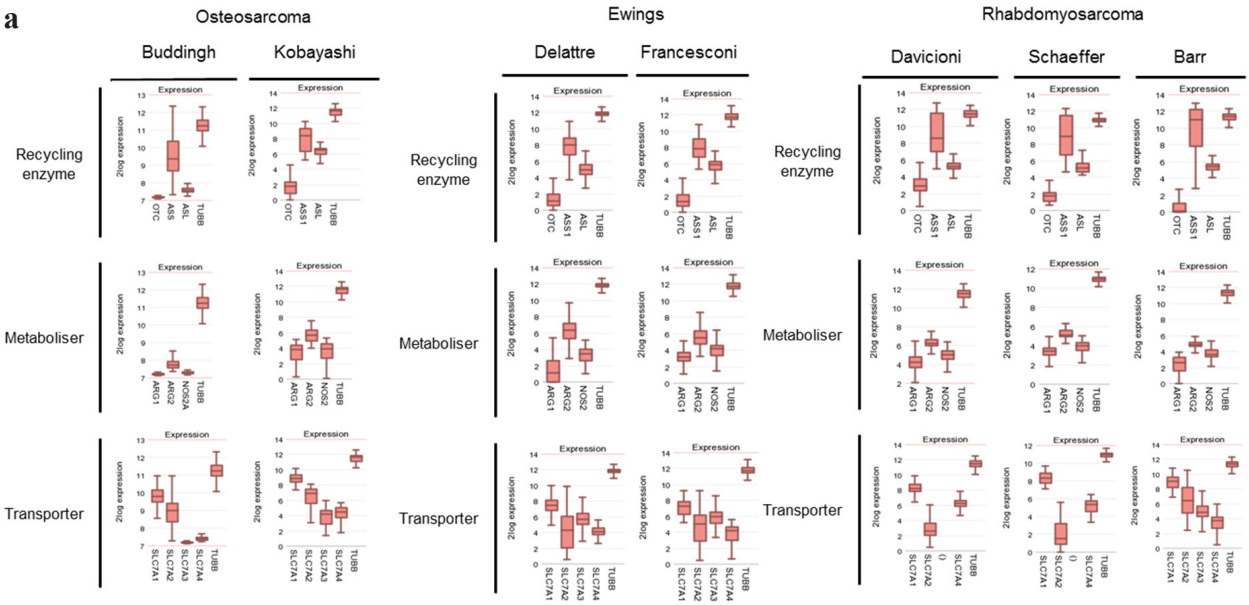
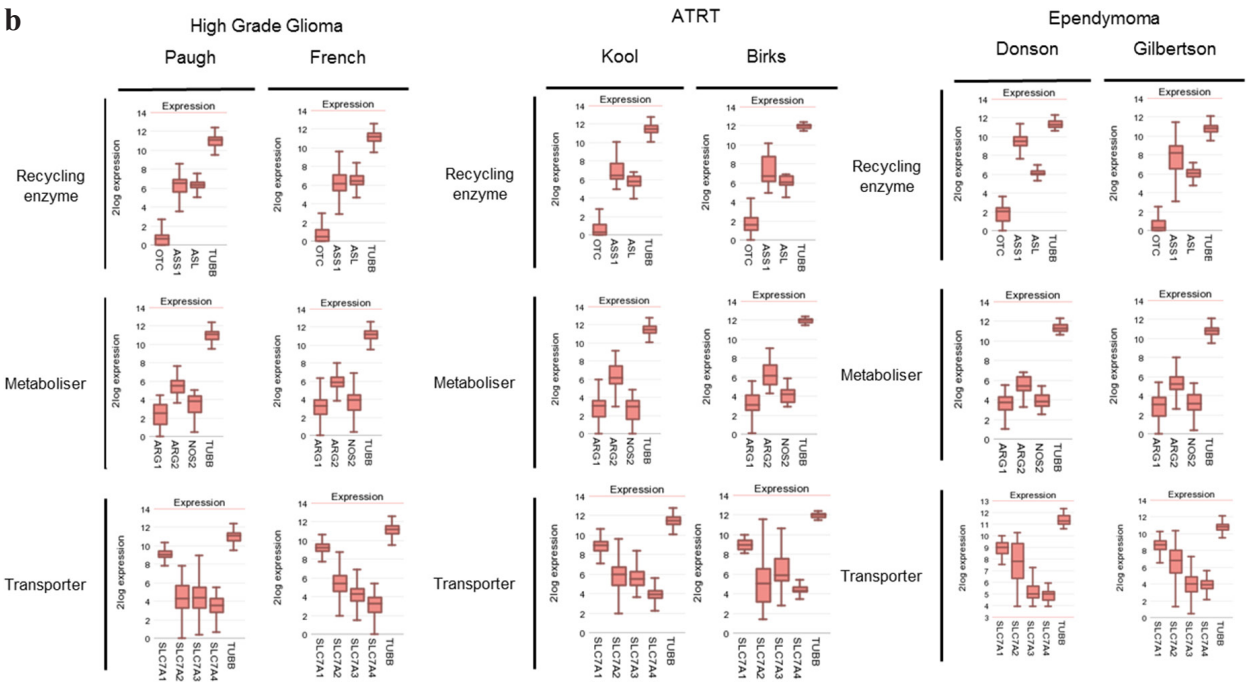
Arginine auxotrophic gene signature in paediatric sarcomas and brain tumours provides a viable target for arginine depletion therapies

SUPPLEMENTARY MATERIALS





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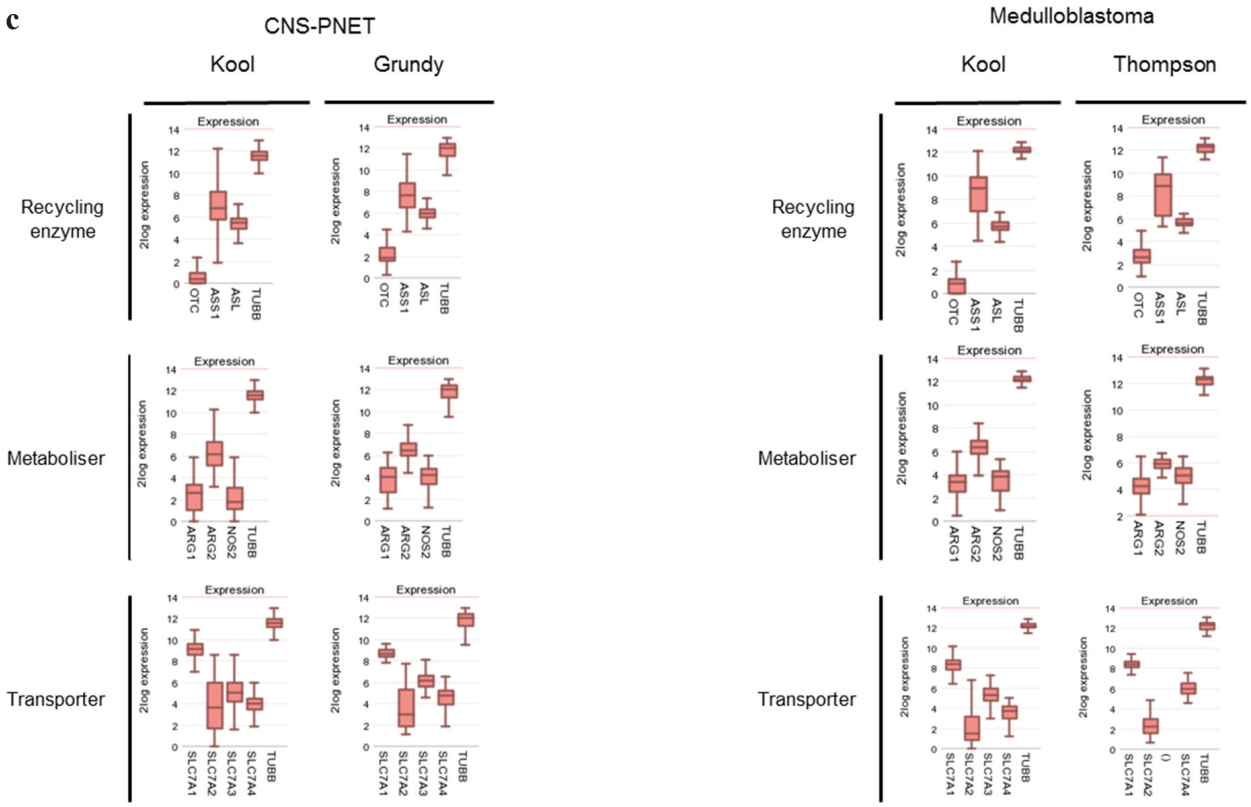
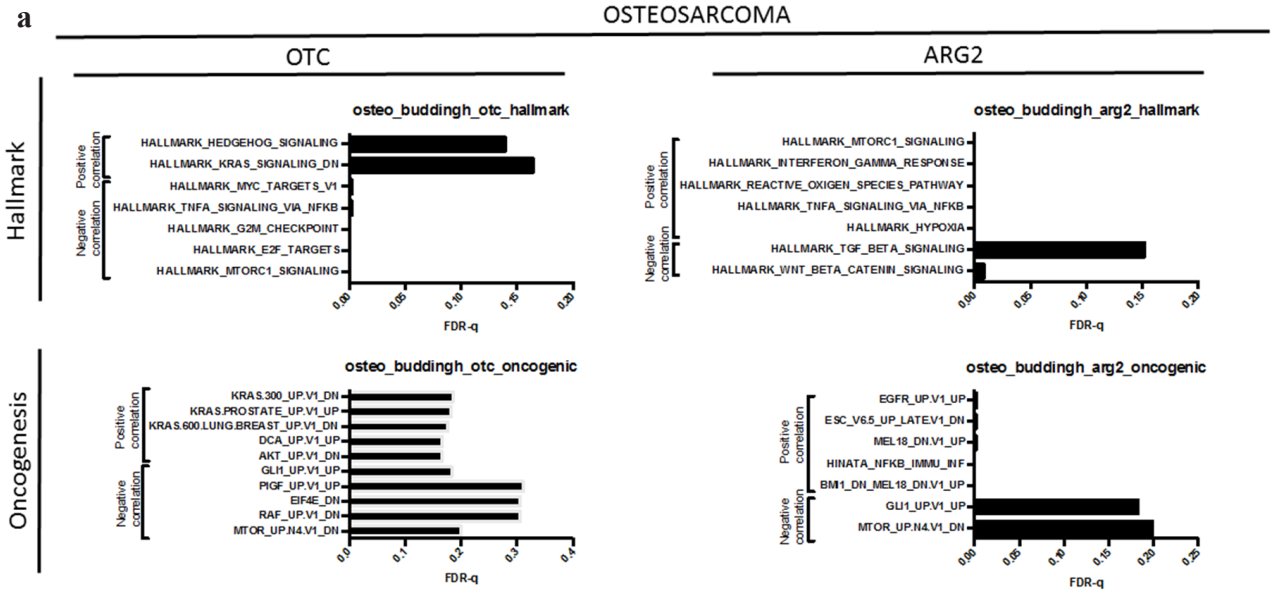
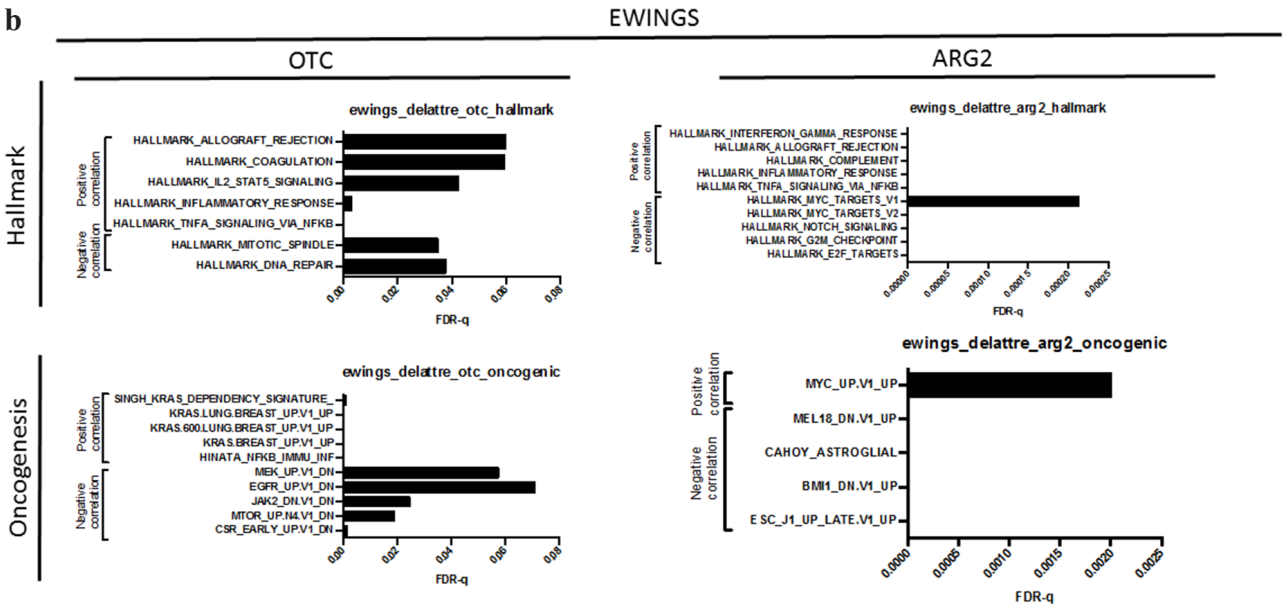


Supplementary Figure 1 (*Continued*): Validation analysis showing arginine auxotrophic gene signature in paediatric tumours. Gene expression profiles of amino acid transporters, arginine catabolic enzymes, and arginine recycling enzymes, compared to Tubulin control for **(a)** sarcomas, and brain tumours **(b** and **c)**.


a
OSTEOSARCOMA
OTC
ARG2
Hallmark
Oncogenesis
osteo_buddingh_otc_hallmark
HALLMARK_HEDGEHOG_SIGNALING
HALLMARK_KRAS_SIGNALING_DN
HALLMARK_MYC_TARGETS_V1
HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_G2M_CHECKPOINT
HALLMARK_E2F_TARGETS
HALLMARK_MTORC1_SIGNALING
Positive correlation
Negative correlation
FDR-q
osteo_buddingh_arg2_hallmark
HALLMARK_MTORC1_SIGNALING
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY
HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_HYPOXIA
HALLMARK_TGF_BETA_SIGNALING
HALLMARK_WNT_BETA_CATENIN_SIGNALING
osteo_buddingh_otc_oncogenic
KRAS.300_UP.V1_DN
KRAS.PROSTATE_UP.V1_UP
KRAS.600.LUNG.BREAST_UP.V1_DN
DCA_UP.V1_UP
AKT_UP.V1_DN
GLI1_UP.V1_UP
PIGF_UP.V1_UP
EIF4E_DN
RAF_UP.V1_DN
MTOR_UP.N4.V1_DN
osteo_buddingh_arg2_oncogenic
EGFR_UP.V1_UP
ESC_V6.5_UP_LATE.V1_DN
MEL18_DN.V1_UP
HINATA_NFKB_IMMU_INF
BMI1_DN_MEL18_DN.V1_UP
GLI1_UP.V1_UP
MTOR_UP.N4.V1_DN


b
EWINGS
OTC
ARG2
Hallmark
Oncogenesis
ewings_delattre_otc_hallmark
HALLMARK_ALLOGRAFT_REJECTION
HALLMARK_COAGULATION
HALLMARK_IL2_STAT5_SIGNALING
HALLMARK_INFLAMMATORY_RESPONSE
HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_MITOTIC_SPINDLE
HALLMARK_DNA_REPAIR
Positive correlation
Negative correlation
FDR-q
ewings_delattre_arg2_hallmark
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_ALLOGRAFT_REJECTION
HALLMARK_COMPLEMENT
HALLMARK_INFLAMMATORY_RESPONSE
HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_MYC_TARGETS_V1
HALLMARK_MYC_TARGETS_V2
HALLMARK_NOTCH_SIGNALING
HALLMARK_G2M_CHECKPOINT
HALLMARK_E2F_TARGETS
ewings_delattre_otc_oncogenic
SINGH_KRAS_DEPENDENCY_SIGNATURE_
KRAS.LUNG.BREAST_UP.V1_UP
KRAS.600.LUNG.BREAST_UP.V1_UP
KRAS.BREAST_UP.V1_UP
HINATA_NFKB_IMMU_INF
MEK_UP.V1_DN
EGFR_UP.V1_DN
JAK2_DN.V1_DN
MTOR_UP.N4.V1_DN
CSR_EARLY_UP.V1_DN
ewings_delattre_arg2_oncogenic
MYC_UP.V1_UP
MEL18_DN.V1_UP
CAHOY_ASTROGLIAL
BMI1_DN.V1_UP
ESC_J1_UP_LATE.V1_UP

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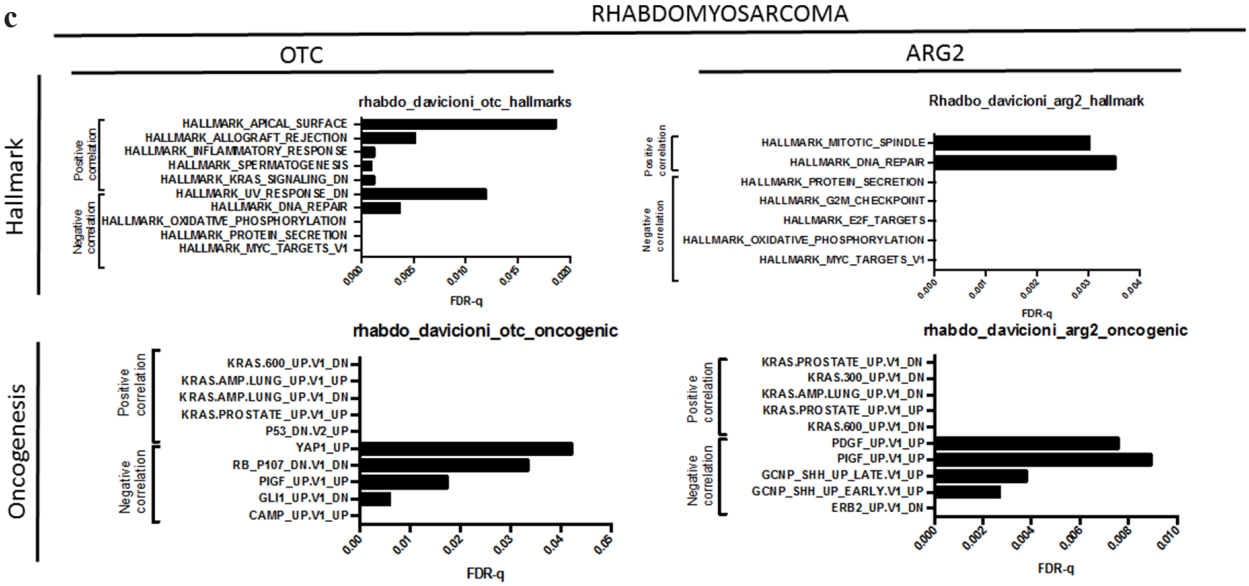
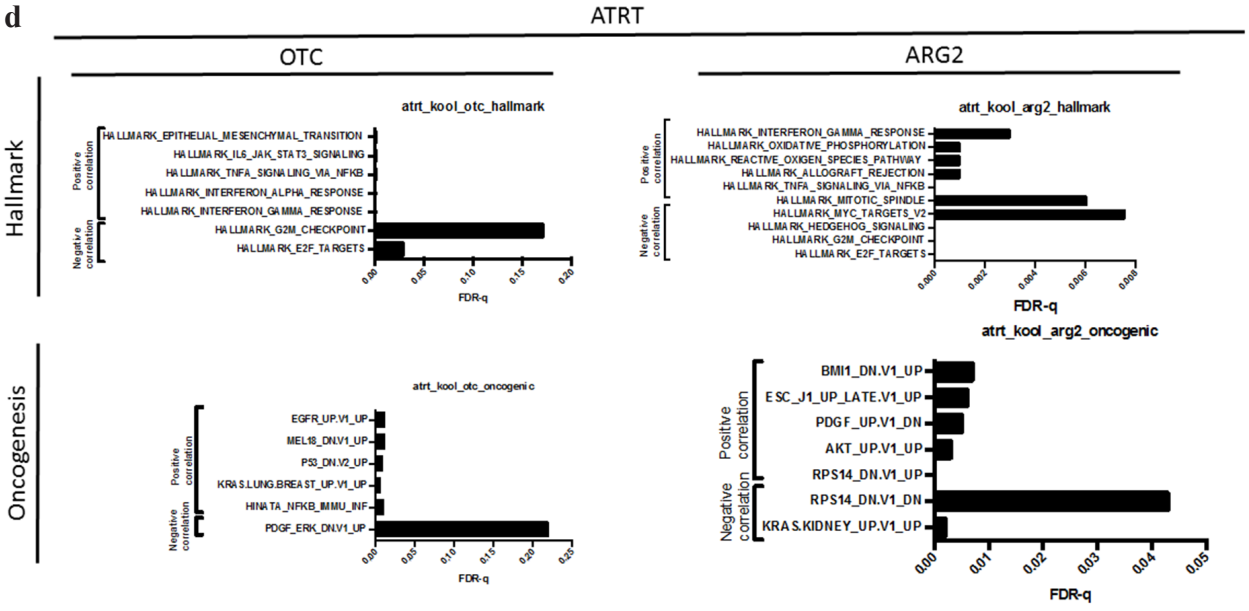
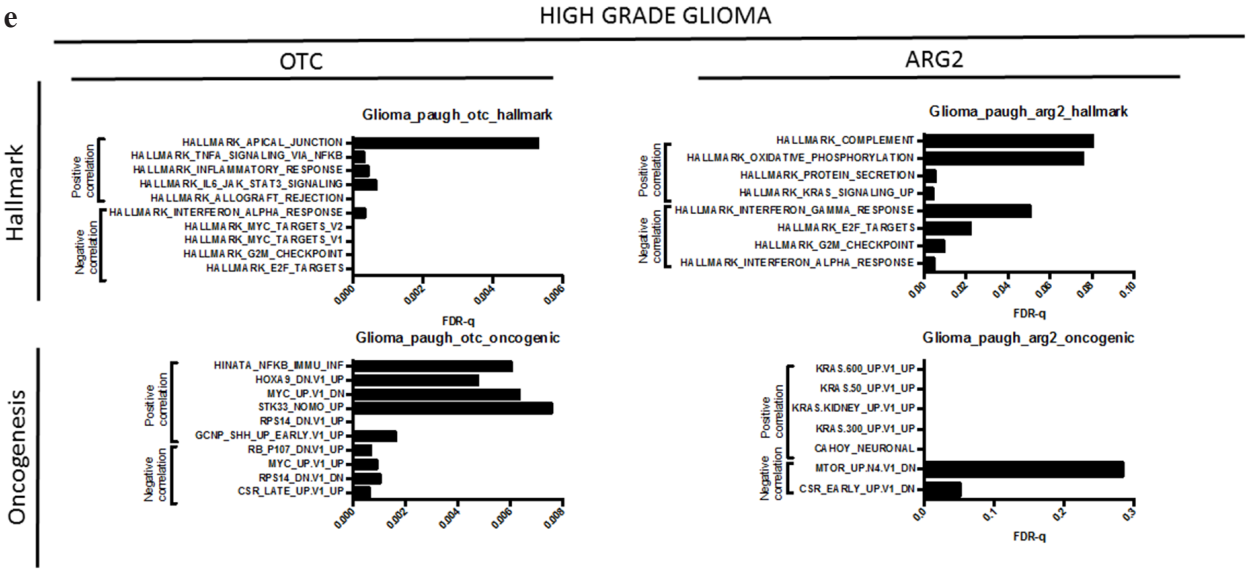

c
RHABDOMYOSARCOMA
OTC
ARG2
Hallmark
Oncogenesis
rhabdo_davicioni_otc_hallmarks
HALLMARK_APICAL_SURFACE
HALLMARK_ALLOGRAFT_REJECTION
HALLMARK_INFLAMMATORY_RESPONSE
HALLMARK_SPERMATOGENESIS
HALLMARK_KRAS_SIGNALING_DN
HALLMARK_UV_RESPONSE_DN
HALLMARK_DNA_REPAIR
HALLMARK_OXIDATIVE_PHOSPHORYLATION
HALLMARK_PROTEIN_SECRETION
HALLMARK_MYC_TARGETS_V1
Positive correlation
Negative correlation
FDR-q
Rhadbo_davicioni_arg2_hallmark
HALLMARK_MITOTIC_SPINDLE
HALLMARK_DNA_REPAIR
HALLMARK_PROTEIN_SECRETION
HALLMARK_G2M_CHECKPOINT
HALLMARK_E2F_TARGETS
HALLMARK_OXIDATIVE_PHOSPHORYLATION
HALLMARK_MYC_TARGETS_V1
rhabdo_davicioni_otc_oncogenic
KRAS.600_UP.V1_DN
KRAS.AMP.LUNG_UP.V1_UP
KRAS.AMP.LUNG_UP.V1_DN
KRAS.PROSTATE_UP.V1_UP
P53_DN.V2_UP
YAP1_UP
RB_P107_DN.V1_DN
PIGF_UP.V1_UP
GLI1_UP.V1_DN
CAMP_UP.V1_UP
rhabdo_davicioni_arg2_oncogenic
KRAS.PROSTATE_UP.V1_DN
KRAS.300_UP.V1_DN
KRAS.AMP.LUNG_UP.V1_DN
KRAS.PROSTATE_UP.V1_UP
KRAS.600_UP.V1_DN
PDGF_UP.V1_UP
PIGF_UP.V1_UP
GCNP_SHH_UP_LATE.V1_UP
GCNP_SHH_UP_EARLY.V1_UP
ERB2_UP.V1_DN


d
ATRT
OTC
ARG2
Hallmark
Oncogenesis
atrt_kool_otc_hallmark
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
HALLMARK_IL6_JAK_STAT3_SIGNALING
HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_INTERFERON_ALPHA_RESPONSE
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_G2M_CHECKPOINT
HALLMARK_E2F_TARGETS
Positive correlation
Negative correlation
FDR-q
atrt_kool_arg2_hallmark
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_OXIDATIVE_PHOSPHORYLATION
HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY
HALLMARK_ALLOGRAFT_REJECTION
HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_MITOTIC_SPINDLE
HALLMARK_MYC_TARGETS_V2
HALLMARK_HEDGEHOG_SIGNALING
HALLMARK_G2M_CHECKPOINT
HALLMARK_E2F_TARGETS
atrt_kool_otc_oncogenic
EGFR_UP.V1_UP
MEL18_DN.V1_UP
P53_DN.V2_UP
KRAS.LUNG.BREAST_UP.V1_UP
HINATA_NFKB_IMMU_INF
PDGF_ERK_DN.V1_UP
atrt_kool_arg2_oncogenic
BMI1_DN.V1_UP
ESC_J1_UP_LATE.V1_UP
PDGF_UP.V1_UP
AKT_UP.V1_UP
RPS14_DN.V1_UP
RPS14_DN.V1_DN
KRAS.KIDNEY_UP.V1_UP

(*Continued*)

e



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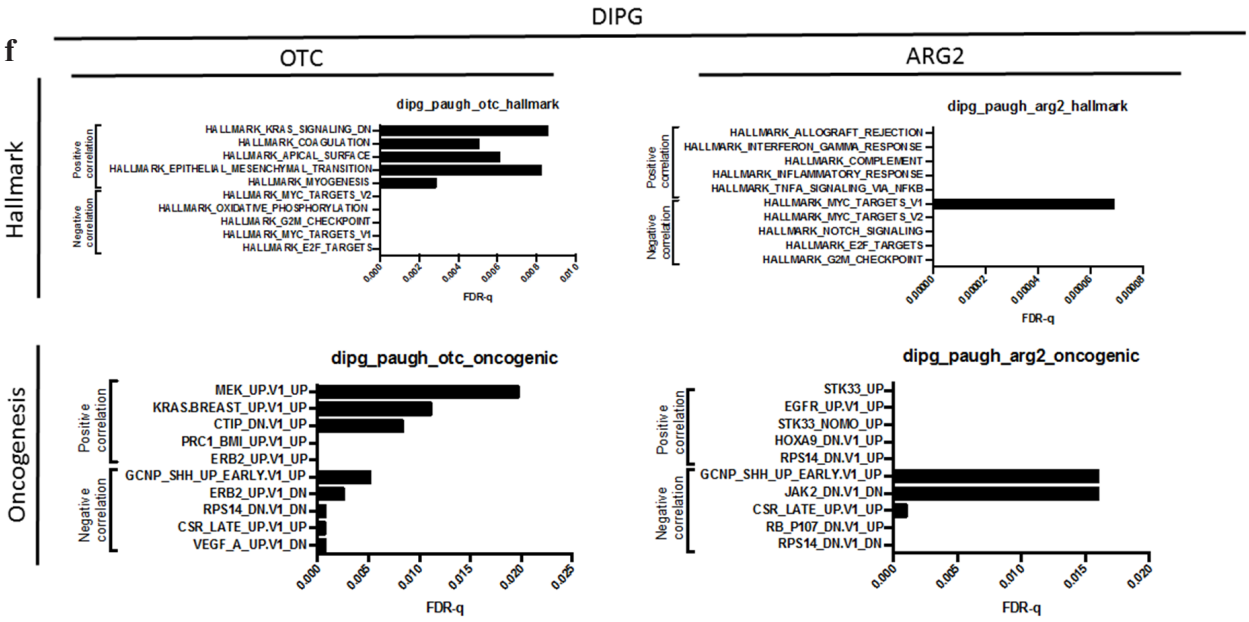

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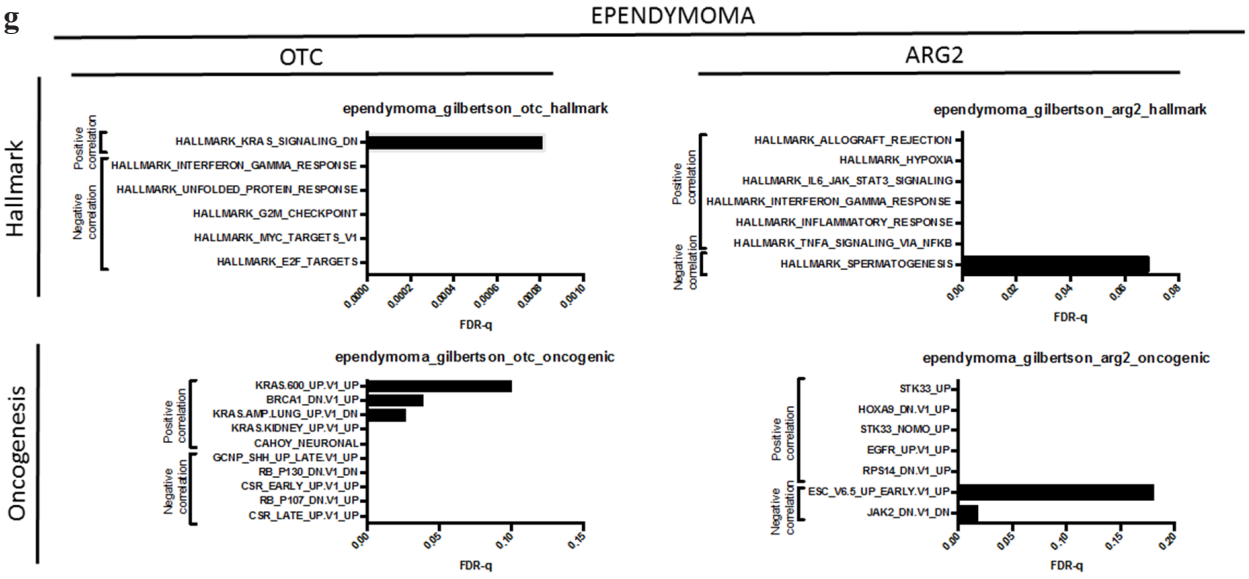
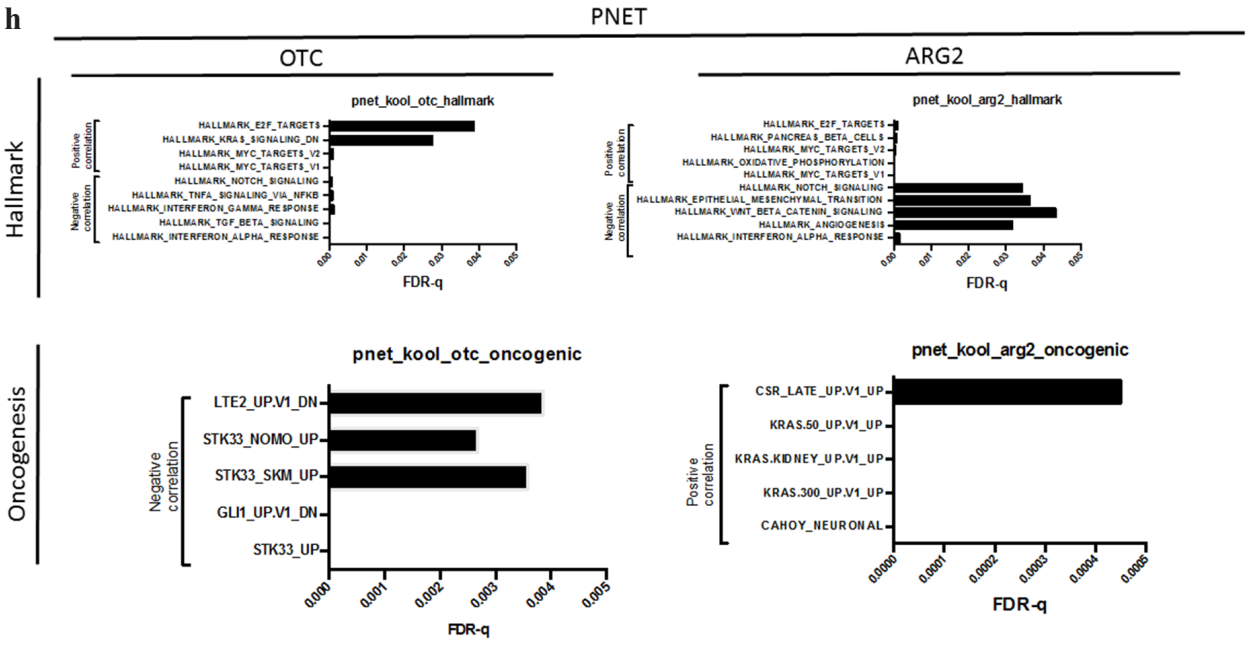
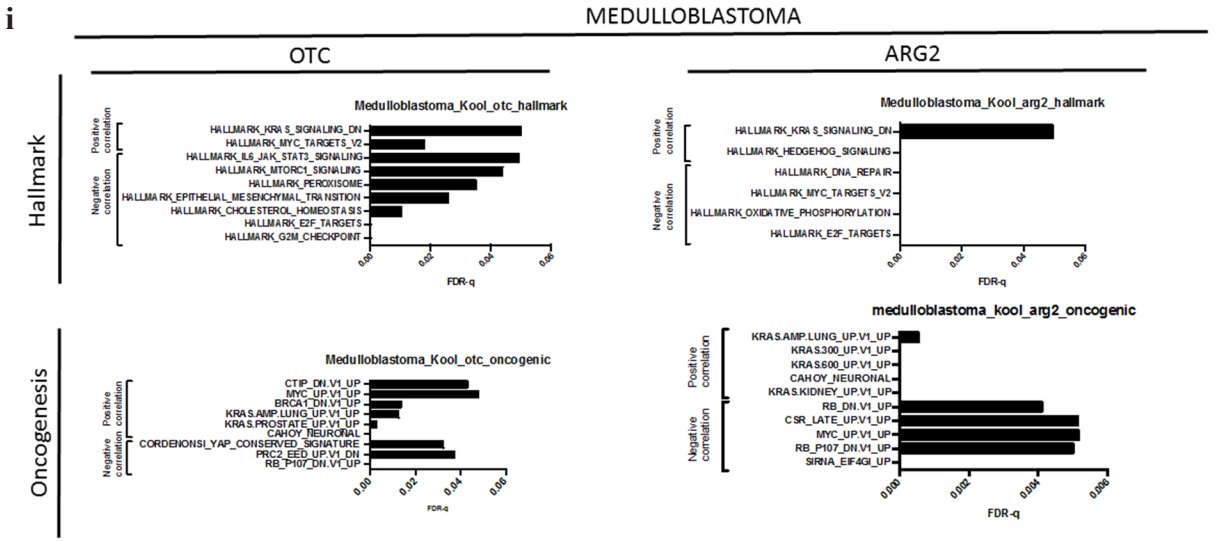
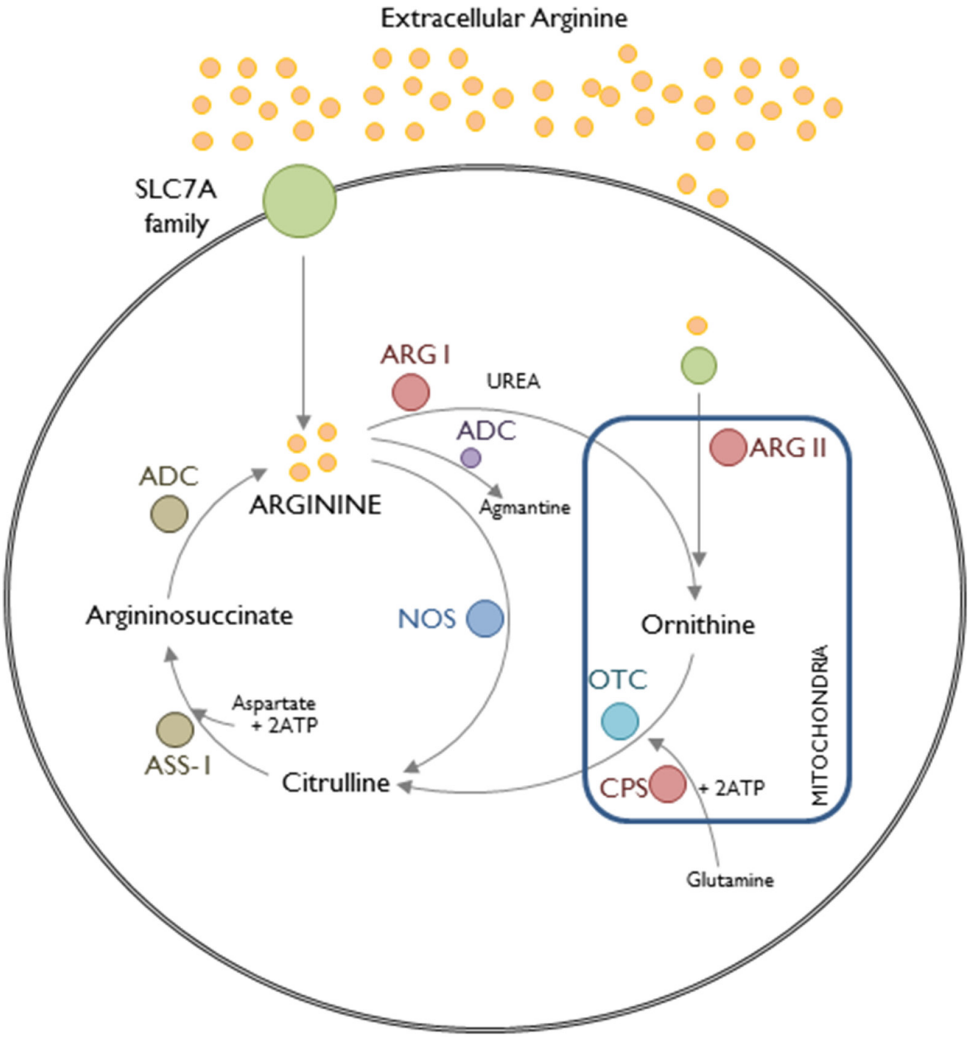

g
EPENDYMOMA
OTC
ARG2
Hallmark
Oncogenesis
ependymoma_gilbertson_otc_hallmark
HALLMARK_KRAS_SIGNALING_DN
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_UNFOLDED_PROTEIN_RESPONSE
HALLMARK_G2M_CHECKPOINT
HALLMARK_MYC_TARGETS_V1
HALLMARK_E2F_TARGETS
Positive correlation
Negative correlation
FDR-q
ependymoma_gilbertson_arg2_hallmark
HALLMARK_ALLOGRAFT_REJECTION
HALLMARK_HYPOXIA
HALLMARK_IL6_JAK_STAT3_SIGNALING
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_INFLAMMATORY_RESPONSE
HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_SPERMATOGENESIS
ependymoma_gilbertson_otc_oncogenic
KRAS.600_UP.V1_UP
BRCA1_DN.V1_UP
KRAS.AMP.LUNG_UP.V1_DN
KRAS.KIDNEY_UP.V1_UP
CAHOY_NEURONAL
GCNP_SHH_UP_LATE.V1_UP
RB_P130_DN.V1_DN
CSR_EARLY_UP.V1_UP
RB_P107_DN.V1_UP
CSR_LATE_UP.V1_UP
ependymoma_gilbertson_arg2_oncogenic
STK33_UP
HOXA9_DN.V1_UP
STK33_NOMO_UP
EGFR_UP.V1_UP
RPS14_DN.V1_UP
ESC_V6.5_UP_EARLY.V1_UP
JAK2_DN.V1_DN


h
PNET
OTC
ARG2
Hallmark
Oncogenesis
pnet_kool_otc_hallmark
HALLMARK_E2F_TARGETS
HALLMARK_KRAS_SIGNALING_DN
HALLMARK_MYC_TARGETS_V2
HALLMARK_MYC_TARGETS_V1
HALLMARK_NOTCH_SIGNALING
HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_TGF_BETA_SIGNALING
HALLMARK_INTERFERON_ALPHA_RESPONSE
Positive correlation
Negative correlation
FDR-q
pnet_kool_arg2_hallmark
HALLMARK_E2F_TARGETS
HALLMARK_PANCREAS_BETA_CELLS
HALLMARK_MYC_TARGETS_V2
HALLMARK_OXIDATIVE_PHOSPHORYLATION
HALLMARK_MYC_TARGETS_V1
HALLMARK_NOTCH_SIGNALING
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
HALLMARK_WNT_BETA_CATENIN_SIGNALING
HALLMARK_ANGIOGENESIS
HALLMARK_INTERFERON_ALPHA_RESPONSE
pnet_kool_otc_oncogenic
LTE2_UP.V1_DN
STK33_NOMO_UP
STK33_SKM_UP
GLI1_UP.V1_DN
STK33_UP
pnet_kool_arg2_oncogenic
CSR_LATE_UP.V1_UP
KRAS.50_UP.V1_UP
KRAS.KIDNEY_UP.V1_UP
KRAS.300_UP.V1_UP
CAHOY_NEURONAL

(*Continued*)



Supplementary Figure 2 (*Continued*): GESA analysis. GSEA figures showing hallmark or oncogenic signatures for OTC and ARG2, in each tumour type **(a-i)**. FDR-q values plotted. 0.00 represents a highly significant correlation.



Supplementary Figure 3: Arginine metabolic pathway in cancer cells. Arginine is imported via the SLC7A family of membrane transporters. Intracellular arginine is metabolized by the enzymes Arginase I (ARGI) in the cytoplasm and/or Arginase II (ARGII) in the mitochondria, converting arginine to urea and ornithine. Ornithine transcarbamylase (OTC) converts ornithine to citrulline. Additional citrulline is produced via the activity of nitric oxide synthases (NOS) on arginine. Cells expressing the enzyme arginosuccinate synthase (ASS-1) and arginosuccinate lyase (ASL) resynthesize arginine from citrulline, to complete the cycle.