

SI Table1: Primers used for the quantitative polymerase chain reaction

Gene Symbol	Gene Name	Primer Sequence (5' - 3')	
<i>HIF1A</i>	<i>Hypoxia-inducible factor-1-alpha</i>	F:	CCT CTG TGA TGA GGC TTA CCA TC
		R:	CAT CTG TGC TTT CAT GTC ATC TTC
<i>NES</i>	<i>Nestin</i>	F:	CTT CCC TCC GCA TCC CGT CA
		R:	AAA GCC AGC ATG TCA CCC TC
<i>SNAI1</i>	<i>Snail family transcriptional repressor-1</i>	F:	CGG TTC CGA TGC CCT GAG GCT C
		R:	CGT CAC ACT TCA TGA TGG AAT TG
<i>TWIST1</i>	<i>Twist basic helix-loop-helix transcription</i>	F:	ACC ATC CTC ACA CCT CTG CAT
		R:	TTC CTT TCA GTG GCT GAT TGG
<i>VEGFA</i>	<i>Vascular endothelial growth factor A</i>	F:	AGG GCA GAA TCA TCA CGA AG
		R:	CAC ACA GGA TGG CTT GAA G
<i>CD44</i>	<i>Cluster of differentiation 44</i>	F:	CGC CAA ACA CCC AAA GAA
		R:	GTG TTG TCC TTC CTT GCA TT
<i>GAPDH</i>	<i>Housekeeping gene</i>	F:	ACC ACA GTC CAT GCC ATC AC
		R:	TCC ACC ACC CTG TTG CTG TA

F: Forward; **R:** Reverse;

SI Table 1: Comparison of Spheroid Culture Techniques in MDA-MB-468 TNBC Cells under Varying Oxygen Conditions in this study

Parameter	Hanging Drop (HD)	Liquid Overlay (ULA/Agarose) *	Scaffold-Based
Spheroid Formation Time	Moderate (24–36 h)	Dependent on HD pre-formation	Fast (24–48 h)
Structural Integrity	Moderate under (NOC)	High (especially ULA under NOC)	Variable (less compact under LOC)
Morphometric Uniformity (Sphericity)	Variable	High under NOC, moderate under LOC	Moderate; perimeter fluctuation observed
Ease of Handling & Imaging	Moderate; prone to disruption	High (ULA) / Moderate (Agarose)	Low (scaffold material obscures visualisation)
Gene Expression Consistency	Moderate	High (notably <i>CD44</i> , <i>TWIST1</i> , <i>VEGFA</i> under NOC)	less responsive under LOC
Hypoxia Simulation (<i>HIF1A</i> , <i>VEGFA</i>)	Poor under LOC	NOC mimics pseudo-hypoxia response	Inconsistent, sometimes inverse expression
Reproducibility	Moderate	High	Moderate

LOC: low oxygen condition; **HD:** hanging drop; **NOC:** normal oxygen condition; **ULA:** ultra-low attachment; *, Rat tail collagen I added to the complete media