Figure 1

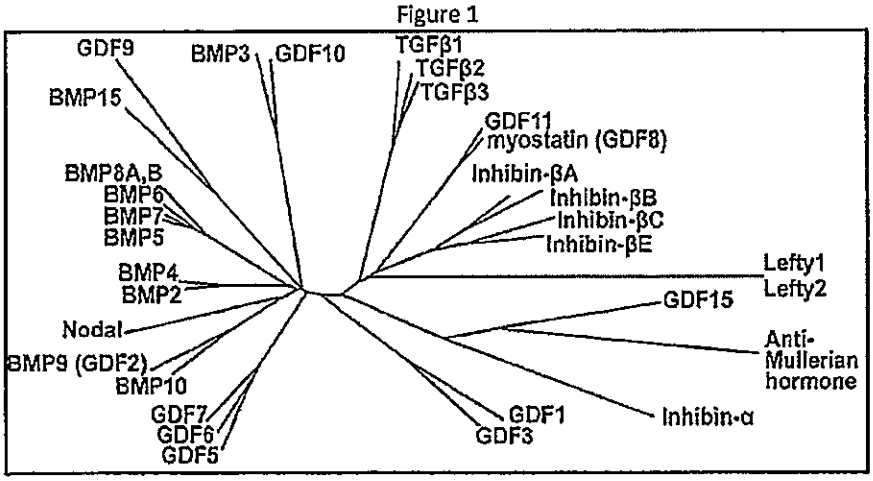

GDF9
BMP3
GDF10
TGFβ1
TGFβ2
TGFβ3
BMP15
GDF11
myostatin (GDF8)
BMP8A,B
Inhibin-βA
BMP6
Inhibin-βB
BMP7
Inhibin-βC
BMP5
Inhibin-βE
Lefty1
BMP4
Lefty2
BMP2
GDF15
Nodal
Anti-
Mullerian
hormone
BMP9 (GDF2)
BMP10
GDF7
GDF1
Inhibin-α
GDF6
GDF3
GDF5

Figure 2

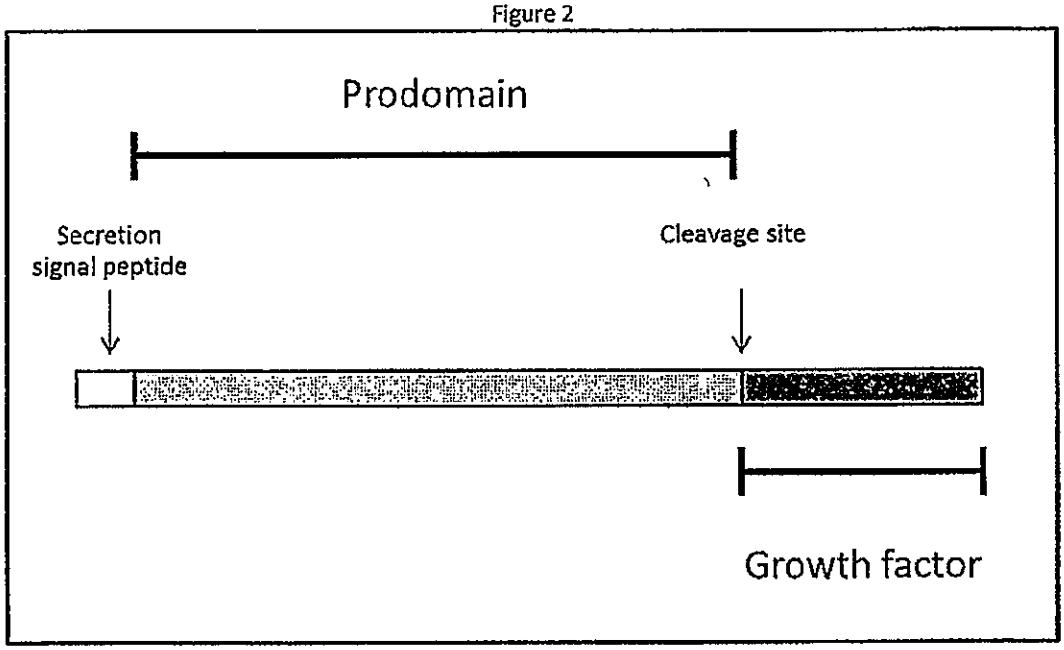

Prodomain
Secretion
signal peptide
Cleavage site
Growth factor

Figure 3

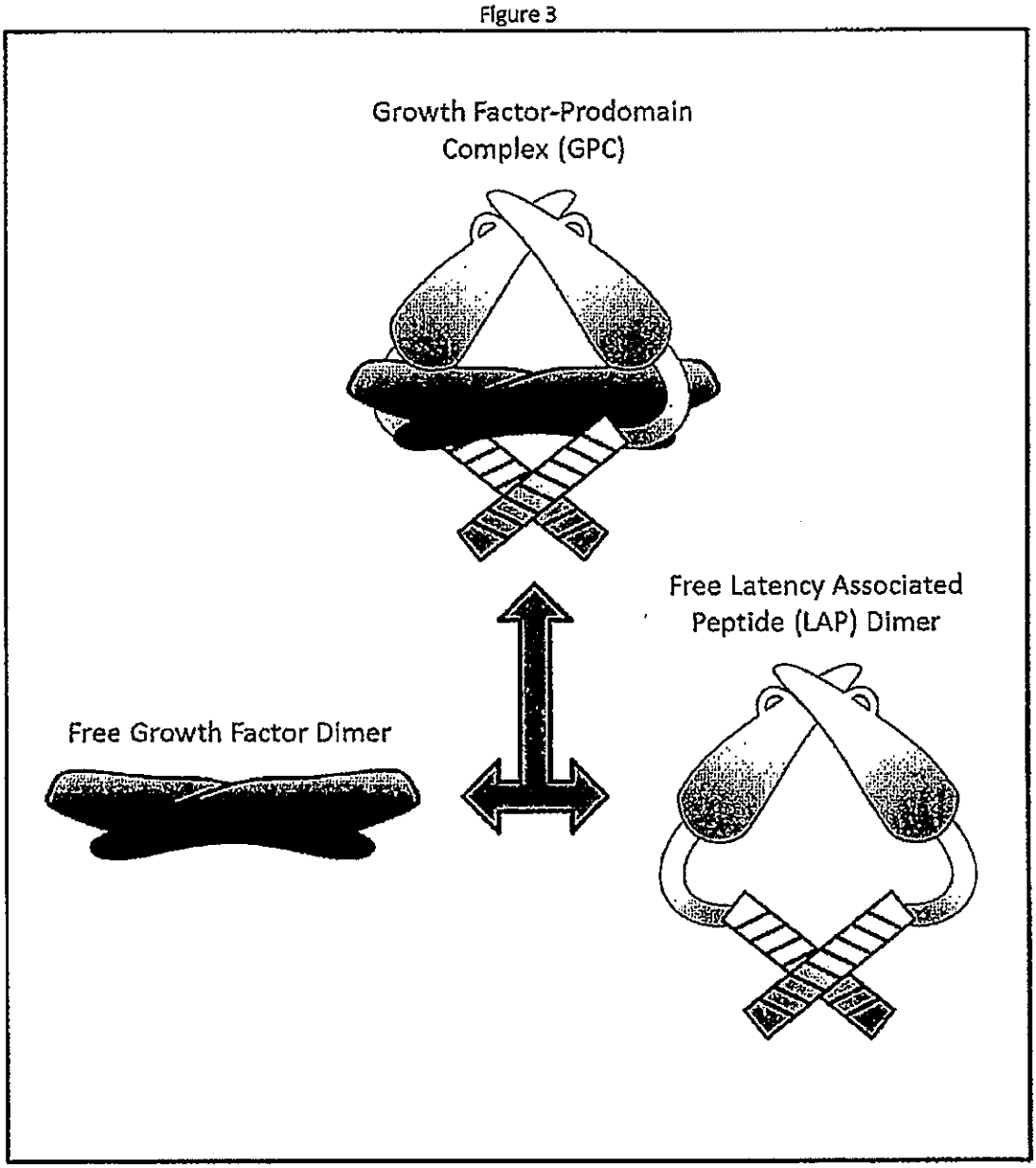

Growth Factor-Prodomain
Complex (GPC)
Free Latency Associated
Peptide (LAP) Dimer
Free Growth Factor Dimer

Figure 4

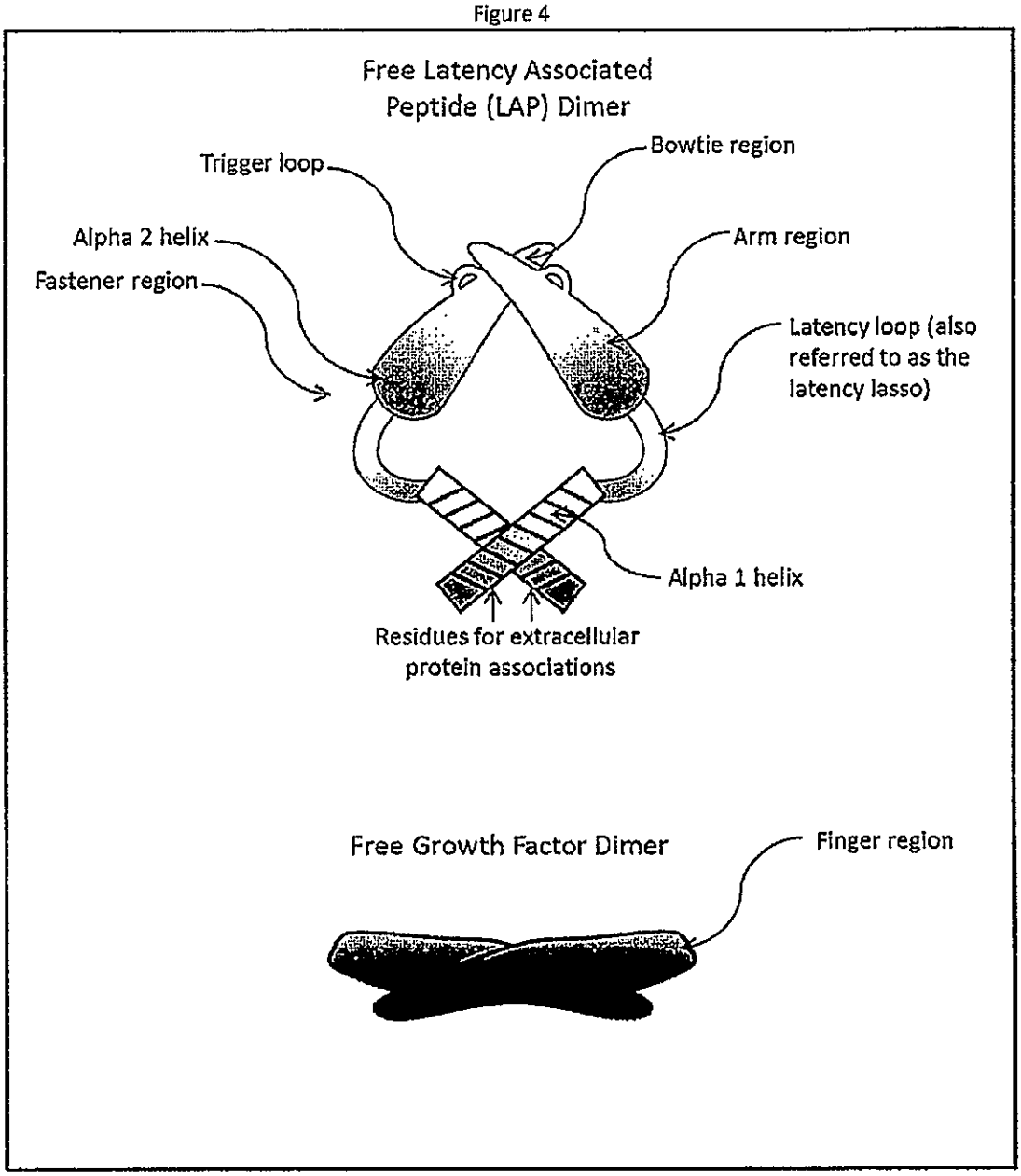

Free Latency Associated
Peptide (LAP) Dimer
Trigger loop
Bowtie region
Alpha 2 helix
Fastener region
Arm region
Latency loop (also
referred to as the
latency lasso)
Alpha 1 helix
Residues for extracellular
protein associations
Free Growth Factor Dimer
Finger region

Figure 5

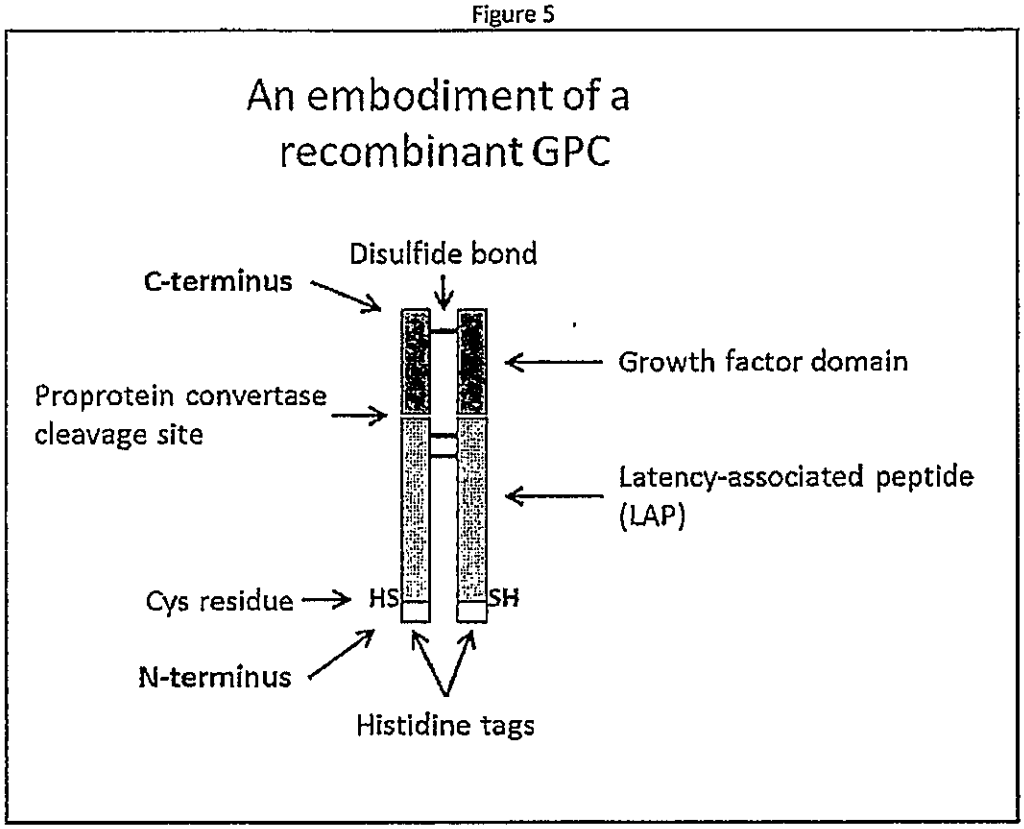
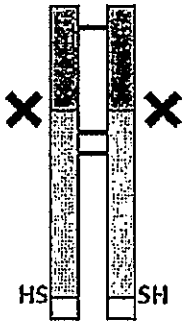

An embodiment of a recombinant GPC
Disulfide bond
C-terminus
Growth factor domain
Proprotein convertase cleavage site
Latency-associated peptide (LAP)
Cys residue
HS
SH
N-terminus
Histidine tags

Figure 6

An embodiment of a proprotein convertase cleavage site mutant (e.g. RXXR → RXG; D2G)



An embodiment of an N-terminal cysteine mutant (e.g. C4S: Cys4→Ser)

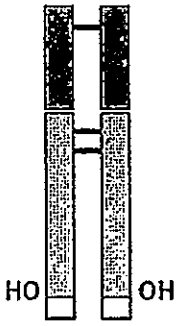


Figure 7

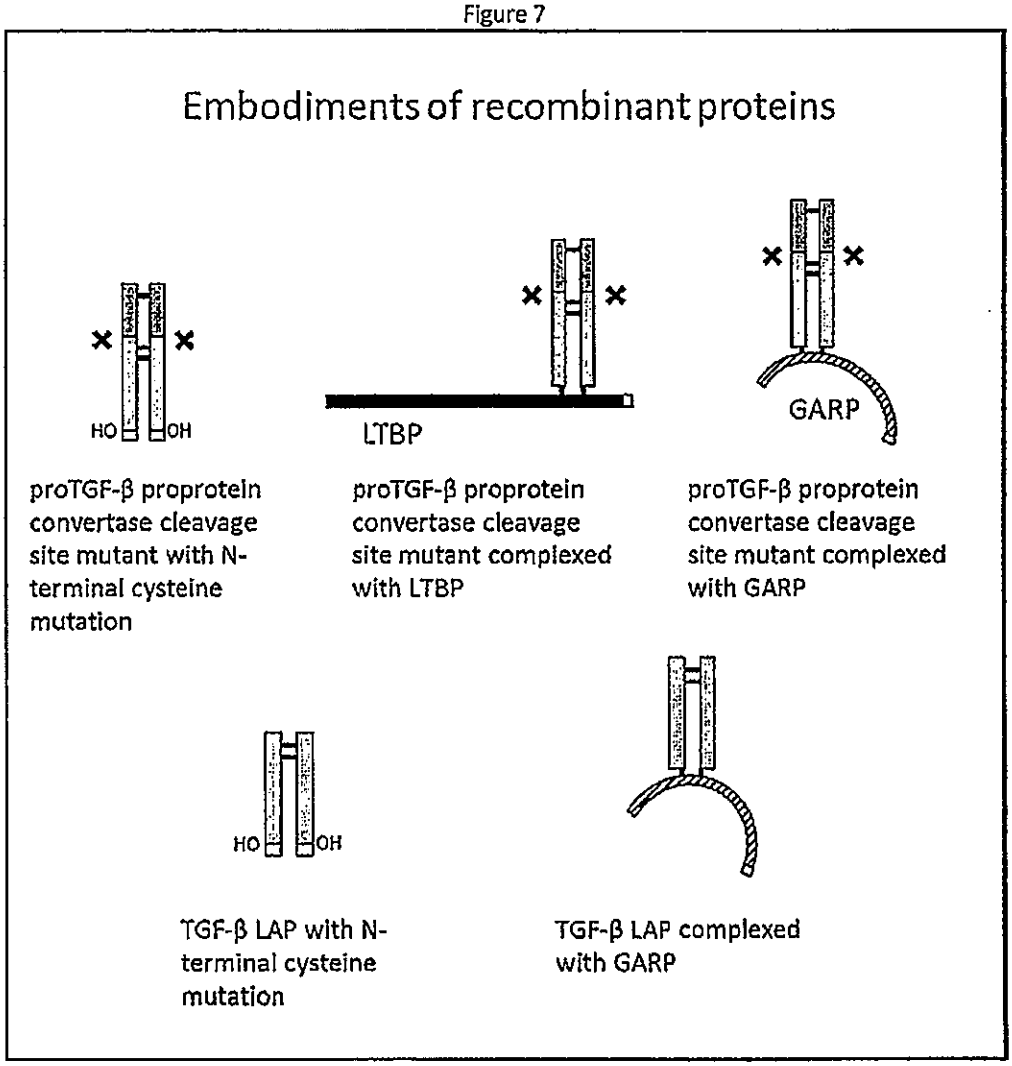

Embodiments of recombinant proteins
HO
OH
LTBP
GARP
proTGF-β proprotein convertase cleavage site mutant with N-terminal cysteine mutation
proTGF-β proprotein convertase cleavage site mutant complexed with LTBP
proTGF-β proprotein convertase cleavage site mutant complexed with GARP
HO
OH
TGF-β LAP with N-terminal cysteine mutation
TGF-β LAP complexed with GARP

Figure 8A

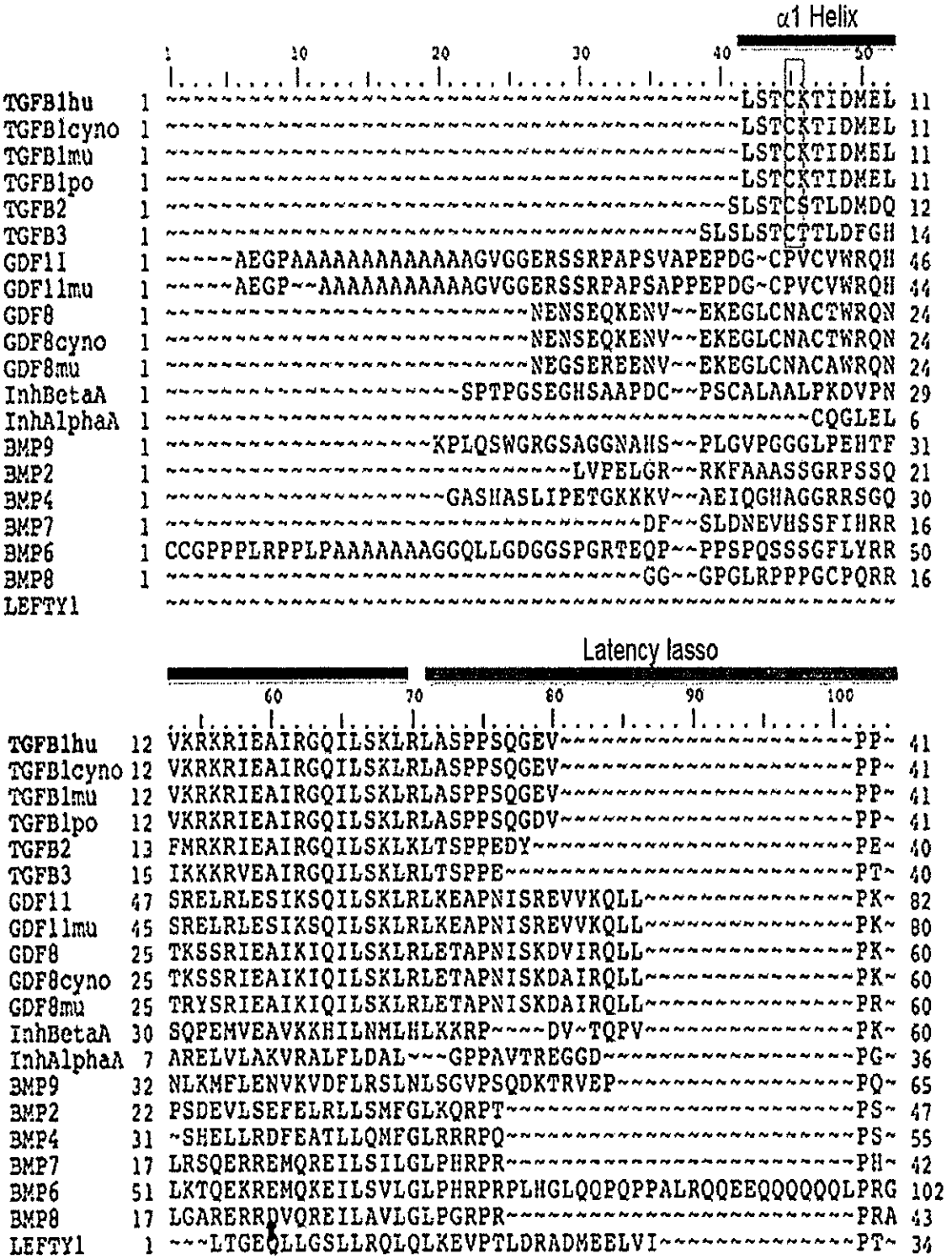

α1 Helix
Latency lasso
TGFB1hu
TGFB1cyno
TGFB1mu
TGFB1po
TGFB2
TGFB3
GDF11
GDF11mu
GDF8
GDF8cyno
GDF8mu
InhBetaA
InhAlphaA
BMP9
BMP2
BMP4
BMP7
BMP6
BMP8
LEFTY1

Figure 8A (continued)

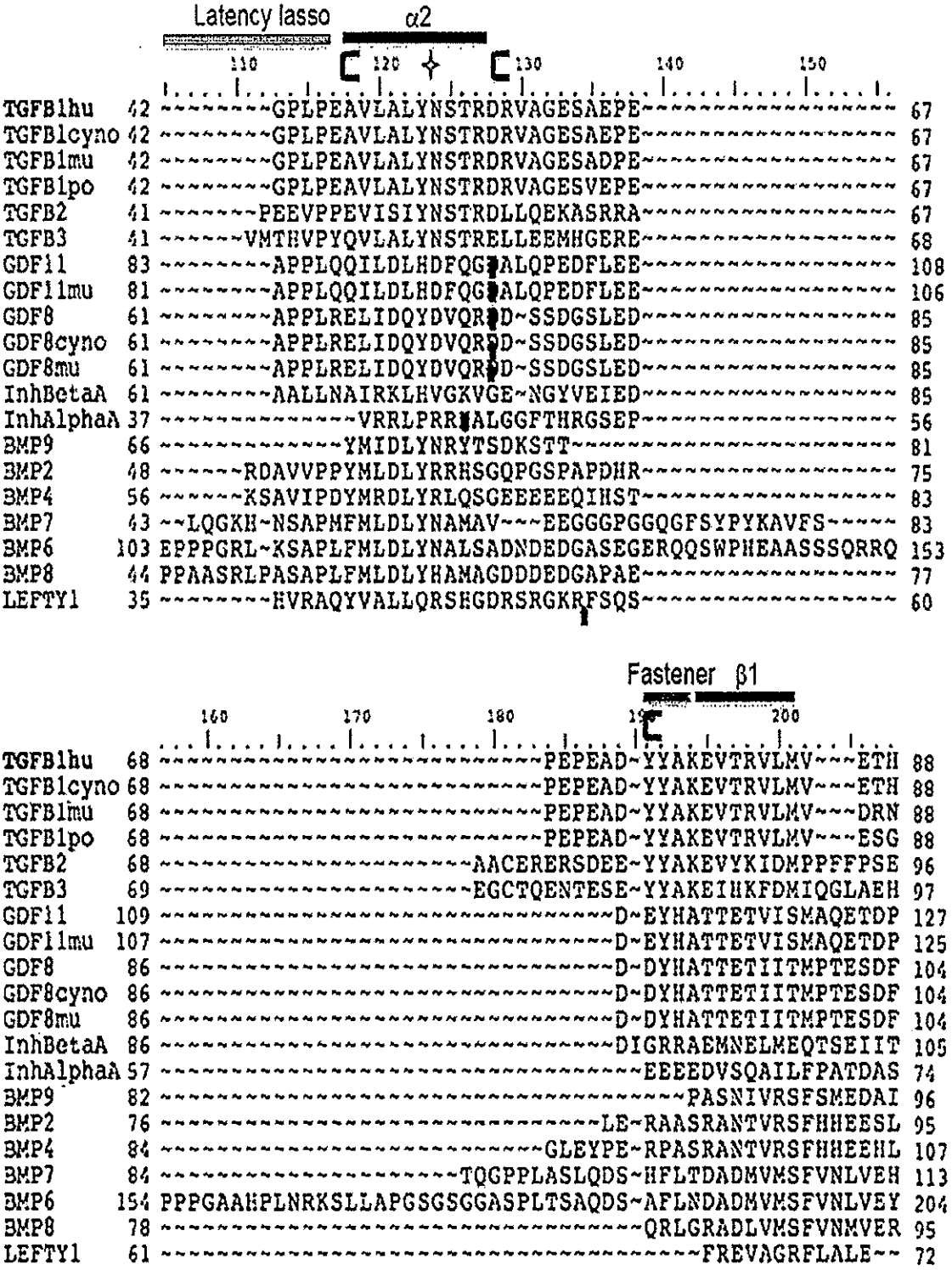


Figure 8B

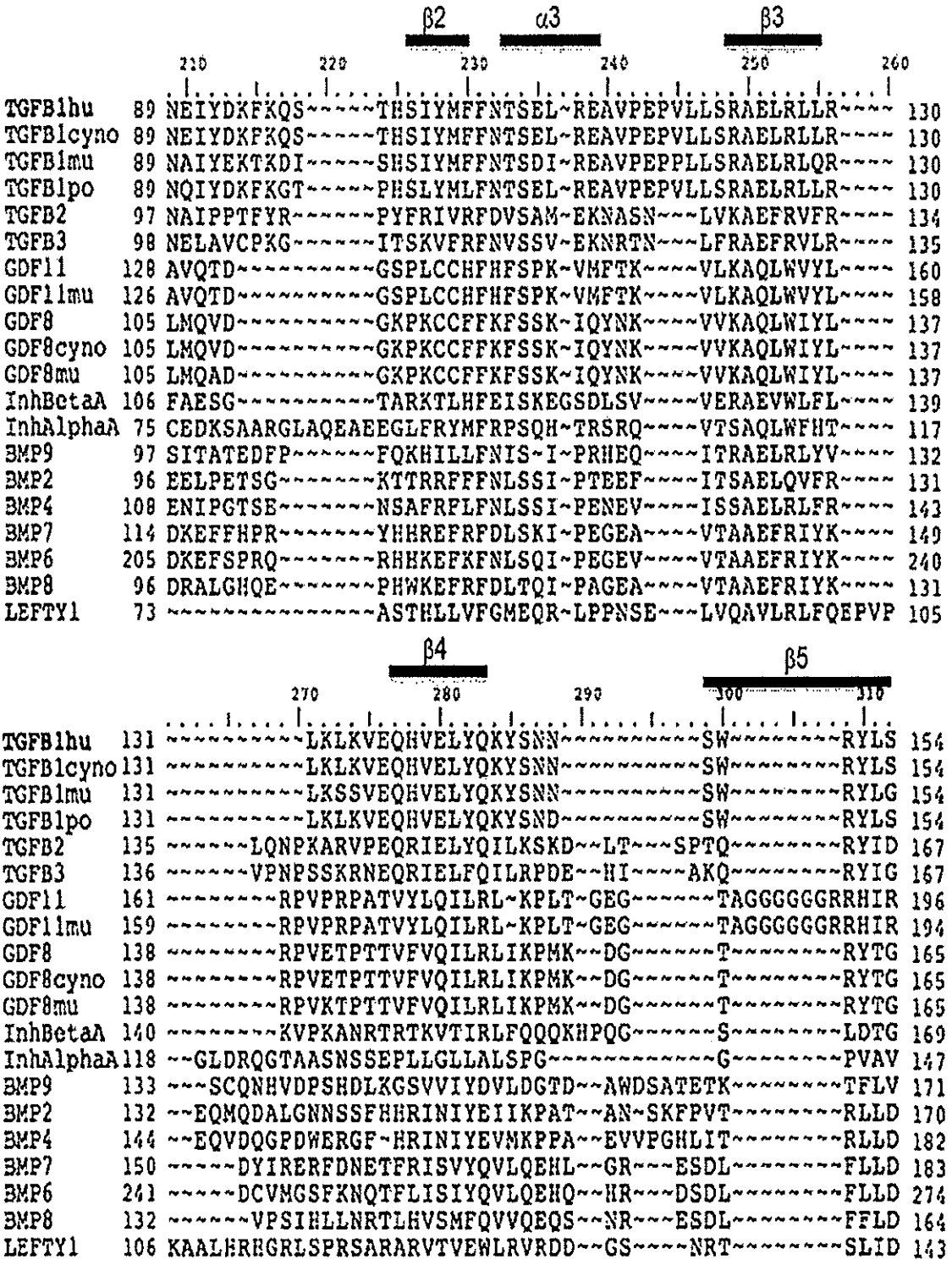

β2
α3
β3
β4
β5
TGFB1hu 89 NEIYDKFKQS~~~~~THSIYMFFNTSEL~REAVPEPVLLSRAELRLLR~~~~ 130
TGFB1cyno 89 NEIYDKFKQS~~~~~THSIYMFFNTSEL~REAVPEPVLLSRAELRLLR~~~~ 130
TGFB1mu 89 NAIYEKTKDI~~~~~SHSIYMFFNTSDI~REAVPEPPLLSRAELRLQR~~~~ 130
TGFB1po 89 NQIYDKFKGT~~~~~PHSLYMLFNTSEL~REAVPEPVLLSRAELRLLR~~~~ 130
TGFB2 97 NAIPPTFYR~~~~~~PYFRIVRFDVSAM~EKNASN~~~LVKAEFRVFR~~~~ 134
TGFB3 98 NELAVCPKG~~~~~~ITSKVFRFNVSSV~EKNRTN~~~LFRAEFRVLR~~~~ 135
GDF11 128 AVQTD~~~~~~~~~~GSPLCCHFHFSPK~VMFTK~~~~VLKAQLWVYL~~~~ 160
GDF11mu 126 AVQTD~~~~~~~~~~GSPLCCHFHFSPK~VMFTK~~~~VLKAQLWVYL~~~~ 158
GDF8 105 LMQVD~~~~~~~~~~GKPKCCFFKFSSK~IQYNK~~~~VVKAQLWIYL~~~~ 137
GDF8cyno 105 LMQVD~~~~~~~~~~GKPKCCFFKFSSK~IQYNK~~~~VVKAQLWIYL~~~~ 137
GDF8mu 105 LMQAD~~~~~~~~~~GKPKCCFFKFSSK~IQYNK~~~~VVKAQLWIYL~~~~ 137
InhBetaA 106 FAESG~~~~~~~~~~TARKTLHFEISKEGSDLSV~~~~VERAEVWLFL~~~~ 139
InhAlphaA 75 CEDKSAARGLAQEAEEGLFRYMFRPSQH~TRSRQ~~~~VTSAQLWFHT~~~~ 117
BMP9 97 SITATEDFP~~~~~~FQKHILLFNIS~I~PRHEQ~~~~ITRAELRLYV~~~~ 132
BMP2 96 EELPETSG~~~~~~~KTTRRFFFNLSSI~PTEEF~~~~ITSAELQVFR~~~~ 131
BMP4 108 ENIPGTSE~~~~~~~NSAFRFLFNLSSI~PENEV~~~~ISSAELRLFR~~~~ 143
BMP7 114 DKEFFHPR~~~~~~~YHHREFRFDLSKI~PEGEA~~~~VTAAEFRIYK~~~~ 149
BMP6 205 DKEFSPRQ~~~~~~~RHHKEFKFNLSQI~PEGEV~~~~VTAAEFRIYK~~~~ 240
BMP8 96 DRALGHQE~~~~~~~PHWKEFRFDLTQI~PAGEA~~~~VTAAEFRIYK~~~~ 131
LEFTY1 73 ~~~~~~~~~~~~~~~ASTHLLVFGMEQR~LPPNSE~~~LVQAVLRLFQEPVP 105
TGFB1hu 131 ~~~~~~~~~~LKLKVEQHVELYQKYSNN~~~~~~~~~~SW~~~~~~~~RYLS 154
TGFB1cyno 131 ~~~~~~~~~~LKLKVEQHVELYQKYSNN~~~~~~~~~~SW~~~~~~~~RYLS 154
TGFB1mu 131 ~~~~~~~~~~LKSSVEQHVELYQKYSNN~~~~~~~~~~SW~~~~~~~~RYLG 154
TGFB1po 131 ~~~~~~~~~~LKLKVEQHVELYQKYSND~~~~~~~~~~SW~~~~~~~~RYLS 154
TGFB2 135 ~~~~~~LQNPKARVPEQRIELYQILKSKD~~LT~~~SPTQ~~~~~~~~RYID 167
TGFB3 136 ~~~~~~VPNPSSKRNEQRIELFQILRPDE~~HI~~~~AKQ~~~~~~~~RYIG 167
GDF11 161 ~~~~~~~~RPVPRPATVYLQILRL~KPLT~GEG~~~~~~TAGGGGGGRRHIR 196
GDF11mu 159 ~~~~~~~~RPVPRPATVYLQILRL~KPLT~GEG~~~~~~TAGGGGGGRRHIR 194
GDF8 138 ~~~~~~~~RPVETPTTVFVQILRLIKPMK~~DG~~~~~~T~~~~~~~~RYTG 165
GDF8cyno 138 ~~~~~~~~RPVETPTTVFVQILRLIKPMK~~DG~~~~~~T~~~~~~~~RYTG 165
GDF8mu 138 ~~~~~~~~RPVKTPTTVFVQILRLIKPMK~~DG~~~~~~T~~~~~~~~RYTG 165
InhBetaA 140 ~~~~~~~~KVPKANRTRTKVTIRLFQQQKHPQG~~~~~~S~~~~~~~~LDTG 169
InhAlphaA 118 ~~GLDRQGTAASNSSEPLLGLLALSPG~~~~~~~~~~~G~~~~~~~~PVAV 147
BMP9 133 ~~~SCQNHVDPSHDLKGSVVIYDVLDGTD~~AWDSATETK~~~~~~~~TFLV 171
BMP2 132 ~~EQMQDALGNNSSFHHRINIYEIIKPAT~~AN~SKFPVT~~~~~~~~RLLD 170
BMP4 144 ~~EQVDQGPDWERGF~HRINIYEVMKPPA~~EVVPGHLIT~~~~~~~~RLLD 182
BMP7 150 ~~~~~DYIRERFDNETFRISVYQVLQEHL~~GR~~~ESDL~~~~~~~~FLLD 183
BMP6 241 ~~~~~DCVMGSFKNQTFLISIYQVLQEHQ~~HR~~~DSDL~~~~~~~~FLLD 274
BMP8 132 ~~~~~~VPSIHLLNRTLHVSMFQVVQEQS~~NR~~~ESDL~~~~~~~~FFLD 164
LEFTY1 106 KAALHRHGRLSPRSARARVTVEWLRVRDD~~GS~~~~NRT~~~~~~~~SLID 143

Figure 8B (continued)

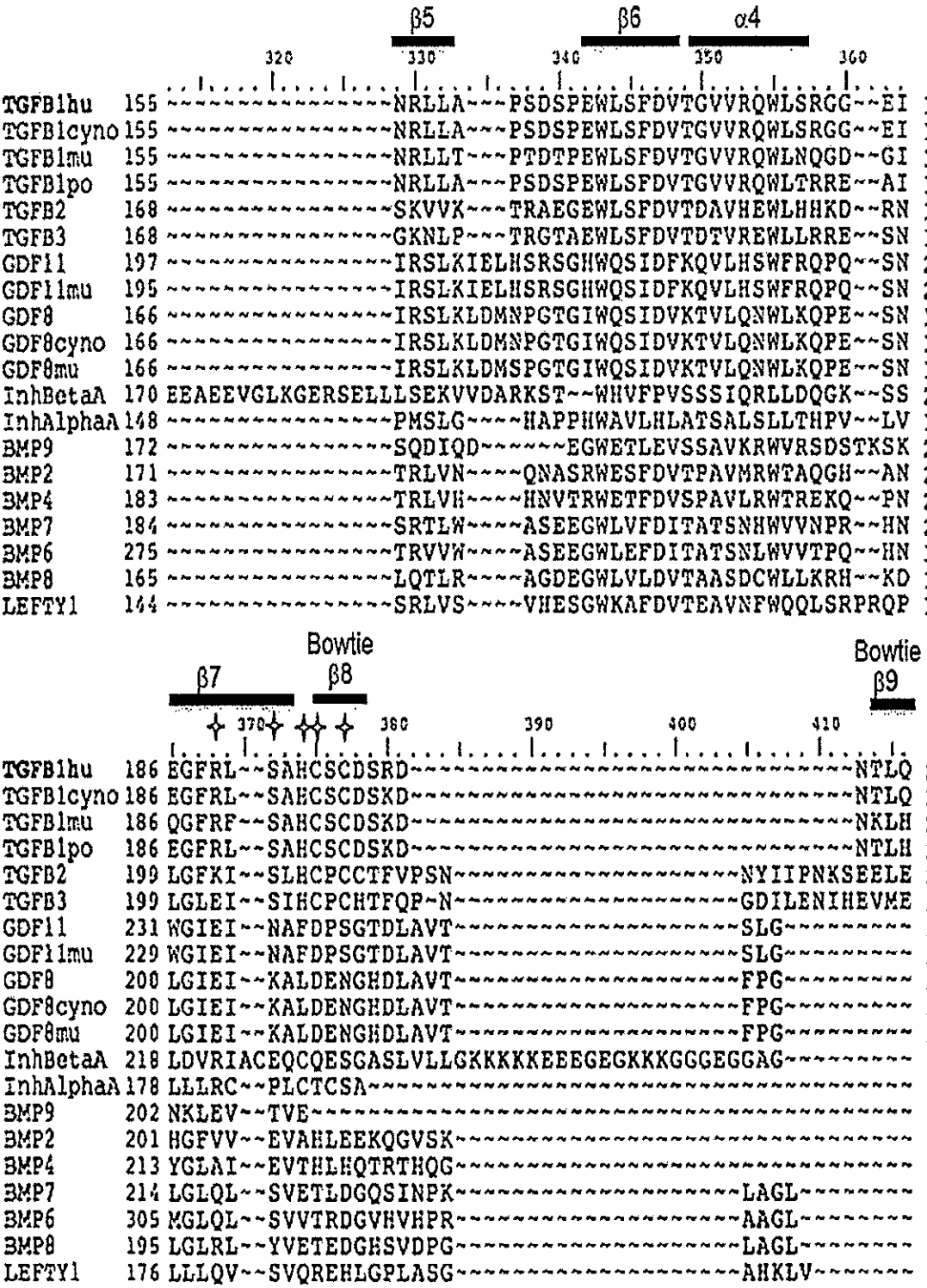


Figure 8C

```
          Bowtie
          β9          Trigger Loop                β10      α5
            420       430       440       450       460
          ...|....|....|....|....|....|....|....|....|....|...
TGFB1hu    205 VDINGFT~~~~~~~~~TGRRGDLATIHG~~~MNRPF~~LLLMATPLERAQH~ 241
TGFB1cyno  205 VDINGFT~~~~~~~~~TGRRGDLATIHG~~~MNRPF~~LLLMATPLERAQH~ 241
TGFB1mu    205 VEINGIS~~~~~~~~~PKRRGDLGTIHD~~~MNRPF~~LLLMATPLERAQH~ 241
TGFB1po    205 VEINGFN~~~~~~~~~SGRRGDLATIHG~~~MNRPF~~LLLMATPLERAQH~ 241
TGFB2      229 ARFAGIDGTSTYTSGDQKTIKSTRKKNS~~~GKTPH~~LLLMLLPSYRLESQ 275
TGFB3      228 IKFKGVDNED~~~DHGRGDLGRLKKQKD~~~HHNPH~~LILMMIPPHRLDNP 271
GDF11      252 ~~~~~~~~~~~~~~~~~~~~~~~~PGAE~~~GLHPF~~MELRVLE~~~~~~~ 267
GDF11mu    250 ~~~~~~~~~~~~~~~~~~~~~~~~PGAE~~~GLHPF~~MELRVLE~~~~~~~ 265
GDF8       221 ~~~~~~~~~~~~~~~~~~~~~~~~PGED~~~GLNPF~~LEVKVTD~~~~~~~ 236
GDF8cyno   221 ~~~~~~~~~~~~~~~~~~~~~~~~PGED~~~GLNPF~~LEVKVTD~~~~~~~ 236
GDF8mu     221 ~~~~~~~~~~~~~~~~~~~~~~~~PGED~~~GLNPF~~LEVKVTD~~~~~~~ 236
InhBetaA   261 ~~~~~~~~~~~~~~~~~~~~~~~~ADEEKEQSHRPFLMLQARQSE~~~~~~~ 281
InhAlphaA  190 ~~~~~~~~~~~~~~~~~~~~~~~~~~RP~~~EATPF~~LVAHTRTRPP~~~~ 206
BMP9       210 ~~~~~~~~~~~SHRKGCDTLDISVPPGS~~~RNLPF~~FVVFSNDH~~~~~~ 239
BMP2       219 ~~~~~~~~~~~RHVRISRSLHQDEHSWS~~~QIRPL~~LVTFGHDGKG~~~~ 250
BMP4       231 ~~~~~~~~~~~QHVRISRSLPQGSGNWA~~~QLRPL~~LVTFGHDGRG~~~~ 262
BMP7       236 ~~~~~~~~~~~~~~~~~~~~~IGRHGPQ~~~NKQPF~~MVAFFKATEVHFRS 261
BMP6       327 ~~~~~~~~~~~~~~~~~~~~~VGRDGPY~~~DKQPF~~MVAFFKVSEVHVRT 352
BMP8       217 ~~~~~~~~~~~~~~~~~~~~~LGQRAPR~~~SQQPF~~VVTFFRASPSPIRT 242
LEFTY1     199 ~~~~~~~~~~~~~~~~~~RFASQGAPAG~~~LGEPQ~~LELHTLDLGDYGAQ 227

                470       480       490       500       510       520
               .|....|....|....|....|....|....|....|....|....|....|
TGFB1hu    242 ~~~LQSSRHRR~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ 249
TGFB1cyno  242 ~~~LQSSRHRR~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ 249
TGFB1mu    242 ~~~LHSSRHRR~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ 249
TGFB1po    242 ~~~LHSSRHRR~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ 249
TGFB2      276 ~~~QTNRRKKR~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ 283
TGFB3      272 ~~GQGGQRKKR~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ 280
GDF11      268 ~~~~NTKRSRR~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ 274
GDF11mu    266 ~~~~NTKRSRR~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ 272
GDF8       237 ~~~~TPKRSRR~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ 243
GDF8cyno   237 ~~~~TPKRSRR~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ 243
GDF8mu     237 ~~~~TPKRSRR~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ 243
InhBetaA   282 ~~~~DHPHRRRRR~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ 290
InhAlphaA  207 ~~~SGGERARR~~~~~~~~~~~~~~~~~~~~~~~~~~~~STPLMSWPWSPSA 227
BMP9       240 ~~~SSGTKETRLELREMISHEQESVLKKLSKDGSTEAGESSHEEDTDGHVAA 288
BMP2       251 ~~~HPL~~HKREKR~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ 259
BMP4       263 ~~~HALTRRRRAKR~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ 273
BMP7       262 IR~STGSKQRSQNRSK~~~~~~~~~~~~~~~~~~~~~~~~~~~TPKNQEALR 285
BMP6       353 TR~SASSRRRQQSRNR~~~~~~~~~~~~~~~~~~~~~~~~~~~STQSQDVAR 376
BMP8       243 PR~AVRPLRRRQP~KK~~~~~~~~~~~~~~~~~~~~~~~~~~~SNELPQANR 265
LEFTY1     228 GD~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ 229
```

Figure 8C (continued)

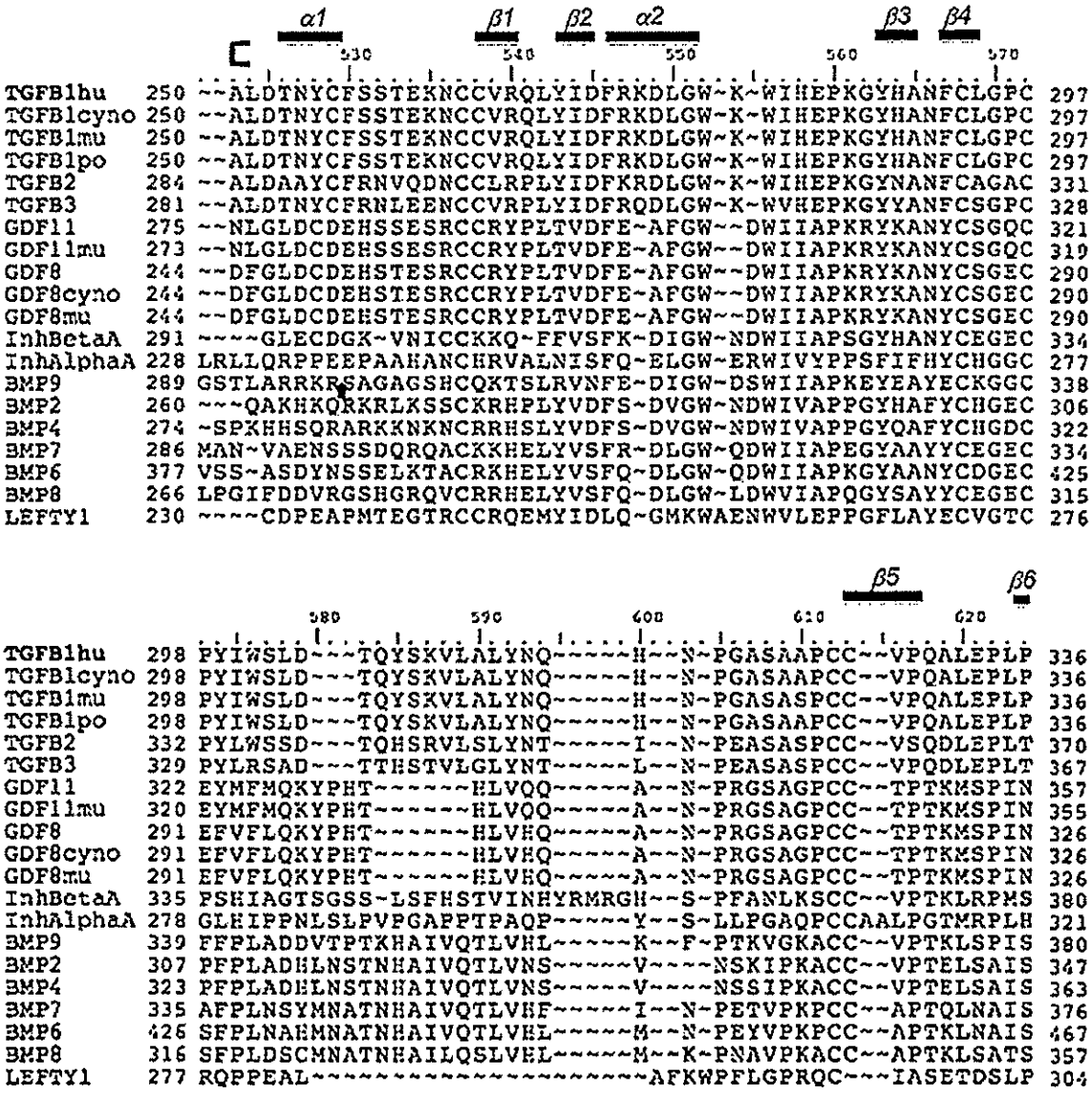
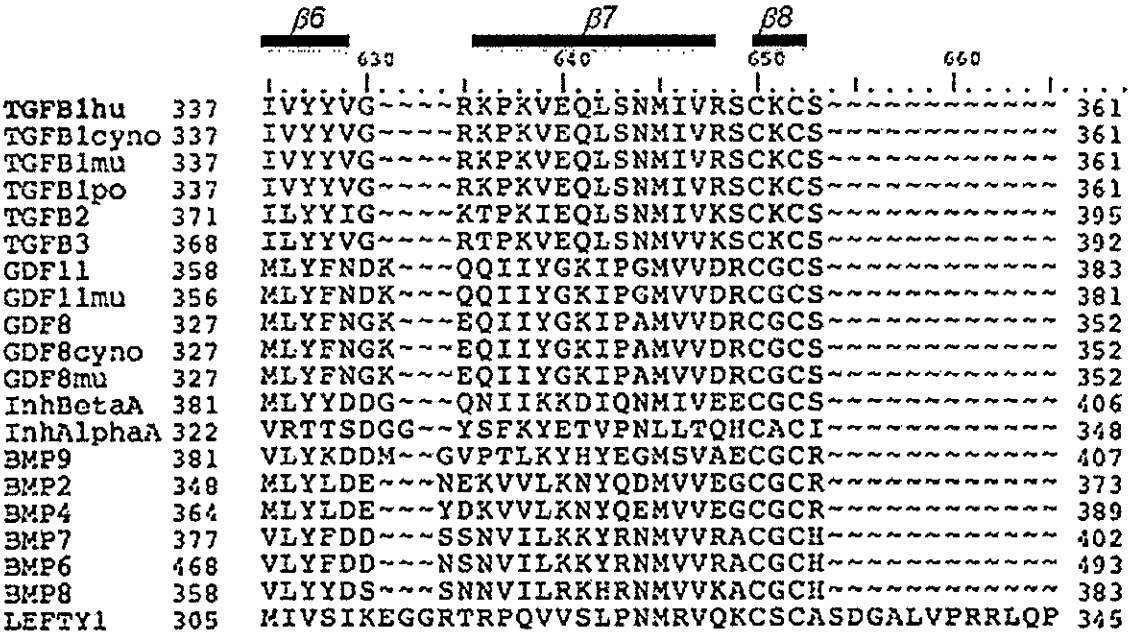


Figure 8C (continued)



Percent identity among TGF-β family member proteins (prodomain and growth factor)

	TGFB1hu	TGFB1po	TGFB2	TGFB3	GDF11	GDF8	InhBetc	nAlph	BMP9	BMP2	BMP4	BMP7	BMP6	BMP8	LEFTY1
TGFB1hu	100	94	42	46	19	22	16	14	14	19	18	18	16	17	15
TGFB1po	94	100	42	46	20	22	15	14	14	19	19	18	15	17	15
TGFB2	42	42	100	55	18	20	13	12	13	21	19	18	15	16	15
TGFB3	46	46	55	100	19	21	14	13	14	19	18	20	17	19	14
GDF11	19	20	18	19	100	61	21	15	15	18	18	17	18	16	13
GDF8	22	22	20	21	61	100	24	14	16	20	18	18	17	19	12
InhBetaA	16	15	13	14	21	24	100	12	15	18	18	17	15	19	9
InhAlphaA	14	14	12	13	15	14	12	100	14	11	12	13	10	13	13
BMP9	14	14	13	14	15	16	15	14	100	24	25	21	20	21	11
BMP2	19	19	21	19	18	20	18	11	24	100	59	26	23	25	15
BMP4	18	19	19	18	18	18	18	12	25	59	100	25	24	26	14
BMP7	18	18	18	20	17	18	17	13	21	26	25	100	54	50	12
BMP6	16	15	15	17	18	17	15	10	20	23	24	54	100	42	11
BMP8	17	17	16	19	16	19	19	13	21	25	26	50	42	100	12
LEFTY1	15	15	15	14	13	12	9	13	11	15	14	12	11	12	100

Percent identity among TGF-β family member growth factor domains

	TGFB1hu	TGFB1po	TGFB2	TGFB3	GDF11	GDF8	InhBetaA	InhAlphaA	BMP9	BMP2	BMP4	BMP7	BMP6	BMP8	LEFTY1
TGFB1hu	100	100	83	86	48	47	44	34	36	43	44	44	46	41	32
TGFB1po	100	100	83	86	48	47	44	34	36	43	44	44	46	41	32
TGFB2	83	83	100	89	50	49	47	37	37	47	47	48	49	46	30
TGFB3	86	86	89	100	50	49	46	36	36	47	46	46	50	44	30
GDF11	48	48	50	50	100	94	52	37	45	47	44	50	50	45	29
GDF8	47	47	49	49	94	100	51	34	44	47	44	49	50	45	27
InhBetaA	44	44	47	46	52	51	100	40	47	53	52	55	56	52	27
InhAlphaA	34	34	37	36	37	34	40	100	40	41	39	42	42	39	26
BMP9	36	36	37	36	45	44	47	40	100	60	56	56	59	57	28
BMP2	43	43	47	47	47	47	53	41	60	100	88	69	71	68	30
BMP4	44	44	47	46	44	44	52	39	56	88	100	66	66	67	29
BMP7	44	44	48	46	50	49	55	42	56	69	66	100	90	78	29
BMP6	46	46	49	50	50	50	56	42	59	71	66	90	100	81	30
BMP8	41	41	46	44	45	45	52	39	57	68	67	78	81	100	31
LEFTY1	32	32	30	30	29	27	27	26	28	30	29	29	30	31	100

Percent identity among TGF-β family member prodomains

	TGFB1hu	TGFB1po	TGFB2	TGFB3	GDF11	GDF8	InhBetaA	InhAlphaA	BMP9	BMP2	BMP4	BMP7	BMP6	BMP8	LEFTY1
TGFB1hu	100	92	30	34	14	17	10	10	9	14	14	13	11	13	13
TGFB1po	92	100	31	35	15	17	10	11	10	14	14	13	10	13	13
TGFB2	30	31	100	46	13	16	7	8	8	17	14	13	10	11	14
TGFB3	34	35	46	100	13	16	9	9	9	15	14	15	11	14	11
GDF11	14	15	13	13	100	50	16	12	9	12	13	11	12	10	13
GDF8	17	17	16	16	50	100	19	11	10	13	13	11	10	13	11
InhBetaA	10	10	7	9	16	19	100	9	9	10	11	9	8	13	8
InhAlphaA	10	11	8	9	12	11	9	100	11	8	9	10	7	11	11
BMP9	9	10	8	9	9	10	9	11	100	16	17	13	12	13	10
BMP2	14	14	17	15	12	13	10	8	16	100	49	17	14	16	14
BMP4	14	14	14	14	13	13	11	9	17	49	100	17	16	17	13
BMP7	13	13	13	15	11	11	9	10	13	17	17	100	46	43	11
BMP6	11	10	10	11	12	10	8	7	12	14	16	46	100	34	8
BMP8	13	13	11	14	10	13	13	11	13	16	17	43	34	100	9
LEFTY1	13	13	14	11	13	11	8	11	10	14	13	11	8	9	100

Figure 10

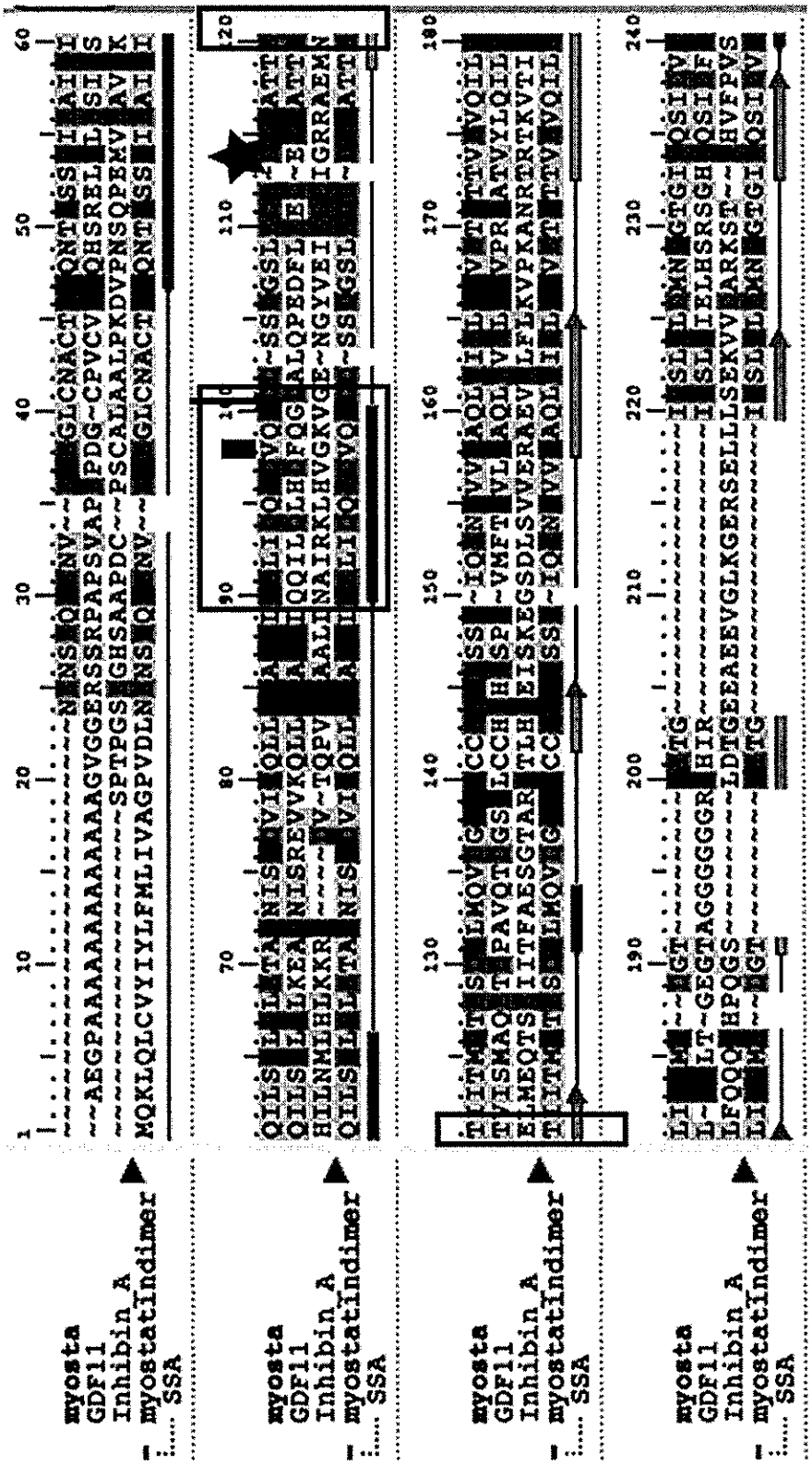


Figure 10 (continued)

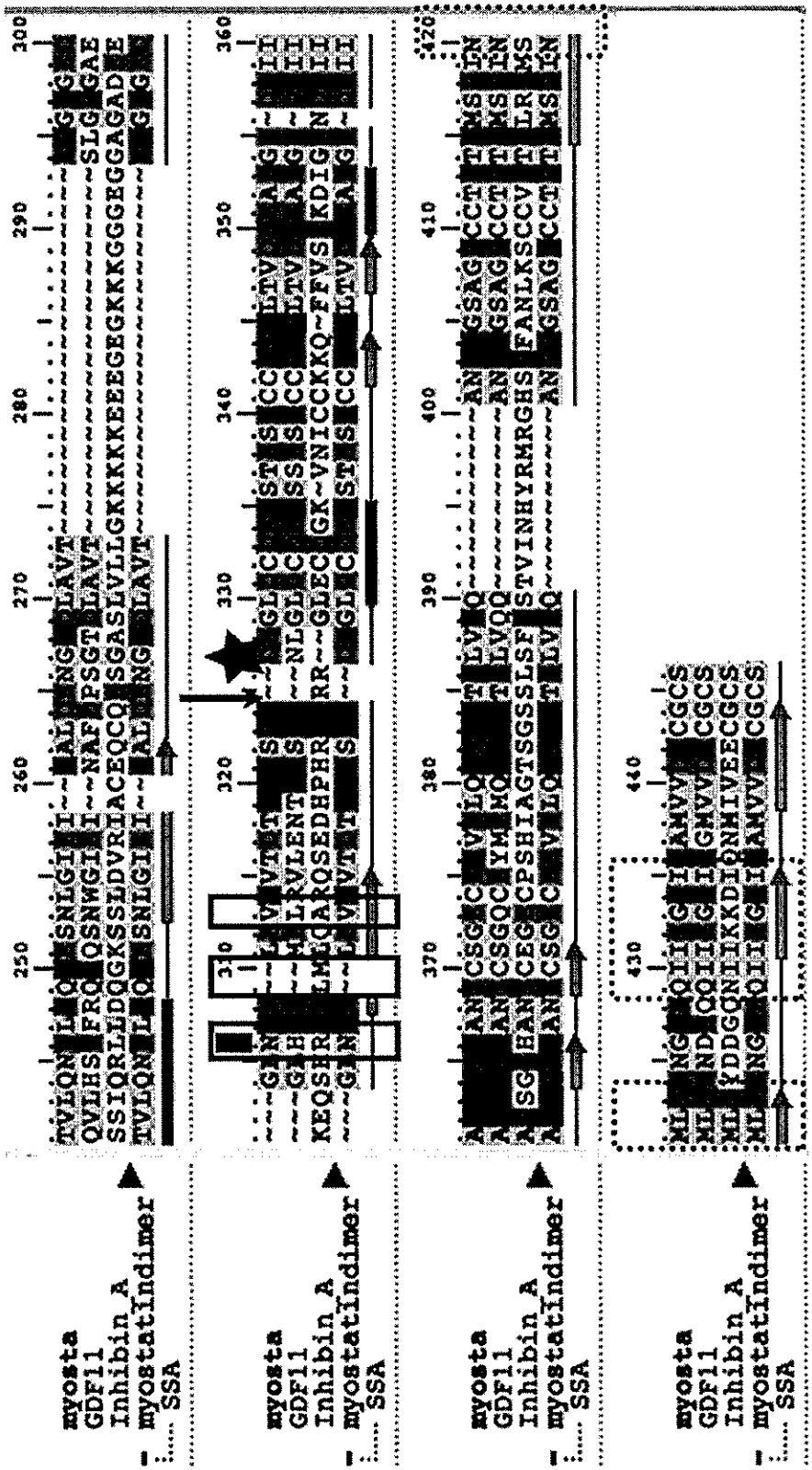
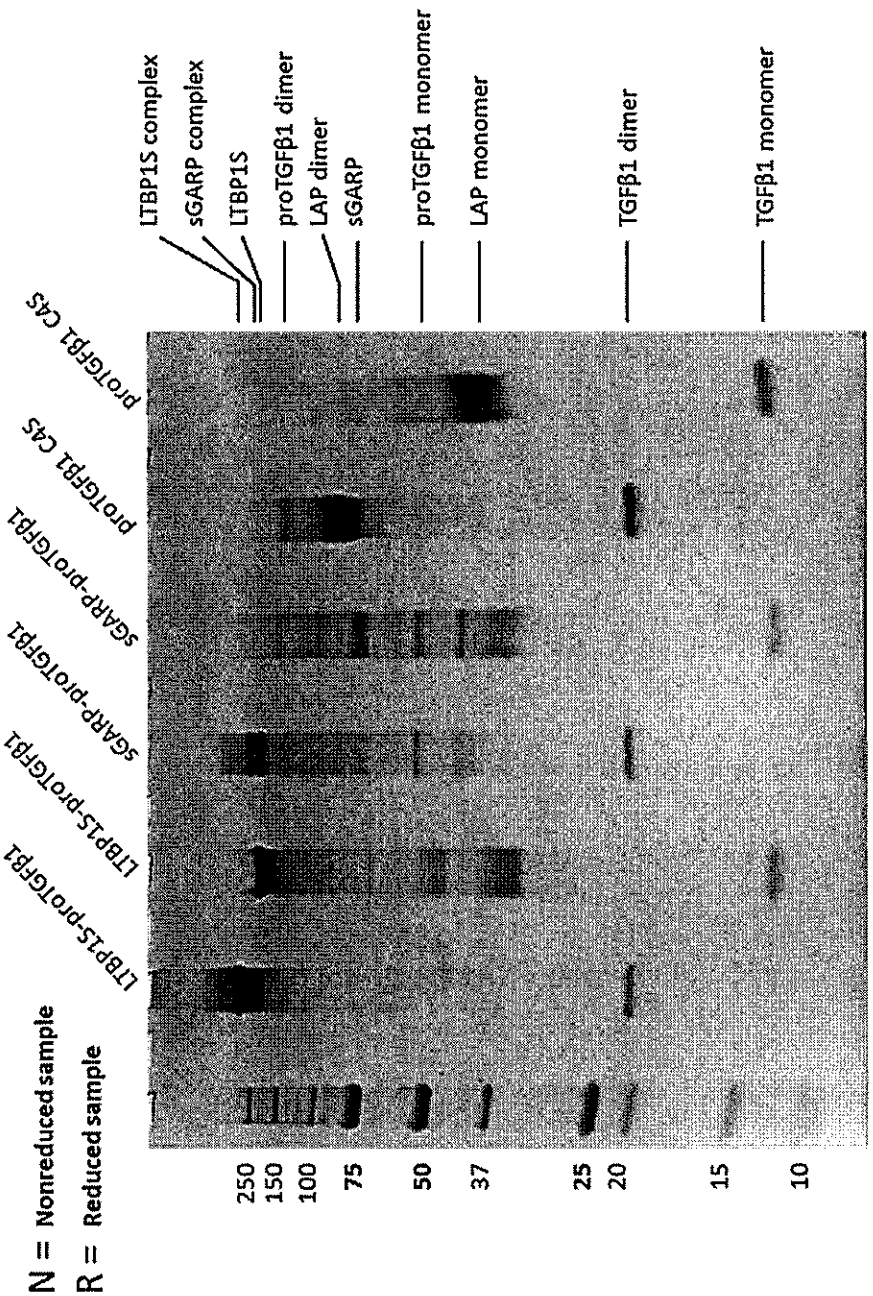


Figure 11


N = Nonreduced sample
R = Reduced sample
N R N R N R
LTBP1S-proTGFβ1
LTBP1S-proTGFβ1
sGARP-proTGFβ1
sGARP-proTGFβ1
proTGFβ1 CAS
proTGFβ1 CAS
LTBP1S complex
sGARP complex
LTBP1S
proTGFβ1 dimer
LAP dimer
sGARP
proTGFβ1 monomer
LAP monomer
TGFβ1 dimer
TGFβ1 monomer
250
150
100
75
50
37
25
20
15
10

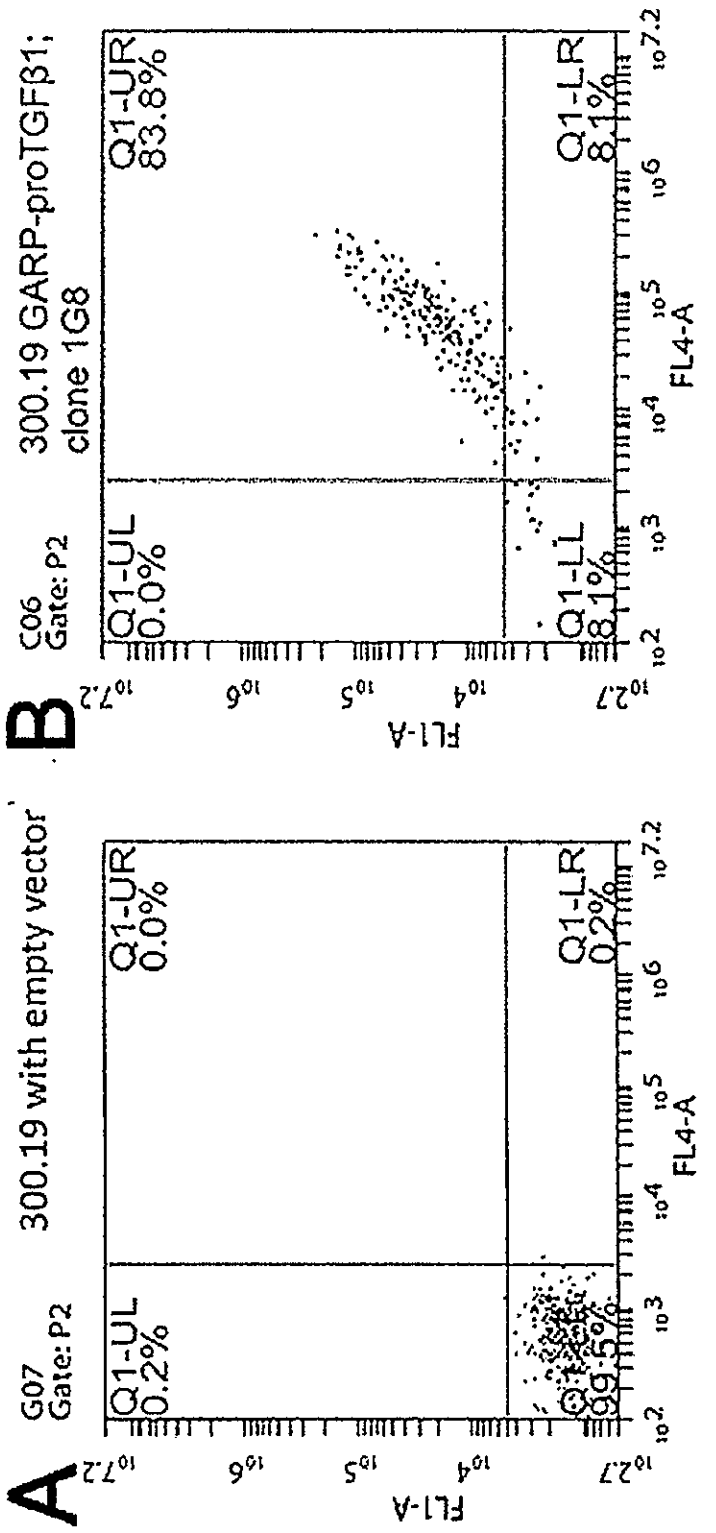

A
G07
Gate: P2
300.19 with empty vector
Q1-UL
0.2%
Q1-UR
0.0%
Q1-LR
0.2%
99.5%
FL1-A
FL4-A
B
C06
Gate: P2
300.19 GARP-proTGFβ1;
clone 1G8
Q1-UL
0.0%
Q1-UR
83.8%
Q1-LL
8.1%
Q1-LR
8.1%
FL1-A
FL4-A

Figure 12

Figure 12 (Continued)

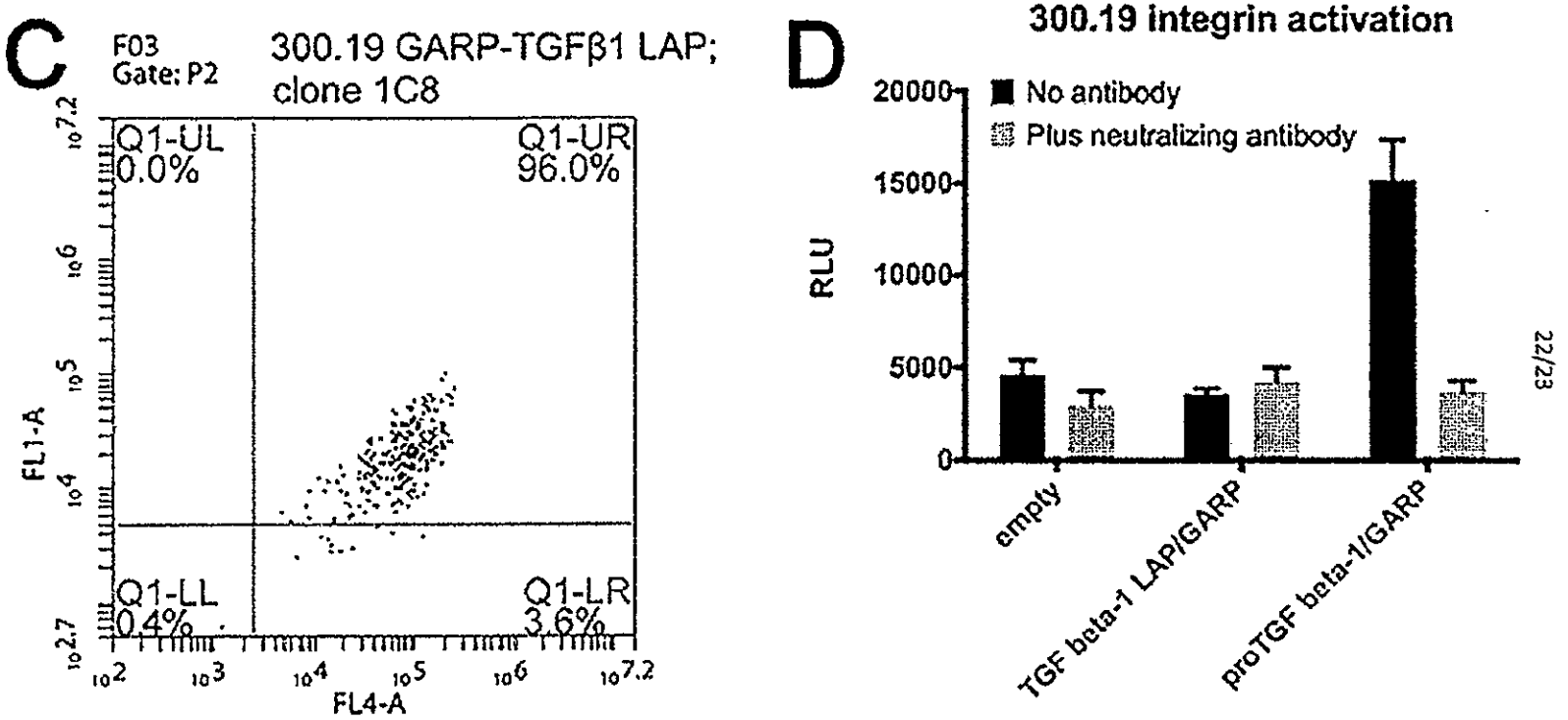
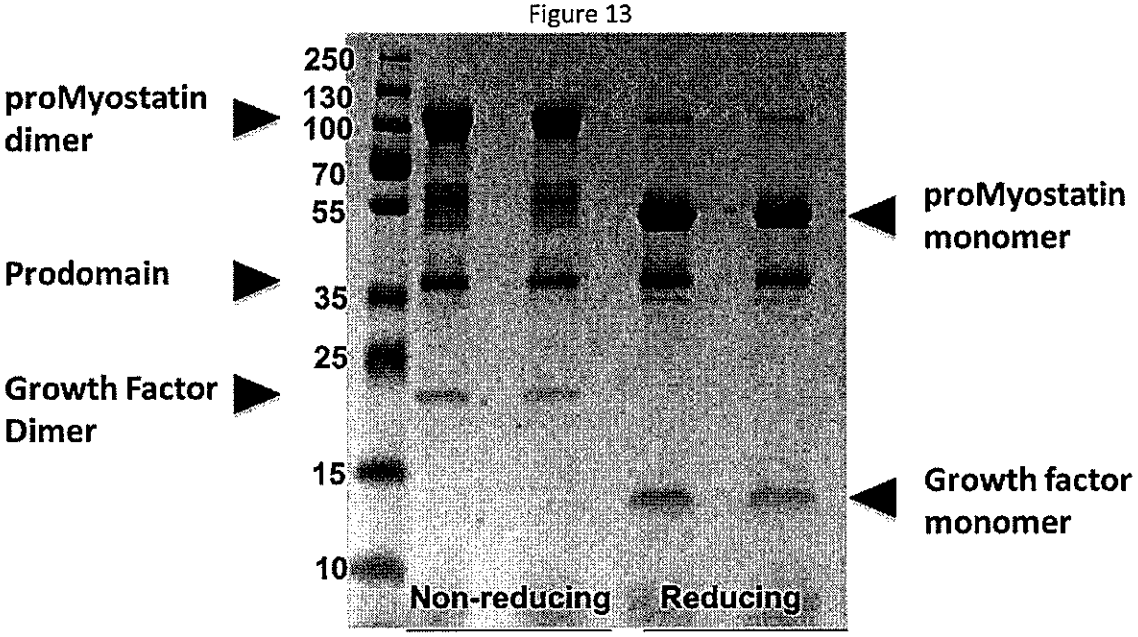


Figure 13


proMyostatin dimer
Prodomain
Growth Factor Dimer
250
130
100
70
55
35
25
15
10
Non-reducing
Reducing
proMyostatin monomer
Growth factor monomer
Colloidal blue stain