**Supplementary Fig. 1**

**Cell cycle distribution of nuclear S100A4.** a HCT116 cells were sorted by fluorescent-activated cell sorting (FACS) before the cells were collected on glass slides and subjected to immunofluorescent analyses using anti-S100A4 (green) and DAPI for nuclear staining. Representative pictures show cells sorted in the three indicated cell cycle phases (scale bar = 50 µm). b Percentage of cells having either strong, intermediate or no expression of nuclear S100A4 were monitored by counting the number of cells in the different phases (mean of n=3 biological experiments). c Nuclear expression of S100A4 were confirmed using Z-stack pictures from confocal laser scanning microscopy, with the red and the green lines indicating the planes from where Z-stack are produced, showing S100A4 staining throughout the nucleus (scale bar = 5 µm)
Supplementary Fig. 2

Lentiviral modified cells. a SW620 cells were modified to either have lower total expression (SW620<sup>shS100A4</sup>) or higher nuclear expression (SW620<sup>S100A4/NLS</sup>) of S100A4. To verify the protein expression, untreated samples were separated into cytoplasmic and nuclear fraction by subcellular fractionation, before protein lysates were made and analyzed with immunoblotting. Lamin B1 was used to validate the quality of the nuclear fraction. Cytoplasmic S100A4 was shown to be lower in cells treated with the shRNA construct (SW620<sup>shS100A4</sup>), while nuclear expression was almost completely abolished. Cells with the nuclear tagged S100A4 variant (SW620<sup>S100A4/NLS</sup>), however, showed enriched...
expression of nuclear protein. This protein is slightly bigger than the endogenous, and visible in the figure around 17 kDa. The untreated SW620, as well as cells treated with non-targeting shRNA (SW620<sup>shNT</sup>), showed similar patterns of expression, with high amounts of S100A4 in the cytoplasmic fraction and low, but evident amounts in the nuclear fraction. RKO cells were modified to have total expression (RKO<sup>S100A4/myc</sup>) or nuclear expression (RKO<sup>S100A4-NLS</sup>) of S100A4. To verify the protein expression, untreated samples were separated into cytoplasmic and nuclear fraction by subcellular fractionation, before protein lysates were made and analyzed with immunoblotting. Lamin B1 was used to validate the quality of the nuclear fraction. Strong cytoplasmic expression was observed in the S100A4/myc cells, with only weak expression of the protein in the nuclear fraction. The protein size just below the 14 kDa band correspond to the size of S100A4, being around 12 kDa, along with the myc-tag slightly larger than 1 kDa. Expression of the protein was visible in both cytoplasmic and nuclear fractions of cells transduced with the nuclear tagged S100A4 variant, however, with slightly higher expression in the nucleus. The protein band was observed around 17 kDa as the sequence in addition to the myc-tag is coupled to the triple NLS sequence of around 2.5 kDa. The control transduced RKO (RKO<sup>empty/myc</sup>) showed no expression of S100A4
Introduction of S100A4 does not lead to changes in proliferation or cell cycle progression. a S100A4 negative cell line RKO was modified to either express total (RKO^{S100A4/myc}) or nuclear (RKO^{S100A4/NLS}) S100A4. However, no changes in cell cycle distribution were seen between the
variants and the control cells (RKO\textsuperscript{empty/myc}) using fluorescent-activated cell sorting (FACS). b No significant changes in proliferation were seen between the variant and the control cell line when cell number were determined after 24, 48 and 72 hours of growth (n=3). c Modified RKO cells were treated with nocodazole before surface attached (+16h), as well as cells in the medium (Med.), were collected for proteins analysis. Expression of selected G2/M markers were examined in the modified cell lines and compared to the control (representative immunoblot from n=2)
Supplementary Fig. 4

Localization of endogenous S100A4 in G2/M. a Proximity ligation assay (PLA) was performed in HCT116 cells, using antibodies against S100A4 and cyclin B1. Positive signals were observed (visualized as black dots) in samples treated with the two antibodies, while no signal was observed in HCT116 cells treated with S100A4 along with an unspecified antibody (Neg. ctrl.; scale bar = 10 µm).

b Glass slides with HCT116 cells were treated with anti-S100A4 (green), anti-pericentrin (PCNT; red), before prometaphase-cells were determined. Enrichment of S100A4 in regions corresponding to the
centrosomal marker PCNT was observed. The same cell is represented in one panel taken by confocal laser scanning microscopy (left panel), as well as z-stack pictures generated from the same picture (right panel; scale bar = 2 µm)