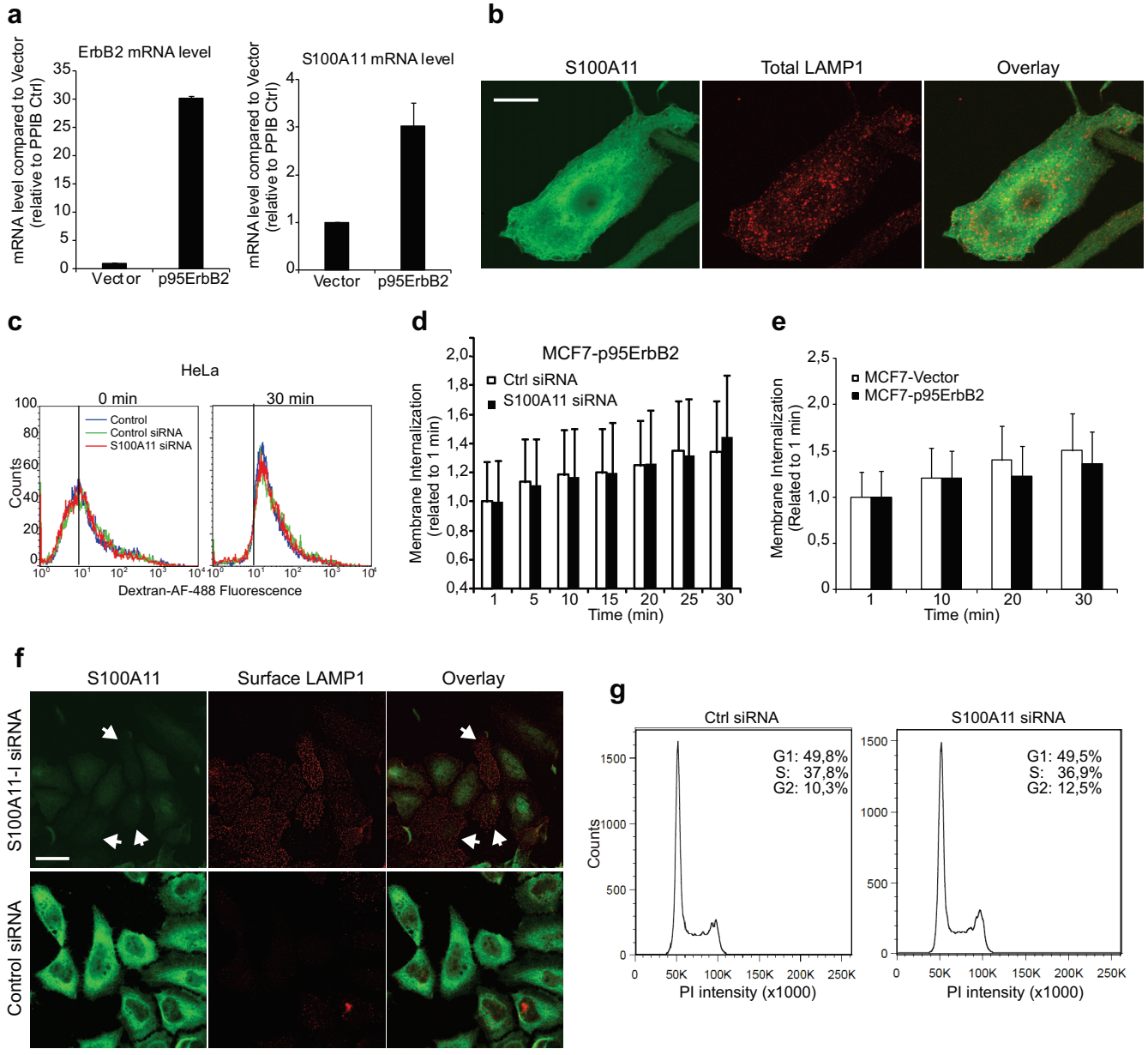
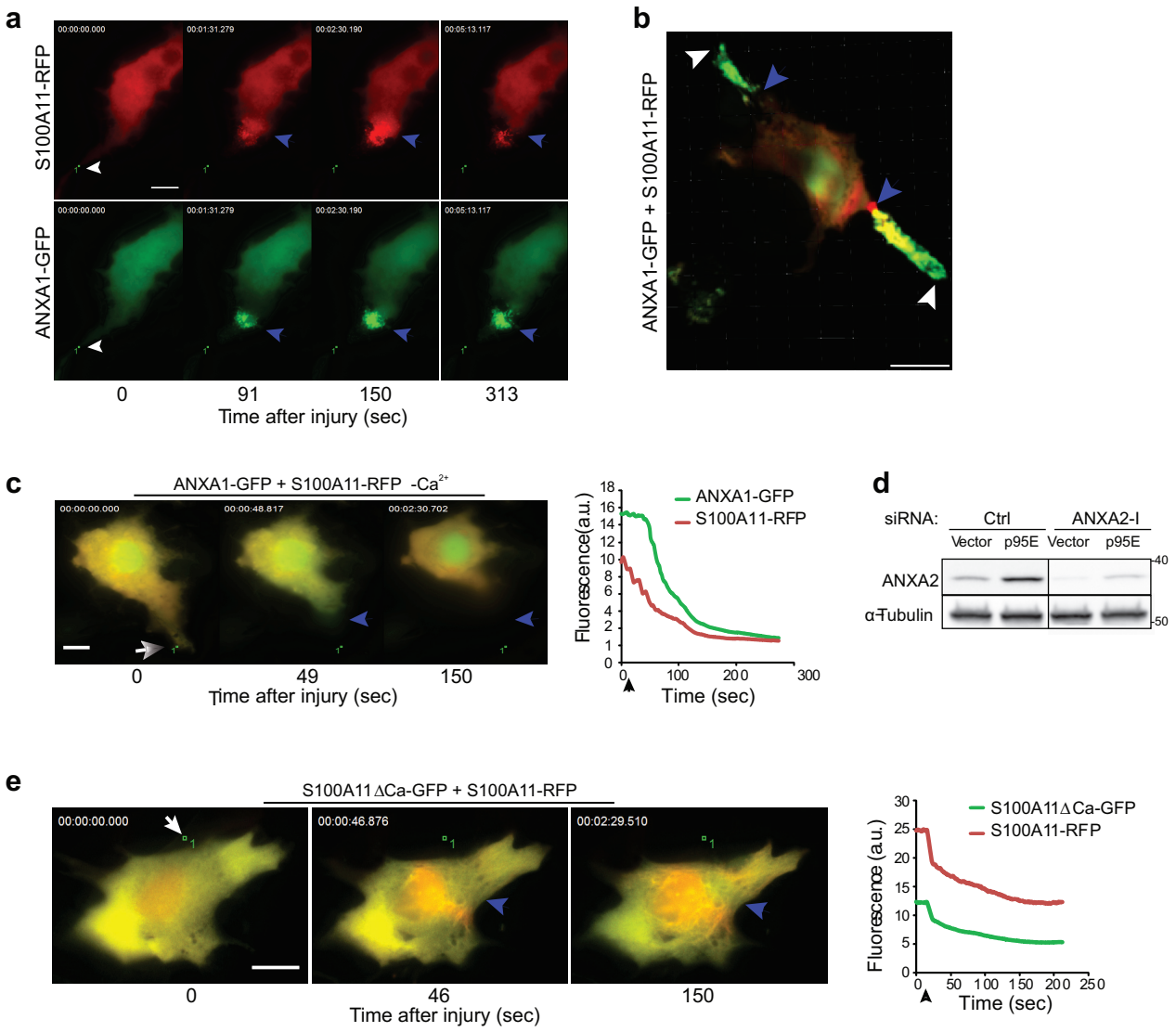
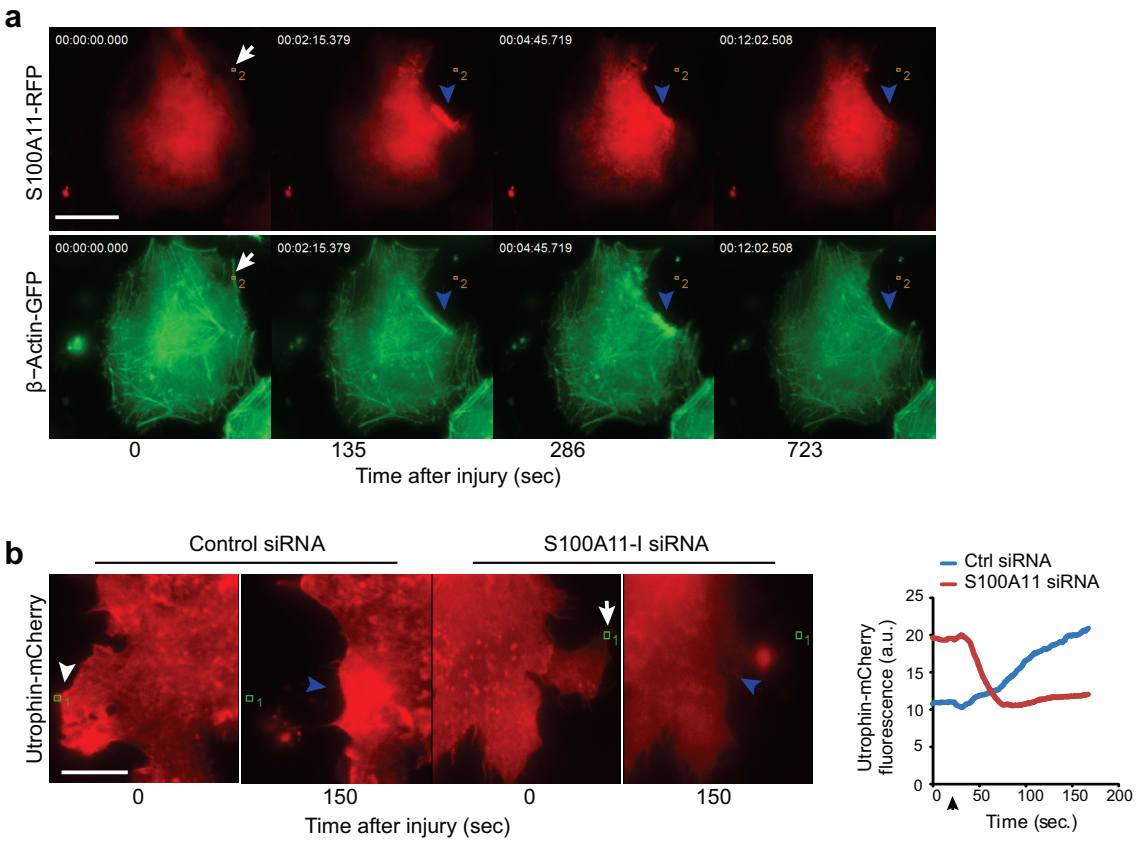


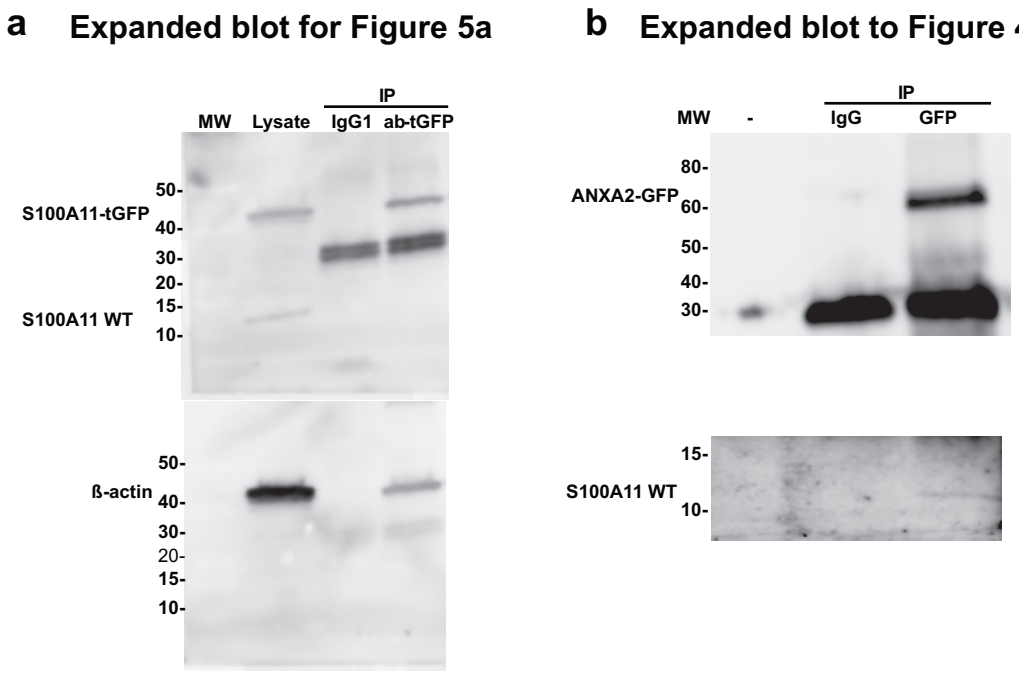
Supplementary Figure 1: S100A11 mRNA levels, immunodetection of S100A11/LAMP1 and endocytosis activity after S100A11 depletion. Related to Fig. 1b, 1f-g, 3b and 3c. (a) qPCR analysis showing relative mRNA expression levels of p95ErbB2 and S100A11 in MCF7-p95ErbB2 cells as compared to vector. PPIB was used as internal reference. SD bars of three replicates. (b) Images of MCF7-p95ErbB2 cells immunostained to show endogenous S100A11 and LAMP1 distribution. Scale bar = 20 µm (c) Representative fluid phase endocytosis activity assay in HeLa cells as measured by incubating cells with 10 kDa fluorescently labeled dextran for indicated time. Prior to FACS analysis cells were depleted for S100A11 or treated with ctrl siRNA for 72 h. (d) Endocytosis activity in p95ErbB2 cells after treatment with S100A11 specific- or ctrl siRNA (72 h) or (e) untreated MCF7-vector and p95ErbB2. Plasma Membranes were stained with FM1-43 dye and subsequently washed. Internalizing membrane vesicles was imaged by time lapse microscopy and quantified by Metamorph software (SD of n=3 experiments). (f) Confocal images of HeLa cells immunostained for cell surface LAMP1 and total S100A11 after treatment with indicated siRNA for 72 h. Scale bars, 20 µm. (g) Asynchronously growing culture of MCF7-p95ErbB2 cells were transfected with control siRNA or S100A11 specific (S100A11) siRNA for 72 hours. Following fixation, cellular DNA was labeled using propidium iodide (PI) and analyzed by flow cytometry. The histogram plot for each cell culture was used to calculate the proportion of cells in each cell cycle stage listed above, which are similar between the two cultures.



Supplementary Figure 2: **S100A11 is recruited to the healing membrane in a Ca^{2+}-dependent manner.** Related to Fig. 4. (a) Fluorescence pictures adapted from Supplementary Movie 3 and Fig. 4a showing independent images for S100A11-RFP and ANXA1-GFP proteins in MCF7-p95ErbB2 cell laser injured (site of injury: white arrows; site of repair: blue arrows). (b) MCF7-p95ErbB2 cell co-expressing ANXA1-GFP and S100A11-RFP was injured at protrusions at the two opposing ends of the cell. These protrusions were eventually excised as part of the healing process and marked with ANXA1. Blue arrowheads indicate excision zones where S100A11 accumulates. (c) A representative cell injured in the absence of Ca^{2+}. Note that S100A11 and ANXA1-GFP failed to translocate in response to injury and the cell failed to heal in the absence of Ca^{2+}. (d) Representative immunoblots of ANXA2 and α-tubulin (loading control) in lysates of MCF7-vector and -p95ErbB2 cells transfected with indicated siRNAs 72 h prior to the lysis. (e) Expression of S100A11ΔCa-GFP in HeLa cells prevents translocation of S100A11-RFP to the injury site. GFP/RFP fluorescence was measured at the site of laser injury (white arrowhead) and quantification is presented in the adjacent plot. Scale bars, 20 µm.



Supplementary Figure 3: **S100A11 is required for actin buildup around the wound perimeter during membrane healing.** Related to Fig. 5. (a) Dynamics of S100A11-RFP recruitment with β-actin-GFP during repair of HeLa cells following laser injury (individual red and green channels extracted from the color merged images in time-lapse series in Fig. 5c; also see Supplementary Movie 5). (b) MCF7-p95ErbB2 cells depleted for S100A11 fail to buildup F-actin at the site of repair. Utrophin-mCherry expressing cells treated (for 48 hours) with S100A11 or control (Ctrl) siRNA were injured by laser. White and black arrows indicate site and time of injury respectively. Blue arrow heads mark the site of repair. Scale bars, 20 μm.



Supplementary Figure 4: **Extended Western blots for co-IP results shown in figures 5a and 4i.**

Supplementary Table 1

siRNA name	siRNA sequence
S100A11-I	5'-ACUCUCUCCAAGACAGAGUUCCUAA-3'
S100A11-II	5’-CCAACAGUGAUGGUCAGCUAGAUUU-3′
S100A11-III	5’-CCUGGUGUCCUUGACCGCAUGAUGA-3’
ANXA1	5’-GUGUUCAAUACCAUCCUUA-3’
ANXA2	5’-GUUACAGCCCUUAUGACAU-3’

siRNAs used in the study: Names and sequence of the siRNAs used in the study to silence expression of S100A11, Annexin A1 and Annexin A2 genes is listed.

Supplementary Table 2

Gene name	Primer sequence
ErbB2	Forward: 5'-CCC ATA TGT CTC CCG CCT TC-3' Reverse: 5'-AGC TCA TCC CCT TGG CAA TC-3'
S100A11	Forward: 5'-ATC GAG TCC CTG ATT GCT GT -3' Reverse: 5'-CCA TCA CTG TTG GTG TCC AG - 3'
PPIB	Forward: 5'- GGG AGA TGG CAC AGG AGG AGG AAA-3' Reverse: 5'- TGG GAG CCG TTG GTG TCT TTG-3'

qPCR primers used in the study: Sequence of the primers used for qPCR analysis of the expression of ErbB2, S100A11, and the control PPIB genes is listed.