



**Supplementary Figure 3. AXL depletion in latent and metachronous brain metastatic cells.**

**A**, Immunoblotting of AXL in HCC1954 and SKBR3 AXL knockdown (AXL<sup>KD</sup>) Lat and M-BM cells. **B**, Relative quantification of oncospheres formed by SKBR3 Lat and M-BM cells upon AXL<sup>KD</sup> and Lapatinib (1 $\mu$ M) or Tucatinib (3 $\mu$ M) treatment for seven days. Data are presented as mean  $\pm$  SEM. One-way ANOVA was used, followed by the Dunnett test. \*, P < 0.05, \*\*, P < 0.01, \*\*\*, P < 0.001, \*\*\*\*, P < 0.0001. **C**, Quantification of GFP+ brain metastatic lesions in mice bearing SKBR3 AXL<sup>KD</sup> Lat cells (n = 5 per group). Mice were on doxycycline feed 3 days post injection until study termination. Data are presented as mean  $\pm$  SEM. Mann–Whitney U test, \*\*\*, P < 0.001. **D-E**, Whole body and ex-vivo brain metastasis incidence as measured by BLI signal from athymic nude mice bearing SKBR3 AXL<sup>KD</sup> M-BM cells that were intracardially injected. (n = 4 per group). Mice were on doxycycline feed 3 days post injection until study termination. Data are presented as mean  $\pm$  SEM. Mann–Whitney U test. \*, P < 0.05, \*\*, P < 0.01. Two representative BLI images of whole-body mice and ex-vivo brains of their respective conditions.