Supplementary Figures

Supplementary Figure A

Reproducibility of separation of phosphoproteins in two-dimensional gels. Images of two-dimensional gels with proteins labeled with [^{32}P]orthophosphate are shown, out of 48 generated gels. Cell lines are indicated on the side of the images, and treatment times with TGFβ1 (5 ng/ml) are indicated on the top of images.

Supplementary Figure B

Phosphorylation profiles of identified proteins. Phosphorylation of identified proteins in MCF-7-vector (- ?-; rhomb), MCF-7-Flag-Smad2 (- ¦ -; square), and MCF-7-myc-Smad3 (- ? -; triangle) cells are shown. Annotation of proteins is as in Table I and Figure 1. Time of treatment of cells with TGFβ1 (5 ng/ml) and fold of changes in phosphorylation values are indicated.

Supplementary Figure C

Clustering of identified proteins according to the effect of overexpression of Flag-Smad2 and myc-Smad3 on phosphorylation pattern of proteins. Annotation of proteins is as in Table I. Five groups are shown. For explanations, see the text.

Supplementary figure D

Phosphopeptide maps of the second band of TFII-I. Under closer examination of TFII-I band which is shown in Figure 3C, two bands could be observed (**A**). These two bands could be observed for 32P-labelled TFII-I as well (**B**). Phosphopeptide mapping of both

of these bands was performed as described in Figure 4 and in the Material and Methods section. Endogenous TFII-I from vector-transfected, or MCF-7-Flag-Smad2, or MCF-7-myc-Smad3 cells, which were treated or not with TGFβ1, was subjected to two-dimensional phosphopeptide mapping (**C**). Arrows show migration position of phosphopeptides which appeared after treatment of cells with TGFβ1. Triangles show sample application points. Directions of electrophoresis and chromatography are shown on the sides of maps. Cell lines are indicated at the side of the maps, and time of the treatment of cells with TGFβ1 is indicated on the top of the maps.

Supplementary Figure E

Peptides containing serine residue at the third position are shown. The calculated migration characteristics in electrophoresis and chromatography, are shown. Peptides #1 and #2 are indicated.

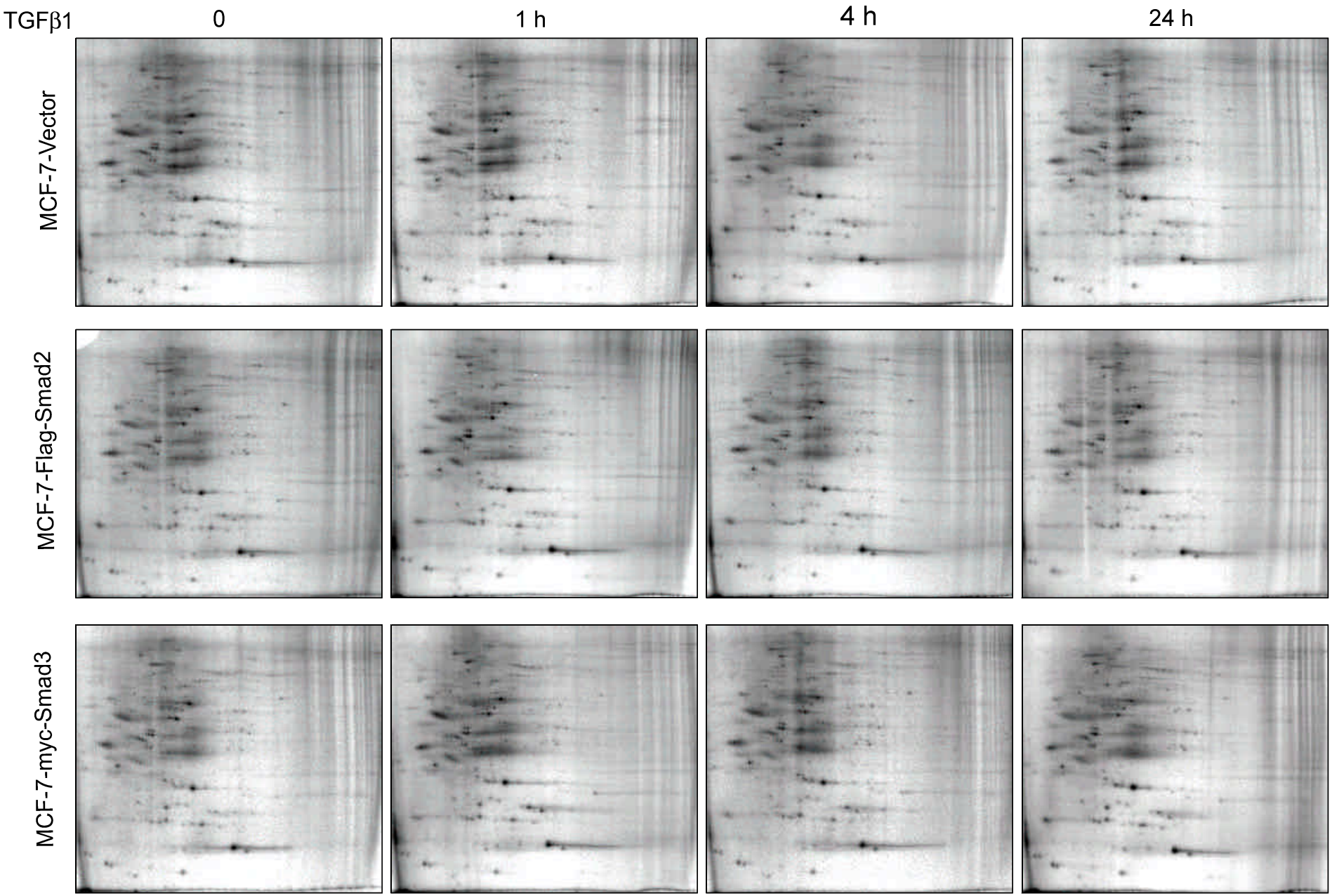
Supplementary Figure F

Stable transfection of wild-type (WT) or Ser371,743Ala mutant (Mut1 and Mut2) of myc-TFII-I had no significant effect on TGFb**1-dependent regulation of cyclin D1 (A), cdk7 (B), and cdk8 (C) expression.** Evaluation of expression of transfected myc-TFII-I constructs in MCF-7 cells is shown in panel A of Figure 6. Cells were treated with TGFβ1, as indicated, and mRNA expression was evaluated as described in Figure 6.

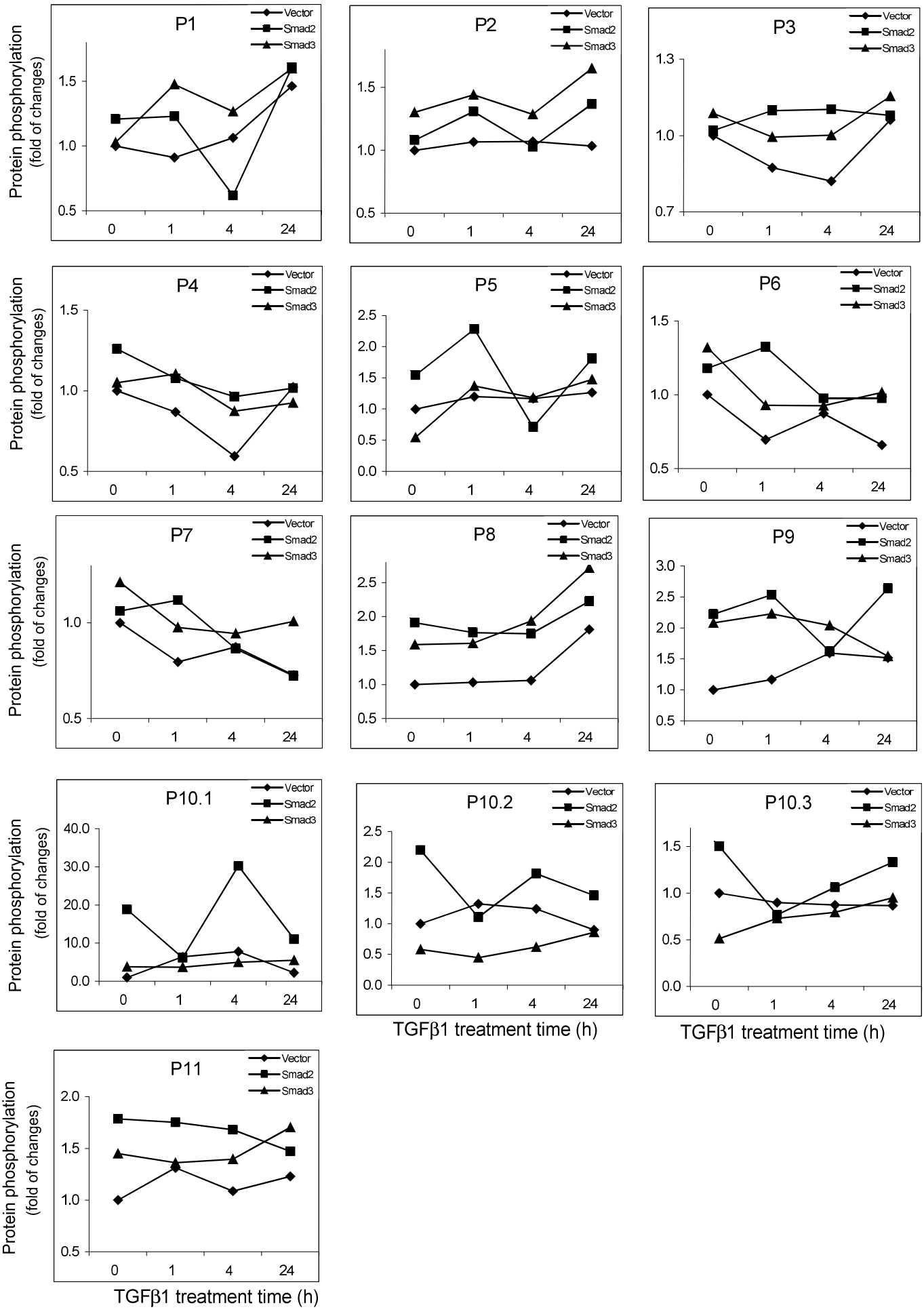
Supplementary Figure G

TGFb**-dependent transcriptional activation of its known targets in preserved in MCF-7 cells.** Fold changes in transcriptional activation in MCF-7 cells are shown side-by-side with results reported by Ota *et al.* (2002). TGFβ-dependent regulation of cyclins D1, F, G1 and G2, cdc2, cdk2, E2F2, E2F3 and GAAD45A are shown. Treatment of cells with TGFβ1 was as indicated. Reference to Ota *et al.*: Ota T, Fuji M, Sugizaki T, Ishii M, Miyazawa K, Aburatani H, Miyazono K (2002). J Cell Physiol. 193, 299-318.

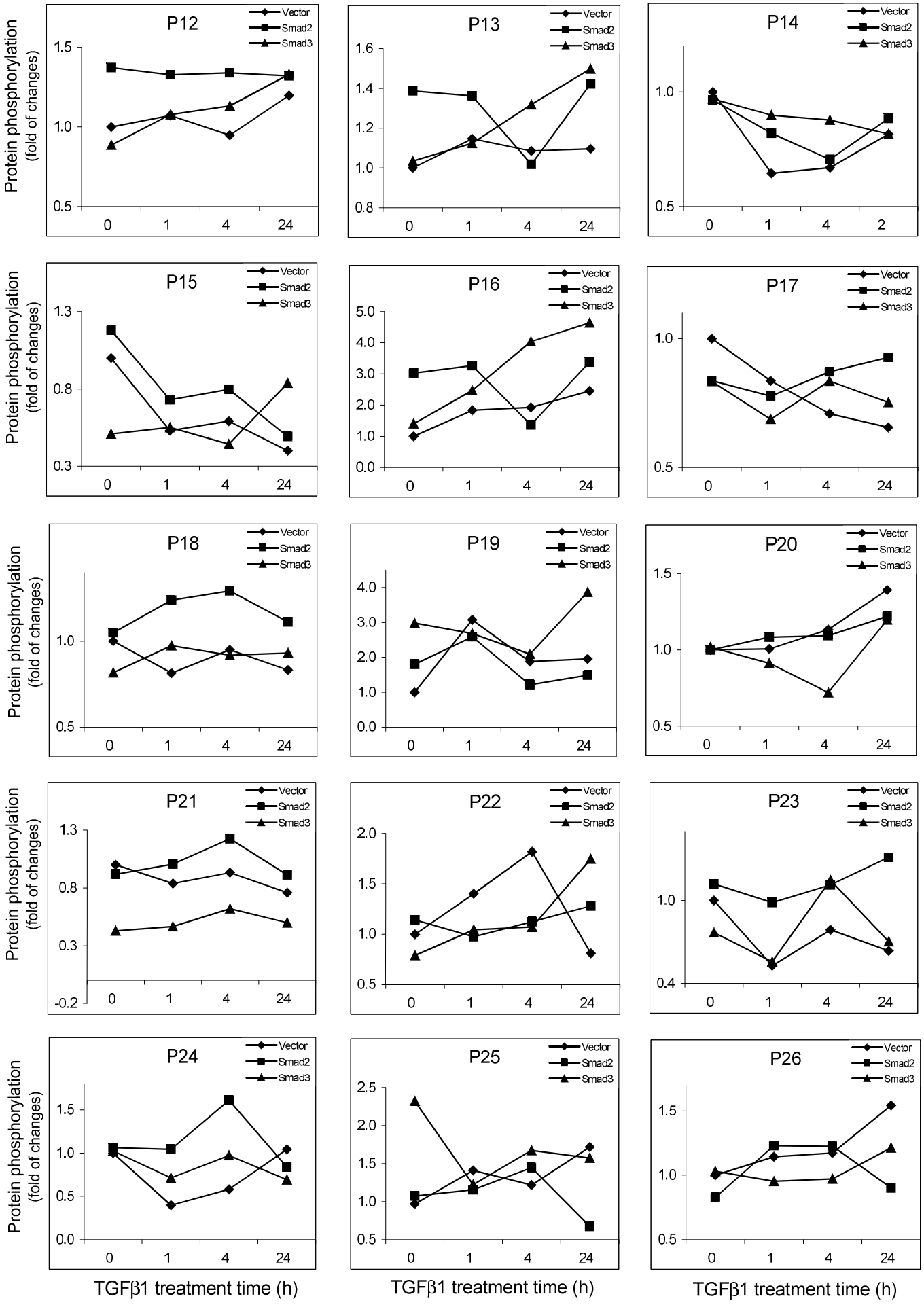
Supplementary Figure A



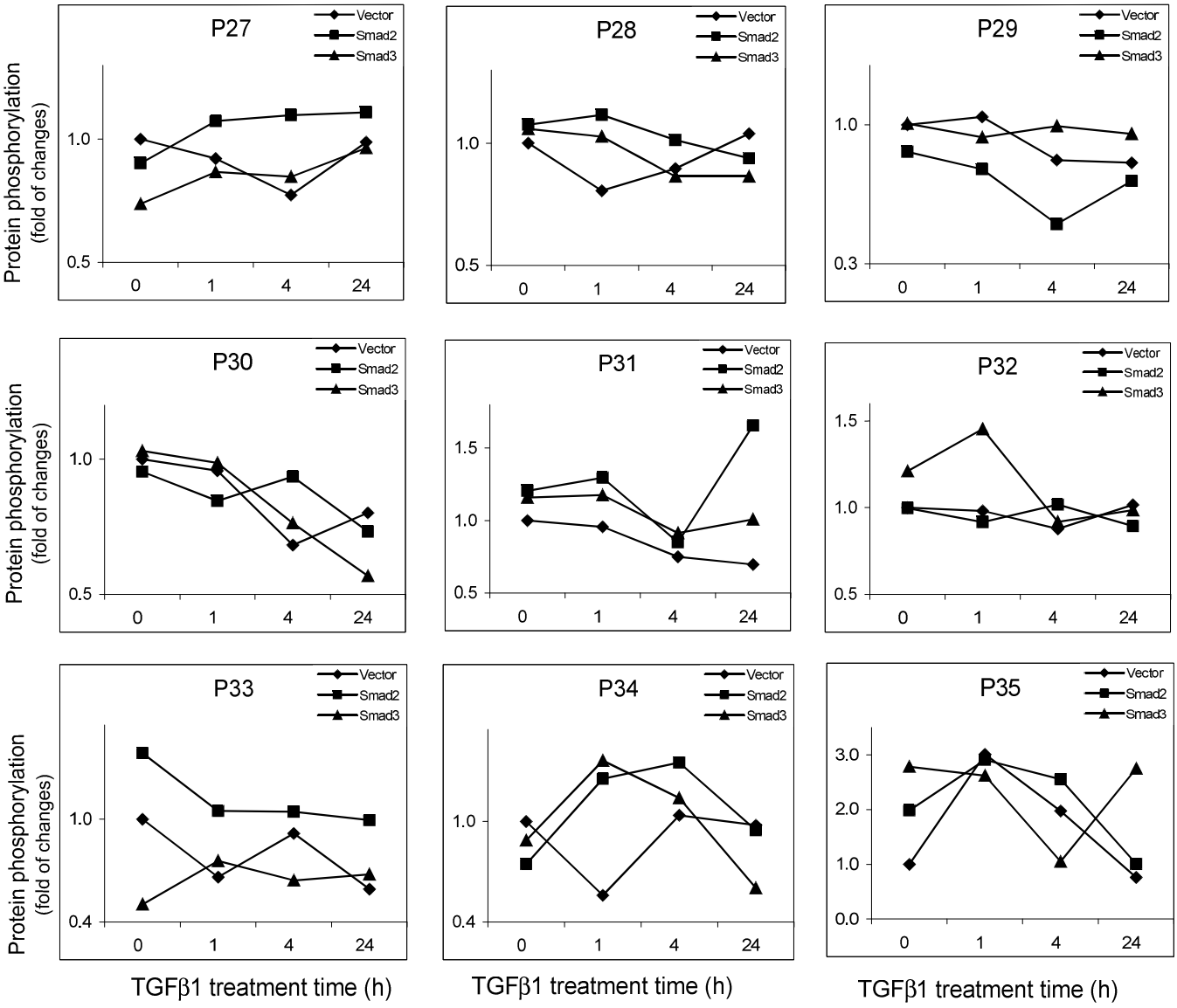
Supplementary figure B-1



Supplementary figure B-2



Supplementary figure B-3



Supplementary Figure C

Group I (vector; Smad2=Smad3)

P2 Eucaryotic translation elongation factor 1 delta
P3 Eucaryotic translation elongation factor 1 beta
P4 Acidic ribosomal phosphoprotein P2
P9 Heterogeneous nuclear ribonucleoprotein K
P17 Tumor necrosis factor, alfa-indused protein 2
P18 Cofilin1 (nonmuscle)
P22 Serologically defined brest canser antigen NY-BR-75
P24 Glucose 6-phosphate Dehydrogenase
P27 HSP 27
P28 BiP protein
P34 Hypothetical protein

Group II (vector=Smad2; Smad3)

P7 Heterogenous nuclear ribonucleoprotein C (C1/C2)
P14 Ras-GTPase-activating protein SH3-domain-binding protein
P15 Rab GDP dissosiation inhibitor, beta
P19 Putative human HLA class II associated protein I
P20 Melanoma antigen gp 75
P21 Serologically defined colon cancer antigen 28
P32 keratin 18, cytoskeletal - human (fragment)
P35 Hypothetical protein

Group III (vector=Smad3; Smad2)

P1 Eucaryotic translation elongation factor 1 delta
P5 60S ribosomal phosphoprotein P0
P6 Heterogenous nuclear ribonucleoprotein C (C1/C2)
P12 Proteasome subunit, alfa type, 3
P23 Enolase 1
P26 HSP 90 - alfa (HSP86)
P29 keratin, 67K type II cytoskeletal - human
P31 keratin 10, type I, cytoskeletal

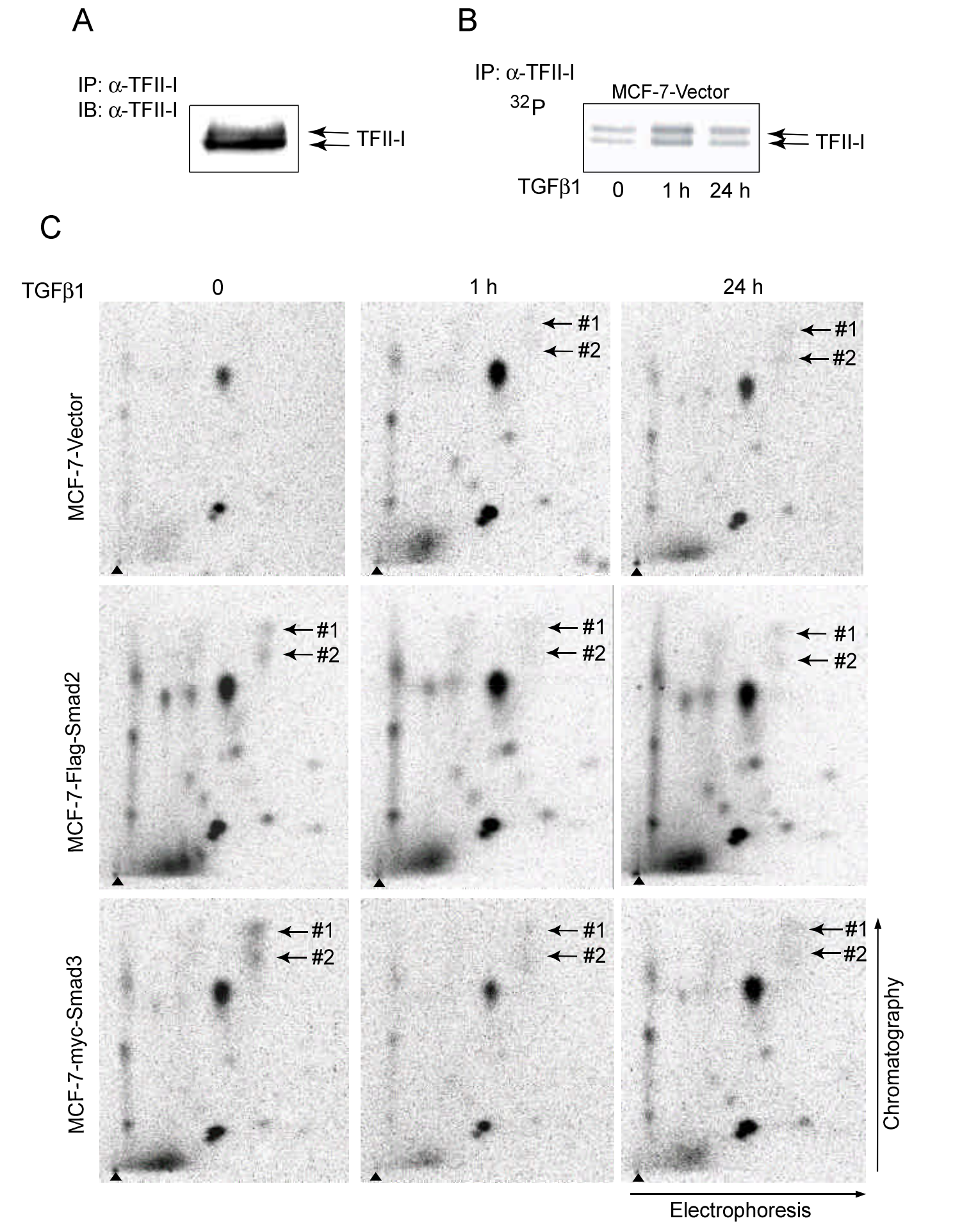
Group IV (vector=Smad2=Smad3)

P8 Heterogeneous nuclear ribonucleoprotein K
P30 Keratin 8, type II cytoskeletal

Group V (vector; Smad2; Smad3)

P10.1, P10.2, P10.3 General transcription factor II-I
P11 RAD23 homolog B, HHR23B
P13 26S proteasome subunit p40.5
P16 dJ25J6.4, Ret finger protein
P25 HSP gp96 precursor
P33 Keratin-like protein - human (fragment)

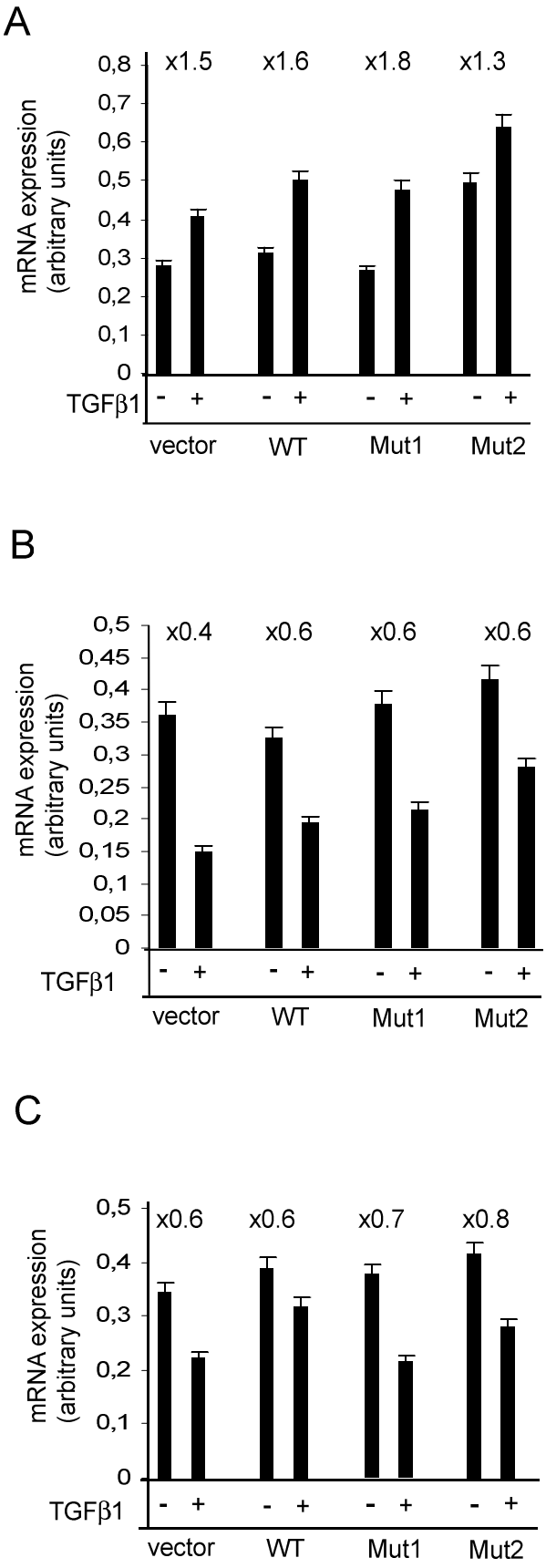
Supplementary figure D



Supplementary figure E

	TFII-I tryptic peptides containing Ser at position #3	e/M (electrophoresis)	Rf (chromatography)
	DQSAVVVQGLPEGVAFK	0.57	0.66
	EESEDPDYYQYNIQGSHHSSEGNEGTEMEVPAEDDDYSPPSK	0.63	0.57
#2	RPSTYGIPR	1.91	0.65
	SPSWYGIPR	0.94	0.65
	LGSTEAK	1.42	0.58
	EFSFEAWNAK	0.81	0.62
#1	RPSTFGIPR	1.94	0.68
	INSSPNVNTTASGVEDLNIIQVTIPDDDNER	1.57	0.50
	APSYLEISSMR	0.80	0.59

Supplementary Figure F



Supplementary Figure G