Apolipoprotein E2 Stimulates Protein Synthesis and Promotes Melanoma Progression

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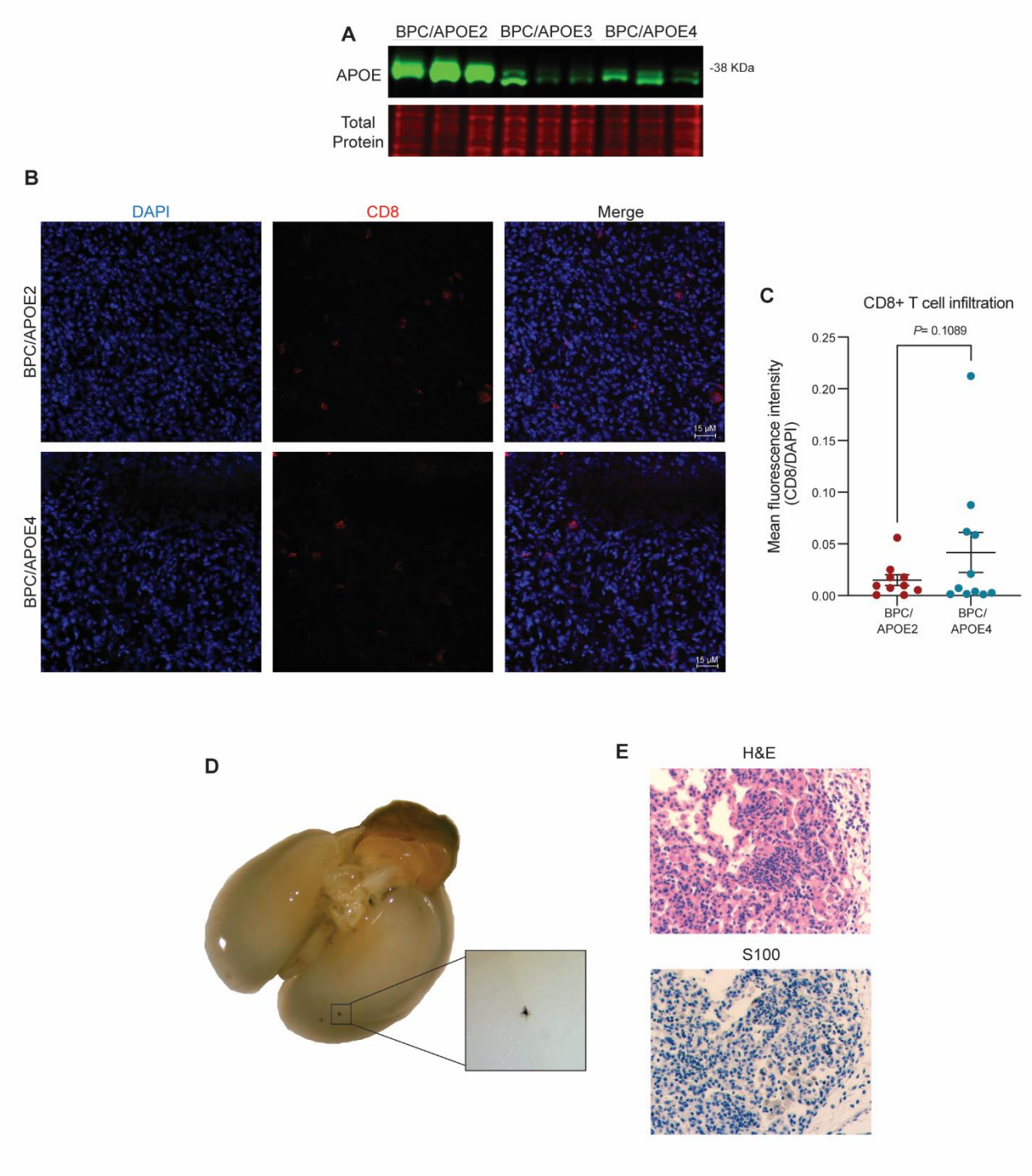
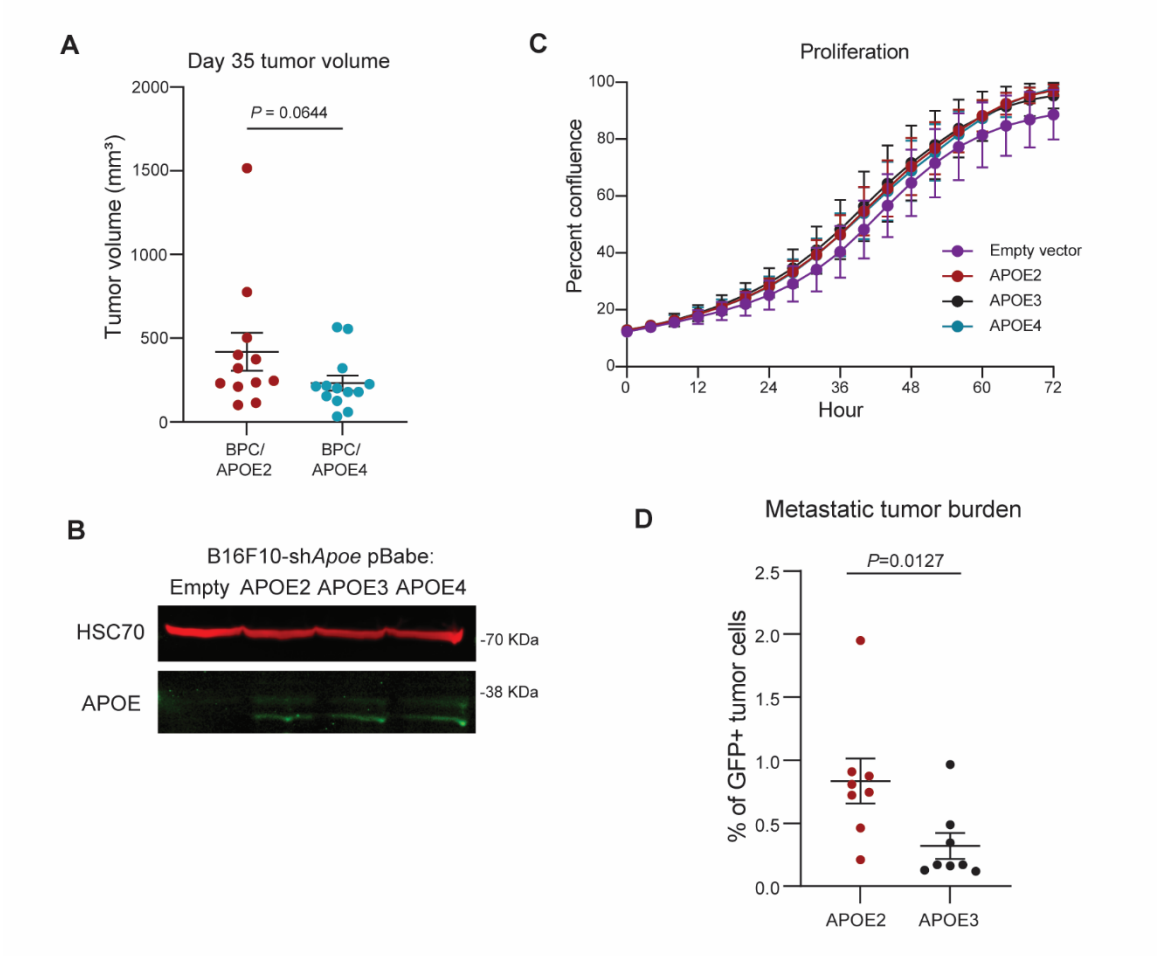
Supplementary Figures S1-S5



Supplementary Figure 1. Characterization of *APOE* allelic GEMM

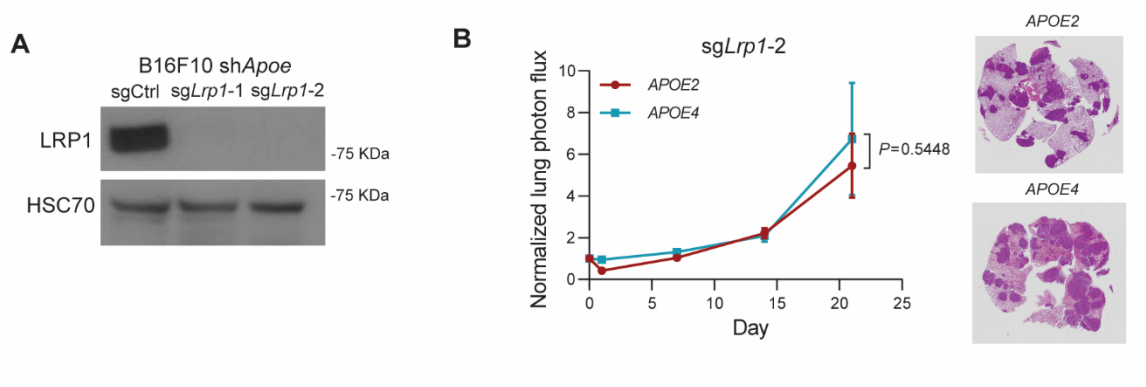
A, Western blot of APOE expression in BPC/APOE2, BPC/APOE3, and BPC/APOE4 tumors (n=3 per group). **B,** Representative immunofluorescence images of CD8 and DAPI nuclear staining in BPC/APOE2 and BPC/APOE4 primary tumors (scale bar = 15 μm). **C,** Quantification of CD8+ T cell infiltration into BPC/APOE2 (n=10) and BPC/APOE4 (n=11) tumors at the day 49 endpoint, normalized to DAPI. Unpaired t-test. **D,** Representative high-

magnification image of a pigmented lung metastasis after neonatal tumor induction. **E,** Representative H&E (top) and S100 (bottom) staining of a lung metastatic focus after neonatal tumor induction.



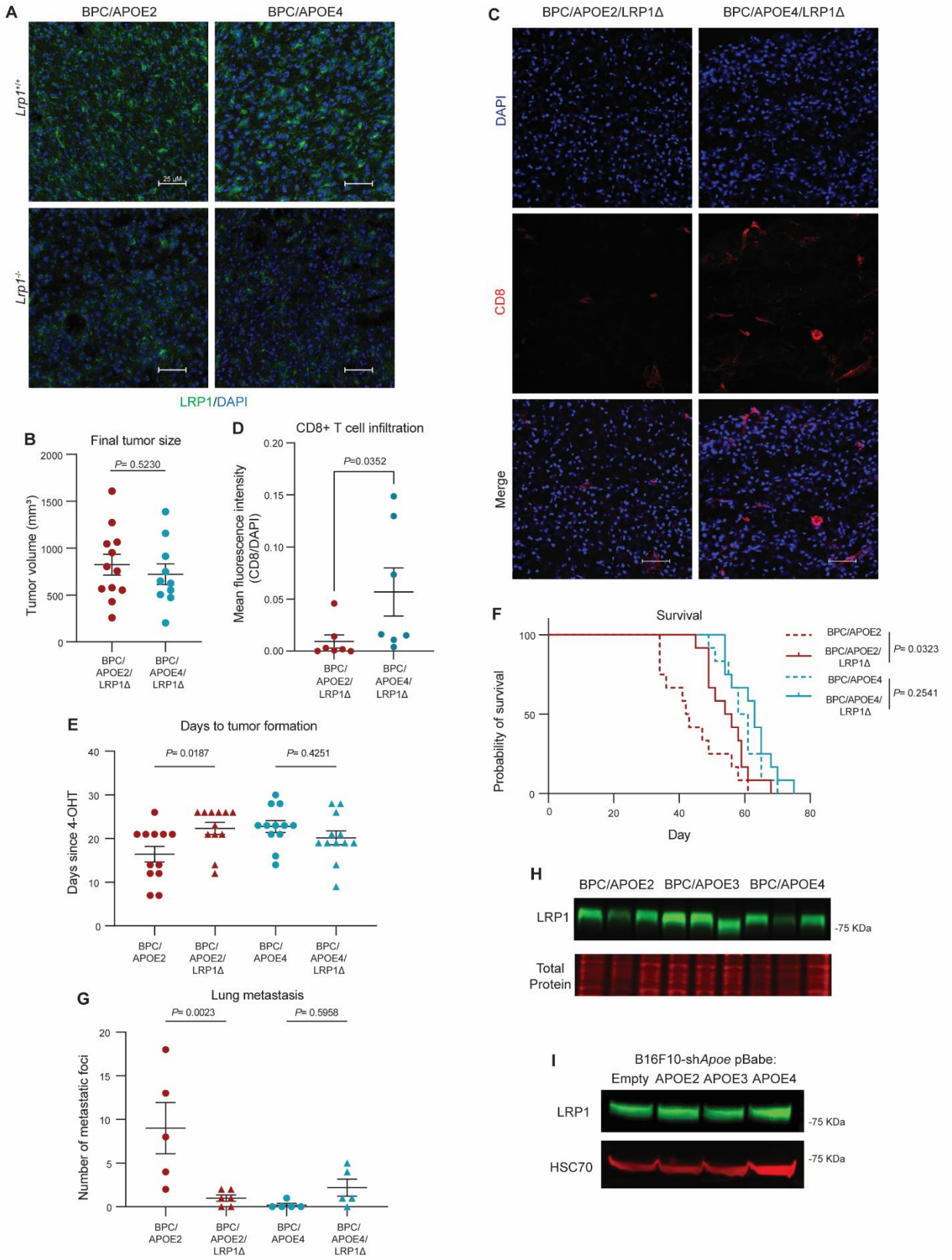
Supplementary Figure 2. *APOE2* genotype promotes translation in melanoma

A, Tumor volumes of BPC/APOE2 (n=12) and BPC/APOE4 (n=13) mice 35 days after 4-OHT administration. Unpaired t-test. **B,** Western blot of APOE expression in B16F10-sh*Apoe* cells transduced with pBabe Empty, APOE2, APOE3, or APOE4 retrovirus. HSC70 served as a loading control. **C,** Proliferation under normal growth conditions of B16F10-sh*Apoe* cells transduced with pBabe Empty, APOE2, APOE3, or APOE4 retrovirus (n=3 independent experiments). **D**, Abundance of GFP^+ B16F10 tumor cells present in the total cell population derived from dissociated lung tissue of *APOE2* (n=8) and *APOE3* (n=8) knock-in mice (unpaired t-test).



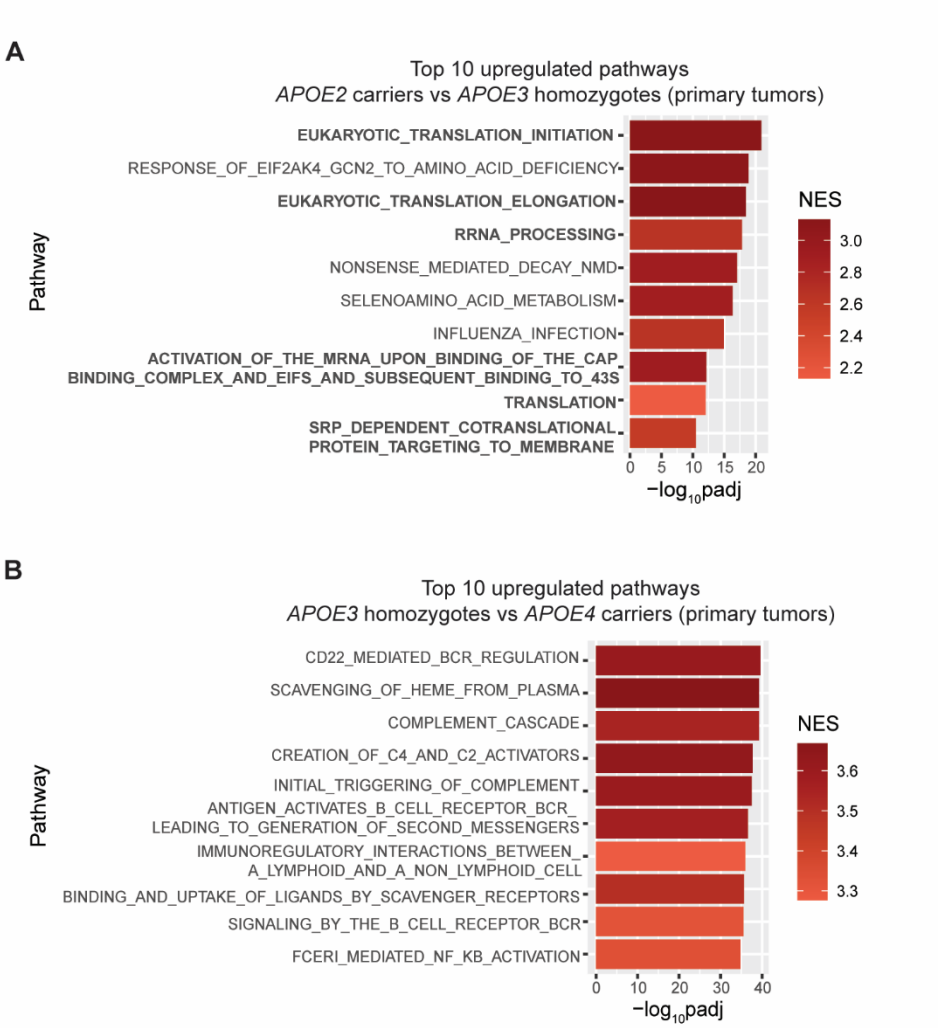
Supplementary Figure 3. Tumoral *Lrp1* deletion abrogates *APOE* genotype-dependent differences in metastatic colonization

A, Western blot of LRP1 expression in B16F10-TR-sh*Apoe* cells transfected with a non-targeting control CRISPR single guide RNA (sgRNA) or two independent *Lrp1*-targeting sgRNAs. HSC70 served as a loading control. **B,** Quantification of lung metastatic progression via bioluminescence imaging of B16F10-TR-sh*Apoe* sg*Lrp1*-2 cells injected via lateral tail vein into *APOE2* and *APOE4* mice. Representative images of H&E-stained lungs taken from mice at the day 21 endpoint (n =8-10 mice per group; representative of two independent experiments; two-way ANOVA).


A
BPC/APOE2
BPC/APOE4
Lrp1+/+
Lrp1-/-
25 uM
LRP1/DAPI
B
Final tumor size
P= 0.5230
Tumor volume (mm3)
2000
1500
1000
500
0
BPC/ APOE2/ LRP1Δ
BPC/ APOE4/ LRP1Δ
D
CD8+ T cell infiltration
P=0.0352
Mean fluorescence intensity (CD8/DAPI)
0.20
0.15
0.10
0.05
0.00
BPC/ APOE2/ LRP1Δ
BPC/ APOE4/ LRP1Δ
E
Days to tumor formation
P= 0.0187
P= 0.4251
Days since 4-OHT
40
30
20
10
0
BPC/ APOE2
BPC/ APOE2/ LRP1Δ
BPC/ APOE4
BPC/ APOE4/ LRP1Δ
G
Lung metastasis
P= 0.0023
P= 0.5958
Number of metastatic foci
20
15
10
5
0
BPC/ APOE2
BPC/ APOE2/ LRP1Δ
BPC/ APOE4
BPC/ APOE4/ LRP1Δ
C
BPC/APOE2/LRP1Δ
BPC/APOE4/LRP1Δ
DAPI
CD8
Merge
F
Survival
Probability of survival
100
50
0
0
20
40
60
80
Day
BPC/APOE2
BPC/APOE2/ LRP1Δ
P= 0.0323
BPC/APOE4
BPC/APOE4/ LRP1Δ
P= 0.2541
H
BPC/APOE2 BPC/APOE3 BPC/APOE4
LRP1
-75 KDa
Total Protein
I
B16F10-shApoe pBabe:
Empty APOE2 APOE3 APOE4
LRP1
-75 KDa
HSC70
-75 KDa

Supplementary Figure 4. Tumoral LRP1 is a mediator of germline *APOE* variant differences in cancer progression and survival in a genetically initiated model of melanoma

A, Representative immunofluorescence images of LRP1 expression and DAPI nuclear staining in BPC/APOE2, BPC/APOE4, BPC/APOE2/LRP1Δ, and BPC/APOE4/LRP1Δ primary tumors (scale bar = 25 μm). **B,** Final tumor volumes of BPC/APOE2/LRP1Δ (n=12) and BPC/APOE4/LRP1Δ (n=10) mice at the experimental endpoint 49 days after topical 4-OHT administration. Unpaired t-test. **C,** Representative immunofluorescence images of CD8 and DAPI nuclear staining in BPC/APOE2/LRP1Δ and BPC/APOE4/LRP1Δ primary tumors (scale bar = 25 μm). **D,** Quantification of CD8+ T cell infiltration into BPC/APOE2/LRP1Δ and BPC/APOE4/LRP1Δ tumors at the survival endpoint, normalized to DAPI (n=7 per group, unpaired t-test). **E,** Aggregated results from **Figs. 1C** and **3C** of number of days after topical 4-OHT administration until visible, palpable tumors were detected in BPC/APOE2, BPC/APOE2/LRP1Δ, BPC/APOE4, and BPC/APOE4/LRP1Δ mice (n=12 per group). One-way ANOVA. **F,** Aggregated Kaplan-Meier survival curves from **Figs. 1D** and **3E** of BPC/APOE2, BPC/APOE2/LRP1Δ, BPC/APOE4, and BPC/APOE4/LRP1Δ mice after topical 4-OHT administration (n=12 per group). Log-rank test. **G,** Aggregated results from **Figs. 1G** and **3F** of lung metastatic foci in BPC/APOE2 (n=5), BPC/APOE2/LRP1Δ (n=6), BPC/APOE4 (n=5), and BPC/APOE4/LRP1Δ (n=5) mice after neonatal tumor induction. One-way ANOVA. **H,** Western blot of LRP1 expression in BPC/APOE2, BPC/APOE3, and BPC/APOE4 tumors (n=3 per group). **I,** Western blot of LRP1 expression in B16F10-sh*Apoe* cells transduced with pBabe Empty, APOE2, APOE3, or APOE4 retrovirus. HSC70 served as a loading control.



Supplementary Figure 5. *APOE2* genotype associates with enrichment of mRNA translation gene expression pathways in human melanoma

A, Top ten pathways upregulated in primary tumors of *APOE2* carrier patients (n=14) relative to *APOE3* homozygotes (n=65) as determined by GSEA and ranked by adjusted p-value (NES, normalized enrichment score; padj, adjusted p-value). **B,** Top ten pathways upregulated in primary tumors of *APOE3* homozygotes (n=65) relative to *APOE4* carrier patients (n=19) as determined by GSEA and ranked by adjusted p-value.