An interplay between extracellular signalling and the dynamics of the exit from pluripotency drives cell fate decisions in mouse ES cells

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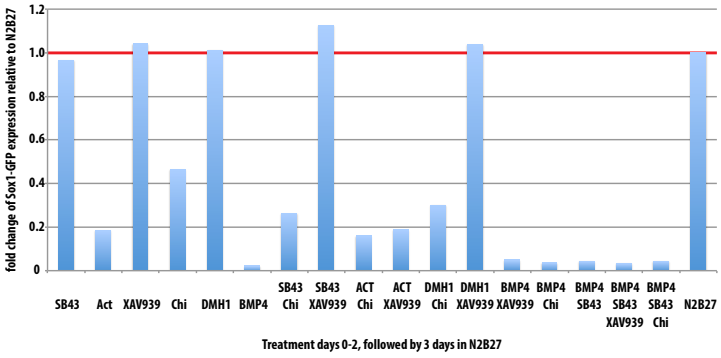
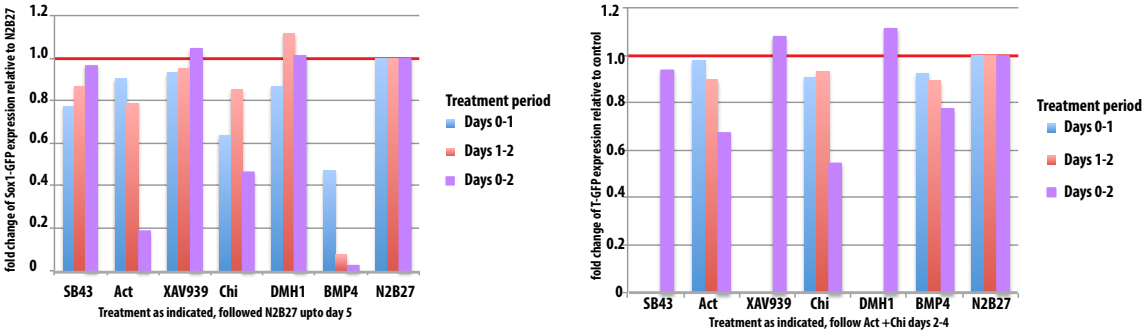
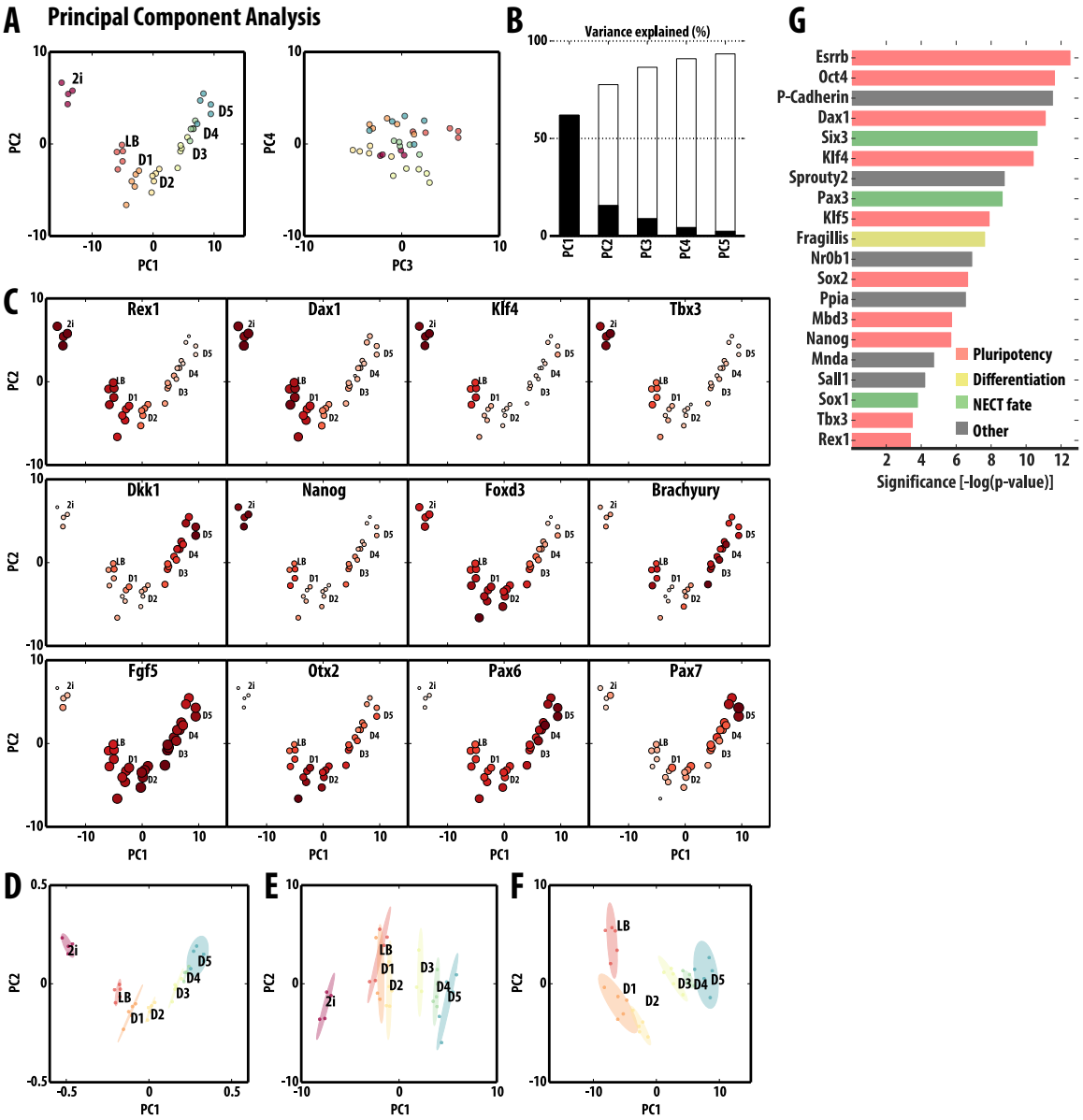
Supplementary Figures



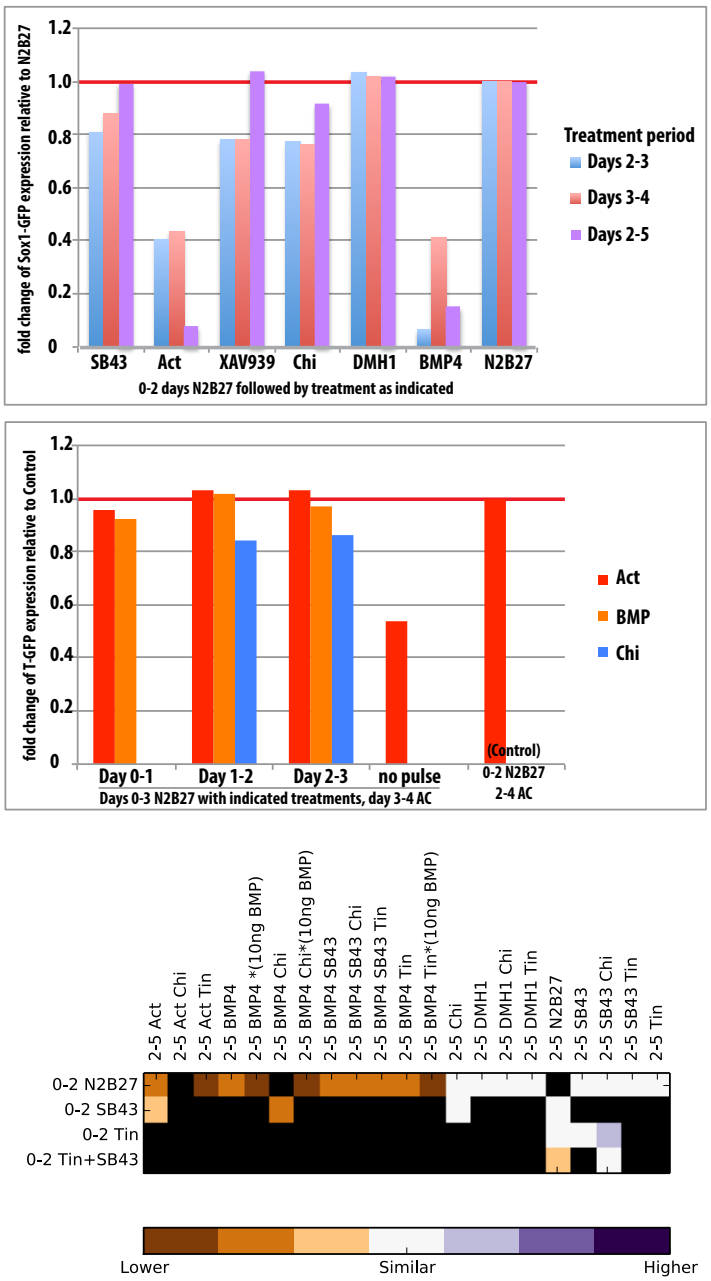


Supplementary Data for Figure 2: Effects of signalling during the exit from pluripotency. (A) Sox1::GFP cells were cultured in the indicated factors days 0-2, followed by days 2-5 in N2B27. (B-C) Sox1::GFP (B) or T::GFP(C) cells were maintained in N2B27 for 5 days (Sox1::GFP) or 2 days in N2B27, followed by 2 days in AC (T::GFP) and exposed to pulses of the treatment as indicated. GFP expression was analyzed by flow cytometry and results were normalized to 5 days in N2B27 (Sox1::GFP) or 2 days N2B27 followed by 2days AC (T::GFP). Note the effect of the factors and treatments have remarkably similar effects on GFP expression in both Sox1::GFP cells and T::GFP cells.

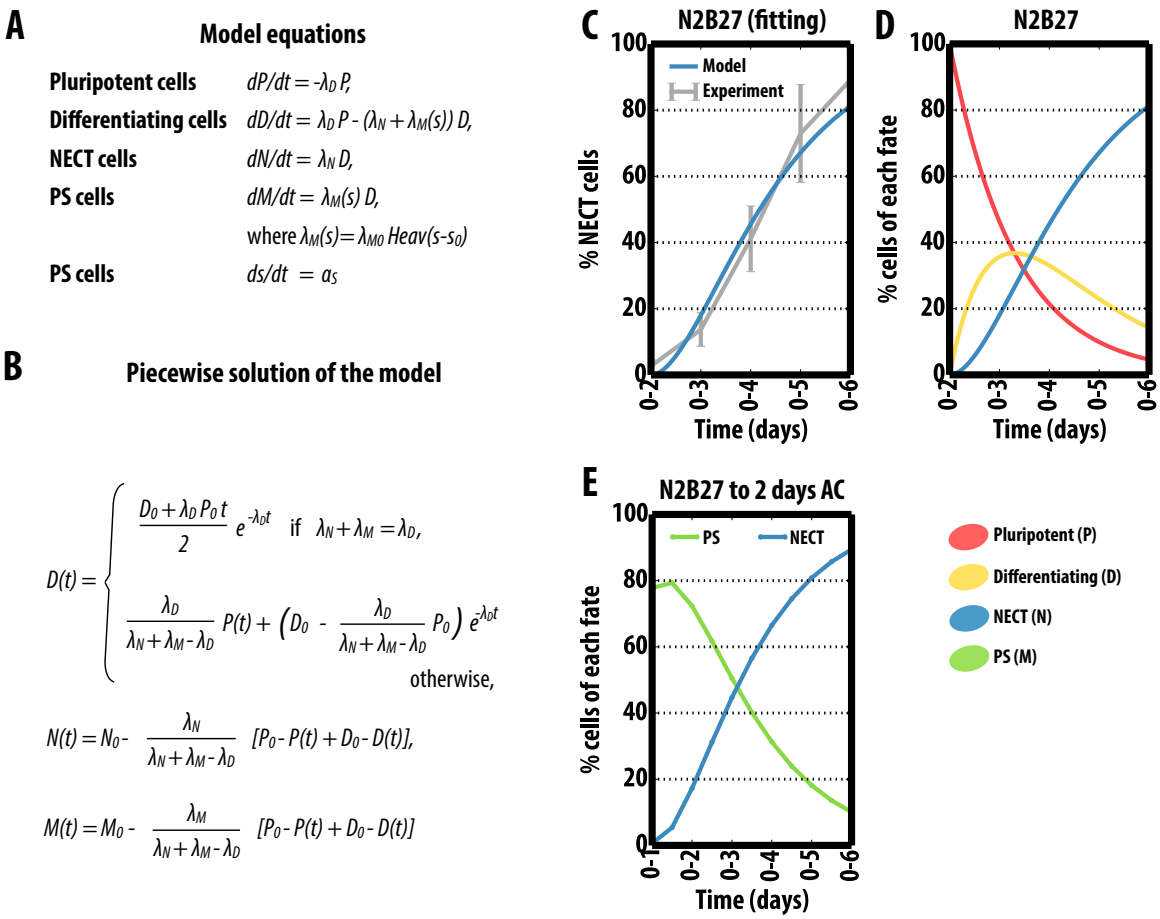
Supplementary Data for Figure 3: A fate restriction point at the exit from pluripotency. (A) Sox1::GFP cells were grown in the indicated medium (ordinate) for 2 days and a further day in N2B27 prior to RNA extraction and RT-qPCR analysis for the indicated genes (graph titles). Data normalised to the house-keeping gene Ppia (see supplemental data for Fig. 3). (B) Live-cell imaging of TNGA Nanog::GFP cells treated with a 2 day pulse of either SB43 or XAV939 before wash-off and medium replacement with N2B27. Scale-bar indicates 100μm.



Supplementary Data for Figure 4: Analysis of gene expression during the exit from pluripotency. (A-F) Principal Component Analysis of bulk gene expression. We performed PCA of the gene expression profiles of 34 genes in 2i, BMP and LIF, and the 5-days differentiation in N2B27. The first four components are shown in (A). Each dot corresponds to a technical repeat of a particular experimental condition (colour coded and labelled in the left panel). PC1 and PC2 cluster data according to each condition (A) and account for more than 75% of the total variance (B). Panel (C) shows the levels of expression (coded in colour and size) of each condition for several genes of interest. (D-E) The results of the PCA are robust to data normalisation procedures. We obtained similar results doing PCA with data normalised with the min-max method (D) or the Z method (E). (F) The results of the PCA are hold when we do not consider the 2i conditions. (G) Single-cell gene expression analysis: top-most significant genes for differences in the distributions of expression levels of 4 days as well as the initial LIF and BMP condition (Kruskal-Wallis one-way test p-values corrected with Bonferroni for multiple testing).



Supplementary Data for Figure 5: Activin activity but not Wnt/β-Catenin activity controls fate decisions between PS and NECT. (A) Sox1::GFP cells were maintained in N2B27 and treated with factors as indicated. GFP expression was assessed by flow cytometry and are normalized to 5 days in N2B27. Note ACT and BMP inhibit GFP expression and the longer the exposure to these factors the stronger their inhibitory effects. SB43, XAV939, DMH1 and surprisingly Chi either do not effect or mildly inhibit GFP expression and this inhibition is reduced the longer the exposure to the factors. (B) T::GFP cells were differentiated for 3 days in N2B27 followed by one day in AC and treated with factors as indicated. Results are normalized to 2 days in N2B27 followed by 2 days in AC. Note both BMP and to a lesser extent Chi are able to enhance GFP expression compared to N2B27 over the same period. (C) Sox1::GFP cells were cultured in N2B27, SB43, XAV939 or SB43 + XAV939 for 0-2 days and as indicated days 2-5. GFP expression was analyzed by flow cytometry, normalized to 5 days in N2B27 and is presented in the form of a heat map.



Supplementary Data for Figure 6: A simple population model for a cell fate decision race. (A) Model equations: The model consists of 4 different cellular types: pluripotent cells (*P*), differentiating cells (*D*), cells committed to NECT (*N*) and cells committed to PS (*M*). We consider initially all cells to be pluripotent. Cells then spontaneously loose pluripotency and start differentiating with a certain rate λ_D. Once each cell abandons the pluripotent state, rapidly and irreversibly adopts a final fate: either NECT or PS. Cells are assumed to acquire either fate at a particular rate: λ_N for NECT and λ_M for PS. In addition to these cellular types and the corresponding transitions, we also consider a signal (*s*) that builds up and once crosses a threshold, biases the rates of fate adoption. In particular, we consider that the rate of PS conversion is negligible in the absence of signal and becomes the largest rate once the signal concentration has reached the threshold which we arbitrarily take to $s_0=1$. We initially consider all cells to be pluripotent ($P=1, D=N=M=0$). The model is linear but non-smooth, with piecewise constant $\lambda_M(s)$ depending on the signal level. The analytic solution of each region of constant λ_M is given in (B). (C-D) Two of the three parameters (λ_D and λ_N) can be fitted to the experimental data on Sox1::GFP in N2B27 by assuming no cells adopt the PS fate ($\lambda_M=0$). (E) The model reproduces to a certain extent the 2-day Activin and Chiron pulse-chase experiment with the parameter values found in (C) and assuming a PS-fate adoption rate much higher than the NECT-fate adoption rate during the pulse (i.e., $\lambda_M >> \lambda_N$).