Fig. S1. Generation of EBRβcatM cells. (**A**) The *Rosa26* locus of EBRβcatM cells. The *Rosa26* locus of the parental EBRTcH3 cells was modified by Cre-dependent gene insertion so that the Ctnnb1(S33Y)-Mer gene was placed under the control of a Tet-repressible CMV promoter (CMV*). A low concentration of Tet will release the expression of the Ctnnb(S33Y)-Mer protein, and 4OHT will activate the protein. To construct such cell lines, the mouse Ctnnb1(S33Y)-Mer DNA fragment was amplified with PCR to introduce a Xho I site at the 5'-end and a Not I site at the 3'-end, and the amplified fragment was inserted into Xho I-Not I site of the pPthC donor plasmid (Masui et al., 2005). The resultant plasmid, pPthC-βcatM, contained Ctnnb1(S33Y)-Mer under the control of the Tet-repressive CMV promoter (CMV*-Ctnnb1(S33Y)-Mer) and a complete transcription unit for the expression of PacΔTK (PGK-PacΔTK-pA). EBRTcH3 ES cells carry a loxP-CMV*-Hph-loxP' cassette on the Rosa26 locus, and grow normally in EBR medium supplemented with 40 μg/ml Hygromycin B (Invitrogen) in the absence of Tet (EBR-Hyg medium) (Masui et al., 2005), which can be readily replaced by a donor cassette containing a different drug-resistant gene: e.g. CMV*-Ctnnb1(S33Y)-Mer with PGK- PacΔTk-pA via Cre-mediated site directed recombination (Masui et al., 2005). The expression vector for NLS-Cre recombinase, pHD2-Cre1, was kindly provided by Dr. Robert Ramsey (Melbourne, Australia). The EBRTcH3 cells were co-transfected with pPthC-βcatM and pHD2-Cre1 using Lipofectamine 2000 (Invitrogen, Carlsbad, CA), and were maintained for 2 days in the EBR medium supplemented with 1 μM Tet. Culture continued in the selection medium of EBR medium supplemented with1 μM Tet and 1.5 μg/ml of Puromycin (EBR-PurTet) for 9–11 days. The resulting colonies (approximately 20) were picked and individually maintained in a 24-well plate in the EBR-PurTet medium. Correct integrants were identified by

their ability to grow in EBR-PurTet medium but not in EBR-Hyg medium. (**B**) Copy-number of Ctnnb1 in EBRβcatM clones. Genomic DNA was purified from cultured EBRTcH3 cells and EBRβcatM clones (Cl3-2, 3-8, 21-3, 21-4, 24-5) using the Genomic DNA purification kit (Qiagen). Absolute Quantification PCR was done on the genomic DNA (primer pairs listed in Table S1) with the ABI 7500 system using the SYBR Green Master mix (Applied Biosystems). Triplicate experiments were performed for each DNA sample with each primer pair. Average Ct was calculated and ΔCt for Ctnnb1 exon-3 and Ctnnb1(S33Y)-Mer were calculated relative to the average Ct of Ctnnb1 intron-5 for each sample. The relative copy number of exon-3 in the isolated clones was calculated using the relative exon copy number of the parental EBRTcH3 cells as the base (i.e. 2 copies), and are plotted (B-catenin, red). All five clones show only 1 copy addition of Ctnnb1, suggesting that no non-specific integration had occurred in the isolated clones. The relative copy numbers of Ctnnb1(S33Y)-Mer in the clones were calculated using Cl3-2 as control (Mer, blue). (**C**) Expression of the Ctnnb(S33Y)-Mer protein in the EBRβcatM clones. EBRβcatM clones (Cl3-2, 3-8, 21-3, 21-4, 24-5) were propagated in EBR medium containing 0.5 μg/ml of Puromycin in the presence and absence of Tet for 3 days, confirmed by FACS to become YFP^+ in the absence of Tet, and subjected to Western blot analysis. Cells were lysed with 100 μl of Lysis Buffer: 50mM Tris-HCl pH8.0, 150mM NaCl, 1% (v/v) Triton X-100 (Sigma), 1x cOmplete Protease Inhibitor cocktail (Roche AS, Indianapolis, IN) at room temperature for 10 min, the extracts cleared, and the protein concentration determined (NanoDrop, Wilmington, DE). Twenty μg of each sample was subjected to SDS-PAGE, and blotted to Immobilon-FL PVDF membrane (Millipore, Billerica, MA). The membranes were blocked with Odyssey Blocking Buffer (Li-COR Biotech, Lincoln NE) for 1 h, then incubated for another 1 h in the presence of the primary antibodies anti-β-catenin (BD) and anti-β-tubulin

(Millipore, Temecula, CA). The blots were washed (x3) with PBST (0.05% [v/v] Tween-20 in PBS), and stained with secondary antibody IRDye700 (0.2 μg/ml) in Odyssey Blocking Buffer for 1 h. The blots were washed again with PBST, and scanned on an Odyssey Imager (Li-COR).

Fig. S2. Characterization of EBRβcatM Cl3-8 and Cl24-5 cells. (**A**) Experimental Scheme. EBRβcatM (Cl24-5 and Cl3-8) ES cells were differentiated by successive reduction in concentration of Tet and increase in concentration of Puromycin and by addition on day 2 of WNT3a (W), 500 ng/ml Noggin (N), BMP4 (B), and/or 4OHT (OHT). (**B**) EBRβcatM (Cl24-5) cells were differentiated with no factor (none, black triangle/line), W (pale pink), N (green), WN (blue), OHT (orange), or OHT+N (pink). EBs were harvested on the indicated days for RT-PCR. (**C**) Supplementary to Fig. 1B. RT-PCR analysis was also conducted using *Uncx* and *Hand2* primers. The preferential conditions for *Uncx* expression resemble those for *Meox1* expression, and the preferential conditions for *Hand2* expression are similar to those for *Foxf1a* expression. (**D**) (Left panel) EBRβcatM clones Cl24-5 and Cl3-8 were differentiated under conditions of OHT+N (brown), OHT+B (green) and OHT (blue) for 6 days. Then $YFP^+Flk1^-Pdgfr\alpha^+(YFP^+P^+)$ and $YFP^+Flk1^-Pdgfr\alpha^-(P^-)$ progeny were isolated by FACS from each condition, and were subjected to RT-PCR. The *Meox1* transcript was always enriched in the YFP^+P^+ progeny, but inclusion of BMP4 during differentiation (OHT+B) suppressed the *Meox1* level. (Right panel) EBRβcatM (Cl24-5) cells were differentiated with OHT+ N and various concentrations of Activin or SB431542 (SB). Then, RT-PCR for *Meox1* expression was performed. The dose-dependent effect of SB is plotted as "−μM" in the negative range. Activin at low concentrations (0 to 2 ng/ml) slightly enhanced the *Meox1* expression, but both SB and a higher concentration of Activin (>2 ng/ml) were strongly inhibitory. (**E**) EBRβcatM (Cl3-8) cells were differentiated

under OHT+N conditions for 6 days, and the $YFP^{+}P^{+}$ progeny were isolated and subjected to micromass culture in the presence of PDGF (P) and TGFβ (T) (Nakayama et al., 2003; Tanaka et al., 2009). The culture was fixed on day12, and stained with acid Alcian Blue. TGFβ enhancement of chondrogenesis (PT vs. P) was observed. Typical dark blue spots (signs of chondrogenesis) are highlighted with yellow arrowheads.

Fig. S3. (A-D) Feedforward system between WNT and BMP and between WNT and Nodal, but not between BMP and Nodal. (**A**) Experimental Scheme. E14, Bry-GFP, and EBRTcH3 ES cells were differentiated in the presence of no factor (none), WNT3a (W), BIO, MeBIO, 2 μg/ml Fzd8CRD-Fc (FZD), Noggin (N) with various concentrations of Activin added on day 2. (**B**) GSK3-inhibitor substitutes for Wnt3a on the *Bmp4* and *Nodal* induction during differentiation. Bry-GFP ES cells were differentiated in the presence of W (purple), BIO (green) and no factor (black) conditions. EBs were harvested on days 4, 5 and 6 and subjected to RT-PCR for *Bmp4* add *Nodal* expression. Compared to BIO, WNT3a induced *Bmp4* and *Nodal* more strongly and sooner than BIO. (**C**) Dose-dependent effects of Activin and WNT3a on *Bmp4* and *Nodal* gene expression during ES cell differentiation. E14 ES cells were differentiated for 5 days under conditions of no factor (black), N (green), W (purple), or WN (blue) with various concentrations of Activin or WNT3a. Note that Activin has no role in WNT3a-induced *Bmp4* expression and Noggin shows no effect on WNT3a-induced *Nodal* expression. (**D**) Nodal/Activin/TGFβ signal-dependent induction of mesoderm is achieved through endogenous WNT functions. EBRTcH3 (EBR) and Bry-GFP (BRY) ES cells were differentiated for 6 days using various concentrations of Activin in the presence of Noggin (N) with (blue) or without (brown, green) FZD. The *Meox1* expression induced by Activin + Noggin, the level of which

was much lower than that achieved by WNT3a + Noggin, was completely blocked by FZD in both cell lines, suggesting that the Nodal/Activin/TGFβ-induced mesoderm specification is mediated via endogenous WNT activity. (**E**) Titration of Noggin and GSK3 inhibitor. EBRTcH3 ES cells were differentiated in the presence of no factor (black triangle), WNT3a (W, purple), BIO (brown) and various concentrations of Noggin (Left two panels), or in the presence of various concentrations of BIO (brown) or MeBIO (black) alone (Right most panel). RNAs were extracted on day 6 and subjected to RT-PCR analysis. A concentration of 100 ng/ml is sufficient to induce *Meox1* and suppress *Foxf1a* expression. BIO-induction of *Meox1* depends on the "active" GSK3-inhibitor (BIO) but not the modified "inactive" version (MeBIO).

Fig. S4. Fine tuning of Nodal/Activin/TGFβ signaling for efficient generation of mesendoderm and mesoderm. (**A**) E14 ES cells were differentiated for 5 days in CDM in the presence of no factor (none, black), 500 ng/ml Noggin (N, red), 25 ng/ml Activin (A, green), or Activin + Noggin (A+N, blue) with various concentrations of WNT3a. RT-PCR was then performed using primers for *Tal1*, *Chrd*, and *Foxa2*. Note that Activin stimulates WNT3a-dependent *Tal1* (hemogenic angioblast marker) expression as well as (WNT3a + Noggin)-dependent *Chrd* and *Foxa2* (axial mesendoderm marker) expression. (**B**) E14 cells were differentiated in the presence of no factor (green), 500 ng/ml Noggin (N, purple), WNT3a (W, brown), and WNT3a + 500 ng/ml Noggin (WN, blue), together with various concentrations of Activin, and RT-PCR was performed using primers for the indicated genes. Note that high concentrations of Activin (>5 ng/ml) increased WNT3a-dependent *Tal1* expression and (WNT3a + Noggin)-dependent *Gsc* (mesendoderm marker) expression, but inhibited (WNT3a + Noggin)-dependent *Meox1* expression. (**C**) E14 ES cells were differentiated for 5 days in CDM in the

presence of 0 (A0) or 25 (A25) ng/ml Activin and 0 (W0), 5 (W5) or 50 (W50) ng/ml WNT3a. EBs were harvested for FACS analysis. Note that the genesis of the $Flk1^{+}Pdgfr\alpha^{-}$ hemogenic cell fraction (Tanaka et al., 2009) depends on high concentrations of Activin (A25) and WNT3a (W50). (**D**) Dose dependent effects of Nodal/Activin/TGFβ signaling on the GSK3-inhibitor induction of lateral plate mesoderm gene expression. EBRTcH3 ES cells were differentiated in the presence of no factor (black), BMP4 (B, purple), and BIO (brown), with various concentrations of SB and Activin, and RT-PCR was performed using primers for *Tal1* and *Foxf1a* (on day 6). Note that BIO-induced differentiation but not BMP-induced differentiation demonstrated the parabolic response to Nodal/Activin/TGFβ signaling. In conclusion, the BMP-dependent specification of lateral plate/extraembryonic mesoderm and hemoangiogenic mesoderm was not affected by the concentrations of SB and Activin tested. The WNT3a-induced specification of hemoangiogenic mesoderm and the (WNT3a + Noggin)-induced specification of axial mesendoderm were enhanced by Activin in a dose-dependent fashion. Thus, the optimal requirement of Nodal/Activin/TGFβ signaling for specification of subtypes of mesodermal and endodermal progeny is dependent on the inducer used.

Fig. S5. (**A**) Experimental Scheme. EBRTcH3 ES cells were differentiated for 6 days in the presence of WNT3a (W) and Noggin (N), or CHIR (C) and LDN (L) with or without SAG (S) added on day 4 (S4). (**B**) On average, the CL condition resulted in higher expression levels of *Meox1* than the WN condition in day 6 EBs (n=4). However, the difference was not statistically significant. P=0.145. (**C**) Supplementary for Fig. 3C. The results from RT-PCR using *Meox1* primers are provided. The E-cadherin^{-}Flk1^{-}Pdgfrα^{+} (P^{+}) progeny developed under CL and CLS4 were enriched in the *Meox1* transcript.

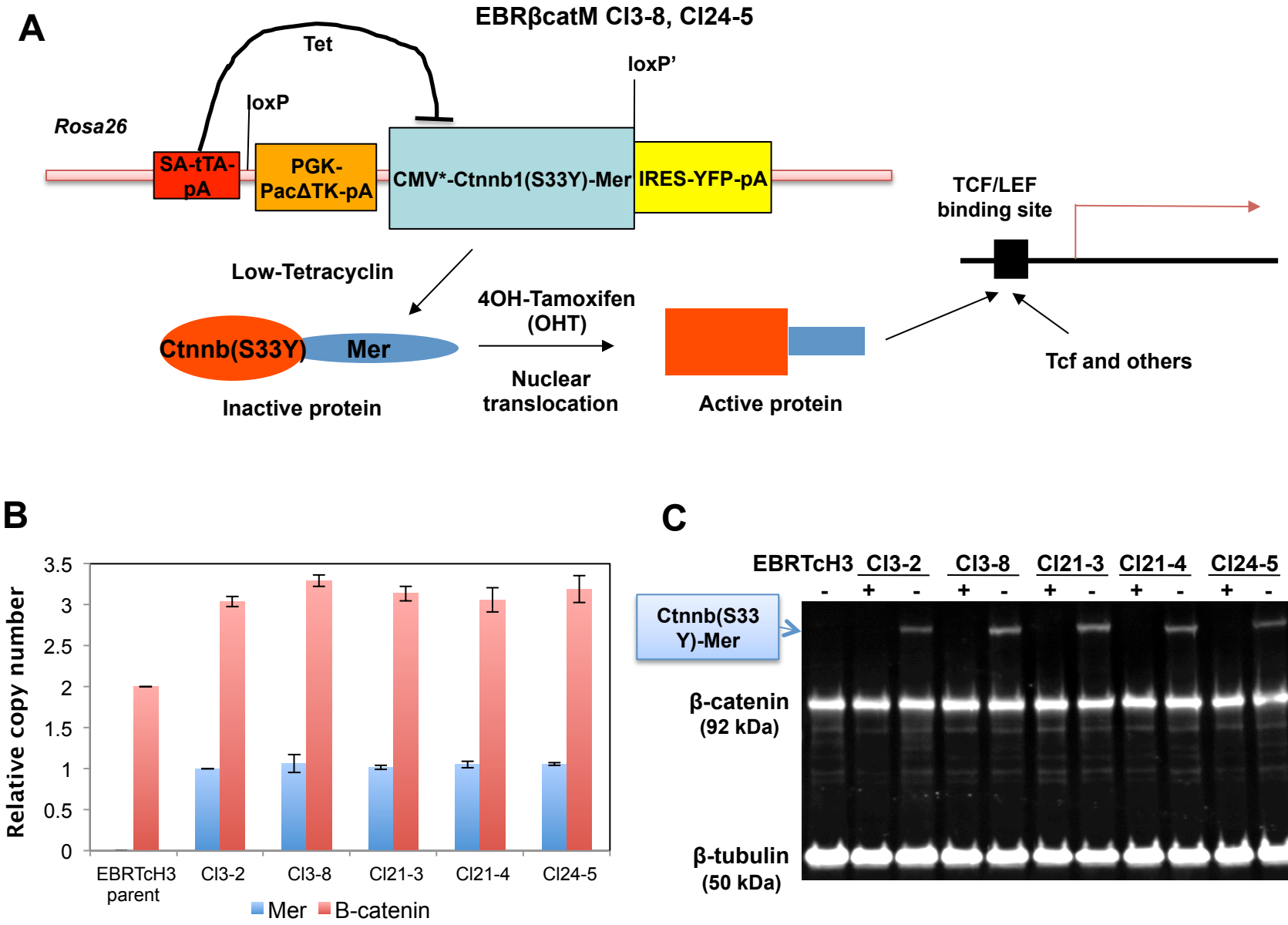
Fig. S6. (**A**) Experimental Scheme. EBRTcH3 ES cells were differentiated for 6 days in the presence of CHIR (C) and LDN (L) and various progeny were isolated by FACS and subjected to micromass culture for 12 days in the presence of various factors. (**B**) Time requirement of PSL treatment. The micromass culture initiated with E-cadherin$^-$Pdgfrα^+ cells under PDGF + SAG + LDN (PSL) was subjected to medium change (→) to PDGF + BMP (PB) on day3 and 6. Note: the standard 6-day treatment with LDN (PSL→PB) showed stronger chondrogenesis (slightly larger dark blue area) compared with the 3-day treatment (PSLday3→PB). (**C**) Supplementary for Fig. 4C. Effect of Noggin (N). The sorted E-cadherin$^-$Flk1$^-$Pdgfrα^+ progeny were subjected to micromass culture under PDGF + SAG (PS) or PDGF + SAG + Noggin (PSN) conditions, and the media were changed (→) to PB on day 6. Note: the 6-day treatment with Noggin enhanced chondrogenesis later, demonstrated by acid Alcian Blue stain. (**D**) Requirement of PSL conditions and E-cadherin$^-$Flk1$^-$Pdgfrα^+ (P$^+$) cells for successful chondrogenesis in micromass culture. Use of either Flk1$^-$Pdgfrα^- (P$^-$) cells, or the PDGF + LDN (PL) or PS conditions resulted in inefficient chondrogenesis as judged by Alcian Blue quantification as in Fig. 4D. Continuous presence of SAG is neither inhibitory nor stimulatory (PSL→PSB). * $P<0.05$. (**E**) Requirement of BMP4 from day 6 of micromass culture. The E-cadherin$^-$Flk1$^-$Pdgfrα^+ progeny were subjected to micromass culture under PDGF (P) or PSL conditions, and the media were changed (→) to P or PB on day 6. Note: Chondrogenesis is dependent on BMP signaling, when micromass culture was initiated under PSL conditions. (**F**) The E-cadherin$^-$Flk1$^-$Pdgfrα^+ progeny were subjected to micromass culture under P, PL, PS, or PSL conditions and the media were changed (→) to P, PB, or PSB on day 6. The cultures were subjected to Alcian Blue quantification as in Fig. 4D. * $P<0.05$. Note: The initial requirement of both SAG and LDN (PSL) and later requirement of

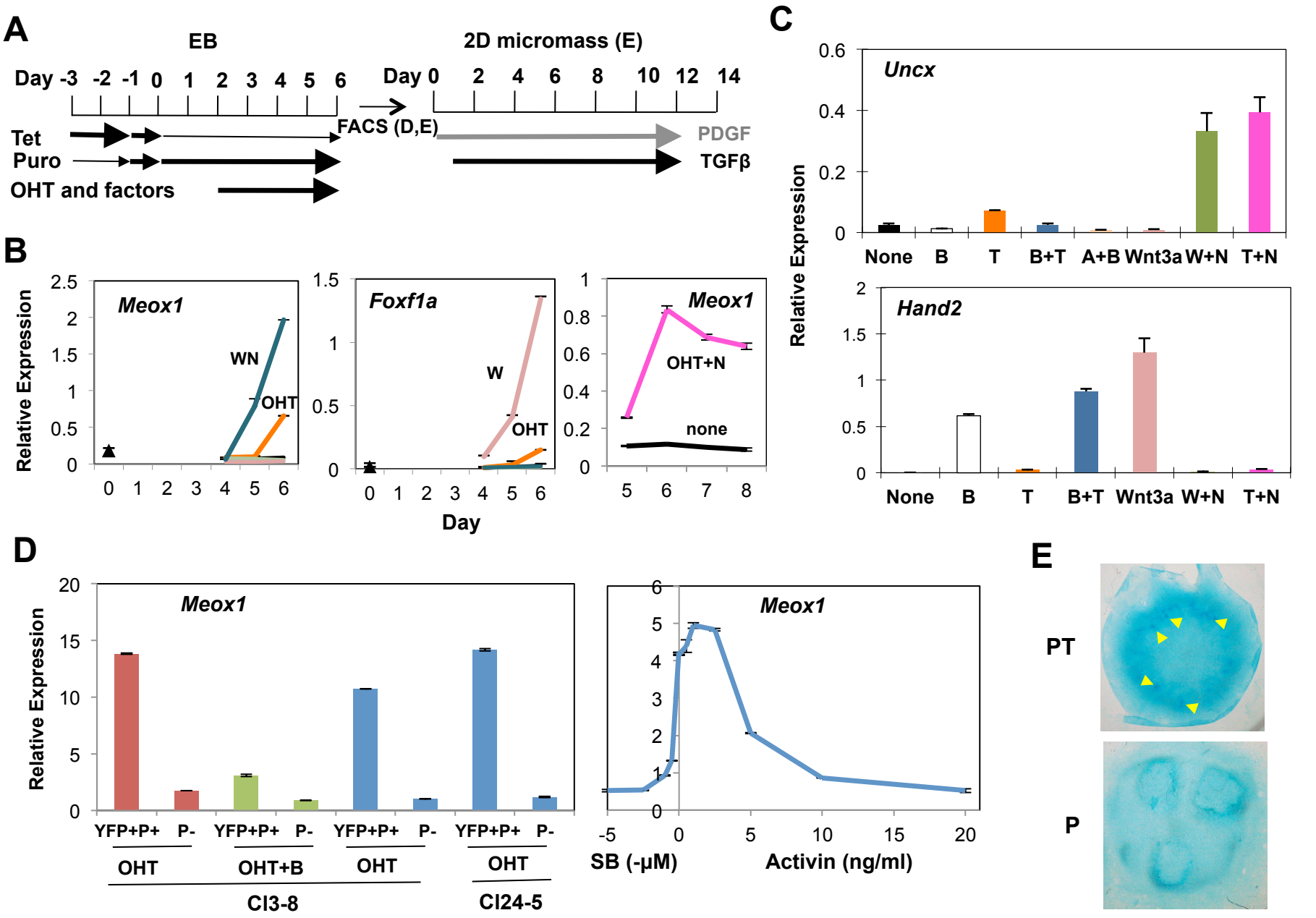
BMP (PB) for successful chondrogenesis was reproducibly shown. Continuous presence of SAG is again neither inhibitory nor stimulatory (PSL→PSB). (**G**) Supplementary to Fig. 4E. Quantification of sGAG and DNA. The total amounts of DNA and sGAG per micromass are displayed. * P<0.05

Fig. S7. Supplementary to Fig. 5C and Fig. 6B. (**A**) Experimental Scheme. (**B**) The same micromass cultures as performed for Fig. 5C were also subjected to RT-PCR with primers for the additional hypertrophic differentiation marker, *Alpl* (alkaline phosphatase gene). The *Bapx1* data are the same as shown in Fig. 5C but displayed without the PSL→PB data. (**C**,**D**) The micromass cultures performed for 14 days under PSN→PB (blue), PT→PTB (brown) and PT→PB (purple) conditions were periodically harvested for RT-PCR with primers for genes representing early chondrogenesis: *Bapx1* and *Sox9* (**C**) and for those representing chondrocytes: *Col2a1*, *Acan*, and *Col10a1* (**D**). Black triangle: freshly sorted cells. Note: (**D**) Continuous presence of TGFβ (after day 6) is neither inhibitory nor stimulatory for expressing (PT→PTB vs. PT→PB).

Fig. S8. (**A**) Experimental Scheme. (**B**) Supplementary to Fig. 5D. Quantification of sGAG and DNA. The micromass cultures performed under PT→PTB (brown), PSN→PB (blue), and PSL→PB (green) conditions were periodically harvested for total DNA and sGAG quantification. The amounts were normalized to one micromass to display μg sGAG/mass or μg DNA/mass. Black triangle: freshly sorted cells. Note: In support of the RNA results shown in Fig. 5C, LDN is a better alternative to Noggin for overall cellular growth (DNA) and cartilage matrix deposition (sGAG) during micromass culture. (**C**) Supplementary to Fig. 6B (upper panel). The levels of *Col10al* and *Col2a1* transcripts in the day 20 micromass developed under the indicated conditions have been used for the calculation. * P<0.05. (**D**,**E**) Supplementary to

Fig. 6C,D. Micromass cultures were initiated under the PSL→PB condition, which were converted to pellet culture on day 7, and continued till day 30. Some cartilage particles were fixed and stained with Toluidine Blue (T-Blue) (**D**) and some were transplanted into NSG mice for 12 weeks after which they were fixed, plastic embedded, and sectioned (**E**). The sections were subjected to von Kossa-van Gieson staining (left) and acid T-Blue staining (right).


A
EBRβcatM Cl3-8, Cl24-5
Tet
loxP
loxP'
Rosa26
SA-tTA-pA
PGK-PacΔTK-pA
CMV*-Ctnnb1(S33Y)-Mer
IRES-YFP-pA
TCF/LEF binding site
Low-Tetracyclin
4OH-Tamoxifen (OHT)
Ctnnb(S33Y)
Mer
Inactive protein
Nuclear translocation
Active protein
Tcf and others
B
Relative copy number
3.5
3
2.5
2
1.5
1
0.5
0
EBRTcH3 parent
Cl3-2
Cl3-8
Cl21-3
Cl21-4
Cl24-5
Mer
B-catenin
C
EBRTcH3
Cl3-2
Cl3-8
Cl21-3
Cl21-4
Cl24-5
- + - + - + - + - + - Tet
Ctnnb(S33 Y)-Mer
β-catenin (92 kDa)
β-tubulin (50 kDa)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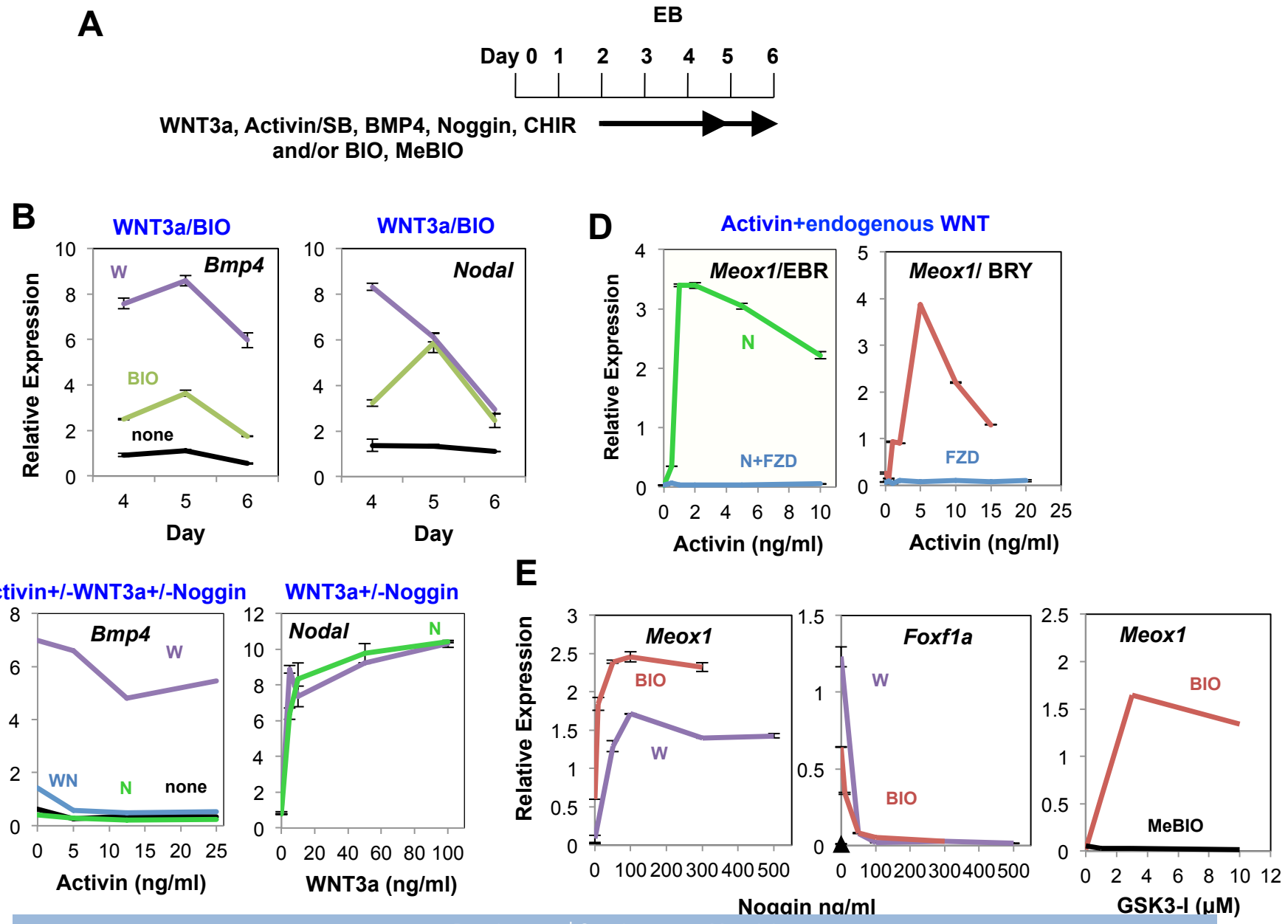
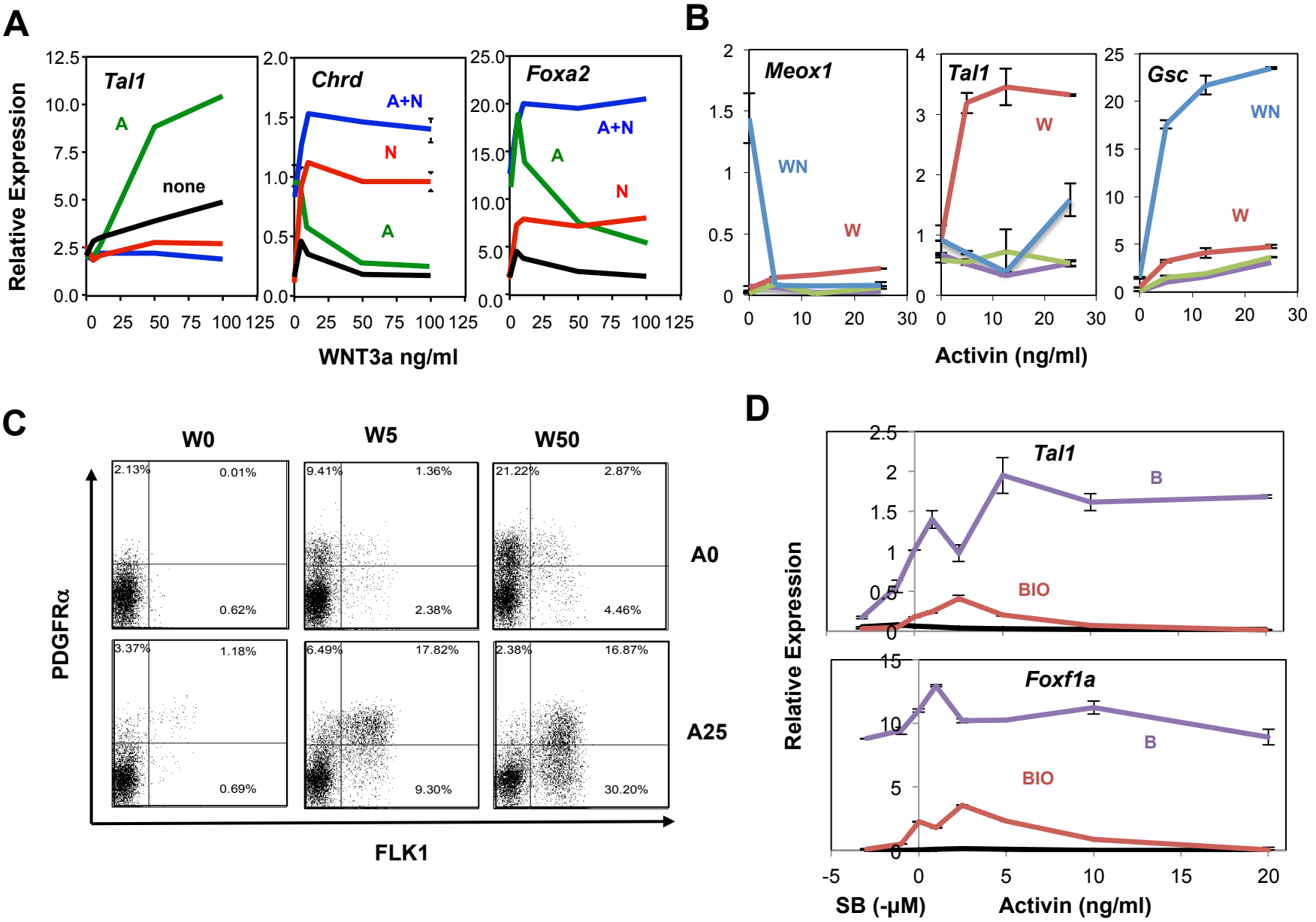
EB
2D micromass (E)
Day -3 -2 -1 0 1 2 3 4 5 6
Day 0 2 4 6 8 10 12 14
FACS (D,E)
Tet
Puro
OHT and factors
PDGF
TGFβ
B
Meox1
WN
OHT
Foxf1a
W
OHT
Meox1
OHT+N
none
Relative Expression
Day
C
Uncx
Hand2
Relative Expression
None B T B+T A+B Wnt3a W+N T+N
D
Meox1
Relative Expression
YFP+P+ P-
OHT
OHT+B
OHT
OHT
CI3-8
CI24-5
Meox1
SB (-μM)
Activin (ng/ml)
E
PT
P


A
EB
Day 0 1 2 3 4 5 6
WNT3a, Activin/SB, BMP4, Noggin, CHIR and/or BIO, MeBIO
B
WNT3a/BIO
Bmp4
W
BIO
none
Relative Expression
Day
WNT3a/BIO
Nodal
Day
D
Activin+endogenous WNT
Meox1/EBR
N
N+FZD
Relative Expression
Activin (ng/ml)
Meox1/ BRY
FZD
Activin (ng/ml)
C
Activin+/-WNT3a+/-Noggin
Bmp4
W
WN
N
none
Relative Expression
Activin (ng/ml)
WNT3a+/-Noggin
Nodal
N
WNT3a (ng/ml)
E
Meox1
BIO
W
Relative Expression
Noggin ng/ml
Foxf1a
W
BIO
Meox1
BIO
MeBIO
GSK3-I (μM)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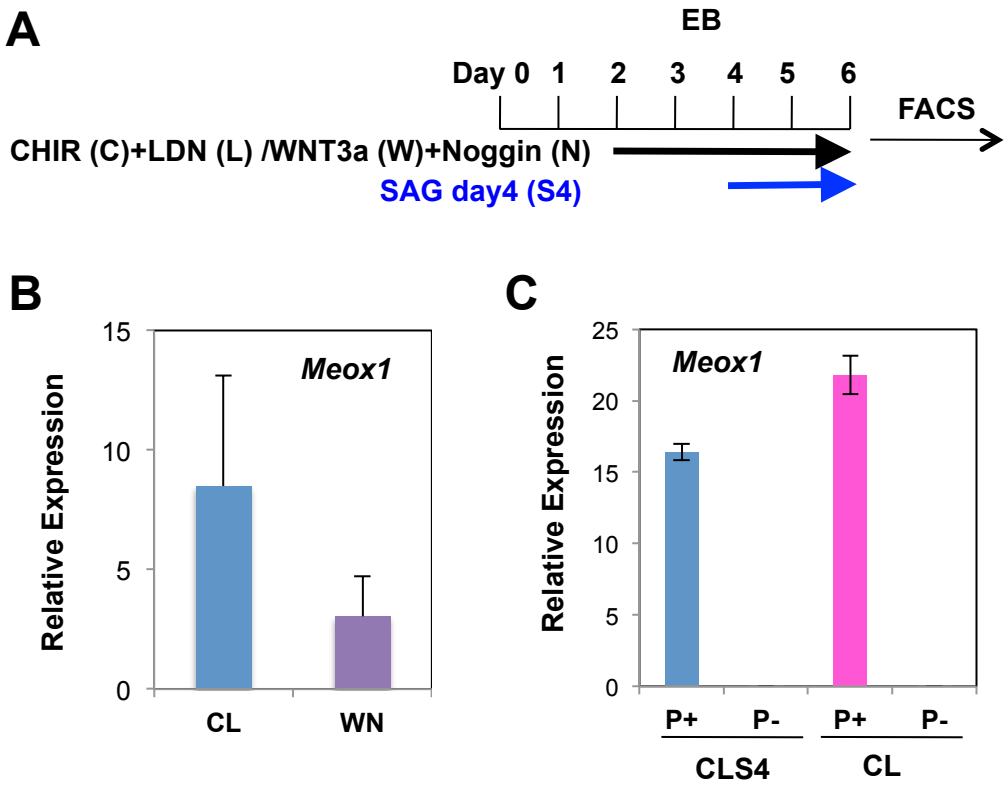
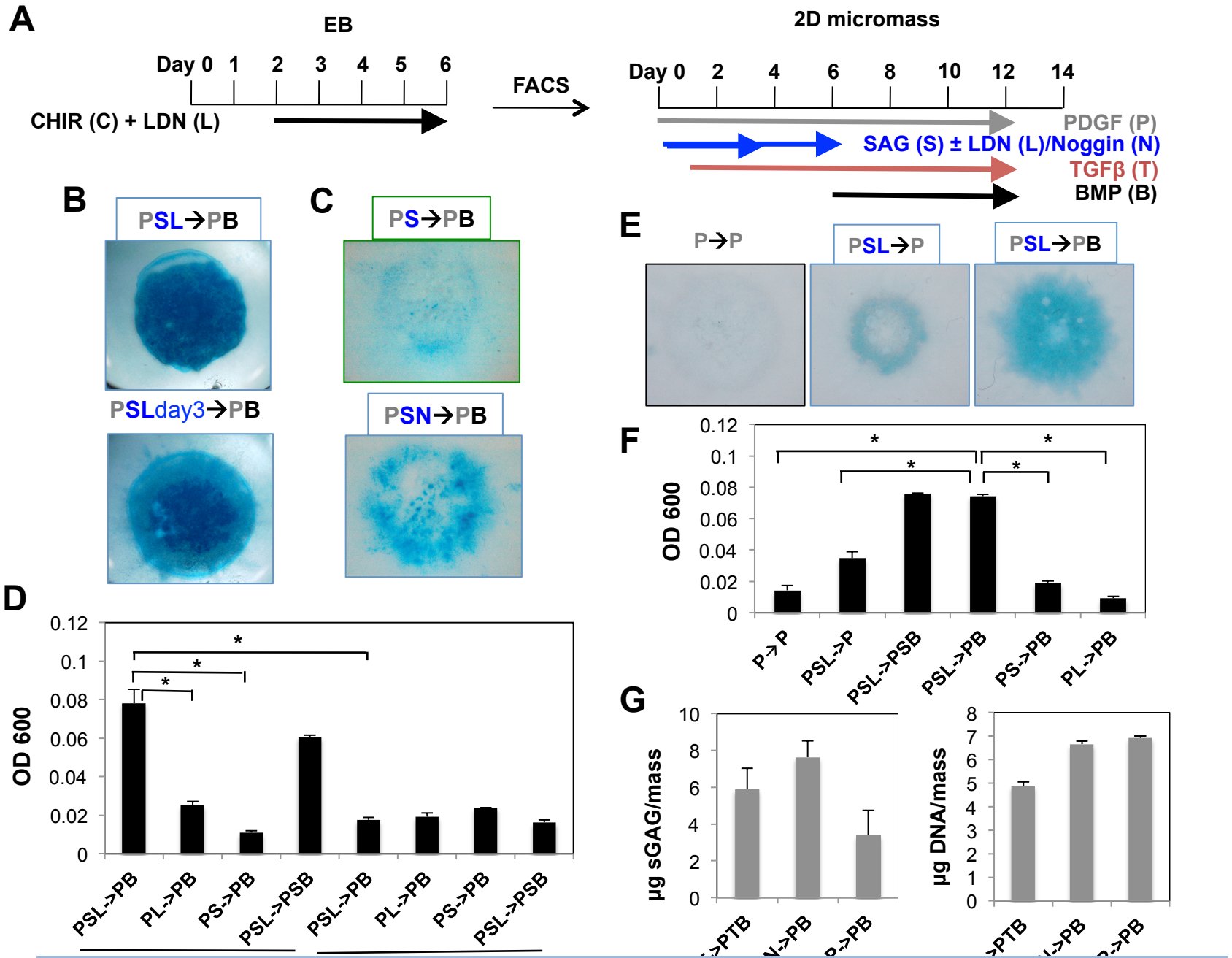
Relative Expression
Tal1
A
none
Chrd
A+N
N
A
Foxa2
A+N
A
N
WNT3a ng/ml
B
Meox1
WN
W
Tal1
W
Gsc
WN
W
Activin (ng/ml)
C
W0
W5
W50
2.13%
0.01%
0.62%
9.41%
1.36%
2.38%
21.22%
2.87%
4.46%
A0
3.37%
1.18%
0.69%
6.49%
17.82%
9.30%
2.38%
16.87%
30.20%
A25
PDGFRα
FLK1
D
Relative Expression
Tal1
B
BIO
Foxf1a
B
BIO
SB (-μM)
Activin (ng/ml)


A
EB
Day 0 1 2 3 4 5 6
FACS
CHIR (C)+LDN (L) /WNT3a (W)+Noggin (N)
SAG day4 (S4)
B
Meox1
Relative Expression
15
10
5
0
CL
WN
C
Meox1
Relative Expression
25
20
15
10
5
0
P+
P-
P+
P-
CLS4
CL


A
EB
Day 0 1 2 3 4 5 6
CHIR (C) + LDN (L)
FACS
2D micromass
Day 0 2 4 6 8 10 12 14
PDGF (P)
SAG (S) ± LDN (L)/Noggin (N)
TGFβ (T)
BMP (B)
B
PSL→PB
PSLday3→PB
C
PS→PB
PSN→PB
E
P→P
PSL→P
PSL→PB
F
OD 600
0.12
0.1
0.08
0.06
0.04
0.02
0
P→P
PSL→P
PSL→PSB
PSL→PB
PS→PB
PL→PB
D
OD 600
PSL->PB
PL->PB
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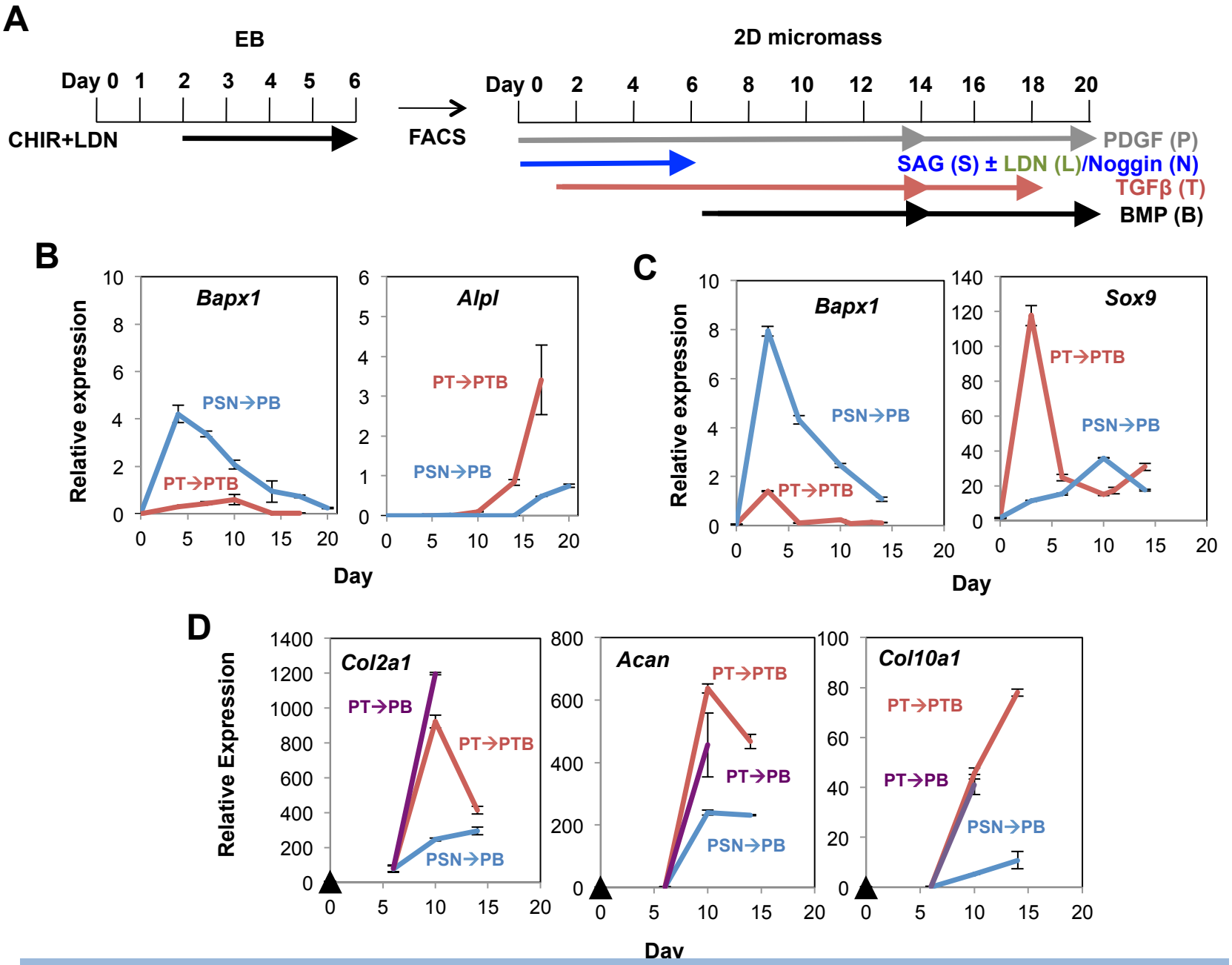
PS->PB
PSL->PSB
G
µg sGAG/mass
µg DNA/mass


A
EB
Day 0 1 2 3 4 5 6
CHIR+LDN
FACS
2D micromass
Day 0 2 4 6 8 10 12 14 16 18 20
PDGF (P)
SAG (S) ± LDN (L)/Noggin (N)
TGFβ (T)
BMP (B)
B
Bapx1
Alpl
PSN→PB
PT→PTB
Relative expression
Day
C
Bapx1
Sox9
PSN→PB
PT→PTB
Relative expression
Day
D
Col2a1
Acan
Col10a1
PT→PB
PT→PTB
PSN→PB
Relative Expression
Day

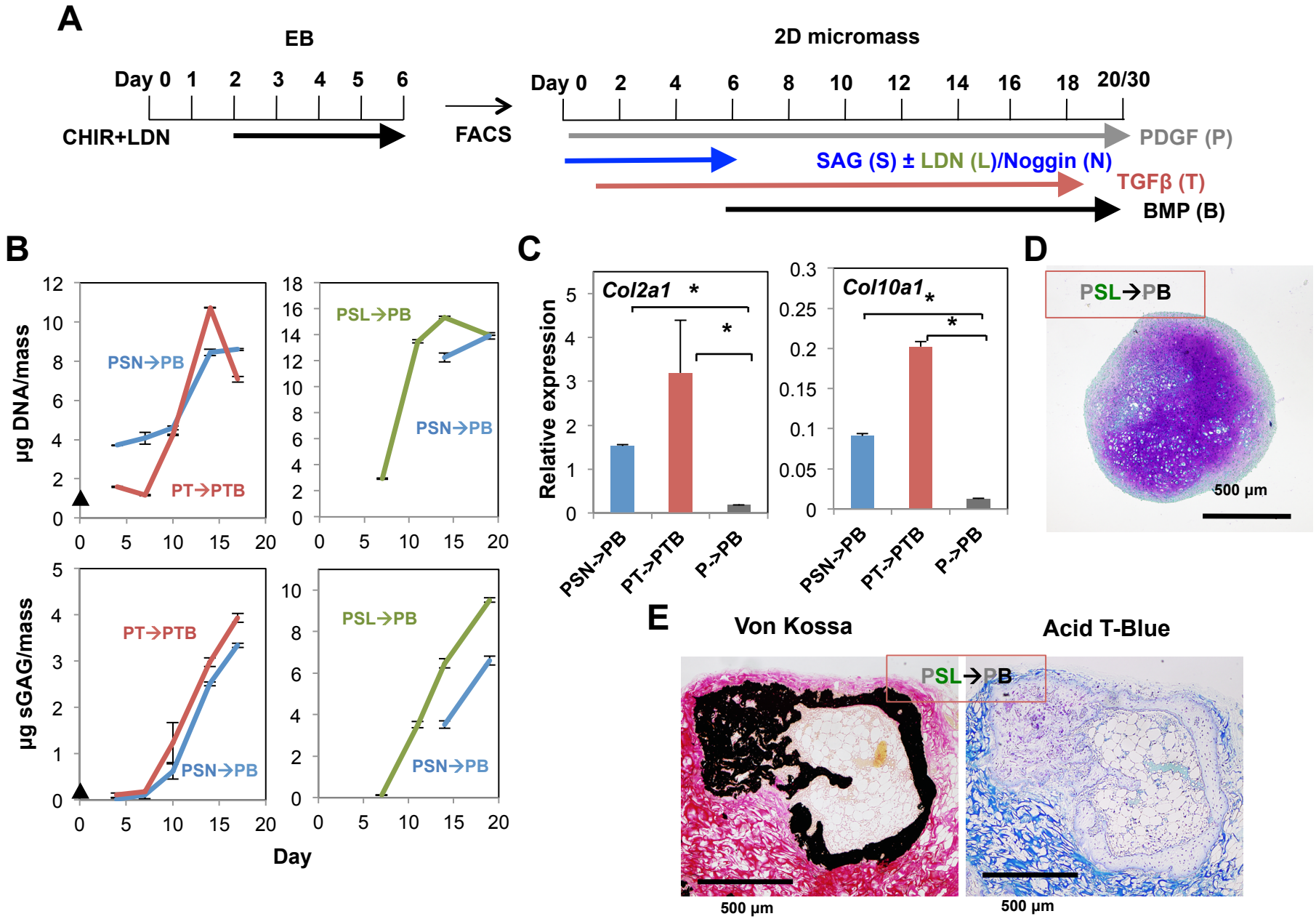

A
EB
Day 0 1 2 3 4 5 6
CHIR+LDN
FACS
2D micromass
Day 0 2 4 6 8 10 12 14 16 18 20/30
PDGF (P)
SAG (S) ± LDN (L)/Noggin (N)
TGFβ (T)
BMP (B)
B
µg DNA/mass
PSN→PB
PT→PTB
PSL→PB
PSN→PB
µg sGAG/mass
PT→PTB
PSN→PB
PSL→PB
PSN→PB
Day
C
Col2a1 *
Col10a1 *
Relative expression
PSN->PB
PT->PTB
P->PB
D
PSL→PB
500 µm
E
Von Kossa
Acid T-Blue
PSL→PB
500 µm
500 µm

Table S1. Primers

DNA PCR Primer	Location	Sequence 5'→3'
mCtnnB1-147F	Exon 3	GGG CAA CCC TGA GGA AGA A
mCtnnB1-210R	Exon 3	AAA GCC TTG CTC CCA TTC ATA A
mCtnnB1-intron5F	Intron 5	GTG GGC CAT GTC TTA AAG CAA
mCtnnB1-intron5R	Intron 5	TGC CTA GCT CAT TAA CTG CTC AAG
mCtnnB1-MerF	β-catenin-COOH	GCT GGC CTG GTT TGA TAC TGA
mCtnnB1-MerR	ER-NH_2	TCC TGA AGC ACC CAT TTC ATT

Gene	RT-PCR Primers	Sequence 5'→3'
Acan	(Realtimeprimers) F	TTC ACT GTA ACC CGT GGA CT
	(Realtimeprimers) R	TGG TCC TGT CTT CTT TCA GC
Col10a1	(Realtimeprimers) F	ATA GGC AGC AGC ATT ACG AC
	(Realtimeprimers) R	TAG GCG TGC CGT TCT TAT AC
Alpl	(Realtimeprimers) F	ACG AAT CTC AGG GTA CAC CA
	(Realtimeprimers) R	TGA GCT TTT GGA GTT TCA GG
Col2a1	F	GAA AAG AAA CAC ATC TGG TTT GGA
	R	TCT GGA CGT TAG CGG TGT TG
Bapx1	F	TGG GAC TTG ACA CAC CTA TCC A
	R	AGC GTC CCG AGG CTT GA
Sox9	F	TCA CAT CTC TCC TAA TGC TAT CTT CAA
	R	CGG CGG ACC CTG AGA TT
Nodal	F	CCT CCA GGC GCA AGA TGT
	R	CGC CCA TAC CAG ATC CTC TTC
Gsc	Taqman (Applied Biosystems)	
Foxf1a	Taqman (Applied Biosystems)	
Uncx	Taqman (Applied Biosystems)	

F: forward, R: reverse. Sequence information of other primers used will be found in (Tanaka et al., 2009).