Supplemental Figure 1. The knock-down efficiency of ADAM10, TACE and BACE siRNAs in Panc-28 (A and B) and HEK293T cells (C and D). The mRNA levels for ADAM10, TACE and BACE in Panc-28 cells (A) and HEK293T cells (C) were estimated by qRT-PCR as described in the MATERIALS and METHODS section. Data are expressed as percentage of the control siRNA (siCTR). Means ± s.e.m (n=3). *** P < 0.001, One way ANOVA with Bonferroni’s multiple comparisons test. The protein levels for ADAM10 or TACE in Panc-28 cells (B) and HEK293T cells (D) were analyzed by Western blotting using ab1997 (Abcam) and 6978S antibodies (Cell Signaling Technologies), respectively. The experiment was performed twice and a representative blot is shown. The same protein samples were used for analyzing the levels of AXL-FL and AXL-CTF (Fig. 3D and 3E). GAPDH served as protein loading control.

Supplemental Figure 2. Single or double mutations in the AXL ectodomain do not affect its proteolytic processing mediated by α- and γ-secretases. HEK293T cells were transiently transfected with plasmids carrying AXL with the L437A, L438A or L437A/L438A mutations. After 16 h of treatment with DAPT, the whole cell lysates were probed for AXL-CTF by Western blot (C-20). The experiment was performed twice and a representative blot is shown. β-actin was used as the protein loading control.
Supplemental Figure 3. Putative γ-secretase cleavage sites in AXL. AXL processing by γ-secretase is not affected by a substitution of the C-terminal sequence in the AXL transmembrane domain (TM) with InsR (Mut4; A), or by partial mutations of the mut1 region (mut1a, 1b or 1c; B), or by the indicated single point mutations (C). HEK293T cells were transiently transfected with the plasmids carrying the indicated AXL mutants and analyzed as described in Fig. 4E. D) The transmembrane domains of mouse and rat AXLs can be cleaved by γ-secretase. HEK293T cells transiently expressing human AXL harboring the mouse or rat transmembrane domains (Fig. 4C) were tested for cleavage by γ-secretase. E) MERTK and TYRO3 are poorly or not at all processed by γ-secretase. HEK293T cells overexpressing MERTK or TYRO3 were treated with DAPT (10 μM) for 16 h and analyzed for proteolytic processing mediated by α- or γ-secretases. AXL was probed with anti-Flag antibody and a representative blot from at least two independent experiments is shown. The levels of β-actin or GAPDH were used as protein loading controls.
Supplemental Figure 4. Potency of erlotinib in NSCLC cells with high expression levels of endogenous AXL-FL. A549 or H1299 cells (high-expression of AXL, Figs. 1 and 3) were incubated for 3 days with the indicated concentrations of erlotinib, and the cell viability was measured as described in MATERIALS AND METHODS. All data are expressed as the percentage of control (DMSO-treated cells) and shown as means ± s.e.m (n=3).

Supplemental Figure 5. The reduced levels of AXL full-length caused by the inhibition of the proteasome can be rescued with an α-secretase inhibitor. Panc-28 cells were treated overnight with 10 μM DAPT, 200 nM Epo, 100 ng/ml PMA, 20 μM TAPI-1 or the indicated combinations. Cell lysates were probed for AXL-FL, -CTF or -ICD by Western blot using the AXL(C-20) antibody. β-actin was used as the protein loading control. All experiments were performed at least twice and a representative blot is shown.

Supplemental Figure 6. The AXL-ICD suppresses NF-κB-dependent gene transcription. HEK293T cells were transfected with the NF-κB firefly luciferase reporter plasmid in presence of AXL-ICD or the empty vector for 48 h. The pGMLR-TK Renilla luciferase reporter was also co-transfected into the same cells as an internal standard for transfection efficiency. Firefly and Renilla luciferase activities in each sample were sequentially recorded and the relative luciferase activity was normalized by dividing the firefly luciferase activity values with the Renilla luciferase activity values. The data were further expressed as a percentage of control (cells transfected with the empty vector and the two luciferase reporter plasmids; 100%). Means ± s.e.m (n=3). The experiment is independently performed three times and one representative result is shown, ***, P<0.001, unpaired Student’s t test.