

Supplemental Figure Legends

Figure S1: (A) Representative trace showing changes of whole cell currents from RWPE cells that were activated by the depletion of intracellular Mg^{2+} under various conditions (1 μM cholesterol treatment and plus 500 μM 2-APB treatment). Outward currents (top curve) were measured at +100mV; whereas inward currents (bottom curve) was measured at -100mV. Average IV curves (developed from maximum currents) under this condition are shown in (B). (C) Changes of whole cell currents under similar conditions from DU145 cells are shown. Outward currents were again measured at +100mV; whereas inward currents were measured at -100mV (bottom line). IV curve of these cells under this condition is shown in (D). (E), (F) Average (8-10 recordings) current intensity at +100mV and -100mV under these conditions is shown. * and ** indicates significance ($p < 0.05$, $p < 0.01$) versus cholesterol treated cells. (G) Ca^{2+} imaging was performed in control and in pretreatment cholesterol (1 μM) for 24 hours in DU145 cells. Analog plots of the fluorescence ratio (340/380) from an average of 40-60 cells are shown. (H) Quantification (mean \pm SD) of fluorescence ratio (340/380). * indicates significance ($p < 0.05$) versus control. (I). Bar diagram representing the densitometry reading showing the activity of phospho form of AKT and ERK, each bar represent percentage of respective pAKT or pERK expression normalized with the total AKT or ERK expression of the respective samples. Each bar gives the mean \pm SEM (N=4, ***, $p < 0.001$). (J) Images showing the wound-healing assay for cellular migration of LNCaP cells treated with 1 μM cholesterol for 24 hours. The pictures are representative of 4 separate experiments.

Figure S2: (A) Representative blots indicating LNCaP cells control, knockdown and overexpressing TRPM7. Cell lysates were resolved on NuPAGE 3-8% Tris-Acetate gels and analyzed by western blotting using TRPM7 antibody (1.0 ± 0.45 , 0.42 ± 0.12 and 1.92 ± 0.26 for mock, LNCaP TRPM7 knockdown and TRPM7 overexpressed cells marked as shTRPM7 and TRPM7⁺, respectively; $p < 0.05$; N= 4) β -actin was used as loading control. Average IV curves (developed from maximum currents) of TRPM7-like currents in LNCaP cells, cells transfected with shTRPM7 and overexpress TRPM7 are shown in (B). Statins and cholesterol pretreatment for 24 hours affect TRPM7-like currents in LNCaP cells which average IV curves under various conditions are shown in (C). (D), (E) Average (8-10 recordings) current intensity at +100mV and -100mV under these conditions is shown. * indicates significance ($p < 0.05$) versus untreated cells. (F) Bar diagram showing the relative absorbance at 450nm of LNCaP (shRNA control non-targeting marked as shC, TRPM7 knockdown marked as shTRPM7 and TRPM7 overexpressed cells marked as TRPM7⁺, respectively) with and without 1 μM cholesterol treatment for 24 hours and 2 hours of BrDU incorporation. Each bar gives the mean \pm SEM of 4 separate experiments. * indicates significance *, $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. (G) Images representing the soft agar colony tumor growth in LNCaP cells and TRPM7 knockdown and overexpressing cells. (H) Bar diagram represents the relative fluorescence

reading at 485/525 nm filters, of control and TRPM7 overexpressing DU145 cells after agar media being solubilized, lysed and detected by the patented CyQuant® GR Dye in a fluorescence plate reader, * indicates significance *, $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. (I) Bar diagram showing relative free cholesterol concentration in RWPE, DU145 and LNCaP cells measured using Wako Cholesterol E measurement Kit. *, $p < 0.05$ and **, $p < 0.01$.

Figure S3: (A) Ca^{2+} imaging was performed in the presence of cholesterol ($1 \mu\text{M}$) in control and statins treated for 24Hour in RWPE cells. Analog plots of the fluorescence ratio (340/380) from an average of 40-60 cells are shown. (B) Quantification (mean \pm SD) of fluorescence ratio (340/380). * indicates significance ($p < 0.05$) versus control. (C), (D) Changes of Ca^{2+} influx under similar conditions from DU145 cells are shown. Statins pretreatment for 24 hours affect TRPM7-like currents cells transfected with shRNA targeting TRPM7 which average IV curves (developed from maximum currents) under various conditions are shown in (E) and (F). Average (8-10 recordings) current intensity at +100mV and -100mV under these conditions is shown in (G) and (H).





