

Expanded View Figures

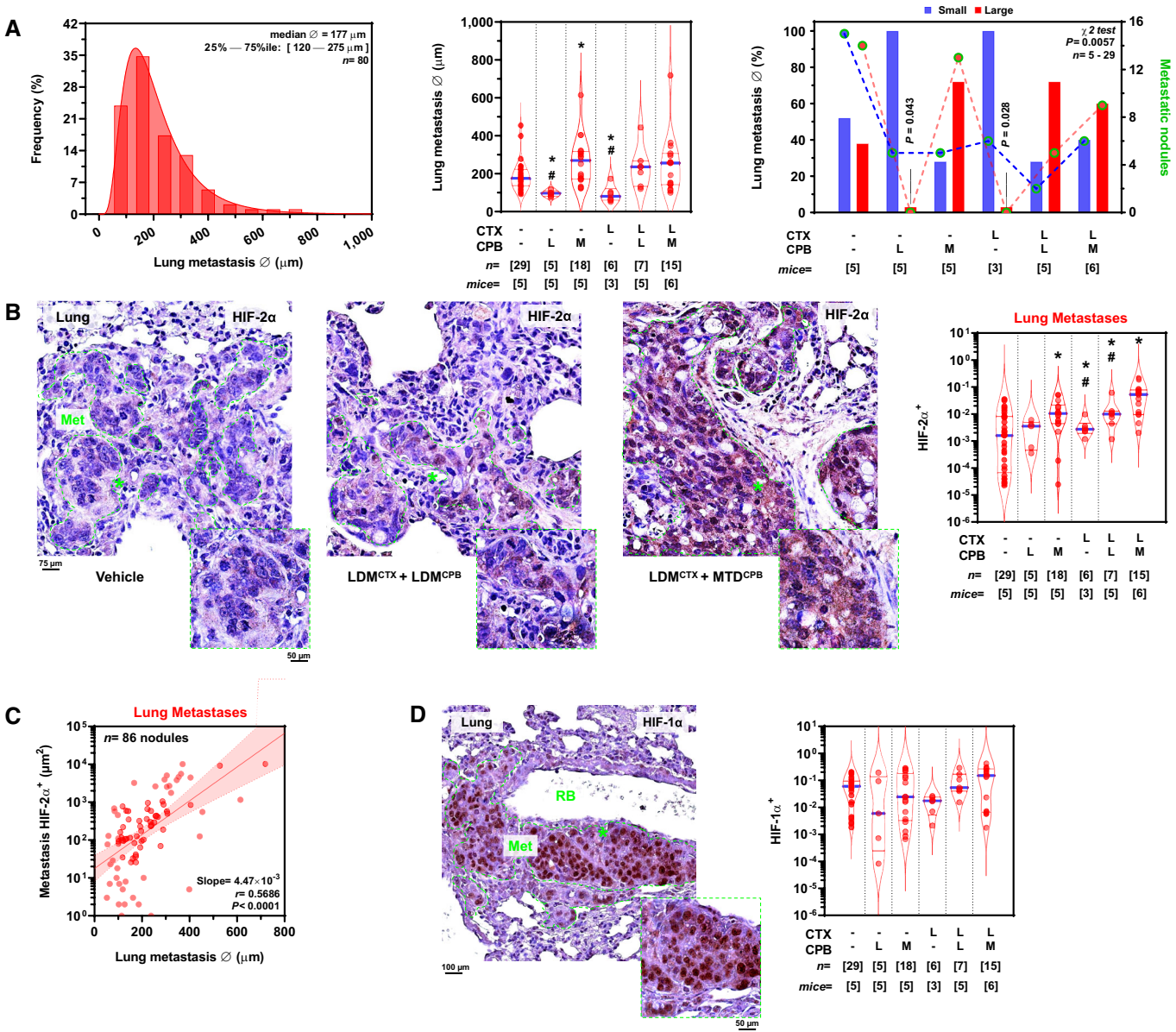


Figure EV1.

Figure EV1. LDM chemotherapy offsets HIF-2 α levels in colon cancer metastases to the lung.

- A Effect of LDM and MTD chemotherapy on HT29 lung metastatic nodule size. *Left*: Histogram of cross-sectional metastatic diameter ($\emptyset$). *Middle*: Metastatic diameter ($\emptyset$) by chemotherapy regimen. $F_{5,43} = 9.066$ and $P < 0.0001$ for overall treatment by Brown–Forsythe ANOVA; $*P < 0.05$ versus vehicle; $^{\#}P < 0.01$ versus LDM^{CTX} + MTD^{CPB} by Benjamini, Krieger, and Yekutieli *post hoc* test. *Right*: Dichotomized metastatic size at median diameter of vehicle-treated tumors. Lung nodule size was classified as small (blue) or large (red). Individual nodule counts per group and size category (small/large) are plotted on the right ordinate. χ^2 (df = 5) = 16.44, $P = 0.0057$ for overall effects on size; χ^2 (df = 1) = 4.10, $P = 0.043$ LDM^{CPB} versus vehicle; χ^2 (df = 2) = 7.00, $P = 0.028$ LDM^{CTX} versus vehicle.
- B HIF-2 α levels in HT29 lung metastatic nodules. *Left*: vehicle-treated controls. *Middle/left*: LDM doublet cyclophosphamide + capecitabine (LDM^{CTX} + LDM^{CPB}). *Middle/right*: LDM cyclophosphamide + MTD capecitabine (LDM^{CTX} + MTD^{CPB}). *Right*: Automatic quantification of the effect of monotherapies or doublet regimens on HIF-2 α^* areas in individual metastatic nodules. $F_{5,64} = 12.38$ and $P < 0.0001$ for overall treatment by Brown–Forsythe ANOVA; $*P < 0.01$ versus vehicle; $^{\#}P < 0.05$ versus LDM^{CTX} + MTD^{CPB} by Benjamini, Krieger, and Yekutieli *post hoc* test.
- C Effect of lung metastatic diameter on HIF-2 α levels. Individual nodule diameter and HIF-2 α^* area (logarithm). Regression line (red) and 95% CI (shaded red area) are shown. $F_{1,84} = 40.18$ and $P < 0.0001$; slope $\neq 0$ by *F*-test.
- D HIF-1 α levels in HT29 lung metastatic nodules. *Left*: Example of HIF-1 α expression in a para-bronchiolar nodule. RB, respiratory bronchiole showing lysed intraluminal erythrocytes. Low power magnification image can be found in Appendix Fig S5B. *Right*: Automatic quantification of the effect of monotherapies or doublet regimens upon intra-metastatic HIF-1 α^* area. $F_{5,14} = 1.670$ and $P = 0.2053$ (not significant) for overall treatment by Brown–Forsythe ANOVA.

Data information: Violin plots present 50th (blue line), 25th, and 75th percentiles (red lines); numbers in brackets indicate number of nodules (*n*) or animals (mice). L, low-dose metronomic; M, maximum-tolerated dose; Met, metastasis; *r*, correlation coefficient. Dashed green lines encircle the histological limit between metastatic nodules and surrounding normal lung parenchymae. *Insets*, high-magnification images of regions marked with asterisks (*) in all microphotographs. Low power magnification images of all experimental conditions of B can be found in Appendix Fig S5A.

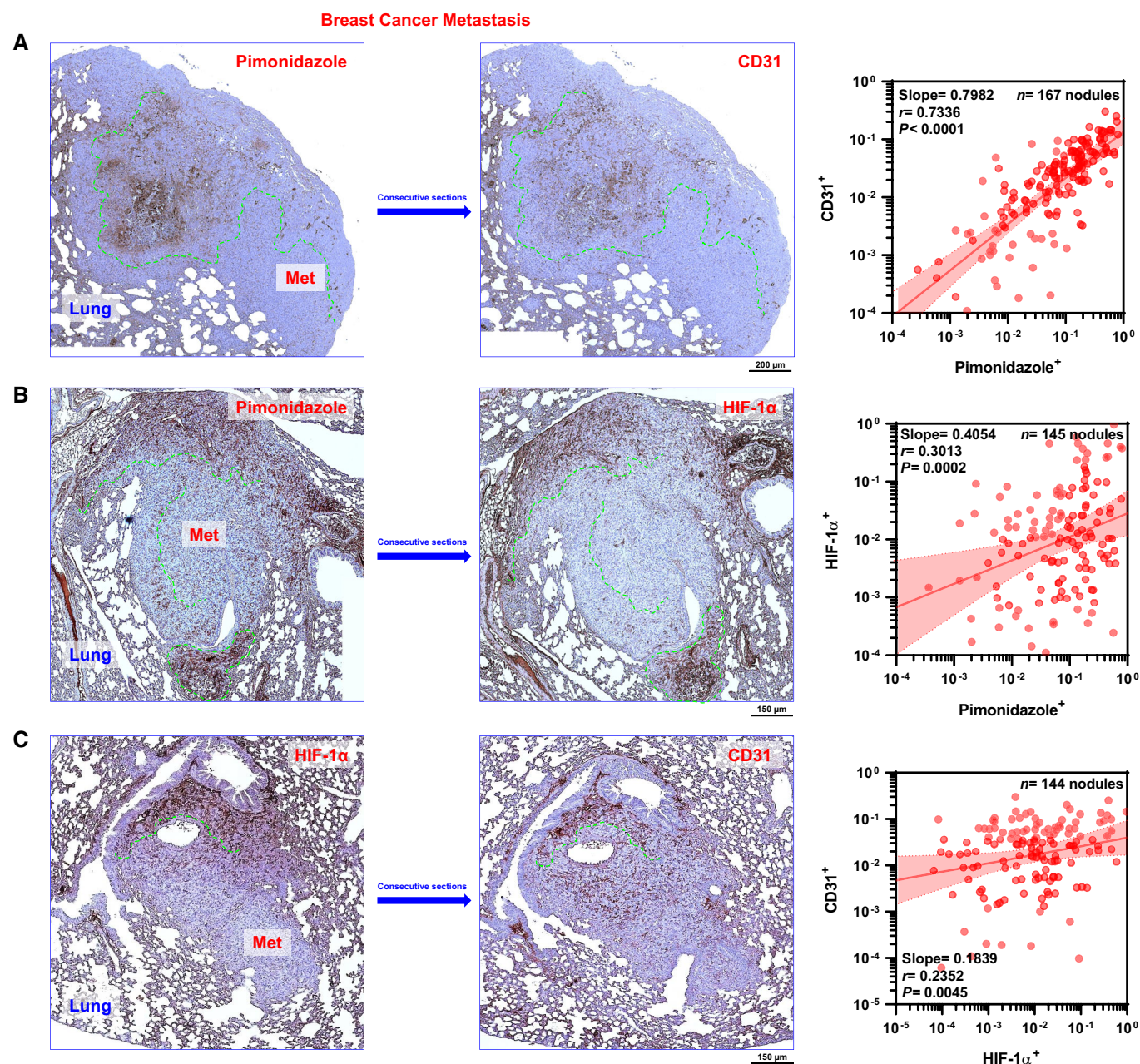


Figure EV2. Relationship among hypoxia, HIF-1 α , and microvessel density in breast cancer metastases to the lung.

- A Intra-metastatic hypoxia (pimonidazole) and microvessel density (CD31). *Left and middle*: Consecutive sections showing pimonidazole (*left*) and CD31 (*middle*) immunoreactivities. *Right*: Correlation between intra-metastatic hypoxia (pimonidazole) and microvessel density (CD31). Points represent median values per nodule. $F_{1,165} = 192.2$ and $P < 0.0001$, slope $\neq 0$ by F -test.
- B Intra-metastatic hypoxia (pimonidazole) and HIF-1 α levels. *Left and middle*: Consecutive sections showing pimonidazole (*left*) and HIF-1 α (*middle*) immunoreactivities. *Right*: Correlation between intra-metastatic hypoxia (pimonidazole) and HIF-1 α . Points represent median values per nodule. $F_{1,143} = 14.3$ and $P = 0.0002$, slope $\neq 0$ by F -test.
- C HIF-1 α levels and microvessel density (CD31). *Left and middle*: Consecutive sections showing HIF-1 α (*left*) and CD31 (*middle*) immunoreactivities. *Right*: Correlation between HIF-1 α and CD31. Points represent median values per nodule. $F_{1,142} = 8.313$ and $P = 0.0045$, slope $\neq 0$ by F -test.

Data information: *Pearson* regression line (red) and 95% CI (shaded red area) are indicated. Immunoreactivities are expressed as fractions of nodular areas. *Met*, metastasis; r , correlation coefficient. Dashed green lines demarcate corresponding intra-metastatic areas of immunoreactivity among pimonidazole, CD31, and HIF-1 α in consecutive histological sections from example specimens (blue frames/arrows).