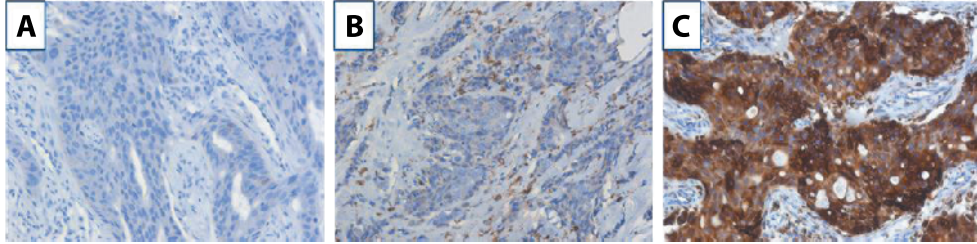
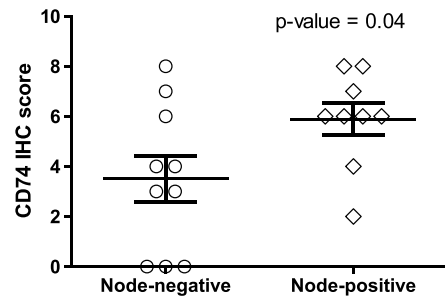


IHC staining of CD74 in TNBC



IHC staining of CD74 in 19 TNBC specimens



CD74 and Scribble spectral counts by LC-MS/MS

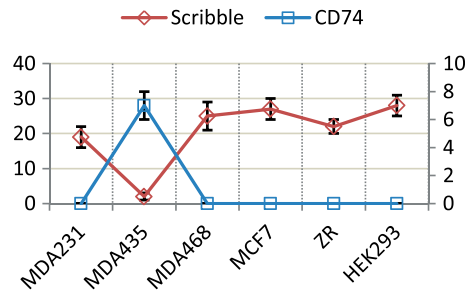


Figure W1. Overexpression of CD74 in lymph node–metastatic triple-negative breast tumors. (Top) Formaldehyde-fixed section from a tumor was stained with secondary antibody only (A); CD74 staining in a section from node-negative tumor showing positive staining in infiltrating lymphocytes and negative malignant cells (B); CD74 staining in a node-positive triple-negative tumor showing strong cytoplasmic staining of the malignant cells (C). (Bottom left) CD74 IHC scores for node-positive ($n = 9$) and node-negative ($n = 10$) tumors. The mean and SEM are indicated by horizontal lines. The P value of .0409 was calculated by one-tailed nonparametric Mann-Whitney t test. A one-tailed Fisher's exact test assuming cut value of 5 returns a P value of .051. (Bottom right) CD74 and Scribble abundances in the membrane fractions of cultured breast cancer cell lines and the HEK293s cell line. The abundance was estimated by the spectral count method in triplicate LC-MS/MS analyses. CD74 spectral counts are plotted on the right y -axis. The protein was only detected in MDA-435-MB. Scribble spectral counts are plotted on left y -axis. Vertical bars represent SDs. Only MS/MS spectra passing the 1% FDR threshold as calculated by MaxQuant were included in the analysis. Scribble is barely detectable with one to two spectral counts in MDA-435-MB.

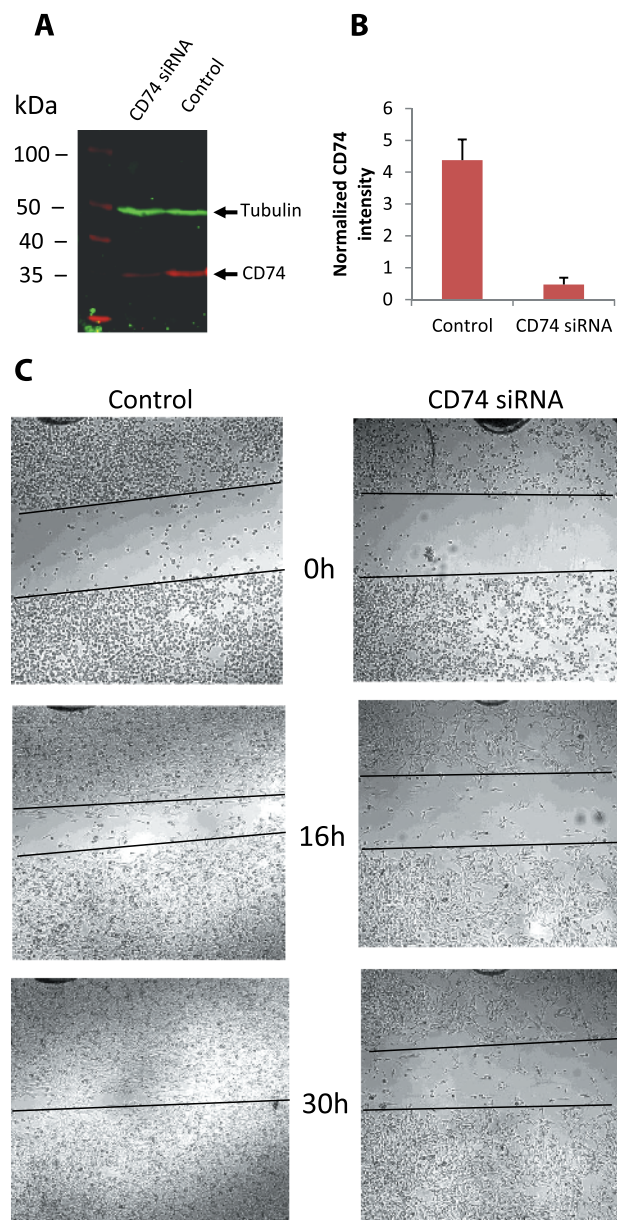


Figure W2. CD74 knockdown affects the wound healing in MDA-435-MB. (A) Western blot analysis of CD74 abundance in cells transfected with control DNA (left) and CD74 siRNA performed 18 hours after transfection. (B) Bar graph is based on two replicate analyses. (C) Wound healing assay with control and CD74 siRNA-transfected cells. The assays were started 18 hours after transfection. Photographs were taken at 0, 16, and 30 hours.

>sp|Q14160|SCRIB_HUMAN Protein scribble homolog OS=Homo sapiens
GN=SCRIB PE=1 SV=4

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PCLELSLLVRR[DPAPPGLR]ELCIQKAPGERLGISIRGGARGHAGNPR[DPTDEGIFISK]VS
PTGAAGRDGRLRVGLR[LELVNQSLGLTHGEAVQLLR]SVGDTLTVLVCDFEASTDAAL
EVSPGVIANPFAAGIGHR[NSLESISSIDRELSPEGPK]EK[ELPGQTLHWGPEATEAAGR]
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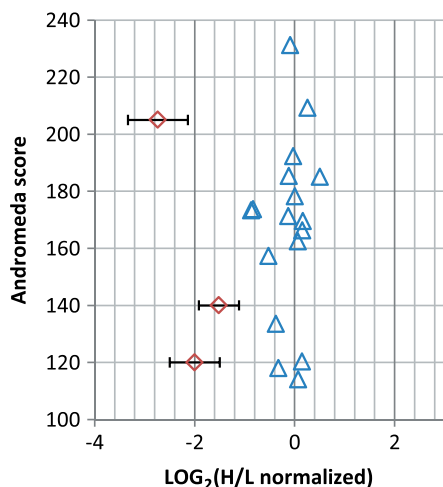


Figure W3. (Top) Scribble peptides and phosphorylation sites detected in immunoprecipitated Scribble by high-resolution mass spectrometry. The detected peptides are shown as boxed sequences highlighted in yellow. The three phosphopeptides affected by CD74 overexpression are highlighted in blue with phosphoserines shown in red. (Left) Measured *H/L* ratios for the high-scoring Scribble peptides (Andromeda score > 100) showing that the unmodified peptides center on a normalized *H/L* ratio of 1 (blue symbols). The three phosphorylated peptides, shown with red symbols ± SD, are significantly decreased in the heavy Scribble molecules coming from CD74-overexpressing cells. For each of the phosphopeptides, the Student's two-tailed *t* test returns a *P* value that is lower than .0001 when calculated against the normalized *H/L* ratios of the unmodified peptides shown with blue triangles.

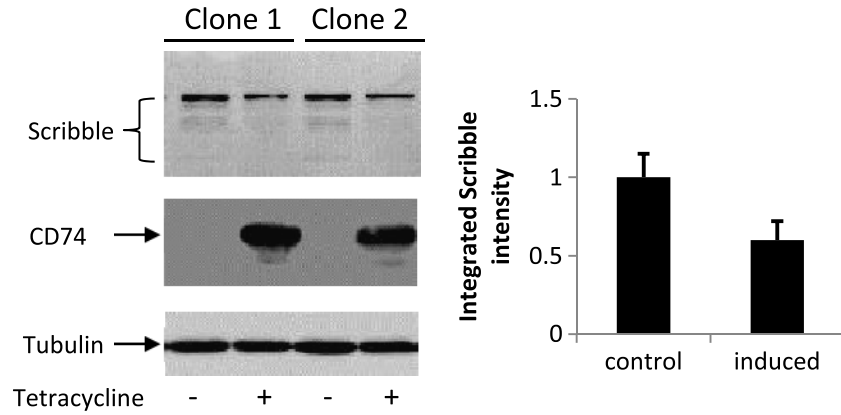


Figure W4. Long-term overexpression of CD74 leads to a decrease of Scribble abundance as determined by Western blot analysis. (Left) Scribble detection in lysates from uninduced TetR/TetO-CD74 cells and cells induced with tetracycline for 48 hours. (Right) Bar graph of the integrated band intensities of Scribble determined by the infrared fluorescent scanner in two independent experiments. The intensities were normalized against tubulin.

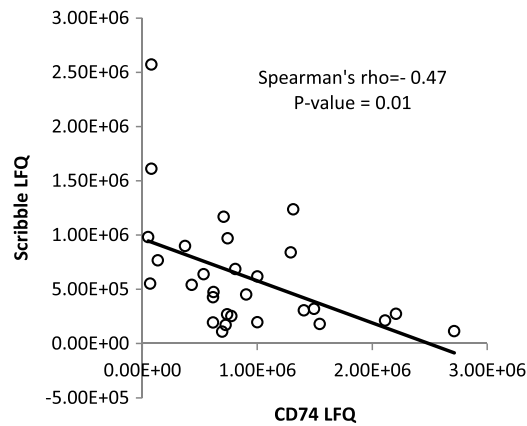


Figure W5. Label-free quantitation (LFQ) of CD74 and Scribble in membrane isolates of 25 breast tumor biopsies. The membrane proteins were isolated by the permeabilization and extraction procedure, digested with trypsin, and analyzed by LC-MS/MS as described in Materials and Methods section. MaxQuant was used to process the LC-MS/MS data and calculate LfQ as described in [1]. The nonparametric Spearman ρ and corresponding P values were calculated in R using cor.test.