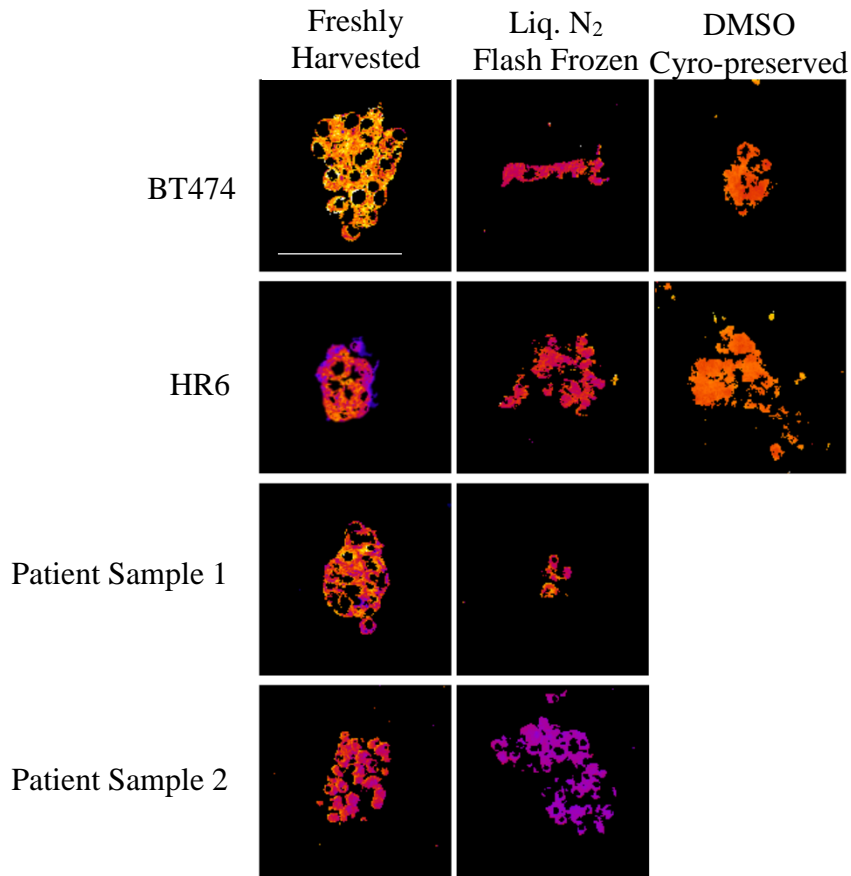


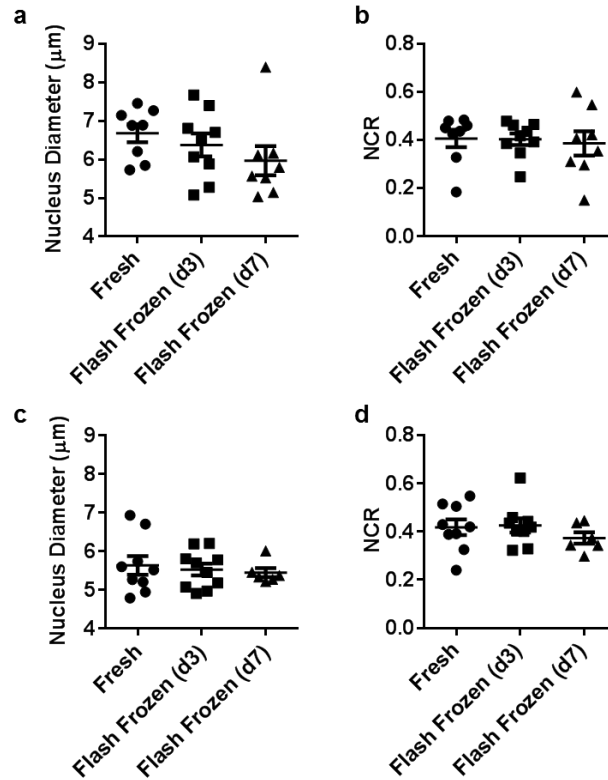
Supplementary Information for

“Drug response in organoids generated from frozen primary tumor tissues”

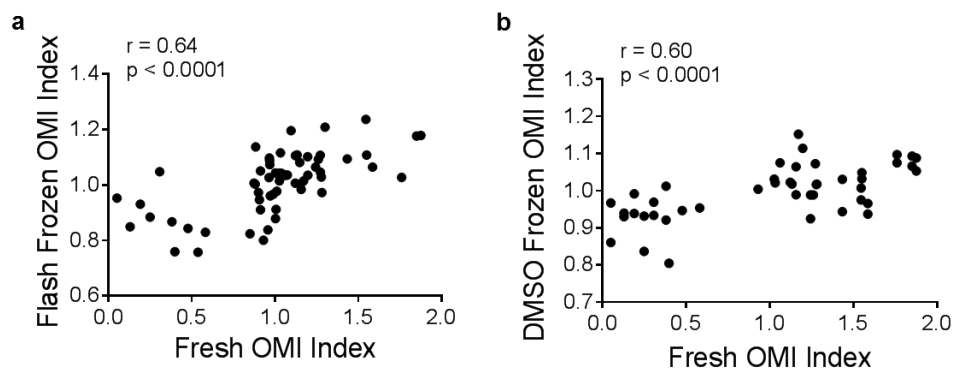
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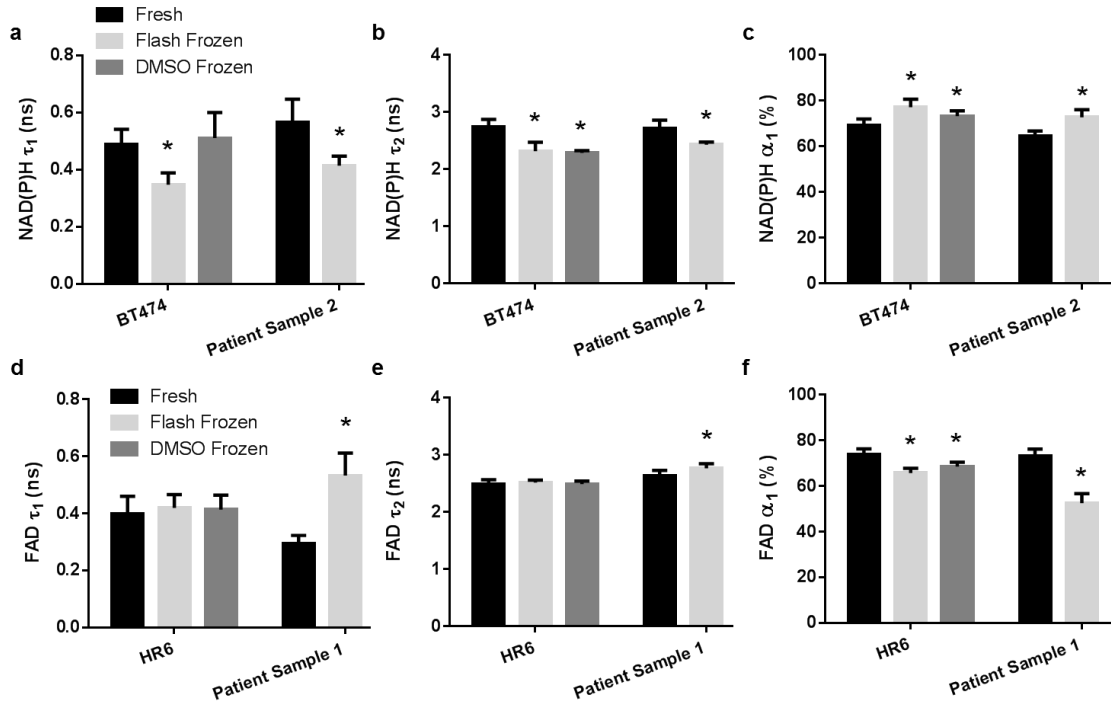
Supplementary Fig. 1: Representative redox ratio images of BT474, HR6, Patient Sample 1, and Patient Sample 2 organoids derived from fresh, flash frozen, and DMSO frozen tissues. Scale bar is 100  $\mu$ m.



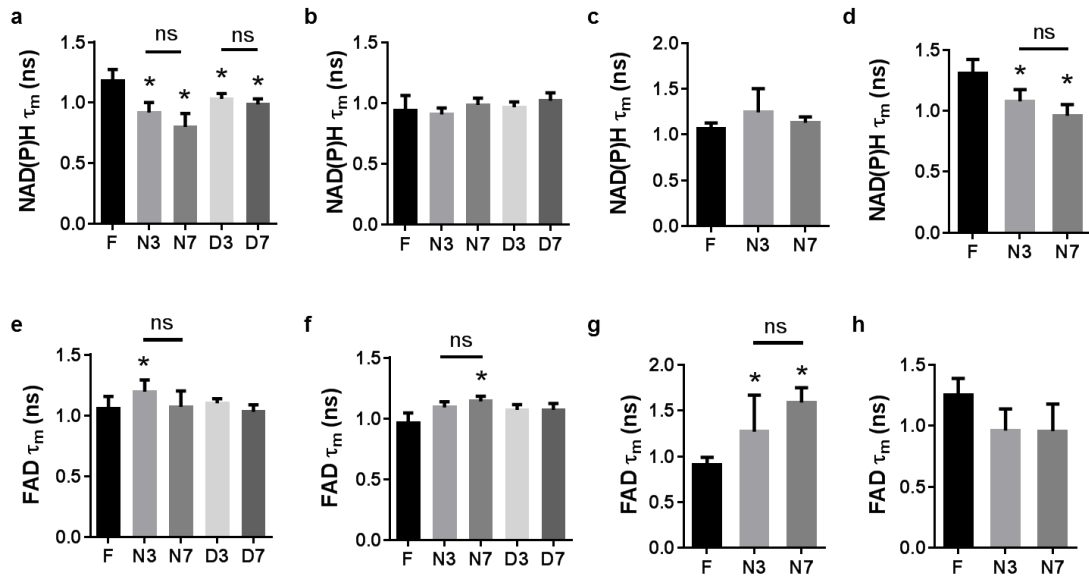
Supplementary Fig. 2: (a) Nucleus diameter and (b) nuclear to cytoplasm ratio (NCR) for organoids derived from fresh and flash-frozen tissues of Patient Sample 1. (c) Nucleus diameter and (d) nuclear to cytoplasm ratio (NCR) for organoids derived from fresh and flash-frozen tissues of Patient Sample 2. d3/d7 represents organoids grown for 3 or 7 days after generation. n = 5-10 organoids.



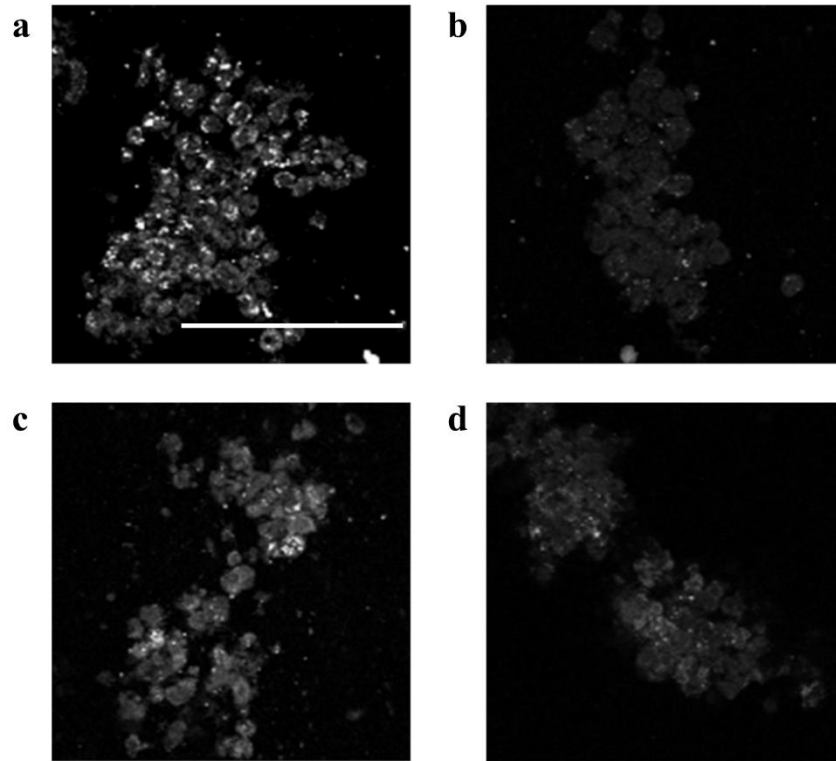
Supplementary Fig. 3: Correlation between OMI index values for drug response studies of organoids derived from fresh and flash-frozen thawed tissue (a) or DMSO frozen tissue (b). r is Spearman's correlation coefficient.



Supplementary Fig. 4: (a) NAD(P)H  $\tau_1$ , (b) NAD(P)H  $\tau_2$ , and (c) NAD(P)H  $\alpha_1$  for organoids derived from fresh tissues and organoids derived from frozen tissues and grown for 7 days. (d) FAD  $\tau_1$ , (e) FAD  $\tau_2$ , and (f) FAD  $\alpha_1$  for organoids derived from fresh tissues and organoids derived from frozen tissues and grown for 7 days. \*  $p < 0.05$  vs. Fresh; mean  $\pm$  SE.  $n = 30-300$  cells.



Supplementary Fig. 5: NAD(P)H  $\tau_m$  of (a) BT474, (b) HR6, (c) Patient Sample 1, and (d) Patient Sample 2 organoids grown from fresh and frozen tissue. FAD  $\tau_m$  of (e) BT474, (f) HR6, (g) Patient Sample 1, and (h) Patient Sample 2 organoids grown from fresh and frozen tissue. d3/d7 represents organoids grown for 3 or 7 days after generation. F = Fresh, N3/7 = Flash frozen and grown for 3/7 days, D3/7 = DMSO frozen and grown for 3/7 days. \*  $p < 0.05$  vs. Fresh; ns = not significant, mean  $\pm$  SE.  $n = 30-300$  cells.



Supplementary Fig. 6: Representative immunofluorescence images of HR6 organoids derived from fresh tissue (a,b) or flash frozen tissue (c,d) and stained for Ki67 (a,c) and cleaved caspase 3 (b,d). Scale bar is 100  $\mu$ m.