

Fig. S1

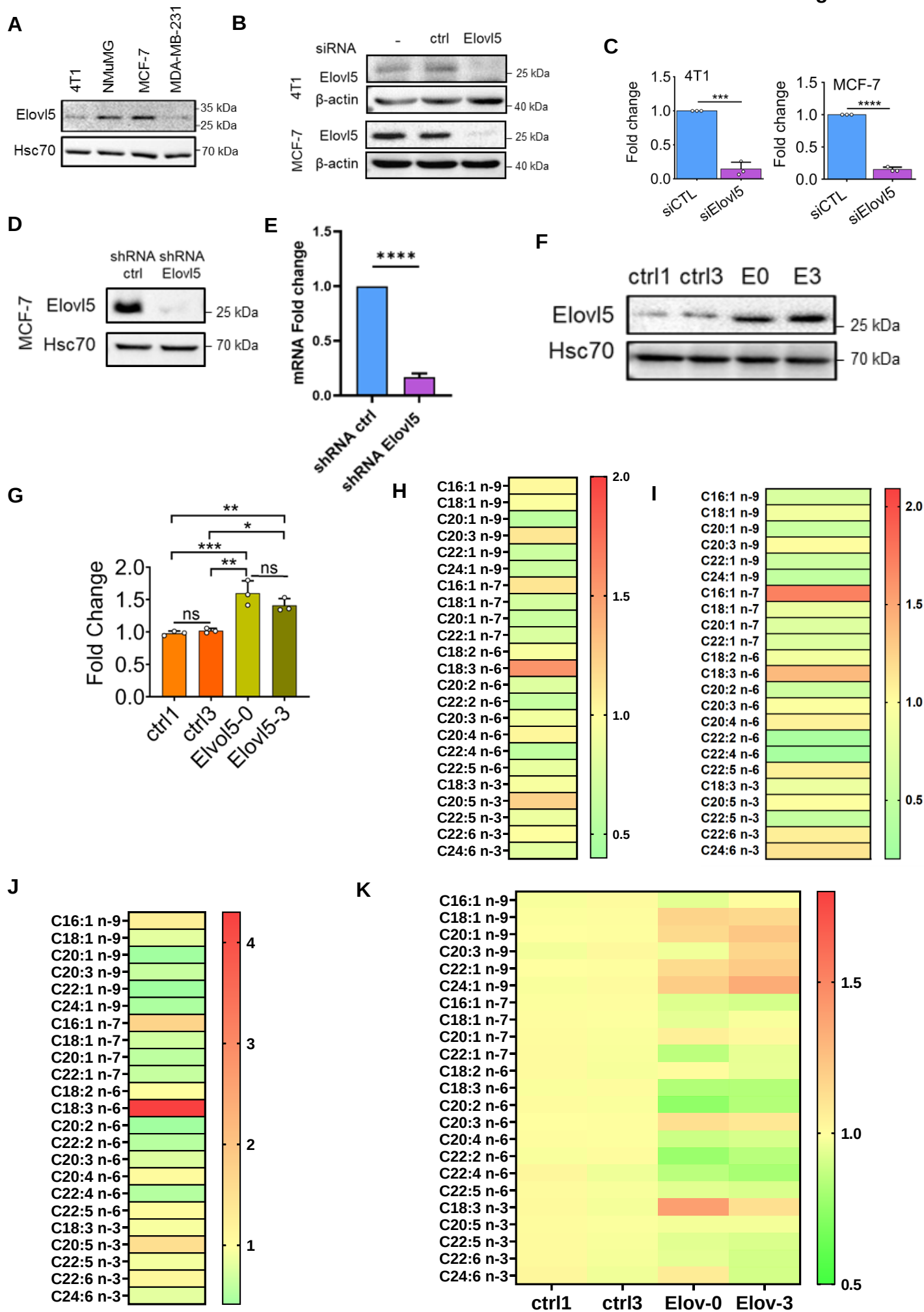


Figure S1: Validation of breast cancer cell models with modulation of Elovl5 expression. A. Elovl5 expression in murine 4T1 breast carcinoma cells, murine NMUMG normal mammary epithelial cells and human (MCF-7 and MDA-MB-231) breast cancer cell lines analyzed by western-blotting. B-C. Elovl5 expression 48 hours after transfection with a control (ctrl) or Elovl5-targeting siRNA analyzed by western-blotting (B) and RT-qPCR (C). *** $p < 0,001$ and **** $p < 0,0001$ (Student's *t* test). D-E. Validation of stable Elovl5 extinction using an Elovl5-targeting shRNA Elovl5 compared to a control shRNA (ctrl) in MCF-7 cells analyzed by western-blotting (D) and RT-qPCR (E). F. Analysis by western-blotting of stable overexpression of Elovl5 in 4T1 cells (Elovl5-0 -E0- and Elovl5-3 -E3) compared to control (ctrl1 and ctrl3) 4T1 cells. G. Expression of Elovl5 mRNA in Elovl5-overexpressing 4T1 cells (Elovl5-0 and Elovl5-3) and control (ctrl1 and ctrl3) 4T1 cells. * $p < 0,05$, ** $p < 0,01$, *** $p < 0,001$ and non-significant (ns) were calculated using a one-way Anova analysis using Tukey's multiple comparison test. H-K. Heatmaps showing the fold change of mono- and polyunsaturated fatty acid content in Elovl5 siRNA-treated MCF-7 cells relative to control siRNA-treated MCF-7 cells (H), in Elovl5 shRNA MCF-7 cells relative to control shRNA MCF-7 cells (I), in Elovl5 siRNA-treated 4T1 cells relative to control siRNA-treated 4T1 cells (J) and in Elovl5-overexpressing 4T1 cells (Elovl-0 and Elovl-3) relative to the mean of control (ctrl1 and ctrl3) 4T1 cells (K).

Fig. S2

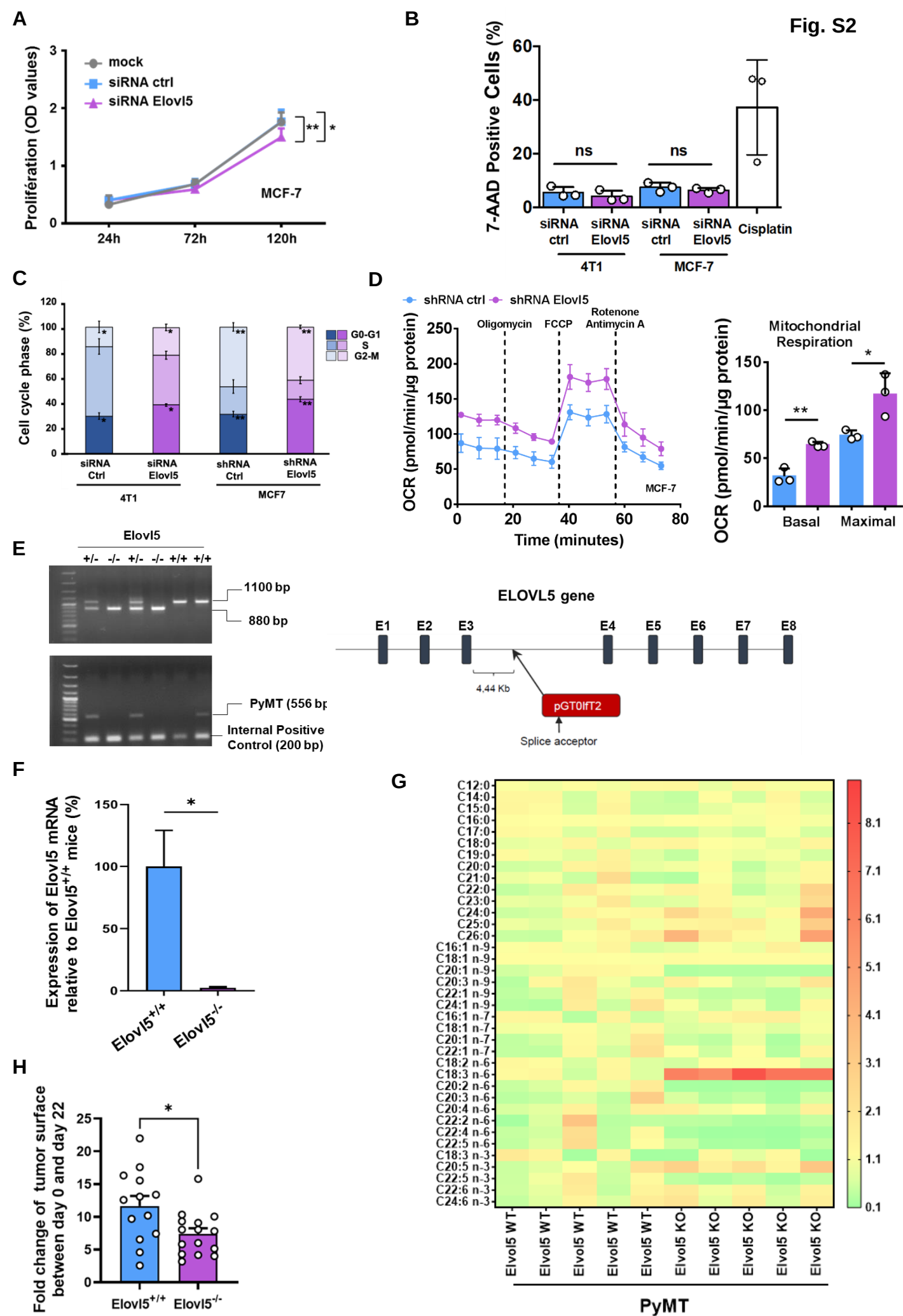


Figure S2: Proliferation and validation of the MMTV-PyMT;Elovl5 murine model. A. Proliferation of transient Elovl5-silenced MCF-7 cells analysed with crystal violet staining. Error bars represent the mean $\pm$ SD of independent experiments (n=5 (A) with * p <0.05, ** p <0.01 and *** p <0.001 using a one-way Anova analysis with Tukey test. B. Cell death analyzed by 7-ADD staining in 4T1 and MCF-7 cells treated for 72 hours with siRNA ctrl or Elovl5. Cisplatin treatment was used as a positive control of cell death. C. Cell cycle analysis in control or Elovl5-depleted 4T1 and MCF-7 cells. Histograms and error bars show the mean $\pm$ SD with * p <0.05 and ** p <0.01 according to Student's t test. D. Oxygen consumption rate measured with Seahorse XFe96 analyzer in stable Elovl5-depleted MCF-7 cells. Error bars represent the mean $\pm$ SD with * p <0.05 and ** p <0.01 according to Student's t test. E. *Elovl5* gene targeting strategy. *Elovl5*^{-/-} mice were obtained from the Mutant Mouse Regional Resource Center at UC Davis and were generated by inserting a genetrap cassette in the exon 3 (E3) of the *Elovl5* gene. Genotyping analysis of C57BL/6 mice by PCR. Elovl5 primers amplified a 1100 bp DNA fragment for wild-type allele and a 880 bp DNA fragment for the knockout allele. PyMT primers amplified a 556 bp DNA fragment in mice with the transgene. F. Relative expression of Elovl5 mRNA in mammary tumors of MMTV-PyMT;Elovl5^{+/+} (n=5) and MMTV-PyMT;Elovl5^{-/-} (n=5) mice. Shown are the mean $\pm$ SEM of 5 mice per group. * p <0.05 defined with Student's t test. G. Heatmap showing the fold change of fatty acid content in MMTV-PyMT;Elovl5 knockout (KO) mice (n=5) relative to MMTV-PyMT;Elovl5 wild-type (WT) mice (n=5). H. Fold change of aggregated tumor surface on day 22 compared to day 0 (day on which the tumor lesion was first palpable and measured) in MMTV-PyMT;Elovl5^{+/+} (Elovl5^{+/+}, n=23) and MMTV-PyMT;Elovl5^{-/-} (Elovl5^{-/-}, n=20) mice. Fold change for each individual mouse is indicated by dots, the histogram and error bars show the mean $\pm$ SEM with * p <0.05 according to Student's t test.

Figure S3: Development of lung metastases depends on Elovl5 expression. A. Number of metastases on the lung surface of 6-month-old MMTV-PyMT;Elovl5^{+/+} (Elovl5^{+/+}, n=13) and MMTV-PyMT;Elovl5^{-/-} (Elovl5^{-/-}, n=15) mice. Shown are the mean±SEM with **p*<0.05 according to Student's *t* test. B. Graph representing the number of lung metastases and the cumulative surface of the primary mammary tumor for each 6-month-old MMTV-PyMT;Elovl5^{+/+} (Elovl5^{+/+}, n=13) and MMTV-PyMT;Elovl5^{-/-} (Elovl5^{-/-}, n=15) mice. C. Representative images of H&E staining of metastases in lungs of Balb-c mice with fat pad injection of Elovl5-overexpressing (Elovl5-0 and Elovl5-3) or control (ctrl1 and ctrl3) 4T1 cells correspondent to Figure 3B. D. Number of metastases on the lung surface of Elovl5-overexpressing (Elovl5-0 and Elovl5-3) and control (ctrl1 and ctrl3) 4T1 cells transplanted in the fourth fat pad of female Balb-c mice. Shown are the mean±SEM with ***p*<0,01 and ****p*<0,001 values determined by a Kruskal-Wallis analysis with Dunn's test. E. Number of metastases on the lung surface in female NMRI-nude mice with tail vein injection of Elovl5 shRNA and control shRNA-expressing MCF-7 cells. Shown are the mean±SEM with **p*<0.05 according to a Mann-Whitney test. F-G. QuPath analysis and number of metastases in lungs of female Balb-c mice with tail vein injection of Elovl5-overexpressing (Elovl5-0 and Elovl5-3) or control (ctrl1 and ctrl3) 4T1 cells. Shown are the mean±SEM with ***p*<0.01, ****p*<0,001, *****p*<0,0001 and non-significant (ns) according to a Kruskal-Wallis analysis with Dunn's test. H. Representative images of Elovl5 IHC staining for H-score analysis. The red insert shows breast tumor tissue and the blue insert shows normal adjacent tissue. Breast cancer subtypes are indicated.

Fig. S3

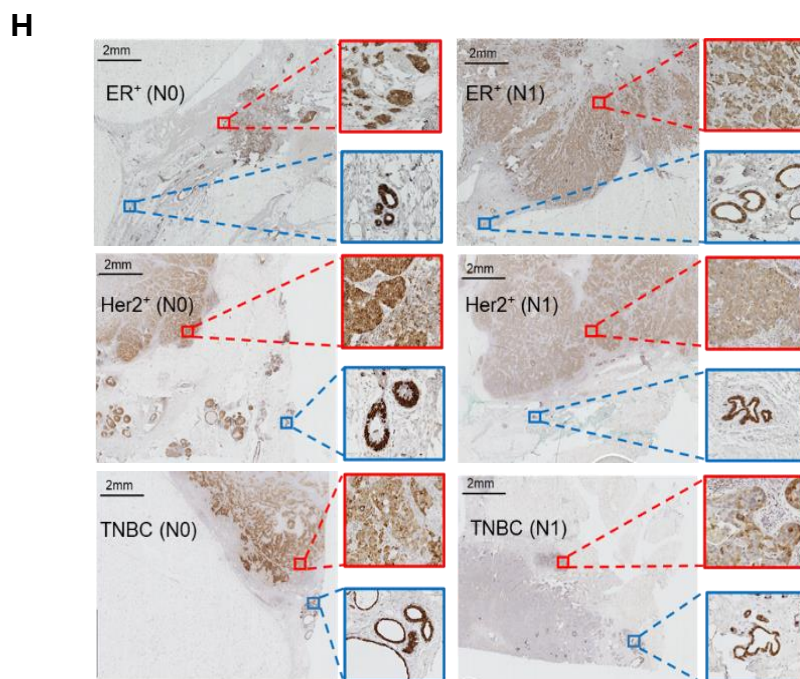
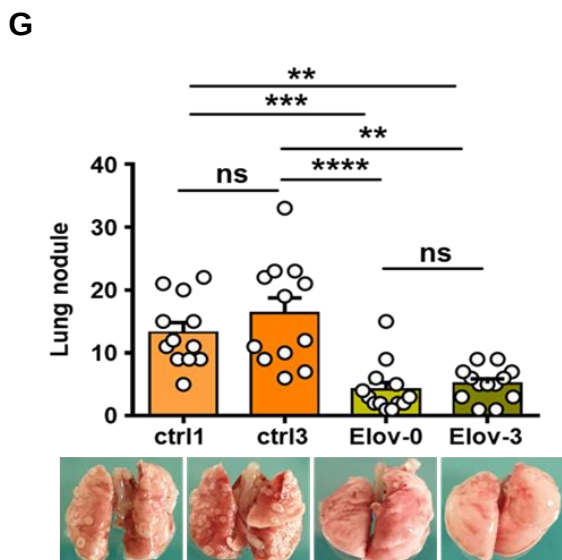
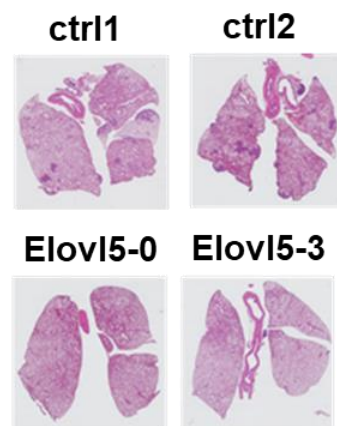
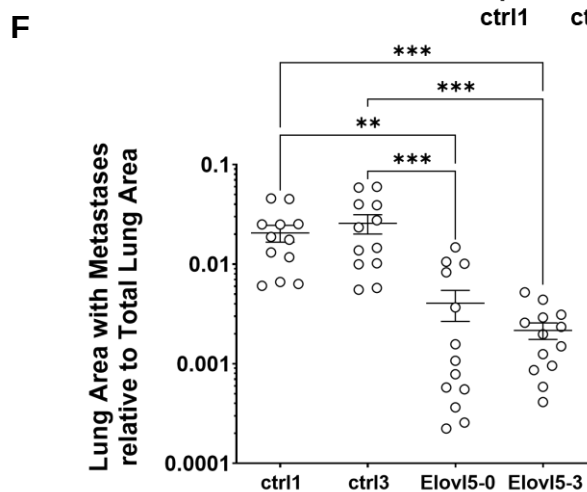
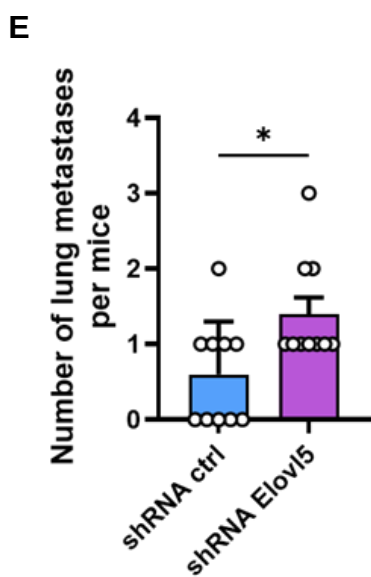
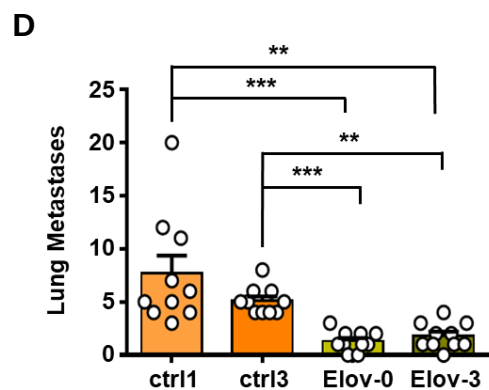
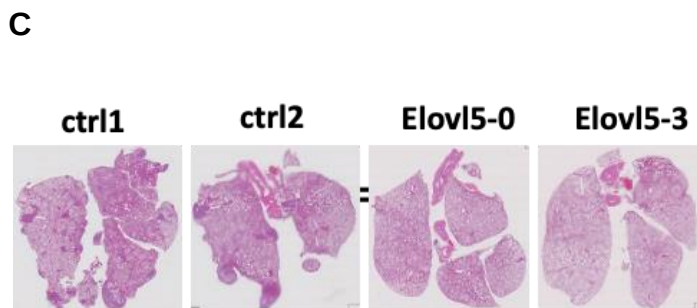
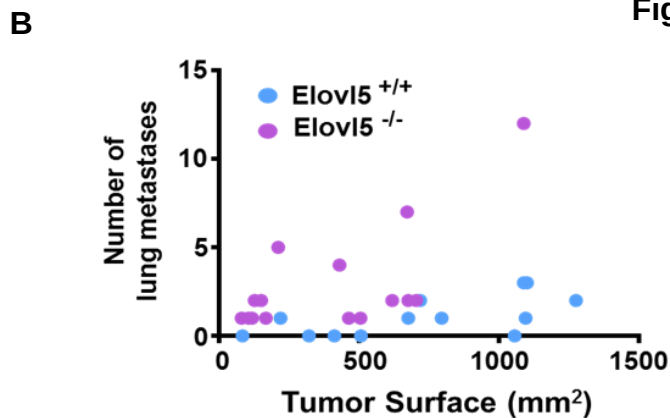
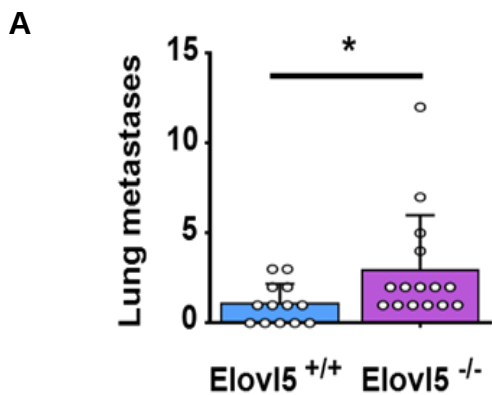


Fig. S4

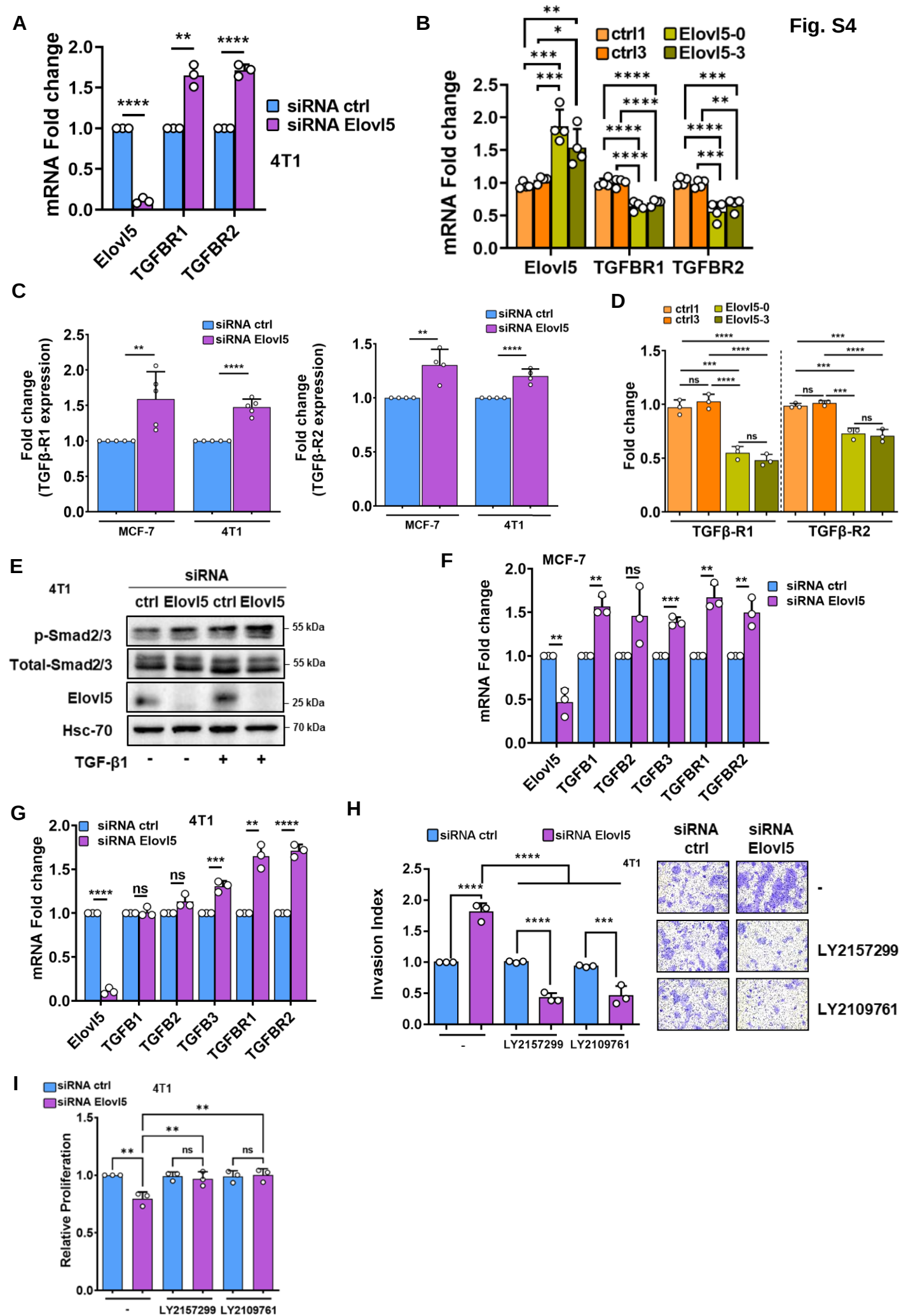
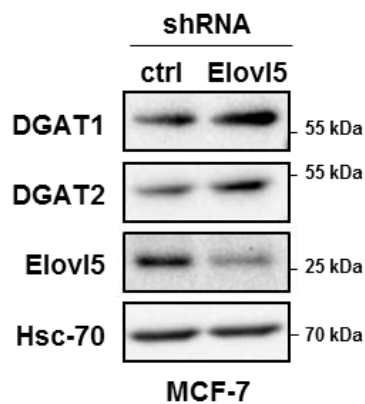


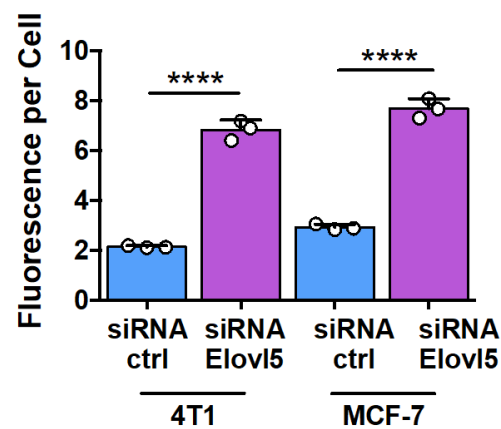
Figure S4: Regulation of the TGF- β pathway by Elovl5. A. Analysis of TGFBR1 and TGFBR2 mRNA expression at 24 hours by RT-qPCR in Elovl5-silenced 4T1 cells using an siRNA against Elovl5 relative to non-targeting siRNA-treated 4T1 cells. Histograms and error bars represent the mean $\pm$ SD of three independent experiments with ** $p < 0.01$ and **** $p < 0.0001$ according to Student's t test. B. Analysis of TGFBR1 and TGFBR2 mRNA expression at 24 hours by RT-qPCR in Elovl5-overexpressing 4T1 cells (Elovl5-0 and Elovl5-3) relative to the mean of control 4T1 cells (ctrl1 and ctrl3). Histograms and error bars represent the mean $\pm$ SD of five independent experiments with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ according to one-way Anova analysis with Tukey's multiple comparison test. C. Analysis of TGF- β receptor 1 and 2 expression at 48 hours by flow cytometry in Elovl5-depleted MCF-7 and 4T1 cells treated using an siRNA. Histograms and error bars represent the mean $\pm$ SD of at least four independent experiments with *** $p < 0.001$ and **** $p < 0.0001$ according to Student's t test. D. Analysis of TGF- β receptor 1 and 2 expression at 48 hours by flow cytometry in Elovl5-overexpressing 4T1 cells (Elovl5-0 and Elovl5-3) relative to the mean of control 4T1 cells (ctrl1 and ctrl3). Histograms and error bars represent the mean $\pm$ SD of three independent experiments with **** $p < 0.001$, **** $p < 0.0001$ and non-significant (ns) according to one-way Anova analysis with Tukey's multiple comparison test. E. Analysis of Smad2/3 phosphorylation (p-Smad2/3) and total Smad2/3 expression in Elovl5-silenced 4T1 cells treated with TGF- β 1 (5 ng/ml) for 30 minutes by western-blotting. F-G. Analysis of TGFB1, TGFB2, TGFB3, TGFBR1 and TGFBR2 mRNA expression by RT-qPCR at 24 hours in Elovl5-silenced MCF-7 (F) and 4T1 (G) cells using an Elovl5 siRNA relative to a control siRNA. Shown are the mean $\pm$ SD of three independent experiments with non-significant (ns), ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ according to Student's t test. H. Analysis of cell invasion through a Matrigel-coated membrane for Elovl5-depleted 4T1 cells with transient siRNA against Elovl5 relative to 4T1 treated with a control siRNA (ctrl).

*** $p < 0.001$ and **** $p < 0.0001$ were determined by one-way Anova analysis with Tukey's test. Histograms and error bars represent the mean $\pm$ SD of three independent experiments. Representative images are shown. I. Analysis of proliferation by crystal violet staining in Elov15-depleted 4T1 cells using an siRNA against Elov15 relative to 4T1 cells treated with a control siRNA (ctrl). ** $p < 0.01$ and non-significant (ns) were determined by one-way Anova analysis with Tukey's test. Histograms and error bars represent the mean $\pm$ SD of three independent experiments.

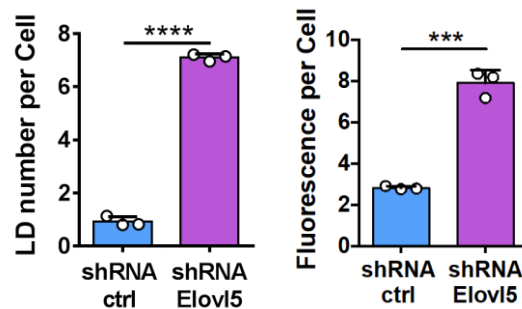
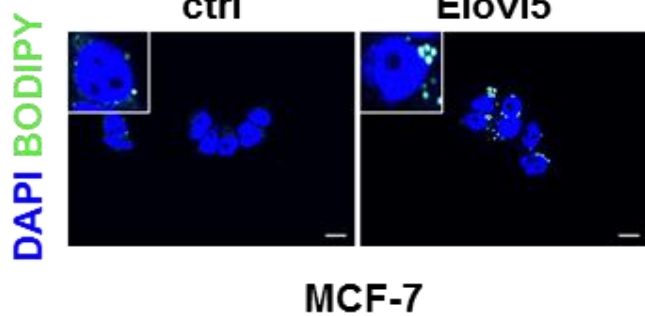
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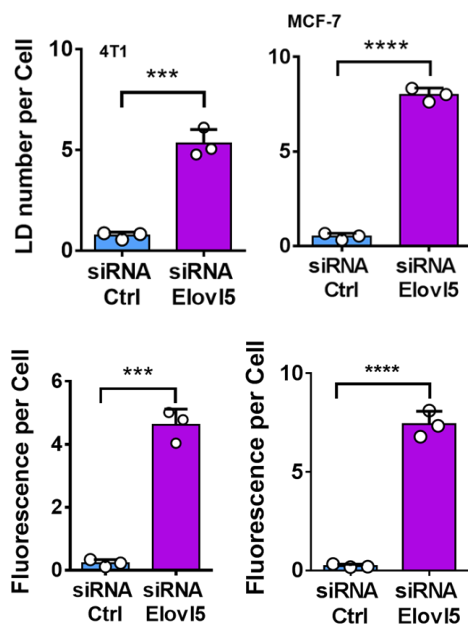
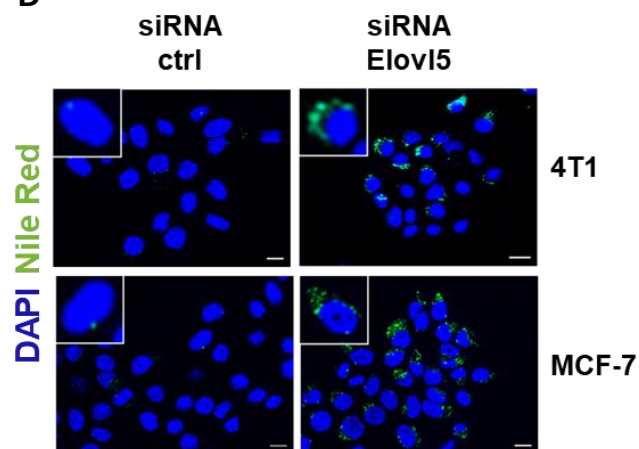


Fig. S5

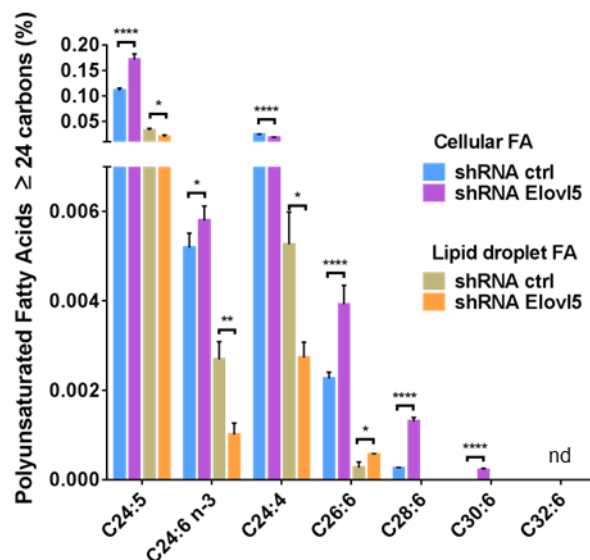
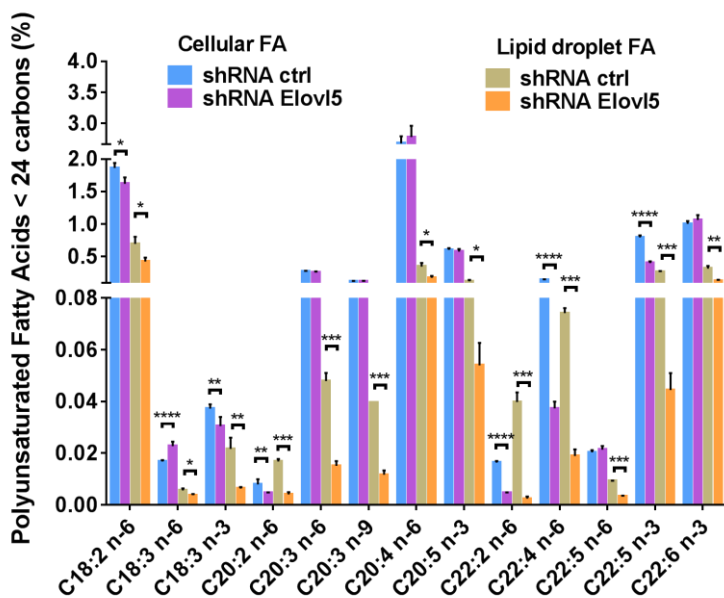
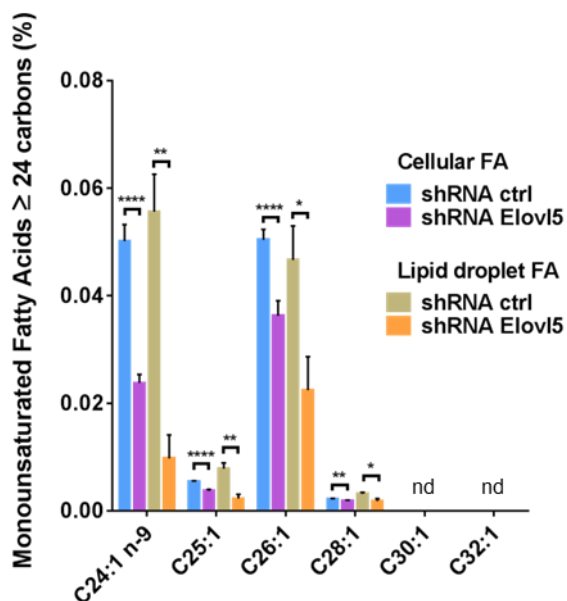
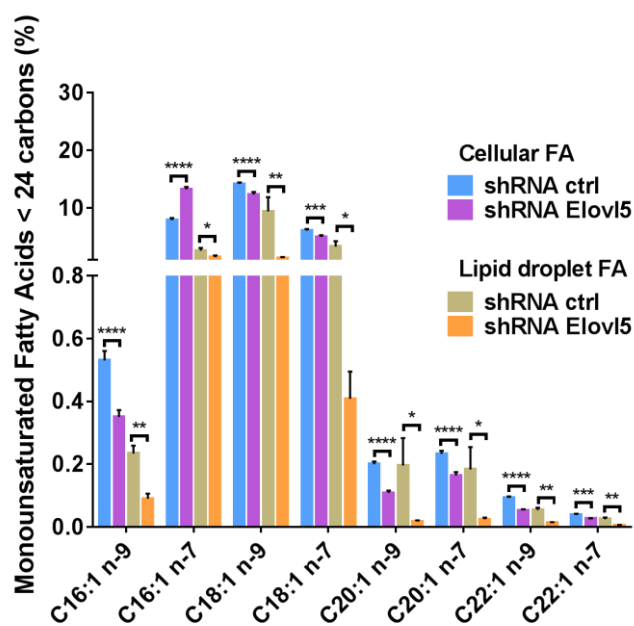
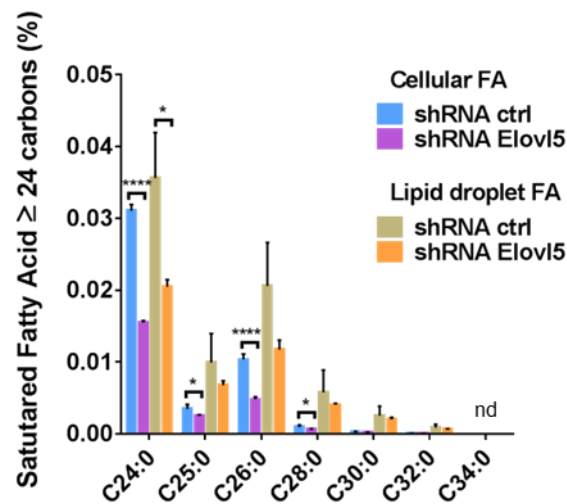
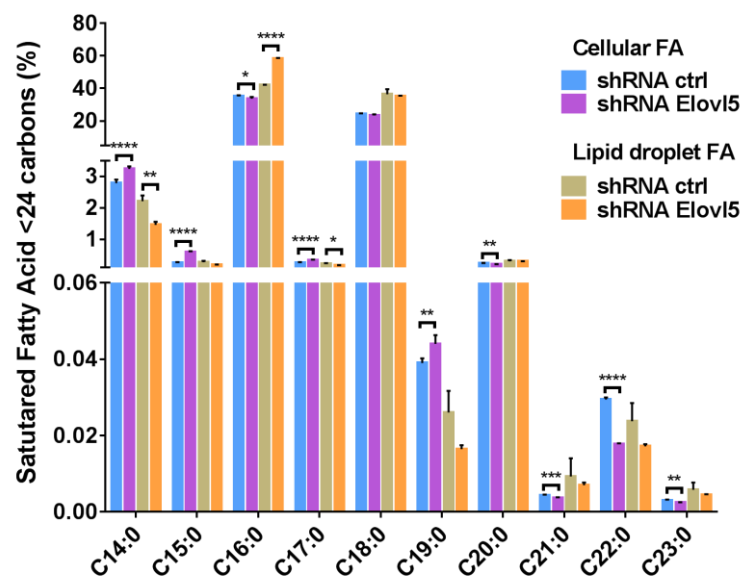


Fig. S5

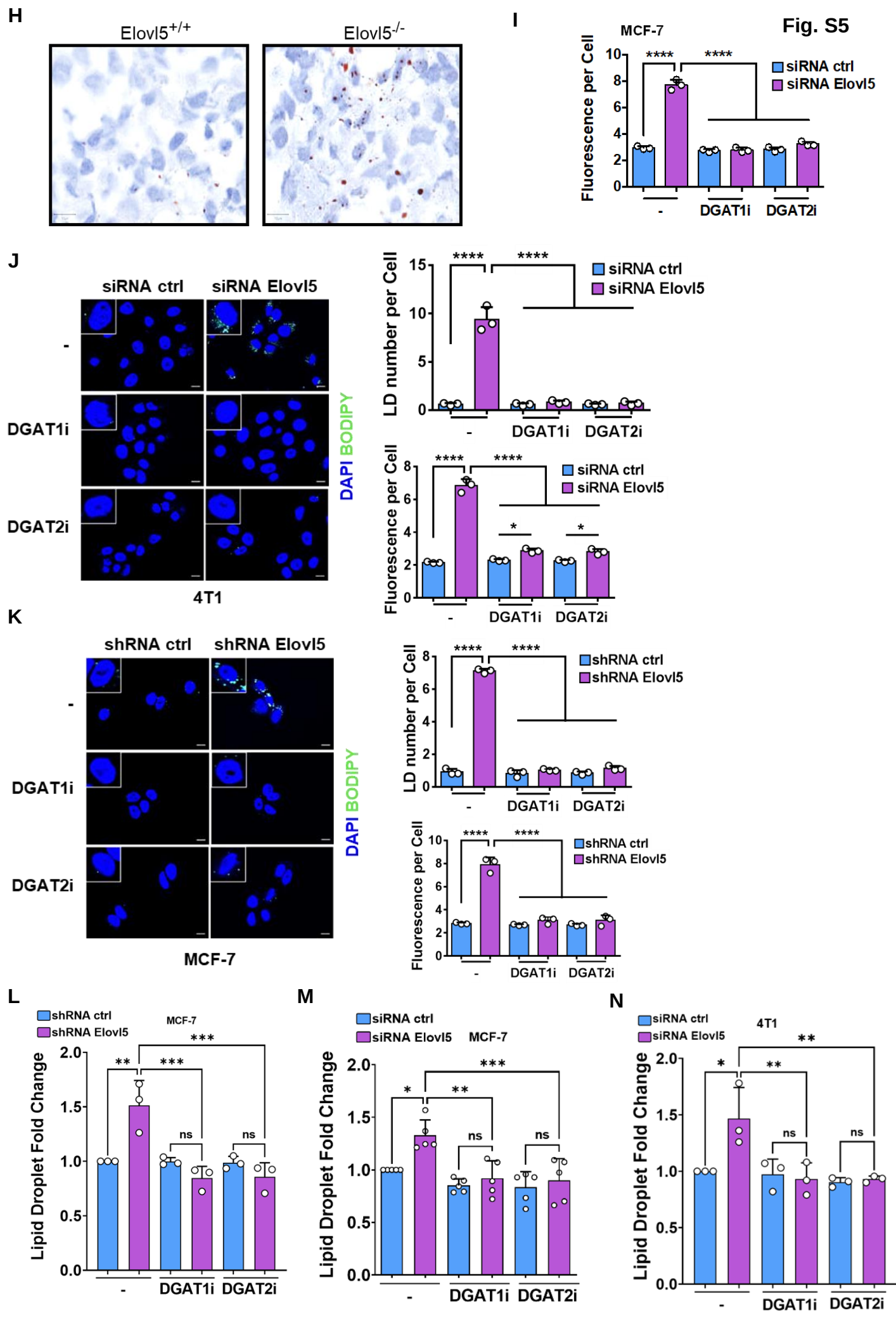
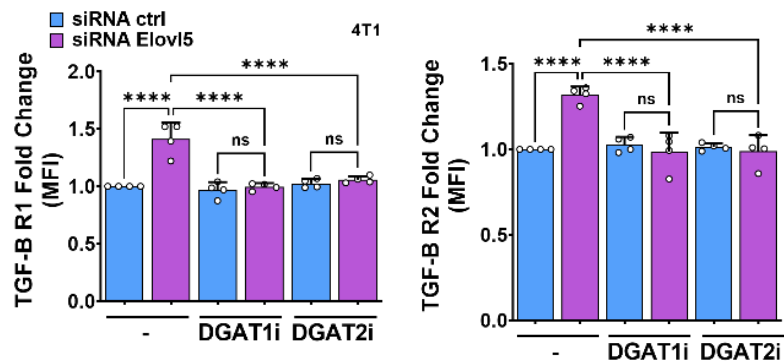


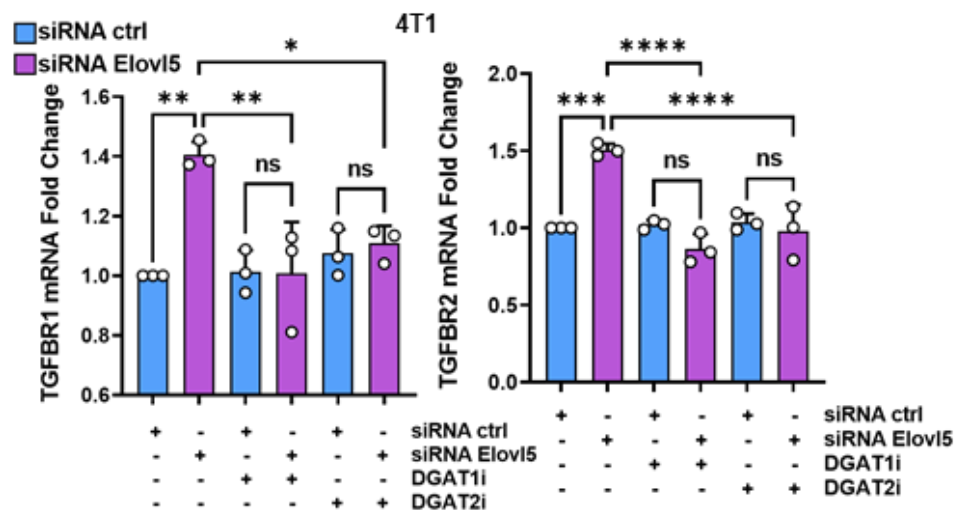
Figure S5: Elovl5 downregulation increases lipid droplet abundance. A. Expression of DGAT1 and DGAT2 enzymes analyzed by western-blotting in MCF-7 cells with stable Elovl5 downregulation. B. Quantification of lipid droplet fluorescence intensity per cell in 4T1 and MCF-7 cells transfected with siRNA ctrl or against Elovl5 after Bodipy 493/503 staining (Images are shown in Fig. 5H). Data are indicated as the mean $\pm$ SD of three independent experiments. **** $p < 0.0001$ was determined by a Student's t test. C. Determination of lipid droplet content in stable Elovl5-depleted and control MCF-7 cells by fluorescence microscopy using a Bodipy 493/503 staining. The lipid droplet number was determined by assessing the number of total lipid droplet and the cell number (minimum 100 cells per experimental condition) in the field for the calculation of the average lipid droplet number per cell. In addition, a quantification of the average of fluorescence intensity per cell was performed in the randomly recorder fields using ImageJ software. Data are expressed as the mean $\pm$ SD of three independent experiments. *** $p < 0.001$ and **** $p < 0.0001$ was determined by a Student's t test. Scale bar: 20 μ m. D. Analysis of lipid droplet content in 4T1 and MCF-7 cells treated with control siRNA and Elovl5 siRNA. The nonpolar lipids of the lipid droplets were detected with a Nile Red staining by fluorescence microscopy. Data are expressed as the mean $\pm$ SD of three independent experiments. *** $p < 0.001$ and **** $p < 0.0001$ was determined by a Student's t test. Scale bar: 20 μ m. E-G. Percentage of total cellular or lipid droplet fatty acids in control and Elovl5-silenced MCF-7 cells. Shown are the mean $\pm$ SD of three independent experiments with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ according to Student's t test. H. Representative images of Oil Red O staining of mammary tumors from MMTV-PyMT;Elovl5^{+/+} (Elovl5^{+/+}) and MMTV-PyMT;Elovl5^{-/-} (Elovl5^{-/-}) mice (Images correspond to the Figure 5J). Scale bar: 10 μ m. I. Lipid droplet content in Elovl5-depleted MCF-7 with DGAT inhibitors or DMSO (control) treatment analyzed by Bodipy 493/503 staining and shown as the average of fluorescence intensity per cell (Images are shown in Figure

5K). Data are expressed as the mean $\pm$ SD of three independent experiments with ****p<0.0001 according to Student's t test. J-K. Analysis of lipid droplet content by Bodipy 493/503 staining in MCF-7 cells with transient (J) and stable (K) Elovl5 depletion treated with or without DGAT inhibitors. Data are presented as average of LD number and fluorescence intensity per cell. Histograms and error bars represent the mean $\pm$ SD of three independent experiments. ****p<0.0001 was determined by one-way Anova analysis with Tukey's test. L-N. Lipid droplet quantification by flow cytometry after Nile red staining in control or Elovl5-depleted MCF-7 (L-M) and 4T1 (N) cells. The detection of Nile Red fluorescence was performed with 488 nm excitation and 575 nm emission wavelengths. Data represent the mean $\pm$ SD of 3-5 independent experiments with *p<0.05, **p<0.01 and non-significant (ns) according to One-way Anova analysis with Tukey's multiple comparison test.

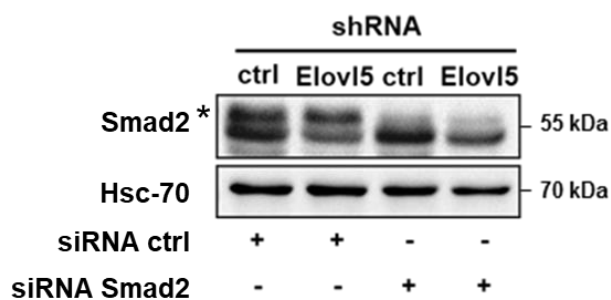
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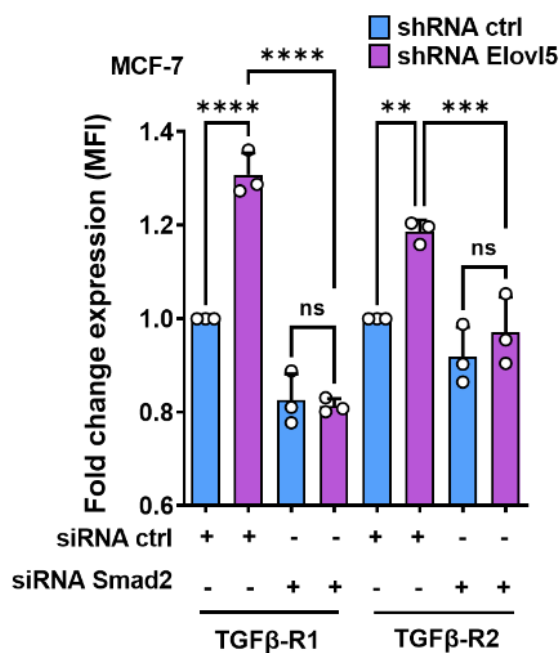
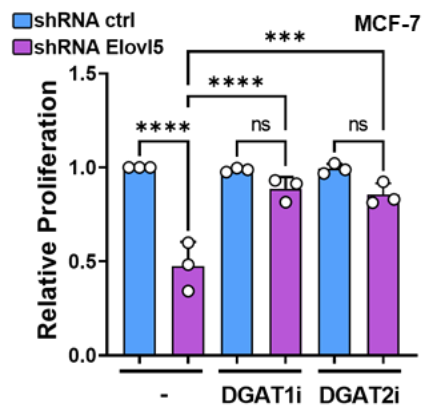


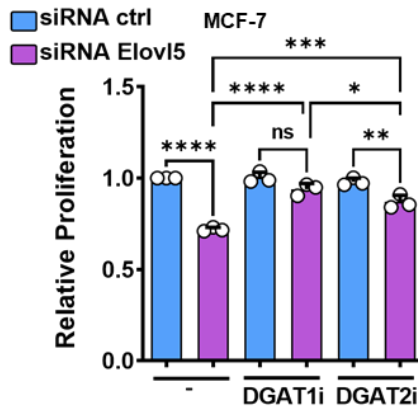
Figure S6: Elovl5 dependent-LD accumulation controls expression of TGF- β receptors.

A. Analysis of TGF β -1 and 2 receptor expression by flow cytometry. The 4T1 cells with transient silencing of Elovl5 expression were treated for 48 hours with DGAT1 or 2 inhibitors. Values are represented as the fold change of mean of fluorescence intensity (MFI) relative to 4T1 cells treated with vehicle (DMSO) and a control shRNA or siRNA (ctrl). Data are expressed as the mean $\pm$ SD of three independent experiments. ****p<0,0001 and non-significant (ns) determined by One-way Anova analysis with Tukey's test. B. Analysis of TGF β 1 and 2 receptor mRNA expression in 4T1 cells by RT-qPCR. 4T1 cells transfected with siRNA were treated for 48 hours with DGAT1 and 2 inhibitors. Values are the fold change of Elovl5 mRNA expression relative to 4T1 cells treated with vehicle (DMSO) and a control siRNA (ctrl). Data are expressed as the mean $\pm$ SD of three independent experiments. *p<0.05, **p<0,01, ***p<0,001, ****p<0,0001 and non-significant (ns) were determined by one-way Anova analysis with Tukey's test. C. Validation of Smad2 silencing by western-blotting in control and Elovl5 shRNA MCF-7 cells. D. Analysis of TGF- β receptor 1 and 2 expressions at 48 hours by flow cytometry in Elovl5-targeting shRNA and control shRNA MCF-7 cells treated by siRNA ctrl or against smad2. Values are the fold change in mean of fluorescence intensity (MFI) relative to shRNA control MCF-7 cells transfected with siRNA control. Histograms and error bars represent the mean $\pm$ SD of three independent experiments with non-significant (ns), **p<0.01, ***p<0.001 and ****p<0.0001 according to one-way Anova analysis with Tukey's multiple comparison test.

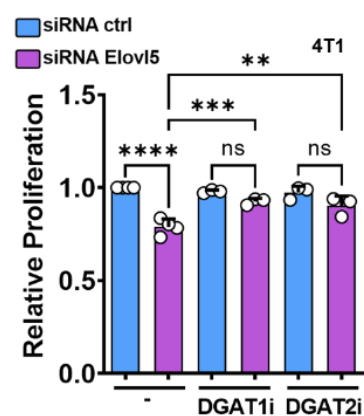
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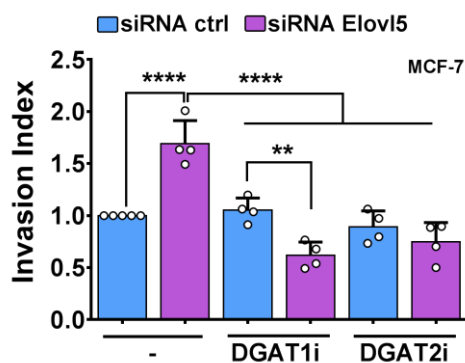
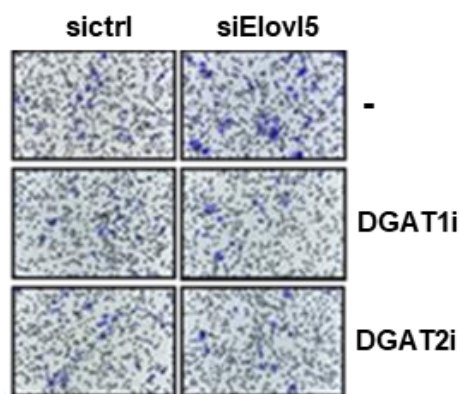
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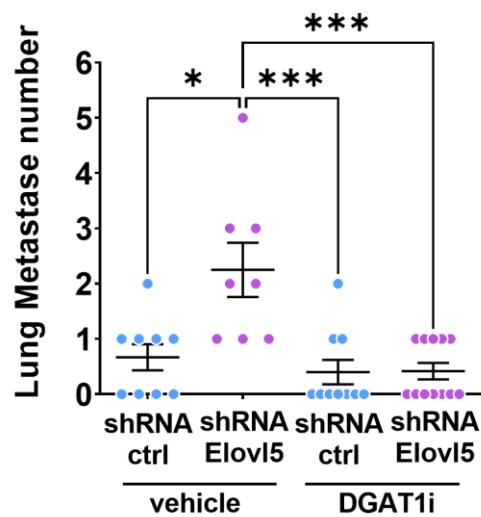


Figure S7: Elovl5 dependent-LD accumulation controls proliferation, invasion and lung metastasis. A-C. Analysis of relative cell proliferation by crystal violet in MCF-7 (A-B) or 4T1 (C) cells with downregulation of Elovl5 and treated with DGAT1 or DGAT2 inhibitors. * $p < 0,05$, ** $p < 0,01$, *** $p < 0,001$, **** $p < 0,0001$ and non-significant (ns) were determined by one-way Anova analysis with Tukey's test. Represented are the mean $\pm$ SD of three independent experiments. D. Analysis of cell invasion through a Matrigel-coated membrane of Elovl5-depleted cancer MCF-7 cells and treated with DGAT inhibitors (DGAT1i and DGAT2i) relative to MCF-7 cells treated with a control siRNA (ctrl) and vehicle (DMSO). ** $p < 0,01$ and **** $p < 0,0001$ were determined by One-way Anova analysis with Tukey's test. Histograms and error bars are the mean $\pm$ SD of three independent experiments. Representative images are shown. E. Number of lung metastases in female NMRI-nude mice with tail vein injection of shRNA Elovl5 or shRNA control (ctrl) MCF-7 cells. DGAT1 inhibitor (10 μ M) was loaded in MCF-7 cells 24 hours before injection. Lungs were collected 35-days post-injection. Data represent the mean $\pm$ SEM with * $p < 0.05$ and *** $p < 0.001$ determined by Kruskal-Wallis analysis with a Dunn's multiple comparison test.