

Fig. S1. Detection of various lineage markers expression in harvested tissues to assess potential contamination from non-ectodermal tissues. (A) RT-qPCR analysis of marker gene expression in tissues separated from E6.5 mouse embryo, including the anterior portion of the embryonic ectoderm (E6.5-Anterior), the posterior portion of the embryonic ectoderm (E6.5-Posterior) and the ExE (E6.5-EXE). (B) RT-qPCR analysis of marker gene expression in tissues obtained from the E7.0 mouse embryo, including the anterior part of the mesoderm and endoderm (Me/En-Anterior), the anterior portion of the ectodermal layer (Anterior), the posterior portion of the ectodermal layer (Posterior) and the posterior part of the mesoderm and endoderm (Me/En-Posterior).

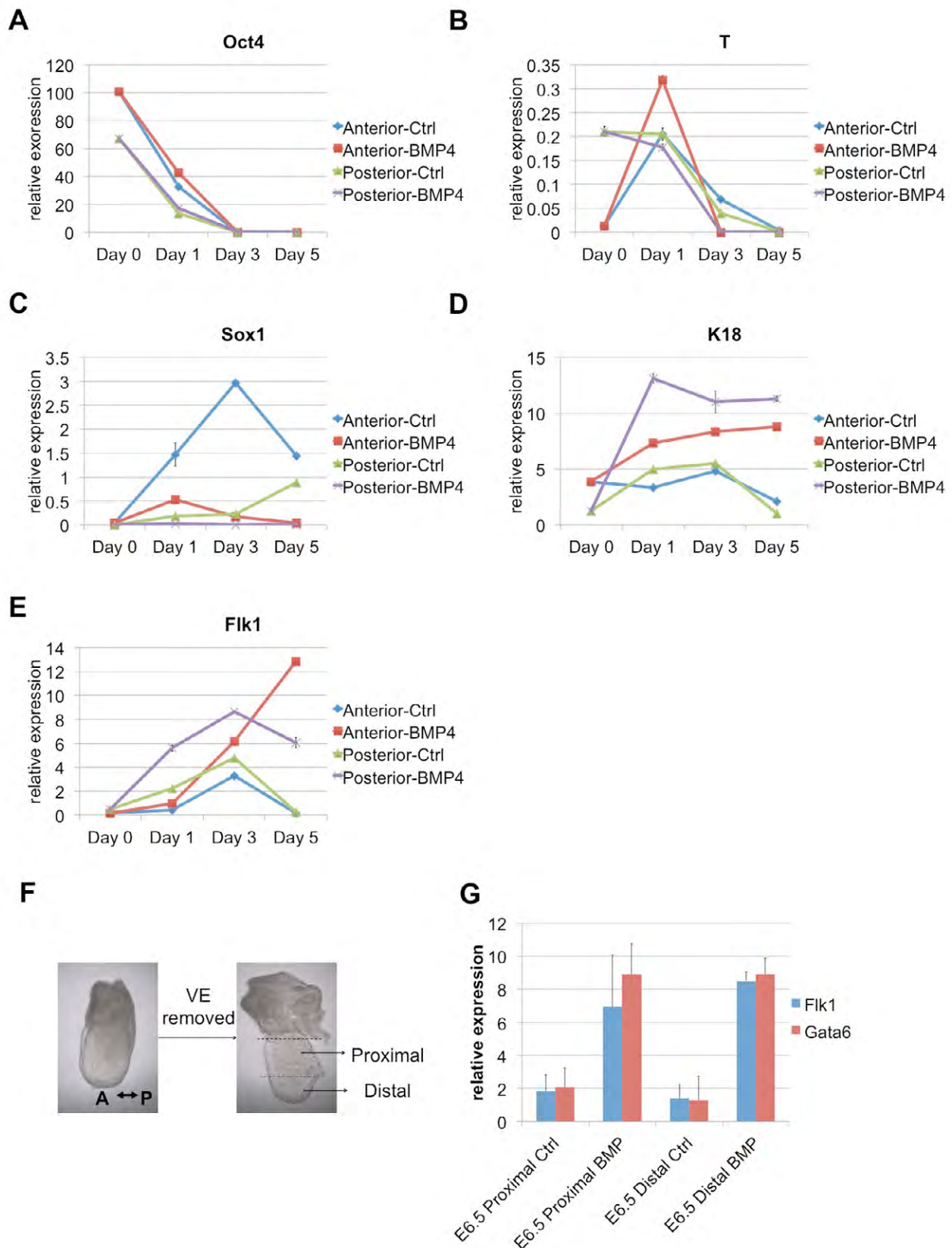


Fig. S2. E6.5 explants culture in control and BMP4-supplemented medium. (A-E) Marker genes expression (*Oct3/4*, *T*, *Sox1*, *K18*, *Flk1*) is analyzed during the 5 days culture of E6.5 anterior and posterior explants in the medium without or with BMP4. Each sample has epiblast tissue from three embryos. (F) A schematic showing how the epiblast is cut into proximal and distal portions. (G) RT-qPCR analysis of *Flk1* and *Gata6* in proximal and distal explants after 5 days culture with or without BMP4. There are three replicates for each group of data.

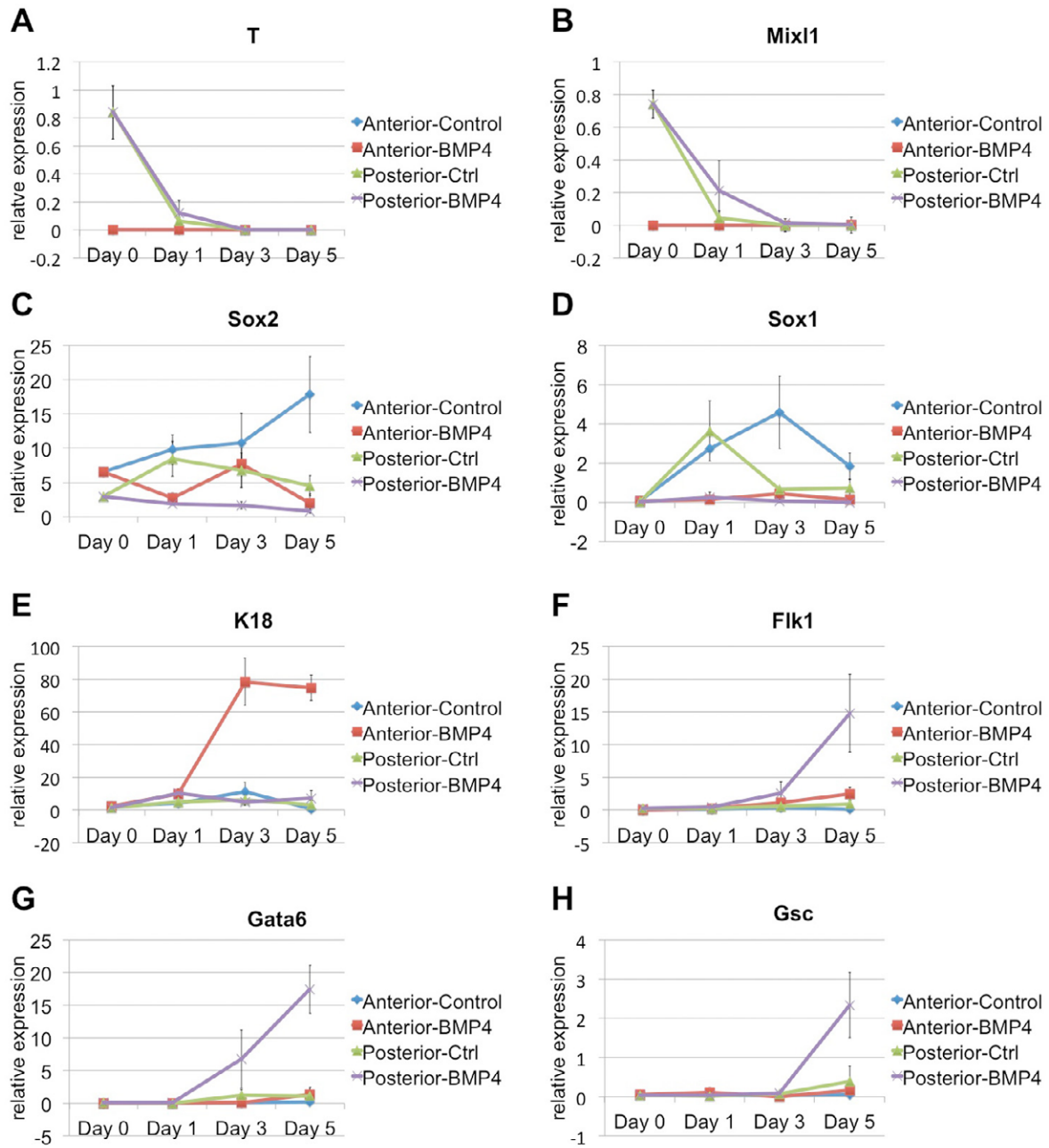


Fig. S3. The lineage progression of E7.0 anterior and posterior explants cultured without or with BMP4 for 5 days. (A-H) The expression of *T*, *Mixl1*, *Sox2*, *Sox1*, *K18*, *Flk1*, *Gata6* and *Gsc* is analyzed through RT-qPCR. There are four replicates for each group of data.

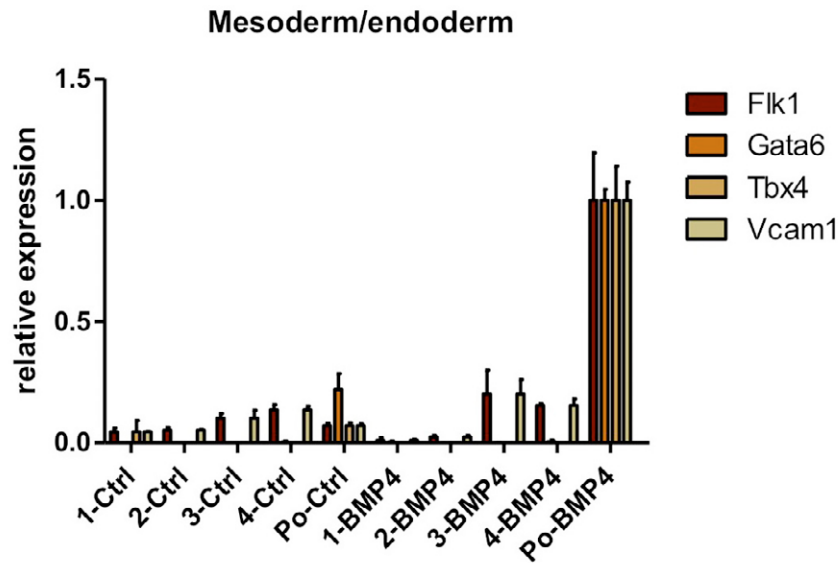


Fig. S4. Mesoderm/endoderm marker genes expression in the derivatives of E7.0 A/P fragments 1, 2, 3, 4. RT-qPCR analysis of mesoderm/endoderm marker genes expression in derivatives of fragments 1, 2, 3, 4 and posterior explants that have been cultured in control or BMP4-added medium for 5 days.

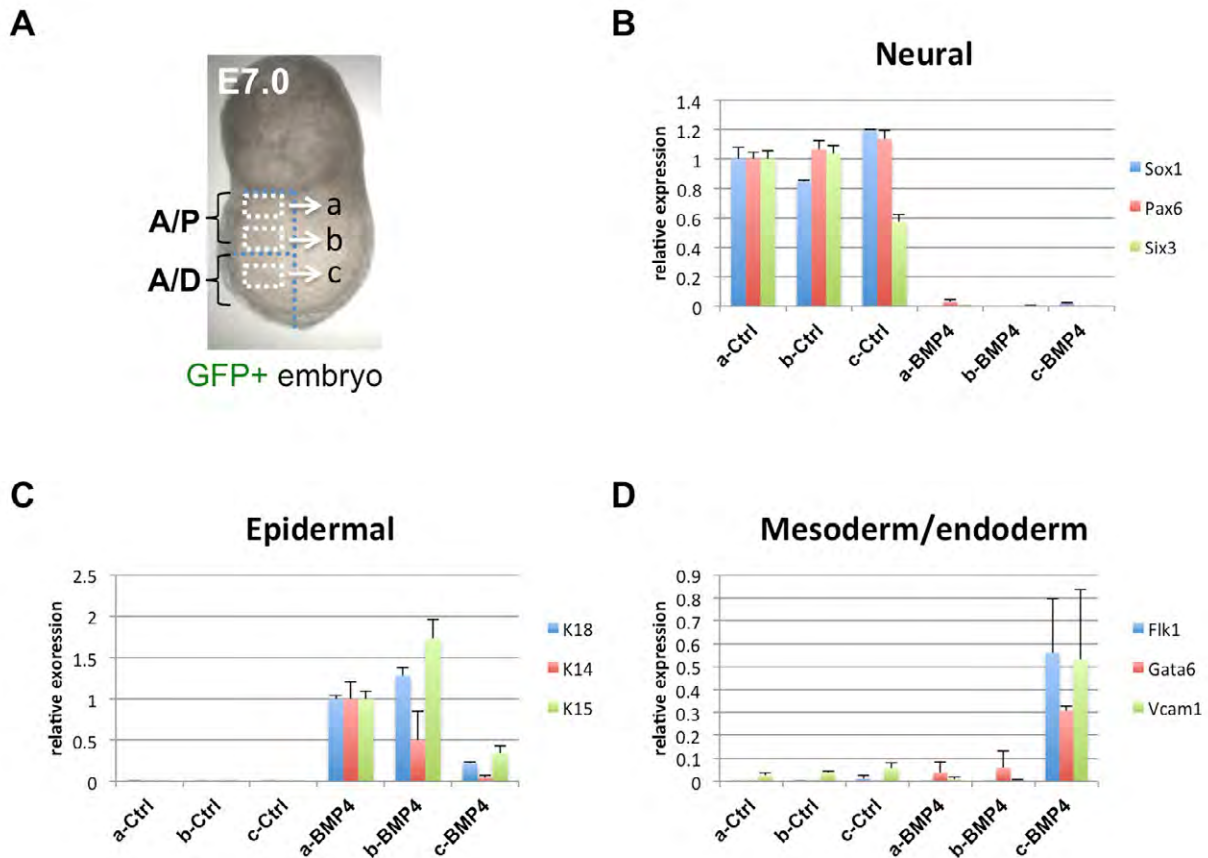


Fig. S5. Regionalization of ectodermal progenitor potential in E7.0 ectodermal layer. (A) Schematic illustrating locations of fragments a, b and c in anterior ectoderm region. (B-D) RT-qPCR analysis of neural (*Sox1*, *Pax6*, *Six3*), epidermal (*K18*, *K14*, *K15*) and mesoderm/endoderm (*Flk1*, *Gata6*, *Vcam1*) marker genes expression in derivatives of fragments a, b and c co-cultured with large anterior ectoderm explants for five days.

Table S1. Primer sequences (5'→3') for RT-qPCR

Genes	5' primer	3' primer
Hhex/Hex	CGGACGGTGAACGACTACAC	CGTTGGAGAACCTCACTTGAC
Pou5f1/Oct4	AGTTGGCGTGGAGACTTTGC	CAGGGCTTTCATGTCCTGG
Pou3f1/Oct6	TCGAGGTGGGTGTCAAAGG	GGCGCATAAACGTCGTCCA
Nanog	TTGCTTACAAGGGTCTGCTACT	ACTGGTAGAAGAATCAGGGCT
Nodal	CCTGGAGCGCATTGGATG	ACTTTTCTGCTCGACTGGACA
Fgf5	GCTGTGTCTCAGGGGATTGT	CACTCTCGGCCTGTCTTTTC
Sox2	GCGGAGTGGAAACTTTTGTCC	CGGGAAGCGTGTACTTATCCTT
Sox1	GCACACAGCGTTTTCTCGG	ACATCCGACTCCTCTTCCC
Krt18/K18	CAGCCAGCGTCTATGCAGG	CTTCTCGGTCTGGATTCCAC
Krt19/K19	GGGGGTTTCAGTACGCATTGG	GAGGACGAGGTCACGAAGC
Brachyury/T	CTCGGATTCACATCGTGAGAG	AAGGCTTTAGCAAATGGGTTGTA
Kdr/Flk1	GGGTCGATTTCAAACCTCAATGT	AGAGTAAAGCCTATCTCGCTGT
Sox17	CGAGCCAAAGCGGAGTCTC	TGCCAAGGTCAACGCCTTC
Gata6	TTGCTCCGGTAAACAGCAGTG	GTGGTCGCTTGTGTAGAAGGA
Gata4	CCCTACCCAGCCTACATGG	ACATATCGAGATTGGGGTGTCT
Eomes	CCTGGTGGTGTTTTGTGTG	TTAATAGCACCGGGCACTC
Cdx2	GCTACGGCGAACTTGGACA	GTGATGGTGCGCGTGGTAT
Krt8/K8	TCCATCAGGGTGA CT CAGAAA	CCAGCTTCAAGGGGCTCAA
Krt5/K5	TCCAGTGTGTCCTTCCGAAGT	TGCCTCCGCCAGAACTGTA
Krt14/K14	AAGGTCATGGATGTGCACGAT	CAGCATGTAGCAGCTTTAGTTCTTG
Krt15/K15	AGCTATTGCAGAGAAAAACCGT	GGTCCGTCTCAGGTCTGTG
ΔNp63	TGTACCTGGAAAACAATGCCCA	GACGAGGAGCCGTTCTGAATCT
Tbx4	TCCCCAGCTACAAGGTAAAAGT	ACCATCCATTTGTTGTCACAGAA

Vcam1	AGTTGGGGATTTCGGTTGTTCT	CCCCTCATTCCCTTACCACCC
Mtap2/MAP2	GCCAGCCTCGGAACAAACA	GCTCAGCGAATGAGGAAGGA
Cer1	CTCTGGGGAAGGCAGACCTAT	CCACAAACAGATCCGGCTT
Krt17/K17	ACCATCCGCCAGTTTACCTC	CTACCCAGGCCACTAGCTGA
Six3	CCGGAAGAGTTGTCCATGTTC	CGACTCGTGTTTGTGATGGC
Cdh1/E-cadherin	CAGGTCTCCTCATGGCTTTGC	CTCCGAAAAGAAGGCTGTCC
Pax6	GCAGATGCAAAAGTCCAGGTG	CAGGTTGCGAAGAACTCTGTTT
Id1	GGTCCGAGGCAGAGTATTACA	CCTGAAAAGTAAGGAAGGGGGA
Id2	ATGAAAGCCTTCAGTCCGGTG	AGCAGACTCATCGGGTCGT
Hand1	GGCAGCTACGCACATCATCA	CCTGGCATCGGGACCATAG
Mesp2	CGGCGTTCTCTCACCGATG	CACCCCACTACTCATGGCTG
Mixl1	ACGCAGTGCTTTCCAAACC	CCCGCAAGTGGATGTCTGG
Gsc	CAGATGCTGCCCTACATGAAC	TCTGGGTACTTCGTCTCCTGG
Sox7	ATGCTGGGAAAGTCATGGAAG	CGTGTTCTGGTCACGAGAGA
MyoD	CCACTCCGGGACATAGACTTG	AAAAGCGCAGGTCTGGTGAG

Table S2. Primer sequences (5'→3') for *in situ* probes

T: Fwd: ATCAAGCTTCTGGGAGCTCAGTTCTTTTCGAGGC
T: Rev: ATCGGATCCGTGGACGAATTCCAGGATTTCAAAG
Hesx1: Fwd: CCGAATTCGGAAGGTGCTCAGCTC
Hesx1: Rev: GCTCTAGAACTGTGATTCTCTACGGGAC
Sox2: Fwd: GGAATTCAAAGGAAAAAAAAATCTCCG
Sox2: Rev: GCTCTAGAACGTTTGCCTTAAACAA
Oct6: Fwd: TTAAACAAAACCAAACACCCG
Oct6: Rev: CGATTTAAATTAAGGGCGCG

Krt18: Fwd: CTCCAGACAAGATGAGCTTCACA
Krt18: Rev: CTCCATCTGTGCCTTGTATCG
Six3: Fwd: GCGCACTACCAGGAGGCCGAGAA
Six3: Rev: GTGTGTATCTGTCTGTGTATCCTGATT
Fgf5: Fwd: CCGGAAGAATGAGCCTGTCCTT
Fgf5: Rev: GAGCATCATCCAAAGCGAACTTC