



Table S1. The effect of apigenin on viability of hFOB 1.19 (A), Saos-2 (B) and HCASMC (C) cells. The cells were incubated with different concentrations (μM) of apigenin for 1, 3, 5, 7, 14 days (hFOB 1.19 and Saos-2 cells) or 2, 4, 7, 14, 21 days (HCASMC cells), and then MTT assay was performed. Data are means \pm S.E. of at least three independent experiments (* $p < 0.05$, ** $p < 0.01$ compared with cells without apigenin).

A					
hFOB 1.19					
Apigenin concentration (μM)	Absorbance				
	1 day	3 days	5 days	7 days	14 days
0	0.136 \pm 0.014	0.161 \pm 0.034	0.224 \pm 0.019	0.273 \pm 0.051	0.385 \pm 0.030
1	0.132 \pm 0.017	0.151 \pm 0.006	0.212 \pm 0.013 *	0.261 \pm 0.030	0.432 \pm 0.024 *
2	0.137 \pm 0.023	0.158 \pm 0.013 *	0.190 \pm 0.021	0.216 \pm 0.029 *	0.351 \pm 0.036
5	0.132 \pm 0.028	0.148 \pm 0.030	0.175 \pm 0.008	0.204 \pm 0.043	0.236 \pm 0.036
10	0.119 \pm 0.018	0.128 \pm 0.010	0.135 \pm 0.007	0.174 \pm 0.043	0.130 \pm 0.024
20	0.121 \pm 0.027	0.113 \pm 0.017	0.115 \pm 0.017	0.121 \pm 0.029	0.113 \pm 0.017
B					
Saos-2					
Apigenin concentration (μM)	Absorbance				
	1 day	3 days	5 days	7 days	14 days
0	0.102 \pm 0.015	0.162 \pm 0.030	0.266 \pm 0.008	0.340 \pm 0.024	0.620 \pm 0.073
1	0.117 \pm 0.018	0.185 \pm 0.028	0.251 \pm 0.035	0.272 \pm 0.031	0.504 \pm 0.061
2	0.109 \pm 0.010	0.179 \pm 0.021 *	0.230 \pm 0.037	0.245 \pm 0.030	0.391 \pm 0.022
5	0.113 \pm 0.027	0.151 \pm 0.030	0.203 \pm 0.015	0.229 \pm 0.028	0.389 \pm 0.016
10	0.116 \pm 0.015	0.118 \pm 0.022	0.162 \pm 0.024	0.191 \pm 0.022	0.269 \pm 0.046
20	0.094 \pm 0.012	0.094 \pm 0.016	0.088 \pm 0.011	0.092 \pm 0.014	0.082 \pm 0.012
C					
HCASMC					
Apigenin concentration (μM)	Absorbance				
	2 day	4 days	7 days	14 days	21 days
0	0.133 \pm 0.019	0.201 \pm 0.098	0.035 \pm 0.015	0.145 \pm 0.042	0.110 \pm 0.048
1	0.245 \pm 0.017 *	0.047 \pm 0.027	0.081 \pm 0.008 *	0.145 \pm 0.002	0.251 \pm 0.071 *
2	0.234 \pm 0.075	0.066 \pm 0.041	0.100 \pm 0.020	0.115 \pm 0.007 *	0.382 \pm 0.036
5	0.149 \pm 0.015	0.071 \pm 0.041	0.103 \pm 0.026	0.051 \pm 0.042	0.385 \pm 0.035
10	0.173 \pm 0.056	0.125 \pm 0.095	0.140 \pm 0.014	0.030 \pm 0.046	0.365 \pm 0.028
20	0.292 \pm 0.024	0.208 \pm 0.081	0.112 \pm 0.012	0.099 \pm 0.020	0.203 \pm 0.244

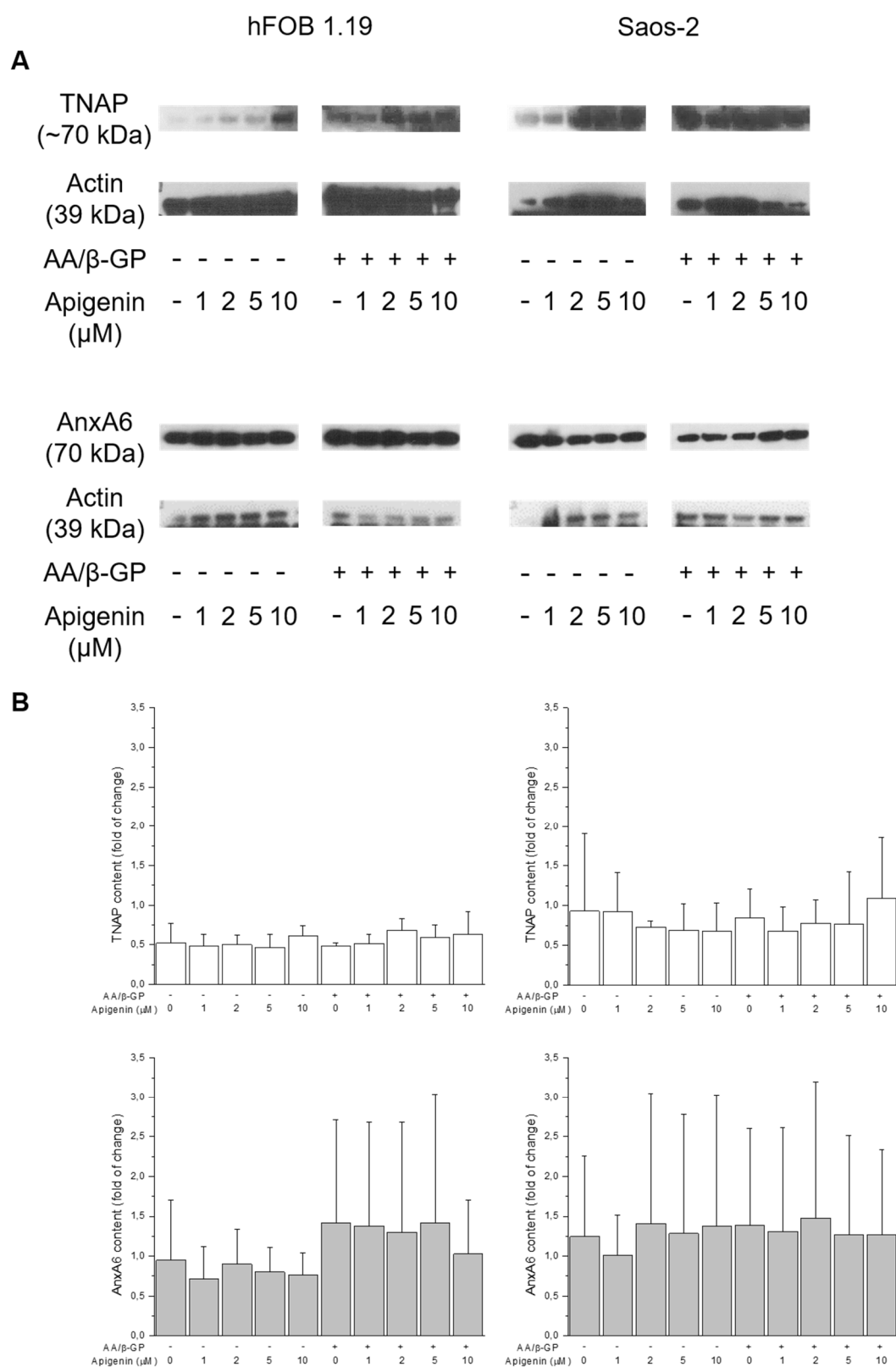


Figure S1. Protein level determined by Western Blot (WB) of TNAP, AnxA6 and actin in hFOB 1.19 and Saos-2 cells under resting conditions (R) or after 7 days stimulation with AA and β -GP (S) in control cells and cells subjected to treatment with different concentrations (μ M) of apigenin. Whole cell lysates were prepared in TLB. **(A)** WB were incubated with rabbit anti-TNAP and mouse anti-AnxA6 primary antibodies followed by appropriate secondary antibodies conjugated with horseradish peroxidase. The proteins content was quantified using inGenius software (Syngene) and calculated per actin content. **(B)** Data are means \pm S.E. of at least three independent experiments.

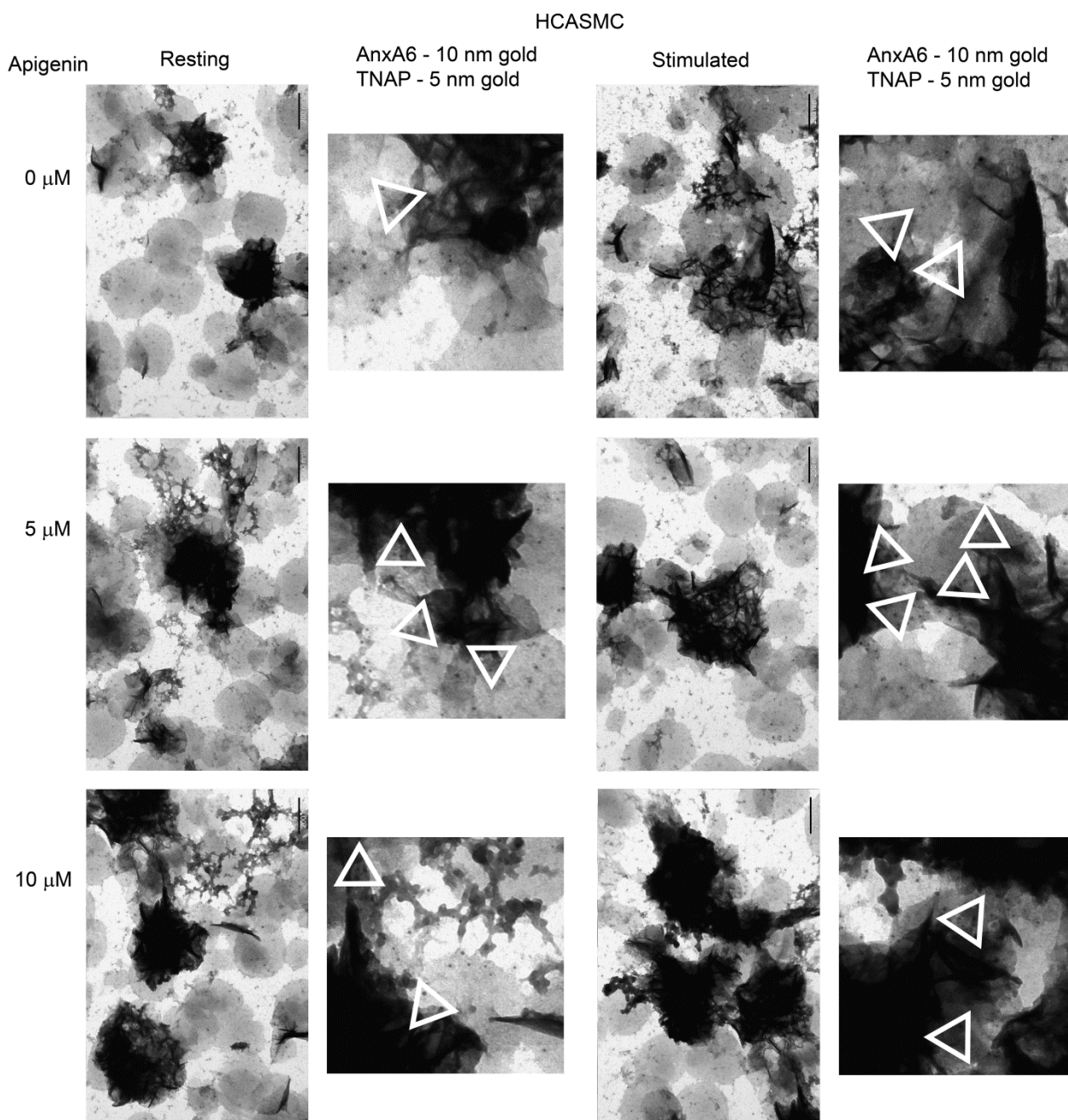


Figure S2. TEM images of co-localization of AnxA6 and TNAP in MVs during mineralization of HCASMC cells under resting conditions or in the presence of stimulators, AA and β -GP. Cells were incubated with different concentrations (μ M) of apigenin for 14 days, lysed and analyzed by TEM (magnification 100,000 \times). Bar: 200 nm. Additional magnifications 300,000 \times . AnxA6 was labelled with anti-AnxA6 primary antibody conjugated with 10 nm colloidal gold secondary antibody. TNAP was labelled with anti-TNAP primary antibody conjugated with 5 nm colloidal gold secondary antibody. Sites of AnxA6 and TNAP co-localization are marked by triangles for HCASMC cells.