Supplementary Figure S1: Seipin silencing and effects of oleic acid loading. A) U251MG cells were transfected with control or seipin siRNAs for 5 days and analyzed for seipin expression by immunoblotting with anti-seipin antibodies (20 µg protein/lane). B) TG content in U251MG cells transfected with control or seipin siRNAs, under standard growth conditions. Bars: TG content normalized to control siRNA cells +/- SEM. N of samples 13 from 5 individual experiments, *p<0.05. Average TG content in control siRNA treated cells was 13 µg/mg of protein. C) TG content in U251MG cells expressing GFP or GFP-fused seipin N88S upon oleic acid loading (0.5 mM overnight). Bars: TG content normalized to control (GFP expressing cells without oleic acid) +/- SEM, samples in triplicate. D) TG content in NSC-34 cells expressing GFP-F or GFP-fused seipin N88S mutant upon oleic acid loading (0.4 mM for 4-18 h). Bars: TG content normalized to control (GFP expressing cells without oleic acid) +/- SEM, N of samples 3-6 from 3 individual experiments. E) U251MG cells were transfected with GFP-fused seipin N88S plasmid, loaded with oleic acid (0.5 mM overnight), then fixed and stained with Oil Red O. Scale bar, 20 µm.
Supplementary Figure S2: Effect ATGL siRNA #2 on tunicamycin-induced CHOP levels. A) NSC-34 cells were transfected with control or ATGL siRNAs #1 or #2 for 3 days and analyzed for ATGL expression by immunoblotting with anti-ATGL antibodies (20 µg protein/lane). B) NSC-34 cells were transfected with control siRNA or ATGL siRNA #2, followed by tunicamycin (TM) treatment for 6 h (siRNA treatment 3 days). Cells were then lysed and immunoblotted with anti-CHOP antibodies (20 µg protein/lane).
Supplementary Figure S3: Effect of seipin N88S expression on TG mass and motor neuron morphology in zebrafish. A) Transmission images of 5 dpf zebrafish +/- microinjection with mRNAs encoding RFP fused seipin N88S. Arrows: yolk. Note the dense mass in N88S expressing fish that is absent from non-injected fish. B) Analysis of TG levels in the whole embryos injected with mRNAs encoding soluble GFP or GFP fused seipin N88S at 3, 5 and 7 dpf. Bars: µg TG/mg protein +/- SEM from duplicate samples (8 embryos per sample). C) Live imaging of 4 and 6 dpf larvae from motor neuron: GFP transgenic fish (see Materials and Methods) with and without (control) microinjection of seipin N88S fused with RFP. Images show a side view of trunk with GFP-labeled spinal motor neurons and peripheral axons.
Supplementary Figure S4: Expression pattern of *gata6SAGFP10A* larvae at 4 dpf. A) Lateral view showing expression in the brain and spinal cord. B) Magnification of trunk segments shown in the inset in A. The dashed line shows the ventral limit of the spinal cord. Ventrally-projecting motor axons are indicated by arrowheads. Approx. 12-14 lateral GFP-positive neurons (asterisks) have axons projecting ventrally to the periphery. GFP-positive interneurons are also observed in this line.
Supplementary Videos: Touch-evoked escape and swimming behavior of seipin N88S (Video 1) or WT seipin (Video 2) expressing zebrafish larva. Videos of 4 dpf zebrafish microinjected with mRNAs encoding RFP fused seipin N88S or GFP fused WT seipin, recorded at 500 frames/s and played at 10 frames/s.