

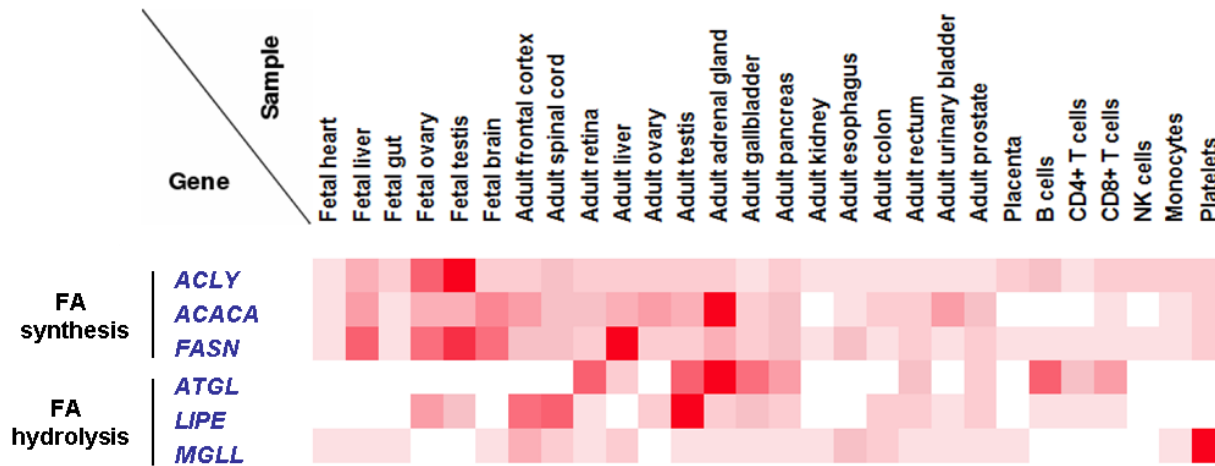
Loss of adipose triglyceride lipase is associated with human cancer and induces mouse pulmonary neoplasia

Supplementary Material

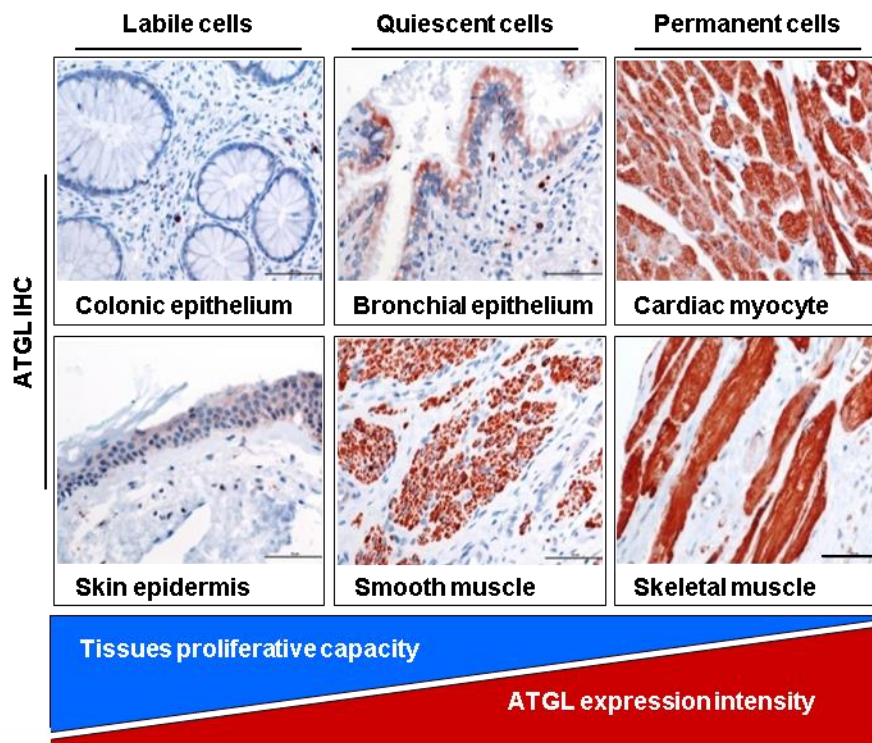
This file includes:

- **Supplementary Figures 1- 6**
- **Supplementary Table**

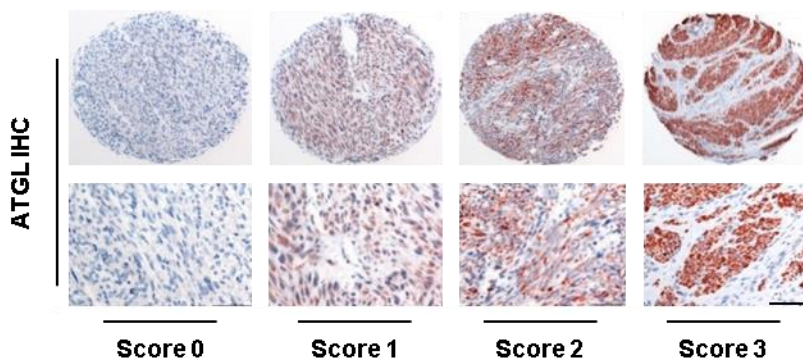
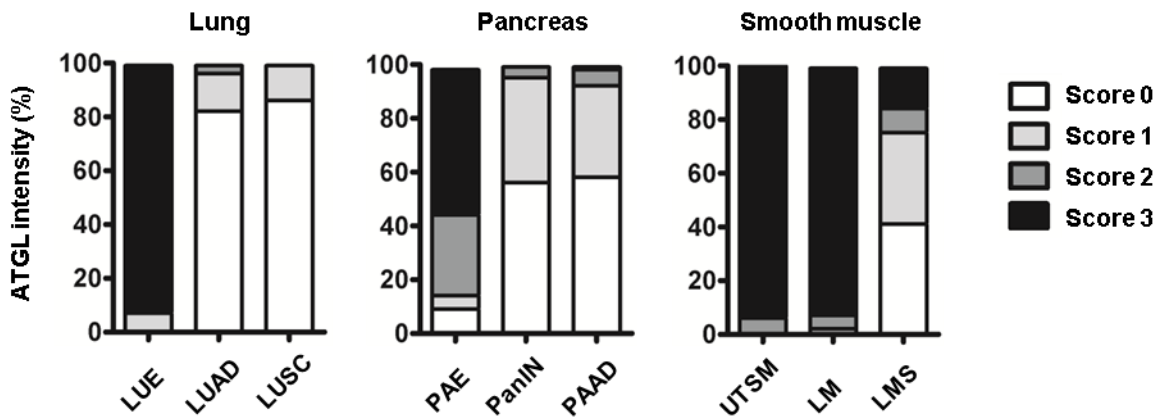
A



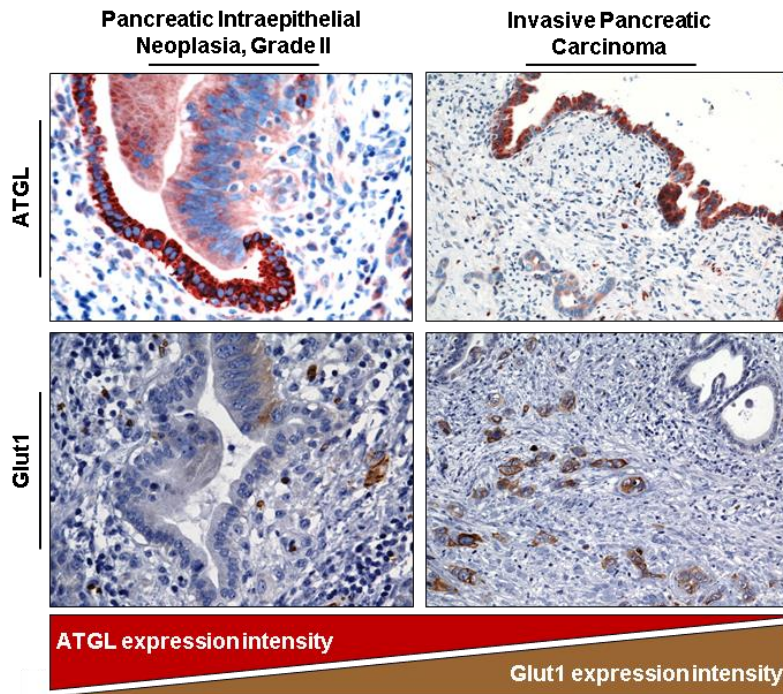
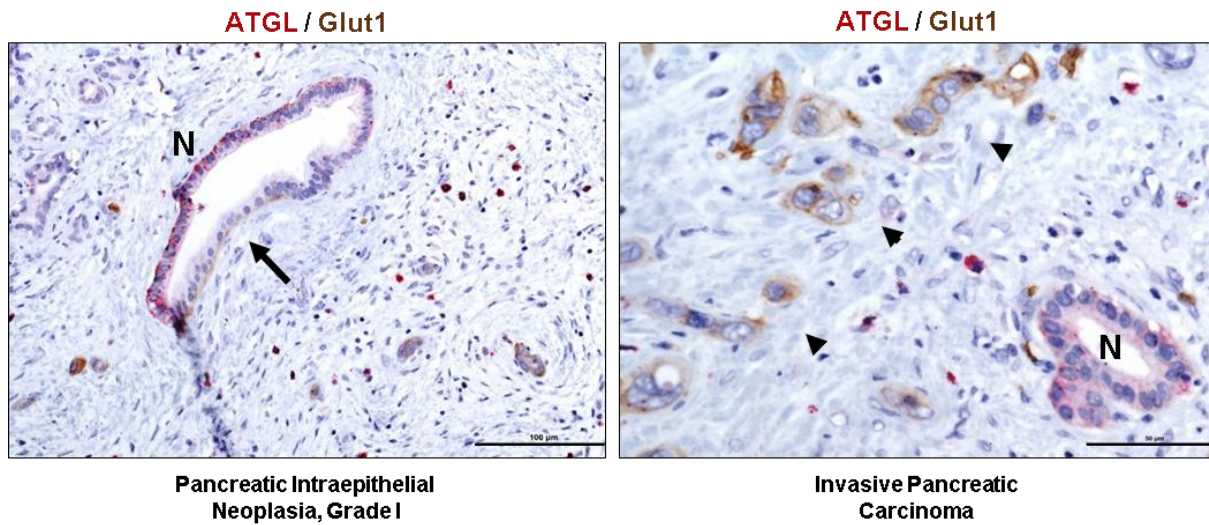
B



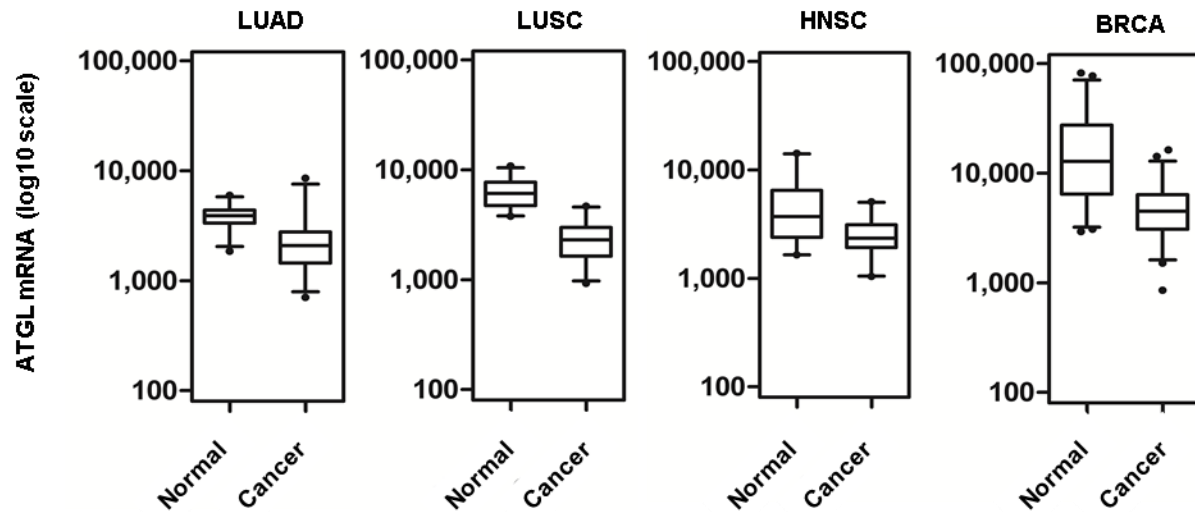
Supplementary Figure 1. ATGL protein expression. **A.** Heat map of protein abundance in fetal and adult tissues. FA, fatty acids; *ACLY*, ATP citrate lyase; *ACACA*, acetyl-CoA carboxylase alpha; *FASN*, fatty acid synthase; *ATGL*, adipose triglyceride lipase (the full name is patatin-like phospholipase domain containing 2, *PNPLA2*); *LIPE*, lipase hormone-sensitive (also known as *HSL*); *MGLL*, monoglyceride lipase (also known as *MGL*). **B.** ATGL protein expression across normal tissues. Scale bar, 50µm.

A**B**

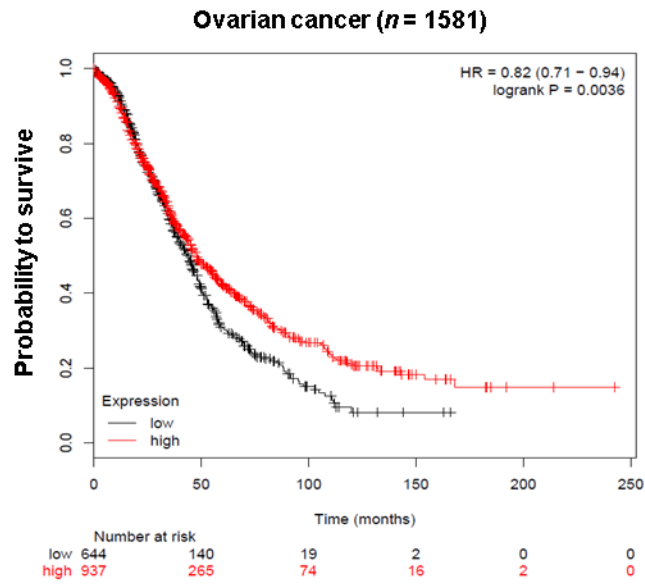
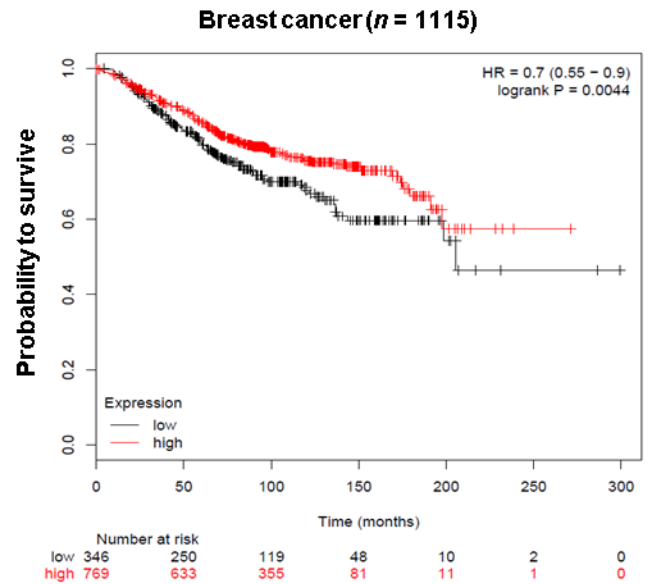
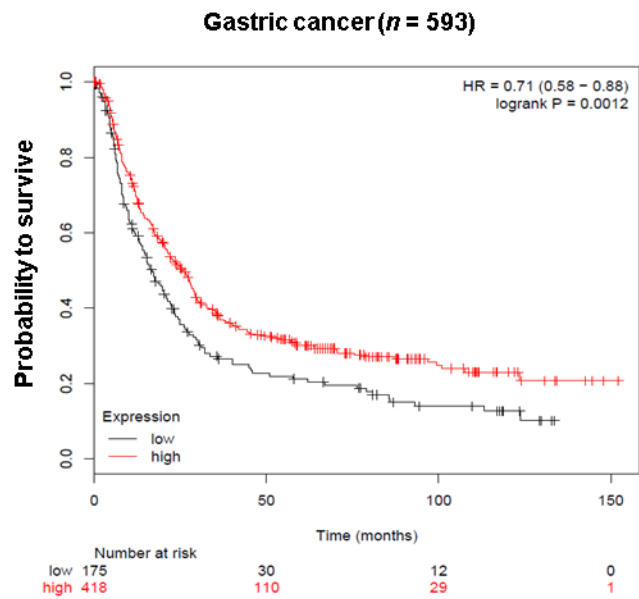
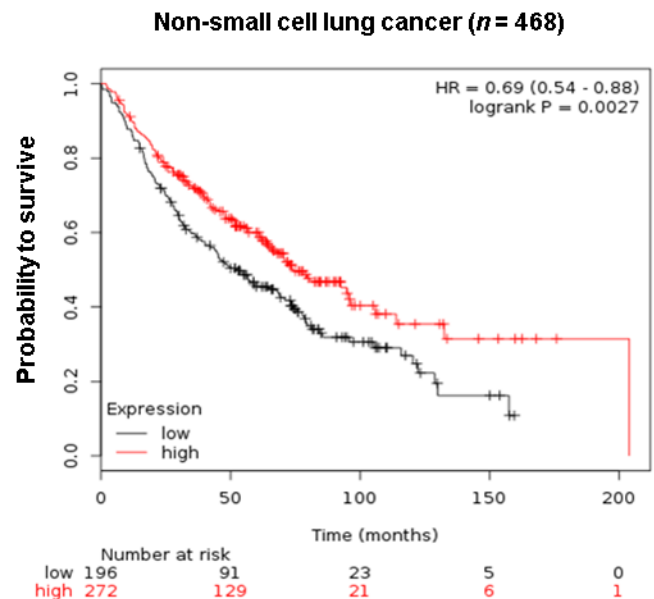
Supplementary Figure 2. ATGL protein is frequently suppressed in human cancer. A. Images illustrating ATGL IHC staining intensity score. Regions from the upper panels are shown in higher magnification in the lower panels. Scale bar, 50µm. **B.** Bar graphs show percentages of ATGL IHC staining intensity in neoplasia of lung epithelium, pancreatic epithelium or smooth muscle (see Fig. 1). LUE, normal lung epithelium; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PAE, normal pancreatic epithelium; PanIN, pancreatic intraepithelial neoplasia; PAAD, pancreatic adenocarcinoma; UTSM, normal uterus smooth muscle; LM, benign smooth muscle tumor (leiomyoma); LMS, malignant smooth muscle tumor (leiomyosarcoma).

A**B**

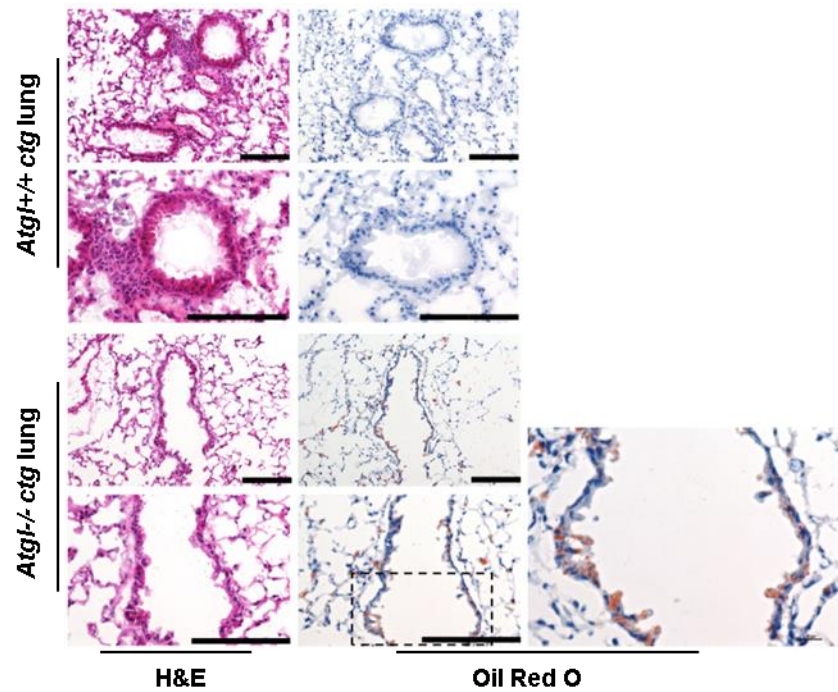
Supplementary Figure 3. Inverse correlation between ATGL and glucose transporter 1 (Glut1). **A.** Immunohistochemical (IHC) staining for ATGL (upper panels), or Glut1 (lower panels). **B.** Double IHC staining for ATGL (signal in red) and Glut1 (signal in brown). N, normal epithelium; arrow points to a neoplastic lesion shows detectable Glut1 signal at the basal domain; arrowheads point to invasive tumor cells. Scale bars, 100 μm (left panel) and 50 μm (right panel).



Supplementary Figure 4. ATGL mRNA expression levels in samples of four types of cancer patients. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; HNSC, head and neck squamous cell carcinoma; BRCA, breast ductal carcinoma. (LUAD: $n = 57$, Log2fold change = -0.85, Adjusted P value = 1.47×10^{-17} ; LUSC: $n = 50$, Log2fold change = -1.41, Adjusted P value = 6.23×10^{-50} ; HNSC: $n = 40$, Log2fold change = -0.72, Adjusted P value = 1.57×10^{-7} ; BRCA: $n = 108$, Log2fold change = -1.61, Adjusted P value = 6.17×10^{-33} .)

A**B****C****D**

Supplementary Figure 5. *ATGL* mRNA expression level is a prognostic factor in multiple cohorts of different types of cancer. Overall survival of 1581 ovarian cancer patients (A), 1115 breast cancer patients (B), 593 gastric cancer patients (C), 486 non-small cell lung cancer patients (CAARRAY cohort) (D) after dichotomization according to the *ATGL* expression levels. HR: hazard ratio.



Supplementary Figure 6. Accumulation of neutral lipids in ATGL deficient bronchial epithelial cells. Representative images show H&E and Oil Red O staining of lung sections. Bronchial epithelium of *Atgl*^{-/-} ctg mice stains positive for Oil Red O whereas bronchial epithelium of *Atgl*^{+/+} ctg mice is negative. Regions from the upper panels are shown in higher magnification in the lower panels. Scale bar, 100µm.

Supplementary Table: Number of animals with neoplastic lesions detected in lungs of *Atgl*+/+ ctg, *Atgl* +/- ctg and *Atgl* -/- ctg mice

Age group (months)	Number of animals with pulmonary neoplasia Neoplasia/Total ^(Statistical significance)		
	<i>Atgl</i> +/+ ctg	<i>Atgl</i> +/- ctg	<i>Atgl</i> -/- ctg
1-3	0/8	0/13 ^(ns)	2/13 ^(ns)
4-6	0/10	1/10 ^(ns)	8/11 ^(***)
7-10	0/10	0/5 ^(ns)	5/5 ^(***)
11-18	1/11	4/12 ^(ns)	12/12 ^(****)
19-30	1/11	4/9 ^(ns)	8/8 ^(****)

^a Symbol meanings: ^{ns} $P > .05$; ^{***} $P \leq .001$; ^{****} $P \leq .0001$

^b Frequency of neoplasia in *Atgl*+/- ctg or *Atgl*-/- ctg mice is compared to frequency of neoplasia in *Atgl*+/+ ctg mice