



Supplementary Figure S3. APP is Specifically Required for Melanoma Brain Metastasis. **A**, Western blot of analysis of 12-273 BM cells transduced with the indicated sh-RNA carrying lentivirus. In addition to sh-APP, the following sh-RNAs were used in the miniscreen: sh-XPNPEP3-c, sh-PRKAR2B-c, sh-SCARB1-c. **B-D**, Fold change in vitro proliferation and western blot analysis of 12-273BM cells transduced with **(B)** sh-Scr vs. sh-PRKAR2B, **(C)** sh-Scr vs. sh-SCARB1, **(D)** sh-Scr vs. sh-XPNPEP3. **E-N**, Intracardiac injection in NSG mice of 12-273 BM with sh-RNA-mediated silencing of selected candidates or sh-Scr (n=10-12 mice per group). **E**, Quantified brain luminescence in mice 35 days post-intracardiac injection. sh-Scr vs. sh-APP (** p<0.005), sh-Scr vs. sh-SCARB1 (* p<0.05). **F**, Quantified body luminescence in mice 35 days post-intracardiac injection. sh-Scr vs. sh-PRKAR2B (* p<0.05), sh-Scr vs. sh-SCARB1 (* p<0.05). **G**, Representative H&E-stained FFPE sections of brains, livers, and kidneys from mice injected with 12-273 BM sh-Scr vs. sh-PRKAR2B. Each column contains images from the same mouse. **H-I**, Ex vivo MRI analysis of brains from mice injected with 12-273 sh-Scr vs. sh-APP. Quantified average brain metastasis size **(H)**, (** p<0.005) and number of brain metastases **(I)**, (**** p<0.00005). **J-N**, Quantification of NuMA+ metastatic cells in FFPE sections of mouse organs. **J**, Number of brain metastases (* p<0.05). **K**, Average brain metastasis size (* p<0.05). **L**, Number of liver metastases. **M**, Average liver metastasis size. **N**, NuMA+ metastatic cells in mouse kidneys. **O**, CRISPR/Cas9-mediated knockdown of APP in 12-273 BM (upper panel) and 5B1 (lower panel) with two independent sg-RNAs (sg-APPa, sg-APPb). **P-Q**, Fold change in-vitro proliferation of 12-273 BM **(P)** and 5B1 **(Q)**. **R-S**, Intracardiac injection in mice of 5B1 cells with CRISPR/Cas9 knockdown (n=11-12 nude mice per group). **R**, Representative images of FFPE brain slides with staining of 5B1 brain metastatic cells by anti-NuMA immunohistochemistry. **S**, Quantification of 5B1 NuMA+ metastatic cells in mouse brains. sg-NTC vs. sg-APPa (* p<0.05), sg-NTC vs. sg-APPb (p=0.12).