

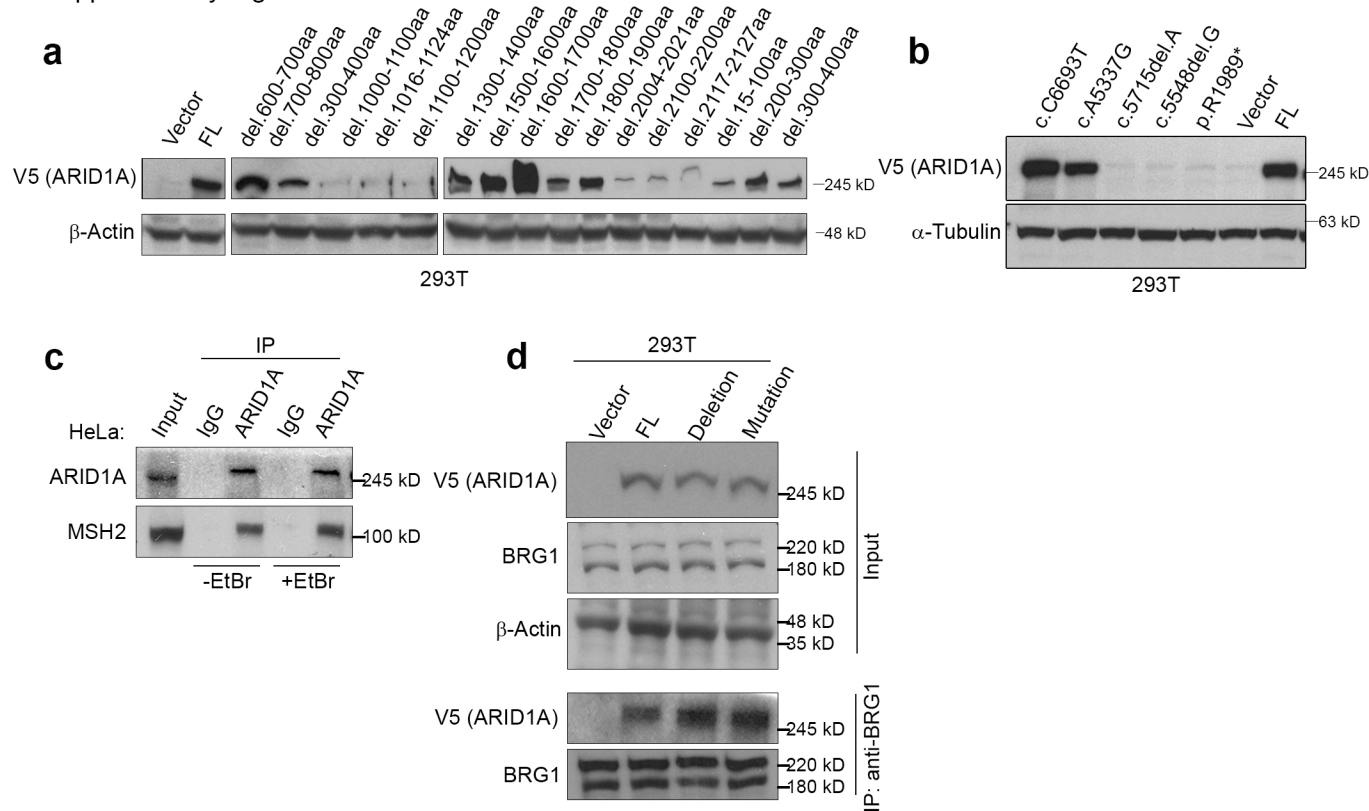
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ARID1A deficiency promotes mutability and potentiates therapeutic antitumor immunity unleashed by immune checkpoint blockade

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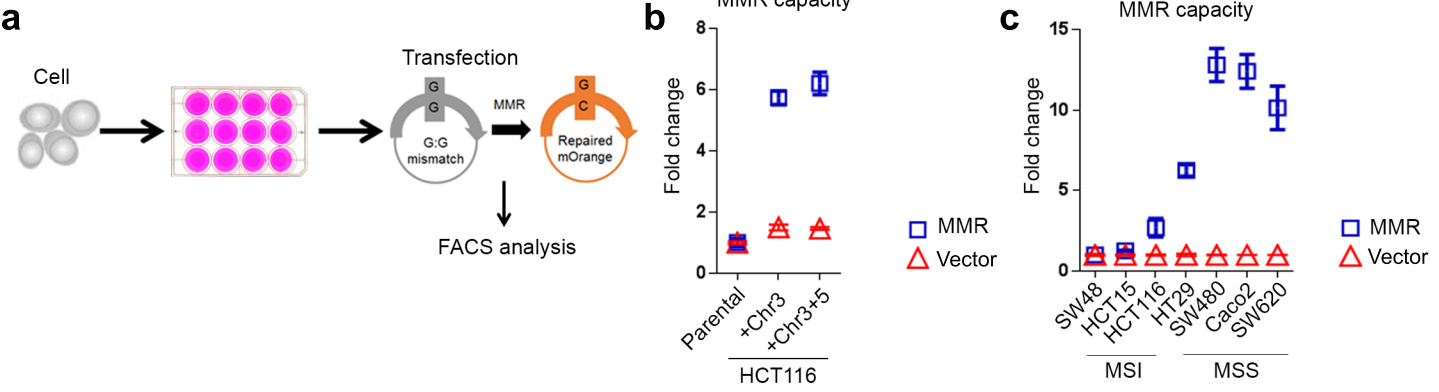
Supplementary Fig. 1



Supplementary Figure 1. Expression of ARID1A constructs.

a, Western blots of ARID1A deletion constructs in 293T cells. The experiments were repeated for three times. **b**, Western blots of ARID1A point mutation constructs in 293T cells. Deletion or mutations, which significantly impaired ARID1A expression, were not suitable for immunoprecipitation and reconstitution analysis. FL, full-length. The experiments were repeated for three times. **c**, ARID1A interacts with MSH2 with or without the addition of EtBr (Ethidium Bromide, 50 µg/ml) before IP. The experiments were repeated for three times. **d**, Western blots of ARID1A constructs interactions with BRG1. Deletion (del 1700-1800aa), Mutation (A5337G). The experiments were repeated for three times.

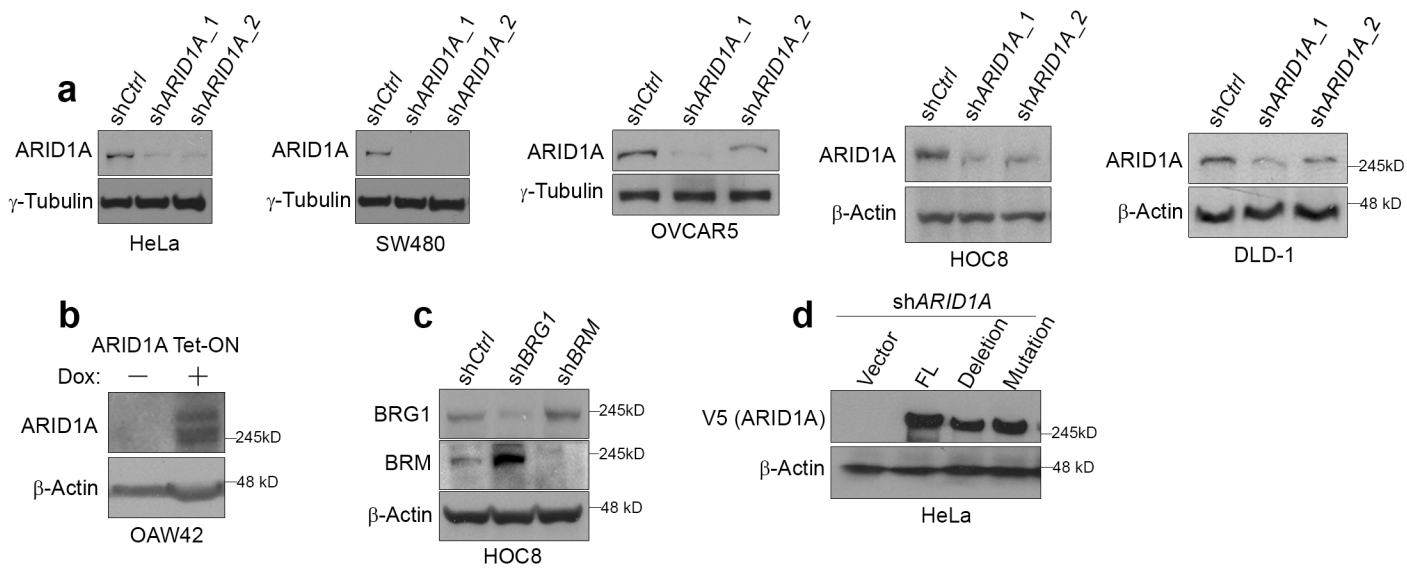
Supplementary Fig. 2



Supplementary Figure 2. MMR capacity assay.

a, Schematic of MMR capacity assay. **b**, Relative MMR capacities in HCT116 parental cells, which have MSI, and MSS HCT116+Chr3 and HCT116+Chr3+Chr5 cells. Results are presented as fold change of MMR compared to vector. Data represent mean $\pm$ s.e.m. of 3 independent experiments. Parental: 1 ± 0.134 ; +Chr3: 5.735 ± 0.218 ; +Chr3+Chr5: 6.205 ± 0.367 . **c**, Relative MMR capacities in colorectal cancer cell lines with known MSI status. Data represent mean $\pm$ s.e.m. of 3 independent experiments. SW48: 1 ± 0.00291 ; HCT15: 1.262 ± 0.0334 ; HCT116: 2.692 ± 0.576 ; HT29: 6.254 ± 0.357 ; SW480: 12.795 ± 1.040 ; Caco2: 12.412 ± 1.05 ; SW620: 10.131 ± 1.351 .

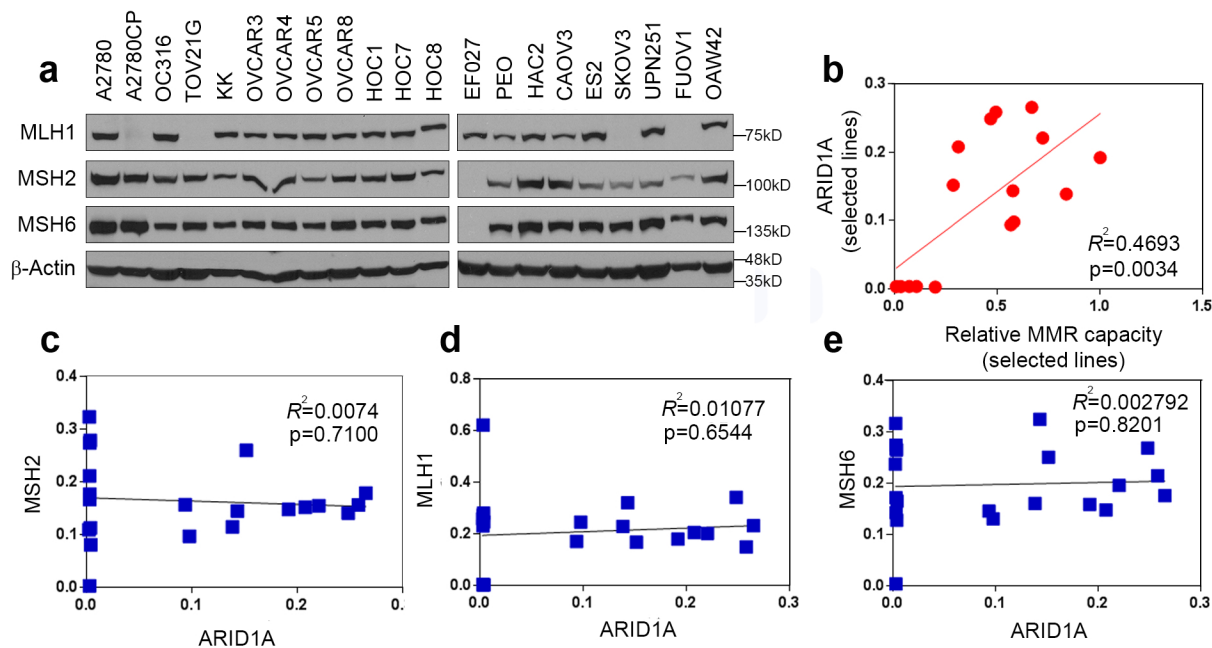
Supplementary Fig. 3



Supplementary Figure 3. ARID1A and BRG1 but not BRM modulate MMR capacity.

a, Western blots of *ARID1A* knockdown efficiency in HeLa, SW480, OVCAR5, HOC8, and DLD-1 cells. The experiments were repeated for three times. **b**, Western blots of ARID1A induction in ARID1A-null OAW42 cells by Doxycycline (Dox, 2 µg/ml, 6 days). The experiments were repeated for three times. **c**, Western blots of *BRG1* and *BRM* knockdown efficiency in HOC8 cells. The experiments were repeated for three times. **d**, Western blots of V5-tagged vector, ARID1A-full-length (FL), and mutants: Deletion (del. 1700-1800aa), Mutation (A5337G) in *ARID1A*-depleted HeLa cells. The experiments were repeated for three times.

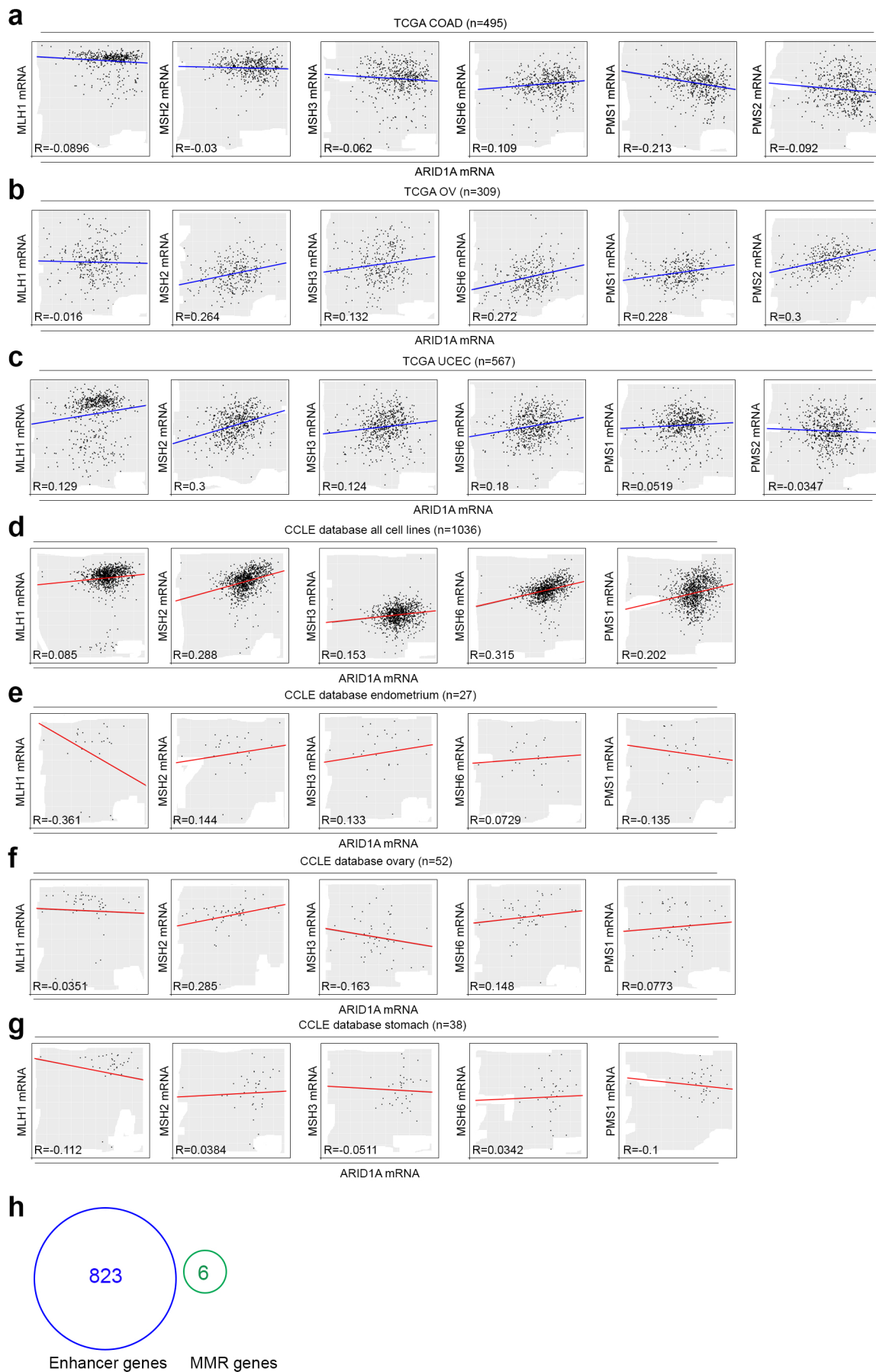
Supplementary Fig. 4



Supplementary Figure 4. ARID1A expression positively correlates with MMR capacity.

a, Western blots of MLH1, MSH2, and MSH6 in 21 ovarian cancer cell lines. The experiments were repeated for three times. **b**, Correlation analysis of ARID1A and MMR capacity in selected ovarian cancer cell lines with intact MLH1, MSH2 and MSH6 including A2780, OC316, KK, OVCAR3, OVCAR4, OVCAR5, OVCAR8, HOC1, HOC7, HOC8, PEO, HAC2, CAOV3, ES2, UPN251 and OAW42. Linear-regression test. **c-e**, Correlation analysis of ARID1A and MSH2, MLH1, and MSH6 protein levels in 21 ovarian cancer cell lines. Linear-regression test.

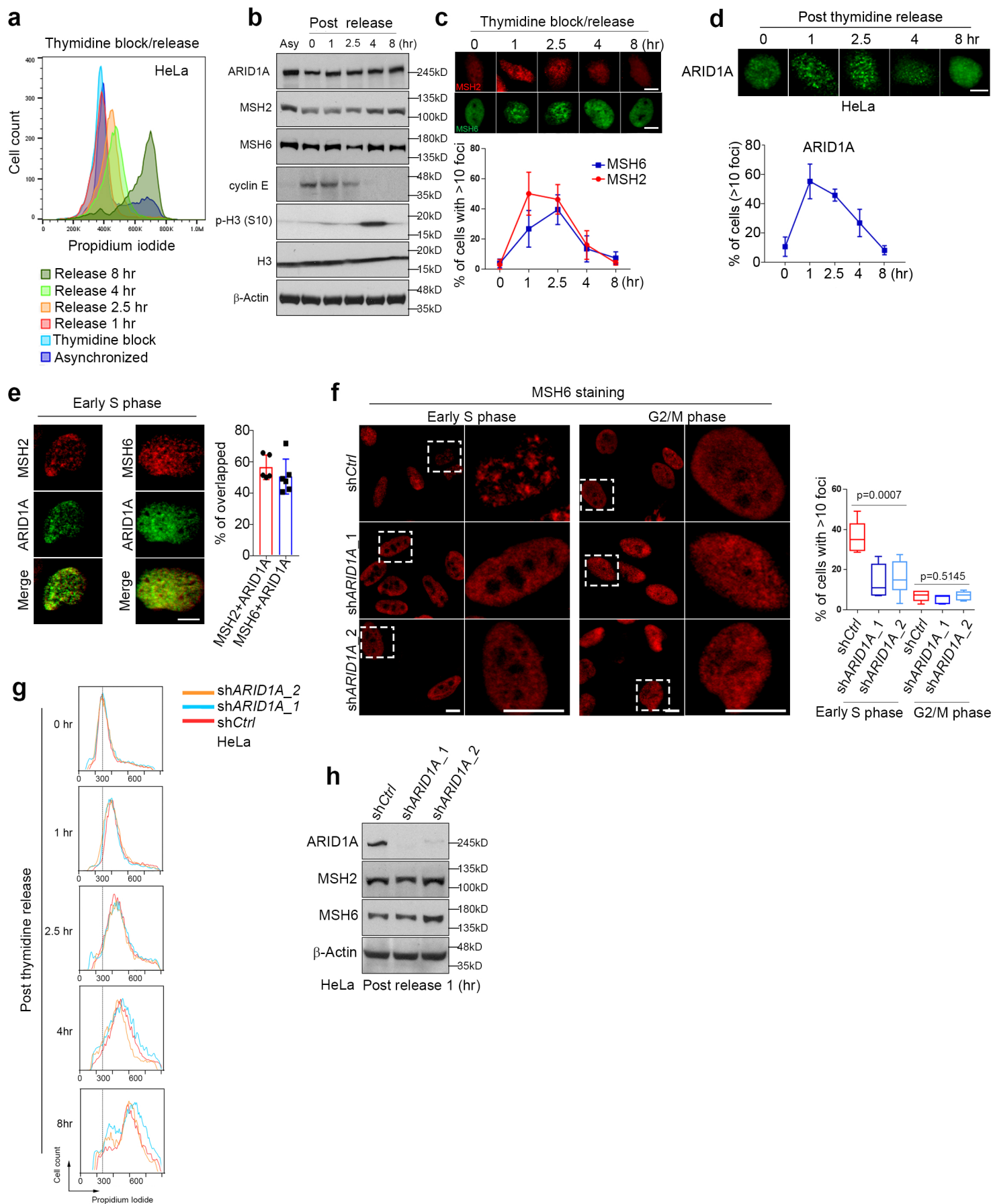
Supplementary Fig. 5



Supplementary Figure 5. The mRNA levels of core MMR genes do not exhibit a significant correlation with *ARID1A* mRNA expression level.

a-c, Correlation analysis of *MLH1*, *MSH2*, *MSH3*, *MSH6*, *PMS1*, and *PMS2* mRNA levels with *ARID1A* mRNA level in colon adenocarcinoma (COAD) (**a**), ovarian cancer (OV) (**b**), and uterine corpus endometrial carcinoma (UCEC) (**c**) patient samples from The Cancer Genome Atlas (TCGA). Linear-regression test. **d-g**, Correlation analysis of *MLH1*, *MSH2*, *MSH3*, *MSH6*, and *PMS1* mRNA levels with *ARID1A* mRNA level in all cell lines (**d**) and endometrial cancer (**e**), ovarian cancer (**f**), and stomach cancer (**g**) cell lines using the human Cancer Cell Line Encyclopedia (CCLE) database. Linear-regression test. **h**, Venn diagram showed no overlap between key MMR genes and genes previously reported to be involved in *ARID1A*-associated enhancer-mediated regulation ⁷.

Supplementary Fig. 6

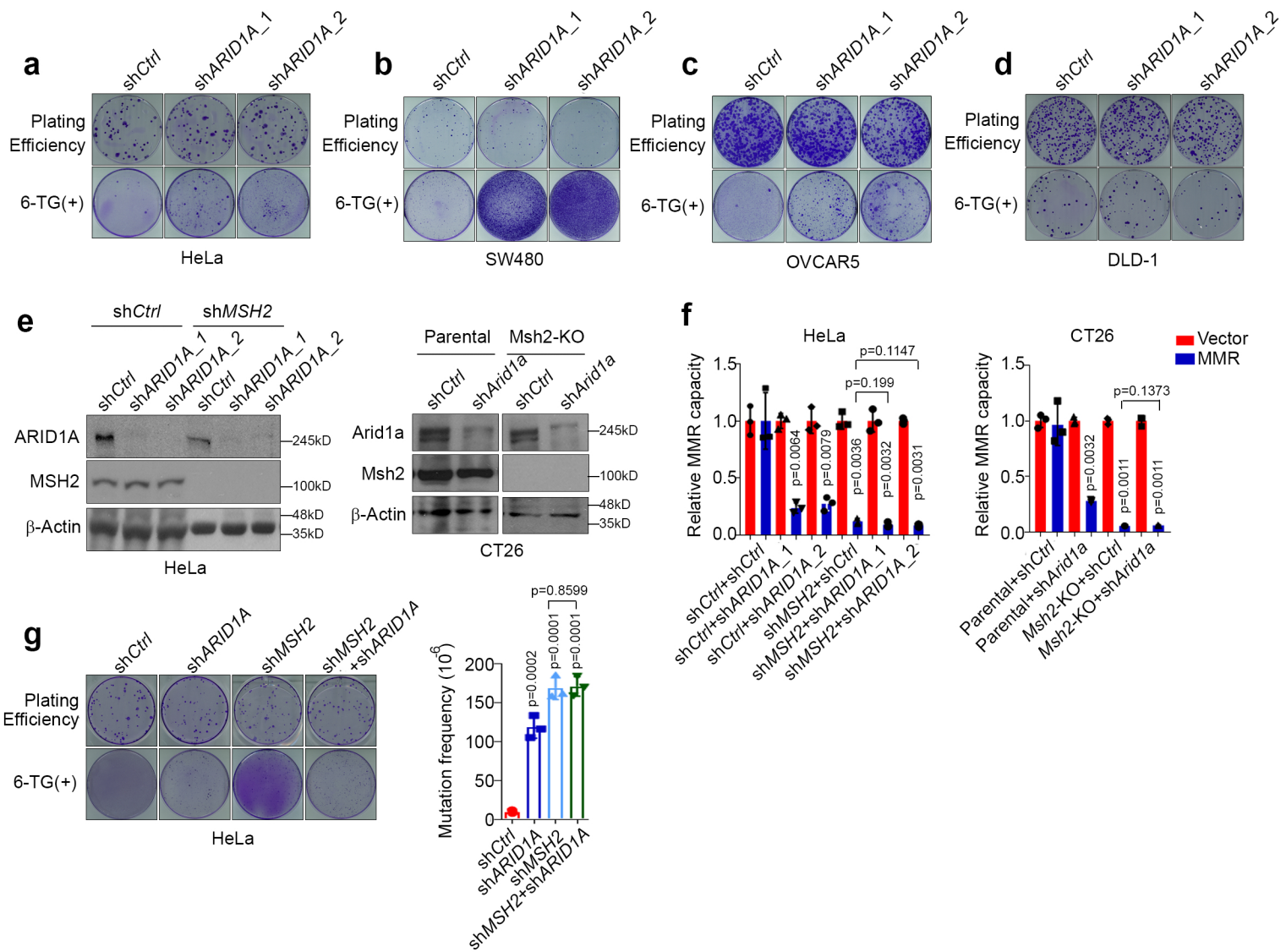


Supplementary Figure 6. Cell cycle–dependent nuclear distribution of MSH2 and MSH6 and regulation of MSH2 and MSH6 by ARID1A.

a, Cell cycle profiling by flow cytometry in HeLa cells using double thymidine block. The experiments were repeated for three times. **b**, Western blots of target genes in cell cycle. The experiments were repeated for three times. **c**, MSH2 and MSH6 immunocytochemistry staining in various cell cycle stages in HeLa cells. MSH2: 0hr, n=214 cells, 3.054 ± 3.351 ; 1hr, n=149 cells, 50.075 ± 14.265 ; 2.5hr, n=165 cells, 46.296 ± 9.797 ; 4hr, n=220 cells, 16.091 ± 9.507 ; 8hr, n=255 cells, 4.254 ± 1.466 ; MSH6: 0hr, n=150 cells, 3.876 ± 2.889 ; 1hr, n=281 cells, 26.743 ± 12.142 ; 2.5hr, n=179 cells, 39.476 ± 9.839 ; 4hr, n=245 cells, 13.514 ± 8.659 ; 8hr, n=263 cells, 7.355 ± 4.158 ; Scale bars, 10 μm . **d**, ARID1A immunocytochemistry staining in various cell cycle stages in HeLa cells. 0hr, n=156 cells, 10.635 ± 6.620 ; 1hr, n=233 cells, 55.210 ± 11.826 ; 2.5hr, n=191 cells, 45.797 ± 4.125 ; 4hr, n=205 cells, 26.857 ± 9.273 ; 8hr, n=95 cells, 8.199 ± 3.0861 ; Scale bars, 10 μm . **e**, Subcellular localization of ARID1A, MSH2, and MSH6 at early S phase in HeLa cells by immunocytochemistry staining. Overlapped ARID1A+MSH2, n=180 cells, 56.54 ± 3.512 , ARID1A+MSH6, n=311 cells, 50.59 ± 4.564 . Scale bars, 10 μm . The experiments were repeated twice. **f**, MSH6 immunocytochemistry staining in control and *ARID1A*-depleted HeLa cells. Scale bars, 10 μm . Early S phase: shCtrl, n=311 cells, 36.43 ± 3.29 ; sh*ARID1A*_1, n=258 cells, 14.22 ± 3.682 ; sh*ARID1A*_2, n=213 cells, 15.92 ± 3.459 , one-way ANOVA; G2/M phase: shCtrl, n=299 cells, 6.985 ± 1.145 ; sh*ARID1A*_1, n=500 cells, 5.521 ± 0.9254 ; sh*ARID1A*_2, n=216 cells, 7 ± 0.9571 , one-way ANOVA. Dashed square, magnification of area. **g**, Cell cycle profiling of control (shCtrl) and *ARID1A*-depleted HeLa cells released from thymidine block. (G1 gate was set at Propidium Iodide 300 fluorescent reading as marked). The experiments were repeated at least for three times. **h**, Western blots of

target genes in control and *ARID1A*-depleted HeLa cells after 1 hr of thymidine block. The experiments were repeated for three times.

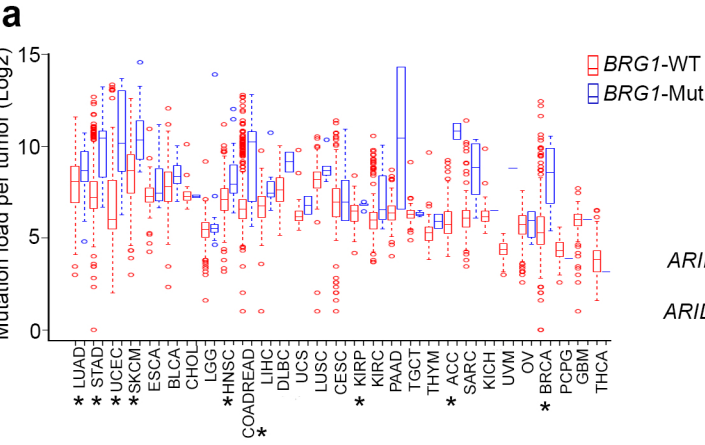
Supplementary Fig.7



Supplementary Figure 7. *ARID1A*-knockdown promotes mutability.

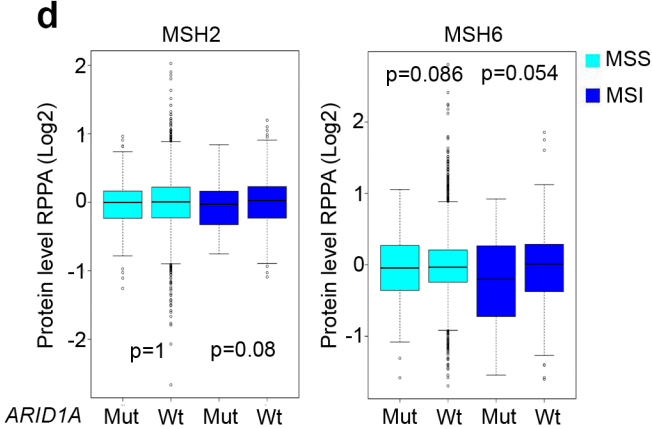
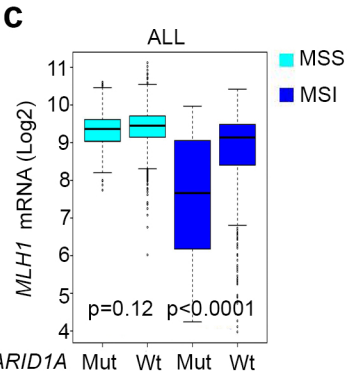
a-d, Representative images of mutability assay in HeLa (**a**), SW480 (**b**), OVCAR5 (**c**) and DLD-1 (**d**) cells. The experiments were repeated for three times. **e**, Western blots of *ARID1A* and *MSH2* in HeLa and CT26 cells with *ARID1A* and/or *MSH2* depletion. KO, knockout. The experiments were repeated for three times. **f**, Relative MMR capacities of HeLa and CT26 cells with *ARID1A* and/or *MSH2* depletion. n=3 independent experiments, two-tailed *t* test compared with shCtrl+shCtrl or Parental+shCtrl. **g**, Mutability analysis of HeLa cells with *ARID1A* and/or *MSH2* depletion. n=3 independent experiments, two-tailed *t* test compared with shCtrl.

Supplementary Fig. 8



b

	MLH1_WT	MLH1_Mut	MSH2_WT	MSH2_Mut	MSH3_WT	MSH3_Mut	MSH6_WT	MSH6_Mut	PMS1_WT	PMS1_Mut	PMS2_WT	PMS2_Mut
ARID1A_WT	262	60	269	53	252	70	247	75	257	65	263	59
ARID1A_Mut	498	32	495	35	491	39	475	55	508	22	499	31
	p=0.0001		p=0.0001		p=0.0001		p=0.0001		p=0.0001		p=0.0001	



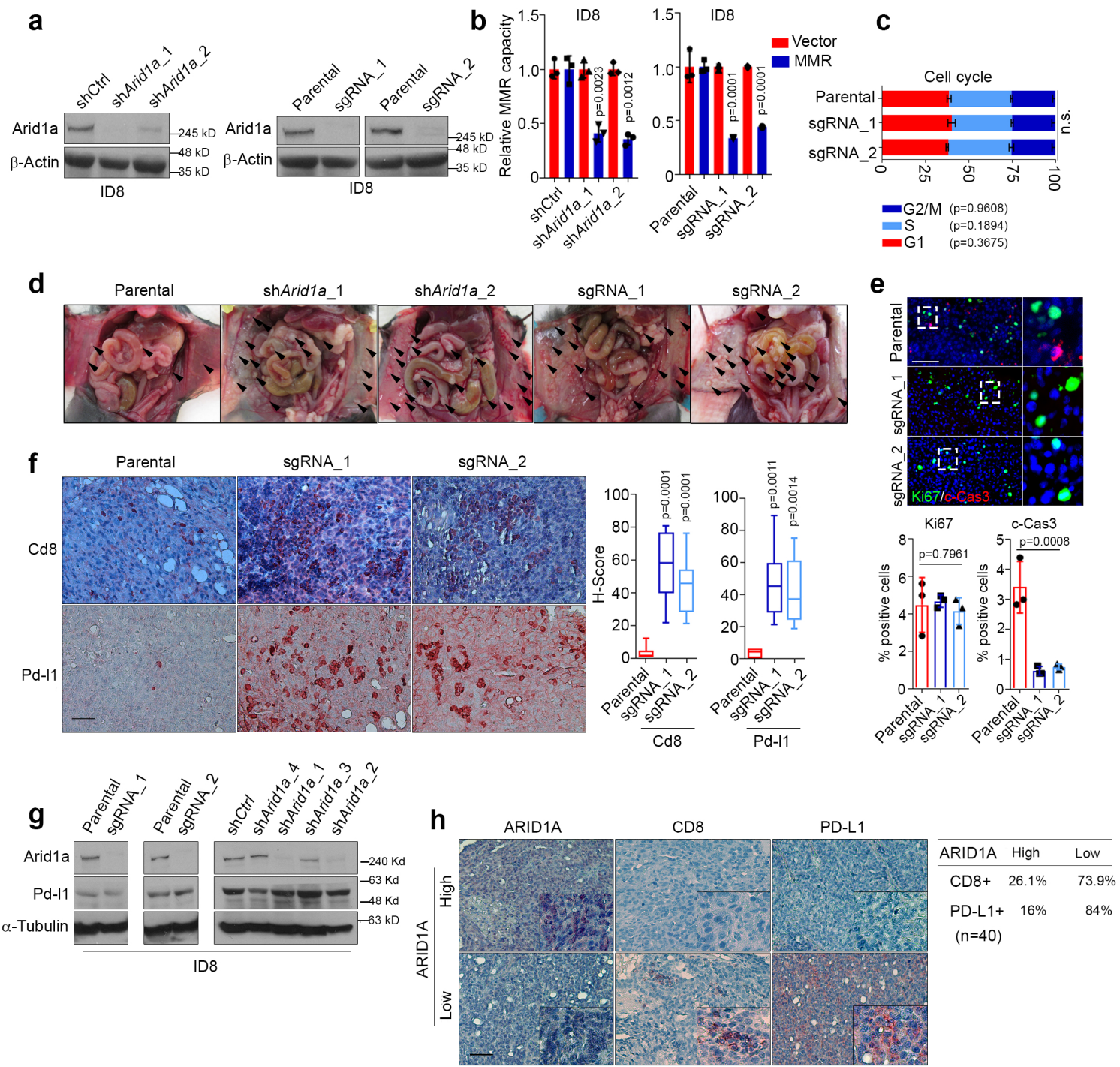
e

	MSS				
	WT	MLH1_Mut	PMS2_Mut	MLH1+PMS2_Mut	
WT	100	26	32	0	Chi-square (p=0.0001)
ARID1A_Mut	254	9	7	2	
MSH2_Mut	23	1	1	0	
ARID1A+MSH2_Mut	6	1	2	0	
	MSI				
	WT	MLH1_Mut	PMS2_Mut	MLH1+PMS2_Mut	
WT	13	8	5	0	Chi-square (p=0.0083)
ARID1A_Mut	54	8	5	0	
MSH2_Mut	4	3	0	0	
ARID1A+MSH2_Mut	7	1	2	1	

Supplementary Figure 8. *BRG1*-mutant tumors show increased mutation load.

a, Mutation load in *BRG1*-wild-type (WT) and *BRG1*-mutant (Mut) tumors. Cancer types with statistical significance between *BRG1*-WT and *BRG1*-Mut were indicated by “*” (two-tailed *t* test). P values were shown in **Supplementary Table 4**. **b**, Mutation status of *ARID1A* and MMR genes in TCGA tumors combining BLCA, COADREAD, STAD and UCEC datasets. *n*=852, chi-square test. **c**, Mutation status of *ARID1A* and *MLH1* mRNA levels in MSS and MSI tumors combining BLCA, COADREAD, STAD and UCEC datasets. MSS (*n*=4060): *ARID1A*_Mut, mean=9.341; *ARID1A*_Wt, mean=9.416, standard error=0.00692, two-tailed *t* test; MSI (*n*=351): *ARID1A*_Mut, mean=7.574, *ARID1A*_Wt, mean= 8.631, standard error=0.0787, two-tailed *t* test. **d**, MSH2 and MSH6 protein levels by RPPA analysis in TCGA tumors combining BLCA, COADREAD, STAD and UCEC datasets. MSH2: MSS (*n*=3095): *ARID1A*_Mut, mean=-0.0114; *ARID1A*_Wt, mean=-0.0113, standard error=0.00726, two-tailed *t* test; MSI (*n*=276): *ARID1A*_Mut, mean=-0.0870, *ARID1A*_Wt, mean=0.0111, standard error=0.0241, two-tailed *t* test. MSH6: MSS (*n*=3095): *ARID1A*_Mut, mean=-0.0599; *ARID1A*_Wt, mean=-0.000464, standard error=0.00835, two-tailed *t* test; MSI (*n*=276): *ARID1A*_Mut, mean=-0.234, *ARID1A*_Wt, mean=-0.051, standard error=0.0368, two-tailed *t* test. **e**, Mutation analysis of *MLH1/PMS2* axis and *ARID1A/MSH2* axis in TCGA tumors combining BLCA, COADREAD, STAD and UCEC datasets. MSS, *n*=464; MSI, *n*=111, chi-square test.

Supplementary Fig. 9

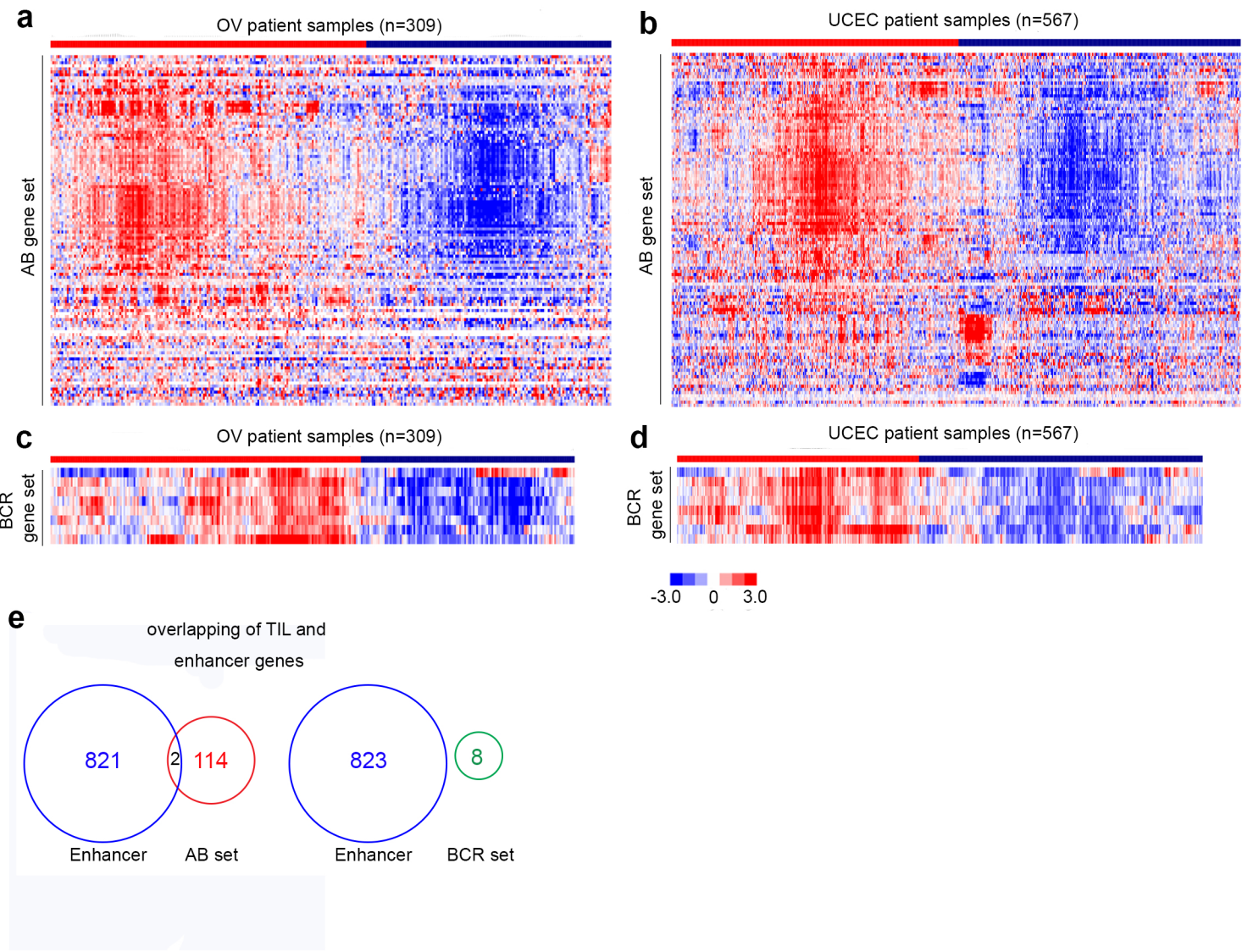


Supplementary Figure 9. Depletion of ARID1A promotes tumor growth and TILs *in vivo*.

a, Western blots of Arid1a in ID8 cells following depletion engineered by either shRNA or CRISPR-Cas9 gene editing technology (sgRNA_1, sgRNA_2). The experiments were repeated for three times. **b**, Relative MMR capacities in control (shCtrl) and *Arid1a*-depleted ID8 cells. Data represent mean $\pm$ s.e.m., n=3 independent experiments, two-tailed *t* test. **c**, Cell cycle profiling of parental and *Arid1a*-depleted ID8 cells. n=3 independent experiments, one-way ANOVA. **d**, Representative images of tumor formation 28 days after i.p. injection of parental and *Arid1a*-depleted ID8 cells. Arrows indicate tumor nodules. The experiments were repeated for three times. **e**, Representative images and statistical analysis of Ki67 and cleaved Caspase 3 (c-Cas3) staining in control and *Arid1a*-depleted ID8 orthotopic tumors. n=3 independent mice, one-way ANOVA. Dashed square, magnification of area. Scale bar, 100 μ m. **f**, Representative images and H-score analysis of Cd8 and Pd-l1 IHC staining in tumors retrieved after i.p. injection of parental and *Arid1a*-depleted ID8 cells. Cd8: Parental, n=10 tumors from 3 independent mice, 3.392 ± 1.082 , sgRNA_1, n=6 tumors from 3 independent mice, 56.74 ± 8.649 , sgRNA_2, n=15 tumors from 3 independent mice, 43.84 ± 4.088 , two-tailed *t* test compared with Parental; Pd-l1: Parental, n=5 tumors from 3 independent mice, 3.463 ± 1.086 , sgRNA_1, n=7 tumors from 3 independent mice, 49.49 ± 8.427 , sgRNA_2, n=7 tumors from 3 independent mice, 44 ± 7.679 , two-tailed *t* test compared with Parental. Scale bar, 100 μ m. **g**, Western blots of Pd-l1 in control and *Arid1a*-depleted ID8 cells. The experiments were repeated for three times. **h**, Representative images of ARID1A, CD8, and PD-L1 staining in an ovarian cancer tissue microarray containing 40 cases (31 serous adenocarcinoma, 5 endometrioid adenocarcinoma, 3 mucinous adenocarcinomas and 1 OCCC). Percentage of specimens with

CD8⁺ and PD-L1⁺ staining in specimens with high and low ARID1A expression. Scale bar, 100 μ m.

Supplementary Fig. 10

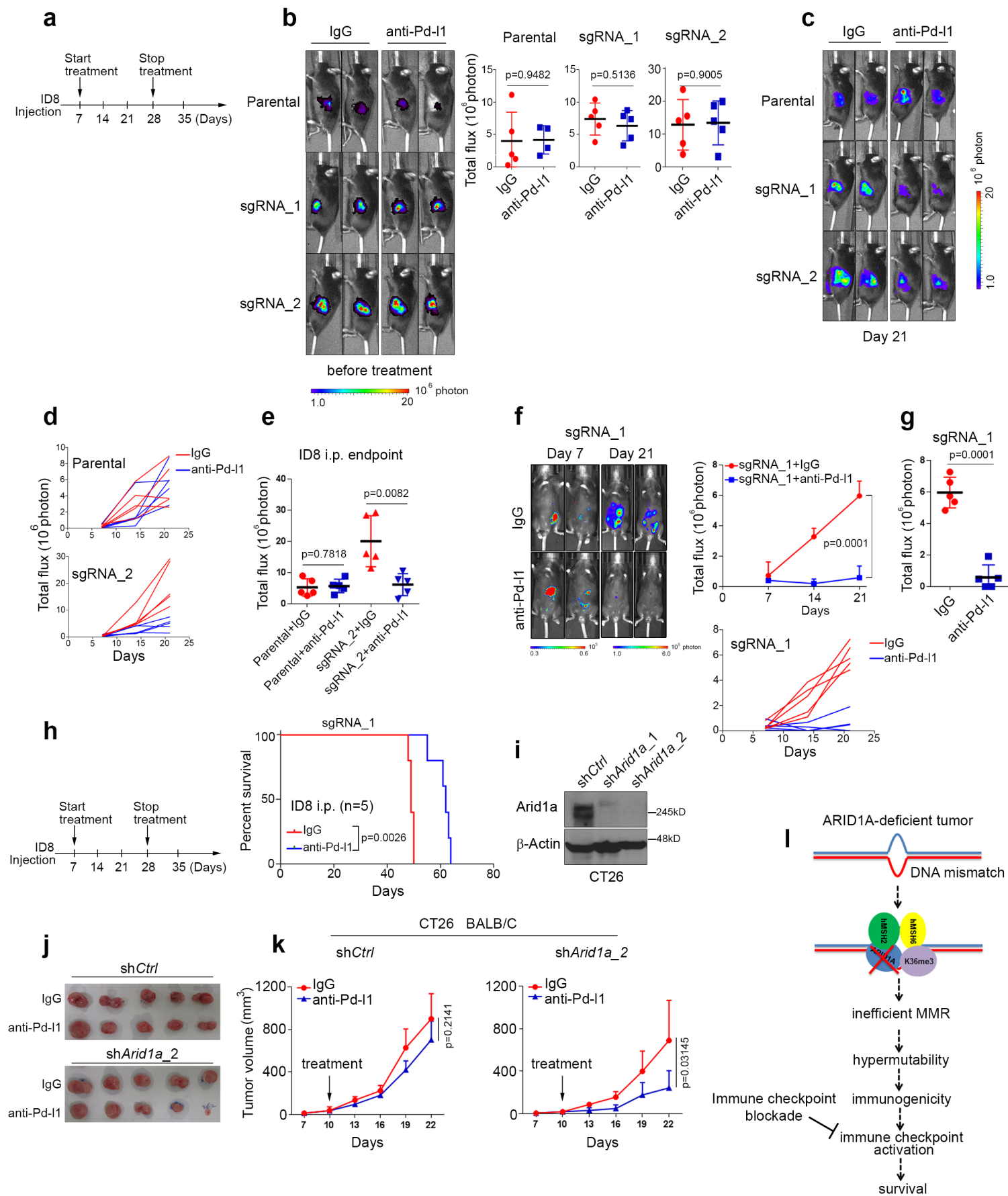


Supplementary Figure 10. ARID1A level negatively associates with TILs.

a-d, Heatmaps of TIL analyses in OV and UCEC samples using AB-set and BCR-set signatures.

e, Overlapping of AB-set and BCR-set genes with ARID1A-associated enhancer genes⁷.

Supplementary Fig. 11



Supplementary Figure 11. Therapeutic effects of anti-PD-L1 treatment.

a, Schematic of isotype control IgG and anti-Pd-l1 antibody treatment. **b**, Bioluminescence of mice with orthotopic ID8 tumors before treatment (Day 7). Parental+IgG, n=5 mice, 4.008 ± 1.982 ; Parental+anti-Pd-l1, n=4 mice, 4.172 ± 1.091 , two-tailed *t* test; sgRNA_1+IgG, n=5 mice, 7.386 ± 1.103 ; sgRNA_1+anti-Pd-l1, n=5 mice, 6.348 ± 1.044 , two-tailed *t* test; sgRNA_2+IgG, n=5 mice, 12.81 ± 3.431 ; sgRNA_2+anti-Pd-l1, n=4 mice, 13.4 ± 2.983 , two-tailed *t* test; **c**, Bioluminescence of mice with orthotopic ID8 tumors after treatment (Day 21). **d**, Spider plots of bioluminescence of parental and sgRNA_2 mice. **e**. Statistical analysis of the endpoint bioluminescence in **Figure 4i**. Parental+IgG, n=5 mice, 5.249 ± 1.236 ; Parental+anti-Pd-l1, n=5 mice, 5.699 ± 0.966 , two-tailed *t* test; sgRNA_2+IgG, n=5 mice, 20.04 ± 3.65 ; sgRNA_2+anti-Pd-l1, n=5 mice, 6.149 ± 1.58 , two-tailed *t* test; **f, g**, Change in bioluminescence over time (**f**) and endpoints (**g**) in mice bearing *ARID1A*-depleted (sgRNA_1) ID8 i.p. tumors. sgRNA_1+IgG, n=5 mice, 5.964 ± 0.4358 ; sgRNA_1+anti-Pd-l1, n=5 mice, 0.5853 ± 0.3511 , two-tailed *t* test. **h**, Survival curves of mice bearing *ARID1A*-depleted (sgRNA_1) ID8 i.p. tumors with treatments. n=5 mice per group, Log-rank test. **i**, Western blots of *Arid1a* knockdown in CT26 murine colorectal cancer cells. The experiments were repeated for three times. **j**, Representative images of CT26 tumors retrieved from BALB/C mice. **k**, Tumor volume of control (shCtrl) and *Arid1a*-depleted (shArid1a_2) CT26 cells in BALB/C mice treated by IgG or anti-Pd-l1. shCtrl+IgG, n=5 mice, 900.46 ± 234.99 ; shCtrl+anti-Pd-l1, n=5 mice, 702.08 ± 176.62 , two-tailed *t* test; shArid1a_2+IgG, n=4 mice, 788.23 ± 357.29 ; shArid1a_2+anti-Pd-l1, n=5 mice, 242.89 ± 162.79 , two-tailed *t* test. **l**, Model for the role of ARID1A in regulating MMR, mutability, immunogenicity, and therapeutic response to immunotherapy using immune checkpoint blockade.

Figure 1b

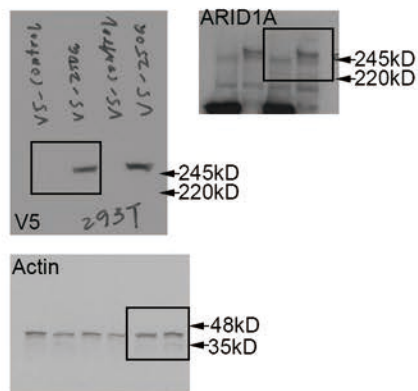


Figure 1c

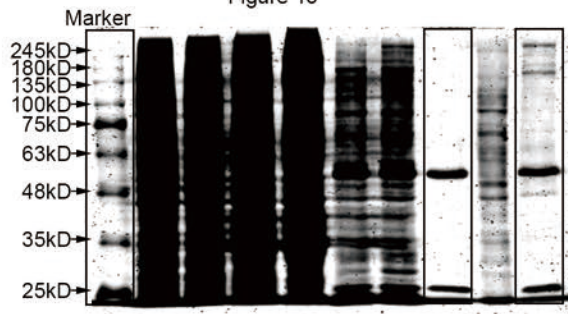


Figure 1e

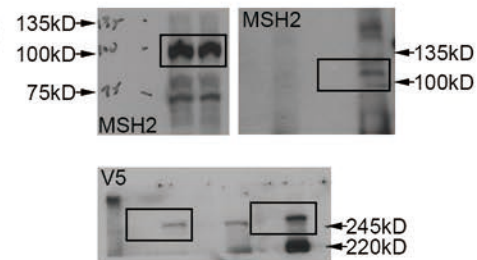


Figure 1f

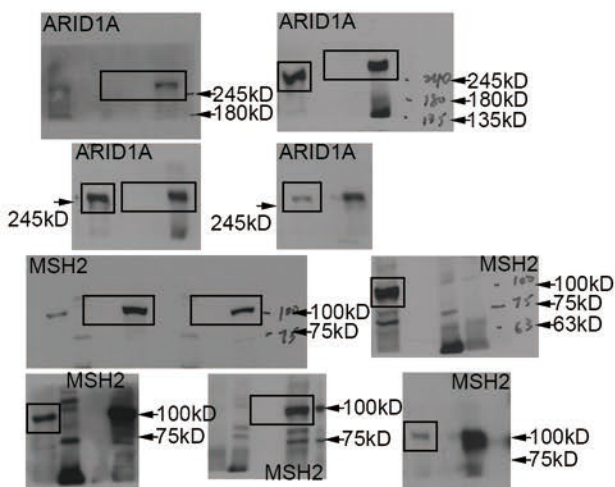


Figure 1g

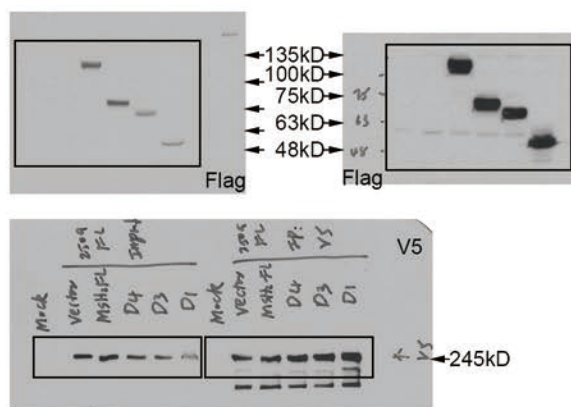


Figure 1h

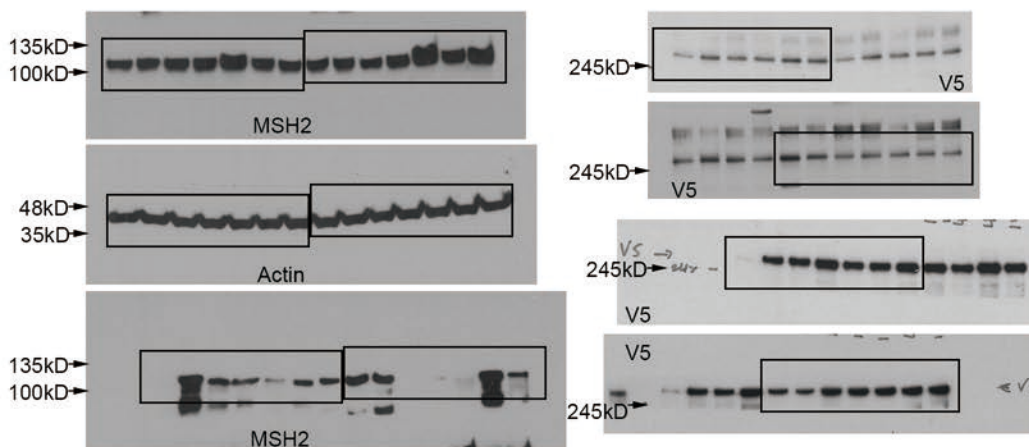
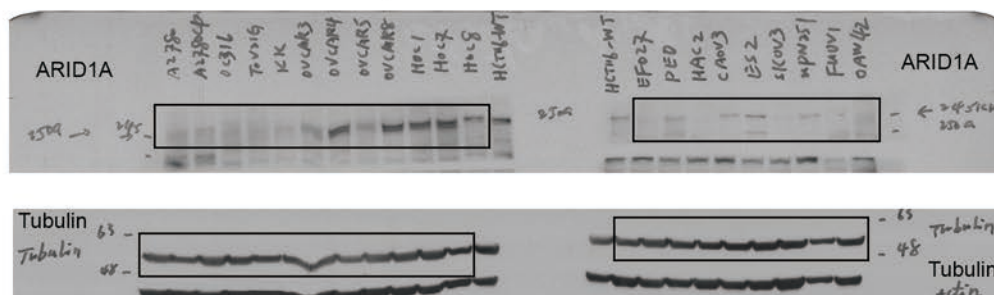
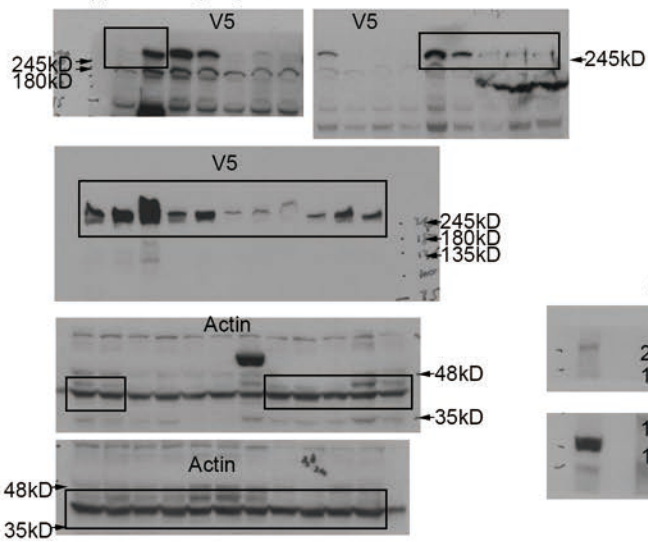


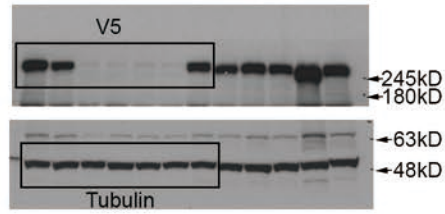
Figure 2e



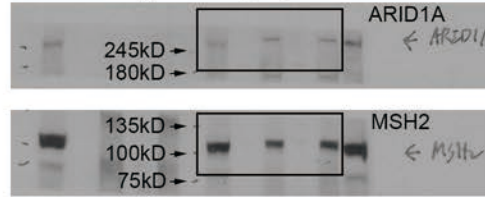
Supplementary Fig. 1a



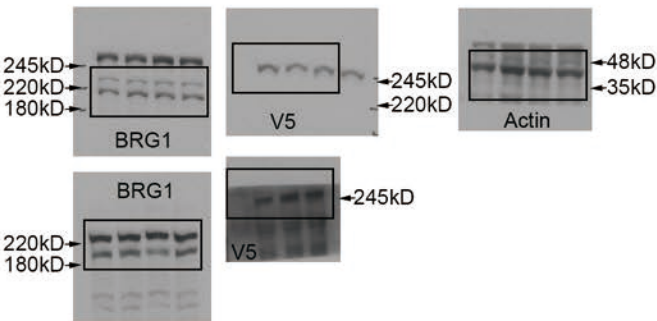
Supplementary Fig. 1 b



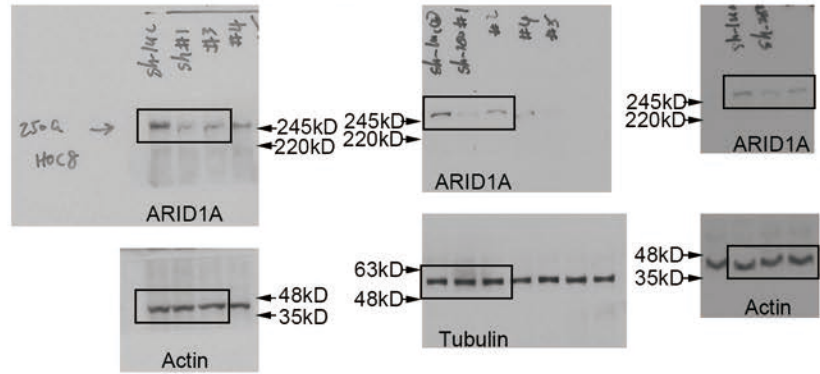
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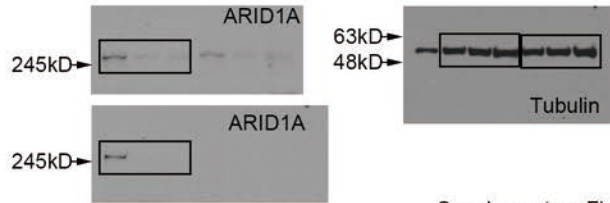
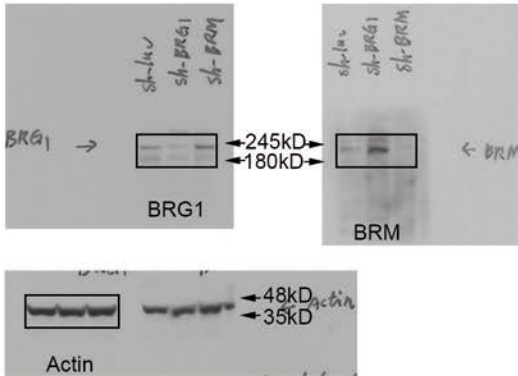
Supplementary Fig. 1d



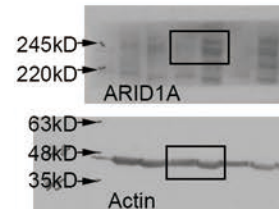
Supplementary Fig. 3a



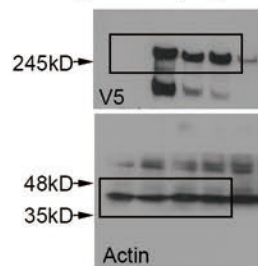
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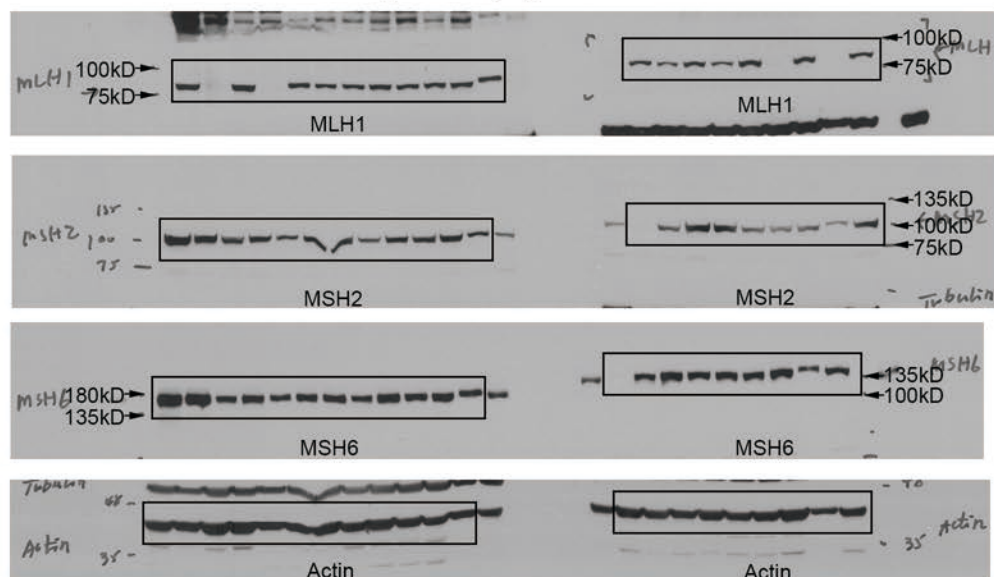
Supplementary Fig. 3b



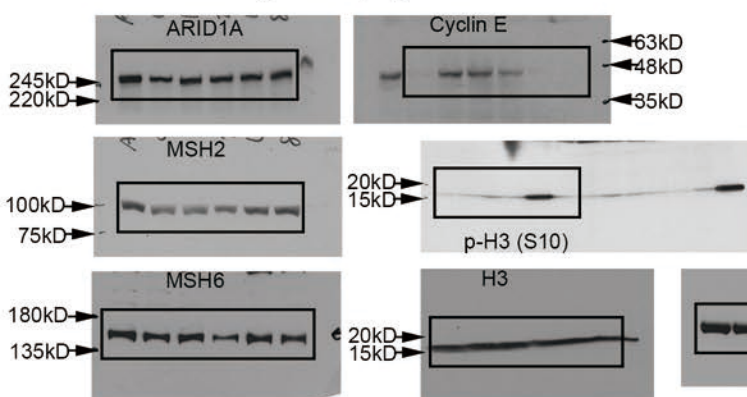
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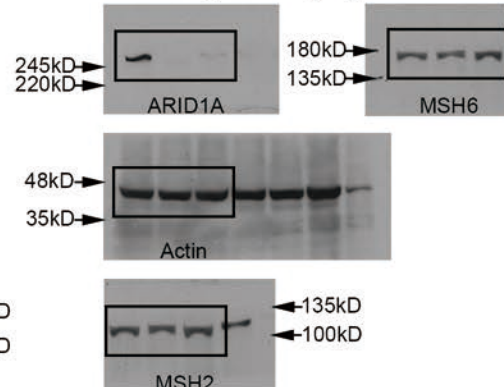
Supplementary Fig. 4a



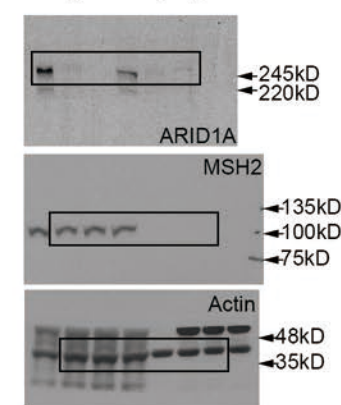
Supplementary Fig. 6b



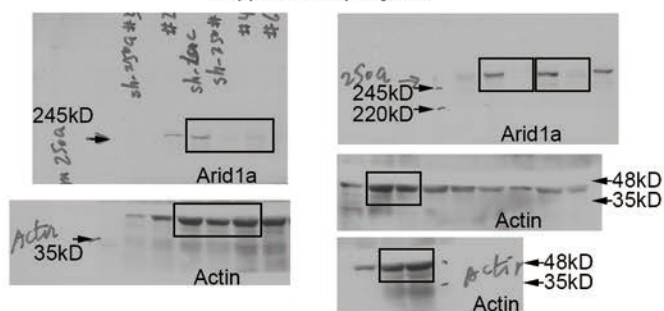
Supplementary Fig. 6h



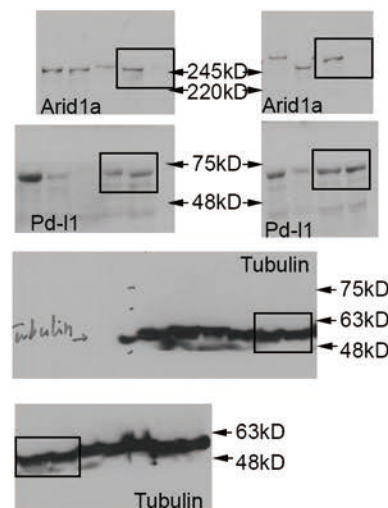
Supplementary Fig. 7e



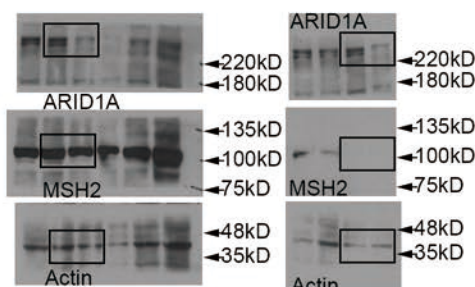
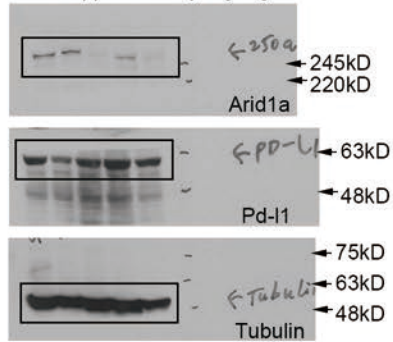
Supplementary Fig. 9a



Supplementary Fig. 9g



Supplementary Fig. 9g



Supplementary Fig. 11i

