## Mammary gland intraductal (MIND) injection of human breast cancer cells

Eight to 12-week-old NSG females were anesthetized by intraperitoneal injection with 10 mg/kg xylazine and 90 mg/kg ketamine (Graeub).

Mice were injected into the cleaved teat with a blunt end Hamilton syringe (cat. No. HAMI80508), specifications: 50 µl 705 N, gauge 30/13 mm/pst3) with 100,000 cells for NST cell lines and 250,000 cells for patient-derived cells, and 400,000 for MDA-MB-134-VI cells.

Dafalgan was administered intraperitoneally Temgesic® (Buprenorphinum) at 100 mg/kg when needed.

Live imaging was performed from the day after injection with Xenogen IVIS Imaging System 200 (Caliper Life Sciences) upon intraperitoneal injection of 100 µl of luciferin (15 mg/ml) (Biosynth, cat# L-8220).

Eight minutes after injection, mice were anesthetized with oxygen combined with 2% isoflurane, and bioluminescence was measured from 12 min after injection.

To examine metastatic spread, mice were injected with 300 µl luciferin (15 mg/ml) (Biosynth, cat# L-8220), and tumors and organs of interest were dissected within 30 min and imaged with IVIS (Perkin Elmer).

For re-transplantation of patient-derived cells, mammary glands were collected on ice-cold 1X PBS, dissociated using parallel razor blades, and enzymatically digested using the tumor dissociation kit (Miltenyi Biotec) to generate single cells (human and mouse).

To enrich for human cells, the mouse cell depletion kit (Miltenyi Biotec) was used according to the manufacturer's instructions. Cells were counted, and $2 \times 10^5$ cells were intraductally injected.

The bioluminescence of treated mice organs was normalized to the average bioluminescence of matched control organs.