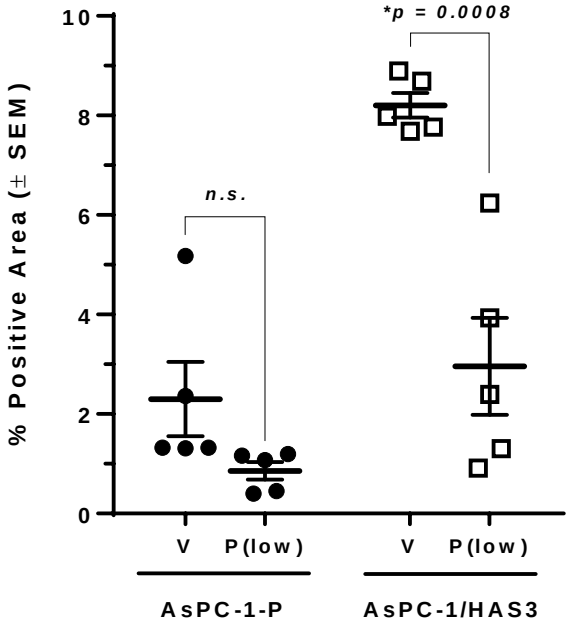
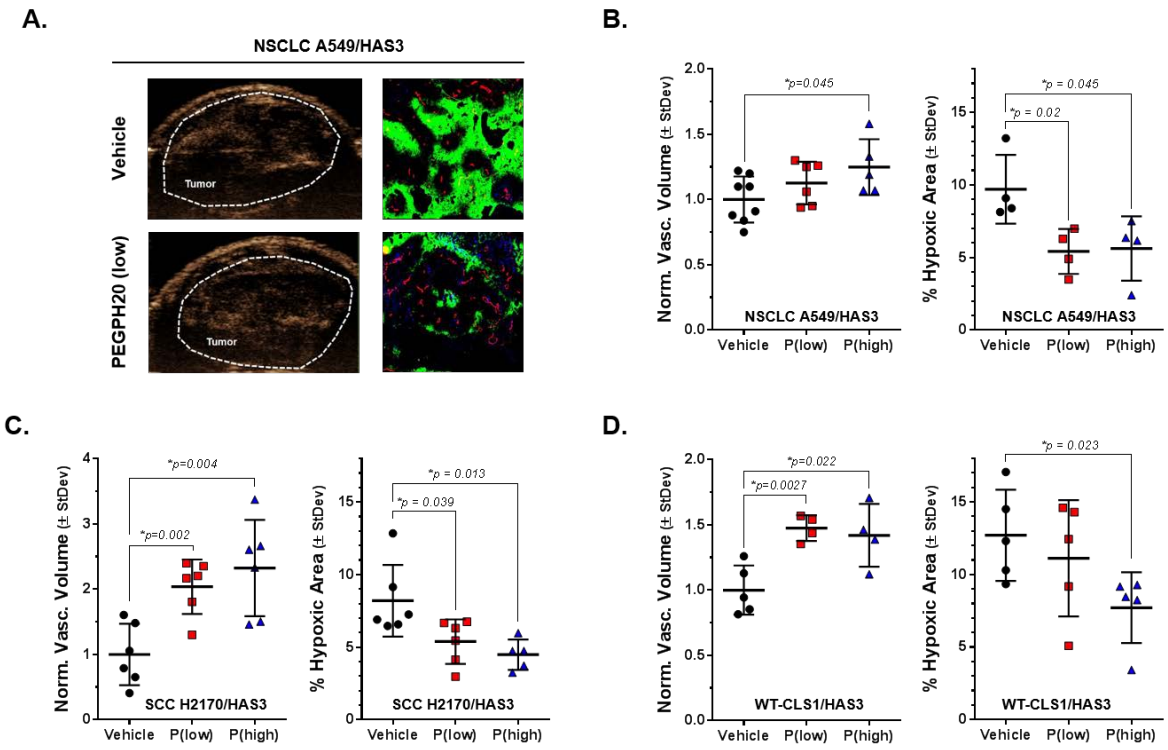


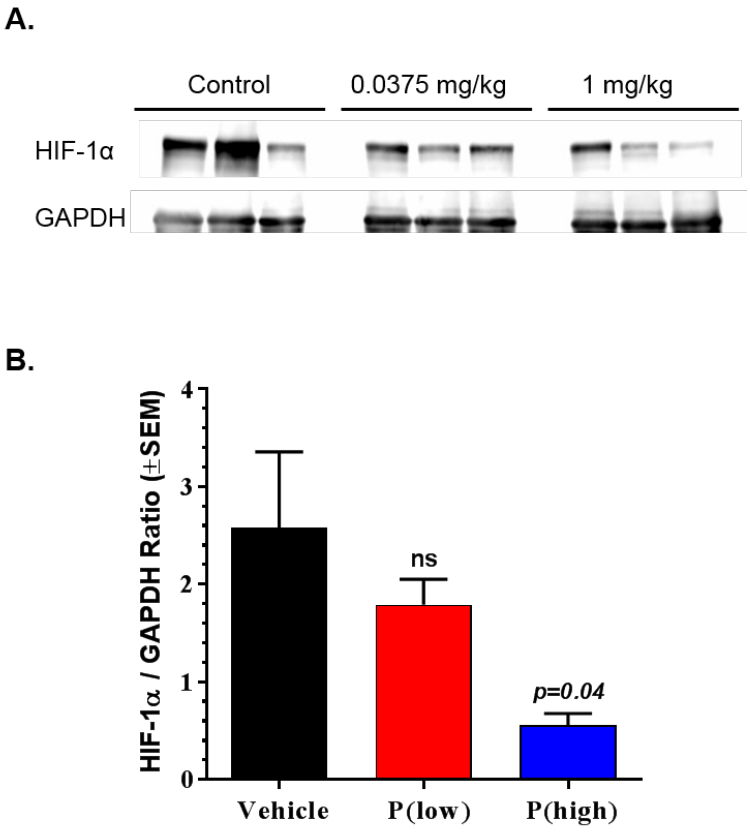
Supplementary Figure S1.

In HA-accumulating AsPC-1/HAS3 tumors, low-dose PEGPH20 decreased tumor HA levels by >64%; whereas the level of tumor HA in the already very HA-low parental AsPC-1 (AsPC-1-P) tumors remained statistically unchanged (n = 10/group). In a modification from the HA quantification methods used in the manuscript, HA staining was detected using TSG-6 and followed by fluorescein-HRP (Vector Labs) and Texas Red (Dako) staining and images were then analyzed with Image-Pro Analyzer 7.0 software (Media Cybernetics). The percent fluorescent positive area was calculated as the fluorescent signal area divided by the entire tumor area (% positive area = positive signal area / total area).



Supplementary Figure S2.

PEGPH20-mediated HA degradation increases tumor vascular area and decreases tumor hypoxia in HA-accumulating tumors. A, Representative high-resolution ultrasound tumor 2D images (left) and hypoxic regions (right, green) of A549/HAS3 tumors treated with vehicle control or low PEGPH20 (0.0375 mg/kg), as described in Materials and Methods. Low- and high-dose PEGPH20 (0.0375 mg/kg, 1 mg/kg) increased tumor vascular area and decreased hypoxia in peritibial NSCLC A549/HAS3 (B) and SCC H2170/HAS3 (C) xenograft tumors; whereas in Wilms' Tumor WT-CLS1/HAS3 (D) tumors, vascular volume was increased at both PEGPH20 doses, but a significant decrease in hypoxia was observed only at the high PEGPH20 dose ($n \geq 5$/group, P value vs. vehicle control).



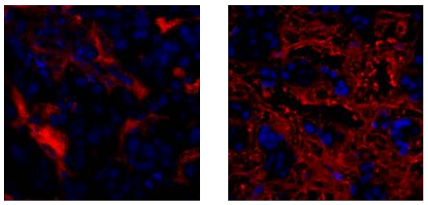
Supplementary Figure S3.

Reduced tumor HA via enzymatic digestion decreases HIF-1α protein expression. A, Representative HIF-1α western blots of whole human xenograft H2170/HAS3 tumor homogenates. B, Repeat PEGPH20 administration (4 doses) at 1 mg/kg (P(high)) decreased HIF-1α protein levels by 78% ($n \geq 5$/group, P value vs. vehicle control). PEGPH20 treatment at 0.0375 mg/kg (P(low)) did not show a show a statistically significant reduction. Immunoblot method: in brief, tumor tissues were homogenized in cold hypotonic buffer and nuclear proteins were extracted using nuclear extraction buffer (25mM Tris.HCl pH7.4, 250 mM NaCl, 10% glycerol, 0.5% Triton X-100, 5 mM MgCl2 with protease & phosphatase inhibitor cocktail). Equal amounts of nuclear protein (15 µg) were separated on SDS-PAGE gel, transferred into a nitrocellulose membrane along with purified protein standard (Full Range Rainbow™ Molecular Weight Markers, from 10 to 250 kDa, GE Healthcare) to insure qualitative accuracy of

analyzed proteins. Membranes were blocked with 5% BSA in PBST (PBS with 0.1% Tween 20) for 2 hours at 4ºC. Membrane was then probed with HIF-1α mouse monoclonal antibody (1:2000; Abcam) overnight at 4ºC under constant shaking. Membranes were next incubated with HRP-conjugated goat anti-mouse IgG antibody (1:10000; Jackson ImmunoResearch) for 2 hours at room temperature. Color was developed by incubated with chemiluminescent reagent (GE Healthcare). All membranes were visualized using GE ImageQuant 400. Bands were quantified using Image-Pro Analyzer 7.0 software.

A.

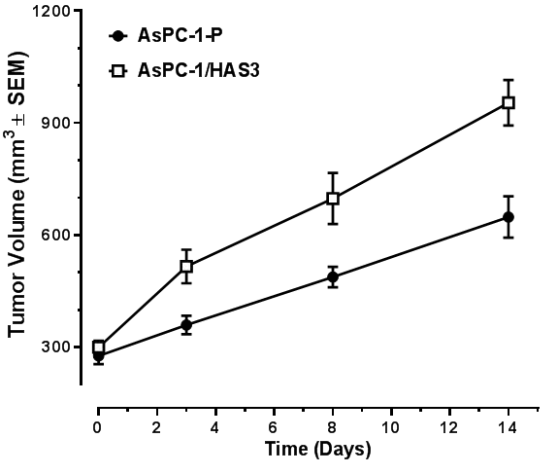
AsPC-1



xenografts	% HA positive (pixels)[1]	Relative HA Level
AsPC-1-P	2.3 ± 1.7	1
AsPC-1/HAS3	8.2 ± 0.6	3.5

[1]Assessed via IHC as described in Materials and Methods (*n* ≥ *5 group*). NOTE – HA in necrotic and/or fibrotic tumor regions excluded from analysis

B.



Supplementary Figure S4.

AsPC-1 parental cells (AsPC-1-P) engineered to overexpress HAS3 (AsPC-1/HAS3, see Materials and Methods) produced more HA in the peritumoral matrix and the stroma. **A,** The proportion of HA-positive staining in whole tumor sections suggested that AsPC-1/HAS3 accumulated ~3.5-fold higher levels of HA within the TME. **B,** HA-accumulating AsPC-1/HAS3 tumors subsequently grew significantly faster than parental AsPC-1-P tumors (*n* = 20/group).