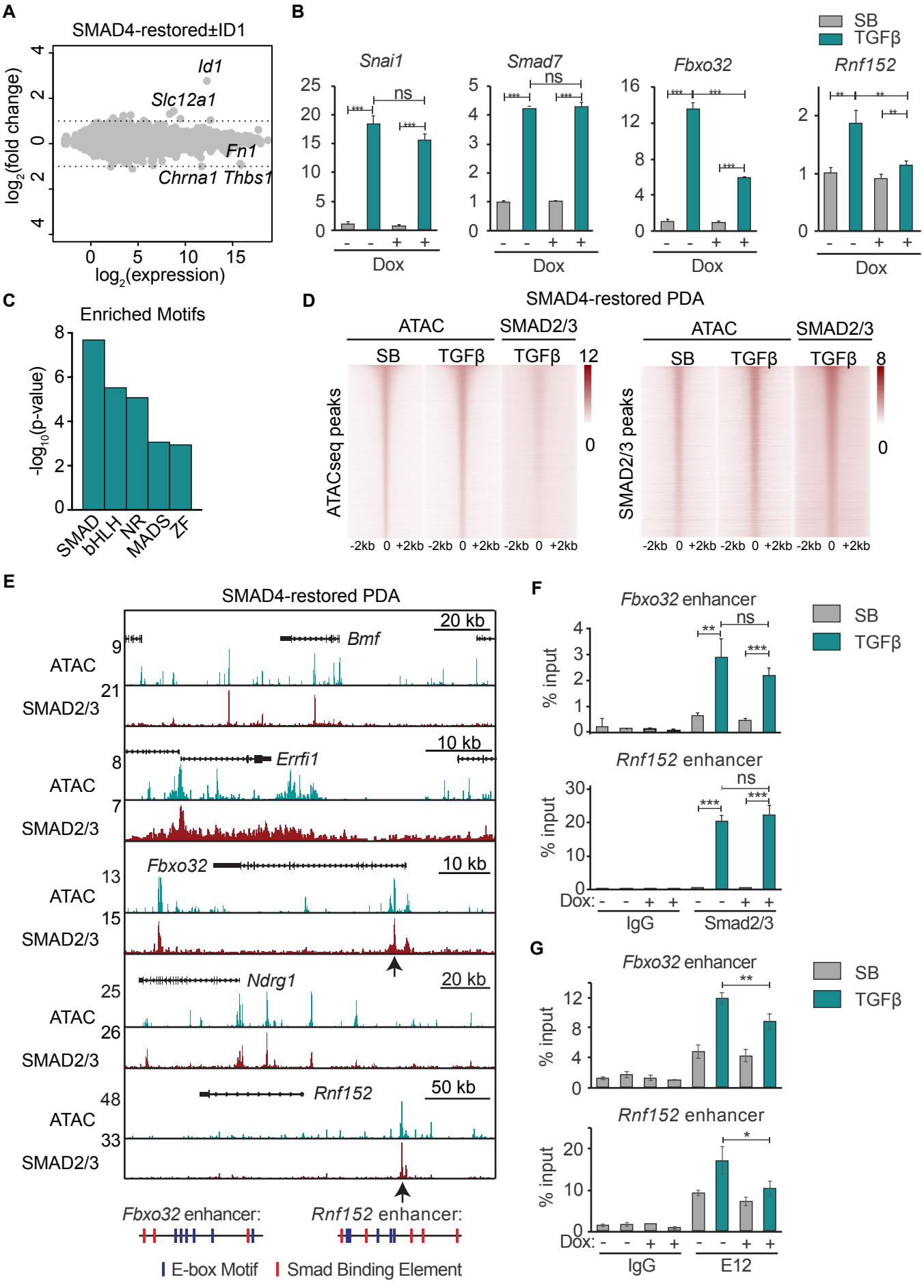
Supplementary Fig. S5



Supplementary Fig. S5: ID1 decouples TGF-β-induced EMT from apoptosis

A) SMAD4-restored *Kras^{G12D};Cdkn2a$^{-/-}$;Smad4$^{-/-}$* mouse PDA cells transduced with a Tet-ON *Id1* vector were treated with or without doxycycline for 12 h and subjected to RNA-seq analysis, n=2 per group. Highlighted genes represent genes with a fold change >4 in response to doxycycline, readcounts >10, and p_{adj}<0.05.

B) The same cells from (A) were treated with or without doxycycline for 24 h and then with 2.5 μM SB505124 or 100 pM TGF-β for 2 h. *Snai1*, *Smad7*, *Fbxo32* and *Rnf152* mRNA levels were deteremined by qRT-PCR. p-values are calculated by two-sided unpaired t-test. ** $p \leq 0.01$; *** $p \leq 0.001$; ns: $p > 0.05$.

C) The SMAD4-restored cells from (A) were subjected to ATAC-seq analysis of accessible chromatin. ATAC-seq peaks were assigned to the nearest transcription start site, and peaks associated with ID1-regulated, pro-apoptotic genes (*Fbxo32, Bmf, Rnf152, Ndrg1, and Errfi1)* were selected. De novo motif enrichment versus a background list of ATAC-seq accessible regions was performed using Homer.

D) SMAD4-restored mouse PDA cells were treated with 2.5 μM SB505124 or 100 pM TGF-β for 1.5 h and subjected to ATAC-seq and SMAD2/3 ChIP-seq analysis. The heatmaps represent ATAC-seq and SMAD2/3 ChIP-seq peak densities in genomic regions ±2 kb from the center of high-confidence ATAC-seq peaks (*left*) and SMAD2/3 binding peaks (*right*).

E) ATAC-seq and SMAD2/3 ChIP-seq gene tracks at ID1-regulated genes in SMAD4-restored mouse PDA cells treated with TGF-β for 1.5h. *Bottom,* schema of SMAD binding elements and E-protein binding elements present in the *Fbxo32* and *Rnf152* enhancers.

F-G) The Smad2/3 occupancy (F) and the E12/E47 occupancy (G) at the enhancer regions of *Fbxo32* and *Rnf152* was measured by ChIP-qPCR. p-values calculated by two-sided unpaired t-test. ** $p \leq 0.01$; *** $p \leq 0.001$; ns, $p > 0.05$.