



Fig. S1. RhoA does not inhibit Nuclear Localization of NFAT. (a) U2OS cells transfected with flag-tagged wild type NFAT with or without constitutively active RhoA were treated with CaCl_2 and ionomycin. NFAT nuclear localization was then measured by fluorescence microscopy. (b) The percentage of nuclear NFAT expression cells was determined by scoring NFAT transfected U2OS cells after stimulation with CaCl_2 and ionomycin. There was no significant difference in the percentage of cells expressing nuclear NFAT after stimulation in the presence of RhoA versus vector. Several hundred NFAT+ cells were counted for each experiment. Data shown is the average of 4 independent experiments. (c) Jurkat T cells were either untransduced or transduced with the RhoA63L retrovirus and stimulated with PHA and ionomycin for 5 hours or left unstimulated. The NFAT binding site from the IL-2 promoter was used as a probe for NFAT binding. Upon activation with PHA and ionomycin, there was binding to the NFAT probe in both vector and RhoA63L cells. The addition of an α -NFATc1 antibody to lysates before incubation with the probe resulted in a size shift of the probe/NFAT complex in both cell lines.