

OMX-0407, a highly potent SIK3 inhibitor, sensitizes tumor cells to cell death and eradicates immune-checkpoint resistant tumors synergistically in combination with PD-1 inhibition

Introduction

Using the iTarg genetic screening platform, salt-inducible kinase 3 (SIK3) was recently identified as a novel epigenetic modulator in cancer therapy. SIK3, a serine/threonine kinase of the AMP-activated protein kinase (AMPK) family, is known for regulating the NF- κ B-driven gene landscape through phosphorylation of class IIa histone deacetylases (HDACs), causing the tumor to evade death receptor-mediated killing. Here we report that OMX-0407, an orally available, single-digit nanomolar inhibitor of SIK3 abates downstream pro-survival signaling of the SIK3-HDAC4-NF- κ B axis and potentiates caspase-mediated cell death in murine and human tumor cell lines. OMX-0407 dose-dependent suppression of intratumoral NF- κ B activity was shown *in vitro* and *in vivo* with an MC38 NF- κ B-luc reporter cell line. OMX-0407 monotherapy results to significant tumor growth inhibition (TGI) as well as prolonged survival in the highly immune-infiltrated syngeneic murine colorectal carcinoma model MC38. Besides its direct inhibitory effects on cancer cells, OMX-0407 repolarizes the tumor microenvironment (TME) by strongly decreasing regulatory T cells (Treg) and M2-polarized macrophages in the tumor bed, while not affecting the peripheral T cell compartment. Using immune checkpoint inhibitor resistant breast (EMT6) and lung (KLN205) cancer models, we demonstrate that OMX-0407 and anti-PD-1 therapy act synergistically by combining the sensitization towards cell death with a reduction in immunosuppressive TME and an increase in cytotoxic T cell activity. OMX-0407 is an outstanding therapeutic option to overcome TNF/TRAIL-induced immune evasion and anti-PD-1 resistance, which will be clinically investigated in the near future.

Results

SIK3 inhibits death receptor mediated apoptosis in tumor cells

- Knockout of SIK3 sensitizes tumor cells to death receptor mediated apoptosis and thereby delays tumor cell growth

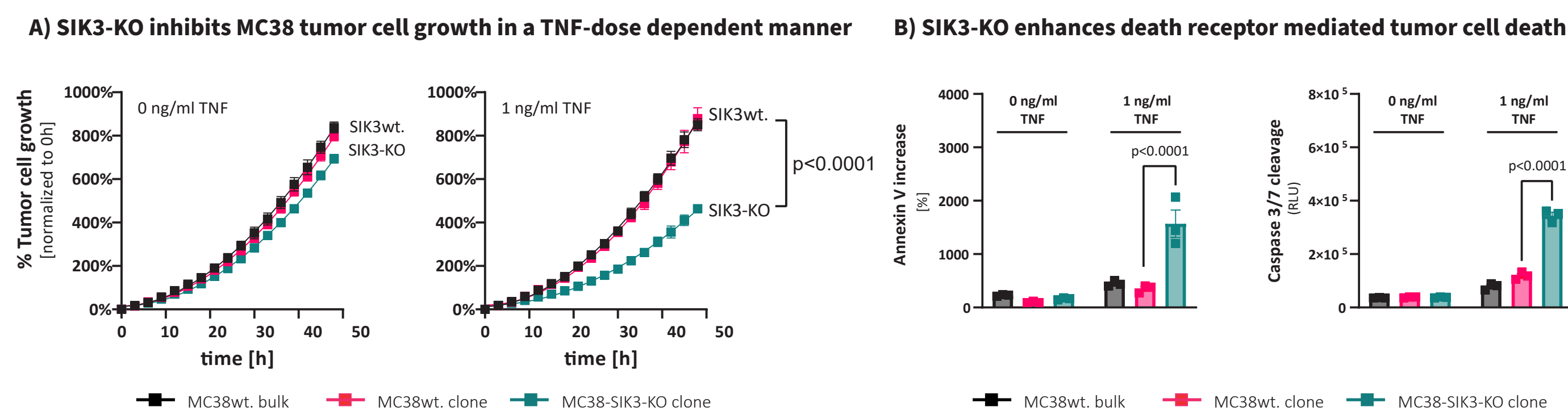


FIGURE 1
MC38-SIK3-KO or MC38 wildtype control clones were generated using CRISPR. MC38-SIK3-KO and wildtype clone as well as MC38 wildtype bulk tumor cells were treated with 1 ng/ml TNF or corresponding controls. **A)** Tumor growth kinetics was monitored for 48h in a life cell imaging system including analysis of phosphatidylserine expression by Annexin V and caspase 3/7 cleavage 8h after therapy start (**B**).

OMX-0407 a potent inhibitor of SIK3, enhances death receptor mediated apoptosis in tumor cell lines of various indications

- OMX-0407 is a single-digit nanomolar inhibitor of salt-inducible kinase 3
- OMX-0407 enhances apoptosis in murine and human tumor cell lines upon TNF- or TRAIL treatment in a dose-dependent manner

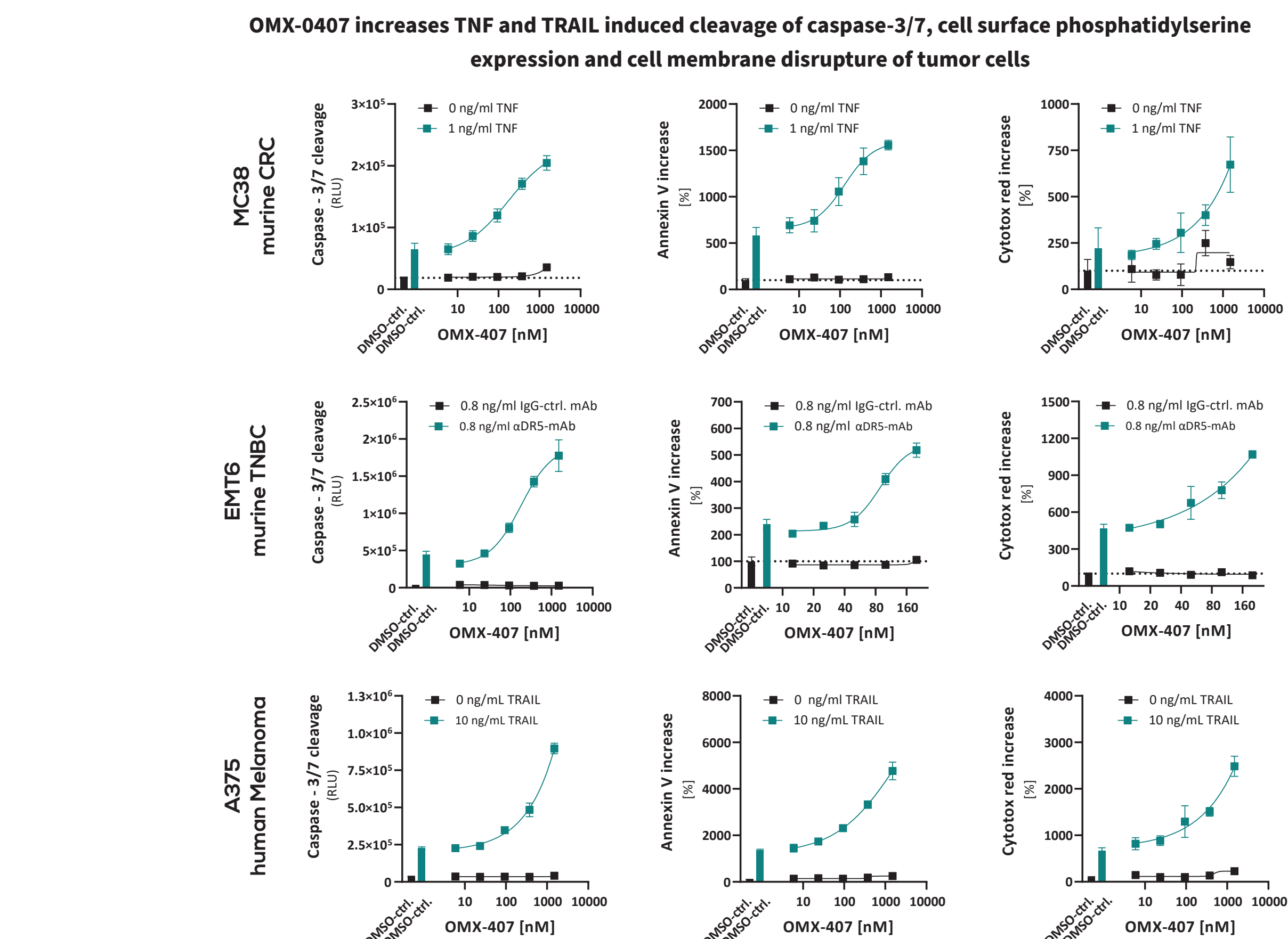


FIGURE 2
Murine colorectal carcinoma cell line MC38, murine triple negative breast cancer cell line EMT6 and human melanoma tumor cell line A375 were treated with 1 ng/ml TNF, 0.8 ng/ml murine death receptor 5 targeting antibody (clone MD5-1), 10 ng/ml complexed TRAIL or appropriate controls in combination with OMX-0407 and an associated DMSO-control. The onset of tumor cell death was monitored 8 hours after treatment initiation by cleavage of effector caspases 3/7, while the increase in extracellular phosphatidylserine expression was analyzed by annexin V and cell membrane disruption by cytotox red staining 24 hours after therapy initiation.

OMX-0407 inhibits SIK3-triggered phosphorylation of HDAC4/5 and the associated transcriptional activity of NF- κ B

- OMX-0407 demonstrates dose dependent dephosphorylation of histone deacetylase 4/5
- OMX-0407 prevents TNF mediated activity of the NF- κ B pathway

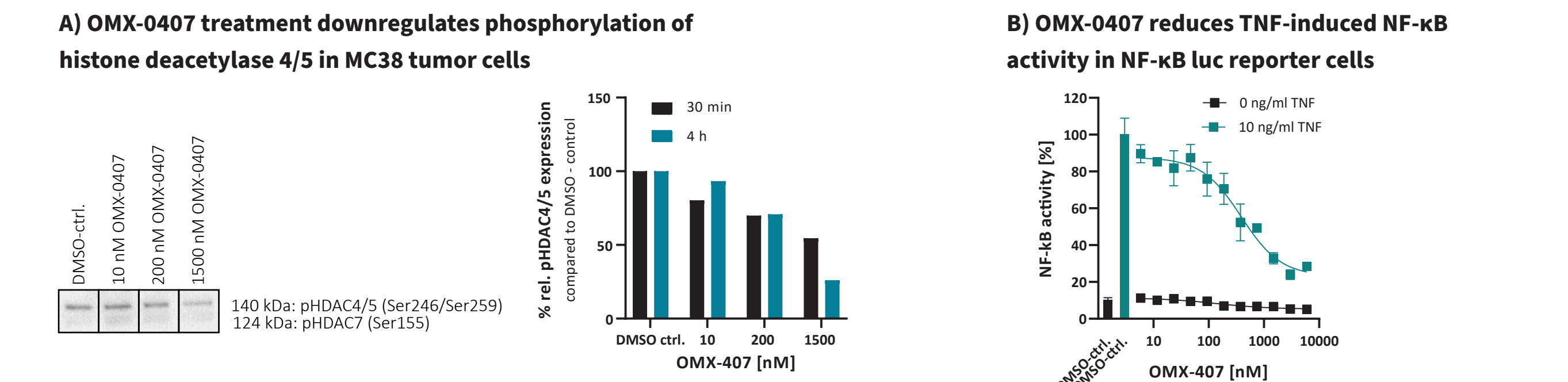


FIGURE 3
MC38wt. (A) and MC38-NF- κ B luc reporter tumor cells (B) were treated *in vitro* with 10 ng/ml TNF or corresponding controls in combination with OMX-0407 and an associated DMSO-control. Tumor cells were lysed 30 min or 4h after treatment start and analyzed for phosphorylated histone deacetylase 4/5/7 by Western Blot. **(A)** Representative Blots are shown for 4 hour treatment. Phosphorylated HDAC4/5 was quantified and normalized to total protein load. Modulation of pHDAC4/5 level compared to DMSO control are shown. **(B)** MC38-NF- κ B luc reporter activity was monitored after 7h treatment with OMX-0407 or an associated DMSO-control.

In vivo pharmacokinetics of OMX-0407 determines dose-dependent pharmacodynamic abrogation of the SIK3 - NF- κ B pathway activity in MC38 tumors

- OMX-0407 demonstrates dose dependent accumulation in tumor and peripheral tissues upon oral application
- OMX-0407 prevents intratumoral, transcriptional activity of NF- κ B in an MC38-NF- κ B reporter model associated to its pharmacokinetics
- OMX-0407 prohibits phosphorylation of HDAC4/5 and NF- κ B subunit p65 and triggers tumor cell death

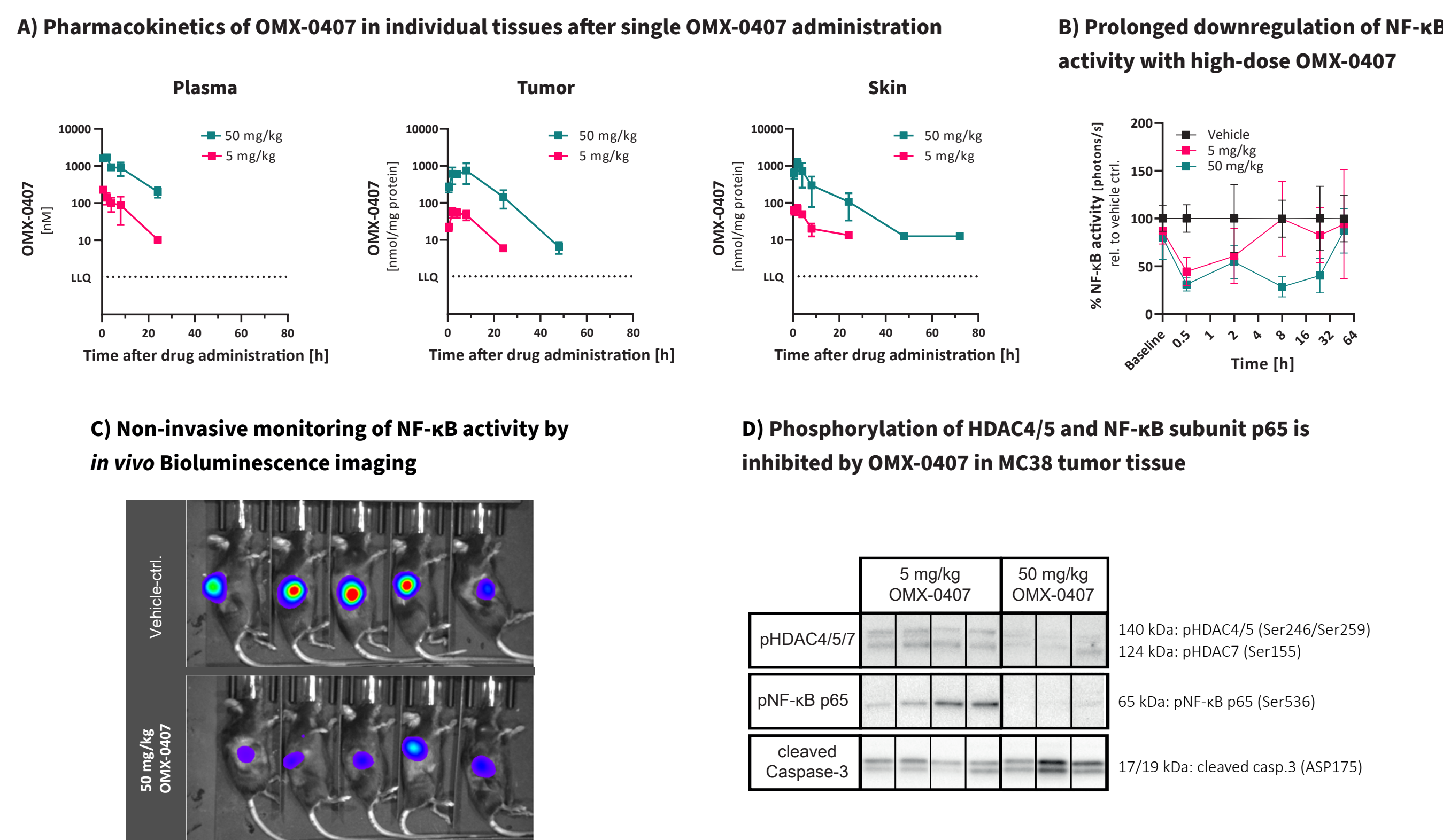


FIGURE 4
MC38-NF- κ B luc reporter tumor cells were subcutaneously implanted in C57Bl6/N mice, randomized at an average tumor volume of 400mm³ and treated with a single dose of 5 mg/kg or 50 mg/kg OMX-0407 or appropriate vehicle control by oral gavage. **A)** OMX-0407 concentration was analyzed by LC-MS in plasma, skin and tumor tissue at the indicated time points. **B)** Transcriptional activity of the NF- κ B luc reporter was monitored non-invasively by bioluminescence imaging (BLI) in eight animals per time point. Mean BLI-signal for the indicated time points is shown normalized to vehicle control. **C)** Representative example of BLI signals 8 hours after start of treatment of animals with vehicle and 50 mg/kg OMX-0407. **D)** MC38 tumor tissue was collected 8h after treatment initiation. Tumor tissue lysates were analyzed *ex vivo* by Western Blot for phosphorylation of HDAC4/5/7 and NF- κ B subunit p65 as well as level of cleaved caspase 3. Representative Blots of the 5 mg/kg and 50 mg/kg therapy group are shown for each analyte.

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Conclusion

- OMX-0407 is a strong inhibitor of SIK3 kinase that inhibits the phosphorylation of HDAC4 in a dose-dependent manner and thereby abrogates the nuclear activity of pro-tumorigenic transcription factor NF- κ B in tumor cells both *in vitro* and *in vivo*
- Downregulation of the SIK3-HDAC4-NF- κ B pathway with OMX-0407 potentiates apoptosis by death receptor ligands, such as TNF or TRAIL, *in vitro* in tumor cell lines of different origin
- Pharmacokinetics of OMX-0407 in tumor tissue correlates with intra-tumoral abrogation of the HDAC4-NF- κ B axis
- OMX-0407 shows strong efficacy as monotherapy in the syngeneic tumor model MC38 with a marked repolarization of the tumor microenvironment towards an anti-tumor immune profile
- OMX-0407 acts synergistically with PD-1 blockade in immune checkpoint inhibitor-resistant syngeneic breast and lung cancer models
- The ability of OMX-0407 to repolarize the tumor microenvironment and sensitize tumors to death receptor-mediated apoptosis holds great potential for patients with high unmet medical need, both as monotherapy and in combination with anti-PD-1/PD-L1 immune checkpoint blockade
- OMX-0407 will enter clinical trials in patients with advanced cancer in 2022

OMX-0407 repolarizes the tumor microenvironment and shows strong anti-tumor efficacy in monotherapy and in combination with PD-1 blockade

- OMX-0407 demonstrates highly significant anti-tumor efficacy and prolonged survival in monotherapy in the colorectal carcinoma model MC38
- OMX-0407 repolarizes the tumor microenvironment by reducing the number of regulatory T cells (Treg) and immunosuppressive myeloid cells and potentiating the cytotoxic T lymphocyte (CTL) activity as well as promoting the CTL to Treg ratio
- Combination treatment of OMX-0407 together with anti-PD-1 exploits the full potential of a repolarized anti-tumor immune environment

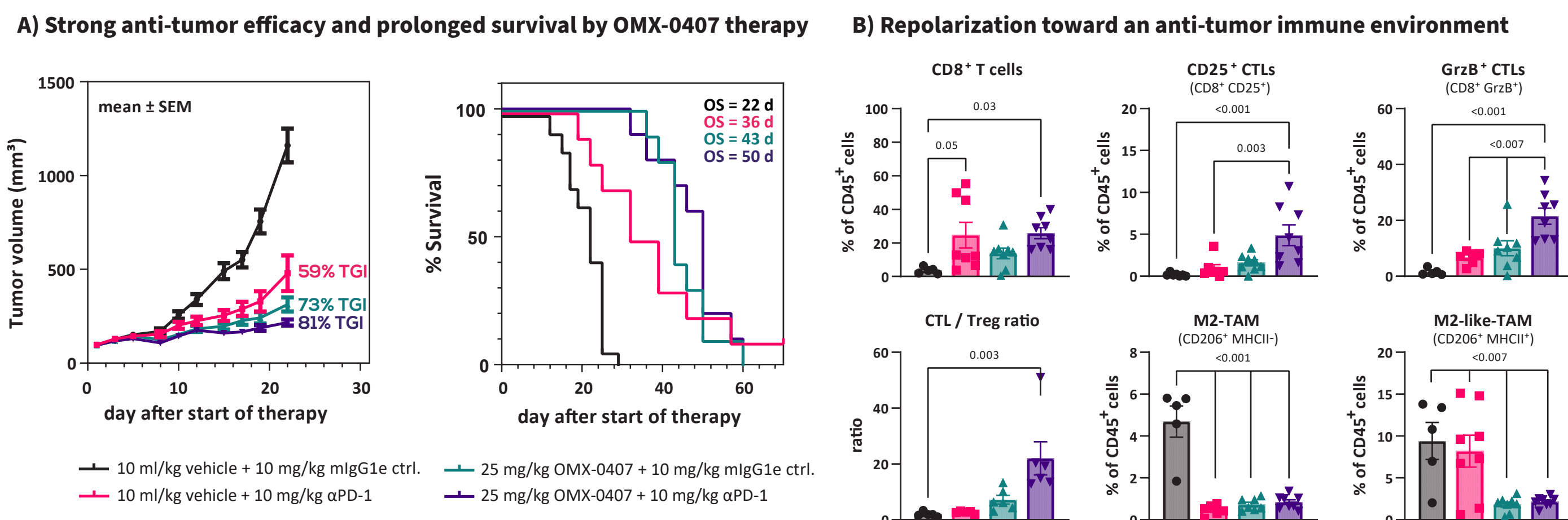


FIGURE 5
MC38 tumor cells were subcutaneously implanted in C57Bl6/N mice, randomized at an average tumor volume of 100 mm³ and tumor bearing mice were treated twice daily with 25 mg/kg OMX-0407 by oral gavage and twice weekly, intraperitoneal with 10 mg/kg anti-PD-1 mAb (clone: RMP1-14) or corresponding controls. **A)** Average tumor growth kinetics and overall survival. **B)** Tumor infiltrating cytotoxic T cells (CTL), CTL to regulatory T cell ratio, tumor associated M2-macrophages (M2-TAM) and M2-like TAM were analyzed by flow cytometry.

OMX-0407 acts synergistically with PD-1 blockade in immune checkpoint-resistant breast and lung cancer models

- Combination therapy of OMX-0407 and PD-1 blockade acts synergistically in the immune-excluded breast cancer model EMT6 and the immune checkpoint-resistant lung squamous cell carcinoma model KLN205
- Complete tumor regression and extension of overall survival were achieved in EMT6 tumors and significantly delayed tumor outgrowth in KLN205 lung cancer

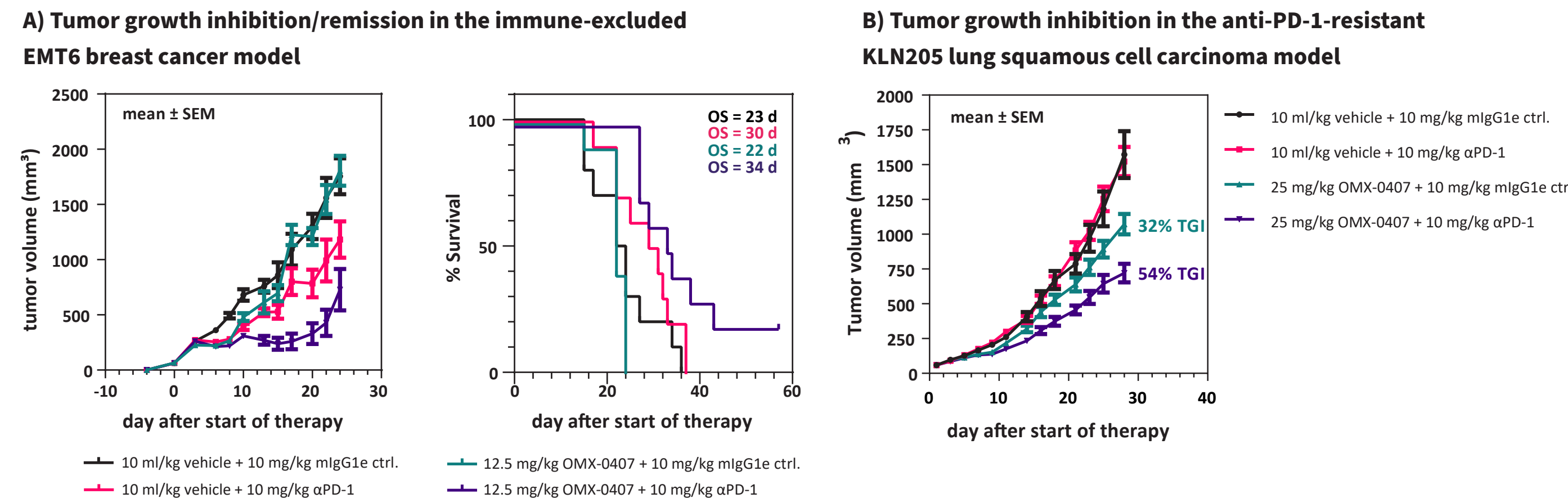


FIGURE 6
EMT6 tumor cells were implanted into mammary fat pad in BALB/c mice and randomized at an average tumor volume of 65mm³. KLN205 tumor cells were subcutaneously implanted in DBA/2 mice and randomized at an average tumor volume of 100mm³. Both animal models were treated twice daily by oral gavage with the indicated doses of OMX-0407 and twice weekly, intraperitoneal with 10 mg/kg anti-PD-1 mAb (clone: RMP1-14) or corresponding controls. **A)** Average tumor growth kinetics and overall survival monitored for the EMT6 tumor model after treatment start. Two out of ten animals demonstrated complete tumor regression. **B)** Average tumor growth kinetics for the KLN205 tumor model upon initiation of therapy.

Statistical information

If not indicated otherwise, graphs are representative data from at least two independent experiments. Sigmoidal dose-response curves were fitted to data using 4-Parameter Logistic (4PL) non-linear curve models for *in vitro* dose responses. Data points show mean $\pm$ SEM. Significance was calculated with two-way ANOVA analysis including Tukeys multiple comparison analysis.

Contact

