

Supplemental Material**HMGA2 and Smads coregulate SNAIL1 expression during induction of epithelial-to-mesenchymal transition**

Sylvie Thuault, E-Jean Tan, Hector Peinado, Amparo Cano, Carl-Henrik Heldin, and Aristidis Moustakas

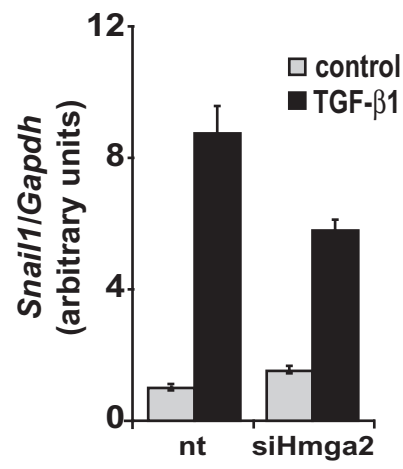
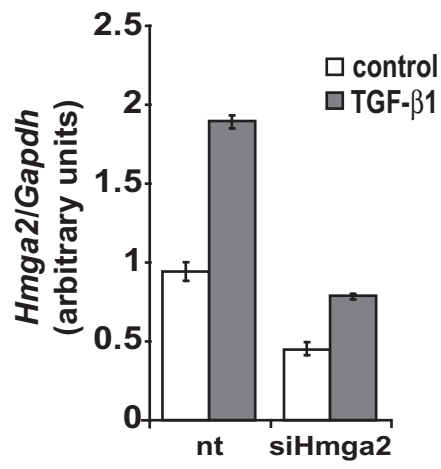
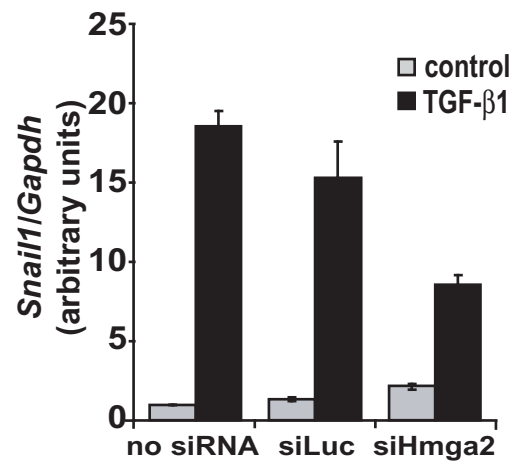
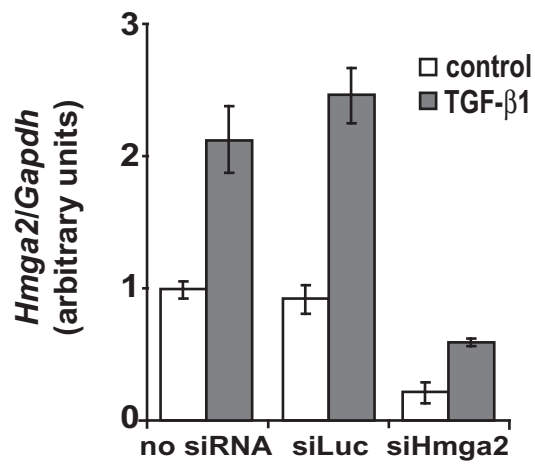
Supplemental Figure Legends

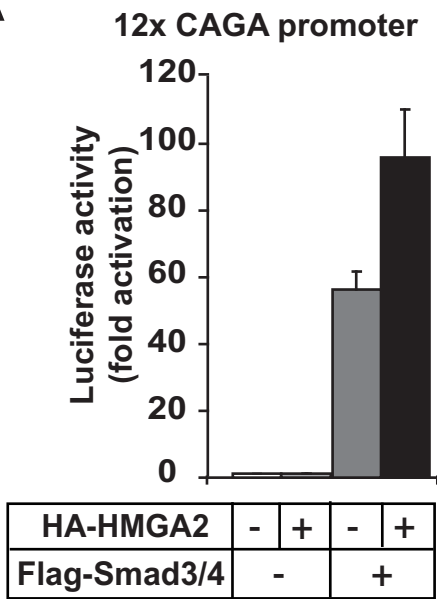
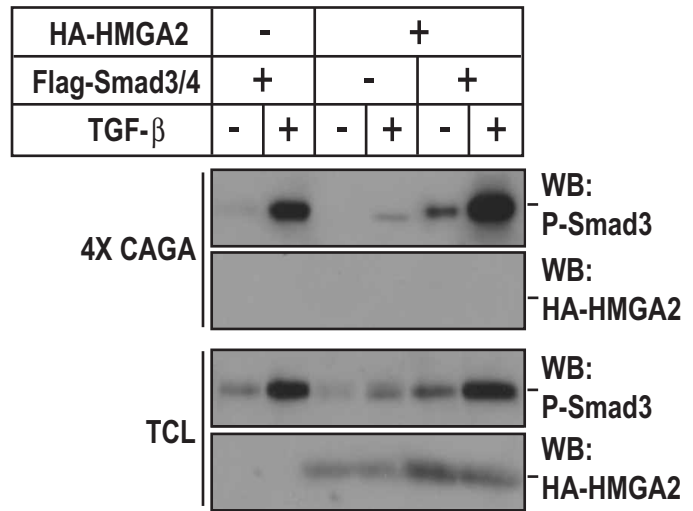
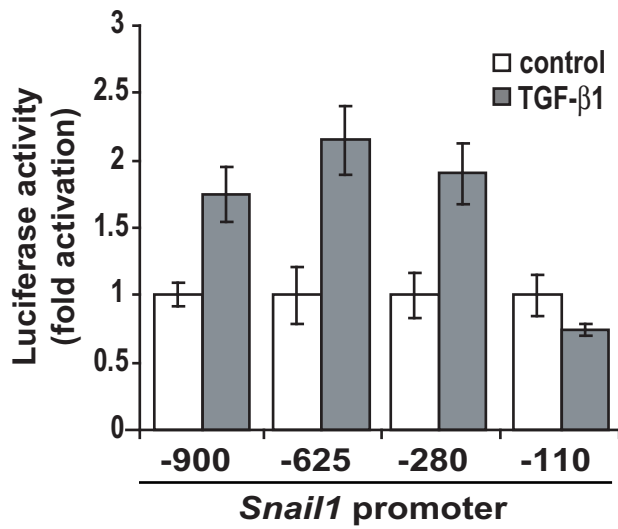
FIG. S1. HMGA2 is required for full induction of *Snail1* expression by TGF- β . (A) and (B) Quantitative RT-PCR analysis of *Hmga2* (left panel) and *Snail1* (right panel) expression in NMuMG cells not transfected (nt), treated with the transfection reagent (no siRNA) or transfected with control (siLuc) or specific siRNA against *Hmga2* (siHmga2) treated with vehicle (white and light gray) or 5 ng/ml TGF- β 1 (dark gray and black) for 2 hours. (A) and (B) are two independent repeats of the experiment.

FIG. S2. HMGA2 enhances Smad transcriptional activity, Smad-DNA binding and TGF- β 1 induces *Snail1* promoter activity.

(A) Luciferase reporter assays of the CAGA₁₂ promoter construct in HepG2 cells transiently transfected with Flag-Smad3, Flag-Smad4 and HA-HMGA2 expression constructs, as indicated. (B) Binding of C-terminally phosphorylated Smad3 (P-Smad3) and HMGA2 (HA-HMGA2) to the 4X CAGA probe was assessed by DNAP experiments using extracts of transiently transfected HepG2 cells with Flag-Smad3, Flag-Smad4 and HA-HMGA2 expression constructs treated (+) or not (-) with 5 ng/ml TGF- β 1 for 2 hours. (C) Luciferase reporter assays of the indicated *Snail1* promoter deletion constructs (see map in Fig. 2E) in HepG2 cells stimulated with 5 ng/ml TGF- β 1 for 24 hours. Luciferase data are plotted as in Fig. 2.

FIG. S3. HMGA2 induces and binds to the *Snail1* promoter. (A) Luciferase reporter assays of wild-type (wt) or mutant (m1) -625 *Snail1* promoter constructs in HepG2 cells transiently transfected with increasing amounts of the HA-HMGA2 expression construct, as indicated by triangles. Luciferase data are plotted as in Fig. 2, and immunoblot of the transfected HMGA2 protein is shown below the graph. (B) Binding of HMGA2 (HA-HMGA2) and C-terminally phosphorylated Smad3 (P-Smad3) to the -230/-92 *Snail1* promoter probe was assessed by DNAP experiments using extracts of transiently transfected HepG2 cells with Flag-Smad3, Flag-Smad4 and HA-HMGA2 expression constructs treated with 5 ng/ml TGF- β 1 for 2 hours. Protein mass markers (in kDa) are shown in lane M.

A**B****Fig.S1 Thuault et al.**

A**B****C****Fig.S2 Thuault et al.**

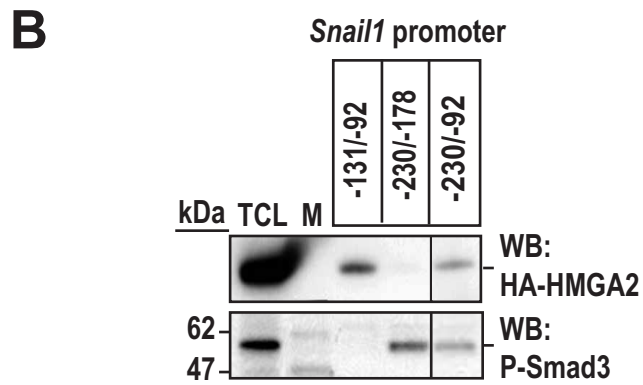
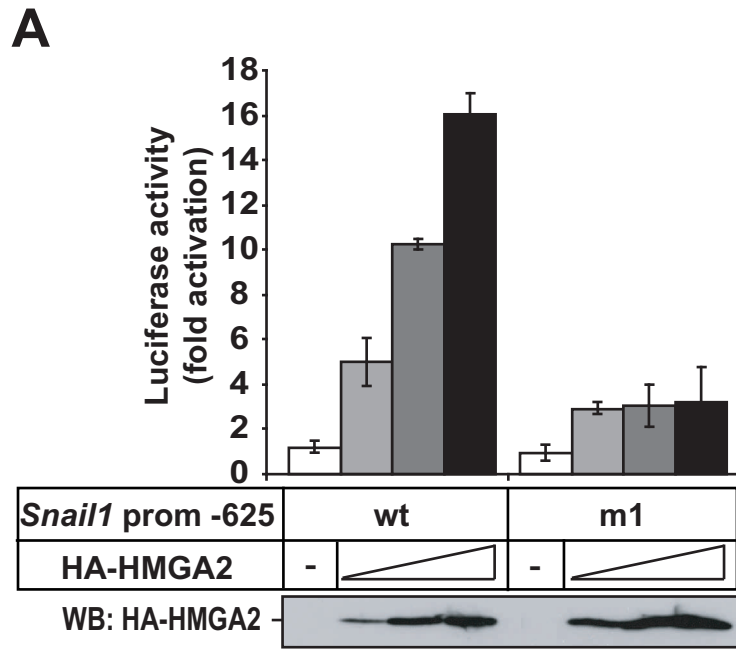


Fig.S3 Thuault et al.