Supporting Information

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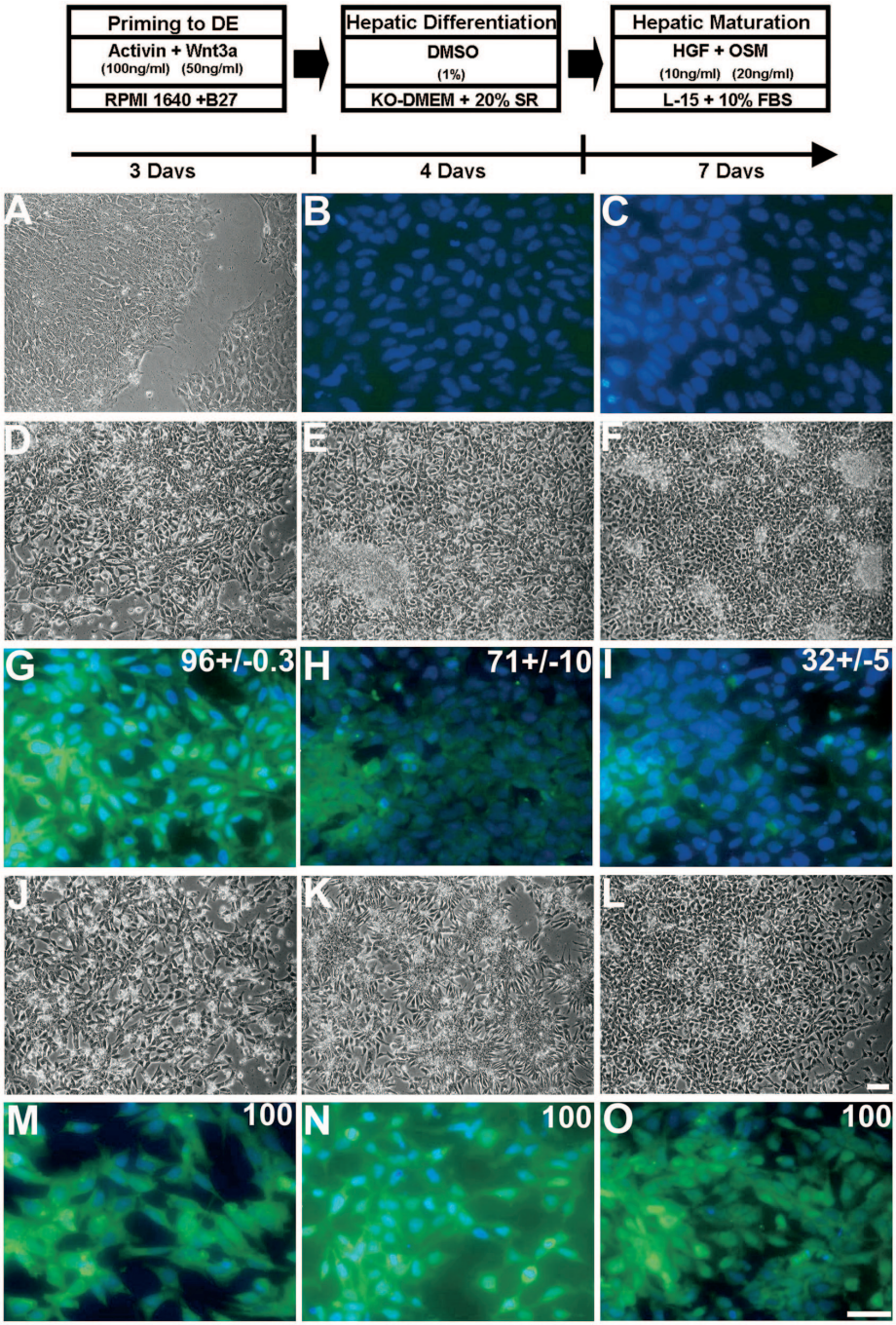

Fig. S1. AW promotes down-regulation of Brachyury in differentiating hESCs. The hESC differentiation protocol is shown at top. hESCs (*A*) exhibited a drastic change in cellular morphology in response to the different media. AW-differentiated cells rapidly formed a confluent monolayer (*D–F*) of cells exhibiting similar morphology. In contrast, AB-differentiated cells took longer to reach confluence (*J–L*). Consistent with different morphology, Brachyury gene expression was differently regulated in both conditions. hESCs did not express Brachyury (*B*). In contrast, cells differentiated with AW showed high levels of Brachyury at day 1, which decreased by day 3 (*G–I*). Cells treated with AB exhibited high-level Brachyury expression throughout the 72-h period (*M–O*). Specificity of staining was assessed using an IgG control (*C*). Phase images were recorded using a Nikon TE300/U, immunostaining was recorded using a Leica DMIRB. Scale bar = 100 μM. HGF, hepatocyte growth factor; OSM, Oncostatin M. Results are presented as a mean of 4 fields of view ± SE.

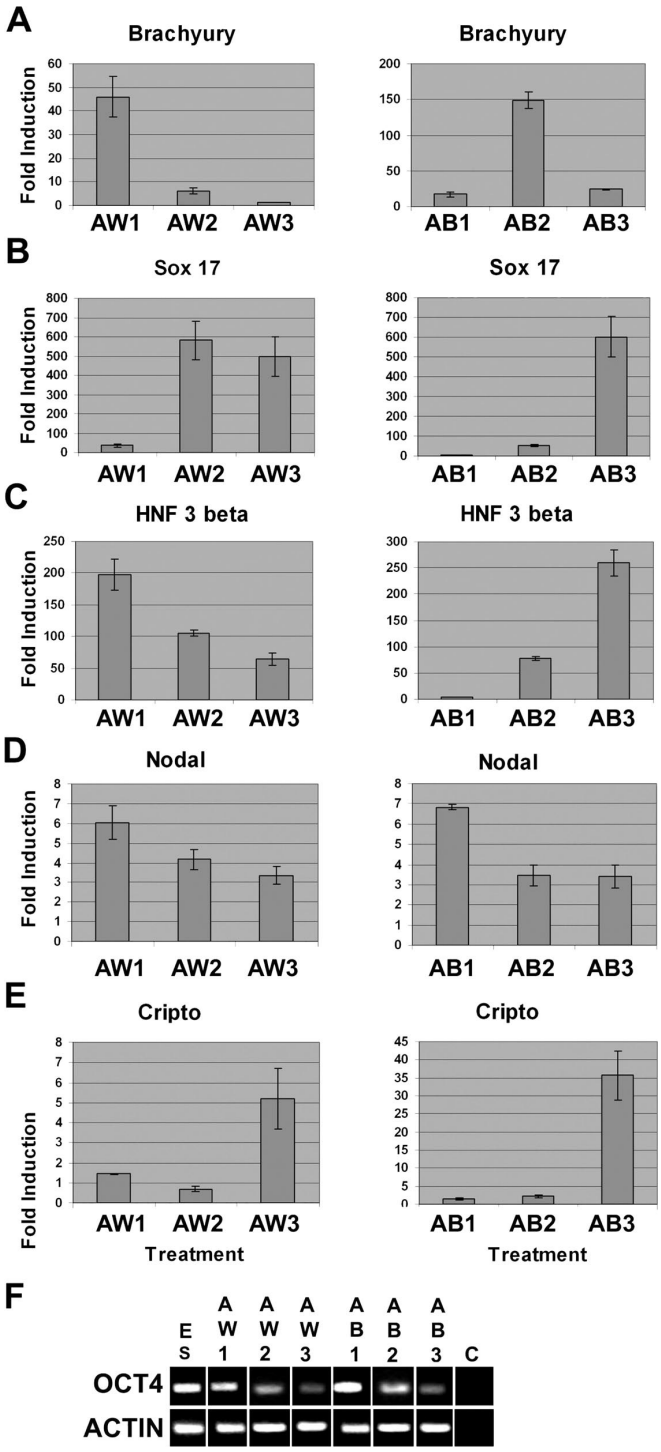

Fig. S2. AW treatment promotes accelerated progression of cells through the PS. hESC-directed mesoderm and endoderm differentiation was monitored by qPCR to *Brachyury*, *SOX17*, and *HNF3β*. Cells differentiated with AW proceeded through mesoendoderm differentiation more rapidly than cells differentiated with AB. AW treatment exhibited rapid down-regulation of the mesoderm marker Brachyury (*A*, AW1–3 and AB1–3) and more rapid progression through the PS (*SOX17*, *HNF3β*) than cells exposed to AB (*B* and *C*, AW1–3 and AB1–3). (*D*) Nodal levels decreased over the 3-day time course in both models (AW1–3 and AB1–3). (*E*) *CRIPTO* levels were maximally expressed at day 3 in both protocols, indicating cellular transition to the endoderm. (*F*) Down-regulation of the hESC marker *OCT4* was observed in both protocols over the 3-day period and absent in the −RT control, C. qPCR amplicons were measured using an ABI Prism 7900.

Table S1. Primer sequences and RT-PCR conditions

Gene	Primer Sequence	Annealing temperature, °C	Cycles	Size, bp
AFP	For-AGAACCTGTCACAAGCTGTG Rev-GACAGCAAGCTGAGGATGTC	55	30	675
ALB	For-CCTTTGGCACAATGAAGTGGGTAACC Rev-CAGCAGTCAGCCATTTCACCATAGG	55	30	354
AAT	For-AGACCCTTTGAAGTCAAGCGACC Rev-CCATTGCTGAAGACCTTAGTGATGC	55	30	358
TO	For-GGCAGCGAAGAAGACAAATC Rev-TCGAACAGAATCCAACTCCC	55	30	218
HNF4α	For-CTGCTCGGAGCCACCAAGAGATCCATG Rev-ATCATCTGCCACGTGATGCTCTGCA	55	30	370
OCT4	For-GACAACAATGAAAATCTTCAGGAGA Rev-TTCTGGCGCCGGTTACAGAACCA	55	30	218
CYP3A4	For-CCTTACATATACACACCCTTTG Rev-GGTTGAAGAAGTCCTCCTAAGCT	50	35	169
TAT	For-ACTGTGTTTGGAAACCTGCC Rev-GCAGCCACTTGTCAGAATGA	55	30	188
APOF	For-GGAAGCGATCAAACCTACCA Rev-ATCAGCCTGACAACCAGCTT	58	35	347
CYP7A1	For- CTGCCAATCCTCTTGAGTTCC Rev- ACTCGGTAGCAGAAAGAATACATC	57	35	387
β-ACTIN	For-TCACCACCACGGCCGAGCG Rev-TCTCCTTCTGCATCCTGTCG	58	25	350

[A]**AFP*, α fetoprotein; *ALB*, albumin; *TO*, tryptophan dioxygenase; *HNF4α*, hepatocyte nuclear factor 4 α; *OCT4*, Octamer 3/4; *CYP3A4*, cytochrome p450 3A4; *TAT*, tyrosine amino transferase; *APOF*, apolipoprotein F; *CYP7A1*, cytochrome p450 7A1; *β-ACTIN*, β actin.

Table S2. qPCR primer sequences and conditions

Gene	Primer/probe sequence
BRACHYURY	Applied Biosystems - hs00610079-ml
HNF3β	Applied Biosystems - hs00936490-ml
SOX17	Applied Biosystems - hs00751752-sl
NODAL	Applied Biosystems - hs01080749-ml
CRIPTO	Applied Biosystems - hs00414425-ml
ALBUMIN	Applied Biosystems - hs 00910225-m1
WNT3	For-TCCTCGCTGGCTACCCAAT
	Rev-CGGCAGAAGCGCAGTTG
	Probe-CCAGGGCCAGGGACCACCAA
WNT3α	For-GCCCGCTCAGCCATGA
	Rev-CCGTGGCACTTGCACTTGA
	Probe-CAGGCCATCGCCAGCCACATG
WNT11	For-ACTTCTGCATGAAGAATGAGAAGGT
	Rev-TCGCTTCCGTTGGATGTCTT
	Probe-CCCACGGGACACAAGACAGGCAG
b2M	For-GAGTATGCCTGCCGTGTG
	Rev-AATCCAAATGCGGCATCT
	Probe-CCTCCATGATGCTGCTTACATGTCTC

WNT, wingless; *HNF3β*, hepatocyte nuclear factor 3 β; *SOX17*, SRY (sex-determining region Y)-box 17; *β-2M*, β 2 microglobulin.

Table S3. Immunostaining: primary and secondary antibodies

Antigen	Type	Source	Dilution
Wnt3a	Mo mono	R&D Systems	1/20
DPPIV	Go poly	R&D Systems	1/1000
Brachyury	Go poly	R&D Systems	1/100
Albumin	Mo mono	Sigma	1/500
Albumin	Rb poly	AbCam	1/600
CK18	Mo mono	DAKO	1/50
CK19	Mo mono	DAKO	1/50
hKi67	Mo mono	DAKO	1/400
Anti-Mo 488	Rb anti-Mo	Molecular Probes	1/500
Anti-Go488	Do anti-Go	Molecular Probes	1/500
Anti-Mo HRP	Rb anti-Mo	DAKO	1/100
Anti-Go HRP	Rb anti-Go	DAKO	1/100

Mo mono, mouse monoclonal antibody; Go poly, goat polyclonal antibody; Rb anti-Mo, rabbit antimouse; Rb-anti-Go, rabbit antigoat; Do anti-Go, donkey antigoat.