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Supplementary Materials for

Convergent deployment of ancestral functions during the evolution of mammalian flight membranes

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The PDF file includes:

Figs. S1 to S3
Tables S1 to S3, S15, and S16
Legends for tables S4 to S14
Legend for movie S1

Other Supplementary Material for this manuscript includes the following:

Tables S4 to S14
Movie S1

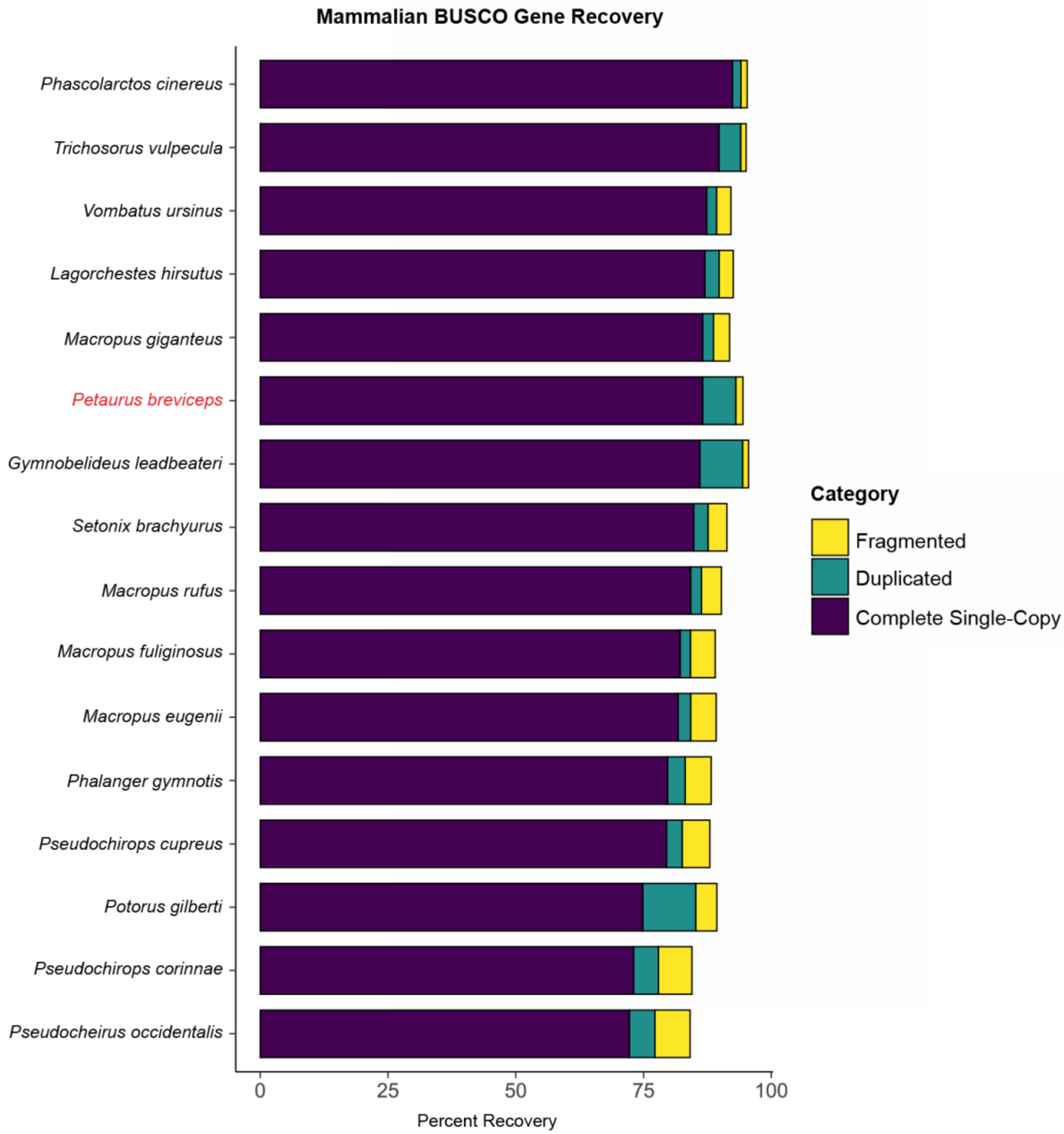

Fig. S1. Comparison of benchmarking universal single-copy ortholog (BUSCO) recovery in the genomes of the sugar glider and related diprotodont marsupials. Species are sorted by the percent of complete-single copy BUSCO genes. The sugar glider assembly presented in this study is shown in red font.

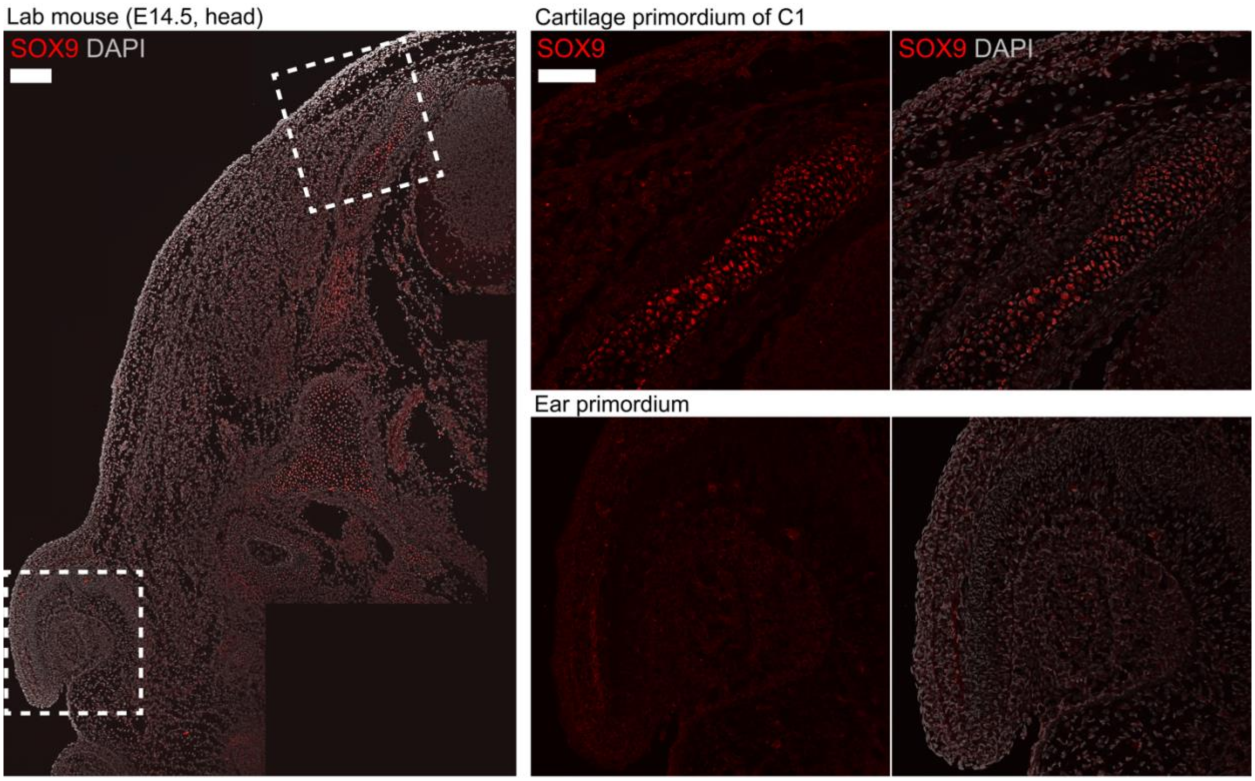

Fig. S2. Chondrogenic cells are present in the developing vertebrae but not in the ear primordium of E14.5 mouse embryos. Head cross sections of E14.5 laboratory mouse stained for the chondrogenic marker SOX9 and DAPI. Dashed boxes outline the C1 cervical vertebrae, which displays SOX9 positive cells, and the ear primordium, which does not show evidence of SOX9 positive cells. These results demonstrate that the E14.5 ear primordium is composed of undifferentiated mesenchyme.

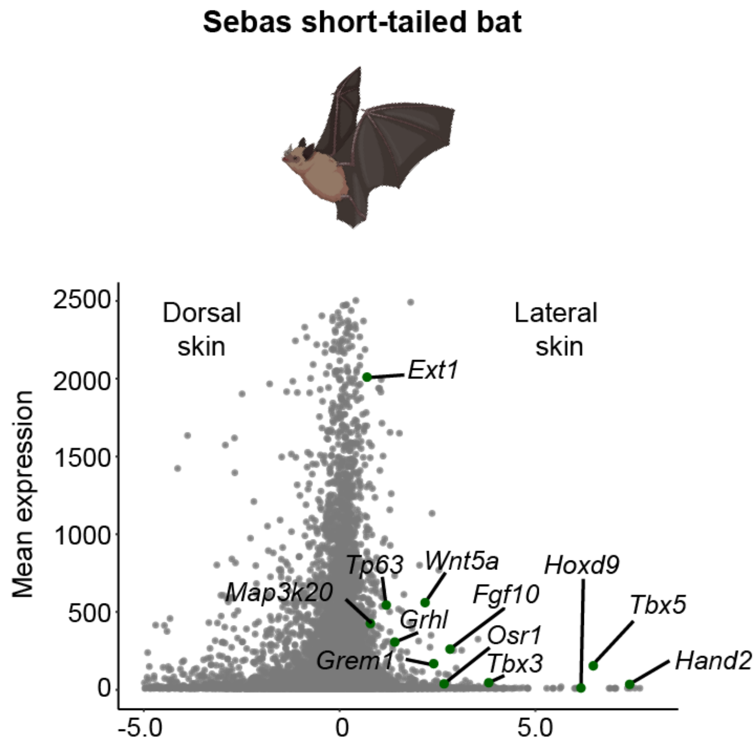

Fig. S3. Volcano plot of genes differentially expressed between the bat plagiopatagium and neighboring dorsal skin during its outgrowth. Genes upregulated in the plagiopatagium and belonging to limb development Gene Ontology terms are labeled in green.

Table S1. Sugar glider genome assembly metrics.

Metric	Value
Scaffold Length	3463.91 Mbp
Gap-Free Length	3344.58 Mbp
Contig/Scaffold Number	87,817 / 1,909
Contig/Scaffold N50	69.63 Kbp / 28.19 Mbp
Contig/Scaffold N90	51.92 Kbp / 20.28 Mbp
Longest Contig/Scaffold	599.68 Kbp / 109.60 Mbp
Gap Percentage	1.89%
G+C Percentage	38.95%

Table S2. Sugar glider genome BUSCO recovery.

BUSCO Reference Database: mammalia_odb10: 9226 genes		
BUSCO category	**Gene Count**	**Percent Recovery (of 9226)**
Complete (total)	8581	93.0
Complete single-copy	7984	86.5
Complete and duplicated	597	6.5
Fragmented	132	1.4
Missing	513	5.6

Table S3. Manifest of RNA-Seq samples used in this study.

Species	Sample	Tissue	Stage	Weight (grams)
P. breviceps	pb_pat_pre-patagium_121	Patagium	pre-patagium	0.22
P. breviceps	pb_pat_pre-patagium_122	Patagium	pre-patagium	0.22
P. breviceps	pb_pat_pre-patagium_130	Patagium	pre-patagium	0.23
P. breviceps	pb_pat_pre-patagium_131	Patagium	pre-patagium	0.25
P. breviceps	pb_pat_pre-patagium_100	Patagium	pre-patagium	0.25
P. breviceps	pb_pat_pre-patagium_137	Patagium	pre-patagium	0.26
P. breviceps	pb_pat_pre-patagium_101	Patagium	pre-patagium	0.3
P. breviceps	pb_pat_pre-patagium_119	Patagium	pre-patagium	0.35
P. breviceps	pb_pat_pre-patagium_138	Patagium	pre-patagium	0.35
P. breviceps	pb_pat_pre-patagium_118	Patagium	pre-patagium	0.35
P. breviceps	pb_pat_early-forming_124	Patagium	early-forming	0.38
P. breviceps	pb_pat_early-forming_129	Patagium	early-forming	0.39
P. breviceps	pb_pat_early-forming_141	Patagium	early-forming	0.4
P. breviceps	pb_pat_early-forming_132	Patagium	early-forming	0.41
P. breviceps	pb_pat_early-forming_140	Patagium	early-forming	0.43
P. breviceps	pb_pat_early-forming_139	Patagium	early-forming	0.49
P. breviceps	pb_pat_early-forming_110	Patagium	early-forming	0.49
P. breviceps	pb_pat_early-forming_102	Patagium	early-forming	0.52
P. breviceps	pb_pat_early-forming_90	Patagium	early-forming	0.53
P. breviceps	pb_pat_early-forming_103	Patagium	early-forming	0.55
P. breviceps	pb_pat_early-forming_111	Patagium	early-forming	0.58
P. breviceps	pb_pat_early-forming_142	Patagium	early-forming	0.59
P. breviceps	pb_pat_early-forming_117	Patagium	early-forming	0.62
P. breviceps	pb_pat_early-forming_105	Patagium	early-forming	0.63
P. breviceps	pb_pat_early-forming_125	Patagium	early-forming	0.63
P. breviceps	pb_pat_early-forming_120	Patagium	early-forming	0.69
P. breviceps	pb_pat_late-forming_97	Patagium	late-forming	0.86
P. breviceps	pb_pat_late-forming_93	Patagium	late-forming	0.89
P. breviceps	pb_pat_late-forming_94	Patagium	late-forming	0.9
P. breviceps	pb_pat_late-forming_108	Patagium	late-forming	0.9
P. breviceps	pb_pat_late-forming_127	Patagium	late-forming	0.92
P. breviceps	pb_pat_late-forming_126	Patagium	late-forming	0.97
P. breviceps	pb_pat_late-forming_115	Patagium	late-forming	1.19
P. breviceps	pb_pat_late-forming_98	Patagium	late-forming	1.68
P. breviceps	pb_dors_pre-patagium_131	Dorsal Skin	pre-patagium	0.25
P. breviceps	pb_dors_pre-patagium_137	Dorsal Skin	pre-patagium	0.26
P. breviceps	pb_dors_pre-patagium_101	Dorsal Skin	pre-patagium	0.3

P. breviceps	pb_dors_pre-patagium_119	Dorsal Skin	pre-patagium	0.35
P. breviceps	pb_dors_pre-patagium_138	Dorsal Skin	pre-patagium	0.35
P. breviceps	pb_dors_pre-patagium_118	Dorsal Skin	pre-patagium	0.35
P. breviceps	pb_dors_early-forming_124	Dorsal Skin	early-forming	0.38
P. breviceps	pb_dors_early-forming_129	Dorsal Skin	early-forming	0.39
P. breviceps	pb_dors_early-forming_141	Dorsal Skin	early-forming	0.4
P. breviceps	pb_dors_early-forming_132	Dorsal Skin	early-forming	0.41
P. breviceps	pb_dors_early-forming_140	Dorsal Skin	early-forming	0.43
P. breviceps	pb_dors_early-forming_139	Dorsal Skin	early-forming	0.49
P. breviceps	sg_shd_pre-patagium_131	Shoulder Skin	pre-patagium	0.25
P. breviceps	sg_shd_pre-patagium_137	Shoulder Skin	pre-patagium	0.26
P. breviceps	sg_shd_pre-patagium_101	Shoulder Skin	pre-patagium	0.3
P. breviceps	sg_shd_pre-patagium_119	Shoulder Skin	pre-patagium	0.35
P. breviceps	sg_shd_pre-patagium_138	Shoulder Skin	pre-patagium	0.35
P. breviceps	sg_shd_pre-patagium_118	Shoulder Skin	pre-patagium	0.35
P. breviceps	sg_shd_early-forming_124	Shoulder Skin	early-forming	0.38
P. breviceps	sg_shd_early-forming_129	Shoulder Skin	early-forming	0.39
P. breviceps	sg_shd_early-forming_141	Shoulder Skin	early-forming	0.4
P. breviceps	sg_shd_early-forming_132	Shoulder Skin	early-forming	0.41
P. breviceps	sg_shd_early-forming_140	Shoulder Skin	early-forming	0.43
P. breviceps	sg_shd_early-forming_139	Shoulder Skin	early-forming	0.49
C. perspicillata	sstb_pat_3_stg16	Plagiopatagium	Stage16	N/A
C. perspicillata	sstb_pat_5_stg16	Plagiopatagium	Stage16	N/A
C. perspicillata	sstb_pat_2_stg17	Plagiopatagium	Stage17	N/A
C. perspicillata	sstb_pat_4_stg17	Plagiopatagium	Stage17	N/A
C. perspicillata	sstb_pat_1_stg18	Plagiopatagium	Stage18	N/A
C. perspicillata	sstb_dors_stg16_3	Dorsal Skin	Stage16	N/A
C. perspicillata	sstb_dors_stg16_5	Dorsal Skin	Stage16	N/A
C. perspicillata	sstb_dors_stg17_2	Dorsal Skin	Stage17	N/A
C. perspicillata	sstb_dors_stg17_4	Dorsal Skin	Stage17	N/A
C. perspicillata	sstb_dors_stg18_1	Dorsal Skin	Stage18	N/A

Table S4. Gene models belonging to sugar glider patagium module 8.

See attached excel document

Table S5: Summary of GO Biological Process terms enriched among PM8 Genes.

See attached excel document

Table S6: PM8 genes belonging to enriched Wnt-signaling GO term GO:0030111.

See attached excel document

Table S7. Differentially expressed genes between the sugar glider patagium and dorsal skin prior to outgrowth.

See attached excel document

Table S8. Differentially expressed genes between the sugar glider patagium and shoulder skin prior to outgrowth.

See attached excel document

Table S9: Differentially expressed genes between outgrowing patagium and dorsal skin in the sugar glider

See attached excel document

Table S10: Differentially-expressed genes between outgrowing plagiopatagium and dorsal skin in Seba's short-tailed bat.

See attached excel document

Table S11: Summary of GO Biological Process terms enriched among genes upregulated in the sugar glider patagium during its early outgrowth.

See attached excel document

Table S12: Sugar glider patagium-upregulated genes belonging to enriched limb development GO terms.

See attached excel document

Table S13: Summary of GO Biological Process terms enriched among genes upregulated in the bat plagiopatagium.

See attached excel document

Table S14: Gene Ontology term enrichment among genes upregulated during plagiopatagium outgrowth in the bat.

See attached excel document

Table S15. Differentially expressed genes shared between sugar glider and bat lateral patagia during their outgrowth.

Gene ID	Differential Expression
ATRNL1	Upregulated
BARX2	Upregulated
BCL2	Upregulated
CCDC3	Upregulated
CREB5	Upregulated
EPHA3	Upregulated
ETV5	Upregulated
GABRA1	Upregulated
GABRB2	Upregulated
GATA6	Upregulated
GFRA1	Upregulated
GREM1	Upregulated
HAND2	Upregulated
HAS2	Upregulated
HECTD2	Upregulated
HGF	Upregulated
ITGA4	Upregulated
ITGA8	Upregulated
MAN1A1	Upregulated
OSR1	Upregulated
PDE3A	Upregulated
TBX1	Upregulated
TBX3	Upregulated
TBX5	Upregulated
TMEM26	Upregulated
WNT5A	Upregulated
CLDN11	Downregulated
FABP7	Downregulated
FMOD	Downregulated
TENM2	Downregulated

Table S16: Expression status of core AER and hair placode genes in the sugar glider (top) and Seba's short-tailed bat (bottom).

Species	AER Genes	Expressed in Patagium	Differentially Expressed Prior to Outgrowth	Differentially Expressed During Outgrowth
Sugar glider	FGF2	TRUE	UP	UP
	FGF4	FALSE	FALSE	FALSE
	FGF8	FALSE	FALSE	FALSE
	FGF9	TRUE	FALSE	FALSE
	FGF10	TRUE	FALSE	FALSE
	FGF17	FALSE	FALSE	FALSE
	Hair Placode Genes	**Expressed in Patagium**	**Differentially Expressed Prior to Outgrowth**	**Differentially Expressed During Outgrowth**
	FGF20	TRUE	DOWN	DOWN
	EDA	TRUE	FALSE	FALSE
	EDAR	TRUE	FALSE	FALSE
	FGFR1	TRUE	FALSE	FALSE
	FGFR2 (Encodes FGFR2B)	TRUE	FALSE	FALSE
	SHH	FALSE	FALSE	FALSE
	DKK4	TRUE	FALSE	FALSE
	CDH3 (Encodes P-cadherin)	TRUE	FALSE	FALSE
	WNT10B	TRUE	FALSE	DOWN

Species	AER Genes	Expressed in Patagium	Differentially Expressed During Outgrowth
Seba's Short-Tailed Bat	FGF2	TRUE	FALSE
	FGF4	FALSE	FALSE
	FGF8	FALSE	FALSE
	FGF9	TRUE	FALSE
	FGF10	TRUE	UP
	FGF17	FALSE	FALSE
	Hair Placode Genes	**Expressed in Patagium**	**Differentially Expressed During Outgrowth**
	FGF20	FALSE	DOWN
	EDA	TRUE	FALSE
	EDAR	FALSE	FALSE
	FGFR1	TRUE	FALSE
	FGFR2 (Encodes FGFR2B)	TRUE	FALSE
	SHH	FALSE	FALSE
	DKK4	N/A	N/A
	CDH3 (Encodes P-cadherin)	TRUE	FALSE
	WNT10B	TRUE	FALSE

Movie S1. Cell proliferation in sugar glider joeys. Light-sheet microscopy of postnatal day 3 sugar glider joey injected with EdU and collected 2hrs later. Visualization of cell proliferation (white signal) was carried using a modified iDISCO and tissue clearing protocol.