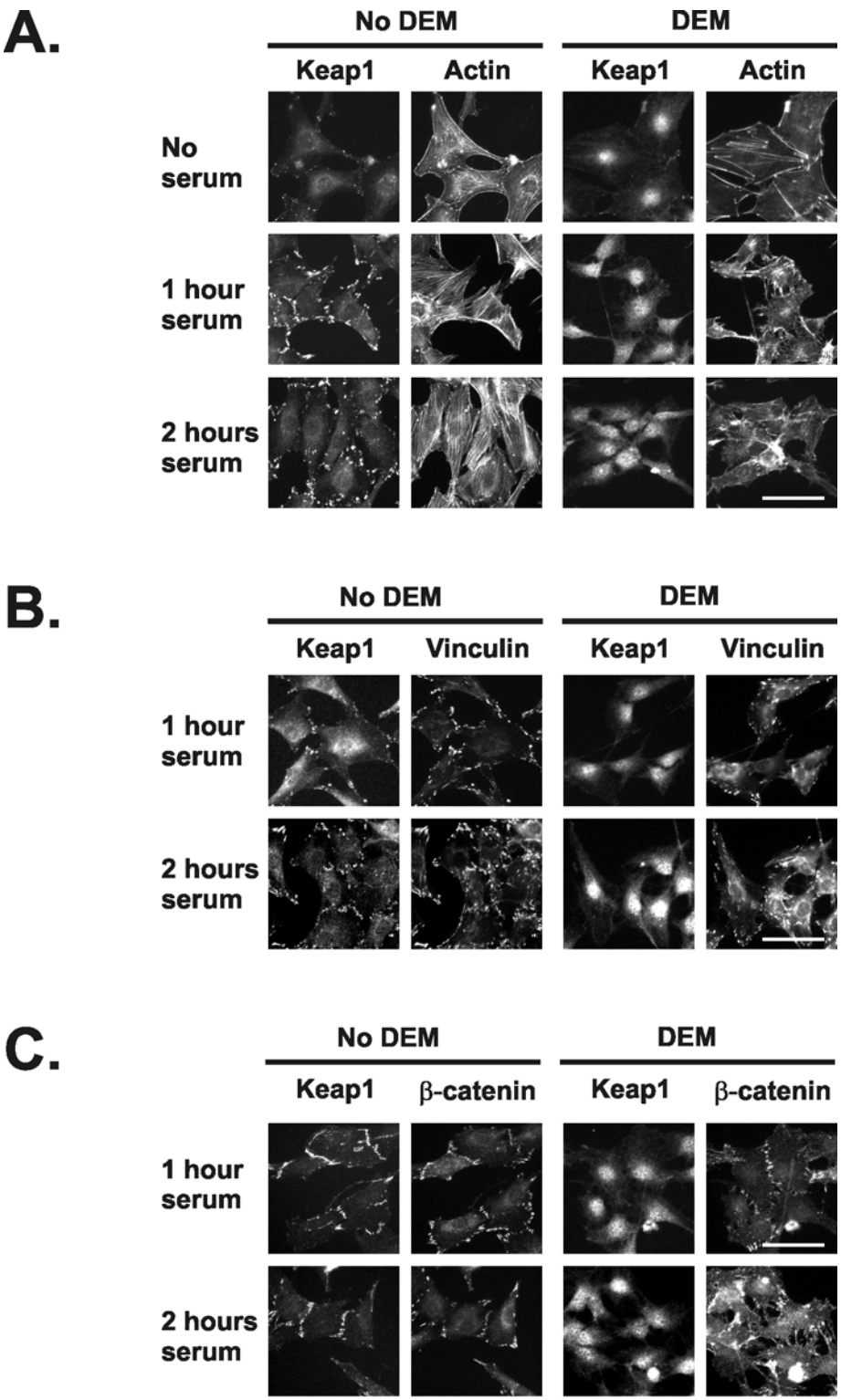
A.
No DEM
DEM
Keap1
Actin
Keap1
Actin
No serum
1 hour serum
2 hours serum
B.
No DEM
DEM
Keap1
Vinculin
Keap1
Vinculin
1 hour serum
2 hours serum
C.
No DEM
DEM
Keap1
β-catenin
Keap1
β-catenin
1 hour serum
2 hours serum

Supplementary Figure 2. Keap1's nuclear localization upon exposure to oxidative agents is not affected by the state of assembly of the actin cytoskeleton

Localization of Keap1 and F-actin in NIH3T3 cells under varying state of cytoskeletal assembly. NIH3T3 cells were grown under different serum conditions (serum starvation for 16 hours and serum starvation followed by addition of serum for 1 and 2 hours) to induce actin filaments disassembly/assembly. Cells from each serum condition were exposed to oxidative stress by addition of 100 μM DEM for an hour and then fixed and stained for Keap1 and actin, vinculin and β-catenin as described for Figure 2A. (A) Immunolocalization of Keap1 and actin. Keap1 and actin were visualized as described in Figure 2A,a. Keap1's nuclear localization after oxidative stress is not affected by the progressive assembly of the actin cytoskeleton. (B) Immunolocalization of Keap1 and vinculin. Vinculin is used as a marker for focal adhesions. Keap1 and vinculin were visualized as described in Figure 2A,b. Vinculin does not exit focal adhesions after DEM treatment suggesting maintenance of focal adhesion integrity after oxidative stress. (C) Immunolocalization of Keap1 and β-catenin (marker for adherens junctions). Keap1 and β-catenin were visualized as described in Figure 2A,c. β-catenin remains associated with the residual adherens junction between adjacent cells while Keap1 concentrates in the nucleus. Scale Bars = 10μm.