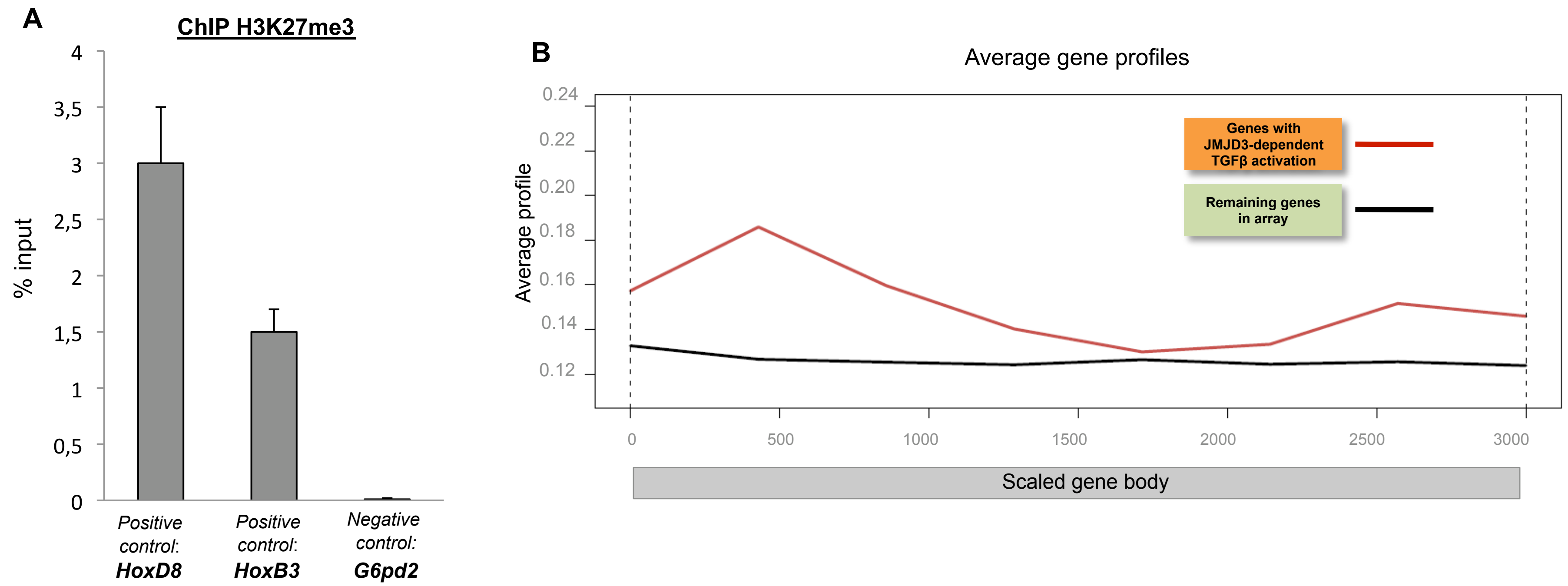
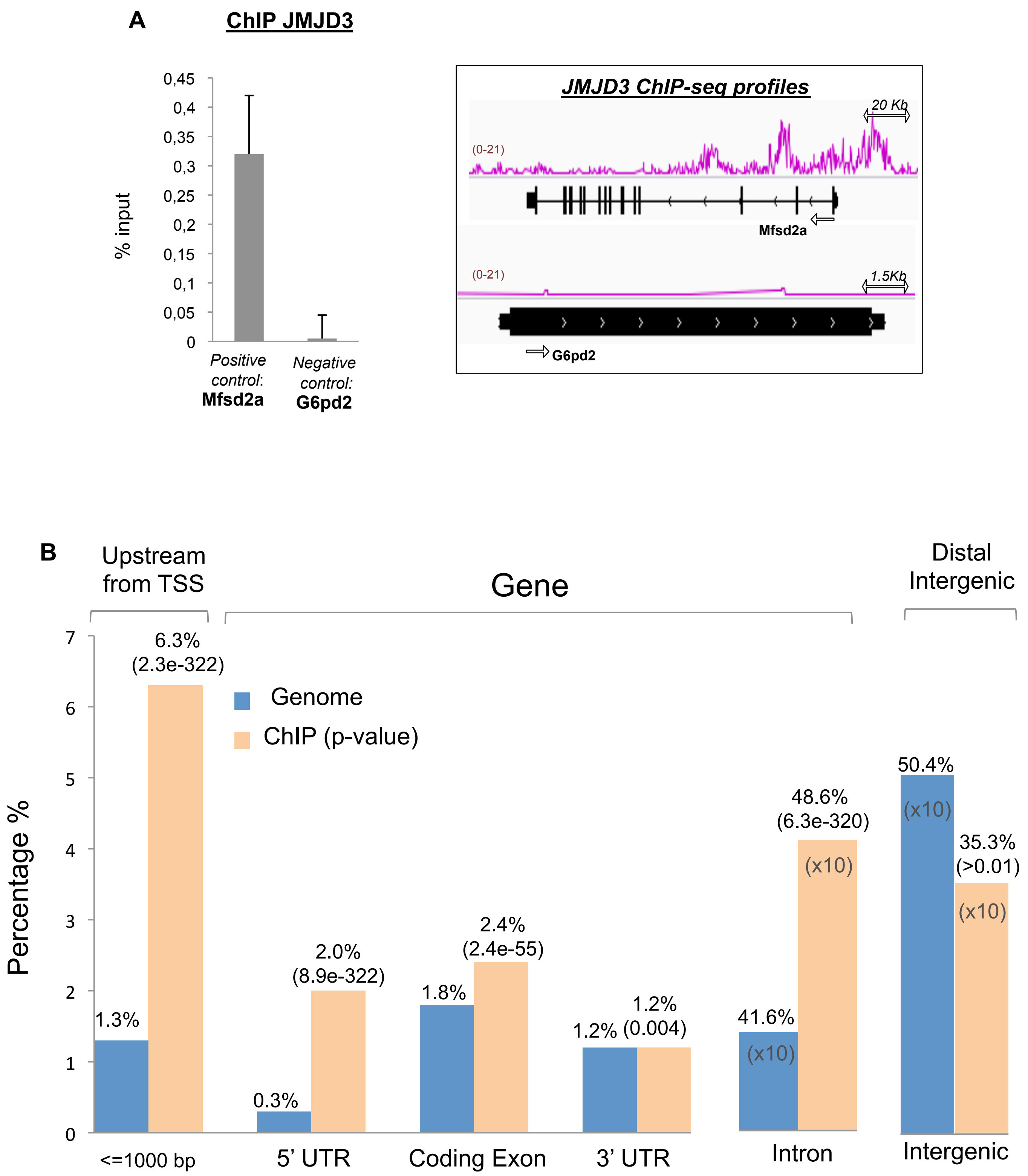
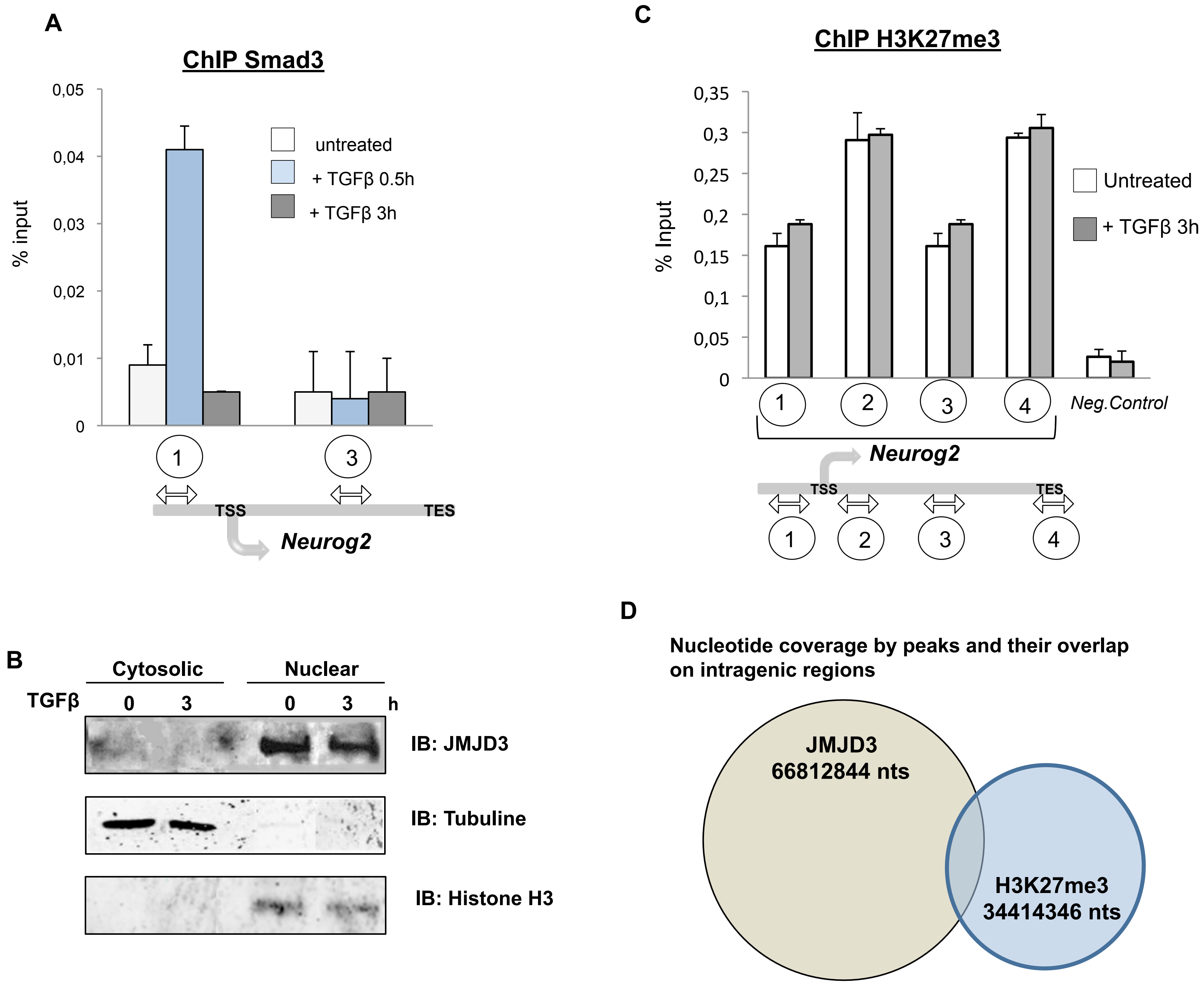
SUPPLEMENTARY FIGURE1



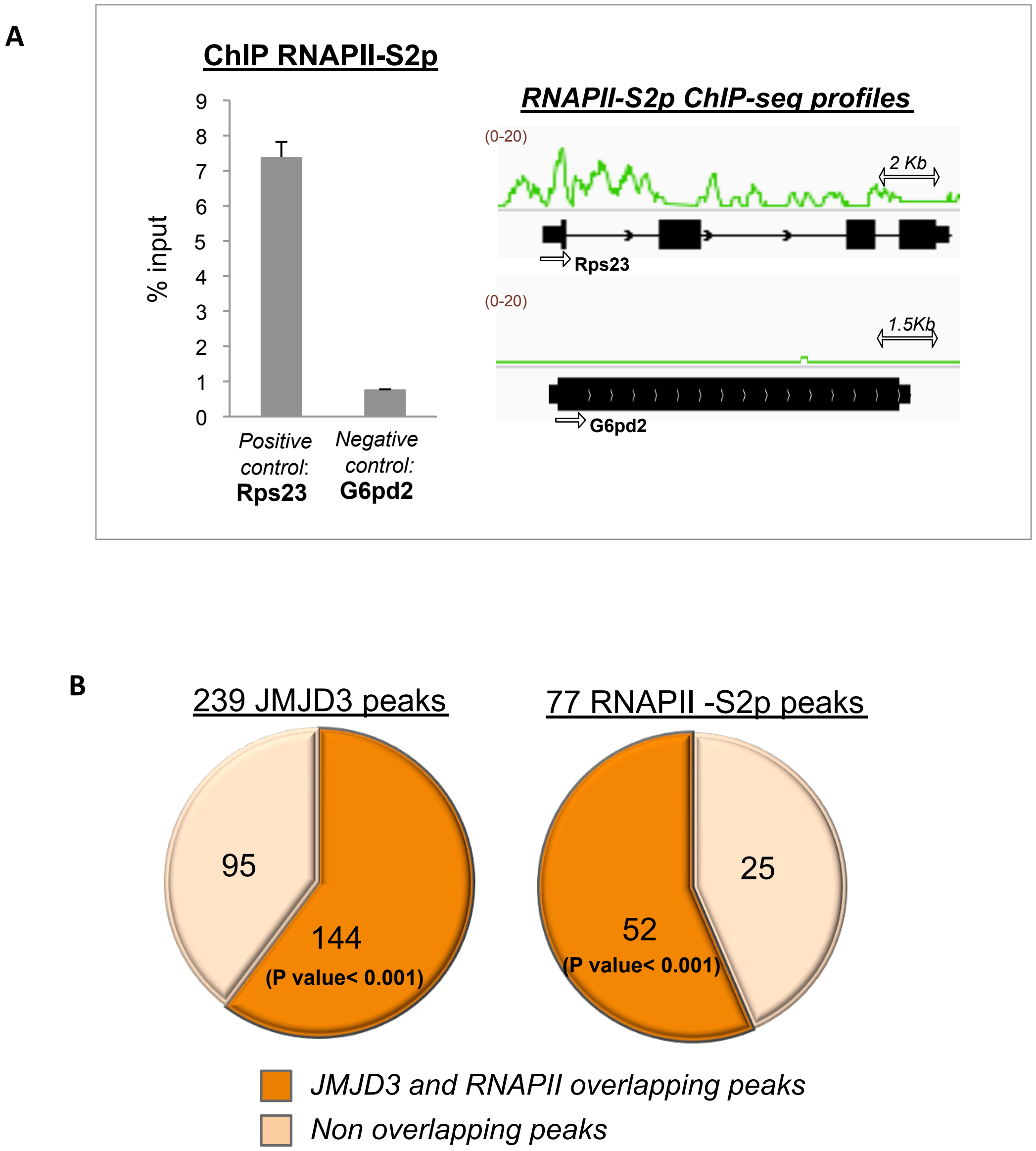
SUPPLEMENTARY FIGURE 2



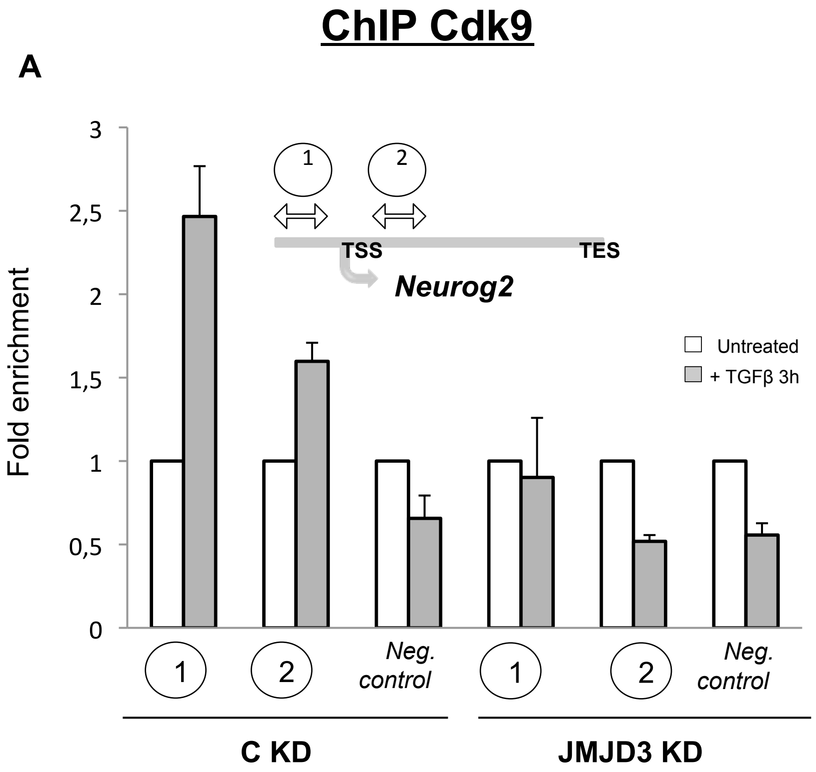
SUPPLEMENTARY FIGURE 3



SUPPLEMENTARY FIGURE 4



SUPPLEMENTARY FIGURE 5



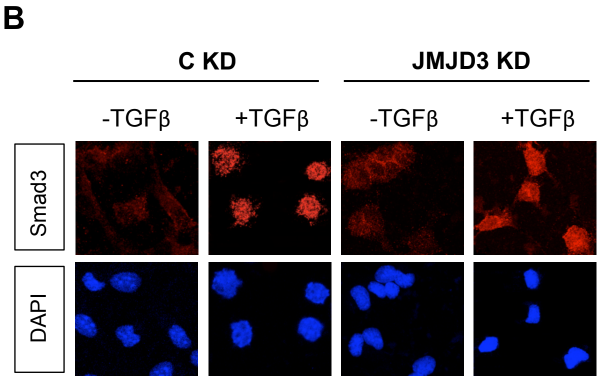


Table S1: List of JMJD3 dependent TGFβ activated genes (JDTA genes)

ID	Gene name	FC C KD	Adj. P value C KD	FC JMJD3 KD	Adj. P value JMJD3 KD
A_55_P2004179	Col2a1	16,24377065	5,17111E-12	11,35083454	2,02622E-11
A_55_P2067707	Mep1a	9,673053053	5,75141E-12	5,207245062	1,29147E-10
A_55_P2000943	Nrip3	8,760586025	5,17111E-12	5,73849171	3,36363E-11
A_51_P277006	Chst8	7,167338724	1,43765E-10	3,671794975	5,33605E-09
A_55_P2004881	D18Ertd653e	6,223014967	3,64733E-08	4,224942641	2,89604E-07
A_55_P2014124	Gjb3	6,105667375	3,05407E-09	2,859304311	3,5372E-07
A_52_P650855	Myo1d	6,048224121	1,25381E-07	3,522676964	3,49575E-06
A_55_P2394308	Fst	5,76162566	5,24848E-11	4,040965267	3,61273E-10
A_66_P124858	Diras2	5,428953616	1,17809E-08	2,918104085	6,4788E-07
A_55_P2143282	Nkd2	4,800835583	3,00506E-10	3,391727965	3,06396E-09
A_55_P2008921	Cdkl4	4,528871302	1,43765E-10	2,806693074	4,31928E-09
A_55_P2076777	Mdfi	3,698805762	0,000214813	1,620520568	0,145976114
A_55_P2020128	Dhrs3	3,369679195	3,70818E-07	2,478987695	6,72899E-06
A_52_P589169	2010111I01Rik	3,237928595	2,02799E-08	2,17557339	8,01831E-07
A_52_P368306	Tmem100	3,089445187	7,05518E-09	2,300495944	9,36617E-08
A_51_P157042	Ctgf	3,084118679	3,95019E-09	1,860099621	8,12844E-07
A_51_P474701	Fbp1	3,015193021	1,89138E-06	1,931896371	0,000240504
A_51_P426270	Mgp	2,924585339	1,32646E-08	1,955743502	8,05297E-07
A_52_P579933	Slc16a6	2,911019452	0,025861137	1,681506986	0,366414703
A_55_P2026734	Rgs4	2,764342889	2,33829E-07	1,901461515	1,9754E-05
A_55_P2186005	Sphk1	2,616375891	8,37817E-07	1,838175679	6,96513E-05
A_51_P419637	Dclk3	2,558304311	2,89895E-07	1,000905063	0,997318218
A_55_P2036693	Cd40	2,526512809	3,8619E-08	1,746196126	4,12245E-06
A_51_P430929	Fam20a	2,482678994	8,54049E-06	1,763493455	0,000655253
A_51_P433615	Klhl6	2,48151149	2,89895E-07	1,67686575	6,48653E-05
A_51_P270904	9930023K05Rik	2,38667137	9,83479E-08	1,682658589	1,13915E-05
A_66_P114528	Trim67	2,371942353	1,28002E-07	1,731564543	8,98887E-06
A_51_P115005	Edn1	2,32039475	1,71961E-07	1,420304275	0,000542891
A_55_P2094262	Tph1	2,314226864	8,87221E-08	1,506988454	6,61741E-05
A_52_P665675	Abca1	2,281425052	7,40917E-08	1,708480827	4,00435E-06
A_52_P59318	Rdh10	2,257786029	1,08852E-07	1,540946918	4,13336E-05
A_51_P370615	Neurog2	2,225207059	2,60523E-07	1,515086898	0,000123853
A_52_P103391	B4galt1	2,189181908	2,02418E-07	1,389214555	0,000618742
A_52_P274496	Tspan18	2,152184291	4,80919E-06	1,423689428	0,004281154
A_55_P2162988	Tmem179	1,990964802	9,92766E-06	1,194350427	0,153195495
A_51_P272876	Fam46a	1,97807362	0,000871338	1,332826082	0,162186072
A_51_P507290	Klf5	1,953562115	1,11204E-05	1,40258016	0,004209651
A_66_P128434	Kit	1,941501294	6,02506E-06	1,403052025	0,002411519
A_52_P220783	Tmem229b	1,933978576	8,73152E-05	1,238846803	0,159249557

A_55_P2150717	Eomes	1,927385624	7,98103E-06	1,20240957	0,101212643
A_55_P2162160	Anp32a	1,862809208	0,024833375	1,186954174	0,645589431
A_55_P2137828	Gprc5b	1,860442937	0,033286694	1,015437376	0,976357317
A_52_P420504	Acta2	1,816676055	4,23994E-05	1,272968737	0,043262924
A_65_P12104	Tmtc1	1,806603733	0,000111006	1,199888975	0,201423888
A_55_P2021505	Adra2a	1,738584867	4,27614E-05	1,18876677	0,136791324
A_55_P2052690	Synm	1,723948723	4,05132E-06	1,252957708	0,008018084
A_52_P547662	P2ry1	1,697997363	0,003909029	1,21760938	0,356420217
A_52_P266132	Fgl2	1,693091619	4,33468E-06	1,227583057	0,01276981
A_51_P100298	Stx3	1,649995849	0,008106837	1,150072101	0,569688875
A_51_P136355	Gng11	1,608031556	0,042883584	1,375742982	0,21570279
A_55_P2066484	Crtc3	1,585150196	0,001979758	1,210165606	0,228389098
A_52_P489295	Adamts1	1,582328466	6,16479E-05	1,074843427	0,558269244
A_55_P1967168	Lrp4	1,565816799	0,021115522	1,119433674	0,66863292
A_51_P361220	Fzd4	1,558765654	0,004990779	1,301308632	0,104895417
A_55_P1985544	Kcnk10	1,551258682	0,04564364	1,202201616	0,512342277
A_55_P2143517	Fstl1	1,533274735	0,001782867	1,211950955	0,173817278
A_55_P1990755	Ppp3r1	1,528974059	0,047556012	-1,001492869	0,997601452
A_52_P502577	S1pr3	1,514379478	0,014303026	1,282293771	0,17530289
A_55_P2122160	Ssfa2	1,513834419	0,001627512	1,158220456	0,31314124
A_66_P110490	Polr3h	1,510900629	4,9525E-05	1,151409852	0,104056425
A_55_P2010116	Rab27b	1,505037552	0,000113448	1,155244668	0,13138029

Table S2. Primers used in the manuscript

Primers used for ChIPs

Mouse Neurog2 region1 Fw:	TTAACTGGAGTGCCTTGG
Mouse Neurog2 region1 Rw:	CTCGTGTGTTGTCGTTC
Mouse Neurog2 region2 Fw:	TAAGGAGAGTCGTGTGCC
Mouse Neurog2 region2 Rw:	GCACTCCAGTTAAAGCCG
Mouse Neurog2 region3 Fw:	CACAACCTAAACGCCGC
Mouse Neurog2 region3 Rw:	TCTTCGTGAGCTTGGCAT
Mouse Neurog2 region4 Rw:	GGGTCATGTTTGATGTGGA
Mouse Neurog2 region4 Rw:	AGGGTTGGTGTTTCAAGTAG
Mouse G6pd2 Fw:	ACAGTCTATGAAGCAGTCAC
Mouse G6pd2 Rw:	CTCCACTATGATGCGGTTA
Mouse MFSD2 Fw:	TGCTTATGAACGCCATCTG
Mouse MFSD2 Rw:	AGCTGTTCTTCTTGTGTCC
Mouse Hoxb3 Fw:	GAAGGATGGGAGAGTTGC
Mouse Hoxb3 Rw:	AACATTACACCACAACCAAG
Mouse Hoxd8 Fw:	CAGTCTCTGGCAGTTCTTT
Mouse Hoxd8 Rw:	CCTGTCCTGTGCTTAACG
Mouse Stx3 FW:	CCTAAAGTTTCACACTCGC
Mouse Stx3 RW:	GCCTATTTCCTTGTCGGT

Primers used for cDNA

Mouse Neurog2 Fw:	CACAACCTAAACGCCGC
Mouse Neurog2 Rw:	TCTTCGTGAGCTTGGCAT
Mouse 23S Fw:	CGTCAGGGTGCAGCTCATTA
Mouse 23S Rw:	GGCACGAACGCTGTGATCTT

SUPPLEMENTARY FIGURE LEGENDS

RNA Polymerase II progression through H3K27me3-enriched gene bodies requires JMJD3 histone demethylase

Figure S1

(A) ChIP assays analyzed by qPCR performed in NSCs using H3K27me3 antibody. Analyzed genes are indicated in the X-axis.

(B) Metagene profiles of H3K27me3 coverage for JDTA genes and for the remaining genes of the array (see orange and green box in legend, respectively).

Figure S2

(A) ChIP assays analyzed by qPCR performed in TGFβ stimulated (3h) NSCs using JMJD3 antibody. Analyzed genes are indicated in the X-axis. Right panel shows representation of JMJD3 coverage by normalized wig files for the indicated RefSeq genes. Right numbers of the panels represent the length of the visualized region. Left numbers indicate the scale of the Y axis (number of reads).

(B) Genomic distribution of JMJD3 in TGFβ stimulated NSCs. Relative enrichment level of JMJD3 regions in different genomic features (orange bars) with respect to the genome background (blue bars). P-values (calculated using one-sided binomial test) show the significance of the relative enrichment with respect to the background. Only percentages of the ChIP regions that reside either upstream from TSS (≤1000 bp) or on gene bodies (5'UTRs, coding exons, 3’UTRs and introns) have a significant enrichment of JMJD3 binding (p values<0,01).

Figure S3

(A) Smad3 ChIP assays analyzed by qPCR performed in NSCs prior to (0h) and after TGFβ treatment (0.5h and 3h). Analyzed gene regions are indicated in the X-axis. Error bars indicate s.d.

(B) Immunoblot of nuclear and cytosolic fractions of NSCs prior to or after TGFβ treatment (3h) using JMJD3, tubuline or histone H3 antibodies.

(C) H3K27me3 ChIP assays analyzed by qPCR performed in JMJD3 KD NSCs untreated (white bars) or TGFβ treated (3h) (grey bars). Regions analyzed for *Neurogenin2* locus and the negative control gene (G6pd2) are indicated in the X-axis. Error bars indicate s.d.

(D) Venn diagram showing the nucleotides occupied by H3K27me3 and JMJD3 within the gene bodies from the set of "remaining genes in array" (see Figure1, green box).

Figure S4

(A) ChIP assays analyzed by qPCR performed in TGFβ stimulated (3h) NSCs or using RNAPII-S2p antibody. Analyzed genes are indicated in the X-axis. Right panel shows representation of RNAPII-S2p coverage by normalized wig files for the indicated RefSeq genes. Numbers on the right of the panels represent the length of the visualized region. Numbers on the left indicate the scale of the Y axis (number of reads).

(B) The total peaks for JMJD3 (239) and for RNAPII (77) found in the coding region of JDTA genes and the overlapping between them.

Figure S5

(A) Cdk9 ChIP experiment analyzed by qPCR performed in C KD and JMJD3 KD NSCs prior to (0h) and after TGFβ treatment (3h). Regions analyzed for *Neurog2* locus and the negative control (Stx3 intergenic region) are indicated in the X-axis. Error bars indicate s.d.

(B) Immunostaining of NSCs and JMJD3 KD NSCs with Smad3 antibody (red) prior to or after TGFβ treatment. Images in blue are DAPI staining (DNA).