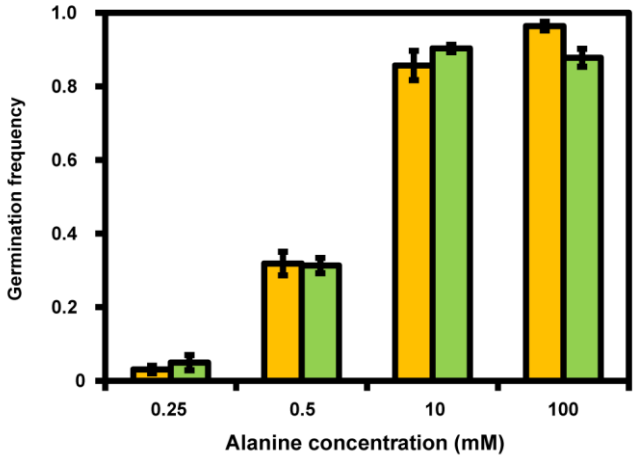
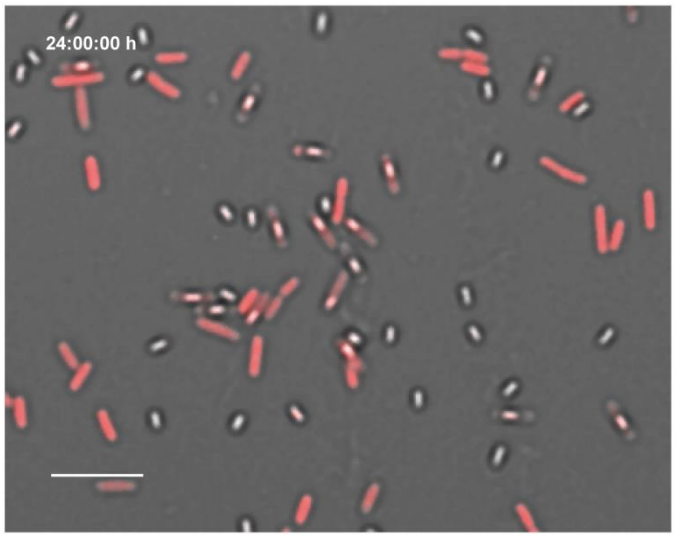
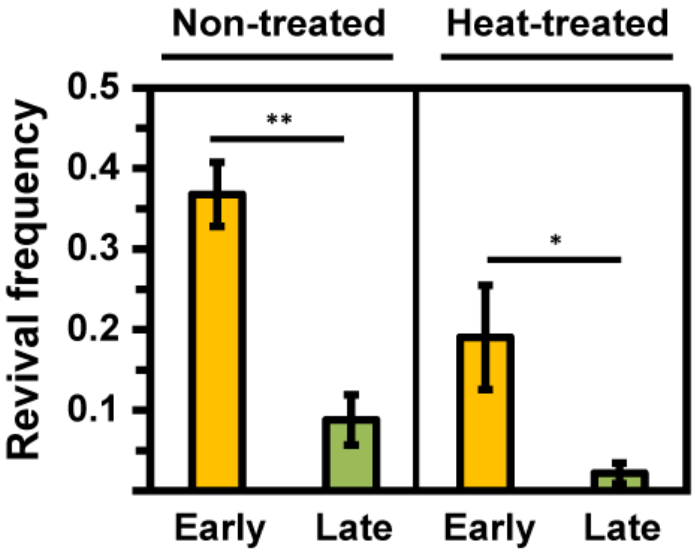
Supplementary Figures



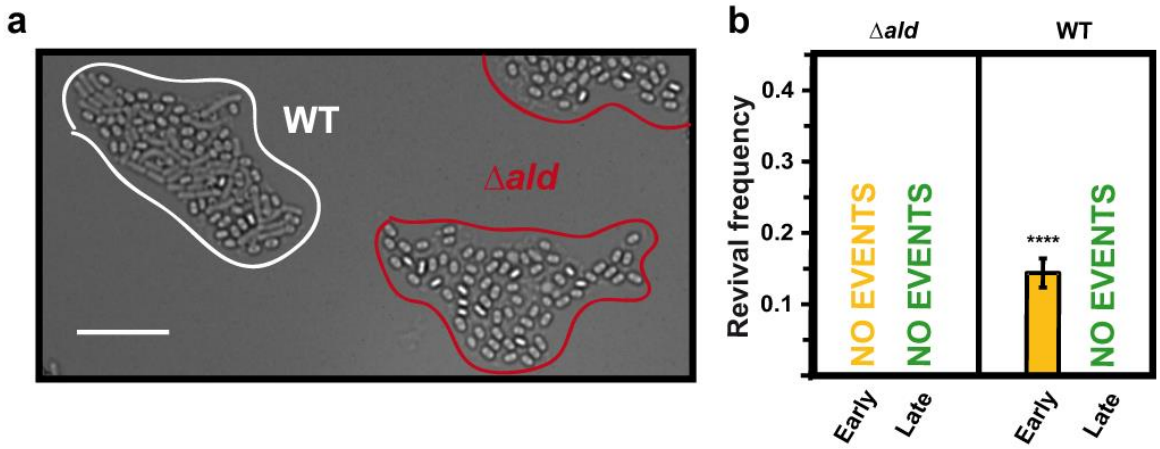
Supplementary Figure 1. *Early* and *late* spores achieve comparable germination frequencies. Germination frequency of *early* (yellow) and *late* spores (green) as a function of the L-alanine concentration that was used for stimulating spores after sporulation on agarose pads. See Methods for details. Results denote the final germination frequency after 2 hours upon which no further germination events were observed in the microcolonies. Strain: BIB1019. Data: mean ± SEM, $n_c = \geq 4$ ($n_s = \geq 200$). Unpaired t-test: the differences between *early* and *late* spores are not significant ($P > 0.05$).



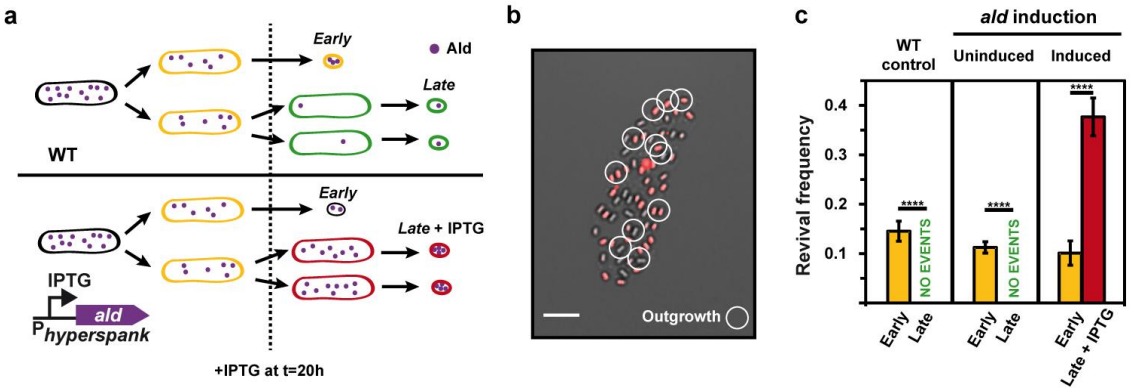
Supplementary Figure 2. Micrograph of P$_{rapA}$-*mCherry* (BIB1126) cells from a culture that was cultivated for 24 h in liquid SM. Spores that have been released from the sporangia do not show any fluorescence (*early* spores). Spores contained within mother cells show some fluorescence, cells that delay sporulation for even longer are strongly fluorescent. Scale bar: 5 µm.



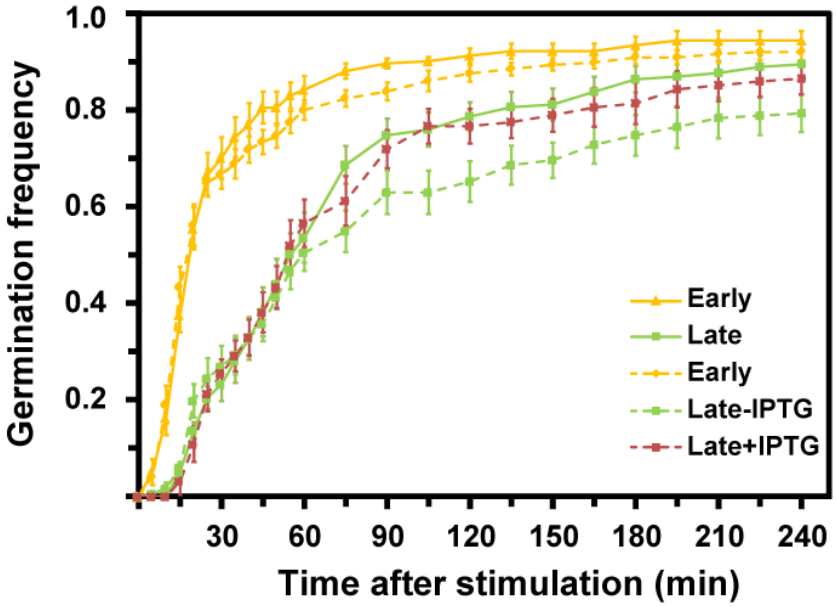
Supplementary Figure 3. Effect of heat-treatment on differential spore revival. Revival frequencies of *early* and *late* spores (BIB1126) which were heat-treated prior to induction with L-alanine on a SM pad. Data: mean ± SEM, n = 4 movies (n_s = ≥ 700), unpaired t-test: * $P \leq 0.05$, ** $P \leq 0.01$.



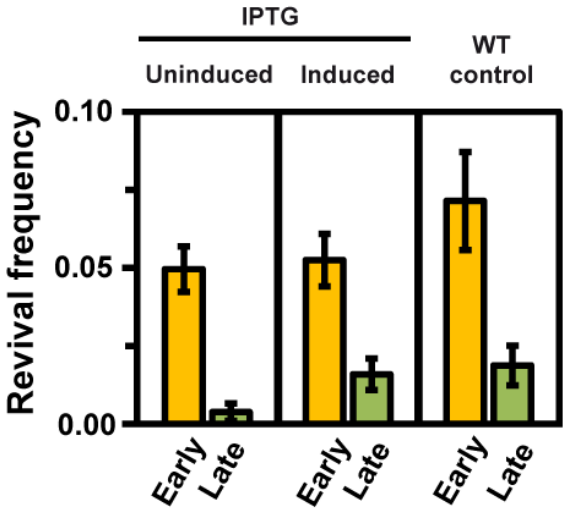
Supplementary Figure 4. Effect of an *ald* gene knock-out. (a) Bright-field image of a colony of an *ald* knock-out (BIB1416) that was co-cultured with a WT strain (BIB224). The *ald* strain carries the P_{trpE*}-*mCherry* promoter fusion to distinguish it from the WT. A representative image of spore colonies at t=10 h *after* stimulation with L-alanine is shown. Several *early* WT spores have grown out successfully, while most *ald* mutant spores have germinated but none have grown out. See also Supplementary Movie 4. Scale bar: 10 µm. **(b)** Corresponding revival frequencies. Strains: BIB1416, BIB224. Data: mean ± SEM, $n_c = \geq 8$ ($n_s = \geq 500$), unpaired t-test: **** $P \leq 0.0001$.



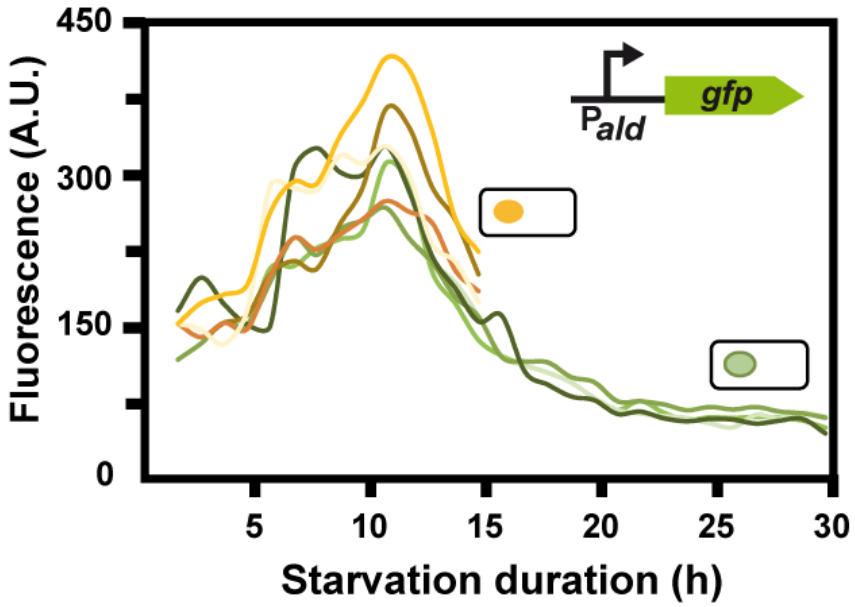
Supplementary Figure 5. Effect of Ald-induction in the progenitor cells of *late* spores. (a) Schematics illustrating the formation of *late* spores carrying low levels of Ald in the WT (top) and higher levels of Ald (bottom) formed by inducing *ald* expression from a σ^A -dependent promoter specifically in the progenitors of *late* spores. **(b)** Micrograph of a spore microcolony showing Ald-induced *late* spores in red. The bright-field image (gray) was overlaid with a fluorescence image of the P_{rapA}-*mCherry* reporter. Spores that grew out in response to L-alanine are circled. See also Supplementary Movie 5. Scale bar: 5 µm. **(c)** Corresponding revival frequencies of *early* and *late* spores in response to L-alanine. Results from left to right show the WT, the uninduced control, and the induced strain as shown in (a) and (b). Strains: BIB1300 and BIB224 (WT), Data: mean ± SEM, $n_c = \geq 11$ ($n_s = \geq 800$), unpaired t-test: **** $P \leq 0.0001$.



Supplementary Figure 6. Ald does not affect the germination response to L-alanine. Germination frequency as a function of time after stimulating spore microcolonies with L-alanine at time $t = 0$. During the nutrient down-shift Ald expression was induced at $t = 30$ h in the progenitor cells of (very) *late* spores (red). Their germination dynamics were comparable to WT *late* spores (green line) or *late* spores that had formed before the induction of Ald expression, i.e. between $t = 20$ h and $t = 30$ h (green dashed line). The germination dynamics of *early* spores (yellow) are included as a reference in each case. Strain: BIB1300 and BIB224 (WT), Data: mean ± SEM, $n_c = \geq 8$ ($n_s = \geq 350$).



Supplementary Figure 7. Ald does not affect the spore revival frequency in response to stimulation with AGFK. Revival frequency (after 8 h) in response to stimulation with AGFK at t = 0 h. During sporulation BIB1300 was induced with IPTG at t = 20 h and compared to the uninduced control. The data for the WT strain is included for reference. Strains: BIB1300, BIB224 (WT). Data: mean ± SEM, $n_c = \geq 12$ ($n_s = \geq 900$). Unpaired t-test: The difference between induced and uninduced spores is not significant ($P > 0.05$).



Supplementary Figure 8. Cells down-regulate the *ald* promoter during starvation. Fluorescence trajectories for single cells carrying a P_{ald}-*gfp* reporter (BIB1213). Yellowish (greenish) denote the trajectories for cells that give rise to *early* (*late*) spores. The trajectories were terminated when a pre-spore became visible.

Supplementary Tables

Supplementary Table 1. Vectors used in this work

Accession Number	Vector	Genotype	Source
EIB380	pGFP_Star	'*amyE cat* T*gyrA* LICS promoterless *gfpmut3 amyE*' *bla* ColE1 origin	Ref. 1
EIB422	pRFP_Star	'*amyE cat* T*gyrA* LICS promoterless *mcherry amyE*' *bla* ColE1 origin	This work
EIB303	pDR111	*'amyE spec^R* T*rrnB* T_0λ *Phyperspank-MCS lacI amyE' bla* ColE1 origin	David Rudner, Harvard Medical School
	pDR110	*'amyE spec^R* T*rrnB* T_0λ *Pspank-MCS lacI amyE' bla* ColE1 origin	David Rudner, Harvard Medical School
EIB7	pSac-KAN	*'sacA kan^R MCS sacA' bla* puc18 origin	Ref. 2
EIB25	pDG1514	*'MCS par tet*^{R} *lacZ*' *bla* pMB1 origin	Ref. 3

Supplementary Table 2. Plasmids used in this work

Accession Number	Plasmid	Genotype	Source
EIB432	pRFP_Star_P$_{rapAII}$	'*amyE cat^R* T*gyrA* P$_{rapAII}$-*mcherry amyE*' *bla* ColE1 origin	This work
EIB404	pGFP_Star_P$_{spoIIE}$	'*amyE cat^R* T*gyrA* P$_{spoIIE}$-*gfpmut3 amyE*' *bla* ColE1 origin	This work
EIB450	pGFP_Star_P$_{ald}$	'*amyE cat^R* T*gyrA* LICS P$_{ald}$-*gfpmut3 amyE*' *bla* ColE1 origin	This work
EIB499	pGFP_Star_P$_{ald}$-*ald-mCherry*	'*amyE cat^R* P$_{ald}$-*ald-mCherry* T*gyrA* P$_{ald}$-*gfpmut3 amyE*' *bla* ColE1 origin	This work
EIB452	pDR111_*ald*	'*amyE spec^R* P$_{hyperspank}$-*ald lacI amyE*' *bla* ColE1 origin	This work
EIB297	pDR111_*rapA*	'*amyE spec^R* P$_{hyperspank}$-*rapA lacI amyE*' *bla* ColE1 origin	This work
EIB419	pDR110_*kinA* / 04F14	'*amyE spec^R* P$_{spank}$-*kinA lacI amyE*' *bla*ColE1 origin	Ref. 4
EIB480	pSac-KAN_ P$_{hyperspank}$-*ald*	'*sacA* P$_{hyperspank}$-*ald lacI kan^R sacA*' *bla* ColE1 origin f1(+) origin	This work
EIB503	pSac-KAN_P$_{ald}$-*ald-mCherry*	'*sacA* P$_{ald}$-*ald-mCherry lacI kan^R sacA*' *bla* ColE1 origin f1(+) origin	This work

Supplementary Table 3. Strains used in this work

Strains	Name	Genotype	Source
E. coli			
	DH5α	*F⁻ φ80lacZΔM15 Δ(lacZYA-argF)U169 recA1 endA1 hsdR17(r_k^-, m_k^+) phoA supE44 thi-1 gyrA96 relA1 λ⁻*	Invitrogen
B. subtilis			
BIB224	168 1A700	*trpC2*	BGSC
BIB182	168 P_{trpE*}-*mcherry* P_{spo0F}-*yfp* / JL028	*amyE*::[P_{spo0F}-*yfp cat*^{R}] *ppsB*::[P_{trpE*}-*mcherry ery*^{R}]	Ref. 5
BIB444	168 P_{trpE*}-*mcherry*	*trpC2 ppsB*::[P_{trpE*}-*mcherry ery*^{R}]	See methods
BIB1019	168 P_{spoIIE}-*gfp* P*trpE-mcherry*	*trpC2* *amyE*::[T*gyrA* P_{spoIIE}-*gfpmut3 cat*^{R}] *ppsB*::[P_{trpE*}-*mcherry ery*^{R}]	BIB444 transformed with EIB404
BIB1126	168 P_{rapAII}-*mcherry*	*trpC2* *amyE*::[T*gyrA* P_{rapAII}-*mcherry cat*^{R}]	BIB224 transformed with EIB432
BIB1416	168 *ald*::*tet*^{R} P_{trpE*}-*mcherry*	*trpC2 ald*::*tet*^{R} *ppsB*::[P_{trpE*}-*mcherry ery*^{R}]	See methods
BIB1213	168 P_{ald}-*gfp*	*trpC2 amyE*::[T*gyrA* P_{ald}-*gfpmut3 lacI cat*^{R}]	BIB224 transformed with EIB450
BIB1300	168 P_{rapAII}-*mcherry* $P_{hyperspank}$-*ald*	*trpC2* *amyE*::[T*gyrA* P_{rapAII}-*mcherry cat*^{R}] *sacA*::[$P_{hyperspank}$-*ald lacI kan*^{R}]	BIB1126 transformed with EIB480
BIB1423	168 P_{ald}-*ald-mcherry* P_{ald}-*gfp*	*trpC2* *amyE*::[P_{ald}-*ald-mcherry cat*^{R} T*gyrA* P_{ald}-*gfpmut3*]	BIB224 transformed with EIB499
BIB1332	168 P_{spank}-*kinA*	*trpC2 amyE*::[P_{spank}-*kinA lacI spec*^{R}]	BIB224 transformed with EIB419
BIB1330	168 $P_{hyperspank}$-*rapA*	*trpC2 amyE*::[$P_{hyperspank}$-*rapA lacI spec*^{R}]	BIB224 transformed with EIB297
BIB1440	168 P_{spank}-*kinA* P_{ald}-*ald-mcherry*	*trpC2* *amyE*::[P_{spank}-*kinA lacI spec*^{R}] *sacA*::[P_{ald}-*ald-mcherry lacI kan*^{R}]	BIB1332 transformed with EIB503
BIB1442	168 $P_{hyperspank}$-*rapA* P_{ald}-*ald-mcherry*	*trpC2* *amyE*::[$P_{hyperspank}$-*rapA lacI spec*^{R}] *sacA*::[P_{ald}-*ald-mcherry lacI kan*^{R}]	BIB1330 transformed with EIB503

Supplementary Table 4. Primers used in this work

Primer	Sequence 5' – 3' (LIC-site underlined; overlap in gray italics; Restriction cut-site in bold)	Purpose
ST162	CATAGTAGTTCCTCCTTCCCGGGAAAGC	pRFP_Star
ST219	*CGGGAAGGAGGAACTACT*ATGGTTTCCAAGGGCGAGG	pRFP_Star
ST220	*CCAAGCTCAGCTAATTAAGC*TTATTTGTACAGCTCATCCATGCCAC	pRFP_Star
ST221	GCTTAATTAGCTGAGCTTGGACTCC	pRFP_Star
ST1	CCGCGGGCTTTCCCAGCTAAAAGAAAGCACGGGTGTTTG	PrapAII up fragment
ST222	*AATCGTCTGCTTCATCCT*TTTGAATTACCCGAGATATGTC	PrapAII up fragment
ST223	AGGATGAAGCAGACGATTCCGTC	PrapAII down fragment
ST224	GTTCCTCCTTCCCACCCCTTCGATGTCTTCTAACAATTCTG	PrapAII down fragment
ST209	CCGCGGGCTTTCCCAGCTCCTAACAAATCGGTTTCTC	PspoIIE
ST210	GTTCCTCCTTCCCACCACCTGTTATATTCGTTGCCTG	PspoIIE
ST16	GGTAAGTTTTCCGTATGTTGC	Seq pGFP_Star
ST17	GTGAATTTAGGAGGCTTACTTG	Seq pXFP_Star
ST243	GTCTGGGTGCCTTCATAC	Seq pRFP_Star
ST133	TCAGCTCGCATACCGAATTAAG	PtrpE-mcherry
ST134	ATGCCTAAACACCAGCCATCC	PtrpE-mcherry
SS34	**TATATAGCTAGC**ATATGACATATCTCGGGTAATTCAAAAGG	rapA_fw
SS37	**TATATAGCATGC**GACCGCAACGAGCAACAAACCTGACATC	rapA_rev
MA34	CCGCGGGCTTTCCCAGCGCGGGCCATTATAATTACTC	Pald_fw
MA35	GTTCCTCCTTCCCACCCCTGTATATGTGATATTTTTAGTGTAGC	Pald_rev
MA37	**ATAGCTAGC**TATCACATATACAGGAGGAGACAG	ald_fw
MA38	**ATAGCATGC**GTCATAATTCGTGAAATGGTCTC	ald_rev
MA47	**ATAACTAGT**TGGCCTTTTTGCGTTTCTAC	Phyperspank_ald_fw
MA48	**ATAGAGCTC**TTTCCTTACGCGAAATACGG	Phyperspank_ald_rev
SONSEQ18	ATGGCAAGAACGTTGCTCGA	Seq pDR111
SONSEQ19	TACGTACGATCTTTCAGCCG	Seq pDR111
MA49	GTGACGATCATTGACTTAAACG	Seq Phyp_ald

MA50	ATATTCAAACGGAGGGAGACG	Seq Phyp_ald
ST39	CTGGTCGGAGATTGGGATGATAG	Check for chromosomal integration into *amyE* locus
ST40	AATTTCCATGTTGCGTAAGTCAG	Check for chromosomal integration into *amyE* locus
ST283	GCTCAATATGTCGTCATTACAGGC	Check for chromosomal integration into *sacA* locus
ST284	GCAGGGCTAATTGCAGATATAGG	Check for chromosomal integration into *sacA* locus
ST129	ATGAGTATTCAACATTTCCGTGTC	Check for single crossover
ST130	TTACCAATGCTTAATCAGTGAGG	Check for single crossover
MA51	GATGAAGATCTGCTGACATTG	ald up_f
MA52	CGAGCGCCTACGAGGAATTTGTATCGGGAACCC CTATGATCATATC	ald up_r
MA53	CCTATCACCTCAAATGGTTCGCTGGATCTAGGCT ATGAGTATGTTCC	ald down_f
MA54	CCAAACAGCCTAAAGACTG	ald down_r
LA54	CAGCGAACCATTTGAGGTGATAGGTCTTGCAAT GGTGCAGGTTGTTCTC	Tet fwd
LA56	CGATACAAATTCCTCGTAGGCGCTCGGGAACTC TCTCCCAAAGTTGATCCC	Tet rev
LA58	CATCGGTCATAAAATCCGTAATGC	Tet check rev
KN1	TCATATCAGCGACGTTCTGC	Ald-up-check
KN4	CTGGTGCTGGAATGAGTTTGCT	Tet check fwd
KN3	AAATGCGGATGGCACATATT	Ald-do-check
MA55	**ATAGGTACC**GCGGGCCATTATAATTACTC	Pald_ald fw
MA56	*GGAACCTCCACCTCC*AGCACCCGCCACAGATGAT	Pald_ald rev
MA57	*GTGCTGGAGGTGGAGGTTCC*GTTTCCAAGGGCGA GGAG	mcherry fw
MA58	**ATAGGATCC**TTATTTGTACAGCTCATCCATGCC	mcherry rev

Supplementary References

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5. Levine, J. H., Fontes, M. E., Dworkin, J. & Elowitz, M. B. Pulsed feedback defers cellular differentiation. *PLoS Biol.* **10,** e1001252 (2012).