А	Induction tre Spikes 7-9 days at 4°C	eatment → Anthers 2 hours at RT	Wash 5 min. at RT	Anther Culture 40 days at 25°C
CLD (control)	Cold (water)	MS3M	MS3M medium	OVPCM medium
C+0T (DMSO control)	Cold (water)	<ul> <li>MS3M + 1% DMSO</li> </ul>	MS3M medium	OVPCM medium
C+xT (x= 0.1, 0.2, 0.4 µM TSA)	Cold (water)	MS3M+xTSA		OVPCM medium
В	Induction tre Anthers 5 days at 25°C —	eatment → Anthers 2 hours at RT	Wash 5 min. at RT	Anther Culture 40 days at 25°C
MAN (control)	0.7 M mannitol	MS3M	MS3M medium	<ul> <li>OVPCM medium</li> </ul>
M+0T (DMSO control)	0.7 M mannitol →	MS3M + 1% DMSO	MS3M medium	<ul> <li>OVPCM medium</li> </ul>
M+xT (x=0.1, 0.2, 0.4 µM TSA)	0.7 M mannitol	► MS3M+xTSA	MS3M medium	► OVPCM medium
	Induction treatment	Wash	Excision and Anther Culture	
С	Spikes 7-9 days at 4°C	5 min. at RT	40 days at 25°C	
CLD (control)	Cold (water)	MS3M medium	► OVPCM medium	
C0T (DMSO control)	Cold (water) + 1% DMSO	MS3M medium	<ul> <li>OVPCM medium</li> </ul>	
CxT (x= 0.1, 0.2, 0.4 µM TSA)	Cold (water) + xTSA	► MS3M medium	→ OVPCM medium	
D	Induction treatment Anthers 5 days at 25°C	Wash 5 min. at RT	Anther Culture 40 days at 25°C	
MAN (control)	0.7 M mannitol	MS3M medium	→ OVPCM medium	
M0T (DMSO control)	0.7 M mannitol + 1% DMSO	MS3M medium	<ul> <li>OVPCM medium</li> </ul>	
MxT (x=0.1, 0.2, 0.4 µM TSA)	0.7 M mannitol + xTSA	MS3M medium	<ul> <li>OVPCM medium</li> </ul>	
E	Induction treatment Anthers 5 days at 25°C <sup>1</sup> or 2 hours a	Wash 1 RT <sup>2</sup> 5 min. at RT	Anther Culture 40 days at 25°C	
MAN (control)	0.7 M mannitol <sup>1</sup>	→ MS3M medium –	► OVPCM medium	
0T (DMSO control)	MS3M + 1% DMSO <sup>2</sup>		► OVPCM medium	
xT (x= 0.1, 0.2, 0.4 µM TSA)	MS3M + xTSA <sup>2</sup>	→ MS3M medium –	<ul> <li>OVPCM medium</li> </ul>	

**Supplementary Figure 1.** Scheme of the diverse strategies used to induce microspore embryogenesis in wheat anther culture by the application of trichostatin A (TSA) in combination with or without different stress treatments. Concentrations of 0.1, 0.2, and 0.4  $\mu$ M TSA dissolved in 1% DMSO (0.1T, 0.2T, and 0.4T), and control cultures with 0% DMSO (0T) were tested in all strategies. Anthers cultured with 0.7 M mannitol for 5 days at 25 °C or spikes at 4 °C for 7–9 days were used as control experiments depending on the strategy used. (A) Application of TSA after cold stress treatment, (B) application of TSA after mannitol stress treatment, C) application of TSA in combination with cold stress treatment, (D) application of TSA in combination with mannitol stress treatment. RT = 25 °C.