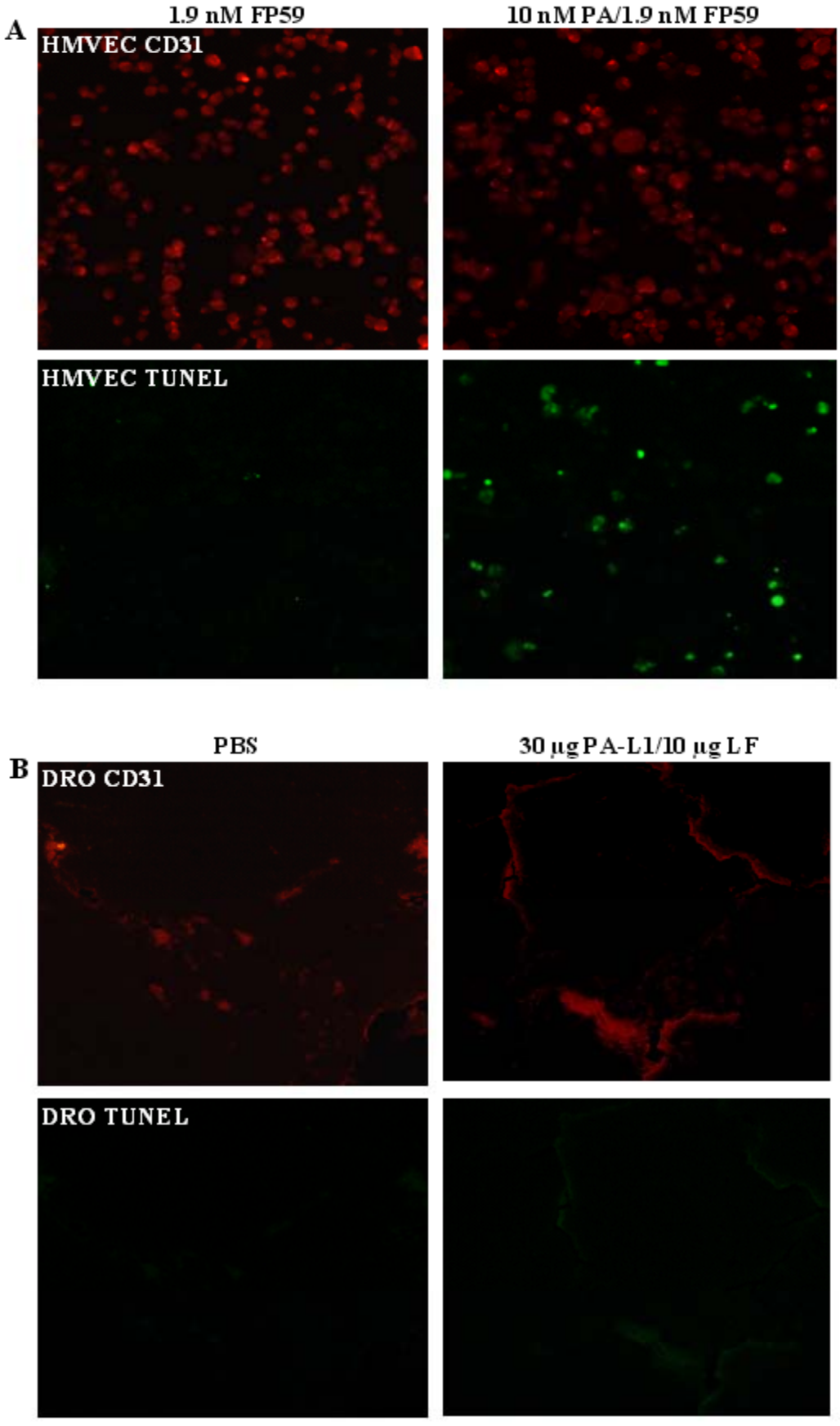
Alfano et al., Supplementary Figure 2



Supplementary Figure 2: CD31/TUNEL costaining in DRO tumor xenografts. The DeadEnd Fluorometric TUNEL system (Promega, Madison, WI) was used to determine apoptosis in endothelial cells. (A) Human dermal microvascular endothelial cells (HMVECs) that were treated with 1.9 nM FP59 alone or in combination with 10 nM PA for 4 hours at 37ºC/5% CO2 served as positive controls. FP59 or PA/FP59-treated HMVECs were trypsinized and pelleted by centrifugation, washed with PBS, resuspended in 100 µl of PBS, and 15 µl were dropped onto a positively charged Superfrost slides. HMVECs were air dried for 15 min and then fixed with 4% paraformaldehyde in PBS. Slides were then washed twice with PBS. Slides were incubated with anti-CD31 antibody overnight at 4ºC followed by 3 PBS washes for 5 min each. Slides were then incubated with a Texas Red-conjugated goat anti-rat secondary antibody for 1 h at room temperature. Slides were then washed with PBS 3 times and refixed in 4% paraformaldehyde in PBS for 10 min followed by 2 PBS washes. Cells were then permeabilized by a .2% Triton X-100 wash for 15 min and TUNEL staining was performed according to the manufacturer's instructions. Both FP59 and PA/FP59-treated HMVECs are positive for CD31 staining while only PA/FP59-treated HMVECs are TUNEL positive. Total magnification 100x. (B) DRO orthotopic xenografts treated with a single dose of either PBS or 30 µg PA-L1/10 µg LF were harvested 18 h later and were stained for CD31 as described in the methods section. Slides were then fixed 4% paraformaldehyde in PBS for 10 min followed by 2 PBS washes and were then permeabilized by a .2% Triton X-100 wash for 15 min. TUNEL staining was performed according to the manufacturer's instructions. Both PBS and PA-L1/LF-treated xenografts demonstrate extensive CD31 positive vasculature while neither treatment induces an increase in TUNEL positive staining. Total magnification 100x.