Supplemental Figure 1
Nutlin-3 inhibits HRMECs proliferation. (A, upper) Racemic Nutlin-3, in the listed concentrations, was added to proliferating HRMECs for 36 hours. (A, lower) Phase contrast images representative of conditions in (A) at 36 hours. (B) Flow cytometry of annexin V and propidium iodide staining of HRMECs in serum enriched medium at 24 hours. (C) Representative images of HRMECs grown in 10% FBS with DMSO (vehicle) (left), Nutlin-3 15 µM (middle) for 24 hours prior to TUNEL staining. Image on the right is 10 minutes prior to TUNEL staining with DNase treatment (positive control). 100x original magnification.
Supplemental Figure 2

p53 is necessary for Nutlin-3 mediated cell death. (A and B). Rat retinal endothelial cells (RREC) were transfected with two different p53 specific siRNA sequences (#1 and #2) or control siRNA. (A) Cell lysates for Western blot analysis were used to confirm knock down of p53 in siRNA transfected RREC. (B) RRECs seeded at 3000 cells/well in 96 well plates were transfected with control siRNA or two different target specific p53 siRNAs (#1 and #2) and then incubated with either Nutlin-3 (7.5 μM) or vehicle (DMSO).
Supplemental Figure 3
Nutlin-3 does not induce apoptosis in HUVEC growth medium. Representative bar graph from three independent experiments ran in duplicate after staining HUVECs with annexin V and propidium iodide were analyzed with flow cytometry. Either 7.5 µM of Nutlin-3A or vehicle was added to HUVECs cultured in growth medium.
Supplemental Figure 4

VEGF-A inhibition does not further impact Nutlin-3 anti-angiogenesis. VEGF-A f/f mice were given either subretinal AAV1-VMD2-Cre or AAV1-VMD2-GFP. n=8, *=p<0.05 and NS= p>0.05
Supplemental Figure 5
Nutlin-3 did not affect lymphangiogenesis in hindlimb ischemia. (A) Quantification (mean ± SEM) of lymphatic capillary density by Lyve1 immunostaining, expressed as a ratio of ischemic to non ischemic hindlimb normalized on myocytes number, shows that Nutlin-3 did not affect lymphangiogenesis in ischemic hindlimb. (B) Representative pictures of Lyve1 immunostaining of capillaries in non ischemic and ischemic muscles of lower limbs revealed no differences at 7 days in the Nutlin-3 treated mice compared to vehicle-treated ischemic hindlimb. Scale bar: 100µm.
Supplemental Figure 6
Characterization of smooth muscle cells in mouse retinal vasculature. Adult mouse retinal wholemounts were stained with lectin and then permeabilized and stained for smooth muscle actin. 40X (left panel) and 200X (right panel) images of a retinal wholemount are displayed. Arrows point to large blood vessels that have smooth muscle actin positive cells around some of the vessels. Notably most of the smaller caliber blood vessels do not have smooth muscle actin positive cells.