

Figure S1. Individual body weight record of all the mice subjected to metastatic xenograft experiments. In Exp 1, 8 mice received either 10-cell or 1-cell inoculations. In Exp 2, 18 mice received both 10- and 1-cell inoculations. In Exp 3, 26 mice received 10- and 1-cell inoculations. Exp, experiment.

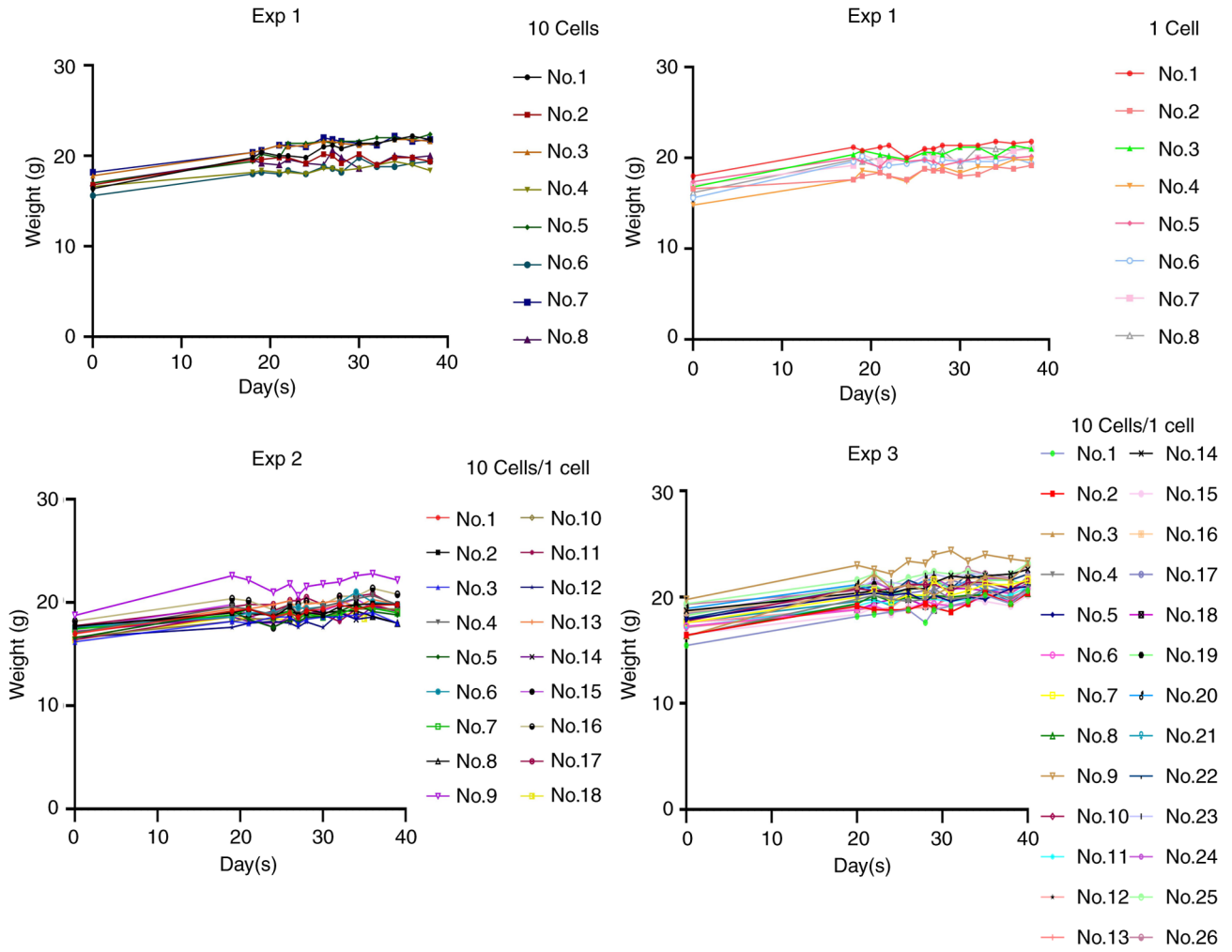


Figure S2. Image of all the xenograft tumors collected with their net weight and estimated volume as measured post-extraction in the metastatic colony incidence experiments. Red frames indicate paired tumors developed from 10-cell inoculates that were grown in the same mouse. Green frames indicate paired tumors developed from 1-cell inoculates that were grown in the same mouse. Scale bar, 5 mm. Exp, experiment.

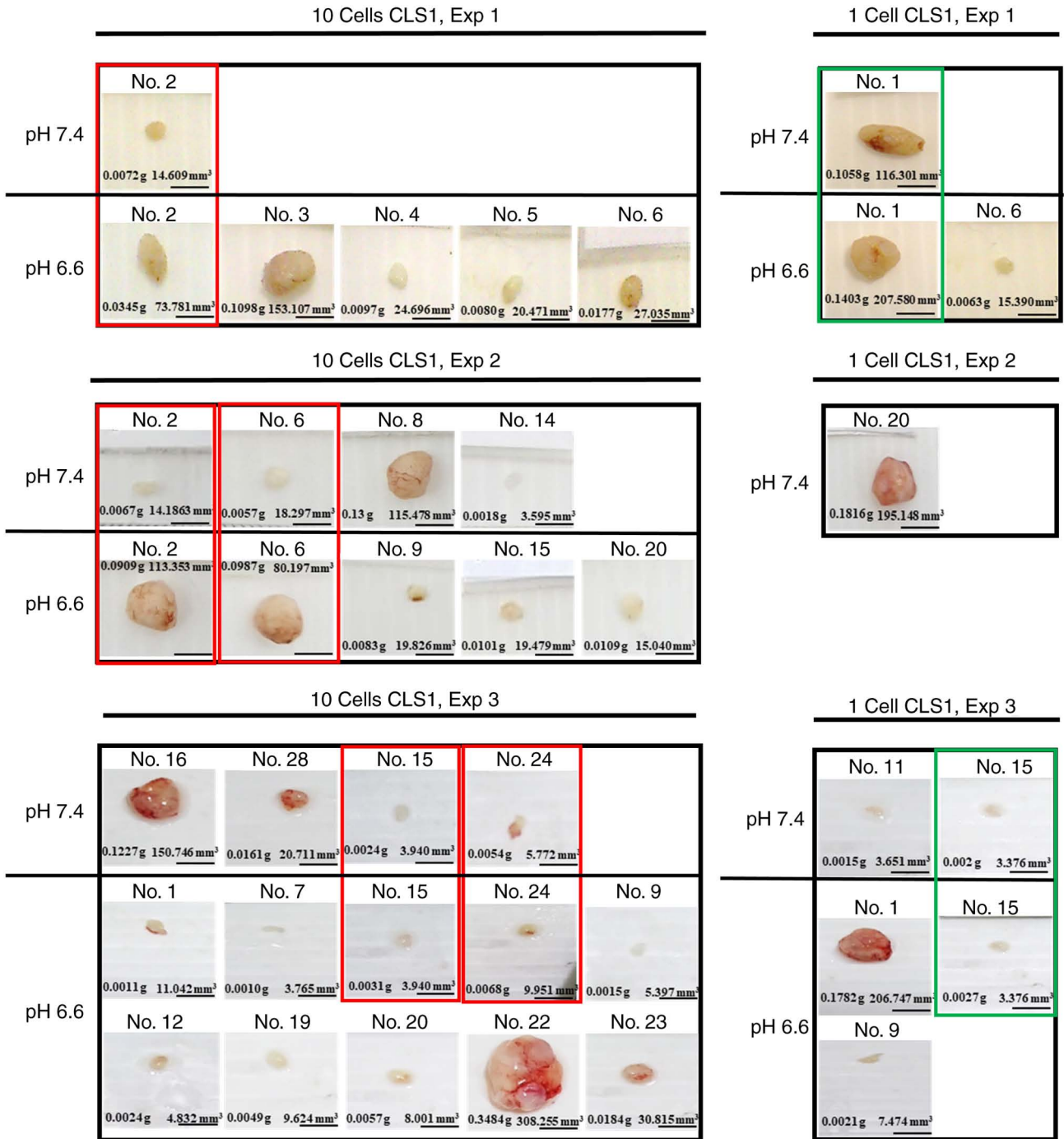


Figure S3. Representative image of an experimental animal that did not develop a tumor. Post-mortem dissection was performed to inspect for any subcutaneous tumors that could not be detected by palpation. A total of 6 small-volume tumors were identified in this manner.

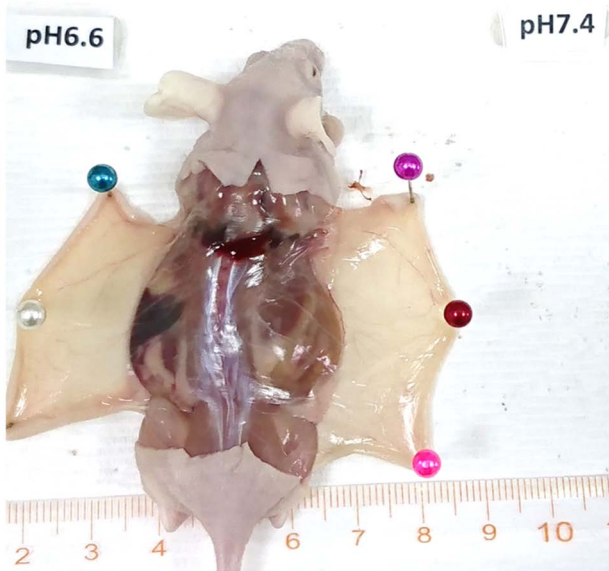


Figure S4. Heatmap showing the 1,735 acidosis-responsive genes. Each column represents a sample, and each row represents a gene. Orange and purple colors indicate the level of upregulation and downregulation of the genes, respectively. Two independent experiments with two biological replicates were performed. Exp, experiment.

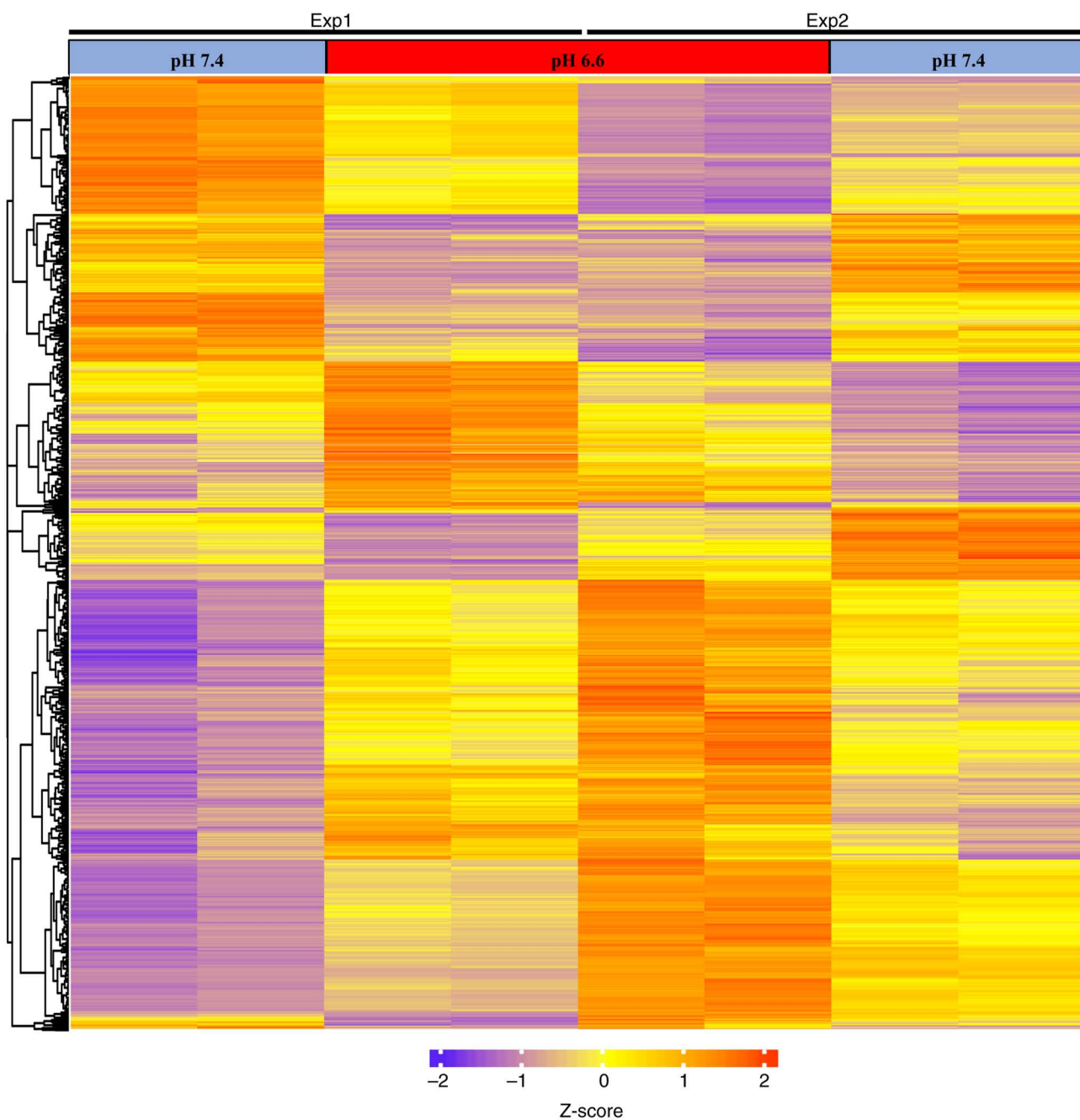


Figure S5. Additional parameters for vasculogenic mimicry activity of acidotic cells. (A) Quantification of vasculogenic mimicry structures from all xenograft tumors grown. *In vitro* vasculogenic mimicry capability of the pH 7.4 or pH 6.6 cells quantified as (B) the percentage of the total loop squared area per well, and (C) the number of tubes detected at 10-h post-seeding. Data are presented as the mean \pm SD of three independent experiments and were analyzed using Student's unpaired t-test. * $P < 0.05$ and **** $P < 0.0001$ compared with pH 7.4. PAS, periodic acid-Schiff.

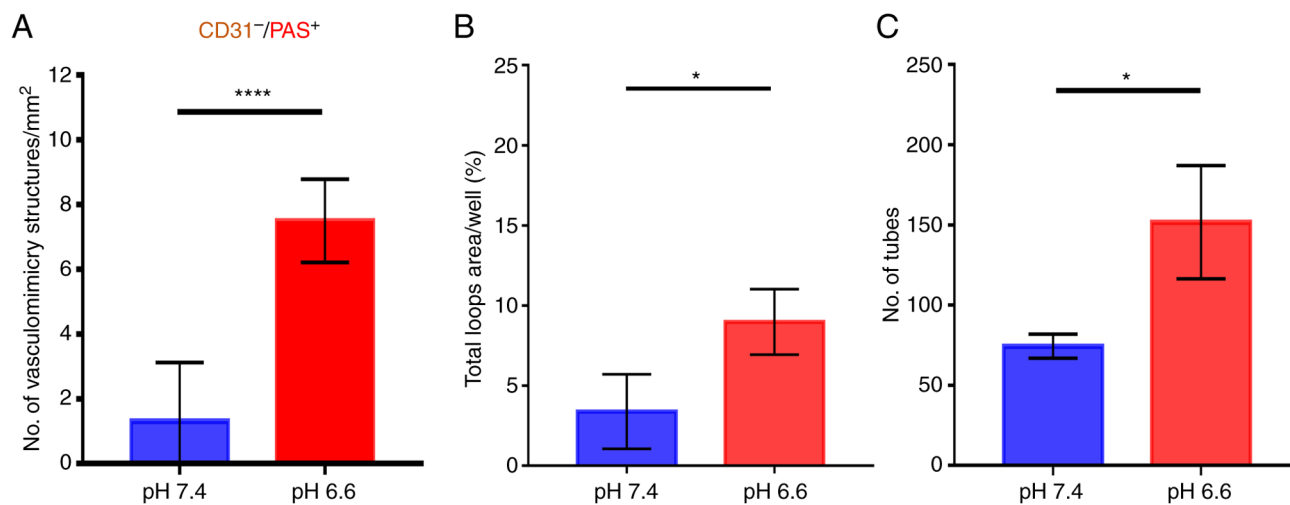


Figure S6. Upregulation of *ITGA4* expression can be elicited upon short-term acid stimulation, and this is further amplified after long-term acidosis. After 24 h, acid stimulation elicited an ~2-fold upregulation of *ITGA4* mRNA read counts, whereas an almost 10-fold upregulation of *ITGA4* expression was observed after 2 months of continuous acidosis. Data from two different groups were compared using Student's unpaired t-test. *** $P < 0.001$ compared with pH 7.4. *ITGA4*, integrin subunit α -4.

