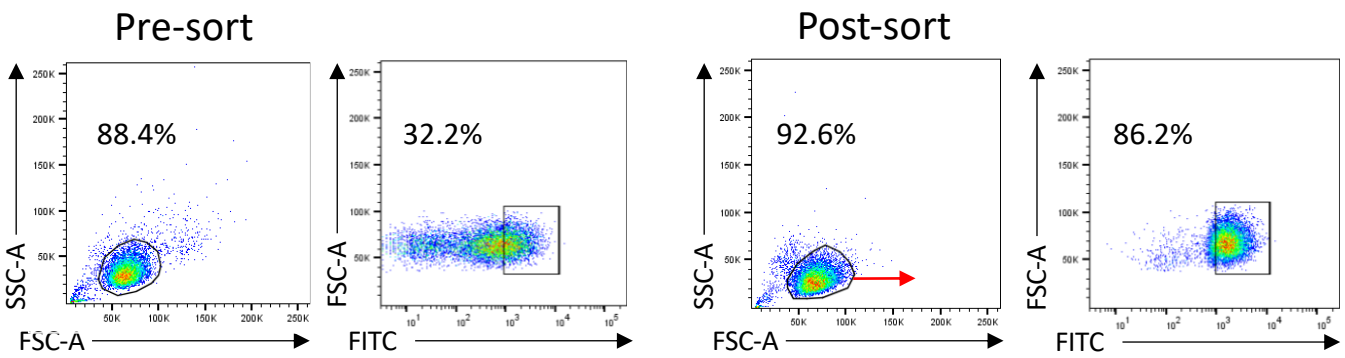
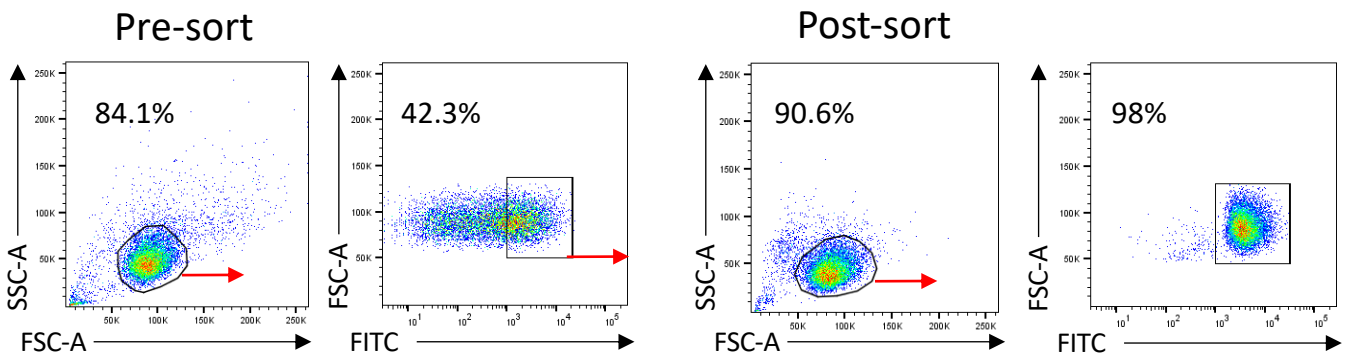
LM008 vector control NSCs

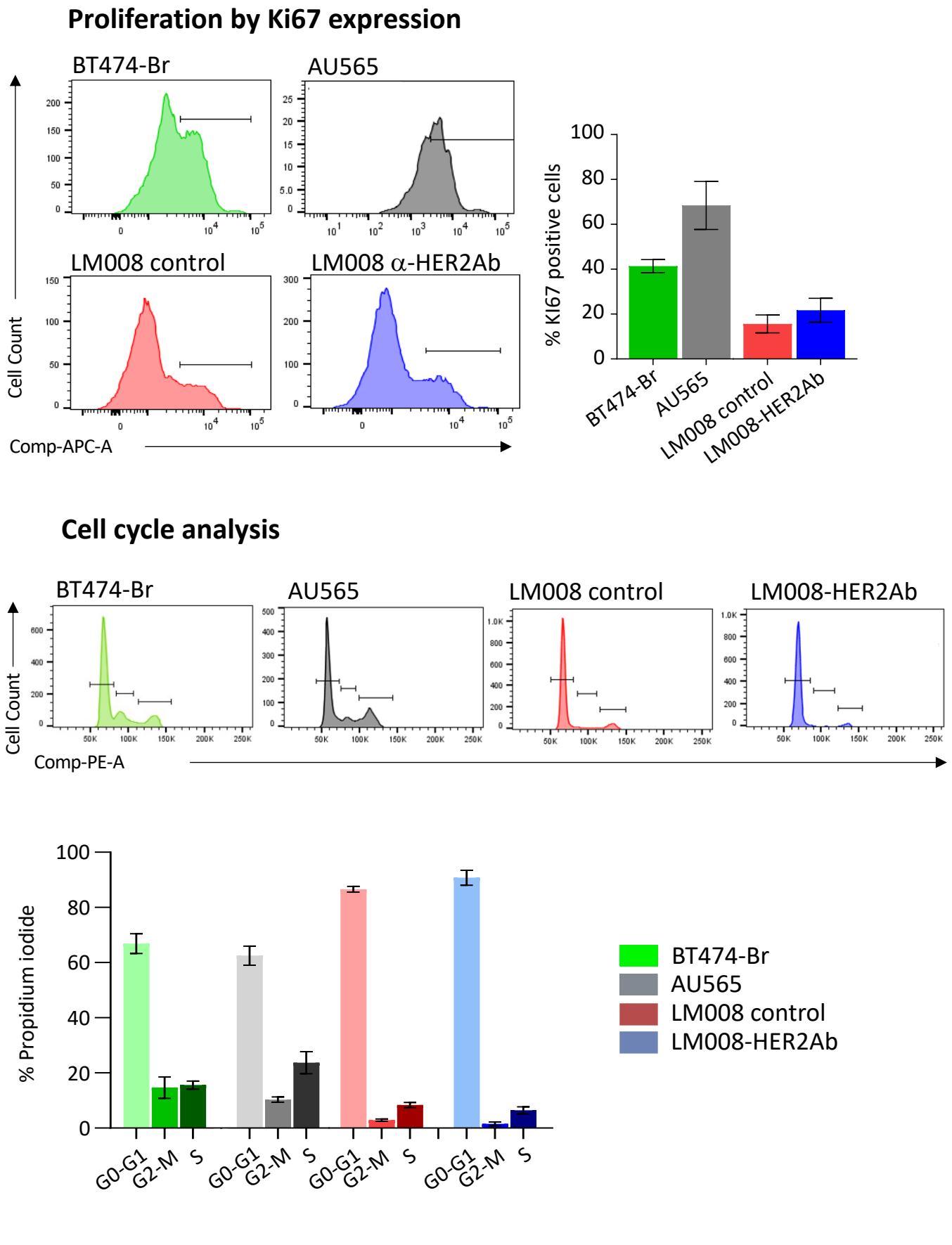


LM008-HER2Ab NSCs



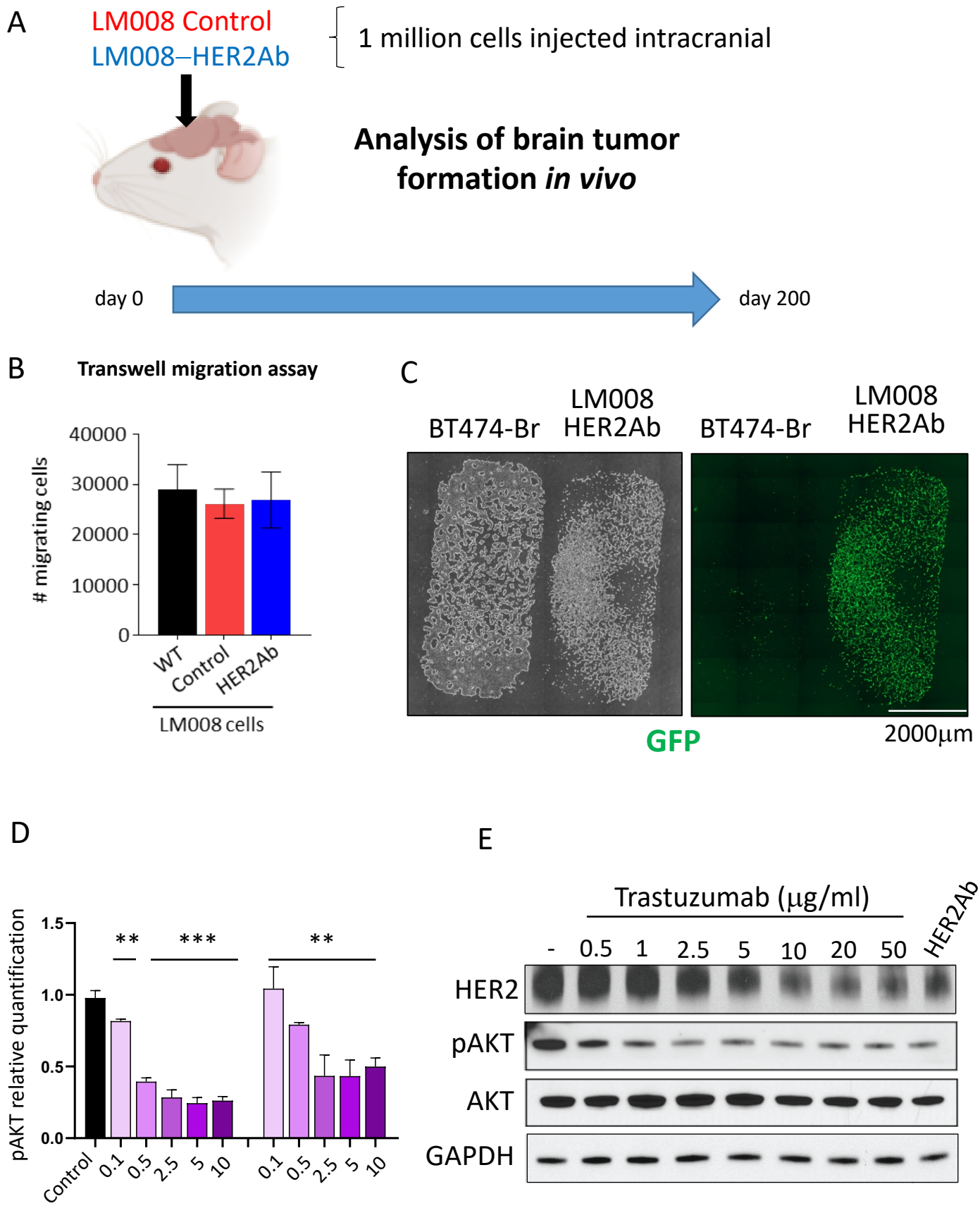
Supplementary figure 1, related to Figure 1. Isolation of transduced LM008 cells. Representative dot blots showing the FACS-mediated isolation and enrichment of LM008 cells transduced with lentivirus containing the vector control (top panel) or the anti-HER2Ab (bottom panel). LM008 cells were sorted twice based on their GFP expression (FITC)

SI Appendix, Fig. S2



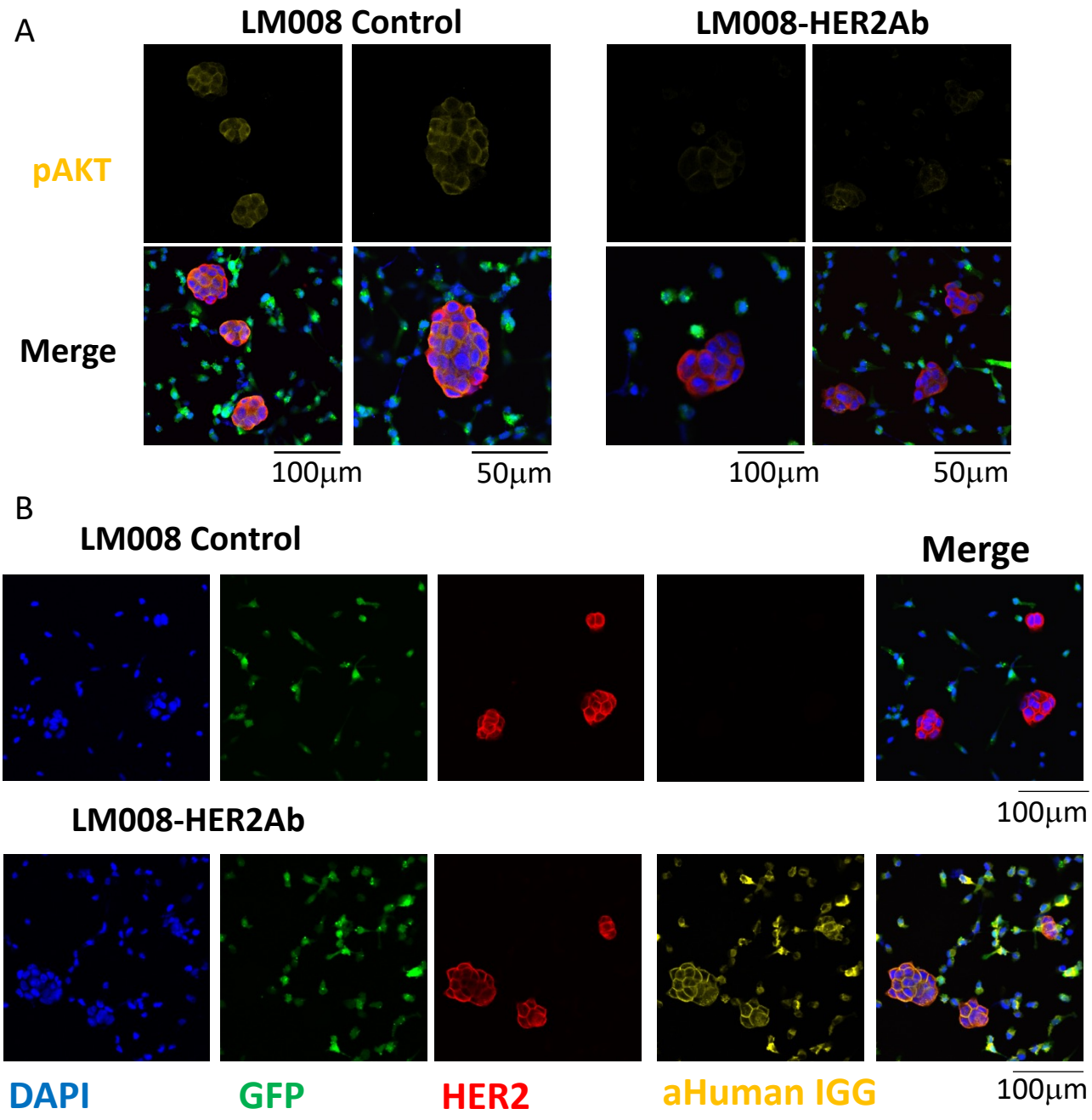
Supplementary figure 2, related to Figure 1. Proliferation and cell cycle analysis in LM008 NSCs and breast cancer cells. Analysis by flow cytometry of proliferation based on Ki67 expression (top panel) and cell cycle (bottom panel) in LM008 control and anti-HER2Ab secreting cells, and in metastatic breast cancer cell lines BT474-Br and AU565.

SI Appendix, Fig. S3



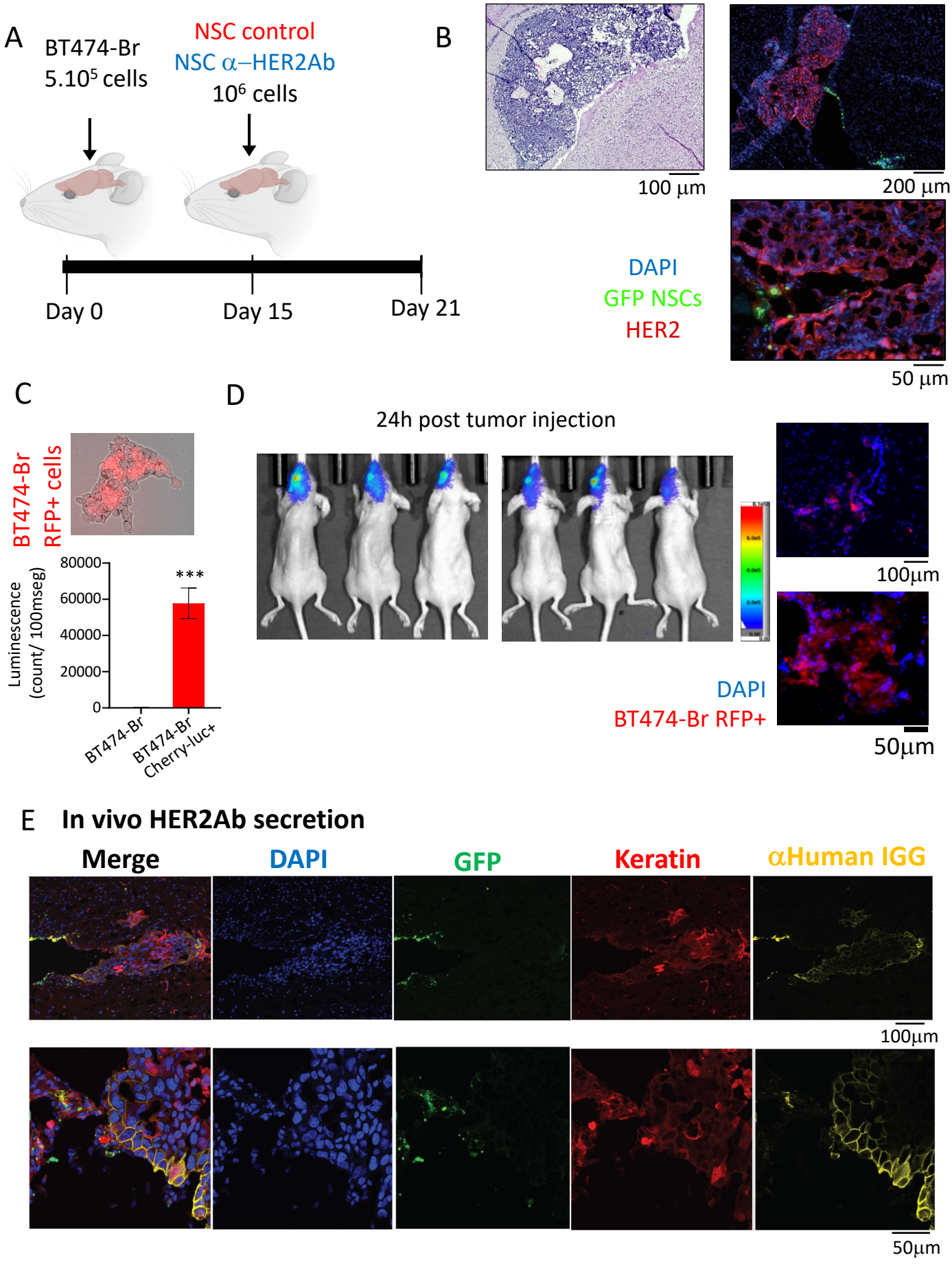
Supplementary figure 3, related to Figure 1. Characterization of LM008 NSCs. A) 10^6 LM008 vector control or LM008-HER2Ab NSCs were injected intracranial in immunodeficient $^{nu/nu}$ mice to test their potential malignant transformation. Our results revealed that no tumors were formed in any of these mice. B-C) Analysis of the migration ability *in vitro* of LM008 control and anti-HER2Ab NSCs. B) 10^5 LM008 WT, vector control or anti-HER2 NSCs were placed on the upper layer of a cell culture insert with a permeable membrane (8 mm pore size). After 22 hours cells that have migrated through the membrane are stained and counted. C) Representative images showing LM008-HER2Ab cells migrating towards BT474-Br cells in wound healing migration assays. D) Analysis of protein expression relative to western blots in figure 1F. Graphs are representative of two independent experiments, and bar graphs represent means ± SD. E) BT474-Br cells cere treated with anti-HER2Ab (15 µg/ml) and a range of concentrations of TZ (0 – 50 µg/ml) as positive control. Cells were then lysed and equal amounts of whole-cell lysates were resolved by SDS-PAGE. Immunoblots were probed with antibodies against HER2, pAKT, total AKT and GAPDH (loading control). Immunoblot images are representative of two independent experiments. Immunoblots are representative of two independent experiments. Statistical analysis was performed using one-way ANOVA with post hoc Tukey's test. *** $p< 0.001$; ** $p< 0.01$.

SI Appendix, Fig. S4



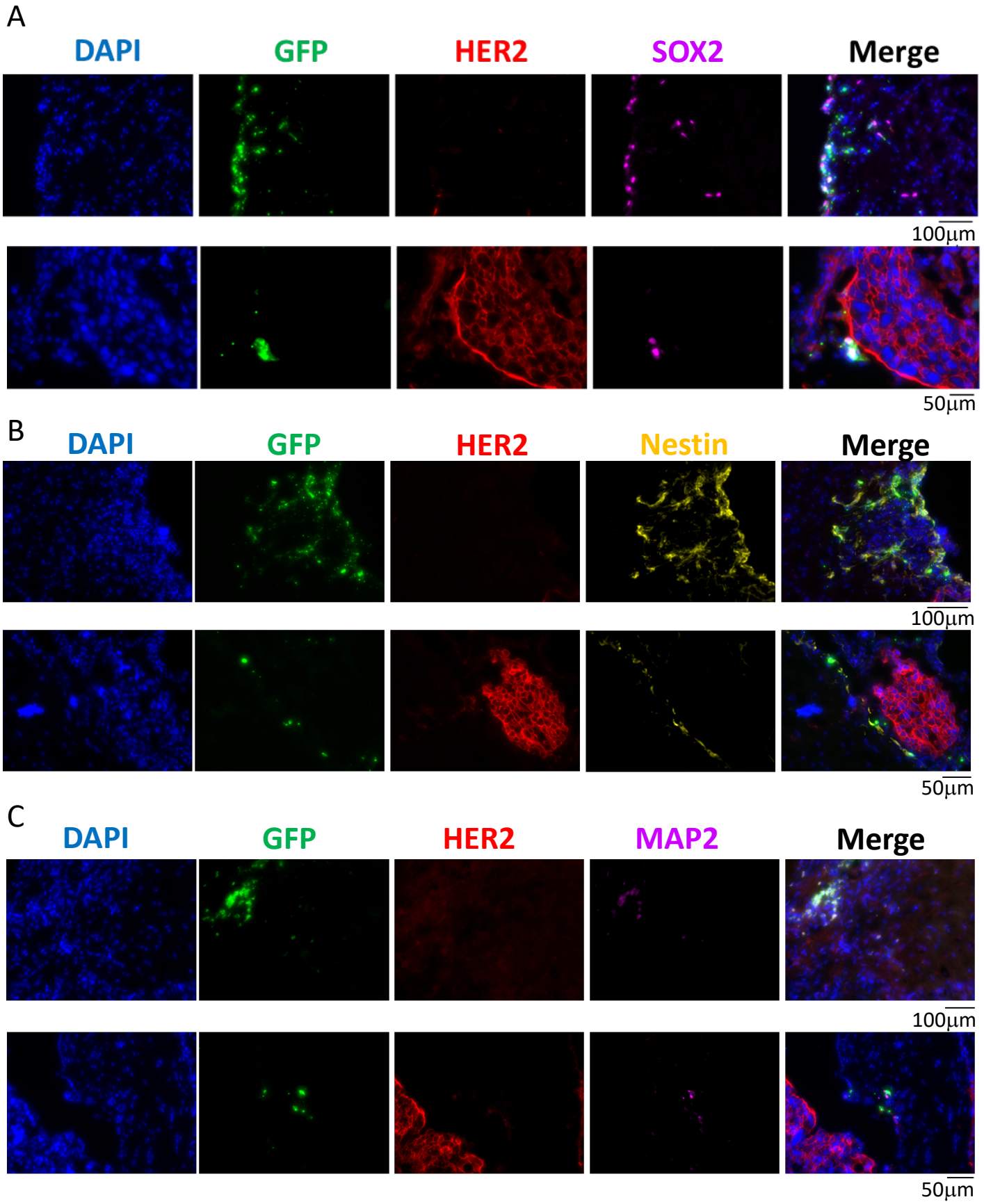
Supplementary figure 4, related to Figure 1. A-B) We established co-cultures of BT474-Br and LM008 Control/HER2Ab cells during 48 hours to determine the effect of the HER2Ab released by NSCs. A) Representative immunostaining, from three independent experiments, showing p-AKT expression (in yellow) in BT474-Br cells. LM008 Control and HER2Ab cells were stained for GFP (in green), and BT474-Br cells were stained for HER2 (in red). Nuclear staining (DAPI) is shown as blue. B) Representative immunostaining, from three independent experiments, showing the antiHER2 antibody release (measured with anti-Human IGG (in yellow)) by LM008-HER2Ab cells. LM008 Control and HER2Ab cells were stained for GFP (in green), and BT474-Br cells were stained for HER2 (in red). Nuclear staining (DAPI) is shown as blue.

SI Appendix, Fig. S5



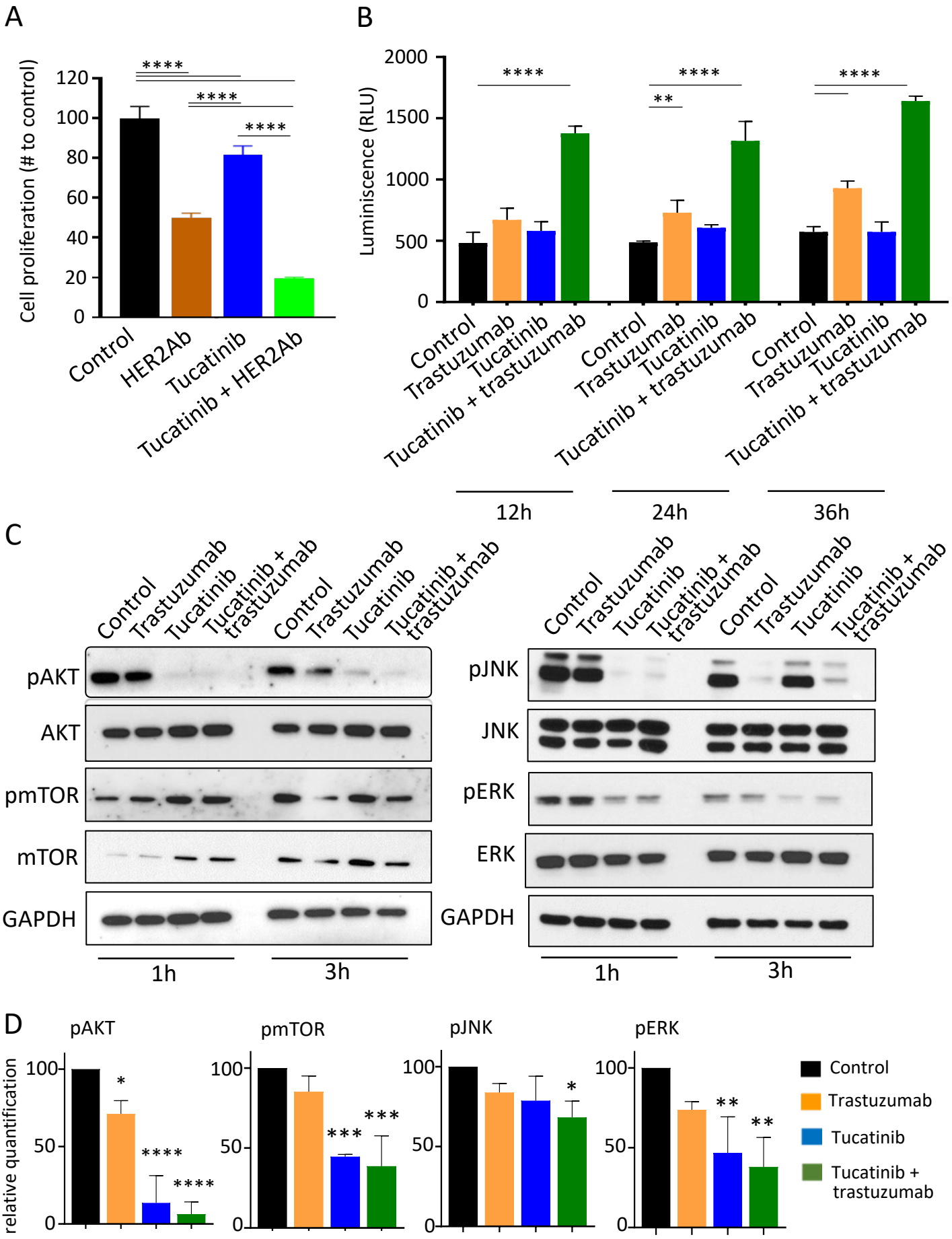
Supplementary figure 5, related to Figure 2. Systemic delivery of LM008-HER2Ab NSCs in a HER2+ overexpressing BCBM model. A) The presence of HER2+ BCBM and LM008 NSCs in the brain was validated *in vivo*. The injection of $5x10^5$ BT474-Br tumor cells in the brain of immunocompromised mice was followed by the injection of 10^6 LM008-HER2Ab NSCs in the same location of the brain. Mice were sacrified 21 days after injecting tumor cells, and brains were harvested and analyzed. B) Representative histology and immunostaining images showing the presence of HER2+ tumor cells brain (in red) in the brain, surrounded by LM008 control or anti-HER2Ab (green, GFP-positive). DAPI is shown as blue. C) Representative image and RFP+ quantification in BT474-Br cells transduced with a lentiviral plasmid containing a mcherry/F-luciferase reporter construct (BT474-Br cherry-luc+ cells). D) Top panel: Representative BLI images showing the presence of metastatic breast cancer cells in the brain of mice only 24h after injecting BT474-Br cherry-luc+ cells. Bottom panel: Representative immunostaining showing BT474-Br cherry-luc+ cells in the brain parenchyma after systemic injection in the carotid. E) Representative immunostainings of brain sections harvested from mice in figure 2D, treated with LM008-HER2Ab NSCs (n=5). Note the presence of NSCs (GFP-positive staining shown in green) surrounding tumor cells (Keratin-positive, shown in red). The amount of antiHER2Ab released by NSCs was measured with antihuman IGGs (in yellow). Nuclear staining (DAPI) is shown in blue.

SI Appendix, Fig. S6



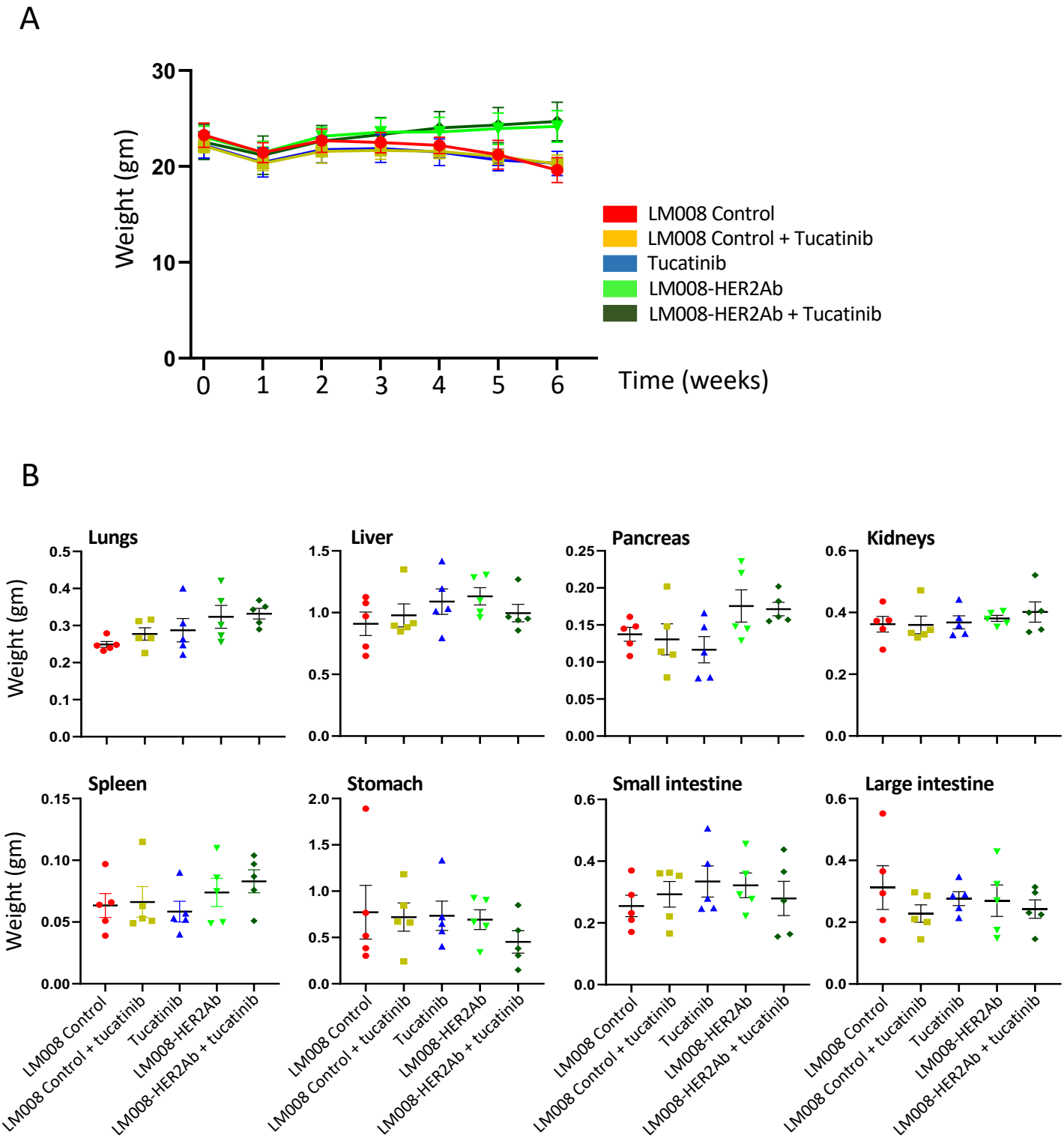
Supplementary figure 6, related to Figure 2. Expression of stem cell and neuronal markers in vivo in LM008 NSCs. A-C) Representative immunostainings of brain sections harvested from mice in figure 2D, treated with LM008-HER2Ab NSCs (n=5). LM008 NSCs (GFP-positive staining shown in green) were stained against human SOX2 (A), human Nestin (B) and human MAP2 (C). BT474-Br cells were stained for HER2 (in red). Nuclear staining (DAPI) is shown as blue.

SI Appendix, Fig. S7



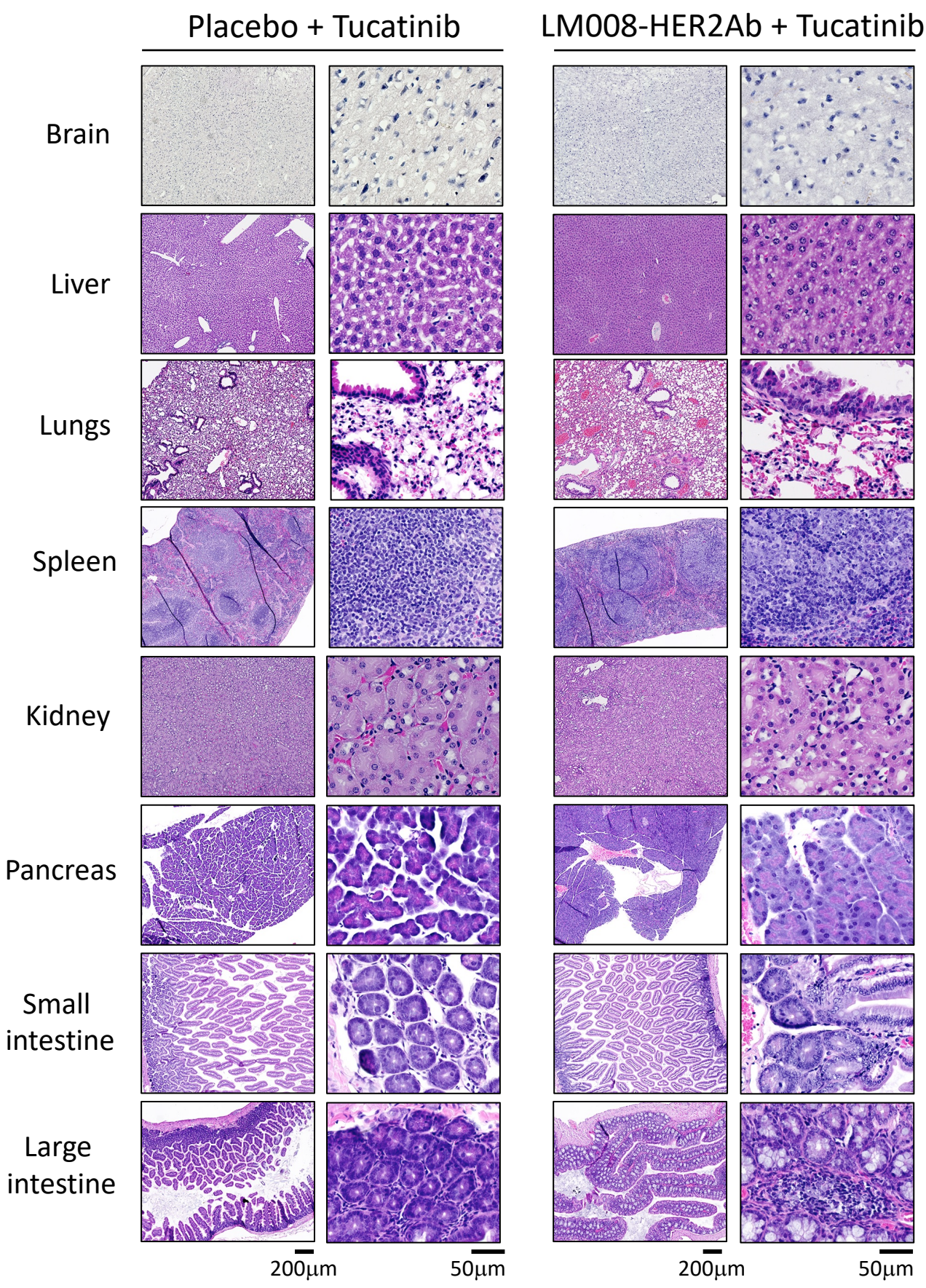
Supplementary figure 7, related to Figure 3. Combined treatment with tucatinib and LM008-HER2Ab NSCs or trastuzumab increase apoptosis and decrease proliferation and PI3K-Akt activation in HER2+ BCBM cells. A) MTT proliferation for BT474-Br treated with antiHER2Ab (10 µg/ml), tucatinib (6.2 nM) or both drugs in combination, relative to the proliferation in untreated BT474-Br controls at 96 hours. B) Cell apoptosis was measured by Caspase3/7 glo in BT474-Br cells treated with trastuzumab (10 µg/ml), tucatinib (6.2 nM) or both drugs in combination, for the indicated time points (12, 24 and 36h). C) BT474-Br cells were treated with trastuzumab (10 µg/ml), tucatinib (6.2 nM) and in combination (tucatinib + trastuzumab) during 1 and 3 hours, and equal amounts of whole-cell lysates were resolved by SDS-PAGE. Immunoblots were probed with antibodies against p-Akt (phosphorylation at S473), total Akt, p-mTOR (phosphorylation at S2448), total mTOR, p-JNK, total JNK, pERK, total ERK and GAPDH (loading control). All graphs and immunoblots are representative of three independent experiments, and bar graphs represent means ± SD. D) Analysis of protein expression relative controls for the western blots in figure 3D. Graphs are representative of three independent experiments, and bar graphs represent means ± SD. Statistical analysis was performed using one-way ANOVA with post hoc Tukey's test. **** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

SI Appendix, Fig. S8



Supplementary figure 8, related to Figure 4. Toxicological evaluation of the combined NSC and tucatinib therapeutic regimen. A) Mice from the survival analysis in figure 4D were monitored for body weight during the first 6 weeks after tumor injection, including the treatment window-period. n=8 mice per group B) The weights of the indicated peripheral organs from 5 different mice per group were analyzed, and no toxicity or side effects were observed.

SI Appendix, Fig. S9



Supplementary figure 9, related to Figure 4. Toxicological evaluation of the combined NSC and tucatinib therapeutic regimen. Representative histopathological images (H&E) from the analysis of the indicated organs harvested from mice treated with PBS + tucatinib (left panel) or LM008-HER2Ab (right panel). No toxicity or side effects were observed.

SUPPORTING VIDEO LEGENDS

Supplementary Movie S1, related to figure 1. Migration of LM008-HER2Ab NSCs towards HER2+ brain metastatic BT474-Br tumor cells. Wound healing assays were performed by plating 7.10^4 BT474-Br and LM008-HER2Ab NSCs in different chambers of the cell culture insert (ibidi, Munich, Germany; www.ibidi.de). The cell culture insert was removed after 8 hours leaving a defined cell-free gap of 500 µm. Images were taken at 10X magnification every 5 minutes during 24 hours. BT474-Br tumor cells are represented in the left, and migrating LM008-HER2Ab NSCs are represented in the right.