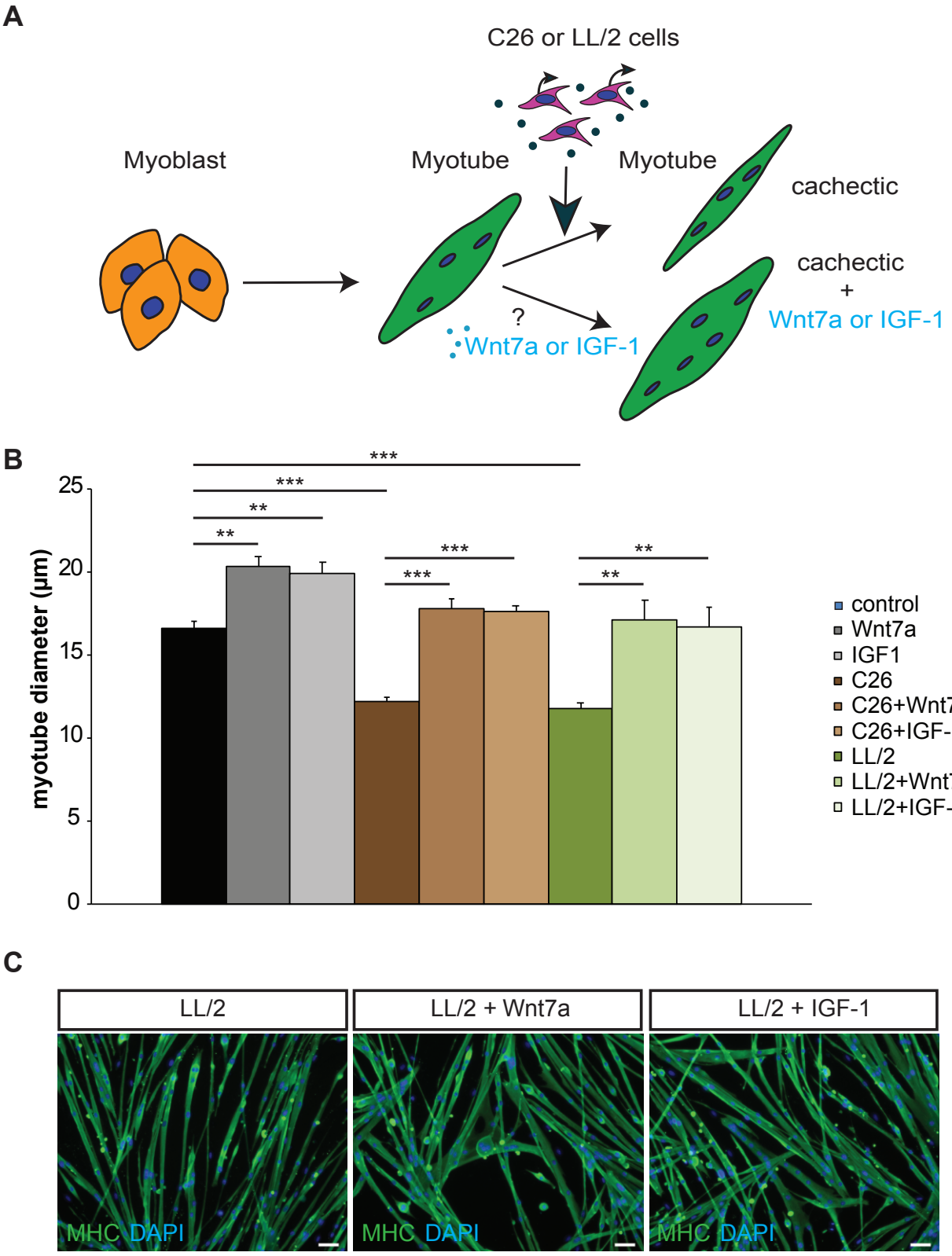
OMTO, Volume *16*

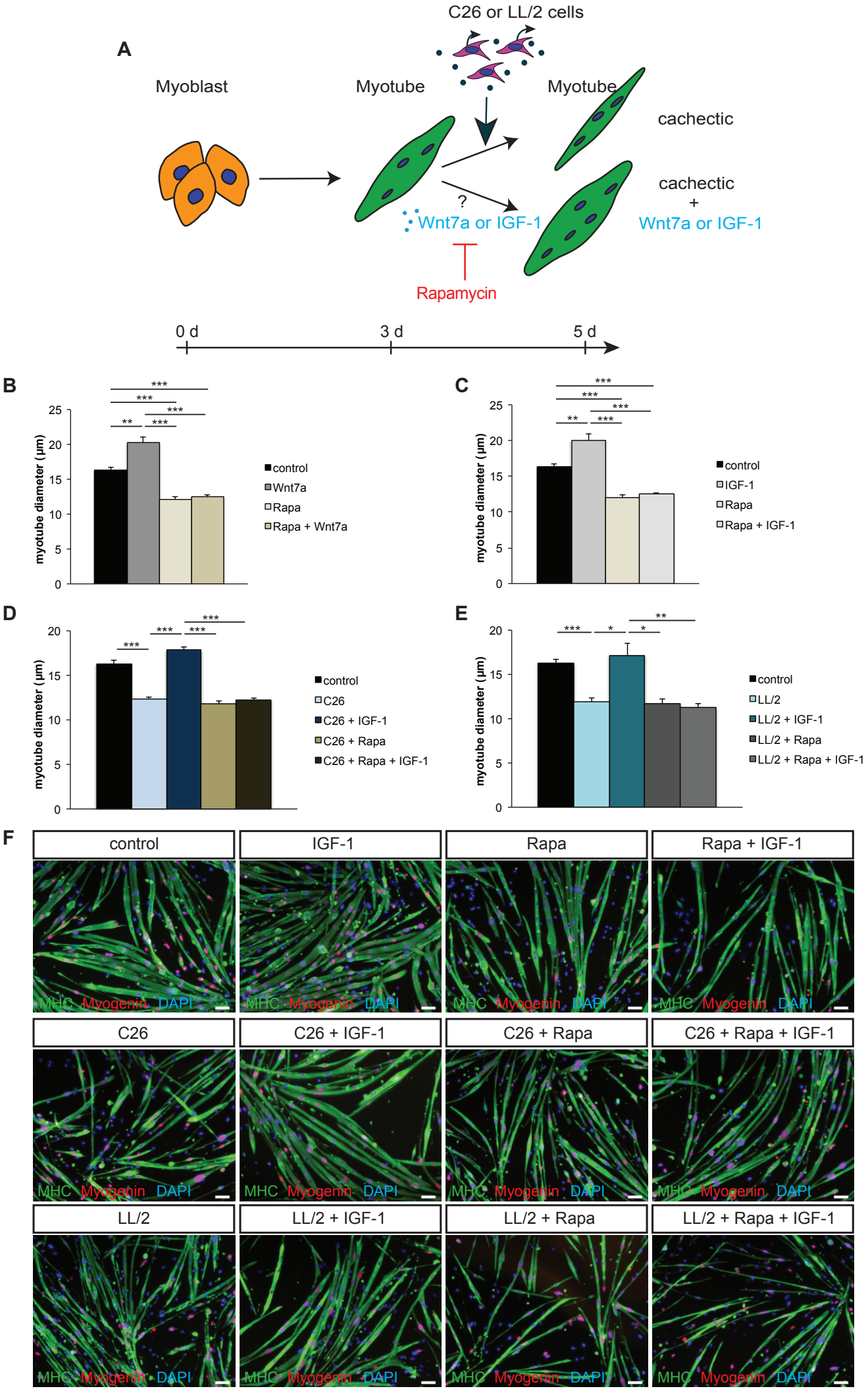
Supplemental Information

Wnt7a Counteracts Cancer Cachexia

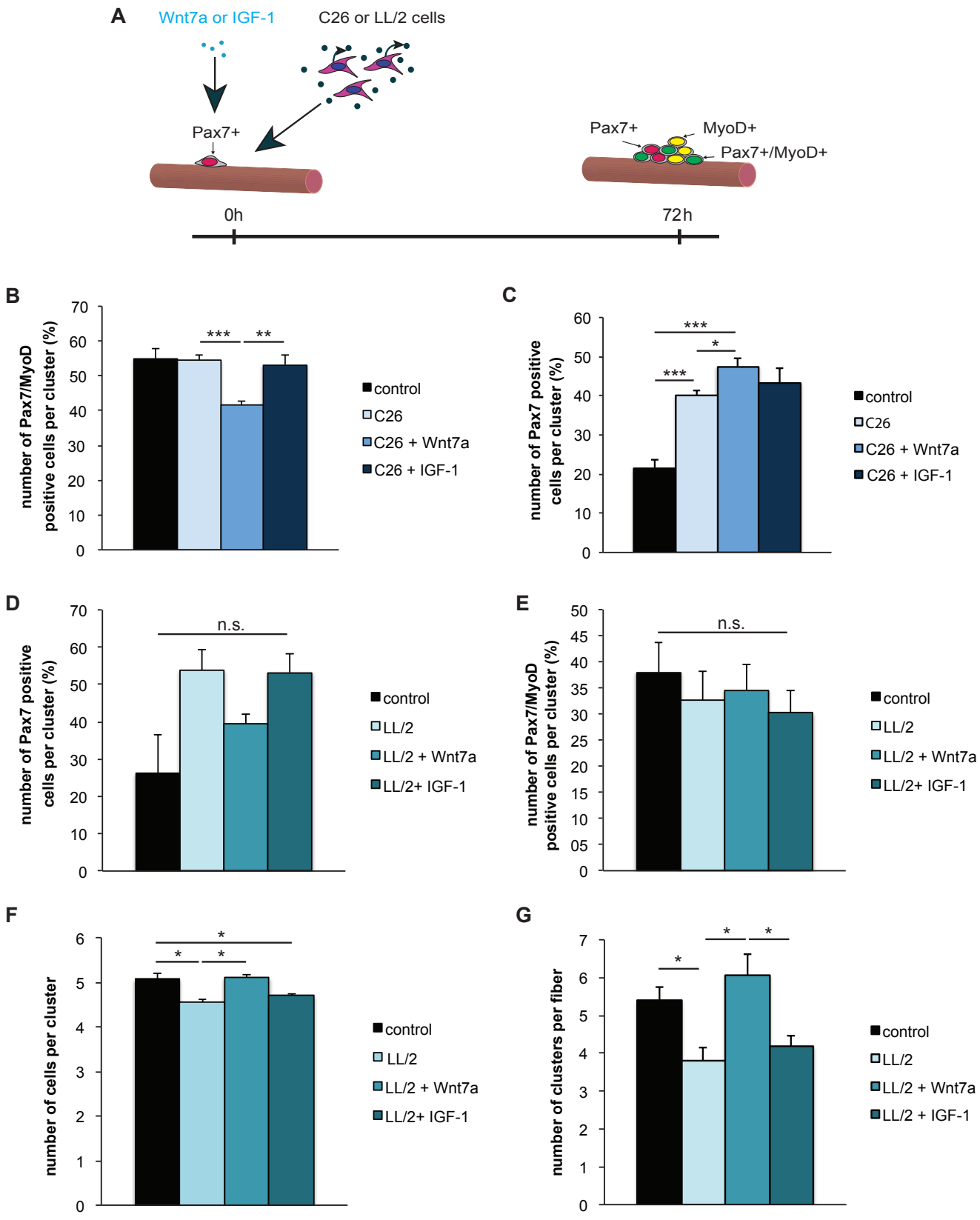
Manuel Schmidt, Christine Poser, and Julia von Maltzahn



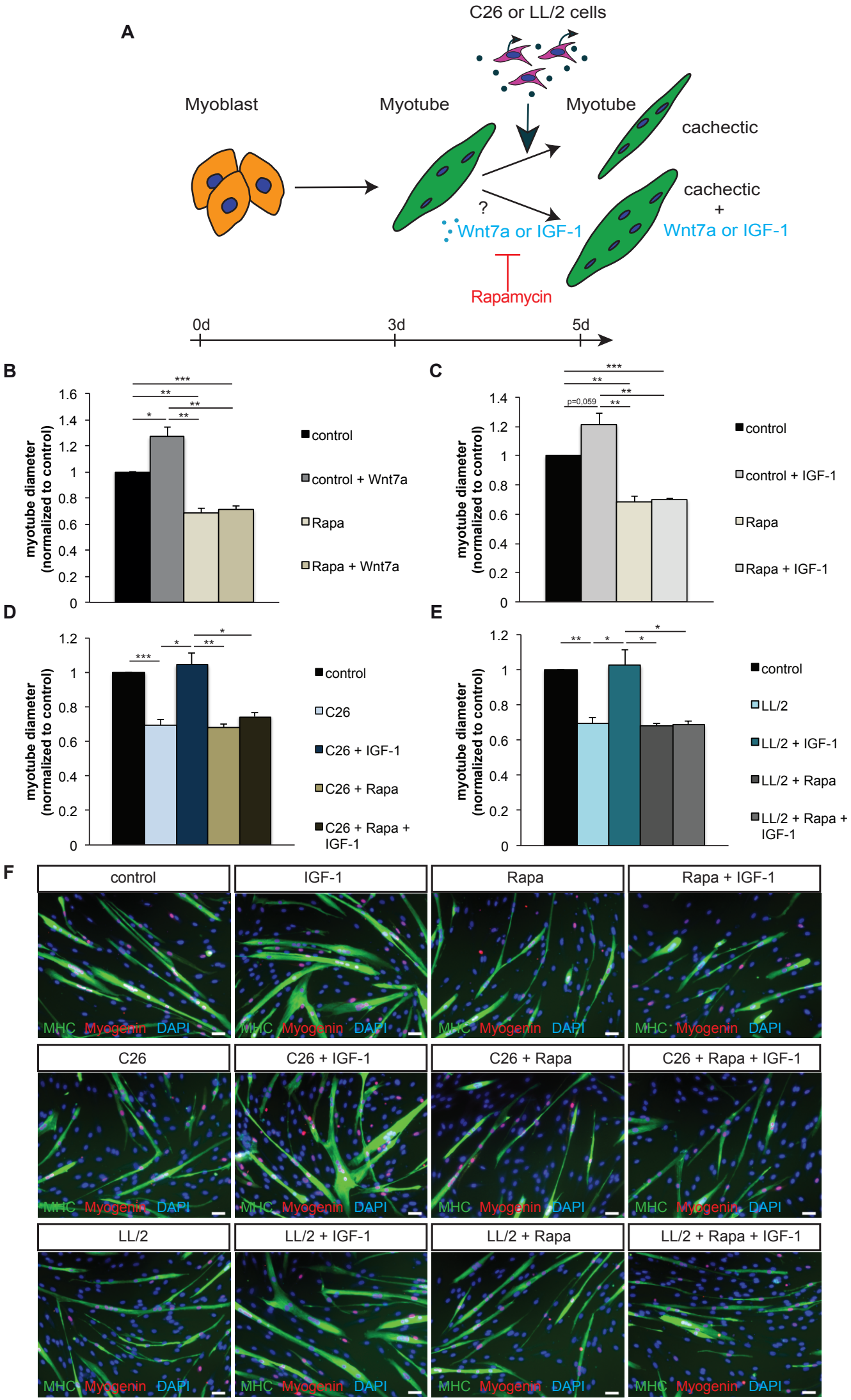
Supplemental figure 1



Supplemental figure 2



Supplemental figure 3



Supplemental figure 4

Supplementary figure legends:

Supplemental Figure 1: Wnt7a prevents myotube atrophy caused by conditioned media from LL/2 lung carcinoma cells.

(A) Experimental schematic outlining induction of myotube atrophy and treatment with Wnt7a or IGF-1 using murine primary myoblasts. (B) Measurement of the maximal myotube diameter (normalized to control) demonstrates that myotube atrophy caused by conditioned medium from LL/2 lung carcinoma cells is prevented by addition of Wnt7a or IGF-1. (C) Representative images of myotubes from control conditions, after induction of myotube atrophy by LL/2 cell conditioned medium with and without Wnt7a or IGF-1 treatment. Images show Myosin heavy chain (in green) and Dapi (in blue). Scale bar = 50μm. n=3. Error bars represent SEM. * = $p<0.05$, ** = $p<0.01$.

Supplemental figure 2: Wnt7a counteracts myotube atrophy through the AKT/mTOR pathway.

(A) Experimental schematic outlining induction of myotube atrophy and treatment with Wnt7a and rapamycin using murine primary myoblasts. (B) Inhibition of mTOR by rapamycin inhibits myotube hypertrophy induced by Wnt7a. (C) Inhibition of mTOR by rapamycin inhibits myotube hypertrophy induced by IGF-1. (D) Counteracting myotube atrophy by IGF-1 caused by conditioned medium from C26 colon carcinoma cells measured as the maximal myotube diameter is dependent on the AKT/mTOR pathway as shown by addition of the mTOR inhibitor rapamycin. (E) Counteracting myotube atrophy

by IGF-1 caused by conditioned medium from LL/2 lung carcinoma cells measured as the maximal myotube diameter is dependent on the AKT/mTOR pathway as shown by addition of the mTOR inhibitor rapamycin. (F) Representative images of myotubes from control conditions, after induction of myotube atrophy by C26 or LL/2 cell conditioned medium with and without IGF-1 treatment and/or addition of rapamycin. Images show Myosin heavy chain (in green), Myogenin (in red) and Dapi (in blue). Scale bar = 50μm. n=3. Error bars represent SEM. * = $p<0.05$, ** = $p<0.01$. Please note that the experiment shown here was performed together with the experiment shown in Fig.2, therefore the panels for control and rapamycin treatment are identical.

Supplemental figure 3: Differentiation of muscle stem cells in cancer cachexia is improved by Wnt7a

(A) Experimental schematic outlining the experimental setup to investigate the muscle stem cell activation and differentiation in control and cancer cachexia conditions induced by conditioned medium from C26 colon carcinoma or LL/2 lung carcinoma cells with or without treatment with Wnt7a or IGF-1. (B) The number of cells per cluster is decreased in cancer cachexia induced by conditioned medium from LL/2 lung carcinoma cells, but can be increased by addition of Wnt7a. (C) Wnt7a but not IGF-1 drives activation and proliferation of muscle stem cells in cancer cachexia caused by conditioned medium from LL/2 lung carcinoma cells measured by the number of clusters per myofiber. n=4. Error bars represent SEM. * = $p<0.05$, ** = $p<0.01$.

Supplemental Figure 4: IGF-1 counteracts myotube atrophy through the AKT/mTOR pathway in human primary myotubes.

(A) Experimental schematic outlining induction of myotube atrophy and treatment with IGF-1 and rapamycin using human primary myoblasts. (B) Inhibition of mTOR by rapamycin inhibits myotube hypertrophy induced by Wnt7a. (C) Inhibition of mTOR by rapamycin inhibits myotube hypertrophy induced by IGF-1. (D) Counteracting myotube atrophy by IGF-1 caused by conditioned medium from C26 colon carcinoma cells measured as the maximal myotube diameter is dependent on the AKT/mTOR pathway as shown by addition of the mTOR inhibitor rapamycin. (E) Counteracting myotube atrophy by IGF-1 caused by conditioned medium from LL/2 lung carcinoma cells measured as the maximal myotube diameter is dependent on the AKT/mTOR pathway as shown by addition of the mTOR inhibitor rapamycin. (F) Representative images of myotubes from control conditions, after induction of myotube atrophy by C26 or LL/2 cell conditioned medium with and without IGF-1 treatment and/or addition of rapamycin. Images show Myosin heavy chain (in green), Myogenin (in red) and Dapi (in blue). Scale bar = 50μm. Error bars represent SEM. n=3. * = $p<0.05$, ** = $p<0.01$. Please note that the experiment shown here was performed together with the experiment shown in Fig.6, therefore the panels for control and rapamycin treatment are identical