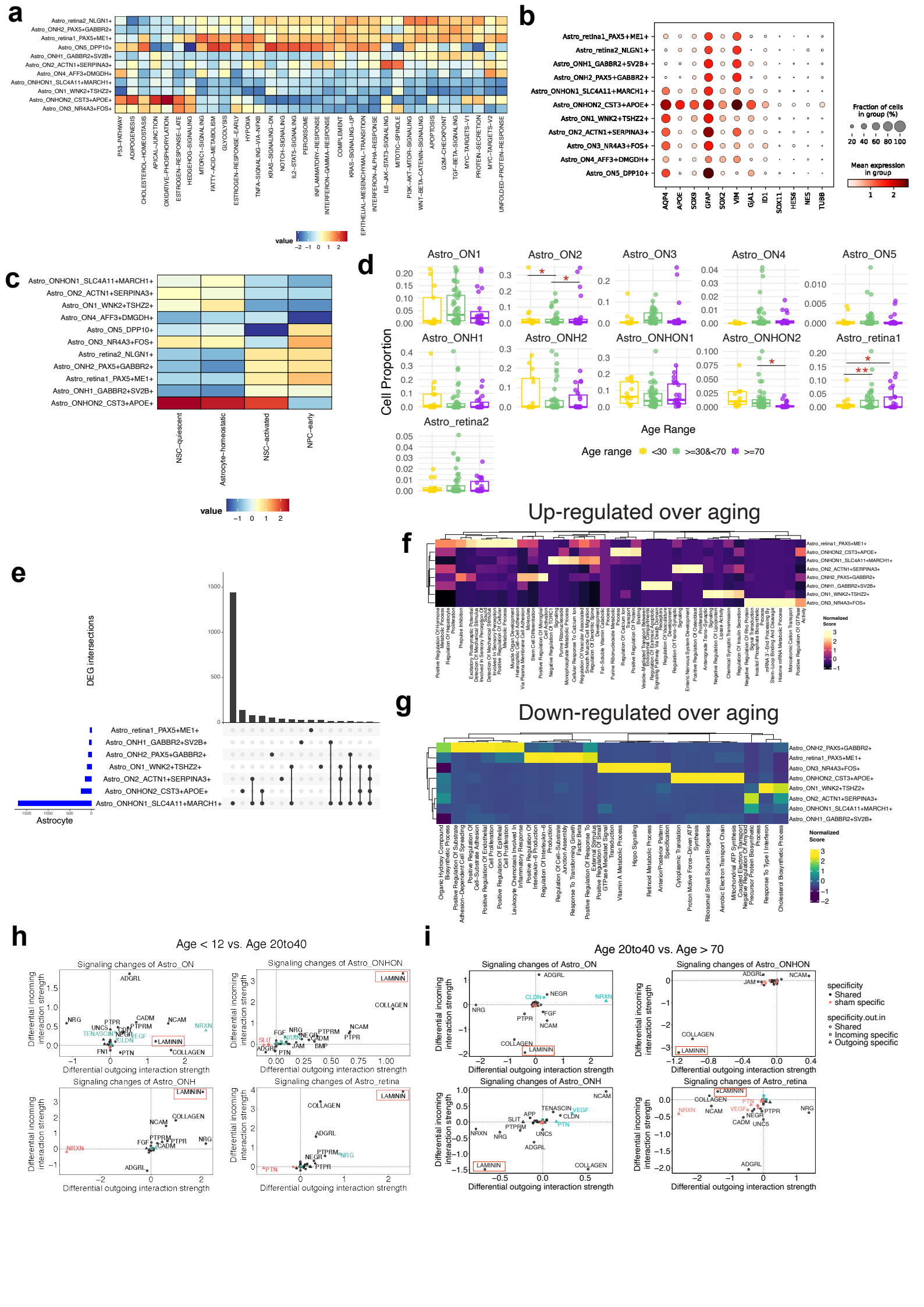
a
Astro_retina2_NLGN1+
Astro_ONH2_PAX5+GABBR2+
Astro_retina1_PAX5+ME1+
Astro_ON5_DPP10+
Astro_ONH1_GABBR2+SV2B+
Astro_ON2_ACTN1+SERPINA3+
Astro_ON4_AFF3+DMGDH+
Astro_ONHON1_SLC4A11+MARCH1+
Astro_ON1_WNK2+TSHZ2+
Astro_ONHON2_CST3+APOE+
Astro_ON3_NR4A3+FOS+
P53_PATHWAY
ADIPOGENESIS
CHOLESTEROL_HOMEOSTASIS
APICAL_JUNCTION
OXIDATIVE_PHOSPHORYLATION
ESTROGEN_RESPONSE_LATE
HEDGEHOG_SIGNALING
MTORC1_SIGNALING
FATTY_ACID_METABOLISM
GLYCOLYSIS
ESTROGEN_RESPONSE_EARLY
HYPOXIA
TNFA_SIGNALING_VIA_NFKB
KRAS_SIGNALING_DN
NOTCH_SIGNALING
IL2_STAT5_SIGNALING
PEROXISOME
INFLAMMATORY_RESPONSE
INTERFERON_GAMMA_RESPONSE
COMPLEMENT
KRAS_SIGNALING_UP
EPITHELIAL_MESENCHYMAL_TRANSITION
INTERFERON_ALPHA_RESPONSE
IL6_JAK_STAT3_SIGNALING
MITOTIC_SPINDLE
PI3K_AKT_MTOR_SIGNALING
WNT_BETA_CATENIN_SIGNALING
APOPTOSIS
G2M_CHECKPOINT
TGF_BETA_SIGNALING
MYC_TARGETS_V1
PROTEIN_SECRETION
MYC_TARGETS_V2
UNFOLDED_PROTEIN_RESPONSE
value
−2 −1 0 1 2
b
Astro_retina1_PAX5+ME1+
Astro_retina2_NLGN1+
Astro_ONH1_GABBR2+SV2B+
Astro_ONH2_PAX5+GABBR2+
Astro_ONHON1_SLC4A11+MARCH1+
Astro_ONHON2_CST3+APOE+
Astro_ON1_WNK2+TSHZ2+
Astro_ON2_ACTN1+SERPINA3+
Astro_ON3_NR4A3+FOS+
Astro_ON4_AFF3+DMGDH+
Astro_ON5_DPP10+
AQP4
APOE
SOX9
GFAP
SOX2
VIM
GJA1
ID1
SOX11
HES6
NES
TUBB
Fraction of cells in group (%)
20 40 60 80 100
Mean expression in group
1 2
c
Astro_ONHON1_SLC4A11+MARCH1+
Astro_ON2_ACTN1+SERPINA3+
Astro_ON1_WNK2+TSHZ2+
Astro_ON4_AFF3+DMGDH+
Astro_ON5_DPP10+
Astro_ON3_NR4A3+FOS+
Astro_retina2_NLGN1+
Astro_ONH2_PAX5+GABBR2+
Astro_retina1_PAX5+ME1+
Astro_ONH1_GABBR2+SV2B+
Astro_ONHON2_CST3+APOE+
NSC–quiescent
Astrocyte–homeostatic
NSC–activated
NPC–early
value
−1 0 1 2
d
Astro_ON1
Astro_ON2
Astro_ON3
Astro_ON4
Astro_ON5
Astro_ONH1
Astro_ONH2
Astro_ONHON1
Astro_ONHON2
Astro_retina1
Astro_retina2
Cell Proportion
Age Range
Age range <30 >=30&<70 >=70
e
DEG intersections
Astro_retina1_PAX5+ME1+
Astro_ONH1_GABBR2+SV2B+
Astro_ONH2_PAX5+GABBR2+
Astro_ON1_WNK2+TSHZ2+
Astro_ON2_ACTN1+SERPINA3+
Astro_ONHON2_CST3+APOE+
Astro_ONHON1_SLC4A11+MARCH1+
Astrocyte
f
Up-regulated over aging
Astro_retina1_PAX5+ME1+
Astro_ONHON2_CST3+APOE+
Astro_ONHON1_SLC4A11+MARCH1+
Astro_ON2_ACTN1+SERPINA3+
Astro_ONH2_PAX5+GABBR2+
Astro_ONH1_GABBR2+SV2B+
Astro_ON1_WNK2+TSHZ2+
Astro_ON3_NR4A3+FOS+
Normalized Score
g
Down-regulated over aging
Astro_ONH2_PAX5+GABBR2+
Astro_retina1_PAX5+ME1+
Astro_ON3_NR4A3+FOS+
Astro_ONHON2_CST3+APOE+
Astro_ON1_WNK2+TSHZ2+
Astro_ON2_ACTN1+SERPINA3+
Astro_ONHON1_SLC4A11+MARCH1+
Astro_ONH1_GABBR2+SV2B+
Normalized Score
h
Age < 12 vs. Age 20to40
Signaling changes of Astro_ON
Signaling changes of Astro_ONHON
Signaling changes of Astro_ONH
Signaling changes of Astro_retina
Differential incoming interaction strength
Differential outgoing interaction strength
i
Age 20to40 vs. Age > 70
Signaling changes of Astro_ON
Signaling changes of Astro_ONHON
Signaling changes of Astro_ONH
Signaling changes of Astro_retina
Differential incoming interaction strength
Differential outgoing interaction strength
specificity
Shared
sham specific
specificity.out.in
Shared
Incoming specific
Outgoing specific

Figure 6. Functional state and age-related changes of Astrocytes
a, Heatmap showing the biological processes and pathways enriched in each astrocyte population. **b,** Dot plot showing the expression of genes associated with stemness across astrocyte populations. **c.** Heatmap showing the enrichment of gene modules associated with neuron stem cell states across astrocyte populations. **d**, Box plot showing cell proportions across three age ranges for each astrocyte population. **e**, UpSet plot showing the number of age-related differentially expressed genes (DEG) identified in each astrocyte population. **f**, Heatmap showing the biological processed enriched among DEGs up-regulated with age. **g**, Heatmap showing the biological processed enriched among DEGs down-regulated with aging. **h,** Scatter plot showing cell-cell communication signaling changes in region-specific astrocyte subclasses in ages 20-40 compared with ages < 20 years. **i,** Scatter plot showing cell-cell communication signaling changes in region-specific astrocyte subclasses in ages >70 compared with ages 20-40 years.