Supplementary information for:

Title: Obesity-associated inflammation promotes angiogenesis and breast cancer via angiopoietin-like 4

Supplementary Table Legends:

Supplementary Table S1. Supplementary table to Figure 4F.

Gene Set Enrichment Analysis of basal-like breast cancer samples comparing *ANGPTL4* high expression tertile (n=66) to *ANGPTL4* low expression tertile (n=66) from GEO datasets GSE76275. Gene sets listed were enriched in the *ANGPTL4* high tertile. Only gene sets with FDRs < 0.25 were considered significantly enriched. Data from GSE76275

Supplementary Table S2. Supplementary table to supplementary Figure S3A.

Gene Set Enrichment Analysis of basal-like breast cancer samples comparing *VEGFA* high expression tertile (n=66) to *VEGFA* low tertile (n=66) from GEO dataset GSE76275. Gene sets listed were enriched in the *VEGFA* high tertile. Only gene sets with FDRs < 0.25 were considered significantly enriched. Data from GSE76275

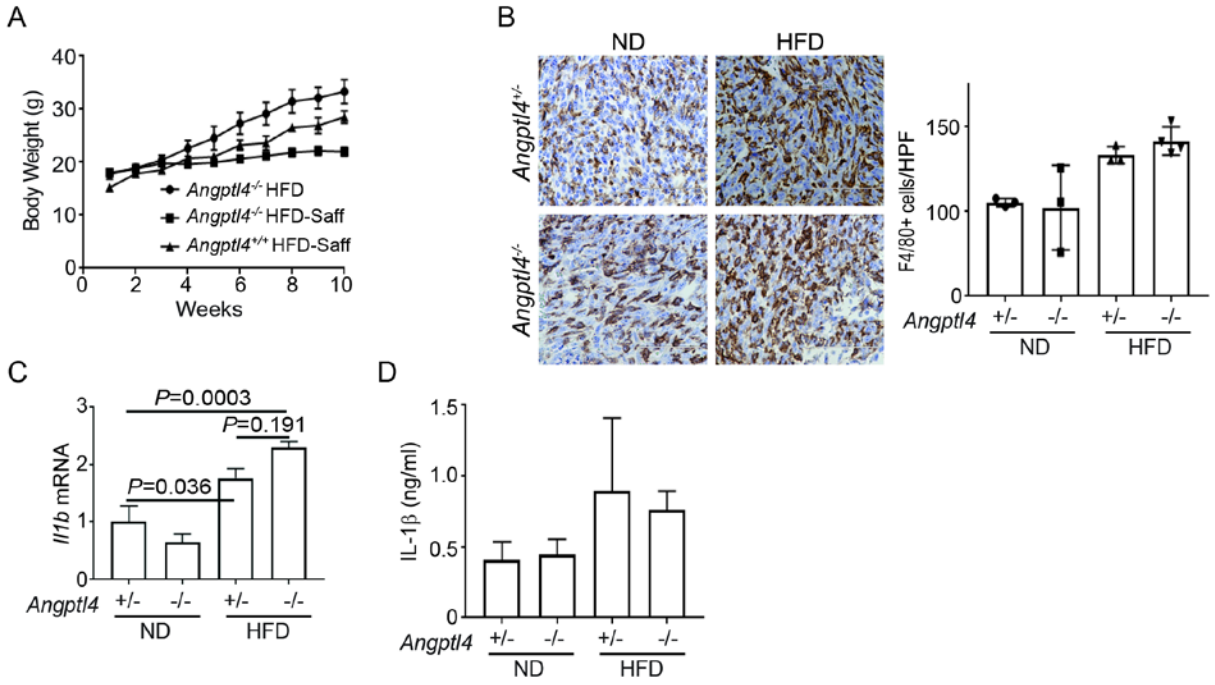
Supplementary Table S3. Supplementary table to supplementary Figure S4B.

Basal-like breast cancer samples were first separated into the *VEGFA* high expressing tertile. Gene Set Enrichment Analysis was then performed comparing *ANGPTL4* high expressing tertile (n=22) to the low expressing tertile (n=22) from *VEGFA* high or low tertiles. Gene sets listed were enriched in the *Angptl4* high tertile. Only gene sets with FDRs < 0.25 were considered significantly enriched. Data from GSE76275

Supplementary Table S4. Supplementary table to supplementary Figure S3B.

Basal-like breast cancer samples were first separated into the *VEGFA* low expressing tertile. Gene Set Enrichment Analysis was then performed comparing *ANGPTL4* high expressing tertile (n=22) to the low expressing tertile (n=22) from *VEGFA* high or low tertiles. Gene sets listed were enriched in the *Angptl4* high tertile. Only gene sets with FDRs < 0.25 were considered significantly enriched. Data from GSE76275

Supplementary Figures and Legends



Supplemental Figure S1 - Supplementary data for Figure 2: ANGPTL4 promotes obesity-driven breast cancer progression and angiogenesis

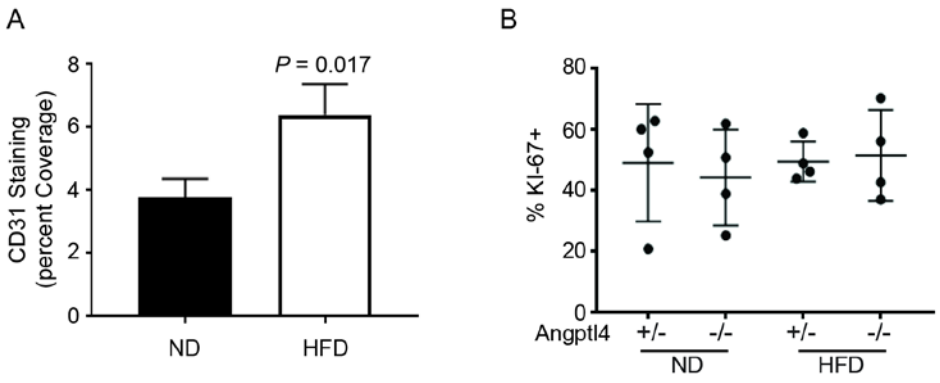
A) The indicated mice were given either the HFD with saturated fatty acid (HFD), or HFD enriched with unsaturated 18:2 fatty acids from safflower oil (HFD-saff). Graph depicts the average body weight of the indicated mice ± s.e.m. (n=5 for all groups)

B) Tumors from the indicated mice from Figure 2B were sectioned and stained for F4/80. Right panel: representative images from the indicated mice. Left panel: graph depicts the average number of F4/80+ cells per high powered field ± s.d. At least 4 images per slide were counted and 3 samples per group were used.

C) Real-time PCR using RNA isolated from tumors from the indicated mice used in Figure 2B. Graph depicts the average *Il1b* mRNA relative to *Ppia* as a fold change compared to *Angptl4*$^{+/-}$ ND ± s.d. N=3 for all groups.

D) Graph depicts the average IL-1β protein level in tumors from the indicated mice from Figure 2B ± s.d. as determined by ELISA. (n=3 for all groups)

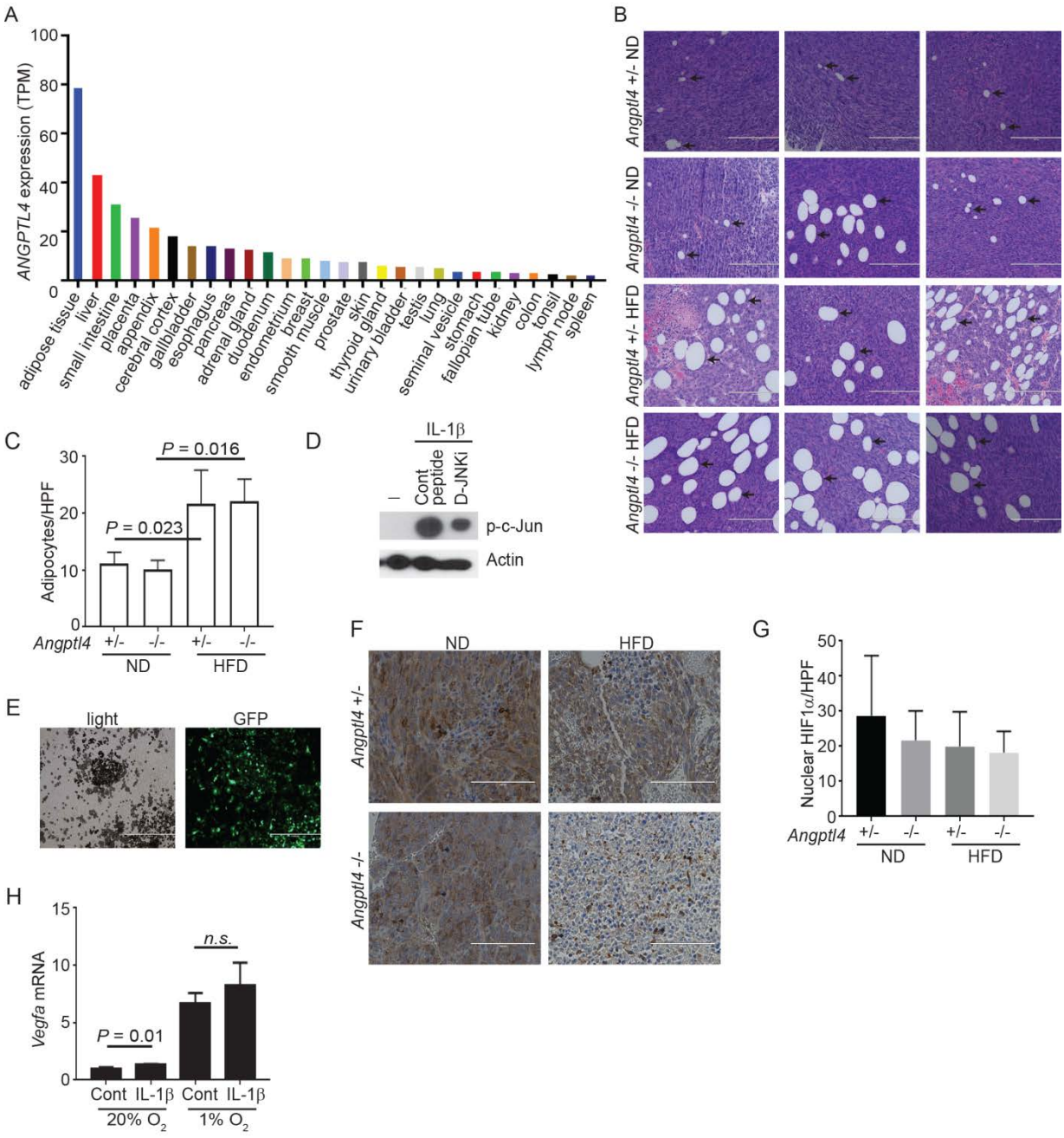
One-way ANOVA with multiple comparisons correction using Dunnett's test was performed to determine significance.



Supplementary Figure S2 - Supplementary data for Figure 2: ANGPTL4 promotes obesity-driven breast cancer progression and angiogenesis

A) Py8119 cells were implanted into the mammary gland of C56BL/6 mice following 10 weeks of either an ND or HFD. Tumors were harvested when they reached 2cm in diameter and stained for CD31. Graph depicts the average CD31 staining ± s.d. as a percent of total pixels (at least 4 images per sample and 3 samples per group were used). Student's t test was used to determine significance.

B) Tumors from Figure 2B were sectioned and stained for Ki-67. Graph depicts the percent of Ki-67+ cells in tumors from the indicated mice. Bar represents the average % of Ki-67+ cells per sample ± s.d. 3 images per sample and 4 samples per group were stained and counted.



Supplementary Figure S3 - Supplementary data for figure 3: IL-1β directly upregulates ANGPTL4 in adipocytes

A) *ANGPTL4* expression in tissues. Data from the Human Protein Atlas.

B-C) H & E staining of Py8119 tumors from the indicated mice. Representative images of intratumoral adipocytes indicted by black arrow (B). Graph (C) depicts the average number of adipocytes per high powered field (HPF) in tumors from the indicated mice ± s.d. At least 10 images per sample and 2 samples per group were counted.

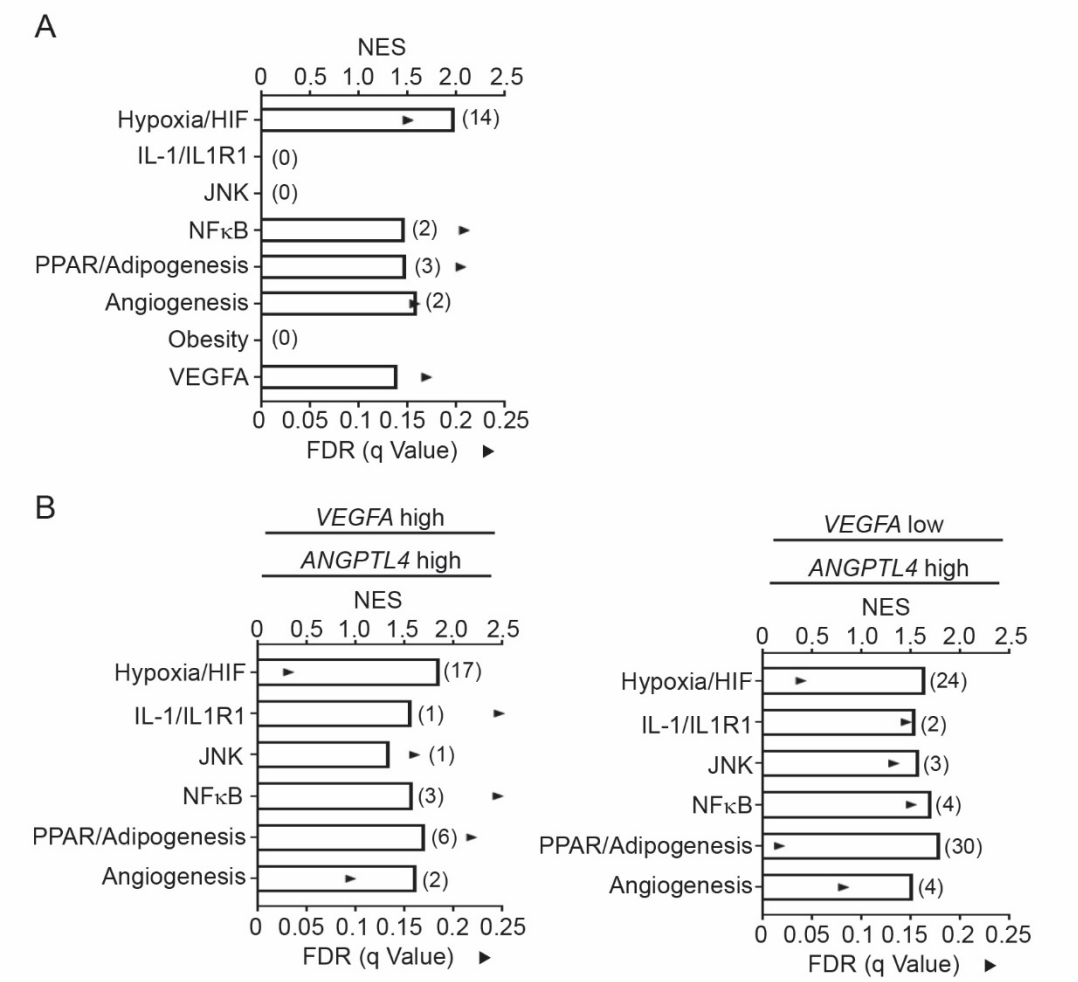
D) Primary mouse adipocytes were treated with D-JNKi or control peptide 1 hour prior to treatment with IL-1β as indicated. Shown are representative images for Western Blotting analysis for phospho-c-JUN and β-Actin.

E) Differentiated human adipocytes were incubated with adenovirus expressing GFP, dominant-negative (DN) JNK or DN IKKβ for 16 hr. Representative image of adipocytes transduced with Ad-GFP with a light microscope were shown using GFP for monitoring transduction efficiency.

F-G) IHC staining for HIF1α of tumor sections from the indicated mice from Figure 2B. Representative images are shown (F). Graph (G) depicts the average number of cells with nuclear HIF1α staining per HPF ± s.d. in tumors from the indicated mice. At least 10 images per sample and 3 samples per slide were counted.

H) Primary adipocytes were treated with or without IL-1β and incubated at 20% or 1% O_2 for 6 hours. Graph depicts the average *Vegfa* mRNA relative to *Ppia* as a fold compared to control (cont) 20% O_2 ± s.d. in the primary mouse adipocytes used in figure 4C. One-way ANOVA was used to determine significance. (n=3 for all groups)

One-way ANOVA with multiple comparisons correction using Dunnett's test was performed to determine significance.

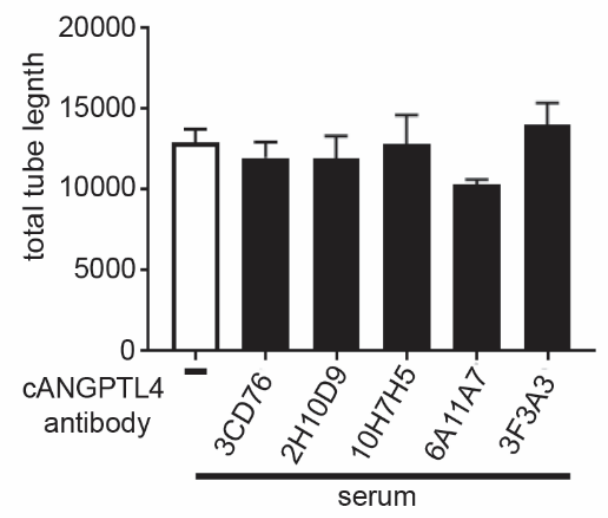


Supplementary Figure S4 - ANGPTL4 is associated with pathways related to angiogenesis, obesity, and inflammation.

A) Gene Set Enrichment Analysis of basal-like breast cancer samples comparing *VEGFA* high expression tertile (n=66) to *VEGFA* low tertile (n=66) from GEO dataset GSE76275. Graph depicts the nominal enrichment score (NES; bar) and FDR (solid triangle) of a representative gene set related to the indicated pathway enriched in the *VEGFA* high group. The number of enriched gene sets related to the indicated pathway is labeled in parenthesis.

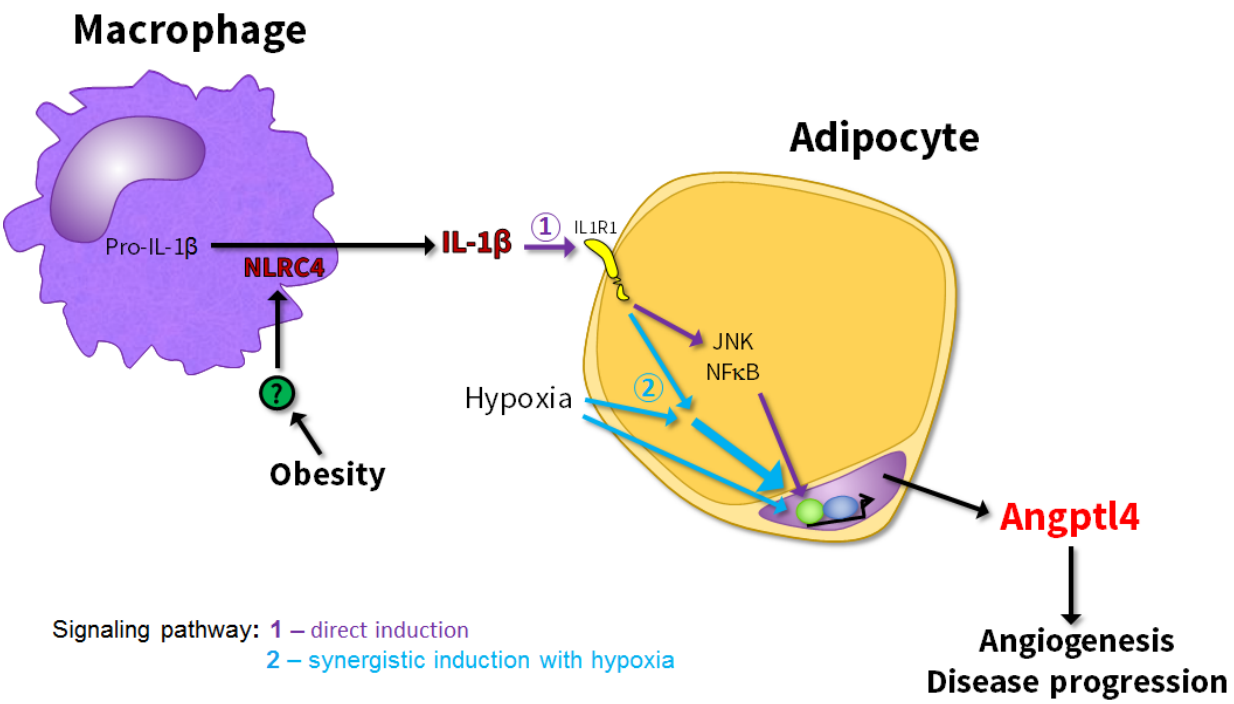
B) Basal-like breast cancer samples were first separated into the *VEGFA* high expressing and low expressing tertiles. Gene Set Enrichment Analysis was then performed comparing *ANGPTL4* high expressing tertile (n=22) to the low expressing tertile (n=22) from *VEGFA* high or low tertiles. Graph depicts the nominal enrichment score (NES; bar) and FDR (solid triangle) of a representative gene sets related to the indicated pathway enriched in the *ANGPTL4* high group. The number of enriched gene sets related to the indicated pathway is labeled in parenthesis.

In (A-B) data is from GSE76275. FDR < 0.25 was considered significant.



Supplementary Figure S5 – Supplementary data for Figure 5 - Targeting cANGPTL4 inhibits obesity-driven breast cancer progression.

Endothelial cell tube formation assay. Endothelial cells were plated in matrigel and treated with serum ± anti-cANGPTL4 antibodies as indicated. After 6 hours cells were stained with Calceim Am and images were analyzed. Graph depicts the average total tube length ± s.d. (n=3 for all groups).



Supplementary Figure S6 - Model.

Here we propose a model depicting how obesity drives angiogenesis and cancer progression. Tumor associated macrophages have activated NLRC4-inflammasome that leads to activation and release of IL-1β. IL-1β induces angiogenic factors such as VEGF and most importantly ANGPTL4 from adipocytes that can drive angiogenesis and cancer progression. IL-1β can induce ANGPTL4 in adipocytes both independently through JNK and NFκB signaling (1:purple) and synergistically with hypoxia (2: blue).