A

PA Lat S-BM M-BM

EGFR

HER2

ACTIN

HCC1954

B

■ DMSO ■ Lap ■ Tuc

Oncosphere Formed (%)

**** / ** / ns / ns / ns / ns

PA Lat M-BM

SKBR3

C

PA vs Lat

-log10 (p-value)

AXL

Log2 FC
HCC1954

D

PA vs M-BM

-log10 (p-value)

AXL

Log2 FC
HCC1954

E

Relative AXL Expression

**** / ns / ****

PA Lat S-BM M-BM

HCC1954

F

PA Lat S-BM1 S-BM2 M-BM1 M-BM2 M-BM3

HCC1954

AXL

ACTIN

G

Relative AXL Expression

**** / ****

PA Lat M-BM

SKBR3

H

PA Lat M-BM

AXL

ACTIN

SKBR3

I

Lat M-BM

Input AXL-IP IgG-IP Input AXL-IP IgG-IP

HCC1954

EGFR

HER2

AXL

J

M-BM

SKBR3

K

HCC1954

Lat Surface / Whole ****

M-BM Surface / Whole ****

Mean Fluorescence Intensity

L

Lat M-BM

CE ME NE CB CE ME NE CB

AXL

GAPDH

TFRC

LAMIN B1

HISTONE 3

SKBR3

Supplementary Figure 1. AXL enrichment in latent and metachronous brain-tropic cells.

A, Immunoblots showing EGFR and HER2 expression in HCC1954 PA, Lat, S-BM and M-BM cells. **B,** Relative quantification of oncospheres formed by SKBR3 PA, Lat and M-BM cells seven days post-treatment with Tucatinib ($3\mu M$) and Lapatinib ($1\mu M$) compared to DMSO control. Data are presented as mean ± SEM. One-way ANOVA was used, followed by the Dunnett test. **, $P < 0.01$. ****, $P < 0.0001$. ns: not significant. **C-D,** Volcano plots showing differentially expressed genes in HCC1954 Lat and M-BM cells. Normalized gene count TPM. **E,** AXL mRNA levels in HCC 1954 Lat, S-BM and M-BM cells normalized to PA. Unpaired Student t test. ****, $P < 0.001$. ns: not significant. **F,** Immunoblot showing AXL expression in HCC1954 PA, Lat and S-BM derivative and M-BM derivative cells. **G,** AXL mRNA levels in SKBR3 Lat and M-BM cells normalized to PA. Unpaired Student t test. ****, $P < 0.001$. **H,** Immunoblot showing AXL expression in SKBR3 PA, Lat and M-BM cells. **I,** Immunoblots show no interaction between immunoprecipitated AXL and EGFR or HER2 in HCC1954 Lat and M-BM cells. **J,** Representative image of AXL IHC on brain metastatic lesion in mice bearing SKBR3 M-BM cells. **K**, Total AXL expression (mean fluorescence intensity of surface expression and surface plus intracellular expression (whole)) in HCC1954 Lat and M-BM cells. **L,** Immunoblot analysis of subcellular fractions from SKBR3 Lat and M-BM cells showing AXL localization and purity of subcellular fractions. GAPDH (CE, cytoplasmic extract), TFRC (ME, membrane extract), Lamin-B1 (NE, soluble nuclear extract) and Histone-3 (CB, chromatic bound extract).