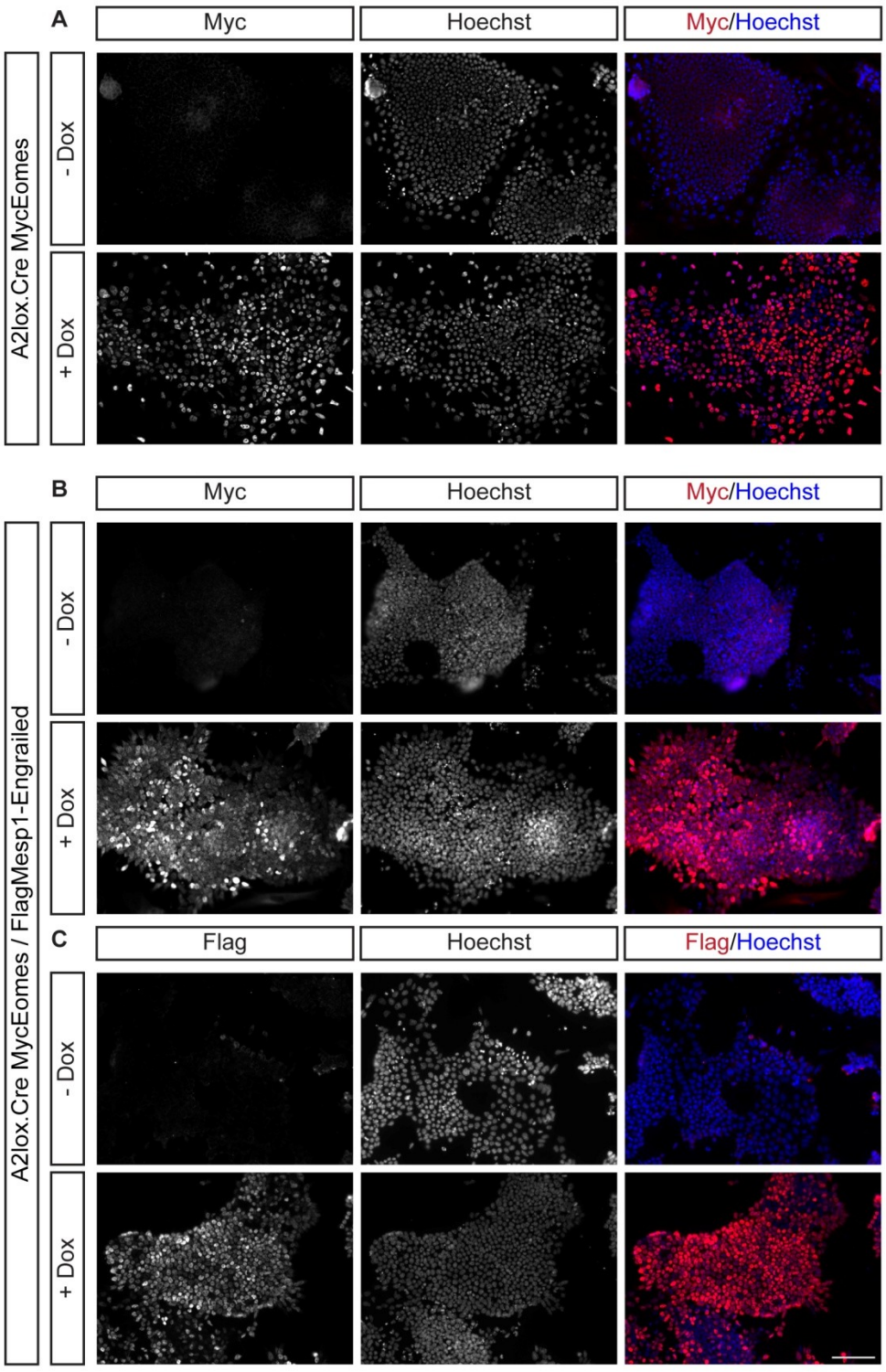
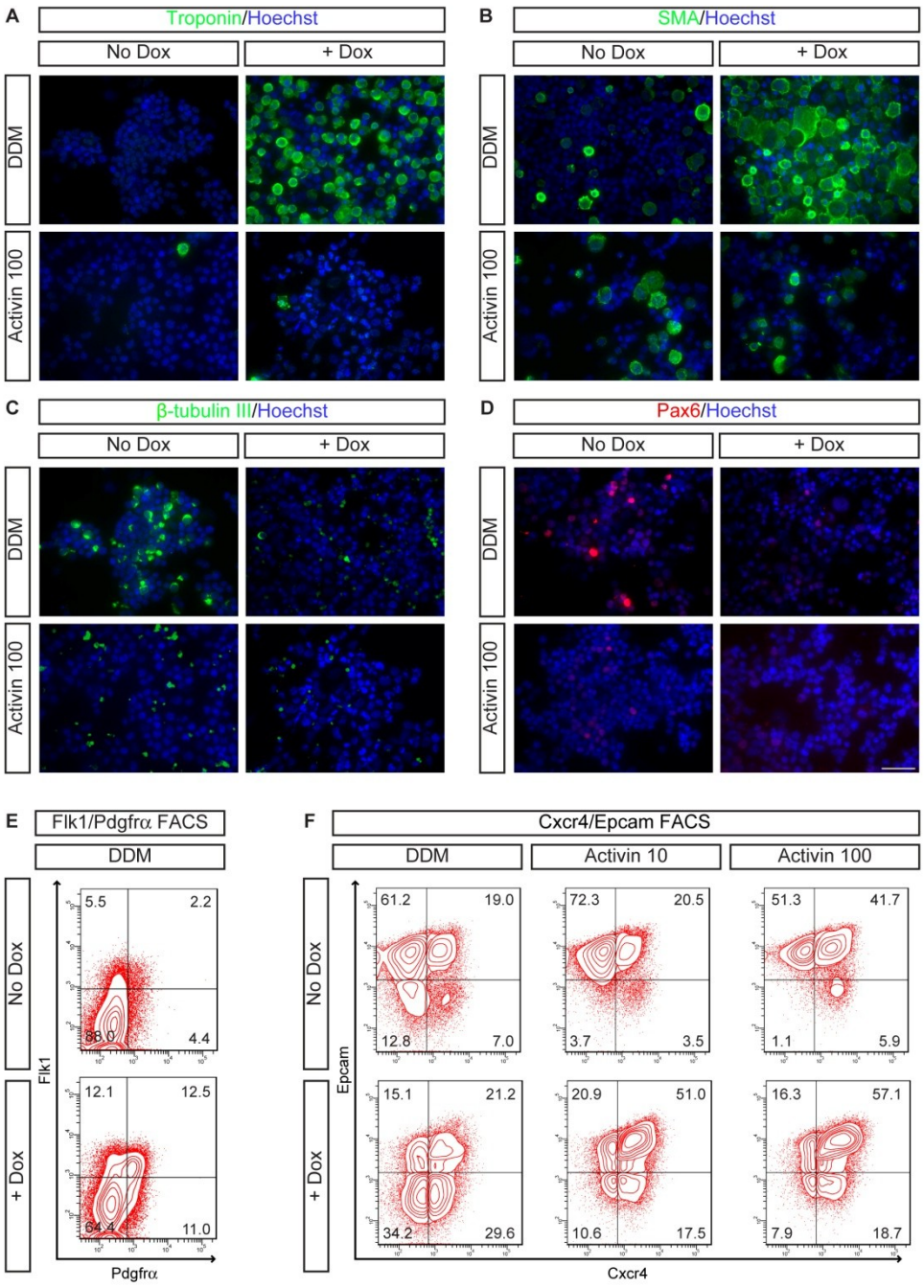


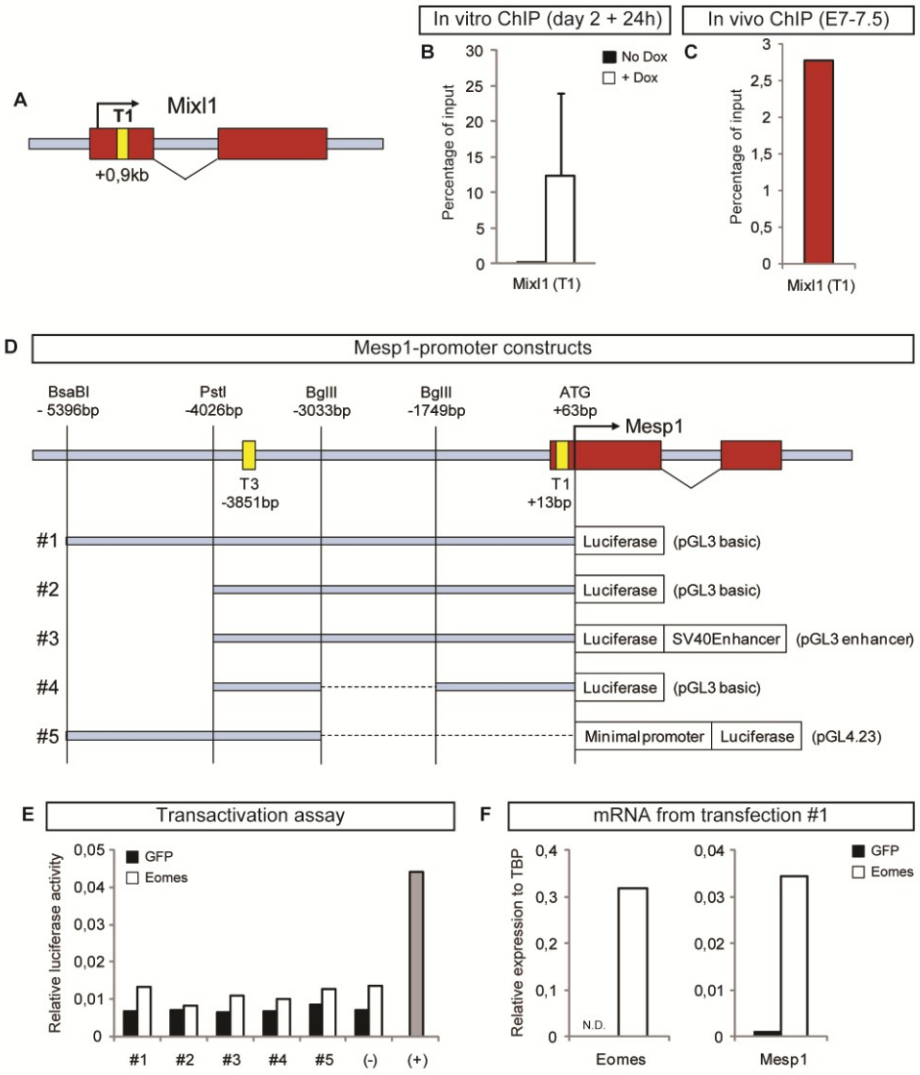
Supplementary figure 1. van den Ameele et al.

Immunostainings against the Myc- or Flag-tag showing expression of MycEomes (A,B) and FlagMesp1Engrailed (C) in A2lox.Cre-MycEomes (A) and A2lox.Cre-MycEomes-FlagMesp1Engrailed (B,C) ESC lines, 24 hours after addition of doxycylin (+Dox). Nuclei are counterstained with Hoechst dye. Scale bar, 100µm.



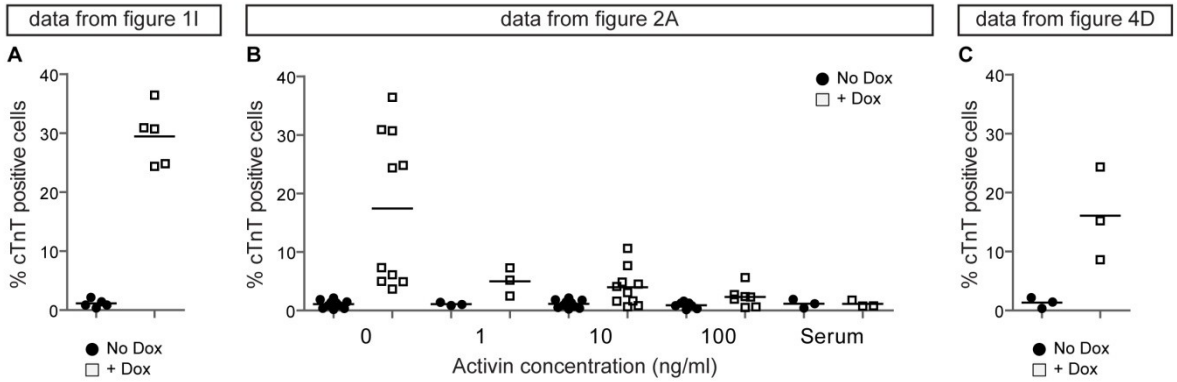
Supplementary figure 2. van den Ameele et al.

(A-D) ESC were cultured in defined default medium (DDM), in the presence or absence of Activin (100ng/ml) from day 0-4 and/or doxycyclin (Dox) at day 2-3. After cytospin at day 10, they were immunostained for cTnT (A), Smooth muscle actin (SMA) (B), β-tubulin III (C) or Pax6 (D). Nuclei are stained with Hoechst dye. Scale bar, 50µm. (E-F) Representative density plots from the experiments depicted in figure 1O (E) and figure 3F (F), where cells were analyzed at day 4, 48 hours after addition of doxycyclin (+Dox) for the expression of Flk1 and Pdgfrα (E) or Epcam and Cxcr4 (F).



Supplementary figure 3. van den Ameele et al.

(A) Representation of the genomic region of Mixl1, showing the exons (red) and the Eomes binding site (yellow, T1) 0,9kb downstream of the start of transcription of Mixl1. (B,C) Quantification as measured by qPCR of DNA fragment enrichment by ChIP on differentiating ESC at day 3 with or without Dox from day 2 (B) or on E7 embryos (C) using anti-Eomes antibody. Data are presented as percentage of the input. In vitro data are presented as mean + sem of two experiments (B). (D) Constructs generated for transactivation assays of the Mesp1-promoter. Various fragments of a 5,6kb Mesp1-promoter were cloned into pGL3 basic, pGL3 enhancer or pGL4.23 vector backbones using the restriction enzymes depicted. (E) Relative luciferase activity of the different constructs shown in (D) 24 hours after transfection into P19 cells, together with pCAG expressing GFP or pCAG-MycEomes-IRES-GFP. pGL3 basic was used as a negative control (-); a validated Hes5-promoter construct together with Notch1ΔE as a positive control (+). (F) qRT-PCR for Eomes and Mesp1 24 hours after transfection of construct #1 with pCAG-GFP or pCAG-MycEomes-IRES-GFP. N.D., transcript not detectable.



Supplementary figure 4. van den Ameele et al.

(A-C) Scatter plots of the FACS quantifications of the percentage of cTnT-expressing cells at day 10 of differentiation of the A2lox.Cre-MycEomes ESC lines. Each scatter plot shows the raw data of the different experiments performed to obtain the graphs depicted in figure 1I (A), figure 2A (B) and figure 4D (C).

qRT-PCR primers

Target gene	Forward sequence	Reverse sequence
Afp	GAGAAATGGTCCGGCTGTGGTG	GGGGGAGGGGCATAGGTTTCA
CD31 (Pecam1)	AATGGCAACTGGAGCGAGCACT	GGAGAAGGCGAGGAGGGTTAGGT
Eomes	CCGGGACAACTACGATTCCA	ACCTCCAGGGACAATCTGATG
Gsc	CCAGCAGTGCTCCTGCGTCC	CGACAGCGTGCCCACGTTCA
Mesp1	TGTACGCAGAAACAGCATCC	TTGTCCCCTCCACTCTTCAG
Sox1	AGGCACCTGGGACCAGCACA	CGCTGCCTCCTCTTGTCGGC
Sox17	GCGGCGCAAGCAGGTGAAG	GGGGCCCATGTGCGGAGAC
Tnnt2	GCGGAAGAGTGGGAAGAGACAGAC	GCACGGGGCAAGGACACAAG
Tubb3	CCAGTGCGGCAACCAGATAGG	AAAGGCGCCAGACCGAACACT

ChIP-qPCR primers

Target site	Forward sequence	Reverse sequence
Mesp1 (T1)	TGCACCTCAGAGCCCCTGCT	GGCCTGACCATTGGGTCCGC
Mesp1 (T2)	CCAGGAACCTGGTGGCCTAGC	ATGGAGCACTCGCTTGGCGT
Mesp1 (T3)	CTCTCACCTCTGTTCTGATGGGG	TGGGTTCCTTTGGGAGCTGCTTGG
Mesp1 (-)	TCAGAATGGCTGGGCAAGGTGC	AGGACACAGGGTGGCCAGGG
Mixl1 (T1)	AAGTTCGCGCCTCCCTCATT	CGCGCGCCTGCAGTTATAG

Supplementary table 1. van den Ameele et al.