

**Supplementary Information**

**Figure S1.....2**

**Figure S2.....3**

**Figure S3.....4**

**Figure S4.....5**

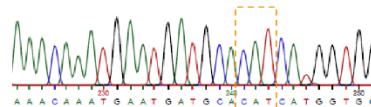
**Video legends.....6**

**Table S1.....7**

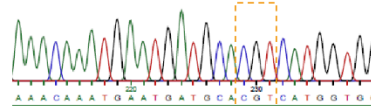
*PIK3CA* H1047R/+

## Sequencing

PIK3CA



*PIK3CA*



### Alignment

WT K Q M N D A E H H G G W T T K M D W I F R  
aaacaaatggaatgatgcacatcgtggtgctggacacaaatggaatggaatctccac  
H1047R aaacaaatggaatgatgcacgtcgtggtgctggacacaaatggaatggaatctccac  
K Q M N D A R H H G G W T T K M D W I F R

+1 bp

-23 bp

**Alignment**

WT  
KO allele #1

cut gRNA

insertion 1bp

Premature stop codon

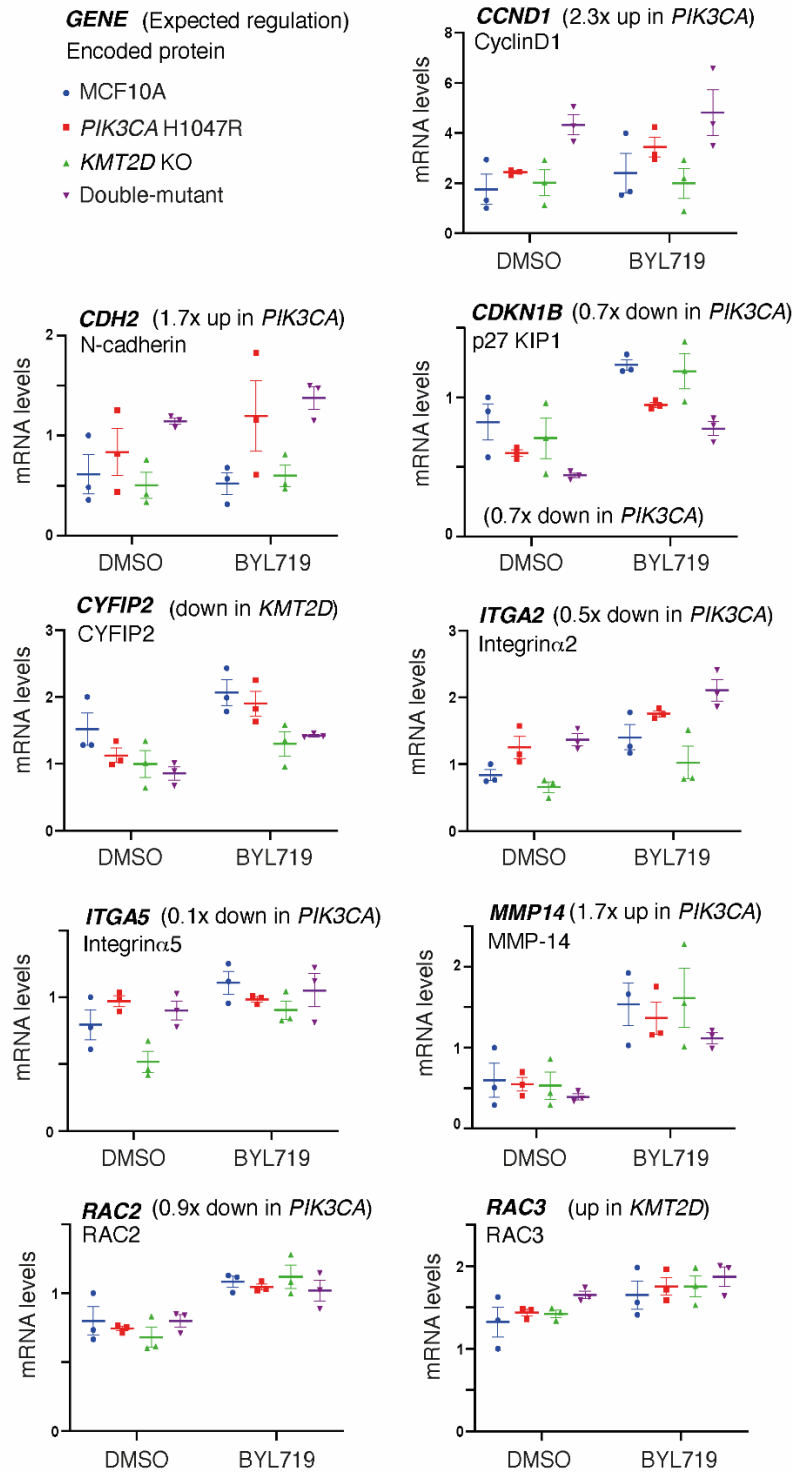
WT  
KO allele #2

cut gRNA

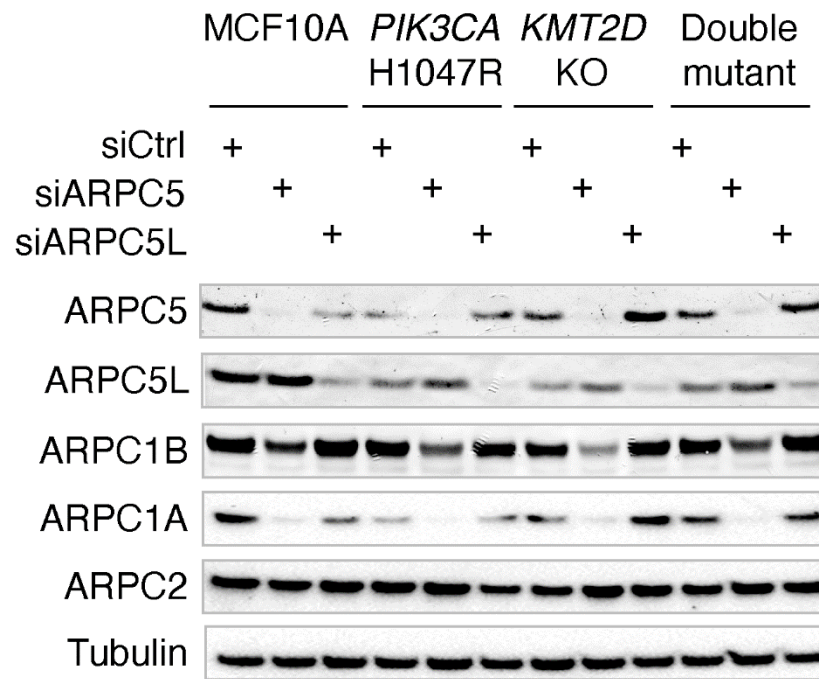
deletion 23bp

Premature stop codon

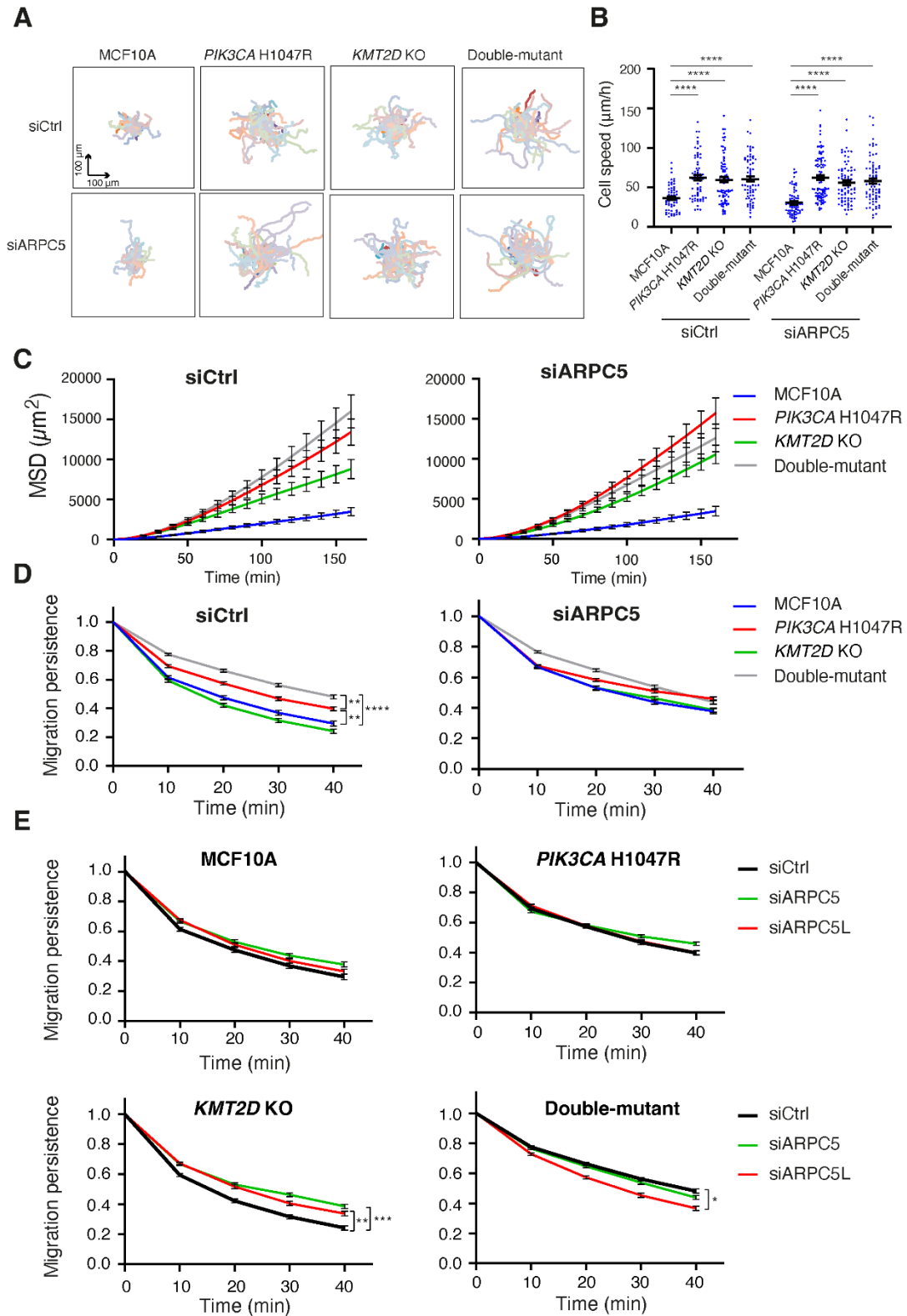
2



**Figure S2.** Genes, whose expression is measured and is not found to be significantly deregulated in MCF10A cells by *PIK3CA* H1047R or *KMT2D* inactivation. qRT-PCR analysis of gene expression is performed from MCF10A, *PIK3CA* H1047R, *KMT2D* KO and double-mutant cells in the presence or absence of 5  $\mu$ M BYL719. Mean  $\pm$  SEM, n=3 biological repeats. The variations that are previously reported in the references cited in the main text are indicated in parentheses.



**Figure S3.** Western blots of MCF10A, *PIK3CA* H1047R, *KMT2D* KO and double-mutant cells when transfected with siRNAs targeting ARPC5 or ARPC5L or non-targeting siRNAs. The same depleted cells are assayed for single cell migration and results are displayed in Figure 6 and Figure S4.



**Figure S4.** Single cell migration analysis. MCF10A, *PIK3CA* H1047R, *KMT2D* KO and double-mutant cells transfected with siRNAs targeting ARPC5 or ARPC5L or non-targeting siRNAs. **A** Trajectories of 56 MCF10A, *PIK3CA* H1047R, *KMT2D* KO and double-mutant cells, depleted of ARPC5 or not. The origin of each track is registered at the center of the plot. **B** Cell speed (mean  $\pm$  SEM). **C** Mean Square Displacement (MSD). **D** Migration persistence. **E** Migration persistence in all cell lines when depleted of either ARPC5 or ARPC5L. Three independent experiments are performed, overall number of cells ranges from 56 to 78 for each condition. \*\*  $p < 0.01$ , \*\*\*\*  $p < 0.0001$  (1-way ANOVA on non linear mixed-effect models for each condition). This supplementary figure displays additional results from the same experiment whose main results are displayed in Figure 6.

## Video legends

**Video S1.** Collective migration of parental MCF10A cells is observed using phase contrast microscopy in the presence of 5  $\mu$ M BYL719 or not. Displacement vectors obtained using Particle Image Velocimetry are overlaid in green.

**Video S2.** Collective migration of *PIK3CA* H1047R cells is observed using phase contrast microscopy in the presence of 5  $\mu$ M BYL719 or not. Displacement vectors obtained using Particle Image Velocimetry are overlaid in green.

**Video S3.** Collective migration of *KMT2D* KO cells is observed using phase contrast microscopy in the presence of 5  $\mu$ M BYL719 or not. Displacement vectors obtained using Particle Image Velocimetry are overlaid in green.

**Video S4.** Collective migration of double-mutant cells is observed using phase contrast microscopy in the presence of 5  $\mu$ M BYL719 or not. Displacement vectors obtained using Particle Image Velocimetry are overlaid in green.

**Video S5.** Single cell migration of parental, *PIK3CA* H1047R, *KMT2D* KO or double-mutant cell lines when depleted or not of ARPC5L.

**Table S1.** Primers used for qRT-PCR of genes described to be regulated by either *PIK3CA* H1047R or *KMT2D* mutations in breast cancer.

| Gene name     | Regulated by    | Primer forward           | Primer reverse            |
|---------------|-----------------|--------------------------|---------------------------|
| <i>CYFIP2</i> | KMT2D mut       | CCCACGTCATGGAGGTGTACTCT  | TAATTGTAGCGTGTGGCTCTCTCA  |
| <i>RAC3</i>   | KMT2D mut       | CGTGGGGAAGACATGCTTGCT    | GGCAGAGTAGTTGTCAAAAACGGTG |
| <i>CCND1</i>  | PI3KCA (H1047R) | GGATGCTGGAGGTCTGCGA      | AGAGGCCACGAACATGCAAG      |
| <i>CDKN1B</i> | PI3KCA (H1047R) | GAGGGATCAAAGCCTGGAACAT   | CGATTCTGTACCTCAACATCCCAT  |
| <i>PLAUR</i>  | PI3KCA (H1047R) | ACGGGGTTAGCGGAGCAAT      | TGTTTTGAGTAGAAGAATCGTCGGT |
| <i>MMP14</i>  | PI3KCA (H1047R) | ACACCTGCGTCCCAGCCTCT     | CGCACTCTTCCACACGGCA       |
| <i>ITGA2</i>  | PI3KCA (H1047R) | CAACATTGGAGGAGACACCCACT  | CCAGGAAGATGTCATTTCCATTCA  |
| <i>ITGA3</i>  | PI3KCA (H1047R) | AGGTGCCTGCAGAAGAATATGGT  | GACAACATCAGAGGGCTCCTGTAT  |
| <i>ITGA5</i>  | PI3KCA (H1047R) | ACCCTGCCGCTCAGATTTCA     | ACCTAAAACCACACGGCCAGTCT   |
| <i>ARPC5L</i> | PI3KCA (H1047R) | CATGCAGCCTTGCGGAACCTCT   | CCCTGGGCTCGCTCCTTCA       |
| <i>RAC2</i>   | PI3KCA (H1047R) | TGGCCAAGGAGATTGACTCGGT   | GGCCTCTCTGGGTGAGAGCTGA    |
| <i>VIM</i>    | PI3KCA (H1047R) | TCAGACAGGATGTTGACAATGCGT | CTGCAGCTCCTGGATTTCTCTT    |
| <i>ZEB1</i>   | PI3KCA (H1047R) | ACAGTGTTACCAGGGAGGAGCAGT | TTTCTTGCCCTTCCTTTCTGTCAT  |