

# Involvement of cancer-derived EMT cells in the accumulation of $^{18}\text{F}$ -fluorodeoxyglucose in the hypoxic cancer microenvironment

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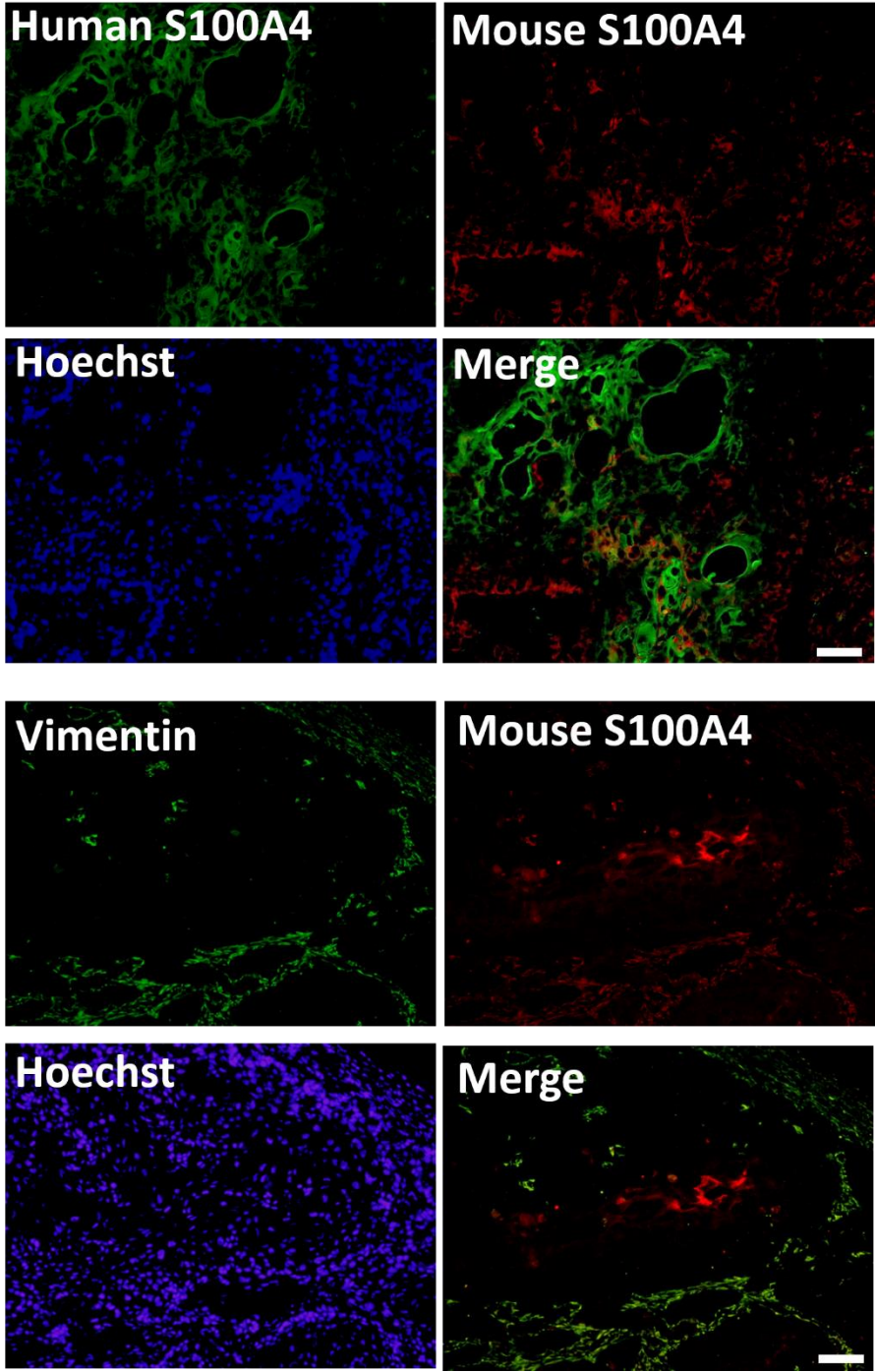
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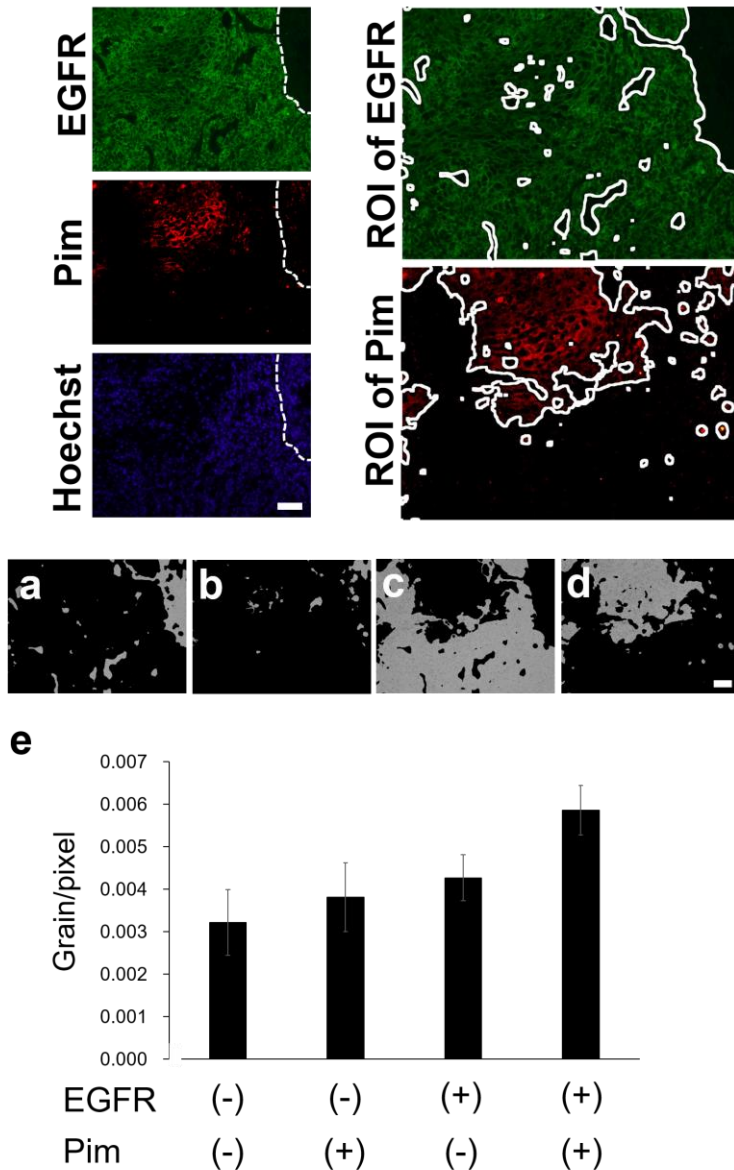
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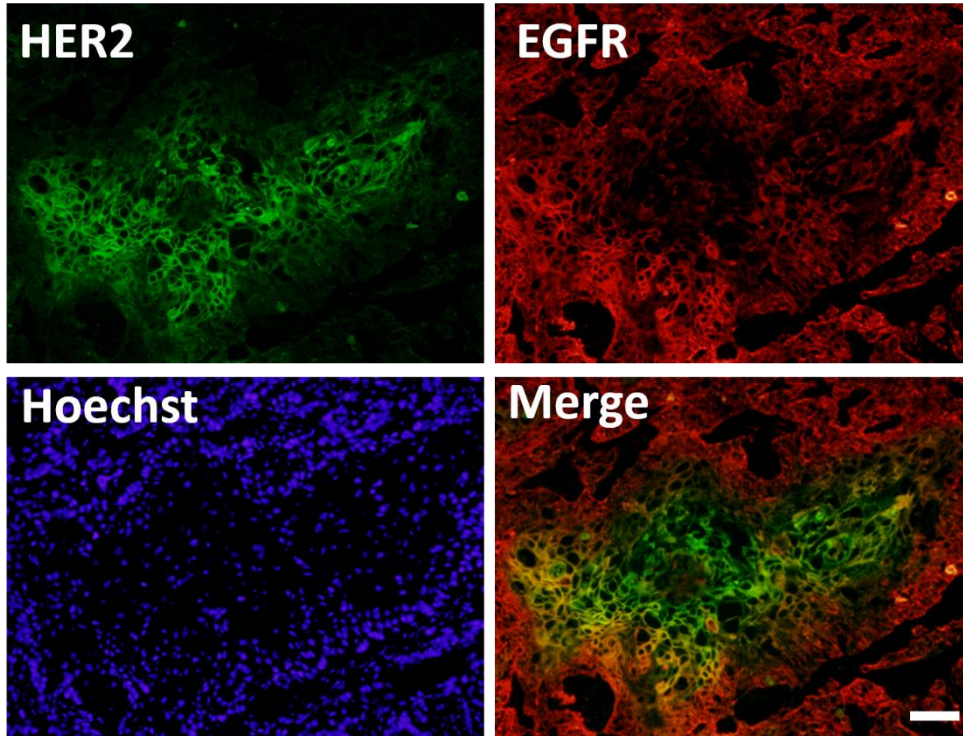
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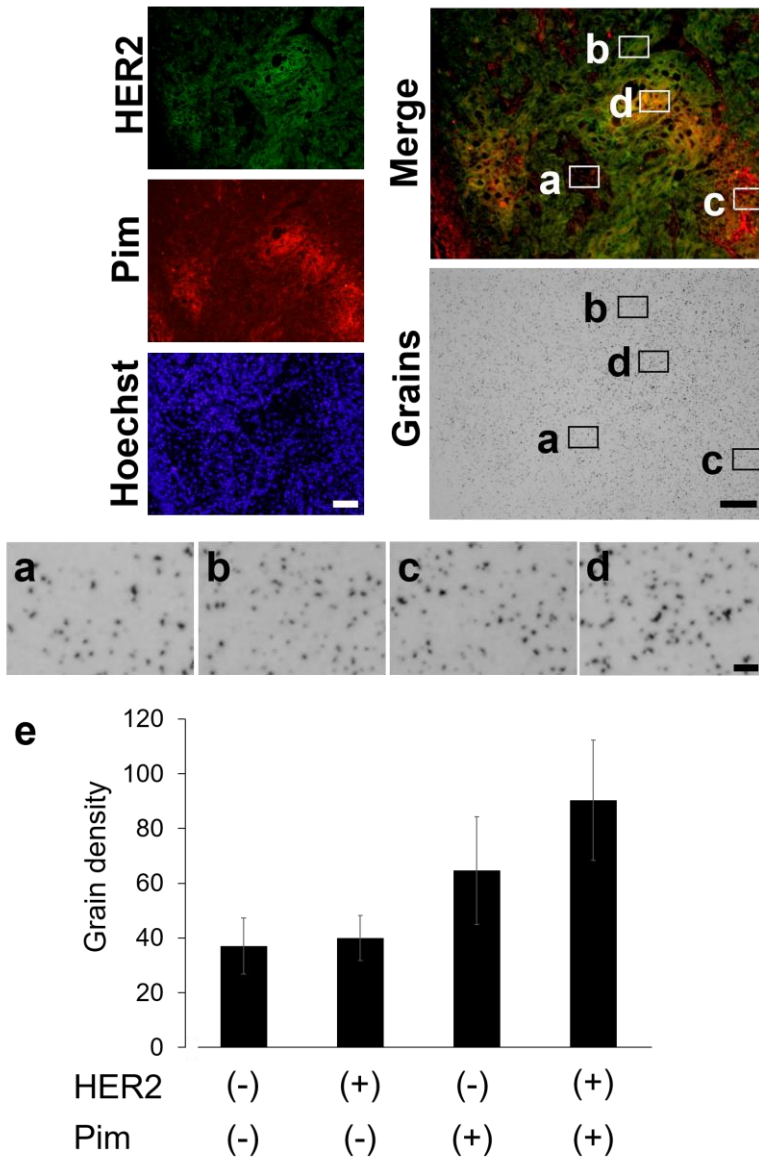
**Supplementary Fig. 1 Human S100A4, Mouse S100A4, and vimentin expression patterns in A431 tumor cells. Bars: 100  $\mu$ m**



**Supplementary Fig. 2  $^{18}\text{F}$ -FDG accumulation in hypoxic cells expressing EGFR.** The dotted line shows the boundary between the tumor and the skin area. The white line shows the computer determined ROI. a) ROI image showing the pimonidazole- and EGFR-negative area, b) pimonidazole-positive and EGFR-negative area, c) pimonidazole-negative and EGFR-positive area, and d) pimonidazole- and EGFR-positive area. e) Determination of the grain density in the pimonidazole- and EGFR-positive or negative area in each ROI (n = 3, mean  $\pm$  SEM). Pim: Pimonidazole. Bars: 100  $\mu\text{m}$

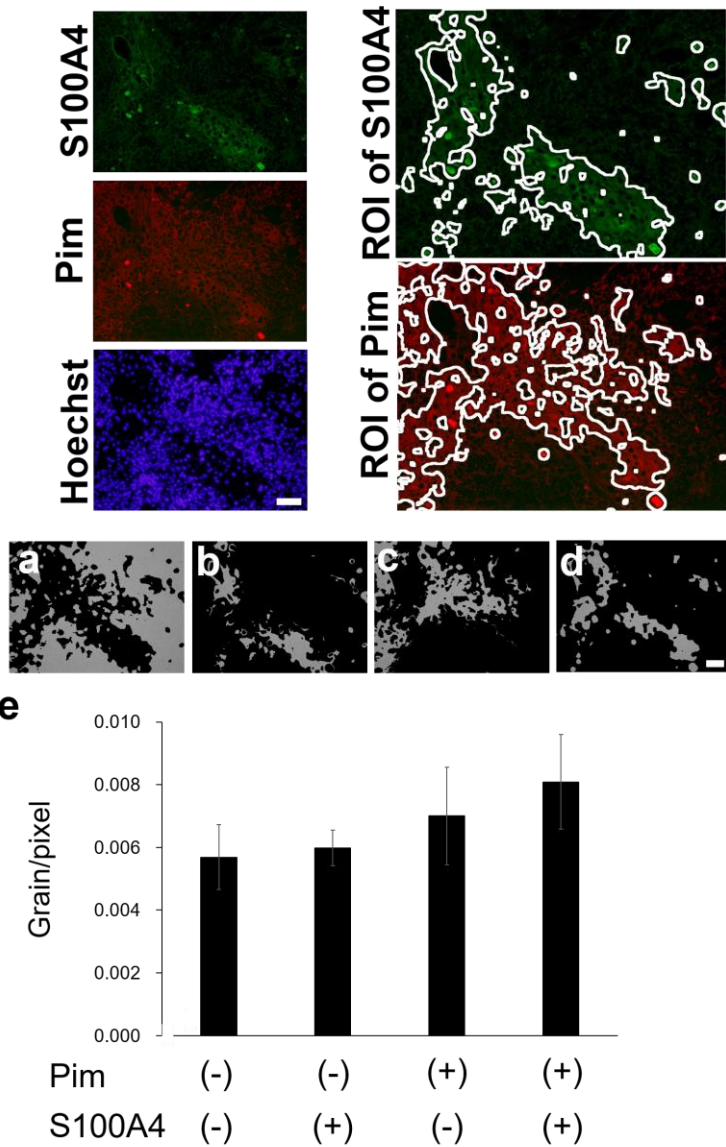


**Supplementary Fig. 3 EGFR and HER2 expression patterns in A431 tumor cells. Bar: 100  $\mu\text{m}$**

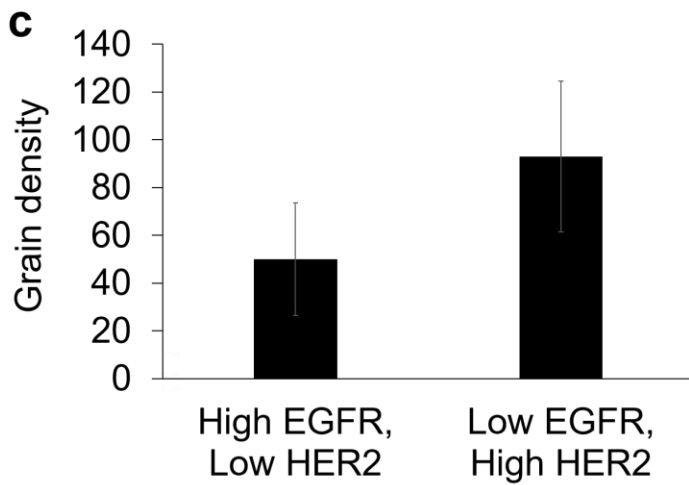
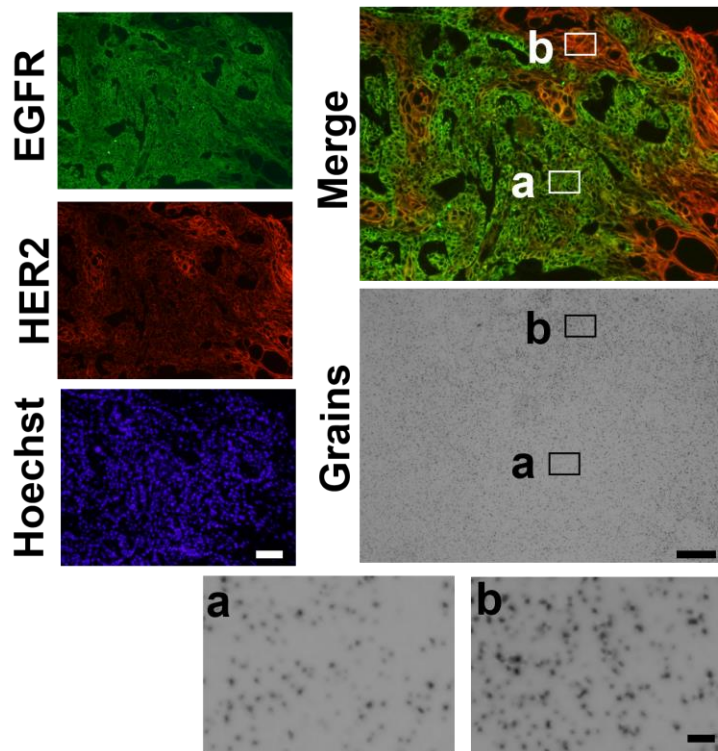


**Supplementary Fig. 4**  $^{18}\text{F}$ -FDG accumulation in hypoxic cells expressing HER2. a)

Magnified view of the micro-autoradiographic image showing the pimonidazole- and HER2-negative area, b) pimonidazole-negative and HER2-positive area, c) pimonidazole-positive and HER2-negative area, and d) pimonidazole- and HER2-positive area. e) Determining the grain density in the pimonidazole- and HER2-positive and negative areas by focusing on ROIs of the same size (n = 3, mean ± SEM). Pim: Pimonidazole. Bars: 100  $\mu\text{m}$  (HER2, Pimonidazole, Hoechst, Merge and Grains), 10  $\mu\text{m}$  (a-d)

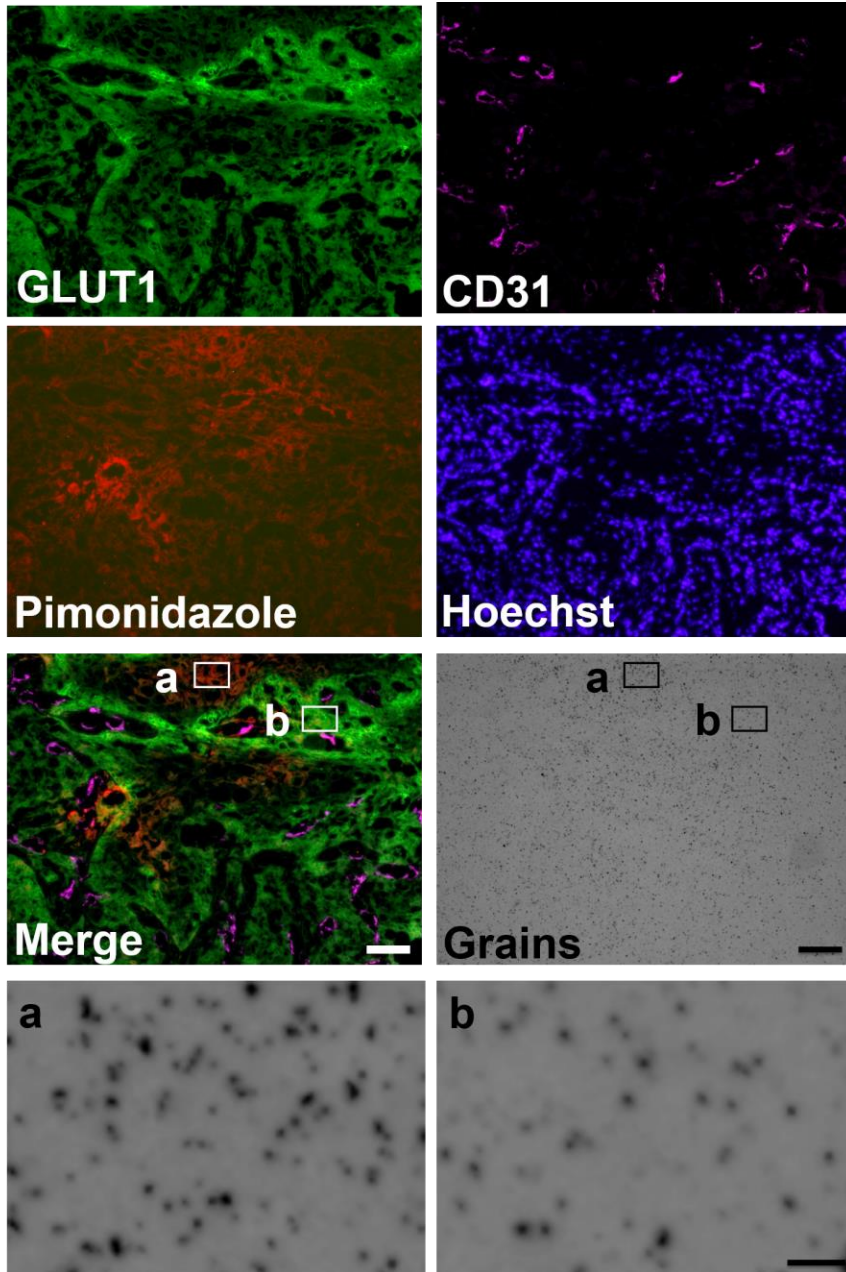


**Supplementary Fig. 5**  $^{18}\text{F}$ -FDG accumulation in hypoxic tumor cells expressing human **S100A4**. The white line shows the computer-determined ROI. a) Merged micro-autoradiography and ROI image showing the pimonidazole- and human S100A4-negative area, b) pimonidazole-negative and human S100A4-positive area, c) pimonidazole-positive and human S100A4-negative area, and d) pimonidazole- and human S100A4-positive area. e) Grain density in the pimonidazole- and human S100A4-positive or negative area in each ROI (n = 3, mean  $\pm$  SEM). Pim: Pimonidazole. Bars: 100  $\mu\text{m}$



**Supplementary Fig. 6  $^{18}\text{F}$ -FDG accumulation in tumor cells expressing EGFR or HER2.**

The ROI images (a, and b) show the regions with intensively stained EGFR and weakly stained HER2 (high EGFR/low HER2, a) and with weakly stained EGFR and intensively stained HER2 (low EGFR/high HER2, b). c) Grain density in the region with high EGFR/low HER2 or low EGFR/high HER2 (n = 3, mean  $\pm$  SEM). Pim: Pimonidazole. Bars: 100  $\mu\text{m}$ . (EGFR, HER2, Hoechst, Merge and Grains), 10  $\mu\text{m}$  (a, b)



**Supplementary Fig. 7  $^{18}\text{F}$ -FDG accumulation in tumor cells expressing GLUT1 and Pimonidazole.** The ROI images (a, and b) show the regions with intensively stained pimonidazole and weakly stained GLUT1 (a) and with weakly stained pimonidazole and intensively stained GLUT1 (b). Bars: 100  $\mu\text{m}$  (GLUT1, Pimonidazole, CD31, Hoechst, Merge and Grains), 10  $\mu\text{m}$  (a, b)