SUPPORTING EXPERIMENTAL PROCEDURES

Cell lines, inhibitors, antibodies, and siRNA:

The following inhibitors were used in Fig. 4A, at the following concentrations: metalloproteinase inhibitor BB94 (10μM; Tocris Bioscience), p38 inhibitor SB203580 (20μM; Selleck Chem), MEK1/2 inhibitor PD0325901 (10μM; LC Labs), MET/AXL inhibitor XL-880 (5μM; Selleck Chem), MEK1/2 inhibitor U0126 (10μM; Selleck Chem), MEK1/2 inhibitor AZD6244 (10μM; Selleck Chem), Aurora kinase inhibitor VX-680 (10μM; Selleck Chem), PI3K inhibitor LY294002 (10μM; Selleck Chem), JNK1/2 inhibitor JNK-IN-8 (3μM; courtesy Nathaniel Gray, Harvard Medical School), JNK inhibitor SP600125 (10μM; LC Labs), EGFR inhibitor gefitinib (1μM; LC Labs), EGFR inhibitor erlotinib (1μM; LC Labs), JNK inhibitor TCS-6o (1μM; Tocris Bioscience), Abl inhibitor imatinib (1μM; LC Labs), EGFR/HER2 inhibitor lapatinib (1μM; LC Labs), AXL inhibitor R428 (3μM; Selleck Chem), Alk5 inhibitor ALX-270-448 (5μM; Enzo life sciences), AXL/MET inhibitor MP470 (5μM; Selleck Chem), myosin light chain kinase inhibitor ML7 (1μM; Sigma), and Glycogen Synthase Kinase 3b inhibitor SB216763 (10μM; Selleck Chem). Inhibitor concentrations were chosen based roughly on exceeding previously published K_i and IC_{50} values. The ADAM10 inhibitor pro-ADAM10 was from Biozyme, Inc. and used at 4μM. GI-254023X was from Tocris Bioscience.

AXL, MET, TNFR1, and EGF-ligand supernatant measurements were performed using duo-set ELISA kits (R&D Systems). Total RTK measurements for HER2 and HER4 were performed using bead-based immunoassays (Millipore). Phospho-protein measurements were performed using bead-based immunoassays (Bio-Rad) for ERK1/2 ($pThr^{185}/pTyr^{187}$, $pThr^{202}/pTyr^{204}$), GSK3 (GSK3α/β $pSer^{21}/pSer^{9}$), JNK ($pThr^{183}/pTyr^{185}$), p38 ($pThr^{180}/pTyr^{182}$), cJun ($pSer^{63}$), STAT3 ($pTyr^{705}$), Akt ($pSer^{473}$), NFkB (p65 $pSer^{536}$), and p70S6 ($pThr^{421}/pSer^{363}$). pTyr-RTK measurements used bead-based immunocapture with a pan-pTyr detection antibody (Millipore). Unless otherwise stated, immunoassays followed manufacturer protocols. Bead-based immunoassays were routinely normalized according to the micro BCA protein assay (Pierce) or cell count (ViCell). ADAM10 western blot Abs were from Sigma. Anti-phospho Histone H3 (Ser10) for measuring mitotic index was from Millipore. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and α-actinin were used for Western blotting and purchased from Cell Signaling Technology. APP Ab was from Abcam. For WB, pAktS473 (CST #9271), pc-JunS63 (Abcam, ab32385), Tubulin (DMA1clone, Sigma). Live-cell immunostaining was performed using R&D Systems Abs. SiRNAs, including non-targeting control siRNA, were packaged as SMARTpool ON-TARGETplus and used with DharmaFECT4 (Thermo Scientific). For ADAM10 western blots, lysis was performed with 50 mM tris-HCl (pH 7.5), 10% glycerol, 150 mM NaCl, and 1% NP-40, with complete protease (Roche) and phosphatase (Boston BioProducts) inhibitors added immediately before use.

Supernatant analysis: For quantification of supernatant protein levels, cell supernatant was collected at the indicated time-point, spun for 5 min at 300g, and frozen at -80^{o}C for

future use. Supernatants were routinely normalized to cell count, determined via trypsinization and ViCell (Beckman Coulter), and volumes were kept consistent across treatments.

RNA microarray analysis: MDA-MB231 cells were plated in 10cm plates at 70% confluency, treated the following day with either DMSO control, BB94, or PD325901, and lysed 24h later. RNA was prepared using the Qiagen RNeasy Mini kit, and samples were analyzed using Affymetrix Primeview arrays. Control and inhibitor treatments had n=4 and n=3 biological replicates, respectively. Data were preprocessed using the R/Bioconductor package "affy" and the RMA normalization routine. One BB94 replicate was excluded as an outlier, as determined using the arrayQualityMetrics function. Differentially expressed genes were determined by student's t-test and as falling below a Storey false-discovery-rate of 0.05 (2). Gene set enrichment analysis was performed as previously described using gene ontology (GO) gene sets.

siRNA knockdown protocol: For all siRNA treatments, 500,000 cells were seeded in 10cm dishes, transfected using 5 uL Dharmafect4 and 125 pmol siRNA, reseeded for knockdown experiments 24h later, and 48h after transfection cells were treated and lysed.

Short term p-AXL up-regulation: MDA-MB231 cells were plated at 90% confluency on 10cm plates, serum starved for 4h the following day, treated with inhibitor in serum-free media for the indicated time, lysed, and analyzed by bead-based immunoassay.

Short term downstream phosphosignaling: MDA-MB231 cells were treated with siRNA as described above and seeded in 96-well plates at equal 90% confluency. 48h post-transfection, cells were serum starved for 4h, treated with inhibitor for 3h, then lysed and analyzed by bead-based immunoassay.

AXL measurements following siRNA and ADAM10 inhibitor treatments: Corresponding to Fig. 6, MDA-MB231 cells were treated with siRNA as described above and were seeded in 96-well plates at equal 90% confluency. Media was changed at 48h post-transfection, and 24h later supernatant was collected and cells were analyzed by live-cell immunostaining. For proADAM10 treatment, cells were seeded in 96-well plates at 90% confluency, treated with inhibitor or buffer control the following day, and supernatant was collected 24h later.

Purified AXL digestion: 15cm plates of MDA-MB231 cells were lysed in 1% NP40 lysis buffer, clarified, precleared with agarose resin, and incubated with protein A/G resin (Pierce) and 10 ug anti-AXL antibody mAb154 (R&D Systems) overnight. After repeated washing, the resin was split into separate samples for digest. ADAM-17 (R&D Systems) was incubated with resin for 4h. Supernatant was collected, and both supernatant and resin were then boiled in denaturing sample buffer. Cleavage products were blotted using an antibody targeted to the intracellular C-terminus of AXL (Santa Cruz Biotechnology). For digestion of recombinant AXL (Abnova), 0.5 ug AXL was digested with 1 ug of recombinant ADAM10 (R&D) or the combination of 1 ug ADAM10, 1 ug ADAM17, and 10 uM BB94. 4h incubations were carried out in 30 ul

buffer containing 25 mM Tris, 2.5 uM ZnCl2, 0.005% Brij-35 (w/v), pH 9.0, at 37^{o}C. Cleavage products were blotted using an antibody targeted to the intracellular C-terminus of AXL (Santa Cruz Biotechnology) and the N-terminus (R&D Systems).

Cell cycle analysis: Cells were trypsinized, rinsed in 4^{o}C PBS, fixed in 70% 4^{o}C ethanol overnight, rinsed, and permeabilized in 0.1% Triton-X-100. Cells were then blocked for 1h in PBS + 3% FBS, incubated with anti-phospho-Histone H3 (pSer^{10}) for 1h, rinsed, incubated with Alexa647-conjugated 2^{o}Ab, and again rinsed. Lastly, cells were finally incubated at 37^{o}C for 1h with 40 ug/ml propidium iodide and 100 ug/ml RNaseA, and again rinsed. Mitotic index was calculated by interpreting DNA-content histograms as described previously (3) in Matlab and gating for G2/M phase cells with high p-Histone-H3 staining.

Proliferation/cytotoxicity and synergy calculation: Throughout this report, proliferation and cytotoxicity were assessed by gently rinsing cells, trypsinizing for 15 min, and immediately counting cells by flow-cytometry. Live/dead staining routinely confirmed the majority (>98%) of counted cells to be alive using this procedure. Synergy across multiple cell lines (Fig. 6A) was calculated using the model of Bliss independence (4), reported here as the ratio of (observed combination effect size) / (predicted combination effect size), such that values >1 indicate super-additive drug effect, or synergy, and values <1 indicate antagonism. Drug concentrations for synergy measurements were chosen to roughly approximate the IC_{30} value averaged across the panel of cell lines for the individual drug treatments, and were 20μM U0126, 7μM PD325901, and 200nM R428.

Loewe synergy calculation: To calculate Loewe synergy, a computational model was used (5) and the synergy term α was computationally inferred using Matlab (Mathworks; Natick, MA). Statistical significance was determined by leave-one-out jackknife error estimation (Efron and Gong, 1983). For synergy calculations, the modeling was fit to data shown in Supplemental Fig. 3S and Fig. 6C. For cell growth assays, 5000 cells were plated per well in a 96-well plate, treated with drugs the following day, and counted at 72h according to either a rezasurin assay (PrestoBlue; Life Tech.) using manufacturer's guidelines, or by flow cytometry following trypsinization and immediate analysis on an LSR-II (BD Biosciences).

Clinical breast cancer serum sample analysis: Serum samples from healthy control individuals, stage I, and stage IV breast cancer patients were purchased from Bioreclamation (Long Island, NY). Samples were analyzed using bead-based immunoassay (for MET, HER2, HER4, and AXL) and ELISA (for AXL). AXL measurements were averaged between the two assay formats. For each analyte, measurement values were divided by the averaged FLU measurement across all control samples. Statistical significance was calculated by Kruskal-Wallis, and two-tailed Student's t-test for all double-, triple-, and quadruple-positive calculations.

Autophagy, apoptosis, and cell death measurements: MDA-MB231 were plated at 90% confluency in 10cm plates O/N; treated the following day with BB94 or PD325901

for 24h; and immediately analyzed by flow-cytometry for autophagy using the CYTO-ID Autophagy Detection Kit (Enzo) or the Membrane Permeability / Dead Cell Apoptosis Kit with YO-PRO-1 and PI for Flow Cytometry (Life Technology), both used according to the manufacturer guidelines.

Live-cell immunostaining: Cells were trypsinized, rinsed in 4^{o}C phospho-buffered saline (PBS) + 3% FBS, incubated with 1^{o}Ab in PBS+3% FBS for 1h, rinsed, and fixed overnight in PBS + 1% formaldehyde at 4^{o}C. The following day, cells were rinsed, incubated with 2^{o}Ab conjugated to either Alexa647 or Alexa546, rinsed, and analyzed by flow cytometry. Fold-change was determined after subtracting the median fluorescence from control cells stained with the IgG control antibody.

Clinical Response. RECIST criteria were used to classify response, and are defined as follows: Complete Response (CR): Disappearance of all target lesions. Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD. Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since initiation of treatment. Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions. Progression free survival (PFS) was defined as the time from commencement of BRAF/MEK inhibitor therapy until disease progression, as per RECIST, or death.

Clinical gene expression correlation. RNA microarray data from matched samples in 21 melanoma patients (Rizos et al., 2015; accession number GSE50509) were analyzed for expression of *AXL*, *MERTK, TYRO3, HER2, HER4,* and *MET*. For RTKs with negative values reported in any sample, data were log-transformed after subtracting the minimum value and adding 1 to correct for negative values, and values were then averaged across all RTKs. The log-difference was calculated between pre- and on-treatment samples. Non-log transformed data showed similar results. Data were stratified according to the median value.

RTK cross-linking: MDA-MB231 cells were plated at 90% confluency on three 10-cm plates for each condition, serum starved for 4h the following day, and treated with inhibitor in serum-free media for 2h. Cells were then cross-linked with 1 mM EGS for 30 min at 4 ^{o}C, lysed, and the receptor cross-linking was measured using a modified MET and HER2 Luminex Bioplex assay, as previously described (6).

qPCR: RNA was prepared as with the RNA microarray experiment, and AXL gene expression was analyzed by qPCR following manufacturer guidelines (PrimePCR™ SYBR® Green Assay: AXL, Human; probe assay qHsaCIP0026819), normalized to GAPDH and quantitated using the ddCt method.

shRNA: Stable knockdown of *ADAM10* and *ADAM17* was performed using MISSION shRNA constructs (Sigma). *ADAM17*-targeted constructs include the following:

TRCN0000294262 (sh1;
CCGGGAAGGTGAATCTAGCTTATTTCTCGAGAAATAAGCTAGATTCACCTTCT
TTTTG), *TRCN0000052168 (sh2;*
CCGGCCAGCAGCATTCGGTAAGAAACTCGAGTTTCTTACCGAATGCTGCTGG
TTTTTG),
TRCN0000052169 (sh3;
CCGGGCAGCGTCAGAATCGTGTTAACTCGAGTTAACACGATTCTGACGCTGC
TTTTTG), TRCN0000052171 *(sh4;*
CCGGCCTGGTTACAACTCATGAATTCTCGAGAATTCATGAGTTGTAACCAGGT
TTTTG), and *TRCN0000052172 (sh5;*
CCGGCCTATGTCGATGCTGAACAAACTCGAGTTTGTTCAGCATCGACATAGGT
TTTTG).
ADAM10-targeted constructs include *TRCN0000006674 (sh6;*
CCGGCCCTACAAATCCTTTCCGTTTCTCGAGAAACGGAAAGGATTTGTAGGGT
TTTT), *TRCN0000006673 (sh7;*
CCGGGCAGGTTCTATCTGTGAGAAACTCGAGTTTCTCACAGATAGAACCTGCT
TTTT), *TRCN0000418144 (sh8;*
CCGGGTTCATACTCCAAGTAGTAATCTCGAGATTACTACTTGGAGTATGAACT
TTTTTG), T*RCN0000006675 (sh9;*
CCGGGCTGTGCAGATCATTCAGTATCTCGAGATACTGAATGATCTGCACAGCT
TTTT), and *TRCN0000419446 (sh10;*
CCGGTTGAATCTGGCCAACCTATTTCTCGAGAAATAGGTTGGCCAGATTCAAT
TTTTTG).
LOX-IMVI cells were infected according to manufacturer's guidelines using HEK293 lentiviral packaging cells, and selected for using 1 ug/ml puromycin treatment. As a control, non-mammalian targeted shRNA was used (*SHC002*;
CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTGT
TTTT).

TIMP1 cell surface binding: MDA-MB231 cells were plated overnight at 90% confluency, and the following day MEKi or DMSO stock was added directly to the cell culture and incubated for the indicated time. Cells were then rinsed with PBS, trypsinized, and analyzed by live-cell flow cytometry for TIMP1 staining as described above. For rTIMP1-fluor experiments, recombinant TIMP1 (Peprotech) was labeled with NHS-VT680XL (Perkin Elmer) at an 8:1 dye:protein molar ratio following manufacturer's guidelines. Remaining free dye was quenched by TBS and removed by repeated (5x) washing using 3kDa molecular weight cutoff filters (Amicon; Millipore). After overnight plating at 90% confluency, cells were rinsed, treated with DMSO or U0126 for 5 min at 37^{o}C, rinsed in cold PBS, and incubated at 4^{o}C with 10nM rTIMP1-fluor for 45 min in PBS + 3% FBS. Cells were then rinsed, trypsinized, and immediately analyzed for VT680XL fluorescence by flow cytometry using an LSR-II (BD).

ADAM10-HA IP: MDA-MB231 cells were transfected with pLenti-GIII-CMV-C-term-HA (ABM Inc.) following manufacturer's guidelines using Lipofectamine LTX

(Invitrogen), and the following day were assessed by live-cell flow cytometry for ADAM10 surface levels (clone 139712; R&D Systems). After changing media at 24h, cells were treated with U0126 for 5 min, rinsed with cold PBS, and incubated with 10nM rTIMP1-fluor for 45 min in PBS + 3% FBS on ice with gentle agitation. Cells were again rinsed with cold PBS, and incubated for 45 min with 10mM EGS in PBS on ice with gentle agitation. Cells were rinsed, lysed, and IP was performed all according to manufacturer's guidelines using an anti-HA immunopurification kit (Sigma). After washing the anti-HA agarose resin, bulk re-suspended resin was analyzed on a fluorescence plate reader (Tecan Safire 2) to measure co-immunoprecipitated fluorescent rTIMP1 (λ_{ex}/ λ_{em} = 660nm / 690nm). Eluted immunoprecipitate was analyzed by western blot for ADAM10 (clone A2851**;** Sigma) and TIMP1 (clone 63515; R&D systems)**,** detected by chemiluminescence.

Multivariate correlation analysis: For correlation analysis following BB94 treatment, 13 cell lines were plated at 70% confluency, treated with following day with BB94. 24h later cells were counted and analyzed by live-cell immunostaining or bead-based immunoassay. Pairwise correlation was calculated between fold-change measurements as they varied across the 13 cell lines. Both Spearman and Pearson correlations were determined from mean-centered and variance-normalized data, with the more significant of the two correlations reported.

Exosome analysis: Patient plasma samples were filtered and rinsed 3x using centrifugal size-exclusion columns (MWCO 100kDa; Millipore Amicon Ultra). Retained sample was analyzed by nanoparticle tracking analysis using Nanosight LM10 (Malvern Instruments, Malvern, UK) and analyzed for protein levels using the nPLEX nano-plasmonic sensor as described previously (7). For analysis of cell-culture microvesicles, 90% confluent cells were grown in the presence or absence of inhibitor for 48h in DMEM supplemented with 10% microvesicle-free FBS (System Biosciences, Mountain View, CA). Microvesicles were then purified by differential ultra-centrifugation as previously described (8). Western blot loading was controlled for by protein content as measured by the microBCA assay (Pierce); nanoparticle counting as determined by Nanosight LM10 yielded similar results. For nPLEX analysis, well-behaved spectra from an initial 5 technical replicates were analyzed for each protein, with exclusion of outliers by Dixonra Q-test 95%.

***In vitro* TIMP1 neutralization:** 5000 cells per well were plated in 96-well plates overnight and treated with 10ug/ml TIMP1 neutralization antibody (T1-NAB; ABD-Serotec / Bio-Rad) or IgG control for 24h. The following day PD325901 was added to a final concentration of either 0.5uM (MDA-MB-231) or 5uM (LOX-IMVI), or vemurafenib was added at 10uM. 72h after PD325901/vemurafenib treatment, cell count was assessed using the PrestoBlue assay (Life Technologies) according to manufacturer's guidelines.

Antibody microarray analysis: MDA-MB231 cells were plated in 10cm plates at 70% confluency, treated the following day with either DMSO or PD325901, and supernatant

was collected 24h later. Using L-1000 RayBio microarrays, supernatant was processed according to manufacturer instructions. Two biological replicate samples were analyzed for the control and MEKi conditions, with one sample undiluted and the other diluted 1:5 in growth media. Each array contains duplicate antibody spots, and so a total of n=4 replicates was used to determine significant changes in supernatant proteins. Only proteins measured more than four standard deviations above background for at least one condition were included in the analysis. Gene set enrichment analysis was performed as previously described using gene ontology (GO) gene sets (9). The transmembrane receptor activity gene set, comprising 13 proteins, exhibited the highest enrichment score for control-treated cells, with a corresponding p-value of 0.06. The other top gene-sets were "intrinsic to membrane", "integral to membrane", "receptor activity", and "intrinsic to plasma membrane". The highest enrichment score in PD325901-treated cells was for "hematopoietin interferon class-D200 domain cytokine receptor binding" comprising 10 non-transmembrane proteins, with a corresponding p-value of 0.003.

Immunohistochemistry. Tumor biopsies were stained with primary antibody to AXL (Cell Signaling 8661S) followed by a secondary antibody for horseradish peroxidase and then 3,3' –diaminobenzidine (DAB), as previously described (10). To correlate with PFS, tumor sections were blindly ranked according to staining intensity and then correlated with PFS by Spearman's correlation.

ADAM activity assays: Live-cell ADAM10 catalytic activities were measured using proteolytic activity matrix analysis (PrAMA; (11)). MDA-MB231 cells were seeded in serum-free media at 5000 cells per well of a 384-well plate, and the following day cells were simultaneously treated with either 10μM U0126, 10μM AZD6244, or 0.1% DMSO, along with one of six different FRET protease substrates (PEPDAB-05, 08, 10, 11, 14, 22; Biozyme, Inc.) at 5μM concentration. Substrate cleavage was monitored over 3h, and average cleavage rates were deconvolved into specific protease activities using Matlab (Mathworks, Natick MA), as previously described (11).

Melanoma patient sample RTK analysis: Frozen aliquots of plasma were analyzed by bead-based immunoassays using duo-set antibodies from R&D Systems for the TAM receptors. Simultaneously, other RTKs were measured using multiplexed bead-based immunoassays from Millipore (RTK Mitogenesis Kit). Measurements were normalized to total protein content, measured by micro-BCA assay (Pierce). Average bead fluorescence from Luminex Flexmap 3D was divided by protein content; divided by mean RTK levels across the entire cohort; and log-transformed. For average measurements, change in log-transformed RTK levels was first calculated for each RTK and then averaged together by the mean. Kaplan-Meier survival analysis was calculated using Matlab, according to the RTK measurement threshold that yielded the most statistically significant result.

Supplemental animal experimental details: Tumor volumes was measured two to three times a week, estimated using the spherical tumor volume formula $V = {}^{4}/_{3}\,\pi r^{3}$, where r is averaged from 4 caliper measurements performed by two blinded researchers. Upon sacrifice, the lungs were inflated with and fixed in 3.7% (wt/vol) formaldehyde for 24 h,

followed by 24 h in 75% (vol/vol) ethanol. For micro-metastasis quantification, the numbers of metastases containing at most 10 cells were counted in paraffin-embedded, H&E-stained sections, by a blinded researcher. Upon sacrifice, blood was collected via cardiac puncture into a heparinized syringe, immediately centrifuged at 2000xg for 20min, and plasma fraction was stored at -80^{o}C for later quantification of receptor levels by ELISA and bead-based immunoassay; plasma RTK measurements were measured from blood taken at end of inhibitor treatment course for both xenograft models. For immunostaining primary tumor sections, tumors were formalin-fixed overnight and paraffin embedded. Tissue sections (5μm thick) were deparaffinized followed by antigen retrieval using Citra Plus solution (Biogenex). Sections were incubated with primary antibodies for AXL (R&D systems, MAB154, 1:50) and MET (R&D systems, AF276, 1:20) overnight at 4°C and fluorescently labeled secondary antibodies (AlexaFluor 594 and AlexaFluor 647, Jackson Immunoresearch)tat room temperature for 2h. Sections were mounted in Fluoromount mounting media and imaged at room temperature. Images (5x5 fields) were captured with a Nikon TE2000 microscope (TE2000, Nikon) with a 20X objective and a Photometrics Coolsnap HQ camera. AXL and MET staining was performed simultaneously across all samples, and imaging was performed in a single session using identical exposure settings. Exposure adjustments were made for DAPI staining shown in Fig. 6C.

MET and AXL levels were quantified by measuring fluorescent staining intensity over a line drawn radially from the tumor edge towards the tumor core. We ignored the very immediate edge of the tumor (0-30um), which generally appeared to contain compacted tissue and/or highly auto-fluorescent adipose tissue with distinct morphology. We therefore defined the beginning of the "tumor edge" here as the tumor region exhibiting regularly spaced nuclei determined through DAPI counter-staining (generally the 2nd or 3rd observable nucleus observable along the radial line by DAPI counter-stain intensity). Regions with morphology characteristic of stroma or necrosis were generally avoided during quantification. Peak intensities along the line were confirmed by visual inspection as corresponding to cell membranes, and were quantified and indexed according to the distance from the tumor edge. This process was repeated for $n \geq 4$ lines per tumor by a blinded researcher, and data were averaged accordingly. This was repeated across $n \geq 3$ separate tumors per treatment group, and average results for each tumor were background-corrected according to control tumor sections that were stained without primary antibody. Average results for each tumor then were used to calculate statistics between treatment groups, using the two-way Student's t-test.

We used exclusion criteria for the mice that were pre-determined at the onset of the experiment. Mice were excluded that failed to ever develop a tumor (n=4/37 for the TNBC model, n=1/60 for the melanoma model confirmed by dissection). All other mice had tumors on dissection. Among mice with successful tumor implantation, no significant difference was observed in pre-drug tumor volume across the four groups. One mouse asphyxiated during gavage treatment, all others survived for the duration of the experiment. Among mice with successful tumor implantation, two were excluded that exhibited a pre-drug tumor volume falling >2 standard deviations from the mean, across

all groups (n=2/33 tumor-developing mice). Final sample sizes for the treatment groups, after exclusions described above, were n=7 controls, n=7 AXLi, and n=8 for the other groups.

SUPPORTING EXPERIMENTAL REFERENCES

1. Minn AJ, Gupta GP, Siegel PM et al. Genes that mediate breast cancer metastasis to lung. Nature. 2005;436:518-24.
2. Storey JD. A direct approach to false discovery rates. J Royal Statistical Society: Series B (Statistical Methodology). 2002;64:479-98.
3. Watson JV, Chambers SH, Smith PJ. A pragmatic approach to the analysis of DNA histograms with a definable G1 peak. Cytometry. 1987;8:1-8.
4. Bliss CI. The toxicity of poisons applied jointly. Annals Appl Biol. 1939;26:585-615.
5. Greco WR, Bravo G, Parsons JC. The search for synergy: a critical review from a response surface perspective. Pharmacol Rev. 1995;47:331-85.
6. Meyer AS, Miller MA, Gertler FB, Lauffenburger DA. The receptor AXL diversifies EGFR signaling and limits the response to EGFR-targeted inhibitors in triple-negative breast cancer cells. Sci Signal. 2013;6:ra66.
7. Im H, Shao H, Park YI et al. Label-free detection and molecular profiling of exosomes with a nano-plasmonic sensor. Nat Biotechnol. 2014;32:490-5.
8. Théry C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Curr Protoc Cell Biol. 2006;Chapter 3:Unit 3.22.
9. Subramanian A, Tamayo P, Mootha VK et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A. 2005;102:15545-50.
10. Konieczkowski DJ, Johannessen CM, Abudayyeh O et al. A melanoma cell state distinction influences sensitivity to MAPK pathway inhibitors. Cancer Discov. 2014;4:816-27.
11. Miller MA, Barkal L, Jeng K et al. Proteolytic Activity Matrix Analysis (PrAMA) for simultaneous determination of multiple protease activities. Integr Biol (Camb). 2011;3:422-38.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. A) U0126 treatment reduces p-Erk1/2 while increasing p-cJUN in a panel of cell lines, measured by bead-based immunoassay. **B)** Most MET and AXL, but not EGFR, is not microvesicle-anchored in cell supernatant. Using bead-based immunoassay, RTKs were measured before and after removing microvesicles by differential ultra-centrifugation (*$p<0.05$; two-tailed t-test, $n\geq2$, mean+/- SEM). **C)** MPi and MEKi significantly reduce supernatant accumulation of AXL but not EGFR in LOX-IMVI cells (*$p<0.05$; two-tailed t-test, n=4). **D)** Breast cancer patients exhibit increased circulating RTKs. Individual RTK levels were normalized to the average value across n=20 control patient samples. **E)** While individual RTK levels did not significantly change with disease, the combined minimum measured across all four RTKs for a given patient was significantly increased with disease (p=0.02; Kruskal-Wallis test; plot shows median +/- interquartile range). **F)** Combined, but not individual, circulating RTK levels are increased in stage IV breast cancer patients in a statistically significant manner. The combined minimum levels of the RTKs indicated at left were determined for each sample, and corresponding p-values were calculated to compare healthy vs. stage IV disease (n=19) groups (two-tailed t-test). The MET/AXL/HER4/HER2 signature at bottom corresponds to the combination signature reported in *E*. **G)** Plasma from melanoma patients assayed for soluble RTK levels before and during treatment with dual BRAFi/MEKi therapy using bead-based immunoassay. Data corresponds to Fig. 1E-H and Fig. 2A. **H)** Melanoma tumors from a subset of patients in the above cohort (*G*) were assessed using immunohistochemistry for AXL C-terminus. Blinded tumors were ranked according to staining intensity and correlated with PFS. This procedure was separately performed for biopsies from both pre- and on-treatment patients, and in both cases no correlation was observed between IHC and PFS (Spearman's rank correlation p-value). **I-J**) Melanoma tumors [Rizos et al., 2014, Clin Cancer Res, 20, 1965-77] were stratified into groups of n=10 and n=11 based on gene expression of six RTKs analyzed in *G* before treatment with BRAFi (*I*). Gene expression was also measured after treatment began, and changes in RTK expression between matched pre- and on-treatment tumors were then used to re-stratify patients (*J*).

Supplemental Figure 2. A) Correlation between plasma RTK changes with MAPKi, PFS, and initial tumor shrinkage (using RECIST) in melanoma patients. Note RECIST poorly correlates with RTK and PFS; patient #9 and #4 exhibit similar RECIST response but distinct RTK levels and PFS. **B)** Immunofluorescence quantification of AXL cytoplasmic domain (C-term) and ectodomain (N-term) from tumor biopsies from patients in S2A, replicated from Fig. 2C but with shaded bars included to denote min and max single-cell staining intensities from each patient to depict intratumoral heterogeneity. **C)** Characterization of melanoma patient exosome size using nanoparticle tracking analysis (Nanosight), corresponding to Fig. 2E. Thick line and shading denote mean +/- SEM ($n\geq2$). **D)** PD325901 treatment does not impact quantity or size of exosomes from MDA-MB231 cells, collected from supernatant after 48h treatment and assessed using nanoparticle tracking analysis (Nanosight; mean +/- SEM, n=3). **E)** Timecourse of surface AXL (live-cell immunostaining) and p-AXL (bead-based immunoassay)

following U0126 treatment in MDA-MB231. **F-G)** MEKi does not reduce gene expression of AXL, measured by RNA microarray (*F*; n≥2) and qPCR (*G;* n=3; geometric mean +/- 95% C.I.). **H)** Representative western blots of lysates obtained from MDA-MB231 cells treated with increasing concentrations of U0126 for 24h, probed with the ectodomain (N-term) and intracellular (C-term) domains of AXL. **I)** Quantification of cell count, pERK1/2, and RTK levels in MDA-MB231 cells treated with U0126 (n≥3).

Supplemental Figure 3. A-C) Corresponds to Fig. 3C, showing U0126 and PD325901 elicit AXL upregulation to a degree that correlates with AXLi/MEKi synergy across a panel of cell lines. **A)** Cell count was measured at 72h following treatment with R428, U0126, PD325901, or a combination thereof. Columns correspond to cell lines listed at bottom. **B)** AXLi/MEKi synergy was determined according to a model of bliss independence, defined here such that values >1 denote synergistic interaction. Cell lines are ordered according to average synergy for the two drug combinations. **C)** Surface AXL was measured by live-cell immunostaining 24h following treatment with either U0126 or PD325901. **D)** BRAF and RAS mutational status of cell lines. **E)** Anti-EGFR antibody treatment (mAb225, non-humanized cetuximab) does not substantially impact proliferation over 72h treatment, measured by a resazurin-based assay (mean +/- SEM, n=3). **F)** MEKi and BRAFi synergistically combine with AXLi, measured by cell count/cytotoxicity using a resazurin assay 72h post-treatment in LOX-IMVI cells. Both treatment combinations were synergistic ($\alpha>0$; $p<0.05$, two-tailed jackknife test; n=16 measurements over n=2 reps). Normalized cell counts are shown by heatmap color and number. Data were fit to a model of Loewe synergy, yielding the synergy interaction term α.

Supplemental Figure 4. A) Corresponding to Ab-microarray results (Fig. 4A), the enrichment score trace for the bottom-ranked gene-set in the GSEA shows an increase in secreted (not shed) cytokines with MEKi; vertical bars below trace indicate location of proteins in the bottom gene-set. **B)** Surface receptor levels measured by live-cell immunostaining and FACS following 3h treatment with various inhibitors (listed as target followed by compound name) in MDA-MB231. **C)** Surface AXL (measured by live-cell immunostaining) and p-AXL (measured by bead-based immunoassay) were measured at various times after MPi using BB94. **D)** Corresponding to Fig. 4C, top two boxes show volcano plots that display statistical significance as a function of fold-change in RNA expression after 24h treatment with BB94 or PD325901 in MDA-MB231. Each dot represents a gene, measured by RNA microarray. The bottom plot shows correlation ($p=3 \cdot 10^{-6}$; two-tailed permutation test) between fold-change in RNA expression following either BB94 or PD325901 treatment, corresponding to microarray data in the top two plots. Statistically significant DEGs are denoted by scatter dots, and insignificant DEGs are represented by colored contour lines to show spatial density. **E)** Statistically significant gene set enrichment scores for gene sets depleted in PD325901-treated cells compared to the control sample, using data in *D*. **F)** Fraction relative to control of MET and AXL surface levels following treatment with either BB94, U0126, or PD325901, measured in 12 cell lines (live-cell immunostaining). Each dot describes changes in AXL or MET in an individual cell line following treatment with BB94 and either U0126 or PD325901. Significant correlation exists between surface receptor upregulation

following either MPi or MEKi (p=0.01, two-tailed permutation test). **G)** Normalized changes in protein surface levels relative to altered RNA expression following 24h inhibitor treatment. Left: Surface protein levels measured by FACS in MDA-MB231 following 24h treatment with either BB94 or PD325901. Middle left: Processed expression values from RNA microarray, using data in *D*. Middle right: Normalized RNA expression values, corresponding to plot at middle left. Right: Surface protein fold-change normalized to RNA fold-change following inhibitor treatment, sorted according to average value across both inhibitor treatments. **H-I)** p-Erk quantification from western blots shown in Fig. 4E-F. **J)** Autophagy, dead cells, and apoptotic cells were quantified by FACS after 24h inhibitor treatment using MDA-MB231 ($n \geq 3$). **K-L)** BB94 reduces supernatant RTK ectodomain levels (*K*), and increases levels of total and phosphorylated RTK in lysate (*L*) ($n \geq 2$), corresponding to Fig. 4I.

Supplemental Figure 5. A) AXL association with MET and HER2 increases following 2h MEKi or MPi treatment in MDA-MB231, determined by s-EGS crosslinking and a bead-based co-I.P. (bars denote pooled t-test, $p<0.05$, $n=3$). **B)** Heatmap displays relative changes in total and phosphorylated levels of RTKs and downstream proteins (measured by bead-based immunoassay), along with total surface levels of AXL and MET (measured by live-cell immunostaining) following 24h treatment with BB94, across a panel of TNBC cell lines. na, not assessed. **C)** From data in *B*, the heat-map shows hierarchical bi-clustering of the spearman correlations between protein drug responses, across the panel of cell lines. Co-clustering with surface-AXL is highlighted in green. **D)** BB94 increases phosphosignaling in an AXL-dependent manner, measured by bead-based immunoassay in MDA-MB231 after 2h treatment with AXL inhibitor R428 ($n=8$). **E)** Validation of siRNA knockdown for AXL and MET using live-cell immunostaining in MDA-MB231 and MDA-MB157 (*$p<0.05$; $n \geq 2$ reps / cond). **F-G)** Synergy between MEKi and JNKi is metalloproteinase dependent. Cells were treated with combinations of MEKi and JNKi for 72h, in the presence or absence of BB94. Normalized cell counts are shown by heatmap color and number (*F*). Data were fit to a model of Loewe synergy, yielding the synergy interaction term α (*G*; *$p<0.05$, two-tailed jackknife test, $n=20$ measurements over $n=2$ reps).

Supplemental Figure 6. A) Validation of ADAM10 and ADAM17 siRNA knockdown in MDA-MB157 and MDA-MB231, assessed by live-cell immunostaining and confirmed at right by western blot in MDA-MB231 (*$p<0.05$). **B)** Live-cell immunostaining shows ADAM10 and ADAM17 siRNA increase surface AXL in MDA-MB157, 72h post-transfection ($n=2$; data are mean +/- SEM). **C-D)** Live-cell immunostaining shows ADAM10, ADAM17, and AXL levels in LOX-IMVI that stably express one of 11 unique shRNAs, pooled across cell lines showing >10% on-target knockdown in *D* (*$p<0.01$; two-tailed t-test; $n=3$). **E)** Supernatant AXL accumulation in cell lines corresponding to *C-D* (*$p<0.05$; two-tailed t-test; $n=4$). **F)** Full-length AXL was immunoprecipitated from cell lysate and incubated with recombinant protease for 4h. Reaction resin was boiled in denaturing sample buffer, run on an SDS-PAGE gel, and blotted for AXL. Bands show full-length (100-150 kDa) and cleaved C-terminal (55 kDa) AXL. **G)** Quantification of *F*, across $n=2$ replicate experiments. **H)** Stable expression of ADAM10-targeting shRNA does not substantially impact growth compared

to parental cells and non-targeting shRNA cells in LOX-IMVI xenograft tumors ($p>0.2$; $n=19$; pooled two-tailed t-test).

Supplemental Figure 7. A) Live-cell immunostaining shows relatively minor changes in ADAM10 and ADAM17 surface levels following MEKi. Measurements were taken 1, 2, and 3h following inhibitor treatment and averaged (*$p=0.02$, $n=3$). **B-C)** Phospho-ADAM17 decreases following 3h MEKi ($p=0.03$, pooled t-test; $n=2$ reps / cond; *C* shows replicate western blots). **D)** Live-cell immunostaining of TIMP-1 and TIMP-3 in MDA-MB231 validates siRNA knockdown (*$p<0.05$; $n\geq2$ reps / cond). **E)** Live-cell immunostaining shows MEKi increases surface TIMP1 in MDA-MB231 and MDA-MB157 (bar denotes $p<0.05$; pooled t-test; $n\geq2$). **F)** MEKi (using PD325901) decreases supernatant TIMP1 in MDA-MB231, measured by Ab-microarray (*$p<0.05$; $n=4$). **G)** Live-cell immunostaining shows surface TIMP1 association depends on ADAM10, measured 72h after siRNA transfection in MDA-MB231 (*$p<0.05$; $n\geq2$). **H)** 24h post-transfection with ADAM10-HA leads to 2-fold increase in cell surface ADAM10, measured by FACS in MDA-MB231 (*$p<0.05$; $n=3$). **I)** 24h pre-treatment with a TIMP1 neutralization antibody (T1-NAB) followed by co-treatment with PD325901 or vemurafenib led to enhanced reduction in cell count at 72h; corresponds to Fig. 7I. **J)** T1-NAB and BRAFi/MEKi treatment groups show no difference in tumor AXL after tumor recurrence, roughly 3 weeks after drug treatment ended in the LOX-IMVI melanoma xenograft model, shown by immunofluorescence quantification and corresponding to Fig. 7K ($n>4$).