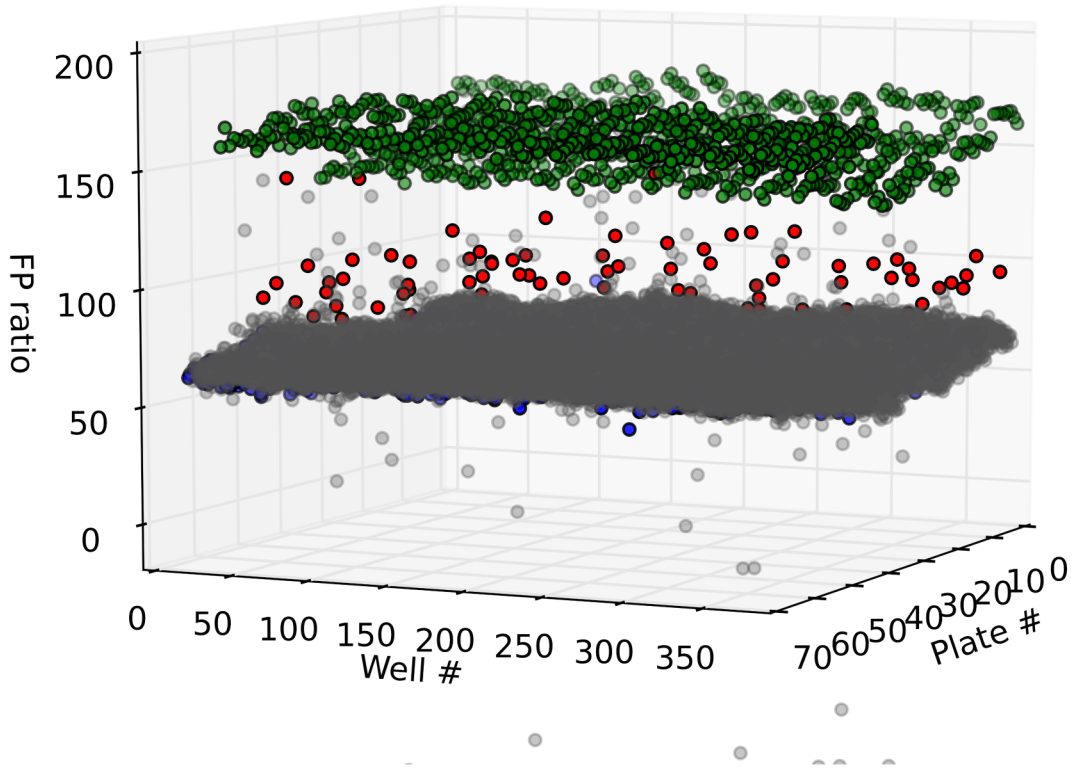
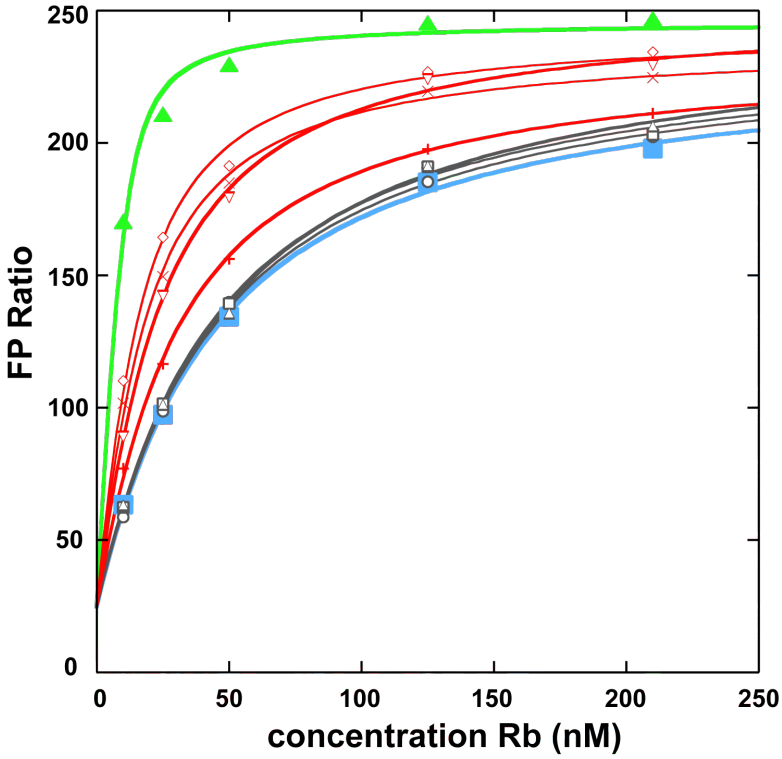
Supporting Figure S1



Supporting Figure 1. FP ratio for each well in the high throughput screen of the ChemDiv library. The wells contain phosphorylated Rb^{NP} and compounds (grey circles), phosphorylated Rb^{NP} and DMSO (blue circles, "negative control"), or unphosphorylated Rb^{NP} (green circles, "positive control"). The red circles are hits that increase the FP ratio of $E2F^{TMR}$ in the presence of phosphorylated Rb^{NP} and satisfy our total fluorescence criterion.

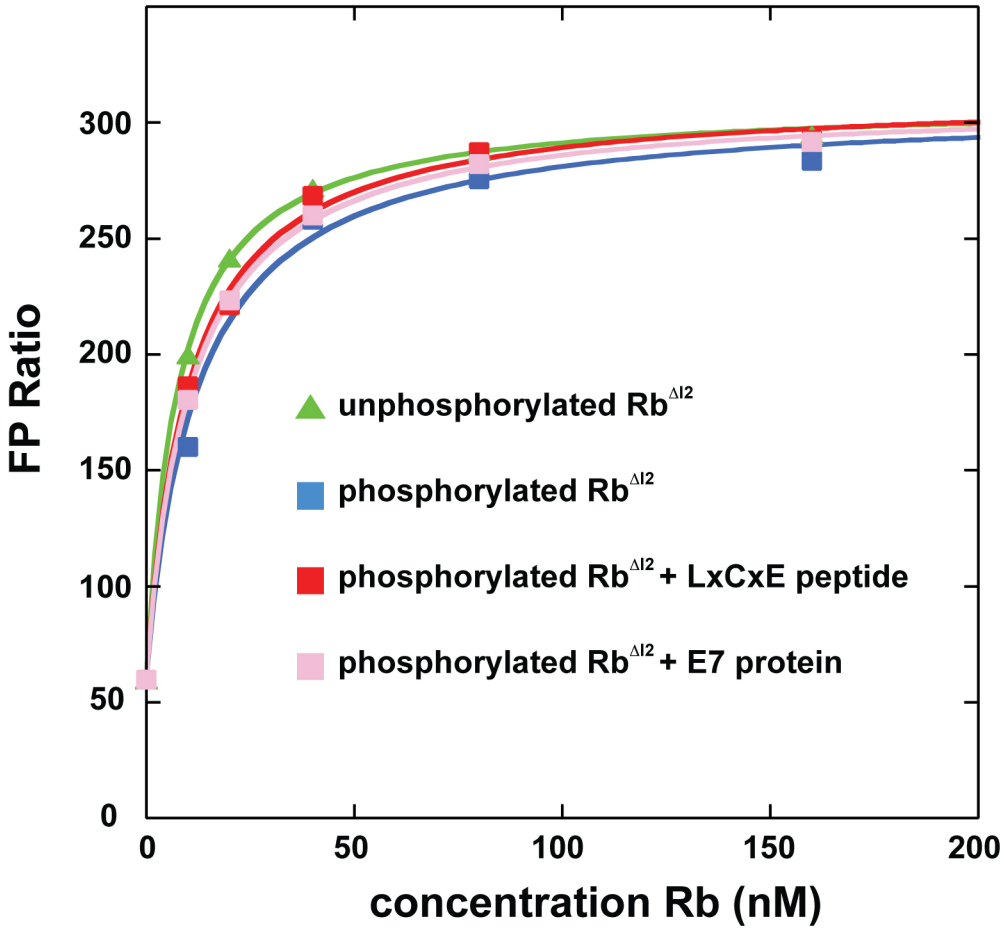
Supporting Figure S2



		$E2F^{TMR}$ K_d (nM)
▲	unphosphorylated Rb^{NP}	2.2 ± 0.6
■	phosphorylated Rb^{NP}	42 ± 3
phosphorylated Rb^{NP} + hit compound #		
○	23	40 ± 2
□	41	39 ± 3
▽	67	19 ± 1
◇	68	11 ± 1
△	69	39 ± 3
+	70	26 ± 2
×	72	13 ± 1

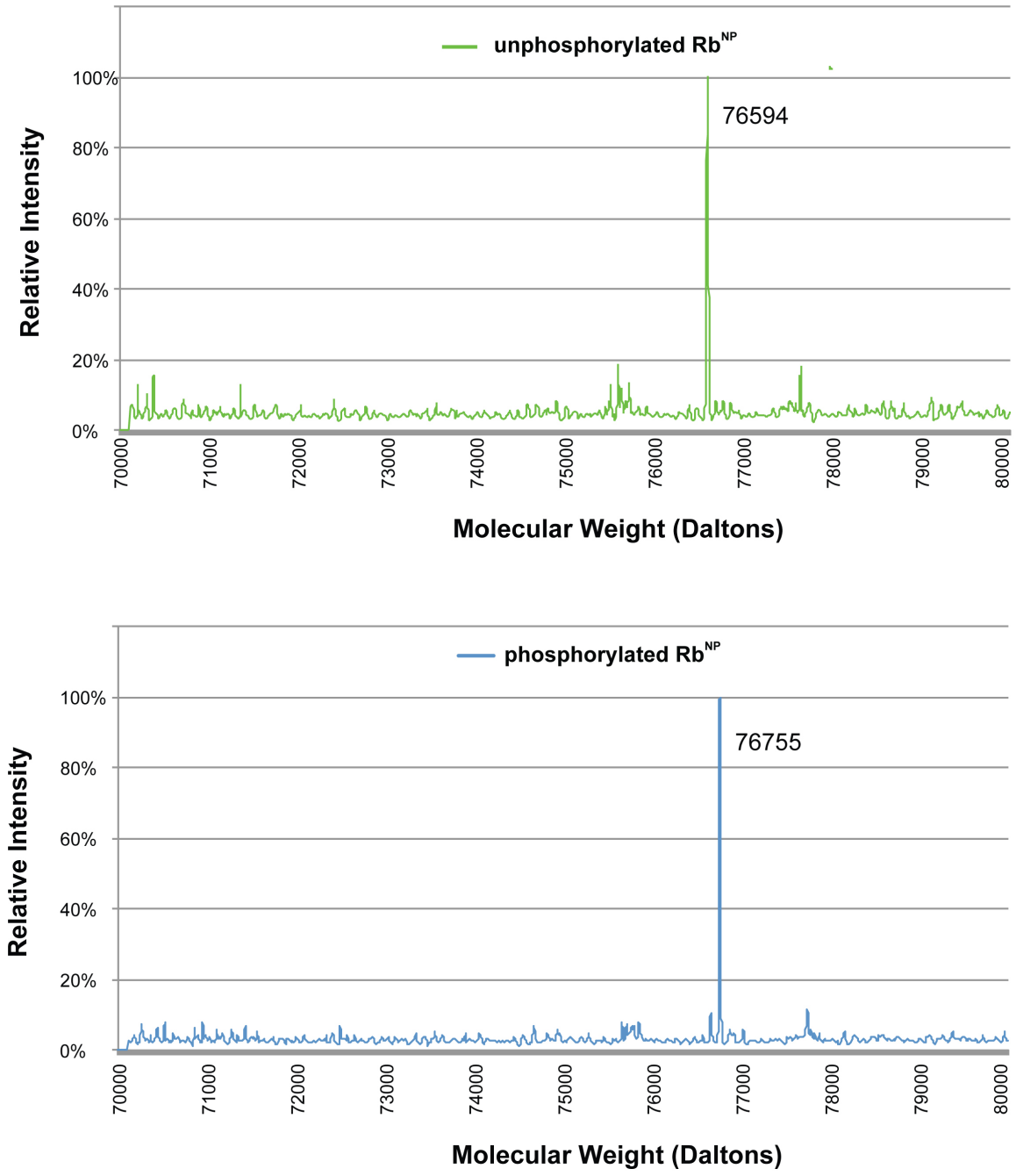
Supporting Figure 2. Effect of validated primary screen hits in a complete Rb^{NP} protein titration. FP ratios were measured and K_d values for $E2F^{TMR}$-Rb^{NP} binding were determined as in Figure 3a.

Supporting Figure S3



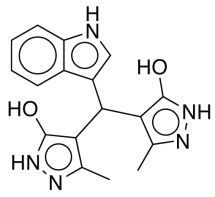
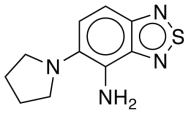
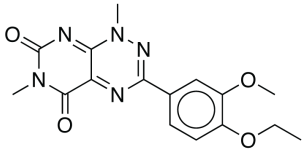
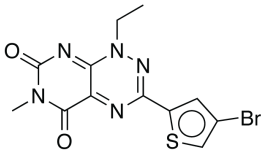
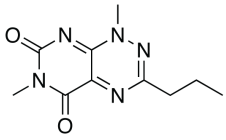
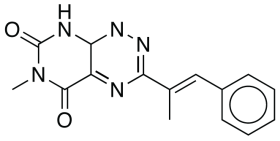
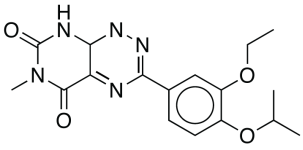
Supporting Figure 3. The LxCxE peptide and E7 protein activators do not affect E2F binding to an Rb mutant in which interdomain docking is disrupted. Binding measurements are shown of $E2F^{TMR}$ to an Rb^{NP} construct containing Q736A and K740A mutations ($Rb^{\Delta I2}$). As described previously[14], E2F binding to the unphosphorylated (K_d=7.5 ± 0.3 nM) and phosphorylated (K_d=12 ± 2 nM) mutant are similar. Addition of 2 μM LxCxE peptide (K_d=10 ± 1 nM) or E7 protein (K_d=10.5 ± 0.3 nM) does not increase E2F affinity, indicating that the activating effect is not independent of interdomain docking. We confirmed using ITC that the LxCxE peptide still binds $Rb^{\Delta I2}$ with K_d=180 ± 20 nM.

Supporting Figure S4



Supporting Figure 4. Electrospray ionization mass spectra of intact samples of unphosphorylated and phosphorylated Rb^{NP}. Samples were prepared by desalting. Each spectrum shows a single, dominant peak at the indicated molecular weight. The ~160 Da increase in the phosphorylated sample corresponds to addition of two phosphates.

Supporting Table

Structure	Hit Compound No.
	**23**
	**41**
	**67**
	**68**
	**69**
	**70**
	**72**

Supporting Table. Structures of validated hits in the primary screen.