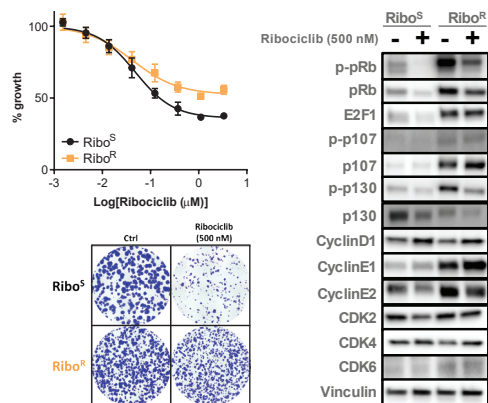


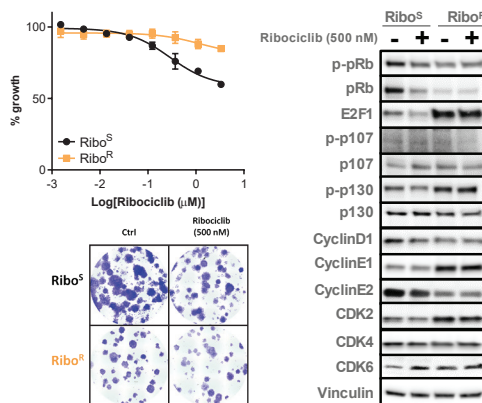
**Supplementary Figure 1.**

**A**

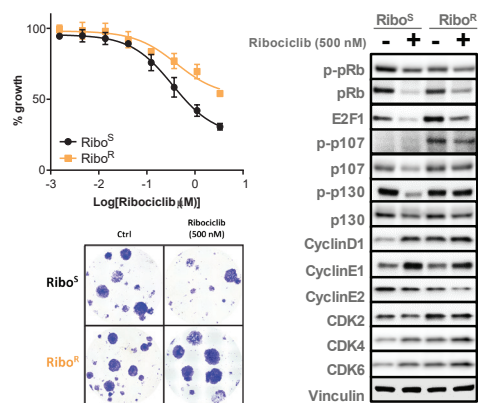
**ESR1<sup>wt</sup>**



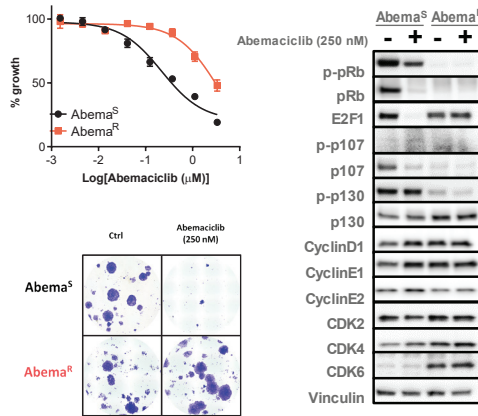
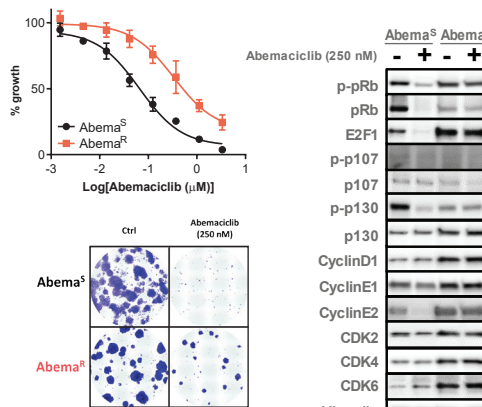
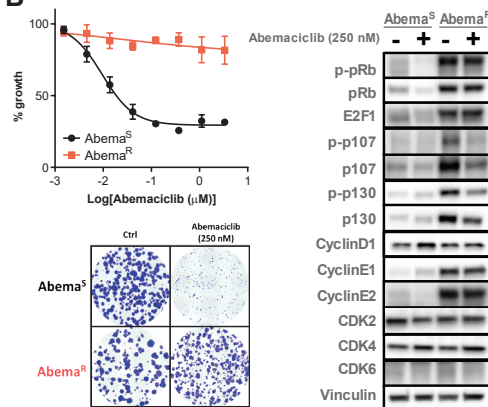
**ESR1<sup>mut</sup>: D538G**



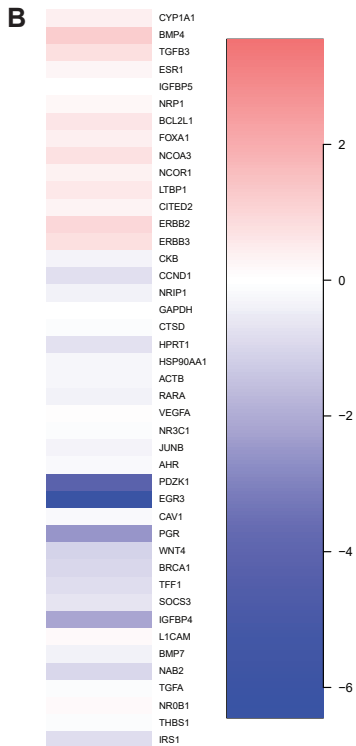
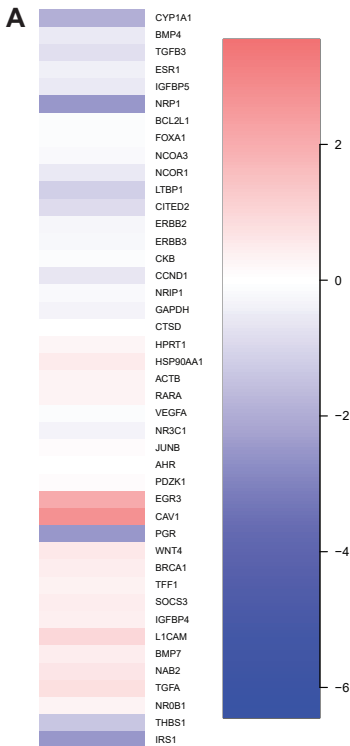
**ESR1<sup>mut</sup>: Y537S**



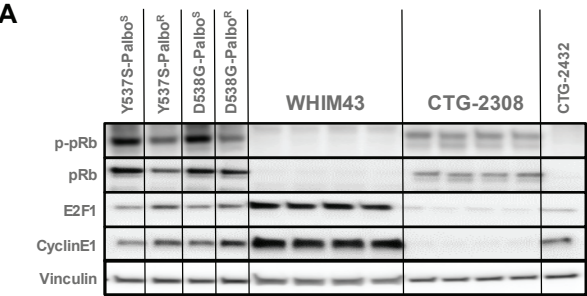
**B**



# Supplementary Figure 2.



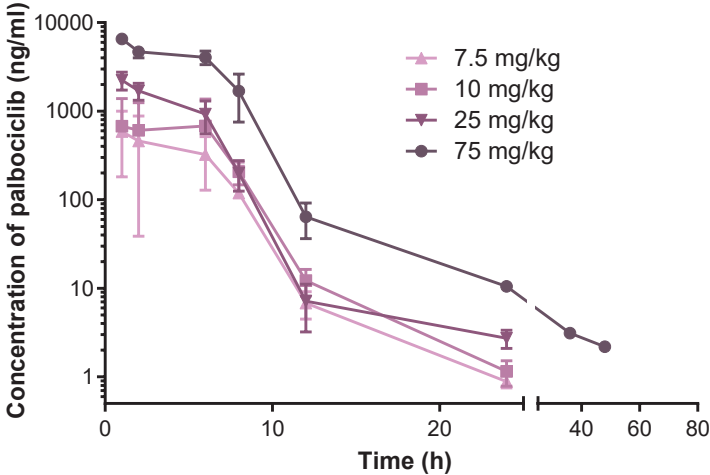
Supplementary Figure 3.



**B**

| PDX      | Treatment history                                           | Palbociclib response in clinic   | Palbociclib response in PDX model |
|----------|-------------------------------------------------------------|----------------------------------|-----------------------------------|
| WHIM43   | Tamoxifen, AI, fulvestrant, chemotherapy (PDX collection)   | not-treated                      | insensitive                       |
| CTG-2308 | Letrozole, letrozole+palbo, chemotherapy (PDX collection)   | no response                      | sensitive                         |
| CTG-2432 | Tamoxifen, fulvestrant+palbo, chemotherapy (PDX collection) | no response                      | insensitive                       |
| PDX-R1   | Tamoxifen, letrozole+palbo (PDX collection)                 | resistant after initial response | sensitive                         |
| ST3932   | Chemotherapy, fulvestrant, letrozole+palbo (PDX collection) | no response                      | insensitive                       |

Supplementary Figure 4.

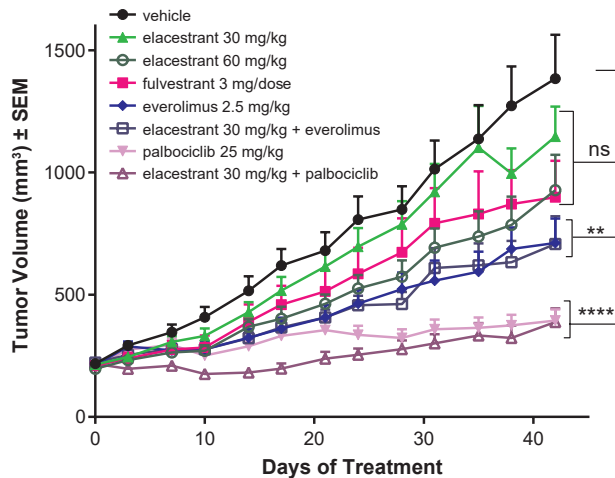


| Palbociclib PK                                     |                   |                      |
|----------------------------------------------------|-------------------|----------------------|
| Treatment                                          | AUC_inf (ng·h/mL) | AUC_inf animal/human |
| Palbociclib 7.5 mg/kg                              | 2880              | 1.16                 |
| Palbociclib 10 mg/kg                               | 4757              | 1.92                 |
| Palbociclib 25 mg/kg                               | 8334              | 3.35                 |
| Palbociclib 75 mg/kg                               | 53719             | 21.6                 |
| HUMAN Palbociclib 125 mg, single dose <sup>1</sup> | 2483              | n/a                  |

<sup>1</sup>Tamura K, et al. *Cancer Sci.* 2016;107:755-63.

**Supplementary Figure 5.**

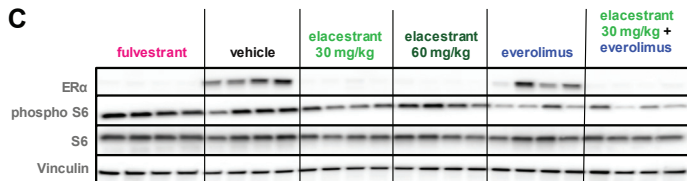
**A**



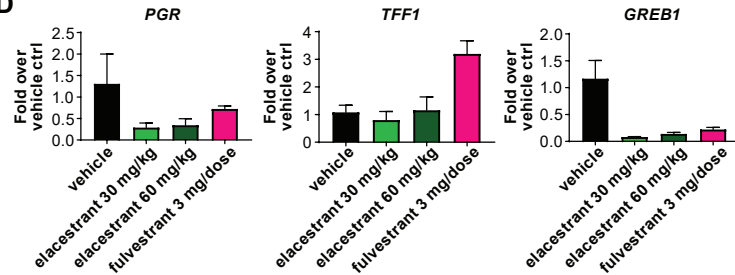
**B**



**C**



**D**



**Supplementary Table 1.**

| CDK4/6i resistant cell line models |                    | Rb and family members – transcriptional activators when phosphorylated |       |      |        |      |        | Transcription regulator | Cyclins – cell cycle transition |           |           | Cyclin-dependent kinases – cell cycle transition |      |      |
|------------------------------------|--------------------|------------------------------------------------------------------------|-------|------|--------|------|--------|-------------------------|---------------------------------|-----------|-----------|--------------------------------------------------|------|------|
|                                    |                    | pRb                                                                    | p-pRb | p107 | p-p107 | p130 | p-p130 | E2F1                    | cyclin D1                       | cyclin E1 | cyclin E2 | CDK2                                             | CDK4 | CDK6 |
| Wild-type                          | Palbo <sup>R</sup> | N.C.                                                                   | N.C.  | ↑    | N.D.   | N.C. | ↓      | N.C.                    | ↑                               | ↑         | N.C.      | N.C.                                             | N.C. | N.D. |
|                                    | Ribo <sup>R</sup>  | ↑                                                                      | ↑     | ↑    | ↑      | ↑    | ↑      | ↑                       | N.C.                            | ↑         | ↑         | N.C.                                             | N.C. | N.D. |
|                                    | Abema <sup>R</sup> | ↑                                                                      | ↑     | ↑    | ↑      | ↑    | ↑      | ↑                       | N.C.                            | ↑         | ↑         | N.C.                                             | N.C. | N.D. |
| D538G                              | Palbo <sup>R</sup> | ↓                                                                      | ↓     | ↓    | N.D.   | N.C. | ↑      | ↑                       | ↓                               | ↑         | ↑         | N.C.                                             | N.C. | ↑    |
|                                    | Ribo <sup>R</sup>  | ↓                                                                      | ↓     | N.C. | N.D.   | N.C. | ↑      | ↑                       | ↓                               | ↑         | ↓         | ↑                                                | N.C. | N.C. |
|                                    | Abema <sup>R</sup> | ↓                                                                      | N.C.  | N.C. | N.D.   | ↑    | ↑      | ↑                       | ↑                               | ↑         | ↑         | N.C.                                             | ↑    | ↑    |
| Y537S                              | Palbo <sup>R</sup> | ↓                                                                      | ↓     | ↑    | N.C.   | N.C. | N.C.   | ↑                       | ↑                               | ↑         | ↑         | N.C.                                             | N.C. | ↑    |
|                                    | Ribo <sup>R</sup>  | N.C.                                                                   | N.C.  | N.C. | ↑      | N.C. | N.C.   | ↑                       | ↑                               | N.C.      | N.C.      | ↑                                                | ↑    | ↑    |
|                                    | Abema <sup>R</sup> | ↓                                                                      | ↓     | ↓    | N.D.   | N.C. | ↓      | N.C.                    | ↑                               | ↑         | N.C.      | N.C.                                             | ↑    | ↑    |

↑, upregulated; ↓, downregulated; N.C., no change; N.D., not detected