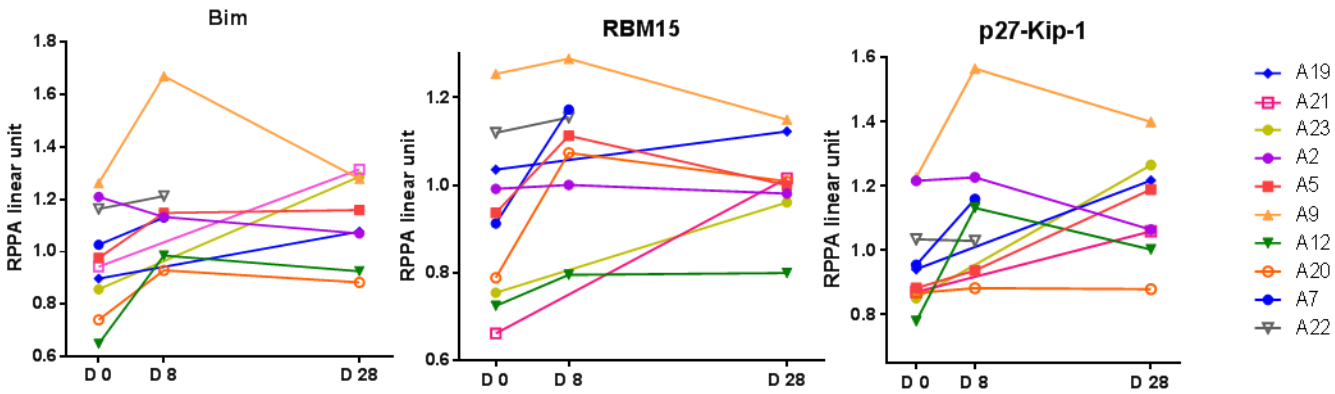
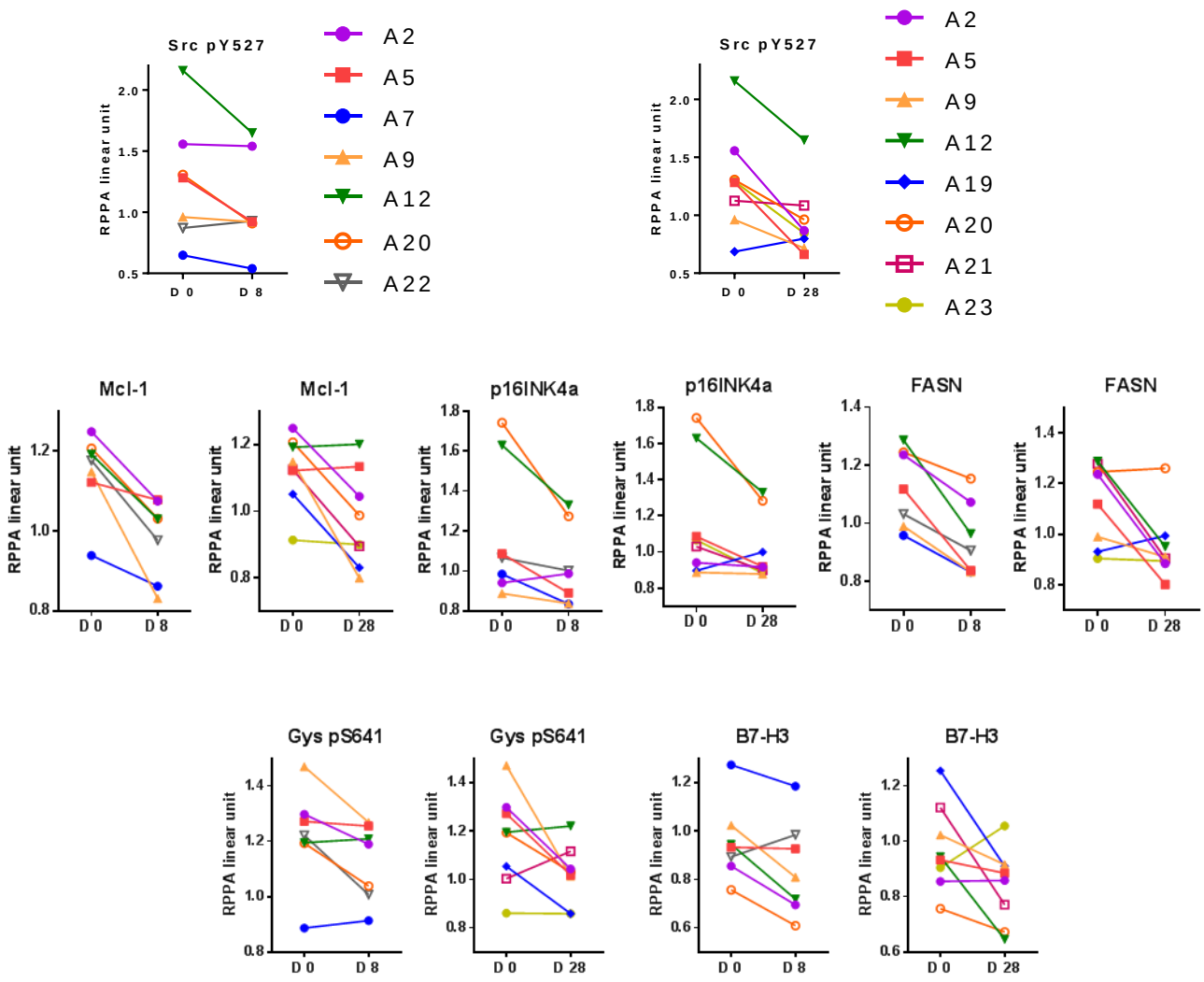
Supplemental Figure 1



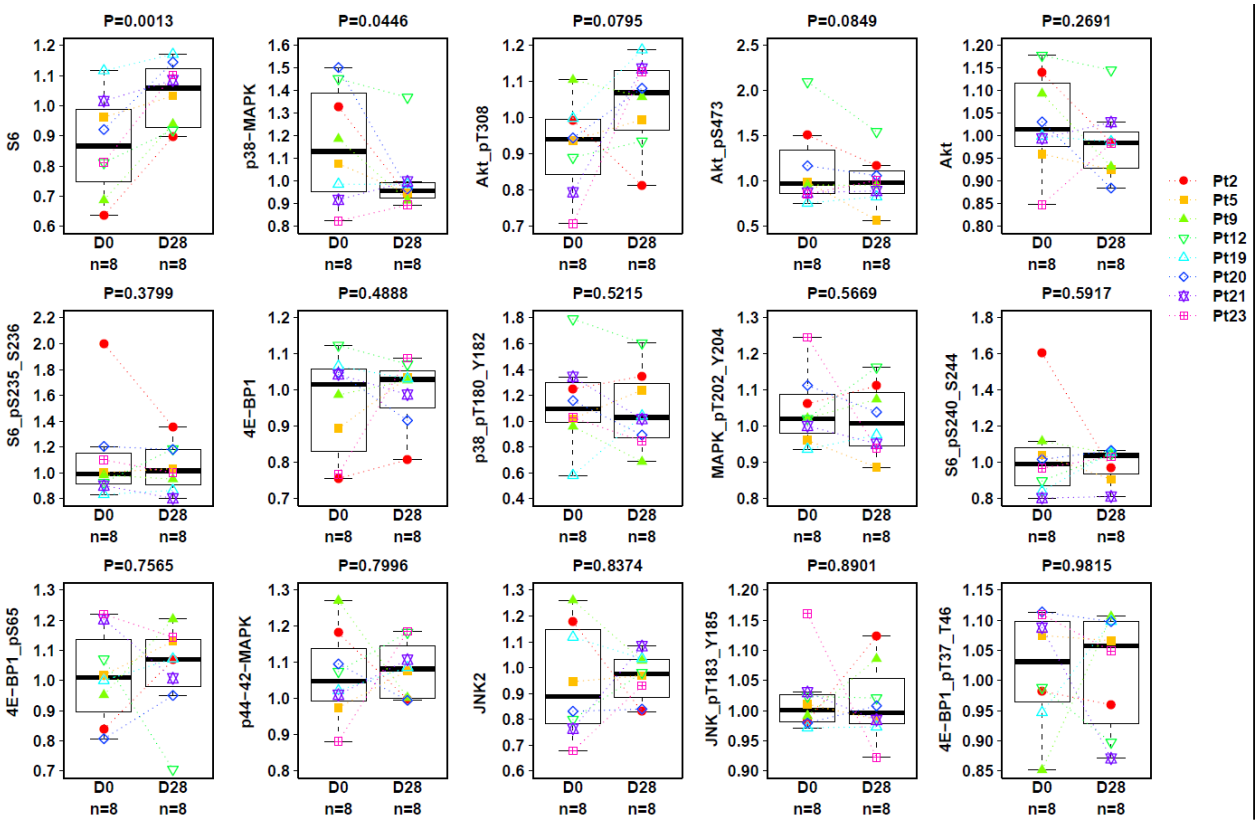
Supplemental Figure 1. Increase in the protein levels during acalabrutinib therapy. Lymphocytes were isolated from CLL patients before and after 8 and 28 days of acalabrutinib therapy. Cell lysates were prepared and RPPA assay was performed. Three proteins (Bim, RBM15, and p27-Kip-1) were significantly increased on both D8 and D28.

Supplemental Figure 2



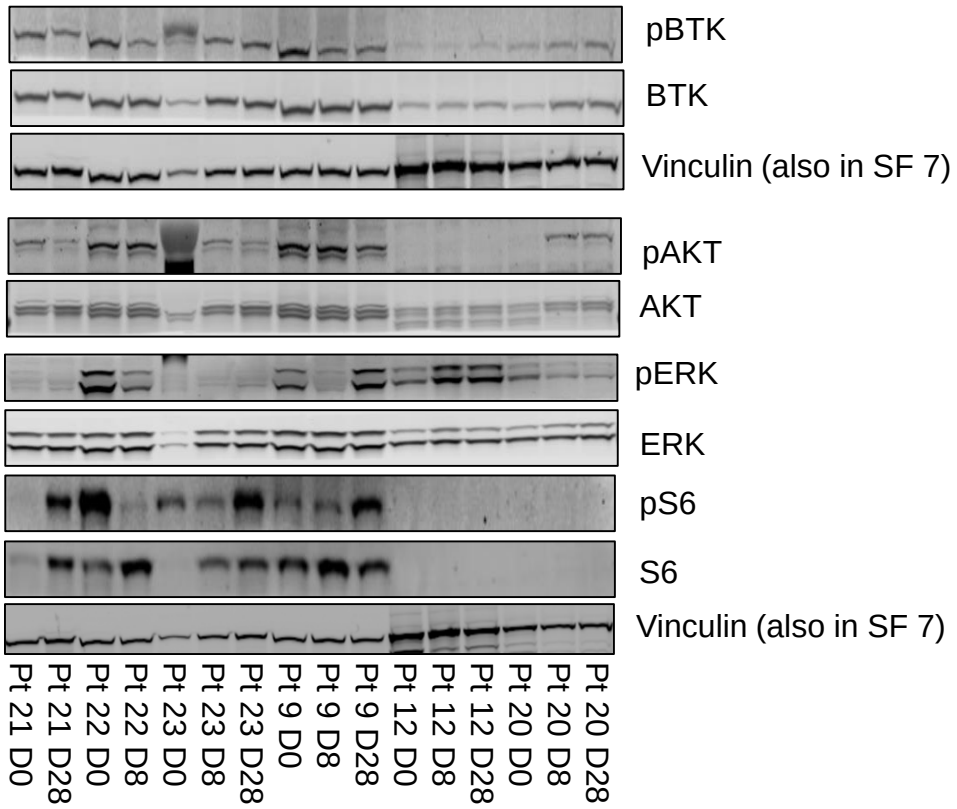
Supplemental Figure 2. Decrease in the protein levels during acalabrutinib therapy. Lymphocytes were isolated from CLL patients before and after 8 and 28 days of acalabrutinib therapy. Cell lysates were prepared and RPPA assay was performed. Six proteins that were decreased both at D8 and D28 are phospho-Src, Mcl-1, p16INK4a, FASN, phospho-Gys, and B7-H3.

Supplemental Figure 3



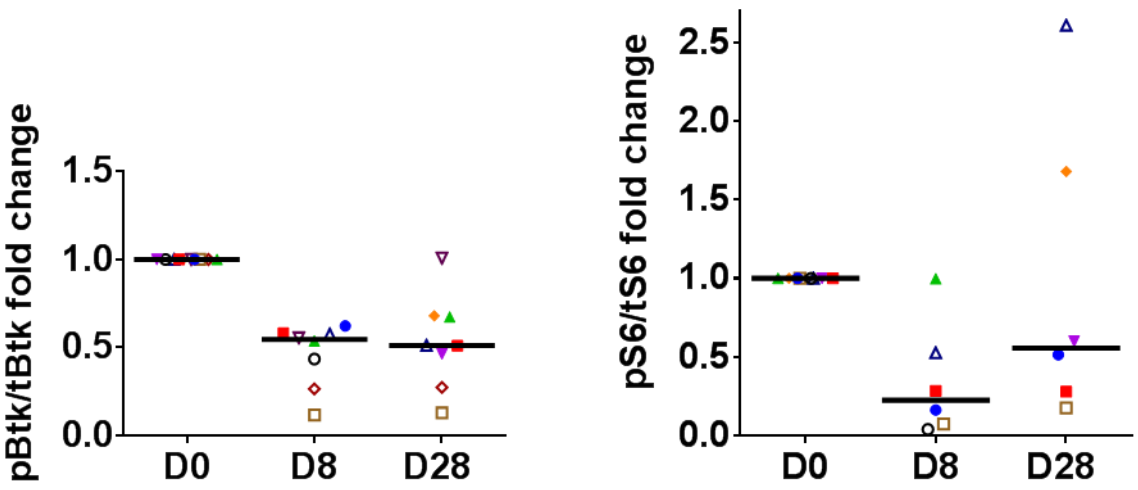
Supplemental Figure 3. Changes in B-cell receptor pathway protein levels during acalabrutinib therapy. Lymphocytes were isolated from CLL patients before and after 28 days of acalabrutinib therapy. Cell lysates were prepared and RPPA assay was performed. Fifteen proteins in this array were relevant to BCR pathway. Panels are arranged based on p values.

Supplemental Figure 4



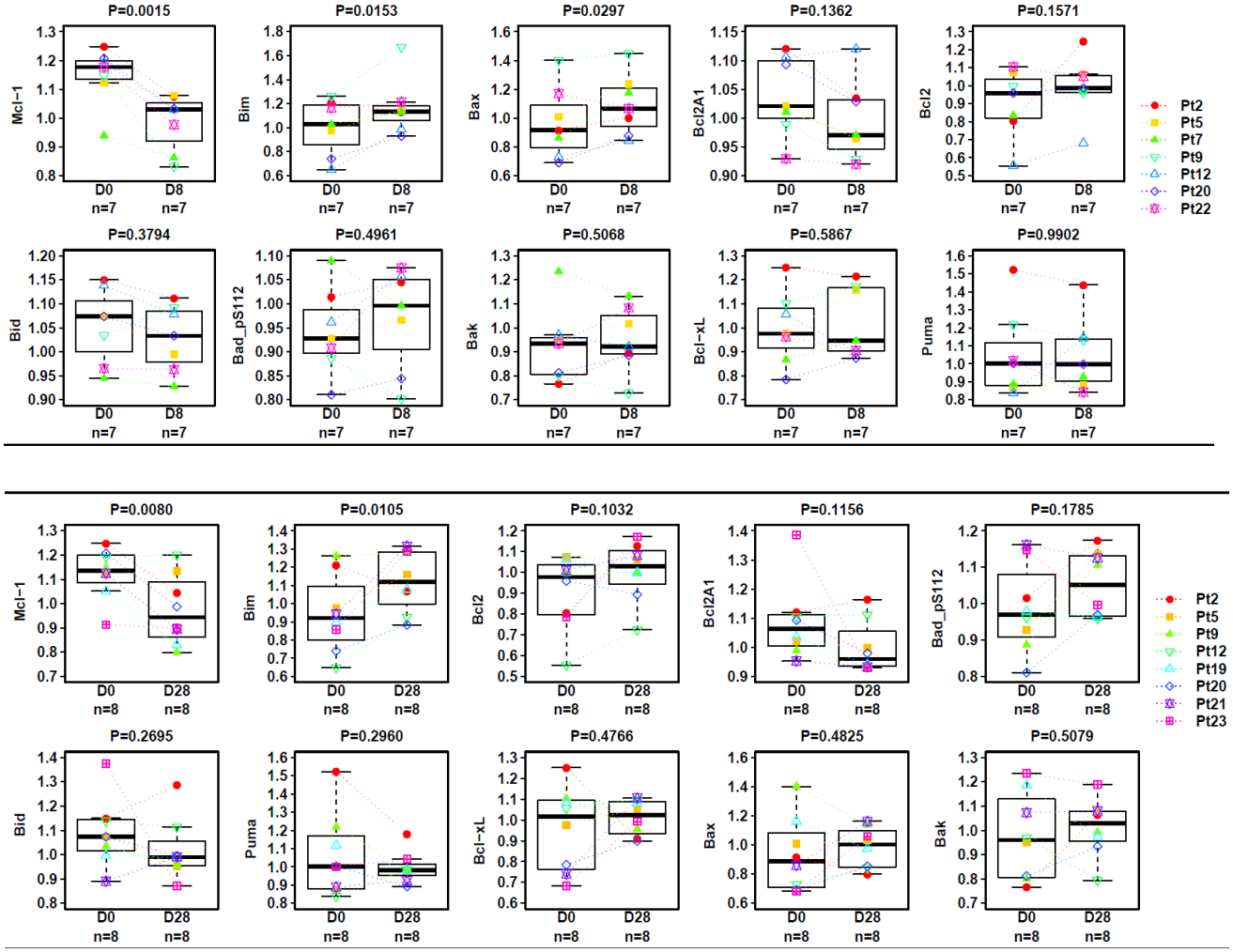
Supplemental Figure 4. Changes in BTK and BCR pathway proteins during acalabrutinib therapy. Lymphocytes were isolated from CLL patients before and after 8 or 28 days of acalabrutinib therapy. Cell lysates were prepared and immunoblotted for respective proteins. Vinculin was used as a loading control. To create this immunoblot, two gels were run. First contained p and total BTK along with Bcl-XL, Mcl-1, Bax and Bim (Lower portion of Supplemental Figure 7). The second gel was used for Bcl-2 and Puma along with total and phospho AKT, ERK, and S6 (Upper portion was used in Supplemental Figure 7). SF = Supplemental Figure.

Supplemental Figure 5



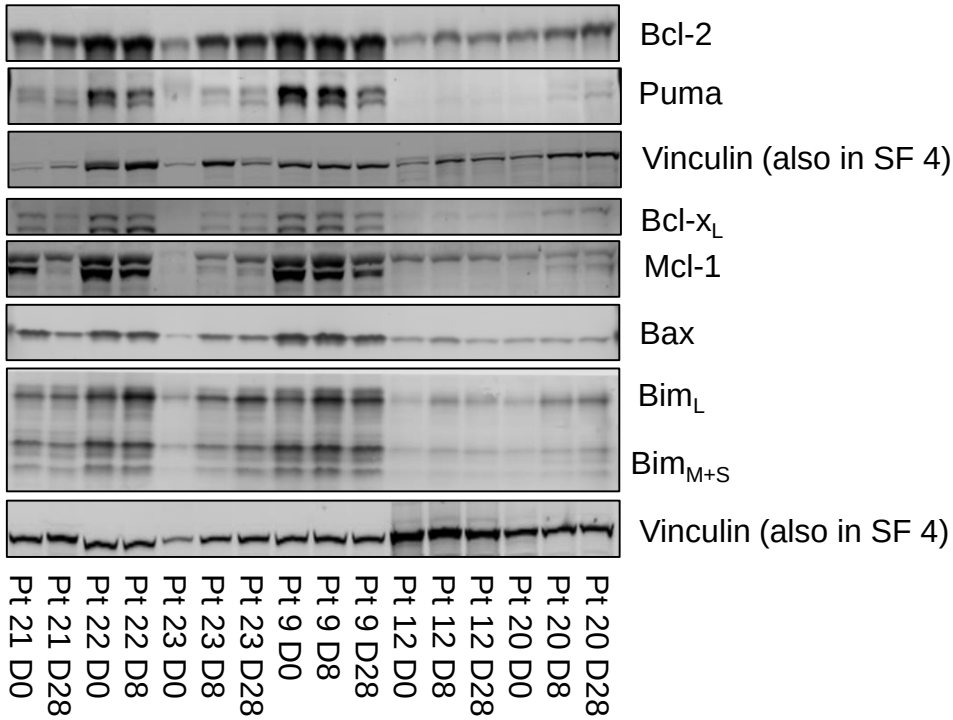
Supplemental Figure 5. Changes in pBTK and pS6 proteins during acalabrutinib therapy. Lymphocytes were isolated from CLL patients before and after 8 or 28 days of acalabrutinib therapy. Cell lysates were prepared and immunoblotted for respective proteins as presented in Supplemental Figure 4. Proteins were quantitated, normalized to vinculin and ratio of phospho to total or vinculin to total is presented.

Supplemental Figure 6



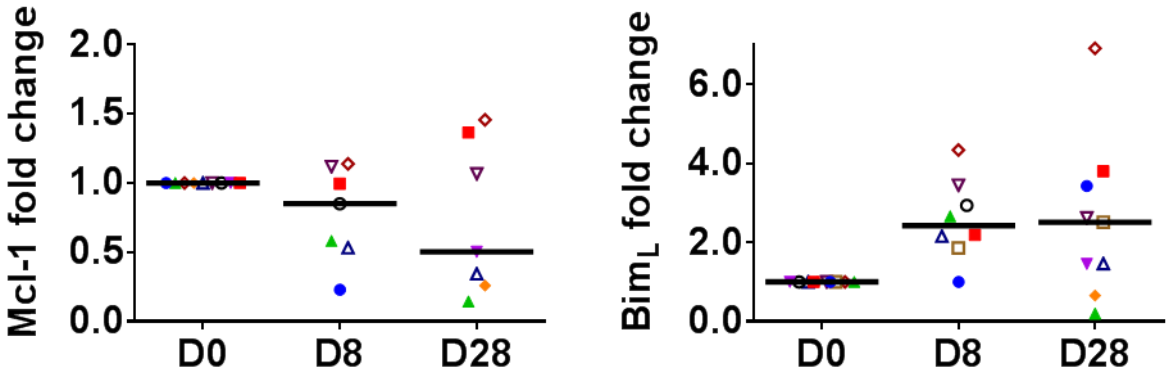
Supplemental Figure 6. Changes in Bcl-2 family protein levels during acalabrutinib therapy. Lymphocytes were isolated from CLL patients before and after 8 or 28 days of acalabrutinib therapy. Cell lysates were prepared and RPPA assay was performed. There were 10 proteins in this array that belong to Bcl-2 family. Top panels of ten proteins are D0 versus D8. Bottom panels of ten proteins are D0 versus D28.

Supplemental Figure 7



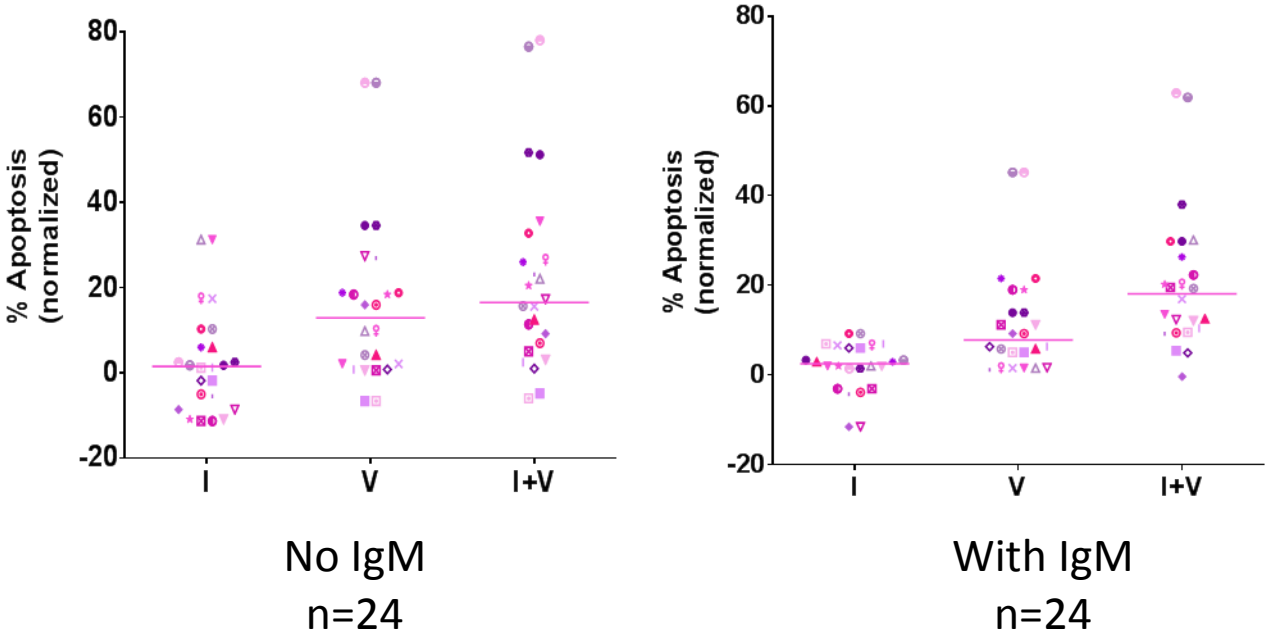
Supplemental Figure 7. Changes in Bcl-2 family protein levels during acalabrutinib therapy. Lymphocytes were isolated from CLL patients before and after 8 or 28 days of acalabrutinib therapy. Cell lysates were prepared and immunoblotted for respective proteins. Vinculin was used as a loading control. To create this immunoblot, two gels were run. First contained p and total BTK along with Bcl-XL, Mcl-1, Bax and Bim (Upper portion of Supplemental Figure 4). The second gel was used for Bcl-2 and Puma along with total and phospho AKT, ERK, and S6 (Lower portion was used in Supplemental Figure 4). SF = Supplemental Figure.

Supplemental Figure 8



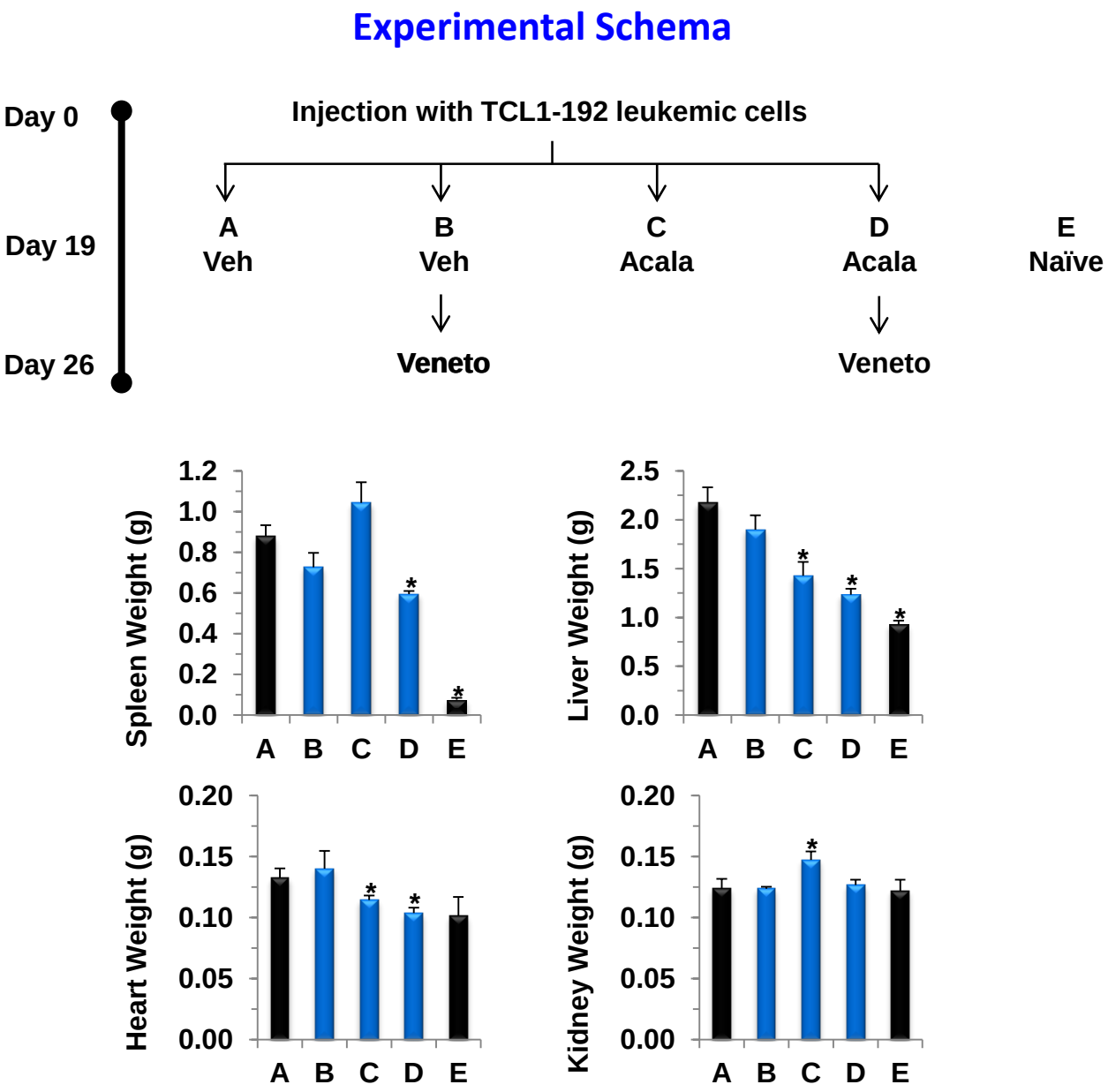
Supplemental Figure 8. Changes in total Mcl-1 and total Bim proteins during acalabrutinib therapy. Lymphocytes were isolated from CLL patients before and after 8 or 28 days of acalabrutinib therapy. Cell lysates were prepared and immunoblotted for respective proteins as presented in Supplemental Figure 7. Proteins were quantitated, normalized to vinculin and expressed as fold change compared to control.

Supplemental Figure 9



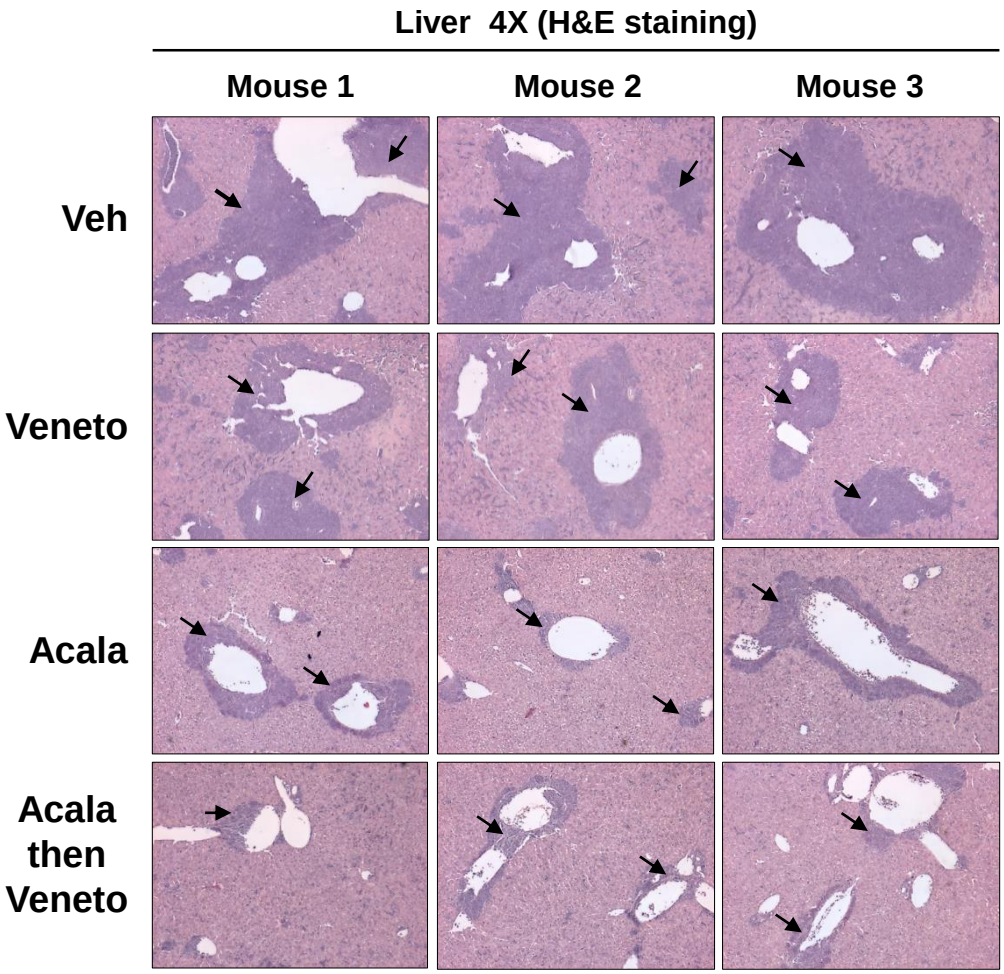
Supplemental Figure 9. In vitro cytotoxicity with ibrutinib (I), venetoclax (V) and combination (I+V). Lymphocytes were isolated from CLL patients (n = 24) and in vitro treated with or without IgM followed by ibrutinib alone, venetoclax alone or in combination for 24 hr. After incubation, cell were stained and analyzed for apoptosis using annexin/PI positivity.

Supplemental Figure 10



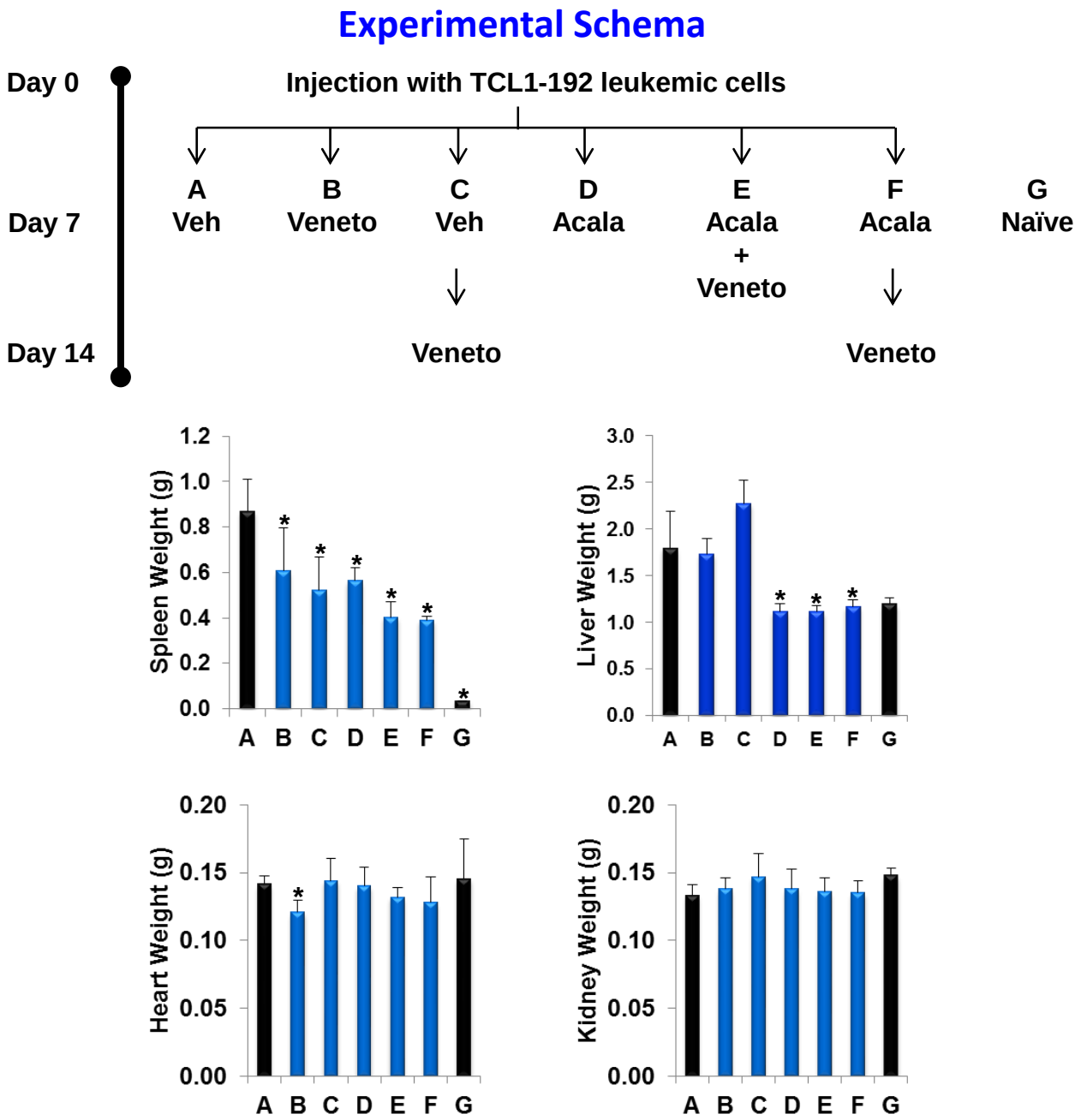
Supplemental Figure 10. Effect of acalabrutinib and venetoclax combination on different organs in the TCL1 adoptive transfer mouse model. SCID mice (n = 40) were injected with CLL TCL1-192 leukemic cells on day 0. On day 19 post-injection, the mice were treated with vehicle (Veh) or acalabrutinib (Acala) and on day 26 venetoclax (Veneto) was administrated as indicated. On Day 35, SCID (naïve) mice and mice from each treatment group (A-E; see Experimental Schema) were sacrificed for necropsy and histology analysis. The indicated organs were harvested and their weights were recorded. **P*<0.05 compared to vehicle.

Supplemental Figure 11



Supplemental Figure 11. Effect of acalabrutinib and venetoclax combination on liver infiltrates with leukemic cells in the TCL1 adoptive transfer mouse model. SCID mice (n = 40) were injected with CLL TCL1-192 leukemic cells on day 0. On day 19 post-injection, the mice were treated with vehicle (Veh) or acalabrutinib (Acala) and on day 26 venetoclax (Veneto) was administrated as indicated. On Day 35, mice from each treatment group were sacrificed for necropsy and histology analysis. Liver was stained with hematoxylin and eosin (H&E). Black arrows indicate infiltration of leukemic cells.

Supplemental Figure 12



Supplemental Figure 12. Effect of acalabrutinib and venetoclax combination on different organs in the TCL1 adoptive transfer mouse model following different scheduling treatments. Experimental schema depicts that SCID mice (n=90) were injected with CLL TCL1-192 on day 0 then divided into 6 groups (n=15). On day 7 and/or day 14, the indicated groups received vehicle (Veh), acalabrutinib (Acala), and/or venetoclax (Veneto). On Day 35, SCID (naïve) mice and mice from each treatment group were sacrificed for necropsy and histology analysis. The indicated organs were harvested and their weights were recorded. **P*<0.05 compared to vehicle.