Figure S1. Phase contrast microscopy for the verification of mesenchymal cell differentiation into adipocytes by the accumulation of large lipid droplets in the cells. (A) 3T3L1, (B) hMSC-Tert, and (C) human primary omental adipocytes. Scale bars, 50 μm. hMSC-Tert, human mesenchymal stem cells immortalized by stable transduction of the catalytic subunit of human telomerase.

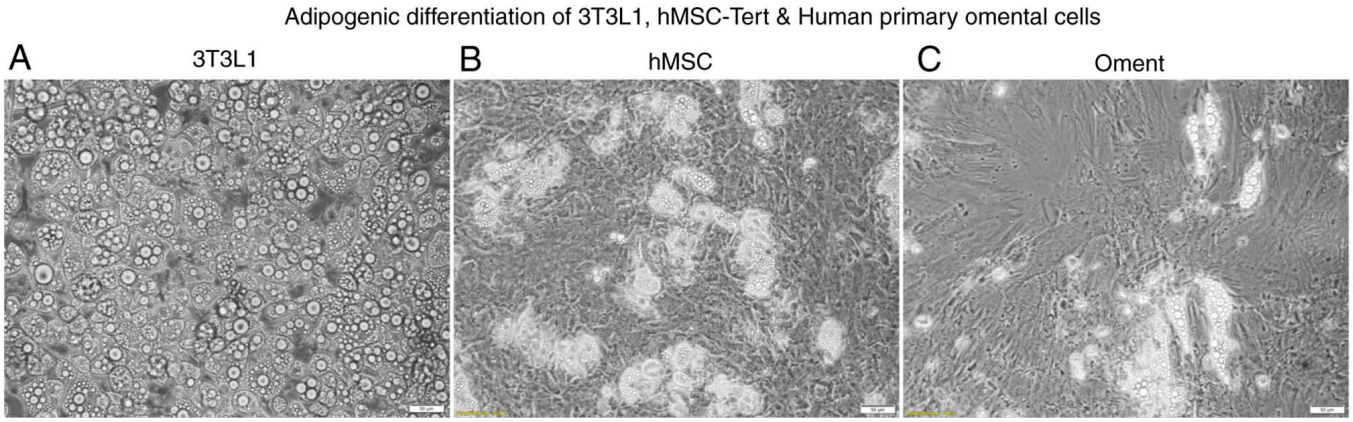

Figure S2. Serum is an efficient chemoattractant for drug-naïve OC cells. A concentration of 5% is already sufficient for maximal attraction. Two drug-naïve red fluorescent OC cell lines ($5x10^4$ mCherry-A2780 cells/insert or $12.5x10^4$ mCherry-OVCAR3 cells/insert) were loaded into 24-well FluoroBlok cell culture inserts (apical chamber) and allowed to migrate for 24 h through 8 μm large pores into the basal chambers of 24-well plates containing media with 0% FCS (control group), or 5 or 30% FCS (experimental groups) as a chemoattractant before determining the red fluorescence in the basal chamber with a fluorometer (upper diagrams) and for mCherry-A2780 with an Olympus IX73 fluorescence microscope (lower micrographs; scale bar, 100 μM). Red fluorescence intensity was quantified and MPI is provided for each micrograph (micrographs not shown for OVCAR3 cells). Further technical details are specified in the Materials and methods section. Plotted data are provided in RFU, normalized to seeded OC cell number/insert. All data are presented as the mean ± SD, n ≥3. Data were analyzed using ANOVA, followed by a Scheffe post hoc test. **P<0.01 and ***P<0.001, relative to 0% FCS (control group). OC, ovarian cancer; FCS, fetal calf serum; MPI, mean pixel intensity; RFU, relative fluorescence units; SD, standard deviation.

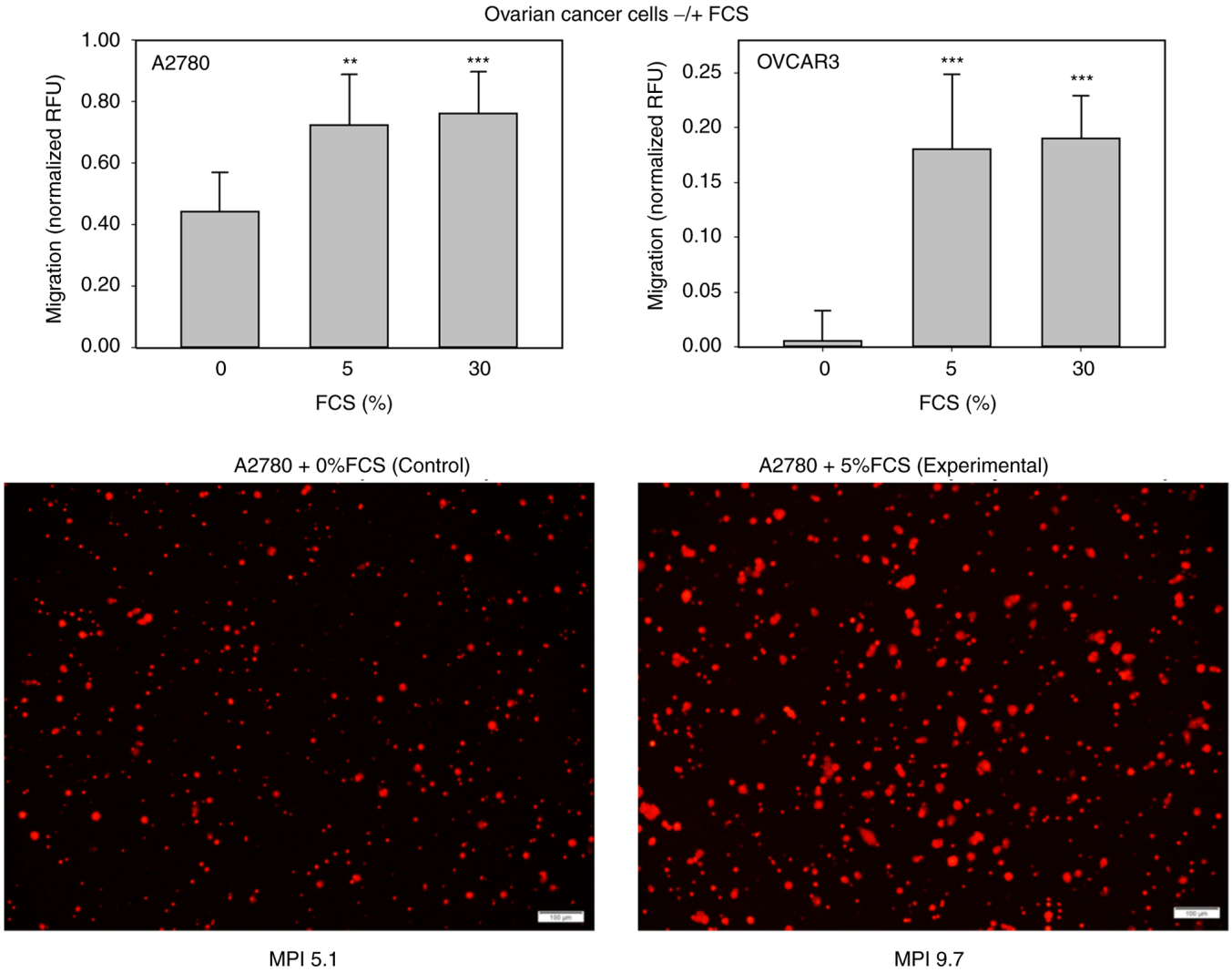

Figure S3. Effects of FASN inhibitors on the migration of OC cells in the presence of FCS or adipocytes. In total, $5x10^4$ red fluorescent mCherry-A2780 OC cells/insert were plated in RPMI-1640 medium containing 5% FCS in 24-well FluoroBlok cell culture inserts (apical chamber) with pores of 8 μm in diameter, which were inserted into the basal chambers of 24-well plates containing media with (A-C) 5% FCS, (D-F) murine 3T3L1 adipocytes, (G-I) human hMSC-Tert adipocytes, or (J-L) primary human omental adipocytes as chemoattractants. Subsequently, solvent (DMSO, final concentration ≤0.1%) without (control groups) (A, D, G and J) or with 10 μM G28UCM (B, E, H and K) or 90 μM Fasnall FASN inhibitors (C, F, I and L) (experimental groups) was added. Fluorescent mCherry-A2780 OC cells were allowed to migrate for 24 h through the pores from the apical chamber down to the basal chamber before microscopic examination of the transmigrated red fluorescent mCherry-A2780 OC cells using an Olympus IX73 fluorescence microscope. Red fluorescence intensity was quantified and MPI is provided for each micrograph. Scale bar, 100 μm. FASN, fatty acid synthase; OC, ovarian cancer; FCS, fetal calf serum; hMSC-Tert, human mesenchymal stem cells immortalized by stable transduction of the catalytic subunit of human telomerase; MPI, mean pixel intensity; SD, standard deviation.

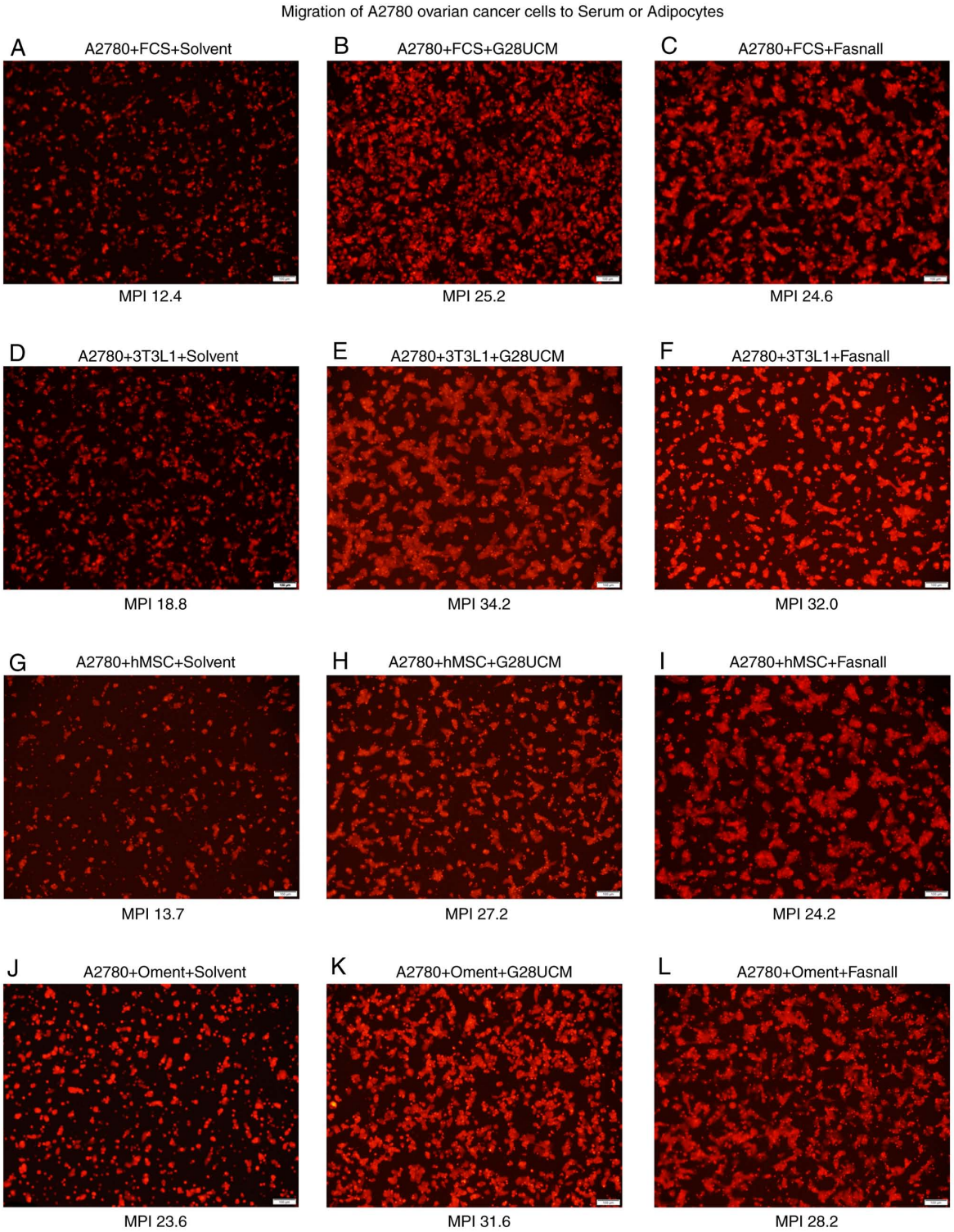

Figure S4. Differentiated murine 3T3L1 adipocytes are efficient chemoattractants for OC cells. Drug-naïve red fluorescent mCherry-A2780 ($5x10^4$ cells/insert, left panel) or mCherry-OVCAR3 ($12.5x10^4$ mCherry-OVCAR3 cells/insert, right panel) were plated in 24-well FluoroBlok cell culture inserts (apical chamber) and allowed to migrate for 24 h through 8 μm large pores into the basal chambers of 24-well plates containing adipocyte media with (black bars, experimental groups) or without (white bars, control groups) differentiated murine 3T3L1 adipocytes as attractant before determining the fluorescence in the basal chamber with a fluorometer (upper diagrams). mCherry-A2780 were also observed in an Olympus IX73 fluorescence microscope (lower micrographs; scale bar, 100 μM). Micrographs for OVCAR3 cells are not shown. Red fluorescence intensity was quantified and MPI is provided for each micrograph. Further technical details are specified in the Materials and methods section. Plotted data are provided in RFU normalized to seeded OC cell number/insert. Data are presented as the mean ± SD, n ≥3. Data were analyzed using one-tailed Student's t-test. ***P<0.001, relative to -3T3L1 (white bars, control groups). OC, ovarian cancer; FCS, fetal calf serum; MPI, mean pixel intensity; RFU, relative fluorescence units; SD, standard deviation.

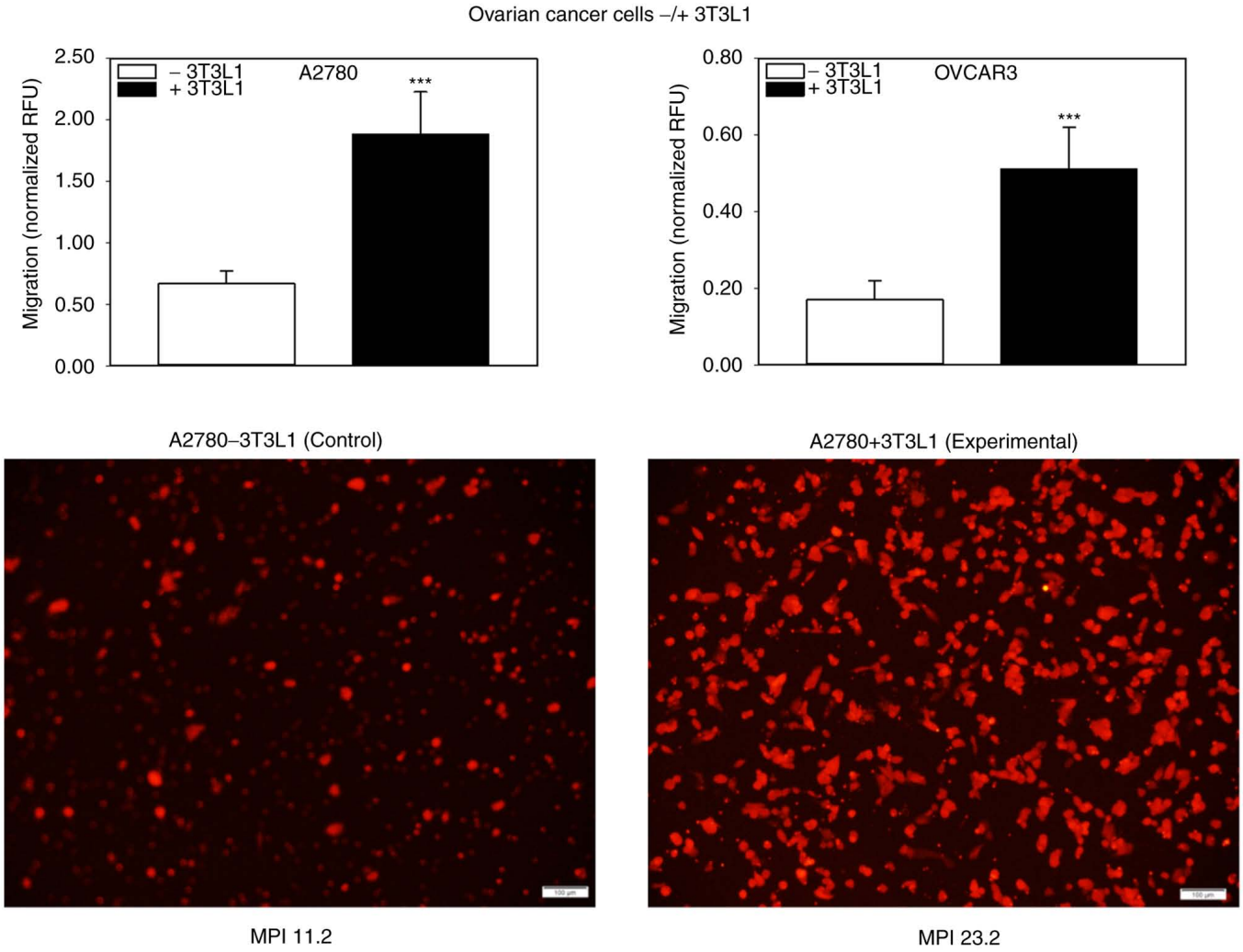

Figure S5. Effects of FASN inhibitors on the transcellular transfer of FA from (A-C) differentiated murine 3T3L1 adipocytes or (D-F) differentiated primary human omental adipocytes into OC cells. In total, $3x10^4$ mCherry-A2780 OC cells per cylinder suspended in RPMI-1640 medium with 5% FCS containing solvent (DMSO, final concentration ≤0.1%) without (solvent control group) (A and D) or with 5 μM FASN inhibitor G28UCM (B and E) or 80 μM Fasnall (C and F) (experimental groups) were plated inside of glass cloning cylinders in the center of the wells of 24-well plates. On the outside of the cloning cylinders the wells contained monolayers of (A-C) $7.5x10^3$ differentiated murine 3T3L1 adipocytes in adipocyte media or (D-F) $6x10^4$ differentiated human omental adipocytes in omental adipocyte media. Following a 24-h drug exposure of mCherry-A2780 OC cells, the adipocyte monolayers were serum deprived for 1 h and then labeled with 1.5 μM of the green fluorescent FA marker Bodipy FL C16. After 4 h, adipocytes were washed once with HBSS with 0.2 % FA-free BSA before the glass cloning cylinders separating OC cells from adipocytes were removed. All cells were then washed again five times with HBSS with 0.2 % FA-free BSA and incubated in RPMI-1640 medium containing (A and D) 1% FCS without FASN inhibitor (control groups), or (B and E) with 5 μM G28UCM or (C and F) 80 μM Fasnall (experimental groups) and co-cultured for 24 h. The experiment was terminated by removing the media and washing the co-cultures once with 1% HBSS with 0.2% FA-free BSA prior to microscopic examination of the green fluorescent Bodipy FA marker that has been transported from peripheral adipocytes into the central mCherry-A2780 OC cells using an Olympus IX73 fluorescence microscope. Green fluorescence intensity of the Bodipy-loaded mCherry-A2780 OC cells was quantified and MPI is provided for each micrograph. Scale bar, 50 μm. FASN, fatty acid synthase; FA, fatty acids; OC, ovarian cancer; HBSS, Hank's balanced solution; BSA, bovine serum albumin; FCS, fetal calf serum; MPI, mean pixel intensity; SD, standard deviation.

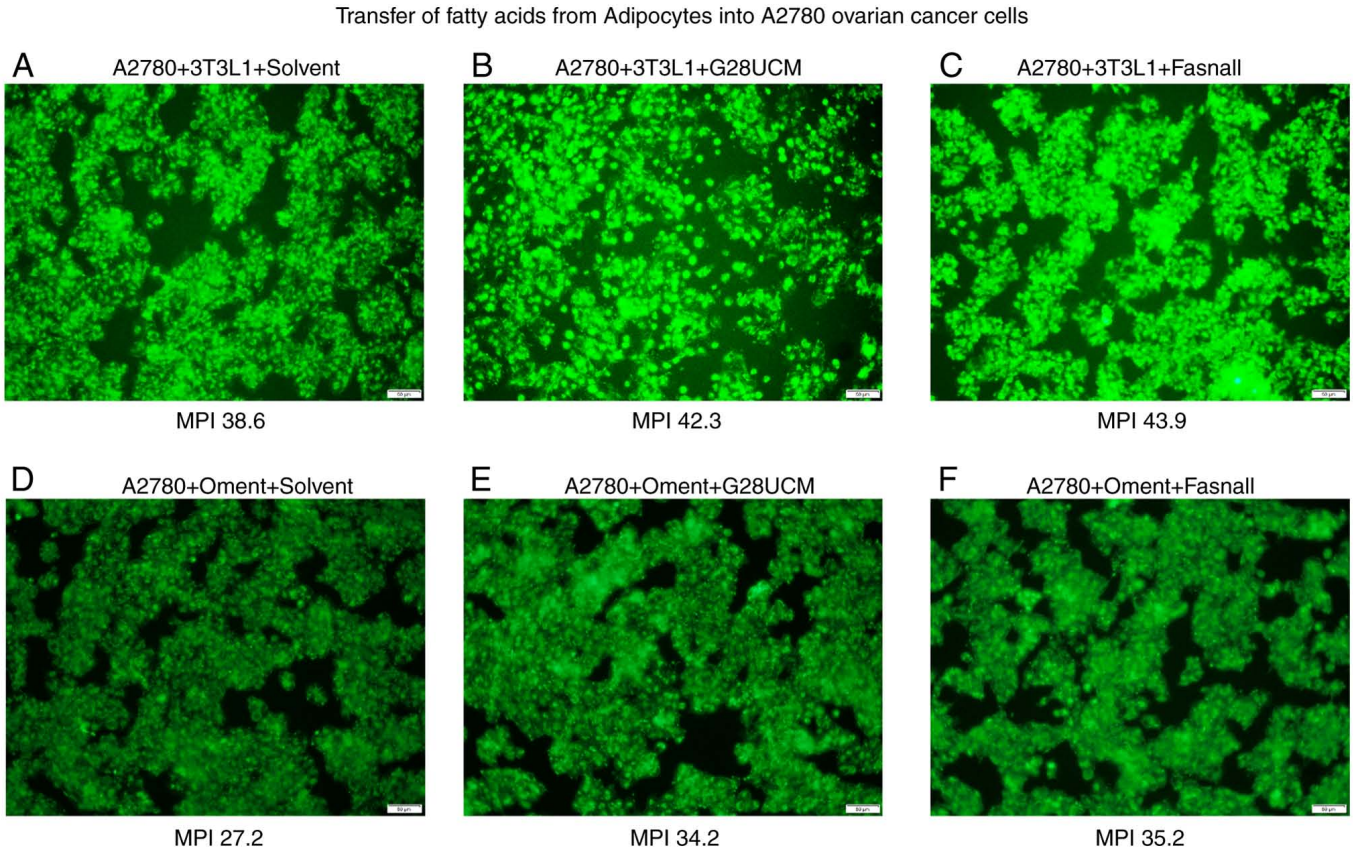

Figure S6. Uptake of exogenous FA in adipocytes is (A-C and J) dependent on the type and origin of the adipocytes, but (D-I) is resistant to FASN inhibitors. Accumulation of the green fluorescent Bodipy FA marker was strong in untreated differentiated murine 3T3L1 adipocytes (3T3L1) (A and J), but was only moderate in untreated human hMSC-Tert adipocytes derived from hMSC (B and J) and even weaker in untreated differentiated primary human omental adipocytes (oment) (C and J). Monolayers of differentiated murine 3T3L1 adipocytes in adipocyte media (A, D-F and J), differentiated hMSC-Tert adipocytes (B, G-I and J), or differentiated human omental adipocytes (C and J) were serum deprived for 1 h and then labeled with 1.5 μM green fluorescent FA marker Bodipy FL C16. After 4 h, adipocytes were washed and cultured for 24 h with solvent (DMSO, final concentration ≤0.1%) (A-C, D, G and J) without (control groups) or with (E and H) 10 μM FASN inhibitor G28UCM or (F and I) 80 μM Fasnall (experimental groups). The experiment was terminated by removing the media and washing the cultures once with 1% HBSS with 0.2% FA-free BSA before microscopic examination of the uptake of the green fluorescent Bodipy FA marker into adipocytes using an Olympus IX73 fluorescence microscope. (A-I) Fluorescence intensity was quantified and MPI is provided for each micrograph. (J) The accumulation of the green fluorescent Bodipy FA marker in untreated 3T3L1, hMSC-Tert (abbreviated in the figure as hMSC for short), and primary human omental adipocytes (oment) was determined with a fluorometer. Data are provided in RFU of the cell monolayers. All data are presented as the mean ± SD, n ≥3. Data were analyzed using ANOVA, followed by a Scheffe post hoc test. *P<0.05 and ***P<0.001, relative to 3T3L1. Scale bars, 50 μm. FA, fatty acids; FASN, fatty acid synthase; hMSC-Tert, human mesenchymal stem cells immortalized by stable transduction of the catalytic subunit of human telomerase; MPI, mean pixel intensity; RFU, relative fluorescence units; SD, standard deviation.

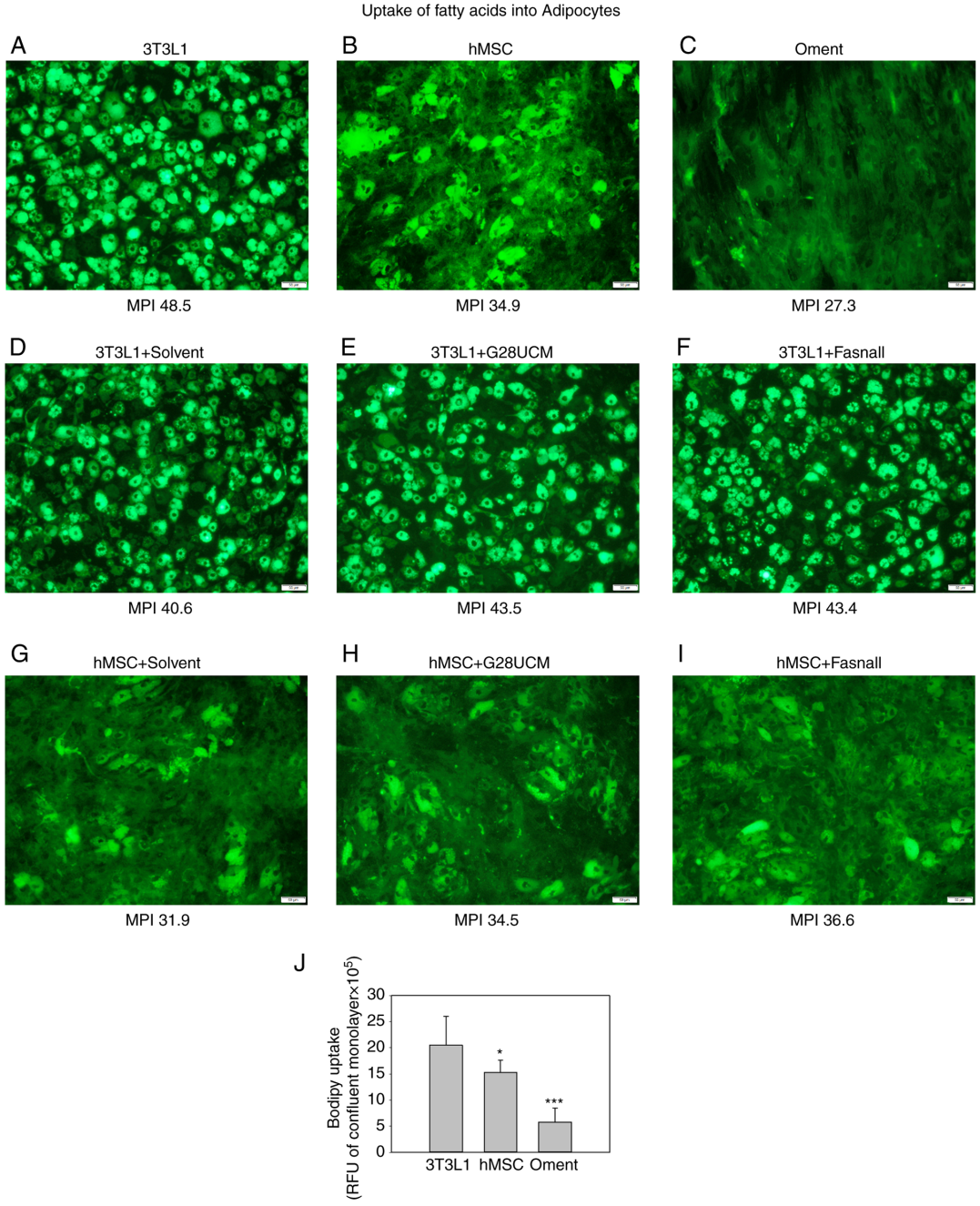

Figure S7. Growth inhibitory effects of FASN inhibitors in OC cells when co-cultured with 3T3L1 adipocytes. In total, $3x10^4$ red fluorescent mCherry-A2780 OC cells/cylinder suspended in RPMI1640 with 5% FCS containing solvent (DMSO, final concentration ≤0.1%) (A) without (control group) or with (B) 10 μM G28UCM or (C) 90 μM Fasnall FASN inhibitors (experimental groups) were plated inside of glass cloning cylinders in the center of the wells of 24-well plates. On the outside of the cloning cylinders the wells contained monolayers of $7.5x10^3$ differentiated murine 3T3L1 adipocytes in adipocyte media. Following a 24-h drug exposure of mCherry-A2780 OC cells the glass cloning cylinders separating OC cells from adipocytes were removed. All cells were then washed again 5 times with HBSS with 0.2 % FA-free BSA and incubated in RPMI-1640 medium containing 1% FCS with 10 μM G28UCM or 90 μM Fasnall and co-cultured for a further 24 h. The experiment was terminated by removing the media and washing the cultures once with 1% HBSS with 0.2% FA-free BSA before microscopic examination of the red fluorescent mCherry-A2780 OC cells in the center of the wells using an Olympus IX73 fluorescence microscope. Red fluorescence intensity was quantified and MPI is provided for each micrograph. Scale bar, 100 μm. FASN, fatty acid synthase; OC, ovarian cancer; HBSS, Hank's balanced solution; BSA, bovine serum albumin; MPI, mean pixel intensity.