Supplemental Data Iyengar et al.

Collagen VI does not affect the formation of metastases in other tissues

Given that collagen VI promotes hyperplasia and primary tumor development, we sought to determine if collagen VI promoted metastasis. PyMT+/ColVI+/+ and PyMT+/ColVI-/- mice were injected with an equal number (10^5) of metastatic tumor cells (Met1 cells) derived from an isogenic PyMT+ mouse. Lung is one of the most prominent tissues for metastatic growth of breast cancer cells. Therefore, the number of lung metastases was determined after 1, 2, 3, 4, and 6 weeks. None of the time points examined showed a difference in the number of lung metastases observed ($p > .05$), suggesting that the homing and growth of metastatic lesions is unaffected by the absence of collagen VI (**Fig. 1**). This is consistent with the observation that lung tissue does not display a particularly prominent expression for collagen VI under normal conditions. Breast tumor cells, once they achieve strong metastatic potential, may therefore no longer critically depend on collagen VI for growth. This conclusion is supported by recent observations that suggest an up-regulation of collagen VIα3 subunit only in primary, nonmetastatic tumors (1).

Late stage tumor growth and formation of metastases no longer critically depends on adipose tissue

The previous results indicate that the collagen VI-/- mice displayed an equally high susceptibility to metastatic spread and growth. This suggests that the tumor cells lose their dependence on the local presence of collagen VI for survival and growth once they become metastatic. In order to test whether this phenomenon is not restricted to collagen VI but can be generalized to other adipocyte-derived factors, we chose a genetic model for late stage loss of adipose tissue. Overall, our aim was to assess how relatively late stage tumor progression was affected by the lack of adipocytes and their secreted factors in the local microenvironment. Transgenic mice expressing low levels of diphteria toxin under the control of the adipocyte-specific aP2 promoter display normal adipose tissue development (2). These mice completely lose their adipocytes between 15 to 20 weeks of age as judged by a dramatic reduction of the adipose specific secretory factor adiponectin in circulation (**Fig. 2A**). The female transgenic fatless mouse has normal ductal development and can reproduce without problem. Even several months after the onset of adipose tissue loss, the overall architecture of the breast ductal epithelium remains intact (**Fig. 2B**). By day 80, however, a loss in some adipocyte membrane integrity can be observed (**Fig. 2B**), and at later stages, adipose tissue has morphologically completely disappeared (**Fig. 2C**). **Fig. 2D** represents magnetic resonance images taken of the DPT+ and wild type transgenic mice, illustrating how effectively adipose tissue mass has been reduced and almost completely eliminated at about 20 weeks post birth. The ductal architecture remained preserved after the loss of adipocytes (**Fig. 2E**).

This transgenic line was crossed into the MMTV- PyMT line. The relatively late onset of fat loss allows the primary tumors to fully develop under conditions similar to the local environment in a wildtype mouse. Indeed, primary tumor growth in aP2-DpTx / MMTV-PyMT mice was comparable to mice carrying the MMTV-PyMT transgene after 80 days (**Fig. 2F**). The

aP2-DpTx / MMTV-PyMT double-transgenic mice allow us to answer the question as to whether primary tumors that have grown beyond early hyperplasia still require the sustained presence of adipose tissue-derived factors for further progression and metastases. Work from a number of laboratories suggests that early hyperplasias require mammary fat pads to form foci and progress through tumorigenesis. However, cell lines and transplanted mammary ducts that have progressed to increased levels of malignancy are not as strictly dependent upon this adipose-rich microenvironment. Consistent with these observations, once adipose tissue is ablated in the aP2-DpTx / MMTV-PyMT double-transgenic mice (**Fig. 2E**), we failed to observe a difference in the rate of foci formation, primary tumor expansion, and growth compared to MMTV-PyMT littermates that do not lose the adipose tissue (**Fig. 2F**). In addition, loss of adipose tissue had no effect on the number, size, and histological grade of metastases to the lungs **(Fig. 2 G, H)**.

References

1. Wang, W., Wyckoff, J.B., Frohlich, V.C., Oleynikov, Y., Huttelmaier, S., Zavadil, J., Cermak, L., Bottinger, E.P., Singer, R.H., White, J.G., et al. 2002. Single cell behavior in metastatic primary mammary tumors correlated with gene expression patterns revealed by molecular profiling. *Cancer Res* **62**:6278-6288.

2. Ross, S.R., Graves, R.A., and Spiegelman, B.M. 1993. Targeted expression of a toxin gene to adipose tissue: transgenic mice resistant to obesity. *Genes Dev* **7**:1318-1324.

Figure Legends

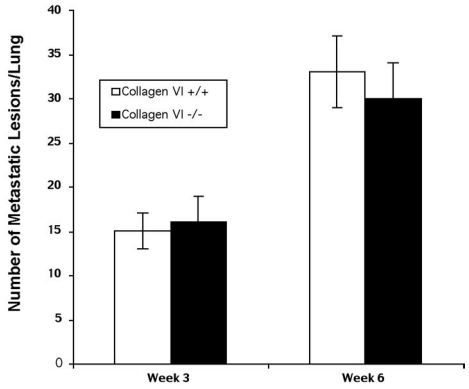
Suppl. Fig. 1. Collagen VI -/- mice do not display a reduced number of metastatic lesions. $1x10^5$ Met1 cells were tail vein injected into 5 mice per group of either wild type collagen +/+ (white bars) or collagen VI -/- mice (black bars). Mice were sacrificed at 1, 2, 3, 4, and 6 weeks post injection. Lungs were subsequently inflated and fixed in formalin for H&E staining. The metastatic lesions were counted.

Suppl. Fig. 2. A model of late onset lipoatrophy, the aP2-DTA mouse, does not display reduced tumor growth. A. *Ablation of adipocyte-derived serum markers:* Levels of the adipocyte-secreted protein adiponectin in the serum of 140 day old aP2-DTA mice compared to wildtype littermates. n=5; Asterisks denote significant differences with $p< 0.05$. **B.** *The aP2-DTA transgene ablates adipocytes around 10 to 12 weeks of age:* H&E stain of mammary fat pad sections taken from aP2-DTA mice at 50 days of age (just prior to fat loss) and 75 days of age (during fat ablation). **C.** *Absence of morphologically distinct adipose tissue at late age:* Mammary sections taken from aP2-DTA mice (358 days old; top) and an age-matched wild type (bottom) littermate. Note the complete absence of adipose tissue at this stage. **D.** *Lack of adipose tissue as judged by MRI imaging:* MRI images of wild type and aP2-DTA mice (140 days old). Transverse sections of the abdomen are shown. Fat appears white in the images. **E.** *Lack of*

adipose tissue does not significantly alter the ductal epithelial structure: Whole mounts of mammary glands taken from wild type and aP2-DTA mice (140 days old mice; low and high magnifications are shown). **F.** *Late stage fat loss does not affect number of foci or primary tumor size:* Number of lesions (left panel) and primary tumor sizes (right panel) from MMTV-PyMT mice transgenic mice (white bars) or MMTV-PyMT / aP2-DTA double transgenic at 13 weeks of age (lesions) and 13 and 16 weeks of age (primary tumor size). n=5. **G.** *Late stage fat loss does not affect number of metastatic lesions:* MMTVPyMT mice transgenic mice (white bars) or MMTV-PyMT / aP2-DTA double transgenic at 10, 13 and 16 weeks of age were analyzed for the number of metastatic lesions found in lungs. n=5. **H.** H&E stain of lung lesions show no pathologic difference in type of metastases found in the presence or absence of adipose tissue post primary tumor development.

Suppl. Table I Transcriptional changes specifically induced by collagen VI

Supplemental figure 1



Supplemental figure 2

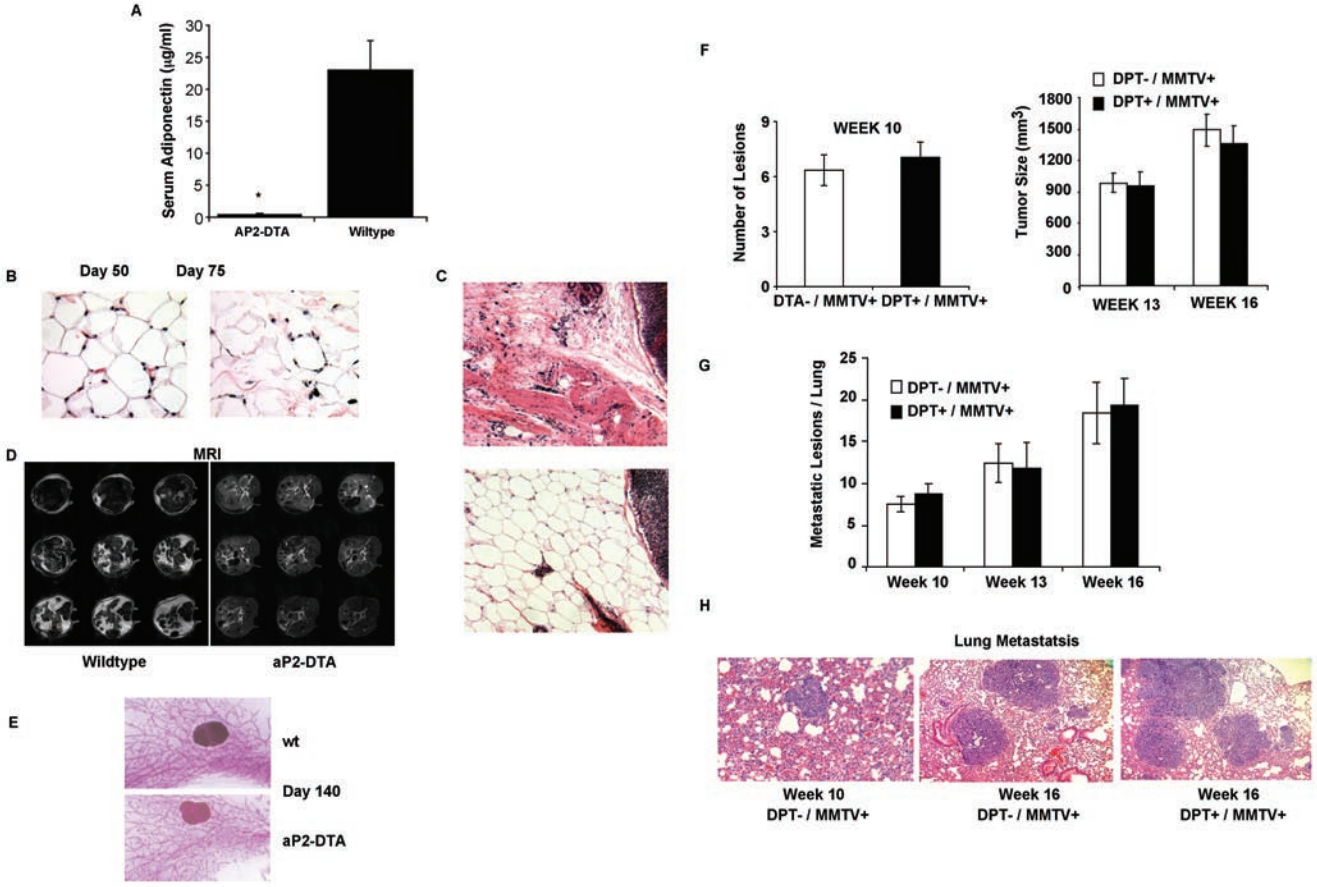


Table I
Iyengar et al.

Collagen VI vs. Collagen I Driven Gene Induction Patterns in MCF-7 Cells - Microarray Analysis

EGR2	Transcription Factor	**UP 34.1 FOLD +/- 5**
EST	Accession Number H77714	**UP 18.1 FOLD +/- 2.3**
Metallothionein-1f	Metal Binding Protein; Consistently Up-regulated in Stage II & III Tumors; Associated with Tumor Invasion; Regulates p53 function; Promotes Chemoresistance	**UP 18 FOLD +/- 2.1**
Metallothionein-1e	Metal Bindind Protein; Also Up-regulated in Late Stage Tumors; Involved in Chemoresistance	**UP 14 FOLD +/- 1.7**
EST	Accesssion Number H54796	**UP 9.71 FOLD +/- 3.7**
EST	Accession Number T50230	**UP 7.38 FOLD +/- 2.6**
ATF3/4	Transcription Factor that Promotes Tumor Metastatic Potential and Malignancy	**UP 6.81 FOLD +/- 1.9**
ETR101	Transcription Factor - No Known Function	**UP 4.2 FOLD +/- .75**
PC4 Homolog	Growth Factor	**UP 3.1 FOLD +/- .45**
Tyk 2	Protein Tyrosine Kinase	**UP 2.9 FOLD +/- .87**
CDC28 PK1	Protein Kinase - Increases Cell Proliferation via p27 Kip1 Degradation	**UP 2.8 FOLD +/- .32**
VEGF	Pro-angiogenic Molecule	**UP 2.6 FOLD +/- .25**
Calpain 4	Up-regulated During Tumor Progression	**UP 2.5 FOLD +/- .33**
IL-8	Cytokine Modulating Tumor Proliferation	**UP 2.3 FOLD +/- .42**
Angiopoitein-2	Promoter of Cell Proliferation	**UP 2.21 FOLD +/- .22**
HSP90	Chaperone	**DOWN 2.25 FOLD +/- .3**
Apolipoprotein C-III	Metabolic Packaging Protein	**DOWN 3.31 FOLD +/- .4**