Supplementary Figure Legend

Supplementary Figure S1. Production and characterization of the anti-SCUBE2 specific antibody. A, Specificity of anti-SCUBE2 antibody by western blot analysis. The anti-SCUBE2 antibody could specifically recognize the recombinant SCUBE2 but not SCUBE1 or SCUBE3 protein expressed in HEK-293T cells (top panel). As a control for the protein loading, expression of the FLAG-tagged SCUBE protein was confirmed by anti-FLAG antibody (bottom panel) B, The anti-SCUBE2 antibody specifically detects SCUBE2 protein in formalin-fixed, paraffin-embedded HEK-293T cells. The anti-SCUBE2 antibody stained HEK-293T cells expressing SCUBE2 but not SCUBE1 or 3 (brown color, arrowhead, top panel). Immunostaining with anti-FLAG antibody verified the protein expression of the respective FLAG-tagged SCUBE protein (brown color, arrow, second panel). Staining specificity was confirmed by use of pre-immune serum or omission of the anti-SCUBE2 antibody (PBS) showing no specific staining (third and bottom panels, respectively). Bar = 50 μm.

Supplementary Figure S2. Localization of SCUBE2 protein expression in normal breast tissue or invasive breast tumor by immunohistochemistry. A, Expression of

SCUBE2 protein in normal breast tissue. Anti-SCUBE2 immunoreactive staining was found on the luminal surface of normal breast ducts (arrow) and vascular endothelial cells (arrowheads). B, Specificity of the anti-SCUBE2 staining. The luminal membrane of ductal epithelial and endothelial cells was immunoreactive (arrow and arrowhead, left panel). Consecutive sections were stained with anti-SCUBE2 antibody pre-absorbed with the respective peptide antigen (+ peptide, right panel). Pre-absorption of the anti-SCUBE2 antibody with the corresponding peptide immunogen resulted in no immunostaining, which confirmed the specificity of the anti-SCUBE2 immunoreactive signal in the breast tissue. C and D, Expression of SCUBE2 protein in breast tumors by immunohistochemistry. Representative images of negative (C) and positive tumor staining (D) for SCUBE2. Despite no tumor staining in Panel C, intratumor vascular endothelial cells remained positive for anti-SCUBE2 antibody (arrowhead), which serves as an internal control for immunohistochemistry. Bar = 50 μM.

Supplementary Figure S3. Overexpression of SCUBE2-FL (full-length) and D4 mutant protein suppresses MCF-7 breast-cancer cell proliferation *in vitro*. A, Induction of ectopic SCUBE2-FL or -D4 protein in MCF-7 Tet-off stable clone cells.

MCF-7 Tet-off SCUBE2-FL or -D4 cells were cultured in the medium with or without doxycycline, (+) or (-) Dox, for 5 days. The induction of ectopic FLAG-tagged SCUBE2-FL or -D4 protein expression was determined by western blot analysis using anti-FLAG antibody. B, Effect of SCUBE2 protein overexpression on MCF-7 breast-cancer cell proliferation. The MCF-7 Tet-off Vector, SCUBE2-FL, or SCUBE2-D4 stable cells were cultured in medium without doxcycline, (-) Dox, to induce the expression of SCUBE2 protein. Cell proliferation was measured over the next 9 days by MTT assays. *, $p < 0.01$ (Vector vs SCUBE2-FL or -D4).

Supplementary Figure S4. Growth of the MC-7 Tet-off Vector or the MCF-7 Tet-off SCUBE2-FL tumors in nude mice in the presence of doxycycline. The MCF-7 Tet-off Vector or MCF-7 Tet-off SCUBE2-FL clone cells were injected into nude mice. The mice continued to receive doxycycline in drinking water. Tumor growth was compared and plotted as a function of time until the termination of experiments (n=5 in each group).

Supplementary Figure S5. *In vitro* cleavage of SCUBE2 by purified recombinant matrix metalloprotease 2 (MMP2). Cell lysates from HEK-293T cells transfected with

an expression plasmid producing the N-terminal FLAG-tagged SCUBE2-FL protein (FLAG.SCUBE2-FL) were prepared and incubated with purified recombinant human MMP2 (500 ng) at 37 ºC for 2 h in the absence of presence of a broad-spectrum MMP inhibitor (GM6001, 20 μM). Recombinant SCUBE2 and its cleaved product were analyzed by SDS-PAGE and western blot analysis using an anti-FLAG antibody (A) or anti-CR polyclonal antibody (B), respectively. Bands corresponding to uncleaved, full-length product (arrows in panel A and B), N-terminal fragment (asterisk in panel A), or C-terminal fragment (triangle in panel B) are indicated to the right of each gel. The processing of SCUBE2 by MMP2 appears to be specific since addition of an inhibitor for MMP (GM6001) completely blocked the cleavage of recombinant SCUBE2 protein (in panel A).