†Electronic Supplementary Information (ESI) available. See DOI: 10.1039/b000000x/

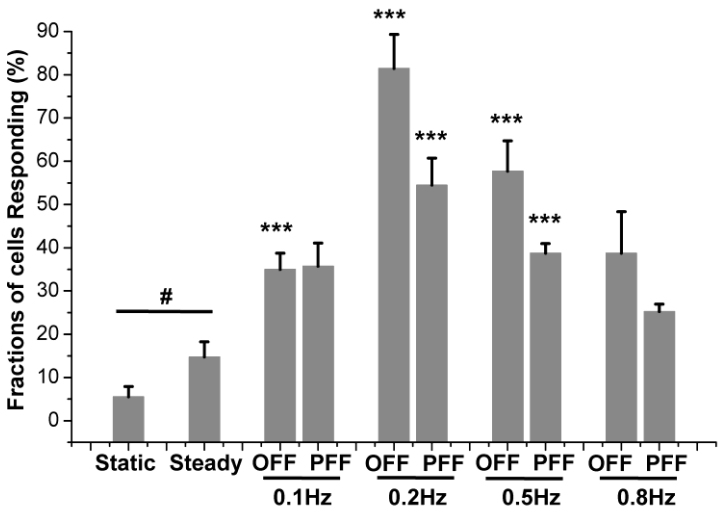

Fig. S1. Response of mouse primary osteoblast cells to different flow. All frequencies studied oscillating flow were more stimulatory than pulsatile or steady flow. Note that after flow application those cell have 1.2 fold increase in global intracellular calcium (F/F_0) counted as responsive cell. Data are expressed as mean and SD.; number of cell analyzed for each group from left to right was; n = 49, 53, 61, 30, 51, 31, 43, 53, 35, 42; #P<0.1 versus static and ***P < 0.001 versus the steady condition.

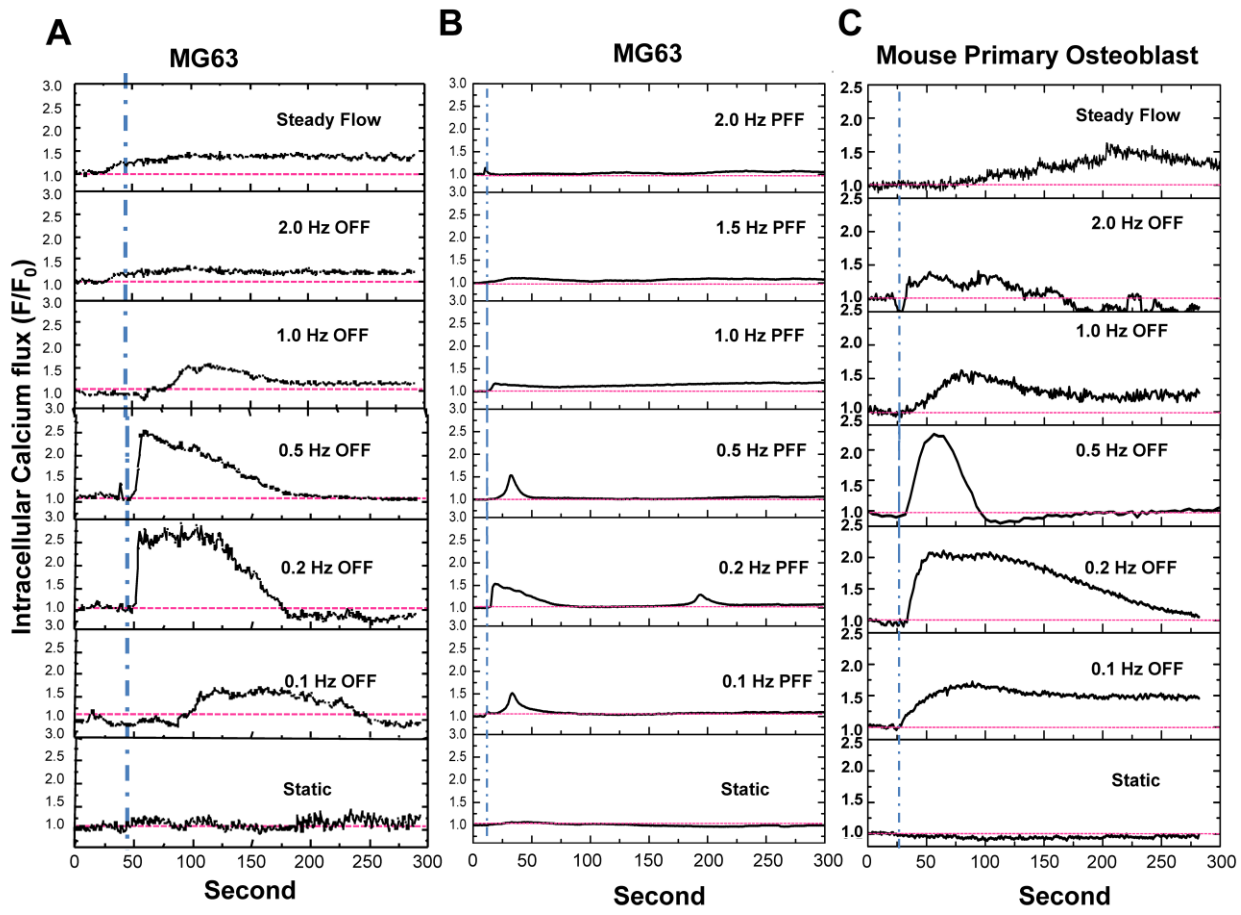

Fig. S2. 0.2 Hz oscillatory flow is more stimulatory than pulsatile or steady flow in both MG63 and mouse primary osteoblast. Cells were labeled with Fluo-4, subjected to fluid flow (steady, pulsatile, or oscillatory) conditions, and imaged for 5 minutes. Cases in which cells were not treated and imaged in the same way, but were not exposed to fluid flow, were labeled static. These served as the baseline control. The average global calcium influx amplitudes corresponding to each flow condition was plotted as intracellular calcium flux (F/F_0). Within the experimental range of frequency, both in MG63 cells (A, B) and in primary osteoblasts (B), oscillating flow was more stimulatory than pulsatile or steady flow. Notably, response was maximum at 0.2 Hz frequency in both oscillatory and pulsatile flow type. Blue dotted lines depict the onset of flow. Each plot represents the average fold increase of intracellular calcium level of several cells (n=10-17) under each flow condition. 1.2 fold increase of F/F_0 was set as threshold (red dotted line) to distinguish the responding cells from the non-responding cells.

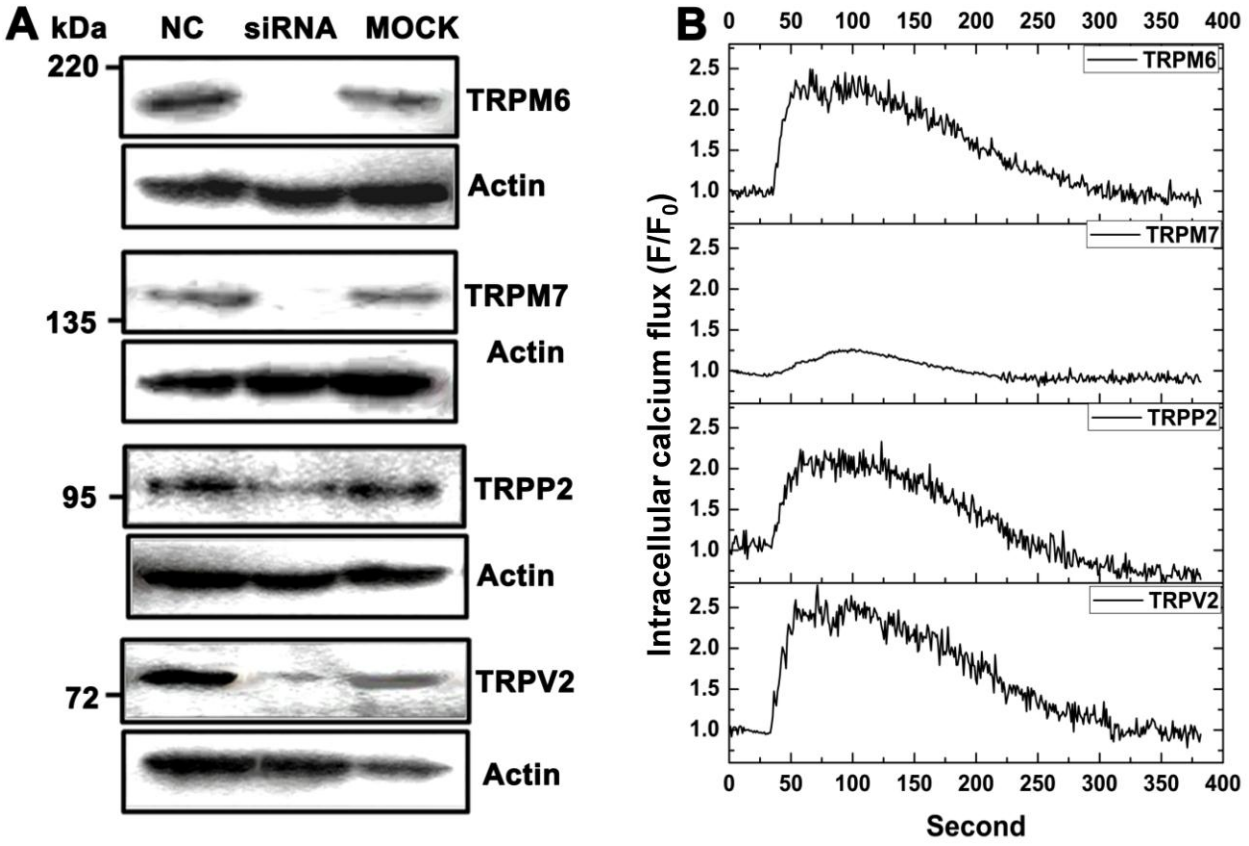

Fig. S3. TRPM7 is main route of oscillatory flow induced Ca influx. (A) RNAi mediated knockdown assay of TRPM6, TRPM7, TRPP2 and TRPV2 by immunoblotting. NC: negative control RNA; siRNA: corresponding siRNA sequences; MOCK: without siRNA. (B) MG63 cells were cultured on microchannel and knockdown of TRPM6, TRPM7, TRPP2 or TRPV2 by corresponding siRNA. Cells were pre-labeled with Fluo4 and then were subjected to 0.2 Hz oscillatory flow conditions. The average global calcium influx amplitudes corresponding to knockdown condition was plotted as Intracellular calcium flux (F/F_0). Oscillatory flow induced calcium-influx was abolished by TRPM7 knockdown condition not by TRPC6, TRPP2 or TRPV2 knockdown condition. Note that onset of flow application was at t= 30s; n=15–20 cells in each group.

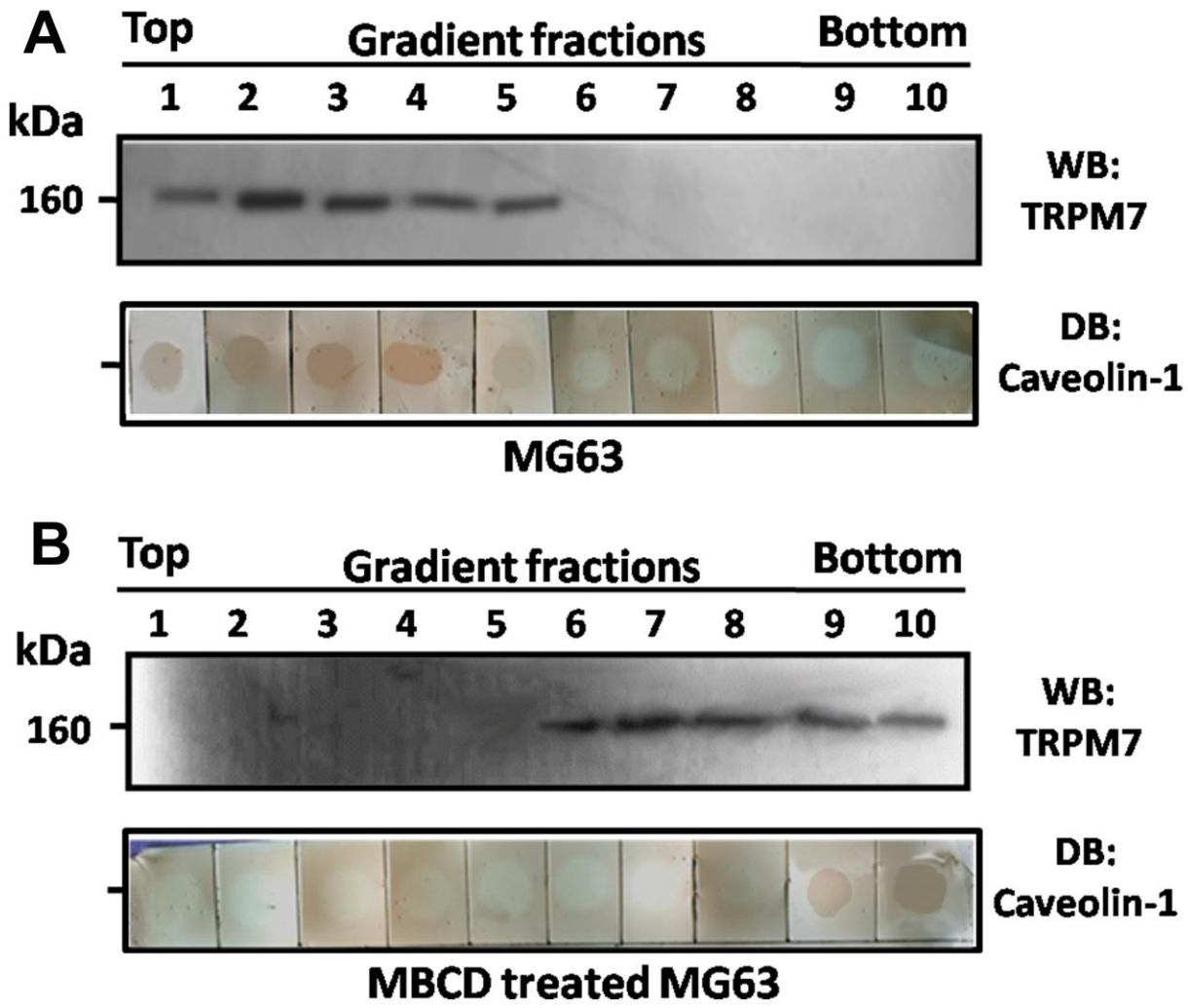

Fig. S4. TRPM7 channels segregate into lipid rafts in MG63 cells. (A) MG63 cells were cultured on microchannel and subjected to oscillatory fluid flaw. Low-density detergent insoluble raft fractions were isolated from the top fractions of ultracentrifuged sucrose density gradient, preloaded with the MG63 cell lysates. Presence of TRPM7 in each fraction was probed by immunoblotting (Western Blot: WB) with TRPM7 antibody. Presence of caveolin-1, an endogenous marker of lipid rafts in the same fractionswasprobed by immunoblotting (Dot Blot: DB) (bottom panel). Immunoreactive bands corresponding to TRPM7 was observed in the top fractions (fraction 1-5) and similar pattern was observed for the raft-marker protein caveolin-1 (B) To observe whether disintegration of raft domains prevents TRPM7 segregation, MG63 cells were pre incubated with10mM MBCD for 30minutes at 37 °C and subjected to oscillatory fluid flow followed by lipid rafts isolation was done as above. The localization of both TRPM7 in lipid rafts was observed. The results shown are representative of three to five independent experiments. Due to cholesterol depletion, the immunoreactive bands of TRPM7 and

caveolin-1were shifted at the bottom fractions (fraction 6-10) as expected for the raft-associated protein. This lipid raft dependent flotation of TRPM7 demonstrated theTRPM7 localization in membrane lipid rafts.

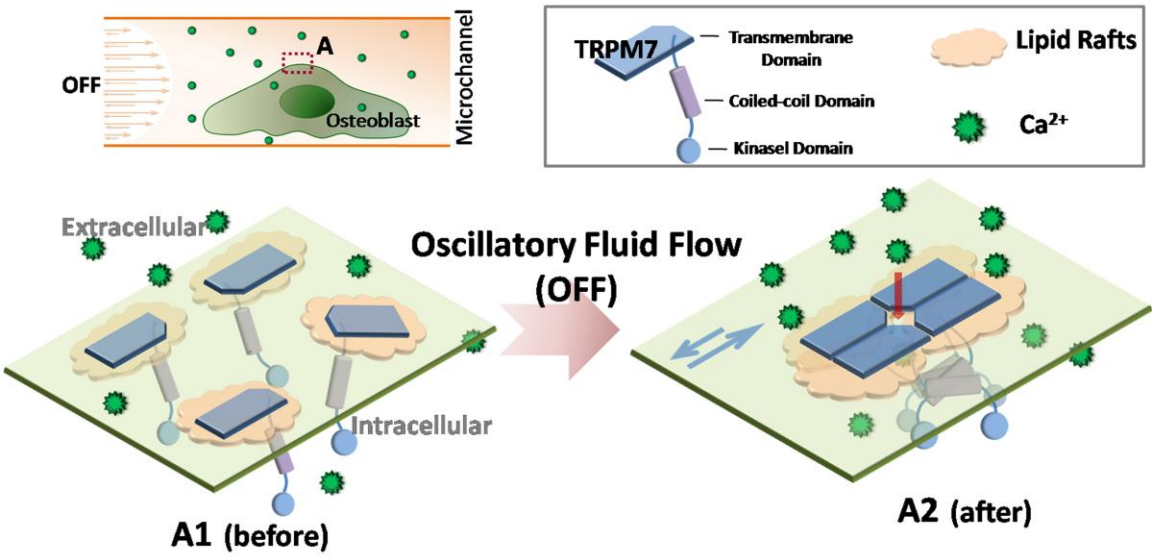

Fig. S5. Both Lipid Raft Domains and Oscillatory Shear Stress are Necessary for TRPM7 Channel Opening. Schematic diagram summarizing the experimental observations and hypothesis on oscillatory shear stress induced lipid raft mediated stimulation of calcium flickers in osteoblast. Oscillatory shear stress helps more TRPM7 segregation at the membrane raft region by increased spatial clustering of lipid rafts through cholesterol agglomeration. This cholesterol enriched and densified TRPM7 channel protein microenvironment within LRs might be favorable for the pore forming tetrameric anti-parallel coiled-coil domain assembly between four TRPM7 as a result of Ca-influx in the form of Ca-flickers. Calcium influx in form of calcium flickers might then be mediated by this assembly. Collectively, the results conclude that for the generation of the maximum level of TRPM7 mediated Ca-flicker, both cholesterol rich raft domains and oscillatory shear stress are necessary.

Table S1. Experimental flow parameters

	Oscillatory	**Pulsatile**	**Steady**
Micrchannel (X×Y×Z)mm	I-shaped (40×15×0.075)	Y-shaped (40×15X0.075)	I-shaped (40×15×0.075)
Inlet Flow Rate (µL/min)	95.78	Inlet1:143.67 Inlet2:0	233.40
Shear stress (Pa)	-0.2→0.8→2	1→1.5→2	2
Reynolds Number (Re)	0.58→5.87	2.93→5.87	5.87
Dynamic viscosity(µ) (N-s/m^2)×10^{-3}	0.723	0.723	0.723

$$\text{Shear stress } (\tau) = \frac{6Q\mu}{WH^2} \qquad Re = \frac{\rho u D_h}{\mu} = \frac{2Q}{vW}$$

Where, Q: flow Rate; W width; H: height of microchannel; μ:dynamic viscosity; ρ:density; v:kinematic viscosity.

Supplementary Movies:

Movie S1: **Flow induced calcium influx in MG63 cells**. Calcium influx was observed immediate after application of 0.2 Hz OFF (max. shear stress of 2 Pa) at T= 13s in presence of extracellular Ca^{2+}. Cells were cultured on microchannel and cells were prelabeled with Fluo-4 and then were subjected to oscillaory fluid-flow.

Keywords to tag the video: Calcium influx, oscillatory fluid-flow, shear stress, MG63 cells, fluo-4

Movie S2: Generation Ca flickers in MG63 cells: The spatial flicker generation was analyzed by customized Matlab program. The difference of Fluo-4 intensity (ΔF) between two consecutive images captured at 0.5s interval for 150s was plotted in the matlab analyzed contour plot. The colourbar of the images is as same as Fig.5. Note that 0.2Hz OFF was applied at t = 5s and cell outline is watermarked.

Keywords to tag the video: Calcium flicker, Spatio-temopral calcium influx, oscillatory shear stress, Calcium imaging using Matlab.