Figure S1

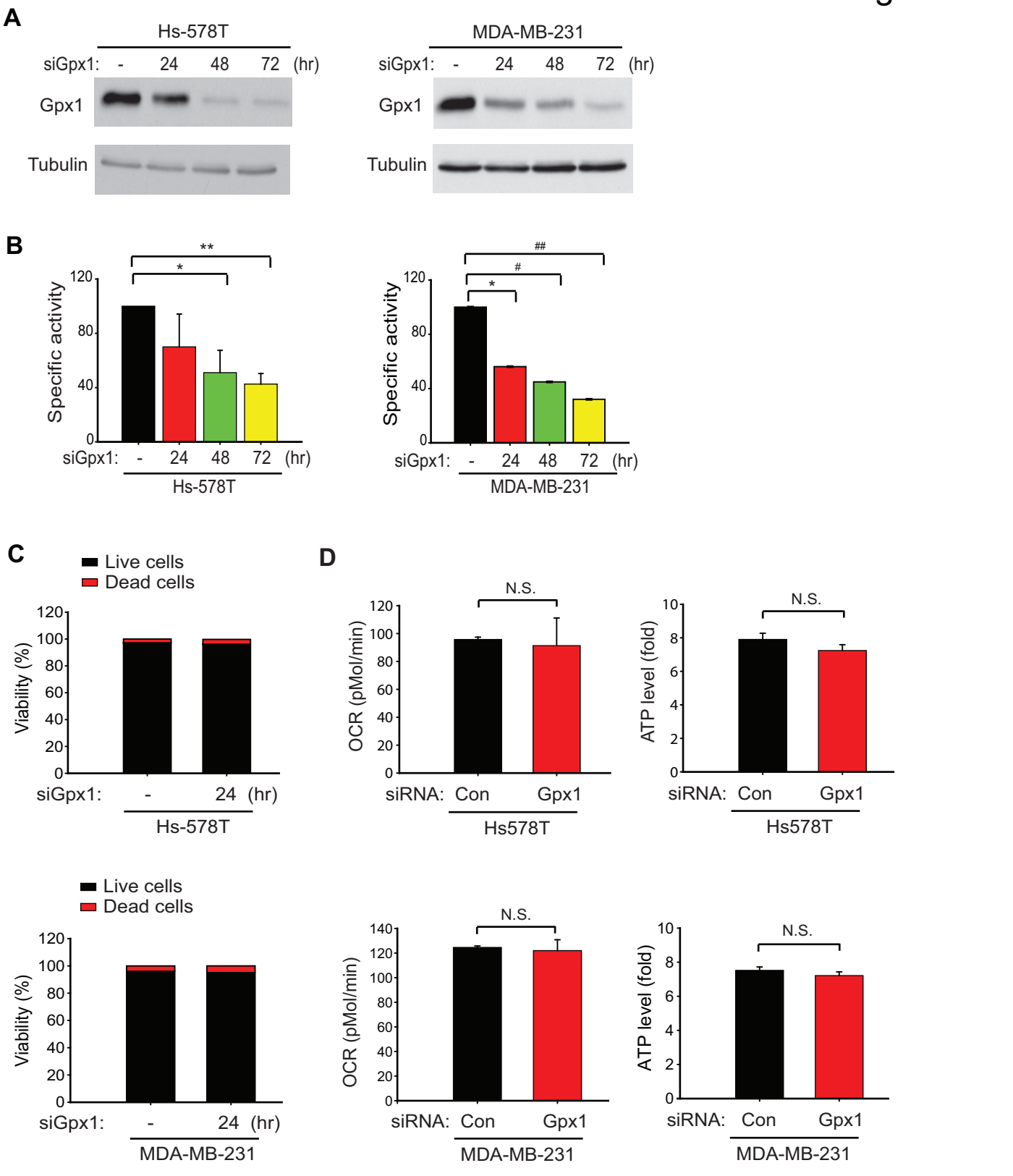

Figure S1. Characterization of Gpx1 depletion in the TNBC cells.
A. Knockdown of Gpx1 expression by the specific siRNA transfection.
B. GSH-dependent peroxidase activity in the cell extracts of the Gpx1-depleted TNBC cells prepared in panel A. Data in the graph are means ± SD of the specific activity (n = 3, $^{*}P < 0.005$, $^{**}P < 0.001$, $^{\#}P < 0.0001$, $^{\#\#}P < 0.00005$)
C. Tryphan blue assay for cell death with the Gpx1-depleted TNBC cells. Data in the graph are means ± SD of the percent of live and dead cells versus total cells (n = 3).
D. Metabolic activity in the Gpx1-depleted TNBC cells. ATP level and oxygen consumption rate (OCR) were measured in the control and Gpx1-depleted TNBC cells by the luciferase-based assay and live-cell metabolic assay (Seahorse XF analyzer, Agilent), respectively. OCR is measured with 2 x 10^4 cells. Data in the graph are means ± SD of the fold change or O_2 consumption rate (n = 3). N.S., not significant.

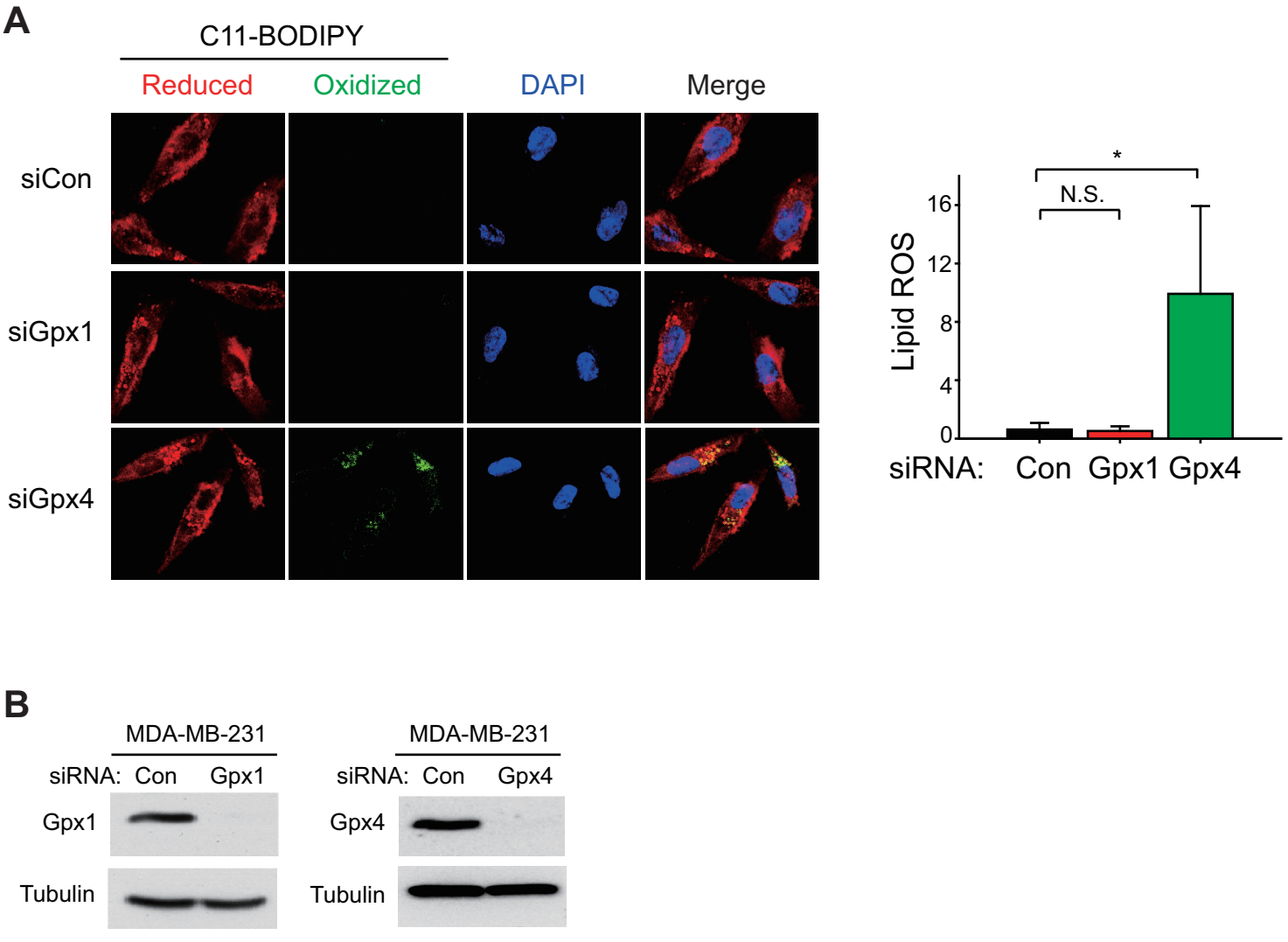

Figure S2. Level of lipid hydroperoxide in the Gpx1- or Gpx4-depleted MDA-MB-231 cells (Related to Fig. 3). The level of lipid hydroperoxide (LOOH) was measured using a LOOH-specific fluorescent probe, C11-BODIPY$^{581/591}$, in the MDA-MB-231 cells (**A**). Cells were transfected with either control, Gpx1, or Gpx4 siRNA (siCon, siGpx1, or siGpx4) for 48 hr and treated with C11-BODIPY (1 μM). The Gpx depletion was verified by immunoblotting (**B**). Data in the graph are means ± SD of the relative rations of green versus red fluorescence intensities averaged from 40 cells (n = 3, *P < 0.0001). Representative images are shown. N.S., not significant.

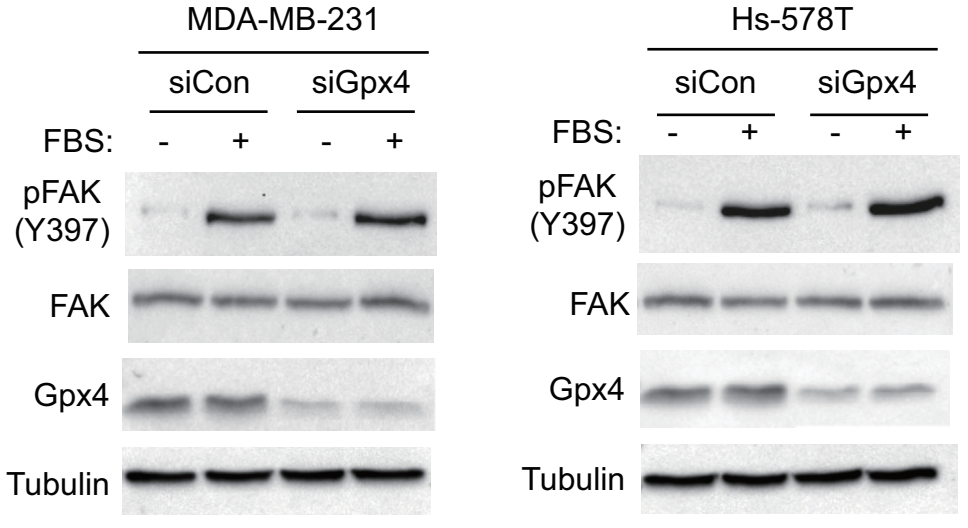

Figure S3. Effect of Gpx4 depletion on serum-induced FAK kinase activation (Related to Fig. 4). The activation of FAK kinases was examined by immunoblotting with the phosphorylation-specific antibodies in the control- and Gpx4-depleted MDA-MB-231 and Hs578T cells. The experiment was carried out as done for Gpx1. Representative blots are shown (*n* = 2).

Figure S4

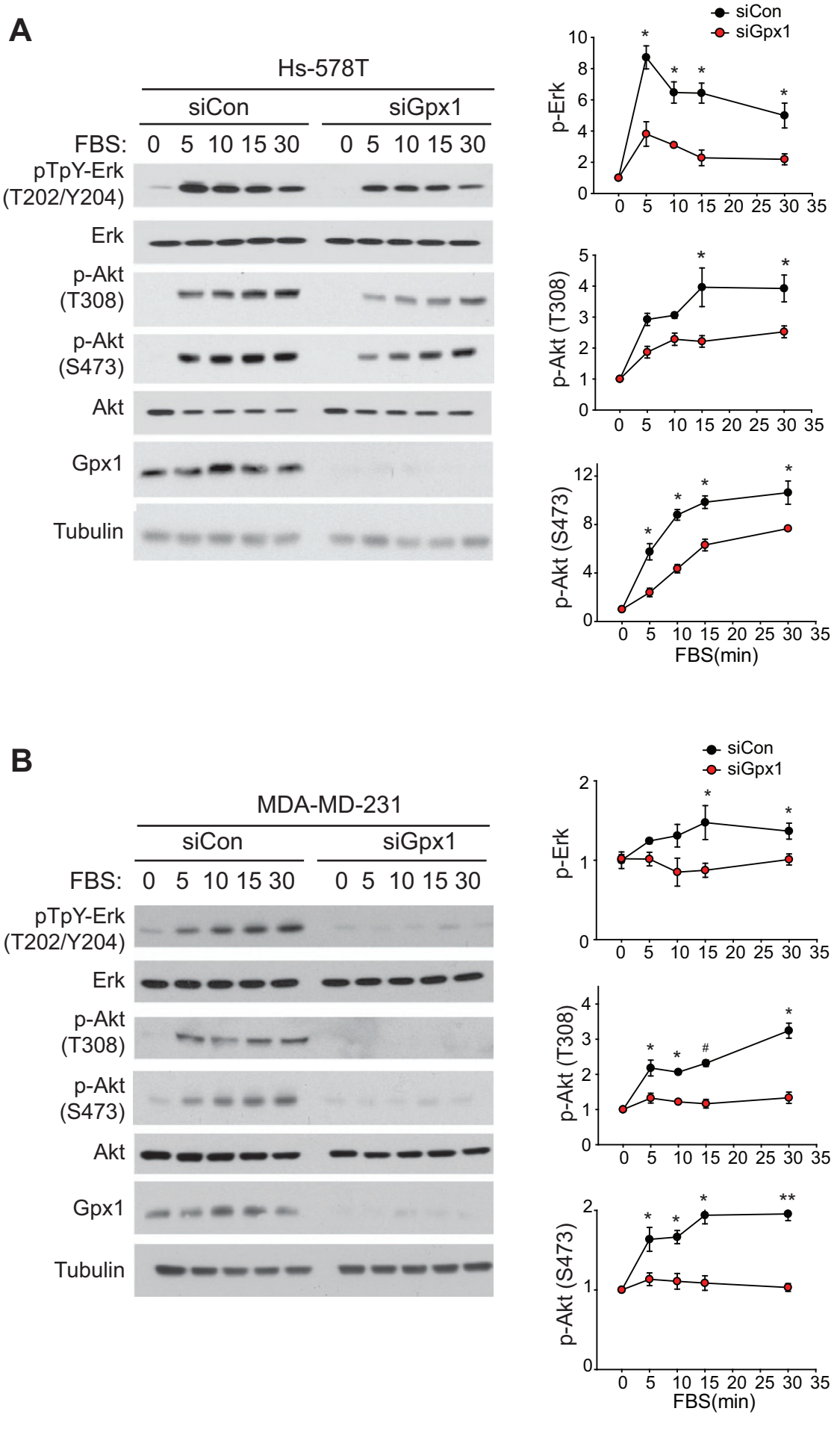

Figure S4. Effect of Gpx1 depletion on serum-induced kinase activation.
The activation of ERK and Akt kinases was examined by immunoblotting with the phosphorylation-specific antibodies in the Gpx1-depleted MDA-MB-231 (**A**) and Hs578T (**B**) cells. Data in the graph are means ± SD of the fold increase (n = 3, *P < 0.05, **P < 0.001, $^{\#}P$ < 0.0005).

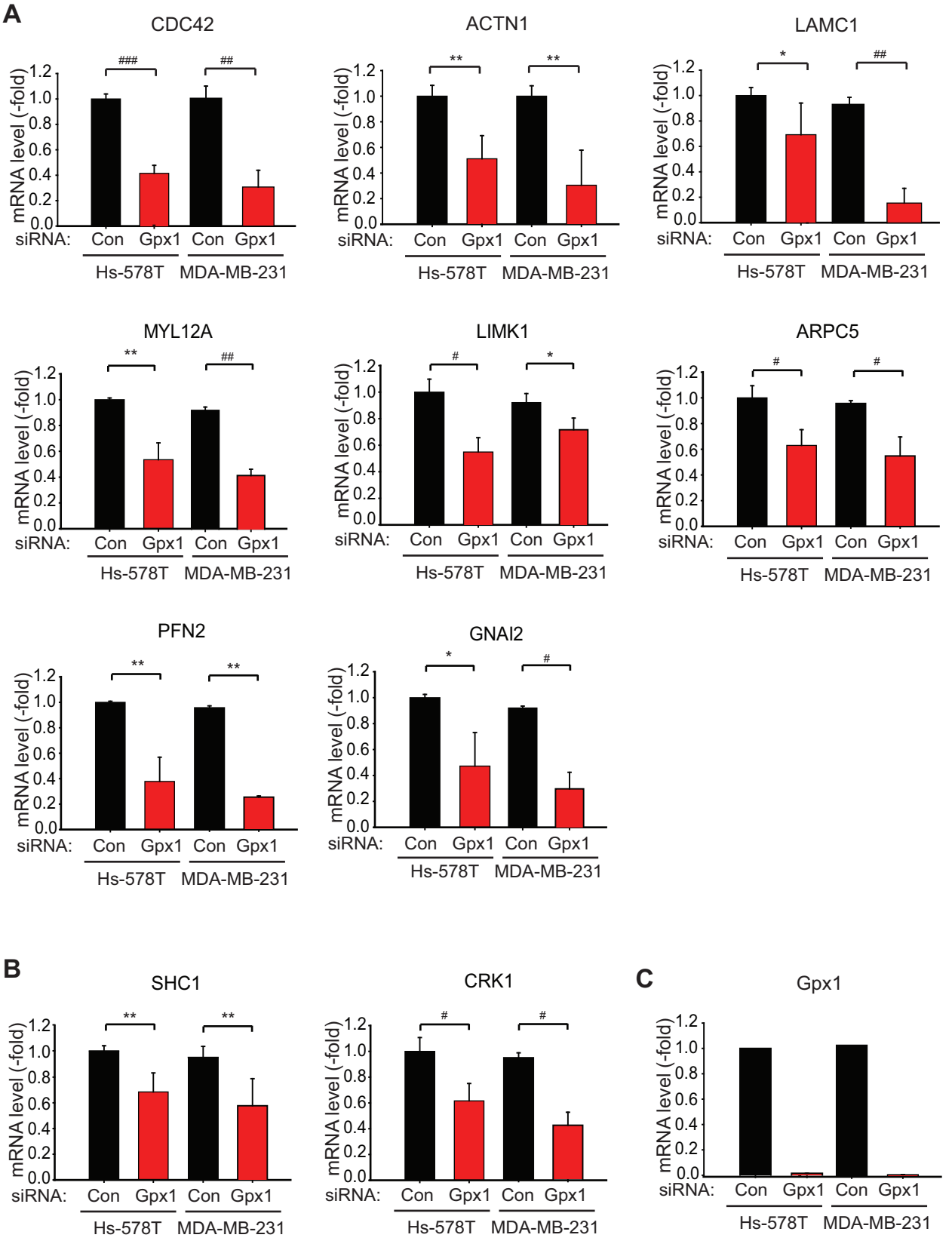

Figure S5. Expression levels of some genes down-regulated by the Gpx1 depletion.
The expressions of the cell junction/adhesion-related genes (**A**) and proliferation-related genes (**B**) were measured by quantitative real-time PCR (qPCR) in the Gpx1-depleted Hs578T and MDA-MB-231 cells. Data in the graph are means ± SD of the fold change (n = 3, $^{*}P < 0.05$, $^{**}P < 0.001$, $^{\#}P < 0.0001$, $^{\#\#}P < 0.00005$).
The levels of Gpx1 expression and knockdown were also evaluated by the qPCR (**C**).