The American Journal of Human Genetics, Volume *93*

Supplemental Data

SAMS, a Syndrome of Short Stature, Auditory-Canal Atresia, Mandibular Hypoplasia, and Skeletal Abnormalities Is a Unique Neurocristopathy Caused by Mutations in Goosecoid

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SUPPLEMENTAL DATA

SUPPLEMENTAL FIGURES

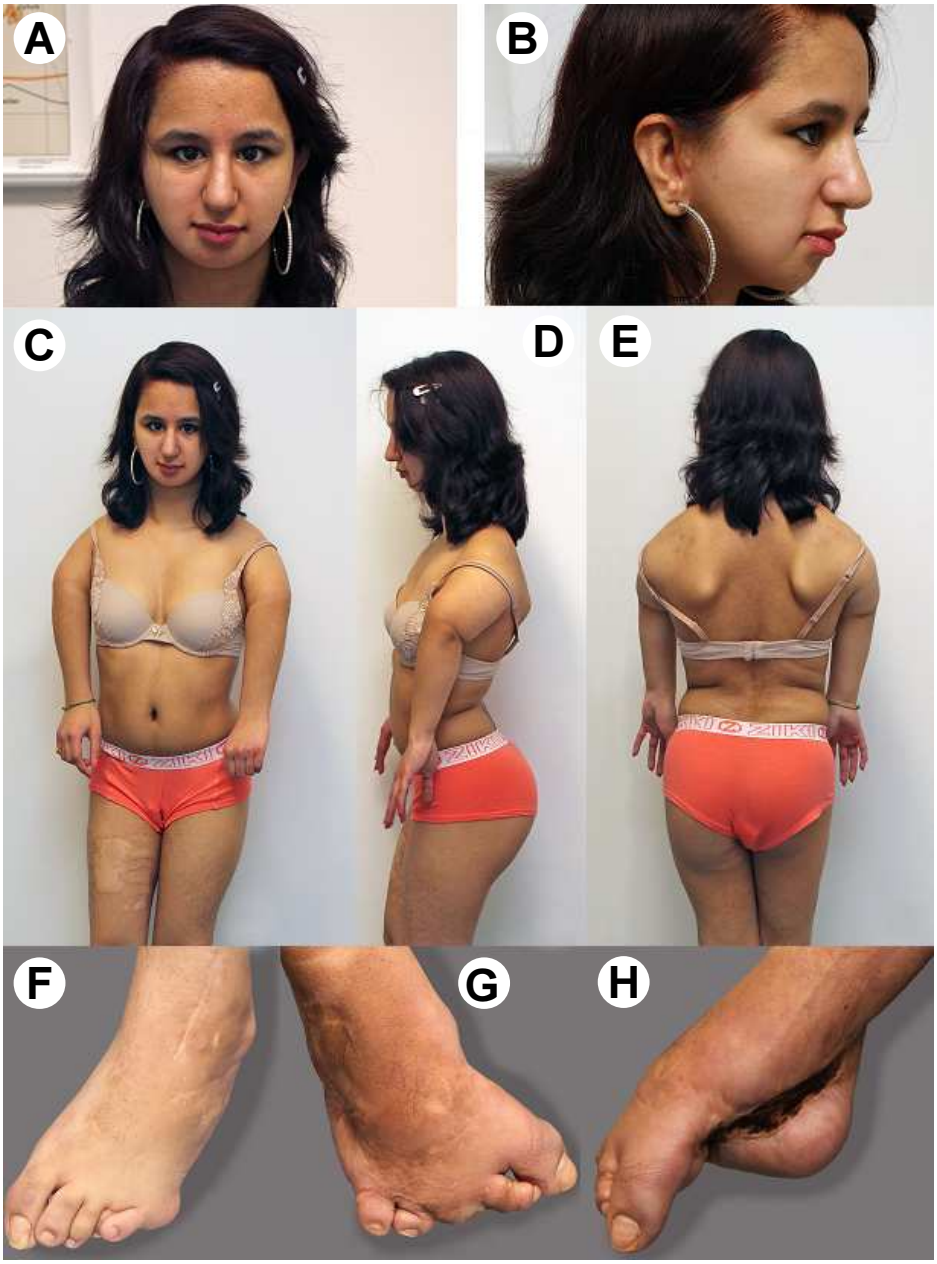

Figure S1: Clinical features of SAMS in individual B

(A-B) Individual B at age of 19 years, presenting with micrognathia, asymmetrical facial features, auditory canal atresia, **(C-E)** shortening of both humeri with bilateral scapulo-humeral synostosis, lumbar hyperlordosis and central dislocation of both hips. **(F)** Left and **(G-H)** right foot after repeat clubfeet surgery. Walking is only possible with the use of orthopaedic shoes.

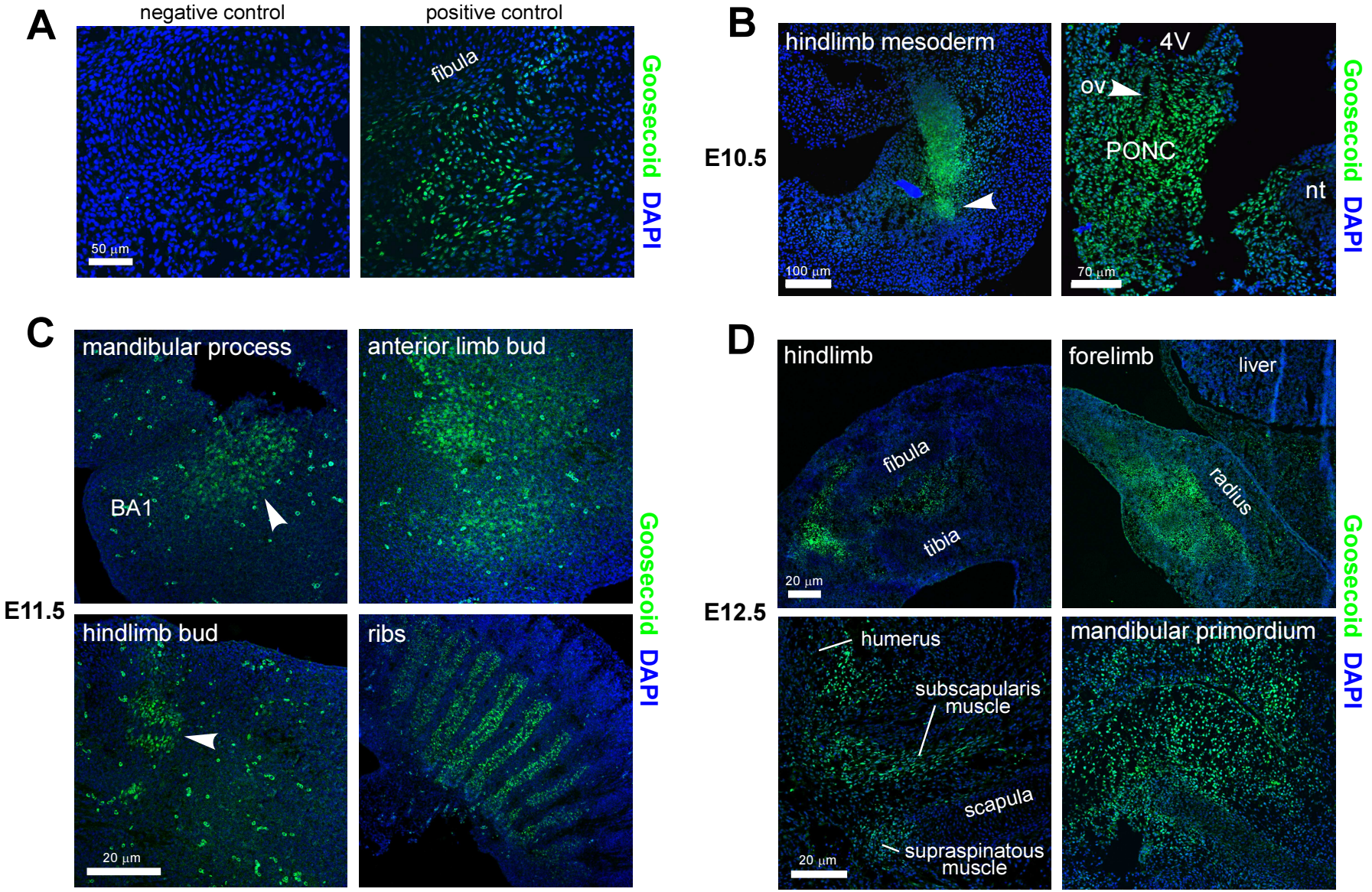
A
negative control
positive control
fibula
50 μm
Goosecoid DAPI
B
E10.5
hindlimb mesoderm
4V
ov
PONC
nt
100 μm
70 μm
Goosecoid DAPI
C
E11.5
mandibular process
BA1
anterior limb bud
hindlimb bud
ribs
20 μm
Goosecoid DAPI
D
E12.5
hindlimb
fibula
tibia
20 μm
forelimb
liver
radius
humerus
subscapularis muscle
scapula
supraspinatous muscle
20 μm
mandibular primordium
Goosecoid DAPI

Figure S2: Goosecoid localization in mouse embryonic tissues

Immunofluoresence (IF) confocal microscopy of mouse embryonic sections for the indicated tissues stained for Goosecoid (green) and nuclei (DAPI, in blue). **(A)** E11.5 distal hindlimb stained following peptide competition (left panel) and with anti-Goosecoid only (right panel) to confirm the specificity of the antibody reagent. Note the nuclear localization of Goosecoid in connective tissue by the fibula. Para-sagittal section; scale bar = 50μm. **(B)** Specific, high levels of Goosecoid localization in E10.5 hindlimb mesodermal condensation (left panel), including the probable femoral head (arrowhead). Goosecoid localization also surrounded the neural tube (right panel; nt) and was in the post-otic neural crest cells (PONC) surrounding the otic vesicle (ov; arrowhead) but not in neuronal tissues; 4V, fourth ventricle. Para-sagittal and horizontal sections, respectively; scale bars = 100 and 70 μm, as indicated. **(C)** High levels of Goosecoid expression (arrowhead) in E11.5 mouse embryonic mandibular process of the first branchial arch (BA1), anterior and hind limb buds (arrowhead) and developing ribs. Midline and para-sagittal sections, scale bar = 20μm for all panels. **(D)** Goosecoid expression in E12.5 embryonic connective tissue but not bone primordia of hindlimb and forelimb, muscles and connective tissue of the shoulder joint, and the mandibular primordium. Midline and para-sagittal sections, scale bars = 20μm.

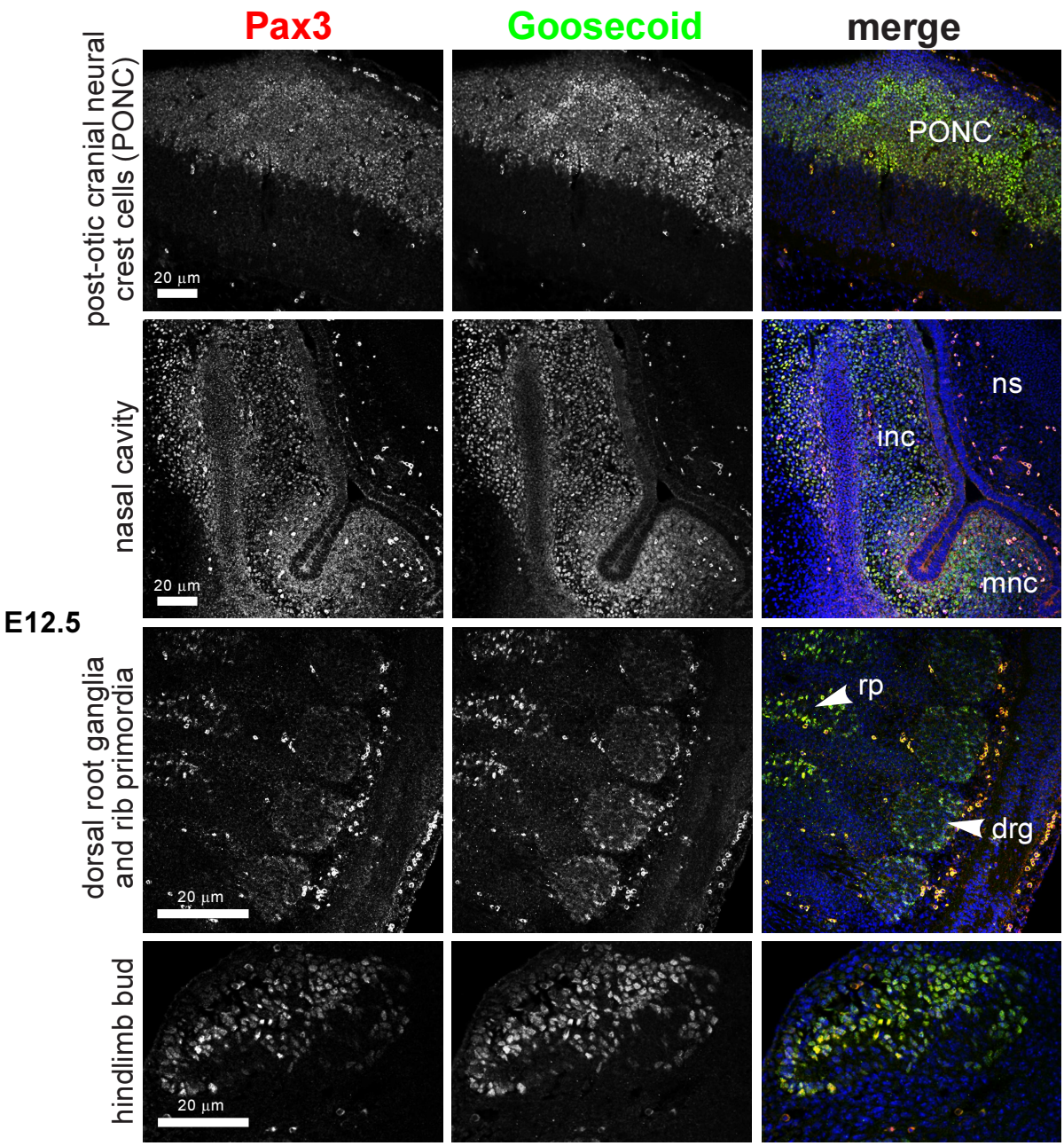

Figure S3: Goosecoid co-localizes with Pax3 in neural crest derivatives and bone primordia

IF microscopy for mouse E12.5 embryonic horizontal and para-sagittal sections for the indicated tissues stained for Goosecoid (green), Pax3 (red) and nuclei (DAPI, in blue). Goosecoid is co-expressed with Pax3 in neural crest tissue derivatives in post-otic cranial neural crest cells (PONC), nasal cavity, dorsal root ganglia (drg) and rib primordia (rp), and hindlimb bud. Abbreviations: inc, inferior nasal concha; mnc, middle nasal concha; ns, nasal septum.

SUPPLEMENTAL TABLES

variant filtering conditions:	genes with potential biallelic variants in patient C…	segregating in mother…	...and father	...and unaffected sibling
with non-synonymous, indel or splice site variants	3815	1365	591	482
dbSNP, 1000 Genomes and EVS MAF < 1% and not in dbSNP129 (or earlier)	165	67	24	27
not in 53 local UK-Pakistani exomes	50	26	16	13
not in Gujarati exomes	38	19	11	8
predicted damaging by Polyphen2	20	8	4	2

Table S1: Variant filtering in individual C and family

The number of candidate genes with variants compatible with autosomal recessive inheritance and passing quality control filters are given under various filtering conditions. Columns show the number of variants after removal of homozygous variants in either unaffected family members, or potential compound heterozygous variant combinations in unaffected family members. Abbreviations: EVS, Exome Variant Server; MAF, minor allele frequency.

exon	forward primer	reverse primer
1	CTCTCTTTCGGTTTGGTCGG	GGAGTTGCAAGAGGAGCAAA
2	TCTAAGTGGAAGAGGGTCGG	TGTTTTTAAAGTGCGGGGAG
3	AGTTGAATGAAAACGCGGAA	GCGTGTGCAAGAAAGTAGCA

Table S2: Oligonucleotide primers used for sequence analysis of *GSC*

The forward and reverse primers used for PCR and Sanger sequencing of all the exons in *GSC* are indicated.