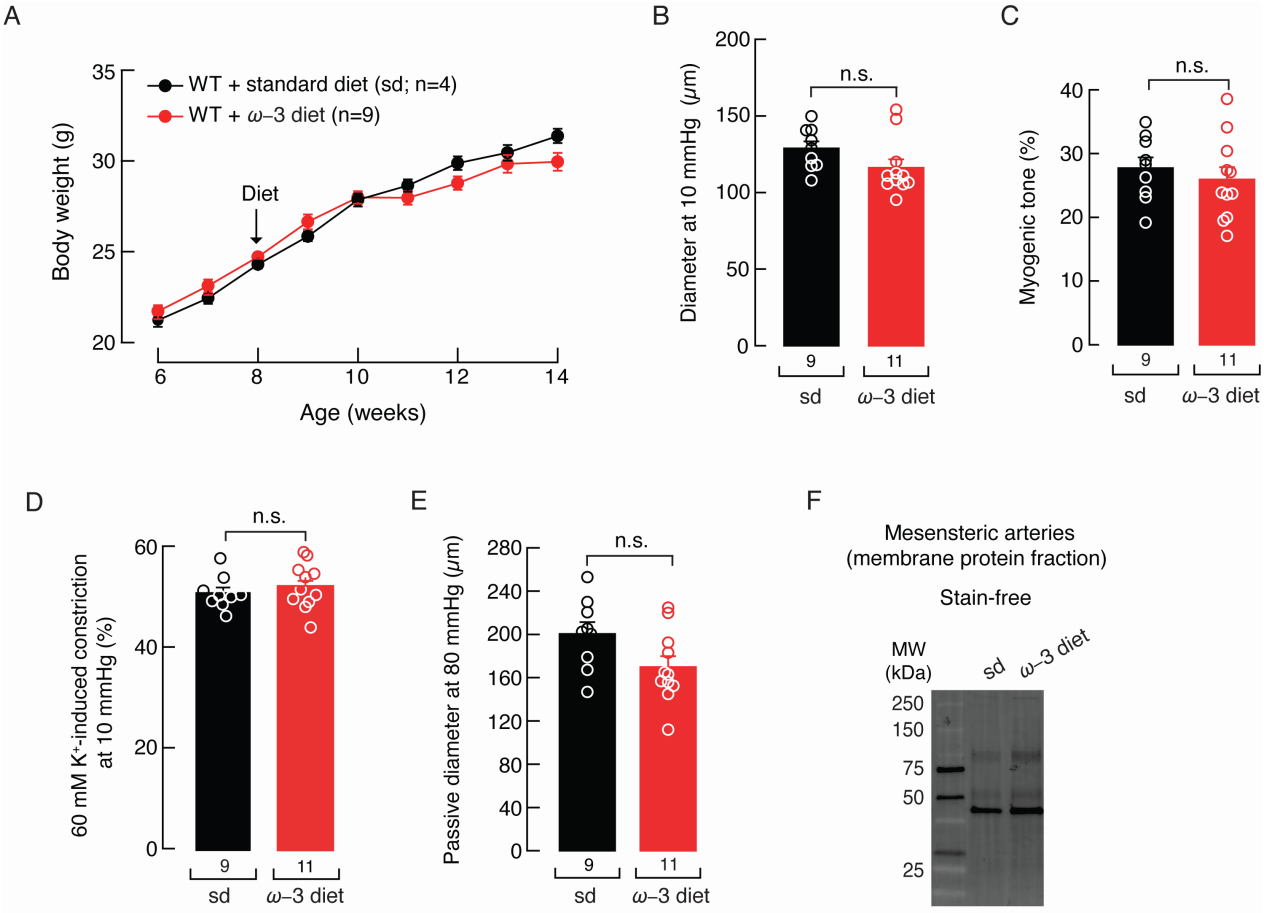

Figure S4. Related to Figure 4. Mesenteric arteries of mice fed with an *ω*-3 fatty acid-enriched diet feature normal physical parameters.

(A) Body weight measurements were recorded every week at similar times from mice of 6 to 14 weeks of age. The arrow indicates the beginning of the *ω*-3 fatty acid-enriched diet. Symbols denote the mean ± SEM. Bar graphs show mean ± SEM for active diameter at 10 mm Hg **(B)**, myogenic tone **(C)**, 60 mM K^+-induced constriction at 10 mm Hg **(D)**, and passive diameter at 80 mm Hg **(E)**, in mesenteric arteries of WT mice fed with standard or *ω*-3 fatty acid-enriched diets. n is denoted below the bars. Two-tailed unpaired *t* test. n.s. indicates values not significantly different from the standard diet. **(F)** Representative Stain-Free™ gel (Bio-Rad) of the total chemically labeled proteins of the membrane fractions from the mesenteric arteries of WT mice fed with standard or *ω*-3 fatty acid-enriched diets.

Supplementary Figure 5

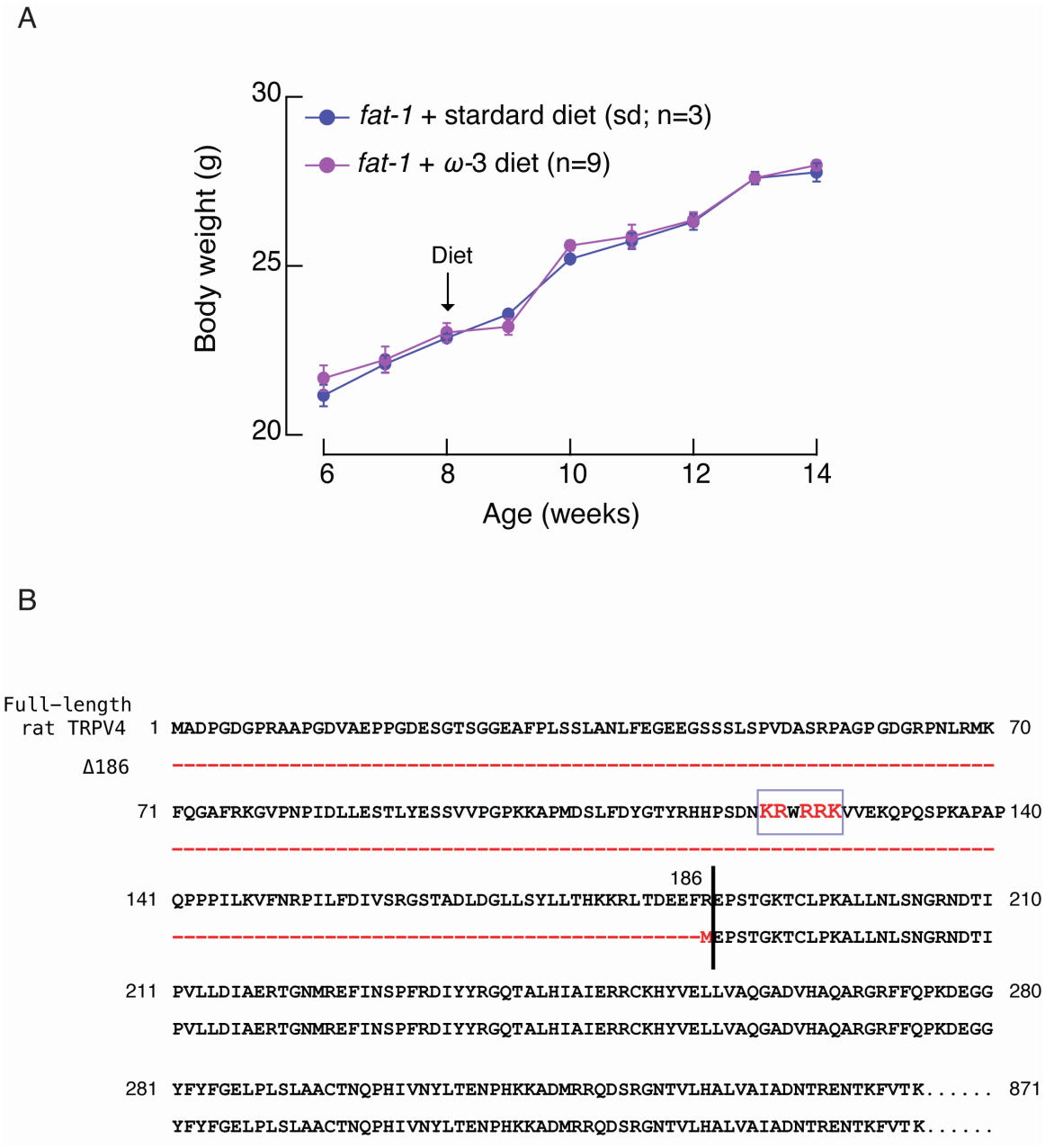

Figure S5. Related to Figures 5 and 7. *fat-1* mice body weight in standard or *ω*-3 fatty acid-enriched diets and TRPV4 constructs protein sequences.
(A) Body weight measurements were recorded every week at similar times from mice of 6 to 14 weeks of age. The arrow indicates the beginning of the *ω*-3 fatty acid-enriched diet. Symbols denote the mean ± SEM. **(B)** Protein sequences highlighting the first 186 amino acid residues deleted from the TRPV4 N terminus in the Δ186 construct and the five positively charged residues (in box) mutated to alanine.