Supplemental Figure

Fig. S1

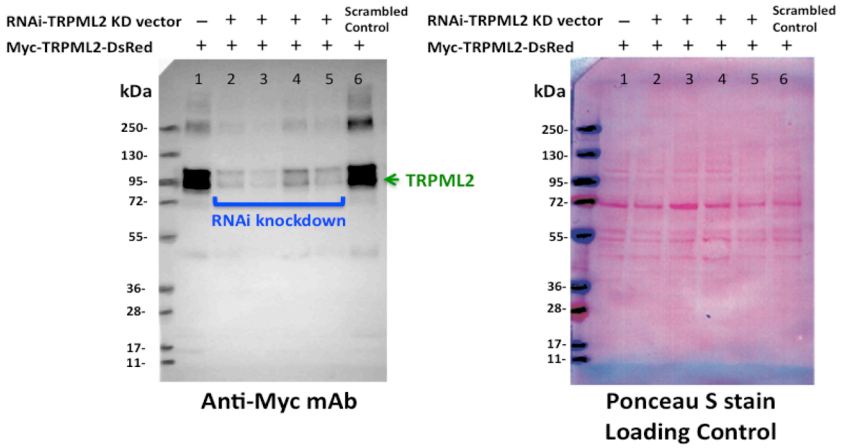
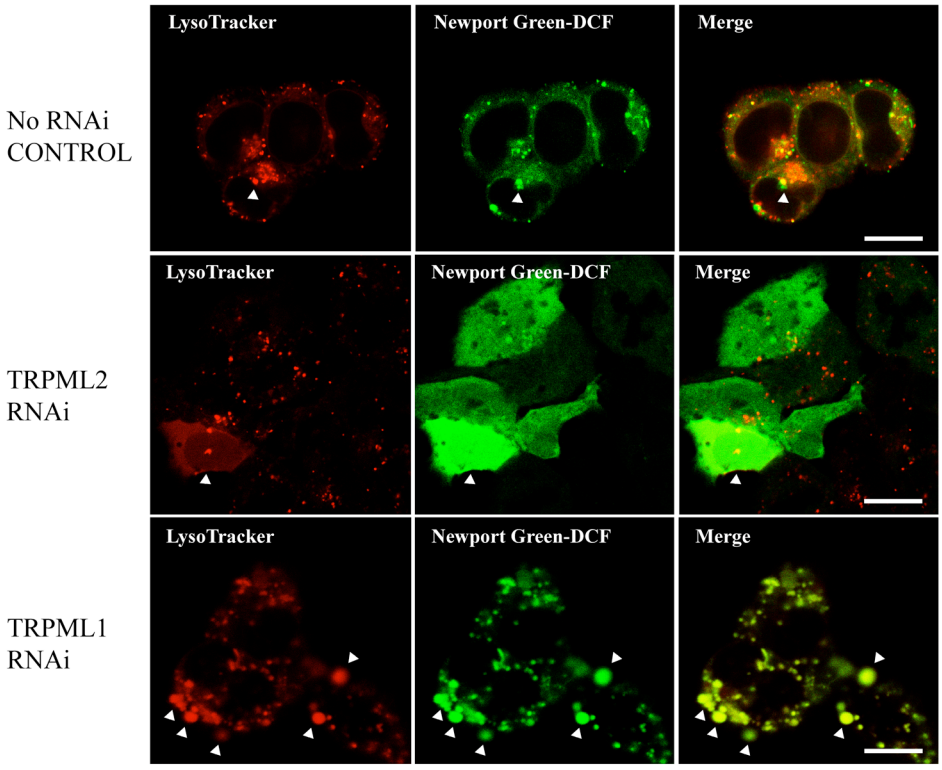


Fig. S2



Supplemental Figure Legend

Fig. S1. Western blot analysis of TRPML2 RNAi-treated cells over-expressing human TRPML2 plasmid. HEK-293 cells over-expressing HsTRPML2 (Myc-TRPML2-dsRed) were transfected with short-hairpin RNAi plasmid (RNAi TRPML2 KD vector). The samples were run on SDS-PAGE and blotted with an anti-myc antibody (*left panel*). Lane 1: no RNAi control; Lanes 2-5: RNAi TRPML2 KD treatments; Lane 6: Scrambled RNAi treatment control. To show equal protein loading between lanes, the blot was stained with Ponceau S (*right panel*). The TRPML2 protein bands indicated by the *green arrow* run at approximately 95 kDa, since the TRPML2 expression plasmid contained both DsRed fluorescent protein and myc peptide tags. A standard Western blot was performed using chemiluminescence. The primary

anti-myc mAb (monoclonal antibody 9E10) was obtained from Developmental Hybridoma Bank (University of Iowa).

Fig. S2. Representative confocal micrographs of Lysotracker- and Newport Green DCF-stained cells. The image shows untreated control (*top panel*), TRPML2 RNAi-treated (*middle panel*), and TRPML1 RNAi-treated HEK-293 cells (*bottom panel*). Lysosomes (red) that are positive for LysoTracker fluorescence are also positive for chelatable Zn^{2+} using Newport Green DCF. TRPML1 RNAi-treated cells exhibit many enlarged lysosomes, and that these large lysosomes are more likely to be positive for chelatable Zn^{2+} fluorescence (*arrowheads*). Scale bar = 10 μm.