

Supplementary figure legends

Supplementary Figure S1. Celecoxib induces MAGI1 in colorectal carcinoma

cells. (a) The colorectal carcinoma-derived cell lines HCT116, SW620, DLD1, T84 and HT29 were treated with 50 μ M celecoxib for 4 days and then analyzed for MAGI1 by Western blotting. Celecoxib treatment induced MAGI1 expression in all cell lines except in DLD1 cells. **(b)** The same cell lines were analyzed by Western blotting for COX-2 expression. The DLD1 cell line does express very low level of COX-2. Actin immunoblotting demonstrates equivalent protein loading across different lanes. **(c)** The same cell lines were analyzed by real time RT-PCR for COX-2 expression to confirm low level of COX-2 expression in DLD1 cells. Numbers on top of the columns indicate levels of COX-2 mRNA relative to HT29. **(d)** SW480 (S) and HCT16 cells (H) were analyzed for E-prostane receptor (EP) 1-4 expression by RT-PCR. SW480 and HCT16 cells express mostly EP4. **(e)**. Treatment of SW480 cells for 24 hours with the stabilized PGE₂ analogue 16, 16 dimethyl PGE₂ (dm PGE₂) decreases MAGI1 mRNA levels (mean values \pm S.D.). *p<0.001 to untreated.

Supplementary Figure S2. Celecoxib suppresses SW480 tumor growth. (a)

One million SW480 cells were injected s.c into Swiss nu/nu mice. At day 7 mice were randomized and one group was treated with celecoxib mixed with a powdered rodent chow diet at a concentration of 1000 ppm. Tumors were measured at the indicated time points. Celecoxib treatment slows tumor growth. Results represent mean values \pm S.D. *p<0.001 to control. (N=7). **(b)** Western blotting analysis of MAGI1 expression in tumors derived from subcutaneously implanted SW480 cells. Here shown are

control (mice 1-4) and mice treated with celecoxib (mice 5-8). Tumors growing in celebrex-treated mice have increased levels of MAGI1 protein. Actin immunoblotting demonstrates equivalent protein loading across different lanes.

Supplementary Figure S3. MAGI1 overexpression and silencing in SW480 and

HCT116 cells. (a) Western blotting analysis of MAGI1 expression in wild type

SW480 (WT), SW480 overexpressing MAGI1 (MAGI1) and SW480 expressing 3 different MAGI1 shRNA (sh1, sh2, sh3). Clone sh3, was used for further analyses.

(b) Western blotting analysis of MAGI1 expression in wild type HCT116 (WT) and

HCT116 expressing 3 different MAGI1 shRNA (sh1, sh2, sh3). Clone 2 was used for further analyses. NS, cells expressing non-silencing sh constructs. **(c)** Relative levels

of MAGI1 overexpressed in SW480 cells compared to MAGI1 levels induced by 4-

day culture in the presence of celecoxib. MAGI1 cDNA expression gives rise to a

protein smaller than endogenous MAGI1, consistent with published results (Ide *et al.*, 1999). Actin is detected to demonstrate equal loading.

Supplementary Figure S4. Effect of MAGI1 on HCT116 cell migration and

invasion. (a) MAGI1 overexpression reduces HCT116 cell migration through

uncoated Transwell filters (left panel) and invasion through Matrigel-coated

(0.5mg/ml) Transwell filters (right panel). Results represent the average number of cells per field \pm S.D. counted on the lower side of the insert membrane. $p < 0.001$ to

WT. (N=3). **(b)** MAGI1 overexpression slightly reduces SW480 cell proliferation

under standard culture conditions, while MAGI silencing slightly promotes it. $*p < 0.02$

to WT. WT, wild type cells; MAGI1, MAGI1 overexpressing cells; shMAGI1, MAGI1 silenced cells, shNS, non-silenced cells.

Supplementary Figure S5. Chracterization of SW480 integrin-mediated

adhesion of matrix proteins (a) Effect of MAG1 levels on cell surface integrin

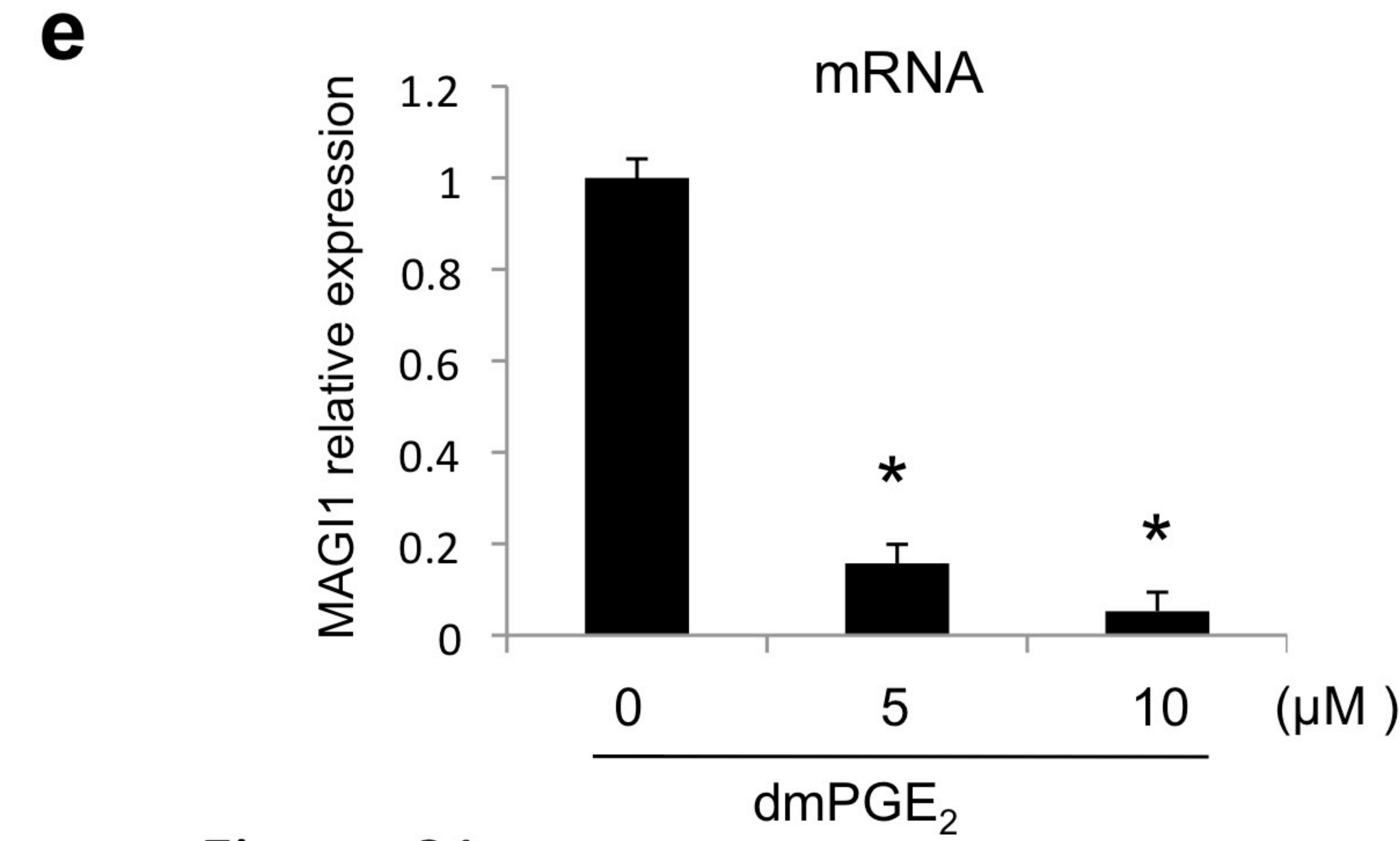
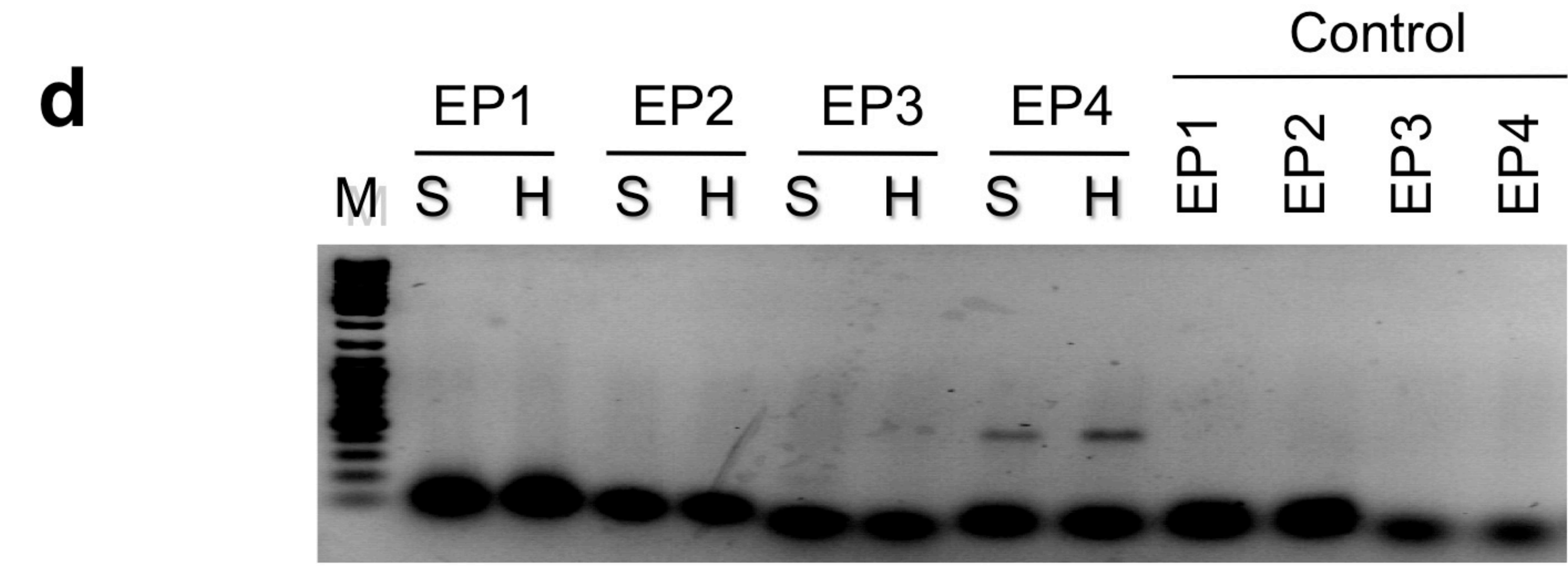
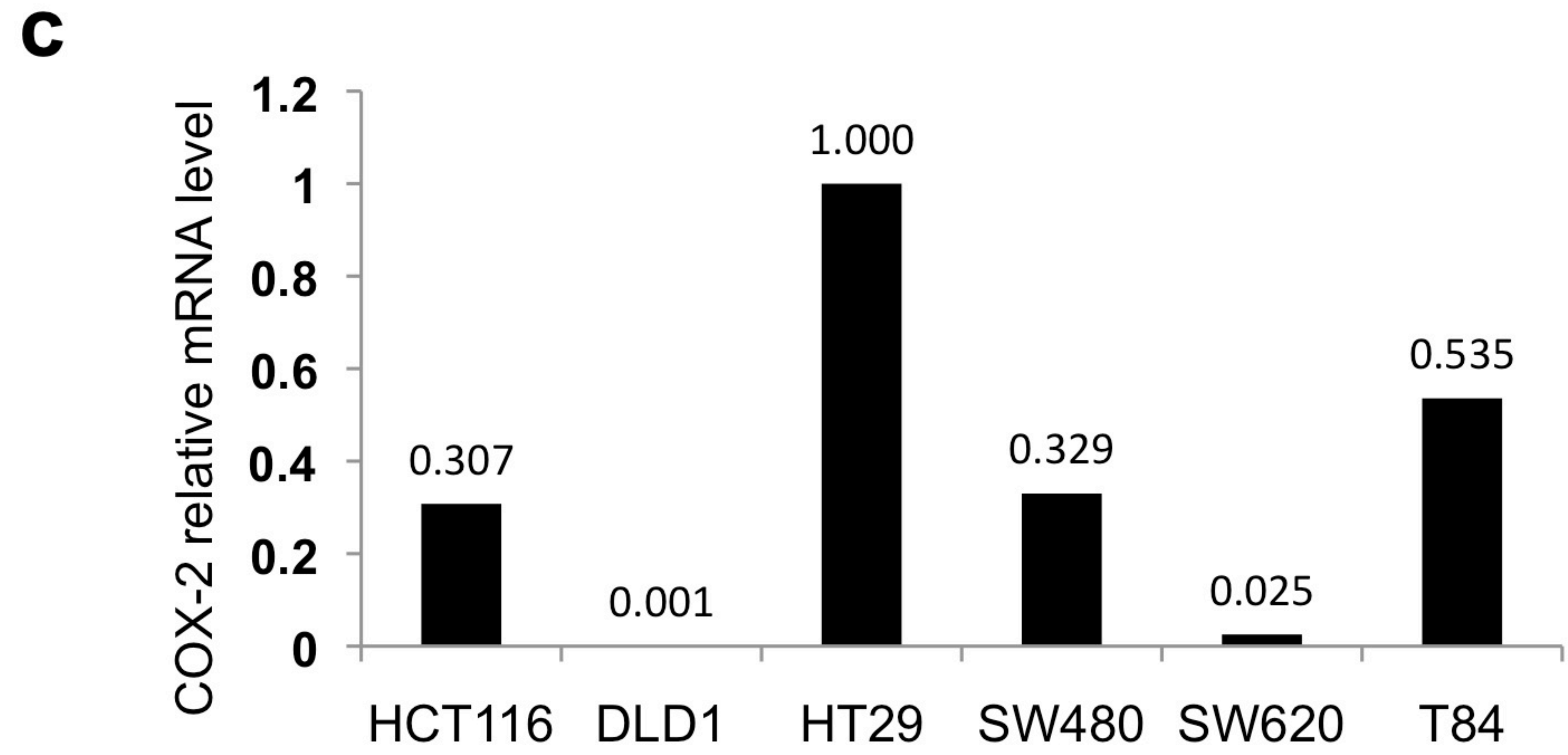
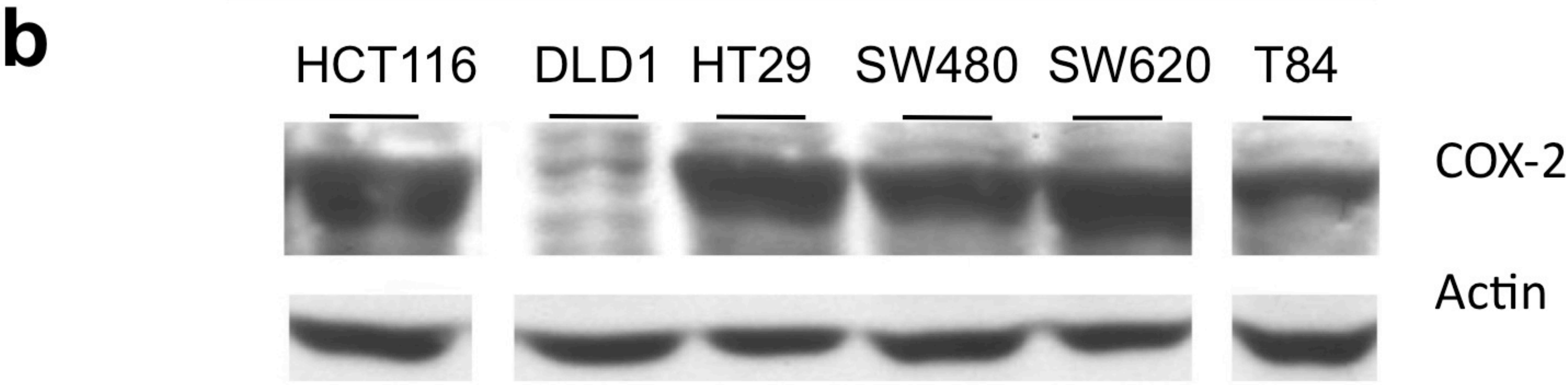
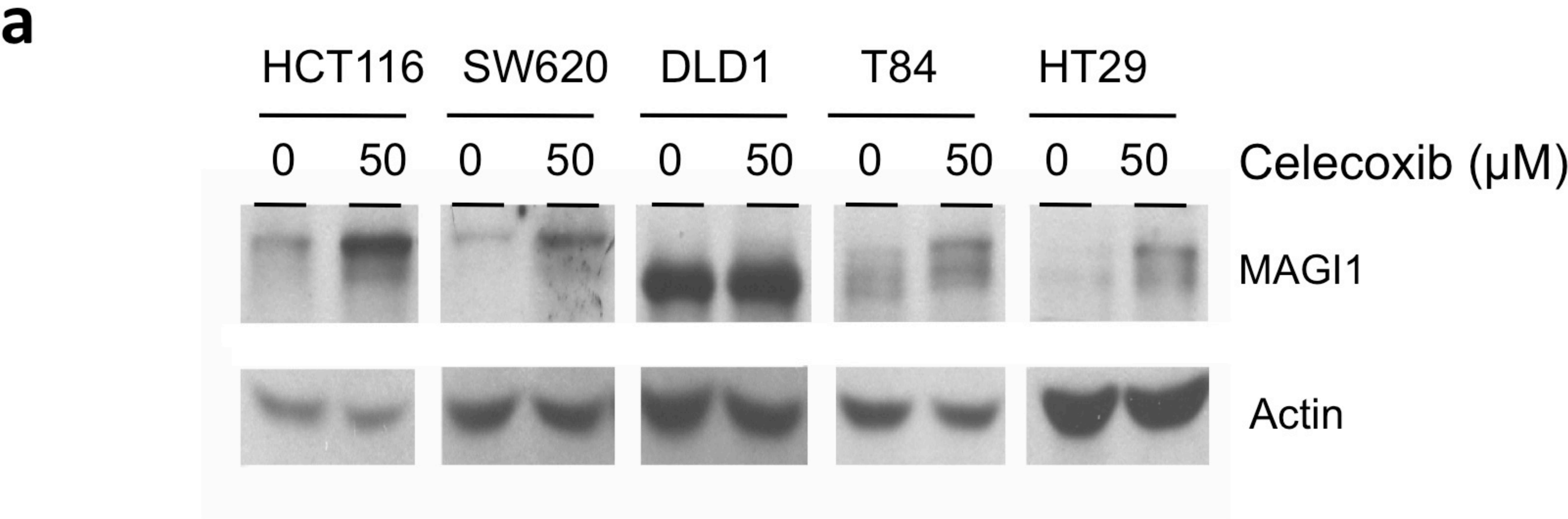
expression. To determine integrin expression, wild type, MAGI1 overexpressing and MAGI1 silenced SW480 cells were stained by indirect immunofluorescence with anti-integrin antibodies specific for the subunits $\alpha 4$, $\alpha 5$, $\alpha 6$, $\beta 1$ and the integrin complex $\alpha V\beta 3$, and analyzed by flow cytometry. Modulation of MAGI1 had no impact on cell surface in integrin expression. **(b) Antibody blocking of integrin-mediated adhesion.** SW480 cells were plated on PLL (as integrin-independent adhesion control), on laminin, fibronectin and collagen I in the absence or presence of anti-integrin blocking mAbs as indicated. Results are given and mean \pm S.D. of triplicate determinations and are shown as O.D. of crystal violet-stained wells. MAGI1-enhanced cell adhesion was fully dependent on integrin. GoH3, anti- $\alpha 6$; GI9, anti- $\alpha 2$; Lia1/2, anti- $\beta 1$; SAM1, anti- $\alpha 5$; LM609 $\alpha\beta$. WT, wild type; MAGI1, MAGI1 overexpressing; shMAGI1, MAGI1 silenced.

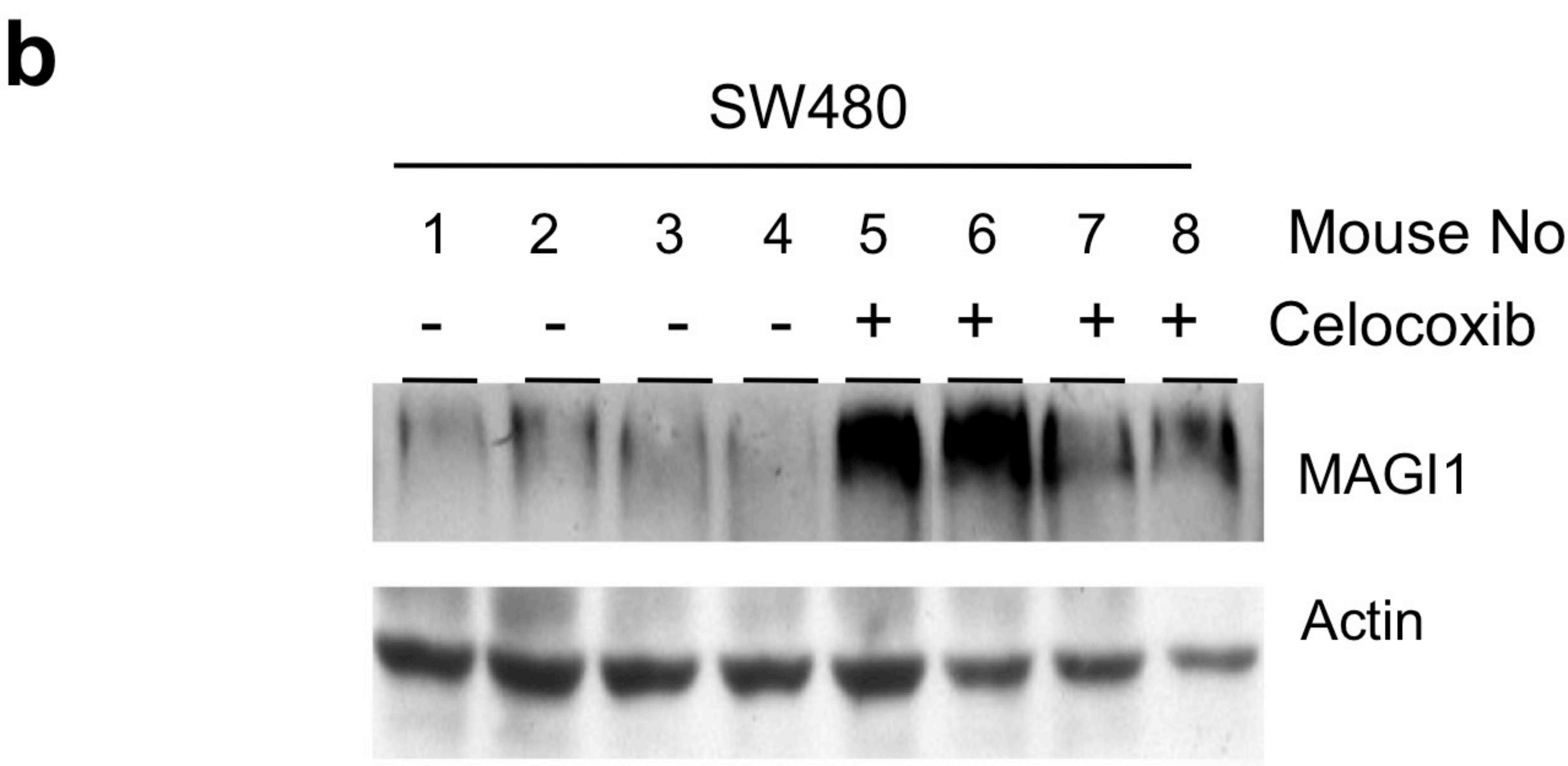
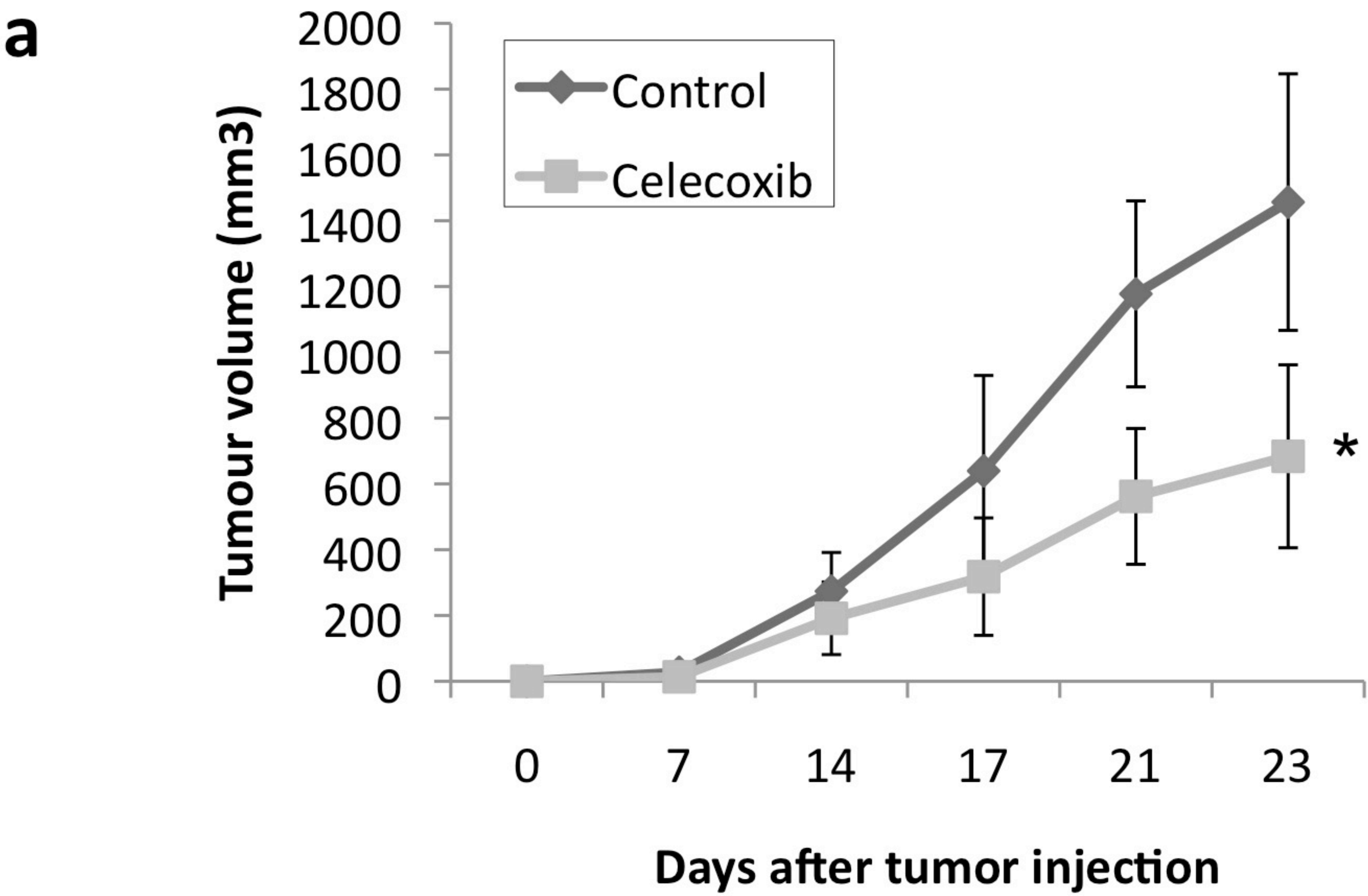
Supplementary Figure S6. MAGI1 overexpression inhibits Wnt signaling in

HCT116 cells. (a) Measurement of TCF/LEF transcriptional activity in HCT116 cells using the TOP/FOP Flash activity assay. Cells were transfected with Flash plasmids expressing either the β -catenin responsive elements (TOP) or and control sequence (FOP). Activities were normalized to co-transfected Renilla luciferase and expressed

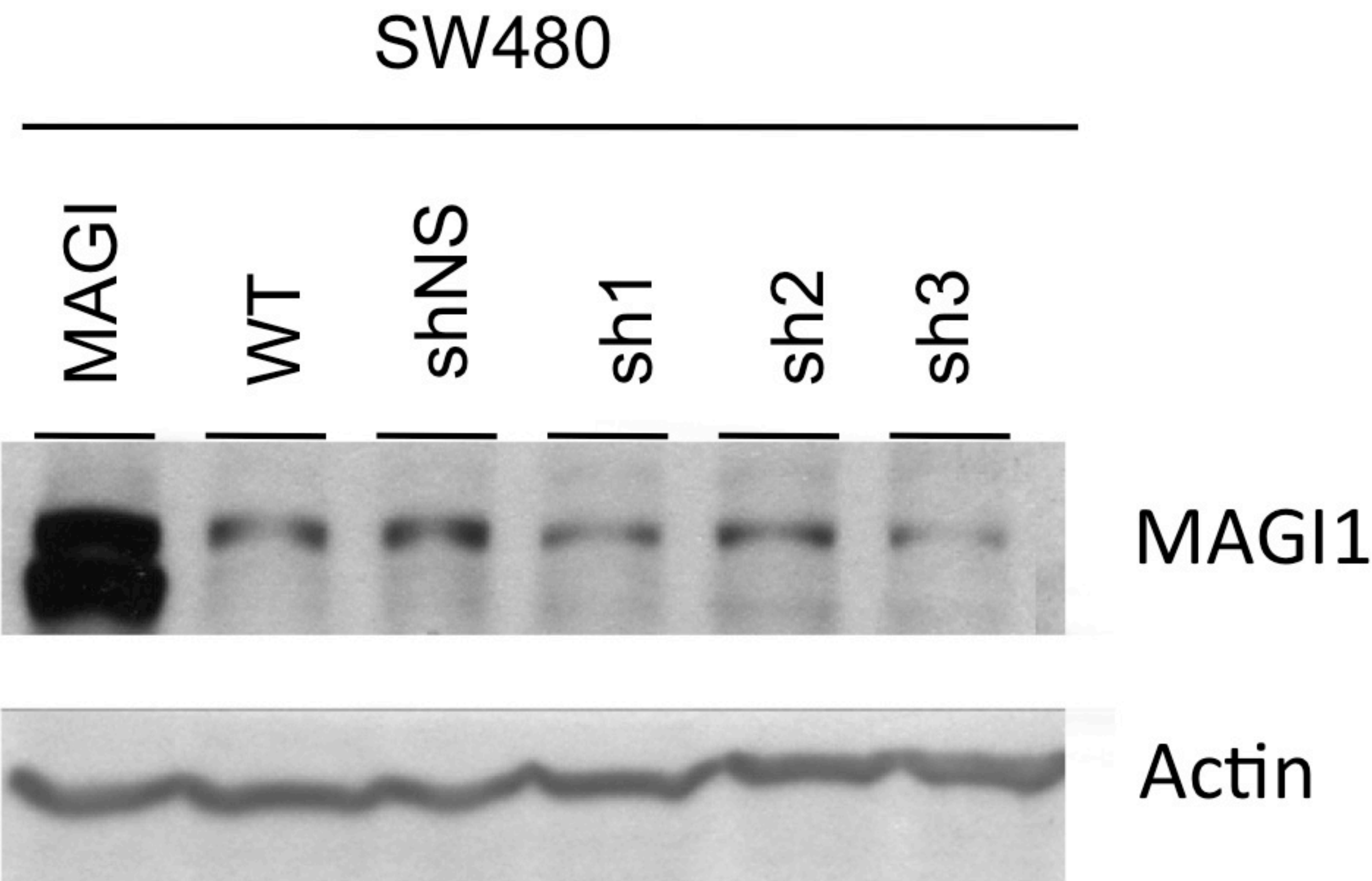
here as the TOP/FOP ratio. MAGI1 silencing (shMAGI) enhanced TOPFlash activity, while MAGI1 overexpression (MAGI) decreased it. WT, HCT116 wild type cells, shNS, HCT116 cells expressing a non-silencing shRNA. * $p < 0.001$ to WT. (N=3). **(b)** Measurement of Axin2 mRNA levels by real-time RT-PCR in the same cells of panel A. MAGI1 silencing (shMAGI) enhanced Axin2 level, while MAGI1 overexpression (MAGI) decreased it. Results represent mean values \pm S.D. and are shown as the fold difference relative to Axin2 level in SW480 WT cells normalized to GAPDH levels. * $p < 0.001$ to WT. (N=3).

Supplementary Figure S7. Microvascular density in celecoxib treated tumors, and MAGI1 effect on orthotopic SW480 tumor growth. (a) Microvascular density (MVD) in celebrex-treated SW480 tumors and untreated tumors was determined by CD31 staining and vessel count. Celebrex significantly reduces MVD, * $p < 0.001$ to corresponding untreated tumors. **(b)** Luciferase-expressing wild type, MAGI1 overexpressing and MAGI1 silenced SW480 cells (1×10^6) were injected orthotopically into the cecum of NSG mice and tumor growth was monitored non-invasively by detecting *in vivo* luciferase activity. Results represent mean photon values within the tumor region of interest. Data are the same as those shown in Figure 5d, except that results are given in a logarithmic scale. * $p < 0.05$, ** $p < 0.001$ to WT. (N=4). WT, wild type; MAGI1, MAGI1 overexpressing; shMAGI1, MAGI1 silenced.

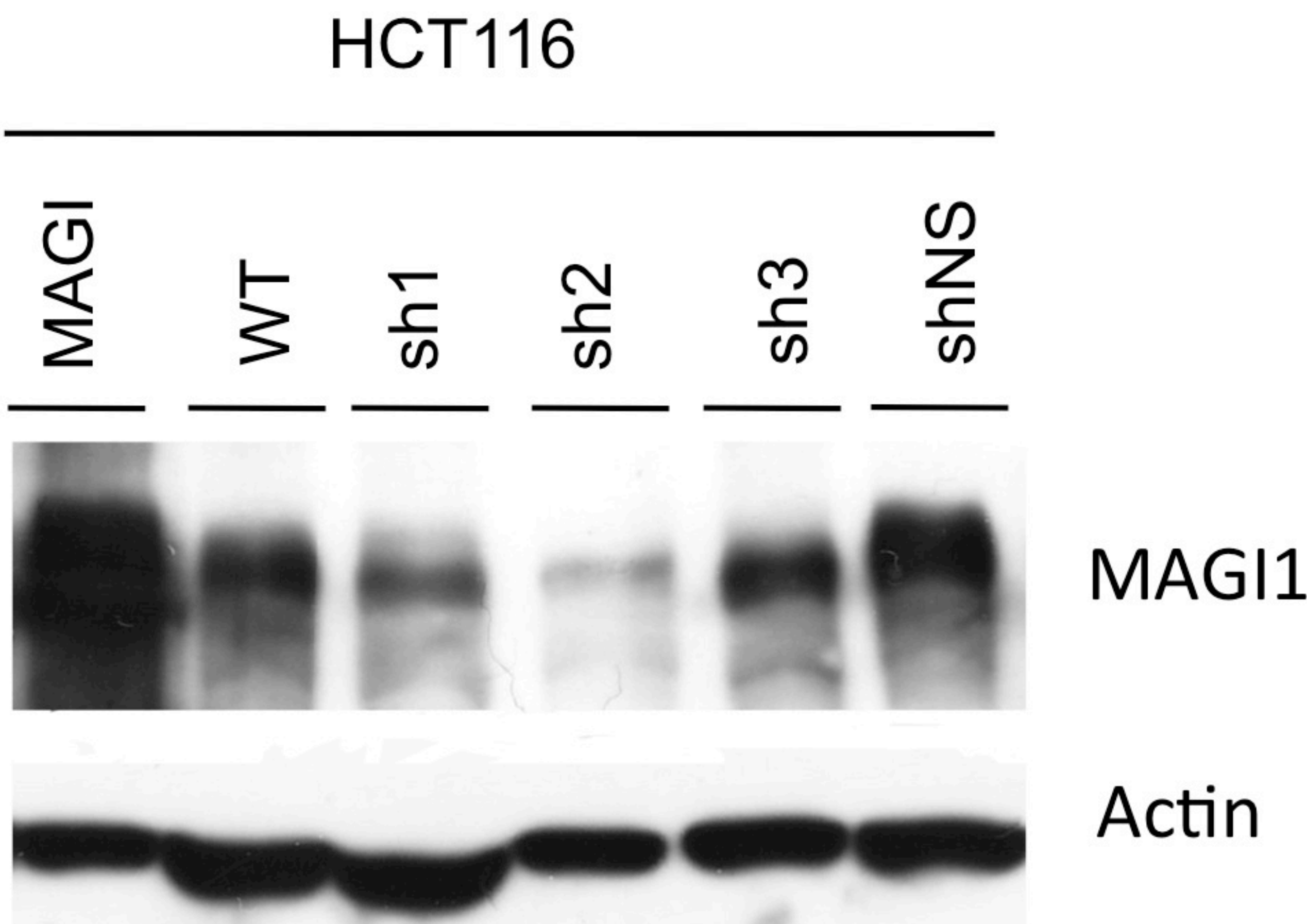




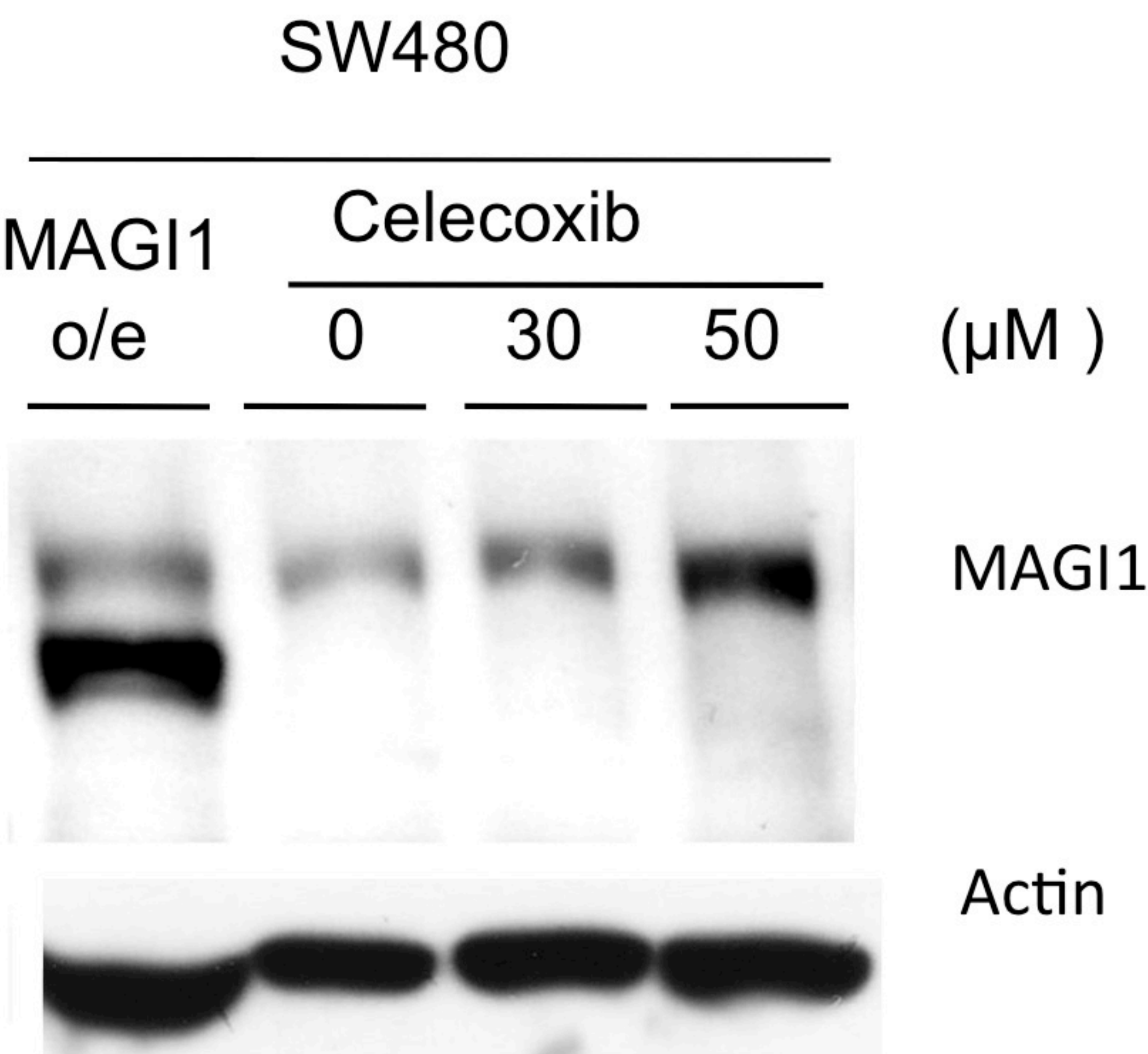
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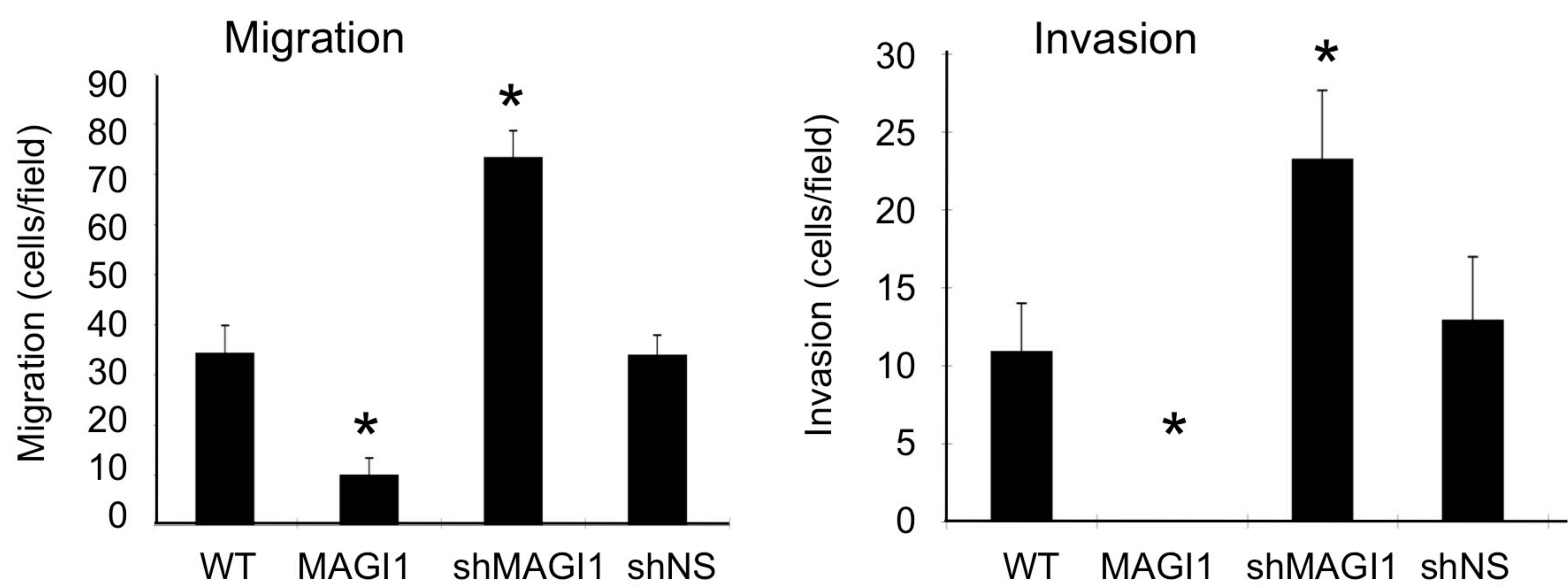
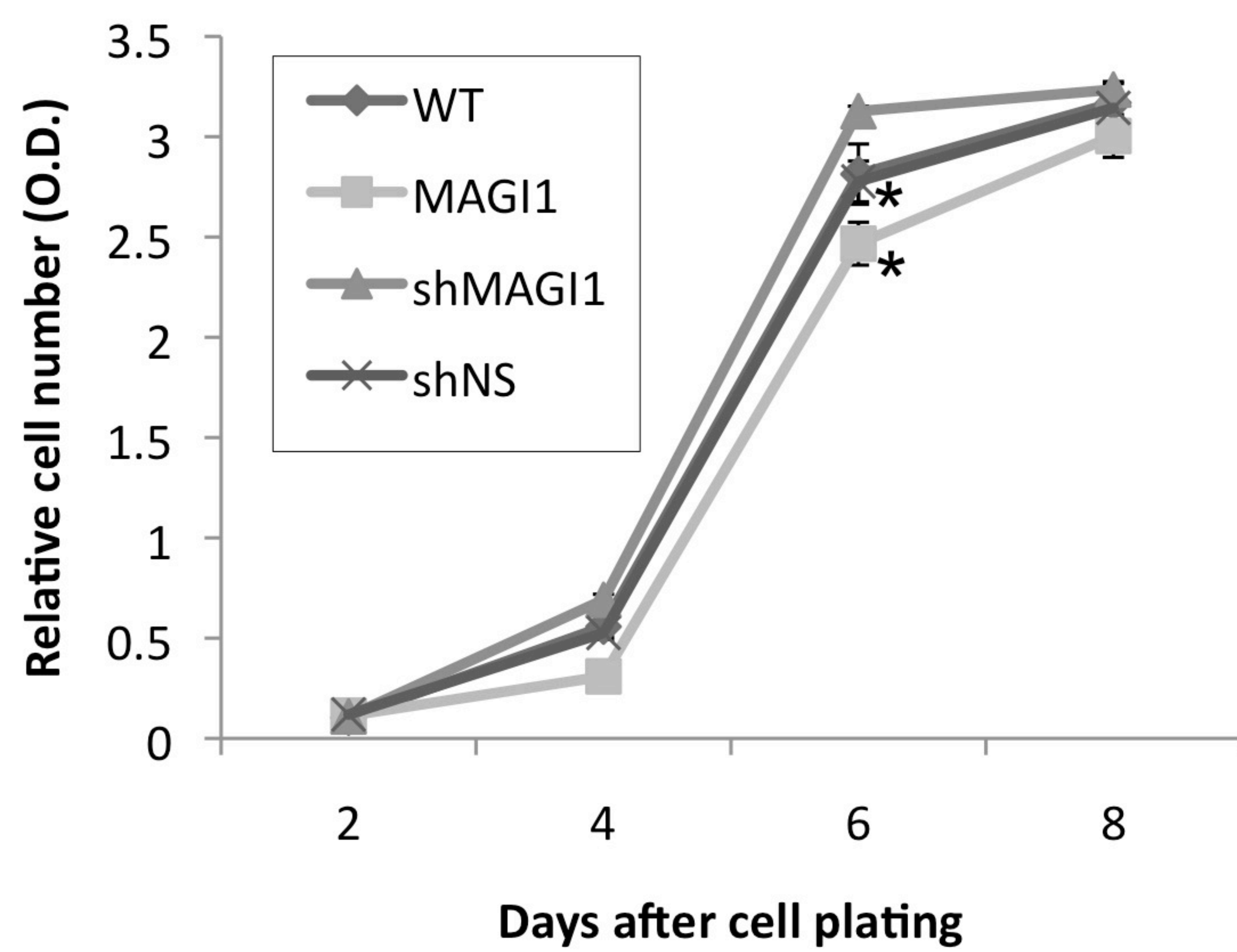


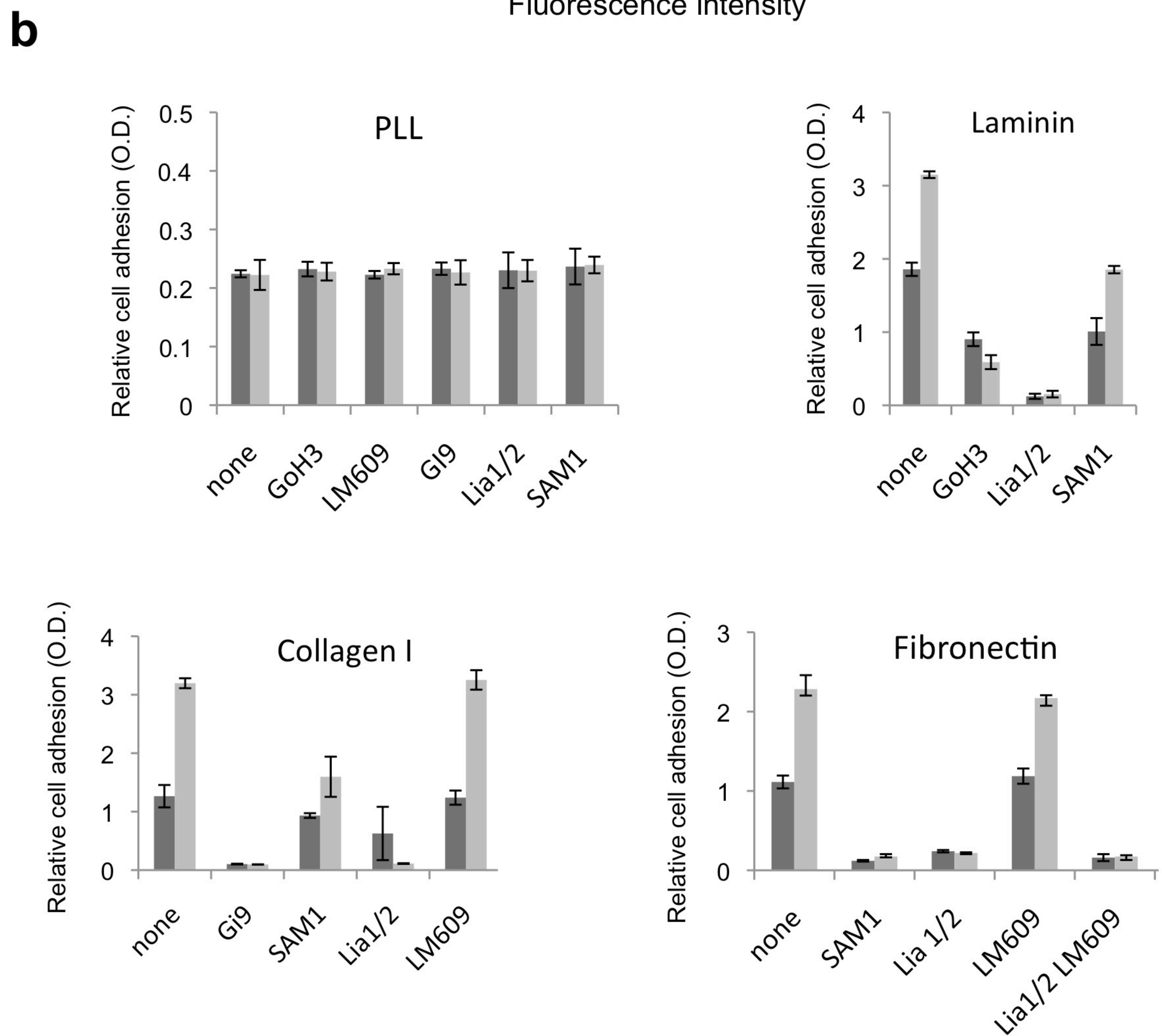
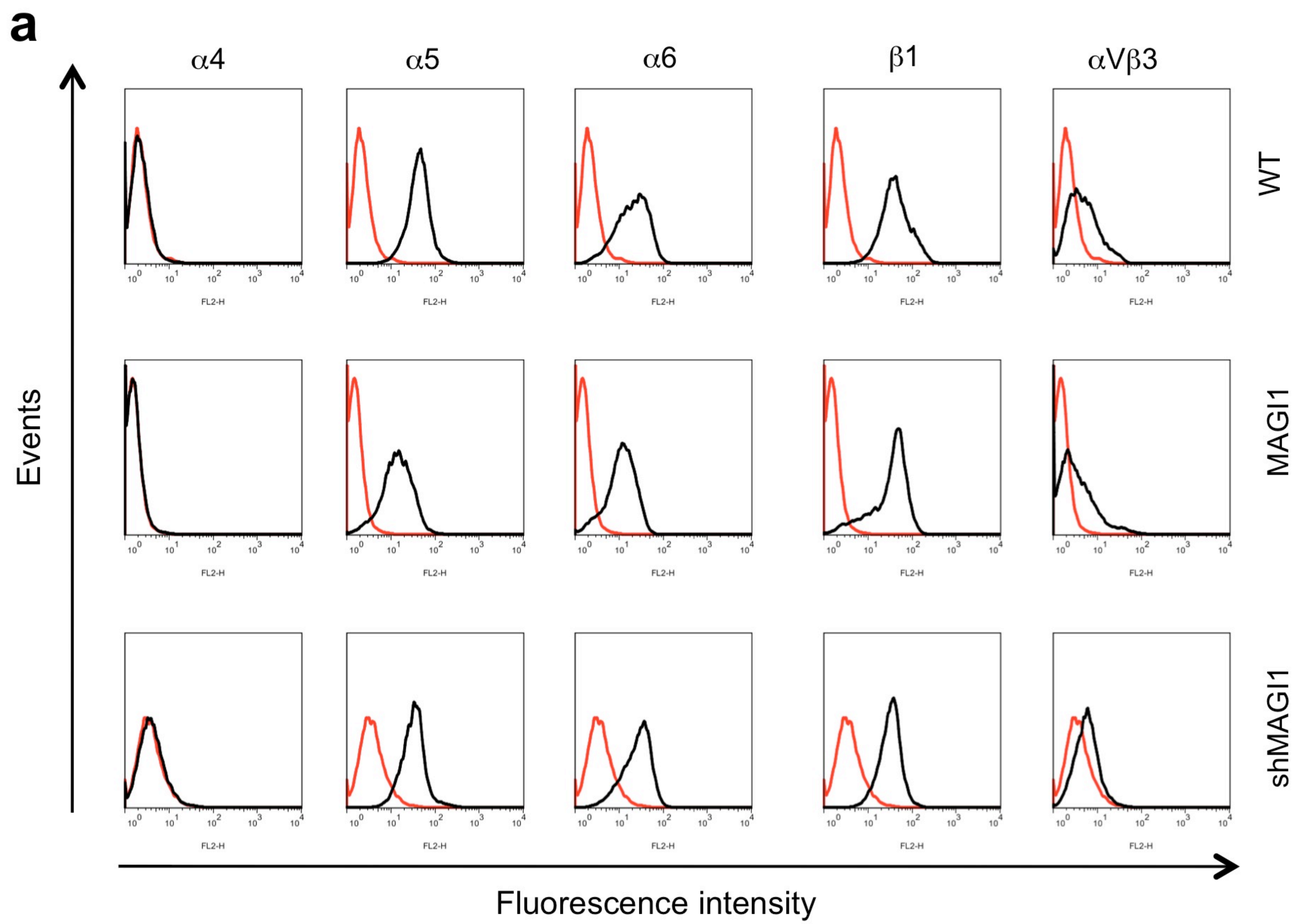
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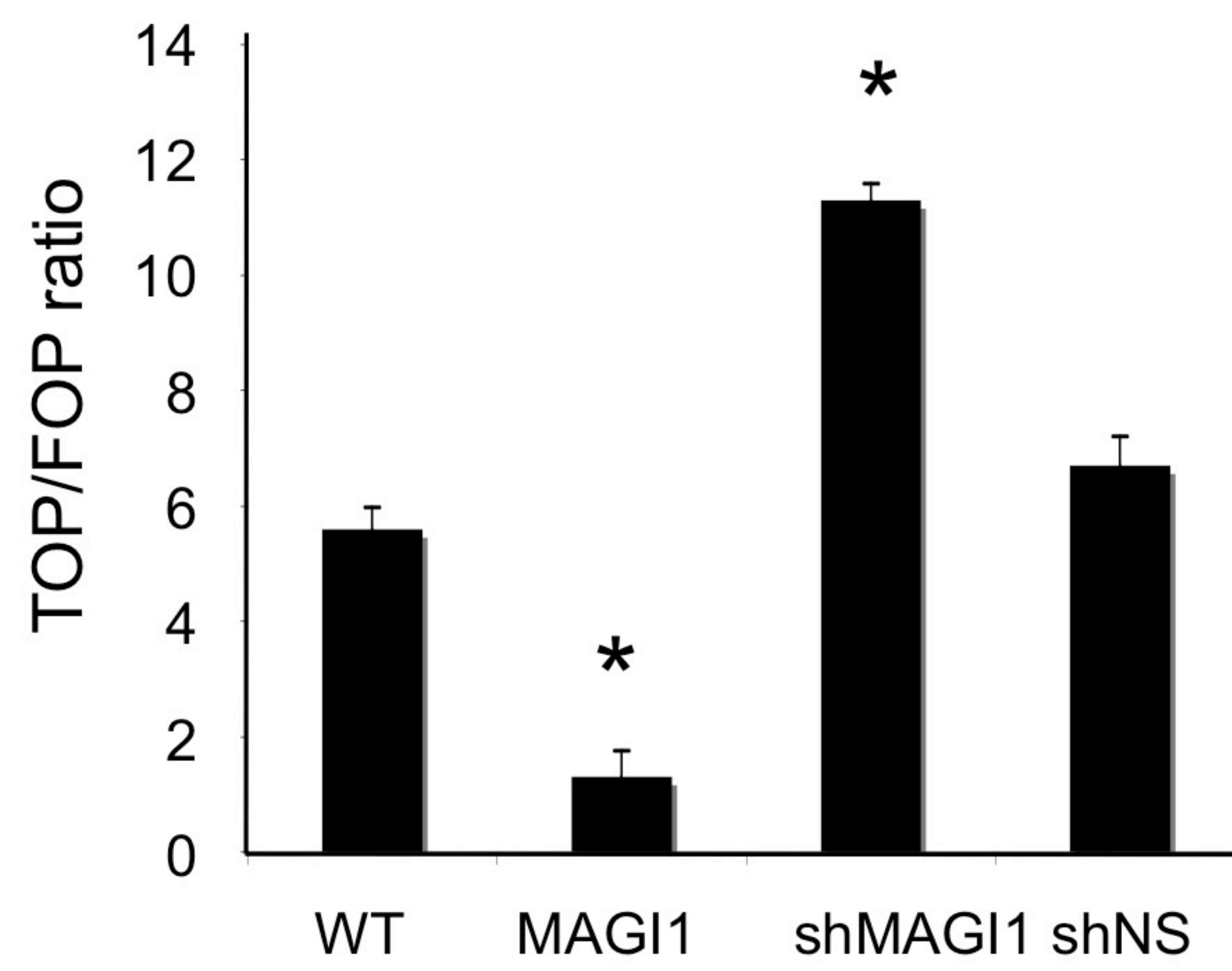
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